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HYPOTHETICAL MODELS OF DOPAMINE D-1 RECEPTOR

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โมเดลทางทฤษฎีของโดปามีน ดี-1 รีเซพเตอร์

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ภาควิชาเภสัชเคมี คณะเภสัชศาสตร์ มหาวิทยาลัยขอนแก่น จ.ขอนแก่น 40002,
ภาควิชาเภสัชเคมี มหาวิทยาลัยเปอร์ดู เมืองเวสตลาฟาแยตต์ รัฐอินเดียน่า สหรัฐอเมริกา

โมเดลของโดปามีนรีเซพเตอร์หลายรูปแบบ ได้ข้อมูลจากสารประกอบทางเคมีประเภทต่าง ๆ และระบบการทดสอบฤทธิ์ทางเภสัชวิทยาที่แตกต่างกัน โมเดลของแมคเดอร์เมีด ซึ่งเป็นที่ยอมรับมากในปัจจุบัน และเป็นรูปแบบตัวอย่างของโดปามีน ดี-1 และ ดี-2 รีเซพเตอร์ ได้เสนอว่า ส่วนของรีเซพเตอร์ที่จับกับสาร มี 2 แห่ง (sites) ที่จำเป็นต่อการออกฤทธิ์ และอีก 1 แห่ง ที่เป็นบริเวณซึ่งทำให้เกิดผลสเตอริคขึ้น ในการศึกษานี้ เป็นการเปรียบเทียบฤทธิ์ทางเภสัชวิทยาของสารประกอบในกลุ่มเบต้าเฟนิลโดปามีนและสารอนุพันธ์ที่มีคอนฟอร์เมชั่นคงที่ โดยคำนึงถึงความแตกต่างในด้านความคงตัวของคอนฟอร์เมชั่น และระนาบของโมเลกุล โดยทำการเปรียบเทียบฤทธิ์ทางเภสัชวิทยาในแง่ ดี-1 และ ดี-2 ข้อมูลจากความสัมพันธ์ระหว่างฤทธิ์ทางเภสัชวิทยากับสูตรโครงสร้างทางเคมี ทำให้ได้ข้อเสนอแนะว่าอาจจะมีบริเวณที่ยาจับกับรีเซพเตอร์อีกแห่งหนึ่ง (accessory site) สำหรับโดฟามีนรีเซพเตอร์ ดี-1 บริเวณนี้น่าจะมีคุณสมบัติเป็นไฮโดรโฟบิค (Hydrophobic region) และเชื่อว่าเป็นบริเวณที่อาจจะมีปฏิกิริยากับกลุ่มเฟนิลของสารประกอบพวก 1-เฟนิล-3-เบนซาซีปีน หรือกลุ่มเฟนิลที่ไม่มีกลุ่มแทนที่ของสารอนุพันธ์พวกไดไฮดรอกซีนอมิเฟนซีนและอนุพันธ์ในกลุ่มเบต้าเฟนิลโดปามีน

Abstract.

A number of dopamine receptor models have been proposed based on diverse chemical classes of compounds and different pharmacological systems. The McDermed receptor model,

the most well accepted one, which may be considered as a prototype of current models of both D-1 and D-2 receptor provided the suggestion of two essential binding sites and a zone of steric occlusion. In this study, a series of beta-phenyldopamine and its conformationally restricted dopamine analogs are compared, on the basis of conformation rigidity and planarity of the molecule, with the pharmacological evaluation for D-1 and DA-1 activities. The structural activity relationship data provides the suggestion that there may be an accessory site for D-1 receptor subtype. This site may be considered as hydrophobic region which is believed to be complimentary with phenyl moiety of 1-phenyl-3-benzazepine analogs or the unsubstituted phenyl ring of dihydroxynomifensine derivatives as well as beta-phenyldopamine analogs.

Introduction

The emergence of neurotransmitter role of dopamine and significant progress on specific dopaminergic agents have actively stimulated the concept of dopamine receptors during the past two decades. It is now widely accepted that multiple classes of dopamine receptors are present in vertebrate and invertebrate nervous and peripheral systems. The hypothesis currently accepted by most investigators is that there are two subtypes of dopamine receptors, as proposed by Kebabian and Caln in 1979.[1] They are classified into D-1 and D-2 receptors based on pharmacological criteria and the regulation of adenylate cyclase activity. The dopamine D-1 receptor mediates the stimulation of adenylate cyclase, while dopamine D-2 receptors are either unassociated with this enzyme or mediate its inhibition. Moreover, the peripheral dopamine receptor subtype concept was proposed by Goldberg and Kohli based on the pharmacological differentiation in the cardiovascular and renovascular system.[2] The DA-1 receptor, upon activation, causes a relaxation of vascular smooth muscle in renal blood vessels and an increase in vascular cAMP content. Stimulation of the DA-2 receptor inhibits the stimulated release of norepinephrine from postganglionic sympathetic nerve terminals, inhibits ganglionic transmission, and does not increase cAMP formation.[3]

A large number of dopamine receptor models have been proposed from consideration of structure-activity relationship (SAR) data from various classes of dopamine analogues, particularly dopamine agonists[4,5,6,7,8], antagonist[9]. The most well accepted hypothetical mofel of dopamine receptor, which may be considered as a prototype model of the D-1 and D-2 receptor was proposed by McDermed in 1979[7]. Seiler and Markstein[10] proposed a D-1 receptor model based on the SAR data of a series of monohydroxy-2-aminotetralins. Dihydroxynomifensine derivatives were also employed as a probe for D-1 and DA-1 receptor by Dandridge[6]. However, a diversity in structural requirements for D-1 activities leads the urge to search for the models based on the various classes. Nichols proposed that a series of beta-phenyl dopamine derivatives may be useful as another probe for D-1 receptor model and hence as another lead for design of more selective agents.[11]

Therefore, a series of beta-phenyldopamine analogs was designed and the pharmacological evaluations are performed to determine the dopamine D-1 or DA-1 activities. Compound [1], 3',4'-dihydroxy-4-phenyl-1,2,3,4-tetrahydroisoquinoline; compound [2], 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine; compound [3], beta-methyldopamine and compound [4], beta-phenyldopamine were evaluated by the rat retinal adenylate cyclase assay. Compound [1] was also tested for its renal vasodilating property as indicated by changes in renal blood flow, a parameter for DA-1 activity. In addition, compound

[5]; hexahydrobenzo[a]phenanthridine is preliminarily studied by radioligand binding assay for D-1 and D-2 receptors.

METHODS

A wide variety of test systems have been developed over the years to determine the activity and degree of selectivity of compounds at dopamine receptor subtypes. Generally, D-1 activity is evaluated by the ability to stimulate dopamine-sensitive adenylate cyclase activity for agonists or to inhibit dopamine-induced adenylate cyclase for antagonists,[1] and radioligand binding assay employing ^{3}H-R-SCH 23390[12]. Dopamine DA-1 activity is tested by the vasodilation effects in dog renal and mesenteric vascular bed or rabbit splenic artery[13].

Adenylate Cyclase Assay Method

The rat-retinal dopamine-sensitive adenylate cyclase assay was performed to examine the dopamine D-1 activity of test compounds. The procedures were as follows:

Rat retinas were homogenized in 150 vol/wt of 2.0 mM Tris HCl (pH 7.4) with 2 mM EDTA using a Teflon-glass homogenizer. Each reaction mixture contained the following final concentrations in a volume of 0.2 mL : 2 mM $MgSO_4.7H_2O$, 0.5 mM EGTA, 1 mM IBMX, 0.01 mM GTP, 80 mM Tris HCl (pH 7.4), 0.5 mM ATP with approximately 5 x 10^6DPM and ^{32}P-ATP and 20-30 micrograms of retinal homogenate protein. Following an incubation of 20 min at 30 degree the reaction was terminated by adding 200 microlitres of a solution containing 1% SDS, 20 mM ATP, 0.7 mM cyclic AMP with 1.0 x 10^4 DPM ^{3}H-cyclic AMP in 80 mM Tris HCl, pH 7.4 and heating to 85 degree for 2 min. Cyclic AMP was isolated from the mixture using the column chromatographic technique of Salomon and measured by CPM. Control values were 40-45 pmoles/mg protein/min.

DA-1 Activity Assay Method

A frank incision and retroperitoneal dissection of 20-25 kg pentobarbital-anesthesized adult male dog was performed and the right renal artery was isolated and prepared for measurement of renal blood flow using an electromagnetic flow probe. Drug administration was operated through a 25-gauge needle bent at about an 80 degree angle and placed into the renal artery proximal to the flow probe. Phenoxybenzamine, 5-10 mg/kg, was infused intraarterially over a 15 to 20-min period. Complete alpha-adrenergic blockade was confirmed by abolition or reversal of the vasoconstrictor effect of injection of 12 nmol of noradrenaline. Under these circumstances, dopamine in doses of 12-190 nmol contained in 0.2 ml injection volume produced short-lived reversible increases in renal blood flow. After standard responses to dopamine were obtained, the test drugs were injected in increasing doses into the renal artery. The result was expressed in millilitre per minute increase in blood flow. Then, agonists which exhibited vasodilator were also tested with sulpiride to determine the degree of inhibition. A dose of 0.5 mg of sulpiride in 0.2 mL volume was combined with a dose of 750 nmol of the test drug in 0.2 mL and simultaneously injected. In order to determine whether the dilation was beta-adrenergic in origin, propanolol,2.5-5 mg/kg, was infused intraarterially over a 20-min period. Beta-adrenergic blockade was established by abolition of the vasodilator effects of 3 nmol isoproterenol.

RADIOLIGAND BINDING ASSAY

[^{3}H]-SCH 23390 Binding. Striatal tissue is homogenized in 50 mL of 50 mM HEPES

buffer (pH 7.4) per gram of tissue, using a PCU-2-polytron at a setting 6 for 20 seconds. EDTA (1mM) and $MgSO_4$ (4mM) were included in all experiments where competition for binding is examined. Following homogenization, tissue was centrifuged at 27,000g for 10 minutes, the supernatant is discarded, and the pellet resuspended in buffer and centrifuged. After repeating this wash step, the final pellet is suspended in buffer at a concentration of 1 mg tissue (original wet weingt)/mL buffer or less.

Culture tubes were used with a final incubation volume of 1.0 mL. This includes 300 uL buffer, 100 uL [^{3}H]SCH 23390 in buffer, 500 uL tissue homogenate, and 100 uL of added drug or vehicle. Nonspecific binding of [^{3}H] SCH 23390 was defined by adding unlabelled SCH 23390 at a final concentration of 1 uM. Culture tubes and tissues were maintained separately on ice prior to being mixed. Incubations were at 30 degree for 15 minutes unless stated otherwise, and binding is terminated by filtration on Gelman GF/C glass fiber filter. Filters were allowed to air dry, and radioactivity was quantified by liquid scintillation spectrometry after additon of 10 mL Scintiverse E using a LKB Rack Beta liquid scintillation counter with 50% counting efficiency for tritium. Samples are run in triplicate. IC50 values were derived from linear regression of log-logit transformations.

[^{3}H]-Spiperone Binding. Striatal tissue is homogenized in approximately 100 volumes of ice cold 50 mM HEPES buffer, pH 7.55 at 25 degree, with a polytron at a setting of 5 for 20 seconds. The striatal homogenate is then centrifuged at 30,000 g for 10 minutes. Two additional washes were performed by resuspending the pellet in fresh HEPES buffer and centrifuging. The reaction is started by the addition of tissue (2.5 mg/mL) to each tube containing [^{3}H]-spiperone and test compounds dissolved in 0.1% tartaric acid. Tubes are then incubated for 20 minutes at 37 degree and filtered under vacuum through glass fiber filters. Each tube is rinsed three times with a 5 mL of cold HEPES buffer. The filters are then placed in glass vials, allowed to dry, 10 mL of scintillation cocktail is added, and the sample subjected to liquid scintillation spectrometry. The concentration of each drug that produces a 50% inhibition of specific [^{3}H]-spiperone binding is calculated by linear regression of a log-logit analysis.

RESULTS AND DISCUSSION

In Figure 2, dopamine was considerable more potent than Compound [1] with more than 10 folds of potency difference. The enantioselective study of this compound was shown in Table 1. Dopamine itself demonstrated less selectivity for D-1 than Compound [1] whereas the S-enantiomer was alightly more potent than its racemate for the D-1 activity and the R-form was inactive. All compound [2] tested required relatively high doses to attain th required D-2 affinities (Table 2). In this case, the enantioselectivity for D-1 resided in the R-enantiomer. In table 3, the racemic beta-methyldopamine [3] showed very low activity, whereas, surprisingly, the R and S enantiomers were essentially equipotent in stimulating dopamine-sensitive adenylate cyclase and possessed about 20% of the activity of dopamine. Beta-phenyldopamine [4] was more potent having one-sixth the activity of dopamine at 0.1 to 1.0 micromolar and half of the potency of dopamine at 10 micromolar. From Table 4, Compound [5], hexahydrobenzo[a]phenantridine showed the significant D-1 affinity in the trans ring junction form and D-1 inactivity in the cis-form. It also exhibited the selectivity for D-1 over the D-2 subtypes.

McDermed demonstrated that the R-isomer of 6,7-ADTN was a more potent dopamine agonist, where as in the 5-mono-

hydroxyaminotetralins, the S-isomer was more potent. McDermed receptor model suggested, based on enantioselectivity of aporphine and a number of hydroxylated 2-amino-1,2,3,4-tetrahydronaphthalene derivatives as shown in Figure 3 (bottom), two essential binding sites and a zone of steric intolerance or steric occlusion.[8] One of the primary binding sites accommodates the hydroxyl group 'meta' to the ethylamine sidechain of the dopaminergic pharmacophore, and a second is in a position to interact with the properly spatially-oriented amino group. A zone of steric occlusion is believed to account for the inactivity of a number of agents, e.g. isoapomorphine.

It is likely from this study that there may be an additional site for interacting with the receptor as proposed by the hydrophobic region, designated as "H" in Figure 3. The activity of beta-phenyldopamine may be rationalized by the binding of the free-phenyl group to the proposed hydrophobic site on the D-1 receptor, thus enhancing D-1 agonist activity. Conversely, the decreased activity and the lack of enantioselectivity of beta-methyldopamine might be rationalized by the inability of the smaller methyl group to bind adequately to the hydrophobic site. However, the beta-methyl group still increases steric bulk and, therefore, may decrease overall affinity for the receptor. When benzo[f]quinoline, a D-2 agonist was considered to align with the proposed model, it is probable that the major part of the molecule is directed far beyond the hydrophobic region. This similar interaction also occurred with the D-1 inactive cis-hexahydrobenzo[a]phenanthridine as illustrated in Figure 4. In Figure 5, the D-1 active trans-hexahydrobenzo[a]phenanthridine interaction with the proposed receptor in such a way that the hydrophobic region could accommodate the hydrophobic moiety of the molecule. Based on the interaction of all compounds together with the previously proposed model[6,7], it is proposed that the hydrophobic binding site may be located above the plane of the catechol ring. This site can accommodate the beta-phenyl group, whose angle relatively to the plane of the catechol is 60 degree or less. This indicates a range of angles between the two phenyl rings is permissible and that potency and selectivity may be dependent on choices of appropriate frameworks.

However, the role of the beta-phenyl group is not yet entirely clear. The beta-phenyl ring may serve two important roles : to interact favorably with an accessory hydrophobic site on the dopamine D-1 receptor, and to exclude interaction with the D-2 site or alpha-adrenergic receptor. More investigation is obviously required to clarify this point.

Acknowledgement

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TABLE 1. Enantioselectivity of 4-phenyl- Dihydroxy 4-phenyl-1,2,3,4-tetrahydroisoquinoline [1].

	A.C.STIMULATION EC50, uM	INHIBITION OF D-2 BINDING, uM
DOPAMINE	**3.5**	**5.34**
(R,S)	**2.88**	**92.0**
(R)	**INACTIVE**	**99.1**
(S)	**1.87**	**85.8**

TABLE 2. D-1 Activity of 2,3,4,5-Tetrahydro-7, 8-dihydroxy-1-phenyl-1H-3-benzazepine [2].

	A.C. STIMULATION EC50, nM	INHIBITION OF D-2 BINDING, uM
DOPAMINE	3500	5.34
(R,S)	71	34.43
(R)	32	33.86
(S)	>27000	197.4

TABLE 3. D-1 Activity by Adenylate cyclase model of Beta-methyl- dopamine [3] and Beta-phenyldopamine [4].

Compound	Conc (micromolar)	% Stimulation
Dopamine	0.1	31.4 ± 3.4
	1.0	106.4 ± 9.3
	10.0	205.4 ± 10.3
(R,S)-[3]	0.1	5.9 ± 4.6
	1.0	3.5 ± 9.6
	10.0	10.5 ± 1.8
(R)-[3]	0.1	1.5 ± 10.4
	1.0	6.0 ± 3.2
	10.0	41.0 ± 8.9
(S)-[3]	0.1	1.6 ± 8.5
	1.0	11.7 ± 3.7
	10.0	39.1 ± 6.3
[4]	0.1	4.9 ± 2.9
	1.0	16.3 ± 3.6
	10.0	97.1 ± 4.2

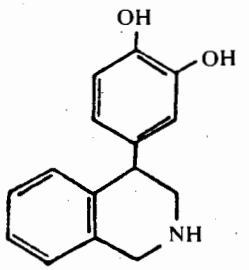
TABLE 4. Radioligand binding assay of Hexahydrobenzo [a] phenanthridine [5]. IC50 is reported as a indication for binding affinities to the appropriate receptors

	IC50 (nM)	
	D-1	D-2
cis Form	>10000	>10000
Trans Form	110	4000

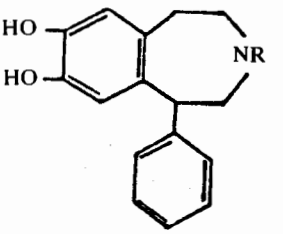
A mixture of rat striatal homogenates, [^{3}H]-Spiperone and a varying concentration of test compounds was incubated at 37 degree for 20 minutes. After filtration through glass filter, radioactivity counting was monitored by liquid scintillation spectrometry. The concentration of test compounds that produced a 50% inhibition of specific [^{3}H]-SCH 23390 or [^{3}H]-spiperone binding, IC50 is calculated by linear regression of a log -logit analysis.

TABLE 5. The relationship of angles between the beta-phenyl and catechol ring and D-1 activity of some dopamine analogs

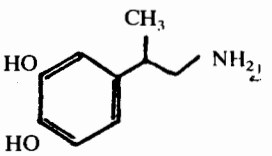
Compound	Angle	D-1 activity
SKF 38393	25	active
4-Phenyl-tetrahydroisoquinoline	60	active
cis-hexahydrobenzo [a] phenanthridine	70	inactive



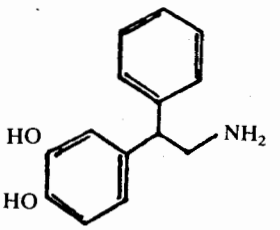
[1]
Dihydroxy-4-phenyl-
1,2,3,4-tetrahydroisoquinoline



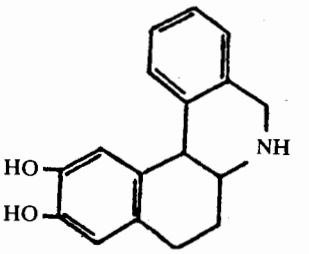
[2]
2,3,4,5-tetrahydro-7,8-
dihydroxy-1-phenyl-1H-3-benzazepine



[3]
Beta-methyldopamine



[4]
Beta-phenyldopamine



[5]
Hexahydrobenzo[a]
phenanthridine

Figure 1 A series of Beta-phenyldopamine analogs.

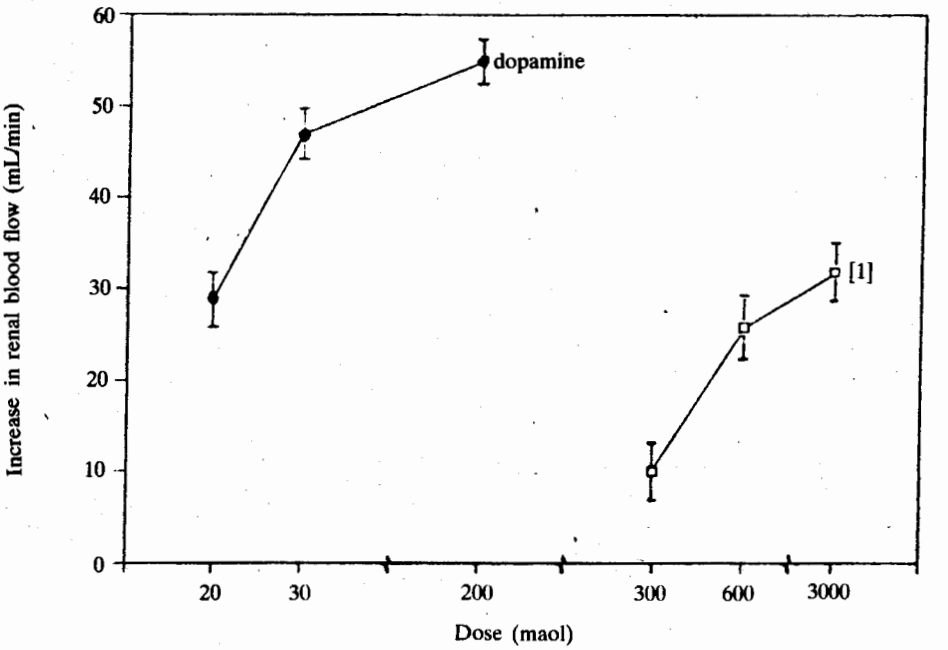
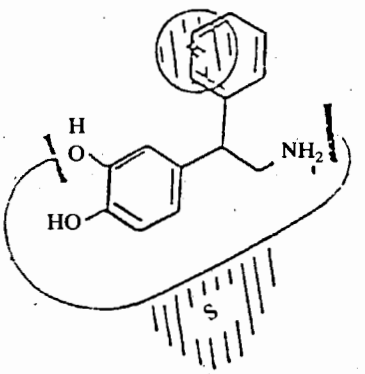
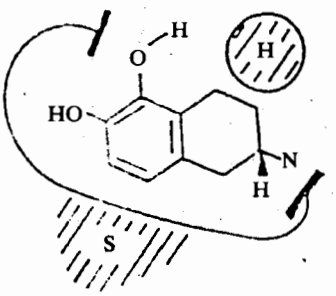


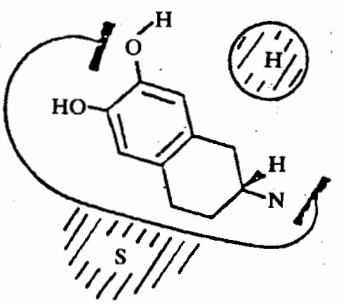
Figure 2. Effects of dopamine and [1] on renal blood flow. Points plotted with bars represent the mean plus or minus SEM.



Beta-phenyldopamine



(S)-5,6-Dihydroxy-2-aminotetralin



(R)-6, 7-Dihydroxy-2-aminotetralin

Figure 3. The proposed D-1 receptor model based on the aminotetralin derivatives and Beta-phenyldopamine analogs.

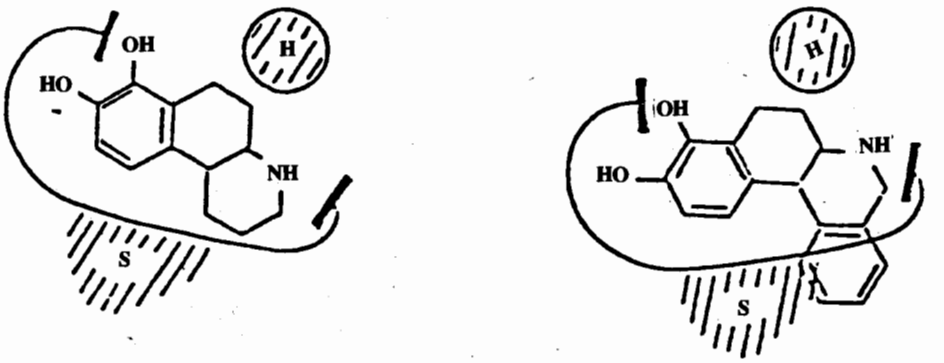


Figure 4. The proposed D-1 receptor model based on benzo[f]quinoline and failure of interaction with cis-hexahydrobenzo[a]phenantridine.

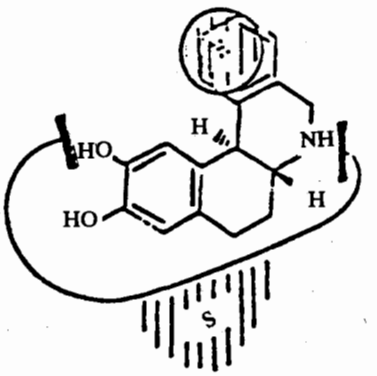


Figure 5. The proposed interaction between trans-hexahydrobenzo[a] phenanthridine and the receptor.