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Metformin and glitazones: does similarity in biomolecular mechanism originate from tautomerism in these drugs?

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This theoretical study attempts to find out similarity between metformin and glitazone class of antidiabetic drugs. It was found that some tautomeric forms of both metformin and thiazolidinedione ring of glitazones have similar molecular electrostatic potential (MESP) surface and may bind to a common complementary surface. Complexation and docking studies were also carried out in order to support this hypothesis. Copyright © 2007 John Wiley & Sons, Ltd.

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Keywords: metformin; glitazones; tautomerism; mechanism of action; PPARγ; AMPK

INTRODUCTION

Metformin **1** and Glitazones (e.g. Rosiglitazone **2**) belong to insulin sensitizer class of antidiabetic drugs (Fig. 1). Molecular target for metformin is not yet clearly established. Metformin has been suggested to inhibit complex-I of respiratory chain and impairs both mitochondrial functions and cell respiration.[1] It is also shown that complex-I inhibition may be the cause of anti-hyperglycemic effect of metformin.[1a] Glitazones are known to activate peroxisome proliferator activated receptor gamma PPARγ and induce hypoglycemic response.[2] However, there are evidences of possibility of other molecular mechanisms for glitazones.[3]

Recently, Brunmair *et al*. demonstrated that glitazones also inhibit respiratory complex-I, like metformin. The authors, thus suggested a common mechanism for both drugs starting from inhibition of complex-I, leading to activation of adenosine mono phosphate kinase (AMPK) and finally producing anti-diabetic effect *via* a cascade of events.[4] Moreover, it was discovered that a high PPARγ binding affinity is associated with complex-I inhibition, independent of whether the individual ligand is a receptor agonist or antagonist.[4] The authors, thus stated that same 'moleuclar properties' could be responsible for both PPARγ and complex-I binding.

The above reports suggest that complex-I may be the common target for both glitazones and metformin, and raise the question regarding the similarities between the two moieties. In order to address this issue, we took up the detailed electronic structure study of both the drugs using density functional theory (DFT). Since, thiazlodinedione ring is the key-binding moiety in glitazone class of molecules that makes three hydrogen bonds with PPARγ,[5] methylthiazolidinedione (**3**) was taken for the calculations to represent the glitazones (Fig. 2). *Ab initio* MO studies on metformin (**1**) and methylthiazolidinedione (**3**) revealed that there are remarkable similarities between the tautomeric structures of both molecules and they are capable of binding to a common complementary surface. The results are presented below.

METHODS OF CALCULATIONS

Ab initio MO and density functional theory (DFT) calculations have been performed using Gaussian03 software.[6a] Complete optimisation of **1** and **3** and their tautomers were carried out using B3LYP/6-31+G* method[6b–d] and the final energies of some important isomers were obtained using high accuracy G2MP2 method.[6e] Analytical frequencies of all the minima studied in this work have been estimated by estimating second derivatives of energy to ascertain all the structures are minima on their respective potential energy (PE) surfaces. The molecular electrostatic potential (MESP)[7a,b] plots of the isomers considered in this work were obtained using SPARTAN software.[7c,d] The complexation energies of systems with MESP complementarity were studied using B3LYP/6-31+G*. Molecular docking analysis of a selected set of compounds (**2** and **12**) with PPARγ has been carried out to estimate the binding affinity of these systems with the bimolecular target. Initially Flex X[8] based molecular docking analysis as incorporated in SYBYL6.9 software[9] has been employed to get the best possible poses of the ligand–receptor complexes using the crystal structure with the protein data bank (PDB) code: 2PRG. Energy minimisation of the resulting

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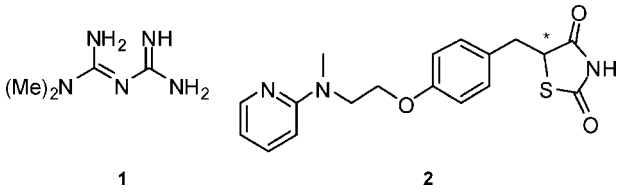

Figure 1. Structures of metformin and rosiglitazone

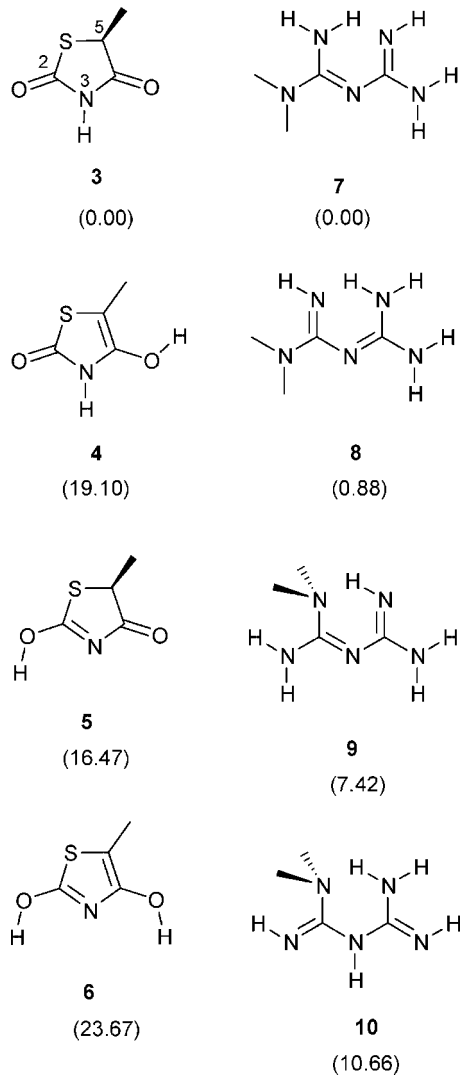

Figure 2. Few important tautomers of methylthiazolidinedione and metformin with relative energies at G2MP2 in the parentheses

complexes was carried out using Tripos force fields using SYBYL6.9 software package on an SGI Octane2 system. The stabilisation energies are estimated as a comparison of energies of the energy minimised ligand–receptor complex with that of the sum of energies of ligands and the apo-protein.

RESULTS AND DISCUSSION

Tautomerism in thiazolidinediones

Thiazolidinediones are chiral but rapidly racemizing cyclic systems. Painstaking separation of R and S isomers was found to be futile because these undergo rapid racemization.[10] Keto-enol tautomerism, in these systems was mainly implicated for the observed racemization. Our earlier studies indicated that the probability of keto-enol tautomerism in thiazolidinediones is not very high.[11a] Following the suggestion by Hulin *et al.*[11b] and the computational[11a] and metabolism studies,[11c] it is currently believed that rapid racemization in thiazolidinedione may involve S-oxide formation. Alternatively, a pathway involving two tautomeric rearrangements first an amide-iminol tautomerism followed by keto-enol tautomerism can be considered. *Ab initio* MO and density functional calculations on 5-methylthizolidin-2,4-dione (**3**), show that the amide-iminol tautomerism involving N3—C2═O unit is much more favourable than the keto-enol tautomerism involving C5—C4═O unit. Methylthiazolidinedione (**3**) can exist in more than 10 tautomeric forms (see supporting information), Fig. 2 shows a few important tautomers (**3–6**). Energy difference between the important tautomers **3** and **4** is 19.1 kcal/mol at G2MP2 level, illustrates that direct keto-enol tautomerisation in **3** is not a favourable process.[12] Amide tautomerism in **3**, leads to **5**, much easily feasible because of the smaller ΔE (16.5 kcal/mol). Also, this process can be assisted by solvent; in the presence of single water molecule, the ΔE between **3** and **5** get reduced by 4.3 kcal/mol (B3LYP), thus indicating that the amide-iminol tautomerism is quite favourable in **3** under polar solvent conditions. Water assistance is not found to favour the **3**–**4** tautomerisation step. Tautomer **6** is only about 7.20 kcal/mol less stable than **5**, showing that the keto-enol tautomerisation is facilitated by the initial amide-iminol tautomerisation in thiazolidinedione derivatives. This double tautomeric action $3 \rightleftharpoons 5 \rightleftharpoons 6$, is likely responsible for the rapid racemization of thiazolidinedione derivatives. Delocalisation of 6π electron in **6** ensures stability to this tautomer and makes it available for binding with receptors *in vivo*.

Tautomerism in metformin

Biguanide was shown to adopt at least 10 different tautomeric forms.[13] Metformin, *N,N*-dimethyl biguanide, can in principle adopt about 28 tautomeric forms (see supporting information); Fig. 2 shows structures of a few important tautomers (**7–10**). Stable tautomers of metformin (**7–9**) possess a C═N—C═N conjugated unit and intramolecular H-bond, which is consistent with our previous electronic structure studies on biguanide.[13] Tautomers **7** and **8** are related with each other with 1,5-H shift with a barrier of about 4 kcal/mol (G2MP2), and thus are expected to exist in equilibrium at room temperature. Tautomer **9** adopts an arrangement which is typical of many biguanide derivatives and is only about 7.42 kcal/mol higher in energy in comparison to tautomer **7**, such a tautomeric state is expected to be an easily accessible state as this energy is much lower than many energies of tautomeric equilibria.[14] Tautomer **10** is another important tautomer of metformin which is only about 10.66 kcal/mol less stable than **7**. Tautomer **10** does not possess the C═N—C═N conjugative interaction but it is still not a very high-energy tautomer because of the intramolecular hydrogen bond. Tautomer **10** is related to **9** in terms of two 1,3-H shifts and has electronic structure complimentary to **9**. All the tautomers of **7** are characterised by delocalisation of lone pair of electrons from the NH_2 groups in addition to conjugative delocalisation as in biguanide.[13] All the tautomers of **7** are thus expected to be thermodynamically accessible in equilibrium, with any of them

being responsible for the therapeutic effect. More significantly, one tautomeric form may produce pharmacological effect with one target while another tautomer may cause a different pharmacological effect on a different target. This factor may be mainly responsible for the lack of clear information regarding the biomolecular target for metformin. This probably is also responsible for the toxic side effects of these classes of compounds—Troglitazone withdrawn from market during hepatotoxicity and metformin is known to show side effects due to lactic acidosis.

Comparison of tautomers

Some of the tautomeric forms of metformin are structurally comparable to the tautomeric forms of methylthiazolidinedione. For example, tautomer **3** of methylthiazolidinedione is structurally analogous to the tautomer **10** of metformin. Likewise, the tautomer **6** of methylthiazolidinedione is structurally comparable to that of tautomer **9** of metformin. Figure 3 shows the 3D structures of these tautomers along with the molecular electrostatic potentials (MESP[15]) of these tautomers. MESP plots of structures **3** and **10** and that of structures **6** and **9** are quite alike, indicating that not only the structural parameters, but also the electronic surface of these systems possesses enough similarities.

The MESP of tautomer **3** of methylthiazolidinedione is complementary to that of tautomer **9** of metformin and MESP of tautomer **3** is also complementary to that of **6**. Complexation between these systems provide information whether the above observed similarities can have any bearing on the ligand–drug

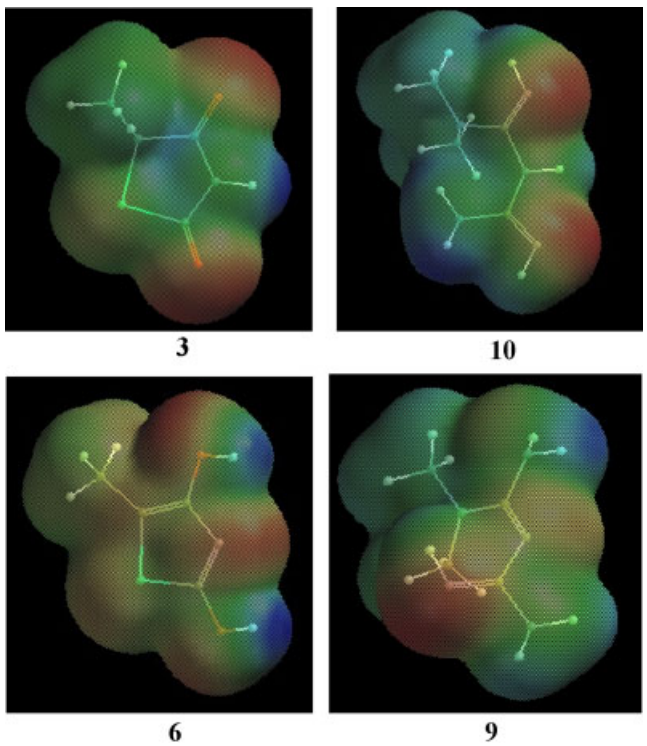

Figure 3. A comparison of molecular electrostatic potentials (MESP) of metformin and methylthiazolidinedione This figure is available in colour online at www.interscience.wiley.com/journal/poc

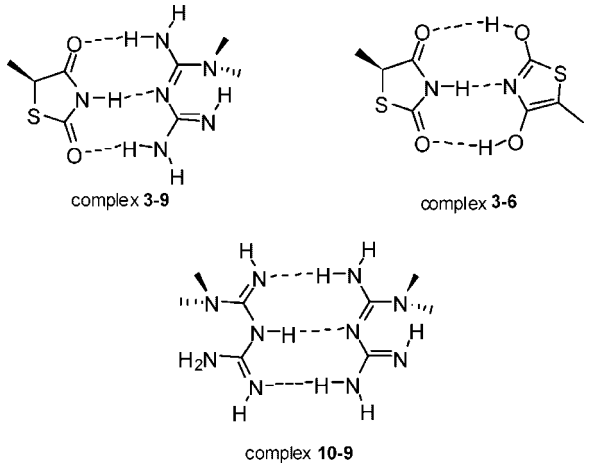

Figure 4. Structures of complexes between the tautomers of metformin and methylthiazolidinedione showing three H-bonds

interactions. Stabilisation energy due to complexation between the structures **3–9** and **3–6**, (Fig. 4) respectively are −13.53 and −13.79 kcal/mol (at B3LYP/6-31+G* level), which are quite comparable. The close comparisons of these values indicate that the binding strengths of **6** and **9** are quite similar. Likewise, **3–9** and **10–9** complexation energies (−13.53 and −10.95 kcal/mol, respectively) differ only by about 2.6 kcal/mol, indicating that the binding affinities of **3** and **10** are also comparable. All these complexes are characterised by three hydrogen bonds each. This data confirm that the comparisons shown using molecular electrostatic potentials get reflected in their binding affinities.

Molecular docking

To further obtain evidence of similar binding strength of the tautomers to a common surface, molecular docking studies were performed (using Flex X module of Sybyl) using PPARγ (crystal structure PDB code: 2PRG).[5] A biguanide derivative **11** was designed, whose tautomer **12** (Fig. 5) has similar electronic structure at thiazolidinedione ring of Rosiglitazone (**2**). Both **2** and **12** were docked into the active site of PPARγ. The docked

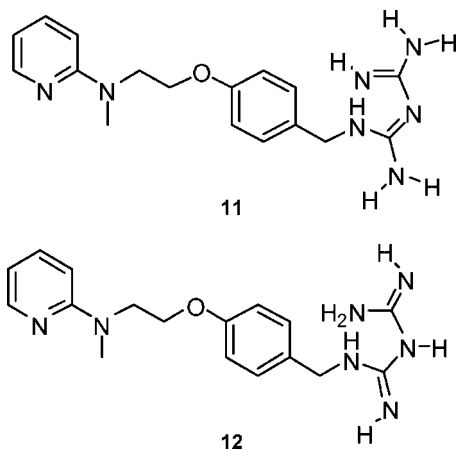

Figure 5. Rosiglitazone molecule with biguanide framework

conformations of **2** and **12** with PPARγ were energy minimised using molecular mechanics methods. As expected, **12** forms strong hydrogen bonding interactions with His323, His449, and Tyr473, similar to that of **2**[16] also as reported in the X-ray structure reports of complex of **2** with PPARγ.[5] The estimated ΔE were found to be quite comparable (−76.94 and −83.14 kcal/mol for **2** and **12**, respectively), thus further supporting the argument that tautomers of metformin and thiazolidinedione ring are capable of binding to a common complementary surface.

CONCLUSIONS

It is the electronic structure and electronic surface of the drug that is important for binding to its biological target. Biomolecules should also possess proper complementary surface for binding. Metformin and glitazones belong to totally different chemical classes with no structural similarity, however, a common molecular target is suggested for these drugs. Tautomerisation is frequently observed phenomenon under biological conditions and both glitazones and biguanides can exist in many tautomeric states. This study reveals that many tautomers of metformin and thiazolidinedione should be available in equilibrium because the energy differences among tautomers are within the known energetic limits of tautomeric equilibria. A few tautomers in both categories are similar and complementary to each other. Furthermore, comparison of MESPs, complexation studies and docking studies indicates that a common binding surface is possible for the tautomers of these drugs. This study finds remarkable similarities among the tautomers of metformin and thiazolidinedione derivatives which belong to two different classes of antidiabetic drugs. Such considerations may play an important role in understanding the drug action (also toxicological action) of therapeutic agents and in identifying biological targets for drugs like metformin.

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