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ARTICLE

Synthesis and Characterization of Thiadiazole Pyrazolene Anthranilic Acid Derivatives as Potent Anti-inflammatory Agents

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ABSTRACT

Several new substituted thiadiazole pyrazolene anthranilic acid derivatives were synthesized. These compounds also evaluated for their anti-inflammatory and analgesic activities. Compound 2-((5-(3-(2,6-dichloro)acrylamido)-1,3,4-thiadiazol-2-yl)methyl amino)-benzoic acid (**5b**) and 2-((5-(1-acetyl-5-(2,6-dichloro)-4,5-dihydro-1*H*-pyrazol-3-ylamino)-1,3,4-thiadiazol-2-yl)methyl amino)benzoic acid (**6b**) were found to be most active compounds of this series, which exhibits 38.10 & 48.50% anti-inflammatory activity while, 36.24 & 40.10 % analgesic activity, respectively. The structures of all the compounds were characterized by analytical data, IR, ^{1}H NMR and mass spectrometry.

KEYWORDS

Thiadiazoles, Pyrazolene, Anthranilic acid derivatives, Analgesic activity, Anti-inflammatory activity.

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INTRODUCTION

Treatment of inflammation with steroids (*i.e.* glucocorticoids) is associated with side effects leading, at times, to liver, heart and kidney [1]. Presently, non-steroidal anti-inflammatory drugs (NSAIDs) are preferred for the treatment of pain, acute and chronic inflammation and different types of arthritis. The fenamates [2-11] are a family of non-steroidal anti-inflammatory drugs (NSAIDs), which are derivatives of N-phenyl anthranilic acid. They include mefenamic acid, meclofenamic acid, flufenamic acid, tolfenamic acid and etofenamic acid, *etc.* which are useful agents for clinical treatment of inflammatory disorders. The fenamates have anti-inflammatory, antipyretic and analgesic properties [12-23]. 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 cyclooxygenase. 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 [17-21] 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 cardiovascular activities [24].

Pyrazolines [25,26] are the most important representatives of hydrazine in both the synthetic and theoretical respect. Compounds of these classes are widely used as photographic developers, dyes, herbicides and medicinal agents with 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 activity. In the light of 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.

EXPERIMENTAL

The melting points were determined in open capillaries with the help of thermionic melting point apparatus and are uncorrected. The purity of all the newly synthesized compounds was 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. 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. Tetramethyl silane (TMS) was used as internal reference standard. Elemental analysis 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.

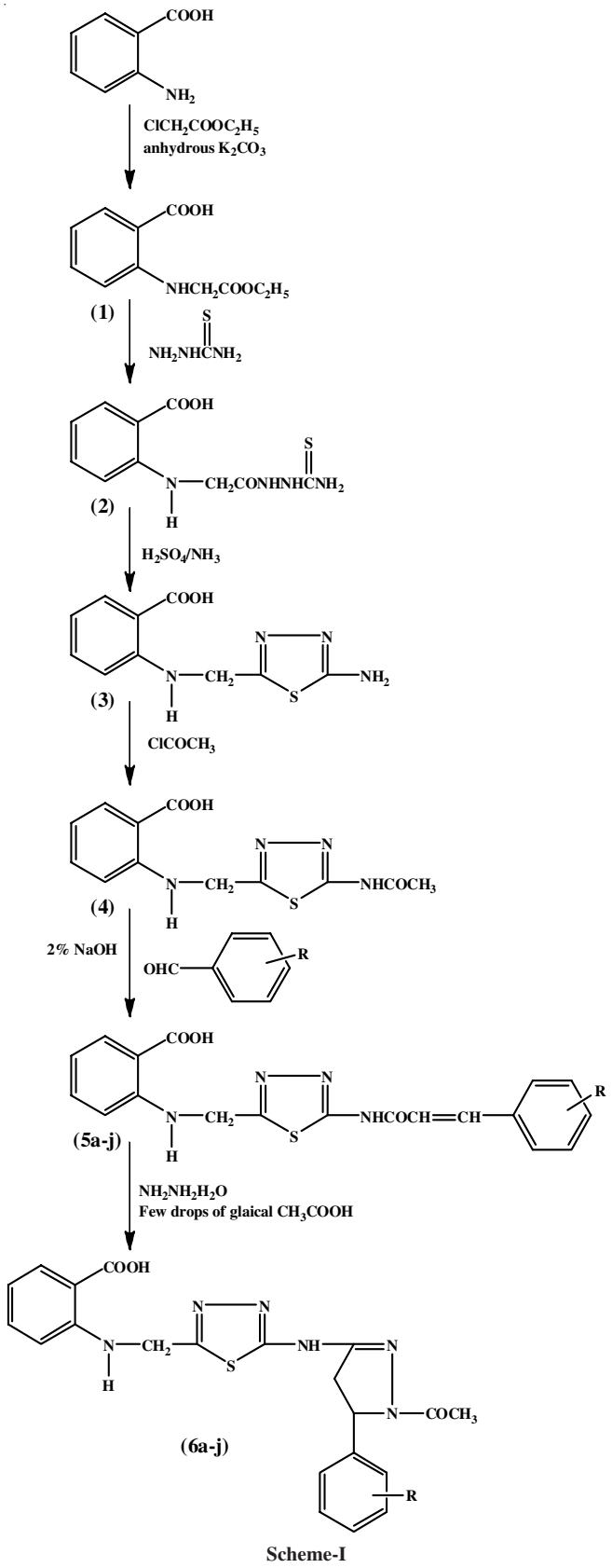
2-(2-Ethoxy-2-oxo ethyl amino)benzoic acid (1): A mixture of anthranilic acid (0.l mol), ethyl chloro acetate (0.1 mol) and anhydrous K_2CO_3 (5.0 g) in acetone (80 mL) was refluxed for 12 h on a steam bath. The excess of solvent was distilled off under reduced pressure and the resulting solid mass was poured into ice-cold water, filtered. The separated solid was recrystallized from methanol-water to give compound **1**. Yield: 55%, m.p.: 115 ºC. Elemental analysis (%) calcd. (found) of $C_{11}H_{13}NO_4$: C, 59.19 (59.32); H, 5.87 (5.85); N, 6.27 (6.30). IR (KBr, ν_{max}, cm^{-1}): 3500 (O-H), 3150 (N-H), 3040 (C-H aromatic), 2935 (CH_2), 1725 (C=O), 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-oxoethylamino)-benzoic acid (2): A mixture of 2-(2-ethoxy-2-oxoethyl amino)-benzoic acid (0.02 mol) and thiosemicarbazide (0.02 mol) in methanol (50 mL) was refluxed for 8 h. 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**. Yield: 78%, m.p.: 126 ºC. Elemental analysis (%) calcd. (found) of $C_{10}H_{12}N_4O_3S$: C, 44.77 (44.89); H, 4.51 (4.50); N, 20.88 (20.95); IR (KBr, ν_{max}, 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)methylamino)-benzoic acid (3): A mixture of 2-(2-(2-carbamothioyl hydrazinyl)-2-oxoethyl amino)benzoic acid (0.05 mol) and conc. H_2SO_4 (20 mL) was kept overnight at room temperature. Then, the reaction mixture was poured into cold-water and neutralized with liquid NH_3 and filtered. The product thus obtained was recrystallized from ethanol-water to furnish compound **3**. Yield: 60%, m.p.: 136 ºC. Elemental analysis (%) calcd. (found) of m.f.: $C_{10}H_{10}N_4O_2S$: C, 47.99 (48.23); H, 4.03 (4.07); N, 22.39 (22.16). IR (KBr, ν_{max}, 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 a solution of 2-((5-amino-1,3,4-thiadiazol-2-yl) methyl amino)benzoic acid (0.01 mol) in dry benzene (50 mL), acetyl chloride (0.01 mol) was added drop by drop at 0-5 ºC with constant stirring. The reaction mixture was further stirred for 3 h at room temperature and refluxed for 5 h 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**. Yield: 62%, m.p.: 158 ºC. Elemental analysis (%) calcd. (found) of $C_{12}H_{12}N_4O_3S$: C, 49.31 (49.15); H, 4.14 (4.19); N, 19.17 (19.28). IR (KBr, ν_{max}, cm^{-1}): 3475 (O-H), 3182 (N-H), 3075 (CH_2), 2932 (C-H aliphatic), 2835 (C-H of $COCH_3$), 1725 (C=O), 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 mol) in methanol was refluxed with 2,4-dibromo benzaldehyde (0.01 mol) in the presence of few drops of 2% NaOH solution for 8 h, 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** (**Scheme-I**). Yield: 60%, m.p.: 97 ºC, Elemental analysis (%) calcd. (found) of $C_{19}H_{14}N_4O_3SBr_2$: C, 42.40 (42.61); H, 2.62 (2.61); N, 10.41 (10.48). IR (KBr, ν_{max}, cm^{-1}): 3496 (O-H of carboxylic proton), 3150 (N-H), 3020 (C-H aromatic), 2930



Scheme-I

(C-H aliphatic), 1720 (C=O of -COOH), 1627 (C=N), 1525 (C⎓C of aromatic ring), 1177 (C-N), 1028 (N-N), 555 (C-Br). ^{1}H NMR ($CDCl_3$) δ in ppm: 12.40 (s, 1H, carboxylic proton, exchangeable with D_2O), 8.45 (s, 1H, NHCO, exchangeable with D_2O), 8.15 (s,1H, =CH-Ar), 7.80-7.21 (m, 7H, Ar-H), 6.80 (s, 1H, -COCH), 5.90 (s, 1H, NH, exchangeable with D_2O), 4.68 (s, 2H, N-CH_2). MS: $[M]^+$ at *m/z* 538.

Other compounds **5b-e** were also synthesized similarly and their physical and analytical data are given in Table-1 while spectral data *i.e.* IR, ^{1}H NMR and mass are given in Table-2.

2-((5-(1-Acetyl-5-(2,4-dibromophenyl)-4,5,dihydro-1*H*-pyrazol-3-ylamino)-1,3,4,-thiadiazol-2-yl)methyl amino)benzoic acid (6a): A mixture of 2-((5-(3-(2,4-dibromo phenyl)acrylamido)-1,3,4-thiadiazol-2-yl)methyl amino)-benzoic acid (0.01 mol) in absolute ethanol (50 mL), hydrazine hydrate (99%, 0.01 mol) was added dropwise with constant stirring in presence of glacial acetic acid. The reaction mixture was refluxed for 9 h, distilled in vacuum and cooled. The separated solid was filtered, washed with petroleum ether and recrystallized from DMF-water to give compound **6a** (**Scheme-I**). Yield: 52%, m.p.: 128 ºC. Elemental analysis (%) calcd. (found) of $C_{21}H_{18}N_6O_3SBr_2$: C, 42.44 (42.66); H, 3.05 (3.04); N, 14.14 (14.18). IR (KBr, ν_{max}, cm^{-1}): 3480 (O-H of carboxylic proton), 3155 (N-H), 3000 (C-H aromatic), 2910 (C-H aliphatic), 2840 (C-H of $COCH_3$), 1705 (C=O of -COOH), 1600 (C=N), 1528 (C⎓C of aromatic ring), 1150 (C-N), 1028 (N-N), 715 (C-S-C), 555 (C-Br). ^{1}H NMR ($CDCl_3$) δ ppm: 12.22 (s, 1H, carboxylic proton, exchangeable with D_2O), 7.60-7.15 (m, 7H, Ar-H), 6.80 (t, 1H, =CH-Ar), 6.27 (brs, 1H, NH, exchangeable with D_2O), 5.84 (s,1H,NH, exchangeable with D_2O), 5.28 (d, 2H, CH_2), 4.63(s, 2H, N-CH_2), 2.35 (s, 3H, $COCH_3$). MS: $[M]^+$ at *m/z* 594.

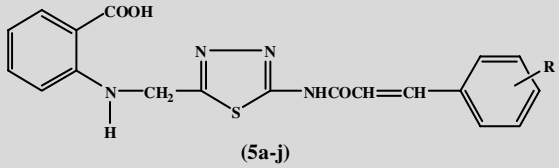
Other compounds (**6b-e**) were synthesized similarly and their physical and analytical data are given in Table-1 while spectral data *i.e.*, IR, ^{1}H NMR and mass are given in Table-2.

Animal ethics: The animals were housed under standard conditions and received a diet of commercial food pellets and water *ad libitum* during the captivity, but were entirely fasted during the experiment period. Each group was composed of 6-15 animals. The experiments were conducted with the recommendations in the Guidelines for the Care and Use of Laboratory Animals of the 393 Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) 394 in India. All the animal studies were conducted in adherence to experimental guidelines and 395 procedures approved by the Institutional Animal Care and Use Committee (MCLMC/IAEC/2020-39420/MCL-1/A4) of the Medicinal Chemistry Laboratory, Department of Chemistry, Meerut College, Meerut, India.

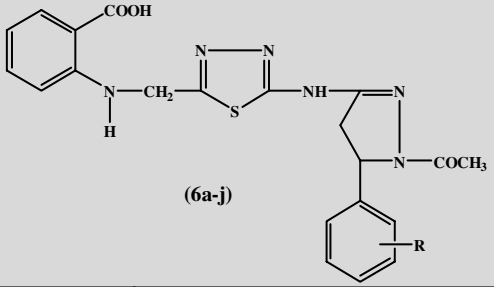
Biological activity of newer substituted thiadiazolyl and pyrazolyl anthranilic acid derivatives: All the newly (substituted anthranilic acid) derivatives have been synthesized and screened for their inflammation inhibition and analgesic activities at a dose of 50 mg/kg p.o.

Pharmacology: The pharmacological studies were performed on albino rats of Charles Foster strain (either sex) of 70 to 95 days weighing 75-150 g, albino mice weighing 20-25 g (pregnant female rats) were excluded from studies. All included rats and mice were divided into three groups (control, standard and drug treated) of six animals each. All the animals had access to food and water *ad libitum.* They were kept in rooms at 20-25 ºC with 12 h (light/dark cycle) and relative humidity up to 50-60%. The test derivatives and reference

TABLE-1
PHYSICAL AND ANALYTICAL DATA OF 2-((5-(3-(SUBSTITUTED PHENYL)ACRYLAMIDO)-1,3,4-THIADIAZOL-2-YL)-METHYLAMINO)BENZOIC ACID (**5a-e**) AND 2-((5-(1-ACETYL-5-(SUBSTITUTED PHENYL)-4,5-DIHYDRO-1*H*-PYRAZOL-3-YLAMINO)-1,3,4-THIADIAZOL-2-YL) METHYLAMINO)BENZOIC ACID (**6a-e**)



(5a-j)



(6a-j)

Compd.	R	m.p. (ºC)	Yield (%)	Recrystallization solvent	m.f.	Elemental analysis (%): Calcd. (found)		
						C	H	N
5a	2,4-Br_2	97	60	DMF	$C_{19}H_{14}Br_2N_4O_3S$	42.40 (42.61)	2.62 (2.61)	10.41 (10.48)
5b	2,6-Cl_2	84	68	Acetone	$C_{19}H_{14}Cl_2N_4O_3S$	50.79 (50.91)	3.14 (3.13)	12.47 (12.52)
5c	2,6-Br_2	88	62	Methanol	$C_{19}H_{14}Br_2N_4O_3S$	42.40 (42.58)	2.62 (2.61)	10.41 (10.46)
5d	2-CH_3	119	54	Ethanol	$C_{20}H_{18}N_4O_3S$	60.90 (60.69)	4.60 (4.61)	14.20 (14.26)
5e	4-CH_3	115	52	Methanol	$C_{20}H_{18}N_4O_3S$	60.90 (60.75)	4.60 (4.61)	14.20 (14.24)
6a	2,4-Br_2	128	52	Acetic acid	$C_{21}H_{18}Br_2N_6O_3S$	42.44 (42.66)	3.05 (3.04)	14.14 (14.18)
6b	2,6-Cl_2	125	50	Acetone	$C_{21}H_{18}Cl_2N_6O_3S$	49.91 (59.99)	3.59 (3.60)	16.63 (16.56)
6c	2,6-Br_2	112	65	DMF	$C_{21}H_{18}Br_2N_6O_3S$	42.44 (42.66)	3.05 (3.03)	14.14 (14.17)
6d	2-CH_3	139	58	Acetone	$C_{22}H_{22}N_6O_3S$	58.65 (58.83)	4. 92(4.94)	18.65 (18.59)
6e	4-CH_3	135	60	Ethanol	$C_{22}H_{22}N_6O_3S$	58.65 (58.46)	4.92 (4.90)	18.65 (18.69)

TABLE-2
SPECTRAL DATA OF COMPOUNDS (**5b-e** & **6b-e**)

Compd. No.	IR (KBr, ν_{max}, cm^{-1})	^1H NMR ($CDCl_3$) (δ, ppm)	MS:$[M]^+$ at *m/z*
5b	3485 (O-H of carboxylic proton), 3140 (N-H), 3020 (C-H aromatic), 2925 (C-H aliphatic), 1715 (C=O of -COOH), 1615 (C=N), 1520 (C...C of aromatic ring), 1160 (C-N), 1020 (N-N), 540 (C-Br).	12.46 (s, 1H, carboxylic proton, exchangeable with D_2O), 8.50 (s, 1H, NHCO, exchangeable with D_2O), 8.22 (s, 1H, =CH-Ar), 7.80-7.20 (m, 7H, Ar-H), 6.82 (s, 1H, -COCH), 5.88 (s, 1H, NH, exchangeable with D_2O), 4.69 (s, 2H, N-CH_2).	449
5c	3490 (O-H of carboxylic proton), 3150 (N-H), 3024 (C-H aromatic), 2930 (C-H aliphatic), 1722 (C=O of -COOH), 1620 (C=N), 1520 (C...C of aromatic ring), 1166 (C-N), 1025 (N-N), 542 (C-Br).	12.48 (s, 1H, carboxylic proton, exchangeable with D_2O), 8.47 (s, 1H, NHCO, exchangeable with D_2O), 8.20 (s, 1H, =CH-Ar), 7.74-7.18 (m, 7H, Ar-H), 6.80 (s, 1H, -COCH), 5.92 (s, 1H, NH, exchangeable with D_2O), 4.70 (s, 2H, N-CH_2).	538
5d	3505 (O-H of carboxylic proton), 3178 (N-H), 3035 (C-H aromatic), 2948 (C-H aliphatic), 1730 (C=O of -COOH), 1640 (C=N), 1545 (C...C of aromatic ring), 1188 (C-N), 732 (C-S-C).	12.30 (s, 1H, carboxylic proton, exchangeable with D_2O), 8.38 (s, 1H, NHCO, exchangeable with D_2O), 8.17 (s, 1H, =CH-Ar), 7.80-7.28 (m, 8H, Ar-H), 6.75 (s, 1H, -COCH), 5.80 (s, 1H, NH, exchangeable with D_2O), 4.60 (s, 2H, N-CH_2), 2.40 (s, 3H, CH_3).	394
5e	3596 (O-H of carboxylic proton), 3170 (N-H), 3030 (C-H aromatic), 2940 (C-H aliphatic), 1725 (C=O of -COOH), 1630 (C=N), 1540 (C...C of aromatic ring), 1180 (C-N), 725 (C-S-C).	12.27 (s, 1H, carboxylic proton, exchangeable with D_2O), 8.35 (s, 1H, NHCO, exchangeable with D_2O), 8.20 (s, 1H, =CH-Ar), 7.80-7.30 (m, 8H, Ar-H), 6.72 (s, 1H, -COCH), 5.81 (s, 1H, NH, exchangeable with D_2O), 4.62 (s, 2H, N-CH_2), 2.37 (s, 3H, CH_3).	394
6b	3480 (O-H of carboxylic proton), 3150 (N-H), 3005 (C-H aromatic), 2905 (C-H aliphatic), 2845 (C-H of $COCH_3$), 1700 (C=O of -COOH), 1605 (C=N), 1525 (C...C of aromatic ring), 1145 (C-N), 1025 (N-N), 735 (C-Cl), 715 (C-S-C).	12.32 (s, 1H, carboxylic proton, exchangeable with D_2O), 7.70-7.30 (m, 7H, Ar-H), 6.82 (t, 1H, =CH-Ar), 6.30 (brs, 1H, NH, exchangeable with D_2O), 5.85 (s, 1H, NH, exchangeable with D_2O), 5.30 (d, 2H, CH_2), 4.65 (s, 2H, N-CH_2), 2.40 (s, 3H, $COCH_3$).	504
6c	3485 (O-H of carboxylic proton), 3150 (N-H), 3010 (C-H aromatic), 2912 (C-H aliphatic), 2845 (C-H of $COCH_3$), 1710 (C=O of -COOH), 1610 (C=N), 1530 (C...C of aromatic ring), 1145 (C-N), 1025 (N-N), 718 (C-S-C), 545 (C-Br).	12.20 (s, 1H, carboxylic proton, exchangeable with D_2O), 7.65-7.20 (m, 7H, Ar-H), 6.80 (t, 1H, =CH-Ar), 6.28 (brs, 1H, NH, exchangeable with D_2O), 5.80 (s, 1H, NH, exchangeable with D_2O), 5.25 (d, 2H, CH_2), 4.65 (s, 2H, N-CH_2), 2.37 (s, 3H, $COCH_3$)	594
6d	3490 (O-H of carboxylic proton), 3158 (N-H), 3020 (C-H aromatic), 2915 (C-H aliphatic), 2850 (C-H of $COCH_3$), 1720 (C=O of -COOH), 1622 (C=N), 1540 (C...C of aromatic ring), 1150 (C-N), 1034 (N-N), 725 (C-S-C).	12.10 (s, 1H, carboxylic proton, exchangeable with D_2O), 7.48-7.14 (m, 8H, Ar-H), 6.65 (t, 1H, =CH-Ar), 6.10 (brs, 1H, NH, exchangeable with D_2O), 5.70 (s, 1H, NH, exchangeable with D_2O), 5.10 (d, 2H, CH_2), 4.45 (s, 2H, N-CH_2), 2.30 (s, 3H, $COCH_3$), 2.40 (s, 3H, CH_3)	451
6e	3480 (O-H of Carboxylic Proton), 3165 (N-H), 3010 (C-H aromatic), 2915 (C-H aliphatic), 2850 (C-H of $COCH_3$), 1730 (C=O of -COOH), 1615 (C=N), 1550 (C...C of aromatic ring), 1150 (C-N), 1055 (N-N), 725 (C-S-C).	12.12 (s, 1H, carboxylic proton, exchangeable with D_2O), 7.50-7.15 (m, 8H, Ar-H), 6.66 (t, 1H, =CH-Ar), 6.12 (brs, 1H, NH, exchangeable with D_2O), 5.76 (s, 1H, NH, exchangeable with D_2O), 5.15 (d, 2H, CH_2), 4.46 (s, 2H, N-CH_2), 2.31 (s, 3H, $COCH_3$), 2.40 (s, 3H, CH_3)	451

drug were dissolved in propylene glycol. Phenyl butazone, a potent anti-inflammatory drug, was used as reference drug for comparison.

Acute toxicity study: The test derivatives were examined for their acute toxicity (ALD_{50}) in albino mice, according to the method of Smith [27]. The test derivatives were provided orally at different dose levels in separate groups of rats. After 24 h of drug administration, percent mortality in each group was observed. ALD_{50} was calculated from the data observed.

Ulcerogenic activity: Ulcerogenic activities of all the newly synthesized derivatives were checked with the help of method of Verma *et al.* [28]. Albino rats were fasted for 24 h prior to drug administration. All tested animals were sacrificed 8 h after drug treatment and their stomachs and small intestines were microscopically evaluated to assess the incidence of hyperemia, shedding of epithelium, Petechial and frank hemorrhages and erosion or discrete ulceration with or without perforation. The appearance of any one of these criteria was considered to be an evidence of ulcerogenic activity.

Anti-inflammatory activity: The study was carried out by following the procedure of Winter *et al.* [29]. All the rats were divided into three groups *i.e.* (control, drug treated and standard drug) of six animals each. A freshly prepared solution of carrageenan (1% in 0.9% saline). 0.05 mL was injected under the planter aponeurosis of the right hind paw of each rat. Synthesized derivatives and standard drug were administered orally to the rats of drug treated groups and the standard drug group, respectively 1hr before the carrageenan injection. The rat paw volume of each treated rat was measured before 1 and after 3 h of carrageenan treatment with the help of a plethymometer. The % anti-inflammatory activity was calculated according to the formula given below:

$$\text{Inhibition of oedema (\%)} = \left(1 - \frac{V_t}{V_c}\right) \times 100$$

where, V_t and V_c are the mean increase in paw volume of rats of the treated and the control groups, respectively. Results obtained were statistically analyzed.

Analgesic activity: Analgesic activity obtained with the help of method of Berkowitz *et al.* [30] performed this activity. This procedure is based on the fact of the test derivatives to antagonize the phenyl quinone-induced pain syndrome in mice. Groups of five mice were injected intraperitonely with 0.25 mL of (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 to 10 min) after injection of an irritant. The analgesic observations were expressed as percent protection in comparison to control.

$$\text{Protection (\%)} = \left(1 - \frac{\text{Mean no. of writhes in mice of test groups}}{\text{Mean number of writhes in mice of control group}}\right) \times 100$$

RESULTS AND DISCUSSION

The proposed mass spectral fragmentation of 2-((5-(5-(2,6-dichloro phenyl)-1-(2-oxopropyl)-4,5-dihydro-1*H*-pyrazol-3-ylamino)-1,3,4-thiadiazol-2-yl)methyl amino)-benzoic acid (6b) is illustrated in **Scheme-II**. The percent relative intensities of molecular ion, base peak and some other principal peaks are listed in Table-3. The 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.

TABLE-3

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

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** in **Scheme-II** was observed. This give rises to fragment ion $[e]^+$ at *m/z* 328, which on ejection of acetyl radical ($COCH_3$) 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 $C_6H_4Cl_2$ 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 Fig. 1.

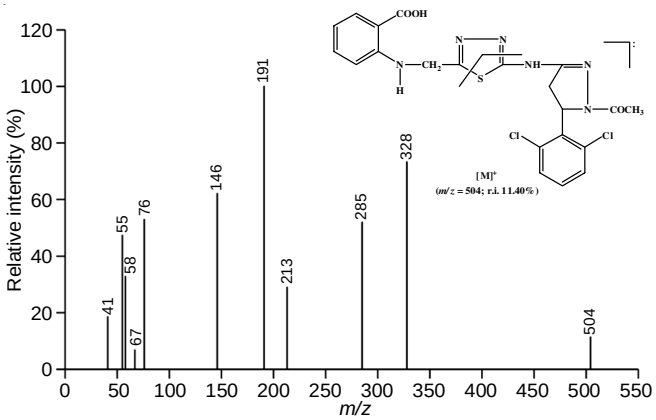
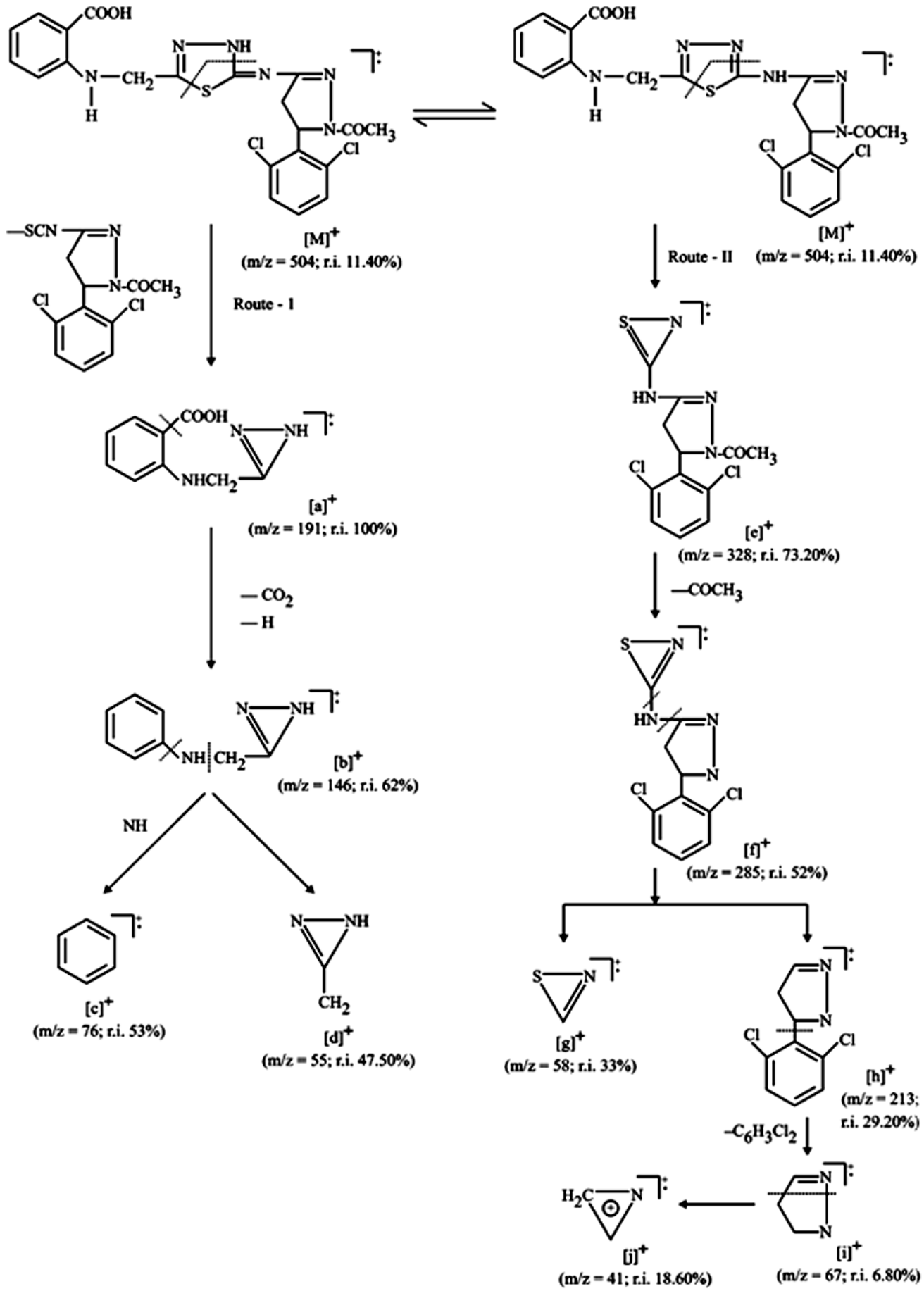


Fig. 1. Mass spectra of 2-((5-(5-(2,6-dichlorophenyl)-1-(2-oxopropyl)-4,5,dihydro-1*H*-pyrazol-3-ylamino)-1,3,4,-thiadiazol-2-yl)methyl amino)benzoic acid (**6b**)

Biological activity: Among all the synthesized derivatives, only two compounds, **5b** and **6b** were found to have more potent anti-inflammatory activity in comparison to phenyl butazone. Compound **5b** which was substituted with 2,6-dichloro group at 2nd & 6th position of phenyl ring have exhibited 38.10% of


[M]+
(m/z = 504; r.i. 11.40%)
Route - I
Route - II
[a]+
(m/z = 191; r.i. 100%)
— CO2
— H
[b]+
(m/z = 146; r.i. 62%)
NH
[c]+
(m/z = 76; r.i. 53%)
[d]+
(m/z = 55; r.i. 47.50%)
[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
[i]+
(m/z = 67; r.i. 6.80%)
[j]+
(m/z = 41; r.i. 18.60%)

Scheme-II

TABLE-4
BIOLOGICAL DATA OF 2-((5-(3-(SUBSTITUTED PHENYL)ACRYLAMIDO)-1,3,4-THIADIAZOL-2-YL)-METHYLAMINO)BENZOIC ACID (**5a-e**) AND 2-((5-(1-ACETYL-5-(SUBSTITUTED PHENYL)-4,5-DIHYDRO-1*H*-PYRAZOL-3-YLAMINO)-1,3,4-THIADIAZOL-2-YL) METHYLAMINO)BENZOIC ACID (**6a-e**)

Compd.	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	2,4-Br_2	50	19.92**	50	16.80*	–	> 800
5b	2,6-Cl_2	25	27.88**	25	15.60**	177.27	> 1400
		50	38.10***	50	36.24***		
		100	66.77***	100	58.18***		
5c	2,6-Br_2	50	21.48**	50	19.24*	–	> 800
5d	2-CH_3	50	8.38*	50	6.20*	–	> 800
5e	4-CH_3	50	10.24*	50	8.38*	–	> 800
6a	2,4-Br_2	50	32.96**	50	30.48***	–	> 800
6b	2,6-Cl_2	25	28.28**	25	18.22**	148.45	> 3200
		50	48.50***	50	40.10***		
		100	70.48***	100	60.19***		
6c	2,6-Br_2	50	33.85***	50	31.57***	–	> 800
6d	2-CH_3	50	26.46**	50	24.21**	–	> 800
6e	4-CH_3	50	27.63**	50	24.93**	–	> 800
Phenyl butazone		25	26.76**	25	14.26**	66.60	
		50	36.50***	50	32.50***		
		100	64.68***	100	54.58***		

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

inhibition of oedema. Compound **5d** which have methyl group at 2nd position of phenyl ring have exhibited least activity *i.e.* 08.38%.

The last step derivatives **6a-e** were elucidated by the presence of pyrazoline ring. All the derivatives of this step have shown promising degree (26.46-48.50%) of anti-inflammatory activity. Derivative **6b** exhibited the maximum percentage of anti-inflammatory activity, (48.50%) at a dose of 50 mg/kg p.o. Taking into consideration, the potentiality of compounds **5b** and **6b** have shown better anti-inflammatory activity (at all three graded doses of 25, 50 and 100 mg/kg p.o.) as compared to phenyl butazone (Table-4).

The pyrazoline congeners (**6a-e**) exhibited better analgesic activity than the thiadiazolyl congeners (**5a-e**). Thiadiazolyl derivatives (**5a-e**) have exhibited moderate to good analgesic activity. Compound **5b** derivative, which was substituted by 2,6-dichloro phenyl ring of anthranilic acid showed (36.24%) analgesic activity at a dose of 50 mg/kg p.o. Most active derivative of this series is **6b**, which have exhibited potent analgesic activity (40.10%) at a dose of 50 mg/kg p.o. When these derivatives were evaluated at three graded doses 25, 50 and 100 mg/kg p.o., it was observed that the analgesic activity of compound **6b** is greater than phenyl butazone (Table-4). The ALD_{50} of all these compounds were greater than 800 mg/kg p.o. except **5b** and **6b**, which have 1400 mg/kg p.o. (Table-4). Therefore, these exhibited good safety margin.

Conclusion

Several new substituted thiadiazole pyrazolene anthranilic acid derivatives were synthesized. Different substituted thiadiazolyl derivatives have exhibited mild to moderate anti-inflammatory activity. Moreover, cyclization of these thiadiazolyl derivatives into their corresponding pyrazolines increases the anti-inflammatory property. Derivatives, **5b** and **6b** possess a 2,6-dichloro phenyl group as substituent's, showed most potent anti-inflammatory and analgesic activities. Thus the results clearly indicate that derivative which exhibited the maximum anti-inflammatory activity also exhibited potent analgesic activity.

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