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***Research Article** (Open Access)*

SYNTHESIS AND CHARACTERIZATION OF SOME NEW ANTHRANILIC ACID DERIVATIVES AS POTENTIAL NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

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ABSTRACT
Newly substituted thiadiazole pyrazolene anthranilic acid derivatives were synthesized. These compounds also evaluated for their anti-inflammatory and analgesic activities. Compound 5e, i.e., 2-((5-(3-(2,4-Dichloro) acrylamido)-1,3,4-thiadiazol-2-yl) methyl amino) benzoic acid and 6e, i.e., of 2-((5-(1-Acetyl-5-(2,4-Dichchloro)-4,5-dihydro-1H-pyrazol-3-ylamino)-1,3,4-thiadiazol-2-yl) methyl amino) benzoic acid were found to be most active compounds of this series, which exhibits 43.98 & 51.22% anti-inflammatory activity while, 40.82 & 45.63 % analgesic activity. The structures of all the newly synthesized compounds were characterized by analytical data, IR, ^{1}H-NMR and Mass spectrometry.

Keywords: *Thiadiazols; Thiadiazole Pyrazolene Anthranilic Acid; NSAIDs & Analgesic Activities*

INTRODUCTION
The fenamates, (Flower, 1985; Verma, 1994; Dubey, 2006), is a family of non-steroidal anti-inflammatory drugs (NSAIDs), which are derivatives of N-phenyl-anthranilic acid. They include mefenamic, meclofenamic, flufenamic tolfenamic and etofenamic acid etc., which are useful agents for clinical treatment of inflammatory disorders. The fenamates have anti-inflammatory, antipyretic and analgesic properties, (Kumar, 2010, Singh, 2011, Kumar, 2011 & Kumar, 2014). Considerable amount of work has been done on structural variation of this sub-class of NSAIDs. They appear to owe these properties primarily to their capacity to inhibit cyclo-oxygenase. It has been observed that the best known NSAIDs are acidic in nature. Substitution pattern at N-position of anthranilic acid play a pivotal role in delineating the anti-inflammatory activity of these agents.

Many fenamate compounds have gained the medicinal importance in the recent years. Among these, thiadiazoles has been the most potent ones. Furthermore, substitution pattern in thiadiazole nucleus plays a pivotal role in delineating the biological activities like anti-inflammatory, anticonvulsant and analgesics.

Pyrazolines (Kumar, 2017, Bansal, 2001) is the most important representatives of hydrazine in both the synthetic and theoretical respect. Compounds of these classes are widely used as potent analgesic and anti-inflammatory activities. They act by inhibiting both cycloxygenase-1 and cycloxygenase-2 enzymes.

Moreover, pyrazoline nucleuses gain medicinal importance because incorporation of this moiety into different heterocyclic nuclei markedly enhances the anti-inflammatory.

In the light of the above report and also in continuation of our experimental work on chemo selective reaction of anthranilic acid derivatives, a new drug strategy has been planned to synthesize several new substituted Anthranilic acid derivatives possessing thiadiazole and pyrazolene moieties with the hope to possess better anti-inflammatory and analgesic activities. All the newly compounds have been screened for their anti-inflammatory, analgesic, alcerogenic and toxicity activities.

MATERIALS AND METHODS

The melting points were determined in open capillaries with the help of thermionic 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 proportions. 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, $\nu_{max.}$ 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 (δ) in ppm. Tetra methyl Silane (TMS) was used as internal reference standard. Elemental analyses were 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-I and table-II.

2-(2-Ethoxy-2-oxo ethyl amino) benzoic acid (1)

A mixture of anthranilic acid (2-amino benzoic acid) (0.l mole), ethyl chloro acetate (0.1 mole) and anhydrous K_2CO_3 (5.0 gm) in acetone (80 ml.) was refluxed for 12hr. on a steam bath. The excess of solvent was distilled off under reduced pressure, and the resulting solid mass was poured into ice-cold water, filtered. The separated solid was recrystallized from methanol-water to give compound (1)

Compound (1): M.P: 115ºC, yield: 55%, mol. formula: $C_{11}H_{13}O_4N$

Elemental analysis;

% C	:	Calcd.	:	59.19	:	Found	: 59.32
% H	:	Calcd.	:	05.87	:	Found	: 05.85
% N	:	Calcd.	:	06.27	:	Found	: 06.30

Spectral analysis:

IR (KBr) $\nu_{max.}$ in cm^{-1} : 3500 (O-H), 3150 (N-H), 3040 (C-H aromatic), 2935 (CH_2), 1725 (C=0), 1590 (C⎓C of aromatic ring).

^{1}H-NMR ($CDCl_3$) (δ) in ppm: 12.43 (s, 1H, -COOH exchangeable with D_2O), 7.52-7.25 (m, 4H, Ar-H), 5.88 (s, 1H, NH, exchangeable with D_2O), 4.65 (s, 2H, N-CH_2), 4.22 (q, 2H,-$COOCH_2CH_3$), 1.35 (t, 3H,-$COOCH_2CH_3$).

MS: $[M]^+$ at m/z 223

2-(2-(2-Carbamothioyl hydrazinyl)-2-oxo ethylamino) benzoic acid (2)

A mixture of 2-(2-Ethoxy-2-oxo ethyl amino) benzoic acid (0.02 mole.) and thiosemicarbazide (0.02 mole.) in methanol (50 ml.) was refluxed for 8hr. The excess of solvent was removed under reduced pressure and the viscous mass poured over ice-water, filtered and recrystallized from methanol-water to afford compound (2)

Compound (2): M.P: 126ºC, yield: 78%, mol. formula: $C_{10}H_{12}N_4O_3S$

Elemental analysis;

% C	:	Calcd.	:	44.77	:	Found	: 44.89
% H	:	Calcd.	:	04.51	:	Found	: 04.50
% N	:	Calcd.	:	20.88	:	Found	: 20.95

Spectral analysis:

IR (KBr) ν_{max} in cm^{-1} : 3475 (O-H); 3130 (N-H), 3065 (C-H aromatic), 2932 (CH_2), 1705 (C=0), 1565 (C⎓C of aromatic ring)

^{1}H-NMR ($CDCl_3$) (δ) in ppm: 12.46 (s, 1H, -COOH exchangeable with D_2O), 8.17 (m, 4H, $NHNHCSNH_2$, exchangeable with D_2O) 7.64–7.42 (m, 4H, Ar-H), 5.80 (s, 1H, NH, exchangeable with D_2O), 4.53 (s, 2H, N-CH_2).

MS: $[M]^+$ at m/z 268

2-((5-Amino-1,3,4-thiadiazol-2-yl) methyl amino) benzoic acid (3) :

A mixture of 2-(2-(2-Carbamothioyl hydrazinyl)-2-oxo ethyl amino) benzoic acid (0.05 mole) and conc. H_2SO_4 (20 ml.) was kept overnight at room temp. Then, the reaction mixture was poured into cold-water

and neutralized with liquid ammonia and filtered. The product thus obtained was recrystallized from ethanol-water to furnish compound (3)

Compound (3): M.P.: 136ºC, yield: 60%, mol. formula: $C_{10}H_{10}N_4O_2S$

Elemental analysis;

% C	:	Calcd.	:	47.99	:	Found : 48.23
% H	:	Calcd.	:	04.03	:	Found : 04.07
% N	:	Calcd.	:	22.39	:	Found : 22.16

Spectral analysis:

IR (KBr) ν_{max} in cm^{-1} : 3472 (O-H); 3352 (NH_2), 3175 (N-H), 3068 (C-H aromatic), 2935 (CH_2), 1728 (C=0), 1595 (C=N), 1580 (C⎓C of aromatic ring) 1215 (C-N), 1050 (N-N), 735 (C-S-C)

^{1}H-NMR ($CDCl_3$) (δ) in ppm: 12.40 (s, 1H, -COOH, exchangeable with D_2O), 7.65-7.35 (m, 4H, Ar-H), 6.38 (brs, 2H, NH_2, exchangeable with D_2O), 5.82 (s, 1H, NH, exchangeable with D_2O), 4.55 (s, 2H, N-CH_2)

MS: $[M]^+$ at m/z 250

2-((5-Acetamido-1,3,4-thiadiazol-2-yl) methyl amino) benzoic acid (4)

To the solution of 2-(5-Amino-1,3,4-thiadiazol-2-yl) methyl amino) benzoic acid (0.01 mole.) in dry benzene (50 ml.), acetyl chloride (0.01 mole.) was added drop by drop at 0-5ºC temp. with constant stirring. The reaction mixture was further stirred for 3hr. at room temperature & refluxed for 5hr. on water bath and then distilled off. The resulting mixture was poured onto crushed ice. The solid thus obtained was recrystallized from ethanol-water to yield compound **(4)**

Compound (4): M.P.: 158ºC, yield: 62%, mol. formula: $C_{12}H_{12}N_4O_3S$

Elemental analysis:

% C	:	Calcd.	:	49.31	:	Found : 49.15
% H	:	Calcd.	:	04.14	:	Found : 04.19
% N	:	Calcd.	:	19.17	:	Found : 19.28

Spectral analysis:

IR (KBr) ν_{max} in cm^{-1} : 3475 (O-H), 3182 (N-H), 3075 (CH_2), 2932 (C-H aliphatic), 2835 (C-H of $COCH_3$), 1725 (C=0), 1590 (C=N), 1570 (C⎓C of aromatic ring) 1205 (C-N), 1046 (N-N), 740 (C-S-C)

^{1}H-NMR ($CDCl_3$) (δ) in ppm: 12.34 (s, 1H, -COOH, exchangeable with D_2O), 8.22 (brs, 1H, NHCO, exchangeable with D_2O), 7.60-7.38 (m, 4H, Ar-H), 5.85 (s, 1H, NH, exchangeable with D_2O), 4.50 (s, 2H, N-CH_2), 2.32 (s, 3H, $COCH_3$).

MS: $[M]^+$ at m/z 292

2-((5-(3-(2-Chloro phenyl) acrylamido)-1,3,4,-thiadiazol-2-yl) methyl amino) benzoic acid (5a) :

To a solution of 2-((5-Acetamido-1,3,4-thiadiazol-2-yl) methyl amino) benzoic acid (0.01 mole.) in methanol was refluxed with 2-chloro benzaldehyde (0.01 mole.) in the presence of few drops of 2% NaOH solution for 8hr., while progress and completion of the reaction was monitored by TLC. The excess of solvent was removed through distillation. The separated solid was poured onto crushed ice and filtered. The product thus obtained was recrystallized from methanol-water to give compound (5a)

Compound (5a): M.P.: 111ºC, yield: 64%, mol. formula: $C_{19}H_{15}ClN_4O_3S$

Elemental analysis:

% C	:	Calcd.	:	55.01	:	Found : 55.13
% H	:	Calcd.	:	03.64	:	Found : 03.63
% N	:	Calcd.	:	13.50	:	Found : 13.57

Spectral analysis:

IR (KBr) $\nu_{max.}$ in cm^{-1} : 3490 (O-H of carboxylic proton), 3160 (N-H), 3022 (C-H aromatic) 2935(C-H aliphatic), 1718 (C=O of -COOH), 1625 (C=N), 1535 (C⎓C of aromatic ring) 1175 (C-N), 725 (C-S-C), 745 (C-Cl).

^{1}H-NMR ($CDCl_3$) (δ) in ppm.: 12.40 (s, 1H, carboxylic proton, exchangeable with D_2O), 8.48 (s, 1H, NHCO, exchangeable with D_2O), 8.20 (s, 1H, = CH-Ar), 7.85-7.30 (m, 8H, Ar-H), 6.85 (s, 1H, -COCH), 5.93 (s, 1H, exchangeable with D_2O), 4.70 (s, 2H, N-CH_2), 3.45 (s, 3H, Ar-OCH_3)
MS: $[M]^+$ at m/z 414

Compound (5b-5e) were prepared similarly and their physical and analytical data are given in table-I, while spectral date i.e., 1R, ^{1}H-NMR and mass are given in table-V.

2-((5-(1-Acetyl-5-(2-chlorophenyl)-4, 5, dihydro-1H-pyrazol-3-ylami- no)-1,3,4,-thiadiazol-2-yl) methyl amino) benzoic acid (6a) :

A mixture of 2-((5-(3-(2-Chloro phenyl) acrylamido)-1,3,4-thiadiazol-2-yl) methyl amino) benzoic acid (0.01 mole.) in absolute ethanol (50 ml.), hydrazine hydrate (99%, 0.01 mole) was added drop by drop with constant stirring in the presence of glacial acetic acid. The reaction mixture was refluxed for 9hr., distilled in vacuum and cooled. The separated solid was filtered, washed with petroleum ether and recrystallized from DMF-water to give compound (6a)

Compound (6a): M.P.: 151ºC, yield: 70%, mol. formula: C21H19ClN6 O3S

Elemental analysis;

% C	:	Calcd.	:	53.56	:	Found	: 53.71
% H	:	Calcd.	:	04.07	:	Found	: 04.09
% N	:	Calcd.	:	17.85	:	Found	: 17.69

Spectral analysis:

IR (KBr) ν_{max} in cm^{-1} : 3480 (O-H of carboxylic proton), 3160 (N-H), 3005 (C-H aromatic) 2910 (C-H aliphatic), 2850 (C-H of $COCH_3$) 1710 (C=O of -COOH), 1600 (C=N), 1540 (C⎓C of aromatic ring) 1145 (C-N), 1030 (N-N), 740 (C-Cl), 720 (C-S-C).
^{1}H-NMR ($CDCl_3$) (δ) in ppm.: 12.24 (s, 1H, carboxylic proton, exchangeable with D_2O), 7.65-7.25 (m, 8H, Ar-H), 6.80 (t, 1H, = CH-Ar), 6.26 (brs, 1H, NH, exchangeable with D_2O), 5.90 (s, 1H, NH, exchangeable with D_2O), 5.30 (d, 2H, CH_2) 4.60 (s, 2H, N-CH_2), 3.44 (s, 3H, Ar-OCH_3), 2.40 (s, 3H, $COCH_3$)
MS: $[M]^+$ at m/z 485

Compounds (6b-6e) were prepared similarly and their physical and analytical data are given in **table-II,** while spectral data i.e., 1R, ^{1}H-NMR and mass are given in **table-V.**

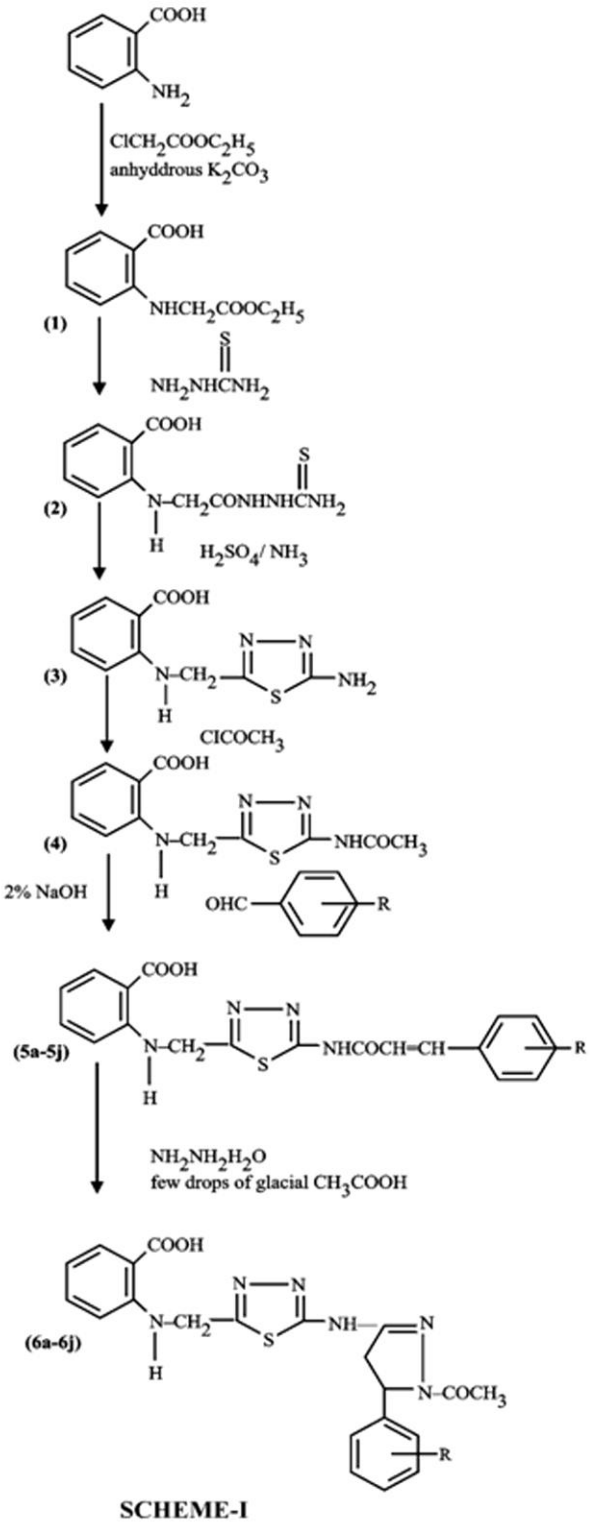
Mass spectral study of 2-((5-(5-(2,4-Dichloro phenyl)-1-(2-oxopropyl)-4,5-dihydro-1H-pyrazol-3-ylamino)-1,3,4-thiadiazol-2-yl) methyl amino) benzoic acid (6b)

The Proposed mass spectral fragmentation of this compound is illustrated in **Scheme-Ia**. The percent relative intensities of molecular ion, base peak and some other principal peaks are listed in **table-VI.**

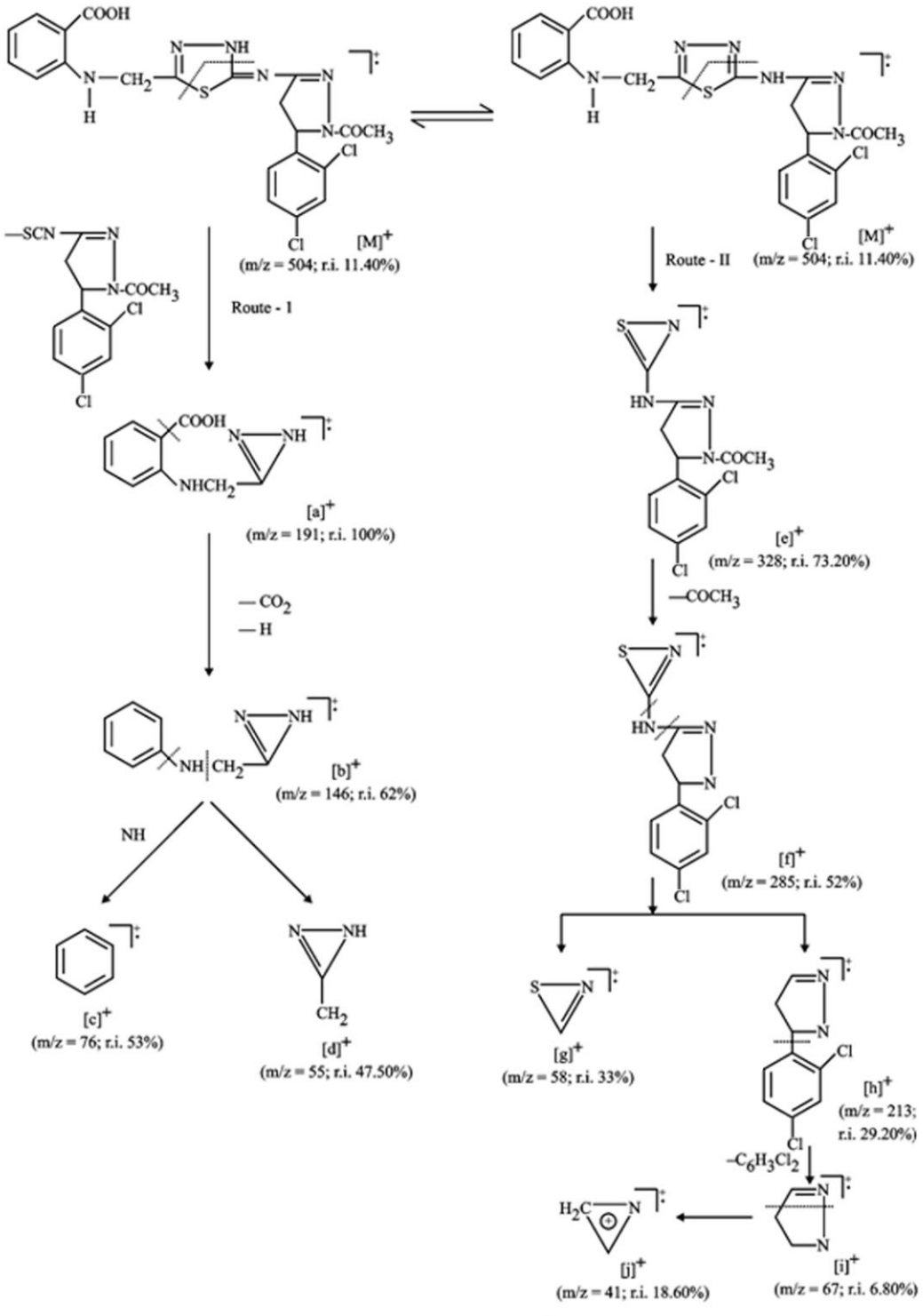
Molecular ion peak was observed at m/z 504 exhibiting tautomerism. So, in general, two distinct modes of decomposition were observed, and in both modes of decomposition splitting across the 1,3,4-thiadizole ring was observed. Similar type of cleavage of this ring has been reported by Bottino *et al.* (1982).

By **Route-I,** the major fragment ion $[a]^+$ appeared at m/z 191 by splitting of 1,3,4-thiadiazole ring, as a base peak, in the mass spectrum of this compound, which further gave CO_2 and a proton yielding radical ion $[b]^+$ with m/z 146. The formation of radical ion $[b]^+$ was found to be important peak, which showed cleavage at two sites giving rise to ion $[c]^+$ and $[d]^+$ at m/z 76 and m/z 55, respectively.

Another kind of splitting of 1,3,4-thiadiazole ring, via Route-II was observed. This give rises to fragment ion [e]+ at m/z 328 which on ejection of acetyl radical (COCH3) resulted in the formation ion [f]+ at m/z 285. Further, fragment [t]+ splitted at two sites and yielded stable cyclic ion [g]+ at m/z 58 along with radical ion [h]+ at m/z 213. Finally radical ion [h]+ on expulsion of C6H4Cl2 gave ion [i]+ which on removal of cyanide radical (CN) resulted in to the ion [j]+ at m/z 41.
The mass spectrum of this compound is given in **figure-I.**


COOH
NH2
ClCH2COOC2H5
anhyddrous K2CO3
NHCH2COOC2H5
(1)
NH2NHCNH2
S
N–CH2CONHNHCNH2
(2)
H2SO4/ NH3
(3)
ClCOCH3
NHCOCH3
(4)
2% NaOH
OHC
R
(5a-5j)
NHCOCH=CH
NH2NH2H2O
few drops of glacial CH3COOH
(6a-6j)
N–COCH3

SCHEME-I


COOH
N—CH2
H
N
NH
S
N
N
N-COCH3
Cl
Cl
[M]+
(m/z = 504; r.i. 11.40%)
—SCN
Route - I
Route - II
COOH
NHCH2
[a]+
(m/z = 191; r.i. 100%)
—CO2
—H
NH
CH2
[b]+
(m/z = 146; r.i. 62%)
NH
[c]+
(m/z = 76; r.i. 53%)
[d]+
(m/z = 55; r.i. 47.50%)
HN
[e]+
(m/z = 328; r.i. 73.20%)
—COCH3
[f]+
(m/z = 285; r.i. 52%)
[g]+
(m/z = 58; r.i. 33%)
[h]+
(m/z = 213;
r.i. 29.20%)
–C6H3Cl2
H2C
[j]+
(m/z = 41; r.i. 18.60%)
[i]+
(m/z = 67; r.i. 6.80%)

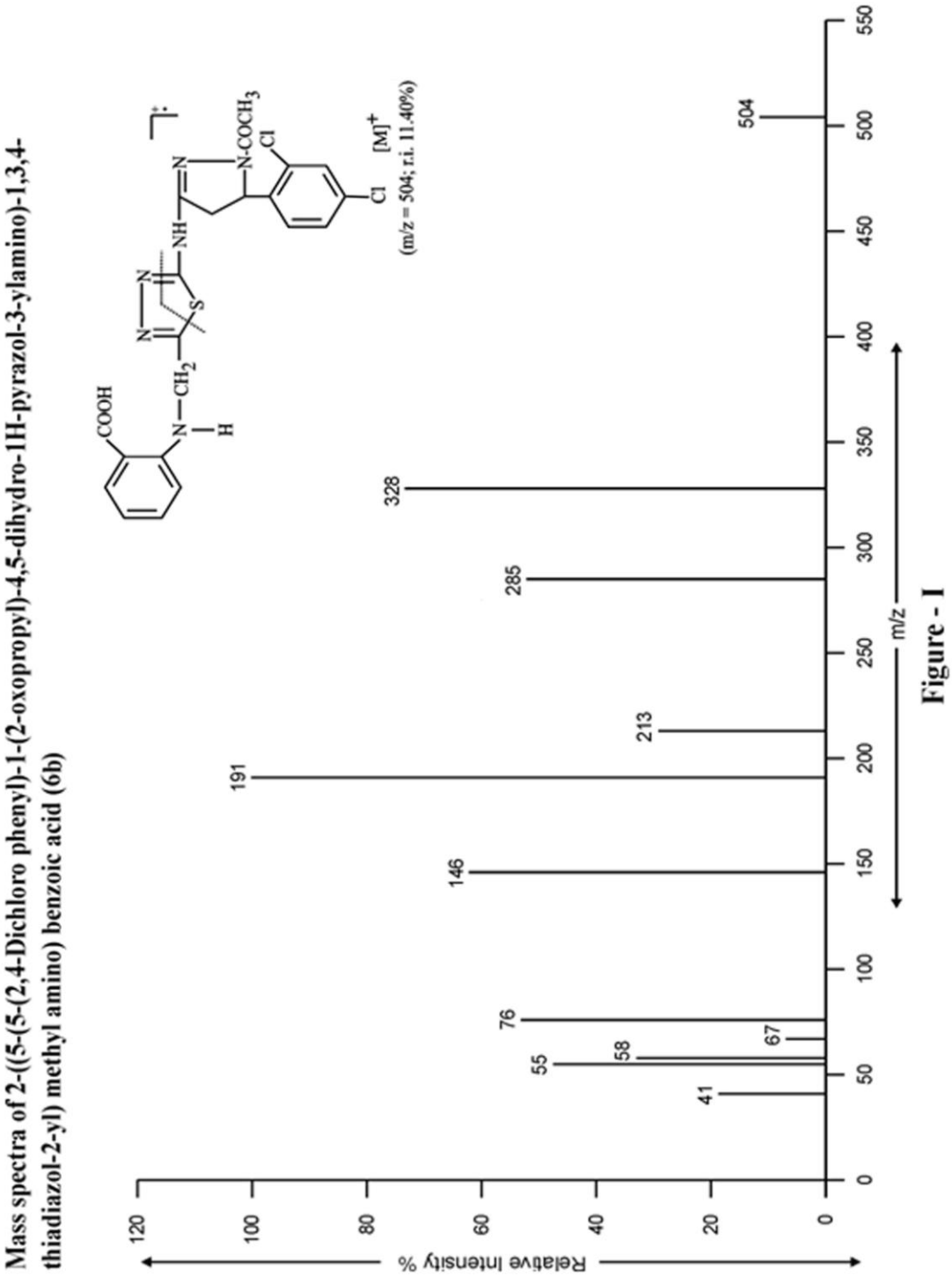
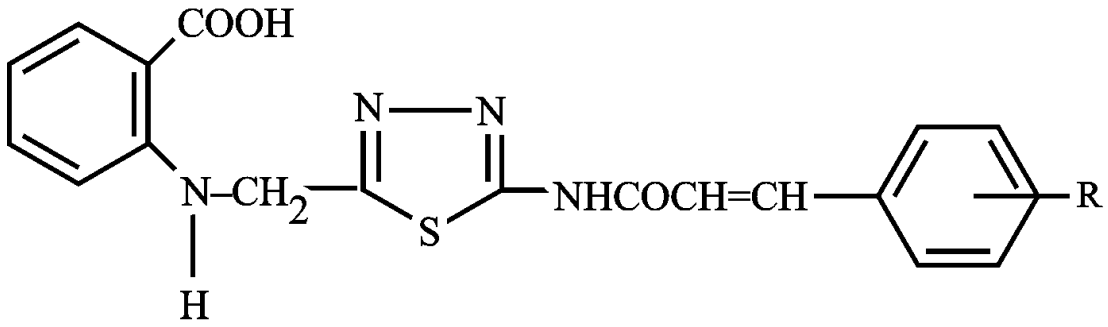

Mass spectra of 2-((5-(5-(2,4-Dichloro phenyl)-1-(2-oxopropyl)-4,5-dihydro-1H-pyrazol-3-ylamino)-1,3,4-thiadiazol-2-yl) methyl amino) benzoic acid (6b)
Relative Intensity %
m/z
504
328
285
213
191
146
76
67
58
55
41
[M]+
(m/z = 504; r.i. 11.40%)

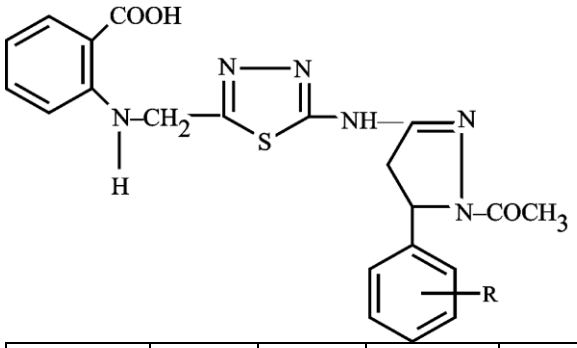
Figure - 1

Table I: Physical and analytical data of 2-((5-(3-(Substituted phenyl) acrylamido)-1,3,4-thiadiazol-2-yl) methyl amino) benzoic acid (5a-5e).



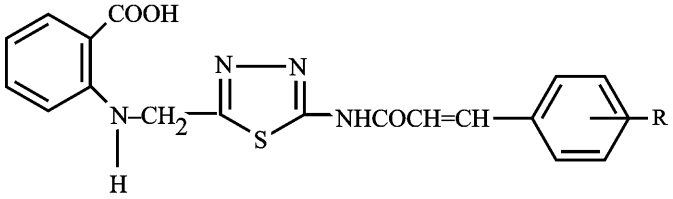
Comp.	R	M.P. (ºC)	Yield (%)	Recrystallization solvent	Molecular formula	Elemental analysis (%)					
						C %		H %		N %	
						Calcd.	Found	Calcd.	Found	Calcd.	Found
5b	4-Cl	108	60	Ethanol	$C_{19}H_{15}ClN_4O_3S$	55.01	55.25	3.64	3.65	13.50	13.45
5c	2-Br	117	55	Ethanol	$C_{19}H_{15}BrN_4O_3S$	49.68	49.80	3.29	3.30	12.20	12.24
5d	4-Br	105	50	Acetone	$C_{19}H_{15}BrN_4O_3S$	49.68	49.77	3.29	3.30	12.20	12.27
5e	2,4-Cl_2	92	65	Methanol	$C_{19}H_{14}Cl_2N_4O_3S$	50.79	50.92	3.14	3.13	12.47	12.40

Table II: Physical and analytical data of 2-((5-(1Acetyl-5-(substituted phenyl)-4,5-dihydro-1H-pyrazol-3-ylamino)-1,3,4-thiadiazol-2-yl) methyl amino) benzoic acid (6a-6e)



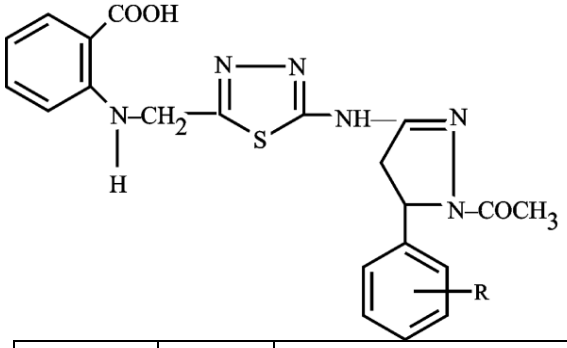
Comp.	R	M.P. (ºC)	Yield (%)	Recrysta-llization solvent	Molecular formula	Elemental analysis (%)					
						C %		H %		N %	
						Calcd.	Found	Calcd.	Found	Calcd.	Found
6a	2-Cl	151	70	DMF	$C_{21}H_{19}ClN_6O_3S$	53.56	53.71	4.07	4.09	17.85	17.69
6b	4-Cl	142	64	Acetone	$C_{21}H_{19}ClN_6O_3S$	53.56	54.66	4.07	4.08	17.85	17.70
6c	2-Br	160	55	Acetone	$C_{21}H_{19}BrN_6O_3S$	48.94	48.78	3.72	3.70	16.31	16.50
6d	4-Br	155	58	Methanol	$C_{21}H_{19}BrN_6O_3S$	48.94	48.76	3.72	3.70	16.31	16.50
6e	2,4-Cl_2	130	60	Ethanol	$C_{21}H_{18}Cl_2N_6O_3S$	49.91	59.69	3.59	3.57	16.63	16.82

Table III: Biological data of 2-((5-(3-(Substituted phenyl) acrylamido)-1,3,4-thiadiazol-2-yl) methyl amino) benzoic acid (5a-5e).



Comp	R	Anti-Inflammatory Activity		Analgesic Activity		UD_{50} (mg./kg.i.p.)	Acute Toxicity ALD_{50} (mg./kg.p.o)
		Dose (mg./kg. p.o.)	% Inhibition of oedema	Dose (mg./kg. p.o.)	% Protection		
5a	2-Cl	50	16.86*	50	13.52*	-	> 800
5b	4-Cl	50	14.64*	50	12.45*	-	> 800
5c	2-Br	50	13.75*	50	11.63*	-	> 800
5d	4-Br	50	11.57*	50	09.24*	-	> 800
5e	$2,4\text{-}Cl_2$	25 50 100	32.27** 43.98*** 65.69***	50	21.08** 40.82*** 62.23***	162.27	> 1400

Table IV: Biological data of 2-((5-(1-Acetyl-5-(substituted phenyl)-4,5-dihydro-1H-pyrazol-3-ylamino)-1,3,4-thiadiazol-2-yl) methyl amino) benzoic acid (6a-6e)



Comp.	R	Anti-Inflammatory Activity		Analgesic Activity		UD_{50} (mg/kg.i.p.)	Acute Toxicity ALD_{50} (mg./kg.p.o)
		Dose (mg./kg.p.o.)	% Inhibition of oedema	Dose (mg./kg.p.o.)	% Protection		
6a	2-Cl	50	33.78***	50	30.52***	-	> 800
6b	4-Cl	50	32.82**	50	29.65**	-	> 800
6c	2-Br	50	30.55**	50	28.48**	-	> 800
6d	4-Br	50	28.56**	50	25.18**	-	> 800
6e	$2,4\text{-}Cl_2$	25 50 100	29.91** 51.22*** 67.84***	50	18.88** 45.63*** 55.36***	141.86	> 3200
Phenyl butazone		25 50 100	26.76** 36.50*** 64.68***	25 50 100	14.26** 32.50*** 54.58***	66.60	

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

Table V: Spectral data of compounds (5b-5e & 6b-6e)

Comp. No.	IR (KBr) $\nu_{max.}$ in cm^{-1}	^{1}H-NMR ($CDCl_3$) δ in ppm.	MS:[M]$^+$ at m/z
5b	3495 (O-H of carboxylic proton), 3155 (N-H), 3025 (C-H aromatic), 2940 (C-H aliphatic), 1725 (C=O of -COOH), 1630 (C=N), 1530 (C⋯C of aromatic ring), 1180 (C-N), 1035 (N-N), 740 (C-Cl), 730 (C-S-C).	12.40 (s, 1H, carboxylic proton, exchangeable with D_2O), 8.45 (s, 1H, NHCO, exchangeable with D_2O), 8.16 (s,1H,=CH-Ar), 7.84-7.28 (m, 8H, Ar-H), 6.83 (s, 1H, -COCH), 5.90 (s, 1H, NH, exchangeable with D_2O), 4.70 (s, 2H, N-CH_2), 3.44 (s, 3H, Ar-OCH_3).	415
5c	3495 (O-H of carboxylic proton), 3160 (N-H), 3030 (C-H aromatic), 2940 (C-H aliphatic), 1728 (C=O of -COOH), 1636 (C=N), 1530 (C⋯C of aromatic ring), 1180 (C-N), 1040 (N-N), 728 (C-S-C), 560 (C-Br).	12.42 (s, 1H, carboxylic proton, exchangeable with D_2O), 8.48 (s, 1H, NHCO, exchangeable with D_2O), 8.20 (s,1H,=CH-Ar), 7.85-7.30 (m, 8H, Ar-H), 6.85 (s, 1H, -COCH), 5.91 (s, 1H, NH, exchangeable with D_2O), 4.73 (s, 2H, N-CH_2).	459
5d	3500 (O-H of carboxylic proton), 3160 (N-H), 3034 (C-H aromatic), 2940 (C-H aliphatic), 1730 (C=O of -COOH), 1640 (C=N), 1530 (C⋯C of aromatic ring), 1188 (C-N), 1040 (N-N), 764 (C-Br).	12.38 (s, 1H, carboxylic proton, exchangeable with D_2O), 8.44 (s, 1H, NHCO, exchangeable with D_2O), 8.15 (s,1H,=CH-Ar), 7.82-7.25 (m, 8H, Ar-H), 6.80 (s, 1H, -COCH), 5.90 (s, 1H, NH, exchangeable with D_2O), 4.70 (s, 2H, N-CH_2).	459
5e	3490 (O-H of carboxylic proton), 3150 (N-H), 3022 (C-H aromatic), 2932 (C-H aliphatic), 1718 (C=O of -COOH), 1620 (C=N), 1524 (C⋯C of aromatic ring), 1170 (C-N), 1025 (N-N), 550 (C-Br).	12.44 (s, 1H, carboxylic proton, exchangeable with D_2O), 8.48 (s, 1H, NHCO, exchangeable with D_2O), 8.20 (s,1H,=CH-Ar), 7.85-7.25 (m, 7H, Ar-H), 6.86 (s, 1H, -COCH), 5.93 (s, 1H, NH, exchangeable with D_2O), 4.72 (s, 2H, N-CH_2).	449
6b	3480 (O-H of carboxylic proton), 3165 (N-H), 3008 (C-H aromatic), 2916 (C-H aliphatic), 2850 (C-H of $COCH_3$), 1715 (C=O of -COOH), 1610 (C=N), 1545 (C⋯C of aromatic ring), 1150 (C-N), 1040 (N-N), 745 (C-Cl), 720 (C-S-C).	12.30 (s, 1H, carboxylic proton, exchangeable with D_2O), 7.65-7.22 (m, 8H, Ar-H), 6.78 (t, 1H,=CH-Ar), 6.25 (brs, 1H, NH, exchangeable with D_2O), 5.90 (s,1H,NH, exchangeable with D_2O) 5.26 (d, 2H, CH_2), 4.61 (s, 2H, N-CH_2), 2.37 (s, 3H, $COCH_3$).	471
6c	3485 (O-H of carboxylic proton), 3165 (N-H), 3010 (C-H aromatic), 2920 (C-H aliphatic), 2852 (C-H of $COCH_3$), 1718 (C=O of -COOH), 1605 (C=N), 1545 (C⋯C of aromatic ring), 1150 (C-N), 1050 (N-N), 722 (C-S-C), 550 (C-Br).	12.20 (s, 1H, carboxylic proton, exchangeable with D_2O), 7.62-7.20 (m, 8H, Ar-H), 6.76 (t, 1H,=CH-Ar), 6.20 (brs, 1H, NH, exchangeable with D_2O), 5.84 (s, 1H, NH, exchangeable with D_2O), 5.25 (d, 2H, CH_2), 4.60 (s, 2H, N-CH_2), 2.35 (s, 3H, $COCH_3$).	515
6d	3485 (O-H of carboxylic proton), 3170 (N-H), 3015 (C-H aromatic), 2913 (C-H aliphatic), 2845 (C-H of $COCH_3$), 1712 (C=O of -COOH), 1600 (C=N), 1545 (C⋯C of aromatic ring), 1152 (C-N), 1050 (N-N), 715 (C-S-C), 555 (C-Br).	12.18 (s, 1H, carboxylic proton, exchangeable with D_2O), 7.60-7.19 (m, 8H, Ar-H), 6.75 (t, 1H,=CH-Ar), 6.20 (brs, 1H, NH, exchangeable with D_2O), 5.80 (s, 1H, NH, exchangeable with D_2O), 5.22 (d, 2H, CH_2), 4.59 (s, 2H, N-CH_2), 2.31 (s, 3H, $COCH_3$).	515
6e	3475 (O-H of carboxylic proton), 3152 (N-H), 3000 (C-H aromatic), 2910 (C-H aliphatic), 2840 (C-H of $COCH_3$), 1708 (C=O of -COOH), 1595 (C=N), 1525 (C⋯C of aromatic ring), 1140 (C-N), 1025 (N-N), 740 (C-Cl), 710 (C-S-C).	12.35 (s, 1H, carboxylic proton, exchangeable with D_2O), 7.70-7.29 (m, 7H, Ar-H), 6.85 (t, 1H, =CH-Ar), 6.30 (brs, 1H, NH, exchangeable with D_2O), 5.95 (s, 1H, NH, exchangeable with D_2O), 5.36 (d, 2H, CH_2), 4.68 (s, 2H, N-CH_2), 2.49 (s, 3H, $COCH_3$).	505

Table VI

Major ion fragments	m/z	Relative intensity (%)
$[M]^+$	504	11.40
$[a]^+$	191	100.00
$[b]^+$	146	62.00
$[c]^+$	076	53.00
$[d]^+$	055	47.50
$[e]^+$	328	73.20
$[f]^+$	285	52.00
$[g]^+$	058	33.00
$[h]^+$	213	29.20
$[i]^+$	067	06.80
$[j]^+$	041	18.60

BIOLOGICAL ACTIVITY OF NEWER SUBSTITUTED THIADIAZOLYL & PYRAZOLYL ANTHRANILIC ACID DERIVATIVES (Scheme)

All the newly ten substituted anthranilic acid derivatives have been synthesized and screened for their anti-inflammatory & analgesic activities at a dose of 50 mg./kg.p.o., the pharmacological data of all the compounds of this series have been reported in **table-III** & **IV**.

RESULTS AND DISCUSSION

Out of twenty compounds only three compounds, **5e,** and **6e** were found to possess more potent anti-inflammatory activity in comparison to phenyl butazone. Compound **5e** which was substituted with chloro group at 2^{nd} & 4^{th} position of Phenyl ring have shown 43.98% of inhibition of oedema. Compound **5d** which possessed bromo group at 4^{nd} position of phenyl ring have shown least activity i.e., 11.57%.

The last step compounds **(6a-6e)** were characterized by the presence of pyrazoline ring. All the compounds of this stage have shown promising degree (28.56-51.22%) of anti-inflammatory activity. Compound **6e,** has shown the maximum percentage of anti-inflammatory activity i.e., 51.22% at a dose of 50mg./kg.p.o. Considering the potentiality of compounds **5e** and **6e** exhibited better anti-inflammatory activity at all three graded doses of 25, 50 and 100 mg/kg p.o. as compared to phenyl butazone **Table-(III).**

The pyrazoline derivatives **(6a-6e)** showed better analgesic activity than thiadiazolyl derivatives **(5a-5e).** Compounds of thiadiazolyl derivatives **(5a-5e)** have shown moderate to good analgesic activity. Compound **5e** which was substituted by 2,4-dichloro phenyl ring of anthranilic acid exhibited 40.82% analgesic activity at 50 mg./kg.p.o.

The most active compound of this series was **6e** which have shown potent analgesic activity i.e., 45.63% at a dose of 50 mg./kg.p.o., Moreover, when these compounds were tested at three graded doses 25, 50 and 100 mg/kg p.o. It was found that analgesic activity of **6e** is greater than phenyl butazone **(Table-IV).**

ALD_{50} of all these compounds were greater than 800 mg./kg.p.o. except **5e** & **6e** which have 1400 mg./kg.p.o. Therefore, these exhibited good safety margin.

Therefore, it may be concluded that:

(a) Different substituted thiadiazolyl nucleus has shown mild to moderate anti-inflammatory activity. Cyclization of these thiadiazolyl derivatives into their corresponding Pyrazolines enhances the anti-inflammatory property.

(b) Compounds 5e and 6e having a 2,4 dichloro Phenyl group as substituent's, exhibited most potent anti-inflammatory and analgesic activities, thus the obtained biological results clearly indicate that compounds which showed maximum anti-inflammatory activity also exhibited potent analgesic activity.

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 ºC 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.,* (1962). 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-

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.,* (1977) 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 liabilities of newly synthesized compounds were checked with method of Verma *et al.,* 1981). 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 without 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 (Smith, 1960). 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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