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ORIGINAL PAPER

Pharmacophoric features of drugs with guanylurea moiety: an electronic structure analysis

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Abstract Several therapeutically important compounds contain guanylurea (GU) moiety. The appropriate tautomeric state of these species has not been explored, preliminary studies indicated that the traditional representation of this class of compounds use a high energy tautomeric state. In this work, quantum chemical studies (HF, B3LYP, MP2, G2MP2 and CBS-Q methods) were performed on the medicinally important GU based drugs so as to identify their stable tautomeric state and to understand the pharmacophoric features of these drugs. Electronic structure studies suggested that **GU-1** is the most stable and preferred isomer among the various ketone and enol isomers of the model GU. This study revealed that the general representation adopted in medicinal chemistry literature (**GU-5**) is about 10 kcal mol^{-1} less stable than the energy minimum tautomeric state; and four other alternate structures are possible with energy less than that of the generally represented structure. Hence, it is advisable to consider the energy minimum tautomeric state (**GU-1**) in all future studies of GU derivatives. Further, the importance of the correct tautomeric representation was demonstrated using a comparative molecular docking analysis of WHR 1049 in α2A adrenergic receptor target.

Keywords *Ab initio* study · Guanylureas · Pharmacophoric features · Tautomerism

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Introduction

Guanylurea (GU) (also known as dicyanodiamidine, dicyandiamidine, carbamoylguanidine, carbamimidoylurea, amidinourea) and its derivatives show extensive medical applications [1–10]. Lidamidine, (**1**) (Fig. 1a) a nonnarcotic, nonanticholinergic drug intended for the treatment of diarrhea, is a GU derivative which acts on peripheral alpha-2 adrenoceptors to show its antisecretary and antidiarroheal action [1–4] and it has been categorized as sympatholytic cardiovascular agent [5]. Eaker et al. reported that WHR 1049 (**2**) is a potent demethylated hepatic metabolite of lidamidine which accounts for antidiarrheal and antimotility effects of the lidamidine majorly [6]. Besides the antidiarroheal activity, GU derivatives are reported to have extensive medical applications such as antispasmodic, antiulcerogenic, anesthetic, antihypertensive and antihistaminic activity [7]. Macrocyclic amidinoureas are recently reported for their antifungal activity [8]. Cloguanamil (**3**) is reported for its antimalarial activity [10]. Biclodil (**4**) is reported to have vasodilator effect in congestive heart failure and is used in the therapy of coccidiosis [9]. Fenobam (**5**) is used as anxioltytic and recently it has been identified as a potential drug for the treatment of fragile X syndrome [9]. Besides pharmaceutical applications, GU derivatives show extensive industrial applications also; *N*-guanylurea-dinitramide has been reported as a new energetic material with low sensitivity for propellants and explosive applications [11]. An electrolytic solution of guanylurea phosphate in organic solvent-water mixture is useful for improving the durability of electrolytic capacitors [12]. Hair bleaching and coloring agents containing high-performance, low-irritant properties were prepared with *N*-guanylurea sulfate hydrate [13]. Salts of GU possess promising optical applications [14]. Guanylurea sulfate has been reported to have nitrogenous

fertilizer value on plant growth [15]. Metallic complexes of guanylurea nitrate are used to generate the gas useful in the inflation of automotive inflatable restraint airbag cushions [16]. Considering the medicinal and industrial applications of GU and its derivatives, several synthetic and process strategies were developed for their efficient generation [17–19].

GUs are basic in nature and hence they can readily form phosphate or sulfate or hydrochloric acid salts. Basic property of GUs has been exploited for the preparation of formulations of lidamidine and these preparations are available as HCl salt commercially. GUs also show acidic property and hence the complexes of GU with the metals B, V, As, Cu, Ni, and Si are reported in the literature [20]. GU is a strong bidentate ligand and hence it has been used extensively in coordination chemistry of transition metals. Considering the importance of organotin medicinal applications, GU derivatives of BuMeSnClHL, PhEtSnClHL, PhMeSnClHL, PhBuSnClHL, BuMeSnL, PhEtSnL, PhMeSnL, and PhBuSnL (where H_2L=GU) were prepared and characterized by elemental analysis, FTIR, and multinuclear spectroscopic techniques [21–23].

In 1985 Begley et al. reported the first crystal structure details of the GU in neutral form as 1-carbamoylguanidine ethanol as well as its protonated salt form 1-carbamoylguanidinium perchlorate [24]. Later on in 1991 Scoponi et al. reported the protonated form of GU as its hydrochloride salt and they have performed spectroscopic analysis (vibrational analysis by FTIR and Raman spectra) of GU hydrochloride salt [25]. Their results revealed that protonated GU is characterized by pronounced electron delocalization on the cation and by the presence of strong intra and inter molecular hydrogen bonding interactions between amino groups belonging to the guanidine moiety and the carbonyl oxygen of the ureic group.

In the medicinal chemistry literature, GU and their derivatives are represented using both the structural representations **6** and **7** (Fig. 1b), which bring ambiguity in understanding the electronic structure of GUs. Ray reported the possible tautomers of GU in ketone, enol as well as zwitter ion like structures [20]. However, there is no conclusion in the report regarding the preferred structure of GU. Molecular modeling studies can be successfully carried out only when a proper understanding of the accurate structure, the detailed electronic charge distribution and physicochemical properties of the drug and their chemical moieties are available. The pharmacophoric features of the drugs are associated with the energy minimum state and the electrostatic surface properties in this state. Several groups highlighted the importance of conformational and tautomeric preferences and their importance in the field of pharmaceuticals. Tautomeric preferences play an important role in drug action, determining their p*K*a/PD values *etc*. [26–30]. Understanding the preferred tautomeric state is essential to know the therapeutic profile of any drug at atomic level [26–30]. Our group reported the detailed electronic structure studies, conformational and tautomeric preferences on aminoguanidines, biguanides, thiazolylguanidines, biurets, thiobiurets, guanylthioureas and sulfonylureas which can have the potential therapeutic value in pharmaceuticals [31–39]. Recently Kuhn et al. reported the importance of intramolecular hydrogen bonding in medicinal chemistry and the relation between the hydrogen bonding interaction with several physiochemical properties of the chemical moieties and drug like molecules [40]. Understanding the electronic structure of the preferred tautomer of drugs is an essential component in various applications such as intermolecular interactions of macromolecules, molecular recognition of drug receptor complexes, plausible protonation and deprotonation sites of molecule, preparation of various salt forms of the molecule, study of different polymorphic forms, chelating properties of the ligands and their applications in various industrial and therapeutic areas.

In this report, an electronic structure and the tautomer preferences of drugs containing GU moiety are reported. Conformational analysis of the neutral, cationic, and anionic forms of GU in gas phase is reported. Solvation models were employed in the study so as to understand the influence of medium on conformational preferences. The protonation and deprotonation energies have been estimated using high-accuracy *ab initio* calculations. Natural bond orbital (NBO) analysis has been carried out to study the electron delocalization in various isomers of GU. MESP calculations have been performed to know the surface properties of these compounds. The results indicate that the best tautomeric presentation of this class of compounds is different from the general trend followed in the medicinal chemistry literature.

Computational details

Ab initio molecular orbital (MO) [41, 42] and density functional (DFT) [43, 44] calculations have been carried out using the Gaussian03 software package [45]. Complete optimizations have been performed on various isomers of model GU to understand the electronic structure using Hartree-Fock (HF),Becke3, Lee, Yang, Parr (B3LYP) [46, 47], and fully correlated Moeller-Plesset perturbation (MP2 (full)) [47, 48] methods with the 6–31+G(d) basis set both in gas phase as well as aqueous phase. Solvent level calculations have been performed using the conductor-like polarizable continuum model (CPCM) method [49] at 6–31+G(d) basis set. Frequencies were computed analytically for all optimized species at all levels to characterize each stationary point as a minimum or a transition state and to estimate the

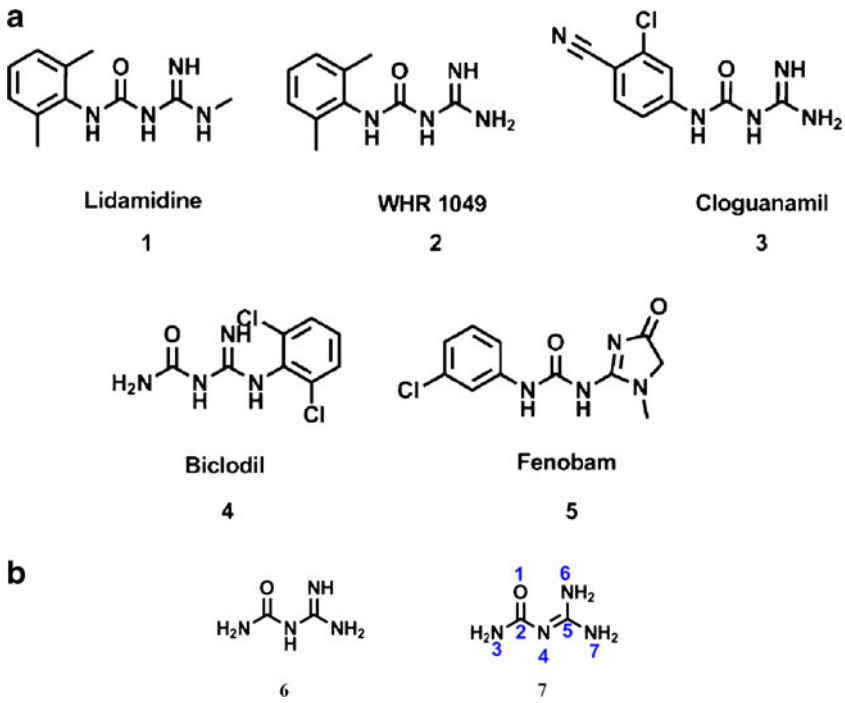


Fig. 1 **a** Several medicinally important guanylurea derivatives, in a representation which is generally adopted in medicinal chemistry literature **b** Structural representation of guanylurea in the literature

zero point vibrational energies (ZPE). The calculated ZPE values (at 298.15 K) have been scaled by a factor of 0.9153, 0.9806, and 0.9661 for the HF, B3LYP, and MP2(full) levels, respectively. The final absolute and relative energies of GU-1 to GU-11 were obtained using the high accuracy Gaussian2-MP2 (G2MP2) [50] and CBS-Q [51] methods. The NBO approach has been employed to quantitatively estimate the second-order interactions [52, 53]. The harmonic oscillator measure of aromaticity (HOMA), a geometry-based aromaticity index, has been applied to quantify the extent of π-electron delocalization of GU and related structures [54–57]. Relative energies and geometrical parameters were obtained using G2MP2 calculations. Intramolecular H-bonding is confirmed by atoms in molecules (AIM) calculations using AIM2000 software package [58, 59]. Electron localization function (ELF) analysis was performed using TopMod software to evaluate the electronic structure of GUs [60].

The molecular electrostatic potentials (MESP) were calculated on the B3LYP/6–31+G(d) optimized geometries of selected GUs and superimposed onto a constant electron density (0.002 e/au^3) to provide a measure of the electrostatic potential at roughly the van der Waals surface of the molecules using SPARTAN'06 software [61]. The color-coded surface provides a location of the positive (deepest blue, most positive) and negative (deepest red, most negative) electrostatic potentials. The regions of positive potential indicate relative electron deficiency (estimated as a function of the repulsion experienced by a positively charged test probe), and regions of negative potential indicate areas of excess negative charge (estimated as a function of the attractive force experienced by a positively charged test probe). Electron localization function (ELF) calculations have been performed on the most stable tautomer **GU-1**. In the discussion, geometrical parameters obtained at B3LYP/6-31+G(d) and energies obtained using G2MP2 method are employed for GU. The discussion on the drugs **1**–**5** is based on B3LYP/6–31+G(d) optimized data.

Molecular docking studies were carried out to demonstrate the importance of tautomeric representation of GU during molecular docking analysis. Molecular docking studies were carried out using FlexX module of SYBYL7.1 installed on a Silicon Graphics workstation running with IRIX6.5 operating system. FlexX program is a fast-automated program based on incremental construction procedure in which the flexibility of the ligands is considered by including several conformations of ligands while maintaining rigid structure for the biomolecule [62]. To carry out the molecular docking study, homology model of α2A adrenergic receptor reported by Halip et al. is considered for the study [63]. Experimentally it has been reported that amino acid Asp113 is the most important residue responsible for molecular recognition of ligands in α2A adrenergic receptor [64]. While creating the receptor description file (rdf), a radius of 6.5 Å around the active site was defined as the area within around the Asp113 to define the active site. The formal charges were assigned to the molecules before submission for FlexX docking calculation. Preliminary docking studies were carried out with a well-known α2A adrenergic agonist clonidine in the α2A adrenergic receptor to verify the experimentally reported binding interaction of Asp113 with α2A adrenergic ligands. Ligand molecules

were built using the builder module of SYBYL7.1 software and these ligands were subjected to minimization to remove close atom or bad van der Waals contacts by using 1000 cycle minimization with standard TRIPOS forcefield. A 0.005 kcal mol^{-1} energy gradient convergence criterion using Powell's method was also used as a final step of energy minimization.

Results and discussion

Conformations of GU drugs

Gas phase geometry optimizations have been performed on medicinally important GU scaffold based drugs at HF/6-31+G(d) and B3LYP/6–31+G(d) levels to verify the conformational preferences. In Fig. 1b it has been shown that structure **6** is the usual representation of GU in scientific literature but the quantum chemical studies are suggesting that structure **7** is the most stable tautomer. On the potential energy surfaces of the drugs **1**–**5**, tautomers represented by structure **7** are energetically more stable than the corresponding tautomers represented by structure **6**. Table 1 lists the energy difference between the two alternate tautomeric states of drugs **1**–**5**, positive values imply that the tautomer represented by structure **6** is less stable. These energy differences are quite significant and lead to the conclusion that the structures shown in Fig. 1a are not the accurate representations of the medicinally important molecules of **GU** based drugs and their appropriate tautomeric representations are shown in Fig. 2a after performing the quantum chemical analysis on these drugs. The 3D structures of the most stable tautomers of **1**–**5** are shown in Fig. 2b. Clearly the observed stability of tautomers in Fig. 2a are due to the π conjugation and strong intramolecular hydrogen bonds. The tautomer preference is smallest (8.35 kcal mol^{-1} at B3LYP/6-31+G(d) level) in fenobam (**5**), presumably due to the cyclic structure of imidazolidinone unit in **5**. This is also reflected in the longer intramolecular hydrogen bond. Maximum tautomer energy difference is shown by cloguanamil (**3**, 15.77 kcal mol^{-1}), followed by lidamidine (**1**, 14.23 kcal mol^{-1}), WHR 1049 (**2**, 12.08 kcal mol^{-1}), biclodil (**4**, 11.98 kcal mol^{-1}). The C$(NR_2)_2$ units (on the right hand sides of central nitrogen) in **1**–**5** are diamino carbenes and probably carry electron donating characteristics as in the case of biguanides [36] and thiazolylgaunides [39]. Drugs lidamidine and biclodil are administered as their HCl salts whereas cloguanamil and fenobam are administered as neutral species. Mir et al. reported that lidamidine.HCl shows rapid clearance from the serum and its metabolite WHR 1049 (neutral form) is responsible for its antidiarrheal and antimotility effects, suggesting that demthylated lidamidine (WHR 1049) is therapeutically active in the neutral form. As these molecules are active in both protonated and neutral form, it is important to establish the site of protonation so as to understand the pharmacophoric features of these molecules. This difference in the practical application indicates that the oral bioavilability of cloguanamil and fenobam are sufficiently high in their neutral state on the other hand the oral bioavailability of lidamidine and biclodil increases upon protonation. This leads to interesting questions on the dipole moment and protonation energy of these drugs. Table 1 also lists the quantum chemically estimated dipole moment and protonation energies of **1**–**5**. The B3LYP/6–31+G(d) estimated dipole moments of lidamidine and biclodil are 4.5 and 4.1 Debye respectively. This low value probably is responsible for their poor bioavailability. Upon protonation, (HCl salts) the dipole moments of these drugs increase to 12.5 and 8.1 respectively. On the other hand, the dipole moments of cloguanamil (11.4) and fenobam (7.5 Debye) are relatively high and thus they are available as neutral formulations. The estimated protonation energies for **1**–**5** are in the range of −214 to−231 kcal mol^{-1}, indicating that these drugs show high nucleophilicity. The preferred site of protonation is the **N4** nitrogen atom. To understand the conformational and tautomeric preferences of GU moiety, potential energy (PE) surface analysis was taken up on a model GU moiety.

Tautomers of guanylurea

The potential energy surface of GU was searched through the electronic structure studies which revealed that the ketone

Table 1 Tautomerization energies (kcal mol^{-1}, ZPE corrected), dipole moment and protonation energies of drugs with guanylurea moiety in gas phase

Drug	Tautomerization energies[a]		Dipole moment	Protonation energy
	HF	B3LYP	B3LYP	B3LYP
Lidamidine	13.54	14.23	4.456	−231.27
WHR-1049	11.93	12.08	3.899	−227.56
Cloguanamil	16.14	15.77	11.419	−214.87
Biclodil	11.72	11.98	4.104	−228.75
Fenobam	9.85	8.35	7.503	−214.42

[a]The basis set used for all optimizations is 6–31+G(d)

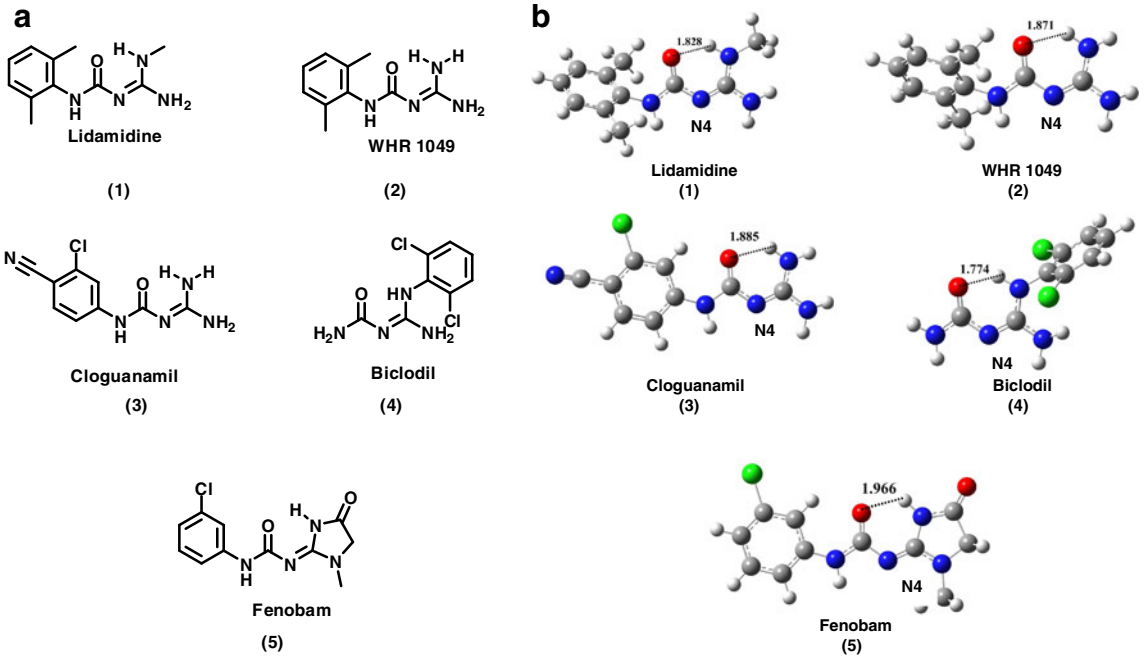


Fig. 2 **a** Corrected structures of drugs **1**–**5** (compare with Fig. 1a). **b** 3D structures of the most stable tautomers of medicinally important guanylurea derivatives. The intramolecular hydrogen bond lengths are given in Å units (B3LYP/6–31+G(d) level of quantum chemical optimization)

isomers (**GU1-GU5**) of GU are more stable than the enol isomers of GU by 15–30 kcal mol^{-1}. **GU-1** was found to be the most stable isomer (global minimium) at all the levels in both the gas as well as in the aqueous phase. The 3D structures of eleven important isomers found on the PE surface of GU are given in Fig. 3. Relative energies of these tautomers are listed in Table 2. **GU-1** has no hydrogen atom on the central nitrogen (N4) and is characterized by O1 H10−N6 hydrogen bonding interaction with a bond length of 1.866 Å. **GU-2** is the next best isomer to **GU-1** on the PE surface with higher 4.92 kcal mol^{-1} energy (G2MP2 data). Solvent phase calculations also support that **GU-1** is the most stable tautomer of GU (See supporting information). 1,3-H shift from N6 to N4 of **GU-1** gives **GU-5**. In scientific literature GU and their derivatives were considered as **GU-5** isomer and molecular modeling studies were reported with this structural representation. Calculations at all quantum chemical levels indicate that **GU-5** is about 9–10 kcal mol^{-1} less stable than **GU-1**. These results are in accordance with the crystal structure studies reported by Begley et al. suggesting that **GU-1** is the correct representation of GU. Presence of hydrogen on central nitrogen (N4) of GU (**GU-5**) causes destabilization to isomer because of the absence of conjugative delocalization of π-electron density. The large energy difference between **GU-1** and **GU-5** clearly suggests that there is insignificant probability of the existence of the tautomer **GU-5** in equilibrium with **GU-1**. This phenomenon is consistent with our earlier reports on biguanides and guanylthioureas suggesting that tautomers characterized by greater electron delocalization are the stable tautomers [31–39]. **GU-2** to **GU-4** are other ketone isomers of **GU-1** which are high energy isomers and such structures cannot be expected to be significant in equilibrium state.

All the enol isomers of GU (**GU-1** to **GU-11**) were observed to be less stable than their respective ketone counterparts (**GU-1** to **GU-5**). At the G2MP2 level, the most preferred enol isomer (**GU-6**), is less stable than **GU-1** by 10.04 kcal mol^{-1}. This energy difference is fairly comparable to that of ketone-enol tautomers of urea (15.04 kcal mol^{-1}) at the same level of theory. More than ten enol isomers of GU were identified on the PE surface but all are with > 10 kcal mol^{-1} relative energy and are ignored for the rest of analysis. Relative stabilities of enol isomers (**GU-6** to **GU-10**) are given in Table 2. The zwitterionic state of **GU-1** (i.e., **GU-11**) is quite high on the relative energy scale.

Electron distribution in guanylurea (GU-1)

To verify the extent of electron delocalization (i) rotational barrier analysis (ii) NBO and AIM analysis and (iii) HOMA analysis were performed on **GU-1**. The rotational barriers across C2-N3, C5-N6 and C5-N7 are 16.13 kcal mol^{-1}, 10.43 kcal mol^{-1} and 10.89 kcal mol^{-1} respectively at G2MP2 level through the rotational transition states (**GUR-1**, **GUR-2** and **GUR-3**) (Table 2). These values represent the extent of π-electron delocalization in GU. N4-C2 (and N4-C5) rotational barrier of **GU-1** is found to

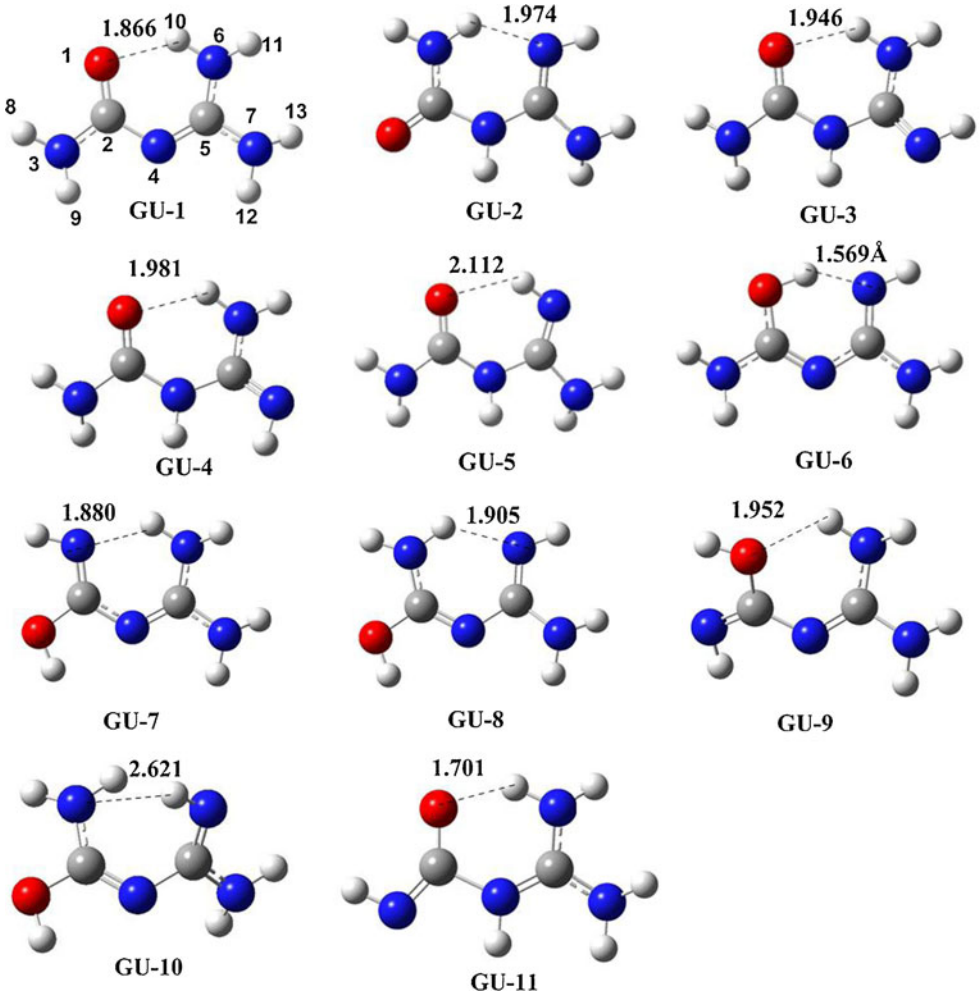


Fig. 3 3D structures of ketone, enol and zwitterionic isomers of guanylurea (**GU**) obtained at B3LYP/6-31+G(d) level of quantum chemical optimization. (**GU-6** conformer geometry is based on MP2 (full)/6–31+G(d) level of quantum chemical optimization). The intramolecular hydrogen bonds given in the figure are in Å units

be 17.75 kcal mol^{-1} (at G2MP2 level) which is comparable to the rotational barrier of biguanide (19.39 kcal mol^{-1}) signifying that delocalization of electrons across the six membered ring (N4−C5−N6−H10···O1−C2) system in **GU-1**. *Ab initio* calculations were performed to explore the 1,3-H shift and 1,5-H shift energetic barriers of **GU-1** (Table S2). Results revealed that the 1,3-H shift is a very high energy (~40–45 kcal mol^{-1}) process. **GU-1** may tautomerise

Table 2 Relative energies (kcal mol^{-1}, ZPE corrected) of various conformers of ketone, enol and zwitterionic isomers of **GU** in gas phase

Structure	Descritpion	HF[a]	B3LYP[a]	MP2(full)[a]	G2MP2	CBS-Q
GU-1	Global minimum among all tautomers	0.00	0.00	0.00	0.00	0.00
GU-2	C2-N4 rotamer with N4-N6 H-shift	7.42	6.57	5.60	4.92	5.29
GU-3	N4-N7 H-shift	7.59	7.14	6.30	5.45	5.84
GU-4	N4-N7 H-shift	10.67	9.73	9.03	7.60	8.13
GU-5	N6-N4 H-shift	11.84	11.71	9.53	9.45	9.98
GU-6	N6-O1 H-shift	15.48	—	12.00	10.04	9.58
GU-7	C2-N4 rotamer with O1-N3 H-shift	19.40	16.95	16.68	14.66	14.86
GU-8	C2-N4 rotamer with O1-N3 H-shift	19.27	17.07	16.71	14.49	14.59
GU-9	N3-O1 H-shift	24.89	22.47	21.70	20.05	20.49
GU-10	C2-N4 rotamer with O1-N6 H-shift	25.94	24.25	22.34	20.40	20.51
GU-11	N3-N4 H-shift	29.98	24.70	24.86	23.85	24.21

[a] The basis set used for all optimizations is 6–31+G(d)

The ZPE corrected absolute energy values are given as supplementary information in Table S1

to **GU-6** *via* a 1,5-H shift with low barrier (10.53 kcal mol^{-1}, MP2 data), but revert back with ease.

Natural bond orbital (NBO) calculations were performed to analyze second order delocalization in **GU-1**. The electron occupancy in the lone pairs at N3, N4, N6 and N7 in **GU-1** are 1.83, 1.92, 1.81 and 1.86 respectively, suggesting greater delocalization from N6. The energy associated with the second-order delocalization $n_{N3} \rightarrow \pi^*_{C2\text{-}O1}$ is 61.65 kcal mol^{-1}. The $E^{(2)}$ associated with $n_{N6} \rightarrow \pi^*_{C5\text{-}N4}$ is 79.67 kcal mol^{-1} and $n_{N7} \rightarrow \pi^*_{C5\text{-}N4}$ is 54.33 kcal mol^{-1}. These values represent strong resonance stabilization of **GU-1** through second order delocalization across the GU molecule.

GU-1 is characterized by a strong intramolecular hydrogen bond which is supported by atoms in molecules (AIM) calculations. An AIM study suggests that for closed shell interactions like ionic bonds and van der Waals interactions (hydrogen bonding interactions) Laplacian of electron density ($\nabla^2\rho$) must be positive. For **GU-1**, Laplacian of electron density ($\nabla^2\rho$) at bond critical point (BCP) was found to be 0.1206 signifying the presence of hydrogen bond between O1··· H10−N6 and total electron density at ring critical point (RCP) was found to be 0.01641 (N4−C5−N6−H10···O1−C2) indicating the presence of six membered ring electronic system due to the presence of strong intramolecular hydrogen bond in GU (MP2(full)/6–31+G(d) level). To measure the extent of electron delocalization quantitatively, HOMA studies were performed and the HOMA parameter for **GU-1** is 0.867 (close to that of benzene). This result suggests H-bond assisted aromaticity [54–57] is present in **GU-1**, which also provides extra stability to this tautomer.

Electron localization function (ELF) analysis [60] showed a bean shaped isosurface at N4 and the population of the V(N1) basin is 3.27 e (see supporting information) signifying electron density accumulation at this center. Protonation studies suggested that protonated GU shows that the preferred site of protonation is N4 but not O1. **GU-1** shows high first protonation energy 224.72 confirming the greater neucleophilicity at N4 center of this species.

Molecular electrostatic potential (MESP) studies give the details regarding the nuclear and electronic charge distribution across the surface of the molecule. MESP color contours provide a location of the positive (blue color) and negative (red color) electrostatic potentials. If the MESP contour shows the deepest blue color then that region is the most positive electrostatic potential. Similarly, if the MESP contour shows the deepest red color then that region is the most negative electrostatic potential. Understanding the MESP maps of drugs and receptors is essential in the computer aided drug discovery, so as to hold potent drug like molecules from the chemical space which can have

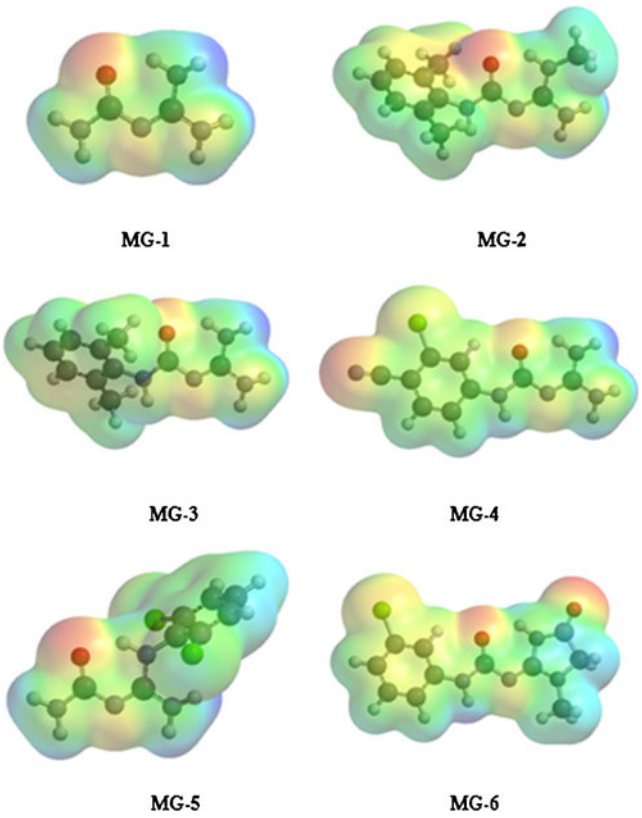


Fig. 4 Molecular electrostatic potential studies on few neutral, protonated and deprotonated guanylureas and the complementary MESP surface of **GU-1** (**MG-1**), lidamidine (**MG-2**), WHR 1049 (**MG-3**), cloguanamil (**MG-4**), biclodil (**MG-5**) and fenobam(**MG-6**).; plotted onto a surface of constant electron density (0.002 e/au^3)

complementary surface to the receptor. Figure 4 shows the MESP of **GU-1** (MG-1) and the five drugs with the GU moiety. MESP surface defines the possible interactive features of GU derivatives with its receptor active site residues and thus represent the pharmacophoric features. The alternative low-high-low electrostatic potentials due to the H_2N-C-N-C-NH_2 unit in the drugs **1**–**5** constitute the pharmacophoric signature of this class of compounds.

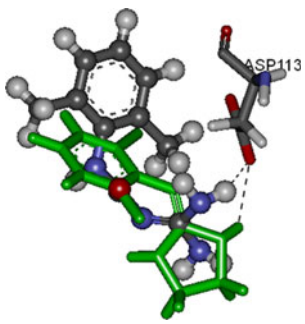
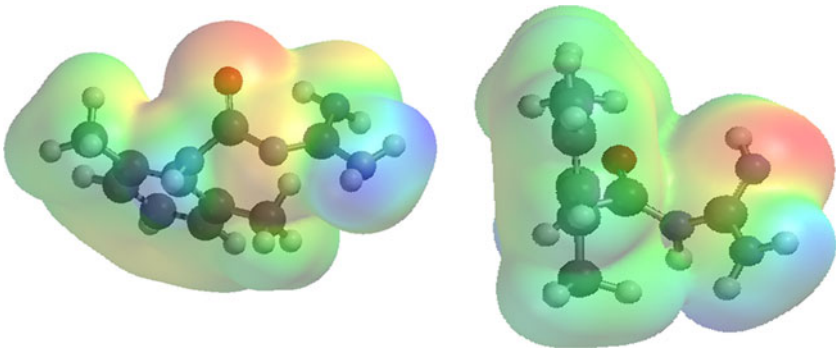


Fig. 5 Docked pose and H-bonding interactions of WHR1049 (**GU-1** isomer, shown in ball and stick model) and clonidine (shown in stick model in green color) with ASP113 of homology model of α2A adrenoceptor



GU-1 conformer of WHR1049 **GU-5** conformer of WHR1049

Fig. 6 MESP surfaces of docked poses of WHR1049 in both conformers **GU-1** and **GU-5**; plotted onto a surface of constant electron density (0.002 e/au^3)

Docking analysis

To demonstrate the importance of tautomeric representation of GU during molecular docking analysis, docking of WHR1049 has been performed with α2A adrenergic receptor. α2A adrenergic receptor interacts with drugs like WHR 1049 and clonidine to produce antidiarrhoeal effect. Halip et al. [62] reported the homology model of α2A adrenergic receptor and employed it in the molecular docking of endogen ligand, norepinephrine. Amino acid Asp113 was reported to be the most important residue responsible for molecular recognition of ligands in α2A adrenergic receptor—both mutation studies and modeling studies—support this hypothesis. The cavity near this aminoacid is characterized by 640 Å volume. WHR1049 can interact with Asp113 in α2A adrenergic receptor cavity producing the therapeutic influence. Molecular docking experiment has been performed using FlexX (version 1.20) module implemented in SYBYL7.1 software package. In order to reproduce the reported binding interaction, validation of docking protocol was performed with clonidine (a well established α2A adrenergic agonist) on α2A adrenergic receptor. Clonidine is able to reproduce the reported binding interaction with ASP133 [64] using FlexX algorithm suggesting the core interaction is reproduced with the software. Later on GU derivative WHR 1049, **GU-1** and **GU-5** isomers were subjected for docking study and it was found that **GU-1** isomer of WHR1049 binds in the cavity in a similar fashion to that of clonidine making a H-bonding interaction with the ASP133 of the α2A adrenoceptor (Fig. 5), however **GU- 5** isomer is not able to show the similar H-bonding interaction with the ASP133 of the α2A adrenoceptor and **GU-5** binds away from the docked site of clonidine signifying that **GU- 5** isomer is not a suitable representation of WHR 1049, explaining the binding of the drug in the cavity of the macromolecule. This analysis clearly suggests that the two tautomers carry different pharmacophoric features and choosing a tautomeric state plays a significant role in the prediction of drug receptor interactions. Further, MESP surfaces of docked poses of WHR1049 were shown in Fig. 6 in both conformers **GU-1** and **GU-5**. MESP contours of WHR1049 shows that the MESP surfaces of the docked poses have similar electronic charge distribution across the surface of the molecule as per the MESP surface of QM optimized structure suggesting that **GU-1** is the most appropriate tautomeric representation for GU rather than **GU-5**.

Conclusions

Ab initio MO and DFT calculations were performed on GU to explore the conformational and tautomeric preferences on the PE surface. Conformational study suggested that there are 11 important conformational minima on the PE surface of model GUs. However, **GU-1** is the most stable isomer and preferred conformer among the various ketone and enol isomers of GU derivatives. **GU-1** is characterized by strong intramolecular hydrogen bond (N3··· H10-N6, 1.891 Å) along with C2−N4−C5−N6 4π-electron conjugation. All other isomers carry decreased electron delocalization followed by weak intramolecular hydrogen bonds. Medicinally important GU derivatives (**1**–**5**) also preferred the **GU-1** conformation with the energy difference of 10–15 kcal mol^{-1} in comparison with **GU-5** isomers in gas phase studies. Molecular docking analysis of the drug WHR 1049 in α2A adrenergic receptor target was performed to demonstrate the importance of correct tautomeric representation. Thus this theoretical study established that correct tautomeric representation is quite essential for drugs [65].

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