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Computer-Automated Structure Evaluation of Flavonoids and Other Structurally Related Compounds as Glyoxalase I Enzyme Inhibitors

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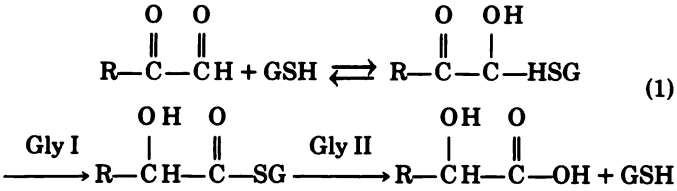
SUMMARY

The Computer-Automated Structure Evaluation (CASE) methodology has been applied to a set of flavonoids and other structurally related compounds tested for glyoxalase I enzyme inhibition. CASE identified several structural features believed to be responsible for activity. A total of five fragments were isolated. The most important structural feature is the α-hydroxy-α,β-unsaturated carbonyl group attached to a fused ring carbon atom. This fragment tautomerizes into a transition state analog of the substrate of the enzyme. Five tested compounds initially removed from the database were submitted to CASE in the predictive mode. The predictions generally matched the tested values for enzyme inhibition. A set of chromones and phenyl-pyrones, although untested, were also submitted to CASE. CASE predicts that, as a class of compounds, chromones would be more effective inhibitors than phenyl-pyrones.

The glyoxalase enzyme system is believed to be involved in the detoxification of α-ketoaldehydes (1–3). Glyoxalase I (Gly.I) and its cofactor glutathione (GSH) catalyze the isomerization of the cytotoxic α-ketoaldehydes whereas glyoxalase II (Gly.II) facilitates their oxidation into the corresponding, metabolically inert, α-hydroxy-acids (4).



(1)

Other components such as magnesium and a base may also be involved in the catalytic reaction (5).

Evidence exists that the glyoxalase enzymes and some of their substrates may be controlling factors in the regulation of cell division and thereby play a fairly important role in the control of rapidly dividing cells and tumor cells (6, 7). α-Ketoaldehydes are cytotoxic and can be used in small quantities to control cell proliferation in cancer chemotherapy. Unfortunately, the glyoxalase enzyme metabolizes them before they can express their cytotoxicity. It is not surprising, therefore, to find that there is interest in identifying agents that could inhibit the activity of these enzymes.

Our database of glyoxalase inhibitors consisted of 53 flavonoids and other structurally related compounds found to inhibit the enzyme to varying degrees. The experimental values were obtained from Drs. Edge and Thomson,[1] and the details of their measurements were published previously (8).

Methods

The CASE program is a fully automated system that analyzes the biological activity of a given set of compounds and identifies structural descriptors believed to be responsible for the activity or inactivity of the compounds (9–14).

The molecular formulae of the compounds of the data set are encoded using KLN, a line notation method that has been described before (15). Each compound to be analyzed is associated with an index of biological activity, in this case, the concentration needed for 50% inhibition of the enzyme glyoxalase I.

Specifically, CASE takes each molecule in a learning set of compounds and breaks it up into all possible fragments of 2 to 10 linearly connected heavy atoms each (12). These fragments are marked as active or inactive depending on whether the parent molecule is active or not. The fragments are then subjected to a series of statistical tests to determine which fragments have a distribution that is markedly skewed towards either activity or inactivity. Those that are selected as significant are subjected to a QSAR analysis to yield a linear regression equation that best describes the activity of the compounds in the data base. The QSAR analysis is based on the frequency of occurrences of the relevant fragments. It also considers the logarithm of the partition

[1] Personal Communication from Drs. C. Edge and C. Thomson, NFCR Regional Workshop, Department of Chemistry, University of St. Andrews. St. Andrews, Fife, KY16 9ST.

ABBREVIATIONS: CASE, computer-automated structure evaluation; QSAR, quantitative structure activity relationship.

coefficient (log P) of the compound in an octanol/water system as a potential descriptor. The log P values are calculated by the program using the charge density method (16). The object of the QSAR analysis is to generate an equation of the form.

$$\text{Activity} = a + \sum b_i(n_iF_i) + c \log P + d \log^2 P \quad (1)$$

where a, b, c, and d are regression coefficients, n_i is the number of times fragment F_i appears in a compound, and log P is the common logarithm of the partition coefficient.

Results and Discussion

The common names, experimental activities, and CASE-calculated activities of the compounds submitted to analysis (compounds **1–48**) are given in Table 1. The structures of the coumarin derivatives used in the analysis are shown in Table 2. A total of 53 compounds were available for CASE analysis. The set included 11 flavonoids (compounds **2–4, 6, 10, 12, 13, 15, 17, 50,** and **51**), 14 coumarin derivatives (compounds **1, 7–9, 11, 16, 19–22, 24, 30, 46,** and **49**), and 11 benzoquinone and hydroquinone derivatives (compounds **34, 36, 37, 40, 42–45, 47, 48,** and **52**). Five compounds were chosen at random and removed from the database submitted for initial analysis. These were to be used later to check the predictive ability of the analysis. The compounds removed from the learning set are listed in Table 3 (compounds **49–53**). Experimental activities are expressed as the concentration in micromolar needed for 50% inhibition (I_{50}) of the enzyme glyoxalase I. The qualitative scale used for the calculated activity is as follows: extremely active, I_{50} below 12 μM; very active, I_{50} from 12 to 35 μM; active, I_{50} from 35 to 100 μM; marginally active, I_{50} from 100 to 300 μM; and inactive, I_{50} above 300 μM.

The analysis requires the coding of all the molecular structures and the recording of their experimental activities.

Of the 48 compounds submitted for analysis, 18 were labeled active, 19 inactive, and 11 marginal. CASE then determined which fragments in these compounds were suitable descriptors. Only the fragments whose distributions were found to have less than 15% probability of being due to chance were kept for analysis. In the QSAR analysis, fragments were selected through a forward stepwise regression analysis and the result is shown in Eq. 2.

$$\log_{10}(1/I_{50}) = -2.615 + 0.887n_I(F_I) + 0.244n_{II}(F_{II}) + 0.235n_{III}(F_{III}) + 0.765n_{IV}(F_{IV}) - 0.162n_V(F_V) + 0.0265 \log^2 P \quad (2)$$

with $r^2 = 0.850$, $s = 0.282$, $n = 48$, and $F_{(6, 41, 0.05)} = 38.63$.

The five fragments selected by the QSAR analysis are shown in Fig. 1. Eq. 2 was used to calculate the activities of the 48 compounds and the results are shown in Table I, *column 3*. With this equation, 16 out of the 18 active compounds and 16 out of the 19 inactive compounds are correctly accounted for. The remaining two active compounds (**14** and **18**) were calculated to be inactive, i.e., false negatives. The incorrect assignment of these two molecules as inactive compounds is due to the fact that most of the active compounds in the database have multiple ring systems whereas these two do not. It is interesting to note, however, that **18** has a fragment similar to **I**, the α-hydroxy-α,β-unsaturated carbonyl group, although the carbonyl carbon is not attached to a fused ring bridging carbon as is the case in the other active molecules in the database. As

TABLE I

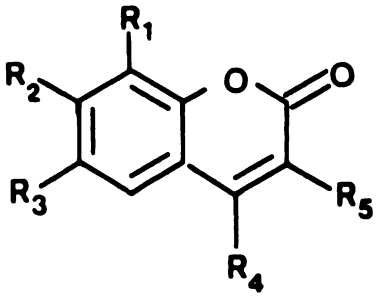
List of compounds submitted for CASE analysis with experimental and calculated activities

++++, extremely active; +++, very active; ++, active; +, marginally active; –, inactive.

No.	Common name[a]	Experimental		Calculated activity[c]
		I_{50}[b]	Activity	
		μM		
1	Coumarin-8	3.5	++++	++++
2	Myricetin	5	++++	++++
3	Quercetin	9	++++	++++
4	3-Hydroxyflavone	9	++++	++++
5	Purpurogallin	9	++++	++++
6	Fisetin	10	++++	++++
7	Coumarin-10	20	++++	+++
8	Coumarin-4	24	++++	++
9	Coumarin-5	30	+++	++
10	Morin	30	+++	+++
11	4-Methylesculetin	50	+++	++
12	Quercitrin	70	++	++
13	Apigenin	70	++	++
14	Pyrogallol	70	++	–
15	Pyrogallol red	70	++	++
16	Coumarin-9	80	++	++
17	Rutin	110	++	+++
18	Squaric acid	120	++	–
19	Isoesculetin	145	+	++
20	Coumarin-1	150	+	++
21	Coumarin-2	200	+	++
22	Esculetin	200	+	+
23	2,3,4-Trihydroxybenzohydroxamic acid	210	+	+
24	Esculin	230	+	+
25	Naringenin	250	+	+
26	Chlorogenic acid	270	+	–
27	Caffeic acid	270	+	–
28	2,3-Dihydroxybenzoic acid	280	+	–
29	D-Catechin	290	+	+
39	Coumarin-3	320	–	++
31	3,4-Dihydroxybenzoic acid	320	–	–
32	Taxifolin	330	–	+
33	3,4-Dihydroxybenzohydroxamic acid	380	–	–
34	2,3-Dimethoxybenzoquinone	390	–	–
35	L-Ascorbate	480	–	–
36	Benzoquinone	480	–	–
37	Methoxybenzoquinone	480	–	–
38	3,4,5-Trihydroxybenzohydroxamic acid	520	–	+
39	2,3-Dihydroxypyridine	530	–	–
40	Tetramethoxybenzoquinone	870	–	–
41	L-Tryptophan	1050	–	–
42	2,5-Dimethoxyhydroquinone	1100	–	–
43	2,6-Dimethoxyhydroquinone	1200	–	–
44	Tetramethoxyhydroquinone	1600	–	–
45	Methoxyhydroquinone	1750	–	–
46	Coumarin	1900	–	–
47	2,3-Dimethoxyhydroquinone	2300	–	–
48	Hydroquinone	4100	–	–

[a] For coumarin derivatives, see Table 2.
[b] Concentration for 50% inhibition.
[c] Using Eq. 2.

TABLE 2
Coumarin derivatives used in analysis



No.	Compound	R_1	R_2	R_3	R_4	R_5
1	Coumarin-8	OH	OH	H	Ph[a]	H
7	Coumarin-10	H	OH	OH	Ph	H
8	Coumarin-4	H	OH	OH	CH_2Ph	H
9	Coumarin-5	H	OH	OH	CH_3	CH_2Ph
11	4-Methylesculetin	H	OH	OH	CH_3	H
16	Coumarin-9	H	OH	OH	$(CH_2)_2CH_3$	H
19	Isoesculetin	H	H	H	OH	OH
20	Coumarin-1	H	CH_3O	CH_3O	Ph	H
21	Coumarin-2	H	CH_3O	OH	Ph	H
22	Esculetin	H	OH	OH	H	H
24	Esculin	H	OH	Glucose	H	H
30	Coumarin-3	H	OH	OH	CH_3	CH_3
46	Coumarin	H	H	H	H	H
49	Coumarin-7	H	OH	OH	4-F-Ph	H

[a] Ph, phenyl.

TABLE 3
Results of qualitative and quantitative predictions of CASE on compounds removed from initial data
For activity scales, see Table 1.

No.	Compound	Experimental[a]		Predicted[b]	Probability[c]
		μM			
49	Coumarin-7	20	++++	+++	98.2%
50	Kaempferol	55	+++	++++	99.9%
51	Myricitrin	185	+	+++	99.8%
52	2,6 Dimethoxybenzoquinone	240	+	–	17.0%
53	Maltol	750	–	–	83.0%

[a] Quantitative activities of I_{50}; see Table I.
[b] From Eq. 2.
[c] Qualitative prediction, probability of being an inhibitor.

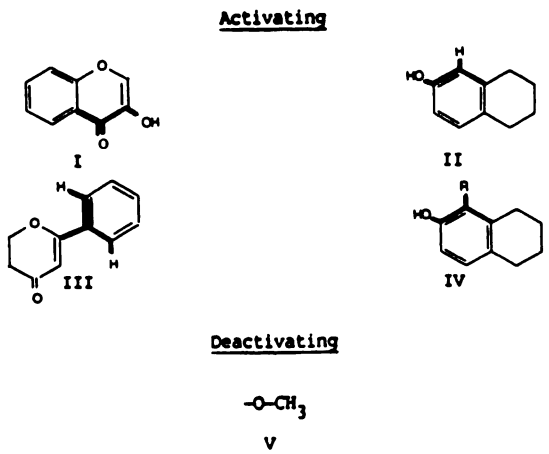


Fig. 1. Activating and deactivating fragments selected for QSAR.

for **14**, there is insufficient information to explain its activity. Of the three inactive compounds one (**30**) is calculated to be active while the other two (**32** and **38**) were recalculated as having marginal activity. The assignment of **30** as active is due to the presence of two fragments of the type **II**, which is associated with activity in at least five other active compounds. Of the 11 marginal compounds, five were correctly assigned, three were incorrectly calculated as active and three as inactive.

Of the fragments selected for QSAR, the most important seems to be fragment **I**, which alone could account for 43% of the data. This particular fragment occurs in six compounds, all of which are active. The emergence of fragment **I** as the most important one can be rationalized by the proposed action of glyoxalase I on its substrate, the glutathione hemimercaptal adduct of methylglyoxal. The transition state of the metabolic reaction is believed to go through an enediol (17–20). The position of fragment **I**, particularly in the flavonoids, makes it particularly facile to tautomerize into a conjugated enolate, which is the polar analog of an enediol (see Fig. 2A). The positive charge forming on the carbon β to the carbonyl will be next to an oxygen atom, the lone pairs of which can stabilize such charge formation. However, this can only be so if there is α-β unsaturation. The importance of this α-β unsaturation can be seen in the case of **32**, which is identical to **3** in all respects except for the missing unsaturation (see Fig. 2B). **3** is extremely active whereas **32** is inactive.

Fragment **III** represents a substitution generally by a phenyl ring at the carbon that bears the positive charge in the tautomer of fragment **I**. Thus, another stabilizing factor for the tautomerization of fragment **I** is the concurrent presence of fragment **III**.

Fragments **II** and **IV** are similar. The only difference is that, in **II**, there is no substituent on the carbon atom next to the bridging carbon. In the four compounds (**1**, **5**, **25**, and **19**) that contain fragment **IV**, this substituent was a hydroxyl group. Both of these fragments are activating. However, it can be noted that the coefficient of fragment **IV** in Eq. 2 is much larger than that of **II**. Thus, although the existence of a hydroxyl group in position β of the naphthalene moiety is sufficient to endow the molecule with some activity, an additional hydroxyl group in the α position greatly enhances this activity. It is observed from the compounds in the database that fragments **II** and **IV** are located far from fragment **I** in the four compounds (**2**, **3**, **6**, and **10**) that contain **I** and either **II** or **IV** (see Fig. 3). Several models can be seen as leading to this observation. One such model is that a two-site interaction is required for the compounds to be active as inhibitors of the

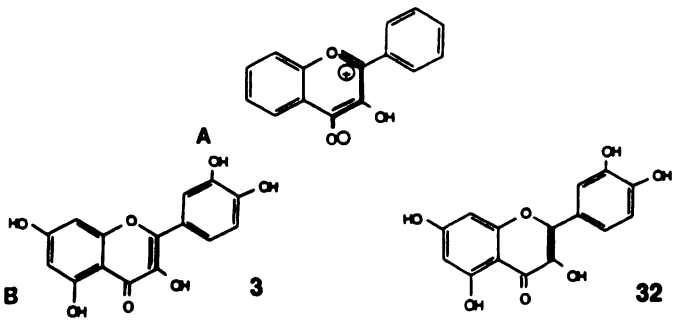


Fig. 2. A, Formation of the polar enediol with fragment **I** in the general flavonoid structure. B, Quercetin (**3**) versus taxifolin (**32**). **3** is extremely active whereas **32** is inactive. Note the lack of α,β-unsaturation in **32**.

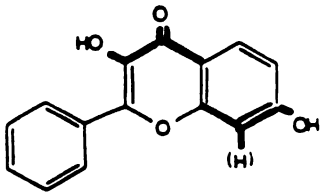


Fig. 3. Relative locations of fragments II or IV, and I, in the general flavonoid structure.

TABLE 4

Results of qualitative and quantitative predictions of CASE on chromones and phenyl-pyrones

For activity scales, see Table 1.

No.	Compound	Predicted[a]	Probability[b]
54	Chromone	–	74.0%
55	2-Methylchromone	–	74.0%
56	3-Hydroxychromone	+++	95.0%
57	3-Hydroxy-2-methylchromone	+++	95.0%
58	2-(4-Hydroxyphenyl)pyrone	++	85.0%
59	2-(3-Hydroxyphenyl)pyrone	++	85.0%
60	3-Hydroxy-2-(4-hydroxy-phenyl)pyrone	+	98.9%
61	3-Hydroxy-2-(3-hydroxy-phenyl)pyrone	+	98.9%

[a] From Eq. 2.
[b] Qualitative prediction, probability of being an inhibitor.

glyoxalase I enzyme. Alternatively, one may invoke a mechanism similar to that used above to link fragments **I** and **III** namely enolization of the keto group across the two rings. In both cases, the hydroxyl group may be involved in some H-bonding, which increases the affinity of the molecule for the active site of the enzyme.

Fragment **V** is the only deactivating group identified by CASE. The selection of this fragment is due to the presence of the methoxy substituent on a number of substituted benzoquinones and hydroquinones, which were generally inactive classes of compounds.

To test the predictive ability of the analysis, the five compounds initially set aside, (**49–53**) were run through CASE in the predictive mode. The compounds were tested for the presence or absence of the activating and inactivating fragments found earlier to be relevant to the qualitative evaluation of activity. If found active, the quantitative activity of the molecule is evaluated from the QSAR equation. Experimentally, we know that compounds **49** and **50** are active, whereas **51** and **52** are marginally active and **53** is inactive. When submitted to evaluation, three of the five molecules were predicted to be active and two inactive. The two active molecules were indeed identified as such with better than 98% certainty. Molecule **51** was also found to be active whereas experimentally it is only found to be marginally active. Both molecules **52** and **53** were correctly calculated to be at best marginally active. Molecule **53** does contain an activating fragment and on this basis is predicted to be active. However, the QSAR equation predicted its activity to be negligible. A summary of these results is given in Table 3.

CASE was also used to evaluate the inhibitory activities of two other structurally related classes of compounds, e.g., phenyl-pyrones and chromones. A total of eight as yet untested structures (compounds **54–61**) were submitted for analysis and the results are shown in Table 4. It was previously found that the presence of fragment **I** in the chromones is generally necessary for activity. Because fragment **I** requires the presence of fused rings, phenyl-pyrones cannot have this functionality. Nonetheless, CASE identified enough structural features on the phenyl-pyrones, e.g., fragment **III**, to predict marginal to moderate activity for this set of compounds. The addition of a hydroxyl substituent α to the carbonyl on the pyrone ring markedly increases the probability of the compound of being an inhibitor. Generally, the fused ring seems to be more important than a phenyl substituent for potent activity. Hence, as a class of compounds, chromones would be more effective than phenyl-pyrones. This suggests that in the flavonoids, the fused ring system plays a more vital role than does the phenyl substituent in the activity of the compound.

Conclusion

CASE was able to identify four structural features in the compounds of the data set that account for the activity of the glyoxalase I enzyme inhibitors. The analysis clearly isolated the most important structural feature, fragment **I**. This was the underlying strategy behind the choice of this particular set of compounds, i.e., to effect enzyme inhibition through the use of transition state analogs.

The successful prediction of the activity of the five compounds initially excluded from the analysis specifically shows that the descriptors selected by the CASE program for this set of compounds are probably related to inhibitory activity of these kinds of molecules. With the knowledge of the important structural features, new molecules can be designed with the specific purpose of obtaining optimal activity.

Acknowledgments

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