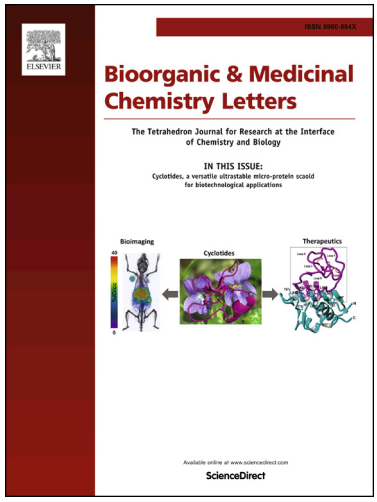

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PII: S0960-894X(18)30201-4
DOI: https://doi.org/10.1016/j.bmcl.2018.03.022
Reference: BMCL 25676

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 6 January 2018
Revised Date: 1 March 2018
Accepted Date: 9 March 2018

Please cite this article as: Veeraswamy, B., Madhu, D., Jitender Dev, G., Poornachandra, Y., Shravan Kumar, G., Kumar, C.G., Narsaiah, B., Studies on synthesis of novel pyrido[2,3-*d*]pyrimidine derivatives, evaluation of their antimicrobial activity and molecular docking, *Bioorganic & Medicinal Chemistry Letters* (2018), doi: https://doi.org/10.1016/j.bmcl.2018.03.022

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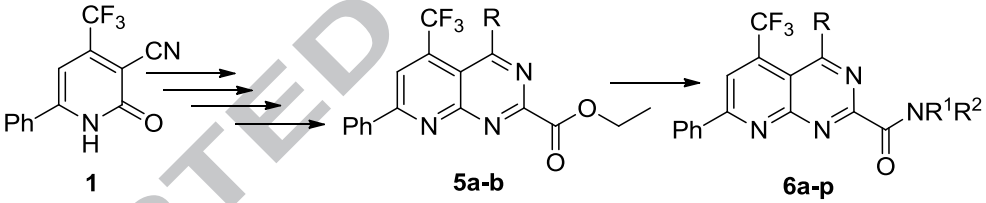

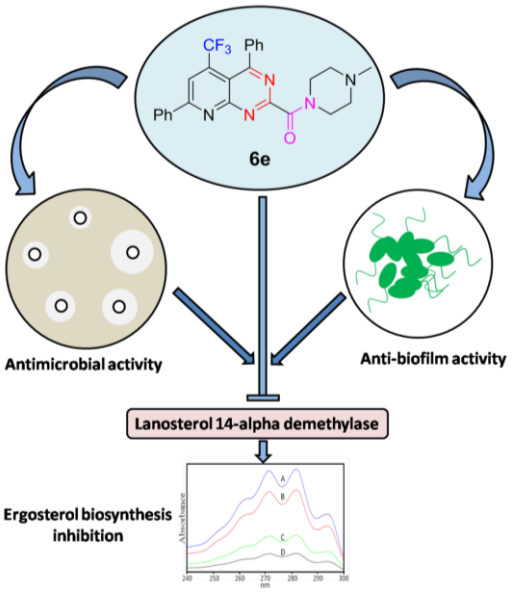

Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

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ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Key words:
Grignard's reaction;
cyclization;
coupling reaction;
antimicrobial activity.

ABSTRACT

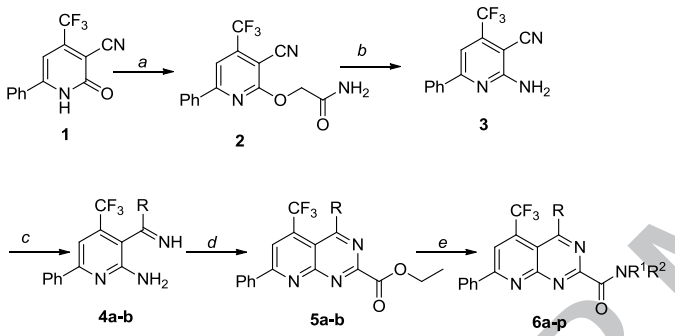
A series of novel pyrido[2,3-*d*]pyrimidine derivatives **6** were prepared starting from 2-amino-3-cyano-4-trifluoromethyl-6-phenyl pyridine **3** via Grignard's reaction, cyclization followed by coupling with aliphatic and cyclic amines. All the compounds **6** were screened for antibacterial, minimum bactericidal concentration (MBC), biofilm inhibition activity as well as antifungal and minimum fungicidal concentration (MFC) activities. Among the screened compounds, the compounds **6e, 6f,** and **6m** which showed exhibiting promising activity have been identified. The results reveal that the compound pyrido[2,3-*d*]pyrimidine derivative **6e** altered the sterol profile which may exert its antifungal activity through inhibition of ergosterol biosynthesis and could be an ideal candidate for antifungal therapy. The molecular docking results also validated the antifungal results.

In most ecological niches, bacterial cells grow on inert or living surfaces as single or multiple-species communities enclosed by a self-produced polymeric matrix, which are so-called the biofilms. The bacterial growth is very poor when floating in water or low in nutrients and show better growth when adherent to surfaces, where they can find a protective and nutrient-rich environment.[1] Biofilms formed by potentially pathogenic bacteria which are commonly encountered in chronic and nosocomial infections provide the bacteria the ability to resist against stress, antibiotics, biocides and host-immunological defenses [2] and in the medical sector where they colonize through bacterial adhesion and biofilm formation on several biomedical implants such as stents, heart valves, vascular grafts and catheters.[3] In this context, the discovery of novel compounds that can specifically target and inhibit the biofilm formation would be of great interest in comparison to the rational use of antibiotics and/or biocides. Such biofilm inhibitors would prove to be of significance for use in the prevention of biofilm formation in various industrial and medical environments. Current biofilm preventive strategies are essentially aimed at the discovery of potential natural anti-biofilm compounds such as 2-aminoimidazole containing alkaloids isolated from marine sponges[4] and Kocuran-functionalized silver glyconanoparticles for use as anti-biofilm coatings on silicone urethral catheters.[5] In this regard, we were interested to explore the anti-biofilm and anti-*Candida* activities against some of the novel molecules having the pyrido[2,3-*d*] pyrimidine framework. In our earlier studies, we also identified novel pyrazolo[3,4-b]pyridine and pyrimidine functionalized 1,2,3-triazole derivatives exhibiting promising antimicrobial and anti-biofilm activities. [6]

In the present context, pyrido[2,3-*d*]pyrimidine derivatives were identified to display promising biological activities,[7,8] and was found to be more specific to exhibit dihydrofolate reductase inhibition, anti-tumor, [9-11] as well as diuretic properties.[12] Some of these compounds also possessed antimicrobial[13-16] and cytotoxic activities.[17,18] The strategically positioned fluorine[19] or trifluoromethyl[20,21] group in the molecule basically influenced the change in the reactivity and the properties of molecule in terms of lipid solubility, oxidative thermal stability and the oral bioavailability. Considering these above facts, the trend is driving more towards the synthesis of fluorinated molecules and the objective of the present study is to identify the promising molecules exhibiting anti-biofilm and anti-*Candida* activities. Keeping in view, the importance of pyrido[2,3-*d*] pyrimidine derivatives and in continuation of our efforts,[22-24] we have synthesized a series of novel pyrido[2,3-*d*] pyrimidine derivatives and screened for antibacterial, minimum bactericidal concentration (MBC), biofilm inhibition, and anti-*Candida* activities. Minimum fungicidal concentration (MFC), and inhibition of ergosterol biosynthesis aspects were also investigated. Sterol 14 alpha-demethylase (CYP51) is one of the enzymes involved in ergosterol biosynthesis pathway, catalyzing C_{14}-demethylation of lanosterol which is critical for ergosterol biosynthesis. It transforms lanosterol into 4,4'-dimethyl cholesta-

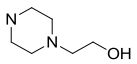
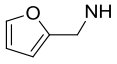
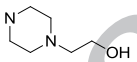
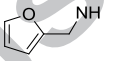
8,14,24-triene-3-beta-ol, which is the target of therapeutic importance for anti-fungal drug development.[25] The compounds which showed promising activity have been identified in each case.

The 2(*1H*) pyridone **1** was reacted with 2-chloroacetamide in acetone using K_2CO_3 as base under reflux condition for 6 h to obtain exclusively 2-((3-cyano-6-phenyl-4-(trifluoromethyl) pyridin-2-yl)oxy)acetamide **2**, and was treated with potassium carbonate in N,N-dimethylformamide at 110-120 °C to form 2-amino-3-cyano-4-trifluoromethyl-6-phenyl pyridine **3**.[26] Compound **3** was further reacted with freshly prepared different aryl magnesium bromides in diethyl ether at room temperature and obtained the 3-(imino(aryl)methyl)-6-phenyl-4-(trifluoromethyl)pyridin-2-amine **4**. The compound **4** was independently reacted with ethyl 2-chloro-2-oxoacetate in DCM using triethylamine as base at room temperature for 1 h which resulted in the formation of ethyl 4-aryl-7-phenyl-5-(trifluoromethyl)pyrido[2,3-*d*]pyrimidine-2-carboxylate **5** and was coupled with different primary aliphatic amines, cyclic secondary amines to obtain products **6a-p**. The reactions are outlined in Scheme 1 and the products are tabulated in Table 1.



Reagents and conditions : (a) 2-Chloroacetamide, NaI, acetone, K_2CO_3, reflux, 6h; (b) K_2CO_3, DMF, 110-120^{O}C, 2h; (c) RMgX, Et_2O, rt, 1h; (d) Ethyl-2-chloro-oxo-acetate, Et_3N, DCM, rt, 1h; (e) amines, 50-60^{o}C, 2-3h

Scheme 1. Preparation of pyrido[2,3-*d*]pyrimidine derivatives.

S. No.	Compound	NR^1R^2	R	M.P (^{o}C)	Yield[a] (%)
1	**6a**	NHC_2H_5	C_6H_5	240	47.39
2	**6b**	NHC_2H_4OH	C_6H_5	248	68.49
3	**6c**	NHC_3H_7	C_6H_5	224	72.63
4	**6d**		C_6H_5	206	78.12
5	**6e**		C_6H_5	117	52.41
6	**6f**		C_6H_5	142	46.02
7	**6g**		C_6H_5	202	77.35
8	**6h**	$C_6H_5CH_2NH$	C_6H_5	236	79.2
9	**6i**	NHC_2H_4OH	4-MeO C_6H_4	219	71.22
10	**6j**	NHC_3H_7	4-MeO C_6H_4	184	57.22
11	**6k**		4-MeO C_6H_4	222	49.40
12	**6l**		4-MeO C_6H_4	176	83.68
13	**6m**		4-MeO C_6H_4	139	42.73
14	**6n**		4-MeO C_6H_4	132	74.48
15	**6o**		4-MeO C_6H_4	210	85.98
16	**6p**	$C_6H_5CH_2NH$	4-MeO C_6H_4	186	87.55

[a] - Isolated yield of the final reaction (i.e., **5** to **6**).

Table 1. Physical properties of compounds **6a-p**

Compounds **6a-p** were screened for antibacterial activity[27] *in vitro* against different Gram-positive and Gram-negative bacterial strains. Among all the compounds screened, compound **6e**, **6f** and **6m** showed promising activity against all the bacterial species. Compounds **6g** and **6k** exhibited promising activity specifically towards *Staphylococcus aureus* MTCC 96, while compound **6l** and **6a** showed promising to moderate activity towards *Klebsiella planticola* MTCC 530. The structure-activity relationship studies revealed that these compounds **6e**, **6f** and **6m** have piperazine moiety with alkyl groups in *para*-position adjacent to carbonyl promoting activity due to enhancement of electron density on carbonyl oxygen which binds to the organism. However, all other compounds (**6c**, **6d**, **6i**, **6j**, **6n**, **6o** and **6p**) did not show antibacterial activity up to the maximum tested concentration of 125 µg/mL. The antibacterial activity results to this regard are tabulated in table 2.

Table 2. Antibacterial activity of the pyrido[2,3-*d*]pyrimidine derivatives

Compd	Minimum inhibitory concentration (µg/mL)						
	BS MTCC 121	*SA* MTCC 96	*SA* MTCC 2940	*ML* MTCC 2470	*KP* MTCC 530	*EC* MTCC 739	*PA* MTCC 2453
6a	-	-[a]	-	-	31.2	-	-
6b	-	31.2	-	-	-	-	-
6c	-	-	-	-	-	-	-
6d	-	-	-	-	-	-	-
6e	15.6	7.8	15.6	15.6	31.2	15.6	31.2
6f	31.2	7.8	15.6	15.6	15.6	15.6	15.6

6g	-	7.8	-	-	15.6	-	-
6h	-	31.2	-	-	-	-	-
6i	-	-	-	-	-	-	-
6j	-	-	-	-	-	-	-
6k	-	7.8	-	-	-	-	-
6l	-	-	-	-	15.6	-	-
6m	7.8	7.8	15.6	15.6	15.6	15.6	7.8
6n	-	-	-	-	-	-	-
6o	-	-	-	-	-	-	-
6p	-	-	-	-	-	-	-
C	0.9	0.9	0.9	0.9	0.9	0.9	0.9

[a]- No activity
BS, *Bacillus subtilis* MTCC 121; SA, *Staphylococcus aureus* MTCC 96; *Staphylococcus aureus* MLS16 MTCC 2940; ML, *Micrococcus luteus* MTCC 2470; KP, *Klebsiella planticola* MTCC 530; EC, *Escherichia coli* MTCC 739; PA, *Pseudomonas aeruginosa* MTCC 2453; **C** Ciprofloxacin (Standard).

Compounds **6b, 6e, 6f, 6g, 6h, 6k, 6l** and **6m** were further evaluated for the minimum bactericidal concentration[28] against all the seven bacterial species in comparison to ciprofloxacin as standard. Compounds **6e, 6f** and **6m** consistently showed promising minimum bactericidal concentration activity. The activity data is tabulated in Table 3.

Table 3. Minimum bactericidal concentration of compound **5a-r**, **6a-h** and **7a-f**

Test Compd	Minimum bactericidal concentration (µg/ml)						
	BS MTCC 121	*SA* MTCC 96	*SA* MLS16 MTCC 2940	*ML* MTCC 2470	*KP* MTCC 530	*EC* MTCC 739	*PA MTCC 2453*
6b	-[a]	62.4	-	-	-	-	-
6e	31.2	15.6	31.2	15.6	62.4	31.2	62.4
6f	31.2	15.6	31.2	31.2	31.2	31.2	31.2
6g	-	15.6	-	-	31.2	-	-
6h	-	31.2	-	-	-	-	-
6k	-	7.8	-	-	-	-	-
6l	-	-	-	-	15.6	-	-
6m	15.6	15.6	15.6	31.2	31.2	31.2	15.6
C	0.58	0.58	1.17	1.17	0.58	0.58	1.17

[a]Not active; BS, *Bacillus subtilis* MTCC 121; SA, *Staphylococcus aureus* MTCC 96; *Staphylococcus aureus* MLS16 MTCC 2940; ML, *Micrococcus luteus* MTCC 2470; KP, *Klebsiella planticola* MTCC 530; EC, *Escherichia coli* MTCC 739; PA, *Pseudomonas aeruginosa* MTCC 2453; **C** Ciprofloxacin (Standard).

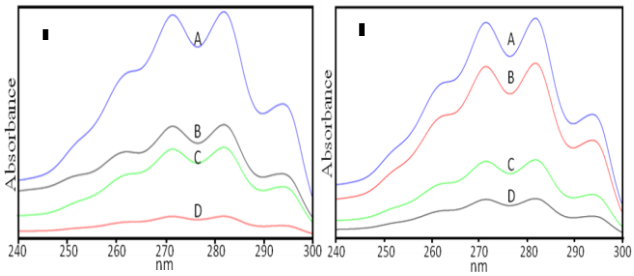
Biofilms are structured consortia of bacteria embedded in a self-produced polymeric matrix causing chronic infections in humans *via* hospital and community environments, especially due to the high antibiotic resistance associated with them as well as the ability to resist phagocytosis and other components of the body's defense system. In several biomedical implants such as stents, heart valves, vascular grafts and catheters, the bacteria colonize through adhesion mechanism and forms biofilms due to the contamination of these biomedical implants during post-surgical infections.[29] Considering these facts, the further step undertaken was to investigate whether these compounds exhibited a specific anti-biofilm activity or whether this observation was simply related to a general toxic effect on the bacterial strains. Thus, the compounds **6b, 6e, 6f, 6g, 6h, 6k, 6l** and **6m** were screened for anti-biofilm activity[30] against five bacterial species such as *Bacillus subtilis* MTCC 121, *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLC 16 MTCC 2940, *Pseudomonas aeruginosa* MTCC 2453 and *Klebsiella planticola* MTCC 530 which are important nosocomial pathogens and have the ability to form biofilms. The results summarized in Table 4, clearly confirmed that the promising activity is related to the presence of piperazine moiety in compounds **6e, 6f** and **6m** with IC_{50} values between 2.7 – 21.6 µg/mL towards all the tested bacterial species. Among them, compounds (**6e, 6f**, **6g, 6k** and **6m**) showed promising activity (IC_{50} values ranging between 2.7–5.4 µg/mL) against *Staphylococcus aureus* MTCC 96, while compounds (**6f**, **6g, 6l** and **6m**) showed promising activity (IC_{50} values ranging between 5.4–10.8 µg/mL) specifically against *Klebsiella planticola* MTCC 530.

In medical environments, the use of contaminated medical devices and prolonged intensive care units showed increased prevalence of fungal diseases. Under such episodes, the most commonly associated fungal strains belong to the genus *Candida*, most notably *Candida albicans*, which causes both superficial and systematic candidiasis.[31] Based on these facts and the observed antibacterial results, *Candida* species are important opportunistic pathogens frequently encountered in infections of immunocompromised patient groups comprising those on cancer chemotherapy, broad-spectrum antibiotics and HIV-infected individuals. Among the many pathogenic *Candida* species, *Candida albicans* is the major fungal pathogen of importance to humans. Due to its versatility, it can behave as a commensal organism posing a major problem from a clinical perspective resulting in infections.[32] Different *Candida* strains have the ability to produce extracellular polymeric substances (EPS) and get encased on this matrix to form biofilms which are known to develop on the surfaces of prosthesis and medical devices, and exhibit resistance to both antifungal and host immune defenses as compared to their free-living planktonic counterparts. This is most likely to be the cause of recalcitrant persistence of *Candida albicans* on inert, inserted surfaces or, superficial mucosae.[33,34] Considering these above facts, we screened some of the selected compounds **6b, 6c, 6d, 6e, 6f, 6h** and **6m** for anti-*Candida* activity against different *Candida* strains. More specifically, compounds **6d, 6e, 6f** and **6m** showed promising activity (MIC values ranging between 3.9–31.2 µg/mL) against *C. albicans* MTCC 3017 and was comparable to the standard miconazole drug. The results on the anti-*Candida* activity are tabulated in Table 5.

Compounds **6b, 6c, 6d, 6e, 6f, 6h** and **6m** were also evaluated for minimum fungicidal concentrations against different *Candida* strains in comparison to the standard miconazole drug. All the compounds showed minimum fungicidal concentration (MFC) values ranging between 3.9 - 62.5 µg/mL). However, the standard miconazole drug exhibited minimum fungicidal concentration values ranging between 7.8 - 15.6 µg/mL. Among them, compound **6e** proved promising and exhibited a lower MFC value of 3.9 µg/mL. The activity data is tabulated in Table 6.

Candida albicans is now recognized as a major cause of hospital-acquired infections.[35] Most of the antifungal drugs currently available to treat *Candida* infections target the ergosterol biosynthetic pathway or its end product ergosterol. Considering this fact, we further investigated the promising test compound **6e** in comparison to the standard miconazole drug to delineate its mode of action in the ergosterol biosynthetic pathway for one of the highly susceptible strain of *C. albicans* MTCC 183. In this

regard, the UV spectral scans of the sterol profiles for one of the representative strain of *C. albicans* MTCC 183 [36] was determined (Figure 1), and later the total ergosterol content was quantified from the data obtained on culturing the *C. albicans* MTCC 183 strain with different concentrations (0, 2, 4, and 16 µg/mL) of the test compound **6e** and the standard miconazole drug (see Table 7). It was noticed that the ergosterol content decreased significantly with an increase in the concentration of test compound **6e**. Similarly, a dose-dependent decrease in ergosterol content was observed when the *C. albicans* MTCC 183 strain was cultured in presence of miconazole. Our findings suggest that the pyrido[2,3-*d*]pyrimidine derivative **6e** altered the sterol profile which may exert its antifungal activity through inhibition of ergosterol biosynthesis. The selective cytotoxic behaviour of this compound hints at its affinity to the specific target site in the ergosterol biosynthetic pathway. The *Candida*-cidal activity of the compound **6e** might also be due to the direct damage to the cell membrane. However, the exact mechanism of action of this compound needs to be further elucidated.



UV spectrophotometric scans of the sterol profiles of *C. albicans* MTCC 183. The culture was grown for 20 h in Sabouraud dextrose broth containing different concentrations of the (I) test compound, **6e** and (II) Miconazole per mL, such as 0 (curve A), 2 (curve B), 4 (curve C) and 16 (curve D) µg. The sterols were extracted from the cells, and the spectral profiles were recorded at the wavelength between 240 and 300 nm

Figure 1

Molecular docking study was performed to validate the antifungal activity results and to explain the binding mode of proteins and ligand complexes. The compound was docked by using Autodock 4.2 software.[37-38] The modeled three dimensional structure of lanosterol 14-alpha demethylase protein was imported to Autodock 4.2 and structurally optimized by adding hydrogens to protein allocated with Kollman charges. After adding the hydrogens, the model was saved in PDBQT format, later ligands were prepared by optimizing the torsion angles and saved them in PDBQT format. Potential binding site for the lanosterol 14-alpha demethylase modeled protein was identified. A grid was generated around active site pocket amino acids (X=25.874, Y=18.831 and Z= 15.131). Lamarckian genetic algorithm (LGA) was selected for freezing, docking and default parameters used in Autodock 4.2.

The amino acid sequence of lanosterol 14-alpha demethylase was retrieved from Uniprot.[39] A three dimensional model was generated for lanosterol 14-alpha demethylase. A sequence similarity search was performed to identify the structural similarity of the query sequence by using Protein BLAST [40] tool by selecting database against Protein Data Bank (PDB) for identifying template for homology model building. 4K0F protein was selected as a template for modeled protein. The template protein was downloaded from RCSB.[41] Comparative sequence alignment studies were performed with query and template structure using Clustal X tool and online Clustal W tools.[42] MODELLER 9.17 software was used to develop the model. It is an automated approach for comparative modeling by satisfaction of spatial restrains.[43] After generating the files, the best model was selected on the basis of Lowest Objective Function. It is also known as normalized Discrete Optimized Molecule Energy (DOPE) score. The generated model was validated by Ramachandran plot and Psi/Phi angles using PROCHECK.[44]
Active Site Prediction: Active site was predicted for the developed model by Sybyl Siteid module. The models showed 18 active site pockets. The pocket which is having higher value was selected as active site. The molecule **6e** was sketched in SYBYL version 6.7 [45] and energetically minimized by adding Gasteiger-Huckel charges. The molecules were then saved in .mol2 format for molecular docking purpose.

Docking of the **6e** synthesized compound is summarized in Table 8. The derivative **6e** was selected for molecular docking has some collective structural features. The comparison of free energies corresponding to binding of title compounds with target protein revealed that maximum of the compounds interacted with the receptor. Compound **6e** exhibited binding energy of -10.70 kcal/mol. It showed interaction with Asp233.

In conclusion, a series of novel pyrido[2,3-*d*]pyrimidine derivatives **6a-p** were prepared and screened for antibacterial, minimum bactericidal concentration (MBC), anti-biofilm, anti-*Candida*, minimum *Candida*-cidal concentration (MFC) and ergosterol inhibition activities. Compounds **6e, 6f,** and **6m** which showed promising activity have been identified. The promising pyrido[2,3-*d*]pyrimidine derivative **6e** exhibited an important inhibitory role in the ergosterol biosynthetic pathway and it was also validated through modeling studies. Further, optimization of structural modification on the pyridopyrimidine derivatives is in progress.

Table 4: Biofilm inhibition assay of the selected pyrido[2,3-*d*]pyrimidine derivatives

Compound	IC_{50} values (in µg/ml)[h]				
	BS MTCC 121[a]	*SA* MTCC 96[b]	*SA* MLS-16 MTCC 2940[C]	*PA* MTCC 2453[d]	*KP* MTCC 530[e]
6b	-	21.6 ± 0.42	-[f]	-	-
6e	10.8 ± 0.15	5.4 ± 0.12	10.8 ± 0.32	21.6 ± 0.33	21.6 ± 0.41
6f	21.6 ± 0.26	2.7 ± 0.11	10.8 ± 0.28	10.8 ± 0.32	5.4 ± 0.22
6g	-	2.7 ± 0.20	-	-	5.4 ± 0.26

6h	-	21.6 ± 0.33	-	-	-
6k	-	2.7 ± 0.19	-	-	-
6l	-	-	-	-	10.8 ± 0.32
6m	2.7 ± 0.13	5.4 ± 0.22	10.8 ± 0.16	2.7 ± 0.16	10.8 ± 0.25
C[g]	0.23 ± 0.14	0.21 ± 0.11	0.24 ± 0.09	0.32 ± 0.12	0.29 ± 0.14

[a]BS, *Bacillus subtilis* MTCC 121; [b]SA, *Staphylococcus aureus* MTCC 96; [c]SA *Staphylococcus aureus* MLS16 MTCC 2940; [d] PA, *Pseudomonas aeruginosa* MTCC 2453; [e]KP, *Klebsiella planticola* MTCC 530; -[f]no activity [g]C Ciprofloxacin (Standard). [h]Biofilm inhibition assay was performed using crystal violet method. All the biofilms were treated with different concentrations of test compounds and the respective IC_{50} values were interpreted from the dose-response curves. Each experiment was performed in triplicate and the results are represented as mean ± S.D (n = 3).

Table 5: Anti-*Candida* activity of the pyrido[2,3-*d*]pyrimidine derivatives.

compd	Minimum inhibitory concentration (µg/mL)													
	C. a MTCC 183	*C. a* MTCC 227	*C. a* MTCC 854	*C. a* MTCC 1637	*C. a* MTCC 3018	*C. a* MTCC 3017	*C. a* MTCC 3958	*C. a* MTCC 4748	*C. a* MTCC 7315	*C. parapsilosis* MTCC 1744	*C. aaseri* MTCC 1962	*C. glabrata* MTCC 3019	*C. krusei* MTCC 3020	*Issatchenkia hanoiensis* MTCC 4755
6b	31.2	-[a]	-	-	15.6	31.2	31.2	62.5	31.2	-	31.2	15.6	31.2	15.6
6c	15.6	31.2	31.2	-	-	15.6	-	-	15.6	-	15.6	7.8	15.6	31.2
6d	7.8	15.6	7.8	7.8	31.2	7.8	31.2	62.5	15.6	15.6	7.8	31.2	-	31.2
6e	3.9	3.9	7.8	7.8	15.6	3.9	7.8	15.6	31.2	31.2	7.8	-	-	15.6
6f	3.9	7.8	7.8	15.6	-	3.9	-	-	62.5	31.2	31.2	15.6	-	15.6
6h	31.2	-	-	-	-	31.2	-	62.5	62.5	31.2	31.2	15.6	15.6	31.2
6m	3.9	31.2	15.6	15.6	31.2	3.9	62.5	62.5	31.2	15.6	15.6	7.8	-	7.8
Miconazole (Standard)	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8

[a]- No activity

Table 6: Minimum Fungicidal Concentration (MFC) of the selected pyrido[2,3-*d*]pyrimidine derivatives

Candida strains	Minimum fungicidal concentration (µg/mL)							
	6b	**6c**	**6d**	**6e**	**6f**	**6h**	**6m**	Miconazole (Standard)
Candida albicans MTCC 183	62.5	31.2	15.6	3.9	7.8	62.5	7.8	7.8
C. albicans MTCC 227	-[a]	31.2	31.2	7.8	15.6	-	31.2	7.8
C. albicans MTCC 854	-	62.5	15.6	15.6	15.6	-	31.2	15.6
C. albicans MTCC 1637	-	-	15.6	15.6	31.2	-	31.2	7.8
C. albicans MTCC 3017	62.5	15.6	15.6	7.8	7.8	62.5	7.8	7.8
C. albicans MTCC 3018	31.2	-	62.5	31.2	-	-	62.5	15.6
C. albicans MTCC 3958	62.5	-	62.5	15.6	-	-	62.5	7.8
C. albicans MTCC 4748	62.5	-	62.5	31.2	-	62.5	62.5	7.8
C. albicans MTCC 7315	62.5	31.2	31.2	62.5	62.5	62.5	62.5	15.6

C. parapsilosis MTCC 1744	-	-	15.6	62.5	62.5	62.5	31.2	7.8
C. aaseri MTCC 1962	31.2	15.6	15.6	15.6	62.5	62.5	31.2	15.6
C. glabrata MTCC 3019	31.2	15.6	31.2	-	31.2	31.2	15.6	7.8
C. krusei MTCC 3020	62.5	31.2	-	-	-	31.2	-	15.6
Issatchenkia hanoiensis MTCC 4755	15.6	62.5	62.5	31.2	31.2	62.5	15.6	15.6

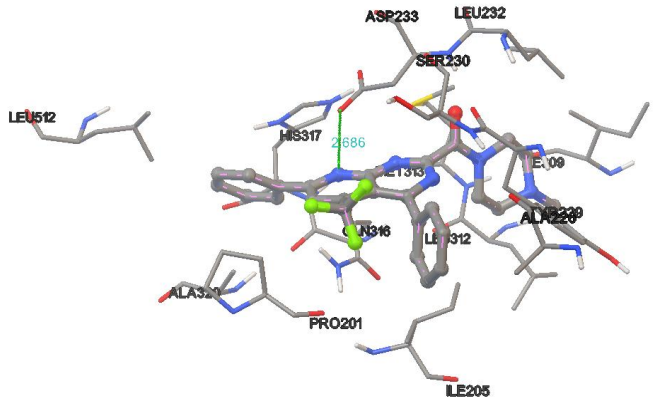
[a]- No activity

Table 7: Inhibition of ergosterol biosynthesis in *C. albicans* MTCC 183 by compound **6e**

Test compounds	Mean ergosterol content of cells grown with test compounds at different concentrations (µg/mL)			
	0	2	4	16
6e	2.27 ± 0.11	1.75 ± 0.12	1.08 ± 0.08	0.41 ± 0.06
Miconazole (Standard)	2.27 ± 0.11	1.81 ± 0.09	1.12 ± 0.10	0.53 ± 0.04

Table 8: Interacting amino acids and their binding energy of compound **6e** with modeled lanosterol 14-alpha demethylase protein

No. of ligand	Interacting amino acids	Grid X-Y-Z coordinates	Binding energy ΔG (Kcal/Mol)	Dissociation constant (kl)
6e	Asp233	25.874, 18.831, 15.131	-10.70	14.42 nM



6e

Figure 3. Molecular docking interactions of compound **6e** with lanosterol 14-alpha demethylase protein

Acknowledgements

Authors are thankful to the Council of Scientific and Industrial Research (CSIR), New Delhi, India for the financial assistance in the form of XII five year plan project DITSF code; CSC-0204. Authors (B.Veeraswamy, G. Jitender Dev) are also thankful to CSIR for providing financial assistance in the form of Research Fellowship and contingency grant.

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