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Kinetic and mechanistic insight into the formation of amphetamine using the Leuckart–Wallach reaction and interaction of the drug with GpC·CpG base–pair step of DNA: A DFT study

Hoda Ostovari1 • Ehsan Zahedi1 • Iraj Sarvi1 • Abolfazl Shiroudi2

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Abstract The Leuckart–Wallach reductive amination reaction in clandestine amphetamine synthesis is the most popular, simple, rapid, and safe non–metal reduction route, in which its mechanism is not known with certainty. The Minnesota 2006 exchange correlation functional M06–2X in conjunction with aug–cc–pVTZ basis set and SMD universal solvation model have been used to elucidate the kinetics and mechanism of the Leuckart–Wallach reaction for the formation of amphetamine via a five–step pathway mechanism in 1–butanol and benzene solvents. The unimolecular and bimolecular rate constants were calculated at the experimentally employed temperature 403.15 K using canonical transition state theory corrected by the quantum tunneling factors. The overall reaction is thermodynamically spontaneous and kinetically second–order (first–order in ammonium formate and first–order in phenyl–2–propane) which is in agreement with experimental results. In the following, drug–
DNA interaction in four different models has been studied in the water solvent using the mPW1B95/6–31G* level of theory. The mPW1B95/6–31G* energies were corrected for the basis set superposition error (BSSE) and the underestimation of London dispersion (DISP) interactions by adding the gCP and D3(BJ) correction terms, respectively. According to the interaction energies, topological analysis of electron localization function (ELF) and localized orbital locator (LOL), interaction of amphetamine with GpC·CpG base–pair step of DNA is non–covalent in nature. Non–covalent interaction index (NCI) plots indicated that there are weak van der Waals and strong stabilizing hydrogen bond attractions between the drug and DNA. Presence of strong stabilizing hydrogen bond attractions is the responsible for the higher negative interaction energies in the interaction models including hydrogen bonds between amphetamine and DNA.

**Keywords** The Leuckart–Wallach reaction • Amphetamine • Kinetics • Reaction mechanism • Density functional theory • DNA

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Introduction

Amphetamine as a contraction of its generic name (\textit{alpha–methyl–phenylethyl–amine}) [1] is a synthetic psychoactive drug with biochemical effects that is abused in many regions of the world. It is a powerful central nervous system (CNS) stimulant and sympathomimetic, which leads to increase of the total level of dopamine in the brain via releasing of dopamine from the nerve cells and blocking the removal of it. Amphetamine also increases the levels of two important brain chemicals norepinephrine and serotonin [2]. In the 1920s and 1930s, amphetamines were prescribed for use as nasal decongestants, in the treatment of hay fever, orthostatic hypotension, epilepsy, parkinsonism, acute and chronic alcoholism, migraine, narcolepsy and as a psycho stimulant. Today, it is proved that the low doses of amphetamine make it an effective treatment for attention deficit hyperactivity disorder (ADHD), narcolepsy, and an aid in weight loss [3,4]. On the other hand, high doses of amphetamine can causes primary clinical syndrome and secondary complications such as neurological, cardiovascular, renal, musculoskeletal, pulmonary, and gastrointestinal effects. Other effects of overdose are tremor, agitation, hyperreflexia, combative behavior, confusion, hallucinations, delirium, anxiety, paranoia, movement disorders, seizures, coma, aortic dissection, vasospasm, cerebral vasculitis, with subsequent intracerebral hemorrhage.
and myocardial infarction [5,6]. From the structural point of view, amphetamine is a methyl homolog of phenethylamine in which attachment of methyl group to its side chain leads to prevention of degradation of amphetamine by the enzyme monoamine oxidase during digestion. The asymmetric carbon atom adjacent to the primary amine of amphetamine is a stereogenic center, which can leads to optical activity of amphetamine. Amphetamine is a racemic 1:1 mixture of two enantiomeric mirror images that can be classified as levorotatory, $(R)$–amphetamine, and dextrorotatory, $(S)$–amphetamine, isomers (see Figure 1). Greater biological activity of $(S)$–amphetamine enantiomer with respect to the $(R)$–amphetamine make it as a racemic drug with one major bioactive enantiomer [3,7].

![Diagram of Amphetamine Isomers](image)

**Fig. 1** The $(S)$ and $(R)$ isomers of amphetamine.
Since the first synthesis of amphetamine in 1887 by the Romanian chemist Lazar Edeleanu [8], numerous legal and illicit methods for synthesis of amphetamine have been reported [9], such as Hofmann [10] Curtius [11,12], and Schmidt [12] rearrangements, heterogeneous reduction [13], Friedel–Crafts alkylation [14], Henry reaction [15], Knoevenagel condensation [16], Ritter reaction [17], and Leuckart–Wallach reductive amination reaction [18,19]. In the clandestine laboratories, the former synthetic route is the most popular because the procedure is simple, rapid, and safe with high efficiency (see Scheme 1).

Scheme 1

The Leuckart–Wallach reaction is a non–metal reductive amination of carbonyl compounds by ammonium or amine salts of formic acid. An original mechanism for the reductive amination of carbonyl compounds with ammonium formate was proposed by Wallach [19-23] (see Scheme 2). The initial step (I) of this mechanism is dissociation of ammonium formate into formic acid and ammonia. Ammonia then nucleophilically attacks, step (II), on the carbonyl carbon to form carbonyl ammonia. In the step (III), water molecule leaves the carbonyl ammonia and the resulting
imine is then, step (IV), reduced by formic acid to the amine. Other mechanism is based on formation of carbenium–immonium cation intermediate [24]. In this mechanism (see Scheme 3) after the formation of carbonyl ammonia in second step, oxygen atom can be protonated, step (III), via an attack of the produced proton from the hydrolysis of formic acid. The carbenium–immonium cation intermediate may be formed in the step (IV) by elimination of water molecule from the protonated carbonyl ammonia. Formic acid as reducing agent attacks to the carbenium–immonium cation intermediate, step (V), and forms a cyclic transition state. The products of this step are carbon dioxide and a protonated amine. Finally, produced formate anion in the side reaction attacks to the protonated amine (VI), to form a formic acid and an amine.

 Scheme 2

![Scheme 2](attachment://Scheme_2.png)
Another interpretation [25] (see Scheme 4) of the Leuckart–Wallach reaction, involves nucleophilic attack of carbonyl oxygen on formic acid in step (II). In step (III) ammonia performs nucleophilic attack to the protonated carbonyl. Protonation of oxygen by abstraction of hydrogen from nitrogen in step (IV), and subsequently elimination of water in step (V) lead to the formation of carbenium–immonium cation intermediate. Reduction (VI) and deprotonation (VII) steps occur similar to the former mechanism.

In spite of the fact that different mechanisms have been proposed for the Leuckart–Wallach reaction, there is not a known mechanism with certainty [26]. Therefore, detailed kinetic study is required to elucidate feasible mechanism for the Leuckart–Wallach reaction. The first purpose of this paper is to establish the kinetics and mechanism of formation of amphetamine from the energetic point of view. Because of this, the molecular level details and mechanistic insights for the possible Leuckart–Wallach mechanisms were explored using the density functional theory.

The other purpose of this work is to describe qualitatively and quantitatively the non–covalent interaction of amphetamine with a small portion of DNA.
Scheme 3

\[ \text{HCOONH}_4 \xrightarrow{\text{I}} \text{HCOOH} + \text{NH}_3 \]

\[ \text{R}^1 \text{C}=\text{O} + \text{NH}_3 \xrightarrow{\text{II}} \text{R}^1 \text{C} (\text{OH}) \text{NH}_2 \]

\[ \text{HCOOH} \xrightarrow{\text{III}} \text{HCOO}^- + \text{H}^+ \text{ Side reaction} \]

\[ \text{R}^1 \text{C} (\text{OH}) \text{NH}_2 + \text{H}^+ \xrightarrow{\text{IV}} \text{R}^1 \text{C} (\text{O}) \text{H} \]

\[ \text{R}^1 \text{C} (\text{OH}) \text{OH} \xrightarrow{\text{V}} \text{R}^1 \text{C} (\text{NH}) \text{H} + \text{H}_2\text{O} \]

\[ \text{C} = \text{N} \text{H} + \text{HCOOH} \xrightarrow{\text{VI}} \text{R}^1 \text{C} (\text{NH}) \text{H} + \text{HCOOH} \]

\[ \text{R}^1 \text{C} (\text{NH}) \text{H} + \text{HCOO}^- \xrightarrow{\text{VI}} \text{R}^1 \text{C} (\text{NH}) \text{H} + \text{HCOOH} \]
Scheme 4

\[
\begin{align*}
\text{HCOONH}_4 & \xrightarrow{\text{I}} \text{HCOOH} + \text{NH}_3 \\
\text{R}^1\text{C} = \text{O} + \text{HCOOH} & \xrightarrow{\text{II}} \text{R}^1\text{C} = \text{O} + \text{HCOO}^\ominus \\
\text{R}^1\text{C} = \text{O}^\ominus + \text{NH}_3 & \xrightarrow{\text{III}} \text{R}^1\text{C} = \text{O}^\ominus \\
\text{R}^1\text{C} = \text{O}^\ominus + \text{NH}_3 & \xrightarrow{\text{IV}} \text{R}^1\text{C} = \text{O}^\ominus \\
\text{R}^1\text{C} = \text{O}^\ominus + \text{OH}_2 & \xrightarrow{\text{V}} \text{R}^1\text{C} = \text{O}^\ominus \\
\text{R}^1\text{C} = \text{O}^\ominus + \text{HCOOH} & \xrightarrow{\text{VI}} \text{R}^1\text{C} = \text{O}^\ominus \\
\text{R}^2\text{C} = \text{N}^\ominus & \xrightarrow{\text{VII}} \text{R}^2\text{C} = \text{N}^\ominus + \text{HCOOH}
\end{align*}
\]
DNA is a presumed target for a wide range of drugs that plays an important role in biological processes. Since DNA carries the hereditary information codes, distortion of its structure induced by drug binding is responsible for changes in essential biological processes such as synthesis of proteins and enzymes which lead to a cascade of biological responses ultimately ending in apoptosis, or programmed cell death [27,28]. Drug–DNA interactions can be classified into covalent and non–covalent interactions. The three major modes of non–covalent interactions are: i) electrostatic interactions between the negatively charged phosphate backbone of DNA and the positively charged ends of drug, ii) groove binding involves hydrogen bonding or van der Waals interactions of drug with nucleic acid bases, and iii) intercalation of drug within the nucleic acid base pairs [29]. Understanding how amphetamine interact with DNA is the interface between chemistry and medicine.

**Results and discussion**

As a part of ongoing work, kinetics and thermodynamics of the amphetamine formation via the Leuckart–Wallach mechanism using ammonium formate 1 and phenyl–2–propane 4 precursors, as depicted in Scheme 5, have been studied. The gas phase optimized geometries of transition states associated with the steps II–V are shown in Figure 2.
Thermodynamic and kinetic parameters associated with all steps of the studied mechanism are calculated and tabulated in Tables 1–3.

Scheme 5

Step I:

\[
\begin{align*}
1 & \quad 2 \quad 3 \\
\text{Step II:} & \quad 4 \quad 2 \quad \overset{\text{TS II}}{\rightarrow} \quad 5 \quad 6 \\
\text{Step III:} & \quad 5 \quad \overset{\text{TS III}}{\rightarrow} \quad 6 \quad 7 \\
\text{Step IV:} & \quad 3 \quad \overset{\text{TS IV}}{\rightarrow} \quad 8 \\
\text{Step V:} & \quad 6 \quad 8 \quad \overset{\text{TS V}}{\rightarrow} \quad \text{amphetamine} \quad \text{amphetamine} \quad 9
\end{align*}
\]
Fig. 2 M06–2X/aug–cc–pVTZ gas phase geometries of transition states associated with the steps II–V and their imaginary vibrational frequencies. The lengths of the breaking/forming bonds are in Å, and imaginary frequencies are in cm$^{-1}$. 
Table 1 Thermodynamic parameters for all steps of the reaction mechanism in the 1–butanol and benzene solvents calculated at 403.15 K at the M06–2X/aug–cc–pVTZ level of theory

<table>
<thead>
<tr>
<th></th>
<th>(\Delta H) (kJ.mol(^{-1}))</th>
<th>(\Delta G) (kJ.mol(^{-1}))</th>
<th>(K)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1–Butanol</strong> ((\varepsilon = 17.3320))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step I</td>
<td>28.24</td>
<td>-19.15</td>
<td>3.03 \times 10^2</td>
</tr>
<tr>
<td>Step II</td>
<td>-27.04</td>
<td>45.34</td>
<td>1.34 \times 10^{-6}</td>
</tr>
<tr>
<td>Step III</td>
<td>33.32</td>
<td>-35.89</td>
<td>4.46 \times 10^4</td>
</tr>
<tr>
<td>Step IV</td>
<td>4.60</td>
<td>4.11</td>
<td>2.93 \times 10^{-2}</td>
</tr>
<tr>
<td>Step V</td>
<td>-67.13</td>
<td>-53.23</td>
<td>7.88 \times 10^6</td>
</tr>
<tr>
<td><strong>Benzene</strong> ((\varepsilon = 2.2706))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step I</td>
<td>40.32</td>
<td>-7.07</td>
<td>8.24 \times 10^0</td>
</tr>
<tr>
<td>Step II</td>
<td>-22.57</td>
<td>49.80</td>
<td>3.53 \times 10^{-7}</td>
</tr>
<tr>
<td>Step III</td>
<td>39.92</td>
<td>-29.28</td>
<td>6.22 \times 10^3</td>
</tr>
<tr>
<td>Step IV</td>
<td>13.44</td>
<td>12.95</td>
<td>2.10 \times 10^{-2}</td>
</tr>
<tr>
<td>Step V</td>
<td>-97.54</td>
<td>-83.63</td>
<td>6.85 \times 10^{10}</td>
</tr>
</tbody>
</table>

Dissociation of ammonium formate \(1\) into formic acid and ammonia is a barrierless step endothermic by 28.24 and 40.32 kJ.mol\(^{-1}\) and spontaneous by -19.15 and -7.07 kJ.mol\(^{-1}\) (see Table 1) in the 1–butanol and benzene solvents, respectively. Negative Gibbs free energy of step I is due to the high employed temperature of procedure and positive effect of entropy. In fact, in the step I of the mechanism endothermicity plays a small role in the balance. Equilibrium constant values and barrierless nature of this step lead to easy formation of formic acid and ammonia. Due to the consumption of them in steps II and IV, there is not unfavorable factor for generation of formic acid and ammonia, and based on Le Chatelier's principle they can be easily produced along the reaction course.
In the step II, 2-amino-1-phenylpropan-2-ol 5 is produced by the nucleophilic attack of the generated ammonia in the previous step on the carbonyl carbon of phenyl-2-propane 4.

**Table 2** Kinetic parameters associated with the steps II–V of the reaction mechanism in the 1-butanol and benzene solvents calculated at 403.15 K by means of CTST theory at the M06-2X/aug-cc-pVTZ level of theory

<table>
<thead>
<tr>
<th></th>
<th>$E^\dagger$ (kJ.mol$^{-1}$)</th>
<th>$E_a$ (kJ.mol$^{-1}$)</th>
<th>$k$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1-Butanol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step I</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Step II</td>
<td>103.01</td>
<td>105.55</td>
<td>$3.0910 \times 10^{-29}$</td>
</tr>
<tr>
<td>Step III</td>
<td>233.13</td>
<td>216.97</td>
<td>$3.3730 \times 10^{-15}$</td>
</tr>
<tr>
<td>Step IV</td>
<td>45.17</td>
<td>42.76</td>
<td>$4.4813 \times 10^7$</td>
</tr>
<tr>
<td>Step V</td>
<td>52.50</td>
<td>54.92</td>
<td>$2.7242 \times 10^{-22}$</td>
</tr>
<tr>
<td><strong>Benzene</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step I</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Step II</td>
<td>126.89</td>
<td>129.42</td>
<td>$2.4866 \times 10^{-32}$</td>
</tr>
<tr>
<td>Step III</td>
<td>232.97</td>
<td>216.77</td>
<td>$3.5395 \times 10^{-15}$</td>
</tr>
<tr>
<td>Step IV</td>
<td>49.51</td>
<td>47.09</td>
<td>$1.3208 \times 10^7$</td>
</tr>
<tr>
<td>Step V</td>
<td>65.39</td>
<td>67.80</td>
<td>$5.8234 \times 10^{-24}$</td>
</tr>
</tbody>
</table>

(a) Activation energies are calculated by the linear fitting of the rate constants against inverse temperature over the temperature range 322–484 K.

(b) The units of $k$ for unimolecular and bimolecular reactions are in terms of s$^{-1}$ and cm$^3$.molecule$^{-1}$.s$^{-1}$, respectively.

Formation of carbonyl ammonia 5 is an exothermic step by −27.04 and −22.57 kJ.mol$^{-1}$ and nonspontaneous by 45.34 and 49.80 kJ.mol$^{-1}$ (see
Table 1) in the 1–butanol and benzene solvents, respectively. This step shows a barrier of 105.55 and 129.42 kJ.mol$^{-1}$ (see Table 2) in the 1–butanol and benzene solvents, respectively. Transition state of this step is associated with simultaneous N–C and O–H bond formation and N–H bond breaking (see Figure 2). Large imaginary frequency of 1368.6i cm$^{-1}$ associated with the TSII implies that the quantum tunneling play a significant role on the rate constant of step II. The calculated rate constant for the step II is of the order of $10^{-29}$ and $10^{-32}$ cm$^3$.molecule$^{-1}$.s$^{-1}$ for reaction in the 1–butanol and benzene solvents, respectively. The supplied rate constant of step II with Wigner and Eckart tunneling corrections is respectively about 1.9 and 3.1 times greater than that without the tunneling corrections. These tunneling correction factors are equal to decrease in activation energy about 3 and 10 kJ.mol$^{-1}$ for Wigner and Eckart tunneling corrections, respectively (see Table 3).

In the step III, the generated 2–amino–1–phenylpropan–2–ol 5 undergoes a 1,2–elimination reaction to extrude a water molecule and produce 1–phenylpropan–2–imine 6. Dehydration reaction of carbonyl ammonia 5 is endothermic by 33.32 and 39.92 kJ.mol$^{-1}$ and spontaneous by $-35.89$ and $-29.28$ kJ.mol$^{-1}$ (see Table 1) in the solvents phases of 1–butanol and benzene, respectively. The barrier height of this step was observed to be 216.97 and 216.77 kJ.mol$^{-1}$ in the solvents phases of 1–
butanol and benzene, respectively, correspond to the rate constant of the order of 10^{-15} \text{s}^{-1} (see Table 2). Simultaneous C–O and N–H bond breaking and O–H bond forming of TSIII is associated with the extremely large imaginary frequency 1775.6i cm\(^{-1}\) (see Figure 2) with significant effect of the quantum tunneling. Wigner and Eckart tunneling correction factors of about 2.6 and 11.1 leads to decrease in activation energy about 4 and 32 kJ.mol\(^{-1}\), respectively (see Table 3).

The produced formic acid in the first step undergoes a trans → cis isomerization via transition state TSIV before the step V to allow the reduction of 1–phenylpropan–2–imine 6. Trans formic acid can isomerize to its cis isomer through a low barrier of 42.76 and 47.09 kJ.mol\(^{-1}\) in the solvent phases of 1–butanol and benzene, respectively, associated to the rate constant of the order of 10\(^7\) \text{s}^{-1} (see Table 2). Imaginary frequency of 584.9i was observed for TSIV which shows that the effect of the quantum tunneling on the rate constant and activation energy of this step is insignificant. As can be seen by comparing of the Tables 2 and 3, quantum tunneling effect leads to increasing of the rate constant less than 1.3 times and decreasing of activation energy less than 2 kJ.mol\(^{-1}\). The endothermicity of this isomerization found to be respectively 4.60 and 13.44 kJ.mol\(^{-1}\) in the solvent phases of 1–butanol and benzene with the Gibbs free energies of 4.11 and 12.95 kJ.mol\(^{-1}\), respectively.
Table 3 Kinetic parameters associated with the steps II–V of the reaction mechanism in the 1–butanol and benzene solvents corrected by Wigner and Eckart tunneling factors calculated at 403.15 K by means of CTST theory at the M06–2X/aug–cc–pVTZ level of theory

<table>
<thead>
<tr>
<th></th>
<th>Wigner tunneling correction</th>
<th>Eckart tunneling correction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$E_a$ (kJ.mol$^{-1}$) (a)</td>
<td>$k$ (b)</td>
</tr>
<tr>
<td>Step I</td>
<td>$-$</td>
<td>$-$</td>
</tr>
<tr>
<td>Step II</td>
<td>102.32</td>
<td>$5.9881 \times 10^{-29}$</td>
</tr>
<tr>
<td>Step III</td>
<td>212.87</td>
<td>$8.6939 \times 10^{-15}$</td>
</tr>
<tr>
<td>Step IV</td>
<td>41.82</td>
<td>$5.6378 \times 10^7$</td>
</tr>
<tr>
<td>Step V</td>
<td>54.14</td>
<td>$3.0702 \times 10^{-22}$</td>
</tr>
<tr>
<td>1–Butanol ($\varepsilon = 17.3320$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step I</td>
<td>$-$</td>
<td>$-$</td>
</tr>
<tr>
<td>Step II</td>
<td>126.19</td>
<td>$4.8172 \times 10^{-32}$</td>
</tr>
<tr>
<td>Step III</td>
<td>212.71</td>
<td>$9.1229 \times 10^{-15}$</td>
</tr>
<tr>
<td>Step IV</td>
<td>46.15</td>
<td>$1.5470 \times 10^7$</td>
</tr>
<tr>
<td>Step V</td>
<td>67.03</td>
<td>$6.5630 \times 10^{-24}$</td>
</tr>
<tr>
<td>Benzene ($\varepsilon = 2.2706$)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(a) Activation energies are calculated by the linear fitting of the rate constants against inverse temperature over the temperature range 322–484 K.

(b) The units of $k$ for unimolecular and bimolecular reactions are in terms of s$^{-1}$ and cm$^3$.molecule$^{-1}$.s$^{-1}$, respectively.
Reduction of the produced 1–phenylpropan–2–imine 6 in step V, leads to the formation of the racemic mixture of two enantiomeric mirror images (R) and (S) of amphetamine. It was observed that the formation of amphetamine in the step V is an exothermic reaction by –67.13 and –97.54 kJ.mol\(^{-1}\) in the 1–butanol and benzene solvents, respectively, with the Gibbs free energies –53.23 and –83.63 kJ.mol\(^{-1}\) (see Table 1). Breaking of C–H and O–H bonds of cis–formic acid and formation of new C–H and N–H bonds in 1–phenylpropan–2–imine 6 (see Figure 2) implies to the formation of amphetamine through TS V (R) or TS V (S) with an imaginary frequency of 503.8 i. This step shows a barrier of 54.92 and 67.80 kJ.mol\(^{-1}\) in the 1–butanol and benzene solvents, respectively, equal to rate constant of the order of \(10^{-22}\) and \(10^{-24}\) cm\(^3\).molecule\(^{-1}\).s\(^{-1}\) (see Table 2). Negative value of Gibbs free energy and low barrier energy indicate that this step is thermodynamically and kinetically favored to be occurred.

The corrected kinetic parameters, in the Table 3, by Wigner and Eckart tunneling corrections show that the quantum tunneling effect of this step is worthless. The quantum tunneling effect on this step causes the reduction in activation energy less than 1 kJ.mol\(^{-1}\) and increasing in the rate constant by less than 1.2 times.

The overall reaction is thermodynamically spontaneous with equilibrium constant of \(4.18 \times 10^6\) and \(2.60 \times 10^7\) in the solvent phases of
1–butanol and benzene, respectively. By applying the steady state approximation with regards to the intermediates, the rate equation can be written as follows:

\[
\text{Rate} = \frac{d[\text{amphetamine}]}{dt} = k_3[6][8] \quad \Rightarrow \quad \text{Rate} = k_3[5]
\]

\[
\frac{d[6]}{dt} = 0 = k_3[5] - k_5[6][8] \quad \Rightarrow \quad \frac{d[5]}{dt} = 0 = k_3[4][2] - k_3[5]
\]

\[
\text{Rate} = k_2[4][2] = k_2[\text{phenyl} - 2 - \text{propane}][\text{NH}_3^-]
\]

Since the concentration of ammonia is proportional to the concentration of ammonium formate 1, the rate equation can be written in form of

\[
\text{Rate} = k[\text{phenyl} - 2 - \text{propane}][\text{NH}_4^+\text{COO}^-].
\]

Therefore, based on the described Leuckart–Wallach mechanism (see Schemes 2 and 5) the overall reaction of formation of amphetamine is second–order which is in agreement with experimental results [30,20]. In the following, we hardly tried to study other dispute mechanisms in Schemes 3 and 4, but our attempts to find the transition states of some steps were not successful.

In case of drug–DNA interaction, small model of DNA namely duplex GpC·CpG base–pair step has been selected. Interaction of (S)– and (R)–amphetamine with DNA have been studied in four different models (see Figure 3): (1) with an “canonical/crossing”+ form amphetamine interact from nitrogen atom of amine group with hydrogen atom of amine
group of cytosine in which the amphetamine and DNA are crossing in + shape, (2) “canonical/crossing×” with the same “canonical/crossing+” but the amphetamine and DNA are crossing in × shape, (3) “canonical/out” with the same “canonical/crossing+” but the amphetamine pointing away from DNA, (4) in “open/in” form, intercalation of amphetamine (insertion between a pair of base pairs).

Fig. 3 Schematic form of the four drug–DNA models.

The drug–DNA interaction energy, which is known to be driven by non–covalent interactions, were calculated from the expression

\[ E_{\text{int}} = E_{\text{DNA-Drug}} - E_{\text{DNA}} - E_{\text{Drug}} \]  

(1)
where the augmented energies by gCP and D3(BJ) corrections $E^{gCP-D3(BJ)}$ are given as

$$E^{gCP-D3(BJ)} = E^{d} + E^{gCP} + E^{D3(BJ)}$$  \hspace{1cm} (2)

Table 4 reports interaction energies of amphetamine with DNA in four different models. The mPW1B95/6–31G* energies and gCP–D3(BJ) corrections are presented in Table S1 (see Supplementary Material).

Table 4 Interaction energies of amphetamine with DNA in four different models in water solvent

<table>
<thead>
<tr>
<th></th>
<th>$E_{int}$ (kJ.mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S$ (1)</td>
<td>–43.7</td>
</tr>
<tr>
<td>$S$ (2)</td>
<td>–33.4</td>
</tr>
<tr>
<td>$S$ (3)</td>
<td>–30.3</td>
</tr>
<tr>
<td>$S$ (4)</td>
<td>–27.2</td>
</tr>
<tr>
<td>$R$ (1)</td>
<td>–39.0</td>
</tr>
<tr>
<td>$R$ (2)</td>
<td>–31.3</td>
</tr>
<tr>
<td>$R$ (3)</td>
<td>–27.7</td>
</tr>
<tr>
<td>$R$ (4)</td>
<td>–24.8</td>
</tr>
</tbody>
</table>

Interaction of ($S$–) and ($R$–)–amphetamine with DNA in three models of (1), (2), and (3) are more energetically favorable than model (4). Since, the interaction energy values are in the range of $-24.8$ to $-43.7$ kJ.mol$^{-1}$, it can be concluded that the nature of drug–DNA interaction must be non–covalent. The calculated interaction energies give the trend of (1) $>$ (2) $>$ (3) $>$ (4). In three models of (1), (2), and (3) a hydrogen bond is formed
between the nitrogen atom of amine group of amphetamine with hydrogen atom of amine group of cytosine. It is well known that the strength of N–H…N hydrogen bond is about 13.0 kJ.mol⁻¹ [31]. Therefore, additional interaction energy values respect to N–H…N hydrogen bond are due to the electrostatic interactions of anionic and cationic charge centers and van der Waals forces between the drug and DNA. When the drug acts an intercalator system, the model of (4), there are greater available number of anionic/cationic charge centers between the drug and DNA. Despite of the fact that more electrostatic and van der Waals interactions in the model of (4) can be found, the forming of the intercalator gap leads to the deformation of the DNA backbone from its canonical orientation and consequently to the disappearance of some attraction interactions in the DNA structure. Since there are many susceptible interaction sites between the drug and DNA, more detail examination of the drug–DNA interaction using graphical tools is indispensable. For this purpose, NCI analysis has been performed to identify and characterize the nature of the drug–DNA interactions in visual manner. The 2D and 3D NCI plots for DNA and drug–DNA complexes are shown in Figure 4 and Figures S1–S9 (see Supplementary Material), respectively.
Fig. 4 2D NCI plots of the RDG, $s(r)$, versus the electron density multiplied by the sign of the second Hessian eigenvalue $\lambda_2 \rho(r)$.

2D NCI plot of the RDG for DNA shows a low-gradient and high-density region with $\lambda_2 > 0$ associated to the non-bonding repulsive interactions indicating the lack of bonding in the center of 5- and 6-membered rings which are indicated by red sign in its 3D NCI plot. Low-gradient, low-density spikes near zero lying at positive and negative electron densities are corresponded to the weak destabilizing and stabilizing interactions, respectively. The van der Waals interactions as weak attractions are indicated by green sign in the 3D NCI plot of DNA. Low-gradient, high-density values larger than 0.05 a.u. with negative $\lambda_2$ are related to the hydrogen bonding as strong stabilizing attractions which are characterized in the 3D NCI plot of DNA by blue sign.
2D NCI plots of the RDG for different drug–DNA models are completely similar to each other and also approximately similar to the raw DNA. This observation can be attributed to the presence of similar non–covalent interactions in the raw DNA and drug–DNA complex, in such a manner that appeared RDGs associated to the drug–DNA interactions are covered by the present non–covalent interactions in raw DNA. Therefore, NCI analysis of raw DNA and drug–DNA complex without studying of 3D NCI plots will be useless effort.

NCI surfaces (Figures S2–S9) of interaction models of (1), (2), and (3) show hydrogen bond between nitrogen atom of amine group with hydrogen atom of amine group of cytosine as strong stabilizing attraction indicated by blue disks. Also, there are van der Waals interactions as weak attractions between the side of drug and DNA which is clearly shown by green isosurfaces. On the other hand, NCI surface of interaction model of (4) only shows van der Waals surfaces. Therefore, it seems that the lower interaction energy in the interaction model of (4) is due to the lack of hydrogen bonds as strong stabilizing attractions, while this interaction is present between the amphetamine and DNA in the interaction models of (1), (2), and (3).

Furthermore, a pictorial view of the ELF and LOL for the drug–DNA interaction models (1), (2), and (3) in the molecular plane of the N–
H…N are visualized and presented in Figures 5 and 6, respectively. According to these figures, the non–covalent interaction between the amphetamine and DNA is displayed by low ELF and LOL indices. Meaning that the region between the drug and DNA is electron depleted and this interaction is electrostatic (non–covalent) in nature.

**Fig. 5** Color–filled maps of ELF for the drug–DNA interaction models (1), (2), and (3) in the molecular plane defined by N–H…N. The ELF values are visualized on a blue–green–red color scale. Blue and red colors indicate respectively no electron and high electron localization.
Fig. 6 Color–filled maps of LOL for the drug–DNA interaction models (1), (2), and (3) in the molecular plane defined by N–H…N. The LOL values are visualized on a blue–green–red color scale. Blue and red colors indicate respectively no electron and high electron localization.

Conclusion

Synthesis of amphetamine (alpha–methyl–phenylethyl–amine) in the clandestine laboratories is most often achieved via non–metal reduction routes. The most popular, simple, rapid, and safe non–metal reduction route for the synthesis of amphetamine is Leuckart–Wallach reductive amination
reaction, in which its mechanism is not known with certainty. As a part of this work, the Minnesota 2006 exchange correlation functional M06–2X in conjunction with aug–cc–pVTZ basis set and SMD universal solvation model have been used to elucidate the kinetics and mechanism of the formation of amphetamine in 1–butanol and benzene solvents via the Leuckart–Wallach reaction. We were successful to find all stationary points of a five–step pathway mechanism including: (I) dissociation of ammonium formate into formic acid and ammonia, (II) nucleophilic attack of ammonia on the carbonyl carbon of phenyl–2–propane, (III) dehydration reaction of the formed 2–amino–1–phenylpropan–2–ol in the former step, (IV) trans → cis isomerization of the formic acid, and (V) formation of amphetamine by reduction of the produced 1–phenylpropan–2–imine using cis–formic acid. The overall reaction is thermodynamically spontaneous; kinetically first–order in ammonium formate and first–order in phenyl–2–propane. Activation parameters revealed that the dehydration of 2–amino–1–phenylpropan–2–ol is the rate determining step. It should be noted that dissociation of ammonium formate is a barrierless step, and quantum tunneling effect in steps II and III are greater than others. In the following, interaction of amphetamine with GpC·CpG base–pair step of DNA in four different models of (1) canonical/crossing+, (2) canonical/crossing×, (3) canonical/out, and (4) open/in have been studied in the water solvent using
the mPW1B95/6–31G* level of theory. All interaction energies were corrected for the basis set superposition error (BSSE) and the underestimation of London dispersion (DISP) interactions by adding the gCP and D3(BJ) correction terms, respectively. According to the interaction energies and topological analysis of electron localization function (ELF) and localized orbital locator (LOL), interaction of amphetamine with DNA is non–covalent in nature. Intercalation of amphetamine (open/in model) is energetically unfavorable with respect to other interaction models due to the lack of strong stabilizing hydrogen bond between the drug and DNA. Also, non–covalent interaction of drug with DNA was investigated using NCI index. 3D NCI plots indicated that there are weak van der Waals attractions between the drug and DNA. Strong stabilizing hydrogen bond attraction is the responsible for higher interaction energies in models (1), (2), and (3) in comparison with model (4) in which present only weak van der Waals attractions.

**Theoretical background and computational details**

In the kinetic and mechanistic section the geometries of reactants, transition states, intermediates and products are optimized using the energy–represented direct inversion in the iterative subspace algorithm (GEDIIS) optimizer [32] of Berny algorithm. All optimizations have been
performed by the Minnesota 2006 exchange correlation functional M06–2X [33,34] in conjugation with the Dunning’s correlation–consistent basis set of triple–ζ quality augmented by diffuse function [35] using the Gaussian 09 package of programs [36]. The M06 suite of density functionals were developed for general–purpose applications, and M06–2X has improved performance for main–group thermochemistry, barrier heights, and non–covalent interactions [37]. Harmonic vibrational frequencies were computed to identify the nature of all stationary points found by geometry optimization. Hessian (force) matrix of minima on the potential energy surface (PES) must have only positive eigenvalues, while for first order saddle points must have one imaginary eigenvalue corresponding to the vibrations of the bonds involved in the reactions. The solvent effect of 1–butanol (ε = 17.3320) and benzene (ε = 2.2706) on the kinetic and thermodynamic parameters of reactions has been taken into account through single–point energy calculations in the framework of self–consistent reaction field (SCRF) based on the integral–equation–formalism polarizable continuum model (IEF–PCM) with radii and non–electrostatic terms from the SMD universal solvation model [38]. It is recently found that the SMD solvent model is a high accurate model for predicting of free energy barriers and its estimated barriers are in reasonable agreement with the experimental data [39]. Kinetic parameters of unimolecular and
bimolecular elementary processes in the reaction mechanism were calculated at 403.15 K, in line with the experimental temperatures [30,20,22,19], using canonical transition state theory (CTST) equations [40]:

\[ k^{\text{TST}}_{\text{uni}}(T) = \kappa(T) \frac{k_B T}{h} \times \frac{Q^+}{Q_R} \times \exp\left(-\frac{E^+}{k_B T}\right) \]  

(3)

\[ k^{\text{TST}}_{\text{bi}}(T) = \kappa(T) \frac{k_B T}{h} \times \frac{Q^+}{Q_{R1} \times Q_{R2}} \times \exp\left(-\frac{E^+}{k_B T}\right) \]  

(4)

where \( k_B \) and \( h \) have the usual meanings of quantum mechanics, \( \kappa(T) \) is the tunneling correction factor, \( T \) is the absolute temperature, and \( E^+ \) is the zero–point excluded potential energy. \( Q_R \) and \( Q^+ \) are the total canonical partition functions of reactant(s) and transition state, respectively, for all the degrees of freedom except of the reaction coordinate in the transition state. The partition functions include the rotational symmetry numbers, also their vibrational part are computed with the zero of energy being the bottom of the well. Anharmonic effects were included in the calculation of partition functions by applying a scale factor of 0.971 to the M06–2X/aug–cc–pVTZ harmonic frequencies [41].

The quantum tunneling correction has been approximated using two different methods: simple Wigner method and asymmetric Eckart potential energy barrier. The Wigner correction is based on the imaginary frequency \( \text{Im}(\nu^+) \) of transition state as [42]:
The Eckart tunneling correction is based on the numerically integration of the transmission probability $p(E)$ of tunneling through the corresponding 1-dimensional barrier at energy $E$ over a Boltzmann distribution of energies, that is [43,44]:

$$
\kappa_{\text{Eckart}}(T) = \frac{\exp\left(\frac{\Delta H_{i}^{+0K}}{k_{B}T}\right)}{k_{B}T} \int_{0}^{\infty} p(E) \exp\left(-\frac{E}{k_{B}T}\right) dE
$$

where $\Delta H_{i}^{+0K}$ is the zero-point corrected energy barrier in the forward direction and $p(E)$ depends on the energy, on the shape of the barrier and on the effective mass for the system [45].

In the Drug–DNA interaction section, the geometries of drug, DNA, and Drug–DNA complexes were optimized using hybrid generalized gradient approximation (GGA) method of mPW1B95 [46] in conjugation with 6–31G* basis set. The mPW1B95/6–31G* energies were corrected for the basis set superposition error (BSSE) and the underestimation of London dispersion (DISP) interactions by adding the geometrical counterpoise, gCP [47,48], and Becke–Johnson–damping dispersion, D3(BJ) [49,50] correction terms, respectively. It is worth noting that the D3(BJ) correction term is not available for M06–2X functional [51]. Solvent effects of water ($\varepsilon = 78.3553$) on interaction energies were estimated through single–point
energy calculations using the SMD universal solvation model. Drug–DNA interaction has also been investigated qualitatively using electron localization function (ELF) [52,53] and localized orbital locator (LOL) [54] as well as non–covalent interaction (NCI) analysis [55]. Localization descriptors ELF and LOL depend on the kinetic energy density and can be used as useful tools for visualizing and analyzing of covalent and non–covalent interactions. The ELF and LOL descriptors are defined as:

\[
\text{ELF}(\mathbf{r}) = \frac{1}{1 + \left( \frac{D_{\sigma}(\mathbf{r})}{D_{\sigma}^0(\mathbf{r})} \right)^2}
\]

(7)

\[
\text{LOL}(\mathbf{r}) = \frac{\tau(\mathbf{r})}{1 + \tau(\mathbf{r})}
\]

(8)

where

\[
D_{\sigma}(\mathbf{r}) = \sum_i \eta_i |\nabla \varphi_i(\mathbf{r})|^2 - \frac{1}{4} \left[ \frac{|\nabla \rho_{\sigma}(\mathbf{r})|^2}{\rho_{\sigma}(\mathbf{r})} + \frac{|\nabla \rho_{\beta}(\mathbf{r})|^2}{\rho_{\beta}(\mathbf{r})} \right]
\]

(9)

\[
D_{\sigma}^0(\mathbf{r}) = \frac{3}{5} (6\pi^2)^{2/3} \left[ \rho_{\sigma}(\mathbf{r})^{5/3} + \rho_{\beta}(\mathbf{r})^{5/3} \right]
\]

(10)

\[
\tau(\mathbf{r}) = \frac{D_{\sigma}(\mathbf{r})}{2 \sum_i \eta_i |\nabla \varphi_i(\mathbf{r})|^2}
\]

(11)

In the above equations, \( \eta_i \) is occupation number of orbital \( i \), \( \varphi_i(\mathbf{r}) \) is orbital wave function, \( \rho \) is the electron density, \( D_{\sigma}(\mathbf{r}) \) has the physical meaning of the excess of local kinetic energy density due to Pauli’s repulsion, and \( D_{\sigma}^0(\mathbf{r}) \) is the Thomas–Fermi kinetic energy density [56]. The
ELF and LOL analysis were performed using Multiwfn suite of tools [56] upon mPW1B95/6–31G* wavefunctions of Drug–DNA complexes in the water solvent.

In addition to ELF and LOL indices, the non–covalent interaction (NCI) index was also adopted for identification and characterization of favorable or unfavorable interactions of various strengths in a semi–quantitative and visual manner. NCI analysis is based on a 2–dimensional plot of reduced density gradient (RDG, \( s(r) \)) and electron density written as [57]:

\[
 s(r) = \frac{\left| \nabla \rho(r) \right|}{2^{1/3} \pi^{1/2} \rho(r)^{1/4}} \tag{12}
\]

where \( s(r) \) is a fundamental dimensionless quantity to describe electron distribution. NCI analysis, plotting of non–covalent interaction regions, and data visualization were carried out by using NCIPLOT code [55] upon promolecular approximation to analyze non–covalent interactions in the Drug–DNA complexes.

Notes The authors declare that there is no conflict of interest.

Supplementary Material Supplementary Material file is available with the article through the web address........
References


