Synthesis of new 4-substitued-1-(4-amino phenyl)-5,6-dihydropyridine-2(1H)-one sulfonamide conjugates and evaluation of their anti-microbial activity

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A new series of substituted sulfonyloxopyridine conjugates are reported for first time. The antibacterial and antifungal activities of the synthesized compounds have been evaluated against known bacterial strains. The obtained data indicated that in particular, compound 7a, i.e. N-(4-(3-(morpholin-2-oxo-5,6-dihydropyridin-1(2H)-yl]phenyl)benzenesulfonamide exhibited activity comparable to the well known antibacterial agents. The previously reported expensive and delicate processes for synthesis of 1-(4-nitrophenyl)piperidine-2-one 3 have also been replaced with novel and efficient processes via lactam ring activation.

**Keywords:** Antimicrobial activity, 1-(4-nitrophenyl)piperidine-2-one, sulfonamide derivative, substituted oxopyridine, sulfonyloxopyridine conjugates

Heteroaryl substituted sulfonamides are extremely useful compounds in pharmaceutical industry since they exhibit wide range of biological activity such as antimicrobial, anti-thyroid, anti-tumor and carbonic anhydrase inhibitor. However, various bacterial strains are slowly developing resistance towards the existing heterocyclic sulfonamide drugs. Another very important class of heterocyclic compounds are substituted oxopyridine derivatives which exhibit antimicrobial, anti-convulsant, anticancer and CB2 cannabinoid receptor agonist-antagonist activities. Encouraged by the diverse activities of the sulfonamides as well as oxopyridine derivatives, we have combined them to generate a library of conjugates exhibiting enhance activity as novel antimicrobial agents. In this article, we have introduced for first time the substituted sulfonyloxopyridine derivatives as a new class of effective antimicrobial compounds. In our studies, we have tried to develop a simple, cost effective and scalable process for the synthesis of target compounds 7a-g. The synthesized compounds have been evaluated for antimicrobial activities against different microbial strains and the results compared with standard drugs.

**Results and Discussion**

**Chemistry**
In 2013, Jian’an and co-worker reported process (Scheme I) for the synthesis of compound (6a) starting from 4-nitroaniline (1) and 5-chlorovaleryl chloride (2a). However, the process was not convenient and economically viable due to high price of 5-chlorovaleryl chloride and low overall yield (~60%) up to compound (6a). Synthesis of 3-morpholino-5,6-dihydropyridin-2(1H)-one (10) as an intermediate of Apixaban was reported by Zhou and co-worker. In that article δ-Valerolactam (7), which was used as a starting material is quite expensive and overall yield of the process was not very high either, only ~ 17% up to compound (10). Method for valerolactam (14) ring construction using bromovaleryl chloride (13) starting from 4-idoaniline (12) was reported in several previously published articles. It was noticed that, both 4-idoaniline and bromovaleryl chloride are pretty expensive starting materials and additionally iodo to amine functional group transformation will not be a suitable process in order to prepare the desired compound (6a). Therefore here we have developed a new process for the synthesis of compound (3) employing inexpensive and easily available gluteric anhydride (Scheme II and Scheme III) which was eventually converted (6a and 6b). In this alternate approach, compound (1) was reacted with glutaric anhydride (2) in presence of catalytic trifluoroacetic acid. Removal of by product water by azotropic distillation using Dean-Stark apparatus produced
piperidinedione intermediate, which was further reacted without isolation with triflic anhydride in presence of 2-nitropyridine at −5°C to generate intermediate (3b). This concept of activation of tertiary amide was gained from previously reported article15. The activated triflate intermediate was reduced using the reducing agent triethylsilane. Addition of triethylsilane was done drop-wise at −5°C to provide compound (3) with 86% yield. During our study we have also found that base plays an important role in activation of tertiary amide (Table I).

It was observed that, when electrophilic activation of piperidinedione intermediate (Figure 1, 3a) was done with bases having lower pKa value, compound (3) could be obtained with high yield. Use of bases like triethylamine (TEA) and dimethyl aminopyridine (DMAP) resulted in lowest yield, whereas picoline and pyridine provided moderate i.e. around 40% yield. Interestingly, higher trend in yield was observed when substituted pyridines with different electron withdrawing groups having lower pKa values were used as a base. By employing bases such as 2-halo-substituted pyridines reaction proceeded well resulting in yields in the range of 76-81%. Best result was obtained with 2-nitropyridine where reaction completed in 30 min with 89% yield. Role of base can be explained by proposed plausible mechanistic pathway (Figure 1).
Chlorination reaction was carried out on compound (3) using phosphorous pentachloride (Scheme II) to produce the dichloro compound (4) which was in situ converted to enamine (5a) using excess morpholine. In another sequence compound (4) was converted to (5b) using 1-methyl piperazine (Scheme III). Corresponding aniline analogues (6a and 6b) were produced after reduction of nitro compounds (5a and 5b) with sodium sulphide nonahydrate. The sulfonation process of amines has also been developed for the final step to get the target compounds 7(a-g). Compound 6a and 6b were reacted with various sulfonyl chlorides in the presence of base to produce corresponding sulfonamide analogues 7(a-g). The reactions were clean and disulfonamide impurity formation was not observed in any case.
**Experimental Section**

All the key starting materials, solvents and reagents were obtained from Sigma Aldrich and Merck Specialities Pvt. Ltd. NMR spectra were recorded on a Bruker spectrometer at 400 MHz for ¹H and ¹³C NMR respectively and the chemical shifts were reported as δ value in ppm relative to TMS as internal standard. Infrared (IR) spectra were obtained using a Nicolet 6700 IR spectrometer. Mass spectra were recorded on Shimadzu mass spectrometer. Melting points were recorded on open capillaries and are uncorrected.

**Procedure for the synthesis of compounds (3, 5a, 5b, 6a, 6b,7a, 7b, 7c, 7d, 7e, 7f, 7g)**

**Synthesis of 1-(4-nitrophenyl)piperidin-2-one, 3:** A mixture of 4-nitroaniline (100 g, 724 mmol), glutaric anhydride (86.73 g, 760.20 mmol) and triflic acid (10.86 g, 72.40 mmol) in toluene (500 mL) was refluxed with azeotropic distillation of water. After 4 h toluene was distilled out. The residue was dissolved in 300 mL dichloromethane followed by addition of 2-nitropyridine (94.34 g, 760.20 mmol) at 0°C. To the mixture was added triflic anhydride (214.48 g, 760.20 mmol) at −5°C drop-wise over a period of 30 min, followed by drop-wise addition of triethylsilane (168.37 g, 1448 mmol). The reaction mass was stirred for 1 h at −5°C. After completion of reaction, water (1.5 L) was added to the reaction mass and stirred for 15 min. Layers separated. Organic layer washed with water (1.5 L), dried over sodium sulfate and concentrated. Isopropyl ether (300 mL) was added to the slurry, filtered, washed with isopropyl ether. The wet cake was dried under vacuum at 50°C (149.87 g, 94 %): Rf = 0.45 (CHCl₃/MeOH = 9:1). m.p.95-96°C.

**Table I — Screening of different bases for the activation of piperidinedione intermediate**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagents</th>
<th>pKa</th>
<th>Temperature (°C)</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>TEA</td>
<td>10.75</td>
<td>0</td>
<td>8.00</td>
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</tr>
<tr>
<td>2</td>
<td>DMAP</td>
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<td>0</td>
<td>8.00</td>
<td>33</td>
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<td>3</td>
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<td>8.00</td>
<td>38</td>
</tr>
<tr>
<td>4</td>
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<td>−5</td>
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</tr>
<tr>
<td>5</td>
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<td>−5</td>
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<td>76</td>
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<tr>
<td>6</td>
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</tr>
<tr>
<td>7</td>
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</tr>
<tr>
<td>8</td>
<td>2-NO₂ pyridine</td>
<td>−2.06</td>
<td>−5</td>
<td>0.30</td>
<td>86</td>
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</table>

**Figure 1 — Plausible reaction mechanism**
Synthesis of 3-(morpholine-1-(4-nitrophenyl))-5,6-dihydropyridin-2(1H)-one, 5a: Phosphorous pentachloride (241.8 g, 1360 mmol) was added to chloroform (500 mL) at 25°C. The reaction mass was heated to 45°C and stirred for 30 min. A solution of 1-(4-nitrophenyl)piperidin-2-one (100 g, 450 mmol) in chloroform (600 mL) was added under stirring at 45-50°C. The reaction mass was stirred for 3 h. After completion of reaction, the mass was concentrated to get slurry. The reaction mass was cooled to 10°C and water (1.2 L) was added slowly below 25°C. The product was extracted in dichloromethane (600 mL). Dried the dichloromethane layer over sodium sulfate and concentrated to get oily residue. Isopropyl alcohol (150 mL) was added and stirred to precipitate out the solid. Filtered the product to get wet cake of 3,3-dichloro-1-(4-nitrophenyl)piperidin-2-one (4). The wet cake was added to 1-Methylpiperazine (200 mL) under stirring at 25°C. The reaction mass was heated to 100-105°C and stirred for 4 h. After completion of reaction, the reaction mass was cooled to 55-60°C and water (100 mL) added slowly. The reaction mass was further cooled to 0-5°C and stirred for 30 min. Reaction mass was filtered to get yellow coloured wet cake.

Synthesis of 3-(4-methylpiperazin-1-yl)-1-(4-nitrophenyl)-5,6-dihydropyridin-2(1H)-one, 6a: 3-(morpholine-1-(4-nitrophenyl))-5,6-dihydropyridin-2(1H)-one (50 g, 160 mmol) was added to ethanol (500 mL) and stirred for 15 min. A solution of sodium sulfide nonahydrate (79.18 g, 320 mmol) in water (150 mL) was charged to the reaction mass. Reaction mass was filtered to get yellowish brown coloured wet cake. The wet cake was washed with water and dried under vacuum at 50°C (101.5 g, 71%): R_f = 0.69 (CHCl_3/MeOH = 9:1). m.p. 150-151°C. IR (KBr): 2809 (C-H aliphatic), 1672 (C-O stretching), 1624 (aliphatic C-C), 1590, 1508 (aromatic C=C), 1487 (N=O stretching), 1347 (C-N stretching), 1109 (C-N stretching), 835 cm^{-1} (Ar-H aromatic bending); ^1H NMR (400 MHz, DMSO-d_6, 25°C, TMS): δ 8.25 (d, J = 8.9 Hz, 2H; H-8, H-10), 7.69 (m, 4H; H-13, H-13', H-14, H-14'), 2.34 (s, 3H, C-16); ^13C NMR (400 MHz, DMSO-d_6, 25°C, TMS): δ 161.10 (C-1), 148.91 (C-2), 145.01 (C-9), 142.90 (C-6), 125.70 (C-7, 11), 124.25 (C-8, 10), 116.21 (C-3), 66.42 (C-13, 14), 50.48 (C-12, 15), 48.37 (C-5), 23.2 (C-4); HRMS (ESI) [M+H]^+ Calcd for C_{18}H_{21}N_2O_4: 304.1297. Found: 304.1296. Anal. Calcd for C_{18}H_{21}N_2O_4: C, 59.39; H, 5.64; N, 13.85. Found: C, 59.40; H, 5.65; N, 13.87%.

Synthesis of 3-(4-methylpiperazin-1-yl)-1-(4-nitrophenyl)-5,6-dihydropyridin-2(1H)-one, 5b: Phosphorous pentachloride (241.8 g, 1360 mmol) was added to chloroform (500 mL) at 25°C. The reaction mass was heated to 45°C and stirred for 30 min. A solution of 1-(4-nitrophenyl)piperidin-2-one (100 g, 450 mmol) in chloroform (600 mL) was added under stirring at 45-50°C. The reaction mass was stirred for 3 h. After completion of reaction, the mass was concentrated to get slurry. The reaction mass was cooled to 10°C and water (1.2 L) was added slowly below 25°C. The product was extracted in dichloromethane (600 mL). Dried the dichloromethane layer over sodium sulfate and concentrated to get oily residue. Isopropyl alcohol (150 mL) was added and stirred to precipitate out the solid. Filtered the product to get wet cake of 3,3-dichloro-1-(4-nitrophenyl)piperidin-2-one (4). The wet cake was added to 1-Methylpiperazine (200 mL) under stirring at 25°C. The reaction mass was heated to 100-105°C and stirred for 4 h. After completion of reaction, the reaction mass was cooled to 55-60°C and water (100 mL) added slowly. The reaction mass was further cooled to 0-5°C and stirred for 30 min. Reaction mass was filtered to get yellowish brown coloured wet cake. The wet cake was washed with water and dried under vacuum at 50°C (101.5 g, 71%): R_f = 0.69 (CHCl_3/MeOH = 9:1). m.p. 150-151°C. IR (KBr): 2809 (C-H aliphatic), 1672 (C-O stretching), 1624 (aliphatic C=C), 1590, 1508 (aromatic C=C), 1487 (N=O stretching), 1347 (C-N stretching), 1109 (C-N stretching), 835 cm^{-1} (Ar-H aromatic bending); ^1H NMR (400 MHz, DMSO-d_6, 25°C, TMS): δ 8.24 (d, J = 8.9 Hz, 2H; H-8, H-10), 7.59 (d, J = 8.9 Hz, 2H; H-7, H-11), 5.68 (t, J = 4.1 Hz, Hz, 1H-3), 3.79-3.70 (m, 2H; H-5, H-5'), 2.35-2.30 (m, 4H; H-13, H-13', H-14, H-14'), 2.39-2.34 (s, 3H, C-16); ^13C NMR (400 MHz, DMSO-d_6, 25°C, TMS): δ 161.10 (C-1), 148.91 (C-2), 145.01 (C-9), 142.90 (C-6), 125.70 (C-7, 11), 124.25 (C-8, 10), 116.21 (C-3), 49.74 (C-13, 14), 50.32 (C-12, 15), 48.03 (C-5), 40.89 (C-16), 23.88 (C-4); HRMS (ESI) [M+H]^+ Calcd for C_{18}H_{21}N_2O_4: 317.1613. Found: 317.1610. Anal. Calcd for C_{18}H_{21}N_2O_4: C, 60.75; H, 6.36; N, 17.71. Found: C, 60.72; H, 6.36; N, 17.75%.
completion of reaction, the reaction mass was concentrated, methanol (250 mL) was added to the slurry, cooled to 0-5°C, filtered, washed the wet cake with chilled methanol (50 mL) and wet cake was dried under vacuum at 50°C (39.6 g, 88%): Rf = 0.41 (CHCl3/MeOH = 9:1). m.p. 178-179°C. IR (KBr): 3428 and 3267 (N-H stretching), 2811 (C-H aliphatic), 1656 (C=O stretching), 1610 (aliphatic C=C), 1519 and 1444 (aromatic C=C), 1358 (C-N stretching), 1165 (C-O stretching), 765 and 748 cm⁻¹ (Ar-H aromatic bending); 1H NMR (400 MHz, DMSO-d₆, 25°C, TMS): δ 7.09 (d, J = 8.4 Hz, 2H; H-7, H-11), 6.68 (d, J = 8.4 Hz, 2H; H-8, H-10), 5.70-5.52 (m, 1H; H-3), 3.90-3.75 (m, 4H; H-5, H-5', NH₂), 3.71-3.53 (m, 4H; H-12, H-12', H-15, H-15'), 2.10-2.83 (m, 4H; H-13, H-13', H-14, H-14'), 2.59-2.42 (m, 2H; H-4, H-4'); 13C NMR (400 MHz, DMSO-d₆, 25°C, TMS): δ 160.6 (C-1), 146.7 (C-2), 143.1 (C-9), 131.9 (C-6), 126.4 (C-7,11), 113.7 (C-8,10), 113.5 (C-3), 65.9 (C-13,14), 49.9 (C-12,15), 48.9 (C-5), 22.9 (C-4); HRMS (ESI) [M+H]+ Calcd for. C₁₅H₁₉N₃O₂: 274.1555. Found: 274.1561. Anal. Calcd for C₁₅H₁₉N₃O₂: C, 65.91; H, 7.09; N, 15.35%.

**Synthesis of 1-(4-aminophenyl)-3-(4-methylpiperazin-1-yl)-5,6-dihydropyridin-2(1H)-one, 6b:**
3-(4-methylpiperazin-1-yl)-1-(4-nitrophenyl)-5,6-dihydropyridin-2(1H)-one (50 g, 160 mmol) was added to ethanol (500 mL) and stirred the reaction mass for 15 min. A solution of sodium sulphide nonahydrate (76.85 g, 320 mmol) in water (150 mL) was charged to the reaction mass. Reaction mass was heated to 50°C and stirred for 5 h. After completion of reaction, the reaction mass was concentrated, methanol (250 mL) was added to the slurry, cooled to 0-5°C, filtered the slurry, washed the wet cake with chilled methanol (50 mL) and wet cake was dried under vacuum at 50°C (34.6 g, 77%): Rf = 0.44 (CHCl3/MeOH = 9:1). m.p. 189-190°C. IR (KBr): 3258 (N-H stretching), 2809 (C-H aliphatic), 1655 (C=O stretching), 1605 (Aliphatic C=C), 1517 and 1438 (Aromatic C=C), 1361 and 1167 cm⁻¹ (C=N stretching); 1H NMR (400 MHz, DMSO-d₆, 25°C, TMS): δ 7.09 (d, J = 8.4 Hz, 2H; H-7, H-11), 6.68 (d, J = 8.4 Hz, 2H; H-8, H-10), 5.70-5.52 (m, 1H; H-3), 3.90-3.75 (m, 4H; H-5, H-5'; NH₂), 3.26-3.10 (m, 4H; H-12, H-12', H-15, H-15'), 2.56-2.43 (m, 6H; H-4, H-4', H-13, H-13', H-14, H-14'), 2.41-2.36 (s, 3H; H-16); 13C NMR (400 MHz, DMSO-d₆, 25°C, TMS): δ: 161.02 (C-1), 148.27 (C-2), 143.21 (C-9), 132.02 (C-6), 125.94 (C-7, 11), 113.2 (C-8, 10), 116.21 (C-3), 49.74 (C-13, 14), 50.32 (C-12, 15), 48.03 (C-5), 40.89 (C-16), 23.88 (C-4); HRMS (ESI) [M+H]+ Calcd for C₁₅H₂₁N₃O₆: 287.1871. Found 287.1873. Anal. Calcd for C₁₅H₂₁N₃O₆: C, 67.11; H, 7.74; N, 19.56. Found: C, 67.18; H, 7.70; N, 19.61%.

**Procedure for the synthesis of N-(4-(3-(morpholin-2-oxo-5,6-dihydropyridin-1(2H)-yl)phenyl)benzenesulfonylamide, 7a:**
1-(4-aminophenyl)-3-morpholino-5,6-dihydropyridin-2(1H)-one (5 g, 18 mmol) followed by pyridine (1.62 mL, 20 mmol) were added to dichloromethane (DCM, 50 mL). Reaction mass was stirred to get clear solution. Benzenesulfonyl chloride (3.56 g, 20 mmol) was added to the reaction mass and stirred for 5 h at 40°C. After completion of reaction, water (50 mL) was added to quench the reaction and layers separated. Organic layer was washed with dilute aq. HCl and water, concentrated to get oil. The compound was column purified using ethyl acetate: hexane (30:70) solvent mixture (5.68 g, 75%): Rf = 0.53 (CHCl₃/MeOH = 9:1). m.p. 240-241°C. IR (KBr): 3265 (SO₂N-H stretching), 2811 (C-H aliphatic), 1656 (C=O stretching), 1610 (Aliphatic C=C), 1519 and 1444 (Aromatic C=C), 1345 and 1165 cm⁻¹ (S=O stretching); 1H NMR (400 MHz, DMSO-d₆, 25°C, TMS): δ 9.41(s, 1H, NH), 7.66 (d, J = 8.4 Hz, 2H; H-7, H-11), 7.52-7.38 (m, 5H; H-17, H-18, H-19, H-20, H-21), 7.02 (d, J = 8.4Hz, 2H; H-8, H-10), 5.67 (m, 1H; H-3), 3.90-3.73 (m, 6H; H-5, H-5', H-12, H-12', H-15, H-15'), 2.92-2.85 (m, 4H; H-13, H-13', H-14, H-14'), 2.43-2.39 (m, 2H; H-4, H-4'); 13C NMR (400 MHz, DMSO-d₆, 25°C, TMS): δ 161.10 (C-1), 149.21 (C-2), 144.21 (C-6), 134.80 (C-9), 138.53 (C-16), 132.70 (C-7, 11), 129.32 (C-19), 125.66 (C-18, 20), 124.28 (C-17, 21), 118.31 (C-8, 10), 114.21 (C-3), 66.82 (C-13, 14), 50.48 (C-12, 15), 48.37 (C-5), 23.90 (C-4); HRMS (ESI) [M+H]+ Calcd for C₂₁H₂₃N₃O₅S: 414.1487 (M+1). Found: 414.1490. Anal. Calcd for C₂₁H₂₃N₃O₅S: C, 61.00; H, 5.61; N, 10.16. Found: C, 61.11; H, 5.60; N, 10.18%.

**Synthesis of 4-methyl-N-(4-(3-(morpholin-2-oxo-3,6-dihydropyridin-1(2H)-yl)phenyl) benzenesulfonamide, 7b:**
1-(4-aminophenyl)-3-morpholino-5,6-dihydropyridin-2(1H)-one (5 g, 18 mmol) followed by pyridine (1.62 mL, 20 mmol) were added to DCM (50 mL). Reaction mass was stirred to get clear solution. Tosyl chloride (3.84 g, 20 mmol) was added to the reaction mass and the reaction was
stirred for 3 h at 40°C. After completion of reaction, water (20 mL) was added to quench the reaction and layer separated. Organic layer was washed with dilute aq. HCl and water, concentrated to get oil. The compound was column purified using ethyl acetate: hexane (30:70) solvent mixture (5.78 g, 74%); \( R_f = 0.55 \) (CHCl₃/MeOH = 9:1). m.p. 248-249°C. IR (KBr): 3265 (SO₂N-H stretching), 2811 (C-H aliphatic), 1656 (C=O stretching), 1610 (aliphatic C=C), 1519 and 1444 (aromatic C=C), 1345 and 1167 cm⁻¹ (S=O stretching); ¹H NMR (400 MHz, DMSO-d₆, 25°C, TMS): δ 8.35 (s, 1H, NH), 7.68 (d, J = 8.4 Hz, 2H; H-7, H-11), 7.53-7.39 (m, 5H; H-17, H-18, H-19, H-20, H-21), 7.03 (d, J = 8.4 Hz, 2H; H-8, H-10). 5.67 (m, 1H; H-3), 3.79-3.70 (m, 2H; H-5, H-5'), 3.25-3.09 (m, 4H; H-12, H-12', H-15, H-15'), 2.63-2.44 (m, 6H; H-13, H-13', H-14, H-14', H-4, H-4'), 2.39-2.34 (s, 3H; H-22, H-22', H-22''); ¹³C NMR (400 MHz, DMSO-d₆, 25°C, TMS): δ 161.10 (C-1), 149.21 (C-2), 144.21 (C-6), 143.80 (C-9), 138.52 (C-16), 132.53 (C-7, 11), 129.70 (C-19), 125.74 (C-18, 20), 124.28 (C-17-21), 118.25 (C-8, 10), 114.98 (C-3), 50.33 (C-12, 15), 49.95 (C-13, 14), 48.03 (C-5), 40.91 (C-22), 23.90 (C-4); HRMS (ESI) [M+H]⁺ Calcd for C₂₂H₂₅N₃O₄S: 427.1812. Found: 427.1812. Anal. Calcd for C₂₂H₂₅N₃O₄S: C, 61.95; H, 5.90; N, 9.81%.

Synthesis of \( N-(4-(3-(4-methylpiperazin-1-yl)-2-oxo-5,6-dihydopyridin-1(2H)-yl)phenyl) naphthalene-2-sulfonamide, 7d\): 1-(4-aminophenyl)-3-morpholino-5,6-dihydopyridin-2(1H)-one (5 g, 18 mmol) followed by pyridine (1.62 mL, 20 mmol) was added to a solution of DCM (50 mL). Reaction mass was stirred to get clear solution. Organic layer was washed with dilute aq. HCl and water, concentrated to get oil. The compound was column purified using ethyl acetate: hexane (30:70) solvent mixture (5.43 g, 64%); \( R_f = 0.52 \) (CHCl₃/MeOH = 9:1). m.p. 273-275°C. IR (KBr): 3262 (SO₂N-H stretching), 2811 (C-H aliphatic), 1656 (C=O stretching), 1610 (aliphatic C=C), 1519 and 1444 (aromatic C=C), 1362 and 1158 cm⁻¹ (S=O stretching); ¹H NMR (400 MHz, DMSO-d₆, 25°C, TMS): δ 9.42 (s, 1H, NH), 8.53 (s, 1H; H-23), 8.04-7.99 (m, 4H; H-22, H-19, H-18, H-17), 7.75-7.66 (m, 3H; H-7, H-11, H-21), 7.38 (m, 1H; H-20), 7.10-7.08 (m, 2H; H-8, H-10), 5.71 (m, 1H; H-3), 3.86-3.82 (m, 6H; H-5, H-5', H-12, H-12', H-15, H-15'), 2.92 (m, 4H; H-13, H-13', H-14, H-14'), 2.51 (m, 2H; H-4, H-4'); ¹³C NMR (400 MHz, DMSO-d₆, 25°C, TMS): δ 161.02 (C-1), 149.22 (C-2), 144.17 (C-6), 143.00 (C-9), 136.02 (C-25), 134.66 (C-16), 132.86 (C-24), 132.17 (C-7, 11), 129.81 (C-18), 129.68 (C-19), 129.23 (C-22), 128.32 (C-20), 128.08 (C-21), 125.70 (C-23), 124.23 (C-17), 118.88 (C-8, 10), 114.83 (C-3), 66.42 (C-13, 14), 50.48 (C-12, 15), 48.37 (C-5), 23.20 (C-4), 21.53 (C-22); HRMS (ESI) [M+H]⁺ Calcd for C₂₂H₂₅N₃O₄S: 464.1644. Found: 464.1640. Anal. Calcd for C₂₂H₂₅N₃O₄S: C, 61.84; H, 5.61; N, 13.11%; 82%): \( R_f = 0.57 \) (CHCl₃/MeOH = 9:1). m.p. 1519 and 1444 (aromatic C=C), 1360 and 1160 cm⁻¹ (S=O stretching); ¹H NMR (400 MHz, DMSO-d₆, 25°C, TMS): δ 9.39 (s, 1H, NH), 7.69 (d, J = 8.4 Hz, 2H; H-7, H-11), 7.53-7.39 (m, 5H; H-17, H-18, H-19, H-20, H-21), 7.03 (d, J = 8.4 Hz, 2H; H-8, H-10). 5.67 (m, 1H; H-3), 3.79-3.70 (m, 2H; H-5, H-5'), 3.25-3.09 (m, 4H; H-12, H-12', H-15, H-15'), 2.63-2.44 (m, 6H; H-13, H-13', H-14, H-14', H-4, H-4'), 2.39-2.34 (s, 3H; H-22, H-22', H-22''); ¹³C NMR (400 MHz, DMSO-d₆, 25°C, TMS): δ 161.10 (C-1), 149.21 (C-2), 144.21 (C-6), 143.80 (C-9), 138.52 (C-16), 132.53 (C-7, 11), 129.70 (C-19), 125.74 (C-18, 20), 124.28 (C-17-21), 118.25 (C-8, 10), 114.98 (C-3), 50.33 (C-12, 15), 49.95 (C-13, 14), 48.03 (C-5), 40.91 (C-22), 23.90 (C-4); HRMS (ESI) [M+H]⁺ Calcd for C₂₂H₂₅N₃O₄S: 427.1812. Found: 427.1812. Anal. Calcd for C₂₂H₂₅N₃O₄S: C, 61.95; H, 6.14; N, 13.13. Found: C, 61.84; H, 6.15; N, 13.11%.
Synthesis of \( N-(4-(3-(\text{morpholino-2-oxo-5,6-dihydropyridin-1(2H)-yl})phenyl)\) methane sulphonamide, 7e: \( 1-(4\text{-aminophenyl})-3\text{-morpholino-5,6-dihydropyridin-2(1H)-one}\) (5 g, 18 mmol) followed by pyridine (1.62 mL, 20 mmol) were added to a solution of DCM (50 mL). Reaction mass was stirred to get clear solution. Methanesulfonyl chloride (2.30 g, 20 mmol) was added to the reaction mass and the reaction was stirred for 3 h at 40°C. After completion of reaction, water (50 mL) was added to quench the reaction and layers separated. Organic layer was washed with dilute aq. HCl and water, concentrated to get oil. The compound was purified using ethyl acetate: hexane (30:70) solvent mixture: (5.43 g, 64%): \( R_f = 0.62 \) (CHCl\( _3/\)MeOH = 9:1). m.p. 240-241°C. IR (KBr): 3250 (SO\textsubscript{2}N-H stretching), 2820 (C-H aliphatic), 1650 (C=O stretching), 1602 (aliphatic C=C), 1351 and 1146 cm\(^{-1}\) (S=O stretching); \( ^1\text{H NMR} \) (400 MHz, DMSO-\( _d_6 \), 25°C, TMS): \( \delta \) 7.65 (d, \( J = 8.4 \text{ Hz}, 2\text{H}; \text{H-7, H-11} \)), 7.03 (d, \( J = 8.4 \text{Hz}, 2\text{H}; \text{H-8, H-10} \)), 6.85 (s, 1\text{H}, NH), 5.67 (m, 1\text{H}; H-3), 3.91-3.72 (m, 6\text{H}; H-5, H-5', H-12, H-12', H-15, H-15'), 2.92-2.85 (m, 4\text{H}; H-13, H-13', H-14, H-14'), 2.43-2.39 (m, 2\text{H}; H-4, H-4'); HRMS (ESI) \([M+H]^+\) Calcd for \( \text{C}_{16}\text{H}_{21}\text{N}_{3}\text{O}_{4}\text{S} \): 352.1311. Found: 352.1312. Anal. Calcd for \( \text{C}_{16}\text{H}_{21}\text{N}_{3}\text{O}_{4}\text{S}: \text{C}, 54.75; \text{H}, 6.11; \text{N}, 11.96. \) Found: C, 54.75; H, 6.11; N, 11.92%.

Synthesis of \( 1,1,1\text{-trifluoro-N-(4-(3-(\text{morpholino-2-oxo-5,6-dihydropyridin-1(2H)-yl})phenyl)\) ethanesulfonamide, 7g: \( 1-(4\text{-aminophenyl})-3\text{-morpholino-5,6-dihydropyridin-2(1H)-one}\) (5 g, 18 mmol) followed by pyridine (1.62 mL, 20 mmol) were added to a solution of DCM (50 mL). Reaction mass was stirred to get clear solution. Ethanesulfonyl chloride (2.58 g, 20 mmol) was added to the reaction mass and the reaction was stirred for 3 h at 40°C. After completion of reaction, water (50 mL) was added to quench the reaction and layers separated. Organic layer was washed with dilute aq. HCl and water, concentrated to get oil. The compound was column purified using ethyl acetate: hexane (30:70) solvent mixture: (5.43 g, 64%): \( R_f = 0.60 \) (CHCl\( _3/\)MeOH = 9:1). m.p. 240-241°C. IR (KBr): 3254 (SO\textsubscript{2}N-H stretching), 2825 (C-H aliphatic), 1651 (C=O stretching), 1605 (aliphatic C=C), 1355 and 1145 cm\(^{-1}\) (S=O stretching); \( ^1\text{H NMR} \) (400 MHz, DMSO-\( _d_6 \), 25°C, TMS): \( \delta \) 7.66 (d, \( J = 8.4 \text{Hz}, 2\text{H}; \text{H-7, H-11} \)), 7.02 (d, \( J = 8.4 \text{Hz}, 2\text{H}; \text{H-8, H-10} \)), 6.34 (m, 1\text{H}; H-3), 3.91-3.72 (m, 6\text{H}; H-5, H-5', H-12, H-12', H-15, H-15'), 2.90-2.84 (m, 7\text{H}; H-13, H-13', H-14, H-14', CH\textsubscript{2}SO\textsubscript{2}), 2.43-2.39 (m, 2\text{H}; H-4, H-4'); Calcd for \( \text{C}_{16}\text{H}_{15}\text{F}_{6}\text{N}_{3}\text{O}_{6}\text{S}_{2} \): 352.1313. Found: 352.1312. Anal. Calcd for \( \text{C}_{16}\text{H}_{15}\text{F}_{6}\text{N}_{3}\text{O}_{6}\text{S}_{2}: \text{C}, 54.68; \text{H}, 6.02; \text{N}, 11.96. \) Found: C, 54.75; H, 6.11; N, 11.92%.

Antibacterial activity

The MIC of the synthesized compounds 6a, b and 7a-g were tested against both Gram positive and Gram negative bacteria (Table II) by Broth dilution method\(^{16,17}\). Broth dilution method is one of the non-automated \textit{in vitro} bacterial susceptibility tests. This classic method provides\(^{18,19}\) a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganism. \textit{Staphylococcus aureus} (MTCC 96), \textit{Streptococcus pyogenes} (MTCC 442) were used as Gram positive group of bacteria and \textit{Escherichia coli} (MTCC 443), \textit{Pseudomonas aeruginosa} (MTCC 1688) as Gram negative group of bacteria. Standard antibiotics like
ampicillin, chloramphenicol, and ciprofloxacin were used as standard anti-biotic drugs against Gram positive and Gram negative bacteria.

Overall inhibition effect of compound (7a) towards the growth of both type of bacterial colony was superior as compared to standard drug ampicillin and almost similar with the other standard drugs like chloramphenicol and ciprofloxacin. Compound (7a) and (7c) were most effective against E. coli. Activity (6.25 µg/mL) of the compound (7a) against E. coli was better than standard drugs like ampicillin (100 µg/mL), chloramphenicol (50 µg/mL) and ciprofloxacin (25 µg/mL). Compound (7c) also possessed more activity (62.5 µg/mL) against E. coli as compared to ampicillin and similar activity as compared to chloramphenicol. Four compounds (7a-7c) and (7d) showed better activity against S. aureus as compared to standard drug ampicillin. Compound (7a) showed better activity against S. pyogenus as compared to ampicillin and almost similar activity as compared to chloramphenicol and ciprofloxacin. Compound (7b) showed similar activity against S. pyogenus as compared to standard drug ampicillin. Three compounds (7e-g) showed lower activities on all the strains as compared to the standard drugs.

**Antifungal activity**

Since the synthesized compounds showed promising anti-bacterial activity, those were also tested against three different pathogenic fungi. The tested fungi were C. albicans (MTCC 227), A. niger (MTCC 282) and A. clavatus (MTCC 1323). Nystatin and greseofulvin have been used as standard antifungal agents (Table III).

<table>
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<th>Compd</th>
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<tr>
<td>Greseofulvin</td>
<td>500</td>
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</tr>
</tbody>
</table>

All the compounds showed low antifungal activity except compound (7c), which was effective against C. albicans as compared to standard drug greseofulvin.

**Structure activity relationship**

The activity of penultimate compounds (6a) and (6b) were also evaluated along with the sulphonamide compounds 7a-g. Almost negligible activity (>1000 µg/mL) were observed for both the compounds (6a) and (6b). The test samples showed considerable activity after introduction of sulphonamide linkage at amine terminal. This important finding indicates that presence of sulfonyl group is important to obtain the activity of the compounds. The reason for the highest activity of compound (7a) against both Gram positive and Gram negative bacterial colony could be due to the presence of aromatic benzene ring at sulfonyl terminal. In case of compound (7b), where the
benzene ring was replaced by \( p \)-toluene ring the activity of the compound drastically reduced against gram negative bacterial colony mainly \( E.\) coli. The activity against gram positive bacterial colony remained almost same. The substituent at 2-position of oxopyridine ring also played an important role towards the activity of the compounds. In case of compound (7c), when the morpholine substituent was replaced by 1-Methylpiperazine group the activity of the compound decreased for both Gram negative and Gram positive bacterial colony as compared to compound (7a). This indicated that presence of morpholine substituent at oxopyridine terminal is essential to retain better activity. Further in compound (7d) when benzene ring was replaced by poly-nuclear aromatic hydrocarbons like naphthalene ring at sulfonamide terminal, keeping the morpholine substituent intact at the oxopyridine terminal, lower activity was observed for both type of bacterial colony. Further, it was noticed that, in case of aliphatic sulfonyl group 7(e-g) lesser activity was observed as compared to aromatic sulfonyl group.

**Plausible mode of action**

It is previously reported in the literature that, dihydropteroate synthase (DHPS) plays an important role in the synthesis of folate via dihydrofolic acid pathway. The folate is required for the prokaryotes to make nucleic acids \( i.e. \) DNA and RNA which in turn helps in cell division. We assumed that similar to earlier reports on mechanism of sulfonamide drugs, the compounds (7a-g) might be acting as the inhibitor of dihydropteroate synthase (DHPS) and thus preventing activities like folate synthesis followed by formation of nucleic acids, which is turn arrest prokaryotic cell growth.

**Conclusion**

The antibacterial and antifungal activities of the prepared compounds were evaluated showing moderate to good activities. Compound (7a) exhibited the highest antimicrobial activity comparable to standard antibiotics. The introduction of these conjugates are expected to become important additions to mankind’s continuous quest for the design and development of more efficient drugs in the fight against harmful microorganisms which are developing resistance to the existing drugs at an alarming rate. The feasibility of the synthetic procedures and the excellent yield of the prepared compounds are the main advantages for the developed protocol. Among the synthesized compounds, (7a) can be identified as potential antimicrobial agent and selected as lead compounds for further studies.

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**References**