Design and Study of Piracetam-like Nootropics, Controversial Members of the Problematic Class of Cognition-Enhancing Drugs

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Abstract- Cognition enhancers are drugs able to facilitate attentional abilities and acquisition, storage and retrieval of information, and to attenuate the impairment of cognitive functions associated with head traumas, stroke, age and age-related pathologies. Development of cognition enhancers is still a difficult task because of complexity of the brain functions, poor predictivity of animal tests and lengthy and expensive clinical trials. After the early serendipitous discovery of first generation cognition enhancers, current research is based on a variety of working hypotheses, derived from the progress of knowledge in the neurobiopathology of cognitive processes.

Among other classes of drugs, piracetam-like cognition enhancers (nootropics) have never reached general acceptance, in spite of their excellent tolerability and safety. In the present review, after a general discussion of the problems connected with the design and development of cognition enhancers, the class is examined in more detail. Reasons for the problems encountered by nootropics, compounds therapeutically available and those in development, their structure activity relationships and mechanisms of action are discussed. Recent developments which hopefully will lead to a revival of the class are reviewed.

INTRODUCTION

Cognitive dysfunction is one of the main symptoms accompanying ageing, stroke, head injury and neurodegenerative diseases like Alzheimer's disease. Cognition enhancers can be defined as drugs able to facilitate attentional abilities and acquisition, storage and retrieval of information, and to attenuate the impairment of cognitive functions associated with age and age-related pathologies [1]. By definition, this class of drugs improve declining cognitive functions but do not change the rate of progression of neurodegeneration [2]. Obviously, drugs that are neuroprotectant or slow down the progression of the disease will very likely improve cognitive functions. The overlapping of these properties is frequent and in the literature distinction among the modes of action is usually neglected.

One of the major challenges of the third millennium will be to restore normal brain functions in individuals that suffer from neurodegenerative diseases or from the physiological decline of brain functions related to ageing. However, cognitive neuropharmacology, which should provide the theoretical support and a set of reliable assays for the discovery of cognition enhancing drugs, is still in its infancy [3]. An extremely complicated neuronal network in the brain regulates the complex mechanism of learning and memory. Receptors [4] and channels [5] are the major players in the interactive framework that eventually produces information acquisition, storage and retrieval and knowledge of their interplay is limited.

Acetylcholine [6], dopamine [7], glutamate [8, 9], histamine [10, 11], neuropeptide [12], and serotonin [13] receptors have been reported to play a fundamental role in cognition. Others like adenosine [14], cannabinoid [15], catecholamine [16], GABA [17], opioid [18], and sigma [19] seem to be involved but their role is, at the moment, less documented. Both receptor-operated and potential-operated ionic channels [20, 21] are also critically involved.

A prominent role has been attributed to cholinergic receptors and this has been the bases for the cholinergic hypothesis [22], that by some scientists has been judged too reductionist [4]. However, considering the complexity of the brain and the number of biological entities involved in cognitive processes [23], singling out any mechanism of action will invariably result in a reductionist approach. On the other hand, since many of the receptors and channels involved in learning and memory eventually produce modulation of cholinergic receptors [24, 25], a pivotal role of the cholinergic system can hardly be disputed and the controversy regarding the cholinergic hypothesis appears of academic interest.
Under these circumstances, it is by no way unexpected that cognition enhancing drugs are at the moment poorly defined and the tests to detect their activity are under some criticism, which slows down the pace of discovery of new molecules in this class of drugs. The fact that cognition enhancers may use more than one neuronal mechanism does contribute to the poor definition of the class.

ASSESSING COGNITION AND COGNITION ENHANCERS

One of the conditions for the design and development of drugs is the availability of simple, reliable, and inexpensive preclinical assays that allow rapid identification of the drug(s) to submit to the clinical tests. These tests, in turn, should be able to provide reliable and univocal information on the therapeutic utility of the drug candidates. From this point of view, the situation of cognition enhancers is rather unsatisfactory.

Many experimental models are currently available for the preclinical evaluation of agents that affect learning and memory processes [3, 26]. In general, they suffer from poor quantitation as, due to their nature, they do not allow an easy construction of dose-effect curves. Mazes are the traditional tool in assessing cognitive performances in animals and both passive avoidance and active avoidance tasks can be planned. Two behavioral tasks that are commonly used are passive avoidance [27] and Morris water

Fig. (1). First generation cognition enhancers: a selection of marketed drugs.
However, they have met much criticism [4, 5, 29] and their usefulness for predicting the efficacy of cognition enhancers in humans seems minimal, as many false positive results have been observed [30]. Nevertheless, since passive avoidance experiments on mice are relatively simple and rapid, they can be used as a quick screening method to select compounds for further studies to confirm passive avoidance results. In fact, it seems a general opinion that conclusions about the cognition enhancing effects of a drug should not rest on the results of one experiment. In general the battery of tests to assess the cognition enhancing activity of new drugs needs to be improved and, as recently pointed out by Eid [5]: "Development of effective treatments for dementia would be considerably aided if the stringency of preclinical behavioral tests could be improved". Of course this applies also to cognition enhancers in general.

As far as clinical tests are concerned, the difficulties facing psycho-pharmacologists are numerous. The task of assessing major cognitive functions such as memory, language, attention, orientation and reasoning is by no means simple and a variety of protocols for dementia assessment have been designed [31, 32]. In addition to global and functional assessment protocols, ADAS Cog (Alzheimer Disease Assessment Scale Cognition Subscale) is the most suitable for cognition enhancing drugs and has been the mainstay of cognitive testing in recent dementia trials [33]. Last but not least, clinical trials to test anti dementia drugs are lengthy and exceedingly expensive.

**COGNITION ENHANCING DRUGS**

Many compounds have been claimed to be endowed with cognition enhancing activity [34-37] (reviewed by Froestl and Maitre [38], for them the terms *metabolic enhancer* or *cerebral stimulants* have also been used) and few of them have reached the market for use in some countries for memory disturbances. Their use has been controversial and there is no general consensus on their efficacy in humans: the case of propentofylline (figure (1)), launched in 1994, may be illustrative. This compound has been recently withdrawn from the Japanese market, following the issue of new guidelines for proof of efficacy of nootropic drugs [39] even if positive reports on its pharmacological efficacy have continued to appear [40]. On the other hand, compounds like nicergoline (figure (1)), an $\alpha_1$ and 5-HT$_{1A}$ receptor ligand used since 1972 in Italy and other countries, memantine (figure (1)), a non competitive NMDA antagonist that has been used for over ten years in Germany, and idebenone (figure (1)), an antioxidant drug discovered and used in Japan, are now under trial for Alzheimer’s disease and look promising [41-43]. In general however, except for a few cases, the mechanism of action of these early cognition enhancers is ill-defined or unknown. In other cases (Nafronyl, Vincamine, Cyclandelate), the nootropic action is indirect, being the consequence of cerebral vasodilation.

In Fig. (1) are reported the structures of some marketed cognition enhancers, except piracetam and piracetam-like
compounds that will be considered in detail below, and in Fig. (2) a selection of other related compounds that are still in development. They can be considered the first generation of cognition enhancers. It must be added that some preparations of natural origin such as Ginkgo biloba extracts [44], cerebrolysin [45] and gangliosides [46] are also available and are being studied for treating dementia and cognitive impairment.

In contrast to previous serendipitous discovery, current research aims to design and synthesise new compounds, on the bases of present information about neuropathobiology of cognitive processes, following the various hypotheses that have been proposed for cognitive decline: among them, the cholinergic hypothesis of Alzheimer's disease has, so far, produced the most useful compounds [47]. Most studies consider ligands of the receptors and channels involved in cognitive processes (see above), but compounds that spare brain neuromediators and neuropeptides (like acetylcholinesterase [31], MAO [48] and prolylendopeptidase inhibitors [49]) are also valuable. Even if they belong more to the disease modifying group [2], drugs that maintain and restore the health of the brain neuronal network (like antioxidants [50], cyclooxygenase inhibitors [51] and NGF inducers [52]) are gaining interest. In this respect, compounds able to interfere with amyloid plaques formation are actively sought and the amyloid hypothesis [53, 54] has flanked the cholinergic hypothesis as a rationale for antidementia drug design.

The effort of the pharmaceutical industry in this field is impressive and a myriad of new potentially useful compounds for cognitive disorders are claimed every year in patent literature, as can be appreciated from the periodic survey of Baudy [55]. In Fig. (3) are reported some selected compounds, acting with different mechanisms, that have been shown to possess cognition enhancing properties: some of them are in development for this use.

**PIRACETAM-LIKE NOOTROPICS**

Piracetam properties were disclosed in 1967 [56] and this stimulated the design and synthesis of a large number of structurally related molecules that were found to be endowed with a similar pharmacological profile. This class of cognition enhancing drugs is often referred to as nootropics and it has been thoroughly reviewed by Gouliaev [57, 58]. Much like the cognition enhancers of the first generation, piracetam-like nootropics revert amnesia induced by scopolamine and other amnesing drugs, electroconvulsive...
Mechanism of Action

In general, piracetam-like nootropics show no affinity for the most important central receptors ($K_i > 10 \, \mu M$). A modest affinity for muscarinic receptors is shown by aniracetam ($K_i = 4.4 \, \mu M$ [58]) and nebracetam ($K_i = 6.3 \, \mu M$ [61]). Nefiracetam is the only one showing affinity in the nanomolar range ($K_i = 8.5 \, nM$ on the GABA_A receptor [58]). As a consequence, they do not seem to act on any well-characterised receptor system, although modulation of most central neurotransmitters, in particular acetylcholine [62, 63] and glutamate [64] has been reported. Neurotransmitter modulation could be due to interactions with ion channels: indeed this has been one of the mechanisms proposed to rationalise activity of some piracetam-like compounds [65]. As can be deduced from the wealth of data presented by Gouliaev [58] and from the extensive and accurate discussion made in the review, a great deal of different biochemical and behavioural findings have been presented for piracetam-like nootropics, but so far, they have been unable to indicate a common molecular mechanism of action. On the other hand, generalisation is made difficult by the fact that, quite often, piracetam-like nootropics do not share the same behaviour in many biological assays [66, 67].

Nevertheless, attempts to find common properties in the class have been made. On the basis of the finding that memory enhancing effects are steroid-sensitive [68], Mondadori has proposed that nootropics exert a modulatory action on protein synthesis [69]. Protein kinase C (PKC) activation as well, could be a general mechanism of action of...
Table 1. Piracetam-like Cognition Enhancers (Nootropics): Drugs Available or in Development

<table>
<thead>
<tr>
<th>Drug</th>
<th>Drug codes</th>
<th>Company</th>
<th>Year of launch/phase</th>
</tr>
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<tbody>
<tr>
<td>Piracetam</td>
<td>UCB-6215</td>
<td>UCB</td>
<td>1973</td>
</tr>
<tr>
<td>Oxiracetam</td>
<td>ISF-2522</td>
<td>ISF</td>
<td>1987</td>
</tr>
<tr>
<td>Aniracetam</td>
<td>Ro-13-5057</td>
<td>Roche</td>
<td>1993</td>
</tr>
<tr>
<td>Pramiracetam</td>
<td>CI-879</td>
<td>Warner-lambert</td>
<td>1993</td>
</tr>
<tr>
<td>Nebracetam</td>
<td>WEB1881FU</td>
<td>Boehringer Ing.</td>
<td>Awaiting approval</td>
</tr>
<tr>
<td>Nefiracetam</td>
<td>DM-9384</td>
<td>Daiichi Seyaku</td>
<td>Awaiting approval</td>
</tr>
<tr>
<td>Fasoracetam</td>
<td>NS-105/LAM-105</td>
<td>Nippon Shinyaku</td>
<td>Phase III</td>
</tr>
</tbody>
</table>

cognition enhancing compounds, as appears from studies on oxiracetam [70], aniracetam [71], and nefiracetam [72]. Very recently, based on the effect of piracetam on the fluidity of mouse brain membranes, Müller and co-workers have proposed that cognition enhancing properties of piracetam can be explained by their effect on brain membrane properties, in particular by modification of membrane-located mechanisms of central signal transduction [73]. However, none of these hypotheses have, so far, gathered general consensus.

It can be concluded that, thirty years after the identification of piracetam, a unifying hypothesis on the mechanism of action is still lacking and that this class of cognition enhancers, despite chemical similarity and close pharmacological profile, uses an amazing variety of molecular mechanisms to produce the final nootropic effect.

Structure-activity relationships

The lack of a common mechanism at a molecular level allows sound structure-activity correlations only with in vivo behavioural assays, which has frustrating consequences on drug design. In fact, the resulting activity is the consequence of both pharmacokinetic and pharmacodynamic properties that may be differently affected by structural modifications. Moreover, since the protocols for behavioural tasks that are commonly used vary widely between investigators [5], making general comparison of the results difficult, and the structures studied are fairly heterogeneous, one might wonder whether SAR’s are meaningful at all. This fact, severely limits the significance of the models of the pharmacophore that have been proposed for piracetam-like compounds [74, 75]. However, most of the compounds containing a 2-oxopyrrolidine structure do present nootropic activity, suggesting that this feature plays a critical role in nootropic activity.

In Fig. (4), the chemical evolution of piracetam-like compounds is shown through some of the most studied and interesting members of the class: except for sunifiram, all contain a 2-oxopyrrolidine ring. The following is a short discussion on structure and pharmacological properties of the compounds that have been used or studied in more detail, starting from previous reviews [34-38], in particular that of Gouliaev and coworkers [57, 58].

**a- N-Side Chain Homologues**

From the several α-substituted derivatives of piracetam synthesised (see Gouliaev [57] and the tables available as Addendum), etiracetam has emerged as the most interesting compound. It shows nootropic activity and its S enantiomer, levetiracetam (Fig. (4), also known as UCB L059(S)), which is responsible of nootropic activity as well (the R enantiomer is inactive [58]) has been approved for the treatment of epilepsy. Apparently, simple alkylation of the side chain results in a definitely different pharmacological and clinical profile [76]. However, in line with the odd behaviour of the compounds of this series, analogous to mechanism of nootropic activity, that of antiepileptic activity, has not been clearly established.

**b- N-Substituted Amides**

Early efforts to modulate activity of piracetam regarded the substitution with different groups of the amide nitrogen. A large array of substituents has been used and hundreds of compounds of this kind have been synthesised. Most of them have been claimed to be active as nootropics, but few have survived the preclinical stage [35, 57].

Pramiracetam (Fig. (4) [77]) was introduced in 1993. The drug has been shown to enhance cholinergic neurotransmission in brain pathways involved in cognitive processes (mainly increasing high affinity choline uptake), to interact with steroids and to inhibit (K<sub>i</sub> = 11 µM) prolyl endopeptidases [58].

Nefiracetam (chart (1)) is awaiting approval. It presents a variety of pharmacological actions as it is reported to activate the cholinergic, GABAergic and other monaminergic systems and to modulate N-type calcium channels [78-81]. It has proven effective also on β-amyloid induced learning impairment [82]. There are indications that these effects may be due to activation of protein kinases A and C (PKA/PKC) followed by modulation of presynaptic nicotinic receptors [80, 83, 84].
Fig. (4). Chemical evolution of piracetam-like nootropics.

Another derivative of this type, SNK-882 (chart (I)) [85], is undergoing phase II clinical trials as cognition enhancing drug [86].

Several derivatives of piracetam were obtained by simultaneous substitution in the ring and at the amide nitrogen [57, 58]. One of them, (I, chart (I)) a pramiracetam derivative, has been recently reported in a patent to be active,
thus showing that the pyrrole ring can be substituted without loss of nootropic activity [87].

**c- Ring Substituted Derivatives**

Only oxiracetam (Fig. (4)) among the numerous synthesised and studied ring-substituted analogues of piracetam [57, 58] survived preliminary and clinical tests and was introduced in 1987. It is used as a racemate. It is interesting to notice that, also for oxiracetam, PKC seem involved in the action at molecular level [88].

More recently, N-acyl derivatives of 4-phenyl-2-oxopyrrolidine endowed with nootropic activity have been reported [89].

**d- N-Side Chain Modified Derivatives**

The original acetamido side chain of piracetam can be modified without loss of nootropic action and a variety of substituents have been inserted on the pyrrole nitrogen, some times with a contemporary introduction of a substituent in the pyrrole ring [38, 57].

Aniracetam (Fig. (4)), introduced in 1993, is one of the most studied. It has been reported to be a potent modulator of the glutamatergic (AMPA) and cholinergics systems and to block the N-type calcium current [90]. The serotoninergic system also seems involved [91]. Action on AMPA receptor, where it seems to act as a positive allosteric modulator, has been confirmed recently by its high potency in the kinurenate test [92]. Its main metabolite, N-anisoyl-γ-aminobutyric acid, where the 2-pyrrolidinone ring has been cleaved, maintains nootropic properties, although with lower potency [93, 94]. Aniracetam has been found to have an extremely rapid onset and washout, which is desirable, but, on the other hand, it works only at high concentration and is hydrolyzed to anisoyl-GABA to a very large extent when administered peripherally. To improve the properties of aniracetam, a series of benzoypiperidines (1-BCP and CX516), benzoylpyrrolidines and policyclic related derivatives (CX614), that also act as positive allosteric modulators of AMPA and are often referred to as ampakines (chart (2)) [95-97], have been designed. This class of compounds represents one of the most significant results of piracetam-like compounds and the drug labeled CX516 is, at present, in phase III development as cognition enhancer [98].

Another member of this family, BMY-21502 (chart (3)) [99], has entered clinical trials but, while generally well tolerated, was not significantly superior to placebo in improving patient conditions [100].

Nebracetam (Fig. (4)), which is waiting approval, presents a quite new structure and has direct cholinomimetic properties as it shows some affinity for muscarinic receptors [61]. However, other neurotransmitter systems, like adrenergic [101] and triptaminergic [102], seem involved. The enantioselectivity of nebracetam enantiomers has been studied. It was found that both isomers increase the release of acetylcholine, the (-) enantiomer being slightly more potent [103].

Ru-47067 (chart (3)) [104] is a member of a series of compounds where a sulfonyl group replaces the carbonyl group of piracetam. However, although they have been
studied for some time [38, 58], this kind of compound apparently did not reach clinical development. They are of interest since sulfonamido function appears also in some new, potent nootropic drugs.

Two compounds, 2 and 3 (chart (3)), also modified at the N-side chain, have been claimed in patents [105, 106] but no other information is available.

e- N-Unsubstituted, Pyrrole Substituted Derivatives

This kind of compound lacks the N-side chain which is a common feature of piracetam-like compounds. Their nootropic action demonstrates that this is not a stringent requirement for activity, as was believed for some time. In general, the compounds of this group are derivatives of pyroglutamic acid.

Fasoracetam (Fig. (4)), which is currently in phase III trials, is the most studied representative. Fasoracetam significantly increased cAMP accumulation in membranes pretreated with pertussis toxin, indicating that it activates both inhibitory and stimulatory G proteins [107]. This effect seems to be mediated by GABA_B receptors, but even cholinergic [108] and metabotropic glutamate [109] receptors have been found involved. No information is available on the influence of stereochemistry on nootropic activity of fasoracetam.

To this group belong two compounds that, although endowed with some nootropic activity, are used or in development for other purposes. Pidotimod (chart (4)) [110] has been used since 1993 as immunomodulator and rolipram (chart (4)) [111], a potent inhibitor of PDE IV, is in development as antidepressant.

f- Ring-Modified Derivatives

Several attempts to modulate nootropic activity through manipulation of the pyrrolidine ring have been reported [35, 38, 58, 112, 113].

The only compound that has been studied in some detail is tenilsetam (chart (5)) [114], where the pyrrolidinone has formally been enlarged into a piperazinone ring. Its nootropic properties (similar to that of piracetam) seem related to inhibition of protein glycosylation [115].

More recently, two other series of products, 4 [116] and 5 [117] (chart (5)), have been reported, but no details on their activity are available.

g- Polycyclic Derivatives

Although a few polycyclic derivatives of piracetam-like compounds (exemplified by 6 and 7, chart (6)) have been reported [35], only rolziracetam has been studied in some details reaching phase II clinical studies [118].

The series of dihydro-1H-pyrrolo[1,2-a]imidazole-2,5(3H,6H)-diones, with the general structure 8 (chart (6)), has been synthesised and some compounds have been reported to be endowed with potent nootropic activity [119].

Pyrrolidinone compounds condensed with an aromatic ring, as represented by linopirdine (Fig. (3)) or compound T-82 (a donepezil analog [120], chart (6)), do show nootropic activity but, very likely, their pharmacological profile is not related to the pyrrolidinone moiety.

Recently, a series of 1,4-diazabicyclo[4.3.0]nonan-9-ones, showing unprecedented potency on mouse passive avoidance tests, has been disclosed [121]. Among the compounds studied, DM 232 (unifiram, see Fig. (4)) is the most potent, being able to revert amnesia induced by scopolamine at the minimal active dose of 0.001 mg/kg. Interestingly, molecular simplification of the structure of 1,4-diazabicyclo[4.3.0]nonan-9-ones to seco derivatives like DM 235 (sunifiram, Fig. (4)) [122], maintained activity and potency, thus suggesting that the pyrrolidinone ring can be mimicked, at least in this series, by a suitable alkanoic acid amide. To the best of our knowledge, there is only one poorly characterised example of a piracetam-like nootropic.
where a similar situation can be found: aloracetam (chart (7)) [101]. On the other hand, sunifiram, being a benzoylamide, can be also related to the aniracetam-derived ampakines mentioned before, which would suggest a similar mechanism of action.

Unifiram and sunifiram were able to revert amnesia induced by a variety of amnesing drugs such as diphenhydramine (antihistaminic), baclofen (GABA B agonist) and clonidine (α2 agonist) with a potency pattern similar to that reported for scopolamine. Moreover, they were active as well in a test (social learning) where there is no deficit in the cognitive functions.

Both unifiram and sunifiram were able to increase the release of acetylcholine in the cerebral cortex of freely moving rats (Fig. (5)).

**Fig. (5).** Dose-response curve for sunifiram (open symbols) and unifiram (closed symbols) on ACh release from rat cerebral cortex. Compounds were injected ip after 60 min as shown by the arrow. All values are expressed as changes over basal output. Vertical lines give S.E.M. Each point represents the mean of 3 experiments. Open squares: sunifiram 0.01 mg/kg; open circles: sunifiram 1 mg/kg; closed circles: unifiram 0.01 mg/kg; closed squares: unifiram 1 mg/kg.
help in restoring impaired cognitive functions, both directly or through the cure of the pathologies that produce cognitive dysfunction.

Among other classes of cognition enhancers, piracetam-like compounds suffer from the lack of a common, generally accepted, mechanism of action; a condition which has precluded, so far, a wide acceptance of these drugs as useful medicines. Ironically, the very low toxicity of this class of compounds is itself a problem, since it has been considered the result of insufficient activity, even if they are active in most preclinical assays and, at least in some clinical trials, their therapeutic efficacy has been found significant. As a consequence, after a period of intense research in the late eighties, interest for this class of drugs has vanished. Nevertheless, research in this field has allowed identification of two new classes of drugs, the ampakines and diacylpiperazines, whose cognition enhancing properties look promising. It is hoped that the new recently disclosed compounds, that seem to be endowed with unprecedented high potency, will contribute to elucidate the mechanism of action of the class and its revival.

In fact, as shown in Fig. (6), where the minimal effective doses on scopolamine induced amnesia of unifiram and its seco derivatives 9-11 are reported, there is a strict correlation between potency and structural and conformational characteristics of the molecules.

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Design and Study of Piracetam-like Nootropics


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