Bioorganic & Medicinal Chemistry Letters, Vol.3, No.6, pp. 1233-1239, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

DETERMINATION OF THE ACTIVE CONFORMATION OF 6-AMINO-α-[(4-DIPHENYLMETHYL-1-PIPERAZINYL)METHYL-9H-PURINE]-9-ETHANOL: A POSITIVE INOTROPIC AGENT

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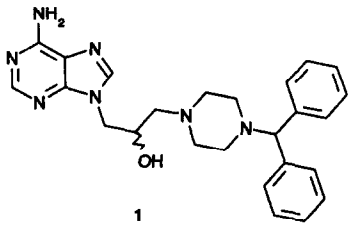
(*Received in USA* 21 *January* 1993)

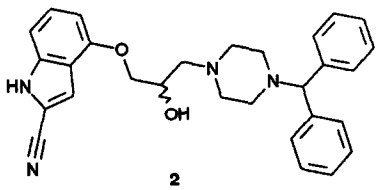
Abstract: Conformationally restricted analogues of the positive inotropic agent 1 have been synthesized in order to determine its biologically active conformation. Comparison of the distance maps (as a tool for the allowed conformations) with the biological activity give new insights in the biologically active conformation of this class of positive inotropic agents.

Introduction

The purine derivative 1 (SDZ 211-500)[1], similar to the indole derivative SDZ DPI 201-106 (2)[2], enhances contractile force of the heart by a mechanism involving fast sodium channels that had not been previously described[3]. The duration of the open state of the sodium channel is prolonged leading to an increase in sodium influx during the cardiac action potential. The sodium is rapidly exchanged with calcium by the Na^+ / Ca^{2+} exchanger[4]. Thus, the positive inotropic effect of these substances is ultimately brought about by an increase in intracellular calcium. Although high intracellular calcium concentrations may be associated with an increased risk for cardiac arrhythmias[5], sodium agonists increase both action potential duration and the functional refractory period (FRP), thus providing antiarrhythmic principles, which might compensate the arrhythmogenic elevation of calcium.

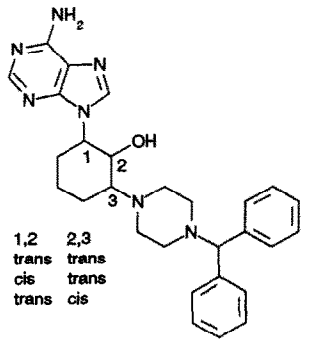


1
SDZ 211-500

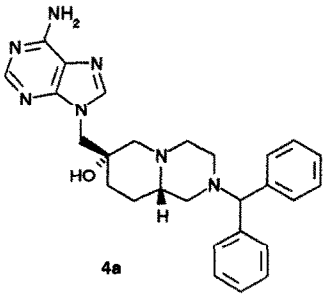


2
SDZ DPI 201-106

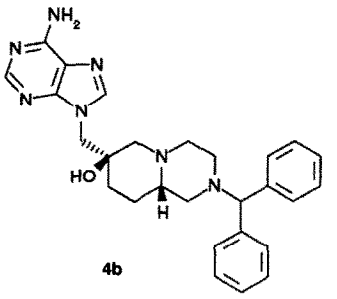
Since the exact binding site of this class of positive inotropic agents to the fast sodium channel is unknown, rational structure based drug design is not possible. The great flexibility of compound **1**, arising from the four rotatable bonds between the adenine and piperazine rings, does not allow the determination of one defined conformation. In order to find the possible biologically active conformation, conformationally restricted compounds with only two rotatable bonds in this region of the molecule have been synthesized. Thus, in the cyclohexanol derivatives **3a - c**, the relative orientation of the adenine and diphenylmethylpiperazine moieties is controlled. In the pyrido[1,2-a]pyrazine derivatives **4a** and **4b** rotation of the benzhydryl piperazine system is frozen[6].



	1,2	2,3
3a	trans	trans
3b	cis	trans
3c	trans	cis



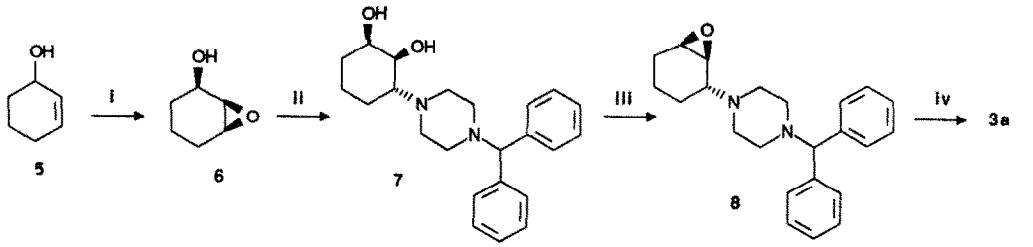
4a



4b

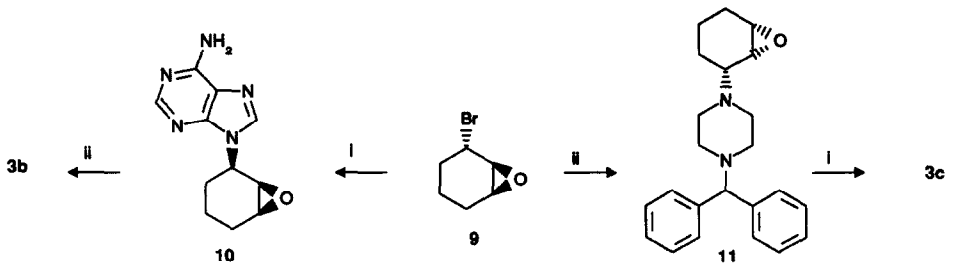
Syntheses

Derivative **3a** was synthesized from *cis*-2,3-epoxy-cyclohexanol (**6**), obtained stereoselectively by oxidation of 2-cyclohexen-1-ol (**5**) using tert-butyl-hydroperoxide and $VO(acac)_2$[7]. Subsequent addition of 4-diphenylmethylpiperazine and conversion of the resulting diol **7** into the epoxide via the Moffatt procedure[8] gave **8**, which was then reacted with adenine to give **3a**.



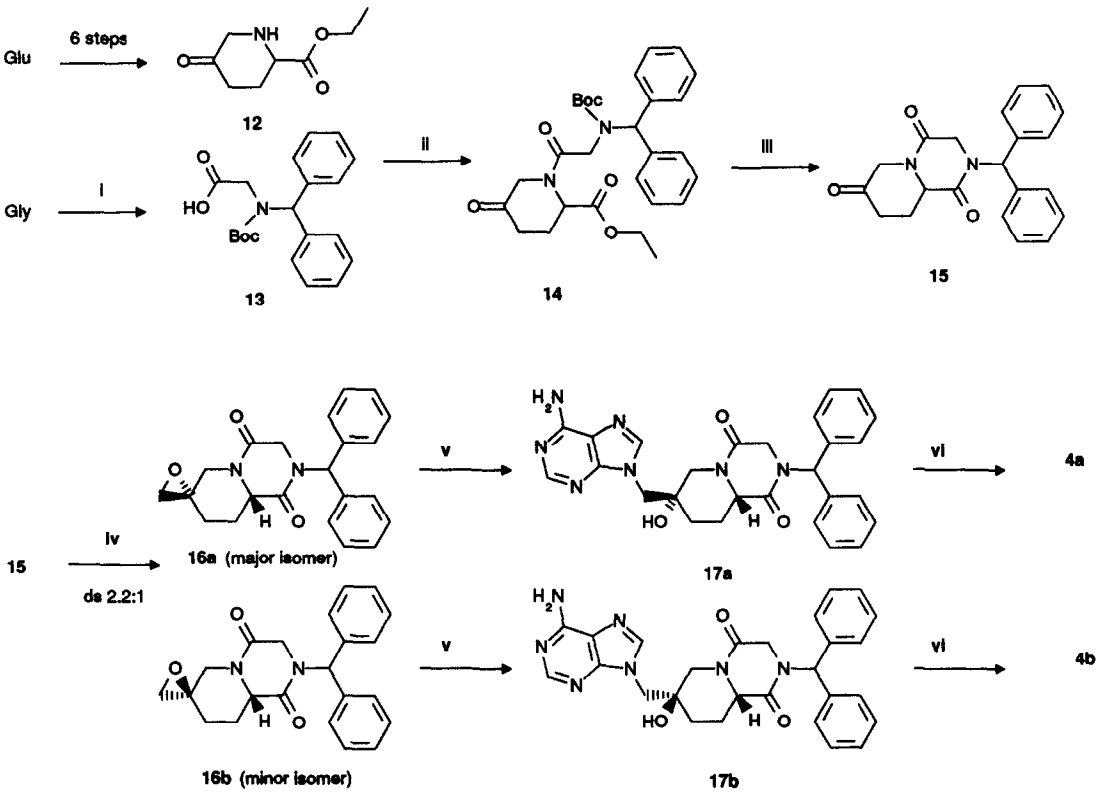
i) ^{t}BuOOH, $VO(acac)_2$, toluene, r.t., 24h; ii) diphenylmethylpiperazine; ^{t}BuOH, refl., 48h; iii) $AcOC(Me)_2COCl$, acetonitrile, refl., 6h; iv) 0.5N NaOH, dioxane, r.t., 16h; v) adenine, K_2CO_3, DMF, 120°C, 16h.

The cyclohexanol derivatives **3b** and **3c** were prepared by successive reaction of adenine and 4-diphenylmethylpiperazine with *trans*-1-bromo-2,3-epoxy-cyclohexane[9] or *vice versa*, respectively.



i) adenine, K_2CO_3, DMF, 100°C, 16h; ii) diphenylmethylpiperazine, DMSO/toluene 2:1, refl., 16h.

The pyrido[1,2-a]pyrazine derivatives **4a** and **4b** were prepared *via* the corresponding diketo piperazines. Thus ethyl 5-ketopipecolate **12** (prepared from glutamic acid in 6 steps according to the procedure of M. E. Freed and A. R. Day[10]) was coupled to BOC protected N-diphenylmethylglycine (derived from glycine *via* reductive amination[11]) to form compound **14**. Following deprotection, cyclisation to **15** was achieved by refluxing in toluene.



i) benzophenone, $NaBH_3CN$, $MeOH/H_2O$ 10:1, refl., 26h; $(BOC)_2O$, 1N NaOH, ^tBuOH, r.t., 23h; ii) **13**, propane phosphonic anhydrate, NMM, DMF, r.t., 4h; iii) HOAc, HCl; toluene, reflux, 5h; iv) trimethylsulfoniumiodide, KO^tBu, DMSO, r.t., 30 min; v) adenine, K_2CO_3, DMF, 100°C, 1h; vi) AlH_3, THF, 1.5h 0°C, r.t., 2h.

Conversion of the keto function into the epoxide, *via* the sulfonium ylide using trimethylsulfonium-iodide, led to isomer **16a** with a diastereoselectivity of 69% arising from attack of the sulfonium ylide at the less hindered face of the concave bicyclic system. Reaction of the epoxides **16a** and **16b** with adenine furnished compounds **17a** and **17b** along with small amounts of the 3-substituted adenines. Reduction of the amide functions with AlH_3 yielded the desired compounds **4a** and **4b**. The relative configuration as well as the regioselectivity of the reaction with adenine were confirmed by NOE- and HCCORR/COLOC experiments.

Biological Evaluation

Biological activity of all substances was evaluated in isolated rat atria. Left atria were dissected and mounted in an organ bath containing modified Krebs-Henseleit solution[12]. The solution was gassed with 95% O_2 / 5% CO_2 and the temperature was maintained at 36±0.1°C. Contractile force (Fc) was measured by connecting the atria to a Statham UC 3 transducer. FRP was determined in atria driven by square wave pulses (double threshold, 3 ms duration) at a basal rate of 1 Hz. Paired pulses of increasing coupling intervals were applied until a positive inotropic response after the test stimulus was seen[13]. The substances were added to the organ bath in stepwise increasing doses (0.1, 0.3, 1.0, 3.0 and 10 µM).
Sodium agonists prolong the open state of the fast sodium channel and thus lead to a positive inward current. This has two main functional consequences that can be determined in our assay: the Na^+-carried inward current prolongs the plateau phase of the action potential and consequently prolongs the FRP. Furthermore, the increased intracellular sodium is exchanged with calcium, which directly increases contractile force.

Computational Chemistry

Compounds **1**, **3a-c** and **4a-b** were modelled using the program SYBYL 5.1[14]. The following characteristic points were chosen for the structures: a dummy atom 1 Å above the center of the purine system on a line normal to it, the hydrogen of the alcohol function, the lone pair of the basic piperazine nitrogen and the benzylic carbon atom. The conformations are sufficiently described by five distances between these four points (2n-3, figure 1). Rotations of the rotatable bonds were carried out using the SEARCH procedure within SYBYL 5.1 (five for compound **1** as highlighted in figure 1, and each three for compounds **3** and **4**, which correspond to bonds a,d,e and a,b,e, resp.; increment: 10°, van-der-Waals factor: 0.85). The allowed distances between these four points are summarized in table 1.

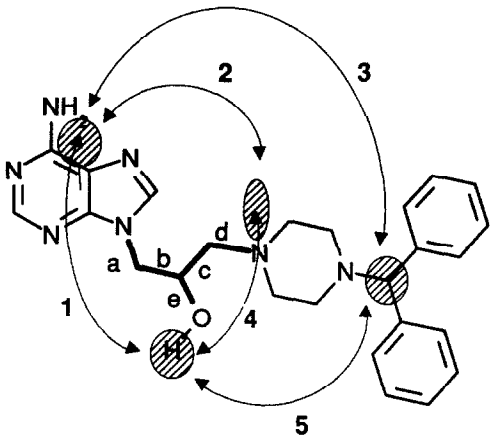


Figure 1. Compound **1**, with the rotatable bonds a - e highlighted and showing the four characteristic points, between which the allowed maximum and minimum distances (1-5) were computed (table 1).

Table 1. EC_{25} values for compounds increasing the functional refractory period (FRP) and contractile force (Fc) and parameters from the distance maps of these compounds:

Nr.	FRP EC_{25} [µM]	Fc EC_{25} [µM]	dist. 1 min. [Å]	dist. 1 max. [Å]	dist. 2 min. [Å]	dist. 2 max. [Å]	dist. 3 min. [Å]	dist. 3 max [Å]	dist. 4 min. [Å]	dist. 4 max. [Å]	dist. 5 min. [Å]	dist. 5 max. [Å]
1	0.2	0.5	2.0	6.5	2.5	7.5	5.5	11.2	1.5	4.8	5.2	8.6
3a	9	1.2	2.4	6.0	5.3	7.8	8.8	11.3	1.6	4.7	5.1	7.4
3b	0.9	1.5	3.2	6.0	5.0	7.1	8.7	10.6	1.4	4.7	5.1	7.3
3c	17	20	5.2	6.5	4.5	7.2	8.0	10.6	1.9	4.9	5.3	7.8
4a	1.8	6.5	2.0	6.5	4.3	7.1	7.8	11.1	1.8	3.4	5.88	7.6
4b	15	15	1.9	6.2	3.4	5.7	6.7	10.0	4.1	4.6	8.15	8.6

Results and Discussion

Considering the correlation between distance 1 and the contractile force of compounds **1** and **3a-c**, it appears that a minimal distance between the purine system and the hydroxy function is required for biological activity[15]. However, this feature alone is not sufficient for activity, since **4a** and **4b** are less active than compound **1**, despite having similar minimal values for distance 1 (2.0 and 1.9 Å). But **4a** and its inactive isomer **4b** differ drastically in the allowed minimal distances between the hydroxy group and the diphenylmethylpiperazine system (distances 4 and 5).

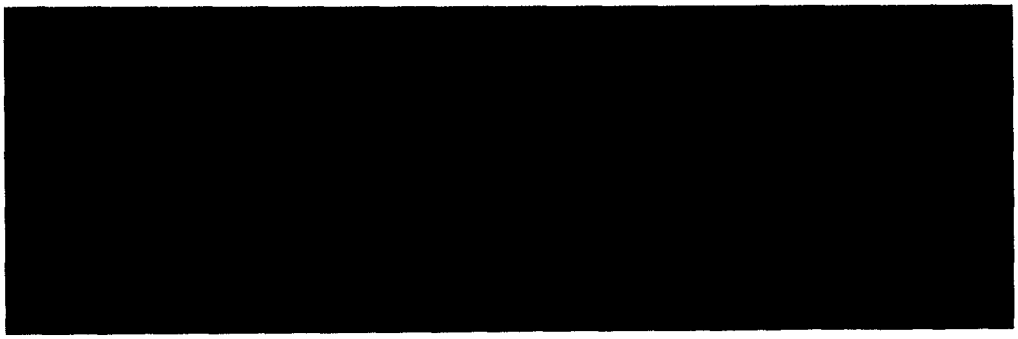


Figure 2: Superimposition of the allowed conformations. Stereoview of one conformation and the positions of the purine nitrogens (blue), the hydroxy group (red / cyan) and the lone pair of the basic piperazine nitrogen (blue / magenta).

Using these results the conformational search was repeated for the parent compound **1** with restrictions in the distances 1 (2 - 3.5 Å), 4 (1.4 - 1.9 Å) and 5 (5 - 6 Å)(increment 30°, van-der-Waals factor 0.85). The resulting 70 conformations have been superimposed (figure 2) thus showing the spatial arrangement of the functional groups in the biologically active conformations: the hydroxy function forms a H-bond to the basic nitrogen of the piperazine ring and the purine system is located in a plane perpendicular to the piperazine ring giving the molecule a overall U-shaped conformation.

Acknowledgment

The authors thank Mrs. Zardin and Mr. Bürkler for the n.m.r. experiments and Mr. M. Sanner and Mr. C. Ehrhardt for advice in the computational chemistry part.

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15 The FRP values correlate to the Fc values, except for compound **3a**, which shows less effect on the FRP.