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Correlation of LUMO* energies and free energies of binding for a series of nifedipine analogues

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A theoretical study was carried out in order to examine the potential of pharmacologically active 1,4-dihydropyridines (DHPs) to react via charge-transfer (CT) interactions with their binding site. For this purpose, the molecular orbitals of nine mainly 3′-substituted DHP derivatives from the nifedipine type were determined by semiempirical (AM1, PM3/tm, MNDO/d), ab initio (STO-3G, RHF 3-21G*, RHF 6-31G**) and electron density function (LSDA) methods. Qualitative analysis of the results revealed that for DHPs exerting high affinity to the receptor site the energetically most favourable lowest unoccupied molecular orbital (LUMO) is found at the 4-phenyl ring, whereas the highest occupied molecular orbital (HOMO) is detected at the DHP heterocycle. In contrast, DHPs with lower binding affinity produce only energetically less favourable unoccupied MOs at the 4-phenyl moiety (designated as LUMO*s) and in addition, also the HOMO is partially localized at this position. A quantitative approach performed by correlating experimentally estimated free energies of binding and calculated LUMO* energies yielded satisfying correlations with correlation coefficients ranging from $R = 0.80$ (RHF 6-31G**) to $R = 0.91$ (AM1). Based on these findings one can conclude that besides the classical binding forces (electrostatic, hydrogen bonding, and van der Waals interactions) also charge-transfer mechanisms should be involved in DHP/binding site stabilization.

1. Introduction

Calcium entry blocking 1,4-dihydropyridines (DHPs) are frequently applied drugs in the therapy of cardiovascular disorders like angina pectoris, certain types of arrhythmias and hypertension [1, 2]. Radiolabelling experiments [3, 4] and site-directed mutagenesis [5–7] unambiguously demonstrated the α_1-subunit of L-type calcium channels as their molecular target. Unfortunately, no 3D coordinates of a DHP/binding site complex are available to clarify the forces involved in specific receptor binding. Structure-activity relationships, however, indicate hydrogen donor properties of the N–H group and (at least) one further hydrogen bond to be accepted by the carbonyl oxygen(s) of the ester side chains combined with electrostatic forces as the most prominent types of binding site interaction [8, 9]. On the other hand, Bolger et al. [10] demonstrated that the presence of these critical elements cannot alone account for high binding site affinity. They determined the binding affinities of 23 nifedipine-type DHPs by radioligand binding experiments with [^{3}H]nitrendipine in a homogenized microsomal fraction of guinea-pig ileal longitudinal smooth muscle. The estimated binding affinities (IC_{50}s) were found in a range of more than five orders of magnitude although the single structural change has been the variation of the substitution pattern of the DHP 4-phenyl ring. Until now it is not yet clarified whether the various substituents interact directly with the binding site or influence particular molecular DHP characteristics. Recently, however, we published [11] an atomistic pseudoreceptor model for pharmacologically active DHPs indicating a putative charge-transfer (CT) interaction for DHP/binding site stabilization.

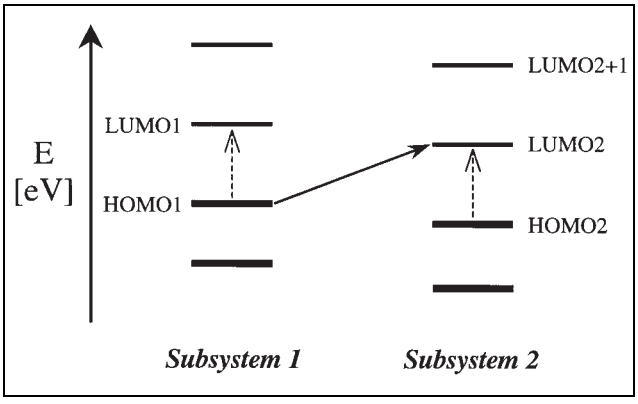


Fig. 1: The solid arrow symbolizes π-electron transfer of a CT interaction between HOMO1 and LUMO2 (▬ occupied molecular orbitals, – unoccupied molecular orbitals). Dashed arrows indicate potential polarization interactions between corresponding HOMOs and LUMOs of one subsystem

In order to evaluate this hypothesis, the present study describes the qualitative and quantitative analysis of molecular orbitals derived from seven different semiempirical and ab initio methods for nine DHP derivatives.

2. Investigations, results and discussion

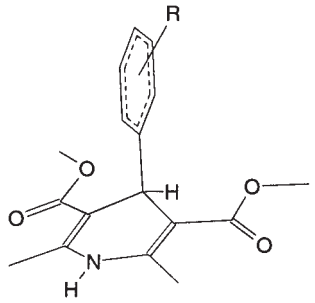
2.1. Qualitative analysis

Charge-transfer (CT- or electron-donor-acceptor) interactions indicate π-electron transfers from the highest occupied molecular orbital of one subsystem (HOMO1) to the lowest unoccupied molecular orbital of a second subsystem (LUMO2) [12]. Although small energy barriers between HOMO1 and LUMO2 increase the probability of CT interactions, two further prerequisites must be fulfilled. First, the corresponding molecular orbitals must be able to overlap, and second, the electron-providing HOMO1 and also LUMO1 (located at the same subsystem) must be energetically less favourable than the corresponding MOs of subsystem 2 (Fig. 1).

In context with the postulated CT interaction for DHP/binding site stabilization, the electron-accepting LUMO should be located at the substituted 4-phenyl ring of DHPs, since highest binding affinities are detected for DHP derivatives with electron-withdrawing substituents at this position (Table 1).

Since quantum chemical calculations are particularly sensitive to molecular distortions, all force field generated

Table 1: Experimentally estimated free energies of binding (ΔG°) and calculated solvation energies (E_{solv}) of the investigated DHP derivatives in their putative bioactive sp/sp/sp-conformations (kcal/mol)



Compd.	R	ΔG°	E_{solv}
a	H	−10.7074	−6.69
b	3′-CH_3	−9.9299	−6.24
c	3′-F	−11.5804	−7.11
d	3′-Cl	−12.6852	−6.98
e	3′-NO_2	−13.5991	−11.35
f	3′-CN	−11.8395	−7.29
g	3′-N_3	−11.8259	−7.37
h	3′-OCH_3	−9.9163	−7.29
i	2′,3′,4′,5′,6′-F_5	−14.1447	−7.19

DHPs (Table 1) were re-optimized applying semiempirical and ab initio methods. This revealed that most of the employed algorithms are able to yield accurate geometries for DHPs. Only in course of the MNDO/d minimizations all ester side chains were rotated to experimentally not detected orthogonal positions relative to the 1,4-dihydropyridine ring. Therefore, this method was not further used.

Subsequently, the molecular orbitals were computed using several quantum mechanical methods (listed in Table 2). In order to correctly assign the unoccupied molecular orbital localized at the 4-phenyl ring (designated as LUMO*), all relevant unoccupied MOs (LUMO, LUMO + 1, LUMO + 2, ...) had to be visualized yielding the information that the LUMO of DHPs usually is found at the 1,4-dihydropyridine scaffold (Fig. 2, left side). Only for the DHP derivatives **e** (3′-NO_2) and **i** (F_5) the LUMO is identical with the electron-accepting LUMO* of the molecules (Fig. 2, right side). For all other investigated DHPs lacking such potent electron-withdrawing substituents only energetically less favourable LUMO*s (LUMO + 1 and LUMO + 2) were observed at the aromatic ring system (Fig. 2, left side).

However, it has to be examined whether besides the LUMO*s also suitable HOMOs are localized at the 4-phenyl moieties because this would favour intramolecular polarization effects (e.g. electron transfer from HOMO1 to LUMO1, see Fig. 1) rather than intermolecular CT interactions.

Analysis of the occupied MOs indicates that the HOMO of the investigated DHPs is most frequently found at the 1,4-dihydropyridine heterocycle (Fig. 3, left side). Only for the DHP congeners **g** and **h** it is (partially) observed at the 4-phenyl ring (Fig. 3, right side).

But despite this common HOMO/LUMO* occurrence at one subsystem CT interactions are not necessarily prevented. The deciding factor to modulate π-electron transfer between two different subsystems is the energy level of the electron-providing HOMOs in relation to the electron-accepting LUMO (i.e. LUMO2 in Fig. 1). If HOMO1 is energetically less favourable than HOMO2 the energy barrier between HOMO1 and LUMO2 is smaller compared to HOMO2/LUMO2, and thereby potential CT interactions will be facilitated (see Fig. 1). Nevertheless, it is evident that compound **h** possessing the strongest electron-providing (3′-methoxy) substituent has the lowest binding affinity of all considered DHPs. This may be interpreted in such a way that electron-providing moieties cause an electron excess at the 4-phenyl ring and thereby induce the formation of occupied MOs at this position. At the same time, the existence of energetically favourable LUMO*s becomes more unlikely.

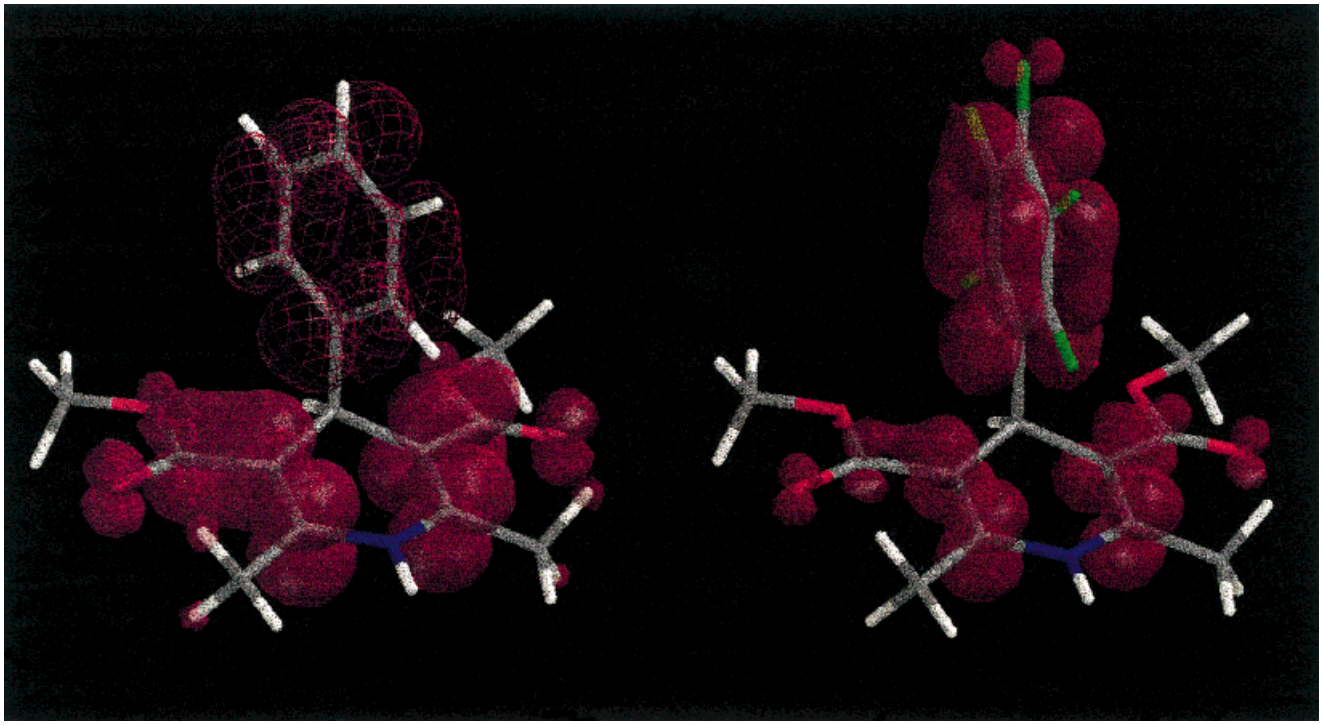


Fig. 2: Unoccupied MOs of the DHP derivatives **a** (left) and **i** (right) derived from semiempirical AM1 calculations. Translucent surfaces indicate the LUMOs and dot surfaces represent the LUMO + 2 as the energetically most favourable LUMO* of compound **a**

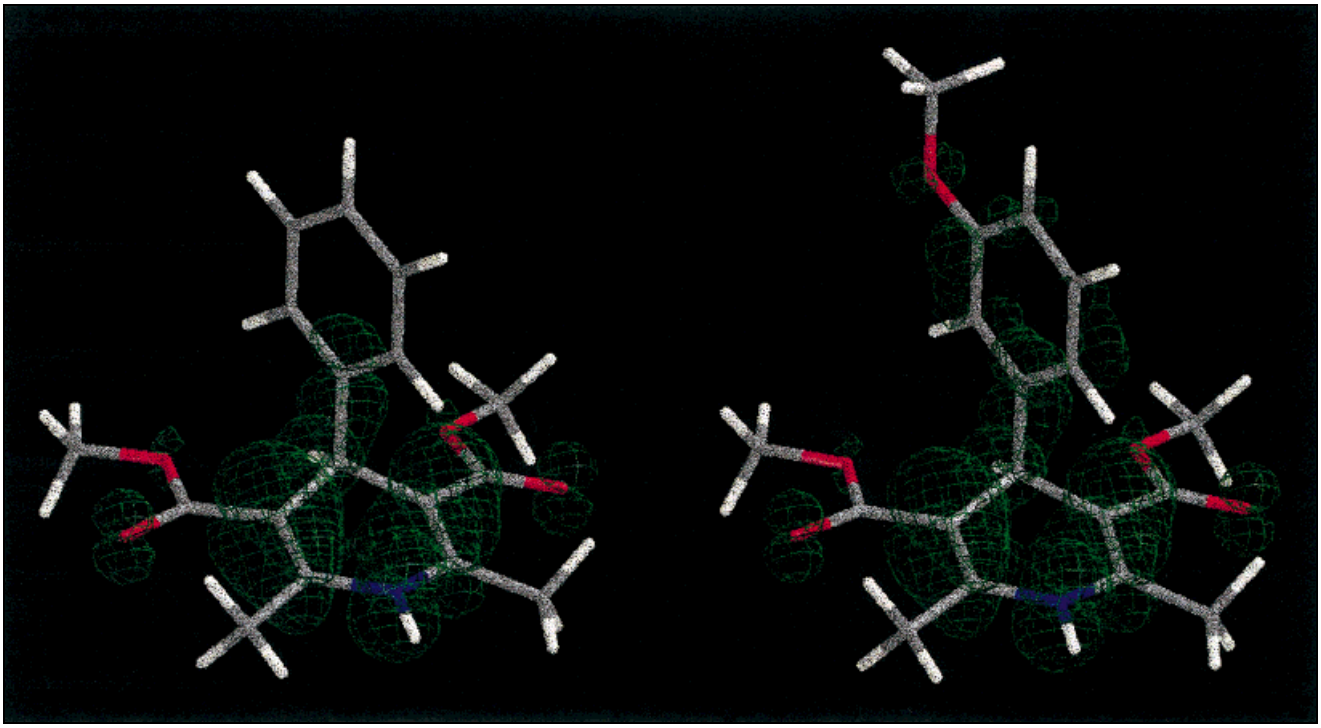


Fig. 3: HOMOs of the DHP derivatives **a** (left) and **h** (right) derived from semiempirical AM1 calculations

2.2. Quantitative analysis

Subsequently to these qualitative reflections, a quantitative investigation should be performed by correlating experimentally derived free energies of binding and calculated LUMO* energies. However, before doing this one has to keep in mind that ΔG° values mirror a multitude of ligand-dependent direct (i.e. hydrogen bonding, electrostatic, van der Waals, hydrophobic, CT interactions) and indirect (solvation energies, entropic terms) factors. Assuming that all investigated DHPs exert almost identical direct interactions to the binding site (with the exception of potential CT interactions), indirect factors could prevent a clear-cut interpretation of the findings. On the other hand, because of the limited structural variation in the set of DHPs studied, almost identical entropy effects may be assumed. At least, the calculation of solvation energies yields quite similar results for the whole series (Table 1) implicating no significant influence of this parameter on the desired correlation.

2.3. Correlation of ΔG° and LUMO energies*

In the next step, the LUMO* energies were determined for all geometry-optimized conformers employing the same method as used for minimization. The following correlation of these energies with the experimentally estimated ΔG° values yielded correlations ranging from $R = 0.82$ (RHF 3-21G*) to $R = 0.91$ (AM1). Subsequently, more time-consuming methods (RHF 6-31G** and electron density function approximations) were performed using the conformer derived from RHF 3-21G* geometry-optimization as input structure (Table 2).

Analysis of the results indicates that all applied quantum mechanical methods yield quite good correlations of ΔG° and LUMO* energies with F-values in a range from 11.30 (STO-3G) to 33.75 (AM1) further corroborating the significance of the findings. It is interesting to note that a better correlation is achieved with semiempirical methods ($R \approx 0.90$) in comparison to the local-spin-density-approximation (LSDA) functions ($R \approx 0.84$) and ab initio procedures ($R \approx 0.80$). The most suitable algorithm in this context being the semiempirical AM1 method [13] yields not only accurate DHP geometries but also the best corre-

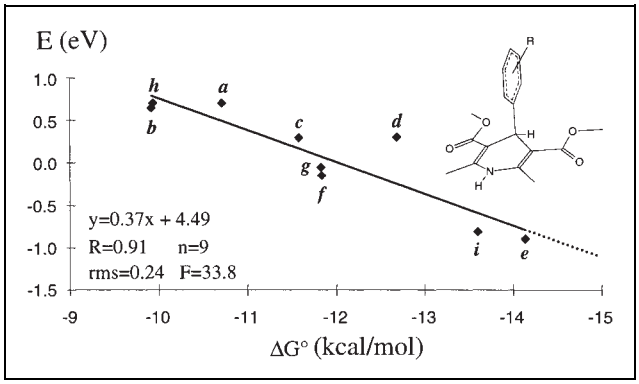


Fig. 4: The graph shows the correlation of experimentally elucidated free energies of binding (ΔG°) and calculated LUMO* energies for all investigated DHPs (**a**–**i**). Geometry optimization of the DHP derivatives and calculation of the molecular orbitals were carried out with the semiempirical AM1 method

Table 2: Correlation coefficients (R) obtained by correlating experimentally derived free energies of binding and calculated LUMO* energies of the DHP compounds a–i

MO Calculation	Optimization Procedure				
Method	AM1	PM3/tm	MNDO/d	STO-3G	3-21G*
AM1	0.91				
Pm3/tm		0.89			
MNDO/d			n. p.		
STO-3G				0.82	
3-21G*					0.82
6-31G**					0.80
LSDA	0.85				0.84

n. p. (not possible) because of non-realistic results in course of the minimization procedure

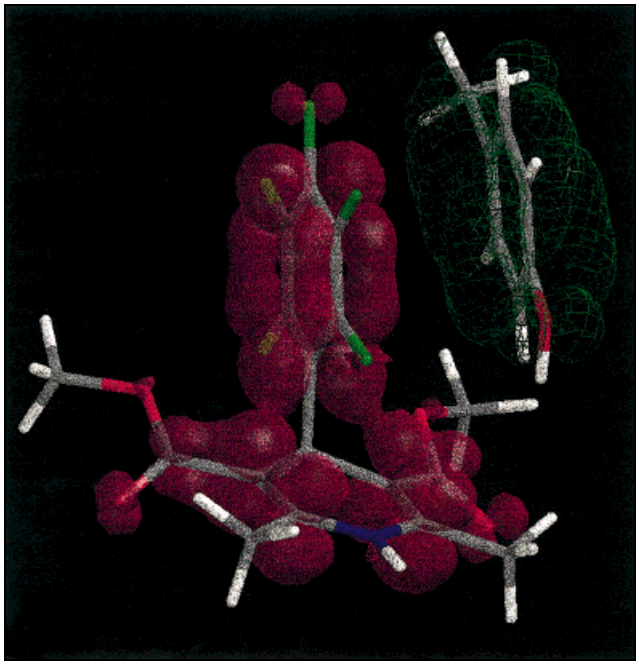


Fig. 5: Hypothetical complex composed of p-cresol, representing a truncated tyrosine of the binding site, and the DHP derivative **i**. The π-electron transfer from the HOMO (green grid) to the LUMO (red cloud) characterizes the postulated CT interaction

lation of ΔG° and LUMO* energies ($R = 0.91$) achieved in this study (Fig. 4).

Although usually the higher sophisticated ab initio calculations provide more reliable results, one explanation for the better correlation obtained with semiempirical methods in this aproach might be the use of experimental data like ionization energies for the development of parameters implemented in semiempirical procedures [13, 14].

2.4. Conclusion

Based on the qualitative findings concerning the HOMO/LUMO* distribution of DHPs, and especially supported by the significant correlation of ΔG° and LUMO* energies it seems to be likely that DHPs may also interact via a CT mechanism with their binding site. If this is true, especially the electron-rich aromatic system of a tyrosine, which as experimentally proven is one of the crucial determinants for binding [6, 7], may be assumed as potential π-electron donor subsystem (Fig. 5).

3. Experimental

3.1. DHP construction and optimization

All investigated DHP derivatives were generated and geometry-optimized within the TRIPOS force field of the SYBYL software package [15] using the conjugate gradients algorithm. In agreement with structure-activity relationships [8, 11] and consideration of energetic aspects derived from ab initio calculations [16], the so-called sp/sp/sp-conformation (synperiplanar) of the substituents at 3-, 4- and 5-position of DHPs was chosen as pharmacophoric geometry. This means, that the coplanar oriented carbonyl oxygens of the ester side chains are located at the same side relative to the double bonds of the 1,4-dihydropyridine ring, and that substituents of the pseudoaxially arranged 4-phenyl ring and the hydrogen atom at C4 are pointing into the same direction (see Table 1).

The force field minimized structures were used as input for further geometry optimizations employing semiempirical (AM1, PM3/tm, and MNDO/d) as well as ab initio (STO-3G and RHF 3-21G*) methods offered by SPARTAN software package [17].

3.2. Calculation of molecular orbital energies

The molecular orbitals (MOs) of the geometry optimized conformers were calculated applying semiempirical (AM1, PM3/tm, MNDO/d) and ab initio (STO-3G, RHF 3-21G*, RHF 6-31G**) algorithms. In addition, the local-spin-density-approximation (LSDA) function obtained by Vosko et al. [18] was applied including a double numerical basis set (DN). Experimental data concerning binding affinities of DHP derivatives were taken from Bolger et al. [10] and solvation energies were determined according to Still et al. [19]. All computations were carried out on SGI Origin2000 and Indigo^2 R 10000 workstations.

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