Synthesis and antimicrobial activity of 1-(benzo[b]thiophen-4-yl)-4-(2-(oxo, hydroxyl, and fluoro)-2-phenylethyl)piperazine and 1-(benzo[d]isothiazole-3-yl)-4-(2-(oxo, hydroxy, and fluoro)-2-phenylethyl)piperazine derivatives

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Abstract: Twenty-two compounds in a series of 1-(benzo[b]thiophen-4-yl)-4-(2-(oxo, hydroxy, and fluoro)-2-phenylethyl)piperazine and 1-(benzo[d]isothiazole-3-yl)-4-(2-(oxo, hydroxy, and fluoro)-2-phenylethyl)piperazine derivatives were synthesized through nucleophilic substitution reaction of phenacyl bromides with hetero arylpiperazine, reduction, and then fluorination. Compound K2 showed potent activity against gram-negative bacterial stain *P. aeruginosa* with minimum inhibitory concentration (MIC) value of 12.5 µg/mL. This compound showed better inhibitory activity than the standard drug chloramphenicol. K4 against *S. aureus*, H2 against *P. aeruginosa*, and F4 against *E. coli* showed good inhibitory activity with MIC values of 62.5 µg/mL. Compounds K1, K2, K4, K8, F1, and F3 showed good inhibitory activity against fungal stain *C. albicans* with MIC values of 250 µg/mL. The crystal structure of F1 was determined by single-crystal XRD (CCDC 1832090).

Key words: Piperazine, benzo[b]thiophene, benzo[d]isothiazole, synthesis, antimicrobial, crystal structure

1. Introduction

The adaptations of bacteria and fungi have continuously resulted in their resistance towards existing antibiotics. There is always a need for new antimicrobial substances, which must be active towards drug-resistant microorganisms. Piperazine derivatives are well-known nitrogen heterocycles that have shown diverse biological activities, such as antimicrobial,1–5 antiproliferative,6 and antidepressant.7 Antibiotic drugs like ciprofloxacin and eperezolid and antifungals like itraconazole, ketoconazole, and posaconazole are used for such purposes (Figure 1).

Recent advancement in biological activities, such as the antimalarial8 and antimicrobial activities9–11 of piperazine derivatives, attracted us towards piperazine-containing antimicrobial compounds. We were inspired by piperazine scaffolds that showed very good antimicrobial activity12–17 and a ciprofloxacin analogue found to be more potent than the drug itself18 (Figure 2).

In the present work we designed a prototype, shown in Figure 3, and checked its antimicrobial activity. We have designed and synthesized a series of piperazine derivatives based on the prototype and checked the antimicrobial activity against two gram-positive bacteria, two gram-negative bacteria, and one fungus.

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Figure 1. Antimicrobial and antifungal drugs with piperazine moiety.

Figure 2. Antimicrobial scaffolds with piperazine moiety.
2. Results and discussion

2.1. Chemistry

The synthesis of the target compounds was designed over three steps: nucleophilic addition of substituted phenacyl bromides (PB1 to PB4) with 1-(benzo[b]thiophen-4-yl)piperazine (PZ1) and 3-(piperazin-1-yl)benzo[d]isothiazole (PZ2) to obtain keto derivatives K1 to K8; reduction with sodium borohydride in ethanol to obtain hydroxyl derivatives H1 to H8; and finally fluorination with DAST to obtain fluoro derivatives F1 to F8. Compounds H2 and H6 did not yield respective fluoro compounds F2 and F6 under fluorination conditions with DAST. Structures of the synthesized compounds are demonstrated in Figure 4. All synthesized compounds were well characterized by $^1$H NMR, $^{13}$C NMR, and electron ionization mass spectroscopy (EI-MS) analyses. A well-known and interesting carbon ($^{13}$C) and fluorine ($^{19}$F) interaction in $^{13}$C NMR spectra during fluorination of hydroxy derivatives (H1–H8) to their consecutive fluoro derivatives (F1–F8) could be seen clearly. $^{19}$F is a highly abundant isotope of fluorine having spin 1/2. Thus, it can couple with carbon nuclei ($^{13}$C, also having spin 1/2), similarly to how two neighboring hydrogen nuclei couple in the $^1$H NMR spectrum. The $^{13}$C NMR pulse sequence without C-F decoupling shows C-F splitting in the carbon spectrum. The $-\text{CH OH}$ carbon peak of H1 at $\delta$68.46 shifts highly downfield to $\delta$93.29 ($\alpha$ carbon of $-\text{CH-F}$) in F1 with peak splitting due to coupling with a highly electronegative fluorine atom, showing high coupling constant $^1J_{CF} = 171.25$. The $\beta$ carbon also couples with fluorine, but with lower coupling constant $^2J_{CF} = 23.75$. Shifting of the $\beta$ carbon peak of $-\text{CH}_2\text{-CH OH}$ in compound H1 from $\delta$66.31 to $\delta$64.53 in F1 was observed. Similarly, in compound H3 to F3 the $\alpha$ carbon peak at $\delta$68.24 splits and shifts downfield to 92.93 (d, $^1J_{CF} = 172.5$) and the $\beta$ carbon peak at $\delta$66.27 splits and shifts to 64.55 (d, $^2J_{CF} = 22.5$). In compound H4 to F4 the $\alpha$ carbon peak at $\delta$68.28 splits and shifts downfield to 92.79 (d, $^1J_{CF} = 175.0$) and the $\beta$ carbon peak at $\delta$65.98 splits and shifts to 64.48 (d, $^2J_{CF} = 22.5$). In compound H5 to F5 the $\alpha$ carbon peak at $\delta$68.46 splits and shifts downfield to 93.34 (d, $^1J_{CF} = 171.25$) and the $\beta$ carbon peak at $\delta$66.38 splits and shifts to 64.49 (d, $^2J_{CF} = 23.75$). In compound H7 to F7 the $\alpha$ carbon peak at $\delta$68.24 splits and shifts downfield to 92.97 (d, $^1J_{CF} = 172.5$) and the $\beta$ carbon peak at $\delta$66.34 splits and shifts to 64.50 (d, $^2J_{CF} = 23.75$). In compound H8 to F8 the $\alpha$ carbon peak at $\delta$68.28 splits and shifts downfield to 92.84 (d, $^1J_{CF} = 175.0$) and the $\beta$ carbon peak at $\delta$66.04 splits and shifts to 64.42 (d, $^2J_{CF} = 23.75$).
Figure 4. 1-(Benzo[b]thiophen-4-yl)-4-(2-(oxo, hydroxy, and fluoro)-2-phenylethyl)piperazine and 1-(benzo[d]isothiazole-3-yl)-4-(2-(oxo, hydroxy, and fluoro)-2-phenylethyl)piperazine derivatives.

The $^{19}$F atoms present in the trifluoromethyl group (–CF$_3$) also interact with the adjacent $^{13}$C atom to produce a quartet. This phenomenon is observed due to spin-spin coupling of $^{19}$F and $^{13}$C atoms having 1/2 spin. Sometimes the –CF$_3$ quaternary carbon does not show this splitting due to the lack of large Overhauser enhancement by proton decoupling that the –CH carbons do. To see a clear quartet for the –CF$_3$ carbon, a larger number of accumulations are needed. In the $^{13}$C NMR spectra of K4 and H4, a quartet of –CF$_3$ is observed at δ125.65 and 125.38, respectively, due to carbon-fluorine interaction. The crystal of compound F1 was grown and characterized by single-crystal XRD. The structure of compound F1 is shown in Figure 5. Please see the supplementary data for spectra of the compounds.

The optimized conditions for the synthesis first involve nucleophilic substitution reaction of heteroaryl piperazines (PZ1 and PZ2) with substituted phenacyl bromides in acetonitrile, addition at 0–5 °C, and reaction at 20–25 °C for 6–8 h. Secondly, reduction of keto compounds is done in ethanol with NaBH$_4$, addition at
0–5 °C, and reaction at 20–25 °C for 10–12 h, and finally, fluorination of hydroxy compounds in dichloromethane at −5 to 0 °C for 2–4 h (Figure 6). The results are summarized in Table 1.

Table 1. Optimized conditions for reaction conversions.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Solvent</th>
<th>Temp (°C)</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
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<tbody>
<tr>
<td>Substitution</td>
<td>Acetonitrile</td>
<td>0 to ambient</td>
<td>6–8</td>
<td>95–98</td>
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<tr>
<td>Reduction</td>
<td>Ethanol</td>
<td>0 to ambient</td>
<td>10–12</td>
<td>88–94</td>
</tr>
<tr>
<td>Fluorination</td>
<td>DCM</td>
<td>−5 to 0</td>
<td>2–4</td>
<td>96–99</td>
</tr>
</tbody>
</table>

Figure 6. Synthesis of derivatives via nucleophilic substitution, reduction, and fluorination.
2.2. Antimicrobial activity

Antimicrobial activities of all synthesized compounds were evaluated against pathogenic gram-positive bacterial strains *Staphylococcus aureus* (MTCC96) and *Streptococcus pyogenes* (MTCC442), gram-negative bacterial strains *Escherichia coli* (MTCC443) and *Pseudomonas aeruginosa* (MTCC1688), and fungus *Candida albicans* (MTCC227) by broth microdilution methods. Ampicillin and chloramphenicol (antibacterials) and griseofulvin (antifungal) were used as standard drugs. Minimum inhibitory concentrations (MICs) of compounds against the above bacterial and fungal strains are summarized in Table 2.

Compounds (K1–K8, H1–H8, and F1–F8) were primarily screened for their in vitro antibacterial activity against gram-positive bacterial stains *Staphylococcus aureus* (SA, MTCC96) and *Streptococcus pyogenes* (SP, MTCC442) and gram-negative bacterial stains *Escherichia coli* (EC, MTCC443) and *Pseudomonas aeruginosa* (PA, MTCC1688) using the agar diffusion method. The antibiotics ampicillin and chloramphenicol were taken as positive controls. Antibacterial screenings of all compounds as well as positive controls were performed at concentrations of 1000 µg/mL, 500 µg/mL, and 250 µg/mL. All compounds showed antibacterial activity against both gram-positive and gram-negative bacterial strains. All compounds except H3, F7, and F8 showed antifungal activity against *Candida albicans* (CA, MTCC227).

In a secondary screening, all compounds were further diluted to 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL concentrations to determine MIC values by serial dilution method. K2 was found to be the most potent antibacterial agent (MIC 12.5 µg/mL) against PA, as it showed four times more activity than chloramphenicol against the PA strain (MIC 50 µg/mL). K4 (MIC 62.5) showed four times more activity than ampicillin (MIC 250 µg/mL) against the SA strain. F2 (MIC 62.5 µg/mL) showed 1.6 times better activity than ampicillin (MIC 100 µg/mL) against the EC strain.

Compound K2 having 4-OH and 3-CONH₂ groups on the phenyl ring showed the most potent activity (MIC: 12.5 µg/mL) against *P. aeruginosa*, and H2 with similar groups on the phenyl ring had reduced activity (MIC: 62.5 µg/mL) against the same bacterial strain. This shows that the 4-OH and 3-CONH₂ groups are essential for antibacterial activity against *P. aeruginosa*. The keto group enhances the activity while the presence of hydroxy group results in reduced antibacterial activity of the compound. Benzo[b]thiophene is also important for the activity as a similar compound, K6, with all functional groups but benzo[b]isothiazole instead of benzo[b]thiophene, results in decreased activity (500 µg/mL). Compound F4 showed moderate activity against *E. coli* (62.5 µg/mL). This suggests that the presence of the fluoro group enhances the antibacterial activity, whereas similar compounds K4 and H4 were found relatively less active, suggesting that keto and hydroxy groups in a compound with a CF₃ group in the phenyl ring result in reduced activity. Compounds K1, K2, K4, F1, F3, and F8 having benzo[b]thiophene moiety showed good antifungal activity against *C. albicans* (MIC: 250 µg/mL), which suggests that benzo[b]thiophene is important for antifungal activity. Moreover, keto is also essential for the activity. The structure-activity relationship is shown in Figure 7 and a comparative activity chart of all synthesized compounds against standard drugs is shown in Figure 8. Compounds K1, K2, K4, K8, F1, and F3 (MIC: 250 µg/mL) showed two times better antifungal activity than griseofulvin (MIC 500 µg/mL) against the CA strain.

2.3. Conclusions

Twenty-two compounds in a series of 1-(benzo[b]thiophen-4-yl)-4-(2-(oxo, hydroxy, and fluoro)-2-phenylethyl) piperazine and 1-(benzo[d]isothiazole-3-yl)-4-(2-(oxo, hydroxy, and fluoro)-2-phenylethyl) piperazine derivatives
Table 2. Minimum inhibitory concentration of 1-(benzo[b]thiophen-4-yl)-4-(2-(oxo, hydroxy, and fluoro)-2-phenylethyl)piperazine and 1-(benzo[d]isothiazole-3-yl)-4-(2-(oxo, hydroxy, and fluoro)-2-phenylethyl)piperazine derivatives and reference drugs against bacteria and fungus.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC µg/mL</th>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
<th>Fungus</th>
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<tr>
<td></td>
<td></td>
<td>SA</td>
<td>SP</td>
<td>EC</td>
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<tr>
<td>K1</td>
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<tr>
<td>GRF</td>
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AMP = Ampicillin, CAM = chloramphenicol, GRF = Gresofulvin
SA = Staphylococcus aureus, SP = Streptococcus pyogenes, EC = Escherichia coli,
PA = Pseudomonas aeruginosa, CA = Candida albicans.

were synthesized. Most of the synthetic compounds showed inhibitory activity against gram-positive and gram-negative bacterial strains and a fungal strain. K2 was found to be a potent lead compound for antibacterial activity against the gram-negative bacterial strain Pseudomonas aeruginosa. K4 was found to be a lead compound for antibacterial activity against gram-positive bacterial strain S. aureus, and compounds H2 and F4 were found as leads for antibacterial activity against gram-negative bacterial strains for further structural optimization as potential antibacterial agents. Compounds K1, K2, K4, K8, F1, and F3 may be considered
as lead compounds for further structural optimization and development as potential antifungal agents for the treatment of microbial infections.

3. Experimental

3.1. Materials and methods
All the required chemicals were purchased from Spectrochem and Aldrich Chemical Company. Precoated aluminum sheets (silica gel 60 F254, Merck) were used for thin-layer chromatography (TLC) and spots were
visualized under UV light. Melting points were determined using an open capillary tube and hot paraffin oil and are uncorrected. IR spectra were recorded on a PerkinElmer-1800 FTIR spectrophotometer in the frequency range of $\nu$ 450–4000 cm$^{-1}$ in Nujol mull and as KBr pellets. $^1$H NMR and $^{13}$C NMR spectra were recorded on Bruker Advance II 400 NMR spectrophotometer at 400 and 125 MHz, respectively, using a solvent and tetramethylsilane as an internal standard. Splitting patterns are designated as follows; s = singlet, d = doublet, dd = double doublet, m = multiplet. Chemical shift (\(\delta\)) values are given in ppm. EI-MS spectra were obtained on a Waters Xevo G2-XS Tof mass spectrometer.

3.2. General procedure for the synthesis of compounds K1–K8

A suspension of heteroaryl piperazine (PZ1 or PZ2) (1.0 mol equiv.) in acetonitrile (20 mL) was cooled to 0–5 °C. Potassium carbonate was added to the suspension and stirred for 15 min at 0–5 °C. Phenacyl bromide (1.0 mol equiv.) was added at 0–5 °C, allowed to warm at ambient temperature, and stirred for 6–8 h. Reaction progress was monitored on TLC using ethyl acetate/hexane (7:3) as the mobile phase and visualized under UV light (254 nm). After completion of the reaction it was filtered to remove inorganic salts. The filtrate was concentrated under vacuum at 45–50 °C to obtain crude product. Crude keto product (K1–K8) was then purified by crystallization in DCM/hexane or purified by column chromatography using 100–200 mesh silica gel and 10–20% ethyl acetate/hexane as the mobile phase. Pure fractions were collected and distilled under vacuum at 45–50 °C to obtain pure solid product (yield 95–98%)

3.2.1. 2-(4-(Benzo[b]thiophen-4-yl)piperazin-1-yl)-1-(4-methoxyphenyl)ethanone (K1)

Beige powder, yield: 95%; C$_{21}$H$_{22}$N$_2$O$_2$S; MW 366.48; mp 92–95 °C; IR (KBr, cm$^{-1}$): 3092 (Ar-H), 2938 (C-H), 1693 (C=O), 1513, 1376 (C-H), 1172 (C-N), 810 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$, 400 MHz): 8.04 (2H, d, $\ J = 8.88$ Hz), 7.69 (1H, d, $\ J = 8.0$ Hz), 7.40 (1H, d, $\ J = 5.16$ Hz), 7.26 (1H, t, $\ J = 15.68$ Hz), 7.06 (2H, d, $\ J = 8.88$ Hz), 6.91 (1H, d, $\ J = 7.48$ Hz), 6.20 (1H, d, $\ J = 8.96$ Hz, Ar-H), 3.65 (2H, s), 3.33 (4H, bs, -CH$_2$-piperazine), 2.73 (4H, bs, -CH$_2$ piperazine); $^{13}$C NMR (DMSO-d$_6$, 125 MHz): 195.00(1C), 163.66(1C), 148.44(1C), 141.13(1C,), 134.12(1C), 130.57 (1C), 129.22(1C), 125.03(2C), 124.97(2C), 121.87(2C), 117.06(1C), 113.74(1C), 112.30(1C), 64.44(2C), 55.49(1C), 53.91(2C), 52.01(2C); EI-MS, m/z: 367.14 [M+H]$^+$.

3.2.2. 5-(2-(4-(Benzo[b]thiophen-4-yl)piperazin-1-yl)acetyl)-2-hydroxybenzamide (K2)

Yellow powder, yield: 94%; C$_{21}$H$_{21}$N$_3$O$_3$S; MW 395.48; mp 320 °C; IR (KBr, cm$^{-1}$): 3550 (O-H phenol), 3430 (N-H amide), 3089 (Ar-H), 2932 (C-H), 1643 (C=O amide), 1692 (C=O keto), 1509, 1375 (C-H), 1171 (C-N), 809 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$, 125 MHz): δ = 10.90 (1H, d, $\ J = 6.20$ Hz, -CONH$_2$), 7.69 (1H, s, Ar-H ), 7.67–7.56 (2H, m, Ar-H), 7.41 (1H, d, $\ J = 5.32$ Hz, Ar-H), 7.27 (1H, t, $\ J_1 = 7.80$ Hz, $\ J_2 = 7.56$ Hz, Ar-H), 6.91 (1H, d, $\ J = 7.64$ Hz, Ar-H), 6.63 (1H, d, $\ J = 6.20$ Hz, Ar-H), 6.20 (1H, d, $\ J = 8.96$ Hz, Ar-H), 3.65 (2H, s, -CH$_2$-C=O), 3.33 (4H, bs, -CH$_2$ piperazine), 2.73 (4H, bs, -CH$_2$ piperazine); $^{13}$C NMR (DMSO-d$_6$, 125 MHz): 192.83(1C), 177.93(1C), 169.91(1C), 148.44(1C), 141.13(1C), 134.12(1C), 130.57 (1C), 129.22(1C), 125.03(2C), 124.97(2C), 121.87(2C), 117.06(1C), 113.74(1C), 112.30(1C), 64.44(2C), 55.49(1C), 53.91(2C), 52.01(2C); EI-MS, m/z: 367.14 [M+H]$^+$.
3.2.3. 2-(4-(Benza[b]thiophen-4-yl)piperazin-1-yl)-1-(4-fluorophenyl)ethanone (K3)

Brown solid, yield: 96%; C_{20}H_{19}FN_{2}OS; MW 354.44; mp 76 °C; IR (KBr, cm\(^{-1}\)): 3087 (Ar-H), 2930 (C-H), 1690 (C=O keto), 1507, 1370 (C-H), 1280 (C-F), 1170 (C-N), 811 cm\(^{-1}\); \(^1\)H NMR (DMSO-d\(_6\), 400 MHz): δ = 8.13–8.11 (2H, m, Ar-H), 7.75 (1H, d, J = 8.12 Hz, Ar-H), 7.62 (1H, d, J = 7.96 Hz, Ar-H), 7.41–7.31 (3H, m, Ar-H), 7.26 (1H, t, J\(_{1} = 7.80\) Hz, J\(_{2} = 7.88\) Hz, Ar-H), 6.91 (1H, d, J = 7.60 Hz, Ar-H), 3.92 (2H, s, -CH\(_2\)-C=O), 3.08 (4H, bs, -CH\(_2\) piperazine), 2.77 (4H, bs, -CH\(_2\) piperazine): \(^{13}\)C NMR (CDCl\(_3\), 125 MHz): 194.97(1C), 166.88(1C), 148.33(1C), 141.16(1C), 134.12(1C), 132.47(2C), 130.97(1C), 125.02(1C), 121.81(1C), 117.15(1C), 115.78(2C), 115.61(1C), 112.32(1C), 64.67(1C), 53.85(2C), 51.97(2C, C-19,C-20); EI-MS, m/z (relative intensity 100%): 355.12 [M+H\(^+\)] (100%).

3.2.4. 2-(4-(Benza[b]thiophen-4-yl)piperazin-1-yl)-1-(4-(trifluoromethyl)phenyl)ethanone (K4)

Yellow powder, yield: 97%; C\(_{21}\)H\(_{19}\)F\(_3\)N\(_2\)OS; MW 404.45; mp 122–125 °C; IR (KBr, cm\(^{-1}\)): 3087 (Ar-H), 2930 (C-H), 1692 (C=O keto), 1508, 1372 (C-H), 1230 (C-F), 1172 (C-N), 809 cm\(^{-1}\); \(^1\)H NMR (DMSO-d\(_6\), 400 MHz): δ = 8.23 (2H, d, J = 8.04 Hz, Ar-H), 7.92 (2H, d, J = 8.12 Hz, Ar-H), 7.70 (1H, d, J = 5.48 Hz, Ar-H), 7.62 (1H, d, J = 8.0 Hz, Ar-H), 7.41 (1H, d, J = 5.48, Ar-H), 7.27 (1H, t, J\(_{1} = 7.8\) Hz, J\(_{2} = 7.84\) Hz, Ar-H), 6.91 (1H, d, J = 7.6 Hz, Ar-H), 4.00 (2H, s, -CH\(_2\)-C=O), 3.08 (4H, bs, -CH\(_2\) piperazine), 2.78 (4H, bs, -CH\(_2\) piperazine): \(^{13}\)C NMR (CDCl\(_3\), 125 MHz): 195.7(1C), 148.28(1C), 141.17(1C), 138.66(1C), 134.12(1C), 128.71(4C), 125.65 (q, J\(_{1} = 2.5\) Hz, J\(_{2} = 70\) Hz, J\(_{3} = 6.35\) Hz, 1C, -CF\(_3\)), 121.77(1C), 117.19(1C), 113.33(1C), 64.97(1C), 53.86(2C), 51.97(2C); EI-MS, m/z: 405.12 [M+H\(^+\)].

3.2.5. 2-(4-(Benza[d]isothiazol-3-yl)piperazin-1-yl)-1-(4-methoxyphenyl)ethanone (K5)

Light yellow powder, yield: 98%; C\(_{20}\)H\(_{21}\)N\(_{3}\)O\(_{2}\)S; MW 367.47; mp 112–114 °C; IR (KBr, cm\(^{-1}\)): 3080 (Ar-H), 2935 (C-H), 1690 (C=O keto), 1508, 1375 (C-H), 1229 (C-F), 1175 (C-N), 809 cm\(^{-1}\); \(^1\)H NMR (DMSO-d\(_6\), 400 MHz): δ = 8.06–8.01 (4H, m, Ar-H), 7.55 (1H, t, J\(_{1} = 7.48\) Hz, J\(_{2} = 7.64\) Hz, Ar-H), 7.43 (1H, t, J\(_{1} = 7.68\) Hz, J\(_{2} = 7.40\) Hz, Ar-H), 7.05 (1H, d, J = 8.68 Hz, Ar-H), 3.87 (2H, s, -CH\(_2\)-C=O), 3.45 (3H, s, -OCH\(_3\)), 3.31 (4H, bs, -CH\(_2\) piperazine), 2.75 (4H, bs, -CH\(_2\) piperazine): \(^{13}\)C NMR (CDCl\(_3\), 125 MHz): 194.98(1C), 163.85(1C), 163.68(1C), 152.80(1C), 130.59(2C), 129.16(1C), 128.04(2C), 127.52(1C), 123.89(1C), 120.57(1C), 113.74(2C), 64.46(1C), 55.48(1C), 53.26(2C), 49.96(2C); EI-MS, m/z: 368.14 [M+H\(^+\)].

3.2.6. 5-(2-(4-(Benza[d]isothiazol-3-yl)piperazin-1-yl)-1-acetyl)-2-hydroxybenzamide (K6)

Yellow powder, yield: 98%; C\(_{20}\)H\(_{20}\)N\(_{4}\)O\(_{3}\)S; MW 396.47; mp 340 °C; IR (KBr, cm\(^{-1}\)): 3548 (O-H phenol), 3428 (N-H amide), 3089 (Ar-H), 2930 (C-H), 1640 (C=O amide), 1690 (C=O keto), 1510, 1370 (C-H), 1169 (C-N), 805 cm\(^{-1}\); \(^1\)H NMR (DMSO-d\(_6\), 400 MHz): δ = 10.90 (1H, bs, CONH\(_2\)), 8.43 (1H, bs, OH), 8.05 (2H, d, J = 5.80 Hz, Ar-H), 7.53–7.53 (2H, m, Ar-H), 7.44–7.40 (1H, m, Ar-H), 6.62 (1H, d, J = 6.0 Hz, Ar-H), 6.20 (1H, d, J = 8.92 Hz, Ar-H), 3.66 (2H, s, -CH\(_2\)-C=O), 3.45 (4H, bs, -CH\(_2\) piperazine), 2.72 (4H, bs, -CH\(_2\) piperazine): \(^{13}\)C NMR (DMSO-d\(_6\), 125 MHz): 192.80(1C), 177.85(1C), 169.87(1C), 164.03(1C), 152.42(1C), 133.70(1C), 132.05(1C), 128.31(1C), 127.82(1C), 124.87(1C), 124.64(1C), 123.02(1C), 121.48(1C), 118.57(1C), 117.58(1C), 63.07(1C), 53.22(2C), 50.11(2C); EI-MS, m/z: 397.13 [M+H\(^+\)].
3.3. 2-(4-(Benzo[d]isothiazol-3-yl)piperazin-1-yl)-1-(4-fluorophenyl)ethanone (K7)

Yellow powder, yield: 97%; C\textsubscript{19}H\textsubscript{18}FN\textsubscript{3}O\textsubscript{3}; MW 355.43; mp 120 °C; IR (KBr, cm\textsuperscript{-1}): 3092 (Ar-H), 2935 (C-H), 1685 (C=O keto), 1507, 1370 (C-H), 1170 (C-N), 810 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}, 400 MHz): δ = 8.13–8.10 (2H, m, ArH), 8.06 (2H, d, J = 8.44 Hz, ArH), 7.55 (1H, t, J\textsubscript{1} = 7.24 Hz, J\textsubscript{2} = 7.32 Hz, ArH), 7.43 (1H, t, J\textsubscript{1} = 7.66 Hz, J\textsubscript{2} = 7.88 Hz, ArH), 7.36 (2H, t, J\textsubscript{1} = 8.88 Hz, J\textsubscript{2} = 8.88 Hz, ArH), 3.94 (2H, s, -CH\textsubscript{2}-C=O), 3.45 (4H, bs, -CH\textsubscript{2} piperazine), 2.76 (4H, bs, -CH\textsubscript{2} piperazine); \textsuperscript{13}C NMR (CDCl\textsubscript{3}, 125 MHz): 195.00 (1C), 166.89 (1C), 164.86 (1C), 163.78 (1C), 152.82 (1C), 131.06 (2C), 130.99 (1C), 127.54 (1C), 123.91 (1C), 123.84 (1C), 120.59 (1C), 115.77 (1C), 115.60 (1C), 64.74 (1C), 53.22 (2C), 49.94 (2C); EI-MS, m/z: 356.12 [M+H]+.

3.3.1. 2-(4-(Benzo[d]isothiazol-3-yl)piperazin-1-yl)-1-(4-(trifluoromethyl)phenyl) ethanone (K8)

Orange crystals, yield: 98%; C\textsubscript{20}H\textsubscript{18}F\textsubscript{3}N\textsubscript{3}O\textsubscript{3}; MW 405.44; mp 90–95 °C; IR (KBr, cm\textsuperscript{-1}): 3086 (Ar-H), 2932 (C-H), 1690 (C=O keto), 1507, 1370 (C-H), 1236 (C-F), 1170 (C-N), 805 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}, 400 MHz): δ = 8.22 (2H, d, J = 7.96 Hz, ArH), 8.06 (2H, d, J = 8.44 Hz, ArH), 7.92 (2H, d, J = 8.24 Hz, ArH), 7.55 (1H, t, J\textsubscript{1} = 7.72 Hz, J\textsubscript{2} = 7.48 Hz, ArH), 7.43 (1H, t, J\textsubscript{1} = 7.92 Hz, J\textsubscript{2} = 7.12 Hz, ArH), 4.02 (2H, s, -CH\textsubscript{2}-C=O), 3.46 (4H, bs, -CH\textsubscript{2} piperazine), 3.31 (4H, bs, -CH\textsubscript{2} piperazine); \textsuperscript{13}C NMR (CDCl\textsubscript{3}, 125 MHz): 195.71 (1C), 163.73 (1C), 152.84 (1C), 138.61 (1C), 128.70 (2C), 127.99 (1C), 127.56 (1C), 125.66 (1C), 125.63 (1C), 124.65 (1C), 123.91 (1C), 123.81 (1C), 122.48 (1C), 120.60 (1C), 64.94 (1C), 53.19 (2C), 49.91 (2C); EI-MS, m/z: 406.11 [M+H]+.

3.4. General procedure for the synthesis of compounds H1–H8

Keto compounds (K1–K8) (1.0 mol equiv.) were suspended in ethanol (10 mL) and cooled to 0–5 °C. NaBH\textsubscript{4} was added (2.0 mol equiv.) at 0–5 °C, allowed to warm at ambient temperature, and stirred for 10–12 h. Reaction progress was monitored on TLC using ethyl acetate/hexane (1:1) as the mobile phase and visualized under UV light (254 nm). After completion of the reaction, the pH was adjusted to 6.0 with acetic acid (0.4 mL) to quench excess NaBH\textsubscript{4}. The mixture was poured into DM water (20 mL) and extracted with dichloromethane (2 ×20 mL). Combined dichloromethane layers were distilled at 40–45 °C to obtain an oily crude residue. The obtained crude hydroxy product (H1–H8) was then purified by column chromatography using 100–200 mesh silica gel and 50% ethyl acetate/hexane to pure ethyl acetate as the mobile phase. Pure fractions were collected and distilled under vacuum at 45–50 °C to obtain pure solid product (yield 88–94%).

3.4.1. 2-(4-(Benzo[b]thiophen-4-yl)piperazin-1-yl)-1-(4-methoxyphenyl)ethanol (H1)

White crystals, yield: 94%; C\textsubscript{21}H\textsubscript{24}N\textsubscript{2}O\textsubscript{2}S; MW 368.50; mp 156 °C; IR (KBr, cm\textsuperscript{-1}): 3390 (sec OH), 3085 (Ar-H), 2930 (C-H), 1455 (CH\textsubscript{2} bend), 1370 (C-H), 1175 (C-N piperazine), 709 (C-S) cm\textsuperscript{-1}; \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}, 400 MHz): δ = 7.69 (1H, d, J = 4.52 Hz, ArH), 7.62 (1H, d, J = 8.12 Hz, ArH), 7.40 (1H, d, J = 4.60 Hz, ArH), 7.29 (3H, d, J = 7.44 Hz, ArH), 6.89 (3H, d, J = 6.4 Hz, ArH), 4.93 (1H, s, -CH\textsubscript{2}-C=O), 3.46 (4H, bs, -CH\textsubscript{2} piperazine), 3.31 (4H, bs, -CH\textsubscript{2} piperazine); \textsuperscript{13}C NMR (CDCl\textsubscript{3}, 125 MHz): 159.17 (1C), 148.30 (1C), 141.19 (1C), 134.11 (1C), 134.08 (1C), 127.16 (2C), 125.07 (1C), 125.02 (1C), 121.80 (1C), 117.18 (1C), 113.87 (2C), 112.27 (1C), 68.46 (1C), 66.31 (1C), 53.31 (2C), 53.41 (1C), 52.26 (2C); EI-MS, m/z: 369.16 [M+H]+.

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3.4.2. 5-(2-(4-(Benzo[b]thiophen-4-yl)piperazin-1-yl)-1-hydroxyethyl)-2-hydroxybenzamide (H2)
White crystals, yield: 88%; C_{21}H_{23}N_{3}O_{3}S; MW 397.49; mp 164–166 °C; IR (KBr, cm⁻¹): 3540 (O-H phenol), 3428 (N-H amide), 3370 (sec OH), 3085 (Ar C-H), 2930 (C-H), 1548 (CH₂ bend), 1643 (C=O amide), 1165 (C-N), 705 (C-S) cm⁻¹; ¹H NMR (DMSO-d₆, 400 MHz): δ = 12.89 (1H, s, -CONH₂), 8.40 (1H, s, phenolic OH), 7.83 (2H, s, ArH & primary OH), 7.69 (1H, d, J = 5.20 Hz, ArH), 7.62 (1H, m, ArH), 7.43–7.39 (2H, m, ArH), 7.27 (1H, t, J₁ = 7.84 Hz, J₂ = 7.72 Hz, ArH), 6.90–6.83 (2H, m, ArH), 5.03 (1H, s, -CH₂), 4.69 (1H, s, -CH₂), 3.06 (4H, s, -CH₂ piperazine), 2.74 (4H, s, -CH₂ piperazine), 2.65–2.60 (1H, -CH, m); ¹³C NMR (CDCl₃, 125 MHz): 172.46(1C), 161.59(1C), 148.17(1C), 141.23(1C), 134.11(1C), 132.54(1C), 125.16(1C), 125.00(1C), 123.65(1C), 121.71(1C), 118.61(1C), 117.30(1C), 113.14(1C), 112.27(1C), 91.00(1C), 67.98(1C), 66.28(1C), 53.39(2C), 52.21(2C); EI-MS, m/z: 398.15 [M+H]+.

3.4.3. 2-(4-(Benzo[b]thiophen-4-yl)piperazin-1-yl)-1-(4-fluorophenyl)ethanol (H3)
Light yellow crystals, yield: 93%; C_{20}H_{21}FN₂O; MW 356.46; mp 110–112 °C; IR (KBr, cm⁻¹): 3394 (sec OH), 3059 (Ar C-H), 2947 (C-H), 1450 (CH₂ bend), 1072 (C-N piperazine), 702 (C-S); ¹H NMR (DMSO-d₆, 400 MHz): δ = 7.69 (1H, d, J = 5.40 Hz, ArH), 7.61 (1H, d, J = 7.80 Hz, ArH), 7.42–7.38 (3H, m, ArH), 7.27 (1H, t, J₁ = 7.76 Hz, J₂ = 7.76 Hz, ArH), 7.14 (2H, t, J₁ = 7.24 Hz, J₂ = 8.72 Hz, ArH), 6.89 (1H, d, J = 7.56 Hz, ArH), 5.12 (1H, s, -CH₂), 4.77 (1H, s, -CH₂), 3.05 (4H, s, -CH₂ piperazine), 2.72 (4H, s, -CH₂ piperazine), 2.61–2.56 (1H, -CH, m); ¹³C NMR (CDCl₃, 125 MHz): 163.26(1C), 161.32(1C), 148.24(1C), 141.21(1C), 137.72(1C), 134.11(1C), 127.54(1C), 125.02(2C), 121.76(1C), 117.24(1C), 115.34(2C), 112.28(1C), 68.24(1C), 66.27(1C), 53.24(2C), 52.24(2C); EI-MS, m/z: 357.14 [M+H]+.

3.4.4. 2-(4-(Benzo[b]thiophen-4-yl)piperazin-1-yl)-1-(4-trifluoromethyl)phenyl) ethanol (H4)
White crystals, yield: 94%; C_{21}H_{21}F₃N₂O; MW 406.47; mp 140–142 °C; IR (KBr, cm⁻¹): 3370 (sec OH), 3079 (Ar-H), 2930 (C-H), 1510, 1370 (C-H), 1169 (C-N), 710 (C-S) cm⁻¹; ¹H NMR (DMSO-d₆, 400 MHz): δ = 7 (3H, m, ArH), 7.62 (3H, m, ArH), 7.40–7.39 (1H, m, ArH), 7.27 (1H, t, J₁ = 7.80 Hz, J₂ = 7.76 Hz, ArH), 6.90 (1H, d, J = 7.64 Hz, ArH), 5.33 (1H, s, -CH₂), 4.87 (1H, s, -CH₂), 3.06 (4H, s, -CH₂ piperazine), 3.05 (4H, s, -CH₂ piperazine), 2.64–2.59 (1H, -CH, m); ¹³C NMR (CDCl₃, 125 MHz): 148.18(1C), 146.22(1C), 141.22(1C), 134.11(1C), 129.93(1C), 129.67(1C), 126.09(1C), 125.38 (q, J₁ = 3.75 Hz, J₂ = 23.75 Hz, J₃ = 17.5 Hz, 1C, -CF₃), 123.12(1C), 121.73(1C), 117.29(1C), 112.29(1C), 68.28(1C), 65.98(1C), 53.41(2C), 52.22(2C); EI-MS, m/z: 407.13 [M+H]+.

3.4.5. 2-(4-(Benzo[d]isothiazol-3-yl)piperazin-1-yl)-1-(4-methoxyphenyl)ethanol (H5)
White crystals, yield: 94%; C_{20}H_{13}N_{3}O₂S; MW 369.48; mp 118–122 °C; IR (KBr, cm⁻¹): 3290 (sec OH), 3065 (Ar-H), 2930 (C-H), 1547 (CH₂ bend), 1560 (C=N), 1170 (C=N), 1055 (C-N piperazine), 685 (C-S) cm⁻¹; ¹H NMR (DMSO-d₆, 400 MHz): δ = 8.06–8.03 (2H, m, ArH), 7.55 (1H, t, J₁ = 7.56 Hz, J₂ = 7.20 Hz, ArH), 7.42 (1H, t, J₁ = 7.12 Hz, J₂ = 7.56 Hz, ArH), 7.29 (2H, d, J = 8.56 Hz, ArH), 6.89 (2H, d, J = 8.56 Hz, ArH), 4.96 (1H, s, -CH₂), 4.71 (1H, s, -CH₂), 3.73 (3H, s, -OCH₃), 3.43 (4H, s, -CH₂ piperazine), 2.71 (4H, s, -CH₂ piperazine), 2.55 (1H, -CH, m); ¹³C NMR (CDCl₃, 125 MHz): 163.81(1C), 159.18(1C), 152.84(1C), 133.99(1C), 127.58(2C), 127.15(2C), 123.94(1C), 123.82(1C), 120.62(1C), 113.87(2C), 68.46(1C), 66.38(1C), 55.31(1C), 52.91(2C), 50.19(2C); EI-MS, m/z: 370.15 [M+H]+.
3.4.6. 5-(2-(4-(Benzo[d]isothiazol-3-yl)piperazin-1-yl)-1-hydroxyethyl)-2-hydroxy benzamide (H6)

Light yellow crystals, yield: 89%; C_{20}H_{22}N_{4}O_{3}S; MW 398.48; mp 98–100 °C; IR (KBr, cm\(^{-1}\)): 3490 (O-H phenol), 3429 (N-H amide), 3355 (sec OH), 3085 (Ar-H), 2930 (C-H), 1456 (CH\(_2\) bend), 1640 (C=O amide), 1560 (C=N), 1175 (C-N), 680 (C-S) cm\(^{-1}\); \(^1\)H NMR (DMSO-d\(_6\), 400 MHz): \(\delta = 12.89\) (1H, s, -CONH\(_2\)), 8.40 (1H, s, phenolic OH), 8.05 (2H, d, \(J = 8.04\) Hz, ArH), 7.83 (2H, s, ArH & secondary OH), 7.57 (1H, t, \(J = 7.36\) Hz, \(J_2 = 7.28\) Hz, ArH), 7.44–7.41 (2H, m, ArH), 6.85–6.83 (1H, m, ArH), 5.05 (1H, s, -CH\(_2\)), 4.69 (1H, s, -CH\(_2\)), 3.44 (4H, s, -CH\(_2\) piperazine), 2.72 (4H, s, -CH\(_2\) piperazine), 2.70 (4H, s, -CH\(_2\) piperazine), 2.63–2.58 (1H, -CH, m); \(^{13}\)C NMR (CDCl\(_3\), 125 MHz): 172.56(1C), 163.73(1C), 161.54(1C), 152.82(1C), 132.50(1C), 127.65(1C), 124.01(1C), 123.82(1C), 123.78(1C), 120.64(1C), 118.56(2C), 113.23(1C), 68.03(1C), 66.28(1C), 60.39(1C), 52.87(2C), 50.13(2C); EI-MS, m/z: 399.15 [M+H]+.

3.4.7. 2-(4-(Benzo[d]isothiazol-3-yl)piperazin-1-yl)-1-(4-fluorophenyl)ethanol (H7)

White crystals, yield: 94%; C_{19}H_{20}FN_{3}OS; MW 357.45; mp 108–110 °C; IR (KBr, cm\(^{-1}\)): 3050 (Ar C-H), 2990 (C-H), 1452 (CH\(_2\) bend), 1560 (C=N), 1168 (C=N), 1040 (C-N piperazine), 682 (C-S) cm\(^{-1}\); \(^1\)H NMR (DMSO-d\(_6\), 400 MHz): 8.05–8.03 (2H, m, ArH), 7.55 (1H, d, \(J = 7.32\) Hz, \(J_2 = 7.20\) Hz, ArH), 7.44–7.39 (3H, m, ArH), 7.14 (2H, t, \(J = 8.88\) Hz, \(J_1 = 8.88\) Hz, ArH), 5.14 (1H, s, -CH\(_2\)), 4.77 (1H, s, -CH\(_2\) piperazine), 3.43 (4H, s, -CH\(_2\) piperazine), 2.72 (4H, s, -CH\(_2\) piperazine), 2.50–2.45 (1H, -CH, m); \(^{13}\)C NMR (CDCl\(_3\), 125 MHz): 163.76(1C), 163.26(1C), 161.31(1C), 152.85(1C), 137.66(1C), 137.64(1C), 127.53(2C), 123.96(1C), 123.80(1C), 120.63(1C), 115.17(2C), 68.24(1C), 66.34(1C), 52.89(2C), 50.17(2C); EI-MS, m/z: 358.13 [M+H]+.

3.4.8. 2-(4-(Benzo[d]isothiazol-3-yl)piperazin-1-yl)-1-(4-(trifluoromethyl)phenyl)ethanol (H8)

Light yellow crystals, yield: 90%; C_{20}H_{20}F_{3}N_{3}OS; MW 407.46; mp 127–129 °C; IR (KBr, cm\(^{-1}\)): 3059 (Ar C-H), 2990 (C-H), 1452 (CH\(_2\) bend), 1555 (C=N), 1165 (C=N), 1040 (C-N piperazine), 682 (C-S) cm\(^{-1}\); \(^1\)H NMR (DMSO-d\(_6\), 400 MHz): \(\delta = 8.06–8.04\) (2H, m, ArH), 7.70 (2H, d, \(J = 7.88\) Hz, ArH), 7.62 (2H, d, \(J = 7.96\) Hz, ArH), 7.55 (1H, t, \(J = 7.24\) Hz, \(J_2 = 7.80\) Hz, ArH), 7.42 (1H, t, \(J = 7.92\) Hz, \(J_2 = 7.28\) Hz, ArH), 5.35 (1H, s, -CH\(_2\)), 4.87 (1H, s, -CH\(_2\)), 3.43 (4H, s, -CH\(_2\) piperazine), 2.73 (4H, s, -CH\(_2\) piperazine), 2.63–2.57 (1H, -CH, m); \(^{13}\)C NMR (CDCl\(_3\), 125 MHz): 163.72(1C), 152.86(1C), 146.13(1C), 129.94(1C), 129.68(1C), 127.97(1C), 127.62(1C), 126.08(1C), 125.38(1C), 125.35(1C), 123.98(1C), 123.77(1C), 123.11(1C), 120.64(1C), 68.28(1C), 66.04(1C), 52.86(2C), 50.45(2C); EI-MS, m/z: 408.13 [M+H]+.

3.5. General procedure for the synthesis of compounds F1–F8

A suspension of hydroxy compounds (H1–H8, excluding H2 and H6) (1.0 mol equiv.) in dichloromethane (10 mL) was cooled at –5 to 0 °C. Diethylaminosulfur trifluoride (DAST) (2.0 mol equiv.) was added slowly to maintain the mixture at a temperature of –5 to 0 °C (exothermicity was observed upon addition of DAST) and stirred at –5 to 0 °C for 2–4 h. Reaction progress was monitored on TLC using ethyl acetate/hexane (1:1) as the mobile phase and visualized under UV light (254 nm). After completion, the reaction was quenched with methanol (1 mL), maintaining a temperature of not more than 10 °C. It was then poured into DM water (10 mL) and separated into layers. The dichloromethane layer was washed with sat. aq. NaHCO\(_3\) solution (5 mL) and then with DM water (5 mL). The dichloromethane layer was distilled at 40–45 °C to obtain crude fluoro...
product (F1–F8) as oil. Crude fluoro product was then purified by column chromatography using 230–400 mesh silica gel and 5%–20% ethyl acetate/hexane as the mobile phase. Pure fractions were collected and distilled under vacuum at 45–50 °C to obtain a pure oily product, which became solid after settling at 2–8 °C overnight (yield 96–99%).

Hydroxy compounds H2 and H6 did not yield respective fluoro compounds F2 and F8 under the above fluorination conditions. On reaction of H2 and H8 with DAST in dichloromethane, the starting material was finished on TLC but no prominent product was obtained.

3.5.1. 1-(Benzo[b]thiophen-4-yl)-4-(2-fluoro-2-(4-methoxyphenyl)ethyl)piperazine (F1)

Beige powder, yield: 99%; C_{21}H_{23}FN_{2}OS; MW 370.48; mp 150 °C; IR (KBr, cm⁻¹): 3055 (Ar C-H), 2988 (C-H), 1456 (CH₂ bend), 1265 (C-F), 1045 (C-N piperazine), 680 (C-S) cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ = 7.41 (1H, m, ArH), 7.39–7.31 (2H, m, ArH), 7.30–7.26 (3H, m, ArH), 6.99–6.90 (3H, m, ArH), 5.75–5.61 (1H, dd, -CH coupled with fluoro), 3.82 (3H, s, -OCH₃), 3.23 (4H, s, -CH₂ piperazine), 3.10–3.00 (1H, m), 2.78 (4H, s, -CH₂ piperazine), 2.77–2.72 (1H, m); ¹³C NMR (CDCl₃, 125 MHz): 159.84 (1C), 148.45 (1C), 141.15 (1C), 134.12 (1C), 131.11 (1C), 130.95 (1C), 127.22 (1C), 127.17 (1C), 125.02 (1C), 121.87 (1C), 117.05 (1C), 113.95 (1C), 112.24 (1C), 93.29 (d, J_{CF} = 171.25, 1C, -CH-F), 64.53 (d, J_{CF} = 23.75, 1C, -CH₂-CH-F), 55.32 (1C), 54.05 (2C), 52.12 (2C); EI-MS, m/z: 371.15 [M+H⁺].

3.5.2. 1-(Benzo[b]thiophen-4-yl)-4-(2-fluoro-2-(4-fluorophenyl)ethyl)piperazine (F3)

Yellow powder, yield: 96%; C_{20}H_{20}F₂N₂S; MW 358.45; mp 120–122 °C; IR (KBr, cm⁻¹): 3059 (Ar C-H), 2980 (C-H), 1455 (CH₂ bend), 1260 (C-F), 1048 (C-N piperazine), 680 (C-S) cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ = 7.55 (1H, m, ArH), 7.48–7.38 (4H, m, ArH), 7.36–7.28 (1H, m, ArH), 7.26–7.06 (2H, m, ArH), 6.92 (1H, d, J = 7.16 Hz, ArH), 5.77–5.63 (1H, dd, -CH coupled with fluoro), 3.22 (4H, s, -CH₂ piperazine), 2.87 (4H, s, -CH₂ piperazine), 3.15–3.00 (1H, -CH, m), 2.86–2.69 (1H, -CH, m); ¹³C NMR (CDCl₃, 125 MHz): 163.14 (1C), 161.77 (1C), 148.39 (1C), 141.15 (1C), 134.12 (1C), 131.11 (1C), 130.95 (1C), 127.22 (1C), 127.17 (1C), 125.02 (1C), 121.87 (1C), 117.11 (1C), 115.57 (1C), 115.39 (1C), 92.93 (d, J_{CF} = 172.5, 1C, -CH-F), 64.55 (d, J_{CF} = 22.5, 1C, -CH₂-CH-F), 55.32 (1C), 54.05 (2C), 52.10 (2C); EI-MS, m/z: 359.13 [M+H⁺] (100%).

3.5.3. 1-(Benzo[b]thiophen-4-yl)-4-(2-fluoro-2-(4-(trifluoromethyl)phenyl)ethyl)piperazine (F4)

White powder, yield: 98%; C_{21}H_{20}F₄N₂S; MW 408.46; mp 138–140 °C; IR (KBr, cm⁻¹): 3055 (Ar C-H), 2989 (C-H), 1450 (CH₂ bend), 1560 (C=N), 1268 (C-F), 1050 (C-N piperazine), 680 (C-S) cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ = 7.67 (2H, d, J = 8.0 Hz, ArH), 7.57 (1H, d, J = 8.04 Hz, ArH), 7.51 (2H, d, J = 8.04 Hz, ArH), 7.42–7.38 (2H, m, ArH), 7.30–7.26 (1H, m, ArH), 6.92 (1H, d, J = 7.52 Hz, ArH), 5.84–5.70 (1H, dd, -CH coupled with fluoro), 3.22 (4H, s, -CH₂ piperazine), 3.09–2.98 (1H, -CH, m), 2.89 (4H, s, -CH₂ piperazine), 2.87–2.72 (1H, -CH, m); ¹³C NMR (CDCl₃, 125 MHz): 148.35 (1C), 142.98 (1C), 142.81 (1C), 141.17 (1C), 1134.12 (1C), 125.80 (1C), 125.74 (1C), 125.50 (1C), 125.47 (1C), 125.02 (1C), 121.82 (1C), 117.14 (1C), 112.25 (1C), 92.79 (d, J_{CF} = 175.0, 1C, -CH-F), 64.48 (d, J_{CF} = 22.5, 1C, -CH₂-CH-F), 54.06 (2C), 52.11 (2C); EI-MS, m/z: 409.13 [M+H⁺].
3.5.4. 3-(4-(2-Fluoro-2-(4-methoxyphenyl)ethyl)piperazin-1-yl)benzo[d]isothiazole (F5)

Light yellow powder, yield: 98%; C_{20}H_{22}FN_3OS; MW 371.47; mp 128 °C; IR (KBr, cm⁻¹): 3055 (Ar C-H), 2989 (C-H), 1450 (CH₂ bend), 1560 (C=N), 1268 (C-F), 1169 (C=N), 1050 (C-N piperazine), 680 (C-S) cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ = 8.0 (1H, m, ArH), 7.92–7.82 (1H, m, ArH), 7.52–7.46 (1H, m, ArH), 7.45–7.32 (3H, m, ArH), 7.12–6.90 (2H, m, ArH), 5.74–5.60 (1H, dd, -CH coupled with fluoro), 3.82 (3H, s, -OCH₃), 3.60 (4H, s, -CH₂ piperazine), 3.08–3.04 (1H, -CH, m), 2.90 (4H, s, -CH₂ piperazine), 2.86–2.67 (1H, -CH, m); ¹³C NMR (CDCl₃, 125 MHz): 163.89(1C), 159.84(1C), 152.81(1C), 131.07(1C), 130.91(1C), 127.52(1C), 127.21(1C), 127.16(1C), 123.89(1C), 120.57(1C), 113.94(1C), 93.34 (d, ¹J_{CF} = 171.25, 1C, -CH-F), 64.49 (d, ²J_{CF} = 23.75, 1C, -CH₂-CH-F), 55.31(1C), 53.48(2C), 50.08(2C); EI-MS, m/z: 372.15 [M+H]^+.

3.5.5. 3-(4-(2-Fluoro-2-(4-(trifluoromethyl)phenyl)ethyl)piperazin-1-yl)benzo[d]isothiazole (F7)

Yellow powder, yield: 99%; C_{19}H_{19}F₂N₃S; MW 359.44; mp 130–132 °C; IR (KBr, cm⁻¹): 3059 (Ar C-H), 2990 (C-H), 1451 (CH₂ bend), 1562 (C=N), 1270 (C-F), 1160 (C-N), 1047 (C-N piperazine), 678 (C-S) cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ = 7.91 (1H, d, J = 8.08 Hz, ArH), 7.82 (1H, d, J = 8.08 Hz, ArH), 7.47 (1H, t, J₁ = 7.08 Hz, J₂ = 7.12 Hz, ArH), 3.59 (4H, s, -CH₂ piperazine), 3.04–2.99 (1H, -CH, m), 2.90 (4H, s, -CH₂ piperazine), 2.75–2.71 (1H, -CH, m); ¹³C NMR (CDCl₃, 125 MHz): 163.85(1C), 152.83(1C), 127.54(1C), 123.89(1C), 120.57(1C), 115.55(1C), 115.38(1C), 92.97 (d, ¹J_{CF} = 172.5, 1C, -CH-F), 64.50 (d, ²J_{CF} = 23.75, 1C, -CH₂-CH-F), 53.47 (2C, piperazine), 50.05 (2C, piperazine), 29.69 (1C); EI-MS, m/z: 360.13[M+H]^+.

3.5.6. 3-(4-(2-Fluoro-2-(4-fluorophenyl)ethyl)piperazin-1-yl)benzo[d]isothiazole (F8)

Yellow powder, yield: 98%; C_{20}H_{19}F₂N₃S; MW 409.44; mp 102–104 °C; IR (KBr, cm⁻¹): 3055 (Ar C-H), 2995 (C-H), 1450 (CH₂ bend), 1560 (C=N), 1271 (C-F), 1165 (C-N), 1112 (CF₃ sym. str.), 1047 (C-N piperazine), 678 (C-S) cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ = 7.91 (1H, d, J = 8.12 Hz, ArH), 7.66 (2H, d, J = 8.0 Hz, ArH), 7.50–7.45 (3H, m, ArH), 5.83–5.70 (1H, dd, -CH coupled with fluoro), 3.60 (4H, s, -CH₂ piperazine), 3.04–2.94 (1H, -CH, m), 2.90 (4H, m, -CH₂ piperazine), 2.75–2.71 (1H, -CH, m); ¹³C NMR (CDCl₃, 125 MHz): 163.83(1C), 152.83(1C), 127.56(2C), 125.73(2C), 123.91(2C), 123.84(1C), 120.59(2C), 92.84 (d, ¹J_{CF} = 175.0, 1C, -CH-F), 64.42 (d, ²J_{CF} = 23.75, 1C, -CH₂-CH-F), 53.48(2C, piperazine), 50.07(2C, piperazine); EI-MS, m/z: 410.12[M+H]^+.

3.6. Screening of antimicrobial activity of synthesized compounds

All the synthesized compounds (K1–K8, H1–H8, and F1–F8) were used for antimicrobial test procedures. All necessary controls like the drug control, vehicle control, agar control, organism control, and known antibacterial drugs control were considered. Mueller Hinton broth was used as a nutrient medium to grow and dilute the drug suspension for the test bacteria. Inoculum size for the test strain was adjusted to 10⁸ cfu/mL by comparing the turbidity. S. aureus MTCC96, S. pyogenes MTCC442, E. coli MTCC443, P. aeruginosa MTCC1688, and C. albicans MTCC 227 standard strains were used for screening of the antibacterial and antifungal activities. The strains were procured from the Institute of Microbial Technology, Chandigarh. DMSO
was used as diluent/vehicle to get the desired concentrations of drugs to test upon standard bacterial strains. Each synthesized compound was diluted, obtaining 2000 μg/mL concentration as a stock solution. In primary screening 1000 μg/mL, 500 μg/mL, and 250 μg/mL concentrations of the synthesized compound were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilutions against all microorganisms. The compounds found active in the primary screening were similarly diluted to obtain 200 μg/mL, 100 μg/mL, 50 μg/mL, 25 μg/mL, 12.5 μg/mL, and 6.25 μg/mL concentrations. The highest dilution showing at least 99% inhibition zone was taken as the MIC. The result of this was significantly affected by the size of the inoculum. The test mixture should contain 108 organisms/mL.

3.7. Procedure for crystallization of 1-(benzo[b]thiophen-4-yl)-4-(2-fluoro-2-(4-methoxyphenyl)ethyl) piperazine (F1)

1-(Benzo[b]thiophen-4-yl)-4-(2-fluoro-2-(4-methoxyphenyl)ethyl) piperazine (F1) (10 mg) was dissolved in 1 mL of dichloromethane in a 10-mL round-bottom flask. Hexane (4 mL) was added to the solution and mixed well. The flask was tightly closed with a stopper and sealed with Teflon tape. The flask was kept inside a closed-cap 250-mL glass container filled with 50 mL of hexane. This container was then kept in a dark place at a temperature of about 18–20 °C. A transparent hexagonal planar crystal was obtained after 15 days. The solvent was carefully decanted from the flask and the crystal was washed several times with hexane. The crystal structure was determined by single-crystal XRD. The crystallographic data are shown in Table 3.

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Acknowledgments
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References
Figure S1. $^1$H NMR and $^{13}$C NMR spectra of 2-(4-(benzo[b]thiophen-4-yl) piperazin-1-yl)-1-(4-methoxyphenyl)ethanone (K1).
Figure S2. $^1$H NMR and $^{13}$C NMR spectra of 5-(2-(4-(benzo[b]thiophen-4-yl)piperazin-1-yl)acetyl)-2-hydroxybenzamide (K2).
Figure S3. $^1$H NMR and $^{13}$C NMR spectra of 2-((4-(benzo[4]thiophen-4-yl)piperazin-1-yl)-1-(4-fluorophenyl)ethanone (K3).
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Figure S5. $^1$H NMR and $^{13}$C NMR spectra of $2$-(4-(benzo[$b$]thiophen-4-yl)piperazin-1-yl)-1-(4-(trifluoromethyl)phenyl)ethanone, $2$-(4-(benzo[$d$]isothiazol-3-yl)piperazin-1-yl)-1-(4-methoxyphenyl)ethanone (K5).
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Figure S17. $^1$H NMR and $^{13}$C NMR spectra of 1-(benzo[b]thiophen-4-yl)-4-(2-fluoro-2-(4-methoxyphenyl)ethyl)piperazine (F1).
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Figure S23. Mass spectra (EI-MS) of 2-(4-(benzo[b]thiophen-4-yl) piperazin-1-yl)-1-(4-methoxyphenyl)ethanone (K1).

Figure S24. EI-MS of 5-(2-(4-(benzo[b]thiophen-4-yl) piperazin-1-yl)acetyl)-2-hydroxybenzamide (K2).
Figure S25. EI-MS of 2-(4-(benzo[b]thiophen-4-yl)piperazin-1-yl)-1-(4-fluorophenyl)ethanone (K3).

Figure S26. EI-MS of 2-(4-(benzo[b]thiophen-4-yl)piperazin-1-yl)-1-(4-(trifluoromethyl)phenyl)ethanone (K4).
**Figure S27.** EI-MS of 2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)-1-(4-methoxyphenyl)ethanone (K5).

**Figure S28.** EI-MS of 5-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)acetyl)-2-hydroxybenzamide (K6).
**Figure S29.** EI-MS of 2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)-1-(4-fluorophenyl)ethanone (K7).

**Figure S30.** EI-MS of 2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)-1-(4-(trifluoromethyl)phenyl)ethanone (K8).
**Figure S31.** EI-MS of 2-(4-(benzo[b]thiophen-4-yl)piperazin-1-yl)-1-(4-methoxyphenyl)ethanol (H1).

**Figure S32.** EI-MS of 5-(2-(4-(benzo[b]thiophen-4-yl)piperazin-1-yl)-1-hydroxyethyl)-2-hydroxybenzamide (H2).
Figure S33. EI-MS of 2-(4-(benzo[b]thiophen-4-yl)piperazin-1-yl)-1-(4-fluorophenyl)ethanol (H3).

Figure S34. EI-MS of 2-(4-(benzo[b]thiophen-4-yl)piperazin-1-yl)-1-(4-(trifluoromethyl)phenyl)ethanol (H4).
Figure S35. EI-MS of 2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)-1-(4-methoxyphenyl)ethanol (H5).

Figure S36. EI-MS of 5-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)-1-hydroxyethyl)-2-hydroxybenzamide (H6).
Figure S37. EI-MS of 2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)-1-(4-fluorophenyl)ethanol (H7).

Figure S38. EI-MS of 2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)-1-(4-(trifluoromethyl)phenyl)ethanol (H8).
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Figure S40. EI-MS of 1-(benzo[b]thiophen-4-yl)-4-(2-fluoro-2-(4-fluorophenyl)ethyl)piperazine (F3).
Figure S41. EI-MS of 1-(benzo[b]thiophen-4-yl)-4-(2-fluoro-2-(4-(trifluoromethyl)phenyl)ethyl)piperazine (F4).

Figure S42. EI-MS of 3-(4-(2-fluoro-2-(4-methoxyphenyl)ethyl)piparazine-1-yl)benzo[d]isothiazole (F5).
Figure S43. EI-MS of 3-(4-(2-fluoro-2-(4-fluorophenyl)ethyl)piperazin-1-yl)benzo[d]isothiazole (F7).

Figure S44. EI-MS of 3-(4-(2-fluoro-2-(4-(trifluoromethyl)phenyl)ethyl)piperazin-1-yl)benzo[d]isothiazole (F8).