Synthetic *N*-[(substitutedsulfamoyl)phenyl]acetamides as moderate chymotrypsin inhibitors

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Abstract: A facile method has been implemented for the synthesis of different *N*-substituted sulfamoylacetamides by reacting 4-acetamidobenzenesulfonyl chloride (1) with different alkyl/aralkyl/aryl amines (2a-q) in basic aqueous media under controlled pH to afford -[(Substitutedsulfamoyl) phenyl]acetamides (3a-q) which were confirmed through spectral analysis like FT-IR, EIMS and ^{1}H-NMR. Moreover, the synthesized derivatives were screened against α-Chymotrypsin. The enzyme inhibitory results revealed that most of the synthesized compounds were found to be moderate enzyme inhibitors.

Keywords: 4-Acetamidobenzenesulfonyl chloride, alkyl/aralkyl/aryl amines, *N*-[(Substitutedsulfamoyl) phenyl] acetamides, spectral characterization, α-Chymotrypsin and Enzyme inhibitory analysis.

INTRODUCTION

For more than 50 years, sulfonamides have been used as therapeutic agents. They are amongst the earliest antibiotics and antibacterial agents; and now they are used to treat other diseases as well (Patrick, 2001).

Sulfonamides bearing SO_2NH- group is the central moiety of many pharmacologically active compounds (Remko and Lieth, 2004). The medicinal properties of sulfonamides are unforeseen, as they emerged as an off shoot of dyes industry from Germany. Sulfonyl groups serve as protecting groups for amino functionality because of their high stability, electron withdrawing power and ease of synthesis (O'Connell *et al.*, 1992). Sulfonamides ($RSO_2NHR^{'}$) are nitrogen bearing compounds and Lewis acid due to strong electron withdrawing character of sulfonyl group (Gordon and Ford, 1972; Lappert *et al.*, 1979). Additionally the sulfonamide linkages are extraordinarily stable and resistant to hydrolyzing, oxidizing and reducing agents (Greene and Wuts, 1991). Sulfonamides were first synthesized from sulfonic acid in pyridine or triethylamine salts in the comportment of an activating agent, triphenylphosphine ditriflate (Zani and Vicini, 1998). Other widely used procedure was the reaction at room temperature between sulfonic acid and isocynate (Maren, 1976). A variety of indole derivatives have been synthesized by using 2-subsituted or unsubstituted *N*-monosulfonyl aromatic amines (Yasuhara *et al*, 1999).

p-Aminobenzoic acid (PABA), a pharmacophore and essential core of sulfonamide, is responsible for the drug action (Crossley *et al*, 1939). Sulfonamides, an important class of drugs are broadly used as anti-microbial, anti-thyroid and anti-inflammatory agents (Perlovich *et al*, 2008). These compounds have extensive spectrum of applications as anti-tumor, anti-cancer and anti-viral agents because they have the tendency to slow down growth of cancer cells (El-Sayed *et al*, 2011). They are also amongst the most widely used veterinary medicines (Gracia-Galan *et al*, 2008). Some sulfonamide derivatives are also used for urinary tract and gastrointestinal infection (Gadad *et al*, 2000). Amongst the un commercialized application of sulfonamides is their tendency to inhibit various enzymes such as carbonic anhydrase, cystein protease, cyclohydrogenase and HIV protease (Supran *et al*, 1998). The most widespread structural design in organic synthesis is *N*-acyl sulfonamide. The current developments in the sulfonamide drugs include therapeutic agents for Alzheimer's disease and in treatment of osteoporosis (Wang *et al*, 2000).

In animal kingdom chymotrypsin belongs to the largest family of enzymes and participates in the digestion of dietary proteins (Tidwell and Bomba, 2001). It is secreted as chymotrypsinogen (a chain of amino acids comprising of 245 amino acids residues) in pancreas (Hartely, 1964) and is activated by hydrolysis of a single peptide bond as chymotrypsin under the catalytic action of trypsin (Desnuelle, 1960). Chymotrypsin cleaves the peptides on the carboxyl side of phenyl alanine, tyrosine and tryptophan residues (Guyonnet *et al*, 1999; Boeris *et al*, 2009). It prevents tissue damage and fibrin clots. Therefore it is used to treat various infections in mammals (Xiu-Zhen *et al*, 2008). The enzyme is involved in many pathological diseases such as destruction of bone in arthritic joints and tumor incursion (Berquin and Sloane, 1996; Bilfinger and Stefano, 2002).

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The present research effort is an extension of our formerly reported work (Aziz-ur-Rehman, Afroz *et al.*, 2012; Aziz-ur-Rehman, Awais-ur-Rehman *et al.*, 2012; Aziz-ur-Rehman, Rasool *et al.*, Aziz-ur-Rehman, Tanveer *et al.*, 2011; 2012; Hira Khalid *et al.*, 2012) and all the findings mentioned in the literature regarding the pharmacological activity of the sulfamoylacetamides encouraged us to synthesize a series of *N*-substituted sulfamoylacetamides exhibiting enhanced chymotrypsin activity.

MATERIALS AND METHODS

The melting points of synthesized derivatives were determined on Griffin and George M.P apparatus by capillary tube method. Thin layer technique (TLC) was conducted on pre coated silica G-25-UV_{254} plates and developed mobile phase comprising of *n*-hexane and ethyl acetate (80:20) to monitor the completion of reaction and to check the purity of the synthesized compounds. IR spectra were recorded by potassium bromide disc method on a Jasco-320-A spectrophotometer (wave number in cm^{-1}). NMR spectra were recorded in CD_3OD on a Bruker spectrophotometer operational at 300MHz (δ ppm). Mass spectra (EIMS) were recorded on a JMS-HX-110 spectrophotometer, with a data system.

Synthesis of N-[4-(N-substitutedsulfamoyl)phenyl] acetamides (3a-q)

Different substituted alkyl/aralkyl/aryl amines (0.0021 mol; 2a-q) were suspended in 50mL distilled water which was basified till pH 10 by the addition of aqueous solution of Na_2CO_3 (10%) at 0-5^{o}C in a 100mL round bottomed flask. 4-acetamidobenzenesulfonyl chloride (0.5g; 0.0021mol; 1) was added slowly to the reaction mixture in 15min. The reaction mixture was stirred at room temperature for 3 hours. Completion of reaction was confirmed by TLC in *n*-hexane: Ethyl acetate (80:20), which was found to be single spot. The reaction was acidified up to pH 3 with dil. HCl and the reaction mixture was kept for half an hour till appearance of precipitates. The collected precipitates were filtered, washed with distilled water, air-dried and recrystallized from methanol to obtain the pure *N*-[4-(*N*-substitutedsulfamoyl)phenyl] acetamides (3a-q).

N-[4-(N-Phenylsulfamoyl)phenyl]acetamide (3a)

Off-white amorphous solid; Yield: 89%; M.P. 250°C; R_f: 0.5; Molecular formula: $C_{14}H_{14}N_2O_3S$; Molecular weight: 290$gmol^{-1}$; IR (KBr, cm^{-1}): ν_{max}: 3117(C-H aromatic stretching), 1660 (C=O amide stretching), 1533 (C=C aromatic ring stretching), 1318(-SO_2 stretching); ^{1}H-NMR (300 MHz, CD_3OD, δ/ppm): 7.21 (br.d, *J*=7.2 Hz, 2H, H-2' & H-6'), 7.17 (br.d, *J*=7.2 Hz, 2H, H-3' & H-5'), 7.07-7.00 (m, 5H, H-2 to H-6), 2.10 (s, 3H, CH_3CON); EIMS: *m/z* 290 [M^+], 226 (100%), 92(32%), 78 (13%), 64 (34%).

N-[4-(N-Benzylsulfamoyl)phenyl]acetamide (3b)

Off-white solid; Yield: 85%; M.P. 150°C; R_f: 0.6; Molecular formula: $C_{15}H_{16}N_2O_3S$; Molecular weight: 304 $gmol^{-1}$; IR (KBr, cm^{-1}): ν_{max}: 3010 (C-H aromatic stretching), 1650 (C=O amide stretching), 1525 (C=C aromatic ring stretching), 1320 (-SO_2 stretching); ^{1}H-NMR (300 MHz, CD_3OD, δ/ppm): 7.74 (br.d, *J*=9.3 Hz, 2H, H-2' & H-6'), 7.71 (br.d, *J*=9.3 Hz, 2H, H-3' & H-5'), 7.23-7.18 (m, 5H, H-2 to H-6), 4.03 (s, 2H, CH_2-7), 2.14 (s, 3H, CH_3CON); EIMS: *m/z* 304 [M^+], 240 (100%), 106 (64%), 91(10%), 64 (33%).

N-[4-(N-Cyclohexylsulfamoyl)phenyl]acetamide (3c)

Off-white powder; Yield: 98%; M.P. 184°C; R_f: 0.8; Molecular formula: $C_{14}H_{20}N_2O_3S$; Molecular weight: 296 $gmol^{-1}$; IR (KBr, cm^{-1}): ν_{max}: 3110 (C-H aromatic stretching), 1647 (C=O amide stretching), 1527 (C=C aromatic ring stretching), 1322 (-SO_2 stretching); ^{1}H-NMR (300 MHz, CD_3OD, δ/ppm): 7.77 (br.d, *J*=8.7 Hz, 2H, H-2' & H-6'), 7.73 (br.d, *J*=8.7 Hz, 2H, H-3' & H-5'), 3.02-2.94 (m, 1H, H-1), 2.14 (s, 3H, CH_3CON), 1.67-1.50 (m, 4H, CH_2-2 & CH_2-6), 1.19-1.13 (m, 6H, CH_2-3 to CH_2-5); EIMS: *m/z* 296 [M^+], 221(100%), 98 (34%), 83 (16%), 64 (33%).

N-[4-(N-Phenylethylsulfamoyl)phenyl]acetamide (3d)

White amorphous solid; Yield: 63%; M.P. 126 °C; R_f: 0.7; Molecular formula: $C_{16}H_{18}N_2O_3S$; Molecular weight: 318 $gmol^{-1}$; IR (KBr, cm^{-1}): ν_{max}: 3100 (C-H aromatic stretching), 1651 (C=O amide stretching), 1530 (C=C aromatic ring stretching), 1321 (-SO_2 stretching); ^{1}H-NMR (300 MHz, CD_3OD, δ/ppm): 7.22 (br.d, *J*=9.8 Hz, 2H, H-2' & H-6'), 7.16 (br.d, *J*=9.8 Hz, 2H, H-3' & H-5'), 7.11-7.08 (m, 5H, H-2 to H-6), 3.05 (t, *J*=7.2 Hz, 2H, CH_2-8), 2.70 (t, *J*=7.2 Hz, 2H, CH_2-7), 2.14 (s, 3H, CH_3CON); EIMS: *m/z* 318 [M^+], 254 (100%), 120 (25 %), 105 (15%), 64 (31%).

N-[4-(N-o-Tolylsulfamoyl)phenyl]acetamide (3e)

Off-white solid; Yield: 70%; M.P. 220°C; R_f: 0.7; Molecular formula: $C_{15}H_{16}N_2O_3S$; Molecular weight: 304 $gmol^{-1}$; IR (KBr, cm^{-1}): ν_{max}: 3112 (C-H aromatic stretching), 1630 (C=O amide stretching), 1527 (C=C aromatic ring stretching), 1315 (-SO_2 stretching); ^{1}H-NMR (300 MHz, CD_3OD, δ/ppm): 7.66 (br.d, *J*=9.0 Hz, 2H, H-3' & H-5'), 7.55 (br.d, *J*=9.0 Hz, 2H, H-2' & H-6'), 7.09-7.04 (m, 4H, H-3 to H-6), 2.13 (s, 3H, CH_3CON), 2.01 (s, 3H, CH_3-2); EIMS: *m/z* 304 [M^+], 240 (100%), 246 (65%), 210(21%), 64 (35%).

N-[4-(N-(2-Ethylphenylsulfamoyl)phenyl]acetamide (3f)

Light purple amorphous solid; Yield: 91%; M.P. 206°C; R_f: 0.8; Molecular formula: $C_{16}H_{18}N_2O_3S$; Molecular weight: 318 $gmol^{-1}$; IR (KBr, cm^{-1}): ν_{max}: 3118 (C-H aromatic stretching), 1659 (C=O amide stretching), 1529 (C=C aromatic ring stretching), 1315 (-SO_2 stretching); ^{1}H-NMR (300 MHz, CD_3OD, δ/ppm): 7.67 (br.d, *J*=9.0 Hz, 2H, H-3' & H-5'), 7.60 (br.d, *J*=9.0 Hz, 2H, H-2' & H-6'), 7.16 (d, *J*=7.8 Hz, 1H, H-3), 7.13 (dt, *J*=7.8, 1.8 Hz, 1H, H-4), 7.03 (dt, *J*=7.8, 1.8 Hz, 1H, H-5), 6.95 (dd,= 7.5, 1.5 Hz, 1H, H-6), 2.45 (q, *J*=7.8 Hz, 2H, CH_3CH_2-2),

2.13 (s, 3H, CH_3CON), 1.01 (t, *J*=7.8 Hz, 3H, CH_3CH_2-2); EIMS: *m/z* 318 [M^+], 254 (100%), 120 (35%), 105 (21 %), 64 (33%).

N-[4-(N-2-Ethyl-6-methylphenylsulfamoyl)phenyl] acetamide (3g)

White solid; Yield: 92%; M.P. 224°C; R_f: 0.8; Molecular formula: $C_{17}H_{20}N_2O_3S$; Molecular weight: 332 $gmol^{-1}$; IR (KBr, cm^{-1}): ν_{max}: 3017 (C-H aromatic stretching), 1649 (C=O amide stretching), 1519 (C=C aromatic ring stretching), 1322 (-SO_2 stretching); ^{1}H-NMR (300 MHz, CD_3OD, δ/ppm): 7.71 (br.d, *J*=8.7 Hz, 2H, H-2' & H-6'), 7.60 (br.d, *J*=8.7 Hz, 2H, H-3' & H-5'), 7.12 (t, *J*=7.8 Hz, 1H, H-4), 7.05 (dd, *J*=7.2, 1.5 Hz, H-5), 6.98 (dd, =7.5, 1.8 Hz, H-3), 2.48 (q, *J*=7.5 Hz, 2H, $CH_3\underline{CH_2}$-2), 2.14 (s, 3H, CH_3CON), 1.97 (s, 3H, CH_3-6), 1.02 (t, *J*=7.5 Hz, 3H, $\underline{CH_3}CH_2$-2); EIMS: *m/z* 332 [M^+], 268 (100%), 134 (35%), 119 (21%), 64 (35%).

N-[4-(N-2-Methyl-6-nitrophenylsulfamoyl)phenyl] acetamide (3h)

Yellow solid; Yield: 87%; M.P. 306°C; R_f: 0.5; Molecular formula; $C_{15}H_{15}N_3O_5S$; Molecular weight: 349 $gmol^{-1}$; IR (KBr, cm^{-1}): ν_{max}: 3119 (C-H aromatic stretching), 1655 (C=O amide stretching), 1528 (C=C aromatic ring stretching), 1317 (-SO_2 stretching); ^{1}H-NMR (300 MHz, CD_3OD, δ/ppm): 7.92 (dd, *J*=8.7, 1.0 Hz, 1H, H-5), 7.69 (br.d, *J*=8.7 Hz, 2H, H-3' & H-5'), 7.60 (br.d, *J*=8.7 Hz, 2H, H-2' & H-6'), 7.30 (dd, *J*=7.2, 1.0 Hz, 1H, H-3), 6.58 (dd, *J*=7.2, 8.7 Hz, 1H, H-4), 2.22 (s, 3H, CH_3CON), 2.13 (s, 3H, CH_3-2); EIMS: *m/z* 349 [M^+], 285 (100%), 134 (45%), 122 (15%), 64 (32%).

N-[4-(N-2-Ethoxyphenylsulfamoyl)phenyl]acetamide (3i)

Light purple solid, Yield 93%, M.P. 152°C; R_f: 0.8; Molecular formula: $C_{16}H_{18}N_2O_4S$; Molecular weight: 334 $gmol^{-1}$; IR (KBr, cm^{-1}): ν_{max}: 3113 (C-H aromatic stretching), 1657 (C=O amide stretching), 1526 (C=C aromatic ring stretching), 1315 (-SO_2 stretching); ^{1}H-NMR (300 MHz, CD_3OD, δ/ppm): 7.60 (br.d, *J*=9.3 Hz, 2H, H-3' & H-5'), 7.55 (br.d, *J*=9.3 Hz, 2H, H-2' & H-6'), 7.41 (dd, *J*=7.8 Hz, H-6), 7.07 (ddd, *J*=1.5, 7.6, 8.0 Hz, H-4), 6.86 (ddd, *J*=1.2, 7.8 Hz, H-5), 6.77 (dd, *J*=1.0, 8.1 Hz, H-3), 3.73 (q, *J*=6.9 Hz, 2H, CH_3CH_2O-2), 2.11 (s, 3H, CH_3CON), 1.16 (t, *J*=6.9 Hz, 3H, CH_3CH_2O-3); EIMS: m/z 334 [M^+], 270 (100%), 136 (47%), 121(20%), 64 (31%).

N-[4-(N-3-Ethoxyphenylsulfamoyl)phenyl]acetamide (3j)

Dark brown solid; Yield: 78%; M.P. 174°C; R_f: 0.75; Molecular formula: $C_{16}H_{18}N_2O_4S$; Molecular weight: 334 $gmol^{-1}$; IR (KBr, cm^{-1}): ν_{max}: 3113 (C-H aromatic stretching), 1657 (C=O amide stretching), 1526 (C=C aromatic ring stretching), 1315 (-SO_2 stretching); ^{1}H-NMR (300 MHz, CD_3OD, δ/ppm): 7.60 (br.d, *J*=9.0 Hz, 2H, H-3' & H-5'), 7.55 (br.d, *J*=9.0 Hz, 2H, H-2' & H-6'), 7.06 (t, *J*=8.1 Hz, 1H, H-5), 6.65 (t, 1H, H-2), 6.63-6.56 (m, 2H, H-4 & H-6), 3.92 (q, *J*=6.9 Hz, 2H, CH_3CH_2O-3), 2.11 (s, 3H, CH_3CON), 1.31 (t, *J*=6.9 Hz, 3H, CH_3CH_2O-3); EIMS: m/z 334 [M^+], 270 (100%), 136 (47%), 121(20%), 64 (31%).

N-[4-(N-4-Ethoxyphenylsulfamoyl)phenyl]acetamide (3k)

Beige colored solid; Yield: 73%; M.P. 194°C; R_f: 0.8; Molecular formula: $C_{16}H_{18}N_2O_4S$; Molecular weight: 334 $gmol^{-1}$; IR (KBr, cm^{-1}): ν_{max}: 3114 (C-H aromatic stretching), 1660 (C=O amide stretching), 1530 (C=C aromatic ring stretching), 1321 (-SO_2 stretching); ^{1}H-NMR (300 MHz, CD_3OD, δ/ppm): 7.62 (br.d, *J*=8.7 Hz, 2H, H-3' & H-5'), 7.56 (br.d, *J*=8.7 Hz, 2H, H-2' & H-6'), 6.92 (br.d, *J*=9.0 Hz, 2H, H-2 & H-6), 6.72 (br.d, *J*=9.0 Hz, 2H, H-3 & H-5), 3.93 (q, *J*=6.9 Hz, 2H, $CH_3\underline{CH_2}O$-4), 2.11 (s, 3H, CH_3CON), 1.32 (t, *J*=7.2 Hz, 3H, $\underline{CH_3}CH_2O$-4); EIMS: *m/z* 334 [M^+], 270 (100%), 136 (25 %), 121 (15%), 64 (33%).

N-[4-(N-2-Methoxycarbonylphenylsulfamoyl)phenyl] acetamide (3l)

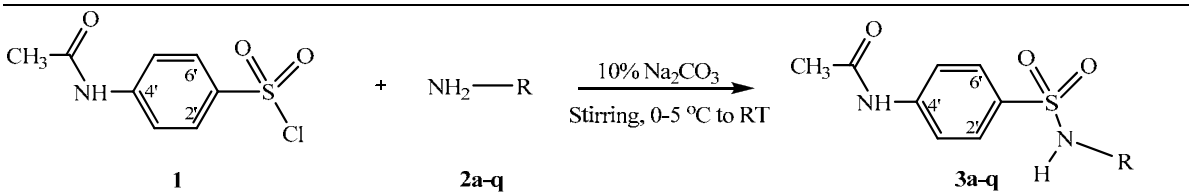
White solid; Yield: 83%; M.P. 146°C; R_f: 0.55; Molecular formula: $C_{16}H_{16}N_2O_5S$; Molecular weight: 348 $gmol^{-1}$; IR (KBr, cm^{-1}): ν_{max}: 3117 (C-H aromatic stretching), 1665 (C=O amide stretching), 1531 (C=C aromatic ring stretching), 1319 (-SO_2 stretching); ^{1}H-NMR (300 MHz, CD_3OD, δ/ppm): 7.90 (dd, *J*=8.0, 1.5 Hz, 1H, H-3), 7.70 (br.d, *J*=9.3 Hz, 2H, H-3' & H-5'), 7.67 (br.d, *J*=9.3 Hz, 2H, H-2' & H-6'), 7.64 (dd, *J*=8.0, 1.5 Hz, 1H, H-6), 7.50 (dt, *J*=8.0, 1.5 Hz, 1H, H-5), 7.10 (dt, *J*=8.1, 1.2 Hz, 1H, H-4), 3.85 (s, 3H, CH_3OCO), 2.10 (s, 3H, CH_3CON); EIMS: *m/z* 348 [M^+], 284 (100 %), 148 (62%), 133 (25 %), 64 (33%).

N-[4-(N-2,3-Dimethylphenylsulfamoyl)phenyl] acetamide (3m)

Light pink solid; Yield: 63%; M.P. 215°C; R_f: 0.75; Molecular formula: $C_{16}H_{18}N_2O_3S$; Molecular weight: 318 $gmol^{-1}$; IR (KBr, cm^{-1}): $\nu_{max:}$ 3013 (C-H aromatic stretching), 1653 (C=O amide stretching), 1525 (C=C aromatic ring stretching), 1317 (-SO_2 stretching); ^{1}H-NMR (300 MHz, CD_3OD, δ/ppm): 7.65 (br.d, *J*=6.9 Hz, 2H, H-3' & H-5'), 7.55 (br.d, *J*=6.9 Hz, 2H, H-2' & H-6'), 7.00 (d, *J*=7.5 Hz, 1H, H-6), 6.92 (t, *J*=7.5 Hz, 1H, H-5), 6.75 (d, *J*=7.5Hz, 1H, H-4), 2.19 (s, 3H, CH_3CON), 2.13 (s, 3H, CH_3-2), 1.97 (s, 3H, CH_3-3); EIMS: *m/z* 318 [M^+], 254 (100%), 120 (35%), 105 (29%), 64 (35%).

N-[4-(N-2,5-Dimethylphenylsulfamoyl)phenyl] acetamide (3n)

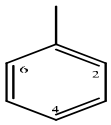
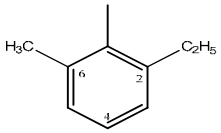
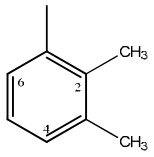
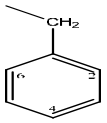
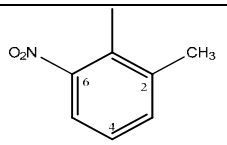
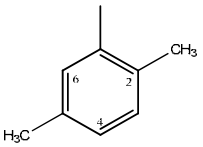
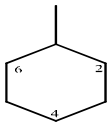
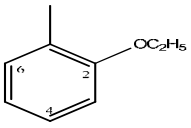
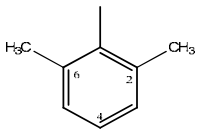
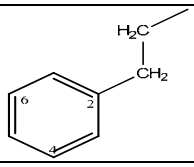
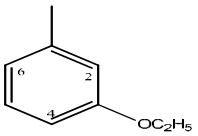
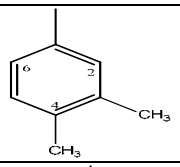
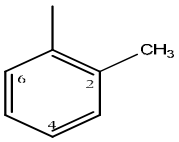
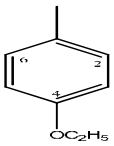
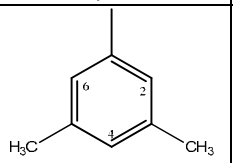
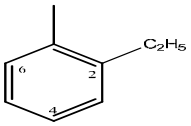
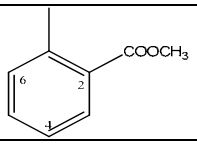
Grey solid, Yield 94%; M.P. 228°C; R_f: 0.72; Molecular formula $C_{16}H_{18}N_2O_3S$; Molecular weight: 318 $gmol^{-1}$; IR (KBr): ν_{max}: 3000 (C-H aromatic stretching), 1645 (C=O amide stretching), 1520 (C=C aromatic ring stretching), 1312 (-SO_2 stretching); ^{1}H-NMR (300 MHz, CD_3OD, δ/ppm): 7.65 (br.d, *J*=9.0 Hz, 2H, H-3' & H-5'), 7.57



1 4-Acetamidobenzenesulfonyl chloride

2a-q Substituted alkyl/aralkyl/aryl amines

3a-q *N*-[4-(*N*-substitutedsulfamoyl)phenyl]acetamides

Compound	R	Compound	R	Compound	R
3a		3g		3m	
3b		3h		3n	
3c		3i		3o	
3d		3j		3p	
3e		3k		3q	
3f		3l			

Scheme 1: Outline for the synthesis of *N*-[4-(*N*-substitutedsulfamoyl)phenyl]acetamides (3a-q)

(br.d, *J*=9.0 Hz, 2H, H-2' & H-6'), 6.96 (d, *J* =7.8 Hz, 1H, H-3), 6.91 (s, 1H, H-6), 6.87 (d, *J* =7.8 Hz, 1H, H-4), 2.18 (s, 3H, CH_3CON), 2.13 (s, 3H, CH_3-2), 1.93 (s, 3H, CH_3-5)); EIMS: *m/z* 318 [M^+], 254 (99%), 120 (39%), 105 (25%), 64 (31%).

N-[4-(N-2,6-Dimethylphenylsulfamoyl)phenyl] acetamide (3o)

Off white solid, Yield 94%, M.P. 226°C; R_f: 0.7; Molecular formula $C_{16}H_{18}N_2O_3S$; Molecular weight: 318 $gmol^{-1}$; IR (KBr): ν_{max}: 3000 (C-H aromatic stretching), 1645 (C=O amide stretching), 1520 (C=C aromatic ring stretching), 1312 (-SO_2 stretching); ^{1}H-NMR (300 MHz, CD_3OD, δ/ppm): 7.70 (br.d, *J*=6.9 Hz, 2H, H-3' & H-5'), 7.61 (br.d, *J*=6.9 Hz, 2H, H-2' & H-6'), 7.06-6.96 (m, 3H, H-3-5), 2.14 (s, 3H, CH_3CON), 2.00 (s, 6H, CH_3-2 and CH_3-6); EIMS: *m/z* 318 [M^+], 254 (94%), 120 (41%), 105 (29%), 64 (27%).

N-[4-(N-3,4-Dimethylphenylsulfamoyl)phenyl] acetamide (3p)

Off white solid; Yield: 93%; M.P. 182°C; R_f: 0.75; Molecular formula: $C_{16}H_{18}N_2O_3S$; Molecular weight: 318 $gmol^{-1}$; IR (KBr, cm^{-1}): ν_{max}: 3120 (C-H aromatic stretching), 1647 (C=O amide stretching), 1525 (C=C aromatic ring stretching), 1318 (-SO_2 stretching); ^{1}H-NMR (300 MHz, CD_3OD, δ/ppm): 7.69 (br.d, *J*=8.7 Hz, 2H, H-3' & H-5'), 7.60 (br.d, *J*=8.7 Hz, 2H, H-2' & H-6'),

7.01 (d, *J*=8.7 Hz, 1H, H-5), 6.98 (dd, *J*=8.7, 2.4 Hz, 1H, H-6), 6.96 (s, 1H, H-2), 2.22 (s, 3H, CH_3CON), 2.13 (s, 3H, CH_3-4), 1.96 (s, 3H, CH_3-3); EIMS: *m/z* 318 [M^+], 254 (100%), 120 (36%), 105 (27%), 64 (35%).

N-[4-(N-3,5-Dimethylphenylsulfamoyl)phenyl] acetamide (3q)

Off white solid; Yield: 67%; M.P. 220°C; R_f: 0.7; Molecular formula: $C_{16}H_{18}N_2O_3S$; Molecular weight: 318 $gmol^{-1}$; IR (KBr, cm^{-1}): ν_{max}: 3110 (C-H aromatic stretching), 1651 (C=O amide stretching), 1515 (C=C aromatic ring stretching), 1312 (-SO_2 stretching); ^{1}H-NMR (300 MHz, CD_3OD, δ/ppm): 7.66 (br.d, *J*=8.7 Hz, 2H, H-3' & H-5'), 7.55 (br.d, *J*=8.7 Hz, 2H, H-2' & H-6'), 6.92 (s, 1H, H-4), 6.86 (s, 2H, H-2 & H-6), 2.22 (s, 3H, CH_3CON), 2.13 (s, 3H, CH_3-3), 1.96 (s, 3H, CH_3-5); EIMS: *m/z* 318 [M^+], 254(100 %), 120 (37%), 105 (29%), 64 (34%).

α-Chymotrypsin Assay

The *α*-Chymotrypsin inhibition assay was carried according to protocol established by (Cannell *et al,* 1988). 100µL of the reaction mixture containing 60µL of 50mM Tris-HCl buffer at pH 7.6, 10µL of 0.5mM test compound and 15µL (0.9 units) of enzyme (Sigma, USA) were mixed thoroughly, pre-incubated for 15 min at 37°C and pre-read at 410 nm. 15µL of 1.3mM substrate was added to initiate the reaction i.e. *N*-succinyl phenylalanine-*p*-nitroanilide (Sigma, USA). Absorbance was measured using Synergy HT micro plate reader at 410 nm after 30-60 min when absorbance values of uninhibited enzyme assay reached 0.7-0.9. The assay included positive and negative controls. All experiments were carried out in triplicate. IC_{50} values were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

The percent inhibition was calculated by equation:

Inhibition (%) = (Control –Test / Control) x 100

RESULTS

The basic purpose of the synthesis was to inaugurate new drug candidates having enzyme inhibitory potential. The current investigation emphasizes on the synthesis of various *N*-substituted sulfamoylacetamides (3a-q) by pairing of 4-acetamidobenzenesulfonyl chloride (1) with a series of substituted/unsubstituted alkyl/aralkyl/aryl primary amines (2a-q) with an equimolar ratio in a basic media. The synthesized compounds include the dual functionalities like sulfamoyl group and amidic linkage. In this synthesis, different substituted/unsubstituted alkyl/aralkyl/aryl primary amines were suspended in 50 mL distilled water at 0-5°C in an ice-bath. The pH was made at 9-10 through the addition of 10% sodium carbonate solution. Then 4-acetamidobenzenesulfonyl chloride was added gradually in 15 minutes with continuous stirring. During the reaction, again the pH was maintained at 9-10 till there was no change in the pH of the reaction mixture. It's necessary to maintain the basic pH because HCl is produced during the reaction which protonates the lone pair of nitrogen of amines. The reaction completion was corroborated via TLC and total conversion was attained in 3 hrs. The products 3a-q was precipitated at an acidic pH of 3 by the addition of dilute hydrochloric acid. Dilute HCl was poured to neutralize the mixture which changes over the salt form of products into acidic form at nitrogen of sulfamoyl group. The precipitates were filtered, washed with distilled water and air-dried to afford pure compounds as demonstrated in Scheme 1. The synthesized *N*-substituted sulfamoyl acetamides 3a-q was further screened against *α*-chymotrypsin to check their enzyme inhibitory potential and was found to be moderately active. The results are tabulated in table 1.

DISCUSSION

The compound 3a was synthesized as an off white solid having melting point 250°C and a yield of 89%. The molecular formula $C_{14}H_{14}N_2SO_3$ was established by counting the number of protons in the P-NMR spectrum and from EIMS showing [M^+] ion peak at *m/z* 290. Infrared spectra revealed the absorption bands at $3117cm^1$, $1660cm^{-1}$, $1533cm^{-1}$ and $1318cm^{-1}$ which confirmed the presence of C-H (aromatic ring stretching), C=O (stretching of acetamide linkage), C=C (aromatic ring stretching) and -SO_2 (stretching of sulfonyl group) respectively. EIMS analysis gave characteristic peaks at *m/z* 226 due to the loss of sulfonyl group and at *m/z* 92 due to the cation of aniline. In the proton nuclear magnetic resonance spectrum, the aromatic signals were observed as two broad doublets at δ 7.21 (*J*=7.2 Hz, 2H, H-2' & H-6') and 7.17 (*J*=7.2 Hz, 2H, H-3' & H-5') which affirmed the presence of *p*-substituted aryl ring bearing sulfamoyl and acetamido groups; and a multiplet was observed at δ 7.07-7.00 (5H, H-2 to H-6) for monosubstituted aromatic ring corresponding to the aromatic ring of the aniline (primary aromatic amine). In the aliphatic region, the signal appeared as a singlet at δ 2.10 (3H, CH_3CON) which corroborated the presence of methyl group of acetamide. On the basis of above stated spectral information, the structure of 3a was found to be *N*-(4-(*N*-phenylsulfamoyl)phenyl)acetamide. Likewise, the structures of other compounds were characterized by ^{1}H-NMR, IR and MS data as described in experimental section.

α-Chymotrypsin activity

The synthesized *N*-substituted sulfamoyl acetamides (3a-q) was screened against *α*-chymotrypsin enzyme and they displayed moderate to weak inhibitory potential against *α*-chymotrypsin as apparent from their IC_{50} values (table 1).

It is obvious that the compound 3a *N*-[4-(*N*-phenylsulfamoyl)phenyl]acetamide and 3n *N*-[4-(*N*-2,5-dimethylphenylsulfamoyl)phenyl]acetamide showed

Table 1: Enzyme Inhibition activity of *N*-[4-(*N*-substitutedsulfamoyl)phenyl]acetamides (3a-q)

Compound	α-Chymotrypsin Inhibition Assay		
	Conc. mg/mL	Inhibition (%)	IC_{50} µg/mL
3a	0.5	75.24±0.11	150.99±0.01
3b	0.5	65.49±0.09	175.11±0.19
3c	0.5	62.81±0.02	250.11±0.12
3d	0.5	66.13±0.12	170.12±0.06
3e	0.5	51.02±0.11	<400
3f	0.5	48.45±0.12	-
3g	0.5	23.37±0.17	-
3h	0.5	75.71±0.01	167.71±0.11
3i	0.5	30.71±0.11	-
3j	0.5	53.38±0.01	<400
3k	0.5	35.78±0.16	-
3l	0.5	50.73±0.11	<400
3m	0.5	55.31±0.31	<400
3n	0.5	75.11±0.12	150.91±0.03
3o	0.5	61.79±0.11	194.31±0.01
3p	0.5	44.60±0.10	-
3q	0.5	36.26±0.31	-
Control	Chymostatin	93.50±0.91	8.24±0.11

better inhibitory potential amongst the series as evident from their IC_{50} values of 150.99±0.01 and 150.91±0.03µmoles/liter respectively. However some compounds 3f-3k, 3p and 3q revealed no activity against α-chymotrypsin. Chymostatin was used as a control having IC_{50} value of 8.24±0.11 µmoles/litre.

CONCLUSION

The predictable structures of *N*-substituted sulfamoyl acetamides 3a-q are well supported by their spectral analysis data. From α-chymotrypsin enzyme inhibition data it might be concluded that the compounds have moderate to weak inhibitory potential against α-chymotrypsin as manifested by their IC_{50} values, comparative to the reference standard used. All the synthesized compounds have acidic hydrogen and so these can be further substituted by the alkyl/aralkyl/aryl groups for the enhancement of their inhibition activity as substitution on sulfonamides can result in changed activity behavior.

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