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# Studies on synthesis of novel pyrido[2,3-*d*]pyrimidine derivatives, evaluation of their antimicrobial activity and molecular docking

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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,

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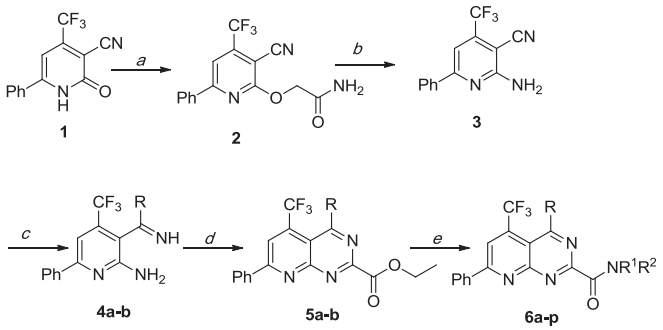
and anti-*Candida* activities. Minimum fungicidal concentration (MFC), and inhibition of ergosterol biosynthesis aspects were also investigated. Sterol 14 alpha-demethylase (CYP51) is one of the enzymes involved in ergosterol biosynthesis pathway, catalyzing $C_{14}$-demethylation of lanosterol which 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]



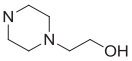
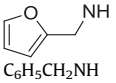
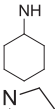
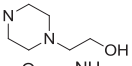
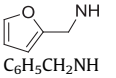
**Scheme 1.** Preparation of pyrido[2,3-*d*]pyrimidine derivatives. Reagents and conditions: (a) 2-Chloroacetamide, NaI, acetone, $K_2CO_3$, reflux, 6 h; (b) $K_2CO_3$, DMF, 110–120 °C, 2 h; (c) RMgX, $Et_2O$, rt, 1 h; (d) Ethyl-2-chloro-oxo-acetate, $Et_3N$, DCM, rt, 1 h; (e) amines, 50–60 °C, 2–3 h.

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.

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.

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 1**
Physical properties of compounds **6a**–**p**.

| S. No. | Compound | $NR^1R^2$ | R | M.P (°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**).