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Some New 2,3,6-Trisubstituted Quinazolinones as Potent Anti-inflammatory, Analgesic and COX-II Inhibitors

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Abstract—Various 2-(substitutedphenylmethyleneimino)aminoacetylmethylene-3-(2′-substitutedindol-3′-yl)-halosubstituted-4(3H)quinazolinones (**5a–5i**) and 2-(substituted phenylaminomethyleneacetyl-4′-oxo-1′-thiazolidinyl-3-(2″-substitutedindol-3″-yl) 4(3H)-quinazolinones (**6a–6i**) have been synthesized in the present studies. The structure of these compounds have been elucidated by elemental (C, H, N) and spectral (IR, ^{1}H NMR and mass) analysis. Furthermore, above said compounds were evaluated for their anti-inflammatory, analgesic, ulcerogenic activities and acute toxicity study. Compound **6d** was found to be most potent. Compound exihibiting less ulcerogenic liability and $ALD_{50} > 2000$ mg/kg po.

Introduction

Recently developed non acidic or weakly acidic NSAIDs like celecoxib[1], rofecoxib[1] and so on have drawn the attention of medicinal chemists as they preferentially act by inhibiting COX-II enzyme and possessed lower incidence of gastric ulcers than the acidic NSAIDs which inhibit both COX-I and COX-II enzyme like indomethacin, aspirin, naproxen and so on. Furthermore, a large number of quinazolinones[2–4] have been reported to possess potent anti-inflammatory and analgesic activities. However, the substitution pattern in the quinazolinone nucleus at 2/3 position[5,6] by different aryl or heteo aryl moieties markedly modulates its anti-inflammatory activity. Moreover, indole[7,8] and thiazolidinone[9,10] derivatives have also been reported to possess potent anti-inflammatory activity. There are very few reports in the literature that quinazolinones derivatives reduce the inflammation in different inflammatory disorders by inhibiting the COX-II enzyme. Hence, it was thought worthwhile to synthesize some new 2,3,6-trisubstituted quinazolinone derivatives with the hope to possess better anti-inflammatory potential and lesser side effect than the already existing COX-II inhibitors. All the compounds were tested pharmacologically for their anti-inflammatory, analgesic, ulcerogenic, toxicity and COX-II inhibiting activity.

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Results

Chemistry

The starting compound, that is monobromoanthranilic acid and 5 iodoanthranilic acid, were prepared according to reported method by Wheelar et al.[11] and Klemme et al.[12] respectively compound 1 was also synthesized by known method.[13] 2-Methyl-3-(2′-substitutedindol-3-yl)-6-halosubstituted-4(3H)-qunizolinones (**2a–2c**) were prepared by the reaction of different acetanthranils with 2-substituted-3-aminoindoles in dry pyridine. These were on treatment with chloroacetyl chloride yielded the compounds (**3a–3c**). The later compounds were refluxed with hydrazine hydrate to obtain 2-hydrazinoacetyl methylene-3-(2′-substitutedindol-3-yl)-6-halosubstituted-4(3H) quinazolinones (**4a–4c**). These compounds were further refluxed with various aromatic aldehydes in the presence of glacial acetic acid to obtain 2-(substituted phenyl methyleneimino)aminoacetylmethylene - 3 - (2′-substituedindol - 3′ - yl) - 6 - halosubstituted quinazolin-4(3H)-ones (**5a–5i**). Furthermore, 2-substitutedphenyl-aminomethylacetyl{4′ - oxo - 1′ - thiazolidinyl}-3-(2″-substitutedindol-3″-yl)-quinazolin-4(3H)-ones (**6a–6i**) were synthesized by reacting compounds **5a–i** with thioglycolic acid in the presence of anhydrous $ZnCl_2$. The synthetic pathway of the above said compounds is shown in Scheme 1. The structure of all the newly synthesized compounds was confirmed on the basis of spectral (IR, ^{1}H NMR and mass) and analytical data.

Pharmacological evaluation

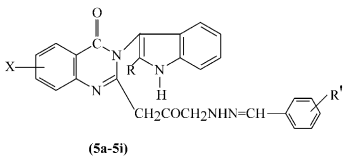
Random screening of the compounds **5a–5i** and **6a–6i** was performed at 50 mg/kg po for their anti-inflammatory and analgesic activities. Compounds **5f** and **6d** were found to possess more potent activities than phenylbutazone, reference drug, and the rest of the compounds of this series. Furthermore, these two compounds and phenylbutazone were evaluated at three graded doses, that is 25, 50 100 mg/kg po for their anti-inflammatory and analgesic activities. All compounds of this series were also screened for their acute toxicity, ulcerogenic, liability and COX-II inhibitory activity interestingly, these two compounds, that is **5f** and **6d** possessed less ulcerogenic liability and showed $ALD_{50} > 1000$ mg/kg po and > 2000, respectively. The results of the biological study are depicted in Table 1.

Discussion

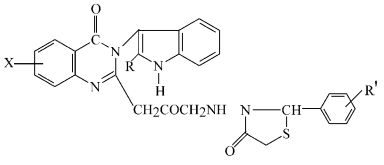
Compounds **5a–5i** and **6a–6i** have been tested for their acute toxicity, anti-inflammatory, analgesic, ulcerogenic and cycloxygenas-II inhibiting activities. The important feature of the compounds **5a–5i** is the presence of 2′-substitutedindol-3′-yl moiety at the 3-position of quinazolinone nucleus and substituted phenyliminoaminoacetyl-methylene chain at the 2-position of the quinazolinone moiety. These compounds **5a–5i** were evaluated at a dose of 50 mg/kg po for their anti-inflammatory and analgesic activities. From the results, it seems that compound **5a** having 2-methoxyphenyl ring showed less potent activities, but exhibited better activities than compound **5b** possessing 4-methoxyphenyl group as substitution. However, compound **5c** exhibited more potent activities than the compounds **5a** and **5b**. Among these compounds, compound **5f** was found to possess most potent anti-inflammatory and analgesic activities and less ulcerogenic potentialities than other compounds.

Furthermore, cyclization of compounds **5a–5i** in to their corresponding compounds **6a–6i** in general enhances the activities. The characteristic feature of compounds **6a–6i** is the presence of thiazolidinyl moiety at the 2-position of quinazolinone nucleus. The compound **6d** having 2-

Table 1. Pharmacological evaluation of compounds **5a–5i** and **6a–6i**

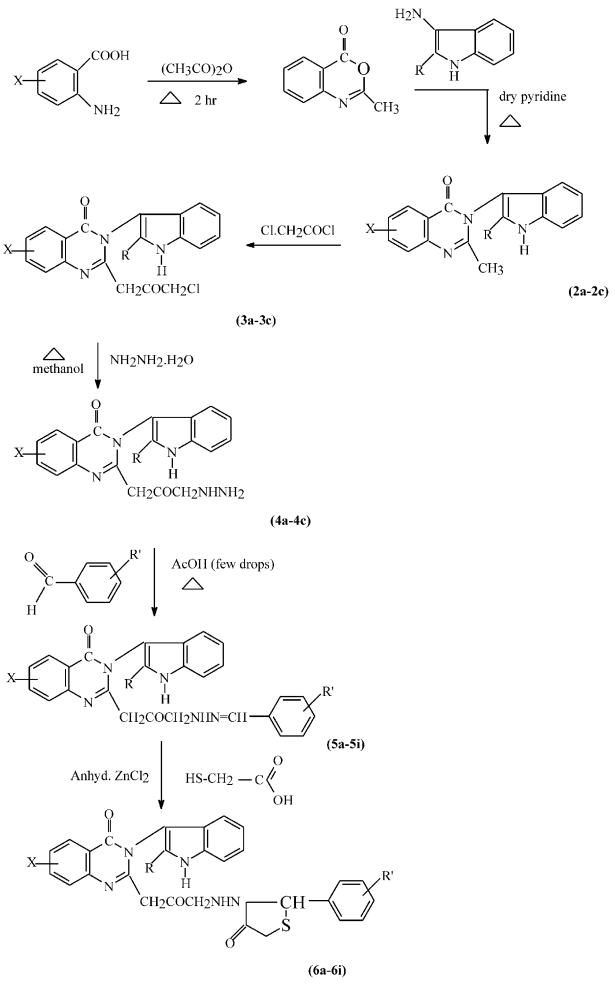


(5a-5i)



(6a-6i)

Comd. No.	X	R	R′	Anti-inflammatory activity		Analgesic activity		Ulcerogenic activity			COX-II inhibitory activity	ED_{50} mg/kg p.o.	ALD_{50} mg/kg p.o.
				Dose (mg/kg p.o.)	% oedema inhibitor relation	Dose (mg/kg p.o.)	% decreases	Dose	% of animal with hypermia	% of animal with ulcer			
5a	H	CH_3	2-OCH_3	50	38.7	50	30	300	60	30	ni	—	> 1000
5b	H	CH_3	4-OCH_3	50	31.6	50	20	300	30	40	ni	—	> 1000
5c	H	CH_3	2-Cl	50	49.2	50	40	300	20	10	ni	—	> 1000
5d	6-I	H	2-OCH_3	50	52.6	50	60	300	100	20	ni	—	> 1000
5e	6-I	H	4-OCH_3	50	44.8	50	50	300	90	30	ni	—	> 1000
5f	6-I	H	4-Cl	25	40.2	25	40	100	50	20			
				50	58.9	50	70	200	70	30	40	35.4	> 1000
				100	79.9	100	90	300	90	40			
5g	6-Br	CH_3	2-OCH_3	50	49.7	50	70	300	60	20	ni	—	> 1000
5h	6-Br	CH_3	N-$(CH_3)_2$	50	43.5	50	60	300	50	40	ni	—	> 1000
5i	6-Br	CH_3	2-Cl	50	55.3	50	80	300	50	50	ni	—	> 1000
6a	H	CH_3	2-OCH_3	50	32.6	50	40	300	80	30	ni	—	> 1000
6b	H	CH_3	4-OCH_3	50	28.2	50	30	300	70	40	ni	—	> 1000
6c	H	CH_3	2-Cl	50	29.7	50	40	300	50	40	ni	—	> 1000
6d	6-I	H	2-OCH_3	25	39.6	25	30	100	30	10			
				50	60.4	50	70	200	60	10	90	35.4	> 2000
				100	82.4	100	80	300	100	10			
6e	6-I	H	4-OCH_3	50	52.7	50	60	300	90	20	ni	—	> 1000
6f	6-I	H	4-Cl	50	58.3	50	50	300	80	30	ni	—	> 1000
6g	6-Br	CH_3	2-OCH_3	50	50.1	50	60	300	100	40	ni	—	> 1000
6h	6-Br	CH_3	N-$(CH_3)_2$	50	46.3	50	60	300	80	50	ni	—	> 1000
6i	6-Br	CH_3	2-Cl	50	57.1	50	70	300	90	40	ni	—	> 1000
Phenyl butazone				25	28.42								
	—	—	—	50	36.4	—	—	—	—	—	—	—	—
				100	58.4								
Indomethacin				5.0	52.2								
	—	—	—	7.0	63.1	—	—	—	—	—	—	—	—
				10	93.2								



Scheme 1.

methoxyphenyl group as substituent was found to be the most potent derivative of the present series. Being a most potent compound, this compound was further screened for their anti-inflammatory and analgesic properties at three graded doses, that is 25, 50 and 100 mg/kg po; interestingly, this compound showed better activities than the standard drugs at all doses tested. As far as the ulcerogenic liability of these compounds is concerned, the compounds **5f** and **6f** exhibit less ulcerogenic potentialities than the rest of the compounds of this series.

All these compounds were also evaluated for COX-II inhibitory activity, and only compounds **5f** and **6d** showed COX-II inhibitory action, that is 40 and 90% inhibition, respectively. Compound **6d** exhibited $ALD_{50} > 2000$ mg/kg po and the rest of the compounds of this series showed $ALD_{50} > 1000$ mg/kg po.

Experimental

Chemistry

Melting points were determined in open capillaries with the help of thermonic melting point apparatus and are uncorrected. Homogeneity of the newly synthesized compounds was checked by thin layer chromatography. IR spectra (KBr/Nujol) were recorded on Beckman Acculab-10-FTIR spectrometers. ^{1}H NMR spectra were recorded by Bruker DRX-300 FTNMR instrument using $CDCl_3$ or DMSO-d_6 as solvent and tetramethyl silane (TMS) as internal reference standard. All chemical shift (δ) values were recorded in ppm. The purity of the compounds was checked by thin-layer chromatography on silica gel G plates of 0.5-mm thickness. The elemental analysis (C, H, N) of the compounds was performed on a Carlo Erba-1108 instrument.

2-Methyl-3-(2′-methylindol-3′-yl)-4(3H)-quinazolinone (2a). To a solution of 2-methyl-3-aminoindole (0.01 mol) in dry pyridine (100 mL) different acetanthranils (0.02 mol) was added. The reaction mixture was refluxed for 10 h. After refluxing, excess of solvent was removed and the residue neutralized with HCl. The solid separated out was washed with water and recrystallized from ethanol to give **2a** (85%): mp 214 °C. IR (KBr) ν 3250, 3045, 1690, 1608 and 1155 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.60–7.95 (8H, m), 9.01 (1H, s), 2.20 (6H, s) ppm. Anal. calcd for $C_{18}H_{15}N_3O$: C, 74.74; H, 5.19; 14.53. Found: C, 74.93; H, 5.37; N, 14.72. The following compounds were prepared using a similar procedure described for **2a**.

2-Methyl-3-(indol-3′-yl)-6-iodo-4(3H)-quinazolinone (2b). Mp 247–248 °C (DMF/water); IR (Nujol) ν 3255, 3040, 2965, 1692, 1610, 1150 and 730 cm^{-1}; ^{1}H NMR δ 8.62–7.95 (7H, m), 9.05 (1H, s), 2.15 (3H, s) ppm. Anal. calcd for $C_{17}H_{11}IN_3O$: C, 50.87; H, 2.75; N, 10.50. Found: C, 51.02 ; H, 2.92 ; N, 10.65.

2-Methyl-3-(2′-methylindol-3′-yl)-6-bromo-4(3H)-quinazolinone (2c). Mp 277–278 °C (acetic acid/water); IR (Nujol) ν 3250, 2960, 1695, 1605, 1155 and 560 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.60–7.98 (7H, m), 9.05 (1H, s), 2.18 (6H, s) ppm. Anal. calcd for $C_{18}H_{14}BrN_3O$: C, 58.69; H, 3.80; N, 11.41. Found: C, 58.72; H, 3.97; N, 11.67.

2-Chloroacetylmethylene-3-(2′-methyl-indol-3′-yl)-4(3H)-quinazolinone (3a). To a solution of 2-methyl-3-(2′-methyl-indol-3′-yl)-4(3H)-quinazolinone **1a** (0.32 mol) in dry THF (200 mL) was added at 0 °C temperature drop by drop along with manual stirring for 2 h. The reaction mixture was further stirred for 4 h at room temperature on mechanical stirrer. After stirring, excess of solvent was distilled off, cooled, poured onto crushed ice and filtered. The solid thus obtained was recrystallized from methanol to yield **3a** (75%): mp 176 °C; IR (KBr) ν 3265, 3062, 2910, 1700, 1610, 1580 and 580 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.55–7.90 (8H, m), 9.10 (1H, s), 2.20 (3H, s), 4.10 (2H, s) 3.90 (2H, s) ppm. Anal. calcd for $C_{20}H_{16}ClN_3O_2$: C, 65.55; H, 4.38; N,11.49. Found: C, 65.73; H, 4.56; N, 11.57. The following compounds were prepared using a similar procedure described for **3a**.

2-Chloroacetylmethylene-3-(indol-3′-yl)-6-iodo-4(3H)-quinazolinone (3b). Mp 202–203 °C (ethanol): IR (KBr) ν 3265, 2960, 1708, 1603, 1585, 575 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.55–7.95 (8H, m), 9.12 (1H, s), 4.08 (2H, s), 3.91(2H, s) ppm. Anal. calcd for $C_{19}H_{13}ClIN_3O_2$: C,

47.75; H, 2.72; N, 8.79. Found: C, 47.92; H, 3.03; N, 8.91.

2-Chloroacetylmethylene - 3 - (2′ - methylindol - 3′ - yl)-6-bromo-4(3H)-quinazolinone (3c). Mp 245–246 °C (acetone/pet. ether); IR (Nujol) ν 3270, 3050, 1710, 1605, 1585, 570 and 550 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.55–7.92 (7H, m), 9.10 (1H, s), 4.10 (2H, s), 2.20 (3H, s), 3.95 (2H, s) Anal. calcd for $C_{20}H_{15}BrClN_3O_2$: C, 53.99; H, 3.37; N, 9.45. Found: C, 54.12; H, 3.26; N, 9.32.

2-Hydrozinoacetylmethylene - 3 - (2′ - methylindol - 3′yl)-4(3H)-quinazolinone (4a). A mixture of compound **2a** and hydrazine hydrate (99%) in absolute ethanol (50 mL) was refluxed for 12 h and the completion of the reaction was monitored by TLC. Excess of solvent was distilled off and the reaction mixture was poured onto crushed ice and then filtered. The solid thus obtained was recrystallized from ethanol/water to yield **4a** (80%): mp 214–215 °C; IR (KBr) ν 3350, 3272, 1710, 1603, 2960, 1580 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.57–7.95 (8H, m), 9.15 (1H, s), 4.10 (2H, s), 3.90 (2H, d), 5.85–5.70 (3H, m), 2.18 (3H, s) ppm. Anal. calcd for $C_{20}H_{19}N_5O_2$: C, 66.48 ; H, 5.26 ; N, 19.39. Found: C, 66.62; H, 5.31; N, 19.47. The following compounds were synthesized by adopting a similar procedure described for **4a**.

2-Hydrozinoacetylmethylene-3-(indol-3′yl)-6-iodo-4(3H)-quinazolinone(4b). Mp 260–261 °C (methanol/water); IR (Nujol) ν 3355, 3270, 3052, 2965, 1712, 1600, 1585, 760 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.60–7.95 (8H, m), 9.12 (1H, s), 4.12 (2H, s), 3.95 (2H, d), 5.86–5.70 (3H, m) ppm. Anal. calcd for $C_{19}H_{16}IN_5O_2$: C, 48.20; H, 3.38; N, 14.79. Found: C, 48.12; H, 3.72; N, 15.05.

2-Hydrozinoacetylmethylene - 3 - (2′ - methylindol - 3′yl)-6-bromo-4(3H)-quinazolinone (4c). Mp 255–256 °C (ethanol); IR (KBr) ν 3272, 3360, 1715, 1600, 1200, 560 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.60–8.00 (7H, m), 9.15 (1H, s), 2.15 (3H, s), 4.11 (2H, s), 3.95 (2H, d), 5.80–5.65 (3H, m) ppm. Anal. calcd for $C_{20}H_{18}O_2N_5Br$: C, 54.55; H, 4.09; N, 15.90. Found: 54.63; H, 4.17; N, 15.60.

2-(*o*-Methoxyphenylmethyleneimino)aminoacetylmethylene-3-(2′-methyl-indol-3′-yl)-4(3H)-qinazolinone (5a). A mixture of compound **3a** and *o*-methoxybenzaldehyde in absolute methanol (50 mL) was refluxed for 8 h in presence of glacial acetic acid (5 mL). The excess of solvent was distilled off and the residue thus obtained washed with a mixture of diethyl ether and water 6:8 and finally recrstallised from benzene/hexane to furnish compound **5a** (60%) mp 215–216 °C. IR (KBr) ν 3270, 3055, 2950, 1710, 605 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.50–7.70 (12H, m) 9.10 (1H, s), 2.18 (3H, s), 4.15 (2H, s), 3.90 (2H, s), 5.60 (1H, t), 6.10 (1, s), 3.15 (3H, s) ppm. Anal. calcd for $C_{28}H_{25}N_5O_3$: C, 70.14; H, 5.21, N, 14.61. Found: C, 70.55; H, 5.63; N, 14.25. The following compounds were prepared using a similar procedure described for **5a**.

2-(*p*-Methoxyphenylmethyleneimino)aminoacetylmethylene-3-(2′-methyl-indol-3′-yl)-4(3H)-quinazolinone (5b). Mp 195–196 °C (ethanol); IR (KBr) ν 3265, 3060, 2965, 1715, 1603 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.50–7.75 (12H, m), 9.15 (1H, s), 2.20 (3H, s), 4.15 (2H, s), 3.18 (3H, s) ppm. Anal. calcd for $C_{28}H_{25}N_5O_3$; C, 70.14; H, 5.21; N, 14.61. Found: C, 70.27; H, 5.57; N, 14.25.

2-(*o*-Chlorophenylmethyleneimino)aminoacetylmethylene-3-(2′-methyl-indol-3′-yl)-4(3H)-quinazolinone (5c). Mp 184–185 °C (methanol/water); IR (KBr) ν 3270, 3065, 2965, 1712, 1603, 1580, 1205, 560 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.55–7.80 (12H, m), 9.16 (1H, s), 2.18 (3H, s), 4.16 (2H, s), 3.90 (2H, d), 5.65 (1H, t), 6.15 (1H, s) ppm. Anal. calcd for $C_{27}H_{22}ClN_5O_2$; C, 67.01; H, 4.55; N, 14.47. Found: C, 66.05; H, 4.26; N, 14.83.

2-(*o*,-Methoxyphenylmethyleneimino)aminoacetylmethylene-3-(indol-3′-yl)-6-iodo-4(3H)-quinazolinone (5d). Mp 155–156 °C (ethanol/water). IR (KBr) ν 3275, 1715, 1600, 1585, 752 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.55–7.75 (12H, m), 9.18 (1H, s), 4.20 (2H, s), 3.90 (2H, d), 5.65 (1H, t), 6.15 (1H, s), 3.20 (3H, s). Anal. calcd for $C_{27}H_{22}IN_5O_3$: C, 54.82; H, 3.72; N, 11.84: Found: C, 54.57; H, 3.59; N, 12.00.

2-(*p*-Methoxyphenylmethyleneimino)aminoacetylmethylene-3-(indol-3′-yl)-6-iodo-4(3H)-quinazolinone (5e). Mp 184–186 °C (DMF/water); IR (Nujol) 3275, 3062, 1720, 1582,750 cm^{-1};^1H NMR ($CDCl_3$) δ 8.60–7.80 (12H, m), 9.20 (1H, s), 4.22 (2H, s), 3.92 (2H, d), 5.62 (1H, t), 6.20 (1H, s), 3.20 (3H, s) ppm. Anal. calcd for $C_{27}H_{22}IN_5O_3$: C, 54.82; H, 3.72; N, 11.84. Found: C, 54.67; H,3.56; N, 12.12.

2-(*p*-Methoxyphenylmethyleneimino)aminoacetylmethylene-3-(indol-3′-yl)-6-iodo-4(3H)-quinazolinone (5f). Mp 197–198 °C (ethylacetate); IR (KBr) ν 3275, 1605, 1710, 1205, 1600, 750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.50–7.76 (12H, m), 9.20 (1H, s), 4.20 (2H, s), 3.91 (2H, d), 5.65 (1H, t), 6.15 (1H, s). Anal. calcd for $C_{26}H_{19}ClIN_5O_2$: C, 60.21; H, 4.30; N,12.54. Found: C, 59.00; H, 4.12; N, 12.66.

2-(*p*-Methoxyphenylmethyleneimino)aminoacetylmethylene-3-(2′-methyl-indol-3′-yl)-6-bromo-4(3H)-quinazolinone (5g). Mp 221–222 °C (ethanol/water); IR (KBr) ν 3270, 3080, 2860, 2950, 1700, 1590, 1480 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.55–7.80 (1H, m) 9.22 (1H, s), 4.25 (2H, s), 3.95 (2H, d), 5.60 (1H, t), 6.18 (1H, s), 2.25 (3H, s) ppm. Anal. calcd for $C_{28}H_{24}BrN_5O_3$: C, 60.21; H, 4.30; N, 12.54. Found: C, 59.90; H, 4.12; N, 12.66.

2-(*p*-Dimethylaminophenylmethyleneimino)aminoacetylmethylene-3-(2′-methyl-indol-3′-yl)-6-bromo-4(3H)-quinazolinone (5h). Mp 211–212 °C (methanol/water), ν 1603, 1210, 2962, 560 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.60–7.90 (11H, m), 9.20 (1H, s), 4.25 (2H, s), 3.95 (2H, d), 5.70 (1H, t), 6.20 (1H, s), 2.95 (6H, s), 2.25 (3H, s) ppm. Anal. calcd for $C_{28}H_{27}BrN_6O_2$: C, 60.10, H, 4.83; N, 15.02. Found: C, 60.44; H, 4.69; N, 15.09.

2-(*o*-Chlorophenylmethyleneimino)aminoacetylmethylene-3-(2′-methyl-indol-3′-yl)-6-bromo-4(3H)-quinazolinone (5i). Mp 235–236 °C (benzene), IR (KBr) ν 3278, 1715, 1600, 1585, 1205, 562 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.65–7.90 (11H, m), 9.22 (1H, s), 4.25 (2H, s), 3.90 (2H, d),

5.58 (1H, t), 6.25 (1H, s), 2.22 (3H, s) ppm. Anal. calcd for $C_{27}H_{21}BrN_5O_2Cl$: C, 57.60; H, 3.73; N, 12.44. Found: C, 57.37; H, 3.26; N, 12.80.

2-(*o*-Methoxyphenylaminomethylacetyl-4′-oxo-1′-thiazolidinyl)-3-(indol-3″-yl)-4(3H)-quinazolinone (6a). Equimolar amount of compound **4a** and thioglycolic acid in absolute ethanol was refluxed for 18 h. The solvent was removed under reduced pressure. The solid thus obtained was treated with saturated solution of $NaHCO_3$ and then extracted three times with chloroform. The organic extract was combined, washed with water, dried over anhydrous sodium sulphate, concentrated in vacuo upto a small volume and then chromatographed. The product finally obtained was recrystallised from ethanol to give **6a** (40%), mp 245–246 °C. IR (KBr) ν 3272, 3075, 2860, 2955, 1710, 1580, 1460 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.55–7.70 (12H, m), 9.12 (1H, s), 4.20 (2H, s), 3.85 (2H, d), 5.55 (1H, t), 4.80 (2H, s), 2.18 (3H, s), 3.20 (3H, s), 6.15 (1H, s) ppm. Anal. calcd for $C_{30}H_{27}N_5O_4S$: C, 65.09; H, 4.88; N, 12.66. Found: C, 65.37; H, 4.66; N, 12.95.

2-(*p*-Methoxyphenylaminomethylacetyl-4′-oxo-1′-thiazolidinyl) - 3 - (2″methyl - indol - 3″ - yl)-4(3H)-quinazolinone (6b). Mp 255–256 °C (methanol–water): IR (Nujol) ν 3275, 3025, 2950, 1710, 1450 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.55–7.22 (12H, m), 9.15 (1H, s), 4.20 (2H, s), 3.85 (2H, d), 5.56 (1H, t), 4.80 (2H, s), 2.18 (3H, s), 3.20 (3H, s), 6.20 (1H, s) ppm. Anal. calcd for $C_{30}H_{27}N_5O_4S$: C, 65.09; H, 4.88; N, 12.66. Found: C, 65.40; H, 5.11; N, 12.87.

2-(*o*-Chlorophenylaminomethylacetyl-4′-oxo-1′-thiazolidinyl)-3-(2″methyl-indol-3″-yl)-4(3H)-quinazolinone (6c). Mp 218–220 °C (benzene): IR (KBr) ν 3280, 3025, 2830, 1710, 1603, 1575, 1220, 2010 cm^{-1}; ^1H NMR δ 8.52–7.22 (12H, m), 9.15 (1H, s), 3.20 (3H, s), 3.82 (2H, d), 4.20 (2H, s), 5.55 (1H, d), 6.20 (1H, s) 4.80 (2H, s) ppm. Anal. calcd for $C_{29}H_{24}O_3N_5ClS$, C, 62.42; H, 4.30; N, 12.56.00 Found: C, 62.65; H, 4.39; N, 12.67.

2-(*o*-Methoxyphenylaminomethylacetyl-4′-oxo-1′-thiazolidinyl)-3-(indol-3″-yl)-6-iodo-4(3H)-quinazolinone (6d). Mp 200–201 °C (benzene); IR (KBr) ν 3280, 3028, 2825, 1710, 1603, 1580, 1220, 1012 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.60–7.20 (12H, m), 4.21 (2H, s), 5.55 (1H, t), 3.80 (2H, d), 4.81 (2H, s), 9.15 (1H, s), 2.20 (3H, s), 6.20 (1H, s) ppm. Anal. calcd for $C_{29}H_{24}N_5O_4IS$: C, 52.33, H, 3.61; N, 10.53. Found: C, 52.21; H, 3.56; N, 10.63.

2-(*p*-Methoxyphenylaminomethylacetyl-4′-oxo-1′-thiazolidinyl)-3-(indol-3″-yl)-6-iodo-4(3H)-quinazolinone (6e). Mp 250–251 °C (benzene); IR (Nujol) ν 3280, 3028, 2825, 1710, 1603, 1580, 1220, 1012, 1108 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.60–7.20 (12H, m), 5.55 (1H, t), 4.80 (2H, s), 3.80 (2H, d), 4.21 (2H, s), 9.15 (1H, s), 2.22 (3H, s), 6.20 (1H, s) ppm. Anal. calcd for $C_{29}H_{24}O_4N_5IS$: C, 52.33; H, 3.61; N, 10.53. Found: C, 52.52; H, 3.66; N, 10.71.

2-(*p*-Chlorophenylaminomethylacetyl-4′-oxo-1′-thiazolidinyl)-3-(indol-3″-yl)-6-iodo-4(3H)-quinazolinone (6f). Mp 212–213 °C (DMF/water); IR (Nujol) ν 3280, 3028, 2825, 1710, 1603, 1580, 1220, 1012, 1108 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.60–7.20 (13H, m), 5.55 (1H, t), 4.80 (2H, s), 3.80 (2H, d), 4.21 (2H, s), 9.15 (1H, s), 6.20 (1H, s) ppm. Anal. calcd for $C_{28}N_{21}O_3N_5ClIS$: C, 50.18; H, 3.13; N,10.45; Found: C, 50.22; H, 3.22; N, 10.48.

2-(*p*-Methoxyphenylaminomethylacetyl-4′-oxo-1′-thiazolidinyl)-3-(2″-methyl-indol-3″-yl)-6-bomo-4(3H)-quinazolinone (6g). Mp 214–215 °C (ethanol/water) IR (KBr) ν 3280, 3028, 2825, 1710, 1603, 1580, 1220, 1012 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.60–7.20 (11H, m), 5.55 (1H, t), 4.80 (2H, s), 3.80 (2H, d), 4.21 (2H, s), 9.15 (1H, s), 6.20 (1H, s), 3.21 (3H, s) ppm. Anal. calcd for $C_{30}H_{26}BrN_5O_4S$: C, 56.96; H, 4.11; N, 11.08. Found: C, 57.12; H, 4.36; N, 11.38.

2-(*p*-Dimethylaminophenylaminomethylacetyl - 4′oxo - 1′ - thiazolidinyl) - 3 - (2″ - methyl-indol-3′-yl)-6-bromo-4(3H)-quinazolinone (6h). Mp 224–225 °C (benzene); IR (Nujol) ν 3280, 3028, 2825, 1710, 1603, 1580, 1220, 1012, 1108 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.60–7.20 (11H, m), 5.55 (1H, t), 4.80 (2H, s), 3.80 (2H, d), 4.21 (2H, s), 9.15 (1H, s), 6.20 (1H, s), 3.21 (3H, s), 2.90 (6H, s) ppm. Anal. calcd for $C_{31}H_{29}O_3N_6BrS$; C, 57.67; H, 4.50; N, 13.02. Found: C, 57.93 ; H, 4.53; N, 13.31.

2-(*o*-Chlorophenylaminomethylacetyl-4′-oxo-1′-thiazolidinyl)-3-(2″-methyl-indol-3″-yl)-6-bromo-4(3H)-quinazolinone (6i). Mp 255–256 °C (methanol/water); IR (KBr) ν 3280, 3028, 2825, 1710, 1603, 1580, 1220, 1012, 1108 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.60–7.20 (11H, m), 5.55 (1H, t), 4.80 (2H, s), 3.80 (2H, d), 4.21 (2H, s), 9.15 (1H, s), 6.20 (1H, s), 3.21 (3H, s) ppm. Anal. calcd for $C_{29}H_{23}BrClN_5O_3S$: C, 54.76; H, 3.61; N, 11.00. Found: C, 54.93; H, 3.63; N, 11.26.

Pharmacological evaluation

Male albino rats and mice of Charles Foster strain species were used in experiments. The animals were kept in the groups (control, treated, standard) under constant temperature (25 ± 1 °C) and 12-h light/dark cycle. They had free access to standard mouse diet and tap water except during the experiment. On the day of the experiment, animals were transferred to individual cages randomly and allowed to acclimatize for 30 min before drug administration. Indomethacin and phenylbutazone were used as standard drugs. Newly synthesized compounds were dissolved in propyleneglycol.

Anti-inflammatory activity

Paw oedema inhibition test was performed on albino rats by adopting the method of Winter et al.[14] Thirty min later, 0.2 mL of 1% carrageenan suspension in 0.9% NaCl solution was injected subcutaneously into the plantar aponeurosis of the hind paw, and the paw volume was measured by a water plethysmometer socrel and then measured again 3 h later. The mean increase of paw volume at each time interval was compared with that of control group at the same time intervals and

percent inhibition values were calculated by the formula given below:

$$\%\ \text{anti-inflammatory activity} = 1 - (V_t/V_c) \times 100$$

where V_t and V_c are tested and control groups, respectively.

Analgesic activity

Acetic acid writhing test was performed on mice by following the method of Davis et al.[15] Test compounds were given to the animals at a dose of 50 mg/kg, 30 min later the animals were injected intraperitoneally with 0.25 mL/mouse of 0.5% acetic acid. The mean number of writhes for each experimental groups and percentage decrease compared with the control group were calculated after 60 min.

Ulcerogenic activity

Ulcerogenic liabilities of newly synthesized compounds was checked by the method of Verma et al.[16] Albino rats were fasted for 24 h prior to drug administration. All animals were sacrificed 8 h after drug treatment, and then their stomachs and small intestines were microscopically examined to assess the incidence of hyperaemia, shedding of epithelium, petechial and frank haemorrhages 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.

Acute toxicity study

Approximate lethal dose (ALD_{50}) of compounds was determined in albino mice. The test compounds were given orally at different dose levels in groups of 10 animals. After 24 h of drug administration, percent mortality in each group was observed and from the data obtained ALD_{50} was calculated by the method of Smith.[17]

Cyclooxygenase activity

This test was carried out in vitro according to reported method[18] on the microsomal fraction of mucosal preparations of rabbit distal colon in order to search out the possible mechanism of the compounds. Colonic mucosa ($\cong$2–3 g), stripped as previously described was minced and homogenized Potter homogenizer in 3 vol of Tris buffer 0.1, pH 8.0. The homogenate was centrifused for 30 min at 10,000 *g*. The resulting supernatent was centrifused for 1 h at 100,000 *g*. The precipitate was suspended in Tris buffer 0.1 M, pH 8.0, and recentrifused for 1 h at 100,000 *g*. The microsomal pellet was used immediately for enzyme assay cyclooxygenase activity was assayed by measuring the rate of conversion of arachidonic acid to PGE_2. Microsomal fractions (50 μL) were incubated with test agents for 5 min at 37 °C in 30 μL Tris–HCl, pH 8.0 containing 2 mM reduced glutathione, 5 mM L-tryptophan, 1 μM hematin. The substrate 20 μM arachidonic acid with tracer amount of [1-^{14}C] arachidonic acid [$\cong$200 (xx) cpm] was then added and the reaction proceeded for 3 min at 37 °C. The reaction was stopped by addition of 0.2 mL of ethyl ether/methanol/citric acid 0.2 M (30:4:1), which was precooled at −25 °C PGE_2, was extracted twice into the same mixture. The solvent was evaporated under an N_2 stream and radiolabelled arachidonic acid was separated from radiolabelled acid was separated from radiolabelled PGE_3 by RP-HBLC. HPLC analysis was performed on a Hitachi spectrophotometer equipped with a flow cell. The sample was injected on an ultrasphere column (Beckman) ODS 5 mm. 4.6 mm×25 cm with 2 nmol unlabelled PGE_2 as an interval standard, PG chromatographic profile was obtained by isocratic elution with 150 mM H_3PO_4 in water, pH 3.5, containing 30% acetonitrile, a flow rate of 1 mL/min monitoring the uv absorption at 214 nm. Radioactivity that coeluted with autheutic PGE_2 was quantified by liquid scintillation spectrometry. Test samples were compared to paired control incubations. The percentage of inhibition was calculated as follows:

$$[(\text{cpm control} - \text{cpm test}/(\text{cpm control}) \times 100]$$

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