Bioorganic & Medicinal Chemistry 17 (2009) 8027–8031

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Theoretical calculations of a model of NOS indazole inhibitors: Interaction of aromatic compounds with Zn-porphyrins

José Elguero [a,*], Ibon Alkorta [a], Rosa M. Claramunt [b], Concepción López [b], Dionísia Sanz [b], Dolores Santa María [b]

[a] Instituto de Química Médica, CSIC, Juan de la Cierva, 3, E-28006 Madrid, Spain
[b] Departamento de Química Orgánica y Bio-Orgánica, Facultad de Ciencias, UNED, Senda del Rey 9, E-28040 Madrid, Spain

ARTICLE INFO

Article history:
Received 30 July 2009
Revised 28 September 2009
Accepted 5 October 2009
Available online 9 October 2009

Keywords:
NOS
Indazole
Porphyrin
DFT
Stacking

ABSTRACT

We report a theoretical approach, at the M05-2x/6-311+G(d) level, to explain the affinity of indazoles for nitric oxide synthases using a simplified model of porphyrin. The theoretical $E_{rel} = E_i$ stacking–E_i apical values correlate with the experimental inhibition percents allowing to predict that 3,7-dinitro-1*H*-indazole should be a good NOS inhibitor.

1. Introduction

According to literature results concerning the structures of indazoles bound to any isoform of NOS (nitric oxide synthase: I or neuronal, II or inducible and III or endothelial), nitroindazoles position themselves above the heme **1** adopting two stacking modes called O–N and N–N.[1,2] These modes differ only on the HBs of the nitroindazole forms with other residues of the protein. In addition, Porubsky, Meneely and Scott reported the structure of indazole coordinated through its lone pair (LP model) to the heme iron (Scheme 1) of cytochrome P-450 2E1 in a similar way to 4-methylpyrazole as well as histidine residues.[3]

Theoretical studies of stacking between different types of aromatic molecules have received considerable attention. These studies include benzene,[4] porphyrins,[5] and many other structures.[6,7] Ligand coordination is a classical problem that has been theoretically approached in the case of zinc porphyrins complexes to sp^3 amines.[8] Computationally, Truhlar functionals like M05-2x, are particularly well adapted to stacking calculations.[9] We have replaced the iron of the heme by zinc also present in many important porphyrins[10,11] to simplify the calculations and besides we removed all the *C*-substituents in **1**. Ellison and Scheidt reported the X-ray structures of Fe-octaethylporphyrins bearing on the metal both an NO ligand and an heterocycle: the distances iron-N(heterocycle) are 1.99 Å (1-methylimidazole), 1.99 Å (pyrazole) and 2.01 Å (indazole).[12]

2. Computational details

The geometry of the systems was initially optimized at the M05-2x/6-311+G(d) computational level.[9,13] This functional has shown to provide a good description for a large variety of molecular interaction complexes.[14] Frequency calculations at this computational level were performed to confirm that the structures obtained correspond to energetic minima. All these calculations were carried out within the Gaussian 03 package.[15]

3. Results and discussion

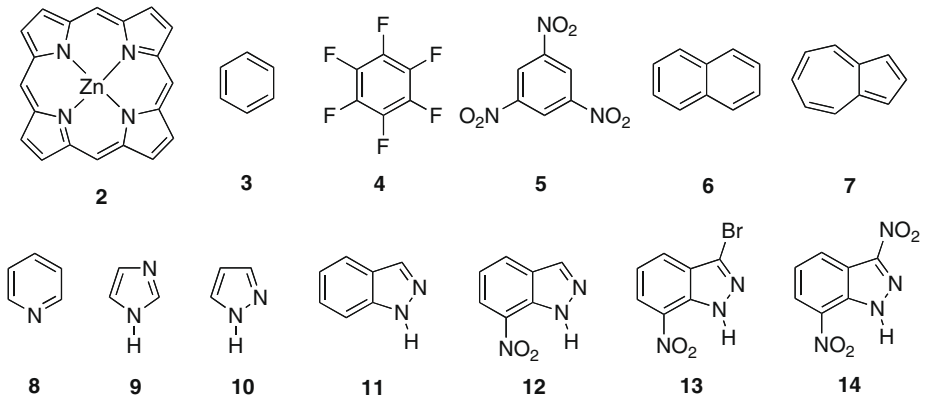
We selected the series of aromatic compounds reported in Scheme 1.

In the last years, we made several contributions to the analogies and differences of the benzene (**3**)/hexafluorobenzene (**4**) pair (aromaticity, stacking, complexes with ions);[16–19] thus, we start by comparing the complexes **2**·**3** and **2**·**4**. Recently, it has been reported that naphthalene-porphyrin and azulene-porphyrin conjugates behave very differently.[20] Therefore, we decided to study the **2**·**6** and **2**·**7** complexes. 1,3,5-Trinitrobenzene (**5**) was selected to understand the behavior of nitroindazoles.

We move then to aromatic heterocycles able to bind either by stacking or by their lone pairs: pyridine (**8**), 1*H*-imidazole (**9**),

* Corresponding author. Tel.: +34 91 4110874; fax: +34 91 5644853.
E-mail address: iqmbe17@iqm.csic.es (J. Elguero).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.10.006



Scheme 1. Zn-porphyrin (**2**) and aromatic ligands **3**–**14**.

Table 1
Geometrical (Å, °) characteristics of the complexes

Complex	Type	Aromatic	Centroid···Zn (Å)	Inclination (°)	N···Zn (Å)	N-Zn-c[a] (°)
2·3	Stacking	C_6H_6	3.12	10.4	—	—
2·4	Stacking	C_6F_6	3.33	4.4	—	—
2·5	Stacking	TNB	3.46	1.2	—	—
2·6	Stacking	Naphthalene	3.12	0.0	—	—
2·7	Stacking	Azulene	3.14	0.2	—	—
2·8	Stacking	Pyridine	3.19	9.8	—	—
2·8	LP	Pyridine	—	—	2.19	90.0
2·9[b]	LP	Imidazole	—	—	2.15	90.8
2·10[b]	LP	Pyrazole	—	—	2.17	90.0
2·11	Stacking	Indazole	3.06	3.5	—	—
2·11	LP	Indazole	—	—	2.18	90.0
2·12	Stacking	7-NO_2-ind	3.31	3.0	—	—
2·12	LP	7-NO_2-ind	—	—	2.21	90.0
2·13	Stacking	3-Br-7-NO_2-ind	3.35	3.0	—	—
2·13	LP	3-Br-7-NO_2-ind	—	—	2.28	90.0
2·14	Stacking	3,7-diNO_2-ind	3.34	2.7	—	—
2·14	LP	3,7-diNO_2-ind	—	—	2.45	90.0

[a] Average value of the four N(heterocycle)-porphyrin-centroid-N(porphyrin) angles (maximum deviation 8° for **2·14**), two of them being always identical.
[b] For complexes **2·9** and **2·10** no minimum corresponding to a stacking disposition has been found.

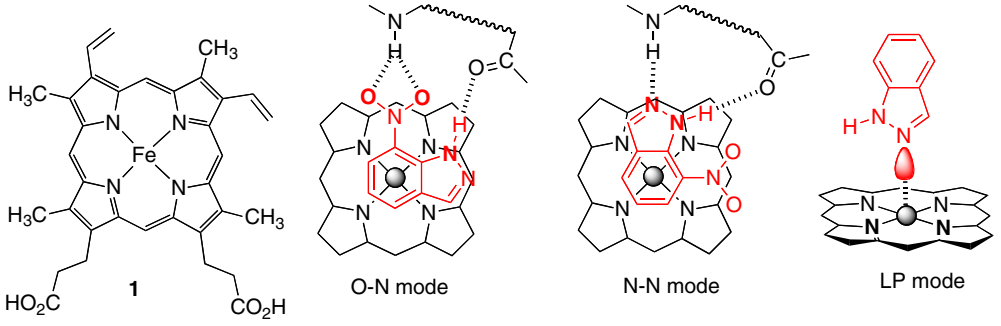


Figure 1. Heme and models of interaction of indazoles.

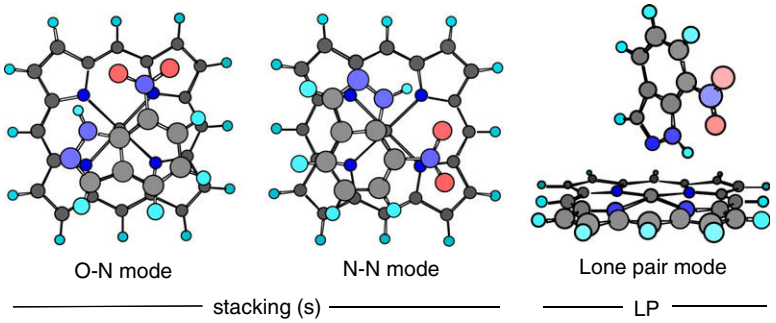


Figure 2. Optimized geometries of **2·12**, the stacking and the lone pair modes. The Zn atom is slightly above the porphyrin plane.

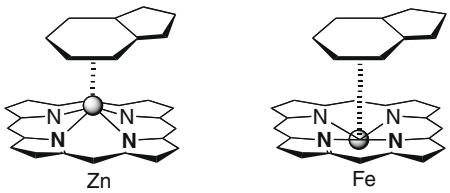


Figure 3. Differences between Zn and Fe porphyrins.

1*H*-pyrazole (**10**) and finally to 1*H*-indazoles **11**, **12**, **13** and **14**. The binding energies of pyridines and a calixarene-capped Zn-porphyrin have been measured.[21] Nitroindazoles **12** and **13** are amongst the strongest ligands of NOS,[1,2] while the 3,7-dinitro derivative **14**, although a known compound[22] has never been tested against any NOS isoform.

The geometries of the different complexes were optimized at the M05-2x/6-311+G(d) level. In Table 1 are some relevant geometrical parameters and in Figure 2 the optimized geometries corresponding to 7-nitroindazole **2**·**12** complex (in the Supplementary

Table 2
Energetic values for the complexes (all values in kJ mol^{-1})

Complex	Mode	E_{rel}	E_i	E_i + BSSE
2·**3**	Stacking	—	−45.9	−35.2
2·**4**	Stacking	—	−54.6	−33.8
2·**5**	Stacking	—	−83.7	−57.7
2·**6**	Stacking	—	−53.3	−36.6
2·**7**	Stacking	—	−52.4	−36.7
2·**8**	Stacking	37.2	−44.1	−33.5
2·**8**	Apical	**0.0**	−81.3	−72.1
2·**9**[a]	Apical	—	−83.9	−75.7
2·**10**[a]	Apical	—	−81.4	−72.5
2·**11**	Stacking	25.4	−52.8	−36.7
2·**11**	Apical	**0.0**	−78.2	−68.5
2·**12**	Stacking	7.7	−63.1	−41.1
2·**12**	Apical	**0.0**	−70.8	−60.0
2·**13**	Stacking	**0.0**	−68.5	−44.9
2·**13**	Apical	0.5	−68.0	−55.7
2·**14**	Stacking	**0.0**	−80.8	−54.4
2·**14**	Apical	22.6	−58.2	−43.5

[a] For complexes **2**·**9** and **2**·**10** no minimum corresponding to a stacking disposition has been found.

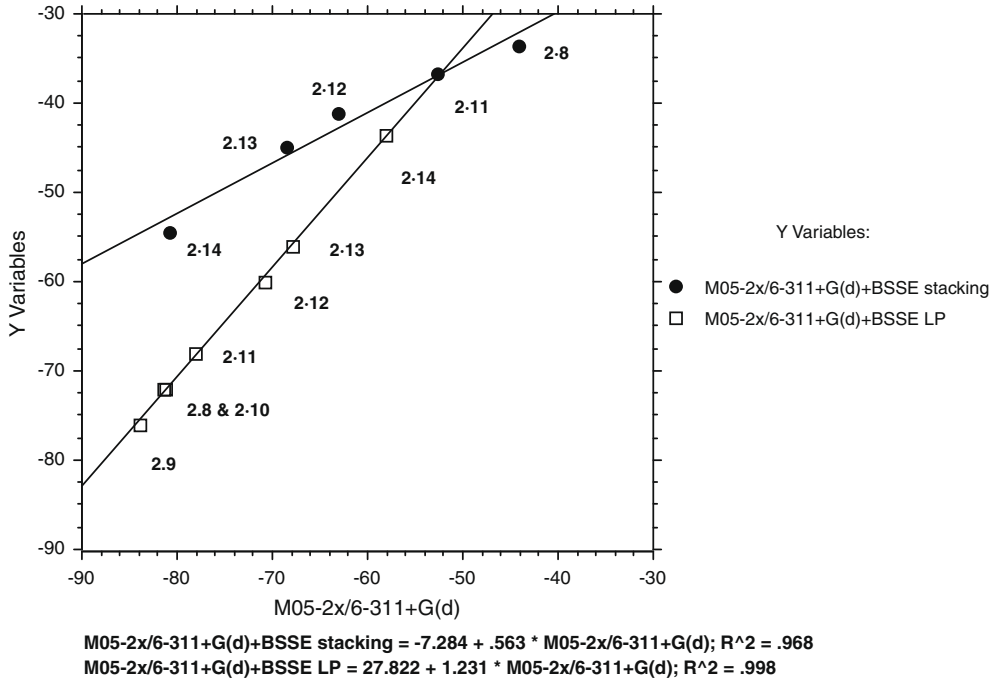


Figure 4. A plot with the trendlines of the BSSE correction.

Table 3
E_i and E_{rel} (E_i stacking–E_i apical) M05-2x/6-311+G(d) calculations including BSSE (in kJ mol^{-1})

Ligand	Stacking	Apical	E_{rel}	PA	NIST[a]	pK_a
8 Pyridine	−33.5	−72.1	38.6	927.8	930.0	5.17[b]
9 1*H*-Imidazole	Not stable	−75.7	—	945.5	942.8	6.99[c]
10 1*H*-Pyrazole	Not stable	−72.5	—	891.4	894.1	2.48[c]
11 1*H*-Indazole	−36.7	−68.5	31.8	898.7	900.8	1.04[c]
12 7-Nitro-1*H*-indazole	−41.1	−60.0	18.9	860.2	—	−0.99[c]
13 3-Br-7-nitro-1*H*-indazole	−44.9	−55.7	10.8	843.3	—	−3.81[d]
14 3,7-Dinitro-1*H*-indazole	−54.4	−43.5	−10.9	793.7	—	—

[a] NIST.[26]
[b] Pyridine.[27]
[c] Azoles.[28]
[d] Estimated additively from the pK_as of **11**, **12** and that of 3-bromo-indazole (−1.78).[28]

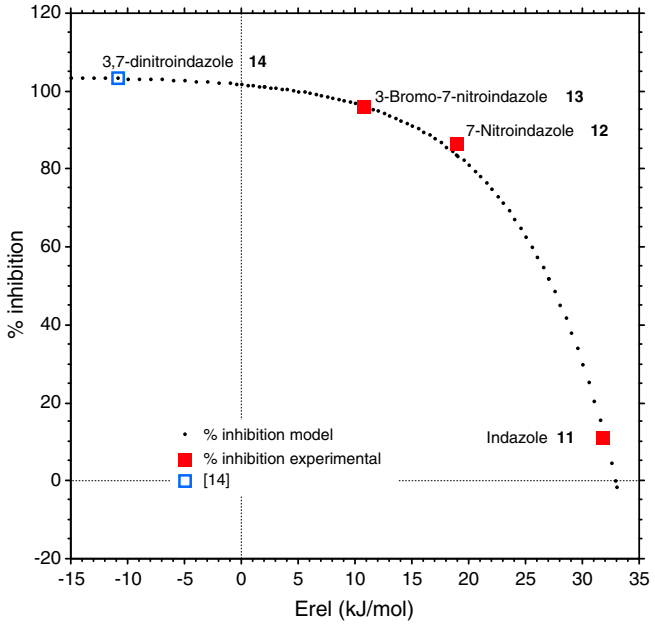


Figure 5. % of inhibition versus E_{rel}. The points were calculated with a fitted exponential model: % inhibition = 104.0–2.21 * $e^{(Erel/8.54)}$.

data are views of all the complexes as well as the corresponding interaction energies).

The N···Zn(II) distances we have found in the optimized geometries lie between 2.15 and 2.28 Å. In the case of pyridine-(Zn)porphyrins, Anderson et al. reported values of 2.15–2.20 Å.[23] A search in the CSD affords 167 hits with an average value of 2.17 Å ranging from 1.99 to 2.49 Å.[24] Note that N···Zn(II) distances are only a little longer than the N(heterocycle)···Fe(porphyrins) distances (about 2.0 Å).[12] The lone pair (LP) complex of Figure 1 shows a N2(indazole)···Fe distance of 2.19 Å.[3]

The geometries of the indazole/heme structures reported in the PDB,[25] differ from those here calculated in the fact that the iron is on the opposite side of the porphyrin ring instead of being on the same side as the indazole in the zinc complexes (Fig. 3).

There are several structures with 7-nitro-1*H*-indazole (**12**) but only one where the indazole is not disordered (PDB Refcode: **1foj**): centroid···Fe = 3.97 Å; inclination = 6.0°. There are four structures for 3-bromo-7-nitro-1*H*-indazole (**13**): **1d0c**: centroid···Fe = 4.01 Å; inclination = 5.0°; **1d0o**: centroid···Fe = 4.15 Å; inclination = 3.9°; **1m9r**: centroid···Fe = 4.18 Å; inclination = 9.5°; **1m97**: centroid···Fe = 4.58 Å; inclination = 18.0° (average 4.23 Å, 9.1°).

We have reported in Table 2 the calculated energies of the complexes.

The BSSE effect is proportional to E_i but it is necessary to put apart the stacking and apical complexes (Fig. 4). In the case of the stacking a curvature is observed.

We have calculated the proton affinities, PA, of all these heterocyclic bases that are well correlated with the experimental PAs from NIST: Calculated PA = –(78 ± 45) + (1.08 ± 0.05) experimental PA, $n = 4$, $R^2 = 0.996$ (Table 3). There is also a good correlation with the pK_as: PA = (877 ± 4) + * (9.6 ± 1.0) pK_a, $n = 6$, $R^2 = 0.96$. That is, to describe the basicity of these heterocycles one can use the experimental PA (gas phase) and pK_a (water) as well as the calculated PAs (gas phase) since the three values are linearly related.

E_{rel} is related to PA by a quadratic expression: E_{rel} = –(1712 ± 232) + (3.6 ± 0.5) * PA –(1.9 ± 0.3) * PA^2/1000, $n = 4$, $R^2 = 1.000$. The difference between E_i stacking and E_i apical decreases when PA decreases.

The % of inhibition (10 M) of indazole **11**, 7-nitro-1*H*-indazole **12** and 3-bromo-7-nitro-1*H*-indazole **13** are 11.2, 86.6 and 96.1, respectively.[29] If we plot these values against E_{rel} we obtain Figure 5. A similar figure is obtained using the stacking energies instead of E_{rel}.

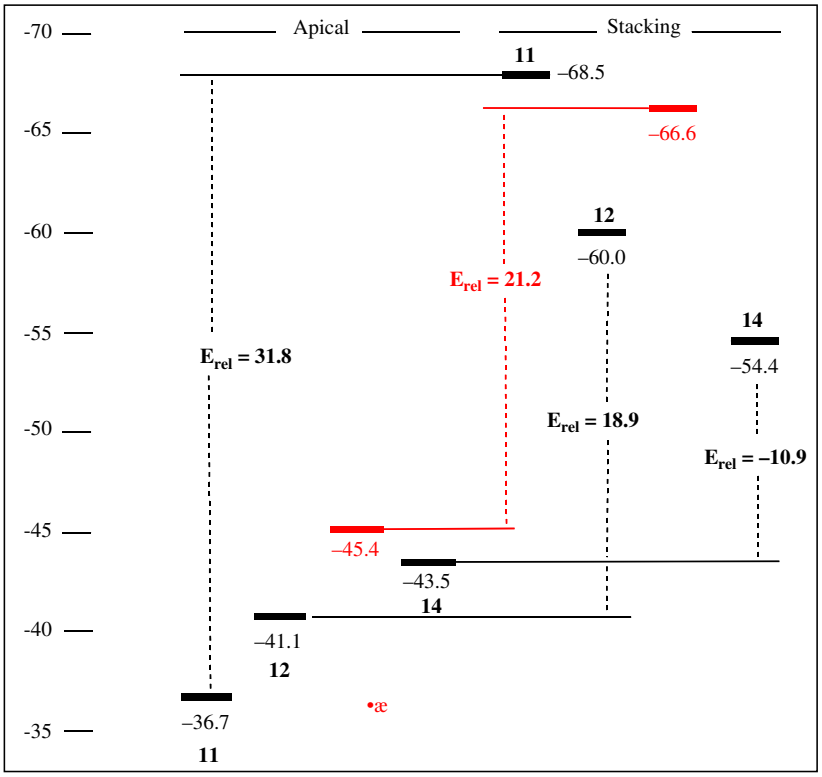


Figure 6. Energy profile of some indazoles (all values in kJ mol^{-1} for E_i + BSSE).

To obtain good indazole ligands for the NOS receptors it is necessary to decrease the apical (LP) interaction decreasing the basicity by using electron-withdrawing substituents (NO_2, F) and to increase the stacking; for this, the nitro groups are needed. The smaller is the difference between the 'apical ligand' and 'stacking' modes of complexation of indazoles the higher the affinity for the NOS receptors. The equation reported in Figure 5 allows to predict for 3,7-dinitro-1*H*-indazole **2**·**14** ($E_{rel} = -10.9$ kJ mol^{-1}) an % inhibition of 103.4%, that is, close to 100%.

To test the validity of our model, which do not distinguish between NOS-I and NOS-II, we have calculated the E_{rel} (kJ mol^{-1}) for 3-methyl-4,5,6,7-tetrafluoro-1*H*-indazole since we reported recently that its % of inhibition are 63% NOS-I and 83% NOS-II.[30] For this indazole (without nitro groups), E_i + BSSE (stacking) = -45.4 kJ mol^{-1}, E_i + BSSE (apical) = -66.6 kJ mol^{-1} and $E_{rel} = 21.2$ kJ mol^{-1}. For this value of E_{rel}, the equation given in Figure 5 predicts a % inhibition = 77.5% which is close to the average value of both NOS inhibition data.

To decrease E_i (Fig. 6) it is necessary to increase E_i apical in absolute value and to decrease E_i stacking in absolute value. Although 3-methyl-4,5,6,7-tetrafluoro-1*H*-indazole (in red) fulfills the first condition it lacks the ability of nitroindazoles to decrease the stacking interaction energy.

4. Conclusions

The interest in NOS inhibition studies continues unabated.[31–34] The work here presented is a first attempt to modelize the inhibitory power of indazoles, a sort of QSAR. According to it, it should be possible to obtain other nitro-1*H*-indazoles similar to 3-bromo-7-nitro-1*H*-indazole as NOS inhibitors but not much better.

Acknowledgments

This work was carried out with financial support from the Ministerio de Educación y Ciencia (Projects Nos. CTQ2009-13129-C02-02 and CTQ2007-62113) and Comunidad Autónoma de Madrid (Project MADRISOLAR, ref. S-0505/PPQ/0225).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.10.006.

References and notes

1. Raman, C. S.; Li, H.; Martásek, P.; Southan, G.; Masters, B. S. S.; Poulos, T. L. *Biochemistry* **2001**, *40*, 13448.
2. Rosenfeld, R. J.; Garcin, E. D.; Panda, K.; Andersson, G.; Åberg, A.; Wallace, A. V.; Morris, G. M.; Olson, A. J.; Stuehr, D. J.; Tainer, J. A.; Getzoff, E. D. *Biochemistry* **2002**, *41*, 13915.
3. Porubsky, P. R.; Meneely, K. M.; Scott, E. E. *J. Biol. Chem.* **2008**, *283*, 33698.
4. Jorgensen, W. L.; Severance, D. L. *J. Am. Chem. Soc.* **1990**, *112*, 4768.
5. Hunter, C. A.; Sanders, K. M. *J. Am. Chem. Soc.* **1990**, *112*, 5525.
6. Alkorta, I.; Blanco, F.; Elguero, J. *Tetrahedron Lett.* **2008**, *49*, 7246.
7. Quiñonero, D.; Frontera, A.; Escudero, D.; Ballester, P.; Costa, A.; Deyà, P. M. *Theor. Chem. Acc.* **2008**, *120*, 385.
8. Gomila, R. M.; Quiñonero, D.; Frontera, A.; Ballester, P.; Deyà, P. M. *Theochem* **2000**, *531*, 381.
9. Zhao, Y.; Schultz, N. E.; Truhlar, D. G. *J. Chem. Theory Comput.* **2006**, *2*, 364.
10. Kimura, M.; Nakano, Y.; Adachi, N.; Tatewaki, Y.; Shirai, H.; Kobayashi, N. *Chem. Eur. J.* **2009**, *15*, 2617.
11. Chang, C.-W.; Luo, L.; Chou, C.-K.; Lo, C.-F.; Lin, C.-Y.; Hung, C.-S.; Lee, Y.-P.; Diau, E. W.-G. *J. Phys. Chem. C* **2009**, *113*, 11524.
12. Ellison, M. K.; Scheidt, W. R. *J. Am. Chem. Soc.* **1999**, *121*, 5210.
13. Frisch, M. J.; Pople, J. A.; Binkley, J. S. *J. Chem. Phys.* **1984**, *80*, 3265.
14. (a) Zhao, Y.; Truhlar, D. G. *J. Phys. Chem. C* **2008**, *112*, 6860; (b) Zhao, Y.; Truhlar, D. G. *Acc. Chem. Res.* **2008**, *41*, 157.
15. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. GAUSSIAN-*03*; Gaussian: Wallingford, CT, 2003.
16. Alkorta, I.; Rozas, I.; Elguero, J. *J. Org. Chem.* **1997**, *62*, 4687.
17. Alkorta, I.; Rozas, I.; Jimeno, M. L.; Elguero, J. *Struct. Chem.* **2001**, *12*, 459.
18. Alkorta, I.; Quiñonero, D.; Garau, C.; Frontera, A.; Elguero, J.; Deyà, P. M. *J. Phys. Chem. A* **2007**, *111*, 3137.
19. Wu, J. I.; Pühlhofer, F. G.; Ragué Schleyer, P. v.; Puchta, R.; Kiran, B.; Mauksch, M.; Eikema Hommes, N. J. R. v.; Alkorta, I.; Elguero, J. *J. Phys. Chem. A* **2009**, *113*, 6789.
20. Xiao, Z.-Y.; Zhao, X.; Jiang, X.-K.; Li, Z.-T. *Org. Biomol. Chem.* **2009**, *7*, 2540.
21. Iwamoto, H.; Yukimasa, Y.; Fukazawa, Y. *Tetraheron Lett.* **2002**, *43*, 8191.
22. Cohen-Fernandez, P.; Habraken, C. L. *J. Org. Chem.* **1971**, *36*, 3084.
23. Anderson, H. L.; Bashall, A.; Henrick, K.; McPartlin, M.; Sanders, J. K. M. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 429.
24. CSD version 5.30 (updated November 2008 & February 2009): Allen, F. H. *Acta Crystallogr., Sect. B* **2002**, *58*, 380; Allen, F. H.; Motherwell, W. D. S. *Acta Crystallogr., Sect. B* **2002**, *58*, 407.
25. RSCB Protein Data Bank, http://www.rcsb.org/pdb/home/home.do.
26. *NIST Chemistry Webbook*; Linstrom, P. J., Mallard, W. G., Eds. NIST Standard Reference Database No. 69; National Institute of Standards and Technology: Gaithersburg, MD, 2003. http://webbook.nist.gov.
27. Hosmane, R. S.; Liebman, J. F. *Struct. Chem.* **2009**, *20*, 693. From: Grandberg, I. I.; Faizova, G. K.; Kost, A. N. *Khim. Geterotsikl. Soedin.* **1966**, 561 (*Chem. Heterocycl. Comp.* **1966**, 421).
28. Catalán, J.; Abboud, J. L. M.; Elguero, J. *Adv. Heterocycl. Chem.* **1987**, *41*, 187.
29. Bland-Ward, P. A.; Moore, P. K. *Life Sci.* **1995**, *57*, 131.
30. Claramunt, R. M.; López, C.; Pérez-Medina, C.; Pérez-Torralba, C.; Elguero, J.; Escames, G.; Acuña-Castroviejo, D. *Bioorg. Med. Chem.* **2009**, *17*, 6180.
31. Sabat, J.; Stuehr, D. J.; Yeh, S.-R.; Rousseau, D. L. *J. Am. Chem. Soc.* **2009**, *131*, 12186.
32. Navati, M. S.; Friedman, J. M. *J. Am. Chem. Soc.* **2009**, *131*, 12273.
33. Lucas, H. R.; Meyer, G. J.; Karlin, K. D. *J. Am. Chem. Soc.* **2009**, *131*, 13924.
34. Rydberg, P.; Olsen, L. *J. Phys. Chem. A*, in press. doi:10.1021/jp9035716.