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Synthesis and antimycobacterial activity of some phthalimide derivatives

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ABSTRACT

Structurally modified phthalimide derivatives were prepared through condensation of phthalic and tetrafluorophthalic anhydride with selected sulfonamides with variable yields. All compounds were screened for their antimycobacterium activity against *Mycobacterium tuberculosis H37Ra* (ATCC 25177) using a micro broth dilution technique. The fluorinated derivatives (compounds **2c, 2d, 2f** and **2h**) had antimycobacterium activity comparable with classical sulfonamide drugs. The minimum inhibitory concentration (MIC) of compounds **2c, 2d, 2f** and **2h** was greater than that of isoniazid (MIC <0.02 μg/mL) and in vitro activity was greater than that of pyrazinamide, another first line antimycobacterium drug (MIC 50–100 μg/mL). The new compounds could be considered new lead compounds in the treatment of multi-drug resistant tuberculosis.

1. Introduction

Tuberculosis (TB) is an illness that results from infection with *Mycobacterium tuberculosis* (MTB). TB is responsible for millions of human deaths annually, claiming more lives than any other single infectious agent.[1,2] Almost one-third to one-half of the world's population is infected with *M. tuberculosis* and approximately 10% of infected individuals will experience active disease at some time in their life.[3] Currently, treatment for active TB includes at least 6 months of therapy with first line drugs such as, isoniazid, rifampin, pyrazinamide and ethambutol.[4–6] Failure of patients to complete a full treatment protocol and the outbreak of *acquired immune deficiency syndrome* (AIDS) has resulted in the emergence of multi-drug resistant (MDR-TB). Recent estimates show that 10% of all new TB infections are resistant to at least one anti-TB drug. Subsequently, the World Health Organization (WHO) has declared TB a global public health emergency.[7] For multi-drug resistance (MDR) and extensively drug resistance (XDR) are used the combination of first line drugs and second line drugs as aminoglycosides, fluoroquinolones, thioamides, cycloserine and *p*-amino salicylic acid.[8] Since the discovery of rifampicin in 1960, there is no more drugs developed to treat tuberculosis. Third line drugs include rifabutin, clarithromicin, linezolid, thiacetone, arginine and vitamin D are still being developed, have less or unproven efficacy and are very expensive.[9] MTB have the cell with a high lipid content which results in a high degree of lipophilicity and resistance to alcohol, acids, alkali and some disinfectants.[10]

Molecular modification is a chemical alteration in a molecule which could be lead compound or a drug aiming to enhance its pharmaceutical, pharmacokinetic or pharmacodynamics. Among the molecular modification used prodrug approach, molecular hybridization and bioisosterism are common methods.[10]

There are several prodrug and hybride compounds with increased lipophilic properties have demonstrated and possess high activity than pyrazinamide against *M. tuberculosis*.[11,12] Other fruitful example is to use molecular hybridization of isoniazide and one quinolone derivative to increases the antimycobacterial activity of the novel compounds.[13] Similar molecular hybridization approach was also performed using a fluoroquinole derivative and pyrazinamide through Mannich bases. The resulting compound demonstrated higher log*P* than pyrazinamide. The log*P* is an important property to be evaluated in novel effective compounds due to the lipophilic charastic of the *M. tuberculosis*.[14]

Phthalimide subunit is important drug candidates with varying biological activities against diseases such as, leprosy, AIDS, cancer, inflammation, multiple myeloma, and became important as COX inhibitors, antidepressants, histon deacetylase inhibitors.

Synthetic compounds, containing a phthalimide subunit also have antimicrobial potential.[15–24] Moreover the pharmaceutical importance of sulfa drugs (sulfonamides) is well established. As early as 1933, sulfonamides were among the first synthetic antimicrobial agents, they are still in use today.[23–25] Dapson (diaminodiphenyl sulfone), has been used for decades to treat leprosy and

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sulfamethoxazole has been shown to have in vitro effects against *M. avium* and *M. introcellulare*.[26,27] Sulfonamides have also been used as carbonic anhydrase inhibitors, anti-cancer and anti-inflammatory agents. In recent years, molecular hybridization of the phthalimide subunit present in thalidomide and sulfonamide drugs has led to the development of antimicrobial compounds against *M. leprea* and *M. tuberculosis*.[25–31]

Considering these results, hybride compounds were prepared having a phthalimide and tetrafluorophthalimide moiety with some sulfonamide drugs in order to increase lipid solubility of the sulfonamide drugs (compounds **1a–2h**) and evaluate their anti-mycobacterial activity against *M. tuberculosis H37Ra* (ATCC 25177). Cytotoxicity was also evaluated with a L929 cell line.

2. Results and discussion

2.1. Chemistry

Compounds were prepared via a condensation reaction between commercially available phthalic anhydride and 4,5,6,7-tetrafluorophthalic anhydride and corresponding sulfonamide derivatives under reflux for 3–4 h in acetic acid with high yield. Then 20 mL of distilled water was added to the reaction medium and the compounds filtered and recrystallized in ethanol.

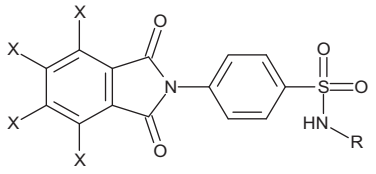
Compound physical properties are described in Table 1. Yields of compounds **2a–2h** were dramatically lower than those of compounds **1a–1c**, most likely because the electron withdrawing potency of fluorine atoms on the phenyl moiety decreases the potency of electrophilic center of the phthalimide ring. IR, ^{1}H NMR, ^{13}C NMR, mass spectroscopy and elementary analysis methods were used for structure elucidation (Scheme 1).

2.2. Antimycobacterial activity

The anti-mycobacterial activity of compounds (**1a–2h**) was tested against *M. tuberculosis H37Ra* (ATCC 25177). The minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for *M. tuberculosis H37Ra* were determined by a micro broth dilution method (Table 1). Compounds (**1a–2h**) were compared to the commercially available sulfonamide drugs, thalidomide and isoniazid (INH) under the same experimental conditions (Table 2).

Compounds, carrying a *N*-[4-(4,5,6,7-tetrafluoro-1,3-dioxo-isoindolin-2-yl)phenyl] basic structure (**2a–2h**), were moderately anti-mycobacterial. Compounds **2c, 2d, 2f** and **2h** inhibited *M. tuberculosis* growth at a concentration of 32 μl/mL. However the antimycobacterial activity of the compounds **1a–1c** was dramatically decreased compared to the activity of sulfonamide drugs.

Table 1
Physical constants and in vitro anti-mycobacterial activities of newly developed compounds (**1a–2h**) and *M. tuberculosis H37Ra* (ATCC 25177)



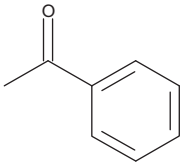
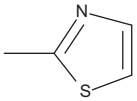
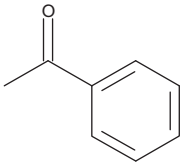
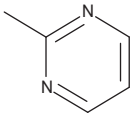
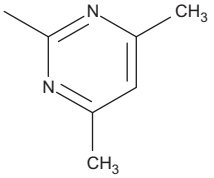
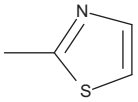
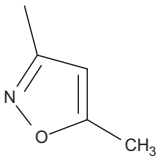
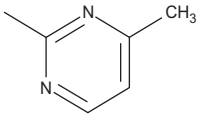
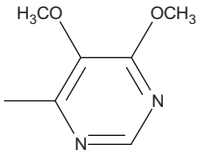
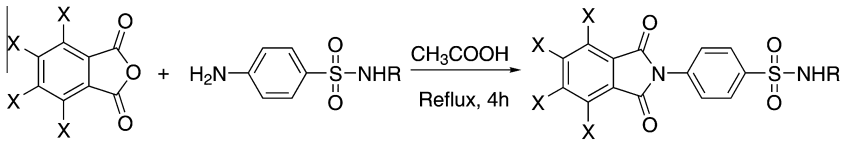
Compound no.	X	R	Yield (%)	Mp(°C)	MIC (μg/mL)	MBC (μg/mL)	*C*Log *P*[a]
1a	H	–$COCH_3$	90	255.9	256	256	0.502
1b	H		90	245.7	128	256	3.241
1c	H		92	287	256	256	2.204
2a	F	–$COCH_3$	40	260.5	64	128	0.904
2b	F		35	264.5	64	128	3.643
2c	F		21	>300	32	64	1.979
2d	F		17	246.9	32	128	2.977

Table 1 (*continued*)

Compound no.	X	R	Yield (%)	Mp(°C)	MIC (μg/mL)	MBC (μg/mL)	*C*Log*P*[a]
2e	F		41	>300	64	64	2.606
2f	F		26	>300	32	128	2.443
2g	F		35	>300	128	256	2.478
2h	F		11	266	32	64	3.111

[a] *C*Log*P* values were calculated by using Chembiodraw v;11.0 (Chembridgesoft, Cambridge, MA, USA).



Scheme 1. General synthesis of the compounds. For compounds **1a–c** X = H and **2a–h** X = F.

Table 2
In vitro anti mycobacterial activities of thalidomide, sulfa drugs and isoniazid a*gainst M. Tuberculosos H37Ra* (ATCC 25177)

Compound	MIC (μg/mL)	MBC (μg/mL)	*C*Log*P*
Thalidomide	128	256	0.528
Sulfacetamide (S1)	64	256	−0.976
Sulfabenzamide (S2)	32	256	1.763
Sulfadiazine (S3)	8	128	0.099
Sulfamethazine (S4)	32	256	1.097
Sulfathiazole (S5)	4	16	0.726
Sulfamethoxazole (S6)	32	256	0.563
Sulfamerazine (S7)	32	256	0.598
Sulfadoxine (S8)	32	256	1.231
Isoniazid	<0.2		−0.668
Pyrazinamide	50–100		−0.676

Our results show that anti-mycobacterial activity of fluorinated phthalimide hybrid molecules is similar to the sulfonamide drugs. Furthermore, isosteric replacement of R groups did not affect anti-mycobacterial activity. Although the MIC values obtained here are greater than those of isoniazid (MIC <0.02 μg/mL), compounds **2c, 2d, 2f** and **2h** showed greater in vitro activity than pyrazinamide, which is another first line drug (MIC of 50–100 μg/mL).[30]

2.3. In vitro cytotoxicity evaluation

A cell line routinely used for cytotoxicity assessments, L929 mouse fibroblast cell line, was used to test the cytotoxicity of compounds (**1a–2h**) at a concentration of 10^{-4} M (Fig. 1).[31,32] Compounds **2d, 2h** and **2f** were more toxic in L929 cells than compounds**2a, 2b, 2c, 2e, 1a, 1b** and **1c**. Compound **2f** showed relatively high toxicity on the viability of L929 cells, followed by compound 2 h, (less than 50%). These results are encouragingly parallel to the anti-tuberculosis data presented in Table 1.

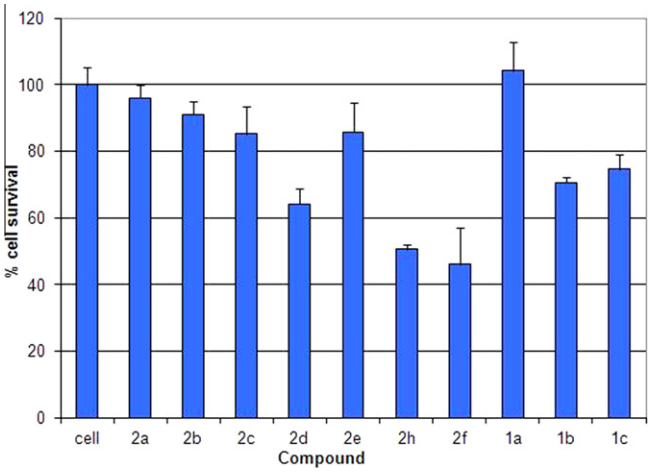


Figure 1. Cytotoxicity of compounds **1a–2h** in L929 mouse fibroblast cells at a concentration of 10^{-4} M.

3. Conclusions

MTB is epidemiologically characterized by high rate infectivity. Since drug resistance to classic anti-TB drugs is increasing at an alarming rate and *M. tuberculosis* continues to be one of the single most infectious agents with the highest morbidity and mortality, new drugs to treat TB are urgently needed.

In this study, new sulfonamide-phthalimide and sulfonamide-tetrafluorophthalimide compounds were prepared as potential anti-mycobacterium agents. The fluorinated compounds (**2c, 2d, 2f** and **2h**) had comparable anti-mycobacterial activity as that of the classic sulfa drugs. Lipophilicity is an important physico-chemical property, which affects the capacity of the compounds to cross membrane. Lipophilicity of compounds (**1a**–**2h**) was higher than drugs such as isoniazide and pyrazinamide.[30] On the other hand hybridization of phthalimide subunit with sulfonamide drugs decreased the antitubercular effect when compared with the correspondent sulfonamides. In this case, increasing log*P* value is not good enough to provide better activity. Maybe these hybrid compounds could not interact well with the target receptors. Further modifications may increase their potential as anti-TB drug candidates.

We measured also whether these potential drug candidates caused significant cytotoxicity in the L929 cell line as a control. When compounds were tested for cytotoxicity in L929 cells at a concentration of 10^{-4} M, compounds **2c, 2d, 2f, 2h** were the most toxic. Further studies such as the TNF-α inhibition properties and antiproliferative activity of the compounds we developed are in progress.

4. Experimental

All materials were commercially available and used without further purification. 4,5,6,7-Tertafluorophthalic anhydride, phthalic anhydride, sulfacetamide, sulfamethoxazole, sulfadiazine and sulfadoxin were purchased from Sigma–Aldrich (St. Louis, MO, USA) Sulfamethazine, sulfathiazole and sulfabenzamide were obtained from Alfa Aesar (Ward Hill, MA, USA) and Acros Organics (New Jersey, USA). Melting points were detected with a Mettler-Toledo FP-62 melting point apparatus (Columbus, USA) and are uncorrected. IR spectra (KBr) were recorded on a Perkin Elmer 1720X FT-IR spectrometer (Beaconsfield, UK). ^{1}H and ^{13}C NMR spectra were obtained with a Varian Mercury 400, 400 MHz FT NMR using DMSO-d_6 as the solvent and tetramethylsilane as an internal standard. All chemical shift values (δ) were recorded in ppm. LC/MS analyses were performed with Waters Alliance and Micromass ZQ by using ESI(+) or ESI(−) technique.

Compound purity was monitored by thin-layer chromatography on silica gel-coated aluminum sheets (Merck, 1.005554, silica gel HF254-361, Type 60, 0.25 mm; Darmstadt, Germany). Elemental analysis of compounds was performed with a LECO CHNS 932 analyzer (LECO Corp., Michigan, and USA). Elemental analysis for C, H, and N were within ±0.4% of theoretical values. ^{1}H and ^{13}C NMR spectra, mass spectroscopy and elemental analyses were performed at the Central Analysis Laboratory of Ankara University, Faculty of Pharmacy, Ankara, Turkey.

4.1. General procedure A: Synthesis of phthalimide derivatives (1a–1c)

The compounds were prepared through condensation reaction between 0.001 mol phthalic anhydride and 0.001 mol sulfonamides in 10 mL acetic acid under reflux at 120 °C for 3–4 h. Then 20 mL distilled water was added into the reaction media. The compounds were filtered and recrystallized in ethanol.[30]

4.2. General procedure B: Synthesis of 4,5,6,7-tetrafluorophthalimide derivatives (2a–2h)

The compounds were prepared through condensation reaction between 0.001 mol tetrafluorophthalic anhydride and 0.001 mol sulfonamides in 5 mL acetic acid under reflux at 120 °C for 3–4 h. Then 20 mL distilled water was added into the reaction media. The compounds were filtered and recrystallized in ethanol.

4.2.1. *N*-(4-(1,3-Dioxoisoindolin-2-yl)phenylsulfonyl)acetamide (1a)

The compound was prepared using general procedure A. Yield 90%, mp: 255.5 °C (lit., mp: 213–215 °C).[33] FT-IR (KBr), cm^{-1}: 3246 (N-H, st), 3050, (C–H, aromatic, st), 2848 (C–H, aliphatic, st), 1745 and 1708 (C=O, imide, st), 1695 (C=O amide) 1370 and 1125 (SO_2, st). ^{1}H NMR (DMSO-d_6), δ: 1.96 (3H, s, $COCH_3$); 7.73 (2H, dd, J = 8 Hz, H2', H3'); 7.90 (2H, d, J = 8 Hz, H2, H3); 8.16 (4H, m, H1', H4', H1, H4); 12.23 (s, 1H, NH). ^{13}C NMR (400 MHz, DMSO-d_6), δ: 168.9; 166.4; 138.1; 136.2; 134.8; 131.4; 128.2; 127.3; 123.6; 23.2 ppm. MS ESI (−) m/z: 343.8 (%100) (M−H). Calcd for $C_{16}H_{12}N_2SO_5$: C, 55.81; H, 3.48; N, 8.14; S, 9.30. Found: C, 55.61; H, 3.446; N, 8.24; S, 9.17.

4.2.2. *N*-Benzoyl-4-(1, 3-dioxoisoindolin-2-yl)benzenesulfonamide (1b)

The compound was prepared using general procedure A. Yield 93%, mp: 245.7 °C. FT-IR (KBr), cm^{-1}: 3269 (N–H, st), 3064 (C–H, aromatic, st), 1735 and 1693 (C=O, imide, st), 1682 (C=O amid), 1377 and 1162 (SO_2, st). ^{1}H NMR (DMSO-d_6), δ: 7.50 (5H, m, H2, H3, H7, H6', H5'); 7.75 (2H, d, J = 7 Hz, H1', H3'); 7.95 (4H, m, H2', H3', H5', H9'); 8.16 (2H, dd, H1, H4); 10.82 (s, 1H, -NH). ^{13}C NMR (400 MHz, DMSO-d_6), δ: 166.4; 165.5; 138.1; 136.3; 134.8; 133.28 131.3; 131.2; 128.5; 128.4; 128.3; 127.2; 123.5 ppm. MS ESI (−) m/z: 405.7 (%100) (M−H). Calcd for $C_{21}H_{14}N_2SO_5$: C, 62.07; H, 3.44; N, 6.89; S, 7.88. Found: C, 62.02 H, 3.49; N, 7.04; S, 7.82.

4.2.3. 4-(1,3-Dioxoisoindolin-2-yl)-*N*-(2-thiazolyl)benzene sulfonamide (1c)

The compound was prepared using general procedure A. Yield 92%, mp: 287 °C. FT-IR (KBr), cm^{-1}: 3063 (C–H, aromatic, st), 1750 and 1710 (C=O, imide, st), 1375 and 1145 (SO_2, st). ^{1}H NMR (DMSO-d_6), δ: 6.80 (1H, d, J = 3 Hz, H2'); 7.29 (1H, d, J = 3 Hz, H1'); 7.61 (2H, d, J = 7 Hz, H2, H3); 7.95(2H, d, J = 8 Hz, H6, H7); 7.85 (2H, d, J = (7 Hz, H5, H8); 8.00 (2H, d, J = 7 Hz, H1, H4); 10.27 (s, 1H, –NH). ^{13}C NMR (400 MHz, DMSO-d_6), δ: 168.9; 166.5; 141.3; 134.8; 134.7; 131.3; 127.3; 126.3; 124.5; 123.4; 108.7 ppm. MS ESI (−) m/z: 384.7 (%100) (M−H). Calcd for $C_{17}H_{11}N_3S_2O_4$: C, 52.99; H, 2.85; N, 10.90; S, 16.62. Found: C, 53.34; H, 2.96; N, 11.11; S, 16.25.

4.2.4. *N*-[4-(4,5,6,7-Tetrafluoro-1,3–dioxo-isoindolin-2-yl) phenyl]sulfonylacetamide (2a)

The compound was prepared using general procedure B. Yield = 39.49%, mp: 260.5 °C. FT-IR (KBr), cm^{-1}: 3247 (N–H, st), 3115 (C–H, aromatic, st), 2844 (C–H, aliphatic, st), 1763 and 1723 (C=O, imide, st), 1705 (C=O amide), 1375 and 1158 (SO_2, st). ^{1}H NMR (DMSO-d_6), δ: 1.96 (3H, s, $COCH_3$); 7.70 (2H, dd, J = 8 Hz, H1, H4); 8.10 (2H, m, H2, H3); 12.23 (s, 1H, NH). ^{13}C NMR (400 MHz, DMSO-d_6), δ: 169.7; 162.0; 146.2; 144.6; 143.6; 142.1; 139.6; 135.9; 129.2; 128.2; 114.6; 23.9 ppm. MS ESI (−) m/z: 415.6 (%100) (M−H). Calcd for $C_{16}H_8F_4N_2SO_5$: C, 46.15; H, 1.92; N, 6.73; S, 7.69. Found: C, 45.73; H, 2.08; N, 6.76; S, 7.77.

4.2.5. *N*-Benzoyl-4-(4,5,6,7-tetrafluoro-1,3-dioxo–isoindolin-2-yl)benzensulfonamide (2b)

The compound was prepared using general procedure B. Yield = 35.26%, mp: 264.5 °C. FT-IR (KBr), cm^{-1}: 3344 (N–H, st) , 3107 (C–H, aromatic, st), 1775 and 1723 (C=O, imide, st), 1373 and 1163 (SO_2, st). ^{1}H NMR (DMSO-d_6), δ: 7.78 (2H, t; J = 7.6 Hz, H3', H4'); 7.65 (1H, t; J = 7.5 Hz, H2'); 7.76 (2H, dd; J = 6.8 Hz, H1', H4'); 7.90 (2H, dd; J = 8.1 Hz, H1, H4) ;8.19 (2H, dd; J = 8.4 Hz, H2, H3). ^{13}C NMR (400 MHz, DMSO-d_6), δ: 172.7;

166.0; 162.4; 152.3; 148.6; 147.6; 145.1; 139.6; 136.6; 134.5; 133.2; 131.9; 129.2; 128.2; 127.6; 114.6 ppm. MS ESI (+) *m*/*z*: 478.3 (%100) (M+H). Calcd for $C_{21}H_{10}F_4N_2SO_5$: C, 52.72; H, 2.09; N, 5.85; S, 6.69. Found: C, 52.62; H, 2.23; N, 5.87; S, 6.58.

4.2.6. 4-(4,5,6,7-Tetrafluoro-1,3-dioxo-isoindolin-2-yl)-*N*-pyrimidin-2-yl-benzenesulfonamide (2c)

The compound was prepared using general procedure B. Yield = 21.37%, mp: >300 °C FT-IR (KBr), cm^{-1}: 3241 (N–H, st), 3034 (C–H, aromatic, st), 1793 and 1738 (C=O, imide, st), 1347 and 1164 (SO_2, st). ^{1}H NMR (DMSO-d_6), δ: 7.01 (1H, t, J = 5 Hz, H6'); 7.80 (2H, d J = 7 Hz, H1, H4); 7.89 (2H, d, J = 7 Hz, H2, H3); 8.49 (2H, m, H5', H7'); 11.19 (s, 1H, NH). ^{13}C NMR (400 MHz, DMSO-d_6), δ: 170.7; 163.0; 158.1; 151.2; 148.6; 147.6; 145.1; 139.6; 136.5; 131.9; 129.2; 125.6; 114.6 ppm. MS ESI (+) *m*/*z*: 453.2 (%100) (M+H). Calcd for $C_{18}H_8F_4N_4SO_4$: C, 47.79; H, 1.77; N, 12.39; S, 7.08. Found: C, 47.98; H, 2.03; N, 12.52; S, 7.14.

4.2.7. *N*-(4,6-Dimethylpyrimidin–2-yl)-4-(4,5,6,7-tetrafluoro-1,3-dioxo-isoindolin-2-yl) benzenesulfonamide (2d)

The compound was prepared using general procedure B. Yield = 16.77%, mp: 246.9 °C. FT-IR (KBr), cm^{-1}: 3068 (C–H, aromatic, st), 2981 (C–H, aliphatic, st), 1765 and 1715 (C=O, imide, st), 1371 and 1142 (SO_2, st). ^{1}H NMR (DMSO-d_6), δ: 2.26 (6H, s, CH_3); 6.76 (1H, s, pyr-H); 7.63 (2H, d, J = 8 Hz, H1, H4); 8.14 (2H, m, H2, H3). ^{13}C NMR (400 MHz, DMSO-d_6), δ: 166.5; 162.2; 155.9; 147.6; 144.4; 140.3; 139.2; 135.6: 134.8; 131.4; 128.5; 126.7; 123.5; 109.5; 23.3 ppm. MS ESI (+) *m*/*z*: 481.3 (%100) (M+H). Calcd for $C_{20}H_{12}F_4N_4SO_4$: C, 50.00; H, 2.50; N, 11.67; S, 6.67. Found: C, 49.63; H, 2.45; N, 11.64; S, 6.82.

4.2.8. 4-(4,5,6,7-Tetrafluoro-1,3-dioxo-isoindolin-2-yl)-*N*-thiazol-2-yl-benzene sulfonamide (2e)

The compound was prepared using general procedure B. Yield 41.20%, mp: >300 °C. FT-IR (KBr), cm^{-1}: 3104 and 3017 (C–H, aromatic, st), 1765 and 1728 (C=O, imide, st), 1382 and 1728, 1151 (O_2, st). ^{1}H NMR (DMSO-d_6), δ: 6.87 (1H, d J = 3 Hz, H6); 7.30 (2H, d, J = 8 Hz, H1, H4); 7.60 (2H, d, J = 8 Hz, H2, H3); 7.98 (1H, d, J = 3 Hz, H5). ^{13}C NMR (400 MHz, DMSO-d_6), δ: 170.97; 168.50; 150.4; 147.6; 144.4; 140.3; 141.34; 139.2; 134.9; 131.38; 127.34; 123.48; 108.7 ppm. MS ESI (+) *m*/*z*: 458.4 (%100) (M+H). Calcd for $C_{17}H_7F_4N_3S_2O_4$: C, 44.64; H, 1.53; N, 9.19; S, 14.00. Found: C, 44.41; H, 1.74; N, 9.20; S, 13.78.

4.2.9. *N*-(5–Methylisoxazol-3–yl)-4-(4,5,6,7-tetrafluoro-1,3-dioxo-isoindolin-2-yl) benzenesulfonamide (2f)

The compound was prepared using general procedure B. Yield 25.90%, mp: >300 °C. FT-IR (KBr), cm^{-1}: 3105 (C–H, aromatic, st), 2995 (C–H, aliphatic, st), 1783 and 1714 (C=O, imide, st), 1376 and 1170 (SO_2, st). ^{1}H NMR (DMSO-d_6), δ: 2.31 (3H, s, CH_3); 6.18 (1H, s, H1', H1, H4); 7.95 (2H, d, H1, H4); 8.05 (2H, m, H2, H3); 11.63 (s, 1H, NH). ^{13}C NMR (400 MHz, DMSO-d_6), δ: 178.2; 171.7; 162.00; 158.05; 155.10; 145.20; 144.66; 142.20; 139.78; 135.70; 121.2; 96.3; 12.76 ppm. MS ESI (+) *m*/*z*: 456.8 (%100) (M+H). Calcd for $C_{18}H_9F_4N_3SO_5$: C, 47.47; H, 1.76; N, 8.20; S, 7.03. Found: C, 47.06; H, 2.01; N, 8.10; S, 7.05.

4.2.10. *N*-(4-Methylpyrimidin-2-yl)-4-(4,5,6,7-tetrafluoro-1,3-dioxo-isoindolin-2-yl) benzenesulfonamide (2g)

The compound was prepared using general procedure A. Yield 35%, mp: >300 °C. FT-IR (KBr), cm^{-1}: 3299 (N–H, st), 3093 (C–H, aromatic, st), 2914 (C–H, aliphatic, st), 1718 and 1679 C=O, imide, st), 1365 and 1174 (SO_2, st). ^{1}H NMR (DMSO-d_6), δ: 2.30 (3H, s, –CH_3), 6.80 (1H, d, J = 5 Hz, H6); 7.85 (2H, d, J = 8 Hz, H1, H4); 8.01(2H, d, J = 8 Hz, H2, H3); 8.20 (1H, d, J = 8 Hz, H7). ^{13}C NMR (400 MHz, DMSO-d_6), δ: 166.8; 162.4; 156.9; 147.6; 144.2; 140.1; 138.9; 135.7; 133.8; 131.6; 129.0; 127.7; 124.5; 110.5; 23.4 ppm. MS ESI (+) *m*/*z*: 467.3 (%100) (M+H). Calcd for $C_{19}H_{10}F_4N_4SO_4$: C, 49.93; H, 2.15; N, 12.01; S, 6.87. Found: C, 50.20; H, 2.40; N, 11.97; S, 6.97.

4.2.11. *N*-(5,6-Dimethoxypyrimidin-4-yl)-4-(4,5,6,7-tetrafluoro-1,3-dioxo-isoindolin-2-yl) benzenesulfonamide (2h)

The compound was prepared using general procedure B. Yield 10.83%, mp: 266 °C. FT-IR (KBr), cm^{-1}: 3283 (N–H, st), 3098 and 3025 (C–H, aromatic, st), 2992 and 2941 (C–H, aliphatic, st), 1784 and 1719 (C=O, imide, st), 1376 and 1170 (SO_2, st). ^{1}H NMR (DMSO-d_6), δ: 3.72 (3H, s, OCH_3); 3.91 (3H, s, OCH_3); 7.66 (2H, d, J = 8 Hz H1, H4); 8.02 (2H, d, J = 8 Hz, H2, H3); 8.18 (1H, s, H7). ^{13}C NMR (400 MHz, DMSO-d_6), δ: 166.2; 163.4; 151.2; 150.4; 146.6; 145.9; 145.2; 142.1; 137.9; 135.7; 132.4; 130.6; 127.5; 124.5; 60.8; 58.1 ppm. MS ESI (+) *m*/*z*:.512.2 (%100) (M+H). Calcd for $C_{20}H_{12}F_4N_4SO_6$: C, 46.86; H, 2.34; N, 10.94; S, 6.25. Found: C, 46.82; H, 2.46; N, 10.67; S, 6.25.

4.3. Pharmacological activity procedure

4.3.1. Antimycobacterial activity

To evaluate the inhibitory efficiency of molecules on Mycobacterium tuberculosis, *M. tuberculosis H37Ra* (ATCC 25177), which is susceptible to all classical anti-TB drugs, was used. The minimal inhibitory concentration (MIC) for *M. tuberculosis H37Ra* for each compound was determined by a micro broth dilution method. All molecules tested were dissolved in dimethylsulfoxide and their ½ dilutions were prepared in 0.2 mL tubes using Middlebrook Broth 7H9. A few colonies from freshly grown *M. tuberculosis* H37Ra were suspended in Middlebrook Broth 7H9 to obtain 1.0 McFarland turbidity and diluted ten times using the same medium. 50 μl of this suspension was added to tubes containing 50 μl of medium with a different concentration of the tested molecule and to a positive control tube containing only Middlebrook Broth 7H9. A negative control tube, which was not inoculated, was also included in the test as a negative control to check that the incubation was completed without any contamination. The final concentration of the molecules ranged from 256 to 1 μg/mL. The tubes were placed in a 37 °C incubator and incubated until mycobacterial growth was clearly observed in the positive control tube as white sediment at the bottom of the tube. Mycobacterial growth was confirmed by preparing a smear from the sediment, staining by Kinyoun stain and observing acid fast staining bacilli. The tube that contained the lowest concentration of molecule which inhibited completely the growth was considered as MIC. After determination of MIC, to determine Minimal Bactericidal Concentration (MBC), 50 ml from each tube was inoculated on Löwenstein Jensen medium and the tubes were incubated at 37 °C until colonies were clearly visible in the positive control tube. The tube without any colonies that referred to the lowest concentration of tested molecule was considered as MBC.

4.3.2. In vitro cytotoxicity evaluation

Cytotoxic effects of samples were evaluated in L929 cell line with a MTT assay. The cell line was subcultured at a concentration of 3×10^3 cells/well in 96-well plates. The 96 well plates were read with Elisa plate reader at 570 nm. Each experiment was repeated three times. Cells were plated in 96-well plates at 3×10^3 cell per well in 100 μl of medium per well. Control wells were prepared by adding culture medium without cells. Wells were treated with synthesized samples for 24 h. A MTT assay was used to quantitate viable cells. Absorbance at 570 nm was recorded with an Elisa plate reader. Cell viability was expressed as the percent absorbance relative to that obtained for cells not exposed to the synthesized compounds.[31,32]

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