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Original article

Synthesis of new substituted azetidinoyl and thiazolidinoyl-1,3, 4-thiadiazino (6,5-b) indoles as promising anti-inflammatory agents

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Abstract

Various *N*-({5-[(arylmethylene)amino]-1,3,4-thiadiazol-2-yl}methyl) [1,3,4] thiadiazino[6,5-b]indol-3-amine (**6a–6h**), 2-aryl-3-{5-[([1,3,4] thiadiazino[6,5-b]indol-3-ylamino)methyl]-1,3,4-thiadiazol-2-yl}-1,3-thiazolidin-4-one (**7a–7h**), and 3-chloro-4-aryl-1-{5-[{[1,3,4]thiadiazino[6,5-b]indol-3-ylamino]methyl]-1,3,4-thiadiazol-2-yl}azetidin-2-one (**8a–8h**) have been synthesized in the present study. The structure of these newly synthesized compounds were confirmed by their analytical and spectral data. These compounds were also evaluated for their anti-inflammatory, ulcerogenic and analgesic activities. Compound **8g** has shown most active anti-inflammatory and analgesic activities with better ulcerogenic activity than phenylbutazone, while this compound was found to be associated with lesser degree of anti-inflammatory and analgesic activities as compared to indomethacin.

Keywords: Benzylidenaminoindoles; Thiazolidinones; Azetidinones; Anti-inflammatory, analgesic, ulcerogenic activities and 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-steriodal anti-inflammatory agents is the only way to fortify against this awful threat. Indole and its analogs constitute the active class of compounds possessing wide spectrum of biological activities, such as anti-inflammatory [1-6], anticonvulsant [7], antimicrobial [8] antibacterial [9,10] antifungal [11,12], Moreover, azetidinones [13,14] and thiazolidinones [15,16] are well famed for their anti-inflammatory activities. In the light of 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 azetidinone and thiazolidinone moiety with the hope to get better anti-inflammatory molecules. All compounds have been screened for their anti-inflammatory, ulcerogenic, analgesic and toxicity activities.

2. Chemistry

The chemical synthesis initiates with the reaction of indole-2,3-dione with thiosemicarbazide to yield 3-thiosemicarbazidoindole-2-one **1**. 2-Amino-1,3,4-thiadiazino (6,5-b) indole-2 was prepared by the cyclization of compound **1** with cold conc. sulphuric acid. Furthermore, compound **2** reacted with chloroethylacetate to yield 2-carboethoxymethylamino-1,3,4,-thiadiazino(6,5-b) indole **3**. The latter compound on reaction with thiosemicarbazide resulted into the formation of 2-(thiosemicarbazidocarbonylmethylamino)-1,3,4,-thiadiazino(6,5-b) indole **4**. Compound **4** on treatment with conc. H_2SO_4 and followed by neutralization with liquid NH_3 gave 2-[5′-aminothiadiazol-2′-yl methylamino]-1,3,4-thiadiazino (6,5-b) indole **5**. Compound **5** when reacted with substituted benzaldehydes yielded *N*-({5-[(arylmethylene)amino]-1,3,4-thiadiazol-2-yl}methyl)[1,3,4] thiadiazino(6,5-b)indol-3-amine **6a–6h**, 2-aryl-3-{5-[([1,3,4] thiadiazino(6,5-b)indol-3-ylamino)

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methyl]-1,3,4-thiadiazol-2-yl}-1,3-thiazolidin-4-one **7a–7h**, were synthesized by cyclo condensation of **6a–6h** with thioglycolic acid in the presence of anhydrous $ZnCl_2$, while 3-chloro-4-aryl-1-{5-[{[1,3,4]thiadiazino(6,5-b)indol-3-ylamino]methyl]-1,3,4-thiadiazol-2-yl}azetidin-2-one **8a–8h** were synthesized by cycloaddition of **6a–6h** with chloroacetylchloride in presence of triethylamine. The structure of all newly synthesized indole derivatives were confirmed on the basis of analytical and spectral data (Scheme 1).

3. Results and discussion

All the newly synthesized compounds of this series were tested for anti-inflammatory, analgesic, and ulcerogenic activities, at a dose of 50 mg/kg p.o. The results of anti-inflammatory and analgesic activities of all the compounds are statistically significant (Table 4). Out of 20 compounds, only three compounds **7g**, **8g** and **8h** were found to possess more potent anti-inflammatory activity in comparison to phenylbutazone. Compound **7g**, which was substituted with chloro group at 2 position of phenyl ring have shown 38.54% of inhibition of oedema. Compound **6a** which possessed phenyl ring have shown least activity, i.e. 14.75%. Compound **7c** which was substituted with 4-methoxy group on phenyl ring, has shown moderate degree of anti-inflammatory activity (28.13%).

The last step compounds **8a–8h** were characterized by the presence of an azetidinone ring (β-lactam). All the compounds of this stage have shown promising degree (30.55–41.23%) of anti-inflammatory activity. Compound **8g**, has shown the maximum percentage of anti-inflammatory activity, i.e. 41.23% at a dose of 50 mg/kg p.o. Considering, the potentiality of compounds **7g** and **8g**, these were studied in detail at three graded doses 25, 50, 100 mg/kg p.o. Compound **8g** exhibited better anti-inflammatory activity at all three graded doses of 25, 50 and 100 mg/kg p.o. as compared to phenylbutazone. However, compound **8g** was less active as compared to indomethacin at the three graded doses. The ulcerogenic liabilities of compounds **7g** (166.66 mg/kg i.p.) and **8g** (133.33 mg/kg i.p.) are much less than that of indomethacin (54.60 mg/kg i.p.) and phenylbutazone (66.6 mg/kg i.p.). The azetidinone derivatives **8a–8h** showed better analgesic activity than thiazolidinones **7a–7h**. Compounds of thiazolidinones **7a–7h** have shown moderate to good analgesic activity. Compound **7g** which was substituted by 2-chlorophenyl ring at 5-position of thiadiazole nucleus exhibited most potent (24.67%) analgesic activity at 50 mg/kg p.o. The most active compound of this series was **8g** which has shown potent analgesic activity, i.e. 38.00% at a dose of 50 mg/kg p.o. Moreever, when these compounds were tested at three (25, 50 and 100) graded doses, it was found that analgesic activity of **7g** was less than phenylbutazone and indomethacin while **8g** exhibited better analgesic activity than phenylbutazone, while it is less active then indomethacin.

All these compounds were also evaluated for COX-1 and COX-2 inhibitory activities. Compound **7g** showed 43.13% COX-1 and 74.64% COX-2 inhibition action while compound **8g** exhibited 69.13% and 93.34% inhibition of action. Moreover, rest of the compounds showed moderate degree of COX-1 and COX-2 inhibitory actions suggesting that these compounds showed anti-inhibitory activity by inhibition of both COX-1 and COX-2 enzymes.

ALD_{50} of all these compounds were >800 mg/kg p.o.

4. Conclusion

1. Azetidinones **8a–8h** possessed more potent anti-inflammatory and analgesic activities than that of their corresponding parent compounds, i.e. *N*-({5-[(arylmethylene)amino]-1,3,4-thiadiazol-2-yl}methyl) [1,3,4] thiadiazino(6,5-b)indol-3-amine **6a–6h**.
2. Azetidinone compounds **8a–8h** have also shown better activities then thiazolidinones **7a–7h**.
3. Compounds **7g** and **8g** having a 2-chlorophenyl group as substituent exhibited most potent anti-inflammatory and analgesic activities. Thus the obtained biological results clearly indicate that compounds which showed maximum anti-inflammatory activity also exhibit potent analgesic activity.
4. All the compounds **6a–6h**, **7a–7h** and **8a–8h** have shown much less anti-inflammatory and analgesic activities, when compared with indomethacin.

5. Experimental

5.1. Chemistry

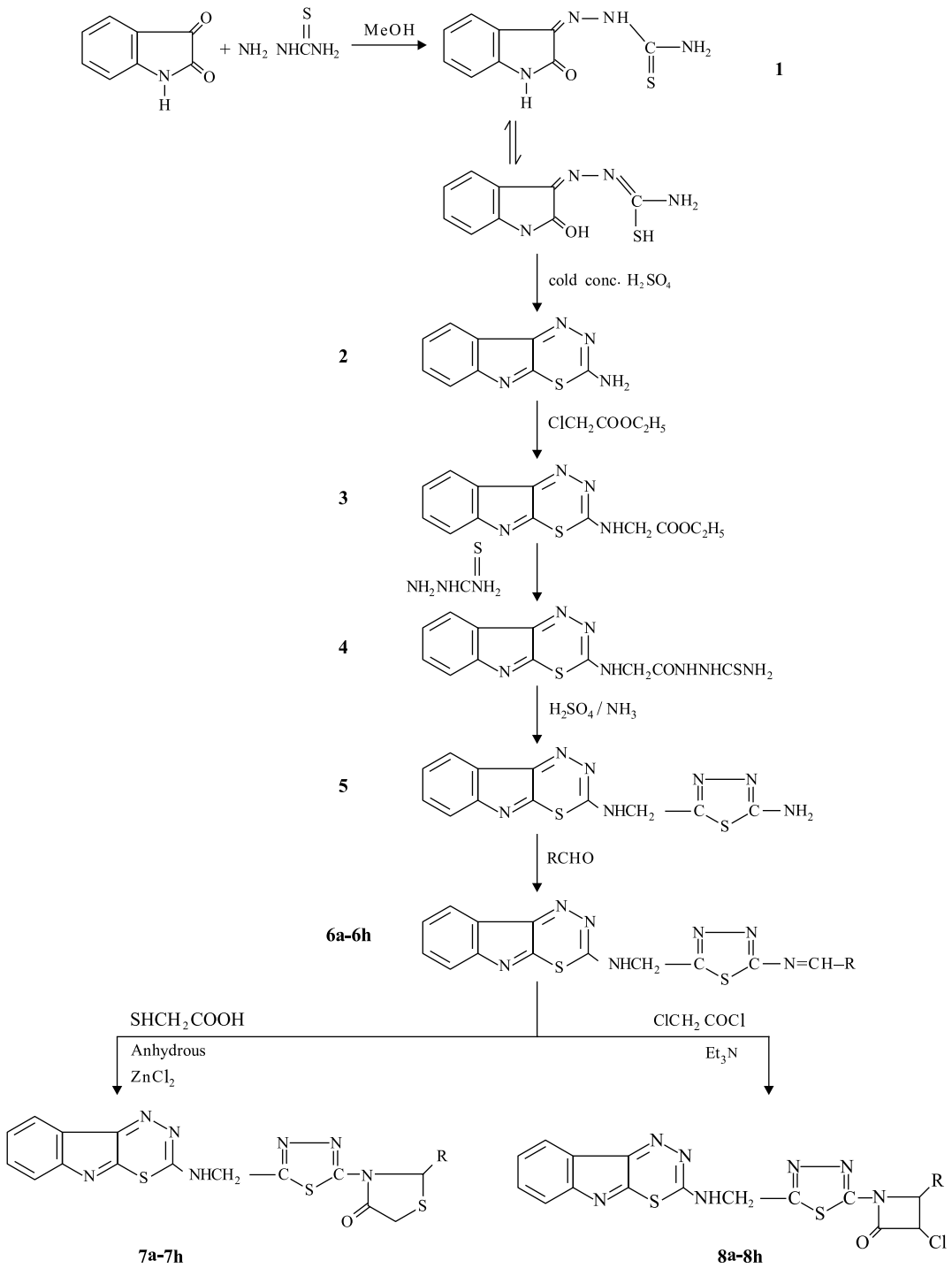
The melting points of the compounds were determined in open glass capillaries with the help of thermonic melting points apparatus and are uncorrected. The homogeneity of all the newly synthesized compounds were routinely checked by TLC on silica gel G plates and spots were located by using iodine chamber. Elemental analysis was performed in Heraeus CHN rapid analyser. The results were found within the ±0.4% of theoretical values. Infrared spectra were recorded on KBr pellets on a Perkin–Elmer system 2000 FTIR spectrometer and ^{1}H NMR spectra on Bruker DPX 200 using TMS as internal standard and chemical shifts expressed in δ ppm.

Compounds 1 and 2 were synthesized according to the method of Panwar et al. [17].

5.1.1. 3-Thiosemicarbazido indole-2-one [1]

A mixture of indole-2,3-dione (0.01 mol), thiosemicarbazide (0.01 mol) in methanol (50 ml) was refluxed for 1 h. The completion of reaction was checked by TLC and excess of methanol was distilled off. The cooled reaction mixture was poured into ice-water, filtered, washed with water, dried and recrystallized from methanol to obtain compound **1** (80%), m.p. 205 °C.

IR (KBr) ν_{max} in cm^{-1}: 1205 (C=S), 1615 (C=C of aromatic ring), 1665 (C=N), 1715 (C=O), 3140 (C–H aromatic), 3420 (NH_2). ^{1}H NMR ($CDCl_3$) δ in ppm: 6.70–6.90 (d, 1H, ArH), 7.10–7.15 (t, 1H, ArH), 7.25–7.30 (t, 1H, ArH), 7.55–7.60 (d, 1H, ArH), 9.42 (bs, 1H, NH indolic),



Scheme 1.

7.75 (bs, 1H, NH), 8.90 (bs, 2H, NH_2). Anal. Calcd for $C_9H_8N_4SO$: C, 49.09; H, 3.63; N, 25.45; Found C, 49.32; H, 3.64; N, 25.56%. MS: $[M]^+$ at *m/z* 220.

5.1.2. 2-Amino-1,3,4-thiadiazino (6,5-b) indole [2]

Compound **1** (0.01 mol) was mixed with cold and conc. H_2SO_4 (0.001 mmol). The reaction mixture was left at room temperature for 16 h. The reaction mixture was poured into ice-cold water, neutralized with liquid ammonia to obtain solid mass, which was filtered, washed with water, dried and recrystallized from methanol to yield compound **2** (70%), m.p. 235 °C. IR (KBr) ν_{max} in cm^{-1}: 670 (C–S–C), 1290 (N–N), 1615 (C=C of aromatic ring), 1585 (C=N), 3140 (C–H aromatic), 3415 (NH_2). ^1H NMR ($CDCl_3$) δ in ppm: 6.85–6.90

(d, 1H, ArH), 7.05–7.10 (t, 1H, ArH), 7.25–7.30 (t, 1H, ArH), 7.55–7.60 (d, 1H, ArH), 8.55 (bs, 2H, NH_2). Anal. Calcd for $C_9H_6N_4S$: C, 53.46; H, 2.98; N, 27.73; Found: C, 53.52; H, 2.97; N, 27.77. MS: $[M]^+$ at *m/z* 202.

5.1.3. 2-Carboethoxymethylamino 1,3,4, thiadiazino (6,5-b) indole [3]

A mixture of compound **2** and chloroethylacetate (0.01 mol) in dry dioxane (50 ml) was refluxed for 8 h. The reaction mixture was further stirred for 1 h, and poured in water. The resulting mixture was filtered and recrystallized from ethanol to yield compound **3** (68%), m.p. 222 °C.

IR (KBr) ν_{max} in cm^{-1}: 1572 (C=N), 728 (C–S–C), 1695 (C=O), 2950 (C–H aliphatic), 3045 (C–H aromatic), 1295 (N–N), 3185 (N–H), 1245 (C–N). ^{1}H NMR ($CDCl_3$) δ in ppm: 6.95–7.15 (m, 4H, Ar-H), 1.45 (t, 3H, $COOCH_2$–CH_3), 4.25 (q, 2H-$COOCH_2$–CH_3), 4.55 (d, 2H, NH–CH_2), 6.10 (t, 1H, NH, exchangeable with D_2O). Anal. Calcd for $C_{13}H_{12}N_4O_2S$: C, 54.16; H, 4.16; N, 19.44; Found: C, 54.12; H, 4.18; N, 19.43. MS: $[M]^+$ at *m/z* 288.

5.1.4. 2-(Thiosemicarbazido carbonylmethylamino)-1,3, 4-thiadiazino (6,5-b) indole [4]

The equimolar mixture (0.02 mol) of compound **3** and thiosemicarbazide (0.02 mol) in methanol (50 ml) was refluxed for 10 h. The excess of solvent was distilled off and viscous mass was poured into water, dried and recrystallized from methanol to yield compound **4** (64%), m.p. 229 °C.

IR (KBr) ν_{max} in cm^{-1}: 1575 (C=N), 722 (C–S–C), 1250 (C–N), 3182 (N–H), 3188 (C–H aromatic), 1705 (C=O), 2975 (C–H aliphatic), 1590 (C=C aromatic ring). ^{1}HNMR ($CDCl_3$) δ in ppm: 6.92–7.22 (m, 4H, Ar-H), 7.32–8.12 (m, 4H, $NHNHCSNH_2$, exchangeable with D_2O), 4.47 (d, 2H, NH–CH_2), 5.60 (t, 1H, NH, exchangeable with D_2O). Anal. Calcd for $C_{12}H_{11}N_7OS_2$: C, 43.24; H, 3.30; N, 29.42; Found: C, 43.32; H, 3.32; N, 29.47. MS: $[M]^+$ at *m/z* 333.

5.1.5. 2-(5′-amino 1,3,4-thiadiazol-2′-yl methylamino)-1,3,4-thiadiazino (6,5-b) indole [5]

A mixture of compound **4** (0.01 mol) and conc. H_2SO_4 (20 ml) was kept overnight at room temperature, poured into ice-cold water, neutralized with liquid ammonia and filtered. The product thus obtained was recrystallized from ethanol water to furnish compound **5** (60%), m.p. 256 °C.

IR (KBr) ν_{max} in cm^{-1}: 3370 (NH_2), 2960 (C–H aliphatic), 1584 (C=C of aromatic ring), 2970 (C–H aromatic), 3170 (NH), 1572 (C=N), 1235 (C–N), 1290 (N–N), 738 (C–S–C). ^{1}H NMR ($CDCl_3$) δ in ppm: 7.05–7.30 (m, 4H, Ar-H), 6.26 (bs, 2H, NH_2), 4.55 (d, 2H, NH–CH_2), 5.75 (t, 1H, NH, exchangeable with D_2O). Anal. Calcd for $C_{12}H_9N_7S_2$: C, 50.89; H, 3.18; N, 34.63; Found: C, 50.97; H, 3.15; N, 34.68. MS: $[M]^+$ at *m/z* 315.

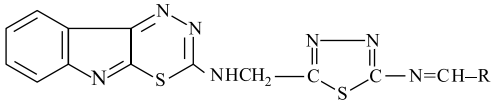
5.1.6. N-({5-[(arylmethylene)amino]-1,3,4-thiadiazol-2-yl}methyl) [1,3,4] thiadiazino(6,5-b)indol-3-amine [6a–6h]

A mixture of compound **5** (0.01 mol) and proper aromatic aldehydes (0.01 mol) in methanol (50 ml) was refluxed for 7 h, in the presence of few drops of glacial acetic acid. The progress and completion of reaction was checked by TLC. The reaction mixtures were distilled off cooled and then poured into ice water, filtered, washed with water and dried. By this procedure, compounds **6a–6h** were obtained starting from benzaldehyde. 4-Chlorobenzaldehyde, 4-methoxybenzaldehyde, 4-hydroxybenzaldehyde, 4-*N*-dimethylbenzaldehyde, 4-methylbenzaldehyde, 2-chlorobenzaldehyde, 2-methoxybenzaldehyde, respectively. The solid thus obtained was recrystallized from appropriate solvent to yield compounds **6a–6h**. Their physical, analytical and spectral data are given in Tables 1 and 5.

5.1.7. 2-aryl-3-{5-[([1,3,4] thiadiazino(6,5-b)indol-3-ylamino)methyl]-1,3,4-thiadiazol-2-yl}-1,3-thiazolidin-4-one [7a–7h]

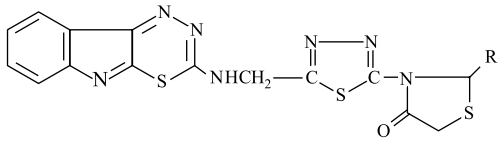
To a solution of compounds **6a–6h** in methanol (50 ml), thioglycolic acid (0.02 mol) was added dropwise in the

Table 1
Physical and analytical data of compounds **6a–6h**



Compound No.	R	M.p. (°C)	Yield	Recrystallization solvent	Molecular formula	Element analysis					
						%C		%H		%N	
						Calcd	Found	Calcd	Found	Calcd	Found
6a	C_6H_5	220	60	Methanol–water	$C_{19}H_{13}N_7S_2$	56.58	56.62	3.22	3.23	24.31	24.35
6b	4-ClC_6H_4	205	55	Methanol–water	$C_{19}H_{12}Cl\ N_7S_2$	52.11	52.18	2.75	2.74	22.40	22.46
6c	4-$OCH_3C_6H_4$	200	58	Ethanol–water	$C_{20}H_{15}N_7OS_2$	55.42	55.36	3.46	3.48	22.64	22.60
6d	4-OHC_6H_4	210	54	Methanol–water	$C_{19}H_{13}N_7OS_2$	54.41	54.48	3.10	3.11	23.38	23.32
6e	4- $N(CH_3)_2C_6H_4$	195	51	DMF–water	$C_{21}H_{18}N_8S_2$	56.50	56.56	4.03	4.02	25.11	25.14
6f	4-$CH_3C_6H_4$	190	56	Methanol–water	$C_{20}H_{15}\ N_7S_2$	57.56	57.51	3.60	3.62	23.50	23.55
6g	2-Cl C_6H_4	195	52	Methanol–water	$C_{19}H_{12}Cl\ N_7S_2$	52.11	52.16	2.75	2.76	22.40	22.36
6h	2-$OCH_3C_6H_4$	180	55	Ethanol–water	$C_{20}H_{15}N_7OS_2$	55.42	55.48	3.46	3.45	22.64	22.61

Table 2
Physical and analytical data of compounds **7a–7h**



Compound No.	R	M.p. (°C)	Yield	Recrystallization solvent	Molecular formula	Element analysis					
						%C		%H		%N	
						Calcd	Found	Calcd	Found	Calcd	Found
7a	C_6H_5	175	50	Methanol–water	$C_{21}H_{15}N_7OS_3$	52.83	52.96	3.14	3.15	20.55	20.61
7b	4-ClC_6H_4	165	45	Methanol–water	$C_{21}H_{14}ClN_7OS_3$	50.26	50.32	2.74	2.75	19.15	19.20
7c	4-$OCH_3C_6H_4$	160	48	Ethanol–water	$C_{22}H_{17}N_7O_2S_3$	52.07	52.15	3.35	3.37	19.32	19.38
7d	4-OHC_6H_4	170	42	Methanol–water	$C_{21}H_{15}N_7O_2S_3$	51.11	51.18	3.04	3.05	19.87	19.82
7e	4- $N(CH_3)_2C_6H_4$	165	46	DMF–water	$C_{23}H_{20}N_8OS_3$	51.49	51.41	3.74	3.75	20.90	20.96
7f	4-$CH_3C_6H_4$	160	44	Methanol–water	$C_{22}H_{17}N_7OS_3$	54.77	54.82	3.46	3.45	19.96	19.92
7g	2-Cl C_6H_4	155	41	Methanol–water	$C_{21}H_{14}ClN_7OS_3$	50.26	50.32	2.74	2.75	19.15	19.19
7h	2-$OCH_3C_6H_4$	150	45	Ethanol–water	$C_{22}H_{17}N_7O_2S_3$	52.07	52.02	3.35	3.37	19.32	19.37

presence of anhydrous zinc chloride and the reaction mixture was refluxed for 10 h. The completion of the reaction was checked by TLC. The excess of methanol was distilled off. The cooled residual mass was diluted with ice-water, filtered, washed with water, dried and recrystallized from suitable solvents to yield compounds **7a–7h**. Their physical, analytical and spectral data are given in Tables 2 and 5.

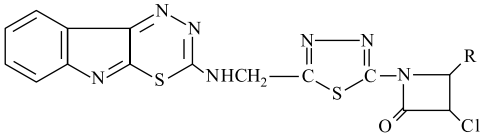
5.1.8. 2-3-chloro-4-aryl-1-{5-[{[1,3,4]thiadiazino(6,5-b) indol-3-ylamino]methyl]-1,3,4-thiadiazol-2-yl}azetidin-2-one [8a–8h]

To the ethanolic solution of the synthesized **6a–6h** (0.01 mol) was added dropwise with constant stirring in presence of triethylamine (0.01 mol) at 0.5 °C. The reaction mixture was refluxed for 8 h. The completion of reaction was checked by TLC and excess of ethanol distilled off. The resulting residual mass was cooled, poured into ice water, filtered, washed with water, dried and recrystallized from suitable solvents to yield compounds **8a–8h**. Their physical, analytical and spectral data are given in Tables 3 and 5.

5.2. Pharmacology

Compounds **6a–8h** (Table 4) were tested for their anti-inflammatory and analgesic activities as well as for their ulcerogenicity and acute toxicity. The experiment was performed with albino rats of Charles–Foster strain of either sex, excluding pregnant females, of 60–90 days weighing 100–120 g. Food (chaw pallet) and water was given to the animals *ad libitum*. The test compounds were dissolved in propylene glycol. Indomethacin and phenylbutazone were used as reference drugs for the comparison of anti-inflammatory, analgesic and ulcerogenic activities.

Table 3
Physical and analytical data of compounds **8a–8h**



Compound No.	R	M.p. (°C)	Yield	Recrystallization solvent	Molecular formula	Element analysis					
						%C		%H		%N	
						Calcd	Found	Calcd	Found	Calcd	Found
8a	C_6H_5	165	40	Ethanol–water	$C_{21}H_{14}ClN_7OS_2$	52.56	52.62	2.92	2.93	20.43	20.46
8b	4-ClC_6H_4	155	35	Methanol–water	$C_{21}H_{13}Cl_2N_7OS_2$	49.02	49.08	2.53	2.52	19.06	19.09
8c	4-$OCH_3C_6H_4$	160	32	Methanol–water	$C_{22}H_{16}ClN_7O_2S_2$	51.82	51.96	3.14	3.16	19.23	19.18
8d	4-OHC_6H_4	150	38	Ethanol–water	$C_{21}H_{14}ClN_7O_2S_2$	50.86	50.72	2.83	2.82	19.78	19.72
8e	4- $N(CH_3)_2C_6H_4$	155	36	DMF–water	$C_{23}H_{19}ClN_8OS_2$	52.83	52.97	3.64	3.62	21.43	21.48
8f	4-$CH_3C_6H_4$	145	31	Methanol–water	$C_{22}H_{16}ClN_7OS_2$	53.49	53.42	3.24	3.26	19.86	19.81
8g	2-Cl C_6H_4	150	34	Methanol–water	$C_{21}H_{13}Cl_2N_7OS_2$	49.02	49.09	2.53	2.54	19.06	19.12
8h	2-$OCH_3C_6H_4$	140	35	Ethanol–water	$C_{22}H_{16}ClN_7O_2S_2$	51.82	51.74	30.14	3.15	19.23	19.27

Table 4
Pharmacology data of compounds (**6a–6h**), (**7a–7h**) and (**8a–8h**)

Compound No.	R	Anti-inflammatory activity		Analgesic activity		UD_{50} (mg/kg/ip)	IC_{50} % of inhibition		ALD_{50}(mg/kg/ip)
		Dose (mg/kg/p.o)	% Inhibition of oedema	Dose (mg/kg/p.o)	% Protection		COX-1	COX-2	
6a	C_6H_5	50	14.75**	50	12.16**	–	22.37*	24.23*	>800
6b	4-ClC_6H_4	50	25.97***	50	16.50**	–	27.43*	33.67**	>800
6c	4-$OCH_3C_6H_4$	50	22.37**	50	15.67**	–	26.16*	29.44*	>800
6d	4-OHC_6H_4	50	24.32**	50	16.87**	–	25.15*	31.47**	>800
6e	4- $N(CH_3)_2C_6H_4$	50	21.36**	50	14.28**	–	24.25*	26.39*	>800
6f	4-$CH_3C_6H_4$	50	20.66**	50	12.67**	–	23.13*	25.40*	>800
6g	2-Cl C_6H_4	50	26.03***	50	18.66**	–	28.67*	34.38**	>800
6h	2-$OCH_3C_6H_4$	50	23.16**	50	22.23***	–	24.13*	26.67*	>800
7a	C_6H_5	50	19.78**	50	9.70*	–	31.89*	36.68**	>800
7b	4-ClC_6H_4	50	18.16**	50	8.78*	–	36.52**	44.12***	>800
7c	4-$OCH_3C_6H_4$	50	28.13***	50	10.76**	–	34.28**	40.06***	>800
7d	4-OHC_6H_4	50	25.27***	50	9.95*	–	34.39**	43.98***	>800
7e	4- $N(CH_3)_2C_6H_4$	50	28.66***	50	18.00**	–	32.22**	38.14**	>800
7f	4-$CH_3C_6H_4$	50	36.97***	50	22.08***	–	33.42**	36.32**	>800
7g	2-C1 C_6H_4	25	22.44***	25	12.32***	166.66	43.13**	45.35***	>800
		50	38.54***	50	24.67***				
		100	44.51***	100	36.36***				
7h	2-$OCH_3C_6H_4$	50	31.34***	50	22.56***	–	33.34**	38.54**	>800
8a	C_6H_5	50	30.55***	50	21.00**	–	37.05***	74.64***	>800
8b	4-ClC_6H_4	50	34.67***	50	25.01***	–	40.94***	72.14***	>800
8c	4-$OCH_3C_6H_4$	50	31.62***	50	21.27**	–	39.53***	66.26***	>800
8d	4-OHC_6H_4	50	32.67***	50	19.95**	–	39.27**	71.09***	>800
8e	4- $N(CH_3)_2C_6H_4$	50	33.60***	50	22.67***	–	37.16**	65.82***	>800
8f	4-$CH_3C_6H_4$	50	32.17***	50	18.76**	–	38.39**	63.56***	>800
8g	2-Cl C_6H_4	25	28.75***	25	19.00***	133.33	69.13**	93.34***	>800
		50	41.23***	50	38.00***				
		100	66.76***	100	58.00***				
8h	2-$OCH_3C_6H_4$	50	36.19***	50	35.00***	–	38.68***	64.28***	>800
	Phenylbutazone	25	26.76***	25	14.26***	66.66	–	–	>800
		50	36.80***	50	32.50***				
		100	64.68**	100	54.58***				
	Indomethacin	5.0	52.20***	5.0	41.30***	54.60	–	–	–
		7.5	63.10***	7.5	59.25***				
		10.0	93.20***	10.0	64.33***				

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
Propylene glycol standard for control group.

5.2.1. Anti-inflammatory activity against carrageenan-induced rat's paw oedema

This study was done following the procedure of Winter et al. [18]. The rats were divided into three groups (control, drug treated, and standard, drug of six animals each). A freshly prepared suspension of carrageenan (1% in 0.9% saline). 0.05 ml was injected under the planter aponeurosis of the right hind paw of each rat. Test compounds and standard drug were administered orally to the animals of drug treated groups and the standard drug group, respectively, 1 h before the carrageenan injection. The paw volume of each rat was measured before 1 and after 3 h of carrageenan treatment with the help of a plethymometer. The percent 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 mean increase in paw volume of rats of the treated and the control group, respectively. Results obtained were statistically analyzed.

5.2.2. Analgesic activity

Analgesic activity was performed following the method of Berkowitz et al. [19]. This method is based on the property of the test compound to antagonize the phenylquinone-induced pain syndrome in mice. Groups of five mice were injected intraperitonealy 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 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.

$$\%\text{protection} = (1 - \text{mean no. of writhes in mice of test groups}/\text{mean number of writhes in mice of control group}) \times 100$$

5.2.3. Ulcerogenic activity

Ulcerogenic labilities of newly synthesized compounds were checked with method of Verma et al. [20]. Albino rats were fasted for 24 h prior to drug administration. All animals

Table 5
Spectral data of compounds (**6a–8h**)

Compound	$[M]^+$ at *m/z*	^1H NMR ($CDCl_3 + DMSOd_6$)	IR (KBr) in cm^{-1}
6a	403	6.82–7.30 (m, 9H, Ar-H), 4.65 (d, 2H, NH–C*H*$_2$), 5.90 (t, 1H, N*H*$_2$), 7.98 (s, 1H, CH-Ar)	3042 (C–H), 1572 (C=N), 3270 (N–H), 1290 (N–N), 728 (C–S–C)
6b	$[M]^+$437 $[M+1]^+$ 438 $[M+2]^+$ 439	6.85–7.28 (m, 8H, Ar-H), 4.68 (d, 2H, NH–C*H*$_2$), 5.94 (t, 1H, N*H*$_2$), 7.95 (s, 1H, CH-Ar)	3045 (C–H), 1570 (C=N), 3275 (N–H), 1295 (N–N), 730 (C–S–C)
6c	433	6.80–7.25 (m, 8H, Ar-H), 4.70 (d, 2H, NH–C*H*$_2$), 5.88 (t, 1H, N*H*$_2$), 7.97 (s, 1H, CH-Ar), 3.28 (s, 3H, Ar-OCH_3)	3050 (C–H), 1575 (C=N), 3278 (N–H), 1285 (N–N), 735 (C–S–C), 1165(C–O–C), 1695 (C=O)
6d	419	6.82–7.20 (m, 8H, Ar-H), 4.78 (d, 2H, NH–C*H*$_2$), 5.80 (t, 1H, NH), 8.02 (s, 1H, CH-Ar), 9.98 (s, 1H, Ar-OH)	3040 (C–H), 1580 (C=N), 3280 (N–H), 1295 (N–N), 725 (C–S–C), 1690 (C=O)
6e	446	7.05–7.35 (m, 8H, Ar-H), 4.72 (d, 2H, NH–CH_2), 5.85 (t, 1H, NH), 8.10 (s, 1H, CH-Ar), 2.94 (s, 6H, Ar-N$(CH_3)_2$)	3035 (C–H), 1585 (C=N), 3285 (N–H), 1280 (N–N), 705 (C–S–C)
6f	417	6.95–7.25 (m, 8H, Ar-H), 4.67 (d, 2H, NH–CH_2), 5.90 (t, 1H, NH), 8.05 (d, 1H, CH-Ar), 3.68 (s, 3H, Ar-CH_3)	3030 (C–H), 1582 (C=N), 3283 (N–H), 1282 (N–N), 698 (C–S–C)
6g	$[M]^+$437 $[M+1]^+$ 438 $[M+2]^+$ 439	7.00–7.28 (m, 8H, Ar-H), 4.75 (d, 2H, NH–CH_2), 5.98 (t, 1H, NH), 7.90 (s, 1H, C*H*-Ar)	3032 (C–H), 3278 (N–H), 1572 (C=N), 1290 (N–N), 725 (C–S–C), 668 (C–Cl)
6h	433	6.90–7.32 (m, 8H, Ar-H), 4.80 (d, 2H, NH–CH_2), 5.92 (t, 1H, NH), 7.87 (s, 1H, CH-Ar), 3.35 (s, 3H, Ar-OCH_3)	3037 (C–H), 1578 (C=N), 3287 (N–H), 1280 (N–N), 720 (C–S–C), 1168 (C–O–C), 1685 (C=O)
7a	477	7.05–7.70 (m, 9H, Ar-H), 4.50 (d, 2H, NH–CH_2), 6.08 (t, 1H, NH), 4.40 (s, 1H, C*H*–N), 2.85 (s, 2H, CH_2)	3030 (C–H), 1590 (C=N), 3295 (N–H), 1270 (N–N), 710 (C–S–C), 1480 (C–N of –N–CH-Ar), 1695 (C=O)
7b	$[M]^+$511 $[M+1]^+$ 512 $[M+2]^+$ 513	7.10–7.75 (m, 8H, Ar-H), 4.60 (d, 2H, NH–CH_2), 6.10 (t, 1H, NH), 4.36 (s, 1H, CH–N), 2.90 (s, 2H, CH_2)	3025 (C–H), 1595 (C=N), 3320 (N–H), 1275 (N–N), 715 (C–S–C), 1485 (C–N of –N–CH-Ar), 670 (C–Cl), 1690 (C=O)
7c	507	7.15–7.80 (m, 8H, Ar-H), 4.57 (d, 2H, NH–CH_2), 6.12 (t, 1H, NH), 4.30 (s, 1H, CH–N), 3.40 (s, 3H, Ar-OCH_3), 2.86 (s, 2H, CH_2)	3035 (C–H), 1585 (C=N), 3325 (N–H), 1280 (N–N), 705 (C–S–C), 1482 (C–N of –N–CH-Ar), 1170 (C–O–C), 1700 (C=O)
7d	493	7.12–7.77 (m, 8H, Ar-H), 4.62 (d, 2H, NH–CH_2), 6.09 (t, 1H, NH), 4.38 (s, 1H, CH–N), 9.95 (s, 1H, Ar-OH), 2.82 (s, 2H, CH_2)	3038 (C–H), 1600 (C=N), 3330 (N–H), 1265 (N–N), 700 (C–S–C), 1475 (C–N of –N–CH-Ar), 3415 (OH), 1680 (C=O)
7e	520	7.10–7.72 (m, 8H, Ar-H), 4.65 (d, 2H, NH–CH_2), 6.15 (t, 1H, NH), 4.42 (s, 1H, CH–N), 2.80 (s, 2H, CH_2), 2.98 (s, 6H, Ar-N$(CH_3)_2$)	3022 (C–H), 1588 (C=N), 3322 (N–H), 1270 (N–N), 720 (C–S–C), 1460 (C–N of –N–CH-Ar), 1688 (C=O)
7f	491	7.06–7.70 (m, 8H, Ar-H), 4.70 (d, 2H, NH–CH_2), 6.12 (t, 1H, NH), 4.36 (s, 1H, CH–N), 2.85 (s, 2H, CH_2), 3.70 (s, 3H, Ar-CH_3)	3020 (C–H), 1592 (C=N), 3328 (N–H), 1278 (N–N), 725 (C–S–C), 1465 (C–N of –N–CH-Ar), 1692 (C=O)
7g	$[M]^+$511 $[M+1]^+$ 512 $[M+2]^+$ 513	6.95–7.62 (m, 8H, Ar-H), 4.54 (d, 2H, NH–CH_2), 6.11 (t, 1H, NH), 4.32 (s, 1H, CH–N), 2.87 (s, 2H, CH_2)	3025 (C–H), 1598 (C=N), 3335 (N–H), 1280 (N–N), 730 (C–S–C), 1472 (C–N of –N–CH-Ar), 1698 (C=O), 675 (C–Cl)
7h	507	6.98–7.75 (m, 8H, Ar-H), 4.55 (d, 2H, NH–CH_2), 6.05 (t, 1H, NH), 3.44 (s, 3H, Ar-OCH_3), 4.34 (s, 1H, CH–N), 2.87 (s, 2H, CH_2)	3015 (C–H), 1594 (C=N), 3320 (N–H), 1285 (N–N), 728 (C–S–C), 1477 (C–N of –N–CH-Ar), 1695 (C=O)
8a	$[M]^+$479 $[M+1]^+$ 480 $[M+2]^+$ 481	7.08–7.68 (m, 9H, Ar-H), 4.50 (d, 2H, NH–CH_2), 6.02 (t, 1H, NH), 4.35 (d, 1H, –N–CH), 4.82 (d, 1H, CH–Cl)	3020 (C–H), 1585 (C=N), 3310 (N–H), 1280 (N–N), 715 (C–S–C), 1485 (C–N of –N–CH-Ar), 1690 (C=O), 675 (C–Cl)
8b	$[M]^+$514 $[M+1]^+$ 515 $[M+2]^+$ 516	7.12–7.73 (m, 8H, Ar-H), 4.52 (d, 2H, NH–CH_2), 6.05 (t, 1H, NH), 4.38 (d, 1H, –N–CH), 4.85 (d, 1H, CH–Cl)	3015 (C–H), 1590 (C=N), 3315 (N–H), 1275 (N–N), 720 (C–S–C), 1480 (C–N of –N–CH-Ar), 670 (C–Cl), 1685 (C=O)
8c	$[M]^+$509 $[M+1]^+$ 510 $[M+2]^+$ 511	7.15–7.70 (m, 8H, Ar-H), 4.55 (d, 2H, NH–CH_2), 6.10 (t, 1H, NH), 4.42 (d, 1H, –N–CH), 4.80 (d, 1H, CH–Cl), 3.42 (s, 3H, Ar-OCH_3)	3025 (C–H), 1580 (C=N), 3320 (N–H), 1290 (N–N), 710 (C–S–C), 1170 (C–O–C), 1695 (C=O), 677 (C–Cl), 1475 (C–N of –N–CH-Ar).
8d	$[M]^+$495 $[M+1]^+$ 496 $[M+2]^+$ 497	7.10–7.65 (m, 8H, Ar-H), 4.60 (d, 2H, NH–CH_2), 6.15 (t, 1H, NH), 4.45 (d, 1H, –N–CH), 4.88 (d, 1H, CH–Cl), 10.12 (s, 1H, Ar-OH)	3010 (C–H), 1600 (C=N), 3325 (N–H), 1265 (N–N), 710 (C–S–C), 1495 (C–N of –N–CH-Ar), 3410 (OH), 1680 (C=O), 665 (C–Cl)
8e	$[M]^+$522 $[M+1]^+$ 523 $[M+2]^+$ 524	7.05–7.72 (m, 8H, Ar-H), 4.57 (d, 2H, NH–CH_2), 6.12 (t, 1H, NH), 4.39 (d, 1H, –N–CH), 4.84 (d, 1H, CH–Cl), 2.92 (s, 6H, Ar-N$(CH_3)_2$	3015 (C–H), 1595 (C=N), 3320 (N–H), 1270 (N–N), 715 (C–S–C), 1490 (C–N of –N–CH-Ar), 3410 (OH), 1690 (C=O), 670(C–Cl)
8f	$[M]^+$493 $[M+1]^+$ 494 $[M+2]^+$ 495	6.97–7.62 (m, 8H, Ar-H), 4.54 (d, 2H, NH–CH_2), 6.18 (t, 1H, NH), 4.33 (d, 1H, –N–CH), 4.89 (d, 1H, CH–Cl), 3.65 (s, 3H, Ar-CH_3)	3020 (C–H), 1590 (C=N), 3315 (N–H), 1275 (N–N), 720 (C–S–C), 1485 (C–N of –N–CH-Ar), 1685 (C=O), 675 (C–Cl)

Table 5 (*continued*)

Compound	$[M]^+$ at *m/z*	^1H NMR ($CDCl_3 + DMSOd_6$)	IR (KBr) in cm^{-1}
8g	$[M]^+$514 $[M+1]^+$ 515 $[M+2]^+$ 516	7.09–7.67 (m, 8H, Ar-H), 4.48 (d, 2H, NH–CH_2), 6.05 (t, 1H, NH), 4.30 (d, 1H, –N–CH), 4.91 (d, 1H, CH–Cl)	3025 (C–H), 1595 (C=N), 3310 (N–H), 1280 (N–N), 715 (C–S–C), 1480 (C–N of –N–CH-Ar), 670 (C–Cl), 1695 (C=O)
8h	$[M]^+$509 $[M+1]^+$ 510 $[M+2]^+$ 511	7.13–7.74 (m, 8H, Ar-H), 4.59 (d, 2H, NH–CH_2), 6.13 (t, 1H, NH), 4.48 (d, 1H, –N–CH), 4.86 (d, 1H, CH–Cl), 3.47 (s, 3H, Ar-OCH_3)	3010 (C–H), 1585 (C=N), 3320 (N–H), 1285 (N–N), 710 (C–S–C), 1170 (C–O–C), 1690 (C=O), 1475 (C–N of –N–CH-Ar), 675 (C–Cl)

were sacrificed 8 h after drug treatment, and their stomachs and small intestines were microscopically examined to assess the incidence of hyperemia, shedding of epithelium, petechial and frank hemorrhages and 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.

5.2.4. Acute toxicity study

The test compounds were investigated for their acute toxicity (ALD_{50}) in albino mice, according to the method of Smith [21]. The test compounds were given orally at different dose levels in separate groups of animals. After 24 h of drug administration, percent mortality in each group was observed. ALD_{50} was calculated from the data obtained.

5.2.5. COX-1 and COX-2 activities

The compounds prepared were tested for cyclooxygenase-1 and cyclooxygenase-2 inhibitory activities. The method of Copeland et al. [22] was followed to determine the IC_{50} values. The enzyme activity is measured using chromogenic assay based on oxidation of *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) during the reduction of prostaglandin G_2 to prostaglandin H_2 by COX-1 and COX-2 enzymes. COX-1 and COX-2 enzymes used in the assay were purified from microsomal fraction.

The compounds were dissolved in DMSO and stock solution was diluted to required assay concentration. The assay mixture consists of Tris–HCl. Tris–HCl buffer (pH 8.0, 100 mM). hematin (15 μM), EDTA (3 μm), enzyme (COX-1 or COX-2, 100 μM) and test compound. The mixture was pre-incubated at 25 °C for 15 min and then the reaction was initiated by the addition of arachidonic acid (100 μM) and TMPD (120 μM) in total volume of 1.0 mL. The enzyme activity was measured by estimating the initial velocity of TMPD oxidation for the first 25 s of the reaction following the increase in absorbance at 603 nm. IC_{50} values are calculated from four parameter least squares non-linear regression analysis of the log dose vs percentage inhibition plot. However, none of the compound studied here exhibited significant inhibitory activity when compared to standard inhibitors indomethacin (for COX-1) and celecoxib (for COX-2).

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