Natural Toxins as Leads in Drug Discovery

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Pharmacological taming of natural toxins

A large number of plants, animals, and micro-organisms produce secondary metabolites showing toxic effects on other organisms. The biosynthetic routes for such natural toxins have in many cases been mapped out, but their physiological roles in the host organisms are frequently unknown. Many organisms do, however, produce such compounds as constituents of venoms which are secreted as defence or capture mechanisms.

Natural toxins belong to different classes of compounds, notably proteins, peptides, alkaloids, or amino acids, and there are numerous examples of protein toxins acting as enzymes. Toxins interact with a broad range of biomechanisms including receptors, ion channels, or enzymes, normally as antagonists or inhibitors, but certain low molecular weight toxins are capable of activating different types of receptors.

It is evident that structure and function of toxins during evolution have been optimized with regard to inactivation or killing of the target organisms, and with a few exceptions, notably botulinum and tetanus toxins (1), such compounds normally can not be used therapeutically. On the other hand, toxins interacting with biomechanisms, which play a key role in disease conditions, are of particular interest to medicinal chemists. In such cases, the challenge to medicinal chemists and molecular pharmacologists is to map out in detail the interaction of the toxin with the disease-related biomechanism. Based on mechanistic studies and the pharmacological profile of the toxin, the objective is to develop a strategy for "taming" of the toxin with the goal of designing compounds acting at the target biomechanism in a desired and controlled manner.

There are many outstanding examples of this kind of drug design or redesign projects, where medicinal chemists through rational and systematic structural modifications of natural toxins have developed analogues suitable for clinical studies and ultimately therapeutic use. In the following sections a few examples of drug design/discovery projects using natural toxins as leads will be described. Hopefully, these briefly described projects will focus the interest of medicinal chemists on the potential of natural products as leads and the fascination and prospects of "pharmacological taming" of toxins.

Angiotensin-converting enzyme inhibitors

Angiotensin-converting enzyme (ACE) is an enzyme belonging to the group of zinc carboxypeptidases. ACE plays a key role in the regulation of blood pressure. It converts the inactive decapeptide angiotensin I into angiotensin II, an octapeptide showing highly potent vasoconstrictor activity, and concomitantly transforms the endogenous

nonapeptide vasodilator, bradykinin into an inactive heptapeptide. In light of this effective dual regulatory mechanism, ACE was identified as a key target in the search for therapeutic agents for the treatment of patients suffering from hypertension.

Teprotide became the primary lead structure in a drug design project aiming at the development of low-molecular weight analogues showing potent ACE inhibition activity and desirable pharmacokinetic properties.

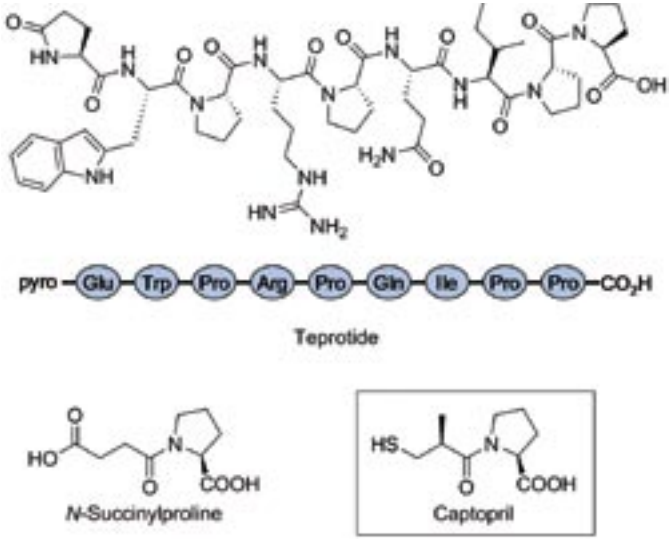

Figure 1

In the 1960s it was observed that the venom of the Brazilian pit viper, *Bothrops jararaca*, contains peptides capable of intensifying responses to bradykinin. These venomous peptides turned out to be inhibitors of kininase II, a bradykinin-inactivating enzyme, which subsequently was shown to be identical with ACE. Based on the sequence of one of the key venomous peptides, the peptide teprotide (Figure 1) was synthesized and shown also to be an inhibitor of ACE. The presence of proline residues and an N-terminal pyroglutamate unit, made teprotide relatively resistant to proteolytic decomposition in vivo, but it was not sufficiently stable for oral administration.

Two structural features were considered essential for activity, namely the presence of a C-terminal proline residue and a functional group capable of co-ordinating effectively with the zinc atom of the enzyme (2).

As a result of the systematic reduction of the molecular weight of teprotide following this strategy and by using bovine carboxypeptidase A as the assay enzyme, N-succinylproline was synthesized and shown to be a moderately potent ACE inhibitor. Using this derivative of proline as the secondary lead structure, captopril (Figure 1) was designed and shown to be a potent carboxypeptidase A and ACE

inhibitor, in which the mercapto group serves as an effective zinc co-ordinating group.

Captopril was marketed as an effective antihypertensive drug and was soon followed by other equally effective "prils".

Nicotinic acetylcholine receptor ligands

The neurotransmitter acetylcholine (ACh) operates through two heterogeneous classes of receptors, the ionotropic nicotinic ACh receptors (nAChRs) and the muscarinic metabotropic muscarinic ACh receptors (mAChRs). The nAChRs are ligand-gated ion channels (LGICs) containing 5 identical (homomeric) or different (heteromeric) protein subunits. So far, 17 different subunits have been cloned and characterized. Neuronal nAChRs are composed of α(α2-α10) and β(β2-β4) subunits, the heteromeric α4β2 and the homomeric α7 receptors being the most abundant (3).

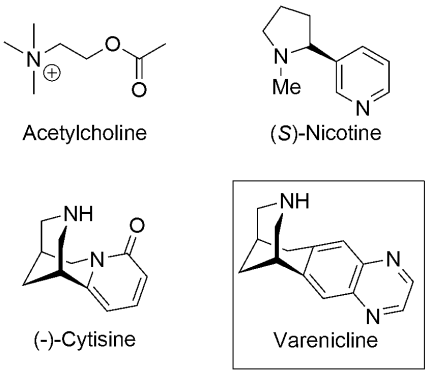

Figure 2

In addition to nicotine (Figure 2), which is an alkaloid isolated from *Nicotiana tabacum* and has given name to the class of nAChRs, a wide variety of nAChR ligands, primarily agonists, have been isolated from natural sources (4,5). This diversity of naturally occurring nAChR ligands probably reflects the sensitivity of nAChRs as targets in the sophisticated chemical warfare in nature. A number of these compounds have been used for the characterization and pharmacological studies of subtypes of nAChRs.

The nAChRs have become key targets for therapeutic approaches to treat pain, cognition disorders, depression, schizophrenia, and nicotine dependence. The last-mentioned condition appears to be mediated by central α4β2 nAChRs, and in a search for an agent for smoking cessation, Dr. J.W. Coe and co-workers focused on the discovery of a nAChR partial agonist showing optimally balanced nAChR agonist/antagonist effects (6). The naturally occurring nAChR ligand, (–)-cytisine (Figure 2) does possess the requested pharmacological profile but poor absorption and limited blood-brain barrier (BBB) penetration probably explain, why (–)-cytisine shows limited and unsatisfactory in vivo pharmacological effects (6). (–)-Cytisine was, however, selected as lead structure in this drug discovery project, and as a result of this rational and systematic approach, the partial α4β2 nAChR agonist, varenicline (Figure 2) has very recently been approved by the US FDA as a drug for smoking cessation (7).

***Amanita muscaria* constituents**

The fly agaric mushroom, *Amanita muscaria* produces muscarine (Figure 3), the classical mAChR agonist, which has played a key role in the pharmacological characterization of this class of AChRs. The presence of a quaternary

ammonium group prevents muscarine from penetrating the BBB, and this toxin exerts its effect through peripheral mAChRs, notably in the heart muscle.

Amanita muscaria also biosynthesizes the heterocyclic glutamate (Glu) bioisostere, ibotenic acid (8), in which the 3-isoxazolol unit mimics the distal carboxyl group of Glu (Figure 3). The dried mushroom also contains muscimol, a zwitterionic 3-isoxazolol bioisostere of γ-aminobutyric acid (GABA), which is formed by decarboxylation of ibotenic acid, a process which is catalyzed by the enzyme glutamate decarboxylase (GAD) (9) (Figure 3).

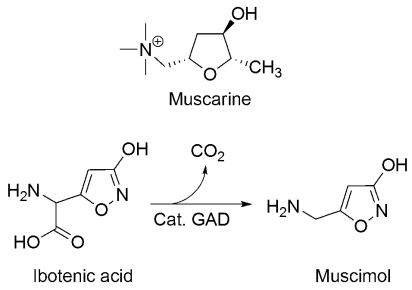

Figure 3

Both of these 3-isoxazolol amino acids are toxic. Ibotenic acid may be an ingeniously designed dual chemical war weapon, which per se acts as an excitotoxin at Glu receptors and also as a prodrug for muscimol, which is a powerful agonist at $GABA_A$ receptors. Both of these classes of receptors are widely distributed in vertebrates as well as invertebrates.

The use of muscimol as well as ibotenic acid as leads in drug discovery projects will be described in the two subsequent sections.

THIP (Gaboxadol), a clinically active $GABA_A$ agonist derived from muscimol

Muscimol, a conformationally restricted bioisostere of GABA (Figure 4), interacts nonselectively with all synaptic mechanisms operated by GABA. Muscimol shows very high affinity and efficacy at $GABA_A$ receptors, which are heteropentameric ionotropic receptors containing a variety of different subunits. Muscimol also interacts with neuronal and glial GABA uptake and is a substrate for GABA transaminase (GABA-T).

Muscimol exerts toxic effects in man, including hallucinations, but the mechanism(s) underlying these effects are not fully understood. It is unlikely that metabolites of muscimol can explain these toxic effects, which may be related to its powerful agonist activity at synaptic $GABA_A$ receptors. Thus, muscimol is likely to cause effective desensitization of these receptors, which mediate fast signal transmission, resulting in "functional antagonism" at $GABA_A$ receptors. These aspects are still under investigation.

Muscimol has been successfully used as a lead in a project aiming at "separation" of the effects of muscimol at $GABA_A$ receptors and GABA uptake and elimination of the substrate affinity for GABA-T. The key steps in this drug design project were the syntheses of the isomeric bicyclic analogues, THIP and THPO (Figure 4) (10). Whereas THIP specifically interacts with $GABA_A$ receptors, acting as a partial agonist at synaptic $GABA_A$ receptors, THPO shows no receptor affinity but interacts with neuronal and glial GABA uptake. Neither THIP nor THPO interact with GABA-T. By retrobioisosteric 3-isoxazolol/

carboxylate considerations, THIP and THPO were converted into the specific amino acid $GABA_A$ agonist, isoguvacine, and GABA uptake inhibitor, nipecotic acid, respectively (Figure 4) (11).

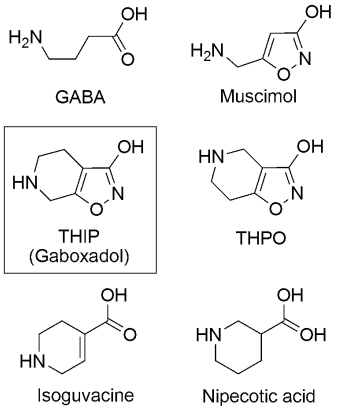

Figure 4

Clinical studies in the early 1980s disclosed potent nonopioid analgesic effects of THIP, but this activity was accompanied by "sedative effects". These latter properties have more recently been studied by Dr. M. Lancel and shown to reflect unique hypnotic effects in man (11). THIP has recently been shown to be a "superagonist" at extrasynaptic $GABA_A$ receptors, which are insensitive to the GABA-modulatory agents, the benzodiazepines (BZDs). Importantly, this class of $GABA_A$ receptors, containing $\alpha 4\beta\delta$ subunits, desensitize very slowly following agonist activation.

Under the company name, Gaboxadol, THIP is now subject to advanced phase III clinical studies as a non-BZD hypnotic agent capable of "re-establishing a normal sleep architecture" (11).

Ibotenic acid, an exitotoxic bioisostere of glutamate

Ibotenic acid is a 3-isoxazolol bioisostere of the central excitatory neurotransmitter Glu, which mediates fast signal transmission through different classes of heterotetrameric ionotropic receptors named NMDA, AMPA, and kainic acid (KA) receptors and a heterogeneous class of metabotropic Glu receptors.

Ibotenic acid, which interacts with different affinity and efficacy with all of these receptor subtypes, is a widely used experimental exitotoxin in neurobiological research, but chemical and stereochemical instability (9) and lack of receptor selectivity limit the utility of ibotenic acid.

Ibotenic acid has been extensively used as a lead for the design of subtype-specific Glu receptor ligands. AMPA (Figure 5) (12) is a chemically and stereochemically stable analogue of ibotenic acid, and AMPA was shown to be a selective and highly potent agonist at the group of ionotropic Glu receptors, iGluR1-4, collectively named AMPA receptors (12). AMPA shows little or no effect at NMDA or KA receptors, the latter of which comprise 3 receptors, iGluR5-7.

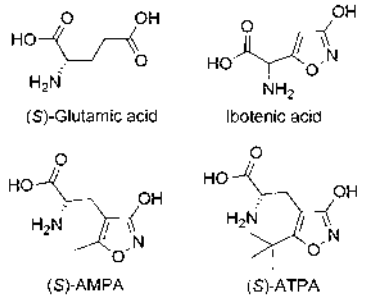

Figure 5

Replacement of the methyl group of AMPA by a tert-butyl group quite dramatically changes the pharmacological profile. Thus, ATPA (Figure 5) shows very limited agonist effect at AMPA receptors, but ATPA has been shown to be a highly potent subtype-selective iGluR5 agonist and is now a standard agonist for studies of this therapeutically interesting Glu receptor (13).

A large number of analogues of AMPA have been synthesized and pharmacologically characterized. Analogues containing heterocyclic substituents in the 5-position of the 3-isoxazolol ring of AMPA have been particularly useful for topographic studies of AMPA receptors, including studies of co-crystallized AMPA ligand/receptor binding domain (13).

Thapsigargin, a unique lead in anticancer drug design

The sesquiterpene lactone, thapsigargin (Tg) (Figure 6) was isolated in the late 1970s from the medicinal plant, *Thapsia garganica*. Tg is an effective inhibitor of the sarco/endoplasmatic reticulum Ca-ATPase (SERCA) causing a rise in the cytosolic calcium level, which eventually leads to cell death.

Tg exerts apoptotic activity in slowly proliferating cells such as prostate cancer cells and offers a unique possibility for designing chemotherapeutics for a broad spectrum of cancer diseases. The drawback of Tg in this regard is, however, that the target of this toxin is the SERCA pump, which is ubiquitous and essential for all cell types. The attempts to target Tg towards prostate cancer cells, was based on the observation that prostate cancer cells excrete a proteolytic enzyme, prostate specific antigen (PSA), showing a unique substrate specificity. This specificity was utilized in the design of a prodrug of Tg, sensitive to PSA, capable of targeting specifically prostate cancer cells.

The initial step in this prodrug synthesis was hydrolysis of the butyrate ester bond of Tg (Figure 6), followed by introduction of a 12-aminodecanoate group. This enabled coupling of an appropriate peptide sequence to the terminal amino group to give the prodrug shown in Figure 6.

PSA efficiently cleaves the peptide sequence, and the remaining part of the molecule penetrates the cell membrane and the cytosol and blocks the SERCA pump. Administration of the prodrug to mice,

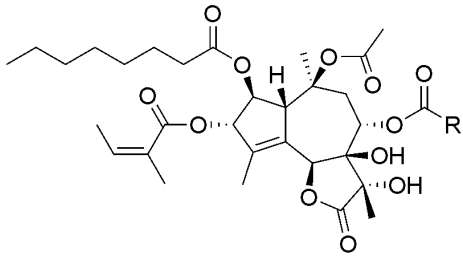

Figure 6

Thapsigargin R = *n*-propyl
Thapsigargin prodrug R = $(CH_2)_{11}$-NH-Leu-Gln-Leu-Lys-Ser-Ser-His-CON

inoculated with prostate cancer tumours, prevented development of the tumours (15).

The described conversion of the naturally occurring compound, Tg showing pronounced general cell toxicity into an effective tissue-specific anticancer experimental drug is a promising example of pharmacological taming and targeting of toxins.

Epilogue

Limitation of space in this minireview has only made it possible to incorporate a very few examples of using natural toxins in drug design and discovery. Several other successful projects in this colourful and scientifically challenging area of medicinal chemistry had deserved to be described, and the number of as yet unexploited exciting natural products is essentially unlimited. Hopefully, intelligent and intuitive exploration of natural toxins will play an increasing and productive role in future drug discovery projects.

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