J. Chem. Sci., Vol. 116, No. 4, July 2004, pp. 235–241.

Basicities of some 9-substituted acridine-4-carboxamides: A density functional theory (DFT) calculation

RAGHAB PARAJULI and C MEDHI*
Department of Chemistry, Gauhati University, Gauhati 781 014, India
e-mail: chitrani@satyam.net.in

MS received 2 August 2003; revised 10 May 2004

Abstract. Acid–base properties of drugs are important in understanding the behaviour of these compounds under physiological condition. In order to understand such behaviour the proton affinities of acridine 4-carboxamides with substitution (R) at the 9-position are theoretically studied, and considered for the basic sites of both the heterocyclic ring as well as side chain nitrogens. In 9-amino acridine 4-carboxamide, the $–NH_2$ group is observed to be an additional basic site. The heterocyclic nitrogen of substituted carboxamides (R = $–NH_2$, –O-methyl, –O-ethyl, and –O-phenyl) is more basic than the side chain nitrogen, however, side chain nitrogen corresponds to more basic site for some carboxamides (R = –OH and –Cl) and the $–NH_2$ group represents the least basic site of 9-amino acridine 4-carboxamide. In addition to presenting the basicities of these drugs an indication of another hydrogen-bond between heterocyclic ring N and carboxamide chain O is observed. The difference of basicities with substituents at 9-position are very narrow and carboxamides with substituents at 9-position are found to be suitable for studying intramolecular H-bonds between the heterocyclic N and carboxamide O. The resultant stabilization of a configuration due to such H-bonding is determined.

Keywords. Carboxamide; DFT method; anticancer drugs; DNA binding.

1. Introduction

Generally, drugs are weakly ionizable molecules, which can efficiently penetrate the plasma membrane in their unionized form.[1–3] In this context, the absorbed drug which may be unionized at low pH (stomach, pH = 2) can easily pass across the membrane but when it reaches the blood (pH = 7·4) reconverts to ionized form and cannot pass back across the membrane. Hence, both the pH and the ionization normally play important roles in pH-partition of many drugs. Moreover, drugs with p*Ka* close to physiological pH can be considered for interesting pharmacological properties.[1–7]

Acridine 4-carboxamides were first reported by Denny.[5] The physiological and biological properties of these drugs are well discussed and the carboxamide group at the 4-position enhances the antitumour properties.[3–9] In fact, most of the acridine 4-carboxamides have p*Ka* values lower than that of acridine 1- and acridine 2-carboxamides and show better anticancer activity. Also, in practice, the *in-vivo* activity of this drug is much less than the *in-vitro* activity. In addition to other conditions of *in-vivo* potency, the p*Ka* value of a drug may essentially be considered a factor for the better understanding of the anticancer property, since biological activity may often depend on its ability to penetrate the cell. While the p*Ka* of this drug is controlled by the presence of carboxamide group at 1, 2 and 4 positions, substituents at the 9-position may also cause significant variations. Indeed, 9-amino acridine 4-carboxamide is reported to have more protonation sites.[9–11] In this case, proton transfer equilibrium is also observed at low pH and some evidence of forming mono-anionic species at higher pH is seen.[9] Further, it also suggests another additional stabilization of free base possibly by hydrogen-bond formation. In addition, acridine 4-carboxamides are known to be biologically active compounds with strong DNA binding ability.[9] On the other hand, the strong affinity does not ensure *in-vivo* antitumour property.[8–13] However, although alteration of carboxamide groups at different position change the p*Ka* of the drug and the substitution of $–NH_2$ at 9-position results in significant change of p*Ka* compared to other groups,

*For correspondence

the main differences among these drugs in terms of their acid or base stabilities are not known. Hence, estimation of basicities of acridine 4-carboxamides with various substituents at the 9-position may be suitable for understanding the p*Ka* of the drug. As noted experimentally, 9-chloro or 9-methoxy derivatives are very reactive but they do not show many differences in DNA binding abilities.[4,6–10] Although the protonation of this drug might take place at more than one basic site, an appropriate approach is required in changing the p*Ka* of a drug. However, the prediction of absolute p*Ka* is extremely difficult. In order to rationalize the most basic site, the quantitative estimation of proton affinities at various positions is necessary. The present study involves the computation of proton affinities (basicities) of acridine 4-carboxamides at different basic sites.

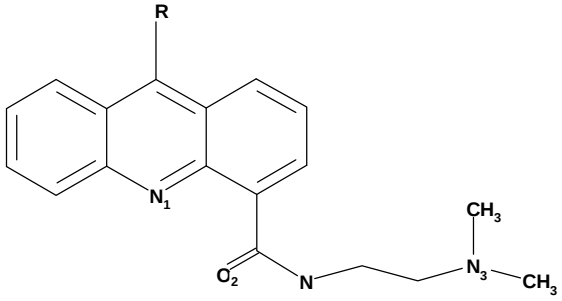
Density functional theory has been considered a better method for studying hydrogen-bonded complexes. Alternatively, the Fukui function also provides donor–acceptor interaction directly by the contribution of HOMO and LUMO if the preferred sites of attack are atoms with large MO coefficients in HOMO and LUMO. Further, the HOMO orbital converges to a specific charge and energy; if the basis set is improved in *ab-initio* calculation, LUMO is determined by the diffused function in the basis set. These concepts play important roles in hard- and soft-acids and -bases in which hard acids prefer to react with hard bases. In this case, the change in electron density in HOMO and LUMO gaps is controlled by the polarizability of another atom or molecule.[14–18] Nevertheless, the electron densities of frontier orbials in analysing reactivity have been reported.[19] There are several descriptors to study intermolecular and intramolecular reactivity. In such cases, the total hardness parameters are reported to give reliable intermolecular reactivity whereas local softness and Fukui function (FF) are used for intramolecular reactivity. It has been found that in some cases, condensed FF can perhaps produce molecular reactivity better than FF[18,19] itself. Hence, the Fukui functions are alternate descriptors for such systems, while the DFT method is very sensitive to population analysis because it deals with the difference in population of N electrons from $N + 1$ or $N - 1$ electrons.[19a–c] Also, both Mulliken population analysis (MPA) and Lowdin population analysis (LPA) are reported to give negative FF indices due to improper charge-partitioning whereas Hirchfield population ensures non-negative FF. This is because of the difference of weight factors in the electron density distribution of MPA and the Hirchfield method. Thus the global hardness value of the system can be alternatively defined from molecular charge and hardness.

In the present calculation, the intermolecular interaction of drug with charged ion (proton) is mainly electrostatic and also the important part in the protonation is the large negative atomic site of drugs which is well accounted for in the DFT method. The adequacy of the density functional method for the study of hydrogen-bonded compounds has been investigated by several researchers.[20–27] Moreover, the B3LYP method has been shown to perform much better than the more computationally demanding UMP2 method.[21,28,29]

Thus the *ab-initio* density functional (DFT) method with diffused functions has been used to reproduce basicities of molecules in accordance with experimental findings.[4,9–13,30,31] Hence, we report here the extensive computation of proton affinities of acridine 4-carboxamides using the density functional method.

2. Method

Ab-initio density functional method is used to study the basicities of 9-substituted acridine 4-carboxamides which are shown in figure 1. All the geometrical parameters are optimized for obtaining ground-state structures of free- and protonated-carboxamides using 6-31G/B3LYP methods adopted in Gaussian programme code.[32] It is not possible to use higher basis functions for such large molecules and the polarization function in 6-31G-basis set may still represent acceptable proton affinities.[30,31] The



N_1 = Ring nitrogen, O_2 = carboxamide oxygen, N_3 = distal nitrogen, R = substituent group

Figure 1. Structure of 9-substituted acridine 4-carboxamide.

Table 1. The computed proton affinities (PA) (6-31G/B3LYP) of drugs at different sites.

Molecules	PA (a.u.) at heterocyclic-N	PA (a.u.) at distal-N
9-Hydroxy acridine 4-carboxamide (S1)	0·42321	0·45019
9-Methoxy acridine 4-carboxamide (S2)	0·42566	0·42558
9-Ethoxy acridine 4-carboxamide (S3)	0·42833	0·42699
9-Amino acridine 4-carboxamide (S4)	0·43483	0·42969
9-Phenoxy acridine 4-carboxamide (S5)	0·42348	0·42287
9-Chloro acridine 4-carboxamide (S6)	0·41261	0·42059

Table 2. The geometrical parameters of hydrogen-bonds in protonated drugs (6-31G/B3LYP optimization).

Protonation of molecules (ring nitrogen)	N–H^+ (Å)	O–H^+ (Å)	f_1^* (°)	f_2^* (°)
S1	1·039	1·700	17·52	120·17
S2	1·039	1·697	16·04	120·83
S3	1·039	1·701	17·42	120·80
S4	1·036	1·712	17·57	120·14
S5	1·041	1·686	15·53	120·76
S6	1·046	1·659	15·59	119·12

f_1^* = out-of-plane angle of O in protonated carboxamides; f_2^* = out-of-plane angle of O in free carboxamides

amendable number of atoms used in the calculation ranges from 41 to 52.

The proton affinities (PA) are calculated using following equation PA = $E_D - E_{DH+}$, where E_D and E_{DH+} represent the ground state total energies of free and protonated carboxamides.

3. Results and discussion

The experimental study has indicated the heterocyclic nitrogen as one of the sites of protonation and initially the models for PA calculation are based on the ability of protonation at this site. The computed proton affinities are shown in table 1. Among carboxamides, 9-amino acridine 4-carboxamide is found to be the most basic, however, the PA's of other carboxamides (R = –OH, –O-Et, and –O-Me) show less variation. Thus the ring nitrogen experiences less effect in changing from H to ethyl of these groups. However, the electron donor like $–NH_2$ enhances PA significantly and the decrease of PA results due to the presence of electron-withdrawing group (–Cl). Ultimately, the electron-donating ability of ring nitrogen is influenced by the substituents.

The optimized geometries of protonated carboxamides at heterocyclic ring N indicate another susceptible site of protonation which is the oxygen atom (O) of carboxamide group. In this structure the H^+ lie at the energetically favored unique position between N and O (figure 3). The H-bond lengths and orientations of H^+ within this region are shown in table 2. Nevertheless in the protonated structure of substituted carboxamides (R = –O-Me, $–NH_2$, O-Et and –O-Ph), the H^+ interaction at the nitrogen of heterocyclic ring is more favorable than O of carboxamide. In the optimized geometries the H^+ is found well attached to the ring nitrogen (figure 3). Hence the electron-donating abilities of –O-Me, –O-Et, and $–NH_2$ groups are responsible for enhancing the H-bonding within N…H^+…O. The variation of H-bonds due to the presence of electron-withdrawing groups (R = –OH and –Cl) is observed. Moreover in the protonated molecule the carboxamide O lies above the ring plane by certain angle (15–17°) whereas in free molecules non-planarity of ~ 120° is observed (figures 2 and 3). The ordering of PAs is of the order of the substituent, $–NH_2$>–O-Et > –O-Me > –O-Ph ~ –OH > –Cl (table 1). Hence $–NH_2$ substituent behaves better electron donor and acquire

more PA, while the electron-withdrawing substituent (–Cl) gives least value.

According to the structure shown in figure 3, the protonation at ring nitrogen atom does not indicate the intrinsic basicity of heterocyclic N only. Similar protonated structures are observed for other carboxamides. Hence the charge drift between the carboxamide oxygen and nitrogen occurred through proton. Table 3 shows the net charges on N and O of both protonated and free carboxamides (figures 2 and 3). The quantities related to PAs are the net charges on heterocyclic N atoms, in fact, the substituents, –O-Et, $–NH_2$, –O-Ph and –Cl should result in noticeable variation of charges among them, however no significant changes are observed. The proton occupies the energetically favoured position between N and O. The NH^+ and OH^+ bond lengths are shown in table 2. The shorter OH^+ due to –Cl substitution indicates the shifting of H^+ towards oxygen since the ring nitrogen acquires less basicity. Similarly, in 9-amino acridine 4-carboxamide the NH^+ bond length is less and greater PA in ring N is indicated. However for all carboxamides, the heterocyclic N atom is one of the susceptible sites for protonation (table 1).

Based on the net charges on N, O and H^+ (table 3), a comparison can be made between protonated and free molecules (figures 2 and 3). The charge density on the N atom in the protonated state is expected to acquire less negative charge compared to that in the free molecule. However the increase of negative charge on N indicates the characteristics of internal charge transfer (table 3). Indeed, such charge transfer occurring within the N…H^+...O region also leads to increase in negative charges on both N and O. The shift of electron densities from N to O depends slightly on the electron-donating abilities of substituents at the 9-position (table 3). In some cases net charges obtained from Mulliken population analysis gives unrealistic values due to equal apportioning of electrons between pairs of atoms. Due to this, the net charges may be accumulated at one atomic centre but Lowdin population analysis gives chemically intuitive values of net charges. So we have computed both the net charges obtained from Mulliken and Lowdin methods to present a brief testing of variability of net charges (table 4). Both the methods are capable of predicting comparable charges of the hydrogen-bonded sites. Here the LPA and MPA methods give the same trend of net charges but for some atomic sites the values obtained from LPA are significantly different from MPA (tables 3 and 4). Moreover, the PA is also not very sensitive to such small increases of electron densities due to substitution (table 1).

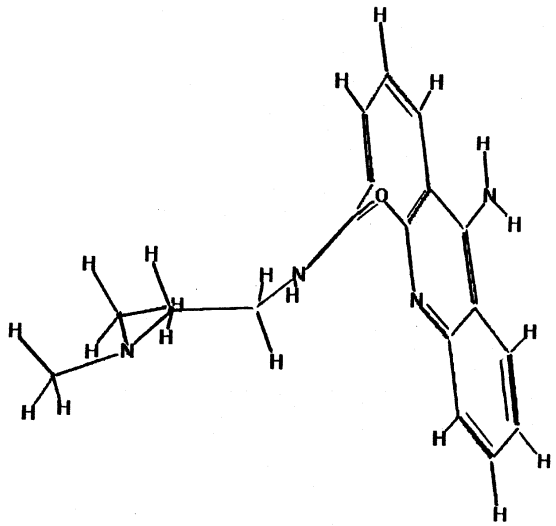


Figure 2. 9-Amino acridine 4-carboxamide.

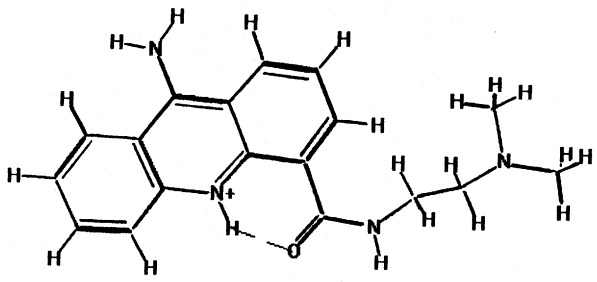


Figure 3. Protonated (ring nitrogen) 9-amino acridine 4-carboxamide.

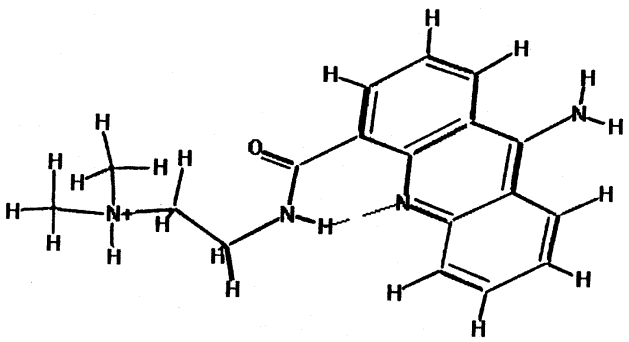


Figure 4. Protonated (distal nitrogen) 9-aminoacridine 4-carboxamide.

Table 3. The computed net charges (Mulliken) on N, O and H^+ in the protonated and free drugs.

	Free molecule		Protonated molecule		
	Net charges on		Net charges on		
Molecule	N	O	N	O	H^+
S1	–0·472	–0·462	–0·857	–0·506	0·405
S2	–0·463	–0·462	–0·857	–0·512	0·405
S3	–0·466	–0·463	–0·859	–0·512	0·403
S4	–0·481	–0·466	–0·863	–0·505	0·400
S5	–0·464	–0·462	–0·853	–0·516	0·405
S6	–0·467	–0·458	–0·855	–0·514	0·413

Table 4. The computed net charges (Lowdin) on N, O and H^+ in the protonated and free drugs.

	Free molecule		Protonated molecule		
	Net charges on		Net charges on		
Molecule	N	O	N	O	H^+
S1	–0·462	–0·611	–0·496	–0·652	0·489
S2	–0·446	–0·611	–0·498	–0·657	0·488
S3	–0·450	–0·612	–0·500	–0·657	0·487
S4	–0·484	–0·614	–0·511	–0·652	0·486
S5	–0·443	–0·611	–0·494	–0·660	0·489
S6	–0·433	–0·607	–0·477	–0·657	0·492

Thus study of the protonation of 9-amino acridine 4-carboxamide may be useful for explaining the experimentally observed pH-dependent kinetic study.[9] Moreover more protonation sites are reported in the ground state of 9-amino acridine 4-caboxamide, probably another one at the distal N atom of the carboxamide side chain. Thus, the distal N-protonation of some selected acridine 4-carboxamides are also analysed (figure 1). The computed PA's may be observed for analysing the sensitivity of protonation at all possible sites. The preference of protons for the distal $–N(CH_3)_2$ of carboxamide rather than heterocyclic-N is seen as due to the presence of electron-withdrawing substituents like –OH and –Cl (table 1). For these carboxamides the distal-N might be considered the dominant site for protonation.

The order of PA for 9-amino acridine 4-carboxamide at these sites is PA (distal-N) < PA (heterocyclic ring). However the PA's at these sites not only allow a general analysis of the basic behaviour, the protonated structure shows a proton well-buried within the $N...H^+...O$ region. The result might be useful for explaining the experimentally observed differences of p*Ka* for the ground and excited states of 9-amino acridine 4-carboxamides (figure 3). In addition, the basicities at distal-N for various carboxamides (R = $–NH_2$, –O-Me, –O-Et and –O-Ph) are computed and unexpectedly proton affinities are less than those of heterocyclic-N. The optimum structures of the distal protonated carboxamides interestingly show different configurations with H bonds between chromophore and carboxamide chain (figure 4). Thus the magnitude of H^+ affinity should necessarily relate to the additional N...H...N bond and not just the intrinsic PA at the distal-N. Unlike the $N...H^+...O$ in a ring-protonated structure the N...H…N bonding is exactly coplanar to the heterocyclic ring. Consequently two protonated structures are indicated for all carboxamides (figures 3 and 4). Indeed, deprotonation of the first H^+ (at the ring N) might not be easy when the H^+ lies at the optimum charge separation of N and O. But the second H^+ (at the distal-N) can deprotonate easily. Figures 5–8 represent the plots of total energies versus the posi-

tions of H^+ between N and O of some carboxamides (R = $–OCH_3$, $–NH_2$, –OH and –Cl). In view of the possibility of H^+ transfer from N to O it is worth analysing the correlation plots. Interestingly, differences in the steepness of energy curves of various carboxamides are observed. For $–NH_2$ substituents, the steep energy plot shows the strong affinity for the ring N whereas shallow curves for carboxamides (R = –OH, –O-Et, –Cl) are due to lower affinities for protons (figures 5–8). Hence it is possible for certain substituents to acquire least energy in transferring protons from N to O, in the sense that the proton can acquire equal affinities for N as well as O. The condition might be necessary for stabilizing the configuration with H^+ well embedded between these two atomic sites, which might be stable even at physiological pH (7·4). Such configuration is likely to acquire better intercalating ability compared to free carboxamides (figure 2) where the O of carboxamide is non-planar and may not be suitable for intercalation. So the effect of substituents not only results in the variation of basicities of carboxamides, the findings indirectly demonstrate the contribution of certain configurations that may indeed be viable in drug action through intercalation.

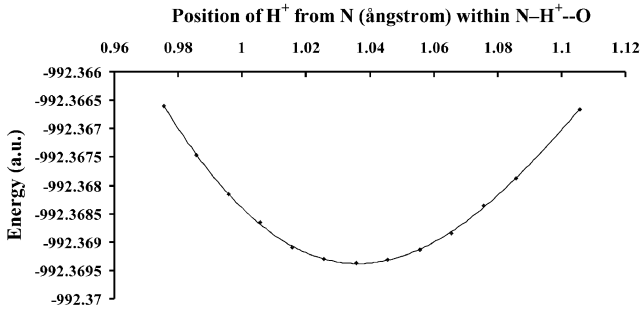


Figure 5. The plot of total energies versus the distances of H^+ from N to O in 9-amino acridine 4-carboxamide derivative.

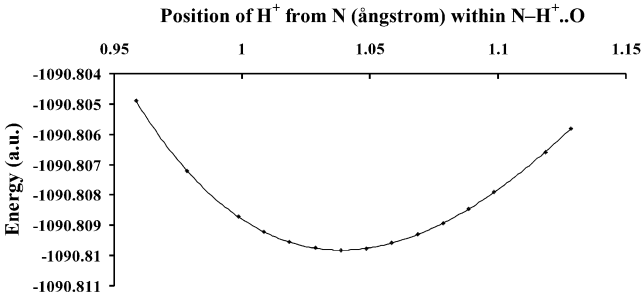


Figure 6. The plot of total energies versus the distances of H^+ from N to O in 9-ethoxy acridine 4-carboxamide derivative.

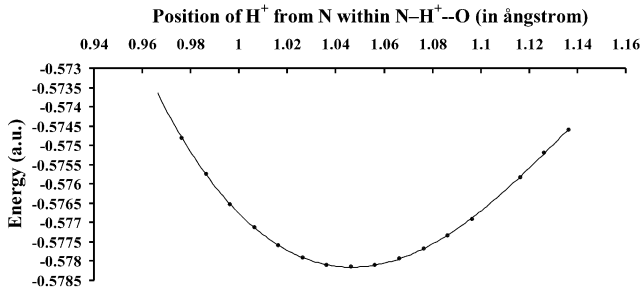


Figure 7. The plot of total energies versus the distances of H^+ from N to O in 9-chloro acridine 4-carboxamide derivative.

4. Conclusion

We have computed the basicities of 9-substituted acridine 4-carboxamides at the heterocyclic ring N and the distal N, but the differences of basicities of these carboxamides are very narrow. The basicities of the ring nitrogens maintain the following order of substituents $–NH_2$ > –O-Et > –OMe > –O-Ph ~ –OH > –Cl. In addition, basicities of distal N and heterocyclic N are quite close, particularly in –O-Me substituted carboxamide, the values are almost equal and the electron-donating substituent enhances proton affinity of ring nitrogen whereas the distal nitrogen is more basic for the carboxamides with electron-withdrawing group at 9-position. The importance of N...H^+...O bonding for stabilizing a proton through intramolecular H-bond between heterocyclic ring N and carboxamide O is indicated in the ring-nitrogen protonated structure, while change of configuration occurs in distal nitrogen-protonated carboxamides. The study indirectly allows the characterization of acidic or basic properties of various

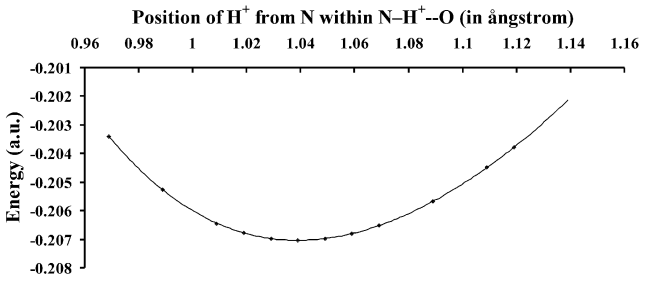


Figure 8. The plot of total energies versus the distances of H^+ from N to O in 9-hydroxy acridine 4-carboxamide derivative

drugs that could ultimately provide better understanding of drug under different pH conditions. The electronic properties of substituents at the 9-position influence the intramolecular hydrogen-bond, N...H^+…O, thereby resulting in the stabilization of a structure that may be related with the biological properties as well.

Acknowledgement

The authors acknowledge the Council of Scientific and Industrial Research, New Delhi for funding in the present research work. We also thank the referee for valuable comments.

References

1. (a) Mason J S and Pickell S D 1997 *Perspect. Drug Des.* **7/8** 85; (b) Albert A 1996 *The acridines* 2nd edn (London: Arnold)
2. Norris D A, Puri N and Sinko P J 1998 *Adv. Drug Deliv. Res.* **34** 135
3. Jani P, McCarthy D E and Florence A T 1992 *Int. J. Pharmaceut.* **86** 239
4. Atwell G J, Cain B F, Baguley B C, Finley G J and Denny W A 1984 *J. Med. Chem.* **27** 1481
5. Denny W A, Atwell G J, Baguley B C and Wallelin L P G 1985 *J. Med. Chem.* **28** 1568
6. Muller W and Crothers D M 1968 *J. Mol. Biol.* **35** 251
7. Zee-Cheng R K Y and Cheng C C 1978 *J. Med. Chem.* **21** 291
8. Atwell G J, Rewcastle G W, Baguley B C and Denny W A 1987 *J. Med. Chem.* **30** 664
9. Spicer J A, Gamage S A, Atwell G J, Baguley B C Finlay G J and Denny W A 1997 *J. Med. Chem.* **40** 1919
10. Chew O P, Deady L W, Baguley B C and Denny W A 1994 *J. Med. Chem.* **37** 593
11. Finlay G J and Baguley B C 1984 *Eur. J. Cancer Clin. Oncol.* **20** 947
12. Smith C A, Chang H C, Struve W S, Atwell G J and Denny W A 1995 *J. Phys. Chem.* **99** 927
13. Baguley B C, Denny W A, Atwell G J and Cain B F 1981 *J. Med. Chem.* **24** 170
14. Liu S B, Ayers P W and Parr R G 1999 *J. Chem. Phys.* **111** 6197
15. Novoa J J and Sosa C 1995 *J. Phys. Chem.* **99** 15837
16. Pearson R G 1963 *J. Am. Chem. Soc*. **85** 3533
17. Profit F D, Liu S and Parr R G 1997 *J. Chem. Phys.* **107** 3000
18. (a) Ayers P W and Parr R G 2000 *J. Am. Chem. Soc*. **122** 2010; (b) Ayers P W, Morrison R C and Roy R K 2002 *J. Chem. Phys.* **116** 8731; (c) Ayers P W and Parr R G 2001 *J. Am. Chem. Soc*. **123** 2007
19. (a) Roy R K, Pal S and Hirao K 1999 *J. Chem. Phys*. **110** 8236; (b) Roy R K, Hirao K and Pal S 2000 *J. Chem. Phys.* **113** 1372; (c) Roy R K, Hirao K, Krishnamurty S and Pal S 2001 *J. Chem. Phys.* **115** 7 2902
20. Zhang Q, Bell R and Truong T N 1995 *J. Phys. Chem.* **99** 592
21. Sim F, St. Amant F A, Papai I and Salahub D R 1992 *J. Am. Chem. Soc*. **114** 4391
22. Sodupe M, Oliva A and Bertran J 1997 *J. Phys Chem.* **A101** 9142
23. Baker J, Muir M and Andzelm J 1995 *J. Chem. Phys*. **102** 2063
24. Cysewski P 1998 *J. Chem. Soc., Faraday Trans*. **94** 3117
25. Gonzalez L, Mo O, Yanez M and Elguero J 1996 *J. Mol. Struct. Theochem.* **371** 1
26. Latajka Z, Bouteiller Y and Scheiner S 1995 *Chem. Phys. Lett*. **234** 159
27. Gill P M W, Johnson B G, Pople J A and Frisch M J 1992 *Int. J. Quan. Chem.* **S26** 319
28. Handly N C, Knowles P Z and Somasundran K 1985 *Theor. Chem. Acta* **68** 87
29. Tozer D J, Handy N C, Amos R D, Pople J A, Nobes R H, Yaoming X and Schaefer H F 1993 *Mol. Phys.* **79** 777
30. Platts J A and Howard S T 1996 *J. Org. Chem.* **61** 4480
31. Medhi C 2002 *Int. J. Chem. Inf. Comput. Sci.* **3** 598
32. Frisch M J *et al* 1995 *Gaussian* 94; Gaussian Inc, Pittsburgh, PA