INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

SYNTHESIS, *IN-VITRO* ANTIOXIDANT, ANTIBACTERIAL ACTIVITIES OF NOVEL SULFONAMIDES FROM 5-AMINOSALICYLIC ACID: PROTECTIVE EFFECT OF SELECTED SULFONAMIDES ON ACETIC ACID INDUCED ULCERATIVE COLITIS IN RATS

Madhavi Kuchana * and Bindu Sree Nadakuditi
Department of Pharmaceutical Chemistry, Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati-517502, Chittoor, Andhra Pradesh, India
*Corresponding Author Email: kuchanamadhavi@yahoo.co.in

Article Received on: 19/10/18 Approved for publication: 28/11/18

DOI: 10.7897/2230-8407.0912302

ABSTRACT

An eco-friendly method has been used to synthesize the novel sulfonamides from 5-aminosalicylic acid and substituted sulfonyl chlorides (**3a-3e**). To study the structure activity relationship (SAR), similar sulfonamides from 3-aminobenzoic acid (**3f-3j**) and 4-aminophenol (**3k-3o**) were synthesized by following the same synthetic route. All the synthesized compounds were characterized by their physical and spectral data. The compounds were evaluated for their *in-vitro* antioxidant properties and antibacterial activity by agar well diffusion method. The selected compounds (**3a, 3b, 3k** and **3l**) were evaluated against acetic acid induced ulcerative colitis in rats. The present work revealed that the sulfonamides synthesized from 5-aminosalicylic acid and 4-aminophenol exhibited excellent *in-vitro* antioxidant properties in both DPPH and nitric oxide free radical scavenging assays. Among the 5-aminosalicylic acid sulfonamides, **3b** and **3e** were found more active than the standard ascorbic acid in DPPH scavenging model. The antibacterial activity data revealed that the compound **3g** showed good activity towards all the tested organisms better than the standard drug sulfanilamide and with greater zone of inhibition against *E.coli, S.aureus* and *B.subtilis* when compared with the standard Amoxicillin. The pharmacological study revealed that the evaluated compounds exhibited reduced levels of colonic lipid peroxides and myeloperoxidase, while increased levels of glutathione content compared to disease control. Further, the importance of various substituents on the sulfonamide pharmacophore was supported by prediction of molecular properties and bioactivity scores using Molinspiration Cheminformatics software. The prediction data indicated that the novel compounds **3b** and **3g** were bioactive molecules as protease and enzyme inhibitors.

Keywords: Sulfonamides, 5-Aminosalicylic acid, Antioxidant activity, Antibacterial activity, Ulcerative colitis.

INTRODUCTION

5-Aminosalicylic acid is a drug of choice for the treatment of inflammatory bowel disease. It has been reported that 5-aminosalicylic acid is extensively absorbed and metabolized in the upper gastrointestinal tract by first pass metabolism and is not made available to the desired site i.e. colon[1]. Various prodrugs of 5-aminosalicylic acid such as Sulphasalazine, Olsalazine, Balsalazide are used for the treatment of inflammatory bowel disease. These 5-aminosalicylic acid derivatives are metabolized by the bacteria present in the colon to 5-aminosalicylic acid. Therefore, the 5-aminosalicylic acid is available at the desired site. Several 5-aminosalicylic acid derivatives are developed to improve its pharmacokinetics and pharmacodynamics properties[2].

In the literature, various sulfonamide derivatives were reported to possess wide variety of pharmacological activities[3-6]. In view of this, the present study aimed to synthesize some sulfonamides of 5-aminosalicylic acid using substituted sulfonyl chlorides. To study the structure activity relationship, it was proposed to synthesize sulfonamides of 3-aminobenzoic acid and 4-aminophenol for comparison of salicylic acid, benzoic acid and phenolic moieties. The study also includes evaluation of synthesized compounds for *in-vitro* antioxidant and antibacterial activities. It was found logical to evaluate the active molecules for the possible pharmacological activity; acetic acid induced ulcerative colitis in rats. Finally, it was also aimed to predict the molecular properties and bioactivity scores of synthesized compounds by using Molinspiration Cheminformatics software.

MATERIALS AND METHODS

All the chemicals were procured from Sigma Aldrich and SD fine chemicals. Melting points were determined in open capillaries on a tempo melting point apparatus and were uncorrected. The purity of compounds was checked by using silica gel coated plates and the spots detected using Iodine vapour. IR spectra (KBr, υ_{max}, cm^{-1}) were run on bruker FTIR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker avance-400MHz spectrophotometer and the values for chemical shift (δ) were expressed in ppm downfield from tetramethylsilane (TMS as internal standard) using DMSO-d_6 as solvent. Mass spectra were recorded on LC-MS, Agilent Technology 1200 infinity series, Apex chromatogram model.

The animal experiments were carried out in accordance with the guidelines of CPCSEA after approval from Institutional Animal Ethics Committee [Regd.No.1677/PO/Re/S/2012/ CPCSEA].

General procedure for the synthesis of substituted sulfonamides (Scheme-I: 3a, 3c-3f, 3h-3k, 3m-3o):

To the solution of substituted aromatic amines (10mM) in aqueous potassium carbonate solution (20mM, 15 %), substituted sulphonyl chlorides(10mM) were added portion-wise with stirring. The mixture was then stirred for one hour at room temperature and heated at 70–80 ºC for another hour. The resulting clear solution was cooled to room temperature and then acidified with 2M HCl in an ice-bath to pH 3. After 30 min., the

precipitated product thus obtained was filtered, washed thoroughly with cold water and dried.

3a: 5-(4-acetamidophenylsulfonamido)-2-hydroxybenzoic acid: Yield 80%; mp 254 °C; IR (KBr) υ_{max}: 3470 cm^{-1} (OH str), 3363 cm^{-1} (NH str), 3156 cm^{-1} (NH Sulfonamide str), 1668 cm^{-1} (C=O str), 1318 cm^{-1} (S=O anti-sym str), 1154 cm^{-1} (S=O sym str); ^{1}H NMR (DMSO-d_6, 400MHz): δ 2.063 (s, 3H, CH_3), δ 6.832- 6.854 (d, 1H, Ar), δ 7.171-7.200 (dd, 1H, Ar), δ 7.456-7.463 (d, 1H, Ar), δ 7.579-7.601 & 7.687-7.709 (2d, 4H, Ar), δ 9.894 (s, 1H, -SO_2NH), δ 10.327 (s, 1H, -CONH-), δ 11.199 (br, 1H, OH); ^{13}C NMR (DMSO, 100MHz): 24.07, 113.08, 117.71, 118.46, 123.48, 127.86, 128.84, 129.99, 132.73, 143.05, 158.28, 168.96, 171.12; Mass m/z: 349 $[M-H]^-$.

3c: 2-hydroxy-5-(4-methylphenylsulfonamido)benzoic acid: Yield 72%; mp 212 °C; IR (KBr) υ_{max}: 3262 cm^{-1} (NH & OH str), 1698 cm^{-1} (C=O str), 1312 cm^{-1} (S=O anti-sym str) & 1153 cm^{-1} (S=O sym str); ^{1}H NMR (DMSO-d_6, 400MHz): δ 2.338 (s, 3H, CH_3), δ 6.804- 6.826 (d, 1H, Ar), δ 7.158-7.187 (dd, 1H, Ar), δ 7.327-7.347 (d, 1H, Ar), δ 7.464 – 7.471 (d, 1H, Ar), δ 7.548-7.569 (2d, 2H, Ar), δ 9.913 (s, 1H, -SO_2NH); ^{13}C NMR (DMSO, 100MHz): 20.90, 117.32, 117.60, 123.40, 126.67, 128.59, 129.56, 136.45, 143.07, 158.42, 171.07; Mass m/z: 306 $[M-H]^-$.

3d: 2-hydroxy-5-(phenylsulfonamido)benzoic acid: Yield 64%; mp 170 °C; IR (KBr) υ_{max}: 3257 cm^{-1} (NH Sulfonamide str), 1662 cm^{-1} (C=O str), 1330 cm^{-1} (S=O anti-sym str) & 1160 cm^{-1} (S=O sym str); ^{1}H NMR (DMSO-d_6, 400MHz): δ 6.803- 6.825 (d, 1H, Ar), δ 7.162-7.191 (dd, 1H, Ar), δ7.456-7.463 (d, 1H, Ar), δ 7.519-7.688(m, 5H, Ar), δ 9.998 (s, 1H, -SO_2NH-); ^{13}C NMR (DMSO, 100MHz): 113.85, 117.57, 123.72, 126.63, 128.33, 129.11, 129.75, 132.75, 139.24, 158.57, 171.08; Mass m/z: 292 $[M-H]^-$.

3e: 2-hydroxy-5-(methylsulfonamido)benzoic acid: Yield 78%; mp 232 °C; IR (KBr) υ_{max}: 3464 cm^{-1} (OH str), 3285 cm^{-1} (NH Sulfonamide str), 1660 cm^{-1} (C=O str), 1317 cm^{-1} (S=O anti-sym str) & 1148 cm^{-1} (S=O sym str); ^{1}H NMR (DMSO-d_6, 400MHz): δ 2.871 (s, 3H, CH_3), δ 6.805- 6.826 (d, 1H, Ar), δ 7.230-7.259 (dd, 1H, Ar), δ 7.632-7.639 (d, 1H, Ar), δ 9.302 (s, 1H, -SO_2NH-); ^{13}C NMR (DMSO, 100MHz): 38.44, 116.28, 117.08, 123.95, 128.10, 128.83, 159.34, 171.13; Mass m/z: 230 $[M-H]^-$.

3f: 3-(4-acetamidophenylsulfonamido)benzoic acid: Yield 78%; mp 252 °C; IR (KBr) υ_{max}: 3357 cm^{-1} (NH str), 3134 cm^{-1} (NH Sulfonamide str), 1691 cm^{-1} (C=O str), 1323 cm^{-1} (S=O anti-sym str) & 1156 cm^{-1} (S=O sym str); ^{1}H NMR (DMSO-d_6, 400MHz): δ 2.054 (s, 3H, CH_3), δ 7.311- 7.722 (m, 8H, Ar), δ 10.326 (s, 1H, -SO_2NH-), δ 10.408 (s, 1H, -CONH-), δ 13.002 (br, 1H, COOH); ^{13}C NMR (DMSO, 100MHz): 24.05, 112.60, 118.57, 120.52, 123.96, 124.66, 127.85, 129.37, 131.69, 132.76, 138.17, 143.23, 166.69, 168.98; Mass m/z: 334 $[M-H]^-$.

3h: 3-(4-methylphenylsulfonamido)benzoic acid: Yield 77%; mp 140 °C; IR (KBr) υ_{max}: 3254 cm^{-1} (NH Sulfonamide str), 1685 cm^{-1} (C=O str), 1337 cm^{-1} (S=O anti-sym str), 1159 cm^{-1} (S=O sym str); ^{1}H NMR (DMSO-d_6, 400MHz): δ 2.331 (s, 3H, CH3), δ 7.099 – 7.702 (m, 8H, Ar), δ 10.476 (s, 1H, -SO_2NH-), δ 12.926 (br, 1H, COOH); Mass m/z: 290 (M-1) $[M-H]^-$.

3i: 3-(phenylsulfonamido)benzoic acid: Yield 89%; mp 192 °C; IR (KBr) υ_{max}: 3257 cm^{-1} (NH Sulfonamide str), 1686 cm^{-1} (C=O str), 1332 cm^{-1} (S=O anti-sym str), 1161cm^{-1} (S=O sym str); ^{1}H NMR (DMSO-d_6, 400MHz): δ 7.346 – 7.778 (m, 9H, Ar), δ 10.543 (s, 1H, -SO2NH-), δ 12.958 (br, 1H, COOH); Mass m/z: 276 $[M-H]^-$.

3j: 3-(methylsulfonamido)benzoic acid: Yield 68%; mp 204 °C; IR (KBr) υ_{max}: 3236 cm^{-1} (NH Sulfonamide str), 1691 cm^{-1} (C=O str), 1317 cm^{-1} (S=O anti-sym str), 1148 cm^{-1} (S=O sym str); ^{1}H NMR (DMSO-d_6, 400MHz): δ 3.012 (s, 3H, CH_3), δ 7.443 –7.819 (m, 4H, Ar), δ 9.978 (s, 1H, -SO_2NH-), δ 12.970 (br, 1H, COOH); Mass m/z: 214 $[M-H]^-$.

3k: N-(4-(N-(4-hydroxyphenyl)sulfamoyl)phenyl)acetamide: Yield 71%; mp 250 °C; IR (KBr) υ_{max}: 3375 cm^{-1} (OH str), 3317 cm^{-1} (NH str), 3129 cm^{-1} (NH Sulfonamide str), 1319 cm^{-1} (S=O anti-sym str), 1147 cm^{-1} (S=O sym str); ^{1}H NMR (DMSO-d_6, 400MHz): δ 2.065 (s, 3H, CH_3), δ 6.582- 6.620 & δ 6.808 - 6.838 (2d, 4H, Ar), δ 7.560-7.582 & δ 7.668 - 7.689 (2d, 4H, Ar), δ 9.278 (s, 1H, SO_2NH -), δ 9.575 (s, 1H, -OH), δ 10.266 (s, 1H, -CONH), ^{13}C NMR (DMSO, 100MHz): 24.05, 115.45, 118.36, 121.15, 122.91, 124.08, 127.85, 128.59, 129.55, 132.71, 136.82, 143.24, 145.08, 168.91; Mass m/z: 305 $[M-H]^-$.

3m: N-(4-hydroxyphenyl)-4-methylbenzenesulfonamide: Yield 62%; mp 194 °C; IR (KBr) υ_{max}: 3339 cm^{-1} (OH str), 3279 cm^{-1} (NH Sulfonamide str), 1371 cm^{-1} (S=O anti-sym str), 1153 cm^{-1} (S=O sym str); Mass m/z: 262 $[M-H]^-$.

3n: N-(4-hydroxyphenyl)benzenesulfonamide: Yield 61%; mp 130 °C; IR (KBr) υ_{max}: 3431 cm^{-1} (OH str), 3291 cm^{-1} (NH Sulfonamide str), 1315 cm^{-1} (S=O anti-sym str), 1155 cm^{-1} (S=O sym str); ^{1}H NMR (DMSO-d_6, 400MHz): δ 6.581- 6.611 (d, 2H, Ar), δ 6.816 - 6.854 (d, 2H, Ar), δ 7.503 - 7.668 (m, 5H, Ar), δ 9.302 (s, 1H, -SO_2NH-), δ 9.723 (s, 1H, OH); ^{13}C NMR (DMSO, 100MHz): 115.48, 124.08, 126.04, 128.39, 128.97, 132.51, 139.57, 154.86; Mass m/z: 248 $[M-H]^-$.

3o: N-(4-hydroxyphenyl)methanesulfonamide: Yield 72%; mp 157 °C; IR (KBr) υ_{max}: 3400 cm^{-1} (OH str), 3255 cm^{-1} (NH Sulfonamide str), 1358 cm^{-1} (S=O anti-sym str), 1136 cm^{-1} (S=O sym str); ^{1}H NMR (DMSO-d_6, 400MHz): δ 2.849 (s, 3H, CH_3), δ 6.711 - 6.750 (d, 2H, Ar), δ 7.019 -7.058 (d, 2H, Ar), δ 9.178 (s, 1H, -SO_2NH-), δ 9.373 (s, 1H, OH); ^{13}C NMR (DMSO, 100MHz): 40.16, 115.63, 123.16, 124.08, 137.38, 154.91; Mass m/z: 186 $[M-H]^-$.

General procedure for the synthesis of substituted p-aminobenzene sulfonamides (Scheme-I: 3b, 3g, 3l):

The compound **3a**/ **3f**/ **3k** (5mM) was added to 30ml of water-ethanol (1:1) mixture containing 10 ml of concentrated HCl. The reaction mixture was kept for reflux until clear solution was obtained. After 1 hr the reaction mixture was cooled to room temperature and poured onto the crushed ice and pH was adjusted to 6-7 using saturated sodium bicarbonate solution. The precipitated product thus obtained was filtered, washed thoroughly with cold water and dried.

3b: 5-(4-aminophenylsulfonamido)-2-hydroxybenzoic acid: Yield 65%; mp 214 °C; IR (KBr) υ_{max}: 3474 & 3372 cm^{-1} (OH & NH str), 3253 cm^{-1} (NH Sulfonamide str), 1674 cm^{-1} (C=O str), 1321 cm^{-1} (S=O anti-sym str) & 1148 cm^{-1} (S=O sym str); ^{1}H NMR (DMSO-d_6, 400MHz): δ 5.967 (s, 2H, NH_2), δ 6.510- 6.532 & δ 7.283-7.305 (2d, 4H, Ar), δ 6.813-6.835 (d, 1H, Ar), δ 7.168-7.197 (dd, 1H, Ar), δ 7.463-7.470 (d, 1H, Ar), δ 9.542 (s, 1H, -SO_2NH-), δ 11.084(br, 1H, OH) ; ^{13}C NMR (DMSO, 100MHz): 112.53, 112.99, 117.48, 122.94, 124.23, 128.60, 129.53, 129.58, 152.72, 157.94, 171.24; Mass m/z: 307 $[M-H]^-$.

3g: 3-(4-aminophenylsulfonamido)benzoic acid: Yield 61%; mp 80 °C; IR (KBr) υ_{max}: 3380 cm^{-1} (NH str), 3250 cm^{-1} (NH Sulfonamide str), 1694cm^{-1} (C=O str), 1372 cm^{-1} (S=O anti-sym str), 1152 cm^{-1} (S=O sym str); ^{1}H NMR (DMSO-d_6, 400MHz): δ 5.743 (s, 2H, NH_2), δ 6.491- 6.512 & δ 7.376-7.397 (2d, 4H, Ar), δ 7.174-7.358 (m, 3H, Ar), δ 7.573(s, 1H, Ar); Mass m/z: 291 $[M-H]^-$.

3l: 4-amino-N-(4-hydroxyphenyl)benzenesulfonamide: Yield 78%; mp 170 °C; IR (KBr) υ_{max}: 3473 cm^{-1} (OH str), 3376 cm^{-1} (NH str), 3206 cm^{-1} (NH Sulfonamide str), 1310 cm^{-1} (S=O anti-sym str), 1150 cm^{-1} (S=O sym str); ^1H NMR (DMSO-d_6, 400MHz): δ 5.894 (s, 2H, NH_2), δ 6.497- 6.519 & 6.574 – 6.596 (2d, 4H, Ar), δ 6.809 – 6.831 & 7.263 – 7.285 (2d, 4H, Ar), δ 9.207 (s, 1H, -SO_2NH-), δ 9.254 (s, 1H, OH); ^{13}C NMR (DMSO, 100MHz): 112.43, 115.30, 123.59, 128.58, 129.35, 130.28, 152.86, 154.47; Mass m/z: 263 $[M-H]^-$.

***In-vitro* antioxidant activities**

The synthesized compounds were evaluated for the *in-vitro* antioxidant properties by scavenging DPPH[7] and nitric oxide[8] free radicals at 100 µM concentrations.

DPPH free radical scavenging

Solutions of various test compounds at 100 µM concentration were added to 100 µM DPPH in absolute alcohol separately. The tubes were kept at an ambient temperature for 20 minutes and absorbance was measured at 517 nm. Ascorbic acid at 100 µM concentration was used as reference standard. The results were expressed as mean of triplicate experiments. The percentage of DPPH free radical scavenging was calculated using the following formula.

Percentage of DPPH free radical scavenging = (Control – Test / Control) x 100

Nitric oxide free radical scavenging

Sodium nitroprusside (5 mM) in phosphate buffer pH 7.4 was added to 100 µM concentration of test compounds dissolved in ethanol. The solutions were incubated at 25 ºC for 150 minutes and 2 ml of incubation solution was added to 2 ml of Griess reagent. The absorbance of chromophore formed during diazotization of nitrite with Sulfanilamide and subsequent coupling with N-(1-Naphthyl)ethelenediamine was read at 546 nm. Control experiment was conducted in similar manner without test compound but with equal amount of solvent. The results were expressed as mean of triplicate experiments and the percentage of nitric oxide free radical scavenging was calculated using the following formula.

Percentage of nitric oxide free radical scavenging = (Control – Test / Control) x 100

Antibacterial activity

In-vitro antibacterial activity of synthesized compounds were evaluated using agar well diffusion method against bacterial strains *E.coli, P.vulgaris, S.aureus* and *B.subtilis* at a concentration of 1000 µg/ml.

The sterile agar medium was poured into sterile petriplates at 40-45 ºC and allowed to solidify. The bacterial inoculums were uniformly spread using sterile cotton swab on solidified nutrient agar medium present in the petriplates. Three cups of 8 mm diameter were made in each petriplate using a sterile borer. The test compounds were dissolved in DMSO (Dimethyl Sulfoxide), then 0.1 ml of the test solutions containing 100 µg was added to each well aseptically with the help of micro pipette and the petriplates were labeled accordingly. Positive control was maintained by employing 0.1 ml of standard solution containing 100 µg Sulfanilamide and Amoxicillin separately. After proper diffusion of test solutions/standard solution into the media the plates were incubated for 24 hrs at 37 ºC under aerobic conditions. The zone of inhibition of bacterial growth was measured in millimeters (mm).

Pharmacological activity: Acetic acid induced Ulcerative colitis in rats

The male Wistar rats (180-200 g) were used for the study. The rats were fed with standard food pellets with *ad libitum* food and water access, controlled temperature and lighting (12 hrs light-dark cycles). The animals were divided into seven groups, each group consisting of four animals.

Group I: Normal control animals (Vehicle control).
Group II: Acetic acid control animals, received 2 ml of 4% v/v acetic acid solution, intrarectally on the 3rd day (Positive control).
Group III: Animals treated with standard, received pretreatment with standard 5-aminosalicylic acid (0.13mmol/kg), *p.o* and 2 ml of 4% acetic acid solution, intrarectally on the 3rd day. Drug treatment was continued till 5th day.
Group IV – Group VII: Animals treated with compound **3a**, **3b**, **3k** and **3l** received pretreatment with compound **3a**, **3b**, **3k** and **3l** (0.13mmol/kg), *p.o* and 2 ml of 4% acetic acid solution, intrarectally on the 3rd day. Drug treatment was continued till 5th day.

The rats were anaesthetized with ether following 24 hrs fast, and then a medical-grade polyurethane tube for enteral feeding was inserted into the anus. Acetic acid (2 ml, 4% v/v) was instilled into the colon and after 30 seconds the fluid was withdrawn. After 72 hrs of acetic acid treatment, animals were sacrificed by cervical dislocation and dissected to remove the colon. Colon was flushed gently with saline and weighed. The tissue homogenate was prepared and the quantification of inflammation was done by measurement of colonic lipid peroxides concentration (LPO), measurement of reduced glutathione (GSH) content and assessment of colonic myeloperoxidase (MPO) activity.

Estimation of Lipid Peroxidation

Colon tissue was homogenized in KCl (1.15%) solution and the supernatant was used for the estimation of MDA levels. The tissue supernatant (200 µl) was added to 200 µl of 8.1% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid solution and 1.5 ml of thiobarbituric acid (0.8%) was added and placed in a boiling water bath for 60 min and then the samples were allowed to cool at room temperature. A mixture of 1.25 ml of butanol: pyridine (15:1), was added, vortexed and centrifuged at 4000 rpm for 10 mins. The coloured layer (500 µl) was measured at 532 nm[9]. The values were expressed as µmoles of MDA formed/mg tissue.

Determination of colonic GSH contents

Reduced glutathione content was measured according to the method of Ellman[10]. Briefly, 0.75 ml of supernatant was mixed with 0.75 ml of 4% sulphosalicylic acid and then centrifuged at 12000 rpm for 5 min. at 4 ºC, from this 0.5 ml of supernatant was taken and added to 4.5 ml of 0.01 M DTNB and yellow colour developed was read spectrometrically at 412 nm immediately. The GSH content was calculated and expressed as µg/mg tissue.

Assessment of colonic MPO activity

The activity of MPO was assessed according to the method of mullane et al.[11], the colon tissue was homogenized in phosphate buffer containing 0.5% hexadecyltrimethyl ammonium bromide to produce 10% w/v homogenate. The samples were centrifuged at 15000 rpm for 30 min at 4 ºC and the resulting supernatant was assayed spectrophotometrically for MPO. To 0.1 ml of the sample, 2.9 ml of phosphate buffer containing 0.167 mg/ml o-dianisidine dihydrochloride and 0.0005% hydrogen peroxide was mixed and shaken vigorously. The change in absorbance of this mixture was measured at 460 nm for 3 min. at an interval of 60

sec. One unit of enzyme activity was defined as the amount of MPO that causes a change in absorbance measured at 460 nm for 3 min. MPO activity was expressed as units/mg tissue.

Statistical Analysis

The results were expressed as mean ± SD. The statistical significance of any difference in each parameter among the groups was evaluated by one-way ANOVA.

Prediction of molecular descriptors and bioactivity scores of synthesized sulfonamides using Molinspiration Cheminformatics software

The structures of resultant products (**3a**-**3o**) and 5-aminosalicylic acid were generated and the molecular descriptors, such as lop P (partition coefficient), molecular weight (MW), the acceptors and donors for hydrogen bonding in a molecule and topological polar surface area (TPSA) were calculated using the online software (http://www.molinspiration.com). These descriptors are strongly associated with membrane permeability and oral bioavailability. The Lipinski rule states that the compounds are more likely to be orally bioavailable[12], if they obey the rule and fulfill following criteria: log P ≤ 5, molecular weight ≤ 500, hydrogen bond acceptors ≤ 10 and hydrogen bond donors ≤ 5. The bioactivity scores of all the synthesized sulfonamides and 5-aminosalicylic acid towards GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors and enzyme inhibitors were predicted using the same Molinspiration Cheminformatics software. The bioactivity scores allow adequate identification of active and inactive molecules.

RESULTS AND DISCUSSION

Chemistry

A series of sulfonamides **3a**-**3o** were synthesized by most commonly used eco-friendly method. The present study mainly concentrated on the formation of sulfonamide linkage between the free amino group of 5-aminosalicylic acid, 3-aminobenzoic acid, 4-aminophenol and sulfonyl group of various substituted sulfonyl chlorides so as to study the structure activity relationship. Among the synthesized sulfonamides, compounds **3a**-**3g** were newly reported for the first time from our laboratory. The synthetic reaction mixture consisting equimolar quantities of substituted aromatic amine and substituted sulfonyl chloride in aqueous potassium carbonate solution was stirred vigorously until all the compounds were dissolved and then it was kept for heating at a temperature 70-80 °C for one hour. The reaction mixture was cooled to room temperature and the solution was acidified to pH 3. The precipitated product was then filtered, washed thoroughly with cold water and dried. Compounds **3b**, **3g** and **3l** were synthesized by hydrolysis of **3a**, **3f** and **3k** respectively. The yield of the compounds ranges from 61% to 89%. The purity of synthesized compounds was confirmed by TLC. The structures of these compounds were characterized by their IR, ^{1}H NMR, ^{13}C NMR and Mass spectra.

The IR spectra of all the compounds **3a**-**3o** showed the presence of bands in the range of 1310-1372 & 1136-1160 cm^{-1} due to anti-symmetric and symmetric stretching vibrations of S=O group. A prominent band was observed in the range of 3129-3291 cm^{-1} due to NH of sulfonamide (–SO_2NH-) indicating the formation of sulfonamide linkage. The spectra of compounds **3a**-**3j** revealed the presence of absorption bands at 1660-1698 cm^{-1} indicating C=O stretching of carboxylic acid group. The IR spectra of compounds **3a**-**3e** & **3k**-**3o** showed phenolic OH stretch in the range of 3339-3476 cm^{-1}. The IR spectra of compounds **3a, 3f** and **3k** showed absorption bands at 3317-3363 cm^{-1}, due to the NH stretching vibrations of amide functional group. The spectra of compounds **3b, 3g** and **3l** showed absorption bands at 3374-3380 cm^{-1} due to NH stretching and 1632-1625 cm^{-1} due to NH deformation indicating the presence of primary amine group.

The IR spectral data was supported by ^{1}H NMR spectral data which confirm the structure of synthesized compounds on the basis that the sulfonamide proton appearing as singlet at δ 9.178-10.543. The ^{1}H NMR spectra of all the compounds showed multiplets in the range δ 6.582-7.819 due to aromatic protons. The amide NH proton in compounds **3a, 3f** and **3k** was observed at δ 10.266-10.408 and three N-acetyl protons were observed at δ 2.054-2.065. The NH_2 protons of compounds **3b, 3g** and **3l** were observed at δ 5.743-5.967. The methyl protons of compounds **3c** and **3h** were observed at δ 2.331-2.338 and the methyl protons of methane sulfonamide derivatives **3e, 3j** and **3o** were observed at δ 2.849-3.012. The spectra of compounds **3f**-**3j** revealed the presence of broad singlets at δ 12.926-13.002 due to carboxylic acid protons. The phenolic OH moiety of compounds **3k**-**3o** was observed at δ 9.254-9.723.

The ^{13}C NMR spectra of compounds **3a**-**3e, 3f, 3k**-**3l** and **3n**-**3o** showed absorption peaks at δ 128.83-138.17 confirming the presence of carbons attached to NH group of 5-aminosalicylic acid, 3-aminobenzoic acid and 4-aminophenol moieties. The spectra also revealed the presence of absorption peaks at δ 124.23-139.57 due to the carbons attached to SO_2 group. All aromatic carbons were observed in the range of δ 112.43-168.91. The spectra of compounds **3a**-**3e** and **3f** showed absorption peaks at δ 166.69-171.24 confirming the presence of COOH carbon. The compounds **3a**-**3e, 3k**-**3l** and **3n**-**3o** showed absorption peaks at δ 145.08-159.34 confirmed the presence of carbon attached to phenolic hydroxyl group of 5-aminosalicylic acid and 4-aminophenol moieties. The spectra of compounds **3a, 3f** and **3k** showed absorption peaks at δ 168.96, 168.98 and 143.24 respectively, indicating the presence of C=O of amide. The spectra of compounds **3b** and **3l** showed absorption peaks at δ 152.72 and 154.47 indicating the presence of carbon attached to primary NH_2 group. The methyl carbons of **3a, 3c, 3f, 3k** and **3e, 3o** appeared at δ 24.05-24.07 and δ 38.44-40.16. The mass spectra of compounds were recorded in negative ion mode and the $[M-H]^-$ peaks indicated the molecular mass of the compounds.

***In-vitro* antioxidant activities**

DPPH free radical scavenging

DPPH, nitrogen centered free radical having an odd electron, characterized as a stable free radical by virtue of delocalization of odd electron over the molecule as a whole and due to the delocalization it gives rise to the deep violet color, with an absorption in ethanol solution at 517 nm. DPPH free radical scavenging method involves the reduction of DPPH in alcoholic solution in the presence of hydrogen/electron donating antioxidant and consequently the violet color changes to yellow due to formation of stable diamagnetic molecule diphenylpicrylhydrazine (DPPH-H) in the reaction. The decolorization is stoichiometric, hence the DPPH free radical scavenging method offers the first approach for evaluating the antioxidant potential of a compound[13]. The synthesized sulfonamides exhibited their ability to reduce DPPH by rapidly converting the unpaired electrons to paired ones. The data presented in Table-1. All the evaluated compounds, except **3f**-**3j** exhibited excellent antioxidant activity in this model. The reason for the better activity of above compounds may be due to the presence of salicylic acid group in **3a**-**3e** and phenol group in **3k**-**3o**. It was evident from the activity data that the presence of salicylic acid group causes increased antioxidant activity. Among the series, 5-aminosalicylic acid sulfonamides **3b** (89.4%) and **3e** (88.2%) showed highest DPPH scavenging activity at 100 µM concentration. The activity of these compounds found greater

than the standard compound ascorbic acid (85.6%). The highest activity of the above compounds may be due to the presence of sulfonamide group along with salicylic acid moiety. In the series, **3a** (84.9%), **3o** (76.9%), **3c** (74.3%), **3d** (73.3%) and **3n** (72.8%) were found the next potent compounds in DPPH scavenging activity in the decreasing order.

On observation of the results, it was found that the replacement of acetamido group of compounds **3a** and **3k** with amine substitution on benzene ring, as in compound **3b** and **3l,** causes significant increase in antioxidant activity. Further, it was found that the replacement of methyl group of compounds **3e** and **3o** with benzene ring, as in compound **3d** and **3n**, causes reduction in antioxidant activity.

The activity data revealed that the removal of carboxylic acid from 5-aminosalicylic acid sufonamides causes reduction in DPPH scavenging activity. Further, it was observed that the acetamido and amine derivatives of 4-aminophenol sulfonamides (**3k** and **3l**) exhibited less DPPH scavenging activity than the benzene sulfonamide derivative of 4-aminophenol **3n**. But, the acetamido and amine derivative of 5-aminosalicylic acid sulfonamides (**3a** and **3b**) exhibited greater DPPH scavenging activity than the benzene sulfonamide derivative of 5-aminosalicylic acid **3d**. This further indicates the importance of substituents on the resultant sulfonamides which influences the capacity of donating hydrogen/electron to DPPH free radical.

Nitric oxide free radical scavenging

In-vitro scavenging of nitric oxide free radical is one of the methods that can be used for the determination of antioxidant potential of natural or synthetic molecules. The method is based on the principle that the sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite detectable by Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions[14].

The nitric oxide scavenging activity data of all the synthesized compounds was given in Table-1. Among the evaluated compounds **3l** (63.38%), **3a** (60.9%), **3g** (58.46%) and **3c** (55.1%) showed prominent activity against nitric oxide scavenging activity in decreasing order. The activity exhibited by these compounds was greater than the standard compound tocopherol (54.3%). The results indicated that the amino group on benzenesulfonyl moiety and phenolic hydroxyl group on aromatic amine moiety of sulfonamide (**3l**) were essential for the better scavenging of nitric oxide free radical. It was observed that when the sulfonamide possess acetamido group on benzenesulfonyl moiety together with phenolic hydroxyl group on aromatic amine moiety, as in compound **3k**, resulted in reduced nitric oxide scavenging activity (38.76%). However, the sulfonamide containing acetamido group on benzenesulfonyl moiety and salicylic acid moiety, as in compound **3a**, resulted in enhanced nitric oxide scavenging activity. Removal of phenolic hydroxyl group in compound **3a** resulted in compound **3f** with reduced nitric oxide scavenging activity (30.76%). The results indicated that hydrolysis of compound **3f** yielded compound **3g**, which exhibited greater nitric oxide scavenging activity. When the amine group of **3g** replaced with methyl group resulted in drastic decrease in the activity (**3h**, 22.46%). On removal of amine/methyl group of **3g**/**3h** leads to compound **3i** with drastic increase in nitric oxide scavenging activity (50.76%) when compared with compound **3h** and slightly reduced activity when compared with compound **3g**.

It was also observed that the hydrolysis of compound **3a** resulted in compound **3b** with reduced nitric oxide scavenging activity (35.38%). The amine group of compound **3b** when replaced with methyl group resulted in increase in the activity (**3c**, 55.07%). On removal of amine/methyl group of **3b**/**3c** leads to compound **3d** with drastic reduction in nitric oxide scavenging activity (8.92%).

Finally, it can be concluded that the nitric oxide free radical scavenging activity was dependent on the accumulation of nitrite, a stable oxidation product of nitric oxide. The assay indicated that the amount of nitrite produced was very low with the compound **3l**, **3a**, **3g** and **3c** indicating the importance of substituents on the sulfonamide pharmacophore.

Antibacterial activity

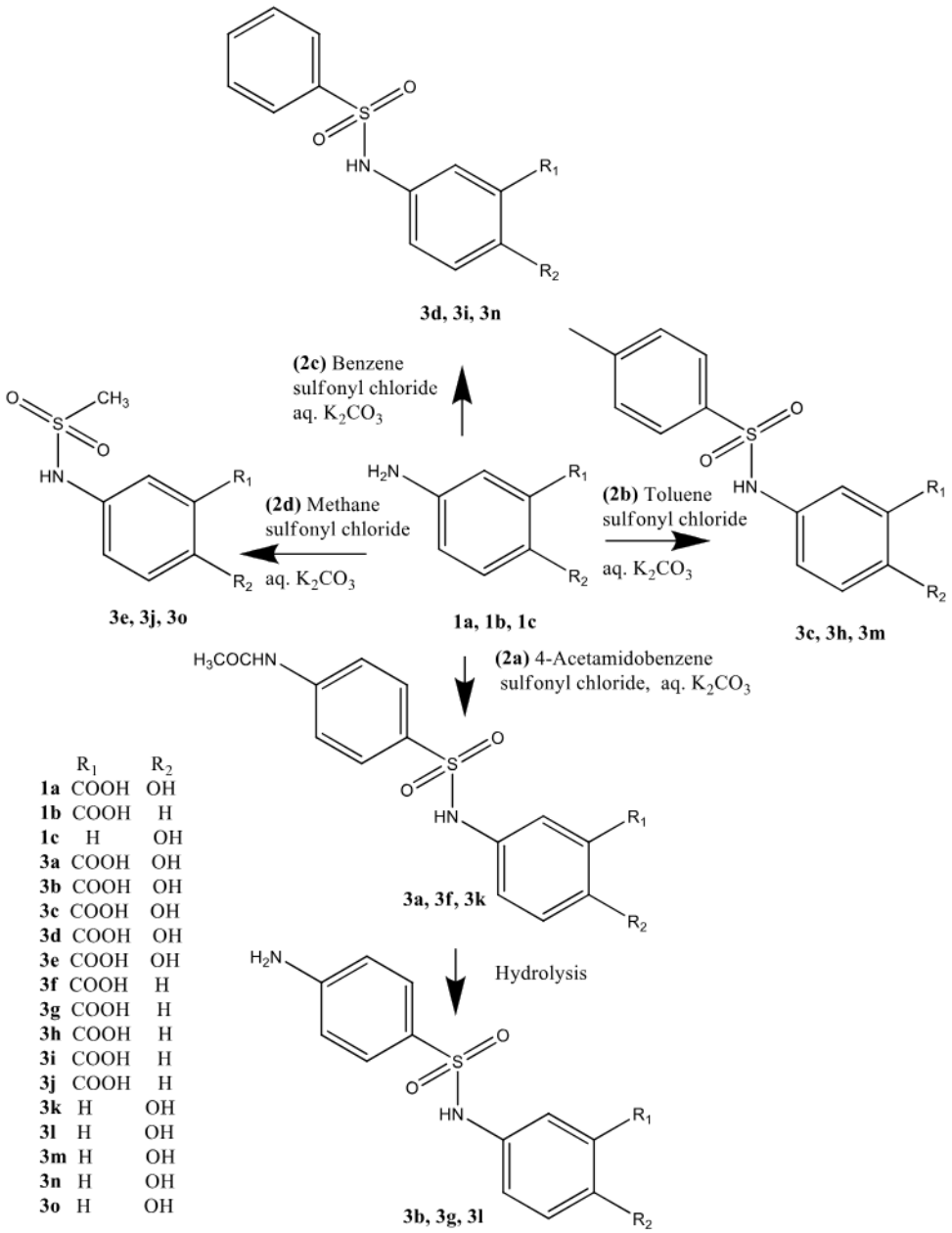
The synthesized compounds were evaluated for *in-vitro* antibacterial activity, using agar well diffusion method against bacterial strains *E.coli, P.vulgaris, S.aureus* and *B.subtilis* at a concentration of 1000 µg/ml. Triplicate results were obtained and the mean values of all the synthesized compounds were rounded to nearest integer and presented in Table-2. The results revealed that the compound **3g** showed excellent antibacterial activity against all the four bacterial strains better than the standard drug sulfanilamide and exhibited greater zones of inhibition against *E.coli, S.aureus* and *B.subtilis* when compared with the standard Amoxicillin. The compound **3h** showed good activity against the gram positive organism *S.aureus* greater than the standard drugs and the antibacterial activity of this compound against *B.subtilis* was comparable to the activity exhibited by the standard drugs employed. The results also indicated that the compound **3b** exhibited good antibacterial activity better than the standard drug sulfanilamide against gram negative bacterial strains *E.coli* and *P.vulgaris*. Further, all the compounds exhibited good to moderate antibacterial activity against the selected bacterial organisms.

Pharmacological activity: Acetic acid induced Ulcerative colitis in rats

Oxidative stress is known to play an important role in initiation and progression of ulcerative colitis. Experimentally induced colitis in animals is characterized by oxidative damage and an imbalance between oxidant and antioxidant levels. The acetic acid induced colitis model is known to cause an injury in the colon of animals due to the excessive generation of free radicals and reactive oxygen species, developing inflammation, a prominent feature of colitis. In the present study, four compounds **3a**, **3b**, **3k**, and **3l** with better antioxidant properties were selected for evaluation against acetic acid induced ulcerative colitis in rats. The mucosal protective effect of these compounds was confirmed by biochemical estimations such as the assay of tissue lipid peroxidation, reduced glutathione and assessment of myeloperoxidase levels. The 5-aminosalicylic acid was used as standard drug for comparing the results and the activity data presented in Table -3.

Estimation of Lipid Peroxidation

Lipid peroxidation was determined by measuring malondialdehyde (MDA). A significant increase in content of MDA was observed in disease control group (DC) when compared with normal control group (48.99 ± 5.98 µmol/mg *vs* 2.35 ± 0.62 µmol/mg $p<0.001$). On comparison to disease control, the levels of MDA were significantly reduced in all test groups and standard treated group. The compounds **3b** and **3a** significantly reduced the colonic lipid peroxides (17.02 ± 1.752 µmol/mg and 22.86 ± 1.53 µmol/mg respectively) compared to the standard 5-aminosalicylic acid (25.07 ± 0.67 µmol/mg). The greater activity of these compounds may be due to their better *in-vitro* antioxidant properties as evidenced in the DPPH free radical scavenging assay.



	R_1	R_2
1a	COOH	OH
1b	COOH	H
1c	H	OH
3a	COOH	OH
3b	COOH	OH
3c	COOH	OH
3d	COOH	OH
3e	COOH	OH
3f	COOH	H
3g	COOH	H
3h	COOH	H
3i	COOH	H
3j	COOH	H
3k	H	OH
3l	H	OH
3m	H	OH
3n	H	OH
3o	H	OH

Scheme-I: Synthesis of substituted sulfonamides (3a-3o)

Table-1: *In-vitro* antioxidant activity of synthesized sulfonamides

Compounds	R, R_1, R_2	% DPPH Scavenging at 100 µM	%Nitric oxide Scavenging at 100 µM
3a	R- C_6H_4-$NHCOCH_3$, R_1- COOH, R_2- OH	84.9	60.9
3b	R- C_6H_4-NH_2, R_1- COOH, R_2- OH	89.4	35.3
3c	R- C_6H_4-CH_3, R_1- COOH, R_2- OH	74.3	55.1
3d	R- C_6H_5, R_1- COOH, R_2- OH	73.3	8.92
3e	R- CH_3, R_1- COOH, R_2- OH	88.2	39.38
3f	R- C_6H_4-$NHCOCH_3$, R_1- COOH, R_2- H	19.4	30.76
3g	R- C_6H_4-NH_2, R_1- COOH, R_2- H	1.88	58.46
3h	R- C_6H_4-CH_3, R_1- COOH, R_2- H	17.6	22.46
3i	R- C_6H_5, R_1- COOH, R_2- H	14.8	50.76
3j	R- CH_3, R_1- COOH, R_2- H	17.1	40.92
3k	R- C_6H_4-$NHCOCH_3$, R_1- H, R_2- OH	63.2	38.76
3l	R- C_6H_4-NH_2, R_1- H, R_2- OH	65.2	63.38
3m	R- C_6H_4-CH_3, R_1- H, R_2- OH	38.2	NA
3n	R- C_6H_5, R_1- H, R_2- OH	72.8	9.2
3o	R- CH_3,R_1- H, R_2- OH	76.9	12.92
Standard	Ascorbic acid	85.6	-
Standard	Tocopherol	-	54.3

NA – Not Active

Table-2: Antibacterial activity of synthesized sulfonamides

Compound	(Gram –ve)		(Gram +ve)	
	E. coli	*P. vulgaris*	*S. aureus*	*B. subtilis*
3a	18	20	22	18
3b	20	21	20	17
3c	17	20	15	17
3d	15	19	13	13
3e	19	18	20	16
3f	18	16	20	19
3g	20	24	22	23
3h	17	19	23	23
3i	17	22	17	22
3j	16	20	16	17
3k	17	16	20	16
3l	18	19	15	19
3m	20	18	18	22
3n	15	16	14	15
3o	18	16	15	19
1a	18	17	17	17
Sulfanilamide	12	21	22	23
Amoxicillin	20	27	21	23

Table-3: Effect of selected sulfonamides on biochemical markers of colitis in colon specimens

Groups	MDA Levels µmol /mg wet tissue	GSH Levels µg/mg wet tissue	MPO Levels U/mg of wet tissue
Disease control	48.99 ± 5.98 +++	1.37± 0.878 +++	0.313 ± 0.009 +++
Control	2.35 ± 0.62***	10.49± 0.319 ***	0.048 ± 0.004***
5-Aminosalicylic acid	25.07 ± 0.67***, +++	8.87± 0.784 ***, +	0.080 ± 0.012***, +++
3a	22.86 ± 1.53***, +++	6.22± 0.292***, +++	0.177 ± 0.014***, +++
3b	17.02 ± 1.75***, +++	7.57± 0.167 ***, +++	0.212 ± 0.006***, +++
3k	35.36 ± 3.05***, +++	4.61± 0.286 ***,+++	0.112 ± 0.006***,+++
3l	27.19 ± 1.89***, +++	5.84± 1.319 ***, +++	0.143 ± 0.005***, +++

Dose: 0.13mM/kg body weight, *p.o*;
Values are expressed as Mean ± SD (n=4)
Analyzed by one-way ANOVA followed by post hoc Dennett's test
***p <0.001 *vs* Control group;
+p < 0.05, +++p < 0.001 *vs* Disease control group

Table-4: Prediction of molecular properties of synthesized sulfonamides and 5-aminosalicylic acid using Molinspiration Cheminformatics software

Compound	mi logP	M.Wt	HBA	HBD	Volume	n Violations	nrotb	TPSA	%ABS
3a	1.83	350.35	8	4	282.25	0	5	132.79	63.19
3b	1.69	308.31	7	5	245.59	0	4	129.72	64.24
3c	3.06	307.33	6	3	250.87	0	4	103.70	73.22
3d	2.61	293.30	6	3	234.31	0	4	103.70	73.22
3e	1.08	231.23	6	3	179.46	0	3	103.70	73.22
3f	1.81	334.35	7	3	274.23	0	5	112.57	70.16
3g	1.67	292.32	6	4	237.58	0	4	109.49	71.22
3h	3.04	291.33	5	2	242.85	0	4	83.47	80.20
3i	2.59	277.30	5	2	226.29	0	4	83.47	80.20
3j	1.06	215.23	5	2	171.44	0	3	83.47	80.20
3k	1.44	306.34	6	3	255.25	0	4	95.50	76.50
3l	1.30	264.31	5	4	218.59	0	3	92.42	77.11
3m	2.67	263.32	4	2	223.87	0	3	66.40	86.09
3n	2.22	249.29	4	2	207.30	0	3	66.40	86.09
3o	0.69	187.22	4	2	152.46	0	2	66.40	86.09
1a	0.92	153.14	4	4	130.35	0	1	83.55	80.17

%ABS = percentage of absorption; MW = molecular weight; HBD = number of H-bond donors; HBA = number of H-bond acceptors; MR = molar refractivity; nrotb = number of rotatable bonds; TPSA = topological polar surface area

Table-5: Prediction of bioactivity scores of synthesized sulfonamides and 5-aminosalicylic acid using Molinspiration Cheminformatics software

Compound	Bioactivity scores					
	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
3a	-0.10	-0.32	-0.26	-0.09	0.01	-0.08
3b	-0.02	-0.16	-0.16	-0.04	0.10	0.13
3c	-0.13	-0.32	-0.33	-0.01	-0.08	-0.05
3d	-0.12	-0.24	-0.32	-0.02	-0.05	0.02
3e	-0.34	0.04	-0.50	-0.13	-0.31	-0.04
3f	-0.13	-0.35	-0.31	-0.16	-0.01	-0.12
3g	-0.09	-0.18	-0.25	-0.15	0.05	0.09
3h	-0.20	-0.35	-0.42	-0.12	-0.14	-0.10
3i	-0.20	-0.28	-0.43	-0.14	-0.12	-0.04
3j	-0.47	-0.02	-0.68	-0.32	-0.41	-0.17
3k	-0.15	-0.31	-0.28	-0.23	-0.09	-0.12
3l	-0.14	-0.11	-0.25	-0.28	-0.07	0.08
3m	-0.27	-0.30	-0.45	-0.25	-0.28	-0.14
3n	-0.28	-0.22	-0.47	-0.30	-0.27	-0.09
3o	-0.61	0.03	-0.76	-0.55	-0.64	-0.29
1a	-0.80	-0.25	-0.81	-0.81	-0.86	-0.18

Determination of colonic GSH contents

The experimentally induced colitis produced a significant decrease in colonic GSH content when compared with normal control group (1.37± 0.878 µg/mg *vs* 10.49± 0.319 µg/mg respectively, $p<0.001$). Significantly increased GSH levels ($p<0.001$) were found in colon tissue of animals treated with test compounds than the disease control. Among the evaluated compounds, **3b** and **3a** showed significant restoration of GSH levels (7.57± 0.167 µg/mg and 6.218 ± 0.292 µg/mg, $p<0.001$) and these values were comparable to the value obtained with standard 5-aminosalicylic acid (8.875± 0.784 µg/mg, $p<0.05$). The compounds **3l** and **3k** also protected GSH depletion induced by acetic acid but to a lower extent than the compounds **3b** and **3a** indicating the importance of salicylic acid moiety.

Assessment of colonic MPO activity

MPO, an inflammatory marker was found to increase in disease control group, which was found to be 0.313 ± 0.009 U/mg of wet tissue. Pre-treatment with test compounds produced a significant reduction in MPO activity ($p<0.001$) as compared to acetic acid group. This indicates the ability of evaluated sulfonamides in prevention of the acetic acid induced ulcerative colitis. Among the tested compounds, acetamido benzene sulfonyl derivative of 4-aminophenol sulfonamide **3k** exhibited greater activity. Interestingly, the results indicated that 5-aminosalicylic acid sulfonamides showed reduced activity when compared to 4-aminophenol sulfonamides. Finally, it was observed that all the compounds showed less activity when compared to the standard 5-aminosalicylic acid.

***In-silico* prediction of molecular properties and bioactivity scores of synthesized sulfonamides**

The series of synthesized sulfonamides **(3a-3o)** and the precursor 5-aminosalicylic acid were considered to calculate the molecular properties using Molinspiration Cheminformatics software. The results presented in Table-4. The Lipinski's rule-of five, widely used as a filter for drug-likeness, estimated from the molecular properties such as partition coefficient (log P), molecular weight (MW), or hydrogen bond acceptors and donors of a molecule. These properties affect their absorption, distribution, metabolism and excretion (ADME) of the compounds and also indicate membrane permeability and bioavailability. The prediction data indicated that the compounds **(3a-3o)** and the precursor 5-aminosalicylic acid obeyed the Lipinski's rule-of five. Topological polar surface area (TPSA), used to predict the transportation properties of drugs like intestinal absorption and blood-brain barrier penetration. The calculated TPSA values were in the range of 66.40 to 132.79. The bioavailability of all the compounds was further supported by percentage absorption calculations[15], which indicated that the compounds **3a** and **3b** had low intestinal absorption (63.19% and 64.24% respectively) than the precursor 5-aminosalicylic acid (80.17%). Hence, more amounts of these compounds may be available locally in the colon compared to 5-aminosalicylic acid. All these results further support the protective effect of compounds **3a** and **3b** on acetic acid induced ulcerative colitis in rats.

The bioactivity score of all the synthesized compounds and the 5-aminosalicylic acid were also calculated based on Molinspiration Cheminformatics software and the data presented in Table-5. It was reported that when a molecule having bioactivity score more than 0.00 then it is active, if the score is between -0.50 to 0.00 then it is moderately active and if less than -0.50, then it is inactive[16]. On observation of predicted values, the compounds **3b, 3g** and **3a** were active as protease inhibitors and the compounds **3b, 3g, 3l** and **3d** were active as enzyme inhibitors. The compounds **3e** and **3o** were active as ion channel modulators. The data revealed that all the sulfonamides were found to have better bioactivity score towards GPCR ligand, nuclear receptor ligand, kinase and protease inhibition compared to standard 5-aminosalicylic acid. Finally, it can be concluded that the compounds **3b** and **3g** were identified as bioactive molecules particularly as protease and enzyme inhibitors.

CONCLUSION

A simple and environmentally benign method was used for the synthesis of novel sulfonamides from 5-aminosalicylic acid and the same method used for the preparation of sulfonamides from 3-aminobenzoic acid and 4-aminophenol to know the structure activity relationship of synthesized sulfonamides. The present study indicated that the compound **3b**, p-aminobenzenesulfonamide with salicylic acid moiety possess excellent *in-vitro* antioxidant properties in both DPPH and nitric oxide free radical scavenging assays compared to the standards employed. This compound also exhibited good *in-vivo* antioxidant activity compared to standard 5-aminosalicylic acid indicated by estimation of colonic LPO and GSH. Nevertheless, these results were not consistent with the assessment of colonic MPO activity. It was noticed that the compound **3g,** p-aminobenzenesulfonamide with benzoic acid moiety exhibited good antibacterial activity towards all the tested organisms, better than the standard drug sulfanilamide and showed greater zone of

inhibition against *E.coli, S.aureus* and *B.subtilis* when compared with the standard Amoxicillin. Although, the results obtained made it difficult to establish a clear relationship between chemical structure and biological activity, the importance of various substituents on the sulfonamide pharmacophore was supported by prediction of molecular properties and bioactivity scores using Molinspiration Cheminformatics software. The prediction data indicated that the novel compounds **3b** and **3g** were bioactive molecules as protease and enzyme inhibitors.

ACKNOWLEDEMENTS

Authors thank the DST-CURIE Center, Sri Padmavati Mahila Visvavidyalayam (Women's University) for providing IR spectra and Laila Implex Research Center, Vijayawada, Andhra Pradesh for providing ^{1}H NMR, ^{13}C NMR and Mass spectra.

REFERENCES

1. Sawarkar S P, Deshpande S G, Bajaj A N, Nikam V S. *In vivo* evaluation of 5-ASA colon-specific tablets using Experimental colitis rat animal model. AAPS PharmSciTech, 2015; 16: 1445.
2. Abdu-Allah H H, EI-Shorbagi A N A, Abdel-Moty S G, EI-Awady R, Abdel-Alim A A N. 5-Aminosalyclic acid (5-ASA): A unique anti-inflammatory salicylate. Medicinal Chemistry (Los Angeles), 2016; 6: 306.
3. Lal J, Gupta S K, Thavaselvam D, Agarwal DD. Biological activity, design, synthesis and structure activity relationship of some novel derivatives of curcumin containing sulfonamides. European Journal of Medicinal Chemistry 2013; 64: 579.
4. Vanparia S F, Patel T S, Dixit R B, Dixit B C. Synthesis and in vitro antimicrobial activity of some newer quinazolinone–sulfonamide linked hybrid heterocyclic entities derived from glycine. Medicinal Chemistry Research 2013; 22(11): 5184.
5. Qadir M A, Ahmed M, Iqbal M. Synthesis, characterization, and antibacterial activities of novel sulfonamides derived through condensation of amino group containing drugs, amino acids, and their analogs. BioMed Research International 2015; Article ID 938486.
6. Shashikala P, Keerthi D S. Chlorosulfonation of acetanilide to obtain an intermediate for the preparation of a sulfa drug. Journal of Chemical and Pharmaceutical Research 2016; 8(11): 204.
7. Blois M S. Antioxidant determination by the use of stable free radical. Nature 1958; 181: 1199.
8. Sreejayan, Rao M N A. Nitric oxide scavenging by curcuminoids. Journal of Pharmacy and Phamcology 1997; 49: 105.
9. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry1979; 95: 351.
10. Ellman G L. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics 1959; 82(1): 70.
11. Mullane K M, Kraemer R, Smith B. Myeloperoxidase activity as a quantitative assessment of neutrophil infiltration into ischemic myocardium. Journal of Pharmacological Methods 1985; 14: 157.
12. Lipinski C A, Lombardo F, dominy B W, Feeney P J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews 1997; 23: 3.
13. Kedare S B, Singh R P. Genesis and development of DPPH method of antioxidant assay. Journal of Food Science and Technology 2011; 48: 412.
14. Marcocci L, Maguire J J, Droy-Lefaix M T, Packer L. The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. Biochemical and Biophysical Research Communications 1994; 201(2): 748.
15. da Silva M M, Comin M, Duarte T S, Foglio M A, de Carvalho J E, Vieira M C, Formagio A S N. Synthesis, antiproliferative activity and molecular properties predictions of galloyl derivatives. Molecules 2015; 20(4): 5360.
16. Verma A. Lead finding from *phyllanthus debelis* with hepatoprotective potentials. Asian Pacific Journal of Tropical Biomedicine 2012; 2(3): S1735.

Cite this article as:

Madhavi Kuchana and Bindu Sree Nadakuditi. Synthesis, in-vitro antioxidant, antibacterial activities of novel sulfonamides from 5-aminosalicylic acid: Protective effect of selected sulfonamides on acetic acid induced ulcerative colitis in rats. Int. Res. J. Pharm. 2018;9(12):105-113 http://dx.doi.org/10.7897/2230-8407.0912302

Source of support: Nil, Conflict of interest: None Declared