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Bioactive natural products as a potential source of new pharmacophores. A theory of memory*

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Abstract: The plant kingdom offers a rich source of structural biodiversity in the form of a variety of natural products. Our work on new bioactive compounds from medicinal plants has led to the isolation and structure elucidation of a number of exciting new pharmacophores. Bioassay-guided fractionation has recently led to the discovery of a series of new acetylcholinesterase, urease and α-glucosidase inhibitors, antioxidants and other classes of bioactive compounds, which will be presented. A theory based on hydrogen bonding on the chemical basis of memory storage in the brain is presented.

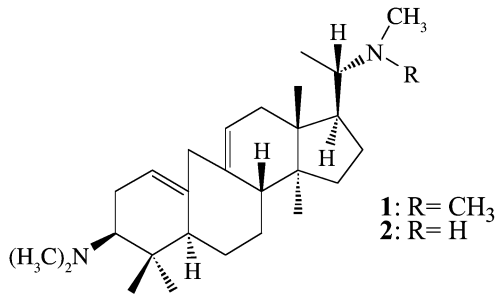
ENZYME INHIBITORS FROM NATURAL SOURCES

Using spectrophotometic enzyme inhibition assays against a number of clinically important enzymes, we have discovered new classes of enzyme inhibitors which are presented below.

Acetylcholinesterase inhibitors

The principal role of acetylcholinesterase is termination of the nerve impulse transmission at the cholinergic synapses by rapid hydrolysis of the neurotransmitter acetylcholine. Inhibition of acetylcholinesterase serves as a promising strategy for the treatment of Alzheimer's disease, senile dementia, ataxia, myasthenia gravis, and Parkinson's disease [1].

Bioassay-directed phytochemical investigations on a number of medicinal plants of Pakistan and Iran have led to the isolation of acetylcholinesterase inhibitors, **1** to **4**.



N_a,N_b-Dimethyl buxapapine (**1**) (IC_{50} 7.28 ± 0.06 μM) Source: *Buxus papillosa* C. K. Schineder
Buxamine-B (**2**) (IC_{50} 7.56 ± 0.008 μM) Source: *Buxus papillosa* and *B. hyrcana*

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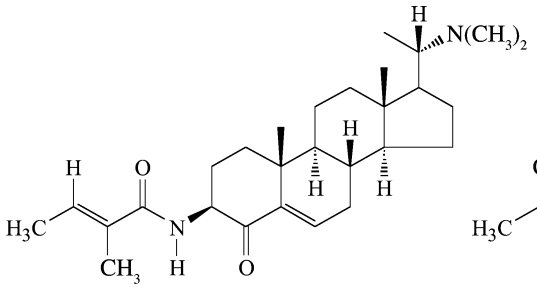
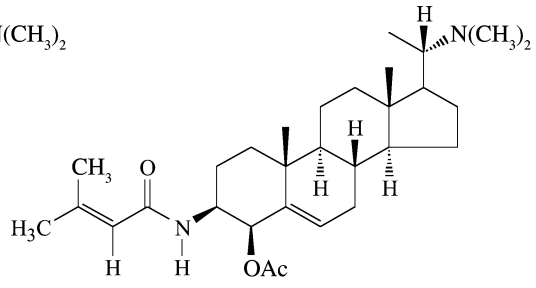


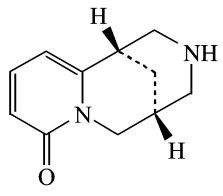
Sarsalignone (**3**) (IC_{50} 7.028 ± 0.007μM)
Source: *Sarcococca saligna* D. Don Muell.



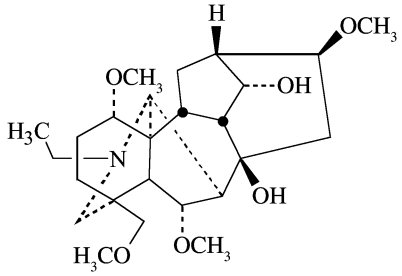
Vaganine (**4**) (IC_{50} 8.59 ± 0.155 μM)
Source: *S. saligna*

Urease inhibitors

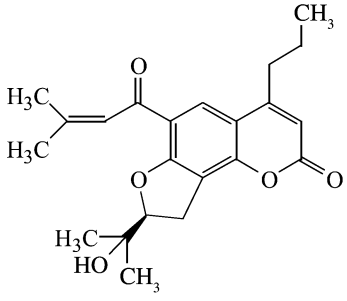
Urease occurs in many bacteria, several species of yeast, and a number of higher plants. The enzyme catalyzes the hydrolysis of urea into CO_2, H_2O, and N_2. Our research on the development of new and safer urease inhibitors developed from natural sources has led to the isolation of a number of new urease inhibitors [2]. The methanolic fractions of the roots of *Delphinium denudatum*, *Sophora mollis* (from Pakistan) and of stem bark of *Mammea africana* (collected from Cameroon) have yielded compounds **5**–**7**, which showed inhibitory activity against the enzyme urease comparable to standard inhibitors.



(-)-Cytisine (**5**) (IC_{50} = 0.92 ± 1.21 mM)
Source: *Sophora mollis* Grah



(+)-Chasmanine (**6**) (IC_{50} = 0.8 ± 0.03 mM)
Source: *Delphinium denudatum* Wall.

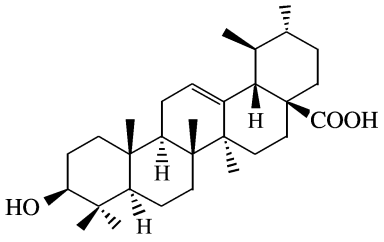


8,9-Dihydro-5-hydroxy-8-(1-hydroxy-1-oxobutyl)-4-phenyl-2H-furo[2,3-H]-1-benzopyran-2-one (**7**) (IC_{50} = 60 ± 0.192 μM) Source: *Mammea africana* Linn.

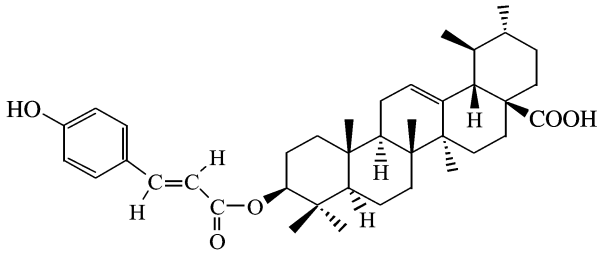
α-Glucosidase inhibitors

α-Glucosidase inhibitors delay digestion of complex carbohydrates by acting as competitive inhibitors of the intestinal enzyme α-glucosidase that hydrolyzes oligosaccharides into monosaccharides. α-Glucosidase inhibitors can hence be used to reduce the postprandial glycemic excursions and decrease postprandial hypoglycaemia [3].

We have identified a series of α-glucosidase inhibitors from a variety of natural sources. These include 3β-hydroxy-12-ursen-28-oic acid (**8**) and 3β-(4-hydroxy cinnamoyl)-12-ursen-28-oic acid (**9**) from the methanolic extracts of *Mimusops elengi* Linn. (Family Sapotaceae).



3β-Hydroxy-12-ursen-28-oic acid (**8**) (IC_{50} = 8.19 μM ± 0.123)

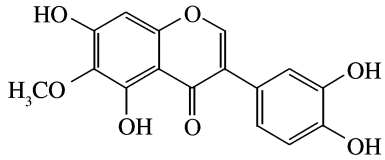


3β-(4'-Hydroxycinnamoyl)-12-ursen-28-oic acid (**9**) (IC_{50} = 11.65 μM ± 0.395)

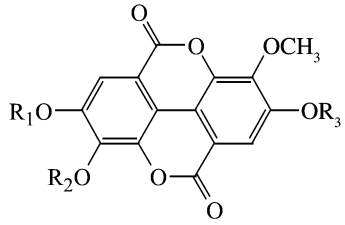
NATURAL ANTIOXIDANTS

Free radicals such as superoxide, hydroxyl, peroxyl, and alkoxyl are produced through oxidative processes within the body by environmental pollutants, e.g., cigarette smoke, pesticides, smog, UV radiation, etc. Once formed, free radicals can damage both the structure and function of a cell membrane in a chain reaction leading to degenerative diseases and conditions such as Alzheimer's, Parkinson's, and Hodgkin's disorders, aging processes, cataracts, acute liver toxicity, cardiovascular diseases, inflammation processes, and DNA damage that can lead to carcinogenesis. Oxidation is also responsible for the deterioration of various organic materials ranging from the biologically important materials (e.g., lipids, foods, and oils) to the industrially important ones (e.g., rubber and lubricants).

By using a battery of *in vitro* antioxidant assays (DPPH radical scavenging, xanthine oxidase inhibition, etc.) we have identified compounds **10–12** as potent antioxidants from natural sources [4].



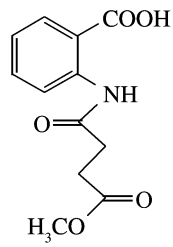
4-*H*-1-Benzopyran-4-one-3-(3',4'-dihydroxyphenyl)-5,7-dihydroxy-6-methoxy (**10**) (89.5% at mM) Source: *Iris bungei* Maxim.



R_1,R_2= CH_2; R_3= Glu: 3,4-Methylenedioxy-3'-*O*-4-glucoside ellagic acid (**11**)
(IC_{50} 1.096 ± 0.066 mM)
R_1= H;R_2= CH_3; R_3= H: 3,4-Methylenedioxy-3',3",3'",4""-tetra-*O*-methyl ellagic acid (**12**)
(IC_{50} 0.389 ± 0.02 mM)
Source: *Pteleopsis hylodandron* Mildbr.

SYNTHESIS OF CHYMOTRYPSIN INHIBITORS

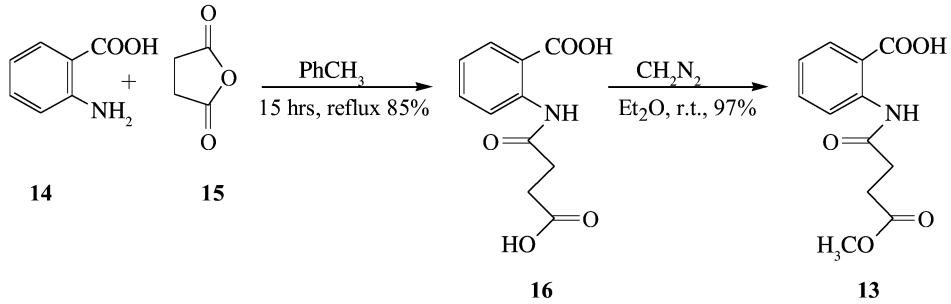
Methyl[2-methoxycarbonyl] succinanilate (**13**), a natural aromatic amide, was isolated for the first time by us from a dark brown alga, *Jolyna laminarioides* Guimaraes (syn. *Endrachne binghamiae*) [5,6].



13

The chymotrypsin inhibitory activity of **13** (IC_{50} 1.14 mM) has also been studied [5]. Chymotrypsins are serine proteases that play critical roles in several physiological processes including digestion, blood coagulation, complement activation, fibrinolysis, and reproduction [7]. Serine proteases are not only a physiological necessity, but can be of potential hazard if present in excessive quantities. For example, blood coagulation serine proteases are responsible for vascular clotting and cerebral and coronary disorders. The excessive activity of this enzyme can also cause skin diseases, glomerulonephritis, pancreatitis, and other disorders that can be controlled by its inhibition [8,9].

Compound **13** occurs in alga in very low (i.e., 0.0002%) concentration. We have therefore synthesized compound **13** for the detailed biological studies by a concise and convenient synthetic route that yielded the desired product **13** in two steps in 97% yield. The commercially available anthranilic acid (**14**) was condensed with succinic anhydride (**15**) by refluxing in dry toluene. The desired condensed product, succinyl anthranilic acid (**16**), was obtained in 85% yield which was then esterified with diazomethane in etheral solution at room temperature to afford **13** in an average yield of 97% (Scheme 1).



14 **15** **16** **13**

Scheme 1

CHEMICAL BASIS OF MEMORY

While a considerable amount of work has been done on the process of long-term potentiation [10], the basis of memory storage at the molecular level remains a mystery. The process of proton transfer plays a key role in many chemical and biological reactions [11–19]. Intramolecular proton transfer in the transition state can play a catalytic role in some reactions and may also influence the physical and chemical properties of compounds. A collective proton transfer can also be important in a number of biological processes such as vision and transportation in biological membrane. The mechanism of proton transfer in hydrogen bonds is related to the proton potential function which is a multidimensional surface depending on the stretching and bending modes of the hydrogen bonds and the hydrogen bond vibrations [16]. This theory of memory depends on the orchestrated formation and breakage of hydrogen bonds across the glycoprotein molecular surfaces in the human brain.

We present here a theory of memory storage based on hydrogen bonding. It is proposed that memory is stored as a specific pattern of hydrogen bonds and other noncovalent interactions involving the hydroxyl groups present in the sugar moieties in the glycoproteins other biochemicals in the human brain. The ease with which hydrogen bonds form and break, as well as the vast, almost infinite, variation in structural possibilities which present themselves through the conformational mobility of sugar moieties in the glycoproteins present in the human brain offer an excellent template for information storage. Hydrogen bonding can freeze sugar molecules in various conformations. The registration of an image in the human brain may correspond to the ability of the human brain to freeze an array of sugar molecules through a controlled and directed process of hydrogen bondings between sugar moieties in glycoproteins, thereby creating images and thought channels. This spectacular and fluid media of hydrogen bonds and other noncovalent interactions can hence provide the requisite powerful matrix for rapid memory storage. The strength of these interactions, represented by the cumulative sum of individual hydrogen bonds, may thus correspond to the strength of the memory storage. The formation of covalent bonds in long-term memory processes is also conceivable.

It is noticeable that hydrogen bonds play a crucial role in life processes. The conformational restriction which can be rapidly imposed through the process of hydrogen bonding in glycoproteins offers the basis of memory storage through the corresponding patterns which can result from such restrictions. The visual recognition of objects may involve the instantaneous formation of patterns in the human brain through the process of hydrogen bonding in the substrate glycoprotein structure. Strong memories may therefore correspond to a correspondingly larger number of intermolecular and intramolecular hydrogen bonds while weaker memories may correspond to a lesser number of such hydrogen bonds. The gradual fading away of memories with time may be due to the corresponding breaking of hydrogen bonds and the gradual destruction of the molecular imprints in the human brain.

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