The Structure–Photosynthesis-Inhibiting Activity Relationships of the Compounds Containing the N-Arylpiperazine Moiety

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Abstract: The research was focused on the in silico characterization and the in vitro biological testing of the series of compounds carrying a N-arylpiperazine scaffold. Their in silico investigation was based on the prediction of electronic, steric and lipohydrophilic features. The in vitro inhibitory effect of those molecules on a photosynthetic electron transport (PET) in spinach (Spinacia oleracea L.) chloroplasts was studied using the Hill reaction. Among the tested N-arylpiperazines, the most promising potential to inhibit the PET was found for 1-[3-(3-ethoxyphenylcarbamoyl)-oxy-2-hydroxypropyl]-4(3-trifluoromethylphenyl)piperazin-1-ium chloride and 1-[3-(4-ethoxy-phenylcarbamoyl)oxy-2-hydroxypropyl]-4(3-trifluoromethylphenyl)piperazin-1-ium chloride. The current study discussed preliminary structure–inhibitory activity relationships considering electronic, steric and lipophilic properties.

Keywords: N-Arylpiperazines; Photosynthesis Inhibition; Electronic Properties; Steric Features; Lipophilicity.

1. Introduction

In medicinal chemistry, N-arylpiperazines have been considered privileged substructures, i.e., they represent a class of the molecules capable of binding to multiple receptors or effector sites with a high affinity [1]. The N-arylpiperazine framework formed the structure of ciprofloxacin (CPX; Figure 1a), a member of a second generation of fluoroquinolone (FQ) antibiotics [2], which has also shown an ability to stereochemically interfere with a catalytic activity of a reaction centre II, the pheophytin–quinone-type centre [3], which has been present in a photosystem II (PS II). It was found that CPX and related FQs might interact with specific photosynthetic bioenergetics pathways. In addition, those antibiotics caused morphological deformities in higher plants [3–5] or inhibited a physiological progress including primary a photochemistry, an electron transport, a photophosphorylation and a carbon assimilation in algae, Selenastrum capricornutum [6]. Regarding the structure–photosynthesis-inhibiting activity (SAR) relationships of some prospective quinolone derivatives, which contained an alkyl chain (alkyl=pentyl to heptadecyl), it was found that their maximal inhibitory potency in the PS II was notably influenced by the length and position of the alkyl substituent on the quinolone ring, steric features and the compounds’ lipophilicity [2–4].

Common structural denominator of the mentioned quinolone-derived molecules and previously investigated phenylcarbamic acid-based compounds was the presence of a hydrocarbon fragment (the R substituent) and the centrum of basicity (the X group; Figure 1b). The screened 2-/3- and 4-alkoxyphenylcarbamates (alkoxy=methoxy to decyloxy) have shown inhibitory activity on a photosynthetic apparatus of plants and algae, namely on a photosynthetic electron transport (PET) in spinach (Spinacia oleracea L.) chloroplasts and on a chlorophyll synthesis in green algae (Chlorella vulgaris) as well [7–9].
Their inhibitory efficiency was strongly dependent on the alkoxy side chain length and increased with its prolongation up to only a certain level. The subsequent prolongation meant the decrease in the potency [9]. That dependence was described as the cut-off effect and it was systematically reviewed by Balgavý and Devínsky [10]. The 2-alkoxy substitution led to the decrease in the inhibitory potency of the in vitro evaluated compounds when compared to their 3- and 4-alkoxy substituted positional isomers. That finding was explained by a secondary steric effect, which was induced by the intramolecular interactions between the 2-alkoxy substituent and the carbamate group [7–9].

![Figure 1](image.png)

*Figure 1. The promising inhibitors of the in vitro photosynthetic processes, which contained: (a) the N-arylpirperazine moiety (ciprofloxacin, CPX); (b) the 2-/3-/4-alkoxyphenylcarbamoyloxy moiety.*

This study has been focused on the compounds, which chemical structure was a combination of both N-arylpirperazine and 2-/3-/4-alkoxyphenylcarbamoyloxy fragments. In the first part of the research, which preceded another presented phase, a biological evaluation in vitro of those 1-[3-/2-/3-/4-alkoxyphenylcarbamoyl]oxy-2-hydroxypropyl]-4-(3-trifluoromethylphenyl)piperazin-1-ium chlorides 1a–f (alkoxy=methoxy or ethoxy; Table 1), an attention was paid on the in silico characterization of their basic forms 1aB–fB to closely investigate the electronic, steric and lipophilic properties. Supposed differences in those features of the 1aB–fB set, chemically 3-[4-(3-trifluoromethylphenyl)piperazin-1-yl]-2-hydroxypropyl (2-/3-/4-alkoxyphenyl)carbamates (alkoxy=methoxy or ethoxy), might influence the compounds’ biological activity.

Next, a very essential objective of the current research was the in vitro investigation of the molecules’s ability to inhibit the PET in the spinach (*Spinacia oleracea* L.) chloroplasts.

After the in silico examination and the in vitro biological testing, a notable aim of the research was to reveal some structural and physicochemical features of the compounds 1a–f (1aB–fB), which might be essential for their inhibitory efficiency and thereby to contribute to comprehensive structure–photosynthesis-inhibiting activity relationships analyses in that class of the compounds.

### 2. Results and Discussion

#### 2.1. Electronic, Steric and Lipohydrophilic Properties of the Compounds 1a–f (1aB–fB)

The common feature of the compounds under the study 1a–f was the presence of a lipophilic 4’-(3’-trifluoromethylphenyl)piperazin-1’-yl as the salt-forming moiety (Table 1). Fluorine-containing group(s) played a pivotal role in a drug discovery process for modulating biological activity of molecules. The effect of a fluorine substitution in organic compounds included the ability of that atom to participate in a hydrogen bonding, either as a hydrogen-bond acceptor or as an inductive activator of a hydrogen-bond donor group. The fluorine substitution on aromatic substructures rendered remaining aromatic hydrogen substituents more acidic, so the capacity of those compounds to act as the hydrogen bridge donors was enhanced. In addition, non-covalent intermolecular interactions of the C–F bond can be important for the affinity of a drug with a macromolecular recognition site [11,12].

It has been well-established that the 3’-CF₃ group has shown a strong electron-withdrawing effect. Those electronic properties might be described by the Hammett substituent constant σ; the more positive value of the σ parameter meant the stronger electron-withdrawing influence of the substituent. As published, the σ output for the 3’-CF₃ moiety (σ₁₃) was set to 0.43 [13]. On the contrary, the alkoxy substituents attached to the aromatic system (Table 1) have shown very weak
electron-withdrawing or moderate electron-donating effect. If concerning the methoxy moiety, the 
α value of 0.12 and -0.27 was related to its 3- and 4-position [13]. Similar readouts were published 
for the ethoxy group [13] attached to the position 3 (0.10) or 4 (-0.24).

The expression of compounds’ electronic properties by the Hammett substituent constant α 
could be more complicated for the 2-substituted derivatives (the molecules 1a and 1b in present 
research) because their α values include a steric contribution. In general, compared to the 
3- or 4-substituents, the 2-ones could lead to conformational changes sometimes being favorable 
for interactions with effecter sites, sometimes being very unfavorable [13]. On those grounds, 
electronic (as well as acidobasic) properties of all the studied N-arylpyrazines could be also 
characterized by the values of dissociation constants [13]. For the series 1a–f, the pKₐs were 
estimated by a potentiometric titration [14,15] and were listed in Table 1.

Table 1. Experimentally observed and calculated values of the dissociation constants (pKₐ) of the 
compounds 1a–f and their bases 1aB–fB as well as the in silico generated molecular volume (MV) 
data related to the basic forms.

<table>
<thead>
<tr>
<th>Comp.</th>
<th>R</th>
<th>pKₐ</th>
<th>pKₐMarvin¹</th>
<th>pKₐACE-O²</th>
<th>MVₐs [Å³]¹</th>
<th>MVₐch [Å³]²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>2-OCH₃</td>
<td>5.83</td>
<td>7.87</td>
<td>7.60</td>
<td>418.25</td>
<td>393.05</td>
</tr>
<tr>
<td>1b</td>
<td>2-OCH₃</td>
<td>6.00</td>
<td>7.87</td>
<td>7.60</td>
<td>436.81</td>
<td>409.85</td>
</tr>
<tr>
<td>1c</td>
<td>3-OCH₃</td>
<td>5.73</td>
<td>7.87</td>
<td>7.60</td>
<td>417.93</td>
<td>393.05</td>
</tr>
<tr>
<td>1d</td>
<td>3-OCH₃</td>
<td>5.35</td>
<td>7.87</td>
<td>7.60</td>
<td>436.49</td>
<td>409.85</td>
</tr>
<tr>
<td>1e</td>
<td>4-OCH₃</td>
<td>5.66</td>
<td>7.87</td>
<td>7.60</td>
<td>417.85</td>
<td>393.05</td>
</tr>
<tr>
<td>1f</td>
<td>4-OCH₃</td>
<td>5.69</td>
<td>7.87</td>
<td>7.60</td>
<td>436.41</td>
<td>409.85</td>
</tr>
</tbody>
</table>

¹ pKₐMarvin, Predicted values of the dissociation constants by the MarvinSketch Online Calculator
(ChemAxon, Budapest, Hungary); ² pKₐACE-O, predicted values of the dissociation constants by the
Achieving Chemistry Excellence: Organic Chemistry applet (Departments of Chemistry and
Computer Sciences, University of Kentucky, KY, USA); ³ MVₐs [Å³], the molecular volume data
(in the Å³ units) calculated by an interactive Molecular Properties Calculator applet (MolSoft LLC.,
San Diego, CA, USA); ⁴ MVₐch [Å³], the molecular volume data (in the Å³ units) calculated by an
interactive Molinspiration Molecular Properties Calculator applet (Molinspiration Cheminformatics,
Slovenský Grob, Slovak Republic). The pKₐMarvin, pKₐACE-O, MVₐs and MVₐch readouts were
calculated for the non-protonated bases 1aB–fB.

It was found that the compounds’ pKₐs were observed in the range from 5.35 (the compound 1d)
to 6.00 (1b). The elongation of an alkoy side chain led to lower pKₐs only for the 3-alkoxy
substituted derivatives (1c, 1d), that course was not observed for the 2- and 4-alkoxy substituted
molecules (Table 1).

The interactive MarvinSketch applet (ChemAxon, Budapest, Hungary) allowed to calculate the
pKₐs only for non-protonated forms 1aB–fB (bases). In addition, that Java-based interface provided
the calculated pKₐ=7.87 for all the investigated basic substances regardless of the position and the
length of a side chain. Furthermore, the Achieving Chemistry Excellence: Organic Chemistry applet
(Departments of Chemistry and Computer Sciences, University of Kentucky, KY, USA) generated
slightly lower pKₐs (7.60) for all the molecules 1aB–fB (Table 1).

The differences in the steric properties of inspected bases 1aB–fB were verified by the
calculation of a molecular volume (MV) using both interactive Molecular Properties Calculator
applets, which were developed by MolSoft LLC. (San Diego, CA, USA) and Molinspiration
Cheminformatics (Slovenský Grob, Slovak Republic).

Regarding the position of the alkoy side chain attached to the aromatic system, the decrease in
the MV data was observed if the MolSoft LLC. applet was used (MVₐs) as follows: 2-positional
isomers > 3-positional isomers > 4-positional isomers (Table 1). The highest MVMS value was related to the compound 3-[4-(3-trifluoromethylphenyl)piperazin-1-yl]-2-hydroxypropyl (2-ethoxyphenyl)carbamate 1BB (436.81 Å³), the lowest MVMS was calculated for the molecule 3-[4-(3-trifluoromethylphenyl)piperazin-1-yl]-2-hydroxypropyl (4-methoxyphenyl)carbamate 1EB (417.85 Å³).

On the contrary, the positional isomerism of the side chain was not reflected in the MV values, which were calculated by the Molispiration Cheminformatics applet (MVMSN). For the methoxy derivatives 1AB, 1CB and 1EB, the MVMSN=393.05 Å³ was generated. Slightly higher MVMSN was related to the ethoxy substituted substances 1BB, 1DB and 1FB (409.85 Å³).

Both Java-based applets did not allow to generate the data for biologically screened salts 1a–f, but it could be supposed that suggested tendency would be maintained.

The lipophilicity has been considered one of the essential factors involved in the structure–photosynthesis-inhibiting activity relationships studies [7–9,16–18]. Experimentally observed values of the partition coefficient for the molecules 1a–f in the octan-1-ol/phosphate buffer (pH=7.4) system (log PexpS) indicated their highly lipophilic nature [14,15,19]. Those log PexpS ranged from 3.57 (1a) to 3.72 (1d; Table 2).

Table 2. Experimentally observed values of the partition coefficient (log Pexp) of the compounds 1a–f in the octan-1-ol/phosphate buffer (pH=7.4) system and the in silico predicted readouts for corresponding non-protonated bases 1AB–1FB by the miLogP 2.2, ALOGP, MLOGP, XLOGP 2.0 and XLOGP 3.0 methods, respectively, as well as the potential of the molecules 1a–f to inhibit the photosynthetic electron transport (PET-I) in the spinach (Spinacia oleracea L.) chloroplasts.

<table>
<thead>
<tr>
<th>Comp.</th>
<th>log Pexp</th>
<th>miLogP 2.2</th>
<th>ALOGP</th>
<th>MLOGP</th>
<th>XLOGP 2.0</th>
<th>XLOGP 3.0</th>
<th>PET-I [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>3.57</td>
<td>3.72</td>
<td>3.78</td>
<td>2.17</td>
<td>3.28</td>
<td>3.37</td>
<td>9</td>
</tr>
<tr>
<td>1b</td>
<td>3.60</td>
<td>4.10</td>
<td>4.13</td>
<td>2.38</td>
<td>3.70</td>
<td>3.74</td>
<td>1</td>
</tr>
<tr>
<td>1c</td>
<td>3.61</td>
<td>3.75</td>
<td>3.78</td>
<td>2.17</td>
<td>3.28</td>
<td>3.37</td>
<td>22</td>
</tr>
<tr>
<td>1d</td>
<td>3.72</td>
<td>4.12</td>
<td>4.13</td>
<td>2.38</td>
<td>3.70</td>
<td>3.74</td>
<td>24</td>
</tr>
<tr>
<td>1e</td>
<td>3.60</td>
<td>3.77</td>
<td>3.83</td>
<td>2.17</td>
<td>3.28</td>
<td>3.37</td>
<td>– (pr.)</td>
</tr>
<tr>
<td>1f</td>
<td>3.71</td>
<td>4.15</td>
<td>4.13</td>
<td>2.38</td>
<td>3.70</td>
<td>3.74</td>
<td>23</td>
</tr>
</tbody>
</table>

1 PET-I [%], the inhibition of the photosynthetic electron transport (PET) by the compounds 1a–f, which was expressed in the percentages; 2 – (pr.), the particular compound has shown no inhibiting effect on the PET in the used concentration due to the precipitation.

The current research provided the log P data calculated in silico by five atomic/fragmental methods, namely miLogP 2.2 (Molispiration Cheminformatics, Slovenský Grob, Slovak Republic), ALOGP [20], MLOGP [21], XLOGP 2.0 [22,23] and XLOGP 3.0 [24], respectively. According to all those procedures, the increase in lipophilicity of the analyzed non-protonated bases 1AB–1FB resulted in higher log Ps (Table 2). The positional isomerism of attached alkoxy side chain was not being reflected in the log P outputs, which were generated by all the approaches based on a substructure principle, excluding the miLogP 2.2 method. Regarding the miLogP 2.2, the increase in the log Ps was as follows: 2-positional isomers > 3-positional isomers > 4-positional isomers (Table 2). The highest calculated level of the compounds’ lipophilicity was connected with the ALOGP approach and that values were in the interval of 3.78 to 4.13. On the other hand, the lowest log Ps were generated by the MLOGP method, which outputs varied from 2.17 to 2.38 (Table 2).

Following the in silico procedures involved, the derivative 3-[4-(3-trifluoromethylphenyl)piperazin-1-yl]-2-hydroxypropyl (4-ethoxyphenyl)carbamate (1FB) was regarded as the most lipophilic (miLogP 2.2=4.15, ALOGP=4.13, XLOGP 3.0=3.74, XLOGP 2.0=3.70) among the analyzed compounds.

According to the averaged absolute residual sums (AARS) values [25], which were calculated by the Microsoft Office Excel 2010 program (Microsoft Corporation, Redmond, WA, USA), following ranking of those programs was observed: XLOGP 3.0 > XLOGP 2.0 > miLogP 2.2 > ALOGP > MLOGP. Almost all the predictors were regarded as acceptable with the AARS in the range
of 0.00 to ±0.49, excluding the MLOGP, which was considered unacceptable and for which the AARS descriptor exceeded ±0.99 (Table 3).

The classification into acceptable, disputable and unacceptable calculations mirrored that view. Counting the negative and positive deviations of calculations from the log P_{sys} has shown a rather equilibrated pattern for the acceptable miLogP 2.2 and ALOGP procedures. On the other hand, a slight difference in that negative and positive deviations was observed between the XLOGP 2.0 (1 case versus 5 cases) and XLOGP 3.0 (3 versus 3) approaches (Table 3).

**Table 3.** Comparative validity check of employed calculation programs.

<table>
<thead>
<tr>
<th>Comp.</th>
<th>miLogP 2.2</th>
<th>ALOGP</th>
<th>MLOGP</th>
<th>XLOGP 2.0</th>
<th>XLOGP 3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>AARS</td>
<td>-0.30</td>
<td>-0.32</td>
<td>1.36</td>
<td>0.15</td>
<td>0.08</td>
</tr>
<tr>
<td>acceptable</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>disputable</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>unacceptable</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&gt;log P_{sys}</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>&lt;log P_{sys}</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

1 Comp., The set of investigated derivatives 1a-f.

### 2.2. Photosynthesis-Inhibiting Potential of the Compounds 1a-f

The PET inhibition by the molecules 1a-f in the spinach chloroplasts was determined spectrophotometrically using an artificial electron acceptor, 2,6-dichlorophenol-indophenol (DCPIP) by the well-known Hill reaction [26,27], as a basic redox reaction of the PS II [28]. The rate of the PET was monitored as a photoreduction of DCPIP, the inhibition (PET-I) was expressed in the percentages.

If concerning the position of attached alkoxyl side chain, the current findings (Table 2) were in agreement with the conclusions of the research articles [7-9]. It was found that the 2-alkoxy substituted compounds (1a, 1b) have shown approximately twice lower activity compared to that of the 3- and 4-alkoxy substituted derivatives (1c-f) at the concentration of c=0.2 mM.

In addition, comparable PET-I data of corresponding 2-/3- and 4-alkoxy positional isomers were observed at the c=1.0 mM. That results would be explained by very limited solubility of the screened molecules, which contained the R substituent in the 3- and 4-position. Moreover, the methoxy group-containing substances 1c and 1e have shown no inhibiting effect on the PET in the particular concentration due to low aqueous solubility and the precipitation (Table 2).

The most promising potential to inhibit the PET was found for 1-[3-(3-ethoxyphenylcarbamoyl)oxy-2-hydroxypropyl]-4-(3-trifluoromethylphenyl)piperazin-1-ium chloride (1d, PET-I=24%) as well as for 1-[3-(4-ethoxyphenylcarbamoyl)oxy-2-hydroxypropyl]-4-(3-trifluoromethylphenyl)piperazin-1-ium chloride (1f, PET-I=23%).

### 2.3. Preliminary Structure–Activity Relationships

The position and the length of the alkoxyl side chain influenced electronic, steric and lipophylic features as well as the *in vitro* inhibitory activity of tested derivatives 1a-f. The presently observed PET-Is indicated (at the c=0.2 mM) that electron-donating effect of the R substituent attached to the phenylcarbamoyl moiety would not be probably the most decisive factor, which could improve the photosynthesis-inhibiting potential of the investigated molecules.

The electron-donating impact of the 4-methoxy substitution on the aromatic system was slightly more pronounced than that of the 4-ethoxy one. In fact, thus electron density enhancement was not positively mirrored in the activity profiles of particular substances (1e versus 1f). Moreover, slightly electron-withdrawing influence of the 3-alkoxy substituents (1c and 1d) was not regarded as inconvenient.

On the basis of observed results it would be suggested that the presence of electron-withdrawing (and sterically bulky) R substituent could be strongly taken into the consideration in order to improve the PET-inhibiting activity.
The positive influence of the 3′-CF₃ group, which was attached to the phenylcarbamoyl moiety of tested 1-hydroxynaphthalene-2-carboxanilides (Figure 2a), on the PET inhibition was also observed [29]. The position changing of that lipophilic group on the phenylcarbamoyloxy fragment (4′-CF₃) of structurally similar naphthalene-1-carboxanilides (Figure 2b) was concerned favorable [30] as well. On the other hand, the PET-inhibiting activity of 8-hydroxyquinoline-2-carboxanilides (Figure 2c) decreased as follows: 3′-CF₃ substituted derivative > 2′-CF₃ substituted derivative > 4′-CF₃ substituted derivative [18]. That dependence was explained by the highest lipophilicity and very limited solubility of the 4′-CF₃ substituted molecule [18].

![Figure 2](image.png)

**Figure 2.** The promising *in vitro* inhibitors of the photosynthetic electron transport with the attached CF₃ group to the phenylcarbamoyl moiety of the: (a) 1-hydroxynaphthalene-2-carboxanilide; (b) naphthalene-1-carboxanilide; (c) 8-hydroxyquinoline-2-carboxanilide pharmacophore.

Furthermore, the most active substances have shown the pKᵢ=5.35 (1d) and 5.69 (1f). According to the present results could be preliminary stated, that the biphasic (bilinear) relationship between the experimentally estimated pKᵢs and the PET-I values was observed (Figure 3).

![Figure 3](image.png)

**Figure 3.** The biphasic relationship between the PET-I outputs (in the percentages), which were estimated at the c=0.2 mM, and the observed pKᵢ values of the compounds 1a–f (excluding the molecule 1e).

It was found that the 3- and 4-alkoxy substituted compounds 1c–f, with the pKᵢ data ranging from 5.35 to 5.73, caused a comparable PET inhibition and their PET-I is were in the interval of 22% to 24%. The subsequent increase in the observed pKᵢ values meant a notable decrease in the PET-inhibiting ability of those derivatives. As indicated (Table 1, Figure 3), the highest pKᵢs were related to the 2-alkoxy substituted substances 1a (pKᵢ=5.83) and 1b (6.00).

For the completeness, it would be important to note that the compound 1e was excluded from that analysis model due to its precipitation at the used concentration (c=0.2 mM). The implementation of the *in silico* algorithms (pKᵢ Marvin, pKᵢ ACE-O), as an integral part of the research, did not provide more explicit conclusions (Table 2).
The distortion of the phenyl ring plane towards that of the carbamate group was probably occurred in the chemical structure of the 2-alkoxy substituted derivatives (1a, 1b). Described process led to the perturbation of the planarity of those molecules and it was also mirrored in the conjugation of the π-bonds of the aromatic system through the N–H moiety up to the C=O group. All those interactions involved the changes in an electron density on the C=O moiety. That secondary steric effect was probably the reason, why the 2-alkoxy substituted compounds have shown lower inhibitory potential compared to that of 3- and 4-alkoxy substituted substances 1c–f. In the chemical structure of the compounds 1c–f thus intramolecular interactions were strongly lowered of practically eliminated [8,9].

The present research suggested that the observed log \( P_{exp} \) could be altered by the in silico outputs from the XLOGP 3.0, XLOGP 2.0, miLogP 2.2 or ALOGP procedure. On the contrary, the Moriguchi’s MLOGP approach was considered unacceptable. Furthermore, the increase in lipophilicity of the evaluated compounds led to their slightly higher in vitro photosynthesis-inhibiting activity.

The study confirmed that inspected derivatives 1a–f have shown only low to moderate efficiency with the PET-Is in the range of 1% to 24% (c=0.2 mM), despite of being highly lipophilic, as the log \( P_{exp} \) values as well as the in silico generated readouts (XLOGP 3.0, XLOGP 2.0, miLogP 2.2 and ALOGP, respectively) indicated. The non-protonated basic forms of the most prospective molecules 1d (PET-I=24%) and 1f (PET-I=23%) have shown the calculated log \( P_s \) in the range of 3.70 to 4.15.

The increase in observed and calculated lipophilicity of the tested 3-alkoxy substituted compounds (1c, 1d) resulted in higher PET-Is at the c=0.2 mM. In addition, that dependence was found for both 2- and 3-alkoxy substituted derivatives at the c=1.0 mM (Table 2). Following the current results, it would be predicted that the presence of relatively longer R substituent (butoxy or higher alk oxy group) together with the sterically bulky electron-withdrawing group(s) attached to the aromatic system within the salt-forming moiety would be more favorable structural modification. In addition, the cut-off effect would be also expected at the certain length of the R side chain.

The mechanism of the PET inhibition by the alk oxy substituted membrane-active compounds might be connected with their ability to penetrate their hydrophobic chain into the lipid membrane and change the orientation of electron donor and acceptor complexes relative to one another [7–9,18]. Due to the direct interaction of such membrane-active molecules with membrane proteins, conformational changes in those proteins could occur.

3. Materials and Methods

3.1. Tested Compounds

The compounds under the current study 1a–f, chemically 1-[3-(2-/3-/4-alkoxyphenylcarbamoyl)oxy-2-hydroxypropyl]-4-(3-trifluoromethylphenyl)piperazin-1-ium chlorides 1a–f (alkoxy=methoxy or ethoxy), were synthesized previously [19], their experimentally observed pK\(_a\) values (Table 1) and the log \( P_{exp} \) (Table 2) were published in the research papers [14,15,19].

3.2. In Silico Investigation

3.2.1. Calculation of Electronic (Acidobasic) Characteristics

The dissociation constant (pK\(_a\)) values of the non-protonated forms 1aB–fB, chemically 3-[4-(3-trifluoromethylphenyl)piperazin-1-yl]-2-hydroxypropyl (2-/3-/4-alkoxyphenyl)carbamates (alkoxy=methoxy or ethoxy), of the biologically screened molecules 1a–f were predicted by the MarvinSketch Online Calculator (ChemAxon, Budapest, Hungary) as well as by the Achieving Chemistry Excellence: Organic Chemistry applet (Departments of Chemistry and Computer Sciences, University of Kentucky, KY, USA).
3.2.2. Calculation of Molecular Volume

The molecular volume (MV) outputs of the non-protonated forms 1a–fB (Table 1) were calculated by the interactive MolSoft’s Molecular Properties Calculator applet (MolSoft L.L.C., San Diego, CA, USA) as well as by the Java-based Molinspiration’s Molecular Properties Calculator interface (Molinspiration Cheminformatics, Slovenský Grob, Slovak Republic).

3.2.3. Prediction of Lipophyrophilic Properties

The values of the logarithm of a partition coefficient related to the non-protonated basic molecules 1a–fB (Table 2) were generated in silico for the octan-1-ol/water partitioning system (log P) by the substructure-based (atomic/fragmental) methods, namely miLogP 2.2 (Molinspiration Cheminformatics, Slovenský Grob, Slovak Republic), ALOGP [20], MLOGP [21], XLOGP 2.0 [22,23] and XLOGP 3.0 [24], respectively.

3.3. Study of Inhibition of Photosynthetic Electron Transport (PET) in Spinach Chloroplasts

The chloroplasts were prepared from spinach (Spinacia oleracea L.) according to the research articles [31,32]. The inhibition of the photosynthetic electron transport (PET) in the spinach chloroplasts was determined spectrophotometrically (Genesys 6, Thermo Scientific, Waltham, MA, USA), using an artificial electron acceptor 2,6-dichlorophenol-indophenol (DCPIP) according to Kráľová et al. [28] and the photosynthetic electron transport was monitored as a photoreduction of DCPIP at the wavelength of λ=600 nm. The measurements were carried out in a phosphate buffer (c=20.0 mM, pH=7.2) containing sucrose (c=400.0 mM), MgCl2; (c=5.0 mM) and NaCl (c=15.0 mM). In those experiments, the chlorophyll content was 30 mg/L and the samples were irradiated (~100 W/m² with the 10-cm distance) with a halogen lamp (250 W) using a 4-cm water filter to prevent warming of the samples (temperature was 25°C). The tested compounds 1a–f were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, Darmstadt, Germany) due to their insolubility in water. The applied DMSO concentration (up to 10%) did not affect the photochemical activity in the spinach chloroplasts (observed differences in the DCPIP photoreduction due to the DMSO addition were within an experimental error). The PET inhibition by the screened derivatives 1a–f was expressed in the percentages (Table 2).

3.4. Statistical Analysis

The validity of the log P predictions was checked for the involved in silico methods via experimental log PexpS for the compounds 1a–f by the averaged absolute residual sums (AARS) values [25]. The AARS for the differences between the experiment and the calculation were given as the statistical criterion. The differences (Δ log P) between the log Pexp and the predicted data in the range of 0.00 to ±0.49 were qualified as acceptable, Δ log P values of ±0.50 to ±0.99 were viewed as disputable and differences exceeding ±0.99 were classified as unacceptable.

The numbers of calculations exhibiting higher or lower values than the log PexpS were counted as well [25], and were listed in Table 3. The AARS were calculated by the Microsoft Office Excel 2010 program (Microsoft Corporation, Redmond, WA, USA).

The figure, which characterized the relationship between the independent variable (the pKaS) and the dependent variable, i.e., the inhibition of the photosynthetic electron transport (the PET-I values expressed in the percentages), were calculated and visualized by the Origin Pro 9.0.0 software (OriginLab Corporation, Northampton, MA, USA).

Author Contributions: “I.M. previously synthesized the compounds, created the concept and designed the study, performed the in silico calculations, analyzed the data related to the in vitro photosynthesis-inhibiting efficiency, interpreted the preliminary SAR results, wrote the paper; F.Š. performed the in vitro photosynthesis-inhibiting activity of the compounds, contributed reagents/materials tools; J.Cs. designed the chemical structure of the compounds under the study, previously synthesized the compounds, contributed reagents/materials tools; J.J. analyzed the data related to the in vitro photosynthesis-inhibiting efficiency; K.K. analyzed the data related to the in vitro photosynthesis-inhibiting activity, interpreted the preliminary SAR results.” The authors have approved the final version of the manuscript.
Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

- $\sigma$: Hammett substituent constant
- AARS: Averaged absolute residual sums
- DMSO: Dimethyl sulfoxide
- $F$: Fisher significance ratio
- FQ: Fluoroquinolone (antibiotics)
- log $P_{exp}$: Experimentally observed values of the partition coefficient in the octan-1-ol/phosphate buffer ($pH=7.4$) system
- $MV_{MCH}$: Molecular volume data calculated by the interactive Molecular Properties Calculator applet of Molinspiration Cheminformatics
- $MV_{MS}$: Molecular volume data calculated by the interactive Molecular Properties Calculator applet of MolSoft L.L.C.
- $PET-I$: Inhibition of the photosynthetic electron transport (the $PET-I$ values were expressed in the percentages)
- $pK_a$ $AEL-O$: Predicted values of the dissociation constants ($pK_a$) by the interactive Achieving Chemistry Excellence: Organic Chemistry applet
- $pK_a$ $Marvin$: Predicted values of the dissociation constants ($pK_a$) by the interactive MarvinSketch Online Calculator interface
- PS II: Photosystem II
- SAR: Structure–activity relationship(s)

References


Sample Availability: Samples of the compounds 1a–f are available from the author Ivan Malik.