Med Chem Res
DOI 10.1007/s00044-013-0861-4

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, anti-inflammatory evaluation, and docking studies of some new thiazole derivatives

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Received: 4 August 2013 / Accepted: 18 October 2013

Abstract A series of new 2-substituted-*N*-(1,3-thiazole-2-yl) acetamide **3–7** and *N*-(benzo[*d*]thiazol-2-yl)-2-(substituted) acetamide **10–13** derivatives have been synthesized and evaluated in vivo (rat paw edema) for their anti-inflammatory activities and in silico(docking studies) to recognize the hypothetical binding motif of the title compounds with the cyclooxygenase isoenzyme (COX-2) employing GLIDE software (Schrodinger Inc.). The compounds, **10–13** were found to have good anti-inflammatory activities [around 84–93 % of the standard: indomethacin]. The binding mode of the title compounds has been proposed based on the docking studies. Further, the predicted ADME properties of all the tested compounds were found to be in the ranges as predicted by QikProp for 95 % of known oral drugs and alsosatisfy the Lipinski's rule of five.

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Keywords Microwave-assisted synthesis · Thiazole derivatives · Anti-inflammatory activity · COX-2 docking studies · ADME prediction

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are important therapeutic agents for the treatment of pain and inflammation of everyday life. Through their anti-inflammatory, antipyretic, and analgesic activities they represent a choice of treatment in various inflammatory diseases such as arthritis and rheumatisms (Stefano *et al.*, 2001). NSAIDs are competitive inhibitors of cyclooxygenase (COX), the enzyme that catalyzes the first step of the biosynthesis of prostaglandins (PGG_2) from arachidonic acid which serves as a precursor for the synthesis of PGs, prostacyclines, and thromboxanes such as TXA_2 that are collectively termed as prostanoids (Vane *et al.*, 1998). It is well established that COX exists in two different isoforms, a constitutive form (COX-1) and an inducible form (COX-2) (Xie *et al.,* 1991). The constitutive COX-1 isozyme is expressed in many tissues and appears to be important for the maintenance of various physiological functions such as cytoprotection of gastric mucosa, regulation of renal blood flow, and platelet aggregation (William and David, 1996). In contrast, COX-2 isozyme is not detected in most normal tissues, but its expression is rapidly induced by stimuli such as mitogenes and oncogenes, growth factors, hormones, and disorders of water-electrolyte homeostasis linking its involvement to pathological processes such as inflammation and various cancer types (Harvey, 1996; Kawamori *et al.,* 1998; Shigeru *et al.,* 2007). The classical NSAIDs act by reducing the production of proinflammatory PGs at the sites of injury via COX-2 inhibition. The side effects associated with these

Published online: 09 November 2013

NSAIDs such as GI ulcer and renal function suppression are due to the inhibition of COX-1 pathway (Allison *et al.*, 1992; Charles and Raymond, 1995). Thus the success of NSAIDs in treatment of various inflammatory disorders depends on the selective inhibition of COX-2 over COX-1 isoenzyme. Moreover, recent studies indicating the role of COX-2 inhibitors in cancer chemotherapy especially colon cancer (Taketo, 1998; Lisa and Dan, 2010) and neurological diseases such as Parkinson (Van Gool *et al.*, 2003) and Alzheimer's (Paul *et al.*, 2003) diseases still continues to attract investigations on development of COX-2 inhibitors. As a consequence, many selective COX-2 inhibitors have been developed (Balakumar *et al.*, 2010). However, the market withdrawal of some COXIBs such as rofecoxib due to its adverse cardiovascular side effects (Jean *et al.*, 2005) imposed a great challenge to the researchers to explore and evaluate alternative templates with selective COX-2 inhibitory activity (Sara and Afshin, 2010).

Recently, a series of 8/10-trifluoromethyl-substituted-imidazo[1,2-*c*]quinazolines (Balakumar *et al.*, 2010), 3-alkoxy-4-methanesulfonamido acetophenone derivatives (Alka *et al.*, 2012) cycloalkyl/aryl-3,4,5-trimethylgallates (Mamta *et al.*, 2013) have been reported from our laboratory as potent anti-inflammatory agents. The literature reveals that thiazole derivatives are very potential for their wide range of pharmacological activities including anti-inflammatory, antiallergic, antibacterial, antitumor, anti-hyperlipidemic activities (Afshin *et al.*, 2007; Masakazu *et al.,* 1998; Michel *et al.*, 1997; Jeffery *et al.*, 1999; Pawan and Sawhney, 1997; Rosaria *et al.*, 2005; Franklin *et al.*, 2008, Prakash *et al.*, 2008; Bharti *et al.,* 2010; Balladka *et al.*, 2010). In continuation of our earlier effort, in the present study, we report the synthesis and evaluation of in vivo anti-inflammatory activity of a series of new 2-substituted-*N*-(1,3-thiazol-2-yl)acetamide **(3–7)** and *N*-(benzo[*d*]thiazol-2-yl)-2-(substituted)acetamide **(10–13)** derivatives. Further, we propose the molecular interactions and the binding mode of these target compounds using COX-2 isoenzyme based on in silico docking studies.

Experimental

Reactions were routinely monitored by thin layer chromatography (TLC) on silica gel (pre-coated F254 103 Merck plates) and visualized the products under UV light (254 nm). The ^{1}H was recorded on Bruker Avance II 400 MHz spectrometer for solutions in $CDCl_3$/DMSO-d_6 in parts per million (δ) downfield from tetramethylsilane as internal standard, and *J* values were given in Hz. The ^{13}C NMR spectra were recorded at 100 MHz. The spin multiplicities are indicated by the symbols, s (singlet), d (doublet), t (triplet), dd (double doublet), m (multiplet), and br (broad). IR spectra were recorded on Perkin Elmer FT-IR Spectrometer (Spectrum RX I) using KBr pellet technique. Melting points were recorded in open capillaries on LABINDIA melting point apparatus and were uncorrected. Mass spectra (ESI) were recorded on Waters Micromass Q-TOF Micro.

General procedure for the preparation of 2-chloro-*N*-(1,3-thiazol-2-yl)acetamide (**2**) and *N*-(benzo[*d*]thiazol-2-yl)-2-chloroacetamide (**9**)

Method A

To a solution of 2-amino-1,3-thiazole **1**/2-amino-1,3-benzothiazole **8** (0.10 mol) in freshly distilled THF, triethylamine (0.10 mol) was added followed by dropwise addition of chloroacetyl chloride (0.12 mol) at 0–5 °C and allowed to stir for 30 min–3 h (Table 1). The reaction mixture was poured into crushed ice and neutralized with 5 % HCl followed by extraction with EtOAc. The organic phase was dried on anhydrous Na_2SO_4 and concentrated under reduced pressure to get the pure compound 2-chloro-*N*-(1,3-thiazol-2-yl)acetamide **2** and *N*-(benzo[*d*]thiazol-2-yl)-2-chloroacetamide **9**.

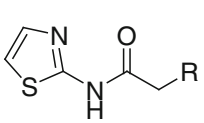
Method B

A mixture of 2-amino-1,3-thiazole 1/2-amino-1,3-benzothiazole **8** (0.001 mol) in freshly distilled THF and triethylamine and chloroacetylchloride (0.001 mol) was heated under microwave irradiation at 560 W for 10–20 s (Table 1). The reaction mixture was worked up following the same process as mentioned in Method A to get the pure compound 2-chloro-*N*-(1,3-thiazol-2-yl)acetamide **2** and *N*-(benzo[*d*]thiazol-2-yl)-2-chloroacetamide **9**.

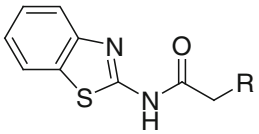
2-Chloro-N-(1,3-thiazol-2-yl)acetamide (2) White solid, Yield: 51 & 80 % (MW), M.P.: 161–162 °C, IR: 3,496, 3,089, 2,926, 1,676, 1,598 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.33 (*s*, 2H, –C<u>H</u>$_2$), 7.11 (*d*, $J = 6.8$, 1H, Ar–<u>H</u>), 7.23 (*d*, $J = 6.8$, 1H, Ar–<u>H</u>), 10.37 (*s*, 1H, –N<u>H</u>); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 45.44 (Cl–<u>C</u>H$_2$), 114.22 (<u>C</u>5-1,3-thiazole), 130.66 (<u>C</u>4-1,3-thiazole), 159.93 (<u>C</u>2-1,3-thiazole), 166.72 (<u>C</u>=O); ESIMS *m/z* 177.68 (M^++1).

N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (9) Slight yellow solid, Yield: 70 & 95 % (MW), M.P.: 162–163 °C, IR: 3,505, 3,048, 2,920, 1,691, 1,646, 1,265 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.37 (*s*, 2H, –C<u>H</u>$_2$–), 7.85 (*m*, 2H, Ar–<u>H</u>), 7.42 (*m*, 2H, Ar–<u>H</u>), 12.63 (*s*, 1H, –N<u>H</u>); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 47.68 (Cl–<u>C</u>H$_2$), 120.20 (<u>benzo</u>[*d*]thiazole), 122.54 (<u>benzo</u>[*d*]thiazole), 125.59 (<u>benzo</u>[*d*]thiazole), 127.67 (<u>benzo</u>[*d*]thiazole), 132.33 (<u>benzo</u>

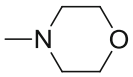
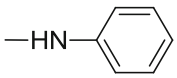
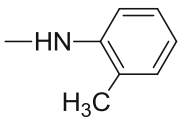
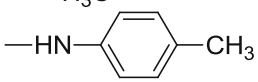
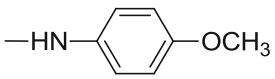
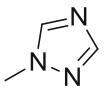
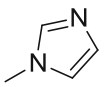
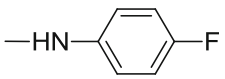
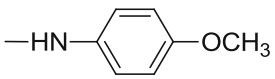
Table 1 Comparative data of conventional (Method A) and microwave-assisted (Method B) synthesis of compounds **2–7** and **9–13**



(2-7)



(9-13)

Compounds	R	M.P.	Conventional (Method A)		Microwave-assisted (Method B)	
		(°C)	Time (h)	% Yield	Time (s) at 560 W	% Yield
2	Cl	160–162	30 min	51	10	80
3		140–142	8 h	35	50	75
4		148–151	8 h	49	50	80
5		160–162	8 h	57	50	80
6		162–164	8 h	60	45	85
7		158–160	8 h	57	50	85
9	Cl	161–162	3 h	70	20	95
10		210–212	2 h	70	30	85
11		190–193	2 h	75	30	87
12		146–148	4 h	65	50	88
13		120–122	4 h	65	50	85

M.P. melting point

[*d*]thiazole), 149.94 (benzo[*d*]thiazole), 167.64 (C=O), 175.55 (C2-benzo[*d*]thiazole); ESIMS *m/z* 227.93 (M^++1).

General procedure for the preparation of 2-(substituted)-*N*-(1,3-thiazol-2-yl)acetamide (**3–7**) and *N*-(benzo[*d*]thiazol-2-yl)-2-(substituted)acetamide (**10–13**)

Method A

A mixture of compound **2/9** (0.10 mol) in freshly distilled THF and triethylamine and appropriate amines (0.10 mol) was heated under reflux for 2–8 h (Table 1). The reaction mixture was poured into crushed ice and neutralized with 5 % HCl followed by extraction with EtOAc. The organic phase was dried on anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc-Hexane 8:2) to afford the desired compound *2-(substituted)-N-(1,3-thiazol-2-yl)acetamide* (**3–7**) and *N-(benzo[d]thiazol-2-yl)-2-(substituted) acetamide* (**10–13**) as a solid.

Method B

A mixture of compound **2/9** (0.001 mol) in freshly distilled THF and triethylamine and appropriate amines (0.001 mol)

was heated under microwave irradiation at 560 W for 30–50 s (Table 1). The reaction mixture was worked up following the same process as mentioned in Method A to get the pure compound **3–7** and **10–13**.

2-Morpholino-N-(1,3-thiazol-2-yl)acetamide ***(3)*** White solid, Yield: 35 & 75 % (MW), M.P.: 140–142 °C, IR: 3,089, 2,928, 1,676, 1,598 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.55 (*t*, $J = 4.6$, 4H, –C$\underline{H}_2$–), 3.19 (*s*, 2H, –C$\underline{H}_2$), 3.70 (*t*, $J = 4.4$, 4H, –C$\underline{H}_2$–), 6.93 (*d*, $J = 6.8$, 1H, Ar–$\underline{H}$), 7.38 (*d*, $J = 6.8$, 1H, Ar–$\underline{H}$), 10.37 (*s*, 1H, –N$\underline{H}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 56.28 (C$\underline{H}_2$), 61.39 (C$\underline{H}_2$–N–C$\underline{H}_2$), 64.23 (C$\underline{H}_2$–O–C$\underline{H}_2$), 114.53 (C5-1,3-thiazole), 130.91 (C4-1,3-thiazole), 160.84 (C2-1,3-thiazole), 166.33 (C=O); ESIMS *m/z* 228.2 (M^++1).

2-(Phenylamino)-N-(1,3-thiazol-2-yl)acetamide ***(4)*** White solid, Yield: 49 & 80 % (MW), M.P.: 148–151 °C, IR: 3,350, 3,030, 2,965, 1,669, 1,610, 1,590 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.87 (*s*, 2H, –C$\underline{H}_2$), 4.12 (*s*, 1H, –NH), 7.01(*d*, $J = 6.9$, 1H, Ar–$\underline{H}$), 7.23 (*m*, 3H, Ar–$\underline{H}$), 7.42 (*d*, $J = 6.9$, 1H, Ar–$\underline{H}$), 7.51 (*d*, $J = 7.2$, 2H, Ar–$\underline{H}$), 10.41 (*s*, 1H, –N$\underline{H}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 54.28 (C$\underline{H}_2$), 111.18 (C5-1,3-thiazole), 112.61 (C3′&C5′-phenyl), 118.76 (C4′-phenyl), 130.33 (C2′&C6′-phenyl), 131.89 (C4-1,3-thiazole), 145.22 (C1′-phenyl), 161.29 (C2-1,3-thiazole), 166.72 (C=O); ESIMS *m/z* 234.1 (M^++1).

2-(o-Toluidino)-N-(1,3-thiazol-2-yl)acetamide ***(5)*** White solid, Yield: 57 & 80 % (MW), M.P.: 160–162 °C, IR: 3,345, 3,029, 2,964, 1,670, 1,620, 1,514 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.2 (*s*, 3H, –C$\underline{H}_3$), 3.90 (*s*, 2H, –C$\underline{H}_2$), 4.01 (*s*, 1H, –N$\underline{H}$), 6.49 (*d*, $J = 7.2$, 1H, Ar–$\underline{H}$), 6.97 (*m*, 4H, Ar–$\underline{H}$), 7.41 (*d*, $J = 7.2$, 1H, Ar–$\underline{H}$), 11.62 (*s*, 1H, –N$\underline{H}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.22 (C$\underline{H}_3$), 55.11 (C$\underline{H}_2$), 113.32 (C5-1,3-thiazole), 118.11 (C2′-phenyl), 119.22 (C3′-phenyl), 121.39 (C5′-phenyl), 125.66 (C4′-phenyl), 127.88 (C6′-phenyl), 131.56 (C4-1,3-thiazole), 144.29 (C1′-phenyl), 161.46 (C2-1,3-thiazole), 166.39 (C=O); ESIMS *m/z* 248.2 (M^++1).

2-(p-Toluidino)-N-(1,3-thiazol-2-yl)acetamide ***(6)*** White solid, Yield: 60 & 85 % (MW), M.P.: 162–164 °C, IR: 3,356, 3,123, 2,913, 1,664, 1,616, 1,521 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.24 (*s*, 3H, –C$\underline{H}_3$), 4.02 (*s*, 2H, –C$\underline{H}_2$), 4.13 (*s*, 1H, –N$\underline{H}$), 6.56 (*d*, $J = 6.8$, 2H, Ar–$\underline{H}$), 7.00 (*d*, $J = 7.1$, 1H, Ar–$\underline{H}$), 7.02 (*d*, $J = 6.8$, 2H, Ar–$\underline{H}$), 7.42 (*d*, $J = 7.1$, 1H, Ar–$\underline{H}$), 10.89 (*s*, 1H, –N$\underline{H}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.43 (C$\underline{H}_3$), 55.34 (C$\underline{H}_2$), 112.94 (C5-1,3-thiazole), 114.32 (C3′&C5′-phenyl), 129.11 (C4′-phenyl), 130.18 (C2′&C6′-phenyl), 131.11 (C4-1,3-thiazole), 145.33 (C1′-phenyl), 161.29 (C2-1,3-thiazole), 167.22 (C=O); ESIMS *m/z* 248.3 (M^++1).

2-(4-Methoxyphenylamino)-N-(1,3-thiazol-2-yl)acetamide ***(7)*** White solid, Yield: 57 & 85 % (MW), M.P.: 158–160 °C, IR: 3,348, 3,041, 2,898, 1,661, 1,515, 1,238, 1,033 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.72 (*s*, 3H, –OC$\underline{H}_3$), 3.95 (*s*, 2H, –C$\underline{H}_2$), 4.13 (*s*, 1H, –N$\underline{H}$), 6.60 (*d*, $J = 7.0$, 1H, Ar–$\underline{H}$), 6.78 (*d*, $J = 7.2$, 2H, Ar–$\underline{H}$), 7.00 (*d*, $J = 7.2$, 2H, Ar–$\underline{H}$), 7.42(*d*, $J = 7.0$, 1H, Ar–$\underline{H}$), 11.12 (*s*, 1H, –N$\underline{H}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 55.26 (CH_2), 56.38 (O$\underline{C}H_3$), 112.72 (C5-1,3-thiazole), 115.45 (C3′&C5′-phenyl), 116.47 (C2′&C6′-phenyl), 131.66 (C4-1,3-thiazole), 138.76 (C4′-phenyl), 154.24 (C1′-phenyl), 162.11 (C2-1,3-thiazole), 167.46(C=O); ESIMS *m/z* 264.1 (M^++1).

N-(benzo[d]thiazol-2-yl)-2-(1H-1,2,4-triazol-1-yl)acetamide ***(10)*** White solid, Yield: 70 & 85 % (MW), M.P.: 240 °C, IR: 3,045, 2,934, 1,668, 1,625, 1,267 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.01 (*s*, 2H, C$\underline{H}_2$), 7.39 (*m*, 2H, Ar–$\underline{H}$), 7.71 (*d*, $J = 6.8$, 1H, Ar–$\underline{H}$), 7.88 (*s*, 1H, N=C$\underline{H}$), 7.98 (*s*, 1H, N = C$\underline{H}$), 8.75 (*d*, $J = 7.0$, 1H, Ar–$\underline{H}$), 12.43 (*s*, 1H, N$\underline{H}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 56.57 (C$\underline{H}_2$), 120.32 (benzo[*d*]thiazole), 122.63 (benzo[*d*]thiazole), 125.16 (benzo[*d*]thiazole), 127.39 (benzo[*d*]thiazole), 132.54 (benzo[*d*]thiazole), 144.14 (C5′-triazole), 148.11 (C3′-triazole), 149.81 (benzo[*d*]thiazole), 169.33 (C2-benzo[*d*]thiazole), 175.44 (C=O); ESIMS *m/z* 260.4 (M^++1).

N-(benzo[d]thiazol-2-yl)-2-(1H-imidazol-1-yl)acetamide ***(11)*** White solid, Yield: 75 & 87 % (MW), M.P.: 230 °C, IR: 3,042, 2,920, 1,662, 1,646, 1,260 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.12 (s, 2H, C$\underline{H}_2$), 7.01 (*d*, $J = 6.6$, 1H, N–C$\underline{H}$=CH), 7.32 (*d*, $J = 7.0$, 1H, N–C$\underline{H}$=CH–), 7.45 (*s*, 1H, N=C$\underline{H}$), 7.56 (*m*, 2H, Ar–$\underline{H}$), 8.01 (*d*, $J = 7.0$, 1H, Ar–$\underline{H}$), 8.12 (*d*, $J = 7.3$, 1H, Ar–$\underline{H}$), 11.98 (*s*, 1H, N$\underline{H}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 39.47 (C$\underline{H}_2$), 119.23 (benzo[*d*]thiazole), 121.18 (C4′-imidazole), 122.30 (benzo[*d*]thiazole), 124.89 (benzo[*d*]thiazole), 126.93 (benzo[*d*]thiazole), 128.49 (C5′-imidazole), 132.36 (benzo[*d*]thiazole), 137.70 (C2′-imidazole), 150.22 (benzo[*d*]thiazole), 168.91 (C2-benzo[*d*]thiazole), 175.12 (C=O); ESIMS *m/z* 259.3 (M^++1).

N-(benzo[d]thiazol-2-yl)-2-(4-fluorophenylamino)acetamide ***(12)*** White solid, Yield: 64 & 88 % (MW), M.P.: 146–148 °C, IR: 3,398, 3,048, 2,930, 1,675, 1,648, 1,380, 1,252 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.01 (*s*, 2H, C$\underline{H}_2$), 4.15 (*s*, 1H, N$\underline{H}$), 7.02 (*d*, $J = 6.9$, 2H, Ar–$\underline{H}$), 7.12 (*d*, $J = 7.0$, 2H, Ar–$\underline{H}$), 7.45 (*m*, 2H, Ar–$\underline{H}$), 7.73 (*d*, $J =$

7.1, 1H, Ar–H), 7.80 (*d*, $J = 7.3$, 1H, Ar–H), 11.12 (*s*, 1H, –NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 54.91 (CH_2), 115.34 (C2′&C6′-4-fluorophenyl), 118.96 (benzo[*d*]thiazole), 119.12 (C3′&C5′-4-fluorophenyl), 122.11 (benzo[*d*]thiazole), 124.84 (benzo[*d*]thiazole), 126.88 (benzo[*d*]thiazole), 132.29 (benzo[*d*]thiazole), 146.32 (C1′-4-fluorophenyl), 152.33 (benzo[*d*]thiazole), 154.11 (C4′-4-fluorophenyl), 169.21 (C2-benzo[*d*]thiazole), 174.93 (C=O); ESIMS *m/z* 302.1 (M^++1).

N-(benzo[d]thiazol-2-yl)-2-(4-methoxyphenylamino)acetamide ***(13)*** White solid, Yield: 65 & 85 % (MW), M.P.: 120–122 °C, IR: 3,389, 3,041, 2,920, 1,692, 1,646, 1,265 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.82 (*s*, 3H, OCH_3), 4.11 (*s*, 2H, CH_2), 5.50 (*s*, 1H, NH), 6.95 (*d*, $J = 6.7$, 2H, Ar–H), 7.51 (*d*, $J = 6.8$, 2H, Ar–H), 7.65 (*m*, 2H, Ar–H), 7.8 (*d*, $J = 7.1$, 1H, Ar–H), 7.91 (*d*, $J = 7.3$, 1H, Ar–H), 12.61 (*s*, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 54.77 (CH_2), 56.13 (OCH_3), 116.52 (C2′&C6′-4-methoxyphenyl), 117.33 (C3′&C5′-4-methoxyphenyl), 119.14 (benzo[*d*]thiazole), 122.20 (benzo[*d*]thiazole), 123.11 (benzo[*d*]thiazole), 125.97 (benzo[*d*]thiazole), 132.67 (benzo[*d*]thiazole), 138.73 (C1′-4-methoxyphenyl), 152.92 (benzo[*d*]thiazole), 152.11 (C4′-4-methoxyphenyl), 169.33 (C2-benzo[*d*]thiazole), 174.74 (C=O); ESIMS *m/z* 314.2 (M^++1).

Pharmacological evaluation: anti-inflammatory activity-carrageenan-induced rat paw edema assay

The in vivo anti-inflammatory screening for the synthesized compounds was performed by using the functional model of carrageenan-induced rat paw edema and is presented as the percentage inhibition of edema at the right hind paw in comparison to the control (Table 2). Carrageenan-induced edema is a nonspecific inflammation, but is highly sensitive to NSAIDs. Indomethacin, a potent NSAID was used as a reference standard. COX-2-mediated increase in prostaglandin E_2 ($PG\text{-}E_2$) production contributes to the severity of the inflammatory and pain responses in this model. Wistar rats (female) weighing between 190 and 260 g, obtained from Central Animal House, Panjab University, Chandigarh, India and were used in the present study. Animals were kept in wire-mesh cages and maintained under constant environmental food and water (ad libitum), in a constant light–dark cycle. During the course of experiment, the general behavior of animals was normal. All the experimental protocols were approved by the Institutional Animal Ethical Committee (IACE), and experiments were conducted in accordance with the standard guidelines. Carrageenan and indomethacin were procured from Sigma Chemical, Co. For statistical analysis, we have used GraphPad Prism 3.0 version (GraphPad Software Inc (2007).

The synthesized compounds were tested for their anti-inflammatory activity against carrageenan-induced paw edema at dose of 10 mg/kg. The percentage inhibition of edema was calculated using the formula given below:

$$\frac{V_c - V_t}{V_c} \times 100$$

where V_c is the increase in paw volume of control (in the absence of test compound), and V_t is the increase in paw volume after administration of the test compound.

The given data are of five animals (female wistar rats) per group and divided into eleven (11) groups. Group 1 referred as control (animals received carrageenan along with the vehicle [0.5 % carboxymethylcellulose (CMC)]) and Group 2 received standard reference (indomethacin) along with the vehicle prior to the administration of carrageenan. Group 3, 4, 5, 6, 7, 8, 9, 10 and 11 received the test compounds (**3–7** and **10–13**), respectively 1 h prior to the administration of carrageenan. All the test compounds were suspended in 0.5 % of CMC and administered orally (10 mg/kg) 60 min prior to the injection of 0.1 ml of freshly prepared carrageenan (1 %) in physiological solution (154 mM NaCl) into the subplanter tissue of hind paw of each rat. The equivalent volume of carrageenan (1 %) in physiological solution was injected into hind paw of the control. The volume was measured three times using water plethysmometer prior to the administration of carrageenan, 2 and 4 h after the injection. The increase in volume of the paw was adopted as a measure of edema (Winter *et al.*, 1962). The antiedematous effects of the compounds were estimated as percentage inhibition of the induced inflammation in comparison with control. Statistical analysis was carried out using a one-way analysis of variance (ANOVA). In all cases, post hoc comparisons of the means of individual groups were performed using Tukey's test. A significance level of $p < 0.001$ denoted significance in all cases.

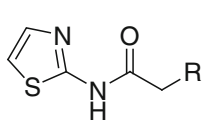
Molecular modeling study

Molecular modeling investigations were carried out using Dell Precision work station T3400 running Intel Core2 Duo Processor, 4 GB RAM, 250 GB hard disk, and NVidia Quodro FX 4500 graphics card. Maestro 9.4, GLIDE v5.9 XP docking program, Schrodinger Inc. (Maestro version and 9.4, 2013; Richard *et al.*, 2004) was employed for the docking studies.

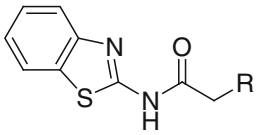
Preparation of protein

PDB structure (www.rcsb.org) **1CX2** (Crystal structure of COX-2) was downloaded, refined, and prepared using Schrodinger protein preparation wizard tool (Glide), which performs the following steps: assigning of bond orders, addition of hydrogens, optimization of hydrogen bonds by

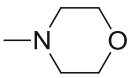
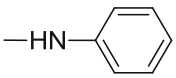
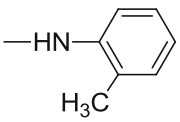
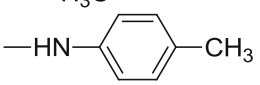
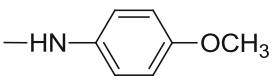
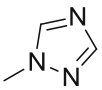
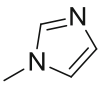
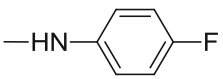
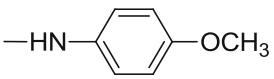
Table 2 Anti-inflammatory data of compounds **3–7** and **10–13** on carrageenan-induced paw edema in rats



(3-7)



(10-13)

Treatment	R	Volume of paw edema (ml)	
		2 h	4 h
Control (carrageenan treated)	–	0.93 ± 0.012	0.90 ± 0.020
Indomethacin[b]	–	0.21 ± 0.023[a] (77.42)	0.19 ± 0.019[a] (78.89)
3		0.36 ± 0.015[a] (61.29)	0.32 ± 0.021[a] (64.44)
4		0.41 ± 0.020[a] (55.91)	0.40 ± 0.035[a] (55.55)
5		0.39 ± 0.042[a] (58.06)	0.37 ± 0.030[a] (58.88)
6		0.37 ± 0.036[a] (60.22)	0.34 ± 0.025[a] (62.22)
7		0.36 ± 0.017[a] (61.29)	0.31 ± 0.030[a] (65.55)
10		0.32 ± 0.019[a] (65.59)	0.29 ± 0.021[a] (67.77)
11		0.29 ± 0.016[a] (68.81)	0.27 ± 0.070[a] (70.00)
12		0.26 ± 0.019[a] (72.04)	0.23 ± 0.090[a] (74.44)
13		0.27 ± 0.023[a] (70.97)	0.25 ± 0.013[a] (72.22)

Values are expressed as mean ± S.E.M ($n = 5$) and analyzed by ANOVA

Values in parenthesis (percentage inhibition of edema), 10 mg/kg

[a] Different from carragenan group ($p < 0.001$)

[b] Reference standard

flipping amino side chains, correction of charges, and minimization of the protein complex. All the bound water molecules, ligands and cofactors, were removed (preprocess) from the proteins which were taken in.mae format. The tool neutralized the side chains that are not close to the binding cavity and do not participate in salt bridges. This step is then followed by restrained minimization of cocrystallized complex, which reorients side chain hydroxyl groups and alleviates potential steric clashes. The complex obtained was minimized using OPLS_2005 force

field (George and Richard, 2001) with Polack-Ribiere Conjugate Gradient (PRCG) algorithm. The minimization was terminated either completion of 5,000 steps or after the energy gradient converged below 0.05 kcal/mol.

Preparation of ligands

Structures of the ligands (**3–7** and **10–13**) were sketched using built panel of Maestro and taken in.*mae* format. LigPrep is a utility of Schrodinger software suit that combines tools for generating 3D structures from 1D (Smiles) and 2D (SDF) representation, searching for tautomers, steric isomers, and perform a geometry minimization of the ligands. Molecular Mechanics Force Fields (OPLS_2005) with default settings were employed for the ligand minimization.

Docking studies

Docking studies were carried out using the above mentioned prepared protein (PDB: **1CX2**) and ligands (**3–7** and **10–13**), by employing Glide XP docking program (Schrodinger Inc.) following the reported procedure (Maestro version 9.4, and Glide v5.9, 2013; Richard *et al.*, 2004).

Prediction of ADME properties

The QikProp module of Schrodinger is a quick, accurate, easy-to-use absorption, distribution, metabolism, and excretion (ADME) prediction program design to produce certain descriptors related to ADME. QikProp predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules, either individually or in batches. QikProp has two modes: normal mode and fast mode. In fast mode, certain time-consuming calculations are omitted; some properties are not predicted, and some have different values. In the present study, QikProp was run in normal processing mode with default options (QikProp, version 3.6, 2013). To set up the calculation, pose viewer file (generated after docking with Glide) was used to consider the receptor and source of ligands. After choosing the receptor and ligands, by using pose viewer file (*pv.maegz*), the program QikProp that generates the descriptors were run with default options that were chosen to produce reasonable descriptors. The selected properties that are known to influence metabolism, cell permeation, and bioavailability are presented in Table 3.

Results and discussion

Chemistry

As illustrated in Scheme 1, target compounds were synthesized from 2-amino-1,3-thiazole **1** which on reaction with chloroacetyl chloride in the presence of tetrahydrofuran (THF) using triehtylamine as base maintaining the temperature between 0 and 5 °C with constant stirring resulted in the formation of 2-chloro-*N*-(1,3-thiazol-2yl)acetamide **2** in quantitative yield (Method A) (Narendra *et al.,* 2010). Similarly, the compound **2** was also synthesized in good yields by irradiating 2-amino-1,3-thiazole **1** with chloroacetyl chloride in the presence of THF using triehtylamine at 560 W hitherto unreported in the literature (Method B). The compound **2** was characterized based on a typical NH absorption at 3,496 cm^{-1}, a strong signal at 1,676 cm^{-1} of the carbonyl (C=O) group, H–Ar absorption at 3,089 cm^{-1}, and $–CH_2–$ absorption at 2,926 cm^{-1}. The ^1H NMR spectrum showed characteristic singlet at δ 3.33 (2H, $–CH_2$) ppm assignable to the two methylene protons (attached to chlorine atom). A broad singlet at δ 10.37 ppm corresponding to amine proton (–NH), and two doublets at δ 7.11 and 7.23 ppm integrated to one proton each and assignable to thiazole protons confirmed the formation of compound **2**. The ^{13}C NMR signal at δ 45.44 and 166.72 ppm indicated the presence of methylene carbon (attached to chlorine atom) and carbonyl carbon (C=O), respectively. The thiazole carbons appeared at δ 114.22 ppm (C5, thiazole), δ 130.66 ppm (C4, thiazole), and δ 159.93 ppm (C2, thiazole) with good intensity further confirmed the formation of the key intermediate compound **2**. The compound **2** on reaction with various aryl amines in basic condition under reflux for 8 h resulted in the formation of target compounds **3–7** in quantitative yields. They were also synthesized in good yields by irradiating compound **2** with aryl amines under similar basic condition at 560 W. Compounds **3–7** were characterized based on a typical NH absorption at 3,240 cm^{-1}, a strong signal at 1,702 cm^{-1} of the carbonyl group and H–Ar absorption at 3,030 cm^{-1} (except in compound **3** with $–CH_2–$ absorption at 2,928 cm^{-1}). Moreover, the appearance of an additional new broad singlet of NH signal around δ 5 ppm (except in compound **3**) along with the NH signal of amide bond around δ 10 ppm and aromatic protons of various aryl amines at around 7.5 ppm confirmed the formation of target compounds (**3–7**). The downfield shift of ^{13}C NMR signal around δ 55 ppm indicated the attachment of aromatic substituents (except compound 3 with morpholine substituent) to methylene carbon. The appearance of downfield shift of ^{13}C NMR signal at δ 61.39 and 64.23 ppm, respectively assignable to methylene carbons of morpholine moiety confirmed the formation of compound **3**. Moreover, the appearance of aromatic carbons around δ 111–140 ppm confirmed the formation of target compounds (**4–7**). The ^{13}C NMR signal at δ 18.22, 20.43, and 56.38 ppm, respectively, assignable to methyl carbon ($–CH_3$) attached to *ortho* and *para* position of aromatic moiety of compound **5** and **6** as well as methoxy

Table 3 Properties of tested compounds calculated by QikProp

Comp No.	QPlogPo/w[a]	QPlogS[b]	QPPCaco[c]	#metab[d]	Percent human oral absorption[e]	Lipinski's rule of five[f]
3	0.753	−2.507	317.043	3	76.122	0
4	0.488	−2.223	297.565	3	74.078	0
5	0.384	−2.045	319.565	3	74.019	0
6	1.58	−3.654	408.183	3	82.927	0
7	3.016	−5.036	603.636	3	94.375	0
10	2.376	−4.548	527.28	3	89.575	0
11	0.736	−3.049	182.19	3	71.714	0
12	2.337	−4.656	267.979	4	84.089	0
13	1.3	−3.228	390.352	3	80.941	0

[a] Predicted log of the octanol/water partition coefficient, range 95 % of drugs (−2 to 6.5)

[b] Predicted log of aqueous solubility S (mol/l), range 95 % of drugs (−6.5 to 0.5)

[c] Caco2 cell permeability in nm/s, range 95 % of drugs (<25 poor, >500 great). Caco-2 cells are a model for the gut-blood barrier. QikProp predictions are for nonactive transport.

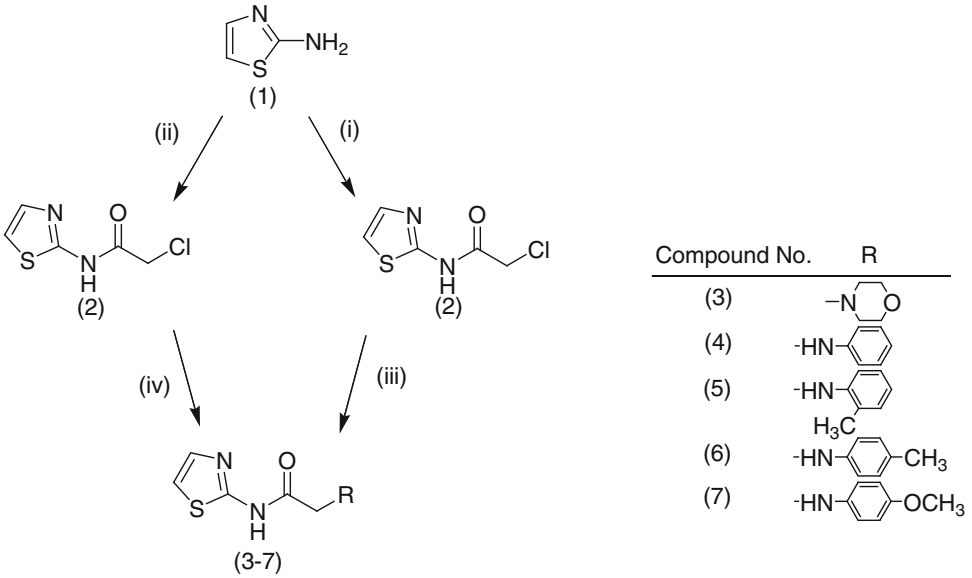
[d] Number of likely metabolic reactions. Range 95 % of drugs (1–8).

[e] Predicted human oral absorption on 0–100 % scale (>80 % is high, and <25 % is poor)

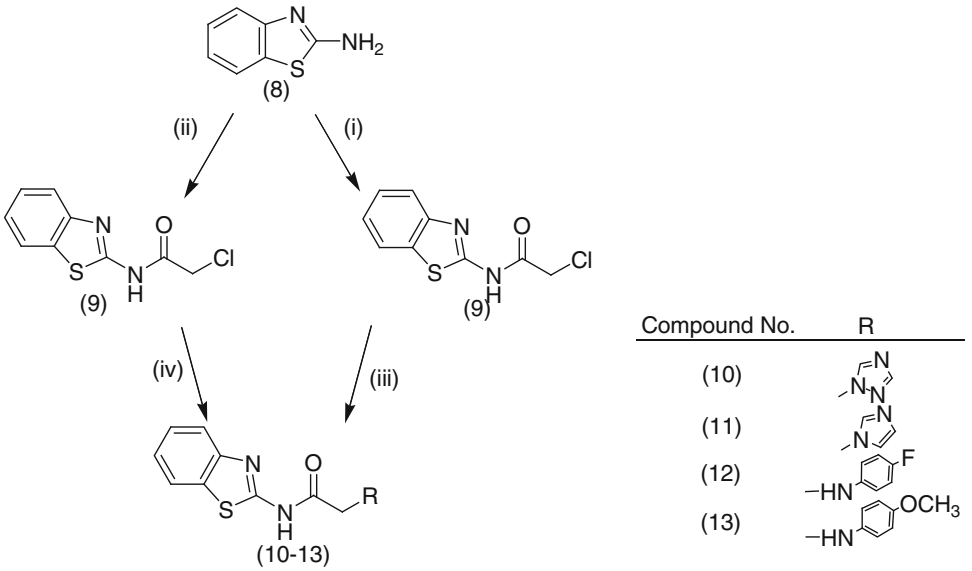
[f] Number of violations of Lipinski's rule of five. The rules are: mol_MW < 500, QPlogPo/w < 5, donorHB ≤ 5, accptHB ≤ 10. Compounds that satisfy these rules are considered druglike. (The "five" refers to the limits, which are multiples of 5.)

carbon ($–OCH_3$) attached to *para* position of compound **7**. Further, molecular ion peak (ESI–MS) corresponding to its molecular formula revealed the formation of all the products.

Similarly, the target compounds **10–13** were synthesized following scheme-2, starting from 2-amino-benzo[*d*]thiazole **8** which on reaction with chloroacetyl chloride in the presence of tetrahydrofuran (THF) using triehtylamine as a base maintaining the temperature between 0 and 5 °C with constant stirring resulted in the formation of *N*-(benzo[*d*]thiazol-2-yl)-2-chloroacetamide **9** in quantitative yield (Method A) (Guangfu *et al.*, 2001). Similarly, the compound **9** was also synthesized in good yields by irradiating 2-amino-benzo[*d*]thiazole **8** with chloroacetyl chloride under similar basic condition at 560 W hitherto unreported in the literature (Method B). The compound **9** was characterized based on a typical NH absorption at 3,505 cm^{-1}, a strong signal at 1,691 cm^{-1} of the carbonyl (C=O) group, H–Ar absorption at 3,048 cm^{-1}, and $–CH_2–$ absorption at 2,920 cm^{-1}. The ^1H NMR spectrum showed characteristic singlet at δ 4.37 (2H, $–CH_2$) ppm assignable to the two methylene protons (attached to chlorine atom). A broad singlet at δ 12.63 ppm corresponding to amine proton (–NH) and two multiplets at δ 7.85 and 7.42 ppm, respectively, integrated to two protons each and assignable to aromatic protons confirmed the formation of compound **9**. The ^{13}C NMR signal at δ 47.68 and 167.64 ppm indicated the presence of methylene carbon (attached to chlorine atom) and carbonyl carbon (C=O), respectively. The benzo[*d*]thiazole carbons appeared at δ 120.20 (benzo[*d*]thiazole), 122.54 (benzo[*d*]thiazole), 125.59 (benzo[*d*]thiazole), 127.67 (benzo[*d*]thiazole), 132.33 (benzo[*d*]thiazole), 149.94 (benzo[*d*]thiazole), 175.55 (C2-benzo[*d*]thiazole) ppm, respectively, with good intensity further confirmed the formation of the key intermediate compound **9**. The compound **9** on reaction with various aryl amines in basic condition under reflux for 2–4 h resulted in the formation of target compounds **10–13** in quantitative yields. They were also synthesized in good yields by irradiating **9** with various aryl amines in the presence of THF and triethylamine at 560 W. Compounds **10–13** were characterized based on a typical NH absorption at 3,505 cm^{-1}, a strong signal at 1,702 cm^{-1} indicating carbonyl group and H–Ar absorption at 3,030 cm^{-1}. Further, the appearance of an additional new broad singlet of NH signal around δ 6.5–7 ppm (compounds **12** & **13**) and typical triazole and imidazole proton peak at δ 7–8 ppm (compounds **10** & **11**) along with the NH signal of amide bond around δ 12 ppm and aromatic protons of various aryl amines at around 7.5 ppm confirmed the formation of target compounds **10–13**. The downfield shift of ^{13}C NMR signal around δ 55 ppm indicated the replacement of chlorine atom and attachment of aromatic substituents to methylene carbon. The appearance of downfield shift of ^{13}C NMR signal at δ 144.14 (C5′-triazole), 148.11 (C3′-triazole) ppm, respectively assignable to triazole carbons confirmed the formation of compound **10**. Similarly, the appearance of ^{13}C NMR signal at δ 121.18 (C4′-imidazole), 128.49 (C5′-imidazole), 137.70 (C2′-imidazole) ppm, respectively assignable to imidazole carbons confirmed the formation of compound **11**. Moreover, the appearance of additional aromatic carbon signals around δ 115–150 ppm confirmed the formation of target compounds **12** and **13**. The



Scheme 1 Synthesis of 2-(substituted)-*N*-(thiazol-2-yl)acetamide derivatives. Reagents and conditions: (*i*) Method A, $ClCH_2COCl$, THF, triethyl amine, 0–5 °C, 30 min; (*ii*) Method B, $ClCH_2COCl$, THF, triethyl amine, MW 560 W, 10 s; (*iii*) Method A, aryl amines, THF, triethyl amine, reflux, 8 h; (*iv*) Method B, Aryl amines, THF, Triethyl amine, MW 560 W, 45–50 s



Scheme 2 Synthesis of *N*-(benzo[*d*]thiazol-2-yl)-2-(substituted)acetamide derivatives. Reagents and conditions: (*i*) Method A, $ClCH_2COCl$, THF, Triethyl amine, 0–5 °C, 3 h; (*ii*) Method B, $ClCH_2COCl$, THF, Triethyl amine, MW 560 W, 20 s; (*iii*) Method A, Aryl amines, THF, Triethyl amine, reflux, 2–4 h; (*iv*) Method B, Aryl amines, THF, Triethyl amine, MW 560 W, 30–50 s

^{13}C NMR signal at δ 56.13 ppm assignable to methoxy carbon ($-OCH_3$) attached to *para* position of compound **13**. Further, molecular ion peak (ESI–MS) corresponding to its molecular formula revealed the formation of the product.

Under classical heating conditions, these reactions have certain disadvantages like long reaction times (30 min–8 h), high energy consumption and the need for large amounts of solvents for work up and purification. The MW-assisted reactions were carried out using a Catalyst Microwave Reactor, under constant irradiation power and by varying the temperature (the so-called "power control"). The best results were obtained when we used 80 % of the full power of the magnetron (560 W). The details of the optimized conditions employed, under MW irradiation as well as under classical heating are presented in Table 1.

Anti-inflammatory activity: Carrageenan-induced rat paw edema assay

All the title compounds were screened for their in vivo anti-inflammatory activity using the carrageenan-induced rat paw edema model and exhibited protection against carrageenan-induced edema (Table 2; Fig. 1a, b). The protection ranged up to 72 %, while the reference drug (indomethacin) showed 77 % at an equivalent dose. Among all the tested compounds, *N*-(benzo[*d*]thiazol-2-yl)-2-(substituted)acetamides **10–13** showed remarkable anti-inflammatory activities compared to other derivatives. In particular, compounds **12** and **13** with 4-fluoroaniline and 4-methoxyaniline substituent showed better activity compare to analogs **10** and **11** with triazole and imidazole

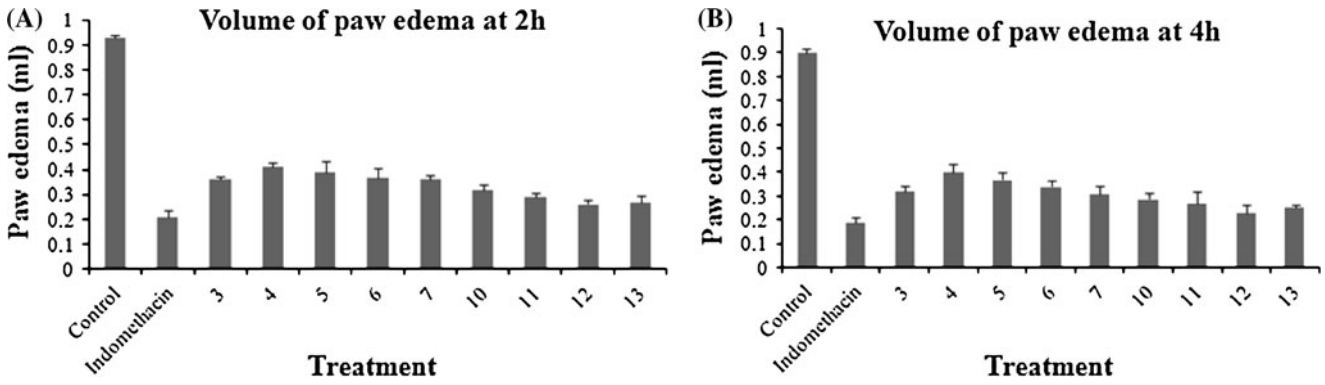


Fig. 1 Treated groups versus paw edema (ml). **a** after 2 h, **b** after 4 h

moieties. It indicates that the presence of benzene ring attached with thiazole moiety along with the presence of NH group attached to the aromatic ring is essential for showing the enhanced activity. Further it has been observed that a fluoro group at a strategic position on the heterocyclic ring enhances the activity of a molecule due to its enhanced lipophilicity (William, 2008) as it can be evident from the most active compound **12** (with 4-fluoroaniline group). The SAR of the title compounds was drawn based on docking studies and their binding mode analyses.

Molecular modeling studies

The final compounds **3**–7 and **10–13** were evaluated in silico (docking) to recognize their hypothetical binding mode using the X-ray crystal structure of COX-2 (PDB ID: 1CX2) and also to rationalize their structure activity relationships. To investigate the ability of molecular docking to reproduce an experimentally observed ligand-binding mode, the cocrystallized ligand **SC-558** (a selective COX-2 inhibitor) has been used as reference ligand (Fig. 2a). It was docked back into its binding site (Fig. 2a) of the crystal structure of the COX-2 using GLIDE docking program (Schrodinger Inc.). The top docked conformations (poses) closely resembled the cocrystallized conformation with a root-mean-square deviation (RMSD) 0.50–0.90 of nonhydrogen atomic positions of the ligand (**SC-558**).

The experimental binding mode of **SC-558** was reproduced and as shown in the Fig. 2a; the trifluoromethyl group ($–CF_3$) formed strong hydrogen bonding interactions with the exocyclic NH_2 group of Arg120 at a distance of 2.087 Å. The same group ($–CF_3$) was also found to be located in the vicinity of Leu531, Val116, Val349, Leu359, and Tyr355. The Val523 permits the location of the phenylsufonamide group of the ligand (**SC-558**) in a cavity formed by Phe518, Leu352, Val523, the backbone of Ser353, and Tyr355. Further sulphonamido group interacted with His90, Arg513, and Gln192 by forming a hydrogen bond which is the key point of interaction for COX-2 selectivity. In particular, sulfonamide $–NH_2$ was found to form hydrogen bonding with the nitrogen atom of imidazole ring of His90 and oxygen atom of Ser353 at a distance of 2.505 and 3.19485 Å, respectively, whereas sulfonamide oxygen (=O) formed hydrogen bond with the NH of Arg513. Pyrazole ring of SC-558 is found to be located within the vicinity of Tyr355 and Val349. Sulphonyl group of the ligand was located around the residues of Gln192, Ser353, Phe518, Arg513, and Val523. Bromophenyl moiety was surrounded by Gly526, Val523, and Ala527. These residues were identified as crucial and involved in the binding which perhaps contributing to the selectivity of the ligand.

Earlier from the docking studies of the indomethacin, a nonselective COX-1/2 inhibitor, it has been observed that the carboxylate ion formed a salt bridge with the Arg120 in COX-2 isoenzyme which gives a more generalized anchoring point for all the classical NSAIDs, thus, limiting their selectivity due to curtailed freedom of movement of the ligand (Balakumar *et al.*, 2010). The present docking study also revealed the similar observation (Fig. 2b). The acetyl CH_2 of indomethacin formed a hydrogen bond with the phenolic OH group of Tyr355 at a distance of 3.256 Å, whereas the chlorophenyl group showed significant π-π stacking interaction with the phenyl ring of Phe518.

Interestingly, all the thiazole as well as benzothiazole derivatives **3–7** and **10–13** docked well into the binding pocket of COX-2 (Fig. 2c–h). The NH group and fluoro group at 4th position of the 4-fluoroaniline moiety of the most active compound **12** were found to form moderate H-bond with the phenolic OH group of Tyr355 (3.108 Å) and exocyclic NH_2 group of Arg120 (3.810 Å), respectively (Fig. 2d, e), as compared to **SC-558**. Similarly, the

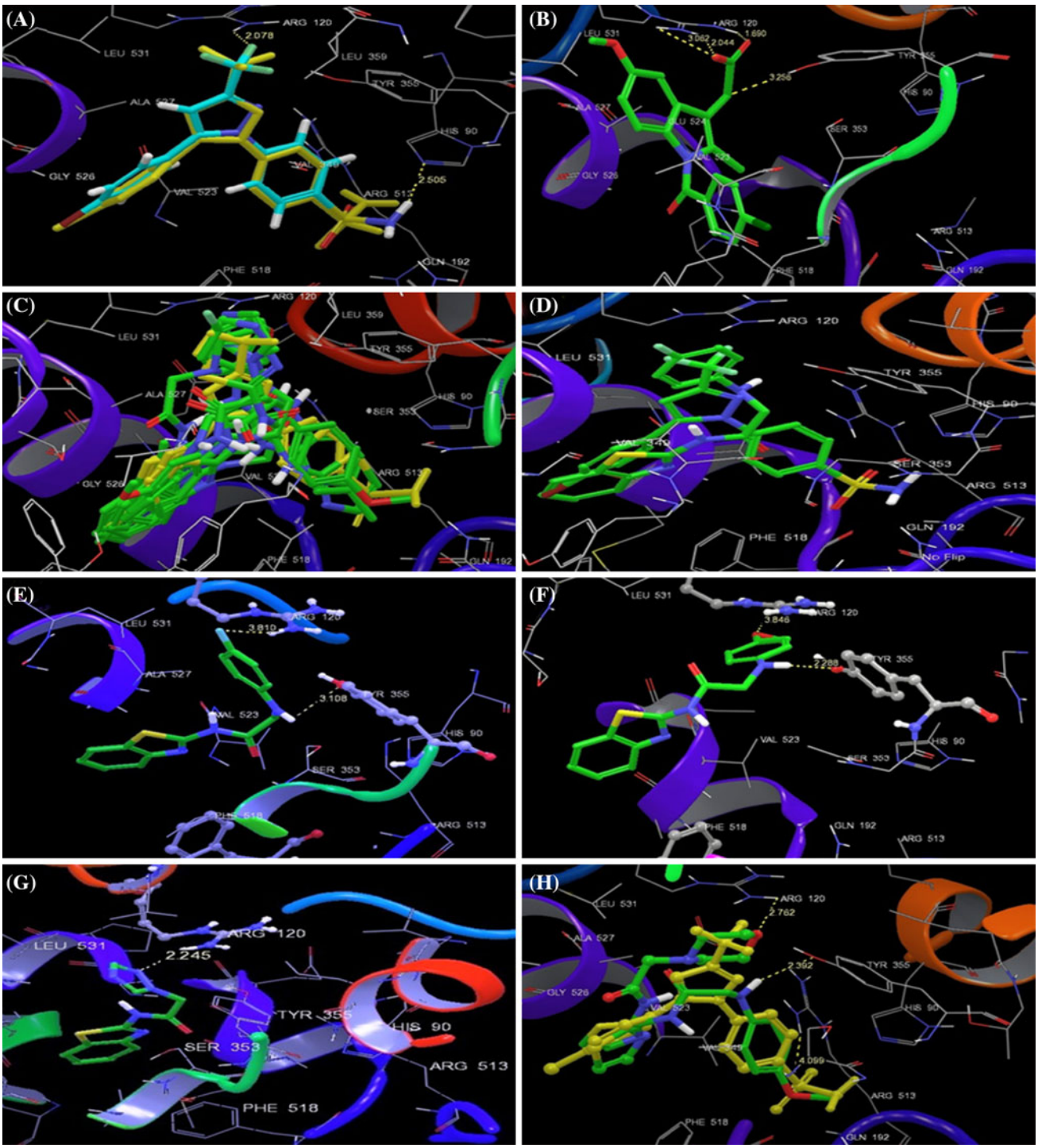


Fig. 2 **a** Superimposition of redocked **SC-558** (*cyan* color) with its original position (*yellow* color) as cocrystal in the binding site of the crystal structure of COX-2 (PDB ID: 1CX2) isoenzyme (rmsd-0.50–0.90) showing H-bond interactions with the amino acid residues His90, Arg120, Tyr355, and Arg513, respectively. **b** Binding orientation of indomethacin within the binding site of COX-2 showing salt bridge formation between the carboxylate ion and Arg120. **c** Superimposition of compounds **3–7, 10–13**, and **SC-558** original position (yellow color) and orientation in the binding site of the crystal structure of COX-2. **d** Superimposition of compound **12** and **SC-558** with their orientations in the binding site of the crystal structure of COX-2. **e** Hypothetical binding motif of compound **12** showing H-bonding interaction between F atom and N–H of fluoroaniline moiety with N–H of Arg120 and OH of Tyr355 at a distance 3.810 and 3.108 Å, respectively. **f** Hypothetical binding orientation of compound **13** showing H-bonding interaction between OH and N–H of *p*-anisidine moiety with N–H of Arg120 and OH of Tyr355 at a distance 3.846 and 2.288 Å, respectively. **g** Binding orientation of compound **10** in the binding site of the crystal structure of COX-2. **h** Superimposition of compounds **3, 7,** and **SC-558** original position (*yellow* color) and orientation in the binding site of the crystal structure of COX-2 (Color figure online)

compound **13** showed significant H-bond between the H-atom of N–H group and O-atom at 4th position of the 4-methoxyaniline group with the phenolic OH group of Tyr355 and exocyclic NH_2 group of Arg120 at a distance 2.288 and 3.846 Å, respectively (Fig. 2f). The thiazole and benzothiazole moieties of all the target compounds were found to be aligned similar to the bromophenyl ring of **SC558**, and the benzene ring of the benzothiazole moiety showed significant π–π stacking interaction with Phe518 (Fig. 2c–g) similar to indomethacin which probably account for their better anti-inflammatory efficacy compared to other analogs **3–7** of the series. Whereas in case of compounds **10** and **11**, the N-atom of triazole and imidazole moiety formed significant H-bond with the exocyclic NH_2 group of Arg120 at a distance 2.245 and 1.959 Å, respectively, (Fig. 2g) but there was no H-bond formation with Tyr355 due to lack of aromatic NH group which probably causes decrease in their activities compared to other benzothiazole derivatives.

The thiazole derivatives (**4–7**, except compound **3**), on the other hand, were found to have different orientations compared to benzothiazole derivatives where the aromatic ring attached to amino moiety was found to be aligned with phenylsulfonamido group of **SC558**. Hence they were unable to form any hydrogen bond with Arg120, but showed significant hydrogen bond formation with other important amino acid residues such as His90 and Arg513 (Fig. 2h). Moreover, these derivatives could not form any π–π stacking interaction with Phe518 which might be the reason for their overall decrease in activity as compared to benzothiazole derivatives **10–13**. Further, none of the compounds showed any kind of salt bridge formation like nonselective indomethacin. Hence it can be deduced that in silico docking, studies can enable us to get an insight and further guide us to rationally design novel and potent anti-inflammatory agents in future.

Prediction of ADME properties

The expected ADME properties of the tested compounds were evaluated with QikProp module of Schrodinger (Table 3). The selected properties are known to influence metabolism, cell permeation, and bioavailability. Almost all the predicted properties of the tested compounds were in the ranges as predicted by QikProp for 95 % of known oral drugs and also satisfy the Lipinski's rule of five to be considered as druglike potential.

Conclusion

In the present study, a series of new 2-substituted-*N*-(1,3-thiazole-2-yl)acetamide **3–7** and *N*-(benzo[*d*]thiazol-2-yl)-2-(substituted)acetamide **10–13** derivatives have been synthesized and evaluated in vivo (rat paw edema) for their anti-inflammatory activities and in silico (docking studies) to recognize the hypothetical binding motif of the title compounds with the cyclooxygenase isoenzyme (COX-2) employing GLIDE software (Schrodinger Inc.). The microwave-assisted synthesis of these compounds was found be economic, less time consuming with better yield as compared to classical heating conditions. Among all the tested compounds, *N*-(benzo[*d*]thiazol-2-yl)-2-(substituted)acetamides **10–13** showed remarkable anti-inflammatory activity compared to other derivatives (around 84–93 % of the standard: indomethacin). In particular, compounds **12** and **13** with 4-fluoroaniline and 4-methoxyaniline substituent showed better activity compared to analogs **10** and **11** with triazole and imidazole moieties. The binding mode of the title compounds has been proposed based on the docking studies. The predicted ADME properties of all the tested compounds were in the ranges as predicted by QikProp for 95 % of known oral drugs and also satisfy the Lipinski's rule of five which signifies a good absorption and hence, good bioavailability so that the observed differences in bioactivity of these compounds may be attributable to the differences in their chemical structures. Further, evaluation of these active compounds for their in vitro COX-2 binding studies is under progress.

Acknowledgments The author PKD is thankful to CSIR, New Delhi for awarding the Senior Research Fellowship [Sanction No. 09/135/(0534)/2008/EMR-I (18/03/2008)]. The authors thankfully acknowledge the Chairman, UIPS, Chandigarh for providing the facilities. Authors are also thankful to Dr. Kanwaljit Chopra, Pharmacology Division and Dr. Maninder Karan Vasisht, Pharmacognosy Division, UIPS, PU for valuable help during pharmacological studies. Authors profusely thank to Avtar Singh, SAIF (CIL), PU for carrying out the NMR studies. Author PKD is thankful to Pharmaceutical Chemistry Division, School of Pharmacy, IMU, Malaysia for providing the facilities to carry out the docking studies. Authors are also thankful to Mr. Raghurangaswamy, Executive Director, Schrodinger, India for providing the evaluation license (14 Jun–16 July, 2013) to carry out the molecular modeling studies.

References

Afshin Z, Leila N, Bahram D, Orkideh GD, Mehdi H (2007) Synthesis of 2,3-diaryl-1,3-thiazolidine-4-one derivatives as selective cyclooxygenase (COX-2) inhibitors. Bioorg Med Chem Lett 17:5634–5637

Alka B, Ruchika O, Pran Kishore D (2012) Synthesis, evaluation and docking studies on 3-alkoxy-4-methanesulfonamido acetophenone derivatives as non ulcerogenic anti-inflammatory agents. Eur J Med Chem 49:397–405

Allison MC, Howatson AG, Torrance CJ, Lee FD, Russell RI (1992) Gastrointestinal damage associated with the use of non-steroidal anti-inflammatory drugs. N Eng J Med 327:749–754

Balakumar C, Lamba P, Pran Kishore D, Lakshminarayana B, Venkat Rao K, Rajwinder K, Raghuram Rao A, Shireesha B, Narsaiah B

(2010) Synthesis, anti-inflammatory evaluation and docking studies of some new fluorinated fused quinazolines. Eur J Med Chem 45:4904–4913

Balladka KS, Bettadapura GK, Chenna GD, Basavapattana RB, Hanumanthappa M (2010) Synthesis, characterization, in vitro and molecular docking studies of new 2,5-dichloro thienyl substituted thiazole derivatives for antimicrobial properties. Eur J Med Chem 45:3490–3496

Bharti SK, Nath G, Tilak R, Singh SK (2010) Synthesis, anti-bacterial and anti-fungal activities of some novel Schiff bases containing 2,4-disubstituted thiazole ring. Eur J Med Chem 45:651–660

Charles EE, Raymond ND (1995) Eicosanoids and the gastrointestinal tract. Gastroenterology 109:285–301

Franklin PX, Ajay DP, Parendu DR, Swapnil Y, Manish N, Harish P, Kamala KV, Sudarsanam V (2008) 2-Amino-5-thiazolyl motif: a novel scaffold for designing anti-inflammatory agents of diverse structures. Eur J Med Chem 43:129–134

George AK, Richard AF (2001) Evaluation and reparametrization of the OPLS-AA force field for proteins via comparison with accurate quantum chemical calculations on peptides. J Phys Chem B 105:6474–6487

GraphPad Software Inc (2007) CA, USA

Guangfu Y, Li X, Aihong Lu (2001) Synthesis and bioactivity of novel triazolo[1,5-*a*]pyrimidine derivatives[3]. Heteroatom Chem 12:491–496

Harvey RH (1996) Prostaglandin synthase 2. Biochem Biophys Acta 1299:125–140

Jean MD, Claudiu TS, Domenico P (2005) Adverse cardiovascular effects of the coxibs. J Med Chem 48:2251–2257

Jeffery SC, Mathew JG, Karen S, Carol MK, Yan Z, John JT (1999) Design and synthesis of sulfonyl-substituted 4,5-diarylthiazoles as selective cyclooxygenase-2 inhibitors. Bioorg Med Chem Lett 9:1167–1170

Kawamori T, Rao CV, Seibert K, Reddy BS (1998) Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, against colon carcinogenesis. Cancer Res 58:409–412

Lisa EY, Dan AD (2010) Posttranscriptional regulation of cyclooxygenase 2 expression in colorectal cancer. Curr Colorectal Cancer Rep 6:60–67

Maestro version 9.4, and Glide v5.9, (2013–1) Schrödinger, LLC, New York, NY

Mamta SD, Pran Kishore D, Renu C, Tejvir S, Maninder K (2013) Synthesis, evaluation and molecular docking studies of cycloalkyl/aryl-3,4,5-trimethylgallates as potent non-ulcerogenic and gastroprotective anti-inflammatory agents. Med Chem Res doi:10.1007/s00044-013-0620-6

Masakazu B, Hiroaki T, Takeo K, Mitsuru T, Kiyotaka S, Akihiko W, Takanari T (1998) Novel antiallergic and antiinflammatory agents. Part I: synthesis and pharmacology of glycolic amide derivatives. Bioorg Med Chem 6:1069–1076

Michel T, Christine B, Chi CC, Wanda AC, Jacques YG, Robert G, Gillian G, Stacia K, Cheuk KL, Yves L, Chun SL, Gary PO, Denis R, Patrick R, Zhaoyin W, Lijing X, Petpiboon P (1997) Synthesis and biological evaluation of 5,6-diarylimidazo[2,1-*b*]thiazole as selective COX-2 inhibitors. Bioorg Med Chem Lett 7:47–52

Narendra S, Uma Shankar S, Niranjan S, Sushil K, Umesh KS (2010) Synthesis and antimicrobial activity of some novel 2-amino thiazole derivatives. J Chem Pharm Res 2:691–698

Paul SA, Kimberly AS, Michael G, Eric P, Mary S, Kenneth ID, Martin RF, Shelia J, Ronald GT, Leon JT (2003) Effects of rofecoxib or naproxen vs placebo on alzheimer disease progression a randomized controlled trial. JAMA 289:2819–2826

Pawan KS, Sawhney SN (1997) Potent antiinflammatory 3-thiazole-4(5)-acetic acids of 1,2-benzisothiazole. Bioorg Med Chem Lett 7:2427–2430

Prakash K, Mari SK, Dasappa JP, Manjathuru M, Bantwal SH, Nalilu SK (2008) Synthesis of some novel 2,4-disubstituted thiazoles as possible antimicrobial agents. Eur J Med Chem 43:261–267

QikProp, version 3.6, (2013) Schrödinger, LLC, New York

Richard AF, Jay LB, Robert BM, Thomas AH, Jasna JK, Daniel TM, Matthew PR, Eric HK, Mee S, Jason KP, David ES, Perry F, Peter SS (2004) Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. J Med Chem 47:1739–1749

Rosaria O, Rosanna M, Maria LB, Giuseppe B, Archimede R, Antonietta R, Giuseppa C, Rosanna DP, Lidia S, Salvatore C, Maria GV (2005) 5-Arylidene-2-imino-4-thiazolidinones: design and synthesis of novel anti-inflammatory agents. Bioorg Med Chem 13:4243–4252

Sara A, Afshin Z (2010) Design, synthesis and biological evaluation of new (*E*)-and (*Z*)-1,2,3-triaryl-2-propen-1-ones as selective COX-2 inhibitors. Eur J Med Chem 45:4013–4017

Shigeru K, Tetsunari T, Ken-ichi Y (2007) Cyclooxygenase-2 and tumor biology. Adv Clin Chem 43:59–78

Stefano F, Rosaria M, Mariarosaria B, Giuseppe C (2001) Dual inhibitors of cyclooxygenase and 5-lipoxygenase. A new avenue in anti-inflammatory therapy? Biochem Pharmacol 62:1433–1438

Taketo MM (1998) COX-2 and colon cancer. Inflamm Res 47:112–116

Van Gool WA, Aisen PS, Eikelenboom P (2003) Anti-inflammatory therapy in Alzheimer's disease: is hope still alive? J Neurol 250:788–792

Vane JR, Bakhle YS, Botting RM (1998) Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol 38:97–120

William KH (2008) The many roles for fluorine in medicinal chemistry. J Med Chem 51:4359–4369

William LS, David LD (1996) Prostaglandin endoperoxide H synthases-1 and -2. Adv Immunol 62:167–215

Winter CA, Risely EA, Nuss GW (1962) Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. Proc Soc Exp Biol Med 111:544–547

Xie WL, Chipman JG, Robertson DL, Erikson RL, Simmons DL (1991) Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. Proc Natl Acad Sci USA 88:2692–2696