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Bioactivities of Triazolium-Based *N*-Heterocyclic Carbene Salts

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Abstract: 1,2,4-Triazolium salts are precursors of *N*-heterocyclic carbenes (NHCs), which have been extensively used as effective catalysts and ligands for both asymmetric and non-enantioselective reactions. Nevertheless, they are also a kind of quaternary ammonium compounds (QACs) that possess amphipathic properties. The unique chemical and physical properties of 1,2,4-triazolium salts have received significant attention from scientists focusing on the development of novel bioactive molecules as pesticides and medicines. It is timely and meaningful to summarize the bioactivities of 1,2,4-triazolium salt derivatives against various bacteria, fungi, cancer cells, and other pathogens in the past 30 years. Meanwhile, the structure-activity relationship (SAR) of 1,2,4-triazolium salts was also summarized. Finally, our perspective on the future development and applications of triazolium salts as agrichemicals or human drugs is presented.

1. Introduction

1,2,4-Triazolium salt derivatives have been extensively studied as *N*-heterocyclic carbene (NHC) catalysts[1] and ligands.[2] Meanwhile, 1,2,4-triazolium salts have also been reported to possess a wide range of significant bioactivities, such as antibacterial,[3] antifungal,[4] anticancer,[5] antiprotozoal,[6] and even worm growth regulation[7] activities. It is well known that triazoles are widely used in the development of agrochemicals and medicines based on their highly efficient and broad-spectrum biological activities.[8] But the drug resistance in the triazole structures has been significantly increased because of the frequent use of triazole drugs.[9] As a result, triazolium salts that bear similar chemical structures to triazoles have attracted considerable attention in recent years.

Some of the triazolium compounds, such as Biapenem[4] and Cresemba,[10] have been approved by U. S. the Food and Drug Administration (FDA) as marketed human drugs (Figure 1). Introducing the cationic triazolium structure into a drug-candidate molecule can improve the aqueous solubility, efficacy, and safety to non-target organisms.[11] For example, the antibacterial activity of Biapenem is superior to its precursor Imipenem in both the *in vivo* and *in vitro* bioactive tests, which is believed to benefit from the cationic triazolium fragment in the Biapenem structure.[12–15] Cresemba has been used in the clinical treatment of invasive aspergillosis and mucormycosis in adults.[10] It can be converted into the effective drug of Isavuconazole in the human body after injection to exert its medicinal effect. The poor solubility of the Isavuconazole in water is significantly improved through the introduction of the triazolium cation.[10]

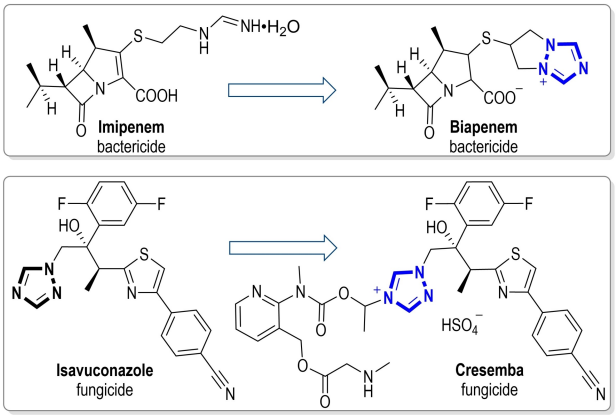

Figure 1. Commercial drug structures of 1,2,4-triazolium salts.

Triazolium salts are conventionally prepared through *N*-alkylation reactions of 1,2,4-triazoles, which can be efficiently obtained through "Click chemistry" between various alkynes and azide compounds.[16] Moreover, the different substituents and substitution patterns around the triazolium compounds can greatly affect their biological activities,[7,11,17–19] and cytotoxicities.[6,20] Therefore, triazolium salts are promising lead structures for the development of novel pesticides and pharmaceuticals. This concept provides a systematic overview on the state-of-the-art in the development of triazolium-derived

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bioactive compounds, which is organized through four aspects: 1) bioactivities of mono-1,2,4-triazolium salts; 2) bioactivities of bis-1,2,4-triazolium salts; 3) bioactivities of metal/NHC complexes; and 4) structure-activity relationship of 1,2,4-triazolium salts.

2. Bioactivities of Mono-1,2,4-Triazolium Salts

2.1. Antifungal activities of mono-1,2,4-triazolium salts

Broad-spectrum injectable antifungal drugs are rare at present due to their poor water solubilities.[12] To address this problem, scientists have used a prodrug approach to develop intravenous formulations that can replace traditional antifungal drugs.[21] Among these antifungal bioactive triazolium salt molecules, Cresemba has been approved by the FDA and widely used as a

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Prof. Chengli Mou received her BS Degree in 2011 from Anshun University. She then entered Guizhou University to pursue her PhD Degree under the guidance of Prof. Yonggui Robin Chi, during which time she visited Nanyang Technological University in Singapore as a joint student for two years. She obtained her PhD Degree in 2017 and joint Guizhou University of Traditional Chinese Medicine at the same year for an independent research career. She has been a visiting scholar at Nanyang Technological University from 2018 to 2019. Her research interests cover the development of fundamentally novel synthetic methodologies for asymmetric synthesis, the discovery of small molecular structures for green pesticide development and the isolation and characterization of novel structures in the components of traditional Chinese medicines.

Prof. Yonggui Robin Chi received his undergraduate training from Tsinghua University and Hong Kong Baptist University during 1998–2002. He obtained Ph.D. in Chemistry at the University of Wisconsin-Madison under the guidance of Prof. Sam Gellman in 2007. After a two-year postdoctoral stay at the University of California-Berkeley with Prof. Jean Frechet, he started his independent career at Nanyang Technological University and was quickly promoted to full professor and chair professor. His current research, supported by students and researchers from Nanyang Technological University and Guizhou University, focuses on the development of new basic activation modes and reactions mediated by organic catalysts, efficient transformations for rapid construction of sophisticated molecules, selective modification of Chinese medicines and biomacromolecules, green reactions and processes for industrial applications, and functional molecules for applications in medicines, agricultural chemicals, and materials.

human drug in the clinical treatment of invasive aspergillosis and mucormycosis (Figure 1).[10] Besides the marketed drug of Cresemba, many 1,2,4-triazolium salts have been developed as prodrugs from triazole fungicides.[4,17,22–24] These 1,2,4-triazolium salts have good water solubility and can be rapidly and safely converted into antifungal triazole drugs in living organisms. In 2001, Ichikawa[4] et al. synthesized a new antifungal prodrug **1** (TAK-457) for injection with sufficient solubility (4–10 mg/mL in 5% glucose), stability (1.8% of TKA-456 after 1 d in 5% glucose), and high *in vivo* inhibitory index against *Candida albicans* TA and *Aspergillus fumigatus* 437, with the ED_{50} (the dose of the test compound which allows 50% survival of the infected mice) values of 0.62 and 4.49 mg/kg (Figure 2). Furthermore, the *in vivo* curative effect of the compound **1** in mice of invasive pulmonary aspergillosis was similar to that of Fluconazole (ED_{50} = 0.22–0.39 and 179 mg/kg, respectively) against *C. albicans* TA and *A. fumigatus* 437.[4]

In 2002, Umeda et al.[23] prepared a set of *N*-benzyltriazolium or *N*-benzylimidazolium salt prodrugs with good solubility in water from highly lipophilic triazole and imidazole antifungals. They could be transformed into their parent triazole and imidazole antifungal drugs through enzymatic activation to work as antifungals. Compound **2** showed good antifungal activities (ED_{50} = 4.6–19 μmol/kg on day 14 against *C. albicans* ATCC48130 and *A. fumigatus* CF437) which could cure the systemic candidiasis, aspergillosis and pulmonary aspergillosis in rats. In addition, the compound **2** is sufficiently chemically stable and water-soluble as a prodrug and is rapidly and quantitatively converted into active substances in human plasma.

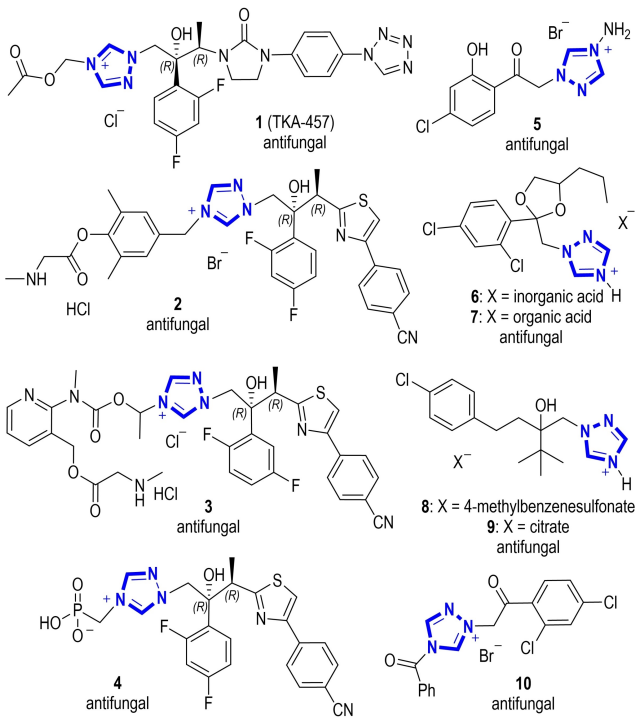

Figure 2. Structures of mono-1,2,4-triazolium salts with antifungal activities.

Subsequently, they[17] also found that the compound **3** has strong antifungal activity against systemic candidiasis, aspergillosis and pulmonary aspergillosis in rats. Compound **3** showed great antifungal activity (ED_{50} = 6.0–14 mmol/kg) and was nearly as active as or better than Itraconazole against *C. albicans* CY1002, CY3003 and *Candida tropicalis* (Fluconazole-resistant strains) through intravenous and oral administration.

In 2003, Ueda and co-workers[24] obtained the phosphonooxymethyl 1,2,4-triazolium salt derivative **4** from Ravuconazole and evaluated its bioactivity and chemical stability. This 1,2,4-triazolium salt was proven to have good solubility in water, which can be transformed into the parent Ravuconazole under the action of alkaline phosphatase in rats. However, the chemical stability of the compound **4** in water does not meet the requirements of an injectable water-soluble prodrug.

Emami et al.[18] synthesized in 2008 the triazolium salts by the quaternization of 4-amino-1,2,4-triazole with α-bromoketone. 1,2,4-Triazolium salt **5** showed higher activity with the minimum inhibitory concentration (MIC) value of 4 μg/mL against *C. albicans* than that of the control drug Fluconazole (MIC = 8 μg/mL). The cytotoxicity of **5** was lower than that of other compounds, and similar to the control drug Fluconazole. It's worth noting that the triazolium salt still displayed antifungal activity at non-cytotoxic concentrations.

In 2015, Pernak and co-workers[25] reacted Tebuconazole and Propiconazole with inorganic and organic acids to efficiently generate a set of new salts. Many of the newly prepared triazolium salts can be described as protic ionic liquids, whose thermal stability and antifungal activities were evaluated during bioassays. The evaluation results showed that the synthetic Tebuconazole- and Propiconazole-based triazolium salts had good inhibitory effects against plant pathogenic fungi. The anions of the propiconazole-based triazolium salts can be both inorganic (the compound **6**) and organic (the compound **7**) acid ions with the antifungal activities (the growth of the fungal species of *Fusarium culmorum, Microdochium nivale, Sclerotinia sclerotiorum* and *Botrytis cinerea* mycelium < 2.1 cm at 10 ppm concentration) remained as excellent commercial drug Propiconazole (the growth of the fungal species of *F. culmorum, M. nivale, S. sclerotiorum* and *B. cinerea* mycelium < 0.6 cm at 10 ppm concentration).

In the same year, Zabielska-Matejuk et al.[26] reported that the antifungal activity of the compound **8** (ED_{50} < 0.1 ppm) with a 4-methylbenzenesulfonate anion against *Trametes versicolor* was better than that of the commercial drug Tebuconazole (ED_{50} = 1 ppm). And thetebuconazole-based triazolium citric acid salt **9** has the same effect on several wood fungi, such as *Coniophora puteana*, *T. versicolor* and *Sclerophoma pithyophila*, as the commercial drug Tebuconazole (ED_{50} = 1 ppm).

In 2022, Macaev and co-workers[27] synthesized fifteen triazolium salts and evaluated their antifungal activities against eight fungi, including *Aspergillus niger* ATCC 6275, *A. fumigatus* ATCC 1022, *Aspergillus versicolor* ATCC 11730, *Penicillium funiculosum* ATCC 36839, *Trichoderma viride* IAM 5061, *Penicillium verrucosum var. cyclopium*, *Aspergillus. ochraceus* and *Penicillium. ochrochloron*. Among these triazolium salts, the

compound **10** showed the best activities with the MIC values of 0.009–0.037 mg/mL and the minimal fungicidal concentration (MFC) values of 0.0125–0.05 mg/mL.

2.2. Antibacterial activities of mono-1,2,4-triazolium salts

In 1992, Miyake et al.[28] synthesized a series of cephalosporin derivatives bearing different fused heterocyclic azolium rings and tested their antibacterial activities (Figure 3). Compound **11** showed potent antibacterial activities, with the MIC values below 0.1 μg/mL against *Escherichia coli* NIHJ JC-2, *Enterobacter cloacae* IFO 12937 and *Serratia marcescens* IFO 12648. Therefore, they proposed that the fused heterocyclic triazolium salts were important structural units that can improve the antibacterial activities and broaden the bactericidal spectrum of Cephalosporins (Figure 3).

Jain et al.[29] prepared a set of *N,N*-dichlorotriazolium compounds in 2009 and evaluated their antibacterial activities through the examinations of minimum bactericidal concentration (MBC). Among them, the compound **12** had good inhibitory activities against *E. coli* ATCC 25922 (MBC = 2 μg/mL), *Staphylococcus aureus* ATCC 29213 (MBC = 32 μg/mL) and *C. albicans* ATCC 10231 (MBC = 16 μg/mL).

2.3. Mono-1,2,4-triazolium salts possessing both antibacterial and antifungal activities

In 2009, Zhou et al.[30] reported that the 1,2,4-triazolium salt compounds **13** and **14** containing di-halophenyl and dodecyl groups had promising antimycotic activities (Figure 4). The MIC values of them were 8.38/4.50 μg/mL and 4.19/4.50 μg/mL against *C. albicans* ATCC 76615 and *A. fumigatus*, respectively. Their antifungal activities were obviously superior to that of Fluconazole used in clinics.

Subsequently, they[31] prepared the 1,2,4-triazolium derivative **15** through the use of 2,4-dichlorobenzyl chloride and examined its bioactivities against bacteria and fungi. The bioassay results showed that the carbazole–triazolium compound **15** had good activities against bacteria and fungi with MIC values of 2–8 μg/mL and 32–64 μg/mL, respectively. Their results also showed that the introduction of the 1,2,4-triazolium group into the carbazole derivatives could improve their antimicrobial activities.

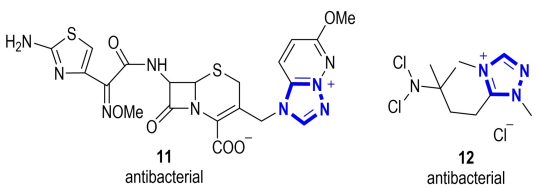

Figure 3. Structures of mono-1,2,4-triazolium salts with antibacterial activities.

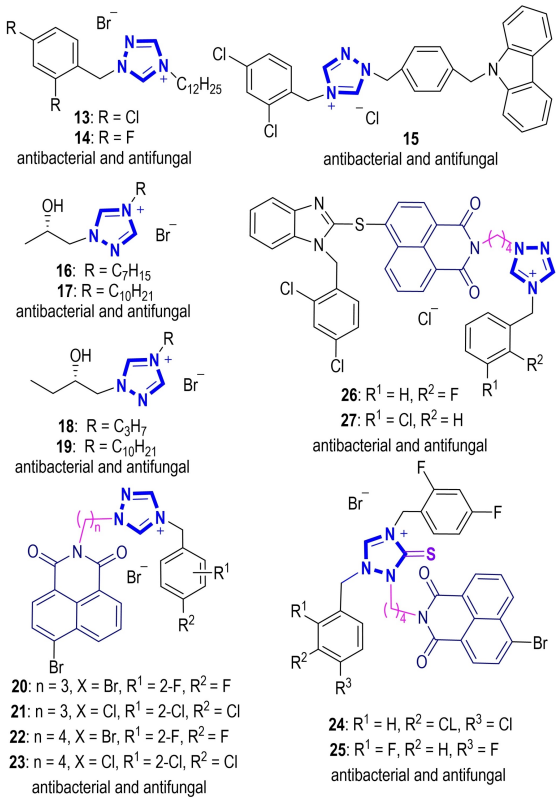

Figure 4. Structures of mono-1,2,4-triazolium salts with antibacterial and antifungal activities.

In 2013, Borowiecki and co-workers[19] obtained a series of new 1,2,4-triazolium salt compounds and studied their bioactivities. The bioassay result demonstrated that the compound **16** has optimal antifungal activities. Its inhibitory rate (%) against *Fusarium oxysporum* MF 5, *Fusarium sambucinum* MF 1, *Fusarium culmorum* MF 18, *Aspergillus brasiliensis* ATCC 16404, *Colletotrichum coccodis* MC 1, *Phytophthora infestans* MP 324, and *Phytophthora infestans* MP 1320 at the concentration of 1 mM was 105.10 ± 2.32, 98.39 ± 5.47, 95.58 ± 2.78, 104.12 ± 1.58, 99.82 ± 3.43, 104.63 ± 7.64 and 92.64 ± 4.45, respectively. The biological activities of the series of compounds showed close relationship to the length of the alkyl chain, and the substituents with 10–16 carbon units had considerably higher toxicities against bacteria. Particularly, the 1,2,4-triazolium salt **17** with a 10-C alkyl chain exhibited great antibacterial activities with the MIC values of 0.5–5.5 mM.

In the same year, they[3] also prepared the 1,2,4-triazolium salt **18** with good activities against phytopathogenic fungi. Its inhibitory rate (%) against *F. oxysporum* MF 5, *F. sambucinum* MF 1, *F. culmorum* MF 18, *A. brasiliensis* ATCC 16404, *C. coccodis* MC 1, *P. infestans* MP 324, and *P. infestans* MP 1320 at the concentration of 1 mM was 93.77 ± 1.33, 104.56 ± 1.92, 101.21 ± 0.93, 100.78 ± 0.78, 99.57 ± 3.29, 90.51 ± 3.76 and 108.39 ± 2.95, respectively. Meanwhile, the 1,2,4-triazolium salt **19** possessed good antibacterial activities against Gram-negative (G-) bacteria (*E. coli* ATCC 8739, *Salmonella typhimurium* ATCC 14028 and *Pseudomonas aeruginosa* ATCC 9027), Gram-positive (G+)

bacteria (*Bacillus subtilis* ATCC 6633 and *S. aureus* ATCC 6538) and yeast (*C. albicans* ATCC 10231) with MIC values of 0.6–4.4 mM. The SAR studies demonstrated that the lengths of the alkyl chains of the triazolium salts are critical to their antibacterial and antifungal activities.

In 2013, Zhou et al.[32] synthesized a series of compounds containing 1,2,4-triazolium structures and examined their bioactivities. The 1,2,4-triazolium salts **20** to **23** bearing 3,4-dichlorobenzene and 2,4-difluorobenzene groups have good antimicrobial effects on all tested strains. The MIC values of the thiotriazolium compounds **24** and **25** against *C. albicans* were both 4 μg/mL. The MIC value of the compound **25** against *Candida mycoderma* was 4 μg/mL.

A short time later, they[33] synthesized a set of benzimidazole-derived naphthalimide triazolium salts and took bioassays for diverse microbial *in vitro*. The results revealed that the triazoliums **26** and **27** containing a 3-fluorobenzene moiety had good antibacterial activities against all tested bacteria strains with MIC values of 2–19 μg/mL. And these compounds were nontoxic to PC12 cell lines at the concentration of 128 μg/mL. In addition, Compound **27** has broad-spectrum antifungal activities against *C. albicans*, *C. mycoderma*, *Candida utilis*, *Saccharomyces cerevisiae*, and *Aspergillus flavus*, with MIC values of 4, 4, 14, 4 and 4 μg/mL, respectively.

2.4. Anticancer activities of mono-1,2,4-triazolium salts

In 2013, Yang and co-workers[5] prepared a series of 2-phenyl-3-alkylbenzofuran triazolium compounds and investigated there *in vitro* anticancer activities (Figure 5). The IC_{50} (50% inhibiting concentration) values of Compound **28** against five strains of human tumor cell lines, including HL-60, SMMC-7721, A549, MCF-7, and SW480, ranged from 1.86 μM to 3.11 μM, which were better than all the other 2-phenyl-3-alkylbenzofuran triazolium compounds they prepared in their study.

3 Years later, they[20] studied a novel set of dibenzo[*b*,*d*]furan-1*H*-1,2,4-triazolium salts in the in vitro anti-tumor activities against five tumor cell lines. Compound **29** showed selective activities against leukemia (HL-60) and breast carcinoma (MCF-7) cell lines with the IC_{50} values of 0.80 and 1.76 μM, respectively. Compound **29** can induce cell cycle arrest at G2/M phase and apoptosis in SMMC-7721 cells. The introduction of a benzyl, 4-bromobenzoyl or naphthylacyl group onto the 1,2,4-triazolium ring at the 4-position was assumed significant in the cytotoxicity of these types of molecules (Figure 5).

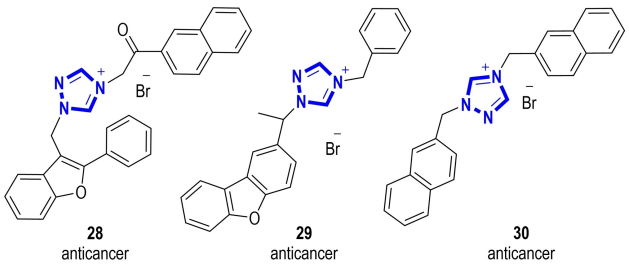

Figure 5. Structures of mono-1,2,4-triazolium salts with anticancer activities.

A series of *N,N′*-bis-substituted 1,2,4-triazolium salts with functional groups at the N1- and N4-position were synthesized by Shelton et al.[34] in 2019. The inhibitory effect of the triazolium salt **30** showed an IC_{50} value of 15.79 μM against breast cancer cells, which was stronger than that of the control drug Tamoxifen (IC_{50} = 22.5 μM). Furthermore, **30** exhibited aqueous solubility and overall hydrophilicity.

2.5. Other bioactivities of mono-1,2,4-triazolium salts

Early in 1987, Bélai et al.[7] prepared the 1,2,4-triazolium salt **31** and found a promising anti-ecdysteroid effect in worms (Figure 6). Compound **31** can not only lead to the delay in pupariation but also cause deaths due to its toxicity at a dose of 0.8 nmole/spec in *Sarcophaga bullata*. Mechanistic studies indicated that the compound **31** could inhibit the biosynthesis of the 20-hydroxyecdysone in worms.

In 2003, their group[35] synthesized the quaternary 1,2,4-triazolium salt **32** and demonstrated its anti-ecdysteroid activity through the *in vivo* tests with the red cotton bug *Dysdercus cingulatus*. Compound **32** could delay the ontogeny of worms and cause developmental abnormalities through interfering the moulting hormone system.

In addition, the 1,2,4-triazolium salts have shown inhibition effects on the growth of protozoans. For instance, Szarek and co-workers[6] synthesized the 1,2,4-triazolium salt **33** in 2009 and found excellent anti-plasmodium activity (IC_{50} = 0.1 μM) against *Plasmodium falciparum* and excellent selectivity (SI = 1430) between *P. falciparum* and CHO cell cultures with this compound (Figure 7).

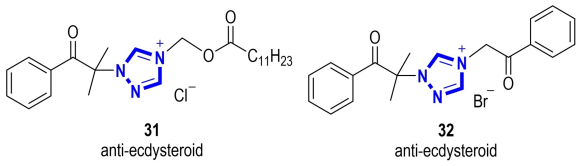

Figure 6. Structures of mono-1,2,4-triazolium salts with anti-ecdysteroid activities.

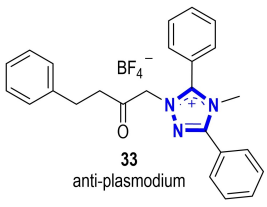

Figure 7. Structure of mono-1,2,4-triazolium salts with anti-plasmodium activities.

3. Bioactivities of Bis-1,2,4-Triazolium Salts

3.1. Bis-1,2,4-triazolium salts with antibacterial activities

In 2012, Zhou and co-workers[36] reported that the bis *N*-hexyltriazolium salt **34** could effectively inhibit the growth of *C. albicans* at a concentration of 2 μg/mL (Figure 8). The inhibitory activities of the hexyltriazolium **34** against *S. aureus* (MIC = 2 μg/mL), *E. coli* (MIC = 1 μg/mL) and *P. aeruginosa* (MIC = 4 μg/mL) were better than that of the control drug Chloramphenicol. In addition, the bis-triazolium salt **34** exhibited excellent antifungal activities with the MIC values of 2–16 μg/mL, which are lower than those of the control drug Fluconazole (0.5–4 μg/mL).

In 2019, Boisbrun et al.[37] prepared a new series of bis-1,2,4-triazoliums compounds and identified the compound **35** with the strongest antibacterial activities with the MIC values ranging from 0.5 μg/mL to 2 μg/mL, and the highest inhibition effect to the eukaryotic cells with an IC_{50} value of 1.8 μg/mL.

3.2. Bis-1,2,4-triazolium Salts with Antifungal Activities

Naphthalimide is an important anticancer group, which is composed of a naphthalene skeleton and a cyclic diimide group. In 2011, Zhou and co-workers[11] reported that the compound **36** was 8 to 16 times more active than Fluconazole against *A. fumigatus* (Figure 9). Note that, the salts with linking chains consisted of 3 and 4 carbon units were the most active.

In 2020, Czerniak et al.[38] reported that **37** and **38** showed good biological activities against fungi and bacteria. Compound **38** has the highest activity against *S. sclerotiorum* at a concentration of 10 ppm. In most cases, its efficacy is higher than 90% or equivalent to the control drug Tebu 250 EW (activity equal to 91.7%). Their bioactivities depended mainly on the concentrations of the compounds. Furthermore, the bactericidal activities of the compounds could be increased along with the length of the linking chain. In most cases, the compounds showed excellent anti-pathogenic activities when the linkers contained 8 to 10 carbon units.

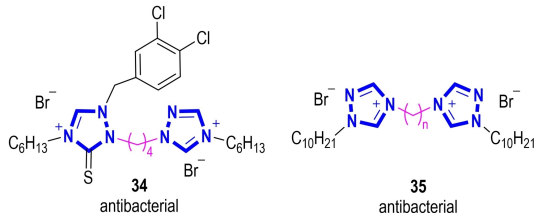

Figure 8. Structures of bis-1,2,4-triazolium salts with antibacterial activities.

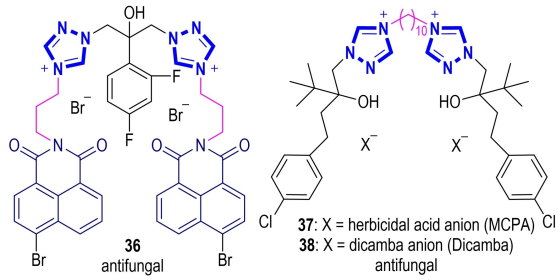

Figure 9. Structures of bis-1,2,4-triazolium salts with antifungal activities.

3.3. Bis-1,2,4-triazolium salts possessing both antibacterial and antifungal activities

In 2017, Voloshina et al.[39] reported that the compound **39**, a 1,3-bis(alkyl)-6-methyl uracil derivative containing bis-1,2,4-triazolium fragments linked to the alkyl chain, had good antibacterial activities, antifungal activities and low cytotoxicities (Figure 10). The MIC values against three bacteria strains of *S. aureus*, *Bacillus cereus* and *E. coli* were 2.0, 0.8 and 8.0 μg/mL, respectively. The MIC values against two fungi strains of *C. albicans* and *Trichophyton mentagrophytes* were 3.1 and 0.8 μg/mL, respectively. No cytotoxicity of the compound **39** was found against human erythrocytes and WI-38 cultured cells within the concentration range that could inhibit the growth of bacteria and fungi.

In the same year, Shtyrlin et al.[40] found that the compound **40** could more effectively inhibit the biofilm growth than Fluconazole even at low concentrations. However, treatments with either Fluconazole or **40** did not lead to disruption of preformed biofilms. Compound **40** had potent antifungal activities against several fungal pathogens including *C. albicans*, *Trichophyton rubrum*, *A. fumigatus*, *R. nigricans*, *C. albicans*, *Candida rubrum*, *Candida fumigatus* and *Candida niger* with the corresponding MIC values ranging from 1.5 μg/mL to 6.25 μg/mL. In addition, Compound **40** showed good antibacterial activities against G+ and G- bacteria with MICs falling in the range of 1 μg/mL to 32 μg/mL, which were comparable or better than those of the control drugs Benzalkonium chloride and Miramistin. In contrast, under the same experimental conditions, Fluconazole was inactive or moderately active against the fungal pathogens. Cytotoxicity studies on human skin fibroblasts and embryonic kidney cells showed that **40** was

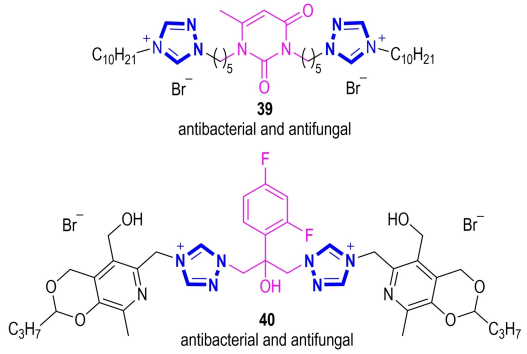

Figure 10. Structures of bis-1,2,4-triazolium salts with antibacterial and antifungal activities.

much less toxic than Mithramycin and Benzalkonium chloride, but was more toxic than Fluconazole and Terbinafine.

3.4. Bis-1,2,4-triazolium salts with anti-plasmodium activities

In 2011, Szarek et al.[41] reported that the bis-1,2,4-triazolium compound **41** was highly active against *P. falciparum* in both *in vivo* and *in vitro* experiments (Figure 11). The IC_{50} value was 8 ± 2 nM against *P. falciparum* and the SI value between *P. falciparum* and normal CHO cells ($IC_{50}=52\pm9$ μM) was 6500. Meanwhile, it exhibited excellent curative activities in the mice infected with Plasmodium.

4. Bioactivities of Metal/NHC Complexes

In 2019, Budagumpi and co-workers[42] prepared a series of silver(I)/1,2,4-triazolium complexes [NHC–Ag–NHC]$^+PF_6^-$ and investigated their bioactivities in cancer treatment. Compound **42** exhibited great anticancer potentials with GI_{50} (growth inhibition of cells by 50%) values of 0.3540 ± 0.032 and 8.5983 ± 0.98 mM obtained against the MCF 7 and HT-29 cell lines, respectively (Figure 12).

Recently, it was found that the silver(I) NHC complexes [NHC–Ag–NHC]$^+PF_6^-$ had strong antioxidant and antihaemolytic properties. Budagumpi et al.[43] synthesized some silver–NHC complexes with 1,2,4-triazoliums through in situ deprotonation strategies. Bis-NHC coordinated silver hexafluorophosphate complexes **43** to **46** displayed 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities with the IC_{50} values ranging from 61 μM to 131 μM. In addition, the antihaemolytic activities of these compounds were carried out at the concentration of 100 μM, and the results showed the lysis of red blood cells with the lowest rate of 2.32 ± 0.05%.

In 2020, the group[44] synthesized the complexes **47** and **48** and demonstrated their promising antioxidant activities. The IC_{50} values of the complexes **47** and **48** were 7.42 ± 0.125 and 7.49 ± 0.702 μM, respectively.

Very recently, Jin et al.[45] synthesized a novel set of Ag–NHC supramolecular assemblies and evaluated their bioactivities via the assay of CCK-8 cancer cell lines. The bioassay results showed that the Ag–NHC supramolecular complex **49** could significantly inhibit the growth of breast cancer cells at a concentration of 5×10^{-5} mol/L.

Figure 11. Structure of bis-1,2,4-triazolium salts with anti-plasmodium activities.

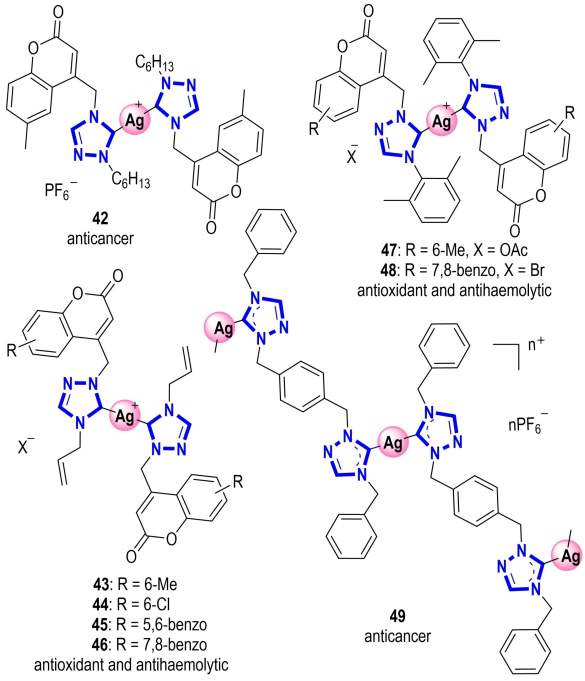

Figure 12. Structures of silver–NHC complexes possessing both anticancer, antioxidant and antihaemolytic activities.

5. Structure-Activity Relationship of 1,2,4-Triazolium Salts

The triazolium cation, alkyl chain, substitution pattern, and the linkers of triazolium rings are several important factors that can greatly affect the bioactivities of the triazolium salt derivatives. It has been proven that different anions of triazolium salts possessed different inhibitory effects on different pathogenic species. However, the study on the effect of anion types on biological activities is still rare.

5.1. Effects of the triazolium electronic properties on bioactivities

Of all the structural factors in triazolium compounds, the electronic properties of the triazolium cations may have the greatest influence on the bioactivities of the compounds. The introduction of triazolium cations can greatly enhance the antibacterial,[29,30,32] antifungal,[18,27] anticancer,[5,20,34] and anti-plasmodium[6] activities of the bioactive compounds. The triazolium cation group with localized positive charge can easily interact with anionic components such as glucans, mannans, proteins, and the lipids on the microbial cell wall to form electrostatic interactions to exhibit antimicrobial activities.[46] The electrostatic interactions between triazolium salts and bio-anion components have a greater impact on microorganisms than the H-bond interactions between triazole and biomolecular targets, which leads to higher inhibition effects of triazolium salt derivatives than electroneutral triazole-containing molecules.[11,27,46] For instance, the results of molecular docking

studies of the compound **10** showed that forming a strong hydrogen bond between its triazolium cation and the heme group of enzymes, which reflected a higher inhibitory activity against fungi than the triazole analogs.[27] In addition, poly-cationic derivatives exhibit stronger activities than commonly used the mono-cation compounds and are less prone to resistance.[45] The compound **49** bearing four triazolium cationic groups showed good anticancer activity against break cancer MCF-7 cells.[45] With the introduction of the thione moiety into the triazolium salt, a sulfur–triazolium salt is formed and the antibacterial activities can be significantly enhanced.[36]

However, the introductions of triazolium cationic units in not all compounds can play a positive role in biological activity. For instance, when electropositive triazoliums were introduced into 3′-azido-3′-deoxythymidine derivatives, all target compounds were negatively effective against Human Immunodeficiency Virus (HIV).[16] Therefore, the introduction of cationic triazolium groups should match with the active functional sites on both the bioactive structures and the pathogen cell membranes.

5.2. Effects of the alkyl chain lengths on bioactivities

Alkyl chain lengths mainly affect the lipophilicity of these compounds.[47] The lipophilic alkyl chain may be beneficial to the electrostatic interactions between the triazolium salt group and the molecule on the cell membrane to enhance the antimicrobial activities.[46,47] However, the mutual balance of lipophilicity and hydrophilicity is the key to high activities, such as the compound **17** and **19**[3,11,19] This balance can be regulated through adjusting hydrophobic substituents of the molecule.[3,19,30] Compounds **16**/**17**,[19] and **18**/**19**[3] with different length alkyl chains reflect different biological activities. Therefore, the appropriate length of the alkyl chain (strong lipophilicity) is more conducive to the biological activity of triazolium salts.[3,19]

In addition, the electron-donating effect of alkyl chains is also an important reason for the improvement of the activity of triazolium salts.[47]

5.3. Effects of the substitution patterns on bioactivities

1,2,4-Triazolium salt groups have four substitutional sites. Attaching different substituents at different sites can affect the bioactivities and selectivity of the triazolium salt compounds. For instance, the *N*,*N*′-bis-substituted 1,2,4-triazolium salt **30** bearing naphthalene methyl substituents at the N1- and N4-position showed the best anticancer activity against breast cancer cells.[34] Besides, 1,2,4-triazolium compound **33** containing a 4-phenylbutan-2-one group at N3- position exhibited excellent selectivity with SI approach 1430, which was higher than the SI of other compounds.[6]

5.4. Effects of the linkers between triazoliums on bioactivities

Alkyl linkers of the bis-triazolium derivatives have significant effects on their antimicrobial activities. In general, alkyl linkers, typically containing 3–12 carbon units, provide the most effective biological activities.[36–38,41,48] For example, among the bis-1,2,4-triazolium derivatives synthesized by Zhou et al., the compound **39** containing a $(CH_2)_3$ linker showed the best antibacterial effect.[39] Moreover, the 1,2,4-triazolium **40** containing a $(CH_2)_3$ linker showed the best antibacterial and antifungal activities compared with the other compounds possessing similar functional groups.[40] Alkyl chain linkers are significant in the modulation of the lip/water partition of the compounds, thereby enhancing the antimicrobial activity and spectrum of the triazolium derivatives.[11,36,40,41]

6. Conclusions and Perspectives

1,2,4-Triazolium-based compounds have shown great potential in agrichemical and medicinal development as antibacterial, antifungal, anticancer, and anti-plasmodium agents with good bioactivity, high selectivity and biocompatibility. 1,2,4-Triazolium derivatives also showed better activity, water-solubility and lower resistance possibility as prodrugs than the corresponding 1,2,4-triazole compounds. At present, the triazolium compounds that have been successfully marketed and widely used in clinical therapies are still limited. Therefore, there is plenty of room for the future development of novel pesticides and human medicines with triazolium salt derivatives.

One of the key reasons for the excellent bioactivities of the 1,2,4-triazolium salt derivatives is the strong electrostatic interactions between the positively charged triazolium cations and the negatively charged functional groups that existed on the cell membranes of microbial. The strong interactions also make it less possible for pathogens to develop drug resistance. Therefore, the application of a variety of triazolium salts as prodrugs might be an effective way to solve the problem of triazole resistance. In addition, multiple triazolium rings connected with an alkyl linker may significantly enhance the bioactivities. However, the action mechanism of triazolium salts is still far from clear. Extensive studies through molecular docking, bioinformatics and other methods are still needed to elucidate the mechanisms of the bioactivities of various triazolium salts. In summary, the development and applications of 1,2,4-triazolium salt derivatives in the development of both agrochemicals and pharmaceuticals are attractive.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Keywords: anticancer · antifungal · antimicrobial · antiprotozoal · NHC precursors · 1,2,4-triazolium

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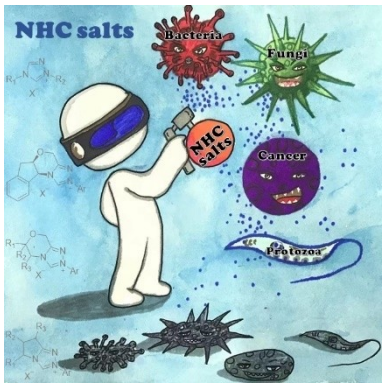
CONCEPT

Biological activity: 1,2,4-Triazolium salt derivatives have been extensively studied as *N*-heterocyclic carbene (NHC) catalysts and ligands. Meanwhile, it has also shown a wide range of highly effective bioactivities, including the activities of antibacterial, antifungal, anticancer, anti-plasmodium and even worm growth regulation. The development and applications of 1,2,4-triazolium salts in the development of both agrochemicals and pharmaceuticals are attractive.



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1 – 10

Bioactivities of Triazolium-Based *N*-Heterocyclic Carbene Salts