Biodiversity—A wonderful source of exciting new pharmacophores. Further to a new theory of memory*

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Abstract: The diverse biological resources in nature are vast factories of chemical diversity. With the recent advances in the biological and chemical sciences it is now easier than ever to explore the potential uses of immense biological resources in a sustainable manner. Our investigations focused on the bioprospecting of both terrestrial and marine organisms have led to the discovery of a number of interesting chemical compounds. The discovery of a new class of cholinesterase, α-glucosidase and phosphodiesterase I inhibitors, several new antioxidants, and other classes of bioactive natural products is presented. The theory of the chemical basis of memory through hydrogen bonding in the brain is further elaborated.

ENZYME INHIBITORS FROM NATURAL SOURCES

In continuation of our work on the discovery of natural enzyme inhibitors by using high-throughput spectrophotometric assays, we have recently discovered several new classes of enzyme inhibitors which are presented below:

First examples of natural inhibitors of phosphodiesterase I

Phosphodiesterase I successively hydrolyzes 5'-mononucleotides from 3'-hydroxyl terminated ribo- and deoxyribo-oligonucleotides. The enzyme has been widely utilized as a tool for structural and sequence studies of nucleic acids. Although its physiological role remains obscure, clinically the activity of this enzyme is markedly elevated in cultured skin fibroblasts from patients of Low’s syndrome [1]. Inhibitors of phosphodiesterase I might also be useful for the treatment of some forms of arthritis [2].

Mitragyna stipulosa (D. C.) O. Kuntze. (Rubiaceae) is a tree widely distributed throughout southern, eastern, and western Africa [3]. Its stem barks have been used to treat neuralgia pain, cancer, and diabetes by the local people of southern Cameroon for a long time.

Known triterpenes quinovic acid (1) and quinovin glycoside C (2) have been isolated from M. stipulosa of Cameroonian origin and screened against snake venom phosphodiesterase I. Cystein was used as standard inhibitor (IC50 = 0.748 mM ± 0.015). Both compounds were found to be the first and most potent natural inhibitors of this enzyme. The results of this study also show some relationship between the chemical composition of this plant and its traditional uses in folk medicine by the local people of southern Cameroon [4].

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New class of cholinesterase inhibitors

According to the cholinergic hypothesis, memory impairment in patients with senile dementia of the Alzheimer's type results from a deficiency in cholinergic function in the brain [5,6]. Hence, the most promising therapeutic strategy for activating central cholinergic functions has been the use of cholinomimetic agents. The enzyme acetylcholinesterase (AchE) catalyzes the hydrolysis of the neurotransmitter acetylcholine, and it has long been an attractive target for the rational drug design and discovery of mechanism-based inhibitors for the treatment of Alzheimer's disease (AD) and for other possible therapeutic applications in the treatment of Parkinson's disease, aging, and myasthenia gravis. The role of butyrylcholinesterase (BchE) in normal aging and brain diseases is still unclear, even though BchE inhibition was considered to be directly associated with the side effects profile of the AchE-inhibiting drugs. Recently, it has been found that BchE is found in significantly higher quantities in Alzheimer's plaques than in plaques of normal age-related nondemented brains. The inclusion of cymserine, which is a very potent selective BchE inhibitor, in the clinical trials for treatment of AD indicate that BchE inhibition may be an effective tool for the treatment of AD and related dementias [7].

*Sarcococca* plants (Buxaceae) have been used since ancient times as folk remedies for ulcers, gastric disorders, and bacterial infections [8–10]. Recently we investigated the inhibition of electric eel acetylcholinesterase (EC 3.1.1.7) and horse serum butyrylcholinesterase (EC 3.1.1.8) by pregnane-type steroidal alkaloids of *Sarcococca saligna* of Pakistani origin. This includes five new alkaloids 3–7 and ten known alkaloids 8–17 isolated through extensive bioassay-guided fractionation and purification. These compounds were found to inhibit both enzymes in a concentration dependent fashion with the IC\textsubscript{50} values ranging from 5.21–227 µM against acetylcholinesterase and 1.89–38.36 µM against butyrylcholinesterase. The majority of these compounds 3, 5, 6, 8, 11, 12, 16, 17 were found to be non-competitive inhibitors of BchE, while compound 15 showed linear mixed inhibition of AchE. The K\textsubscript{i} values were found to be in the range of 2.65–216 µM against acetylcholinesterase and 1.63–26.33 µM against butyrylcholinesterase. Phytostigmine (IC\textsubscript{50} = 40 nM for AchE and IC\textsubscript{50} = 800 nM for BchE) was used as standard inhibitor. The structures of five new alkaloids, saligenamide-C (3), saligenamide-D (4), 2β-hydroxyepipachysamine-D (5), saligenamide-E (6), and saligenamide-F (7), were elucidated with the help of modern spectroscopic techniques [11].
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3  \( R^1 = \text{OH} \)  \( R^2 = \text{NH-Tigloyl} \)  \( R^3 = \text{OAc} \)  \( R^4 = \text{N(CH}_3)_2 \Delta^{14,15} \)

4  \( R^1 = \alpha-\text{OH} \)  \( R^2 = \text{NH-Tigloyl} \)  \( R^3 = \text{H} \)  \( R^4 = \text{N(CH}_3)_2 \Delta^{14,15} \& \Delta^{16,17} \)

5  \( R^1 = \text{OH} \)  \( R^2 = \text{NH-Benzyoyl} \)  \( R^3 = \text{H} \)  \( R^4 = \text{N(CH}_3)_2 \)

6  \( R^1 = \text{H} \)  \( R^2 = \text{NCH}_3\text{COCH}=\text{CCH}_3\text{CH(CH}_3)_2 \)  \( R^3 = \text{H} \)  \( R^4 = \text{N(CH}_3)_2 \Delta^{16,17} \)

7  \( R^1 = \text{H} \)  \( R^2 = \text{NCH}_3\text{COCH}=\text{CCH}_3\text{CH(CH}_3)_2 \)  \( R^3 = \text{H} \)  \( R^4 = \text{N(CH}_3)_2 \)

8  \( R^1 = \text{OH} \)  \( R^2 = \text{NH-Benzyoyl} \)  \( R^3 = \text{OAc} \)  \( R^4 = \text{N(CH}_3)_2 \)

9  \( R^1 = \text{OH} \)  \( R^2 = \text{NH-Tigloyl} \)  \( R^3 = \text{OAc} \)  \( R^4 = \text{N(CH}_3)_2 \)

10  \( R^1 = \text{H} \)  \( R^2 = \text{NHAc} \)  \( R^3 = \text{H} \)  \( R^4 = \text{N(CH}_3)_2 \)

11  \( R^1 = \text{H} \)  \( R^2 = \text{NHCH}_3 \)  \( R^3 = \text{H} \)  \( R^4 = \text{NCH}_3\text{Ac} \)

12  \( R^1 = \text{H} \)  \( R^2 = \text{CH}_3\text{N-\text{Cinnamoyl}} \)  \( R^3 = \text{H} \)  \( R^4 = \text{N(CH}_3)_2 \)

13  \( R^1 = \text{H} \)  \( R^2 = \text{NHCOCH}=\text{C(CH}_3)\text{CH(CH}_3)_2 \)  \( R^3 = \text{H} \)  \( R^4 = \text{N(CH}_3)_2 \)

14  \( R^1 = \text{H} \)  \( R^2 = \text{NH-Senecioy}l \)  \( R^3 = \text{OAc} \)  \( R^4 = \text{N(CH}_3)_2 \)

15  \( R = \text{NH-Benzyoyl} \)  \( \Delta^{2,3} \)

16  \( R = \text{NH-Tigloyl} \)  \( \Delta^{3,6} \)

17  \( R = \text{NH-Tigloyl} \)  \( \Delta^{3,6} \& \Delta^{14,15} \)

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New α-glucosidase inhibitors

*Ferula mongolica* Seud. (Umbelliferae), an important species of genus *Ferula*, grows in Bulgan Somone in the Hovd district of Mongolia. Ancient texts record its usage for abortive purposes, and the crude MeOH extracts of the plant were found to inhibit implantation of fertilized eggs in rats [12]. We have recently reported the α-glucosidase inhibitory activity of compounds isolated from this plant for the very first time. The enzyme α-glucosidase is involved in the hydrolytic cleavage of glucose from disaccharides and oligosaccharides. Inhibition of this enzyme prolongs the overall carbohydrate digestion time and thus reduces the rate of glucose absorption. It is effective in controlling postprandial hyperglycemia and avoids the late complications associated with type-II diabetes [13].

Our work on the MeOH extracts of roots of *F. mongolica* has resulted in the isolation and characterization of four new sesquiterpenoid derivatives 18–21 along with four known compounds 22–25. The compounds 18–25 were evaluated as inhibitors of α-glucosidase from yeast [14].

![Chemical Structures]

18 (IC$_{50}$ = 56.06 mM ± 2.56)

19 11α-Methyl (IC$_{50}$ = 32.21 mM ± 1.38)

23 11β-Methyl (IC$_{50}$ = 20.5 mM ± 1.62)

20 R = H, 11α-Methyl (IC$_{50}$ = 63.68 μM ± 2.686)

21 R = CH$_3$, 11α-Methyl (IC$_{50}$ = 79.87 μM ± 2.970)

24 R = CH$_3$, 11β-Methyl (inactive)

22 (22) R = CH$_3$ IC$_{50}$ = 82.41 mM ± 0.321

25 (25) R = H IC$_{50}$ = 9.31 mM ± 0.150

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New natural antioxidants

The plant *Pteleopsis hylodendron* Mildbr. belongs to the family Combretaceae, commonly found in the forest regions of West and Central Africa [15,16]. The genus *Pteleopsis* is represented in Africa by ten species but only *P. hylodendron* is found in Cameroon [16]. The aqueous decoction of the stem bark of *P. hylodendron* is used to treat sexually transmitted diseases, female fertility, liver and kidney disorders as well as dropsy. In the present study phytochemical investigation on *P. hylodendron* has resulted in the isolation and characterization of two new compounds 3,4-methylenedioxy-3'-O-methyl-4'-O-glycoside ellagic acid (26) and pteleoallagic acid derivative (27), along with three known compounds 28–30. Compounds 26 and 30 exhibited significant antioxidant activity by scavenging the DPPH radicals. Compound 30 was found to be the most effective of the two scavengers (IC$_{50}$ = 0.389 mM). 3-t-Butyl-4-hydroxy anisole was used as a standard antioxidant with IC$_{50}$ = 0.044 mM [17].

Further to the chemical basis of memory

During the 22nd IUPAC Symposium in Sao Carlos, Brazil (3–8 September 2000), one of us presented a new theory of memory based on specific patterns of hydrogen bonding [18]. While much work has been done on the process of long-term potentiation [19], the precise mechanism how thoughts may be stored in the brain and recalled has remained one of the most profound mysteries of nature and represents a “holy grail” of modern neuroscience. Clues as to how this may happen are provided by (a) the “instantaneous” process of memory storage, (b) the potential reversibility of the process (forgetfulness), and (c) the storage mechanism, which must rely on the biomolecules present in the brain that have the potential of providing the requisite “storage space”.

Taking these factors into consideration, the process of hydrogen bonding among glycoproteins offers an appropriate mechanism because of the facile nature of this process. Glycoproteins, which are present in the brain in abundance, are an attractive template as they offer a huge biological space for
information storage. The sugar molecules present on the protein backbone contain a large number of stereocenters bearing hydroxyl groups which can, by hydrogen bonding, freeze the sugar moieties in various conformations. This hydrogen bonding can occur both in an intramolecular and in an intermolecular fashion. Memories may be preserved in the brain by the freezing of sugar moieties located on the glycoprotein backbone, each thought corresponding to a certain unique set of assembly of such structures. The strength of the memory can then be equated with the number and cumulative strength of such hydrogen bonds, and the process of loss of memory may then be explained by the breaking of such bonds. The OH bonds can be protonated to provide positively charged entities, or deprotonated to afford negatively charged species, thereby allowing the process of hydrogen bonding to create a distribution of positive and negative charges on the glycoprotein templates. The disturbance of memory when electric shocks are administered to schizophrenic patients may occur by disturbance of this pattern of charges in the human brain. "Permanent" memories may involve the irreversible formation of such patterns by ethereal bonds between adjacent sugar moieties. The thought process may thus represent a spectacular and dynamically changing process involving the sensing of these patterns stored in the brain along with the formation of new patterns as one thought process leads to another.

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