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Synthesis, pharmacological evaluation and docking studies of *N*-(benzo[*d*]thiazol-2-yl)-2-(piperazin-1-yl)acetamide analogs as COX-2 inhibitors

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ABSTRACT

The existing NSAIDs having number of toxicities emphasises the need for discovery of new non-toxic anti-inflammatory agents. In this Letter, we present the simple two step chemical synthesis, in vivo pharmacological screening and docking study of few *N*-(benzo[*d*]thiazol-2-yl)-2-(piperazin-1-yl)acetamide analogs. Different amino benzothiazoles were chloroacetylated and further reacted with substituted piperazines in presence of a base to get *N*-(benzo[*d*]thiazol-2-yl)-2-(piperazin-1-yl)acetamide analogs (**A1–C4**). These compounds were evaluated for anti-inflammatory activity by carragenan induced paw oedema method. Promising compounds were screened for toxicity by evaluating the ulcerogenic potential. Molecular docking experiments were carried out against COX-2 enzyme using Surflex-Dock GeomX programme of Sybyl software on Dell T-1500 workstation to confirm the mechanism of action of active compounds among the series. In silico study reveal the binding interactions of *N*-(benzo[*d*]thiazol-2-yl)-2-(piperazin-1-yl)acetamide analogs with COX-2 protein and is in agreement with the in vivo anti-inflammatory activity.

Non-steroidal anti-inflammatory drugs (NSAIDs) are one amongst the most frequently prescribed classes of drugs. Both their benefits and side effects arise due to inhibition of cycloxygenase (COX) of which there are two isoenzymes, COX-1 and COX-2. Both COX isoenzymes have a hydrophobic tunnel, through which the substrate accesses the active site. The tunnel is larger in the COX-2 isoenzyme with a side pocket, a property exploited in the development of specific COX-2 inhibitors.[1] The premise of the initial, COX-2 hypothesis was that the gastrointestinal side effects arise due to inhibition of COX-1, whereas their anti-inflammatory or analgesic properties were COX-2 mediated.[2,3] For the past 6–7 years, reports mentioning risk of cardiovascular events with selective COX-2 inhibitors are increasing. In an early study of major gastrointestinal events, an unexpected fivefold increase in the risk of acute myocardial infarction (AMI) with rofecoxib was observed when compared with naproxen.[4] At the time, many suggested and aggressively pursued the hypothesis that the increased frequency of events was a spurious observation not due to any prothrombotic effects of rofecoxib, but the cardioprotective properties of naproxen. However, subsequent placebo-controlled studies of both rofecoxib, and celecoxib in chemoprevention also reported an approximate twofold increase in cardiovascular events with both drugs.[5,6] Thus, there remains a compelling need for effective NSAIDs with an improved safety profile.

Substitutions at 2-position of benzothiazole have emerged in its usage as a core structure in the diversified therapeutic applications.[7–13] The studies of structure–activity relationship interestingly reveal that change of the structure of substituent group at C-2 position commonly results in the change of its bioactivity. Though literature survey reports many therapeutic applications of 2-substituted benzothiazoles, their investigation for anti-inflammatory activity is limited.[8,14–17] Piperazines attached to benzimidazole and indole were found to have potent anti-inflammatory activity.[18] Prompted by recent literature developments and as a part of our continuous search for biologically active compounds, we worked on joining the two active moieties with an intention of getting better biological activity.[19,20] With this concept, we are reporting the pharmacological activity of *N*-(benzo[*d*]thiazol-2-yl)-2-(piperazin-1-yl)acetamide analogs for their anti-inflammatory activity by carragenan induced paw oedema method. Selected compounds were also evaluated for ulcerogenicity index. Molecular modelling studies were performed in order to probe whether COX-2 was a possible target for the synthesised compounds (**A1–C4**).

As depicted in Scheme 1, commercially available substituted amino benzthiazoles are treated with chloroacetylchloride to form

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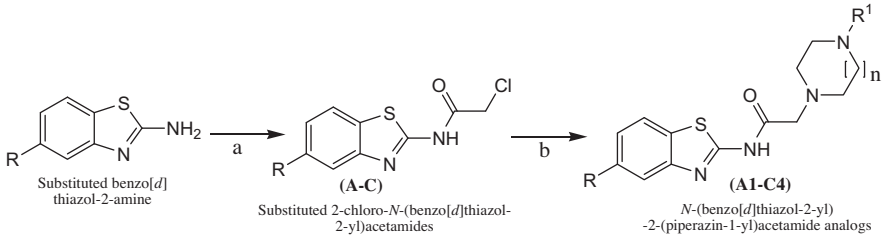

A: R = H
B: R = OCH_3
C: R = NO_2
A1: R = H, R^1 = H, n = 1
A2: R = H, R^1 = C_2H_5, n = 1
A3: R = H, R^1 = H, n=2
A4: R = H, R^1 = C_6H_5, n = 1
B1: R = OCH_3, R^1 = H, n = 1
B2: R = OCH_3, R^1 = C_2H_5, n = 1
B3: R = OCH_3, R^1 = H, n = 2
B4: R = OCH_3, R^1 = C_6H_5, n = 1
C1: R = NO_2, R^1 = H, n = 1
C2: R = NO_2, R^1 = C_2H_5, n = 1
C3: R = NO_2, R^1 = H, n = 2
C4: R = NO_2, R^1 = C_6H_5, n = 1

Scheme 1. Reagents and conditions: (a) $ClCOCH_2Cl$, triethylamine, dioxane (15 °C for 20 min followed by 110 °C for 4 h); (b) substituted piperazine, triethylamine, dioxane 100 °C for 2–6 h.

corresponding substituted 2-chloro-*N*-(benzo[*d*]thiazol-2-yl)acetamides (**A–C**).[21,22] The ^1H NMR signals at 3.5–4.4 due to CH_2 of chloro acetamide group, δ 7–8.5 of benzothiazole; absence of primary amino group IR signals at 3200–3300 cm^{-1}, presence of C–Cl stretching at 720 cm^{-1} and molecular weight determination from Mass spectra confirms the structures of substituted 2-chloro *N*-(benzo[*d*]thiazol-2-yl)acetamides (**A–C**). In the second step, substituted 2-chloro *N*-(benzo[*d*]thiazol-2-yl)acetamides (**A–C**) were reacted with substituted piperazines to yield *N*-(benzo[*d*]thiazol-2-yl)-2-(piperazin-1-yl)acetamide analogs (**A1–C4**).[23] Chemical synthesis and characterisation of **A4** is already reported in the literature.[17] The structures of the final compounds (**A1–C4**) were substantiated on the basis of IR, NMR, and Mass spectral data.[24]

The anti-inflammatory activity of title compounds were evaluated on Wistar rats at (30 mg/kg) by Winter's method[25] and data is given in the Table 1. Compounds **A1** and **C4** were the most active compounds in the series compared to naproxen. Compounds **A2** and **B4** showed moderate activity. The compound **A1** having *N*-benzo[*d*]thiazol-2-yl moiety attached to 2-(piperazin-1-yl) acetamide (% DPV = 41.30) and the compound **C4** having *N*-(5-nitrobenzo[*d*]thiazol-2-yl) moiety attached to 2-(4-phenylpiperazin-1-yl)acetamide (% DPV = 34.78) were found to be the optimum structural features amongst the series to show promising in vivo

Table 1
Percent inhibition of paw volumes of *N*-(benzo[*d*]thiazol-2-yl)-2-(pipierazinyl-1-yl)acetamides analogs (**A1**–**C4**)

Compd	Anti-inflammatory activity[a]	
	Paw volume mean ± SEM[c]	% DPV[d]
Control	0.76 ± 0.049	0
A1	0.58 ± 0.051	41.30*
A2	0.70 ± 0.025	13.04
A3	0.72 ± 0.049	8.60
A4	0.65 ± 0.025	23.91
B1	0.75 ± 0.022	2.17
B2	0.68 ± 0.030	17.39
B3	0.63 ± 0.030	17.39
B4	0.66 ± 0.033	21.70
C1	0.70 ± 0.033	12.30
C2	0.68 ± 0.030	17.39
C3	0.68 ± 0.030	17.39
C4	0.60 ± 0.025	34.78*
Naproxen	0.43 ± 0.021	71.70***
Rofecoxib	0.15 ± 0.031	81.01***

[a] Percent inhibition of paw volumes of compounds at 2 h.
[c] SEM = Standard error of mean.
[d] DPV = Decrease in paw volume.
* P <0.05.
*** P <0.0001.

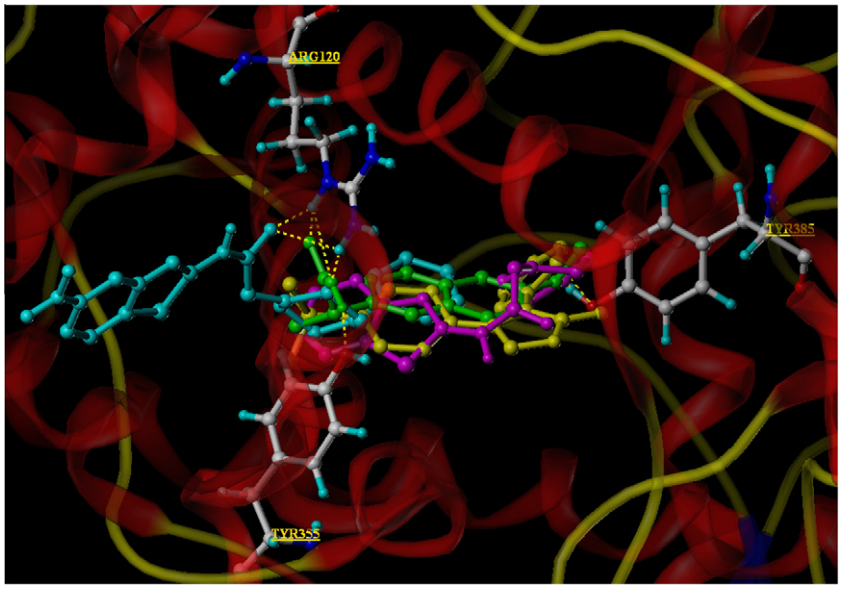

Figure 1. Overlap of ball and stick form of **A1** (magenta colour), **C4** (cyano colour), naproxen (green colour) and rofecoxib (yellow colour) showing hydrogen bonding interaction with amino acids in the active site of COX-2. PDB code 3NT1.

anti-inflammatory activity. Sodium carboxy methyl cellulose (control) alone did not modify the responses to nociceptive stimuli in the rat pedal oedema induced by fresh carrageenan administration.

The compounds **A1** and **C4** endowed with significant anti-inflammatory activity were evaluated for acute gastric ulcerogenic effect in Wistar rats.[26] Six hours after the treatment with a dose of 90 mg/kg, they were sacrificed under deep ether anaesthesia and their stomachs were removed and opened through great curvature for examining lesions or bleedings. Compounds **A1** (Ulcer Index (UI) = 1.24) and **C4** (UI = 1.21) were found to possess less ulcer inducible capacity than naproxen (UI = 2.5).

To understand the mechanism of anti-inflammatory activity of the compounds synthesised, molecular modelling and docking studies were performed on X-ray crystal structure of COX-2 protein (PDB code: 3NT1; resolution 1.73 Å)[27] using Surflex-Dock GeomX (SFXC) programme of Sybyl software on Dell Precision T-1500 workstation.[28] Synthesised compounds (**A1–C4)** occupy the same binding site as that of naproxen and rofecoxib (Fig. 1); naproxen was found to have three hydrogen bonding interactions with Arg120 (2.16, 2.52 and 1.92 Å) and one with Tyr355 (1.86 Å). Compound **A1** forms two hydrogen bonds one with NH of its piperazine moiety and OH of Tyr385 (2.62 Å) and the other with NH of its piperazine moiety and OH of Tyr385 (2.54 Å). Compound **C4** forms hydrogen bonding interaction with its carbonyl oxygen of amide bond and Arg120 (1.60, 2.64 Å). Compound **A1**, **C4**, naproxen and rofecoxib were having hydrophobic interactions with the amino acid residues such as Met522, Val523, Leu352, Leu525, Phe529, Ala527, Val116, Leu531, Leu359, and Val349 (Fig. 2). The rest of the compounds except **A2**, **B2**, **B3**, and **C2** were having inappropriate penetration (crash score >−4.5 kcal/mol) into the binding site resulting in the decreased forces of interaction with the amino acids of the COX-2 protein. However, compounds **A2**, **B2**, **B3**, and **C2** were having low C-score, which can be attributed to their low in vivo anti-inflammatory activity.

Compounds **A1** and **C4** were having better hydrogen bonding, complex (ligand–protein), and internal (ligand–ligand) energies than naproxen, rofecoxib and other compounds of the series (Table 2). Helmholtz free energies of interactions for protein–ligand atom pairs prefers **C4** over the rofecoxib and naproxen followed by **A1**. Charge and van der Waals interactions between the protein and the ligand suggests **C4** and **A1** are a superior ligand than naproxen to bind with COX-2. Scoring of compounds with respect to the reward for hydrogen bonding, lipophilic contact, and rotational entropy, along with an intercept terms reveal that compound **C4** has more interactions with the protein than rofecoxib, naproxen and **A1**. The C-score indicating the summary of all the forces of interaction between the ligands and COX-2 enzyme, including the crash score is in favour of naproxen, followed by **A1**, **C4**, rofecoxib and the rest of the compounds amongst the series.

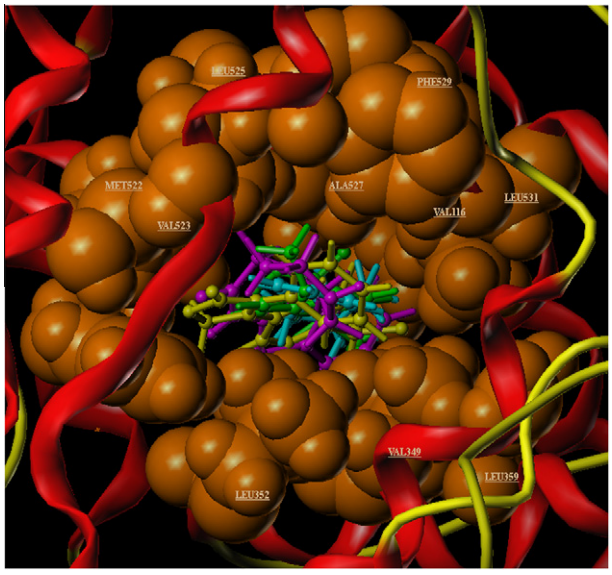

Figure 2. Hydrophobic amino acids (brown coloured space fill) enclosing the ball and stick form of **A1** (magenta colour), **C4** (cyano colour), naproxen (green colour) and rofecoxib (yellow colour) in the active site of COX-2. PDB code 3NT1.

In conclusion, in vivo studies demonstrated that *N*-(benzo[*d*]thiazol-2-yl)-2-(piperazin-1-yl)acetamide analogs (**A1** and **C4**), have promising anti-inflammatory activity and less toxicity than naproxen. Molecular modelling and docking studies suggested that compound **A1** and **C4** interacts with COX-2 enzyme more efficiently than rofecoxib. Therefore, these two compounds can be further developed to improve their anti-inflammatory activity.

Table 2
Surflex-Dock GeomX score (kcal/mol) of compounds

Compd	C-Score[a]	Crash Score[b]	Polar Score[c]	G score[d]	PMF score[e]	D score[f]	Chem score[g]
Naproxen	9.16	−0.39	2.98	−182	−47.9	−102	−29.8
A1	6.48	−3.12	0.00	−281	−30.5	−130	−25.8
C4	6.23	−3.73	1.06	−289	−53.9	−145	−37.0
Rofecoxib	6.13	−2.91	0.00	−268	−48.8	−140	−35.3
A2	5.95	−2.94	1.01	−267	−11.3	−128	−25.6
B4	5.78	−4.53	0.00	−309	−35.3	−141	−27.6
C2	5.36	−2.70	0.52	−285	−39.2	−140	−32.6
A3	5.15	−5.72	0.58	−341	−26.1	−152	−28.4
C1	5.14	−5.18	0.00	−304	−19.9	−145	−27.2
B1	4.52	−5.62	0.42	−289	−23.0	−138	−27.8
B2	4.35	−3.36	0.22	−276	−22.9	−127	−24.7
A4	4.25	−6.52	0.00	−337	−24.5	−157	−39.4
C3	4.02	−5.20	1.06	−283	−34.7	−140	−23.8
B3	3.39	−3.56	0.91	−254	−29.6	−123	−23.3

[a] C-Score (Consensus-score) reports the output of total score.
[b] Crash-score revealing the inappropriate penetration into the binding site.
[c] Polar region of the ligand.
[d] G-score showing hydrogen bonding, complex (ligand–protein), and internal (ligand–ligand) energies.
[e] PMF-score indicating the Helmholtz free energies of interactions for protein–ligand atom pairs (Potential of Mean Force, PMF).
[f] D-score for charge and van der Waals interactions between the protein and the ligand.
[g] Chem-score points for hydrogen bonding, lipophilic contact, and rotational entropy, along with an intercept term.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.12.062.

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23. Synthesis of *N*-(benzo[*d*]thiazol-2-yl)-2-(piperazin-1-yl)acetamide analogs (**A1**–**C4**): A mixture of equimolar quantities of substituted 2-chloro-*N*-(benzo[*d*]thiazol-2-yl)acetamides (**A**–**C**), various piperazine derivatives, dioxane (10 ml) and triethylamine (0.2 ml) were heated at 100 °C with stirring for 2–6 h. The reaction was monitored by TLC using mobile phase petroleum ether/ethyl acetate (2:8). The excess of ethanol was removed by distillation. To the residue add warm distilled water. The solid thus obtained is filtered, washed with 5% $NaHCO_3$ to remove excess acidic impurities, filtered, washed and dried. The product obtained was recrystallized and further purified by flash chromatograph.
24. *Spectral data of N*-(benzo[*d*]thiazol-2-yl)-2-(piperazin-1-yl)acetamide analogs (**A1**–**C4**): *N*-(benzo[*d*]thiazol-2-yl)-2-(piperazin-1-yl)acetamide (**A1**): Yield 22%, mp 196 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.9 (br r, 1H), 1.2 (br r, 8H), 3.36 (s, 2H), 7.35 (d, 2H, J = 7.50 Hz), 7.67 (d, 2H, J = 7.50 Hz), 7.9 (br r, 1H); IR (KBr, ν cm^{-1}): 3282, 3060, 2872, 2848, 1690, 1600, 1533, 842; MS (API-ES) m/z for $C_{13}H_{16}N_4OS$ (M+H)$^+$ 277. *N*-(benzo[*d*]thiazol-2-yl)-2-(4-ethylpiperazin-1-yl)acetamide (**A2**): Yield 28%, mp 132 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.12 (t, 3H, J = 7.0 Hz), 2.3 (q, 2H, J = 6.8 Hz), 2.7 (br r, 8H), 3.33 (s, 2H), 5.5 (br r, 1H), 7.0 (d, 1H, J = 7.4 Hz), 7.15 (t, 1H, J = 7.5 Hz), 7.58 (d, 2H, J = 7.3 Hz); IR (KBr, ν cm^{-1}): 3270, 3030, 2901, 2878, 1685, 1600, 1567, 821; MS (API-ES) m/z for $C_{15}H_{20}N_4OS$ (M+H)$^+$ 305. *N*-(benzo[*d*]thiazol-2-yl)-2-(1,4-diazepan-1-yl)acetamide (**A3**): Yield 30%, mp 227 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.25 (s, 1H), 1.96–2.10 (m, 2H, J = 7.3 Hz), 2.9–3.0 (m, 8H, J = 7.3 Hz), 3.45 (s, 2H), 7.3–7.8 (m, 4H, J = 7.4 Hz), 10.60 (br r, 1H); IR (KBr, ν cm^{-1}): 3323, 3049, 2883, 2823, 1697, 1600, 1529, 827; MS (API-ES) m/z for $C_{14}H_{18}N_4OS$ (M+H)$^+$ 291. *N*-(5-methoxybenzo[*d*]thiazol-2-yl)-2-(piperazin-1-yl)acetamide (**B1**): Yield 26%, mp 119 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.3 (s, 1H), 3.82 (s, 8H), 3.88 (s, 3H), 4.29 (s, 1H), 7.11 (d, 1H, J = 7.4 Hz), 7.45 (d, 1H, J = 7.6 Hz), 7.69 (d, 1H, J = 7.5 Hz); IR (KBr, ν cm^{-1}): 3280, 3095, 2920, 2845, 1690, 1603, 1571, 823; MS (API-ES) m/z for $C_{14}H_{18}N_4O_2S$ (M+H)$^+$ 307. 2-(4-ethylpiperazin-1-yl)-*N*-(5-methoxybenzo[*d*]thiazol-2-yl)acetamide (**B2**): Yield 23%, mp 212 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.2 (s, 3H), 2.0 (br r, 8H), 3.9 (s, 2H), 6.90 (d, 1H, J = 7.1 Hz), 7.1 (d, 1H, J = 7.5 Hz), 7.45 (d, 1H, J = 7.4 Hz), 7.5 (s, 1H); IR (KBr, ν cm^{-1}): 3287, 3095, 2971, 2922, 2848, 1641, 1545, 1466, 821; MS (API-ES) m/z for $C_{16}H_{22}N_4O_2S$ (M+H)$^+$ 335. 2-(1,4-diazepan-1-yl)-*N*-(5-methoxybenzo[*d*]thiazol-2-yl)acetamide (**B3**): Yield 33%; mp 222 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.8 (br r, 2H), 2.00 (q, 4H, J = 6.5 Hz), 2.9-3.04 (m, 4H), 3.45 (t, 2H), 3.89 (s, 3H), 7.01 (d, 1H, J = 7.3 Hz), 7.28 (d, 1H, J = 7.5 Hz), 7.68 (s, 1H); IR (KBr, ν cm^{-1}): 3280, 3095, 2920, 2896, 2848, 1690, 1537, 1470, 820; MS (API-ES) m/z for $C_{15}H_{20}N_4O_2S$ (M+H)$^+$ 321. *N*-(5-methoxybenzo[*d*]thiazol-2-yl)-2-(4-phenylpiperazin-1-yl)acetamide (**B4**): Yield 36%, mp 199 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.70 (s, 4H), 3.19 (s, 4H), 3.43 (s, 2H), 3.79 (s, 3H), 6.78 (t, 1H, J = 7.8 Hz), 6.96 (d, 2H, J = 7.3 Hz), 7.20 (t, 2H, J = 7.3 Hz), 7.58 (s, 1H), 7.62–7.69 (m, 2H, J = 7.5 Hz), 11.9 (s, 1H); IR (KBr, ν cm^{-1}): 3261, 3088, 2987, 2931, 2824, 1699, 1540, 1501, 813; MS (API-ES) m/z for $C_{20}H_{22}N_4O_2S$ (M+Na)$^+$ 405. *N*-(5-nitrobenzo[*d*]thiazol-2-yl)-2-(piperazin-1-yl)acetamide (**C1**): Yield 45%, mp 268 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.3 (s, 1H), 2.62–2.67 (m, 4H, J = 8.5 Hz), 2.50 (s, 4H), 3.40 (s, 2H), 7.90 (d, 1H, J = 7.3 Hz), 8.30 (d, 1H, J = 7.8 Hz), 9.05 (s, 1); IR (KBr, ν cm^{-1}): 3343, 3088, 2937, 2821, 1701, 1573, 1514, 1442, 848; MS (API-ES) m/z for $C_{13}H_{15}N_5O_3S$ (M+H)$^+$ 322. 2-(4-ethylpiperazin-1-yl)-*N*-(5-nitrobenzo[*d*]thiazol-2-yl)acetamide (**C2**): Yield 25%, mp 179 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.40 (t, 3H, J = 7.3 Hz), 3.10 (br r, 4H), 3.25 (q, 2H, J = 7.0 Hz), 3.40 (br r, 4H), 3.62 (s, 2H), 7.78 (d, 1H, J = 7.3 Hz), 8.25 (d, 1H, J = 7.3 Hz), 8.75 (s, 1); IR (KBr, ν cm^{-1}): 3402, 3170, 2876, 2831, 1705, 1573, 1506, 1442, 819; MS (API-ES) m/z for $C_{15}H_{19}N_5O_3S$ (M+H)$^+$ 350. 2-(1,4-diazepan-1-yl)-*N*-(5-nitrobenzo[*d*]thiazol-2-yl)acetamide (**C3**): Yield 38%, mp 229 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.1–1.3 (m, 2H, J = 7.9 Hz), 2.0 (br r, 1H), 2.93–3.0 (m, 4H, J = 7.8 Hz), 3.6 (s, 4H), 3.85 (s, 2H), 7.69 (d, 2H, J = 7.5 Hz), 8.15 (d, 1H, J = 7.5 Hz), 8.8 (s, 1H); IR (KBr, ν cm^{-1}): 3394, 3170, 2918, 2878, 1708, 1573, 1500, 1448, 827; MS (API-ES) m/z for $C_{14}H_{17}N_5O_3S$ (M+H)$^+$ 334. *N*-(5-nitrobenzo[*d*]thiazol-2-yl)-2-(4-phenylpiperazin-1-yl)acetamide (**C4**): Yield 72%, mp 230 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.80 (br r, 4H), 3.12 (s, 2H), 3.20 (br r, 4H), 6.9–7.1 (m, 3H, J = 7.0 Hz), 7.3–7.4 (m, 2H, J = 7.5 Hz), 8.1 (d, 2H, J = 7.3 Hz), 8.5 (s, 1H); IR (KBr, ν cm^{-1}): 3311, 3040, 2916, 2864, 1651, 1570, 1516, 1442, 852; MS (API-ES) m/z for $C_{19}H_{19}N_5O_3S$ (M+H)$^+$ 398.
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28. Molecular modelling and docking simulations: The X-ray crystal structure of COX-2-naproxen complex (PDB code: 3NT1; resolution 1.73 Å) was optimized by deleting the identical B chain of the protein and retaining respective A chain for docking studies. Mislabelled atom types from the pdb file were corrected, subsequently, proline F angles were fixed at 70°, side chain amides were checked to maximize potential hydrogen bonding, side chains were checked for close van der Waals contacts, and essential hydrogens were added. The model was checked for conformational problems using the module ProTable from Sybyl. Ramachandran plot[29] of the backbone torsion angles PHI and PSI, local geometry and the location of buried polar residues/exposed non-polar residues were examined. The protein and synthesised compounds, including naproxen and rofecoxib were subjected to energy minimization following the gradient termination of the Powell method for 3000 iterations using Kollman united force field with non-bonding cut-off set at 9.0 and the dielectric constant set at 4.0.[30,31] The synthesised compounds and the standard compounds tested in this study were docked to COX-2 (PDB code: 3NT1) using Surflex-Dock GeomX programme in Sybyl software by incremental construction approach of building the structure in the active site so as to favour the binding affinity.[32,33] Finally, the docked ligands were ranked based on a variety of scoring functions that have been compiled into the single consensus score (C-score).[34,35]
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