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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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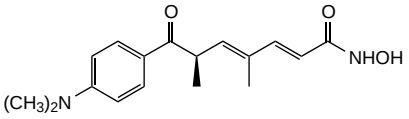
Novel anticancer molecules

New histone deacetylase inhibitors

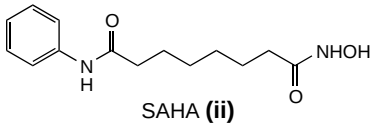
Histone acetylation/deacetylation in eukaryotic cells is a crucial factor in transcriptional regulation. Histone acetylases (HATs) and histone deacetylases (HDACs) are groups of enzymes that catalyze acetylation (associated with transcriptional activation) and deacetylation (associated with transcriptional silencing) of lysine residues in the N termini of core histones. HDACs have been identified as transciptional co-repressors, and HDAC inhibition is being actively pursued as a novel approach to cell-cycle regulation and the treatment of proliferative diseases, such as cancer.

Several small-molecule HDAC inhibitors have shown the ability to inhibit proliferation and induce apoptosis and differentiation in cancer cell lines, and therefore have promise as antitumour agents. Reported HDAC inhibitors include both natural products (e.g. trichostatin A; compound **i**) and synthetic agents (e.g. suberoylanilide hydroxamic acid, SAHA; compound **ii**).

Woo and co-workers (MethylGene; http://www.methylgene.com) have reported the synthesis and antitumour evaluation of a series of new trichostatin A (TSA)-like straight chain hydroxamates [1]. Several of these compounds exhibited potent HDAC inhibitory activity with low nanomolar values (e.g. compound **iii**,



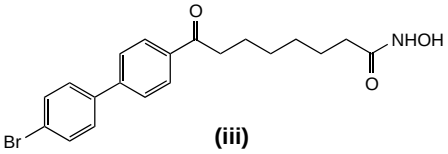
Trichostatin A **(i)**



SAHA **(ii)**

IC_{50} = 2 nM; TSA IC_{50} = 5 nM), and were found to induce hyperacetylation of histones in T24 human cancer cell lines and significantly inhibit proliferation in various human cancer cell lines (sub-micromolar IC_{50} values in T24, HCT116, A549, H446 and MDAmb231 cell lines for compound **iii**). The lead compounds identified in this study could aid in the design of future nonhydroxamate inhibitors of HDAC.

Fournel and co-workers, also from MethylGene, have designed and synthesized novel non-hydroxamate sulfonamide



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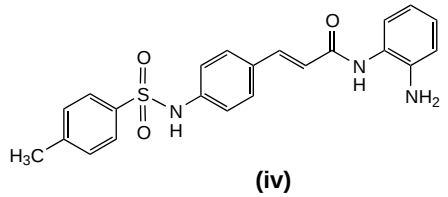

(iv)

anilides, for example compound **iv**, that can inhibit human HDAC enzymes and induce hyperacetylation of histones in human cancer cells [2]. In addition, these compounds selectively inhibit proliferation and cause cell-cycle blocks in human HCT116 colon cancer cells but not in normal HMEC cells. *In vivo*, compound **iv** significantly reduces tumour growth in an HCT116 human colon cancer xenograft model without noticeable toxicity. cDNA microarray analysis has identified downstream genes whose expression is significantly altered by treatment of human cancer cells with compound **iv**; these include upregulation of $p21^{WAF1/Cip1}$ and downregulation of cyclin A and cyclin B1.

1 Woo, S.H. *et al.* (2002) Structurally simple trichostatin A-like straight chain hydroxamates as potent histone deacetylase inhibitors. *J. Med. Chem.* 45, 2877–2885
2 Fournel, M. *et al.* (2002) Sulfonamide anilides, a novel class of histone deacetylase inhibitors, are antiproliferative against human tumors. *Cancer Res.* 62, 4325–4330

Inhibitors of the HIF-1 transciptional activation pathway

Adaptive responses in solid tumours to oxygen deficient (hypoxic) conditions are regulated by the transcription factor hypoxia-inducible factor 1 (HIF-1). The case for HIF-1 as a validated anticancer drug target rests upon the following evidence. HIF-1 is implicated in the regulation of genes involved in angiogenesis

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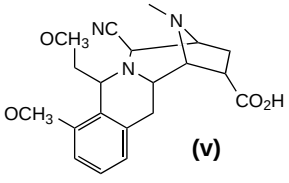
(e.g. vascular endothelial growth factor; VEGF) and anaerobic metabolism (e.g. glycolytic enzymes). In addition, HIF-1 is known to be essential for apoptosis and tumour progression, and the overexpression of subunit HIF-1α has been demonstrated in many common human cancers.

Rapisarda and co-workers [National Cancer Institute Developmental Therapeutics Program (NCI DTP); http://dtp.nci.nih.gov] have reported the development of cell-based HTS for the identification of HIF-1 inhibitors using genetically engineered U251 human glioma cells that consistently express luciferase in a hypoxia- and HIF-1-dependent fashion [3]. A pilot screen of the NCI 'Diversity Set', a collection of ~2000 compounds selected to represent the wide chemical diversity of the NCI chemical repository, uncovered two different classes of compounds that specifically inhibited HIF-1-dependent induction of luciferase. The first of these, NSC-607097 (**v**), is a stable analogue of quinocarmycin that was previously developed to a Phase I trial but was discontinued as a result of unexpected and unpredictable toxicity. The second class of compounds identified in the screen is represented by drugs that inhibit Topo-1 activity, such as the clinically used topotecan (**vi**) and two camptothecin (CPT) analogues, suggesting that topotecan also inhibits VEGF transcription. These results indicate that screening of larger chemical libraries using cancer cell lines engineered with HIF-1 inducible promoters might lead to the identification of HIF-1 inhibitors that could be developed for clinical application. The NCI DTP has undertaken a HIF-1 targeted HTS campaign of a 140,000-compound library (available at the NCI) in the search for such inhibitors.



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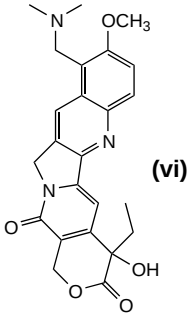


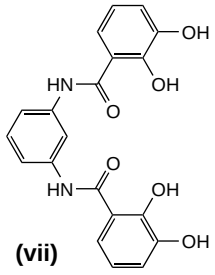
(vi)

3 Rapisarda, A. *et al.* (2002) Identification of small molecule inhibitors of hypoxia-inducible factor 1 transcriptional activation pathway. *Cancer Res.* 62, 4316–4324

Novel telomerase inhibitors

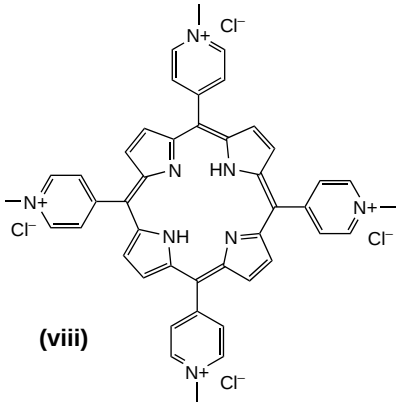
Telomeres are tandem repeat DNA sequences (TTAGGG in humans) at the ends of chromosomes that progressively shorten after each replicative cycle as a result of the 'end-replication problem'. Critically short telomeres act as a signal for replicative senescence and, as such, limit the replicative capacity of each cell. The ribonucleoprotein telomerase complex acts to restore telomeres lost during cell division, and is found to be active in most (80–90%) cancer cells (which usually possess short telomeres that are stable in length) but is absent from most normal somatic cells. Inhibition of telomerase has, for this reason, been pursued as a therapeutic target in the cancer field, mainly through the identification of small-molecule ligands that stabilize the G-quadruplex structure thought to be adopted by the single-stranded telomeric TTAGGG overhang.

One interesting class of telomerase inhibitors, reported previously, is the anticancer tea catechin class, such as epigallocatechin gallate (EGCG). Seimiya and co-workers [Japanese Foundation for Cancer Research, http://www.jfcr.or.jp; University of Tokyo, http://www.u-tokyo.ac.jp; Mitsui Chemicals, http://mitsui-chem.co.jp) have now reported the synthesis of simple structures containing EGCG-related moieties, such as compound **vii**, that are more effective telomerase inhibitors than EGCG [4]. Continuous treatment of human monoblastoid leukaemia U937 cells with a 1–2 μM (nontoxic) dose of **vii** caused progressive telomere shortening and the eventual reduction of growth rate accompanied by induction of the senescence-associated β-galactosidase activity. The precise mechanism by which EGCG and these new compounds inhibit telomerase has not been determined; however, results obtained here suggest that they are unlikely to be G-quadruplex ligands.



(vii)

Cationic porphyrins, such as TMPyP4 (**viii**), have been previously reported to bind to and stabilize G-quadruplexes in human telomere sequences, resulting in the inhibition of telomerase activity [5]. Grand and co-workers (Arizona Cancer Center, http://www.azcc.arizona.edu; University of Arizona, http://www.arizona.edu; Institute for Drug Development, http://www.idd.org) have reported the results of cDNA microarray analysis on cells treated with compound **viii** [6]. c-*MYC*, an oncogene that is nearly ubiquitous in human tumours and that potentially forms a G-quadruplex in its promoter region, and the *hTERT* gene, which encodes the catalytic subunit of telomerase, were among the genes



(viii)

specifically downregulated by **viii**. *In vivo*, compound **viii** was also found to prolong survival and decrease tumour growth rates in two xenograft tumour models. The dual action of the cationic porphyrin **viii** in decreasing both c-MYC protein levels and telomerase activity make this a worthwhile agent for further study.

4 Seimiya, H. *et al.* (2002) Telomere shortening and growth inhibition of human cancer cells by novel synthetic telomerase inhibitors MST-312, MST-295 and MST-199. *Mol. Cancer Therap.* 1, 657–665

5 Izbicka, E. *et al.* (1999) Effects of cationic porphyrins as G-quadruplex interactive agents in human tumor cells. *Cancer Res.* 59, 639–644

6 Grand, C.L. *et al.* (2002) The cationic porphyrin TMPyP4 down regulates c-*MYC* and human telomerase reverse transcriptase expression and inhibits tumor growth *in vivo*. *Mol. Cancer Therap.* 1, 565–573

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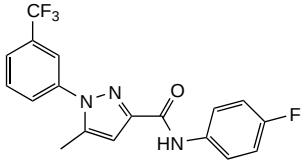
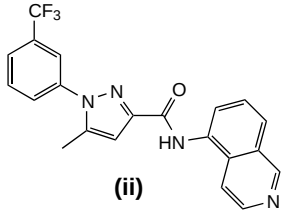
Combinatorial chemistry

Neuropeptide Y5 receptor ligands

Neuropeptide Y (NPY), a 36-amino acid C-amidated peptide, is abundantly expressed in the CNS and has been shown to robustly stimulate feeding. A family of six NPY receptor subtypes belonging to the superfamily of G-protein coupled receptors has been described in the literature ([1] and references therein). NPY Y1 and NPY Y5 are thought to be the most likely subtypes responsible for centrally mediated NPY-induced feeding responses. Antagonists at the NPY Y5 receptor are effective in reducing food intake in animal models of feeding [2]. Consequently, there has been an impetus to discover small-molecule NPY Y5 receptor antagonists to provide new treatments for obesity and other eating disorders [1]. A small library of 28 compounds was synthesized in solution in an attempt to elucidate SAR based on the pyrazole carboxamide lead **i** found from HTS. The library compounds were evaluated for binding affinity to the human NPY Y5 receptor. The assay used a stably transfected HEK293 cell line and measured competitive inhibition of binding of ^{125}I-PYY. One of the most potent compounds found was **ii**, which bound to the human NPY Y5 receptor with an IC_{50} value of 80 nM and was shown to inhibit cumulative food consumption by 43.2%, 2–6 hours after dosing in a fasting induced feeding model in rats. This work has produced an interesting set of compounds with affinity for the human NPY Y5 receptor, and further work in this area is warranted.



(i)



(ii)

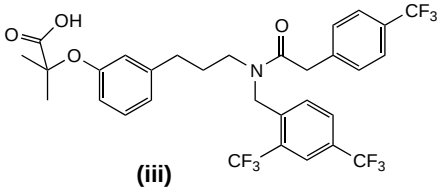
1 Kordik, C. P. *et. al.* (2001) Pyrazolecarboxamide human neuropeptide Y5 receptor ligands with *in vivo* antifeedant activity. *Bioorg. Med. Chem. Lett.* 11, 2287–2290

2 Criscione, L. *et al.* (1998) Food intake in free-feeding and energy-deprived lean rats is mediated by the neuropeptide Y5 receptor. *J. Clin. Invest.* 102, 2136–2145

PPARγ/δ dual agonists

The metabolic syndrome X, which consists of a clustering of several metabolic risk factors in a single patient, contributes significantly to increased mortality. The major components of this syndrome include dyslipidemia, insulin resistance, obesity and hypertension. Although established therapies are available to treat these risk factors individually (e.g. statins and fibrates for dyslipidemia, and metformin and glitazones for insulin resistance), no single drug can treat multiple risk factors of the metabolic syndrome X. Evidence suggests that the hyperlipidemic effects of the fibrate drugs and the antidiabetic effects of the glitazones are a result of activation of the α and γ sub-types, respectively, of the peroxisome proliferator-activated receptor (PPAR).

The PPARs are orphan receptors that belong to the nuclear hormone receptor superfamily of ligand-activated transcription factors. Identification of the metabolic syndrome X has therefore become one of the major efforts in the pharmaceutical industry. PPARδ is a recently discovered molecular target for the treatment of dyslipidemia. Hence, PPARγ/δ dual agonists could provide an efficient treatment for this syndrome by providing dual control of glucose and lipid metabolism [3]. A library of 480 compounds was synthesized on solid phase using SASRIN® resin. The library compounds were screened against all three human PPAR subtypes in a scintillation proximity assay (SPA) binding assay. One of the most potent compounds isolated was **iii**, which possessed a K_i value of 10 nM against hPPARγ, a K_i value of 5 nM against hPPARδ, and over 30-fold selectivity against hPPARα. This work has provided a novel potent PPARγ/δ dual agonist. Compounds with this dual receptor activity could provide a new approach to the development of drugs for the metabolic syndrome X.



(iii)

3 Liu, K. G. *et. al.* (2001) Identification of a series of PPARγ/δ dual agonists via solid-phase parallel synthesis. *Bioorg. Med. Chem. Lett.* 11, 2959–2962

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