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Molecular modeling and QSAR studies on K_{ATP} channel openers of the benzopyran type

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Abstract

The present paper describes our molecular modeling and quantitative structure–activity relationships (QSARs) studies on K_{ATP} channel openers (KCOs) of the benzopyran type. In the first part we performed molecular modeling investigations with seven benzopyrans, varied at the C3- and C4-positions, in order to understand which molecular features at these positions are essentially effecting the biological activity. The impact of C6-substitution on biological activity was studied in the second part via HANSCH analysis. For this purpose physicochemical properties (charge distributions, lowest unoccupied molecular orbital (LUMO) energies, desolvation energies, volumes and dipole moments) were calculated for a set of 50 C6-varied benzopyrans. A QSAR equation was developed showing a relationship between the vasodilator activity and the direction of the dipole vector of the ligands. The conclusion can be drawn that a direct interaction between the C6-substituents and the receptor structure is not of primary importance. However, the substitutents influence the orientation of the whole ligand approaching the binding site. An unfavorably oriented ligand cannot bind to the binding site, thus exhibiting weak activity.

Keywords: K_{ATP} channels; K_{ATP} channel openers; Benzopyrans; Molecular modeling; QSAR

1. Introduction

ATP sensitive potassium channels [1,2], termed K_{ATP} channels, link the electrical activity of cell membranes to cellular metabolism. These channels are heteromultimers of a sulfonylurea receptor (SUR) protein and an inwardly rectifying potassium channel subunit (K_{ir}). The K_{ir} subunit forms the channel pore, whereas the SUR is required for activation and regulation. K_{ATP} channels exist in a variety of tissues including e.g. cardiomyocytes [3], β-cells of the pancreas [4], smooth muscle cells [5] and neurons [6]. Correspondingly, they exhibit a wide variety of physiological functions like the regulation of insulin secretion [7], the control of vessel tone [8] or the release of neurotransmitters [9].

The pharmacological modulation of K_{ATP} channels forms the basis of therapeutic approaches to various diseases of pancreas, cardiac and smooth muscles and of neuronal tissues. The putative therapeutic uses of K_{ATP} channel openers (KCOs) include hypertension, asthma, urinary incontinence or cardiac ischemia, to mention only a few [10]. However, due to the ubiquitous distribution of K_{ATP} channels, the development of novel KCO should be linked to the expression of a high selectivity for a particular channel subtype located on a specific target tissue. Currently available KCO are chemically rather heterogeneous and can be subdivided into a steadily increasing number of structural classes [11]. Most important in this context are the benzopyrans, the cyanoguanidines and the thioformamides, of which the benzopyrans have been most intensively studied. Since the discovery of cromakalim [12] and the elucidation of its mode of action in 1986, a great many benzopyran derivatives have been synthesized and their biological activities have been tested in a variety of biological systems. The analysis of structure–activity relationships has revealed that variations at the C3-, C4- and the C6-position of the benzopyran nucleus are particularly efficacious for modulating the biological activity.

In the first part of the present study we performed molecular modeling investigations with a set of seven benzopyrans in order to understand which pharmacophoric features are essential at the C3- and C4-position. In the second part, we calculated physicochemical properties for a set of 49

Abbreviations: KCOs, K_{ATP} channel openers; MEP, molecular electrostatic potential; SUR, sulfonylurea receptor; QSARs, quantitative structure–activity relationships

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6-varied benzopyrans in order to derive QSAR equations elucidating the impact of C6-substitution on biological activity.

2. Methods

2.1. Test molecules

The seven benzopyrans with variations at the C3- and C4-position are given in Fig. 1A. Their synthesis and pharmacological properties can be found in the literature [12–19]. Syntheses and biological activities of the 50 C6-varied benzopyran derivatives have been described by our group [20–23]. The compounds can be divided into six chemical classes as depicted in Fig. 1B. Details of the substitution pattern are given in Table 1.

2.2. Molecular modeling studies and QSAR analysis

The molecular modeling studies were carried out on Silicon Graphics Indigo2 workstations, using SYBYL6.5 [24]. The conformational behavior of all compounds was

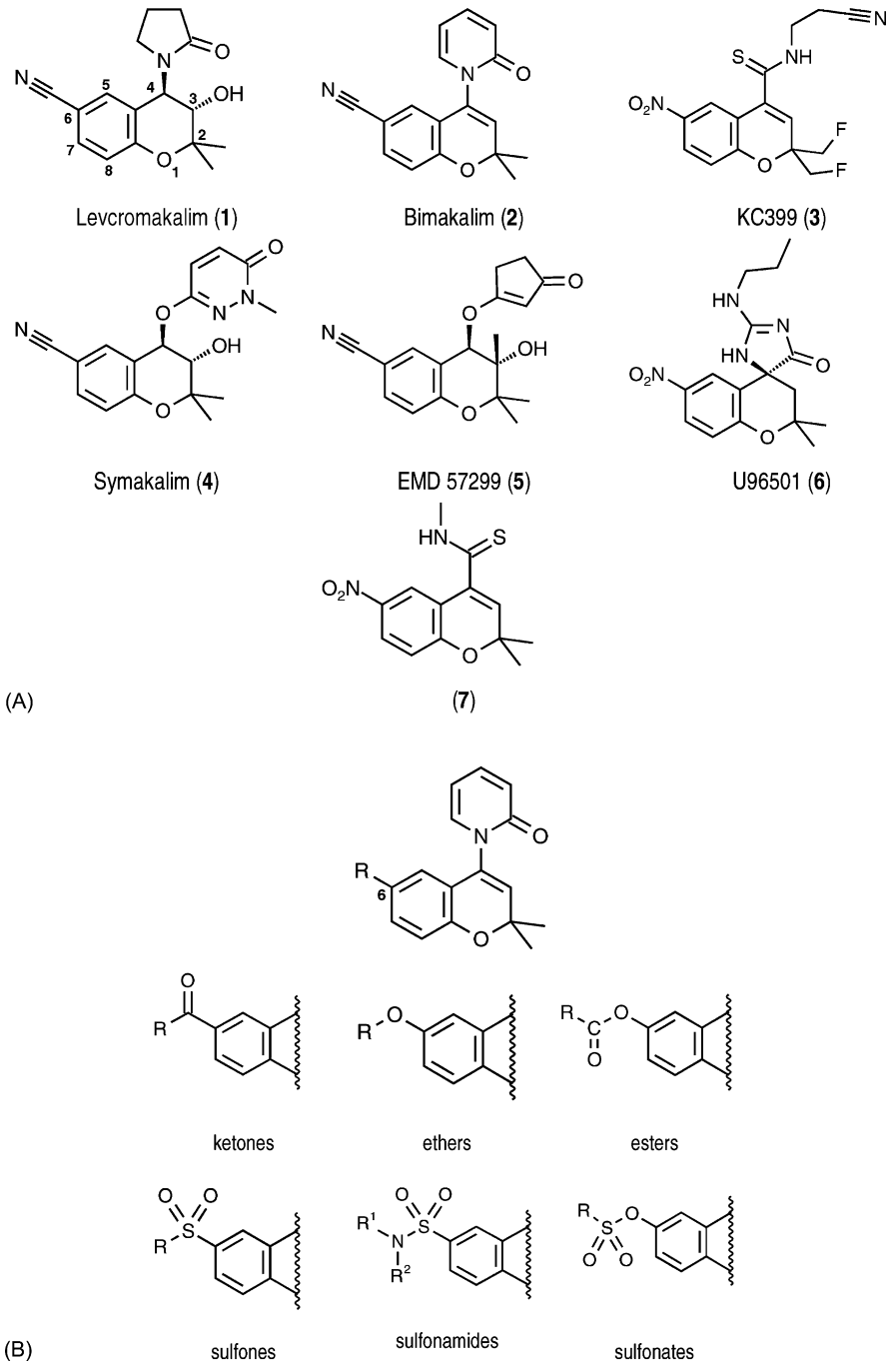
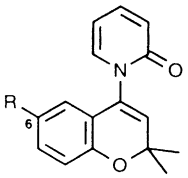


Fig. 1. (A) Benzopyran derivatives with varied C3- and C4-substitution. (B) Classification of the 6-varied bimakalim derivatives.

Table 1
Substitution pattern, vasodilator potency and chemical properties of 6-varied benzopyrans



	R	pEC_{50} aorta	LUMO (benz)[a]	Desolvation[b]	Dipole (total) [c]	Dipole (X-component)[d]	Dipole (Y-component)[d]	Dipole (Z-component)[d]	Volume[e]
AE9	H	5.43	−0.2568	−9.21	3.63	−0.0398	0.2393	0.9701	2.5
AE11	CO–CH_3	7.37	−0.4871	−12.73	4.20	0.4772	−0.2864	0.8308	35
AE15	CO–C_2H_5	6.63	−0.4817	−12.31	4.12	0.4601	−0.2668	0.8468	51.9
AE18	CO–C_6H_{11}	6.33	−0.4806	−11.82	4.10	0.4600	−0.2742	0.8442	105.9
AE20	CO-Ph	6.61	−0.5287	−12.91	4.94	0.2602	−0.2919	0.9204	82.4
AE21	CO–pOH-Ph	6.65	−0.5253	−16.19	3.70	0.0518	−0.2362	0.9703	89.1
AE22	CO-3-furyl	6.49	−0.4898	−16.44	5.26	0.4362	−0.1906	0.8794	65.7
AE23	CO-3-thienyl	6.40	−0.5085	−13.26	5.02	0.3482	−0.2359	0.9072	74.2
AE25	CO–pOCH_3-Ph	6.15	−0.4837	−14.90	4.07	−0.0979	−0.3548	0.9298	104.7
AE26	CO–pNO_2-Ph	6.20	−0.7516	−17.27	7.00	0.7902	0.4526	0.4133	99.7
AE29	CO–oF-Ph	7.08	−0.5652	−13.68	6.38	0.3072	−0.3643	0.8791	85.2
AE30	CO–oNO_2-Ph	6.17	−0.6031	−19.25	8.18	0.0644	−0.3382	0.9389	101.2
AE33	CO–oCH_3-Ph	6.83	−0.4890	−12.08	4.32	0.3381	−0.2878	0.8960	97.5
AE34	CO–oCF_3-Ph	6.76	−0.6724	−15.81	7.72	0.3327	−0.3289	0.8838	106.1
AE44	CHO	6.91	−0.5408	−12.51	4.60	0.6267	−0.1280	0.7687	18.1
AE45	C(=NOH)-NH_2	5.99	−0.4018	−15.58	3.50	0.1975	0.8103	0.5518	42.3
AE47	CH=$C(CN)_2$	6.95	−1.2262	−13.45	9.45	0.7394	0.5707	0.3571	57.4
AE48	N-2,5-$(CH_3)_2$-pyrrolyl	7.27	−0.4757	−9.46	4.50	0.5564	0.3182	0.7676	86.6
AE49	CO–o,o′-F-Ph	6.97	−0.6219	−14.37	4.79	0.1776	−0.5927	0.7856	87.7
Bimakalim	CN	7.67	−0.6119	−11.58	6.37	0.7841	0.2602	0.5635	16.9
EMD 53704	Pyridyl	6.68	−0.4415	−12.90	4.68	0.6269	0.2489	0.7383	62.8
EMD 55491	Br	7.84	−0.4785	−8.99	4.17	0.4587	0.2850	0.8417	19.2
EMD 60893	CF_3	7.61	−0.6183	−10.82	5.34	0.7158	0.2957	0.6326	25.9
EMD 54208	CS–NH_2	6.21	−0.7916	−14.91	6.71	0.3524	−0.3523	0.8670	40.6
EMD 67618	OH	5.44	−0.3632	−12.04	3.35	−0.0233	−0.2264	0.9738	9.7
EMD 67617	OCH_3	6.65	−0.3010	−11.00	3.56	0.3064	−0.0939	0.9472	25.3
CW46	OCONH-Ph	5.30	−0.6789	−15.71	5.49	−0.2880	0.1125	0.9510	102.9
CW47	OCH_2-Ph	4.73	−0.6261	−12.72	4.64	−0.0899	0.3733	0.9233	90.9
CW67	OCO-Ph	5.20	−0.6454	−13.82	5.42	−0.4081	0.1893	0.8931	90.8
CW75	OCO–CH_3	5.60	−0.3809	−13.96	5.17	−0.0865	0.1938	0.9777	42
CW78	OCH_2–o,o′F-Ph	5.27	−0.5899	−13.35	6.77	−0.0306	0.2677	0.9630	96.3
CW80	OCH_2–CO-Ph	5.05	−0.6451	−13.71	5.74	0.0491	−0.5822	0.8115	107.7
CW100	OSO_3^-	4.82	1.8764	−73.82	17.38	0.9663	0.2535	0.0454	34.7
CW104	OSO_2F	7.95	−0.6777	−19.20	4.66	0.7778	0.4895	0.3942	38.8
CW105	OSO_2CF_3	6.53	−0.8078	−16.59	5.46	0.6955	0.6174	0.3675	60.2
CW106	OSO_2Ph	7.63	−0.5586	−18.00	8.35	0.3894	−0.3699	0.8435	102.2
CW107	OSO_2CH_3	6.60	−0.6313	−17.10	6.88	0.4942	−0.3433	0.7987	53.4
CW110	OSO_2NH_2	<4.50	−0.6933	−16.56	7.70	0.4554	−0.3230	0.8296	80.5
CW111	OSO_2Cl	7.09	−0.7791	−16.21	7.42	0.7318	0.4110	0.5436	48.8
CW169	OCH_2SO_2Ph	7.42	−0.7165	−21.66	9.30	0.3510	−0.0628	0.9342	118.8
ES40	SO_2NH–C_2H_5	7.25	−0.5440	−17.61	8.40	0.4403	0.1038	0.8918	75.2
ES41	SO_2NH–CH_2-Ph	6.28	−0.6392	−17.89	8.61	0.3972	0.1160	0.9104	121.8
ES42	SO_2NH_2	7.16	−0.5600	−19.15	8.11	0.4404	0.0962	0.8927	42.6
ES43	SO_2N–$(CH_3)_2$	7.79	−0.5387	−16.94	8.18	0.4196	0.0756	0.9046	75.3
ES44	SO_2NH-Ph	8.51	−0.7399	−18.29	8.59	0.5074	0.1503	0.8485	108.5
ES45	SO_2N–$(CH_2CH_2)_2$–O	7.07	−0.7477	−18.17	8.58	0.5991	0.2151	0.7712	104.5
ES51	SO_2NH–$CH(CH_3)_2$	6.17	−0.6335	−17.61	8.68	0.4233	0.0849	0.9020	91.1
ES62	SO_2NH–CH_3	7.75	−0.5509	−17.76	8.34	0.4396	0.1128	0.8911	58.9
ES64	SO_2-Ph	7.76	−0.8105	−18.46	9.47	0.4390	0.1711	0.8821	95.4
ES70	SO_2F	8.32	−0.7930	−19.44	7.89	0.7768	0.2418	0.5815	33.4
ES71	SO_2N_3	7.79	−0.6418	−16.90	6.96	0.5153	0.3215	0.7944	52.3

The pEC_{50}-values represent the drug concentration, at which precontracted rat aortic rings are half-maximally relaxed.

[a] Energy of molecular orbital (eV).

[b] Desolvation energy (kcal/mol).

[c] Absolute value of the dipole moment (Debye).

[d] Components of the standardized dipole vector.

[e] Volume of the C6-substituent ($Å^3$).

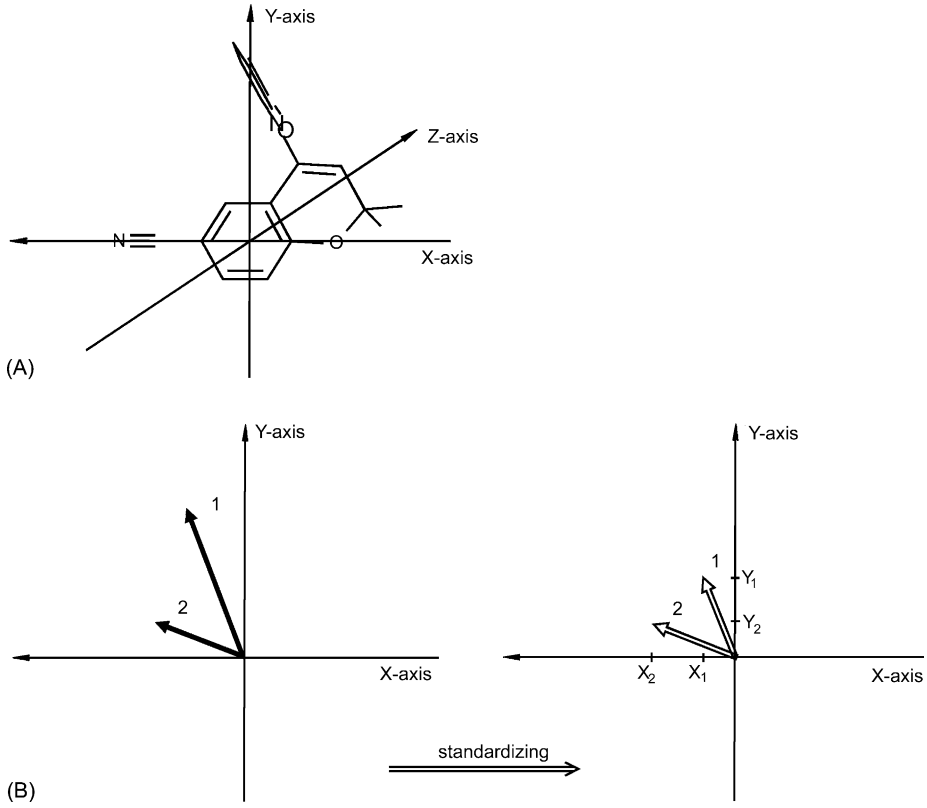


Fig. 2. (A) Positioning of the chromene core of all derivatives in the coordinate system, exemplified for bimakalim. (B) Standardization of the dipole vector to a uniform value. In the right hand scheme both vectors have the same magnitude, but still carry the information about direction.

investigated using molecular mechanics. In general the geometry optimizations were done in vacuo, neglecting the electrostatics inside the Tripos force field [25]. The structures were minimized with the conjugate gradient method until the gradient is <0.01 kcal/mol Å. In the special case of levcromakalim we confirmed its conformation with the MM2* force field as it is included in MacroModel [26], using the implemented solvation model (GB/SA) for water. Quantum mechanical methods were used in order to yield an accurate picture of the charge distributions of all investigated compounds. The wavefunctions were calculated using a HF/3-21G* basis set using the ab initio programme SPARTAN 5.0 [27]. For deriving quantitative structure–activity relationships (QSARs) we calculated desolvation energies, charge distributions, dipole moments and lowest unoccupied molecular orbital (LUMO) energies of C6-varied benzopyrans. The desolvation energies were generated applying a semi-analytical approach, which is implemented in the programme PrGen2.0 [28]. In order to calculate the components of the dipole vector we imported all compounds with their molecular electrostatic potential (MEP) derived point charges into SYBYL. The structures were positioned in the coordinate system, as shown for bimakalim in Fig. 2A. Once the magnitude of the dipole moment was calculated, it was normalized to 1, so that its components, which define the direction of the vector in space, only convey the information about the direction of the vector, but not its magnitude. The scheme in Fig. 2B explains this procedure on the basis of a two-dimensional vector. LUMO energies should be calculated with semi-empirical methods because the energies of virtual molecular orbitals are not well reproduced by Hartree–Fock methods [29]. Thus, the LUMO energies were calculated by the AM1 method, which is also accessible through the SPARTAN software [27]. The above described chemical descriptors of the C6-varied benzopyrans were used in Hansch-analyses for correlations with their dilator potency in rat aorta as biological parameter. The multiple linear regression anaysis program BILIN [30] was used for this purpose.

3. Results and discussion

3.1. Pharmacophoric considerations via modeling studies on C3- and C4-varied benzopyrans

C3- and C4-varied structures [12–19], included in this study, are shown in Fig. 1A. The following section concerns the impact of substituents in C4-position. Gadwood et al. [19] have shown that the *S*-enantiomer of U96501 (compound **6**, Fig. 1A) is the active one (=eutomer) and that the distomer is devoid of activity. This supports our previous

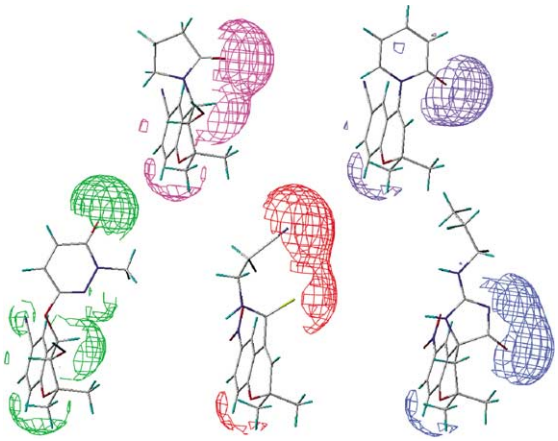


Fig. 3. Bioactive conformations of diverse benzopyrans. First row: levcromakalim (**1**) and bimakalim (**2**); second row: symakalim (**4**), KC399 (**3**) and U96501 (**6**). The most electronegative functional groups of the C4-substituents point backwards with respect to the benzopyran nucleus. The contour maps show the MEPs at −20 kcal/mol.

findings [31] that in the active conformations of the remaining more flexible benzopyrans the electronegative group of the C4-substituents points backwards with respect to the benzopyran nucleus, as illustrated in Fig. 3.

In order to unravel the essential pharmacophoric features for receptor interactions, we investigated the active compounds in more detail. Superimposition of U96501 and levcromakalim (which designates the active enantiomer of cromakalim—in elder publications it is named lemakalim) allows the conclusion that the lone pair of the imine nitrogen in U96501 mimics the 'upward' pointing lone pair of the carbonyl oxygen of levcromakalim and represents one essential feature for receptor interaction. However, inclusion of symakalim into these alignment considerations casts some doubt on the above hypothesis. Symakalim does not have a lone pair in an equivalent position and its own C4-substituent, the 3-pyrazinone ring, would sterically hinder a favorable interaction at the receptor site. In contrast, we assume that one essential feature is the 'downward' pointing lone pair of the carbonyl oxygen of levcromakalim, which is mimicked by the upward pointing lone pair of the carbonyl oxygen of U96501 and the downward pointing lone pair of the unsubstituted pyrazinone nitrogen of symakalim. In Fig. 4 these three molecules are shown with the discussed lone pairs; the arrow marks a position from which a potential receptor group could favorably interact with these different structures.

EMD 57299 exemplifies that structures can also be very active, when they lack the above feature, but possess a carbonyl oxygen, which is superimposable with the carbonyl oxygen of symakalim. This fact led us to hypothesize that strong binding is favored, when a compound is able to induce a negative potential at two different, yet adjacent sites. This idea is supported by the structure–activity relationships, evaluated by Tamura et al. [18]. They demonstrated that the

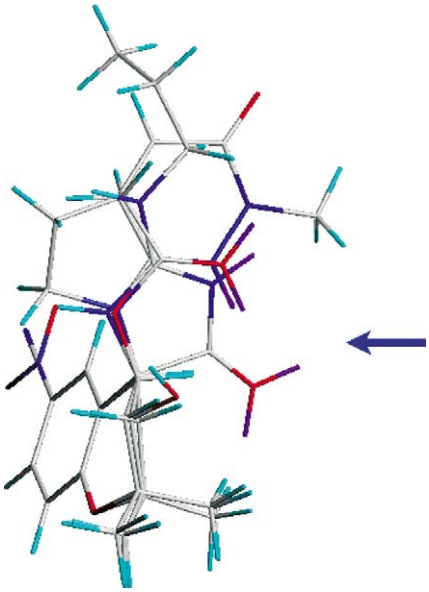


Fig. 4. Superimposition of U96501 (**6**), symakalim (**4**) and levcromakalim (**1**). The discussed lone pairs are displayed and labeled (e.g. LP 6u refers to an upward pointing lone pair of compound **6**). The arrow marks a suitable position for an H-bond-donor.

introduction of the cyanoethyl group to the already effective amide and thioamide compounds (compound **7** in Fig. 1) increases their activity. The superimposition of KC399, EMD 57299 and levcromakalim and their respective MEPs (contoured at a level of −20 kcal/mol) illustrates that KC399 combines the features of the other two molecules (Fig. 5).

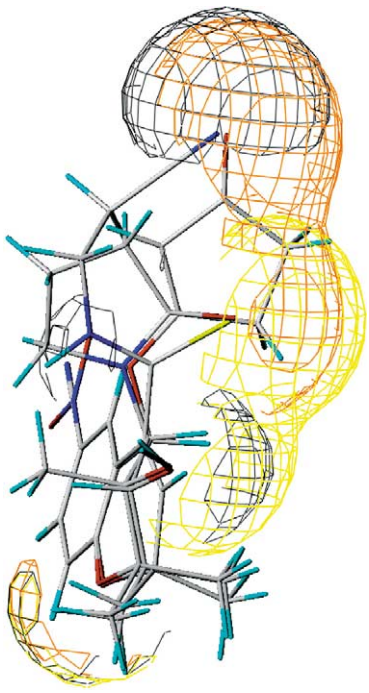


Fig. 5. Superimposition and MEPs of KC399 (orange), EMD 57299 (black) and levcromakalim (yellow).

The importance of the hydroxyl group at C3, as present e.g. in levcromakalim, is ambiguous. Whereas the introduction of hydroxyl in levcromakalim increases the potency 15-fold [32], compounds like bimakalim or KC399, which lack a substituent at C3, are more potent than levcromakalim.

Ecker et al. [33] performed a quantum mechanical conformational analysis of levcromakalim, especially considering the rotation around the carbon(4)–amide-nitrogen bond. They conclude that the bioactive conformation exhibits an intramolecular hydrogen bond between the carbonyl oxygen and the hydrogen of the hydroxyl group, because these conformations are energetically most favorable. Using quantum mechanical calculations we have confirmed that a conformation forming an intramolecular hydrogen bond represents the global minimum; however this is presumably due to the fact that these calculations are performed in vacuo and any solvation effects are neglected. This idea is supported by calculations with the GB/SA method as it is included in the MacroModel software [26]. Making a minimization in water conditions the intramolecular hydrogen bond is not formed whereas the optimization in vacuum favors the conformation with this intramolecular interaction. We can imagine that a favorable complex between a water and a levcromakalim molecule is formed as it is depicted in Fig. 6. So the conformation which we believe being the bioactive one and which is also found in the crystalline state is stabilized. This hypothesis could explain the structure–activity relationships found by Attwood et al. [32]. They are showing that the introduction of the 3-hydroxyl-group accounts for the chiral discrimination in the biological activity of the enantiomers of cromakalim.

These qualitative investigations on benzopyran derivatives with variations at the C3- and C4-positions can be summarized as follows: The hydroxyl group in C3-position seems to stabilize the bioactive conformations. It is rather unlikely that this group contributes fundamentally to a direct interaction with the binding site. C4-substituents must have at least one structural element inducing an electronegative potential, which points backwards with respect to the benzopyran ringsystem. Further increase in activity is yielded by an additional electronegative group, closely located to the first one.

3.2. QSAR studies on C6-varied benzopyrans

The second part of our study concerns the impact of C6-substituents. Substitution at the C6-position of the benzopyran nucleus significantly influences biological activity, as shown by Ding et al. [34] for a series of C6-modified benzopyranylcyanoguanidines. Similar results were obtained for 6-varied derivatives of benzopyran-4-thioamides

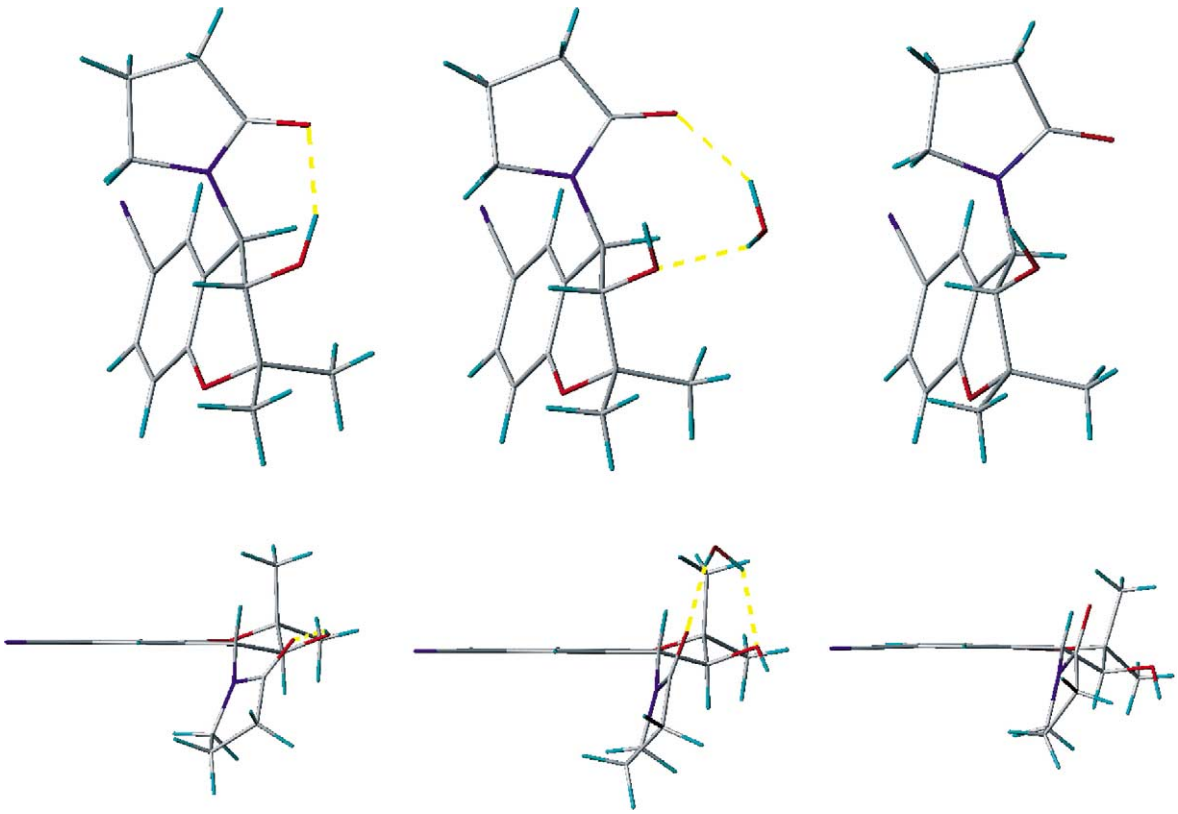


Fig. 6. Three different conformations/complexes of levcromakalim in two views. In the second row, the molecule is seen from above, so the phenyl ring of the benzopyran nucleus is only seen as a line. Left: global energy-minimum conformation of levcromakalim, calculated by quantum mechanical methods (ab initio: 3-21G*). Under these in vacuo conditions, the formation of an intramolecular hydrogen bond is favored. Middle: possible complex of levcromakalim and a water molecule (also optimized with ab initio: 3-21G* method). Right: conformation of levcromakalim in the crystal structure.

and benzopyran-4-carboxamides [35–37]. All these studies indicate that small electronegative substituents such as a cyano group are favorable.

We recently addressed the question whether the C6-substituent directly interacts with the receptor site or influences biological activity by modulating the electronic properties of the aromatic part of the benzopyran nucleus [38]. At that time only a series of 6-acyl derivatives and some compounds with diverse 6-substituents were available. We calculated LUMO energies with the AM1 method to test the following hypothesis: electron withdrawing C6-substituents might enable the aromatic part of the benzopyran ring to serve as binding partner (electron acceptor) in charge transfer complexes. The lack of a correlation between LUMO energies and activity data clearly indicated that the formation of a charge transfer complex cannot underly the drug receptor interaction. On the contrary, we found a relationship between the desolvation energies and the biological activities (relaxation of rat aorta) of the C6-varied benzopyrans. This term represents the amount of energy necessary to remove water molecules from the surface of the ligand to allow direct receptor binding. Binding is favored when the desolvation energy is small, since binding a ligand will result in a larger energy gain. From these studies we concluded that the binding region for C6-substituents appears to contain electropositive moieties, explaining why electronegative C6-substituents are preferred.

In the present study, we extended our investigations to a larger and in particular more diverse database. For this purpose C6-varied benzopyran derivatives were synthesized, using the scaffold of bimakalim as common core structure [20–23]. The relaxant potency of these compounds was measured in rat aorta. Half-maximal potency was derived from dose-response curves as pEC_{50}-values. Relaxation by KCO was measured in isolated rat aortic rings precontracted with 25 mM KCl [38]. Maximum effects resulted in a complete relaxation of aortic rings as compared to basal tension before addition of KCl. The pEC_{50}-values for relaxation were derived from concentration–effect curves normalized to individual maximum effects of the respective compounds. Substitution pattern and the pEC_{50}-values of the test compounds are shown in Table 1.

In a first step we studied the conformational behavior and the volumes of the C6-substituents, attached to a phenyl ring instead of the benzopyran nucleus to save calculation time. Afterwards, all derivatives were superimposed, using the highly potent ES64 as a template. Its 6-substituent, the phenylsulfonyl moiety, exhibits a high volume, but only intermediate conformational flexibility. For all the compounds we chose conformations, which, while energetically permissible, do not necessarily represent the global minimum, and which best fit the template. Furthermore, we calculated charge distributions, dipole moments, LUMO energies and desolvation energies for these compounds (for data see Table 1).

In order to test the hypothesis that the C6-substituent interacts favorably with electropositive elements at the binding site, we investigated the MEPs. The more negative the potential a substituent is able to induce, the more active the compound should be. But on the one hand the highly active compounds ES70 or CW104 induce with their sulfonyl oxygens a less negative potential as for example CW107. And on the other hand the sulfonyl oxygens of CW110 induce strong negative potentials, but this compound shows very low activity. Thus, it is not likely that favorable interactions of electropositive elements of the receptor with electronegative groups determine the activity of the ligands.

While visually examining the dipole vectors of the compounds it was striking that the vectors of active derivatives point in the direction of the C6-substituents. In order to prove the putative impact of the direction of the dipole vector, we correlated the components of a normalized vector (Section 2) and the pEC_{50}-values. First we included only compounds with a volume up to 43 $Å^3$ (omitting however the negatively charged compound CW100). The substituents of this subset (marked with an asterisk in Table 1) are conformationally not very flexible and a correlation is unlikely to be biased by a chance alignment. A highly significant correlation between the X-component of the dipole vector and the dilator activity in the aorta is observed:

$$\begin{aligned} &pEC_{50} = 2.96(\pm 0.78)\text{X-component} + 5.66(\pm 0.40) \\ &n = 14, \quad r = 0.921, \quad s = 0.403, \quad F = 67.5 \end{aligned} \tag{1}$$

Expanding this investigation to all compounds a dependency between the activity data and the X-component could still be confirmed, as shown by the following equation:

$$\begin{aligned} &pEC_{50} = 2.16(\pm 0.35)\text{X-component} + 5.88(\pm 0.16) \\ &n = 49, \quad r = 0.669, \quad s = 0.706, \quad F = 38.1 \end{aligned} \tag{2}$$

These correlations indicate the importance of the way in which the ligand approaches its binding site. It appears that the appropriate preorientation of the entire ligand is a prerequisite for the interaction with the binding site. This preorientation can be set on the way to the binding site by different structures of the protein. On the one hand, the protein induces an electric field and on the other hand it provides steric constraints. Fig. 7 illustrates this hypothesis in some detail; the scheme on the left represents a ligand with an ideally oriented dipole vector. The frequency with which the ligand approaches the binding site in a correct orientation for interaction is increased, because this orientation is energetically favored. On the right in Fig. 7, the dipole vector is not directed along the sterically favored orientation of the ligand. Accordingly, the dipole vector of the ligand assumes its favored position, which is not appropriate for the correct approach to the receptor. The appropriate orientation is energetically disfavored and will rarely exist, resulting in a less active compound.

The observed correlation between the activity data and the X-component of the dipole vector is based on an

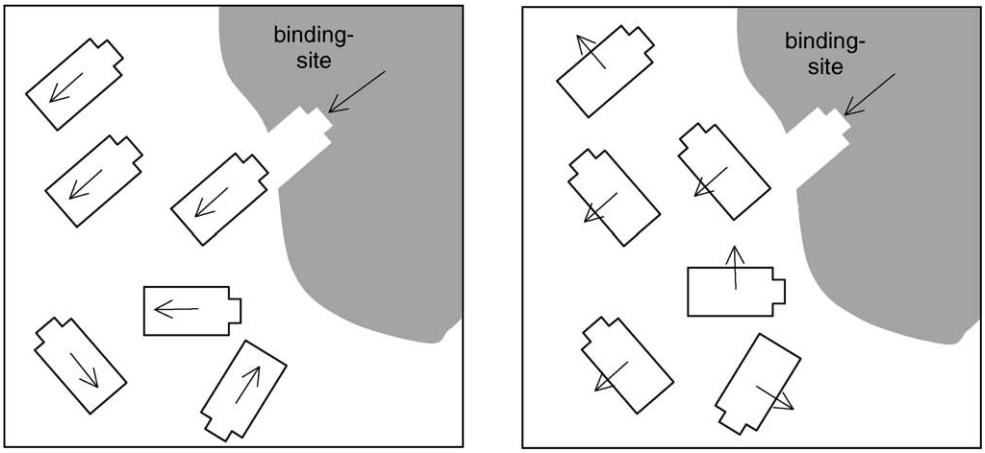


Fig. 7. Scheme of ligands and a binding site. Left: the ligand in the appropriate preorientation is often present. Right: the dipole vector of this ligand positions the ligand in a non-fitting orientation for binding.

energetic effect. The more X-component a ligand has, the more energetically favored is its correct orientation for the approach to the binding site. According to the Boltzmann distribution, a given state is more likely to exist the lower its energy. Taking this hypothesis into account it is possible to explain the poor activity of a compound such as CW47. The 6-substituent of CW47 is conformationally flexible and can adopt conformations, which can be perfectly superimposed on ‘ template ES64. But since the dipole vector points nearly orthogonal to the benzopyran nucleus, CW47 will not reach the binding site. The structure–activity relationships described for pyranopyridines derivatives [39] may also be explained by this idea. It is ideal for activity when the pyridine nitrogen is located at the position 6, the position 7 is also tolerated, but being at the position 8 results in inactive compounds. In this case, steric hindrance could not be the reason for inactivity, but the direction of the dipole vector has changed which—according to our hypothesis—will be responsible for the drop in activity.

The negatively charged compound CW100 was not included in the correlation analysis, because it is rather devoid of activity despite its high value for the X-component of the dipole vector. There are two conceivable explanations for this aberrant behavior. If the binding site is located intracellularly, CW100 will not reach it, because it will exist predominantly in the negatively charged form under physiological conditions. Secondly, if the formation of a charge-transfer complex is a prerequisite for binding, CW100 will be unable to bind, since it is already negatively charged and cannot act as an electron acceptor.

When we first developed this hypothesis, no experimental value from our test system for a benzopyran derivative with a nitro-group at the C6-position was available. It was only known from literature, that such a nitro compound is highly potent. A prediction of the activity should be possible, because the nitro substituent is small and rigid. With its X-component value of 0.837 we predicted a pEC_{50}-value of 8.14; the now available experimental pEC_{50}-value of 8.33 shows that the nitro compound fits into and confirms our model.

Studying the physicochemical properties of 50 C6-varied benzopyran derivatives, a significant correlation between the direction of the dipole vector of the ligands and their pEC_{50}-values was detected. Accordingly, the biological activity is essentially influenced by the correct approach of the entire compound to the binding site. The fact that CW100 is an outlier in this correlation can be explained by its own outstanding structural property being an ionic (negatively charged) molecule.

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