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Monitor: molecules and profiles

Monitor provides an insight into the latest developments in drug discovery through brief synopses of recent presentations and publications together with expert commentaries on the latest technologies. There are two sections: *Molecules* summarizes the chemistry and the pharmacological significance and biological relevance of new molecules reported in the literature and on the conference scene; *Profiles* offers commentary on promising lines of research, emerging molecular targets, novel technology, advances in synthetic and separation techniques and legislative issues.

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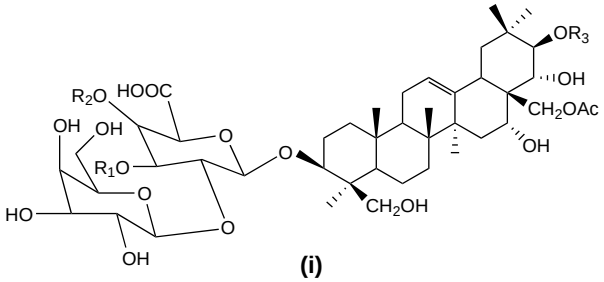
Molecules

Novel triterpenoid saponins with insulin-like activity

In their search for new bioactive natural compounds from Chinese plants distributed in the Yunnan Province, Sakurai and collaborators found that the ethanol extract of the roots of *Aesculus assamica* Griff. showed strong insulin-like activity against both rat and 3T3-L1 adipocytes [1]. These findings prompted them to isolate and characterize the active compound from the plant, which resulted in the identification of two novel triterpenoid saponins, assamicin I and assamicin II (**i**). From an ethanolic extract of the roots (1126.7 mg) of *Aesculus assamica* Griff., 7.8 mg and 2.4 mg of assamicin I and II were obtained, respectively. Their structures were determined on the basis of HR-FABMS and NMR spectra.

The compounds were tested for their insulin-like activity in rats or 3T3-L1 adipocytes. It is well known that insulin inhibits the release of free fatty acids from epinephrine-treated rat adipocytes [2]; Assamicin I and II almost completely inhibited the release of free fatty acids at concentrations of 100 and 25 μg ml^{-1}, respectively. In addition, like insulin, they enhanced glucose uptake into 3T3-L1 adypocytes. In particular, when the cells were incubated in a medium containing [^{3}H]-2-deoxyglucose, the uptake of the radioisotope into the cells was enhanced by 2.5-fold and 3.5-fold after addition of 25 μg ml^{-1} assamicin I and II, respectively, compared with controls.

Alhough the biological activity of saponins contained in *Aesculus* species has been previously reported [3], assamicin I and II are the first saponins whose insulin-like activity has been shown. Work to clarify the structures and biological activity of other saponin constituents of *Aesculus assamica* Griff. is in progress.



(i)

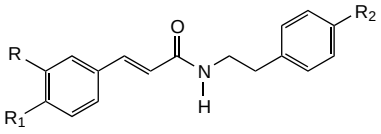
Assamicin I: R_1 = α-Rhamnopyranosyl
Assamicin II: R_1 = α-Rhamnopyranosyl
R_2 = H
R_2 = H
R_3 = Angeloyl
R_3 = 3,4-di-*O*-Angeloyl-6-deoxy-β-glucopyranosyl

1 Sakurai, T. *et al.* (2002) Assamicin I and II, novel triterpenoid saponins with insulin-like activity from *Aesculus assamica* Griff. *Bioorg. Med. Chem. Lett.* 12, 807–810
2 Nakai, M. *et al.* (1995) Mechanism on insulin-like action of vanadyl sulfate: studies on interaction between rat adipocytes and vanadium compounds. *Biol. Pharm. Bull.* 18, 719–725
3 Yang, X.W. *et al.* (1999) Anti-HIV-1 protease triterpenoid saponins from the seeds of *Aesculus chinensis*. *J. Nat. Prod.* 62, 1510–1513

α-Glucosidase inhibitors from *Cuscuta reflexa*

The genus *Cuscuta* belongs to the family *Convolvulaceae* and comprises three species. All of them are leafless, twining parasites with slender yellowish stems. *Cuscuta reflexa* ROXB. has been known for some time as being a depurant and an evacuant (purgative). Long-chain esters of the oleane series and sterol glycosides have been extracted from this plant [4]. The methanolic extract and its subsequent ethyl acetate fraction showed significant inhibition against α-glucosidase, with no activity on several other enzymes (e.g. thrombin, β-glucuronidase). Inhibition of α-glucosidase prolongs the absorption time of glucose in the blood after a meal. Thus, post-prandial hyperglycaemia can be decreased and can enable non-insulin dependent diabetes mellitus (NIDDM) to be managed [5].

PII: S1359-6446(02)02382-6



(ii) R = OH, R_1 = OH, R_2 = OCH_3
(iii) R = OCH_3, R_1 = OH, R_2 = $(CH_2)_3CH_3$

On this basis, Anis and collaborators carried out further studies on this plant [6]. They isolated two new compounds (**ii** and **iii**), together with five other known derivatives, which had never been seen before in this plant species. All of the compounds were isolated from the hexane insoluble residue of the ethyl acetate fraction, obtained by fractionation of the methanolic extract followed by repeated column chromatography over silica gel. The molecular formula of compounds **ii** and **iii** was determined by high resolution MS and then confirmed by fast atom bombardment-MS (FAB-MS). Their relative structures were determined by infrared, ultraviolet, ^{1}H-NMR, and distortionless enhancement by polarization transfer (DEPT)-^{13}C-NMR spectra. Heteronuclear multiple bond connectivity (HMBC) experiments enabled the researchers to establish the position of the methoxy group.

All of the isolated compounds were tested for their inhibition of α-glucosidase type VI using 1-deoxynojirimycin as control (IC_{50} = 0.3 mM). Compounds **ii** and **iii** had IC_{50} values of 103.58 μM and 45.67 μM, respectively. By contrast, they proved to be inactive against thrombin and β-glucuronidase.

4 Anis, E. *et al.* (1999) Sterols and sterol glycosides from *Cuscuta reflexa*. *Nat. Prod. Sci.* 5, 124–126
5 Matsui, T. *et al.* (1996) *In vitro* survey of α-glucosidase inhibitory food components. *Biosci. Biotech. Biochem.* 60, 2019–2022
6 Anis, E. *et al.* (2002) α-Glucosidase inhibitory constituents from *Cuscuta reflexa*. *Chem. Pharm. Bull.* 50, 112–114

Antioxidant activity of novel indole derivatives

Free radicals and reactive oxygen species (ROSs) are implicated in a variety of pathophysiological events, including inflammation, cancer, myocardial infarction, arthritis, and neurodegenerative disorders [7]. ROSs have been suggested to play an important role in the pathophysiology of myocardial reperfusion injury.

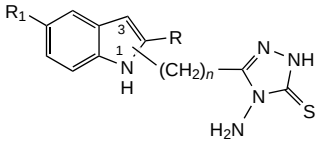
During post-ischemic reperfusion, oxygen enters the cell at high tension (increased flow) combining with hypoxanthine in the presence of xanthine oxidase. This results in the generation of superoxide anions and other free radicals that cause widespread lipid peroxidation and damage to cellular membranes [8]. Therefore, considerable interest is focused on the investigation of the antioxidant properties of pharmacologically active compounds.

The development of hybrid molecules through the combination of different pharmacophores in one frame can lead to compounds with interesting pharmacological profiles. In particular, indole derivatives bearing a thiosemicarbazide moiety, as well as their cyclic triazole analogues, were shown to possess significant antioxidant properties [9]. However, these were poorly soluble because of the presence of highly hydrophobic substituents on the thiosemicarbazide or triazole moiety. This prevented their further *in vivo* investigation.

To circumvent these unfavourable characteristics, the same group has now synthesized two series of less lipophilic indole–triazole derivatives: series 1 with the side-chain at position 1 and series 2 with the side-chain at position 3 [10]. All of the compounds were investigated for their antioxidant and potential anti-ischemic activity.

In studies of *in vitro* non-enzymatic lipid peroxidation of rat hepatic microsomal membranes (by measuring the formation of 2-thiobarbituric acid reactive substances), all of the compounds inhibited lipid peroxidation by >97% at a concentration of 0.25 mM. The only exception was compound **iv** from series 2, which inhibited peroxidation by 73%. At a concentration of 0.05 mM, inhibition was less potent for compounds in series 2. By contrast, compounds in series 1 (with the exception of **v**), retained their high inhibitory potency. In the same test the DL-(α)-tocopherol acetate, used as reference drug, is unable to inhibit lipid peroxidation at a concentration of 0.5 mM.

Compounds **vi**, **vii** and **viii** (from series 1) were then tested *in vivo* to monitor myocardial malondialdehyde (MDA) levels, as an indicator of lipid peroxidation during ischemia–reperfusion. Based on preliminary toxicity studies, the dose was set at 100 μm kg^{-1}. All of the compounds showed a statistically significant reduction of MDA levels compared with the controls.



(iv) R = H, R_1 = H, *n* = 2
(v) R = H, R_1 = Br, *n* = 1
(vi) R = H, R_1 = H, *n* = 1
(vii) R = H, R_1 = Cl, *n* = 1
(viii) R = H, R_1 = NO_2, *n* = 1

7 Halliwell, B. *et al.* eds (1998) Free Radicals in Biology and Medicine. (3rd edn), Oxford University Press
8 Cuzzocrea, S. *et al.* (2001) Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. *Pharmacol. Rev.* 53, 135–159
9 Andreadou, I. *et al.* (2000) Significant antioxidant properties of indole containing derivatives of thiosemicabazide and their cyclic 1,2,4-triazole analogs. *Res. Commun. Biochem. Cell Mol. Biol.* 4, 269–275
10 Andreadou, I. *et al.* (2002) Antioxidant activity of novel indole derivatives and protection of the myocardial damage in rabbits. *Chem. Pharm. Bull.* 50, 165–168

New antimalarial analogues of quinoline alkaloids

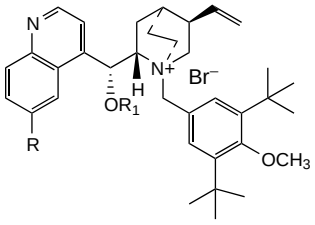
Malaria, one of the most prevalent infectious diseases in the world, is caused by several species of the genus *Plasmodium*, a protozoan parasite that is transmitted to humans by *Anopheles* mosquitoes.

P. falciparum is the most virulent human malaria parasite and is responsible for more deaths in Africa than any other parasitic disease [11]. The incidence of malaria is still increasing, largely because of the development of resistance to available drugs and insecticides by parasites and mosquitoes, respectively [12]. Thus, there is a great need for new antimalarial agents and insecticides, ideally with different modes of action and chemical structures than currently used compounds.

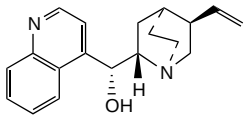
Historically, plant secondary metabolites have played an important role as antimalarial agents. Quinine, a quinoline alkaloid derived from the bark of *Cinchona ledgeriana* Moens ex Trimen, is the oldest known natural antimalarial drug. [13] Lee and co-workers [14] have recently evaluated four naturally occurring quinoline alkaloids (OSL-1–OSL-4) and twelve synthetic analogues (OSL-5–OSL-16) for *in vitro* antimalarial activity against *P. falciparum.* The four natural quinolines showed strong antimalarial activity as determined by an assay of inhibition of ^{3}H-hypoxanthine uptake. By contrast, the synthetic analogues showed relatively lower antimalarial activity compared with their natural parent compounds or chloroquine (IC_{50} = 190 nM).

However, increased antimalarial activity of several synthetic quinolines was seen with an assay that measures the formation of new ring-stage parasites after 48 h of incubation with inhibitors. Two cinchonidine analogues (**ix**, OSL-5, IC_{50} = 160 nM; **x**, OSL-7, IC_{50} = 63 nM) and one quinine analogue (**xi**, OSL-14, IC_{50} = 99 nM) showed potent antimalarial activity, compared with their parent compounds, cinchonidine (**xii**, OSL-1, IC_{50} = 290 nM) and quinine (**xiii**, OSL-4, IC_{50} = 120 nM).

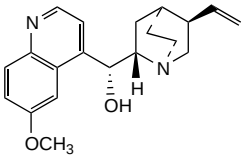
These findings show that the synthetic compounds are active, but not as rapidly potent as the parent compounds. They could represent a useful alternative to currently used drugs in malarial chemotherapy.



(ix) R = H, R_1= H
(x) R = H, R_1= $CH_2C_6H_5$
(xi) R = OCH_3, R_1= H



(xii)



(xiii)

11 Olliaro, P. *et al.* (1996) Malaria, the submerged disease. *J. Am. Med. Assoc.* 275, 230–233

12 Penilla, R.P. *et al.* (1998) Resistance management strategies in malaria vector mosquito control. Baseline data for a large-scale field trial against *Anopheles albimanus* in Mexico. *Med. Vet. Entomol.* 12, 217–233

13 Karbwang, J. *et al.* (1990) Clinical pharmacokinetics of mefloquine. *Clin. Pharmacokinet.* 19, 264–269

14 Park, B.-S. *et al.* (2002) Synthesis and evaluation of new antimalarial analogues of quinoline alkaloids derived from *Cinchona ledgeriana* Moens ex Trimen. *Biorg. Med. Chem. Lett.* 12, 1351–1355

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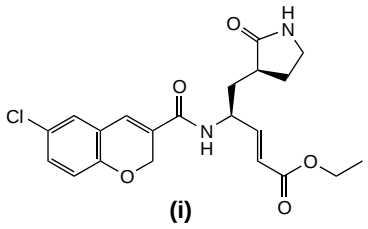
Combinatorial chemistry

Inhibitors of human rhinovirus 3C protease

The human rhinoviruses (HRVs) are members of the picornavirus family and are the most prevalent cause of the common cold. More than 100 serotypes of the virus exist, and so immunisation is an impractical approach to prevent the infection. Rhinoviruses contain a positive-sense strand of RNA that is translated to a large polyprotein in infected cells. This polyprotein is cleaved by viral proteases to yield mature viral enzymes and structural proteins. The 3C protease (3CP) does the majority of the proteolytic processing. Inhibition of this viral protease by a small-molecule agent should stop viral replication and thus control the extent of infection. Small-molecule inhibitors of 3CP have been documented in the literature, such as the isatins and homophthalimides, but these all suffer from problems such as cellular toxicity and modest antiviral activity.

Low molecular weight non-peptidic HRV 3CP inhibitors have been synthesized [1]. Structure based design was used to highlight a set of carboxylic acids that formed part of molecules which were able to dock well into a model of the target protein. A small library of compounds was synthesized, screened and chosen as hits based on their rate of inactivation of the HRV-14 3CP serotype. One of the most potent compounds found was **i** which possessed a K_{obs} value of 19,700 $M^{-1}s^{-1}$ against HRV-14. The use of parallel synthesis guided with information from target structural data has led to the discovery of a new class of HRV-3CP inhibitors.



(i)

1 Johnson, T.O. *et al.* (2002) Structure-based design of a parallel synthetic array directed toward the discovery of irreversible inhibitors of human rhinovirus 3C protease. *J. Med. Chem.* 45, 2016–2023

Sodium channel blockers

It is known that several classes of guanidines act as ion-channel blockers, including *N*-methyl-D-aspartate (NMDA)-activated

1359-6446/02/$ – see front matter PII: S1359-6446(02)02381-4