Comprehensive Review in Current Developments of Imidazole-Based Medicinal Chemistry

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Abstract: Imidazole ring is an important five-membered aromatic heterocycle widely present in natural products and synthetic molecules. The unique structural feature of imidazole ring with desirable electron-rich characteristic is beneficial for imidazole derivatives to readily bind with a variety of enzymes and receptors in biological systems through diverse weak interactions, thereby exhibiting broad bioactivities. The related research and developments of imidazole-based medicinal chemistry have become a rapidly developing and increasingly active topic. Particularly, numerous imidazole-based compounds as clinical drugs have been extensively used in the clinic to treat various types of diseases with high therapeutic potency, which have shown the enormous development value. This work systematically gives a comprehensive review in current developments of imidazole-based compounds in the whole range of medicinal chemistry as anticancer, antifungal, antibacterial, antitubercular, anti-inflammatory, antineuropathic, antihypertensive, antihistaminic, antiparasitic, antiobesity, antiviral, and other medicinal agents, together with their potential applications in diagnostics and pathology. It is hoped that this review will be helpful for new thoughts in the quest for rational designs of more active and less toxic imidazole-based medicinal drugs, as well as more effective diagnostic agents and pathologic probes.

Key words: imidazole; anticancer; antifungal; antineuropathic; antihistaminic

1. INTRODUCTION

Ever since the discovery of imidazole as early as the 1840s, the research and developments of imidazole-based compounds have been quite a rapidly developing and increasingly active field due to their wide potential applications as medicinal drugs, agrochemicals, man-made materials, artificial acceptors, supramolecular ligands, biomimetic catalysts, and so on.\(^{1-5}\) Especially, the
applications of imidazole derivatives in medicinal chemistry have achieved great progress. Many imidazole-based clinical drugs have been playing a vital role in the treatment of various types of diseases, and new imidazole derivatives with medicinal value are being actively exploited worldwide. Imidazole ring, a five-membered aromatic framework containing two nitrogen atoms, is an amphoteric and highly polar heterocycle. It exists in two equivalent tautomeric forms, in which the hydrogen atom can be located on either of the two nitrogen atoms. Furthermore, the electron-rich nitrogen heterocycle could not only readily accept or donate proton, but also easily form diverse weak interactions. These special structural characteristics of imidazole ring are beneficial for its derivatives to readily bind with a variety of enzymes and receptors in biological systems via hydrogen bonds, coordination, ion–dipole, cation–π, π–π stacking, hydrophobic effects, van der Waals forces, and so on, thereby exhibiting broad bioactivities. In fact, imidazole ring is prevalently present in naturally occurring products and a range of bioactive substances in the human metabolism. The phenomenon that nature selects this unique type of imidazole ring in numerous biological molecules such as histamine, vitamin B₁₂, deoxyribonucleic acid (DNA), and hemoglobin for exerting various biological functions shows that the imidazole ring should be vital to the physiological action for important biological activities. These specific physiological properties and unusually important roles in vital processes have been attracting special interest in imidazole-based medicinal chemistry. The presence of imidazole ring in interesting compounds may be favorable for improving water solubility to some extent due to its two nitrogen atoms easily leading to the formation of hydrogen bonds. The imidazole ring has also been identified as an attractive isostere of triazole, oxazole, pyrazole, thiazole, tetrazole, amide, etc., and extensively used to design and develop various bioactive molecules. More importantly, imidazole ring with multiple binding sites is capable of coordinating with a variety of inorganic metal ions or interacting with organic molecules via noncovalent bonds to produce supramolecular drugs, which may have not only bioactivities of imidazoles themselves, but also the advantages of numerous supramolecular drugs, possibly exerting double action mechanisms that are helpful to overcome drug resistances. On the other hand, imidazole ring as an attractive binding site could interact with diverse anion and cation ions as well as biological molecules in the human body. Therefore, imidazole ring has been frequently incorporated into fluorescent skeleton to generate artificial fluorescent compounds as diagnostic agents and pathologic probes to monitor the biochemical process of biologically important ions and molecules in living systems for the understanding of biological phenomena. All the above mentions show the enormous potentiality of imidazole-based compounds in medicinal chemistry and a lot of increasing work has been directing toward their feasible prolific applications in diverse areas. Particularly, a large number of imidazole-based compounds as clinical drugs such as anticancer (dacarbazine, zoledronic acid, azathioprine, and tipifarnib), antifungal ( clotrimazole, miconazole, ketoconazole, and oxiconazole), antiparasitic (metronidazole, benzimidazole, ornidazole, and secnidazole), antihistaminic (cimetidine, imetit, immezip, and thioperamide), antineuropathic (nafimidone, fipamezole, and dexmedetomidine), and antihypertensive (losartan, eprosartan, and olmesartan) drugs have been widely used to treat various types of diseases with high therapeutic potency, which have shown the huge development value. This has been strongly promoting much effort to focus on imidazole-based medicinal agents, and the expanding research and developments have become a rapidly developing and increasingly active topic and almost extended to the whole range of medicinal field. Previously, some imidazole derivatives as medicinal agents were partly reviewed, but up to now no literature has systematically reported on the current situation in the developments of imidazole-based compounds. In view of this, combining with authors’ research and referring other work from literature, this review gives a comprehensive overview in current developments of imidazole-based compounds in the whole range of medicinal chemistry as anticancer, antifungal, antibacterial, antitubercular, antiparasitic, antihistaminic, antineuropathic, antihypertensive, anti-inflammatory, antiobesity, antiviral, and
other medicinal agents, as well as their potential applications in diagnostics and pathology. The successful strategies and structure–activity relationships are discussed. The perspectives of the foreseeable future in the new trend of imidazoles in medicinal chemistry are also presented.

2. IMIDAZOLES AS ANTICANCER AGENTS

Cancer is one of the most serious threats to human health, which has drawn unusual attention all over the world. Extensive research has been devoted to the development of effective anticancer therapeutics, involving an integrated employment of surgical techniques, radiation therapy, and chemotherapy. A lot of breakthroughs and great strides have been made over the past 60 years. In spite of the great quantity of clinically used anticancer drugs including natural products (e.g., camptothecins, taxols) and synthetic compounds such as alkylating agents (e.g., mechloretaminoxide, chlorambucil), porphyrin drugs (e.g., photofrin, visudyne), azole agents (e.g., letrozole, fadrozole), and inorganic metal complexes (e.g., cisplatin, carboplatin), the medicinal need remains largely unmet due to their diverse drawbacks including poor curative effect, low selectivity, high cytotoxicity, and multidrug resistances. Lots of efforts have been therefore made in the identification of novel non-cross-resistant and more tumor-specific therapies able to selectively decrease the migration of cancer cells, and increase the sensitivity of tumor cells to cytotoxic drugs. Much research has revealed that imidazole derivatives as anticancer drugs possess considerable potentiality. Generally, imidazoles could interfere with DNA synthesis via weak interactions such as hydrogen bonds, coordination bonds, and π–π stacking and then halt cell growth and division. Meanwhile, imidazoles could easily bind to protein molecules compared with the other heterocyclic rings. Furthermore, some imidazole drugs, at high concentrations, could directly inhibit the synthesis of essential cell membrane components without interference with sterols and sterol esters. So far, many imidazole derivatives as anticancer drugs such as dacarbazine (1), zoledronic acid (2), azathioprine (3), tipifarnib (4), and nilotinib (5) have been widely used in the clinic (Fig. 1). They have been playing important roles in the treatment of various cancers. More importantly, continuous effort has been directing toward developing new imidazole-based anticancer agents targeting various enzymes or receptors such as topoisomerases, microtubule, cytochrome P450 enzyme, rapidly accelerated fibrosarcoma (RAF) kinases, transforming growth factor-β

![Figure 1. Some clinical imidazole-based anticancer drugs.](image-url)
(TGF-β), farnesyltransferase, and DNA. They have great potentiality to overcome the diverse drawbacks of currently available clinical drugs and to be developed as anticancer drugs.\textsuperscript{40–42}

\textbf{A. Imidazoles as Topoisomerase Inhibitors}

Topoisomerase (TOP) has been recognized as a valuable and particularly intriguing target for chemotherapy agents because of its critical roles in cell progression, apoptosis, transcription, and other cellular regulation. TOP targeting agents could not only stabilize the cleavable complex formed between enzyme and DNA, but also control the replication and transcription of DNA in malignant tumor cells. Many TOP inhibitors such as topotecan and irinotecan have been successfully developed and used in the clinic. However, the poor solubility, short action duration, and high toxicity associated with drug resistance hinder their continuous use. Much effort has been done to search for safer and more effective TOP inhibitors. Imidazole ring with two typical nitrogen atoms has the ability to form hydrogen bonds and thus is favorable to improve the water solubility of target compounds. For this reason, imidazole ring has been considered as a valuable structural fragment and been frequently introduced into other bioactive skeletons. The structures for imidazoles as TOP inhibitors are shown in Figure 2. Imidazole-containing indimitecan (6) is a clinical anticancer candidate with better water solubility to treat refractory solid tumors. In comparison to conventional Top I inhibitors, indimitecan without the labile lactone moiety has improved stability. Moreover, it could induce long-lasting DNA breaks and Top I cleavage at unique genomic positions, bind with different targeting site, and cause cell cycle arrest at both S and G(2)-M phases,\textsuperscript{43} which demonstrates robust potency to overcome drug resistance.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{imidazoles.png}
\caption{Imidazoles as topoisomerase inhibitors.}
\end{figure}

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Camptothecin (7a, CPT), a plant alkaloid from *Camptotheca acuminata*, is the first effective small molecule-type TOP I inhibitor. However, its poor water solubility largely limits the drug administration.\textsuperscript{44} Introduction of ethyl and imidazolyl groups into camptothecin 7a at 7- and 10-position yielded compound 7b. It was observed to possess improved water solubility and better TOP I inhibitory ability in comparison to camptothecin. Imidazole derivative 7b gave low IC\textsubscript{50} (50% inhibitory concentration) values of 0.16 and 0.06 \(\mu\)M against breast cancer cell line MCF-7 and colon cancer cell line HCT-8, which was comparable to topotecan (0.15 and 0.07 \(\mu\)M) but inferior to camptothecin (0.02 and < 0.01 \(\mu\)M), respectively. This phenomenon suggested that TOP I inhibitory activity was not absolutely correlated with the anticancer efficacy.\textsuperscript{45}

Research has disclosed that the E-lactone ring in camptothecin is instable and easily produces non-anticancer even toxic carboxylates in the human body. Camptothecin derivatives 8a and 8b with imidazolyl group at 20-position were found to have the ability to increase the stability of lactone ring and decrease the toxicity. Both of them displayed stronger anticancer potency than camptothecin toward human cancer cell lines PC-3, MCF-7, and HCT-8.\textsuperscript{46} This might be responsible for the convenience of their transport across lipid barriers and cell membranes as a result of the increased lipophilicity. The results provide practical guidance for the development of more stable and less toxic anticancer camptothecin derivatives.

Another strategy to overcome the lactone instability of camptothecin is to search for non-CPT TOP I inhibitors with similar specificity and potency.\textsuperscript{47,48} Aromathecins are a kind of important non-CPT TOP I inhibitors obtained via the replacement of hydroxyl lactone by benzene ring. Imidazole-modified aromathecin 9 was found to bind in the TOP I cleavage complex as interfacial inhibitor in a “camptothecin-like” pose. However, research also showed that the chlorine atom in this compound was unfavorable for its interaction with TOP I.\textsuperscript{49} Further work is necessary to investigate imidazole-based aromathecins with the aim to improve TOP I targeting affinity.

Indenoisoquinolines without the instable hydroxyl lactone are also a kind of important non-CPT TOP I inhibitors. Imidazole-derived indenoisoquinoline 10a displayed better water solubility and affinity toward DNA than indenone analog 10b due to the interaction of the incorporated NH moiety with the phosphates of DNA backbone. Nevertheless, compound 10a displayed weaker TOP I inhibitory activity than 10b, which was worthy of in-depth investigation.\textsuperscript{50,51}

Norindenoisoquinolines are also found to be a novel class of TOP I inhibitors with poor solubility. Imidazole-incorporated norindenoisoquinoline 11 showed good water solubility and improved anticancer efficacy toward MCF-7, IGROVI, HCT-116, and SN12C and the removal of imidazole ring resulted in low potency. The fact indicated that structural modification of norindenoisoquinoline by imidazole ring is an effective strategy to improve the physicochemical property and bioactivity. However, compound 11 displayed moderate Top I inhibitory activity, which further indicated that the TOP I inhibitory activity was not absolutely correlated with the anticancer efficacy as above mentioned.\textsuperscript{52}

It is well established that acridine-based compounds, such as amsacrine, are a class of DNA-TOP II inhibitors. They could intercalate into DNA base pair, thereby leading to cell cycle arrest and apoptosis. Acridine-based imidazole 12 was able to satisfy the stereochemical and electronic requirements of target receptor in a suitable way. It exhibited good anticancer activity toward human cancer cell lines MCF-7, HEP-2, COLO-205, HCT-15, A-549, and IMR-32 at a concentration of 10 \(\mu\)M in comparison with adriamycin.\textsuperscript{53} This study suggests that the introduction of imidazole ring into 9-amino acridine is an effective approach to exploit new anticancer drugs.

Podophyllotoxin-based compounds have shown encouraging clinical efficacy toward several types of neoplasms by inhibiting DNA-TOP II. Nitroimidazole-modified podophyllotoxin
13 exhibited more potent anticancer activities toward human cell lines HeLa, K562, and K562/ADM than etoposide. Contrast tests manifested that the demethylation of methoxy group on the benzene ring obviously decreased anticancer efficacy. The action mechanism was currently unclear. \(^\text{54}\)

It has been reported that imidazolyl polyamides could competitively inhibit nuclear factor-Y (NF-Y) binding to ICB\(^2\) site in Top II\(\alpha\) promoter region, cause upregulation of Top II\(\alpha\), and thus lead to good anticancer efficacy. \(^\text{55}\) Polyamide 14 is an analog of the naturally occurring distamycin A. It could selectively target the ICB\(^2\) site with slightly enhanced binding affinity (\(K_{eq} = 5 \times 10^5 \text{ M}^{-1}\)) to cognate site 50-TACGAT-30 than its monomer. \(^\text{56}\) However, compound 14 could not enter the nucleus and showed no upregulation effect toward Top II\(\alpha\), which presumably resulted from its bulky skeleton. Further research is thus worthwhile to focus on the design of new polyamides as cellular gene control agents through addressing the problem of nuclear uptake.

**B. Imidazoles as Microtubule Polymerization Inhibitors**

Microtubules, as major components of cytoskeleton, are indispensable for the formation and disappearance of the mitotic spindle, which is responsible for the separation of duplicated chromosomes during cell division. The abortive formation of microtubules can result in cell-cycle arrest and cell death. Therefore, the discovery of potential microtubule inhibitors is an attractive strategy for cancer therapy. Although tubulin-targeting compounds including taxanes, vinca alkaloids, and combretastatins have been widely used in the treatment of many cancers, \(^\text{57, 58}\) the poor bioavailability and multidrug resistance of these drugs compel researchers to exploit novel inhibitors toward microtubule polymerization with low side effect, little drug resistance, and good oral activity. \(^\text{59, 60}\) The structures for imidazoles as microtubule polymerization inhibitors are shown in Figure 3.

The \textit{cis}-configuration of the double bond in combretastatin A-4 (15) is a critical structural requirement for its antitubulin activity. \(^\text{61, 62}\) Bioisosteric replacement of the olefinic linker by a rigid imidazole ring was able to not only effectively restrain the isomerization, but also endow products with better water solubility, pharmacokinetic property, and oral bioavailability. \(^\text{63–65}\) N-Methylimidazole bridged analogs 16a and 16b exhibited good inhibitory activities for tubulin polymerization and tube formation in human umbilical vein endothelial cells. Notably, dibromide 16b displayed unique selective inhibition toward KB-3–1 cervix and PC-3 prostate cancer
cells, which indicated its potential antiangiogenic use in solid tumors. Additionally, the Br or I atom at the m-position on the phenyl group played a positive role in exerting the excellent activity, since their exchange by F atom decreased activity.\textsuperscript{66, 67}

The hydrochloride of disubstituted imidazole \textsuperscript{17} bearing naphthyl moiety at 5-position showed comparable vascular disruption efficacy but less anticancer potency in comparison to combretastatin A-4, which implied no direct correlation between vascular disruption activity and anticancer potency. More importantly, it could cause a rapid central necrosis for the multidrug resistance MDA-MB-435 tumors in mice.\textsuperscript{68} The results also disclosed the possibility of employing naphthyl moiety to replace the phenyl group in designing more potent tubulin inhibitors.

Carbonyl linked imidazole \textsuperscript{18a} bearing 4-methyl benzene showed significant anticancer activity by the inhibition of tubulin polymerization toward melanoma and prostate cancer cell lines. Specifically, it was able to effectively inhibit the growth of the multidrug resistance cancer cell lines MDA-MB-435, MDA-MB-435/LCC6MDR1 with IC\textsubscript{50} values of 67 and 83 nM, respectively, and possessed better drug resistance indices (\(R = 0.9\)) than paclitaxel (IC\textsubscript{50} = 465 and 16 nM, \(R = 29\)). However, in vitro metabolic studies indicated that the N-methyl group on the imidazole ring was not helpful for metabolic stability.\textsuperscript{69} Demethylation of compound \textsuperscript{18a} yielded its analog \textsuperscript{18b} with good anticancer efficacy. Compared to reference drug colchicine (IC\textsubscript{50} values in the range of 11–20 nM), compound \textsuperscript{18b} displayed superior anticancer potency toward A375, LNCaP, PC-3, and PPC-1 with IC\textsubscript{50} values between 9 and 12 nM. The impressive antiproliferative potency possibly resulted from the effective hydrogen-bond interactions between compound \textsuperscript{18b} and the binding sites ASP251, LEU252, and THR179 in tubulin.\textsuperscript{70}

Amino imidazoles have been paid special attention as potential anticancer drugs since they could enhance liposolubility and pharmacokinetic properties.\textsuperscript{71, 72} Compound \textsuperscript{19a} with a phenyl group and \textsuperscript{19b} with a pyridinyl moiety were found to be active microtubule-destabilizing anticancer agents. They could prolong the lifespan of murine leukemic P388 cells-inoculated mice in both orally and intravenously administered routes. Especially, compound \textsuperscript{19b} gave comparable tubulin polymerization inhibition with an IC\textsubscript{50} value of 0.27 \(\mu\)M to colchicine (IC\textsubscript{50} = 0.29 \(\mu\)M). Structure–activity relationship demonstrated that the chlorine substituent on 4-position of phenyl moiety played a critical role in exerting the anticancer efficacy, and the replacement of chlorine with bromine decreased the bioactivities.\textsuperscript{73}

It was reported that imine group could be metabolized by liver microsomal enzymes in vivo,\textsuperscript{74} which limited the extensive use of amino imidazoles. As the bioisosteric product of compound \textsuperscript{19a}, aminimidazole derivative \textsuperscript{20} without the imine liker was orally absorbable, and could improve pharmacological property and metabolic stability. Moreover, this compound could distribute efficiently to the tumor tissues at effective concentrations and significantly prolong the lifespan of the leukemia mice. Contrast tests revealed that the replacement of thiophene and phenyl groups by other fragments would decrease the anticancer potency.\textsuperscript{75} These amino imidazole-based tubulin-targeting compounds are worthy to be further investigated as potential candidates for cancer chemotherapy.

\textbf{C. Imidazoles as Cytochrome P450 Enzymes Inhibitors}

Cytochrome P450 (CYP) is a large family of hemoproteins present in most forms of life (plants, bacteria, and mammals). They are concerned with metabolism in vital processes and many of them with the ability to activate carcinogens have been implicated as risk factors for cancers. Azole-based CYP inhibitors such as fadrozole (21), YM-116 (22), and letrozole (23a) are of great interest to medicinal chemists. Especially, fadrozole and letrozole have been recommended as first-line drugs in the therapy of breast cancer by the Food and Drug Administration.
The effectiveness of theseazole inhibitors in inhibiting aromatization activity is largely attributed to their ability to coordinate with iron ion in aromatase heme through azole rings. However, CYP enzymes inhibitors have the possibility to cause significant systemic side effects due to the poor selectivity among CYP enzymes. Thus, the exploitations of novel CYP inhibitors with good selectivity and low toxicity are necessary. Fortunately, the discovery of bioactive YM-116 (22) with CYP inhibitory activity gives a new hope of imidazole derivatives as anticancer drugs. The structures for imidazoles as cytochrome P450 enzymes inhibitors are shown in Figure 4.

Substitution of the triazole ring in letrozole (23a) by an imidazole moiety produced compound 23b, which gave better CYP19 inhibitory activity with an IC$_{50}$ value of 4 nM than letrozole (IC$_{50}$ = 8 nM). It also displayed stronger anticancer efficacy (40% inhibition) against human epithelial cancer cell line H295R than letrozole (10% inhibition) at the concentration of 10 nM. Further study pointed out that the nitrogen atom at 3-position of the imidazole ring was crucial for aromatase inhibition, while the two para-cyano phenyl groups were helpful for good anticancer activity.

The above results attracted more effort toward diverse sets of imidazole-based letrozole analogs with the aim to improve their CYP inhibitory efficacy. Research revealed that benzoxazole ring was helpful for CYP26A1 inhibitory efficacy in homology modeling studies using human P450 templates. Introduction of benzoxazole ring in compound 23b through
amino bridge produced analog 24. It showed much better CYP26A1 inhibitory efficacy ($IC_{50} = 0.9 \mu M$) than liarozole ($IC_{50} = 7 \mu M$). Comparative study discovered that the replacement of benzoxazole moiety by phenyl or methyl group or the substitution of imidazoyl group by triazole or tetrazole moiety would decrease the inhibitory efficacy.\(^{85}\)

Styryl fragment was also found to play an important role in improving CYP inhibitory activity. Imidazole derivative 25 possessed stronger efficacy with an $IC_{50}$ value of 0.30 $\mu M$ than ketoconazole ($IC_{50} = 0.52 \mu M$) against human CYP24A1 hydroxylase. Structure–activity relationship manifested that the replacement of styryl group by benzofuran, 4-bromobenzene, or methylendibenzene moiety resulted in almost the total loss of activity. This demonstrated the importance of styryl moiety for anticancer efficacy.\(^{86}\)

CYP17 inhibitors are able to block androgen biosynthesis and thus are commonly regarded as promising agents to treat prostate cancer. Compound 26, a ring-opening derivative of fadrozole (21), showed good inhibitory efficacy against 17$\alpha$-hydroxylase ($IC_{50} = 0.4 \mu M$) but weak potency toward 17,20-lyase ($IC_{50} = 10.7 \mu M$) of CYP17 enzymes in comparison to ketoconazole (0.21 and 2.66 $\mu M$, respectively). Its 2-methyl benzyl analog was also discovered to exhibit good bioactivity against 17$\alpha$-hydroxylase with an $IC_{50}$ value of 0.4 $\mu M$. However, compound 26 possessed weak selectivity over other CYP enzymes.\(^{87}\) Therefore, it is worthy for further modification of compound 26 to increase selectivity and binding affinity toward CYP17.

Research found that steroid mimetic structures such as biphenyl and naphthalene ring were suitable lipophilic moieties for CYP17 inhibitors and attracted special interest in anticancer drug discovery. The (-)-enantiomer of biphenyl imidazole 27 showed much stronger CYP17A1 inhibitory efficacy ($IC_{50} = 131 nM$) than ketoconazole ($IC_{50} = 2780 nM$) with good selectivity over CYP3A4. Particularly, this compound exhibited high bioavailability and a long plasma half-life (12.8 hr) in rat.\(^{88}\)

As the analogs of compound 27, chiral imidazoles 28a-b displayed superior inhibitory activities ($IC_{50} = 14$ and 26 nM, respectively) to compound 27 against 17,20-lyase with excellent selectivity over CYP3A4. Particularly, this compound exhibited high bioavailability and a long plasma half-life (12.8 hr) in rat.\(^{88}\)

A lot of literature has revealed that the incorporation of sulfonate group usually results in good inhibitory activity against CYP17. Phenyl imidazole 29a ($IC_{50} = 0.10$ and 0.01 $\mu M$, respectively) and its isopropyl derivative 29b ($IC_{50} = 1.21$ and 0.03 $\mu M$, respectively) exhibited stronger inhibitory efficacy than ketoconazole ($IC_{50} = 2.66$ and 0.21 $\mu M$) against 17$\alpha$-hydroxylase and 17,20-lyase of CYP17, respectively. The greater inhibitory potency for compound 29a could be explained by the reduced conformational flexibility that made the additional phenyl group unable to undergo any steric interaction with the active site.\(^{90}\) Nitro derivative 29c possessed weak inhibitory activity toward 17$\alpha$-hydroxylase, but gave superior lyase inhibitory potency to ketoconazole. Moreover, it could reduce side effects because of no interference with corticosteroid synthesis.\(^{91}\)

Some literature reported that structural modification by large conjugated framework was also an effective strategy to enhance the CYP inhibitory activities of target compounds. Naphthyl imidazole 30 was identified to be an excellent CYP26A1 inhibitor with an $IC_{50}$ value of 3 nM in comparison to liarozole ($IC_{50} = 540 nM$). It showed good stability in human liver microsomes as well as absence of mutagenic potentiality in Ames assay. Research showed that replacement of NH linker by O or CH$_2$ moiety resulted in a substantial loss of activity. This fact demonstrated the importance of NH group for bioactivity.\(^{92}\) Additionally, indanone-based
imidazole 31 displayed 50 times more potent CYP 19 inhibitory activity (IC\textsubscript{50} = 0.55 \mu M) than aminoglutethimide. These results suggested that imidazole derivatives bearing large conjugated system should have excellent potentiality to bind with the active site of aromatase, thereby exerting a positive impact on the anticancer activity.

It has been reported that aromatic heterocycles such as coumarin-, xanthone-, and acridone-derived imidazoles could exert high CYP19 inhibitory activities and the related research is quite active. Coumarins are an important type of heterocyclic compounds with diverse bioactivities. Compound 32 was obtained by the introduction of imidazole ring into the 7-substituted coumarin scaffold. It displayed stronger CYP19 inhibitory activity (IC\textsubscript{50} = 47 nM) than fadrozole (IC\textsubscript{50} = 52 nM) with excellent selectivity over CYP11B1 and CYP11B2. Xanthone-imidazole hybrids 33a and 33b showed notable CYP19 inhibitory efficacy with IC\textsubscript{50} values of 5.59 and 3.98 nM, respectively, in comparison to fadrozole (IC\textsubscript{50} = 52 nM). Interestingly, the nitro group was essential for high inhibitory potency in the absence of ketone moiety. Acridone-derived imidazole 33c was identified to be an excellent inhibitor against both CYP19 and CYP11B1 with high selectivity over CYP11B2 and CYP17, especially toward CYP19 even more effective than letrozole. Further study manifested that the position of imidazole ring on xanthone framework exerted an important effect on the bioactivity. Therefore, this class of compounds deserve further exploitation as new aromatase inhibitors.

D. Imidazoles as RAF Inhibitors

RAF kinases (A, B, and C) are regarded as attractive biological targets in the discovery of anticancer drugs because of their relations with cell growth, differentiation, and proliferation. Sorafenib, as a currently available RAF inhibitor, has been clinically validated for the treatment of renal cancer. Its imidazole analog 34a with amide linker and 34b bearing urea bridge were found to possess remarkable CRAF inhibitory efficiency and superior anticancer activities with GI\textsubscript{50} values of 0.62 and 0.65 \mu M, respectively, to sorafenib (GI\textsubscript{50} = 0.78 \mu M) toward melanoma cancer cell line WM3629. Especially, compound 34a showed 99% inhibitory efficacy toward CRAF at a concentration of 10 \mu M with high selectivity. Surprisingly, for compound 34b, the substitution of chlorine atom by a CF\textsubscript{3} group on 3-position of the benzene ring or its removal on 4-position substantially decreased the bioactivities. All these findings revealed the great potentiality of pyrimidine imidazoles as potent and selective RAF inhibitors for the treatment of melanoma. The structures for imidazoles as RAF inhibitors are shown in Figure 5.

Triaryl imidazoles can easily exert diverse weak interactions with enzymes and have been actively investigated in medicinal chemistry as basic scaffolds to target active kinase RAF. Compound 35 was identified to be an excellent RAF inhibitor and exhibited superior anticancer activities toward A549 and DLD-1 cell lines to sorafenib. Notably, impressive antiproliferative results were also obtained when tested against MV4–11 cells (acute myeloid leukemia) in comparison with sorafenib. Modeling studies manifested that the imidazole ring in compound 35 could occupy the ribose position of ATP-binding pocket and was necessary for anticancer activity.

Pyrazole multicyclic fragment has also attracted considerable attention as a new hinge binder for RAF inhibitor. Imidazole-incorporated pyrazole bicycle 36 displayed 96% inhibitory activity toward CRAF at the concentration of 10 \mu M and good antiproliferative activities with GI\textsubscript{50} values of 2.24 and 0.86 \mu M, respectively, in contrast to sorafenib (GI\textsubscript{50} = 5.58 and 0.65 \mu M) against human melanoma cell lines A375P and WM3629. Further investigations of this type of compounds as CRAF inhibitors to treat melanoma should be worthwhile.

Imidazole-containing pyrazole tricyclic derivative 37 was also reported to be an active BRAF inhibitor for melanoma with good cellular activity (IC\textsubscript{50} = 0.24 \mu M) and
pharmacokinetic profile. Further study indicated that both pyrazole tricyclic moiety and imidazole ring played a crucial role in exerting the bioactivity. The replacement of tricyclic pyrazole by tricyclic triazole or six-membered ring decreased inhibitory potency, this might be attributed to the steric clash or unfavorable electrostatic interaction with BRAF binding pocket.  

**E. Imidazoles as TGF-β Inhibitors**

TGF-β is a kind of protein that modulates cell proliferation and differentiation through activin receptor-like kinase 5 (ALK5). Exploitation of small molecule inhibitors of ALK5 is one strategy to reduce TGF-β induced cancers. A lot of imidazole-based ALK5 inhibitors generally bearing pyridine ring as hinge-binding group are under preclinical development. It has been found that the pyridine and imidazole fragment play an important role in exerting ALK5 inhibitory efficacy. Recently, much work has been devoted to the design and development of pyridine-imidazole hybrids as promising ALK5 inhibitors. The structures for imidazoles as TGF-β inhibitors are shown in Figure 6.
Previous research showed that quinoxaline ring was helpful to improve the potency and selectivity toward ALK5. Quinoxaline-imidazoles 38a and 38b possessed more than 90% inhibitory activity against ALK5 at the concentration of 0.5 μM with high selectivity over p38α MAP kinase that is the only kinase to be significantly affected by the ALK5 inhibitor SB-431542 in vitro. However, the sulfonamide group could not interact with the ATP-binding pocket of ALK5 as favorably as the amide moiety. The amide derivative 39 displayed excellent ALK5 inhibitory potency (IC$_{50}$ = 0.034 μM) with high selectivity versus other kinases (>$100$-fold). Docking studies disclosed that compound 39 should fit well into the active site cavity of ALK5, and the imidazole ring might form hydrogen bonds with Asp351 that facilitated its deep binding with the active site. The in vitro metabolism test in human CYP supersomes revealed that the quinolinyl compound 39 was also more stable than the corresponding quinoxalinyl analog. Structure–activity relationship pointed out that the ethyl or methyl group on the pyridine ring in both 38 and 39 was indispensable for the selectivity and inhibitory activity. These results are helpful for further modifications of this type of compounds as ALK5 inhibitors.

Research revealed that pyridinyl imidazole 40 could enter very well into the cavity of ALK5. This compound displayed comparable ALK5 inhibitory activity (69% inhibition at 1 μM) to the well-known ALK5 inhibitor SB431542 (76% inhibition at 1 μM) with high selectivity versus ALK4 and ALK7 (tenfold). Interestingly, bis-pyridinyl derivative 41 was found to be a potent and selective TGF-β inhibitor. It gave 50% inhibition activity at the concentration of 100 nM. Comparative test demonstrated that the imidazole ring was helpful for good bioactivity because replacement of the imidazole ring by phenyl, quinolinyl, or piperidyl group lowered the TGF-β inhibitory potency and selectivity. Further investigation is necessary to develop these compounds as potent TGF-β inhibitors.

Thiazolyl imidazole 42 showed good water solubility, it not only gave excellent and selective inhibitory efficacy against ALK5 with an IC$_{50}$ value of 8.2 nM, but also had good activity (IC$_{50}$ = 32 nM) against TGF-β induced Smad 2/3 phosphorylation at a cellular level. Docking studies indicated that benzothiazole ring could bind by hydrogen bonds with NH of His-283 in the ATP-binding site of ALK5, while the thiazolyl group could form water-mediated networks of hydrogen bonds with the carboxy oxygen of Glu-245, the hydroxyl hydrogen of Tyr-249, and the backbone NH of Asp-351. This was in concordance with the typical hydrogen bond acceptor.

**F. Imidazoles as Farnesyltransferase Inhibitors**

The farnesylation of some particular proteins exhibits an essential effect on intracellular signal transduction, cell proliferation, and apoptosis. Since the identification of this function, farnesyltransferase (FTase) has emerged as a promising target in the discovery of anticancer drugs. Plenty of FTase inhibitors have been successfully developed as anticancer agents with high efficacy and low toxicity. Imidazole ring, which can interact with the zinc ion at the active site in FTase, makes a great contribution to the biological potency. The structures for imidazoles as farnesyltransferase inhibitors are shown in Figure 7. Tipifarnib (4) is an excellent imidazole-based FTase inhibitor in clinical trials to treat blood cancers, its success has provoked an increasing effort to investigate the potentiality of imidazole derivatives as anticancer agents.

Tipifarnib analog 43 was obtained by the replacement of quinolinone moiety with benzofoxuran ring. This imidazole derivative could significantly inhibit FTase with an IC$_{50}$ value of 1.1 nM and showed strong anticancer efficacy in vivo without noticeable loss of body weight in the QG56 human nonsmall-cell lung carcinoma (NSCLC) xenograft model in mice. Further study suggested that the cyano group on benzofoxuran ring, which might form hydrogen bonds through a water molecule with the amide backbone of Phe360B, was essential for the excellent

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Figure 7. Imidazoles as farnesyltransferase inhibitors.

inhibitory potency.\textsuperscript{116} Compound 43 could be employed for further optimization to search for potential anticancer agents.

Literature reported that ethylenediamine-containing compounds could gain simultaneous access toward four subpockets in the active site of \emph{Plasmodium falciparum} farnesyltransferase (Pf FTase) and thus possessed potent Pf FTase inhibitory activity. Compound 44 exhibited good inhibitory efficacy (IC\textsubscript{50} = 25 nM) toward hFTase with high selectivity (113-fold) over geranyltransferase-I. It also showed notable whole-cell inhibition activity (IC\textsubscript{50} = 90 nM) for H-Ras processing. Further study indicated that the para-nitrile phenyl moiety could be stabilized by the Y361\(\beta\) residue via \(\pi-\pi\) stacking interaction, and was important structural fragment for the high activity.\textsuperscript{117} The results provide facile access to further design more potent and selective FTase inhibitors.

Azaheptapyridines are another kind of FPT inhibitors and some of them are being evaluated in clinical trials. Imidazole-modified azaheptapyridines 45a and 45b, containing a conformationally constrained hydroxyl group, which easily forms hydrogen bonds, were reported to have good FPT inhibitory activity. (R)-stereomer 45a demonstrated a 20-fold much better enzyme inhibitory efficacy (IC\textsubscript{50} = 0.38 nM) than (S)-stereomer 45b (IC\textsubscript{50} = 12 nM). Further work revealed that the strong interaction between imidazole 3-N and catalytic Zn\textsuperscript{2+} ion in enzyme made an important contribution to the high bioactivity. However, the methylation of hydroxyl group led to a lower inhibitory efficacy.\textsuperscript{118}

It was observed that most of FTase inhibitors were also competitive farnesylpyrophosphate (FPP) or CaaX inhibitors. Structural modification of farnesyl chain with the ability to improve the binding affinity to both FPP and CaaX sites was favorable for FTase inhibitors to exhibit better specificity and affinity. Imidazole-linked tripeptide 46 displayed good affinity toward FPP with an IC\textsubscript{50} value of 0.24 \(\mu\text{M}\), but weak potency for CaaX-binding site. The results implied that the relative position of farnesyl and tripeptide chains in this compound was not appropriate enough to fit both FPP and CaaX binding sites. In addition, comparative study pointed out that the removal of farnesyl chain would result in almost 300-fold decreased bioactivity.\textsuperscript{119}

\section*{G. Imidazole-Based Metal Supermolecules as Anticancer Agents}

Metal supermolecules with distinct medicinal values are in general formed by two or more molecules via coordination bonds, and many of them have been widely used in the clinic for the treatment of various cancer diseases in recent years.\textsuperscript{19,120} For instance, the well-known
anticancer drug cisplatin cis-[Pt(NH₃)₂Cl₂] is an inorganic supramolecular aggregate of one PtCl₂ compound and two NH₃ molecules through coordination bonds. Particularly, this type of drugs with a wide range of geometries and coordination numbers allow for the organization of different anions and organic ligands in more appropriate spatial distributions, thus providing better modalities to attack targets. Furthermore, the redox potentiality of metal compounds can interact with the balanced cellular redox state. It was able to cause significant modification of cell viability through either a direct way or the conversion of a relatively inert compound into an activated one, therefore tune the inherent toxicity of drugs. Additionally, metal ions can protect the ligands against possible enzymatic degradation before reaching the target and have a synergic effect with ligands to gain better hydrophilicity/hydrophobicity ratio. On the other hand, metal complexes are relatively convenient, cheap, and easy for their preparation and thus might have a larger possibility as clinical anticancer drugs. All the above mentions make metal-based supermolecules exhibit extensive potentiality such as higher safety, lower toxicity, less adverse effect, higher bioavailability, better biocompatibility, fewer drug resistances, and better curative effects as clinical drugs. Imidazole ring with multiple binding sites such as carbon or electron-donating nitrogen atoms is capable of interacting with a variety of metal ions or organic molecules to produce supramolecular drugs via coordination bonds, hydrogen bonds, ion–dipole, cation–π, π–π stacking, hydrophobic effects, and van der Waals forces, etc.121–125 These virtues have been powerfully encouraging numerous researchers to engage in the developments of imidazole-based metal supramolecular anticancer drugs.126,127 The structures of imidazole-based anticancer metal supramolecular complexes are shown in Figure 8.

1. Noble Metal-Based Supermolecules as Anticancer Agents
Since the clinical use of cisplatin, the first noble metal supramolecular anticancer drug, the developments of noble metal-based supramolecules as effective anticancer agents have unusually become more and more active in drug discovery.128–131 Currently, platinum complexes are the most widely used metal supramolecular anticancer drugs. They could effectively treat many types of cancers such as breast, colorectal, ovarian carcinoma, and metastatic cancers.132,133 However, some problems in platinum-containing anticancer therapy including side effects and drug resistance phenomena have stimulated an increasing effort to search for novel platinum- and other metal-based complexes as cytostatic agents and many excellent achievements have been acquired.

Clotrimazole is the earliest imidazole antifungal agent with significant curative effect on superficial fungal infection. The nitrogen atoms of imidazole ring in clotrimazole provide the possibility to coordinate with metal ions. Clotrimazole platinum(II) supramolecular complex 47 was formed by two clotrimazole molecules and one platinum dichloride through coordination of nitrogen atom in imidazole ring. It was able to interact with the minor groove of DNA and effectively inhibit the growth of MCF-7, SKBR-3, HT-29, and B16/BL6.134 This result expanded the application of clotrimazole in cancer therapy and opened a novel space of using antifungal agents to develop metal-based anticancer agents.

The anticancer potentiality of imidazole-based metal complexes has inspired researchers to be engaged in their development. Research discovered that 2-phenylpyridine complex could be easily dissociated in the biological systems, which was favorable for reducing side effects and cell resistance. However, organoplatinum(II) imidazole 48 showed weak anticancer effects toward Jurkat leukemia and Raji cell lines with IC₅₀ values of 12 and 14 μg/mL, respectively, in comparison with cisplatin (IC₅₀ = 9.9 and 9.0 μg/mL).135 This work presents some points for the development of more potent organometallic anticancer complexes compatible with biological systems.
Platinum(II) complexes with bulky bis-imidazole ligands were reported to have the ability to decrease side effects and regulate hydrophilicity/hydrophobicity ratio, which make them easy to penetrate into biofilms, thus leading to better curative effect. Supermolecule 49 could directly interact with DNA without further activation steps and had superior activity against cisplatin-sensitive human cancer cell line A2780 with an IC$_{50}$ value of 18.7 μM to carboplatin (IC$_{50}$ = 19.9 μM). Specifically, its IC$_{50}$ value toward cisplatin-resistant cell line A2780Cp8 was 20.8 μM, which was much lower than that of carboplatin (IC$_{50}$ = 78.3 μM). The results suggested that development of this type of platinum(II) complexes was an effective strategy to overcome cisplatin resistance.

Nitrogen-heterocyclic carbene (NHC) ligands can form strong metal-C bond with metals. They have unique physical property and good stability against biological reduction and ligand

**Figure 8.** Imidazole-based anticancer metal supramolecular complexes.
exchange reaction. Literature revealed that NHC ligands could still confer platinum(II) complexes with the ability to interact with other cell targets except for DNA. Platinum(II)-imidazole 50 was found to have a synergistic effect with cisplatin against human cancer cell lines HeLa, HepG2, SUNE1, and CCD-19Lu with low IC\textsubscript{50} values ranging from 0.057 to 0.77 \(\mu\)M and possessed different anticancer mechanism from cisplatin. Particularly, in vivo test displayed that this complex could obviously inhibit tumor growth in mice without significant reduction in body weight and acute toxicity.

A lot of literature revealed that ruthenium complexes have great potentiality as anticancer metallodrugs with less toxicity and higher water solubility than cisplatin. Different from platinum complexes, they could target other biomolecules besides DNA, which were expected to reduce tumor resistance. Moreover, ruthenium has the ability to mimic iron due to its chemical similarity and ability to bind with transferrin. Imidazole-based Ru(III) complex 51 exhibited high activity and selectivity against metastases of solid tumors with moderate in vitro cytotoxicity. It had successfully entered clinical trials as a promising anticancer candidate.

Bis-imidazole-Ru(III) complex 52 was constructed by coordination of Ru(III) trichloride with two nitrogen atoms in bis-imidazoles and one sulfur atom in dimethylsulfoxide. It exhibited pronounced inhibitory activity against cyclin-dependent kinases (cdk) with different modes of action. The IC\textsubscript{50} values were much lower (between 78.4 and 142.3 \(\mu\)M) toward cancer cell lines HepG2, MCF-7, HeLa, and 95-D than those of NAMI-A (in the range of 564.4–750.4 \(\mu\)M). Complex 52 also exerted excellent inhibitory effects on cell metastasis in comparison with NAMI-A. Additionally, ruthenium (II) complex 53 with three bis-imidazole molecules also displayed superior efficacy with an IC\textsubscript{50} value of 18 mM to cisplatin (IC\textsubscript{50} = 35 mM) toward human breast cancer cell line MDA-MB-45S.

The specific structural feature of naphthalimide ring with strong hydrophobicity and large desirable \(\pi\)-conjugated backbone makes naphthalimide-based derivatives have spacious potential application in medicinal chemistry, supramolecular recognition and assembly, and material science. Some naphthalimides as anticancer drugs have displayed good efficiencies by the interactions with DNA. Naphthalimide-ruthenium(II) complex 54 was formed by the coordination of ruthenium(II) with one nitrogen atom of imidazole ring and one methylbenzene by \(\pi\)–\(\pi\) stacking. It showed significant anticancer activities toward cisplatin-resistant human ovarian carcinoma A2780 through a double-action mechanism. Further study demonstrated that naphthalimide skeleton was the dominant interaction moiety with DNA, whereas the ruthenium(II) was preferred to bind to proteins. However, this complex did not display good selectivity.

It was revealed that alkyl chain with the ability to modulate the hydrophobicity could increase the anticancer potency. A series of ruthenium(II) complexes bearing alkyl chains were discovered to display promising anticancer activity, in which phosphorylated complex 55 having suitable water solubility exhibited good antiproliferative efficacy against both cisplatin-sensitive and cisplatin-resistant A2780 cell lines with high selectivity. The preliminary biological studies showed that the antiproliferative activity of these complexes was related to their lipophilicity.

Several recent papers revealed that gold complexes also showed reproducible and significant activity in murine tumor models in vivo, even toward cisplatin-resistant cell lines. Imidazole-coordinated gold(I) complex 56 bearing pentafluorophenyl group displayed comparable anticancer potency with an IC\textsubscript{50} value of 0.55 \(\mu\)M to cisplatin (IC\textsubscript{50} = 0.45 \(\mu\)M) against cervical carcinoma cell line. Tetrahedral gold(I) complex 57, where the four coordination is filled by the four phosphor atoms in bis-imidazoles, exhibited a different mode of action from traditional metallodrugs. It showed considerably higher activities in both cisplatin-sensitive and cisplatin-resistant A2780 cell lines with IC\textsubscript{50} values of 0.40 and 0.81 \(\mu\)M, respectively, than
cisplatin (1.32 and 13.4 μM, respectively). Importantly, this complex possessed relatively low cytotoxicity against both human colon carcinoma and rat hepatoma cell lines and almost no cytotoxicity toward the leukemia cell line K562.\textsuperscript{158}

Silver(I) supramolecular complexes have also been investigated very well for their anticancer activity in recent years.\textsuperscript{159,160} Metal silver derivatives 58a-c were identified to have strong cytotoxicity toward renal cancer cell line Caki-1. Silver(I) imidazoles 58a and 58b displayed comparable anticancer efficacy with IC\textsubscript{50} values of 3.2 and 3.3 μM, respectively, to cisplatin (IC\textsubscript{50} = 3.3 μM). However, the replacement of imidazole carbene by 4,5-dichlorine imidazole carbene or benzimidazole carbene caused approximately eight- and 11-fold loss of potency.\textsuperscript{161,162} Accordingly, the introduction of para-cyano group on the benzyl group in compound 58c resulted in twofold decrease in activity (IC\textsubscript{50} = 6.2 μM).\textsuperscript{163} These results indicated that the substituted pattern on imidazole and phenyl groups could exert a substantial effect on their anticancer activities.

2. Non-Noble Metal-Based Supermolecules as Anticancer Agents

Non-noble metal complexes have also been found to possess considerable potentiality in treatment of cancers. Metal copper plays a crucial role in organisms due to its endogenous availability. Lots of copper supermolecules with less side effects have been exhaustively exploited as potential anticancer agents.\textsuperscript{164–168} Nitroimidazoles are a kind of radiosensitizers used in cancer treatment,\textsuperscript{169,170} and their copper(II) complexes have been confirmed to be able to increase the sensibility to cancer cells.\textsuperscript{171} Imidazole copper(II) complex 59 with high lipophilicity, as a consequence of coordination with antiamoebic ornidazole drug, exhibited a significant increase in radiosensitization with respect to the free ornidazole itself against human larynx cancer cell line Hep2. Further study indicated that the metal and ornidazole ligands exerted a significant effect on the radiosensitizer activity.\textsuperscript{172} This type of complexes set a good starting point to develop the metallodrugs of antiamoebic ornidazole for tumor cells.

Cobalt is a natural trace element and a necessary component of vitamin B\textsubscript{12}. Many Co(III) complexes were reported to provide the desirable reductive environment for the target hypoxic cells and can be successfully served as prodrugs for drug delivery. Mononuclear Co(III) supermolecule 60 with two imidazolyl ligands showed to be an effective hypoxia selective anticancer agent.\textsuperscript{173} It could be reduced into more active Co(II) species under hypoxic conditions, and consequently display comparable capacity to inhibit cellular growth with an IC\textsubscript{50} value of 0.50 mM to cisplatin (IC\textsubscript{50} = 0.60 mM) when using Saccharomyces cerevisiae cells as eukaryotic model. It was special to emphasize that neither the imidazolyl ligand nor its oxidized form presented any expressive inhibitory effect. Probably, complex 60 in the reduced form might react endogenously within cells and increase the production of free radicals, thus leading to inhibition of cell growth. The results revealed the potentiality of 60 as a bioreductively activated prodrug.\textsuperscript{174–176}

As mentioned previously, bioreductive compounds bearing nitroimidazole group have the potential use as radiopharmaceuticals toward hypoxic tissue diagnosis. Based on this observation, tridentate \textsuperscript{99m}Tc(I)-tricarbonyl nitroimidazole 61 was prepared, in which metal Tc(I) ion was coordinated with tricarbonyl (CO), a tightly bound ligand to stabilize metals in low oxidation states. This nitroimidazole complex had favorable tumor/muscle ratio and high stability in human plasma, and could be selectively uptaken and retained in tumor cells. It was a promising candidate for further evaluation as a hypoxia imaging agent in tumors.\textsuperscript{177}

The above mentions show that imidazole-based metal complexes as anticancer agents are versatile subjects of medicinal chemistry in view of their substitutional behavior, rich redox activity, and various coordination geometries. Many studies have been directing toward exploring the mechanistic pathways of these metal-based drugs. Additionally, it has been confirmed that organometallic complexes often generate a synergistic effect, providing the rationality for
coupling the different types of anticancer agents. However, an understanding of structure–activity relationships has not yet reached a level that allows extrapolation to provide general rules. Thus, future developments of metal complexes for treating cancers are needed to elucidate the relationship. In addition, many anticancer complexes studied so far appear to have more than one biomolecular target, hence systematic mode of action studies on anticancer metal complexes should continue to be carried out. As predicting, it is inevitable that the research and development of imidazole-based metal supramolecular complexes as anticancer drugs will become increasingly active in future.

**H. Imidazole-Containing Natural Products as Anticancer Agents**

Natural products from plants, animals, and microorganisms, etc., are one of the most important sources of medicinal drugs with relatively less toxic and side effects in comparison to traditional synthetic agents. They play a positive role in the discovery of new lead compounds. Imidazole-containing natural products commonly have novel chemical structures that usually exert different mechanism of action from other kinds of anticancer agents. Imidazole ring with multiple binding sites is capable of coordinating with a variety of inorganic metal ions or interacting with enzymes and receptors in biological systems through noncovalent interactions such as hydrogen bonds, coordination bonds, \( \pi-\pi \) stacking, and so on. Therefore, imidazole ring in natural products not only helpfully leads to good water solubility, but also beneficially exerts multiple action mechanisms that are helpful to overcome drug resistances. The special structural characteristic of imidazole ring endows imidazole-based natural products underlying development value. In fact, imidazole ring frequently exists in a great number of natural bioactive products. Natural imidazole derivatives have been extensively investigated as anticancer agents and some of them exhibited different action mechanisms from clinical drugs. However, nonfused imidazole rings, excluding simple histamines, are less common among natural products. Fortunately, some isolated natural nonfused imidazoles have been recently reported and represented promising potentialities in drug discovery. The structures of imidazole-based anticancer natural products are shown in Figure 9. Bleomycin (62) is a kind of well-known nonfused imidazole-containing peptide antibiotic. It has been proved to display anticancer efficacy via interaction with DNA, which encourages numerous workers to contribute to develop the potent activity of this type of natural products against cancers.

Marine environment provides a potential source of novel anticancer drugs. Steroidal imidazole 63a was isolated from marine sponge *Phorbas amaranthus* in which the highly charged sulfate group restricted the cell permeability. Acid hydrolysis of this compound afforded imidazole derivative 63b. This compound exhibited a significantly increased cytotoxicity with an IC\(_{50}\) value of 4.4 \( \mu \)g/mL toward human colon tumor cell line HCT-116 in comparison with 63a (IC\(_{50}\) > 32 \( \mu \)g/mL). Natural product 64 was isolated from a deep ocean sediment-derived fungus *Penicillium* sp. It displayed moderate cytotoxicity against cancer cell lines A-549 and HL-60. Moreover, this compound was revealed to be a potent tubulin polymerization inhibitor. Thus, further modification to improve its anticancer efficacy is worthy of an in-depth investigation. Imidazole alkaloids naamidine G from bright yellow sponge *leucetta chagosensis* also showed anticancer activity. Analogs 65a and 65b with the replacement of imidazole ring by triazine one still showed potentiality against cancer cell lines. All these natural imidazole-containing compounds deserve to be investigated in depth as anticancer agents.

**I. Imidazole Anticancer Agents Directly Acting on DNA**

DNA damage is the underlying cause of mutations leading to cancer. DNA sequence selective binding agents are potentially useful for targeting and modulating the expression of genes associated with cancer cell growth. Imidazole ring containing two nitrogen atoms and
Desirable \( \pi \)-conjugated backbone endows its derivatives to easily exert diverse weak interactions with DNA by \( \pi-\pi \) stacking and hydrogen bonds, which is helpful to improve the anticancer efficacy. On the other hand, compounds bearing heteroatoms such as nitrogen, sulfur, and oxygen could increase the interaction of complexes by forming hydrogen bonds with DNA and thus disrupt the double helix of DNA.\(^{187}\) The strategy that combines imidazole ring with heteroatom-containing groups in a single compound has been demonstrated to be effective to generate anticancer compounds with synergistic improvement via interactions with DNA.\(^{188}\) The structures for imidazole-based anticancer compounds directly acting on DNA are shown in Figure 10.

Some triaryl-imidazoles have recently been reported to be potent telomeric G-quadruplex ligands and display remarkable anticancer activities. Piperazinyl imidazole 66a showed good...
binding ability \( (K_a = 2.19 \times 10^6 \text{ M}^{-1}) \) and stabilizing activity to G-quadruplex DNA with excellent selectivity over duplex DNA (8.7-fold).\(^{189}\) Thiadiazole derivative 66b could cleave the DNA of HT-29 cancer cell and displayed significant anticancer activity. Moreover, it also had the potency to inhibit the DNA synthesis of HT-29 and MCF-7 cell lines with IC\(_{50}\) values of 2.7 and 3.2 μg/mL, respectively, which was comparable to cisplatin (IC\(_{50}\) = 1.7 and 2.6 μg/mL). In addition, the substitution of thiadiazole ring by a triazole or tetrazole moiety maintained the bioactivity, but the transposition of piperazine one decreased the anticancer activity. These suggested the importance of azole rings for the biological efficacy.\(^{190}\)

Aminoimidazolyl polyamides have great potentiality as DNA sequence selective binding agents in gene control associated with cancer cell growth. Very recently, diamino imidazole 67 with low molar mass and appreciable solubility in aqueous media was discovered to be able to bind with DNA sequence specifically with higher affinity \( (K_{eq} = 1.5 \times 10^7 \text{ M}^{-1}) \) over its monoamino/monocationic counterpart \( (K_{eq} = 4.8 \times 10^6 \text{ M}^{-1}) \). Importantly, this diamino polyamide might readily be taken up by cells due to its small molecule size.\(^{191}\) Therefore, it was worthwhile for further study as a potential new anticancer agent.

DNA alkylating agents represent an important class of anticancer drugs used in chemotherapy. They can exert interactions between compounds and DNA through van der Waals, hydrogen bonds, polarization, or hydrophobic effects.\(^{192}\) Combination of alkylating unit with imidazole intercalator gave biological compound 68. It could significantly inhibit the growth of HeLa cell in vitro. However, the substitution of imidazole ring by pyrimidine, pyridazine, pyridine, or benzimidazole moiety all decreased the anticancer activity.\(^{193}\)

### J. Other Imidazole-Based Anticancer Agents

Other imidazole-based anticancer agents are also currently being actively investigated.\(^{194–200}\) The structures are shown in Figure 11. Some N-alkyl imidazoles have been reported to display highly potent inhibitory activity against heme oxygenase (HO-1 and HO-2) related with certain types of cancers.\(^{201,202}\) Compound 69 was found to be a potent HO-1 inhibitor with notable bioactivity \( (IC_{50} = 4.4 \mu \text{M}) \) and high selectivity over HO-2 (21-fold). Structure–activity relationship revealed that the sulfur atom had a positive impact on HO-1 inhibitory potency and the substitution of sulfur atom by oxygen or carbon atom would decrease the HO-1 inhibitory efficacy. Further study indicated that the length of linker between the imidazole and phenyl group exerted a crucial influence in enhancing the specificity for HO-1 inhibitory activity.\(^{203}\)

It was reported that biphenyl group could interact better with the hydrophobic pocket of heme oxygenase, thus was beneficial for improving inhibitory activity. Imidazole 70 was identified as the first azole-based HO-2 inhibitor \( (IC_{50} = 0.43 \mu \text{M}) \) with slight selectivity over HO-1 (0.29-fold). It was a potential lead compound for further development as a HO-2 selective inhibitor.\(^{204}\)

Catechol is a useful medicinal fragment in drug discovery and its introduction is able to endow imidazoles with high affinity and good selectivity against placental alkaline phosphatase (PLAP) isozyme expressed in a variety of human cancers. Derivative 71 was an active PLAP inhibitor \( (IC_{50} = 2.1 \mu \text{M}) \) with excellent selectivity over tissue-nonspecific AP (32-fold). Structure–activity relationship pointed out that the presence of a phenyl group at 2-position of the imidazole ring resulted in a fivefold loss of PLAP inhibitory potency and introduction of bromine atom into 4-position of the imidazole ring led to a sevenfold reduction of efficacy.\(^{205}\) This type of compounds should be useful for understanding the physiological role of PLAP in biology and pharmacology in cancer treatment.

E-cadherin is a transmembrane protein and can maintain intercellular contacts and cell polarity in epithelial tissue. Loss of E-cadherin has been observed in a variety of human tumors.
However, compounds directly targeting E-cadherin restoration have rarely been developed. A recent research provided critical insights that the presence of hydrogen-bond acceptor might be a key structural feature for design of E-cadherin-targeted small molecules to treat cancer. Compound 72 with the incorporated imidazole ring as hydrogen-bond acceptor could effectively restore E-cadherin expression in cell line SW620 and reduce the invasion of cancer cells without

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affecting cellular proliferation.\textsuperscript{206} This compound might represent a starting point for the development of imidazole-based E-cadherin targeting agents.

Somatostatin (SST) is widely distributed in the human body where it exhibits various biological functions including anticancer activity. Particularly, SST\textsubscript{5} antagonists have been found to be able to activate and upregulate NMDA receptor function and to control hormonal secretions. Imidazole-incorporated nicotinamide \textsuperscript{73} was revealed to be an excellent SST\textsubscript{5} antagonist ($K_i = 11$ nM) with high selectivity over SST\textsubscript{1} (213-fold). Interestingly, the SST\textsubscript{5} receptor was strongly enantiomer-discriminated because all compounds with ($R$)-configuration were much more potent than the corresponding ($S$)-isomers. Moreover, compound \textsuperscript{73} possessed favorable pharmacokinetic properties, which was worthy to be further exploited as an SST\textsubscript{5} receptor antagonist.\textsuperscript{207}

Aminoquinazoline, especially 4-aminoquinazoline, was an attractive pharmacological fragment present in many anticancer drugs such as iressa and tarceva.\textsuperscript{208} Combination of 4-aminoquinazoline moiety with benzyl imidazole skeleton via hydrazine linker produced hybrid \textsuperscript{74}. It resulted in a synergistic contribution to anticancer activities toward H-460 and HT-29 cancer cell lines with IC\textsubscript{50} values of 0.031 and 0.015 \(\mu\)M, respectively, which were much better than reference iressa (IC\textsubscript{50} = 5.59 and 3.36 \(\mu\)M). Comparative study proved the significance of the imidazole ring in anticancer activity.\textsuperscript{209} These observations highlight that further development of 4-aminoquinazoline-based imidazoles as anticancer agents is of great necessity.

Gonadotropin releasing hormone (GnRH) antagonists have achieved positive clinical effectiveness in prostate cancer due to their ability to inhibit the release of gonadotropins luteinizing hormone. Compound \textsuperscript{75} with good solubility was found to be an orally active antagonist toward human GnRH with an IC\textsubscript{50} value of 7 nM and could elicit plasma suppression of serum luteinizing hormone in rats. Replacement of the imidazole ring by other heterocycles such as thiophene, furan, pyrrole, or thiazole all led to weak affinity, which implied the actual benefit for introduction of the imidazole ring to gain good biological activity.\textsuperscript{210}

Epidermal growth factor receptor (EGFR) has been identified as an intriguing anticancer target as it is involved in the regulation of cell growth and differentiation. Recently, literature has reported the importance of metronidazole for the EGFR inhibitory activity and a series of metronidazole-sulfonamide derivatives targeting EGFR have been studied. Compound \textsuperscript{76} displayed potent EGFR inhibitory efficacy (IC\textsubscript{50} = 0.39 \(\mu\)M) and antiproliferative activity (IC\textsubscript{50} = 1.26 \(\mu\)g/mL) toward cancer cell line A549 in comparison with erlotinib (IC\textsubscript{50} = 0.03 \(\mu\)M and 0.13 \(\mu\)g/mL, respectively). Comparative study indicated that the substitution of fluorine atom on benzene ring by a methyl group significantly decreased the inhibition, which manifested the importance of fluorine substituent for the bioactivity.\textsuperscript{211}

Inosine monophosphate dehydrogenase (IMPDH) inhibitors have emerged as important medicinal agents for leukemic therapy due to their ability to inhibit the biosynthesis of de novo purine nucleotide, which is indispensable for cell growth and division. Imidazole derivative \textsuperscript{77} showed highly selective anticancer activity toward human T-cell acute lymphoblastic leukemia cells through inhibition of IMPDH without toxicity in human diploid fibroblasts.\textsuperscript{212} These results showed that this molecule should be a potential lead compound for further development to treat leukemia.

Betulinic acid can induce apoptosis via activation of caspases and thus is regarded as a melanoma-specific cytotoxic agent. Nevertheless, its poor aqueous solubility limits the exploitation of its potential application. Recently, literature has reported that compounds bearing imidazole ring at the C-28 position of betulinic acid possessed the ability to improve anticancer activity. Derivative \textsuperscript{78} with the introduced imidazole ring and carbonyl group by the oxidation of the hydroxyl group in betulinic acid gave much better anticancer activities (IC\textsubscript{50} = 0.8, 1.4, and 2.0 \(\mu\)M, respectively) than betulinic acid (13- to 49-fold) toward cancer cell lines.
HepG2, Jurkat, and HeLa. Structure–activity relationship disclosed that the oxidation of the hydroxyl group in betulinic acid to a ketone at C-3 position remarkably influenced the cytotoxic potentiality.\textsuperscript{213}

It has been revealed that type I 17β-hydroxysteroid dehydrogenase (17β-HSD1) involved in the biosynthesis of estradiol was an attractive target to treat estrogen-induced cancers. Steroidal imidazole 79a was found to be a potent 17β-HSD1 inhibitor. Specially, the bis-methylene bridge between C-nucleoside and imidazole ring was of biological importance and its removal affording analog 79b resulted in substantial loss of inhibitory efficacy.\textsuperscript{214}

Inhibitors toward hedgehog pathway are able to influence tissue patterning, growth, and differentiation, and thereby are considered as potent anticancer agents. However, many inhibitors toward hedgehog pathway also gave potent p38 MAP kinases inhibitory potency. Imidazole 80 showed a selective and significant inhibitory efficacy against the hedgehog pathway in both Gli reporter cell assay and shh-stimulated cell differentiation assay. It showed no inhibitory activity against p38 MAP kinase in a whole blood cell assay and gave excellent in vivo pharmacokinetics and oral bioavailability.\textsuperscript{215} Therefore, this compound warranted further developments for the treatment of cancer.

Nek2 is of great interest as a potential target for anticancer drugs as it plays an important role in bipolar spindle assembly protein. Combination of the imidazole ring with oxindole backbone yielded their hybrid 81. This compound exhibited excellent affinity toward Nek2 ($IC_{50} = 0.77 \mu M$) without affecting mitotic kinases Cdk1, Aurora B, or Plk1 ($IC_{50} > 20 \mu M$). Particularly, this compound did not perturb the bipolar spindle assembly of human cells. Further study demonstrated that the nitrogen atom in oxindole moiety, which could form hydrogen bonds with Nek2 hinge region, was essential for the inhibitory activity.\textsuperscript{216}

Histone deacetylases (HDACs) are a large family of enzymes involved in acetylation of histones in cells that are related to cell differentiation, growth arrest, and apoptosis.\textsuperscript{217, 218} Carboxamide moiety as zinc-binding group is a potential pharmaceutical fragment for HDACs inhibitory activity. Carboxamide derivative 82 was the first selective HDAC1 inhibitor. It showed excellent bioactivity ($IC_{50} = 30 \text{nM}$) with good selectivity over HDAC2 (sevenfold) and HDAC3 (tenfold). Notably, it not only displayed comparable inhibition of the growth of HeLa cell line with an $IC_{50}$ value of 460 nM to vorinostat ($IC_{50} = 440 \text{nM}$), but also exerted equally in vivo anticancer effect in mice to vorinostat with less than 5% body weight loss in a HCT116 xenograft study.\textsuperscript{219}

Carbonic anhydrases (CAs) belong to a common class of metalloenzymes. They have critical influence on respiration, transport of CO$_2$ and protons, pH balancing of blood and other biological events. Their inhibitors possess a variety of pharmacologic applications including the treatment of cancer. Pyridinium-based imidazole 83 was the first reported CA activator with distinct binding model from previous activators, however, it would turn into an effective inhibitor after a longer contact with enzyme, and showed strong activity toward transmembrane CA isoforms including hCA IX, XII, and XIV with affinity values in the range of 0.08–0.96 μM. X-ray crystal structure of compound 83 evidenced that the pyridinium ring was fixed deep inside the enzyme cavity by a large number of hydrophobic interactions. The imidazolyl moiety interfered with the catalytic mechanism of the enzyme, thereby transforming the compound from a CA activator into an inhibitor.\textsuperscript{220}

A great many imidazoliums with positive center have been reported to be helpful in reinforcing the affinity and membrane permeability, and thus display high anticancer activities. Alkyl imidazoliums 84a and 84b could significantly inhibit the growth of leukemia cell lines with very low toxicity. Comparative study pointed out that the length of the alkyl chain at N-3 position of the imidazole ring had an important influence on bioactivity, and the most optimal chain length was 17 methylenes.\textsuperscript{221} While phenacyl imidazolium 85 showed better anticancer activity with $IC_{50}$ values in the range of 1.5–4.7 μM against HL-60, A431, Skov-3, K562,
SMMC-7721, Hep-2, and GLC-15 than cisplatin (IC$_{50}$s = 1.5–9.2 μM). Large conjugated imidazolium 86 also displayed superior anticancer potency with IC$_{50}$ values between 1.6 and 10.9 μM against SMMC-7721, SW480, MCF-7, and A549 to cisplatin (IC$_{50}$s = 8.8–16.6 μM). Especially, it was 5.4-fold more potent than cisplatin against SMMC-7721 with an IC$_{50}$ value of 1.6 μM, which demonstrated that the naphthyl acyl group was the most suitable one for modulating anticancer potency. Thus, imidazoliums might be considered as a promising type of leads for further structural modifications as anticancer drugs.

Insulin-like growth factor 1 receptor (IGF-1R) is involved in the emergence and proliferation of various tumors. Its inhibitors have become important agents to treat cancer. Imidazolone 87 showed potent IGF-1R inhibitory potency in HTRF (IC$_{50}$ = 98 nM) and ELISA (IC$_{50}$ = 7 nM) assay and displayed remarkable anticancer efficacy (IC$_{50}$ = 8 nM) in IGF-1-dependant cell assay. The good activity of compound 87 might be attributed to the free NH group, which was not only beneficial for the solubility but also helpful for the pronounced bioactivity. Additionally, imidazolone 88 was also able to strongly suppress the migration of prostate cancer (PC-3) (IC$_{50}$ = 8.1 μM) without obvious toxicity up to 200 μM in MTT assay. Therefore, compound 88 provided a chemical structural skeleton for future modifications in the design of new anticancer molecules.

3. IMIDAZOLES AS ANTIFUNGAL AGENTS

Azole compounds such as imidazoles and triazoles are the first class of synthetic antifungal agents. It is commonly considered that the imidazole ring could efficiently coordinate with the iron(II) ion of heme to restrain the biosynthesis of ergosterol thus inhibiting the growth of fungi. The structures of some clinical imidazole-based antifungal drugs are shown in Figure 12. The well-known clotrimazole (89a) was launched in 1972. It had a broad-spectrum antifungal
effect and was mainly used for the treatment of cutaneous Candida infective diseases. Since then, many ongoing efforts have been made to exploit novel imidazole-based antifungal agents. So far, a great number of antifungal imidazole drugs have been used in the clinic such as flutrimazole (89b), bifonazole (89c), croconazole (90a), oxiconazole (91b), climbazole (92a), ketoconazole (93c), miconazole (96a), fenticonazole (96d), sertaconazole (96g), and sulconazole (96h) (Table I). Some of them have historically been used in frontline antifungal therapies. Particularly, new types of imidazole antifungal drugs luliconazole (94) and eberconazole (95) were also marketed in 2005. All these have shown their large development value and wide potentiality as antifungal agents. However, along with the widespread use of current antifungal drugs, the increasing fungal resistances have largely influenced their therapeutic effects. Thus, the pursuit of structurally novel imidazoles with more effective, less toxic, and fewer resistances remains to be a highly challenging task and has aroused great interest in medicinal chemistry.

A. Structural Modification of Clinical Antifungal Azole Drugs

Structural modification of clinical antifungal azole drugs is an effective strategy to increase the biological activities and broaden active spectrum of current drugs in clinic. The structures for imidazoles modified compounds based on clinical azole antifungal drugs are shown in Figure 13. Enantiomerically pure molecules are highly demanded in pharmaceutical applications since many receptors are chiral, and single enantiomer agents generally display good activities. Importantly, the administration doses of single enantiomer are lower than racemic one, which suffers from minor risks of side effects and unspecific toxicity. (R)-miconazole 97 showed much stronger bioactivity with minimum inhibitory concentration (MIC) values ranging from 0.84 to 6.09 μM against C. krusei, Cryptococcus neoformans, P. chrysogenum, and Aspergillus niger than (S)-enantiomer (MIC values in the range of 16.34–48.06 μM).

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Research revealed that modification of miconazole with ester moiety led to different degrees of enhanced antifungal activities in contrast to the parent. A sevenfold enhanced anti-\textit{Candida} activity (MIC = 1.5 $\mu$g/mL) of compound 98a was observed after the esterification. Introduction of chlorine substituent in compound 98a produced analog 98b leading to a fivefold increased efficacy against \textit{Candida} with an MIC value of 0.3 $\mu$g/mL even stronger than miconazole (MIC = 0.5 $\mu$g/mL). On the contrary, introduction of strong electron-withdrawing nitro group resulted in a dramatic decrease for anti-\textit{Candida} activity. These results disclosed that the chlorine substituent was suitable to modulate the electronic effects on the benzene ring, thus was beneficial for the anti-\textit{Candida} efficacy. Removal of one methylene group in compounds 98a-b yielded analogs 98c-d. They still showed excellent antifungal efficacy in comparison to fluconazole toward both \textit{C. albicans} and non-\textit{albicans Candida} species with low cytotoxicity. Remarkably, pure enantiomers (-)-98c and (-)-98d displayed much higher anti-\textit{C. krusei} potency (IC$_{50}$ = 0.13 and 0.37 $\mu$g/mL, respectively) than fluconazole (IC$_{50}$ = 12 $\mu$g/mL). This difference might be explained by the conformation of these two compounds that was beneficial for antibacterial target rather than antifungal one. Interestingly, replacement of imidazole fragment by triazole ring would reduce the inhibitory effect.

The common structural feature of azole antifungal agents such as miconazole, oxiconazole, ketoconazole, itraconazole, and voriconazole is characterized by an ethyldiene chain bridged \textit{N}-(phenethyl)azole skeleton. Based on this scaffold, a number of oxiconazole analogs were designed and showed significant antifungal activities. Compounds 100a and 100b displayed superior anti-\textit{C. albicans} efficacy (MIC = 4 and 8 $\mu$g/mL, respectively) to
fluconazole (MIC = 8 μg/mL).\textsuperscript{239} Excellent anti- \textit{Microsporum gypseum} activity (MIC = 2 μg/mL) was also found on their analogs \textit{101a} and \textit{101b} with 16-fold more efficacy than fluconazole (MIC = 32 μg/mL). Further study demonstrated that the high antifungal potency especially for compounds \textit{101a} and \textit{101b} was related to their steric configurations, which were beneficial for imidazole ring to bind with the enzyme active site.\textsuperscript{240}

Since the discovery of bifonazole that is derived from clotrimazole, but contains no halogen atoms in the therapy of various dermatomycoses, many structural modifications on bifonazole have been carried out with the goal of increasing the antifungal potency and selectivity and improving the bioavailability. Imidazole derivative \textit{102a} bearing 2-chlorophenyl group displayed superior anti- \textit{Trichophyton rubrum} and anti- \textit{A. niger} effect (MIC = 0.5 and 4 μg/mL, respectively) to bifonazole (MIC = 4 μg/mL).\textsuperscript{241} While the MIC value of 4-chloride \textit{102b} (MIC = 0.5 μg/mL) toward \textit{C. albicans} was fourfold lower than that of fluconazole (MIC = 2 μg/mL). However, replacement of diphenyl group in bifonazole by benzoxazolone ring (\textit{103}) led to completely lost anti- \textit{Candida} efficacy.\textsuperscript{242} The high antifungal potency of \textit{102a-b} might be attributed to their small size, which made them convenient to penetrate into fungi cell, and the chlorine atoms with a positive effect was helpful for improving affinity toward the heme binding site.\textsuperscript{243}

Clotrimazole is the earliest imidazole antifungal drug used clinically. It is effective for the treatment of diverse fungal infections such as \textit{epidermophytosis}, \textit{trichophyton}, \textit{aspergillus}, \textit{chromatium}, \textit{cryptococcus}, and \textit{candida}. Its analog \textit{104} showed comparable antifungal activities to clotrimazole toward \textit{T. mentagrophytes}, \textit{M. gypseum}, \textit{C. albicans}, \textit{T. rubrum}, \textit{M. canis}, and \textit{Epidermaphyton floccosum}. Studies manifested that one methoxy group on the benzene ring was helpful for antifungal efficacy, while the introduction of another methoxy group would decrease the potency.\textsuperscript{244}

The action mechanism of ketoconazole-like drugs could be better explained by the “channel 2 opened” enzyme conformation. Replacement of phenyl-piperazine chain in ketoconazole by 1,4-benzoxazine gave analogs \textit{105a} and \textit{105b}. They could form hydrogen bonds with the backbone of Tyr505 and Met508 in the same manner as fluconazole, thus making them quite sensitive to \textit{C. albicans}. Especially, \textit{cis-105a} and \textit{trans-105b} displayed significant antifungal efficacy against high capsulated \textit{C. neoformans} strain CAP-67 and obviously in vivo bioactivity. Importantly, both compounds could cross the fungi membrane without difficulty resulting from their good balance of hydrophilic and hydrophobic properties.\textsuperscript{245} Therefore, structural modification of ketoconazole is a meaningful strategy to develop more effective antifungal agents.

B. New Types of Imidazole Antifungal Agents

Apart from the research of structural modifications of clinical drugs, development of imidazole antifungal compounds with new structural skeleton is another significative direction. Recently, considerable new skeleton-based imidazoles with good antifungal activities have been developed.\textsuperscript{246–250} The structures for new types of imidazole-based antifungal compounds are shown in Figure 14. A lot of \textit{N}-alkyl imidazoles that are able to exert stronger interaction with the amino acid residues of the target enzymes are being actively investigated for their great potentiality as antifungal agents. Electron-withdrawing groups like nitro and bromo moieties are helpful to strengthen antifungal efficacy because they can easily generate hydrogen-bond interactions with key amino acids Ser378 and His377 of CYP51. Imidazoles \textit{106a-c} with para-bromo, nitro, or phenyl group showed comparable antifungal potency to ketoconazole toward \textit{C. albicans}, \textit{A. niger}, and \textit{P. chrysogenum}.\textsuperscript{251}

Recent literature provided evidence that the incorporation of phenethyl ether group could enhance the antifungal ability. Imidazoles \textit{107a} and \textit{107b} were obtained by the introduction
Figure 14. New types of imidazole-based antifungal compounds.

of phenethyl ether group into compound 106. They gave equipotent anti-\textit{Candida} potency to fluconazole. Especially, dichloride 107b displayed superior anti-\textit{S. cerevisiae} efficacy with an MIC value of 4 $\mu$g/mL to fluconazole (MIC = 64 $\mu$g/mL) as well as better anti-\textit{A. niger} (MIC = 32 $\mu$g/mL) and anti-\textit{M. gypseum} (MIC = 8 $\mu$g/mL) activities than fluconazole (no activity at concentrations of less than 128 $\mu$g/mL). Contrast test demonstrated that chloro-substituted derivative exhibited more potent antifungal activity than others. Notably, compound 107b had a positive drug-likeness value of 4.49 revealing its potential use as a safe lead compound.

Piperidone-based imidazole derivative 108 could inhibit the growth of \textit{A. niger} and \textit{C. neoformans} with IC$_{50}$ values of 12.5 and 6.25 $\mu$g/mL, respectively, which was more effective than amphotericin-B (IC$_{50}$ = 50 and 25 $\mu$g/mL). Comparative study pointed out that replacement of the imidazole ring by benzotriazole group lowered the antifungal potency, which manifested the important role of the imidazole ring for the good bioactivity. Therefore, this kind of compounds might be used as templates to generate better antifungal drugs.

Pyridines are an important class of compounds and possess a broad spectrum of biological activities. Much effort has been devoted to exploring their pharmacological efficacy.
as antifungal agents. Bis-imidazole modified 1,4-dihydropyridines 109a and 109b showed comparable anti-*C. albicans* activity to clotrimazole. They are worthy of further investigation for the treatment of fungal diseases.²⁵⁴

Coumarin nucleus with low toxicity profile and convenient synthesis is present in many bioactive compounds and has attracted quite interest in the development of antifungal drugs.²⁵⁵–²⁵⁹ Coumarin-derived imidazoles 110a and 110b were able to strongly inhibit the growth of *C. albicans* and *C. krusei*.²⁶⁰ Further study manifested that electron-donating groups on the phenyl group were not favorable for the antifungal potency. Deep investigations of this type of compounds might be a rational approach to afford drug candidates. Chromenes, as coumarin analogs, could kill microbes or block their active sites. Chromene-based imidazole 111 displayed comparable anti-*C. albicans* activity with an MIC value of 12.5 μg/mL to ketoconazole (MIC = 12.5 μg/mL).²⁶¹ The low MIC value of this compound makes it possess considerable possibility as an antifungal agent.

Chalcones are a large type of important bioactive molecules with remarkable biological activities.²⁶²–²⁶⁴ Imidazole-incorporated chalcones 112a and 112b had comparable anti-*A. fumigatus* efficacy to nystatin. Specially, the *p*-chloride 112a showed significant superoxide anion radical scavenging activity (90.06%) comparable to the standard *n*-propyl gallate (91.3%).²⁶⁵ These observations demonstrated that imidazole-containing chalcones had great potentiality to treat aspergillosis caused by *A. fumigatus*.

It has been disclosed that bis-phenyl ether group was an efficient pharmacophore and was widely used in pesticide such as difenoconazole. Imidazoles 113a and 113b bearing bis-phenyl
ether moiety displayed much better anti-*C. albicans* (MIC = 0.06 μg/mL) and anti-*A. niger* (MIC = 1.00 μg/mL) activities than amphotericin B (1.00 and 6.00 μg/mL) and miconazole (0.12 and 2.00 μg/mL), respectively. Structure–activity relationships showed that the para-chloro or para-bromo group on the benzene ring was beneficial for improving antifungal efficacy.

Benzimidazole derivatives with a variety of biological activities showed large potentiality in medicinal chemistry. Much research suggested that the incorporation of heterocycles such as thiophene, furan, and thiadiazole into benzimidazole scaffold could improve antimicrobial efficacy. Imidazole-modified benzimidazole showed superior anti-*C. albicans* activity and comparable anti-*C. tropicalis* and anti-*C. globrata* efficacies to ketoconazole with safety LC50 (50% lethal concentration) > 1000 μg/mL. It is worthy of further investigations as a potential antifungal agent.

*N*-cyanocarboxamides have great potentiality to be developed as antifungal agents, however, their synthesis is remaining a challenge. Recent literature revealed a convenient method for the synthesis of imidazole derivative through ring-opening rearrangement without using highly toxic reagents. Notably, it was observed to display much better anti-*Rhizoctonia solani* activity with an EC50 (50% effective concentration) value of 2.63 μg/mL than triadimefon (EC50 = 14.68 μg/mL). This finding provides an opportunity to further exploit antifungal *N*-cyanocarboxamides.

Literature disclosed that the 5-nitroimidazole moiety in secnidazole was favorable for tissue penetration and essential for biological activity. However, the reactive intermediates formed in microorganisms by the reduction of nitro group in nitroimidazole could covalently bind with DNA and trigger the adverse effect. Esterification of the hydroxyl group in secnidazole was an effective way to sterically protect the nitro moiety and thus led to the improved metabolism and physicochemical property. Therefore, secnidazole derivatives and showed superior anti-*A. niger* and anti-*C. albicans* efficacy to secnidazole.

Fluconazole is a well-known first-line antifungal drug recommended by World Health Organization (WHO). Its exceptional therapeutic record for *Candida* infections has received special attention. However, several disadvantages including the increasing fluconazole-resistant *C. albicans* isolates along with the extensive clinical use of fluconazole, low water solubility, no effectiveness against invasive aspergillosis, and nonfungicide limit its clinical use. Therefore, a large amount of work has been devoted to further structural modification of fluconazole. It is well known that tertiary amino group could not only easily form hydrogen bonds, but also readily accept proton to produce quaternary salts with facility to improve water solubility. Thus, bis-imidazole fluconazole analogs were prepared by the bioisoster replacement of the tertiary alcohol moiety in fluconazole with a tertiary amino group, substitution of 2,4-difluorophenyl group by halobenzyl moieties, replacement of methylene bridge between tertiary alcohol group and triazolyl moiety by ethylene chain, and substitution of triazole ring by different imidazole rings. Bioactive test demonstrated that all compounds displayed biological activity against *C. albicans* and *A. fumigatus*. When one imidazole ring in compound was substituted by a berberine fragment, the resulting berberine fluconazole analogs displayed antifungal activity with MIC values of 32 μg/mL against *C. albicans*, *C. mycoderma*, *C. utilis*, and *Beer yeast*. However, their biological activities were weaker than fluconazole. This work provides a new type of structural skeleton of fluconazole analog, it is worthy for further investigations as new antifungal agents.

Natural glucose with multiple hydroxyl groups is widely used in prodrug developments in order to improve the water solubility and tissue penetration. Natural glucose-based imidazoles and with good water solubility exhibited potent antifungal efficacy toward *C. albicans*. Bis-phenyl derivative could significantly inhibit the growth of *C. albicans*, even much better than clotrimazole. Structure–activity relationship demonstrated that the number
of phenyl groups on the imidazole ring had an important impact on improving their antifungal activities.

Benzodiazepines as an important class of heterocyclic anticonvulsant agents such as chlor Diazepoxide have attracted much attention. Interestingly, a recent research showed that imidazole modified benzodiazepines 119a and 119b displayed comparable anti-A. niger activity to nystatin. Comparative study revealed that the halo and hydroxyl substituents on the benzene ring were beneficial for antifungal efficacy.

Antimicrobial peptides (AMPs) are a large class of natural peptides with a broad spectrum of bioactivities and show higher activities in slightly acidic areas of the human body. Amphiphilic imidazoles 120a-c displayed strong anti-A. fumigatus and anti-C. neoformans activities at pH 5.5 with nontoxic activity, but weak efficacy at pH 7.5. The pH-dependent activity was most likely due to the increased amphiphilicity resulting from the positively charged histidine residues at pH 5.5. Interestingly, they were selectively active against fungi but not bacteria. The research indicated that imidazole-modified AMPs had the potentiality to become the next generation of clinical antifungal agents.

Cyclopeptide present in many natural products is a special bioactive group of compounds with interesting pharmacological and biochemical properties. Imidazole-substituted peptide 121 exhibited comparable anti-C. krusei and anti-C. neoformans efficacy with MIC90 (90% MIC) values of 64 and 4 μg/mL, respectively, to fluconazole (MIC90 = 64 and 4 μg/mL, respectively). Further study suggested that cyclic peptides had generally better antifungal activities than their linear analogs.

Motivated by the bioactivities of macrocyclic compounds, many researchers have devoted to the synthesis and bioevaluation of macrocyclic bioactive molecules. Sulfone derivatives are well known for their broad-spectrum biological activities. Sulphonophane imidazoli-ums 122a-c presented equivalent anti-C. albicans efficacy (MIC = 5 μg/mL) to clotrimazole (MIC = 5 μg/mL) via synergistic effects. Specially, the number of sulfone groups had a major effect on the bioactivity. Compound 122a bearing two sulfone moieties could exert strong hydrogen-bond interactions with the side chain of the DNA gyrase B. Such a good inhibitor of DNA gyrase B could be considered as a lead compound for further investigation as an antifungal agent.

4. IMIDAZOLES AS ANTIBACTERIAL AGENTS

The development of antibacterial agents to treat infections has been one of the most notable medical achievements in the past century. The structures of some clinical imidazole-based antibacterial drugs are shown in Figure 15. Metronidazole (123a), ornidazole (123b), secnidazole (123c), nimorazole (123d), and tinidazole (123e) are well-established nitroimidazole drugs in widespread clinical use to treat diseases caused by protozoa and anaerobic bacteria. Particularly, structurally simple metronidazole as an effective synthetic compound introduced in 1960.

![Figure 15. Some clinical imidazole-based antibacterial drugs](image-url)
possesses strong inhibitory efficacies against Gram-negative anaerobic bacteria such as *Helicobacter pylori* and protozoa such as *Giardia*, *Lamblia*, and *Entamoeba histolytica*. In the case of ornidazole, the (S)-isomer is solely responsible for the therapeutic effect toward anaerobic bacteria, protozoan, and trichomonas infections. Specially, despite of their long-term clinical use, the incidence of resistance in anaerobic bacteria is still very low. This encourages continuous research to focus on the development of such nitroimidazoles with potential medicinal application. Recently, many efforts have been made to identify new imidazole antibacterial agents with novel structures and a great number of imidazoles have been discovered to possess broad-spectrum antibacterial activities. It has demonstrated that imidazole derivatives should possess infinite space to be developed as antibacterial agents with better curative effect, lower toxicity, and less side effects. The structures for imidazole-based antibacterial compounds are shown in Figure 16.

### A. Alkyl-Linked Imidazoles as Antibacterial Agents

Introduction of an alkyl chain in the target molecule should be favorable for modulating physicochemical properties such as binding affinity and water solubility, thus be conductive for antimicrobial activity and beneficial for increasing biological activities. Lots of research showed that structural modification of the imidazole ring by suitable alkyl side chain was favorable for effective interaction with the hydrophobic parts of target enzymes and thereby resulted in enhanced antibacterial activities. Numerous imidazole derivatives characterized with a certain length of alkyl linkers between the imidazole ring and an additional function group have been reported to manifest good antibacterial activities.

Pyridinone derivatives have attracted much attention owing to their interesting pharmacological properties. Combination of a pyridinone group with an imidazole ring through a trimethylene bridge yielded hybrid 124. It was identified as an effective anti-*Proteus vulgaris* A161 agent (MIC = 250 μg/mL) in comparison with maxipine (MIC = 125 μg/mL). Further study demonstrated that the 3-hydroxyl and 4-carbonyl groups in pyridinone moiety, which could selectively chelate with Fe³⁺ ion in bacteria, played a vital role in improving bioactivities.

Carbazole is a kind of aromatic heterocycle present in a variety of naturally occurring and medicinally active substances such as antibiotics carbazomycins and murrayafoline. Carbazole-based imidazoles 125a and 125b bearing alkyl or aralkyl linker displayed comparable or even superior antibacterial activities with MIC values in the range of 1–8 μg/mL to chloramphenicol and norfloxacin toward *S. aureus*, methicillin-resistant *S. aureus* (MRSA), *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *B. proteus*. Specially, compound 125a bearing a (CH₂)₄ or (CH₂)₆ bridge showed similar anti-*B. proteus* potency with an MIC value of 1 μg/mL to norfloxacin (MIC = 1 μg/mL). It was much better than the corresponding phenyl derivative 125b (MIC = 4 μg/mL). However, replacement of imidazole ring by other heterocycles resulted in low antibacterial activity.

Berberine has been commonly used in the clinic as a therapeutic agent to treat infectious diseases such as acute gastroenteritis, cholera, and bacillary dysentery for many years. A series of hybrids of berberine with clinical metronidazole as new type of antimicrobial agents were developed. The introduction of berberine fragment into metronidazole is helpful to spatially protect the nitro group in metronidazole with the aim to improve the metabolism and physicochemical property, the tertiary amino group is beneficial to improve the water solubility and binding affinity, thereby increase their biological activities and broaden active spectrum. It was found that 5-nitroimidazole derivative 126a gave low inhibitory concentration toward *S. dysenteriae* and *P. vulgaris* with MIC values of 4 μg/mL, which were comparable to or even better than the reference drugs chloromycin, berberine, and norfloxacin. The transportation...
behavior of human serum albumin (HSA) to compound 126a showed that nitroimidazole derivative 126a could be effectively stored and carried by HSA. Contrast test revealed that the antibacterial activities should be closely related to nitroimidazole moiety and halobenzyl group to some extent. Difluorobenzyl group was more helpful for increasing the antibacterial efficacy in comparison to other halobenzyl ones. Additionally, 126a was more active than compound 126b.\(^\text{16}\)

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Amino imidazoles have aroused widespread attention in medicinal chemistry for that they are able to inhibit and disperse bacterial biofilms via a nonmicrobicidal mechanism to keep the bacteria within more sensitive planktonic state. In this regard, amino imidazole was designed and displayed notable biofilm dispersal and antibacterial activities toward *E. coli*, *S. epidermidis*, methicillin sensitive *Staphylococcus aureus* (MDRAB), MRSA, vancomycin-resistant *enterococci* (VRE), and methicillin sensitive *Staphylococcus aureus* (MSSA). Structure–activity relationship demonstrated that the length of alkyl linker had a crucial influence in modulating activity, and the 5-methylene compound was the most active one. These results demonstrated that amino imidazoles might be the basis to gain compounds with either good antibiotic or antibiofilm efficacy.

Dihydroimidazole moiety is widely found in many biologically active compounds with diverse biological and pharmacological properties. A great deal of work has been engaged in the exploration of novel dihydroimidazole derivatives with promising antibacterial activities. The reaction between 2-guanidinobenzimidazole and halogenated methylene afforded compounds and . Compared with chloride , compound exerted obviously increased inhibitory efficacy toward *B. cereus*, *B. subtilis*, *E. coli*, *Micrococcus luteus*, *S. aureus*, *P. aeruginosa*, and *M. roseus*. Further study revealed that the substitutions on imidazole ring made a significant contribution to the antibacterial activities.

### B. Acyl-Linked Imidazoles as Antibacterial Agents

A number of acyl imidazole derivatives displayed good antibacterial activities, which possessed large development value as medicinal agents. Specially, amide linkage with favorable properties such as high polarity, good stability, and conformational diversity, prevalently in peptides and proteins, is one of the most popular and reliable functional groups in the versatile and widely used synthetic drugs. The acyl linkage also endues target compounds with large conjugated system and the ability to bind easily with various enzymes and receptors in biological systems, thereby leading to good antibacterial activities. Carbonyl-linked imidazoles were reported to have excellent antibacterial activities. Compound bearing a pyridine group had equivalent anti-*S. aureus*, anti-*B. subtilis*, and anti-*E. coli* efficacy (MIC = 0.002 μM/mL) to norfloxacin (MIC = 0.002 μM/mL). Notably, diphenyl imidazole displayed two times more effective antitubercular potency (MIC = 0.002 μg/mL) than ciprofloxacin (MIC = 0.004 μg/mL). However, the removal of the carbonyl group significantly lowered the antimicrobial activity, implying the significance of the carbonyl group for bioactivity.

Bis-phenyl piperidines have been identified as highly active antimicrobial agents. Compound was prepared by the introduction of the imidazole ring into such skeleton. It was two times more potent anti-*E. coli* agent than linezolid (MIC = 128 μg/mL). Notably, imidazole derivative also displayed excellent activity toward vancomycin- and linezolid-resistant *Enterococcus faecalis* with MIC values of 64 μg/mL in comparison with trovafloxacin (MIC = 128 μg/mL) and linezolid (MIC = 64 μg/mL). These observations indicated that bis-phenyl piperidine-based imidazoles had the potentiality to improve antibacterial efficacy particularly to overcome multidrug resistant problem, thereby being worthy of in-depth investigations.

Histidinol dehydrogenase is essential for bacterial growth without counterpart in mammals. It constitutes a therapeutic target for the development of anti-infectious treatment against intracellular pathogens. Histidine-derived compound showed good inhibitory efficacy with an IC value of 24 nM toward histidinol dehydrogenase. Substitution of the hydroxy group in compound by a sulfhydryl moiety caused a 100-fold reduction of inhibitory activity, indicating the biological importance of the hydroxy group. This type of compounds might provide valuable candidates for the potential development of novel nonclassical antibacterial agents.
Bacterial topoisomerases have been an attracting target for antibacterial activity since the discovery of *E. coli* topoisomerase II (DNA gyrase) and its role in the action mechanism of nalidixic acid. Thiosemicarbazide derivatives with imidazole and benzoyl groups were expected to possess good antibacterial activity via inhibition of bacterial topoisomerase. Compound 132 was a potent topoisomerase IV inhibitor and gave high therapeutic potentiality toward Gram-positive bacteria, while inactive against Gram-negative bacteria. It had the same or superior potency toward *M. luteus* (MIC = 0.98 μg/mL), *S. epidermidis* (MIC = 3.91 μg/mL), and *B. subtilis* (MIC = 7.81 μg/mL) in comparison to cefuroxime (MIC = 0.98, 0.98, and 125 μg/mL, respectively). This finding indicates that imidazole-modified thiosemicarbazides might be potential candidates for further structural optimization in developing novel gyrase inhibitors.

Metronidazoles have been developed as selective inhibitors of porphyrin auxotroph bacterium *Porphyromonas gingivalis* through the recognition by HA2 receptor. Research implied that capture by HA2 was crucial for their uptake into cell and thus led to the improved bioactivity. Compounds without the ability to be recognized by HA2 receptor were ineffective *P. gingivalis* inhibitors. Porphyrin-metronidazole adducts 133a and 133b with the same selective anti-*P. gingivalis* activity showed that changing the positions of the protons on the vinyl face of porphyrin macrocycle did not affect the recognition by HA2.

**C. Imidazole Conjugates as Antibacterial Agents**

Much research has shown that combination of the imidazole ring with conjugated heterocyclic moiety could endow compounds with a larger conjugated system, which might be helpful for binding with enzymes and receptors in biological systems and therefore lead to the enhancement of bioactivity. Meanwhile, heterocyclic compounds bearing nitrogen, sulfur, or oxygen atom could provide multiple action sites and noncovalent interactions. They are characterized with a better hydrophilicity/hydrophobicity ratio, making them easy to penetrate into biofilms, thus leading to a better curative effect. All of these provoked great interest to investigate the potentiality of conjugated imidazole heterocycles as a new type of antibacterial agents.

Quinolinone derivatives have become one of hot topics in medicinal chemistry in view of their wide-range biological activities. 6-Fluoroquinolinones, as highly effective antibacterial molecules, have been widely used to treat infectious diseases. Imidazole derivative 134 gave comparable anti-*E. coli* potency in comparison to ciprofloxacin. Structure–activity relationship displayed that substitution of the imidazole ring by piperazine, morpholine, piperidine, or pyrrolidine moiety all had a negative effect on bioactivity.

Dibenzothiazepines with eminent biological activities in the central nervous system (CNS) are of great interest in drug discovery and development. Dibenzothiazepine-based imidazole 135 was considerably effective against bacterial strains *Salmonella typhi*, *Shigella dysenteriae*, *E. coli*, *S. aureus*, *Klebsiella pneumoniae*, *B. cereus*, *P. aeruginosa*, and *Serratia marcescens*. Further study manifested that the imidazole ring contributed greatly to the improved antibacterial potency and broadened antimicrobial spectrum. Replacement of the imidazole ring with other moieties such as benzimidazole, mercapto benzimidazole, 2-amino ethanol, benzyl amine, and cyclopropyl amine resulted in obviously decreased bioactivities.

Oxazolidinones are a relatively new kind of orally active and totally synthetic antibacterial agents. Eperezolid and linezolid are two potent antibacterial agents of this class with different action mechanisms from existing antibacterial drugs. Structural modification of linezolid yielded its analog 136. This compound could significantly inhibit the growth of all tested Gram-positive bacteria with MIC values in the range of 0.024–0.195 μg/mL at noncytotoxic concentrations. Notably, this compound displayed superior anti-*M. luteus* (MIC = 0.195 μg/mL) and anti-*S. warneri* (MIC = 0.006 μg/mL) efficacy to linezolid (MIC = 0.781 and 0.781 μg/mL) and...
ciprofloxacin (MIC = 3.125 and 0.195 μg/mL), respectively. Structure–activity relationship showed that nitroimidazole ring had a positive impact on the biological potency.\(^{344}\)

It was found that 1,4-dihydropyridine derivatives exhibited antibacterial potentiality.\(^{345, 346}\) Substitution of classical structure of 3,5-diester in 1,4-dihydropyridines by 3,5-diamide was a good pathway to combat cloxacillin-resistant strains. Compound 137 had a synergistic effect with cloxacillin leading to the enhanced antibacterial activity, but it did not show any bioactivity in the absence of cloxacillin. Comparative study discovered that the chlorine atom on the benzene ring was essential for good efficacy.\(^{347}\) However, the reason for synergistic effect with cloxacillin was not known and required further investigations.

The widespread biological activities of triphenyl imidazole derivatives have attracted much attention.\(^{348}\) Compound 138 was twofold more active toward \(S.\) \(aureus\) (MIC = 0.5 mg/mL) and \(P.\) \(aeruginosa\) (MIC = 0.5 mg/mL) than tetracyclin (MIC = 1 mg/mL).\(^{349}\) Incorporation of triazole-thiol moiety\(^{350}\) into triphenyl imidazole skeleton gave compound 139a with strong anti-\(S.\) \(aureus\) efficacy in comparison to ofloxacin.\(^{351}\) Additionally, some acyl-bridged triphenyl imidazole adducts were also reported to show good antibacterial activities. Pyrrolyl imidazole 139b displayed equivalent anti-\(E.\) \(coli\) potency with an MIC value of 250 μg/mL to tetracycline (MIC = 250 μg/mL).\(^{352}\) Schiff base derivative 139c could effectively inhibit the growth of \(E.\) \(coli\) and \(S.\) \(aureus\) with MIC values in the range of 0.5–0.25 mg/mL, which was comparable to tetracycline (MIC = 0.25 mg/mL).\(^{353}\) The results demonstrate that combination of triphenyl imidazole with heterocycles is an effective strategy to produce new types of antibacterial agents.

### D. Imidazole-Modified Macrocyclic Natural Products as Antibacterial Agents

Natural products acquired from microbial and plant sources have served well as antibacterial agents for more than 50 years.\(^{354}\) However, the increasing number of multidrug resistant strains has become a growing public health problem in many regions. In order to reduce the emergence of resistant organisms, various chemotherapy regimens have been adopted. Combination therapy enhances the uptake resulting in improved antimicrobial activity. Such a combinatorial regime has shown synergistic bactericidal effects against the most resistant organisms. Imidazole ring, a naturally occurring moiety, has provoked great interest to investigate its potential role in natural products. The structures for imidazole-based macrocyclic antibacterial compounds are shown in Figure 17. A recent marketed drug telithromycin (140) containing imidazole ring has shown promising antibacterial potency. Much research has revealed that introduction of imidazole ring into natural skeleton could remarkably enhance the antimicrobial activities and had the potentiality to overcome drug resistance. Incorporation of imidazole ring into clarithromycin produced compound 141. It displayed excellent anti-\(Haemophilus influenzae\) activity (MIC = 0.06 μg/mL) and superior efficacy (MICs = 0.25–4 μg/mL) toward clindamycin-resistant strains \(Streptococcus pneumoniae\) to telithromycin (MICs = 4–64 μg/mL). Moreover, compound 141 also exhibited comparable in vivo potency to telithromycin in murine systemic infection model using \(S.\) \(pneumoniae\) 3579.\(^{355}\) These results suggest that it should be worthwhile for further studies on clarithromycin-derived imidazoles as a new type of antimicrobial agents for their potentiality to overcome drug resistance.

Thiopeptide-based natural product GE2270 A was described to have the ability to inhibit the growth of MRSA and VRE with significant in vitro potency (MIC < 1 μg/mL). However, its imidazole analogs 142a–c gave moderate activities with MIC values in the range of 4–8 μg/mL against \(E.\) \(faecalis\) and \(S.\) \(aureus\) and no activity toward \(Enterrococcus faecium\) and \(S.\) \(pyogenes\). The reduced antibacterial efficacy might be attributed to their weaker cell penetration due to the ionizable nature of these imidazole-carboxylic acids. Interestingly, both compounds 142a and 142c displayed equivalent potency to the natural product using in vitro bacterial cell extract assay.\(^{356}\)
Gramicidin S with the ability to inhibit a broad range of Gram-negative bacteria has become increasingly attractive for the development of antibacterial compounds with good activity and lower toxicity. Substitution of the phenyl group in gramicidin S with an imidazole ring gave its analog 143. It showed superior antibacterial abilities with MIC values in the range of 4–16 μg/mL to gramicidin S (MIC = 4–32 μg/mL) against both Gram-positive and Gram-negative bacteria including S. aureus, CNS 5277, E. faecalis, P. aeruginosa, and S. mitis BMS. Especially, this compound also displayed significantly diminished hemolytic potency. Further research implied that the high potency of 143 might arise from the higher basicity of the imidazole ring (pK_a = 7.0), and replacement of the imidazole ring with weaker basic triazole ring (pK_a = 1.2) decreased the bioactivity.357

E. Imidazolium Salts as Antibacterial Agents

Azolium salts with potent antimicroorganism activity have drawn much attention in medicinal chemistry.358,359 Positive imidazolium salts are not only helpful in reinforcing affinity, water solubility, and membrane permeability, but also have the ability to prevent migration leading to improving antimicrobial efficacy.360,361 These special properties forecast a brilliant future for their derivatives to be developed as antimicrobial agents in anti-infective field. Transformation of imidazole ring into imidazolium would result in more potent antibacterial compounds with broadened spectrum, which attracts lots of interest to study imidazolium-based antibacterial agents. The structures for imidazolium-based antibacterial compounds are shown in Figure 18.

Selenium is a well-known important bioessential element and able to interact with DNA, RNA, and proteins. Naturally, selenium-containing compounds offer large possibilities as therapeutic agents.362 Selenium imidazolium 144 gave quite low MIC values of 0.06 and
0.03 μg/mL against *E. coli* and *S. aureus*, respectively.\(^{363}\) This suggested that this kind of selenium imidazolium should have large potentiality as a new type of antibacterial drugs.

Literature has reported the importance of water-soluble polymers for the antimicrobial activity as they could disrupt the cell membrane of microorganisms. Sulfonic acid modified imidazole polymer \(145\) with good water solubility could more effectively suppress the growth of *B. coagulans* BTS-3 with an MIC value of 0.062 mg/mL than penicillin (MIC = 0.25 mg/mL). Further research manifested that the OH\(^-\) at N\(^+\) ion center might have the ability to influence the alignment of polymer chains and affect the interaction between target molecules and microbes.\(^{364}\)

Imidazoliums bearing alkyl chain are favorable for interacting with target enzymes, thus incorporation of these structures in polymer might enhance antibacterial activities. Imidazolium ionomer \(146\) bearing a 16-membered carbochain with different counterions showed comparable anti-*S. aureus* efficacy but lower anti-*E. coli* potency to triclosan. Moreover, it was more stable than the corresponding chloride and bromide salts.\(^{365}\) This type of compounds deserve to be investigated in-depth as selective antibacterial agents against Gram-positive pathogens.

Cyclophanes, especially imidazole-based dicationic ones, have recently received much attention\(^{366}\) because the dicationic imidazolophanes with various spacers such as pyridine, \(m\)-terphenyl, oxadiazole,\(^{367}\) quinoline, and carbazole\(^{368}\) units could act as bactericidal agents with high potency and good selectivity. Compound \(147\) gave stronger antibacterial activities with MIC values in the range of 10–20 μg/mL toward *S. aureus*, *P. aeruginosa*, *Shigella* sp., *K. pneumoniae*, *E. coli*, and *Vibrio cholera* than streptomycin (MIC values in the range of 20–45 μg/mL).\(^{369}\) It implied that this type of compounds should be worthy to be deeply investigated.
F. Imidazolone Derivatives as Antibacterial Agents

Imidazolone derivatives as inhibitors of serine protease and liver glycogen phosphorylases have shown good prospect for the exploitation of novel antibacterial agents with good bioactivity.\textsuperscript{370–375} Imidazolone bearing a reactive $\alpha,\beta$-unsaturated keto functionality that can be altered depending on the type and position of substituents on the aromatic rings acts an essential role in certain biologically important compounds. Particularly, numerous efforts have been devoted to the synthesis of N-aryl imidazolone derivatives with promising antibacterial activities due to their excellent bioactivity. The structures for imidazolone-based antibacterial compounds are shown in Figure 18.

Quinoline derivatives with desirable large $\pi$-conjugated aromatic nitrogen heterocyclic backbone are being actively developed for their versatile therapeutic activities.\textsuperscript{376} Quinoline-derived imidazolone \textit{148a} exhibited comparable anti-\textit{B. megaterium} efficacy to streptomycin.\textsuperscript{377} Piperazine-modified compound \textit{148b} was capable of inhibiting the growth of \textit{E. coli} with equivalent potency to ampicillin. Structure–activity relationship revealed that the methoxy group in the quinoline ring was of biological importance.\textsuperscript{378}

Fluorobenzothiazoles are a kind of effective therapeutic agents and have been widely employed in the development of novel pharmacologically active agents. Imidazolone \textit{149} gave an equivalent anti-\textit{S. aureus} efficacy to streptomycin. Further research confirmed the positive impact of fluorine atom on antibacterial potency.\textsuperscript{379}

It has been reported that pyrazole derivatives possessed widespread biological activities.\textsuperscript{380} A series of pyrazole incorporated imidazoles were reported to possess promising antibacterial activities. Imidazole thione \textit{150} was identified to have comparable anti-\textit{Clostridium profingens} potency to streptomycin. Particularly, this compound was safe up to 3000 mg/kg, making it have the potentiality to be developed as an ideal antibacterial drug.\textsuperscript{381}

G. Imidazole-Based Metal Complexes as Antibacterial Agents

Metal chelates play an important role in the biological systems, as they are essential parts of metalloproteins and enzymes. Over the past few years, the development of metal complexes-type drugs has stimulated increasing interest in the possibility of imidazole-based metal complexes as antibacterial agents.\textsuperscript{382} Imidazoles possess multiple nitrogen atoms that are available as chelating ligands and could easily interact with metal ions through weak interactions. Moreover, they are easy to be synthesized and modified in multiple ways allowing a rational design of metal complexes with improved stability, redox potentiality, membrane permeability, and biological activity. The structures of imidazole-based antibacterial metal supramolecular complexes are shown in Figure 19.

Plenty of lipophilic imidazole-copper(II) complexes that favor permeation through the bacterial membrane have been observed to display high antibacterial activities. Copper(II) complex \textit{151} was prepared by coordination of Cu(II) ion with Schiff-base ligand through two nitrogen and one oxygen atoms. It was able to effectively inhibit the growth of \textit{E. coli} and \textit{B. subtilis}.\textsuperscript{383} Copper(II) supermolecule \textit{152} could cleave DNA in the presence of biological reductant and exhibited good anti-\textit{S. aureus} potency.\textsuperscript{384} Additionally, copper(II) imidazole \textit{153} also exerted high antimicrobial efficacy toward \textit{S. aureus}, \textit{E. coli}, \textit{K. pneumaniae}, \textit{P. vulgaris}, and \textit{P. aeruginosa}.\textsuperscript{385}

Nickel is also an essential element in the life process and plays an important role in the absorption of iron element, increase of red corpuscle, and synthesis of some amino enzymes in the human body.\textsuperscript{386} Nickel(II) complex \textit{154} was formed by the coordination of Ni(II) ion with two imidazoles and two L-tyrosine ligands through four nitrogen and two oxygen atoms. It was
able to significantly suppress the growth of *P. verrucosu* and possessed a remarkably stronger antibacterial efficacy than single imidazole ligands.\textsuperscript{387}

Biomimetic tripodal bis-imidazole thioether ligands are characterized by distinct basicity and donor-acceptor properties from traditional nitrogen ligands. This type of ligands provides an encumbered environment that could prevent the formation of bimetallic species and have a synergic effect with Ag\textsuperscript{+} ion. Silver(I) complex 155 displayed high antibacterial activities toward *E. coli*, *B. cereus*, *S. aureus*, *S. cerevisiae*, and *Almonella tiphymurium* with low cytotoxicity, which motivated further investigations to this kind of complexes as potential antibacterial agents with broad spectrum. Notably, distinguished from previously reported imidazole coordinated complexes, complex 155 was formed through coordination of sulfur and oxygen atom in trifluoromethanesulfonate and bis-imidazole thioether ligand, while the imidazole ring was not coordinated, which provided the diverse coordinated forms of imidazole derivatives.\textsuperscript{388}

Taken altogether, the research and developments of imidazole-based supramolecular complexes in medicinal chemistry are still in its infancy. Special attention should be paid toward this new orientation for the development of imidazole-based metal drugs. Further efforts toward more imidazole-based metal compounds with good curative effects, low toxicity, and broad antimicrobial spectrum are worthy to be done.

5. IMIDAZOLES AS ANTITUBERCULAR AGENTS

Tuberculosis (TB) especially that caused by *Mycobacterium tuberculosis* (MTB) has become one of the most serious prevalent diseases threatening public health worldwide. Some clinically available anti-infectious agents such as isoniazide and rifampin have a vital effect on the treatment of TB over past decades. However, the global prevalence of drug-resistant TB (XDRTB) and multidrug resistant TB (MDRTB) made these traditional anti-TB agents with limited efficacy. Unfortunately, no novel anti-TB drugs have been developed in the past four decades. Thus there is an emergent need to develop more effective drugs. Research on imidazoles as anti-TB agents is active. Nitroimidazopyran PA-824 (156) is an active agent against both the replicating and the latent mycobacterium and is in advanced stage of clinical trials for

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure19}
\caption{Imidazole-based antibacterial metal supramolecular complexes.}
\end{figure}
the treatment of TB. This inspired numerous researchers to devote to the searches for new imidazole-based anti-TB agents and many of them have been reported recently. The structures of imidazole-based antitubercular compounds are shown in Figure 20.

Pyrazinamide (PZA) with excellent sterilizing effect on tubercle bacillus could be considered as an attractive starting point for the development of anti-TB drugs. Imidazole-modified PZA derivative 157 was found to act through a different action mechanism from that proposed to PZA. It showed stronger anti-*M. tuberculosis* ATCC 27294 effect (IC$_{50}$ = 50 μg/mL) than PZA (IC$_{50}$ > 100 μg/mL) using the microplate Alamar Blue assay. Especially, it was not cytotoxic in noninfected or infected macrophages of *Mycobacterium bovis* Bacillus Calmette-Guerin.

The NADH-dependent enoyl-acyl carrier protein reductase (enoyl-ACP reductase) encoded by the mycobacterium gene inhA has been validated as a target receptor of the antitubercular drug isoniazid. Quinolyl hydrazone-based imidazole 158 was a promising enoyl-ACP reductase inhibitor and displayed good activity toward *M. tuberculosis*. Quinoline imidazole 159 could target the proton pump of bacterial ATP synthase and gave comparable antimycobacterial activity to isoniazid. The impressive antimycobacterial potency likely resulted from the effective binding affinity of compound 159 to the binding site of ATP synthase. Interestingly, the large conjugated quinoline imidazole 160 was also found to exhibit comparable antitubercular efficacy to isoniazid without cytotoxicity to host cells. Further studies for compounds 158–160 manifested that the incorporated imidazole ring had a crucial impact on modulating the binding affinity to target protein. These results suggested some directions to design and synthesize novel imidazole-based quinolines with antitubercular potency.

Recent studies suggested that the commercially availableazole drugs could bind tightly with MTB-CYP121, which might be the reason why they possessed antimycobacterial activity. A lot of anti-MTB agents with polycyclic structures, which resemble the classical antifungalazole drugs were designed and synthesized. Imidazole derivative 161 bearing p-methoxybenzyl group, which could increase the lipid solubility of target compound, displayed good anti-*M. tuberculosis* H37Rv activity. The bioactivity was obviously decreased along with the substitution.
of \( p \)-methoxybenzyl moiety by 2-furyl group.\(^{394}\) The 1,2,3-triazole unit is generally accepted as a moderate linker between two separate fragments to improve water solubility and pharmacokinetic property, which is helpful for bioactivity.\(^{395-397}\) 1,2,3-Triazole inserted compound \( 162 \) with \((R)\)-configuration was found to be capable of inhibiting the growth of \( M. \) tuberculosis \((\text{MIC} = 16 \, \mu\text{g/mL})\) and gave comparable potency to econazole \((\text{MIC} = 12.5 \, \mu\text{g/mL})\) and clotrimazole \((\text{MIC} = 20.4 \, \mu\text{g/mL})\). The fact that replacement of the Br atom by Cl or F substituent would decrease the antimycobacterial activity demonstrated that the bioactivity of compound \( 162 \) should considerably depend on the Br atom at the 4-position of benzene ring.\(^{398}\)

Econazole is a well-established antifungal drug in the clinic and has been known to be active against MDRTB. Investigations of the econazole-derived nitroimidazoles led to the discovery of derivative \( 163 \). It showed much better anti-\( M. \) tuberculosis activity with an MIC value of 0.5 \( \mu\text{g/mL} \) than econazole \((\text{MIC} = 16 \, \mu\text{g/mL})\) and particularly displayed identical efficacy to clinical antitubercular agent PA-824 toward nonreplicating \( M. \) tuberculosis. Structure–activity relationship pointed out that the methoxyl group on nitroimidazole moiety was helpful to increase the antitubercular activity. Noticeably, this econazole analog did not show any antifungal activity implying a different action mechanism from econazole.\(^{399}\)

It has established that FtsZ protein targeting compounds such as carbamoyl-bearing pyridines possess significant antitubercular properties. Structural modification of classical 1,4-dihydropyridines with diamides is a good access to fight against cloxacillin-resistant strains. Interestingly, imidazole-substituted 1,4-dihydropyridines \( 164a \) and \( 164b \) displayed significant anti-\( M. \) tuberculosis \( H37R V \) efficacy with MIC values of 2 and 1 \( \mu\text{g/mL} \), respectively, which were comparable to rifampicin \((\text{MIC} = 2 \, \mu\text{g/mL})\). They were also associated with weak cytotoxicity when compared with doxorubicin.\(^{400}\) Additionally, bis-imidazole \( 165 \) still exhibited very good in vitro antitubercular activity toward virulent strain \( M. \) tuberculosis \( H37R V \).\(^{401}\) This structurally simple compound may be served as a lead for further optimization.

6. IMIDAZOLES AS ANTIPARASITIC AGENTS

Parasitic infections such as trypanosomiasis, leishmaniasis, malaria, and Chaga’s disease caused by contaminated food or water, etc., constitute one of the most widespread human health problems. Since parasites are eukaryotic, they share many common features with their mammalian host, making the development of effective and selective drugs a challenging task. Despite of the great effort that has been made in the discovery of unique and selective targets, many drugs used today have serious side effects. Imidazole derivatives such as megazol \((166)\), benzimidazole \((167a)\), and metronidazole \((123a)\) are traditional antiprotozoal agents. Megazol, synthesized in 1968, is an antimicrobial agent, and later as a powerful trypanocide agent, but has been discarded because of its mutagenic risk. Benzimidazole is an important drug for Chaga’s disease and has been used in other parasitic diseases. Metronidazole is the only FDA-approved drug for trichomonasias and is currently the most effective antiamoebic agent.\(^{402}\) The common nitroimidazole core in these compounds has been accepted as an important pharmacophore for anti-infectious chemotherapy toward anaerobic bacteria and parasites. In the process of development of more safe and effective drugs against these protozoan infections, a lot of imidazole antiparasitic agents have been reported.\(^{403-409}\) The structures of imidazole-based antiparasitic compounds are shown in Figure 21.

Research revealed that nitroimidazole exerted its antiparasitic effect through the production of free radicals that were toxic to the microbe and the nitro reduction was crucial in this process to activate the nitroimidazole. A recent work manifested that the position of nitro group played an important role in the biological activity. Bioisosteric replacement of the 2-nitroimidazole
ring in benznidazole with 2-methyl-4-nitroimidazole group produced analog 167b. It was three times more potent (IC\textsubscript{50} = 5.61 μM) than benznidazole (IC\textsubscript{50} = 18.62 μM) toward \textit{Trichomonas vaginalis} with noncytotoxicity.\textsuperscript{410}

Solitary imidazole usually exhibits weak or even no antiparasitic activity, but often plays a synergistic role with other organic fragments. Phenyl nitroimidazoles 168a-b displayed comparable anti-\textit{trypanosomal} efficacy (IC\textsubscript{50} = 0.16 and 0.10 mM, respectively) to megazol (IC\textsubscript{50} = 0.10 mM) without genotoxicity in mammalian cells. Importantly, compound 168b possessed remarkable curative capacity when given orally at a low dose of 50 mg/kg for 5 days in chronic mouse models, even better than clinical anti-\textit{trypanosomal} drug fexinidazole (100 mg/kg). The result confirmed that it was a promising lead compound for further development to treat human African trypanosomiasis.\textsuperscript{411}

Benzoyl nitroimidazole 169 showed stronger anti-\textit{T. vaginalis} activity (IC\textsubscript{50} = 25 μg/mL) in comparison to the marketed spermicide nonoxynol-9 (IC\textsubscript{50} = 37.4 μg/mL) with high level of safety profile toward normal vaginal flora (\textit{Lactobacillus}) and human cervical (HeLa) cells. Notably, it caused 100% immobilization of human sperm at 1% concentration comparable to nonoxynol-9 (100%) and also exhibited anti-\textit{Candida} activities. Further research disclosed that the relative position of the carbonyl group and nitroimidazole ring was optimal for dual activity and safety.\textsuperscript{412} Compound 169 provides the possibility to be developed as a potent dually active antimicrobial spermicide.

Recently, a lot of studies suggested that modification on the 2-position of imidazole ring that tune the redox potentiality was thought to be critical for the antimicrobial activity, selectivity, and mechanisms of action, which might lead to effective antiparasitic agents even with the

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possibility to overcome drug resistance. Styryl nitroimidazole 170 was identified to have a weaker activity ($\text{PEC}_{50} = 6.28$) than metronidazole ($\text{PEC}_{50} = 4.35$) toward metronidazole-resistant $G. lamblia$ accompanied with high selectivity ratio and no toxicity. Introduction of small substituents such as methyl, chloro, bromo, or iodo group on the benzene ring increased the potency, while larger substituents including OMe, OCF$_3$, and phenyl groups markedly diminished the efficacy.\textsuperscript{413} Dibromide 171 displayed 14-fold more potent anti-metronidazole-resistant $G. lamblia$ activity with an $\text{ID}_{90}$ (90% inhibitory dose) value of 0.4 $\mu$M than metronidazole ($\text{ID}_{90} = 6.2$ $\mu$M).\textsuperscript{414} Normally, the mechanism of metronidazole activation in $Giardia$ apparently involves reduction to toxic radicals by ferredoxin oxidoreductase (PFOR). However, compound 171 presented a different mechanism of action since the tested resistant lines had normal levels of PFOR, which might reflect a practical strategy to handle the drug resistance problem.

Piperazine ring is an attractive pharmacological fragment present in a variety of marketed drugs.\textsuperscript{415} Introduction of the piperazine ring can effectively accommodate physicochemical and pharmacokinetic properties of target compounds. Piperazine-imidazole 172 with low cytotoxicity and good water solubility could effectively inhibit the growth of $Trypanosomiasis brucei$ with an $\text{IC}_{50}$ value of 0.67 $\mu$M. Further study demonstrated that the methyl and nitro group could exert a balanced electronic effect on the imidazole ring that was beneficial for the enhanced efficacy. The replacement of nitro group on the imidazole ring with other electron-withdrawing groups such as Cl, Br, and SO$_3$H failed to give any improved anti-$T. brucei$ activity even increased cytotoxicity. However, the exact mechanism of action of this compound was still unknown.\textsuperscript{416}

Some antifungal azole drugs such as ketoconazole, miconazole, and itraconazole have been used to treat cutaneous leishmaniasis with variable success rates.\textsuperscript{417,418} Through modifications of miconazole, many analogs were obtained and showed various degrees of antileishmanial activities. Furyl compound 173 displayed superior in vitro antiprotozoal efficacy ($\text{IC}_{50} = 3.04$ $\mu$M, selective index (SI) = 19.80) against amastigote to miconazole ($\text{IC}_{50} = 6.00$ $\mu$M, SI = 1.66).\textsuperscript{419} Diphenyl imidazole 174 exhibited significant in vitro bioactivity (94–100%) against promastigotes at the concentration of 10 $\mu$g/mL and potent in vivo inhibitory efficacy (58–60%) at 50 mg/kg × 10 i.p. dose.\textsuperscript{420} This class of imidazoles deserve to be deeply investigated including both good in vitro and in vivo antileishmanial activities. They have the possibility to become new members of effective antileishmanial agents.

Various $N$-substituted imidazole derivatives bearing phenyloxy or benzyloxy moieties have also been reported with promising antileishmanial activity. Tetralin imidazole 175 displayed remarkable anti-$Leishmania donovani$ ($L. donovani$) efficacy with an $\text{IC}_{50}$ value of 0.64 $\mu$g/mL and an SI value of 34.78 toward amastigotes in comparison with sodium stibogluconate ($\text{IC}_{50} = 46.54$ $\mu$g/mL, SI = 5.78) and paromomycin ($\text{IC}_{50} = 24.79$ $\mu$g/mL, SI = 2.78). Notably, it showed significant in vivo inhibition activity (83.33%) against $L. donovani$/Hamster model. Comparative research indicated that the benzyloxy group was beneficial for the good selectivity, and its substitution by phenyloxy or cyclohexyl group all resulted in weak efficacy.\textsuperscript{421} Cyclohexyl-bridged bis-imidazole 176 showed much stronger antileishmanial efficacy with an $\text{IC}_{50}$ value of 1.17 $\mu$g/mL toward amastigotes than sodium stibogluconate ($\text{IC}_{50} = 46.54$ $\mu$g/mL) and pentamidine ($\text{IC}_{50} = 12.11$ $\mu$g/mL) with high selectivity (SI > 30). Moreover, it was also identified to have good in vivo inhibitory activity (77.9%). Structure–activity relationship pointed out that the methylene imidazole groups greatly contributed to the remarkable activity, and their substitution by other moieties such as bis-imidazole, monomethylene imidazole, or monoimidazole all would weaken the bioactivities.\textsuperscript{422}

Chagas’ disease is caused by the protozoan $Trypanosoma cruzi$ ($T. cruzi$) that affects a lot of people in Latin America.\textsuperscript{423} The current available drugs such as nifurtimox or benznidazole are only effective against the acute phase, but exhibit very limited efficacy in the chronic stage and even have high toxicity and severe side effect. Much attention therefore has been
paid on the developments of new anti-\textit{T. cruzi} drugs with improved efficacy and decreased side effects.\textsuperscript{424,425} Iron superoxide dismutase (Fe-SOD) has emerged as a promising target for anti-\textit{T. cruzi} therapy due to its scavenging ability for the superoxide anion. A recent study revealed that introduction of the imidazole ring into the flexible side chain of benzophthalazine skeleton afforded compounds with enhanced anti-\textit{T. cruzi} activity and Fe-SOD enzyme inhibitory activity. Compound 177 with significant ability to inhibit Fe-SOD enzyme (IC\textsubscript{50} = 1.3 \textmu M) displayed superior anti-\textit{T. cruzi} activity (IC\textsubscript{50} = 13.7 \textmu M) to benznidazole (IC\textsubscript{50} = 15.8 \textmu M) toward epimastigotes. Even more interestingly, it also exhibited much lower in vitro toxicity against Vero cells than the reference benznidazole.\textsuperscript{426} This study implied that competitive complexation for the metal ion of SOD should be an effective way to inhibit the protective effect of the enzyme, which affected both the growth and survival of parasitic cells.

Through cell-based screening, pyrimidine-imidazole 178 with favorable physiological and pharmacokinetic properties was obtained. Notably, this compound exhibited excellent anti-\textit{P. falciparum} activity against both wild-type and drug-resistant parasite strains, especially toward \textit{P. falciparum} 3D7 and D10 strains with EC\textsubscript{50} values of 15 and 34 nM, respectively. Contrast test manifested that the 4,6-diamine pyrimidine skeleton with free NH was essential for the good potency.\textsuperscript{427} This study might provide a potential possibility to solve drug-resistant problem.

Thiadiazole-bridged imidazole 179 displayed significant anti-\textit{Toxoplasma gondii} activity with LD\textsubscript{50} (50\% lethal dose) values of 0.6 mM for infected cells and 0.05 mM for parasites as well as low cytotoxicity (LD\textsubscript{50} > 10 mM), indicating a more effective action than standard drugs sulfadiazine (LD\textsubscript{50} > 10, 0.5 mM) and hydroxyurea (LD\textsubscript{50} > 10, 6 mM). Structure–activity relationship revealed that the electron-withdrawing or electron-donating substituents on the phenyl group did not dramatically alter the anti-\textit{T. gondii} efficacy.\textsuperscript{428} This kind of compounds provided interesting leads for anti-\textit{T. gondii} drug discovery and merited in-depth exploration.

7. IMIDAZOLES AS ANTIHISTAMINIC AGENTS

Histamine is a kind of biogenic amine that influences a variety of physiological and pathophysiological processes in the body via stimulation of four histamine receptor (HR) subtypes. The H\textsubscript{1}R and H\textsubscript{2}R antagonists have been used successfully in the clinic for the treatment of allergic conditions and gastric ulcer.\textsuperscript{429} The H\textsubscript{3}R subtype is mainly expressed in CNS and modulates the release of several neurotransmitters. Development of H\textsubscript{3}R targeting drugs has received a major boost and several H\textsubscript{3}R ligands are being under clinical trials for a wide variety of applications in the medical field, including anti-Alzheimer’s disease (AD), antiobesity, and antiepilepsy.\textsuperscript{430} The H\textsubscript{4}R subtype is preferentially expressed in hematopoietic and immune cells, which was not discovered until the year 2000. Currently, the application of H\textsubscript{4}R ligands in the treatment of asthma, allergic rhinitis, and pruritis is being actively investigated in preclinical studies.\textsuperscript{431} Imidazole ring is an important biological building block present in histidine, but does not appear in the most common H\textsubscript{1}R and H\textsubscript{2}R antagonists, presumably due to its metabolic vulnerability, except for cimetidine (180, H\textsubscript{2}R antagonist). Due to the structural similarity of H\textsubscript{3}R and H\textsubscript{4}R, numerous H\textsubscript{3}R ligands, for example, imetit (181), thioperamide (182), and clobenpropit (183) also have significant affinity for the H\textsubscript{4}R. In recent years, more attention has been focused on the potential therapeutic applications of selective H\textsubscript{3}R and H\textsubscript{4}R ligands.\textsuperscript{432–434} The structures of imidazole-based antihistaminic compounds are shown in Figure 22.

Despite of the high potency and clinical studies of H\textsubscript{3}R ligands, none of them has entered the market as clinical drug due to the undesired inhibition of CYP enzymes, low oral bioavailability, and poor brain penetration. Some alkyl imidazole carbamates were discovered to have shown selective H\textsubscript{3}R antagonistic activity with good oral bioavailability and brain penetration.

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Amongst, compound 184a gave excellent H₃R affinity (Kᵢ = 8.3 nM) with a selectivity index of 20 over H₄R. Replacement of branched alkyl side chain by unsaturated alkyl group yielded compounds 184b-c, which were highly selective H₃R antagonists (Kᵢ = 27, 14 nM). Importantly, they could cross the blood-brain barrier and exhibited in vivo H₃R antagonistic activity after peroral administration to Swiss mice. Especially, compound 184b showed remarkable H₃R antagonistic activity in the CNS and could increase the N⁺-methylhistamine levels in mice with an ED₅₀ (50% effective dose) value of 0.55 mg/kg after oral administration, which was about 0.5-fold lower than thioperamide (ED₅₀ = 1.0 mg/kg).

Replacement of amino group in histamine by sulfur-bridged phenyl moiety yielded an H₃R ligand 185 (Kᵢ = 0.17 nM, EC₅₀ = 0.75 nM) with similar affinity toward H₄R (Kᵢ = 1.1 nM, Kᵢ(H₄)/Kᵢ(H₃) = 6.5) and high selectivity over H₁R and H₂R. However, it showed good pharmacokinetic profiles in rat, but unfavorable profile in the inhibition of CYP enzymes.

Literature revealed that piperidine ring could provide appropriate conformational restriction, and thus was beneficial for highly selective H₃R affinity. Piperidine-modified imidazole derivative 186 could interact with the key residues Asp114 and Glu206 using a different manner from histamine. It was identified as a potent H₃R agonist with good selectivity over H₁R (20-fold) and H₄R (93-fold). Particularly, (S)-enantiomer 186 was favorable for binding with the hydrophobic region in TM6 and exhibited much higher affinity (13-fold) than (R)-enantiomer. These observations indicated that human H₃R receptor recognized the strictly enantiomeric structure of ligands.

Notably, imidazole derivative 187 with piperidine group was also a potent H₃R ligand (Kᵢ = 3 nM) and displayed significant brain uptake activity without appreciable affinity for CYP enzymes. Contrast test disclosed that replacement of the carbonyl group by alcohol or methylene unit, and either shortening or lengthening the distance between the imidazole ring
and the piperidine group all lowered H₃R affinity. These findings might be helpful for the development of new agents targeting H₃R.

Another piperidine-bridged imidazole derivative 188 was a highly selective human H₃R agonist over H₄R (574-fold). Further study showed that replacement of the piperidine ring by a piperazine heterocycle dramatically diminished the H₃R affinity. Additionally, the agonistic activity was also regulated by the substituents on the benzene ring, and small para-substituents were favorable for the potency, whereas meta- and bulky para-groups reduced the efficacy.

Developments of selective H₄R antagonists are of large interest and very useful in confirmation of postulated roles of the H₄R. Some cyclohexyl-based 1H-imidazoles have been recently found to possess highly selective H₄R affinity. Cyclohexyl amide derivative 189, as conformationally restricted histamine analog, displayed good H₄R affinity (Kᵢ = 45 nM) with excellent selectivity over H₃R (125-fold). Structure–activity relationship pointed out that amide bridge was necessary for bioactivity, and its replacement by carbamate, urea, ester, ketone, or ether linker had negative effect on affinities toward both H₃R and H₄R subtypes. As isostere of amide, triazole ring was also proved to exert a significant influence on the affinities and selectivity toward H₄R. Incorporation of a 1,2,3-triazole ring by click reaction between cyclohexyl and imidazole ring afforded compound 190. It exhibited remarkable H₄R affinity (pKᵢ = 8.08) with a selectivity index of 15 over H₃R. In addition, comparative study revealed that either insertion of one nitrogen atom or removal of one methylene group between imidazole and triazole ring resulted in dramatically decreased H₄R affinity.

Cyanoguanidine moiety is a common group present in many H₄R agonists such as antinociceptive drug improgan. Recently, many cyanoguanidines have been prepared with the aim to develop highly selective agonists toward the H₄R. Cyanoguanidine-type imidazole 191 was identified as a highly potent H₄R agonist (pEC₅₀ = 7.47, R = 0.93) with significant selectivity over H₁R (450-fold), H₂R (70-fold), and H₃R (30-fold) in GTPase assay. Binding mode test indicated that the cyanoguanidine moiety could form hydrogen bonds with the conserved Asp-94 and the hH₄R-specific Arg-341 residue. Notably, unlike previously described selective H₄R agonists, compound 191 was devoid of agonistic activity to other HR subtypes. The result implied that compound 191 might be a valuable pharmacological agent to study the biological functions of H₄R.

Isothiourea is a well-known fragment and widely exists in many H₃R agonists such as imetit (181) and antagonists such as clobenpropit (183). Interestingly, isothiourea-incorporated imidazoles 192a-c were discovered to have high affinity toward H₄R, and their bioactivities could be modulated by the substituents on the phenyl group. Iodide 192a displayed full agonistic activity against H₄R (αH₄R = 0.98) while 4-chlorine analog 192b had partial agonistic efficacy (αH₄R = 0.83). Introduction of an additional chlorine atom produced a full H₄R agonist 192c (αH₄R = 1), which resulted in highly increased H₄R affinity (pKᵢ value from 8.0 to 8.8) and decreased H₃R affinity (pKᵢ value from 8.5 to 8.2). Structure–activity relationship showed that elongating the spacer between isothiourea and phenyl group lowered the affinity for both H₃R and H₄R. These results provided critical insights into the design of more potent and selective H₄R agonists.

8. IMIDAZOLES AS ANTINEUROPATHIC AGENTS

The research of imidazole compounds as potential antineuropathic agents has been developed for a long time. Many imidazole-based antineuropathic drugs are prevalently used in the clinic. Dexmedetomidine (193) with sedative, analgesic, sympatholytic, and anxiolytic effects has been widely applied in perioperative period since its approval by the FDA in 2000. It could reduce the requirements for volatile anesthetics, sedatives, and analgesics without causing significant
respiratory depression. Recently, the research on imidazoles as antineuropathic agents is quite active. A lot of imidazoles have been demonstrated to possess good biological activities toward some neuropathic-relative diseases such as epilepsy, AD, Parkinson’s disease, schizophrenia, dementia, anxiety, and depression.\(^{445-453}\) The structures of imidazole-based antineuropathic compounds are shown in Figure 23.

### A. Imidazoles as Anticonvulsant Agents

Epilepsy is one of the most frequent neurological diseases affecting more than 50 million people worldwide. The majority of currently clinical drugs have many disadvantages such as staggering cost treatment and dose-related side effects, which promotes continuous efforts to develop safe and more efficacious agents to treat this devastating disease. The utilization of substituted imidazoles in the treatment of epilepsy was brought with the introduction of nafimidone (194) in 1981.\(^{22}\) Whereafter, much attention has been paid to develop more potent and low toxic imidazole-based anticonvulsant drugs.\(^{454}\)

Recently, some nafimidone analogs obtained by the reduction of the carbonyl group have been discovered to show potent anticonvulsant activities. The esterified product 195 possessed comparable anti-maximal electroshock seizure (MES) activity in mice with an ED\(_{50}\) (50% effective dose) value of 38.46 mg/kg to nafimidone (ED\(_{50} = 34\) mg/kg) devoid of neurotoxicity. Further study disclosed that the imidazole ring and the ester group in the alkyl chain were important for the anticonvulsant potency.\(^{455}\) Schiff base derivative 196 not only gave remarkable anti-MES efficacy with an ED\(_{50}\) value of 30 mg/kg, but also had a suitable lipophilicity for crossing the blood–brain barrier. The meta-cholo substitution of benzene ring was vital since activity disappeared with para-substitution.\(^{456}\) The results confirmed the value for further optimization of these compounds as anticonvulsant agents.

Thiazole ring is an important fragment present in many anticonvulsant agents. Recent research has reported the ability of thiazole-modified imidazoles to combat with convulsion. Among them, imidazole derivative 197 showed significant anticonvulsant activity at 100 mg/kg dose in both pentylenetetrazole seizure (100%) and MES (100%) tests. Importantly, compound 197 possessed favorable physicochemical properties for acting as a CNS drug, which might become a potential agent for epilepsy therapy.\(^{457}\)

It was reported that (S)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazol-yl) propionic acid (AMPA) receptors had been considered as promising drug targets to treat convulsant diseases. Compound 198 displayed high binding affinity with an IC\(_{50}\) value of 82 nM toward AMPA receptor. It was worthy to note that the introduction of imidazole ring was helpful for not only enhancing the affinity but also improving the selectivity toward the AMPA receptor. Unfortunately, this compound was devoid of oral activity and required high intraperitoneal doses to inhibit E-shock induced convulsions in mice.\(^{458}\)

Literature survey displayed that triphenyl imidazoles were also a kind of anticonvulsant agents. Imidazole 199 having optimum lipophilicity showed comparable anticonvulsant activity at 30 mg/kg in MES screening to standard drugs phenytoin and carbamazepine. Furthermore, it had low toxicity at 300 mg/kg in comparison with carbamazepine (300 mg/kg) and phenytoin (100 mg/kg).\(^{459}\)

### B. Imidazoles as Anti-AD Agents

AD is a progressive neurodegenerative disease that leads to synaptic failure and neuronal death as consequence of the accumulation of amyloid-\(\beta\) (A\(\beta\)). A\(\beta\) is generated by sequential proteolytic cleavage of amyloid precursor protein (APP) through \(\beta\)- and \(\gamma\)-secretase. Therefore, the \(\beta\)- and \(\gamma\)-secretase have been identified as compelling targets for the treatment of AD.
Figure 23. Imidazole-based antineuropathic compounds.
Literature revealed that the 3-N atom in the imidazole ring could interact with the side chains of Asp228 and Thr232 of the β-secretase APP cleaving enzyme (BACE-1) by stable electrostatic and hydrogen bonds.\textsuperscript{460} Compound 200 with the ability to cross the blood–brain barrier was revealed to be a good BACE-1 inhibitor (IC\textsubscript{50} = 7.4 μM) and could effectively inhibit Aβ\textsubscript{38}, Aβ\textsubscript{40}, and Aβ\textsubscript{42} secretion (IC\textsubscript{50} = 15, 23, and 19 μM, respectively) with moderate toxicity at 25 μM using cellular assays.

DAPT (201) is the first reported centrally active γ-secretase inhibitor with instable t-butyl ester group. Many imidazoles as replacements for the ester functionality in DAPT were found to possess potent activity against γ-secretase. Imidazole 202a could strongly suppress γ-secretase secretion and inhibit the production of toxic Aβ-peptides in whole-cell and cell-free assay with IC\textsubscript{50} values of 0.4 and 1.1 nM, respectively.\textsuperscript{461} Further modification of 202a led to the discovery of compound 202b (PF-3084014). It displayed good γ-secretase inhibitory activities in fetal thymic organ culture (EC\textsubscript{50} = 1.83 μM) and whole-cell assay (IC\textsubscript{50} = 1.2 nM), and was currently under clinical trials. Structure–activity relationship showed that the α-branched side chain on the imidazole ring was of biological significance and the substitution of branched alkyl group with monomethyl amine moiety resulted in 90-fold reduction of potency. These might be ascribed to the fact that branched alkyl chain was less lipophilic than the corresponding straight alkyl chain due to the larger molar volumes and shapes.\textsuperscript{462}

cdk play a critical role in cell cycling. Inhibition of the aberrant cdk5/p25 complex is a viable target for treating AD. Amino imidazoles 203a and 203b were active (IC\textsubscript{50} = 6 nM) against cdk5/p25 enzyme with excellent selectivity over cdk2/cyclin E (34- and 18-fold). Specialiy, methyl derivative 203b showed significant whole-cell activity (IC\textsubscript{50} = 230 nM). However, compound 203b was a substrate for p-glycoprotein with limited brain penetration in mouse.\textsuperscript{463} Continuous effort is necessary to develop new inhibitors with cellularly active and selective properties as well as acceptable brain penetration.

Adenosine receptor is reported to have four subtypes (A\textsubscript{1}, A\textsubscript{2A}, A\textsubscript{2B}, and A\textsubscript{3}), and some of the selective agonists or antagonists have entered clinical trials or gained the FDA approval for diagnostic or therapeutic uses. Particularly, the A\textsubscript{2A} subtype is a very attractive target for drug discovery in neurodegenerative diseases. Imidazole-modified chromene 204 showed remarkable antagonistic potency (pK\textsubscript{i} = 6.2) toward human adenosine A\textsubscript{2A} receptor in cAMP assays with marked selectivity over A\textsubscript{1}, A\textsubscript{2B}, and A\textsubscript{3} (pK\textsubscript{i} > 20). However, the removal of 8-methoxyl group on chromene ring or the replacement of fluorine atom on benzene ring by methoxyl or cyano group would dramatically decrease the affinity.\textsuperscript{464}

Butyrylcholinesterase (BChE) with the ability to regulate the levels of depleted acetylcholine in human brain has recently gained increasing attention as an alternative target in AD therapy. Study showed that compounds with dual ability to inhibit BChE and impede Aβ fibril formation could potentiate BChE with a neuroprotective ability while facilitating cholinergic restoration. Compound 205 was proved to be a bifunctional inhibitor of BChE hydrolase (IC\textsubscript{50} = 0.10 μM, K\textsubscript{i} = 0.073 μM) and Aβ fibril formation (IC\textsubscript{50} = 5.8 μM). Comparative study indicated that the thiophene ring with high electron density was beneficial for the BChE inhibitory activity and its substitution by a phenyl group resulted in substantially decreased potency.\textsuperscript{465}

C. Imidazoles as Anti-Parkinson’s Disease Agents

Parkinson’s disease is a chronic and progressive neurodegenerative disorder associated with a loss of dopaminergic neurons. However, rare treatment has been observed to prevent the progression of the disease in spite of tremendous efforts currently. Fipamezole (206) has shown positive effects in phase II trial for reducing l-dopa-induced dyskinesias in Parkinson’s disease.
Additionally, $\alpha_2$ antagonists have been proposed as therapeutic agents not only to relieve symptoms but also to retard the evolution of neurodegenerative disorders. Compound (+)-207 was regarded a potent antagonist on presynaptic $\alpha_{2A/C}$ receptor. It could effectively trigger the release of cortical noradrenaline in mouse after acute systemic administration, which was 30-fold more potent than fipamezole and atipamezole. Interestingly, it could substantially increase the blood pressure in pithed rat but exert minimal cardiovascular effect in intact and anesthetized rat, suggesting its opposed central and peripheral actions.\(^{466}\)

Inhibition of phosphodiesterases (PDEs) in cellular signaling pathways has been considered as a novel therapeutic method to treat Parkinson’s disease. Compound 208 was manifested to be a highly selective PDE10A inhibitor with an IC\(_{50}\) value of 16 nM. Specially, compared with the corresponding benzimidazole compound (IC\(_{50}\) = 12 nM), imidazole derivative 208 showed obvious improvement in water solubility and brain penetration.\(^{467, 468}\) This interesting class of compounds were worthy of further development as novel agents for Parkinson’s disease.

Opioid receptor-like 1 (ORL1) receptor antagonists were identified as potent agents for the treatment of Parkinson’s disease. Imidazoles 209a-b were found to be excellent ORL1 receptor antagonists with good permeability (Papp = 18 and 29) and P-gp efflux susceptibility (B-A/A-B ratio = 1.0 and 1.4), respectively. Notably, compound 209b with high ORL1 binding affinity (IC\(_{50}\) = 7.6 nM) had CNS drug-like profile, which ended it with potential therapeutic use as a neuroprotective agent to treat Parkinson’s disease.\(^{469}\)

**D. Others**

Acetylcholine esterase (AChE) is able to catalyze the hydrolysis of neurotransmitter acetylcholine at synaptic cleft and facilitate nerve impulse transmission across the synaptic gap. Many CB\(_1\) receptor antagonists were revealed to increase ACh release in certain brain areas. The impact of both AChE inhibition and CB\(_1\) receptor antagonism on cognitive deficits and drug addiction prompted the development of dual acting AChE inhibitors/CB\(_1\) receptor antagonists. Tacrine-based imidazole 210 not only displayed good CB\(_1\) receptor affinity ($K_i$ = 48 nM) with high selectivity over CB\(_2\) (>20-fold) but also gave comparable AChE inhibitory activity (pIC\(_{50}\) = 5.9) to tacrine (pIC\(_{50}\) = 6.6). Molecular modeling studies disclosed that this compound could nicely accommodate the binding pocket in the AChE enzyme.\(^{470}\)

Fluoxetine was a potent agent in inhibiting the uptake of [2,5,6-$^3$H] dopamine, which would be useful with regard to substituting for cocaine and minimizing its abuse. Replacement of the N-methyl group in fluoxetine by imidazole ring produced its analog 211. It could significantly inhibit [2,5,6-$^3$H] dopamine uptake with an IC\(_{50}\) value of 25.5 $\mu$M, which was more potent than fluoxetine (IC\(_{50}\) = 205 $\mu$M).\(^{471}\) Therefore, this type of structural modification of fluoxetine might be an effective approach in the exploitation of dopamine transporter inhibitors.

Serotonin (5-HT) is a neurotransmitter involved in many physiological functions and pathological disorders including sensory reflexes and cardiac arrhythmias. It revealed that 5-HT\(_3\) receptor might play a role in perception of cardiac pain during ischemia in humans. Quinoxaline-based ligands 212a-b with the imidazole ring as proton exchange system showed selectively high binding affinity toward 5-HT\(_3\) receptor. Particularly, compound 212a was discovered to be a brain-penetrating agent. It might be a new and useful tool to investigate the role of 5-HT\(_3\) receptor at the cardiac level. However, replacement of the imidazole ring by phenyl or other five-membered heterocyclic groups resulted in at least tenfold reduction of affinity, and the change of substituted position on the imidazole ring from C-4 to C-2 also led to eightfold lowered activity.\(^{472}\)

Free radical gas nitric oxide (NO) as signaling molecule in various tissues can be produced by NO synthase (NOS), while overproduction of NO, especially nNOS and iNOS, is associated with various neurological diseases. Thus, the inhibition of NOS has great therapeutic
potentiality for the treatment of neurological diseases. Research revealed that compound 213 was a good nNOS inhibitor with high selectivity over eNOS, iNOS, and cytochrome P450. It also had good capacity to scavenge free radicals and to reduce lipid peroxidation. This type of compounds might be useful for the therapy of brain diseases caused by overproduction of radical species.

Cannabinoid CB$_2$ receptors are considered as attractive analgesic targets since they have potentiality to retain the analgesic efficacy with fewer adverse effects associated with CB$_1$ receptors. Imidazole 214a was identified to display high human CB$_2$ affinity ($K_i = 42$ nM) with good selectivity over CB$_1$ ($K_i = 1$ $\mu$M). It also displayed potent agonistic activity (EC$_{50} = 9$ nM) in the cyclic adenosine monophosphate (cAMP) assay. Specially, methyl substitution on the 2-position of morpholine ring (compound 214b) led to slightly reduced agonistic activity (EC$_{50} = 25$ nM) but twofold enhancement of plasma exposure (AUC (area under curve): 1989 nM h) in comparison to that of 214a (AUC: 866 nM h) after oral administration in rats. In addition, structure–activity relationship pointed out that the introduction of substituent on N-1 position of the imidazole ring would decrease the bioactivity.

A recent work revealed that some imidazole-4-carboxamide compounds were discovered to be potent and highly selective CB$_2$ receptor antagonists. The most active compound 215 showed significant CB$_2$ receptor affinity ($K_i = 1.03$ nM) with excellent selectivity for CB$_2$ over CB$_1$ (> 9708-fold). It also possessed good lipophilicity and molecular polar surface area values, indicating its favorable pharmacokinetic property as a drug candidate.

The A$_3$ adenosine (h) and dopamine D$_1$ (h) receptors are important actors in the CNS. Their ligands play major roles in the treatments of diverse neurological disorders. Macrocyclic imidazole 216 showed high binding affinity with A$_3$ adenosine (h) receptor and dopamine D$_1$ (h) receptor with IC$_{50}$ values of 0.85 and 1.1 $\mu$M, respectively. Further study manifested that the flexibility of macrocycles exerted an important effect on the binding affinity, in which the 17-membered macrocycle gave the highest potency.

9. IMIDAZOLES AS ANTIHYPERTENSIVE AGENTS

Hypertension is a major risk factor for heart attacks, heart failure, and peripheral arterial disease. The challenge that hypertension brings to health care is obviously striking. Therefore, the development of new antihypertensive drugs with higher curative effects and lower side effect is quite necessary. Since losartan (217), the first imidazole angiotensin II receptor (type AT1) antagonist, successfully launched in 1995, many researchers have been devoted to exploiting novel orally active losartan congeners as antihypertensive agents. Eprosartan (218), marketed in 1997, is generally better tolerated than other angiotensin II receptor antagonists, especially for the elderly. Olmesartan (219), launched in 2002, is also a kind of angiotensin II receptor antagonists. It works by blocking the action of certain natural substances that tighten the blood vessels. It has been reported that losartan, eprosartan, and olmesartan have become the top 200 best-selling drugs of recent years worldwide. These successfully marketed drugs impel an increasing effort to discover new imidazole-based antihypertensive agents with good curative effect, long action time, and low toxicity. The structures of imidazole-based antihypertensive compounds are shown in Figure 24.

The angiotensin II is not only beneficial for lowering blood pressure, but also plays a significant role in the progression of tissue damage in cardiovascular diseases. In this context, many compounds were designed and synthesized with the aim to increase their ability to prevent tissue damage while retain their antihypertensive potency. The hybrid of losartan and hydrocaffeic acid with dual action receptors exhibited stronger affinity than one bioreceptor alone. Compound 220 with both angiotensin II block and antioxidant potency could effectively...
reduce arterial pressure in rats, even better than losartan. The significant activity might result from the synergistic effect with an extra tissue protection through its antioxidant property. Further study manifested that the hydroxyl group on benzene ring and the ester linker between the phenol group and the imidazole ring contributed to the synergistic effect.

Losartan analog 221 with butyl and hydroxymethyl groups at the 2- and 5-positions of imidazole ring was found to be selectively active angiotensin II type 1 (AT1) receptor antagonist with an IC$_{50}$ value of 53.8 nM, which was threefold lower activity than losartan (IC$_{50}$ = 16.4 nM). Docking studies clearly showed that the 2-hydroxymethyl and 5-butyl groups were of primary importance to the high binding affinity to the AT1 receptor.

Replacement of the hydroxymethyl group in losartan by dihydropyridine moiety and then bioisoster substitution of the tetrazole ring with a carboxyl group gave its derivatives 222a-b. Both of them were potent dual calcium channel blockers and angiotensin antagonists in isolated rat aorta. Especially, their effects on AT1 receptors were 1000 and 100,000 times higher than losartan. Notably, compound 222a showed much better activity than methyl imidazole 222b, which exerted a comparable effect on L-type calcium channel to nifedipine.

Compound 223 is a new structural imidazole derivative with antihypertensive potentiality. Its preparation underwent the replacement of the o-nitrophenyl group in nifedipine with 4-chloro-5-imidazole moiety. This compound exhibited more potent calcium channel antagonistic activity than nifedipine. Structure–activity relationship pointed out that the increase or decrease of the chain length in C-3 and C-5 ester substituents was unfavorable for the bioactivity. Molecular docking studies displayed that the oxygen of ester and the N-3 of imidazole ring formed hydrogen-bond interactions with the NH of HIS 363 and the NH of
LYS354, respectively, on the active site of L-type calcium channel. These findings make this kind of imidazole-modified nifedipines deserve to be further studied as potential antihypertensive agents.

α2-Adrenoceptor is considered as an attractive therapeutical target for the treatment of cardiovascular diseases. Many imidazoles were observed to have the ability to interact with α2-adrenoceptor to lower systemic blood pressure. Triphenyl imidazole 224 was also reported to display better antihypertensive efficacy than clonidine with safe ALD50 (50% approximate lethal dose) > 1000 mg/kg in mice.484 This study suggested that compound 224 might be applicable for further optimization to treat hypertension.

10. IMIDAZOLES AS ANTI-INFLAMMATORY AGENTS

Inflammation is a complicated disease related to the response of the host to tissue injury or infection caused by trauma or biochemical stimulation, which causes a range of uncomfortable symptoms.485 Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used in the reduction of pain and inflammation. Recently, much work has been devoted to searching for more effective and safe NSAIDs.486–489 Imidazoles especially triphenyl imidazoles have attracted increasing interest in treating inflammatory disease and shown large potentiality as new member of anti-inflammatory drugs.490–495 The structures of imidazole-based anti-inflammatory compounds are shown in Figure 25.

Cyclooxygenase-2 (COX-2) selective inhibitors have been discovered as useful agents to treat acute pain and chronic inflammatory diseases. Imidazole derivatives 225a-c were capable of inhibiting COX-2 with high potency toward lipopolysaccharide (LPS)-induced RAW 264.7 cells. Structure–activity relationship showed that the para-substituted halogen and mesyl group on the benzene ring exerted a positive impact on the bioactivity, but the dihalo substituent would lower the efficacy.496

Triphenyl imidazoles 226a and 226b were also found to display significant anti-inflammatory activity with 59% and 52% inhibitory activity, respectively, in comparison with indomethacin (68%). Specially, they also showed excellent antifungal potency.497 Therefore, these two compounds might have future commitment to be developed as safe bifunctional anti-inflammatory and antifungal agents.

It was reported that the combination of triphenyl imidazole nucleus with nitrogen-containing heterocyclic moiety was an effective strategy to produce potent COX-2 inhibitors. Compared to celecoxib (IC50 = 150 nM), both bis-imidazoles 227a and 227b exhibited comparable but less-selective inhibitory efficacy against COX-2 with IC50 values of 170 and 175 nM, respectively. Notably, they showed equivalent in vivo anti-inflammatory activities in comparison with the reference drug celecoxib. Further study revealed that the benzyl group on the imidazole ring could interact with the big hydrophobic pocket at the distant terminal of helix 6 and exerted an important effect on the high potency.498

Sulfonamides are an important class of drugs with widespread bioactivities.499, 500 Some research suggested that the incorporation of sulfuryl group into triphenyl imidazoles, which produced compounds 228a-b, could result in good anti-inflammatory activities. Comparative study showed that both the imidazole ring and the sulfonyl group had a positive effect on the anti-inflammatory potency.501 Further introduction of a phenyl group in compound 228a afforded imidazole derivative 229, which displayed comparable anti-inflammatory activity to indomethacin.502, 503 These above mentions pointed out that sulfuryl imidazoles were actually potential to be developed as more potent anti-inflammatory compounds.

Research has revealed that p38α mitogen-activated protein kinase (MAPK) is the key enzyme in regulating the biosynthesis and release of proinflammatory cytokines.504–506
Inhibition of p38\(\alpha\) MAPK is therefore a promising therapeutic strategy for the treatment of cytokine-related disorders. Recently, a lot of imidazole derivatives with pyridine ring were revealed to be potent p38\(\alpha\) MAPK inhibitors, in which the pyridine ring was essential as it could generate crucial hydrogen bonds with the backbone amino group of Met109. Compound 230a showed an IC\(_{50}\) value of 0.079 \(\mu\)M against p38\(\alpha\) MAPK. Substitution of the imidazole ring with furan, pyrrole, or carbocyclic cyclopentene group all obviously diminished the efficacy. This might be explained by the fact that imidazole ring could interact with the amino group in the side chain of Lys53 by hydrogen bonds.\(^5\)\(^0\)\(^7\) Notably, imidazole derivative 230b gave much
better inhibitory activity with an IC\(_{50}\) value of 0.058 \(\mu\)M than compound 230a due to the additional acylated fragment that is able to interact with the kinase's hydrophobic region II or sugar pocket.\(^{508}\) Acylates 231a-b obtained by acylation of the pyridine ring were also highly active against p38\(\alpha\) MAPK with IC\(_{50}\) values of 0.003 and 0.048 \(\mu\)M, respectively. Particularly, compound 231b gave high metabolic stability in the in vitro metabolism assay.\(^{509,510}\)

Interestingly, 4-hydroxycyclohexyl imidazolone 232 also gave superior p38\(\alpha\) MAPK inhibitory activity with an IC\(_{50}\) value of 23 nM to reference SB203580 (IC\(_{50}\) = 44 nM) and exhibited similar efficacy (IC\(_{50}\) = 2.92 \(\mu\)M) toward LPS-stimulated tumor necrosis factor (TNF)-\(\alpha\) to SB203580 (IC\(_{50}\) = 1.76 \(\mu\)M). Structure–activity relationship disclosed that 4-hydroxycyclohexylamino moiety contributed to the good activity and its removal resulted in 20-fold reduction of bioactivities.\(^{511}\)

Microsomal prostaglandin E\(_2\) synthase (mPGES-1) represents a potential target for novel analgesic and anti-inflammatory agents. Imidazole 233 with good pharmacokinetic property exhibited significant and only three- and 1.5-fold less inhibitory activity with an IC\(_{50}\) value of 1.6 \(\mu\)M than rofecoxib (IC\(_{50}\) = 0.53 \(\mu\)M) and etoricoxib (IC\(_{50}\) = 1.1 \(\mu\)M) against mPGES-1 in human whole blood assay. Moreover, it gave excellent bioavailability (127\%) and the half-life value was 4.8 hr in rats. Structure–activity relationship showed that the 5-bromo imidazolyl group was necessary for the good activity and its substitution by the triazole ring would decrease the potency.\(^{512}\)

Fatty acid amide hydrolase (FAAH) and cytosolic phospholipase A\(_2\) \(\alpha\) (cPLA\(_2\)\(\alpha\)) with several common features have emerged as attractive targets for the treatment of inflammatory diseases. Compound 234 was a good dual inhibitor of FAAH (IC\(_{50}\) = 0.76 \(\mu\)M) and cPLA\(_2\)\(\alpha\) (IC\(_{50}\) = 0.50 \(\mu\)M) with ability to inhibit the formation of proinflammatory and degradation of anti-inflammatory lipid mediators at the same time. However, the presence of a carboxy group on the imidazole ring was unfavorable for the activity.\(^{513}\)

\(S\)-Nitrosoglutathione reductase (GSNOR) has emerged as an attractive therapeutic target for inflammatory diseases. Recently, some imidazole derivatives were reported to have potent and selective effects in animal models to treat inflammatory disease by reducing the levels of \(S\)-nitrosoglutathione (GSNO) and bioavailable NO. Currently, compound 235 (N6022), a potent and reversible GSNOR inhibitor (IC\(_{50}\) = 20 nM), has been in clinical trials as a potential agent for the treatment of acute asthma. Specially, the study manifested that the imidazole ring in this compound could favorably bind with Zn\(^{2+}\) ion in GSNOR.\(^{514}\)

PDE4 inhibitors with the ability to inhibit the production of proinflammatory cytokines are an important class of anti-inflammatory agents. Imidazole-modified phthalazinone 236 showed good PDE4 inhibitory efficacy with an IC\(_{50}\) value of 0.7 nM and potent TNF-\(\alpha\)-suppressing activity with an IC\(_{50}\) value of 0.5 nM. Further study demonstrated that the inhibitory activities were strongly dependent on the substituent at 2-position of phthalazinone, and the removal of benzylimidazole moiety led to 150-fold reduction of activity.\(^{515}\)

Lipoxygenase (LOX) is an important kind of enzymes in membrane lipid peroxidation by forming hydroperoxides in the lipid bilayer and their inhibitors have already attracted much attention for the treatment of inflammatory diseases. Pyrrolidinone-derived imidazoles 237a-b were found to be excellent LOX inhibitors with IC\(_{50}\) values of 0.08 and 0.07 mM, respectively. Especially, compound 237a displayed higher in vivo anti-inflammatory efficiency (41\%) than carboxyl derivative 237b toward rat paw edema, comparable to indomethacin (47\%). Comparative study pointed out that the carboxylic group was not favorable for improving bioactivity and imidazole 237a could be served as a lead compound for the design of more promising anti-inflammatory agents.\(^{516}\)

NF kappa B (NF-\(\kappa\)B) plays a vital role in innate immune system and is instrumental in induction of genes leading to a proinflammatory response. Selective inhibitors of IkB kinases \(\beta\) (IKK\(\beta\)) capable to activate NF-\(\kappa\)B are very promising candidates to treat inflammatory diseases.
Quinoline-imidazole hybrid 238 was observed to be an excellent IKKβ inhibitor with an IC_{50} value of 0.01 μM. Modeling studies revealed that the N atoms of NH₂ group and 3-position of the imidazole ring acted as a respective H-bond donor and acceptor to the hinge carbonyl of Gln-100 and Cys-99 of IKKβ in ATP-binding domain. However, this type of compounds with −CSNH−N=C− moiety suffered from poor pharmacokinetic characteristics in contrast with semicarbazones. Therefore, further structural optimization of this compound to improve its water solubility is worthwhile.

Methyl ester of 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO-Me) is currently being developed in late phase II clinical trials for the treatment of severe chronic kidney disease. Its N-acylimidazole derivative (CDDO-Im) was more potent and less toxic in rodents but with unstable property. Modification of the N-acylimidazole with acetylene afforded a more stable compound 239. It had nearly equivalent activity (IC_{50} = 1.4 and 0.4 nM) to CDDO-Im (IC_{50} = 0.6 and 0.2 nM) in the inhibition of NO and induction of cytoprotective enzyme quinine oxidoreductase 1 (NQO1) assays, respectively. These findings suggested that compound 237 was a very promising anti-inflammatory and cytoprotective agent.

Colony-stimulating factor-1 receptor (CSF-1R or FMS) inhibitors have shown therapeutic applications for immune-modulated inflammation. Compounds 240a-b displayed potent inhibitory efficacy (IC_{50} = 1.1 and 2.4 nM, respectively) against FMS but with poor oral bioavailability and undesirable effects in guinea pig right atrium assay. Further investigations by modulating the basicity of compounds to mitigate off-target ion channel effects led to the discovery of anti-inflammatory candidate 240c (JNJ-28312141), which had entered phase I clinical trials to treat rheumatoid arthritis. This compound not only showed good and selective FMS inhibitory activity (IC_{50} = 0.69 nM), but also could effectively reduce inflammation and bone erosion in rat adjuvant and streptococcal cell wall induced models of arthritis. Contrast test disclosed that the cyanoimidazole group had an important influence on bioactivity with approximately tenfold improved affinity over either furan or pyrrole analogs.

11. IMIDAZOLES AS ANTIOBESITY AGENTS

Obesity is one of the most active therapeutic areas in medicine that has become a major global health issue and is growing rapidly throughout the world. It also causes complications such as hypertension, type 2 diabetes mellitus, cardiovascular disease, cancer, and arthritis. Currently, sibutramine and orlistat are two well-known drugs approved for the chronic treatment of obesity with suboptimal tolerability and limited efficacy. Hence, the investigations of imidazoles as antiobesity agents are necessary to validate new molecular targets for more effective obesity treatment, and many potent antiobesity imidazoles have been studied. The structures of imidazole-based antiobesity compounds are shown in Figure 26.

As excess food intake would lead to obesity, compounds with ability to suppress food intake might thus be potent to treat obesity. Since rimonabant, a CB₁ receptor antagonist, was reported to be able to modulate food intake, CB₁ receptor has been considered as a new therapeutic target in the treatment of obesity. Replacement of hydrazide moiety in rimonabant by an imidazole ring yielded analog 241, which possessed potent and selective binding affinity to rCB₁ receptor (K_i = 5.3 nM). Notably, this compound could exert significant reduction (44%, p < 0.01) in food intake over a 4 hr test period at the concentration of 10 mg/kg in comparison with rimonabant (31%, p < 0.01 at 3 mg/kg). Compound 242 (BPR-890), an imidazole-thione analog of rimonabant, was identified to be a highly potent CB₁ inverse agonist (IC_{50} = 12.0 nM) with excellent selectivity over CB₂ (396-fold). Moreover, it displayed remarkable in vivo effect on weight reduction in diet-induced obese mouse model. Imidazole
Figure 26. Imidazole-based antiobesity compounds.

243 also displayed high and selective binding affinity toward CB$_1$ receptor with an IC$_{50}$ value of 1.91 nM.$^{522}$ From these results, imidazole-modified rimonabant analogs possessed great therapeutic possibility as CB1 receptor antagonists to treat obesity.

Research has demonstrated that bombesin receptor subtype-3 (BRS-3) might be a potential target for treating obesity, since mice lacking of BRS-3 were found to cause metabolic defects and obesity. Biphenyl imidazole 244a showed significant potency toward both rodent and human BRS-3 (EC$_{50}$ = 9.6 and 25 nM, respectively) with good pharmacokinetic profiles. Further study disclosed that the two methylene linker between imidazole and phenyl group was optimal for good activity. Either shortening the linkage to methylene or rigidifying with a trans-ethylenic linkage would completely abolish the bioactivity.$^{523}$ Pyridine 244b as analog of 244a showed more active binding affinity (IC$_{50}$ = 18 nM) toward human BRS-3 with high selectivity over hNMB-R (400-fold) and hGRP-R (355-fold). Moreover, this compound had sufficient exposure in diet induced obese mice after oral administration, which demonstrated its efficacy in lowering food intake and body weight via BRS-3 activation.$^{524}$

12. IMIDAZOLES AS ANTIVIRAL AGENTS

Viral infections are common obligate parasites, which severely threaten the health of human beings. Nucleoside analogs are currently in clinical use for the treatment of viral infections through inhibiting viral replication. However, their poor solubility results in a major challenge for the synthesis of novel derivatives of nucleosides. Therefore, extensive work has been done in exploring non-nucleoside compounds as antiviral agents and some imidazoles showed good antiviral activities.$^{525,526}$ The structures of imidazole-based antiviral compounds are shown in Figure 27.

Since the first reported sulfanyltriazole as potent non-nucleoside reverse transcriptase inhibitor (NNRTIs) with high potency and low toxicity toward human immunodeficiency virus type-1 (HIV-1), much attention has been drawn in the development of novel NNRTIs based on this scaffold.$^{527}$ Isosteric replacement of the triazole ring in sulfanyltriazole with an imidazole ring gave analogs 245a-b. Both compounds exhibited superior anti-HIV-1 activities (EC$_{50}$ = 0.20 and 0.18 μM, respectively) with high selectivity to sulfanyltriazole (EC$_{50}$ = 2.05 μM), nevirapine (EC$_{50}$ = 0.20 μM), and delavirdine (EC$_{50}$ = 0.32 μM). Molecular modeling studies indicated that the naphthalene ring could fit into the aromatic-rich binding pocket, which was favorable for the high binding affinity.$^{528}$

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It has been disclosed that bicyclic pyrimidine derivatives are potent HIV integrase inhibitors through coordinative interaction with metal ions for enzyme catalysis. Imidazole-incorporated bicyclic pyrimidine 246 possessed pronounced efficacy with an EC\(_{50}\) value of 6 nM in single-round HIV-1-based infective assays. Replacement of imidazole ring by thiazole or oxazole moiety would decrease the inhibitory potency suggesting the importance of nitrogen but not sulfur or oxygen atom for metal chelation. These demonstrated that imidazoles might be regarded as useful metal chelator to produce more effective HIV integrase inhibitors.

Hepatitis B virus (HBV) infection is one of the most prevalent viral diseases in the world. However, all the nucleotide analogs are facing resistant HBV variants in antiviral therapy. It is thus necessary to develop anti-HBV agents with novel structures different from the current nucleotide drugs. Imidazole 247 displayed obvious in vitro anti-HBV activity (IC\(_{50}\) = 1.7 μM) with excellent selectivity (SI = 60.3) in comparison with lamivudine (IC\(_{50}\) = 240 μM, SI = 9.1). Further research showed that the imidazole ring was not only favorable for improving inhibitory efficacy, but also for decreasing toxicity. Replacement of the imidazole ring by morpholine, piperidine, or pyrrolidine group failed to give enhanced anti-HBV activity even increased toxicity.

13. IMIDAZOLES AS OTHER MEDICINAL AGENTS

Apart from the above mentions, imidazole derivatives have been also found to exhibit potential applications in other medical fields, such as thromboxane synthase inhibitors, antioxidant agents, acid pump antagonists, bronchodilator, agents against Cushing’s syndrome, and myocardium in ischemia. The structures for imidazoles as other medicinal agents are shown in Figure 28.

Selective cortisol synthase CYP11B1 inhibitors are potential candidates for the treatment of Cushing’s syndrome and related diseases. However, the high sequence identity between cortisol synthase CYP11B1 and aldosterone synthase CYP11B2 makes it difficult to obtain selective enzyme inhibitors. Compound 248, the first reported CYP11B1 inhibitor, showed comparable activity (IC\(_{50}\) = 152 nM) to ketoconazole (IC\(_{50}\) = 127 nM) with remarkable selectivity over CYP11B2, CYP17, and CYP19. This compound provides valuable information for the development of drugs to treat cortisol-dependent disease.

Serotonin 5-HT\(_{2B}\) receptor has attracted much attention as a potential therapeutic target for the drug discovery to treat chronic heart failure. Biphenyl imidazole 249 displayed excellent binding affinity with an IC\(_{50}\) value of 0.2 nM toward 5-HT\(_{2B}\) receptor with high selectivity (SI = 35) and favorable pharmacokinetic property. This result should be helpful to design more superior 5-HT\(_{2B}\) antagonists for chronic heart failure.
Reversible imidazopyridine $\text{H}^+/\text{K}^+$ ATPase inhibitors (acid pump antagonists) could rapidly inhibit gastric acid secretion with potentially long-lasting property. Biphenyl imidazole 250 was found to be an excellent $\text{H}^+/\text{K}^+$ ATPase inhibitor with pIC$_{50}$ value of 6.1. Further research disclosed that the position of methoxy group on the middle benzene ring had a great influence on the bioactivity. The displacement of the methoxy group from 6- to 2-position resulted in complete loss of biological activity.

Inhibition of the activity of $\text{Na}^+$/H$^+$ exchanger type-1 (NHE-1) would be an effective access to prevent the damage of myocardium in ischemia reperfusion. Compounds 251a-b were identified to have superior NHE-1 inhibitory activities with IC$_{50}$ values of 0.10 and 0.24 μM, respectively, to cariporide (IC$_{50}$ = 1.20 μM). Moreover, they also displayed good in vitro and in vivo cardioprotective efficacy against ischemia/reperfusion injury.

It has been reported that nitrotyrosine is the biomarker of oxidative and nitrosative stress in many allergic diseases, and the inhibition of nitration toward bovine serum albumin (BSA) can inhibit the formation of nitrotyrosine. Seleno-imidazole 252 exhibited notable inhibitory activity toward peroxynitrite-mediated nitration of BSA. Further study showed that the inhibitory potency could be enhanced by the incorporation of more than one selenourea moiety into a single molecule. The existence of zwitterionic forms with significant negative charge on the selenium atoms endowed this compound with highly nucleophilic property and thus acted as an effective scavenger of peroxynitrite. This kind of compounds have the potentiality to be developed as potential antihyperthyroidism agents.

14. IMIDAZOLES AS DIAGNOSTIC AGENTS AND PATHOLOGIC PROBES

Cations, anions, and amino acids are involved in a variety of biochemical functions by affecting the activity of enzymes, coenzymes, and cofactors in biological aspects. Both their deficiency and excess in biological systems have an important influence on human health. Currently, a great deal of interest has been directing toward the design and synthesis of artificial receptors capable of binding guest molecules or ions to monitor biological events. Imidazole ring has been frequently incorporated into fluorescent skeleton to provide artificial molecules suitable for the selective recognition of ions and biological molecules in the human body, which have manifested the potent capacity of imidazoles as diagnostic agents and pathologic probes. The intracellular applications of imidazoles, particularly as ion receptors, are being actively
under investigation. The structures of imidazole-based diagnostic agents and pathologic probes are shown in Figure 29.

### A. Imidazoles as Anion Receptors

Anions are ubiquitous species in biological systems, as over 70% of enzyme substrates and cofactors in biology are in fact anionic, and they play essential roles in the activity of enzymes, the transport of hormones, protein synthesis, and DNA regulation.\(^{544}\) The binding between the receptor and the anion is typically labile and reversible, and is involved in different types of interactions such as electrostatic interactions, hydrogen-bond, and metal–ligand interactions.\(^{545}\) The electron-rich nitrogen atoms present in the imidazole derivatives could provide multiple interaction sites toward the guest molecules. Recently, many imidazole-based anion receptors with high selectivity and excellent efficiency and sensitivity have been developed.

#### 1. Imidazoles as Inorganic Anion Receptors

Fluoride (F\(^{-}\)) is an essential anion in nature and has a significant role in food processing, clinical analysis fluorination of water supplies, and dental care for the treatment of osteoporosis. Much effort has been devoted to developing fluorescence sensors for fluoride detection. However, current sensors still lack the sensitivity and selectivity for the robust detection of fluoride. Imidazole-naphthalimide 253 as inorganic anion receptor could recognize fluoride anion with high sensitivity and selectivity. Fluorescence titration of compound 253 suggested that it formed a 1:1 stoichiometric complex with F\(^{-}\) ion when indicated by the decrease of fluorescence at 442 nm with an association constant of log\(K_a = 5.31 \pm 0.07\). Further study indicated the proposed mechanism that the F\(^{-}\) ion could effectively disrupt the intramolecular hydrogen bond between imidazole \(H\) and carbonyl \(O\) atoms, resulting in a noncoplanar geometry with fluorescence quenching.\(^{546}\)

Ruthenium(II) complex 254 was also able to selectively recognize F\(^{-}\) ion with strong binding interaction (log\(K = 7.61\)), and it could form a 1:1 adduct with F\(^{-}\) ion by significant quenching with a redshift of the band from 716 to 760 nm. Further study revealed that the anion sensing mechanism was mediated by hydrogen bonds. The free N-H group in benzimidazole could form hydrogen bonds with the pendant carboxylate oxygen while the imidazole N-H was responsible for possible hydrogen-bond interaction with the anions.\(^{547}\) It has been found that transition metals can be used as structural elements to build up anion receptors, enhancing hydrogen-bond donor tendencies and favoring the assembling of the molecular framework.

Cholesterol is an essential metabolite required for major biological functions such as the construction of cell membrane and the integral part of the lipid bilayers. It also provides the structural scaffold for the biosynthesis of bile and bile acid salts to construct supramolecular systems for molecular recognition. Imidazolium-based cholestane receptor 255 bearing anthracene group as fluorophore showed high binding affinity toward dihydrogen phosphate (\(K_a = 1.6 \times 10^5 \text{ M}^{-1}\)).\(^{548}\) This type of compounds bearing membrane-compatible steroid framework with the ability to translate the anion recognition into biological activity, having definite advantages to be used as steroid-based receptors in biology.

#### 2. Imidazoles as Organic Anion Receptors

Many exciting and significant results have been obtained by applying imidazole receptors to various organic anionic targets such as ATP, DNA. Specially, imidazolium derivatives have been paid much attention due to their capability to bind anions through strong and unique hydrogen bonds between the imidazolium and various anions. They could normally be observed via fluorescence quenching effects and have been studied extensively in recent

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Figure 29. Imidazole-based diagnostic agents and pathologic probes.
Recognition of DNA is helpful for the diagnosis of diseases that are caused by genetic disorders. Recently, literature has reported an anthracene-type bis-imidazolium 256, which could be used for sensing culf thymus (CT) DNA and monitoring the activity of DNase. Notably, this compound displayed a large and selective fluorescent quenching effect toward CT DNA duplex with an association constant of $8.9 \times 10^6$ M$^{-1}$, similar to commercially available intercalating agent proflavine. Further study suggested that the ionic hydrogen-bond interactions between imidazolium groups and phosphate backbone of DNA. The intercalations between compound 256 and DNA were helpful to strengthen the sensing efficacy. This work showed that the bis-imidazolium receptors should be nice candidates for sensing DNA.

Imidazole-containing polyamides, known for their ability to bind in the minor groove of DNA, showed potential use as regulators of gene expression. Imidazole polyamide 257 was capable of crossing certain cellular and nuclear membranes and exhibited an increase in fluorescence at 370 nm upon binding to DNA with high sequence specificity and binding affinity. The $p$-anisyl benzimidazole carboxamido fluorophore in this compound with favorable stabilizing property to the DNA sequence was revealed to be effective DNA recognition element. Such imidazole-based receptors with simple molecular structure and synthetic methods provide a direct and simple way to monitor DNA recognition processes in cells.

Nucleotides ATP and ADP are basic and important components in bioenergetic conversion processes of organisms and necessary for understanding and evaluating several key biological processes. A luminescence imidazole 258 could selectively sense the nucleoside polyphosphates ADP in living cells through a “turn-on” manner, and formed a 1:3 stoichiometry of the host–guest with the association constant (log$K$) calculated as 10.44. Notably, no remarkable fluorescence change was observed when incubated with ATP suggesting the possible distinguishability of ADP from ATP in the living cells. Further study suggested that the complementary interactions including $\pi$–$\pi$ stacking and hydrogen bonds between adenosine and compound were a paramount source of unique selectivity. These results suggest that compound 258 might be a useful probe for the fluorescence detection of ADP with high selectivity and strong binding affinity.

The $D$-myo-inositol 1,4,5-trisphosphate (IP$_3$) plays a pivotal role as a second messenger in intracellular signal transduction and regulates many calcium-dependent cellular functions. Compound 259 was the first reported imidazolium-modified fluorescent receptor for IP$_3$ with a large and selective fluorescent quenching effect, and the association constant for IP$_3$ was $1.6 \times 10^5$ M$^{-1}$. This relatively simple synthetic receptor showed promising practical use for imaging of specific IP$_3$ with better selectivity.

Imidazole-based protein labeling in living cells was reported to be useful for understanding natural protein functions. Compound 260 beard a methotrexate moiety as a fluorescein and an affinity ligand for folate receptor (FR) connected through an imidazole linker. It was capable of selectively converting endogenous FR to a fluorescent biosensor on the living cell surface. The labeling reaction was driven by ligand–protein recognition, and Lys32 was uniquely modified as a single labeling site. Comparative study displayed that substitution of the imidazole ring by tosylate moiety led to 12-fold less potent activity, demonstrating the imidazole ring had an important impact in facilitating to produce labeled active protein.

Generally, among the various ion receptor systems, the anionic ones are rather limited. This may be attributed to the varied size, shape, and charge characteristics of the anions, which demand higher and specific interactions with the receptors for the recognition, as compared to the almost spherical cationic species. Therefore, great efforts are required to develop suitable artificial receptors for anions.
B. Imidazoles as Cation Receptors

Cations such as potassium, sodium, and ferri ions are very essential for maintaining the health of human beings. The electron-rich imidazole ring endows its derivatives with the ability to interact with various cations. Many imidazole-based derivatives as cation receptors have been prepared, and they have exhibited a promising application prospect in clinical medicine.

Fluorescent compounds that can selectively monitor specific metal ions in living cells have become indispensable tools for the understanding of biological phenomena. Potassium ion (K\(^+\)) plays an important role in biological systems including its ability to stabilize guanine (G)-rich oligonucleotide sequences to form G-quadruplex. Perylene-like imidazolium 261 was reported to have the ability to transform the G-rich DNA sequences to G-quadruplex through trapping K\(^+\) ion with high selectivity and sensitivity, leading to fluorescence enhancement. Without any doubt, this compound could be readily used to monitor the signal for K\(^+\) ion recognition. The study revealed that compound 261 should have large potentiality in diagnostic and therapeutic application toward human telomere and cancer cells.

The development of artificial fluorescent receptors for the sensing and recognition of transition-metal ions is currently of great interest because of their highly sensitive, convenient, and inexpensive properties. Imidazole bearing tetraphenylethylene 262 was a fluorescent probe for Fe\(^{3+}\) ion in tetrahydrofuran-water mixture with highly stable constant (log\(K_a = 25.1\)). It could be completely quenched by Fe\(^{3+}\) ion due to the coordination between the imidazole ring and the Fe\(^{3+}\) ion. Considering that aggregation-caused fluorescence quenching often takes place in traditional receptors when dispersed in aqueous media, this type of fluorescent probes, to some extent, expand the application of sensors in biological systems and environmental determinations.

Imidazolium-based ionic liquid 263, a water-soluble and highly photostable dye, could be employed as a Fe\(^{2+}\)-selective receptor for live cell imaging involving an attack of the hydroxyl radical to the four-membered ring of squaraine. It could permeate through the cell membrane and preferentially accumulate around the perinuclear region with low toxicity. This compound should be valuable in revealing the roles of Fe\(^{2+}\) ion in biological systems under either in vitro or in vivo conditions.

A recent study reported that nonconjugated polymers with degradable properties could also maintain the excellent fluorescent properties of conjugation. Nonconjugated polymer 264 was a kind of degradable water-soluble fluorescent compound, and displayed slow degradation pattern with the enhancement of fluorescence in physiological environment (pH 7). Since it was easy to remove from animal or human body by metabolic paths, this polymer had potential in vivo applications in biological and biomedical fields such as drug delivery and cell imaging.

Some studies also revealed that many imidazole-based neutral anion receptors as well as fused imidazoles also exhibited potential application in diagnostics and pathology. Since neutral anion receptors are often lack of water solubility, which prevents binding studies in aqueous solution, the incorporation of imidazole ring could possibly endow the neutral receptors with sufficient water solubility. Evidently, the functionalized imidazoles are good candidates for the selective recognition of ions and molecular species. Continuous development of imidazole-based diagnostic agents and pathologic probes and their potential application in preclinical animal models and humans should provide mechanistic insight into basic biology and disease, and further improve disease detection and treatment in the clinic.
15. CONCLUSIONS AND PERPECTIVES

The above mentions have clearly shown that the structurally simple imidazole ring plays an important role in medicinal chemistry and the related research has been being unusually active subjects. A large amount of work has been made toward imidazole-based medicinal chemistry. Numerous outstanding achievements revealed that imidazole-based compounds possess extensively potential application as medicinal drugs, diagnostic agents, and pathologic probes. In particular, a large number of imidazole-based compounds as clinical anticancer, antibacterial, antifungal, antineuropathic, antihypertensive, antihistaminic, antiparasitic agents, and so on have been successfully developed, marketed, and extensively used in the clinic in preventing and treating various types of diseases with low toxicity, high bioavailability, and good biocompatibility and curative effects. An expanding effort from all over the world has been directly focusing on imidazole-based compounds for possible clinical application in the diagnosis and treatment of various types of diseases. Excitingly, an increasing number of imidazole derivatives have been becoming clinical drug candidates in actively ongoing research and developments. All these have strongly suggested the infinite potentiality of imidazole derivatives in medicinal field.

Though a lot of progress has been acquired, it is undoubted that the developments of all possible application of imidazole-based compounds as medicinal drugs, diagnostic agents, and pathologic probes will continuously be an overwhelmingly attractive topic in quite a long time. The extensive medicinal potentiality will ineluctably draw more and more researchers to engage in the medicinal research of imidazole derivatives. The new trends in imidazole-based medicinal chemistry might mainly appear in the following active topics:

1. Much effort will contribute to structural modification of clinical imidazole drugs to retain the advantages of these drugs and overcome their shortcomings.
   One important strategy is to employ some functional groups or structural fragments that are helpful for improving physicochemical properties and affinity with target sites to modify clinical imidazole drugs. This intention is to increase their biological activities, broaden active spectrum, and overcome drug resistances.

2. Research of imidazole ring as an isostere to replace triazole, oxazole, pyrazole, thiazole, tetrazole, and amide, etc., to modify other marketed drugs will become a hot topic.
   This will be an attractive and prevalent strategy to develop new imidazole drugs. Distinguished from its other isosteres, imidazole ring is highly polar and could not only readily accept or donate proton, but also easily form hydrogen bonds that are beneficial for endowing its derivatives with the ability to improve water solubility, bioavailability, metabolic stability, and bioactivity.

3. Exploitation of structurally novel types of imidazole derivatives for all possibly medicinal application will become an actively important direction.
   Structurally novel imidazole derivatives might exert a new mechanism of action that might exhibit different bioactivity. This strategy includes the combination of imidazole ring with other pharmacophores and the development of new structural imidazoles with distinct skeleton from traditional clinical drugs. This conation is to hopefully discover new lead imidazole-based compounds and open a new pathway to develop novel structural types of drugs, thereby solving the increasingly serious problem of drug resistances.

4. Isolation and bioactive evaluation as well as action mechanism investigation of naturally occurring imidazole products from natural source like plants will continuously attract special interest.
   The structurally new types of natural imidazole derivatives especially those with wholly new structural skeleton different from traditional clinical drugs are an important
source or new leads to develop new medicinal drugs generally with enormous potentiality in the treatment of diverse diseases.

5. Developments of imidazole-based chiral molecules will become important demand for chiral target sites.

The chiral receptors and corresponding drug-receptor complexes might involve different binding sites, which might result in undesirable side effects and toxicity. Importantly, some single enantiomer agents generally display better activities and their administration doses are lower than racemic ones, which suffer from minor risks of side effects and unspecific toxicity.

6. Imidazole-based supramolecular drugs will draw increasing attention and become more and more active subjects.

The overwhelming advantages of supramolecular drugs, including easy preparation, low cost, high safety, low toxicity, less adverse effect, high bioavailability, fewer drug resistances, good biocompatibility, and curative effects, etc., will attract numerous efforts toward their research and developments. The unique structure of imidazole ring with multiple binding sites undoubtedly provides plenty of natural source as a building block with a variety of inorganic metal ions or interacting with organic molecules via diverse noncovalent interactions such as coordination bonds, hydrogen bonds, π–π stacking, hydrophobic effects, and van der Waals forces to produce imidazole-type supramolecular drugs. These research include the construction of imidazole-based supramolecular drugs and developments of imidazole-based highly drug-loaded hosts such as imidazole macrocycles (e.g., crown ether, cyclophane, etc.) being able to load more drugs as well as the research of these hosts as drug carriers. Further investigation in imidazole supramolecular drugs principally covers their structure–activity relationships, action mechanism, solubility, stability, drug dissolution, selectivity, as well as safety, etc.

7. More and more work will contribute to imidazole-based artificial receptors and fluorescent molecules for diagnosis and pathologic probe in biological systems.

Imidazole ring with attractive poly-binding sites is able to interact with a variety of anion and cation ions as well as biological molecules in the human body. Reasonably, much work will contribute to the design and synthesis of novel imidazole-based artificial receptors and fluorescent probes with poly-coordination or poly-binding sites such as poly-nitrogen and poly-sulfur ones as well as their research of coordination behavior, and their application as diagnostic agents and pathologic probes to monitor biologically important ions and molecules of biochemical process in biological systems.

Convincingly, with the continuous efforts directly toward imidazole-based medicinal chemistry and the progress in other disciplines such as cell biology, molecular biology, pharmacology, and materials science. A growing number of imidazole-based drugs with better efficacy, lower toxic and superior pharmacokinetic characteristics, and effective diagnostic agents and pathologic probes will be used in the clinic and make remarkable contributions for the protection of human health.

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