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The use of a pharmacophore model for identification of novel ligands for the benzodiazepine binding site of the $GABA_A$ receptor

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Abstract

A Catalyst pharmacophore model has been developed for the benzodiazepine site within the $GABA_A$ receptor complex. The model is based on a pharmacophore model originally proposed by Cook and co-workers (Drug Des. Discovery 1995, 12, 193–248) and further developed by Kahnberg et al. (J. Med. Chem. 2002, 45, 4188–4201). The Catalyst pharmacophore model has been validated by using a series of flavonoids with varying affinities for the benzodiazepine receptor and has then been used as a search query in database searching with the aim of finding novel structures which have the possibility to be modified into novel lead compounds. Five of the hits from the database searching were purchased and their affinities for the benzodiazepine site of the $GABA_A$ receptor were determined. Two of the compounds displayed K_i values below 10 μM. The substance showing highest potency in-vitro displayed an affinity of 121 nM making it an interesting compound for optimization. The false positive compounds (K_i values >10 μM affinities) have been analysed in terms of conformational energy penalties and possibilities for hydrogen bond interactions. The analysis clearly demonstrates the need for post processing of Catalyst hits.

Keywords: Catalyst; $GABA_A$; Database searching; Pharmacophore; Benzodiazepine site; Flavones; Conformational analysis; Hydrogen bonding

1. Introduction

The major inhibitory neurotransmitter in the mammalian central nervous system, γ-aminobutyric acid (GABA), exerts its physiological effects by binding to three major classes of receptors, the $GABA_A$, $GABA_B$ and $GABA_C$ receptors. The $GABA_A$ [1,2] and $GABA_C$ [3] receptors are ligand-gated ion channels, whereas the $GABA_B$ [4] receptor belongs to the G-protein coupled receptor superfamily. In addition to GABA binding sites, the $GABA_A$ receptor complex contains binding sites for compounds that allosterically modify the activity of GABA, such as benzodiazepines, β-carbolines and barbiturates [5–8]. Due to their pharmacological effects (anxiolytic, anticonvulsant, muscle relaxant and sedative-hypnotic) the benzodiazepines are the most important $GABA_A$ receptor modulating drugs in clinical use [9].

A pharmacophore model for the benzodiazepine site of the $GABA_A$ receptor has been developed by Cook and co-workers [10]. In previous studies [11,12] we have validated and further developed this pharmacophore model employing a large number of flavone derivatives not included in the original development of the pharmacophore model. On the basis of the results of these studies and of the refined pharmacophore model, 5′-bromo-2′-hydroxy-6-methylfla-

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vone was designed. Among the flavone derivatives this compound shows the highest binding affinity to the benzodiazepine receptor in-vitro reported so far [12].

In the present investigation we have converted this pharmacophore model into a Catalyst pharmacophore model (hypothesis) [13–17]. This Catalyst pharmacophore model has then been used as a search query for 3D database searching [18–25] in an attempt to find new classes of compounds with affinity for the benzodiazepine site of the $GABA_A$ receptor. Some of the hit-compounds from the database searches have been purchased and tested pharmacologically and the relationships between pharmacological results and the Catalyst mappings of the hits are being discussed.

2. Methods

2.1. Validation of the Catalyst pharmacophore

Thirty-eight flavone derivatives (Table 1) were used to build a database for the validation of the Catalyst pharmacophore. The receptor binding affinities (K_i values) for these compounds range between 2 and 7300 nM (Table 1). The database conformations were generated with the best conformation model generation method. The energy cut-off was set to 20 kcal/mol and the maximum number of conformations was set to 250. Searching the database was performed with the fast flexible search method. The hits from the database searching were saved and the compare/fit method was used to calculate a fit value using the best fit option.

2.2. Pharmacophore mapping and database searching

The Catalyst program version 4.6 was used for mapping pharmacophore features and for database searching using the fast flexible search method [26,13–17]. The Maybridge and ACD databases were used in searching for compounds fitting the pharmacophore model. The hits from the database searching were saved and the compare/fit method was used to calculate a fit value using the best fit option. All features in the pharmacophore have equal weight (set to 1.0). For the pharmacophore with two features for hydrogen bond acceptors, one for hydrogen bond donors and two for hydrophobic groups this gives a maximum fit value of 5. For the pharmacophore with four features the maximum fit value is 4.

2.3. Receptor binding

The determination of binding affinities of hits from the database searching were performed using ^{3}H-Flumazenil (^{3}H-Ro 15-1788) binding to rat cortical membranes in vitro. Clonazepam was used to determine non-specific binding. Tissue preparation, the ^{3}H-Flumazenil binding assay and calculations of IC_{50} and K_i values were performed as previously reported [12].

2.4. Conformational analysis and calculation of conformational energy penalties

Conformational analyses of hits from the database searching were performed using the MMFF94s force field [27–33] and the monte carlo multiple minimum (MCMM) method [34] implemented in the MacroModel program version 7 [35,36]. The calculations were performed for aqueous solution using the Generalized Born/solvent accessible surface area (GB/SA) dielectric continuum solvation model [37] implemented in the MacroModel program. The conformational searches were continued until all low energy minima had been found multiple times. The energy minimizations were carried out employing the truncated newton conjugate gradient (TNCG) algorithm in MacroModel.

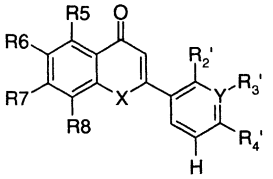
The conformational energy penalty of Catalyst hits, i.e. the energy required for a Catalyst hit to adopt the conformation found in the database searching was calculated by subtracting the internal (steric) energy of the calculated preferred conformation in aqueous solution (the energy of the calculated global energy minimum in aqueous solution excluding the hydration energy) from the calculated internal energy of the conformation of the Catalyst hit [38]. If the Catalyst hit-conformation was not present in the output of the conformational search, the conformation was generated by modifying dihedral angles about rotatable bonds to those displayed by the Catalyst hit, and the energy was then calculated by applying torsional constraints.

3. Results and discussion

3.1. Generation of the Catalyst pharmacophore

The pharmacophore model used as the basis for the development of the Catalyst pharmacophore model (hypothesis) is shown in Fig. 1. The figure shows 6-methylflavone (**11**, Table 1) and the high-affinity 2-aryl-pyrazolo[3,4-c]quinolin-3-one compound CGS-9896 (Scheme 1) fitted to the model [11,12]. The pharmacophore model consists of a hydrogen bond acceptor site (A2), a hydrogen bond donor site (H1), a bifunctional hydrogen bond donor/acceptor site (H2/A3), three lipophilic regions (L1–L3) and five receptor-essential volumes (S1–S5). The template structure shown in Scheme 1 was used for mapping of pharmacophore features in Catalyst. This template structure can be seen as a hybride molecule between the flavonoids (Table 1) [11,12] and CGS-9896 (Fig. 1, Scheme 1). In terms of the pharmacophore model in Fig. 1, the flavone carbonyl group of the template compound interacts with the hydrogen bond donor site H1 and the oxygen atom in position 1 is interacting with the hydrogen bond donor site H2. These groups were both mapped as hydrogen bond acceptors in Catalyst as displayed in Fig. 2. The NH group in the template molecule is interacting with the hydrogen bond acceptor site A2 and was mapped as a hydrogen bond donor. The fused benzene ring

Table 1
Chemical structures of the flavonoids used for optimisation of the Catalyst pharmacophore and the binding affinities



Compound	X	Y	R_2'	R_3'	R_4'	R5	R6	R7	R8	K_i (nM)
1	O	C	H	NO_2	H	NO_2	CH_3	H	H	1.9
2	O	C	H	NO_2	H	H	CH_3	H	H	5.6
3	O	C	H	NO_2	H	H	Br	H	H	16
4	O	C	H	NO_2	H	H	NO_2	H	H	26
5	O	C	H	CH_3	H	H	CH_3	H	H	29
6	S	C	H	NO_2	H	H	CH3	H	H	33
7	O	C	H	H	H	H	Br	H	H	70
8	O	C	H	OCH_3	H	H	CH_3	H	H	100
9	O	N	H	–	H	H	CH_3	H	H	120
10	O	C	H	H	H	H	CH_2CH_3	H	H	120
11	O	C	H	H	H	H	CH_3	H	H	125
12	O	C	H	$CO_2CH(CH_3)_2$	H	H	CH_3	H	H	180
13	O	C	H	OH	H	H	CH_3	H	H	180
14	O	C	H	CO_2CH_3	H	H	CH_3	H	H	240
15	S	C	H	H	H	H	CH_3	H	H	310
16	O	C	H	$CO_2CH_2CH(CH_2C_3)2$	H	H	CH_3	H	H	350
17	O	C	H	H	H	H	$(CH_2)_2CH_3$	H	H	350
18	O	C	H	H	H	H	OH	H	H	400
19	O	C	H	H	H	H	$CH(CH_3)2$	H	H	570
20	O	C	H	H	H	H	OCH_3	H	H	570
21	O	C	H	H	H	H	H	H	CH3	>590
22	O	C	H	$O(CH_2)3CH3$	H	H	CH3	H	H	600
23	O	C	NH_2	H	H	H	CH3	H	H	690
24	O	C	H	$OCH_2CH(CH_2CH_3)2$	H	H	CH3	H	H	690
25	O	C	OCH_3	H	H	H	CH_3	H	H	750
26	O	C	H	H	OH	OH	H	OH	H	770
27	O	C	H	H	H	OH	H	OH	H	920
28	O	C	NO_2	H	H	H	CH_3	H	H	1000
29	O	C	H	NH_2	H	H	CH_3	H	H	1200
30	O	C	H	H	H	BENZO		H	H	1480
31	O	C	H	H	NO_2	H	NO_2	H	H	>1700
32	O	C	H	CH_3	CH_3	H	CH_3	H	H	>1700
33	O	C	H	H	H	OH	H	H	H	1900
34	O	C	H	H	NH_2	H	CH_3	H	H	2600
35	O	C	H	H	H	H	H	H	H	4200
36	O	C	H	H	H	H	H	OH	H	4200
37	O	C	H	H	CH_3	H	CH_3	H	H	4400
38	O	C	H	H	NO_2	H	CH_3	H	H	7300

and the 2-phenyl ring are part of the core structure in the flavones and these ring systems were mapped as hydrophobic features in Catalyst. The hydrogen bond accepting, hydrogen bond donating and the hydrophobic features were all mapped with default tolerance values (1.5 Å). Finally, the shape of the molecule was added to the pharmacophore using the option to convert a molecule to a shape in Catalyst.

In order to allow for substituents in different positions in the flavone structures, a bromo substituent in position 6 (for numbering system see Table 1), a nitro group in position 5 and an isopropyl ester in position 3′ were included in the template structure. A nitro group in position 5 and a bromo substituent in position 6 were chosen because these groups have been found favorable for the binding affinity in the flavonoid series of compounds [11]. An isopropyl ester group was added in position 3′ in order to describe a proposed partly lipophilic channel-like region (Fig. 1) which may exist at the interface between an α- and a γ-subunit in the $GABA_A$ receptor where the benzodiazepine binding site most likely is located [12]. A 3′-isopropyl ester group only slightly decreases the affinity in the flavone series [12]. A tolerance value between 1.0 (max.) and 0.6 (min.) was used for the shape. In order to take the most important of the previously deduced steric constraints of the binding pocket into account (Fig. 1), exclusion spheres were added close to positions 7, 8 and 4′ in the flavone structure and above and

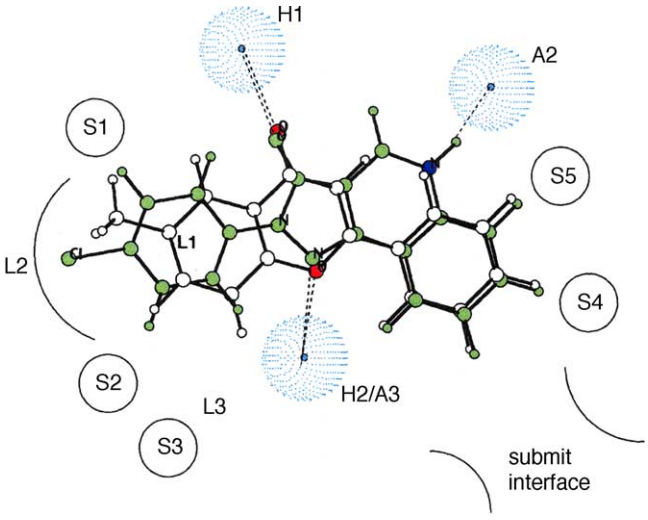
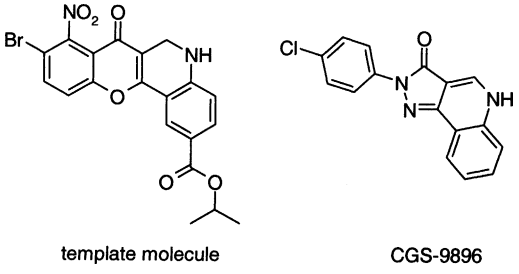


Fig. 1. The pharmacophore model used as the basis for the generation of a Catalyst pharmacophore.



Scheme 1.

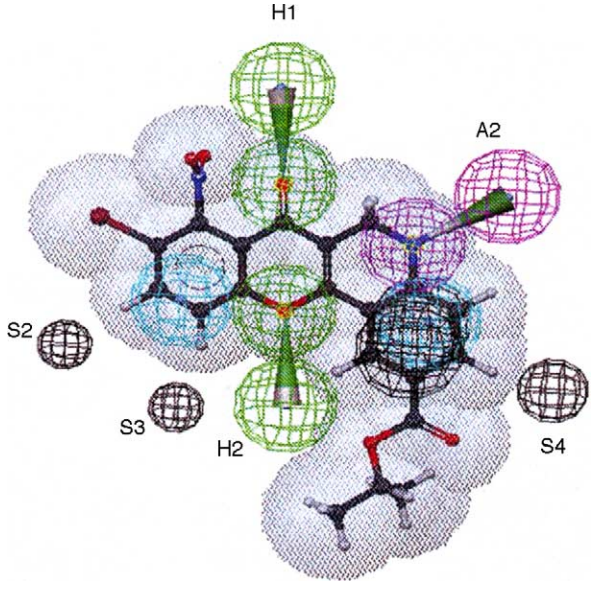


Fig. 2. The template structure mapped to the Catalyst pharmacophore with two hydrogen bond acceptors (green), one hydrogen bond donor (magenta), two hydrophobic features (blue), shape (light grey) and five exclusion spheres (black).

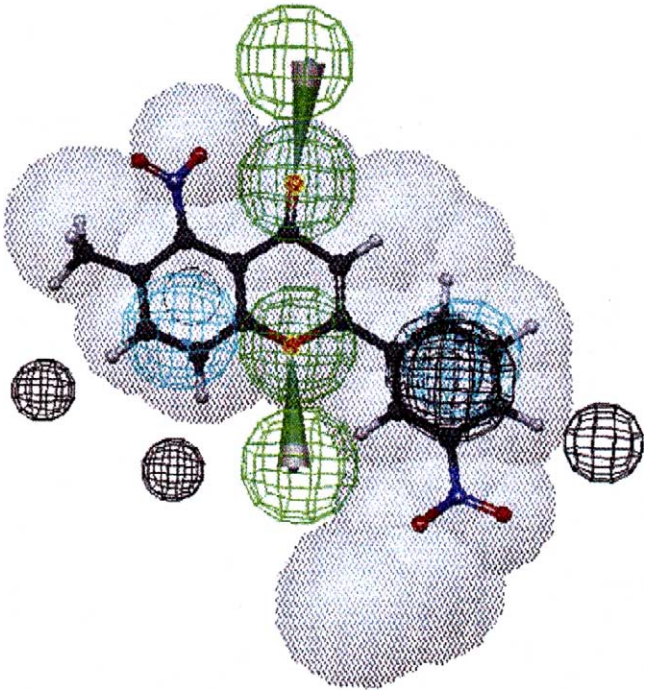


Fig. 3. Fitting of 3′, 5-dinitro-6-methylflavone (1) to the Catalyst pharmacophore with two hydrogen bond acceptors (green), two hydrophobic features (blue), shape (light grey) and five exclusion spheres (black).

below the 2-phenyl ring. The exclusion spheres above and below the phenyl ring both have a radius of 1.5 Å, the sphere at the 4′-position has a radius of 1.0 Å and the two spheres at the 7- and 8-position both have a radius of 0.8 Å. The reason to include exclusion spheres above and below the phenyl ring is that a planar or close-to-planar geometry is required for ligands with potent activity [10]. The optimal position and size of each exclusion sphere were determined as described in the next section. The template structure fitted to the manually developed Catalyst pharmacophore model is shown in Fig. 2.

A Catalyst pharmacophore model excluding the hydrogen bond donating feature was also generated. This pharmacophore model was used to investigate if a search through a database of known flavone derivatives is able to preferentially return the more active compounds as hits, as described in the next section. The hydrogen bond donating feature was removed since none of the flavone derivatives in Table 1 contains a functionality at an appropriate position able to interact as an hydrogen bond donor. A fit of 3′, 5-dinitro-6-methylflavone (**1**, Table 1), a high affinity flavone derivative, to this reduced pharmacophore model is shown in Fig. 3.

3.2. Validation of the generated Catalyst pharmacophore

In order to validate the pharmacophore, a database with the 38 different flavone derivatives shown in Table 1 was generated. Their affinites for the benzodiazepine site of the $GABA_A$ receptors receptor are given in Table 1. The reduced

Table 2
K_i values of flavones used in the validation of the Catalyst pharmacophore and fit values for the hits when searched with the Catalyst pharmacophore

Compound	K_i (nM)[a]	Hits from search 1[b]	Fit value 1[b]	Hits from search 2	Fit value 2[c]
1	1.9	√	3.3	√	2.3
2	5.6	√	2.4	√	2.1
3	16	√	3.9	√	3.9
4	26	√	3.4	√	2.9
5	29	√	2.5	√	1.9
6	33	√	3.1	√	2.9
7	70	√	3.9	√	3.8
8	100	√	2.7	√	2.1
9	120	√	2.8	√	2.0
10	120	√	3.5	√	2.1
11	125	√	3.9	√	3.8
12	180				
13	180	√	2.6	√	1.9
14	240	√	2.4	√	2.2
15	310				
16	350				
17	350				
18	400	√	3.9	√	3.8
19	570				
20	570	√	2.7	√	1.9
21	>590				
22	600	√	3.9	√	3.9
23	690				
24	690				
25	750				
26	770				
27	920				
28	1000				
29	1200	√	2.1	√	1.8
30	1480	√	3.9	√	3.8
31	>1700				
32	>1700	√	1.6		
33	1900	√	3.8	√	3.8
34	2600				
35	4200				
36	4200	√	0.5		
37	4400				
38	7300				
Total hits		21 (55%)[d]		19 (50%)[d]	
Hits with $K_i < 250$ nM		13 (93%)[e]		13 (93%)[e]	
Hits with $K_i \geq 1000$ nM		5 (45%)[e]		3 (27%)[e]	

[a] K_i values from [3H]Flumazenil binding [11,12]
[b] Default tolerance values for the hydrogen bond features (1.5 Å).
[c] Tolerance values for the hydrogen bond features set to 1.0 A.
[d] Percentage of total number of compounds.
[e] Percentage of total hits.

Catalyst pharmacophore without the feature for hydrogen bond interaction with site A2 (Fig. 3) was used to search the database with the flavone derivatives. A few test runs were first performed in order to determine the optimal position and size of each exclusion sphere. The size and position of each sphere were manually modified until an optimal separation between high affinity flavone derivatives ($K_i <$ 250 nM) and low affinity ones ($K_i >$ 1000 nM) was obtained. The final search resulted in 21 hits (Fit Value 1, Table 2). A pharmacophore is only useful as a predictive model if it is able to find the active compounds and as few as possible of the low- or inactive compounds. The database which is used for validation of the pharmacophore contains 14 high affinity compounds with a K_i value less than or equal to 250 nM and 13 of these are found in the database search. The database contains 11 low affinity compounds having a K_i value larger than 1000 nM and five of these are found as hits in the database search. The result from the database search shows a clear enrichment of high affinity compounds amongst the hits. Compound **12** is the only false negative in the database search. This is due to the size of the shape. If the maximum size of the shape is increased from 1.0 to 1.1,

Table 3
Hits of compounds from searching the ACD and Maybridge databases with the Catalyst pharmacophore including two hydrogen bond acceptors, two hydrophobic features, shape and five exclusion spheres (Fit value 1) and the same pharmacophore with more constrained hydrogen bond acceptor features (Fit value 2) K_i values, calculated conformational energy penalties and hydrogen bond qualities are included in the table

Compounds	Hits from search 1	Fit value 1[a]	Hits from search 2	Fit value 2[b]	Conformational energy penalty (kcal/mol)	Hydrogen bond[c]	K_i (nM)
39	√	2.0	√	1.6	0.0	good	121
40	√	3.5	√	2.0	12.0	good	6400
41	√	1.9			1.7	poor	>10,000
42	√	0.7			1.2	poor	>10,000
43	√	3.0			0.0	poor	>10,000

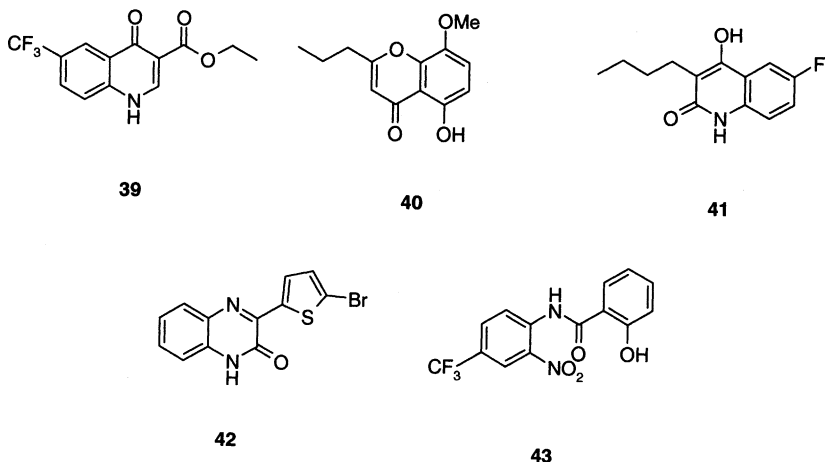
[a] Default tolerance values for the hydrogen bond features (1.5 Å).
[b] Tolerance values for the hydrogen bond features set to 1.0 Å.
[c] Hydrogen bonds are described as poor if the hydrogen bond distances in fitting of the Catalyst hit to the orginal pharmacophore modelo (Fig. 1) are all 1 Å longer or shorter than an ideal hydrogen bond distance, taken to be 2.8 Å between the heavy atoms.

compound **12** comes out as a hit. However, as the number of hits of compounds with a K_i value larger than 1000 nM also increases with this pharmacophore, the pharmacophore with the maximum value of 1.0 for the shape was chosen. Compounds **29**, **30**, **32**, **33** and **36** are false positive hits. Compounds **30** and **33** are hits because there is no exclusion sphere in the Catalyst pharmacophore corresponding to S1 and this allows the compounds to map with the benzo group and the hydroxyl hydrogen atom, respectively, in this area. Compound **29** has a NH2 group in position 3′. The area about the 3′-position is only characterized by a shape. Thus, the pharmacophore cannot take the electronic properties of the 3′-substituent into account. Compounds **32** and **36** have low fit values.

3.3. Searching the Maybridge and ACD databases

The Maybridge and ACD data bases in Catalyst were searched using the Catalyst pharmacophore including the feature for interaction with site A2 as shown in Fig. 2. The search gave 22 hits from the Maybridge database and 76 hits from the ACD database. Among these 98 hits, 5 compounds (**39–43,** Table 3 and Scheme 2) with a significant diversity of the molecular scaffolds were selected. The compounds that were selected were purchased and tested in the brain benzodiazepine receptor binding assay in vitro. The two most potent compounds are compounds **39** and **40** with K_i values of 121 and 6400 nM, respectively (Table 3). Compound **39** is mapped to the Catalyst pharmacophore with the phenyl ring corresponding to the 2-phenyl ring in the flavones and the ethyl group corresponding to the fused benzene ring in the flavones as shown in Fig. 4. The carbonyl group in the six membered ring fits to the hydrogen bond acceptor interacting with site H2 whereas the ester carbonyl group fits to the hydrogen bond acceptor interacting with site H1. The NH group fits to the hydrogen bond donor interacting with site A2. The high affinity of compound **39** makes it an interesting novel lead compound. To our knowledge the class of compounds represented by **39** has not previously been tested for affinity for the benzodiazepine binding site of the $GABA_A$ receptor.

Compound **40** is mapped to the pharmacophore with the phenyl ring close to the 2-phenyl ring in the flavones and the propyl group corresponding to the fused benzene ring in the flavones, Fig. 5. The carbonyl group fits to the hydrogen bond acceptor interacting with site H1, the ether oxygen



Scheme 2.

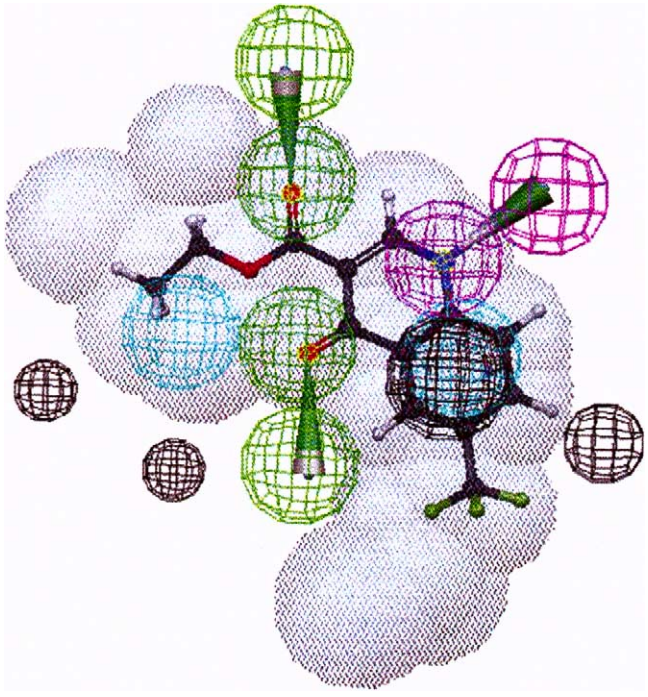


Fig. 4. Compound **39** mapped to the Catalyst pharmacophore with two hydrogen bond acceptors (green), one hydrogen bond donor (magenta), two hydrophobic features (blue), shape (light grey) and five exclusion spheres (black).

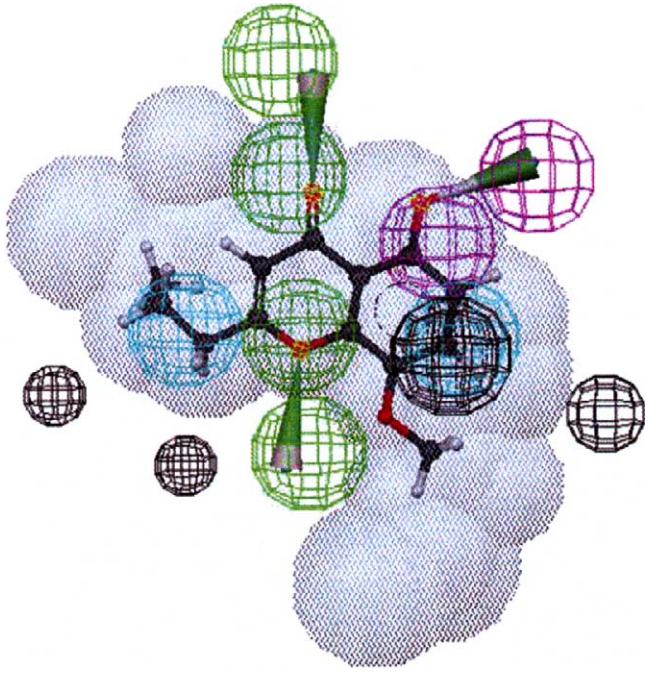
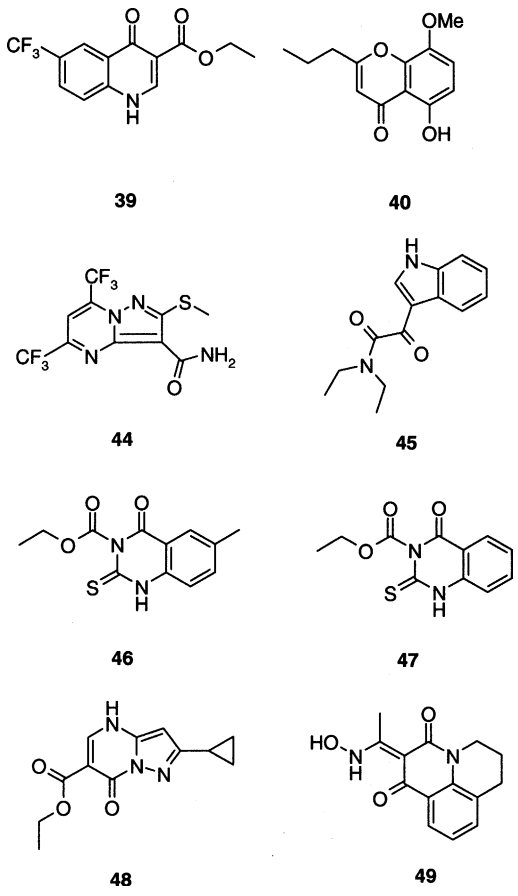


Fig. 5. Compound **40** mapped to the Catalyst pharmacophore with two hydrogen bond acceptors (green), one hydrogen bond donor (magenta) and two hydrophobic features (blue), shape (light grey) and five exclusion spheres (black).

atom fits to the hydrogen bond acceptor interacting with site H2 and the hydroxyl group fits to the hydrogen bond donor interacting with site A2.

3.4. Analysis of the database hits

In order to analyse the selected database hits, a conformational analysis of each of the compounds was performed as described in the Section 2. The compounds were fitted to the original pharmacophore (Fig. 1) and the conformational energy penalty of the conformation obtained in the database search was calculated as described in the Section 2. The results of these calculations are shown in Table 3. Compound **39**, which is the most active compound, and compound **43** fit the pharmacophore in their calculated global energy minimum and compounds **41** and **42** both display small conformational energy penalties. In contrast, compound **40** fits to the pharmacophore in a high energy conformation The hydroxyl group in compound **40** fits as a hydrogen bond donor interacting with site A2 (Fig. 5). In its calculated global energy conformation the hydroxyl group displays an intramolecular hydrogen bond to the carbonyl group. In order to interact as a hydrogen bond donor with site A2, the internal hydrogen bond between the hydroxyl group and the carbonyl group must be broken resulting in a



Scheme 3.

high conformational energy. Thus, the energy gained by interacting with pharmacophore sites H1 and A2 (Fig. 1) are counteracted by the energy increase due to the breaking of a strong intramolecular hydrogen bond. The exact molecular nature of sites H1 and A2 are unknown, but most likely the net free energy of binding of 40 due to interactions with H1 and A2 is very low, rationalizing its low affinity. It is clear that the energetics of the conformational properties of 40 is not properly taken into account by Catalyst.

Analyses of the hydrogen bonding for compounds **39**–**43** by fitting the Catalyst hits to the original pharmacophore model show that compounds **41**–**43** all display very long or very short hydrogen bonds to the pharmacophore sites H1, A2 and H2 (Fig. 1). The hydrogen bond distances for these molecules are all more than 1 Å longer or shorter than the normal hydrogen bond distance displayed by the template structure (Scheme 1), ca. 2.8 Å. These abnormal hydrogen bond distances are most likely the reason for the low affinity of the compounds.

Compound **39** which has a reasonable fit value, a low conformational energy penalty and display reasonable hydrogen bond lengths is found to be the most active compound (Table 3). In the set of five purchased and tested compounds only compound **39** satisfies these conditions. Based on these observations, the tolerance values for the hydrogen bond features were changed from the default value of 1.5 Å to 1.0 Å and the database with the flavones was researched. The results are shown as fit value 2 in Table 2. It can be seen in Table 2, that the search with a more constrained pharmacophore does not significantly change the number of hits.

Compounds **39**–**43** which were purchased and pharmacologically tested were then searched using the more constrained Catalyst pharmacophore. The only compounds which came out as hits from this search were compounds **39** and **40**, which are the two substances with the highest affinities. The search with the more constrained pharmacophore shows that the hits with bad hydrogen bond interactions can be filtered from with this pharmacophore. If the search in ACD and Maybridge is repeated with the more constrained pharmacophore the result is 8 hits (Scheme 3) and compound **39** and **40** are among these. Compound **45** belongs to a class of compounds which have affinity to the benzodiazepine binding site [39–41]. Compounds **44**, **46**–**49** could not be purchased. This shows that the majority of the hits have bad hydrogen bond interactions. By changing the tolerance level for hydrogen bond, compounds with good or bad hydrogen bond interactions can be selected.

4. Conclusion

A manually developed Catalyst pharmacophore has been validated with a series of flavone derivatives with known affinities for the benzodiazepine site of the $GABA_A$ receptor complex. The validated pharmacophore has been used to search commercially available databases (Maybridge and ACD) and virtual hits have been identified. From the virtual hits, five compounds were purchased and tested for affinity for the benzodiazepine site of the $GABA_A$ receptor. The validity of the pharmacophore has been confirmed by identification of active compounds with a core structure different from the compounds used to generate the pharmacophore. The highest affinity compound displays a K_i value of 121 nM. To the best of our knowledge the class of compounds represented by this compound has not previously been tested for affinity for the benzodiazepine site of the $GABA_A$ receptor. This finding makes the compound an interesting lead for further optimization. The importance of post-processing of database hits to identify compounds, which require high conformational energies in order to fit to the pharmacophore and therefore most likely will display low affinities has been shown. Post-processing of Catalyst hits in terms of hydrogen bond distances to hydrogen bonding pharmacophore elements in the search query is shown to be an efficient way of pruning the hit list.

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References

[1] L.E. Rabow, S.H. Russek, D.H. Farb, From ion currents to genomic analysis: recent advances in $GABA_A$ receptor research, Synapse 21 (1995) 189–274.
[2] R.L. Macdonald, R.W. Olsen, $GABA_A$ receptor channels, Annu. Rev. Neurosci. 17 (1994) 569–602.
[3] G.A.R. Johnston, $GABA_C$ receptors: relatively simple transmitter-gated ion channels, TiPS 17 (1996) 319–323.
[4] K. Kaupmann, K. Huggel, J. Heid, P.J. Flor, S. Bischoff, S.J. Mickel, G. McMaster, C. Angst, H. Bittiger, W. Froestl, B. Bettler, Expression cloning of $GABA_B$ receptors uncovers similarity to metabotropic glutamate receptors, Nature 386 (1997) 239–246.
[5] P.J. Whiting, R.M. McKernan, K.A. Wafford, Structure and pharmacology of vertebrate $GABA_A$ receptor subtypes, 38th ed. Academic Press, New York, 1995.
[6] W. Sieghart, Structure and pharmacology of γ-aminobutyric $acid_A$ receptor subtypes, Pharmacol. Rev. 47 (1995) 181–234.
[7] C.R. Gardner, W.R. Tully, J.R. Hedgecock, The rapidly expanding range of neuronal benzodiazepine ligands, Prog. Neurobiol. 40 (1993) 1–61.
[8] J.H.M. Medina, H. Violoa, C. Wolfman, M. Marder, C. Wasowskj, D. Calvo, A.C. Paladini, Neuroactive flavonoids; new ligands for the benzodiazepine receptors, Phytomedicine. 5 (1998) 235–243.
[9] A. Doble, I.L. Martin, The $GABA_A$/benzodiazepine receptor as a target for psychoactive drugs, Springer-Verlag, Heidelberg, Germany, 1996.
[10] W. Zhang, K.F. Koehler, P. Zhang, J.M. Cook, Development of a comprehensive pharmacophore model for the benzodiazepine receptor, Drug Des. Discovery 12 (1995) 193–248.
[11] K. Dekermendjian, P. Kahnberg, M.-R. Witt, O. Sterner, M. Nielsen, T. Liljefors, Structure-activity relationships and molecular modeling

analysis of flavonoids binding to the benzodiazepine site of the rat brain $GABA_A$ receptor complex, J. Med. Chem. 42 (1999) 4343–4350.

[12] P. Kahnberg, E. Lager, C. Rosenberg, J. Schougaard, L. Camet, O. Sterner, E.Ø. Nielsen, M. Nielsen, T. Liljefors, Refinement and evaluation of a pharmacophore model for flavone derivatives binding to the benzodiazepine site of the $GABA_A$ receptor, J. Med. Chem. 45 (2002) 4188–4201.

[13] J. Green, S. Kahn, H. Savoj, P. Sprague, S. Teig, Chemical function queries for 3D database search, J. Chem. Inf. Comput. Sci. 34 (1994) 1297–1308.

[14] A. Smellie, S.D. Kahn, S. Teig, Analysis of conformational coverage. 1. Validation and estimation of coverage, J. Chem. Inf. Comput. Sci. 35 (1995) 285–294.

[15] A. Smellie, S.D. Kahn, S. Teig, An analysis of conformational coverage 2. Applications of conformational models, J. Chem. Inf. Comput. Sci. 35 (1995) 295–304.

[16] A. Smellie, S.L. Teig, P. Towbin, Poling: promoting conformational variation, J. Comp. Chem. 16 (1995) 171–187.

[17] M. Hahn, Three-dimensional shape-based searching of conformationally flexible compounds, J. Chem. Inf. Comput. Sci. 37 (1997) 80–86.

[18] P.W. Sprague, Automated Chemical Hypothesis Generation and Database Searching with Catalyst, in: K. Miiller (Ed.), Perspectives in Drug Discovery and Design, vol. 3, ESCOM Science Publishers B. V, 1995, pp. 1–20.

[19] Y. Kurogi, O.F. Güner, Pharmacophore modeling and three-dimensional database searching for drug design using Catalyst, Curr. Med. Chem. 8 (2001) 1035–1055.

[20] M. Grigorov, J. Weber, J.M.J. Tronchet, C.W. Jefford, W.K. Milhous, D. Maric, A QSAR study of the antimalarial activity of some synthetic 1,2,4-Trioxanes, J. Chem. Inf. Comput. Sci. 37 (1997) 124–130.

[21] E.A. Hecker, C. Duraiswami, T.A. Andrea, D.J. Diller, Use of Catalyst pharmacophore models for screening of large combinatorial libraries, J. Chem. Inf. Comput. Sci. 42 (2002) 1204–1211.

[22] G.C. Chen, C.-S. Chang, W.M. Kan, C.-L. Chang, K.C. Wang, J.-W. Chern, Novel lead generation through hypothetical pharmacophore three-dimensional database searching: discovery of isoflavanoids as nonsteriodal inhibitors of rat 5α-Reductase, J. Med. Chem. 44 (2001) 3759–3763.

[23] Y. Koide, T. Hasegawa, A. Takahashi, A. Endo, N. Mochizuki, M. Nakagawa, A. Nishida, Development of novel EDG3 antagonists using a 3D database search and their structure-activity relationships, J. Med. Chem. 45 (2002) 4629–4638.

[24] R. Griffith, R. Chanphen, S.P. Leach, P.A. Keller, New anti-malarial compounds from database searching, Bioorg. Med. Chem. Lett. 12 (2002) 539–542.

[25] Y. Kurogi, K. Miyata, T. Okamura, K. Hashimoto, K. Tsutsumi, M. Nasu, M. Moriyasu, Discovery of novel mesangial cell proliferation inhibitors using a three-dimensional database searching method, J. Med. Chem. 44 (2001) 2304–2307.

[26] Catalyst, version 4.6; Accelrys Inc., 9685 Scranton Road, San Diego, CA 92121, www.accelrys.com.

[27] T.A. Halgren, Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94, J. Comput. Chem. 17 (1996) 490–519.

[28] T.A. Halgren, Merck molecular force field. II. MMFF94 van der waals and electrostatic parameters for intermolecular interactions, J. Comput. Chem. 17 (1996) 520–552.

[29] T.A. Halgren, Merck molecular force field. III. Molecular geometries and vibrational frequencies for MMFF94, J. Comput. Chem. 17 (1996) 553–586.

[30] T.A. Halgren, R.B. Nachbar, Merck molecular force field. IV. Conformational energies and geometries for MMFF94, J. Comput. Chem. 17 (1996) 587–615.

[31] T.A. Halgren, Merck molecular force field. V. Extension of MMFF94 using experimental data, additional computational data, and empirical rules, J. Comput. Chem. 17 (1996) 616–641.

[32] T.A. Halgren, MMFF VI. MMFF94s option for energy minimization studies, J. Comput. Chem. 20 (1999) 720–729.

[33] T.A. Halgren, MMFF VII. Characterization of MMFF94, MMFF94s, and other widely available force fields for conformational energies and for intermolecular–interaction energies and geometries, J. Comput. Chem. 20 (1999) 730–748.

[34] G. Chang, W.C. Guida, W.C. Still, An internal coordinate Monte Carlo method for searching conformational space, J. Am. Chem. Soc. 111 (1989) 4379–4386.

[35] Schrödinger Inc. www.schrodinger.com

[36] F. Mohamadi, N.G.J. Richards, W.C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W.C. Still, MacroModel—An integrated software system for modeling organic and bioorganic molecules using molecular mechanics, J. Comp. Chem. 11 (1990) 440–467.

[37] W.C. Still, A. Tempczyk, R.C. Hawley, T. Hendrickson, Semianalytical treatment of solvation for molecular mechanics and dynamics, J. Am. Chem. Soc. 112 (1990) 6127–6129.

[38] J. Boström, P.-O. Norrby, T. Liljefors, Conformational energy penalties of protein-bound ligands, J. Computer-Aided Mol. Design. 12 (1998) 383–396.

[39] A.D. Settimo, G. Primofiore, F.D. Settimo, A.M. Marini, E. Novellino, G. Greco, C. Martini, G. Giannaccini, A. Lucacchini, Synthesis, structure-activity relationships, and molecular modeling studies of *N*-(Indol-3-ylglyoxylyl)benzylamine derivatives acting at the benzodiazepine receptor, J. Med. Chem. 39 (1996) 5083–5091.

[40] A.D. Settimo, G. Primofiore, F.D. Settimo, A.M. Marini, E. Novellino, G. Greco, M. Gesi, C. Martini, G. Giannaccini, A. Lucacchini, *N′*-Phenylindol-3-ylglyoxylohydrazide derivatives: synthesis, structure-activity relationships, molecular modeling studies, and pharmacological action on brain benzodiazepine receptors, J. Med. Chem. 41 (1998) 3821–3830.

[41] G. Primofiore, F.D. Settimo, S. Taliani, A.M. Marini, E. Novellino, G. Greco, A. Lavecchia, F. Besnard, L. Trincavelli, B. Costa, C. Martini, Novel *N*-(Arylalkyl)indol-3-ylglyoxylylamides targeted as ligands of the benzodiazepine receptor: synthesis, biological evaluation, and molecular modeling analysis of the structure-activity relationships, J. Med. Chem. 44 (2001) 2286–2297.