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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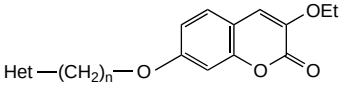
Molecules

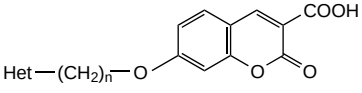
Novel coumarin derivatives as lipid lowering agents

Atherosclerotic cardiovascular disease is the leading cause of death in developed countries. A variety of independent risk factors contribute to coronary artery disease (CAD), in particular, high cholesterol and triglyceride concentrations [1,2]. In addition, oxidative stress has been implicated in the pathogenesis of CAD [3]. Therefore, antioxidative agents, such as coumarins, could represent a potential therapeutic target. On these bases, Madhavan and collaborators have recently reported [4] on several coumarin 3-ethoxy- (**i**, **a–i**) and 3-carboxyl-derivatives (**ii**, **a,c**), where different heterocycles are attached to the coumarin moiety through a one or two carbon chain.

The plasma triglyceride lowering activity of the compounds was tested on Swiss Albino mice, at 3 mg kg^{-1} day *per os*, according to a previously reported procedure [5]. The most potent compound was **ic** [Het = 1(2H)phthalazinone, n = 2], which showed a 45% reduction in triglycerides. Compounds **ia** (Het = 2-phenyl-5-methyl oxazole; n = 2) and **ig** (Het = 1H-indole; n = 2) showed values of 25% and 27% reduction in triglycerides, respectively. None of the other compounds had any significant activity. In the same model, the reference standard, fenofibrate, showed a 36% reduction in plasma triglyceride levels at 30 mg kg^{-1} day^{-1}.



(i) a–i



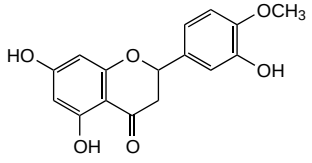
(ii) a,c

1 La Rosa, J.C. (1991) Cholesterol screening. The saga continues. *Circulation* 83, 1456–1457
2 Staels, B. *et al.* (1998) Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation* 98, 2088–2093
3 Balz, F. (1994) Natural antioxidants in human health and disease. Academic Press, San Diego
4 Madhavan, G. R. *et al.* (2003) Novel coumarin derivatives of heterocyclic compounds as lipid-lowering agents. *Bioorg. Med. Chem. Lett.* 13, 2547–2551
5 Reddy, K.A. *et al.* (1999) Novel antidiabetic and hypolipidemic agents. 5. Hydroxyl versus benzyloxy containing chroman derivatives. *J. Med. Chem.* 42, 3265–3278

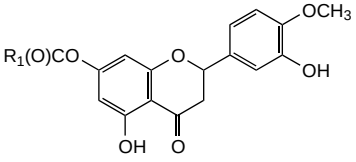
Hesperetin derivatives as hypocholesterolemic agents

Flavonoids, whose daily intake in humans is estimated to be 2–3 g in cases of a high dietary intake of herbs, show a broad range of biological activities [6]. In the course of screening of several flavonoids, with the intention of identifying novel hypocholesterolemic agents, hesperetin (**iii**; the aglycon of hesperidin) showed a significant cholesterol-lowering activity in cholesterol fed mice [7].

On these bases, Lee and collaborators performed a thorough SAR study on this substrate. In particular, they reasoned



(iii)



(iv) a–e

that the introduction of a lipophilic group would be beneficial. Therefore, they synthesized and tested two series of compounds (**iv**, **a–e**; **v**, **a–e**) possessing a long alkyl chain linked to the 7-hydroxyl position of hesperetin by an ester or ether functionality [8].

The compounds were tested for their cholesterol lowering activity in high cholesterol-fed C59BL/6J mice. The plasma total cholesterol levels were measured after feeding a high cholesterol diet supplemented with 0.05% (w/w) of the test compound for 10 days. All the derivatives, with the exception of **iiia** and **iva** ($R_1 = CH_3$ and $R_2 = C_2H_5$, respectively), showed a better activity than hesperetin.

The data clearly indicates that compounds possessing a long alkyl chain were better than those with short chains. In addition, ether derivatives were generally more potent than ester derivatives. Finally, it was found that unsaturation in the lipophilic chain significantly enhanced activity. The most interesting compounds

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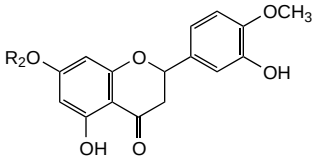
were **vb** ($R_2 = C_{12}H_{25}$) and **ve** ($R_2 = Z$-$C_8H_{17}CH{=}CHC_8H_{16}$), which at the end of the experiment (day 10) showed values of total cholesterol (mg dl^{-1}) of 189 ± 16 and 192 ± 18, respectively. For the control, the corresponding value was 223 ± 29. Further studies on these compounds are in progress.



(v) a–e

6 Hertog, M.G.L. (1998). Flavonols in wine and tea and prevention of coronary heart disease. *Colloques - Institut National de la Recherche Agronomique* 87(Polyphenols 96), 117–131

7 Lee, S.H. *et al.* (1999) Cholesterol-lowering activity of naringenin via inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase and acyl coenzyme A:cholesterol acyltransferase in rats. *Ann. Nutr. Metab.* 43, 173–180

8 Jeong, T.-S. *et al.* (2003) Hypocholesterolemic activity of Hesperetin derivatives. *Bioorg. Med. Chem. Lett.* 13, 2663–2665

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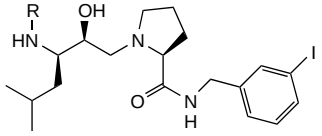
Combinatorial chemistry

Protease inhibitors

Human T-cell leukaemia virus type-1 (HTLV-1), isolated in the 1980s, was the first exogeneous retrovirus shown to be associated with adult T-cell leukaemia and several other chronic diseases. The genome of HTLV-1 is approximately 9 kb in length and contains several open reading frames that encode *gag, pol, env* and regulatory proteins. As in other retroviruses, such as γHIV, HTLV-1 proteins are initially translated as large precursor polyproteins that undergo proteolytic processing by the viral protease during virion assembly and maturation. The protease is an aspartic protease that is encoded in a separate reading frame overlapping the *gag/pol* coding sequence of the virus genome. The protease itself is autoprocessed from a precursor protein and thus the function of the mature 1256-amino acid-long HTLV-1 protease is crucial for virus replication.

Although several studies have reported expression of the stabilized HTLV-1 protease in *Escherichia coli*, as well as its kinetic characterization, the biochemical properties of the HTLV-1 protease have not been well-described because of difficulties obtaining sizeable amounts of the protease. Research has led to the first solid-phase synthesis of HTLV-1 protease inhibitors containing the transition-state isostere mimetic and evaluation of the stereo-SAR of the inhibitors [1].

A small library of four compounds was synthesized on MBHA resin (Novabiochem). Several potent analogues were obtained, with compound **i** being one of the most potent, possessing a K_i of 38 nM – a 250-fold increase in potency over pepstatin. This work is the first solid-phase synthesis of HLTV-1 protease inhibitor containing a hydroxyethylamine isostere backbone. Several potent inhibitors were generated and further work in this area is warranted.

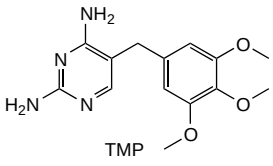


(i) R = H-Pro-Val-Ile

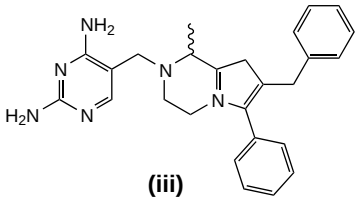
1 Akaji, K. *et. al.* (2003) Solid-phase synthesis of HTLV-1 protease inhibitors containing hydroxyethylamine dipeptide isostere. *J. Org. Chem.* 68 4755–4763

Dihydrofolate reductase inhibitors

The spread of antibiotic resistance has reached alarming proportions in some species, and one of the most worrying trends is the increasing incidence of methicillin resistant *Staphylococcus aureus* (MRSA) in hospitals and multiresistant *Streptococcus pneumoniae* in the community. Therefore, there is an urgent need for effective antibacterial agents to treat infections caused by these organisms.



(ii)



(iii)

The enzyme dihydrofolate reductase (DHFR) has been established in the clinic as a proven target for chemotherapy. The DHFR inhibitor trimethoprim (TMP; compound **ii**), was introduced primarily for the treatment of community-acquired infections and urinary tract infections, with emphasis on Gram-negative pathogens. The enzyme remains an under-exploited target in the antibacterial field, and no optimization of inhibitors against Gram-positive pathogens has been performed. Recent work has been conducted that is aimed at improving the pharmacokinetic properties of DHFR inhibitors [2].

A library of 1392 compounds was synthesized in solution. The compounds were evaluated for inhibition of human DHFR and the bacterial enzymes from TMP-sensitive *S. aureus* (AT25923) and TMP-resistant *S. pneumoniae* (1/1). Several potent inhibitors were found, with one of the most potent being compound **iii**, possessing IC_{50} values of 42 nM against *S. aureus* and 550 nM against *S. pneumoniae.* This work has generated rapid SAR and identified novel and potent inhibitors of DHFR and further work in this area is warranted.

2 Wyss, P. C. *et. al.* (2003) Novel dihydrofolate reductase inhibitors. Structure-based versus diversity-based library design and high-throughput synthesis and screening. *J. Med. Chem.*. 46, 2304–2312

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