ELSEVIER

Eur. J. Med. Chem. 37 (2002) 689–697

EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY

www.elsevier.com/locate/ejmech

Short communication

Newer N-substituted anthranilic acid derivatives as potent anti-inflammatory agents

Shalabh Sharma [1], Virendra Kishor Srivastava, Ashok Kumar *

Medicinal Chemistry Division, Department of Pharmacology, Lala Lajpat Rai Memorial Medical College, Meerut, U.P. 250 004, India

Received 12 July 2001; accepted 23 January 2002

Abstract

The new 5-bromo-*N*-[2′-amino(1″-acetyl-5″substitutedaryl-2″-pyrazolin-3″-yl)-1′,3′,4′-oxadiazol-5′-ylmethyl]anthranilic acids **7a**–**7e** and *N*-[2′-amino-(1″-acetyl-5″-substiutedaryl-2″-pyrazolin-3″-yl)-1′,3′,4′-thiadiazol-5′-ylmethyl]anthranilic acids **6′a**–**6′c** have been synthesised from 5-bromo-*N*-(2′-aminosubstituedbenzylideneacetyl-1′,3′,4′-oxadiazol-5′-ylmethyl)anthranilic acids **6a**–**6e** and *N*-(2′-aminosubstitutedbenzylideneacetyl-1′,3′,4′-thiadiazol-5′-ylmethyl)anthranilic acids **5′a**–**5′e**, respectively. All these compounds have been screened in vivo for their anti-inflammatory and acute toxicity. Compounds **7b** and **6′b** were found to be potent member of this series, which showed 50.66 and 47.56%, respectively, inflammation inhibitory activity at a dose of 50 mg kg^{-1} p.o., while standard drug, phenylbutazone, exhibited 45.52% anti-inflammatory activity at the same dose. However, 5-bromo-*N*-{2′-amino-[1″-acety1-5″-(*para*-methoxyphenyl)-2″-pyrazolin-3″-yl]-1′,3′,4′-oxidiazol-5′-ylmethyl}anthranilic acid (**7b**) was found to be the most active and less ulcerogcnic compound than the standard drag mid rest of the compounds of this series. The structures of these compounds have been established by IR, ^{1}H-NMR spectroscopic data and elemental analyses.

Keywords: N-substituted anthranilic acid-oxadiazolyl-pyrazolines; Thiadiazolyl-pyrazolines; Synthesis; Albino rats; Acute toxicity; Anti-inflammatory activity; Ulcerogenic activity

1. Introduction

Mefenamic acid and meclofenamates [1], both *N*-phenylanthranilic acid derivatives, have been used as anti-inflammatory agents in therapy. A considerable amount of work has been done on the structural variation of this subclass of drugs broadly known as Non-Steroidal anti-inflammatory Drugs (NSAIDs). It has been observed that best known NSAIDs are acidic in nature. In view of this, our attention has been directed to the variation at 2-position of anthranilic acid by incorporating different acidic functional five membered heterocyclic rings with a view to synthesise new analogies with improved anti-inflammatory effects. Recent literature shows that substitutions at 2-position of anthranilic acid (2-amino benzoic acid) by different substitutedaryl or heteroaryl moieties markedly modulate the anti-inflammatory activity [2,3]. Furthermore, looking into the structure activity relationship of various NSAIDs, it is obvious that pyrazoline [4–6], oxadiazole [7,8] and thiadiazole [9,10] have also been found to possess strong anti-inflammatory activity. Hence, it is not irrelevant to speculate that incorporating these pyrazoline, oxadiazole, thiadiazole rings into the 2-position of anthranilic acid may enhance the anti-inflammatory activity of such compounds. Therefore, it was thought worthwhile to synthesise some new N-substituted anthranilic acid derivatives by incorporating oxadiazolyl, thiadiazolyl and pyrazolinyl moieites with the hope to get better anti-inflammatory molecules.

2. Chemistry

The synthetic routes of compounds are outlined in Figs. 1 and 2. In the first synthetic route, 5-bromo antharnilic acid was reacted with ethyl chloroacetate to give compound **2** i.e. 5-bromo-*N*-ethylacetoanthranilic

* Correspondence and reprints
E-mail address: rajput_ak@indiatimes.com (A. Kumar).
[1] This paper is a part of the Ph.D. thesis of Shalabh Sharma.

0223-5234/02/$ - see front matter
PII: S0223-5234(02)01340-5

acid, which was further reacted with semicarbazide hydrochloride to yield the compound **3**. The compound **3** i.e. 5-bromo-*N*-(semicarbazido carbonyl methyl)-anthranilic acid on dehydration with conc. H_2SO_4 afforded 5-Bromo-*N*-(2′-amino-1′,3′,4′-oxadiazol-5′-ylmethyl)anthranilic acid. This compound further reacted with acetylchloride to give compound **5** i.e. 5-bromo-*N*-(2′-aminoacetyl-1′,3′,4′-oxadiazol-5′-ylmethyl)anthranilic acid, which further condensed with proper aromatic aldehydes yielding 5-bromo-*N*-(2′-aminosubstitutedbenzylidene acetyl-1′,3′,4′-oxadiazol-5′-ylmethyl)-anthranilic acids (**6a**–**6e**), which on cyclization with 99% hydrazine hydrate in the presence of few drops of glacial acetic acid gave final compounds **7a**–**7e** i.e. 5-bromo-*N*-[2′-amino-(1″-acetyl-5″-substitutedaryl-2″-pyrazolin-3″-yl)-1′,3′,4′-oxadiazol-5′-ylmethyl]anthranilic acids.

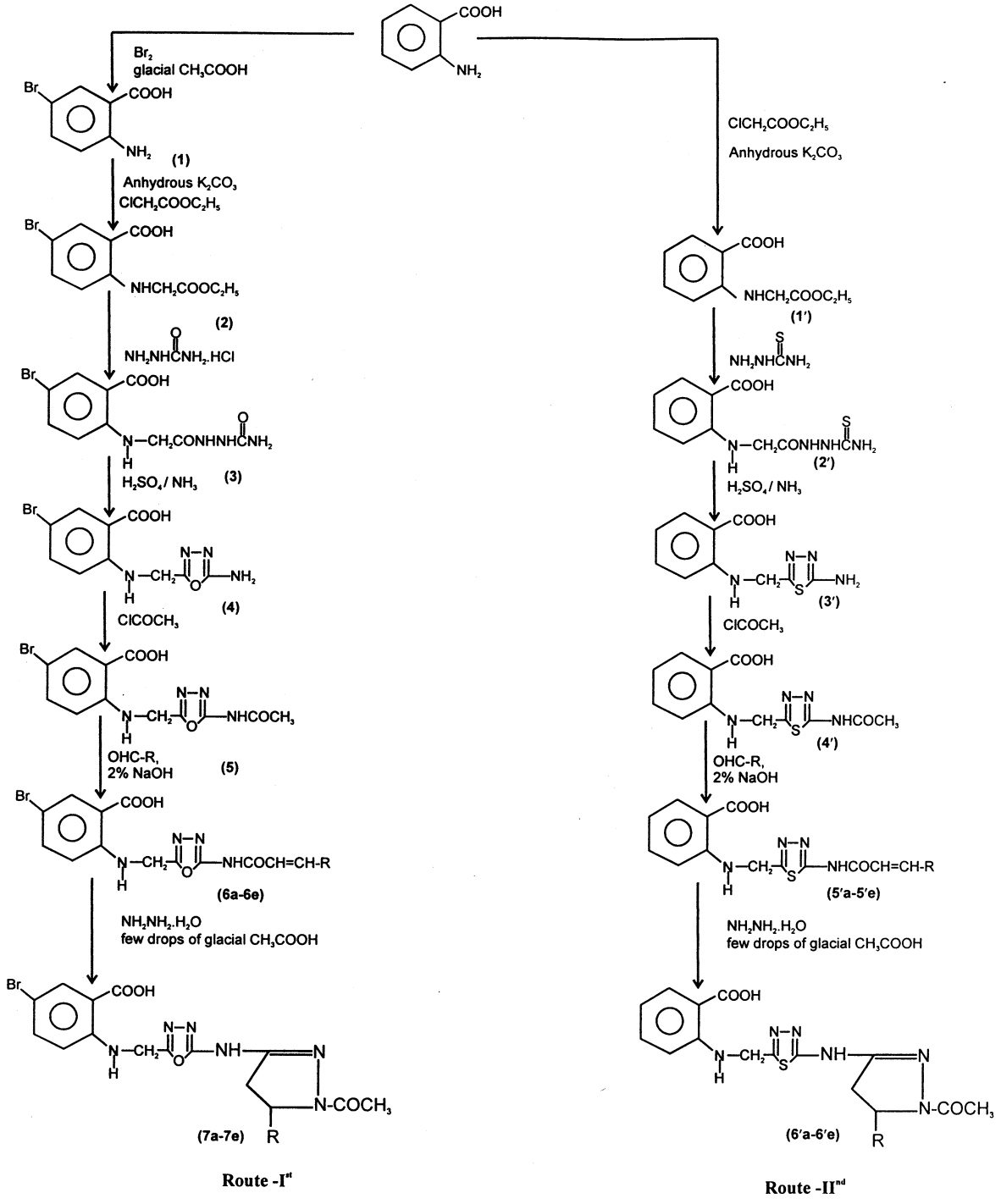


Fig. 1.

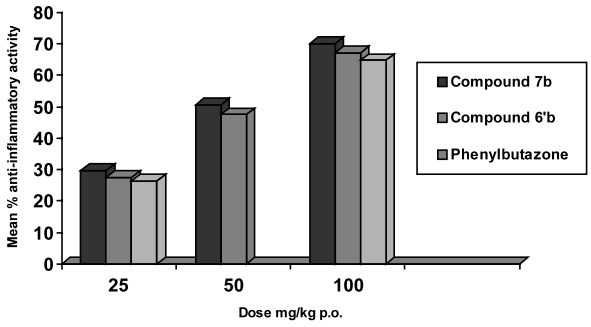


Fig. 2. The bar diagram showing mean percent anti-inflammatory activity of the most potent compounds **7b**, **6′b** and reference drug, Phenylbutazone, at the three graded doses.

In the second synthetic route, anthranilic acid reacted with ethyl chloroacetate to yield compound *N*-ethylacetoanthranilic acid (**1′**), which on condensation with thiosemicarbazide yielded the compound **2′**. This compound was dehydrated with conc. H_2SO_4 to give compound **3′** i.e. *N*-(2′-amino-1′,3′,4′-thiadiazol-5′-ylmethyl)anthranilic acid. Compound **3′** was further acetylated with acetyl chloride to afford *N*-(2′-arninoacetyl-1′,3′,4′-thiadiazol-5′-ylmethyl)anthranilic acid (**4′**). This acetylated product was reacted with proper aromatic aldehydes giving *N*-(2′-aminosubstitutedbenzylideneacetyl-1′,3′,4′-thiadiazol-5′-ylmethyl)anthranilic acids (**5′a**–**5′e**). Finally, compounds **5′a**–**5′e** were cyclised with 99% hydrazine hydrate in the presence of few drops of glacial acetic acid to give *N*-[2′-amino-(1″-acetyl-5″-substitutedaryl-2″-pyrazolin-3″-yl)-1′,3′,4′-thiadiazol-5′-ylmethyl]anthranilic acids (**6′a**–**6′e**).

3. Results

3.1. Acute toxicity study

All the compounds of this series showed $ALD_{50} >$ 800 mg kg^{-1} p.o., with maximum in compound **7b** and **6′b** (1600 mg kg^{-1} p.o). All the results are depicted in Table 3.

3.2. Anti inflammatory activity

Random screening of compounds [(**6a**–**6e**; (**7a**–**7e**); (**5′a**–**5′e**) and (**6′a**–**6′e**)] and reference drug, phenylbutazone, was performed at 50 mg kg^{-1} p.o. The two compounds **7b** and **6′b** were found to possess the most potent anti-inflammatory activity (50.66% and 47.56 and, respectively) at 50 mg kg^{-1} p.o. in comparison with reference drug, phenylbutazone, which showed 45.52% of inhibition of oedema at same dose. Furthermore, the two test compounds **7b**, **6′b** and phenylbutazone were subjected to screening at three graded doses of 25, 50 and 100 mg kg^{-1} p.o. for anti-inflammatory activity. These two compounds showed better anti-inflammatory activity at all three tested doses than the reference drug. However, compound **7b** exhibited maximum percent anti-inflammatory activity.

3.3. Ulcerogenic activity

The two most active compounds **7b**, **6′b** and reference drug, phenylbutazone, were screened for their ulcerogenic activity. The UD_{50} of compounds **7b**, **6′b** and phenylbutazone are 170.52, 156.2 and 66.6 mg kg^{-1} i.p., respectively.

4. Discussion

The characteristic feature of route-first and route-second is that 5-bromoanthranilic acid and anthranilic acid were substituted at *N*-position with five membered ring structure oxadiazolyl and thiadiazolyl, respectively. Furthermore, these compounds were converted in their corresponding substituted benzylidene derivatives, which were finally cyclized into pyrazoline derivatives.

All these new compounds (**6a**–**6e**; **7a**–**7e**; **5′a**–**5′e**; and **6′a**–**6′e**) were tested in vivo in order to evaluate their pharmacological activity. The pharmacological data are given in Table 3. These compounds were screened for their anti-inflammatory profile tested at 50 mg kg^{-1} p.o, exhibited substantive anti-inflammatory property. It was observed that compounds, **6a** and **5′a**, having phenyl group as a substituent showed the least percent inhibition of oedema i.e. 32.57 and 30.2%, respectively, while the compounds, **6b** and **5′h**, substituted with *para*-methoxyphenyl group exhibited the maximum anti-inflammatory activity, 40.39 and 39.72%, respectively. On the other hand, compounds, **6c** and **5′c**, having *meta*-methoxy-*para*-hydroxyphenyl group as a substituent showed interesting anti-inflammatory activity.

It is clear from the results obtained, that cyclisation of substituted benylidene derivatives, **6a**–**6e** and **5′a**–**5′e**, into their corresponding pyrazoline derivatives, **7a**–**7e** and **6′a**–**6′e**, enhanced the anti-inflammatory activity and compounds, **6a**–**6e** and **7a**–**7e**, having oxadiazolyl moiety generally showed better anti-inflammatory property in comparison to the compounds, **5′a**–**5′e** and **6′a**–**6′c**, contained thiadiazolyl moiety.

Compounds **7b** and **6′b** (50.66 and 47.53%, respectively) exhibited more potent anti-inflammatory activity than that of Phenylbutazone (45.52%). However, 5-bromo-*N*-{2′-amino-[1″-acetyl-5″-(*para*-methoxyphenyl)-2″-pyrazolin-3″-yl]-1′,3′,4′-oxadiazol-5′-ylmethyl] anthranilic acid (**7b**) was found to be the most active compound of the present series, which has oxadiazolyl moiety. On considering their potentialities compounds, **7b**, **6′b** and reference drug, Phenylbutazone, were

further tested at three graded doses i.e. 25, 50 and 100 mg kg^{-1}, p.o., and these two compounds, **7b** and **6′b**, showed higher anti-inflammatory activity than the reference drug at all three tested doses. Furthermore, these two compounds and reference drug have been tested for ulcerogenic liability, and these compounds, **7b** and **6′b**, exhibited less ulcerogenie potentiality as compared with reference drug, Phenylbutazone (UD_{50} of **7b** = 170.52 mg kg^{-1} i.p.; UD_{50} of **6′b** = 156.2 mg kg^{-1} i.p.; UD_{50} of phenylbutazone = 66.6 mg kg^{-1} per i.p.). Moreover compounds **7b** and **6′b** have shown $ALD_{50} > 1600$ mg kg^{-1} p.o.

Hence, it may be concluded:

(a) Substituted benzylidene derivatives exhibit mild to moderate the anti-inflammatory profile.
(b) Cyclization of these substituted benzylidene derivatives into pyrazoline derivatives enhance the inflammation inhibiting property.
(c) Presence of *para*-methoxyphenyl or *meta*-methoxy-*para*-hydroxyphenyl as a substituent elicit a remarkable increase in anti-inflammatory activity. However, *para*-methoxy isomer showed better anti-inflammatory activity than *meta*-methoxy isomer.
(d) Compounds having oxadiazolyl moiety generally showed better anti-inflammatory property in comparison to thiadiazolyl. Similar results have also been reported by Mazzone et al. [14].
(e) Presence of electronegative atom, Br, may play a pivotal role in the modulation of anti-inflammatory activity.

5. Experimental protocols

5.1. Chemistry

Melting points of newly synthesised compounds were taken in open capillaries with help of thermonic melting point apparatus and are uncorrected. The purity of the compounds was checked by thin layer chromatography on silica gel-G, eluent was a mixture of methanol–benzene in 2:8 proportion and spots were located by iodine. The structure of these compounds was elucidated by IR, ^{1}H-NMR and elemental analyses. The IR (KBr) spectra were recorded on FTIR Paragon 500 (Perkin–Elmer), ν max in cm^{-1}. The ^{1}H-NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on Brucker-300 FT instrument, chemical shift (δ) in ppm. Tetramethylsilane (TMS) was used as internal reference standard. The carbon, hydrogen and nitrogen analyses were performed on Heracus, Carlo Erba-1108 analyser at Central Drug Research Institute (CDRI) at Lucknow (UP), India. The carbon, hydrogen and nitrogen analyses were found within $\pm 0.4\%$ of the theoretical value.

5.1.1. 5-Bromoanthranilic acid (1)

It was prepared by following the method of Wheeler et al. [15], bromine (0.8 mol) in acetic acid (20 mL) was added to the solution of anthranilic acid (0.4 mol) in absolute acetic acid. The reaction mixture was kept overnight. Then, 200–250 mL coldwater was added into it. The solid product was filtered off, washed with water and dried. It was recrystallised with ethanol-water m.p., 220 °C, yield 55%, while Wheeler et al., reported m.p. 210 °C and yield was 60%.

5.1.2. 5-Bromo-N-ethylacetoanthranilic acid (2)

A mixture of 5-bromoanthranilic acid (**1**) (0.1 mol), ethyl chloroacetate (0.1 mol) and anhydrous K_2CO_3 (5.0 g) in acetone (80 mL) was refluxed for about 15 h. The excess of solvent was distilled off under reduced pressure and the resulting solid mass poured into ice water, filtered and the separated solid recrystallised from ethanol–water to give compound **2**, Compound **2**: m.p. 125 °C, yield 70%, $C_{11}H_{12}O_4NBr$ [Calc. (Found): C, 43.71 (43.82); H, 3.97 (3.60); N, 4.64 (4.79)]%. IR (KBr) ν in cm^{-1}: 3480 (O–H); 3125 (N–H); 3030 (C–H aromatic); 2925 (CH_2); 1710 (C=O); 1590 (C···C of aromatic ling), 660 (C–Br). ^{1}H-NMR ($CDC1_3$). δ in ppm: 7.60–7.40 (m, 3H, Ar–H), 1.25 (t, 3H, $J = 7$ Hz, –$COOCH_2CH_3$) 4.20 (q, 2H, $J = 7$ Hz, –$COOCH_2CH_3$), 4.50 (s, 2H, N–CH_2), 5.8 (s, 1H, NH, exchangeable with D_2O), 11.2 (s, 1H, –COO*H*, exchangeable with D_2O).

5.1.3. 5-Bromo-N-(semicarbazido carbonyl methyl)anthranilic acid (3)

5-Bromo-*N*-ethylaceto-anthranilic acid (**2**) (0.02 mol) and semicarbazide hydrochloride (0.02) in methanol (50 mL) in the presence of anhydrous NaOH (5.0 g), was refluxed for 10 h. The excess of solvent removed under reduced pressure and the viscous mass recrystallised from ethanol–water to afford compound **3**. Compound **3**: m.p. 140 °C. yield 62%, $C_{10}H_{11}O_4N_4Br$ [Calc. (Found): C, 36.25 (36.10); H, 3.32 (3.56); N, 16.92 (16.63)]%. IR (KBr) ν in cm^{-1}: 3475 (O–H); 3130 (N–H); 3020 (C–H aromatic); 2920 (CH_2); 1715 (C=O); 1575 (C···C of aromatic ring), 665 (C–Br). ^{1}H-NMR ($CDCl_3$) δ in ppm: 7.62–7.45 (m, 3H, Ar–*H*), 8.40 (m, 4H, $NHNHCONH_2$, exchangeable with D_2O), 4.70 (s, 2H, N–CH_2). 5.70 (s, 1H, NH, exchangeable with D_2O), 11.31 (s, 1H, –COO*H*, exchangeable with D_2O).

5.1.4. 5-Bromo-N-(2′-amino-1′,3′,4′-oxadiazol-5′-ylmethyl)anthranilic acid (4)

A mixture of compound **3** (0.05 mol) and conc. H_2SO_4 (20 mL) was kept overnight at room temperature., poured into ice cold water, neutralised with liquid ammonia and filtered. The product obtained was recrystallised from methanol–water. Compound **4**: m.p. 150 °C, yield, 50%, $C_{10}H_9O_3N_4Br$ [Calc. (Found): C,

38.33 (38.59); H, 2.88 (2.60); N, 17.89 (17.71)]%. IR (KBr) ν in cm^{-1}: 3485 (O–H): 3140 (N–H), 3330 (NH_2); 3025 (C–H aromatic); 2920 (CH_2); 1720(C=O); 1585 (C···C of aromatic ring), 1600 (C=N), 1215 (C–N), 1040 (N–N), 655 (C–Br); ^{1}H-NMR ($CDC1_3$) δ in ppm; 7.65–7.40 (m, 3H, Ar–*H*), 6.25 (bs, 2H, NH_2, exchangeable with D_2O). 4.75 (s, 2H, N–CH_2), 5.95 (s, 1H, NH, exchangeable with D_2O), 11.25 (s, 1H, –COO*H*, exchangeable with D_2O).

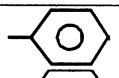
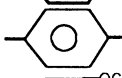
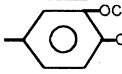
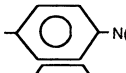
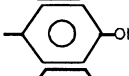
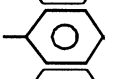
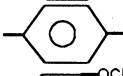
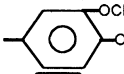
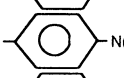
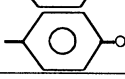
*5.1.5. 5-Bromo-N-(2′-aminoacetyl-l′,3′,4′-oxadiazol-5′-ylmethyl)anthranilic acid (**5**)*

To a solution of compound **4** (0.01 mol) in dry benzene (50 mL), acetyl chloride (0.01 mol) was added drop by drop at 0–5 °C temperature. The reaction mixture was stirred for 2 h, refluxed for 6 h and then distilled off and poured onto crushed ice. The solid thus obtained was recrystallised from methanol–water and purity of product was checked by TLC. Compound **5**; m.p. 133 °C, yield 70%, $C_{12}H_{11}O_4N_4Br$ [Calc. (Found); C, 40.56 (40.39); H, 3.09 (3.27); N, 15.77 (15.50)]%. IR (KBr) ν in cm^{-1}: 3475 (O–H), 3150 (N–H); 3030 (C–H aromatic); 2925 (CH_2); 2840 (C–H of $COCH_3$), 1710 (C=O); 1580 (C···C of aromatic ring), 1565 (C=N), 1210 (C–N), 1040 (N–N), 650 (C–Br); ^{1}H-NMR ($CDC1_3$) δ in ppm; 7.60–7.40 (m, 3H, Ar–*H*), 8.35 (bs, 1H, N*H*CO, exchangeable with D_2O), 4.60 (s, 2H, 5N–CH_2), 5.70 (s, 1H, NH, exchangeable with D_2O), 11.20 (s, 1H, –COO*H*, exchangeable with D_2O), 2.30 (s, 3H, –$COCH_3$).

*5.1.6. 5-Bromo-N-(2′-aminosubstitutedbenzylidene-acetyl-1′,3′,4′-oxadiazol-5′-ylmethyl)anthranilic acids (**6a–6e**)*

A solution of compound **5** (0.01 mol) in absolute ethanol (60 mL) with proper aromatic aldehydes (0.01 mol) in the presence of 2% NaOH was refluxed for 10 h, while progress and completion of the reaction was monitored by TLC. The reaction mixture was distilled off, cooled and then poured into ice–water and filtered. The solid thus obtained was recrystallised from the appropriate solvent giving compound **6**. By this procedure, compounds **6a–6e** were obtained starting from benzaldehyde, 4-methoxybenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde, 4-dimeihylaminobenzaldehyde and 4-hydroxybenzaldehyde, respectively. Their physical and analytical data are given in Table 1. Compound **6b**: m.p. 120 °C, yield 68%, $C_{20}H_{17}O_5N_4Br$ [% Calc. (Found): C, 50.74 (50.40); H, 3.59 (3.88); N, 11.84 (11.65)]%. IR (KBr) ν in cm^{-1}: 3485 (O–H); 3155 (N–H); 3060 (C–H aromatic); 2920 (CH_2), 1710(C=O); 1590 (C···C of aromatic ring), 1600 (C=N), 1200 (C–N), 1124 (C–O–C), 1040 (N–N), 550 (C–Br). ^{1}H-NMR ($CDC1_3$) δ in ppm: 7.65–7.20 (m, 7H, Ar–*H*), 8.SO (bs,

Table 1
Physical and analytical data of compounds **6a–6e** and **7a–7e**

Compd No.	R	M.P. (°C)	Yield (%)	Recrystallisation solvent	Mol. Formula	Elemental analyses [a] % C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
6a		152	62	DMF-water	$C_{19}H_{15}O_4N_4Br$	51.47	51.22	3.39	3.57	12.64	12.90
6b		120	68	ethanol-water	$C_{20}H_{17}O_5N_4Br$	50.74	50.40	3.59	3.88	11.84	11.65
6c		110	70	ethanol-water	$C_{20}H_{17}O_6N_4Br$	49.08	49.40	3.48	3.62	11.45	11.20
6d		98	55	methanol-water	$C_{21}H_{20}O_4N_5Br$	51.85	51.60	4.12	4.39	14.40	14.12
6e		138	58	methanol-water	$C_{19}H_{15}O_5N_4Br$	49.67	49.92	3.27	3.50	12.20	12.37
7a		170	70	acetone	$C_{21}H_{19}O_4N_6$ Br	50.50	50.80	3.81	3.60	16.83	16.64
7b		192	65	methanol-water	$C_{22}H_{21}O_5N_6$ Br	49.90	49.58	3.97	4.21	15.88	16.15
7c		183	62	ethanol-water	$C_{22}H_{21}O_6N_6$ Br	48.44	48.17	3.85	3.99	15.41	15.18
7d		167	55	ethanol-water	$C_{23}H_{24}O_4N_7$ Br	50.92	51.25	4.43	4.18	18.08	18.32
7e		175	44	acetic acid	$C_{21}H_{19}O_5N_6$ Br	48.93	48.70	3.69	3.80	16.31	16.01

[a] C, H, N were found within ±0.4% of theoretical value.

1H, N*H*CO, exchangeable with D_2O), 4.50 (s, 2H, N–CH_2), 5.60 (s, 1H, NH, exchangeable with D_2O), 11.40 (s, 1H, –COO*H*, exchangeable with D_2O), 3.47 (s, 3H, Ar–OCH_3), 6.85 (d, 1H, –COC*H*), 8,20 (d, 1H, =C*H*–Ar).

*5.1.7. 5-Bromo-N-[2′-amino-(1″-acetyl-5″-substitute-daryI-2′-pyrazolin-3″-yl)-1′,3′,4′-oxadiazol-5′-ylmethyl]-anthranilic acids (**7a**–**7e**)*

To a solution of the proper compound **6** (0.02 mol) in absolute ethanol (50 mL), 99% hydrazine hydrate (0.04 mol) was added drop by drop with constant stirring in the presence of few drops of glacial acetic acid. The reaction mixture was refluxed for 12 h, distilled off and cooled. The separated solid was filtered, washed with pet.ether and recrystallised from the appropriate solvent to give compound **7**, By this procedure compounds **7a**–**7e** were obtained starting from **6a**–**6e**, respectively. The physical and analytical data of compounds **7a**–**7e** are given in Table 1. Compound **7b**: m.p. 192 °C, yield 65%, $C_{22}H_{21}O_5N_6Br$ [Calc. (Found): C, 49.90 (49.58); H, 3.97 (4.21); N, 15.88 (16.15)]%. IR (KBr) ν in cm^{-1}: 3480 (O–H); 3160 (N–H); 3065 (C–H aromatic); 2923 (CH_2), 2850 (C–H of $COCH_3$), 1690 (C=O); 1550 (C···C of aromatic ring), 1580 (C=N), 1220 (C–N), 1115 (C–O–C), 1030 (N–N), 555 (C–Br). ^{1}H-NMR ($CDC1_3$) δ in ppm: 7.90–7.25 (m, 7H, Ar–*H*), 6,15 (bs, 1H, NH, exchangeable with D_2O), 4.55 (s, 2H, N–CH_2), 5.65 (s, 1H, NH, exchangeable with D_2O), 11.15 (s, 1H, –COO*H*, exchangeable with D_2O), 3.45 (s, 3H, Ar–OCH_3), 5.25 (d, 2H, CH_2), 6.95 (t, 1H, –C*H*–Ar) 2.35 (s, 3H, $COCH_3$).

*5.1.8. N-ethylacetoanthranilic acid (**1′**)*

A mixture of anthranilic acid (0.1 mol), ethyl chloroacetate (0.1 mol) and anhydrous K_2CO_3 (5.0 g) in acetone (80 mL) was refluxed for about 15 h. The excess of solvent was distilled of under reduced pressure and the resulting solid mass poured into ice water, filtered and the separated solid recrystallised from methanol–water to give compound **1′**. Compound **1′**: m.p. 115 °C, yield 60%, $C_{11}H_{13}O_4N$ [Calc. (Found): C, 59.19 (59.37); H, 5.83 (5.58); N, 6.28 (6.12)]%. IR (KBr) ν in cm^{-1}; 3500 (O–H); 3150 (N–H); 3040 (C–H aromatic); 2930 (CH_2); 1720 (C=O); 1595 (C···C of aromatic ring), ^{1}H-NMR ($CDC1_3$) δ in ppm: 7.50–7.25 (m, 4H, Ar–*H*), 1.35 (t, 3H, $J = 7$ Hz, –$COOCH_2CH_3$), 4.25 (q, 2H, $J = 7$ Hz, –$COOCH_2CH_3$), 4.65 (s, 2H, N–CH_2), 5.90 (s, 1H, NH, exchangeable with D_2O), 12.40 (s, 1H, –COO*H*, exchangeable with D_2O).

*5.1.9. N-(thiosemicarbazido carbonyl methyl)anthranilic acid (**2′**)*

A mixture of *N*-ethylacetoanthranilic acid (**1′**) (0.02 mol) and thiosemicarbazide (0.02) in methanol (50 mL) was refluxed for 10 h. The solvent was removed under reduced pressure and the viscous mass poured over ice–water, filtered and recrystallised from methanol–water to afford compound **2′**. Compound **2′**: m.p. 128 °C, yield 75%, $C_{10}H_{12}O_3N_4S$ [Calc. (Found): C, 44.78 (44.52); H, 4.48 (4.10); N, 22.90 (20.70)]%. IR (KBr) ν in cm^{-1}: 3475 (O–H); 3135 (N–H); 3060 (C–H aromatic); 2930 (CH_2); 1700 (C=O), 1560 (C···C of aromatic ring), ^{1}H-NMR ($CDCl_3$) δ in ppm: 7.60–7.45 (m, 4H, Ar–*H*), 8.15 (m, 4H, $NHNHCSNH_2$, exchangeable with D_2O), 4.55 (s, 2H, N–CH_2), 5.82 (s, 1H, NH, exchangeable with D_2O), 12.45 (s, 1H, –COO*H*, exchangeable with D_2O).

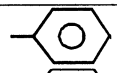
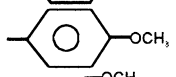
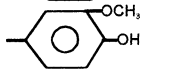
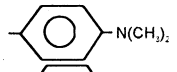
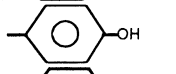
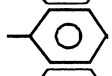
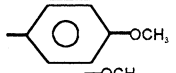
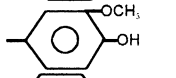
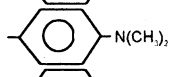
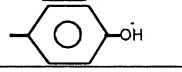
*5.1.10. N-(2′-amino-1′,3′,4′-thiadiazol-5′-ylmethyl)-anthranilic acid (**3′**)*

A mixture of compound **2′** (0.05 mol) and cone, H_2SO_4 (20 mL) was kept overnight at room temperature, then poured into cold water, neutralised with liquid ammonia and filtered. The product thus obtained was recrystallised from ethanol–water. Compound **3′**; m.p. 137 °C, yield 62%, $C_{10}H_{10}O_2N_4S$ [Calc. (Found): C, 48.0 (48.39); H, 4.0 (3.88); N, 22.4 (22.12)]%. IR (KBr) ν in cm^{-1}: 3475 (O–H); 3175 (N–H), 3350 (NH_2); 3065 (C–H aromatic); 2930 (CH_2); 1720(C=O); 1585 (C···C of aromatic ring), 1595 (C=N), 1210 (C–N), 1045 (N–N), 730 (C–S–C); ^{1}H-NMR ($CDC1_3$) δ in ppm; 7.62–7.35 (m, 4H, Ar–*H*), 6.35 (bs, 2H, NH_2, exchangeable with D_2O), 4.60 (s, 2H, N–CH_2), 5.80 (s, 1H, NH, exchangeable with D_2O), 12.35 (s, 1H, –COO*H*, exchangeable with D_2O).

*5.1.11. N-(2′-aminoacetyl-1′,3′,4′-thiadiazol-5′-ylmethyl)anthranilic acid (**4′**)*

To a solution of compound **3′** (0.01 mol) in dry benzene (50 mL), acetyl chloride (0.01 mol) was added drop by drop at 0–5 °C temperature with constant stirring. The reaction mixture was further stirred for 2 h at room temperature and refluxed for 6 h, then distilled off. The resulting mixture was poured onto crushed ice. The solid thus obtained was recrystallised from methanol–water to afford pure compound **4′**. Compound **4′**: m.p. 160 °C, yield 65%, $C_{12}H_{12}O_3N_4S$ [Calc. (Found): C, 49.32 (49.13); H, 4.11 (4.30); N, 19.18 (19.42)]%. IR (KBr) ν in cm^{-1}: 3480 (O–H); 3180 (N–H); 3070 (CH_2); 2935 (C–H aliphatic); 2840 (C–H of $COCH_3$), 1715 (C=O); 1565 (C···C of aromatic ring), 1590 (C=N), 1180 (C–N), 1040 (N–N), 730 (C–S–C); ^{1}H-NMR ($CDC1_3$) δ in pprn: 7.50–7.30 (m, 4H, Ar–*H*), 8.20 (bs, 1H, N*H*CO, exchangeable with D_2O), 4.55 (s, 2H, N–CH_2), 5,85 (s, 1H, NH, exchangeable with D_2O), 12.22 (s, 1H, –COO*H*, exchangeable with D_2O), 2.35 (s, 3H, $COCH_3$).

Table 2
Physical and analytical data of compounds **5′a–5′e** and **6′a–6′e**

Compd No.	R	M.P. (°C)	Yield (%)	Recrystallisation solvent	Mol. Formula	Elemental analyses [a] % C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
5′a		148	50	methanol-water	$C_{19}H_{16}O_3N_4S$	60.00	59.80	4.21	4.37	14.74	14.92
5′b		125	60	ethanol-water	$C_{20}H_{18}O_4N_4S$	58.54	58.30	4.39	4.59	13.66	13.80
5′c		90	55	ethanol-water	$C_{20}H_{18}O_5N_4S$	56.34	56.12	4.23	4.50	13.15	13.34
5′d		105	50	ethanol-water	$C_{21}H_{21}O_3N_5S$	59.57	59.39	4.96	4.77	16.55	16.20
5′e		118	65	ethanol-water	$C_{19}H_{16}O_4N_4S$	57.58	57.78	4.04	4.28	14.14	14.40
6′a		133	60	methanol-water	$C_{21}H_{20}O_3N_6S$	57.80	57.43	4.59	4.75	19.27	19.50
6′b		145	72	methanol-water	$C_{22}H_{22}O_4N_6S$	56.65	56.50	4.72	4.96	18.03	18.27
6′c		165	54	ethanol-water	$C_{22}H_{22}O_5N_6S$	54.77	54.54	4.56	4.80	17.43	17.22
6′d		150	50	acetic acid-water	$C_{23}H_{25}O_3N_7S$	57.62	57.88	5.22	5.06	20.46	20.70
6′e		171	65	benzene-pet. ether	$C_{21}H_{20}O_4N_6S$	55.75	55.90	4.42	4.20	18.58	18.19

[a] C, H, N were found within ±0.4% of theoretical value.

*5.1.12. N-(2′-aminosubstitutedbenzylideneacetyl-1′,3′,4′-thiadiazol-5′-ylmethyl)anthranilic acids (**5′a–5′e**)*

A solution of compound **4′** (0.01 mol) in methanol (60 mL) was refluxed with proper aromatic aldehyde (0.01 mol) in the presence of 2% NaOH for 10 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, filtered. The product thus obtained was recrystallised from the appropriate solvent giving compound **5′**, By this procedure compounds **5′a–5′e** were synthesized starting from methoxybenzaldchyde, 4-dimethylaminobenaldehyde and 4-hydroxybenzaldehyde, respectively. The physical and analytical data of compounds **5′a–5′e** are given in Table 2. Compound **5′b**: m.p. 125 °C, yield 60%, $C_{20}H_{18}O_4N_4S$ [Calc. (Found): C, 58.54 (58.30); H, 4.39 (4.59); N, 13.66 (13.80)]%. IR (KBr) ν in cm^{-1}: 3490 (O–H); 3180 (N–H); 3025 (C–H aromatic); 2910 (CH_2), 1700 (C=O); 1570 (C···C of aromatic ring), 1605 (C=N), 1190 (C–N), 720 (C–S–C), 1035 (N–N), ^{1}H-NMR ($CDCl_3$) δ in ppm; 7.70–7.15 (m, 8H, Ar–*H*), 8.55 (bs, 1H, N*H*CO, exchangeable with D_2O), 4.75 (s, 2H, N–C*H*$_2$), 5.90 (s, 1H, NH exchangeable with D_2O), 12.10 (s, 1H, –COO*H*, exchangeable with D_2O), 6.55 (d, 1H, –COC*H*), 8.15 (d, 1H, =C*H*–Ar) 3.40 (s, 3H, Ar–OC*H*$_3$).

*5.1.13. N-[2′-amino(1″-acetyl-5″-substitutedaryl-2″-pyrazolin-3″-yl)-1′,3′,4′-thiadiazol-5′-ylmethyl]-anthranilic acids (**6′a–6′e**)*

To a solution of the proper compound **5′** (0.02 mol) in absolute ethanol (50 mL), 99% hydrazine hydrate (0.04 mol) was added drop by drop with constant stirring in the presence of glacial acetic acid. The reaction mixture was refluxed for 12 h, then distilled in vacuum and cooled. The separated solid was filtered, washed with pet.ether and recrystallised from the appropriate solvent to give compound **6′**. By this procedure, compounds **6′a–6′e** were obtained starting from **5′a–5′e**, respectively. The physical and analytical data of compounds **6′a–6′e** are depicted in Table 2. Compound **6′b**: m.p. 145 °C, yield 72%, [Calc. (Found): C, 56.65 (56.50); H, 4.72 (4.96); N, 18.0.3 (18.27)]%. IR (KBr) ν in cm^{-1}: 3500 (O–H); 3180 (N–H); 3020 (C–H aromatic); 2900 (CH_2), 2860 (C–H of $COCH_3$), 1715(C=O); 1555 (C···C of aromatic ring), 1600 (C=N), 735 (C–S–C), 1035 (N–N); ^{1}H-NMR ($CDCl_3$) δ in ppm: 7.65–7.10 (m, 8H, Ar–*H*), 6.20 (bs, 1H, N*H*, exchangeable with D_2O), 4.60 (s, 2H, N–CH_2), 5.95 (s, 1H, NH, exchangeable with D_2O), 12.15 (s, 1H, –COO*H*, exchangeable with D_2O), 3.45 (s, 3H, Ar–OCH_3,), 2.40 (s, 3H, $COCH_3$), 5.20 (d, 2H, CH_2), 6.70 (t, 1H, =C*H*–Ar).

5.2. Pharmacology

The compounds [**6a**–**6e**; **7a**–**7e**; **5′a**–**5′e** and **6′a**–**6′c**] were evaluated for their anti-inflammatory activity and acute toxicity. Phenylbutaone, a potent anti-inflammatory compound, was used as reference drug for comparison. The experiments were performed on albino rats of the Charles Foster strain of either sex of 70–95 days weighing 80–140 g, and pregnancy was excluded in females. The animals were maintained at the following conditions 25 ± 2 °C, 50 ± 5% relative humidity, 12 h light/dark cycle. Food and water were freely available up to the time of experiments. Acute toxicity was tested in albino mice (20–25 g) maintained at above mentioned conditions. The test compounds were dissolved in propylene glycol.

5.2.1. Acute toxicity study

The test compounds were administered orally at different dose levels in separate groups of animals. After 24 h of drug administration percent mortality in each group was observed. From the data obtained, Approximate Lethal Dose (ALD_{50}) was calculated by the method of Smith [11].

5.2.2. Anti-inflammatory activity

This activity was performed by the following the procedure of Winter et al. [12] on groups of six animals each. A freshly prepared suspension of carrageenan (1.0 in 0.9% saline; 0.05 mL) was injected under the planter aponeurosis of right hind paw of the rat. One group was kept as control and treated with propylene glycol. The animals of standard drug and drug treated groups were pretreated with standard drug and test compounds given orally 1 h before the carrageenan injection, respectively. The volume of foot was measured before 1 and 3 h after carrageenan treatment, with help of pleythysmometer. 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.

Table 3
Pharmacological data of compounds **6a**–**6e**, **7a**–**7e**, **5′a**–**5′e** and **6′a**–**6′e**

Compound number	Acute toxicity (ALD50 mg kg^{-1} p.o.)	Anti-inflammatory activity		Ulcerogenic activity (UD_{50} mg kg^{-1} i.p.)
		mg kg^{-1} p.o.	% Inhibition of oedema	
6a	>800	50	32.57 [a]	–
6b	>800	50	40.39 [b]	–
6c	>800	50	38.7	–
6d	>800	50	24.78 [b]	–
6e	>800	50	28.23 [a]	–
7a	>800	50	40.2 [a]	
7b	>1600	25	29.75 [b]	
		50	50.66 [c]	170.52
		100	70.18 [b]	
7c	>800	50	44.7 [a]	–
7d	>800	50	32.92 [b]	–
7e	>800	50	36.3 [b]	–
5′a	>800	50	30.2	
5′b	>800	50	39.72 [b]	
5′c	>800	50	35.92 [a]	–
5′d	>800	50	21.52 [a]	–
5′e	>800	50	29.35 [a]	–
6′a	>800	50	39.71 [b]	–
6′b	>1600	25	27.52 [a]	
		50	47.56 [b]	156.2
		100	67.20 [b]	
6′c	>800	50	41.72 [a]	
6′d	>800	50	36.8 [b]	–
6′e	>800	50	38.75 [c]	–
Phenylbutazone	>280	25	26.56 [a]	
		50	45.52 [c]	66.6
		100	64.70 [b]	

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

5.2.3. Ulcerogenic activity

This activity was done according to the method of Verma et al. [13]. In this method, adult albino rats, fasted 24 h prior to the administration of drugs, were divided into groups of ten animals each. Water was allowed ad libitum to the animals. The test compounds and standard drugs were given intraperitoneally and the animals 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 haemorrhage 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.

Acknowledgements

We are thankful to CDRI, Lucknow, for elemental and spectral analyses of newly synthesised compounds.

References

[1] P.A. Insel, in: J.G. Hardman, L.G. Limberd, P.B. Molinoff, R.W. Ruddon, A.G. Qilman (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw Hill, New York, 1996, pp. 638–657.
[2] B. Goel, T. Ram, R. Tyagi, E. Bansal, A. Kumar, D. Mukherjee, J.N. Sinha, Eur. J. Med. Chem. 34 (1999) 265–269.
[3] A. Kumar, B.P. Jaju, J.N. Sinha, Ind. J. Pharm. Sci. 1 (1990) 257–260.
[4] R.H. Udupi, S.N. Rao, A.R. Bhat, Ind. J. Hetrocycl. Chem. 7 (1998) 217–220.
[5] E. Bansal, V.K. Srivastava, A. Kumar, Ind. J. Pharm. Sci. (1999) 385–388.
[6] F. Manna, F. Chimenti, A. Bolasco, M.L. Cenicola, M.O. Amico, C. Parrillo, F. Rossi, E. Marmo, Eur. J. Med. Chem. 27 (1992) 633–639.
[7] F.A. Omar, N.M. Mahfoax, M.N. Rahaman, Eur. J. Med. Chem. 31 (1996) 819–825.
[8] L.V.G. Nargund, G.R.N. Reddy, V. Hariprassad, J. Pharm. Sci. 83 (1994) 246–248.
[9] S.K. Srivastava, S. Srivasiava, S.D. Srivastava, Ind. J. Chem. 38B (1999) 183–187.
[10] B.R. Rani, U.T. Bhalerao, M.F. Rahman, Ind. J. Chem. 29B (1990) 995–998.
[11] Q.E. Smith, Medicinal Chemistry, vol. 1, Butterworth, London, 1960.
[12] C.A. Winter, E.A. Fisley, G.W. Nuss, Proc. Soc. Exp. Biol. New York 111 (1962) 544.
[13] M. Verma, J.N. Sinha, V.R. Gujrati, Pharmacol. Res. Commun. 13 (1981) 967–969.
[14] G. Mazzone, F. Bonina, G. Pulisi, R.A. Reina, D. Consentinoc, G. Balandino, Farmaco. Ed. Sci. 37 (1982) 685–700.
[15] A.A. Wheeler, W.M. Oats, J. Am. Chem. Soc. 32 (1910) 770.