Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 43 (2008) 2597–2609
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Preliminary communication

Thiazolyl/oxazolyl formazanyl indoles as potent anti-inflammatory agents

Nisha Singh[1], Sudhir Kumar Bhati, Ashok Kumar*

Medicinal Chemistry Division, Department of Pharmacology, L.L.R.M. Medical College, Gargh Road, Meerut 250004, Uttar Pradesh, India

Received 21 March 2007; received in revised form 22 September 2007; accepted 11 December 2007
Available online 5 January 2008

Abstract

A series of 3-(2′-substituted indolidene aminothiazol-4′-yl)-2-(4-chlorophenyl) indoles (**3a**–**3d**), 3-(2′-substituted indolidene amino oxazol-4′-yl)-2-(4-chlorophenyl) indoles (**3a′**–**3d′**) and 3-[2′-(1′-substituted phenyl-3′-substituted indolyl formazan-4′-yl) thiazol-4′-yl]-2-(4-chlorophenyl) indoles (**4a**–**4h**), 3-[2′-(1′-substituted phenyl-3′-substituted indolyl formazan-4′-yl) oxazol-4′-yl]-2-(4-chlorophenyl) indoles (**4a′**–**4h′**) were synthesized and evaluated for their anti-inflammatory activity against carrageenan induced oedema in albino rats at a dose of 50 mg/kg p.o. The structure of all these compounds were established on the basis of elemental and spectral (IR, ^{1}H NMR and mass spectral data) studies. All the compounds of this series show moderate to good activity. The most active compound of this series 3-(2′-methyl indolidene aminothiazol-4′-yl)-2-(4-chlorophenyl) indole (**3b**) is found to be the most potent and has shown higher percent of inhibition of oedema, lower ulcerogenic liability and acute toxicity than the reference drug phenyl butazone.

Keywords: Substituted indoles; Oxazole; Thiazole; Formazan; Anti-inflammatory activity; Ulcerogenic activity; Acute toxicity studies

1. Introduction

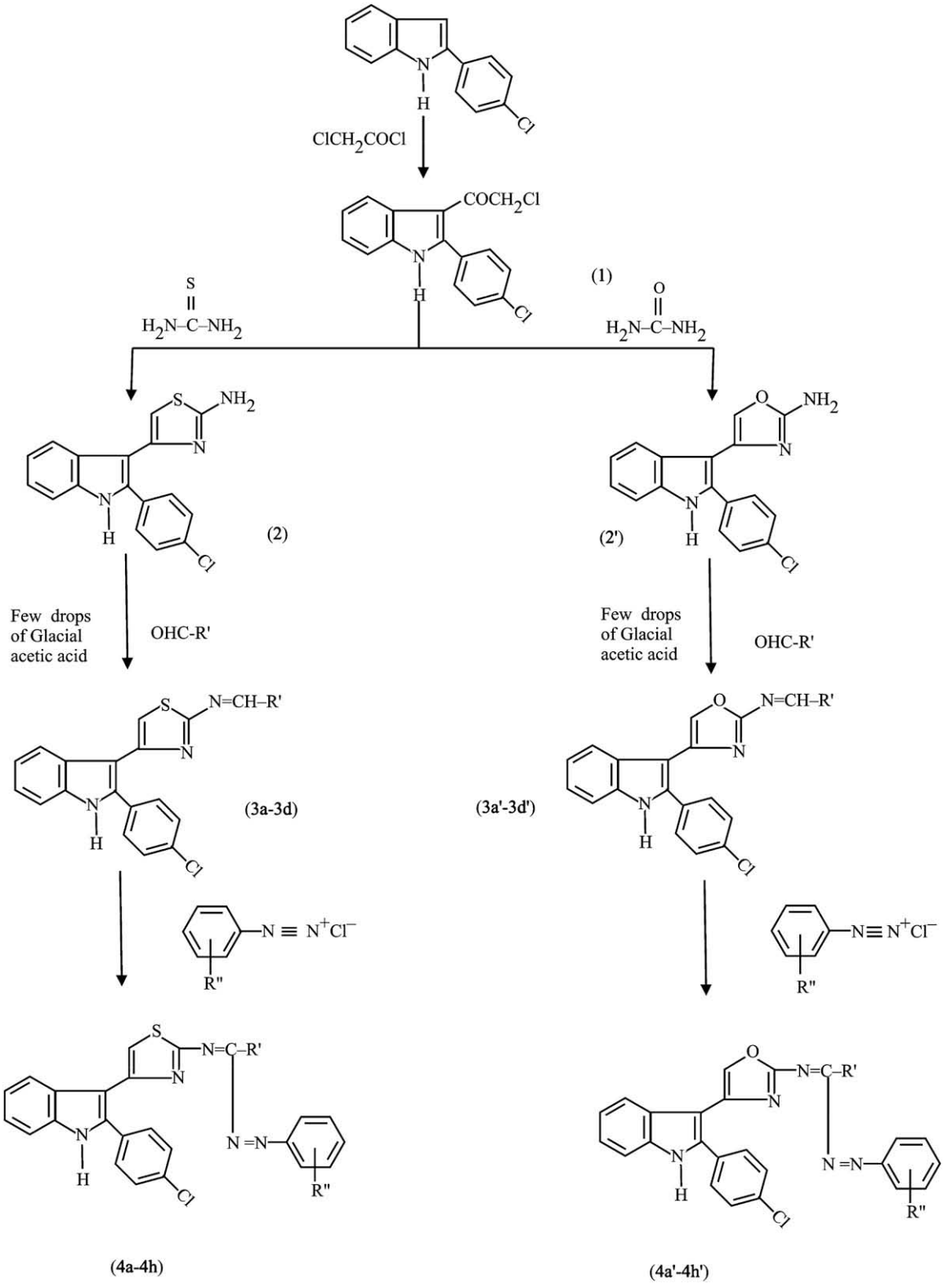
Acute and chronic inflammation and different type of arthritis are the inflammatory disorders which are a big blow to humanity and continual search for newer non-steroidal anti-inflammatory agents is the only way to fortify against this awful threat. The discovery of indomethacin [1] as a successful agent for clinical treatment of anti-inflammatory disorders has led to the exploration of indole moiety to obtain better anti-inflammatory agents. Furthermore indole and its analogs constitute the active class of compounds possessing wide spectrum of biological activities, such as anti-inflammatory [2–12], anti-microbial [13–15], anti-bacterial [16,17], anti-convulsant [18–21], and cardiovascular [22,23]. Moreover, thiazoles [24–27], oxazoles [28], formazanes [29,30] are well famed for their anti-inflammatory activities. In the light of the above report and also in continuation of our laboratory work on chemoselective reaction of indole derivatives, a drug strategy has been planned to synthesize several indole derivatives possessing thiazole, oxazole and formazan moieties with the hope to get better anti-inflammatory molecules. All compounds have been screened for their anti-inflammatory, ulcerogenic, analgesic and acute toxicity activities.

2. Chemistry

The synthetic route of compounds is shown in Scheme 1. Reaction of 2-(4-chlorophenyl) indole and chloroacetyl chloride yielded the starting compound **1** i.e. 3-chloroacetyl-2-(4-chlorophenyl) indole. This compound on reaction with thiourea and urea yielded compounds **2** and **2′**, respectively. These compounds on refluxing with 2-substituted-3-indolealdehyde in the presence of glacial acetic acid result in the next compounds i.e. 3-(2′-substituted indolidene aminothiazol-4′-yl)-2-(4-chlorophenyl) indoles (**3a**–**3d**) and 3-(2′-substituted indolidene amino oxazol-4′-yl)-2-(4-chlorophenyl) indoles

* Corresponding author. Tel.: +91 0121 2764084; mobile: +91 9917053074.
E-mail address: rajputak@gmail.com (A. Kumar).
[1] Part of Ph.D. thesis.

0223-5234/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2007.12.024


ClCH2COCl
COCH2Cl
(1)
H2N–C(=S)–NH2
H2N–C(=O)–NH2
(2)
(2')
Few drops of Glacial acetic acid
OHC-R'
N=CH–R'
(3a-3d)
(3a'-3d')
N≡N+Cl−
R"
N=C–R'
N=N
(4a-4h)
(4a'-4h')

Scheme 1.

(**3a′–3d′**). By diazotising compounds **3a–3d** and **3a′–3d′** with substituted anilines yielded the next compounds **4a–4h** and **4a′–4h′**.

3. Result and discussion

3.1. Anti-inflammatory activity against carrageenin induced oedema

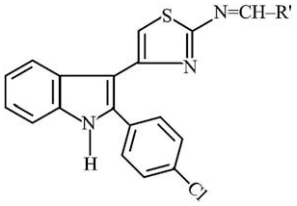
The anti-inflammatory activity of all the synthesized compounds are shown in Tables 1–4.

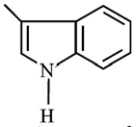
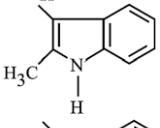
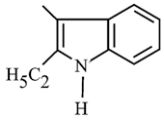
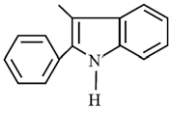
Compounds **3a–3d** have shown anti-inflammatory activity from 19.7 to 53.3%. When the compound was substituted with indole having methyl group at the second position of thiazol moiety (**3b**) it showed better anti-inflammatory activity (53.3%) than reference drug (38.8%) at the dose of 50 mg/kg p.o. Due to the potentiality, this compound and reference drug were tested at the three graded doses (25, 50 and 100 mg/kg p.o.). Compound **3b** have shown better activity at 25 and 50 mg/kg p.o. (36.4, 53.3%, respectively) than reference drug (15.2, 38.8%, respectively) while the same compound possessed almost equal degree of anti-inflammatory activity (68.2%) at the dose of 100 mg/kg p.o. as compared to standard drug (65.4%). Compound **3a**, which was substituted by plane indole also showed good activity (31.8%) but it is less active than standard drug. When the compound was substituted by ethyl group at 2-position of indole moiety (**3c**) it was found to be less active (25.8%) than **3a** and **3b** while compound **3d**, having phenyl group at the second position, exhibited lesser degree of anti-inflammatory activity (19.7%) than **3a–3c** (Table 1).

Compounds **4a–4g**, which were formed by the substitution of hydrogen of azomethine group of **3a–3d** with 4-methoxy phenyl azo (**4a**, **4c**, **4e** and **4g**) and 4-chlorophenyl azo (**4b**, **4d**, **4f** and **4h**) groups, respectively, have shown mild degree of anti-inflammatory activity (9.6–23.1%). Compound **4h**, which was substituted by 4-chlorophenyl azo group, has shown good anti-inflammatory activity (23.1%) among these eight compounds, while compound **4b**, which was also substituted by 4-chlorophenyl azo group and having unsubstituted indole exhibited mild degree of activity (9.6%). The other compounds of this step have shown the activity ranging between 11.5 and 19.2% (Table 2).

Indolidene oxazolyl indoles (**3a′–3c′**) exhibited lesser degree of inhibition of oedema (17.3–22.7%, Table 3) as

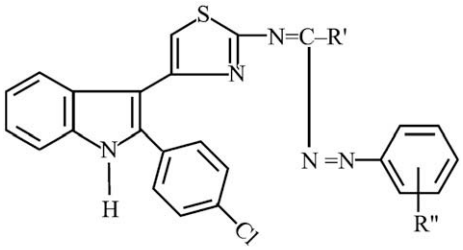
Table 1
Biological data of 3-(2′-substituted indolidene aminothiazol-4′-yl)-2-(4-chlorophenyl) indoles (**3a–3d**)

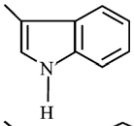
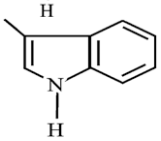
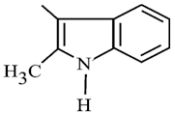
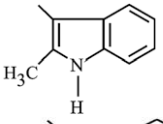
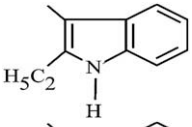
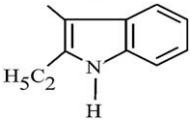
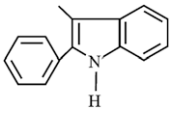
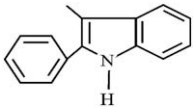


Compound	R′	Mean increase in paw vol ± SE	Anti-inflammatory activity %	Analgesic activity %	Ulcerogenic activity (UD_{50}) mg/kg i.p.	ALD_{50}
Control		0.66 ± 0.011	–			–
3a		0.45 ± 0.011	31.8[b]	30.6		>800
3b		0.42 ± 0.009 0.31 ± 0.013 0.21 ± 0.015	36.4[a] 53 3[b] 68.2[c]	35.1 51.4 64.6	199.9	>1000
3c		0.49 ± 0.013	25.8[b]	23.2		>800
3d		0.53 ± 0.008	19.7[b]	17.5		>800
Phenyl butazone	–	0.44 ± 0.015 0.31 ± 0.02 0.25 ± 0.12	15.2[a] 38.8[b] 65.4[c]	13.7 37.2 62.3	66.66	

[a] Tested at a dose of 25 mg/kg p.o.
[b] Tested at a dose of 50 mg/kg p.o.
[c] Tested at a dose of 100 mg/kg p.o.

Table 2
Biological data of 3-[2′-(1′-substituted phenyl-3′-substituted indolyl formazan-4′-yl) thiazol-4′-yl]-2-(4-chlorophenyl) indoles (**4a**–**4h**)



Compound	R′	R″	Mean increase in paw vol ± SE	Anti-inflammatory activity %	Analgesic activity %	ALD_{50}
Control			0.52 ± 0.009	–	–	–
4a		4-OCH_3	0.42 ± 0.013	19 2[a]	18.8	>800
4b		4-Cl	0.47 ± 0.009	9.6[a]	8.5	>800
4c		4-OCH_3	0.44 ± 0.009	15 4[a]	13.7	>800
4d		4-C1	0.41 ± 0.010	21.2[a]	19.8	>800
4e		4-OCH_3	0.45 ± 0.011	13.5[a]	12.3	>800
4f		4-C1	0.43 ± 0.013	17.3[a]	16.2	>800
4g		4-OCH_3	0.46 ± 0.008	11.5[a]	10.5	>800
4h		4-C1	0.40 ± 0.011	23.1[a]	21.9	>800

[a] Tested at a dose of 50 mg/kg p.o.

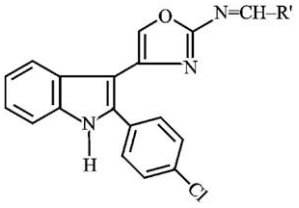
compared to indolidene thiazolyl indoles (**3a**–**3c**). On the contrary compound **3d′** which was substituted by 2-phenyl indole possesses greater activity (25.3%) than thiazolyl indole (**3d**, 17.5%) which was also substituted by same moiety but was less active than phenyl butazone.

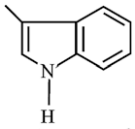
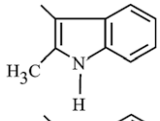
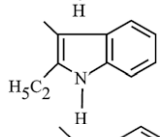
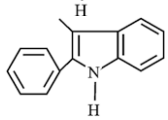
However, oxazolyl foarmazanes (**4a′**–**4h′**) were formed by diazotisation of indolidene oxazolyl indoles with 4-methoxy phenyl azo (**4a′**, **4c′**, **4e′**, **4g′**) and 4-chlorophenyl azo groups (**4b′**, **4d′**, **4f′**, **4h′**), respectively. The activity of these compounds range between 16.2 and 27.9%. It is interesting that 4-chlorophenyl azo substituted compounds (**4d′**, **4f′** and **4h′**) were found to be more active than 4-methoxy azo substituted compounds (**4c′**, **4e′** and **4g′**) but **4a′**, substituted with 4-methoxy phenyl azo group was found to be more active (20.6%) than **4b′** (16.2%), which was substituted by 4-chlorophenyl azo group. (Table 4).

All these compounds are generally more active than thiazolyl formazanes (9.6–23.1%).

Fig. 1 shows the bar diagram of anti-inflammatory activity of compound **3b** and reference drug phenyl butazone.

Table 3
Biological data of -3-(2′-substituted indolidene amino oxazole-4′-yl)-2-(4-chlorophenyl) indoles (**3a′–3d′**)



Compound	R′	Mean increase in paw vol ± SE	Anti-inflammatory activity %	Analgesic activity %	ALD_{50}
Control		0.75 ± 0.014	–	–	–
3a′		0.58 ± 0.011	22.7[a]	20.9	>800
3b′		0.59 ± 0.009	21.3[a]	19.9	>800
3c′		0.62 ± 0.014	17.3[a]	16.2	>800
3d′		0.56 ± 0.015	25.3[a]	23.7	>800

[a] Tested at a dose of 50 mg/kg p.o.

3.2. Analgesic activity

All the indolidene thiazolyl indoles (**3a–3d**) have shown moderate to good analgesic activity (17.5–51.4%). The compound which is substituted with indole having methyl group at the second position of thiazole moiety (**3b**), showed the most potent analgesic activity (51.4%). Thus it was studied in detail at the three graded doses i.e. 25, 50 and 100 mg/kg p.o. and it showed better activity at 25 and 50 mg/kg p.o. (35.1, 51.4%, respectively) than the reference drug phenyl butazone (13.7, 37.2%, respectively) but found to be almost equal at the dose of 100 mg/kg p.o. (64.6%). Formazanes of these compounds (**4a–4h**) generally showed a low degree of analgesic activity (8.5–21.9%). Furthermore, indolidene oxazolyl indoles (**3a′–3c′**), showed lesser activity (16.2–20.9%) as compared to the corresponding thiazolyl indoles. However, **3d′** was found to be more potent (23.7%) than compound **3d** (17.5%). Formazanes of these compounds (**4a′–4h′**) showed a little bit better activity (14.7–26.3%) than formazanes of thiazoles (**4a–4h**).

Fig. 2 shows the bar diagram of analgesic activity of compound **3b** and reference drug phenyl butazone.

3.3. Ulcerogenic activity

Considering the potentiality of compound **3b**, it was studied for ulcerogenic liability. The result clearly shows that the ulcerogenic activity of compound **3b** and phenyl butazone was dose dependent. However, the active compound (**3b**) had much less ulcerogenic liability as compared to phenyl butazone (UD_{50} of compound **3b** = 199.9 mg/kg i.p. and UD_{50} of phenyl butazone = 66.6 mg/kg i.p.)

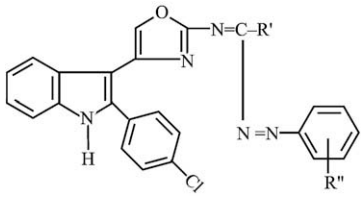
3.4. Acute toxicity

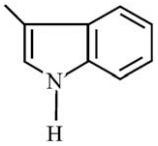
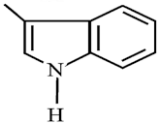
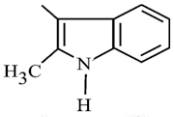
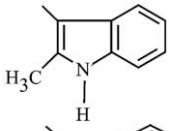
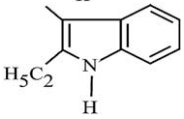
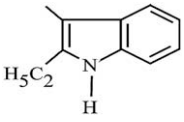
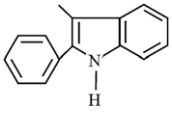
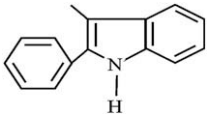
All the compounds showed $ALD_{50} > 800$ mg/kg p.o. However, the most potent compound **3b** showed $ALD_{50} >$ 1000 mg/kg p.o.

4. Conclusion

1. Formazanes were found to be less anti-inflammatory as well as analgesic agents than the corresponding indolidenes.
2. Indolidene thiazolyl indoles (**3a–3d**) were more potent than indolidene oxazolyl indoles (**3a′–3d′**).
3. Oxazolyl formazanes (**4a′–4h′**) have shown a little bit better anti-inflammatory and analgesic activity than thiazolyl formazanes (**4a–4h**).
4. Compound having methyl ($-CH_3$) group at the second position of indole moiety (**3b**) was found to be the most potent anti-inflammatory as well as analgesic agent (53.3 and 51.4%, respectively)

Table 4
Biological data of 3-[2′-(1′-substituted phenyl-3′-substituted indolyl formazan-4′-yl) oxazol-4′-yl]-2-(4-chlorophenyl) indoles (**4a′–4h′**)



Compound	R′	R″	Mean increase in paw vol + SE	Anti-inflammatory activity %	Analgesic activity %	ALD_{50}
Control			0.68 ± 0.009	–	–	–
4a′		4-OCH_3	0.54 ± 0.009	20.6[a]	19.2	>800
4b′		4-Cl	0.57 ± 0.011	16.2[a]	14.7	>800
4c′		4-OCH_3	0.55 ± 0.013	19.1[a]	17.8	>800
4d′		4-Cl	0.53 ± 0.015	22.1[a]	21.2	>800
4e′		4-OCH_3	0.51 ± 0.009	25 2[a]	23.9	>800
4f′		4-Cl	0.52 ± 0.010	23.5[a]	21.5	>800
4g′		4-OCH_3	0.56 ± 0.013	17.6[a]	15.2	>800
4h′		4-OCH_3	0.49 ± 0.015	27.9[a]	26.3	>800

[a] Tested at a dose of 50 mg/kg p.o.

5. The most active compound (**3b**) had much less ulcerogenic liability (UD_{50} = 199.9 mg/kg p.o.)

5. Experimental

5.1. Chemistry

The melting points were determined in open capillaries with the help of thermonic melting point apparatus and are uncorrected. The homogeneity of all newly synthesized compounds was routinely checked by TLC on silica gel-G coated plates. Elemental analysis of all the synthesized compounds were determined by a Perkin–Elmer 2400 elemental analyzer and results were found within the ±0.4% of theoretical values. IR spectra were recorded in KBr on a Perkin–Elmer spectrum RX-I spectrometer. ^{1}H NMR spectra were recorded by Bruker AC-300F instrument using DMSO-d_6 as solvent and TMS as internal reference standard. All chemical shift values were recorded as δ (ppm). Mass spectra were determined on a VG-70-S instrument.

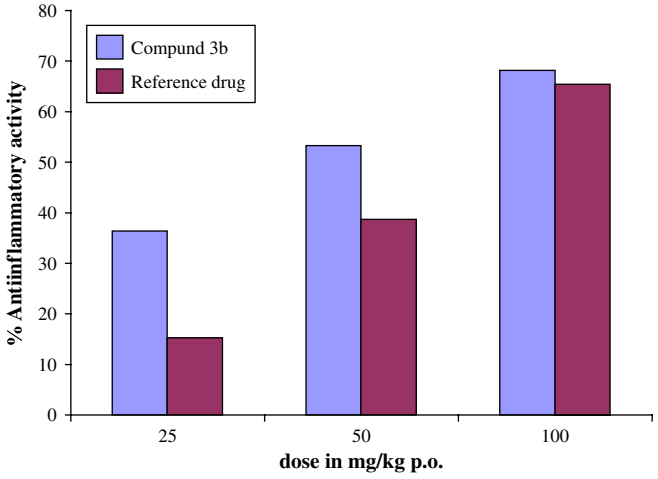


Fig. 1. Showing the bar diagram of anti-inflammatory activity of compound **3b** and reference drug (phenyl butazone).

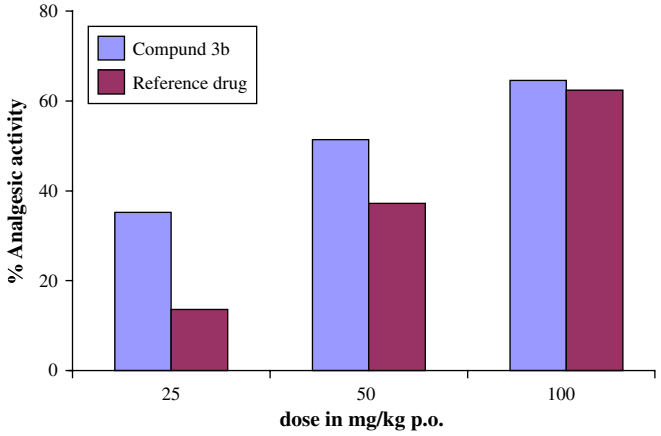


Fig. 2. Showing the bar diagram of analgesic activity of compound **3b** and reference drug (phenyl butazone).

Table 5
Spectral data of compounds **1**, **2**, **2′**, **3a–3d**, **3a′–3d′**, **4a–4h**, **4a′–4h′**

Compound	IR (KBr)ν_{max} in cm^{-1}	^1H NMR ($CDCl_3$) δ in ppm	MS: $[M]^+$ *m/z*
1	665 (C–Cl), 760 (C–C), 1245 (C–N), 1540 (C=C of aromatic ring), 1720 (C=O), 3040 (aromatic C–H)	3.40 (s, 2H, $-CH_2Cl$), 7.65–6.85 (m, 8H, Ar–H), 9.40 (s, IH, NH of indole exchangeable with D_2O)	304
2	660 (C–Cl), 682 (C–S–C), 765 (C–C), 1240 (C–N), 1545 (C=C of aromatic ring), 1580 (C=N), 3045 (aromatic C–H), 3160 (NH), 3335 (NH_2)	6.10 (s, 2H, $-NH_2$), 7.45–6.45 (m, 9H, Ar–H), 9.40 (s, IH, NH of indole exchangeable with D_2O).	325
2′	665 (C–Cl), 765 (C–C), 1075 (C–O–C), 1230 (C–N), 1535 (C=C of aromatic ring), 1585 (C=N), 3050 (aromatic C–H), 3145 (NH), 3360 (NH_2)	6.20 (bs, 2H, $-NH_2$), 7.45–6.50 (m, 9H, Ar–H), 9.50 (s, 1H, NH of indole exchangeable with D_2O)	309
3a	670 (C–Cl), 685 (C–S–C), 760 (C–C), 1240 (C–N), 1555 (C=C or aromatic ring), 1585 (C=N), 3040 (aromatic C–H), 3145 (NH)	7.65–6.45 (m, 14H, Ar–H), 8.15 (s, 1H, N=CH–Ar), 9.40 (2 × 1H, NH of indole exchangeable with D_2O).	452
3b	660 (C–Cl), 688 (C–S–C), 670 (C–C), 1250 (C–N), 1540 (C=C of aromatic ring), 1570 (C=N), 3050 (aromatic C–H), 3150 (NH)	2.36 (s, 3H, CH_3), 7.70–6.55 (m, 13H, Ar–H), 8.20 (s, 1H, N=CH–Ar) 9.36 (2 × 1H, NH of indole exchangeable with D_2O).	466
3c	670 (C–Cl), 682 (C–S–C), 765 (C–C), 1245 (C–N), 1560 (C=C of aromatic ring), 1580 (C=N), 3045 (aromatic C–H), 3155 (NH)	2.28 (t, 3H, CH_3), 4.20 (q, 2H, CH_2), 7.40–6.25 (m, 13H, Ar–H), 8.18 (s, 1H, N=CH–Ar), 9.28 (2 × 1H, NH of indole exchangeable with D_2O).	480
3d	665 (C–Cl), 685 (C–S–C), 760 (C–C), 1235 (C–N), 1555 (C=C of aromatic ring), 1585 (C=N), 3040 (aromatic C–H) 3145 (NH).	7.75–6.30 (m, 18H, Ar–H), 8.24 (s, 1H, N=CH–Ar), 9.30 (2 × 1H, NH of indole exchangeable with D_2O)	528
3a′	660 (C–Cl), 760 (C–C), 1055 (C–O–C), 1230 (C–N), 1570 (C=C of aromatic ring), 1585 (C=N), 3050 (aromatic C–H), 3135 (NH)	7.65–6.45 (m, 14H, Ar–H), 8.15 (s, 1H, N=CH–Ar), 9.50 (s, 2 × 1H, NH of indole exchangeable with D_2O).	436
3b′	655 (C–Cl), 750 (C–C), 1065 (C–O–C), 1245 (C–N), 1565 (C=C of aromatic ring), 1590 (C=N), 3045 (aromatic (C–H), 3140 ((NH)	2.38 (s, 3H, CH_3), 7.45–6.35 (m, 13H, Ar–H), 8.19 (s, 1H, N=CH–Ar), 9.36 (2 × 1H, NH of indole exchangeable with D_2O)	450
3c′	670 (C–Cl), 745 (C–C), 1080 (C–O–C), 1250 (C–N), 1570 (C=C of aromatic ring), 1595 (C=N), 3055 (aromatic C–H), 3150 (NH)	2.25 (t, 3H, CH_3), 4.20 (q, 2H, CH_2), 7.35–6.20 (m, 13H, Ar–H), 8.16 (s, 1H, N=CH–Ar), 9.30 (2 × 1H, NH of indole exchangeable with D_2O)	464
3d′	660 (C–Cl), 750 (C–C), 1065 (C–O–C), 1230 (C–N), 1560 (C=C of aromatic ring), 1610 (C=N), 3045 (aromatic C–H), 3155 (NH)	7.65–6.25 (m, 18H, Ar–H), 8.22 (s, 1H, N=CH–Ar), 9.45 (2 × 1H, NH of indole exchangeable with D_2O)	512
4a	665 (C–Cl), 685 (C–S–C), 750 (C–C), 1240 (C–N), 1430 (N=N), 1565 (C=C of aromatic ring), 1580 (C=N), 3065 (aromatic ring), 3065 (aromatic C–H), 3150 (NH)	3.39 (s, 3H, $-OCH_3$), 7.85–6.40 (m, 18H, Ar–H), 9.50 (s, 2 × 1H, NH of indole exchangeable with D_2O)	586

(*continued on next page*)

Table 5 (*continued*)

Compound	IR (KBr)ν_{max} in cm^{-1}	^1H NMR ($CDCl_3$) δ in ppm	MS: $[M]^+$ *m/z*
4b	650 (C–Cl), 670 (C–S–C), 755 (C–C), 1235 (C–N), 1445 (N=N), 1550 (C=C of aromatic ring), 1590 (C=N), 3060 (aromatic C–H), 3140 (NH)	7.75–6.25 (m, 18H, Ar–H), 9.48 (s, 2 × 1H, NH of indole exchangeable with D_2O)	591
4c	645 (C–Cl), 680 (C–S–C), 745 (C–C), 1245 (C–N), 1440 (N=N), 1545 (C=C of aromatic ring), 1595 (C=N), 3045 (aromatic C–H), 3125 (NH)	2.38 (s, 3H, $-CH_3$), 3.35 (s, 3H, $-OCH_3$), 7.65–6.30 (m, 17H, Ar–H), 9.45 (s, 2 × 1H, NH of indole exchangeable with D_2O)	600
4d	630 (C–Cl), 685 (C–S–C), 730 (C–C), 1230 (C–N), 1430 (N=N), 1555 (C=C of aromatic ring), 1580 (C=N), 3035 (aromatic C–H), 3130 (NH).	2.35 (s, 3H, $-CH_3$), 7.95–6.55 (m, 17H, Ar–H), 9.46 (s, 2 × 1H, NH of indole exchangeable with D_2O)	605
4e	640 (C–Cl), 675 (C–S–C), 740 (C–C), 1245 (C–N), 1445 (N=N), 1540 (C=C of aromatic ring), 1575 (C=N), 3040 (aromatic C–H), 3145 (NH)	2.24 (t, 3H, $-CH_3$), 3.32 (s, 3H, $-OCH_3$), 4.28 (q, 2H, CH_2), 7.85–6.45 (m, 17H, Ar–H) 9.42 (s, 2 × 1H, NH of indole exchangeable with D_2O)	614
4f	660 (C–Cl), 680 (C–S–C), 725 (C–C), 1240 (C–N), 1460 (N=N), 1535 (C=C of aromatic ring), 1570 (C=N), 3050 (aromatic C–H), 3135 (NH)	2.25 (t, 3H, $-CH_3$), 4.26 (q, 2H, CH_2), 7.80–6.45 (m, 17H, Ar–H), 9.38 (s, 2 × 1H, NH of indole exchangeable with D_2O)	619
4g	650 (C–Cl), 670 (C–S–C), 735 (C–C), 1245 (C–N), 1465 (N=N), 1560 (C=C of aromatic ring), 1555 (C=N), 3040 (aromatic C–H), 3125 (NH)	3.30 (s, 3H, $-OCH_3$), 6.90–6.25 (m, 22H, Ar–H), 9.36 (s, 2 × 1H, NH of indole exchangeable with D_2O)	662
4h	650 (C–Cl), 670 (C–S–C), 735 (C–C), 1245 (C–N), 1465 (N=N), 1560 (C=C of aromatic ring), 1555 (C=N), 3040 (aromatic C–H), 3125 (NH).	8.10–6.40 (m, 22, Ar–H), 9.36 (s, 2 × 1H, NH of indole exchangeable with D_2O)	667
4a′	660 (C–Cl), 750 (C–C), 1070 (C–O–C), 1250 (C–N), 1450 (N=N), 1550 (C=C of aromatic ring), 1570 (C=N), 3040 (aromatic C–H), 3145 (NH)	3.38 (s, 3H, $-OCH_3$), 8.05–6.55 (m, 18H, Ar–H), 9.45 (s, 2 × 1H, NH of indole exchangeable with D_2O)	570
4b′	655 (C–Cl), 740 (C–C), 1085 (C–O–C), 1240 (C–N), 1460 (N=N), 1535 (C=C of aromatic ring), 1585 (C=N), 3055 (aromatic C–H), 3135 (NH)	8.10–6.65 (m, 18H, Ar–H), 9.48 (s, 2 × 1H, NH of indole exchangeable with D_2O)	575
4c′	640 (C–Cl), 765 (C–C), 1060 (C–O–C), 1260 (C–N), 1445 (N=N), 1540 (C=C of aromatic ring), 1605 (C=N), 3040 (aromatic C–H), 3120 (NH)	2.36 (s, 3H, $-CH_3$), 3.36 (s, 3H, $-OCH_3$), 7.85–6.50 (m, 17H, Ar–H), 9.46 (s, 2 × 1H, NH of indole exchangeable with D_2O)	584
4d′	635 (C–Cl), 760 (C–C), 1065 (C–O–C), 1255 (C–N), 1435 (N=N), 1550 (C=C of aromatic ring), 1610 (C=N), 3025 (aromatic C–H), 3130 (NH)	2.34 (s, 3H, $-CH_3$), 7.90–6.50 (m, 17H, Ar–H), 9.42 (s, 2 × 1H, NH of indole exchangeable with D_2O)	589
4e′	650 (C–Cl), 750 (C–C), 1080 (C–O–C), 1240 (C–N), 1440 (N=N), 1545 (C=C of aromatic ring), 1595 (C=N), 3035 (aromatic C–H), 3145 (NH)	2.28 (t, 3H, $-CH_3$), 3.30 (s, 3H, $-OCH_3$), 4.32 (q, 2H, CH_2), 7.25–6.35 (m, 17H, Ar–H), 9.46 (s, 2 × 1H, NH of indole exchangeable with D_2O)	598
4f′	660 (C–Cl), 735 (C–C), 1075 (C–O–C), 1245 (C–N), 1450 (N=N), 1530 (C=C of aromatic ring), 1600 (C=N), 3055 (aromatic C–H), 3140 (NH)	2.25 (t, 3H, CH_3), 3.34 (q, 2H, $-CH_2$), 7.60–6.25 (m, 17H, Ar–H), 9.42 (s, 2 × 1H, NH of indole exchangeable with D_2O)	603
4g′	670 (C–Cl), 745 (C–C), 1060 (C–O–C), 1250 (C–N), 1460 (N=N), 1540 (C=C of aromatic ring), 1585 (C=N), 3045 (aromatic C–H), 3125 (NH)	3.35 (s, 3H, $-OCH_3$), 8.25–6.55 (m, 22H, Ar–H), 9.36 (s, 2 × 1H, NH of indole exchangeable with D_2O)	646
4h′	665 (C–Cl), 740 (C–C), 1070 (C–O–C), 1240 (C–N), 1450 (N=N), 1555 (C=C of aromatic ring), 1560 (C=N), 3055 (aromatic C–H), 3130 (NH)	8.10–6.45 (m, 22H, Ar–H), 9.32 (s, 2 × 1H, NH of indole exchangeable with D_2O)	651

*5.1.1. 3-Chloroacetyl-2-(4-chlorophenyl) indole (**1**)*

3-Chloroacetyl-2-(4-chlorophenyl) indole has been prepared by following the method of Arya et al. [31]. A solution of chloroacetyl chloride (0.02 mol) in dry dioxane (50 mL) was added dropwise during 1 h to a well stirred solution of indole (0.01 mol) and dry dioxane (150 mL) at 60 °C. The reaction mixture was further stirred for 1 h, cooled and poured into ice cold water. The resulting mixture was filtered to afford an orange solid which was recrystallised from ethanol. Anal. Calcd for $C_{16}H_{11}NCl_2O$: C, 63.15; H, 3.61; N, 4.60. Found: C, 63.09; H, 3.60; N, 4.61, M.p. 253 °C. Spectral data of this compound is given in Table 5.

*5.1.2. 3-(2′-Aminothiazol-4′-yl)-2-(4-chlorophenyl) indole (**2**)*

The solution of compound **1** i.e. 3-chloroacetyl-2-(4-chlorophenyl) indole (0.01 mol) in absolute ethanol (250 mL) was added to thiourea (0.01 mol). The reaction mixture was refluxed for 10–12 h, concentrated and filtered off. The solid thus obtained was washed with Na_2CO_3 solution and then with water to liberate the base completely, dried and recrystallised from ethanol/water to give compound **2**. Anal. Calcd for $C_{17}H_{12}N_3SCl$: C, 62.67; H, 3.68; N, 12.90. Found: C, 62.83; H, 3.67; N, 12.94, M.p. 268 °C. Spectral data of this compound is given in Table 5.

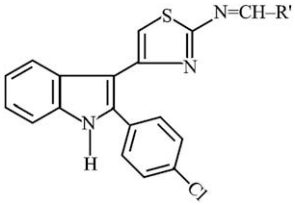
*5.1.3. 3-(2′-Amino oxazol-4′-yl)-2-(4-chlorophenyl) indole (**2′**)*

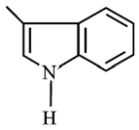
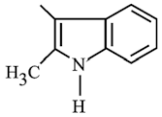
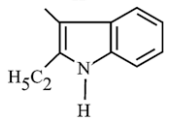
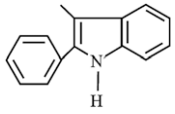
A mixture of 3-chloroacetyl-2-(4-chlorophenyl) indole (0.01 mol) in ethanol and urea (0.01 mol) were refluxed for 10 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the product was filtered off. The precipitate was washed with Na_2CO_3 solution and water. When the base was completely liberated from the precipitate, it was dried and recrystallised from ethanol/water. Anal. Calcd for $C_{17}H_{12}N_3OCl$: C, 65.91; H, 3.87; N, 13.57. Found: C, 66.03; H, 3.86; N, 13.60, M.p. 246 °C. Spectral data of this compound is given in Table 5.

*5.1.4. 3-(2′-Substituted indolidene aminothiazol-4′-yl)-2-(4-chlorophenyl) indoles (**3a–3d**)*

To a solution of compound **2** (0.01 mol) in ethanol (70 mL), 2–3 drops of glacial acetic acid and 2-substituted-3-indolealdehydes (0.01 mol) were added. The reaction mixtures were refluxed for 6 h. The solvents were distilled off, then the cooled reaction mixtures were poured onto ice, filtered off and recrystallised from a suitable solvent. The physical and analytical data of compounds (**3a–3d**) are shown in Table 6 and spectral data in Table 5.

Table 6
Physical and analytical data of 3-(2′-substituted indolidene aminothiazol-4′-yl)-2-(4-chlorophenyl) indoles (**3a–3d**)



Compound	R′	M.p. °C	Yield %	Mol. wt	Recrystallising solvent	Molecular formula	Elemental analysis					
							% C		% H		% N	
							Calcd	Found	Calcd	Found	Calcd	Found
3a		165	61	452.5	Ethanol	$C_{26}H_{17}N_4SCl$	68.95	69.18	3.75	3.76	12.37	12.41
3b		169	63	466.5	Ethanol/water	$C_{27}H_{19}N_4SC1$	69.45	69.59	4.07	4.08	12.00	12.03
3c		172	58	480.5	Benzene	$C_{28}H_{21}N_4SC1$	69.92	69.99	4.37	4.38	11.65	11.61
3d		176	56	528.5	Methanol	$C_{32}H_{21}N_4SCl$	72.65	72.54	3.97	3.96	10.59	10.56

*5.1.5. 3-(2′-Substituted indolidene amino oxazole-4′-yl)-2-(4-chlorophenyl) indoles (**3a′–3d′**)*

A solution of compound **2′** (0.01 mol) in ethanol and 2-substituted-3-indolealdehydes (0.01 mol) was refluxed in the presence of a few drops of glacial acetic acid for about 4–6 h. The reaction mixtures were concentrated, cooled and poured into crushed ice. The solids were filtered off and recrystallised from suitable solvent. The physical and analytical data of compounds (**3a′–3d′**) are shown in Table 7 and spectral data in Table 5.

*5.1.6. 3-[2′-(1′-Substituted phenyl-3′-substituted indolyl formazan-4′-yl) thiazol-4′-yl]-2-(4-chlorophenyl) indoles (**4a–4h**)*

To substituted aniline (0.01 mol) dissolved in glacial acetic acid (5 mL) was added conc. HCl (3 mL) at 0–5 °C. Then sodium nitrite solution (1 g in 5 mL of water) was added dropwise. Prepared diazonium salt solution was added in solutions of compounds **3a–3d** (0.01 mol) in ethanol dropwise with stirring in pyridine (50 mL) below 0 °C. The reaction mixtures were kept at room temperature for 2–3 days and then poured into ice cold water (250 mL). The resulting solids were washed with water and recrystallised from suitable solvents. Their physical and analytical data are depicted in Table 8 and spectral data in Table 5.

*5.1.7. 3-[2′-(1′-Substituted phenyl-3′-substituted indolyl formazan-4′-yl) oxazol-4′-yl]-2-(4-chlorophenyl) indoles (**4a′–4h′**)*

Concentrated HCl (3 mL) at 0–5 °C was added to substituted aniline (0.01 mol) dissolved in glacial acetic acid (5 mL). A solution of sodium nitrite (1 g in 5 mL of water) was then added dropwise. Prepared diazonium salt solutions were added to the solutions of compounds **3a′–3d′** (0.01 mol) in ethanol dropwise with stirring in pyridine (50 mL) below 0 °C. The reaction mixtures were kept at room temperature for 2–3 days and poured onto ice. The separated solids were washed with water and recrystallised from suitable solvent. Their physical and analytical data are depicted in Table 9 and spectral data in Table 5.

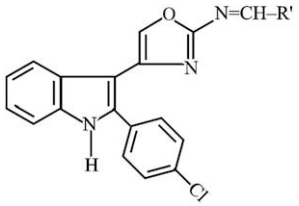
5.2. Pharmacology

5.2.1. Anti-inflammatory activity

Preliminary study at all the three tested doses (25, 50, 100, mg/kg) were compared with standard drug, phenyl butazone and acetyl salicylic acid. These compounds were administered either by oral or intraperitoneal route.

Rats of either sex weighing 75–125 g were divided into groups of 6 animals each. A freshly prepared suspension of carrageenin (1.0% in 0.9% saline) 0.05 mL, was injected under the planter aponeurosis of right paw of the

Table 7
Physical and analytical data of 3-(2′-substituted indolidene amino oxazole-4′-yl)-2-(4-chlorophenyl) indoles (**3a′–3d′**)



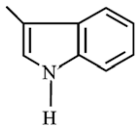
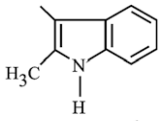
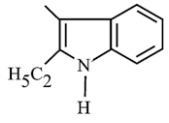
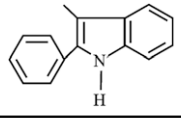
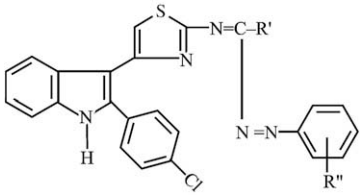
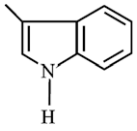
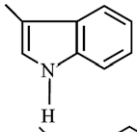
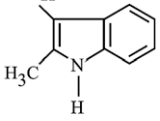
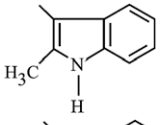
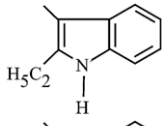
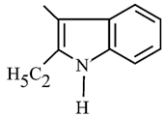
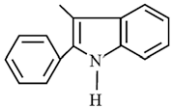
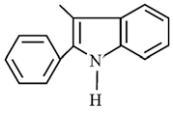
Compound	R′	M.p. °C	Yield %	Mol. wt	Recrystallising solvent	Molecular formula	Elemental analysis					
							%C		% H		%N	
							Calcd	Found	Calcd	Found	Calcd	Found
3a′		188	63	436.5	Methanol/water	$C_{26}H_{17}N_4OCl$	71.47	71.31	3.89	3.90	12.82	12.85
3b′		193	59	450.5	Acetic acid	$C_{27}H_{19}N_4OCl$	71.92	72.03	4.21	4.20	12.43	12.42
3c′		197	56	464.5	Benzene	$C_{28}H_{21}N_4OCl$	72.33	72.35	4.52	4.53	12.05	12.06
3d′		202	60	512.5	Ethanol	$C_{32}H_{21}N_4OCl$	74.92	74.98	4.09	4.08	10.92	10.89

Table 8
Physical and analytical data of 3-[2′-(1′-substituted phenyl-3′-substituted indolyl formazon-4′-yl) thiazol-4′-yl]-2-(4-chlorophenyl) indoles (**4a–4h**)



Compound	R′	R″	M.p. °C	Yield %	Mol. wt	Recrystalizing solvent	Molecular Formula	Elemental analysis					
								%C		%H		%N	
								Calcd	Found	Calcd	Found	Calcd	Found
4a		4-OCH_3	232	58	586.5	Ethanol	$C_{33}H_{23}N_6SOCl$	67.51	67.53	3.92	3.92	14.32	14.30
4b		4-C1	248	55	591	Benzene/hexane	$C_{32}H_{20}N_6SCl_2$	64.97	65.14	3.38	3.39	14.21	14.17
4c		4-OCH_3	236	52	600.5	Methanol/water	$C_{34}H_{25}N_6SOC1$	67.94	68.01	4.16	4.15	13.98	14.01
4d		4-Cl	252	48	605	Ethanol/water	$C_{33}H_{22}N_6SCl_2$	65.56	65.43	3.64	3.65	13.88	13.91
4e		4-OCH_3	239	46	614.5	Acetone	$C_{35}H_{27}N_6SOC1$	68.34	68.19	4.39	4.38	13.66	13.67
4f		4-Cl	257	51	619	Benzene/pet. ether	$C_{34}H_{24}N_6SC1_2$	65.91	66.02	3.87	3.86	13.57	13.59
4g		4-OCH_3	243	48	662.5	Methanol	$C_{39}H_{27}N_6SOC1$	70.64	70.78	4.07	4.08	12.67	12.63
4h		4-Cl	262	50	667	Methanol/water	$C_{38}H_{24}N_6SC1_2$	68.36	68.24	3.59	3.58	12.59	12.62

rat by the method of Winter et al. [32]. One group was kept as control and the animals of other group were pre-treated with the test drugs suspended in gum acacia, given orally 1 h before the carrageenin injection. The volume of foot was measured before 1 and 3 h after carrageenin treatment by the micro-pipette method as described by Buttle et al. [36] in 1957. The mean increase of paw volume in each group was measured and percentage anti-inflammatory activity was calculated according to the formula given below

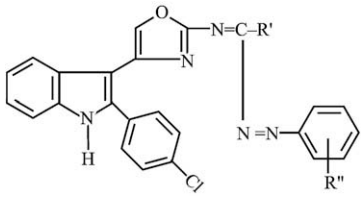
$$\text{Percentage of inhibition of oedema} = (1 - V_t/V_c) \times 100$$

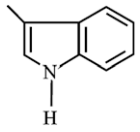
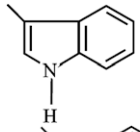
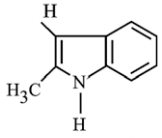
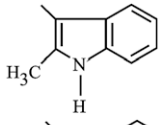
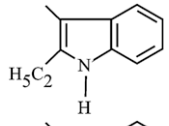
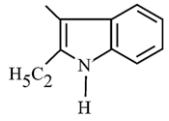
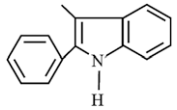
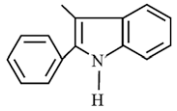
where V_t and V_c are the volumes of oedema in drug treated and the control groups. Phenyl butazone was used as the standard drug for comparison.

5.2.2. Analgesic activity

This activity was performed by following the method of Berkowitz et al. [33]. This method is based on the property of the test compound to antagonize the phenyl quinone-induced pain syndrome in mice. Groups of 5 mice were injected intraperitoneally with 0.25 mL of a 0.02% solution of phenylquinone in ethanol (5%) 1 h after oral administration of the test compound. The number of writhes induced in each

Table 9
Physical and analytical data of 3-[2′-(1′-substituted phenyl-3′-substituted indolyl formazon-4′-yl) oxazol-4′-yl]-2-(4-chlorophenyl) indoles (**4a′–4h′**)



Compound	R′	R″	M.p. °C	Yield %	Mol. wt	Recrystalizing solvent	Molecular formula	Elemental analysis					
								%C		% H		%N	
								Calcd	Found	Calcd	Found	Calcd	Found
4a′		4-OCH_3	238	56	570.5	Acetone	$C_{33}H_{23}N_6O_2C1$	69.41	69.18	4.03	4.02	14.72	14.70
4b′		4-Cl	250	54	575	Methanol	$C_{32}H_{20}N_6OCl_2$	66.78	66.71	3.47	3.46	14.60	14.59
4c′		4-OCH_3	242	46	584.5	Ethanol	$C_{34}H_{25}N_6O_2C1$	69.80	69.88	4.27	4.27	14.37	14.39
4d′		4-Cl	254	55	589	Benzene/pet. ether	$C_{33}H_{22}N_6OC1_2$	67.23	67.20	3.73	3.74	14.26	14.25
4e′		4-OCH_3	245	48	598.5	Methanol/water	$C_{35}H_{27}N_6O_2C1$	70.17	70.02	4.51	4.50	14.03	14.0
4f′		4-Cl	259	53	603	Methanol/water	$C_{34}H_{24}N_6OCl_2$	67.66	67.83	3.98	3.97	13.93	13.91
4g′		4-OCH_3	250	50	646.5	Ethanol	$C_{39}H_{27}N_6O_2C1$	72.38	72.44	4.17	4.16	12.99	13.01
4h′		4-C1	264	52	651	Ethanol/water	$C_{38}H_{24}N_6OCl_2$	70.04	70.23	3.68	3.69	12.90	12.88

mouse was counted for 5 min (between 5 and 10 min) after injection of an irritant. The analgesic effect was expressed as percent protection in comparison to control.

$$\%\text{Protection} = (1 - \text{mean number of writhes in mice of test groups}/\text{mean number of writhes in mice of control group}) \times 100.$$

5.2.3. Ulcerogenic activity

Adult albino rats of either sex were divided into group of 10 animals each. Pregnancy was excluded in the female rats and they were fasted 24 h prior to the administration of drugs. Water was allowed at libitum to the animals. The compounds (which have shown the promising anti-inflammatory activity) and phenyl butazone were given intraperitoneally and the animals sacrificed 8 h after drug treatment. The stomach, duodenum and jejunum were removed and examined with a hand lens for any evidence of (a) shedding of epithelium

(b) red spots below skin and bleeding and (c) erosion or discrete ulceration with or without perforation. The presence of any one of these criteria was considered to be an evidence of ulcerogenic activity Djahanguiri [34].

5.2.4. Acute toxicity study

Approximate 50% lethal dose (ALD_{50}) of the promising compounds was determined in albino mice. The mice of either sex weighing between 20 and 28 g were used for the study. The drugs were injected by intraperitoneal (i.p.) route at different dose levels in separate groups of animals. After 24 h of drug administration, percent mortality in each group was observed. From the data obtained, ALD_{50} was calculated by the method of Smith [35].

References

[1] R.J. Flower, S. Moncada, J.R. Vane, Goodman and Gilman's The Pharmacological Basis of Therapeutics, seventh ed.). (1985) p. 695.
[2] U. Misra, A. Hitkari, A.K. Saxena, S. Gurtu, K. Shanker, Eur. J. Med. Chem. 31 (1996) 629–634.
[3] A. Andreani, M. Rambaldi, A. Locatelli, G. Pifferi, Eur. J. Med. Chem. 29 (1994) 903–906.
[4] M.Y. Ebeid, S.M. Lashine, S.M. El-Ad, K.M.I. Abou, Zagazig, J. Pharm. Sci. 3 (1994) 40–48.
[5] P. Rani, V.K. Srivastava, A. Kumar, Eur. J. Med. Chem. 39 (2004) 449–452.
[6] P.K. Dubey, T. Venkateshwar Kumar, P. Raddanna, K. Anil Kumar, Indian J. Chem. 45B (2006) 2128–2132.
[7] R. Agarwal, C. Agarwal, C. Singh, V.S. Misra, J. Chem. Soc. Pak. 6 (1984) 89.
[8] M. Verma, M. Tripathi, A.K. Saxena, K. Shanker, Eur. J. Med. Chem. 29 (1994) 941–946.
[9] A. Kumar, A. Kumar, A.K. Saxena, K. Shanker, Pharmazie 43 (1998) 45–46.
[10] H. Inion, H.De Vogelaer, M. Descamps, J. Bauthier, M. Colot, J. Richard, R. Charlier, Chem. Abstr. 88 (1978) 601.
[11] A.A. Mohamed Radwan, E.A. Ragab, N.M. Sabry, S.M. El, Shenawy, Bioorg. Med. Chem. 15 (2007) 3832–3841.
[12] M.kF. Zheng, M. Zheng, D.Y. Deng, S. Oils, X. Luo, K. Chen, H. Liu, H. Jiang, Bioorg. Med. Chem. Lett. 17 (2007) 2414–2420.
[13] P. Sharma, A. Kumar, P. Pandey , Indian J. Chem. 45B (2006) 2077–2082.
[14] R.S. H.PanwarVerma, V.K. Srivastava, A. Kumar, Indian J. Chem. (2006) 2099–2104.
[15] S.R. Bhusare, A.B. Shinde, R.P. Pawar, Y.B. Vibhute, Indian J. Pharm. Sci. 3 (2004) 228–231.
[16] A. Dandia, V. Sehgal, P. Singh, Indian J. Chem. 32 B (1993) 1288–1291.
[17] B.S. Holla, K.V. Udupa, J. Indian Chem. Soc. 65 (7) (1988) 524.
[18] Archna, P. Rani, K. Bajaj, V.K. Srivastava, R. Chandra, A. Kumar, Arzneim.-Forsch./Drug Res. (2003) 301–306.
[19] Archna, V.K. Srivastava, A. Kumar, Indian J. Pharm. Sci. 65 (4) (2003) 356–362.
[20] A.C. Bajji, K.P. Channabasavaraj, K.M.K. Swamy, Indian Drugs 31 (6) (1994) 269–272.
[21] A. El.-Gendy Adel, A. Abdou Naida, Z.S. El-Taber, A. El-Banna Hosny, Alexandria, J. Pharm. Sci. 7 (1997) 99–103.
[22] A. Kumar, K.K. Saxena, S. Gurtu, J.N. Sinha, K. Shanker, Indian Drugs 24 (1986) 1–5.
[23] N. Bru-Magniez, T. Guenger, J.M. Tenton, (Laboratories UPSA. Fr.) U.S. U 5,480,983 (Cl. 536-27, 62; C07H19/167), 2 Jan 1996, FR Appl. 92/138, 8 Jan 1992; 30 pp. Cont-in- part of U.S. 5,229,505 (Eng.) Chem. Abstr. 124 (17) (1996).
[24] S.P. Hiremath, A.C. Bajji, J.S. Biradar, Indian J. Chem. 28B (1989) 824–828.
[25] P.K. Sharma, S.N. Sawhney, A. Gupta, G.B. Singh, S. Bani, Indian J. Chem. 37B (1998) 376–381.
[26] B. Holla, Shivarama, K.V. Malini, B.S. Rao, B.K. Sarojini, N.K.I. Suchetha, Eur. J. Med. Chem. 38 (2003) 313.
[27] A. Aldo, R. Mirella, C. Patricia, G. Lucedia, S. Pierlugi, J. Heterocycl. Chem. 26 (1989) 525.
[28] K.V. Reddy, G. Sabitha, A.V.S. Rao, Indian J. Chem. 37B (1998) 697–703.
[29] E. Bansal, V.K. Srivastava, A. Kumar, Indian J. Chem. 39B (2000) 357–362.
[30] I.P. Singh, S. Gurtu, A. Kumar, J.N. Sinha, K.P. Bhargava, K. Shanker, Arch. Pharm. 317 (1984) 609–614.
[31] V.P. Arya, J. David, R.S. Grawal, C.L. Kaul, R.H. Mizoni, S. Rajappa, S.J. Shenoy, Indian J. Chem. 15B (1977) 174–181.
[32] C.A. Winter, E.A. Risley, G.W. Nuss, Proc. Soc. Exp. Biol. 111 (1962) 544–550.
[33] B.A. Berkowitz, A.D. Finck, S.H. Ngai, J. Pharmacol. Exp. Ther. 203 (1977) 539–547.
[34] B. Djahanjuiri, J. Pharm. Pharmacol. 21 (1969) 341–345.
[35] Q.E. Smith, Pharmacological Screening Test Progress in Medicinal Chemistry, I. Butterworths, London, 1960, pp. 1–33.
[36] G.A.H. Buttle, P.F. Arcy, E.M. Howard, D.N. Kellett, Nature 179 (1957) 629.