References

1. J. H. Nelson and H. B. Jonassen, *Coord. Chem. Rev.*, **6**, 27 (1971).
2. F. R. Hartley, *Chem. Rev.*, **69**, 799 (1969); *Angew. Chem.*, **84**, 657 (1972).
3. R. S. Dickson and P. J. Fraser, *Adv. Organomet. Chem.*, **12**, 323 (1973).
4. B. L. Shaw and A. J. Stringer, *Inorg. Chim. Acta. Rev.*, **7**, 1 (1973).
5. R. Baker, *Chem. Rev.*, 73, 487 (1973).
6. S. D. Ittel and J. A. Ibers, *Adv. Organomet. Chem.*, **14**, 33 (1976).
7. S. Otsuka and A. Nakamura, *Adv. Organomet. Chem.*, **14** 245 (1976).
8. H. C. Clark, G. Feruson, A. B. Goel, E. G. Janzen, H. Ruegger, P. Y. Siew, and C. S. Wong, *J. Am. Chem. Soc.*, **108**, 6961 (1986).
9. J. M. Huggins and R. G. Bergman, *J. Am. Chem. Soc.*, **103**, 3002 (1981).
10. C. Bianchini, P. Innocenti, D. Masi, A. Meli, and M. Sabat, *Organometallics*, **5**, 72 (1986).
11. A. Nakamura and S. Otsuka, *J. Am. Chem. Soc.*, **94**, 1886 (1972).
12. A. Nakamura and S. Otsuka, *J. Mol. Catal.*, **1**, 285 (1975/76).
13. W. R. Cullen, D. S. Dowson, and G. E. Styan, *Can. J. Chem.*, **43**, 3365 (1965).
14. A. J. Leusink and H. A. Budding, *J. Organomet. Chem.*, **11**, 533 (1968).
15. H. C. Clark and C. S. Wong, *J. Am. Chem. Soc.*, **99**, 7073 (1977).
16. G. E. Herberich and W. Barlage, *Organometallics*, **6** 1924 (1987).
17. J. S. Bradley and G. Wilkinson, *Inorg. Chem.*, **17**, 73 (1977)
18. I. M. Lee, D. W. Meek, and J. Gallucci, *Bull. Korean Chem. Soc.*, **13**, 491 (1992).
19. (a) A. D. Dobson, D. S. Moore, S. D. Robinson, M. B. Hursthouse, and L. New, *J. Organomet. Chem.*, **177**, C8 (1979); (b) G. Jia, A. L. Rheingold, and D. W. Meek, *Organometallics*, **8**, 1378 (1989).
20. I. M. Lee, D. W. Meek, and J. Gallucci, *Bull. Korean Chem. Soc.*, **13**, 498 (1992).
21. P. B. Critchlow and S. D. Robinson, *Inorg. Chem.*, **17**, 1902 (1978).
22. R. A. Sanchez-Delgado and G. Wilkinson, *J. Chem. Soc. (Dalton Trans.)*, 804 (1977).
23. T. B. Marder, D. Zargarianl, T. H. Herskovitz, and D. Milstein, *J. Chem. Soc. Chem. Commun.*, 1484 (1987).
24. J. P. Collman and W. R. Roper, *Adv. Organomet. Chem.*, **7**, 53 (1968).
25. N. S. Isaacs, "Reactive Intermediates in Organic Chemistry", John Wiley & Sons, London (1974).
26. M. R. Torres, A. Santos, and S. Solans, *Organometallics*, **6**, 1091 (1987).
27. J. J. Leu Sink, H. A. Budding, and W. Drenth, *J. Organomet. Chem.*, **11**, 541 (1968).
28. J. M. Huggins and R. G. Bergman, *J. Am. Chem. Soc.*, **101**, 4410 (1979).
29. S. Otsuka, A. Nakamura, Y. Tatsuno, and M. Miki, *J. Am. Chem. Soc.*, **94**, 3761 (1972)
30. H. G. Alt, U. Freytag, M. Herberhold, and H. I. Hayen, *J. Organomet. Chem.*, **336**, 361 (1987) and reference therein.

Structure-Activity Relationship Study on Cephalosporins with Mechanism-based-Descriptors

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The polarizability and the transition state energy of a cephalosporin are assumed to be theoretical indices of the permeability through the outer membrane and of reactivity of β-lactam ring with penicillin binding proteins, respectively, in Gram-negative bacteria. They are computed by AM1 method and used as variables of quantitative structure-activity relationship study. The results justify quadratic dependence of the activity on the variables. The intersection of difference volumes between β-lactamase stable cephalosporins and unstable ones manifests that the steric hindrance of 7-side chain is responsible for the β-lactamase stability.

Introduction

The cephalosporins, a series of β-lactam antibiotics, inhibit bacterial growth by acylation of the penicillin binding protein (PBP) involved in biosynthesis of the peptidoglycan layer of bacterial cell walls.[1] The bacteria can be classified as two groups, Gram-negative and Gram-positive. Generally the former has outer membrane in it, but the latter does not. The

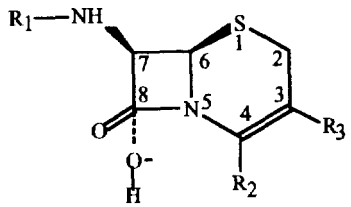


Figure 1. Transition state of complex. The complex consists of cephalosporin and model nucleophile OH^- corresponding to fragment of key residue Ser-OH near active site of PBP.

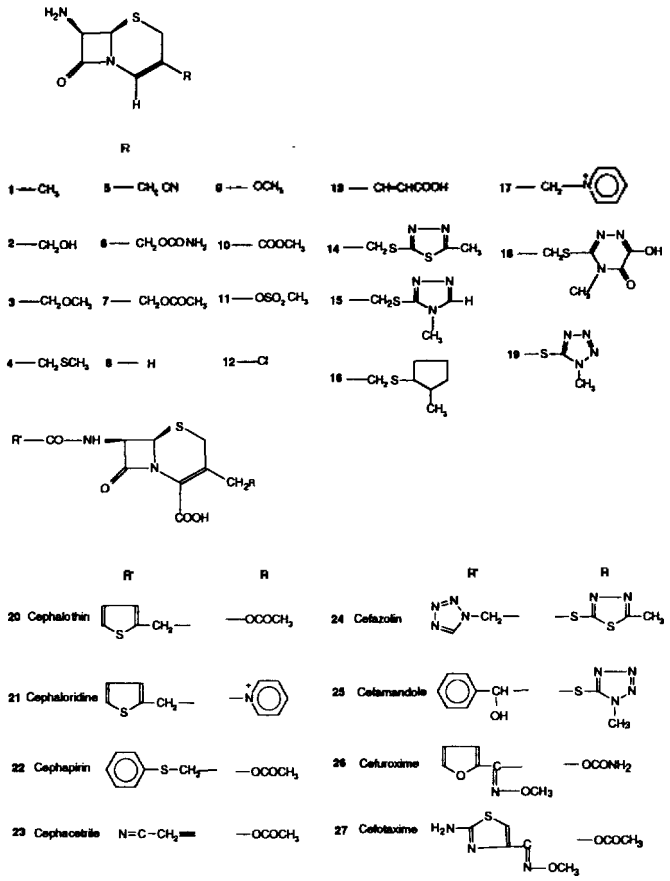


Figure 2. Compounds used as testing set. The 27 compounds consist of nineteen 7-NH_2-3-R-3-cephem and eight drugs.

mechanism of action of β-lactam antibiotics in the Gram-negative has the following cascade to inhibit the PBP.[2,3] Firstly, the antibiotic must penetrate the outer bacterial membrane into the sites of action.[4-6] Secondly, the antibiotic must resist the hydrolysis caused by β-lactamase.[7,8] Thirdly, it must encounter and react with PBP.

We choose the theoretical indices related to mechanism of ation of β-lactam antibiotics such as permeability, stability to β-lactamase and reactivity with PBP. The polarizability of antibiotics is adopted as a descriptor of the permeability since it is a factor related to hydrophobicity.[9] It is also known that the stability to β-lactamase result from steric hindrance of side chain of antibiotics.[10,11] The intersection volume, defined as the common one in difference of van der Waals volumes[12] between unstable and stable cephalosporins, is selected as an indicator of steric hindrance responsible for β-lactamase stability. The reactivity with PBP can be described by transition state energy (TSE),[13-16] which is defined as the difference in total energies of transition state in Figure 1 and each reactant in infinite separation. Thus,

$$\mathrm{TSE} = \mathrm{E(TS)} - \mathrm{E(\beta\text{-lactam reactant})} - \mathrm{E(OH^-)}. \quad (1)$$

Using the polarizability and the transition state energy as variables, quantitative structure-activity relationship (QSAR) is obtained. Also using the intersection volume, structure-actibity relationship (SAR) is acquired qualitatively.

Table 1. Calculated Values of Polarizability and TSE

No	Polarizability (Å^3)	TSE (kJ/mol)
1	18.1	−168
2	18.8	−138
3	21.2	−145
4	22.1	−138
5	20.1	−159
6	22.8	−147
7	23.5	−143
8	15.8	−130
9	19.2	−134
10	22.3	−751
11	26.3	−735
12	19.3	−781
13	25.2	−788
14	32.0	−775
15	30.9	−766
16	30.2	−801
17	49.9	−211
18	35.5	−787
19	28.0	−781
20	39.2	−220
21	45.9	−601
22	44.1	−249
23	32.6	−215
24	47.4	−263
25	48.1	−228
26	42.5	−169
27	45.4	−426

Computational Method & Results

We calculate 27 compounds in Figure 2, which contain nineteen 7-NH_2-3-R-3-cephem and eight drugs in market. Anti-bacterial activity is expressed in terms of averaged minimum inhibitory concentration (MIC) against five test organisms: *S. sonnei, E. coli, K. pneumoniae, E. aerogenes* and *S. heidelberg*.[13,17] Polarizability and TSE are taken as variables of QSAR, and the intersection volume is used as an indicator of steric hindrance. Quadratic dependence of activity on both polarizability and TSE is conjectured as follows:[13,18]

$$\mathrm{MIC} = a\mathrm{POL}^2 + b\mathrm{POL} + c, \quad (2)$$

and

$$\mathrm{MIC} = a\mathrm{TSE}^2 + b\mathrm{TSE} + c. \quad (3)$$

Table 2. Results of Regression Analysis

Variable	n	r	S	F	Signif. F	a	b	c
Polarizability	17	0.672	11.37	9.886	0.0007	5.55×10^{-2}	−4.45	89.4
TSE	27	0.589	12.41	6.380	0.0060	1.85×10^{-4}	-1.97×10^{-1}	46.3

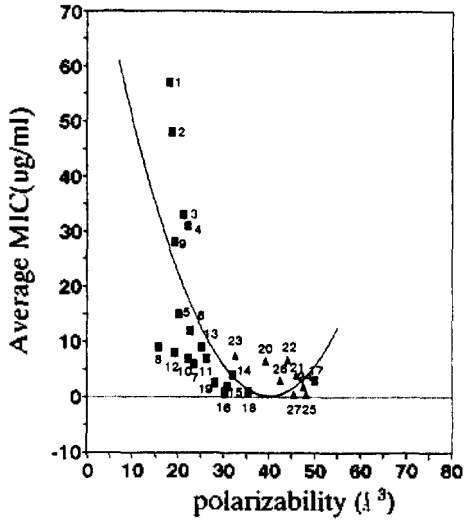


Figure 3. Average *in vitro* Gram-negative MIC *vs* Polarizability. The ■ and ▲ indicate 7-NH_2-3-R-3-cephem and eight drugs respectively. The polarizability of eight drugs are concentrated near the minimum of parabolic curve.

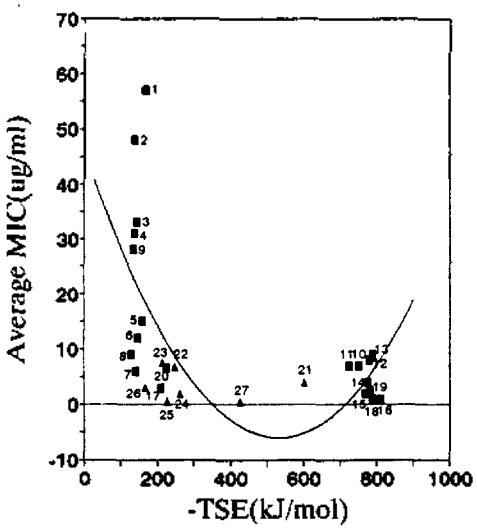
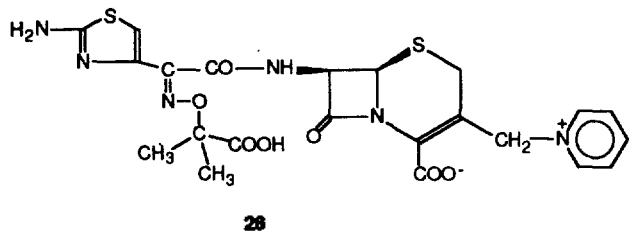


Figure 4. Average *in vitro* Gram-negative MIC *vs* TSE. The ■ and ▲ indicate 7-NH_2-3-R-3-cephem and eight drugs respectively. The TSEs of eight drugs nearly lie between those of 7-NH_2-3-R-3-cephems. The cefotaxime **27** and cephaloridine **21** have TSEs near the minimum of parabolic curve.

The coefficients a, b and c are calculated by SPSS^X.[22]

We take an initial geometry from X-ray crystallography,[19] and optimize it using molecular mechanics in DTMM.[20] The geometry is further optimized and then the polarizability, which is the average of its three principal components, and TSE are calculated by the AM1 method in GEOMOS.[21] The



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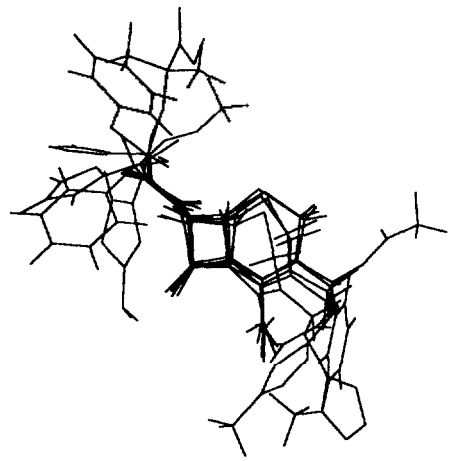


Figure 5. Superimposition of five cephalosporins. The five cephalosporins contain three β-lactamase unstable cephalosporines: cephapirin, cefazolin and cefamandole, and two β-lactamase stable cephalosporins: cefuroxime and ceftazidime.

results of calculation and regression are collected in Tables 1 and 2, respectively. Polarizability and TSE correlate to MIC with 99.93% and 99.40% confidence limits respectively. Activity *vs.* polarizability and Activity *vs.* TSE are plotted in Figures 3 and 4, respectively. The ■ indicates 7-NH_2-3-R-3-cephem and the ▲ depicts eight drugs. It is noted that the polarizabilities of eight drugs in Figure 3 are concentrated in the range of 30 and 50 Å^3-good drug must have optimum polarizability. The highly potent cefotaxime **27** lies near minimum of both parabolic curves.[17]

The intersection volume is calculated to find the essential geometrical feature responsible for β-lactamase stability. The β-lactamase unstable cephalosporins are cephapirin **22**, cefazolin **24** and cefamandole **25**. The β-lactamase stable ones are cefuroxime **26** and ceftazidime **28**. We use Insight*II* program of the BIOSYM[23] to find the intersection volume. The starting geometries of five cephalosporins are generated in the BUILDER module of the Insight*II* using molecular fragments obtained from the crystallographic fragment library available within 3D CONVERTER and fully minimized using DISCOVER CVFF force field. The further geometry optimization have been carried out by AM1 method in the MOPAC 6.0. Using TRANSFORM module of Insight*II*, the five cepha-

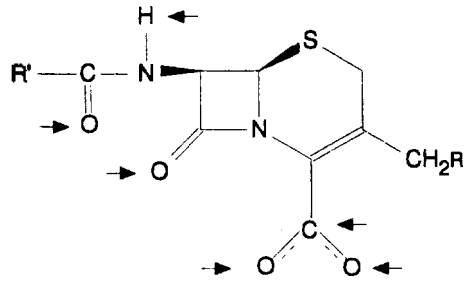


Figure 6. Reference sites in superimposition. The six atoms are used as reference sites, which play a role of binding with β-lactamase.

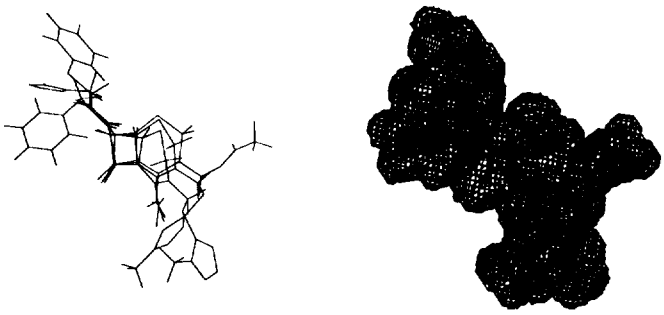
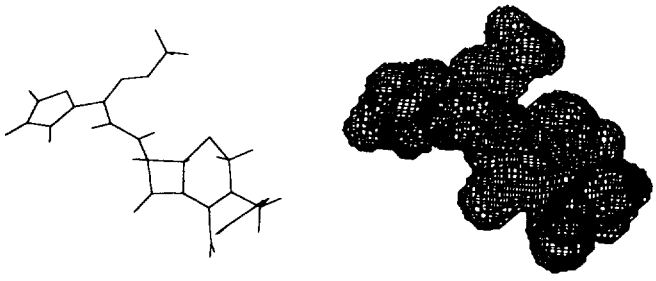


Figure 7. Union volume of three β-lactamase unstable cephalosporins: cephapirin, cefazolin and cefamandole.



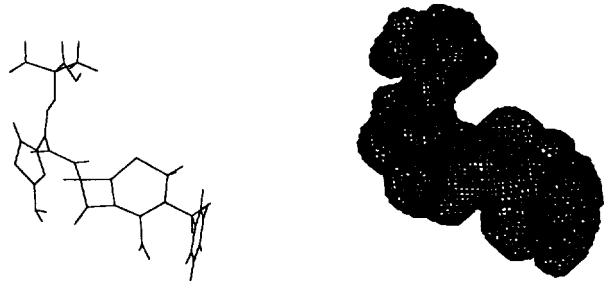


Figure 8. Volume of β-lactamase stable cephalosporins. (a) Cefuroxime, (b) Ceftazidime.

losporins are superimposed (Figure 5). The six atoms of ceftazidime, having a good β-lactamase stability, are used as a reference in the superimposition (Figure 6). To calculate the intersection volume, SEARCH/COMPARE module of Insight*II* is used. The union volume of β-lactamase unstable cephalosporins is calculated (Figure 7), and the volumes of the β-lactamase stable ones are calculated respectively (Figures 8(a), 8(b)). Then the difference volumes between volumes of β-lactamase stable cephalosporins and the union volume of unstable ones are calculated respectively (Figures 9(a), 9(b)). These difference volumes give useful information on geometrical structure responsible for β-lactamase stability. To obtain essential geometrical structure for the stability, the intersection volume between two difference volumes is calculated (Figure 10). The intersection volume confirms the claim of previous reports[24] that the steric hindrance of certain region of 7-side chain of cephalosporin plays a crucial role in β-lactamase stability.

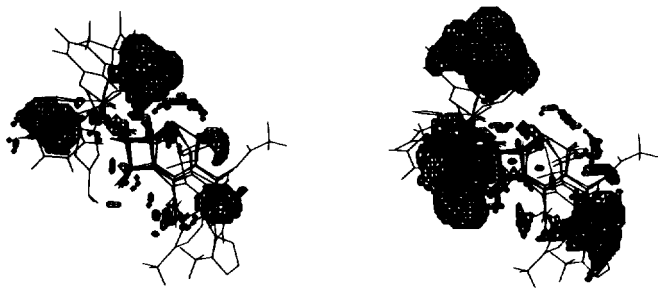


Figure 9. Difference in volume of β-lactamase stable cephalosporins and union volume of β-lactamase unstable ones. (a) Difference volume of cefuroxime, (b) Difference volume of ceftazidime.

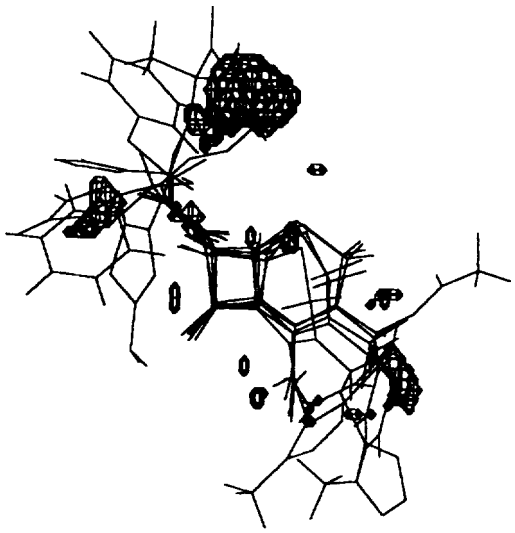


Figure 10. Intersection between difference volumes. The intersection between two difference volumes of Figures 9(a) and 9(b) is obtained. The intersection volume represents the essential geometrical feature responsible for β-lactamase stability.

Discussion

Polarizability and TSE are found as a reliable parameters directly related with the mechanism of action. Intersection volume is proved to be a indicator representing β-lactamase stability. From QSAR with polarizability and TSE, and SAR with intersection volume, it is concluded that the potent drug, with its MIC in range of 0 and 10 μg/ml, should satisfy

the following properties. Firstly, the polarizability of antibiotic must have value in the range of 27 and 53 Å^3 to permeate the outer membrane of Gram-negative bacteria. Secondly, the TSE of antibiotic has to lie between −830 and −240 kJ/mol^{-1} to inhibit with PBP. Thirdly, the intersection volume has to be contained in antibiotic not to be easily hydrolyzed against β-lactamase.

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References

1. R. R. Yocum, D. J. Wasman, J. R. Rasmussen, J. L. Strominger, *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 2730 (1979).
2. A. Tomasz, *Annu. Rev. Microbiol.*, **33**, 113 (1979).
3. R. Wise, *Clin. Pharmacokinet.*, **11**, 470 (1986).
4. J. Trais and H. Nikaido, *Antibicrob. Agents Chemother.*, **34**, 52 (1990).
5. M. J. Miller, *Chem. Rev.*, **89**, 1563 (1989).
6. H. Nikaido, *Pharmacol. Ther.*, **27**, 197 (1985).
7. R. P. Ambler, *Philos. Trans. R. Soc. London, Ser. B*, **289**, 321 (1980).
8. A. A. Medeiros, *Br. Med. Bull.*, **40**, 18 (1984).
9. 'Theoretical drug design methods', R. Franke, Academie-Verlag Press, Berlin, 1984, pp. 132-134.
10. 'Recent Advances in the Chemistry of β-lactam Antibiotics', ed. J. Elks, Special Publication No. 28, The Chemical Society, London, 1977, p. 145.
11. T. A. Tartaglione and R. E. Dolk, *Drug Intell. Clin. Pharm.*, **19**, 188 (1985).
12. S. Y. Sit, R. A. Parker, I. Motoc, W. Han, N. Balasubramanian, J. D. Catt, P. J. Brown W. E. Harte, M. D. Thompson, and J. J. Wright, *J. Med. Chem.*, **33**, 2982 (1990).
13. D. B. Boyd, D. K. Herron, W. H. Lunn, and W. A. Spitzer, *J. Am. Chem. Soc.*, **102**, 1812 (1980).
14. D. B. Boyd, *Ann. N. Y. Acad. Sci.*, **367**, 531 (1981).
15. D. B. Boyd, *J. Med. Chem.*, **26**, 1010 (1983).
16. 'Frontiers of Antibiotic Research', ed. H. Umezawa, Academic Press, New York, 1987, pp. 339-356.
17. 'Antibiotics, an intergrated view', ed. G. C. Lancini, Springer-Verlag Press, New York, 1982, pp. 108-112.
18. C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, **86**, 1616 (1964).
19. R. M. Sweet and L. F. Dahls, *J. Am. Chem. Soc.*, **92**, 5489 (1970).
20. M. James, C. Crabbe, program Desktop Molecular Modeller, Version 1.2.
21. D. Rinaldi, P. E. Hoggan, and A. Cartier, GEOMOS program QCPE No. 584.
22. SPSS^X User's Guide 2nd. ed., SPSS Inc, Chicago, 1986.
23. Insight*II*, Ver. 2.1.2., Biosym Technologies Inc., San Diego, 1992.
24. 'Comprehensive Medicinal Chemistry', Vol. 2, ed. P. G. Sammes, Pergamon Press, 1990, pp. 631-632.