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Prodrugs and mutual prodrugs: synthesis of some new pyrazolone and oxadiazole analogues of a few non-steroidal anti-inflammatory drugs

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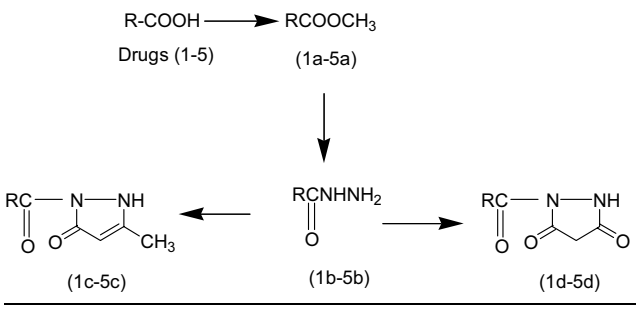
Naproxen, probenecid, diclofenac, ibuprofen and indomethacin were converted to hydrazide derivatives via their methyl ester by reacting with hydrazine hydrate, which were further condensed with β-keto esters to get the pyrazolone derivatives. The hydrazide derivatives of probenecid and diclofenac were also reacted with biphenyl acetic acid while naproxen hydrazide was reacted with *p*-chloro benzoic acid besides biphenyl acetic acid to synthesize their oxadiazole analogues. Some selected members of the compounds prepared were screened for their anti-inflammatory and analgesic activity.

1. Introduction

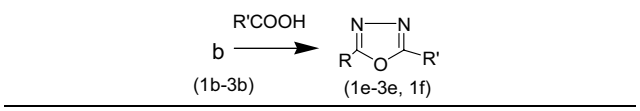
Designing prodrugs or modifications of the marketed formulations of the current NSAIDs has been an area of considerable research with the prime focus on reducing their gastrointestinal toxicity [1–10]. In order to improve the therapeutic index through prevention of gastrointestinal toxicity, the derivatization of the carboxylate function of representative NSAIDs, i.e. naproxen (1), probenecid (2), diclofenac sodium (3), ibuprofen (4) and indomethacin (5) as starting templates has been undertaken.

These studies incorporate the esterification of the drugs **1**–**5** to their respective methyl esters **1a**–**5a** followed by reaction with hydrazine hydrate to obtain the hydrazides **1b**–**5b**, which were condensed with ethyl acetoacetate and diethyl malonate separately in order to get their pyrazolone derivatives (Scheme 1). The hydrazides of naproxen (**1b**), probenecid (**2b**) and diclofenac (**3b**) were condensed with biphenyl acetic acid in presence of phosphoryl chloride under refluxing conditions to give the oxadiazole analogues (Scheme 2). Naproxen hydrazide was also condensed with *p*-chloro benzoic acid to give the oxadiazole derivative (**1f**). Anti-inflammatory and analgesic activity of some selected members of the compounds prepared was investigated.

Scheme 1



Scheme 2



2. Investigations, results and discussion

2.1. Chemistry

Naproxen (**1**), probenecid (**2**), diclofenac (**3**), ibuprofen (**4**) and indomethacin (**5**) were methylated by refluxing with absolute methanol in presence of sulphuric acid to give the methyl esters **1a**–**5a** or alternatively, the methylation was done by refluxing with dimethyl sulphate in dry acetone in presence of anhydrous potassium carbonate. The esters **1a**–**5a** were crystallized from methanol to give TLC pure compounds.

Attempted preparation of the hydrazide derivatives **1b**–**5b** by refluxing **1a**–**5a** with hydrazine hydrate succeeded only with **1b** to **4b**. Instead of the expected **5b** the compound obtained from **5a** was identified as *p*-chloro benzoyl hydrazide. Further reaction of **1b** to **4b** with ethyl acetoacetate in the ratio of 1 : 2 succeeded only with **1b**. The product from **3b** was identified as uncyclized hydrazone (**3c′**) whereas **2b** and **4b** were inert to the reaction. Similar reaction of **1b** to **4b** with diethyl malonate in equimolar ratio gave **1d** and **4d**, while **2b** and **3b** were inert to the reaction. Condensation of **1b** to **3b** with biphenyl acetic acid in presence of $POCl_3$ yielded the oxadiazole **1e**, **2e**, and **3e**. **1b** also underwent condensation with *p*-chloro benzoic acid to give **1f**. Physico-chemical data for the synthesized compounds are summarized in Tables 1 and 2.

2.2. Pharmacology

Compounds **1c**–**1f**, **3c′** and **3e** were tested for their anti-inflammatory activity by the methods of Winter et al. [11]. The mean percent change in paw volume in control and

Table 1: Physico-chemical properties of the compounds synthesized

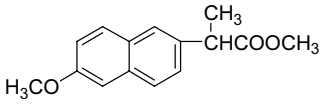
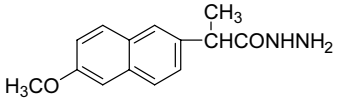
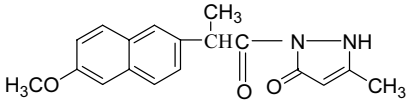
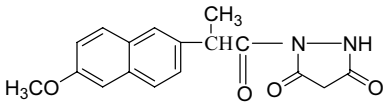
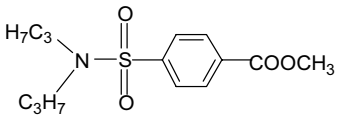
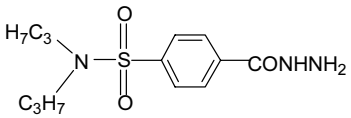
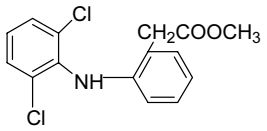
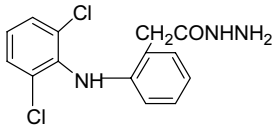
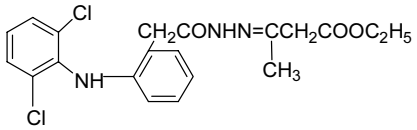
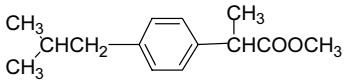
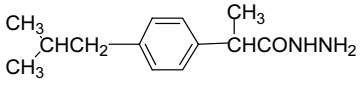
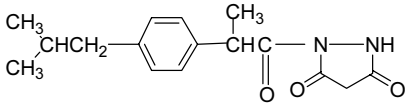
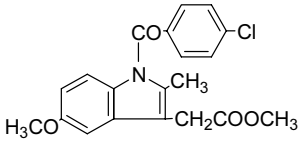
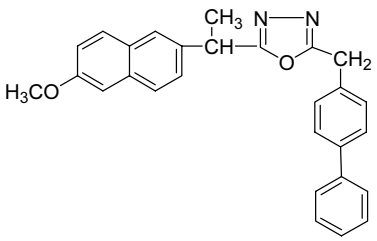
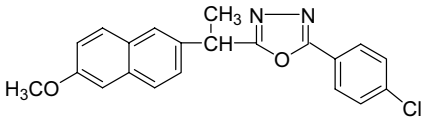
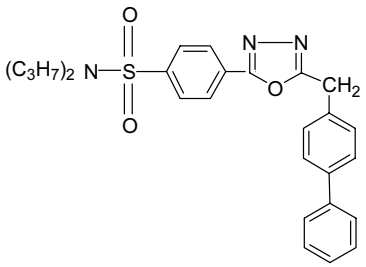
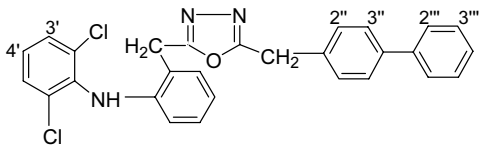
	Compd.	Rf value	Yield (%)	M.P. (°C)	Formula
1a		0.92	83	86–08	$C_{15}H_{16}O_3$
1b		0.55	70	118–20	$C_{14}H_{16}N_2O_2$
1c		0.52	73	220	$C_{18}H_{18}N_2O_3$
1d		0.3	70	126–28	$C_{17}H_{15}N_2O_4$
2a		0.9	85	64	$C_{14}H_{21}NO_4S$
2b		0.53	80	160	$C_{13}H_{21}N_3O_3S$
3a		0.9	85	122–24	$C_{15}H_{13}NCl_2O_2$
3b		0.45	80	160	$C_{14}H_{13}N_3Cl_2O$
3c′		0.6	52	138–39	$C_{20}H_{21}N_3Cl_2O_3$
4a		0.9	61	Semi-Solid	$C_{14}H_{20}O_2$
4b		0.76	60	73–05	$C_{13}H_{20}N_2O$
4d		0.77	52	118	$C_{16}H_{19}N_2O_3$
5a		0.66	80	94–06	$C_{20}H_{18}NO_4Cl$

Table 2: Physico-chemical properties of oxadiazole analogues

	Compd.	Rf value	Yield (%)	M.P. (°C)	Formula
1e		0.65	65	128–30	$C_{28}H_{24}N_2O_2$
1f		0.9	65	218–20	$C_{21}H_{17}N_2O_2Cl$
2e		0.59	58	126–30	$C_{27}H_{29}N_3SO_3$
3e		0.88	68	162–64	$C_{28}H_{21}N_3OCl_2$

drug treated animals were compared and expressed as percent edema inhibition. After the first hour, compound **1e** had almost double the activity, while **1d**, **1f** and **3e** were significantly more active than the parent molecule naproxen and diclofenac at the same dose level. Since the molecular weights of these compounds are higher, the relative activity at the same molar concentration would be even higher (Table 3).

Compounds **1e**–**1f**, **3c′** and **3e** were tested for their analgesic activity by tail flick method [12]. Out of the six compounds tested, compounds, **1d** and **3c′** showed good analgesic activity as compared to the corresponding reference drugs at the same dose levels.

3. Experimental

3.1. Synthetic studies

The melting points of all the compounds were taken on a liquid paraffin bath in open capillary tubes and are uncorrected. Purity of the compounds was tested on TLC plates (silica gel G, E. Merck) with the solvent systems benzene-methanol (8 : 2) except for compounds **1c** and **1d**, where benzene-acetone (8 : 2) was used. TLC solvent system used for all the oxadiazole derivatives was benzene-ethanol (8 : 2). The spots were located under UV light and/or by exposure to iodine vapours. ^{1}H NMR spectra of all the compounds were recorded on 60 MHz, 90 MHz or 300 MHz instruments using TMS as internal standard in solvent $CDCl_3$ or a mixture of $CDCl_3$ with DMSO-d_6 due to solubility reasons. The chemical shift values have been reported in δ values. MS of the compounds were recorded on Jeol JMS-D 300 instrument. All the compounds gave satisfactory elemental analysis.

Table 3: Anti-inflammatory and analgesic properties of pyrazolone and oxadiazole analogues

Compd.	Dose mg/kg	Anti-inflammatory activity		Analgesic activity		
		Percentage Inhibition against carageenan induced edema		Tail flick latency		
		At 60 min	At 180 min	At 30 min	At 60 min	At 120 min
1c	70	5	69	3.6 ± 0.47	3.9 ± 3	4 ± 3.3
1d	70	67	43	4.5 ± 2.6	3.1 ± 3	2.75 ± 0.5
1e	70	90	51	3.25 ± 1.3	3.5 ± 0.58	3.13 ± 1.09
1f	70	65	15	3.25 ± 1.26	3 ± 0.58	3.25 ± 1.26
3c′	10	60	25	5 ± 2.8	7.5 ± 0.58	2.75 ± 0.82
3e	10	94	15	3.1 ± 1.09	3.2 ± 0.4	3.25 ± 0.95
Naproxen	70	48	62	4 ± 1.29	5 ± 0.87	5 ± 0.87
Diclofenac	10	75	40	4 ± 1.29	5 ± 0.87	4.75 ± 0.96
Normal saline	1 ml/kg	–	–	2.75 ± 1.5	2±0.53	2.5±1.29

3.1.1. Synthesis of methyl esters **1a**–**5a***: General procedure*

The methyl esters were synthesized by the standard procedures either by refluxing appropriate drugs having carboxylic acid group with dry methanol in presence of a few drops of sulphuric acid or by refluxing in dry acetone solution with dimethyl sulphate in presence of anhydrous potassium carbonate. Usual work up of the reaction mixture gave desired esters **1a**–**5a**, characterized by NMR data. All these compounds were checked for their purity on TLC plates (Table 1).

3.1.1.1. Methyl ester of naproxen (**1a**)

^{1}H NMR (δ, ppm): 1.57 (d, CH$\underline{\text{CH}}_3$), 3.6 (s, $COOCH_3$), 3.86 (q, CH), 3.9 (s, OCH_3), 7.11 (d, J = 3 Hz, H-5), 7.14 (m, H-7), 7.38 (d, J = 9 Hz, 3 Hz, H-3), 7.6 (d, J = 3 Hz, H-1), 7.69 (m, H-4, H-8).

3.1.1.2. Methyl ester of probenecid (**2a**)

^{1}H NMR (δ, ppm): 0.87 (t, 2 × CH_3), 1.53 (h, 2 × CH_2), 3.1 (t, 2 × CH_2), 3.96 (s, $COOCH_3$), 7.8 (d, J = 9 Hz, A_2/B_2), 8.1 (d, J = 9 Hz, A_2/B_2).

3.1.1.3. Methyl ester of diclofenac (**3a**)

^{1}H NMR (δ, ppm): 3.66 (s, CH_2 + NH), 3.87 (s, OCH_3), 6.51 (d, H-6), 6.9 (t, H-4), 6.97 (t, H-4′), 7.09 (t, H-5), 7.28 (m, H-3), 7.34 (d, H-3′, H-5′).

3.1.1.4. Methyl ester of ibuprofen (**4a**)

Semisolid in nature. NMR was not recorded.

3.1.1.5. Methyl ester of indomethacin (**5a**)

^{1}H NMR (δ, ppm): 2.38 (s, $-CH_3$), 3.67 (s, CH_2), 3.70 (s, $-OCH_3$), 3.83 (s, $-COOCH_3$), 6.6 (dd, H-6), 6.85(d, H-7), 6.95 (d, H-4) 7.4(d, A_2/B_2), 7.66(d, A_2/B_2).

3.1.2. Synthesis of the hydrazide derivatives **1b**–**4b***: General procedure*

The methyl ester **a** (0.01 mol) was refluxed for 4 h with hydrazine hydrate (99%) in slight excess of the molar ratio in ethanol (purified by distilling over NaOH) (20 ml). After completion of the reaction, ethanol was distilled off to get a solid mass, which was crystallized and checked on a TLC plate for purity. It was characterized on the basis of spectral data.
Alternatively the methyl ester **a** (0.01 mol) was dissolved in ethanol (20 ml). Hydrazine hydrate (99%) was added in slight excess of the molar ratio. The contents were stirred first at room temperature for 1–2 h and then refluxed for further 2 h. After completion of the reaction, the contents were concentrated to a small volume. On leaving at room temperature a product crystallized out which was filtered, checked for its purity by TLC and characterized on the basis of NMR data (Table 1).

3.1.2.1. Hydrazide derivative of naproxen (**1b**)

^{1}H NMR (δ, ppm): 1.54 (d, CH$\underline{\text{CH}}_3$), 3.67 (q, −CH), 3.9 (s, OCH_3), 7.11 (d, J = 3 Hz, H-5), 7.15 (dd, J = 9 Hz, 3 Hz, H-7), 7.35 (dd, J = 9 Hz, 3 Hz, H-3), 7.65 (d, J = 3 Hz, H-1), 7.7 (d, J = 9 Hz, H-4), 7.72 (d, J = 8 Hz, H-8).

3.1.2.2. Hydrazide derivative of probenecid (**2b**)

^{1}H NMR (δ, ppm): 0.86 (t, 2 × CH_3), 1.53 (hex, 2 × CH_2), 3.08 (t, 2 × CH_2), 7.8 (d, J = 9 Hz, A_2/B_2), 8.1 (d, J = 9 Hz, A_2/B_2). Molecular formula $C_{13}H_{12}N_3O_3S$ (M^+) 299 (m/z) 270, 199, 135, 104.

3.1.2.3. Hydrazide derivative of diclofenac (**3b**)

^{1}H NMR (δ, ppm): 3.66 (s, CH_2 + NH), 6.51 (d, H-6), 6.9 (t, H-4), 6.97 (t, H-4′), 7.09 (t, H-5), 7.28 (m, H-3), 7.34 (m, H-3′, H-5′).

3.1.2.4. Hydrazide derivative of ibuprofen (**4b**)

^{1}H NMR (δ, ppm): 0.89 (d, 2 × $-CH_3$), 1.51 (d, $-CH-\underline{CH}_3$), 1.84 (m, −CH), 2.45 (d, CH_2), 3.51 (q, $-\underline{CH}-CH_3$), 3.89 (bs, NH_2), 6.92 (bs, NH), 7.09 (d, J = 9 Hz, A_2/B_2), 7.18 (d, J = 9 Hz, A_2/B_2). Molecular formula $C_{13}H_{20}N_2O$ (M^+) 220 (m/z) 188, 161, 119, 105, 91.

3.1.3. Synthesis/attempted synthesis of the pyrazolone derivative (**c**) *from the hydrazide derivative by condensing with ethyl acetoacetate: General procedure*

A mixture of hydrazide **b** (0.01 mol) and ethyl acetoacetate (0.02 mol) was refluxed for 4–5 h in methanol, solvent was then distilled off and the resulting mass was poured into ice cold water. A solid mass, which separated out, was filtered, washed with water and crystallized from methanol. It was examined for purity on TLC plate and characterized on the basis of NMR data.

3.1.3.1. Pyrazolone derivative of naproxen (**1c**)

^{1}H NMR (δ, ppm): 1.58 (d, $-CH_3$), 1.59 (s, $-CH_3$) 3.73 (q, −CH), 3.87 (s, $-OCH_3$), 7.1 (m, H-5, H-7), 7.32 (dd, H-3), 7.65 (m, H-4, H-8, H-1), 8.09 (ring olefenic proton).

3.1.3.2. Pyrazolone derivative of diclofenac (**3c**′)

Usual work up of the reaction mixture gave a compound which was identified as uncyclized hydrazone (**3c**′). ^{1}H NMR (δ, ppm): 1.2 (t, $-C\underline{H}_3CH_2$), 4.2 (q, $-CH_3-\underline{CH}_2$), 1.92 (s, CH_3), 3.4 (s, CH_2), 4.16 (s, CH_2 + NH), 6.51 (d, H-6), 6.9 (t, H-4), 6.97 (t, H-4′), 7.09 (t, H-5), 7.25 (m, H-3), 7.33 (d, H-3′, H-5′), 8.57 (s, NH).

3.1.4. Synthesis/attempted synthesis of the pyrazolone derivative (**d**) *from the hydrazide derivative by condensing with diethyl malonate: General procedure*

A mixture of hydrazide **b** and diethyl malonate in equimolar ratio was refluxed in dry pyridine for 3–4 h. The reaction mixture was then poured onto crushed ice. A solid mass, which separated out, was filtered, dried, and crystallized from methanol. It was characterized on the basis of NMR data.

3.1.4.1. Pyrazolone derivative of naproxen (**1d**)

^{1}H NMR (δ, ppm): 1.54 (d, CH$\underline{\text{CH}}_3$), 3.68 (s, CH_2), 3.85 (q, $-\underline{\text{CH}}CH_3$), 3.87 (s, OCH_3), 7.11 (m, H-5, H-7), 7.70 (m, H-4, H-8), 7.37 (dd, H-3), 7.6 (d, H-1).

3.1.4.2. Pyrazolone derivative of ibuprofen (**4d**)

^{1}H NMR (δ, ppm): 0.86 (d, 2 × CH_3), 1.4 (d, CH$\underline{\text{CH}}_3$), 1.82 (septet, CH), 2.5 (d, CH_2), 3.2 (s, CH_2 from diethyl malonate moiety), 3.5 (q, $CH_3\underline{\text{CH}}$), 7.04 (d, A_2/B_2), 7.26 (d, A_2/B_2).

3.1.5. Synthesis of 1,3,4-oxadiazole derivatives **1e**, **1f**, **2e**, **3e** *from hydrazide derivatives of NSAIDs* (**b**) *and aromatic acids-biphenyl acetic acid and p-chlorobenzoic acid*

A mixture of appropriate acid (2 mmol) and hydrazide derivative **b** of NSAIDs (2 mmol) was refluxed in phosphoryl chloride (5 ml) on an oil bath for 5–6 h. The reaction mixture was cooled to room temperature and poured onto crushed ice in small portions while stirring. The contents were basified with sodium bicarbonate solution and the resulting solid mass was filtered, dried, and crystallized to give the corresponding 1,3,4 oxadiazole derivative. The physical properties and characterization data have been given in Table 2.

3.1.5.1. 1,3,4-oxadiazole derivative of naproxen (**1e**) by condensing **1b** with biphenyl acetic acid

^{1}H NMR (δ, ppm): 1.8 (d, CH_3), 3.91 (s, OCH_3), 4.2 (s, CH_2), 4.4 (q, CH), 7.12 (m, H-5, H-7), 7.31, 7.44 (d each A_2/B_2), 7.36 (m, H-3′, 4′, 5′), 7.4 (m, H-3), 7.54 (d, H-1), 7.55 (m, H-2′, 6′), 7.63 (d, H-8), 7.7 (d, H-4).

3.1.5.2. 1,3,4-oxadiazole derivative of naproxen (**1f**) by condensing **1b** with *p*-chlorobenzoic acid

^{1}H NMR (δ, ppm): 1.8 (d, CH_3), 3.91 (s, OCH_3), 4.4 (q, CH), 7.14 (m, H-5, H-7), 7.4 (m, H-3), 7.46 (d, A_2/B_2), 7.7 (m, H-1, H-4), 7.92 (m, H-8), 8.03 (d, A_2/B_2).

3.1.5.3. 1,3,4-oxadiazole derivative of probenecid (**2e**) by condensing **2b** with biphenyl acetic acid

^{1}H NMR (δ, ppm): 0.86 (t, 2 × CH_3), 1.52 (hex, 2 × CH_2), 3.10 (t, 2 × CH_2), 4.34 (s, CH_2), 7.34 (m, 1 H), 7.42 (m, 4 H), 7.58 (m, 4 H), 7.91 and 8.15 (each d, J = 9 Hz, 2 × A_2/B_2, H-2, 6; H-3, 5).

3.1.5.4. 1,3,4-oxadiazole derivative of diclofenac (**3e**) by condensing **3b** with biphenyl acetic acid

^{1}H NMR (δ, ppm): 3.97 (s, CH_2), 4.27 (s, CH_2), 7.04 (d, H-2″ and H-6″, J = 7 Hz, A_2/B_2), 7.27 (m, H-3, 6), 7.35 (m, H-4, 5), 7.44 (m, H-3″, 5″ and H-3‴, 5‴), 7.59 (m, H-2‴, 6‴ and H-3′, 4′, 5′), 7.69 (m, H-4‴).

3.2. Pharmacological studies

All the test compounds and drugs were suspended in 2% gum acacia and were prepared on the day of experiment. The parent drugs, naproxen (70 mg/kg) and diclofenac (10 mg/kg) were used as the standard reference compounds and injected intraperitoneally. Doses of all the test compounds were calculated with reference to the standard drug and injected intraperitoneally. 0.9% NaCl (1 ml/kg, i.p) solution was injected as control.

3.2.1. Anti-inflammatory activity

The anti-inflammatory activity of the synthesized compounds was investigated according to Winter et al. The studies were done on healthy rats of either sex, divided in groups of six each, weighing between 120–200 g. The paw volume was determined plethysmographically at 60 and 180 min after carrageenan injection and the anti-inflammatory activity was calculated in comparison to the standard drug at the same dose level. Results are expressed as percentage inhibition of the edema.

3.2.2. Analgesic activity

The analgesic activity of the test compounds was evaluated by the tail immersion method. Albino mice of either sex, weighing 25–30 g were selected for testing by immersing the tail in hot water at 55 ± 5 °C and the basal reaction time was noted. Each group was administered different test compound and standard drug, as 2% acacia suspension intaperitoneally. To one group normal saline was injected which acted as control. Immediately after administration of the drug and at intervals of 30, 60 and 120 min, the reaction time was recorded.

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