Synthesis and evaluation of [N-(Substituted phenyl)-2-(3-substituted) sulfamoyl) phenyl] acetamide derivatives as anticancer agents

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FT Target activity
Amide coupling
Sulfonamide
Anticancer activity
Cell lines

A B S T R A C T

A series of molecules containing sulfonyl and amide coupling structure were developed, synthesized and evaluated. Total 21 compounds having sulfonamide and amide groups are synthesized. The structures of the synthesized compounds were elucidated and confirmed by 1H NMR, 13C NMR, Mass spectrum and the purity was checked through HPLC analysis. All synthesized compounds (4a-4u) were tested for their 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 revealed that most of the tested compounds showed good to moderate anticancer activity. Compounds 4d, 4k and 4s show promising anticancer activity in different cell lines.

Introduction

The fused ring nucleus having sulfonamide and amide coupling is an important constituent for an enormous variety of therapeutic agents, including anticancer, antiproliferative, antimarial, antifungal and antibacterial agents [1–5]. The sulfonamide act as matrix metalloproteinase inhibitors it is a significant pharmacophore and its coupling with other rings could provide new biologically active compounds [6]. Lately, the applications of amide coupled with naphthyl rings were found to show antimicrobial agents and biofilm inhibitors [7]. The compounds like some esters and amide coupled compounds act as anti-inflammatory drugs as cyclooxygenase-2-inhibitors [8] while some act as antimalariails [9]. The sulfonamide coupled with pyrimidine shows antimicrobial and anticancer activities [10]. Inhibitors of 5-Lipoxygenase [11], Yersinia enterococci Y ohp tyrosine phosphatase inhibitors [12], antimalasezia [13], antimicrobial and antimalarial activities [14], inhibitor of phosphodiesterase type 4 [15], antihypertensive [16]. Some sulfonamides linked compounds act as hepatitis-C virus as nonstructural protein 3 protease inhibitors [17].

The sulfonamide coupled with thiourea shows antiinflammatory and antimicrobial activities [18]. Some dihydopyrazole sulfonamide derivatives act as potential COX-1/COX-2 inhibitors [19]. Literature revealed some chromone-based sulfonamide derivatives shows carbolic anhydrase inhibition and cytotoxic activity [20], while the heterocyclic sulfonamides act as sphingosine 1-phosphate receptor 1 (S1P1) antagonists [21].

Following a wide literature exploration, it was observed that, different coupling of sulfonamide and amide compounds shows different activities like carbonic anhydrase inhibitors [22], Antimycobacterial [23], some sulfonamides act as sphingosine-1-phosphate (S1P1) receptor [24]. 11-beta-hydroxysteroid dehydrogenase type 1 (11β-HSD1) inhibitors for the treatment of metabolic disorders [25], sulfonamide-1,2,4-triazoles, 1,3,4-thiadiazoles and 1,3,4-oxadiazoles, as potential antibacterial and antifungal agents [26]. The structurally related compounds having sulfonamide and amide linkage in combinations derivatives show selectively SIRT-2 inhibiting activity [27].

From above references it is clear that sulfonamide and amide coupled with different group compounds show considerable varied activities. All above references indicate that the probability of potent anticancer activity of the derivatives containing benzene sulfonamide and phenyl amide coupled compound increases considerably. So we have synthesized compounds having benzene sulfonamide and phenyl amide compounds which coupled at meta positions of benzene to each other. We have also developed simplified reaction conditions for all the steps so we can avoid costly reagents, tedious purifications, and all the synthesized compounds also have good purity. We here report the synthesis of new substituted sulfonamide derivatives (Scheme 1) with the aim of investigating their anticancer activity. The synthetic methods adopted for the preparation of the title compounds (4a-4u) [28] are depicted in the scheme presented below.
Reagents and conditions: (step 1) Chlorosulfonic acid, DCM 0 °C-rt; (step 2) substituted amine, pyridine, DCM, 0 °C-rt; (step 3) Li(OH), THF, EtOH, H2O, rt; (step 4) substituted amine, EDCI, DIPEA, DCM, rt.

From above (Scheme 1, Table 1 & 2 See supporting information) here we have optimized the condition for aromatic chlorosulfonation in the presence of ester group. The reactivity changes according to the equivalent of chlorosulfonic acid used. We have carried out 10 different combinations and optimized the reaction condition which reduces the efforts of tedious work up and purifications of intermediate for first time. For all the reactions we have kept time constant. It is confirmed that when we use neat excess of chlorosulfonic acid without solvent there is 60% formation of required product (entry 10), then we have used excess chlorosulfonic acid with DCM then yield is 40% (entry 9). From above these two conditions it is clear that we have to use chlorosulfonic acid in equivalents along with in neat and in DCM solvent conditions. The varied results are shown in Table 1. The (entries 1, 2, 3 and 4) shows there is formation product along with side products, the yields are 30%–55%. When we consider (entries 5, 6, 7 and 8) the yields are increasing from 40% to 80%. From above these two conditions it is clear that we have to use chlorosulfonic acid in equivalents along with in neat and in DCM solvent conditions.

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| R1     | R2     | R1     | R2     |
|--------|--------|--------|--------|
| 4a     | 2-CH3  | 2,4-CH3| 4l     | 2-CH3-CH2 | 4-C(CH3)3 |
| 4b     | 2-CH3-CH2| 2,4-CH3| 4m     | 2-CF3   | 2-C(CH3)3 |
| 4c     | 2-CF3  | 2,4-CH3| 4n     | 2-C(CH3)3| 2-C(CH3)3 |
| 4d     | 2-C(CH3)3| 2,4-CH3| 4o     | Indoline | 2-C(CH3)3 |
| 4e     | Indoline| 2,4-CH3| 4p     | 2,4-CH3  | 2-CH3    |
| 4f     | 2-CH3  | 2-CH3  | 4q     | 2,4-CH3  | 2-CH3-CH2|
| 4g     | 2-CH3-CH2| 2-CH3  | 4r     | 2-CH3   | 2-CH3-CH2|
| 4h     | 2-CF3  | 2-CH3  | 4s     | 2,4-CH3 | 2-CH3    |
| 4i     | 2-C(CH3)3| 2-CH3  | 4t     | 2,4-CH3 | 2-CH3-CH2|
| 4j     | Indoline| 2-CH3  | 4u     | 2,4-CH3 | 2-CH3-CH2|
| 4k     | 2-CH3  | 4-C(CH3)3|        |         | 2-OCH3   |

In Scheme 2 and Scheme 3 first we have optimized the reaction solvent and base, from initial screening we have finalized DCM as the solvent and pyridine as the base that we have tabulated in Table 2. From Table 2 it is confirmed that when we used equivalents of pyridine and DCM the yield is 90% Table 4 entries 5.

There are many reports for the formations of sulfonamide so initially we have screened different bases by taking DCM and THF as solvents. In entry 5 with 2.5 equiv. of pyridine and in DCM the yield is 70% from entries 1 to 4 the yield ranges from 30% to 60%, in entries 6–10 the yield is 25%–60% also the reaction time for all entries is from 6 to 16 h. Time monitored on the bases on consumption of starting material. We have faced isolation problem in all the cases, like extraction needed for all the examples and obtained compounds are not cleaner so need to modify the yields. In entry 5 we have optimize work up condition so that we have to avoid rigorous extractions. In Table 3 we have varied the equivalents of pyridine and we have come to conclude that if we use equivalent volumes of pyridine along with DCM solvent then yield is 90% (entry 5). We have used pyridine as base and modified the...
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 [29]. The response parameter calculated was the IC_{50} value, which corresponds to the concentration required for 50% inhibition of cell viability. The results are presented in Table 1, where all compounds exhibit moderate to good activity compared to 5-fluorouracil as positive control.

In the case of the human lung cancer cell line (A549) compounds 4a, 4b, 4d, 4k and 4s were the most potent, with IC_{50} values ranging from 1.81 to 2.11 μM. On the HeLa cell line the compounds which showed potent activity were 4b, 4d, 4k, 4n, 4p and 4s (IC_{50} = 1.92–2.52 μM, respectively). In case of the MCF-7 breast cancer cell line, the potent compounds were 4b, 4d, 4k and 4s. (IC_{50} = 2.12–2.52 μM). Moderate activity was observed for the synthesized compounds on the Du-145 prostate cancer cell line, where the most potent candidates were compounds 4k, 4p and 4s (IC_{50} = 2.12–2.76 μM). Generally, the lung (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 1, reveals that compounds 4d, 4k and 4s were the most active, showing effectiveness toward the four cell lines.

The 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 indole group decreases the anticancer activity and despite steric hindrance 4d, 4k and 4s shows promising activity because electron donating tendency. Most of the compounds show promising anticancer activity with electron donating groups on the ring than electron withdrawing groups.

### Table 1

| Cpd. No. | A549 (Lung Cancer cell) | HeLa (Cervical) | MCF-7 (Breast Cancer cell) | DU-145 (Prostate Cancer cell) |
|----------|-------------------------|-----------------|---------------------------|-------------------------------|
| 4a       | 1.98 ± 0.12             | 2.83 ± 0.16     | 3.12 ± 0.06               | 2.86 ± 0.16                   |
| 4b       | 1.81 ± 0.13             | 1.92 ± 0.08     | 2.32 ± 0.22               | 2.82 ± 0.12                   |
| 4c       | 4.81 ± 0.11             | 6.32 ± 0.04     | 4.32 ± 0.06               | 3.73 ± 0.12                   |
| 4d       | 1.82 ± 0.11             | 1.99 ± 0.22     | 2.36 ± 0.12               | 2.52 ± 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                   |
| 4h       | 8.48 ± 0.14             | 9.12 ± 0.08     | 7.82 ± 0.08               | 9.12 ± 0.06                   |
| 4i       | 3.82 ± 0.08             | 4.13 ± 0.12     | 3.13 ± 0.11               | 3.52 ± 0.08                   |
| 4j       | 4.13 ± 0.12             | 5.16 ± 0.08     | 6.12 ± 0.12               | 4.52 ± 0.11                   |
| 4k       | 2.06 ± 0.12             | 2.12 ± 0.08     | 2.52 ± 0.16               | 2.12 ± 0.08                   |
| 4l       | 2.52 ± 0.11             | 3.52 ± 0.11     | 3.48 ± 0.08               | 4.08 ± 0.11                   |
| 4m       | 4.48 ± 0.08             | 4.98 ± 0.11     | 5.17 ± 0.22               | 6.18 ± 0.18                   |
| 4n       | 2.73 ± 0.08             | 2.12 ± 0.12     | 3.12 ± 0.08               | 3.12 ± 0.04                   |
| 4o       | 4.15 ± 0.18             | 5.12 ± 0.08     | 6.17 ± 0.08               | 7.15 ± 0.06                   |
| 4p       | 2.11 ± 0.08             | 2.52 ± 0.08     | 2.98 ± 0.06               | 2.76 ± 0.12                   |
| 4q       | 2.82 ± 0.12             | 3.15 ± 0.18     | 3.98 ± 0.08               | 4.12 ± 0.08                   |
| 4r       | 3.12 ± 0.08             | 3.48 ± 0.08     | 3.82 ± 0.11               | 3.52 ± 0.06                   |
| 4s       | 2.02 ± 0.11             | 2.12 ± 0.08     | 2.12 ± 0.08               | 2.32 ± 0.11                   |
| 4t       | 2.82 ± 0.12             | 3.16 ± 0.21     | 4.28 ± 0.06               | 2.82 ± 0.18                   |
| 4u       | 3.62 ± 0.16             | 3.98 ± 0.11     | 4.12 ± 0.18               | 4.52 ± 0.08                   |
| 5-FU     | 1.71 ± 0.11             | 1.82 ± 0.13     | 1.91 ± 0.08               | 1.82 ± 0.08                   |
the synthesis of some sulfonamide and amide coupling derivatives by simple reaction steps. The method is economical in the sense that no expensive reagents are required, no any tedious purification is needed and all the compounds synthesized are obtained in good yields. The advantages of this method are mild reaction conditions, shorter reaction time and potential anticancer activity. The compounds (4d, 4k and 4s) show potent anticaner activity in all the four cell lines tested.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ejbas.2017.09.001.

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[12] Biological Methods
[13] Cell Culture
[14] Human cancer cell lines HeLa (cervical), A549 (lungs) and DU-145 (prostate) were grown in DMEM + GlutaMax (Invitrogen, Carlsbad, CA, USA), and MCF-7 (breast) were grown in DMEM-F12 + GlutaMax) medium (Invitrogen), supplemented with 10% heat-inactivated FCS and grown overnight. Compounds were dissolved in dimethyl sulfoxide and grown overnight. Then extracted it with 20 ml of DCM. Progress reaction was monitored by TLC and LCMS, after completion of reaction, evaporation reaction mixture under reduced pressure to obtain gummy material. Added 10 ml of water in it and extracted it with diethyl ether (10 mL). Collected aqueous layer and adjust its pH to 4 by using 6N aqueous HCl. Precipitation occurs stirred it for 30 min. Filtered the obtained solid and wash it with excess of water, cold diethyl ether and cold pentane to obtain as white solids. Yield- (54 g, 81%).
[15] Step-4: General experimental procedure for preparation of 4a-4u
[16] The acid (1 equiv.) was dissolved in DCM and treated as white solids. Yield- 80%-90%.
[17] Step-3: General experimental procedure for preparation of 3a-3f
[18] A 10 ml of water in it and extracted it with diethyl ether (10 mL). Collected aqueous layer and adjust its pH to 4 by using 6N aqueous HCl. Precipitation occurs stirred it for 30 min. Filtered the obtained solid and wash it with excess of water, cold diethyl ether (10 mL) and cold pentane (10 mL) to obtain desired compounds as white solids. Yield- 80%–90%.
[19] Step-4: General experimental procedure for preparation of 4a-4u
[20] The acid (1 equiv.) was dissolved in DCM and treated as white solids. Yield- 80%-90%
[21] Further reading
[22] General experimental procedure for the synthesis of compound 4a-4u:Preparation of 2-(3-chlorosulfonylphenyl)acetate (1a)To a stirred solution of methyl 2-phenylacetate (40 g, 266 mmol) in DCM (100 mL) RM was cooled to 0 °C and chlorosulfonic acid (3.4 g, 283 mmol) was added drop wise followed by stirring at room temperature for 1 h. The reaction was monitored by LCMS and TLC, after completion of reaction, evaporation reaction mixture under reduced pressure to obtain gummy material was washed with excess of hexane and it is crystallized in 20% ethanol to obtain white solid as 2-(3-chlorosulfonylphenyl)acetate (1a) which is used further for sulfonamide coupling reaction, Yield: 54 g, 81%.
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[26] Step-3: General experimental procedure for preparation of 3a-3f
[27] A 10 ml of water in it and extracted it with diethyl ether (10 mL). Collected aqueous layer and adjust its pH to 4 by using 6N aqueous HCl. Precipitation occurs stirred it for 30 min. Filtered the obtained solid and wash it with excess of water, cold diethyl ether (10 mL) and cold pentane (10 mL) to obtain desired compounds as white solids. Yield- 80%–90%.
[28] Further reading
[29] Biological Methods

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culture media. The final concentration of DMSO never exceeded 0.1% in the treatment doses. Six different doses of compounds (400, 200, 100, 50, 25 and 10 μM) were further prepared by diluting the stocks in culture media, and cells were treated (in triplicate/dose). 5-fluorouracil was included as standard reference drug (positive control) and untreated culture was considered as negative control. The cultures were further incubated for 48 hrs. At 48 h post-treatment, cell viability test was performed using TACS MTT Cell Proliferation and Viability Assay Kit (TACS) as per manufacturer’s instructions. The optical density (OD) was recorded at 570 nm in a microplate reader (ELx800, BioTek, Winooski, VT, USA) and cell survival fraction was determined. The cell survival fraction was calculated as [(A-B)/A], where A and B are the OD of untreated and of treated cells, respectively. The concentration required for 50% inhibition of cell viability (IC50) was calculated and compared with the reference drug 5-fluorouracil and the results are given in Table 1.