ChemInform Abstract: Thiazolo[3,2-b]-1,2,4-triazole-5(6H)-one Substituted with Ibuprofen: Novel Non-Steroidal Antiinflammatory Agents with Favorable Gastrointestinal Tolerance.

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Original article

Thiazolo[3,2-*b*]-1,2,4-triazole-5(6*H*)-one substituted with ibuprofen: Novel non-steroidal anti-inflammatory agents with favorable gastrointestinal tolerance

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ABSTRACT

In an effort to establish new candidates with improved analgesic and anti-inflammatory activities and lower ulcerogenic risk, a series of thiazolo[3,2-*b*]-1,2,4-triazole-5(6*H*)-one derivatives of ibuprofen were synthesized. All compounds were evaluated for their *in vivo* anti-inflammatory and analgesic activities in mice. Furthermore, the ulcerogenic risks of the compounds were determined. In general, none of the compounds represent a risk for developing stomach injury as much as observed in the reference drugs ibuprofen and indomethacin. The compounds carrying a 3-phenyl-2-propenylidene (**1a**), (biphenyl-4-yl) methylidene (**1f**) and (1-methylpyrrol-2-yl)methylidene (**1n**) at the 6th position of the fused ring have been evaluated as potential analgesic/anti-inflammatory agents without a gastrointestinal side effect. These new compounds, therefore, deserve further attention to develop new lead drugs.

1. Introduction

Inflammation is a spontaneous biological response of vascular tissues to harmful stimuli, such as pathogens, chemicals, thermal and mechanical injuries. It is produced and controlled by the interaction of a wide range of inflammatory mediators such as histamine, 5-hydroxytryptamine, various chemotactic factors such as bradykinin, leukotrienes, prostaglandins [1]. Therefore, a variety of drugs that antagonize the chemical mediators of inflammation, individually or in combination, could be effective to alleviate the signs of it. However, prostaglandin synthesis inhibitors such as non-steroidal anti-inflammatory drugs (NSAIDs) and steroidal anti-inflammatory drugs (glucocorticoids) are extensively used in the treatment of inflammation. The NSAIDs such as acetylsalicylic acid, indomethacin and ibuprofen inhibit early steps in the biosynthesis pathway of prostaglandins by the inhibition of cyclooxygenase (COX) enzymes, and are the main drugs used to reduce the adverse consequences of inflammation [1]. However, the side effects including gastrointestinal (GI) perforation, ulceration, bleeding and renal toxicity of currently available NSAIDs pose a major problem in their clinical use. The gastrointestinal damage from NSAIDs is generally attributed to two factors: local irritation by the carboxylic acid moiety, common to most of NSAIDs (topical effect), and decreased cytoprotective prostaglandin production [2–5]. The use of steroidal drugs as anti-inflammatory agents is also becoming highly controversial due to their multiple side effects [6]. Thus, in spite of the tremendous advances in the last decade, the design and development of a safe, effective and economical therapy for treating inflammatory conditions still presents a major challenge.

To develop novel compounds that are biologically effective but devoid of side effects inherent in traditional NSAIDs, several strategies have been reported on the modification of well-known nonselective NSAIDs. Studies describe that the derivatization of the carboxylate function of representative NSAIDs result in increased anti-inflammatory activity with reduced ulcerogenic potential [7–12]. Furthermore, certain compounds bearing 1,3,4-oxadiazole/thiadiazole and 1,2,4-triazole nucleus have emerged as a promising group of substances [13–19].

In view of the aforementioned reports, the decision was made to synthesize new compounds by combining thiazolo[3,2-*b*]-1,2,4-triazole ring with ibuprofen, (*S*)-naproxen and flurbiprofen, which are members of the 2-arylpropionic acid (profen) family of NSAIDs, in order to improve their safety profile while maintaining full anti-inflammatory/analgesic activity. One of the most interesting characteristics of these novel compounds is their non-acidic structure,

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which differentiates them from the classic acidic nonsteroidal anti-inflammatory/analgesic agents. The preliminary screening test results have revealed that the incorporation of the free carboxylic acid group of NSAIDs into the thiazolo[3,2-*b*]-1,2,4-triazole ring is often accompanied by decreased gastric toxicity *in vivo* [20–22].

Prompted by these promising results, and in continuation of the efforts toward the development of new molecules exhibiting analgesic/anti-inflammatory activity, it is worthwhile to carry on further structural modification on these hybrid compounds. In this study, new analogs of thiazolo[3,2-*b*]-1,2,4-triazole-5(6*H*)-one carrying ibuprofen residue were synthesized by bioisosteric replacement of substituted benzylidene at the 6th position of the ring with various arylalkyls, a bulky ring (biphenyl and napthyl) or a heterocyclic ring. The present report provides an extended study of the chemistry and evaluation of analgesic/anti-inflammatory activities, as well as the gastric risk of a new series of these compounds.

2. Chemistry

Over the last fifteen years, our research group has reported the general synthesis of 3-substituted-1,2,4-triazole-5-thiones, which are fascinating 1,3-dinucleophilic building blocks, and have demonstrated their use in the preparations of a wide range of heterocyclic compounds. In this study, the synthesis of desired compounds, Scheme 1 was followed. The starting compound, (±)-3-[1-(4-(2-methylpropyl) phenyl)ethyl]-1,2,4-triazole-5-thione (**1**) was prepared via dicyclohexylcarbodiimide (DCC)-promoted amid formation reaction starting from ibuprofen in three steps by employing the previously reported procedure [20]. In brief, ibuprofen reacted with *N*-hydroxysuccinimide in the presence of DCC to obtain *N*-[2-(4-(2-methylpropyl)phenyl)propanoyloxy]succinimide. The resulting ester reacted with thiosemicarbazide to yield 1-acylthiosemicarbazide. Cyclization of the intermediate thiosemicarbazide by heating with NaOH 10% under reflux, followed by the acidification with concentrated hydrochloric acid, gave rise to the desired (±)-3-[1-(4-(2-methylpropyl)phenyl)ethyl]-1,2,4-triazole-5-thione (**1**). The melting point of the compound was in accordance with the literature. Therefore, the next step of the reaction was carried out without any further analysis [20].

6-Substituted thiazolo[3,2-*b*]-1,2,4-triazole-5(6*H*)-ones (**1a–1o**) were synthesized by the cyclization of the mercaptotriazole (**1**) with chloroacetic acid and relevant aldehydes in the presence of acetic acid, acetic anhydride and anhydrous sodium acetate in one step in 10–51% yields.

During the reaction of 1,2,4-triazole-5-thione with various dielectrophiles, two isomeric cyclization products, such as thiazolo [3,2-*b*]-1,2,4-triazole or thiazolo[2,3-*c*]-1,2,4-triazole, can be obtained. The mode of cyclization and the configuration around the C=C double bond of similar compounds was assigned on the basis of X-ray crystallographic analysis earlier [23,24], and it was proven that cyclocondensation proceeded regioselectively in the N1 nitrogen atom, and the phenyl ring was located in a *cis* (*Z*) configuration at the 6th position.

All new condensed compounds were characterized by their melting points, elementary analysis, IR, ^{1}H and APT ^{13}C NMR and mass spectra. The spectral data are in agreement with the proposed structures (Tables 1–3). The structure of compounds **1a–1o** was confirmed by the presence of a band between 1762 and 1724 cm^{-1} for C=O of the lactam group in their IR spectra. In the ^{1}H NMR spectra of the compounds, the methylidene proton has been exhibited at δ 7.50–8.56 ppm as a singlet, and sometimes in the aromatic region together with aromatic protons. All the other protons were observed accordingly to the expected chemical shift and integral values (Table 2). The APT ^{13}C NMR spectra of the compounds showed the characteristic lactam C=O carbon of the condensed ring at approximately 176 ppm (Table 3). The mass spectroscopic analysis of the compounds was studied under electron spray ionization. Either quasimolecular ions [M$^+$ + H] or [M$^+$ + Na] peaks confirmed the molecular weights of the examined compounds.

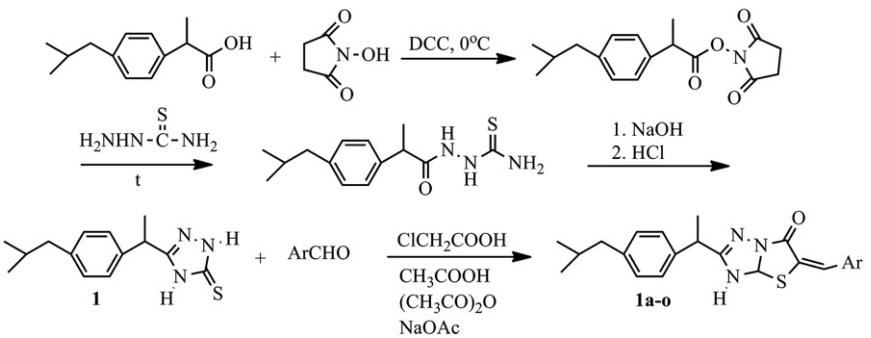
3. Pharmacology

In pharmacological studies, anti-inflammatory and analgesic activity as well as the ulcerogenic risk of the synthesized compounds were investigated. In order to screen the anti-inflammatory profile, the carrageenan-induced hind-paw edema model in mice was used [25]. The analgesic activity of the compounds was studied using both tail-flick assay and the hot plate test [26,27]. In the tail-flick test, latency until the mouse flicked its tail away from the heat source was measured. In the hot plate test, latency of forepaw licking or jump response was evaluated for each animal as an index of nociception. The evaluation of anti-inflammatory and analgesic activities was carried out orally at two dose levels of 50 and 100 mg/kg. To observe the maximum possible effect, the experiments began with a dose level of 100 mg/kg. However, the dose was lowered by half to 50 mg/kg in order to investigate the therapeutic effects of the compounds. The gastric safety on acute administration was also conducted by microscopic examination, and this test was carried out after the carrageenan-induced hind-paw edema test. For the purpose of comparison, two nonselective COX inhibitors, indomethacin (10 and 50 mg/kg) and ibuprofen (50 and 100 mg/kg), and an opioid analgesic oxycodone (50 and 100 mg/kg), which acts on a different mechanism than COX inhibition, were used as the positive control.

4. Results and discussion

4.1. Anti-inflammatory activity

Fig. 1 shows the results of the anti-edematous effect of orally administered test compounds on carrageenan-induced paw edema



Scheme 1. The reaction leading to thiazolo[3,2-*b*]-1,2,4-triazole derivatives **1a–1o**.

Table 1
Melting points, reaction yields and formulas of the compounds synthesized.

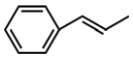
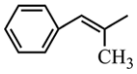
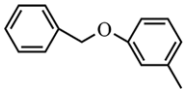
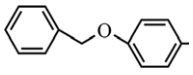
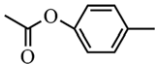
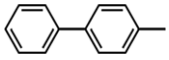
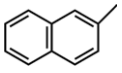
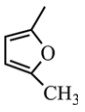
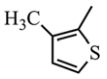
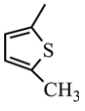
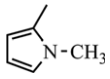
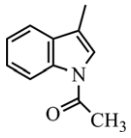
Compounds	*R*	Yield %	M.p. (°C)	Formula (M.W. g/mol)
1a		30	203–205	$C_{25}H_{25}N_3OS$ (415)
1b		14	148–150	$C_{26}H_{27}N_3OS$ (429)
1c		46	123–125	$C_{30}H_{29}N_3O_2S$ (495)
1d		41	180–182	$C_{30}H_{29}N_3O_2S$ (495)
1e		35	140–142	$C_{25}H_{25}N_3O_3S$ (447)
1f		23	160–162	$C_{29}H_{27}N_3OS$ (465)
1g		22	195–196	$C_{27}H_{25}N_3OS$ (439)
1h		51	146–148	$C_{21}H_{21}N_3O_2S$ (379)
1i		43	131–133	$C_{22}H_{23}N_3O_2S$ (393)
1j		45	185–187	$C_{21}H_{21}N_3OS_2$ (395)
1k		41	198–200	$C_{22}H_{23}N_3OS_2$ (409)
1l		26	178–180	$C_{22}H_{23}N_3OS_2$ (409)

Table 1 (*continued*)

Compounds	*R*	Yield %	M.p. (°C)	Formula (M.W. g/mol)
1m		49	242–244	$C_{21}H_{20}BrN_3OS_2$ (475)
1n		10	150 (dec.)	$C_{22}H_{24}N_4OS$ (392)
1o		30	165–167	$C_{27}H_{26}N_4O_2S$ (470)

in mice at 50 and 100 mg/kg doses. In the oral administration of a 50 mg/kg dose, all compounds except **1**, **1a** and **1e** showed moderate to high anti-inflammatory activity, ranging from 30% to 56%, while ibuprofen, indomethacin and oxycodone references showed 55%, 53% and 1% of anti-inflammatory activity, respectively. Compounds **1b**, **1c**, **1d**, **1g**, **1j**, **1n** and **1o** were found as potent as ibuprofen and indomethacin (not statistically significant compared to references). Compounds **1c**, **1d** and **1o** have the highest anti-inflammatory activity among all. The compounds, when administered in a two-fold dose (100 mg/kg, p.o.), exhibited a higher activity profile compared to the 50 mg/kg dose level (except **1**, **1d**, **1l**, **1n** and **1o**). All derivatives except **1** and **1l** showed noticeable anti-inflammatory activity compared to ibuprofen at 100 mg/kg. Especially compounds **1a**, **1c**, **1f** and **1m** showed the most remarkable activity at this dose. In this test, oxycodone, which acts on opioid receptors and has no COX inhibitory activity, exhibited no anti-inflammatory activity in both doses as expected, whereas ibuprofen and indomethacin (non-selective COX inhibitors) showed the highest anti-inflammatory activity.

4.2. Analgesic activity

4.2.1. Tail-flick test

In analgesic activity experiments, most of the compounds exhibited some degree of analgesic activity in both the hot plate and tail-flick tests (Figs. 2 and 3). Oral administration of all synthesized derivatives produced markedly increased latency of the tail-flick response compared to vehicle control at the 50 mg/kg dose level (except compounds **1**, **1e**, **1h** and **1k**). Especially compounds **1f**, **1l** and **1n** have attracted attention with higher or almost equivalent activity to ibuprofen (50%) with percentage activity values **53**%, **46**% and **47**%, respectively, at the 50 mg/kg dose level. When the administered dose was increased two-fold, some of the compounds (compounds **1**, **1a**, **1b**, **1d**, **1h**, **1i**, **1j**, **1l** and **1n**) increased the latencies of the tail-flick response compared to vehicle control. Compounds **1a**, **1b**, **1i**, **1j**, **1l** and **1n** exhibited a similar activity profile with ibuprofen at this dose level. None of the test compounds reached the analgesic activity of narcotic analgesic oxycodone (Fig. 2).

4.2.2. Hot plate test

All synthesized derivatives except **1**, **1g**, **1h** and **1k** produced significant inhibition of the hot plate paw-licking response compared to the vehicle control at a dose of 50 mg/kg. Among the compounds, compounds **1j** (41%) and **1n** (44%) possessed the most prominent and consistent activity compared to ibuprofen (46%) at 50 mg/kg. With an oral dose of 100 mg/kg, all synthesized derivatives except compound **1** showed statistically significant analgesic activity compared to vehicle control. However, derivatives **1d**, **1f**, **1h**, **1j**, **1l** and **1n** were found as potent as ibuprofen. Interestingly, increasing the dose to 100 mg/kg did not cause a dramatic increase in analgesic activity (except **1d** and **1h**). Similar to the tail-flick test, none of the compounds reached the analgesic activity of oxycodone (Fig. 3).

When compared to the results of the tail-flick and hot plate tests, all test compounds (except compounds **1b** and **1l** at the 50 mg/kg dose; **1b** and **1i** at the 100 mg/kg dose) exhibited similar analgesic activity profile in both tests. Some of the compounds with notable anti-inflammatory properties coupled with a good analgesic activity profile (such as **1j**, **1n** and **1o** at the 50 mg/kg dose; **1a**, **1d** and **1f** at the 100 mg/kg dose).

As a result, unlike oxycodone (the effect of which is mediated by opioid receptors), all test compounds showed a similar activity profile to ibuprofen with respect to tail-flick and hot plate tests, as well as carrageenan-induced paw edema test. Therefore, it could be concluded that the test compounds might act on the inhibition of the prostaglandin production.

4.3. Acute ulcerogenesis

The stomachs of mice were examined for lesions in gastric mucosa by using a dissecting microscope. The quantification of gastric mucosal lesions was scored according to their number and size in a scale from 0 up to 7 points, as given in detail in the methods section [28]. As seen in Fig. 4, in spite of the high gastric ulcer incidence in reference compounds ibuprofen (50 and 100 mg/kg doses) and indomethacin (10 and 50 mg/kg doses), all of the test compounds were generally found safe from the viewpoint of ulcer induction at both 50 and 100 mg/kg dose levels. Particularly the ulcerogenic effect of the compounds **1a**, **1b**, **1f**, **1i**, **1l**, and **1n** was appreciably lesser than ibuprofen ($p < 0.01$) and indomethacin in both doses. Additionally, **1h** at the 100 mg/kg and **1j** at 50 mg/kg doses were found safe as well ($p < 0.01$). Furthermore, some of the aforementioned test compounds (**1a**, **1f**, **1n**) are also among the compounds that exhibited marked analgesic and anti-inflammatory activity. The ulcerogenic score of oxycodone was similar to the vehicle control. The analgesic effects of traditional NSAIDs, such as ibuprofen and indomethacin, have been attributed to the inhibition of COX-2, while the GI

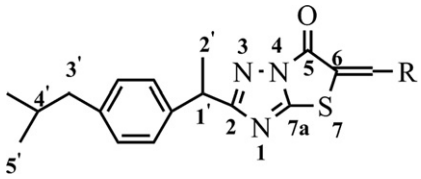
Table 2
IR and ^{1}H NMR and mass spectroscopic data of the compounds **1a**–**1o**.

Compounds	IR (KBr): ν (1/cm)	^{1}H NMR (DMSO-d_6) (δ (ppm); J in Hz)	Mass spectra (ESI)
1a	1742 (C=O); 1605 (C=N)	0.82 (d, 6H, 2× CH_3, J = 6.57 Hz), 1.68 (d, 3H, CH_3, J = 7.19 Hz), 1.73–1.82 (m, 1H, CH), 2.36 (d, 2H, CH_2, J = 7.11 Hz), 4.25 (q, 1H, CH, J = 7.20 Hz), 6.74–6.81 (dd, 1H, CH, J_1 = 11.58 Hz, J_2 = 15.17 Hz), 7.03 (d, 2H, ArH, J = 8.05 Hz), 7.13 (d, 1H, CH, J = 15.17 Hz), 7.22 (d, 2H, ArH, J = 8.05 Hz), 7.34–7.37 (m, 3H, ArH), 7.48–7.51 (m, 2H, ArH), 7.83 (d, 1H, CH, J = 11.54 Hz).	416 [M^+ + H], 438 [M^+ + Na]
1b	1757 (C=O); 1607 (C=N)	0.82 (d, 6H, 2× CH_3, J = 6.60 Hz), 1.68 (d, 3H, CH_3, J = 7.21 Hz), 1.73–1.80 (m, 1H, CH), 2.24 (d, 3H, CH_3, J = 0.94 Hz), 2.36 (d, 2H, CH_2, J = 7.14 Hz), 4.24 (q, 1H, CH, J = 7.20 Hz), 7.03 (d, 2H, ArH, J = 8.05 Hz), 7.08 (s, 1H, CH), 7.19 (d, 2H, ArH, J = 8.05 Hz), 7.26–7.38 (m, 5H, ArH), 7.89 (d, 1H, CH, J = 0.57 Hz).	430 [M^+ + H], 452 [M^+ + Na]
1c	1734 (C=O); 1602 (C=N)	0.82 (d, 6H, 2× CH_3, J = 6.61 Hz), 1.68 (d, 3H, CH_3, J = 7.22 Hz), 1.73–1.80 (m, 1H, CH), 2.37 (d, 2H, CH_2, J = 7.14 Hz), 4.24 (q, 1H, CH, J = 7.18 Hz), 5.06 (s, 2H, CH_2), 7.02–7.06 (m, 4H, ArH), 7,11 (d, 1H, ArH, J = 7.77 Hz), 7.22 (d, 2H, ArH, J = 8.05 Hz), 7.29 (d, 1H, ArH, J = 6.79 Hz), 7.32–7.38 (m, 5H, ArH), 8.06 (s, 1H, CH).	496 [M^+ + H], 518 [M^+ + Na]
1d	1727 (C=O); 1587 (C=N)	0.82 (d, 6H, 2× CH_3, J = 6.61 Hz), 1.69 (d, 3H, CH_3, J = 7.22 Hz), 1.73–1.80 (m, 1H, CH), 2.36 (d, 2H, CH_2, J = 7.15 Hz), 4.25 (q, 1H, CH, J = 7.21 Hz), 5.08 (s, 2H, CH_2), 7.02–7.04 (m, 4H, ArH), 7.22 (d, 2H, ArH, J = 8.03 Hz), 7.29–7.37 (m, 5H, ArH), 7.48 (d, 2H, ArH, J = 8.81 Hz), 8.07 (s, 1H, CH).	496 [M^+ + H], 518 [M^+ + Na]
1e	1765 (C=O); 1731 (C=O); 1597 (C=N)	0.82 (d, 6H, 2× CH_3, J = 6.61 Hz), 1.69 (d, 3H, CH_3, J = 7.23 Hz), 1.73–1.80 (m, 1H, CH), 2.27 (s, 3H, CH_3), 2.37 (d, 2H, CH_2, J = 7.14 Hz), 4.25 (q, 1H, CH, J = 7.34 Hz), 7.04 (d, 2H, ArH, J = 8.03 Hz), 7.21–7.23 (m, 4H, ArH), 7.54 (d, 2H, ArH, J = 8.66 Hz), 8.10 (s, 1H, CH).	448 [M^+ + H], 470 [M^+ + Na]
1f	1736 (C=O); 1595 (C=N)	0.82 (d, 6H, 2× CH_3, J = 6.61 Hz), 1.70 (d, 3H, CH_3, J = 7.21 Hz), 1.73–1.80 (m, 1H, CH), 2.37 (d, 2H, CH_2, J = 7.16 Hz), 4.26 (q, 1H, CH, J = 7.15 Hz), 7.04 (d, 2H, ArH, J = 8.03 Hz), 7.23 (d, 2H, ArH, J = 8.03 Hz), 7.36 (d, 1H, ArH, J = 7.28 Hz), 7.43 (t, 2H, ArH, J = 7.73 Hz), 7.57 (d, 2H, ArH, J = 10.33 Hz), 7.59 (d, 2H, ArH, J = 11.48 Hz), 7.69 (d, 2H, ArH, J = 8.37 Hz), 8.16 (s, 1H, CH).	466 [M^+ + H], 488 [M^+ + Na]
1g	1739 (C=O); 1601 (C=N)	0.82 (d, 6H, 2× CH_3, J = 6.61 Hz), 1.70 (d, 3H, CH_3, J = 7.23 Hz), 1.73–1.82 (m, 1H, CH), 2.37 (d, 2H, CH_3, J = 7.13 Hz), 4.27 (q, 1H, CH, J = 7.22 Hz), 7.04 (d, 2H, ArH, J = 8.11 Hz), 7.23 (d, 2H, ArH, J = 8.10 Hz), 7.45–7.59 (m, 3H, ArH), 7.79–7.91(m, 3H, ArH), 8.03 (s, 1H, ArH), 8.29 (s, 1H, CH).	440 [M^+ + H], 462 [M^+ + Na]
1h	1729 (C=O); 1620 (C=N)	0.82 (d, 6H, 2× CH_3, J = 6.58 Hz), 1.68 (d, 3H, CH_3, J = 7.14 Hz), 1.71–1.80 (m, 1H, CH), 2.36 (d, 2H, CH_2, J = 7.11 Hz), 4.24 (q, 1H, CH, J = 7.20 Hz), 6.56 (dd, 1H, furan ring, J_1 = 1.56 Hz, J_2 = 3.30 Hz), 6.87 (d, 1H, furan ring, J = 3.54 Hz), 7.03 (d, 2H, ArH, J = 7.94 Hz), 7.17–7.22 (m, 2H, ArH), 7.69 (d, 1H, furan ring, J = 1.16 Hz), 7.83 (s, 1H, CH).	380 [M^+ + H], 402 [M^+ + Na]
1i	1734 (C=O); 1611 (C=N)	0.82 (d, 6H, 2× CH_3, J = 6.61 Hz), 1.68 (d, 3H, CH_3, J = 7.23 Hz), 1.73–1.80 (m, 1H, CH), 2.36 (d, 2H, CH_2, J = 7.17 Hz), 2.39 (s, 3H, CH_3), 4.24 (q, 1H, CH, J = 7.17 Hz), 6.20 (d, 1H, furan ring, J = 4.03 Hz), 6.80 (d, 1H, furan ring, J = 3.47 Hz), 7.03 (d, 2H, ArH, J = 8.06 Hz), 7.22 (d, 2H, ArH, J = 8.06 Hz), 7.76 (s, 1H, CH).	394 [M^+ + H], 416 [M^+ + Na]
1j	1727 (C=O); 1603 (C=N)	0.82 (d, 6H, 2× CH_3, J = 6.60 Hz), 1.68 (d, 3H, CH_3, J = 7.20 Hz), 1.73–1.80 (m, 1H, CH), 2.36 (d, 2H, CH_2, J = 7.14 Hz), 4.42 (q, 1H, CH, J = 7.14 Hz), 7.03 (d, 2H, ArH, J = 7.98 Hz), 7.15–7.22 (m, 3H, 2xArH, thiophene ring), 7.44 (d, 1H, thiophene ring, J = 3.64 Hz), 7.68 (d, 1H, thiophene ring, J = 4.76 Hz), 8.27 (s, 1H, CH).	418 [M^+ + Na]
1k	1725 (C=O); 1589 (C=N)	0.82 (d, 6H, 2× CH_3, J = 6.61 Hz), 1.69 (d, 3H, CH_3, J = 7.20 Hz), 1.73–1.80 (m, 1H, CH), 2.36 (d, 2H, CH_2, J = 7.15 Hz), 2.42 (s, 3H, CH_3), 4.25 (q, 1H, CH, J = 7.11 Hz), 6.98 (d, 1H, CH, J = 5.00 Hz), 7.03 (d, 2H, ArH, J = 8.00 Hz), 7.22 (d, 2H, ArH, J = 7.98 Hz), 7.60 (d, 1H, CH, J = 4.94 Hz), 8.33 (s, 1H, CH).	410 [M^+ + H], 432 [M^+ + Na]
1l	1715 (C=O); 1592 (C=N)	0.82 (d, 6H, 2× CH_3, J = 6.60 Hz), 1.61 (d, 3H, CH_3, J = 7.26 Hz), 1.73–1.79 (m, 1H, CH), 2.37 (d, 2H, CH_2, J = 7.15 Hz), 3.70 (s, 3H, CH_3), 4.13 (q, 1H, CH, J = 7.27 Hz), 6.70 (d, 1H, thiophene ring, J = 3.64 Hz), 7.02 (d, 2H, ArH, J = 8.06 Hz), 7.09 (d, 2H, ArH, J = 8.07 Hz), 7.24 (d, 1H, thiophene ring, J = 3.65 Hz), 8.24 (s, 1H, CH).	410 [M^+ + H]
1m	1722 (C=O); 1601 (C=N)	0.82 (d, 6H, 2× CH_3, J = 6.60 Hz), 1.68 (d, 3H, CH_3, J = 7.20 Hz), 1.73–1.80 (m, 1H, CH), 2.37 (d, 2H, CH_2, J = 7.16 Hz), 4.24 (q, 1H, CH, J = 7.15 Hz), 7.03 (d, 2H, ArH, J = 8.00 Hz), 7.21 (d, 2H, ArH, J = 8.11 Hz), 7.32 (s, 1H, thiophene ring), 7.54 (s, 1H, thiophene ring), 8.14 (s, 1H, CH).	498 [M^+ + Na]
1n	1722 (C=O); 1596 (C=N)	0.82 (d, 6H, 2× CH_3, J = 6.60 Hz), 1.68 (d, 3H, CH_3, J = 7.22 Hz), 1.73–1.79 (m, 1H, CH), 2.36 (d, 2H, CH_2, J = 7.13 Hz), 3.74 (s, 3H, CH_3), 4.25 (q, 1H, CH, J = 7.19 Hz), 6.30–6.32 (dd, 1H, pyrrole ring, J_1 = 2.51 Hz, J_2 = 4.16 Hz), 6.73 (d, 1H, pyrrole ring, J = 4.14 Hz), 6.90 (d, 1H, pyrrole ring, J = 1.18 Hz), 7.03 (d, 2H, ArH, J = 7.96 Hz), 7.23 (d, 2H, ArH, J = 8.05 Hz), 7.97 (s, 1H, CH).	415 [M^+ + Na]
1o	1722 (C=O); 1598 (C=N)	0.82 (d, 6H, 2× CH_3, J = 6.61 Hz), 1.70 (d, 3H, CH_3, J = 7.21 Hz), 1.74–1.80 (m, 1H, CH), 2.37 (d, 2H, CH_2, J = 7.15 Hz), 2.70 (s, 3H, CH_3), 4.26 (q, 1H, CH, J = 7.04 Hz), 7.04 (d, 2H, ArH, J = 8.03 Hz), 7.23 (d, 2H, ArH, J = 8.04 Hz), 7.36–7.44 (m, 2H, indole ring), 7.74 (d, 1H, indole ring, J = 7.14 Hz), 7.75 (s, 1H, indole ring), 8.34 (s, 1H, CH), 8.37 (d, 1H, indole ring, J = 8.12 Hz).	493 [M^+ + Na]

side effects are thought to result from the inhibition of COX-1. COX-2-specific inhibition was expected to preserve effective anti-inflammatory activity while causing little or no associated GI toxicity. Therefore, test compounds showing a lower ulcerogenic risk suggest the possibility of high selectivity on COX-2 enzyme over COX-1.

It is well known that quantum–chemical methods and molecular modeling techniques enable the definition of a large number of molecular and local quantities characterizing the reactivity, shape and binding properties of a complete molecule, as well as of molecular fragments and substituents. The electrophilicity index (ω) is one of the calculated quantum chemical descriptors and

Table 3
The APT ^{13}C data of representative carbon atoms of the compounds **1a**–**1o**.



Compounds	APT-NMR ($CDCl_3$, δ (ppm))								
	1′	**2′**	**3′**	**4′**	**5′**	**2**	**5**	**6**	**7a**
1a	40.15	22.41	45.07	30.16	19.78	155.89	176.29	140.48	158.80
1b	40.17	22.42	45.07	30.17	19.86	156.58	176.35	140.46	159.96
1c	40.17	22.41	45.07	30.16	19.82	156.19	176,60	140.51	159.32
1d	40.17	22.41	45.07	30.16	19.84	156.60	176.35	140.47	159.73
1e	40.17	22.41	45.07	30.16	19.78	156.17	176.65	140.54	159.74
1f	40.19	22.42	45.08	30.17	19.81	156.33	176.58	140.53	159.83
1g	40.19	22.41	45.08	30.16	19.84	156.27	176.56	140.51	159.89
1h	40.16	22.40	45.08	30.14	19.84	156.35	176.39	140.44	160.63
1i	40.15	22.42	45.07	30.17	19.93	156.60	176.15	140.40	160.59
1j	40.17	22.40	45.07	30.14	19.82	156.26	176.47	140.47	159.14
1k	40.16	22.41	45.07	30.16	19.85	156.55	176.31	140.45	159.30
1l	38.00	22.35	44.99	30.17	20.17	149.60	166.79	140.93	166.22
1m	40.17	22.41	45.06	30.16	19.77	155.84	176.75	140.56	158.83
1n	40.14	22.42	45.07	30.17	19.88	156.92	175.94	140.41	159.09
1o	40.19	22.41	45.07	30.18	19.85	155.85	176.58	140.54	158.36

defines a quantitative classification of the global electrophilic nature of a molecule within a relative scale [29]. According to the electrophilicity index generated using the semiempirical PM3 method basis sets from the Gaussian 03 package, the compounds **1a**, **1f** and **1n** displayed an enormous similarity to selective COX-2 inhibitors celecoxib and rofecoxib (Table 4). The possibility that these compounds would be selective COX-2 inhibitors will be investigated in future studies.

4.4. Acute toxicity

In the acute toxicity study, mortality was not observed in either the 50 mg/kg or 100 mg/kg doses. During the 14-day observation period, animals showed no weight loss, no behavioral alteration or toxicity signs at any of the dose levels tested. However, indomethacin at a 50 mg/kg dose caused mortality for six of seven mice (86%) in the first 12 h of observation.

5. Conclusion

In conclusion, a series of 6-substituted thiazolo[3,2-*b*]-1,2,4-triazole-5(6*H*)-one derivatives of ibuprofen were prepared with the objective of developing better analgesic/anti-inflammatory molecules with minimum ulcerogenic risk. Although it is not possible to draw an exact conclusion according to the results of *in vivo* experiments, which substituent at the 6th position is more active, it could be claimed that the condensing of (±)-3-[1-(4-(2-methylpropyl)phenyl)ethyl]-1,2,4-triazole-5-thione (**1**) with a thiazole ring provides a superior result in both analgesic and anti-inflammatory activity. As a general consideration, in accordance with previous results, the incorporation of the free carboxylic acid group of ibuprofen into thiazolo[3,2-*b*]-1,2,4-triazole provides a simple strategy for generating much safer analgesic/anti-inflammatory compounds when compared with ibuprofen (50 and 100 mg/kg) and indomethacin (10 and 50 mg/kg). It is likely that the marked reduction of gastrointestinal toxicity of the molecules is due to the masking of the free carboxylic acid group of ibuprofen and/or the selective inhibition of the COX-2 enzyme. Therefore, it was concluded that thiazolo[3,2-*b*]triazol derivatives of ibuprofen might provide a safer alternative to ibuprofen for the treatment of inflammatory disease and pain.

6. Experimental protocol

6.1. Chemistry

Ibuprofen was supplied by Atabay Pharmaceuticals (Istanbul, Turkey). All chemicals were from Aldrich Chemical Co. (Steinheim, Germany). Melting points were measured in sealed tubes using an electrothermal digital melting point apparatus (Gallenkamp) and are uncorrected (London, UK). IR spectra (KBr) were recorded on a Perkin Elmer spectrumOne, Nicolet 520 FTIR spectrometer (Perkin–Elmer, USA). APT ^{13}C and ^{1}H NMR spectra were obtained by a Bruker instrument DPX-400, 400 MHz High Performance Digital FT–NMR Spectrometer (Bruker, Fällanden, Switzerland)

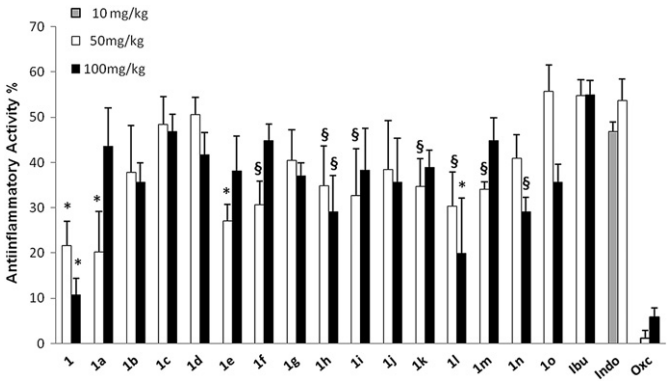


Fig. 1. The anti-inflammatory activity results of the compounds **1**–**1o**, ibuprofen (Ibu) and oxycodone (Oxc) at 50 mg/kg (□) and 100 mg/kg (■); indomethacin (Indo) at 10 mg/kg (▩) and 50 mg/kg (□) doses. $^{*}p < 0.01$ vs ibuprofen, $^{§}p < 0.05$ vs ibuprofen; data expressed as mean ± SEM, one-way ANOVA followed by *post hoc* Dunnett's test, $n = 7$ per group.

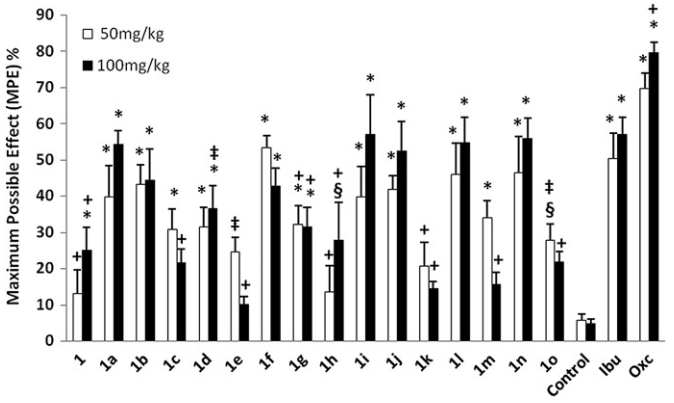


Fig. 2. The analgesic activity results of the tail-flick experiment for compounds **1**–**1o**, ibuprofen (Ibu) and oxycodone (Oxc) at 50 mg/kg (□) and 100 mg/kg (■) doses. $^{*}p < 0.01$ vs vehicle control group; $^{\S}p < 0.05$ vs vehicle control group; $^{+}p < 0.01$ vs ibuprofen; $^{\ddagger}p < 0.05$ vs ibuprofen; data expressed as mean ± SEM, one-way ANOVA followed by *post hoc* Dunnett's test, $n = 7$ per group.

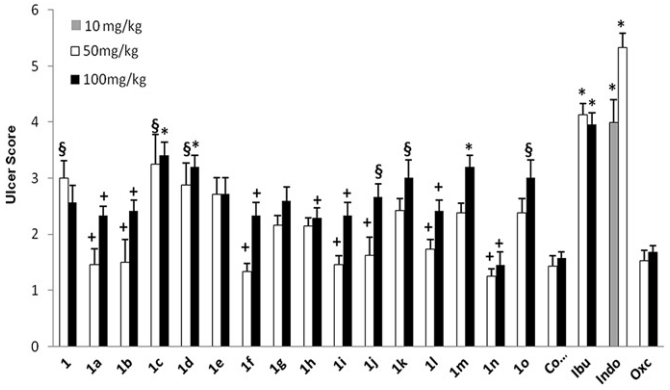


Fig. 4. The ulcerogenic scores of the compounds **1**–**1o**, ibuprofen (Ibu) and oxycodone (Oxc) at 50 mg/kg (□) and 100 mg/kg (■) doses; indomethacin (Indo) at 10 mg/kg (▒) and 50 mg/kg (□). $^{*}p < 0.01$ vs vehicle control; $^{\S}p < 0.05$ vs vehicle control; $^{+}p < 0.01$ vs ibuprofen; data expressed as mean ± SEM, Kruskal–Wallis test followed by *post hoc* Dunn's test, $n = 7$ per group.

using DMSO-d_6 and tetramethylsilane as an internal standard. All chemical shift values were recorded as δ (ppm). The purity of the compounds was controlled by thin layer chromatography on silica gel-coated aluminum sheets (Merck, 1.005554, silicagel $HF_{254-361}$, Type 60, 0.25 mm, Darmstadt, Germany). Mass spectra were obtained with the positive ion electrospray technique using a Micromass ZQ MS Spektrometer and MassLynx 4.1 software (Manchester, UK). The elementary analysis of the compounds was performed on a Leco CHNS 932 analyzer (Philadelphia, USA) at the Central Instrumental Analysis Laboratory of Ankara University, Faculty of Pharmacy. Elementary analysis for C, H, N and S were within ±0.4% of theoretical values.

6.1.1. General procedures

6.1.1.1. Synthesis of (±)-3-[1-(4-(2-methylpropyl)phenyl)ethyl]-1,2,4-triazole-5-thione (1). The compound (**1**) was prepared to the method reported earlier [20].

*6.1.1.2. Synthesis of 6-substituted thiazolo [3,2-b]-1,2,4-triazole-5(6H)-ones (**1a**–**1o**).* The equimolar amounts of (±)-3-[1-(4-(2-methylpropyl)phenyl)ethyl]-1,2,4-triazole-5-thione (**1**), the corresponding aldehyde, chloroacetic acid and sodium acetate were added to 6 ml of acetic acid and 8 ml of acetic anhydride. Reaction time was determined by TLC-monitoring the consumption of the starting material. After completion of the reaction, the reaction mixture was poured on ice, and the crude precipitate was filtered and dissolved in dichloromethane and washed with a sodium bicarbonate and saturated sodium chloride solution, respectively. The organic layer was evaporated to dryness and the crude product then recrystallized from methanol [20–22]. The characterization data of synthesized novel 6-substituted thiazolo[3,2-*b*]-1,2,4-triazole-5(6*H*)-ones are given in Tables 1–3.

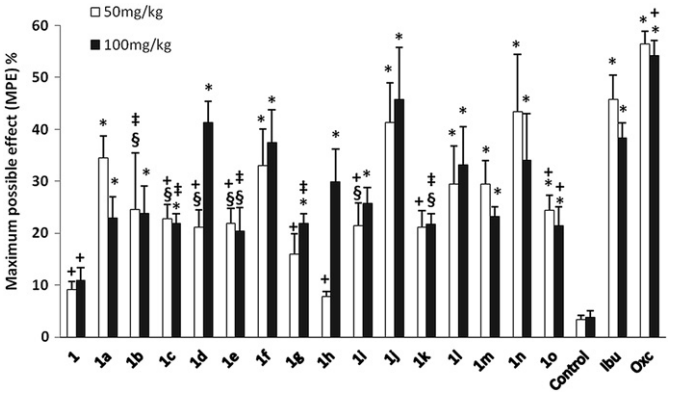


Fig. 3. The analgesic activity results of the hot plate experiment for the compounds **1**–**1o,** ibuprofen (Ibu) and oxycodone (Oxc) at 50 mg/kg (□) and 100 mg/kg (■) doses. $^{*}p < 0.01$ vs vehicle control group; $^{\S}p < 0.05$ vs vehicle control group; $^{+}p < 0.01$ vs ibuprofen; $^{\ddagger}p < 0.05$ vs ibuprofen; data expressed as mean ± SEM, one-way ANOVA followed by *post hoc* Dunnett's test, $n = 7$ per group.

6.2. Computations

All structures were drawn and optimized using Gaussian 03 software with the semiempirical PM3 method to calculate electrophilicity index (ω).

6.3. Pharmacology

6.3.1. Animals

Swiss albino mice of both sexes, weighing 20–35 g, were housed at a room temperature of 22 °C with a 12/12 h light/dark cycle. All animal procedures were conducted in accordance with the institutional guidelines for care and use of laboratory animals, and were approved by the Hacettepe University Animal Ethics Committee (2008/21-5). Animals were kept for one week to acclimatize to laboratory conditions before starting the experiment. Twelve hours before experiments, animals received only water, in order to avoid food interference with substance absorption. Animals were divided into groups ($n = 7$), and two doses of the test compounds in 0.5% carboxymethyl cellulose (CMC) solution (50 and 100 mg/kg) were intragastrically administered to the test groups. The control group received the vehicle control (0.5% CMC solution only) and ibuprofen (Ibu, 50 and 100 mg/kg, p.o.) as the reference drug, respectively. Oxycodone (Oxc, 50 and 100 mg/kg, p.o.) was used in the analgesic tests as an opioid reference drug. Also, indomethacin (Indo, 10 mg/kg and 50 mg/kg) was used as an additional reference drug in determining gastric side effects.

6.3.2. Carrageenan-induced paw edema test

The synthesized compounds were evaluated by carrageenan-induced paw edema described by Winter et al. [25] with modifications. The synthesized compounds (50 and 100 mg/kg) were compared with the vehicle control and reference ibuprofen (50 and 100 mg/kg). Edema was induced by injecting 0.01 ml of a 2% carrageenan suspension into the subplantar region of the right

Table 4
Calculated electrophilicity values (ω) for compounds.

Compounds	**1a**	**1f**	**1n**	Ibuprofen	Indomethacin	Celecoxib	Rofecoxib
ω	1.898	1.926	1.775	1.183	1.368	1.938	1.840

hind-paw of the mice with a Hamilton injector. The carregeenan injection was applied 1 h after the gavage. The thickness of the paws was measured by a dial thickness gauge (0.01–1 mm, Ozaki Co., Japan) immediately before (T0) and 2 h (Tt) after the injection. The edema was calculated as the increase in thickness (mm) of the paw after treatment subtracted from the basal volume ($\Delta T = \mathrm{Tt} - T0$). For each animal, the anti-inflammatory effect of the drugs was expressed as a percentage of edema inhibition [30].

Inhibition (%) = [(Control ΔT − Test ΔT)/Control ΔT] × 100.

6.3.3. Tail-flick test

For the tail-flick test, an automated tail-flick apparatus (TF 0703, 8 V/50 W Tail-flick Commat Ltd.-Ankara) was used, which elicited a response by applying radiant heat to the dorsal surface of the tail. For the experiment, each mouse was gently held and an automatic timer measured latency until the mouse flicked its tail away from the source of the light. The heat stimulus was set to provide a pre-drug tail-flick response time of 6–8 s. The cut-off time for the heat stimulus was set at 15 s to avoid tissue damage [26,31]. After the baseline readings ($T0$) were taken, the mice were treated with CMC as a control, oxycodone or ibuprofen as reference drugs, or the test compounds of 50 or 100 mg/kg doses. Response latencies ($T1$) were measured 2 h after the application of drugs or vehicle. The analgesic activity was calculated as the percentage maximum possible effect (MPE) using the following formula:

%MPE = $(T1 - T0)/(T2 - T0) \times 100$, where the cut-off time ($T2$) was 15 s.

6.3.4. Hot plate test

For determination of antinociceptive activity, a conventional hot plate test was performed according to the method of Eddy and Leimbach [27]. Mice ($n = 7$) were placed individually on a hot plate (9601 Analgesic Hot Plate Commat Ltd.-Ankara) set at 50 ± 0.5 °C and a plexiglass cylinder (12 cm diameter, 20 cm height) was used to confine the mouse on the heated surface of the plate. The time of licking the forepaws or jump response (whichever appears first) were recorded as an index of nociception. Only mice that showed a nociceptive response within 25 s were used in the experiment. Maximum cut-off time was chosen as 25 s to avoid tissue damage. After the baseline readings ($T0$) were taken, the mice were treated with CMC as a control, oxycodone or ibuprofen as reference drugs or the test compounds of 50 or 100 mg/kg doses. Response latencies ($T1$) were measured 2 h after the application of the drugs or vehicle. The effects of the compounds on nociception were determined by the percentage of maximal possible effect (%MPE) was calculated as below:

%MPE = $(T1 - T0)/(T2 - T0) \times 100$, where the cut-off time ($T2$) was 25 s.

6.3.5. Gastric ulceration studies

Ulcerogenic activity was investigated after the carrageenan-induced paw edema test. All groups were starved for 12 h, but water was provided *ad libitum*. Food was allowed 2 h post-administration of the drugs. Two hours following the last doses, mice were euthanized. The stomachs of each mouse were removed, opened along the greater curvature, rinsed with 0.9% sodium chloride (isotonic solution) and stretched by pins on a cork board. The lesions in gastric mucosa were determined by using a dissecting microscope. The quantification of gastric mucosal lesions was scored according to their number and size in a scale from 0 up to 7 points, adapted from Magistretti et al. [28] as follows: (0) without injury, (1) color modification, (2) few petechia/alterations of villous, (3) 1–3 small injuries (≤1 mm length), (4) 1–3 large injuries (≤1 mm length), (5) 1–3 large injuries (>1 mm), (6) more than three small injuries, (7) more than three large injuries or massive bleeding or digested blood in stomach.

6.3.6. Acute toxicity study

Test compounds, vehicle control (CMC), reference drugs (ibuprofen, oxycodone) in 50 mg/kg and 100 mg/kg doses, were administered orally in 0.5% CMC. Indomethacin was tested at a 50 mg/kg dose. However, indomethacin at a 50 mg/kg dose caused high mortality in the first 12 h of observation, therefore, the dose was lowered to 10 mg/kg. The animals were observed continuously for 6 h after treatment, then intermittently for 72 h, and thereafter for over a period of 14 days after administration [32] for behavioral changes, signs of toxicity and/or death.

6.3.7. Statistical analysis of data

All data are expressed as mean ± SEM. Results of carrageenan-induced paw edema experiments are also expressed as a percentage of change from control (pre-drug) values. Differences between vehicle control, reference drugs and treatment groups were tested using a one-way analysis of variance (ANOVA) followed by a *post hoc* Dunnett's test. Ulcer scores were analyzed using the Kruskal–Wallis test followed by a *post hoc* Dunn's test.

Conflict of interest

Additionally, the authors have declared no conflict of interest.

Acknowledgments

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