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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF VARIOUS SUBSTITUTED BENZOTHIAZOLE DERIVATIVES

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A series of 2-(benzo[d]thiazol-2-ylthio)-N-(2-oxoindolin-3-ylidene)acetohydrazide (3a-3g), 2-(benzo [d]thiazol-2-ylthio)-N-(2,4'-dioxospiro[indolin-3,2'-thiazolidin)-3'-yl)acetamide (4a-4g) and 2'-((benzo[d]thiazol-2-ylthio)methyl)spiro[indoline-3,5'-thiazolo[4,3-b][1,3,4]oxadiazol]-2-ones (5a-5g) have been synthesized and screened for their anti-inflammatory, analgesic and antibacterial activities. The most potent anti-inflammatory and antibacterial compound of this series was compound 5d and most potent analgesic compound was compound 5e. Structures of all the compounds were established by elemental and spectral (IR and ^{1}H NMR) analysis.

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Keywords: Benzothiazoles, Indoles, Thiazolidinones, Oxadiazoles, Anti-inflammatory Activity, Analgesic activity, Antibacterial activity, Acute toxicity

1. Introduction

Bacterial infections often produce inflammation and pain. In normal practice, two groups of agents (chemotherapeutic, analgesic and anti-inflammatory) are prescribed simultaneously. The compounds prossessing all three activities are not common benzothiazole derivatives have received the attention of medicinal chemists due to their wide range of biological activities which include anti-inflammatory [1], analgesic [2], antibacterial [3] and antiviral [4] activities. Literature survey reveals that indole and thiazolidinone derivatives are important for anti-inflammatory [5, 6], analgesic [7,8], antibacterial [9,10], and antipsychotic [11,12] activities. Also several oxadiazole derivatives have been found to be of interest with potential activities including anti-inflammatory [13], analgesic [14] and antibacterial [15], insecticidal [15] activities. In the present study it was envisaged that a drug molecule possessing the above mentioned pharmacophore could be of advantage since it might possess analgesic, anti-inflammatory and antibacterial activities.

2. Results and discussion

Results

Chemistry

Synthetic route of benzothiazole derivatives is outlined in scheme 1. Accordingly reaction of 2-mercaptobenzothiazole with ethyl chloro acetate in dry acetone in presence of K_2CO_3 afforded the Ethyl 2-(benzothiazolylthio) acetate (1). Compounds 1 was treated with hydrazine hydrate in ethanol to afford compound 2 which was reacted with various substituted indole-2,3-diones in refluxing methanol to afford compounds 3a-3g which were cyclized with mercaptoacetic acid into corresponding thiazolidinone derivatives i. e. compounds 4a-4g. Compounds 4a-4f on reaction with sulfuric acid in methanol yielded compounds 5a-5g.

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Pharmacology

Anti-inflammatory activity against carrageenan induced oedema

All the newly synthesized compounds and reference drug phenylbutazone and aspirin have been examined for their anti-inflammatory activity. The pharmacological results of synthesized compounds have been reported in Table 1. All the compounds have shown anti-inflammatory activity ranging from 10.8-44.8% at the dose of 50 mg/kg p.o. It is clear that when the compounds as substituted with 5-chloro indole moiety (i.e. compound 3d 17.8%) showed better anti-inflammatory activity than compounds substituted with 7-chloroindole moiety (i.e. compound 3e). The compound 3f and 3g substituted by 5-bromo and 7-bromoindole moieties respectively were less active than compounds 3d but more active than compounds 3a-3c.

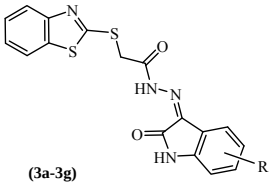
Table 1. Physical and analytical data of compounds 1, 2, 3a-3g, 4a-4g and 5a-5g.

Com. No.	R	MP	Molecular Formula	Elemental analysis Calc. (Found) %		
				C	H	N
1	-	58	$C_{11}H_{11}NO_2S_2$	52.18 (52.17)	4.22 (4.20)	5.40 (5.42)
2	-	193	$C_9H_9N_3OS_2$	45.22 (45.23)	3.10 (3.09)	17.60 (17.65)
3a.	5-OCH_3	210	$C_{18}H_{14}N_4O_3S_2$	54.26 (54.27)	3.54 (3.53)	14.06 (14.05)
3b.	5-CH_3	220	$C_{18}H_{14}N_4O_2S_2$	56.53 (56.50)	3.69 (3.67)	14.65 (14.69)
3c.	7-CH_3	214	$C_{18}H_{14}N_4O_2S_2$	56.53 (56.52)	3.69 (3.65)	14.65 (14.66)
3d.	5-Cl	230	$C_{17}H_{11}ClN_4O_4S_2$	50.68 (50.67)	2.75 (2.74)	13.91 (13.92)
3e.	7-Cl	228	$C_{17}H_{11}ClN_4O_4S_2$	50.68 (50.69)	2.75 (2.75)	13.91 (13.92)
3f.	5-Br	222	$C_{17}H_{11}BrN_4O_2S_2$	45.64 (45.63)	2.48 (2.49)	12.52 (12.55)
3g.	7-Br	225	$C_{17}H_{11}BrN_4O_2S_2$	45.64 (45.66)	2.48 (2.48)	12.52 (12.53)
4a.	5-OCH_3	251	$C_{20}H_{16}N_4O_4S_3$	50.83 (50.86)	3.41 (3.40)	11.86 (11.83)
4b.	5-CH_3	246	$C_{20}H_{16}N_4O_3S_3$	52.61 (52.65)	3.53 (3.55)	12.27 (12.30)
4c.	7-CH_3	252	$C_{20}H_{16}N_4O_3S_3$	52.61 (52.64)	3.53 (3.55)	12.27 (12.26)
4d.	5-Cl	269	$C_{19}H_{13}ClN_4O_3S_3$	47.84 (47.80)	2.75 (2.79)	11.75 (11.76)
4e.	7-Cl	263	$C_{19}H_{13}ClN_4O_3S_3$	47.84 (47.81)	2.75 (2.79)	11.75 (11.75)
4f.	5-Br	258	$C_{19}H_{13}BrN_4O_3S_3$	43.76 (43.77)	2.51 (2.56)	10.74 (10.70)
4g.	7-Br	260	$C_{19}H_{13}BrN_4O_3S_3$	43.76 (43.75)	2.51 (2.54)	10.74 (10.72)
5a.	5-OCH_3	280	$C_{20}H_{14}N_4O_3S_3$	52.85 (52.87)	3.10 (3.12)	12.33 (12.37)
5b.	5-CH_3	286	$C_{20}H_{14}N_4O_2S_3$	54.78 (54.72)	3.22 (3.27)	12.78 (12.78)
5c.	7-CH_3	282	$C_{20}H_{14}N_4O_2S_3$	54.78 (54.74)	3.22 (3.26)	12.78 (12.73)
5d.	5-Cl	298	$C_{19}H_{11}ClN_4O_2S_3$	49.72 (49.70)	2.42 (2.43)	12.21 (12.24)
5e.	7-Cl	290	$C_{19}H_{11}ClN_4O_2S_3$	49.72 (49.73)	2.42 (2.47)	12.21 (12.23)
5f.	5-Br	295	$C_{19}H_{11}BrN_4O_2S_3$	45.33 (45.30)	2.20 (2.23)	11.13 (11.15)

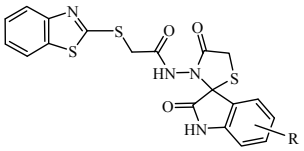
5g.	7-Br	290	$C_{19}H_{11}BrN_4O_2S_3$	45.33 (45.30)	2.20 (2.22)	11.13 (11.16)

It is interestingly enough, the anti-inflammatory activity of 2-(benzo[d]thiazol-2-ylthio)-N-(2,4'-dioxospiro[indolin-3,2'-thiazolidin)-3'-yl)acetamides (4a-4g) were more (15.9-27.2%) than that of their parent compounds i.e. compounds 3a-3g. Among the compounds 4a-4g, compound 4d (having 5-chloroindole moiety) exhibited 27.2% protection against carrageenan induced oedema. Cyclization of compounds 4a-4g into their corresponding oxadiazole ring (i.e. compounds 5a-5g), very clearly showed an increase in anti-inflammatory activity (28.6-44.8%) at dose of 50 mg/kg p.o. Compound 5d and 5e were also tested at three graded doses i.e. 25, 50 and 100 mg/kg p.o. The compound 5d showed 31.4% anti-inflammatory activity at a dose of 25 mg/kg p.o. and 44.8 and 72.2% anti-inflammatory activity at the dose of 50 mg/kg and 100mg/kg p.o respectively. Moreover, compound 5e showed 30.6, 36.4 and 68.8% anti-inflammatory activities at the dose of 25, 50 and 100 mg/kg p.o. respectively.

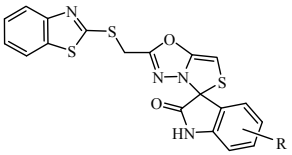
Table 2. Anti-inflammatory and analgesic activity of compounds 3a-3g, 4a-4g and 5a-5g.



(3a-3g)



(4a-4g)



(5a-5g)

Comp. No.	Dose (mg/kg p.o.)	Antiinflammatory activity % oedema inhibition relative to control	Analgesic activity % decrease of writhes in 60 min after treatment relative to control	∑ π (P value)	ALD_{50} mg/kg i.p.
3a.	50	10.8*	12.6*	-12	>1000
3b.	50	13.5*	8.8*	1.29	>1000
3c.	50	11.5**	10.8**	1.25	>1000
3d.	50	17.8**	14.7**	2.00	>1000
3e.	50	15.6**	12.4**	-11	>1000
3f.	50	14.4**	12.2**	2.00	>1000
3g.	50	12.8**	11.8**	1.29	>1000
4a.	50	15.9**	13.2***	1.27	>1000
4b.	50	18.4**	14.5**	2.15	>1000
4c.	50	24.6**	14.8**	1.29	>1000
4d.	50	27.2***	16.6**	3.53	>1000
4e.	50	20.7**	18.6**	3.38	>1000
4f.	50	26.1***	18.6**	3.53	>1000
4g.	50	18.6**	16.3**	2.67	>1000
5a.	50	31.2***	25.2***	1.29	>1000
5b.	50	31.4***	25.9***	1.27	>1000
5c.	50	28.6***	29.5***	2.15	>1000
5d.	25 50 100	31.4*** 44.8*** 72.2***	30.1 *** 32.2*** 55.5***	3.38	>1400
5e.	25 50 100	30.6*** 36.4*** 68.8***	33.6*** 43.4*** 69.2***	3.53	>1400
5f.	50	32.2***	27.6***	3.53	>1000
5g.	50	30.8***	27.3***	2.67	>1000
Phenyl Butazone	25 50 100	31.4*** 40.6*** 63.4***	31.0*** 32.5*** 42.6***		
Aspirin	25	30.25***	30.2***		

	50 100	38.4*** 60.8***	45.5*** 59.3***		

*P < 0.05, **P < 0.01, ***P < 0.001

Analgesic activity

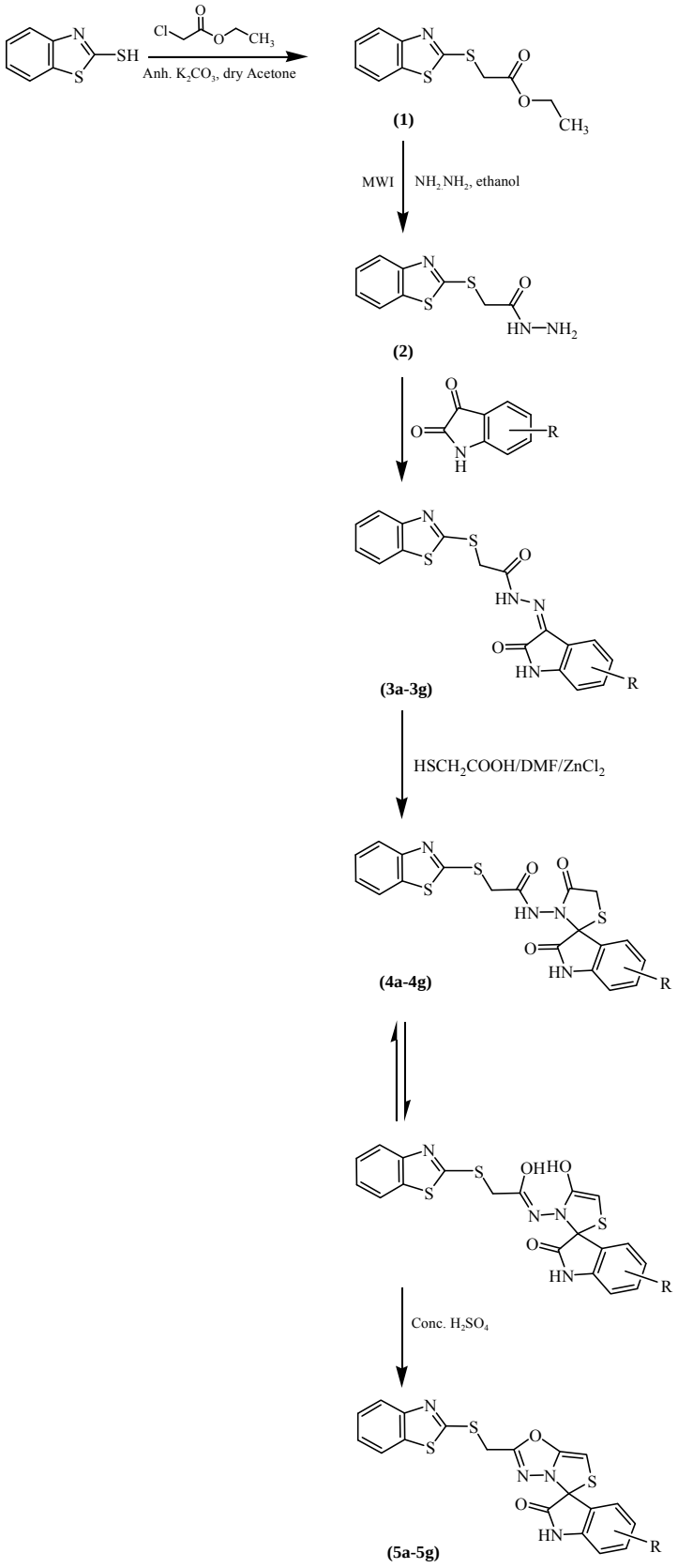
All the newly synthesized compounds 3a-3g, 4a-4g and 5a-5g were screened for their analgesic activity at a dose of 50 mg/kg p.o. From the results it is clear that the compounds (i.e. compound 5d) which exhibited better inflammatory activity and was associated with less analgesic activity (32.2 and 55.5%) at the dose of 50 and 100 mg/kg respectively. Furthermore, compound 5e (having 7-chloro indole moiety) showed 43.4 and 69.2% analgesic activity at the dose of 50 and 100 mg/kg p.o.

Table 3. Antibacterial activity of compounds 3a-3g, 4a-4g and 5a-5g.

Com. No.	R	Zone of inhibition (diameter in mm)		
		K. Pneumoniae	S. aureus	E. coli
Control	-	Nil	Nil	Nil
Ciprofloxacin	-	26	23	25
3a.	5-OCH_3	-	-	8
3b.	5-CH_3	12	-	12
3c.	7-CH_3	10	-	
3d.	5-Cl	11	10	12
3e.	7-Cl	13	12	-
3f.	5-Br	8	-	10
3g.	7-Br	10	17	20
4a.	5-OCH_3	-	18	-
4b.	5-CH_3	15	12	-
4c.	7-CH_3	-	18	20
4d.	5-Cl	18	17	-
4e.	7-Cl	17	-	22
4f.	5-Br	20	14	24
4g.	7-Br	-	-	25
5a.	5-OCH_3	18	10	15
5b.	5-CH_3	-	16	18
5c.	7-CH_3	16	-	19
5d.	5-Cl	28	24	25
5e.	7-Cl	22	22	20
5f.	5-Br	21	19	16
5g.	7-Br	19	20	16

Antibacterial activity

All the newly synthesized compounds were also tested for their antibacterial activity against K. pneumoniae, S. aureus and E. coli. From the table it is clear that compound 5d namely2'-((benzo[d]thiazol-2-ylthio)methyl)-5-chlorospiro[indolin-3,5'-thiazolo[4,3-b] [1,3,4]oxadiazol]-2-one exhibited maximum antibacterial activity (i.e. 28, 24 and 25mm zone of inhibition) against all the bacterial strains in comparison to the other compounds. Moreover, compound 5e having 7-chloroindole moiety showed good antibacterial activity against K. pneumoniae, S. aureus and E. coli (i.e. 22, 22 and 20mm zone of inhibition respectively). Compounds 3a and 4g were devoid of antibacterial activity against K. pneumoniae and S. aureus.



Scheme 1: Synthetic route of Benzothiazole derivatives

3. Conclusions

- Benzothiazole derivatives with chloroindole moieties give the interesting activity (as the electro negativity of chloro group increase the activity) increases.

- The bromo subtituents also showed good activity.
- 5-chloroindolylbenzothiazole derivative (i.e. compound 5d) exhibited most potent anti-inflammatory and antibacterial activities, while 7-chloroindolylbenzothiazole derivative (i.e. compound 5e) exhibited most potent analgesic activity.
- The oxadiazole ring is essential for high anti-inflammatory, analgesic and antibacterial activities

4. Experimental

All reagents and solvents used were of HIMEDIA, CDH and Merck. The melting points were determined in open capillaries tubes on a Jyoti Laboratories melting point apparatus and were uncorrected. The purity of the compounds was confirmed by TLC using silica gel G as stationary phase, using two solvent; by TLC using silica gel G as stationary phase, using two solvent systems; Benzene:Ethanol (9:!) and Toluene:Ethyl formate: Formic acid (5: 4:1) and visualized in iodine. The IR spectra were recorded in potassium bromide on a Perkin Elmer IR spectrometer. ^{1}H-NMR spectra were recorded in $CDCl_3$ and $DMSOd_6$ at 300 MHz on Bruker spectrometer and all chemical shifts were given in ppm relative to tetramethylsilane. The elemental analyses (C, H, N) were performed using Perkin-Elmer model 240c analyzer. The animal research study was approved by the animal ethical committee (CPCSEA).

Preparation of Ethyl 2-(benzothiazolylthio)acetate (1)

Dissolve the 2-Mercaptobenzothiazole (2.0 mol) in methanol and ethyl chloro acetate (2.0 mol) was added dropwise in presence of K_2CO_3 (8 g) in the mixture with stirring. The resulted mixture was refluxed for 10 hours and the reaction mixture poured into ice cold water and neutralized with dil HCl. The semisolid thus obtained was washed several times with water and left in water for 72 hours. The crystals formed were filtered, washed thoroughly with water and dried. The completion of the reaction was mentioned on T.L.C. by using silica Gel G coated plates by using ethyl acetate and petroleum ether (1:1) as the eluent and observed in UV light and Ethyl-2-(1H-indol-3'-yl) acetate was obtained.

Preparation of 2-(benzo[d]thiazol-2-ylthio)acetohydrazide (2)

A mixture of 253.3 g **1** (1 mol,) and hydrazine hydrate (0.4 mol) and ethanol (40 ml) was taken RBF placed in microwave oven and irradiated for 4 min. After completion of reaction (monitored by TLC), mixture was cooled and the resulting solid was filtered, dried and recrystallized from ethanol to yield compound **2**.

Preparation of 2-(benzo[d]thiazol-2-ylthio)-N-(2-oxoindolin-3-ylidene)acetohydrazide (3a-3g)

A mixture of 191.4 g of 2 (0.8 mol) and various substituted indole-2,3-dione (0.8 mol) in methanol (60 ml) in the presence of a catalytic amount of gl. acetic acid was heated under reflux for 30 min. The solid that separated on cooling was filtered, washed with cold methanol and recrystallized from methanol to give compounds 3a-3g.

Preparation of 2-(benzo[d]thiazol-2-ylthio)-N-(2,4'-dioxospiro[indolin-3,2'-thiazolidin)-3'-yl) acetamides (4a-4g)

A mixture of 119.5 g of **3a-3g** (0.3 mol) and mercaptoacetic acid (0.3 mole) in DMF (100 ml) containing a pinch of anhyd. $ZnCl_2$ was heated under reflux for 6-8 h. The reaction mixture was cooled and poured onto crushed ice. The solid thus obtained was filtered, washed with water and recrystallized from DMF to afford **4a-4g**.

Preparation of 2'-((benzo[d]thiazol-2-ylthio)methyl)spiro[indolin-3,5'-thiazolo[4,3-

b][1,3,4] oxadiazo l]-2-ones (5a-5g)

Compound 70.8 g **4a-4f** (0.15 mol) was added slowly to conc. H_2SO_4 (10 ml) in the cold. The reaction mixture was kept for 6 h. at room temperature, poured onto crushed ice and neutralized with ammonia solution. The precipitate thus obtained was filtered, washed with water and recrystallized from DMF to furnish compounds **5a-5g**.

IR and ^{1}H-NMR data of compounds 1, 2, 3a-3g, 4a-4g and 5a-5g:

***Ethyl 2-(benzothiazolylthio)acetate* (1)**

Yield: 86%; IR (KBr): 3020 1720, 1612, 696 cm^{-1}. ^{1}H-NMR: δ (ppm): 1.23 (3H, t), 4.13 (2H, q), 4.46 (2H, s), 6.79-7.87 (4H, m).

***2-(benzo[d]thiazol-2-ylthio)acetohydrazide* (2)**

% Yield: 84, IR (KBr): 3020, 1720, 1612, 694 cm^{-1}. ^{1}H-NMR: δ (ppm): 4.42 (2H, s), 4.80 (2H, s), 6.70-7.80 (4H, m), 7.89 (1H, s).

***2-(benzo[d]thiazol-2-ylthio)-N-(5-methoxy-2-oxoindolin-3-ylidene)acetohydrazide* (3a)**

% Yield: 76; IR (KBr): 3207, 1732, 1665, 1608, 698 cm^{-1}. ^{1}H-NMR: δ (ppm): 3.50 (3H, s), 4.83 (2H, s), 6.73-7.81 (7H, m), 8.13 (1H, s), 8.80 (1H, s).

***2-(benzo[d]thiazol-2-ylthio)-N-(5-methyl-2-oxoindolin-3-ylidene)acetohydrazide* (3b)**

% Yield: 70; IR (KBr): 3220, 1730, 1666, 1605, 690 cm^{-1}. ^{1}H-NMR: δ (ppm): 1.10 (s, 3H, CH_3), 4.79 (2H, s), 6.78-7.88 (7H, m), 8.16 (1H, s), 8.83 (1H, s).

***2-(benzo[d]thiazol-2-ylthio)-N-(7-methyl-2-oxoindolin-3-ylidene)acetohydrazide* (3c)**

% Yield: 73; IR (KBr): 3208, 1725, 1668, 1610, 695 cm^{-1}. ^{1}H-NMR: δ (ppm): 1.11 (3H, s), 4.75 (2H, s), 6.74-7.78 (7H, m), 8.18 (1H, s), 8.81 (1H, s).

***2-(benzo[d]thiazol-2-ylthio)-N-(5-chloro-2-oxoindolin-3-ylidene)acetohydrazide* (3d)**

% Yield: 78; IR (KBr): 3207, 1720, 1667, 1607, 1006, 699 cm^{-1}. ^{1}H-NMR: δ (ppm): 4.78 (2H, s), 6.79-7.98 (7H, m), 8.18 (1H, s), 8.83 (1H, s)

***2-(benzo[d]thiazol-2-ylthio)-N-(7-chloro-2-oxoindolin-3-ylidene)acetohydrazide* (3e)**

% Yield: 74; IR (KBr): 3212, 1721, 1664, 1609, 1004, 696 cm^{-1}. ^{1}H-NMR: δ (ppm): 4.80 (2H, s), 6.87-7.94 (7H, m), 8.20 (1H, s), 8.90 (1H, s).).

***2-(benzo[d]thiazol-2-ylthio)-N-(5-bromo-2-oxoindolin-3-ylidene)acetohydrazide* (3f)**

% Yield: 73; IR (KBr): 3215, 1732, 1668, 1608, 694, 611 cm^{-1}. ^{1}H-NMR: δ (ppm): 4.75 (2H, s), 6.98-7.98 (7H, m), 8.25 (1H, s), 8.84 (1H, s).

***2-(benzo[d]thiazol-2-ylthio)-N-(7-bromo-2-oxoindolin-3-ylidene)acetohydrazide* (3g)**

% Yield: 70; IR (KBr): 3217, 1731, 1666, 1606, 695, 614 cm^{-1}. ^{1}H-NMR: δ (ppm): 4.77 (2H, s), 6.86-7.99 (7H, m), 8.15 (1H, s), 8.84 (1H, s).

***2-(benzo[d]thiazol-2-ylthio)-N-(5-methoxy-2,4'-dioxospiro[indolin-3,2'-thiazolidin)-3'-yl)acetamide* (4a)**

% Yield: 74; IR (KBr): 3320, 1730, 1692,), 1600, 694 cm^{-1}. ^{1}H-NMR: δ (ppm): 3.56 (3H, s), 3.82 (2H, s), 4.81 (2H, s), 6.83-7.91 (7H, m), 8.14 (1H, s), 8.70 (1H, s).

***2-(benzo[d]thiazol-2-ylthio)-N-(5-methyl-2,4'-dioxospiro[indolin-3,2'-thiazolidin)-3'-yl)acetamide* (4b)**

% Yield: 69; IR (KBr): 3323, 1730, 1692, 1602, 690 cm^{-1}. ^{1}H-NMR: δ (ppm): 1.12 (3H, s), 3.86 (2H, s), 4.74 (2H, s), 6.79-7.98 (7H, m), 8.17 (1H, s), 8.83 (1H, s).

***2-(benzo[d]thiazol-2-ylthio)-N-(7-methyl-2,4'-dioxospiro[indolin-3,2'-thiazolidin)-3'-yl)acetamide* (4c)**

% Yield: 71; IR (KBr): 3330, 1736, 1695, 1600, 691 cm^{-1}.^{1}H-NMR: δ (ppm): 1.14 (3H, s), 3.80 (2H, s), 4.72 (2H, s), 6.76-7.75 (7H, m), 8.18 (1H, s), 8.81 (1H, s).

***2-(benzo[d]thiazol-2-ylthio)-N-(5-chloro-2,4'-dioxospiro[indolin-3,2'-thiazolidin)-3'-yl)acetamide* (4d)**

% Yield: 77; IR (KBr): 3333, 1737, 1695, 1604, 1004, 693 cm^{-1}. ^{1}H-NMR: δ (ppm): 3.81 (2H, s), 4.73 (2H, s), 6.97-7.89 (7H, m), 8.33 (1H, s), 8.86 (1H, s).

***2-(benzo[d]thiazol-2-ylthio)-N-(7-chloro-2,4'-dioxospiro[indolin-3,2'-thiazolidin)-3'-yl)acetamide* (4e)**

% Yield: 71; IR (KBr): 3326, 1740, 1691, 1605, 1007, 691 cm^{-1}. ^{1}H-NMR: δ (ppm): 3.82 (2H, s), 4.84 (2H, s), 6.78-7.94 (7H, m), 8.20 (1H, s), 8.91 (1H, s).

2-(benzo[d]thiazol-2-ylthio)-N-(5-bromo-2,4'-dioxospiro[indolin-3,2'-thiazolidin)-3'-yl)acetamide **(4f)**

% Yield: 73; IR (KBr): 3315, 1743, 1682, 1603, 689, 612 cm^{-1}. ^{1}H-NMR: δ (ppm): 3.89 (2H, s), 4.79 (2H, s), 6.89-7.89 (7H, m), 8.28 (1H, s), 8.89 (1H, s).

2-(benzo[d]thiazol-2-ylthio)-N-(7-bromo-2,4'-dioxospiro[indolin-3,2'-thiazolidin)-3'-yl)acetamide **(4g)**

% Yield: 75; IR (KBr): 3317, 1732, 1687, 1607, 690, 613 cm^{-1}. ^{1}H-NMR: δ (ppm): 3.84 (2H, s), 4.76 (2H, s), 6.68-7.91 (7H, m), 8.26 (1H, s), 8.87 (1H, s).

2'-((benzo[d]thiazol-2-ylthio)methyl)5-methoxyspiro[indolin-3,5'-thiazolo[4,3-b][1,3,4]oxadiazol]-2-one **(5a)**

% Yield: 75; IR (KBr): 3330, 1690, 1610, 700 cm^{-1}. ^{1}H-NMR: δ (ppm): 3.58 (3H, s), 4.80 (2H, s), 6.85-7.94 (7H, m), 8.14 (1H, s), 8.76 (1H, s).

2'-((benzo[d]thiazol-2-ylthio)methyl)-5-methylspiro[indolin-3,5'-thiazolo[4,3-b][1,3,4]oxadiazol]-2-one **(5b)**

% Yield: 68; IR (KBr): 3343, 1720, 1691, 1612, 698 cm^{-1}. ^{1}H-NMR: δ (ppm): 1.15 (3H, s), 4.78 (2H, s), 6.77-7.96 (7H, m), 8.17 (1H, s), 8.84 (1H, s).

2'-((benzo[d]thiazol-2-ylthio)methyl)-7-methylspiro[indolin-3,5'-thiazolo[4,3-b][1,3,4]oxadiazol]-2-one **(5c)**

% Yield: 71; IR (KBr): 3340, 1726, 1694, 697 cm^{-1}. ^{1}H-NMR: δ (ppm): 1.16 (3H, s), 4.79 (2H, s), 6.75-7.76 (7H, m), 8.15 (1H, s), 8.85 (1H, s).

2'-((benzo[d]thiazol-2-ylthio)methyl)-5-chlorospiro[indolin-3,5'-thiazolo[4,3-b][1,3,4]oxadiazol]-2-one **(5d)**

% Yield: 70; IR (KBr): 3344, 1730, 1692, 1614, 1007, 702 cm^{-1}. ^{1}H-NMR: δ (ppm): 4.78 (2H, s), 6.95-7.86 (7H, s), 8.38 (1H, s), 8.84 (1H, s).

2'-((benzo[d]thiazol-2-ylthio)methyl)-7-chlorospiro[indolin-3,5'-thiazolo[4,3-b][1,3,4]oxadiazol]-2-one **(5e)**

% Yield: 75; IR (KBr): 3336, 1728, 1691, 1608, 1004, 705, cm^{-1}. ^{1}H-NMR: δ (ppm): 4.84 (2H, s), 6.76-7.94 (7H, m), 8.24 (1H, s), 8.96 (1H, s).

2'-((benzo[d]thiazol-2-ylthio)methyl)-5-bromospiro[indolin-3,5'-thiazolo[4,3-b][1,3,4]oxadiazol]-2-one **(5f)**

% Yield: 70; IR (KBr): 3335, 1735, 1689, 1613, 699, 615 cm^{-1}. ^{1}H-NMR: δ (ppm): 4.77 (2H, s), 6.89-7.87 (7H, m), 8.20 (1H, s), 8.89 (1H, s).

2'-((benzo[d]thiazol-2-ylthio)methyl)-7-bromospiro[indolin-3,5'-thiazolo[4,3-b][1,3,4]oxadiazol]-2-one **(5g)**

% Yield: 69; IR (KBr): 3337, 1730, 1697, 1610, 696, 613 cm^{-1}. ^{1}H-NMR: δ (ppm): 4.78 (2H, s), 6.67-7.97 (7H, m), 8.24 (1H, s), 8.86 (1H, s).

Pharmacology

All the newly synthesized compounds were studied for anti-inflammatory, analgesic and antibacterial activities and compared with reference drugs.

Anti-inflammatory activity

This study was done by following the procedure of Winter et al [16]. The rats were divided into three groups (control, test compounds 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. The compound and standard drug were administered orally to the animals of drug treated groups and the standard drug group, respectively, 1h before the carrrageenan treatment with the help of a plethysmometer. The percent anti-inflammatory activity was calculated according to the formula given below.

Percentage of inhibition of oedema = 1-Vt/Vc) X 100

Where Vt and Vc are the volume of edema in drug, treated and control group, respectively.

Analgesic activity

The analgesic activity was performed by following the method of Berkowitz et al [17]. This method is based on the property of the test compound to antagonize the phenyl quinine-induced pain syndrome in mice. Groups of five mice injected interaperitoneally with 0.25 ml of a 0.02% solution of phenyquinone in ethanol (5%) 1 h after oral administration of the test compounds. The test 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.

% protection = (1-mean no. of writhes in mice of test group/mean number of writhes in mice of control group) x 100

Antibacterial activity

Antibacterial activity was determined by agar cup plate method [18] at a concentration of 100mg/ml using DMF as a solvent against the following organism- Escherichia coli, Staph. Aureus, Klebsiella pneumoniae and B. sublitis. The zone of inhibition of each strain was recorded. The activity has been compared with known standard drug ciprofloxacin at 10 μg/ml concentration. The biological results were analysed statistically by student‘t’ test. Propylene glycol treated group served as control.

Study of lipophilicity as a function of toxicity

The partition coefficient has been determined by measuring the concentration of the compound in two immiscible solutions according to the method of Hansch et al [19]. The partition coefficient is as follows:

$$P = C(\text{octanol}) / C(\text{water})$$

The concentration of the compound in the two solutions was measured by photoelectric calorimeter. The hydrophobic constant (p) was evaluated by the method of Blaney et al. [20]
The substitutent constant for hydrophobic effect

$$\pi = \log P_X - \log P_H$$

Where P_X = The partition coefficient of the substituted compounds
P_H = The partition coefficient of the unsubstituted compounds

The hydrophobic parameter (π) or Hansch ∑ π value was used and measure of hydrophobiicity is the sum of л for substitutent.

There is good correlation between the lipophilicity and toxicity of the drugs. The positive value of P (sum of π) suggests that compound is good from activity point of view. Moreover, it has been reported [21] that the compounds which are more lipid soluble possess more toxicity.

Acute toxicity

Acute lethal doses (ALD_{50}) of all the compounds were investigated by the method of Smith Q.E. [22].

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