

## DFT Study and Biological Activity of Some Methylxanthines

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### Article info

Received: 04/02/2014

Accepted: 29/05/2014

### Keywords:

methylxanthines,  
density functional theory,  
biological activity

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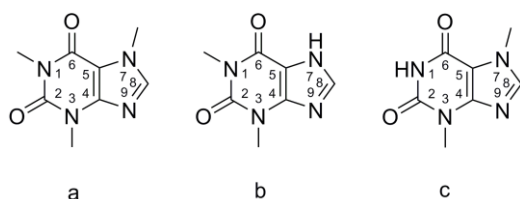
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**Abstract:** Using quantum chemical methods (density functional theory level), biologically active methylxanthines were investigated. All calculations were performed at B3LYP/6-31G\* level of theory. The electronic chemical potential, highest occupied molecular orbital (HOMO)/lowest unoccupied molecular orbital (LUMO) gap, dipole moment, vibration frequencies, electrophilicity, chemical hardness and logP were calculated. Obtained results correspond well with some of the pharmacological properties.

Calculated properties could be useful for quantitative structure-activity relationship (QSAR) analysis which will be performed in the future.

## INTRODUCTION

Methyl derivatives of xanthine are a group of alkaloids commonly used for their effects as mild stimulants on various organ systems such as cardiovascular and CNS (Central Nervous System), and skeletal muscles. The naturally occurring methylxanthines are caffeine, theophylline and theobromine (Figure 1) (Beale, 2011).



**Figure 1.** The 2D structures of caffeine (a), theophylline (b) and theobromine (c)

Caffeine is a widely used CNS stimulant. It acts as a cortical stimulant and facilitates clear thinking and wakefulness, promotes an ability to concentrate on the task at hand and lessens fatigue (Nehlig, Daval, Debry, 1992; Fredholm, Battig, Holmen, et al., 1999).

Theophylline has some medical use as a CNS stimulant, but its main use is in therapy for respiratory diseases such as chronic obstructive pulmonary disease (COPD) and asthma, and infrequently as a diuretic (Barnes, 2006; Beale, 2011).

Theobromine has very little CNS activity, similar to caffeine and theophylline but on a much lower scale (probably because of poor physicochemical properties necessary for distribution in the CNS).

Short review of relative pharmacological potencies of the xanthines is summarized in Table 1 (Beale, 2011).

**Table 1.** Relative pharmacological potencies of the xanthines

Xanthine	CNS Stimulation	Respiratory Stimulation	Diuresis	Coronary Dilatation	Cardiac Stimulation	Skeletal Muscle Stimulation
Caffeine	1 <sup>a</sup>	1	3	3	3	1
Theophylline	2	2	1	1	1	2
Theobromine	3	3	2	2	2	3

<sup>a</sup>1, most potent

The CNS-stimulating effects of the methylxanthines were once attributed to their phosphodiesterase-inhibiting ability. This action is probably irrelevant at therapeutic doses. Evidence indicates that the overall CNS-stimulant action is related more to the ability of these compounds to antagonize adenosine at A<sub>1</sub> and A<sub>2A</sub> receptors (Fredholm, Battig, Holmen, et al., 1999; Daly, 1982; Williams, Huff, 1983; Snyder, Katims, Annau, et al., 1981).

Caffeine and theophylline have pharmaceutically important chemical properties. Both are weak Brønsted bases. Basicity is the result of the imino nitrogen at position 9. Theophylline lacks methyl group at position 7 compared to caffeine, having instead a proton that can be donated. Caffeine cannot donate a proton from position 7. Caffeine does have electrophilic sites at positions 1, 3 and 7. In addition to its Brønsted acid site at 7, theophylline has electrophilic sites at 1 and 3. In condensed terms, both compounds are electron-pair donors, but only theophylline is a proton donor in most pharmaceutical systems (Beale, 2011).

Theobromine differs from caffeine by only one methyl group. However, that single methyl group confers such different properties to caffeine. As a CNS stimulant and diuretic, theobromine is notably less active than caffeine (Pavia, 1973).

Among quantum chemical methods for evaluation of pharmacological effects, DFT has shown significant promise and appears to be adequate for pointing out the changes in electronic structure responsible for pharmacological action (Špirtović-Halilović, Salihović, Džudžević-Čančar, et al., 2013; Stanchev, Mitkov, Georgieva, et al., 2013).

## EXPERIMENTAL

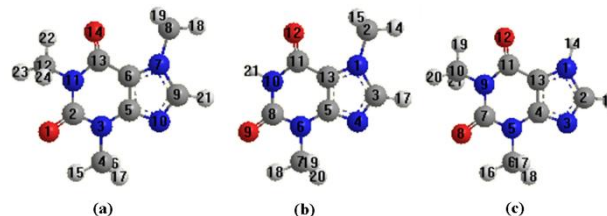
### Density function theory (DFT) study

In order to explore the theoretical-experimental consistency, quantum chemical calculations were performed with complete geometry optimizations using standard Spartan 10 software. Geometry optimization was carried at B3LYP/6-31G\* level of theory. The chemical reactivity descriptors calculated using DFT are: total energy (E), chemical hardness ( $\eta$ ), electronic chemical potential ( $\mu$ ) and electrophilicity ( $\omega$ ).

## RESULTS AND DISCUSSION

### Structural and electronic properties

DFT calculations were performed for caffeine, theobromine and theophylline. Optimized molecular structures of the most stable form are shown in Figure 2.



**Figure 2.** The 3D structures of caffeine (a), theobromine (b) and theophylline (c)

Molecular orbital calculations provide a detailed description of orbitals including spatial characteristics, nodal patterns and individual atom contributions. The contour plots of the frontier orbitals for the ground state are shown in Figure 3, including the Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO).

It is interesting to see that both orbitals are substantially distributed over the conjugation plane. It can be seen from the Figure 3 that the HOMO orbitals are located on the substituted molecule while LUMO orbitals resemble those obtained for the unsubstituted molecule and therefore the substitution has an influence on the electron donation ability, but only a small impact on electron acceptance ability. It can be seen that the energy gaps between HOMO and LUMO of caffeine is 5.12 Hartree, for theobromine is 5.13 Hartree and for theophylline is 5.14 Hartree. The lower value of the HOMO and LUMO energy gap explain the eventual charge transfer interaction taking place within the molecules (Kadhum, Al-Amiery, Musa, et al., 2011).

Chemical hardness is associated with the stability and reactivity of a chemical system, it measures the resistance to change in the electron distribution or charge transfer. On the basis of frontier molecular orbitals, chemical hardness corresponds to the gap between the HOMO and LUMO. Chemical hardness is approximated using equation 1 (Peters, Lanzilotta, Lemon, et al., 1998).

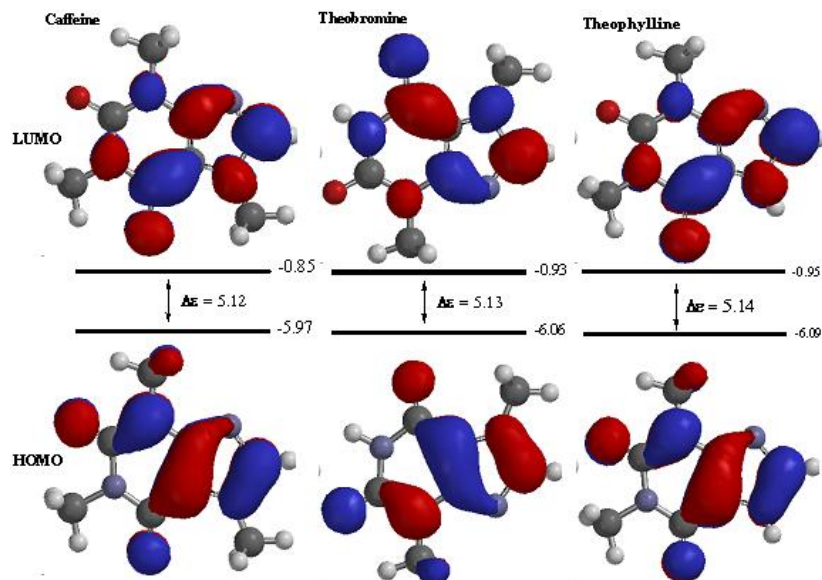
$$\eta = (\epsilon\text{LUMO} - \epsilon\text{HOMO})/2 \quad (1)$$

where  $\epsilon\text{LUMO}$  and  $\epsilon\text{HOMO}$  are the LUMO and HOMO energies.

The larger the HOMO–LUMO energy gap, the harder and more stable/less reactive the molecule (Chattaraj, Maiti, 2003; Liu, 2005; Kadhum, Al-Amiery, Shikara, et al., 2011).

Electronic chemical potential is defined as the negative of electronegativity of a molecule and calculated using equation 2.

$$\mu = (\epsilon\text{HOMO} + \epsilon\text{LUMO})/2 \quad (2)$$



**Figure 3.** Frontier molecular orbitals of caffeine, theobromine and theophylline

Physically,  $\mu$  describes the escaping tendency of electrons from an equilibrium system.

Global electrophilicity index ( $\omega$ ), is calculated using the electronic chemical potential and chemical hardness as shown in equation 3.

$$\omega = \mu^2/2\eta \quad (3)$$

This index measures the propensity or capacity of a species to accept electrons. It is a measure of the stabilization in energy after a system accepts additional amount of electronic charge from the environment (Špirtović-Halilović, Salihović, Džudžević-Čančar, et al., 2013).

Table 2 (row 8) contains the computed chemical hardness values for caffeine, theobromine and theophylline. The results indicate that theophylline is harder and less reactive than theobromine which is harder and less reactive than caffeine. The values of  $\mu$  for caffeine, theobromine and theophylline are presented in Table 2 (row 7). The trend in electronic chemical potential for methylxanthines is caffeine > theobromine > theophylline.

**Table 2.** Global chemical reactivity indices of caffeine, theobromine and theophylline

	Caffeine	Theobromine	Theophylline
E (au)	-680.3764	-641.0661	-641.0621
HOMO (eV)	-5.97	-6.06	-6.09
LUMO (eV)	-0.85	-0.93	-0.95
Dipole moment (debye)	3.80	4.30	3.51
logP	0.17	-0.06	-0.06
Energy gap ( $\Delta$ ) (eV)	5.12	5.13	5.14
$\mu$ (eV)	-3.41	-3.49	-3.52
$\eta$ (eV)	2.560	2.565	2.570
$\omega$ (eV)	2.271	2.374	2.410

The greater the electronic chemical potential, the less stable or more reactive is the compound. Therefore, caffeine is the more reactive than theobromine and theophylline. Theophylline is the least reactive. The electrophilicity values (Table 2, row 9) for the caffeine, theobromine and theophylline are 2.271 eV, 2.374 eV and 2.410 eV. Among the compounds, caffeine is the strongest nucleophile while theophylline is the strongest electrophile.

### Electrostatic potential charges and related quantum chemical properties

The distribution of the electronic density (electrostatic potential charges), related quantum chemical parameters [dipole moment (Table 2, row 4), HOMO/LUMO gap (Table 2, row 6) and the partition coefficients of the compounds (logP; Table 2, row 5)] were calculated for observed methylxanthines. These values and properties are very useful and can be used in order to evaluate chemical properties and possibilities for interaction of methylxanthines with biological macromolecules (receptors, enzymes) as well as their transport through the cell membranes and brain-blood barrier. All these structural, electronic parameters and logP can be used for a building of quantitative structure-activity relationship (QSAR) model, because all of them are closely related to pharmacokinetics (absorption, distribution, metabolism and excretion) and pharmacodynamics (binding with the target proteins) (Stanchev, Mitkov, Georgieva, et al., 2013).

Redox potential can be measured from HOMO/LUMO gap; it is substantial for the metabolism (oxidative reactions) of these compounds involving cytochrome P450 enzymes. The observed HOMO/LUMO gap of 5.12 – 5.14 eV (Table 2) is used to estimate redox potential. The lower negative HOMO energy and the negative values of LUMO energies indicate that these compounds have the general properties of the reductors. This information may be significant for drug metabolism, as the oxidation (and sometimes the reduction) is the main metabolic pathway for many drug molecules.

All three derivatives are polar molecules, judging by their electric dipole moments (Table 2, row 4). The most polar molecule is theobromine. Theobromine differs from caffeine by only one methyl group. However, that single difference confers such different properties. Free nitrogen at position 1 is part of an imide group. This polarity of theobromine hinders passage through blood-brain barrier and explains its notably lesser activity than caffeine as a CNS stimulant and diuretic. Caffeine is strictly basic compound, while theobromine behaves both as an acid and a base depending on conditions (imide and imino groups). Caffeine is found to be the most lipophilic according to the logP values (Table 2, row 5).

Difference in the substituent at the position 7 between caffeine and theophylline gives caffeine this advantage, thus reputedly achieves higher brain concentrations. The half-life of caffeine is 5 to 8 hours, and that of theophylline, about 3.5 hours (Beale, 2011).

#### Atomic charges for caffeine, theobromine and theophylline

These data of caffeine, theobromine and theophylline show that the atomic charges has been affected by the presence of substituent of rings (Peters, Lanzilotta, Lemon, et al., 1998; Kadhum, Mohamad, Al-Amiery, et al., 2011; Kadhum, Wasmı, Mohamad, et al., 2012) as shown in the Tables 3, 4 and 5.

**Table 3.** Atomic charges of caffeine

Atom	Atom Type (MM2)	Charge (MM2)	Charge Huckel	Atom	Atom Type (MM2)	Charge (MM2)	Charge Huckel
O (1)	O Carbonyl	0	-0.953	C (13)	C Carbonyl	0	0.305
C (2)	C Carbonyl	0	0.367	O (14)	O Carbonyl	0	-0.865
N (3)	N Enamine	0	0.460	H (15)	H	0	0.034
C (4)	C Alkane	0	-0.064	H (16)	H	0	0.036
C (5)	C Alkane	0	0.161	H (17)	H	0	0.037
C (6)	C Alkane	0	-0.063	H (18)	H	0	0.037
N (7)	N Enamine	0	0.441	H (19)	H	0	0.040
C (8)	C Alkane	0	-0.065	H (20)	H	0	0.040
C (9)	C Alkane	0	0.053	H (21)	H	0	-0.000
N (10)	N Imine	0	-0.458	H (22)	H	0	0.033
N (11)	N Amide	0	0.398	H (23)	H	0	0.035
C (12)	C Alkane	0	-0.046	H (24)	H	0	0.036

**Table 4.** Atomic charges of theobromine

Atom	Atom Type (MM2)	Charge (MM2)	Charge Huckel	Atom	Atom Type (MM2)	Charge (MM2)	Charge Huckel
O (1)	O Carbonyl	0	-0.950	O (13)	C Carbonyl	0	-0.852
C (2)	C Carbonyl	0	0.384	H (14)	H	0	0.034
N (3)	N Enamine	0	0.457	H (15)	H	0	0.036
C (4)	C Alkane	0	-0.064	H (16)	H	0	0.037
C (5)	C Alkane	0	0.169	H (17)	H	0	0.037
C (6)	C Alkane	0	-0.064	H (18)	H	0	0.041
N (7)	N Enamine	0	0.445	H (19)	H	0	0.041
C (8)	C Alkane	0	-0.065	H (20)	H	0	-0.000
C (9)	C Alkane	0	0.056	H (21)	H	0	0.100
N (10)	N Imine	0	-0.457				
N (11)	N Amide	0	0.285				
C (12)	C Carbonyl	0	0.332				

**Tabela 5.** Atomic charges of theophylline

Atom	Atom Type (MM2)	Charge (MM2)	Charge Huckel	Atom	Atom Type (MM2)	Charge (MM2)	Charge Huckel
N (1)	N Enamine	0	0.331	C (13)	C Alkane	0	-0.042
C (2)	C Alkane	0	0.089	H (14)	H	0	0.085
N (3)	N Imine	0	-0.444	H (15)	H	0	-0.001
C (4)	C Alkane	0	0.168	H (16)	H	0	0.034
N (5)	N Enamine	0	0.463	H (17)	H	0	0.037
C (6)	C Alkane	0	-0.065	H (18)	H	0	0.037
C (7)	C Carbonyl	0	0.366	H (19)	H	0	0.033
O (8)	O Carbonyl	0	-0.953	H (20)	H	0	0.036
N (9)	N Amide	0	0.403	H (21)	H	0	0.035
C (10)	C Alkane	0	-0.046				
C (11)	C Carbonyl	0	0.300				
O (12)	O Carbonyl	0	-0.868				

As a reference compounds, the data for minimized geometry and the 3D-geometrical structures are shown in Figure 3. The Table 3 for caffeine shows that the high estatomic charge in ligand molecule is [O(1) -0.953], next charge value is at [O(14) -0.865] and [N(10) -0.458]. The Table 4 for theobromine shows that the high estatomic charge in ligand molecule is [O(1) -0.950], next charge value is at [O(13) -0.852] and [N(10) -0.457].

## CONCLUSIONS

Caffeine, theophylline and theobromine were investigated, using the hybrid DFT method at B3LYP/6-31G\* level of theory. Judging by their electric dipole moments all three derivatives are polar molecules. The most polar molecule is theobromine. Caffeine is found to be lipophilic, according to the logP values and therefore can easily diffuse through the cell membranes and blood-brain barrier.

Polarity of molecules and negative charges are vital for interaction with serum and target proteins by van der Waals forces and electrostatic interactions. Redox potential of all compounds is about 5.12 – 5.14 eV. According to the corresponding HOMO and LUMO values the analyzed structures may be considered as reductors. All these parameters are closely related to pharmacokinetics and pharmacodynamics of biologically active methylxanthines and can be used for a building of quantitative structure-activity relationship (QSAR) model in the future.

The Table 5 for theophylline shows that the high estatomic charge in ligand molecule is [O(8) -0.953], next charge value is at [O(12) -0.868] and [N(3) -0.444]. These data clearly show that these three atoms of caffeine, theobromine and theophylline are the most reactive towards substitution reactions. Angles determined by the bond and the twist angles and the 3D geometric structure indicates that these molecules are planar.

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## Summary / Sažetak

Koristeći kvantno hemijske metode (teorija funkcionala gustine) istraživani su biološki aktivni metilksantini. Svi proračuni su izvedeni na B3LYP/6-31G\* teorijskom nivou. Izračunati su elektronski hemijski potencijal, razlika u HOMO/LUMO energiji, dipolni moment, vibraciona frekvencija, elektrofilnost, hemijska tvrdoća i logP. Dobiveni rezultati se dobro slažu sa nekim farmakološkim osobinama.

Izračunata svojstva bi mogla biti korisna za analizu kvantitativnog odnosa strukture i aktivnosti (QSAR) koja će se obaviti u budućnosti.