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MARINE SPONGE BIS(INDOLE) ALKALOIDS THAT DISPLACE LIGAND BINDING TO α1 ADRENERGIC RECEPTORS[1]

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Abstract: The α1 adrenergic receptor assay guided fractionation of an extract from the marine sponge *Spongosorites* sp. led to the isolation of topsentin-B2 (bromotopsentin) as the active compound. Topsentin-B2 and five related bis(indole) marine sponge metabolites were shown to displace ligand binding to α1a and α1b adrenergic receptors with K_i values for the α1b receptor ranging from 0.08 to 1.15 μM. All six compounds show selectivity for α1b relative to α1a adrenergic receptors.

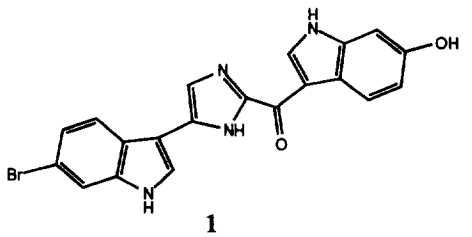
α-Adrenergic receptors have been subdivided into two classes, α1 and α2, based on pharmacological distinctions. The α1 adrenergic receptors have been further subdivided into α1a (previously denoted α1C), α1b, and α1d (previously α1A/D) receptors. The relatively high expression of the α1a subtype in the human prostate[2] has led to interest in selective α1a receptor antagonists to reduce contraction of prostatic smooth muscle and relieve urethal obstruction associated with benign prostatic hyperplasia (BPH).[3] Similarly, the relatively high expression of the α1b receptor in the human aorta[4] is indicative of the importance of this subtype in mediating vascular hypertension. The α1 adrenergic antagonists, such as prazosin and terazosin, which are currently used to treat hypertension and BPH are relatively nonselective for these α1 receptor subtypes.[3] Consequently, the discovery of agents selective for either α1a or α1b adrenergic receptors presents an opportunity for the development of a new generation of drugs to treat BPH or vascular hypertension with reduced side effects.

Chemistry

For the purpose of high throughput screening, small samples of frozen marine organisms were extracted with ethanol by blending in a Waring blender. The extract from the marine sponge *Spongosorites* sp. (HBOI sample number 21-V-93-3-001) was found to be active in an α1a adrenergic receptor assay. The sponge specimen was collected on May 21, 1993, at a depth of 630 m using the *Johnson Sea Link I* submersible. The collection location was west of Cape Santa Maria, Bahamas (23 41.117 N latitude, 75 22.182 W longitude). The sponge was frozen shortly after removal from the water, and stored at -20 °C until workup.

Subsequently, a larger scale extraction was carried out by blending the sponge with ethanol, and the resulting mixture was allowed to stand for 1 h at room temperature. The mixture was then blended again and vacuum filtered. The filter cake was returned to the blender and the above procedure repeated three times. A final extraction was completed by allowing the sponge/ethanol to steep overnight. All of the filtered ethanol extracts were combined, concentrated under reduced pressure, and solvent partitioned between ethyl acetate and water. Bioassay showed the α1a activity to be in the ethyl acetate partition fraction. The ethyl acetate fraction was then subjected to HPLC using a YMC ODS-A column, which was eluted with methanol (70%) and 0.05% aqueous trifluoroacetic acid (30%). The α1a activity correlated to the major UV absorbing peak which, by photodiode array analysis, consisted of a single component (**1**).

The ^{1}H NMR spectra of **1** in CD_3OD and CD_3OD/TFA were indicative of bis(indole) natural products, showing only a cluster of aromatic/olefinic peaks between 6.8 and 8.5 ppm, with peak doubling and peak shape being highly pH dependent. The FABMS gave an $(M+H)^+$ molecular clusters at 421 and 423 daltons, suggestive of topsentin-B2.[5] High resolution FABMS gave the topsentin-B2 molecular formula $C_{20}H_{13}N_4O_2Br$ (observed $(M+H)^+$ mass 421.0301, calc. 421.0300). Difficulties in observing all of the carbon signals in the ^{13}C NMR spectrum were encountered as reported previously.[6] To confirm the identity of compound **1** a co-injection of compound **1** with an authentic sample of topsentin-B2, and analysis by HPLC-photodiode array detection, gave a single symmetrical homogeneous peak.



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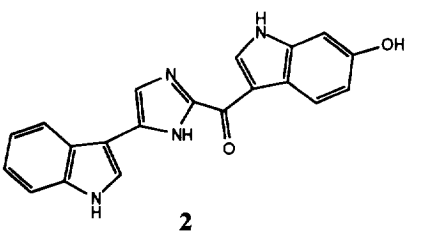

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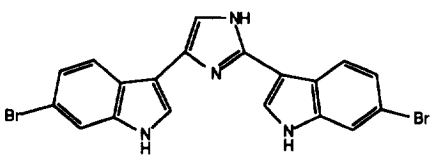

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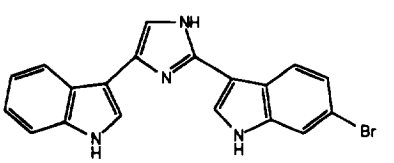

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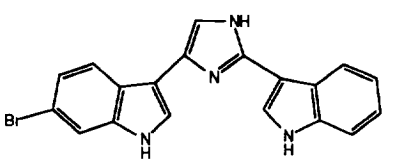

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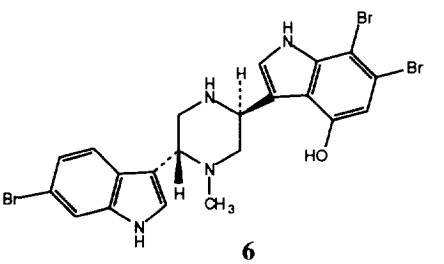

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Pharmacology

The potency of compounds in binding α1a and α1b receptors was assessed by their ability to displace the specific binding of 0.15 nM [^{125}I]-HEAT (2-β-(4-hydroxyphenyl)-ethylaminomethyltetralone).[7] Membranes were prepared by differential centrifugation from COS7 cells stably overexpressing human α1a receptors, or from Rat-1 cells stably overexpressing hamster α1b receptors. Specific binding was defined by displacement with 1 μM prazosin. Incubations in glycylglycine buffer, pH 7.8 were carried out in 96-well MultiScreen plates on a rotary shaker at 25 °C for 1 h. Bound radioligand was separated from free radioligand by filtration. Filters were punched out and radioactivity was quantitated in a γ-counter. K_i values were calculated from IC_{50} values using K_d values of 338 pM (α1a) and 113 pM (α1b), which we had previously determined. The data shown (Table 1) are average K_i values from three separate experiments.

Table 1. α1 K_i values for compounds **1-6**

Compound	α 1a K_i (μM)	α 1b K_i (μM)	Selectivity α1a K_i/α1b K_i
bromotopsentin (**1**)	12.59	0.74	17.0
topsentin (**2**)	4.74	1.15	4.1
nortopsentin-A (**3**)	2.14	0.52	4.1
nortopsentin-B (**4**)	0.77	0.13	5.9
nortopsentin-C (**5**)	0.55	0.17	3.2
dragmacidin (**6**)	0.14	0.08	1.8

The discovery of α1 receptor binding displacement by bromotopsentin (**1**) prompted us to evaluate the α1 activity of several related sponge metabolites that were available from the Harbor Branch Oceanographic Institution pure compound collection. Table 1 shows the potencies at α1a and α1b receptors for topsentin-B2 (bromotopsentin) (**1**) and topsentin (**2**) isolated from a *Spongosorites* sp.,[6] nortopsentins A (**3**), B (**4**), and C (**5**) isolated from *S. ruetzleri*,[8] and dragmacidin (**6**) isolated from a *Dragmacidon* sp.[9] All six compounds bound preferentially to the α1b receptor with topsentin-B2 showing the greatest selectivity. Dragmacidin was the most potent compound with K_i values of 78 nM and 138 nM at α1b and α1a adrenergic receptors, respectively. As can be seen from these data, even among this limited series of compounds, structural variations alter the potency at the α1b receptor by an order of magnitude, and at the α1a receptor by approximately two orders of magnitude.

References and Notes

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