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SYNTHESIS AND CARDIOTONIC ACTIVITY OF 2,9-DIHYDRO-6-(5-METHYL-3-OXO-2,3,4,5-TETRAHYDROPYRIDAZIN-6-YL)PYRAZOLO[4,3-b][1,4]BENZOXAZINE

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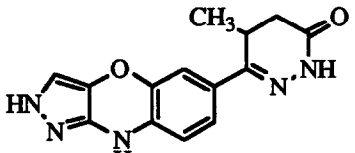
Abstract. 2,9-Dihydro-6-(5-methyl-3-oxo-2,3,4,5-tetrahydropyridazin-6-yl)pyrazolo[4,3-b][1,4]benzoxazine (**1**) is prepared in two steps from a precursor of bemoradan (**2**). The compound displays a maximum increase in cardiac force of 256% at 75 μg/kg intravenously in an anesthetized dog model and has an ED_{50} of 15 μg/kg.

Our laboratories have been involved in the synthesis of phosphodiesterase type III inhibitors and positive inotropes which has resulted in the discovery of bemoradan [1] (**2**) and its analogs [2] as well as several other novel structural types including bemarinone and its analogs [3], a series of quinazolin-3-oxides [4] and short-acting isoquinoline positive inotropes [5]. We now report the synthesis and cardiotonic activity of a bemoradan related compound (**1**)[6] which bears an additional heterocyclic ring while retaining the structural features needed for enzyme inhibiting as well as cardiotonic activity as established by Bristol et al [7].



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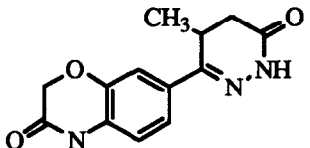


2

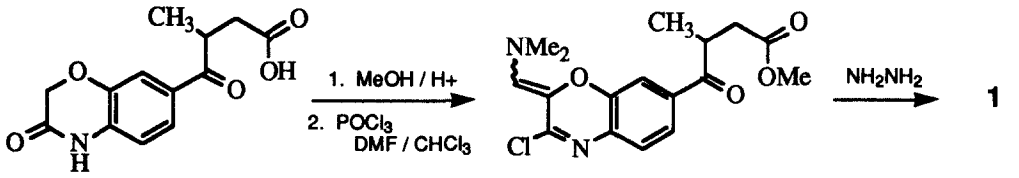
During our search for a backup to the potent cardiotonic, bemoradan, then undergoing human clinical evaluation, we decided to examine substitutions on the benzoxazine ring. The presumption that steric bulk on the 2-position of the benzoxazine ring is well tolerated and perhaps even beneficial is well supported by our previous work [1] as well as by examination of other cardiotonic agents such as indolidan [8], and pimobendan [9]. A ring fused to the benzoxazine ring would maintain a rigid framework with additional valences available for further substitution.

There are two reports in the literature on the synthesis of the pyrazolo[4,3-b][1,4]benzoxazine ring system. The basic ring system was first synthesized from 1,4-benzoxazine under Vilsmeier-Haack conditions in 1971,[10] while substituted analogs are described in a Bayer A.-G. patent in 1983 and are claimed as lipoxygenase inhibitors and antiinflammatory agents [11]. There have been no reports of rings of any kind attached to the pyrazolobenzoxazine nucleus.

It was felt that this modification to the benzoxazine ring of bemoradan would maintain the required polar group, which is perhaps necessary for hydrogen bonding to the enzyme active site, while preserving the overall planarity of the molecule and not adding steric bulk in a prohibited region. In addition, it was felt that the new nitrogen containing ring would increase the solubility of the drug.

The scheme outlines the process for the preparation of **1** from 4-oxo-4-(3,4-dihydro-3-oxo-[1,4]-2H-benzoxazin-7-yl)butyric acid **3**. The methyl ester is made by a routine procedure and is subjected to Vilsmeier-Haack conditions giving the substituted imino chloride **4**. Treatment with two equivalents of hydrazine affords the desired pyrazolobenzoxazine, **1** with loss of water, methanol, hydrogen chloride and dimethylamine.

Scheme



3 4

Using procedures described previously [1] compound **1** demonstrated an intravenous (iv) ED_{50} of 15 μg/kg in the anesthetized dog model with a maximum increase in cardiac force of 256% at 75 μg/kg. The maximal rise in dp/dt was 119% and mean arterial blood pressure dropped 21% as heart rate climbed 51% at the highest dose. This compares quite favorably with bemoradan which is more potent than **1** with an iv ED_{50} of 5.4 μg/kg but has a lower maximal effect of 127% at the 75μg/kg dose. The high peak activity observed with **1** is closer to that seen with the (-) enantiomer of bemoradan which has a peak activity of nearly 200% at a dose of 35μg/kg iv in dogs. The drug (**1**) has a solubility of about 0.3 mg/mL in 1M HCl while bemoradan is essentially insoluble in dilute aqueous acid or base.

In summary, the synthesis of a novel pyrazolo fused analog of bemoradan which displays potent positive inotropic action in vivo and has more than twice the peak activity of the parent compound is described. In addition, a site is available for further substitution on the pyrazolo nitrogen which substantially enhances the solubility of the drug due to the ability to form salts.

Experimental

Melting points were determined on a Hoover Capillary Melting Point Apparatus and are uncorrected. Proton NMR spectra were taken in DMSO-d6 with tetramethylsilane as the internal standard and recorded at 300 MHz on a General Electric QE-300 instrument. Microanalyses were performed on a Perkin Elmer Model 240c Elemental Analyzer and infrared spectra were taken on a Perkin Elmer 1430 Ratio Recording Spectrometer as KBr pellets.

Methyl 4-oxo-4-(3,4-dihydro-3-oxo-[1,4]-2H-benzoxazin-7-yl)butyrate

4-Oxo-4-(3,4-dihydro-3-oxo-[1,4]-2H-benzoxazin-7-yl)butyric acid[1] **3** (5 g) was suspended in methanol (50 mL) and acetyl chloride (0.5 mL) added. The mixture was heated on a steam bath until all of the solid dissolved. The solvent was evaporated at reduced pressure providing the ester as a white foam which was recrystallized from ethyl acetate-methanol to give two crops (5 g total, 95%) of white crystals with mp 95-98°C. Calc for $C_{14}H_{15}NO_5$: C, 60.63; H, 5.46; N, 5.05. Found: C, 60.72; H, 5.52; N, 5.17.

Methyl 4-oxo-4-(3-chloro-2-dimethylaminomethylene-[1,4]-2H-benzoxazin-7-yl) butyrate (4)

Methyl 4-oxo-4-(3,4-dihydro-3-oxo-[1,4]-2H-benzoxazin-7-yl)butyrate (10 g) was added to chloroform (100 mL) containing $POCl_3$ (3.5 mL) and dimethylformamide (2.8 mL) at 0°C. The mixture was warmed to reflux for 3 h then stirred at room temperature overnight. The solvent was removed at reduced pressure and the residue basified with 10% NaOH solution and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated to dryness. The residue was chromatographed on silica gel eluting with methylene chloride. The fractions containing the product were evaporated to dryness and the residue used without further purification.

2,9-Dihydro-6-(5-methyl-3-oxo-2,3,4,5-tetrahydropyridazin-6-yl)pyrazolo[4,3-b][1,4]benzoxazine (1)

Anhydrous hydrazine (2 equiv.) was added to **4** (2.5 g) in ethanol (100 mL). After heating at reflux for 1 h the product was isolated by filtration as tan crystals and washed with ethanol to yield 1.65g of **1** with mp 304-306°C. Calc for $C_{14}H_{13}N_5O_2$: C, 59.35; H, 4.63; N, 24.73. Found: C, 58.96; H, 4.63; N, 24.64.

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