ELSEVIER

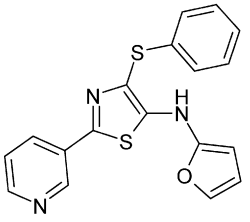
monitor

MOLECULES

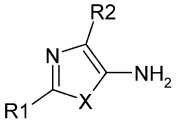
Application of technologies and parallel chemistry for the generation of actives against biological targets

2,4-Diphenylthiazoles and 2,4-diphenyloxazoles as potential novel prion disease therapeutics

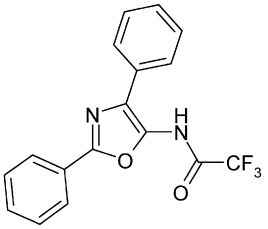
Prion diseases, or transmissible spongiform encephalopathies (TSEs), are invariably fatal neurodegenerative disorders affecting humans and animals. As yet, no effective curative or prophylactic therapy exists. Recent and prominent examples of prion diseases include bovine spongiform encephalopathy (BSE, in cattle) and scrapie (in sheep) [1]. Since the discovery of a new variant of the human TSE, Creutzfeldt-Jacob disease (vCJD), prion diseases have been brought into the focus of current research. These diseases represent a highly significant risk to public health due to transmission, both to and between humans. Iatrogenic transmission has been reported via contact with contaminated neurosurgical instruments [2]. TSEs are associated with a post-translational conversion of the cell-surface glycosylphosphatidylinositol-anchored protein PrP^{C} (or Pr^{Psen}) to a partially protease-resistant isoform denoted PrP^{Sc} (or Pr^{Pres}). Recent work has disclosed efforts towards obtaining small drug-like molecules that interact with human PrP^{C} ($huPrP^{C}$) and/or reduce levels of $PrPS^{c}$ in persistently infected cells, with the aim of identifying novel prion disease therapeutics. A vHTS study, followed by the screening of sourced compounds by surface plasmon resonance (SPR) [3], led to the identification of structure (i) as binding to $huPrP^{C}$ [4]. Further, 5-aminooxazole derivative (ii) also showed binding to $huPrP^{C}$. In following up these results, these workers designed a synthetic route to allow access to a library of amide derivatives at the 5-position of aminothiazoles and 5-aminooxazoles through derivatization of the free 5-amino compound, depicted in generic chemotype (iii). A solution phase library of 18 compounds was synthesized as singletons based on the 5-aminothiazole template (iii; X = S) and 20 compounds based on the 5-aminooxazole template (iii; X = O). Compounds synthesized were then screened with SPR using a BIAcore 3000 (BIAcore, Uppsala, Sweden) equipped with a CM5 sensor chip (carboxymethylateddextran) [3]. Interactions were measured with two forms of prion protein, full-length human ($huPrP^{C}$) and full-length murine ($moPrP^{C}$). Binding affinities are expressed as %RUmax. That is, as a percentage of the theoretical maximum response for 1:1 protein–ligand binding. Compounds were also screened for inhibition of PrP^{Sc} formation in SMB cells of mesodermal origin. From these libraries, several binders to $huPrP^{c}$ and $moPrP^{c}$ were obtained. One of the compounds with the highest binding affinity was (iv), which possessed a %RUmax of 48% for $huPrP^{c}$. Although it was not possible to establish any direct correlation between PrP^{C} binding affinity and cell line activity from this work, it is encouraging to note that all of the active compounds reported in this work showed binding to the full-length human PrP^{C} and all but one to the murine protein. This work is therefore of interest because we have here the first reported instance of antiprion activity in compounds of this type exemplified by chemotype (iii). A number of compounds synthesized as part of the library were found to bind to PrP^{C}. Further work in this area warranted as chemotype (iii) is of interest for further development, with a view to both improving activity and establishing more SAR in these series (X = S, O).



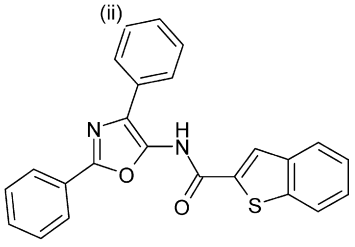
(i)



(iii: X = S, O)



(ii)

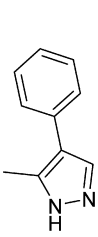


(iv)

1359-6446/06/$ - see front matter © 2008 Published by Elsevier Ltd. doi:10.1016/j.drudis.2008.02.004

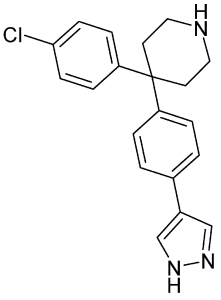
Inhibitors of protein kinase B using fragment-based lead discovery

The serine/threonine kinase protein kinase B (PKB or Akt) plays a central role in the regulation of a variety of signal transduction pathways necessary for the control of cell growth, differentiation and division [5]. Overexpression and disregulation of PKB has been demonstrated in a number of human malignancies, thus giving a potential mechanism for the survival and progression of tumour cells [5]. Thus, inhibition of PKB is considered to be a potentially valuable approach to the treatment of cancers. One of the most oft-used strategies for inhibiting PKB is targeting of the kinase domain by competitive inhibition of the ATP binding site. The recent availability of the three-dimensional crystal structure of active, phosphorylated PKBβ (Akt-2) [6] has provided further opportunities for the development of novel ATP-competitive inhibitors. Its application, however, to iterative structure-based design has, so far, been limited by the lack of a robust, soakable crystal form. Methodology and technology to aid in the production of X-ray crystal structures with small molecule inhibitors soaked in would, therefore, be of value. Recent work has disclosed the application of X-ray crystallography for the rapid development of a potent series of ATP competitive inhibitors of PKB [7]. Fragment screening [8] was carried out using the 'Pyramid' approach [9]. This approach identified fragment (v) as an X-ray hit when it was soaked into crystals of PKA-PKB. Inspection of how the fragment binds suggested ideas for elaboration of the inhibitor, allowing a concomitant increase in potency. Several iterative rounds of compound synthesis, biological testing, soaking and X-ray analysis on a PKA-PKB 'chimera' followed by inhibitor inspection and further design led to a number of potent inhibitors of PKB. One of the most potent was (vi), which possessed an IC_{50} against PKB of 18 nM. The use of fragment-based drug discovery facilitated the rapid identification of novel, low molecular weight PKB/Akt inhibitors. By applying protein–ligand crystallography to iterative rounds of structure based design, elaboration of the initial hit was successfully carried out to provide nanomolar PKBβ inhibitors. Further work in this area is warranted in further improving the properties of this series of inhibitors.



(v)

PKB IC_{50} = 80 μM



(vi)

PKB IC_{50} = 18 nM

1 Prusiner, S.B. (1998) Prions. *Proc. Natl. Acad. Sci. U. S. A.* 95, 13363–13383

2 Brown, P. *et al.* (2000) Iatrogenic Creutzfeldt-Jakob disease at the millennium. *Neurology* 55, 1075–1081

3 Touil, F. *et al.* (2006) Screening a library of potential prion disease therapeutics against cellular prion proteins and studying their mode of biological activities by surface plasmon resonance. *J. Pharm. Biomed. Anal.* 40, 822–832

4 Heal, W. *et al.* (2007) Library synthesis and screening: 2, 4-diphenylthiazoles and 2,4-diphenyloxazoles as potential novel prion disease therapeutics. *J. Med. Chem.* 50, 1347–1353

5 Hill, M.M. and Hemmings, B.A. (2002) Inhibition of protein kinase B/Akt. Implications for cancer therapy. *Pharmacol. Ther.* 93, 243–251

6 Yang, J. *et al.* (2002) Crystal structure of an activated Akt/protein kinase B ternary complex with GSK3-peptide and AMP-PNP. *Nat. Struct. Biol.* 9, 940–944

7 Saxty, G. *et al.* (2007) Identification of inhibitors of protein kinase B using fragment-based lead discovery. *J. Med. Chem.* 50, 2293–2296

8 Rees, D.C. *et al.* (2004) Fragment-based lead discovery. *Nat. Rev. Drug Discov.* 3, 660–672

9 Hartshorn, M.J. *et al.* (2005) Fragment-based lead discovery using X-ray crystallography. *J. Med. Chem.* 48, 403–413

P. Edwards

paul.edwards@graffinity.com