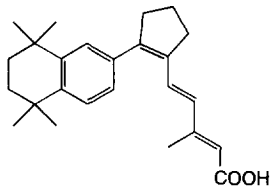
Monitor: molecules and profiles

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.

Retinoid X receptor ligands

Retinoids induce gene transcription in cells by binding to and activating retinoid receptors. These receptors are subdivided into the retinoic acid receptors (RAR) and the retinoid X receptors (RXR). Within each subclass, there are three known receptor subtypes: α, β and γ. Although the ability of retinoids to regulate cell growth and differentiation has led to their use in the treatment of acne, psoriasis and cancer, many of these compounds have been found to have undesirable side-effects. This toxicity may be attributed to the interaction of these compounds with multiple receptors in the target tissue.



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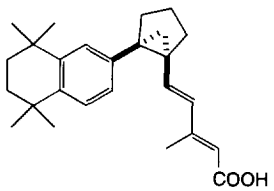

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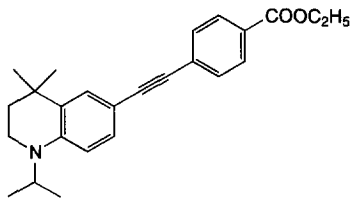
In an attempt to develop more-potent and selective RXR agonists, Farmer, L.J. and co-workers have identified a novel class of RXR selective retinoids based on conformationally strained analogues of 9-*cis*-retinoic acid [*Bioorg. Med. Chem. Lett.* (1997) 7, 2393–2398]. The lead compound (**1**) showed strong binding (K_i = 20–50 nM) and transactivation (EC_{50} = 40–50 nM) of the RXR subtype of retinoid receptor. Further modifications to the geometry of the cyclopentane ring moiety led to the identification of **2**, one of the most potent RXR agonists reported to date (K_i = 3–8 nM, EC_{50} = 3–4 nM).

Another group has reported the synthesis and biological activity of a novel series of potent RAR retinoids substituted with either 1,2,3,4-tetrahydroquinoline (THQ) or 3,4-(1*H*)-dihydroquinolin-2-one (DHQ) moieties [Beard, R.L. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 2373–2378]. Two of the THQ analogues (**3** and **4**) were found to be potent inhibitors of tumour-promoter-induced ornithine decarboxylase (ODC) activity in hairless mouse skin. Interestingly, the group also found that THQ and DHQ analogues with similar receptor binding and transactivation profiles had different effects on ODC activity in the skin. This suggests that these retinoids may have unique pharmacokinetic profiles, offering advantages over currently available retinoids with respect to increased efficacy and reduced toxicity.



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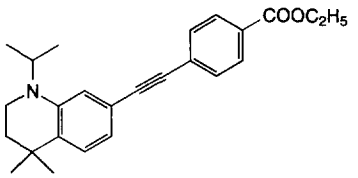

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Selective dopamine ligands

The search for more-selective dopamine receptor ligands continues to dominate the literature.

He, S.X. and co-workers [*Bioorg. Med. Chem. Lett.* (1997) 7, 2399–2402] have prepared a series of 3-[4-arylpiperaz-1-yl)alkylamino]-2*H*-1,4-benzoazines and determined their affinities for cloned human D_2, D_3 and D_4 dopamine receptor subtypes. This study led to the identification of **5**, **6** and **7** as high-affinity D_4 receptor antagonists possessing greater than 60-fold selectivity for the D_4 receptor over the other two subtypes.

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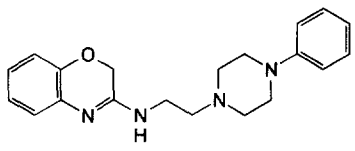

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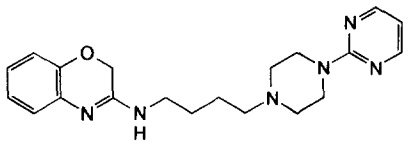

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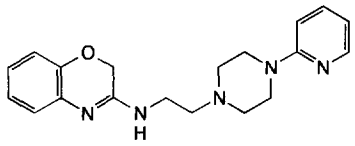

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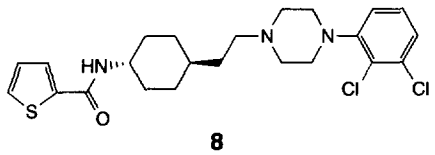
An attempt by a group at Parke-Davis Pharmaceutical Research (Ann Arbor, MI, USA) to develop dopamine D_3 ligands for the treatment of schizophrenia, by modifying the D_2-selective ligand PD137557, has led to the discovery of **8** and **9** as selective D_3 receptor ligands [Belliotti, T.R. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 2403–2408]. Compound **9** was the more selective of these compounds, with a 270-fold greater affinity for the D_3 receptor over the D_2 receptor, while compound **8** had the greater affinity for the D_3 receptor (K_i = 0.02 nM).



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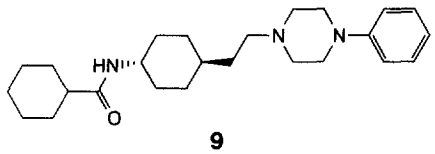
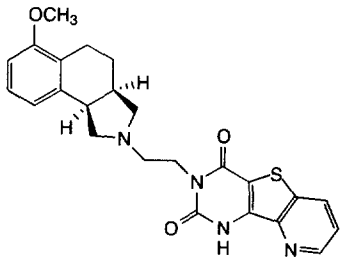


9

Uroselective α_{1A} adrenoceptor antagonists

Although benign prostatic hyperplasia (BPH) is most effectively treated by administration of α_1 antagonists, which relax the prostatic smooth muscle, existing α_1 antagonists give rise to cardiovascular side-effects. Recent studies have shown that antagonist inhibition of norepinephrine- or phenylephrine-induced contraction of human prostate tissue is associated with affinity for the α_{1A} adrenoceptor subtype. Therefore, selective α_{1A} adrenoceptor antagonists may offer advantages over existing drugs for the effective treatment for BPH. In attempting to investigate this hypothesis, Meyer, M.D. and co-workers have prepared and pharmacologically characterized compound **10** (A131701) as a potential uroselective α_{1A} adrenoceptor antagonist [*J. Med. Chem.* (1997) 40, 3141–3143]. This compound has been shown to have high affinity and moderate selectivity for the α_{1A} adrenoceptor. In *in vitro* models predictive of α_1 subtype specificity and in *in vivo* models, **10** also exhibited a higher degree of uroselectivity than tamsulosin, the only uroselective α_1 adrenoceptor antagonist currently in clinical use for the treatment of BPH.



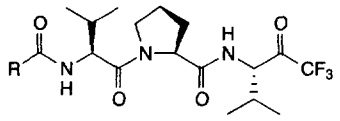
10

Human leukocyte elastase inhibitors

Human leukocyte elastase (HLE) is a potent serine protease that has been implicated in the chronic tissue destruction observed in several disease states, including pulmonary emphysema and acute respiratory distress syndrome. Under normal conditions, the activity of released HLE is controlled by various endogenous inhibitors. However, under certain conditions, an imbalance occurs as a result of either the oversecretion of HLE or the impairment of the regulatory mechanisms. A number of recent papers have reported the development of various inhibitors of HLE as potential therapeutic agents for the treatment of diseases in which HLE is implicated.

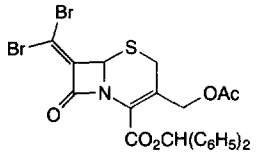
Veale, C.A. and co-workers have described the development of a series of peptidyl trifluoromethyl ketone inhibitors of HLE with excellent pharmacological profiles [*J. Med. Chem.* (1997) 40, 3173–3181]. Two compounds (**11** and **12**) were found to be orally active in a number of species and this has led to the development of **12** for clinical evaluation.

A group from Southern Methodist University (Dallas, TX, USA) has investi-



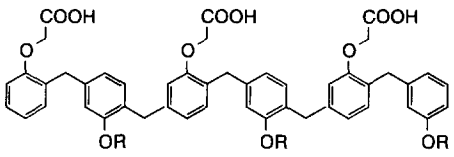
11, R = 4-$CH_3OC_6H_5$-
12, R = CH_3O-

gated the ability of a series of benzhydral 7-alkylidenecephalosporinates and 7-vinylidenecephalosporinates to inhibit HLE [Buynak, J.D. *et al. J. Med. Chem.* (1997) 40, 3423–3433]. The 7-(haloalkylidene)cephalosporins, exemplified by **13**, were found to be potent irreversible inhibitors of HLE.



13

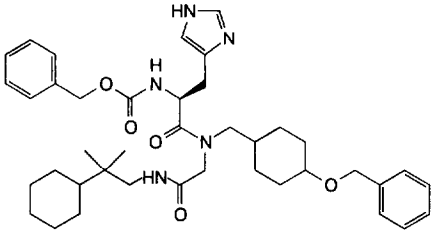
In an alternative approach, Regan, J. and co-workers have synthesized a series of diphenylmethane-based oligomers, containing both anionic and lipophilic functionalities, as potential HLE inhibitors [*J. Med. Chem.* (1997) 7, 3408–3422]. Compounds such as **14**, containing three phenoxyacetic acid groups and three alkyl ethers, were found to be specific competitive inhibitors of HLE (K_i = 20 nM). The authors suggest that compounds of this nature act by forming multidentate interactions with the surface of the HLE near the active site, thereby preventing substrate access to the catalytic site.



14, R = alkyl

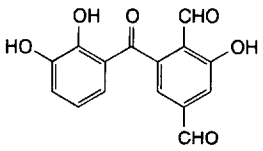
Ras farnesyltransferase inhibitors

In order for the Ras protein to stimulate cell growth, it must bind to the inside of the cell membrane. This involves a cascade of enzyme modifications, the first of which is catalysed by the enzyme farnesyltransferase. Mutation in the Ras protein has been implicated in several cancers and, since mutated Ras protein requires farnesylation in order to transform cells into a malignant state, inhibitors of farnesyltransferase have been suggested as potential anticancer agents. MacNamara, D.J. and coworkers [*J. Med. Chem.* (1997) 40, 3319–3321] have reported the identification of a series of farnesyltransferase inhibitors, exemplified by **15**, with enhanced enzymatic and cellular activities *in vitro*, and *in vivo* anticancer activity in mice.



15

Another group, from Schering-Plough Research Institute (Kenilworth, NJ, USA), have reported a novel farnesyltransferase inhibitor (**16**) that was isolated from an unidentified fungus collected in Equador (culture MYCO-2139). The compound exhibited an IC_{50} of 3.5 μM against farnesyltransferase and of 70 μM against geranylgeranyltransferase.

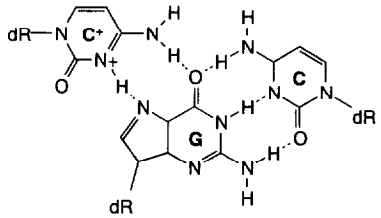


16

Antigene oligonucleotides

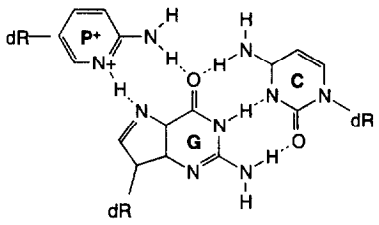
Increasing interest in treating diseases at the gene level (e.g. gene therapy) has furthered the need to develop novel approaches to inhibiting the transcription and translation of specific genes. Generally, groups have focused on the use of antisense approaches in which single-stranded oligonucleotides inhibit gene expression during translation by specifically binding to mRNA targets via Watson–Crick base pairing. Although there has been limited success in a number of clinical trials using this approach, there are clearly problems associated with effective delivery of antisense oligonucleotides. An alternative approach is to target the specific gene directly using single-stranded antigene oligonucleotides. Such oligos are designed to form stable triplexes with the native duplex DNA. The effectiveness of this approach relies on identifying new sequence recognition elements that ideally have binding affinities similar to Watson–Crick base pairing between single-stranded oligonucleotides. The specific formation of Hoogsteen H-bonds between single-stranded oligos and duplex DNA is of limited use for the antigene approach because of two limitations: only adenine and guanine bases of the duplex target serve as recognition elements for the third strand and targeting guanine bases requires low pH.

In order to be effective, antigene oligonucleotides must form stable triplexes at physiological pH. Leumann's group [Hildbrand, S. *et al. J. Am. Chem. Soc.* (1997) 119, 5499–5511] has recently described the identification of pyridine-*C*-nucleoside oligonucleotides that display high-affinity and sequence-specific binding to duplex DNA at physiological pH. This research was based on the known observation that formation of C^+--G-C triplexes (**17**) via Hoogsteen hydrogen bonds between cytosine (**C**) of a single-strand oligo and guanosine (**G**) of a duplex DNA requires protonation of the 3-position of **C**.



17, C^+ - - G - C

At physiological pH (~7.4), **C** is largely unprotonated (pK_a ~ 4.3). These researchers therefore hypothesized that oligos in which one or more **C**s were replaced by an intrinsically more basic heterocycle would generate a derivative with a higher proportion in the ionized form at physiological pH; upon exposure of such an oligo to a target gene at pH 7.4, formation of a more stable Hoogsteen hydrogen-bonded triplex (e.g. **18**) would occur.



18, P^+ - - G - C

Using very efficient synthetic methods, the researchers prepared a variety of 15mer oligos containing pyridine-*C*-nucleosides which, upon protonation, would act as isosteres of protonated **C** derivatives. Included in these structures was the 2-aminopyridine-2'-deoxy-*C*-nucleoside **P** (pK_a ~ 6.3) as a mimic of **C**. One of the major findings of this study was that, relative to the corresponding **C**-containing oligo, a 15mer containing five **P** groups [5'-d(TTTTT-**PTPTPTPTP**T)] exhibited higher sequence specificity and far higher affinity for the complementary target DNA in the pH range 6.0–8.0; this effect was most pronounced at pH 7.0 and above.

This research addresses one of the most important deficiencies of previously reported antigene oligos: namely, that they require a low pH (<6.0) to support formation of stable Hoogsteen hydrogen bonds. This may ultimately lead to the development of specific antigene oligos that inhibit the expression of the specific genes that are responsible for a wide range of human diseases.

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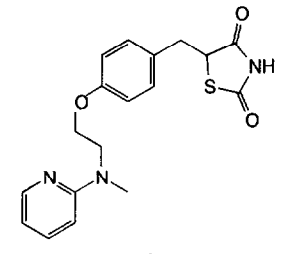
Combinatorial chemistry

Novel PPARγ ligands

Peroxisome proliferator activated receptors (PPARs) are nuclear hormone receptors that appear to recognize fatty acids. Synthetic ligands for these receptors could be useful agents to control lipid metabolism and glucose levels. A recent paper describes the use of solid-phase combinatorial chemistry in the synthesis of a library of human PPARγ ligands [Tomkinson, N.C.O. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 2491–2496].

A thiazolidinedione structure (**1**) had previously been demonstrated to be a PPARγ ligand (K_i = 49 nM), and it was hypothesized that this compound might mimic the structures of naturally occurring fatty acids. Consequently, thiazolidinedione–fatty acid hybrids (**2**) were synthesized using a solid-phase synthetic approach and tested as potential ligands. The thiazolidinedione was linked through the nitrogen to 2-chlorotrityl resin, and a range of amides were individually synthesized using carbodiimide-catalysed couplings. Cleavage from the resin and purification by solid-phase extraction gave a number of analogues that were screened for their affinity for human PPARγ. Several of these compounds, especially those with the longer fatty acid chains, were found to be potent ligands for PPARγ with K_i values as low as 18 nM.



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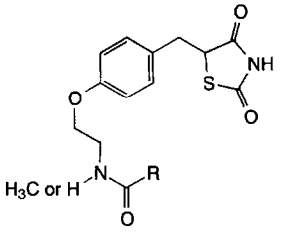
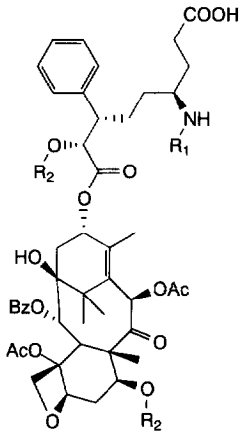


2

Radio-frequency-encoded taxoid library

Radio-frequency-encoded combinatorial chemistry uses porous microreactors that contain both the resin solid support and a tag that can be labelled with a unique radio-frequency code. The microreactors are the individual units in a 'split and pool' library preparation; the sorting of the units is not statistical, but rather directed by the radio-frequency codes, leading to multimilligram quantities of all compounds.

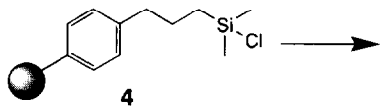
This approach has been used in the preparation of a 400-component library of taxoids with the intention of modifying solubility and biological activity [Xiao, X-Y. *et al. J. Org. Chem.* (1997) 62, 6029–6033]. The core structure was loaded onto 2-chlorotrityl resin and three positions were varied through acylation reactions on amine or hydroxy groups. The products (**3**) were generated in 2–4 mg amounts, and purity was judged by TLC and HPLC to be 50–100%. Biological screening of this structurally complex library is ongoing and will hopefully be reported shortly.



3

A traceless library approach

Several groups have now described methods by which it is possible to cleave library compounds from the solid support, leaving no trace of the point of attachment. One such group is the arylsilyl linker, which generates an aryl hydrogen via protodesilylation or treatment with fluoride. However, a serious limitation of this strategy is the need to pre-form the linker–substrate complex before it can be loaded onto the resin support. As this limits the degree of flexibility of the library synthesis, there has been a search for ways of loading the substrate directly onto a silyl-substituted resin.



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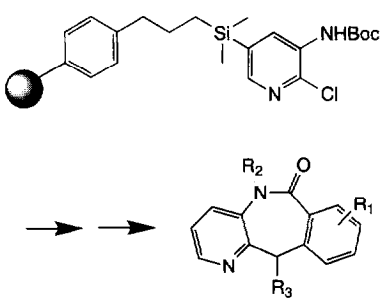

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This has now been achieved in an approach to pyridine-based tricycles related to the HIV reverse transcriptase inhibitor nevirapine [Woolard, F.X. *et al. J. Org. Chem.* (1997) 62, 6102–6103]. The chlorosilane linker (**4**) attached to a polystyrene solid support can be functionalized by the addition of pyridine anions. Further solid-phase chemistry was used to convert the pyridine to a library of tricyclic compounds (**5**). Cleavage of the heterocyclic products from the solid phase was readily achieved by treatment with tetrabutylammonium fluoride.

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