Pharmacophore Models of Group I and Group II Metabotropic Glutamate Receptor Agonists. Analysis of Conformational, Steric, and Topological Parameters Affecting Potency and Selectivity

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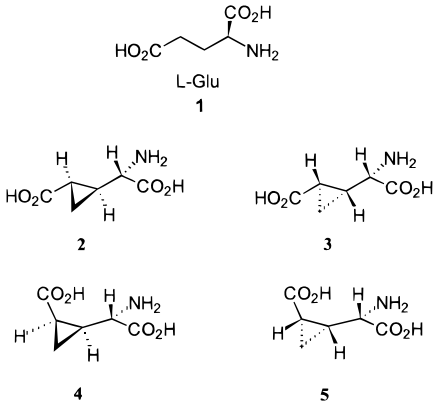
A wide variety of conformationally constrained glutamate analogues, active as group I or group II metabotropic glutamate receptor agonists, were employed in a molecular modeling study aimed at the definition of group I and group II agonist pharmacophoric models. The results of this study can be summarized as follows: (i) Recognition sites of both group I and group II mGluRs can adequately be described by five-point pharmacophores. (ii) An extended conformation of glutamate is required for interaction with both group I and group II mGluRs. Group I receptors, however, can also be activated by a more folded conformation if only four pharmacophore points are considered. (iii) Conformational preferences are, however, not sufficient to explain the potency and selectivity of the whole set of ligands. Volume comparison analysis allowed us to define steric environments for group I and group II mGluRs. Group I mGluRs are characterized by a region of allowed volume in proximity of the distal acidic function, whereas group II mGluRs are characterized by a small polar pocket whose occupancy confers high potency and selectivity. Finally, our study points out the necessity of a careful analysis of the energetic requirements needed to attain the putative bioactive conformations and of explicitly considering the conformational mobility of carboxylate groups.

Introduction

The synaptic actions of L-Glutamic acid (L-Glu, **1**, Chart 1), the major excitatory neurotransmitter in the CNS of vertebrates, are mediated by two main families of receptors, namely ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs). Ionotropic glutamate receptors are constituted by a heterogeneous family of integral membrane spanning cation channels and are pharmacologically divided into NMDA and non-NMDA (AMPA and KA) receptors, whose main action is the fast depolarization of the postsynaptic membrane. Metabotropic glutamate receptors constitute, together with the Ca^{2+}-sensing receptor, the $GABA_B$ receptor and a putative pheromone receptor, a new family of the G-protein coupled receptor superfamily. To date, eight (and several spliced variants) mGluR subtypes have been identified and classified into three groups, according to signal transduction mechanisms, sequence homology, and pharmacology.[1] When expressed in heterologous systems, group I mGluRs (mGluR1 and mGluR5) are coupled to the activity of phospholipase C (PLC), and their activation results in an increased phosphoinositide turnover and intracellular calcium mobilization. Group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6−mGluR8) are both negatively coupled to the activity of adenylyl cyclase (AC), but they share a low sequence homology and are endowed with a different pharmacology and a different localization. A potential role for mGluRs modulators in the treatment of either chronic or degenerative CNS diseases has long been postulated. The molecular diversity of the mGluR family offers indeed an excellent opportunity for the development of subtype-selective modulators, endowed with specific actions and reduced side effects.

Chart 1



L-Glu (**1**) is a highly flexible molecule, and it may be conceivable that different conformations are able to activate different receptor subtypes. Conformationally constrained analogues of L-Glu (**1**), such as carboxycyclopropylglycines (**2**−**5**, Chart 1), have been used extensively in the past as chemical probes for detecting conformational requirements of L-Glu (**1**) acting at individual glutamate receptors. On the basis of a molecular modeling study using (2*S*, 1′*S*,2′*S*)-carboxycyclopropylglycine (L-CCG-I, **2**) as a semirigid template,

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Chart 2. Ligands Used in Group I Pharmacophore Definition

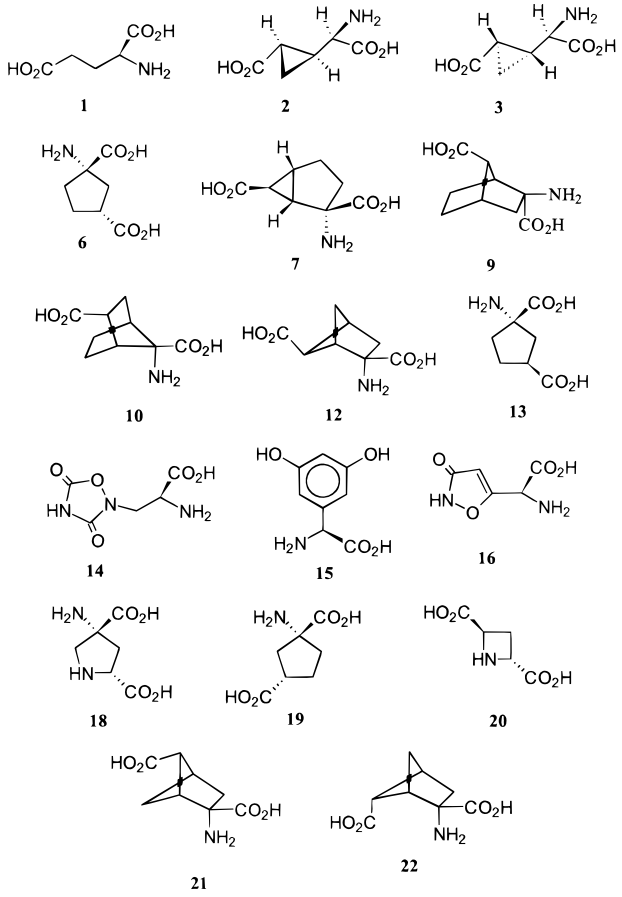
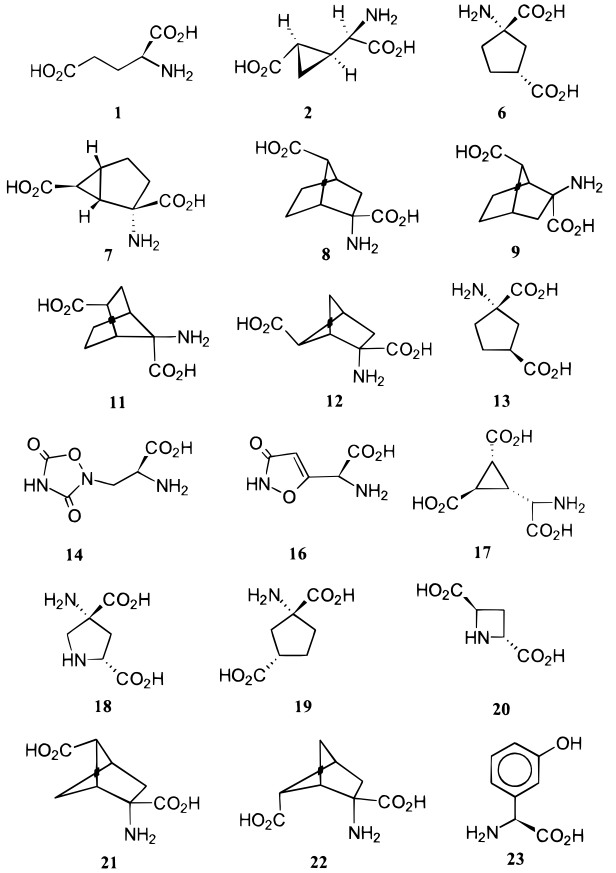


Chart 3. Ligands Used in Group II Pharmacophore Definition



we proposed in 1993 that an extended conformation of L-Glu (**1**) is needed for interacting with group II mGlu receptors.[2] In subsequent studies, other authors have confirmed our finding and extended the conformation−activity/selectivity relationships. In particular, in 1996 Ofhune et al. concluded that L-Glu (**1**) is likely to interact with ionotropic receptors in a folded conformation[21] and with metabotropic receptors in an extended one.[3] Again, in 1997 Girault et al.,[4] in a study based on a combination of NMR and molecular mechanics calculations, pointed out the preference for an extended (*tt*) conformation of L-Glu when acting at group II mGluRs and a more folded one (g^+t) when acting at group I mGluRs, in agreement with our results on docking of (1*S*,3*R*)-ACPD (**6**) on a homology model of mGluR1.[5] The preference of mGluRs, and in particular of group II mGluRs, for an extended conformation of L-Glu (**1**) has been definitively confirmed by the synthesis and biological evaluation of several rigid analogues of L-Glu (**1**). Among these, LY354740 (**7**), introduced in 1997,[6] embeds a fully extended disposition of pharmacophoric groups and is a very potent and selective group II agonist, with no activity at group I or group III receptors. In 1998, Tellier et al.[7] reported a series of aminobicyclo[2.2.1]heptane dicarboxylic acid derivatives (**8**−**11**) which embed either an extended or a folded conformation of L-Glu (**1**) and concluded that the extended disposition is required for both group I and group II receptor subtypes. The same year, Kozikowski et al.[8] introduced aminobicylo[2.1.1]hexane dicarboxylic acid (ABHxD-I, **12**) as another conformationally constrained L-Glu analogue which was shown to be a potent agonist at group I, II, and III receptor subtypes. By molecular modeling comparison with LY354740 (**7**), (1*S*,3*R*)-ACPD (**6**), and (1*S*,3*S*)-ACPD (**13**), the same authors clearly pointed out for the first time that the need for an extended disposition of pharmacophoric groups is necessary for activity but not sufficient to explain selectivity among individual mGluR groups. They indicate that the source of selectivity between the different groups may be their different steric environments.

All the above results provide medicinal chemists with relatively simple operational frameworks that can be used in the design of new, more potent and selective mGluR agonists. However, the predictive potential of these schemes must be proved in terms of their ability to rationalize all the available data, including the activity of structurally diverse compounds or the inactivity of closely related derivatives. With this objective in mind, we engaged ourselves in the determination of pharmacophore models for group I and group II agonists with the aim of elucidating the subtle combination of steric, conformational, and energetic requirements that determine potency and selectivity toward one or the other group. In our approach, we tried to simultaneously consider several parameters that may influence potency and selectivity. Particular attention is given to the energetic cost necessary for a given ligand to attain the putative bioactive conformation and to the conformational properties of the carboxylate moieties of ligands, parameters that seldom are explicitly considered in molecular modeling exercises on glutamate analogues. The results of our study are reported herein.

Methods

A large ensemble of L-Glu (**1**) analogues showing agonist activity toward group I (Chart 2) or group II (Chart 3) mGlu receptors were collected from the literature. Their activity values are reported in Tables 1 and 2, respectively.

It should be reminded that standardized radioligand binding assays for mGluRs are so far unavailable, and the activity values here reported are functional data, often coming from different sources and methods. Hence, the definition of terms "active", "selective", and "inactive", may lead to some ambiguity and deserves some explanation. Quisqualic acid (**14**, Chart 2) and LY354740 (**7**, Chart 3) were chosen as prototypes of potent and selective group I and group II agonists, respectively. Ligands endowed with an EC_{50} within 2 orders of magnitude from the above prototype compounds were defined as "active" compounds. Those displaying a difference in EC_{50}s between the two groups of more than 4 orders of magnitude were also referred to as "selective". These include 3,5-DHPG (**15**), IBO (**16**), L-CCG-I (**2**), (1*S*,3*R*)-ACPD (**6**), and ABHxD-I (**12**) (the latter three being nonselective) for group I and DCG-IV (**17**), (2*R*,4*R*)-APDC (**18**), L-CCG I (**2**), ABHDx-I (**12**), and (1*S*,3*R*)-ACPD (**6**) (again the latter three being nonselective) for group II.

"Moderately active" compounds are those endowed with a measured EC_{50} more than 2 orders of magnitude higher than the prototypes, but still characterized as producing a significant effect at micromolar concentrations (i.e., compounds **3**, **19**, **20** for group I; **6**, **16**, **13**, **19** for group II). Finally, "inactive" compounds were also included. Although the explanation of the inactivity of a given compound may be outside of the scope of pharmacophore modeling, we decided to include in this set those compounds whose structural similarity with known group I or group II agonists might have led one to predict them as potentially active. The inclusion of such compounds (i.e. compounds **7**, **18**, **13**, **21**, **22**, **9**, **10** for group I; **14**, **20**−**22**, **9**, **8**, **11**, **23** for group II) is expected to shed light on subtle steric and/or conformational factors that regulate the activation of group I and II mGluRs.

Once we defined the set of active, moderately active, and inactive compounds for both group I and group II mGlu receptors, the next step was the definition of pharmacophoric points to be used in the pharmacophore search. While automated methods for pharmacophore search (such as Catalysts or DISCO) produce the pharmacophore's dimensionality as the result of the analysis (including as many compounds as possible into the model), we felt that the existing structure−activity relationships for mGluR (and GluR in general) ligands are sufficient to impose the model's dimensionality: the nonrespondence to the defined rule must therefore be a criterion to exclude a possible solution. More specifically, all the mGluR (and GluR in general) ligands are characterized by a zwitterionic amino acid group and a distal acidic function, whose presence is indispensable for the activity. Accordingly, these three moieties must be simultaneously present in a pharmacophore model. Moreover, since both the amino acidic and the distal carboxylate groups are likely to interact with receptor sites by hydrogen bonds (or electrostatically reinforced hydrogen bonds), the carboxylate's oxygens should explicitly be considered in the pharmacophore definition in order to take into account the strong directionality of this interaction. Hence, a five-points pharmacophore should be required for both group I and group II mGluRs. Finally, the possibility that the distal carboxy-

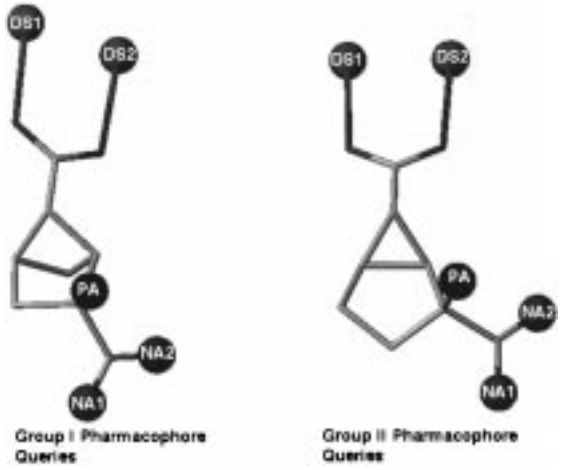


Figure 1. Schematic representation of pharmacophore queries. PA is a positively charged site, NA1 and NA2 are negatively charged sites, and DS1 and DS2 are lone-pair donating sites.

Table 1. Ligands Used for Group I Pharmacophore Definition

name	code	EC_{50} (μM)	def[c]	fit (RMS)	ΔE local (kcal/mol)	ΔE global (kcal/mol)	ref
quisqualate	**14**	0.9	A, S	0.244	1.13	1.82	9
ABHxD-I	**12**	1.6 ± 0.14	A	0.0	0.91	0.91	8
(*S*)-3,5-DHPG	**15**	6.6 ± 3.1	A, S	0.674	2.49	2.49	10
(1*S*,3*R*)-ACPD	**6**	9.3 ± 2.0	A	0.303	0.04	0.97	11
ibotenate	**16**	6.0	A, S	0.847	2.94	2.94	9
L-Glu	**1**	4.9 ± 2.0	A	0.246	0.91	1.63	8
L-CCG-I	**2**	5	A	0.245	2.89	2.89	12
(1*R*,3*S*)-ACPD	**19**	127 ± 15	M	0.435	0.34	0.45	11
(2*R*,4*R*)-ADA[a]	**20**	189.4 ± 6.4	M	1.051	1.16	1.16	13
L-CCG-II	**3**	20	M	0.321	3.69	5.85	12
(1*S*,3*S*)-ACPD	**13**	>300	I	0.301	0.55	0.90	11
ABHxD-II	**21**	121 ± 10	I	0.631	0.15	0.15	8
ABHxD-III	**22**	232 ± 23	I	0.519[d]	4.07	4.07	8
(2*R*,4*R*)-APDC	**18**	>1000	I	0.226	2.77	3.22	7
ABHD-II[a]	**9**	12%[b]	I	0.123	6.35	6.35	7
ABHD-V	**10**	10%[b]	I	0.445	1.96	1.96	7
LY354740	**7**	>1000	I	0.413	0.06	2.95	6

[a] Partial agonist. [b] Percentage of glutamate maximal response. [c] Pharmacological profile: A, active; S, selective; M, moderately active; I, inactive. [d] Fitted on four points of the five queried (PA, NA1, NA2, DS1).

Table 2. Ligands Used for Group II Pharmacophore Definition

name	code	EC_{50} (μM)	def[c]	fit (RMS)	ΔE local (kcal/mol)	ΔE global (kcal/mol)	ref
LY354740	**7**	0.032	A, S	0.0	0.0	2.89	6
DCG-IV	**17**	0.1	A, S	0.333	0.56	2.88	15
L-CCG-I	**2**	0.3	A	0.329	0.57	3.04	16
(2*R*,4*R*)-APDC	**18**	3.5 ± 0.5	A, S	0.272	2.82	3.27	14
L-Glu	**1**	0.29 ± 0.07	A	0.130	0.91	1.63	8
ABHxD-I	**12**	0.33 ± 0.06	A	0.282	0.26	0.26	8
(1*S*,3*S*)-ACPD	**13**	13 ± 3	M	0.224	0.22	0.57	11
(1*S*,3*R*)-ACPD	**6**	18 ± 1	M	0.270	1.86	1.86	11
(1*R*,3*S*)-ACPD	**19**	110 ± 10	M	0.476	0.56	0.66	11
ibotenate	**16**	50	M	0.580	2.06	2.06	17
ABHD-I	**8**	50%[b]	I	0.289	0.61	0.61	7
ABHD-II	**9**	50%[b]	I	0.253	8.41	8.41	7
ABHD-VI	**11**	30%[b]	I	0.637	3.92	3.92	7
(2*R*,4*R*)-ADA[a]	**20**	>100	I	1.227	1.86	1.86	13
(*S*)-3-HPG[a]	**23**	<15%[b]	I	0.141	2.52	2.52	18
quisqualate	**14**	100	I	0.472	1.13	1.82	9
ABHxD-III	**22**	38 ± 10	I	0.590[d]	4.07	4.07	8
ABHxD-II	**21**	54 ± 9	I	0.354	0.03	0.03	8

[a] Partial agonist. [b] Percentage of glutamate maximal response. [c] Pharmacological profile: A, active; S, selective; M, moderately active; I, inactive. [d] Fitted on four point of the five queried (PA, NA1, NA2, DS1).

late groups interact with the same site on the receptor but come from slightly different positions should also be taken into account, so that explicit receptor point projections could be added as dummy atoms to distal carboxylate oxygens. These projections were thus considered as pharmacophoric points instead of the "true" oxygen atoms. The resulting pharmacophore queries are schematized in Figure 1.

After having defined a suitable query, the pharmacophore definition was accomplished by performing a

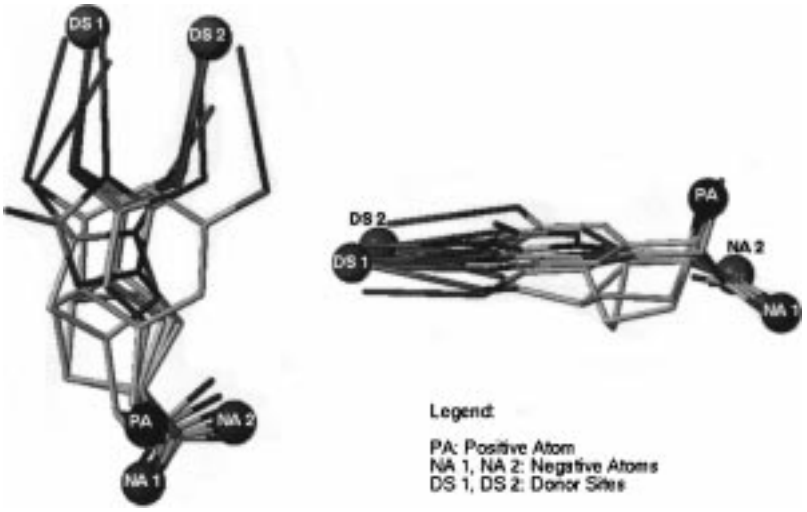


Figure 2. Group I pharmacophore.

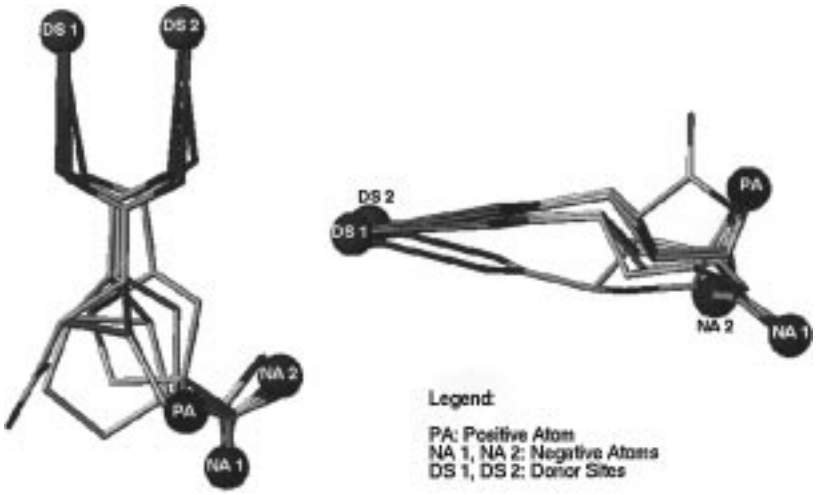


Figure 3. Group II pharmacophore.

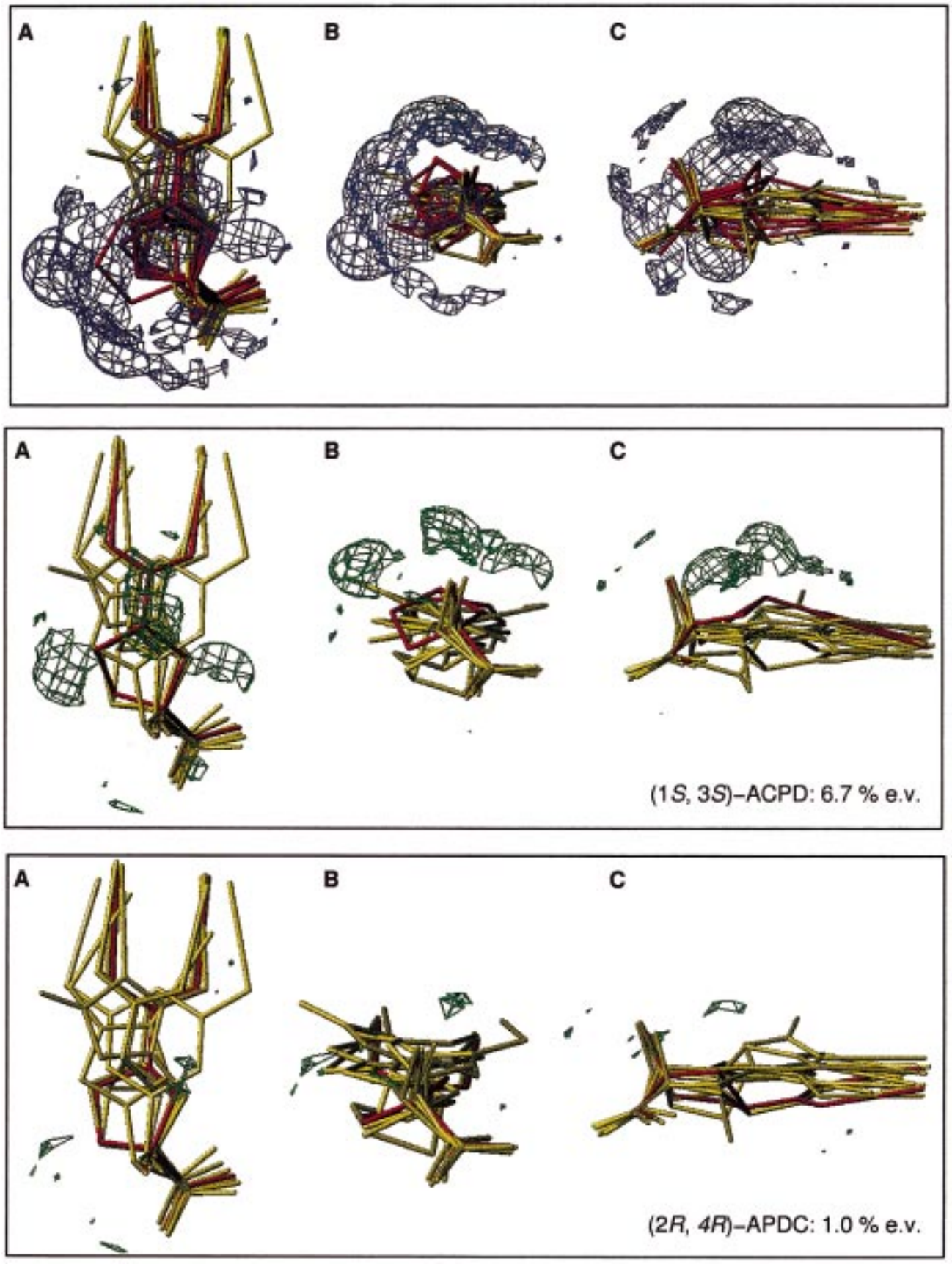


Figure 4a. Excluded volumes in group I pharmacophore. Top: the excluded volume of inactive (red) vs active (yellow) compounds is reported in blue. Middle: the relative contribution of (1*S*,3*S*)-ACPD (red) to the total excluded volume is reported in green. B ottom: the relative contribution of (2*R*,4*R*)-APDC (red) to the total excluded volume is reported in green.

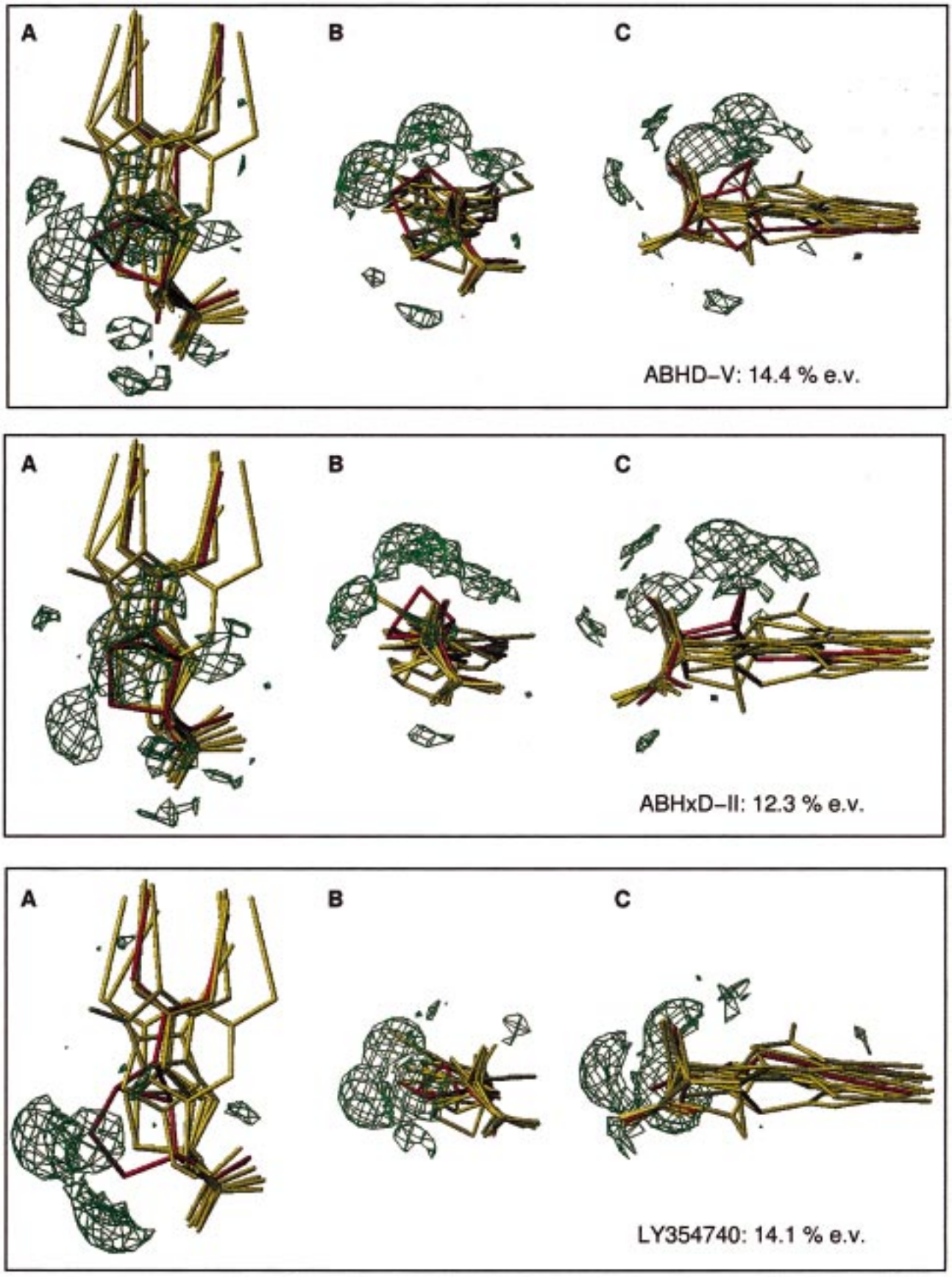


Figure 4b. Excluded volumes in group I pharmacophore. Top: the relative contribution of ABHD-V (red) to the total excluded volume is reported in green. Middle: the relative contribution of ABHxD-II (red) to the total excluded volume is reported in green. Bottom: the relative contribution of LY354740 (red) to the total excluded volume is reported in green.

grid search on all the rotatable bonds of all the active ligands. Single bonds were analyzed at 10° increments. Because of the symmetry, the conformational profile of both amino acidic and distal carboxylate groups was analyzed only in the 0−180° range. When necessary, the conformational mobility of ring systems was also taken into account. For each compound, all the resulting conformers laying within 3 kcal/mol from the global minimum were stored and used in the subsequent multifit procedure. Briefly, all the selected conformers for each compound were superimposed to all the conformers of all the other compounds. The previously defined five pharmacophoric points were used for the superimposition. This procedure was separately carried out on the active ligands of group I and group II. The most rigid structures ABHxD-I (**12**) and LY354740 (**7**) were used as templates for group I and II, respectively. If a given conformer did not produce a reasonable fit (i.e., RMS > 1), it was directly excluded from the calculation. In such a way we succeeded in identifying at least one low energy conformer for each active ligand which could be superimposed to at least one conformer of the other ligands. The final superimposition of the selected conformers has allowed us to extract the geometric requirements in terms of distances between pharmacophoric points. All the remaining compounds, i.e., the moderately active and the inactive ones, were superimposed to the pharmacophore schemes through a distance-constrained conformational search by selecting the appropriate distances between pharmacophore points. The conformers obtained were then checked for their energy from the respective global minimum. Minimization processes and energy calculations were conducted using the BFGS method as implemented in the Sybyl 6.3 software package. Atomic charges were calculated from the Gasteiger−Huckel dictionary, and the dielectric function was set to a constant with a value of 80. Geometry optimization was achieved at 0.05 kcal $mol^{-1}A^{-2}$ of gradient convergence. All calculations were performed on an SGI O2 R5000 workstation.

Results

The results of the grid search analysis and multifit procedure are reported in Tables 1 and 2 for groups I and II, respectively. The 3D depictions of the resulting pharmacophore schemes are shown in Figures 2 and 3 for groups I and II, respectively.

Comparison of the pharmacophore models clearly reveals that, as previously pointed out,[8] distance and conformational requirements are not sufficient to explain the agonist selectivity between group I and group II. Indeed, both group I and II pharmacophores are characterized by distances between amino acidic and distal points which correspond to a nearly extended conformation (*ag*+) of L-glutamic acid (**1**), and the two models are very similar to each other. On the basis of these pharmacophores alone, all the group I agonists would be expected to be also active on group II and vice versa. The selectivity for either group must therefore result from the presence of regions with different topological and steric requirements. This could be inferred by analyzing the occurrence of extra volumes endowed by group I agonists not active on group II and vice versa. Thus, ABHxD-II (**21**), (1*S*,3*S*)-ACPD (**13**), ABHD-V (**10**), (2*R*,4*R*)-APDC (**18**), and LY354740 (**7**), which are endowed with a low energy conformation that neatly fits the group I pharmacophore, were superimposed to all the active group I agonists. The sum of the volumes occupied by active ligands was subtracted by the sum of the volumes occupied by inactive ligands. The results of the volume operations are shown in Figure 4.

Figure 5. Superposition of the putative bioactive confomer (green) of ABHD-II on the global minimum conformation (ΔE = 6.35 kcal/mol). The different spatial disposition of the amino acidic carboxylate group is marked by a red circle.

Two clear regions of forbidden volume can be recognized. The first one is located on the upper side of ABHxD-I (**12**) and is responsible for the lack of activity at group I receptors of ABHD-V (**10**); the second one is located between the amino acidic moiety, and the ring closure and is mainly responsible for the inactivity of LY354740 (**7**). The presence of these two regions of forbidden volume is not sufficient, however, to explain the inactivity of other ligands. It is interesting to note that the conformations of other inactive ligands are substantially too high in energy from the global minimum to achieve a significant binding interaction, yet they fit the group I pharmacophore (RMS < 0.6) and are sterically not much larger than other active ligands. This observationparticularly applies to ABHD-II (**9**) (RMS fit = 0.12, 1.7% of excluded volume, ΔE = 6.6 kcal/mol), L-CCG-II (**3**) (RMS fit = 0.3, excluded volume 3.4%, ΔE = 5.8 kcal/mol), and (2R, 4R)-APDC (**18**) (RMS fit = 0.22, excluded volume 1%, ΔE = 3.2 kcal/mol). It should be noted, moreover, that the very high ΔE of ABHD-II (**9**) is only due to the rotation of the amino acidic carboxylate group (Figure 5).

This would imply that the relative orientation of the carboxylate group is important for binding, in agreement with the accepted mode of binding of group I agonists. They are hypothesized to interact with serine and threonine residues[19] through highly directional hydrogen bonds rather than through isotropic ionic interactions, for which the relative orientation of the carboxylate group could have been neglected. Finally, the low activity of ABHxD-III (**22**) should be ascribed to the fact that it encompasses a folded disposition of glutamate, and a reasonable fit could be achieved only by using four of the five pharmacophoric points. This result, coupled with the moderate activity of **22** as mGluR1 agonist, seems to imply that the folded conformation of glutamate is also tolerated in the group I active site.

The same procedure was applied to inactive ligands at group II receptors. Thus, ABHD-I (**8**), ABHxD-II (**21**), quisqualate (**14**), and (*S*)-3-HPG (**23**), which are endowed with low energy conformations that fit (RMS < 0.6) the group II pharmacophore, were superimposed to all the active ligands, and the volume operations were performed as described above. Again, two areas of excluded volumes can be recognized (see Figure 6). The first one is located on the upper side of LY354740 (**7**) and is responsible for the inactivity of ABHD-I (**8**) and ABHxD-II (**21**), the second one is located near to the distal carboxylate group of group II agonists and is responsible for the inactivity of quisqualate (**14**) and (*S*)-3-HPG (**23**).

As in the case of group I agonists, the inactivity of the rigid compounds ABHD-II (**9**), ABHD-VI (**11**), and ABHxD-III (**22**) can be better explained in terms of the energy cost needed to attain the putative bioactive conformation or by the folded conformation assumed by the latter rather than by excluded volume. It should be noted, however, that also the bioactive conformation of (2*R*,4*R*)-APDC (**18**), L-CCG-I (**2**), DCG-IV (**17**), and even LY354740 (**7**) have a moderate gap from their global minimum, and this fact deserves some explanation. First of all, we investigated the behavior of the highly potent LY354740 (**7**). If we consider the cyclopentane moiety, there are basically two possible conformations that correspond to the flip-flop of one methylene above and below the plane (see Figure 7).

The conformation that corresponds to a pseudoequatorial disposition of the amino group is lower by about 2.5 kcal/mol than the one corresponding to a pseudoaxial disposition of the amino group. However, due to an A_{1-4} interaction, the most stable conformation of the cyclopentane ring forces the amino acidic carboxylate group in a disposition which is not compatible with all the other active group II ligands. It is worthy to note that the 4-amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid (**24**, Chart 4) is claimed to have a potency superior to LY354740 (**7**) as a mGluR2 agonist.[20]

The replacement of the methylene group with the oxygen atom precludes the unfavorable A_{1-4} interaction, thus lowering the conformational gap that eventually penalizes the activity of LY354740 (**7**) (Figure 7), although the oxygen atom may play as such a more specific role (vide infra). The activity of (2*R*,4*R*)-APDC (**18**) and DCG-IV (**17**) can be explained by assuming the presence of a hydrophilic, polar area in the receptor cavity that can conveniently accommodate the basic nitrogen of (2*R*,4*R*)-APDC (**18**) and the carboxylate group of DCG-IV (**17**). Interestingly, the same area can accommodate polar atoms or groups of known group II agonists not included in this study, such as the methoxy group of MCG-I (**25**, Chart 4), the fluoro atoms of L-F_2-CCG-I (**26**, Chart 4), and the endocyclic oxygen atom of 4-amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid (**24**).

Discussion

The comparative analysis of a series of active, moderately active, and inactive agonists at group I and group II mGluRs subtypes has allowed us to clarify the conformational, steric, and energetic requirements that lead to the selective activation of these two groups of mGluRs. The results of our indirect modeling study indicate that the recognition sites of group I and group II receptor subtypes share a number of similarities. Both recognition sites require an *S*-amino acid moiety and a distal carboxylate group in an extended disposition. The distal carboxylate moiety can be substituted by other acidic functions in group I agonists [i.e., quisqualate (**14**), ibotenate (**16**), and 3,5-DHPG (**15**)] but not in group II agonists. Our computational scheme explicitly considers, through the definition of dummy atoms simulating receptor point projections, the directionality of hydrogen bonds, thus accounting for the presence of isosteric replacements. Since in the case of group II ligands there was no difficulty in superimposing the receptor point projections of the carboxylate group and those of the other acidic functionalities (such as the 3,5-dihydroxy phenyl ring of **15** or the heterocycle rings of **14** and **16**), the lack of bioisosterism can only be ascribed to the larger steric requirements of the latter groups with respect to the carboxylate group. This result indicates a difference in the steric environment between the agonist binding site of group I and group II receptors, with the group I recognition site much more sterically accessible in the region of the distal acidic function.

The analysis of a series of bicyclic derivatives, either active or inactive, featured by the required extended disposition of the pharmacophoric groups has allowed the further clarification of the topological environment of the two recognition sites. As previously pointed out by Kozikowski et al.,[4c] two regions can be identified that impact potency and selectivity. The first one is apparently responsible for the high affinity of group II agonists and is inaccessible to group I (region A, according to Kozikowski et al.),[4c] the second one (region B) is equally accessible to either group I or group II but does not confer high affinity to group II agonists. Region A is clearly characterized as a polar, hydrophilic environment. The introduction into molecules of polar atoms or groups that can productively interact with this environment appears to significantly increase their affinity for group II mGlu receptors (when compared to molecules lacking this functionality). Interestingly, the same region A, when occupied by bulky, hydrophobic substituents, is responsible for the antagonist character of the most potent group II antagonists. Other regions of excluded volume, highlighted in Figures 4 and 6, could be identified by comparing conformationally constrained inactive ligands and can be instrumental in the design of new selective ligands. The most interesting result is the observation that in the case of conformationally constrained ligands the lack of activity at both receptor subtypes must be ascribed to the higher energy required by the ligand to achieve the putative bioactive conformation.

Conclusion

In this work we have defined pharmacophoric models for group I and group II mGluR agonists.[22] Five pharmacophoric points, one positively charged, two negatively charged, and two hydrogen bond donating sites, are required for activity at both group I and group II

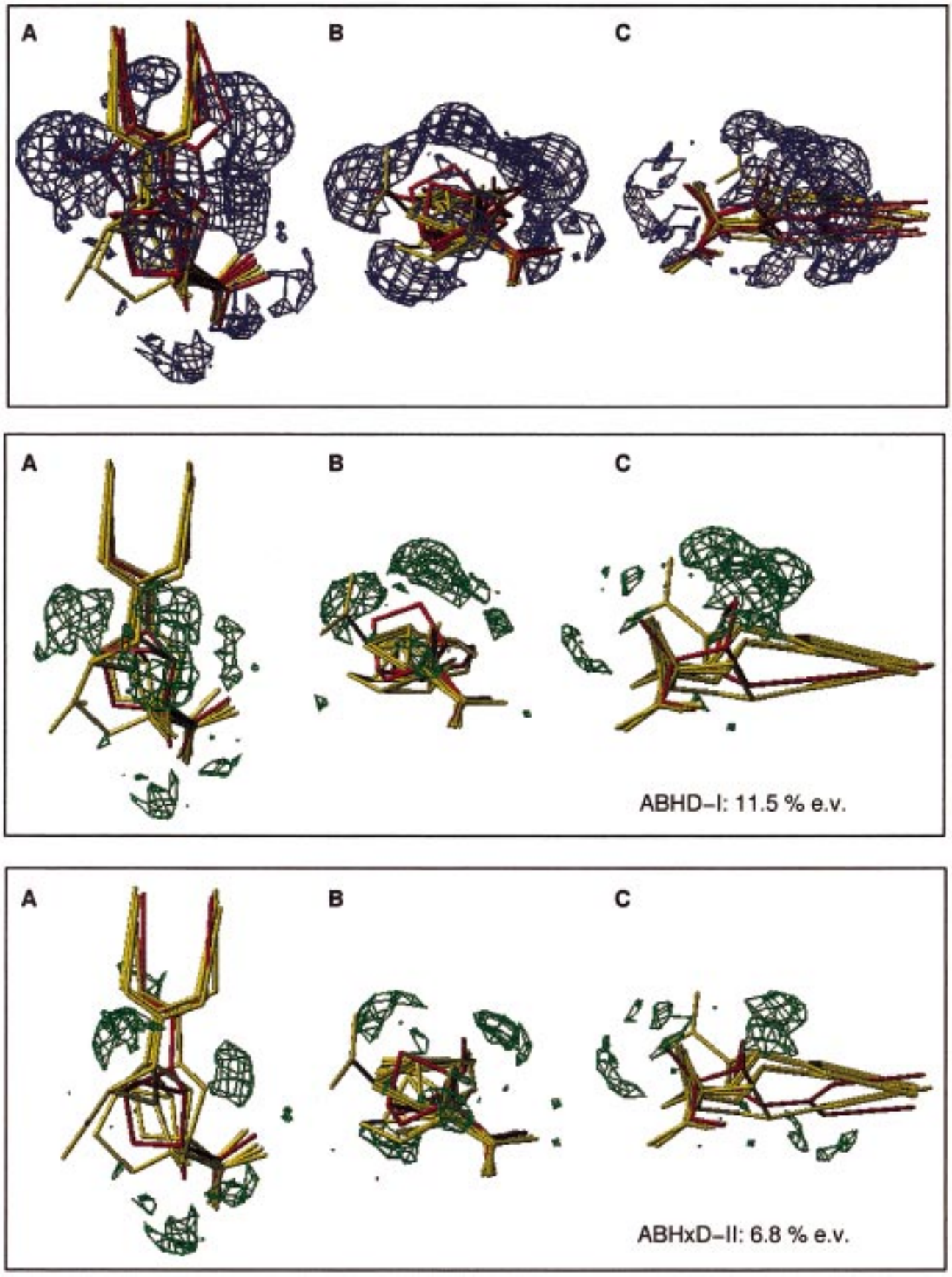


Figure 6a. Excluded volumes in group II pharmacophore. Top: the excluded volume of inactive (red) vs active (yellow) compounds is reported in blue. Middle: the relative contribution of ABHD-I (red) to the total excluded volume is reported in green. Bottom: the relative contribution of ABHxD-II (red) to the total excluded volume is reported in green.

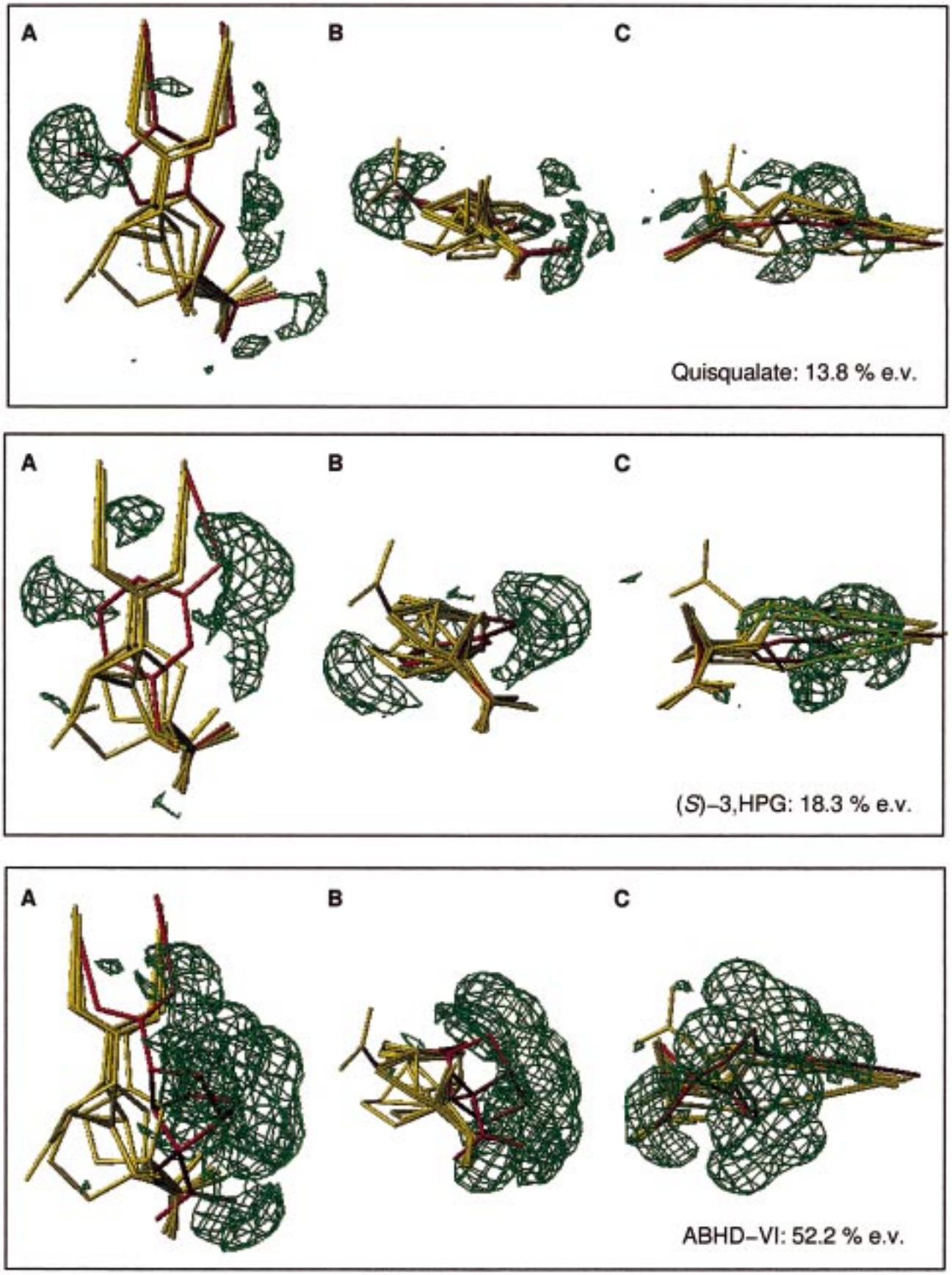


Figure 6b. Excluded volumes in group II pharmacophore. Top: the relative contribution of quisqualate(red) to the total excluded volume is reported in green. Middle: the relative contribution of (*S*)-3-HPG (red) to the total excluded volume is reported in green. Bottom: the relative contribution of ABHD-VI (red) to the total excluded volume is reported in green. Although ABHD-VI presents a 52.2% of e.v., it could be possible that this forbidden region is not informative due to the following evidences: ABHD-VI has a poor fit to the group II pharmacophoric queries (RMS >0.6), its bioactive conformation has a quite high energy gap from its global minimum (ΔE = 3.92 kcal/mol), and finally it is the only compound whose backbone covers such a region.

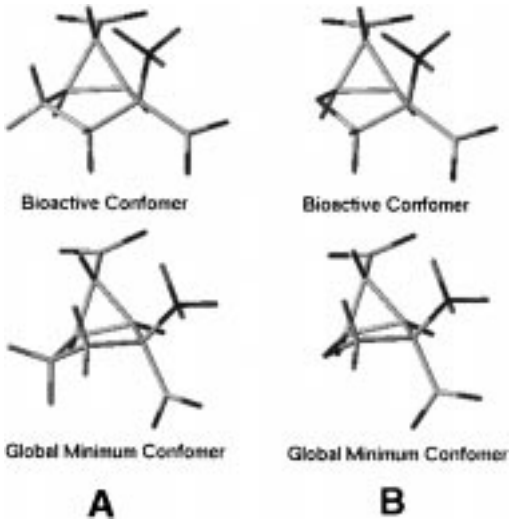
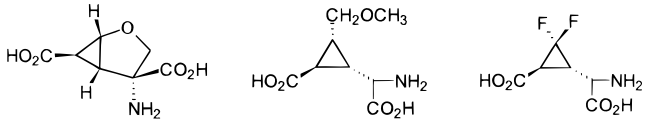


Figure 7. Comparison between LY354740 (A) and 4-amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid (B): energy gap between the bioactive conformation and the global minimum is 2.89 kcal/mol for LY354740 and 1.3 kcal/mol for 4-amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid (see text for details).

Chart 4



24 25 26

mGluRs. L-Glutamic acid (**1**) can be superimposed onto these models in an extended conformation, although group I mGlu receptors can also tolerate a more folded conformation if only four points are considered. Conformational preferences are, however, not sufficient to explain the selectivity of compounds for one group over the other. Excluded volume studies allowed us to map the different steric environment of the two active sites. Region A is clearly characterized as a polar, hydrophilic environment. The introduction into molecules of polar atoms or groups that can productively interact with this environment appears to significantly increase their affinity for group II mGlu receptors (when compared to molecules lacking this functionality). Polar atoms or groups in this region increase affinity toward group II mGlu receptors. Finally, it is worth noting that our results point out the importance of the conformational energetic penalty necessary for attaining the putative bioactive conformation. Taken together, these observations may have valuable impact on the design of new selective agonists for individual mGluR subgroups.

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