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Synthesis and anti-inflammatory activity of some heterocyclic derivatives of phenothiazine

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Some new 10-{[5′-amino-(1″-acetyl-5″-substituted aryl-2″-pyrazolin-3″-yl)-1′,3′,4′-thiadiazol-2′-yl] methyl}-phenothiazines **(11–16)** and 10-{[5′-amino-(1″-acetyl-5″-substituted aryl-2″-pyrazolin-3″-yl)-1′,3′,4′-oxadiazol-2′-yl]methyl}phenothiazines **(26–31)** have been synthesized from 10-{[5′-substituted benzylideneacetylamino-(1′,3′,4′-thiadiazol-2′-yl)]methyl}phenothiazines **(5–10)** and 10-{[5′-substituted benzylideneacetylamino-(1′,3′,4′-oxadiazol-2′-yl)]methyl}phenothiazines **(20–25)**, respectively. All these compounds of the present series have been screened *in vivo* for their anti-inflammatory and acute toxicity. Compounds **16** and **31** were found to be potent members of the present series, which showed 46.2% and 48.0% anti-inflammatory activity, respectively, at a dose of 50 mg/kg p.o., while standard drug, phenylbutazone, exhibited 44.52% anti-inflammatory activity at same dose. However, 10-{[5′-amino-(1″-acetyl-5″-(*o*-methoxyphenyl)-2″-pyrazolin-3″-yl)-1′,3′,4′-oxadiazol-2′-yl]methyl}phenothiazine (**31**) was found to be most active and less ulcerogenic compound of this series. The structure of these compounds have been elucidated by IR, ^{1}H NMR, mass spectroscopy and elemental analysis.

1. Introduction

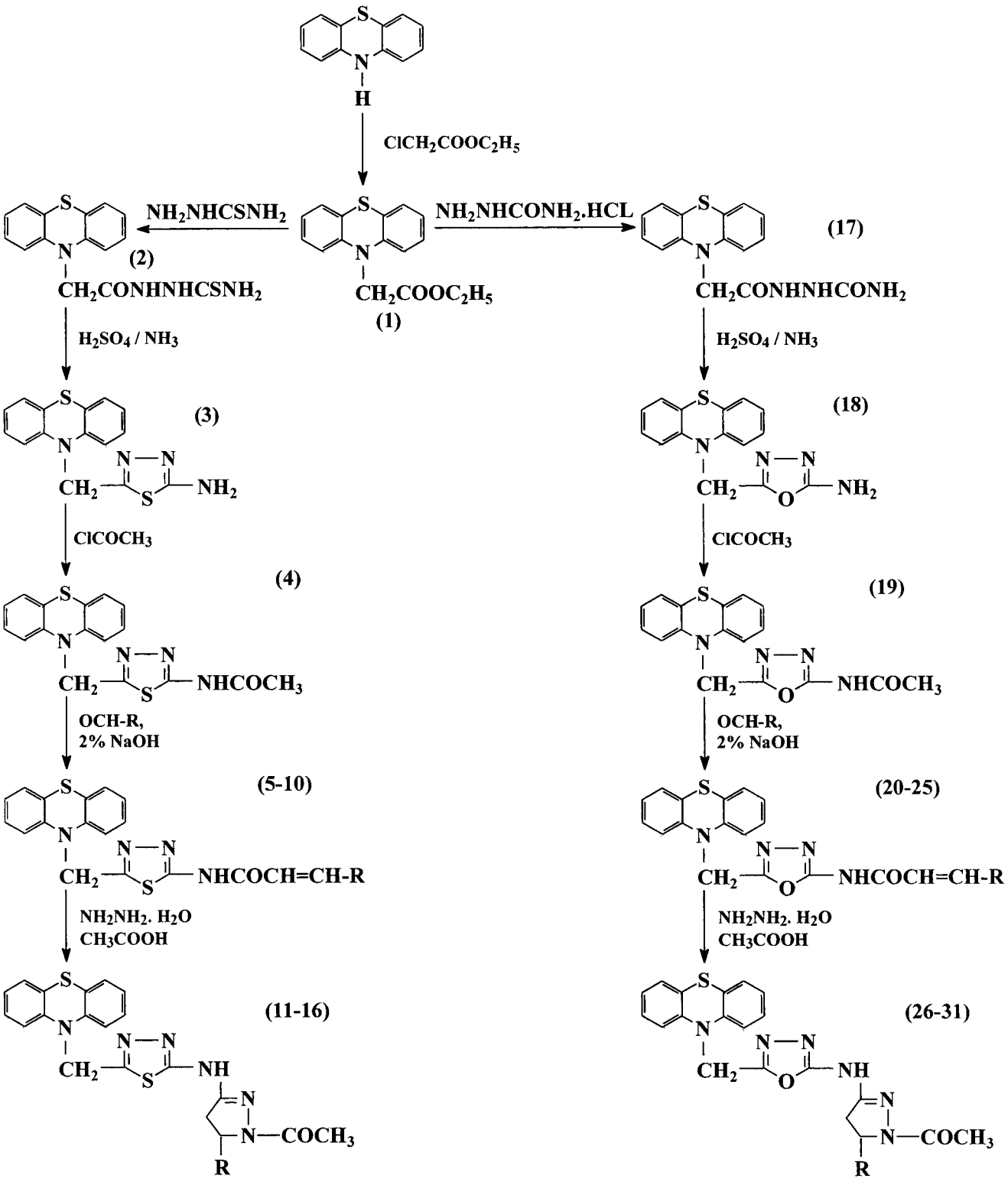
Phenothiazine derivatives have been reported to show a broad spectrum of biological activities. These include anti-inflammatory (Bansal and Kumar 1999; Kumar et al. 1998; Mishra et al. 1997), anti-psychotic (Baldessarini 2001), cardiovascular (Kumar et al. 1983), fungicidal (Jain and Srivastava 1994) activities etc. Furthermore, derivatives of pyrazoline (Udupi et al. 1998; Mann et al. 1992), 1,3,4-oxadiazole (Omar et al. 1996; Nargund et al. 1994) and 1,3,4-thiadiazole (Srivastava et al. 1999; Rani et al. 1990) have also been found to possess promising anti-inflammatory activity. In view of these observations, we thought that it would be interesting to synthesize new 10-{[5′-amino-(1″-acetyl-5″-substituted aryl-2″-pyrazolin-3″-yl)-1′,3′,4′-thiadiazol-2′-yl]methyl} phenothiazines **(11–16)** and 10-{[5′-amino-(1″-acetyl-5″-substituted aryl-2″-pyrazolin-3″-yl)-1′,3′,4′-oxadiazol-2′-yl]methyl}phenothiazines **(26–31)** by incorporating thiadiazolyl, oxadiazolyl and pyrazolinyl moieties at 10-position of the phenothiazine nucleus, and these compounds were evaluated for anti-inflammatory activity and acute toxicity. Their structural assignments were based on elemental analysis and spectral data.

2. Investigations and results

The reaction sequence leading to the formation of different phenothiazine derivatives is outlined in the Scheme. The reaction of phenothiazine with ethyl chloroacetate yielded the desired ethylphenothiazine-10-acetate (**1**), which was converted into 10-(thiosemicar-bazidoacetyl)-phenothiazine (**2**) and 10-(semicarbazidoacetyl)phenothiazine (**17**) on treatment with thiosemicarbazide and semicarbazide hydrochloride, respectively. Compounds **2** and **17** on dehydration with concentrated sulfuric acid afforded 10-{[5′-amino-(1′,3′,4′-thiadiazol-2′-yl)]methyl}phenothiazine (**3**) and 10-{[5′-amino-(1′,3′,4′-oxadiazol-2′-yl)]methyl}phenothiazine (**18**), respectively. Compounds **3** and **18** on acetylation with acetyl chloride furnished 10-{[5′-acetylamino-(1′,3′,4′-thiadiazol-2′-yl)]methyl}phenothiazine (**4**) and 10-{[5′-acetylamino-(1′,3′,4′-oxadiazol-2′-yl)]methyl}phenothiazine (**19**), respectively. Compounds **4** and **19**, when treated with various aromatic aldehydes, separately, resulted in the formation of 10-{[5′-substituted benzylideneacetylamino-(1′,3′,4′-thiadiazol-2′-yl)]methyl}-phenothiazines (**5–10**) and 10-{[5′-substituted benzylideneacetylamino-(1′,3′,4′-oxadiazol-2′-yl)]methyl} phenothiazines (**20–25**), respectively. Finally, these compounds were cyclized to give 10-{[5′-amino-(1″-acetyl-5″-substituted aryl-2″-pyrazolin-3″-yl)-1′,3′,4′-thiadiazol-2′-yl]methyl}phenothiazines (**11–16**) and 10-{[5′-amino-(1″-acetyl-5″-substituted aryl-2″-pyrazolin-3″-yl)-1′,3′,4′-oxadiazol-2′-yl]methyl}phenothiazines (**26–31**), respectively.

All the pharmacological results of the present study are shown in Tables 1 and 2. Screening of compounds **5–16** and **20–31** and the reference drug, phenylbutazone, was performed at 50 mg/kg p.o. for anti-inflammatory activity. The two test compounds **16** and **31** were found to possess almost the same anti-inflammatory activity (46.2% and 48.0%, respectively) at 50 mg/kg p.o. in comparison to the reference drug, which showed 44.52% of inhibition of oe-

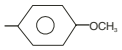
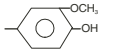
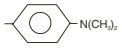
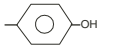
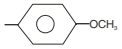
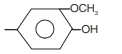
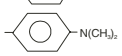
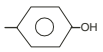
Scheme 1



dema at the same dose. Furthermore, these two test compounds and phenylbutazone were subjected to screening for anti-inflammatory activity at doses of 25, 100 mg/kg p.o. These two compounds showed better anti-inflammatory activity at all three tested doses than the reference drug. However, compound **31** exhibited maximum anti-inflammatory activity. The Fig. 1 illustrates the anti-inflammatory activity of compounds **16**, **31** and phenylbutazone.

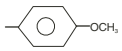
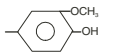
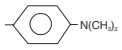
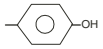
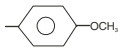
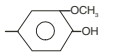
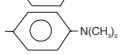
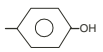
These two most active compounds **16**, **31** and the reference drug were also evaluated for ulcerogenic liabilty and UD_{50} values were 99.9, 168.5 and 66.6 mg/kg. i.p., respec-

Table 1: Physical, analytical and biological data of compounds 5–16

Compd.	R	M.p. °C	Yield %	Recrystallization Solvent	Molecular Formula [a]	Dose (mg/kg p.o)	% Anti-inflam-matory acivity	Ulcerogenic activity (UD_{50}mg/kgi.p.)	Acute toxicity (ALD_{50} mg/kg p.o.)
5		138	55	acetic acid-water	$C_{24}H_{18}ON_4S_2$	50	24.5*	–	> 800
6		162	50	ethanol-water	$C_{25}H_{20}O_2N_4\ S_2$	50	33.4*	–	> 800
7		125	44	DMF	$C_{25}H_{20}O_3N_4\ S_2$	50	30.0**	–	> 800
8		134	60	acetone	$C_{26}H_{23}ON_5\ S_2$	50	22.3*	–	> 800
9		118	52	benzene	$C_{24}H_{18}O_2N_4\ S_2$	50	25.3*	–	> 800
10		150	48	DMF	$C_{25}H_{20}O_2N_4\ S_2$	50	36.7**	–	> 800
11		195	48	methanol-water	$C_{26}H_{22}ON_6\ S_2$	50	26.7*	–	> 800
12		205	50	ethanol-water	$C_{27}H_{24}O_2N_6\ S_2$	50	38.7**	–	> 800
13		170	45	acetic acid-water	$C_{27}H_{24}O_3N_6\ S_2$	50	41.8**	–	> 800
14		190	40	DMF	$C_{28}H_{27}ON_7\ S_2$	50	28.3*	–	> 800
15		182	60	acetone	$C_{26}H_{22}O_2N_6\ S_2$	50	32.7**	–	> 800
16		210	45	methanol-water	$C_{27}H_{24}O_2N_6\ S_2$	25 50 100	27.80* 46.2** 66.5***	99.9	> 800

[a] C, H, N were found within ± 0.4% of theoretical value
*P < 0.05; **P < 0.01; ***P < 0.001

Table 2: Physical, analytical and biological data of compounds 20–31

Compd.	R	M.p. °C	Yield %	Recrystallization Solvent	Molecular Formula [a]	Dose (mg/kg p.o)	% Anti-inflam matory acivity	Ulcerogenic activity (UD_{50}mg/kgi.p.)	Acute toxicity (ALD_{50} mg/kg p.o.)
20		110	65	methanol-water	$C_{24}H_{18}O_2N_4S$	50	26.3*	–	> 800
21		80	60	ethanol-water	$C_{25}H_{20}O_3N_4\ S$	50	35.2**	–	> 800
22		100	50	DMF	$C_{25}H_{20}O_4N_4\ S$	50	33.82*	–	> 800
23		85	45	acetic acid	$C_{26}H_{23}O_2N_5\ S$	50	25.9*	–	> 800
24		95	48	methanol-water	$C_{24}H_{18}O_3N_4\ S$	50	27.5**	–	> 800
25		102	55	methanol-water	$C_{25}H_{20}O_3N_4\ S$	50	38.7**	–	> 800
26		115	48	DMF	$C_{26}H_{22}O_2N_6\ S$	50	27.8*	–	> 800
27		160	55	methanol-water	$C_{27}H_{24}O_3N_6\ S$	50	42.32*	–	> 800
28		82	60	acetic acid	$C_{27}H_{24}O_4N_6\ S$	50	40.55**	–	> 800
29		120	52	ethanol-water	$C_{28}H_{27}O_2N_7\ S$	50	30.63*	–	> 800
30		132	55	ethanol-water	$C_{26}H_{22}O_3N_6\ S$	50	35.2*	–	> 800
31		112	45	methanol-water	$C_{27}H_{24}O_3N_6\ S$	25 50 100	29.74* 48.0*** 68.2**	168.5	> 1600
Phenylbutazone		–	–	–	–	25 50 100	26.5** 44.52* 63.7**	66.6	–

[a] C, H, N were found within ± 0.4% of theoretical value
*P < 0.05; **P < 0.01; ***P < 0.001

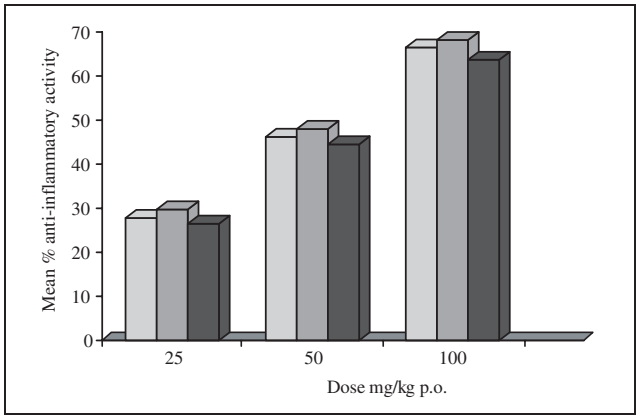


Fig.: Mean% anti-inflammatory activity of most potent compounds **16, 31** and reference drug, phenylbutazone at three doses
Compound 16
Compound 31
Phenylbutazone

tively. All the compounds of this series showed LD_{50} values > 800 mg/kg p.o., with a maximum LD_{50} > 1600 mg/kg p.o. exhibited by compound **31**.

3. Discussion

The majority of the compounds synthesized exhibited statistically significant anti-inflammatory activity ranging from 22.3% to 48.0%. Structure activity relationship of these compounds revealed that conversion of different substituted benzylidenes (**5**–**10** and **20**–**25**) into their corresponding pyrazoline congeners (**11**–**16** and **26**–**31**) markedly enhanced the anti-inflammatory activity. It is clear from the results, which are given in Table 1 and Table 2, that when the compounds **5, 20, 11** and **26** were substituted with a phenyl group a minimum percentage of inhibition of oedema (24.5%, 26.3%, 26.7 and 27.8%, respectively) was seen. It is interesting to point out that the compounds having an *ortho*- or *meta*- or *para*-methoxyphenyl group as a substituent, elicited a remarkable increase in anti-inflammatory activity. Moreover, it has also been observed that ortho-derivatives (**10, 25, 16** and **31**) exhibited more potent anti-inflammatory activity (36.7%, 38.7%, 46.2%, and 48.0%, respectively) than *meta*- and *para*-isomers. Compounds **16** (46.2%) and **31** (48.0%) were found to be equipotent as compared to the reference drug, phenylbutazone (44.52%). These two compounds are substituted with an *ortho*-methoxyphenyl at 5-position of the pyrazolinyl moiety. Hence, it seems that substitution with an *ortho*-methoxyphenyl group at 5-position of the pyrazoline ring (**10, 25, 16 and 31**) is beneficial for anti-inflammatory activity. Moreover, compounds **16** and **31** exhibited less ulcerogenic liability as compared to phenylbutazone.
Therefore, it may be concluded that

a) the differently substituted benzylidenes (**5**–**10** and **20**–**25**) shows mild to moderate anti-inflammatory activity. Cyclisation of these benzylidene congeners (**5**–**10** and **20**–**25**) into their corresponding pyrazoline congeners (**11**–**16** and **26**–**31**) enhances the anti-inflammatory property.
b) compounds with a phenyl group having a methoxy group at either *ortho* or *meta* or *para*-position show promising inflammation inhibiting activity. *Ortho* derivatives possess the better anti-inflammatory properties.
c) phenothiazino-oxadiazolyl-pyrazolines (**26**–**31**) are more active anti-inflammatory compounds than phenothiazino-thiadiazolyl-pyrazolines (**11**–**16**).

4. Experimental

4.1. Chemistry

Melting points of newly synthesized compounds were determined in open capillaries with a thermonic melting point apparatus and are uncorrected. The purity of the newly synthesized compounds was determined by TLC on silica gel-G, eluent was a mixture of methanol-benzene in different proportions and spots were located by iodine. The structure of these compounds were confirmed by IR, ^{1}H NMR, mass and elemental analysis. The IR (KBr) spectra were recorded on Paragon 500 FTIR and ν in cm^{-1}. The ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Brucker DRX-300 FTNMR instrument and chemical shif (δ) is expressed in ppm. Tetramethylsilane (TMS) was used as internal reference standard. Mass spectra were recorded on a Jeol D-300 spectrometer. Analytical data of C, H, N were found within ± 0.4% of theoretical values. The physical and analytical data of compounds are given in Table 1 and Table 2. The required compounds, ethylphenothiazine-10-acetate (**1**), 10-(thiosemicarbazidoacetyl) phenothiazine (**2**) and 10-{[5′-amino-(1′,3′,4′-thiadiazol-2′-yl)]methyl}phenothiazine (**3**) were prepared by a known method Rawat and Srivastava 1998.

4.1.1. 10-{[5′-Acetylamino-(1′,3′,4′-thiadiazol-2′-yl)]methyl}phenothiazine (**4**)

To a solution of compound **3** i.e. 10-{[5′-amino-(1′,3′,4′-thiadiazol)]methyl}phenothiazine (0.02 mol) in dry chloroform (50 ml), acetyl chloride (0.02 mol) was added dropwise at 0–5 °C under constant stirring. The reaction mixture was further stirred for 2 h at room temperature. Furthermore, it was refluxed for 4 h and an excess of solvent was removed by distillation. The product thus obtained was washed with cold water and recrystallized from methanol-water. Compound **4**: m.p. 160 °C; yield 60%; IR (KBr) ν (cm^{-1}): 3345 (N–H), 3020 (C–H aromatic), 2933 (C–H aliphatic), 1715 (C=0), 1610 (C=N), 1160 (C–N), 715 (C–S–C); ^{1}H NMR ($CDCl_3$) (δ ppm.): 7.70–7.20 (m, 8 H, Ar-*H*), 8.50 (s, 1 H, N*H*CO), 4.30 (s, 2 H, N–C*H*$_2$), 2.20 (s, 3 H, COC*H*$_3$).
$C_{17}H_{14}ON_4S_2$

4.1.2. 10-{[5′-Substituted benzylideneacetylamino-(1′,3′,4′-thiadiazol-2′-yl)]methyl}phenothiazines (**5**–**10**)

A solution of compound **4** (0.02 mol) in absolute ethanol (50 ml) with different aromatic aldehydes (0.02 mol) in the presence of 2% NaOH was refluxed, separately, for 10 h. Then, the reaction mixtures were concentrated, cooled, poured over crushed ice, filtered and recrystallized from suitable solvents. The physical and analytical data of compounds **5**–**10** are shown in Table 1. Compound **6**: m.p. 150 °C; yield 48%; IR (KBr) ν (cm^{-1}): 3345 (N–H), 3025 (C–H aromatic), 2950 (C–H aliphatic), 1720 (C=0), 1590 (C=N), 1572 (C⎓C of aromatic ring), 1475 (C=C), 1160 (C–N), 1035 (N–N), 715 (C–S–C); ^{1}H NMR ($CDCl_3$) (δ ppm.): 7.85–7.12 (m, 12 H, Ar-*H*), 8.45 (s, 1 H, N*H*CO), 4.30 (s, 2 H, N–C*H*$_2$), 3.45 (s, 3 H, Ar-OC*H*$_3$), 6.70 (d, 1 H, COC*H*), 8.15 (d, 1 H, C*H*-Ar); MS: $[M]^+$ m/z 472.
$C_{25}H_{20}O_2N_4S_2$

4.1.3. 10-{[5′-Amino-(1′′-acetyl-5′′-substituted aryl-2′′-pyrazolin-3′′-yl)-1′,3′,4′-thiadiazol-2′-yl]met hyl}phenothiazines (**11**–**16**)

To the solutions of different substituted benzylidenes (**5**–**10,** 0.02 mol) in absolute ethanol (50 ml), hydrazine hydrate (99%, 0.04 mol) was added in the presence of few drops of glacial acetic acid. Then, the reaction mixtures were refluxed for 12 h, distilled, cooled and poured into cold water. The separated solids were washed with petroleum ether (40–60 °C) and recrystallized from suitable solvents. The physical and analytical data of compounds **11**–**16** are given in Table 1. Compound **16**: m.p. 210 °C; yield 45%; IR (KBr) ν (cm^{-1}): 3355 (N–H); 3038 (C–H aromatic), 2940 (C–H aliphatic), 2860 (C–H of $COCH_3$), 1715 (C=O), 1592 (C=N), 720 (C–S–C), 1155 (C–N); ^{1}H NMR ($CDCl_3$) (δ ppm.): 7.80–7.05 (m, 12 H, Ar-*H*), 6.22 (brs, 1 H, N*H*), 4.30 (s, 2 H, N–C*H*$_2$), 3.35 (s, 3H, Ar-OC*H*$_3$), 2.35 (s, 3 H, COC*H*$_3$), 5.35 (d, 2 H, C*H*$_2$ of pyrazoline ring), 6.60 (t, 1 H, C*H*-Ar of pyrazoline ring); MS: $[M]^+$ m/z 528.
$C_{27}H_{24}O_2N_6S_2$

4.1.4. 10-(Semicarbazidoacetyl)phenothiazine (**17**)

Compound **1** (0.01 mol) and semicabazide hydrochloride (0.01 mol) in ethanol (60 ml) were heated under reflux in the presence of anhydrous NaOH (4.0 g) for 10 h. The ethanol was distilled off and the viscous mass was poured onto crushed ice, filtered and washed several times with water and finally recrystallized from methanol to afford compound **17:** m.p. 165 °C; yield 72%; IR (KBr) ν (cm^{-1}): 3355 (NH_2), 3150 (N–H), 3048 (C–H aromatic), 1200 (C–N), 1675 (CONH), 1575 (C⎓C of aromatic ring); ^{1}H NMR ($CDCl_3$) (δ ppm.): 7.60–7.10 (m, 8 H, Ar-H), 8.35 (m, 4 H, N*H*NHCON*H*$_2$), 4.30 (s, 2 H, N–CH_2).
$C_{15}H_{14}O_2N_4S$

4.1.5. 10-{[5′-Amino-(1′,3′,4′-oxadiazol-2′-yl)]methyl}phenothiazine (**18**)

A mixture of 10-(semicarbazidoacetyl)phenothiazine (**17**, 0.05 mol) and concentrated sulphuric acid (15 ml) was allowed to stand for 24 h at room temperature and then the reaction mixture was poured over ice-water and neutralized with NH_4OH. The product was filtered and washed with cold water. This solid product was recrystallized from methanol-water to give compound **18**: m.p. 152 °C; yield 65%; IR (KBr) ν (cm^{-1}): 3330 (NH_2), 3025 (C−H aromatic), 2910 (C−H aliphatic), 1605 (C⋯C of aromatic ring), 1590 (C=N); ^{1}H NMR ($CDCl_3$) (δ ppm.): 7.62–7.15 (m, 8 H, Ar-*H*), 6.20 (brs, 2 H, N*H*$_2$), 4.35 (s, 2 H, N−C*H*$_2$).
$C_{15}H_{12}ON_4S$

4.1.6. 10-{[5′-Acetylamino-(1′,3′,4′-oxadiazol-2′-yl)]methyl}phenothiazine (**19**)

Acetyl chloride (0.02 mole) was added to a solution of compound **18** (0.02 mole) in dry benzene (50 ml) dropwise at 0–5 °C under constant stirring. The reaction mixture was further stirred for 2 h at room temperature and refluxed for 4 h. Then, benzene was distilled off. The solid thus obtained was washed with cold-water and recrystallized from ethanol-water. Compound **19**: m.p. 175 °C; yield 62%; IR (KBr) ν (cm^{-1}): 3345 (N−H), 3050 (C−H aromatic), 2920 (C−H aliphatic), 2845 (C−H of $COCH_3$), 1700 (C=O), 675 (C−S−C), 1075 (C−O−C); ^{1}H NMR ($CDCl_3$) (δ ppm.): 7.65–7.10 (m, 8 H, Ar-*H*), 8.45 (brs, 1 H, N*H*CO), 4.40 (s, 2 H, N−C*H*$_2$), 2.25 (s, 3 H, COC*H*$_3$).
$C_{17}H_{14}O_2N_4S$

4.1.7. 10-{[5′-Substituted benzylideneacetylamino-(1′,3′,4′-oxadiazol-2′-yl)]methyl}phenothiazines (**20**–**25**)

A solution of compound **19** (0.02 mol) in methanol (50 ml) with different aromatic aldehydes (0.02 mol) in the presence of 2% NaOH was refluxed, separately, for 10 h, then concentrated, cooled poured over crushed ice, filtered and recrystallized from suitable solvents. The physical and analytical data of compounds **20**–**25** are given in Table 2. Compound **21**: m.p. 102 °C; yield 55%; IR (KBr) ν (cm^{-1}): 3333 (N−H), 3010 (C−H aromatic), 2928 (C−H aliphatic), 1710 (C=O), 1590 (C=N), 1565 (C⋯C of aromatic ring), 1130 (C−N), 1040 (N−N), 1100 (C−O−C), ^{1}H NMR ($CDCl_3$) (δ ppm.): 7.90–7.15 (m, 12 H, Ar-*H*), 8.50 (brs, 1 H, N*H*CO), 4.40 (s, 2 H, N−C*H*$_2$), 3.45 (s, 3 H, Ar-OC*H*$_3$), 6.80 (d, 1 H, COC*H*), 8.20 (d, 1 H, C*H*-Ar); MS: $[M]^+$ m/z 456.
$C_{25}H_{20}O_3N_4S$

4.1.8. 10-{[5′-Amino-(1″-acetyl-5″-substituted aryl-2″-pyrazolin-3″-yl)-1′,3′,4′-oxadiazol-2′-yl] methyl} phenothiazines (**26**–**31**)

To the solutions of different substituted benzylidenes (**20**–**25,** 0.02 mol) in absolute ethanol (50 ml), hydrazine hydrate (99%, 0.04 mol) was added in the presence of few drops of glacial acetic acid, and the reaction mixtures were heated under reflux for 12 h. The purity of these compounds was checked by TLC. The physical and analytical data of compounds **26**–**31** are shown in Table 2. Compound **31**: m.p. 210 °C; yield 45%; IR (KBr) ν (cm^{-1}): 3338 (N−H), 3020 (C−H aromatic), 2925 (C−H aliphatic), 2855 (C−H of $COCH_3$), 1710 (C=O), 1590 (C=N), 1560 (C⋯C of aromatic ring), 1110 (C−O−C), 1160 (C−N), 1030 (N−N); ^{1}H NMR ($CDCl_3$) (δ ppm.): 7.85–7.12 (m, 12 H, Ar-*H*), 6.20 (brs, 1 H, N*H*), 4.55 (s, 2 H, N−C*H*$_2$), 3.30 (s, 3 H, Ar-OC*H*$_3$), 2.40 (s, 3 H, COC*H*$_3$), 5.30 (d, 2 H, C*H*$_2$ of pyrazoline ring), 6.82 (t, 1 H, C*H*-Ar of pyrazoline ring); MS: $[M]^+$ m/z 512.
$C_{27}H_{24}O_3N_6S$

4.2. 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 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.

4.2.1. Acute toxicity

The acute toxicity was determined in albino mice. The test compounds were administered orally at different dose levels in separate groups of animals. After 24 h of drug administration the percent mortality in each group was observed. From the data obtained, the approximate lethal dose (LD_{50}) was calculated by the method of Smith (1960).

4.2.2. Anti-inflammatory activity

This study was done according to the method of Winter et al. (1962). A freshly prepared suspension of carrageenan (1% in 0.9% saline) 0.05 ml was injected under the planter aponeurosis of right hind paw of the rats. One group was kept as control and treated with propylene glycol. The standard drug group and test drug groups pretreated with phenylbutazone and test compound, respectively, 1 h prior to carrageenan challenge. All the drugs were administered orally. The volume of foot was measured before, 1 and 3 h after carrageenan treatment by means of a plethysmometer. The percent anti-inflammatory activity was calculated according to formula given below:

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

Where, V_t and V_c are the volumes of oedema in drug treated and the control groups respectively.

4.2.3. Ulcerogenic activity

This activity was determined according to the method of Verma et al. (1981). In this method, adult albino rats of either sex, fasted for 24 h prior to the administration of test compounds and standard drugs were divided into groups of 6 animals each. The test compounds and standard drugs were given interaperitoneally and the animals were sacrificed 8 h after drugs treatment. The stomach, duodenum and jejunum were removed and examined with a hand lens for any evidence of (a) shedding of epithelium (b) petechial and frank hemorrhages and (c) erosion or discrete ulceration with or without perforation. The presence of any one of these criteria was considered to be an evidence of ulcerogenic activity.

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