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Novel 3,3a,4,5,6,7-Hexahydroindazole and Arylthiazolylpyrazoline derivatives as Anti-inflammatory Agents

A novel series of 7-benzylidene-3,3a,4,5,6,7-hexahydro-3-phenyl-2*H*-indazole substituted at the 2-position were synthesized. The reaction of 2,6-bis-benzylidenecyclohexanone (**1**) with thiosemicarbazide in the presence of NaOH afforded a mixture of the 3-H, 3a-H *trans* **2** and *cis* **2a** diastereoisomers which have been separated by fractional recrystallization. Interaction of the first intermediate **2** with substituted phenacyl bromides, aromatic aldehydes and chloroacetic acid in presence of a mixture of acetic acid and acetic anhydride, and 2,3-dichloroquinoxaline yielded the corresponding 7-benzylidene-3,3a,4,5,6,7-hexahydro-3-phenyl-2*H*-indazole derivatives substituted at the 2-position with 4-aryl-2-thiazolyl **3 a, b**, 5-arylidene-4,5-dihydro-4-oxo-2-thiazolyl **4 a, b** and thiazolo[4,5-b]quinoxalin-2-yl **5,** respectively. Moreover, the other intermediates 3,5-diaryl-1-thiocarbamoyl-2-pyrazolines **7 a**–**d** were reacted with the previously-mentioned reagents and gave the corresponding 3,5-diaryl-1-(4-aryl-2-thiazolyl)-2-pyrazolines **8 a**–**h,** 3,5-diaryl-1-(5-arylidene-4,5-dihydro-4-oxo-2-thiazolyl)-2-pyrazolines **9 a**–**d** and 3,5-diaryl-1-(thiazolo[4,5-b]quinoxalin-2-yl)-2-pyrazoline derivatives **10 a, b**, respectively. Some of the newly prepared compounds were subjected to evaluation for their anti-inflammatory activity. The structures of the new compounds were confirmed by elemental analyses as well as ^{1}H-NMR, IR, and MS data.

Keywords: Arylthiazolylpyrazoline; Hexahydroindazole; Cyclooxygenase-2-inhibitor; Anti-inflammatory activity

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Introduction

Prostaglandins (PGs) elicit a variety of important beneficial and untoward biological responses. Among the undesirable properties of prostaglandins is their ability to induce pain, fever, and symptoms associated with the inflammatory response. Non-steroidal anti-inflammatory drugs (NSAIDs) block the formation of prostaglandins and have analgesic, antipyretic, and anti-inflammatory activity [1]. However, treatment with NSAIDs, particularly chronically, often leads to disruption of beneficial prostaglandin-regulated processes [2, 3]. The principal side effect associated with chronic administration of NSAIDs are significant gastrointestinal irritations [4] and the formation of life-threatening gastrointestinal ulcers, which considerably limits the therapeutic potential of this class of drugs. Alternative treatment of inflammation with glucocorticoids may also lead to a variety of undesirable side effects, particularly during extended periods of therapy [5].

A major mechanism of action of NSAIDs is lowering prostaglandin production through inhibition of cyclooxygenase (COX) [6, 7]. This enzyme exists in two isoforms, one constitutive (COX-1), and the other inducible (COX-2) [8, 9]. The COX-1 isoform is constitutively expressed in most tissues, and is involved in the physiological production of prostaglandins, which are responsible for gastric cytoprotection. The COX-2 isoform is induced by cytokines, mitogens, and endotoxins [10, 11] in inflammatory cells, and is responsible for the elevated production of prostaglandins during inflammation.

Current NSAIDs inhibit both forms of the enzyme, with many demonstrating a selectivity for COX-1. It is believed that it is the inhibition of COX-1 that causes the side effects seen with NSAIDs. The discovery of COX-2 led to the recognition that selective inhibitors of COX-2 would constitute a novel approach to the treatment of inflammation with diminished or no gastric side effects [12].

The chemical structures of COX-2 inhibitors are heterogenic leading to a further classification of this group.

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Full Paper

Contrary to the classic NSAIDs, this new class of enzyme inhibitors is lacking a carboxylic group, thus affecting COX-2 affinity by a different orientation within the enzyme without formation of a salt bridge in the hydrophobic channel of the enzyme. Selective COX-2 inhibitors belong to different structural classes, one of which is the vicinal diaryl heterocycles e.g., Celecoxib, Rofecoxib, SC57666, and Dup-697 (Figure 1). These compounds are characterized by a central carbocyclic or heterocyclic ring system bearing two vicinal aryl moieties, for optimal activity one aromatic ring substituted with methylsulfonyl or sulfonamide substituent. These compounds represent the most important group of COX-2 inhibitors. Occasionally, this structure is defined in the literature as a "tricyclic compound" which is not really appropriate according to chemical nomenclature. It can be assumed that the heterocycle is responsible for the appropriate orientation to the aromatic rings in space and finally for the binding to the enzyme. A wide variety of heterocycles can serve as a template for COX-2 inhibitors i.e., thiazole, furan, thiophene, and pyrrole but, at the moment, pyrazole and cyclopentenone seem to be the most appropriate tools for COX-2 specificity [13–23].

Pyrazolines [24–30], bicyclic pyrazolines [31], and quinoxalines [32] are also well-known for their pronounced anti-inflammatory activity. Our interest in this area has developed novel selective inhibitors of COX-2 that have improved therapeutic properties relative to currently used NSAIDs [33, 34]. In the present work, we detail our strategy to obtain selective COX-2 inhibitors based on the modification of the structure of the known potent vicinal diaryl carbocyclic and heterocyclic systems. Toward that goal, it was thought worthwhile to synthesize new series of pyrazoline and hexahydroindazole derivatives to evaluate them as selective COX-2 inhibitors which ultimately led to the identification of anti-inflammatory agents with a greatly improved safety profile.

Results and discussion

Chemistry

The synthetic routes of the proposed compounds are outlined in Schemes 1 and 2. Our initial goal in this study was to prepare the N-substituted hexahydroindazole derivatives **3**–**5**. This was achieved through the preparation

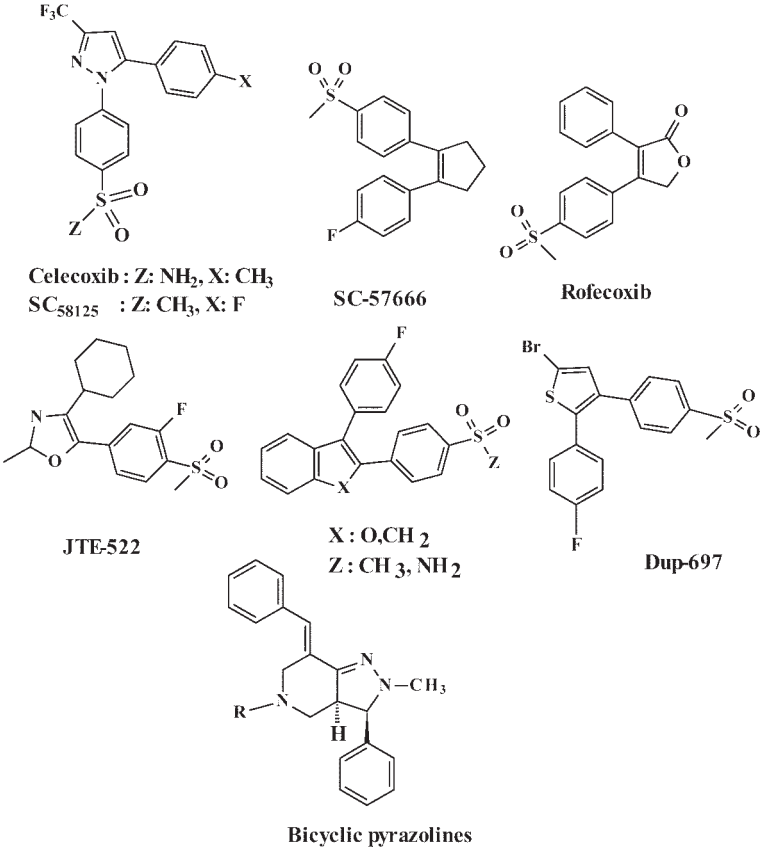
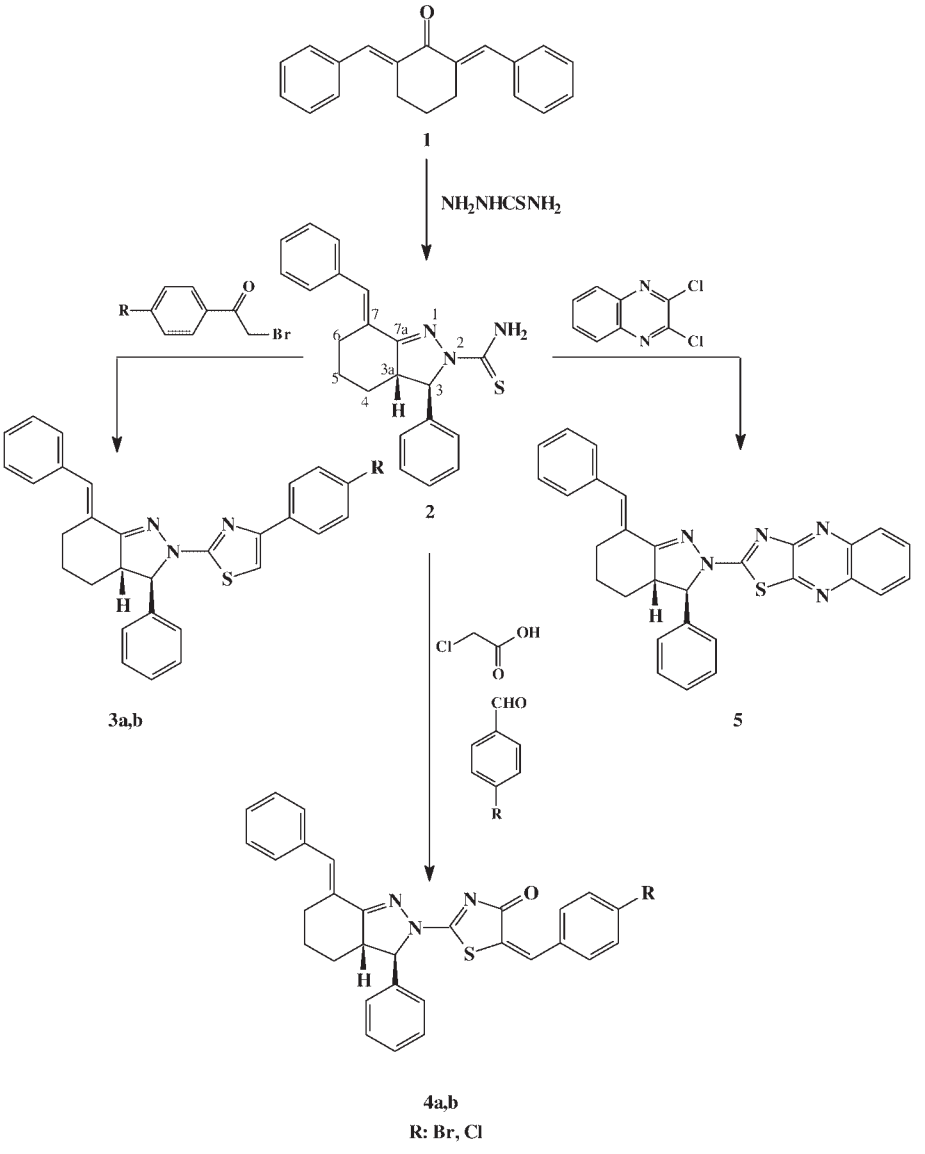
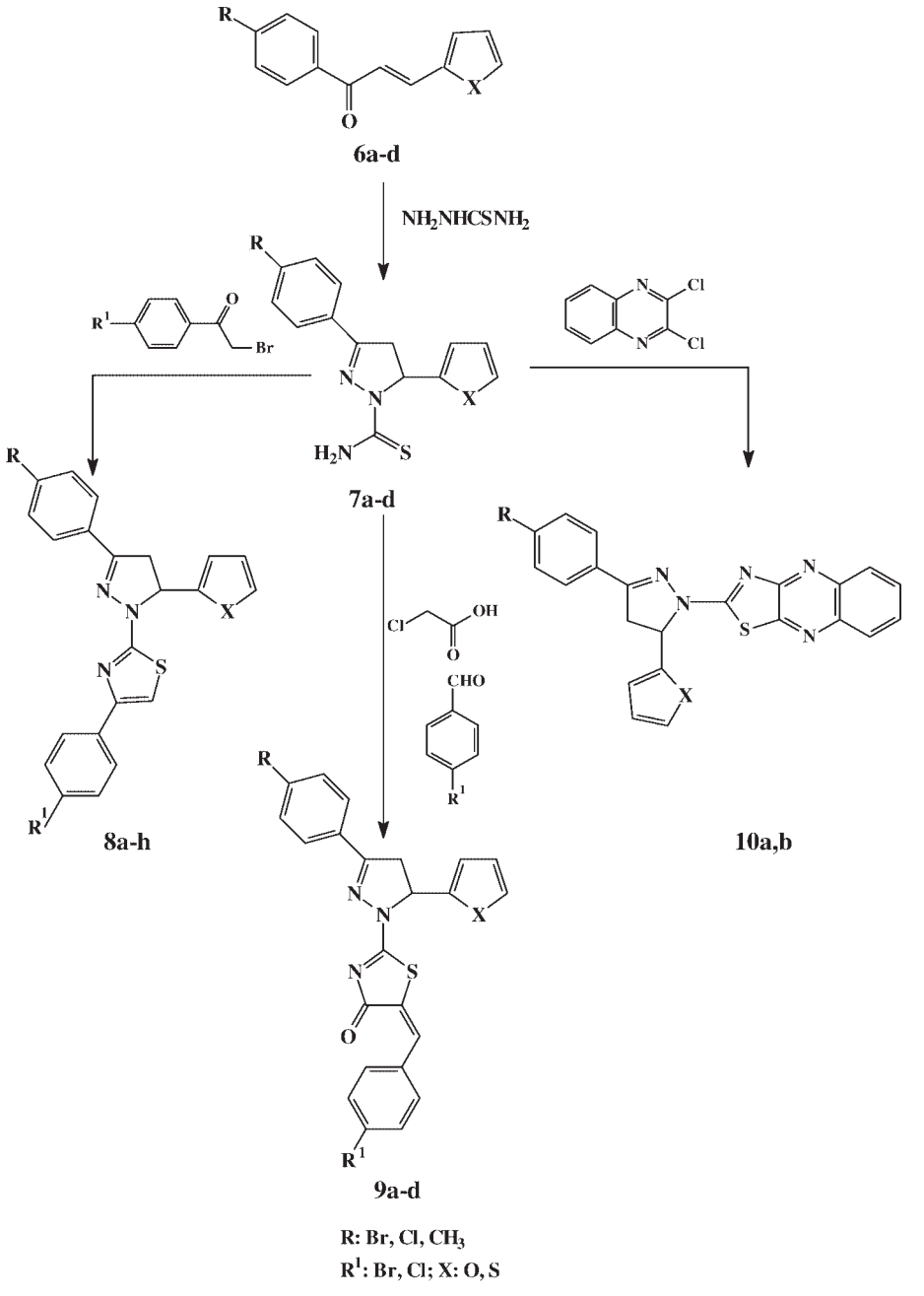


Figure 1. Representative examples of selective COX-2 inhibitors and bicyclic pyrazoline derivatives as anti-inflammatory agents.



Scheme 1.

of the starting compound 7-benzylidene-3,3a,4,5,6,7-hexahydro-3-phenyl-2-thiocarbamoyl-2*H*-indazole (**2**) (Scheme 1). It was reported that the reaction of 2,6-diarylidenecycloalkanones with hydrazine and substituted hydrazines yielded unsubstituted and 2-substituted bicyclic dihydropyrazoles having 3 and 3a-H of *trans* configuration [35, 36]. In contrast to the above results, Jacquier and Maury synthesized dihydropyrazoles derivatives in a two-step reaction of 2-alkylidene and 2-benzylidenecycloalkanones with hydrazine and obtained both the *cis* and *trans* diastereoisomers [37]. Moreover, reactions of 2,6-diarylidenecycloalkanones with thiosemicarbazide under acidic catalysis yielded only one diastereoisomer of the 3-H, 3a-H *cis*, while the reaction with semicarbazide afforded a mixture of *trans* and *cis* diastereoisomers, which have been separated [38, 39]. In the present work, compound **2** was synthesized by the reaction of 2,6-bis-benzylidenecyclohexanone (**1**) [40] with thiosemicarbazide in presence of NaOH afforded a mixture of the 3-H, 3a-H *trans* (major) **2** and *cis* (minor) **2 a** diastereoisomers which have been separated by fractional recrystallization from methanol. However, column-chromatographic separation was a suitable tool to isolate both components in a pure form. Moreover, they were identified by thin-layer chromatography (TLC) analysis of the basic catalysis reaction products with an



Scheme 2.

authentic sample of the *cis* **2 a** diastereoisomer prepared through the acid catalyzed reaction procedure [39]. One of the two product components, apparently the minor one, was matched properly with the *cis* isomer **2 a** and the other component, which seemed to be the major one, is considered to be the *trans* diastereoisomer **2**. The confirmation of the *trans* isomer can also be achieved by the melting point differences between the two products **2** and **2 a** and by oxidation of the 2-thiocarbamoyl group in compound **2 a** with hydrogen peroxide according to the previously described method [41] leading to the published compound *trans* 7-benzylidene-2-carbamoyl-3,3a,4,5,6,7-hexahydro-3-phenyl -2*H*-indazole (**2 c**) [38].

Two further aspects of stereo structures should be considered, the geometrical isomerism of the benzylidene

moiety and the conformation of the partly saturated condensed ring. Regarding the geometrical isomers, the Z-isomer (S-*cis* Ar and N-1) is to be precluded due to unfavorable steric structure. The S-*cis* (to N-1) position of the hydrogen in the exocyclic =CH- group reveals a high downfield shift of its ^{1}H-NMR signal (this appears to overlap with the multiplets of the phenyl hydrogens) due to anisotropic deshielding of the non-bonded electron pair on N-1 [42]. The condensed, partly saturated six-membered ring can exsit in two preferred conformations. These chair and boat-like forms with out-of-plane C-5 and C-7a atoms are both distorted approaching the sofa-arrangement with coplanar C-7a. It is to be noted that the inversion of the six-membered ring hardly influences the envelope form of the pyrazoline ring (with an out-of-plane C-3 atom) bearing the aryl (phenyl, substituted phenyl, thienyl) group in a quasi-equatorial position [38].

The thiocarbamoyl moiety in compound **2** was subjected to cyclocondensation reactions using different reagents. Firstly, reaction of **2** with substituted phenacyl bromide gave the corresponding 2-(4-aryl-2-thiazolyl)-7-benzylidene-3,3a,4,5,6,7-hexahydro-3-phenyl-2*H*-indazole analogues **3 a, b**. On the other side, compound **2** reacted with a variety of aromatic aldehydes and chloroacetic acid in presence of a mixture of acetic acid and acetic anhydride to produce the corresponding 2-(5-arylidene-4,5-dihydro-4-oxo-2-thiazolyl)-7-benzylidene-3,3a,4,5,6,7-hexahydro-3-phenyl-2*H*-indazole derivatives **4 a, b**. Furthermore, refluxing compound **2** with 2,3-dichloroquinoxaline [43] in absolute ethanol, gave the corresponding fused 7-benzylidene-3,3a,4,5,6,7-hexahydro-3-phenyl-2-(thiazolo[4,5-b]quinoxalin-2-yl)-2*H*-indazole (**5**).

The second starting compounds 3,5-diaryl-1-thiocarbamoyl-2-pyrazolines (**7 a**–**d**) (Scheme 2) were prepared. Refluxing the appropriate α,β-unsaturated carbonyl compounds **6 a**–**d** [44–46] with thiosemicarbazide in presence of NaOH provided the requisite **7 a**–**d** in good overall yields. Similar reactions of **7 a**–**d** with substituted phenacyl bromides, different aromatic aldehydes and chloroacetic acid, and 2,3-dichloroquinoxaline afforded the corresponding 3,5-diaryl-1-(4-aryl-2-thiazolyl)-2-pyrazolines **8 a**–**h**, 3,5-diaryl-1-(5-arylidene-4,5-dihydro-4-oxo-2-thiazolyl)-2-pyrazolines **9 a**–**d**, and 3,5-diaryl-1-(thiazolo[4,5-b]quinoxalin-2-yl)-2-pyrazoline derivatives **10 a, b**, respectively.

Table 1. Physicochemical data of the new compounds.

	R/(X)	R^1	Mp °C	Rec. Sol*	Yield (%)	Mol. Form.
3 a	Br	–	182–185	A	68	$C_{29}H_{24}BrN_3S$
3 b	Cl	–	175–177	E	61	$C_{29}H_{24}ClN_3S$
4 a	Br	–	170–172	E	58	$C_{30}H_{24}BrN_3OS$
4 b	Cl	–	180–182	E	60	$C_{30}H_{24}ClN_3OS$
7 a	Br(O)	–	213–215	A	71	$C_{14}H_{12}BrN_3OS$
7 b	Cl(O)	–	218–220	Ac	74	$C_{14}H_{12}ClN_3OS$
7 c	CH_3(O)	–	280–282	EA	72	$C_{15}H_{15}N_3OS$
7 d	Cl(S)	–	155–157	A	78	$C_{14}H_{12}ClN_3S_2$
8 a	Br(O)	Br	225–227	A	68	$C_{22}H_{15}Br_2N_3OS$
8 b	Br(O)	Cl	208–210	EA	65	$C_{22}H_{15}BrClN_3OS$
8 c	Cl(O)	Br	220–222	A	62	$C_{22}H_{15}BrClN_3OS$
8 d	Cl(O)	Cl	203–205	M	63	$C_{22}H_{15}Cl_2N_3OS$
8 e	CH_3(O)	Br	295–297	E	65	$C_{23}H_{18}BrN_3OS$
8 f	CH_3(O)	Cl	167–169	M	67	$C_{23}H_{18}ClN_3OS$
8 g	Cl(S)	Br	200–202	Ac	66	$C_{22}H_{15}BrClN_3S_2$
8 h	Cl(S)	Cl	175–177	E	68	$C_{22}H_{15}BrCl_2N_3S_2$
9 a	Br(O)	Br	188–190	E	52	$C_{23}H_{15}Br_2N_3O_2S$
9 b	Br(O)	Cl	110–112	A	55	$C_{23}H_{15}BrClN_3O_2S$
9 c	Cl(O)	Cl	235–237	AC	58	$C_{23}H_{15}Cl_2N_3O_2S$
9 d	Cl(S)	Br	115–117	A	60	$C_{23}H_{15}BrClN_3OS_2$
10 a	Cl(O)	–	235–237	E	72	$C_{22}H_{14}N_5OS$
10 b	Cl(S)	–	210–212	A	75	$C_{22}H_{14}ClN_5S_2$

*: Recryst. Solv.; A: acetone; Ac: aqueous acetic acid; E: ethanol; EA: ethyl acetate.

For the physicochemical data of these new compounds see Table 1.

Biological screening

Some of the newly synthesized compounds were tested *in vivo* in order to evaluate their anti-inflammatory activity against carrageenin induced paw edema at dose of 100 mg/kg using ketoprofen as a reference standard (Table 2). With the objective of identifying compounds with selective COX-2 inhibition, we distinguished the selective compounds of the present study by assessing *in vivo* ulcerogenic effects. One common feature of many of the previously reported selective COX-2 inhibitors (Figure 1) is the vicinal diaryl moities to a central carbocyclic or heterocyclic ring, for example: pyrazole, thiophene, cyclopentene, and benzofuran . In the present work, the central ring is constructed so as to be either pyrazoline or hexahydroindazole substituted in vicinal positions by two carbocyclic, heterocyclic or fused heterocyclic residues. It is interesting to point out that some compounds of the present series exhibit good activity relative to the standard as shown in Table 2, ranging from 37 to 64 % edema reduction, and some of them show low activity, with 17–18 % edema reduction. The major exception proved to be **7 c** being almost inactive, with 7 % edema reduction. Generally speaking, compounds with a central ring hexahydroindazole are less potent than those with a pyrazoline ring. Compound **2** with a thiocarbamoyl moiety in vicinal position to a phenyl ring has little anti-inflammatory activity. Moreover, cyclization of the thiocarbamoyl group to thiazole ring did not appreciably affect the potency as in **3 b**. On the contrary, cyclization to give thiazolo[4,5-b]quinoxaline moiety resulted in a significant increase in potency with 37 % edema reduction as in **5**. On the other side, with the second analogous series (Scheme 2), most compounds showed good anti-inflammatory activity, except compound **7 c**. Using celecoxib as a "template", numerous sites of modification were explored. The initial modification was the replacement of the vicinal phenyl groups in 1- and 5-positions by two heterocyclic rings and, furthermore, the replacement of the trifluoromethyl group at the 3-position by a phenyl ring substituted by electron-withdrawing lipophilic group (chloro or bromo) or electron donating group (methyl). With the exception of **7 c**, compounds **7 b**–**d** exhibited good anti-inflammatory activity. Among the biologically active members of this series, compound **7 d** shows high anti-inflammatory activity with 50 % edema reduction. The structural features of this compound are characterized by having a thienyl group at the 2-position of the pyrazoline ring vicinal to the thiocarbamoyl moiety in addition to 4-chlorophenyl group at the 3-position. Replacement of a thienyl moiety by a furyl one decreased the activity as in **7 b**. Moreover, changing the substituent on the phenyl ring from electron-withdrawing to electron donating, it nearly abolished the activity as in **7 c**. It is worthy to note that there is an analogy between the hexahydroindazole series and the pyrazoline one. Thus, cyclization of the thiocarbamoyl group of compounds **7** into a thiazole nucleus to give **8** did not alter the anti-inflammatory activity. However, **7 c** gave an anomalous result where cyclization greatly improved the anti-inflammatory activity giving compound **8 f** with high potency. On the contrary, cyclization of **7 b, d** to give **9 c, d,** respectively**,** decreased the activity. Moreover, the potency of **7 b, d** was increased by cyclization of the thiocarbamoyl moiety to thiazolo[4,5-b]quinoxaline in **10 a, b**.

Table 2. Anti-inflammatory activity of the tested compounds assessed in comparison to ketoprofen as reference.

Compound	Mean % increase in paw weight ± SE	% Reduction of paw endema from control group
Control	52 ± 4	–
2	42 ± 3	18
3 b	42 ± 3	17
5	32 ± 2*	37
7 b	31 ± 2*	39
7 c	48 ± 4	7
7 d	25 ± 3*	50
8 d	31 ± 2*	39
8 f	28 ± 3*	44
8 h	26 ± 2*	50
9 c	42 ± 3	17
9 d	32 ± 2*	37
10 a	26 ± 2*	49
10 b	18 ± 1*	64
Ketoprofen	18 ± 1	63

All the test compounds and ketoprofen were given orally in a dose of 100 mg/kg.

* Significantly different from the control group using student's *t*-test ($p < 0.05$).

Considering the potentialities, compound **10 b** was studied in detail for its anti-inflammatory activity. Figure 2 shows the activity of three graded doses (25, 50, 100 mg/kg) of compound **10 b** and the standard drug ketoprofen. It was found that **10 b** has more or less comparable efficacy with that of ketoprofen. The ulcerogenic liability data are compiled in Table 3. The selected compounds, which are the most active in edema reduction, were tested against celexocib, ketoprofen, and indomethacin. The

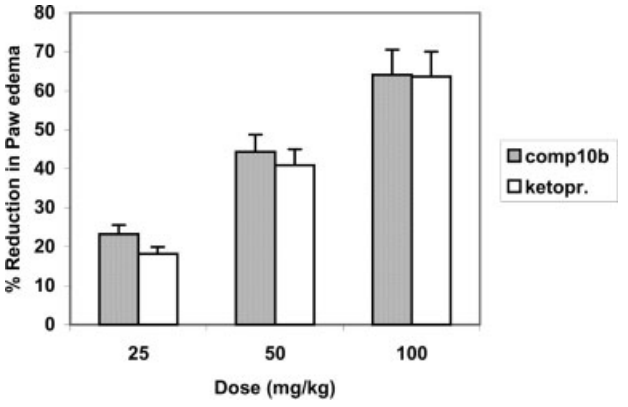


Figure 2. The anti-inflammatory activity of ketoprofen and compound **10 b** at three graded doses (25, 50, 100 mg/kg) expressed as % reduction in paw edema ± SE. No significant difference between **10 b** and ketoprofen-treated groups using student's t-test.

latter was used as a potent anti-inflammatory drug with substantial ulcerogenic effect. It is obvious that indomethacin produced high incidence of gastric ulceration while ketoprofen produces less ulcerogenic effect, and the selective COX-2 inhibitor celecoxib produced minimal ulcerogenic effect. The synthesized compounds were generally found to be safer from the view point of ulcer induction except for compounds **7 b**, **8 d**, and **8 f**. It should be pointed out that compounds **8 h** and **10 a, b** have significantly less ulcerogenic effect than ketoprofen. Moreover, their ulcerogenic effects were not significantly different from the celecoxib-treated group. Meanwhile, the ulcerogenic effect of compound **5** is low, but higher than ketoprofen.

Therefore, it may be concluded that the compounds with the most potent and selective COX-2 inhibitor as compared with ketoprofen and celecoxib are characterized by a central pyrazoline ring and have in vicinal positions thiocarbamoyl, thiazolyl, or thiazolo[4,5-b]quinoxalinyl moieties at the 1-position and the thiophene ring on the adjacent carbon. In addition, it has in the 3-position a phenyl ring substituted with an electron-withdrawing group, e.g. a Cl atom, as in compounds **7 d**, **8 h,** and **10 b**. Replacement of thiophene ring by furan, somewhat decreased the activity as in compounds **7 b**, **8 d,** and **10 a**. Furthermore, displacement of the electron-withdrawing group on the phenyl ring in position 3 by an electron donating markedly decreased the activity as in **7 c**. In addition, changing the arylthiazole moiety in position 2 by arylideneoxothiazole decreased the activity as in **9 c**. Moreover, replacement of the central pyrazoline ring by hexahydroindazole moiety decreased the activity as in compounds **2**, **3 b**, and **5**. Compound **10 b** gave minimal gastric ulceration and is almost equipotent with ketoprofen, thus it is considered to be a lead compound with a potent anti-inflammatory and selective COX-2 inhibitory activities.

Table 3. Potential ulcerogenic effect of the test compounds compared with ketoprofen, indomethacin, and celecoxib.

Compound	Dose (mg/kg)	Mean ulcer index ± S.E.
Control	Vehicle	0
Ketoprofen	50	4.9 ± 0.51*
Indomethacin	5	11.2 ± 1.40
Celecoxib	50	1.3 ± 0.20*$
5	50	5.4 ± 0.43*
7 b	50	6.1 ± 0.51*
7 d	50	3.9 ± 0.26*
8 d	50	5.7 ± 0.56*
8 f	50	6.2 ± 0.57*
8 h	50	2.3 ± 0.18*$
9 d	50	4.9 ± 0.35*
10 a	50	2.2 ± 0.19*$
10 b	50	2.1 ± 0.20*$

* ($p < 0.05$) significantly different from indomethacin-treated group using student's t-test.

$ ($p < 0.05$) significantly different from ketoprofen-treated group using student's t-test.

Experimental

Chemistry

Melting points (°C, uncorrected) were determined on a Fischer-Johns apparatus (Fischer-Scientific, Pittsburgh, PA, USA). IR spectra (KBr) were recorded on a Pye-Unicam SP1000 Spectrometer (í in cm^{-1}) (Pye-Unicam Ltd., Cambridge, UK). ^{1}H-NMR spectra were recorded on a Varian EM-360 (90 MHz) instrument (Varian Inc., Palo Alto, CA, USA) using TMS as internal standard (chemical shifts in ppm, δ units). Mass spectral data were recorded on a Varian MaT 112 A spectrophotometer. Analytical TLC was applied to monitor the reactions using precoated plates (Silica gel 60 F-245, Merck, Darmstadt, Germany) and spots were visualized with UV light. The results of elemental analyses (C,H,N) were within ± 0.4 % of the theoretical values. Thin layer chromatography was performed on silica gel GLF plates, 250 microns.

trans-7-Benzylidene-3,3a,4,5,6,7-hexahydro-3-phenyl-2-thiocarbamoyl-2H-indazole (**2**)

A mixture of 2,6-bis-benzylidenecyclohexanone (**1**) (2.74 g, 0.01 mol), thiosemicarbazide (1.82 g, 0.02 mol), and NaOH (1.23 g, 0.025 mol) was refluxed in ethanol (25 mL) for 5 h. The precipitate was filtered, dried and washed with cold ethanol.

Diastereoisomers **2** and **2 a** were separated by fractional recrystallization from methanol to yield (48 %) **2**: mp 165–167 °C; IR: 3410 (NH_2), 1630 (C=N), 1580 (C=C), 1320 (C=S); ^{1}H-NMR (DMSO-d_6): 1.21–3.12 (m, 6 H, CH_2), 3.41 (m, 1 H, 3a-H), 4.49 (br d, 1 H, 3-H), 7.40–8.21 (m, 11 H, ArH, CH=C), NH_2 not identifiable. MS: m/z (%) M^+ 347(3.9), 273 (100), 246 (13.1), 217 (26.2), 179 (34.8), 141 (25.6), 115 (48.9), 91 (26.0), 76 (42.4).

trans-2-(4-Aryl-2-thiazolyl)-7-benzylidene-3,3a,4,5,6,7-hexahydro-3-phenyl-2H-indazoles (**3 a**, **b**)

A mixture of compound **2** (3.47 g, 0.01 mol), 4-substituted phenacyl bromide (0.01 mol) in absolute ethanol (20 mL) was refluxed for 6 h. On cooling, the solid separated was filtered, dried and recrystallized from the suitable solvent. **3 a**: MS: m/z (%) M^+ 526 (2.9), 359 (27.9), 256 (83.0), 212 (19.5), 174 (100.0), 89 (32.3). **3 b**: ^{1}H-NMR (DMSO-d_6): 1.72–3.00 (m, 6 H, CH_2), 3.41 (m, 1 H, 3a-H), 5.12 (br d, 1 H, 3-H), 7.20–8.21 (m, 16 H, ArH, CH=C). MS: m/z (%) M^+ 481 (2.7), 313 (30.9), 210 (100.0), 168 (49.5), 77 (41.1).

trans-2-(5-Arylidene-4,5-dihydro-4-oxo-2-thiazolyl)-7-benzylidene-3,3a,4,5,6,7-hexahydro-3-phenyl-2H-indazoles (**4 a**, **b**)

A mixture of **2** (1.39 g, 4 mmol), chloroacetic acid (0.57g, 6 mmol), various aromatic aldehydes (4 mmol) and anhydrous sodium acetate (0.54 g) was refluxed in acetic anhydride (8 mL) and acetic acid (10 mL) for 6 h. The mixture was poured into ice-water, and the precipitate was collected by filtration, dried, and recrystallized from the suitable solvent. **4 a**: IR: 1703 (C=O), 1609 (C=N), 1585 (C=C). ^{1}H-NMR (DMSO-d_6): 1.26–3.23 (m, 6 H, CH_2), 3.38 (br d, 1 H, 3a-H), 5.35 (m, 1 H, 3-H), 6.85–8.21 (m, 12 H, ArH, 2CH=C). MS: m/z (%) M^+ 510 (4.7), 263 (37.4), 221 (52.7), 179 (25.3), 162 (17.4), 144 (20.9), 121 (25.9), 104 (58.1), 76 (100.0). **4 b**: IR: 1705 (C=O), 1630 (C=N), 1585 (C=C).

trans-7-Benzylidene-3,3a,4,5,6,7-hexahydro-3-phenyl-2-(thiazolo[4,5-b]quinoxalin-2-yl)-2H-indazole (**5**)

A mixture of compound **2** (3.47 g, 0.01 mol), 2,3-dichloroquinoxaline (1.99 g, 0.01 mol) in absolute ethanol (15 mL) was refluxed for 8 h. The solvent was evaporated under reduced pressure. The residue was recrystallized from acetone to yield 2.93 g (62 %) of **5**, mp 190–192 °C . IR: 1615 (C=N), 1590 (C=C); ^{1}H-NMR (DMSO-d_6): 1.52–2.39 (m, 6H, CH_2), 3.40 (m, 1 H, 3a-H), 4.55 (d, 1 H, 3-H), 7.24–8.22 (m, 15 H, ArH, CH=C).

3,5-Diaryl-1-thiocarbamoyl-2-pyrazolines (**7 a**–**d**)

A mixture of compounds (**6 a**–**d**) (0.01 mol), thiosemicarbazide (0.91 g, 0.01 mol), and NaOH (1.23 g, 0.025 mol) was refluxed in absolute ethanol (25 mL) for 4 h. On cooling, the product obtained was collected by filtration, dried and recrystallized from the appropriate solvent. **7 a**: IR: 3415 (NH_2), 1630 (C=N), 1580 (C=C), 1295 (C=S). ^{1}H-NMR (DMSO-d_6): 3.36 (d, 2 H, CH_2), 5.52 (t, 1 H, CH), 6.85–7.71 (m, 7 H, ArH), 7.80–8.00 (br s, 2 H, NH_2; D_2O exchangeable). **7 d**: IR: 3450 (NH_2), 1620 (C=N), 1580 (C=C), 1229 (C=S). ^{1}H-NMR (DMSO-d_6): 3.31 (d, 2 H, CH_2), 5.22 (t, 1 H, CH), 6.80–7.71 (m, 7 H, ArH), 7.85–8.10 (br s, 2 H, NH_2; D_2O exchangeable).

3,5-Diaryl-1-(4-aryl-2-thiazolyl)-2-pyrazolines (**8 a**–**h**)

These compounds were prepared following the same procedure used for preparation of **3 a**, **b** except that compound **2** was replaced by **7 a**–**d**. ^{1}H-NMR (DMSO-d_6): **8 a**: 3.39 (d, 2 H, CH_2), 5.60 (t, 1 H, CH), 6.85–7.71 (m, 12 H, ArH). **8 g**: 3.30 (d, 2 H, CH_2), 5.32 (t, 1 H, CH), 6.74–7.78 (m, 12 H, ArH).

3,5-Diaryl-1-(5-arylidene-4,5-dihydro-4-oxo-2-thiazolyl)-2-pyrazolines (**9 a**–**d**)

These compounds were prepared from **7 a**–**d** following the same procedure used for preparation of **4 a**, **b**. **9 a**: IR: 1705 (C=O), 1630 (C=N), 1595 (C=C). MS: m/z (%) M^+ 529 (64.8), 348 (26.9), 274 (23.2), 209 (31.5), 173 (56.5), 102 (21.5), 65 (100.0).

3,5-Diaryl-1-(thiazolo[4,5-b]quinoxalin-2-yl)-2-pyrazoline (**10 a**, **b**)

Compounds **10 a**, **b** were prepared following the same procedure used for preparation of **5** except that compound **7 a**–**d** was used instead of compound **2**. **10 a**: ^{1}H-NMR (DMSO-d_6): 3.34 (d, 2 H, CH_2), 5.51 (t, 1 H, CH), 6.80–7.74 (m, 11 H, ArH), 7.82–8.10 (br s, 2 H, NH_2; D_2O exchangeable).

Biological screening

Anti-inflammatory screening

The preliminary screening was done applying the procedure of Winter et al. [47] using groups of albino rats weighing 100–120 g each, 6 rats in each group. The compounds were suspended in 0.5 % carboxymethylcellulose (CMC) and given to the rats orally in a dose of 100 mg/kg. Ketoprofen was used as a positive control (reference standard) given orally in a dose of 100 mg/kg. Another animal group serving as a negative control received equivalent volume of the vehicle (0.5 % CMC) orally. One hour after drug administration, each rat was injected with 0.05 mL of 1 % carrageenin solution into the plantar tissue of the right hind paw. Four hours after carrageenin injection, the animals were killed by cervical dislocation and both the right and left hind paws were cut at a standard point and weighed. The difference in weight between right and left paws was recorded for each animal. The percentage increase in weight of the carrageenin-injected paw over the other paw was calculated and percentage reduction of edema from the control group was calculated as a measure of activity.

Ulcerogenic liability

It was determined in male albino rats weighing 150-180 g following the method of Adami et al. [48]. The rats were divided into 13 groups, 6 rats each, were fasted for 12 h prior to the administration of drugs while allowing free access to water. Indomethacin suspended in 0.5 % CMC was given orally in a dose of 5 mg/kg whereas ketoprofen and the other compounds were administered in a dose of 50 mg/kg. The dose was repeated daily for 5 days. The animals were sacrified 8 h after the last drug treatment by overdose of ether. The stomach was removed, rinsed with physiological saline, opened along the greater curvature and studied with hand lens (× 10 magnification). Ulcer index of each animal was expressed according to the sum of the length of lesions on the basis of Adami et al.

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