

Importance of sulfoxidation in rapid racemisation of glitazones

Majid Ahmed, Smriti Khanna & Prasad V Bharatam*

Department of Medicinal Chemistry, National Institute of Pharmaceutical Education & Research (NIPER),
S. A. S. Nagar (Mohali) 160 062, Punjab, India

E-mail: pvbharatam@niper.ac.in

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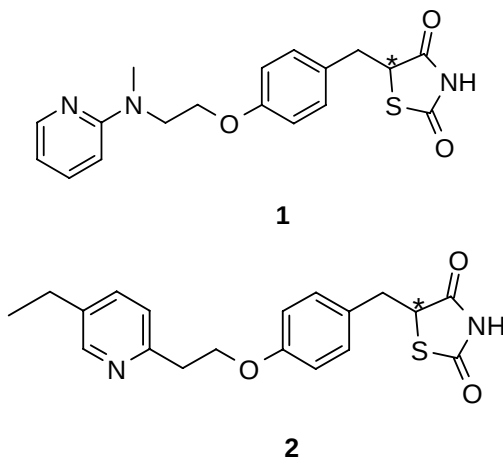
AM1 calculations on equilibrium and transition state geometries of keto and enol forms of glitazones were carried out and compared with those of some carbonyl compounds. The results show that the energy requirements for enolisation of glitazones are much higher than the standard carbonyl systems. Neither the ring system, nor the additional functional groups in thiazolidinedione terminal ring facilitate the keto-enol tautomerisation, hence keto-enol tautomerisation cannot be considered as the cause for rapid racemisation in glitazones. Thio compounds are known to undergo reversible oxidation and the barrier for 1, 3-H shift in S-oxide form of thiazolidinediones is significantly lower than all the other agents studied. This study strongly indicates the involvement of S-oxidation in the enhanced rate of racemisation of thiazolidinediones.

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Chiral sulfoxides have been shown to be important in synthesizing chemicals with higher stereoselectivity¹⁻³. Sulfoxide functional groups increase the acidity at the adjacent chiral carbon centre with –CH bond. Since the sulfoxides can be easily eliminated under thermal conditions and they can be enzymatically reduced under biological conditions, the reversible S-oxide formation has been recognised as a chiral relay process.

Glitazones (**1**) are a class of insulin sensitizers, which contain sulphur atom in the five membered thiazolidinedione terminal ring. They are agonists for the peroxisome proliferator activated receptor gamma (PPAR- γ)—a member of the nuclear receptor superfamily involved in lipid homeostasis⁴. Several hundreds of glitazones have been synthesised and tested for antidiabetic activity and of them two compounds i.e. Rosiglitazone⁵ **1** and Pioglitazone⁶ **2** are presently in clinical use in US and Japan.

These agents contain a chiral centre at the 5th position in the thiazolidinedione terminal ring. S-Enantiomer has been found to be more potent than the R-enantiomer⁷. But utilisation of the pure eutomer for therapeutic purpose turned futile when it was observed that it underwent rapid racemisation under physiological conditions, giving no net advantage of the tedious racemic deconvolution. Sohda *et al.*⁸ have attributed the process of rapid racemization observed in glitazones to occur by enolisation process, however



there is no evidence available to show the existence of enols of thiazolidinediones.

Similar studies on non-thiazolidinedione insulin sensitizers like oxazolidinediones⁹, alkoxypropionic acid derivatives¹⁰ and tyrosine based¹¹ PPAR- γ agonists have revealed the differential activity of the two enantiomers. In fact, the racemisation problem in glitazones led to the diversion of drug design efforts towards non-thiazolidinedione agents, which do not undergo such rapid racemisation.

Hulin *et al.*¹² have proposed that reversible S-oxide formation *in vivo* enzymatically in thiazolidinediones to form sulfoxides or sulfones may be causing the observed difference. Hence, it is important to know which of these processes, enolisation or sulfoxide

formation in glitazones is responsible for rapid racemisation. We have carried out semi-empirical AM1 calculations on glitazones and some non-thiazolidinedione derivatives to understand the importance of sulfoxides in the rapid racemisation. This is in continuation of our work on electronic structure studies of organo-sulfur compounds.

Computational methods

Semi-empirical AM1¹³ calculations have been carried out on a series of glitazones using MOPAC package. AM1 method is ideally suited for studying the systems under consideration because it accounts for the electronic effect as well as the study becomes cost-effective in terms of computer time. The other alternate choices (i) *ab initio* and density functional methods are very costly for the molecules of this size and (ii) force field methods do not account for electronic effects. The racemisation study is a study of bond breaking and bond formation and thus involves the electronic changes and hence demands the use of electronic structure methods. So, we opted for the semi-empirical AM1 method for the calculations. However, since the semi-empirical methods involve approximations, it is ideal to verify whether they are applicable in a given situation. For this purpose, we have studied the keto-enol tautomerisation in the model compound methyl thiazolidinedione using AM1, HF/6-31+G* and B3LYP/6-31+G* methods (**Table I**). The results indicate that the estimated energy differences between the tautomers and the 1,3-H shift barriers obtained using AM1 method are quite

comparable to the results obtained using HF/6-31+G* and B3LYP/6-31+G* levels. Hence, this study was continued using AM1 method. The structural features of important structures, their keto and enol tautomers and the 1,3-H shift transition states are given in **Figure 1**.

Result and Discussion

Extensive studies on tautomerism in carbonyl compounds, imines, acetylenic linkages etc. have been carried out and a large number of reports related to gas phase calculations on tautomerism and related proton transfer reactions are present in literature¹⁴⁻²⁴. **Table II** shows the diverse range of proton transfer reactions in which *ab initio* studies at G2 level have been reported. AM1 estimated tautomerisation energies of the tautomeric processes reported in **Table II** also are comparable to the higher level estimates, confirming the validity of the AM1 method for the current study. The energy difference (ΔE) between the two tautomers is generally correlated to the extent of tautomerisation, lower ΔE representing higher tautomerisation, and feasibility is indicated by overall lower 1,3-H shift barrier (E_a). The energy estimates support the correlation between ΔE of the tautomers and the percentage enol content. Experimentally the percentage enol content is taken as a measure of keto-enol tautomerisation. **Table III** shows a correlation between the percentage enol content and the ΔE between keto-enol tautomers of some carbonyl compounds obtained using AM1 method.

It is in general, expected that the rate of racemisation in thiazolidinediones would be directly proportional to the feasibility of keto-enol tautomerism. Similar energy estimations at semi-empirical AM1 level were carried out on a set of glitazones **G1-G8** (**Figure 2**). The relative energies of keto-enol tautomers of glitazones **G1-G8** are 9.8, 10.0, 9.7, 11.1, 10.0, 9.4, 9.4 and 10.9 kcal/mol

Table I — Relative energies (ΔE), 1,3-H shift barrier in kcal/mol at AM1, HF and B3LYP methods using 6-31+G* basis set for 5-methylthiazolidinedione (**G1**)

Energy parameters	AM1	HF	B3LYP
ΔE	13.20	27.92	23.35
1,3-H shift barrier	85.77	98.20	79.23

Table II — Comparison of energy difference between tautomers (ΔH_f , ΔE) and activation energy for 1,3-H shift (E_a) of some tautomeric pairs at AM1 and MP2(full)/6-31G* and G2 levels (in kcal/mol)

Tautomeric process	Model system	AM1		MP2/6-31G*		G2 ΔE
		ΔH_f	E_a	ΔE	E_a	
Keto – enol	$\text{CH}_3\text{-CHO} \rightleftharpoons \text{CH}_2\text{CHOH}$	08.03	81.69	16.82	76.09	10.8
Imine – enamine	$\text{CH}_3\text{-CH=NH} \rightleftharpoons \text{CH}_2\text{=CH-NH}_2$	00.55	74.59	07.83	74.24	3.9
Amide – imidic	$\text{NH}_2\text{-CHO} \rightleftharpoons \text{NH=CHOH}$	13.48	62.68	13.74	48.34	11.5
Nitro - aci-nitro	$\text{CH}_3\text{-NO}_2 \rightleftharpoons \text{CH}_2\text{=NOH}$	12.18	72.57	22.89	73.64	14.1

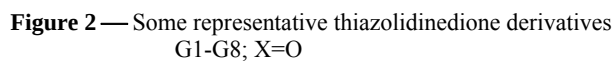
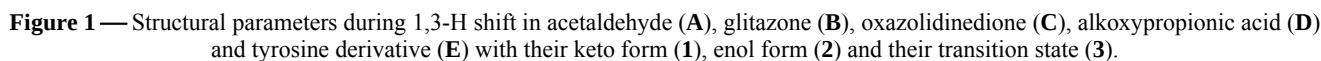


Table III — Relative energies (ΔH_f), enolisation barriers (E B) in kcal/mol with enol content for some carbonyl compounds using AM1 method

Sl No	Molecule	% Enol content	log (Enol content)	ΔH_f	E B
1	Acetophenone	6×10^{-7}	-6.22	10.26	79.62
2	Acetaldehyde	6×10^{-5}	-4.22	8.04	81.69
3	Cyclohexanone	4×10^{-5}	-4.40	7.44	80.28
4	Dimethylacetaldehyde	1.4×10^{-2}	-1.85	2.92	78.33
5	Diphenylacetaldehyde	9.1	0.96	-0.37	72.37
6	Acetylacetone	80	1.9	-1.09	66.79

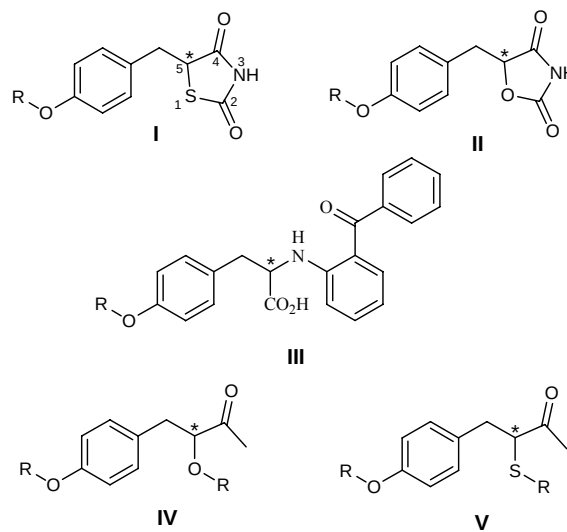
respectively (**Table IV**). These values are comparable to keto-enol tautomer differences in acetaldehyde (8 kcal/mol) and acetophenone (10.2 kcal/mol), indicating that the energy requirement of the keto-enol tautomerism in glitazones is similar to that of simple aldehydes and ketones which do not show rapid racemization. The energy barriers for 1,3-H shift in **G1-G8** are 83.1, 83.2, 81.2, 83.5, 83.0, 84.6, 83.4 and 83.5 kcal/mol respectively. These values are high enough to prevent unimolecular hydrogen shift. Also these values are comparable to the 1,3-shift barrier in acetaldehyde (81.7 kcal/mol) and acetophenone (79.6 kcal/mol), whose enol content is very very small (**Table III**). This indicates that neither ring strain due to five membered ring nor the presence of active centres (two C=O groups and one NH group) in thiazolidinediones, accelerate tautomerism in glitazones. The ΔH_f and the 1,3-H shift barriers of glitazones **G1-G8** are much higher than that of dimethylacetaldehyde ($\Delta H_f = 2.9$, $\Delta H_{bar} = 78.3$ kcal/mol) and diphenylacetaldehyde ($\Delta H_f = -0.36$, $\Delta H_{bar} = 72.4$ kcal/mol) whose enol content is relatively high, indicating that the enol content of glitazones should not be expected to be significant.

The above energy analysis suggests that keto-enol tautomerism might not be responsible for the rapid racemization observed in glitazones based anti-hyperglycemic agents. Racemisation is not known to occur in non-thiazolidinediones. In order to find the differences in the energy parameters between racemising and non-racemising systems similar calculations were performed on some representative examples of non-thiazolidinediones **II-V** (**Figure 3**).

Oxazolidinediones (**II**), are the oxygen analogs of thiazolidinediones. The energy estimates show a considerable increase in relative energies between the two tautomers of oxazolidinediones as compared to glitazones. The relative energies of oxazolidinediones **O1-O5** are of the order of 22.0 kcal/mol (**Table S-I**)

Table IV — Relative energies (ΔH_f), 1,3-H shift barriers (E B) in kcal/mol for glitazones

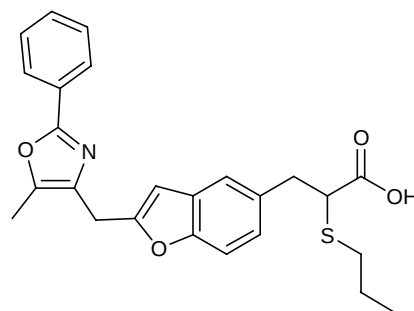
Sr No	Molecule	ΔH_f	E B
1	G1	9.82	83.11
2	G2	10.04	83.25
3	G3	9.69	81.16
4	G4	11.11	83.47
5	G5	10.02	83.02
6	G6	9.40	84.62
7	G7	9.38	83.43
8	G8	10.99	83.53

**Figure 3** — Non-thiazolidinediones category of insulin sensitizers (**II-V**)

almost 12 units more than glitazones. The 1,3-H shift barriers also show an increase in the energy values up to 96.5 kcal/mol. This indicates that replacement of sulfur with the oxygen atom in the ring decreases the probability of keto-enol tautomerism in oxazolidinediones making them less vulnerable to racemization. The results are consistent with the fact

that they show lesser tendency for racemization^{9b} when compared to glitazones. Semi-empirical calculations on some representative non-thiazolidinedione tyrosine derivatives (**III**) **T1-T5** showed relative energies to be ~8 kcal/mol, energy barriers to be ~72 kcal/mol (**Table S-II**). The ΔH_f values determined for a set of five different alkoxy propionic acid derivatives, dual activators of PPAR- γ and PPAR- α , **A1-A5** are ~15 kcal/mol, and energy barriers are ~78 kcal/mol (**Table S-III**). From the data in **Table IV** and **Tables S-I** to **S-III** it is clear that there is no direct correlation between tautomerisation energies and rapid racemisation in the anti-hyperglycemic agents. For example, tyrosine derivatives, which show no racemisation, show less ΔH_f between tautomers of glitazones, which show rapid racemisation. Hence, it may be concluded that inter/intramolecular keto-enol tautomerisation in glitazones is not responsible for the rapid racemisation in these systems.

Hulin *et al.*^{12,25} have reported α -alkylthio carboxylic acid and α -alkoxy carboxylic acid derivatives as PPAR- γ agonists. The ether series was apparently more potent than the thioether series. To reason out the same they tried to find out the effect of stereochemistry at the α -position to the carboxylic acid. Individual enantiomers of one compound of each series were examined. The two enantiomers of **St3**, for which the absolute configuration was not established for α -alkylthio carboxylic acid, were found to be equipotent in their ability to lower glucose *in vivo*. It is ascribed that the inactive antipode readily racemizes either directly by *in vivo* proton exchange as observed in englitazone²⁵ and 2-arylpropionic acids like ibuprofen²⁶ or by indirect racemization via reversible *in vivo* oxidation of sulphide to sulfoxide at physiological pH. Examination of the intrinsic potency of the two enantiomers indicated (-)-**St3** to be more potent than (+)-**St3** with EC₅₀ of 0.2 and 20nM respectively. The result is consistent with the racemization taking place *in vivo* in case of sulfur compounds. The drug candidate **Ot2** from the α -alkoxy carboxylic acid series for which racemization would not be expected, its activity was found to reside entirely in (S) enantiomer. The EC₅₀ value for (S) antipode was estimated to be 5nM and 100nM for the (R) antipode. The same phenomenon is observed for thiazolidinediones, which have greater tendency for racemization compared to oxazolidinediones. This finding strongly indicates the obvious role of S atom in the rapid rate of racemisation. Thus, Hulin

**St3**

et al.^{12,25}, have indicated the proneness of the sulphide to reversible S-oxidation under physiological conditions and have anticipated the enhancement of racemisation in the oxidized state.

To explore the role of S-oxidation in thiazolidinediones, a set of semi-empirical AM1 calculations were carried out on oxidized form of glitazones **G1-G8** (i.e. **Gs1-Gs8**) (**Table V**). Owing to the pyramidal nature of the S atom in the oxidized form, it is chiral and the more stable 1*R*, 5*S* diastereomer is considered in further calculations. In this series the enolisation process can occur two ways; either with adjacent carbonyl group or with the sulfoxide group (**Figure 4**). The results obtained are strongly biased for enolisation of the sulfoxide group (**Table V**). Enolisation of the sulfoxide group brings down the relative energies to 5 kcal/mol and more prominently the barrier for enolisation, to as low as 45 kcal/mol. On the other hand, the results for enolisation of the carbonyl group do not show as much variation from the non-oxidized form. The relative energies between the two tautomers are found to be 10 kcal/mol. The 1,3-H shift barriers also remain unaffected with a value of 77 kcal/mol. This suggests that reversible S-oxidation as proposed by Hulin *et al.*^{12,25} seems to

Table V—Relative energies (ΔH_f), 1,3-H shift barriers (E B) in kcal/mol for sulfoxide derivatives of glitazones **G1-G8** (X=O)

Sulfoxides	$\Delta H_{f \text{ enol-keto}}$	$\Delta H_{f \text{ SO-SOH}}$	E B _{enol}	E B _{SOH}
Gs1	8.84	5.22	76.87	40.89
Gs2	9.63	4.91	76.62	44.23
Gs3	9.29	4.28	76.69	47.63
Gs4	9.52	4.98	77.48	43.17
Gs5	9.82	5.12	77.53	43.59
Gs6	9.28	5.06	77.49	44.72
Gs7	9.63	4.89	77.32	46.17
Gs8	9.65	4.87	77.96	46.86

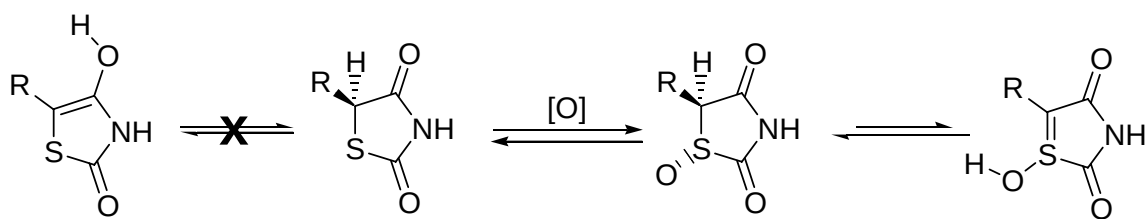


Figure 4—Tautomeric processes in sulfoxides of thiazolidinediones

play a major role in making feasible *in vivo* rapid racemization observed in glitazones. Further verification for the probable role of S-oxidation was done by carrying out semi-empirical AM1 level calculations on sulfoxides of α -alkylthio carboxylic acid series **St1-St6** (Table S-IV) as proposed by Hulin *et al.*^{12,25} and compared to the alkyl ether carboxylic acids **Ot1-Ot6**. The results achieved for this series were akin to those obtained for **G1-G8**. Hence, reversible *in vivo* oxidation of sulphur to sulfoxides seems to be a logical reasoning for the rapid racemization observed in glitazones.

Supplementary material consisting of four **Tables S-I to S-IV** can be obtained from the authors.

Conclusions

Semi-empirical AM1 calculations on a representative set of glitazones and related anti-hyperglycemic agents indicate that keto-enol tautomerisation does not play role in the rapid racemisation of glitazones, contrary to the general expectations. The racemisation process most likely occurs by the sulfoxide pathway, which is found to reduce the energy estimates considerably so as to make it feasible under physiological conditions.

References

- (a) Damani L A, *Drug Metabolism-from Molecules to Man* Benford, D J Bridges, J W Gibson, G G Eds Taylor & Francis: London, **1987**, 581.
(b) Thompson H J, Jiang C, Lu J, Mehta R G, Piazza G A, Paranka N S, Pamukcu R & Ahnen D J, *Cancer Res*, **2**, **1997**, 267.
(c) Swanson B N, Mojaverian P, Boppana V K & Dudash M R, *Drug Metab Dispos*, **6**, **1981**, 499.
- Holland H L, *Chem Rev*, **88**, **1998**, 473.
- Fernandez I & Khair N, *Chem Rev*, **103**, **2003**, 3651.
- Lehmann J M, Moore L B, Smith-Oliver T A, Wilkinson W O, Willson T M & Kliewer S A, *J Biol Chem*, **270**, **1995**, 12953.
- Cantello B C C, Cawthorne M A, Cottam G P, Duff P T, Haigh D, Hindley R M, Lister C A, Smith S A & Thurlby P L, *J Med Chem*, **37**, **1994**, 3977.
- Sohda T, Momose Y, Meguro K, Kawamatsu Y, Sogiyama Y & Ikeda H, *Arzneim -Forsch/ Drug Res*, **40**, **1990**, 37.
- Parks D J, Tomkinson N C O, Villeneuve M S, Blanchard S G & Willson T M, *Bioorg Med Chem Lett*, **8**, **1998**, 3657.
- Sohda T, Mizuno K & Kawamatsu Y, *Chem Pharm Bull*, **32**, **1984**, 4460.
- (a) Dow R L, Bechle B M, Chou T T, Clark D A, Hulin B & Stevenson R W, *J Med Chem*, **34**, **1991**, 1538.
(b) Momose Y, Maekawa T, Yamano T, Kawada M, Odaka H, Ikeda H & Sohda T, *J Med Chem*, **45**, **2002**, 1518.
- (a) Buckle D R, Cantello B C C, Cawthorne M A, Coyle P J, Dean D K, Faller A, Haigh D, Hindley R M, Jecott L J, Lister C A, Pinto I L, Rami H K, Smith D G & Smith S A, *Bioorg Med Chem Lett*, **6**, **1996**, 2121.
(b) Buckle D R, Cantello B C C, Cawthorne M A, Coyle P J, Dean D K, Faller A, Haigh D, Hindley R M, Jecott L J, Lister C A, Pinto I L, Rami H K, Smith D G & Smith S A, *Bioorg Med Chem Lett*, **6**, **1996**, 2127.
(c) Sauerberg P, Pettersson I, Jeppesen L, Bury P S, Mogensen J P, Wassermann K, Brand C L, Sturis J, Woldike H F, Fleckner J, Andersen A T, Mortensen S B, Svensson L A, Rasmussen H F, Lehmann S V, Polivka Z, Sindelar K, Panajotova V, Ynddal L & Wulff E M, *J Med Chem*, **45**, **2002**, 789.
- (a) Henke B R, Blanchard S G, Brackeen M F, Brown K K, Cobb J E, Collins J L, Harrington W W, Hashim (Jr) M A, Hull-Ryde E A, Kaldor I, Kliewer S A, Lake D H, Leesnitzer L M, Lehmann J M, Lenhard J M, Orband-Miller L A, Miller J F, Mook R A, Noble S A, Oliver W, Parks D J, Plunket K D, Szewczyk J R & Willson T M, *J Med Chem*, **41**, **1998**, 5020.
(b) Collins J L, Blanchard S G, Boswell G E, Charifson P S, Cobb J E, Henke B R, Hull-Ryde E A, Kazmierski W M, Lake D H, Leesnitzer L M, Lehmann J, Lenhard J M, Orband-Miller L A, Gray-Nunez Y, Parks D J, Plunket K D & Tong W, *J Med Chem*, **41**, **1998**, 5037.
(c) Cobb J E, Blanchard S G, Boswell G E, Brown K K, Charifson P S, Cooper J P, Collins J L, Dezube M, Henke B R, Hull-Ryde E A, Lake D H, Lenhard J M, Oliver W, Oplinger J, Pentti M, Parks D J, Plunket K D & Tong W, *J Med Chem*, **41**, **1998**, 5055.
- Hulin B, Newton L S, Lewis D M, Generoux P E, Gibbs E M & Clark D A, *J Med Chem*, **39**, **1996**, 3897.
- Dewar M J S, Zoebisch E G, Healy E F & Stewart J J P, *J Am Chem Soc*, **107**, **1985**, 3902.
- Bernhard S H, Peter G & Eugene F M, *J Am Chem Soc*, **104**, **1982**, 5347.
- Apeloig Y, Arad D & Rappoport Z, *J Am Chem Soc*, **112**, **1990**, 9131.
- Smith B J, Nguyen M T, Bouma W J & Radom L, *J Am Chem Soc*, **113**, **1991**, 6452.

- 17 Lammertsma K & Prasad B V, *J Am Chem Soc*, 115, **1993**, 2348.
- 18 Lammertsma K & Prasad B V, *J Am Chem Soc*, 116, **1994**, 642.
- 19 Harris N J & Lammertsma K, *J Am Chem Soc*, 118, **1996**, 8048.
- 20 Lammertsma K & Bharatam P V, *J Org Chem*, 65, **2000**, 4662.
- 21 Bharatam P V & Khanna S, *J Phys Chem*, 108, **2004**, 3784.
- 22 Wong W M, Wiberg K B & Frisch M J, *J Am Chem Soc*, 114, **1992**, 1645.
- 23 Long J A, Harris N J & Lammertsma K, *J Org Chem*, 66, **2001**, 6762.
- 24 Bharatam P V, *Ind J Chem, A*, 39, **2000**, 100.
- 25 Hulin B & McCarthy P A, *Curr Pharm Des*, 2, **1996**, 85.
- 26 Knihinicki R D, Williams K M & Day R O, *Biochem Pharmacol*, 38, **1989**, 4389.