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Identification and synthesis of novel inhibitors of mycobacterium ATP synthase

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ABSTRACT

A non-diaryl quinoline scaffold 6,7-dihydropyrazolo[1,5-a]pyrazin-4-one was identified by screening of diverse set of compounds against *M. smegmatis* ATP synthase. Herein, we disclose our efforts to develop the structure activity relationship against *Mycobacterium tuberculosis* (*Mtb*.H37Rv strain) around the identified hit **1**. A scaffold hopping approach was used to identify compounds **14a**, **14b** and **24a** with improved activity against *MTb*.H37Rv.

Tuberculosis (TB) is a disease of antiquity which is thought to have evolved sometime between the seventh and sixth millennia BC.[1] According to World Health Organization (WHO) there were 9.6 million incident cases of TB and 1.5 million deaths in 2014 making TB a global public health issue.[2] The global health impact of *Mtb.* is further aggravated in AIDS patients whose immune system is compromised.[3] Comorbidity with HIV and the emergence of multidrug resistant (MDR) TB; extensively drug-resistant (XDR) TB or totally drug-resistant (TDR) TB has worsened the TB treatment.[4–7] New drugs are needed to tackle the growing global problem of multidrug-resistant and extensively drug-resistant tuberculosis.[8] Patients with MDR TB need a combination of second-line and third-line anti-tuberculosis drugs,[9,10] which are much more expensive and more toxic compared to standard drug treatment regimen, and treatment may last much longer.

In recent years, persistent efforts in this direction have led to several lead molecules progressing to clinical trials which exhibited promising activities against drug-sensitive and drug-resistant strains of the causative organism *Mtb*. It is hard to treat the persistent infections with antibiotics that target biosynthetic processes; thus in recent years people have targeted bacterial membrane and enzymes involved in anaerobic respiration. This emerging concept involving disruption of energy metabolism in dormant bacteria is exemplified by the efficacy of Bedaquiline, which inhibits membrane-bound ATP synthase.[11] Bedaquiline (Fumarate){also known as TMC-207, diarylquinoline (DARQ)} was the first novel antitubercular drug approved by the US Food and Drug Administration (FDA) specifically for the treatment of MDR-TB in the past 40 years.[12–17] It is found effective against all states of *Mycobacterium tuberculosis* like active, dormant, replicating, non-replicating, intracellular and extracellular.[12,18,19] Although, Bedaquiline has been launched, the drug possesses serious adverse effects such as cardiac arrhythmias, phospholipidosis.[13,20] There were more deaths in the Bedaquiline group than in the placebo group with no causal pattern evident.[21]

Adverse effects of bedaquiline, such as phospholipidosis and cardiovascular risks, may be related to molecular features like cationic, amphiphilic properties and high logP (7.52).[15,22] Especially, an *N*-desmethyl metabolite (“M2”) was reported to be more toxic.[15,23] These observed adverse effects of Bedaquiline suggests the necessity of new chemical entities as selective ATP Synthase inhibitors without any potential adverse effects. It is expected that new class of compounds may have a different toxicological profile. Therefore, screening of a chemically diverse small molecule library against *M. smegmatis* ATP synthase was carried out (see Fig. 1).

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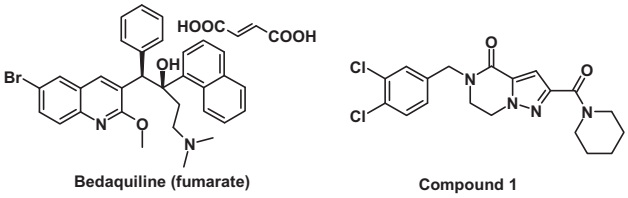

Fig. 1. Structures of Bedaquiline (Fumarate) and hit obtained from screening campaign.

An automated screening of 10,000 compounds was performed using Tecan Robot.

Using this assay, compounds were screened at a single inhibitor concentration of 10 μM and 151 potential actives – (hit rate: 1.51%) were identified. Dose-response curves, using phosphate assays, led to identification of 24 hits (hit rate: 0.24%). This effort led to the identification of hit from a non-DARQ template which exhibited activity of 0.27 μM in biochemical assay and 16 μg/mL against *Mtb*. H37Rv. (see Fig. 2)

Based on the activity in biochemical, whole cell and cytotoxicity assays compound **1** was identified as a hit for further optimization. Compounds from this series were screened using the procedure reported by Koul et. al.[24]

To generate the structure activity relationship we divided compound **1** in three regions viz benzylic region, amide region and the central scaffold (Fig. 3).

Initially our focus was to develop an SAR around the benzylic and amidic region. The strategy for the synthesis of *N*-substituted 6, 7-dihydropyrazolo [1,5-a] pyrazin-4-one (Z = N) derivatives **7a–7l** is summarized in synthetic Scheme 1. The alkylation of commercially available diethyl pyrazole-3,5-dicarboxylate followed by transformation to azide **4**. Reduction of azide **4** using Staudinger reaction[25] furnished the ring closure to lactum **5** as reported in literature.[26,27] This intermediate **5** was converted to **6** by the trimethylaluminium mediated amidation reaction. The compounds **1** and **7a–7n** were synthesized either by alkylation with different benzyl bromides or by Buchwald-Hartwig cross coupling reaction.[28,29] Diverse amide derivatives **9a–9h** were synthesized using similar reaction sequence.

After establishing an initial SAR, scaffold hopping[30,31] was done around "6,7-dihydropyrazolo[1,5-a]pyrazin-4-one" as shown in Fig. 3. We explored five scaffolds viz. 3,4-dihydropyrrolo[1,2-a]pyrazin-1(2H)-one (scaffold A), 5,6,7,8-tetrahydro-4H-pyrazolo[1,5-a][1,4]diazepin-4-one (scaffold B), pyrazolo[1,5-a]pyrazin-4(5H)-one core (scaffold C), 7-hydroxy 6,7-dihydropyrazolo[1,5-a]pyrazin-4-one core (scaffold D) and imidazo[1,2-a]pyrazin-8(7H)-one (scaffold E).

Scaffold A and Scaffold B: Following similar reaction sequence, the amide derivatives of 3,4-dihydropyrrolo[1,2-a]pyrazin-1(2H)-one and 5,6,7,8-tetrahydro-4H-pyrazolo[1,5-a][1,4]diazepin-4-one (**7m** and **7n** respectively) were synthesized as outlined in synthetic Scheme 1.

Scaffold C: The amide derivatives of pyrazolo[1,5-a]pyrazin-4(5H)-one core **14a–14b**, were prepared from commercially available diethyl pyrazole-3,5-dicarboxylate over five steps as outlined in synthetic Scheme 2. Trimethylaluminium mediated monoamidation followed by treatment with p-TSA afforded the cyclized product **11**. De-hydration using methane sulphonic acid yielded compound **12** which upon reaction with trimethylaluminium and piperidine afforded piperidinyl amide **13** in good yield. Reaction of amide **13** using sodium hydride as a base and substituted benzyl bromides furnished the final compounds **14a–14b**.

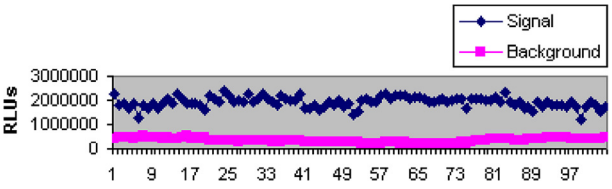

Fig. 2. Signal background (S:B) ratio of ATP synthase screening – assay.

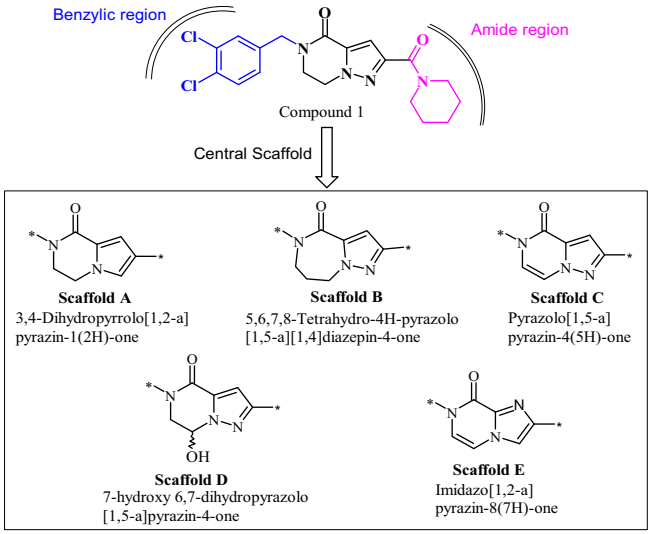

Fig. 3. Derivatization strategy for hit obtained from screening campaign.

Scaffold D: Further derivatives of 7-hydroxy 6,7-dihydropyrazolo[1,5-a]pyrazin-4-one core were synthesized using synthetic Scheme 3. The hydroxyl group of cyclized product **11** was protected using TBDMS group and then it was converted to amides **17a–17b** using similar reaction conditions. The TBDMS deprotection using TBAF afforded compounds **18a–18b**.

Scaffold E: The synthesis of amides **24a–24b** of imidazo[1,2-a]pyrazin-8(7H)-one scaffold was achieved using commercially available 2,3 dichloropyrazine in five steps (Scheme 4). Intermediate **22** was synthesized following similar procedure reported by Ager *et.al.*[32] Trimethylaluminium mediated amidation with piperidine followed by *N*-benzylation afforded the amides **24a–24b**.

The compounds were screened in biochemical assay to confirm the MOA and in whole cell screens for activity against *Mtb.*H37Rv. Three regions of the hit (compound **1**) viz. benzylic region, amide region and the central scaffold were explored. To understand the SAR at benzylic region of 6,7-dihydropyrazolo[1,5-a]pyrazin-4-one core; a set of diverse compounds with cycloalkyl, aryl or substituted-benzyl were explored by fixing the piperidinyl group at amide region. Except for appropriately substituted benzyl substitution, all other substitutions led to loss in ATP synthase activity (**7a–7d**; Table 1). The substitution at *ortho* position (**7i**) of benzyl group led to loss in activity in biochemical assays. Different electron withdrawing and neutral groups were tolerated at *meta* and *para* position of the benzyl ring. However, the substitution at *meta* position (**7e**, **7h**, **7j**) was preferred over *para* substitution. These compounds had a modest whole cell activity of 8 μg/mL against *M. tuberculosis* H37Rv and activity results are summarized in Table 1. Any modification at amide region led to loss in activity in the biochemical assays and thus confirmed the necessity of piperidin-1-yl amide at this position (Results can be referred in Supplementary data). Table 2

Although we identified compounds with potent ATP synthase inhibitory activity, they showed modest whole cell activity. The SAR around the benzylic region and amide region was very narrow