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Synthesis of newer substituted thiadiazolyl and pyrazolyl phenothiazines as potent anti-inflammatory agents

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ABSTRACT

N-(5-((10H-phenothiazin-10-yl)methyl)-1,3,4-thiadiazol-2-yl)-3-(substituted phenyl) acryl- amide (5a-5j) and 1-(3-(5-((10H-phenothiazin-10-yl)methyl)-1,3,4-thiadiazol-2-yl)-5-(sub- stituted phenyl)-4,5-dihydro-1H-pyrazol-1-yl) ethanone (6a-6j), were screened for their anti-inflammatory and analgesic activities at a dose of 50 mg/kg,p.o. The synthesized compounds were screened for their in vitro anti-inflammatory activity against for carrageenan induced rat *paw oedema*. The compounds were also tested for their analgesic activity against phenyl butazone against induced pain syndrome in mice. Compounds 5e and 6e were found to be most active compounds of this series, which shows 45.68% and 48.63% inflammation inhibitory and 35.86% and 45.15% analgesic activities respectively. The structural assignments of compounds have been made on the basis of elemental analysis, IR.^{1}H-NMR and mass spectral data.

Key words: Thiadiazolyl phenothiazine; Pyrazolyl phenothiazine; Anti-inflammatory; Analgesic; Toxicity studies.

INTRODUCTION

Phenothiazines constitute one of the most active class of compounds possessing diversified biological application. It has been reported to show a broad spectrum of biological activities of these include anti-inflammatory[1–3]. Cardiovascular[4], and fungicidal[5] activities etc. Furthermore, derivatives of Pyrazoline[6–7] and 1,3,4–thiadiazole[8–10] have also been reported to possess promising anti-inflammatory activities when substituted at 10-Position of the phenothiazine nucleus. In light of these finding, a new drug strategy has been planned to synthesize several new Phenothiazine derivatives possessing thiadiazole and Pyrazoline moieties at 10–Position of the Phenothiazine nucleus with the hope to possess better anti-inflammatory and analgesic activities. All the newly synthesized compounds have been screened for their anti-inflammatory, analgesic and toxicity activities.

Chemistry

The reaction sequence leading to the formation of different Phenothiazine derivatives is out lined in the scheme. Reaction of ethyl chloroacetate and Phenothiazine yielded the starting compound 1 i.e. ethyl 2-(10H-Phenothiazine-10-yl) acetate. This compound on reaction with thiosemicarbazide yielded compound 2. Compound 2 on dehydration with concentrated sulfuric acid converted into 5-((10H-Phenothiazin-10-yl) methyl)-1,3,4-thiadiazol-2-amine (3). This compound on acetylation with acetyl chloride result in the next compound i.e. N-(5-((10H-Phenothiazin-

10-yl)methyl)-1,3,4-thiadiazol-2-yl) acetamide (4).Compound 4, When treated with various substituted aromatic aldehydes, separately, afforded N-(5-((10H-Phenothiazin-10yl)methyl)-1,3,4-thiadiazol-2-yl)-3-(Substituted Phenyl) acrylamide (5a–5j). Finally,these compounds were cyclized with hydrated hydrazine in the presence of glacial acetic acid separately, resulted in the formation of 1-(3-(5-((10H-Phenothiazin-10-yl) methyl)-1,3,4-thiadiazol-2-ylamino)-5-(Substituted Phenyl)-4,5-dihydro -1-H-Pyrazol-1- yl) ethanone (6a-6j).

RESULTS AND DISCUSSION

All the newly synthesized compounds of this series i.e., 5a-5j and 6a-6j, were evaluated for their anti-inflammatory, analgesic and ulcerogenic activities at a dose of 50 mg/kg p.o. and the pharmacological data of all the compounds of this series have been reported in Table-V. All the compounds of this series 5a-5j and 6a-6j have shown varying degree of anti-inflammatory activity (9.67-45.68 %). The active compounds of this series 5e and 6e were found to possess more potent anti-inflammatory activity in the comparision of Phenyul butazone. The compound 6e, which substituted with chloro group at 2^{nd} & 4^{th} position have shown 48.63% of inhibition of oedema. The compound 5j, which possesed N,N-dimethyl group at 4^{th} position of phenyl ring has shown least activity i.e. 9.67% The compound 6e, i.e., 1-(3-(5-((10H-phenothiazin-10-yl)methyl)-1,3,4-thiadiazol-2-ylamino)-5-(2,4-dichoro phenyl)-4,5-dihydro-1H-pyrazol-1 yl)ethanone and 5e, i.e., N-(5-((10H-Phenothiazin-10-yl)methyl)-1,3,4,-thiadiazol-2-yl)-3-(2,4-dichlorophenyl) acrylamide, have shown the better anti-inflammatory activity i.e. 48.63% and 45.68%, at a dose of 50 mg/kg p.o. as compared to Phenylutazone. figure-1 showed the bar diagram of anti-inflammatory activity at three graded doses (25, 50 and 100 mg/kg p.o.) of compounds 5e, 6e and Phenylbutazone. At all the three graded dose levels compounds 5e & 6e showed more inhibitory activity than that of Phenylbutazone. The newly synthesized compounds of the present series showed analgesic activity variying from 8.92-45.15%. The active compounds of this series 5e and 6e were found to possessed better analgesic activity i.e. (35.86 and 45.15%) at the dose of 50 mg/kg P.O. Considering Potentiality of compound 5e and 6e, these were screened in details at three graded doses, 25, 50 and 100 mg/kg P.O. The compound 6e have shown better analgesic activity at all three graded doses as compaired to Phenyl butazone. Compound 5e (145.0 mg/kg i.p.) and 6e (168.5 mg/kg i.p.) were also evaluated for their ulcerogenic activity and found to be less ulcerogenic liability as compared to Phenyl butazone (66.70%), of all compounds of this series showed > 1000 mg/kg P.O. except 5e and 6e which showed > 1500 mg/kg P.O. It indicates a good safety margin. Approximate lethal dose (ALD_{50}

Therefore, it may be concluded that –

(a) Different substituted chalkones moities have shown mild to moderate anti-inflammatory and analgesic activities. Cyclization of these chalkone moities into their corresponding Pyrazolines enhances the anti-inflammatory and analgesic Properties.

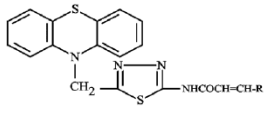
(b) Compounds 5e and 6e having a 2,4-dichloro phenyl ring as substituents, exhibited most potent anti-inflammatory and analgesic activities thus the obtained biological results clearly indicate that compounds which showed max anti-inflammatory activity also exhibited potent analgesic activity.

EXPERIMENTAL

General

The melting points were determined in open capillaries with the help of thermonic melting point apparatus and are uncorrected. The purity of all newly synthesized compounds was routinely checked by thin layer chromatography on silica Gel-G coated plates, eluent was a mixture of methanol-benzene in 2:8 proportion. The structure of these compounds was elucidated by IR, ^{1}H-NMR, Mass and elemental analysis. The IR (KBr) spectra were recorded on perkin-Elmer spectrum RX-1 spectrometer, $½_{max}$. in Cm^{-1}. The ^{1}H-NMR' spectra were recorded by Brucker AC-300 FT instrument using $CDCl_3$ as solvent. Chemical shift values were recorded as (´) in ppm. Tetramethyl Silane (TMS) was used as internal reference standard. Elemental analysis was performed on Perkin-Elmer 2400 elemental analyzer and results were found within the ± 0.4% of theoretical values. Mass spectra were determined on a VG 70-S instrument. The Physical and analytical data of compounds are given in table-

Table 1: Physical and analytical data of N-(5-((10 H-Phenothiazin-10-yl) methyl)-1,3,4- thiadiazol-2yl)-3 (Substituted Phenyl) acrylamide (5b-5j)



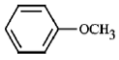
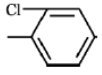
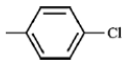
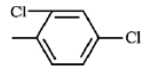
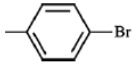
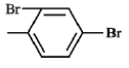
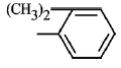
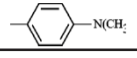
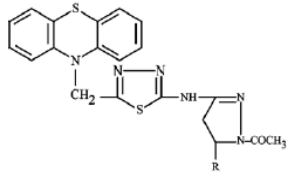
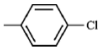
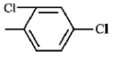
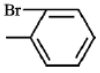
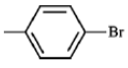
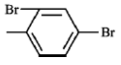
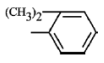
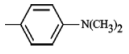
Comp. No.	R	m.p. (°C)	Yield (%)	Recrystallistion Solvent	Molecular Formula	Mol wt.	Elemental Analysis (%)					
							C %		H %		N %	
							(Calcd.)	Found	(Calcd.)	Found	(Calcd.)	Found
5b		153	50	Ethanol	$C_{25}H_{20}N_4O_2S_2$	472.58	(63.54)	63.70	(04.27)	04.31	(11.86)	11.93
5c		206	54	Acetone	$C_{24}H_{17}ClN_4OS_2$	477.00	(60.43)	60.64	(03.59)	03.56	(11.75)	11.80
5d		225	51	Acetone	$C_{24}H_{17}ClN_4OS_2$	477.00	(60.43)	60.31	(03.59)	03.62	(11.75)	11.82
5e		218	65	Methanol	$C_{24}H_{16}Cl_2N_4OS_2$	511.45	(56.36)	56.45	(03.15)	03.18	(10.95)	10.90
5f		238	60	Acetic acid	$C_{24}H_{17}BrN_4OS_2$	521.45	(55.28)	55.40	(03.29)	03.32	(10.74)	10.70
5g		229	63	Methanol	$C_{24}H_{17}BrN_4OS_2$	521.45	(55.28)	55.42	(03.29)	03.30	(10.74)	10.80
5h		247	68	DMF	$C_{24}H_{16}Br_2N_4OS_2$	600.35	(48.01)	48.29	(02.69)	02.72	(09.33)	09.30
5i		186	48	Acetic Acid	$C_{26}H_{23}N_5OS_2$	485.62	(64.30)	64.61	(04.77)	04.80	(14.42)	14.48
5j		193	45	DMF	$C_{26}H_{23}N_5OS_2$	485.62	(64.30)	64.13	(04.77)	04.80	(14.42)	14.45

Table 2: Physical and analytical data of 1-(3-(5-((10 H-Phenothiazin-10-yl) methyl)-1,3,4-thiadiazol-2-ylamino)-5-(Substituted Phenyl)-4,5-dihydro-1 H-Pyrazol-1-yl) ethanone (6b-6j)



Comp. No.	R	m.p. (°C)	Yield (%)	Recrystallistion Solvent	Molecular Formula	Mol wt.	Elemental Analysis (%)					
							C %		H %		N %	
							(Calcd.)	Found	(Calcd.)	Found	(Calcd.)	Found
6b		211	45	Ethanol	$C_{27}H_{24}N_6O_2S_2$	528.65	(61.34)	61.50	(04.58)	04.54	(15.90)	15.98
6c		182	50	Acetone	$C_{26}H_{21}ClN_6OS_2$	533.07	(58.58)	58.73	(03.97)	03.93	(15.77)	15.60
6d		176	47	Acetic Acid	$C_{26}H_{21}ClN_6OS_2$	533.07	(58.58)	58.70	(03.97)	03.92	(15.77)	15.65
6e		202	61	DMF	$C_{26}H_{20}Cl_2N_6OS_2$	567.51	(55.03)	55.22	(03.55)	03.59	(14.81)	14.90
6f		226	55	Methanol	$C_{26}H_{21}BrN_6OS_2$	577.52	(54.07)	54.25	(03.67)	03.70	(14.55)	14.50
6g		217	58	Ethanol	$C_{26}H_{21}BrN_6OS_2$	577.52	(54.07)	54.24	(03.67)	03.65	(14.55)	14.48
6h		236	60	Acetone	$C_{26}H_{20}Br_2N_6OS_2$	656.41	(47.57)	47.71	(03.07)	03.11	(21.80)	21.88
6i		131	46	Ethanol	$C_{28}H_{27}N_7OS_2$	541.69	(62.08)	62.30	(05.02)	05.06	(18.10)	18.05
6j		118	50	DMF	$C_{28}H_{27}N_7OS_2$	541.69	(62.08)	62.25	(05.02)	05.00	(18.10)	18.12

I and table-II. The required compounds, ethyl 2-(10H-Phenothiazin-10-yl) acetate (1), 2–(2–(10H–Phenothiazin-10yl) acetyl) hydrazine carbothioamine (2) and 5-((10H-Phenothiazin-10-yl) methyl)-1,3,4-thiadiazol-2-amino (3) were synthesized according to Rawat and Srivastava (1998) method.

Synthesis of ethyl 2-(10H-Phenothiazin-10-yl)acetate (1)

It was prepared by following the method of Rawat and Srivastava (1998).

A mixture of 10H-Phenothiazine (0.1 mole) in acetone (100 ml.), ethylchloroacetate (0.1 mole) and anhydrous K_2Co_3 (5 gm.) were added. The reation mixture was refluxed for 10 hour. on steam bath. The excess of solvent was removed under reduced pressure and resulting solid mass poured over ice-water, filtered, and separated product was recrystallized from ethanol to furnish cream coloured crystals of compound (1) m.p. : 204°, yield : 80 %, mol. formula : $C_{16}H_{15}NO_2S$.

Synthesis of 2-(2-(10H-Phenothiazin-10-yl) acetyl) hydrazine carbothioamide (2)

To the solution of ethyl 2-(10H-Phenothiazin-10-yl) acetate (0.075 mole) and thiosemicarbazide (0.075 mole) in methanol (50 ml.) was refluxed on steam bath for about 10h. The excess of solvent was removed under reduced pressure and the viscous mass poured into ice-cold water, filtered and recrystallized from ethanol to give yellow leaflets of compound (2) m.p. 162°, yield : 75%, mol. formula : $C_{15}H_{14}N_4OS_2$. This compound was synthesized according to the method of Rawat and Srivastava (1998) and reported m.p. 162°, yield : 81%.

Synthesis of 5-((10H-Phenothiazin-10-yl) methyl)-1,3,4-thiadiazol-2-yl)amine(3)

It was prepared by following the method of Rawat and Srivastava (1998).

To a solution of 2-(2-(10H-Phenothiazin-10-yl) acetyl) carbothioamide, (0.05 mole) and concentrated H_2SO_4 was kept at room temperature, poured into ice-cold water, Neutralized with liquid ammonia and filtered. The product thus obtained was recrystallized from methanol to get compound (3) M.P. 118°, yield: 85 %, mol. formula : $C_{15}H_{12}N_4S_2$.

Synthesis of N-(5-((10H-Phenothiazin-10-yl)methyl)-1,2,4-thiadiazol-2-yl) acetamide (4)

To the solution of 5-((10 H-Phenothiazin-10-yl) methyl)-1,3,4-thiadiazol-2-amine (0.02 mole) in dry chloroform (50 ml.), acetyl chloride (0.02 mole) added drop by drop at 0-5°C temperature with constant stirring. Furthermore, reaction mixture was also stirred for 1h. at room temperature and refluxed for 5h. Excess of solvent was removed through distillation. The product obtained was washed with cold-water and recrystallized with methanol to give compound (4). m.p 161°, yield: 60%, mol. formula : $C_{17}H_{14}N_4OS_2$. Elemental analysis: Calcd. % C : 57.61, % H : 03.98, % N : 15.81 : Found : %C 57.80, % H : 03.90, % N : 15.95. IR (KBr) $\lambda_{max.}$ in cm^{-1} : 3340 (N-H), 3025 (C-H aromatic) 2960 (C-H aliphatic). 1740 (C=0), 1600 (C=N), 1575 (C-C of aromatic ring), 1470 (C=C), 1170 (C-N), 735 (C-S-C), ^{1}H-NMR ($CDCl_3$) ´ in ppm. : 8.60 (S, 1H, NHCO), 8.25 (d, 1H,=CH-Ar) 7.83-7.20 (m, 12H, Ar-H), 4.33 (S, 2H, N-CH_2), 2.38 (S, 3H, $COCH_3$), MS : $[M]^+$ at m/z 354.45.

Synthesis of N-(5-((10H-Phenothiazin-10-yl) methyl)-1,3,4-thiadiazol-2-yl)-3-(2-methoxy Phenyl) acrylamide (5a)

To a solution of N-(5-((10H-Phenothiazin-10-yl) methyl)-1,3,4-thiadiazol-2-yl) acet amide (0.02 mole) in absolute ethanol (50 ml.) with 2-mithoxy benzaldehyde (0.02 mole) in the presence of 10% NaOH, refluxed for 8h. The reaction mixture was concentrated, cooled, poured onto crushed ice, filtered, washed with water and recrystallized from ethanol to afford compound (5a) m.p 170°, yield : 55 %, mol. formula : $C_{25}H_{20}N_4O_2S_2$. Elemental analysis : Calcd. % C : 63.54, % H : 04.27, % N : 11.86 : Found : %C 63.70, % H : 04.35 , % N : 11.72, IR (KBr) $\lambda_{max.}$ in cm^{-1} 3350 (N-H), 3045 (C-H aromatic) 2950 (C-H aliphatic), 1735 (C=O), 1605 (C=N), 1570 (C....C of aromatic ring), 1482 (C=C), 1230 (-OCH_3), 1168 (C-N), 1040 (N-N), 742(C-S-C). ^{1}H-NMR ($CDCl_3$), ´ in ppm. : 8.52 (S, 1H, NHCO), 8.24 (d, 1H=CH-Ar), 7.80-7.18 (m, 12H, Ar-H), 6.64 (d, 1H, COCH=), 4.30 (S, 2H, N-CH_2), 3.51 (S, 3H, Ar-OCH_3), MS : $[M]^+$ at m/z 472.58.

The other N-(5-((10H-Phenothiazin-10-yl)methyl)-1,3,4-thiadiazol-2-yl)-3-(Substitut- ed

Table 3: Spectral data of compounds (5b – 5j) & (6b – 6j)

Comp. No	IR (KBr) νmax. in cm^{-1}	^{1}H-NMR ($CDCl_3$) δ in ppm	$[M]^+$ at m/z
5b	3350 (N-H), 3030 (C-H aromatic), 2950 (C-H aliphatic), 2860 (C-H of $COCH_3$), 1725 (C=0), 1590 (C=N), 1575 (C....C of aromatic ring), 1485 (C=C), 1160 (C-N), 1040 (N-N), 725 (C-S-C), 1222(-OCH_3)	8.50 (S, 1H, NHCO, exchangeable with D_2O), 8.21 (d, 1H, CH-Ar), 7.90 – 7.15 (m, 12, Ar-H), 6.70 (d, 1H, COCH =), 4.35 (S, 2H, N-CH_2), 3.48 (S, 3H, Ar – OCH_3).	472
5c	3340 (N-H), 3022 (C-H aromatic), 2938 (C-H aliphatic), 2845 (C-H of $COCH_3$), 1717 (C=O), 1580 (C=N), 1564 (C....C of aromatic ring), 1470 (C=C), 1155 (C-N), 1028 (N-N), 716 (C-S-C), 755 (C-Cl)	8.43 (S, IH, NHCO, exchangeable with D_2O), 8.16 (d, IH, CH-Ar), 7.86-7.11 (m, 12H, Ar-H), 6.67 (d, 1H, COCH =), 4.29 (S, 2H, N-CH_2)	477
5d	3345 (N-H), 3025 (C-H aromatic), 2942 (C-H aliphatic), 2855 (C-H of $COCH_3$), 1720 (C=O), 1586 (C=N), 1570 (C....C)of aromatic ring), 1475 (C=C), 1156 (C-N), 1035 (N-N), 720 (C-S-C), 750 (C-Cl)	8.45 (S, IH, NHCO, exchangeable with D_2O), 8.18 (d, IH, CH-Ar), 7.85-7.10 (m, 12H, Ar-H), 6.65 (d, 1H, COCH=), 4.30 (S, 2H, N-CH_2).	477
5e	3330 (N-H), 3016 (C-H aromatic), 2930 (C-H aliphatic), 2840 (C-H of $COCH_3$), 1710 (C=O), 1575 (C=N), 1555 (C....C) of aromatic ring), 1460 (C=C), 1145 (C-N), 1020 (N-N), 715 (C-S-C), 735 (C-Cl)	8.32 (S, IH, NHCO, exchangeable with D_2O), 8.02 (d, IH, CH-Ar), 7.73 - 7.04 (m, 11H, Ar-H), 6.48 (d, 1H, COCH=), 4.16 (S, 2H, N-CH_2),	511
5f	3350 (N-H), 3325 (C-H aromatic), 2942 (C-H aliphatic), 2855 (C-H of $COCH_3$), 1725 (C=0), 1585 (C=N), 1575 (C....C)of aromatic ring), 1480 (C=C), 1155 (C-N), 1045 (N-N), 730 (C-S-C), 615 (C-Br)	8.41 (S, IH, NHCO, exchangeable with D_2O), 8.10 (d, IH, CH-Ar), 7.82 - 7.07 (m, 11H, Ar-H), 6.68 (d, 1H, COCH=), 4.27 (S, 2H, N-CH2)	521
5g	3355 (N-H), 3032 (C-H aromatic), 2950 (C-H aliphatic), 2860 (C-H of $COCH_3$), 1730 (C=O), 1595 (C=N), 1580 (C....C of aromatic ring), 1490 (C=C), 1160 (C-N), 1045 (N-N), 735 (C-S-C), 605 (C-Br)	8.40 (S, IH, NHCO, exchangeable with D_2O), 8.12 (d, IH, CH-Ar), 7.86 - 7.08 (m, 12H, Ar-H), 6.58 (d, 1H, COCH=), 4.24 (S, 2H, N-CH_2)	521
5h	3355 (N-H), 3020 (C-H aromatic), 2935 (C-H aliphatic), 2848 (C-H of $COCH_3$), 1715 (C=O), 1582 (C=N) 1565 (C....C of aromatic ring), 1470 (C=C), 1150 (C-N), 1030 (N-N), 720 (C-S-C), 590 (C-Br)	8.36 (S, IH, NHCO, exchangeable with D2O), 8.08 (d, IH, CH-Ar), 7.79 - 7.05 (m, 11H, Ar-H), 6.50 (d, 1H, COCH=), 4.21 (S, 2H, N-CH_2)	600
5i	3370 (N-H), 3050 (C-H aromatic), 2965 (C-H aliphatic), 2880 (C-H of $COCH_3$), 1750 (C=O), 1610 (C=N), 1595 (C....C of aromatic ring), 1515 (C=C), 1340 (N(CH3)2), 1180 (C=N), 1070 (N-N), 750 (C-S-C)	8.68 (S, IH, NHCO, exchangeable with D_2O), 8.36 (d, IH, CH-Ar), 7.95-7.24 (m, 12H, Ar-H), 6.88 (d, 1H, COCH=), 4.50 (S, 2H, N-CH_2) 2.20 [S, 6H, Ar-N (CH_3)$_2$]	485
5j	3360 (N-H), 3040 (C-H aromatic), 2962 (C-H aliphatic), 2875 (C-H of $COCH_3$), 1755 (C=O), 1602 (C=N), 1590	8.70 (S, IH, NHCO, exchangeable with D_2O), 8.35 (d, IH, CH-Ar), 7.92-7.21 (m, 12H, Ar-H), 6.90 (d, 1H, COCH=),	485

Table 3. Contd.....

	(C....C of aromatic ring) 1500 (C=C), 1330 ($N(CH_3)_2$), 1180 (C-N), 1065 (N-N), 745 (C-S-C)	4.47 (S, 2H, N-CH_2) 2.17 [S,6H,Ar–N $(CH_3)_2$]	
6b	3360 (N-H), 3045 (C-H aromatic), 2940 (C-H aliphatic), 2865 (C-H of $COCH_3$), 1725 (C=0), 1590 (C=N), 1245 (-OCH_3), 1165 (C-N), 725 (C-S-C)	7.84-7.10 (m, 12H, Ar-H), 6.63 (t, 1H, CH-Ar of Pyrazoline ring), 6.20 (brs, 1H, NH, exchangeable with D_2O), 5.40 (d, 2H, CH_2 of Pyrazoline ring), 4.30 (S, 2H, N – CH_2), 2.35 (S, 3H, $COCH_3$)	528
6c	3355 (N-H), 3050 (C-H aromatic), 2935 (C-H aliphatic), 2860 (C-H of $COCH_3$), 1730 (C=0), 1580 (C=N), 1162 (C-N), 750 (C-Cl), 718 (C-S-C)	7.75-7.08 (m, 12H, Ar-H), 6.60 (t, 1H, CH-Ar of Pyrazoline ring), 6.12 (brs, 1H, NH, exchangeable with D_2O), 5.35 (d, 2H, CH_2 of Pyrazoline ring), 4.24 (S, 2H, N-CH_2), 2.30 (S, 3H, $COCH_3$)	533
6d	3350 (N-H), 3040 (C-H aromatic), 2930 (C-H aliphatic), 2858 (C-H of $COCH_3$), 1725 (C=0), 1570 (C=N), 1160 (C-N), 715 (C-S-C), 755 (C-Cl)	7.72-7.05 (m, 12H, Ar-H), 6.57 (t, 1H, CH-Ar of Pyrazoline ring), 6.10 (brs, 1H, NH, exchangeable with D_2O), 5.31 (d, 2H, CH_2 of Pyrazoline ring), 4.25 (S, 2H, N-CH_2), 2.33 (S, 3H, $COCH_3$)	533
6e	3330 (N-H), 3025 (C-H aromatic), 2920 (C-H aliphatic), 2835 (C-H of $COCH_3$), 1710 (C=0), 1550 (C=N), 1148 (C-N), 705 (C-S-C), 735 (C-Cl)	7.60-7.01 (m, 11H, Ar – H), 6.35 (t, 1H, CH-Ar of Pyrazoline ring), 5.92 (brs, 1H, NH, exchangeable with D_2O), 5.12 (d, 2H, CH_2 of Pyrazoline ring), 4.11 (S, 2H, N-CH_2), 2.14 (S, 3H, $COCH_3$)	567
6f	3360 (N-H), 3045 (C-H aromatic), 2935 (C-H aliphatic), 2855 (C-H of $COCH_3$), 1735 (C=0), 1575 (C=N), 1170 (C-N), 715 (C-S-C), 610 (C-Br)	7.78-7.10 (m, 12H, Ar-H), 6.65 (t, 1H, CH-Ar of Pyrazoline ring), 6.15 (brs, 1H, NH, exchangeable with D_2O), 5.40 (d, 2H, CH_2 of Pyrazoline ring), 4.30 (S, 2H, N-CH_2), 2.36 (S, 3H, $COCH_3$)	577
6g	3355 (N-H), 3050 (C-H aromatic), 2940 (C-H aliphatic), 2855 (C-H of $COCH_3$), 1740 (C=0), 1570 (C=N), 1175 (C-N), 720 (C-S-C), 620 (C – Br)	7.80-7.10 (m, 12H, Ar-H), 6.68 (t, 1H, CH-Ar of Pyrazoline ring), 6.14 (brs, 1H, NH, exchangeable with D_2O), 5.43 (d, 2H, CH_2 of Pyrazoline ring), 4.34 (S, 2H, N-CH2), 2.40 (S, 3H, $COCH_3$)	577
6h	3340 (N-H), 3030 (C-H aromatic), 2925 (C-H aliphatic), 2838 (C-H of $COCH_3$), 1715 (C=0), 1550 (C=N), 1150 (C-N), 710 (C-S-C), 645 (C-Br)	7.70-7.06 (m, 11H, Ar-H), 6.42 (t, 1H, CH-Ar of Pyrazoline ring), 5.95 (brs, 1H, NH, exchangeable with D_2O), 5.18 (d, 2H, CH_2 of Pyrazoline ring), 4.16 (S, 2H, N – CH_2), 2.21 (S, 3H, $COCH_3$)	656
6i	3380 (N-H), 3070 (C-H aromatic), 2955 (C-H aliphatic), 2875 (C-H of $COCH_3$), 1745 (C=0), 1595 (C=N), 1345 [$N(CH_3)2$], 1180 (C-N), 745 (C-S-C)	7.95-7.20 (m, 12H, Ar-H), 6.80 (t, 1H, CH-Ar of Pyrazoline ring), 6.43 (brs, 1H, NH, exchangeable with D_2O), 5.52 (d, 2H, CH2 of Pyrazoline ring), 4.51 (S, 2H, N-CH2), 2.52 (S, 3H, $COCH_3$), 1.95 (S, 6H, Ar-N $(CH_3)2$)	541
6j	3385 (N-H), 3075 (C-H aromatic), 2955 (C-H aliphatic), 2885 (C-H of $COCH_3$), 1752 (C=0), 1590 (C=N), 1335 [$N(CH_3)2$], 1185 (C-N), 750 (C-S-C)	7.88-7.17 (m, 12H, Ar-H), 6.76 (t, 1H, CH-Ar of Pyrazoline ring), 6.45 (brs, 1H, NH, exchangeable with D_2O), 5.50 (d, 2H, CH2 of Pyrazoline ring), 4.55 (S, 2H, N-CH_2), 2.58 (S, 3H, $COCH_3$), 1.98 (S, 6H, Ar-N $(CH_3)2$)	541

Phenyl) acrylamide (5b-5j) were synthesized according to the above mentioned method. Their physical and analytical data are given in table-I while spectral data i.e, IR, ^{1}H-NMR and mass are given in Table-III.

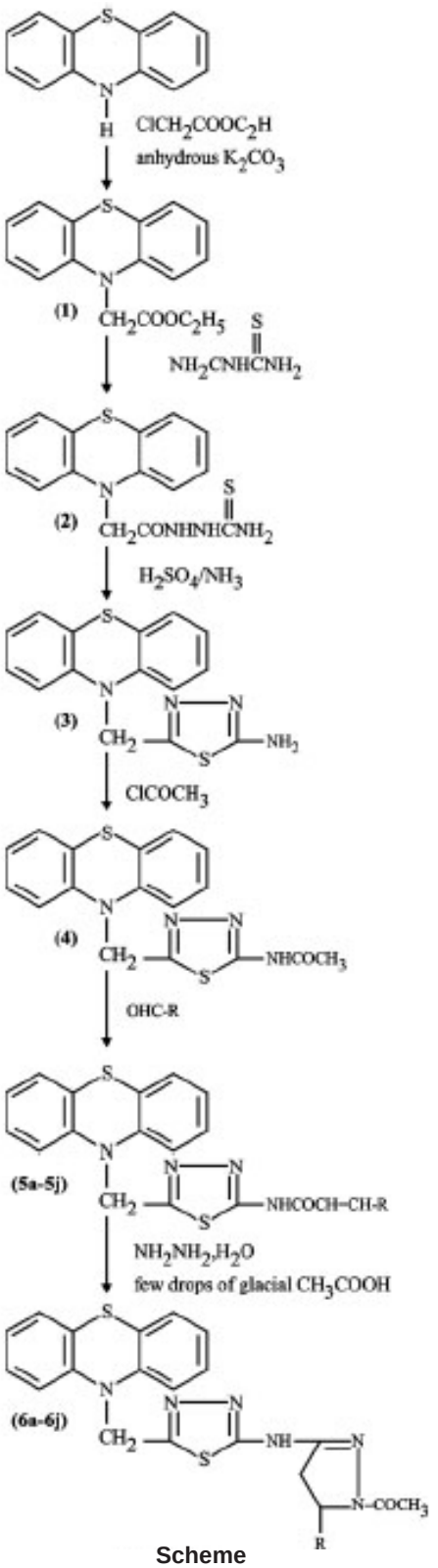
Synthesis of 1-(3-(5-((10H-Phenothiazin-10-yl)methyl)-1,3,4-thiadiazol-2-yl amino)-5-(2-meth-oxy Phenyl)-4-5-dihydro-1H-Pyrazol)-4-5-dihydro-1H-Pyrazol-1-yl) ethanone (6a)

To the solution of N-(5-((10H-Phenothiazin-10-yl) methyl) -1,3,4- thiadiazol-2-yl)-3-(2-methoxy phenyl) acrylamide (0.02 mole) in absolute ethanol (50 ml), hydrazine hydrate (99%, 0.04 mole) was added in the presence of few drops of glacial acetic acid. The reaction mixture was refluxed for 10h, distilled cooled and poured into ice-cold water. The separated solid was washed with petroleum ether at 50°^{C} and recrystallized from ethanol to yield compound (6a) m.p 206°, yield: 65%, mol. formula: $C_{26}H_{24}N_6O_2S_2$. Elemental analysis: Calcd. % C : 61.34, % H : 05.58, % N : 15.90 : Found : %C 61.55, % H : 05.50, % N : 16.00, IR (KBr) λ_{max}. in cm^{-1} : 3365 (N-H), 3050 (C-H aromatic), 2940 (C-H aliphatic), 2872 (C-H of $COCH_3$), 1725 (C=0), 1590 (C=N), 1560 (C...C aromatic), 1220 (-OCH_3), 1155 (C-N), 1045 (N-N), 725 (C-S-C) ^{1}H-NMR ($CDCl_3$) ´ in ppm : 7.80-7.15 (m, 12 H, Ar-H), 6.70 (t, 1H, CH-Ar of Pyrazoline ring), 6.28 (brs, 1H, NH exchangeable with D_2O), 5.42 (d, 2H, CH_2 of Pyrazoline ring) 4.25 (S, 2H, N-CH_2) 3.50 (S, 3H, Ar-OCH_3). 2.30 (S,3H, $COCH_3$). MS: $[M]^+$ at m/z 528.65

The Various other 1-(3-(5-((10H-Phenothiazin-10-yl) methyl)-1,3,4-thaidiazol-2-ylamino)-5-(Substituted Phenyl)-4,5-dihydro-1H Pyrazol-1-yl) ethanone (6b- 6j) have been synthesized in the similar manner. Their physical and analytical data are given in table-II while spectral date i.e. IR, ^{1}H-NMR and mass are given in Table 3.

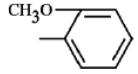
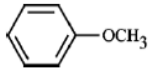
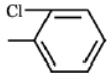
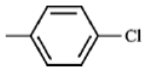
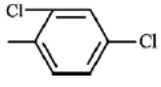
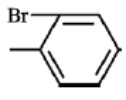
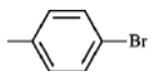
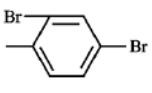
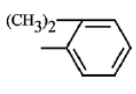
Pharmacology

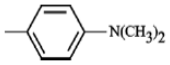
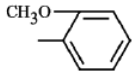
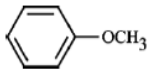
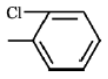
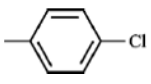
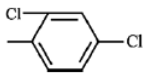
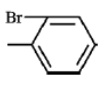
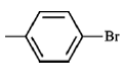
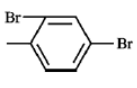
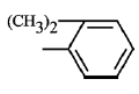
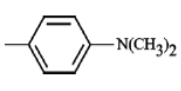
The experiments were performed on *albino* rats of *Charles Foster strain* of either sex of 70 to 95 days weighing 80-140 g, *albino* mice weighing 20-25 g. Pregnant female rats were excluded. These rats and mice were divided into different groups (control, standard and drug treated) of six animals each. The animals had access to food and water ad



Scheme

Table 5: Anti-inflammatory, analgesic ulcerogenic and toxicity data of compounds (5a – 5j) and (6a – 6j)

Comp.	R	Anti-inflammatory activity		Analgesic activity		Ulcerogenic	Acute To
		Dose (mg./kg p.o.)	% Inhibition of oedema	Dose (mg./kg p.o.)	% Protection (mg/kg i.p.)	activity UD_{50}	A L D_{50} (mg/kg p.o)
5a		50	20.50	50	18.52		> 1000
5b		50	16.56	50	15.80		> 1000
5c		50	16.85	50	14.57		> 1000
5d		50	22.66	50	19.43		> 1000
5e		25 50 100	28.30 45.68 66.15	25 50 100	16.37 35.86 58.18	145	> 1500
5f		50	13.73	50	11.56		> 1000
5g		50	19.82	50	15.50		> 1000
5h		50	25.47	50	22.82		> 1000
5i		50	12.18	50	10.30		> 1000

5j		50	9.67	50	08.92		> 1000
6a		50	31.12	50	28.57		> 1000
6b		50	30.35	50	27.26		> 1000
6c		50	27.92	50	25.50		> 1000
6d		50	34.10	50	29.66		> 1000
6e		25 50 100	29.45 48.63 71.36	25 50 100	26.88 45.15 63.67	168.5	> 1500
6f		50	25.77	50	23.40		> 1000
6g		50	32.10	50	30.48		> 1000
6h		50	33.64	50	29.16		> 1000
6i		50	28.40	50	25.52		> 1000
6j		50	26.57	50	23.18		> 1000
Phenylbutazone		25 50 100	26.20 44.52 63.75	25 50 100	14.26 32.50 54.58	66.70	

libitum. They were housed in rooms at 20-25 °C with 12 h light/dark cycle and relative humidity 50-60%. The test compounds and reference drug were dissolved in propylene glycol. phenylbutazone, a potent anti-inflammatory compound, was used as reference drug for comparison.

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

This study was done by following the procedure of Winter *et al*.[14]. 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 mn 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. 1h 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-

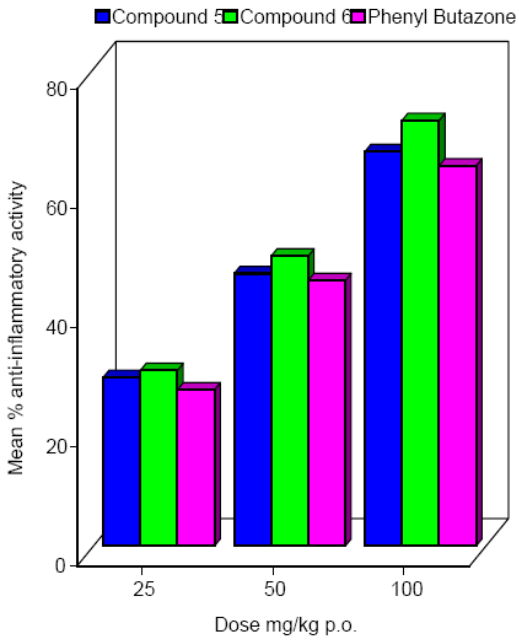


Fig. 1: The diagram showing mean % anti-inflammatc of the most potent compound 5e, 6e & reference d

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.

Analgesic activity

Following the method of Berkowitz *et al.*[15] performed this activity. This method is based on the property of the test compound to antagonize the phenyl quinone-induced pain syndrome in mice. Groups of five mice were injected intraperitonely with 0.25 ml of a 0.02% solution of phenylquinone in ethanol (5%) 1 h after of 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.

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

Ulcerogenic activity

Ulcerogenic labilities of newly synthesized compounds were checked with method of verma *et al.,* [13]. Albino rats were fasted for 24 h prior to drug administration. All animals 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 with out perforation. The presence of any one of these criteria was considered to be an evidence of ulcerogenic activity.

Acute Toxicity study

The test compounds were investigated for their acute toxicity (ALD_{50}) in albino mice, according to the method of Smith [12]. 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.

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