Synthesis, Characterization and Analgesic Activity of New 4-Arylhydrazono-3-methoxymethyl-2-pyrazolin-5-ones

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Summary

New 4-arylhydrazono-3-methoxymethyl-2-pyrazolin-5-ones (5a-j) and 4-arylhydrazono-3-methoxymethyl-1-phenyl-2-pyrazolin-5-ones (6a-j) were synthesized by the reaction of ten novel methyl 2-arylhydrazono-4-methoxy-3-oxobutanoates (4a-j) with hydrazine hydrate and phenylhydrazine, respectively. Treatment of 5a with acetic anhydride yielded 1-acetyl-4-arylhydrazono-3-methoxymethyl-2-pyrazolin-5-one (7). The structures of the tautomerically dynamic compounds were established by spectral data (IR, ^{1}H-NMR, ^{13}C-NMR, EIMS and CIMS) and elemental analysis. The analgesic activity of the title compounds was determined by the modified Koster's test. The most active compound was 4-[(4-chlorophenyl)hydrazono]-3-methoxymethyl-1-phenyl-2-pyrazolin-5-one (6c) demonstrating twice the activity (60 %) exerted by the reference drug acetylsalicylic acid (ASA, 27 %). The majority of the tested compounds were found to be more effective than ASA.

Zusammenfassung

Synthese, Charakterisierung und analgetische Aktivität von neuen 4-Arylhydrazon-3-methoxymethyl-2-pyrazolin-5-onen

Neue 4-Arylhydrazon-3-methoxymethyl-2-pyrazolin-5-one (5a-j) und 4-arylhydrazon-3-methoxymethyl-1-phenyl-2-pyrazolin-5-one (6a-j) wurden durch Reaktion von zehn neuen Methyl-2-arylhydrazon-4-methoxy-3-oxobutanoaten (4a-j) mit Hydrazinehydrat und Phenylhydrazin synthetisiert. Reaktion von 5a mit Acetanhydrid ergab 1-Acetyl-4-arylhydrazon-3-methoxymethyl-2-pyrazolin-5-on (7). Die Strukturaufklärung dieser tautomerisch dynamischen Verbindungen erfolgte mit Hilfe von Spektraldaten (IR, ^{1}H-NMR, ^{13}C-NMR, EI- und CI-Massenspektroskopie) sowie durch Elementaranalyse. Die analgetische Wirkung der synthetisiertenVerbindungen wurde mittels modifierter Kosters-Methode bestimmt. Die aktivste Substanz 4-[(4-Chlorophenyl)hydrazon]-3-methoxymethyl-1-phenyl-2-pyrazolin-5-on) (6c) war doppelt so wirksam (60 %) wie die Vergleichssubstanz Acetylsalicylsäure (ASS, 27 %). Die meisten Substanzen erwiesen sich als wirksamer als ASS.

Key words

- **Arylhydrazones, analgesic activity, synthesis, tautomerism**
- **2-Pyrazolin-5-ones, analgesic activity, synthesis, tautomerism**

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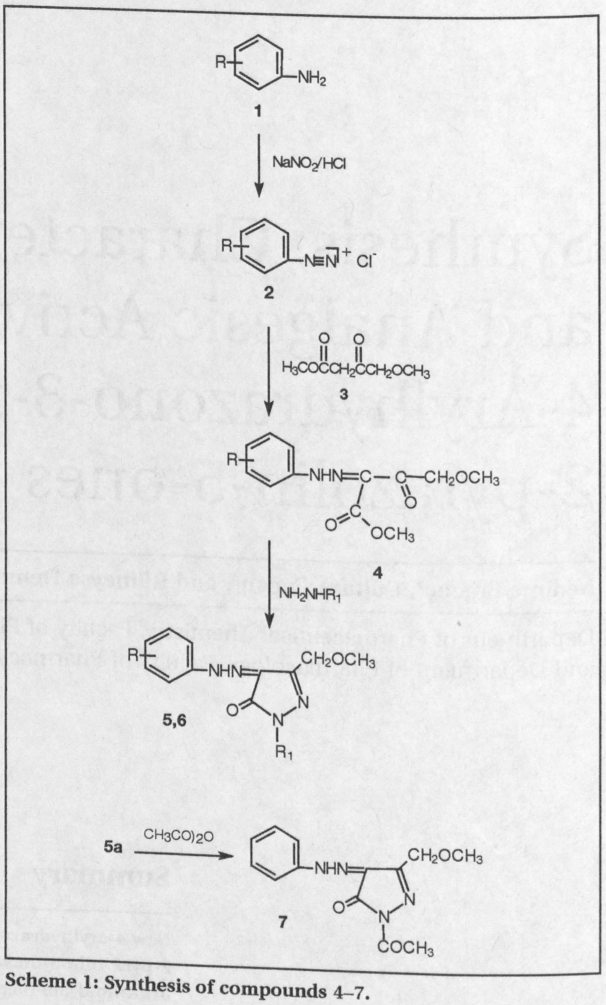
1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are the main therapeutic agents used for the relief of acute and chronic pain which affects a great part of the population. The common mechanism of action of this broad class of drugs is believed to be the inhibition of cyclooxygenase, which is a key enzyme for the conversion of arachidonic acid into prostaglandins. The major drawbacks of the NSAIDs, severe side effects including gastrointestinal ulceration and supression of renal function, make investigation of new anti-inflammatory and analgesic agents a continuing challenge.

Research focused on the pyrazole skeleton after the discovery of the analgesic properties of antipyrine, 2,3-dimethyl-1-phenyl-3-pyrazolin-5-one, has yielded many pyrazole, pyrazoline-3/5-one and pyrazolidin-3,5-dione derivatives like epirizole, benzpiperylone, morazone, nifenazone, ramifenazone, phenylbutazone and suxibuzone which found clinical application as anti-inflammatory and analgesic agents [1]. Several 4-arylazo-pyrazolin-5-one derivatives with anti-inflammatory properties have also been reported [2–4]. A recent report deals with the contribution of the arylhydrazono moiety to the analgesic and anti-inflammatory activity [5]. Based on these findings and in continuation of our work on the synthesis and biological evaluation of arylazo substituted five membered heterocycles [6–8] we designed new 4-arlyhydrazono-3-methoxymethyl-2-pyrazolin-5-one derivatives expecting to achieve improved analgesic activity profiles.

The synthesis of the compounds were made according to Scheme 1.



Scheme 1: Synthesis of compounds 4–7.

2. Materials and methods

2.1. Chemistry

IR spectra were run on a Perkin-Elmer 570 (Grating) spectrophotometer (CT, USA). ^{1}H-NMR and ^{13}C-NMR (proton coupled and decoupled) spectra were taken on Bruker AC 200 (Rheinstätten, Germany) at 200 MHz and 50.3 MHz using $CDCl_3$ or DMSO-d_6 (E. Merck, Darmstadt, Germany) as the solvent. EIMS (Electron Impact Mass Spectrometry, 70 eV) and CIMS (Chemical Ionization Mass Spectrometry, CH_4) were recorded at Freie Universität, Berlin (Germany) and Sittingbourne Research Centre (Sittingbourne, UK), respectively. Elemental analyses were performed on a Perkin-Elmer Model 240 elemental analyzer or provided by Sittingbourne Research Centre. Melting points (m.p.) were determined on a Büchi melting point apparatus (Flawil, Switzerland) and are uncorrected.

2.1.1. Methyl 2-arylhydrazono-4-methoxy-3-oxobutanoates (4a-j)

A solution of an appropriate amine (0.01 mol) in 4 % HCl (40 ml) and EtOH (40 ml) was diazotized at °C by the addition of 8 % $NaNO_2$ (10 ml) solution. The diazonium solution was added dropwise to a stirred and cooled mixture of methyl 4-methoxy-3-oxobutanoate (3) (0.0l mol), NaOAc (15 g) and 50 % EtOH (50 ml). The arylhydrazone formed was filtered off, washed with water and recrystallized from 70 % EtOH.

Spectral data of **4a**; IR [KBr, ν, cm^{-1}]: 3420 (N-H), 3040 (=C-H), 1705, 1640 (C=O). ^{1}H-NMR [200 MHz, δ, ppm, $CDCl_3$]: 3.51 (s, 3H, CH_2OCH_3), 3.87, 3.92 (2s, 3H, CO_2CH_3), 4.62 (s, 2H, CH_2OCH_3), 7.16–7.46 (m, 5H, ar), 13.01, 14.90 (2s, 1H, NH). EIMS [m/z (%)]: 250 (M^+, 30), 205 (100). CIMS [CH_4, m/z (%)]: 251 (MH^+, 100).

Spectral data of **4b**; IR [KBr, ν, cm^{-1}]: 3060 (=C-H), 1705, 1635 (C=O). ^{1}H-NMR [200 MHz, δ, ppm, $CDCl_3$]: 3.50 (s, 3H, CH_2OCH_3), 3.87, 3.91 (2s, 3H, CO_2CH_3), 4.65, 4.67 (2s, 2H, CH_2OCH_3), 7.20, 7.32 (2dd, J: 8.90, 2.05 Hz, 2H, ar), 7.52 (dd, J: 8.90, 2.05 Hz, 2H, ar), 12.95, 14.89 (2s, 1H, NH). CIMS [CH_4, m/z (%)]: 331 ($(MH+2)^+$, 98), 329 (MH^+, 100).

Spectral data of **4g**; IR [KBr, ν, cm^{-1}]: 3200, 3160 (N-H), 3080 (=C-H), 1712, 1710, 1650 (C=O). ^{1}H-NMR [200 MHz, δ, ppm, $CDCl_3$]: 1.42 (t, J: 6.90 Hz, 3H, $CO_2CH_2CH_3$), 3.50, 3.52 (2s, 3H, CH_2OCH_3), 3.89, 3.91 (2s, 3H, CO_2CH_3), 4.44 (q, J: 6.80 Hz, 2H, $CO_2CH_2CH_3$), 4.68, 4.69 (2s, 2H, CH_2OCH_3), 7.34, 7.46 (2dd, J: 8.85, 2.17 Hz, 2H, ar), 8.09 (dd, J: 8.85, 2.17 Hz, 2H, ar), 12.95, 14.80 (2s, 1H, NH) (D_2O exchange). CIMS [CH_4, m/z (%)]: 323 (MH^+, 100).

Spectral data of **4h**; IR [KBr, ν, cm^{-1}]: 3300, 3220 (N-H), 3100 (=C-H), 1712, 1710, 1650 (C=O), 1335, 1150 (SO_2). ^{1}H-NMR [200 MHz, δ, ppm, DMSO-d_6]: 3.33 (s, CH_2OCH_3, and solvent H_2O), 3.80, 3.85 (2s, 3H, CO_2CH_3), 4.57, 4.68 (2s, 2H, CH_2OCH_3), 7.32, 7.36 (2s, 2H, SO_2NH_2), 7.57, 7.68 (2dd, J: 8.82, 1.89 Hz, 2H, ar), 7.82 (dd, J: 8.82, 1.89 Hz, 2H, ar), 11.89, 14.03 (2s, 1H, NH) (D_2O exchange). CIMS [CH_4, m/z (%)]: 330 (MH^+, 100).

Spectral data of **4i**; IR [KBr, ν, cm^{-1}]: 3120 (N-H), 3080 (=C-H), 1700, 1675 (C=O), 1580, 1340 (NO_2). ^{1}H-NMR [200 MHz, δ, ppm, $CDCl_3$]: 3.51, 3.52 (2s, 3H, CH_2OCH_3), 3.92, 3.99 (2s, 3H, CO_2CH_3), 4.68, 4.69 (2s, 2H, CH_2OCH_3), 7.19–7.28 (m, ar with solvent), 7.72 (t, J: 7.70 Hz, 1H, ar), 7.97–8.19 (m, 1H, ar), 8.25 (dd, J: 8.50, 2.12 Hz, 1H, ar), 14.28, 15.56 (2s, 1H, NH). CIMS [CH_4, m/z (%)]: 296 (MH^+, 100).

2.1.2. 4-Arylhydrazono-3-methoxymethyl-1-unsubstituted/phenyl-2-pyrazolin-5-ones (5a-j and 6a-j)

A mixture of **4a-j** (0.005 mol), hydrazine hydrate or phenylhydrazine in glacial acetic acid (4–6 ml) was refluxed on a water bath for 5–30 min. The crude product thus obtained was filtered off and recrystallized from an appropriate solvent (**5a-g**, **5j-6f**, **6i** and **6j** were recrystallized from 70 % EtOH, **5h** from dioxane, **5i** and **6h** from ethyl acetate and **6g** from benzene).

Spectral data of **5a**; IR [KBr, ν, cm^{-1}]: 3170 (N-H), 3040 (=C-H), 1690, 1650 (C=O). ^{1}H-NMR [200 MHz, δ, ppm, $CDCl_3$]: 3.49 (s, 3H, CH_2OCH_3), 4.50 (s, 2H, CH_2OCH_3), 7.21–7.44 (m, 5H, ar), 9.16 (s, 1H, NH), 13.48 (s, 1H, NH) (D_2O exchange). CIMS [CH_4, m/z (%)]: 233(MH^+, 100).

Spectral data of **5b**; IR [KBr, ν, cm^{-1}]: 3250 (N-H), 3080 (=C-H), 1670 (C=O). ^{1}H-NMR [200 MHz, δ, ppm, $CDCl_3$]: 3.48 (s, 3H, CH_2OCH_3), 4.48 (s, 2H, CH_2OCH_3), 7.31 (dd, J: 9.00, 2.20 Hz, 2H, ar), 7.52 (dd, J: 9.00, 2.20 Hz, 2H, ar) 9.45 (s, 1H, NH), 13.45 (s, 1H, NH). ^{13}C-NMR [50.3 MHz, δ, ppm, $CDCl_3$]: 58.52 (CH_2OCH_3), 65.89 (CH_2OCH_3), 118.96 (C4), 117.46 (C2 and C6), 126.66 (C=N, exocyclic), 132.65 (C3 and C5), 140.01 (C1), 147.66 (C=N, ring), 160.53 (C=O). EIMS [m/z (%)]: 312 ($(M+2)^+$, 39), 310 (M^+, 40). CIMS [CH_4, m/z (%)]: 313 ($(MH+2)^+$, 98), 311 (MH^+, 100).

Spectral data of **5g**; IR [KBr, ν, cm^{-1}]: 3250 (N-H), 3080 (=C-H), 1705, 1680 (C=O). ^{1}H-NMR [200 MHz, δ, ppm, $CDCl_3$]: 1.41 (t, J: 7.00 Hz, 3H, $CO_2CH_2CH_3$), 3.50 (s, 3H, CH_2OCH_3), 4.38 (q, J: 7.00 Hz, 2H, $CO_2CH_2CH_3$), 4.50 (s, 2H, CH_2OCH_3), 7.46 (dd, J: 8.85, 1.97 Hz, 2H, ar), 8.09 (dd, J: 8.85, 1.97 Hz, 2H, ar), 9.40 (s, 1H, NH), 13.44 (s, 1H, NH) (D_2O exchange). EIMS [m/z (%)]: 304(M^+, 30), 165(100). CIMS [CH_4, m/z (%)]: 305(MH^+, 100).

Spectral data of **5h**; IR [KBr, ν, cm^{-1}]: 3250 (N-H), 3080 (=C-H), 1670 (C=O), 1340, 1160 (SO_2). ^{1}H-NMR [200 MHz, δ, ppm, DMSO-d_6]: 3.33 (s, CH_2OCH_3 and solvent H_2O), 4.36 (s, 2H, CH_2OCH_3), 7.36 (s, 2H, SO_2NH_2), 7.68 (dd, J: 8.85, 1.99 Hz, 2H, ar), 7.85 (dd, J: 8.85, 1.99 Hz, 2H, ar), 11.96 (s, 1H, NH), 13.20 (s, 1H, NH) (D_2O exchange). CIMS [CH_4, m/z (%)]: 312 (MH^+, 30), 173 (100).

Spectral data of **5i**; IR [KBr, ν, cm^{-1}]: 3160–2800 (N-H and = C-H), 1668 (C=O), 1540, 1335 (NO_2). ^{1}H-NMR [200 MHz, δ, ppm, DMSO-d_6]: 3.48 (s, 3H, CH_2OCH_3), 4.50 (s, 2H, CH_2OCH_3), 7.26 (t, J: 6.80 Hz, 2H, ar), 7.71 (t, J: 7.70 Hz, 2H, ar), 8.16 (d, J: 7.60 Hz, 1H, ar), 8.28 (d, J: 7.60 Hz, 2H, ar), 8.96 (s, 1H, NH), 14.68 (s, 1H, NH). CIMS [CH_4, m/z (%)]: 278 (MH^+, 85), 139 (100).

Spectral data of **6a**; IR [KBr, ν, cm^{-1}]: 3070 (=C-H), 1660 (C=O). ^{1}H-NMR [200 MHz, δ, ppm, $CDCl_3$]: 3.53 (s, 3H, CH_2OCH_3), 4.59 (s, 2H, CH_2OCH_3), 7.23–7.27 (m, 2H, ar), 7.40–7.45 (m, 6H, ar), 7.95 (dd, J: 8.54, 2.47 Hz, 2H, ar), 13.72 (s, 1H, NH) (D_2O exchange). CIMS [CH_4, m/z (%)]: 309 (MH^+, 100).

Spectral data of **6b**; IR [KBr, ν, cm^{-1}]: 3040 (=C-H), 1655 (C=O). ^{1}H-NMR [200 MHz, δ, ppm, $CDCl_3$]: 3.50 (s, 3H, CH_2OCH_3), 4.53 (s, 2H, CH_2OCH_3), 7.24–7.57 (m, 7H, ar), 7.94 (d, J: 7.44 Hz, 2H, ar), 13.60 (s, 1H, NH). ^{13}C-NMR [50.3 MHz, δ, ppm, $CDCl_3$]*: 58.57 (CH_2OCH_3), 65.89 (CH_2OCH_3), 117.39 (C2 and C6), 118.66 (C8 and C12), 118.93 (C4), 127.52 (C=N, exocyclic), 128.80 (C9 and C11), 132.58 (C3 and C5), 137.66 (C7), 139.88 (C1), 147.10 (C=N, ring), 157.51 (C=O). CIMS [CH_4, m/z (%)]: 389 ($(MH+2)^+$, 98), 387 (MH^+, 100). * (C7 was assigned to N1-C of N1-C_6H_5).

Spectral data of **6g**; IR [KBr, ν, cm^{-1}]: 3080 (=C-H), 1720, 1663 (C=O). ^{1}H-NMR [200 MHz, δ, ppm, $CDCl_3$]: 1.41 (t, J: 6.90 Hz, 3H, $CO_2CH_2CH_3$), 3.52, 3.54 (2s, 3H, CH_2OCH_3), 4.38 (q, J: 7.00 Hz, 2H, $CO_2CH_2CH_3$), 4.57, 4.59 (2s, 2H, CH_2OCH_3), 7.20–7.28 (m, ar and solvent), 7.39–7.51 (m, 4H, ar), 7.95 (dd, J: 8.54, 2.12 Hz, 2H, ar), 8.10 (dd, J: 8.54, 2.12 Hz, 2H, ar), 13.67 (s, 1H, NH) (D_2O exchange). CIMS [CH_4, m/z (%)]: 381 (MH^+, 100).

Spectral data of **6h**; IR [KBr, ν, cm^{-1}]: 3330–3220 (N-H), 3040 (=C-H), 1660 (C=O), 1365, 1150 (SO_2). ^{1}H-NMR [200 MHz, δ, ppm, DMSO-d_6]: 3.41(s, 3H, CH_2OCH_3), 4.53 (s, 2H, CH_2OCH_3), 7.26 (t, J: 6.90 Hz, 1H, ar), 7.39 (s, 2H, SO_2NH_2), 7.50 (t, J: 7.00 Hz, 2H, ar), 7.76–7.94 (m, 6H, ar), 13.32 (s, 1H, NH) (D_2O exchange). EIMS [m/z (%)]: 387 (M^+, 68), 77(100). CIMS [CH_4, m/z (%)]: 388 (MH^+, 10), 173 (100).

Spectral data of **6i**; IR [KBr, ν, cm^{-1}]: 3140 (N-H), 3080 (=C-H), 1660 (C=O), 1545, 1360 (NO_2). ^{1}H-NMR [200 MHz, δ, ppm, DMSO-d_6]: 3.53 (s, 3H, CH_2OCH_3), 4.61 (s, 2H, CH_2OCH_3), 7.27 (m, 2H, ar), 7.44 (t, J: 6.70 Hz, 2H, ar), 7.73 (t, J: 7.70 Hz, 1H, ar), 8.00 (d, J: 8.50 Hz, 2H, ar), 8.20 (dd, J: 8.48, 1.16 Hz, 1H, ar), 8.30 (dd, J: 8.44, 1.48 Hz, 1H, ar), 14.88 (s, 1H, NH). CIMS [CH_4, m/z (%)]: 354 (MH^+, 85), 139 (100).

2.1.3. 1-Acetyl-4-arlyhydrazono-3-methoxymethyl-2-pyrazolin-5-one (7)

5a (0.0025 mol) and acetic acid anhydride (3 ml) were refluxed on a water bath for 30 min. The precipitate formed was filtered off and recrystallized from 70 % EtOH.

Spectral data of **7**; IR [KBr, ν, cm^{-1}]: 3160 (N-H), 3060 (=C-H), 1740, 1665 (C=O). ^{1}H-NMR [200 MHz, δ, ppm, $CDCl_3$]: 2.61 (s, 3H, CH_3CO), 3.50 (s, 3H, CH_2OCH_3), 4.56 (s, 2H, CH_2OCH_3), 7.23–7.46 (m, 5H, ar), 13.47 (s, 1H, NH).

2.2. Analgesic activity (modified Koster's test [9])*

Female albino mice weighing 22 ± 2 g were used (local breed). The animals were housed in groups of eight at ambient temperature and humidity with food and water ad libitum and were allowed to get accustomed to their environment for at least two days before the experiments. The animal experiments were conducted after the approval of Hacettepe University Animal Research Ethics Comittee. Each compound was suspended in 5 % gum arabic syrup and given orally to mice in groups of eight at a dose level of 100 mg/kg. Two control groups (n=6), one receiving acetylsalicylic acid (ASA) suspended in 5 % gum arabic syrup (100 mg/kg), the other receiving only gum arabic syrup 1 h prior to injection of acetic acid, were employed. 1 h after this administration pain was induced by i.p. injection of 3 % solution of acetic acid at 300 mg/kg. No pecularities which might have resulted from the side effects of the test compounds were observed during this 1 h period. Animals were placed in glass cages 5 min after acetic acid injec-

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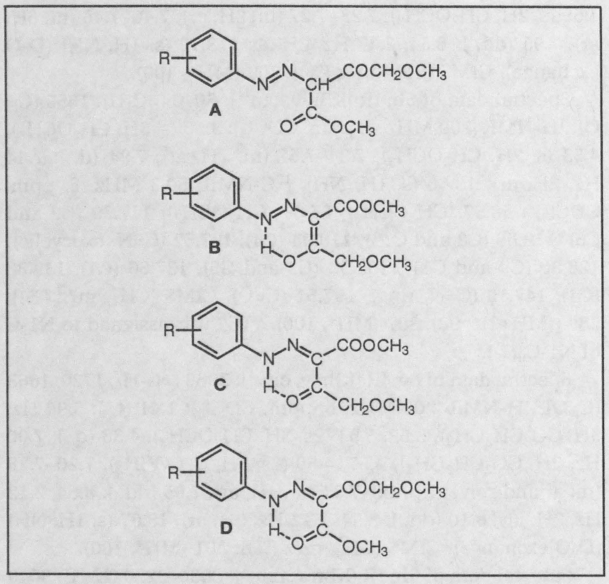

Fig. 1: Tautomeric structures of 4.

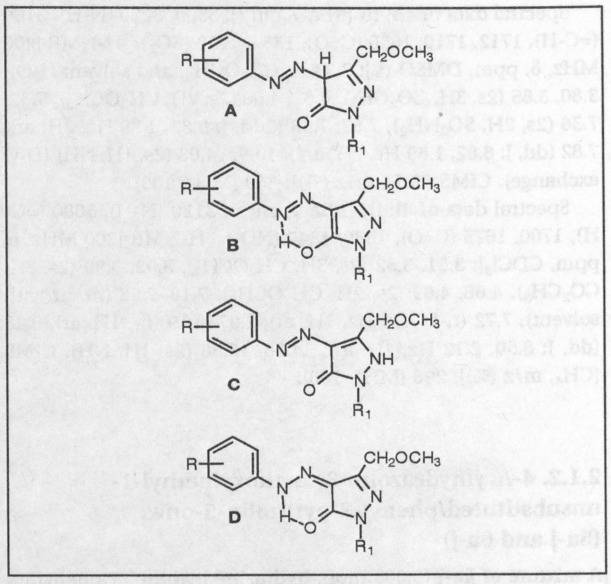

Fig 2: Tautomeric structures of 5 and 6.

tion and the number of "stretchings" per animal was recorded during the following 10 min period. Percent analgesic activity was calculated by the following formula:

$$\text{analgesic activity} = \frac{[n - n']}{n} \times 100$$

where n is the average number of "stretchings" of the control group and n' is the average number of "stretchings" of the test group.

* Blinding was thought unnecessary and therefore was not used during the experiments.

3. Results and discussion

3.1. Chemistry

The acidic proton of β-keto esters (3) may easily be abstracted and a resonance stabilized anion results which is a good nucleophile. Nucleophilic attack of the negatively charged carbon atom towards the diazonium cation affords 2-arylazo/hydrazono derivatives (4) which in turn may be cyclized with nucleophilic agents like hydrazine hydrate or phenylhydrazine to afford 4-arylazo/hydrazono substituted 5-pyrazolones (5 and 6)

Table 1: Physicochemical data and isomeric ratios of compounds 4.

Comp.	R	C/D[a]	Yield (%)	M.p. (°C)	Formula (MW)	Analysis calc./found		
						C	H	N
4a	H	5 : 6	87	89–91	$C_{12}H_{14}N_2O_4$ (250.35)	57.59 58.09	5.63 5.67	11.19 11.66
4b	4-Br	9 : 10	75	119–20	$C_{12}H_{13}BrN_2O_4$ (329.16)	43.78 44.21	3.98 3.87	8.51 8.62
4c	4-Cl	1 : 1	90	105-6	$C_{12}H_{13}ClN_2O_4$ (284.71)	50.62 50.19	4.60 4.56	9.83 9.45
4d	4-I	1 : 1	88	109–10	$C_{12}H_{13}IN_2O_4$ (376.15)	38.31 37.94	3.48 3.46	7.44 7.41
4e	4-CH_3	9 : 10	80	101–2	$C_{13}H_{16}N_2O_4$ (264.28)	59.08 59.00	6.10 6.20	10.59 11.00
4f	4-OCH_3	3 : 2	75	103–4	$C_{13}H_{16}N_2O_5.H_2O$ (298.26)	52.34 52.40	6.00 5.60	9.39 9.30
4g	4-$COOC_2H_5$	3 : 4	95	132–3	$C_{15}H_{18}N_2O_6$ (322.32)	55.89 55.90	5.62 5.80	8.69 8.80
4h	4-SO_2NH_2	1 : 4	60	178–80	$C_{12}H_{15}N_3O_6S$ (329.33)	43.76 43.79	4.55 4.80	12.76 12.50
4i	2-NO_2	1 : 6	90	132–3	$C_{12}H_{13}N_3O_6$ (295.25)	48.80 48.60	4.43 4.50	14.23 13.80
4j[b]	4-NO_2		79	130	$C_{12}H_{13}N_3O_6$ (295.25)	48.80 48.70	4.43 4.30	14.23 14.90

[a] Calculated from the integral values of the NH proton. C (low field resonance) and D (high field resonance). [b] Very broad, could not be integrated.

(Scheme 1, Tables 1 and 2). The structures of the arylhydrazono precursors as well as their cyclization products – pyrazolones – have been the subject of much debate as they may exist in several tautomeric forms. Accordingly **4a-j** can exist in the C-azo (**A**) the azo-enol (**B**) or the hydrazone (**C** and **D**) forms (Fig. 1). **A** was ruled out on the basis of ^{1}H-NMR evidence. Although resonances appearing at about δ 3.50, 3.90 or 4.65 ppm could be attributed to C2-H [10] these absorptions integrated to give exact proton counts for CH_2OCH_3, $COOCH_3$ and CH_2OCH_3, respectively. **4a-j** did not give coloration with $FeCl_3$, neither did they display IR absorptions at about 3700 cm^{-1} or 2700 cm^{-1} indicative of a free OH group or a chelated enolic structure. Furthermore the ^{1}H-NMR spectra of **4a-j** did not display absorbtions which may be atrributed to the chelated (δ 16.5 ppm) or free (δ 10–11 ppm) enolic proton [11, 12]. In view of the foregoing considerations and supportive evidence provided by literature [13–15] it was concluded that **4a-j** existed in the chelated hydrazone form (**C** or **D**). ^{1}H-NMR spectra indicated the presence of both forms (**C** and **D**) in almost equal amounts (except for **4f-i**, Table 1). Restriction of rotation about the C=N bond and hydrogen bonding between the N-H and C=O groups stabilized the molecule in certain conformations [16–18]. Thus the NH (δ 15.56–12.95 ppm), CH_2OCH_3 (δ4.69–3.33 ppm) and $COOCH_3$ (δ3.99–3.80 ppm) protons of the two conformers gave rise to double singlets whereas the H2 and H6 protons of the phenyl ring resonated as two separate double doublets (δ 7.68–7.20 ppm).

5a-j and **6a-j** are also tautomerically dynamic compounds [19–23] (Fig. 2). Presence of C=O absorptions

Table 2: Physicochemical data of compounds 5–7.

Comp.	R	R_1	Yield (%)	M.p. (°C)	Formula (MW)	Analysis calc./found		
						C	H	N
5a	H	H	55	155–6	$C_{11}H_{12}N_4O_2$ (232.24)	56.88 56.46	5.20 5.39	24.12 24.17
5b	4-Br	H	59	159–60	$C_{11}H_{11}BrN_4O_2$ (311.13)	42.46 42.56	3.56 3.54	18.00 18.03
5c	4-Cl	H	62	188–9	$C_{11}H_{11}ClN_4O_2$ (266.68)	49.54 50.07	4.15 3.99	21.00 21.00
5d	4-I	H	25	187	$C_{11}H_{11}IN_4O_2$ (358.13)	36.89 37.44	3.09 3.00	15.64 16.07
5e	4-CH_3	H	30	126–8	$C_{12}H_{14}N_4O_2$ (246.26)	58.52 59.21	5.73 5.96	22.75 23.26
5f	4-OCH_3	H	65	125–6	$C_{12}H_{14}N_4O_3$ (262.26)	54.96 55.04	5.38 5.80	21.37 21.05
5g	4-$COOC_2H_5$	H	69	145–7	$C_{14}H_{16}N_4O_4$ (304.30)	55.25 54.86	5.29 5.26	18.41 17.96
5h	4-SO_2NH_2	H	50	210–3	$C_{11}H_{13}N_5O_4S$ (311.31)	42.44 43.11	4.21 4.53	22.49 22.36
5i	2-NO_2	H	40	219	$C_{11}H_{11}N_5O_4$ (277.24)	47.65 47.30	3.99 4.00	25.26 24.90
5j	4-NO_2	H	39	238	$C_{11}H_{11}N_5O_4$ (277.234)	47.65 47.58	3.99 3.91	25.26 25.26
6a	H	C_6H_5	65	147	$C_{17}H_{16}N_4O_2$ (308.33)	66.22 65.80	5.23 5.50	18.17 18.10
6b	4-Br	C_6H_5	65	160–5	$C_{17}H_{15}BrN_4O_2$ (387.22)	52.72 52.58	3.90 3.75	14.46 14.68
6c	4-Cl	C_6H_5	70	158	$C_{17}H_{15}ClN_4O_2$ (342.77)	59.56 59.83	4.41 4.22	16.34 16.64
6d	4-I	C_6H_5	49	158	$C_{17}H_{15}IN_4O_2$ (434.22)	47.02 46.80	3.48 3.50	12.90 12.90
6e	4-CH_3	C_6H_5	43	143–5	$C_{18}H_{18}N_4O_2$ (322.35)	67.06 67.27	5.62 5.38	17.38 17.24
6f	4-OCH_3	C_6H_5	60	130–1	$C_{18}H_{18}N_4O_3$ (338.35)	63.89 63.62	5.36 5.22	16.55 16.42
6g	4-$COOC_2H_5$	C_6H_5	70	144	$C_{20}H_{20}N_4O_4$ (380.39)	63.14 63.70	5.29 5.50	14.72 15.20
6h	4-SO_2NH_2	C_6H_5	50	244–5	$C_{17}H_{17}N_5O_4S$ (387.41)	52.79 53.00	4.42 4.50	18.07 18.10
6i	2-NO_2	C_6H_5	65	186	$C_{17}H_{15}N_5O_4$ (353.33)	57.78 57.40	4.27 4.20	19.82 19.30
6j	4-NO_2	C_6H_5	75	179–80	$C_{17}H_{15}N_5O_4$ (353.33)	57.78 57.70	4.27 4.20	19.82 19.90
7	H	$COCH_3$	59	156	$C_{13}H_{14}N_4O_3$ (274.27)	56.92 56.60	5.14 5.10	20.42 20.30

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(1720–1655) in the IR spectra and absence of resonances at about δ 3–6 ppm [24] and δ 16.5 ppm or δ 10–11 ppm [11, 12] which could be assigned to the C4-H of the pyrazolone ring and to the enolic proton, respectively, ruled out the azo-CH (**A**) and azo-enol (**B**) forms. Absorption position of the hydrazone NH (δ 13.86–13.20 ppm), except 2-NO_2 substituted derivatives **5i** (δ 14.68 ppm) and **6i** (δ 14.88 ppm), was in accordance with the values given for the chelated hydrazone NH [19, 25, 26] and eliminated the azo-NH (**C**) form [27]. Further confirmation for the hydrazone form (**D**) was obtained by comparing the ^{13}C-NMR data of **5b** and **6b** with those of a model compound, 3-methyl-1-phenylpyrazol-4,5-dione-4-phenylhydrazone, reported by Lycka et al. [28, 29]. They observed the exocyclic C=N at δ 128.31 ppm, ring C=N at δ 148.30 ppm and the C=O function at δ 157.55 ppm. They also studied ^{15}Nα- ^{15}Nβ and ^{15}Nα-^{13}C couplings of the hydrazono group to attain a definite assignment. ^{13}C-NMR values of **6b** (exocyclic C=N: δ 127.52 ppm; ring C=N: δ 147.10 ppm; C=O: δ 157.51 ppm) were very close to those reported. The values observed for **5b** were within ± 0.5 ppm of **6b** for all the C atoms only C=N (exocyclic) was observed at δ 126.66 and C=O was observed at δ 160.53 ppm.

Molecular (M^+) or quasi-molecular (MH^+) ions in the EIMS or CIMS of **4, 5** and **6** confirmed their molecular weights. The major fragmentation route followed in **5** and **6** was in accordance with those cited for pyrazolones and azopyrazolones and primarily involved the breaking of the hydrazono NH-N bond [30–33].

Further spectral details are presented in the Materials and methods section.

3.2. Pharmacology

The analgesic activity of selected members from the 2-pyrazolin-5-one series (**5b, 5c, 5f-i, 6a-f, 6h-j** and **7**) was investigated employing the modified Koster's test [9]. To determine the structural features required for analgesic activity the substituents and/or the substitution pattern of the 5-pyrazolinone ring and the arylhydrazono moiety were varied and many potent compounds were obtained. As can be seen in Table 3 the most active compound among the N1-unsubstituted derivatives was **5f**, bearing the electron releasing 4-OCH_3 group on the phenyl ring of the arylhydrazono moiety. Electron withdrawing substituents and halogens decreased activity in this series. Presumably the contribution of the resonance effect of the OCH_3 function and the inductive effect of the halogens were the dominating factors which caused a significant difference in the whole molecule and thus led to the difference in activity.

N1-Phenyl substituted derivatives were generally more active than N1-unsubstituted entries; **6a, 6c, 6d** and **6f** being the four most active of both series exerted about twice the analgesic activity of that of the reference drug ASA. The potency of the unsubtituted derivative **6a** (58 %) was further enhanced by the introduction of the 4-Cl substituent into the phenyl ring of the arylhydrazone moiety (**6c**, 60 %). Electron withdrawing substituents decreased activity also in **6** and yielded compounds **6h-j** with reduced potency (Table 3).

Table 3: Analgesic activity of 5b, 5c, 5f–i, 6a–f, 6h–j and 7.

Compund	Analgesic activity (%)	Compound	Analgesic activity (%)
5b	27	**6d**	55
5c	31	**6e**	54
5f	52	**6f**	56
5g	31	**6h**	40
5h	16	**6i**	29
5i	17	**6j**	39
6a	58	**7**	42
6b	49	ASA	27
6c	60		

N1-Acetyl substituted derivative **7**, which was unsubstituted on the phenyl ring of the arylhydrazone group, was found to be more active than the N1-unsubstituted derivatives except **5f** (Table 3).

From the above results it may be concluded that N1-substitution, especially N1-phenyl substitution, enhances analgesic activity. Electron releasing substituents at the 4-position of the arylhydrazono group increase the activity, whereas electron withdrawing groups have a negative effect. Halogens may either enhance or lead to a reduction in activity depending upon the contribution of the halogen to the electronic properties of the molecule.

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