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Experimental-Computational Evaluation of Antimicrobial Activity of Some β-Lactams: Advantages and limitations

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ABSTRACT

Experimental-computational evaluation of in-vitro antimicrobial activity of mono and bicyclic β-lactams is described. The compounds prepared were tested against various Gram-positive and Gram-negative bacterial species such as Escherichia coli, Stapylococcus citrus, Klebsiella pneumonae and Bacillus subtillis. Some of these compounds showed potential antimicrobial activities. This study would greatly help to pharmacomodultate the antibiotics.

Keywords: β-lactam antibiotics; virtual screening, Petra; Osiris and Molinspiration (POM), bioinformatics.

INTRODUCTION

The β-lactam skeleton is a key structure element for most of widely used class of antimicrobial agents [1]. Various methods [2, 3] have been used for constructing this four-membered ketene-imine ring system. Hashimoto and coworkers [4] have used chiral imines derived from erythro 2-methoxy-1,2-diphenylethylamine and aromatic aldehydes to prepare β-lactams in good yield with high diastereoselectivity. Recently asymmetric induction has been reported for the preparation of monocyclic β-lactam [5-7]. Following the same route, we have synthesized some diastereoselective mono and bicyclic β-lactams using asymmetric induction by imine as shown in Scheme 1 and wish to report the theoretical analysis of their antimicrobial activity findings against some pathogenic Gram-positive and Gram-negative bacterial species such as *Escherichia coli, Stapylococcus citrus, Klebsiella pneumonae* and *Bacillus subtillis* [7].

The latter presents, to the best of our knowledge, the only record in the literature on the evaluation of antibacterial activity of mono and bicyclic β-lactams of these series.

We present here the results of our virtual screening investigation into possible alternative structures for these compounds. A comparison between experiment and theoretical predictions of the antibacterial activity has enabled us to identify alternative combined pharmacophore sites structures. The nature of pharmacophore site assignment of the β-lactams compounds was based on their POM analyses.

MATERIALS AND METHODS

General

All compounds described here have been previously prepared and well characterized by ^{1}H-NMR, IR spectroscopy and Masse Spectra analysis, as given here just for supplementary information [7].

RESULTS AND DISCUSSION

Reaction of amino acid, D-phenylalanine ethyl ester (**1**) with cinnamaldehyde gave chiral Schiff base (**2**), which underwent an asymmetric Staudinger cycloaddition with phthalimidoacetyl chloride to give the monocyclic β-lactam (**3**) as a single stereoisomer[7]. Ozonolysis of (**3**) into (**4**) followed by reduction with lithium aluminum tri(tert-butoxy) hydride afforded the hydroxymethyl β-lactam (**5**). Treatment of (**5**) with methansulfonyl chloride gave the mesylated monocyclic β-lactam (**6**), which was converted to the bicyclic β-lactam (**7**) upon treatment with 1,8-diazabicyclo [5, 4] undec-7-ene (DBU). Deprotection of the phthalimido group in β-lactams (**3**) and (**7**) by methylhydrazine and subsequent acylation of the free amino β-lactams with different acyl chlorides in the presence of pyridine afforded mono and bicyclic β-lactams (**8a-d**) and (**10a-d**) respectively (Scheme 1).

Table 1. Antimicrobial activity of the synthetic monocyclic and bicyclic β-lactams [7]

Compd.	R	Microorganism			
		B. subtilis	*K. pneumoniae*	*E. coli*	*S. citrus*
(3)	----------	+++	-	-	++
(4)	----------	-	-	-	-
(5)	----------	-	-	-	-
(7)	----------	-	-	-	-
(8a)	Ph	-	-	-	-
(8b)	Bn	+++	-	-	++
(8c)	CH_2OPh	+++	-	-	++
(8d)	CH=CHPh	-	-	-	-
(10a)	Ph	-	-	-	-
(10b)	Bn	-	-	-	-
(10c)	CH_2OPh	-	-	-	-
(10d)	CH=CHPh	-	-	-	-

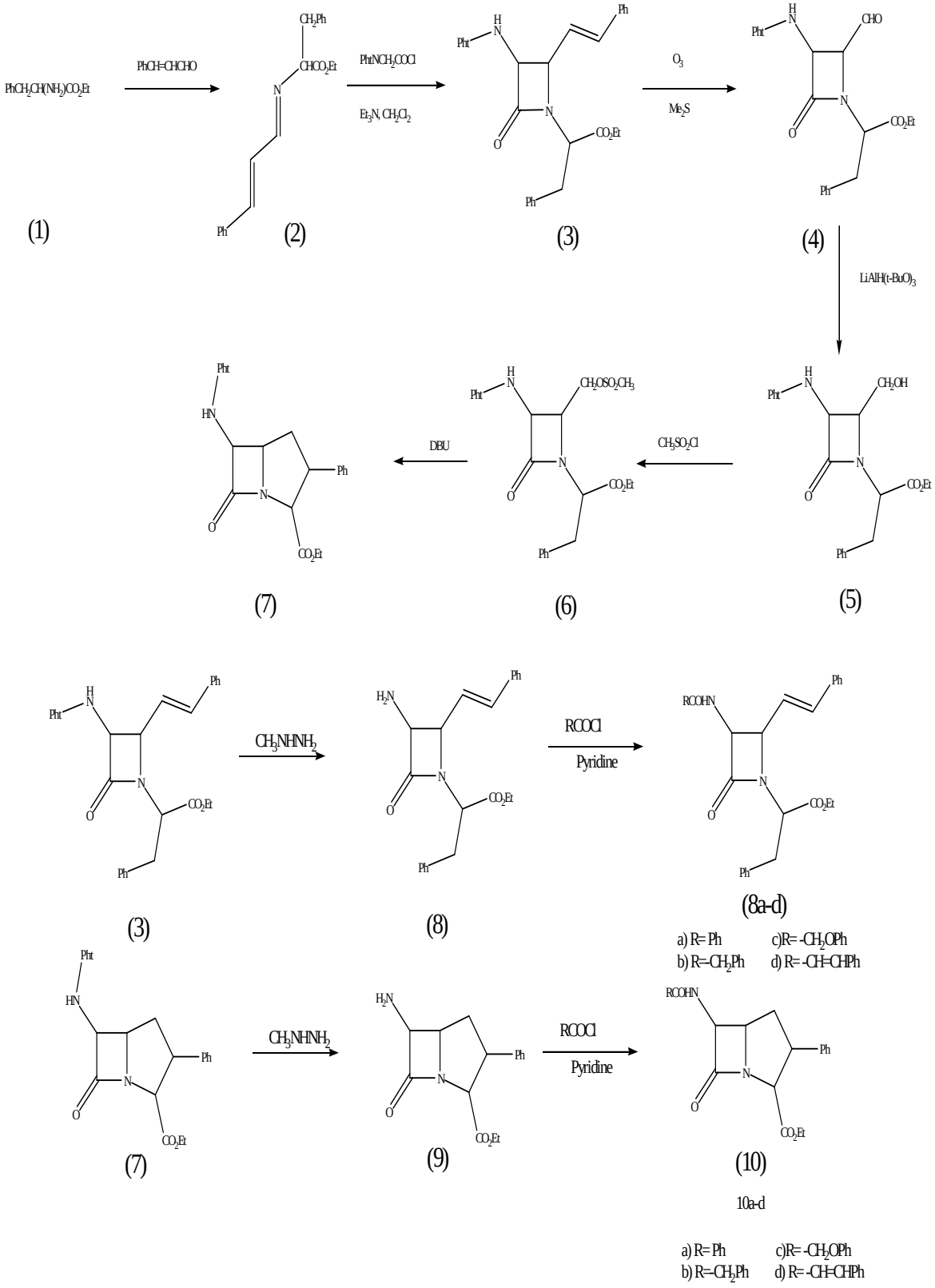
Highly active +++ (inhibition zone>12 mm); Moderately active ++ (inhibition zone 9-12 mm); Slightly active + (inhibition zone 6-9 mm); Inactive - (inhibition zone<6 mm).

Biological Screening

In-vitro antimicrobial activity tests

The antimicrobial activity test was performed by disk diffusion method [8] using ampicillin and gentamycin as reference compounds. The prepared compounds: (**3, 4, 5, 7, 8a-d** and **10a-d**) were tested against Gram positive (*Staphylococcus citrus, Bacillus subtilis*) and Gram negative (*Escherichia coli, Klebsiella pneumonae*) bacterial species. As shown in Table 1, it was found

that compounds (**3**, **8b** and **8c**) were highly active against *Bacillus subtilis* and moderately active against *Staphylococcus citrus*. Other compounds were found to be inactive (Table 1).



Scheme 1. General synthesis of monocyclic (3, 4, 5, 8) and bicyclic β-lactams (7, 9, 10) [7].

Theoretical calculations of molecular properties of (6-15)

Various investigators have used computational methods in understanding efficacy and efficiency of natural products and other sources of drug leads. Modern drug discovery is largely based on screening of small molecules against macromolecular disease targets requiring molecular screening libraries of drug-like or lead-like compounds. We have analyzed known standard references (SR) for drug-like and lead-like properties which would establish a strategy in designing specific drug-like or lead-like β-lactam products.

Petra calculations

The synthesized series of β-lactams (**3-10**) have been subjected to delocalized-charge calculations using Petra method of the non-hydrogen common atoms (Figure 1) obtained from partially pi-charged heteroatoms that have been used as model in the bioactivity against bacterial species.

PETRA is a program package comprising of various empirical methods used for the calculation of physicochemical properties of organic molecules. All methods are empirical in nature and have been developed [9] over the last 20 years. The chemical effects such as heat of formation, bond dissociation and delocalization energies, sigma and □-charge distribution, inductive, resonance and polarizibility effects are then quantified.

It is found that alternative positive and negative charges of the terminal heteroatoms of antibacterial pharmacophore site contribute significantly in favour of antibacteriral activity more than antiviral activity, and this is in good agreement with the mode of antibacterial action of the compound involving electrostatic interaction ($X^{\delta-}$---$Y^{\delta+}$) of the target drug molecule. It was hypothesized that difference in charges between two heteroatoms of the same pharmacophore site ($X^{\delta-}$---$Y^{\delta+}$) may be responsible to facilitate the inhibition of bacteria more than viruses. It is further found that the activity increases with increase in negative charge of the heteroatoms of the common pharmacophore fragment of the molecule. This is related to the possible secondary electronic interaction with the positively charged side chains of the bacteria target(s).

Table 2. Selected Petra calculations of compounds 3-10

Compd	Partial π-charge of heteroatoms (in e-)							
	N_1	N_2	O_1	O_2	O_3	O_4	O_5	O_6
(3)	0.193	0.138	-0.163	-0.163	-0.159	-0.118	...	...
(4)	0.193	0.136	-0.163	-0.163	-0.157	-0.118	-0.026	...
(5)	0.193	0.138	-0.163	-0.163	-0.159	-0.118	...	0.0
(7)	0.193	0.139	-0.163	-0.163	-0.160	-0.118	...	...
(8a)	0.131	0.138	-0.199	...	-0.159	-0.118	...	...
(8b)	0.137	0.138	-0.158	…	-0.159	-0.118	…	...
(8c)	0.137	0.138	-0.156	...	-0.159	-0.118	...	...
(8d)	0.126	0.138	-0.233	…	-0.159	-0.118	…	...
(10a)	0.131	0.1391	-0.199	...	-0.160	-0.118	...	...
(10b)	0.137	0.1391	-0.158	…	-0.160	-0.118	…	...
(10c)	0.1376	0.1391	-0.156	...	-0.160	-0.118	...	...
(10d)	0.1258	0.1391	-0.233	…	-0.160	-0.118	…	...

An attempt was made to incorporate steric and indicator parameters which emerged as important contributors from previous pharmacological analysis. The present results support the previous observations that bulky phenyl ring substituents and a three-member pharmacophore site attached to the lactam/pthalaldehyde bridge-containing ring are condusive to the activity. The Petra software calculations confirmed that all compounds (**3-10)** have a clear preference for

forming antiviral pharmacophore sites although their estimated partial π charges for O and N atoms contain negative charges (-0.163/ -0.159 e-). The Petra calculations with the other β-lactams (**4-10**) are summarised in Table 2.

On the basis of the above observations, it is tentatively suggested that compounds (**3-10)** show two antibacterial and antiviral pharmacophore sites in which the N and O heteroatoms act as ligation centres and possibly accommodate themselves between the metal atom in such a way that a stable complex of the pharmacophore site is formed thus providing a moderate to inactive structure to the tested antibacterial candidates.

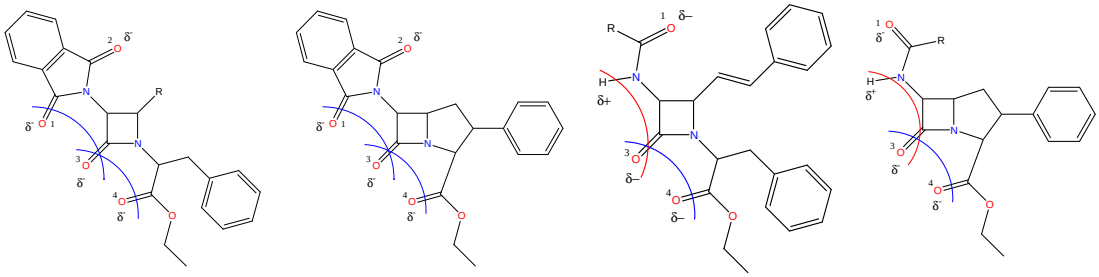
Osiris calculations

Structure based designing is now a fairly routine procedure and many potential drugs do not qualify for clinic practice because of ADME-Tox liabilities. One very important class of enzymes, responsible for many

ADMET problems, is the cytochromes P450. Inhibition of these or production of unwanted metabolites can result in many adverse drug reactions. Of the most important program, Osiris is already available online [10] for its designing/prediction of various activities.

In our recent publication [11-14] of the drug designing of various pharmacophore sites by using spiro-heterocyclic structure, we have predicted activity and/ or inhibition with increasing success in two targets, *Mycobacterium Tuberculosis* and HIV. This is done by using a combined electronic/structure docking procedure. The remarkable mutagenicity of divers synthetic molecules classified in data base of CELERON (Switzerland), can be used to quantify the role of various organic groups in promoting or interfering the way a drug can associate with DNA [10].

The OSIRIS Property Explorer shown in this page is an integral part of Actelion's inhouse substance registration system. It allows drawing chemical structures and also calculates various drug-relevant properties whenever a structure is valid. Prediction results are color coded in which the red colour shows high risks with risks with undesired effects like mutagenicity or a poor intestinal absorption and green colour indicates drug-conform behaviour (Table 3).



(3, 4, 5, 6) **(7)** **(8a-d)** **(10a-d)**

Figure 1. Repartition of partial π-charges of N and O atoms of compounds. The possible antibacterial and antiviral pharmacophores are marked respectively in red and blue colour.

Molinspiration calculations

CLogP (octanol/water partition coefficient) is calculated by the methodology developed by Molinspiration as a sum of fragment-based contributions and correction factors [15].

The method is very robust and is able to process practically all organic and organometallic based molecules. Molecular Polar Surface Area TPSA is calculated by the methodology published by

Ertl et al. [15] as a sum of fragment contributions. O- and N- centered polar fragments are considered. PSA has been shown to be a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability and blood-brain barrier penetration. Prediction results of compounds (**3-10)** with molecular properties (TPSA, GPCR ligand and ICM) are recorded in Table 4.

Table 3. Osiris calculations of compounds 3-10

Compd.	Toxicity Risks				Osiris calculations				
	MUT	TUMO	IRRI	REP	MW	CLP	S	DL	D-S
(3)					494	4.08	-4.89	-10.72	0.12
(4)					420	1.53	-3.06	-12.03	0.15
(5)					422	1.53	-2.84	-9.51	0.19
(7)					404	2.5	-3.53	-9.61	0.18
(8a)					468	4.28	-4.88	1.13	0.45
(8b)					482	4.07	-4.85	1.49	0.47
(8c)					498	3.73	-4.66	1.91	0.5
(8d)					494	4.4	-5.25	-0.39	0.31
(10a)					378	2.69	-3.53	2.27	0.75
(10b)					392	2.49	-3.49	2.93	0.76
(10c)					408	2.15	-3031	3.04	0.77
(10d)					404	2.82	-3.9	-0.2	0.53
Ampicillin					349	-0.04	-1.57	10.72	0.91
Gentamycin					477	-4.03	-1.18	4.88	0.77

MUT: mutagenic; TUMO: tumorigenic; IRRI: irritant; REP: reproductive effective; CLP: cLogP; S: Solubility; DL: Druglikness and DS: Drug-Score.

Table 4. Molinspiration calculations of compounds 3-10

Compd.	Physico-Chemical Properties Calculations [a]						Drug-likeness [b]			
	MW g/mol	cLogP	TPSA	ONI	NV	Volume	GPCRL	ICM	KI	NRL
(3)	494	5.1	85.69	0	1	443.7	-0.12	-0.52	-0.45	-0.57
(4)	420	2.6	102.8	0	0	363.9	-0.27	-0.42	-0.37	-0.76
(5)	422	2.1	105.9	1	0	369.7	-0.06	-0.29	-0.29	-0.64
(7)	404	3.1	85.7	0	0	350.9	-0.12	-0.02	-0.39	-0.59
(8a)	468	4.7	75.7	1	0	435.2	-0.07	-0.36	-0.37	-0.36
(8b)	482	4.8	75.7	1	0	452.0	-0.08	-0.38	-0.47	-0.41
(8c)	498	4.7	84.9	1	0	460.9	-0.11	-0.54	-0.62	-0.46
(8d)	494	5.3	75.7	1	1	462.6	-0.07	-0.45	-0.59	-0.34
(10a)	378	2.7	75.7	1	0	342.4	-0.07	0.02	-0.35	-0.38
(10b)	392	2.8	75.7	1	0	359.2	-0.05	0.09	-0.45	-0.38
(10c)	408	2.7	84.9	1	1	368.2	-0.10	-0.04	-0.59	-0.45
(10d)	404	3.4	75.7	1	0	369.8	-0.06	0.08	-0.63	-0.27
AMP [c]	349	-0.9	112.7	4	0	298.9	-0.56	-0.55	-0.90	-0.87
GENT [c]	476	-4.6	205.5	12	2	453.9	-0.46	-0.24	-0.76	-1.05

[a] TPSA: Total Polar Surface Area; ONI: OH-NH Interraction; NV: Number of Violation. [b] GPCRL: GPCR ligand; ICM: Ion Channel Modulator; KI: Kinase Inhibitor; NRL: Nuclear Receptor Ligand. [c] AMP: Ampicillin; GENT: Gentamycin.

In fact, the hydrolytic action of β-lactamases is the primary mechanism of bacterial resistance to β-lactam antibiotics. Within the past several years a variety of new β -lactam drugs have been developed that show resistance to the action of these enzymes. For example, carbapenems constitute a group of such β–lactamase-resistant molecules, and they possess potent activity against a wide spectrum of bacteria. Studies on the mechanism of action of class a β-lactamases with carbapenems by Knowles and colleagues have indicated a biphasic profile for hydrolysis of carbapenems, with an initial fast phase for substrate turnover leading to a slower one within minute. It was demonstrated previously [16] that the highly conserved arginine 2446 is the essential source of proton for tautomerization of carbapenem antibiotics. For all the reasons, the coordination of lactams entities will be beneficial to give more stability and lipophilicity to β-lactam drugs.

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