RESEARCH PAPER

Accepted 5 June 1998
Received 4 February 1998
Indian J. Pharm. Sci., 1998, 60(6) pp. 353-357

A Quantitative Analysis of N-phenyl-6,11-dihydrodibenzo[b,e]oxepin-11-carboxamides and Related Derivatives as the Inhibitors of Acyl-CoA Cholesterol Acyl Transferase (ACAT)

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The ACAT inhibitory activity, -log IC_{50} (molar), of N-phenyl-6,11-dihydrodibenzo[b,e]oxepin-11-carboxamides and related derivatives, has been quantitatively correlated with structural and electronic substituent parameters; the sum of molar refraction, ΣMR for the R_1, the Kier's Δ parameter and a hydrogen bond count parameter, HB for 2-R_2 and the sum of Δ parameters, ΣΔ' for the 3-R_2, R_3, X, and Y-Z. The analysis has revealed that for good ACAT inhibitory activity, the substituent R_1 should have a large MR value while 2-R_2 should have a higher Δ but a lower HB. The remaining variations at 3-R_2, R_3, X, and Y-Z parts should be such that these give a lower ΣΔ' values.

THE enzyme, acyl-CoA cholesterol acyl transferase (ACAT, EC 2.3.1.26), is mainly responsible for the intracellular esterification of cholesterol and lipoprotein secretion by the liver[1-5]. It is believed to play an important role in the absorption of dietary cholesterol from the intestine, the metabolism of cholesterol in liver, and the accumulation of cholesteryl esters in arterial lesions[5,6]. Reportedly, ACAT activity is enhanced in the intestinal mucosal cells when cholesterol is ingested[7] and in arterial cells undergoing atherosclerosis[8]. Inhibition of arterial macrophage ACAT enzyme can directly intervene in the progression of lesions. Therefore, it would be beneficial to reduce plasma cholesterol concentration, to reduce the secretion of very low density lipoproteins (VLDL) into the plasma, and to prevent the formation of foam cells in the arterial walls. In view of this, the ACAT inhibitors provide potential as hypocholesterolemic and antiatherosclerotic agents[9,10].

During the course of their search for potent ACAT inhibitors of novel structures, Kumazawa et al.[11] have reported a series of N-phenyl-6,11-dihydrodibenzo [b,e]oxepin-11-carboxamides and related analogues (Fig. 1). These were assayed for their ability to inhibit ACAT *in vitro* and to decrease serum total cholesterol *in vivo*. The compounds, their *in vitro* inhibitory actions, as determined by incubation with [^{14}C]oleolyl-CoA and liver microsomes from cholesterol-fed rabbits, are listed in Table 1.

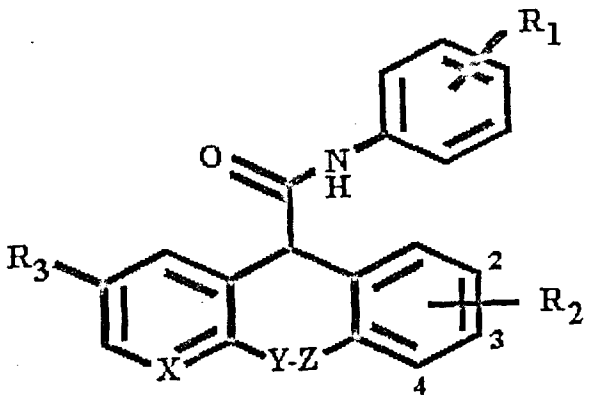

Fig. 1: Structures of N-phenyl-6,11-dihydrodibenzo[b,e] oxepin-11-carboxamides and related derivatives

The title compounds (Fig. 1) have substituents at five different sites, viz., R_1, R_2, R_3, X, and Y-Z. Further, their activities, -log IC_{50}'s (molar basis), show considerable variation, with compound 27 behaving as the most potent

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member of the series. We were, therefore, temped to derive quantitative structure-activity correlations for this series of compounds using multiple regression analysis (MRA) to obtain the best-fit of biological data with certain substituent parameters, described herein. It is intended to throw some light on the physicochemical basis of their biological actions. The various substituent parameters are also given in Table 1.

Parameterization

Amongst the large number of alternatives, those consisdered the most appropriate predictor parameters for explaining the observed inhibitory actions of the title compounds, are the sum of the molar refractions[12,13] ΣMR for the R_1 substituents, and the Kier's Δ parameter[14,15] and a hydrogen bond count parameter[16], HB, for the R_2's. The MR reports on the bulk of the substituents. However, it is also known that MR parameter measures the electronic effects and may also reflect dipole-dipole interaction[13] at the active site. The Kier's Δ parameter is given by Eq. (1).

$$\Delta = (\delta^v - \delta) / N^2 \qquad (1)$$

Where δ^v and δ are the valence and simple deltas, respectively, and are the counts of electrons in σ,π or lone-pair orbitals. N is the principal quantum number (e.g., 1, 2, 3, ..., etc). Kier and Hall[15] have obtained a highly significant linear correlation between Δ and the Mulliken's electronegativities for 19 atoms (considering these in different hybridisation states) of the first, second, and third rows of periodic table. Therefore, Δ parameter is believed to report upon the electronic and other related effects. Since very few variations occur at 3-R_2, R_3 X, andY-Z, the sum of their Kier's Δ values, ΣΔ' instead of an indicator or any other parameter, was used for deriving the follow up correlations. Lastly, the HB parameter is based on a consideration of the number of atoms in a group that can be involved in the hydrogen-bonding interaction, either as a H-bond donor or H-bond acceptor[16].

RESULTS AND DISCUSSION

MRA on the data of Table 1 returned the correlation as given by Eq. (2)

$$-\log IC_{50} = 0.412(\pm 0.056)\Sigma MR + 1.267(\pm 0.268)\Delta - 0.335 (\pm 0.074)HB - 1.104(\pm 0.284)\Sigma\Delta' + 6.116$$

n=43, s=0.268, R=0.840, EV=0.676, $F_{4,38}$=22,875 (2).

Where n is the data-size, s, the standard error of estimates, R, the multiple correlation cofficient, EV, the explained variance in the calculated activity data, and F, the F-ratio between the variances of calculated to observed activities. The ± data are the 95% confidence interval associated with the individual regression coefficients. ΣMR accounts for the bulk at R_1 in the phenyl parts. No other attempted parameters for this position could replace ΣMR. However, the van der Waals volume, V_w, also a measure of size, was found to be perfectly correlated with Σ MR (r=0.994; s=0.026; $F_{1,41}$ = 3223.7) and its use in place of MR gave a comparable EV and R but a higher ± data then the ones in Eq. (2). The variables Δ and HB stand to account respectively for electronegativity based property and H-bonding (both donor and acceptor) property of the 2-substituents. This position seems to be of critical and decisive importance and demands two separate predictors to distinguish between the types of R_2's at this position, specially the halogens. The R_2 substituents having a sulphur atom or one bigger than it, were arbitrarily selected for Δ calculations (Eq. 1) while those being smaller in size but more electronegative than sulphur were retained for the HB count parameter. For example, for R_2=2-F and 2-Cl (compound numbers 20 and 21, the substituents being smaller and more electronegative than sulphur) the HB parameter is important while for R_2 = 2-Br and 2-I (compound numbers 22 and 24, which are bigger and less electronegative than sulphur) the Δ parameter is very necessary. Further, the value of Kier's parameter are the function of the principal quantum number, N (Eq. 1), therefore, these values of Δ are also dependent on the type of halogen. Besides the halogen at 2-R_2, the other substituents such as R_2 = 2-CF_3, 2-OMe and 2-CN (compound numbers 25, 26 and 28) and R_2 = 2-COOMe, 2-$CONMe_2$, 2-CH_2OH and 2-NO_2 (compound numbers 29-32) are considered for HB parameter, according to the method suggested by other workers[16]. The electronic σ, field F, resonance R, or the hydrophobicity π, were all tried but to no avail. Finally, for the 3-R_2, R_3, X and the Y-Z positions also, ΣΔ' was found convincing.

Though Eq. (2) is sound statistically as is evinced from a low, s, a reasonably good R and EV as well as a F-value that is significant at 99% level [$F_{4,38}$(0.01)=3.86], there is still need for its betterment. Using this equation, the calculated activities (omitted here) for compounds 7, 14, 16 and 28, were found to deviate much from the observed ones. Also, these compounds are not active (see Table 1).

Table 1: Predictor Variables and Cholesterol Acyltransferase Inhibition Actions of N-Phenyl-6, 11-dihydrodibenzo[b,e]oxepin-11-carboxamides and Related Derivatives (Fig. 1 for structures)

S. No.	R_1	R_2	R_3	X	Y-Z	ΣMR[a]	Δ	HB	ΣΔ'	-log IC_{50}(M) Obsd[b]	Calcd[c]
1	2,4-F_2	2-Br	H	CH	CH_2O	0.29	0.38	0	0.25	6.24	6.54
2	2,6-Cl_2	2-Me	H	CH	CH_2O	1.31	0	0	0.25	6.72	6.45
3	2,6-Cl_2	2-Br	H	CH	CH_2O	1.31	0.38	0	0.25	7.13	6.95
4	2,6-Cl_2	3-Br	H	CH	CH_2O	1.31	0	0	0.63	6.03	5.97
5	2,6-Cl_2	2-^{t}Bu	H	CH	CH_2O	1.31	0	0	0.25	6.38	6.45
6	2,6-Br_2	2-Me	H	CH	CH_2O	1.88	0	0	0.25	6.72	6.68
7	2,4,6-OMe_3	2-Me	H	CH	CH_2O	2.36	0	0	0.25	6.24[d]	—
8	2,6-Me_2	2-Me	H	CH	CH_2O	1.23	0	0	0.25	6.46	6.42
9	2,6-Me_2	2-^{i}Pr	H	CH	CH_2O	1.23	0	0	0.25	6.44	6.42
10	2,6-Me_2	2-^{t}Bu	H	CH	CH_2O	1.23	0	0	0.25	6.30	6 42
11	2,4,6-Me_3	2-Me	H	CH	CH_2O	1.70	0	0	0.25	6.96	6.61
12	2,6-Et_2	2-Me	H	CH	CH_2O	2.16	0	0	0.25	6.57	6.80
13	2,6-Et_2	2-^{i}Pr	H	CH	CH_2O	2.16	0	0	0.25	6.30	6.80
14	2-Cl,6-Me	2-Br	H	CH	CH_2O	2.16	0.38	0	0.25	6.60[d]	—
15	2-^{i}Pr	2-Me	H	CH	CH_2O	1.27	0	0	0.25	6.59	6.43
16	2,6-$^i Pr_2$	2-Me	H	CH	CH_2O	1.70	0	0	0.25	6.14[d]	—
17	2,6-$^i Pr_2$	H	H	CH	CH_2O	3.10	0	0	0.25	7.03	7.17
18	2,6-$^i Pr_2$	2-Me	H	CH	CH_2O	3.10	0	0	0.25	7.30	7.17
19	2,6-$^i Pr_2$	2-Et	H	CH	CH_2O	3.10	0	0	0.25	6.89	7.17
20	2,6-$^i Pr_2$	2-F	H	CH	CH_2O	3.10	0	1	0.25	6.68	6.83
21	2,6-$^i Pr_2$	2-Cl	H	CH	CH_2O	3.10	0	1	0.25	6.82	6.83
22	2,6-$^i Pr_2$	2-Br	H	CH	CH_2O	3.10	0.38	0	0.25	7.64	7.68
23	2,6-$^i Pr_2$	3-Br	H	CH	CH_2O	3.10	0	0	0.63	6.74	6.70
24	2,6-$^i Pr_2$	2-I	H	CH	CH_2O	3.10	0.24	0	0.26	7.46	7.50
25	2,6-$^i Pr_2$	2-CF_3	H	CH	CH_2O	3.10	0	1	0.25	6.80	6.83
26	2,6-$^i Pr_2$	2-OMe	H	CH	CH_2O	3.10	0	1	0.25	6.85	6.83
27	2,6-$^i Pr_2$	2-SMe	H	CH	CH_2O	3.10	0.44	0	0.25	8.15	7.91
28	2,6-$^i Pr_2$	2-CN	H	CH	CH_2O	3.10	0	1	0.25	6.31[d]	—
29	2,6-$^i Pr_2$	2-COOMe	H	CH	CH_2O	3.10	0	2	0.25	6.74	6.48
30	2,6-$^i Pr_2$	2-$CONMe_2$	H	CH	CH_2O	3.10	0	2	0.25	6.40	6.48
31	2,6-$^i Pr_2$	2-CH_2OH	H	CH	CH_2O	3.10	0	2	0.25	6.24	6.48
32	2,6-$^i Pr_2$	2-NO_2	H	CH	CH_2O	3.10	0	2	0.25	6.62	6.48

S. No.	R_1	R_2	R_3	X	Y-Z	ΣMR[a]	Δ	HB	ΣΔ'	-log IC_{50}(M) Obsd[b]	Calcd[c]
33	2,6-$^iPr_2$	2,3-Me_2	H	CH	CH_2O	3.10	0	0	0.25	7.19	7.18
34	2,6-$^iPr_2$	2-Me,4-COOMe	H	CH	CH_2O	3.10	0	0	0.25	7.57	7.18
35	2,6-$^iPr_2$	2-Me,4-Br	H	CH	CH_2O	3.10	0	0	0.25	7.00	7.18
36	2,6-$^iPr_2$	H	Br	CH	CH_2O	3.10	0	0	0.63	6.72	6.70
37	2,6-$^iPr_2$	2-Me	Br	CH	CH_2O	3.10	0	0	0.63	6.92	6.70
38	2,6-$^iPr_2$	2-Br	Br	CH	CH_2O	3.10	0.38	0	0.63	7.24	7.20
39	2,6-$^iPr_2$	H	H	N	CH_2O	3.10	0	0	0.75	6.19	6.54
40	2,6-$^iPr_2$	2-Br	H	N	CH_2O	3.10	0.38	0	0.75	7.11	7.04
41	2,6-$^iPr_2$	H	H	CH	CH_2CH_2	3.10	0	0	0.25	7.40	7.18
42	2,6-$^iPr_2$	2-Me	H	CH	CH_2CH_2	3.10	0	0	0.25	7.26	7.18
43	2,6-$^iPr_2$	2-Br	H	CH	CH_2CH_2	3.10	0.38	0	0.25	7.41	7.68

[a] The MR values are scaled to 0.1; [b]the IC_{50} data are from ref.11; [c] calculated using Eq. (3), and [d] the 'outlier' compounds, not included in deriving Eq. (3).

However, no specific reason is immediately becoming apparent for their 'outlier' behaviour. Thus the MRA, ignoring these four data points, provided a much improved correlation shown in Eq. (3).

$$-\log IC_{50} = 0.407(\pm0.044)\Sigma MR+1.343(\pm0.222)\Delta-0.348(\pm0.059)HB-1.270(\pm0.226)\Sigma\Delta'+6.234$$
$$n=39,\ s=0.211,\ R=0.903,\ EV=0.793,\ F_{4,34}=37.474 \quad (3)$$

As can be seen from Eq. (3), the R, EV and F-values [$F_{4,34}$ (0.01)=3.93] have been sharply raised while the ± data have been lowered. The EV value now accounts for 79% variance in the calculated -log IC_{50} values. Furthermore, the predictor variables of this equation are mutually orthogonal as shown by the intercorrelation matrix given in Table 2. This equation reveals that for a compound to be an effective inhibitor of ACAT, both the ΣMR (for R_1 substitutions) and the Δ value (for 2-R_2 substitution) should be large positive, the later should preferably be >0.5, while both the HB and ΣΔ should be smaller. This is in agreement with the observation of Kumazawa *et al.*[11] that at 2- and 6-R_1, potency is higher for bulky group (hence, a more MR value) while substitution at R_3 (hence, a larger ΣΔ') leads to reduced potency. They had further observed that 3-bromo analogues were less potent than 2-bromo analogues (thus, demanding a lower ΣΔ' at 3-R_2). For the 2-halo derivatives,

Table 2: The Intercorrelation Matrix Among the Predictor Variable of Eq. (3)

	ΣMR	Δ	HB	ΣΔ'
ΣMR	1.000	0.004	0.318	0.181
Δ		1.000	0.236	0.106
HB			1.000	0.220
ΣΔ'				1.000

Values shown above are the correlation coefficients between predictor variables.

potency increase in the order F, Cl, I, Br, while for the alkyl substituents, methyl analogues are more active than the ethyl or isopropyl analogues. Compounds 22, 24, 27 and 34, which all fulfil these criteria, are in fact, reported to more active than many other of Table 1. Using Eq. (3) the calculated -log IC_{50} values listed in Table 1 are, in most cases, close to the observed ones. Therefore, this equation may be applied in computing the theoretical activity of a potential ACAT inhibitor before its actual synthesis and bioassay. These inferences may, therefore, form the basis for a more rational substituent-selection in future drug-design strategy.

ACKNOWLEDGEMENTS

One of us (PS) thankfully acknowledges the financial assistance received from the University Grants Commission, Central Regional Office, Bhopal.

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