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Quantitative structure-activity relationship analysis of 5-[[2-(ω-carboxyalkoxy)aryl]methylene]-4-oxo-2-thioxothiazolidine derivatives as aldose reductase inhibitor: a Fujita-Ban and Hansch approach

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Discovery of a selective, potent and safe inhibitor of aldose reductase (AR) capable of potentially blocking the excess glucose flux through the polyol pathway that prevails under diabetic condition has been a long standing challenge. In response, we did a quantitative structure-activity relationship (QSAR) study, based on Fujita-Ban and classical Hansch approach (Hansch & Leo 1979), performed on 5-[[2-(ω-carboxyalkoxy)aryl]methylene]-4-oxo-2-thioxothiazolidine derivatives (Murata et al 1999) to gain structural insight into the binding mode of the molecules to the aldose reductase enzyme. The biological activity data (IC_{50} in μM) was converted to negative logarithmic mole dose (pIC_{50}) for generation of quantitative expressions employing VALSTAT (Gupta et al 2004). Two subsets of the series were subjected to Fujita-Ban approach in order to estimate the de novo contribution of substituents to the activity of the molecules as

$$pIC_{50} = 6.686 + 0.907[Br] + 0.790[Cl] + 0.539[MeO] + 0.463[Me] + 0.070[CH_2COOH]$$
$$n = 12,\ r = 0.871,\ r^2 = 0.759,\ SE = 0.322 \quad (1)$$

$$pIC_{50} = 6.440 - 0.766[Me] + 1.056[N_1] + 1.022[N_2]$$
$$n = 6,\ r = 0.842,\ r^2 = 0.708,\ SE = 0.691 \quad (2)$$

The subsets were combined to find out the importance of benzylidine/naphthylmethylene ring system.

$$pIC_{50} = 7.055 + 0.906[Br] + 0.789[Cl] + 0.538[MeO] + 0.049[Me] - 0.137[CH_2COOH] + 0.199[Ring] - 0.361[N_1] - 1.023[N_3]$$
$$n = 18,\ r = 0.812,\ r^2 = 0.660,\ SE = 0.538 \quad (3)$$

The Fujita-Ban analysis of first subset of the series, suggested that electro-negative substituents at R position i.e. Br and Cl group is crucial for activity. At R position, these groups might be imparting for the electronic interaction with the receptor. The less favorable effect of MeO may be attributed to electron-releasing nature of it. Comparison of subset of equations revealed that, benzylidine thiazolidine derivatives might be having the optimum shape and size in comparison to the naphthylmethylene thiazolidine derivatives for the interaction with the receptor. The combined model depicted that benzylidine ring is favorable for the activity. Using the Hansch approach we correlate the activity of 5-(2-carboxymethoxybenzylidine) thiazolidine derivatives as,

$$pIC_{50} = 6.690 + 2.000[F] + 0.266[Iv]$$
$$n = 12,\ r = 0.798,\ r^2 = 0.637,\ SE = 0.322,$$
$$F = 7.907,\ ICAP < 0.001,\ Chance < 0.015 \quad (4)$$

The *F* in this equation refers to the electronic parameter Swain Lupton field constant and I_V is indicator parameters used for R_1-substituents. I_V taken as Zero for $R_1 = H$ and 1 for any other substituent. Eqn-4 expresses that an R_1-substituent will have positive effect on the activity. The positive coefficient of Swain-Lupton field constant signifies the role of electronic interaction in inhibition of aldose reductase. The QSAR analysis gave insight to some common important structural feature (i.e. presence of substitution at nitrogen atom of thiazolidine ring is important for ARI activity and could interact through hydrogen bond formation with the enzyme while electron-withdrawing group at benzylidene ring of nucleus play a significant role in the interaction with enzyme). De novo analysis inferred that benzylidene ring can be explored for optimization of the analogs.

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Rationalization of physicochemical characters of 1,5-diarylpyrazole analogs as dual (COX-2/LOX-5) inhibitors: A QSAR approach

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Arachidonic acid metabolizing enzymes cyclooxygenases and lipoxygenases lead to the formation of various eicosanoids involved in variety of human diseases, like inflamma-

tion, fever, pain, rheumatic and osteoarthritis, etc. (Charlier et al 2003). Non-steroidal anti-inflammatory drugs are useful tools in the treatment of prostaglandin mediated complications. The development of dual inhibitors may prevent a drift of arachidonic acid metabolism towards the other pathway, leading to potential side effects. Hence emphasis was focused on quantification of structure activity relationship, with a view to delineating the influence of key COX-2/LOX-5 activity, to explore structural insights to aid the designing of safer dual inhibitors. The inhibitory activity data (IC_{50} in μM) of 1,5-diarylpyrazole derivatives (Pommery et al 2004) were converted to negative logarithmic mole dose (pIC_{50}) for QSAR analysis. The energy minimization was performed through MM2 force field and semi-empirical modeling application MOPAC-2000. Each minimized structure was added to a molecular database to compute various physicochemical properties using Dragon program while the regression analysis was carried out on VALSTAT (Gupta et al 2004). Rationalization of physicochemical properties with COX-2 inhibitory activity gave statistically significant model (eqn 1).

$$\begin{aligned} pIC_{50(COX-2)} &= 1.230\,(\pm 0.224)\,Mor14v - .258\,(\pm 0.397) \\ &\quad Mor22m + 9.971\,(\pm 1.508)\,Hy + 13.597 \\ n = 20,\ r = 0.870,\ SEE = 0.233,\ F = 16.591,\ q^2 &= 0.400, \\ S_{DEP} = 0.327,\ r^2_{bs} &= 0.713,\ \text{Chance} < 0.001 \end{aligned} \quad (1)$$

To find any major differences in the properties of the molecules showing COX-2 inhibitory activity over COX-1, we searched physicochemical properties which are responsible for the COX-1 inhibitory activity (eqn. 2)

$$\begin{aligned} pIC_{50(COX-1)} &= -0.307\,(\pm 0.116)\,Mor04u - 5.084\,(\pm 0.779)\,Mor17v + 2.938 \\ n = 14,\ r = 0.893,\ r^2 &= 0.797,\ SEE = 0.438,\ F = 21.552 \end{aligned} \quad (2)$$

In search of the physicochemical properties, responsible for the selectivity of 1,5-diarylpyrazole analogs for COX-2 over COX-1 that is of paramount importance in designing of novel selective COX-2 inhibitors, regression gave significant expression which explain more than 77.6% variance in the selectivity (eqn. 3).

$$\begin{aligned} \log Sel_{(COX-1/COX-2)} &= 5.349\,(\pm 1.596)\,Mor24v \\ &\quad + 23.949\,(\pm 3.924)\,Hy + 16.859 \\ n = 14,\ r = 0.881,\ SEE = 0.579,\ F = 19.048,\ q^2 &= 0.648, \\ S_{DEP} = 0.643,\ r^2_{bs} &= 0.795,\ \text{Chance} < 0.001 \end{aligned} \quad (3)$$

In case of LOX-5 inhibitory activity model showed correlation coefficient value equivalent to 0.941, which explained 88.5% variance in the activity (eqn. 4).

$$\begin{aligned} pIC_{50(LOX-5)} &= 1.670\,(\pm 0.278)\,Mor17v \\ &\quad - 0.314\,(\pm 0.076)\,Mor11m + 7.572 \\ n = 11,\ r = 0.941,\ SEE = 0.114,\ F = 30.890,\ q^2 &= 0.729, \\ S_{DEP} = 0.150,\ r^2_{bs} &= 0.591,\ \text{Chance} < 0.001 \end{aligned} \quad (4)$$

The QSAR analysis gave insight to some common important structural feature i.e. hydrophilic factor (*Hy*) contributed positively to the COX-2 activity as well as COX-1/COX-2 selectivity ratio. *Mor17v* (3D molecular representation of structure based on electron diffraction code) is the dominant structure feature, which is decisive in explaining the LOX-5 inhibitory activity and also played key role in explaining the COX-1 inhibitory activity, but contributed negatively to it. These structure features may be helpful in development of more selective and potent dual COX-2/LOX-5 inhibitors.

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QSAR analysis of indazole estrogens as selective β-estrogen receptor ligands: rationalization of physicochemical properties

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Estrogen has many important physiological and pharmacological activities; recent study showed that two type of receptors are mediators of the estrogenic response (i.e. ER_α and ER_β). The tissue distribution of ER_α and ER_β are quite different; ER_α is predominant in the uterus, liver, mammary gland, bone and cardiovascular system, whereas ER_β is mainly occur in the prostate, ovary and urinary tract (Kuiper et al 1996). Finding a second estrogenic receptor, with distinct tissue distribution and different gene regulation, opens new possibilities for the novel selective ER_β ligands. It might have some of the benefits of traditional hormone therapy without the undesired stimulatory effects on uterine and breast tissues. QSAR analysis may be help in exploring molecular in site, which are responsible for selectivity. The logarithmic data of relative binding affinity and selectivity of some indazole estrogen analogs (De Angelis et al 2005) were considered for QSAR analysis using VALSTAT (Gupta et al 2004). Fujita-Ban analysis of α and β estrogenic activity of indazole analogs inferred that the 3rd position (R_1) of indazole nucleus might be play key role in the development of β estrogen agonist as compare to α estrogen agonist. Substitution of halogen moiety like Iodo (I), Bromo (Br) and Chloro (Cl) at this position would be selective to ward β estrogenic agonist activity. Hansch analysis was performed in order to rationalize substituent constant, which is responsible for β estrogenic activity (Eqn 1). Similarly correlation was explored for α estrogenic agonist activity (Eqn 2).

$$\begin{aligned} ER_\beta &= 1.226\,(\pm 0.255)\,I_{2OH} + 2.877\,(\pm 0.527)\,F_1 - 1.345\,(\pm 0.388) \\ &\quad I_{5Cl} + 2.661\,(\pm 0.544)\,I_{6OH} - 3.826 \\ n = 21,\ r = 0.893,\ SE &= 0.508,\ F = 15.801 \end{aligned} \quad (1)$$

$$\begin{aligned} ER_\alpha &= 0.694\,(\pm 0.204)\,I_{2OH} + 1.773\,(\pm 0.436) \\ &\quad F_1 + 2.211\,(\pm 1.004)\,R_1 + 1.192\,(\pm 0.446)\,I_{6OH} - 2.926 \\ n = 21,\ r = 0.812,\ SE &= 0.417,\ F = 7.752 \end{aligned} \quad (2)$$

Hansch analysis suggested that in multi-variant expression; the three substituents contributed α and β estrogenic activity in similar fashion i.e. indicator variable (I_{2OH}) at R_2 position, indicator variable (I_{6OH}) at R_6 position and electronic parameter ($\mathcal{F}_1$) at R_1 position. While fourth variant is different i.e. indicator variable (I_{5Cl}) at R_5 position contributed negatively to the β estrogenic activity and electronic parameter ($\mathcal{R}_1$) at R_1 position contributed positively to α estrogenic activity. The correlations were sought between $ER_{\beta/\alpha}$ ratio activity and various substituents constants, multi variant model with 21 compounds showed moderate correlation (Eqn 3).

$$\begin{aligned} ER_{\beta/\alpha} &= 1.059\,(\pm 0.517)\,F_1 - 1.900\,(\pm 1.194) \\ &\quad R_1 - 0.562\,(\pm 0.370)\,I_{5Cl} + 1.190\,(\pm 0.526)\,I_{6OH} - 0.285 \\ n = 21,\ r = 0.659,\ SE &= 0.495,\ F = 3.064 \end{aligned} \quad (3)$$

The Eqn 3 did not explain the calculated selectivity of compound no. 7 on z-score test hence after removing of compound regression expression was regenerated (Eqn 4) which showed good correlation.

$$\begin{aligned} ER_{\beta/\alpha} &= 1.372\,(\pm 0.414)\,F_1 - 2.341\,(\pm 0.937)\,R_1 - 0.644\,(\pm 0.289) \\ &\quad I_{5Cl} + 1.369\,(\pm 0.413)\,I_{6OH} - 0.480 \\ n = 20,\ r = 0.805,\ SE &= 0.386,\ F = 6.891 \end{aligned} \quad (4)$$

The quantification of structure activity by *de novo* and Hansch approach suggested that the 3rd position of indazole nucleus (R_1) is decisive for the selectivity of molecules towards the β-estrogenic receptor over the α-estrogenic receptor. The study also depicted that the substitution of polar group at R_1 position might be helpful in the β-estrogenic receptor selectivity ($ER_{\beta/\alpha}$).

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Design and synthesis of novel diacyl lipospermines

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The use of an efficient vector for poly-nucleic acid (genes or siRNA) delivery is one of the determining factors for a successful therapy for difficult-to-treat diseases. Among non-viral gene delivery systems, non-liposomal cationic lipids are promising, non-toxic gene carriers (Ahmed et al 2005). The synthesis of the lipopolyamine

dioctadecylamidoglycyl spermine (DOGS, Transfectam), by Behr et al (1989), as an efficient non-viral transfection agent, encouraged several laboratories to focus on the synthesis of novel cationic lipids based on the naturally occurring polyamine spermine (Blagbrough et al 2003). In an effort to improve the efficiency and control of DNA delivery, we are investigating novel spermine-based cationic lipid formulations within our approach to molecular pharmaceutics for molecular medicine. Our model for designing novel diacyl lipospermine vectors consists of the following three moieties: hydrophobic domain (long aliphatic chain or a steroid of e.g. the cholestane class), linkers (amides, esters, or carbamates) and a cationic head group (polyamine or guanidine). In our synthetic schemes, we used different primary amine protecting groups (e.g. trifluoro-acetyl (via ethyl trifluoroacetate), phthalimide (via *N*-carbethoxyphthalimide) and 2,5-dimethylpyrrole (via hexan-2,5-dione, acetonylacetone)). Then different hydrophobic domains were introduced by reacting these diprotected spermines (e.g. N^1,N^{12}-diphthalimidospermine) with decanoyl chloride and lauroyl chloride, finally removing the protecting group by heating under reflux with ethanolic hydrazine hydrate to yield N^4, N^9-didecanoyl spermine and N^4, N^9-dilauroyl spermine, respectively. In our design to synthesize lipospermines having cholestane-type hydrophobic domains, we reacted N^1, N^{12}-diphthalimidospermine with cholesteryl chloroformate in dichloromethane-triethylamine, which gave a mono-carbamate steroid conjugate. Trying to add another cholesteryl moiety or even another long-chain hydrophobic domain to the remaining free secondary amine functional group was unsuccessful presumably due to the steric effect of the added cholesteryl moiety. We have also investigated different linkers by reacting N^1,N^{12}-diphthalimidospermine with various halo esters (e.g. methyl chloroformate, ethyl chloroacetate) to increase the distance between the carbonyl groups and the central secondary amines of spermine so that their basic character (pK_a) will increase. This may be helpful in increasing the efficiency of DNA condensation and subsequent transfection. We are investigating a series of heterocyclic cationic head groups (e.g. imidazole, benzimidazole, and pyridine) to get a final amine pK_a of around 6.5 (5–7) to assist in swelling via the proton sponge effect. Here the import of protons accompanied by water molecules, and possibly by chloride counter ions, leads to swelling and eventually rupture of the sub-cellular organelle, the endosomal compartment, thus helping to avoid lysosomal degradation and leading to more effective transfection. Therefore, we are encouraged by the promising results we obtained with N^4, N^9-dioleoyl spermine (Ahmed et al 2005), to design and synthesize N^1,N^{12}-di(2-imidazolyl)-N^4,N^9-dioleoyl spermine, N^1,N^{12}-di(2-benzimidazolyl)-N^4,N^9-dioleoyl spermine, and N^1,N^{12}-di(2-pyridyl)-N^4,N^9-dioleoyl spermine.

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Structure-function studies of the Zonula Occludens Toxin C-terminal and N-terminal fragments

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The intestinal epithelial tight junctions play a pivotal role in the paracellular transport of molecules. Zonula Occludens Toxin (Zot) is a minor exotoxin secreted by *Vibrio cholerae* which reversibly modulates ("loosens") the intestinal tight junctions (Fasano et al 1991). The C-terminal fragment of Zot binds a putative receptor found at the cell-cell contacts which mediates a transient increase in paracellular permeability (Di Pierro et al 2001; Lee et al 2003). The objective of this study was to obtain structure-function information for the N- and C-terminal fragments of Zot. The N-terminus of Zot was cloned into pQE80-L (Qiagen) and expressed in *E.coli* strain UT5600 (LBL medium, 22°C) as the His-tagged protein to facilitate nickel affinity purification. The C-terminal fragment was expressed as the maltose binding protein (MBP) fusion product (pMAL-c2x, NEB) in *E.coli* strain TB1 (Terrific Broth, 22°C) and purified by amylose affinity chromatography. Purified protein fragment sequences were confirmed by SDS-PAGE gel analysis and subsequent mass spectrometry. Guanidine.HCl (GuHCl) unfolding of N-terminal fragment was measured by the change in tryptophan (Trp) fluorescence intensity and peak emission wavelength (λex. 295 nm). One transitional shift was observed with a [GuHCl] around 1.8 M, concomitant with a decrease in fluorescence intensity and red shift of the peak emission wavelength; suggesting exposure of Trp residues to the protein surface upon unfolding. Biological activity of the Zot fragments were measured by the transepithelial electrical resistance (TEER) and tight junction reorganization of Caco-2 cell monolayers. Cells were seeded at 1×10^5 cells/ml (DMEM, 10% foetal calf serum, 2 mM glutamax) onto polycarbonate membrane Transwell plates (0.4 μm pore diameter, 12-well, Costar) and grown between 14–21 days until the TEER values were 300–600 ω. Cell monolayers were incubated with ZOT fragments (8.3 μM) for 120 min and washed, TEER measurements made at 20-min intervals up to 30 min post-washing. Surprisingly, neither C- nor N-terminal Zot fragments decreased the cell monolayer TEER. Reorganization of actin and occludin was visualised by immuno-fluorescence cell staining. Caco-2 monolayers were grown on cover slips as above and on day 7 treated with Zot C- and N-terminal fragments (8.3 μM), cytochalasin D (2 μM) and MBP (control) for 90 min. Cells were fixed and stained with FITC-phalloidin (1:500), or incubated with anti-occludin FITC antibody overnight, then rinsed, mounted and viewed by CLSM. No reorganisation of actin filaments was observed, although staining of cells with FITC-labelled Zot C-terminus did suggest a localisation to the cell-cell contacts as previously described (Lee et al 2003). While our data has not confirmed previous work (Di Pierro et al 2001), this may be due to incorrect folding of the C-terminal fragment. Further information will be gained by studying the structure of the Zot C-terminus.

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Synthesis of cyclic sulfoximine mimics of 2-deoxyribosides as enzyme inhibitors in cancer

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Thymidine phosphorylase (TP) is an enzyme associated with angiogenesis where the angiogenic mediator is 2-deoxyribose, the end-product of the phosphorolysis of thymidine; tumour growth relies on angiogenesis to acquire nutrients and oxygen. Poly(ADP-ribose) polymerase-1 (PARP-1) is responsible for cellular DNA repair and high levels in tumors confer resistance to DNA-damaging chemotherapy and hence lead to treatment failure. Inosine-5′-monophosphate dehydrogenase (IMPDH) is involved in the *de novo* biosynthesis of guanine nucleotides, which support the upregulated tumor proliferation. Both PARP-1 and IMPDH use NAD^+ as their substrates, and inhibition of these target enzymes is of great clinical interest in cancer therapy. The sulfoximine functional group is configurationally and chemically stable and possesses a chemical versatility. It is tetrahedral and, when the two carbon substituents attached to the sulfur atom are different, is chiral. The imino nitrogen is weakly basic. This study is focused on the design of potential enzyme inhibitors incorporating the sulfoximine group; they are mimics of 2-deoxyribose and 2-deoxyribonucleotides in which the anomeric carbons are replaced by sulfoximine. This area of sulfoximine chemistry is highly innovative and novel. The syntheses of three target compounds will be described. Lead compound for both a 2-deoxyribose analogue and a NAD^+ transition state analogue, the novel (±)-3-benzyloxytetrahydrothiophene-4-carboxamide was obtained with moderate yield. Dieckmann cyclisation of acrylonitrile and methyl mercaptoacetate with sodium and anhydrous methanol gave rise to (±)-3-oxotetrahydrothiophene-4-carbonitrile (Baraldi et al 1985). Subsequent reduction of the ketone with sodium borohydride in cold ethanol afforded the corresponding hydroxyl compound. Separation of the diastereoisomers was achieved by column chromatography and the stereochemistry was determined by X-ray crystallography of the amide compound, synthesised by acid-hydrolysis of the nitrile. An interesting base-catalysed ring-opened product was observed during attempts of O-benzylation and the reaction mechanisms are discussed. Work is underway to explore different opportunities to develop a cyclic sulfoximine based on this novel lead compound. Approaches to a NAD^+ analogue have been successful, 4*S*,5*S*-4-benzyloxymethyl-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane was obtained in good yield. A model reaction for the synthesis of this substrate analogue was executed via a Mitsunobu reaction, involving a dehydration reaction between the sulfur nucleophile, thiophenol, and the hydroxyl group on the dioxolane compound (Mitsunobu 1981). The sulfoximine moiety was introduced to the resulting condensation product according to literature methods (Okamura & Bolm 2004). Acid-hydrolysis of the acetonide gave the corresponding diol. Intramolecular ring closure mediated by a modified Mitsunobu reaction, using tributylphosphine and 1,1′-(azodicarbonyl)-dipiperidine, was attempted. Current work consists of establishing the stereochemistry at the sulfur centre by means of X-ray crystallography and effecting the synthesis of the target compound using a 3-substituted thiophenol as the nucleophilic component. In conclusion, we have herein described two highly novel and efficient reaction sequences that allow the syntheses of cyclic sulfoximine derivatives of 2-deoxyribosides, the enzyme inhibitory profiles of which will be investigated by biological assays with the corresponding target enzymes.

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Synthesis and in vitro cytotoxicity evaluation of novel iodocryptolepine analogues

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Cryptolepine is a tetracyclic aromatic natural product, isolated from West African shrubs of the Cryptolepis species, consisting of fused indol and quinoline rings. Unusually its synthesis was reported before its isolation from nature. In 1906, Fichter and Boehringer published a synthetic route through their research into new dyestuffs. However it was not until 1929 that Clinquart identified it in extracts of Cryptolepis triangularis from West Africa. In the past, African tribes have used botanical extracts containing cryptolepine as a dye and also for ethnomedical uses. Cryptolepine has in vitro antiplasmodial activity due to a chloroquine-like action, as well as cytotoxic properties that are due to its ability to intercalate into DNA, inhibit topoisomerase II and prevent DNA synthesis in mammalian cells (Wright et al 2001). Previous work by this group has shown that when halogen substituents are incorporated, the cytotoxic activity of cryptolepine against certain cancer cell lines can be increased. For example 11-chlorocryptolepine has an IC50 value of 31 μM whereas the parent natural product displays an IC50 of 67 μM against MAC15A cells (Wright et al 2001) Further studies have shown this aromatic system to have a unique mode of DNA intercalation however there is yet to be a strong rationale relating structure with activity (Lisgarten et al 2002). To investigate further, we have synthesised and evaluated a range of novel halogen substituted cryptolepine derivitaves. In this work we investigated the effect of introducing a bromine or an iodine atom at the 11 position. Target compounds were synthesised using modifications of the methodologies from Takeuchi et al (1992) and Wright et al (2001). Anthranilic acid was converted to 2-(bromoacetamido)-benzoic acid via reaction with bromoacetyl bromide, which was then converted to a 2-[(N-phenylamino)-acetamido] benzoic acid derivative, via nucleophilic substitution using an appropriate aniline analogue. Cyclisation in polyphosphoric acid yielded quindolone derivatives, which were then brominated in neat phosphorus tribromide. These were methylated using methyl iodide in tetramethylene sulfone, which unexpectedly yielded 11-iodocryptolepines due to exchange of bromine by iodine. To achieve the corresponding 11-bromo derivatives, the alternative methylating agent, methyl triflate in toluene, was employed. To date, cytotoxicity studies have been carried out with the 11-iodocryptolepines on MAC15A mouse adenocarcinoma cells using the MTT assay with each determination being carried out in triplicate (Table 1). Despite the relatively large size of the iodine atom, which may be predicted to have an adverse affect on DNA intercalation, these compounds show favourable activity in comparison with other previously tested analogues (Wright et al 2001). Further biophysical studies will be employed to investigate the mode of binding which these molecules have with DNA and hence create a greater understanding of the link between mode and strength of binding and resulting cytotoxicity. Cryptolepine and especially its halogeno analogues continue to be of interest as potential novel anticancer agents. We have shown μM cytotoxic activity in 11-iodocryptolepine derivatives and we aim to produce a fuller profile of these compounds' structure-activity relationships in the near future.

Table 1 Activity of cryptolepine derivatives in the MTT assay against MAC15A cells

Cryptolepine derivative	IC50 μM ± s.d.
Cryptolepine	67 ± 26
11-Iodo	10.6 ± 4.5
7-Chloro, 11-iodo	6.2 ± 0.3
8-Chloro, 11-iodo	5.9 ± 2.6
9-Chloro, 11-iodo	4.9 ± 0.1

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Antibiotics research in the postgenomic age: glycosyltransferases as potential targets for novel cell wall biosynthesis inhibitors

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The continuous rise in bacterial strains resistant to standard anti-infective drugs has created a strong need for the development of novel antibiotics. Complete genome sequences are now available for many human pathogens, providing a wealth of opportunities for the identification of novel targets. On the other hand, making sense of this vast amount of sequence data, annotating individual genes and assigning biological function to the corresponding gene products, still represents an enormous challenge. This is particularly true for protein families that comprise a large number of members with closely related and sometimes overlapping biological function. Glycosyltransferases are glycoprocessing enzymes that catalyse the transfer of a monosaccharide unit from an activated donor, frequently a sugar-nucleotide, onto a suitable acceptor molecule (e.g. a lipid, protein or carbohydrate). At the gene level, more than 14 000 glycosyltransferase sequences in 78 families have been described (Coutinho & Henrissat 1999), but structural data for this class of enzymes is scarce, and our understanding of glycosyltransferase donor/acceptor selectivity remains fragmented. Glycoconjugates resulting from glycosyltransferase activity play a critical role in many biological processes during the bacterial life cycle, and glycosyltransferases therefore constitute promising novel targets for anti-infective therapy. In *Mycobacterium tuberculosis*, for example, the biosynthesis of cell wall lipoarabinomannans requires the concerted action of a host of mannosyltransferases (Briken et al 2004). To date, only a few of these mannosyltransferases have been identified and characterised, and no mannosyltransferase inhibitors are known. As a variety of mycobacterial mannosyltransferases use GDP-mannose as their donor substrate, structural analogues of GDP-mannose are promising tools for the identification and characterisation of presently unknown mycobacterial mannosyltransferases, for the investigation of mannosylation pathways in mycobacteria as well as other pathogens, and as templates for inhibitors. Guided by structural differences in the nucleotide binding domain of different mannosyltransferases as predicted from sequence data (Coutinho & Henrissat 1999), we have designed a series of GDP-mannose analogues as potential mannosyltransferase-selective donor analogues. The structural key feature of these novel non-natural sugar-nucleotides are additional hydrophobic substituents at positions 6 and 8 of the nucleobase. The installation of such substituents is complicated by the presence of multiple functional groups, the relative chemical instability of the glycosidic and pyrophosphate bonds, and the water solubility of the unprotected sugar-nucleotide substrates. To overcome these difficulties, we have developed reaction conditions suitable for palladium-catalysed cross-coupling chemistry (Suzuki-Miyaura, Sonogashira) in aqueous solution. Herein, we report the design and synthesis of these novel GDP-mannose analogues, discuss preliminary results on their bioactivity, and outline potential biological applications.

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In-silico design, synthesis and evaluation of novel inhibitors of thymidine phosphorylase

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Thymidine phosphorylase (TP) catalyses the reversible conversion of thymidine to thymine and 2-D-α-deoxyribose-1-phosphate. TP is identical to platelet-derived endothelial cell growth factor (PD-ECGF), which is implicated in the growth of solid tumours thus making it a target for anti-cancer chemotherapy. The best TP inhibitors (nanomolar) are 5-chloro-6-[1-(2-iminopyrrolidinyl)methyl]uracil (TPI) and 5-bromo-6-[(2-aminoimidazoyl)methyl]uracil. However, these are associated with poor pharmacokinetic properties. In a recent virtual screening study from this laboratory using a homology model of TP, 1-methyl-2,5-dioxo-4-pentyl-4-imidazolidinecarbaldehyde semicarbazone was identified as a non-uracil inhibitor of TP (McNally et al 2003). The availability of the X-ray coordinates of human TP (Norman et al 2004) prompted further virtual screening efforts based on this lead structure. Furthermore, a de-novo approach has suggested furan-based analogues as TP inhibitors. Therefore lead compounds from both approaches have been further investigated. Databases of hydantoin- and imidazole-based structures were prepared from the National Cancer Institute (NCI) and Available Chemical database (ACD). In-silico screening of these databases with the X-ray structure of human TP using flexible ligand docking via DOCK 4.0 identified active site inhibitors [e.g. 3-(2-methylbenzyl)-2,4,5-trioxoimidazolidine-1-acetic acid] of TP, which were higher in potency than the initial lead. These compounds and their analogues were then synthesised by condensation of aromatic amines with urea (heating at reflux for four hours) to give the alkylated urea in good yields (~90%). These ureas in tetrahydrofuran were treated with oxalyl chloride at 0°C to give 1-(2-methylbenzyl)-2,4,5-imidazolidinetriones in good yields (~80%). The imidazole compounds were reacted with ethyl bromoacetate or bromoproprionamide at reflux for approximately 8 h to

obtain the acetate and proprionamide analogues, respectively, in excellent yields (~90%). The desired carboxylic acids were obtained in good yields (~65%) by the acid-catalysed hydrolysis of the ethyl esters. The 3-(2-furyl)-2-mercaptopropenoic acid lead was prepared in two synthetic steps from commercially available 2-furaldehyde. Initial reaction of 2-furaldehyde with rhodanine in glacial acetic acid with stirring at 200°C gave furfuralrhodanine in excellent yield (~98%). Subsequent treatment with 15% aq sodium hydroxide and heating to ~100°C followed by 10% aq hydrochloric acid afforded the desired product in excellent yield (~98%). Biological evaluation of these imidazole and furan analogues (35 compounds) against *E. coli* thymidine phosphorylase has shown that 3-(2,4,5-trioxo-3-phenethyl-imidazolidine)-propionamide (IC_{50} 40 ± 3 μM) (n = 3) and 3-(2-furyl)-2-mercaptopropenoic (IC_{50} 77 ± 8 μM) (n = 3) are novel inhibitors of TP with moderate activity. These inhibitors are not uracil-based and therefore create a new platform for design and synthesis of new thymidine phosphorylase inhibitors with potentially improved pharmacokinetic properties.

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In silico study of human hedgehoge pathway by means of homology modeling and docking

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The Hedgehog system is a conserved signaling system that plays an important role in cell-cell signaling during growth and development. Indian hedgehog (IHH) is expressed in the visceral endoderm both in the visceral yolk sac in vivo and in embryonic stem (ES) cell-derived embryoid bodies. The patterning activity of hedgehog pathway is revealed in ventral forebrain, eye, craniofacial structures and floor plate formation. Loss of function mutations in proteins involved in this pathway results in medial cleft lip and palate, single maxillary incisor, frontal CNS lobe fusion, cyclopia and death. This cascade is initiated by attachment of IHH to its receptor, Patched. Homology modeling and docking in addition to the theory of evolution method was used to predict 3D structures of the proteins and to find proper means to artificially initiate and regulate the cascade in case of mutation. The sequences of the proteins were calculated based on cDNA sequence. (Swiss-Prot accession number: Q14623 for IHH and Q9Y6C5 for patched.) Similarity search via Psi and Phi-BLAST (Altschul et al 1997) yielded two templates for IHH, each covering 50% of the query. Thus, a multi segment approach was selected for IHH. The two segments were carefully studied regarding their folding and family via CATH and SCOP servers. Having found no proper templates from pairwise alignment for patched, threading method was applied through FUGUE (Shi et al 2001). Homology modeling was done by means of MODELLER 8v2 on a Windows PC platform. Before submission to evaluation sites, models were investigated in SPDBViewer and ViewerLite programs checking amino acids making clashes, Phi-Psi angles, secondary structure matching the secondary structure prediction etc. The Models were then sent to evaluation sites such as ERRAT, VERIFY3D, WHAT_CHECK, WHAT_IF and iMOLTALK. Ramchandran plot was also contrived for the models. The models were refined according to evaluation scores. The ERRAT score was as well as 84 and more than 99% of amino acids were in acceptable areas of the Ramachandran plot. Docking was carried out using Hex v4.2. According to Theory of evolution, the active site was proposed to be on amino acids No. 140–150 of the sequence, though, no predispositions or any rotation angle limitations were applied to the docking to double check previous findings and the final complex structure was chosen due to its minimum energy content only. The final docking complex satisfies previous findings according to the possible active sites and orientation of enzyme and substrate. This structure also clarifies that mutations clinically reported to be more harmful have been the mutations occurring in the active sites of these proteins. The active site mapping can be used for further de novo drug design.

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Larger molecules penetrate membranes more readily

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Many QSARs have been developed to model penetration of membranes such as skin (Moss et al 2002). Most of these involve a positive hydrophobicity term and a negative molecular size term, thus suggesting that hydrophobicity aids penetration but that larger molecules penetrate less readily. The latter inference seems intuitively reasonable, since one would expect larger molecules to have to overcome greater resistance to penetration. However, QSAR work by Abraham and co-workers (Abraham et al 1999; Platts et al 2000) indicates that larger molecular size aids membrane penetration. For skin penetration they found:

$$\log K_p = -5.132 + 0.439\,R - 0.489\,\pi^H - 1.478\sum\alpha^H - 3.442\sum\beta^H + 1.941\,V_x$$
$$n = 53 \quad r^2 = 0.958 \quad s = 0.213 \quad F = 213 \qquad (1)$$

where K_p = permeability coefficient, R = excess molar refractivity, π^H = a polarity term, α^H and β^H = hydrogen bond donor and acceptor abilities, respectively, and V_x = the McGowan characteristic molecular volume. A similar correlation was reported for blood-brain barrier penetration (Abraham et al 1999), with the V_x term there having a coefficient of +0.995. Abraham et al (1999) also modelled hydrophobicity with the same descriptors, and found:

$$\log P = 0.088 + 0.562\,R - 1.059\,\pi^H + 0.034\sum\alpha^H - 3.460\sum\beta^H + 3.814\,V_x$$
$$n = 613 \quad r^2 = 0.995 \quad s = 0.116 \quad F = 23162 \qquad (2)$$

where P is the octanol-water partition coefficient. The size term in equation 2 is much larger than is that in equation 1, and we suggest that this is the reason for the negative size term in correlations of penetration versus log P and molecular size. That is, the negative size term in the latter correlations is simply a compensation for the large positive size requirement of log P itself. This requirement is probably related to dispersive interactions. However, in membrane penetration there is an additional factor, namely the diffusion of molecules through the membrane once they have partitioned into it. Since diffusion is inversely proportional to the square root of molecular mass, the overall size contribution to membrane penetration is less positive than is its contribution to partitioning. Platts et al (2000) also correlated penetration into *Nitella* cells. For living cells they found:

$$\log k_{Nit} = -1.969 + 0.516\,R - 1.267\,\pi^H - 3.409\sum\alpha^H - 2.094\sum\beta^H + 0.980\,V_x$$
$$n = 63 \quad r^2 = 0.881 \quad s = 0.462 \quad F = 84 \qquad (3)$$

However, for penetration into dead *Nitella* cells they found:

$$\log k_{Nit(dead)} = -3.103 - 0.319\,V_x$$
$$n = 64 \quad r^2 = 0.934 \quad s = 0.035 \quad F = 876 \qquad (4)$$

They attributed this to penetration into dead *Nitella* cells being a purely diffusion-controlled process, with no specific chemical interactions between molecule and cell, and we concur with this. One can in fact envisage dead *Nitella* cell membranes as having open-pore outer and inner walls filled with the same fluid as that outside the cells. The finding that larger molecules penetrate living membranes more readily has significant implications for drug design.

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Synthesis, kinetic studies and pharmacological screening of colon specific mutual prodrug of 5-aminosalicylic acid with D-glucosamine for inflammatory bowel disease

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Inflammatory bowel disease (IBD) is characterised by chronic inflammation in the mucosal membrane of the small and/ or large intestine. The primary goal of drug therapy for IBD is to reduce inflammation in the colon requiring frequent intake of antiinflammatory drugs at higher doses. 5-Aminosalicylic acid (5-ASA) is very

effective in IBD but it is absorbed so quickly in upper gastrointestinal tract (GIT) that it usually fails to reach the colon leading to significant adverse effects. To achieve successful colonic delivery, a drug needs to be protected from the formidable environment of upper GIT and then be abruptly released into proximal colon. Prodrug approach is one of the important approaches for targeting drugs to colon by the utilization of high activity of certain enzymes in that area relative to non- target tissues for prodrug to drug conversion. This approach has been successfully utilised in sulfasalazine for targeting drugs to colon. But majority of side effects associated with sulfasalazine like hepatotoxicity and severe blood disorders is due to its carrier sulfapyridine. So the need for safer prodrug still remains. This work reports the synthesis, physico-chemical characterisation, in-vitro kinetic studies and pharmacological evaluation of mutual azo prodrug of 5-ASA with nutrient carrier D-glucosamine for its colon targeted delivery, which is safer with better activity than plain 5-ASA. D-Glucosamine was selected as carrier because of its protective and antiinflammatory effect on colonic mucosa. It is reported that the levels of N-acetyl glucosamine and glucosamine synthetase go down during the attacks of ulcerative colitis. Azo prodrug of 5-ASA with D-glucosamine was synthesised by coupling its diazonium salt with salicylic acid (Furniss et al 1978). The structure was confirmed by elemental and spectral analysis. The IR spectra of the synthesised compound showed absorption band in the range of 1500–1480 cm^{-1}, for unsymmetrical p-substituted azo bond where as H^1 NMR spectra showed chemical shifts for protons of tetrahydropyran and aromatic ring, which are characteristic of anticipated structure. In-vitro kinetic studies in HCl buffer (pH 1.2) showed negligible release of 5-ASA whereas in phosphate buffer (pH 7.4) only 18% release was observed over a period of 7 h. In rat faecal matter, the release of 5-ASA was almost complete (89.6%) with a half-life of 163 min, following zero order kinetics. Biological evaluation of the synthesized compound was carried out in the Department of Pharmacology, Poona College of Pharmacy and its animal facility is approved by CPCSEA (Reg. No. 100/ 1999/ CPCSEA). The experimental protocols for the same have been approved by the Institutional Animal Ethical Committee. The azo conjugate was evaluated for its ulcerogenic potential by Rainsford's cold stress method (Rainsford et al 1975). Therapeutic efficacy of the carrier system and the mitigating effect of the azo conjugate were evaluated in trinitrobenzenesulphonic acid-induced experimental colitis in rats (Yamada et al 1992). The synthesised prodrug was found to be equally effective in mitigating the colitis in rats as that of sulfasalazine without the ulcerogenicity of 5-aminosalicylic acid.

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