Journal of Industrial Microbiology, 8 (1991) 187–192
Elsevier

SIM 00365

Sch 42029, a naturally produced dopamine receptor ligand: taxonomy, fermentation, isolation and structure

Vinod R. Hegde[1], Mahesh G. Patel[1], Ann C. Horan[1], Jeffrey L. Schwartz[1], Richard Hart[1], Mohindar S. Puar[2], Vincent P. Gullo[1] and Smriti Iyengar[3]*

[1]*Microbial Products,* [2]*Molecular Spectroscopy, and* [3]*Molecular Pharmacology, Schering-Plough Research, Bloomfield, New Jersey, U.S.A.*

(Received 14 March 1991; revision received 25 May 1991; accepted 29 May 1991)

Key words: Dopamine receptor; Agonist and antagonist; Ligand; Dihydroxy acetanilide

SUMMARY

A natural product, Sch 42029, isolated from the fermentation of an *Actinoplanes* sp. (SCC 1971) was found to displace Sch 23390 from the dopamine-1 (D_1) receptor. The compound was isolated from the fermentation broth by adsorption of the filtrate on XAD-16 resin, elution with water–methanol, followed by purification by gel-permeation chromatography and HPLC. Using spectroscopic analysis, the structure was determined to be 2,5-dihydroxy acetanilide. The pure compound displaced Sch 23390, a D_1-selective ligand, at a K_i of 1.6 μm and spiperone, a D_2-selective ligand, at a K_i of 200 μm.

INTRODUCTION

A number of CNS-related disorders as well as motor impairments (e.g. Parkinson's diseases, Schizophrenia) have been linked to improper functioning of dopaminergic pathways in the brain. Based on extensive pharmacological studies, two types of dopamine receptors have been described in the central nervous system: D_1 and D_2 [9,14]. While a number of drugs acting at dopamine receptors have been developed, most of these have been shown to selectively exert their action at D_2 receptors, some as antagonists (haloperidol, spiperone, sulpiride) that are useful neuroleptics and some as agonists (apomorphine, ADTN). Some neuroleptics (flupentixol, piflutixol) have equal affinity for both D_1 and D_2 receptors. However, most of the existing neuroleptics have extensive motor, endocrine and autonomic side effects. D_1 and D_2 receptors have been proposed to be functionally interactive. Since the description of the D_1 receptor is recent [3], very few specific D_1 agonists (SKF 38393) and one specific D_1 antagonist (Sch 23390; Fig. 1, structure 3) have been reported [1,20]. Pharmacological studies with Sch 23390, a selective antagonist, indicate that compounds selective for this subtype are potential antipsychotics with less liability of the side effects associated with D_2 selective antagonists [1].

Since Sch 23390 was found to be weakly active orally and had a short duration of action in primary models, we undertook screening secondary metabolites of soil microorganisms for novel D_1 receptor ligands. Recently while screening actinomycetes for secondary metabolites with pharmacological activity, we have isolated a novel D_1-receptor ligand, Sch 42029, (Fig. 1, structure 1) with 125-fold selectivity for the D_1 receptor. The producing microorganism, identified as an *Actinoplanes* sp. by taxonomical studies, was isolated from a West African soil sample. This report describes the preliminary taxonomy of the producing strain, the production of the active compound, its isolation, structure determination and pharmacological activity.

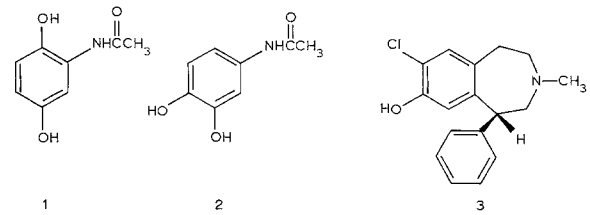

Fig. 1. Structures of Sch 42029(1), 3,4-Dihydroxy acetanilide(2), Sch 23390(3).

Correspondence: V.P. Gullo, Microbial Products, Schering-Plough Research, Bloomfield, NJ 07003, U.S.A.
* Present address: Searle Research and Development, Division of G.D. Searle & Co., c/o Monsanto Company, 700 Chesterfield Village Parkway, St. Louis, MO 63198, U.S.A.

MATERIALS AND METHODS

Taxonomy

The producing culture was isolated from a West African soil sample using a modification of Makkar & Cross' technique for the isolation of motile-spored actinomycetes as reported by Berrie [2,17]. Growth characteristics were observed on ATCC medium 172 [7] after 21–28 days at 30 °C. Morphological observations were made on water agar (agar, 15 g; tap water 1000 ml; pH 7.0) at 7, 14 and 21 days at 30 °C. Biomass production and whole-cell analysis followed the procedures of Lechevalier [15].

Fermentation

Stock cultures were maintained as frozen broths at − 20 °C in final concentration of 10% glycerol. A 250-ml Erlenmeyer flask containing 50 ml of germination medium was inoculated with 2.5 ml stock culture. The flask was incubated at 30 °C on a rotary shaker at 300 rpm for 72 hours. The germination medium consisted of glucose 1%, trehalose 1%, casein 0.5%, soy flour 0.5%, yeast extract 0.5%, $CaCO_3$ 0.2%, and Dow-Corning antifoam B 1 ml per liter of tap water. The pH was adjusted to 7.2 prior to autoclaving. 20 ml of this germination culture was used to inoculate a 2-l Erlenmeyer flask containing 350 ml of the same germination medium and incubated as above. Twenty ml of this second-stage germination inoculum were used to inoculate a 2-l Erlenmeyer flask containing 350 ml of fermentation production medium consisting of PD 650 dextrin 3%, pea flour 1.5%, maltose 0.5%, fructose 0.5%, yeast extract 0.3%, N-Z-Amine A 0.3%, sea salts 0.01%, and 1 ml of Dow-Corning antifoam B per 1 l of tap water. The pH was adjusted to 7.5 prior to autoclaving. The fermentation was carried out at 30 °C, on a rotary shaker at 300 rpm for 72 h. A typical time-course study is shown in Fig. 2. The production of Sch 42029 was monitored using the D_1-receptor binding assay [3] and antimicrobial activity against *Micrococcus luteus* ATCC 9341. Harvested samples were passed through a centriprep filter (10 000 molecular mass cut-off filter) prior to assay. For determination of packed cell volume (PCV) 15 ml of whole broth was centrifuged for 15 min at 4750 rpm on a Fisher Scientific Centrifuge.

Isolation

The steps leading to the isolation and purification of Sch 42029 are outlined in Fig. 3. After fermentation, the cultured broth was filtered (folded cellulose filters from Schleicher & Schuell) to remove cells. The compound was adsorbed onto XAD-16 resin and the inactive spent filtrate decanted. The charged resin was washed with water and eluted with water–methanol (1 : 1). The methanol was evaporated under reduced pressure and the active eluate was freeze-dried. Further purification was accomplished on a Sephadex LH-20 column. Preliminary studies indicated that Sch 42029 is an unstable compound and can be stabilized in acidic medium. Hence, final purification of the major active compound was achieved by preparative HPLC on a reverse-phase C18 column, eluting with a mixture of methanol and acidic water (pH 3.0, HCl) 5 : 95. The eluate on freeze-drying afforded 45 mg of Sch 42029. The HPLC profile of the pure compound is shown in Fig. 4.

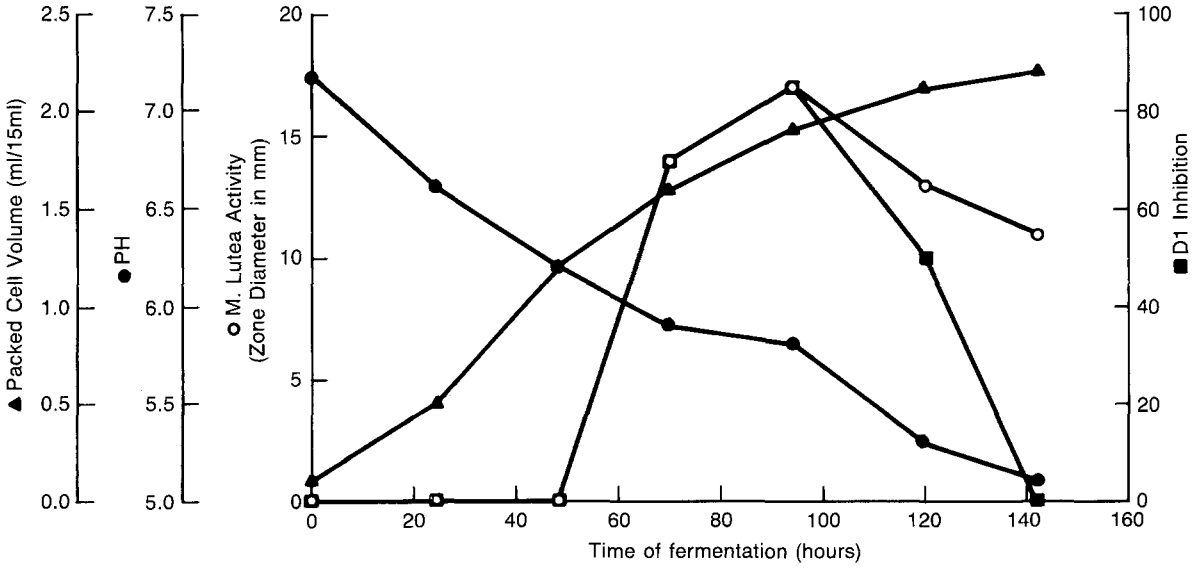

Fig. 2. Fermentation profile of culture SCC 1971.

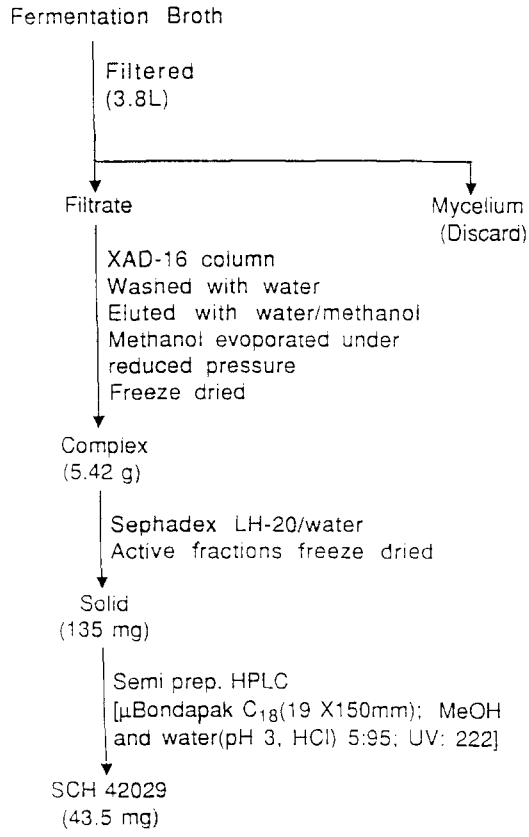

Fig. 3. Scheme for isolation of Sch 42029.

Biological activity

Binding of ^{3}H-Sch 23390, a D_1-selective antagonist was determined using a modification of the method of Billard et al. [3]. Bovine brains were obtained from a local slaughterhouse, and stored on ice. Striata were dissected on ice and membranes prepared as described earlier [3] for rat brain. Briefly, the striata were homogenized in 20 volumes of Tris · HCl (50 mM) buffer, pH 7.4, using a Polytron (Brinkmann) homogenizer, setting 7; membranes were washed by centrifugation (20000 × *g*) and resuspended in buffer, twice, and finally resuspended in assay buffer (Tris · HCl, 50 mM, pH 7.4, containing 120 mM NaCl, 5 mM KCl, 2 nM $CaCl_2$, 1 mM $MgCl_2$). At least 3 to 4 different pools of membrane were processed and stored at −80 °C.

Binding analysis of ^{3}H-Sch 23390 (D_1-binding) (Amersham, MN) and ^{3}H-spiperone (D_2-binding) (NEN, MA) were performed in triplicate using a final incubation volume of 500 μl with Tris · HCl buffer (50 mM, pH 7.4, containing 120 mM NaCl, 5 mM HCl, 2 nM $CaCl_2$, 1 mM $MgCl_2$) and containing 2 mg of tissue per tube. Nonspecific binding was defined with 1 μM of Sch 23390 for D_1-binding and 1 μM of (+) butaclamol for D_2-binding, respectively. For competition studies, several concentrations of test drug or standard were included, ranging from 10^{-4} to 10^{-13}. Tubes were incubated, 30-min incubations at 37 °C were terminated by rapid filtration under vacuum, on to GF/B filters presoaked in 0.3% polyethyleneamine (PEI), with three 5-ml washes. Kinetic analyses for the inhibition constant (K_i) were performed using the LUNDON 2 computer program for competition analysis (LUNDON Software Inc., OH) based on Cheng and Pruschoff [5] and Feldman [8]. Protein was measured by Lowry [16].

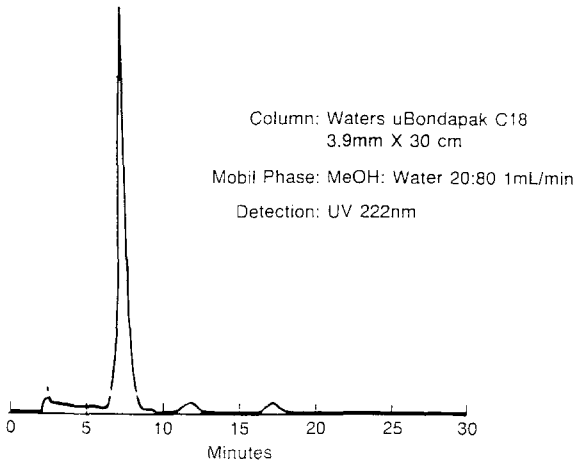

Fig. 4. Analytical HPLC profile of Sch 42029.

RESULTS AND DISCUSSION

Taxonomy

On ATCC medium 172 the culture forms orange-brown to brown substrate mycelia and orange-brown to brown soluble pigments. Aerial mycelia are not formed. Reddish-brown amorphous material is deposited deep in the agar and at the agar surface. Numerous globose sporangia are formed on both water agar and ATCC medium 172. Upon addition of water the sporangia dehise releasing numerous motile spores. Whole-cell analysis indicates the presence of hydroxy and meso-diaminopimelic acid along with arabinose and xylose as the characteristic whole-cell sugars. Based on morphological and chemical analysis the producing culture has been identified as a species of *Actinoplanes*. The culture has been deposited in the Schering Central Culture Collection under accession number SCC 1971.

Structure determination

Sch 42029 is a white unstable solid which decomposes on contact with air. Decomposition can be avoided under nitrogen atmosphere by storing the compound at 5 °C.

TABLE 1

Physico-chemical properties of Sch 42029

UV(MeOH)λ_{max}nm:	225(51900), 296(30400)
IR(KBr)ν_{max} cm^{-1}:	3400, 1660, 1620, 1550, 1470, 1200
CIMS(NH_4Cl):	168$(M + H)^+$, 185$(M + NH_4)^+$
^1H NMR(D_2O)δ:	6.8(d, J = 3Hz, 1H), 6.7(d, J = 8Hz, 1H), 6.5(dd, J = 3.8Hz. 1H), 2.00(s, 3H)
^{13}C NMR(D_2O)ppm:	24.24(d), 114.25(d), 116.26(d), 118.47(d), 126.80(s), 144.51(s), 150.89(s), 175.55(s)

Sch 42029 also decomposes rapidly in solution above pH 6.0, however, it can be purified without decomposition in acidic (pH 2–4) solution. It is soluble in water, methanol and dimethyl sulfoxide, fairly soluble in ethyl acetate, chloroform and insoluble in hexane. The physico-chemical properties of Sch 42029 are shown in Table 1.

The UV spectrum (Fig. 5) of Sch 42029 shows two maxima at 226 and 296. The IR spectrum reveals the presence of an amide (1660, 1550 cm^{-1}). The chemical ionization mass spectrum using ammonia as a carrier displayed two intense peaks at 168 $(M + H)^+$ and 185 $(M + NH_4^+)$, indicating the molecular mass to be 167. ^1H NMR in D_2O showed three peaks due to aromatic protons and one methyl group. The coupling patterns of the aromatic protons revealed that the two protons are on the adjacent carbon atoms and the third *meta* to one of these protons.

^{13}C NMR shows eight carbons and the APT ^{13}C NMR experiment revealed one methyl, three methines and four quaternary carbons. Evaluation of these data leads to the following two possible structures (Fig. 1, structures 1 and 2).

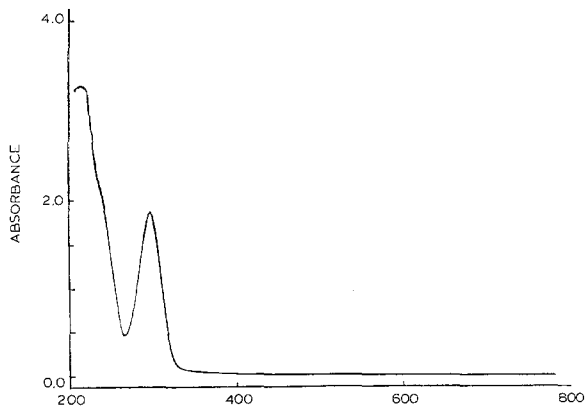

Fig. 5. UV spectrum of Sch 42029.

TABLE 2

Kinetic analysis of dopamine receptor selectivity

Drug	K_i (μM)	
	D_1	D_2
SCH 23390	0.0008	0.8
(+) Butaclamol	0.015	0.001
SCH 42029	1.6	200

The structure of Sch 42029 was established by forming an acetyl derivative and its comparison with the similar derivatives obtained from the commercially available compounds. Sch 42029 affords a diacetyl derivative on stirring with a mixture of acetic anhydride and pyridine at 0 °C. This diacetate was compared with the acetyl derivative of 2,5-dimethoxy aniline and 3,4-dimethoxy aniline. The ^1H NMR spectrum of these compounds revealed a striking similarity between aromatic protons of the diacetate of Sch 42029 and these of 2,5-dimethoxy acetanilide. This observation was further strengthened by NOE experiment. The methyl groups of the amides, for the diacetate of Sch 42029 and the 2,5-dimethoxy acetanilide, were irradiated (Fig. 6) and showed a positive NOE between the methyl protons, -NH and *ortho* proton. These experiments established the structure as shown in Fig. 1 for Sch 42029.

Biological activity

Several comprehensive reviews of the pharmacology [6,10,13,18,21,22] and chemistry [11,12,19,20,24] of dopamine receptors have been published. The most exciting developments in this field have come from recent findings with the benzazepines as the first chemical class to show selectivity for dopamine-receptor subtypes, both as agonists and antagonists [1]. The D_1 and D_2 receptors are biochemically and pharmacologically distinct. Agonists acting at the D_1 receptor elicit an increase in intracellular cAMP levels through stimulation of adenylate cyclase [23]. In the rat corpus striatum and anterior pituitary, the D_2 receptor has been shown to be negatively linked to adenylate cyclase [4].

The affinity for Sch 42029 for dopamine receptors is described in Table 2. The data demonstrated that Sch 42029 is 125-fold selective for the D_1 receptor. Though analogs of dopamine with similar structural subtypes have been reported as antagonists of D_2 receptors, Sch 42029 appears to be the first reported natural product that is selective for the D_1 receptor. This compound represents a novel structural lead which could potentially be exploited synthetically to enhance D_1 potency and selectivity.

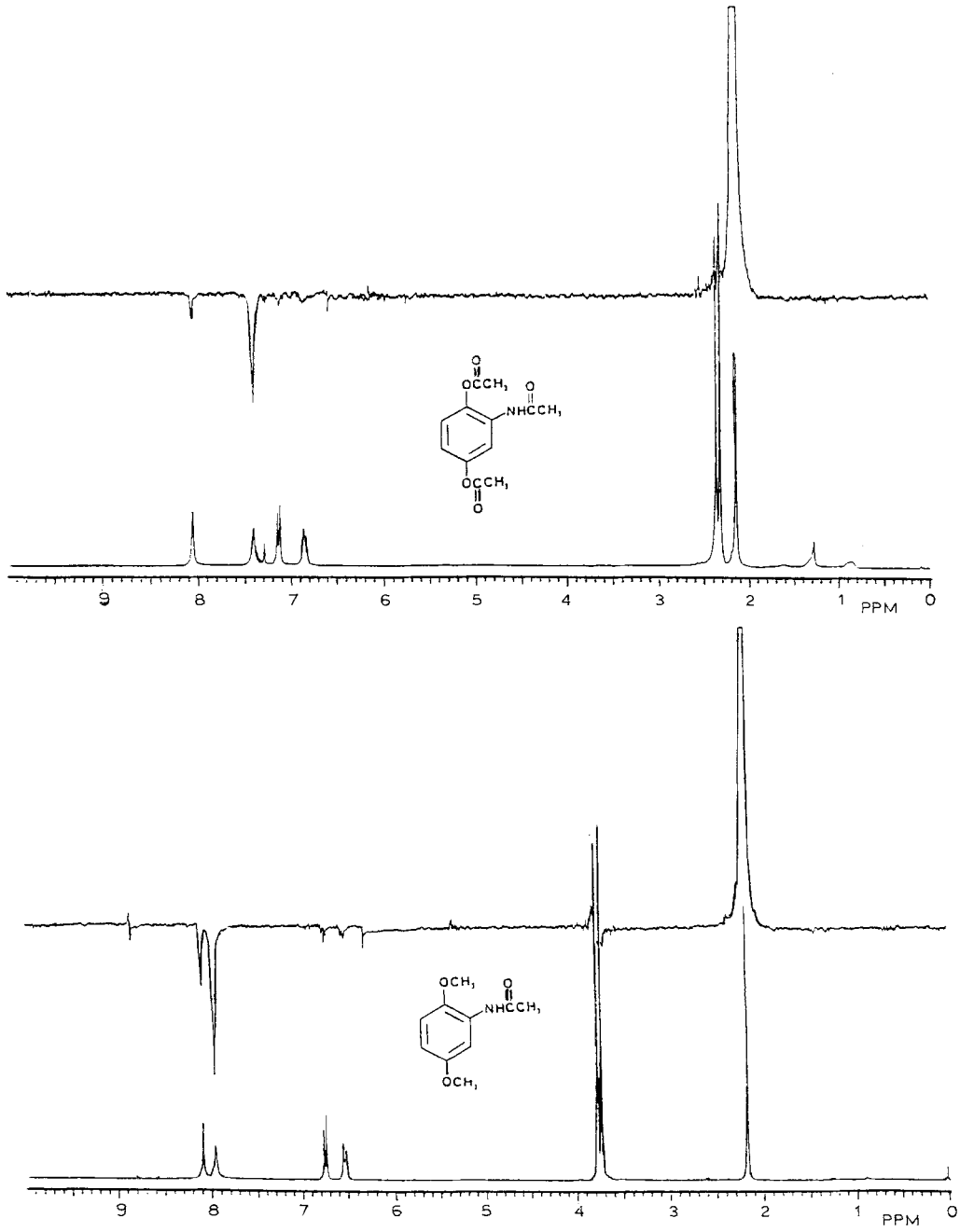

Fig. 6. Difference NOE spectrum of diacetate of Sch 42029 and 2,5-dimethoxy acetanilide.

REFERENCES

1 Barnett, A. 1986. Review on dopamine receptors. Drug of the Future. 11: 49–56.

2 Berrie, R. 1988. A new method for the isolation of motile-spored actinomycetes. Abst., Ann. Meeting of Soc. Industr. Microbiology.

3 Billard, W., V. Ruperto, G. Crosby, L.C. Iorio, and A. Barnett. 1985. Characterization of the binding of ^{3}H-Sch 23390, a selective D_1 receptor antagonist ligand, in rat striatum. Life Sci. 35: 1885.

4 Carboni, E., M. Memo and P.F. Spano. 1983. Pharmacol. Res. Commun. 15: 697.

5 Cheng, Y.C. and W.H. Pruschoff. 1973. Relationship between the inhibition constant (Ki) and the concentration of inhibitor that causes 50 per cent inhibition (150) of an enzymatic reaction. Bio. Chem. Pharmacol. 22: 3099–3108.

6 Clark, D., S. Hjorth, and A. Carlson. 1985. Dopamine recep-

tor agonists: mechanisms underlying autoreceptor selectivity. J. Neural Transm. 62: 1.
7 Cote, R., P.-M. Daggett, M.J. Gantt, R. Hay, S.-C. Hay and P. Pienta. 1984. ATCC media handbook, 1st. edn., American Type Culture Collection, Rockville, MD.
8 Feldman, H.A. 1972. Mathematical theory of complex ligand-binding systems at equilibrium: some methods of parameter fitting. Anal. Biochem. 48: 317–338.
9 Goldberg L.J. and J.D. Kohl. 1983. Differentiation of dopamine receptors in the periphery. In: American Chemical Society Symposium series No. 224, Dopamine Receptors (C. Kaiser and J.W. Kababian, eds.), p. 101.
10 Horn A.S., M.G.P. Feenstra, C.J. Grol, H. Rollema, J.C. Vanoene, and B.H.C. Westrink. 1981. Multiple dopamine receptors: facts, fiction or confusion? Pharm. Weekly 3: 1021.
11 Kaiser, C. 1984. Dopamine Receptor Antagonist (G. Poste and S. Crooke, eds.), Plenum Press, New York, NY.
12 Kaiser, C. and T. Jain. 1985. Dopamine Receptors: Function, Subtypes and Emerging Concepts. Med. Res. Rev. 5: 145–229.
13 Kaiser C. and J. Kebabian (eds.). 1983. Several reviews on dopamine receptors. In: American Chemical Society Symposium, Series 224. American Chemical Society, Washington, D.C.
14 Kebabian, J. and D.B. Calne. 1979. Multiple receptors for dopamine, Nature (Lond). 277: 93.
15 Lechevalier, M.P. 1968. Identification of aerobic actinomycetes of clinical importance. J. Lab. Clin. Med. 71: 934–944.
16 Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with folin-phenol reagent. J. Biol. Chem. 193: 265–175.
17 Makkar, N.S. and T. Cross. 1982. Actinoplanetes in soil and on plat liter for fresh water habitats, J. Appl. Bacteriol. 52: 209–218.
18 Meltzer, H.Y. 1980. Relevance of dopamine autoreceptors for psychiatry: Preclinical and clinical studies. Schizophrenia Bull. 6: 456.
19 New, J.S. and K.S. Takaki. 1988. Antipsychotic agents, Ann. Rep. Med. Chem. 23: 1–10.
20 Schaus, J.M. and Clemens, J.A. 1985. Dopamine receptors and dopaminergic agents. Ann. Rep. Med. Chem. 20: 41–50.
21 Seeman, P. 1980. Brain dopamine receptor. Pharmacol. Rev. 32: 229.
22 Seeman, P., D. Grigoriadis, S.R. George and M. Watanabe. 1984. Dopaminergic systems and their regulation (G.N. Woodruff, I. Creese, G.L. Gessa, O. Hornykiewicz, J.A. Poat and P.J. Roberts, eds.), Macmillan Press, London.
23 Stoof, J.C. and J.W. Kebabian. 1984. Life Sci. 35: 2281.
24 Vinick, F.J. and J.H. Heym. 1987. Antipsychotic Agents. Ann. Rep. Med. Chem. 22: 1–10.