Journal of Enzyme Inhibition and Medicinal Chemistry, June 2007; 22(3): 277–286

QSAR of aromatic substances: MAO inhibitory activity of xanthone derivatives

OMAR DEEB[1], SHERIN ALFALAH[1], & BRIAN W. CLARE[2]

[1]*Chemistry and Chemical Technology Department, Al-Quds University, P.O. Box 20002 Jerusalem, Palestine, and* [2]*School of Biomedical and Chemical Sciences, The University of Western Australia, 35 Stirling Highway, Crawley WA 6009, Australia*

(Received 29 August 2006; in final form 3 November 2006)

Abstract
The flip regression procedure that we used earlier for handling the dibenzofuran system has been applied to xanthones. The MAO-A inhibitory activity expressed as IC_{50} of the xanthones is known to correlate with E-state, molecular connectivity, shape indices and in this contribution it is shown that the orientation of nodes in their occupied π orbitals explain a further large portion of the variance in their inhibitory activity.

Keywords: *Aromatic, QSAR, flip-regression, orbital nodes, xanthones*

Introduction

Monoamine oxidase (MAO) plays a critical role in the regulation of monoamine neurotransmitters such as serotonin, nor adrenaline and dopamine. MAO isoenzymes are classified on the basis of their substrate preference, sensitivity towards specific inhibitors, and tissue distribution into MAO-A and MAO-B. Selective MAO-A inhibitors have been used clinically in the treatment of depression and anxiety, while MAO-B inhibitors have been used in the treatment of Parkinson's and Alzheimer's diseases. Many plant-derived and synthetic compounds such as isoquinoline, xanthones have been identified as MAO inhibitors.

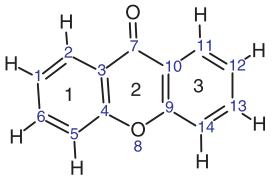
The xanthones (9H-xanthen-9-ones) of natural and synthetic origin are of biological and pharmacological interest. Recently in the literature, a special issue of "Current Medicinal Chemistry" was dedicated to xanthones [1–5].

It has been demonstrated that in most cases the orientations of nodes in π -like orbitals of aromatic molecules are a critically important feature in understanding their activity. This was first found in phenylalkylamine hallucinogens [6], carbonic anhydrase, trypsin, thrombin and bacterial collagenase inhibitors [7], tryptamine hallucinogens [8] as well as polychlorodibenzofurans [9]. The present contribution extends this to some xanthone derivatives.

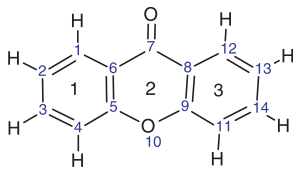
A QSAR study among the MAO-A inhibitory activity of xanthones series was recently studied [10] and shown to correlate with descriptors like the E-state index, molecular connectivity and shape. In this contribution it is hoped to improve the correlation by including the nodal orientations. The calculation of nodal orientation is done with the program NODANGLE [11], which has been described previously. NODANGLE calculates the angle between the nodes in π-like orbitals and a reference point on the aromatic ring. NODANGLE works by for each ring analytically least-squares fitting the coefficients of the p_z orbitals on the ring atoms to those of the degenerate HOMO and LUMO of benzene. The 10 highest occupied and 10 lowest unoccupied orbitals of the compound in question are searched, and an error term is calculated for each - a scaled sum of squares of the difference between the coefficients of the p_z orbitals of the compound and those of benzene with the same nodal orientation as the ring in question. For an exact match to benzene this error term is zero. For an exact match for an orbital of the wrong symmetry it is unity. NODANGLE prints out angles

Correspondence: B. W. Clare, School of Biomedical and Chemical Sciences, The University of Western Australia, 35 Stirling Highway, Crawley WA 6009, Australia. E-mail: bwc@theochem.uwa.edu.au

ISSN 1475-6366 print/ISSN 1475-6374 online © 2007 Informa UK Ltd.
DOI: 10.1080/14756360601161966



Labeling 1: Xanthone labeling for rings **1** and **3**



Labeling 2: Xanthone labeling for ring **2**

Figure 1. Numbering of xanthones skeleton used in the HyperChem and NODANGLE calculations. (compound **1** in Table I).

and orbital energies for those orbitals for which the error term is less than 0.5. These are π-like orbitals, and provided the error term is small have nodal orientations that closely match benzene of the appropriate nodal orientation. This calculation is done for each of the three rings of the xanthones.

For the xanthones, calculating the angles in the three rings can be accomplished in two MOPAC calculations by entering the atom as numbered in Figure 1. In labeling 1, rings 1 and 3 are 6-membered rings numbered 1–6 and 9–14 for ring 1 and 3, respectively. In labeling 2, ring 2 is also a 6-membered ring numbered 5–10. The angles calculated by NODANGLE are then Θ1, Θ2 and Θ3 in that figure, measured at atoms 1, 9 (in labeling 1) and 5 (in labeling 2), respectively. For final interpretation, the final angles are related to the calculated angles, assuming the rings are regular polygons, $\Phi 2 = \Theta 2 - 120$, $\Phi 1 = \Theta 1 - 60$ and $\Phi 3 = 120 - \Theta 3$ as shown in Figure 2.

Table I summarizes the MAO-A inhibitory activity of 42 xanthones derivatives [10] expressed as IC_{50} values, where C is the effective concentration of the compound to achieve 50% MAO-A inhibition in a micro molar range.

A problem arises from the symmetry of the parent molecule and to deal with this problem, we use the program FLIPSTEP, a component of the MARTHA [12] statistical package, which has been described previously [12,13]. FLIPSTEP calculates regressions for all possible combinations of compounds with Φ1 exchanged with Φ3 and Φ2 with – Φ2 and selects that combination with the regression with the best Fisher F-ratio, after eliminating descriptors that are either collinear with other descriptors or are of poor statistical significance.

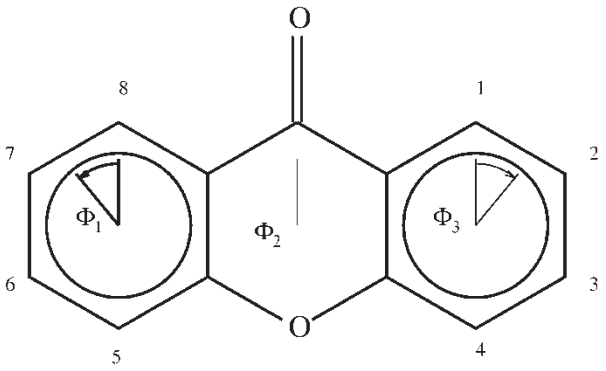


Figure 2. Numbering of the xanthones skeleton and angles used in the interpretations. Angles shown are for compound **1**.

Calculations

The molecules were setup with HyperChem [14] and optimized at the AM1 level with MOPAC 6 [15]. An AM1 optimization was considered adequate for these compounds, as AM1 was developed and parameterized for common organic structures, and also because the calculated angles (but not the orbital energies) are extremely insensitive to the level of theory. A NODANGLE calculation was run on the MOPAC output file to identify the relevant orbital and obtain the angles and corresponding orbital energies. The angles and orbital energies were correlated with the activities taken from the literature [10] with the program FLIPSTEP.

Results

In this study the HOP (highest occupied π orbital) is not identical to HOMO and also LUP (lowest unoccupied π orbital) is not identical to LUMO. In general we restrict our attention to π – like orbitals and, in particular, to those four orbitals that most resemble the degenerate HOMO and LUMO of benzene. SHOP and SLUP refer to the energies of the second highest occupied and second lowest unoccupied π-orbital respectively, and they are not necessarily the same for all rings, hence HOP1, HOP2 and HOP3 refer to orbital energies for the relevant orbitals for the three rings of the xanthones. Table II summarizes the orbital energies and angles of the compounds in Table I.

NODANGLE does not print out values of angles or energies for non π – like orbitals for which the error term for the angle exceeds 0.5. Table III summarizes the calculated angles and their error terms. The different descriptors used in this study are summarized in Table IV.

The best model as given in [10] after excluding compounds **12,16,18,33,37,38,40** and **41** was:

$$IC_{50} = \sum(a_i X_i) - 1071.22 \qquad (1)$$

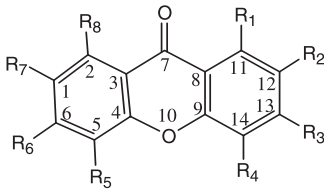
$$n = 34,\ r^2 = 0.847,\ S = 8.069,\ F = 9.72,$$

$$r^2_{cv} = 0.734,\ S^2_{cv} = 1.558$$

where n is the number of compounds used in the fit, r^2 is the squared correlation coefficient, S is the standard deviation, and F is the overall F-statistics for the addition of each successive term. The a_i values are at 90% confidence limit of each coefficient. The data X_i are the E-state for atoms level (S_i), molecular connectivity (χ), shape (κ) indices and finally the constant term.

In flip regression in the present case, flipping consist of changing the sign of Φ_2 and swapping Φ_1 and Φ_3,

Table I. MAO Inhibitory activities of xanthone derivatives



.

No.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	IC_{50} MAO-A (μM)
1	H	H	H	H	H	H	H	H	0.84 ± 0.08
2	OH	H	H	H	H	H	H	H	0.31 ± 0.05
3	MeO	H	H	H	H	H	H	H	0.9 ± 0.1
4	H	OH	H	H	H	H	H	H	3.8 ± 0.3
5	H	MeO	H	H	H	H	H	H	5.3 ± 0.4
6	H	H	OH	H	H	H	H	H	1.1 ± 0.3
7	H	H	MeO	H	H	H	H	H	0.18 ± 0.03
8	H	H	H	OH	H	H	H	H	1.3 ± 0.1
9	H	H	H	MeO	H	H	H	H	30 ± 3.2
10	OH	H	H	H	OH	H	H	H	0.73 ± 0.1
11	H	H	OH	H	OH	H	H	H	4.5 ± 0.2
12	H	H	OH	H	MeO	H	H	H	23 ± 1.4
13	OH	H	MeO	H	H	H	H	H	0.11 ± 0.01
14	MeO	H	MeO	H	H	H	H	H	20.2 ± 0.48
15	H	H	MeO	H	MeO	H	H	H	36 ± 2.9
16	MeO	H	H	H	OH	H	H	H	51 ± 7.8
17	H	H	MeO	OH	H	H	H	H	18 ± 3.1
18	H	H	OH	MeO	H	H	H	H	65 ± 6.8
19	H	H	MeO	MeO	H	H	H	H	31 ± 4.8
20	OH	H	OH	H	OH	H	H	H	3.8 ± 0.25
21	OH	H	MeO	H	OH	H	H	H	0.04 ± 0.005
22	OH	H	MeO	H	MeO	H	H	H	29 ± 4.3
23	MeO	H	MeO	H	MeO	H	H	H	58 ± 6.8
24	OH	H	OH	Me	H	H	H	H	4.3 ± 0.4
25	OH	Me	OH	H	H	H	H	H	3.7 ± 0.2
26	OH	Me	OH	Cl	H	H	H	H	27 ± 1.1
27	OH	Me	OH	Br	H	H	H	H	14.9 ± 0.6
28[a]	OH	H	OH	$C_{10}H_{17}$	OH	H	H	H	37 ± 5.5
29[b]	OH	C_5H_9	H	OH	OH	H	H	H	3.3 ± 0.2
30[c]	OH	H	C_5H_9	OH	OH	H	H	H	40 ± 3.7
31	OH	MeO	OH	H	OH	H	H	H	2.7 ± 0.4
32	OH	MeO	OH	H	MeO	H	H	H	51 ± 11
33	MeO	MeO	MeO	H	MeO	H	H	H	37 ± 2.0
34	OH	H	OH	H	H	H	OH	H	8 ± 1.2
35	OH	H	OH	H	OH	H	H	OH	13 ± 1.4
36	OH	H	MeO	H	OH	H	H	OH	0.66 ± 0.06
37	OH	H	OH	H	H	H	OH	OH	24 ± 4.6
38	OH	H	MeO	H	H	H	OH	OH	8.5 ± 0.8
39	OH	H	MeO	H	H	H	MeO	MeO	19 ± 1.0
40	OH	H	OH	H	H	OH	OH	H	25 ± 3.4
41	MeO	H	H	Me	OH	H	MeO	H	24 ± 7.0
42	OH	MeO	OH	H	MeO	OH	H	H	32 ± 5.0

[a] $C_{10}H_{17}$ is $Me_2C = CH–CH_2–CH_2–C(Me) = CH–CH_2$; [b] C_5H_9 is $CH_2 = CH–CMe_2$; [c] C_5H_9 is $Me_2 = CH–CH_2$.

Reprinted from Bioorg. Med. Chem. Lett. 14, M.B. Núñez, F.P. Maguna, N.B. Okulik and E.A. Castro, "QSAR Modelling of MAO inhibitory activity of xanthones derivatives", 5611-5617, Copyright (2004), with permission from Elsevier

and also swapping HOP1 with HOP3, SHOP1 with SHOP3, LUP1 with LUP3 and SLUP1 with SLUP3.

Compounds **28** and **41** has no nodal angle with error term (α) less than 0.5 which indicates that the symmetry of the π-like orbitals in ring 2 in these compounds is not close to that of the π orbitals for benzene ring. Hence, two regression analyses was performed, one using all of the compounds and only the ring1 and ring 3 variables, and the second using all variables for the three rings with all compounds except compounds **28** and **41**, for which some values were not available. Two runs of FLIPSTEP, that performs a backward-stepwise variable selection, were carried out using the default setting of VIFMAX value of 35 and a reduced VIFMAX value of 15. VIFMAX is the criterion for excluding variables that are collinear with

Table II. Orbital energies (eV) and angles (°) of the compounds in Table 1.

Compound	HOP1	SHOP1	LUP1	SLUP1	Φ_1H	Φ_1L	HOP3	SHOP3	LUP3	Compound	SLUP3	Φ_3H	Φ_3L	HOP2	SHOP2	LUP2	SLUP2	Φ2H	Φ2L	IC_{50}(obs)
1	−9.191	−10.109	−0.709	−0.249	39.5	20.3	−9.191	−10.109	−0.709	**1**	−0.249	39.5	20.3	−10.109	−10.166	−0.249	1.520	0.0	30.0	0.84
2	−9.074	−9.796	−0.841	−0.353	35.2	20.5	−9.074	−9.796	−0.841	**2**	−0.353	62.8	17.9	−10.241	–	−0.353	1.352	4.0	30.2	0.31
3	−9.021	−9.553	−0.551	−0.194	34.4	22.4	−9.021	−9.553	−0.551	**3**	−0.194	61.5	16.4	−10.053	−10.151	−0.194	1.663	15.0	30.2	0.9
4	−8.958	−9.919	−0.802	−0.280	42.0	20.4	−8.958	−9.919	−0.802	**4**	−0.280	34.3	21.8	−9.919	−13.536	−0.280	1.451	7.0	30.7	3.8
5	−9.842	−10.132	−0.732	−0.219	34.0	20.2	−8.853	−9.842	−0.732	**5**	−0.219	38.3	21.1	−9.842	−10.132	−0.219	1.509	11.0	31.0	5.3
6	−9.250	−9.681	−0.712	−0.286	42.0	19.5	−9.250	−9.681	−0.712	**6**	−0.286	18.0	21.9	−9.681	−10.299	−0.286	1.478	−5.0	28.9	1.1
7	−9.176	−9.579	−0.654	−0.232	43.0	19.8	−9.176	−9.579	−0.654	**7**	−0.232	16.0	21.2	−9.579	−10.231	−0.232	1.536	−3.0	29.1	0.18
8	−9.073	−9.807	−0.812	−0.354	36.5	20.7	−9.073	−9.807	−0.812	**8**	−0.354	66.6	17.0	−10.255	–	−0.354	1.430	7.0	30.6	1.3
9	−8.882	−9.704	−0.685	−0.236	38.4	22.3	−8.882	−9.704	−0.685	**9**	−0.236	60.9	17.3	−10.095	–	−0.236	1.539	11.0	30.9	30
10	−9.067	−9.577	−0.942	−0.455	62.0	17.3	−9.067	−9.577	−0.942	**10**	−0.455	57.7	18.2	−10.390	−13.028	−0.455	1.265	−5.0	29.6	0.73
11	−9.129	−9.910	−0.813	−0.388	69.0	16.2	−9.129	−9.728	−0.813	**11**	−0.388	21.3	22.4	−10.424	−13.045	−0.388	1.389	11.0	28.2	4.5
12	−8.946	−9.817	−0.691	−0.266	63.0	16.5	−8.946	−9.565	−0.691	**12**	−0.266	24.7	24.2	−10.288	−12.414	−0.266	1.499	13.0	28.1	23
13	−9.131	−10.016	−0.770	−0.316	33.3	20.0	−9.131	−9.507	−0.770	**13**	−0.316	72.1	18.9	−10.016	−11.614	−0.316	1.383	8.0	29.4	0.11
14	−9.088	−9.874	−0.490	−0.171	35.2	21.4	−9.088	−9.317	−0.490	**14**	−0.171	54.2	17.4	−9.874	−11.399	−0.171	1.675	6.0	29.3	20.2
15	−8.893	−9.764	−0.636	−0.214	62.0	16.6	−8.893	−9.455	−0.636	**15**	−0.214	23.2	23.7	−10.228	–	−0.214	1.554	14.0	28.1	36
16	−8.972	−9.442	−0.648	−0.298	64.0	18.5	−8.972	−9.442	−0.648	**16**	−0.298	53.2	17.0	−10.253	–	−0.298	1.573	−3.0	29.7	51
17	−9.372	−10.149	−0.749	−0.332	47.0	20.3	−8.940	−9.372	−0.749	**17**	−0.332	95.4	18.1	−10.149	−11.995	−0.332	1.455	5.0	29.6	18
18	−9.244	−10.199	−0.750	−0.367	38.0	19.9	−9.244	−9.503	−0.750	**18**	−0.367	56.5	21.7	−10.199	–	−0.367	1.390	8.0	27.8	65
19	−9.176	−10.139	−0.698	−0.294	37.1	19.5	−9.176	−9.385	−0.698	**19**	−0.294	62.8	22.6	−10.139	–	−0.294	1.475	5.0	28.0	31
20	−9.156	−9.573	−0.923	−0.488	65.0	17.1	−9.156	−9.573	−0.923	**20**	−0.488	56.6	18.4	−10.273	−11.989	−0.488	1.236	17.0	28.7	3.8
21	−9.095	−9.478	−0.867	−0.414	64.0	16.7	−9.095	−9.478	−0.867	**21**	−0.414	59.4	19.3	−11.717	–	−0.414	1.298	−23.2	28.6	0.04
22	−8.900	−9.704	−0.749	−0.300	58.0	17.0	−8.900	−9.341	−0.749	**22**	−0.300	62.0	21.4	−11.560	–	−0.300	1.403	−22.0	28.1	29
23	−8.821	−9.533	−0.471	−0.157	60.0	17.3	−8.821	−9.236	−0.471	**23**	−0.157	44.0	20.8	−9.958	−11.345	−0.157	1.692	18.0	28.0	58
24	−10.018	−10.314	−0.816	−0.354	35.2	19.7	−9.068	−9.516	−0.816	**24**	−0.354	71.5	19.5	−10.018	−11.695	−0.354	1.326	9.0	29.1	4.3
25	−9.921	−10.282	−0.800	−0.378	38.8	20.5	−9.067	−9.530	−0.800	**25**	−0.378	57.5	17.8	−9.921	−11.777	−0.378	1.330	15.0	29.2	3.7
26	−9.989	−10.372	−0.933	−0.505	39.5	19.8	−9.069	−9.582	−0.933	**26**	−0.505	57.6	19.4	−9.989	−11.612	−0.505	1.181	16.0	28.2	27
27	−9.995	−10.379	−0.938	−0.514	39.7	19.3	−9.118	−9.610	−0.938	**27**	−0.514	52.7	20.7	−9.995	−11.456	−0.514	1.165	16.0	27.3	14.9
28	−9.072	−9.454	−0.886	−0.426	61.0	16.3	−9.072	−9.454	−0.886	**28**	−0.426	60.8	20.2	–	–	−0.426	1.270	–	31.8	37
29	−9.505	−10.180	−1.025	−0.546	81.0	17.5	−8.770	−10.180	−1.025	**29**	−10.051	71.2	15.5	−10.368	–	−0.546	1.184	−8.8	29.9	3.3
30	−8.842	−10.084	−1.005	−0.511	81.0	17.6	−8.842	−9.471	−1.005	**30**	−0.511	74.8	15.9	−10.313	−11.836	−0.511	1.197	13.0	30.4	40
31	−9.077	−9.619	−0.979	−0.550	63.0	17.0	−9.077	−9.619	−0.979	**31**	−0.550	43.8	18.3	−11.988	−12.981	−0.550	1.155	26.0	31.4	2.7
32	−8.892	−9.494	−0.858	−0.435	58.0	17.2	−8.892	−9.494	−0.858	**32**	−0.435	45.7	20.4	−11.822	–	−0.431	1.263	25.0	32.1	51
33	−8.739	−9.398	−0.511	−0.231	58.0	17.9	−8.739	−9.257	−0.511	**33**	−0.231	42.2	19.7	−9.426	−11.736	−0.292	1.502	16.0	32.6	37
34	−9.032	−9.881	−0.918	−0.418	32.9	21.8	−9.032	−9.596	−0.918	**34**	−0.418	64.9	18.3	−9.881	−12.884	−0.418	1.256	8.0	31.5	8
35	−8.899	−10.438	−1.073	−0.582	76.0	14.7	−9.612	−9.939	−1.073	**35**	−0.582	79.5	19.5	−12.514	–	−0.593	1.108	−5.0	30.6	13
36	−8.821	−10.377	−1.003	−0.515	76.0	14.8	−9.469	−9.879	−1.003	**36**	−0.515	93.1	19.7	−12.194	–	−0.515	1.140	−1.0	31.7	0.66
37	−8.875	−10.266	−1.058	−0.523	48.0	20.0	−9.578	−9.935	−1.058	**37**	−0.523	97.1	18.5	−12.732	–	−0.523	1.093	3.0	28.3	24
38	−8.823	−10.218	−1.003	−0.448	48.0	19.7	−9.479	−9.835	−1.003	**38**	−0.448	100.9	19.3	−12.497	–	−0.448	1.155	9.0	28.2	8.5
39	−8.848	−10.164	−0.783	−0.290	43.0	22.0	−8.848	−9.340	−0.783	**39**	−0.290	60.8	19.1	−9.684	–	−0.290	1.389	17.0	31.2	19
40	−9.075	−9.679	−0.899	−0.465	16.8	22.4	−9.075	−9.613	−0.899	**40**	−0.465	72.0	18.0	−9.679	−12.695	−0.465	1.232	9.0	30.0	25
41	−8.722	−9.281	−0.652	−0.251	47.0	19.1	−8.722	−9.281	−0.652	**41**	−0.251	60.4	18.1	–	–	−0.251	1.560	–	31.5	24
42	−9.563	−9.804	−0.932	−0.540	−45.2	22.1	−9.139	−9.563	−0.932	**42**	−0.540	51.8	16.3	−9.566	–	−0.542	1.166	81.2	31.2	32

Energy of: HOP, Highest Occupied π Orbital; SHOP, Second Highest Occupied π Orbital; LUP, Lowest Unoccupied π Orbital; SLUP, Second Lowest Unoccupied π Orbital; H, HOP; L, LUP, IC_{50}(obs), MAO-A inhibitory activity; Φ angles (as in Figure 2).

Table III. Calculated angles and their error terms.

Compound	Φ_1H	α_1H	Φ_1L	α_1L	Φ_3H	α_3H	Compound	Φ_3L	α_3L	Φ2H	α2H	Φ2L	α2L
1	39.5	0.0582	20.3	0.0813	39.5	0.0582	**1**	20.3	0.0813	0.0	0.1348	30.0	0.0102
2	35.2	0.0806	20.5	0.0909	62.8	0.0431	**2**	17.9	0.1359	4.0	0.0471	30.2	0.0078
3	34.4	0.0607	22.4	0.0716	61.5	0.0569	**3**	16.4	0.0756	15.0	0.3544	30.2	0.0167
4	42.0	0.0995	20.4	0.0943	34.3	0.0214	**4**	21.8	0.0704	7.0	0.1599	30.7	0.0047
5	34.0	0.0049	20.2	0.0904	38.3	0.0158	**5**	21.1	0.0564	11.0	0.2688	31.0	0.0040
6	42.0	0.0555	19.5	0.0737	18.0	0.0640	**6**	21.9	0.1063	−5.0	0.2287	28.9	0.0235
7	43.0	0.0614	19.8	0.0704	16.0	0.0627	**7**	21.2	0.1164	−3.0	0.2631	29.1	0.0216
8	36.5	0.0845	20.7	0.0812	66.6	0.0368	**8**	17.0	0.0806	7.0	0.0793	30.6	0.0065
9	38.4	0.0912	22.3	0.0960	60.9	0.0559	**9**	17.3	0.0656	11.0	0.2012	30.9	0.0076
10	62.0	0.0453	17.3	0.0899	57.7	0.0538	**10**	18.2	0.1374	−5.0	0.0230	29.6	0.0051
11	69.0	0.0334	16.2	0.0727	21.3	0.0919	**11**	22.4	0.1065	11.0	0.3520	28.2	0.0177
12	63.0	0.0508	16.5	0.0581	24.7	0.1026	**12**	24.2	0.1226	13.0	0.3111	28.1	0.0171
13	33.3	0.0654	20.0	0.0826	72.1	0.0555	**13**	18.9	0.1798	8.0	0.2204	29.4	0.0158
14	35.2	0.0524	21.4	0.0599	54.2	0.0718	**14**	17.4	0.1109	6.0	0.1820	29.3	0.0233
15	62.0	0.0530	16.6	0.0555	23.2	0.0965	**15**	23.7	0.1353	14.0	0.3107	28.1	0.0185
16	64.0	0.0376	18.5	0.0720	53.2	0.0805	**16**	17.0	0.0763	−3.0	0.0147	29.7	0.0123
17	47.0	0.0492	20.3	0.0750	95.4	0.0558	**17**	18.1	0.1070	5.0	0.1076	29.6	0.0160
18	38.0	0.0532	19.9	0.0814	56.5	0.0780	**18**	21.7	0.0965	8.0	0.1226	27.8	0.0410
19	37.1	0.0562	19.5	0.0781	62.8	0.0693	**19**	22.6	0.0998	5.0	0.1297	28.0	0.0377
20	65.0	0.0381	17.1	0.0850	56.6	0.0840	**20**	18.4	0.1733	17.0	0.2665	28.7	0.0127
21	64.0	0.0400	16.7	0.0814	59.4	0.0753	**21**	19.3	0.1822	−23.2	0.3618	28.6	0.0119
22	58.0	0.0595	17.0	0.0673	62.0	0.0765	**22**	21.4	0.2031	−22.0	0.3065	28.1	0.0134
23	60.0	0.0508	17.3	0.0454	44.0	0.1066	**23**	20.8	0.1345	18.0	0.2951	28.0	0.0183
24	35.2	0.0036	19.7	0.0791	71.5	0.0456	**24**	19.5	0.1806	9.0	0.2307	29.1	0.0208
25	38.8	0.0059	20.5	0.0851	57.5	0.0449	**25**	17.8	0.1759	15.0	0.3920	29.2	0.0190
26	39.5	0.0064	19.8	0.0932	57.6	0.0377	**26**	19.4	0.1536	16.0	0.4213	28.2	0.0318
27	39.7	0.0064	19.3	0.0915	52.7	0.0452	**27**	20.7	0.1592	16.0	0.4232	27.3	0.0445
28	61.0	0.0449	16.3	0.0760	60.8	0.0574	**28**	20.2	0.1883	–	–	31.8	0.0165
29	81.0	0.0222	17.5	0.0904	71.2	0.0107	**29**	15.5	0.1346	−8.8	0.0295	29.9	0.0049
30	81.0	0.0219	17.6	0.0902	74.8	0.0167	**30**	15.9	0.1273	13.0	0.1670	30.4	0.0037
31	63.0	0.0462	17.0	0.0851	43.8	0.0507	**31**	18.3	0.1609	26.0	0.4333	31.4	0.0146
32	58.0	0.0684	17.2	0.0699	45.7	0.0533	**32**	20.4	0.1815	25.0	0.3638	32.1	0.0148
33	58.0	0.0604	17.9	0.0497	42.2	0.0627	**33**	19.7	0.1141	16.0	0.3099	32.6	0.0276
34	32.9	0.0206	21.8	0.0752	64.9	0.0866	**34**	18.3	0.1926	8.0	0.0917	31.5	0.0106
35	76.0	0.0147	14.7	0.1245	79.5	0.0615	**35**	19.5	0.1990	−5.0	0.0589	30.6	0.0017
36	76.0	0.0146	14.8	0.1253	93.1	0.0591	**36**	19.7	0.2034	−1.0	0.0073	31.7	0.0115
37	48.0	0.0185	20.0	0.1206	97.1	0.0590	**37**	18.5	0.2110	3.0	0.0315	28.3	0.0094
38	48.0	0.0180	19.7	0.1170	100.9	0.0647	**38**	19.3	0.2201	9.0	0.0898	28.2	0.0081
39	43.0	0.0132	22.0	0.0501	60.8	0.0865	**39**	19.1	0.1982	17.0	0.2939	31.2	0.0177
40	16.8	0.0391	22.4	0.1037	72.0	0.0746	**40**	18.0	0.1832	9.0	0.2655	30.0	0.0234
41	47.0	0.0223	19.1	0.0674	60.4	0.0828	**41**	18.1	0.0846	–	–	31.5	0.0103
42	−45.2	0.0465	22.1	0.1082	51.8	−9.5630	**42**	16.3	−0.5400	81.2	0.3235	31.2	0.0409

H, HOP; α, error; Φ, angle (as in Figure 2).

Table IV. Descriptors used in this study.

Name	Descriptor
HOP1	Energy of highest occupied π orbital for ring 1
SHOP1	Energy of second highest occupied π orbital for ring 1
LUP1	Energy of lowest unoccupied π orbital for ring 1
SLUP1	Energy of second lowest unoccupied π orbital for ring 1
HOP2	Energy of highest occupied π orbital for ring 2
SHOP2	Energy of second highest occupied π orbital for ring 2
LUP2	Energy of lowest unoccupied π orbital for ring 2
SLUP2	Energy of second lowest unoccupied π orbital for ring 2
HOP3	Energy of highest occupied π orbital for ring 3
SHOP3	Energy of second highest occupied π orbital for ring 3
LUP3	Energy of lowest unoccupied π orbital for ring 3
SLUP3	Energy of second lowest unoccupied π orbital for ring 3
$S2\Phi_1H$	Sin(2* the nodal angle in the highest occupied π orbital in ring 1)
$C2\Phi_1H$	Cos(2* the nodal angle in the highest occupied π orbital in ring 1)
$S4\Phi_1L$	Sin(4* the nodal angle in the lowest unoccupied π orbital in ring 1)
$C4\Phi_1L$	Cos(4* the nodal angle in the lowest unoccupied π orbital in ring 1)
$S2\Phi_2H$	Sin(2* the nodal angle in the highest occupied π orbital in ring 2)
$C2\Phi_2H$	Cos(2* the nodal angle in the highest occupied π orbital in ring 2)
$S2\Phi_3H$	Sin(2* the nodal angle in the highest occupied π orbital in ring 3)
$S4\Phi_3L$	Sin(4* the nodal angle in the lowest unoccupied π orbital in ring 3)
$C2\Phi_3H$	Cos(2* the nodal angle in the highest occupied π orbital in ring 3)
$C4\Phi_3L$	Cos(4* the nodal angle in the lowest unoccupied π orbital in ring 3)

other variables in the regression. The variance inflation factor (VIF) is defined for each independent vatriable i as $1/(1 - R_i^2)$, where R_i^2 is the R^2 for independent variable i regressed on all of the other independent variables. VIFMAX is the value of VIF above which a variable will be removed from the regression early in the procedure. In general a value of VIF above 10 is cause for concern. By default VIFMAX is set to 35. With this value, the maximum VIF in the final equation is usually much less than 35.

Using VIFMAX value of 15 resulted in removing $C4\Phi_1L$, $C4\Phi_3L$, SLUP1, SLUP2, and LUP1 due to colinearity. LUP1 and HOP2 are removed because they are statistically insignificant.

FLIPSTEP stepwise regression with VIFMAX = 15 gives:

$$\begin{aligned}\text{Log}(IC_{50}) = {} & 48.828 + 1.18550 * \text{HOP1}(2.58) + 2.45240 * \text{SHOP1}(5.40) + 0.40112 * \text{HOP3}(1.49) \\ & - 1.20130 * \text{SHOP3}(3.65) + 0.11963 * C2\Phi_1H(0.55) + 2.78190 * S2\Phi_1H(5.59) \\ & + 1.67690 * C2\Phi_3H(5.51) - 2.50110 * S2\Phi_3H(7.34) + 0.25396 * S4\Phi_1L(0.07) \\ & - 24.55900 * S4\Phi_3L(5.55) + 1.77190 * C2\Phi_2H(4.24) + 0.18188 * S2\Phi_2H(0.77) \end{aligned} \quad (2)$$

$n = 42$, $F = 11.24$, $R^2 = 0.8333$, $Q^2 = 0.6051$, $S = 0.4042$

Here, R^2 is the square of the multiple correlation coefficients. F is the Fisher variance ratio. S is the standard error of estimate. Q^2 is the square of the multiple correlation coefficients based on the leave-one-out residuals. The numbers in parentheses are Student's t values; a value greater than approximately 2 is indicative of significance at the 0.05 level.

Using VIFMAX = 35, SLUP1 and LUP2 were removed because of colinearity. SHOP3 and SHOP1 were removed because they are statistically insignificant. FLIPSTEP stepwise regression with VIFMAX = 35 gives:

$$\begin{aligned}\text{Log}(IC_{50}) = {} & 61.350 + 0.1316 * \text{HOP1}(0.57) + 5.0528 * \text{LUP1}(3.21) + 1.4713 * \text{HOP3}(3.60) \\ & + 0.2411 * \text{HOP2}(2.59) - 4.9822 * \text{SLUP2}(3.23) + 1.6484 * C2\Phi_1H(4.04) \\ & - 1.6282 * S2\Phi_1H(5.63) - 0.6597 * C2\Phi_3H(2.85) + 1.9054 * S2\Phi_3H(3.83) \\ & - 0.3810 * C4\Phi_1L(0.21) - 28.5590 * S4\Phi_1L(4.87) - 5.3481 * C4\Phi_3L(2.97) \\ & - 12.3010 * S4\Phi_3L(1.65) + 0.7984 * C2\Phi_2H(2.45) - 0.3460 * S2\Phi_2H(1.77) \end{aligned} \quad (3)$$

$n = 40$, $F = 11.65$, $1\,R^2 = 0.8792$, $S = 0.3648$,

$Q^2 = 0.7178$, $\alpha = 1.13 \times 10^{-1}$

Here α is the statistical significance of the regression based on 5000 randomizations of the dependent variable [9]. Equation (3) has a higher R^2 than that for Equation (2), which implies that using VIFMAX = 35 gives a better model than that obtained by using VIFMAX = 15, at the cost of introducing a small colinearity.

Reduced Model:

Flip regression was applied on the reduced model, in which variables for rings 1 and 3 for all compounds were entered in regression analysis. Removing ring 2 variables from the descriptors set would result in a set of 12 variables that are: HOP1, SHOP1, LUP1, SLUP1, HOP3, SHOP3, LUP3, SLUP3, Φ_1H, Φ_1L, Φ_3H, Φ_3L. These variables are then flipped. Flipping consists of interchanging ΦH and ΦL for ring 1 with the parallel angles for ring 3. However, the nodal angles are then converted to sin $2\Phi_H$, cos $2\Phi_H$, sin $4\Phi_L$ and cos $4\Phi_L$ and ΦH and ΦL are removed from the variables set. As a result of the flipping process, we will have a set of variables which consists of: HOP1, SHOP1, LUP1, SLUP1, HOP3, SHOP3, LUP3, SLUP3, C2Φ_1H, S2Φ_1H, C2Φ_3H, S2Φ_3H, C4Φ_1L, S4Φ_1L, C4Φ_3L, S4Φ_3L. These variables are defined in Table IV. Applying FLIPSTEP on the reduced model resulted in removing LUP1 due to colinearity and removing S4Φ_3L and S4Φ_1L because they are statistically insignificant. Table V shows the progress of FLIPSTEP for the reduced model. The variables SLUP1 and SLUP3 are a flipped pair. One is of poor significance and the other very poor, so they can advantageously be deleted, reducing the number of variables.

The results for the reduced model with ring 1 and ring 3 variables for all compounds as well as the results for the model with all variables for all compounds but **28** and **41**, were checked by running the regression 5000 times with the dependent variable randomized (randomization test). Statistical significance was tested for using MARTHA routine FLIPRAND [7]. For the set using the rings 1 and 3 variables significance was satisfactory (2.47×10^{-6}), and for the run using all of the variables the regression was totally nonsignificant (1.13×10^{-1}).

Table V. Progress of FLIPSTEP for the reduced model.

Variable	Coefficient	T	Significance	VIF
HOP1	1.0745	4.21	0.0002	2.38
SHOP1	−1.2541	4.50	0.0001	2.31
SLUP1	−0.1016	2.10	0.0447	1.52
HOP3	0.8591	1.85	0.0749	2.37
SHOP3	2.1089	5.12	0.0000	3.62
SLUP3	−0.3177	0.38	0.7049	2.86
C2Φ_1H	2.4483	5.95	0.0000	12.13
S2Φ_1H	−2.9982	7.18	0.0000	9.28
C2Φ_3H	−0.0525	0.22	0.8300	4.42
S2Φ_3H	2.3561	7.36	0.0000	3.75
C4Φ_1L	7.0778	5.68	0.0000	8.64
C4Φ_3L	−0.8675	1.18	0.2478	4.83

$F = 13.59$, $R^2 = 0.8490$, $S = 0.3773$

Finally, a multi-linear regression analysis was carried out on the variables selected by FLIPSTEP regression using the program multlr from the Martha package [12]. Multilinear regression of Log(CI_{50}) with the π-like HOP and LUP energies as well as the converted angles trigonometric functions gives:

$$\begin{aligned}\mathrm{Log}(IC_{50}) = {} & 23.174(\pm 3.8367) + 1.0926 * \mathrm{HOP1}(\pm 0.24625) - 1.2590 * \mathrm{SHOP1}(\pm 0.28991) \\ & + 0.87881 * \mathrm{HOP3}(\pm 0.48252) + 1.8147 * \mathrm{SHOP3}(\pm 0.40331) + 2.4558 * \mathrm{C2}\Phi_1\mathrm{H}(\pm 0.42812) \\ & - 2.9127 * \mathrm{S2}\Phi_1\mathrm{H}(\pm 0.42632) - 0.14216 * \mathrm{C2}\Phi_3\mathrm{H}(\pm 0.23909) + 2.1736 * \mathrm{S2}\Phi_3\mathrm{H}(\pm 0.31586) \\ & + 7.3919 * \mathrm{C4}\Phi_1\mathrm{L}(\pm 1.2651) - 0.76220 * \mathrm{C4}\Phi_3\mathrm{L}(\pm 0.75546)\end{aligned} \quad (4)$$

$$n = 42,\ R^2 = 0.825184,\ F = 14.633,$$
$$S = 0.39264,\ Q^2 = 0.6322\ R = 0.908396,$$
$$\alpha = 2.47 \times 10^{-6}$$

Again, n is the number of compounds used, R^2 is the square of the multiple correlation coefficients, F is the Fisher variance ratio, S is the standard error of estimate and α is the statistical significance of the regression based on 5000 randomizations of the dependent variable. The numbers in parentheses are standard errors of estimates, or approximate 90% confidence intervals. It should be noted that in contrast to Equation (1) no compound has here been deleted.

Table VI shows the flip status and flip significance from FLIPSTEP carried out on the reduced model (with ring 1 and ring 3 variables for all compounds) and the first model with all variables for all compounds but compounds **28** and **41**. A value of the significance greater than 0.05 is indicative that flipping the corresponding compound makes little difference to the quality of the regression. A flip status of 1 indicates that the compound has not flipped in the final regression, and −1 that it has. This has only relative significance, and flipping all of the compounds in a flip regression has no effect.

Equation (4) is reasonably good but could be improved by incorporating more descriptors from the classical work done [10], but in this case the number of descriptors will be too large. Equation (4) and Table V show that R^2 obtained from FLIPSTEP is better than that obtained from multilinear regression analysis while F is better for the latter. However, the differences between the two regressions are not large.

Figure 3 shows HOMO orbitals for two sample compounds of the 42 compounds. HOMO orbitals for the 42 compounds are similar to the molecular orbitals shown in either **(a)** or **(b)**. As Figure 3 shows, p_z orbital on furan's oxygen prevents the formation of a

Table VI. Flip status and flip significance for Equations (3) and (4).

	Equation (3) (all variables)		Equation (4) (all compounds)	
Compound	Flip status	Flip significance	Flip Status	Flip Significance
1	−1	0.9813	1	0.9917
2	1	0.8576	−1	0.8265
3	1	0.0013	−1	0.0007
4	1	0.9752	−1	0.5680
5	−1	0.0003	−1	0.0000
6	−1	0.0007	1	0.2435
7	−1	0.0003	−1	0.1014
8	−1	0.1266	−1	0.0153
9	−1	0.0014	1	0.0359
10	1	0.3309	−1	0.1572
11	1	0.4451	1	0.0124
12	1	0.1609	1	0.6900
13	1	0.0598	1	0.0303
14	−1	0.0606	1	0.0426
15	−1	0.9706	−1	0.4480
16	1	0.0196	1	0.0007
17	−1	0.1748	−1	0.0100
18	−1	0.0144	−1	0.2926
19	−1	0.0031	−1	0.1022
20	1	0.3413	−1	0.0678
21	−1	0.0001	1	0.0001
22	1	0.0001	−1	0.0000
23	−1	0.0035	−1	0.0086
24	−1	0.0045	−1	0.0000
25	1	0.9672	−1	0.0042
26	−1	0.0440	−1	0.0001
27	−1	0.0014	−1	0.0000
28			−1	0.0000
29	−1	0.9045	1	1.0000
30	−1	0.0742	1	0.0010
31	−1	0.5134	1	0.5296
32	−1	0.0050	−1	0.0020
33	1	0.5627	1	0.4992
34	−1	0.8743	−1	0.5724
35	1	0.0006	−1	0.0015
36	1	0.3274	1	0.0006
37	1	0.0659	1	0.0004
38	1	0.0518	1	0.0080
39	−1	0.2376	−1	0.6804
40	−1	0.0024	−1	0.0691
41			1	0.8922
42	1	0.7589	−1	0.4872

nodal plane on ring 2. Hence, the withdrawal of ring 2 variables is supported.

Table VII summarizes the observed activity as well as the estimated activity according to the reduced model for the 42 compounds of xanthones in Table I, while Figure 4 shows the plot of the logarithm of the observed activity against the logarithm of the estimated activity.

Comparison with the QSAR of Castro et al.

The quality of a QSAR is assessed on a number of criteria. The commonest is goodness of fit, usually assessed as R^2. As well as having a high R^2, all of the terms in a QSAR should be statistically significant, conventionally at the 0.05 level. Further, it is necessary to exclude colinearities between independent variables for a QSAR to be reliable. It is also highly desirable, but not essential, that there be an intuitively apparent relationship between the variables considered and the dependent variable. Finally, from a statistical viewpoint, it is desirable to have fewer rather than more independent variables. It is unreasonable however to expect that an outcome such as inhibition of an enzyme would be well described by very few variables. This could only be the case if the structural variation in the inhibitors is strictly limited, so that the variation in activity is confined to a narrow aspect of the variation between the drugs.

Because we are considering the same group of drugs the structural diversity in the two studies is identical. Our R^2 for Equations (2) and (4) are 0.833 and 0.825, with 12 and 10 variables respectively. Castro et al. get 0.586 [10] with 10 variables and 42 compounds in Equation (1), but increased this to 0.847 only at the cost of discarding 8 compounds, which we consider not to be a legitimate procedure. Thus we obtain a much better fit with a similar number of variables than does Castro et al.

Castro et al. controlled colinearity by excluding descriptors with a pairwise R^2 greater than 0.80. This procedure however does not pick up colinearities that

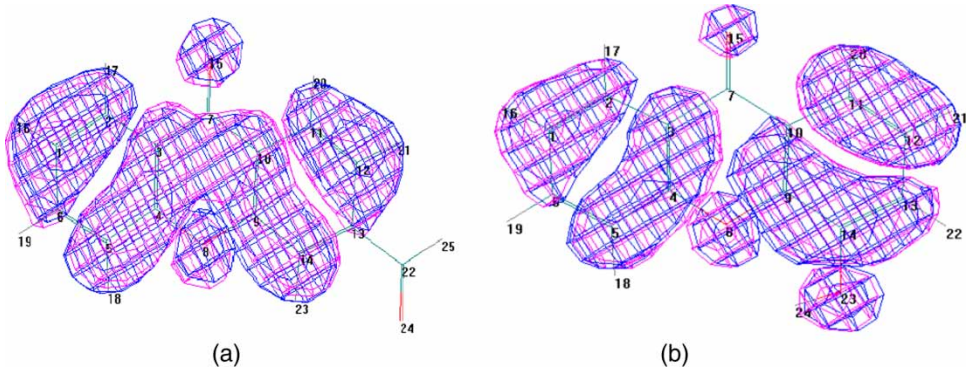


Figure 3. HOMO orbitals for xanthones.

Table VII. Observed Log IC_{50} versus estimated.

Compound	$LogIC_{50}$(Obs)	$LogIC_{50}$(Calc)
1	−7.57E-02	−0.1565
2	−0.50864	0.27088
3	−4.58E-02	−0.10925
4	0.57978	0.57756
5	0.72428	0.94419
6	4.14E-02	−0.41498
7	−0.74473	−0.62585
8	0.11394	9.28E-02
9	1.4771	1.1997
10	−0.13668	8.93E-02
11	0.65321	0.73208
12	1.3617	1.3959
13	−0.95861	−1.0924
14	1.301	1.2701
15	1.5563	1.4892
16	1.7076	1.1112
17	1.2553	0.9801
18	1.8129	1.6629
19	1.4914	2.0751
20	0.57978	0.55389
21	−1.3979	−0.62082
22	1.4624	1.7905
23	1.7634	1.9745
24	0.63347	0.74732
25	0.5682	0.51014
26	1.4314	0.8154
27	1.1761	1.1358
28	1.5682	1.1675
29	0.51851	−0.12478
30	1.6021	1.3739
31	0.43136	0.81976
32	1.7076	1.4114
33	1.5682	1.6192
34	0.90309	0.64607
35	1.1139	1.158
36	−0.18046	0.3633
37	1.3802	1.1962
38	0.92942	1.3671
39	1.2788	0.84322
40	1.3979	1.6668
41	1.3802	1.2964
42	1.5051	1.411

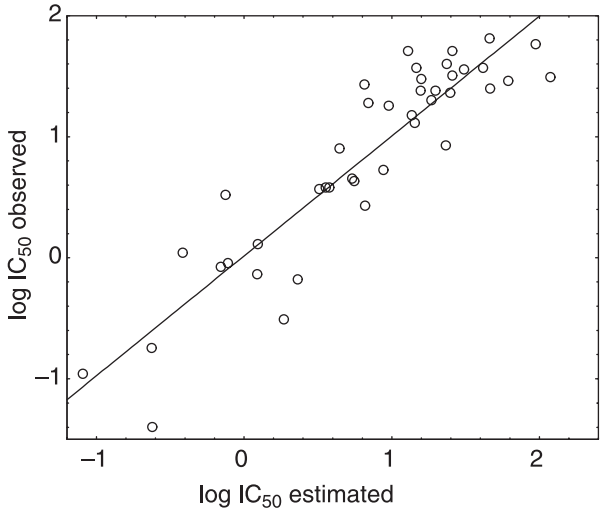


Figure 4. Observed Log IC_{50} versus estimated.

involve 3 or more descriptors. In contrast, we control colinearity using the variance inflation factor (VIF). This procedure picks up all colinearities. It is conventional to regard with suspicion any variable with a VIF greater than 10. In our Flipstep program we use a criterion VIFMAX, which by default is set to 35. Variables with a VIF greater than VIFMAX are excluded early in the stepwise regression. This often results in a regression in which the maximum VIF is much less than VIFMAX, and valuable descriptors can be lost. This is why we do not routinely set VIFMAX to 10.

Castro et al. do not cite values that allow us to assess the individual statistical significance of their terms in their equations, but do state that they are better than 10% confidence. We use a value of 5% confidence, but with a proviso that is uniquely relevant to flip regression. This is that if one of a pair of flipped variables is significant and the variable is included, then so is its partner, even if not significant. If this is not done it is impossible to use flip regression predictively. The significance of the terms in our regressions may be gauged from the t values in Equations (2)–(4). A value greater than approximately 2 is significant at the 5% level.

Finally, the variables used by Castro et al. include e-state, shape, size, connectivity and topological descriptors. These are legitimate descriptors, but are obscure in meaning, especially when many of them are used together in the same equation. The e-state variables are probably a measure of polarization of charge in a molecule. In other studies we have used quantum chemically calculated descriptors such as mean absolute charge or local dipole index, which are probably measures of the same thing, but we usually cannot use both of these in the same equation. They show too much colinearity. We have not used them here because they would expand by too much the number of descriptors used. We do use as descriptors the orientation of nodes in the π orbitals of the drugs, and also the energies of some of the π orbitals. We believe, and hope to show in a future publication, that these are intimately related to intermolecular forces between two aromatic molecules. It is not however as simple as alignment of nodes in an orbital in the drug with one in the receptor. At least two orbitals on each are involved in most cases. Since we have been using these descriptors we have not encountered a single example of a series of aromatic drugs in which at least one of them is not a major term in the QSAR equation.

Conclusions

The nodal orientation terms have a powerful explanatory value in that they account for much more of the variance in activity than is possible using the classical descriptors alone. Were it not for the large number of descriptors already in the equation in

comparison to the number of molecules, a combination of the classical descriptors and the nodal orientation terms would probably give an even better explanation of the MAO-A inhibitory activity of the xanthones.

This study has used two relatively new techniques. The first is flip regression, for handling the symmetry of the xanthone system, which is C_{2v}, like the dibenzofurans studied earlier and the phenylalkylamines for which the method was devised. A similar approach was pioneered 25 years ago by Kishida and Manabe [16] in their study of the QSAR of benzenedisulfonamide carbonic anhydrase inhibitors. The second is the use of the orbital nodal angle descriptors. This is based on the concept that the stability of stacked aromatic systems is highly orientation dependent, and is also dependent on the energies of the orbitals in the two aromatic systems that resemble the degenerate HOMO and LUMO of benzene. It is envisaged that the benzene rings of the xanthones are interacting with aromatic systems on the receptor (here MAO), and that alignment occurs between the π-orbital nodes on the pair. Precisely which rings are involved becomes apparent from the identity of the descriptors that remain in the equations. We hope to publish in the near future a computational and theoretical study of these interactions.

References

[1] Vieira LMM, Kijjoa A. Curr Med Chem 2005;12:2413.
[2] Sila AMS, Pinto DCGA. Curr Med Chem 2005;12:2481.
[3] Gales L, Damas AM. Curr Med Chem 2005;12:2499.
[4] Pinto MMM, Sousa ME, Nascimento MSJ. Curr Med Chem 2005;12:2517.
[5] Riscoe M, Kelly JX, Winter R. Curr Med Chem 2005;12:2539.
[6] Clare BW. J Med Chem 1998;41:3845–3856.
[7] Supuran CT, Clare BW. J Enz Inhib Med Chem 2004;19:237–248.
[8] Clare BW. THEOCHEM 2004;712:143–148.
[9] Clare BW. THEOCHEM 2006;763:207–215.
[10] Nunez MB, Maguna FP, Okulik NB, Castro EA. Bioogan Med Chem Lett 2004;14:5611.
[11] Clare BW. nodangle.zip http://www.chem.biomedchem.uwa.edu.au/staff/homepages/BrianClare 2005.
[12] Clare BW. martha.zip http://www.chem.biomedchem.uwa.edu.au/staff/homepages/BrianClare 2005.
[13] Clare BW, Supuran CT. Bioorg Med Chem 2005;13:2197–2211.
[14] Hypercube Hyperchem 1115 NW 4th Street, Gainesville, Florida 32601-4256 U.S.A.
[15] Stewart JJP. Q.C.P.E. Bull 1990;10:86.
[16] Kishida K, Manabe R. Med J Osaka Univ 1980;30:95–100.