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PASS-predicted design, synthesis and biological evaluation of cyclic nitrones as nootropics

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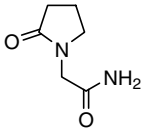
Abstract—Out of 400 virtually designed imidazoline *N*-oxides, five cyclic nitrones were selected on the basis of PASS prediction as potent nootropics and were evaluated for their biological activities in albino mice. The selected *N*-alkyl and aryl-substituted nitrones were found to be excellent nootropics. A series of lead compounds acting as cognition enhancers have been provided, which can be further exploited in search of such New Chemical Entities (NCEs).

One of the major challenges of the third millennium will be to restore normal brain functions in individuals suffering from neurodegenerative diseases like Alzheimer's disease, Parkinson's disease or from cognitive dysfunction, one of the main symptoms accompanying ageing, stroke, acute as well as chronic cerebrovascular diseases, inflammation, traumatic brain injury, intoxication and others.[1–4] Nootropics, also known as smart drugs or cognition activators, are the drugs that enhance mental functions especially under conditions of disturbed neural metabolism resulting from the lack of oxygen, electroshock or age-related induced changes and facilitate information flow between cerebral hemispheres. This category of drugs is becoming especially critical on account of the rapidly growing segment of the victimized population.

Antioxidants are gaining a paramount significance as a panacea for a large number of life-style diseases like ageing, cancer, diabetes, cardiovascular and other degenerative diseases, etc. Nitrones, by acting as excellent spin traps, have been known to oppose the oxidative damage and the associated challenges.[5] However, the mechanism with which nitrones operate as antioxidants does not lie solely in their spin-trapping tendency. Other mechanisms of action, well documented in the literature, involve inhibition of Nitric Oxide Synthase (iNOS) expression, as well as cytokine production, modulation of calcium homeostasis, glutamate regulation and apoptosis.[5a] The piracetam group of antiamnesic compounds works by several mechanisms to revitalise neural function. By supplying glutamic acid analogues to the Kreb's cycle they enhance glucose utilization in aerobic respiration, the major means by which animal cells extract chemical energy from sugars via ATP formation. This, in turn, raises phospholipid cAMP levels, thereby enhancing the function of dopamine and acetylcholine neurons.



Piracetam - the original smart drug

2-Oxo-1-pyrrolidine

Piracetam like nootropics revert amnesia induced by scopolamine and other amnesic drugs, electroconvulsant shocks and hypoxia by an unknown mechanism. They also function as antioxidants (structure comparable to vitamin C) and retard lipofuscin formation. But none of the mechanisms has, so far, gathered general consensus. Thus, years after the identification of piracetam, a unifying hypothesis on the mechanism of action is till lacking and this class of cognition enhancers, despite

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chemical similarity and close pharmacological profile, uses an amazing variety of molecular mechanisms to produce the final nootropic effect. The excellent cognitive properties[6] of piracetam, its neuropatholobiology and the known antioxidant properties of nitrones provided us an impetus for the design, synthesis and biological evaluation of the nitrones (structurally related to piracetam), endowed with a similar pharmacological profile. The chemical and biochemical properties of nitrones as well as their brain penetration and toxicity are tremendously influenced by the substitution pattern of these molecules.

In an effort to optimize their biological profile, a wide structural diversity of nitrones typified by **3**, possessing *p*-nitro phenyl moiety as a silent feature of their chemical structure, have been virtually designed, synthesized and investigated for the foresaid pharmacological activity. To the best of our knowledge, there are no reports concerning the cognition-enhancing activity of such nitrones. The compounds chosen for activity determination were selected on the basis of their best prediction values for crossing the blood–brain barrier from each set of the virtually screened compounds as well as ease of their synthesis. The design was initiated with unsubstituted nitrones followed by the incorporation of various substituents on the nitrogen as well as on the carbon atom of the Schiff's bases and also on the carbon atom of nitrosoalkenes from which they are synthesized. In order to accelerate search for potent New Chemical Entities (NCEs), the assistance of computer-aided drug discovery program PASS (Prediction of Biological Activity Spectra)[7] was used to predict the cognition-enhancing action for different imidazoline *N*-oxides.

Contrary to many other existing methods of SAR/QSAR/QSPR/molecular modelling methods focused on predicting a single type of biological activity within the same chemical series, computer-aided program PASS predicts not only for the desirable pharmacological effect but also for molecular mechanisms of action and different unwanted side effects like mutagenicity, carcinogenicity, teratogenicity and embryotoxicity. Such analysis of heterogeneous sets increases considerably the chance of discovering NCEs (e.g., cognition enhancers). The technique of PASS is based on the analysis of SARs for the training set currently including about 46,000 drugs, drug candidates and lead compounds whose biological activities are determined experimentally. The set of MNA (Multilevel Neighbourhood atoms) descriptors is generated on the basis of structural formulas presented in the MOL-file (SDF-file) form. Since MNA descriptors are generated for each compound de novo, new descriptors can be obtained upon presentation of a novel structural feature in the compound under study. Based on the statistics of MNA descriptors for active and inactive compounds from the training set, two probabilities are calculated for each activity: P_a—probability of compound being active and P_i—probability of compound being inactive. Being probabilities, the P_a and P_i values vary from 0.000 to 1.000 and in general $P_a + P_i < 1$, since these probabilities are calculated independently. The PASS predictions can be interpreted and used in a flexible manner—(i) only activities with $P_a > P_i$ are considered as possible for a particular compound. (ii) If $P_a > 0.7$—the chance to find the activity experimentally is high. But, in many cases the compound may occur to be a close analogue of known pharmaceutical agents. (iii) If $0.5 < P_a < 0.7$—the chance to find the activity experimentally is less, but the compound is probably not so similar to known pharmaceutical agents. (iv) If $P_a < 0.5$—the chance to find the activity experimentally is even less, but the chance to find a structurally new compound, that is, NCEs increases.

Recently, an Internet version of PASS[7c] has been made available at the PASS developer's web site. The user can submit the MOL-file of the molecule under study and obtain the predicted biological activity spectrum on their computer immediately. This new internet version of PASS provides access to prediction of 783 kinds of biological activity, in contrast to an earlier version that predicted 319 activities.

Selection of possible cognition enhancers. Prediction of biological activity spectra was made for about 400 virtually designed variedly substituted structures from the theoretical calculations on the basis of PASS prediction. The compounds screened were the structures formed from the various combinations of R (H, CH_3, C_2H_5, C_6H_5, *p*-CH_3—C_6H_4, *p*-OCH_3—C_6H_4, $C_6H_5CH_2$, cyclohexyl, *iso*-propyl, *n*-butyl, furyl), R^1 (H, CH_3, C_2H_5, C_6H_5, *p*-NMe_2—C_6H_4) and R^2 (H, C_6H_5 *p*-CH_3—C_6H_4, *p*-NO_2—C_6H_4) groups in the nitrone moiety. On the basis of prediction results from the database analysis, potential cognition enhancers were selected. The following criteria were used for selection:

1. Those compounds were selected, which had cognition enhancing activity with $P_a > 0.4$ (intended to find NCEs) in their predicted activity spectra.
2. In case a number of structures in a certain chemical series were predicted to be active, only a few representatives were selected.

The relationship between the number of compounds predicted as probable nootropics and representative values of probability to be active P_a is shown in Figure 1.

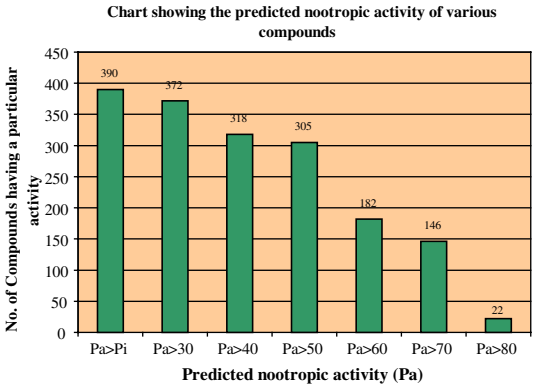


Figure 1. The number of compounds predicted as probable cognition enhancers versus calculated probability to be active P_a.

Out of the 400 compounds chosen for PASS prediction, 318 from were predicted as cognition enhancers with $P_a > 40\%$. On the basis of the above-mentioned criteria, five potential nitrones were selected for testing their nootropic activities. The compounds screened above were further subjected to 'Lipinski rules of five'.[8] The MIPC (Molnspiration Property Calculator) program has been utilized (www.molinspiration.com) for calculating the Lipinski descriptors. The log *P* values and the associated parameters for the five compounds under consideration have been provided in Table 1 and the probabilities of the test compounds for being active (P_a) are provided in Table 2. On the basis of above-mentioned criteria and with a goal to provide the diversity of potent cognition enhancers, the chosen compounds were synthesized and experimentally tested as nootropics.

General procedure for the synthesis of nitrones **3**. The acyclic aryl and alkyl imines (Schiff's bases) **1** derived from the condensation of aryl/alkyl amines and aldehyde, reacted with α-nitrosoalkenes **2** (1.2 equiv), generated in situ from α-bromooxime and sodium bicarbonate, in dry dichloromethane at room temperature under stirring conditions for 24–28 h to give the desired nitrones **3** (Scheme 1), characterized as 1-alkyl/aryl-4-(4-nitrophenyl)-2-aryl-2,5-dihydro-1*H*-imidazole 3-oxide on the basis of their spectral and analytical data.[9a]

This methodology is a facile and direct itinerary towards the synthesis of novel and biologically potent heterocyclic *N*-oxides containing an α-hetero atom.[9b]

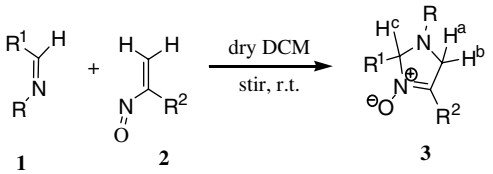
Animals. Albino mice of either sex weighing around 18–25 g (older ones, aged 28 weeks) were used in the present study. The animals were housed in central animal house

Table 1. Log *P* values and the associated parameters for the compounds under consideration

	*M*log *P*	TPSA	N atoms	M_W
A	2.32	84.767	32	433.49
B	2.449	72.295	27	360.39
C	2.411	72.295	27	366.44
D	1.341	85.435	27	364.38
E	2.019	75.533	31	417.49
	*n*ON	*n*OHNH	*n*Violations	Nrotb
A	8	1	0	6
B	6	1	0	4
C	6	1	0	4
D	7	1	0	5
E	7	1	0	6

Table 2. The probabilities of being active P_a of the test compounds on the basis of PASS prediction

Compound	P_a	P_i
A	50.1	11.4
B	69.2	3.3
C	51.8	9.9
D	40.9	19.3
E	56.7	7.0



1 2 3

A. R = C_6H_4-OCH_3(*p*); R^1 = C_6H_4- NMe_2(*p*)
B. R = Ph; R^1 = Ph
C. R = Cyclohexyl; R^1 = Ph
D. R = Furyl; R^1 = Ph
E. R = Benzyl; R^1 = C_6H_4- NMe_2(*p*)
R_2 = C_6H_4-NO_2(*p*)

Scheme 1.

of Guru Nanak Dev University, had free access to food and water and maintained under 12:12 h light and dark cycles. All experiments were carried out during day time from 09:00 to 14:00 h. Institutional Animals Ethics Committee (IAEC) approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA, Dept. of Animal Welfare, Govt. of India.

Drugs and chemicals. Scopolamine hydrobromide (Sigma–Aldrich, USA) piracetam (Nootropil®, UCB India Pvt. Ltd, Vapi, Gujarat) were suspended in 1% CMC solution. Volume of oral and ip administration was 1 ml/100 g.

Behavioural paradigm to evaluate learning and memory—Elevated plus maze. The elevated plus maze served as the exteroceptive behavioural model (wherein the stimulus existed outside the body) to evaluate learning and memory in mice. The apparatus consisted of two open arms (16×5 cm) and two covered arms ($16 \times 5 \times 12$ cm). The arms extended from a central platform (5×5 cm), and maze was elevated to a height of 25 cm from the floor. On the first day, each mouse was placed at the end of open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by mouse to move into one of the covered arms with all its four legs. TL was recorded on the first day. If the animal did not enter into one of the covered arms within 90 s, it was gently pushed into one of the two covered arms and the TL was assigned as 90 s. The mouse was allowed to explore the maze for 10 s and then returned to its home cage. Memory retention was examined 24 h after the first day trial on the second day.[10]

Group I: represented control group for young mice ($n = 6$). 10 ml/kg of distilled water (DW), po, was administered. TL was noted after 30 min of administration on and after 24 h, that is, on next day.

Group II: piracetam, 200 mg/kg, ip, was injected to both young and aged mice, respectively. TL was noted after 30 min of injection and after 24 h.

Groups III–VII: test compounds (**A–E**) were administered orally at a dose of 75 mg/kg, except test compound **D** at a dose of 90 mg/kg, 30 min before subjecting the

Table 3. Effect of transfer latency (TL)

S. No.	Treatment group	Dose (mg/kg)	*n*	% Age decrease in TL
1	Control	—	6	11.2 + 2.78
2	Piracetam	200	6	51.3 + 6.82*
3	**A**	75	6	40.4 + 8.38*
4	**B**	75	6	36.4 + 7.77*
5	**C**	75	6	51.6 + 9.28*
6	**D**	90	6	64.2 + 6.66*
7	**E**	75	6	10.8 + 5.66

* $P < 0.05$ as compared to control.

animals to elevated plus maze test. TL was noted again after 24 h.

The dose of the test compounds was selected as molecular weight equivalents to piracetam.

Statistical analysis. All the results were expressed as means (±SEM). The data from elevated plus maze and passive avoidance tasks were analyzed using ANOVA followed by Student's (Unpaired) '*t*' test.[10] Utility of an elevated plus maze for the evaluation of nootropics, scopolamine and electroconvulsive shock,[11] Ascorbic acid: a promising memory enhancer in mice.[12]

Effect of test compounds on facilitation of passive behaviour. The mice showed higher transfer latency (TL) values on first day as compared to second day (after 24 h), piracetam (200 mg/kg, ip) pretreatment significantly decreased transfer latency on as compared to control group, indicating improvement in both learning and memory. The pretreatment with test compounds **A–E** significantly reduced the TL as compared to control group. Piracetam, **A**, **B**, **C**, and **D** are all significantly different than control. Compounds **A** and **C** are within the standard deviations of piracetam. However **A** showed lower % TL as compared to **C**, although still within the SEM. The compound **C** showed comparable to and compound **D** exhibited a better effect than piracetam (Table 3).

Structure–activity relationship. The resulting biological activities of the compounds studied are the consequence of both pharmacokinetic and pharmacodynamic properties that may be differently affected by structural modifications indicating that there is a strict correlation between potency and structural as well as conformational characteristics of the molecules. Comparison of cognitive activities of the test compounds **A–E** (Table 3) shows that the electronic nature of the substituents present on N1 (i.e., alkyl and aryl) and C2-atoms could be responsible for the potency of the compounds and this is substantiated by the known scavenging mechanism of the nootropics. Electron-withdrawing nature on C-2 position has been thought to be responsible for the better activities of **C** and **D** compared to **A** and **E** (*p*-methoxyphenyl group in **A** and **E** is electron-donating relative to bare phenyl in **C** and **D**). Further *N*-alkyl substituted nitrones show much better activities in our results compared to *N*-aryl substituted ones. Thus **A**, **C** and **D** compounds represent the most significant results of this chemical series. The compound **E**, the only compound with non-hydrogen substituents at R^1 and R^2, shows significantly less activity compared to other compounds **A–D**. This may be perhaps because the addition makes the region too bulky for efficient scavenging to occur. Although the activity under study is whole-animal behaviour, so many other properties of the compounds could also be responsible for differences in observed activity, for example, susceptibility of particular functional groups to metabolism, secondary effects from certain groups, etc. Since, the mechanism of action (MOA) of nitrones for their nootropic activity is well documented in their abilities to scavenge free radicals, the underlying phenomena of mechanism are thought to be influenced by electronic nature of the substituents on the nitrone moiety. However, the detailed studies are still underway to establish the exact MOA.

Thus, innovative computer-assisted approaches have been applied in a search for new cognition enhancers. We have used virtual combinatorial design of highly diverse chemical compounds to increase the probability of finding new chemical entities. Different types of imidazoline *N*-oxides were analyzed during the project. The most likely compounds (presumably NCEs) were selected, synthesized and tested as potential cognition enhancers. Five compounds from the library of 400 compounds (predicted as nootropics) were selected as potential cognition enhancers, out of which, three compounds have comparable or greater effect in considerably less concentration in comparison with the classic cognition enhancer, piracetam. Thus we have provided a novel series of lead compounds acting as cognition enhancers, which can further be expanded a lot to exploit such NCEs. It is anticipated that these drugs when taken in large concentrations can further improve their nootropic activities. Thus, our investigation has shown an increased probability of compounds to be biologically active in the subset of compounds selected on the basis of PASS prediction.

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9. (a) {4-[1-(4-Methoxy-phenyl)-4-(4-nitrophenyl)-3-oxy-2,5-dihydro-1H-imidazol-2-yl]-phenyl}-dimethyl-amine (**3a**). Red crystalline solid; yield 88%; mp: 166–167 °C; ν_{max}/cm^{-1} (KBr): 1220 (N—O), 1542 and 1591 (C=N); ^{1}H NMR (200 MHz): δ 2.96 [(s, 6H, —N(CH$_3$)$_2$], 3.73 (s, 3H, OCH$_3$), 4.79 (dd, J = 14.1 and 3.1 Hz, 1H, —CH$_2$), 5.15 (dd, J = 14.1 and 5.4 Hz, 1H, —CH$_2$), 6.08 (dd, J = 3.1 and 5.4 Hz, 1H, methine), 6.59 (d, J = 8.8 Hz, 2H, ArH), 6.70 (d, J = 8.7 Hz, 2H, ArH), 6.81 (d, J = 8.8 Hz, 2H, ArH), 7.44 (d, J = 8.7 Hz, 2H, ArH), 8.29 (d, J = 8.8 Hz, 2H, ArH), 8.49 (d, J = 8.8 Hz, 2H, ArH); ^{13}C NMR: 40.3 (—N(CH$_3$)$_2$), 52.7 (—CH$_2$), 55.5 (—OCH$_3$), 90.7 (methine), 112.1, 113.7, 114.9, 122.2, 123.9, 127.2, 128.8, 132.9, 135.2, 138.3, 147.8, 151.7 and 152.8; MS: m/z 432 (M$^+$) and 416 (M$^+$−16); Anal. Calcd for C$_{24}$H$_{24}$N$_4$O$_4$: C, 66.69; H, 5.59; N, 12.96; found: C, 66.63; H, 5.52; N, 12.99; (b) Marwaha, A.; Singh, P.; Mahajan, M. P. *Tetrahedron* **2006**, *62*, 5477.
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