Chemistry

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A comparison of commercially available software for the prediction of pKa

J. C. Dearden, M. T. D. Cronin and D. C. Lappin

School of Pharmacy & Chemistry, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK. E-mail: j.c.dearden@ljmu.ac.uk

Objectives The pKa value of a drug is a key factor in its solubility and other ADME properties, including receptor-binding. Consequently, numerous attempts have been made to predict pKa values from structural properties. Most of these attempts have been based on the Hammett constant, and so have involved congeneric series. There have, however, been a few attempts, such as those of Klopman & Fercu (1994) and of Klamt et al (2003), to model pKa values of diverse data-sets. Recently, commercial software has become available for the prediction of pKa values. However, no comparison of the performance of pKa software has been published. In an attempt to rectify this situation, we have tested the predictive ability of ten such software programs, using a large test-set.

Methods Our test-set comprised 653 compounds, and included a large number of drugs. There were about 40 tautomeric compounds in the test-set. Many of the compounds had multiple ionisation sites, but we used only the primary measured pKa values, as these were considered to be the most accurate. In addition, some of the software programs calculated only the primary pKa value of a compound. The software programs tested were: ACD/Labs (www.acdlabs.com), ADME Boxes (www.ap-algorithms.com), ADMET Predictor (www.simulationsplus.com), ChemAxon (www.chemaxon.com), CSpKa (www.chemsilico.com), PALLAS (www.compudrug.com), Pipeline Pilot (www.scitegic.com), QikProp (www.schrodinger.com), SPARC (ibmlc2.chem.uga.edu/sparc) and VCCLAB (www.vcclab.org). Two of the software programs (SPARC and VCCLAB) are freely usable on-line, and two programs were already in use in our laboratory. Results from the other software programs were kindly provided by the software companies.

Results Some of the programs did not predict pKa values for all of the test-set compounds. The predictive abilities of the ten programs are shown in Table 1. The r^2 value is the coefficient of determination for the correlation between observed and predicted pKa values, and MAE is the mean absolute error of prediction.

Conclusions There is wide variation between the predictive abilities of the software programs. The weak performance of the CSpKa software could be due, in part at least, to the fact that this was the only software for which we could be certain that none of our test-set compounds was in the training set used to develop each software program. If a calculated pKa is required, it is recommended that predictions be obtained from three sources, and the mean taken.

Table 1 The predictive abilities of the ten programs

Software	No of compounds predicted	r^2	MAE
ADME Boxes	627	0.959	0.32
VCCLAB	610	0.931	0.40
Pipeline Pilot ADMET	626	0.852	0.43
Predictor	653	0.899	0.67
SPARC	644	0.846	0.78
ChemAxon	653	0.778	0.90
QikProp	645	0.768	0.93
ACD/Labs	644	0.678	1.07
PALLAS	646	0.656	1.17
CSpKa	642	0.565	1.48

r^2 value, coefficient of determination for the correlation between observed and predicted pKa values; MAE, mean absolute error of prediction.

Klamt, A., et al (2003) *J. Phys. Chem. A* **107**: 9380–9386
Klopman, G., Fercu, D. (1994) *J. Comput. Chem.* **15**: 1041–1050

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The design, synthesis and biological evaluation of novel prodrugs for the treatment of cystinosis

G. Kay, D. Cairns, B. McCaughan and B. Warasiha

The Robert Gordon University, School of Pharmacy, Schoohill, Aberdeen, AB10 1FR, UK. E-mail: g.kay@rgu.ac.uk

Objectives Cystinosis is a rare autosomal recessive disease characterised by raised intracellular levels of the amino acid, cystine. Treatment for cystinosis is the administration of cysteamine, an aminothiol that possesses an offensive taste and smell; it and its metabolites are excreted in breath and sweat causing halitosis and body odour. It also causes gastric irritation. As a result, patient compliance may be poor (Cairns et al 2002). The purpose of this investigation was to synthesise, characterise

and biologically evaluate a series of odourless and tasteless prodrug forms of cysteamine.

Methods A library of water insoluble fatty amide prodrugs of cysteamine (and its disulphide cystamine) was synthesised. Both solid and solution phase organic techniques were employed. The compounds were purified by multiple recrystalisations and then fully characterised by mass spectroscopy and ^{1}H NMR. Their cytotoxic effect on MCF-7 cells was evaluated by Alamar blue assay. Due to the insoluble nature of the prodrugs final drug concentrations in the assay were 0.3 μM. Assays were carried out in triplicate (Table 1).

Results See Table 1.

Conclusions A number of water insoluble fatty (alkyl chain length 10–18) amide prodrugs of cysteamine were synthesised and fully characterised. Their toxicological effect was evaluated. The results indicated that at 0.3 μM the toxic effect of these compounds was similar to that of the vehicle alone; therefore, it may be concluded that the inhibition of growth was due to the challenge presented to the cells by the DMSO and not the prodrugs, at this concentration. Further experiments examining the effect of more concentrated solutions are on-going. Moreover, the prodrugs are now progressing through in-vitro proof of concept studies.

Table 1 Percentage change in cell growth, Alamar blue assay when compared to the control

	12 h	24 h	48 h	96 h
DMSO	63.90	76.99	83.85	86.05
CD	50.10	63.38	69.42	80.96
CL	51.04	63.83	69.86	76.73
CP	69.73	76.10	81.71	88.24
CPD	47.10	62.66	72.84	79.42
CS	57.60	68.51	74.27	84.53

Cystamine Decanoate (CD), Cystamine Pentadecanoate (CPD), Cystamine Stearate (CS), Cystamine Laurate (CL) and Cystamine Palmitate (CP).

Cairns, D., et al (2002) *J. Pharm.* **269**: 615–616

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Predicting relative binding affinities for the ER-α estrogen receptor from molecular structure

T. Suzuki[1] and S. Shapiro[2]

[1]Natural Science Laboratory, Toyo University, 5-28-20 Hakusan, Bunkyo-ku, Tokyo 112-8606, Japan and [2]Basilea Pharmaceutica AG, Grenzacherstrasse 487, Postfach, CH-4005 Basel, Switzerland. E-mail: suzuki@toyonet.toyo.ac.jp

Objectives Estrogen receptors are extensively characterised pharmaceutical targets for which many ligand analogues have been identified. Steroids and other compounds that interact with hormone receptors also can form inclusion complexes with natural or modified β-cyclodextrins (CDs) in aqueous milieux. Despite differences in cavity size and shape between receptor and β-CDs, similar steric and energetic interactions are likely to drive both 17β-estradiol complexation by β-CD and 17β-estradiol binding by the estrogen receptor ER-α, in which case (xeno)estrogen binding by β-CD may be able to serve as a predictive surrogate for ER-α binding. 2D- and 3D-QSAR/QSPR have been applied in an effort to identify descriptors governing interactions in β-CD:(xeno)extrogen inclusion complexes and to predict the thermodynamic stability of these complexes (Katrizky et al 2004). Nonlinear group contribution models (GCMs) for calculating binding constants or free energies of complexation of guest molecules with natural α- and β-CDs have been reported (Suzuki 2001). Here we present a group contribution method (GCM) for *in silico* screening the potential estrogenic activity of chemicals as a practical alternative to receptor-ligand binding assays and animal experimentation.

Methods Binding affinities for rat uterine cytosolic estrogen receptor ER-α were determined by competitive binding assay with [^{3}H]17β-estradiol (E_2) (Shi et al 2001). The relative binding affinity (RBA) for a compound "X" is defined as 100 times the molar ratio E_2/X required to decrease E_2 binding by 50%. It was assumed that RBA can be expressed as:

$$\log_{10} \mathrm{RBA} = c_1 \cdot D + c_2 \cdot D^2 + \Sigma(n_i \cdot G_i) + c_0 \quad (1)$$

where D is a molecular descriptor representing molecular bulk or size, c_1 and c_2 are regression coefficients for D and D^2, respectively, G_i is value assigned to ligand structural fragment i, n_i is the number of times molecular fragment i occurs in the ligand, and c_0 is a regression constant.

Results A GCM for calculating $\log_{10}$ RBA values of structurally diverse ligands for ER-α was developed. Least squares regression was used to derive a quadratic correlation equation containing the first-order molecular connectivity index $^1\chi$ as a measure of molecular bulk and weighted structural fragments as a measure of molecular forces between the receptor and ligand. This model showed a good correlation ($r^2 = 0.82$) between experimental and calculated $\log_{10}$ RBAs for a training set comprised of 129 compounds. Similarities between ER-α:(xeno)estrogen binding and formation of β-cyclodextrin:(xeno)estrogen inclusion complexes were explored by comparing experimental free energies of (xeno)estrogens bound to ER-α with those for the same ligands complexed with natural and 2-hydroxpropyl-β-cyclodextrin. Correlations with r^2 values of ~0.6 were obtained for the receptor and β-cyclodextrin systems.

Conclusions A GCM for predicting the estrogenic activity of chemicals was developed. Results from a large training set demonstrated that prediction of RBAs of molecules for rat uterine ER-α can be achieved with reasonable accuracy using the first-order molecular connectivity index $^1\chi$ in combination with molecular fragments. This method should prove of great value for preliminary screening of chemicals for their potential to cause endocrine disruption.

Katrizky, A. R., et al (2004) *J. Chem. Inf. Comput. Sci*. **44**: 529–541
Shi, L. M., et al (2001) *J. Chem. Inf. Comput. Sci*. **41**: 186–195
Suzuki, T. (2001) *J. Chem. Inf. Comput. Sci*. **41**: 1266–1273

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Design, synthesis and biological evaluation of novel DNA binding agents

G. Kay[1], M. Melville[1], B. Warasiha[1] and D. J. Mincher[2]

[1]The Robert Gordon University, School of Pharmacy, Schoolhill, Aberdeen, AB10 1FR and [2]Napier University, School of Life Sciences, 10 Colinton Road, Edinburgh, EH10 5DT, UK. E-mail: g.kay@rgu.ac.uk

Objectives A large number of compounds are known that bind to DNA in various ways. The ability of medicinal chemists to manipulate molecules to enhance DNA-binding affinity makes DNA a viable target for chemotherapeutic intervention. The aim of this research was the design and synthesis of novel aminoanthraquinones and the subsequent *in vitro* evaluation of their DNA binding and cytotoxic effect.

Methods A number of amino substituted anthraquinones were synthesised and fully characterised by mass spectrometry and ^{1}H MMR. Spectrofluorimetry was used to determine the mode of binding to DNA. The measure of the ability to bind to DNA was expressed as either a QE_{50} or QH_{50} value: the concentration required to reduce the initial fluorescence intensity of the DNA-bound ethidium bromide (QE_{50}) (intercalative binding) or Hoechst stain (QH_{50}) (groove binding) complexes by 50%. The compounds ability to inhibit the growth of MCF-7 (breast cancer) cells was determined using an Alamar blue assay.

Results All compounds were more potent groove binders than intercalators (Table 1). Their QH_{50} values were similar to the clinically active comparator, netropsin. Their cytotoxic effect was determined via an Alamar Blue assay. It was apparent that, in general, all compounds did not display significant cytotoxicity at 3 μM; however, GP3 displayed 63% growth inhibition (20 nM doxorubicin 75%) of the MCF-7 cells.

Table 1 Summary DNA-Binding data

Compound	QE_{50}	QH_{50}
Mitoxantrone	0.4	—
Netropsin	—	0.7
NU:UB 131	1.6	0.7
NU:UB 132	0.9	0.5
NU:UB 136	1.0	0.7
GP3	3.6	1.2

mean values, n = 3.

Conclusions A number of amino substituted anthraquinones were synthesised and fully characterised by mass spectrometry and ^{1}H NMR. Their cytotoxic effect was evaluated. The results indicated that compound GP3 was the most potent in terms of growth inhibition. Furthermore, GP3 was the least potent in terms of DNA binding, suggesting that its cytotoxic effect may not be due solely to DNA binding.

Interestingly, GP3 has a different substitution pattern, substituted in the 2 position of the anthraquinone ring, whereas the other analogues are substituted in the 1 position. Further studies are on-going to elucidate GP3's mechanism of growth inhibition / cell kill.

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In silico screening of non-kinase inhibitors of c-Src-SH3: targeting protein-protein interactions

Noor Atatreh[1], Sally Freeman[1], Caroline Dive[2] and Richard A. Bryce[1]

[1]School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Manchester, M13 9PL and [2]Paterson Institute for Cancer Research, University of Manchester, Manchester, M20 4BX, UK.
E-mail: noor.atatreh@postgrad.manchester.ac.uk

Objectives Src signalling and transduction is directly involved in the processes of cell growth, cell cycle, malignant transformation and cell migration. It is linked to different diseases, including epithelial cancers as well as osteoporosis. c-Src levels are elevated in colon cancer cells compared to non-malignant cells (Frame 2002). c-Src consists of an N-terminus connected to the cytoplasmic membrane, followed by two non-catalytic SH3 and SH2 domains, SH2-kinase linker segment, tyrosine kinase domain and catalytic C-terminus (Williams et al 1997). However, component sequences and domains of the Src primary structure among the SFKs have shown some diversity and the noncatalytic SH3 and SH2 domains have been discovered in many other intracellular proteins.

Methods Here, *in silico* screening for the discovery of non-kinase inhibitors of c-Src is employed, focusing on interference of Src-SH3 protein-protein interactions. Based on the recently solved crystal structure of c-Src in its active conformation (PDB code 1Y57), the proline binding site on the SH3 domain was targeted (Figure 1). The genetic algorithm-based docking program GOLD was used to screen the ZINC 3D compound database.

Results Virtual screening was followed by several post-docking analyses involving considerations of solubility, lipophilicity, polarity, key functional groups and active site interactions. The initial 2.7 million compounds (comprising the ZINC database) were reduced to 3500 potential hits. After filtering, a set of compounds have been selected for future biological evaluation and 16 have been obtained to test their ability to inhibit Src-SH3 domain interaction with small peptide ligands.

Conclusion Computer-aided molecular design (CAMD) was pursued to screen for novel non-kinase Src inhibitors. Initially, several potential hits were discovered and are expected to have potential binding capabilities. These compounds will be the focus of future biological analyses with Src, inhibitors of which may be of importance for cancer chemotherapy.

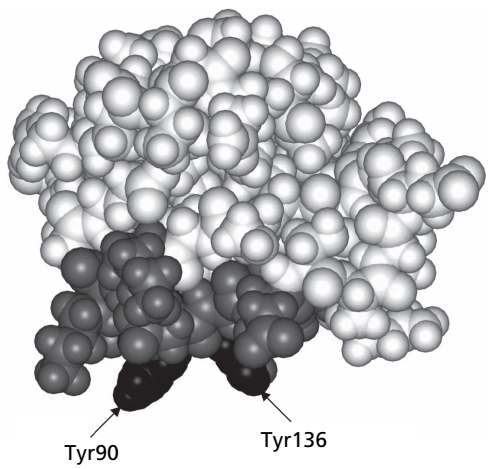

Figure 1 SH3 domain in active Src conformation (PDB code 1Y57, resolution 1.91 Å). Docking active site (dark grey). Tyr90 and Tyr136 that form the proline-rich ligand binding pocket (black).

Frame, M. C. (2002) *Biochim. Biophys. Acta* **1602**: 114–130
Williams, J. C., et al (1997) *J. Mol. Biol*. **274**: 757–775

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Design and synthesis of 5-(4-substituted benzyl)-2-methylsulfonyl-1,3,4-oxadiazoles: a novel class of cyclooxygenase-2 inhibitors

B. Mokarami[1] and A. Zarghi[2,1]

[1]Tehran Azad University, Pharmaceutical Sciences Dept., No. 99, Gholhak St., Shariati Ave, Tehran, 19419 and [2]Shahid Beheshti University, Pharmaceutical Sciences Dept., Tavanir St, Tehran, 19395, Iran. E-mail: golbahari@yahoo.com

Objectives Cyclooxygenase (COX) inhibitors, such as rofecoxib and celecoxib, which selectively inhibit the inducible COX-2 isozyme that causes inflammation, rather than the constitutive COX-1 isozyme that provides gastroprotection and maintains vascular homeostasis, are clinically effective nonulcerogenic anti-inflammatory drugs. Accordingly, there is still a need for the design of COX-2 inhibitors with a greater safety profile for the treatment of arthritis (Zarghi et al 2007). In this regard, new 1,3,4-oxadiazole derivatives (Figure1) were designed and synthesized as COX-2 selective inhibitors.

Methods The docking study was performed using Autodock tools software. The quality of the docked structures was evaluated by measuring the intermolecular energy of the ligand-enzyme assembly. The synthesis was started from 4-substituted phenyl acetic acid. After esterification of this acid by methanol, the obtained ester was converted to corresponding hydrazide by reaction with hydrazine hydrate in DMF. The reaction of hydrazide with carbon disulfide under basic condition gave 5-(4-substituted benzyl)-2-mercapto-1,3,4-oxadiazole. Alkylation with suitable alkyl halide and then oxidation by oxone afforded 5-(4-substituted benzyl)-2-methylsulfonyl-1,3,4-oxadiazole products. Molecular structures of the synthesized compounds were determined by IR, ^{1}H NMR, Mass spectra and CHN analysis. The ability of the test compounds to inhibit COX-1 and COX-2 was determined using an enzyme immuno assay (EIA) kit.

Results A group of 5-(4-substituted benzyl)-2-methylsulfonyl-1,3,4-oxadiazoles were synthesized and the substituents on the C-4 benzyl moiety were simultaneously varied (H,Me,OMe,F,Cl) to determine the combined effects of steric and electronic substituent properties upon COX-1 and COX-2 inhibitory potency and selectivity.COX-1/COX-2 inhibition studies showed that the compounds possessing fluorine or methyl substituent at para position of benzyl moiety had the best potency and selectivity as COX-2 inhibitors. Moreover, molecular modelling studies display that the methylsulfonyl pharmacophore group is oriented into COX-2 secondary pocket.

Conclusions This study indicates that the oxadiazole moiety is a suitable template to design COX-1/COX-2 inhibitors. In this novel class of oxadiazoles, the methylsulfonyl group proved to be a suitable COX-2 pharmacophore and COX-1/COX-2 inhibition was sensitive to the nature of the substituents at para position of benzyl ring.

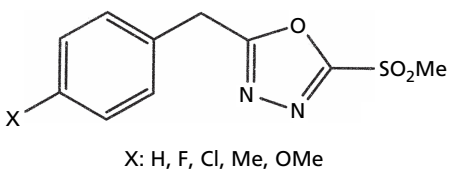

Figure 1 1,3,4-Oxadiazole derivatives.

Zarghi, A., et al (2007) *J. Pharm. Pharm. Sci*. **10**: 29–37

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Design and synthesis of new diaryl sulfonamides as selective COX-2 inhibitors

Parisa Tabaraki[1,2] and Afshin Zarghi[2,1]

[1]School of Pharmacy, Azad University, Yasaman Alley, Yakhchal St., Shariati Ave, Tehran, P.O. BOX: 19419 and [2]School of Pharmacy, Shaheed Beheshti University, 10, Shams Alley, Vali-e-Asr Ave,Tehran, P.O. BOX: 14155-6153, Iran.
E-mail: parisa_t1981@yahoo.com

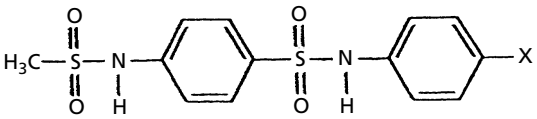
Objectives Nonsteroidal anti-inflammatory drugs (NSAIDs) represent the standard therapy for the management of inflammation and pain. However, because NSAIDs inhibit both isoforms of cyclooxygenase, they are associated with well-known side effects at the gastrointestinal level. On the other hand, selective COX-2 inhibitors can provide anti-inflammatory agents devoid of the undesirable effects associated with classical, nonselective NSAIDs (Biava et al 2005). In this regard, new diaryl sulfonamides were designed and synthesized as selective COX-2 inhibitors.

Methods The docking experiment was performed using Autodock tools software. The quality of the docked structures was evaluated by measuring the

intermolecular energy of the ligand-enzyme assembly. The synthesis was started from p-acetamidobenzenesulfonylchloride. The nucleophilic reaction of this compound with the appropriate aromatic amine in the presence of triethylamine afforded the sulfonamide intermediate. The acetamido group was transformed into the corresponding amine using acidic hydrolysis, which on subsequent reaction with methanesulfonylchloride afforded the expected product. Molecular structures of the synthesized compounds were determined by IR, ^{1}H NMR, Mass spectra and CHN analysis. The ability of the test compounds to inhibit COX-1 and COX-2 was determined using an enzyme immuno assay (EIA) kit.

Results A group of p-methanesulfonamido-N-(phenyl) benzenesulfonamides was synthesized and the substituents on the C-4 phenyl ring were simultaneously varied (H, Me, OMe, F, Cl) to determine the combined effects of steric and electronic substituent properties upon COX-1 and COX-2 inhibitory potency and selectivity. COX-1/COX-2 inhibition studies showed that some of these compounds are potent selective COX-2 inhibitors. Moreover, molecular modelling studies showed that the p-$MeSO_2NH$ pharmacophore is well oriented into the COX-2 secondary pocket and one of the O-atoms of the p-$MeSO_2NH$ moiety can form a hydrogen bond with Tyr^{355}.

Conclusions This study indicates that the sulfonamide moiety is a suitable scaffold to design COX-1/COX-2 inhibitors. In this novel class of diaryl sulfonamides, the p-$MeSO_2NH$ group proved to be a suitable COX-2 pharmacophore and COX-1/COX-2 inhibition was sensitive to the electronic nature of the substituents on the phenyl ring.



X = H, Me, OMe, F, Cl

Biava, M., et al (2005) *J. Med. Chem*. **48**: 3428–3432