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Synthesis and evaluation of indole-based new scaffolds for antimicrobial activities—Identification of promising candidates

Palwinder Singh [a,*], Puja Verma [a], Bhawna Yadav [b], Sneha S. Komath [b]

[a] Department of Chemistry, Guru Nanak Dev University, Amritsar 143 005, India
[b] School of Life Sciences, Jawaharlal Nehru University, New Delhi 160 067, India

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ABSTRACT

Search for new antimicrobial agents led to the synthesis of series of *N*-1, *C*-3 and *C*-5 substituted bis-indoles. Their evaluation for antifungal and antibacterial activities resulted in the optimization of pyrrolidine/morpholine/*N*-benzyl moiety at the *C*-3 end and propane/butane/xylidine groups as linkers between two indoles for significant inhibition of microbial growth. Preliminary investigations have identified three highly potent antimicrobial agents. Dockings of these molecules in the active sites of lanosterol demethylase, dihydrofolate reductase and topoisomerase II indicate their strong interactions with these enzymes.

The increased emergence of microbial infections has led to massive increase in the rate of mortality, especially in the immunocompromised individuals, those suffering from tuberculosis, cancer or AIDS. Fungal and bacterial infections constitute large proportion of the infectious diseases resulting in 13 million deaths each year worldwide.[1] Although a number of antimicrobial drugs are available, the smartness of microbes in developing mutant strains, emergence of drug resistance,[2–6] lack of specificity and narrow spectrum are major obstacles in current microbial therapy. Hence the development of more efficacious, safe and target specific new antimicrobial agents is an issue of current medicinal importance. As per the present status; most of the currently used antimicrobial drugs (Chart 1) target the microbe either at plasma/cell membrane or ribosomes or DNA. Amphotericin B,[7] naftifine,[8,9] terbinafine,[9,10] miconazole,[11] fluconazole[12] target the plasma membrane; caspofungin,[13] ampicillin,[14] cefepime[15] disturb cell wall biosynthesis; clarithromycin,[16] linezolid[17] bind to ribosomes and hinder protein biosynthesis while flucytosine,[18] norfloxacin,[19] trimethoprim[20] block DNA replication. Further exploration of these drugs revealed that: (1) antifungal drugs of azole class interact with iron(II) of protoporphyrin IX in the active site of lanosterol demethylase[21], (2) trimethoprim and its analogues show H-bond interactions with nitrogens of arginine in the active site of dihydrofolate reducatse[22] and (3) quinolone class of antibacterial drugs interact at carbonyl oxygen and nitrogen of serine in the active site of topoisomerase II.[23] Despite a number of antimicrobial agents[24–27] being reported, new compounds are required to meet increasing demands for managing infections in the complex patient population.

Since selective toxicity is a fundamental law for the development of anti-infective agents- destroying only one form of life (microbe) without harming the host, it was kept in mind to develop new molecules with non-toxicity to host. As indole carries a unique place in the biological systems playing crucial roles in the form of amino acid, growth hormone and alkaloids and its hydrophobic nature[28] makes the tryptophan to be present at or near the catalytic/molecular recognition sites of enzymes; a library of *N*-1, *C*-3 and *C*-5 substituted bis-indoles was procured. Some bis- and tris-indole alkaloids isolated from marine organisms, with significant biological activities have been reported.[29,30] 2-(1*H*-Indol/5-bromo-1*H*-indol-3-yl)-2-oxoacetyl chloride **(2)**[31,32] was obtained from the reaction of 1*H*-indole/5-bromo-1*H*-indole **(1)** with oxalyl chloride at 0–5 °C using dry ether as the reaction medium. Treatment of **2** with various amines in presence of K_2CO_3 in acetonitrile gave compounds **3a–g** in 53–89% yield which on further reaction with 0.5 equiv of alkane dibromides in presence of NaH in dry acetonitrile at 0'–5 °C gave target compounds **4a–o** in 16–30% yields (Scheme 1) along with the formation of compounds **5** (2–5%) and **6** (4–8%). Unreacted **3** (15–20%) was also recovered from these reactions. Percentage yields of compounds **4** did not increase on increasing the equivalence of alkyl dibromides to 1 w.r.t. **3**. Moreover, amount of **5** and **6** builds up if more time was given to the reaction. To see the effect of decreased flexibility between the two indole rings on their biological profile, they were linked through xylyl group. Reaction of compounds **3** with

* Corresponding author. Tel.: +91 183 2258802 09x3495; fax: +91 183 2258819.
E-mail address: palwinder_singh_2000@yahoo.com (P. Singh).

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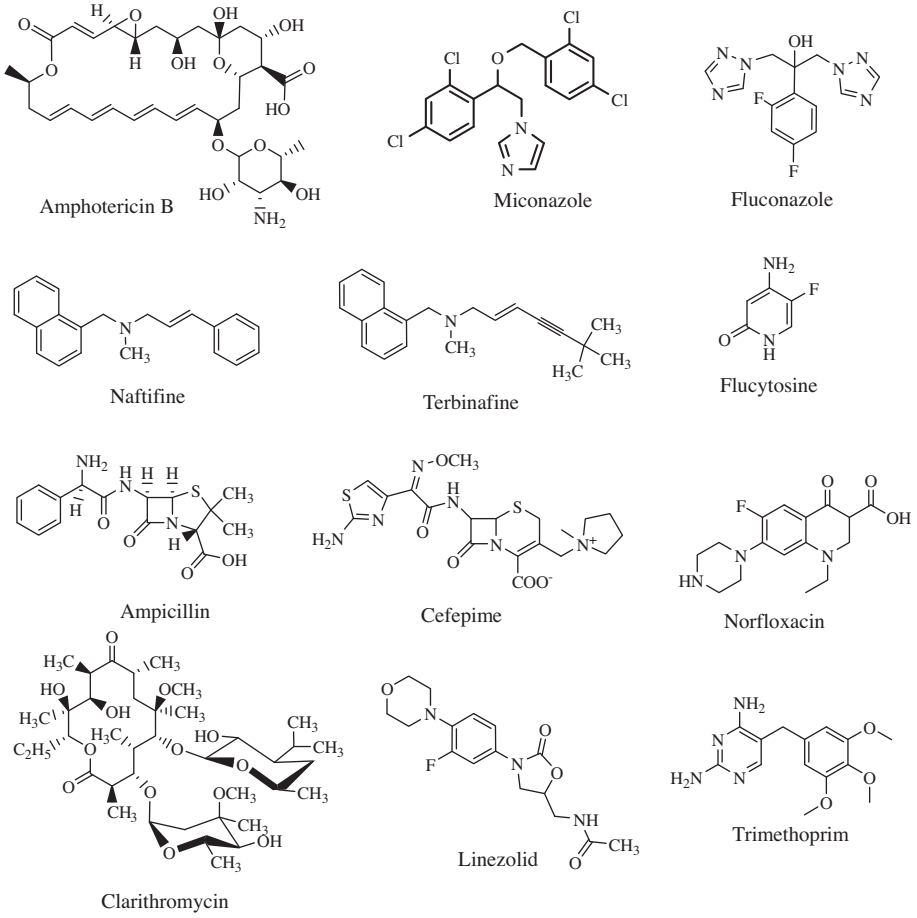

Chart 1. Antimicrobial drugs.

α,α′-dibromo-*p*-xylene using NaH as base provided compounds **7a**–**f** (35–55%) (Scheme 1). It was observed that reactions of **3** with α,α′-dibromo-*p*-xylene were faster than the reactions of **3** with alkyl dibromides. All the compounds were characterized by NMR spectra, mass spectra and CHN analysis.[33]

Antifungal activities of these compounds were determined on *Candida albicans* in solid as well as liquid phase using disc diffusion and 96 well plate assays, respectively. Disc diffusion assay was performed in YEPD medium either alone or in presence of cell wall disrupting agents (SDS, CR, CFW).[34,35] A specific number of cells of *C. albicans* was spread over the medium and discs of 0.5 cm diameter were placed over it. Solution of test compound and reference solvent were spotted over the discs. After incubation at 30 °C, the diameter of inhibition zone was measured (Table 1, *Fig.* S1). We also tested whether the compounds would enhance the toxic effect of Rhodamine 6G (R6G), an antifungal drug and specific substrate of two ATP-dependent drug pumps of *C. albicans*, Cdr1p and Cdr2p.

Compound **4f** showed excellent fungal growth inhibitory profile, having higher zone of inhibition alone as well as in combination with CFW, CR, SDS than the solvent control besides enhancing the toxic effects of R6G. Compound **7d** showed most promising inhibition of fungal growth (12.5 mm zone of inhibition) with 4 mm higher inhibition zone in comparison to the solvent (8.5 mm). Other compounds contributing to the toxicity of R6G were **4a**, **4b**, **4c**, **4d**, **4g**, **4i**, **4j**, **4o** and **7b**. Compounds **4b**, **4c**, **4d**, **4j**, **4l**, **7b** and **7d** proved to be antifungal candidates with their own inhibitory profiles as well as in combination with one or two of the cell wall disruptants while compounds **4a**, **4i**, **4n**, **4o** and **7a** inhibited the fungal growth only in combination with cell wall disruptants. There was no noticeable zone of inhibition in case of compounds **4e**, **4h**, **4k** and **7c**. Therefore, amongst a series of indole derivatives, compounds **4b**, **4d**, **4f**, **4j** and **7d** were found to inhibit the growth of *C. albicans* either alone or in combination with cell wall/plasma membrane disrupting agents. It was observed that compounds with piperidine moiety at the end of C-3 substituent do not exhibit much antifungal activities. Better antifungal activity of compound **4f** than that of **4b**, which differ only in the length of spacer group between two indoles, indicates the role of spacer group as well. However, further increase in the chain length of the linker in case of compounds **4i** and **4l** did not improved their antifungal activities. Replacement of pyrrolidine moieties of **4b** with benzylamine improved the antifungal activity of compound **4d** while its further modification resulted in much better antifungal profile of compound **7d**. Hence, a proper combination of the amine at *C*-3 with linkage group between two indoles is desirable for an appreciable fungal growth inhibition. Remarkably, fungal growth inhibition zone diameter of compound **7d** is comparable to that of miconazole. Compounds **4b**, **4d**, **4f**, **4j** and **7d** exhibit IC_{50} 465 μM, 121 μM, 78 μM, 340 μM and 20 μM, respectively (Table 1).[36]

Antibacterial activities of compounds under present investigation were tested using *Escherichia coli* strain, having an ampicillin resistance gene encoding plasmid. The results in terms of inhibition zone diameter (mm) are given in Table 2 and the pictures of inhibition zones in comparison with the solvent system are shown in Figure S2.

It was observed that presence of compounds **4a**, **4b**, **4d**, **4k** and **4m** in the culture medium increased the zone inhibition diameter