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In Silico Study of the Selective Inhibition of Bacterial Peptide Deformylases by Several Drugs

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Abstract

To counter increasing levels of pathogen resistance new classes of antibiotics are needed without delay. The metalloenzyme peptide deformylase (PDF) correspond to one of the most promising bacterial targets in the search for novel mode of antibiotics action and was firstly selected as a specific bacterial target. Peptide analogs were developed as inhibitors containing a hydroxamate or formyl-hydroxylamine as metal interacting group, and used as inhibitors with in vitro activity against a broad spectrum of organisms and successful antibacterial activity in vivo that is harmonizing with good pharmacokinetic properties and excellent tolerability in diverse species, but a human homologue was recently discovered. A new strategy for selecting highly efficient compounds with low inhibition effect against human PDF was developed. An original class of small, non-peptidic inhibitors of peptide deformylase (PDF) as potent antibiotics such as indol-group and its derivatives with the same mode of action in vivo as previously identified PDF inhibitors but without the apoptotic effects of these inhibitors in human cells, has been discovered. This study has confirmed the selective action of these compounds on bacterial PDFs by docking method using the autodock program. Indeed, a good correlation between IC50 and deltaG values of different complexes PDF-inhibitors was observed. The evaluation of the various molecular properties of these inhibitors lets us conclude that all these compounds are most likely drugable.

Keywords: Antibiotics; Peptide deformylase; Human homologue; Docking; Autodock; molecular properties; Lipinski's rule

Intriduction

For a long time, it has been widely established that eukaryotes have no peptide deformylase (Mazel, et al., 1994; Yuan et al., 2001). Since this enzyme is essential in bacteria and in several human parasites, peptide deformylase (PDF) was then acknowledged as an exceptional target for the design of new antibacterial (Yuan et al., 2001; Giglione et al., 2000; Pei, 2001) and antiparasitic agents (Meinnel et al., 2000).

Several studies of PDFs in complex with an inhibitor has given guidelines for the design of high affinity PDF inhibitors (Guilloteau et al., 2002; Hao et al., 1999). Actinonin, a natural pseudotripeptide hydroxamate coumpound and many of its derivatives have been shown to display powerful antibiotic activity (Chen et al., 2000; Gordon et al., 1962; Boularot et al., 2004; Chikhi et al., 2006). Phase I clinical studies were recently completed for two such potent peptide deformylase inhibitors derived from actinonin (Ramanathan-Girish et al., 2004; Fritsche et al., 2005; Bush et al., 2004), which have now gone on to phase II and III trials.

Though recent studies have led to the identification of peptide deformylases in eukaryotes. These enzymes are targeted to the plastids and mitochondria of plants and to animal mitochondria (Giglione and Meinnel, 2001; Giglione et al., 2000). Two PDFs have been identified in plants and one in humans. Since they do not contain the two insertions typical of PDF2 molecules, all eukaryotic PDFs are without ambiguity of type 1 (PDF1). However, the amino acid sequence of the PDF specific to mitochondria differs from those of other PDF1s in some specific features. There are some changes in the proximity of the active site (Serero et al., 2003). PDF1 molecules therefore form two classes: PDF1A and PDF1B. PDF1Bs correspond to bacterial and plastid PDFs, whereas PDF1As correspond to mitochondrial PDFs. Unlike PDF1B, which is specific to plants, PDF1A is found in almost all eukaryotes. The PDF2 molecules belong exclusively to bacteria.

Likewise the process of deformylation has been shown to be an essential process in eukaryotes (Giglione et al., 2003; Lee et al., 2004). Unlike eukaryotic PDF1Bs, which do not differ significantly from bacterial PDFs in terms of their biochemistry (Serero et al., 2001) or three-dimensional structures (Kumar et al., 2002), PDF1As have a number of specific features.

Enzymatic studies have also shown that PDF1As from plants and animals differ from bacterial PDFs in a number of ways. These differences were investigated, and guidelines for the design of PDF-Inhibitors without anti-PDF1A activity were developed (Serero et al., 2003; Serero et al., 2001).

Docking plays an important role in the rational design of drugs (Kitchen et al., 2004). Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking.

Effectively, several studies estimating and comparing the accuracies of protein-ligand programs like Autodock, ICM, Gold... have been reported (Perola et al., 2004; Bursulaya et al., 2003; Chikhi and Bensegueni, 2008). Autodock4.0 is a set of closely related programs and algorithms developed at the Scripps

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Research Institute and the University of California at San Diego. It was used in this study for the evaluation of the binding energies of the various complexes pdf-inhibitors.

The aim of this study was to check and then confirm by docking method the activity of new compounds that would selectively inhibit both types of bacterial PDFs (PDF1B and PDF2) without significantly inhibiting human PDF (PDF1A) and to estimate the drug-likeness of these new molecules by evaluation of the Lipinski's Rule of Five.

Methods

AutoDock4.0 explores the conformational space of the ligand using the Lamarkian genetic algorithm (LGA), which is a hybrid of a genetic algorithm (GA) with an adaptive local search (LS) method (Morris et al., 1998). In this approach, the ligand's state is represented as a chromosome, which is composed of a string of real-valued genes describing the ligand location (three coordinates), orientation (four quaternions) and conformation (one value for each torsion). The simulation is started by creating a random population of individuals. It is followed by a specified number of generation cycles, each consisting of the following steps: mapping and fitness evaluation, selection, crossover, mutation and elitist selection. Each generation cycle is followed by a local search. The solutions are scored using an energy-based scoring function, which includes terms accounting for short-ranged Van Der Waals and electrostatic interactions, loss of entropy upon ligand binding, hydrogen bonding and solvation.

AutoDock requires the receptor and ligand coordinates in MOL2 format. Nonpolar hydrogen atoms were removed from the receptor file and their partial charges were added to the corresponding carbon atoms. The program Mol2topdbqs was used to transform the receptor MOL2 file into the PDBQS format file containing the receptor atom coordinates, partial charges and solvation parameters. The program AutoTors was used to transform the ligand MOL2 file into a PDBQ file, merge nonpolar hydrogen atoms and define torsions. The grid calculations were set up with the utility Mkgpf3 and maps were calculated with the program AutoGrid. The grid maps were centered on the ligand's binding site and were of dimension 61 × 61 × 61 points. The grid spacing was 0.375 Å yielding a receptor model that included atoms within 22.9 Å of the reference binding site center. The default parameter settings generated by the program Mkdpf3 were used for docking. For each complex 10 dockings were performed. The initial population was set to 50 individuals; maximum number of energy evaluations was 2.5×105; maximum number of generations was 27,000. The other parameters provided by the default setting were the same as in the followed reference (Morris et al., 1998).

We have selected the twelve compounds (among twenty four cited by (Boularot et al., 2007) (*see Supplementary* Table) which act on bacterial PDFs without significantly effect on human PDF (Figure 1). Bacterial PDFs are represented by *Escherichia coli* PDF1B (PDB ID = 1LRU) and *Bacillus cereus* PDF2 (PDB ID = 2OKL). Plant PDF is represented by *Arabidopsis thaliana* AtPDF1B (PDB ID = 3CPM) and human PDF by HsPDF1A (PDB ID = 3G5K). The structures of ligands are represented in the Figure 1.

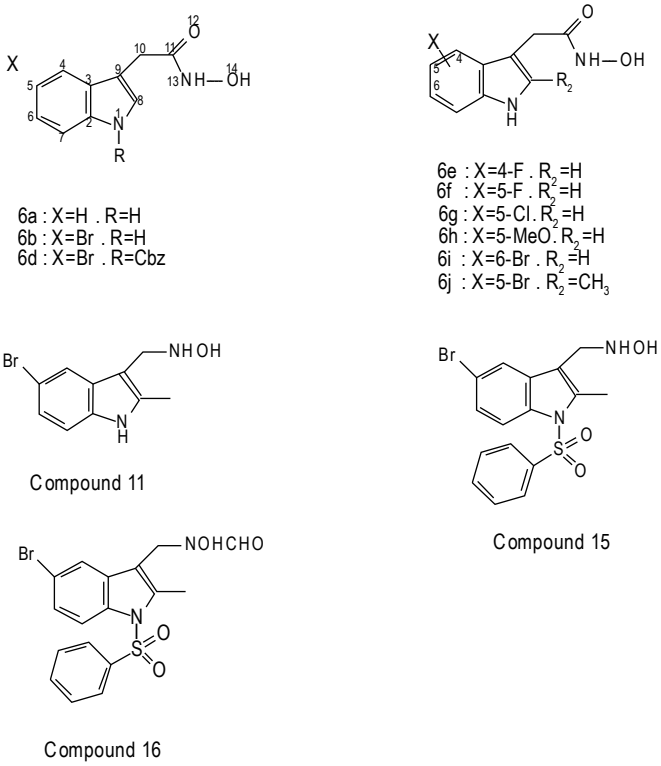

Figure 1: Structure formulas of the twelve inhibitors.

Results and Discussion

All results are gathered in the following figures. The Figure 2, Figure 3 and Figure 4 show the relationship between IC50 and DeltaG values of diverses complexes PDF-inhibitors. A good correlation was observed between IC50 and DeltaG values for both bacterial PDFs (PDF1B and PDF2) and plant AtPDF1B with r=0.83, r=0.90 and r=0.84 respectively. Interesting interactions were detected between PDF1B, PDF2, AtPDF1B and diverses inhibitors with sufficiently high DeltaG values especially for three compounds 6b, 6d and 16 with -38.1, -42.0 and -40.02 Kj/mol respectively.

The interactions between one of the best inhibitors (compound 6b) and diverses PDFs are represented in the following Figures.

On a total of six H-bonds, two amino acids act as H-bonds donors among which two are realized by NH of Leu91 and one by NE2 of Gln50 with O12 of 6b compound. Others take place between atoms of three amino acids His132 (NE2), Glu133 (OE2)

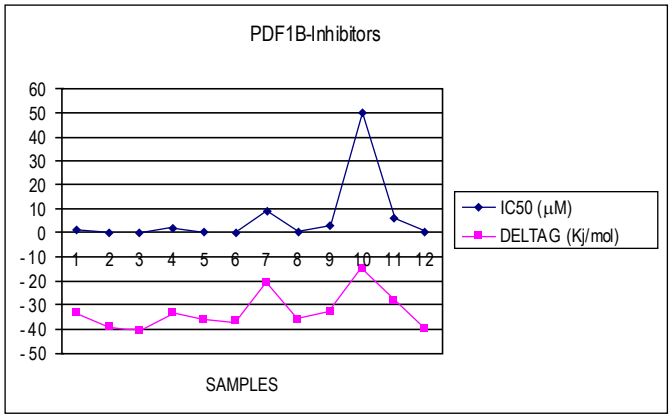

Figure 2: Relationship among IC50 and DeltaG values of diverses complexes PDF1B-Inhibitors.

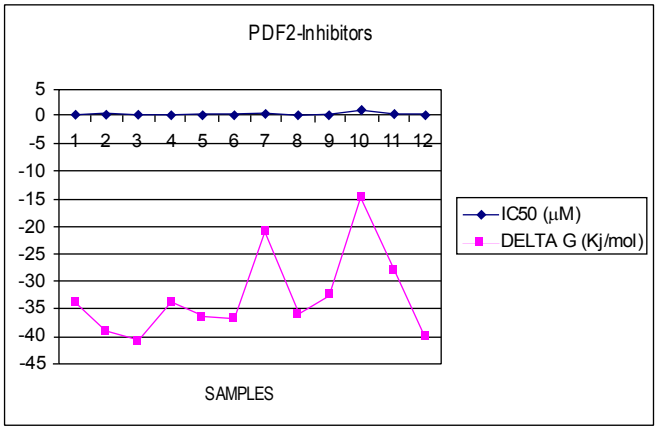

Figure 3: Relationship among IC50 and DeltaG values of diverses complexes PDF2-Inhibitors.

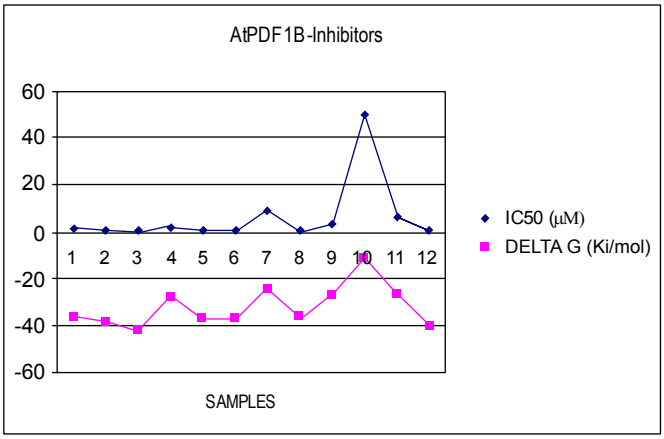

Figure 4: Relationship among IC50 and DeltaG values of diverses complexes PDF1B-Inhibitors.

and His136 (NE2) who intervene as H-bonds acceptors and OH14, NH13, and OH14 of 6b compound respectively.

Over the 5 H-bonds, three are made by OE1 and OE2 atoms of the same amino acid Glu133 which acts as H-bonds acceptor with NH13 and OH14 of 6b compound while two others are realized by NH of Leu91 and NE2 of Gln50 who react as H-bonds donors with O12 of 6b compound.

The docking's results denote that the indol group and its derivatives act on the various bacterial PDFs (Figure 5 and Figure 6) but not on the human PDF (Figure 7).

Lipinski's Rule of Five is a rule of thumb to evaluate drug-

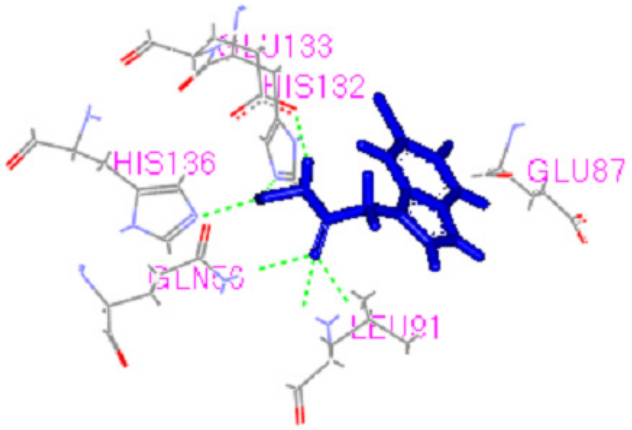

Figure 5: Complexe PDF1B-6b : Broken lines (green) represent the H-bonds realized between the 6b inhibitor (blue) and the amino-acids of the enzyme's active site.

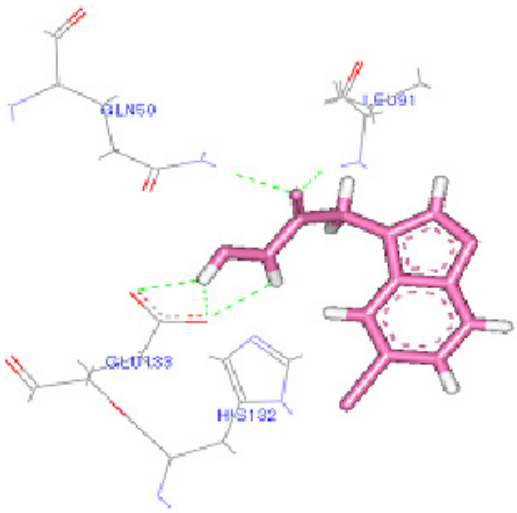

Figure 6: Complexe pdf2-6b : Broken lines (green) represent the H-bonds realized by the 6b inhibitor (mauve) with the amino-acids of the active site.

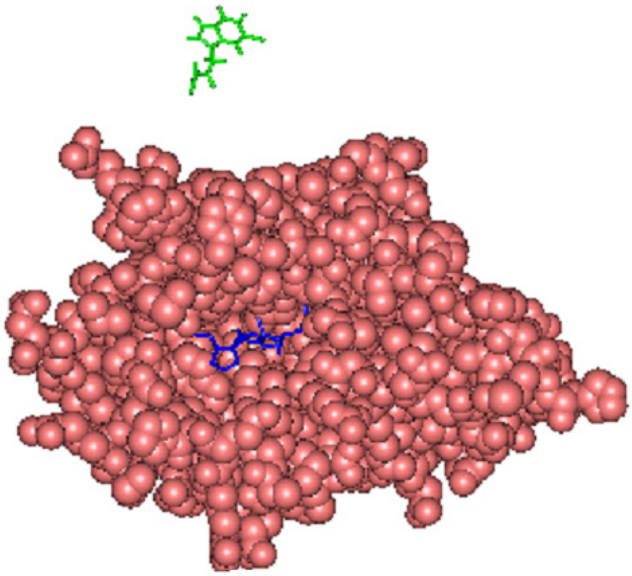

Figure 7: Complexe HsPDF1A-actinonin-6b : Actinonin (blue), a natural inhibitor of all PDFs including human PDF, is shown in the center of the active site of the enzyme while the 6b inhibitor (green) is widely out of the enzyme.

likeness, or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A Lipinski (Lipinski et al., 1997).

The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion ("ADME"). The rule is important for drug development where a pharmacologically active lead structure is optimized step-wise for increased activity and selectivity, as well as drug-like properties as described by Lipinski's rule.

Lipinski's Rule of Five states that, in general, an orally active drug has:

- Not more than 5 hydrogen bond donors (OH and NH groups)
- Not more than 10 hydrogen bond acceptors (notably N and O)
- Not more than 15 rotatable bonds (rotb)
- A molecular weight (M.W) under 500 g/mol

Compounds	n.atoms	M.W	n.O,N	n.OH,NH	n.rot.b	mi.LogP
6a	14	190.202	4	3	2	1.056
6b	15	269.098	4	3	2	1.841
6d	25	403.232	6	2	5	3.353
6e	15	208.192	4	3	2	1.172
6f	15	208.192	4	3	2	0.223
6g	15	224.647	4	3	2	1.710
6h	16	220.228	5	3	3	1.089
6i	15	269.098	4	3	2	1.841
6j	16	283.125	4	3	2	2.063
11	14	255.115	3	3	2	2.336
15	22	364.219	5	3	3	3.861
16	24	392.229	6	2	3	3.489

Table 1: Molecular properties of the inhibitors.

- A partition coefficient log P (mi.LogP) less than 5

Molinspiration cheminformatics package was used for the determination of the inhibitors' molecular properties.

These results show that the Lipinski's rule is respected for all the compounds and that these molecules are accepted to be orally bioavailable.

Conclusion

This theoretical study confirms clearly the experimental results and shows that autodock program can be used to predict enzyme-inhibitors' interactions. Our results prove that the autodock program does a rational job in docking and should assist significantly the drug discovery process. This study also shows that indol-group and its derivatives can represent a novel class of inhibitors specifically active on bacterial PDFs. We think therefore that the discovery of the orthologue of a given target in humans should not lead us to discard the target or slow down the search for efficient, bioavailable drugs against it. Rather, this data should be taken into account in the research strategy in order to minimise the effects of the drugs against the human orthologue. For example, the success of the fluoroquinolones shows that high selectivity can be achieved even when a mammalian orthologue of the target exists.

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