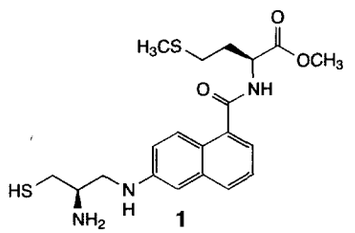
Monitor: molecules and profiles

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.

Farnesyl protein transferase inhibitors

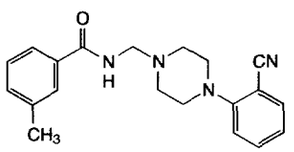
As described previously, farnesyl protein transferase inhibitors may have use as chemotherapeutic agents by preventing the association of the Ras p21 proteins with the cell membrane, thereby blocking the signal transduction pathway leading to unregulated cell proliferation and malignant transformation in various carcinoma cells [*Drug Discovery Today* (1997) 2, 123]. A group from Rhône-Poulenc Rorer (Vitry-sur-Seine, France) have described molecular modelling studies that have led to the identification of novel, conformationally extended naphthalene-based inhibitors of farnesyl protein transferase exemplified by **1** (RPR114334) [Burns, C.J. *et al. J. Med. Chem.* (1997) 40, 1763–1767]. These compounds demonstrated potent cellular activity against Ras processing and inhibited the anchorage-independent cell growth of several cell lines. Compound **1** was also shown to prevent the ability of activated Ha-*ras* and Ki-*ras* transformed cell lines to form colonies in soft agar.



1

Dopamine D_4 agonists

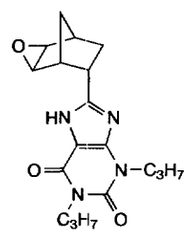
A recent paper from a group from Parke-Davis Pharmaceutical Research (Ann Arbor, MI, USA) claims to describe the first selective D_4 agonist reported in the literature [Glase, S.A. *et al. J. Med. Chem.* (1997) 40, 1771–1772]. The cellular uptake of [^{3}H]-thymidine by D_4-transfected CHO pro-5 cells was used to evaluate the agonist activity of a series of [(4-phenylpiperazinyl)methyl]benzamides. Structural optimization led to the identification of compound **2**, an agonist with high affinity for the D_4 receptor (K_i = 8.7 nM) and >300-fold selectivity for the D_4 receptor over the D_3 receptor and >400-fold selectivity for the D_4 receptor over the D_2 receptor. This compound may be a useful tool for evaluating the physiological and pathological roles of the D_4 receptor.



2

Selective A_1-adenosine antagonist

Pfister, J.R. and coworkers [*J. Med. Chem.* (1997) 40, 1773–1778] have described the synthesis and evaluation of the individual enantiomers of the potent and highly selective racemic A_1-adenosine antagonist 1,3-dipropyl-8-[2-(5,6-epoxynorbornyl)]xanthine. The binding affinities of the individual enantiomers and the racemate were compared using guinea pig, rat and cloned human A_1- and A_{2A}-adenosine receptor subtypes. Although the binding affinities of the enantiomers were similar for the human and guinea pig A_1-adenosine receptor, the *S*-enantiomer (**3**) appeared to bind approximately eight times more strongly to the rat receptors. The *S*-enantiomer was also shown to be more selective than the *R*-enantiomer for the A_1-adenosine over the A_{2A}-adenosine receptor subtype in both the rat and human. Intravenous administration of both enantiomers to saline-loaded rats increased urine and sodium output in a dose-dependent manner through antagonism of renal A_1-adenosine receptors. A recent phase I study of **3** in humans demonstrated a dose-dependent increase



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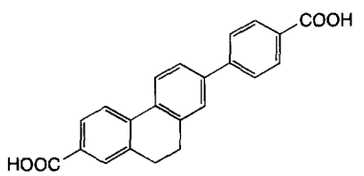
  1359-6446/97/$17.00. PII: S1359-6446(97)01107-0

in sodium, chloride and uric acid excretion without a concomitant increase in potassium loss, suggesting that this agent may be useful as a potent, potassium-sparing diuretic for the treatment of congestive heart failure associated oedema.

Human steroid 5α-reductase inhibitors

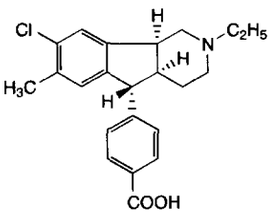
Human steroid 5α-reductase is a membrane-bound, NADPH-dependent enzyme that catalyses the stereospecific reduction of testosterone to dihydrotestosterone, a potent androgen that has been implicated in skin disorders, such as acne and hirsutism, and prostate cancer. The recent identification of two isozymes (type 1 and type 2) has focused research in this field towards the development of isozyme-selective and dual isozyme inhibitors of steroid 5α-reductase. Abell, A.D. and coworkers [*J. Chem. Soc., Perkin Trans. 1* (1997) 1663–1667] have described the synthesis and *in vitro* evaluation of 9,10-dihydrophenanthrene-2-carboxylic acids, exemplified by **4**, as potential nonsteroidal inhibitors of human steroid 5α-reductase. By incorporating the structural features of type 1 and type 2 selective, nonsteroidal inhibitors of human steroid 5α-reductase the group has shown that it is feasible to develop nonsteroidal dual isozyme inhibitors of steroid 5α-reductase.



4

Potent antispermatogenic compounds

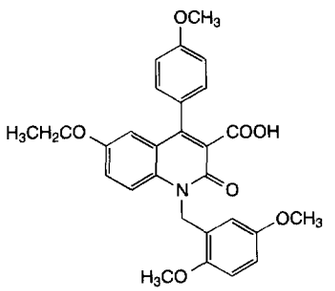
Although a number of nonsteroidal compounds have been evaluated for use in male fertility control, most of them exhibit unacceptable side effects. Previous studies by Cook, C.E. and coworkers [*J. Med. Chem.* (1995) 38, 753–763] have shown that the antispermatogenic activity of 5-arylhexahydroindeno[1,2-c]pyridines is highly stereo-, enantio- and chemoselective. In a recent paper [Cook, C.E. *et al. J. Med. Chem.* (1997) 40, 2111–2112] the group has described studies showing that the antispermatogenic properties of these compounds are enhanced about 40-fold by halogenation of the hexahydroindenopyridine system to give compounds such as **5**. The potent oral activity of these compounds should enhance the possibility of developing effective male oral contraceptives.



5

Endothelin receptor antagonists

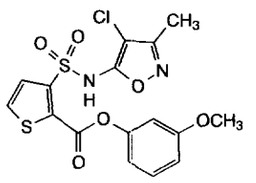
The G-protein-coupled endothelin receptors, ET_A and ET_B, have a role in regulating vascular tone and blood pressure and have been implicated in a number of vascular disease states. Both ET_A-selective and mixed ET_A/ET_B nonpeptide antagonists have been described previously; however, it is not clear which of these two types has the greatest therapeutic value. Mederski, W.W.K.R. and coworkers [*Bioorg. Med. Chem. Lett.* (1997) 7, 1883–1886] have described the discovery, synthesis and structure–activity relationships of a series of novel 1,4-diaryl-2-oxo-1,2-dihydroquinoline-3-carboxylic acids,



6

exemplified by **6**, as non-selective ET_A/ET_B receptor antagonists. Compound **6** was shown to be the most potent of the compounds investigated with IC_{50} values of 260 nM and 200 nM for the ET_A and ET_B receptors, respectively.

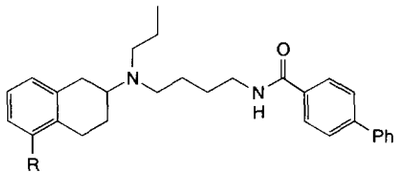
A recent report from Immuno-Pharmaceutics (San Diego, CA, USA) describes the synthesis and evaluation of a series of 2-aryloxycarbonylthiophene-3-sulphonamides as endothelin receptor antagonists [Raju, B. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 2093–2098]. These studies have led to the identification of **7** as a highly selective (1000-fold selectivity for the ET_A receptor over the ET_B receptor), potent (IC_{50} = 8.3 nM), low molecular weight, nonpeptide ET_A receptor antagonist.



7

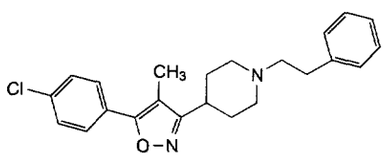
Selective dopamine D_3 and D_4 receptor antagonists

The antipsychotic effects of some drugs used in the treatment of schizophrenia are believed to be partially mediated through the inhibition of the D_2, D_3 and D_4 dopamine receptors. Recent studies have shown that the extra-pyramidal side effects associated with many of these drugs may be attributed to the blockade of the D_2 receptor subtype and that selective dopamine D_3 and/or D_4 receptor antagonists may offer an antipsychotic therapy without side effects. Boyfield, I. and coworkers [*Bioorg. Med. Chem. Lett.* (1997) 7, 1995–1998] have described the synthesis and evaluation of a series of novel *N*-[4-(4-phenylbenzoylamino)butyl]-1,2,3,4-tetrahydro-2-naphthylamines with high affinity and selectivity for the dopamine D_3 receptor. Compounds **8**, **9** and **10** were found to be the highest affinity (pK_i = 8.6–8.9) and most selective (200- to 300-fold selectivity for the D_3 receptor over the D_2 receptor) dopamine D_3 receptor antagonists yet reported.



8 R = -OCH_2c-C_3H_5
9 R = -OSO_2CH_3
10 R = -OSO_2CF_3

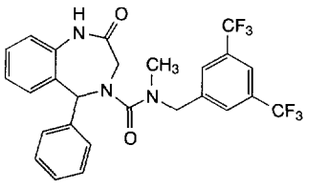
In another recent paper, a group at Merck Sharp and Dohme (Harlow, UK) have reported the discovery of 5-(4-chlorophenyl)-4-methyl-3-[1-(2-phenylethyl)piperidin-4-yl]isoxazole (**11**) as a potent (K_i = 3.5 nM) D_4 receptor antagonist with >500-fold and >200-fold selectivity for the human D_4 receptor over the human D_2 and D_3 receptors, respectively [Rowley, M. *et al. J. Med. Chem.* (1997) 40, 2374–2385]. Furthermore, compound **11** has been found to have a good pharmacokinetic profile with high bioavailability (38%), duration of action ($t_{1/2}$ = 2h) and CNS penetration. This compound will therefore be a useful tool for future studies into the relevance and importance of these receptors in the pathophysiology and treatment of CNS disorders.



11

Novel NK_1 receptor antagonist

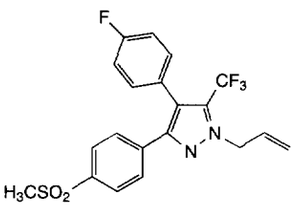
The structural similarities between the G-protein-coupled 7-transmembrane receptors, such as the gastrin/cholecystokinin (CCK) and neurokinin (NK) receptors, have led a group from Glaxo Wellcome Medicines Research Centre (Stevenage, UK) to investigate the use of the 1,4-benzodiazepines, which have previously been optimized to give selective CCK antagonists, as templates for the development of benzodiazepine-derived NK_1 receptor antagonists. This programme has led to the identification of a series of 1,4-benzodiazepin-2-one-derived NK_1 receptor antagonists [Armour, D.R. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 2037–2042]. Compound **12** was shown to be the most potent of these compounds, with a pK_i of 8.0.



12

Cyclooxygenase-2 inhibitors

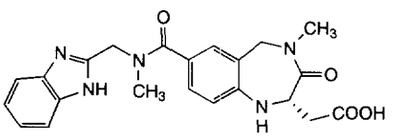
Recent studies have suggested that the side effects associated with the use of nonsteroidal anti-inflammatory inhibitors may be attributed to the inhibition of cyclooxygenase-1 (COX-1); the use of selective cyclooxygenase-2 (COX-2) inhibitors may therefore reduce these side effects. Penning, T.D. and coworkers have described the synthesis and evaluation of a series of 3,4-diarylpyrazoles as potential COX-2 inhibitors [*Bioorg. Med. Chem. Lett.* (1997) 7, 2121–2124]. A number of these compounds, exemplified by **13**, were found to be potent, selective inhibitors of COX-2 and shown to have oral anti-inflammatory activity in a rat carrageenan-induced foot pad oedema assay.



13

Potent nonpeptide vitronectin receptor antagonist

The vitronectin receptor ($\alpha_v\beta_3$) is a member of the integrin family of receptors that is expressed on the surface of various cell types including osteoclasts, vascular smooth muscle cells, endothelial cells and tumour cells. The $\alpha_v\beta_3$ integrin is involved in cell–cell and cell–substrate adhesion and communication and has been shown to be involved in a number of physiological processes including the adhesion of osteoclasts to the bone matrix, vascular smooth muscle migration and angiogenesis. Vitronectin receptor antagonists may therefore have application in the treatment of a wide variety of disease states. Keenan, R.M. and coworkers have reported the discovery of **14**, a highly potent (K_i = 2 nM) and selective non-peptide $\alpha_v\beta_3$ antagonist, following an analysis of the conformations of constrained RGD peptides and peptidomimetics [*J. Med. Chem.* (1997) 40, 2289–2292]. In particular, the group has capitalized on the use of a 1,4-benzodiazepine structure to act as a conformationally constrained Gly-Asp mimic. Further studies have shown that **14** is a potent inhibitor of both human osteoclast-mediated bone resorption and vitronectin-induced haptotaxis of human endothelial cells.

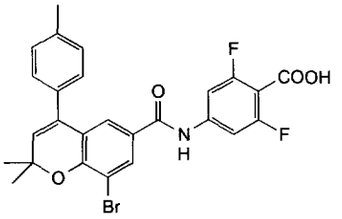


14

α-Retinoic acid receptor antagonists

Retinoid hormones have important roles in cell differentiation, cell proliferation and apoptosis through regulation of gene transcription. There are two families of retinoid receptors, the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs), each having three distinct subtypes (α, β and γ). Although retinoids are used extensively in dermatology and have shown therapeutic potential in the treatment of other disease states, the clinical use of non-selective retinoids invariably results in a broad spectrum of toxic side effects. As the different RAR subtypes have distinct tissue distributions and appear to regulate different subsets of genes, compounds that are selective for a particular subset may be more pharmacologically specific and have a broader therapeutic index. Workers from Allergan (Irvine, CA, USA) have reported the synthesis and full

retinoid receptor characteristics of a novel series of α-retinoic acid receptor antagonists, with compound **15** being the most selective in both binding and functional antagonism assays [Teng, M. *et al. J. Med. Chem.* (1997) 40, 2445–2451]. These compounds will have particular use as tools to further our understanding of the physiology associated with this particular RAR subtype and may also serve as useful agents for the treatment of diseases associated with this particular receptor subtype.



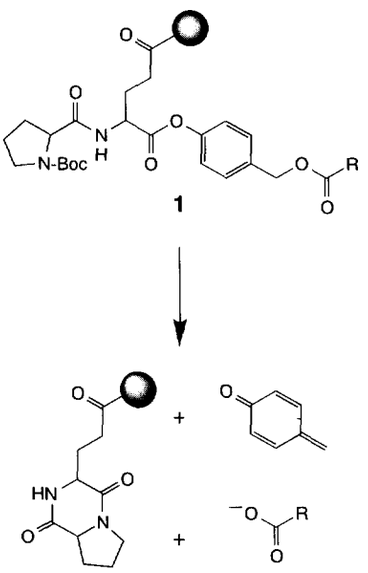
15

Combinatorial chemistry

A pH-cleavable linker for library screening

Linkers play a pivotal role in the solid-phase generation of combinatorial libraries prepared for biological screening. If the library is to be assayed in solution, there is a prerequisite for a step in which the compounds are cleaved from the solid support. However, very few linkers permit cleavage under biological assay-compatible conditions. Many require extremes of pH, and thus the library has to be isolated following cleavage to remove traces of incompatible cleavage reagents. A recent paper describes a new linker that allows the cleavage of library compounds from resin beads at pH 8 [Atrash, B. and Bradley, M. *J. Chem. Soc., Chem. Commun.* (1997) 1397–1398].

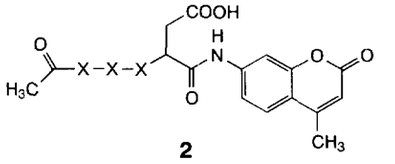
The linker (**1**) contains a key Pro-Glu dipeptide group attached to the solid phase. Removal of the Boc protecting group from this dipeptide reveals the nucleophilic proline amine. Adjusting the solution to pH 8 deprotonates the amine and initiates diketopiperazine formation and product elimination. This linker is especially useful for compound libraries that are tested in zone diffusion assays. The activated linker rapidly cleaves in buffered solution, but at a rate that permits initial distribution of the beads into agarose gel.



Caspase substrate specificities

The caspases are a family of cysteine proteases that include the enzymes interleukin-1β converting enzyme (ICE) and CED-3. These enzymes have been shown to play an essential role in apoptosis, the process of programmed cell death necessary for morphogenesis, tissue homeostasis and host defence. A recent study has used positional scanning combinatorial peptide libraries to determine the preferred substrates and thereby establish functional relationships between the enzymes [Thornberry, N.A. *et al. J. Biol. Chem.* (1997) 272, 17907–17911]. Three sublibraries of 8000 compounds each of the structure **2** were prepared. The libraries were constructed such that in each mixture one of the three amino acid residues was held constant while the others were an equimolar mixture of all of the naturally occurring monomers. By observing which mixtures were the best substrates for each of the enzymes, preferred substrate sequences could be inferred.

It was found that caspases 2, 3 and 7 and CED-3 preferred the tetrapeptide substrate sequence, DEXD (X is a variable amino acid residue), while caspases 6, 8 and 9 and granzyme B had a preference for (I/L/V)EXD. These results suggest functional relationships between the various enzymes, and could lead to the design and synthesis of selective enzyme inhibitors that may further elucidate the enzymes' biological function.

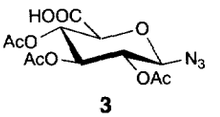


2

Carbohydrate-modified enkephalins

The addition of glucuronic acid onto morphine has been demonstrated to enhance its analgesic properties 10–50 times. A solid-phase synthetic route has been used to synthesize glucuronic acid enkephalin derivatives with the objective of exploring the effect of this modification on δ-opioid receptor agonist activity [Drouillat, B. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 2247–2250]. An azidoglucuronic acid (**3**) was used as the starting point for the solid-phase synthesis of these glycopeptides. Following attachment of the glucuronic acid to 2-chlorotrityl resin, reduction of the azide permitted solid-phase peptide synthesis on the free amine leading to the synthesis of C-terminal-modified enkephalins.

In particular, glycopeptide **4** was a potent δ-opioid receptor agonist, showing inhibition of electrically stimulated



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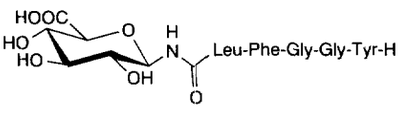

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