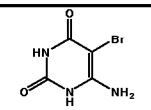
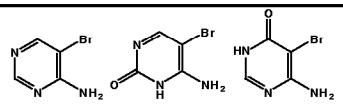
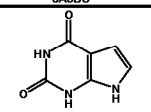
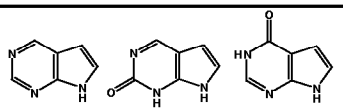
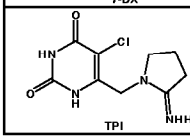
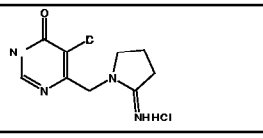
phorylase (TP; PD-ECGF; EC 2.4.2.4) catalyses the reversible phosphorolysis of thymidine to thymine and 2-deoxy-a-D-ribose-1-phosphate, which is dephosphorylated to give 2-deoxyribose, an angiogenic factor. TP is elevated in several hypoxic tumours, promoting both angiogenesis and metastasis, and suppressing apoptosis. Inhibitors of TP are therefore of significant interest in cancer chemotherapy. TP inhibitors are attractive targets for the XO prodrug strategy, since both enzymes are regulated by hypoxia and expressed in the cytoplasmic regions in mammary and colorectal tumours. The prodrugs synthesised were deoxygenated analogs of the known TP inhibitors, 6-amino-5-bromouracil (6A5BU), 7-deazaxanthine (7-DX) and 5-chloro-6-[1-(2-imino-pyrrolidinyl)methyl]uracil hydrochloride (TPI) (Table 1). These prodrugs were designed to exploit the oxidative hydroxylation reaction catalysed by XO.

Table 1. TP inhibitors and XO prodrugs synthesised and evaluated for TP inhibition and XO activation

TP INHIBITOR	XO PRODRUGS
 6A5BU	
 7-DX	
 TPI	

The 6A5BU prodrugs were synthesised by bromination of the appropriate 6-aminopyrimidines. The 7H-pyrrolo[2,3-d]pyrimidine prodrugs of 7-DX were obtained by cyclisation of 4-aminoprimidinyl-acetaldehydes or by Stille coupling of the relevant 5-bromo-6-aminopyrimidine. Coupling of 5-chloro-6-chloromethylpyrimidin-4-one with 2-iminopyrrolidine afforded the desired prodrug of TPI. The inhibition of recombinant E. coli TP by these prodrugs and inhibitors was determined using a spectrophotomeric assay. The XO prodrugs displayed no inhibition of TP, whereas the TP inhibitors showed the expected activity. The biotransformation of these prodrugs to the desired TP inhibitor has been evaluated using bovine XO by UV assay. In utilising the XO prodrug approach it is hoped that the prodrugs synthesised may display improved bioavailability with tumour and hypoxic specificity.

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D-ring modified steroids as potent oestrone sulphatase inhibitors

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Oestrogens, and to a lesser extent androgens, play a major role in the promotion and development of hormone-dependent breast cancers. Regulation and control of the level of active steroids can be achieved via the inhibition of one or several enzymes of steroidogenesis. The enzyme oestrone sulphatase (STS), which converts oestrone sulphate to oestrone, is now considered as a key therapeutic target for depleting oestrogenic stimulation to tumours. Several potent steroidal inhibitors of STS have been reported, of which oestrone-3-O-sulphamate (EMATE) is the benchmark inhibitor. It irreversibly inhibits the enzyme in a time- and concentration-dependent manner but was found to be oestrogenic *in vivo*. In the search for new potent inhibitors of STS that are devoid of oestrogenicity, we synthesised a number of D-ring modified derivatives of EMATE. A methodology for ring expansion was developed where the D-ring of oestrone was cleaved, via a haloform reaction, to a dicarboxylic acid derivative and that was then closed by thermal condensation with urea. The N-atom of the resulting piperidinedione moiety is designed to act as a versatile anchor for the introduction of a variety of side-chains through alkylation.

Upon *in vitro* biological evaluation of the analogues, compounds 2 and 3 have been identified as the two most potent inhibitors in the series. Their

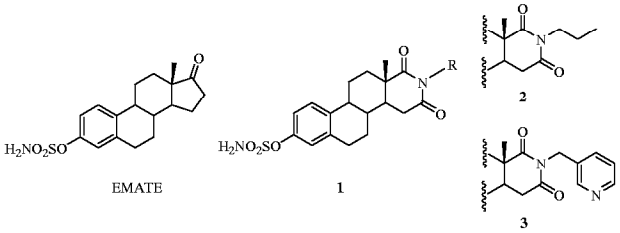

Figure. Structures of EMATE and its piperidinedione analogue **1**. The two most potent compounds are **2** and **3**.

IC_{50}s obtained from a placental microsomes preparation were both found to be 1 nM which is about 18-fold lower than that of EMATE. Unlike EMATE, these compounds are non-oestrogenic since they did not stimulate uterine growth in ovariectomised female Wistar rats at an oral dose of 10mg/kg/day administered over a period of 5 days. The crystal structure of 2 has also been determined which will provide structural information for this series of EMATE analogues. This work therefore represents a new strategy for the design of potent, orally active and non-oestrogenic oestrone sulphatase inhibitors that are structurally distinctive from previously known active compounds. This work is funded by Sterix Ltd. LWLW, BVLP, AP and MR are stockholders of Sterix.

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Complex pattern of molecular targets for antineoplastic pteridines

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7-Benzylamino-6-chloro-2-piperazino-4-pyrrolidino-pteridine (E481) is a potent inhibitor of cAMP-specific phosphodiesterase (PDE4), inhibiting the growth of tumor cell lines in the low micromolar range. Structure-activity studies indicated that in addition to PDE4-inhibition, other cellular effects might be of relevance for the growth inhibitory activity of pteridines. The mitogen-activated protein kinase (MAPK) cascade plays an important role in the regulation of cell proliferation. We therefore investigated the influence of substituted pteridines on the MAPK cascade. The potency of the compounds to inhibit the tyrosine kinase activity of the epidermal growth factor receptor (EGFR) was studied using EGFR isolated from A431. The tyrosine kinase activity was determined by ELISA. The known EGFR inhibitor tyrphostin AG 1478 was used as a positive control. We found that the potency of E481 to inhibit EGFR kinase (IC_{50} = 48 μM) is several orders of magnitude weaker than PDE4 inhibition (IC_{50} = 16 nM). Structure-activity studies showed that modulation of the piperazino residue in position 2 of the pteridine ring system strongly affects EGFR inhibitory properties. A 2-(2-aminoethylamino)-substituent was found to strongly increase the inhibition of the EGFR. Concomitantly, PDE4 inhibitory properties were nearly eliminated. In contrast, the 6-dechloroanalogue did not show EGFR inhibitory potency, whereas PDE4 inhibitory properties were retained. We furthermore investigated the consequence of EGFR or PDE4 inhibition on downstream signaling pathways, such as the MAPK cascade, known to be crucial for cell proliferation. One of the nuclear substrates of MAP kinase is the transcription factor Elk-1. We transiently transfected A431 cells with a luciferase reporter gene plasmid whose expression depends on the phosphorylation of a GAL4-Elk1 fusion protein. For substituted pteridines, we found that not only EGFR inhibitors result in a reduction of Elk-1 phosphorylation, but also effective PDE4 inhibitors, lacking EGFR inhibitory properties. The results indicate, that the transmission of the mitogenic signal, measured as phosphorylation of Elk-1, is substantially influenced by crosstalks between the MAPK cascade and other cellular pathways like for example the cAMP pathway, which is potently affected by several members of this class of compounds.