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CONFORMATIONAL ANALYSIS OF NON-SULFONYLUREA HYPOGLYCEMIC AGENTS OF THE MEGLITINIDE FAMILY

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Abstract—Non-sulfonylurea hypoglycemic agents of the meglitinide family such as S3075, repaglinide, KAD-1229, and A-4166, were found to display a comparable U-shaped conformation by molecular modelling, with hydrophobic cycles placed at the extremity of each branch and a peptidic bond placed at the bottom of the U. A comparable conformation was observed with the hypoglycemic sulfonylureas glibenclamide and glimepiride. A different conformation with a greater distance between the hydrophobic cycles at the extremity of each branch was found, however, with the biologically inactive enantiomers of A-4166 and repaglinide and the poorly efficient insulinotropic agent meglitinide. The identification of a common conformation of these hypoglycemic agents may help in the design of highly active compounds and provide an imprint of their postulated target receptor on the pancreatic B-cell plasma membrane.

Key words: hypoglycemic agents; meglitinide; repaglinide; glibenclamide; glimepiride; conformational analysis

We have recently drawn attention to the analogy in chemical structure of several new non-sulfonylurea hypoglycemic agents proposed as tools for the treatment of non-insulin-dependent diabetes mellitus [1]. These agents, which include R‡, KAD-1229, and A-4166, also display structural analogy with meglitinide and its analog S3075 [1]. The major aim of the present study was to explore, by conformational analysis, the possible existence of molecular determinants common to these non-sulfonylurea agents and conceivably responsible for their insulinotropic action.

In searching for such a common pharmacophore, attention was paid to the following considerations. First, the insulinotropic efficiency of distinct hypoglycemic agents in the meglitinide family spans almost two orders of magnitude. Second, the enantiomers of both repaglinide and A-4166 are known to be virtually devoid of hypoglycemic activity. Third, meglitinide represents the non-sulfonylurea moiety of glibenclamide. Hence, the comparison between distinct meglitinide analogs, between active and inactive enantiomers, and between non-sulfonylurea and sulfonylurea hypoglycemic agents provides further opportunity to identify the biologically relevant conformation of these molecules.

METHODS

The conformational analysis of the drugs depicted in Fig. 1 is based on a semi-empirical procedure. In this approach, the total conformational energy is calculated as the sum of all contributions due to the Van der Waals energy, torsional potential, electrostatic interactions [2], and hydrophobic interactions [3]. These hydrophobic interactions are taken into account through the calculation of the solvation energy, based on a semi-empirical equation [3]. This equation describes the free energy of solvation between atoms i and j as follows:

$$Etrij = \delta ij(|E_{tri}f_{ij} + E_{trj}f_{ij}|)\exp[(ri + rj - dij)/2rsol)],$$

where δij is equal to -1 when atoms i and j are either both hydrophobic or both hydrophilic and equal to $+1$ otherwise. E_{tri} and E_{trj} are the free energies of transfer from a hydrophobic to a hydrophilic phase, ri and rj are the radii of atoms i and j, respectively, dij is the distance between atomic centers, and $rsol$ the radius of a solvent molecule. The parameter f_{ij} represents the portion of atom i covered by atom j, and is between 0 and 1 [3].

The total interaction energy between atoms i and j is equal to the sum of the Van der Waals energy, electrostatic energy, torsional potential, and the mutual solvation energy of atoms i and j and that of the solvent. The free energy of solvation consists of two components, namely, the interaction energy between solute and solvent molecules and that between the atoms of the solute molecule. Any atom interacting with another atom does not actually distinguish whether this atom belongs to the solute or to the solvent molecule. Each atom will tend to interact with another having the same hydrophobic/hydrophilic properties, independently of the concept of solute or solvent.

The conformation of the molecules is first analyzed by a systematic analysis structure tree applied on each torsional angle. The lowest conformational energy of the most probable structure resulting from this step is subsequently obtained by energy minimization using the Simplex method [4].

The values used for the valence angles, boundary length, atomic charges, torsional potential, and energy values for the Van der Waals interactions are those used in the Hyperchem program (version 3.0), as the molecules were built using this program. They were then returned to the PC-PROT+ program for systematic and energy minimization steps.

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‡ Abbreviations: SH_1, glibenclamide; SH_2, glimepiride; M, meglitinide; K, KAD-1229; R, repaglinide; S, S3075; R′, non-insulinotropic enantiomer of R; A′, non-insulinotropic enantiomer of A.

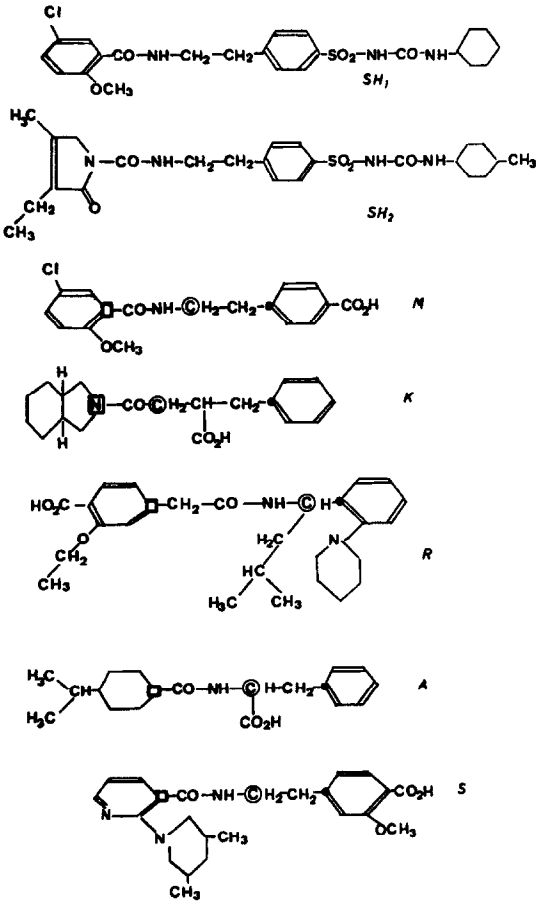

Fig. 1. Chemical formulae of SH_1, SH_2, M, K, R, A, and S. The atoms indicated by a black dot or surrounded by either a square or a circle were used as landmark in the fitting procedure.

The systematic and energy minimization procedures were performed in a medium with a dielectric constant of 1, representative of a medium with no water molecules. This method was previously applied to calculate the conformation of KAD-1229 [5].

Calculations were performed on an Olivetti CP 486 microcomputer equipped with an Intel arithmetic coprocessor, using the PC-PROT+ (Protein Analysis Programs) procedure. Graphs were drawn with the PC-MGM+ (Molecular Graphics Manipulation) and the WinMGM [6] programs.

The following symbols are used to identify each molecule: SH_1 (glibenclamide), SH_2 (glimepiride), M (meglitinide), K (KAD-1229), R (repaglinide), A (A-4166), and S (S3075). The symbols R′ and A′ refer to the non-insulinotropic enantiomers of R and A.

RESULTS

Figure 1 depicts the chemical structure of the seven hypoglycemic agents considered in the present study. In the case of R, it is the S(+) enantiomer that is biologically active. In the case of A, however, the S-enantiomer (or L-enantiomer) is approximately 60 times less potent as a hypoglycemic agent than A itself [7].

As the major aim of our investigation was to identify analogies between M and its non-sulfonylurea analogs (K, R, A, and S), it was first noticed that these drugs display a common backbone. It consisted of a first hydrophobic cycle followed by a peptidic bond, itself linked to an aliphatic chain containing one or more $-CH_2-$ (or $-CH\langle$) group(s), and eventually bound to another hydrophobic cycle. The hypoglycemic agents SH_1 and SH_2 were not included in this scheme, since these molecules present, beyond the second hydrophobic cycle, an elongated chain including the sulfonylurea function. It should be noted that in the case of R, a $-CH_2-$ group is located between the first hydrophobic cycle and the peptidic bond, whereas in the case of K, the peptidic bond occurs between the N atom and belonging to the first hydrophobic cycle and the adjacent carbonyl group.

The most stable conformation of each molecule, including SH_1 and SH_2 as well as A′ and R′, as obtained after energy minimization, is illustrated in Fig. 2.

Considering the common backbone for meglitinide and its analogs, their most stable conformers were fitted to each other by pair. Three atoms were chosen as reference in such a fitting (i.e., the C and O atoms belonging to the carbonyl group of the peptidic bond and the first carbon belonging to the phenyl cycle) (shown as a black circle in Fig. 1). Figure 3 pro\ les so\ ? examples of such fittings.

To quantify the fittings, three representative distances

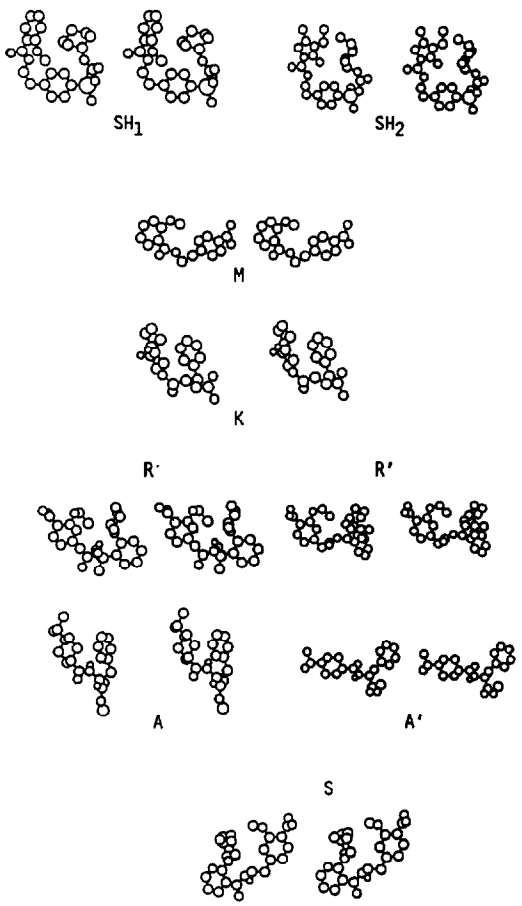

Fig. 2. Stereoscopic view of the most probable conformation of each molecule, as obtained after energy minimization. Atoms are represented as spheres.

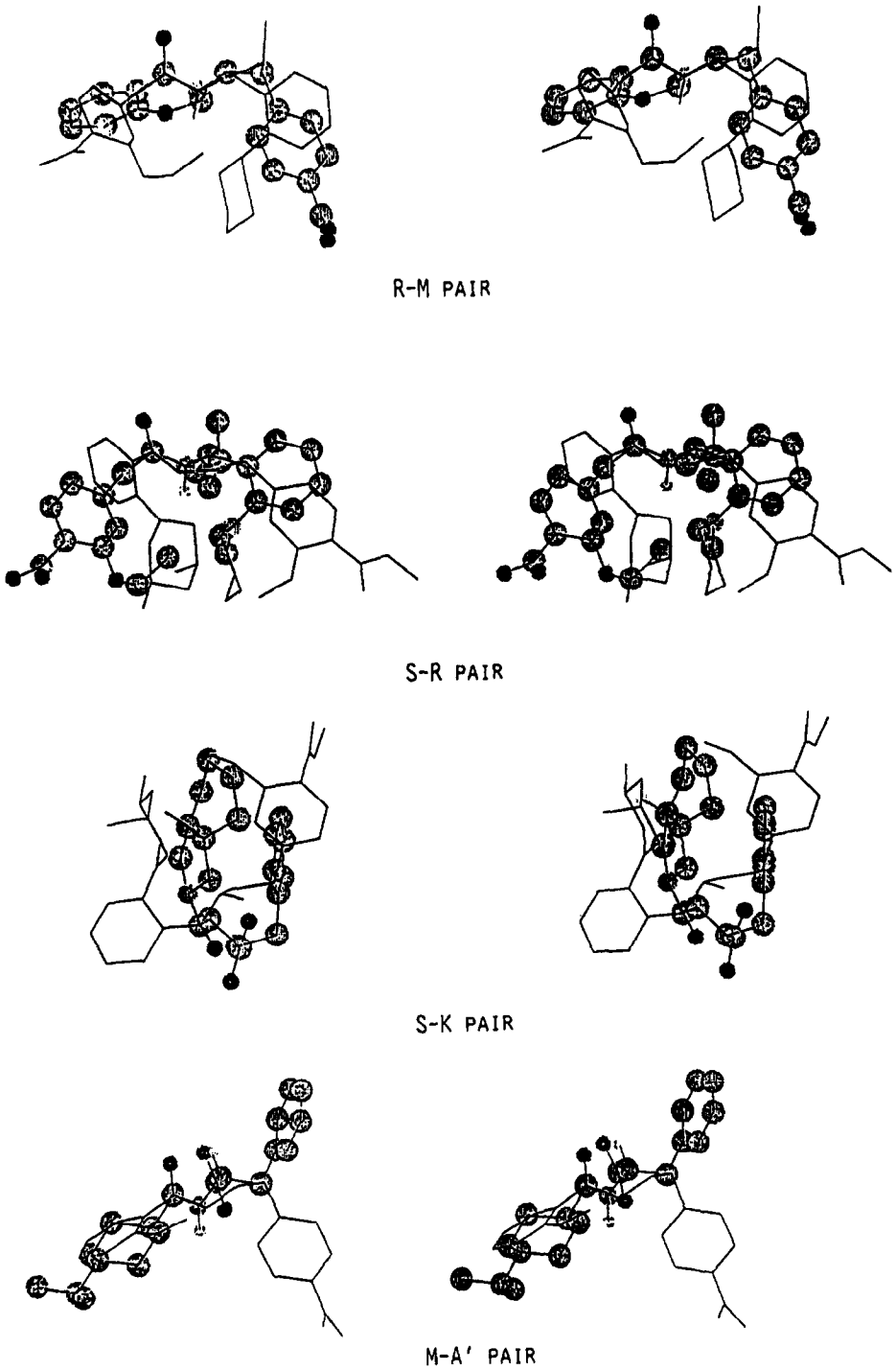

Fig. 3. Paired fitting between R (skeleton) and M (balls), between S (skeleton) and R (balls), between S (skeleton) and K (balls), and between M (skeleton) and A′ (balls). Large grey balls refer to C and N atoms, small and light grey balls to H atoms, and small and dark grey balls to O atoms.

were measured within each molecule pair. They correspond to the distance between the reference first C atoms of the phenyl cycle (shown as a black circle in Fig. 1), the distance between the first atoms of the hydrophobic cycle located close to the peptidic bond (surrounded by a square in Fig. 1), and the distance between the first carbons of the aliphatic chain (surrounded by a circle in Fig. 1).

Table 1 documents that a satisfactory fit was observed for several pairs such as M–K, M–R, M–A, and M–S, R–K, R–A, and R–S, and A–S. However, three molecules (K, A′, and R′) often did not fit the other molecules as well, as indicated by the presence of two distances equal to or in excess of 2 Å. This means that at least one cycle is shifted in the pair of molecules under consideration, as illustrated in Fig. 3 for the S–K and M–A′ pairs.

Table 1. Paired fitting of meglitinide analogs. The distances (expressed in Å) within each pair refer to the first C atom of the phenyl group (C_{phenyl}), the first C or N atom of the other hydrophobic cycle (C_{cycle}), and the first atom of the aliphatic chain (C_{chain}) adjacent to the –CO–group of the peptidic bond

Fair fitting				Poor fitting			
Pair	C_{phenyl}	C_{cycle}	C_{chain}	Pair	C_{phenyl}	C_{cycle}	C_{chain}
M–K	1.6	1.9	2.1	M–A′	2.6	1.0	2.3
M–R	0.7	2.2	0.5	K–R′	1.6	2.1	2.7
M–R′	0.8	1.2	1.5	K–A	0.9	2.3	3.7
M–A	0.9	0.6	1.1	K–S	2.1	1.9	2.1
M–S	0.6	0.0	2.0	R′–A	1.6	2.1	2.2
K–R	0.8	1.3	2.5	A–A′	2.3	1.5	2.8
K–A′	1.5	0.9	1.1	A′–S	3.1	2.0	1.1
R–R′	0.3	2.0	1.1				
R–A	0.4	1.5	2.8				
R–A′	2.2	1.3	1.7				
R–S	1.3	2.2	1.5				
R′–A′	1.9	1.3	0.7				
R′–S	1.6	1.6	1.2				
A–S	1.5	2.5	0.6				

When the most probable conformation of each molecule was further examined, a common shape was observed for all hypoglycemic agents, including SH_1 and SH_2 (Fig. 4). This common conformation, depicted schematically in Fig. 5, can be described as a U shape, in which each branch of the U carries a hydrophobic cycle at its extremity, and the bottom of the U contains the peptidic bond (–CO–N=).

It should be underlined that the non-hypoglycemic drug A′ did not yield the same U conformation. Moreover, the U-shaped aspect appeared less pronounced in the case of R′, which is also devoid of insulinotropic action, and M, which is the least active compound among all hypoglycemic agents under consideration [8].

As depicted in Fig. 6, the mean distance between the two hydrophobic cycles located at the extremity of each branch of the U conformation was estimated from the arithmetic mean value of D1 and D4 when both cycles were made up of 6 atoms (Fig. 6, upper panel), and from the mean value D2, D3, D5, and D6 in the case of K (Fig. 6, lower panel). As shown in Table 2, such a mean distance was close to 5.0 ± 0.5 Å for most drugs under

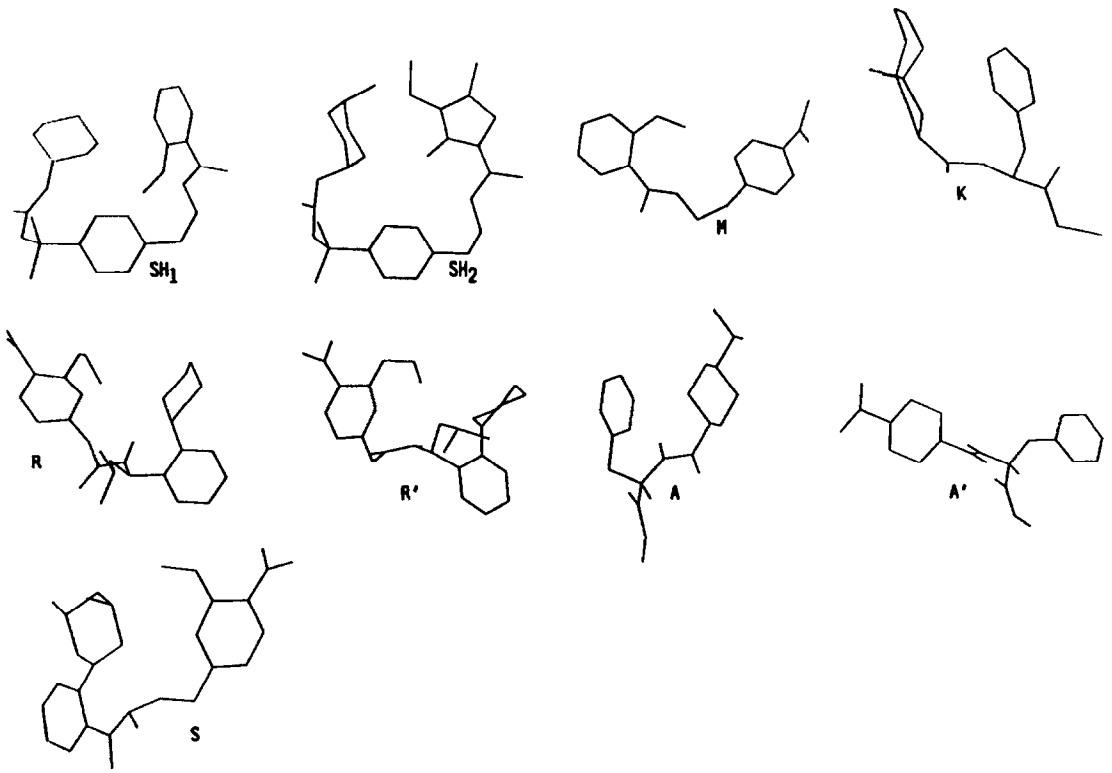

Fig. 4. Skeleton representation of the most probable conformation of SH_1, SH_2, M, K, R, R′, A, A′, and S.

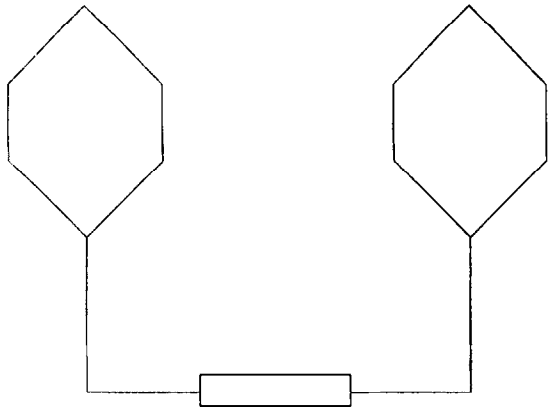

Fig. 5. Schematic representation of the U shape of hypoglycemic agents. The hexagons refer to hydrophobic cycles, and the rectangle to the peptidic bond.

consideration, including SH_1 and SH_2. In the case of M, A′, and R′, however, the mean distance between the two hydrophobic cycles was higher, in the 7.0 to 7.8 Å range.

In view of the less satisfactory fit between K and some other meglitinide analogs (see above), it was considered that such a poor fitting might be due to an ill-inspired choice of the atoms used as reference in the fitting procedure. Indeed, K is the only molecule with a double hydrophobic non-plane cycle at the extremity of one branch of the U conformation. Moreover, the peptidic bond in K occurs between the N atom, which belongs to the double hydrophobic cycle, and the adjacent carbonyl group. As shown in Fig. 7, a much better fitting between K and A was obtained when the atoms chosen as reference consisted of the C atoms belonging to the acidic carboxylic function, the first C of the phenyl group (shown as a black circle in Fig. 1), and the first C atom (in the case of A) or N atom (in the case of K) of the other hydrophobic cycle. The C_{phenyl}, C_{cycle}, and C_{chain} distances, as defined in Table 1, now amounted to 0.8, 0.1, and 1.1 Å, respectively.

Table 2. Mean distance (expressed in Å) between hydrophobic cycles located at the extremities of the U branches in distinct molecules

Distance ≤ 5.5		Distance ≥ 7.0	
SH_1	5.2	M	7.0
SH_2	5.4	R′	7.0
K	4.6	A′	7.8
R	5.5		
A	4.7		
S	5.4		

DISCUSSION

The present study reveals that hypoglycemic agents in both the sulfonylurea series (glibenclamide and glimepiride) and non-sulfonylurea meglitinide family (KAD-1229, repaglinide, A-4166, and S3075) display common conformational characteristics. They all have a typical U shape with hydrophobic cycles located at the extremity of the U branches and a peptidic bond at the bottom of the U. Moreover, the biologically active meglitinide analogs fit each other.

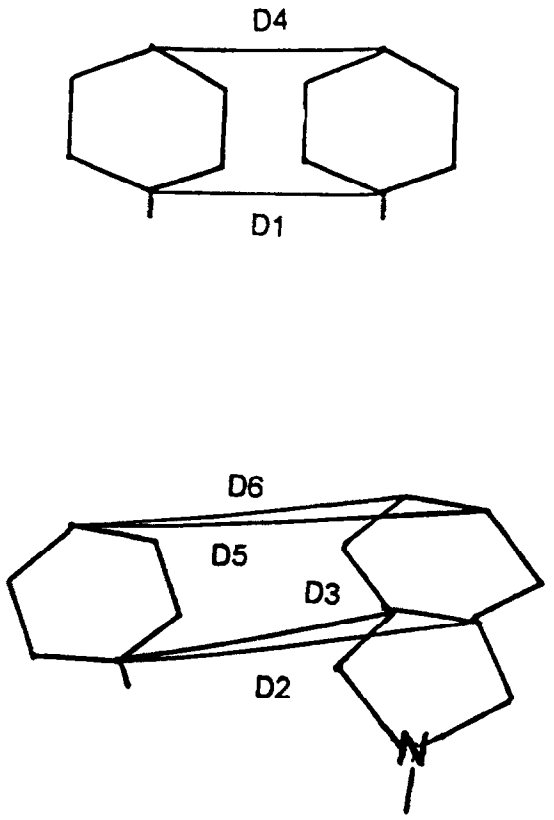

Fig. 6. Indication of the distances (D1 to D6) used to calculate the mean interval between hydrophobic cycles in the case of K (lower scheme) or other drugs (upper scheme).

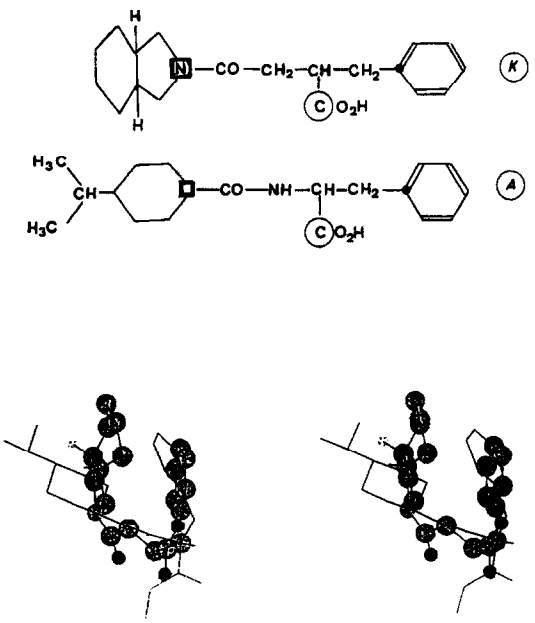

Fig. 7. Fitting between A and K. In the upper panel, the atoms chosen as reference are indicated by a black dot or surrounded by either a square or circle. In the lower panel, the skeleton refers to A and the balls to K. Same presentation as in Fig. 3.

The non-insulinotropic enantiomers of both repaglinide and A-4166 show a poor fitting with the other drugs, and are not well U-shaped. This is confirmed by a higher mean distance between hydrophobic cycles.

These observations thus suggest a link between the U conformation and the insulinotropic or hypoglycemic activity of these molecules. The U shape/activity relationship is also supported by the comparison between meglitinide and its analog S3075. Although these two drugs are close in terms of their chemical formulae, they display vastly different insulinotropic efficiency, S3075 being at least one hundred times more potent than meglitinide. This coincides with the fact that the mean distance between hydrophobic cycles is much larger in the case of meglitinide than S3075. The conformation difference between these two molecules is apparently attributable to a decreased hydrophobic interaction between the cycles located at the U extremities. This is caused by the existence of a $-OCH_3$ group bound to one of the phenyl cycles in meglitinide and its substitution by a hydrophobic cycle in S3075. In the latter case, the hydrophobic interaction is strengthened, whereas, in meglitinide, the $-OCH_3$ group induces a steric hinderance that minimizes the hydrophobic interaction between the two cycles.

It should be stressed that this hydrophobic interaction between cycles is responsible for the U-shaped configuration of the drugs. Indeed, if the free energy of solvation is omitted in the calculation of conformational energy and energy minimization, all molecules lose the U shape, adopting a more linear conformation (data not shown).

The hydrophobic effect might be important in the interaction between the hypoglycemic drugs and their receptor on islet B-cell plasma membrane, in a manner comparable to that previously proposed for propanolol [9].

In conclusion, the finding of a common specific conformation for the hypoglycemic drugs described in this report might help in understanding the molecular determinants of their interaction with the so-called sulfonylurea receptor. It might also be useful in the design of new highly active compounds.

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