Bioorg. Med. Chem. Lett. 40 (2021) 127924

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The optimization and characterization of functionalized sulfonamides derived from sulfaphenazole against *Mycobacterium tuberculosis* with reduced CYP 2C9 inhibition

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ARTICLE INFO

Keywords:
Sulfonamide compounds
Optimization
Antimycobacterial activity
CYP 2C9
Cytotoxicity

ABSTRACT

In this study, a series of sulfonamide compounds was designed and synthesized through the systematic optimization of the antibacterial agent sulfaphenazole for the treatment of *Mycobacterium tuberculosis* (*M. tuberculosis*). Preliminary results indicate that the 4-aminobenzenesulfonamide moiety plays a key role in maintaining antimycobacterial activity. Compounds **10c**, **10d**, **10f** and **10i** through the optimization on phenyl ring at the R^2 site on the pyrazole displayed promising antimycobacterial activity paired with low cytotoxicity. In particular, compound **10d** displayed good activity (MIC = 5.69 μg/mL) with low inhibition of CYP 2C9 (IC_{50} > 10 μM), consequently low potential risk of drug-drug interaction. These promising results provide new insight into the combination regimen using sulfonamide as one component for the treatment of *M. tuberculosis*.

Tuberculosis (TB) is a chronic disease that results from infection with *M. tuberculosis*. TB is one of the top 10 causes of death worldwide, and the leading cause of mortality stemming from a single infectious pathogen.[1] In addition, the emergence of multidrug-resistant (MDR) TB and extensively drug-resistant (XDR) TB have worsened the situation, particularly in low-income countries.[2,3] Renewed efforts have therefore been focused on developing new antibiotics against TB.[4] Meanwhile, repurposing of efficacious antibiotics has emerged as an effective way to discover new pharmacophores, resulting in the discovery of potentially new drugs against *M. tuberculosis*.[5]

Sulfonamides have been intensively investigated since their discovery as the first potent antibacterial agents, and notably, are still in use today.[6–10] Sulfonamides generally act as structural analogues of 4-aminobenzoic acid and therefore inhibit dihydropteroate synthase in bacteria.[11–13] The 4-aminobenzenesulfonamides with antibacterial activity are represented as sulfamethoxazole (SMX), sulfadiazine (SD), sulfisoxazole (SIZ) and sulfaphenazole (SPA).

The antibacterial activity observed upon combining SMX with trimethoprim (TMP) is a result of the sequential inhibition of the biosynthesis of tetrahydrofolic acid within bacteria, which is essential for bacterial viability. Furthermore, the combination of SMX and TMP (SXT) has emerged as a potential treatment regimen to combat drug-resistant TB.[14] Notably, SXT has been successfully used to treat isolated cases of drug-resistant tuberculosis,[15] with further research showing that several other related sulphonamide derivatives also displayed pronounced anti-TB effects.[7,16,17] All these results encouraged us to further evaluate sulfonamide derivatives against *M. tuberculosis*.

The initial hit compound, SPA, discovered through screening our in-house library of clinically relevant sulfonamide compounds, displayed good *in vitro* efficacy against *M. tuberculosis* $H_{37}Rv$ (Fig. 1). However, SPA is also a selective, competitive inhibitor of CYP 2C9, which can potentially lead to drug-drug interactions.[18–20] In this work, a series of sulfonamide compounds derived from SPA was designed and synthesized to establish and explore structure–activity relationships (SARs), with the aim to design-out the unwanted CYP 2C9 activity. Systematic optimization led to compound **10d** which displayed good antimycobacterial activity and, importantly, a reduced CYP 2C9 inhibitory profile.

The synthesis of compounds **5a-i**, **10a-k**, **12a-c**, **16a-f**, **17** and **18a-g** is outlined in Schemes 1–4. Sulfonylation of commercially available 5-

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https://doi.org/10.1016/j.bmcl.2021.127924
Received 22 December 2020; Received in revised form 8 February 2021; Accepted 25 February 2021
Available online 9 March 2021

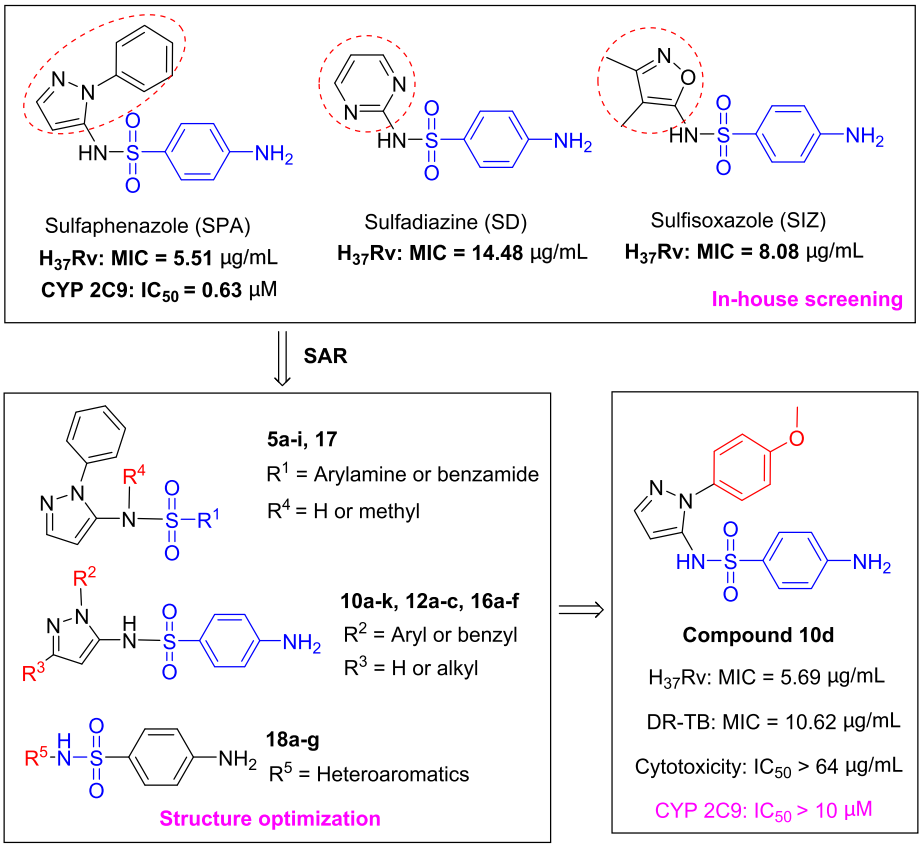
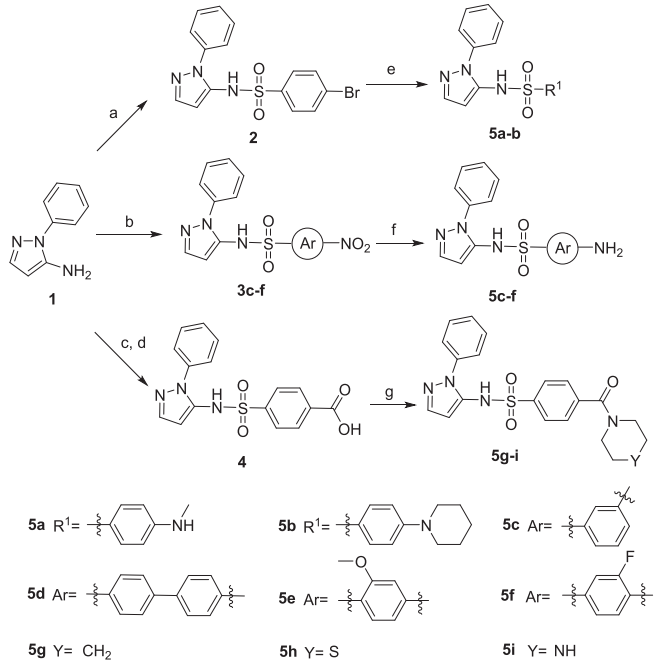


Fig. 1. Structure optimization through in-house screening.



Scheme 1. Synthesis of the target compounds **5a-i**. Reagents and conditions: (a) 4-Bromobenzenesulfonyl chloride, pyridine, reflux; (b) Arylsulfonyl chloride, pyridine, reflux; (c) Methyl 4-(chlorosulfonyl)benzoate, pyridine, reflux; (d) 2 N NaOH aqueous solution, reflux; (e) Methylamine, Cu, microwave (MW), 110℃ for **5a**; piperidine, $Pd_2(dba)_3$, *rac*-BINAP, $K_3PO_4{\cdot}H_2O$, DMF, reflux for **5b**; (f) H_2, Pd/C, MeOH, rt; (g) Alicyclic amines, EDCI, HOBt, Et_3N, DMF, rt for **5g**, **5h** and *N*-Boc **5i**, then TFA, DCM, rt for **5i**.

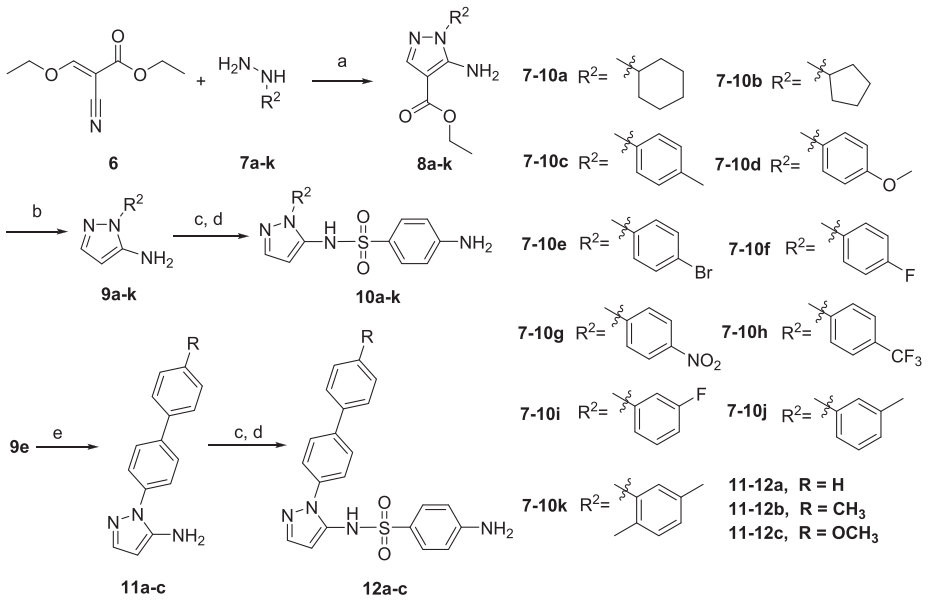
amino-1-phenylpyrazole **1** with various sulfonyl chlorides afforded intermediates **2** and **3c-f**, respectively. The desired products **5a-b** were obtained from **2** via cross-coupling reactions.[21,22] Hydrogenation of **3c-f** led to the desired products **5c-f**. Intermediate **4** was formed by the sulfonylation of **1** followed by simple hydrolysis. The condensation reactions of **4** with various alicyclic amines afforded the corresponding products **5g-h** in the presence of EDCI (*N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride) and HOBt (1-hydroxybenzotriazole) at room temperature. The condensation of **4** with Boc-piperazine followed by deprotection of Boc group provided the target compound **5i** (Scheme 1).

Ethyl (*E*)-2-cyano-3-ethoxyacrylate **6** was treated with hydrazines **7a-k** to form the pyrazole intermediates **8a-k**. Decarboxylation of **8a-k** in the presence of phosphoric acid afforded the intermediates **9a-k**,[23] and subsequent sulfonylation with 4-acetamidobenzene sulfonyl chloride followed by the hydrolysis of acetyl group provided the target compounds **10a-k**. Intermediate **9e** was subjected to Suzuki cross-coupling conditions with the corresponding phenylboronic acids to afford the desired biphenyl intermediates **11a-c**. The subsequent sulfonylation of **11a-c** followed by hydrolysis of the acetyl group generated the target compounds **12a-c** (Scheme 2).

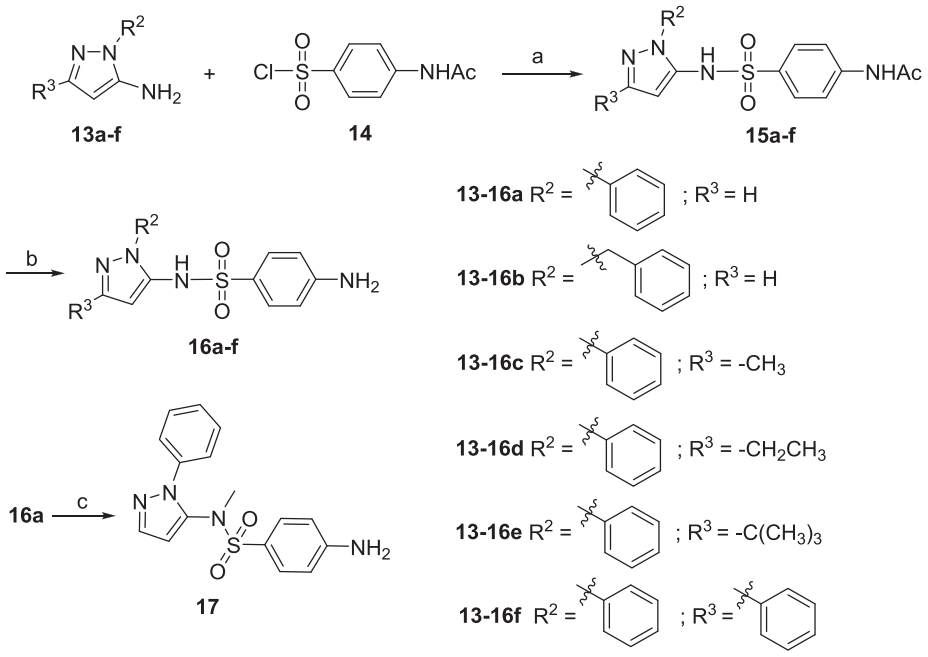
Sulfonylation of intermediates **13a-f** with 4-acetamidobenzene sulfonyl chloride **14** afforded intermediates **15a-f**, which following hydrolysis provided the desired compounds **16a-f**. Methylation of **16a** (SPA) with methyl iodide generated the target compound **17** (Scheme 3).

Various aryl amines were treated with 4-acetamidobenzene sulfonyl chloride **14** followed by the hydrolysis of acetyl group to produce the target compounds **18a-g** (Scheme 4).

The synthesized library was screened for activity against *M. tuberculosis* $H_{37}Rv$ and selected compounds were tested against an XDR-TB isolated clinical strain. The minimum inhibitory concentration



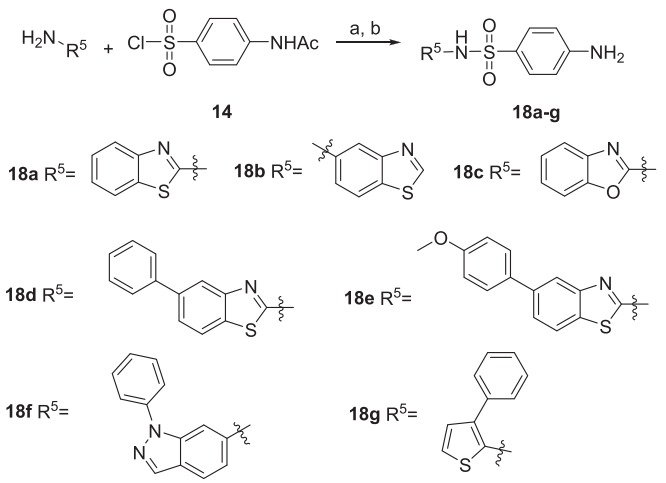
Scheme 2. Synthesis of the target compounds **10a-k** and **12a-c**. Reagents and conditions: (a) EtOH, reflux; (b) H_3PO_4, 170 ℃; (c) 4-Acetamidobenzene sulfonyl chloride, pyridine, reflux; (d) 2 N NaOH aqueous solution, reflux; (e) Phenylboronic acids, Na_2CO_3, $PdCl_2$(dppf), dioxane/H_2O, reflux.



Scheme 3. Synthesis of the target compounds **16a-f** and **17**. Reagents and conditions: (a) 4-Acetamidobenzene sulfonyl chloride, pyridine, reflux; (b) 2 N NaOH aqueous solution, reflux. (c) CH_3I, Cs_2CO_3, MeCN, rt.

(MIC) was defined as the lowest concentration effecting a reduction in fluorescence of ≥90% relative to the mean of replicate bacterium-only controls.[24] Tables 1–3 summarize the MIC data for all synthesized sulfonamide derivatives. Selected compounds were further tested activity against one drug-resistant TB strain and mammalian cell cytotoxicity using Vero cells measured at a concentration inhibiting 50% cell growth (IC_{50}) as compared to a no-treatment control in Table 5. Sulfaphenazole (SPA) and isoniazid (INH) were used as the reference compounds.

Our initial MIC-based SAR studies against *M. tuberculosis* around the hit compound SPA focused on exploring the 4-aminobenzenesulfonamide moiety on antitubercular activity. As shown in Table 1, the replacement of the terminal amino group with the secondary amine in **5a** as well as the bulky cyclic amine in **5b** resulted in the loss of potency. Compared to the parent SPA, an amino group at the meta-position displayed a markedly decreased potency against *M. tuberculosis* (**5c**), while the introduction of a methyl group at the R^4 site (**17**) also resulted in a drop in potency. Furthermore, a phenyl group was not tolerated on the 4-aminobenzenesulfonamide moiety (**5d**). The introduction of electronically dissimilar substituents, via the installation of a methoxy or fluoro substituent on the phenyl ring, led to inactive compounds (**5e** and **5f**). To improve the potency and avoid CYP 2C9 inhibition by blocking the NH_2 group binding to CYP 2C9 iron, we attached benzamide fragment with specific lipophilicity at the R^1 site to produce compounds **5g-i** and to our delight, compound **5g** showed moderate antimycobacterial activity (MIC = 30.15 μg/mL). A bioisosteric replacement strategy to replace the methylene (**5g**) with sulfur or nitrogen heteroatom (**5h** and



Scheme 4. Synthesis of the target compounds **18a-g**. Reagents and conditions: (a) 4-Acetamidobenzene sulfonyl chloride, pyridine, reflux; (b) 2 N NaOH aqueous solution, reflux.

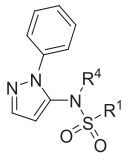
5i) caused a loss of potency. In addition, the acetylation of the amino group (**15a**) also led to a drop in potency. The above results clearly indicate that the 4-aminobenzenesulfonamide moiety plays a key role in maintaining antimycobacterial activity.

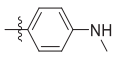
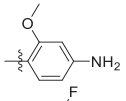
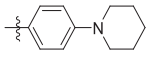
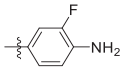
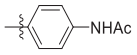
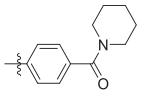
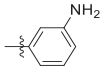
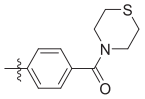
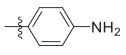
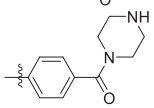
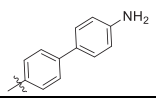
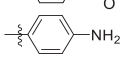
Keeping the 4-aminobenzenesulfonamide moiety as the side chain, we then evaluated the effects of various substituents at the R^2 site. As shown in Table 2, the replacement of benzene in SPA with cyclohexane (**10a**) and cyclopentane (**10b**) led to an obvious decrease in activity. We then introduced various substituents onto the phenyl ring to more deeply explore the SAR. Compounds bearing methyl and methoxy groups on the phenyl ring (**10c** and **10d**) showed good anti-mycobacterial activities (MIC = 7.70 and 7.92 μg/mL), and similar to the parent SPA. Introducing a fluoro substituent onto the phenyl ring afforded compound **10f** with a MIC of 7.94 μg/mL, which showed better potency than that of **10e** with a bromo substituent at the same position. Nitro substituted compound **10g** displayed moderate activity with a MIC of 10.31 μg/mL, while the introduction of a trifluoromethyl group (**10h**) led to the loss of potency. We then turned our attention onto the position of substitutions, with compound **10i,** bearing a meta-fluoro group, demonstrating equal activity to that of para-position **10f** (MIC = 7.93 vs 7.94 μg/mL). In addition, compound **10j** with meta-methyl group, demonstrated moderate activity (MIC = 12.22 μg/mL) while the introduction of the larger dimethyl group (**10k**) led to the loss of potency. The addition of a simple phenyl group caused an obvious decrease in activity (**12a-c**). Finally, we observed that introduction of a methylene bridge between the pyrazole and phenyl ring (**16b**) was not tolerated.

Since the substitution at the R^2 site has been investigated in detail, our attention shifted to exploring substitution at the R^3 site. As shown in Table 2, a methyl substituent at the pyrazole ring (**16c**), provided a moderately active derivative with a MIC of 15.38 μg/mL. The introduction of larger substitutions, as exemplified by ethyl, *tert*-butyl and phenyl groups, led to inactive compounds (**16d-f**).

We then turned our attention onto replacing the phenyl pyrazole scaffold with various aromatic rings via a truncated scaffold hopping strategy. As shown in Table 3, compound benzothiazole **18a** demonstrated moderate activity (MIC = 14.26 μg/mL), while altering the position in which the phenyl ring is attached (**18b**) led to the loss of potency. The replacement of benzothiazole (**18a**) with benzoxazole (**18c**) resulted in a loss of potency. Introduction of phenyl groups derived from **18a**, such as **18d** and **18e**, led to an obvious decrease in activity. Furthermore, addition of a phenyl ring between the pyrazole and sulfonamide was not tolerated (**18f**). Replacing the pyrazole motif in sulfaphenazole with thiophene (**18g**) caused a loss of antimycobacterial activity. The above results indicated that the pyrazole core directly connected with 4-aminobenzenesulfonamide moiety is favor of anti-TB potency.

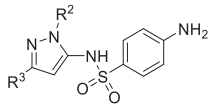
Table 1
SAR of sulfonamide compounds at R^1 and R^4 sites.

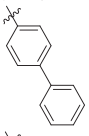
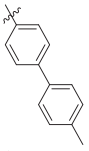
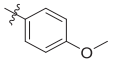
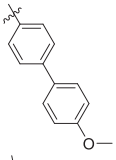
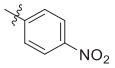
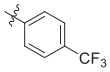
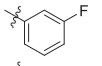


Compds.	R^1	R^4	MIC[a] (μg/mL)	Compds.	R^1	R^4	MIC[a] (μg/mL)
5a		H	>32	**5e**		H	>32
5b		H	>32	**5f**		H	>32
15a		H	>32	**5g**		H	30.15
5c		H	>32	**5h**		H	>32
17		CH_3	>32	**5i**		H	>32
5d		H	>32	**SPA**		H	5.51

[a] MIC against *M. tuberculosis* $H_{37}Rv$.

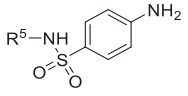
Table 2
SAR of sulfonamide compounds at R^2 and R^3 sites.

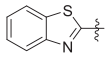
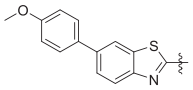
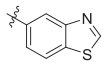
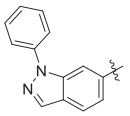
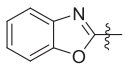
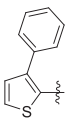
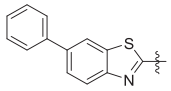
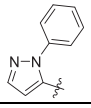


Compds.	R^2	R^3	MIC[a] (μg/mL)	Compds.	R^2	R^3	MIC[a] (μg/mL)
10a		H	>32	**10k**		H	>32
10b		H	>32	**12a**		H	>32
10c		H	7.70	**12b**		H	31.04
10d		H	7.92	**12c**		H	>32
10e		H	14.54	**16b**		H	>32
10f		H	7.94	**16c**		CH_3	15.38
10g		H	10.31	**16d**		CH_2CH_3	>32
10h		H	>32	**16e**		$C(CH_3)_3$	>32
10i		H	7.93	**16f**			>32
10j		H	12.22	**SPA**		H	5.51

[a] MIC against *M. tuberculosis* $H_{37}Rv$.

Table 3
SAR of sulfonamide compounds at R^5 site.



Compds.	R^5	MIC[a] (μg/mL)	Compds.	R^5	MIC[a] (μg/mL)
18a		14.26	**18e**		>32
18b		>32	**18f**		>32
18c		>32	**18g**		>32
18d		>32	**SPA**		5.51

[a] MIC against *M. tuberculosis* $H_{37}Rv$.

Table 4
CYP 2C9 inhibition of the selected compounds.

Compds.	CYP 2C9	
	Inhibition (%)[a]	IC_{50} (μM)
5g	0.2	–
10c	52.3	–
10d	9.1	11.5
10f	45.7	–
12b	2.2	–
16c	45.0	–
18a	4.8	–
SPA	48.3	0.63

[a] Test concentration: 0.5 μM.

The selected sulfonamide compounds with anti-TB potency and the reference compound SPA were further evaluated for inhibition of CYP 2C9 (Table 4). The replacement of the free amino group with a benzamide fragment (**5g**) led to a distinct loss of inhibition of CYP 2C9 as expected through negating the amine-metal binding event between the active compound and CYP 2C9. The compounds bearing relatively small substituents at R^2 and R^3 sites remained similar inhibitory activity against CYP 2C9 (**10c**, **10f**, **16c**) compared to SPA. To our delight, the involvement of methoxy (**10d**) or methylphenyl (**12b**) groups at R^2 site resulted in an obvious decrease of the inhibitory effects toward CYP 2C9 with <10% inhibition at the tested concentration of 0.5 μM. The introduction of benzothiazole at R^5 site (**18a**) also led to reduced inhibition of CYP 2C9. In particular, compound **10d** with good anti-TB activity showed a 18-fold reduction of CYP 2C9 inhibition relative to SPA (IC_{50} = 11.5 vs 0.63 μM). We therefore predict that compound **10d** will lead to low potential risk of drug-drug interactions.

Representative sulfonamide compounds **10c**, **10d**, **10f**, SPA and the reference compound INH were further tested for activity against XDR-TB as well as for cytotoxicity against Vero cells. As shown in Table 5, these compounds displayed equivalent potency against an XDR-TB isolated clinical strain compared to SPA. Additionally, compounds **10c**, **10d**, **10f** and SPA displayed very low cytotoxicity against Vero cells (IC_{50} > 64 μg/mL). The results indicated that sulfonamide compounds with good anti-TB activity have the potential as the component in a combination regimen for the treatment of TB including MDR or XDR-TB.

In summary, we report the design, synthesis and SAR of a series of sulfonamide compounds that have the potential to act as anti-TB agents. All synthesized compounds were evaluated for their *in vitro* activities against *M. tuberculosis* $H_{37}Rv$ and the established SAR indicated that the 4-aminobenzenesulfonamide moiety was most favorable for antimycobacterial activity. We observed that large structural modification of the sulfaphenazole core caused a significant loss of potency. More nuanced optimization, through substituted phenyl derivatives at the R^2 site on the pyrazole fragment resulted in the identification of compounds **10c**, **10d**, **10f** and **10i** with promising antimycobacterial activity. Moreover, compound **10d** containing a 4-methoxy phenyl fragment, demonstrated good *in vitro* activity against both drug-susceptible and drug-resistant tuberculosis, low cytotoxicity as well as low inhibition of CYP 2C9. These promising results laid the foundation for applying sulfonamide as the component in the combination regimen for the treatment of *M. tuberculosis*.

Table 5
In vitro activity of selected compounds against drug-resistant TB strain and Vero cells.

Compds.	MIC (μg/mL)		Vero IC_{50} (μg/mL)
	$H_{37}Rv$	14862[a]	
10c	7.33	11.93	>64
10d	5.69	10.62	>64
10f	6.88	10.88	>64
SPA	6.87	12.59	>64
INH	0.02	>10	–

[a] Resistance to isoniazid (INH), streptomycin (SM), rifampicin (RFP), ethambutol (EMB), paza-aminosalicylate (PAS), prothionamide (1321) and capreomycin (CPM).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by the CAMS Innovation Fund for Medical Sciences (CAMS-2016-I2M-1-010).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127924.

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