Bioorganic & Medicinal Chemistry 20 (2012) 2535–2539

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis of new sulfonamides as lipoxygenase inhibitors

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ARTICLE INFO

Article history:
Received 1 January 2012
Revised 22 February 2012
Accepted 23 February 2012
Available online 3 March 2012

Keywords:
Lipoxygenase
Lipoxygenase inhibitors
Sulfonamides
4-(Toluene-4-sulfonylamino)-benzoic acids

ABSTRACT

The present study describes a convenient method for the synthesis of new lipoxygenase inhibitors, 4-(toluene-4-sulfonylamino)-benzoic acids from *p*-amino benzoic acid. Reaction of *p*-amino benzoic acid with *p*-toluenesulfonyl chloride provided thirteen N- and O-alkylation products **4a–4m** in moderate to good yields. Lipoxygenase inhibition of newly formed sulfonamide derivatives was investigated and some of these compounds **4m, 4g, 4e, 4f** and **4j** showed good lipoxygenase inhibitory activities with IC_{50} values ranged between 15.8 ± 0.57 and 91.7 ± 0.61 μmol whilst all other compounds exhibited mild anti-lipoxygenase activities with IC_{50} values ranged between 139.2 ± 0.75 and 232.1 ± 0.78 μmol. N-alkylated products were more active against the enzyme than O-alkylated or both N- and O-alkylated ones. All synthesized sulfonamides were recrystallized in chloroform to give these title compounds which were characterized using FTIR, ^{1}H NMR, ^{13}C NMR, elemental analysis and single crystal X-ray diffraction techniques.

1. Introduction

Sulfonamide derivatives are well known pharmaceutical agents since this group has been the main functional part of the most of the drug structures due to stability and tolerance in human beings.[1] These molecules have gained much attention due to their diverse biological activities in pharmaceutical[2] as well as in agricultural[3] areas. These are used in urinary tract infections, meningitis, streptococcal pharyngitis, bacillary dysentery, trachoma, chancroid, malaria, toxoplasmosis, nocardiasis and conjunctivitis.[4–6] Sulfonamides have a number of biological activities such as antibacterial,[7–9] insulin releasing,[10] carbonic anhydrase inhibitory,[11,12] anti-inflammatory,[13] and antitumor[14] activities. Antibacterial sulfonamides as sulfadiazinealone, and also diuretics, such as hydrochlorothiazide, are being therapeutically used for many decades.[15] Other commercial drugs with a sulfonamide structure are the antihypertensive agents like bosentan,[16] antiviral HIV protease inhibitor, amprenavir,[17] and the phosphodiesterase-5 inhibitor, sildenafil.[18] Recently, sulfonamide group has been found to be the key constituent of a new class of cyclooxygenase inhibitors such as celecoxib[19] and valdecoxib[20] as in Figure 1. The sulfonamide partial structure with versatile medicinal applications continues to play a fundamental role in medicinal chemistry.[21]

Such molecular architecture of sulfonamides attracted attention and keeping in view of their biological activity, synthesis of new diversely N- and O-alkylated 4-(toluene-4-sulfonylamino)-benzoic acids was carried out in search for new lead compounds as potential inhibitors of lipoxygenase. The data shows that **4m**, **4g**, **4e**, **4f** and **4j** are good inhibitors of lipoxygenase enzyme.

2. Results

2.1. Chemistry

The present approach successfully combined the formation of sulfonamide and its subsequent alkylation in the synthesis of a range of sulfonamides as outlined in Schemes 1a and b.

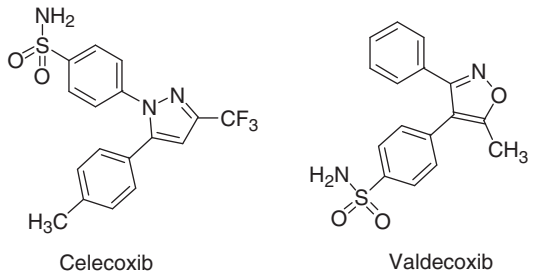

Figure 1. Chemical structures of celecoxib and valdecoxib.

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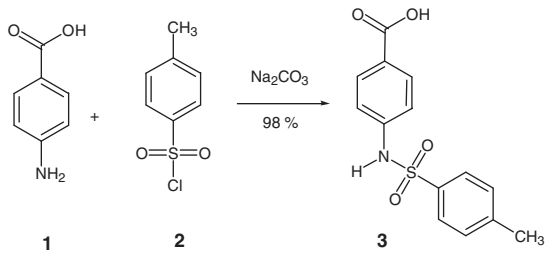
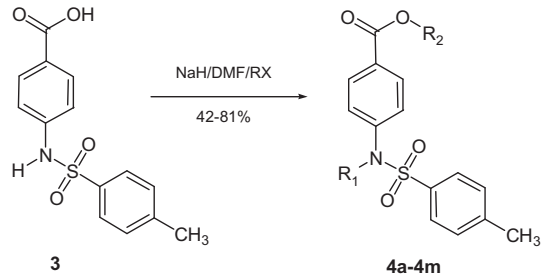
doi:10.1016/j.bmc.2012.02.055



Scheme 1a. Synthesis of precursor sulfonamide.



Scheme 1b. N-alkylation and O-alkylation product.

The synthesis of compound **3** was carried out under basic condition from the reaction of *p*-amino benzoic acid with *p*-toluenesulfonyl chloride and subsequent reaction with various alkylating or acylating agents that afforded new thirteen sulfonamide derivatives in moderate to good yields. Compound 4-(toluene-4-sulfonylamino)-benzoic acid **3** was subjected to alkylation in the presence of sodium hydride using DMF (dimethyl formamide) as solvent to produce fourteen various N- and O- alkylated sulfonamide derivatives **4a–4m**.

Two reactive sites carboxylic acid and amino group in the precursor's molecule are available towards alkylation. We began our investigation by installing different substituents. Under described reaction conditions, three kinds of products could be obtained. After screening a number of conditions, sulfonamide **3** could be then further functionalized, in a selective manner, to either O-alkylation product or N-alkylation product by using slightly different reaction conditions. We found that deprotonation of **3** with 2.0 equiv sodium hydride in the presence of DMF was followed by the addition of electrophiles which successfully yielded alkylation products. Both amide and ester functionality could be installed simultaneously in **4a**, **4b** and **4i** if the reaction time was extended up to 15 hours. Smaller size of alkylating agents was found to favor the reaction at room temperature towards both functional group and resulted **4a** and **4b** in good yields, respectively while **4i** was formed in 65% yield when the reaction was performed at 90 °C for 15 h.

The second type of products such as **4c–f**, **4g**, **4j–l** and **4m** were obtained in which an alkylating agent was reacted with the amino group to produce N-alkylated sulfonamides. Compound **4c** was formed by heating at 80 °C for 5 h while **4d** and **4f** were obtained in moderate yields when reaction conducted at room temperature for 3 h. Compound **4g** was prepared by heating the reaction mixture at 100 °C for 6 h while **4j** and **4k** were obtained when reaction was carried out at 80–90 °C for 12–18 h. Compounds **4l** and **4m** were obtained by simple heating at 60 °C. Acid halides were only reacted with amine group and failed to react with the carboxylic acid because the resulting anhydride was unstable. In general, the reaction of larger size of alkyl halide with sulfonamide **3** was found to occur slowly. The third type of product like **4h** were also obtained as an ester of sulfonamide **3** under the given specific reaction conditions. Consequently, the different product distribution observed on increasing reaction time and/or temperature is indicative of the reactivity of electrophile and stability of the product which favored particular course of reaction. Steric and electronic effects due to the larger groups were apparent in the reactions of **3**.

2.2. Crystallography

The suitable crystals for X-ray diffraction were grown under slow evaporation at room temperature in chloroform. Single crystal X-ray diffraction data were collected at 296 (2) K on a BRUKER KAPPA diffractometer equipped with an APEX II CCD detector (Bruker-AXS) using Mo K_α radiation (0.71073 Å). The crystal structure was solved by direct methods using SHELXS, and all structural refinements were conducted using either SHELXL-97.[22] PLATON[23] and WinGX[24] were used for molecular graphics. All the non-hydrogen atoms were refined with anisotropic displacement parameters. All the hydrogen atoms were placed at calculated positions or located via difference map and were refined using a riding model with co-ordinates and isotropic displacement parameters being dependent upon the atoms to which they are attached.

Crystal data: (**4b**) $C_{18}H_{21}N_1O_4S_1$, M = 347.4 g mol^{-1}, white, needle like, 0.34, 0.17, 0.17 mm^3, monoclinic, space group *P21/n*, a = 8.2114(6), b = 19.4939 (13), c = 11.3291(7) Å, β = t100.078(4)°, V = 1785.49(13), Z = 4, Dc = 1.29 g cm^{-3}, F(000) = 735.9, Mo K_α radiation, λ = 0.71073 Å, T = 296 (2) K, $2\theta_{max}$ = 57.8°, 16948 reflection collected, 4565 unique (R_{int} = 0.086), Final *GooF* = 0.997, $R1$ = 0.072, wR_2 = 0.115, R indices based on 1724 reflections with $I>2\sigma(I)$ (refinement on F^2) 220 parameters, Lp and absorption correction applied, μ = 0.202 mm^{-1}.

In the crystal structure of **4b** (Fig. 2), two aromatic rings are twisted at dihedral angle of 35.61(10)°. The ester group attached to phenyl ring (C7–C13) is planar with the root mean square deviation of 0.0092 Å and it is inclined at 5.06 (18)° with the ring (C7–C13). No classical hydrogen bonding interactions have been observed in the molecules. Only C–H···O type interaction connects the molecule with the symmetry operation (½ + *x*, ½ − y, ½ + *z*) and generate six memebered ring motif which can be represented mathematically as $R_2^1(6)$.[25]

2.3. Lipoxygenase assay

Lipoxygenase (LOX) activity was assayed according to the method of Tappel (1953) with minor modifications.[26] A total volume of 200 μL lipoxygenase assay mixture contained 150 μL sodium phosphate buffer (100 mM, pH 8.0), 10 μL test compound and 15 μL

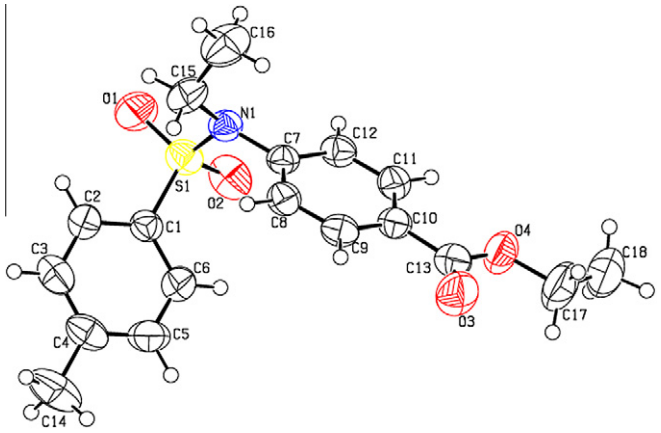


Figure 2. *ORTEP* diagram of **4b**, thermal ellipsoids drawn at 50% probability level.

purified lipoxygenase enzyme (600 units well^{-1}, Sigma Inc. USA). The contents were mixed and pre-read at 234 nm and preincubated for 10 min at 25 °C. The reaction was initiated by the addition of 25 μL substrate solution of linoleic acid. The change in absorbance was observed after 6 min at 234 nm using 96-well plate reader Synergy HT, Biotek, USA. All reactions were performed in triplicates. The positive and negative controls were included in the assay. Baicalin and quercetin (0.5 mM well^{-1}) were used as standard inhibitors. The percentage inhibition was calculated by the following formula. IC_{50} values of selected compounds exhibiting >50% activity at 0.5 mM were calculated after suitable dilutions using EZ–Fit Enzyme Kinetics software (Perrella Scientific Inc. Amherst, USA). All the measurements were taken in triplicate and statistical analysis was performed by Microsoft Excel 2003. Results are presented as mean ± SEM.

$$\text{Inhibition}\ (\%) = \frac{\text{abs of control} - \text{abs of test comp}}{\text{abs of control}} \times 100$$

3. Discussion

A series of structurally related sulfonamide derivatives (**4a–4m**) were prepared according to Scheme 1a by reacting *p*-amino benzoic acid with *p*-toluenesulfonyl chloride which on subsequent reaction with various alkylating or acylating agents provided thirteen N- and O-alkylation products in moderate to good yields. All reactions were carried out at varied temperatures (25–100 °C) and reaction times (2–18 h). The new compounds were in yields of 42–81%. Alkylation of **3** was carried out in the presence of sodium hydride using DMF as solvent at the two reactive sites of carboxylic acid and amino groups. Different reaction conditions yielded either O-alkylation or N-alkylation products. Deprotonation of **3** with sodium hydride in DMF in the presence of various electrophiles gave alkylation products. Both amide and ester functionality could be carried out simultaneously in **4a**, **4b** and **4i** if the reaction is carried out for 15 h. Smaller size of alkylating agents favored reactions at room temperature giving **4a** and **4b** in good yields, 74 and 81%, respectively whilst **4i** was formed at 90 °C for 15 h in 65% yield.

Alkylation at amino group gave N-alkylated sulfonamides, that is, **4c**, **4d**, **4e, 4f**, **4g**, **4j**, **4k**, **4l** and **4m**. The reaction of larger size of alkyl halide with sulfonamide **3** was occurred slowly. Acid halides only reacted with amine group and failed to react with the carboxylic acid because the resulting anhydride was unstable. Compound **4h** was made as ester of sulfonamide **3** under the given reaction conditions with increasing reaction time and temperature. The reactivity of electrophile and stability of the product also had impact in the synthesis of **4**. Steric hindrance and electronic effects due to the larger groups were apparent in the reactions of **3**.

All synthesized compounds were tested for their ability to act as inhibitors against lipoxygenase. Enzyme inhibition data is given in Table 1 as percent inhibition at 0.5 mM and IC_{50} values (concentration to inhibit 50% enzyme activity); the lower the IC_{50} value, the highest the inhibitory activity of the compound. Baicalein and quercetin were used as standard inhibitors. The compound **4m** showed the lowest IC_{50} value of 15.8 ± 0.57 μmol compared with the standard inhibitor baicalein (22.4 ± 1.3 μmol) or quercetin (37.12 ± 0.07 μmol). This was the most active compound against the enzyme and even better than the standard inhibitors. This compound is N-alkylated with $CH_2CH{=}CH(CH_3)_2$ alkyl group (Table 1). N-alkylation with $-CH_2$-Ph group (**4g**) was the second most active compound with IC_{50} value of 59.1 ± 0.87 μmol. Similar values were recorded for another N-alkylated ($-C_5H_{11}$) compound **4f** with IC_{50} value of 68.6 ± 0.43 μmol and **4e** (IC_{50} value of 61.4 ± 0.69 μmol). Compound **4j**, other N-alkylated product, showed slightly increased IC_{50} values of 91.7 ± 0.61 μmol. These studies reveal that N-alkylation at R_1 in the above compounds increased their enzyme inhibition action compared to those compounds which had both O- and N-alkylation (**4a**, **4b**, **4i**, increased IC_{50} values) or N-alkylation with –C(O)-Ph-Cl (**4k**) or $-CH_2-CH{=}CH_2$ (**4l**). N-alkylation with C_2H_4Cl (**4c**) or C_3H_7 (**4d**) abolished the enzyme inhibition potential of the compounds. On the other hand, N-alkylation at R_1 with C_4H_9 (**4e**) had increased inhibitory activity with IC_{50} value of 61.4 ± 0.69 μmol compared with the compound **4h**, wherein substitution of CH_2–CN at R_2 site increased value to 232.1 ± 0.78 μmol.

4. Conclusion

The present work shows that N-alkylation at R_1 yielded some good enzyme inhibitors with IC_{50} values compared with the standard inhibitors. Addition of other alkylating agents at N-site may give more compounds with lower IC_{50} values. This was found especially in butyl (**e**), pentyl (**f**), benzyl (**g**) wherein more enzyme inhibition was found and in that of dimethylallyl (**m**). Substitution at R_2 site with CH_2–CN was the only active compound with O-ester bond. Substitution at both O- and N-sites had little effect on the inhibitory profile of these compounds. Work is in progress on these lines.

Table 1
Reaction conditions and inhibition studies of N- and O-alkylated sulfonamides

Product	R_1	R_2	Alkyl/Acyl halide (R–X)	Rec. Temp (°C)	Rec. Time (Hrs.)	Yield	Lipoxygenase	
							Inhibition at 0.5 mM (%)	IC_{50} (μmol)
3	H	H	–	25	2	98	99.44 ± 0.95	139.2 ± 0.75
4a	CH_3	CH_3	CH_3–I	25	3	74	88.50 ± 0.87	189.1 ± 0.62
4b	C_2H_5	C_2H_5	C_2H_5–I	25	3	81	97.62 ± 0.93	231.3 ± 0.81
4c	C_2H_4Cl	H	Cl–C_2H_4–Cl	80	5	66	21.88 ± 1.09	>600
4d	C_3H_7	H	C_3H_7–I	25	3	68	40.01 ± 0.99	>500
4e	C_4H_9	H	C_4H_9–I	25	4.5	58	99.42 ± 0.88	61.4 ± 0.69
4f	C_5H_{11}	H	C_5H_{11}–I	25	5	55	98.60 ± 0.94	68.6 ± 0.43
4g	CH_2–C_6H_5	H	Cl–CH_2–C_6H_5	100	6	63	85.13 ± 0.79	59.1 ± 0.87
4h	H	CH_2–CN	Cl–CH_2–CN	80	3	55	97.05 ± 0.84	232.1 ± 0.78
4i	$CH_2CH{=}CH$–C_6H_5	$CH_2CH{=}CH$–C_6H_5	Cl–$CH_2CH{=}CH$–C_6H_5	90	15	65	26.88 ± 0.95	>600
4j	COC_6H_5	H	Cl–COC_6H_5	80	15	42	99.03 ± 1.03	91.7 ± 0.61
4k	COC_6H_4Cl	H	Cl–COC_6H_4Cl	80	18	45	98.08 ± 0.97	176.5 ± 0.48
4l	$CH_2CH{=}CH_2$	H	Br–$CH_2CH{=}CH_2$	60	5	63	96.19 ± 0.81	218.6 ± 0.69
4m	$CH_2CH{=}CH(CH_3)_2$	H	Br–$CH_2CH{=}CH(CH_3)_2$	60	5	57	98.71 ± 0.68	15.8 ± 0.57
Quercetin	–	–		–	–	–		37.12 ± 0.07
Baicalein								22.4 ± 1.3

Enzyme inhibition assays are mean of three independent experiments (mean ± SEM, $n = 3$).

5. Experimental

5.1. General methods

All the chemicals used were purchased from Merck and Fluka or BDH and were used without purification except solvents which were purified through distillation. Reaction progress and product purity was checked by precoated TLC silica gel plates (0.2 mm, 60 HF_{254}, Merck). Spots were visualized under short and long wavelength uv light. Melting points were checked on an electrothermal (Griffin 1090) apparatus and are reported as uncorrected. IR spectra were recorded on a Perkin Elmer 1600-FT spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker (300, 400 and 600 MHz) AMX spectrometer. Elemental analysis was done on Vario Micro CHNS Analyzer.

5.2. Synthesis of 4-(toluene-4-sulfonylamino)-benzoic acid (3)

To a solution of *p*-amino benzoic acid (100 mmol, 13.71 g) and water (50 mL), sodium carbonate (1 N) was added to adjust the pH 8. Then added *p*-toluenesulfonyl chloride (120 mmol, 22.87 g) and the mixture was stirred at room temperature keeping the pH of the mixture up to 8.0 with occasional addition of sodium carbonate solution. Progress and completion of the reaction was confirmed by TLC and conversion of suspension into clear solution. After 2 h, whole mixture was poured into a beaker and the pH was adjusted to 2.0 by 1 N HCl. White precipitates were produced which were filtered and washed with distilled water. Colorless crystals were produced after recrystallization from methanol.

Yield 98%, mp 231 °C; (Lit. mp 230 °C).[27] Anal. Calcd for $C_{14}H_{13}NO_4S$: C, 57.72; H, 4.50; N, 4.81; S, 11.01. Found: C, 57.36; H, 4.52; N, 4.83; S, 10.98. IR (KBr), cm^{-1}: 1117 (SO_2), 1334(SO_2), 1705 (C=O), 3215 (OH) 3585 (NH). ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD): δ (ppm) = 2.24 (s, 3H, CH_3), 7.02 (d, J = 8.4 Hz, 2H, Ar-H), 7.11 (d, J = 8.1 Hz, 2H, Ar-H), 7.58 (d, J = 8.1 Hz, 2H, Ar-H), 7.76 (d, J = 8.4 Hz, 2H, Ar-H), 9.43 (s, 1H, NH).

5.3. General procedure for N- and O-alkylation (4a–4n)

4-{[(4-Methylphenyl)sulfonyl]amino}benzoic acid (10 mmol, 2.91 g), DMF (10 mL) and *n*-hexane washed sodium hydride (30 mmol, 0.72 g) were stirred at room temperature for 40 min followed by the addition of alkylating reagent (alkyl iodide/bromide or acid chloride, 10 mmol). The whole reaction mixture was stirred till the completion of the reaction (Table 1 for reaction details) and poured into crushed ice in a beaker. The pH of the mixture was adjusted to 4.0 with 1 N HCl. White precipitates were produced, filtered and washed twice with distilled water.

5.3.1. 4-[Methyl-(toluene-4-sulfonyl)-amino]-benzoic acid methyl ester (4a)

White crystals; Yield 74%, ratio 1.0, mp 166–167 °C. Anal. Calcd for $C_{16}H_{17}NO_4S$: C, 60.17; H, 5.37; N, 4.39; S, 10.04. Found: C, 60.09; H, 5.41; N, 4.26; S, 10.01. IR (KBr), cm^{-1}: 1155 (SO_2), 1338 (SO_2), 1703 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD): δ (ppm) = 2.39 (s, 3H, CH_3), 3.17 (s, 3H, *CH*$_3$–N), 3.90 (s, 3H, *CH*$_3$–O), 7.10 (d, J = 8.7 Hz, 2H, Ar-H), 7.39 (d, J = 8.1 Hz, 2H, Ar-H), 7.69 (d, J = 8.1 Hz, 2H, Ar-H), 7.96 (d, J = 8.4 Hz, 2H, Ar-H).

5.3.2. 4-[Ethyl-(toluene-4-sulfonyl)-amino]-benzoic acid ethyl ester (4b)

White crystals; Yield 81%, ratio 1.0, mp 126–127 °C; (Lit. mp 126 °C).[28] Anal. Calcd for $C_{18}H_{21}NO_4S$: C, 62.23; H, 6.09; N, 4.03; S, 9.23. Found: C, 61.83; H, 6.01; N, 3.96; S, 9.16.IR (KBr), cm^{-1}: 1091 (SO_2), 1337 (SO_2), 1690 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD): δ (ppm) = 0.96 (t, J = 7.2 Hz, 3H, CH_3), 1.24 (t, J = 6.9 Hz, 3H, CH_3), 2.39 (s, 3H. CH_3), 3.50 (q, J = 7.2 Hz, 2H. *CH*$_2$–N), 4.29 (q, J = 6.9 Hz, 2H, *CH*$_2$–O), 7.07 (d, J = 8.4 Hz, 2H, Ar-H), 7.14 (d, J = 7.8 Hz, 2H, Ar-H), 7.62 (d, J = 7.8 Hz, 2H, Ar-H), 7.83 (d, J = 8.4 Hz, 2H, Ar-H).

5.3.3. 4-[(2-Chloro-ethyl)-(toluene-4-sulfonyl)-amino]-benzoic acid (4c)

White crystalline powder; Yield 66%, ratio 1.0, mp 143–144 °C. Anal. Calcd for $C_{16}H_{16}ClNO_4S$: C, 54.31; H, 4.56; N, 3.96; S, 9.06. Found: C, 54.19; H, 4.36; N, 3.81; S, 9.01. IR (KBr), cm^{-1}: 1157 (SO_2), 1338 (SO_2), 1701 (C=O), 3219 (OH). ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD): δ (ppm) = 2.25 (s, 3H, CH_3), 3.66 (t, J = 7.2 Hz, 2H, *CH*$_2$–Cl), 4.39 (t, J = 7.2 Hz, *CH*$_2$–N), 7.03, (d, J = 8.4 Hz, 2H, Ar-H), 7.12 (d, J = 7.8 Hz, 2H, Ar-H), 7.59 (d, J = 8.1 Hz, 2H, Ar-H), 7.77 (d, J = 8.4 Hz, 2H, Ar-H).

5.3.4. 4-[Propyl-(toluene-4-sulfonyl)-amino]-benzoic acid (4d)

Colorless crystals; Yield 67%, ratio 0.78, mp 149–150 °C (Lit. mp 147 °C).[29] Anal. Calcd for $C_{17}H_{19}NO_4S$: C, 61.24; H, 5.74; N, 4.20; S, 9.62. Found: C, 61.08; H, 5.82; N, 4.31; S, 9.42. IR (KBr), cm^{-1}: 1158 (SO_2), 1338 (SO_2), 1701 (C=O), 3252 (OH). ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD): δ (ppm) = 0.96 (t, J = 7.2 Hz, 3H, CH_3), 1.21–1.31 (m, 2H, *CH*$_2$–CH_3), 2.25 (s, 3H, CH_3), 4.16–4.27 (m, 2H, *CH*$_2$–N), 7.03 (d, J = 8.4 Hz, 2H, Ar-H), 7.12 (d, J = 7.8 Hz, 2H, Ar-H), 7.59 (d, J = 8.1 Hz, 2H, Ar-H), 7.75 (d, J = 8.7 Hz, 2H, Ar-H).

5.3.5. 4-[Butyl-(toluene-4-sulfonyl)-amino]-benzoic acid (4e)

Yellow crystals; Yield 57%, ratio 0.83, mp 127–128 °C. Anal. Calcd for $C_{18}H_{21}NO_4S$: C, 62.23; H, 6.09; N, 4.03; S, 9.23. Found: C, 62.09; H, 6.11; N, 4.00; S, 9.19. IR (KBr), cm^{-1}: 1157 (SO_2), 1339 (SO_2), 1689 (C=O), 3487 (NH). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) = 0.96 (t, J = 7.8 Hz, 3H, CH_3), 1.41–1.49 (m, 2H, CH_2), 1.69–1.76 (m, 2H, CH_2), 2.38 (s, 3H, CH_3), 4.27 (t, J = 7.2 Hz, 2H, *CH*$_2$–N), 7.13 (d, J = 8.7 Hz, 2H, Ar-H), 7.25 (d, J = 8.4, 2H, Ar-H), 7.72 (d, J = 8.4 Hz, 2H, Ar-H), 7.91(d. J = 8.7 Hz, 2H, Ar-H); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) = 13.76, 19.23, 21.58, 30.70, 64.88, 118.95, 126.49, 127.24, 129.85, 131.02, 135.68, 140.82, 144.40, 166.03.

5.3.6. 4-[Pentyl-(toluene-4-sulfonyl)-amino]-benzoic acid (4f)

White crystals; Yield 55%, ratio 0.88, mp 207–208 °C. Anal. Calcd for $C_{19}H_{23}NO_4S$: C, 63.13; H, 6.41; N, 3.88; S, 8.87. Found: C, 62.79; H, 6.11; N, 3.64; S, 8.82. IR (KBr), cm^{-1}: 1157 (SO_2), 1339 (SO_2), 1689 (C=O), 3217 (OH). ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD): δ (ppm) = 0.78 (t, J = 7.8 Hz, 3H, CH_3), 1.16–1.28 (m, 4H), 1.60–1.64 (m, 2H, CH_2), 2.26 (s, 3H, CH_3), 4.14 (t, J = 7.2 Hz, 2H, *CH*$_2$–N), 7.04 (d, J = 7.8 Hz, 2H, Ar-H), 7.33 (d, J = 8.1, 2H, Ar-H), 7.60 (d, J = 8.1 Hz, 2H, Ar-H), 7.76 (d, J = 8.4 Hz, 2H, Ar-H).

5.3.7. 4-[Benzyl-(toluene-4-sulfonyl)-amino]-benzoic acid (4g)

Light yellowish powder; Yield 63%, ratio 0.9, mp 107–108 °C. Anal. Calcd for $C_{21}H_{19}NO_4S$: C, 66.12; H, 5.02; N, 3.67; S, 8.41. Found: C, 65.92; H, 4.62; N, 3.69; S, 8.37. IR (KBr), cm^{-1}: 1158 (SO_2), 1314 (SO_2), 1639 (C=O), 3231 (OH). ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD): δ (ppm) = 2.28 (s, 3H, CH_3), 5.22 (s, 2H, *CH*$_2$–N), 7.01–7.31 (m, 7H, Ar-H), 7.42 (d, J = 7.8 Hz, 2H, Ar-H), 7.62 (d, J = 8.1 Hz, 2H, Ar-H), 7.83 (d, J = 8.1 Hz, 2H, Ar-H).

5.3.8. 4-(Toluene-4-sulfonylamino)-benzoic acid cyanomethyl ester (4h)

Clay color crystals; Yield 55%, ratio 0.9, mp 169–170 °C. Anal. Calcd for $C_{16}H_{14}N_2O_4S$: C, 58.17; H, 4.27; N, 8.48; S, 9.71. Found: C, 58.03; H, 4.14; N, 8.38; S, 9.62. IR (KBr), cm^{-1}: 1156 (SO_2), 1338 (SO_2), 1638 (C=O), 3528 (NH). ^{1}H NMR (600 MHz, $CDCl_3$: DMSO): δ (ppm) = 2.37 (s, 3H, CH_3), 4.93 (s, 2H, *CH*$_2$–CN), 7.25 (d, J = 8.4 Hz, 2H, Ar-H),7.25 (d, J = 8.7 Hz, 2H, Ar-H), 7.74 (d,

J = 8.4 Hz, 2H, Ar-H), 7.86 (d, J = 8.7 Hz, 2H, Ar-H), 10.44 (s, 1H, NH); ^{13}C NMR (150 MHz, $CDCl_3$: DMSO): δ (ppm) = 21.10, 48.28, 114.33, 117.71, 121.81, 126.61, 129.26, 130.90, 136.04, 143.23, 143.39, 164.01.

5.3.9. 4-[(3-Phenyl-allyl)-(toluene-4-sulfonyl)-amino]-benzoic acid 3-phenyl-allyl ester (4i)

Colorless crystals; yield 65%. ratio 0.82, mp 112–113 °C. Anal. Calcd for $C_{32}H_{29}NO_4S$: C, 73.40; H, 5.58; N, 2.67; S, 6.12. Found: C, 73.29; H, 5.51; N, 2.64; S, 6.02. IR (KBr), cm^{-1}: 1157 (SO_2), 1343 (SO_2), 1685 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) = 2.43 (s, 3H, CH_3), 4.37 (dd, J = 6.6, 1.2 Hz, 2H, CH_2–N), 4.96 (dd, J = 6.6, 1.2 Hz, 2H, CH_2–O), 6.05 (t, J = 6.6 Hz, 1H, CH), 6.37 (t, J = 6.00 Hz, 1H, CH), 6.39 (t, J = 6.6 Hz, 1H, CH), 6.73 (s, 1H, CH) 7.18–7.22 (m, 5H, Ar-H), 7.24–7.28 (m, 5H, Ar-H), 7.33 (t, J = 7.8 Hz, 2H, Ar-H), 7.42 (d, J = 7.8 Hz, 2H, Ar-H), 7.49 (d, J = 8.4 Hz, 2H, Ar-H), 8.00 (d, J = 8.4 Hz, 2H, Ar-H); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) = 21.59, 52.75, 65.74, 122.94, 123.38, 126.42, 126.63, 127.63, 127.96, 128.10, 128.16, 128.52, 128.62, 129.07, 129.61, 130.36, 134.23, 134.51, 134.91, 135.98, 136.06, 143.46, 143.87, 165.65.

5.3.10. 4-[Benzoyl-(toluene-4-sulfonyl)-amino]-benzoic acid (4j)

White powder; Yield 42%, ratio 1.0, 218 °C. Anal. Calcd for $C_{21}H_{17}NO_5S$: C, 63.79; H, 4.33; N, 3.54; S, 8.11. Found: C, 62.57; H, 4.37; N, 3.49; S, 7.03. IR (KBr), cm^{-1}: 1158 (SO_2), 1285 (SO_2), 1687 (C=O), 3216 (OH). ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD): δ (ppm) = 2.28 (s, 3H, CH_3), 7.04–7.08 (m, 3H, Ar-H), 7.15 (d, J = 8.1 Hz, 2H, Ar-H), 7.34–7.45 (m, 2H, Ar-H), 7.62 (d, J = 8.1 Hz, 2H, Ar-H), 7.75 (d, J = 7.8 Hz, 2H, Ar-H), 7.81 (d, J = 8.7 Hz, 2H, Ar-H).

5.3.11. 4-[(4-Chloro-benzoyl)-(toluene-4-sulfonyl)-amino]-benzoic acid (4k)

Colorless crystals; Yield 45%, ratio 1.0, mp 198–200 °C. Anal. Calcd for $C_{21}H_{16}ClNO_5S$: C, 58.67; H, 3.75; N, 3.26; S, 7.46. Found: C, 58.61; H, 3.77; N, 3.19; S, 7.45 IR (KBr), cm^{-1}: 1160 (SO_2), 1334 (SO_2), 1675 (C=O), 3224 (OH). ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD): δ (ppm) = 2.25 (s, 3H, CH_3), 7.03 (d, J = 8.7 Hz, 2H, Ar-H), 7.12 (d, J = 8.1 Hz, 2H, Ar-H), 7.30 (d, J = 8.4 Hz, 2H, Ar-H), 7.59 (d, J = 8.1 Hz, 2H, Ar-H), 7.77 (d, J = 8.7 Hz, 2H, Ar-H), 7.86 (d, J = 8.4 Hz, 2H, Ar-H).

5.3.12. 4-[Allyl-(toluene-4-sulfonyl)-amino]-benzoic acid (4l)

Yellowish brown thick paste; Yield 63%, ratio 0.93, mp 40 °C. Anal. Calcd for $C_{17}H_{17}NO_4S$: C, 61.61; H, 5.17; N, 4.23; S, 9.68. Found: C, 61.43; H, 4.98; N, 4.19; S, 9.59. IR (KBr), cm^{-1}: 1158 (SO_2), 1341 (SO_2), 1695 (C=O), 3239 (OH). ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD): δ (ppm) = 2.28 (s, 3H, CH_3), 4.06 (d, J = 6.0 Hz, 2H, CH_2–N), 4.61 (d, J = 6.0 Hz, 1H), 4.66 (d, J = 6.0 Hz, 1H), 5.10–5.29 (m, 1H, CH), 7.09 (d, J = 8.4 Hz, 2H, Ar-H), 7.32 (d, J = 8.1 Hz, 2H, Ar-H), 7.56 (d, J = 8.1 Hz, 2H, Ar-H), 7.74 (d, J = 8.4 Hz, 2H, Ar-H).

5.3.13. 4-[(3-Methyl-but-2-enyl)-(toluene-4-sulfonyl)-amino]-benzoic acid (4m)

Brown powder; Yield 57%, ratio 0.97, mp 172 °C. Anal. Calcd for $C_{19}H_{21}NO_4S$: C, 63.49; H, 5.89; N, 3.90; S, 8.92. Found: C, 63.37; H, 5.97; N, 3.82; S, 8.91. IR (KBr), cm^{-1}: 1158 (SO_2), 1383 (SO_2), 1690 (C=O), 3234 (OH). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) = 1.76 (s, 6H, CH_3), 2.38 (s, 3H, CH_3), 4.77 (d, J = 7.2 Hz, 2H), 5.42 (t, J = 7.2 Hz, 1H), 7.11(d, J = 8.4 Hz, 2H, Ar-H), 7.24 (d, J = 8.7 Hz, 2H, Ar-H), 7.71 (d, J = 8.4 Hz, 2H, Ar-H), 7.92 (d, J = 8.7 Hz, 2H, Ar-H); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) = 18.11, 21.57, 25.82, 61.91, 118.46, 119.00, 126.53, 127.24, 129.83, 131.08, 135.63, 139.35, 140.75, 144.41, 165.96.

Acknowledgment

Authors acknowledge Higher Education Commission of Pakistan for financial support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2012.02.055. These data include MOL files and InChiKeys of the most important compounds described in this article.

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