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Synthesis and Antiinflammatory Activity of Novel Indazolones

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In this study, a series of new N^2 substituted 1,2-dihydro-3*H*-indazol-3-ones (**3a-d**) as well as their condensed pyrazolo, pyridazino derivatives such as pyridazino[1,2-*a*]indazole-6,9,11-triones (**4a-h**) and 3,9-dioxo-3*H*,9*H*-pyrazolo[1,2-*a*]indazole (**7**) were synthesized. The antiinflammatory activity of some synthesized compounds was determined by carrageenan-induced rat paw edema technique using diclofenac as reference drug. The pharmacological data showed that most of the tested compounds exhibited a significant long lasting antiinflammatory activity, which in the case of compound **3b** was superior to that of diclofenac.

Key words: Pyridazino[1,2-*a*]indazole-6,9,11-triones, 3,9-Dioxo-3*H*,9*H*-pyrazolo[1,2-*a*]indazole, Antiinflammatory activity

INTRODUCTION

Non steroidal antiinflammatory drugs (NSAIDs) are the most widely used medications in the world; 300 million people worldwide are estimated to use NSAIDs (Mutschler and Derendorf, 1995). The pharmacological activity of these agents is related to the suppression of prostaglandin biosynthesis from arachidonic acid by inhibiting the activity of the enzyme cyclooxygenases (COX-1 and COX-2) (Schindler *et al.*, 1998). Recently, it was discovered that most of the NSAIDs in the market show greater selectivity for COX-1 which provides cytoprotection in the gastrointestinal (GI) tract than COX-2 which mediates inflammation. Therefore chronic use of these non-selective agents may elicit appreciable gastrointestinal ulceration and bleeding. The incidence of clinically significant GI side effects due to NSAIDs is high (over 30%) and causes some patients to abandon NSAID therapy (Lombardino, 1985). Synthetic approaches based upon NSAID chemical modification have been taken with the aim of improving their safety profile (Wallace *et al.*, 1994). One such approach has been to mask the NSAID carboxylic acid moiety into NSAID ester prodrugs that hydrolyze in vivo to release the active parent NSAID (De Caprariis, 1994). In recent years highly selective COX-2 inhibitors have recently been developed and marketed as promising gastroprotective agents (Talley, 1999). Many of these selective agents have been developed and marketed such as celecoxib and refecoxib which have been extensively studied and show exceptional antiinflamatory properties with reduced GI toxicity (Vane and Botting, 1998). Later on, some potential limitations of long term COX-2 inhibitor therapy include ulcer exacerbation in high-risk patients, delayed gastroduodenal ulcer healing, thrombosis due to prostacyclin deficiency and kidney toxicity (Wolfe *et al.*, 1999). Thus COX-2 inhibitor have not eliminated the need for improved drugs in the NSAID area (Bandarage, 2000).

Among these agents, indazole derivatives known was benzydamine, which was the first reported indazole derivative recognized in the management of pain and inflammation topically (Modeer and Yucel, 1999). In this direction, our efforts were focused on the development of new NSAIDs with indazole ring system since the very limited work have been made in this area (Mosti *et al.*, 1988; 1992; 2000; Wrzeciono *et al.*, 1993; Tse *et al.*, 1996; Badawey *et al.*, 1998; Schindler *et al.*, 2000). Later on several orally active indazole derivatives such as triazolo-[1,2-*a*]indazoles triazepino[1,2-*a*]indazoles and pyrazolo-[1,2-*a*]indazole-1,3,9-trione derivatives were reported to possess 5-lipoxgenase inhibition with antiinflammatory, analgesic activities (Badran *et al.*, 1999a; Badran 1999b). On the other hand, other fused polycyclic hetero ring system such as diftalone, a phthalazino[2,3-*b*]phthalazine-5,12(7*H*,14*H*)-dione has been used as NSAID (Schiatti *et al.*, 1974).

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The antiinflammatory property exhibited by indazole, pyrazole, pyridazinone derivatives prompted us to undertake the synthesis of certain indazolone derivatives fused with pyrazolone or pyridazinone ring systems. Also, 4-oxo-4-(3-oxo-1,3-dihydroindazol-2-yl)butyric acid and derivatives, structurally similar to the well known antiinflammatory drugs, febufen (Child *et al.*, 1980) and flobufen (Kuchar *et al.*, 1997) were designed with view to evaluating their antiinflammatory activity.

MATERIALS AND METHODS

Chemistry

Melting points were obtained on a Graffin apparatus and are uncorrected. Microanalyses for C, H and N were carried out at the microanalytical center, Cairo University. IR spectra were recorded on a Shimadzu 435 Spectrometer, using KBr discs.^{1}H-NMR spectra were performed on a Jeol NMR FXQ-200 MHZ Spectrometer, using TMS as internal standard. Mass spectra were recorded on a GCMS-QP 1000 EX, Mass Spectrometer. Progress of the reactions was monitored by TLC using precoated aluminum sheets silica gel MERCK 60 F 254 and was visualized by UV lamp.

4-Oxo-4-(3-oxo-1,3-dihydroindazol-2-yl)butyric acid and derivatives (3a-d)

General procedure: To a stirred solution of (1) (0.01 mol) in glacial acetic acid (20 ml), the respective acid anhydride (0.01 mol) (viz; succinic anhydride (**2a**), *N*-trifluoroacetylaspartic anhydride (**2b**), 2,2-dimethylsuccinic anhydride (**2c**), and 2-phenylsuccinic anhydride (**2d**) was added and the mixture was refluxed for 3 h. The solvent was evaporated under reduced pressure to half its volume, cooled and poured onto water, the precipitate thus formed was filtered, dried and recrystallized from the appropriate solvent (Table I, II).

Ring closure of (3a-d) to the corresponding pyridazino[1,2-*a*]indazole-6,9,11-triones (4a-d)

General procedure: A solution of respective acid (**3a-d**, 0.001 mol) in acetic anhydride (10 ml) was refluxed for 2 h. The excess acetic anhydride was evaporated under reduced pressure and the residue was triturated with ice-cold water. The formed precipitate was filtered, washed with water and crystallized from the appropriate solvent (Table I, II).

Pyridazino[1,2-*a*]indazole-6,9,11-trione. (4e), 6a,7,8,9,10,10a-Hexahydroindazolo[1,2-*b*]phthalazine-6,11,13-trione (4f), Indazolo[1,2-*b*]phthalazine-6,11,13-trione (4g), and 4b,7,10a-triazabenzo[b]fluorene-5,10,11-trione (4h)

General procedure: A solution of **1** (1.34 g, 0.01 mol) and the respective acid anhydride (**2e-h**) (viz,. maleic anhydride (**2e**) 1,2-cyclohexane dicarboxylic anhydride (**2f**), phthalic anhydride (**2g**) and 3,4-pyridine dicarboxylic anhydride (**2h**) (0.01 mol) in glacial acetic acid (10 ml) was stirred under reflux for 3 hours. The reaction mixture was cooled, poured into water (50 ml), the precipitate thus formed was filtered, washed with 5% sodium bicarbonate solution (50 ml) and crystallized from the appropriate solvent (Table I, II).

2-(3-Oxo-1,3-dihydroindazol-2-ylmethylene)malonic acid diethyl ester (5)

A mixture of **1** (2.68 g, 0.02 mol) and 2-ethoxymethylenemalonic acid diethyl ester (4.32 g, 0.02 mol) was heated in oil bath at 120°C for 2 h. The reaction mixture was cooled and triturated with ethanol (20 ml). The formed precipitate was filtered, washed with ethanol, dried and crystallized from the appropriate solvent (Table I, II).

2-Ethoxycarbonylpyrazolo[1,2-*a*]indazole-3,9-dione (6)

A mixture of 1 (2.68 g, 0.02 mol) and 2-ethoxymethylenemalonic acid diethyl ester (15 ml) was heated while stirring in an oil bath at 170°C for 6 h. The reaction mixture was cooled in ice, the yellow precipitate thus formed was filtered, washed with ethanol, dried and crystallized from ethanol affording yellow crystalline solid (Table I, II).

2-(3-Oxo-1,3-dihydroindazol-2-ylmethylene)malonic acid monoethyl ester (VIIa)

A solution of 4 (2 g, 0.0077 mol) in sodium hydroxide (0.62 g. 0.15 mol) and 10 ml water was stirred at room temperature for 2 h. The solution was then acidified with conc. hydrochloric acid and the formed precipitate was filtered, washed with water and dried. The solid was crystallized from appropriate solvent to give (**7a**) as pale yellow crystals (Table I, II).

2-Benzylcarbamoyl-3-(3-oxo-1,3-dihydroindazol-2-yl)acrylic acid ethyl ester (7b)

To solution of **6** (0.5 g, 0.0019 mol) in ethanol (20 ml), benzylamine (0.2 ml, 0.0019 mole) was added and the mixture was refluxed for 2 h. The solvent was evaporated under reduced pressure. The residue was triturated with dil HCl, filtered, washed with water, dried and crystallized from appropriate solvent affording a white solid (Table I, II).

2-Aminocarbonyl-3-(3-oxo-1,3-dihydroindazol-2-yl)acrylic acid ethyl ester (VIIc)

To a solution of **6** (0.5 g, 0.002 mol) in absolute ethanol (5 ml), conc. ammonium hydroxide solution (1 ml) was added and the mixture was stirred at room temperature for 1 h. The precipitate formed was filtered, dried and

Table I. Physical, analytical and IR spectral data of the synthesized compounds

Comp No.	m.p. (°C) (Sovent of crystallization)	Yield %	Mol. Form. (Mol. Wt.)	Elemental Analysis %			IR KBr (cm^{-1})
				C	H	N	
3a	190 (ethanol)	60	$C_{11}H_{10}N_2O_4$ (234)	56.41 56.70	4.30 4.60	11.96 11.80	3100-3000 (NH, OH) 1710, 1680,1660 (3 C=O)
3b	225 (ethanol)	50	$C_{13}H_{10}F_3N_3O_5$ (345)	45.23 45.00	2.92 2.80	12.17 11.90	3100-3000 (NH, OH), 2900-2700 (CH, aliph) 1710, 1700, 1660, 1620 4 C=O
3c	200 (ethanol)	55	$C_{13}H_{14}N_2O_4$ (262)	59.54 59.60	5.38 5.40	10.68 10.50	3100-3000 (NH, OH), 2900-2800 (CH, aliph) 1700, 1680-1620) (3 C=O)
3d	220 (ethanol)	65	$C_{17}H_{12}N_2O_3$ (310)	65.8 65.60	4.55 4.30	9.03 8.80	3100-3000 (NH, OH), 2900-2700 (CH, aliph.) 1720, 1700, 1620 (3 CO)
3a	108 (ethanol)	45	$C_{11}H_8N_2O_3$ (216)	61.11 61.30	3.73 3.60	12.96 12.90	2900 (CH aliph.) 1780, 1760, 1760 (3 C=O)
4b	110 (ethanol)	40	$C_{13}H_8F_3N_3O_4$ (327)	47.72 47.60	2.46 2.20	12.84 12.90	3100 (NH), 2900 (CH aliph) 1780, 1770, 1720, 1620 (4 C=O)
4c	110 (ethanol)	45	$C_{13}H_{14}N_2O_3$ (244)	63.93 64.10	4.95 5.00	11.47 11.40	2900 (CH aliph.), 1760, 1700, 1680 (3 C=O)
4d	102 (ethanol)	52	$C_{17}H_{12}N_2O_3$ (292)	69.86 69.90	4.14 4.10	9.58 9.40	2900 (CH aliph.), 1780, 1770, 1730 (3 C=O)
4e	190 (ethanol)	50	$C_{11}H_6N_2O_3$ (214)	61.69 61.60	2.82 3.20	13.08 12.90	2900 (CH aliph.), 1780, 1760, 1700 (3 C=O)
4f	170 (ethanol)	60	$C_{15}H_{14}N_2O_3$ (270)	66.66 66.40	5.22 5.10	10.36 10.40	2900-2600 (CH aliph.), 1780, 1760, 1700 (3 C=O)
4g	265 (ethanol)	80	$C_{15}H_8N_2O_3$ (264)	68.18 68.00	3.05 2.90	10.60 10.70	1760, 1740, 1680 (3 C=O)
4h	230 (ethanol)	55	$C_{14}H_7N_3O_3$ (265)	63.39 63.50	2.64 2.50	15.84 15.70	1770, 1760, 1680 (3 C=O)
5	122 (ethanol)	50	$C_{15}H_{16}N_2O_5$ (304)	59.21 59.20	5.30 5.20	9.21 9.30	3500-3300 (NH), 3100, 2950 CH aliph), 1780-1760, 1720-1700 (4 C=O)
6	215 (ethanol)	45	$C_{13}H_{10}N_2O_4$ (258)	60.46 60.40	3.90 3.60	10.85 10.60	1760 (ester), 1720-1710 (2 C=O)
7a	178 (ethanol)	37	$C_{13}H_{12}N_2O_5$ (276)	56.52 56.40	4.38 4.30	10.14 10.30	3200, 3100 (NH, OH) 1720, 1710, 1680 (C=O ester, amides)
7b	120 (benzene)	40	$C_{20}H_{19}N_3O_4$ (365)	65.75 65.40	5.20 5.00	11.50 11.70	3400 (NH), 2950 (CH aliph),1720, 1700, 1680 (C=O ester, amides)
7c	245 (acetonitrile)	63	$C_{13}H_{13}N_3O_4$ (275)	56.72 56.80	4.72 4.70	15.27 15.30	3450, 3350 (NH, NH_2), 2950 (CH aliph),1760, 1720, 1680 (C=O ester, amides)
7d	208 (ethanol)	55	$C_{13}H_{14}N_4O_4$ (290)	53.79 53.50	4.86 4.60	19.31 19.10	3300, 3200 (NH, NH_2), 2950 (CH aliph),1700, 1690, 1640 (C=O ester, amides)
8a	108 (benzene/pet.ether)	60	$C_{20}H_{17}N_4O_4F$ (396)	60.60 60.60	4.32 4.30	14.13 14.40	3400 (NH), 2900 (aliph CH.) 1720, 1700, 1660 (three C=O)
8b	90 (benzene/pet.ether)	62	$C_{21}H_{20}N_4O_5$ (408)	61.76 61.90	4.90 4.80	13.72 13.80	3400 (NH), 2900 (aliph CH.) 1720, 1700, 1660 (three C=O)
8c	110 (benzene/pet.ether)	59	$C_{22}H_{22}N_4O_6$ (438)	60.27 60.30	5.02 4.80	12.78 12.70	3400-3450 (NH), 2950 (aliph CH.) 1720, 1700, 1660 (three C=O)

crystalized from the appropriate solvent to give a white crystaline product (Table I, II).

2-Hydrazinocarbonyl-3-(3-oxo-1,3-dihydroindazol-2-yl)acrylic acid ethyl ester (7d)

To a solution of **6** (1 g, 0.0038 mol) in ethanol (5 ml) hydrazine hydrate 99% (0.24 ml, 0.0076 mol) was added and the mixture was stirred at ambient for 1.5 h. The precipitate thus formed was filtered affording white fluffy solid which was crystallized from appropriate solvent (Table I, II).

Table II. ^{1}H-NMR and EIMS Spectral data of the synthesized novel compounds

Compound No.	Spectral Data
3a	**^{1}H-NMR (DMSO-d_6):** δ 2.60 (s, 4H, $2CH_2$), 7.38 (t, 1H, *J*=7.5 Hz, aromatic H), 7.60 (t, 1H, *J*=7.5 Hz, aromatic H),7.78 (d, 1H, J=7.5, aromatic H), 8.25 (d, 1H, J=7.5, aromatic H), 12.00 (br 1H, NH, D_2O exchangeable).
3b	**^{1}H-NMR (DMSO-d_6):** δ 2.60 (d, 2H, CH_2) , 3.60 (br, 1H, NH, D_2O exchangeable) 4.40 (m 1H, CH), 7.37 (t, 1H, *J*=7.5 Hz, aromatic H),7.60 (t, 1H, *J*=7.5 Hz, aromatic H), 7.77 (d, 1H, *J*=7.5, aromatic H), 8.24 (d, 1H, *J*=7.5 Hz, aromatic H), 11.8 (br 1H, NH, D_2O exchangeable).
4a	**^{1}H-NMR(DMSO-d_6):** δ 2.70 (s, 4H, $2CH_2$), 7.45 (t, 1H, *J*=7.5, aromatic H), 7.75 (t, 1H, *J*=7.5 Hz, aromatic H),7.80 (d, 1H, *J*=7.5 Hz, aromatic H), 8.35 (d, 1H, *J*=7.5 Hz, aromatic H).
4b	**^{1}H-NMR (DMSO-d_6):** δ 2.48 (d, 2H, CH_2) , 3.6 (br s, 1H, NH, D_2O exchangeable) 4.41-4.50 (m, 1H, CH), 7.45 (t, 1H, *J*=7.5 Hz, aromatic H), 7.70 (t, 1H, *J*=7.5 Hz, aromatic H), 7.77 (d, 1H, *J*=7.5 Hz, aromatic H), 8.34 (d, 1H, *J*=7.5 Hz, aromatic H). **EIMS m/z:** 328, M+1 (0.04%), 176 (100%).
4e	**^{1}H-NMR (DMSO-d_6):** δ 7.17 (dd, 2H, *J*=13.5 Hz, pyridazindione H), 7.53 (t, 1H, *J*=7.5 Hz, aromatic H), 7.91 (t, 1H, *J*=7.5 Hz, aromatic H), 8.01 (d, 1H, *J*=7.5 Hz, aromatic H), 8.34 (d, 1H, *J*=7.5 Hz, aromatic H). **EIMS m/z:** 214, M^+ (100%), 186 (39%), 149 (38%).
4g	**^{1}H-NMR (DMSO-d_6):** δ 7.54 (t, 1H, *J*=7.5 Hz, aromatic H), 7.76 (t, 1H, *J*=7.5 Hz, aromatic H), 8.02-8.15 (m, 3H, aromatic H), 8.31 (m, 2H, aromatic H), 8.50 (d, 1H, *J*=7.5 Hz, aromatic H).
4h	**^{1}H-NMR (DMSO-d_6):** δ 7.62 (t, 1H, *J*=7.5 Hz, aromatic H), 8.03 (t, 1H, *J*=7.5 Hz, aromatic H), 8.06 (d, 1H, *J*=7.5 Hz aromatic H), 8.20 (d, 1H, *J*=7.5 Hz aromatic H), 8.50 (d, 1H, *J*=7.5 Hz, aromatic H), 9.23 (c, 1H, *J*=7.5 Hz, aromatic H). 9.52 (s, 1H, aromatic H). **EIMS m/z:** 265, M^+ (83%), 236 (7%), 105 (100%).
5	**^{1}H-NMR ($CDCl_3$):** δ 1.35 (t, 6H, *J*= 7.2 Hz, $OCH_2\underline{CH_3}$,), 4.29 (q, 4H, $OC\underline{H_2}CH_3$, *J*= 7.2 Hz), 7.45-7.48 (t, 1H, *J*=8.0 Hz, aromatic H), 7.56-7.58 (t, 1H, *J*=8.0 Hz, aromatic H),7.82 (d, 1H, *J*=8.0 Hz, aromatic H), 7.97 (d, 1H, *J*=8 Hz, aromatic), 12.02 (br, 1H, NH).
6	**^{1}H-NMR($CDCl_3$):** δ 1.22 (t 3H, *J*= 7.2 Hz, $OCH_2\underline{CH_3}$,), 4.30 (q 2H, *J*= 7.2 Hz, $OC\underline{H_2}CH_3$,), 7.39-7.46 (m, 2H, aromatic H), 7.80 (t, 1H, *J*=8 Hz, aromatic H), 8.03 (d,1H, *J*=8 Hz, aromatic H), 8.47 (s,1H, pyrazolone H). EIMS m/z: 258, M^+ (55.66%), 213 (100%), 186 (89%), 158 (8.2%).
7a	**EIMS m/z:** 276, M^+ (17.07 %), 232 (55%),186 (100%).
7b	**^{1}H-NMR, ($CDCl_3$):** δ1.36 (t, 3H, *J*=7.0, $OCH_2\underline{CH_3}$), 4.28 (q, 2H, *J*=7.0, $O\underline{CH_2}CH_3$), 4.47 (d, 2H, *J*=5.8 Hz, $\underline{CH_2}$-NH), 6.54- (br s, 2H, 2 NH, D_2O exchangeable), 7.17-7.22 (m, 5H, aromatic H), 7.45 (t, 1H, *J*=8.0 Hz, aromatic H), 7.48-7.74 (m, 3H, aromatic H),7.82 (s, 1H, azomethine H).
7c	**EIMS (m/z):** 275, M^+ (43.8 %), 229 (100%).
7d	**^{1}H-NMR (DMSO-d_6):** δ 1.21 (t, 3H, *J*=7.2 Hz, $OCH_2\underline{CH_3}$), 4.20 (q, 2H, *J*=7.2 Hz, $OC\underline{H_2}CH_3$), 7.41-7.56 (m, 4H, aromatic H), 8.18 (s, 1H, =C$\underline{H}$ azomethine), 9.47 (br, $NHNH_2$, D_2O exchangeable), 12.01 (NH, indazolone, D_2O exchangeable).
8b (*E* &*Z*)	**^{1}H-NMR ($CDCl_3$):** δ 1.11 (t, 3H, *J*=7.2 Hz, $OCH_2\underline{CH_3}$), 3.70 (s, 3H, $OC\underline{H_3}$) 4.15 (q, 2H, *J*=7.2 Hz, $OC\underline{H_2}CH_3$), 6.85 (d, 2H, aromatic H), 7.26 (d, 2H, aromatic H), 7.52-7.65 (m, 4, aromatic H), 7.80 (s, 1H, N-C$\underline{H}$=C), 8.14 (s, 1H, N=C$\underline{H}$), 10.51 (br, NH, D_2O exchangeable), 11.50 (s, indazolone NH, D_2O exchangeable). δ 1.12 (t, 3H, *J*=7.2 Hz, $OCH_2\underline{CH_3}$), 3.81 (s, 3H, $OC\underline{H_3}$) 4.20 (q, 2H, *J*=7.2 Hz, $OC\underline{H_2}CH_3$), 6.90 (d, 2H, aromatic H), 7.48 (d, 2H, aromatic H), 7.50-7.65 (m, 4 H, aromatic H), 7.80 (s, 1H, N-C$\underline{H}$=C), 8.25 (s, 1H, N=C$\underline{H}$), 10.50 (br., NH, D_2O exchangeable), 11.70 (s., indazolone NH, D_2O exchangeable).

2-(2-Arylidene-hydrazinocarbonyl)-3-(3-oxo-1,3-dihydroindazol-2yl)acrylic acid ethyl ester (8a-c)

General procedure: A mixture of (**7d**) (0.5 g, 0.0017 mol) and the respective aromatic aldehyde (viz., 4-fluorobenzaldehyde, 4-anisaldehyde and 3,4-dimethoxybenzaldehyde) (0.0017 mol) in ethanol (10 ml) was refluxed for 3 h. The solvent was evaported in vacuo and the residue was crystallized from the appropriate solvent to give (**8a-c**) (Table I, II).

Evaluation of antiinflammatory activity

Acute inflammation depends on the release of chemical mediators which bring about oedema formation as a result of extravasation of fluid and proteins from the local microvasculature and accumulation of polymorphonuclear leukocytes at the inflammatory site. (Winter *et al.,* 1962), represented an inflammatory reaction induced in rat hind paw by the subplantar injection of carrageenan. This model of inflammation is considered the most conventional one for acute inflammation. In our study this model was used to assess the antiinflammatory activity of seven compounds (**3a**, **3b**, **4f**, **6**, **7a**, **7b** and **8a**) in comparison with the reference antiinflammatory drug, diclofenac.

Methods

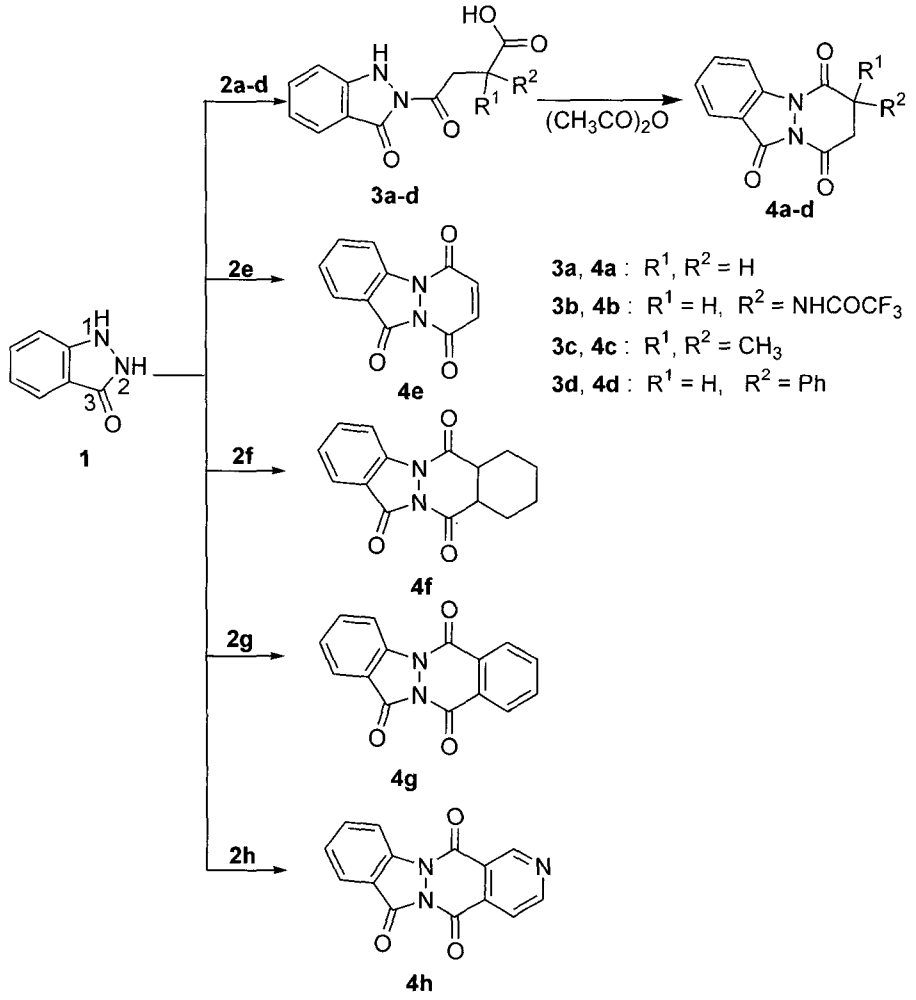
Male Wistar rats (170-200 g, National Research Centre, Giza, Egypt), were housed under suitable laboratory conditions through the period of investigation. Animals were fed standard pellet chow (El-Nasr Chemical Company, Cairo, Egypt) and allowed free access to water. Rats were divided into ten groups each of 6 animals. Group 1 served as normal control and received only vehicle, with paw size ranging between 1 and 1.2 mm. The second group recieved a subplantar injection of carrageenan (0.1 ml of a 1% suspension in a 0.85% saline) into the right hind paw. The other eight groups (3, 4, 5, 6, 7, 8, 9 and 10)

were injected with a single dose (2 mg/kg) of either diclofenac or one of the 7 tested compounds, respectively and concurrently with the injection of carrageenan. Paw volume was measured with the plethysmometer (Ugo-Basile, Varese, Italy) immediately after the injection. Subsequent readings of the volume of the same paw were carried out at 1, 2, 3, 4 and 24 h compared to the initial readings.

RESULTS AND DISCUSSION

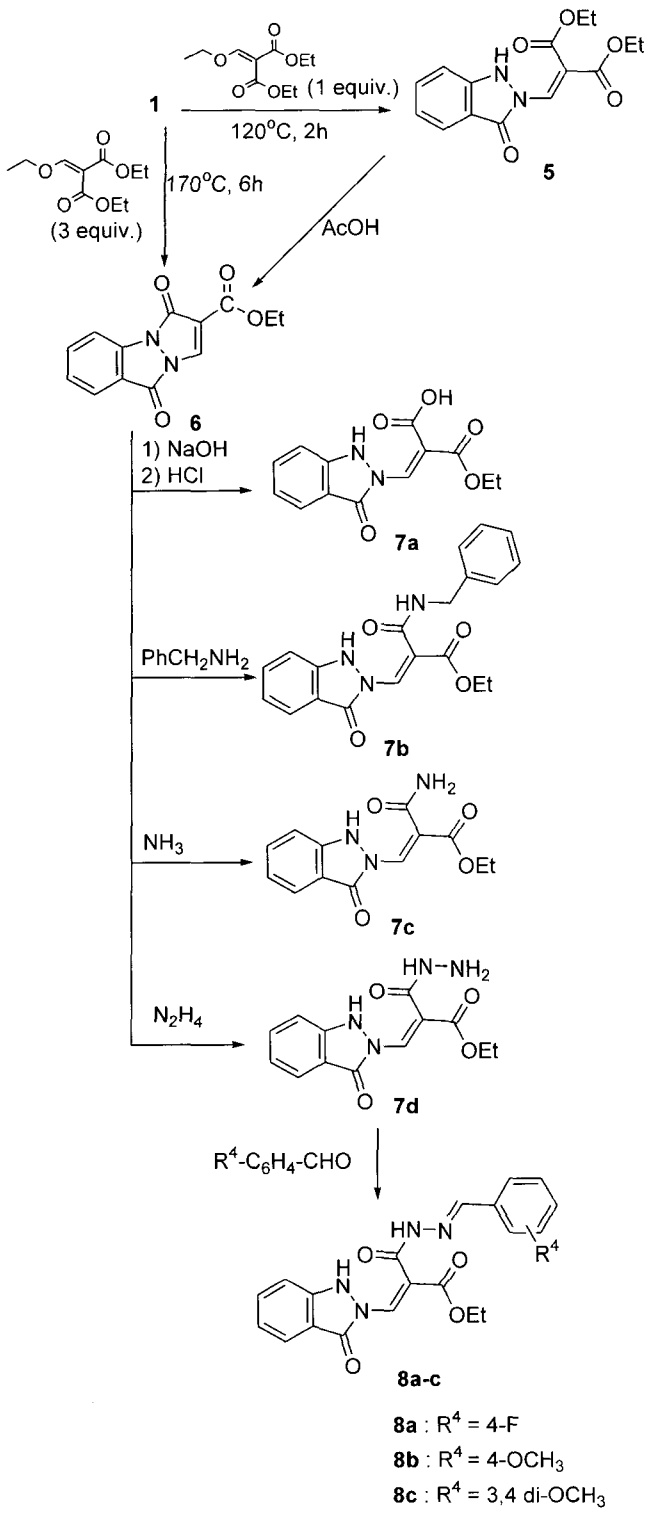
Chemistry

The starting material, 1,2-dihydro-3*H*-indazol-3-one (**1**) was prepared from anthranilic acid using literature methods (Baiocchi *et al.*, 1978; Dumitrascu *et al.*, 1991). Also, *N*-trifluoroacetylaspartic anhydride (**2b**) was prepared as reported by reacting aspartic acid with trifluoroacetic anhydride in trifluoroacetic acid (Moretti and Foresta, 1998). In scheme 1, Acylation of **1** with certain cyclic anhydrides (**2a-d**) in acetic acid afforded the 4-oxobutyric acid derivatives (**3a-d**). Cyclocondensation of these acids with acetic anhydride afforded the pyridazino[1,2-*a*]indazole-6,9,11-triones derivatives (**4a-d**). On the other hand, acylation and concomitant cyclization took place with maleic anhydride (**2e**), cyclohexane-1,2-dicarboxylic anhydride (**2f**), phthalic anhydride (**2g**) and 3,4-pyridine dicarboxylic acid anhydride, (**2h**) gave pyridazino[1,2-*a*]indazole-6,9,11-tri-



2a = succinic anhydride
2b = trifluoroacetylaspartic anhydride
2c = 2,2-dimethylsuccinic anhydride
2d = 2-phenylsuccinic anhydride
2e = maleic anhydride
2f = 1,2-cyclohexane dicarboxylic anhydride
2g = phthalic anhydride
2h = 3,4-pyridine dicarboxylic anhydride

Scheme 1. Synthesis of compound **4a-h**



Scheme 2. Synthesis of compound **7a-d** and **8a-c**

one (**4e**), 6a,7,8,9,10,10a-hexahydroindazolophthalazine-6,11,13-trione (**4f**), indazolo[1,2-*b*]phthalazine-6,11,13-trione (**4g**) and 4b,7,10a-triazabenzo[b]fluorene-5,10,11-trione (**4h**) respectively.

Scheme 2 represents Michael addition of **1** to 2-ethoxymethylenemalonic acid diethyl ester (DEEM) affording the diester (**5**). 3,9-Dioxo-3*H*,9*H*-pyrazolo[1,2-*a*]indazole (**6**) was obtained either from the diester (**5**) via acid catalyzed cyclization of the diester (**5**) or in one step reaction of the indazolone **1** with excess DEEM.

Ring opening of the pyrazole ring of **6** with aqueous NaOH, benzylamine, ammonium hydroxide, and hydrazine hydrate took place affording the acrylic acid esters (**7a-d**). Arylidene derivatives (**8a-c**) were obtained by reacting the hydrazide (**7d**) with certain aromatic aldehydes.

The synthetic routes followed for obtaining the target compounds are outlined in Scheme 1 and 2.

Monoacylation of the indazolone (**1**) took place at N^2 upon the reaction with succinic anhydride (**2a**), N-trifluoroacetylaspartic anhydride (**2b**), 2,2-dimethylsuccinic anhydride (**2c**), acid 2-phenylsuccinic anhydride (**2d**), with least sterically hindered carbonyl group and the substituent trifluoroacetylamino, dimethyl and phenyl groups far from the acylation site affording the corresponding 4-oxobutyric acid derivatives (**3a-d**). Upon cyclodehydration of the latter acids (**3a-d**) with acetic anhydride, the corresponding cyclized pyridazinoindazolone (**4a-d**) were obtained. The cyclized pyridazinoindazolone (**4e-g**) were obtained in one step without separation of their intermediate acids via the reaction of the indazolone (**1**) with the cyclic anhydrides (**2e**, **2f**, and **2g**). On the other hand, upon acylation of the indazolone (**1**) with 3,4-pyridine dicarboxylic anhydride, the more electrophilic carbonyl group para to pyridine-N was thought to be attacked by *N*-2 of the indazolone and therefore the regioisomer (**4h**) was obtained exclusively.

In scheme **2**, Michael addition of the N^2 of indazolone (**1**) to the β carbon of the diethyl ethoxymethylenemalonate reagent affording smooth formation of the diester derivative (**5**).

3,9-Dioxo-3*H*,9*H*-pyrazolo[1,2-*a*]indazole-2-carboxylic acid ethyl ester (**6**) was obtained either upon acid catalyzed cyclization of the **5** with acetic acid or via one-pot reaction of the indazolone (I) with excess diethyl ethoxymethylenemalonate reagent. Similarly to acylation, the more nucleophilic *N*-2 nitrogen preferentially adds to the β carbon of the DEEM reagent affording the diester (**5**) followed by cyclization and elimination of C_2H_5OH. The IR spectrum of the latter pyrazoloindazolone (**6**) demonstrated the absence of the -NH stretching band at 3300-3400 cm^{-1}. Furthermore, facile ring opening of (**6**) with various bases such as NaOH, benzylamine, ammonia, or hydrazine hydrate took place at the pyrazolone C=O to give **7a**, **7b**, **7c** and **7d** respectively. Upon reacting the hydrazide (**7d**) with certain aldehydes, a mixture of almost equal amounts of E and Z -arylidene isomers (**8a-c**) were obtained in each case which could be identified from ^{1}H-NMR spectral data.

Table III. Changes in hind paw oedema thickness (mm) at different time intervals (0, 1, 2, 3, 4 & 24 h), after treatment with a single dose (2 mg/kg, i.p) of either diclofenac or one of the seven tested compounds in carrageenan induced inflamed rats (means 6 animals ± SEM)

Groups under investigation	Thickness of hind paw oedema after treatment					
	At zero time	1 h	2 h	3 h	4 h	24 h
Inflamed control	3.60 ± 0.16	6.18 ± 0.08	6.28 ± 0.08	6.46 ± 0.08	6.56 ± 0.06	7.33 ± 0.17
Diclofenac	3.83 ± 0.11	4.68 ± 0.08*	4.81 ± 0.08*	4.66 ± 0.06*	4.03 ± 0.04	3.83 ± 0.08*
Compound **3a**	4.31 ± 0.15	6.08 ± 0.04!	6.10 ± 0.03!	5.83 ± 0.05*,!	5.16 ± 0.11*,!	3.4 ± 0.14*
Compound **3b**	4.00 ± 0.09	5.00 ± 0.02*,!	5.00 ± 0.05*	4.75 ± 0.04*	4.16 ± 0.11*	3.03 ± 0.04*,!
Compound **4f**	3.65 ± 0.07	5.10 ± 0.11*,!	5.10 ± 0.09*	4.93 ± 0.04*	4.33 ± 0.08*,!	3.33 ± 0.21*
Compound **6**	4.16 ± 0.12	5.30 ± 0.09*,!	5.38 ± 0.09*,!	5.23 ± 0.07*,!	4.83 ± 0.08*,!	3.33 ± 0.21*
Compound **7a**	3.93 ± 0.05	5.50 ± 0.14*,!	5.63 ± 0.15*,!	5.43 ± 0.15*,!	4.76 ± 0.07*,!	3.66 ± 0.17*
Compound **7b**	3.81 ± 0.07	5.60 ± 0.14*,!	5.78 ± 0.13*,!	5.60 ± 0.14*,!	4.73 ± 0.07*,!	3.16 ± 0.08*,!
Compound **8acd	3.50 ± 0.13	5.50 ± 0.13*,!	5.53 ± 0.18*,!	5.33 ± 0.18*,!	4.83 ± 0.08*,!	3.33 ± 0.21*

As compared with inflamed control group (*) and diclofenac treated group (!) (one-way ANOVA followed by Duncan test), $p<0.05$.
**Compound 8a as E/Z regio mixture.

Table IV. Inhibition (%) of hind paw oedema compared to the control at different time intervals, after treatment with a single dose (2 mg/kg, i.p) of either diclofenac or one of the seven tested compounds in carrageenan-induced inflamed rats (means 6 animals)

Compound	Time Intervals				
	1 h	2 h	3 h	4 h	24 h
Diclofenac	24.27	23.41	27.86	38.57	47.75
3a	1.62	2.87	9.75	21.34	53.62
3b	19.09	20.38	26.47	36.59	58.66
4f	17.48	18.79	23.68	33.99	54.57
6	14.24	14.33	19.04	26.37	54.57
7a	11.00	10.35	15.94	27.44	50.07
7b	9.39	7.96	13.31	27.90	56.89
8a	11.00	11.94	17.49	26.37	54.57

**Compound 8a as E/Z regio mixture.

Results of antiinflammatory activity

The effect of the seven tested compounds and diclofenac on carrageenan-induced oedema at different time intervals is depicted in tables (**III**, **IV**). At zero time, none of the compounds used was able to affect the paw oedema thickness. After 1 h, all the tested groups showed a small, yet significant decrease in the oedema size, ranging between 14% for compound **6** to 19% for compound **3b**. However, this reduction was still significant from that caused by diclofenac (24%). Compound **3a** was the only one that failed to reduce the inflammation significantly, as compared with the inflamed control rats. As shown in table III, IV , the tested compounds **3b**, **4f**, **7**, **7a**, **7b** and **8a** were found to have significant activity in descending order. However, compound **3a** showed the least effect while diclofenac was the most effective along the 4 h interval. After 24 h, the inflammation reached the highest size (7.33 mm) and all the tested compounds were able to decrease the paw oedema thickness, an effect which was mounted to about 45%. Compounds **3b** and **7b** were the most powerful ones in minimizing the inflammation size (≈58%), even more than diclofenac itself (47%). The slow onset of action of the tested compounds might be attributed to the slow absportion of these compounds at the site of injection.

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