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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Amide derivatives of [6-acyl-2-benzothiazolinon-3-yl] acetic acids as potential analgesic and anti-inflammatory compounds

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Abstract In this study, we investigated the analgesic and anti-inflammatory activities of [6-acyl-2-benzothiazolinon-3-yl]acetic acids by the derivatization of the carboxylate moiety into amides. We have tested the analgesic and anti-inflammatory activities of the synthesized compounds in vivo by using *p*-benzoquinone-induced writhing test and carrageenan-induced hind paw edema model, respectively. Compounds **4h**, **4i**, **4n**, and **4o** showed comparable analgesic and anti-inflammatory activities to the references without gastric lesions in the tested animals. In addition, all compounds also tested for their inhibitory activity against cyclooxygenase (COX)-1, COX-2 and 5-lipoxygenase (LOX), but no significant inhibition was observed under assayed conditions.

Keywords Benzothiazolinone · Analgesic · Anti-inflammatory · 5-LOX · COX-1 · COX-2

Introduction

Arachidonic acid (AA) stored in cell membranes is metabolized by two enzymatic families namely cyclooxygenases (COX-1, -2, and -3) and lipoxygenases (5-, 8-, 12-, and 15-LOX). These enzymes convert AA into prostaglandins, prostacyclines, and leukotrienes, which are involved in physiological processes as well as pathological responses such as inflammation and cancer formation (Funk 2001). Currently used

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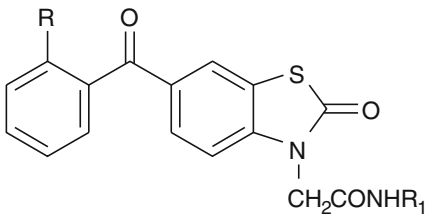


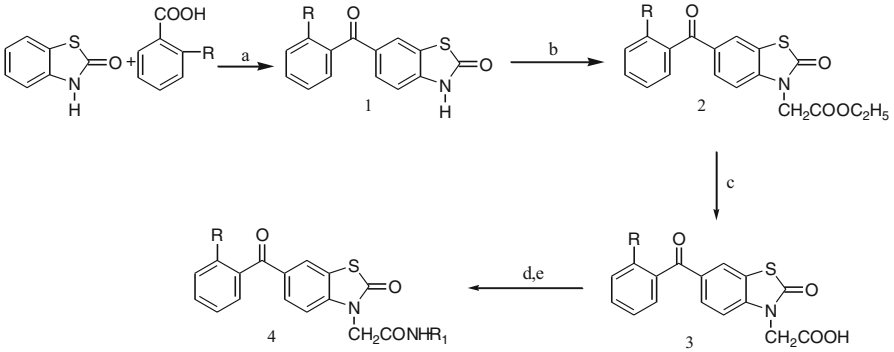
Fig. 1 General structure of the synthesized compounds

nonsteroidal anti-inflammatory drugs (NSAIDs) act through the nonselective inhibition of cyclooxygenase isoforms (COX-1 and COX-2) and show some side-effects including gastrointestinal (GI) toxicity, which appears to occur as a result of the inhibition of COX-1 isoenzyme which is involved in many physiological processes including gastric cytoprotection (Meyer-Kirchrath and Schrör 2000). Moreover, COX inhibition alone may lead to upregulation of the 5-LOX pathway, causing various side-effects especially in the GI tract and kidney (Charlier *et al*., 2003). Therefore, developing potential drugs with high analgesic and anti-inflammatory activities lacking the general side-effects of currently used NSAIDs is still a debate.

We have long been interested in developing compounds with potent analgesic and anti- inflammatory activity without GI liabilities of currently utilized NSAIDs (Banoglu *et al*., 2003; Gülcan *et al*., 2003; Cakır *et al*., 1999). Our recent studies indicated that (2-benzothiazolinon-3-yl)acetamides (Onkol *et al*., 2000) and (2-benzothiazolinon-3-yl)propionamides (Onkol *et al*., 2001, 2004) alleviated the induced pain and suppressed the induced inflammation with no observed acute gastric toxicity in the tested animals. Furthermore, 5-chloro-6-acyl-2-benzoxazolinone nucleus as an aryl/heteroaryl ketone class was determined as important structure for potent analgesic and anti-inflammatory activity. Considering that amide derivatives of certain NSAIDs such as indomethacin and ketoprofen showed some preference for COX-2 over COX-1 (Kalgutkar *et al*., 2000a), we designed and synthesized a series of compounds (Fig. 1) which might lead to further studies to develop better candidates for selective inhibition of COX-2 and 5-LOX to obtain compounds with better analgesic and anti-inflammatory activities and less gastric side-effects.

Results and discussion

In the synthesis of resulting amide derivatives, 2-benzothiazolinone**,** 6-acyl-2-benzothiazolinones (**1a–s**) and (6-acyl-2-benzothiazolinon-3-yl)acetic acids (**3a–s**) were prepared according to the previously published procedures (Fife *et al*., 1975;



Scheme 1 Synthetic route of the title compounds. **a** benzoic acid, PPA, **b** ethyl bromoacetate, potassium carbonate, acetone, **c** HCl, **d** oxalyl chloride, benzene, **e** primer amine, potassium carbonate, THF

Unlü *et al*., 2003a). Compounds **3a–s** were then treated with oxalyl chloride to prepare the corresponding acid chlorides, which were then reacted without subsequent purification with appropriate amine derivatives to obtain resulting novel amide derivatives (**4a–s**). The synthetic route for the resulting amide derivatives (**4a–s**) is outlined in Scheme 1.

The ketone function as a link between an aryl and a heterocyclic ring is long known in the structural classes of NSAIDs such as indomethacin and ketoprofen. In addition, extensive structure–activity relationship (SAR) studies have been done by preparing amide derivatives of these type of compounds and found that, by preparing certain amide derivatives, it was possible to achieve more potent antiinflammatory compounds which inhibited COX enzymes (Kalgutkar *et al*., 2000a, b, 2002, 2005). Moreover, antiinflammatory and analgesic activity of the benzoxazolinone ring and its 6-acyl derivatives have been described previously (Gökhan *et al*., 1999; Palaska *et al*., 1995; Dogruer *et al*., 1998). Therefore, we have used the bioisosteric 6-acyl-benzothiazolinone ring as a building block which might have advantage with regard to metabolic stability as compared with benzoxazolinone ring, and synthesized amide derivatives to obtain compounds with potential activity.

Analgesic activity of the synthesized amide derivatives was determined by *p*-benzoquinone-induced writhing test (Okun *et al*., 1963). As seen in Table 1, four of the amide derivatives of the (6-acyl-2-benzothiazolinon-3-yl)acetic acids (**4h**, **4i**, **4n**, and **4o**) resulted in compounds with comparable analgesic activities to aspirin. Especially, [6-(2-fluorobenzoyl)-2-benzothiazolinon-3-yl]acetamide derivatives having 4-chlorophenyl and 3-chlorophenyl substituents at the amide portion (**4h** and **4i**) exhibited analgesic activity equal to that of aspirin in *p*-benzoquinone-induced writhing test. The same was also true for compounds having the 2-chlorobenzoyl substituents in place of 2-flurobenzoyl at 6 position of benzothiazolinone ring (**4n** and **4o**). Interestingly, replacement of fluoro or chlorobenzoyl with unsubstituted benzoyl at 6-position led to a decrease in the analgesic activity. Apart from the benzamide derivatives, the amide derivatives prepared with 2-aminopyridine demonstrated lower activity, indicating that benzamides were more

Table 1 Biological activities of the title compounds

Compound	R	R_1	Analgesic activity, inhibition of writhing (%)	Antiinflammatory activity, inhibition of edema (%)				Ratio of ulceration	COX-1 inhibition (%)	COX-1 inhibition (%)	5-LOX inhibition (%)
				90 min	180 min	270 min	360 min				
4a	H	Ph	17.3	3.6	3.5	12.5	–	0/6	40.39	1.47	6
4b	H	Ph(4-Cl)	26.5	6.6	15.2	22.9	16.5	0/6	NI	23.06	NI
4c	H	Ph(3-Cl)	23.1	5.6	10.7	10.8	8.0	0/6	NI	NI	46
4d	H	2-Pyridyl	NI	NI	NI	NI	NI	0/6	49.8	NI	NI
4e	H	4-Me-2-Pyridyl	NI	NI	NI	NI	NI	2/6	50.04	6.83	11
4f	H	6-Me-2-Pyridyl	NI	NI	NI	NI	NI	1/6	NI	NI	26
4g	F	Ph	26.8	11.3	23.5	22.6	19.5	0/6	5.61	NI	24
4h	F	Ph(4-Cl)	54.3	25.9	29.0	30.3	31.7	0/6	10.36	NI	NI
4i	F	Ph(3-Cl)	49.9	15.8	18.4	25.5	28.2	0/6	20.54	19.27	31
4j	F	2-Pyridyl	18.7	NI	NI	NI	NI	0/6	17.77	NI	NI
4k	F	4-Me-2-Pyridyl	27.0	2.8	6.8	6.1	4.6	3/6	1.98	NI	19
4l	F	6-Me-2-Pyridyl	22.2	1.7	3.7	1.2	2.3	0/6	27.98	12.63	20
4m	Cl	Ph	20.6	5.3	11.0	10.9	10.5	0/6	3.42	NI	16
4n	Cl	Ph(4-Cl)	47.3	25.8	28.4	31.9	33.2	0/6	39.18	53.13	NI
4o	Cl	Ph(3-Cl)	41.8	23.9	22.0	30.4	31.3	0/6	7.27	NI	35
4p	Cl	2-Pyridyl	15.5	NI	NI	NI	NI	0/6	45.21	28.88	3
4r	Cl	4-Me-2-Pyridyl	8.1	3.8	3.5	5.1	3.7	1/6	10.05	NI	11
4s	Cl	6-Me-2-Pyridyl	15.9	NI	NI	NI	NI	0/6	9.84	2.21	28
Aspirin	–	–	50.1	–	–	–	–	3/6	–	–	–
Indomethacin	–	–	–	25.4	30.7	36.2	40.1	–	–	–	–

Table 1 continued

Compound	R	R_1	Analgesic activity, inhibition of writhing (%)	Antiinflammatory activity, inhibition of edema (%)				Ratio of ulceration	COX-1 inhibition (%)	COX-1 inhibition (%)	5-LOX inhibition (%)
				90 min	180 min	270 min	360 min				
Dup-697	–	–	–	–	–	–	–	–	100	NI	–
SC-560	–	–	–	–	–	–	–	–	NI	92	–

Analgesic and anti-inflammatory activity of the compounds were tested at 100 mg/kg doses. Analgesic activity of aspirin was tested at 100 mg/kg and anti-inflammatory activity of indomethacin was tested at 10 mg/kg dose as described in the "Experimental" section. $P < 0.05$ was found for all testing in comparison with control group. Inhibitor activity of the compounds at 10 μM concentrations on COX-1 and COX-2 were tested by using in vitro human whole blood assay as described in the "Experimental" section. Dup-697 and SC-560 was used as COX-2 and COX-1 selective references. 5-LOX inhibitory activities was done in Merck Researxh Laboratories (Canada)

important in terms of the analgesic activity and no significant differences between the [6-(2-fluorobenzoyl)- or [6-(2-chlorobenzoyl)-2-benzothiazolinon-3-yl]acetamide derivatives were observed.

Anti-inflammatory activities of the title amide derivatives were assessed by utilizing carrageenan-induced hind paw edema model (Kasahara *et al*., 1985). Since carrageenan edema has been used in the development of indomethacin, many researchers adapted this procedure for in vivo screening of the potential anti-inflammatory activity of compounds. It is known that an edema produced by carrageenan is a biphasic event and it is reported that the inhibitory effects of agents which act on the first stage of the carrageenan-induced hind paw inflammation are attributable to the inhibition of the chemical mediators such as histamine, serotonin, and bradykinin, while the second stage of the edema might be related to the arachidonic acid metabolites since it is inhibited by aspirin, indomethacin, and other cyclooxygenase inhibitors (Vinegar *et al*., 1969, 1987).

Anti-inflammatory activities of the synthesized compounds also demonstrated parallel results with their corresponding analgesic activities in which the compounds **4h**, **4i**, **4n**, and **4o** demonstrated comparable activity to indomethacin (Table 1). There were no considerable differences between the 6-(2-chlorobenzoyl)- or 6-(2-fluorobenzoyl)- derivatives of the title amide derivatives in terms of their anti-inflammatory activities, as seen with their analgesic activities. Table 1 shows that these compounds exhibited increasing anti-inflammatory activities in the second phase of carrageenan-induced edema, indicating that these compounds may exert their activities through the inhibition of enzymes which are important in the arachidonic acid cascade, thereby preventing the formation of inflammatory prostaglandins from arachidonic acid. Additionally, microscopic examination of the stomachs of tested animals showed no obvious gastric lesions or bleeding after treatment with most of the compounds in acute phase, also supporting this hypothesis.

Besides cyclooxygenases (COXs), 5-lipoxygenase (5-LOX) is also an important metabolic enzyme for formation of leukotrienes from AA, leading to inflammation and other pathological responses. Products of the 5-LOX pathway, LTB_4 and LTE_4, are indicated in inflammation and inflammation related-diseases such as atherosclerosis and cerebrovascular diseases (Werz and Steinhilber 2006; Radmark *et al*., 2007). Combined with the earlier studies that COX inhibition alone may lead to an upregulation of AA metabolism by the 5-LOX pathway, it is now appreciated that dual inhibitors against both COXs and 5-LOX might present an enhanced anti-inflammatory potency without risks of serious side-effects including gastric damage (Coruzzi *et al*., 2007; Leone *et al*., 2007). Therefore, a single agent inhibiting both enzymes has been of interest to medicinal chemists. Based on these results, we decided to screen the title amide derivatives for possible inhibitory activity on 5-LOX and COXs at 10 μM screening concentrations. Unfortunately, none of the compounds resulted in considerable inhibition of both enzymes at the tested high screening concentrations (Table 1).

We conclude that benzamide derivatives with 2-chloro or 2-fluorobenzoyl substituents in the acyl portion exhibited analgesic and anti-inflammatory activities in vivo at the second stage of inflammation in carrageenan-induced

edema model. The higher biological activity of 2-substituted benzoyl derivatives might be attributed to the effect of halogen substituent, which may force the phenyl ring to take out-of-plane conformation to the benzothiazolinone ring, causing a structure resembling indomethacin derivatives as hypothesized previously (Unlü *et al*., 2003b). Further studies are underway in our laboratory in order to investigate the effect of different alkanoic acid derivatives of the synthesized compounds on chronic inflammatory models and in vitro inhibition on COXs and 5-LOX.

Experimental

Chemistry

Benzoic acid, 2-chlorobenzoic acid, 2-fluorobenzoic acid, ethyl bromoacetate, polyphosphoric acid (PPA), and oxalyl chloride were obtained from Aldrich Co. (Germany). 2-Oxo-benzothiazoline, 6-acyl-2-benzothiazolinone (**1**), and [6-(2-chloro/fluorobenzoyl)-2-benzothiazolinon-3-yl]acetic acids (**3**) were prepared according to the previously published procedures (Unlü *et al*., 2003a; Cakır *et al*., 1997; Dalaeva *et al*., 1994; Petrov *et al*., 1994). Infrared (IR) spectra were recorded on a Bruker Vector 22 IR (Opus Spectroscopic Software Version 2.0) spectrometer (KBr, σ, cm^{-1}). ^{1}H-nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400 FT-NMR spectrometer using trimethylsilane (TMS) as an internal standard in $CDCl_3$ or dimethyl sulfoxide (DMSO)-d_6. All chemical shifts were reported as δ (ppm) values. Elemental analyses were performed with Leco-932 (C, H, N, S, O Elemental Analyzer) at the Instrumental Analysis Center of the Scientific and Technical Research Council of Turkey (Ankara, Turkey) and were within the range of $\pm 0.4\%$ of the theoretical value.

*Synthesis of 3-(6-acyl-2- benzothiazolinone-3-yl)acetamide (**4a–s**)*

(6-acyl-2-benzothiazolinon-3-yl)acetic acid (5 mmole) was dispersed in benzene (50 mL), and oxalyl chloride (1.5 mmole) was added. The final mixture was refluxed for 3 h and stirred for another 1 h at room temperature. After evaporating to dryness, tetrahydrofuran (50 mL) was added to the crude (6-acyl-2-benzothiazolinon-3-yl)acetyl chloride derivative. After adding potassium carbonate (15 mmole) and the appropriate primer amine derivative (15 mmole), the reaction mixture was refluxed for 3–8 h and then poured into ice-water. The crude product precipitated was filtered and crystallized from the appropriate solvents.

*N-(Phenyl)-2-[6-(benzoyl)-2-benzothiazolinone-3-yl]acetamide (**4a**)* Recrystallized from ethanol (yield 69%). mp 241–243°C. ^{1}H-NMR ($CDCl_3$) δ; 10.49 (1H, s, NH), 8.17 (1H, d, H^7), 7.75–7.73 (3H, m, H^5, benzoyl-$H^{2,6}$), 7.66 (1H, t, benzoyl-H^4), 7.58–7.54 (4H, m, phenyl-$H^{2,6}$, benzoyl-$H^{3,5}$), 7.47 (1H, d, H^4), 7.31 (2H, d,

phenyl-$H^{3,5}$), 7.06 (1H, t, phenyl-H^4), 4.91 (2H, s, CH_2) – IR ν_{max} cm^{-1} (KBr): 3315,1686, 1645 – Anal.: $C_{22}H_{16}N_2O_3S$.

N-(4-Chlorophenyl)-2-[6-(benzoyl)-2-benzothiazolinone-3-yl]acetamide ***(4b)*** Recrystallized from ethanol (yield 68%). mp 237–239°C. ^{1}H-NMR ($CDCl_3$) δ; 10.65(1H, s, NH), 8.17 (1H, dd, H^7), 7.75–7.72 (3H, m, H^5, benzoyl-$H^{2,6}$), 7.67 (1H, t, benzoyl-H^4), 7.60 (2H, d, phenyl-$H^{2,6}$), 7.56 (2H, t, benzoyl-$H^{3,5}$), 7.47 (1H, d, H^4), 7.38 (2H, d, phenyl-$H^{3,5}$), 4.91 (2H, s, CH_2) – IR ν_{max} cm^{-1} (KBr): 3321, 1693, 1636– Anal.: $C_{22}H_{15}ClN_2O_3S$.

N-(3-Chlorophenyl)-2-[6-(benzoyl)-2-benzothiazolinone-3-yl]acetamide ***(4c)*** Recrystallized from ethanol–toluene (yield 50%). mp 205–207°C. ^{1}H-NMR (DMSO-d_6) δ; 10.71(1H, s, NH), 8.17 (1H, d, H^7), 7.77–7.73 (4H, m, H^5, benzoyl-$H^{2,6}$, phenyl-H^2), 7.67 (1H, t, benzoyl-H^4) 7.56 (2H, t, benzoyl-$H^{3,5}$), 7.47 (1H, d, H^4), 7.43 (1H, d, phenyl-H^6), 7.35 (1H, t, phenyl-H^5), 7.14 (1H, d, phenyl-H^4), 4.92 (2H, s, CH_2) – IR ν_{max} cm^{-1} (KBr): 3312, 1693, 1644 – Anal.: $C_{22}H_{15}ClN_2O_3S$.

N-(2-Pyridyl)-2-[6-(benzoyl)-2-benzothiazolinone-3-yl]acetamide ***(4d)*** Recrystallized from Methanol (yield 48%). mp 232–234°C. ^{1}H-NMR (DMSO-d_6) δ; 11.00 (1H, s, NH), 8.35 (1H, d, pyr-H^6), 8.17 (1H, d, H^7), 7.96 (1H, d, pyr-H^3), 7.79–7.72 (4H, m, H^5, benzoyl-$H^{2,6}$, pyr-H^4), 7.66 (1H,t, benzoyl-H^4), 7.55 (1H, t, benzoyl-$H^{3,5}$), 7.48 (1H, d, H^4), 7.12 (1H, t, pyr-H^5), 4.99 (2H, s, CH_2) – IR ν_{max} cm^{-1} (KBr): 3253, 1677, 1655 – Anal.: $C_{21}H_{15}N_3O_3S$.

N-(4-Methyl-2-Pyridyl)-2-[6-(benzoyl)-2-benzothiazolinone-3-yl]acetamide ***(4e)*** Recrystallized from Ethanol (yield 55%). mp 248°C. ^{1}H-NMR (DMSO-d_6) δ; 10.92 (1H, s, NH), 8.20 (1H, d, pyr-H^6), 8.17 (1H, d, H^7), 7.83 (1H, d, pyr-H^3), 7.74–7.72 (3H, m, H^5, benzoyl-$H^{2,6}$), 7.67 (1H, t, benzoyl-H^4), 7.56 (2H, t, benzoyl-$H^{3,5}$), 7.46 (1H, d, H^4), 6.97 (1H, t, pyr-H^5), 4.98 (2H, s, CH_2), 1.73 (3H, s, CH_3) – IR ν_{max} cm^{-1} (KBr): 3208, 1682, 1644 – Anal.: $C_{22}H_{17}N_3O_3S$.

N-(6-Methyl-2-Pyridyl)-2-[6-(benzoyl)-2-benzothiazolinone-3-yl]acetamide ***(4f)*** Recrystallized from Ethanol (yield 48%). mp 200–203°C. ^{1}H-NMR (DMSO-d_6) δ; 10.96 (1H, s, NH), 8.16 (1H, d, H^7), 7.74–7.72 (4H, m, H^5, pyr-H^3, benzoyl-$H^{2,6}$), 7.67–7.65 (2H, m, benzoyl-H^4, pyr-H^4), 7.55 (2H, t, benzoyl-$H^{3,5}$), 7.45 (2H, t, H^4), 6.99 (1H, d, pyr-H^5), 4.97 (2H, s, CH_2), 2.42 (3H, s, CH_3) – IR ν_{max} cm^{-1} (KBr): 3263, 1671, 1655 – Anal.: $C_{22}H_{17}N_3O_3S$.

N-(Phenyl)-2-[6-(2-Fluorobenzoyl)-2-benzothiazolinone-3-yl]acetamide ***(4g)*** Recrystallized from Ethanol (yield 54%). mp 245–246°C. ^{1}H-NMR (DMSO-d_6) δ; 10.49 (1H, s, NH), 8.20 (1H, s, H^7), 7.75 (1H, d, H^5), 7.67–7.65 (1H, m, benzoyl-H^4), 7.59 (1H, d, benzoyl-H^6), 7.55 (2H, d, phenyl-$H^{2,6}$), 7.46 (1H, d, H^4), 7.40–7.36 (2H, m, benzoyl-$H^{3,5}$), 7.31 (2H, t, phenyl-$H^{3,5}$), 7.06 (1H, t, phenyl-H^4), 4.90 (2H, s, CH_2) – IR ν_{max} cm^{-1} (KBr): 3339, 1666 – Anal.: $C_{22}H_{15}FN_2O_3S$.

N-(4-Chlorophenyl)-2-[6-(2-Fluorobenzoyl)-2- benzothiazolinone -3-yl]acetamide ***(4h)*** Recrystallized from acetone (yield 46%). mp 247–249°C. ^{1}H-NMR (DMSO-d_6) δ; 10.64 (1H, s, NH), 8.20 (1H, s, H^7), 7.75 (1H, d, H^5), 7.67–7.65 (1H, m, benzoyl-H^4), 7.59–7.56 (3H, m, benzoyl-H^6, phenyl-$H^{2,6}$), 7.45 (1H, d, H^4), 7.40–7.36 (4H, m,benzoyl-$H^{3,5}$, phenyl-$H^{3,5}$), 4.90 (2H, s, CH_2) – IR ν_{max} cm^{-1} (KBr): 3336, 1685, 1661 – Anal.: $C_{22}H_{14}ClFN_2O_3S$.

N-(3-Chlorophenyl)-2-[6-(2-Fluorobenzoyl)-2-benzothiazolinone -3-yl]acetamide ***(4i)*** Recrystallized from acetone (yield 82%). mp 241-243°C. ^{1}H-NMR (DMSO-d_6) δ; 10.71 (1H, s, NH), 8.20 (1H, s, H^7), 7.76–7.74 (2H, m, H^5, phenyl-H^2), 7.67–7.65 (1H, m, benzoyl-H^4), 7.57 (1H, m, benzoyl-H^6), 7.47 (1H, d, H^4), 7.43 (1H, d, phenyl-H^6), 7.40–7.33 (3H, m, benzoyl-$H^{3,5}$, phenyl-H^5), 7.13 (1H, dd, phenyl-H^4), 4.91 (2H, s, CH_2) – IR ν_{max} cm^{-1} (KBr): 3334, 1667 – Anal.: $C_{22}H_{14}ClFN_2O_3S$.

N-(2-Pyridyl)-2-[6-(2-Fluorobenzoyl)-2-benzothiazolinone-3-yl]acetamide ***(4j)*** Recrystallized from ethanol (yield 27%). mp 249–251°C. ^{1}H-NMR (DMSO-d_6) δ; 11.00 (1H, s, NH), 8.35 (1H, d, pyr-H^6), 8.19 (1H, s, H^7), 7.95 (1H, d, pyr-H^3), 7.78–7.73 (2H, m, H^5, pyr-H^4), 7.68–7.63 (1H, m, benzoyl-H^4), 7.57 (1H, t, benzoyl-H^6), 7.47 (1H, t, H^4), 7.39–7.36 (2H, m, benzoyl-$H^{3,5}$), 7.13–7.11 (1H, m, pyr-H^5), 4.98 (2H, s, CH_2) – IR ν_{max} cm^{-1} (KBr): 3251, 1671 – Anal.: $C_{21}H_{14}FN_3O_3S$.

N-(4-Methyl-2-Pyridyl)-2-[6-(2-Fluorobenzoyl)-2-benzothiazolinone-3-yl]acetamide ***(4k)*** Recrystallized from acetone (yield 36%). mp 231–233°C. ^{1}H-NMR (DMSO-d_6) δ; 10.92 (1H, s, NH), 8.19 (1H, s, H^7), 8.18 (1H, s, pyr-H^6), 7.82 (1H, d, pyr-H^3), 7.74 (1H, d, H^5), 7.68–7.64 (1H, m, benzoyl-H^4), 7.57 (1H, t, benzoyl-H^6), 7.45 (1H, d, H^4), 7.40–7.36 (2H, m, benzoyl-$H^{3,5}$), 6.96 (1H, d, pyr-H^5), 4.97 (2H, s, CH_2), 2.26 (3H, s, CH_3) – IR ν_{max} cm^{-1} (KBr): 3208, 1680, 1655 – Anal.: $C_{22}H_{16}FN_3O_3S$.

N-(6-Methyl-2-Pyridyl)-2-[6-(2-Fluorobenzoyl)-2-benzothiazolinone-3-yl]acetamide ***(4l)*** Recrystallized from acetone (yield 45%). mp 263–265°C. ^{1}H-NMR (DMSO-d_6) δ; 10.96 (1H, s, NH), 8.19 (1H, d, H^7), 7.74 (1H, s, pyr-H^3), 7.73 (1H, s, H^5), 7.66–7.63 (2H, m, pyr-H^4, benzoyl-H^4), 7.57 (1H, t, benzoyl-H^6), 7.45 (2H, d, H^4), 7.39–7.35 (2H,m, benzoyl-$H^{3,5}$), 6.98 (1H, d, pyr-H^5), 4.96 (2H, s, CH_2), 2.41 (3H, s, CH_3) – IR ν_{max} cm^{-1} (KBr): 3262, 1671, 1654 – Anal.: $C_{22}H_{16}FN_3O_3S$.

N-(Phenyl)-2-[6-(2-chlorobenzoyl)-2-benzothiazolinone-3-yl]acetamide ***(4m)*** Recrystallized from ethanol (yield 66%). mp 214-215°C. ^{1}H-NMR (DMSO-d_6) δ; 10.48 (1H, s, NH), 8.15 (1H, s, H^7), 7.69 (1H, dd, H^5), 7.61–7.54 (4H, m, benzoyl-$H^{4,5}$, phenyl-$H^{2,6}$), 7.50 (2H, d, benzoyl-$H^{3,6}$), 7.46 (1H, d, H^4), 7.30 (2H, t, phenyl-$H^{3,5}$), 7.05 (1H, t, phenyl-H^4), 4.89 (2H, s, CH_2) – IR ν_{max} cm^{-1} (KBr): 3311, 1684, 1644 – Anal.: $C_{22}H_{15}ClN_2O_3S$.

N-(4-Chlorophenyl)-2-[6-(2-chlorobenzoyl)-2-benzothiazolinone-3-yl]acetamide ***(4n)*** Recrystallized from ethanol (yield 57%). mp 218–219°C. ^{1}H-NMR (DMSO-d_6) δ; 10.64 (1H, s, NH), 8.15 (1H, d, H^7), 7.69 (1H, dd, H^5), 7.61–7.56 (4H, m, benzoyl-$H^{4,5}$, phenyl-$H^{2,6}$), 7.50 (2H, d, benzoyl-$H^{3,6}$), 7.46 (1H, d, H^4), 7.37 (2H, d, phenyl-$H^{3,5}$), 4.90 (2H, s, CH_2) – IR ν_{max} cm^{-1} (KBr): 3260, 1690, 1667 – Anal.: $C_{22}H_{14}Cl_2N_2O_3S$.

N-(3-Chlorophenyl)-2-[6-(2-chlorobenzoyl)-2-benzothiazolinone-3-yl]acetamide ***(4o)*** Recrystallized from ethanol (yield 61%). mp 235–236°C. ^{1}H-NMR (DMSO-d_6) δ; 10.71 (1H, s, NH), 8.16 (1H, d, H^7), 7.75 (1H, s, phenyl-H^2), 7.69 (1H, dd, H^5), 7.61–7.57 (2H, m, benzoyl-$H^{4,5}$), 7.50 (2H, d, benzoyl-$H^{3,6}$), 7.46 (1H, d, H^4), 7.42 (1H, d, phenyl-H^6), 7.34 (1H, t, phenyl-H^5), 7.13 (2H, d, phenyl-H^4), 4.90 (2H, s, CH_2) – IR ν_{max} cm^{-1} (KBr): 3291, 1699, 1650 – Anal.: $C_{22}H_{14}Cl_2N_2O_3S$.

N-(2-Pyridyl)-2-[6-(2-chlorobenzoyl)-2-benzothiazolinone-3-yl]acetamide ***(4p)*** Recrystallized from ethanol (yield 38%). mp 184–185°C. ^{1}H-NMR (DMSO-d_6) δ; 11.00 (1H, s, NH), 8.34 (1H, d, pyr-H^6), 8.15 (1H, d, H^7), 7.93 (1H, s, pyr-H^3), 7.76 (1H, t, pyr-H^4), 7.68 (1H, dd, H^5), 7.61–7.56 (2H, m, benzoyl-$H^{4,5}$), 7.50 (2H, d, benzoyl-$H^{3,6}$), 7.46 (1H, d, H^4), 7.12 (1H, t, pyr-H^5), 4.98 (2H, s, CH_2) – IR ν_{max} cm^{-1} (KBr): 3253, 1676, 1649 – Anal.: $C_{21}H_{14}ClN_3O_3S$.

N-(4-Methyl-2-pyridyl)-2-[6-(2-chlorobenzoyl)-2-benzothiazolinone-3-yl]acetamide ***(4r)*** Recrystallized from ethanol (yield 34%). mp 203–205°C. ^{1}H-NMR (DMSO-d_6) δ; 10.32 (1H, s, NH), 8.19 (1H, d, pyr-H^6), 8.15 (1H, d, H^7), 7.82 (1H, d, pyr-H^3), 7.67 (1H, dd, H^5), 7.59–7.57 (2H, m, benzoyl-$H^{4,5}$), 7.50 (2H, d, benzoyl-$H^{3,6}$), 7.45 (1H, d, H^4), 6.96 (1H, d, pyr-H^5), 4.96 (2H, s, CH_2), 2.26 (3H, s, CH_3) – IR ν_{max} cm^{-1} (KBr): 3205, 1685, 1654 – Anal.: $C_{22}H_{16}ClN_3O_3S$.

N-(6-Methyl-2-pyridyl)-2-[6-(2-chlorobenzoyl)-2-benzothiazolinone-3-yl]acetamide ***(4s)*** Recrystallized from acetone (yield 55%). mp 250°C. ^{1}H-NMR (DMSO-d_6) δ; 10.96(1H, s, NH), 8.15 (1H, d, H^7), 7.73 (1H, s, pyr-H^3), 7.68 (1H, dd, H^5), 7.64 (1H, t, pyr-H^4), 7.61–7.56 (2H, m, benzoyl-$H^{4,5}$), 7.50 (2H, d, benzoyl-$H^{3,6}$), 7.44 (1H, d, H^4), 6.98 (1H, d, pyr-H^5), 4.95 (2H, s, CH_2), 2.41 (3H, s, CH_3) – IR ν_{max} cm^{-1} (KBr): 3264, 1692, 1669 – Anal.: $C_{22}H_{16}ClN_3O_3S$.

Pharmacology

Animals

Male Swiss albino mice (20–25 g) were used for all experiments. The animals were kept in colony cages (six mice each), maintained on standard pellet diet, water ad libitum, and left for 2 days for acclimatization before the experimental session. The food was withdrawn on the day before the experiment, but free access of water was allowed. All experiments were carried out according to the suggested ethical guidelines for the care of laboratory animals.

Preparation of test samples for bioassay

Test samples were suspended in a mixture of distilled H_2O and 0.5% sodium carboxymethyl cellulose (CMC) and were given orally to the test animals. The animals of the control group received the same experimental handling except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. Either indomethacin (10 mg/kg) or acetyl salicylic acid (ASA) in 0.5% CMC (100 mg/kg) was used as reference drug.

p-Benzoquinone-induced writhing test (Okun et al., 1963)

Sixty minutes after oral administration of test samples, the mice were injected intraperitoneally with 0.1 mL/10 g body weight 2.5% (v/v) *p*-benzoquinone (PBQ) solution in distilled H_2O (PBQ, Merck, Darmstadt, Germany). Control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for observation and the total number of abdominal contractions (writhing movements) was counted for the next 15 min, starting on the fifth min after the PBQ injection. The data represent average values of the total number of writhes observed. The analgesic activity was expressed as percentage change from writhing controls.

Carrageenan-induced hind paw edema test

The test was performed according to the method of Kasahara *et al.* (1985). The difference in footpad thickness between the right and left foot was measured with a pair of dial thickness gauge calipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods. Sixty minutes after the oral administration of the test sample or dosing vehicle each mouse was injected with freshly prepared (0.5 mg/25 mL) suspension of carrageenan (Sigma, St. Louis, Mo, USA) in physiological saline (154 mM NaCl) into subplantar tissue of the right hind paw and 25 μL saline solution was injected into that of the left hind paw as secondary control. Measurements were done and evaluated every 90 min during 360 min after induction of inflammation, as described above.

Gastric side ulceration effects

After 2 h the analgesic activity experiment was done; mice were killed under deep ether anesthesia and stomachs were removed. Then the abdomen of each mouse was opened through great curvature and examined under dissecting microscope for lesion or bleedings.

Acute toxicity

Animals employed in the carrageenan-induced paw edema experiment were observed during 48 h and mortality was recorded where present for each group at the end of observation period.

Statistical analysis of data

Data obtained from the animal experiments were expressed as the mean standard error ± standard error on the mean (SEM). Statistical differences between the treatments and the control were tested by analysis of variance (ANOVA) test. Data with p-value < 0.05 was considered to be significant.

COX-1 and COX-2 inhibitory activity

All compounds were tested for their ability to inhibit human COX-1 and COX-2 using in vitro human whole blood assay as described by Patrignani *et al*. (1994). Each drug was evaluated at 10 μM concentration in triplicate determinations. DuP-697 and SC-560 were used as selective COX-2 inhibitor and selective COX-1 inhibitor references, respectively, and assayed at 1 μM for both COX-2 and COX-1 assay.

COX-1 activity in human whole blood

Fresh blood from healthy volunteers who had not taken any NSAIDs for at least 7 days prior to blood extraction was collected in tubes containing no anticoagulants. Aliquots of 500 μL blood were incubated either with 1 μL vehicle (DMSO) or 1 μL test compound solution (10 μM) for 1 h at 37°C. Plasma was separated by centrifugation (5 min at 13,000 rpm, 4°C) and TXB_2 levels were measured using the Correlate-EIA^{TM} TXB_2 Enzyme Immunoassay Kit from Assay Design Inc. (Ann Arbor, MI, USA).

COX-2 activity in human whole blood

Fresh blood from healthy volunteers who had not taken any NSAIDs for at least 7 days prior to blood extraction was collected in ethylene diamine tetraacetic acid (EDTA)-containing tubes. Aliquots of 500 μL blood were incubated either with 1 μL vehicle (DMSO) or 1 μL test compound solution (10 μM) in presence of LPS (10 μg/mL) for 24 h at 37°C to induce COX-2 expression. Plasma was separated by centrifugation (5 min at 13000 rpm, 4°C) and PGE_2 levels were measured using the Correlate-EIA^{TM} PGE_2 Enzyme Immunoassay Kit from Assay Design Inc. (Ann Arbor, MI).

5-LOX inhibitory activity

The inhibitor activity of the title compounds was screened at 10 μM in Merck Research Laboratories, Canada as a courtesy of Dr. Zhaoyin Wang.

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