

# 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-ethoxyphenylcarbamoyl)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.

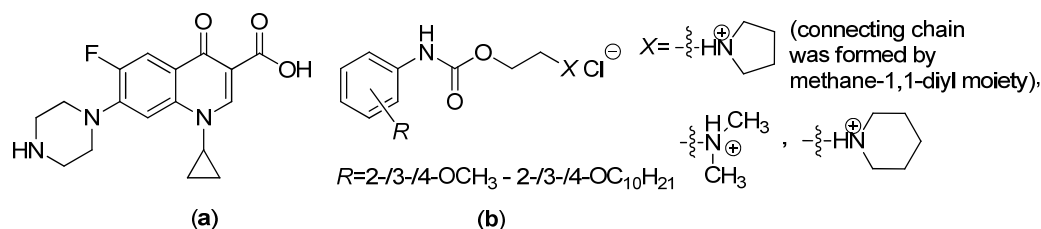
**Keywords:** *N*-Arylpiperazines; Photosynthesis Inhibition; Electronic Properties; Steric Features; Lipophilicity.

## 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.** The promising inhibitors of the *in vitro* photosynthetic processes, which contained: (a) the *N*-arylpiperazine 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*-arylpiperazine 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' 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'-CF<sub>3</sub> group has shown a strong electron-withdrawing effect. Those electronic properties might be described by the Hammett substituent constant  $\sigma$ ; the more positive value of the  $\sigma$  parameter meant the stronger electron-withdrawing influence of the substituent. As published, the  $\sigma$  output for the 3'-CF<sub>3</sub> moiety ( $\sigma_{\text{CF}_3}$ ) 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  $\sigma$  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  $\sigma$  could be more complicated for the 2-substituted derivatives (the molecules **1a** and **1b** in present research) because their  $\sigma$  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 effector sites, sometimes being very unfavorable [13]. On those grounds, electronic (as well as acidobasic) properties of all the studied *N*-arylpiperazines could be also characterized by the values of dissociation constants [13]. For the series **1a–f**, the  $pK_a$ s 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 ( $pK_a$ ) 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.

Comp.	R	$pK_a$	$pK_{a \text{ Marvin}}^1$	$pK_{a \text{ ACE-O}^2}$	$MV_{MS} [\text{\AA}^3]^3$	$MV_{MCh} [\text{\AA}^3]^4$
<b>1a</b>	2-OCH <sub>3</sub>	5.83	7.87	7.60	418.25	393.05
<b>1b</b>	2-OC <sub>2</sub> H <sub>5</sub>	6.00	7.87	7.60	436.81	409.85
<b>1c</b>	3-OCH <sub>3</sub>	5.73	7.87	7.60	417.93	393.05
<b>1d</b>	3-OC <sub>2</sub> H <sub>5</sub>	5.35	7.87	7.60	436.49	409.85
<b>1e</b>	4-OCH <sub>3</sub>	5.66	7.87	7.60	417.85	393.05
<b>1f</b>	4-OC <sub>2</sub> H <sub>5</sub>	5.69	7.87	7.60	436.41	409.85

<sup>1</sup>  $pK_{a \text{ Marvin}}$ , Predicted values of the dissociation constants by the MarvinSketch Online Calculator (ChemAxon, Budapest, Hungary); <sup>2</sup>  $pK_{a \text{ ACE-O}}$ , 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); <sup>3</sup>  $MV_{MS} [\text{\AA}^3]$ , the molecular volume data (in the  $\text{\AA}^3$  units) calculated by an interactive Molecular Properties Calculator applet (MolSoft L.L.C., San Diego, CA, USA); <sup>4</sup>  $MV_{MCh} [\text{\AA}^3]$ , the molecular volume data (in the  $\text{\AA}^3$  units) calculated by an interactive Molinspiration Molecular Properties Calculator applet (Molinspiration Cheminformatics, Slovenský Grob, Slovak Republic). The  $pK_{a \text{ Marvin}}$ ,  $pK_{a \text{ ACE-O}}$ ,  $MV_{MS}$  and  $MV_{MCh}$  readouts were calculated for the non-protonated bases **1aB–fB**.

It was found that the compounds'  $pK_a$  were observed in the range from 5.35 (the compound **1d**) to 6.00 (**1b**). The elongation of an alkoxy side chain led to lower  $pK_a$ s 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  $pK_a$ s only for non-protonated forms **1aB–fB** (bases). In addition, that Java-based interface provided the calculated  $pK_a=7.87$  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  $pK_a$ s (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 L.L.C. (San Diego, CA, USA) and Molinspiration Cheminformatics (Slovenský Grob, Slovak Republic).

Regarding the position of the alkoxy side chain attached to the aromatic system, the decrease in the *MV* data was observed if the MolSoft L.L.C. applet was used ( $MV_{MS}$ ) as follows: 2-positional

isomers > 3-positional isomers > 4-positional isomers (Table 1). The highest  $MV_{MS}$  value was related to the compound 3-[4-(3-trifluoromethylphenyl)piperazin-1-yl]-2-hydroxypropyl (2-ethoxyphenyl)carbamate **1bB** (436.81 Å<sup>3</sup>), the lowest  $MV_{MS}$  was calculated for the molecule 3-[4-(3-trifluoromethylphenyl)piperazin-1-yl]-2-hydroxypropyl (4-methoxyphenyl)carbamate **1eB** (417.85 Å<sup>3</sup>).

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 ( $MV_{MCh}$ ). For the methoxy derivatives **1aB**, **1cB** and **1eB**, the  $MV_{MCh}$ =393.05 Å<sup>3</sup> was generated. Slightly higher  $MV_{MCh}$ s were related to the ethoxy substituted substances **1bB**, **1dB** and **1fB** (409.85 Å<sup>3</sup>).

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 ( $\log P_{exp}$ ) indicated their highly lipophilic nature [14,15,19]. Those  $\log P_{exp}$ s ranged from 3.57 (**1a**) to 3.72 (**1d**; Table 2).

**Table 2.** Experimentally observed values of the partition coefficient ( $\log P_{exp}$ ) 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-fB** 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.

Comp.	$\log P_{exp}$	miLogP 2.2	ALOGP	MLOGP	XLOGP 2.0	XLOGP 3.0	PET-I [%] <sup>1</sup>	
							c=0.2 mM	c=1.0 mM
<b>1a</b>	3.57	3.72	3.78	2.17	3.28	3.37	9	12
<b>1b</b>	3.60	4.10	4.13	2.38	3.70	3.74	1	15
<b>1c</b>	3.61	3.75	3.78	2.17	3.28	3.37	22	– (pr.)
<b>1d</b>	3.72	4.12	4.13	2.38	3.70	3.74	24	17
<b>1e</b>	3.60	3.77	3.78	2.17	3.28	3.37	– (pr.) <sup>2</sup>	9
<b>1f</b>	3.71	4.15	4.13	2.38	3.70	3.74	23	15

<sup>1</sup> PET-I [%], The inhibition of the photosynthetic electron transport (PET) by the compounds **1a-f**, which was expressed in the percentages; <sup>2</sup> – (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-fB** resulted in higher  $\log P$ s (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  $\log P$ s 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  $\log P$ s 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  $\pm 0.49$ , excluding the MLOGP, which was considered unacceptable and for which the AARS descriptor exceeded  $\pm 0.99$  (Table 3).

The classification into acceptable, disputable and unacceptable calculations mirrored that view. Counting the negative and positive deviations of calculations from the  $\log P_{\text{exp}}$ s 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.

Comp. <sup>1</sup>	miLogP	ALOGP	MLOGP	XLOGP	XLOGP
	2.2			2.0	3.0
AARS	-0.30	-0.32	1.36	0.15	0.08
acceptable	3	3	0	6	6
disputable	3	3	0	0	0
unacceptable	0	0	6	0	0
> $\log P_{\text{exp}}$	6	6	0	1	3
< $\log P_{\text{exp}}$	0	0	6	5	3

<sup>1</sup> 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 alkoxy side chain, the current findings (Table 2) were in the 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).

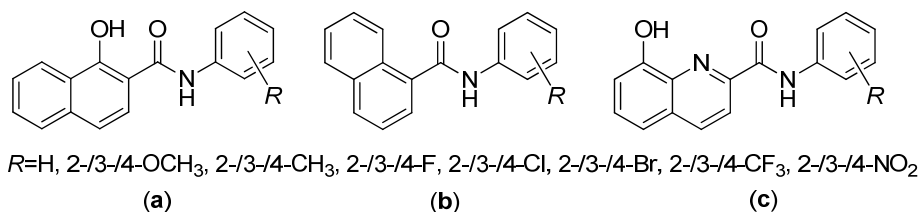
The most promising potential to inhibit the PET was found for 1-[3-(3-ethoxyphenyl-carbamoyl)oxy-2-hydroxypropyl]-4-(3-trifluoromethylphenyl)piperazin-1-ium chloride (**1d**, *PET-I*=24%) as well as for 1-[3-(4-ethoxyphenylcarbamoyl)oxy-2-hydroxypropyl]-4-(3-trifluoromethylphenyl)piperazin-1-ium chloride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

## 2.3. Preliminary Structure–Activity Relationships

The position and the length of the alkoxy side chain influenced electronic, steric and lipohydrophilic features as well as the *in vitro* inhibitory activity of tested derivatives **1a–f**. The presently observed *PET-I*s indicated (at the  $c=0.2$  mM) that electron-donating effect of the *R* substituent attached to the phenylcarbamoyloxy 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.

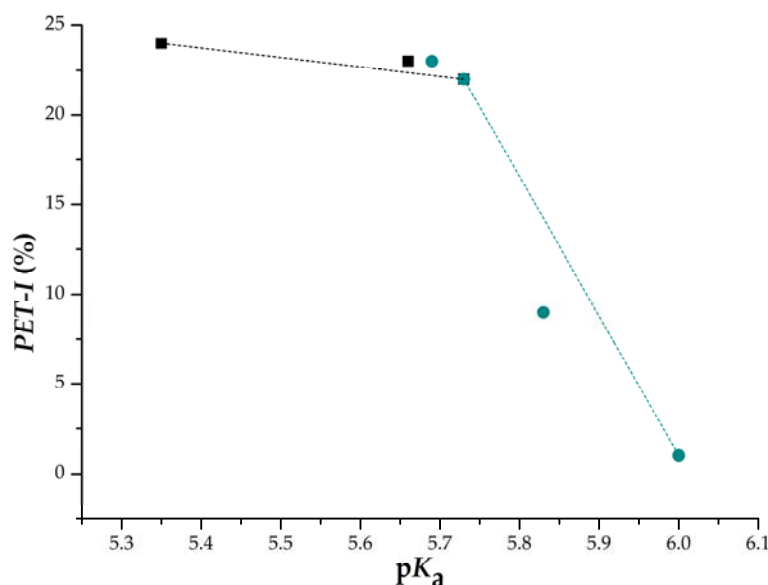
On the basis of observed results it would be suggested that the presence of electron-withdrawing (and sterically bulky) *R* substituent could be strongly taken into the consideration in order to improve the PET-inhibiting activity.

The positive influence of the 3'-CF<sub>3</sub> 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'-CF<sub>3</sub>) 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'-CF<sub>3</sub> substituted derivative > 2'-CF<sub>3</sub> substituted derivative > 4'-CF<sub>3</sub> substituted derivative [18]. That dependence was explained by the highest lipophilicity and very limited solubility of the 4'-CF<sub>3</sub> substituted molecule [18].



**Figure 2.** The promising *in vitro* inhibitors of the photosynthetic electron transport with the attached CF<sub>3</sub> 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 pK<sub>a</sub>=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 pK<sub>a</sub>s and the PET-I values was observed (Figure 3).



**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 pK<sub>a</sub> 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 pK<sub>a</sub> data ranging from 5.35 to 5.73, caused a comparable PET inhibition and their PET-I<sub>s</sub> were in the interval of 22% to 24%. The subsequent increase in the observed pK<sub>a</sub> values meant a notable decrease in the PET-inhibiting ability of those derivatives. As indicated (Table 1, Figure 3), the highest pK<sub>a</sub>s were related to the 2-alkoxy substituted substances **1a** (pK<sub>a</sub>=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 (pK<sub>a</sub> Marvin, pK<sub>a</sub> 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  $\pi$ -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 or practically eliminated [8,9].

The present research suggested that the observed  $\log P_{\text{exp}}$  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  $\log P_{\text{exp}}$  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  $\log Ps$  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 alkoxy 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 alkoxy 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-alkoxyphenyl)carbamoyloxy-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  $\log P_{\text{exp}}$  (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 **1aB–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 Lipohydrophilic Properties

The values of the logarithm of a partition coefficient related to the non-protonated basic molecules **1aB–fB** (Table 2) were generated *in silico* for the octan-1-ol/water partitioning system ( $\log P_s$ ) 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 rate of the photosynthetic electron transport was monitored as a photoreduction of DCPIP at the wavelength of  $\lambda=600$  nm. The measurements were carried out in a phosphate buffer ( $c=20.0$  mM,  $pH=7.2$ ) containing sucrose ( $c=400.0$  mM),  $MgCl_2$  ( $c=5.0$  mM) and NaCl ( $c=15.0$  mM). In those experiments, the chlorophyll content was 30 mg/L and the samples were irradiated ( $\sim 100$  W/m<sup>2</sup> with the 10-cm distance) with a halogen lamp (250 W) using a 4-cm water filter to prevent warming of the samples (suspension 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  $\log P_{\text{exp}}$  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 ( $\Delta \log P$ ) between the  $\log P_{\text{exp}}$  and the predicted data in the range of 0.00 to  $\pm 0.49$  were qualified as acceptable,  $\Delta \log P$  values of  $\pm 0.50$  to  $\pm 0.99$  were viewed as disputable and differences exceeding  $\pm 0.99$  were classified as unacceptable.

The numbers of calculations exhibiting higher or lower values than the  $\log P_{\text{exp}}$  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  $pK_{\text{as}}$ ) 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
<i>F</i>	Fisher significance ratio
FQ	Fluoroquinolone (antibiotics)
$\log P_{\text{exp}}$	Experimentally observed values of the partition coefficient in the octan-1-ol/phosphate buffer (pH=7.4) system
$MV_{\text{MCh}}$	Molecular volume data calculated by the interactive Molecular Properties Calculator applet of Molispiration Cheminformatics
$MV_{\text{MS}}$	Molecular volume data calculated by the interactive Molecular Properties Calculator applet of MolSoft L.L.C.
<i>PET-I</i>	Inhibition of the photosynthetic electron transport (the <i>PET-I</i> values were expressed in the percentages)
$pK_{\text{a ACE-O}}$	Predicted values of the dissociation constants ( $pK_{\text{a}}$ ) by the interactive Achieving Chemistry Excellence: Organic Chemistry applet
$pK_{\text{a Marvin}}$	Predicted values of the dissociation constants ( $pK_{\text{a}}$ ) by the interactive MarvinSketch Online Calculator interface
PS II	Photosystem II
SAR	Structure–activity relationship(s)

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