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Synthesis and evaluation of some 4,5-disubstituted 6-phenyl-3(2*H*)-pyridazinones as hypotensive agents

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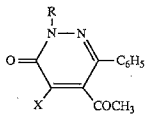
4,5-disubstituted 6-phenyl-3(2*H*)-pyridazinones / hypotensive activity

Introduction

6-Aryl-3(2*H*)-pyridazinones and their 4,5-dihydro derivatives display a wide range of pharmacological effects on the cardiovascular system, such as reduction of blood pressure, platelet aggregation inhibition and positive inotropic activity [1]. These pharmacological properties arise largely from the ability of these compounds to increase the intracellular levels of cyclic nucleotides by inhibiting phosphodiesterases (PDE), which are the enzymes responsible for their degradation [2].

Previous publications [3,4] from our laboratory have dealt with the synthesis of a series of 4,5-functionalized 6-phenyl-3(2*H*)-pyridazinones, some of which are endowed with significant platelet antiaggregatory and positive inotropic activity. On these grounds, we considered it of interest to evaluate the hypotensive activity of the 4,5-functionalized 3(2*H*)-pyridazinones **1a–d**, which are representative compounds of this series [3, 4], and the new products **1e–h** substituted at position 4 with either electron-withdrawing or electron-releasing groups.

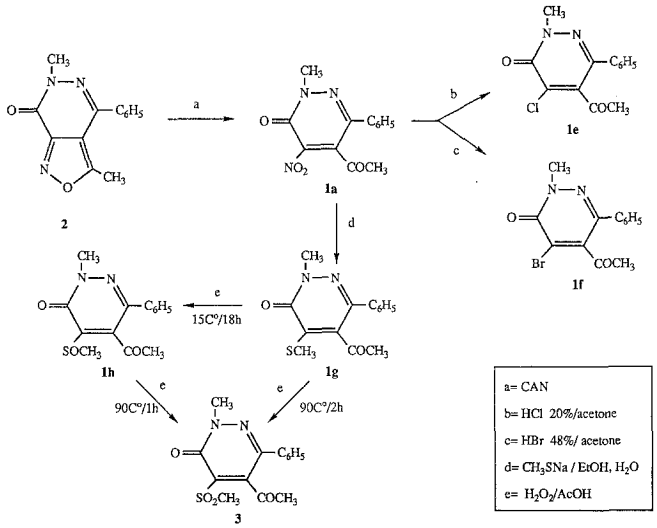


1a-h

1a: R=CH_3, X=NO_2
1b: R=H, X=NH_2
1c: R=CH_3, X=NH_2
1d: R=CH_3, X= 
1e: R=CH_3, X=Cl
1f: R=CH_3, X=Br
1g: R=CH_3, X=SCH_3
1h: R=CH_3, X=$SOCH_3$

Chemistry

As shown in scheme 1, the key intermediate for the synthesis of the new compounds **1e–h** is the previously described 4-nitro-3(2*H*)-pyridazinone **1a** which, in turn, was obtained through oxidative opening of the 5-membered ring of derivative **2** [5] by ceric ammonium nitrate (CAN) [6]. The nitro group of **1a** has been indicated by our earlier studies as a very good leaving group, which is easily replaced by a variety of nucleophiles [3, 7]. Accordingly, compounds **1e** and **1f** were smoothly obtained in good yields by briefly heating **1a** with the appropriate



Scheme 1.

hydrohalic acid in acetone, whereas treatment of **1a** with sodium methanthiolate in aqueous ethanolic medium at room temperature afforded the methylthio-derivative **1g** in moderate yield. Treatment of **1g** with peracetic acid under very carefully controlled conditions (15°C) afforded the methylsulfoxide **1h**, whereas when **1g** was treated with peracetic acid at 60°C the methylsulfone **3** was obtained. Compound **3** was also formed by further oxidation of the sulfoxide **1h**. Finally, compounds **1b–d** were prepared according to previously reported methods [3, 8, 9].

Pharmacology

The hypotensive activity of compounds **1a–h** was determined after ip administration to normotensive anaesthetized rats, with hydralazine as a reference drug. Because of the low water solubility of the test compounds, injectable emulsions were prepared. The effects of the tested compounds at the dose of 20 mg/kg were measured every 10 min up to 60 min. The blood pressure values after 10, 30 and 60 min are shown in table I.

Results and discussion

The majority of compounds were weakly active or inactive except for the methylsulfinyl derivative **1h**. This compound, whose hypotensive effect was also evaluated at the dose of 10 mg/kg, displayed a dose-dependent effect with a maximum reduction in blood pressure of 35% after 60 min at a dose of 20 mg/kg (fig 1).

Table I. Hypotensive effect of compounds **1a–h** in normotensive rats.

Compd[a]	P_{10}[b]	P_{30}[b]	P_{60}[b]
Control[c]	101.9 ± 2.4	103.1 ± 4.2	99.8 ± 5.4
1a	118.9 ± 5.7	90.2 ± 3.0	89.2 ± 4.4
1b	104.8 ± 2.6	94.4 ± 1.2	91.0 ± 1.1
1c	92.3 ± 4.4	87.1 ± 3.8	91.0 ± 1.8
1d	103.2 ± 2.8	101.0 ± 2.9	100.1 ± 5.4
1e	91.4 ± 2.6	88.0 ± 2.8	83.8 ± 7.0
1f	100.5 ± 3.9	96.1 ± 5.6	94.9 ± 0.4
1g	97.0 ± 4.7	98.4 ± 3.6	98.4 ± 1.8
1h[d]	90.5 ± 1.3	79.2 ± 1.1	64.7 ± 2.1
	97.6 ± 1.9	86.2 ± 1.8	79.7 ± 4.4
Hydralazine[e]	66.5 ± 3.2	68.4 ± 3.2	67.7 ± 2.7

[a]All compounds were tested at a dose of 20 mg/kg ip; [b]P_{10}, P_{30} and P_{60} are pressure values determined 10, 30 and 60 min after administration; values are mean ± SEM of at least 4 experiments; [c]controls represent animals that received an emulsion without compound; [d]compound **1h** was tested at a dose of 10 mg/kg; [e]hydralazine was given at a dose of 10 mg/kg ip.

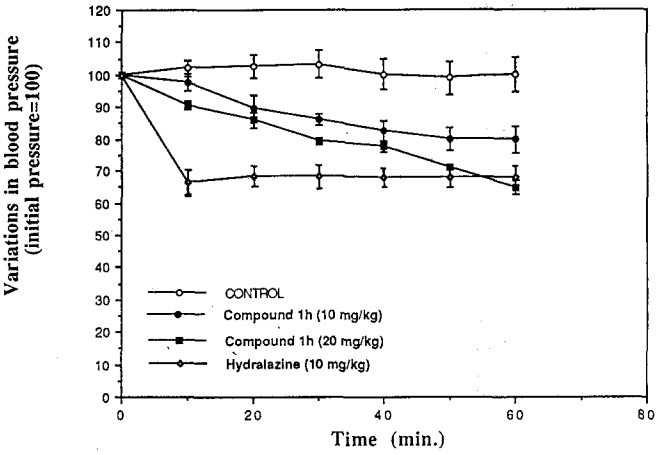
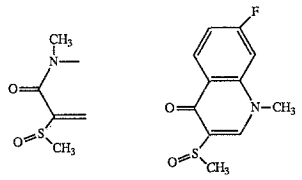


Fig 1. Hypotensive effect of compound **1h** (10 and 20 mg/kg ip) and hydralazine (10 mg/kg) on systolic blood pressure in normotensive rats in comparison with vehicle (control). Symbols and error bars indicate mean ± SEM (n = 4–6).

Nevertheless compound **1h** was less active than hydralazine, which reduced the blood pressure to the same extent at a dose of 10 mg/kg. At this dose level **1h** still showed a moderate hypotensive effect (20% of blood pressure reduction). While a maximum hypotensive effect was reached by hydralazine after only 10 min, the hypotensive effect of compound **1h** increased continuously after administration. This different behaviour could be due to the difference in pharmacokinetics and/or action mechanism. Since the corresponding methylsulfide **1g** is completely devoid of activity, the degree of sulfur oxidation seems to play a crucial role in the hypotensive activity of **1h**. It is interesting to observe that a similar relationship between activity and oxidation degree of the 4-position substituent has been established for 4-nitro and 4-amino-3(2*H*)-pyridazinones **1a** and **1c** in which platelet aggregation inhibition and positive inotropic activity were closely connected to the presence of the NO_2 function at the 4-position [3, 4].

The presence of the substructure **4** embodied in a 6-membered nitrogen heterocycle similar to the ring present in flosequinam **5**, an inotropic and vasodilator agent recently launched in the UK [10], could, at least partially, explain the hypotensive activity of compound **1h**.

Compound **1a** showed a bi-phasic effect: a significant initial hypertensive effect (+20%), 10 min after



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administration, followed by a weak hypotensive effect (–10%) after 30 min with respect to controls. This behaviour could be explained on the basis of the metabolic instability of this compound, which we suggested in a previous paper [3].

The significant activity in human-platelet aggregation inhibition [4], coupled with the absence of hypotensive effects in normotensive rats, made compounds **1e** and **1f** very interesting. Although only preliminary data are available, and bearing in mind differences between species and the pharmacokinetics and/or metabolism involved in the experiments considered, the presence of a chlorine or bromine substituent at position 4 in this group of 3(2*H*)-pyridazinones seems to play an important role in selectively affecting the platelets.

Further studies are in progress to obtain new information about the structural requirements necessary for a more selective activity and to clarify the action mechanism of this series.

Experimental protocols

Chemistry

All melting points were determined with a Buchi 510 melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded as nujol mulls with a Perkin–Elmer spectrometer. Nuclear magnetic resonance (^{1}H-NMR) spectra were obtained using a Gemini 200 spectrometer in $CDCl_3$. Chemical shift values are reported in ppm (δ). Analyses indicated by the symbols of the elements or function were within ± 0.4% of the theoretical values. Silica-gel plates (Merck F254) and silica gel 60 (Merck 70-230 mesh) were used for analytical TLC and column chromatography, respectively. Extracts were dried over sodium sulfate and solvents were removed under reduced pressure.

*5-Acetyl-4-chloro-2-methyl-6-phenyl-3(2H)-pyridazinone **1e***
A mixture of **1a** [6] (0.2 g, 0.73 mmol), 6 M hydrochloric acid (4 ml) and acetone (4 ml) was refluxed under stirring for 1.5 h. The solution was allowed to cool to room temperature and water (50 ml) was added. Compound **1e** thus precipitated (80 mg), was filtered off and the solution exhaustively extracted with CH_2Cl_2. Evaporation of the solvent afforded a further 45 mg of **1e** (overall yield = 65%); mp 100–101°C (ethanol). Anal $C_{13}H_{11}ClN_2O_2$ (C,H,N); IR (cm^{-1}): 1730 (CO), 1670 (CO); ^{1}H-NMR δ 2.10 (s, 3H, $COCH_3$), 3.92 (s, 3H, NCH_3), 7.45 (s, 5H, C_6H_5).

*5-Acetyl-4-bromo-2-methyl-6-phenyl-3(2H)-pyridazinone **1f***
Following the above procedure, starting from **1a** (0.25g, 0.81 mmol), hydrobromic acid (4 ml, 48% w/w) and acetone (4 ml), **1f** (0.2 g) was obtained after 15 min reflux (71% yield); mp 128–130°C (ethanol). Anal $C_{13}H_{11}BrN_2O_2$ (C,H,N); IR (cm^{-1}) 1720 (CO), 1680 (CO), 650 (C-Br); ^{1}H-NMR δ 2.18 (s, 3H, $COCH_3$), 3.92 (s, 3H, NCH_3), 7.43 (s, 5H, C_6H_5).

*5-Acetyl-2-methyl-4-methylthio-6-phenyl-3(2H)-pyridazinone **1g***
To a stirred suspension of **1a** (0.20 g, 0.73 mmol) in ethanol/water 1:1 (20 ml), sodium methanthiolate (0.14 g, 2 mmol) was added portionwise over 10 min at room temperature. After further stirring for 20 min, water (70 ml) was added and the solution exhaustively extracted with dichloromethane. Evaporation of the solvent afforded 0.096 g of **1g** (48% yield); mp 106–107°C (ethanol/water 1:1). Anal $C_{14}H_{14}N_2O_2S$ (C,H,N); IR (cm^{-1}): 1720 (CO), 1650 (CO); ^{1}H-NMR δ 2.18 (s, 3H, $COCH_3$), 2.67 (s, 3H, SCH_3), 3.90 (s, 3H, NCH_3), 7.47 (s, 5H, C_6H_5).

5-Acetyl-2-methyl-4-methylsulfinyl-6-phenyl-3(2H)-pyridazinone **1h**
To a suspension of **1g** (0.20 g, 0.73 mmol) in acetic acid (20 ml, 50% w/w) was added hydrogen peroxide (0.7 ml, 30% w/w). The mixture was stirred at 15°C for 18 h. After dilution with water (100 ml), the mixture was exhaustively extracted with CH_2Cl_2. Evaporation of the solvent afforded **1h** (0.13 g, 61% yield); mp 128-130°C (cyclohexane). Anal $C_{14}H_{14}N_2O_3S$ (C,H,N); IR (cm^{-1}): 1705 (CO), 1650 (CO), 1070 (SO); ^{1}H-NMR δ 2.12 (s, 3H, $COCH_3$), 3.10 (s, 3H, $SOCH_3$), 3.88 (s, 3H, NCH_3), 7.30–7.50 (m, 5H, C_6H_5).

5-Acetyl-2-methyl-4-methylsulfonyl-6-phenyl-3(2H)-pyridazinone **3**
Method A. A mixture of **1g** (0.2 g, 0.73 mmol) and hydrogen peroxide (8 ml, 30% w/w) in acetic acid (20 ml, 50% w/w) was heated at 90°C for 2 h. After cooling the mixture was diluted with water (50 ml). The precipitate **3** (163 mg, 73% yield) was filtered off.
Method B. A mixture of **1h** (0.1 g, 0.34 mmol) and hydrogen peroxide (4 ml, 30% w/w) in acetic acid (10 ml) were heated at 90°C for 1 h. After cooling and dilution with water (20 ml) the solution was exhaustively extracted with CH_2Cl_2. Evaporation of the solvent afforded **3** (92 mg, 87% yield); mp 182–183°C (ethanol). Anal $C_{14}H_{14}N_2O_4S$ (C,H,N); IR (cm^{-1}): 1730 (CO), 1665 (CO), 1140 and 1320 (SO_2); ^{1}H-NMR δ 2.17 (s, 3H, $COCH_3$), 3.40 (s, 3H, SO_2CH_3), 3.90 (s, 3H, NCH_3), 7.42 (s, 5H, C_6H_5).

Pharmacology

Preparation of compound-containing emulsions
Compounds were administered ip as injectable emulsions prepared according to a modification of the procedure proposed by Yu *et al* [11], which is based on a spontaneous emulsification process. For this purpose, 5–10 mg of the test compounds were dissolved in 10 µl DMSO and 100 µl glyceryl caprylate/caprate PEG-4 complex (Labrafac Hydro®, Gattefosse, USA). Then 5 ml ethanol containing 20 mg L-*a*-

phosphatidylcholine extracted from soybean (Sigma Chemical Co, USA) was added. This oily alcoholic solution was slowly added to 10 ml 0.25% non-ionic surfactant aqueous solution (Synperonic® F68, ICI, UK) under moderate magnetic stirring. The resulting emulsion was concentrated to a final volume of 2 ml by removal of ethanol and water under reduced pressure at 35–40°C. Test compounds were omitted from emulsions destined for control animals.

Evaluation of hypotensive effects

Male Sprague-Dawley normotensive rats weighing between 200 and 250 g were anaesthetized by ip injection of urethane (1.25 g/kg) and kept warm (37°C) with an overhead lamp. A cannula was inserted in the trachea to facilitate spontaneous respiration. Systolic and diastolic arterial pressures were recorded continuously on a Letica Uni-graph 1000–506 polygraph using a TRA 021 Letica pressure transducer connected to a cannula inserted in the right carotid artery. Following a 20-min period of stabilization, compounds were administered (4 ml/kg ip) and pressure variations were measured every 10 min, up to 60 min. In order to avoid individual variations, initial blood pressure was taken as 100 in all animals. Changes induced in this value by the administration of compounds therefore represent the percentage pharmacological response. Hydralazine was used as a standard drug.

Acknowledgments

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