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

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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

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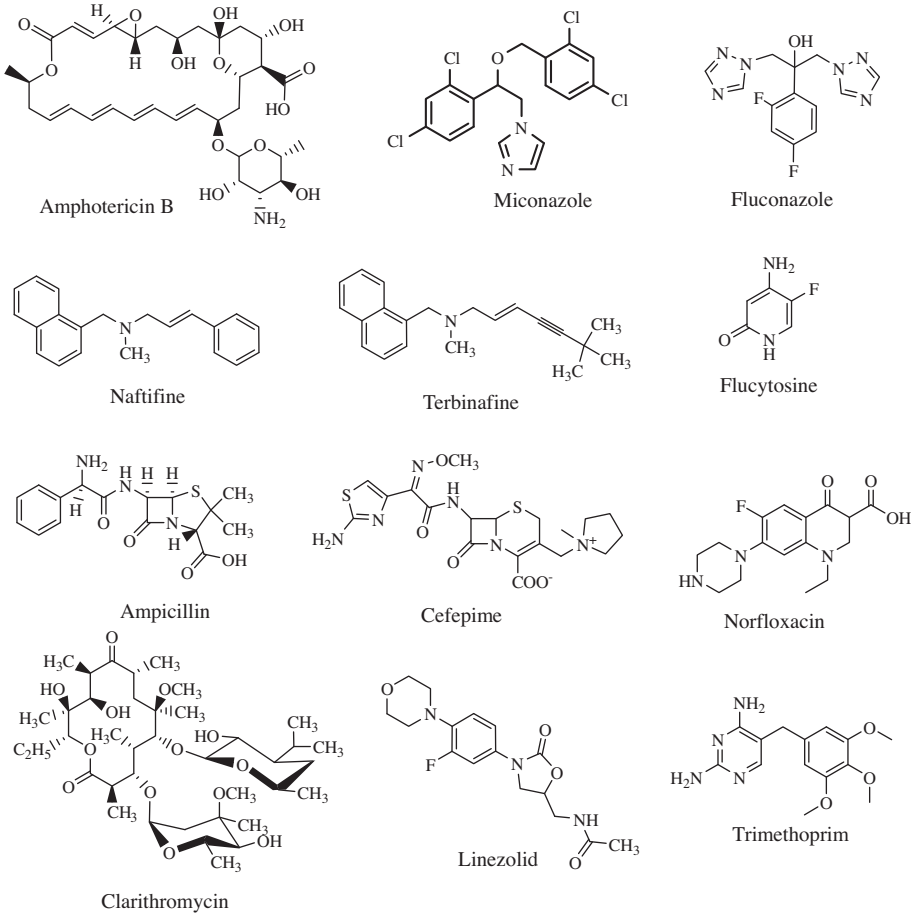


Chart 1. Antimicrobial drugs.

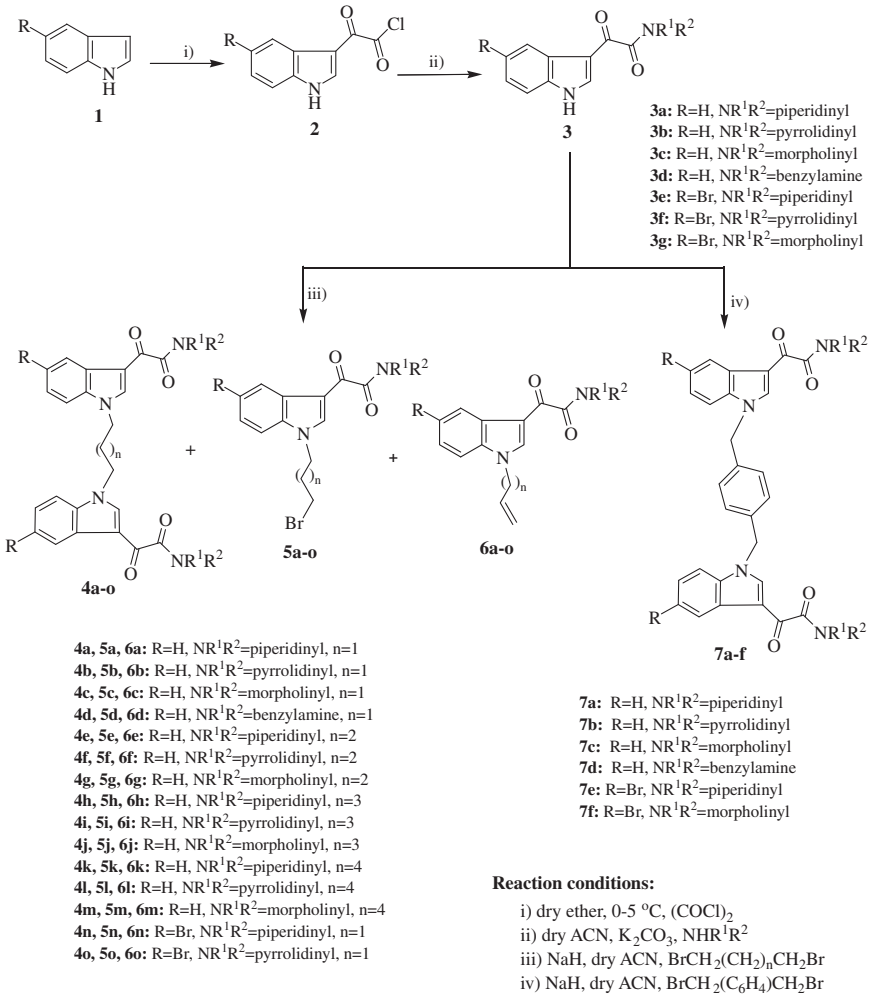
α,α′-dibromo-*p*-xylene using NaH as base provided compounds **7a**–**f** (35–55%) (Scheme 1). It was observed that reactions of **3** with α,α′-dibromo-*p*-xylene were faster than the reactions of **3** with alkyl dibromides. All the compounds were characterized by NMR spectra, mass spectra and CHN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



Scheme 1.

Table 1
Candida albicans growth inhibition

Compd (concn μg/ml)/solvent	YEPD	YEPD+CFW(100 μg/ml)	YEPD+CR (150 μg/ml)	YEPD+SDS (0.01%)	YEPD+R6G(20 μg/ml)	IC_{50} (mM)
4a (374)	–	–	6.5	7.5	6.5	–
Solvent[a]	6.5	7.0	7.0	7.0	6.0	
4b (428)	7.5	6.5	5.5	6.5	6.5	0.465
Solvent[b]	6.5	6.0	7.0	6.0	6.0	
4c (375)	7.0	6.5	8.0	8.5	7.5	561.6
Solvent[a]	6.5	7.0	7.0	7.0	6.0	
4d (250)	9.0	7.0	8.0	8.5	7.0	0.121
Solvent[a]	8.0	9.0	9.0	7.5	6.0	
4e (150)	6.0	6.0	6.0	6.5	–	566
Solvent[a]	6.5	6.5	7.5	6.5	7.0	
4f (375)	7.5	7.5	8.0	8.0	7.5	0.078
Solvent[a]	6.5	7.0	7.0	7.0	6.0	
4g (250)	6.0	7.5	5.0	7.0	7.5	365
Solvent[a]	6.5	6.5	7.5	6.5	7.0	
4h (450)	6.0	6.5	7.0	6.5	6.5	874.7
Solvent[a]	6.5	6.5	7.5	6.5	7.0	
4i (375)	5.5	5.5	8.0	6.0	6.5	627.1
Solvent[a]	6.5	7.0	7.0	8.0	6.0	
4j (375)	7.5	7.5	7.0	7.0	8.0	0.340
Solvent[a]	6.5	6.5	7.5	6.5	7.0	
4k (238)	6.5	–	6.0	6.5	5.5	163.7
Solvent[b]	7.5	7.0	7.5	7.0	6.0	
4l (545)	14.5	11.0	10.5	15.5	12.5	–
Solvent[c]	13.5	15.0	12.5	15	14.5	
4m (357)	5.5	6.5	6.5	–	–	553.3

(*continued on next page*)

Table 1 (*continued*)

Compd (concn μg/ml)/solvent	YEPD	YEPD+CFW(100 μg/ml)	YEPD+CR (150 μg/ml)	YEPD+SDS (0.01%)	YEPD+R6G(20 μg/ml)	IC_{50} (mM)
Solvent[b]	6.0	6.0	6.5	6.0	6.5	
4n (500)	6.0	6.5	9.5	8.0	7.5	401
Solvent[b]	7.5	7.0	7.0	7.5	9.5	
4o (257)	11.5	11.5	11.5	11.5	12.0	29.2
Solvent[b]	11.5	12.5	11.5	11.5	11.0	
7a (300)	—	—	7.5	7.5	6.0	407
Solvent[b]	8.0	6.0	5.0	8.0	6.5	
7b (187)	7.5	6.5	7.0	8.0	7.0	363.3
Solvent[a]	6.5	7.0	7.0	7.0	6.0	
7c (250)	6.5	6.0	6.0	6.5	6.0	376
Solvent[a]	6.5	6.5	7.5	6.5	7.0	
7d (600)	12.5	11.5	10.5	12.5	11.0	0.02
Solvent[b]	8.5	11.0	13.0	12.0	11.0	
Miconazole (300)	12.0	—				
Solvent[d]	7.0	—				
Fluconazole (300)	15.0	—				
Solvent[d]	7.0	—				

Diameter (mm) of zone of inhibition in disc diffusion assay and IC_{50} of compounds **4** and 7.
[a] DMSO.
[b] DMSO+acetone.
[c] Acetone+$CHCl_3$.
[d] CH_3OH.

Table 2
Anti-bacterial activity

Comp (concn μg/ml)/solvent	Inhibition zone diameter (mm)	Comp (concn μg/ml)/solvent	Inhibition zone diameter (mm)
4a (250)	9.5	**4k** (238)	7.5
Solvent[a]	8.5	Solvent[b]	6.5
4b (428)	8.0	**4l** (545)	11.5
Solvent[b]	7.0	Solvent[c]	13.0
4c (500)	6.0	**4m** (357)	7.5
Solvent[a]	10.0	Solvent[b]	6.5
4d (250)	12.0	**4n** (500)	9.0
Solvent[a]	10.0	Solvent[b]	11.0
4e (300)	10.5	**4o** (257)	13.5
Solvent[a]	10.0	Solvent[b]	9.5
4f (500)	10.5	**7a** (300)	9.0
Solvent[a]	10.0	Solvent[b]	8.5
4g (333)	10.0	**7b** (187)	7.0
Solvent[a]	9.5	Solvent[a]	8.5
4h (450)	8.5	**7c** (250)	10.5
Solvent[a]	10.0	Solvent[a]	10.0
4i (500)	8.0	**7d** (600)	9.0
Solvent[a]	10.0	Solvent[b]	10.0
4j (375)	8.5		
Solvent[a]	9.5		

Diameter (mm) of zone of inhibition of compounds **4** and **7** on LB-Amp agar plate.
[a] DMSO.
[b] DMSO+acetone.
[c] Acetone+chloroform.

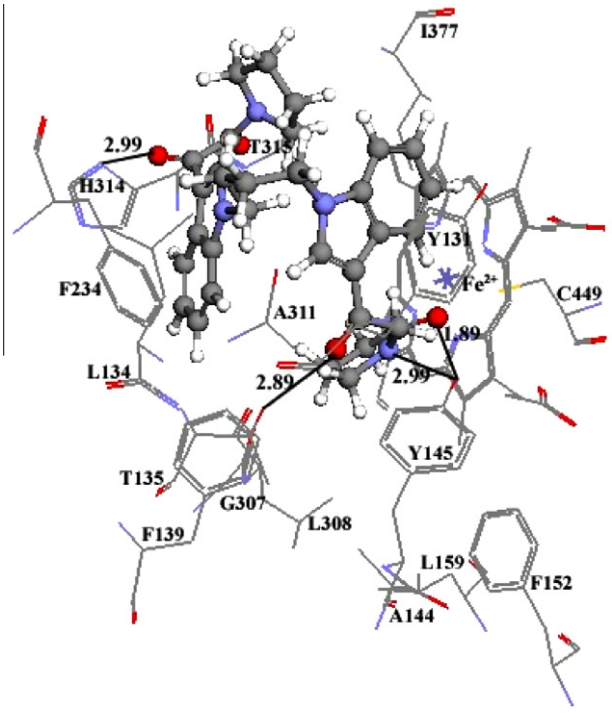


Figure 1. Compound **4f** docked in the active site of lanosterol demethylase. H-bond distances are given in Å. Hs' are omitted for clarity.

by 1 mm, while that of compounds **4e**, **4f**, **4g**, **7a** and **7c** increased the zone inhibition diameter by 0.5 mm in comparison to the solvent controls. Compound **4o**, with an increase of 4 mm in inhibition zone diameter was found to be most promising candidate for antibacterial activity. Comparison of antibacterial activity of **4b** and **4o** indicates that presence of 5-Br substituent in **4o** might be responsible for higher antibacterial activity of this compound. No noticeable zone of inhibition was observed in case of other compounds. Results showed that antibacterial properties of these compounds are less sensitive to the changes in *C*-3 substituents than the antifungal ones. It was observed that compounds **4e**, **4f** and **4g** with four carbon spacer between two indoles have equal antibacterial activity while compounds **4h**, **4i** and **4j** with five carbon spacer did not show antibacterial activity. Significant inhibition zone diameters of compounds **4d** (12 mm) and **4o** (13.5 mm) which are comparable to the inhibition zone diameters of clinically used antibacterial drugs apalcillin and piperacillin (⩽12 mm to ⩽17 mm)[37] indicates the suitability of these compounds as antibacterial agents.

Although the cellular target is not defined in the experimental antimicrobial investigations of these molecules, it was desirable to investigate the interactions of the active compounds with the enzymes targeted by clinically used antimicrobial drugs viz., squalene epoxidase, β-1,3-D-glucan synthase, lanosterol demethylase, dihydrofolate reductase, topoisomerase II, trans-peptidase and DNA dependent polymerase. Search of Protein Data Bank showed the availability of crystal structures of lanosterol demethylase,

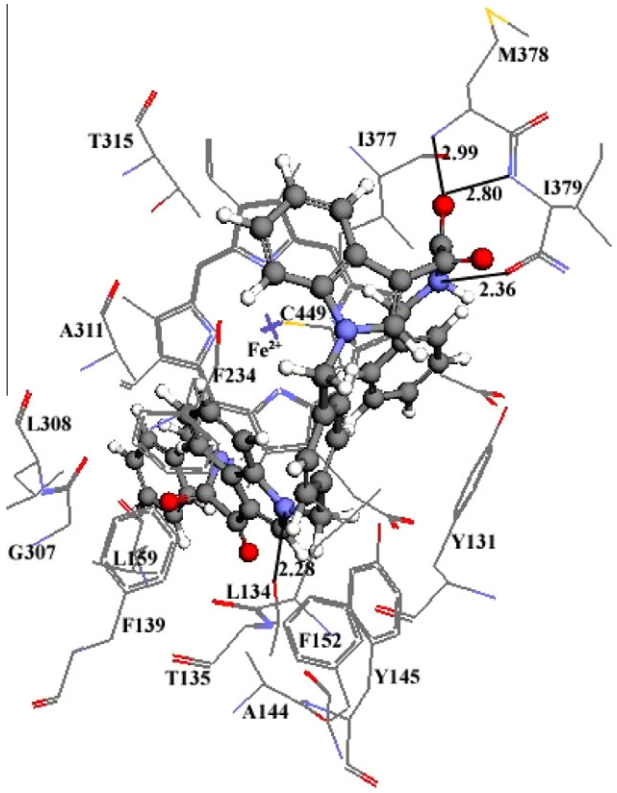


Figure 2. Compound **7d** docked in the active site of lanosterol demethylase. H-bond distances are given in Å. Hs' are omitted for clarity.

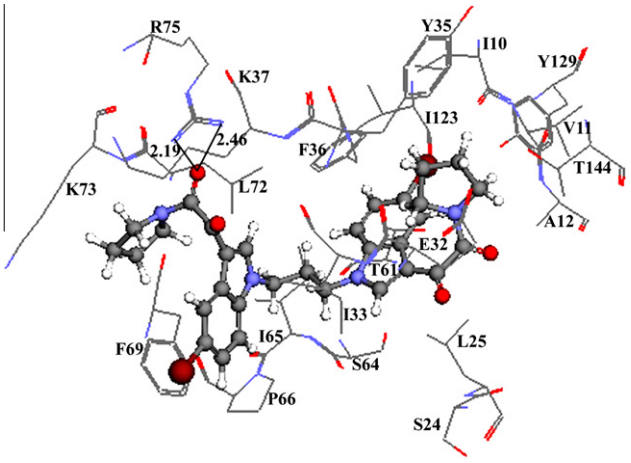


Figure 3. Compound **4o** docked in the active site of dihydrofolate reductase. H-bond distances are given in Å. Hs' are omitted for clarity.

dihydrofolate reductase and topo II in combination with econazole, trimethoprim analogue and dextrazoxazone, respectively. Docking[38] of **4f**, **7d** in the active sites of lanosterol demethylase showed that these compounds interact through H-bond formation between their carbonyl oxygens and H314, Y145, T135 and I379, M378 amino acids, respectively (Figs. 1 and 2). Compound **4f** also showed H-bond interactions through its pyrrolidinyl nitrogen with Y145 and compound **7d** through its amino nitrogen and indole nitrogen with I379 and T135 amino acids, respectively.

Upon docking in the active site of dihydrofolate reductase, compound **4o** showed H-bond interactions through its carbonyl oxygens with R75, just like that of trimethoprim analogue, D2J

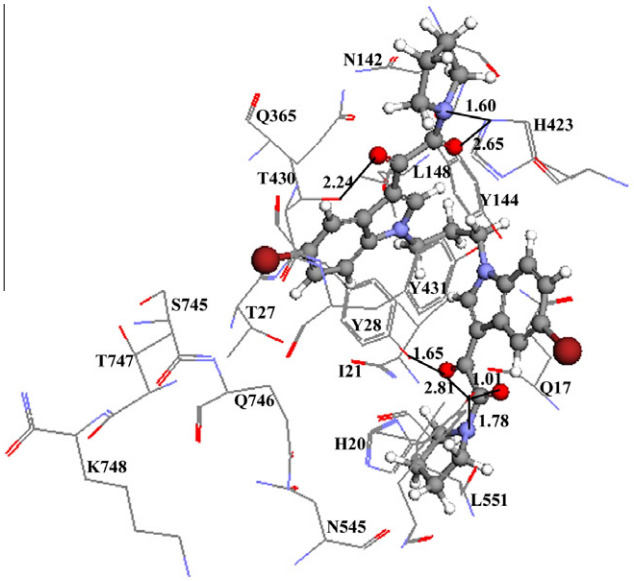


Figure 4. Compound **4o** docked in the active site of topoisomerase II. H-bond distances are given in Å. Hs' are omitted for clarity.

(Fig. 3). In the active site of TOPO II, compound **4o** showed a number of H-bond interactions using all the carbonyl oxygens and pyrrolidinyl nitrogens with T430, H423, Y28 and Y547 amino acids (Fig. 4). Docking results indicate the probability of these compounds to interfere with sterol biosynthesis in *C. albicans* resulting into fungal growth arrest and DNA replication leading to bacterial death.

In conclusion, amongst a series of *N*-1, *C*-3 and *C*-5 substituted indoles, preliminary investigations have identified three highly potent antifungal and antibacterial compounds. Compounds **4o** and **7d** with 4 mm higher zone of inhibition than the solvent control and **4f**, **7d** with IC_{50} 78 μM and 20 μM were found to be most promising antibacterial and antifungal candidates. Work is undergoing to further improve the potencies of these compounds and explore their modes of action.

Acknowledgements

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Supplementary data

Supplementary data (experimental details, ^{1}H NMR spectra of compounds, bio-evaluation procedures, photographs of disc diffusion assays and docking procedure) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.04.001.

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33. *Experimental data for selected compounds*: Compound **4f**: Light brown solid, Yield: 29%; mp 165 °C. IR (KBr, cm^{-1}): 1626 (C=O); UV (THF + HEPES buffer) λ_{max} (ε) 224 (17,320), 251 (22,620), 314 (18,160); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.90 (m, 12H, 6 × CH_2), 3.57–3.65 (m, 8H, 4 × CH_2 of pyrrolidine), 4.11 (m, 4H, 2 × CH_2), 7.24–7.33 (m, 6H, ArH), 8.08 (s, 2H, ArH), 8.35–8.39 (m, 2H, ArH); ^{13}C NMR (normal/DEPT-135) (75 MHz, $CDCl_3$): δ 23.86 (−ve, CH_2), 26.24 (−ve, CH_2), 27.18 (−ve, CH_2), 45.89 (−ve, NCH_2), 46.71 (−ve, NCH_2), 47.38 (−ve, NCH_2), 109.84 (+ve, ArCH), 113.46 (absent, ArC), 122.71 (+ve, ArCH), 123.26 (+ve, ArCH), 123.96 (+ve, ArCH), 126.87 (absent, ArC), 136.57 (absent, ArC), 138.73 (+ve, ArCH), 164.72 (absent, C=O), 184.91 (absent, C=O); Anal. Calcd for $C_{32}H_{34}N_4O_4$: C, 71.35; H, 6.36; N, 10.40. Found C, 71.12; H, 6.11; N, 10.00. MS (FAB) 539 (M^++1).
Compound **7d**: White solid, Yield: 49%; mp 184 °C. IR (KBr, cm^{-1}): 1628 (C=O), 1681 (C=O), 3123 (N–H), 3349 (N–H); UV (THF + HEPES buffer) λ_{max} (ε) 220 (26,220), 259 (19,660), 330 (17,400); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.54 (d, 4H, J = 6 Hz, 2 × NCH_2), 5.33 (s, 4H, 2 × NCH_2), 7.11 (s, 4H, ArH), 7.26–7.27 (m, 4H, ArH), 7.30–7.35 (m, 12H, ArH), 7.84 (br s, 2H, NH), 8.40 (d, 2H, J = 7.5 Hz, ArH), 9.07 (s, 2H, ArH); ^{13}C NMR (normal/DEPT-135) (75 MHz, $CDCl_3$): δ 43.33 (−ve, NCH_2), 50.73 (−ve, NCH_2), 110.49 (+ve, ArCH), 112.46 (absent, ArC), 122.75 (+ve, ArCH), 123.58 (+ve, ArCH), 124.11 (+ve, ArCH), 127.62 (+ve, ArCH), 127.67 (absent, ArC), 127.74 (+ve, ArCH), 128.79 (+ve, ArCH), 135.63 (absent, ArC), 136.38 (absent, ArC), 137.37 (absent, ArC), 141.54 (+ve, ArCH), 162.32 (absent, C=O), 179.93 (absent, C=O); Anal. Calcd for $C_{42}H_{34}N_4O_4$: C, 76.58; H, 5.20; N, 8.51. Found C, 76.80; H, 5.38; N, 8.34. MS (FAB) 659 (M^++1).
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36. *Evaluation of IC_{50}*: Ten serial dilutions of the compounds and the corresponding solvent control were made with synthetic dextrose minimal medium (SDMM) in duplicate in 96-well flat bottom transparent cell culture ELISA plate. *Candida* cells which were already grown in culture medium for 24 h, at 30 °C were resuspended in a 0.9% saline solution to give an optical density of 0.1 at 600 nm (OD_{600}). The cells were then diluted 100 times in SDM medium. 100 μl of diluted cells was added to each well except column 11, which was our media control to monitor contamination during the study. Column 12 contained the growth medium and the fungal cells only to serve as positive control. The plate was incubated at 30 °C for 48 h and OD_{600} was monitored using a micro plate reader. The percent fungal growth inhibition of the compounds were determined with respect to positive control after subtracting the percent inhibition by the solvent control. The IC_{50} values were determined by plotting graph of percent inhibition versus concentration of the compound using Graphpad Prism software.
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