Drugs of the Future 1999, 24(7): 740-746

CCC: 0377-8282/99

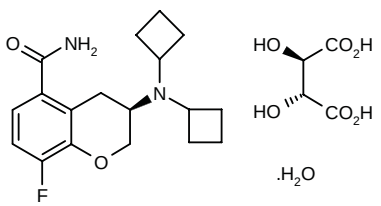
Robalzotan Tartrate Hydrate

Prop INNM

Antidepressant
5-HT$_{1A}$ Antagonist

NAD-299

3(*R*)-(*N*,*N*-Dicyclobutylamino)-8-fluoro-3,4-dihydro-2*H*-1-benzopyran-5-carboxamide (*R*,*R*)-tartrate (1:1) hydrate



$C_{18}H_{23}FN_2O_2.C_4H_6O_6.H_2O$ Mol wt: 486.4899

CAS: 177255-04-8
CAS: 208516-87-4 (anhydrous)
CAS: 169758-66-1 (anhydrous, free base)
CAS: 189311-42-0 (oxalate)
CAS: 189311-40-8 (as citrate)
CAS: 189311-38-4 (as sulfate)
CAS: 189311-37-3 (as phosphate)
CAS: 189311-36-2 (as monoacetate)
CAS: 189311-35-1 (as lactate)
CAS: 189311-34-0 (as monohydrobromide)
CAS: 184674-99-5 (as monohydrochloride)

EN: 230573

Synthesis

Robalzotan has been obtained by two different ways:

1) The esterification of 4-fluoro-3-hydroxybenzoic acid (I) with trimethyl orthoformate and sulfuric acid gives the methyl ester (II), which is condensed with propargyl bromide (III) by means of K_2CO_3 in acetone, yielding the corresponding ether (IV). The cyclization of (IV) by heating at 220 °C in *N*,*N*-diethylaniline affords 8-fluoro-2*H*-1-benzopyran-5-carboxylic acid methyl ester (V), which is hydrolized with NaOH in refluxing ethanol to the corresponding free acid (VI). The reaction of (VI) with thionyl chloride and then with ammonia affords the carboxamide (VII), which is nitrated with $NaNO_2$ and iodine, giving the 8-fluoro-3-nitro-2*H*-1-benzopyran-5-carboxamide (VIII). The hydrogenation of the double bond of (VIII) with $NaBH_4$ in isopropanol yields the 3,4-dihydro compound (IX), which is then further reduced at the nitro group with H_2 and RaNi in ethanol/THF, providing racemic 3-amino-8-fluoro-3,4-dihydro-2*H*-1-benzopyran-5-carboxamide (X). Optical resolution of (X) with L-(+)-tartaric acid gives the 3(*R*)-amino derivative (XI), which is alkylated with cyclobutanone (XII) and sodium cyanoborohydride in methanol/acetic acid to afford robalzotan (XIII) (1). Finally, this compound is treated with L-(+)-tartaric acid (XIV) in THF/ethyl ether (2). Scheme 1.

2) The bromination of 3(*R*)-amino-5-methoxy-3,4-dihydro-2*H*-1-benzopyran (XV) with Br_2 in acetic acid gives the 8-bromo compound (XVI), which is protected with benzyl bromide and K_2CO_3 in acetonitrile, yielding the dibenzylamino compound (XVII). The reaction of (XVII) with *N*-fluorobenzenesulfonimide and BuLi in THF provides the corresponding 8-fluoro derivative (XVIII), which is deprotected with H_2 over Pd/C in methanol/THF, giving 3(*R*)-amino-8-fluoro-5-methoxy-3,4-dihydro-2*H*-1-benzopyran (XIX). The alkylation of (XIX) with cyclobutanone (XII) and sodium cyanoborohydride in methanol gives the dicyclobutylamino compound (XX), which is demethylated by means of boron tribromide in dichloromethane to afford the 5-hydroxy compound (XXI). The triflation of (XXI) with trifluoromethanesulfonic anhydride and collidine in dichloromethane yields the triflate (XXII), which is treated with carbon monoxide, palladium acetate and methanol to give methyl 3(*R*)-(dicyclobutylamino)-8-fluoro-3,4-dihydro-2*H*-1-benzopyran-5-carboxylate (XXIII). The hydrolysis of (XXIII) with refluxing 6M aqueous HCl yields the free acid (XXIV), which by reaction with $SOCl_2$ is converted into the acyl chloride (XXV). Finally, this compound is treated with ammonia in dichloromethane to give robalzotan (XIII) (3, 4). Scheme 2.

Description

Free base: white crystals, m.p. 138-9 °C, $[\alpha]_D^{22}$ –134° (c 0.006, CH_2Cl_2) (1); white solid, m.p. 141.2-2.2 °C, $[\alpha]_D^{21}$ –151.5° (c 0.1, $CHCl_3$) (3, 4). Tartrate: white crystals m.p. 174-80 °C (2).

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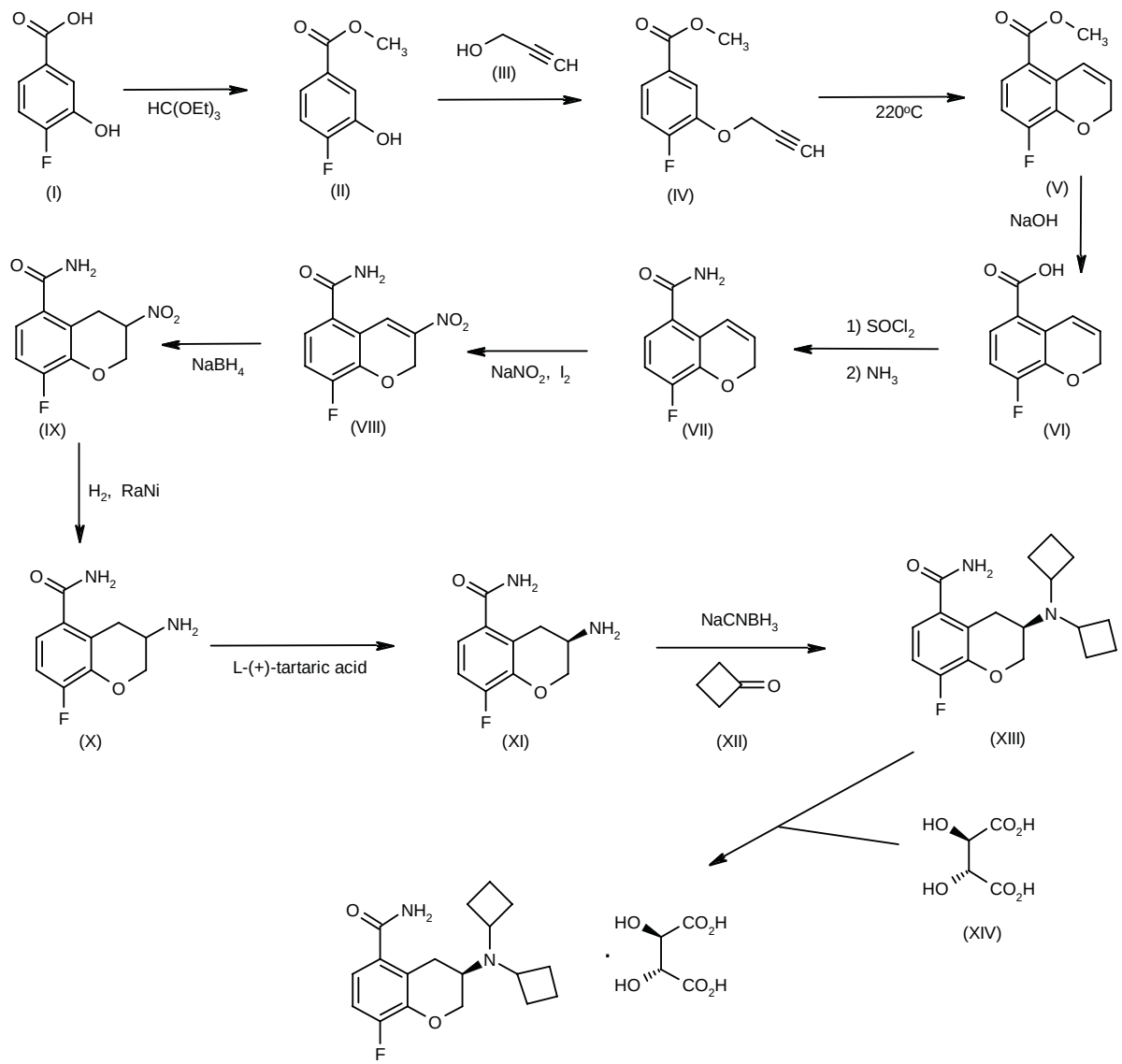
Scheme 1: Synthesis of Robalzotan



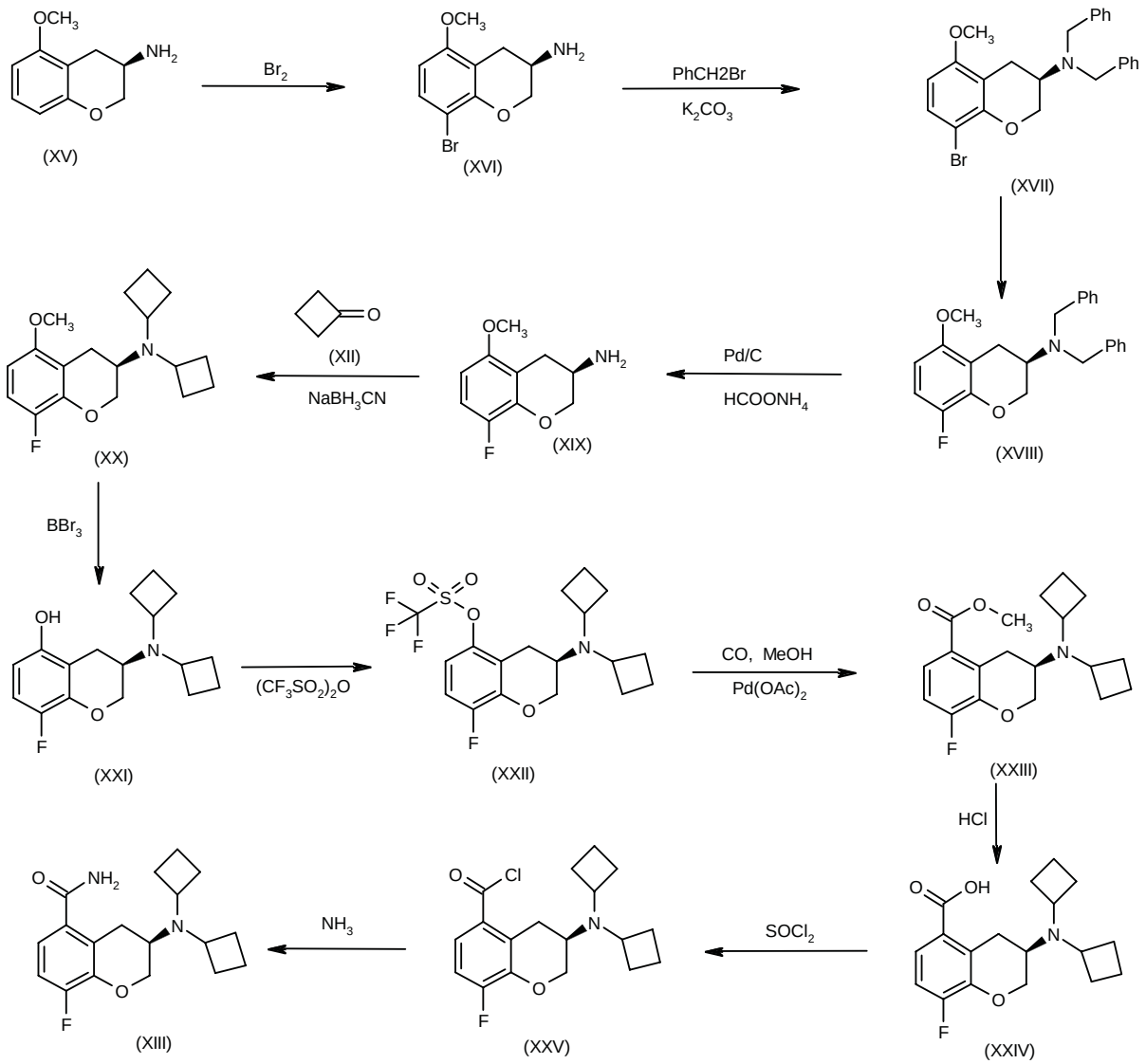
Introduction

After its discovery in the 1930s as a vasoconstrictive substance, serotonin (5-HT) was chemically characterized and synthesized in 1948 and 1951, respectively. Over the past quarter of a century, 5-HT has been found to play a major role in neurotransmission and to affect such diverse systems as the cardiovascular and gastrointestinal systems, in addition to the CNS.

Recent progress in 5-HT research has been stimulated by the characterization of multiple 5-HT receptor subtypes which have been classified according to their pharmacological properties and molecular structure. Among these subtypes, the 5-HT_{1A} receptor is one of the targets proposed for the design of new antidepressant and anxiolytic drugs (5-7).

WAY-100135 (8-10) and the more selective WAY-100635 (11-13) have been described as potent and selective 5-HT_{1A} receptor antagonists devoid of intrinsic activity. Both compounds, and WAY-100635 in particular, have been used as tools for the characterization of 5-HT_{1A} receptor function. Several compounds have been described as 5-HT_{1A} receptor antagonists. Among these, NAD-299 (robalzotan tartrate) was shown to possess high affinity for 5-HT_{1A} receptors and an even higher selectivity than WAY-100635. As shown in Table I, only

Scheme 2: Synthesis of Robalzotan



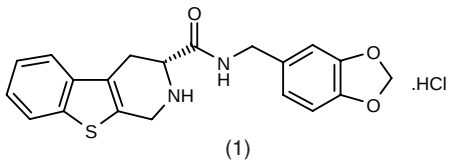
four such compounds were reported to be in development, including the title compound, S-15535 (Servier) (14), DU-125530 (Duphar) (15) and AP-521 (Asahi) (16). Other selective 5-HT_{1A} antagonists that have been reported in preclinical studies include SDZ-216-525 (17) and NAN-190 (18).

Pharmacological Actions

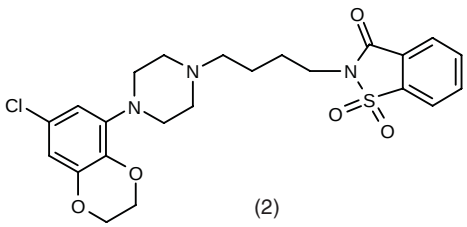
Robalzotan is a substituted chroman that acts as a potent and selective 5-HT_{1A} receptor antagonist which appears to have potential as an antidepressant. Studies characterizing robalzotan pharmacologically *in vitro* and *in vivo* showed that the agent had a high affinity for 5-HT_{1A} receptors in rat hippocampus *in vitro* with a K_i value of 0.6 nM (Tables II and III). The only other receptors for which robalzotan had affinity of < 1 μM were α_1- and β-adrenoceptors with K_i values of 260 and 340 nM, respectively, indicating 400 times more selectivity of the agent for 5-HT_{1A} receptors; WAY-100635 had a higher affinity for alpha1-adrenoceptors (K_i = 45 nM) in addition to dopamine D_2 and D_3 receptors (K_i = 79 and 67 nM, respectively). Robalzotan was shown to competitively block 5-HT-induced inhibition of vasoactive intestinal peptide-stimulated cAMP production in GH4ZD10 cells, with

Table I: Chemical structures of 5-HT$_{1A}$ receptor antagonists.

1. AP-521
2. DU-125530
3. Robalzotan tartrate hydrate
4. S-15535[1]



(1)



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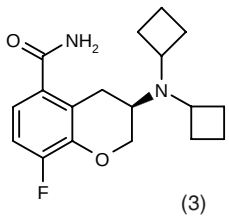

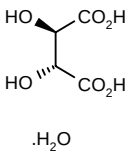

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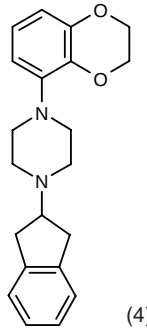

(4)

[1]Antagonist at postsynaptic 5-HT$_{1A}$ receptors and agonist at 5-HT$_{1A}$ autoreceptors

Table II: Receptor binding profile of selected 5-HT$_{1A}$ receptor antagonists (Prous Science MFline® database).

Compound	Ki (nM) 5-HT$_{1A}$	5-HT$_{1B}$	D$_2$	Refs.
DU-125530	0.7	890	5.20	34
NAN-190	1.2*	>1000	11.1*	34-37
NDL-249	2.5*	—-	>1000	38, 39
Pindolol	22.3*	>1000	>10000	34, 40, 41
Robalzotan	0.6	>1000	>1000	19
S-15535	1.3*	>1000	534*	34, 36, 40, 42
SDZ-216525	1.1*	472*	20.4	36, 37, 43
Spiperone	104*	>10000	0.16*	36, 40, 44-50
WAY-100135	22.9*	>10000	245	34, 36, 40, 43
WAY-100635	0.6	>10000	156*	19, 34, 35, 40, 51-53

*Mean from different studies.

no intrinsic activity observed and in a manner similar to WAY-100635. Both agents also competitively antagonized 8-OH-DPAT-induced behavioral effects in rats in vivo, including hypothermia, corticosterone secretion and inhibition of passive avoidance behavior, with no intrinsic actions of their own. The effective dose of robalzotan was determined to be 0.03-0.35 μmol/kg (s.c.), depending on the test and dose of 8-OH-DPAT (19).

The intrinsic efficacy of robalzotan was further investigated in a study examining receptor-mediated control of brain monoamine synthesis via monitoring of the accumulation of DOPA and 5-hydroxytryptophan (5-HTP) in the ventral neostriatum and ventral hippocampus in rats treated with NSD-1015, a cerebral aromatic L-amino acid decarboxylase inhibitor. Although S(–)-UH-301 (2-32 μmol/kg) decreased 5-HTP accumulation in the neostriatum and hippocampus and decreased neostriatal DOPA accumulation, robalzotan (0.75-12 μmol/kg) only slightly increased neostriatal but not hippocampal 5-HTP accumulation while neostriatal or hippocampal DOPA levels were unaffected. In contrast, WAY-100635 increased neostriatal DOPA accumulation. Results indicated that robalzotan was the most selective and specific 5-HT$_{1A}$ receptor antagonist since intrinsic efficacy was observed with S(–)-UH-301 at brain 5-HT$_{1A}$ and D$_{2/3}$ receptors and WAY-100635 acts as a D$_{2/3}$ receptor antagonist (20).

A study using autoradiography examined the anatomical distribution and specificity of [^{11}C]-robalzotan binding to 5-HT$_{1A}$ receptors in postmortem human brain whole-hemisphere cryosections. Results demonstrated the specificity of binding which was observed in high levels in the hippocampus, raphe nuclei and neocortex; the CA1 region of the hippocampus showed the highest amount of binding. Binding of the radioligand could be inhibited by

Table III: Functional pharmacology of selected 5-HT$_{1A}$ receptor antagonists (Prous Science MFline® database).

	Inhibition				
	OH-DPAT-induced ED_{50} (mg/kg s.c.)			Spontaneous ED_{25} (mg/kg s.c.)	Spontaneous ED_{50} (mg/kg i.v.)
Compound	Hypothermia	Body flattening	5-HTP accum.	5-HTP-accum.	Neuronal firing
NAN-190	0.565* (36, 54)	0.250 (36)	–	0.17 (36)	0.004 (36)
NDL-249	–	–	–	–	0.250 (29)
Pindolol	0.860 (54)	–	–	–	–
Robalzotan	0.034 (19)	0.077 (19)	0.097 (19)	–	0.435 (29)
S-15535	1.44 (36)	0.480 (36)	–	0.20 (36)	0.007 (36)
SDZ-216525	0.055* (36, 54)	<2.5 (36)	–	>2.5 (36)	>0.4 (36)
Spiperone	9.255* (36, 54)	0.880 (36)	–	>10.0 (36)	>2.0 (36)
WAY-100135	1.925* (36, 54)	2.81 (36)	–	>40.0 (36)	>2.0 (36)
WAY-100635	0.004 (19)	0.042 (36)	0.010 (19)	–	0.330 (29)
8-OH-DPAT	–	–	–	0.06 (36)	0.0009* (29, 36)

8-OH-DPAT has been included as the standard 5-HT$_{1A}$ receptor agonist. Inhibition of 8-OH-DPAT-induced hypothermia and body flattening reflects postsynaptic 5-HT$_{1A}$ receptor antagonism. Inhibition of striatal 5-HTP accumulation induced by 8-OH-DPAT indicates antagonistic effects on 5-HT$_{1A}$ autoreceptors, whereas inhibition of spontaneous striatal 5-HTP accumulation indicates agonistic effects on 5-HT$_{1A}$ autoreceptors. Inhibition of spontaneous dorsal raphe neurons firing indicates agonistic effects on 5-HT$_{1A}$ autoreceptors. *Mean from different studies using similar methodology. References in parentheses.

the addition of a 5-HT$_{1A}$ receptor ligand (*i.e.*, WAY-100635, pindolol, 5-HT, (±)-8-OH-DPAT and buspirone). In addition to demonstrating binding of robalzotan to the 5-HT$_{1A}$ receptors in human brain *in vitro*, results also indicated that the agent may be used as a potential radioligand for positron emission tomography examination of 5-HT$_{1A}$ receptors *in vivo* (21).

Studies have demonstrated that [^{3}H]-robalzotan was highly useful for labeling 5-HT$_{1A}$ receptors in mouse (22) and rat (23) brains *in vivo* and *in vitro*. Moreover, 5-HT$_{1A}$ receptor occupancy was determined in the cynomolgus monkey brain *in vivo* after i.v. injection of robalzotan, with results showing that the agent binds in a saturable manner. Furthermore, the curvilinear function observed between drug concentrations and 5-HT$_{1A}$ receptor occupancy could be used to predict suitable doses of robalzotan for initial studies in man (24).

Chronic 21-day treatment of rats with robalzotan (0.03, 0.3 and 3 μmol/kg s.c. b.i.d.) showed no consistent effect on the sensitivity of rats to 8-OH-DPAT challenge, indicating no change in responsiveness of 5-HT$_{1A}$ receptors during long-term treatment (25). Furthermore, subchronic treatment with robalzotan (1.5 mg/kg s.c. b.i.d.) or fluoxetine (10 mg/kg p.o.) in rats did not produce any changes in 5-HT$_{2A}$ or β-adrenoceptors, in contrast to the tricyclic antidepressants (*i.e.*, amitriptyline and desipramine) which reduced the density of both receptor-type binding sites (26).

A review of the general pharmacodynamic effects of robalzotan demonstrated that the agent prevented electroshock-induced seizures and raised the threshold for pentylenetrazole-induced seizures when given to mice in high doses (1 mmol/kg or more). However, only minor effects of robalzotan were observed on analgesia, locomotor activity, the gastrointestinal system and cardiovascular system, with the most notable being an increase in heart rate (27).

Microdialysis studies in rat brain demonstrated that robalzotan acts as a potent 5-HT$_{1A}$ autoreceptor antagonist as indicated by its ability to reverse the 5-HT release-suppressing and 5-HIAA-lowering effects of the selective 5-HT$_{1A}$ receptor agonist 8-OH-DPAT and by its ability to enhance the 5-HT-elevating action of citalopram (28).

An electrophysiological study comparing the effects of robalzotan, WAY-100635, *p*-MPPI and NDL-249 on dorsal raphe cell firing in anesthetized rats found that all agents at high doses (> 0.1 μmol/kg) dose-dependently suppressed dorsal raphe cell firing with ED_{50} values of 0.6 ± 0.2, 0.7 ± 0.3 and 0.9 ± 0.4 μmol/kg, respectively; these values were at least 30-fold higher than the doses required to antagonize the effects of 8-OH-DPAT. Results confirmed that all agents fulfilled the criteria for 5-HT$_{1A}$ receptor antagonists lacking intrinsic efficacy in the dorsal raphe system, with robalzotan exhibiting antagonistic potency comparable to WAY-100635 (29).

Since ejaculatory problems and anorgasmia are common adverse effects of selective serotonin reuptake inhibitor antidepressants (SSRIs), a study examined the role of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors in the mediation of male rat ejaculatory behavior. Although robalzotan was ineffective alone (0.75-3 μmol/kg s.c.), it did antagonize the 8-OH-DPAT (0.25-4 μmol/kg)-stimulated facilitation of ejaculatory behavior at a dose of 1 μmol/kg (30).

Pharmacokinetics

Pharmacokinetic studies in rats and dogs showed that robalzotan was rapidly absorbed (0.1-0.9 h) and eliminated (0.5-0.7 h), with an oral bioavailability in rats (25 or 250 μmol/kg) and dogs (5 or 25 μmol/kg) of 4-25% and 20-

40%, respectively. The agent was predominately eliminated in urine as metabolites indicated by 4-8 peaks of radioactivity, with < 0.3% of the dose found as unchanged compound. Extensive metabolism was also noted *in vitro* using S9 liver fractions from mouse, rat, dog, monkey and humans, showing that mono-dealkylated robalzotan was the main metabolite (about 80% in humans) (31).

An *in vitro* study using liver microsomes from rat, dog and humans obtained preliminary apparent K_m and V_{max} values for robalzotan. The V_{max} values were 2.2, 1.5 and 2.6 nmol/min/mg protein, respectively, with corresponding K_m values of 23, 50 and 19 µmol/l for rat, dog and humans, respectively (32).

Robalzotan has advanced to phase II clinical testing as an antidepressant agent (33).

Manufacturer

AstraZeneca plc (UK).

References

1. Hanson, S., Johansson, L., Sohn, D.D. (Astra AB). *A new process.* WO 9846586.

2. Nyqvist, H., Sohn, D.D. (Astra AB). *A new salt.* WO 9854166.

3. Evenden, J., Hammarberg, E.M., Hansson, H.S. et al. (Astra AB). *(R)-5-Carbamoyl-8-fluoro-3-N,N-disubstituted-amino-3,4-dihydro-2H-1-benzopyrans.* US 5616610.

4. Evenden, J., Hammarberg, E.M., Hansson, H.S. et al. (Astra AB). *Novel (R)-5-Carbamoyl-8-fluoro-3-N,N-disubstituted-amino-3,4-dihydro-2H-1-benzopyrans.* EP 725779, JP 97504287, WO 9511891.

5. Dourish, C.T. *Brain 5-HT_{1A} receptors and anxiety.* In: Brain 5-HT_{1A} Binding Sites with G Regulatory Proteins, C.T. Dourish, S. Ahlenius, P.H. Hurson (Eds.), Ellis Horwood, Chichester, U.K., 1987, 261-78.

6. Charney, D.S., Krystal. J.H., Delgado, P.L., Heninger, G.R. *Serotonin-specific drugs for anxiety and depressive disorders.* Annu Rev Med 1990, 41: 437-46.

7. Saxena, P.R. *Serotonin receptors: Subtypes, functional responses and therapeutic relevance.* Pharmacol Ther 1995, 66: 339-68.

8. Cliffe, I.A., Brightwell, C.I., Fletcher, A., Forster, E.A., Mansell, H.L., Reilly, Y., Rourledge, C., White, A.C. *(S)-N-tert-Butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-pheylpropanamide [(S)-WAY-100135): A selective antagonist at presynaptic and post-synaptic 5-HT_{1A} receptors.* J Med Chem 1993, 1509-10.

9. Fletcher, A., Bill, D.J., Bill, S.J., Cliffe, I.A., Dover, G.M., Forster, E.A., Haskins, J.T., Jones, D., Mansell, H.L., Reilly, Y. *WAY100135: A novel, selective antagonist at presynaptic and postsynaptic 5-HT_{1A} receptors.* Eur J Pharmacol 1993, 237: 283-291.

10. *WAY-100135.* Drug Data Rep 1992, 14(1): 8.

11. Fletcher, A., Bill, D.J., Cliffe, I.A., Forster, E.A., Reilly, Y. *A pharmacological profile of WAY-100635, a potent and highly selective 5-HT_{1A} receptor antagonist.* Br J Pharmacol 1994, 112(Suppl.): 91P.

12. Khawaja, X., Evans, N., Reilly, Y., Ennis, C., Minchin, M.C.W. *Characterisation of the binding of [^{3}H]WAY-100635, a novel 5-hydroxytryptamine$_{1A}$ receptor antagonist to rat brain.* J Neurochem 1995, 64: 2716-26.

13. *WAY-100635.* Drug Data Rep 1993 15(10): 899.

14. *S-15535.* Drug Data Rep 1992 14(10): 855.

15. *DU-125530.* Drug Data Rep 1997, 19(8): 691.

16. *AP-521.* Drug Data Rep 1995, 17(1): 20.

17. *SDZ-216-525.* Drug Data Rep 1992, 14(6): 477.

18. *NAN-190.* Drug Data Rep 1989, 11(6): 443.

19. Johansson, L., Sohn, D., Thorberg, S.-O. *The pharmacological characterization of a novel selective 5-hydroxytryptamine$_{1A}$ receptor antagonist, NAD-299.* J Pharmacol Exp Ther 1997, 283: 216-25.

20. Ahlenius, S., Henriksson, I., Magnusson, O., Salmi, P. *In vivo intrinsic efficacy of the 5-HT_{1A} receptor antagonists NAD-299, WAY-100,635 and (S)-(–)-UH-301 at rat brain monoamine receptors.* Eur Neuropsychopharmacol 1999, 9: 15-9.

21. Sandell, J., Halldin, C., Hall, H., Thorberg, S.O., Werner, T., Sohn, D., Sedvall, G., Farde, L. *Radiosynthesis and autoradiographic evaluation of [^{11}C]NAD-299, a radioligand for visualization of the 5-HT_{1A} receptor.* Nucl Med Biol 1999, 26: 159-64.

22. Stenfors, C., Werner, T., Ross, S.B. *In vivo labelling of the mouse brain 5-hydroxytryptamine$_{1A}$ receptor with the novel selective antagonist ^{3}H-NAD-299.* Naunyn-Schmied Arch Pharmacol 1998, 357: 500-7.

23. Jerning, E., Svantesson, G.T., Mohell, N. *Receptor binding characteristics of [^{3}H]NAD-299, a new selective 5-HT_{1A} receptor antagonist.* Eur J Pharmacol 1998, 360: 219-25.

24. Farde, L., Andrée, B., Ginovart, N., Halldin, C., Thorberg, S.-O. *PET-determination of robalzotan occupancy of 5-HT_{1A} receptors in the monkey brain.* Eur Neuropsychopharmacol 1998, 8(Suppl. 2): Abst P.1.095.

25. Jackson, D.M., Wallsten, C.E., Larsson, L.-G., Lindgren, L.-M., Bengtsson, A., Ross, S.B. *No change in responsiveness of 5-HT_{1A} receptor after long-term treatment with the novel 5-HT_{1A} receptor antagonist robalzotan (NAD-299) in rats.* Eur Neuropsychopharmacol 1998, 8(Suppl. 2): Abst P.1.096.

26. Jerning, E., Mohell, N. *The effects of sub-chronic treatment with the selective 5-HT_{1A} receptor antagonist robalzotan, 8-OH-DPAT and antidepressive drugs on 5-HT_{1A}, 5-HT_{2A}, DA D_2 and β-adrenergic receptors.* Eur Neuropsychopharmacol 1998, 8(Suppl. 2): Abst P.1.208.

27. Öjteg, G. *The selective 5-HT_{1A} receptor antagonist robalzotan: General pharmacodynamic effects.* Eur Neuropsychopharmacol 1998, 8(Suppl. 2): Abst P.1.035.

28. Hjorth, S. *In vivo rat brain microdialysis studies of the new 5-HT_{1A} receptor antagonist robalzotan.* Eur Neuropsychopharma-col 1998, 8(Suppl. 2): Abst P.1.113.

29. Martin, L.P., Jackson, D.M., Wallsten, C., Waszczak, B.L. *Electrophysiological comparison of 5-hydroxytryptamine$_{1A}$ receptor antagonists on dorsal raphe cell firing.* J Pharmacol Exp Ther 1999, 288: 820-6.

30. Hillegaart, V., Ahlenius, S. *Facilitation and inhibition of male rat ejaculatory behaviour by the respective 5-HT_{1A} and 5-HT_{1B} receptor agonists 8-OH-DPAT and anpirtoline, as evidenced by use of the corresponding new and selective receptor antagonists NAD-299 and NAS-181.* Br J Pharmacol 1998, 125: 1733-43.

31. Gagner Milchert, I., Berg, A., Berin, B., Breuer, O., Knowles, S., Lindgren, J.-E., Martinsson, S., Roberts, D., Sokolnicka, A.,

Wikström, S.-A. *Pharmacokinetics of robalzotan in rats and dogs including in vitro and in vivo data on metabolism.* Eur Neuropsychopharmacol 1998, 8(Suppl. 2): Abst P.1.140.

32. Gagner Milchert, I., Gabrielsson, J., Linde, J., Terelius, Y. *In vitro-in vivo extrapolation of robalzotan metabolic parameters: An interspecies comparison.* Eur Neuropsychopharmacol 1998, 8(Suppl. 2): Abst P.1.139.

33. *AstraZeneca updates status of robalzotan.* DailyDrugNews.com May 5, 1999.

34. Mos, J. et al. *The putative 5-HT$_{1A}$ receptor antagonist DU125530 blocks the discriminative stimulus of the 5-HT$_{1A}$ receptor agonist flesinoxan in pigeons.* Eur J Pharmacol 1997, 325: 145.

35. Kleven, M.S., Assié, M.-B., Koek, W. *Pharmacological characterization of in vivo properties of putative mixed 5-HT$_{1A}$ agonist/5-HT$_{2A/2C}$ antagonist anxiolytics. II. Drug discrimination and behavioral observation studies in rats.* J Pharmacol Exp Ther 1997, 282: 747.

36. Millan, M.J. et al. *Novel benzodioxopiperazines acting as antagonists at postsynaptic 5-HT$_{1A}$ receptors and as agonists at 5-HT$_{1A}$ autoreceptors: A comparative pharmacological characterization with proposed 5-HT$_{1A}$ antagonists.* J Pharmacol Exp Ther 1994, 268: 337.

37. Schoeffter, P. et al. *SDZ 216-525, a selective and potent 5-HT$_{1A}$ receptor antagonist.* Eur J Pharmacol 1993, 244: 251.

38. Jerning, E. et al. *Receptor binding characteristics of [^{3}H]NAD-299, a new selective 5-HT$_{1A}$ receptor antagonist.* Eur J Pharmacol 1998, 360: 219.

39. Jackson, D.M. et al. *Two selective 5-HT$_{1A}$ receptor antagonists, WAY-100635 and NDL-249, stimulate locomotion in rats acclimatised to their environment and alter their behaviour: A behavioural analysis.* Psychopharmacology 1998, 139: 300.

40. Newman-Tancredi, A. et al. *Labeling of recombinant human and native rat serotonin 5-HT$_{1A}$ receptors by a novel, selective radioligand, [^{3}H]-S 15535: Definition of its binding profile using agonists, antagonists and inverse agonists.* Naunyn-Schmied Arch Pharmacol 1998, 357: 205.

41. Ismaiel, A.M. et al. *2-(1-Naphthyloxy)ethylamines with enhanced affinity for human 5-HT$_{1D\beta}$ (h5-HT$_{1B}$) serotonin receptors.* J Med Chem 1997, 40: 4415.

42. Millan, M.J. et al. *S 15535, a novel benzodioxopiperazine ligand of serotonin (5-HT)$_{1A}$ receptors: I. Interaction with cloned human (h)5-HT$_{1A}$, dopamine hD$_2$/hD$_3$ and hα_{2A}-adrenergic receptors in relation to modulation of cortical monoamine release and activity in models of potential antidepressant activity.* J Pharmacol Exp Ther 1997, 282: 132.

43. Pauwels, P.J. et al. *The 5-HT$_{1A}$ receptor antagonists SDZ 216,525, S(–)-WAY 100,135 and WAY 100,635 display agonist activity at cloned human 5-HT$_{1D}$ receptor sites.* Pharmacol Commun 1996, 7: 309.

44. Durcan, M.J. et al. *Is clozapine selective for the dopamine D4 receptor?* Life Sci 1995, 57: PL 275.

45. Millan, M.J. et al. *S 16924 ((R)-2-(1-[2-(2,3-dihydrobenzo[,4]dioxin-5-yloxy)-ethyl]-pyrrolidin-3yl)-1-(4-fluorophenyl)-ethanone), a novel, potential antipsychotic with marked serotonin (5-HT)$_{1A}$ agonist properties: I. Receptorial and neurochemical profile in comparison with clozapine and haloperidol.* J Pharmacol Exp Ther 1998, 286: 1341.

46. Hidaka, K. et al. *In vitro pharmacological profile of YM-43611, a novel D$_2$-like receptor antagonist with high affinity and selectivity for dopamine D$_3$ and D$_4$ receptors.* Br J Pharmacol 1996, 117: 1625.

47. Lee, J.-C., Matsubara, S., Meltzer, H.Y. *Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin$_2$ pK$_i$ values.* J Pharmacol Exp Ther 1989, 251: 238.

48. Schipper, J., Tulp, M.T.M., Sijbesma, H. *Neurochemical profile of eltoprazine.* Drug Metab Drug Interact 1990, 8: 85-115.

49. Pauwels, P.J., Palmier, C. *Functional effects of the 5-HT$_{1D}$ receptor antagonist GR 127,935 at human 5-HT$_{1D\alpha}$, 5-HT$_{1D\beta}$, 5-HT$_{1A}$ and opossum 5-HT$_{1B}$ receptors.* Eur J Pharmacol Mol Pharmacol Sect 1995, 290: 95.

50. Schoeffer, P., Hoyer, D. *Centrally acting hypotensive agents with affinity for 5-HT$_{1A}$ binding sites inhibit forskolin-stimulated adenylate cyclase activity in calf hippocampus.* Br J Pharmacol 1988, 95: 975.

51. Nielsen, E.B. et al. *NNC 19-1228 and NNC 22-0031, novel neuroleptics with a "mesolimbic-selective" behavioral profile.* Psychopharmacology 1997, 129: 168.

52.Yabuuchi, K. et al. *Effects of tandospirone, a novel anxiolytic agent, on human 5-HT$_{1A}$ receptors expressed in Chinese hamster ovary cells (CHO cells).* Jpn J Pharmacol 1998, 76(Suppl. 1): Abst P-297.

53. Van Steen, B.J. et al. *Functional characteristics of a series of N^4-substituted 1-(2,3-dihydro-1,4-benzodioxin-5-yl)piperazines as 5-HT$_{1A}$ receptor ligands. Structure-activity relationships.* Bioorg Med Chem Lett 1998, 8: 2457.

54. Millan, M.J. et al. *Induction of hypothermia as a model of 5-hydroxytryptamine$_{1A}$ receptor-mediated activity in the rat: A pharmacological characterization of the actions of novel agonists and antagonists.* J Pharmacol Exp Ther 1993, 264: 1364.