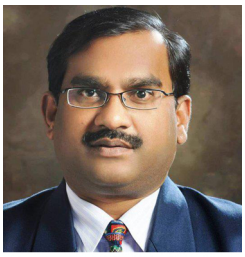
Send Orders for Reprints to reprints@benthamscience.ae

90 *Medicinal Chemistry*, **2016**, *12*, **90-100**

Molecular Modeling, Synthesis and Pharmacological Evaluation of 1,3,4-Thiadiazoles as Anti-inflammatory and Analgesic Agents

Anas M. H. Shkair[1], Ashok K. Shakya[1,*], Nulgumnalli M. Raghavendra[2] and Rajashri R. Naik[1]

[1]*Faculty of Pharmacy and Medical Sciences, Al-Ahliyya Amman University, PO Box 263, Amman 19328, Jordan;* [2]*Department of Pharmaceutical Chemistry, Gokaraju Rangaraju College of Pharmacy, Osmania University, Hyderabad 500 090, Telangana, India*



Ashok K. Shakya

Abstract: A series of novel substituted 2-amino-5-(1-(4-isobutylphenyl)ethyl)-1,3,4-thiadiazoles were designed, synthesized and evaluated as anti-inflammatory and analgesic agents. Compounds were characterized by elemental and spectroscopic analysis. Compounds possessing significant activities were screened for ulcerogenic activity. Compound-**5** *(2-(4-isobutylphenyl)-N-(5-(1-(4-isobutylphenyl)ethyl)-1,3,4-thiadiazol-2-yl)-propanamide)* produces significant *in vitro* anti-inflammatory activity (72.5%) as compared to ibuprofen (47.7%), while compound-**3f** *(2-(N-cyclohexyl-N-methylamino)-N-(5-(1-(4-isobutylphenyl)-ethyl)-1,3,4-thiadiazol-2-yl)-acetamide)* showed 64.1 % activity. Results indicate that compound-**4** *(N-(5-(1-(4-isobutyl-phenyl)-ethyl)-1,3,4-thiadiazol-2-yl)-acetamide)* exhibited highest analgesic activity (69.8%), where as compound-**5** possessed 65.5% activity. Structure based drug design was also investigated to reveal the mechanism of action and specificity of our compounds against COX-2 enzyme. Anti-inflammatory activity and ulcerogenic potential were in agreement with the molecular modeling studies carried out on cycloxygenase enzyme.

Keywords: 1,3,4-thiadiazole, molecular modeling, docking studies, anti-inflammatory activity, analgesic activity, CADD, ulcerogenic potential.

1. INTRODUCTION

Non-steroidal anti-inflammatory drugs are among the most widely used of all therapeutic agents. These drugs are often taken without prescription for minor aches, inflammation and pains. Inflammation is a normal and essential response to any noxious stimulus. The most widely used non-steroidal anti-inflammatory drugs (like aspirin, naproxen, ibuprofen, mefenamic acid, flurbiprofen, and diclofenac sodium) inhibit the cyclooxgenase (COX) enzyme and control the release of inflammatory mediators responsible for inflammation. Generally, all currently available anti-inflammatory agents have more or less unwanted effects in different patients [1]. Currently available non-steroidal anti-inflammatory drugs (NSAIDs) suffer from common drawbacks of gastric bleeding, ulceration, nephrotoxicity due to presence of free carboxylic acid group, poor ionization and inhibition of protective cyclooxygenase-1 (COX-1) enzyme. Inhibition of cycloxygenase (COX) produces different types of biological effects, as there are different isoenzymes COX-1 and COX-2. Both types of cycloxygenases have a hydrophobic tunnel or cavity, 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 [2]. Studies suggest that the ulcerogenic and side effects of the NSAIDs can be reduced by derivatization of the carboxylate function. Different synthetic approaches and chemical modification of NSAID have been studied to improve the safety profile [3-6]. From the exhaustive literature survey, it was observed that a large number of thiadiazoles [7-19] have been reported to have different pharmacological activities. The marketed drugs having 1,3,4-thiadiazole ring system are acetazolamide, methazolamide (diuretic) and megazol (trypanocidal agent) [18]. The compounds which are bearing thiadiazole ring possess anti-inflammatory and analgesic activity with reduced ulcerogenic index and side effects. Therefore, it was thought to design and synthesize some novel hybrid compounds of ibuprofen, in which the COOH group is replaced with 2-acetamido/amido-1,3,4-thiadiazole, which can interact with the possible binding site (Tyr-355, Arg-120) of the COX-2 enzyme. With this thought, in this paper, we are reporting the synthesis and evaluation of N-(5-(1-(4-isobutyl-phenyl)-ethyl)-1,3,4-thiadiazol-2-yl)-acetamide analogs as possible anti-inflammatory and analgesic agents. Ulcerogenicity index of a few selected compounds was also evaluated. Molecular modeling studies were carried out in order to prove whether COX-2 was a possible target for the synthesized compounds or not. The molecular docking approach is one of the most rational and authentic approaches in the drug design and discovery for studying the molecular interaction of small molecules. Additionally, we have also calculated the

*Address correspondence to this author at the Faculty of Pharmacy and Medical Sciences, Al-Ahliyya Amman University, PO Box 263, Amman 19328, Jordan; Tel: +962-79-5065060; Fax: +962-6-5335169; E-mails: ashokkumar2811@gmail.com; shkair_anas@hotmail.com

1875-6638/16 $58.00+.00

binding affinities of these compounds as compared to reference.

2. MATERIAL AND METHODS

2.1. Chemistry

Ibuprofen (99.0%) was received as a gift sample from JoRiver Pharmaceutical Co., Amman, Jordan. Starting material, reagents and solvents were purchased from Sigma Aldrich Chemical Co., USA. Nuclear magnetic resonance spectra (NMR) were recorded on Bruker, Avance DPX-300 spectrometer (300 MHz) using $CDCl_3$ or DMSO-d_6. Tetramethylsilane (TMS) was used as the internal standard. Chemical shifts are reported in ppm. Infra-red (IR) spectra were recorded in potassium bromide (Merck, Dermstadt, Germany) discs, using Thermo Nicolet FT-IR spectrophotometer (Thermo Fischer Scientific Inc., USA). Stuart Scientific electro-thermal melting point apparatus (UK) was used for recording the melting points of synthesized compounds. Mass spectra were recorded on a LC-MS-LCQ (Agilent, Advantage-*Max*, USA) instrument equipped with electrospray ion (ESI) source. Microanalysis was performed on a Carlo Erba 1106 elemental analyzer (Milan, Italy). Thin layer chromatography (TLC) was performed (on silica gel GF_{254}, Merck, Germany) to monitor progress of the reaction and purity of the compounds. Spot being located using either iodine vapors or UV light (UV-GL 58, Handheld UV Lamp, Cambridge, UK). Mobile phase mixtures were: (**1**): Chloroform: methanol: acetic acid (90:10:0.5; v/v/v), (**2**) Chloroform: methanol: acetic acid (70:30:0.5; v/v/v) and (**3**) Petroleum ether: ethyl acetate (4:1; v/v). Compounds were purified using column chromatography on silica gel (100-200 mesh). Chloroform/ dichloromethane and methanol in different composition were used as eluent.

2.1.1. Synthesis of 5-(1-(4-isobutylphenyl)ethyl)-1,3,4-thiadiazol-2-amine (1) [20]

A mixture of thiosemicarbazide (2.28g, 25mM), ibuprofen (5.15g, 25mM) and concentrated poly phosphoric acid (50 g) was heated to 80 °C. The temperature was maintained to 80-90 °C for 10-12h. After cooling to 50 °C it was poured on to crushed ice and neutralized with liquid ammonia (27% v/v) with constant stirring. The precipitated crude product was collected, washed with ice cold water. Ethanol (70%) was used for recrystallization. Yield 75%, m.p. 105-106 ^oC (105-107 ^oC, [11]), UV (λ_{max})-255nm, IR ν_{max} (KBr) cm^{-1}: 3385, 3365, 3105, 2980, 2850, 1640, 1588, 1485, 1415, 1395, 865. ^{1}HNMR ($CDCl_3$, 300MHz): δ p.p.m.: 0.89 (d, 6H, *J*=6.3 Hz, -CH-(C*H*$_3$)$_2$); 1.70-1.90 (d, 3H, *J*=7.2Hz, >CH-C*H*$_3$ overlapped with, m, 1H, -C*H*-(CH$_3$)$_2$); 2.45 (d, 2H, *J*=6.9 Hz, >CH-C*H*$_2$-); 4.36 (q, 1H, *J*= 14.4Hz, >C*H*-CH$_3$); 5.33 (s, 2H, N*H*$_2$, D_2O exchangeable); 7.10 (d, 2H, *J*=7.5 Hz, H_3, H_5 ArH), 7.2 (d, 2H, *J*=7.5Hz, H_2, H_6 ArH). Anal. Calcd. for $C_{14}H_{19}N_3S$: C, 64.33; H, 7.33; N, 16.08. Found: C, 64.36; H, 7.32; N, 16.05. TLC-(Solvent system-1) R_f = 0.51.

2.1.2. Synthesis of 2-chloro-N-(5-(1-(4-isobutylphenyl)-ethyl)-1,3,4-thiadiazol-2-yl) acetamide (2)

Compound **1** (20 mM) was dissolved in 1,4-dioxan (25 ml). Chloroacetyl chloride (22 mM) in small portions was added to it during 30 mins. Contents were stirred for 2h at room temperature and then refluxed for 6h. Reaction was terminated by pouring content on crushed ice. The precipitated product was collected, washed with 1% (w/v) potassium carbonate solution and ice cold water. Dried in vacuum and recrystallized from dichloromethane. Yield: 80.1%, m.p. 104 ^oC, UV (λ_{max})-258nm; IR ν_{max} (KBr) cm^{-1}: 3185, 2957, 2868, 1905, 1697, 1600, 1581, 1511, 1448, 1345, 784, 681. ^{1}HNMR (300 MHz, DMSO-d_6): δ 0.81 (d, 6H, *J*=6.6Hz, -CH-(C*H*$_3$)$_2$); 1.65(d, 3H, *J*=7.0Hz, >CH-C*H*$_3$); 1.78(m, 1H, -C*H*-(CH$_3$)$_2$); 2.05 (s, 1H, NH, D_2O exchangeable); 2.38 (d, 2H, *J*=7.08 Hz, -C*H*$_2$-CH-(CH$_3$)$_2$); 4.36 (s, 2H, -CO-C*H*$_2$-Cl); 4.53(q, 1H, *J*= 14.05 Hz, >C*H*-CH$_3$); 7.09 (d, 2H, *J*=7.8Hz, H_3, H_5, ArH); 7.21 (d, 2H, *J*=7.8Hz, H_2, H_6, ArH). Mol. Formula: $C_{16}H_{20}ClN_3OS$; LC-MS/MS (ESI, positive mode) *m/z* 337 (M+); 339 (M+2). Anal. Calcd. for $C_{16}H_{20}ClN_3OS$: C, 56.88; H, 5.97; N, 12.44. Found: C, 56.85; H, 5.98; N, 12.43. TLC- (solvent system-1) R_f = 0.78.

2.1.3. General Method for the Synthesis of Acetamide (3a-h)

A mixture of **2** (5 mM) in dry benzene (50 ml) and respective secondary amine (10 mM) was refluxed for 6-8 h. On cooling, the amine hydrochloride crystallized out which was filtered and separated. The organic phase was washed with distilled water and dried on sodium sulphate (exsiccated). Benzene was removed under vacuum; product was collected and recrystallized using ethanol (90%). The semi-solid compounds were converted to hydrochloride salt using HCl gas in dry ether for melting point determination and pharmacological studies.

2.1.3.1. 2-(Dimethylamino)-N-(5-(1-(4-isobutyl-phenyl)ethyl)-1,3,4-thiadiazol-2-yl)-acetamide (3a)

Yield: 60.5%, m. p. 170 ^oC (as HCl salt), UV (λ_{max})-256 nm IR ν_{max} (KBr) cm^{-1}: 3450, 3105, 2980, 2850, 1680, 1615, 1588, 1485, 1415, 1395, 865. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.83-0.85 (d, 6H, *J*=7.5Hz, CH-(C*H*$_3$)$_2$); 1.83 (d, 3H, *J*=6.0Hz, >CH-C*H*$_3$); 1.86-1.98 (m overlapped with doublet, 1H, -C*H*-(CH$_3$)$_2$); 2.02-2.07 (br, 1H, NH, D_2O exchangeable); 2.42 (d, 2H, *J*=6.0Hz, -C*H*$_2$-CH<); 2.56 (s, 6H, -N-(C*H*$_3$)$_2$); 3.57 (s, 2H, -C*H*$_2$-CO-); 4.47 (q, 1H, *J*=12.0Hz, >C*H*-CH$_3$); 7.08 (d, 2H, *J*=9.0Hz, H_3,H_5, ArH) 7.17 (d, 2H, *J*=9.0Hz, H_2,H_6, ArH). Mol. Formula: $C_{18}H_{26}N_4OS$; LC-MS/MS (ESI, positive mode) *m/z* 346 (M+); 347 (M+1). Anal. Calcd. for $C_{18}H_{26}N_4OS$: C, 62.40; H, 7.56; N, 16.17. Found: C, 62.37; H, 7.55; N, 16.16. TLC- (solvent system-2) R_f = 0.17.

2.1.3.2. 2-(Diethylamino)-N-(5-(1-(4-isobutyl-phenyl)ethyl)-1,3,4-thiadiazol-2-yl)-acetamide (3b)

Yield. 70%, m.p. 168 ^oC (as HCl salt), UV (λ_{max})-258 nm, IR ν_{max} (KBr) cm^{-1}: 3387, 3050, 2955, 2880, 1695, 1610, 1575, 1463, 1400, 1355 and 868. ^{1}HNMR (300 MHz, $CDCl_3$): δ 0.85-0.87 (d, 6H, *J*=6Hz, -CH-(C*H*$_3$)$_2$); 1.00-1.05 (t, 6H, *J*=5Hz, -CH$_2$-C*H*$_3$), 1.75-1.77 (d, 3H, *J*=6.2Hz, >CH-C*H*$_3$); 1.77-1.80 (m, overlapped, 1H, >C*H*-CH$_2$-); 2.05-2.10 (s, 1H, NH); 2.30-2.34 (q, 4H, *J*=5.5Hz, -C*H*$_2$-CH$_3$), 2.39-2.46 (d, 2H, *J*=Hz, -C*H*$_2$-CH<); 3.42 (s, 2H, -C*H*$_2$-CO-); 4.40-4.55 (q, 1H, *J*=Hz, >C*H*-CH$_3$); 7.03-7.06 (d, 2H, *J*=9Hz, H_3,H_5, ArH); 7.10-7.13 (d, 2H, *J*=Hz, H_2,H_6, ArH). Mol. Formula: $C_{20}H_{30}N_4OS$; LC-MS/MS (ESI, positive

mode) *m/z* 374 (M+); 375 (M+1). Anal. Calcd. for $C_{20}H_{30}N_4OS$: C, 64.14; H, 8.07; N, 14.96. Found: C, 64.12; H, 8.06; N, 14.95. TLC- (solvent system-2) R_f = 0.33.

2.1.3.3. 2-(Dipropylamino)-N-(5-(1-(4-isobutyl-phenyl)ethyl)-1,3,4-thiadiazol-2-yl)-acetamide (3c)

Yield. 70%, m.p. 133 °C (as HCl salt), UV (λ_{max})-255nm, IR ν_{max} (KBr) cm^{-1}: 3385, 3090, 2990, 2870, 1695, 1625, 1590, 1453, 1400, 1395, 868. ^{1}HNMR (300 MHz, $CDCl_3$): δ 0.85 (d, 6H, *J*=6.8Hz, CH(C*H*$_3$)$_2$); 1.00 (t, 6H, *J*=7.0Hz, N-(CH$_2$-CH$_2$-C*H*$_3$)$_2$); 1.7-2.0 (d/m, 8H, >CH-C*H*$_3$, N-(CH$_2$-C*H*$_2$-CH$_3$)$_2$, -C*H*-(CH$_3$)$_2$); 2.25 (s/br, 1H, NH); 2.40 (d, 2H, *J*=7.2Hz, -C*H*$_2$-CH(CH$_3$)$_2$); 3.25-3.40 (br/m, 4H, N-(C*H*$_2$-CH$_2$-CH$_3$)$_2$); 3.45 (s, 2H, -CO-CH$_2$-); 4.50 (q, 1H, *J*=13.5Hz, >C*H*-CH$_3$); 7.08 (d, 2H, *J*=8.2Hz, H$_3$,H$_5$, ArH); 7.15 (d, 2H, *J*=8.3Hz, H$_2$,H$_6$, ArH). Mol. Formula: $C_{22}H_{34}N_4OS$; LC-MS/MS (ESI, positive mode) *m/z* 402 (M+); 403 (M+1). Anal. Calcd. for $C_{22}H_{34}N_4OS$: C, 65.63; H, 8.51; N, 13.92. Found: C, 65.62; H, 8.51; N, 13.90. TLC- (solvent system-2) R_f = 0.73.

2.1.3.4. 2-(Dibutylamino)-N-(5-(1-(4-isobutyl-phenyl)ethyl)-1,3,4-thiadiazol-2-yl)-acetamide (3d)

Yield. 65%, m.p. 128 °C (as HCl salt), UV (λ_{max})-256 nm, IR ν_{max} (KBr) cm^{-1}: 3385, 3115, 3050, 2970, 2860, 1693, 1615, 1500, 1456, 1400, 1352, 861, 771. ^{1}HNMR (300 MHz, $CDCl_3$): δ 0.85 (d, 6H, *J*=6.5 Hz, -CH-(C*H*$_3$)$_2$); 1.00 (t, 6H, *J*=7.2 Hz, N-(CH$_2$-CH$_2$-CH$_2$-C*H*$_3$)$_2$); 1.40-2.10 (br/m 12H, >CH-C*H*$_3$, N-(CH$_2$-C*H*$_2$-C*H*$_2$-CH$_3$)$_2$ and >C*H*-CH$_2$); 2.30 (br, 1H, NH); 2.45 (d, 2H, *J*=6.0Hz, >CH-C*H*$_2$-); 3.25-3.40 (br/m, 4H, N-(C*H*$_2$-CH$_2$-CH$_2$-CH$_3$)$_2$); 3.40 (s, 2H, -CO-CH$_2$-); 4.50 (q, 1H, *J*=13.5Hz, >C*H*-CH$_3$); 7.08 (d, 2H, *J*=8.1Hz, H$_3$,H$_5$, ArH); 7.15(d, 2H, *J*=8.2Hz, H$_2$,H$_6$, ArH). Mol. Formula: $C_{24}H_{38}N_4OS$; LC-MS/MS (ESI, positive mode) m/z 430 (M+); 431 (M+1). Anal. Calcd. for $C_{24}H_{38}N_4OS$: C, 66.94; H, 8.89; N, 13.01. Found: C, 66.95; H, 8.90; N, 13.00. TLC- (solvent system-2) R_f = 0.88.

2.1.3.5. N-(5-(1-(4-isobutylphenyl)ethyl)-1,3,4-thiadiazol-2-yl)-2-morpholinoacetamide (3e)

Yield. 65%, m.p. 130 °C (as HCl salt), UV (λ_{max})-257nm, IR ν_{max} (KBr) cm^{-1}: 3390, 3110, 2970, 2860, 1695, 1625, 1590, 1485, 1450, 1395, 1250, 875. ^{1}HNMR (300 MHz, $CDCl_3$): δ 0.87 (d, 6H, *J*=6.6Hz, -CH-(C*H*$_3$)$_2$); 1.75-1.90 (m/br, 4H, >CH-C*H*$_3$ and >C*H*-CH$_2$-); 2.45 (d, 2H, *J*=6.8 Hz, >CH-C*H*$_2$-); 2.61-2.81 (br, 4H, morpholine); 3.40 (s, 2H, -CO-C*H*$_2$-); 3.70-3.90 (br, 4H, morpholine); 4.51 (q, 1H, *J*=13.0Hz, >C*H*-CH$_3$); 7.10 (d, 2H, *J*=8.3Hz, H$_3$,H$_5$, ArH); 7.20 (d, 2H, *J*=8.2 Hz, H$_2$,H$_6$, ArH). Mol. Formula: $C_{20}H_{28}N_4O_2S$; LC-MS/MS (ESI, positive mode) m/z 388 (M+); 389 (M+1). Anal. Calcd. for $C_{20}H_{28}N_4O_2S$: C, 61.83; H, 7.26; N, 14.42. Found: C, 61.85; H, 7.25; N, 14.41. TLC- (solvent system-1) R_f = 0.73.

2.1.3.6. 2-(N-cyclohexyl-N-methylamino)-N-(5-(1-(4-isobutylphenyl)ethyl)-1,3,4-thiadiazol-2-yl) acetamide (3f)

Yield. 60%, m.p. 171 °C (as HCl salt), UV (λ_{max})-258nm, IR ν_{max} (KBr) cm^{-1}: 3375, 3090, 2980, 2850, 1697, 1615, 1593, 1450, 1407, 1395, 1300, 866, 840. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.81-0.92 (d, 6H, *J*=7.5Hz, -CH(C*H*$_3$)$_2$); 1.15-1.30 (br, 6H, cyclohexyl); 1.40-1.70 (br/m, 5H, cyclo hexyl); 1.75-1.09 (m, 4H, >C*H*-CH$_2$- and >CH-C*H*$_3$); 2.35-2.50 (s/d overlapped, 5H, -N-C*H*$_3$ and >CH-C*H*$_2$-); 3.33 (s, 2H, -CO-C*H*$_2$-); 4.50 (q, 1H, *J*=14.4Hz, >C*H*-CH$_3$); 7.10 (d, 2H, *J*=8.01Hz, H$_3$,H$_5$, ArH); 7.20 (d, 2H, *J*=8.15 Hz, H$_2$,H$_6$, ArH). Mol. Formula: $C_{23}H_{34}N_4OS$; LC-MS/MS (ESI, positive mode) *m/z* 414 (M+); 415 (M+1). Anal. Calcd. for $C_{23}H_{34}N_4OS$: C, 66.63; H, 8.27; N, 13.51. Found: C, 66.61; H, 8.28; N, 13.52. TLC- (solvent system-1) R_f = 0.82.

2.1.3.7. N-(5-(1-(4-isobutylphenyl)ethyl)-1,3,4-thiadiazol-2-yl)-2-(4-methylpiperidin-1-yl)-acetamide (3g)

Yield. 65%, m.p. 182 °C (as HCl salt), UV (λ_{max})-255nm, IR ν_{max} (KBr) cm^{-1}: 3400, 3100, 2989, 2872, 2855, 1680, 1610, 1582, 1480, 1450, 1400, 870. ^{1}HNMR (300 MHz, $CDCl_3$): δ 0.95-1.10 (m, 9H, -CH-(C*H*$_3$)$_2$ and -C*H*$_3$); 1.50-1.90 (m/br, 8H, >CH-C*H*$_3$ and pipd.); 2.10-2.30 (m, 5H, pipd. and >C*H*-CH$_2$-); 2.50 (d, 2H, *J*=6.75 Hz, >CH-C*H*$_2$-); 3.30 (s, 2H, -CO-C*H*$_2$-); 4.45 (q, 1H, *J*=14.0Hz, >C*H*-CH$_3$); 7.11 (d, 2H, *J*=8.0Hz, H$_3$,H$_5$, ArH); 7.19 (d, 2H, *J*=8.0 Hz, H$_2$,H$_6$, ArH). Mol. Formula: $C_{22}H_{32}N_4OS$; LC-MS/MS (ESI, positive mode) *m/z* 401 (M+); 402 (M+1). Anal. Calcd. for $C_{22}H_{32}N_4OS$: C, 65.96; H, 8.05; N, 13.99. Found: C, 65.95; H, 8.05; N, 14.00. TLC- (solvent system-1) R_f = 0.12.

2.1.3.8. N-(5-(1-(4-isobutylphenyl)ethyl)-1,3,4-thiadiazol-2-yl)-2-(4-methylpiperazin-1-yl)acetamide (3h)

Yield. 65%, m.p. 210 °C (as HCl salt), UV (λ_{max})-256nm, IR ν_{max} (KBr) cm^{-1}: 3395, 3056, 2985, 2870, 2850, 1690, 1615, 1585, 1480, 1454, 1390, 1310, 1255, 865. ^{1}HNMR (300MHz, $CDCl_3$): δ 0.90 (d, 6H, *J*=6.3Hz, -CH(C*H*$_3$)$_2$); 1.75-1.90 (m, 4H, >CH-C*H*$_3$ and >C*H*-CH$_2$-); 2.30 (s, 3H, N-C*H*$_3$); 2.45 (d, 2H, *J*=7.05 Hz, >CH-C*H*$_2$-); 2.61-2.81 (br, 8H, pipz.); 3.40 (s, 2H, -COC*H*$_2$-); 4.51 (q, 1H, *J*=13.0Hz, >C*H*-CH$_3$); 7.10 (d, 2H, *J*=8.3Hz, H$_3$,H$_5$, ArH); 7.20 (d, 2H, *J*=8.2 Hz, H$_2$,H$_6$, ArH). Mol. Formula: $C_{21}H_{31}N_5OS$; LC-MS/MS (ESI, positive mode) *m/z* 401 (M+); 402 (M+1). Anal. Calcd. for $C_{21}H_{31}N_5OS$: C, 62.81; H, 7.78; N, 17.44. Found: C, 62.83; H, 7.78; N, 17.43. TLC- (solvent system-1) R_f = 0.10.

2.1.4. Synthesis of N-(5-(1-(4–isobutylphenyl)-ethyl)-1,3,4–thiadiazol-2-yl)-acetamide (4)

To a stirred solution of compound **1** (10mM) in 20 ml benzene, acetic anhydride (10 mM) was added slowly. The contents were stirred for 5h at 40 °C. After completion of reaction, the excess acetic anhydride was removed under reduced pressure. Dichloromethane was used for recrystallization. Yield. 75.8 %, m.p. 137 °C, UV (λ_{max})- 220nm, IR ν_{max} (KBr) cm^{-1}: 3448, 3163, 2956, 2922, 2786, 1685, 1559, 1445, 1372, 1309, 1265, 1235, 1045, 1010, 961, 845, 665. ^{1}HNMR (300 MHz, $CDCl_3$): δ 0.85-0.95 (d, 6H, *J*=6.9Hz, -CH-(C*H*$_3$)$_2$); 1.76-1.78 (d, 3H, *J*=6.0Hz, >CH-C*H*$_3$); 1.80-1.89 (m, 1H >C*H*-CH$_2$-); 2.40-2.52 (m, 5H, -CO-C*H*$_3$ and >CH-C*H*$_2$-); 4.47 (q, 1H, *J*=15.0 Hz, >C*H*-CH$_3$); 7.09 (d, 2H, *J*=9.0Hz, H$_3$,H$_5$, ArH); 7.21 (d, 2H, *J*=9.0Hz, H$_2$,H$_6$, ArH); 13.11 (s, 1H, N*H*, D_2O exchangeable). Mol. Formula: $C_{16}H_{21}N_3OS$; LC-MS/MS (ESI, positive mode) *m/z* 303 (M+); 304 (M+1). Anal. Calcd. for $C_{16}H_{21}N_3OS$: C, 63.33; H, 6.98; N, 13.85. Found: C, 63.35; H, 6.97; N, 13.83. TLC- (solvent system-3) R_f 0.11.

2.1.5. Synthesis of (2-(4-isobutylphenyl)-N-(5-(1-(4-isobutylphenyl)ethyl)-1,3,4-thiadiazol-2-yl)-propanamide (5)

Compound **1** (10 mM), anhydride of ibuprofen (10mM, 5a, supplementary data), zinc dust (0.010 g), glacial acetic acid (10 mM), and dioxane (15 ml) were placed in 100 ml flask. Contents were refluxed for about 2 h with constant stirring. Then, it was poured in ice cold water (50mL) and the precipitated product was collected. Ethyl acetate/methanol was used for recrystallization. Yield. 50%, m.p. 150 ^{o}C, UV (λ_{max})-255 nm, IR (KBr) cm^{-1}: 3157, 2955, 2926, 2869, 1696, 1552, 1512, 1449, 363, 1299, 1218, 1169, 1049, 846. ^{1}HNMR (300 MHz, $CDCl_3$): δ 0.60-0.90 (m, 12H, 2 x -CH-(CH_3)$_2$); 1.50-1.70 (m, merged with doublet, 4H, -C*H*-(CH_3)$_2$ and -CO-CH(C*H*$_3$)-Ar); 1.70-1.90 (m, 4H, >C*H*-CH_2- and >CH(C*H*$_3$)-); 2.38 (d, 2H, *J*= 7.12Hz, -CH(CH_3)-C_6H_4-C*H*$_2$-); 2.43 (d, 2H, *J*=7.2Hz, >CH-CH_2-); 4.10-4.20 (q, 1H, *J*=14.0Hz, -CO-C*H*(CH_3)-); 4.50 (q, 1H, *J*=14.1Hz, >C*H*(CH_3)-); 7.01(d, 2H, *J*=7.0Hz, H_3, H_5, ArH); 7.08 (d, 2H, *J*=7.1 Hz, H_2, H_6,); 7.22 (d, 2H, *J*=7.2 Hz, H_3, H_5, ArH); 7.33(d, 2H, *J*=6.2 Hz, H_2, H_6, ArH,), 12.26(s, 1H, NH). Mol. Formula: $C_{27}H_{35}N_3OS$; LC-MS/MS (ESI, positive mode) *m/z* 449 (M+); 450 (M+1). Anal. Calcd. for $C_{16}H_{21}N_3OS$: C, 63.33; H, 6.98; N, 13.85. Found: C, 63.35; H, 6.97; N, 13.83. TLC- (solvent system-3) R_f = 0.41.

2.2. Pharmacology

For the pharmacological studies, solutions (100 mg/ml) of synthesized compounds were prepared in normal saline. For all studies, healthy Wistar rats (150-200g) and Swiss mice (20-25 g) (approximately of the same age for each species) of either sex were used. They were grouped and placed into clean polypropylene cage and were maintained on a balanced ratio obtained from the Hamoudeh Co., Amman, Jordan. Fresh drinking water was offered to the animals daily *ad libitum*. Standard drug and synthesized compounds were administered orally to the animals by using oral feeding cannula. All experiments were carried out at Faculty of Pharmacy and Medical Sciences, Al-Ahliyya Amman University, Amman, Jordan with the consent of provisional animal ethical committee. All the experiments were conducted under the controlled condition of temperature (23 ± 2^{o} C), relative humidity (50 ± 5 %).

2.2.1. Anti-inflammatory Activity

Carrageenan-induced paw edema model was used for studying anti-inflammatory activity [21]. The animals (albino rats) were divided into groups of four. Synthesized compounds were given orally (30 mg/kg b.wt.) and the paw volume determined plethysmographically (Ugo-Basel, Italy). The control group received equivalent volume of normal saline. The reference group received ibuprofen (20 mg/kg b.wt. p.o.). After half an hour the carrageenan (0.1 mL of 1.0% w/v solution) in sterile saline was injected into the sub plantar tissue of the rat's right hind paw. The paw volume was determined at hourly intervals for 3 hours (0, 1, 2, 3 h). The percent of inhibition of inflammation was determined using formula: *% inhibition = 100 x(1-V_s/V_c)*, where 'V_c' represents edema volume in control and 'V_s' edema volume in the group treated with sample.

2.2.2. Peripheral Analgesic Activity [22]

This experiment was carried out as described by Siegmund *et al.* (1957) with slight modification. Female mice (20-25 g, n=5 each group) were grouped as control, treated with ibuprofen (20 mg/kg), and groups treated with compounds. All animals were treated orally (with compounds 30 mg/kg) 1 h prior to the injection of acetic acid. The control group received normal saline. The effect of the compounds on writhing was observed in comparison to control. Acetic acid (3%, 300 µl/kg) solution was used intraperitoneally to induce writhing in test animals. Mice were placed in big glass cylinder separately and writhing episodes were counted for 20 min and the percent activity was calculated.

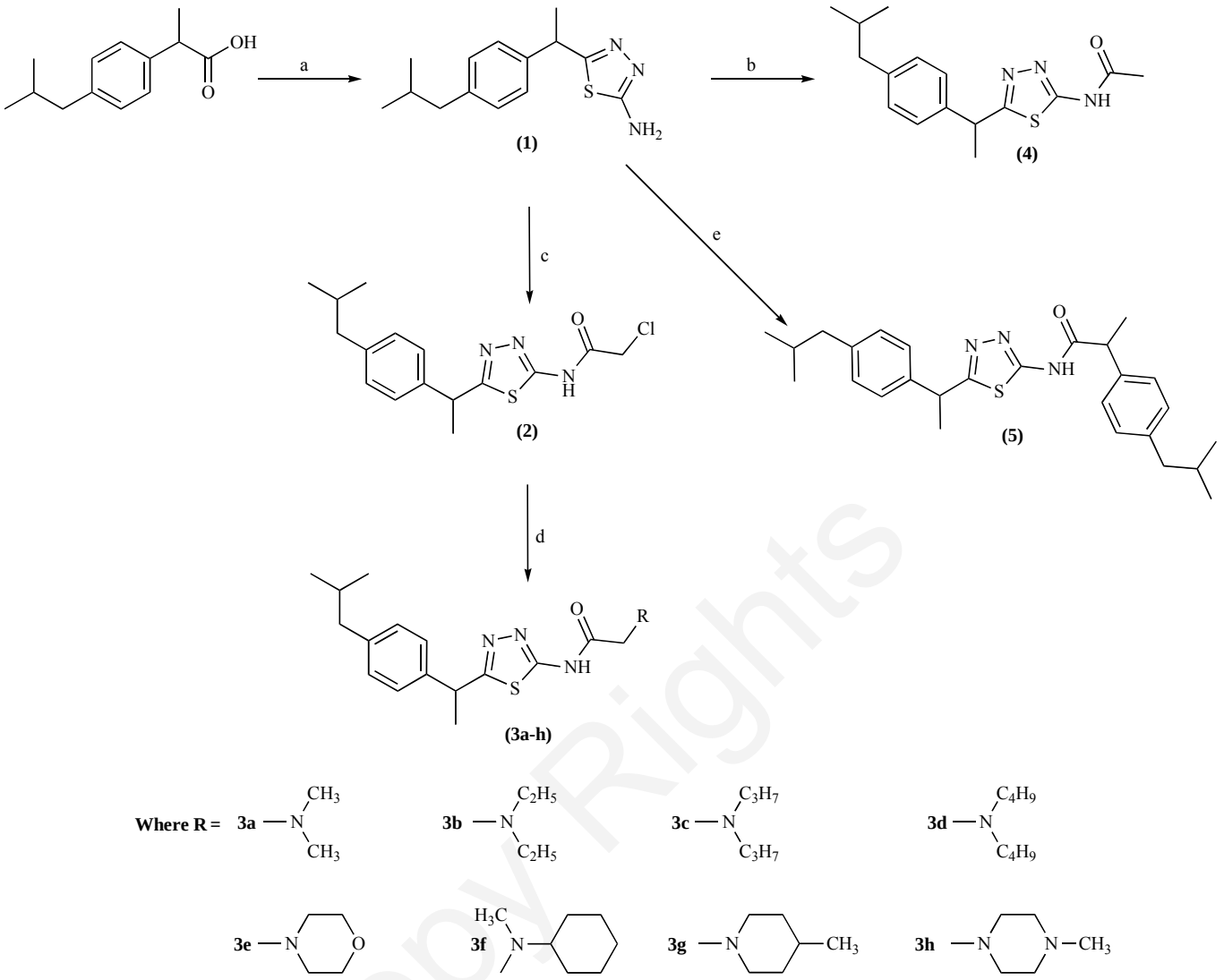
2.2.3. Acute Ulcerogenic Activity [23]

Healthy Wistar rats (150–200 g) were used in the present experiment. Three times of the anti-in ammatory dose of standard/compounds were used. Rats (n=5) were divided into different groups. Compounds (90 mg/kg b.wt.) or ibuprofen (60 mg/kg b.wt.) were given orally. Control group received only normal saline. The animals were fasted 18 h prior to administration of test compounds. After 6 h of the drug treatment the rats were sacrificed. Stomach was removed and opened along the greater curvature. Inner lining was washed with normal saline and examined by magnifying glasses (5x). The number of ulcers and severity index was calculated using scores: 0= no lesions; 1= superficial ulcers; 2= deep ulcers; 3=perforation; 4=severe perforation.

2.3. Structure Based Drug Design

2.3.1. Molecular Modeling and Docking Studies

The protein (COX-2, PDB code: 3NT1) was downloaded from Protein Data Bank (PDB) [24]. The protein was prepared by removing B chain, nonreactive water molecules, and ligand molecules other than crystal ligand (naproxen in COX-2). The ionized protein having the lowest penalty was energy minimized using the OPLS 2005 force field by Impref tool of Glide programme to finally prepare processed 3NT1 protein. The grid was generated in the processed protein by excluding the crystal ligand in the active site using receptor grid generation tool of Glide programme (van der Waals radius scaling factor was limited to 1.0 with a partial charge cut off of 0.25). The ligands were subjected to Ligprep simulations to generate energy minimized 3 D structures (300 steps) by investigating tautomeric, stereo chemical and ionization variations. The ligprep out ligands were docked flexibly in the protein grid using Glide-extra precision (XP) simulations [5, 25]. Compounds having 300 atoms and 50 rotatable bonds were docked using 5 poses per ligand and 10,000 poses per docking run. Energies of residues within 12Å of grid were used for simulations. Finally the docked ligands were scored based on non-bonded interactions such as lipophilic pair term, hydrophobic enclosure reward, hydrogen bond, and electrostatic rewards (Figs. **1-3** and Table **4**).



Scheme 1. Synthetic pathway of the targeted compounds **3a-h**, **4** and **5**. Reagent and conditions: (a) Thiosemicarbazide, PPA, 80-90 °C, 10-12h, then neutralize with NH_4OH; (b) C_6H_6, acetic anhydride, stirring 40 °C, 5h; (c) dioxane, chloroacetylchloride, rt, 2h then reflux 6h; (d) dry benzene, amines, reflux, 6-8h; (e) dioxane, Zn dust, gl. acetic acid, anhydride of ibuprofen, reflux 2h.

3. RESULTS AND DISCUSSION

3.1. Chemistry

The required starting material 5-(1-(4-isobutylphenyl)-ethyl)-1,3,4-thiadiazol-2-amine (**1**) was synthesized using the modified method [20] (Scheme **1**). In this ibuprofen and thiosemicarbazide were condensed using polyphosphoric acid between 80-90 °C. New derivatives containing 1,3,4-thiadiazoles were prepared and characterized by elemental analysis, IR, NMR and LC-MS. The compounds synthesized were purified using the different solvent or with the help of column chromatography using chloroform and methanol as mobile phase.

Acetylation of compound **1** with chloroacetyl chloride in dry benzene gave 2-chloro-N-(5-(1-(4-isobutylphenyl)-ethyl)-1,3,4-thiadiazol-2-yl)-acetamide (**2**). Later the chloro-acetyl chloride derivative was converted into the substituted acetylamino derivatives (**3a-3h**) utilizing the different amines. The compound (N-(5-(1-(4-isobutyl-phenyl)-ethyl)-1,3,4-thiadiazol-2-yl) acetamide, **4**) was prepared by acetylation of compound **1**, using the acetic anhydride in dry condition. Compound **5** (2-(4-isobutylphenyl)-N-(5-(1-(4–isobutylphenyl)ethyl)-1,3,4- thiadiazol-2-yl)-propanamide) was synthesized utilizing the 5-(1-(4-isobutyl phenyl)ethyl)-1,3,4-thiadiazol-2-amine (**1**) and anhydride of ibuprofen in presence of zinc dust, glacial acetic acid, and dioxane. The anhydride of the ibuprofen was prepared utilizing DCC at room temperature. Synthesized compounds showed characteristic absorption bands at their assigned places in IR spectra. The presence of characteristic absorption band for -NH-CO- linkage between 1670 to1700 cm^{-1} indicates the synthesis of the compounds. Compounds also gave the characteristic signal for the presence of NH stretching between 3350 to 3420 cm^{-1}.

^{1}H-NMR spectra of all prepared compounds are in agreement with the suggested structures; ^{1}H-NMR spectra of compounds showed signals corresponding to aromatic, aliphatic, acetamide, and NH protons. Methylene (CH_2) proton

Table 1. Anti-inflammatory activity of the synthesized compounds.

Compd.	Carrageenan Induced Rat paw edema	
	Mean Increase in paw vol. ± SD (mL)	% Inhibition
Control	1.28±0.37	-
Diclofenac sodium (10mg/kg b.wt.)	0.69±0.10	46.39#
Ibuprofen (20mg/kg b.wt.)	0.67±0.15	47.65#
3a	0.93±0.17	27.87*
3b	0.88±0.07	31.38*
3c	0.64±0.19	50.10#
3d	0.78±0.25	38.98$
3e	0.51±0.30	60.62#
3f	0.46±0.05	64.13#
3g	0.65±0.11	49.12$
3h	0.83±0.17	35.47*
4	0.60±0.20	53.22#
5	0.35±0.03	72.51#

Data represents the Mean ± SD for 4 mice. P values were compared with control group (3 hours after inducing edema) (Tukey's Test), Anti-inflammatory activity was assessed as the percent inhibition of carrageenan-induced edema between animals of control group and animals pretreated with reference drug or synthesized compounds (30mg/kg b.wt.). *p>0.05 (no significant difference), $p< 0.05 (significant difference) , #p<0.001 (significant difference)

of -NHCOCH$_2$- were observed between 3.4-3.6 *ppm*, which indicates the synthesis of the target compounds (**3a-3h**), while in case of intermediate compound **2** (2-chloro-N-(5-(1-(4–isobutylphenyl)-ethyl)-1,3,4-thiadiazol-2-yl)- acetamide), the signal was desheilded to 4.36 *ppm* (presence of –Cl). The NH protons were observed as D_2O exchangeable protons. The signals for the methine proton (>C*H*-CH$_3$), were recorded as quartet between 4.40-4.60 *ppm*. In case of compound (**5**), methine protons were observed between 4.15 and 4.50 *ppm* in different environment. Methine proton attached to -NH-CO- functional group was slightly desheilded. The methine (-CH$_2$C*H*-(CH$_3$)$_2$) protons of the isobutyl group were observed as multiplet between 1.70-2.20 *ppm*, while aromatic protons were observed between 6.90 to 7.20 *ppm* as doublet, with coupling constant (*J value*) ~ 7-9Hz. In case of the (1-(4-isobutylphenyl)-ethyl)-1,3,4-thiadiazol-2-amine, the data are in agreement of the data reported earlier [11]. The absence of COOH proton (~10-12 *ppm*) indicates the complete utilization of ibuprofen in the cyclization of 1,3,4-thiadiazole. The LC-MS-MS data also supports the synthesis of compounds.

3.2. Pharmacology

3.2.1. Anti-inflammatory Activity

Compounds (**3a-h)** were evaluated for anti-inflammatory activity on Wistar rats; ibuprofen was used as standard compound. The compound **5** (2-(4-isobutylphenyl)-N-(5-(1-(4-isobutylphenyl)-ethyl)-1,3,4-thiadiazol-2-yl)-propanamide) containing –NHCO- group was the most active compound (72.5%, p≤0.001). It was followed by compound **3f** (N-cyclohexyl-N-methylamino analog, percent inhibition in paw oedema = 64.1%), **3e** (*morpholino analog*, 60.6%), **4** (N-acetyl derivative, 53.2%), **3c** (*dipropyl amino analog*, 50.1%), **3g** (4-methylpiperidine analog, 49.1%) and **3d** (dibutyl analog, 39.0 %). Compounds **3a**, **3b**, and **3h** did not produce significant anti-inflammatory activity (p≥0.05). Present study suggests that the replacement of carboxylic functional group with substituted thiadiazole ring containing acetamide group or amide analogs retains the activity. The results are presented in Table **1.**

3.2.2. Analgesic Activity

The tested compounds showed analgesic activity in the range of 33.8- 69.8%, whereas ibuprofen showed 73.0% inhibition (Table **2**). Compounds **4** (N-(5-(1-(4-isobutylphenyl)-ethyl)-1,3,4-thiadiazol-2-yl)acetamide), and compound **5** (2-(4-isobutylphenyl)-N-(5-(1-(4–isobutylphenyl)-ethyl)-1,3,4- thiadiazol - 2-yl)-propanamide) showed better analgesic activity (65.5-69.8%) compared with other 1,3,4-thiadiazole derivatives **3a–h** (33.8%–61.2%). It was observed that compound **5** possessing highest anti-inflammatory activity also showed excellent analgesic activity 65.5%. In compound **3f** (N-cyclohexyl-N-methylamino analog, 61.2%) and **3e** (morpholino analog, 58.3%) were also found to have a excellent analgesic activity. Compounds **3c** (dipropyl analog, 53.2%), **3g** (4-methylpiperidine, 44.6%) and **3b** (diethyl analog, 41.0%) showed moderate activity while compound **3h** (4-methylpiperazine analog, 39.6%), **3d** (dibutyl analog, 37.4%) and **3a** (dimethyl analog, 33.8%) showed weak activity. Table **2** indicates that all the synthesized compounds significantly inhibited the writhing counts and are capable of controlling the peripheral pain. The acetic acid-induced writhing is a standard test to analyze the pain

Table 2. Effect of synthesized compounds (30 mg/kg b.w. p.o.) on the acetic acid induced writhing in mice.

Treatment	Number of Writhing Counts /20 min.	Percent Inhibition
Normal saline	27.8 ± 5.8	-
Diclofenac sodium (5 mg/Kg b.w.)	3.4 ± 1.7	87.8*
Ibuprofen (10 mg/Kg b.w.)	7.5 ± 1.5	73.0*
3a	18.4 ± 3.9	33.8$
3b	16.4 ± 3.4	41.0*
3c	13.0 ± 1.4	53.2*
3d	17.4 ± 3.3	37.4*
3e	11.6 ± 2.1	58.3*
3f	10.8 ± 3.6	61.2*
3g	15.4 ± 3.6	44.6*
3h	16.8 ± 4.5	39.6*
4	8.4 ± 2.7	69.8*
5	9.6 ± 1.5	65.5*

Data represent the Mean ± SD for 5 mice. P values were compared with control group (20 min) (Tukey's Test), Analgesic activity was assessed as the percent inhibition of writhing episode between animals of control group and mice pretreated with reference drug or synthesized compounds (30 mg/kg b.wt.). #p>0.05 (no significant difference), $ p< 0.05 (moderate significant difference), * p<0.001 (significant difference)

Table 3. Ulcerogenic activity of selected synthesized compounds in wistar rats.

Treatment	Ulcerogenic activity* (Severity Index#) (Mean ± SD)
Normal saline	-
Ibuprofen	3.20 ± 0.37
3e	1.60 ± 0.19
3f	1.40 ± 0.40
4	1.67 ± 0.22
5	1.40 ± 0.37

* Ulcerogenic activity was assessed with reference drug (ibuprofen, 60 mg/kg b.wt.) and synthesized compound (90 mg/kg b.wt.)
# Severity index = Mean score of treated group - Mean score of the control group

sensitivity to opiates as well as to non-opiate analgesics. The peripheral analgesic effect tested by this model was reflected in a significant decrease in writhing counts by the synthesized compounds.

3.2.3. Anti Ulcerogenic Activity

The ulcerogenic activity of ibuprofen and compounds (having significant anti-inflammatory activity) were carried out according to Cioli *et al.* [23]. Severity index of selected compounds were ranged from 1.40 to 1.67, while the ibuprofen exhibited severity index of 3.20 (Table **3**). It is clear from the table 3 that the ulcerogenic effects of tested compounds were appreciably lower than the ibuprofen. The reduction in the activity might be due to weak interactions of our compounds with COX-1 enzyme as we have replaced COOH group by thiadiazole moiety (compound **3e**, **3f**, **4** and **5**) or due to the non-specific spasmolytic activity of the compounds (data not shown).

3.3. Structure Based Drug Design

Molecular modeling and docking studies were carried out using Schrodinger software (Maestro 9.1, USA) installed on Dell Precision T-1500 workstation (Intel® Core™ i7 CPU 860 @ 2.80GHz, 12 GB RAM, 1 TB HD) to investigate the mechanism of action. Docking methodology was validated by redocking the crystal ligand (naproxen) in the binding site of (3NT1; COX-2) [24]. Results indicate that the ligand orientation and position of the crystal ligand and the redocked ligand were similar, including the interactions with the conserved residues in the binding site of 3NT1. As expected, synthesized compounds, **3a-h, 4** and **5** which are actually derived from ibuprofen, were also found to have similar interactions with 3NT1 (Figs. **1-3**). Compounds **5**, **3h, 3f,** and **3e** were having stronger binding interactions with the residues of COX-2 protein than the rest of the series. Increased docking scores are due to enhanced occupancy of thiadiazole moiety of our compounds in the side pocket of binding site of COX-2 created by Val-434; which are further supported by high lipophilic Van der Waals interactions, higher hydrophobic enclosure with the residues of the COX-2 protein (Table **4**). The ketone functional group of naproxen and ibuprofen were having hydrogen bond interaction with side chain of Arg-120 and Tyr-355. The nitrogen of 1,3,4-thiadiazole ring and ketone functional group of side chain of our compounds were having similar hydrogen bond interaction with Arg-120 and Tyr-355 (Fig. **1**). The compound **5**

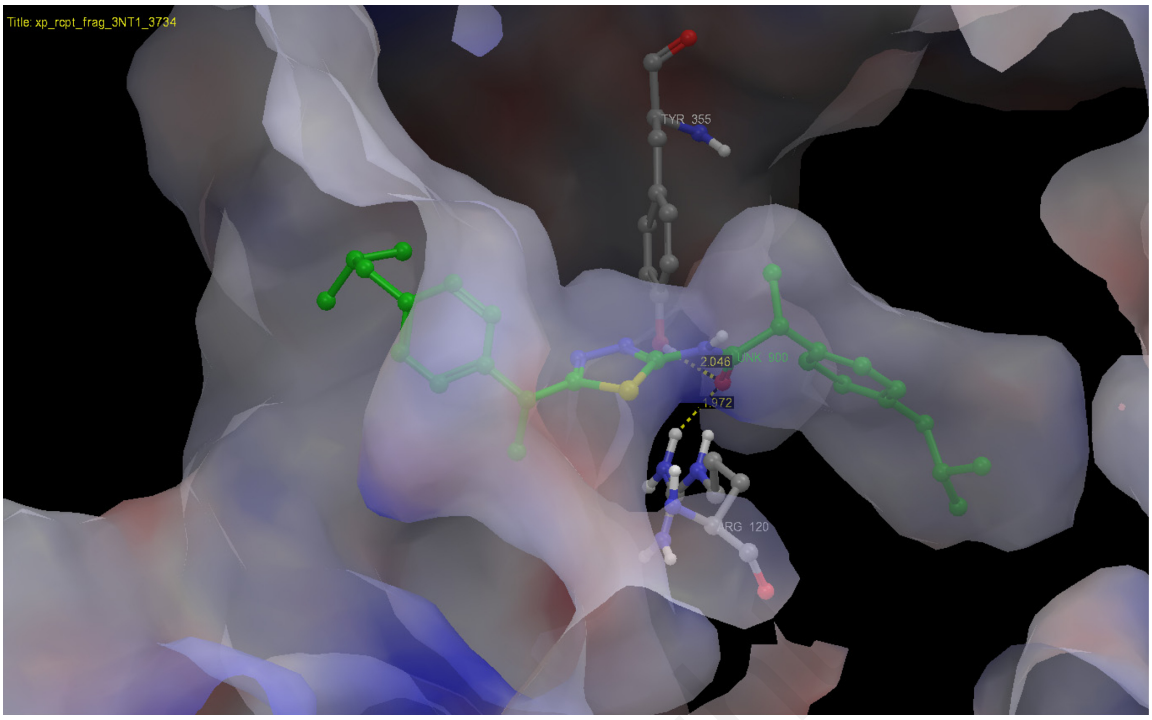


Fig. (1). Compound 5 having interaction with Arg-120 and Tyr-355 in the active site of COX-2 (PDB code 3NT1).

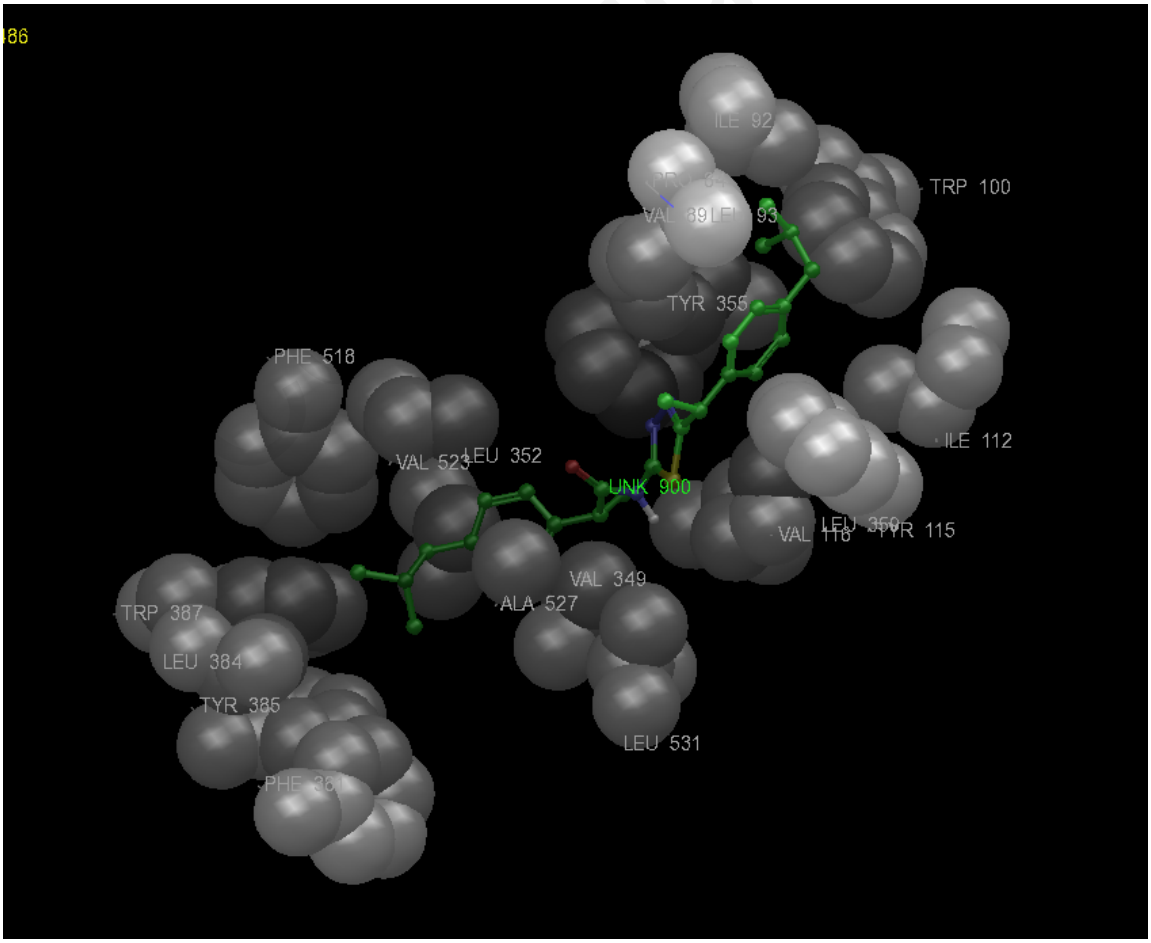


Fig. (2). Hydrophobic amino acids (space filled) enclosing the ball and stick form of Compound **5** in the active site of COX-2 (PDB code 3NT1).

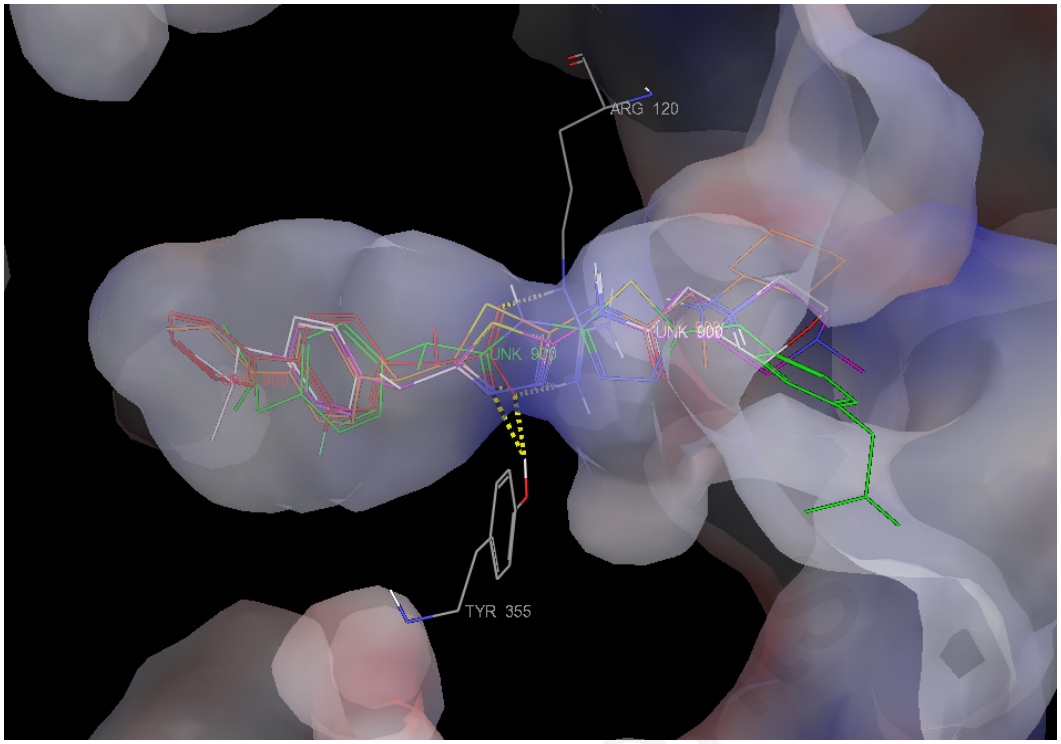


Fig. (3). Hydrogen bonding interaction with Compound **5** (green), **3h** (pink), **3f** (orange), **3e** (white), ibuprofen (red colored line) with Arg-120 and Tyr-355 of COX-2 protein (Surface diagram, Pdb code: 3NT1). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper).

forms H-bonding interactions at thiadiazole: NH of Arg-120 (1.972 Å) and with OH of Tyr-355 (2.046Å). The aromatic ring attached to chain of compound **5** towards the apex of the COX active site and forms Van der Waals interactions with Trp-387, Phe-518, Leu-352, Phe-381, Leu-384, Val-523 and Val-349. The isopropy-methyl-phenyl group was surrounded by Trp-100, Ile-112, Ile-92, Val-89, Pro-84 and Leu-93. The nitrogen atoms of thiadiazole ring were covered by Val-116. The carbonyl side chain was surrounded by non polar amino acids such as Leu-359, Leu-531 and Ala-527 (Fig. **2**).

In silico interactions of **3a-i, 5** and ibuprofen with COX-1 enzyme (Pdb code: 3N8Z) was also investigated to confirm the possible COX-1 related side effects. Earlier studies have suggested that the residues that form the substrate binding channel, the catalytic sites, and the residues immediately adjacent of COX-1 and COX-2 are all identical except for two small variations [3, 26]. Ile in COX-1 is exchanged for Val in COX-2 at positions 434 and 523. The Val substitution in COX-2, at residue 523 contributes to the opening of the side pocket, which is hydrophilic in nature. Coincidently our compounds which are more hydrophilic because of thiadiazole than flubiprofen fails to bind strongly with hydrophobic binding site of COX-1 compared to flubiprofen. COX-2 binding site having a hydrophilic side pocket makes a comfortable site for our compounds to bind. Compound **5, 3c, 3d, 3f, 3g,** and **3h** failed to bind with COX-1, while others showed weak binding; with an exception of better binding interaction of **3b** (docking score: -12.41) with COX-1 protein. With all these evidences it shows that our compounds are selective for COX-2 protein which is in agreement with anti-inflammatory and ulcerogenic activity.

3.4. Structure Activity Relationship (SAR)

On the basis of the current pharmacological data we observed that the COOH group of the ibuprofen can be replaced with the 1,3,4-thiadiazol-2-acetamide. These compounds were having the similar interaction with the COX-2, like the parent compounds. It was observed that the acetamide derivative **4**, and amide derivative **5**, were more effective as anti-inflammatory and analgesic agent than the other compounds. Compound **5** was having the highest NSAID activity among the entire compounds tested. The substituents on side chain can influence the NSAID activity. The compounds having small substituent like dimethyl- (**3a**) and diethyl-(**3b**), dibutyl (**3d**) and methyl piperazinyl (**3h**) were inactive. The optimum activity was recorded with compounds having substituent like dipropyl (**3c**), morpholino (**3e**), N-methyl-N-cyclohexyl (**3f**), methyl piperidinyl (**3g**) and acetamide (**4**).

CONCLUSION

The present study reports the molecular modeling, synthesis, anti-inflammatory activity, analgesic and ulcerogenic potential studies of 2-(substituted)-N-(5-(1-(4-isobutylphenyl)ethyl)1,3,4-thiadiazol-2yl)-acetamide **3a–g**, **4** and 2-(4-isobutylphenyl)-N-(5-(1-(4-isobutylphenyl)-ethyl)-1,3,4-thiadiazol-2-yl)-propanamide **5**. The pharmacological evaluation showed that, compound **5** possessed highest anti-inflammatory (72.5%) and where as compound **4** possessed highest analgesic activity (69.8%). Tested compounds **5** and **4** showed marked reduction in ulcerogenic activity (ulcerogenic index 1.40 and 1.67 respectively) compared with the

Table 4. Scoring data (kcal/mol) of Glide-XP docking of Schrodinger (Maestro 9.1) against COX-2 protein.

Ligand*	Docking Score[a]	Lipophilic EvdW[b]	PhobEn[c]	HBond[d]	Electro[e]	Sitemap[f]	RotPenal[g]
Flurbiprofen	-13.51	-5.75	-2.12	-2.15	-0.84	-0.40	0.25
5	-13.18	-7.52	-2.61	-1.05	-0.34	0.00	0.34
3h	-12.99	-5.91	-2.24	-1.92	-0.96	-0.18	0.36
3f	-12.92	-6.02	-2.10	-1.84	-0.98	-0.20	0.34
3e	-12.80	-5.57	-2.24	-1.94	-0.99	-0.19	0.33
4	-12.12	-5.08	-2.45	-1.81	-0.51	-0.22	0.40
3g	-12.01	-5.41	-1.99	-1.73	-0.82	-0.27	0.36
3b	-11.92	-5.14	-2.18	-1.73	-0.79	-0.24	0.41
3c	-11.75	-5.81	-1.80	-1.36	-0.84	-0.24	0.46
Ibuprofen	-11.65	-4.72	-1.73	-2.15	-0.88	0.00	0.34
Naproxen	-11.63	-5.39	-0.88	-2.15	-0.85	0.00	0.14
3d	-11.40	-6.75	-2.70	-1.05	-0.33	-0.01	0.50
3a	-11.15	-5.46	-2.10	-1.08	-0.48	-0.10	0.41
Aspirin	-8.14	-3.10	-1.31	-0.30	-0.49	-0.65	0.21
Celecoxib	-7.07	-2.27	-1.49	-0.52	-0.29	-0.46	0.11

[a]An estimate of ligand-COX-2 binding energy; [b]Lipophilic van der Walls interactions of ligand-COX-2 complex; [c]Hydrophobic enclosure reward for ligands in the binding site of COX-2; [d]Hydrogen-bonding term; [e]Electrostatic interactions of ligand- COX-2 complex; [f]Score indicating the effective occupancy of ligands in the binding site of 3NT1 protein; [g]Rotational penalty causing the decreased.

ibuprofen (ulcerogenic index 3.20). Similarly, compounds **3e**, **3f** exhibited moderate anti-inflammatory and analgesic activity with less ulcerogenic index. Molecular modeling also supports that compounds **5**, **3f** and **3e** have significant anti-inflammatory activity and less ulcerogenic index due to their affinity and binding towards COX-2 rather than COX-1 enzyme. In conclusion, compound **5** having thiadiazole moiety attached to the propanamide chain of ibuprofen was found to have encouraging anti-inflammatory activity with less toxicity than ibuprofen and is in agreement with molecular modeling studies against COX enzymes.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

ACKNOWLEDGEMENTS

Author wish to thank Dean, Faculty of Pharmacy and Medical Sciences; Dean, Higher Education and Scientific Research, Al-Ahliyya Amman University, Amman, Jordan and Gokaraju Rangaraju Educational Society, Hyderabad, India for providing necessary facilities.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers Web site along with the published article.

REFERENCES

[1] Hamor, G.H. Nonsteroidal anti-inflammatory drugs. In *Principles of Medicinal Chemistry*; W.O. Foye, 3rd Ed.: Philadelphia: Lea and Febiger, **1989**.

[2] Kimmey, M.B. NSAID, ulcers, and prostaglandins. *J. Rheumatol. Suppl.* **1992**, *36*, 68-73.

[3] Kurumbail, R.G.; Stevens, A.M.; Gierse, J.K.; McDonald, J.J.; Stegeman, R.A.; Pak, J.Y.; Gildehaus, D.; Miyashiro, J.M.; Penning, T.D.; Seibert, K.; Isakson, P.C.; Stallings, W.C. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature*, **1996**, *384*(6610), 644-48.

[4] Cocco, M.T.; Congiu, C.; Onnis, V.; Morelli, M.; Cauli, O. Synthesis of ibuprofen heterocyclic amides and investigation of their analgesic and toxicological properties. *Eur. J. Med. Chem.,* **2003**, *38*, 513-18.

[5] Raghavendra, N.M.; Ramakrishna, K.; Sirisha, V.; Divya, P.; Venkateswara Rao. A. Computer aided discovery of potential anti-inflammatory (s)-naproxen analogs as COX-2 inhibitors. *Med. Chem*., **2013**, *9*, 553-59.

[6] Kalgutkar, A.S.; Marnett, A.B.; Crews, B.C.; Remmel, R.P.; Marnett, L.J. Ester and amide derivatives of the nonsteroidal antiinflammatory drug, indomethacin, as selective cyclooxygenase-2 inhibitors. *J. Med. Chem.,* **2000**, *43*, 2860–70.

[7] Yusuf, M.; Khan, R.A.; Khan, M.; Ahmed, B. An interactive human carbonic anhydrase-II (hCA-II) receptor-pharmacophore molecular model and anti-convulsant activity of the designed and synthesized 5-amino-1,3,4-thiadiazole-2-thiol conjugated imine derivatives. *Chem. Biol. Drug. Des.,* **2013**, *81*(5), 666-73.

[8] Dubey, V.; Pathak, M.; Bhat, H.R.; Singh, U.P. Design, facile synthesis, and antibacterial activity of hybrid 1,3,4-thiadiazole-1,3,5-triazine derivatives tethered *via* -S-bridge. *Chem. Biol. Drug. Des.,* **2012**, *80*(4), 598-604.

[9] Aggarwal, N.; Kumar, R.; Dureja, P.; Khurana, J.M. Synthesis of novel nalidixic acid-based 1,3,4-thiadiazole and 1,3,4-oxadiazole

derivatives as potent antibacterial agents. *Chem. Biol. Drug. Des.,* **2012**, *79*(4), 384-97.

[10] Chawla, G.; Kumar, U.; Bawa, S.; Kumar, J. Syntheses and evaluation of anti-inflammatory, analgesic and ulcerogenic activities of 1,3,4-oxadiazole and 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole derivatives. *J. Enzyme Inhib. Med. Chem.,* **2011**, *27*(5), 658-65.

[11] Shirote, P.J.; Bhatia, M.S. Synthesis, Characterization and Anti-inflammatory Activity of 5-{[((5-Substituted-aryl)-1,3,4-thiadiazol-2-yl)thio]-n-alkyl}-1,3,4-oxadiazole-2-thiol. *Chin. J. Chem.,* **2010**, *28*, 1429-36.

[12] Sainy, J.; Mishra, G.P.; Sharma, R.; Chaturvedi, S.C. 2-Amino-5-sulfanyl-1,3,4-thiadiazoles: a novel series of anti-inflammatory and analgesic agents. *Pharm. Chem. Jr.,* **2009**, *43*, 19-24.

[13] Yavuz, S.; Ünal, Y.; Pamir, Ö.; Y lmazer, D.; Kurtipek, Ö.; Kavutçu, M.; Arslan, M.; Ark, M.; Y ld r r, Y. Synthesis and pharmacological evaluation of some novel thebaine derivatives: N-(tetrazol-1H-5-yl)-6,14-endoethenotetrahydrothebaine incorporating the 1,3,4-oxadiazole or the 1,3,4-thiadiazole moiety. Arch. Pharm. (Weinheim), **2013**, *346*(6), 455-62.

[14] Palaska, E.; Sahin, G.; Kelicen, P.; Durlu, N.T.; Altinok, G. Synthesis and anti-inflammatory activity of 1-acylthiosemicarbazides, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazole-3-thiones. *Farmaco*., **2002**, *57*, 101-107.

[15] Shakya, A.K.; Mishra, P.; Patnaik, G.K.; Shukla, R.; Srimal, R.C. Synthesis and biological evaluation of novel 2-[substituted acetyl]-amino-5-alkyl-1,3,4-thiadiazoles. *Arch. Pharm. Res.,* **1998**, *21*(6), 753-58.

[16] Li, Y.; Geng, J.; Liu, Y.; Yu, S.; Zhao, G. Thiadiazole-a promising structure in medicinal chemistry. *Chem. Med. Chem.,* **2013**, *8*(1), 27-41.

[17] Matysiak, J. Biological and Pharmacological Activities of 1,3,4-Thiadiazole Based Compounds. *Mini Rev. Med. Chem*., **2015**, April 17. [Epub ahead of print].

[18] Jain, A.K.; Sharma, S.; Vaidya, A.; Ravichandran, V.; Agrawal, R.K. 1,3,4-thiadiazole and its derivatives: a review on recent progress in biological activities. *Chem. Biol. Drug. Des.,* **2013**, *81*(5), 557-76.

[19] Rostom, S.A.; el-Ashmawy, I.M.; Abd el Razik, H.A.; Badr, M.H.; Ashour, H.M. Design and synthesis of some thiazolyl and thiadiazolyl derivatives of antipyrine as potential non-acidic anti-inflammatory, analgesic and antimicrobial agents. *Bioorg. Med. Chem.*, **2009**, *17*, 882-95.

[20] Turner, S.; Myers, M.; Gadie, B.; Nelson, A.J.; Pape, R.; Saville, J.F.; Doxey, J.C.; Berridge, T.L. Antihypertensive thiadiazoles. 1. Synthesis of some 2-aryl-5-hydrazino-1,3,4-thiadiazoles with vasodilatoractivity. *J. Med. Chem*., **1988**, *31*(5), 902-06.

[21] Winter, C.A.; Risley, E.A.; Nuss, G.W. Anti-inflammatory and antipyretic activities of indomethacin, 1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid. *J. Pharmacol. Exp. Ther.,* **1963**, *141*, 369-76.

[22] Siegmund, E.; Cadmus, R.; Lu, G. A method for evaluating both non-narcotic and narcotic analgesics. *Proc. Soc. Exp. Biol. Med.,* **1957**, *95*, 729-31.

[23] Cioli, V.; Putzolu, S.; Rossi, V.; Barcellona, P.S.; Corradino, C. The role of direct tissue contact in the production of gastrointestinal ulcers by anti-inflammatory drugs in rat. *Toxicol. Appl. Pharmacol*., **1979**, *50*, 283-89.

[24] Duggan, K.C.; Walters, M.J.; Musee, J.; Harp, J.M.; Kiefer, J.R.; Oates, J.A.; Marnett, L.J. Molecular basis for cyclooxygenase inhibition by the non-steroidal anti-inflammatory drug naproxen. *J. Biol. Chem.,* **2010**, *285*, 34950-59.

[25] Raghavendra, N.M.; Jyothsna, A.; Venkateswara Rao, A.; Subrahmanyam, C.V.S. Synthesis, pharmacological evaluation and docking studies of N-(benzo[d]thiazol-2-yl)-2-(piperazin-1-yl)-acetamide analogs as COX-2 inhibitors. *Bioorg. Med. Chem. Lett*., **2012**, *22*, 820-23.

[26] Vane, J.R.; Bakhle, Y.S.; Botting, R.M. Cyclooxygenases 1 and 2. *Annu. Rev. Pharmacol. Toxicol.,* **1998**, *38*, 97-120.

Received: September 15, 2014 Revised: June 03, 2015 Accepted: June 03, 2015