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Chapter

Synthesis and Anticancer Evaluation of Benzenesulfonamide Derivatives

Dattatraya Navnath Pansare and Rohini Narayan Shelke

Abstract

A highly efficient protocol was developed for the synthesis of 3-(indoline-1-carbonyl)-N-(substituted) benzene sulfonamide analogs with excellent yields. The new 3-(indoline-1-carbonyl)-N-(substituted) benzene sulfonamide derivatives (4a-g and 5a-g) were evaluated in vitro anticancer activity against a series of different cell lines like A549 (lung cancer cell), HeLa (cervical), MCF-7 (breast cancer cell) and Du-145 (prostate cancer cell) respectively. The results of the anticancer activity data revealed that most of the tested compounds showed IC_{50} values from 1.98 to 9.12 μM in different cell lines. Compounds 4b, 4d, 5d, and 5g were the most potent, with IC_{50} values ranging from 1.98 to 2.72 μM in different cell lines.

Keywords: indoline, sulfonamide, anticancer

1. Introduction

Antibiotic resistant bacteria are rapidly emerging worldwide [1]. The various biological active heterocyclic compounds, the indole derivatives are the key structural feature commonly found in natural products [2, 3] and bioactive molecules, such as tryptophan [4], tryptamine [5], and auxin [6]. Furthermore, it has been reported that sharing of the indole 3-carbon in the formation of spiroindoline derivatives highly enhances biological activity [7]. Moreover, some of the compounds containing benzenesulfonamide moiety also show broad spectrum biological properties such as elastase inhibitors [8], carboxic anhydrase inhibitors [9], clostridium histolyticum collagenase inhibitors [10] as well as herbicides and plant growth regulators [11]. Sulfonamides are common motifs in many drugs and medicinal compounds and play an important role in their bioactivity since the development of sulfa antibiotics in the 1930s [12]. Common drugs such as glibenclamide [13], sultiame [14], and COX-II inhibitors Piroxicam [15], Ampiroxicam [16], and Celecoxib [17] containing a sulfonyl moiety, which displays potential activity across a variety of biological targets. The sulfonamides are organic sulfur compounds which have attracted the attention for their better pharmacological activity [18–20]. It is interesting to note that the sulfonamide containing moiety is known to have some biological and pharmaceutical properties, such as, antitumor, antibacterial, thrombin inhibition, and antifungal activities [21–23].

In view of the above considerations, in continuation of our previous work on triazoles, pyrimidine, thiazoles and thiazolidinones of pharmaceutical interest [24–30] we report here on the synthesis and anticancer activity of new 3-(indoline-1-carbonyl)-N-(substituted) benzene sulfonamide analogs.
2. Results and discussion

2.1 Chemistry

The aim of this work was to design and synthesize a novel series of benzenesulfonamide incorporating biologically active indoline moieties to evaluate their anticancer activity. We have synthesized new derivatives containing sulfonamide linkage in framework. The synthetic methods adopted for the preparation of the N-(substituted phenyl)-3-(indoline-1-carbonyl)benzenesulfonamide derivatives (5a-g) in Figure 1. We herein report the synthesis of new substituted sulfonamide derivatives with the aim of investigating their anticancer activity (Table 2). The synthetic methods adopted for the preparation of the title compounds (5a-g) are presented below. We have tried to develop simplified reaction conditions for all the steps by avoiding costly reagents, tedious purifications and longer reactions times, we have screened peptide coupling condition in Table 1 to obtain better yield, good purity, shorter reaction time, avoiding costly reagents and mainly reproducibility of yields.

For synthesis of compound 2 was done by using 1 treated with sulfonyl chloride at 0°C in DCM for 30 min and at room temperature for 1 h. The reaction mixture was evaporated under reduced pressure and the obtained gummy material was washed with excess of n-hexane. The material was crystallized using 20% ethyl acetate: n-hexane mixture, no purification was required and the pure compound is obtained as yellow solid. This was used further used for sulfonamide reaction.

Figure 1.
Synthesis of N-(substituted phenyl)-3-(indoline-1-carbonyl)benzenesulfonamide. Reagents and conditions: (a) sulfonyl chloride, dichloromethane (DCM) 0°C-rt; (b) substituted amine, pyridine, DCM, 0°C-rt; (c) lithium hydroxide (LiOH), tetrahydrofuran (THF), water (H2O), rt.; (d) indoline, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI), diisopropylethylamine (DIEPA), DCM, rt.
For the synthesis of compounds from 3a-g by sulfonamide coupling, different substituted amines were coupled with 2 in presence of pyridine as base and DCM as solvent at room temperature for 4 h. The reaction mass was treated with cold 2N aqueous HCl and stirred for 30 min., the solid precipitates out in most of cases which was filtered and washed with cold diethyl ether and cold pentane, all the intermediates obtained were white solids. For intermediates 3a-g the reaction yield was 85–95%.

For synthesis of 4a-4g requires hydrolysis of 3a-g using lithium hydroxide, tetrahydrofuran and water at room temperature for 10 h. Work up of reactions were modified, and wash in basic conditions and later acidifying it to get desired product as white solids with required purity. The acids obtained were in pure state so that it can be directly used for next amide coupling with indoline. All reaction intermediates 4a-g yield up to 80–85%.

For synthesis of 5a-g we have done series of screenings by varying different coupling reagents, different bases, solvents and time. We have varied the equivalents of reagents and bases used to get better yield and purity by avoiding column purifications. The results of screenings are explained in Table 1. In entry 1 and 2 we have used 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) as coupling reagent and DMF as solvent we have varied bases triethylamine and diisopropylethylamine after 14 h we got product 57 and 55% respectively. In entries 3 and 4 we have used benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphat (PyBOP) as coupling reagent and THF as solvent and TEA and DIPEA as base to obtain yields 45 and 50% respectively. In entries 5, 6, 7, and 8 we have used the EDCI and hydroxybenzotriazole (HOBt) as coupling reagents with DMF as solvent along with TEA and DIPEA as base in entries 5 and 6 we have used 2.5 equiv. of base and in entries 7 and 8 we have increased base as 4 equiv. for 14 h. The yields obtained are 62, 72, 78, and 67% respectively. Same results like entry 1 and 2 are obtained in entry 9 and 10 when we used propylphosphonic anhydride (T3P) as coupling reagent and TEA and DIPEA as bases in DCM to get 50 and 60% yield respectively. In entry 11 it was observed that when EDCI (1.5 equiv.) when used along with DIPEA (2.5 equiv.) in DCM the yields was 95%, highlighted bold in Table 1.

Work up requires extraction, and later on washing with 2N aqueous hydrochloric acid (HCl) to

| Sr. no. | Coupling reagent | Base | Solvent | Time (h) | Yield (%) |
|---------|------------------|------|---------|----------|-----------|
| 1.      | HATU (1.1 equiv.) | TEA (1.2 equiv.) | DMF      | 14        | 57        |
| 2.      | HATU (1.1 equiv.) | DIPEA (1.2 equiv.) | DMF      | 14        | 55        |
| 3.      | PyBOP (1.1 equiv.) | TEA (1.2 equiv.) | THF      | 14        | 45        |
| 4.      | PyBOP (1.1 equiv.) | DIPEA (1.2 equiv.) | THF      | 14        | 50        |
| 5.      | EDCI (1.5 equiv.) | TEA (2.5 equiv.) | DMF      | 14        | 62        |
| 6.      | EDCI (1.5 equiv.) | DIPEA (2.5 equiv.) | DMF      | 14        | 72        |
| 7.      | EDCI (1.5 equiv.) | TEA (4 equiv.) | DMF      | 14        | 78        |
| 8.      | HOBr (1.5 equiv.) | DIPEA (4 equiv.) | DMF      | 14        | 67        |
| 9.      | T3P (1.2 equiv.) | TEA (2.5 equiv.) | DMF      | 10        | 50        |
| 10.     | T3P (1.2 equiv.) | DIPEA (2.5 equiv.) | DCM      | 10        | 60        |
| 11.     | EDCI (1.5 equiv.) | DIPEA (2.5 equiv.) | DCM      | 10        | 95        |

Acid (1 equiv.) and indoline (1.2 equiv.)

Table 1. Optimization of peptide coupling reaction (5a-g).
obtain solid compounds. Which was washed with 5% DCM: hexane, cold diethyl ether and cold pentane gives the desired compounds in yield highest yields and with 95% and above purity for 5a-g. We have not used HOBt in entry 11 and 90% yield obtain after 10 h only. The advantage of peptide coupling screenings are no need of column chromatography, no costly reagents required, no prep purification required. All obtained compounds are with 95% and above purity and are directly used for anticancer testing.

2.2 Biological evaluation: anticancer activity

The synthesized compounds were evaluated for their in vitro anticancer activity against human lung cancer cell line (A549), cervical (HeLa) cancer cell line, breast cancer cell line (MCF-7) and prostate cell line (DU-145) using 5-fluorouracil as reference drug [31].

5-Flourouracil is used for anal, breast, colorectal, esophageal, stomach, pancreatic and skin cancers mainly. The response parameter calculated was the IC₅₀ value, which corresponds to the concentration required for 50% inhibition of cell viability. The results are presented in Table 2, where all compounds exhibit moderate to good activity compared to 5-fluouracil as positive control. In the case of the human lung cancer cell line (A549) compounds 4a, 4b, 4d, 4f, 5d, and 5g were the most potent, with IC₅₀ values ranging from 1.98 to 2.82 μM. On the HeLa cell line the compounds which showed potent activity were 4b, 4d, 5d, and 5g (IC₅₀ = 1.99–2.92 μM). In case of the MCF-7 breast cancer cell line, the potent compounds were 4d, 5d, and 5g with IC₅₀ activity of 2.12–2.52 μM. Lower activity was observed for the synthesized compounds on the Du-145 prostate cancer cell line, where the most potent candidates were compounds 5g with IC₅₀ activity in the range of 2.12 μM. Generally, the lung

| Compound | A549 (lung cancer cell) | HeLa (cervical cancer cell) | MCF-7 (breast cancer cell) | Du-145 (prostate cancer cell) |
|----------|-------------------------|----------------------------|----------------------------|-----------------------------|
| 4a       | 1.98 ± 0.12             | 3.83 ± 0.16                | 3.52 ± 0.06                | 3.86 ± 0.16                 |
| 4b       | 2.81 ± 0.13             | 2.92 ± 0.08                | 2.32 ± 0.22                | 3.82 ± 0.12                 |
| 4c       | 4.81 ± 0.12             | 6.32 ± 0.04                | 4.32 ± 0.06                | 3.73 ± 0.12                 |
| 4d       | 2.82 ± 0.11             | 1.99 ± 0.22                | 2.36 ± 0.12                | 3.52 ± 0.11                 |
| 4e       | 3.86 ± 0.08             | 4.38 ± 0.06                | 3.63 ± 0.12                | 6.52 ± 0.22                 |
| 4f       | 2.72 ± 0.11             | 3.87 ± 0.08                | 4.12 ± 0.06                | 3.86 ± 0.22                 |
| 4g       | 3.14 ± 0.14             | 3.98 ± 0.12                | 4.86 ± 0.11                | 4.57 ± 0.11                 |
| 5a       | 8.48 ± 0.14             | 9.12 ± 0.08                | 7.82 ± 0.08                | 9.12 ± 0.06                 |
| 5b       | 3.82 ± 0.08             | 4.13 ± 0.12                | 3.13 ± 0.11                | 3.52 ± 0.08                 |
| 5c       | 4.13 ± 0.12             | 5.16 ± 0.08                | 6.12 ± 0.12                | 4.52 ± 0.11                 |
| 5d       | 2.06 ± 0.12             | 2.12 ± 0.08                | 2.52 ± 0.16                | 5.12 ± 0.08                 |
| 5e       | 2.52 ± 0.11             | 3.52 ± 0.11                | 4.48 ± 0.08                | 4.08 ± 0.11                 |
| 5f       | 4.48 ± 0.08             | 4.98 ± 0.11                | 5.17 ± 0.22                | 5.18 ± 0.18                 |
| 5g       | 2.73 ± 0.08             | 2.12 ± 0.12                | 2.12 ± 0.08                | 2.12 ± 0.04                 |
| 5-FU     | 1.61 ± 0.12             | 1.72 ± 0.18                | 1.81 ± 0.10                | 1.89 ± 0.12                 |

Table 2.
In vitro anticancer screening of the synthesized compounds against four cell lines, data are expressed as IC₅₀ (μM) SD (n = 3).
(A549) and cervical (HeLa) cancer cell lines were the most sensitive to the synthesized compounds. With regard to broad spectrum anticancer activity, close examination of the data presented in Table 2, reveals that compounds 4b, 4d, and 5g were the most active, showing effectiveness toward the four cell lines. The structure activity relationship (SAR) can be explained on the basis of substitutions on both the aromatic rings less hindered substitution like methyl and ethyl on ortho and para position of rings increases the anticancer activity in all four cell lines, interestingly ortho trifluoromethyl and indoline group decreases the anticancer activity and despite steric hindrance 4b, 4d, 5d, and 5g shows promising activity because of electron donating tendency. Most of the compounds show promising anticancer activity with electron donating groups on the ring than electron withdrawing groups.

2.3 General experimental procedure for the synthesis of N-(substituted phenyl)-3-(indoline-1-carbonyl)benzenesulfonamide (5a-g)

2.3.1 Step-1: preparation of ethyl 3-(chlorosulfonyl)benzoate (2)

To a stirred solution of ethyl benzoate (10 g, 67 mmol) in DCM (25 mL). RM was cooled to 0°C and chloro sulfonic acid (9 g, 73 mmol) was added drop wise and stirred for 1 h at same temperature followed by stirring at room temperature for 1 h. After completion of reaction, evaporate reaction mixture under reduced pressure and obtained gummy material is washed with excess of hexane and it is crystalized from 20% ethyl acetate: hexane mixture to obtain white solid as ethyl 3-(chlorosulfonfyl)benzoate (2) which is used further for sulfonamide coupling reaction. Yield 54 g (81%).

2.3.2 Step-2: preparation of ethyl 3-(N-(o-tolyl)sulfamoyl)benzoate (3a-g)

To a stirred solution of ethyl 3-(chlorosulfonyl)benzoate (2) (3 g, 10.1 mmol) in DCM (5 ml) was added pyridine (5 ml) the mixture was stirred at room temperature for 10 min. RM was cooled to 0°C and 2-methyl aniline (1.6 g, 15.16 mmol) was added drop wise followed by stirring at room temperature for 3 h. The reaction was monitored by TLC and LCMS, after completion of reaction poured reaction mass on cold 2N aqueous HCl (10 ml) and stirred RM it for 30 min. Precipitation formed in RM. Filtered the obtained solid and wash it with excess of water and cold diethyl ether (10 ml) and cold pentane (10 ml) to obtain ethyl 3-(N-(o-tolyl)sulfamoyl)benzoate 2 as white solid. Yield 2.8 g (90%).

2.3.3 Step-3: preparation of 3-(N-(o-tolyl)sulfamoyl)benzoic acid (4a-g)

To a stirred solution of ethyl 3-(N-(o-tolyl)sulfamoyl)benzoate (3a-g) (2 g, 5.40 mmol) in THF (10 ml) added water (2 ml), and lithium hydroxide (0.377 g, 18.2 mmol) and stirred reaction mixture for 4 h. Progress reaction was monitored by TLC and LCMS. After the completion of reaction evaporate reaction mixture under reduced pressure to obtain gummy material. Added 10 ml of water in it and extracted it with diethyl ether (10 ml) and cold diethyl ether (10 ml) and cold pentane (10 ml) to obtain desired 3-(N-(o-tolyl)sulfamoyl)benzoic acid 4a as white solids. Yield 1.6 g (90%).
2.3.4 Step-4: N-(substituted phenyl)-3-(indoline-1-carbonyl)benzenesulfonamide (5a-g)

The compound 3-(N-(o-tolyl)sulfamoyl)benzoic acid 4a-g (0.2 g, 0.65 mmol) was treated with EDCI (0.188 g, 0.98 mmol), DIPEA (0.34 ml, 1.96 mmol) in DCM (10 ml). Then added 2,4-dimentyl aniline (0.238 g, 1.96 mmol) and stirred RM at room temperature for 4 h. The reaction was monitored by TLC. Added 10 ml of cold water and stirred for 10 min, then extracted it with 10 ml of DCM. Collected organic layer wash it with 1N aqueous HCl and washed with brine (10 ml). To evaporate the organic layer to obtained the compound with 90% purity (5a-g).

Purification done by washing with 5:95% of DCM: hexane. Obtained solid washed with cold diethyl ether (20 ml) and cold pentane (20 ml) to obtain compounds (5a-g). N-(2,4-dimethylphenyl)-3-(N-(o-tolyl)sulfamoyl)benzamide (5a): (0.240 g, 90%) as white solid, LC-MS m/z (%): 395 (M + H). 1H NMR (400 MHz, DMSO-d6) δ 10.05 (s, 1H), 9.70 (s, 1H), 8.26 (s, 1H), 8.22 (d, J = 7.6 Hz, 1H), 7.81 (d, J = 8 Hz, 1H), 7.7 (d, J = 8 Hz, 1H), 7.18–7.13 (m, 2H), 7.1–7.08 (m, 3H), 7.01 (d, J = 8.4 Hz, 1H), 6.95–6.92 (m, 1H), 2.28 (s, 3H), 2.15 (s, 3H), 2.02 (s, 3H). HPLC-98.25% RT -5.68 min. 13C NMR (CDCl3, 100 MHz): 17.65, 17.79, 20.54, 126.09, 126.38, 126.40, 126.43, 126.58, 129.23, 129.42, 130.82, 130.89, 131.38, 133.42, 133.62, 134.27, 134.65, 135.40, 135.41, 135.45, 141.09, 163.93.

3. Conclusion

An effective method was developed which provided an easy access to a new series N-(substituted phenyl)-3-(indoline-1-carbonyl)benzenesulfonamide (5a-g) analogs. The mild reaction conditions, good to excellent yields, ease of workup and easily available substrates make the reactions attractive for the preparation of compounds. The compounds (4b, 4d, 5d, and 5g) show potent anticancer activity in all the four cell lines tested. The compounds are easy, simple and reproducible to synthesize in normal conditions and no additional conditions or expensive chemicals are required for the reaction. The cell-lines with maximum IC50 values are the important in the study.

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Author details

Dattatraya Navnath Pansare* and Rohini Narayan Shelke
Department of Chemistry, Deogiri College, Aurangabad, MS, India

*Address all correspondence to: dattatraya.pansare7@gmail.com

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