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Report

Antihypertensive Activity of 2[(2-Hydroxyphenyl) amino]-4(3H)-quinazolinone

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Two 2-substituted 4(3H)-quinazolinones were synthesized through an easy one-step synthetic procedure. One of them (1), which was synthesized by the reaction of 1-(2-hydroxyphenyl)-2-thiourea and isatoic anhydride, contained a guanidino moiety and showed antihypertensive activity following cumulative intravenous administration to anesthetized spontaneously hypertensive rats. The other compound (2) does not contain the guanidino group and did not exhibit antihypertensive activity.

KEY WORDS: 4(3H)-quinazolinones; synthesis; guanidino moiety; antihypertensive.

INTRODUCTION

Although distinct mechanisms underlie the mode of action of a large number of antihypertensive agents (1), it is interesting to note that many of them share the guanidino-like group as a structural functionality. For example, the alpha-1/alpha-2 receptor agonist clonidine, the alpha-1 receptor antagonist prazosin, the adrenergic neuron blocker guanethidine, and the general vasodilator minoxidil all possess the guanidino-like functionality in their vastly distinct chemical structures. It is not known whether such a structural design may form a template from which biological targets can be uniquely activated by the various substituents attached to it. To explore this intriguing structure further, we have synthesized and evaluated two compounds containing the quinazolinone structure (Fig. 1), one of which contains the guanidino moiety (1). Quinazolinones have been reported to have antihypertensive properties (2,3). In the present study, the antihypertensive effects of the two compounds and the possible mechanism(s) of action of the active compound are examined.

Reaction of benzamides with isatoic anhydride has been shown to form 2-aryl-4(3H)-quinazolinone (4,5). When the benzamide carried a 2-hydroxy substituent, e.g., salicylamide, the reaction at elevated temperatures in a melt proceeded with a 65% yield (5), and in *N,N*-dimethylformamide (DMF) using dimethylaminopyridine the reaction yield was 75% (M. A. Hussain, unpublished results). In this publication, the reaction of isatoic anhydride with 1-(2-hydroxyphenyl)-2-thiourea and 3-hydroxypicolinamide was employed to prepare 2-substituted 4(3H)-quinazolinones with or without the guanidino moiety in their structures, respectively.

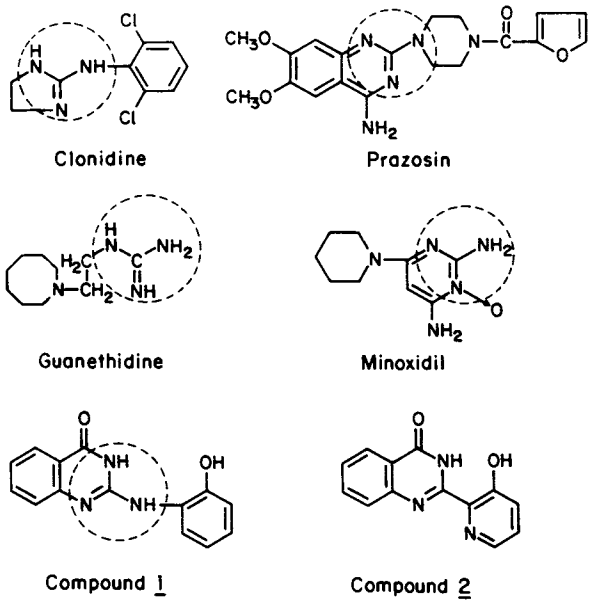

Fig. 1. Structures of some known antihypertensive drugs containing the guanidino-like function, Compound 1, and Compound 2.

MATERIALS AND METHODS

Isatoic anhydride and 4-dimethylaminopyridine were purchased from Fluka AG. *N,N*-Dimethylformamide was purchased from J. T. Baker Chemical Company. 3-Hydroxypicolinamide was purchased from Aldrich Chemical Company. 1-(2-Hydroxyphenyl)-2-thiourea was purchased from Trans World Chemicals Inc.

Melting points were determined on a Fisher–Johns

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melting-point apparatus and are uncorrected. Proton NMR spectra were recorded with a WP200SY NMR spectrometer, Bruker (IBM). Elemental analyses were performed by Atlantic Microlabs, Inc. (Atlanta, Ga.).

Synthesis: 2[(2-Hydroxyphenyl)amino]-4(3H)-quinazolinone (1) and 2-(3-Hydroxy-2-pyridinyl)-4(3H)-quinazolinone (2) (See Fig. 1)

A mixture of 1-(2-hydroxyphenyl)-2-thiourea (1.68 g, 0.01 mol) or 3-hydroxypicolinamide (1.38 g, 0.01 mol), isatoic anhydride (1.95 g, 0.012 mol), and 4-dimethylaminopyridine (1.46 g, 0.012 mol) in *N,N*-dimethylformamide (25 ml) was refluxed for 3 hr. The reaction mixture was then added to 200 ml of water and the pH was adjusted to 7.0 with 1N HCl. The precipitate obtained was then filtered and air dried.

For Compound **1**, the precipitate was triturated twice with 10 ml of methanol and crystallized from DMF–H_2O (1:1) to provide the title compound (15% yield) as a tan solid, decomposed at 320°C. ^1H NMR (Me_2SO-d6): δ 6.86–8.5 (m, 8H, aromatic); 8.33 (s, 1H, N–H) exchanged with D_2O; 10.16, and 11.38 each (s, 1H) phenolic OH and O = C—N–H both exchanged with D_2O. *Anal.* Calcd for $C_{14}H_{11}N_3O_2 \cdot 1/4\ H_2O$: C, 65.24, H, 4.49; N, 16.3. Found: C, 65.31; H, 4.48; N, 16.21.

For Compound **2**, the precipitate was crystallized from DMF–H_2O (4:1) to provide the title compound (60% yield) as a yellow solid, mp 200–202°C. ^1H NMR (Me_2SO-d6): δ 7.5–8.33 (m, 7H, aromatic); 11.97 (broad s, 1H) and 13.47 (broad s, 1H), N–H and O–H, both exchanged with D_2O. *Anal.* Calcd for $C_{13}H_9N_3O_2$: C, 65.27, H, 3.80; N, 17.56. Found: c, 65.25; H, 3.81; N, 17.53.

PHARMACOLOGY

Male spontaneously hypertensive rats (Charles River Laboratories, Portage, Mich.), weighing 280–350 g, were distributed into three groups. One group received the vehicle, while the other two received the test compounds. The rats were anesthetized with a mixture of pentobarbital–barbital sodium, and both the jugular vein and the carotid artery were cannulated with polyethylene tubing (P.E. 50). The rats were then placed on a warm metal plate to maintain the body temperature at 37°C. Blood pressure was measured through the carotid artery using a Statham pressure transducer (Gould P23ID) and was recorded on a Grass polygraph. Heart rate was determined using a tachygraph. The arterial cannula was kept patent using a flush of heparin at 5 U/ml of a 0.9% saline solution.

After the rats' blood pressures and heart rates had stabilized, control mean blood pressures and heart rates were recorded at successive intervals of 5 min for 15 min. Subsequent to this control period, vehicle or test compounds were administered intravenously every 15 min on a cumulative dosing schedule (see Figs. 2 and 3). The compounds in this study were dissolved in a minimum volume of 0.1 *N* sodium hydroxide solution and diluted to the desired volume with a 5% dextrose solution. Mean blood pressure and heart rate were recorded before and at 5-min intervals after each dose and plotted graphically. The ED_{30} (the dose lowering blood pressure by 30 mm Hg) for the compounds was graphically determined. Blood pressures and heart rates after treatments were compared to the control values at time 0 for statistically significant changes by Student's *t* test.

The alpha-1 adrenoceptor binding assay was performed with rat brain membranes according to a described procedure (6). In brief, the reaction mixture contained partially purified rat brain membranes and 0.2 n*M* [^3H]prazosin, with or without test compound, in 0.05 *M* Tris buffer. The mixture was incubated for 60 min at 25°C and subsequently terminated by rapid filtration through a glass-fiber filter using a cell harvester apparatus. Receptor-bound [^3H]prazosin trapped in the filter was quantitated by scintillation counting. Nonspecific binding was defined by the residual amount of radioactivity nondisplaceable by 10^{-6} *M* of phentolamine. Results are expressed as the IC_{50} value, which represents the concentration of compound inhibiting the specfic binding by 50%.

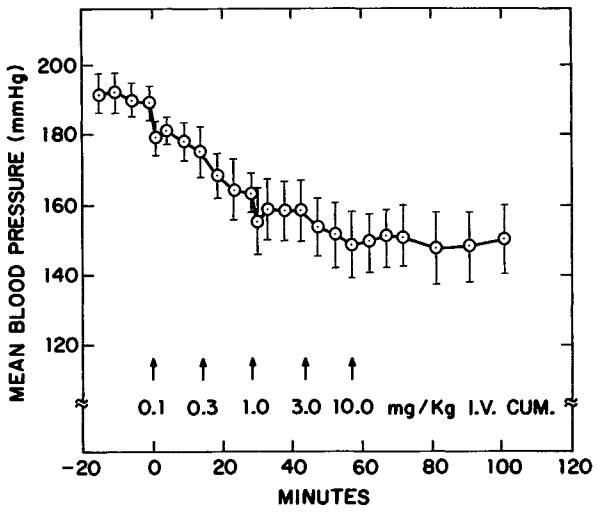

Fig. 2. Effect of Compound 1 on blood pressure in anesthetized spontaneously hypertensive rats after cumulative i.v. administration. Symbols represent values ± SE (N = 4).

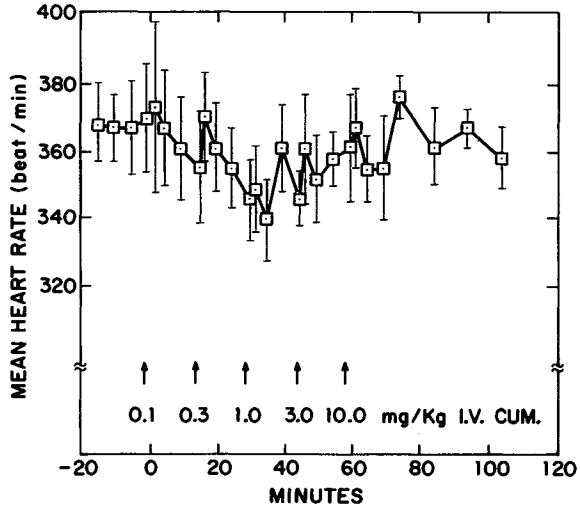

Fig. 3. Effect of Compound 1 on heart rate in anesthetized spontaneously hypertensive rats after cumulative i.v. administration. Symbols represent values ± SE (N = 4).

RESULTS AND DISCUSSION

Compounds 1 and 2 were tested for their antihypertensive properties in spontaneously hypertensive rats. When administered intravenously at cumulative doses from 0.1 to 10 mg/kg, Compound 1 produced a significant dose-dependent reduction of the mean arterial blood pressure as shown in Fig. 2. The ED_{30} for Compound 1 was about 1 mg/kg cumulatively. Unlike a typical vasodilator, such as minoxidil (7), the hypotensive effect of Compound 1 developed slowly and progressively. The maximal effect was seen at doses between 3 and 10 mg/kg. At the termination of drug administration, the maximum hypotensive effect was maintained for at least another 40 min. Again, in contrast to the general vasodilators, Compound 1 elicited no tachycardia during the entire period of drug administration. In fact, the heart rate was not significantly different from that in the predrug control period (Fig. 3). Under the same experimental conditions, the vehicle or Compound 2, which lacks the guanidino functionality, produced no significant alteration of either mean arterial blood pressure or heart rate (data not shown).

It is interesting to note that the hypotensive and the heart-rate profiles of Compound 1 resembled those of the alpha-1 adrenoceptor blocker, prazosin (8). To determine whether or not Compound 1 was an alpha-1 adrenoceptor antagonist, its affinity for the alpha-1 receptor was evaluated using the [^{3}H]prazosin binding assay. In this preparation, prazosin inhibited the binding of the radioactive ligand at an IC_{50} of 2×10^{-9} *M*. Compounds 1 and 2 had no affinity for this receptor even at concentrations of up to 10^{-4} *M* (data not shown).

Although the mechanism(s) for the hypotensive action of Compound 1 is presently not known, we can at least exclude the following possibilities. Since Compound 1 showed no overt central nervous system (CNS) side effects in conscious rats at doses up to 81 mg/kg given either subcutaneously or orally (unpublished data) and exhibited no significant effect on heart rate, we may be able to exclude the possibilities that it is a centrally acting alpha-2 agonist, like clonidine (9) (sedation and bradycardia produced at hypotensive doses), a general vasodilator, like minoxidil (tachycardia), or an adrenergic neuron-blocker (bradycardia), like guanethidine (10). Nevertheless, the actual mechanism of action remains to be determined. The quinazolinone structure of Compound 1 is unique among all the antihypertensive drugs, while the guanidino moiety is similar in many of the antihypertensives mentioned earlier. Based on this limited structure study, further analoguing of this quinazolinone compound may improve the efficacy and potency of this potential antihypertensive agent and, in addition, may shed more light on the importance of the guanidino functionality in drug design.

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