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Anilinopyrimidines as Novel Antituberculosis Agents

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Abstract—A selection of novel anilinopyrimidine analogues have been found to have micromolar activity against *Mycobacterium tuberculosis*. This could potentially generate new lead compounds in the fight against multi-drug resistant tuberculosis.

Tuberculosis (TB) is the leading infectious cause of death today[1] and is rapidly becoming a global epidemic.[2] It is estimated that worldwide 100 million people are infected annually.[3] Approximately ten million develop the disease, with five million of these progressing to the infectious stage and ultimately three million dying.[4] The increase in TB is a result of two major factors; the susceptibility of people infected with the acquired immune deficiency syndrome (AIDS), which augments the risk of developing TB 100-fold,[5] and the increase in resistant strains of the disease[6] with some shown to be resistant to as many as nine drugs.[5]

Although one possible long term solution to the problem is a better vaccine, in the short term, the major reliance will be on chemotherapy[4] requiring the development of novel, effective, non-toxic antituberculosis agents.[1,4,7]

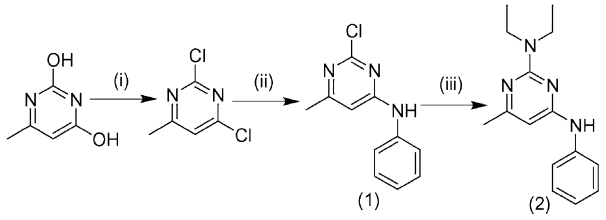
Our work focused upon the synthesis and biological testing of some novel anilinopyrimidine compounds. These compounds were generated via two simple substitution reactions of substituted amines onto a chloropyrimidine derivative.[8] An example reaction is outlined in Scheme 1.

Seven of these synthesized derivatives were tested against malaria and tuberculosis. None of these showed any activity against *Plasmodium falciparum*, however, six of the analogues showed micromolar activity against *Mycobacterium tuberculosis*. The details of our preliminary results are outlined in Table 1.

The short synthetic pathway involved can lead to the generation of a large range of these anilinopyrimidine analogues quickly and easily so they may be tested against TB in order to determine an accurate structure–activity relationship for these compounds.

To the best of our knowledge, there has been no previous reports of anilinopyrimidines as antituberculosis agents. However, there are numerous examples of nitrogen containing heterocycles being used to treat TB, with example structures shown in Table 2. These compounds provide structural precedence that our anilinopyrimidine analogues may lead to the generation of novel anti-TB therapeutics.

The mechanism of action of both isoniazid and pyrazinamide is known to be inhibition of cell-wall synthesis. Isoniazid acts as an inhibitor of InhA, a NADH-specific enoylase-reductase, involved in the synthesis of



(i) $POCl_3$, Δ, 90%; (ii) aniline, H_2O, Δ, 42%; (iii) diethylamine, THF, Δ, 79%.

Scheme 1. Pathway for the synthesis of anilinopyrimidine analogues.

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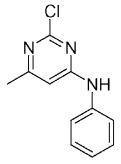
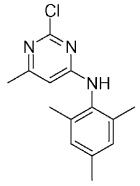
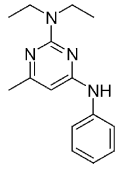
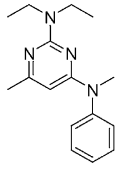
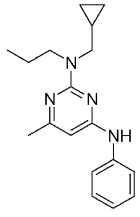
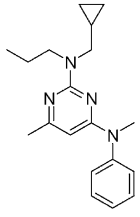
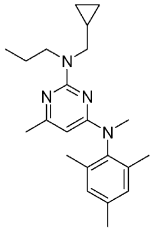
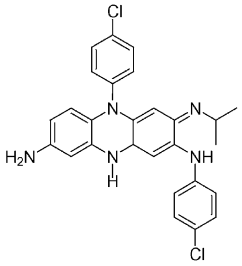
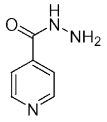
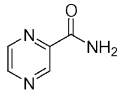
Table 1. The structure and anti-TB activity (MIC) against *Mycobacterium tuberculosis* for a range of anilinopyrimidine compounds

Compds	Structure	MIC μg/mL
1		50
3		Inactive
2		25
4		50
5		12.5
6		12.5
7		3.12

fatty acids in mycobacteria.[10] Initially this compound is converted into its active form via KatG catalase-peroxidase enzyme.[11] The active component then inhibits mycolic acid biosynthesis via the InhA enzyme. Mycolic acids have been shown to be a key component in the mycobacterial cell wall.[12] Pyrazinamide has been shown

Table 2. Nitrogen containing heterocycles as known anti-TB agents

Drug	Structure	Remarks	Ref
Clofazimine		Active against MDR-TB, MIC 2.0 μg/mL	7
Isoniazid		Most widely used agent, however it is hepato-toxic.	6,9
Pyrazinamide		MIC 20 μg/mL	9

to inhibit fatty acid synthase type I, which provides the precursors for mycolic acid biosynthesis.[12] At this stage, it is too premature to predict the mode of action of our anilinopyrimidine compounds.

Biological Testing

The antimycobacterial activity was assessed against *M. tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA). Standard drugs, isoniazid (MIC of 0.040–0.090 μg/mL) and kanamycin sulfate (MIC of 2.0–5.0 μg/mL) were used as reference compounds for the antimycobacterial assay. The cytotoxicity assay against the BC (human breast cancer cells), KB (oral human epidermal carcinoma) and NCl-187 (human small cell lung cancer) was performed employing the colorimetric method,[13] the reference substance was ellipticine with an IC_{50} of 0.1–0.4 μg/mL. The antimalarial activity was evaluated against the parasite *P. falciparum* (K1, multidrug-resistant strain), which was cultured continuously according to the method of Trager and Jensen.[14] Quantitative assessment of antimalarial activity in vitro was determined by means of the microculture radioisotope technique based upon the method described by Desjardins et al.[15] The inhibitory concentration (IC_{50}) represents the concentration that cause 50% reduction in parasite growth as indicated by the in vitro uptake of [^{3}H]-hypoxanthine by *P. falciparum*. An IC_{50} value of 1 ng/mL was observed for the standard compound, artemisinin, in the same test system.

Conclusions

We have synthesised a novel series of anilinopyrimidine compounds, via a short and easy three step procedure. Six of these analogues have been found to have reason-

able activity against tuberculosis. These compounds while not active enough to be considered as therapeutics are definitely lead compounds in the search for novel agents to combat resistance.

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8. A general synthetic procedure for the preparation of anilinopyrimidine derivatives is outlined here. A suspension of 2,4-dichloro-6-methylpyrimidine (2.02 g, 12.4 mmol) in distilled water (15 mL) was stirred at room temperature for 1 h to promote solubilisation. The solution was heated to 100 °C, aniline (1.20 mL, 13.2 mmol) was added and the resulting mixture was heated at reflux for 40 min. The cooled reaction mixture was extracted with DCM (4×30 mL) and the combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The crude residue was purified via gravity silica column chromatography, and elution with 10% ethyl acetate:hexanes gave 4-anilino-2-chloro-6-methylpyrimidine (**1**) (0.86 g, 4.0 mmol, 42%) as a pale yellow solid, mp. 116–117 °C. MS (CI); *m*/*z* 222 (32%) $[M+H\ ^{37}Cl]^+$; 220 (100) $[M+H\ ^{35}Cl]^+$; HRMS for $C_{11}H_{11}N_3{}^{35}Cl$, calc 220.0642, found 220.0635; ^1H NMR δ 7.64, bs, 1H, NH; 7.32, m, 5H, ArH2′,3′,4′,5′,6′; 6.43, s, 1H, ArH5; 2.32, s, 3H, CH_3; ^{13}C NMR δ 168.7, ArC2; 163.0, ArC1′; 160.1, ArC6; 137.1, ArC4; 129.6, ArC2′,6′; 125.9, ArC5; 123.5, ArC3′,5′; 100.7, ArC4′; 23.8, CH_3. To a solution of 4-anilino-2-chloro-6-methylpyrimidine (**1**) (0.12 g, 0.53 mmol) in dry THF (5 mL) was added diethylamine (0.6 mL, 0.54 mmol). The reaction mixture was flushed with nitrogen, and stirred in a sealed tube at 160 °C for 24 h. The reaction mixture was concentrated in vacuo. The crude residue was subjected to gravity silica column chromatography, and elution with 10% ethyl acetate:hexanes gave 4-anilino-2-diethylamino-6-methylpyrimidine (**2**) (0.10 g, 0.39 mmol, 79%) as beige crystals, mp. 78–80 °C. MS (CI); *m*/*z* 257 (100%) $[M+H]^+$; HRMS for $C_{15}H_{21}N_4$, calc 257.1766, found 257.1768; ^1H NMR δ 7.33, m, 5H, ArH2′,3′,4′,5′,6′; 6.40, bs, 1H, NH; 5.82, s, 1H, ArH5; 3.61, q, $J=7.2$ Hz, 4H, $C\underline{H}_2CH_3$; 2.21, s, 3H, Ar–CH_3; 1.81, t, $J=7.2$ Hz, $CH_2C\underline{H}_3$; ^{13}C NMR δ 166.6, ArC4; 161.9, ArC1′; 161.2, ArC2; 139.7, ArC6; 129.0, ArC2′,6′; 122.9, ArC4′; 120.8, ArC3′,5′; 92.8, ArC4; 41.4, $\underline{C}H_2CH_3$; 24.4, Ar–CH_3; 13.3, $CH_2\underline{C}H_3$.
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