Design of New Cognition Enhancers: From Computer Prediction to Synthesis and Biological Evaluation

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To discover new cognition enhancers, a set of virtually designed synthesizable compounds from different chemical series was investigated using two computer-aided approaches. One of the approaches is prediction of biological activity spectra for substances (PASS) and the second is prediction of toxicity, mutagenicity, and carcinogenicity (DEREK). To increase the probability of finding new chemical entities, we investigated a heterogeneous set of highly diverse chemicals including different types of heterocycles: five-membered (thiophenes, thiazoles, imidazoles, oxazoles, pyrroles), six-membered (pyridines, pyrimidines), seven-membered (diazepines, triazepines), fused five+six-membered heterocycles (indoles, benzothiazoles, purines, indolizines, neutral, mesoionic, and cationic azolopyridines). A database including 5494 structures of compounds was created. On the basis of the PASS and DEREK prediction results, eight compounds with the highest probability of cognition-enhancing effect were selected. The cognition-enhancing activity testing showed that all of the selected compounds had a pronounced antiamnesic effect and were found to reduce significantly scopolamine-induced amnesia of passive avoidance reflex (PAR). The action of compounds at doses of 1 and 10 mg/kg caused a statistically significant increase in latent time of reflex and in the number of animals, which did not enter the dark chamber when testing the PAR. Therefore, on the basis of computer prediction, new cognition-enhancing agents were discovered within the chemical series, in which this activity was not known previously.

Introduction

Loss of cognitive functions (such as memory, attention, concentration, comprehension, orientation) is of great importance in a wide spectrum of clinical conditions, such as acute and chronic cerebrovascular diseases, inflammation, traumatic brain injury, intoxication (including alcohol coma), mental retardation in children, age-related and neurodegenerative brain damage (Alzheimer's disease, Parkinson's disease), and others.[1−3] The treatment of patients with Alzheimer's and Parkinson's disease is becoming especially critical, taking into account that in the beginning of the 21st century the number of people aged 65 or over will be higher than ever before. Those over the age of 80 are the fastest growing segment of the population, and at least 25% of this group suffer serious deterioration in mental capacity. Another problem of great concern is stroke, which ranks third (after cancer and heart diseases) as the cause of death in industrial countries, and is the major reason for handicap in the adult population.

Cognition-enhancing (nootropic) agents are represented by a wide spectrum of substances, including Ca-channel blockers, GABA- and glutamate-ergic drugs, free radical scavengers and antioxidants, various cholinomimetics, the mnemotropic neuropeptides, and others.[4−7] However, each of these well-known cognition enhancers has repeatedly been reported to have serious side effects. Therefore, there is an urgent need to search for substances able to restore damaged cognitive functions without causing significant adverse reactions.

To reduce the time and cost required in the search for NCEs with cognition-enhancing action, two computer-aided drug discovery technologies were applied. The computer program PASS[8−12] was used to predict the cognition-enhancing action for compounds from different chemical series. The computer program DEREK[13] was used to predict carcinogenicity, mutagenicity, and skin sensitization for the compounds investigated. It provides a criterion to exclude potentially harmful substances at the stage of compounds' selection for synthesis and experimental testing. Computer-aided analysis of structure−activity relationships and molecular modeling are widely used in discovery of new leads by pharmaceutical chemists.[14] The majority of currently available molecular modeling methods are designed to study the ligand−receptor interaction for one particular biological target at a time, while QSAR analysis is mostly ap-

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plicable to the optimization of lead compounds' properties within the same chemical series. In contrast to these methods, PASS predicts simultaneously several hundreds types of biological activity for druglike substances from different chemical classes on the basis of their structural formulas. It estimates probabilities not only for the desirable pharmacological effect but also for molecular mechanisms of action and different unwanted side effects. Such analysis of heterogeneous sets increases considerably the chance of discovering NCEs (e.g, new cognition enhancers). On the basis of PASS prediction, we selected eight potential cognition enhancers belonging to thyazolyl, oxazolidine, and isatin classes from 5494 compounds designed by chemists.

It is well-known that the thiazolyl group is of great importance in biological systems. Alkyl/aryl-aminoacetyl derivatives of 2-amino-4-phenylthiazolyl,[15] 2-aminobenzothiazolyl,[16] 2-amino(substituted)-benzothiazolyl,[17] 2-phenylamino-4-phenylthiazolyl,[18] 2-amino-4-methylthiazolyl,[19] and in general 2-(N-substituted or *N*,*N*-disubstituted)-acetamido derivatives were found to have a potent local anaesthetic activity.[20] Antiinflammatory, analgesic, and antipyretic activities for some thiazolyl and benzothiazolyl derivatives are also known.[21,22] Meloxicam, for example, is a new NSAID with a thiazole group. A number of thiazolylamino ketones as well as thiazolyl amides were found to be strong antiinflammatory agents.[23,24] Also in a series of hydrazine-thiazoles and their "open" analogue thiosemicarbazide derivatives inhibitory activity on MAO rat liver mitochondria was found.[25]

A number of compounds with a wide spectrum of activity can be synthesized from β-amino alcohols and carbonyl compounds. Derivatives containing the oxazolanes ring are of particular interest because they are widely present in drugs. Oxazolidines possess various biological activities, inhibit tubulin's polymerization activity,[26] and also exhibit antiviral[27] and antibacterial activities.[28]

However, so far as we are aware, there are no publications concerning the cognition-enhancing activity in compounds from these chemical series.

Results and Discussion

Selection of Possible Cognition Enhancers. Prediction of biological activity spectra was made for all 5494 structures from the database. On the basis of prediction results, we selected potential cognition enhancers. The following criteria were used for selection:

1. Compounds were selected if they had cognition-enhancing activity in their predicted activity spectra with Pa > 0.5.

2. If probability of any unwanted/toxic effect for a compound was high, then this compound was deselected.

3. When too many structures were predicted to be active for a certain chemical series, than only several representatives were selected.

The relationship between the number of compounds predicted as probable cognition enhancers and respective values of probability to be active Pa is shown in Figure 1. Only 34 from 5494 compounds were predicted as cognition enhancers with Pa > 60%. On the basis of the above-mentioned criteria and with a goal to provide the diversity of studied compounds, five compounds with

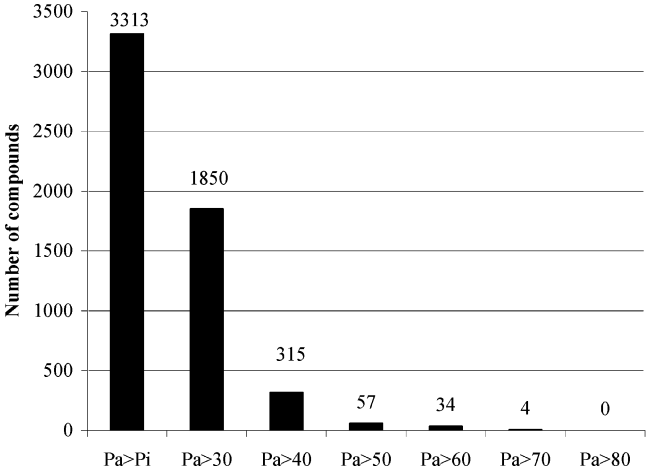


Figure 1. The number of compounds predicted as probable cognition enhancers vs calculated probability to be active Pa.

60% < Pa < 70% and three compounds with Pa > 70% were selected for testing (compounds I−III and V−VIII from the Aristotle University of Thessaloniki and compound IV from the Institute of Chemistry of Moldova Academy of Science). The structures of these compounds were run through DEREK, for most of them, carcinogenicity, mutagenicity, and skin sensitization were predicted as being plausible. This level of probability of possessing these undesirable effects is not an obstacle to further investigation of the compounds. Thus, all selected compounds were synthesized and experimentally tested as cognition enhancers.

Chemistry. General Procedure A. The substituted thiazol-2-ylamine (5 mmol), a slight excess of the aldehyde (6 mmol) and a few drops of piperidine, in ethanolic solution (15 mL), were refluxed in a water bath for 3 h. After cooling, the final products were precipitated, filtered, and recrystallized.

General Procedure B. A solution of benzothiazol-2-ylamine (5 mmol) and the appropriately substituted benzaldehyde (6 mmol) in anhydrous benzene (25 mL) was refluxed for 6 h. The mixture kept overnight at room temperature gave a precipitate, which was removed by filtration and purified by recrystallization.

All reactions proceeded smoothly in good yield. In the synthesis of the more bulky derivatives of the benzothiazolyl series, problems were overcome by heating the reactants in a Dean−Stark apparatus, using *p*-4-toluenesulfonic acid as a catalyst. Under these experimental conditions, we succeeded in obtaining higher yields of the benzothiazolyl Schiff bases.

We obtained the following NMR spectra for these compounds:

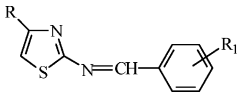
I (4-(Benzothiazol-2-yliminomethyl)phenol): δ in region of 7.3−8.1 (br m, aromatic protons and H azomethine), 9.0 (H, of phneolic hyroxyl).

II 4-(1,3-thiazol)-2-yliminomethyl)phenol: δ 6.91−7.4 (m, 1H azomethine and 3H aromatic), 8.84 (s, 1H hydroxy).

III *N*-(4-Phenyl-1,3-thiazol-2-yl)phenylmethanimine: δ 6.7 (s, 1H thiazole, CHS), 7.3−8.8 (m, 10H aromatic and 1H azomethine).

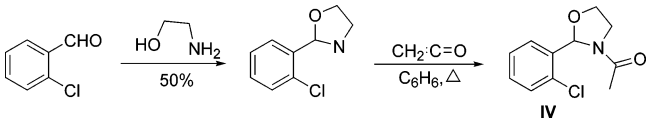
V ((3-Chlorobenzylidene)thiazol-2-ylamine): δ 6.3 (s, 1H thiazole CHS), 6.8−8.7 (m, 1H azomethine and 1H thiazole, CHN, 4H aromatic).

Table 1. Mass Spectra of Compounds I−III and V−VIII



compd	R	R_1	molecular formula	mass spectra, *m/z* (relative intensity %)
I	-	OH	$C_{14}H_{10}N_2SO$	255(65), 254(51), 253(77), 252(71), 227(56), 226(22), 224(44), 150(53), 137(16), 136(34), 135(86), 133(29), 127(43), 120(22), 119(29), 109(29), 108(95), 107(16), 96(20), 91(33), 90(25), 82(28), 78(14), 77(32), 75(14), 69(54), 65(31), 63(35), 58(12), 51(30), 50(15), 45(26), 44(60), 39(37), 32(100)
II	H	OH	$C_{10}H_8N_2SO$	205(68),204(85), 203(85), 187(24), 177(28), 163(13), 149(13), 145(12), 132(13), 121(12), 120(17), 119(20), 111(10), 107(12), 105(21), 102(31),101(24), 100(94),94(13), 93(16), 92(14), 91(26), 86(16), 85(54), 84(17), 78(17), 77(53), 76(19), 73(39), 71(16), 69(21),63(26), 60(36), 59(48), 58(99), 57(46), 55(35), 51(45), 45(63), 43(100), 42(78), 40(67), 39(53), 38(20),32(98)
III	Ph	H	$C_{16}H_{12}N_2S$	264(29), 265(8), 263(22), 177(11), 176(93), 134(100), 108(11), 104(17), 102(24), 90(29), 89(42), 77(26), 76(13), 69(12), 63(23), 51(32)
V	H	*m*-Cl	$C_{10}H_7N_2SCl$	224(21), 223(57), 222(54), 221(82), 131(12), 111(23), 102(18), 100(76), 89(18), 85(55), 77(13), 75(35), 72(35), 63(24), 62(18), 60(26), 59(40), 58(100), 57(48), 50(27), 46(16), 45(61), 43(18), 18(24)
VI	CH_3	*p*-NO_2	$C_{11}H_9N_3SO_2$	247(20), 232(9), 231(41), 230(21), 202(20), 201(25), 200(15), 161(15), 160(19), 159(12), 152(31), 151(35), 150(33), 144(36), 143(19), 141(11), 130(11), 121(13), 117(16), 116(32), 115(97), 114(100), 105(13), 100(10), 99(17), 89(48), 87(84), 86(44), 85(40), 84(29), 77(82), 76(27), 75(24)
VII	Ph	*p*-NO_2	$C_{16}H_{11}N_3SO_2$	310(54), 309(44), 263(12), 176(18), 134(100), 102(72), 90(31), 89(36), 84(14), 77(20), 76(15), 69(11), 63(15), 51(19)
VIII	H	*m*-NO_2	$C_{10}H_7N_3SO_2$	233(38), 232(78), 217(15), 187(15), 186(27), 142(19), 112(13), 99(15), 89(18),86(63), 85(37), 84(54), 76(17), 75(17), 64(18), 63(28), 60(14), 59(67),58(100), 57(28), 52(72), 51(33), 45(46)

Scheme 1. Synthesis of 2-(*m*-Chlorophenyl)-3-acetyl-1,3-oxazolidine



VI ((4-Methylthiazol-2-yl)(4-nitrobenzylidene)-amine): δ 2.47 (s, 3H CH_3), 6.92 (s, 1H, thiazole, CHS), 8.14−9.1 (m, H azomethine and 4H aromatic).

VII ((4-Nitrobenzylidene)(4-phenylthiazol-2-yl)-amine): δ 7.36−7.48 (m, 1H thiazole), 7.9−8.3 (m, 1H azomethine and 10H aromatic).

VIII ((3-Nitrobenzylidene)thiazol-2-ylamine): δ 6.3 (s, 1H, thiazole CHS), 6.8−8.7 (m, 1H azomethine, 1H thiazole, and 4H aromatic).

Mass spectra of compounds I−III and V−VIII are represented in Table 1.

Synthesis of Compound IV (2-(*m*-Chlorophenyl)-3-acetyl-1,3-oxazolidine). Compound IV was synthesized according to Scheme 1. Condensation of *m*-chlorobenzaldehyde with monoethanolamine in benzene gave 2-(*m*-chlorophenyl)-1,3-oxazolidine in a small yield. The use of standard methods for protection of amino group always leads to ring opening. At the end it was found that reaction of 2-(*m*-chlorophenyl)-1,3-oxazolidine with ketene in benzene gave 2-(*m*-chlorophenyl)-3-acetyl-1,3-oxazolidine (IV) in good yield.

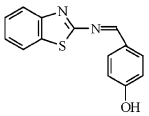
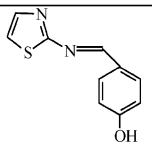
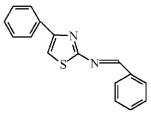
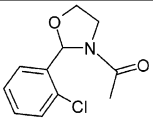
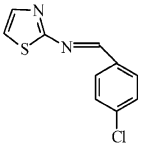
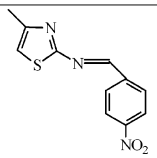
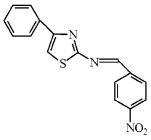
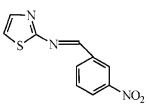
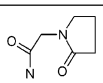
Experimental Testing of Selected Compounds. Investigation of the selected compounds as cognition enhancers showed that several substances had a pronounced cognition-enhancing effect and were found to reduce significantly scopolamine-induced PAR amnesia. The substances coded as I, II, VII in doses of 1 mg/kg and III, IV, V, VI, VIII in doses of 10 mg/kg caused a statistically significant increase in the major behavioral parameter, latent time of reflex (Table 2).

Substances coded as I, VI, V, VII showed a statistically significant increase for another parameter, the number of animals which did not enter the dark chamber when testing the PAR. Novel substances have more potent cognition-enhancing effect than piracetam (Figure 2). Thus, the data obtained demonstrate the high predictive ability of PASS in the field of cognition-enhancing activity: for all selected compounds, cognitive enhancing properties were detected. Mostly, these properties were reflected in the increase of reflex latent time. But it is known, that the index of animals staying out of the dark cell during the reflex reproduction is a more rigorous criterion of the antiamnesic activity on the PAR scopolamine amnesia model.[7,29] Only 4 out of 8 compounds significantly increased the index. Thus, though all the studied compounds possessed antiamnesic activity, only 50% of them showed the full deep effect.

Probable mechanisms of cognition-enhancing effect for the studied compounds were analyzed by PASS. It was shown that all compounds have structural similarity with compounds, acting through the cholinergic system (Table 2). In this regard, cognition-enhancing activity analysis was carried out on scopolamine amnesia level. At the same time, according to the PASS prediction in antiamnesic activity, the mechanism of the studied compounds can involved some other systems, such as those responsible for training and memory processes. The prediction makes it possible to hypothesise that the compounds studied may possess GABA, MAO A, and 5 HT 2B receptors activity.

Similarity Assessment. We used the similarity procedure of ISIS/Base 2.1.1[30] to compare the selected compounds with compounds from the MDDR 99.2 database. It is considered that compounds exhibit the same biological activity if their structural similarity is more than 70%. There are two compounds in the MDDR database with 70% similarity to compound I. One of them has "Antisecretory, Gastric" activity and is an "H2 antagonist". Another has "Antiinflammatory", "Anti-allergic/Antiasthmatic", "Leukotriene Antagonist", and "Phospholipase A2 Inhibitor" activities. Twenty-four known cognition enhancers from the MDDR database have about 45% similarity to compound I. Two compounds with 55% similarity to compound II are in the MDDR database. One of them has "Sedative/Hypnotic"

Table 2. Evaluation of Tested Compounds in Model of Scopolamine-Induced PAR Amnesia

Compound	Structure	Prediction			PAR retrieval after 24 hrs n=12		
		Pa	Pi	Activity	Dose, mg/kg	Reflex latent time, (sec)	Number of animals not entering to the dark chamber, %
Control					—	156±17	83
Scopolamine					1,0	53±8 ••	17&&
I		0.661	0.011	Cognition disorders treatment	1	104±18 *	58 ##
		0.387	0.202	Acetylcholine release stimulant			
		0.158	0.157	MAO A inhibitor	10	15±3	0
		0.149	0.096	Acetylcholinesterase inhibitor			
II		0.673	0.010	Cognition disorders treatment	1	98±6 *	42
		0.418	0.163	Acetylcholine release stimulant	10	41±6	17
III		0.754	0.007	Cognition disorders treatment	1	12±2	0
		0.384	0.206	Acetylcholine release stimulant	10	68±10*	33
IV		0.752	0.007	Cognition disorders treatment	1	25±4	0
		0.465	0.137	Acetylcholine release stimulant	10	103±17 *	58#
Control						160±13	83
Scopolamine					1,0	58±8 •	25 &&
V		0.713	0.008	Cognition disorders treatment	1	61±9	25
		0.469	0.102	Acetylcholine release stimulant	10	123±15 *	66 #
		0.273	0.063	GABA receptor agonist			
VI		0.629	0.007	Cognition disorders treatment	1	76±12	42
		0.175	0.064	Acetylcholinesterase inhibitor	10	126±10 *	50
VII		0.643	0.007	Cognition disorders treatment	1	157±8 *	58 #
		0.164	0.124	5 Hydroxytryptamine 2B antagonist	10	88±13	42
		0.156	0.086	Acetylcholinesterase inhibitor			
VIII		0.653	0.007	Cognition disorders treatment	1	68±10	42
		0.171	0.107	5 Hydroxytryptamine 2B antagonist	10	129±18 *	50
		0.149	0.096	Acetylcholinesterase inhibitor			
Piracetam		0.725	0.08	Cognition disorders treatment	400	75±7	33
					600	92±11*	58#

[a] Results are expressed as mean ± SEM. Statistical comparisons between the saline-treated group and the experimental group with scopolamine: • $P \leq 0.05$; •• $P \leq 0.01$ (Mann−Whitney U-test), & $P \leq 0.05$; && $P \leq 0.01$ (χ^2); between scopolamine group and animals with scopolamine plus substances. * $P \leq 0.05$; ** $P \leq 0.01$ (Mann−Whitney U-test); # $P \leq 0.05$; ## $P \leq 0.01$ (c^2).

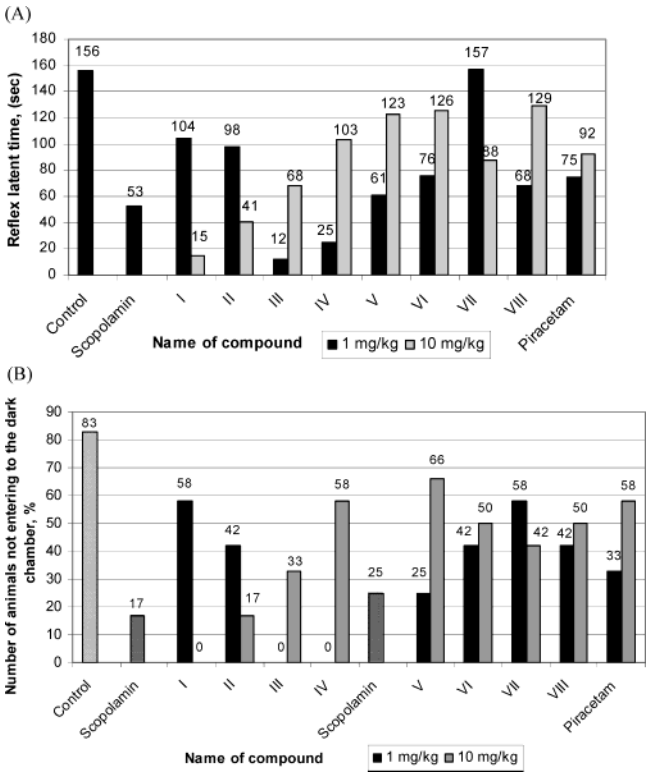


Figure 2. Cognition-enhancing activity of tested compounds. (A) Reflex latent time. (B) Number of animals not entering the dark chamber.

activity; another has "Antianginal" and "Restenosis, Agent for" activities. There is just one cognition enhancer in the MDDR database, which is slightly similar to compound II (45%). One compound from the MDDR database has 70% similarity to compound III. However, its activity is "Sedative/Hypnotic". Five compounds from the MDDR database have 55% similarity to compound IV. Three of them are "Cognition Disorders, Agent for" and two are "Prolylendopeptidase Inhibitor". One compound from the MDDR database has 55% similarity to compound V. Its activity is "Sedative/Hypnotic". Nineteen compounds from MDDR have 55% similarity to compound VI. They reveal different types of biological activity from "Antiarthritic" (4) and "Antiulcerative" (4) to "Antidiabetic, Symptomatic" (3). None of them has cognition-enhancing activity. Twelve compounds from the MDDR database have 60% similarity to compound VII. They reveal twelve different types of biological activity including "Antidiabetic, Symptomatic" (3), "Cyclooxygenase Inhibitor" (3), "Platelet Antiaggregatory", and others. None of them has cognition-enhancing activity. Six compounds from the MDDR database have 50% similarity to compound VIII. There is none among them with cognition-enhancing activity. It is generally accepted that more than 70% similarity of compounds leads to the same biological activity. There are no compounds among those tested with considerable similarity to cognition enhancers from the MDDR database. Thus, compounds discovered within the framework of this project may be new chemical entities.

Conclusions

Innovative computer-assisted approaches have been applied in a search for new cognition enhancers. We used virtual combinatorial design of highly diverse chemical compounds to increase the probability of finding new chemical entities. Different types of heterocycles: thiophenes, thiazoles, imidazoles, oxazoles, pyrroles, pyridines, pyrimidines, diazepines, triazepines, indoles, benzothiazoles, purines, indolizines, neutral, mesoionic, and cationic azolopyridines were analyzed during the project. The most likely compounds (presumably, NCEs) were selected, synthesized, and tested as potential cognition enhancers. Eight compounds from 5494 available were selected as potential cognition enhancers. For all of them, predicted activity was confirmed by experimental testing. Four compounds, (4-(benzothiazol-2-yliminomethyl)phenol), ((3-chlorobenzylidene)thiazol-2-yl-amine), ((4-methylthiazol-2-yl)(4-nitrobenzylidene)amine), and ((3-nitrobenzylidene)thiazol-2-ylamine), have comparable or greater effect in considerably less concentration in comparison with the classic cognition enhancer, piracetam. Thus, our investigation has shown how to increase significantly the number of active compounds in a subset selected for testing due to the application of biological activity spectra prediction.

Methods

Database of Studied Compounds. The chemists who have participated in the INTAS project No 00-071 designed many potentially synthesizable compounds from different chemical classes. These are namely five-membered (thiophenes, thiazoles, imidazoles, oxazoles, pyrroles), six-membered (pyridines, pyrimidines), seven-membered (diazepines, triazepines), fused five+six-membered heterocycles (indoles, benzothiazoles, purines, indolizines, neutral, mesoionic, and cationic azolopyridines). The complete database contains 5494 structures.

PASS Method. Prediction of biological activity spectra for investigated compounds was performed by PASS.[8−12] The current version of PASS predicts simultaneously 900 types of biological activity with mean accuracy of 85%. The PASS prediction result for a compound is presented as a list of activity names and probability values for the appropriate activity to be either active (Pa) or inactive (Pi), respectively. The total list of the predicted activity types that can be predicted by PASS is given on the web-site.[9] PASS uses MNA descriptors ("Multilevel Neighbourhoods of Atoms") for representation of a structural formula of compound.[31] Any structure is replaced by a set of MNA descriptors. The calculation of the biological activity spectrum is based on structure−activity relationships that are obtained by the training procedure and stored in the SAR knowledge base. The training set of PASS (version 1.703) contains 45660 substances, which are represented by 41644 different MNA descriptors. We consider that Pa values should be interpreted by the following way. If $Pa > 0.7$, then the chance of finding this activity in experiments is high, but in many cases the compound may be a close analogue of known pharmaceutical agents. If $0.5 < Pa < 0.7$, the chance of finding the activity in experiments is less, but the compound is not so similar to known pharmaceutical agents. If $Pa < 0.5$, the chance of finding the activity in experiments is even less; the compound has a weak similarity to the compounds from the training set, and if this activity is confirmed by experiments a compound may become a NCE.

On the basis of the theory of PASS approach and previous experience of its applications, we suggest that no correlation exists between the potency of a particular compound and the calculated probability Pa. Instead, Pa is considered as a measure of belonging to the class of active compounds.

Table 3 gives a list of activities associated with cognition-enhancing activity and molecular mechanisms of cognition-enhancing action.

The prediction accuracy of the cognition-enhancing effect estimated by leave-one-out cross validation is 76.7%, while the

Table 3. Biological Activities Associated with Effects of Cognition Enhancers

number	MPA, %	activity
1226	76.7	cognition disorders treatment
232	92.4	5-hydroxytryptamine 1A agonist
126	91.2	5-hydroxytryptamine 1A antagonist
60	86.2	5-hydroxytryptamine 2C antagonist
242	93.6	5-hydroxytryptamine 3 antagonist
69	94.2	5-hydroxytryptamine 4 agonist
219	86.9	5-hydroxytryptamine uptake stimulant
348	85.0	acetylcholine agonist
169	91.0	acetylcholine M1 receptor agonist
5	69.0	acetylcholine M2 receptor agonist
25	80.0	acetylcholine M2 receptor antagonist
215	87.2	acetylcholine muscarinic agonist
98	84.7	acetylcholine nicotinic agonist
27	69.9	acetylcholine release stimulant
256	87.1	acetylcholinesterase inhibitor
68	91.2	adenosine A1 receptor antagonist
132	90.7	adenosine receptor antagonist
547	86.2	adrenaline agonist
119	83.4	adrenaline uptake inhibitor
234	87.8	α-1 adrenoreceptor antagonist
98	90.5	α-2 adrenoreceptor agonist
115	85.6	α-2 adrenoreceptor antagonist
19	89.4	AMPA receptor agonist
147	92.0	AMPA receptor antagonist
16	90.7	benzodiazepine inverse agonist
3	83.3	*β*-amyloid precursor protein antagonist
6	89.4	butyrylcholinesterase inhibitor
18	89.9	calpain inhibitor
4	95.0	carnitine acetyltransferase stimulant
6	90.1	choline acetyltransferase stimulant
47	96.8	dopamine D1 agonist
9	88.2	endopeptidase inhibitor
10	84.7	excitatory amino acid agonist
66	79.2	excitatory amino acid antagonist
33	78.0	free radical scavenger
12	94.5	GABA B receptor antagonist
209	83.1	GABA receptor agonist
104	91.0	glutamate receptor agonist
35	97.7	histamine H3 receptor antagonist
42	84.2	MAO A inhibitor
45	91.1	MAO B inhibitor
22	69.3	neurotrophic factor
414	89.3	NMDA receptor antagonist
7	80.5	NMDA receptor glycine site agonist
64	94.8	prolyl endopeptidase inhibitor
10	87.9	protein synthesis stimulant
35	95.1	thiol protease inhibitor
17	96.6	thyrotropin releasing hormone agonist

[a] "Number" is the number of compounds from the PASS training set exhibiting a particular activity type. [b]MPA is the minimal prediction accuracy (calculated by leave-one-out procedure) for every type of activity from the PASS training set.

prediction accuracy for mechanisms of cognition enhancers varies from 67.9% to 97.7%. Among 47 mechanisms of cognition-enhancing action predicted by PASS only three are predicted worse than "Cognition enhancer" activity, including "Acetylcholine M2 receptor agonist", "Acetylcholine release stimulant", and "Neurotropic factor". Their moderate value of prediction accuracy may be explained by the heterogencity and relatively small number of compounds in the training set. In the selection process, we take into account not only the predicted probability of cognition-enhancing activity but also the predicted probabilities of its mechanisms of action.

DEREK Method. Prediction of toxicity and carcinogenicity by the computer program DEREK[13] provides the basis to avoid the experimental study of potentially harmful substances. It is an expert system for the prediction of toxicity developed by Lhasa Ltd. (Leeds, UK). DEREK predicts carcinogenicity, mutagenicity, and skin sensitization and indicates the likelihood of a compound possessing such toxicities by classification into one of six categories, namely certain, probable, plausible, implausible, improbable, or impossible. Only categories "certain" and "probable" being predicted for a particular compounds are considered as a reason to stop its further study.

Cognition-Enhancing Study. Model of Scopolamine-Induced Passive Avoidance Reflex (PAR) Amnesia. Adult male out bred rats obtained from the "Stolbovaja" animal clinics (Russian Academy of Medical Sciences) were used. Rats weighed 180−200 g. Animals were housed in group plastic cages (8−10/cage) in a 12L:12D cycle in a temperature controlled room (22.0 ± 2.5 °C). All behavioral tests were randomly run during the light phase from 10.00 a.m. to 2.00 p.m. Food and water were available ad lib (except in the case of the conflict test; see below). All animals and experimental procedures were authorized and approved by the Local Ethical Committee; procedures were performed in compliance with the NIH Guide for the Care and Use of Laboratory Animals.

All compounds tested were checked for their ability to prevent memory decline evoked by scopolamine in the passive avoidance step-through paradigm (equipment of Lafayette Instrument Co.).[7,29,32] The experimental device consists of a 40 × 40 × 40 cm dark chamber with black walls supplied with a floor constructed of 4 mm stainless steel rods spaced 1.4 cm apart. A 6 × 25 cm^2 wire-mesh-covered platform lit with a 40 W bulb is attached to the large chamber. In an acquisition trial the rat was placed on the platform facing away from the open door (6 × 6 cm) toward the dark box. When the rat, due to the natural preference for a dark environment, in these experiments comes to the box, it receives an electric shock through an electrifiable grid floor with the current of 0.45 mA and duration of 10 s. Immediately after the punishment, the rat was removed from the dark chamber. On the retention test performed 24 h after the training, the animal was again placed on the hanging platform faced away from the opening to the dark box; the latency of the first entry to the dark compartment and number of animals not entering the dark chamber in percent were measured up to the maximum 180 s. Amnesia of PAR was produced by scopolamine (Sigma, St. Louis, MO, 2 mg/kg, intraperitoneally) administration 15 min before learning. Substances were suspended in a 0.5% solution of Tween-80 and injected intraperitoneally (ip) at a volume of 2 mL/kg in doses 1 and 10 mg/kg 30 min before PAR learning. Control groups of animals received vehicle. There were 12 rats in each group.

The latency for each experimental group was calculated as means ± SEM. The latency to enter the dark compartment was analyzed by nonparametric ANOVA (Kruskal-Wallis One-Way Analysis by Ranks). If this analysis produced a level of significance of 0.05 or less, further pairwise comparisons were made using the Mann−Whitney U-test. The χ^2 test was used to analyze statistically the number of animals not entering the dark chamber.

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