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TRICYCLIC BRIDGED β-LACTAMS AS TRANSITION STATE ANALOGUES: A STUDY USING SEMI-EMPIRICAL MOLECULAR ORBITAL METHODS

Gordon Bruton
SmithKline Beecham Pharmaceuticals, Department of Medicinal Chemistry, Brockham Park, Betchworth, Surrey, England.

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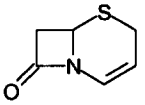
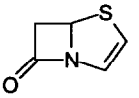
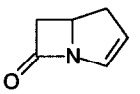
Abstract. The effect of ring strain on nitrogen pyramidality and carboxylate orientation was measured for six novel β-lactam nuclei using both the AM1 and PM3 methods within MOPAC. Comparison with X-ray data resulted in novel carba -cephem and -penem nuclei being proposed as transition state analogues of peptide hydrolysis.

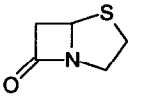
Understanding the molecular basis of enzyme-inhibitor interactions, and the subsequent de novo creation of inhibitors, is increasingly led by computer aided molecular design.[1] Prompted by a recent disclosure,[2] this report describes the application of molecular modelling to the design of novel penicillin binding protein (PBP's) inhibitors, by identifying and manipulating key enzyme recognition parameters.

Each β-lactam nucleus **1-4** exhibits a unique fingerprint of inhibition for the membrane bound transpeptidase, D,D-carboxypeptidase and endopeptidase enzymes (PBP's), involved in cell growth (cell elongation), maintenance of cell shape and cell division (septum formation).[3] The objective was to study the key enzyme recognition parameters of these nuclei, enhancing them for carbapenems (exhibiting a broad PBP inhibition profile) and to create carbapenem bioisosteres from cephem and penam nuclei.


1. Cephem

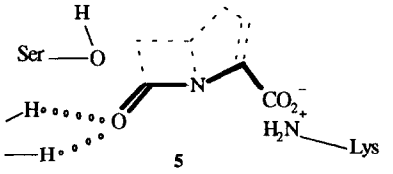

2. Penem


3. Carbapenem

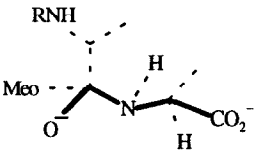

4. Penam

Rao's and Cohen's excellent early work examining nuclei and side chain conformations[4] has identified the degree of β-lactam nitrogen pyramidality and the position of the carboxylate group, especially the 'Cohen' distance (fig. 1), as pivotal features for PBP inhibition. This is in keeping with the molecular basis of β-lactam/PBP interaction[5] and forms the cornerstone of this study. The free carboxylates of β-lactam antibiotics anchor the nuclei electrostatically, by binding to an unstabilised lysine or histidine residue in the basal cleft, while an oxyanion hole locates and activates the β-lactam carbonyl oxygen atom with two hydrogen bonds as in **5**. These primary anchorages present the carbonyl carbon atom to the active site serine hydroxyl, prior to acylation and formation of the final covalent tether. The significance of the carboxy and lactam carbonyl disposition, as argued by Cohen, can thus be rationalised: where the 'Cohen' distance is >3.95 Å, the inactivity is due to an inability to fit the triad of active site residues, unstabilised lysine, oxyanion hole and serine hydroxyl. The contribution of lactam pyramidality to penam, penem and carbapenem nuclei is usually interpreted as increasing the reactivity towards nucleophilic attack, by perturbation of lactam amide resonance. The cephems pyramidality is marginal at 2-13°, but the lactam is activated, to a lesser degree, by enamine resonance of its unshared electrons into the adjacent dihydrothiazine π system. However, the degree of lactam pyramidality is a measure of its resemblance to the transition states for both D-ala-D-ala hydrolysis,

and aminolysis of the acyl D-ala-enzyme intermediate of the transpeptidases. It was thus hypothesized that the active nuclei are not D-ala-D-ala mimics, but represent different stages during the incremental development of the transition state for peptide formation and/or cleavage, therein rationalising the differing PBP inhibition profiles. Supportive preliminary work[6a] indicates a high degree of similarity between the transition state **6** (peptide methanolysis) and carbapenem nucleus **5**; the lactam C=O, nitrogen and carboxyl carbon atoms superimposing upon the transition states C-O⁻, nitrogen and carboxylate atoms respectively.



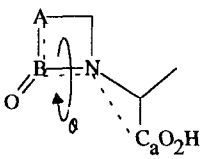
5



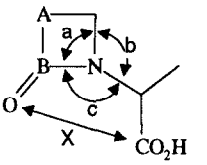
6

A sequence of structures **7-12** was chosen for study in which a bridging section would affect the relative orientation of the β-lactam and adjacent ring.[6b] The intention was to control the pyramidality of the nuclei, monitor the subsequent effects upon carboxylate disposition and maximize transition state mimicry. After initial building and geometry optimisation using the molecular mechanics within CHEM-X,[7] the structures were submitted for full geometry optimisation with MOPAC using both AM1 and PM3 Hamiltonians. For systems with conformationally flexible bridging rings, **8** and **11**, both chair and boat forms were examined. No significant influence upon the key enzyme recognition parameters was noted and only one set of data is reported. Three variables were consistently measured (fig.1) for comparison with crystal data; the Cohen distance X, the pyramidality, as defined by 360° - (a+b+c), and the through space torsion angle ϕ across $ABNC_a$. This measurement of pyramidality differs from those usually described,[8] but is independent of bond lengths and skew β-lactam rings. Data is displayed in Table 1 for novel nuclei, and in Table 2 for X-ray structures.

fig. 1



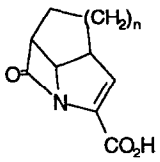
X = Cohen distance in Å
ϕ = Torsion angle about $ABNC_a$
360°-(a+b+c) = Pyramidality



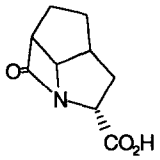
From the results in Table 1 an AM1/PM3 differential emerges. As ring strain increases, **11<10<12<9<8<7**, PM3 gives larger values for the Cohen distance X (0.1-0.25 Å). AM1, however, produces a greater gradient for increasing pyramidality with increasing ring strain than does PM3, such that the significantly higher value obtained with PM3 for the least strained system **11** (pyramidality circa 20°) graduates to lower values with the most strained **7** (pyramidality circa 47°). When compared to crystal data for carbapenems, Table 2, AM1 is shown to overestimate and PM3 to underestimate pyramidality.

The bridging ring generally increases ring strain and consequently the lactam pyramidality. For the carbapenam nucleus **9** this has the effect of conformational locking into the inactive 'closed' species.[9] The resulting Cohen distance of 4.3Å, or more, precludes this as a putative inhibitor. Cohen distances for the remaining nuclei all lie within the required enzyme recognition range.

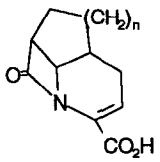
Penem mimicry is achieved to a greater and lesser extent by the carbacephems **10** and **11** respectively.



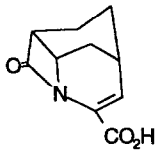
7 n=1
8 n=2



9



10 n=1
11 n=2



12

Table 1

Structure	7		8		9		10		11		12	
Method	AM1	PM3	AM1	PM3	AM1	PM3	AM1	PM3	AM1	PM3	AM1	PM3
X Å	3.7	3.8	3.7	3.8	4.3	4.55	3.5	3.5	3.4	3.4	3.6	3.8
360°-(a+b+c)	49	45	43	40	42	39	24	25	18	22	37	37
θ	140	140	144	144	147	147	159	159	153	153	151	151

Table 2

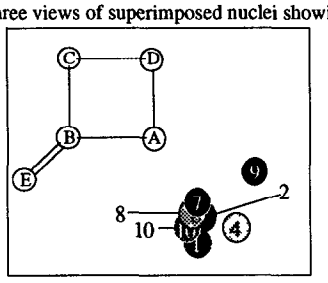
Structure	4, Penam-Open[7]	4, Penam-Closed[7]	1, Cephem		2, Penem	3, Carbapenem		
Method	X-ray	X-ray	X-ray	PM3	X-ray	X-ray	PM3	AM1
X Å	3.9	4.2	3.2-3.6	3.7	3.6	3.6	3.8	3.8
360°-(a+b+c)	22	21	2.5-13	17	27	36	30	42
θ	-176	175	178	176	160	163	141	142

The degree of pyramidality for **10** (24°) is about twice that of the normal cephem nucleus **1** and approaches penem status, **2** (27°). The greater ring strain exhibited by **12** increases pyramidality still further for the carbacephem nucleus which now exhibits carbapenem **3** characteristics of 3.6Å Cohen distance with 37° pyramidality. However, in a related bridged oxacephem system AM1 was found to overestimate the lactam pyramidality compared to X-ray data.[10] Bridging the carbapenem nucleus **3** to give **7** and **8** has little effect on Cohen distance or torsion angle, but pyramidality is increased. Thus the carbapenem nucleus appears to accommodate a bridging ring without significant changes in geometry.

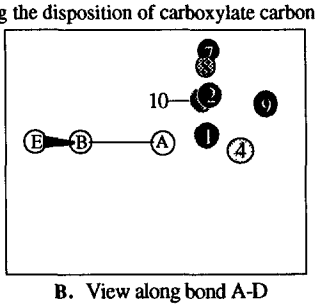
The torsion angle θ defines the carboxylate position relative to the plane of the β-lactam amide bond. The significance of θ is illustrated graphically in fig.2, where the β-lactam rings of nuclei **7,8,9,10,1,2** and **4** were rigid fitted in CHEM-X, with weightings of 10 for atoms A,B,E and 8 for C and D to accommodate skew β-lactams. Extraneous atoms and bonds have been deleted for clarity, leaving the β-lactam carboxylate carbon atoms, numbered according to parent structure, for comparison. The inappropriate positioning of the carboxylate of **9** is evident, as is the effect of the bridging system, forming an arc of carboxylates rising

fig.2

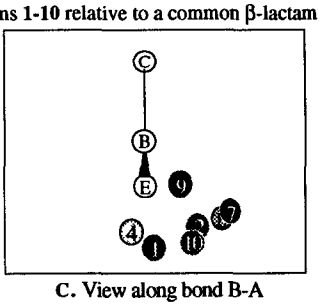
Three views of superimposed nuclei showing the disposition of carboxylate carbon atoms **1-10** relative to a common β-lactam ring



A. View from above β-lactam ring



B. View along bond A-D

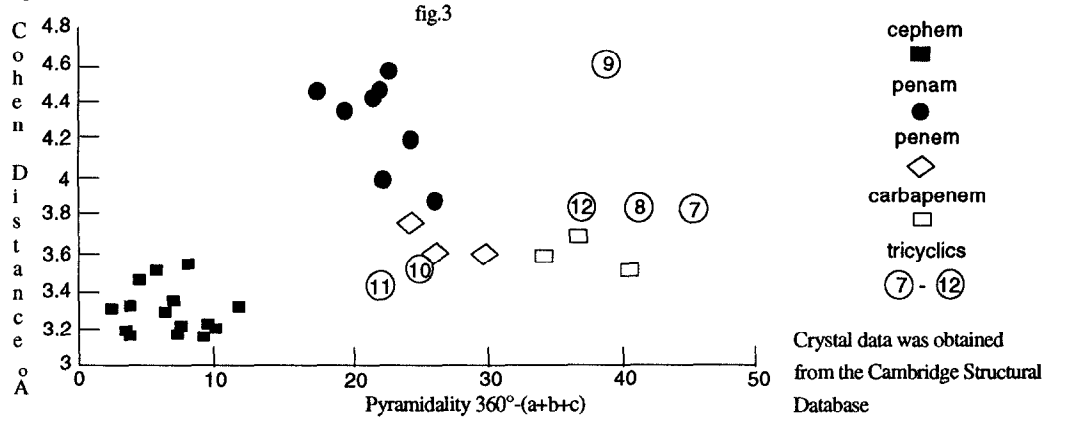


C. View along bond B-A

from the penam **4** to tricycle **7**. Tricycles **7** and **8** represent the peak of achievable pyramidality and torsion angle, while penem **2** mimicry by the carbacephem **10** is self evident.

Plotting out the key parameters (fig.3) drawn from available X-ray data separates out the nuclei according to type. Although the penams are nearly all fixed into their inactive form in the crystal, it is possible to classify these β–lactams into structural groups. The bridged systems **7-12** do not obviously fall into the group of their indigenous class, a feature that should be reflected in the biological properties that they would express.

fig.3



The modelling methods employed may be overstating the degree of pyramidality for the tricyclic structures, suggesting that enhancements using shorter bridges or alternative bond hybridisation may be applicable. However, the principle of penem and carbapenem mimicry by manipulating carbacephems has been demonstrated. Penams have been found unsuitable for this purpose and carbapenems have been found capable of accommodating a bridging ring, without the introduction of excessive ring strain.

Acknowledgment

I would like to acknowledge Dr. Tim Mitchell and Dr. Mike Pearson for their guidance and encouragement during the course of this study.

References and Notes

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6. (a) Bruton, G. Unreported results. (b) Parallel series bearing hydrophylic bridges gave similar results.
7. Davies, F. K., Murrall, N. M. Computers Chem. **1989**, *13*, 149.
8. Usual measurements reported are (a) *h* - The height of the lactam nitrogen from the plane formed by the surrounding atoms. (b) The torsion angle across the β-lactam amide bond of the carbon atom bearing the carboxylate group.
9. See 4. (b) and references therein. Penam carboxylate disposition is dependent upon the thiazolidine ring conformation. "Open" produces an equatorial carboxylate, "Closed" produces an axial carboxylate.
10. Nagata, W. *Pure and Appl. Chem.* **1989**, *61*, 325.