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AM1 heats of formation for the reaction of alcohols with a series of β-lactams and aza-β-lactams: Design of novel β-lactamase inactivators

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Abstract. AM1 heats of formation (H_f) for the reaction of MeOH and EtOH with a series of β-lactams and aza-β-lactams are calculated. The ΔH_f values for hemi-acetal and acyl/carbamoyl-enzyme intermediate formation are compared in the two series. These model calculations predict that the reaction of the β-lactamase active site serine hydroxyl with aza-β-lactams will afford carbamate intermediates which should be refractory to hydrolysis, thereby inhibiting the enzyme. Therefore, novel aza-β-lactams are putative β-lactamase inactivators based on semi-empirical computations.

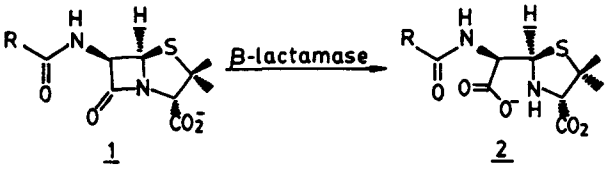
Keywords. Enzyme inhibitor; aza-β-lactam; β-lactamase; AM1.

1. Introduction

The antibacterial activity of β-lactam antibiotics arises from their ability to disrupt cross-linking of D-Ala-D-Ala fragments with peptidoglycan strands (Lancini and Parenti 1982). Certain resistant strains of bacteria have evolved that produce β-lactamase enzymes (Knowles 1985) which hydrolyse active penicillin 1 to inactive penicilloic acid 2 (scheme 1).

Acylation of β-lactam antibiotic 3a by β-lactamase active site serine-OH generates an acyl-enzyme intermediate 4a which hydrolyses to the inactive form 5, and the enzyme is regenerated (scheme 2). We reasoned that if the acyl-enzyme intermediate is rendered sluggish to hydrolytic attack, the enzyme should be transiently trapped as a metastable intermediate. This will result in mechanism-based inactivation of β-lactamase (Walsh 1982; Rando 1984; Knowles 1985; Silverman 1988), an important goal in enhancing the efficacy and versatility of penicillin antibiotics. A carbamate/urethane linkage appears to be a potential candidate for enzymatic inhibition because of the higher resistance of the carbamoyl-enzyme intermediate 4b (X = NR), as compared to the acyl-enzyme intermediate 4a (X = CHR), towards hydrolysis. This leads to 1,3-diazetidin-2-ones 3b (aza-β-lactams) (Marchand-Brynaert and Ghosez 1985; Marchand-Brynaert *et al* 1988) as a novel class of β-lactamase inactivators compared to the ubiquitous and well-studied azetidin-2-ones (β-lactams) (Walsh 1982; Rando 1984; Knowles 1985; Silverman 1988).

In a recent investigation we concluded that aza-β-lactams 17b–20b and 23b (X = NH) are stable molecules with a highly electrophilic carbonyl group based on AM1 heats of formation (H_f) and atomic charges (q), respectively (Nangia 1991). Although these computations define the minimum requirements of stability and



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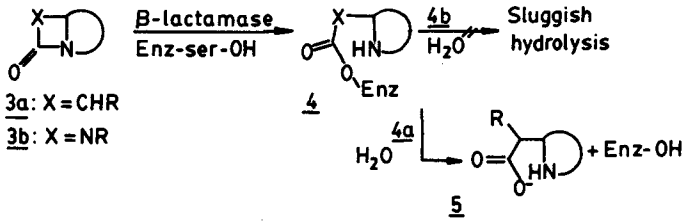
Scheme 1. Action of β-lactamase enzyme on penicillin 1.



Scheme 2. Reaction of β-lactamase with β-lactams 3a and aza-β-lactams 3b (proposed).

electrophilicity necessary in aza-β-lactams for enzyme inhibition, the expected sluggish hydrolysis of carbamoyl-enzyme intermediate 4 because of partial amino-donation is as yet an untested hypothesis. In order to theoretically estimate the inhibitory activity of aza-β-lactams, we decided to compare ΔH_f values for the reaction of serine-OH with substrates 3 to generate intermediates 4 (scheme 2).

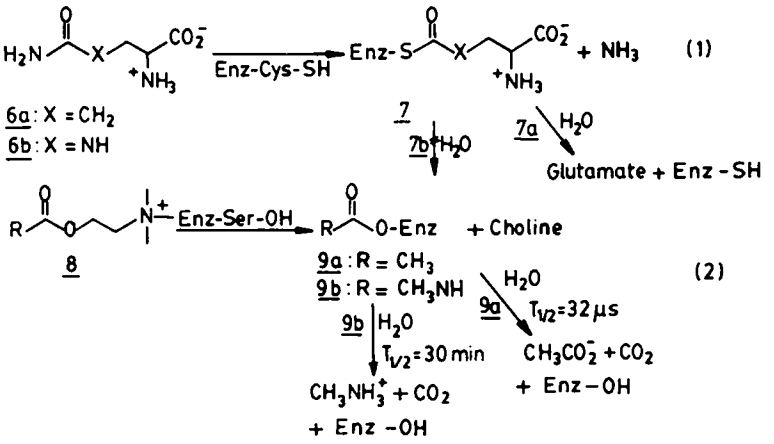
2. Design of enzyme inactivators

The idea of synthesizing aza isosteres for inhibition of protease and esterase enzymes has been exploited earlier. We are aware of at least two biochemical reactions in which substitution of CH_2 with NH adjacent to a carbonyl results in enzyme inhibition. The two enzyme systems chosen as bench-marks for comparison of ΔH_f values with β-lactamase are formylglycinamide ribonucleotide amidotransferase and acetylcholinesterase (scheme 3).

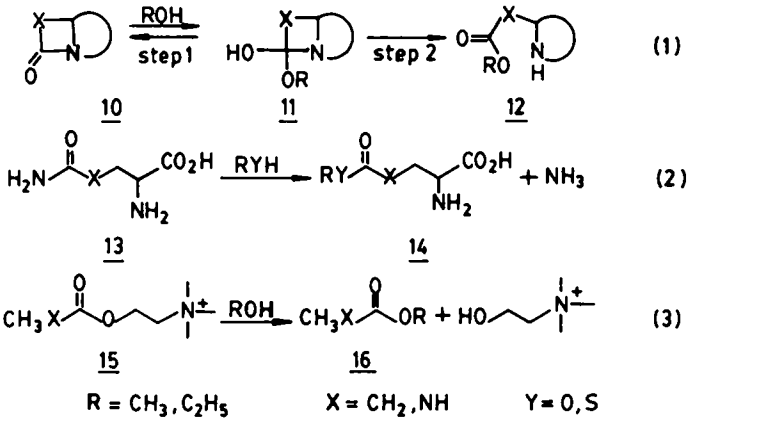
The hydrolysis of glutamine 6a ($X = CH_2$) to glutamate is catalysed by formylglycinamide ribonucleotide amidotransferase (Buchanan 1973, 1978) which has a cysteine-SH residue in the active site ((1) of scheme 3). The protease is inhibited by albizziin 55 6b ($X = NH$) which is structurally similar to glutamine except that the acetamido moiety is replaced by a urea moiety. The thiocarbamate 7b cannot hydrolyse easily because of partial amino-donation.

Acetylcholinesterase (Froede and Wilson 1971) terminates the activity of neurotransmitter acetylcholine 8 ($R = CH_3$) by hydrolysis to inactive choline ((2) of scheme 3) via covalent catalysis with active site serine-OH ($T_{1/2} = 32\ \mu s$). Physostigmine is a potent inhibitor of the enzyme by forming carbamoyl-enzyme intermediate 9b ($R = CH_3NH$) which hydrolyses with great difficulty ($T_{1/2} = 30$ min).

We reasoned that if the differences in ΔH_f values due to $CH_2 \rightarrow NH$ substitution in (1) and (2) of scheme 3 are of the same order of magnitude as the differences in ΔH_f values for the same substitution in the proposed equation (scheme 2), then the extent of amino-donation in the three reactions should be comparable. In other words, the differences in ΔH_f values for $CH_2 \rightarrow NH$ substitution are taken as a



Scheme 3. Inhibition of formylglycinamide ribonucleotide amidotransferase and acetylcholinesterase by aza isosteres 7b and 9b.



Scheme 4. Model reactions for calculation of H_f and ΔH_f values.

measure of hydrolytic stability of carbamates (4b, 7b, 9b) as compared to esters (4a, 7a, 9a). With this objective we calculated AM1 ΔH_f values for the reactions of MeOH and EtOH with β-lactams 10a (and aza-β lactams 10b) to form intermediates 12a (and 12b), (1), and compared them with calculated ΔH_f values for reaction with glutamine 13a (and 13b), (2), and acetylcholine 15a (and 15b), (3) (scheme 4). The results of this theoretical investigation are reported in this paper.

3. Methods of calculation

A detailed understanding of organic reactions using computational techniques has to meet several criteria. Not only must the technique reproduce molecular geometries and energies to a high degree of accuracy, but it must also do so in reasonable computer time and cost. Although *ab initio* methods are, in principle, capable of furnishing quantitative information, they require excessive time on super computers

for systems containing more than half a dozen "heavy" (non-hydrogen) atoms. However, semi empirical tools, such as AM1 (Dewar *et al* 1985), offer a computationally accessible approach for investigating organic reactions of practical interest to chemists and biochemists.

The modelling of active site serine-OH in proteinases as MeOH to avoid prohibitively long computations is reported in quantum-chemical calculations on biochemical systems (Naray-Szabo and Surjan 1986). In our calculations, the serine-OH of β-lactamase and acetyl cholinesterase is mimicked by MeOH/EtOH and the cysteine-SH of formylglycinamide ribonucleotide amidotransferase by MeSH/EtSH. A comparison of ΔH_f values in the carba vs aza series is meaningful only if the substitution $CH_2 \rightarrow NH$ is uniformly maintained in (1)–(3) of scheme 4. In order to calibrate the substrates for the same change in number and nature of atoms between the carba and aza series, the acetyl group in 8 ($R = CH_3$) was replaced by propanoyl in 15a ($X = CH_2$). Equation (1) (scheme 3) involves attack by cysteine-SH instead of serine-OH. Because the AM1 method is not well-parametrized for sulphur atoms, calculations in (2) (scheme 4) were carried out with MeSH/EtSH as well as MeOH/EtOH models. Since zwitterionic amino acids exist as free non-ionic molecules in the gas phase (Jensen and Gordon 1991), H_f values in (2) are computed on the non-ionic forms of glutamine 13 and glutamate 14.

Geometry optimisation of all the structures was performed using AM1 procedure (Dewar *et al* 1985) (AMPAC version 2.1) on a DEIL VAX 3300 computer. All geometries were optimised with respect to all geometrical parameters, without making any assumptions. Positive force constants for a few prototype structures (17a, b; 24a, b; 38a, b) characterised the geometries as local minima.

4. Results and discussion

AM1 H_f values of structures 17–59 (figure 1, table 1) and reactants MeOH, EtOH, MeSH, EtSH and by-products NH_3, $HOCH_2CH_2N^+(CH_3)_3$ (table 2) were calculated using the standard protocol described above.

Perusal of table 1 clearly reveals that hemi-acetals 24a–37a are more stable than their corresponding aza analogs 24b–37b by ≈ 23 kcal/mol based on AM1 ΔH_f values. This difference is somewhat higher than the ΔH_f of ≈ 15 kcal/mol for β-lactams 17a–23a vs aza-β-lactams 17b–23b (Nangia 1991) and esters 38a–51a vs carbamates 38b–51b. The higher ΔH_f between the carba vs aza substrates for hemi-acetals when compared with lactams and esters is attributed to the additional 1,3-diaxial repulsion between the non-bonded electrons on nitrogens in aza acetals 11b. This destabilising interaction is absent in aza lactams 10b because of lone-pair resonance with the sandwiched carbonyl group. The opening of the four-membered heterocycle and resonance with the adjacent carbonyl avoids repulsion between lone-pairs in carbamates 12b (figure 2).

A ΔH_f of ≈ 23 kcal/mol between carba and aza hemi-acetals is in accord with $\Delta H_f \approx 20$ kcal/mol from Benson's thermochemical tables (Benson *et al* 1969; Lowry and Richardson 1981) as the difference in heats of formation between CH_2 and NH groups flanked by carbons. An error of 3 kcal/mol between the calculated and estimated ΔH_f values is well within the mean absolute error of 5.9 kcal/mol in AM1 formalism (Dewar *et al* 1985).

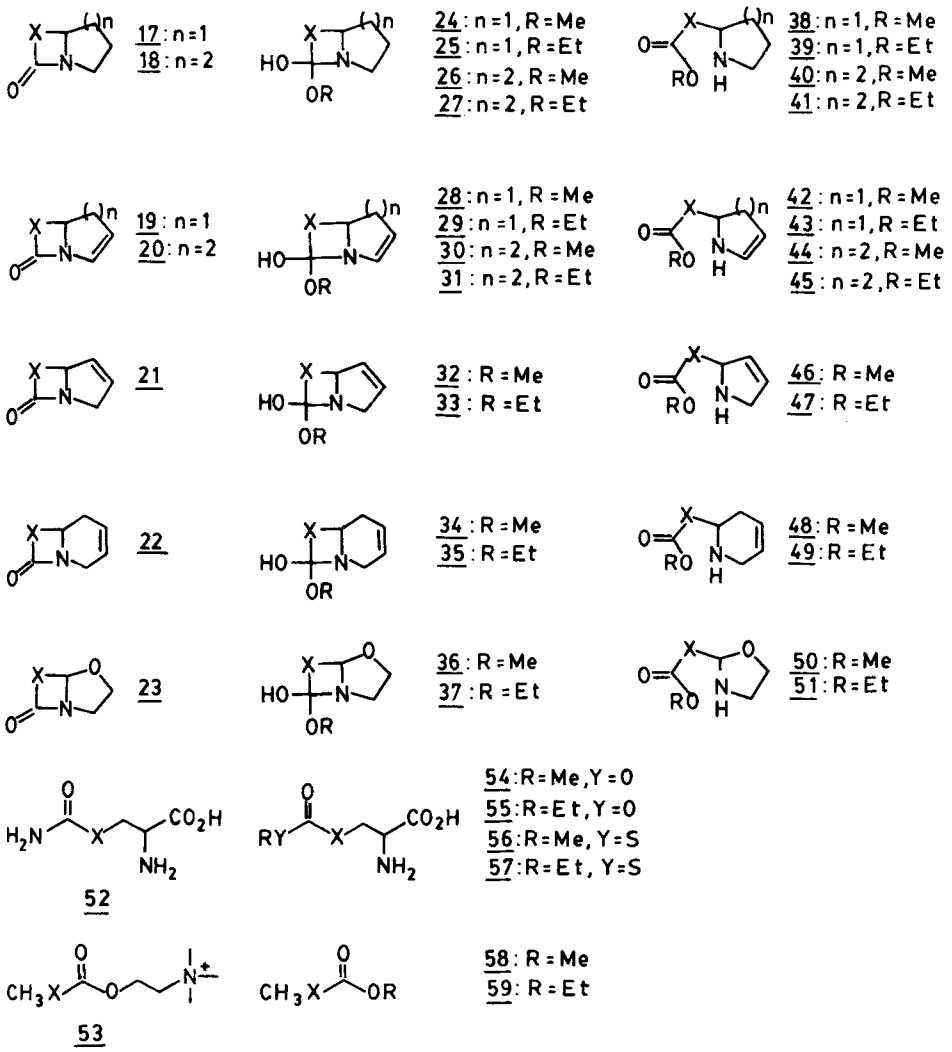


Figure 1. Structure of molecules 17a, b–59a, b (a: X = CH_2; b: X = NH).

The calculated ΔH_1 values for the reaction of MeOH and EtOH with substrates 17–23 to provide hemi-acetals 24–37 ((1), scheme 4) are listed in table 3. The formation of methoxy and ethoxy hemi-acetal adducts 24a–37a from β-lactams 17a–23a is generally exothermic (0–6 kcal/mol), except in a few cases of EtOH addition (to 18a, 20a, 22a) where it is moderately endothermic (0–1 kcal/mol). Compared to this, ΔH_1 values for aza analogs 17b–23b are consistently endothermic in the range of 3–9 kcal/mol. At this juncture it is important to realise that hemi-acetal formation is reversible; and that collapse of sp^3 tetrahedral intermediates 11 to sp^2 planar species 12 is the irreversible step in the inactivation cascade. Indeed, it is obvious from the ΔH_2 values in table 3 that the second step of (1) is far more exothermic for the aza isosteres (38–44 kcal/mol) when compared with the corresponding carba substrates (30–34 kcal/mol). The endothermicity of alcohol addition to aza lactams (step 1) is

Table 1. AM1 H_f values of substrates 17a–59a, aza analogs 17b–59b, and their differences, ΔH_f values (in kcal/mol).

Carba substrate	H_f	ΔH_f	H_f	Aza analog
17a	−7·76	15·15	7·39	17b
18a	−19·95	15·95	−4·00	18b
19a	25·14	14·95	40·09	19b
20a	5·18	15·86	21·04	20b
21a	28·27	15·61	43·88	21b
22a	8·95	15·94	24·89	22b
23a	−34·63	15·03	−19·60	23b
24a	−69·94	23·82	−46·12	24b
25a	−75·42	23·80	−51·62	25b
26a	−77·81	23·19	−54·62	26b
27a	−83·37	23·24	−60·13	27b
28a	−37·11	24·22	−12·89	28b
29a	−42·58	24·15	−18·43	29b
30a	−52·65	23·84	−28·81	30b
31a	−58·06	23·63	−34·43	31b
32a	−34·06	24·75	−9·31	32b
33a	−39·68	26·78	−12·90	33b
34a	−50·10	23·26	−26·84	34b
35a	−55·18	22·93	−32·25	35b
36a	−97·71	24·81	−72·90	36b
37a	−103·25	24·66	−78·59	37b
38a	−100·76	15·76	−85·00	38b
39a	−106·18	15·24	−90·94	39b
40a	−109·62	15·18	−94·44	40b
41a	−115·36	15·21	−100·15	41b
42a	−69·95	13·45	−56·50	42b
43a	−75·67	13·58	−62·09	43b
44a	−86·61	15·62	−70·99	44b
45a	−92·33	15·58	−76·75	45b
46a	−64·16	13·40	−50·76	46b
47a	−70·10	13·50	−56·60	47b
48a	−81·22	15·59	−65·63	48b
49a	−87·13	15·72	−71·41	49b
50a	−129·26	14·87	−114·39	50b
51a	−135·10	14·88	−120·22	51b
52a	−149·51	13·57	−135·94	52b
53a	−52·36	15·46	−36·72	53b
54a	−194·91	12·87	−182·04	54b
55a	−200·46	12·90	−187·56	55b
56a	−138·81	7·77	−131·04	56b
57a	−143·76	7·10	−136·66	57b
58a	−102·43	15·12	−87·31	58b
59a	−108·22	15·15	−93·07	59b

almost compensated during the formation of carbonyl species (step 2) such that the overall reaction is exothermic to the same extent in the two series ($\Delta H_{R\times N} \approx 31$–40 kcal/mol). Therefore, the reaction of MeOH and EtOH with aza-β-lactams 10b is as facile as with β-lactams 10a based on comparison on AM1 ΔH_f values. Since the

Table 2. H_f values of reactants and by-products (in kcal/mol).

Compound	H_f
MeOH	−57·02
EtOH	−64·21
MeSH	−3·35
EtSH	−9·50
NH_3	−7·28
$HOCH_2CH_2N^+(Me)_3$	−9·27

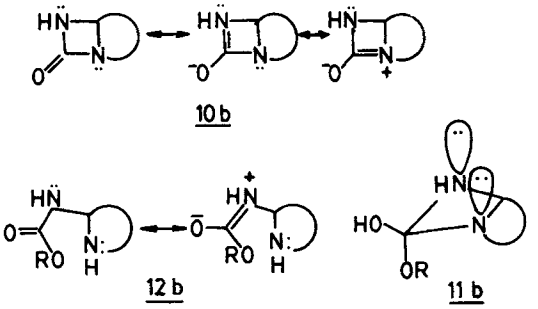


Figure 2. 1,3-Diaxial long-pair repulsion in aza acetals 11b; resonance structures showing delocalisation of lone-pair in lactams 10b, and carbamates 12b.

facile formation of acyl-enzyme intermediates 4a is a documented reaction (Knowles 1985), the proposed attack of β-lactamase serine-OH on aza-β-lactams 3b to furnish carbamoyl-enzyme intermediates 4b should also be favoured.

In order to estimate the extent of stabilisation because of partial amino-donation, it behoved us to compare differences in ΔH_f values in (1) (table 3) with the two reported examples ((2) and (3) table 4) delineated in scheme 4. Accordingly, $\Delta(\Delta H_f$ values) between the carba and aza series (aza-carba) were calculated (table 5). It is clear from the large number of negative values of table 5 (except 17)* that formation of carbamates 40b–51b from aza-β-lactams 18b–23b is more exothermic by 0–2 kcal/mol than the corresponding formation of 40a–51a from 18a–23a. More interestingly, similar values of $\Delta(\Delta H_f)$ are obtained for the transformations 52 → 54, 55 (−0·70, −0·67 kcal/mol) and 53 → 58, 59 (−0·52, −0·49 kcal/mol) (table 5), the bench-marks from reported examples on inactivation of proteases and esterases (Froede and Wilson 1971; Buchanan 1973, 1978). Therefore, semi-empirical $\Delta(\Delta H_f$ values) predict the postulated refractory hydrolysis of carbamates 12b with a hydrolytic stability comparable to 14b and 16b. Given the inhibition of hydrolytic enzymes under the aegis of amino donation in 7b and 9b, the prospect of β-lactamase inactivation by carbamates 4b appears promising. It is likely that the large negative $\Delta(\Delta H_f$ values) for the conversion 52 → 56, 57 with thiol nucleophiles (−5.80, −6·47 kcal/mol) are due to lack of parametrization for the sulphur atom in the AM1 package†.

*The reason for a small positive $\Delta(\Delta H_f)$ for 17 is not clear to us.

†Another deviation is found in table 1: the ΔH_f for 56, 57 is 7–8 kcal/mol; the average value is ≈15 kcal/mol.

Table 3. ΔH_f values for hemi-acetal (ΔH_1), ester/carbamate (ΔH_2) formation, and overall reaction ($\Delta H_{R \times n}$) of lactams 17–23 (in kcal/mol).

Substrate	ΔH_{1Me}	ΔH_{1Et}	ΔH_{2Me}	ΔH_{2Et}	$\Delta H_{R \times nMe}$	$\Delta H_{R \times nEt}$
17a	−5·16	−3·45	−30·82	−30·76	−35·98	−34·21
18a	−0·84	0·79	−31·81	−31·99	−32·65	−31·86
19a	−5·23	−3·51	−32·84	−33·09	−38·07	−36·60
20a	−0·81	0·97	−33·96	−34·27	−34·77	−33·30
21a	−5·31	−3·74	−30·10	−30·42	−35·41	−34·16
22a	−2·03	0·08	−31·12	−31·95	−33·15	−31·87
23a	−6·06	−4·41	−31·55	−31·85	−37·61	−36·26
17b	3·51	5·20	−38·88	−39·32	−35·37	−34·12
18b	6·40	8·08	−39·82	−40·02	−33·42	−31·94
19b	4·04	5·69	−43·61	−43·66	−39·57	−37·97
20b	7·17	8·74	−42·18	−42·32	−35·01	−33·58
21b	3·83	7·43	−41·45	−43·70	−37·62	−36·27
22b	5·29	7·07	−38·79	−39·16	−33·50	−32·09
23b	3·72	5·24	−41·49	−41·65	−37·77	−36·41

Table 4. ΔH_f values for the reaction of MeYH/EtYH with glutamine 52 and acetylcholine 53 (in kcal/mol).

Substrate	Y	$\Delta H_{R \times nMe}$	$\Delta H_{R \times nEt}$
52a	O	4·34	5·98
52a	S	6·77	7·97
53a	O	−2·32	−0·92
52a	O	3·64	5·31
52b	S	0·97	1·50
53b	O	−2·84	−1·41

Table 5. $\Delta(\Delta H_f$ values) (aza-carba) for the reaction of MeYH/EtYH with substrates 17–23 and 52, 53 (in kcal/mol).

Substrate	Y	$\Delta(\Delta H_{R \times nMe})$	$\Delta(\Delta H_{R \times nEt})$
17	O	0·61	0·09
18	O	−0·77	−0·08
19	O	−1·50	−1·37
20	O	−0·24	−0·28
21	O	−2·21	−2·11
22	O	−0·35	−0·22
23	O	−0·16	−0·15
52	O	−0·70	−0·67
52	S	−5·80	−6·47
53	O	−0·52	−0·49

5. Conclusions

In conclusion, AM1 heats of formation predict that (i) attack of β-lactamase serine-OH on aza-β-lactams will be as facile as on β-lactams, and (ii) extent of hydrolytic stabilisation due to partial amino-donation in carbamates derived from aza-β-lactams is comparable to that for aza analogues of glutamine and acetylcholine. Thus, theoretical calculations suggest that facile reaction of β-lactamase serine-OH with aza-β-lactams will yield carbamoyl-enzyme intermediates which will be refraetory to hydrolysis, thereby resulting in enzyme inactivation. Further work on the role of aqueous solvation in biological systems, conformation of active site serine hydroxyl, acid/base catalysis, electrostatic and allosteric effects will provide deeper insight into the pharmaceutically important subject of β-lactamase inactivation. The synthesis of novel aza-β-lactam molecules is currently under progress in our laboratory and these results will be reported elsewhere.

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