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Solvent Effect on the Stability and Reverse Substituent Effect in Nitropurine Tautomers

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Abstract: The solvent effect on the stability and electron-accepting properties (EA) of the nitro group attached to the C2, C6, or C8 position of nitropurine NH tautomers is investigated. For this purpose, the density functional theory (DFT) and the polarizable continuum model (PCM) of solvation in a wide range of solvents ($1 < \varepsilon < 109$) are used. We show that the EA properties of the NO_2 group, described by the charge of the substituent active region (cSAR) model, are linearly dependent on the reciprocal of the solvent dielectric constant; in all cases, solvation enhances the EA properties of this group. Furthermore, the sensitivity of EA properties of the nitro group to the solvent effect depends on the proximity effects. It has been shown that the proximity of two endocyclic N atoms (two repulsive interactions) results in higher sensitivity than the asymmetric proximity of the endocyclic N atom and NH group (one repulsive and one attractive interaction). To explain this phenomenon, the geometry of the nitro group in coplanar form and after forcing its rotation around the CN bond is discussed. Relative stabilities of nitropurine tautomers in different solvents are also presented. Differences in the stabilities and solvation energies are explained by aromaticity, electronic structure, and intramolecular interactions of the nitropurine tautomers.

Keywords: purine; nitro group; solvent effect; substituent effect; cSAR; HOMA

Citation: Jezuita, A.; Wieczorkiewicz, P.A.; Szatylowicz, H.; Krygowski, T.M. Solvent Effect on the Stability and Reverse Substituent Effect in Nitropurine Tautomers. *Symmetry* **2021**, *13*, 1223. https://doi.org/10.3390/sym13071223

Academic Editor: Enrico Bodo

Received: 30 April 2021
Accepted: 11 June 2021
Published: 7 July 2021

1. Introduction

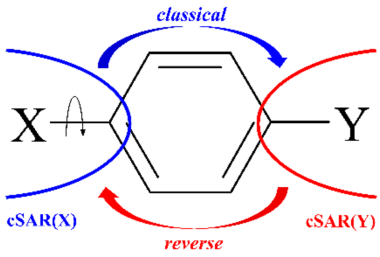
Purine is an important heterocyclic system for life that is present in nucleic acids (DNA and RNA) as a part of two nucleobases: adenine and guanine [1]. Furthermore, purine derivatives are present in various alkaloids such as theophylline, theobromine, and caffeine. Purine exists in the form of nine prototrophic tautomers (four NH and five CH isomers). In the gas phase, the most stable tautomers are 9H, 7H, 3H, and 1H, in which one proton is transferred between four endo-nitrogen atoms [2,3]. Most of the theoretical research on purine has focused on its structural parameters, energetic stabilities, and tautomerism in neutral and protonated form [4]. Additionally, the aromaticity of purine tautomers has also been studied [5].

Due to their importance in bioorganic chemistry, tautomerization and intramolecular interactions in purine nucleobases have been studied in terms of solvation effects. These effects on tautomeric equilibria between all possible nine neutral tautomers of purine, as well as their oxidized and reduced forms, were analyzed theoretically in the aqueous solution and the gas phase [4]. It has been shown that PCM hydration affects the relative energies of purine tautomers (i.e., tautomeric preferences), their geometries, and, to a small extent, the π-electron delocalization. In the gas phase, the tautomeric preferences of neutral and oxidized purine forms are very similar [2,6], with the N9H tautomer being preferred in both cases. In water, the NH tautomers are also more stable than CH, but for the oxidized species, the N1H tautomer is preferred. However, when purine gains one electron in

the reduction processes, the stability of these tautomers changes dramatically. The CH tautomers appeared to be more stable in the gas phase (C6H and C8H dominate), while the N3H tautomer in water. Thus, in electron transfer reactions, the tautomeric purine system can be very sensitive to the environment [4]. The same applies to the stability of the neutral and redox forms of the adenine (6-NH_2-purine) tautomers [3,7].

In the case of purine, in the gas phase, the N9H tautomer is the most stable, while in water, two tautomers, N7H and N9H, have similar energies, and they dominate in the tautomeric mixture [4,8]. Moreover, it was found that, in the gas phase, there are large energetic barriers ($\geq$60 kcal·mol^{-1}) for intramolecular proton transfer, which is another (kinetic) reason for the above-mentioned preference for the N9H tautomer [9]. Solvation, by the hydrogen bonding with water molecules, results in a reduction of the height of these barriers (by 30–40 kcal·mol^{-1}), due to the concerted multipole proton transfer effect. The decrease in the kinetic barrier for proton transfer and relative Gibbs energies may explain why both the N9H and N7H tautomers coexist in the aqueous phase, which was also confirmed experimentally by Raman and ^{15}N NMR spectroscopy [10,11]. Moreover, both of these tautomers also coexist in methanol and dimethylformamide, as shown by low-temperature ^{1}H and ^{13}C NMR spectroscopy [12]. The nature of the solvent only slightly affects their populations. However, their amounts can be significantly changed by substitution at the 6-position of the purine [13,14]. Therefore, apart from tautomerism, the structure of purine and its chemical properties are also significantly influenced by substituent effects.

The substituent effect (SE) is one of the most important effects affecting the chemical, physical, and biochemical properties of chemical compounds. The classical substituent effect is most often described using the Hammett approach [15], which describes the dependence of various properties of the "reaction site" Y (the fixed group in a given series) on the properties of substituents (X) in disubstituted X-R-Y system. Classically, SE is described by the substituent constants, which are primarily derived from dissociation constants of substituted benzoic acids and characterize the electron-withdrawing/donating properties of substituents. Another way of understanding the SE concept is the description of mutual dependences between various properties of the reaction site Y, caused by changes of substituents X. A further aspect of SE is the influence of substituents X (or both X and Y) on the properties of the transmitting moiety, R. One more important aspect of understanding the SE is the reverse SE (Scheme 1) [16].



Scheme 1. Classification of substituent effects.

The reverse substituent effect describes the impact of the reaction site (Y) or the substituted moiety (R, R-Y) on the electronic properties of the substituent (X) in X-R-Y systems. This was already noted by Hammett [15] and represented, for example, by the different values of substituent constant for *para* and *meta* positions. However, for the first time, the concept of "reverse" SE has only recently been presented and documented [17]. It has been demonstrated that this effect may be well characterized by the cSAR(X) approach (charge of substituent active region) proposed by Sadlej-Sosnowska [18–20]. In many articles, cSAR(X) has been shown to correlate linearly with the Hammett constants, σ, and can reliably describe the electronic properties of the substituent [17,20]. In contrast to the substituent constants, which characterize the electronic properties of a substituent by comparison to the reference X-substituted benzoic acid, the cSAR values take into

account the reverse SE of a system to which X is attached. Thus, the cSAR allows the quantification of changes in the electronic properties of the substituent X. Negative values of cSAR(X) correspond to the electron-withdrawing, whereas positive values correlate to the electron-donating character of X.

The reverse SE is dependent on a few factors, such as the transmitter type and the reaction site (Y), but also the proximity effects. Proximity effects, also termed the '*ortho* effect' [21], take into account the existence of intramolecular hydrogen bonds, steric hindrances between atoms, steric inhibition of the resonance effect, and short-range polar effects, which may significantly influence the electron properties of the substituent. These effects make the Hammett equation unsuitable for describing the SE in *ortho*-substituted systems [22]. Moreover, any structural modification leading to a distortion of coplanarity between Y and X groups sterically weakens π-electron delocalization, affecting the SE. This mainly applies to substituents that exhibit a strong steric hindrance with the reaction center, which leads to a significant torsion angle [23–25].

The reverse SE has been examined mainly in hydrocarbon derivatives [16,17,26–28]. For example, in X-substituted 1-, 2-, and 9-anthrol derivatives, where X = NO_2, CN, H, OH, and NH_2, it has been shown that the reverse SE of the OH group is stronger when the number of bonds between two substituents, through which SE is transmitted, is odd [29]. This is some generalization of the well-known SE from the *meta*/*para* positions. The reverse SE of the BH_2 group on X substituents in *para*-substituted phenylboranes was also investigated, as well as the influence of external disturbance in the form of intermolecular interactions on the characteristics of this effect [30]. In heterocyclic systems, the reverse SE was analyzed for 9H, 7H, 3H, and 1H adenine tautomers. It was shown that the values of cSAR(X) are well correlated with the Hammett substituent constants (σ_{para}), similarly to the case of benzene derivatives [31].

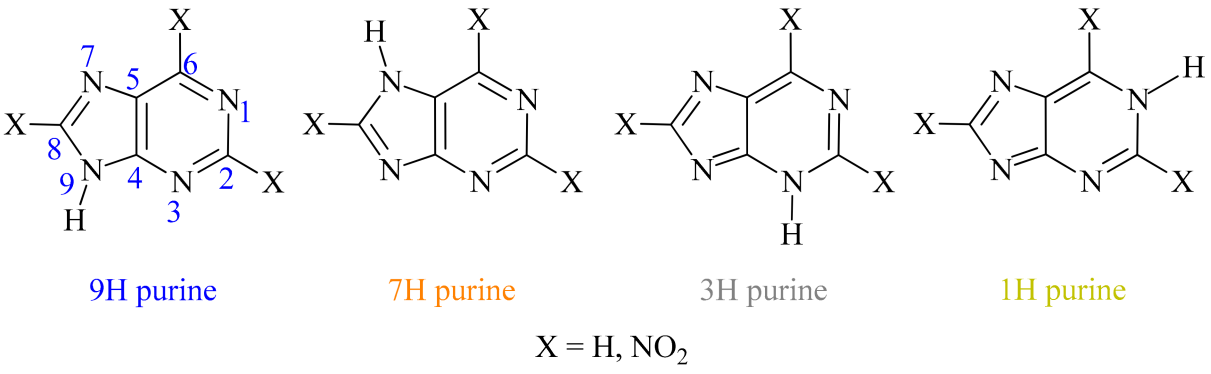
One of the most important substituents in organic chemistry, which can significantly change its electronic properties due to position in the molecule and the nature of the reaction site, is the nitro group. The easiest way to show this is with different values of its substituent constants for the *para* and *meta* positions, 0.778 and 0.710, respectively, or the experimentally obtained acidity constants (pK_a) of *meta*- and *para*-nitrobenzoic acids (3.493 and 3.425, in water at 25 °C) from which these constants were evaluated [15,32]. More pronounced changes in the properties of the nitro group result from the nature of the fixed group (Y) in the reference reactions. This is confirmed by the NO_2 substituent constant: σ (determined in a series of *para*-substituted benzoic acids) and σ^- (determined in a series of *para*-substituted phenol derivatives), which are 0.778 and 1.27, respectively. Higher electron-accepting power of the nitro group in *para*-nitrophenol is the result of strong inductive and particularly resonance effects, caused by intramolecular charge transfer between the NO_2 group and an electron-donating (ED) substituted moiety. Moreover, the resonance effects weaken with the rotation of the nitro group, as evidenced by the changes in the sigma value due to the rotation. This value decreases from 1.27 for coplanar form down to 0.70 for perpendicular one, as shown by Dobrowolski et al. [33] for *para*-nitrophenolate.

To our knowledge, the classical SE of the nitro group, contrary to the reverse SE, has been widely studied in various systems (aliphatic and aromatic) [31,34–40]. The reverse SE, i.e., changes in the properties of the nitro group resulting from the nature of the Y-substituted transmitting moiety, was studied by means of structural and electronic parameters, taking into account coplanar and perpendicular NO_2 conformations [36]. For example, in Li-nitrophenolate, the introduction of the -OLi group leads to a significant increase in Bader's CN bond ellipticity, ϵ_{CN}, which is ~2.6 times higher compared to unsubstituted nitrobenzene. The opposite effect, i.e., a decrease in ϵ_{CN}, was observed in the case of twisting of the nitro group at an angle of 90°. The most fruitful analysis of the reverse SE of the NO_2 group comes from its electronic cSAR parameter. Such analyses have been performed for nitro-X-substituted benzene, cyclohexa-1,3-diene, and bicyclo[2.2.2]octane derivatives, as well as for monosubstituted systems [26,41–45]. It has been documented that the nitro group can change its electron-accepting (EA) properties even by 40% depending

on the type of transmitter and the transmitting method. Furthermore, in nitro-cyclohexa-1,3-diene systems, the nitro group at the fourth position is about 10% more EA than at the third position. In X-nitrobenzene derivatives, the presence of the ED substituent (NH_2, NH^-, OH) in the ring leads to the up to 50% increase in the EA character of the nitro group in the *para* position and 30% in the *meta* position. Moreover, the nitro group loses even 30% of its electron-accepting ability due to rotation in the *para*-nitroaniline system [46]. Hence, it is interesting how factors such as the substitution position, type of tautomer, and resulting proximity effects influence the strength of the reverse SE in heterocyclic systems.

A new aspect of the reverse SE is its dependence on solvation. It is well known that the solvent nature may affect changes in the EA/ED properties of the substituent. Moreover, the solvent has been shown to enhance the substituent effect [47]. The polarizable continuum model (PCM) of solvation was used to predict changes in solvation energies, the stability of tautomeric and anion forms of tetrazole derivatives [48], and the aromaticity of studied compounds. Thus, it can be successfully used to describe relationships between solvating media and the electronic properties of a substituent [49].

As mentioned above, the results of both calculations and experiments show that in purine, NH tautomers are more stable than CH. In this work, we have investigated changes in the electronic structure and mutual stability of NH purine tautomers due to the substituent and solvent effects. The four most stable purine tautomers, i.e., N9H, N7H, N3H, and N1H (shorter notations are used hereafter: 9H, 7H, 3H, and 1H), substituted at C2, C6, or C8 positions by the NO_2 group (Scheme 2) have been examined; changes in the substituent properties (reverse SE) were also realized by rotation of the nitro group around the CN bond. As processes in real biological systems [50,51] and chemistry take place in non-polar and polar environments, a wide range of solvent polarity was selected. For this purpose, the DFT-D method at B97D3/aug-cc-pVDZ level of theory and the polarizable continuum model (PCM) were used. The electron-accepting (EA) properties of the nitro group were characterized using the cSAR index and structural parameters.



Scheme 2. Structures of C2-, C6- or C8-NO_2-substituted 9H, 7H, 3H, and 1H purine tautomers.

2. Methodology

To investigate the reverse substituent effect (Scheme 1) of the nitro group, various positions (C2-, C6-, and C8-X) of the nitro group in purine tautomers were considered (Scheme 2). Moreover, the electron-accepting properties of the nitro group were modified by its rotation around the CN bond by 45 and 90 degrees. The influence of the solvent on the strength of the reverse SE was estimated using 9 solvents of various polarity and the gas phase, as shown in Table 1.

Table 1. Dielectric constant values, ε, of studied solvents.

Solvent/Medium	Acronym	ε	1/ε
Formamide	FA	108.94	0.0092
Water	H_2O	78.36	0.0128
DMSO		46.83	0.0214
Ethanol	EtOH	24.85	0.0402
Pyridine	Py	12.98	0.0771
THF		7.43	0.1347
o-cresol	o-Cr	6.76	0.1479
Chloroform	ClF	4.71	0.2123
Toluene	Tol	2.37	0.4212
Gas phase	GP	1.00	1.00

For studied systems, optimization without any symmetry constraints was performed (in the gas phase and solution) using the Gaussian09 program [52], except in the case of C6-NO_2 substitution in 9H and 3H tautomers, in which the coplanarity of the nitro group with the purine ring was forced, as well as when the NO_2 group was rotated 45° and 90°. According to the results of our previous research [53], calculations were carried out using the DFT-D method at B97D3/aug-cc-pVDZ level of theory [54,55]. The vibrational frequencies were calculated at the same level of theory to confirm that structures optimized without constraints correspond to the minima on the potential energy surface. In the case of C6-NO_2 substituted 3H tautomer with the NO_2 rotated by 90°, no imaginary frequencies were found, contrary to the other systems with the NO_2 group rotated by 90°. Forcing the 45° rotation of the NO_2 group resulted in no imaginary frequencies. The solvent effect was investigated using the polarizable continuum model (PCM) [56–58], using the integral equation formalism variant (IEFPCM). In this method, the solvent is modeled by a continuum of uniform permittivity (ε). The PCM model describes only the solvation effects that result from the mutual solute–solvent electrostatic polarization; specific solute–solvent molecule interactions are not taken into account.

Electronic properties of the nitro group were characterized by the cSAR (charge of the substituent active region) approach [18–20]. cSAR(X) of the nitro group was calculated as a sum of charges at all atoms of the NO_2 group and the charge at the ipso carbon atom:

$$\mathrm{cSAR}(\mathrm{NO_2}) = q(\mathrm{NO_2}) + q(\mathrm{C_{ipso}}) \quad (1)$$

Hirshfeld method of atomic charge assessment [59] was applied to calculate all cSAR values.

For the description of changes in π-electron delocalization in both purine rings, two indices were considered: the geometry-based HOMA (Harmonic Oscillator Model of Aromaticity) [60] and the magnetic NICS (Nucleus-Independent Chemical Shift) [61]. A comparison of the HOMA and NICS values obtained for the 5- and 6-membered rings of imidazole (IM), pyrimidine (PYR), and purine (PU) is presented in Table 2. As NICS values depend on the ring size, the HOMA index was used in our study. It is defined as:

$$\mathrm{HOMA} = 1 - \frac{1}{n}\sum_{i}^{n}\alpha_{\mathrm{j}}\left(d_{\mathrm{opt,j}} - d_{\mathrm{j,i}}\right)^2 \quad (2)$$

where n is the number of bonds taken into account when carrying out the summation, j means the type of bond (e.g., CC or CN), α_{j} is an empirical normalization constant, $d_{\mathrm{opt,j}}$ is the optimal length of a given bond assumed to be realized for full aromatic systems, and $d_{\mathrm{j,i}}$ is an actual bond length in the studied system.

The values of HOMA were calculated using the Multiwfn program [62], according to Equation (2); constants (α_{j} and $d_{\mathrm{opt,j}}$) were taken from Krygowski's paper [63].

Table 2. The HOMA and NICS values for five- and six-membered rings (5_{MR}, 6_{MR}) of imidazole (IM), pyrimidine (PYR), and purine (PU). Data taken from [64].

	HOMA		NICS	
	5_{MR}	6_{MR}	5_{MR}	6_{MR}
IM	0.866		−12.772	
PYR		0.976		−5.000
9H PU	0.757	0.926	−10.971	−7.690
7H PU	0.752	0.915	−11.146	−7.952
3H PU	0.680	0.761	−10.029	−7.749
1H PU	0.548	0.602	−9.555	−7.051

3. Results and Discussion

As mentioned above, the reverse SE, which can be well described with the cSAR model, is very susceptible to various factors. Among them are proximity effects, substitution position, and the type of system. Furthermore, the solvent may also influence the strength of the substituent effect as well as the stability of the studied nitropurine tautomers. Thus, the obtained results are presented in the sections devoted to particular factors influencing the reverse SE of the nitro group (its electron-withdrawing character). Finally, changes in the relative stability of nitropurine tautomers due to solvation are discussed. The cSAR values obtained for the C2-, C6-, and C8-NO_2 substituted purine (PU) 9H, 7H, 3H, and 1H tautomers in various solvents are shown in Tables S1–S3 (Supplementary Materials). Coplanar and rotated (by 45° and 90°) forms of the nitro group were taken into account.

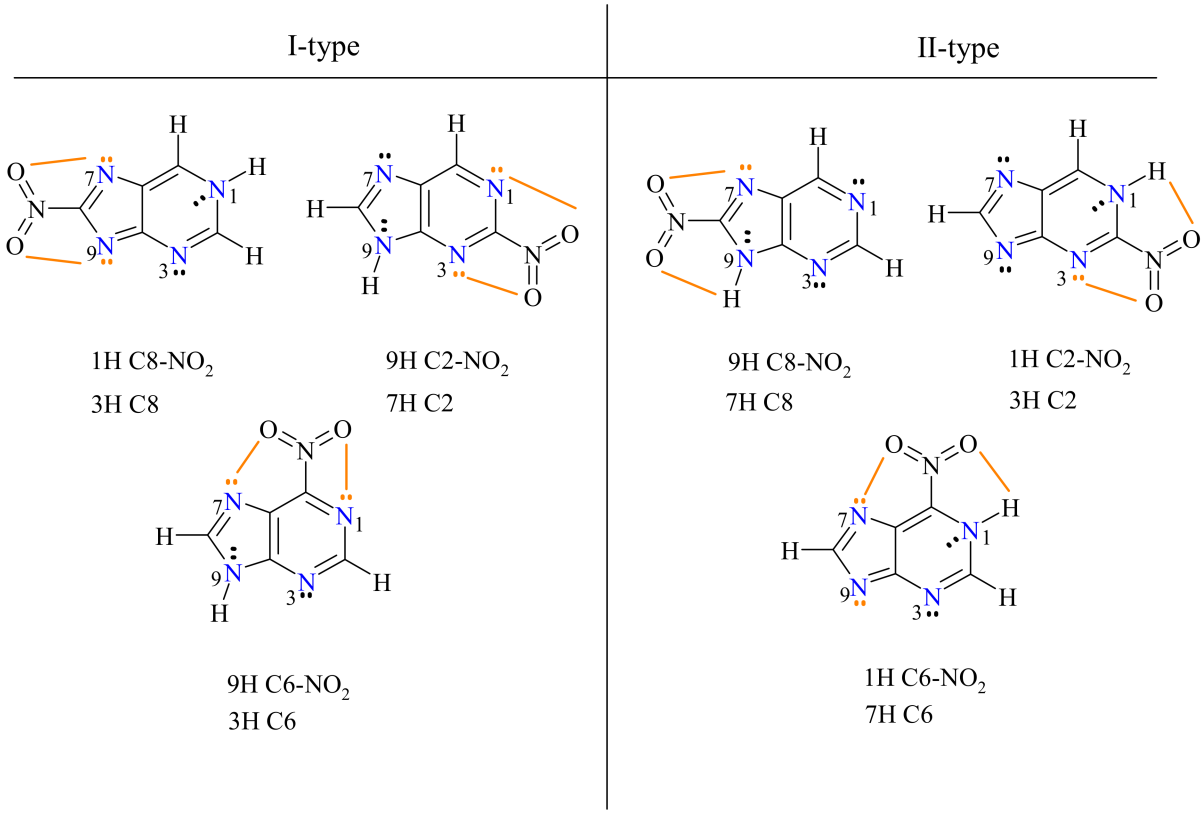
First of all, it should be emphasized that for all analyzed structures, the EA character of the NO_2 group increases with the polarity of the solvent. The cSAR(NO_2) values range from −0.108 to 0.034. This shows that the nitro group may change its EA character by as much as 0.142e, due to both the considered effects: solvation and reverse SE. Moreover, the EA character is strongly dependent on the rotation of the nitro group and is about 20% smaller when NO_2 is perpendicular to the ring. This proves that apart from the resonance effect, the inductive one also plays an important role.

3.1. Dependence of cSAR on the Proximity Effect

The electronic properties of the group/substituent can be significantly modified by proximity effects. In the investigated nitropurine systems, the proximity effects are associated with through-space intramolecular interactions of the nitro group with the neighboring hydrogen atom or with lone electron pairs. Specifically, in 9H, 7H, 3H, and 1H nitropurine tautomers, the nitro group at the C2, C6-, or C8- position can interact in two possible ways, as shown in Scheme 3:

(i) with two pyridine-type N atoms, i.e., with lone pairs in the plane of the molecule (I-type proximity),
(ii) with one pyridine-type N atom, and one pyrrole-type NH group (II-type proximity).

In the case of I-type proximity, the two O atoms of the nitro group interact with adjacent N atoms of the ring. In the II-type of proximity, one of the O atoms of the NO_2 group interacts as in the case of I-type, while the other O atom interacts with the NH group by forming the intramolecular hydrogen bond. From the cSAR(NO_2) values, presented in Tables S1–S3 and their average shown in Table 3, many general differences between the described types of proximity can be observed. The average values of cSAR(NO_2) show that, in general, the nitro group is more electron-withdrawing for I-type than II-type proximity, independently of the nitro group rotamer considered.



Scheme 3. Possible proximities (I and II-type) of the NO_2 group in C2-, C6-, or C8-NO_2-substituted 9H, 7H, 3H, and 1H purine tautomers.

Table 3. Values of cSAR(NO_2), averaged for all studied solvents and their ranges of variability, Δ, for coplanar (∥) and perpendicular (⊥) nitro group; systems with I-type proximity in bold.

		NO_2 (∥)		NO_2 (⊥)		*avg.*⊥−*avg.*∥		$\Delta_\perp/\Delta_\parallel$	Proximity Type
		avg.∥	$\Delta_\parallel$	*avg.*⊥	$\Delta_\perp$		$\Delta_\perp-\Delta_\parallel$	/%	
C2	**9H**	**−0.0627**	**0.0682**	**−0.0404**	**0.0551**	**0.0222**	**−0.0131**	**80.8**	**I**
	7H	**−0.0667**	**0.0745**	**−0.0481**	**0.0615**	**0.0186**	**−0.0130**	**82.6**	
	3H	0.0237	0.0131	0.0470	0.0023	0.0233	−0.0108	17.6	II
	1H	0.0158	0.0137	0.0402	0.0023	0.0244	−0.0114	16.8	
C6	**9H**	**−0.0908**	**0.0661**	**−0.0540**	**0.0532**	**0.0368**	**−0.0129**	**80.5**	**I**
	3H	**−0.0830**	**0.0613**	**−0.0557**	**0.0497**	**0.0273**	**−0.0116**	**81.1**	
	7H	−0.0722	0.0246	−0.0411	0.0161	0.0311	−0.0085	65.4	II
	1H	−0.0303	0.0104	0.0121	0.0047	0.0424	−0.0057	45.2	
C8	9H	−0.0136	0.0300	0.0285	0.0104	0.0421	−0.0196	34.7	II
	7H	0.0001	0.0280	0.0350	0.0094	0.0349	−0.0186	33.6	
	3H	**−0.0819**	**0.0896**	**−0.0400**	**0.0662**	**0.0418**	**−0.0234**	**73.9**	**I**
	1H	**−0.0830**	**0.0960**	**−0.0461**	**0.0739**	**0.0369**	**−0.0221**	**77.0**	

$\Delta = cSAR(NO_2)_{GP} - cSAR(NO_2)_{FA}$.

Comparison of the cSAR(NO_2) ranges, the variability due to solvation (Δ in Table 3), shows a distinctive difference in the way the solvent affects the EA properties of the NO_2 group in I- and II-type systems. In all cases, cSAR(NO_2) values are lower in formamide than in the gas phase; thus, the EA properties are enhanced by the polarity of solvents. When the nitro group is attached to the C2 position, the clearest changes are for the 9H and 7H tautomers, accordingly, when O···N interactions work (marked in bold). The same can be observed for the C6 substitution in the 9H and 3H tautomers and for the C8 position in the 3H and 1H tautomers. Thus, in the case of the I-type proximity, which concerns only

O···N interactions, the variability of cSAR(NO_2) is greater than for the II-type. This shows that the weakening of the repulsive Coulombic interactions between negatively charged N and O atoms due to the increase in ε of the environment has a substantial strengthening effect on the EA properties of the NO_2 group. Our recent results (yet unpublished) on the solvent effect in nitro- and amino-substituted adenine show that the amino group at the C6-position not only enhances the EA properties of the nitro group but also further increases their sensitivity to the solvent effect [65].

Rotation of the nitro group changes both its characteristic as a substituent and its interactions with neighboring atoms. In the coplanar conformation, it induces resonance and inductive substituent effects, while in the 90° rotated form, the resonance is disrupted. The latter is confirmed by the data in Table 3. The average cSAR(NO_2) values for the nitro group rotated by 90° are higher than for the coplanar conformation, so rotation reduces its EA power. On average, for all solvents, 90° rotation causes a weakening of the EA character of the nitro group as measured by cSAR(NO_2) by ~0.034e for C6 substitution, ~0.039e for C8, and ~0.022e for C2. For particular tautomers, the smallest cSAR(NO_2) differences between the coplanar and perpendicular rotamers are always observed for the C2 substitution (Tables S1 and S2). Interestingly, compared to 90°, the 45° rotation results in lower cSAR(NO_2) values (stronger EA effect) for the C2 and C8 substituted tautomers, and slightly higher or comparable for C6, than for the coplanar nitro group (Table S3). This may be caused by the weakening of repulsive intramolecular interactions with neighboring atoms without fully disrupting the mesomeric effect. As shown later in this work (geometric parameters of the nitro group), for the I-type proximity, the CN bond lengths slightly decrease due to rotation. Hence, in the case of 45° rotation of NO_2, the shortening of this bond may have even a stronger (enhancing) effect on the π-electron delocalization between the NO_2 group and purine moiety than the disruption by rotation. This is evidenced by the above-mentioned stronger EA character of the 45° rotated nitro group. Moreover, the ranges of cSAR(NO_2) variability due to the solvent effect are also smaller for non-coplanar rotamers. The reason for this is the weakening of the repulsive N···O interactions due to rotation, and thus, counteraction to the electrostatic part of this repulsion by increasing ε of the environment is less pronounced. As shown later in the text, CN bonds in coplanar systems are shortened to a greater extent than in systems with rotated NO_2, which is another exemplification of this effect.

3.2. Dependence of cSAR on the Type and Position in Tautomer

Other factors that influence the EA ability of the nitro group are the substitution position and the type of tautomer. As mentioned above, the electron-accepting character of the nitro group is usually stronger for the I-type proximity than for the II-type. Moreover, the data in Table 3 suggest that the changes in EA properties of the nitro group depend strongly on the position of NO_2 in the ring. The most negative averaged value of cSAR(NO_2), hence the strongest EA effect, is observed for the C6 substitution, whilst the weakest is observed for the C2 position. Moreover, the EA ability of the NO_2 group at the I-type C8 position is more sensitive to the solvent effect than that of C6 and C2, as indicated by the Δ values. The same trends are observed for a rotated nitro group. Another important conclusion regarding the influence of the substitution position on the EA properties of NO_2 comes from the comparison of the cSAR(NO_2) values for C2, C6, and C8 positions of NO_2 in three solvents, as shown in Table 4. In the gas phase, the NO_2 group is the most electron-accepting for the C6 substitution, regardless of the tautomer. However, the presence of solvent with ε of about 10 (e.g., tetrahydrofuran) may lead to changes in the most EA position in the purine system. This is observed for the 3H and 1H tautomers, in which the NO_2 group becomes the most electron-withdrawing at the C8 position, as well as for the 7H tautomer, in which the NO_2 at the C2 position has the highest EA character. Only in the case of the 9H tautomer are such changes not observed, and the NO_2 group at C6 remains the most electron-withdrawing, regardless of the polarity of the solvent.

Table 4. cSAR(NO_2) values obtained in the gas phase (GP), tetrahydrofuran (THF), and water media.

		NO_2 Coplanar			NO_2 Perpendicular		
		GP	**THF**	**H_2O**	**GP**	**THF**	**H_2O**
9H	C2	−0.0111	−0.0660	−0.0789	0.0018	−0.0434	−0.0530
	C6	−0.0414	−0.0937	−0.1070	−0.0138	−0.0566	−0.0666
	C8	0.0092	−0.0151	−0.0206	0.0369	0.0277	0.0266
7H	C2	−0.0107	−0.0700	−0.0847	−0.0013	−0.0512	−0.0625
	C6	−0.0530	−0.0737	−0.0775	−0.0425	−0.0572	−0.0585
	C8	0.0212	−0.0012	−0.0066	0.0426	0.0342	0.0332
3H	C2	0.0340	0.0229	0.0210	0.0487	0.0465	0.0473
	C6	−0.0370	−0.0858	−0.0979	−0.0035	−0.0436	−0.0529
	C8	−0.0146	−0.0859	−0.1037	0.0105	−0.0435	−0.0554
1H	C2	0.0267	0.0148	0.0131	0.0401	0.0399	0.0411
	C6	−0.0218	−0.0313	−0.0321	0.0098	0.0117	0.0140
	C8	−0.0117	−0.0878	−0.1071	0.0101	−0.0500	−0.0633

Substitution of the purine by the nitro group may lead to changes in the π-electron structure of its rings. As recently documented [64], the electronic structures of the five- and six-membered purine rings either follow the Hückel's 4N+2 rule (tautomers 7H and 9H) or not (3H and 1H). In consequence, those that follow exhibit higher aromaticity (7H and 9H) than the others, as shown in Table 5 (for the coplanar nitro group) and Table S5 (for the rotated nitro group). Moreover, the presence of a polar solvent does not change these dependences. In general, the substitution of purine with the nitro group reduces the aromaticity of both its rings, except for the C8-NO_2 substitution in the 3H and 1H tautomers (see Table 5). The increase in π-electron delocalization in these systems in the gas phase, as compared to the unsubstituted molecule, reveals the substantial influence of the attachment of the nitro group to the five-membered ring, and thus the I-type proximity. A further increase in aromaticity due to the solvent polarity confirms the strongly electron-withdrawing nature of the NO_2 group at the C8 position, as documented by the determined cSAR(NO_2) range values (Table 3 and Table S1).

Table 5. HOMA values of five- and six-membered rings (5_{MR}, 6_{MR}), as well as calculated for all bonds (sum), and differences in HOMA between those obtained in formamide (FA) and gas phase (GP) for coplanar purine derivatives; systems with I-type proximity in bold.

		GP			**FA**			**FA-GP**	**FA-GP**
		6_{MR}	**5_{MR}**	**sum**	**6_{MR}**	**5_{MR}**	**sum**	**6_{MR}**	**5_{MR}**
	9H *	0.926	0.757	0.866					
	7H *	0.915	0.752	0.867					
	3H *	0.761	0.680	0.805					
	1H *	0.602	0.548	0.725					
C2	**9H**	**0.922**	**0.761**	**0.874**	**0.909**	**0.777**	**0.882**	**−0.014**	**0.016**
	7H	**0.910**	**0.749**	**0.870**	**0.904**	**0.784**	**0.887**	**−0.006**	**0.036**
	3H	0.704	0.622	0.782	0.742	0.661	0.806	0.038	0.039
	1H	0.600	0.530	0.730	0.697	0.624	0.786	0.097	0.093
C6	**9H**	**0.876**	**0.724**	**0.850**	**0.861**	**0.735**	**0.857**	**−0.015**	**0.010**
	7H	0.882	0.735	0.861	0.874	0.747	0.868	−0.008	0.013
	3H	**0.661**	**0.560**	**0.754**	**0.703**	**0.605**	**0.785**	**0.042**	**0.045**
	1H	0.425	0.313	0.629	0.547	0.438	0.700	0.122	0.125
C8	9H	0.904	0.770	0.870	0.902	0.790	0.879	−0.002	0.019
	7H	0.896	0.763	0.868	0.903	0.804	0.887	0.007	0.041
	3H	**0.782**	**0.730**	**0.835**	**0.833**	**0.780**	**0.869**	**0.051**	**0.050**
	1H	**0.626**	**0.601**	**0.753**	**0.742**	**0.702**	**0.819**	**0.115**	**0.102**

* Unsubstituted purine tautomers, data taken from Reference [64].

The data in Table 5 show that the π-electron structure of purine rings is most affected by the C6-NO_2 substitution independently of the medium, which is documented by the lowest HOMA values. Interestingly, in the polar medium such as formamide, the HOMA values of the five-membered ring always increase, while for the six-membered ring, they either increase (for 1H and 3H) or decrease (for 7H and 9H). Furthermore, the increases are much more pronounced than the decreases, with a maximum decrease of 0.015, while increases can be as high as 0.125 on the HOMA scale. Larger increases occur when the *endo* NH group is in the six-membered ring, i.e., when this ring contains 7π electrons; for both rings of 1H tautomers, it is about 0.1 on the HOMA scale and only 0.05 for 3H tautomers. The same is observed for aromaticity changes in 5- and 6-membered purine rings for rotated (45° and 90°) nitro systems (Table S4). Moreover, both in the gas phase and in formamide, a clear influence of the rotation of the nitro group on the aromaticity of the rings was found in the case of the C6 substitution. The π-electron delocalization in both rings increases with the rotation of the nitro group. This is due to the disappearance of the resonance effect of the nitro group. Greater increases in HOMA values were observed for the 1H and 3H tautomers than for 7H and 9H. In addition, for perpendicular NO_2 in the gas phase, the greatest increase in the HOMA value occurs for the 1H tautomer, for six- and five-membered rings by 30% and 61%, respectively (with respect to the coplanar system). In formamide, the aromaticity of these rings increases by 21% and 37%, respectively, while for the coplanar system, the transition from the gas phase to formamide increases HOMA by 29% and 40%. Thus, in the case of C6-substitution, the rotation of the nitro group and the solvent more significantly affect the aromaticity of the five- than the six-membered ring of the 1H tautomer. The same, only with less variability, is observed for the 3H tautomer.

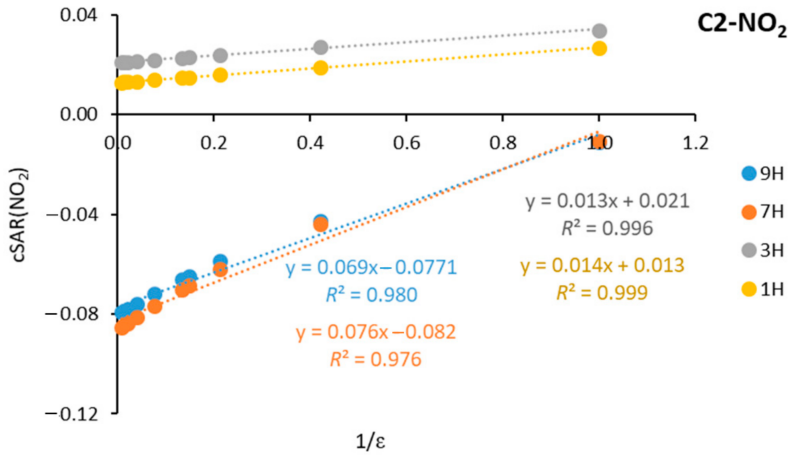
For II-type proximity nitropurines, ranges of cSAR(NO_2) variability seem to be related to aromaticity. Nonaromatic, according to the Huckel's 4N+2 rule and characterized by low HOMA values (Table 5), C2-NO_2 3H and 1H purines have the ranges of 0.013e and 0.014e, respectively. The aromatic C8-NO_2 9H and 7H purines have the ranges of 0.030e and 0.028e, respectively. Among C6-NO_2 purine tautomers, the aromatic 7H tautomer shows a 2.37 times higher range of cSAR(NO_2) variability than the nonaromatic 1H. It follows that the higher aromatic character and therefore better electron delocalization support the nitro group's ability to withdraw electrons from the ring.

In addition, representations of the HOMO and LUMO orbitals in the gas phase and in the most polar solvent (formamide) are shown in Figures S1 and S2, respectively. Only in the cases of 3H and 1H nitropurine tautomers, solvation-induced changes in the HOMO orbitals shapes are observed (Figure S1). In the gas phase, they are mostly localized on the atoms of the 5-membered ring, while in formamide, they spread over both rings, indicating extended electron delocalization. Simultaneously, rings of 1H and 3H tautomers exhibit a relatively large increase in aromaticity, by 0.09–0.12 and 0.04–0.05 units on the HOMA scale, respectively (Table 5). An exception occurs for 1H C6-NO_2, where the HOMO shape does not change significantly. However, this derivative is the least aromatic (Table 5), which may explain this phenomenon. In the 9H and 7H tautomers, no changes in HOMO shapes occur, and the changes in their aromaticity are smaller.

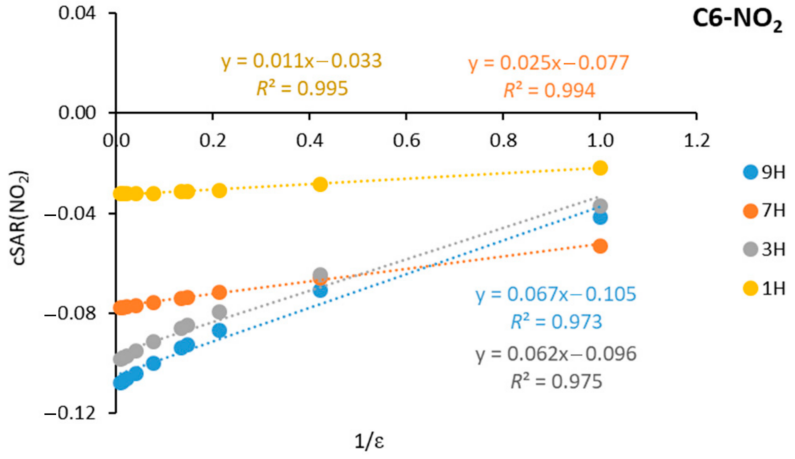
3.3. Dependence of cSAR on Polarity of Solvent

As mentioned above, the polarity of the solvent can significantly affect the strength of the reverse substituent effect. In the case of the studied nitropurine systems, variability of the EA properties of the nitro group to the solvent effect depends on a considered range of dielectric permittivity, ε. This is shown by the ranges of cSAR(NO_2) variability (Table S1); in less polar solvents (ε < 10), they are even 6–25 times greater than in more polar solvents (ε > 10). However, plotting cSAR(NO_2) versus reciprocal of the dielectric constant, $1/\varepsilon$, gives well-correlated linear relationships ($R^2 > 0.95$). These relations are presented in Figure 1; the slopes and R^2 values of the linear relations are collected in Table S5. The slopes inform about the sensitivity of the EA properties of the nitro group to the polarity of the solvent, but as ε of the solvents considered are constant, these slopes are dependent

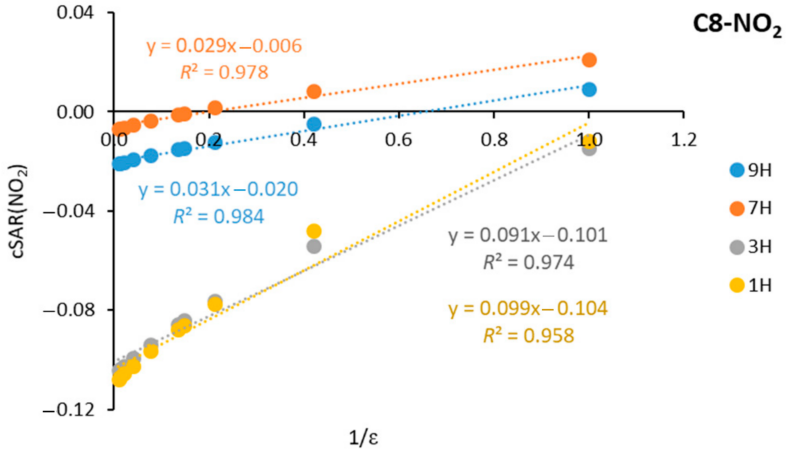
only on the ranges of cSAR(NO_2) variability. Therefore, slopes provide similar information to the ranges already discussed above. Thus, the EA properties of the nitro group in C8-substituted 3H and 1H tautomers are the most sensitive to the solvent effect, while the lack of the resonance effect and N···O interactions (systems with the perpendicular nitro group) lowers its sensitivity by about 25% (slopes in Table S5).



(**a**)



(**b**)



(**c**)

Figure 1. Relationships of cSAR(NO_2) on $1/\varepsilon$ for C2- (**a**), C6- (**b**), and C8-NO_2 (**c**)-substituted 9H, 7H, 3H, and 1H purine tautomers.

3.4. Geometric Parameters of the Nitro Group

The nitro group can be characterized by its structural parameters (d_{CN}, d_{NO}, ∠ONO). For monosubstituted nitropurines, the CN bonds are longer in the case of I-type proximity than in II-type, both in the gas and solvent phase, as shown in Table 6. This means that the repulsive electrostatic interactions of O atoms from the NO_2 group with N atoms of the pyridine-type lead to an elongation of the CN bond. Moreover, for the I-type, the CN bond lengths decrease with increasing solvent polarizability to a greater extent than for the II-type (by an average factor of 1.9). When the nitro group interacts according to I-type and its rotation increases from 0 to 90°, the CN bond lengths decrease, which is due to the weakening of the repulsive N···O interactions in the case of the perpendicular nitro group. An interesting exception from this rule is a slight elongation of CN bonds due to rotation, observed in the 1H and 3H C8-NO2 systems in tetrahydrofuran and formamide solution. The substitution of the C8 atom in the five-membered ring leads to weaker repulsive N···O interactions due to the longer distance between the N and O atoms (Table S8), caused by a geometry of the five-membered ring, namely its smaller internal angles. Consequently, the CN bonds in the 1H and 3H C8-NO_2 derivatives are shorter than in the other I-type systems. In tetrahydrofuran and formamide, the N···O interactions in these systems are weakened to such an extent that in the case of NO_2 rotation, the d_{CN} value is governed mostly by the disruption of the π-electron resonance with the purine rings, rather than further weakening of the N···O interactions. On the other hand, for the II-type proximity, rotation disturbs the intramolecular H-bond, which stabilizes the coplanar structure, which causes the CN bond elongation, as in the case of coplanar and perpendicular rotamers in nitrobenzene (Table 6).

Table 6. Average values of CN bond length, d_{CN}, (in Å) for pairs of tautomers of the same type of proximity (I-type marked in bold) in the gas phase (GP), tetrahydrofuran (THF) and formamide (FA), for coplanar (0°), and rotated by 45° and 90° nitro group *.

		GP			THF			FA		
		0°	**45°**	**90°**	**0°**	**45°**	**90°**	**0°**	**45°**	**90°**
C2	**9H 7H**	**1.5311**	**1.5129**	**1.5048**	**1.5225**	**1.5100**	**1.5082**	**1.5197**	**1.5090**	**1.5091**
	3H 1H	1.4929	1.4923	1.5011	1.4885	1.4902	1.5011	1.4874	1.4898	1.5012
C6	**9H 3H**	**1.5142**	**1.5001**	**1.4955**	**1.5057**	**1.4974**	**1.4980**	**1.5033**	**1.4965**	**1.4985**
	7H 1H	1.4854	1.4908	1.4941	1.4807	1.4848	1.4944	1.4796	1.4842	1.4945
C8	9H 7H	1.4655	1.4688	1.4800	1.4596	1.4651	1.4799	1.4580	1.4643	1.4800
	3H 1H	**1.4891**	**1.4806**	**1.4803**	**1.4780**	**1.4757**	**1.4824**	**1.4747**	**1.4740**	**1.4828**

* d_{CN} nitrobenzene ∥ = 1.4863 Å d_{CN} nitrobenzene ⊥ = 1.4872 Å.

In the case of NO bonds' lengths, smaller values are observed when O atoms participate in N···O interactions, as shown in Table 7. Thus, increasing the CN bond length is related to the shortening of the NO bond. In contrast, the NO bonds, in which the oxygen atom forms O···HN intramolecular hydrogen bond, are longer. As expected, the differences between the two d_{NO} bond lengths are the greatest for the coplanar nitro group (Table 7a), smaller for those rotated by 45° (Table S6), and fully vanish for 90° rotated (Table 7b). This is due to the decrease in the strength of the competing O···N and O···HN interactions and the symmetry of the interactions in the group rotated by 90°.

Table 7. Obtained NO bond lengths, d_{NO}, (in Å) for coplanar (**a**) and perpendicular (**b**) forms of NO_2 substituted purine tautomers in the gas phase (GP), tetrahydrofuran (THF), and formamide (FA); $d_{NO\cdots(H)}$ depicts NO bond whose oxygen atom is an acceptor of the H-bond systems with I-type proximity in bold.

	(a)	**GP**			**THF**			**FA**		
		$d_{NO\cdots(H)}$	d_{NO}	**aver**	$d_{NO\cdots(H)}$	d_{NO}	**aver**	$d_{NO\cdots(H)}$	d_{NO}	**aver**
C2	**9H**	**1.2266**	**1.2284**	**1.2275**	**1.2302**	**1.2311**	**1.2306**	**1.2312**	**1.2317**	**1.2315**
	7H	**1.2279**	**1.2267**	**1.2273**	**1.2305**	**1.2302**	**1.2303**	**1.2313**	**1.2313**	**1.2313**
	3H	1.2427	1.2205	1.2316	1.2400	1.2244	1.2322	1.2391	1.2256	1.2324
	1H	1.2461	1.2197	1.2329	1.2417	1.2247	1.2332	1.2401	1.2263	1.2332
C6	**9H**	**1.2255**	**1.2313**	**1.2284**	**1.2293**	**1.2332**	**1.2312**	**1.2303**	**1.2339**	**1.2321**
	3H	**1.2277**	**1.2294**	**1.2285**	**1.2303**	**1.2324**	**1.2313**	**1.2308**	**1.2334**	**1.2321**
	7H	1.2447	1.2199	1.2323	1.2412	1.2250	1.2331	1.2401	1.2266	1.2334
	1H	1.2465	1.2234	1.2350	1.2423	1.2281	1.2352	1.2408	1.2297	1.2352
C8	9H	1.2423	1.2244	1.2334	1.2403	1.2291	1.2347	1.2397	1.2306	1.2352
	7H	1.2426	1.2228	1.2327	1.2396	1.2279	1.2338	1.2386	1.2296	1.2341
	3H	**1.2297**	**1.2315**	**1.2306**	**1.2338**	**1.2347**	**1.2342**	**1.2351**	**1.2356**	**1.2353**
	1H	**1.2306**	**1.2300**	**1.2303**	**1.2339**	**1.2338**	**1.2339**	**1.2349**	**1.2351**	**1.2350**
	(b)	**GP**			**THF**			**FA**		
		$d_{NO\cdots(H)}$	d_{NO}	**aver**	$d_{NO\cdots(H)}$	d_{NO}	**aver**	$d_{NO\cdots(H)}$	d_{NO}	**aver**
C2	**9H**	**1.2278**	**1.2278**	**1.2278**	**1.2293**	**1.2293**	**1.2293**	**1.2296**	**1.2296**	**1.2296**
	7H	**1.2278**	**1.2278**	**1.2278**	**1.2295**	**1.2295**	**1.2295**	**1.2299**	**1.2299**	**1.2299**
	3H	1.2278	1.2277	1.2277	1.2274	1.2276	1.2275	1.2274	1.2274	1.2274
	1H	1.2280	1.2282	1.2281	1.2278	1.2277	1.2277	1.2277	1.2276	1.2277
C6	**9H**	**1.2274**	**1.2274**	**1.2274**	**1.2289**	**1.2289**	**1.2289**	**1.2293**	**1.2293**	**1.2293**
	3H	**1.2270**	**1.2270**	**1.2270**	**1.2285**	**1.2285**	**1.2285**	**1.2289**	**1.2289**	**1.2289**
	7H	1.2291	1.2295	1.2293	1.2293	1.2292	1.2293	1.2293	1.2293	1.2293
	1H	1.2284	1.2283	1.2283	1.2282	1.2281	1.2281	1.2280	1.2279	1.2279
C8	9H	1.2289	1.2288	1.2288	1.2289	1.2288	1.2288	1.2289	1.2289	1.2289
	7H	1.2288	1.2286	1.2287	1.2287	1.2287	1.2287	1.2287	1.2287	1.2287
	3H	**1.2285**	**1.2285**	**1.2285**	**1.2301**	**1.2301**	**1.2301**	**1.2305**	**1.2305**	**1.2305**
	1H	**1.2285**	**1.2285**	**1.2285**	**1.2302**	**1.2302**	**1.2302**	**1.2306**	**1.2306**	**1.2306**

The analysis of another structural parameter of the NO_2 group, which is ∠ONO (Table S7), shows subtle and hard to interpret differences between pairs of tautomers of similar type. However, a conspicuous difference of 0.39° (in the gas phase), which is more than twice the difference between other tautomers of a similar type, exists between the II-type C6-NO_2 7H and 1H tautomers. This difference may be a consequence of the fact that the NO_2 group in the 7H tautomer, adjusting its ONO angle, forms a favorable quasi-six-membered ring intramolecular hydrogen-bonded system. In the 1H tautomer, the NO_2 group forms a quasi-five-membered system with the neighboring NH group, which is less favorable geometry-wise. Thus, for the 1H tautomer, ∠ONO is similar to that of the other II-type nitropurines. However, in systems with a rotated nitro group, the above difference is even greater (0.58° and 0.51° for 45°- and 90°-rotated NO_2 groups, respectively). Furthermore, in all cases of substitution, increasing rotation of the nitro group leads to a slight increase in ∠ONO, while an increase in the polarity of the solvent lowers its value. A much greater reduction in the angle occurs in the case of I-type systems and when changing the gas phase to tetrahydrofuran.

Comparison of O···N and O···NH distances (in Å) between systems containing I and II-type proximity of the nitro groups shows an interesting difference between these two. For interacting I-type systems (Table S8) in the gas phase, the O···N distances are between 2.64 and 2.72 for the C2- and C8-NO_2 substitution, respectively. In the case of C6 substitution, the O···N distances are varied and are ~2.65 for the N atom of the 6-membered ring and ~2.93 for the 5-membered ring. Additionally, the solvent slightly

influences the O···N distance, as shown by small differences (0.001–0.005) in the O···N distance between the gas phase and formamide. However, for II-type systems (Table S9), the O···N distances are longer, between 2.72 and 3.07. In the case of attractive O···HN contacts, the distances are from 2.09 for C2- substitution, 2.11 and 2.37 for C6- in the 1H and 7H tautomers, respectively, to the maximum value of 2.44 for C8-substitution. These distances, in II-type systems, also show much higher variability due to the solvent polarity than in I-type systems. In formamide, the O···N distances can be shortened by 0.014 up to 0.032 compared to the gas phase, whereas the O···HN distances show even higher changes, between 0.035 and 0.071.

3.5. Influence of Solvent on the Stability of Substituted Purine Tautomers

Solvent effects are important for tautomeric equilibria. They can affect the stability of individual tautomers to a different extent, eventually changing tautomeric preferences. Understanding the relative stabilities between tautomeric forms of heterocyclic compounds is a significant problem in bioorganic chemistry. Figure 2 shows the relative energies (E_{rel}), with respect to the 9H tautomer, obtained in all considered environments for systems with coplanar and perpendicular nitro groups; their values are summarized in Tables S10 and S11, and also in Table 8 for the gas phase and formamide.

In the gas phase, the 9H or 7H tautomers are the most stable for all types of substitutions, regardless of the rotation of the nitro group. For C2- and C8-NO_2-substituted purine, stability decreases in order from the most stable tautomer 9H through 7H, 3H, to 1H, similar to unsubstituted purine [4,5,66]. This may be due to the intramolecular interaction between the *endo* N9-H group and the lone electron pair on the N3 atom, making the 9H tautomer favored in the gas phase and in non-polar solvents [67]. In turn, when the nitro group is attached to the C6 atom and coplanar with the purine, the 7H tautomer is more stable than the 9H tautomer. This can be explained by its high aromaticity, as shown by the HOMA values (Table 5). It should be emphasized that in the gas phase, the most stable tautomers are also the most aromatic.

Table 8. Electronic energies relative to the 9H tautomer (in kcal/mol) for particular substitution (C2, C6, C8) in the gas phase (GP) and formamide (FA). Coplanar and rotated by 90° NO_2 groups taken into account. Tautomers more stable than 9H are marked in bold.

		Coplanar NO_2		**90° NO_2**	
	Tautomer	**GP**	**FA**	**GP**	**FA**
C2	9H	0.00	0.00	0.00	0.00
	7H	3.33	**−0.34**	2.97	**−0.50**
	3H	8.17	11.62	15.29	15.85
	1H	9.69	9.35	17.83	14.08
C6	9H	0.00	0.00	0.00	0.00
	7H	**−4.06**	**−2.11**	2.84	1.49
	3H	10.16	7.97	9.79	7.69
	1H	10.34	9.80	18.26	14.25
C8	9H	0.00	0.00	0.00	0.00
	7H	3.67	0.87	3.30	0.29
	3H	8.96	2.06	2.87	**−0.84**
	1H	11.55	**−0.16**	4.82	**−3.43**

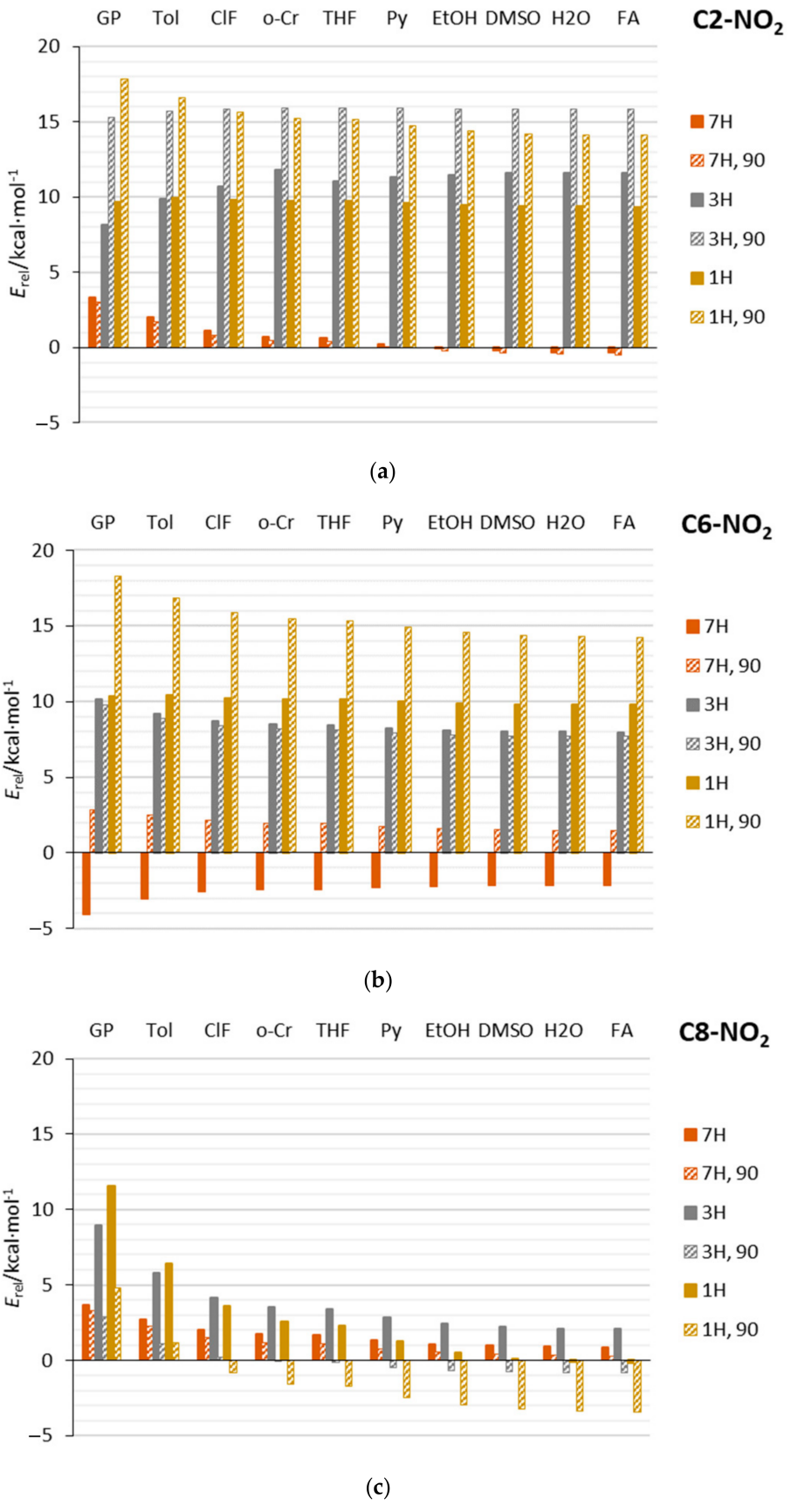


Figure 2. Relative energies (E_{rel}), with respect to the 9H tautomer for studied tautomers of nitropurine for the C2 (**a**), C6 (**b**), and C8 (**c**) substitution.

Taking into account the E_{rel} values and their variability ranges, the 7H tautomers of coplanar and perpendicular systems are generally the least sensitive to environmental changes. However, in all solvents, the coplanar C6-substituted 7H tautomers are more stable than the 9H tautomers (Figure 2), and the opposite is true for perpendicular systems. This can be explained by an intramolecular H-bond with a quasi-six-membered ring geometry in the first case. Moreover, irrespective of the rotation of the nitro group in ethanol and more polar solvents, the C2-substituted 7H tautomers become slightly more stable than the 9H forms (Table 8 and Figure 2). This may be due to the enhancement of their dipole moments by solvents of increasing polarity (see Tables S12 and S13).

The greatest sensitivity of the relative energies of all studied tautomers to the increase in solvent polarity was found for the C8 substitution (Figure 2); the lowering of the E_{rel} values is observed for all tautomers. As shown above, these systems are characterized by the greatest changes in the electronic structure of the nitro group (Table 3), while for the 1H and 3H tautomers (I-type proximity effect) they are in the π-electron delocalization of both purine rings (Table 5 and Table S4) due to the solvent effect. Therefore, among coplanar systems, the 1H tautomer is slightly more stable than 9H in water and formamide (Table 8 and Table S10) due to the stabilization of the strongly dipolar structure (Table S12) by the solvent. Moreover, in the case of perpendicular systems, the 1H tautomer is much more stable than 9H in chloroform, while 3H is slightly more stable than 9H in o-cresol, and more polar solvents (Table S11). For the 1H and 3H tautomers, in the gas phase, the nitro group attached to the five-membered ring significantly increases its aromaticity as well as of the six-membered ring, compared to unsubstituted systems (Table 5). The solvent effect further enhances these aromaticity changes (Table S4), which may explain the particular stability of these 1H and 3H tautomers. Moreover, their strongly dipolar structures (Tables S12 and S13) are stabilized by the solvent.

Summarizing, it should be stressed that in all cases, in more polar solvents, the differences between the energies of the 9H and 7H tautomers are small (e.g., <2.2 kcal/mol in ethanol). This means that both tautomers can coexist in the solution. Interestingly, for the C8-NO_2 coplanar purine in DMSO, water, and FA, the range of the relative energy variability is also 2.2 kcal/mol; thus, all tautomers can be present in solution. Moreover, in the case of systems with perpendicular nitro groups, the stability decreases in the order from the most stable 1H tautomer through 3H, 9H, to 7H already in chloroform and more polar solvents. In water, the 1H and 3H tautomers are more stable than 7H by 3.70 and 2.56 kcal/mol, respectively.

Additional information on the influence of the solvent on the properties of tautomers is provided by their solvation energies, E_{sol} (Tables S14 and S15), and dipole moments, μ (Tables S12 and S13). The extent to which their values change with increasing solvent polarity, ε, can be expressed by slopes, *a*, of the linear relations: E_{sol} vs. $1/\varepsilon$ and μ vs. $1/\varepsilon$, shown in Table 9. Determination coefficients of these relationships are higher than 0.95. The ratios of E_{sol} sensitivity to solvation between the most (1H C8-NO_2) and the least sensitive (9H C8-NO2) purine derivative are approx. 2.41 and 1.98, respectively, for coplanar and perpendicular systems. The highest *a* coefficients can be observed for the I-type proximity effect 7H C2-NO_2, 3H C6-NO_2, 1H, and 3H C8-NO_2 purine derivatives. This is due to the superior enhancement of their dipole moments by solvents of increasing polarity, which is confirmed by the slope values of the μ vs. $1/\varepsilon$ dependence (Table 9). This is also consistent with the solvent effect on the electronic structure of the nitro group (Figure 1, Table S5).

Table 9. Parameters of $E_{sol} = a \cdot (1/\varepsilon) + b$ and $\mu = a \cdot (1/\varepsilon) + b$ linear equations for NO_2 substituted purine derivatives, in their coplanar (**a**), and perpendicular (**b**) forms, and ratios between slopes *a* of particular tautomers. Nitropurines characterized by I-type of proximity are marked in bold.

(a)		$E_{sol} = a \cdot (1/\varepsilon) + b$			$m = a \cdot (1/\varepsilon) + b$		
		a	R^2	**ratio**	***a***	R^2	**ratio**
9H	**C2**	**12.9**	**0.979**	**1.53**	**−2.703**	**0.976**	**2.84**
	C6	**11.6**	**0.968**	**1.38**	**−2.853**	**0.971**	**2.99**
	C8	8.42	0.974	1.00	−0.953	0.965	1.00
7H	**C2**	**16.7**	**0.971**	**1.55**	**−3.858**	**0.971**	**2.14**
	C6	10.8	0.968	1.00	−1.799	0.960	1.00
	C8	11.3	0.963	1.05	−2.228	0.950	1.24
3H	C2	9.36	0.958	1.00	−0.257	0.914	1.00
	C6	**13.6**	**0.966**	**1.45**	**−2.845**	**0.969**	**11.07**
	C8	**15.4**	**0.976**	**1.65**	**−3.484**	**0.974**	**13.55**
1H	C2	13.3	0.966	1.00	−2.531	0.952	1.00
	C6	13.3	0.964	1.00	−2.543	0.953	1.00
	C8	**20.3**	**0.973**	**1.53**	**−5.009**	**0.966**	**1.98**
(b)		$E_{sol} = a \cdot (1/\varepsilon) + b$			$\mu = a \cdot (1/\varepsilon) + b$		
		a	R^2	**ratio**	***a***	R^2	**ratio**
9H	**C2**	**10.6**	**0.982**	**1.23**	**−2.254**	**0.981**	**2.84**
	C6	**10.3**	**0.979**	**1.20**	**−2.404**	**0.977**	**3.02**
	C8	8.6	0.977	1.00	−0.795	0.942	1.00
7H	**C2**	**14.1**	**0.974**	**1.21**	**−3.424**	**0.975**	**1.84**
	C6	11.7	0.971	1.00	−1.856	0.960	1.00
	C8	11.7	0.967	1.00	−2.109	0.950	1.14
3H	C2	10.0	0.976	1.00	−0.660	0.894	1.00
	C6	**12.5**	**0.977**	**1.25**	**−2.568**	**0.971**	**3.89**
	C8	**12.4**	**0.980**	**1.24**	**−2.824**	**0.981**	**4.28**
1H	C2	14.4	0.961	1.00	−2.687	0.955	1.00
	C6	14.4	0.967	1.00	−2.783	0.954	1.04
	C8	**17.0**	**0.975**	**1.18**	**−4.336**	**0.978**	**1.61**

Changing the solvent has a different effect on the stability of each tautomer, but the position of the substituent in the tautomer is also important. The comparison of E_{sol} and μ sensitivities to the solvent effect for the considered substitution positions (C2-, C6- and C8-NO_2) is presented in Table 9. For the same tautomer, the substitutions leading to the I-type proximity have much greater slope values than those leading to the II-type. In the case of the E_{sol}, for coplanar systems, the slopes are on average 51% higher, and for perpendicular systems, they are higher by 22%. As already mentioned, NO_2 groups with I-type proximity exhibit greater changes in cSAR(X) due to solvation, i.e., the higher accumulation of negative charge on this group due to solvation (Table 3). Consequently, the more negative NO_2 group contributes more strongly to the molecular dipole moment. Hence, the superior enhancement of μ can be observed, which in turn leads to the higher sensitivity of E_{sol} on $1/\varepsilon$. The rotation of the NO_2 group substantially weakens its ability to withdraw electrons from the rings. Thus, the increase in μ due to the accumulation of charge on NO_2 in polar solvents is lower, which is evidenced by the smaller values of *a* coefficient

in these systems. Generally, results of the obtained Gibbs energies (Tables S16 and S17) lead to the same conclusions as those derived from electronic energies.

4. Conclusions

In this work, we examined changes in the electronic structure and mutual stability of the four most stable purine tautomers (9H, 7H, 3H, and 1H), substituted at the C2, C6, or C8 positions by the nitro group, due to substituent and solvent effects. Moreover, changes in the substituent properties were also realized by rotation of the NO_2 group around the CN bond. For this purpose, the DFT-D method and the polarizable continuum model (PCM) were used. The electron-accepting (EA) properties of the nitro group were characterized by the cSAR index and structural parameters. The research was carried out in a wide range of solvent properties (from the gas phase, $\varepsilon = 1$, to formamide, $\varepsilon = 109$, nine solvents).

First of all, it should be emphasized that for all analyzed structures, the EA character of the NO_2 group increases with the polarity of the solvent. Moreover, its electronic properties are highly dependent on the substitution position and the proximity type and therefore on the type of tautomer. For the I-type proximity (both O···N interactions), the variability of cSAR(NO_2) is greater than for II-type (O···N and O···HN interactions). Thus, the weakening of the repulsive Coulombic interactions between negatively charged N and O atoms, due to an increase in ε of the environment, has a substantial strengthening effect on the EA properties of the NO_2 group. Considering the position of the substitution, the EA properties of the nitro group in the C8-substituted 3H and 1H tautomers are the most sensitive to the solvent effects. The exclusion of the resonance effect and N···O interactions, by rotating the nitro group by 90°, lowers this sensitivity by about 25%. This shows that besides the resonance effect, the inductive effect also plays an important role. Changes in the electronic structure properties of the nitro group are consistent with changes in its geometry.

Furthermore, the substitution of the NO_2 group to the five-membered ring (C8 position) of the 1H and 3H tautomers increases the π-electron delocalization in these systems compared to the unsubstituted molecule. This is indicated by the HOMA index values for both purine rings in the gas phase. Additionally, the strongly electron-withdrawing nature of the NO_2 group in formamide further enhances their aromaticity. In other cases, in general, the substitution of purine with the nitro group reduces the aromaticity of both its rings.

The stability of individual tautomers can be influenced by both substituent and solvent effects. In the gas phase, the 9H or 7H tautomers are the most stable for all types of substitutions, regardless of the rotation of the nitro group. In the case of the C2- and C6- substituted systems, the difference between their energy decreases with the solvent polarity, and in toluene, it is 2.0 and 3.0 kcal/mol, respectively. This means that both tautomers can coexist in a solution. The 7H tautomers with the C2-NO_2 substitution are slightly more stable than the 9H analogs in ethanol and more polar solvents, while the 7H tautomers with the coplanar NO_2 group at the C6 position are more stable in all considered environments. However, the results obtained show that the purine nitration at C8- leads to small differences in the stability (<2.2 kcal/mol in water) between the NH tautomers. Thus, in an aqueous solution, the mixture may contain a significant amount of all tautomers. Moreover, in the case of systems with the perpendicular nitro group, the stability decreases in the order from the most stable 1H tautomer through 3H, 9H, to 7H, already in chloroform and more polar solvents. In water, the 1H and 3H tautomers are more stable than 7H by 3.70 and 2.56 kcal/mol, respectively.

The substitution of the purine molecule by the nitro group, especially to its five-membered ring and the I-type proximity, increases the molecular dipole moment, which then increases with increasing solvent polarity. This results in different tautomeric preferences in nitro-substituted and unsubstituted purines in the gas phase and their changes with increasing solvent polarity.

Supplementary Materials: The following are available online at http://www.mdpi.com/xxx/s1: cSAR(NO_2) values for C2-, C6-, C8-NO_2 substituted purine tautomers for in-plane (Table S1) and rotated NO_2 group (Tables S2 and S3); HOMA values of five- and six-membered rings(5_{MR}, 6_{MR}), obtained in formamide (FA) and gas phase (GP) for analyzed systems (Table S4); Statistic data of linear relations of cSAR(NO_2) vs. reciprocal of solvent permittivity, $1/\varepsilon$, for analyzed systems (Table S5); d_{NO} values and values of ONO angle for C2-, C6-, C8 NO_2 substituted purine tautomers in the gas phase, tetrahydrofuran (THF) and formamide (FA) for in-plane and rotated NO_2 group (Tables S6 and S7). Distance between O atom from NO_2 and N atom from the purine ring, O···N, with I-type proximity and distance between O atom from NO_2 and N atom from the purine ring, as O···N distance, and O···HN with II-type proximity, in-plane conformation (0°) for all cases substituted tautomers in the gas phase (GP), tetrahydrofuran (THF), and formamide (FA) (Tables S8 and S9); Relative energies of C2-, C6-, C8-NO_2 coplanar and perpendicular substituted purines (Tables S10 and S11); Dipole moments, μ, of C2-, C6-, C8-NO_2 coplanar and perpendicular substituted purines (Tables S12 and S13); Solvation energies of C2-, C6-, C8-NO_2 coplanar and perpendicular substituted purines. (Tables S14 and S15); Solvation Gibbs free energies of C2-, C6-, C8-NO_2 coplanar substituted purines (Table S16); Relative Gibbs free energies of C2-, C6-, C8-NO_2 coplanar substituted purines (Table S17); Representations of HOMO orbitals (Figure S1); Representations of LUMO orbitals (Figure S2).

Author Contributions: Conceptualization, H.S. and T.M.K.; Methodology, H.S. and T.M.K.; Investigation, A.J. and P.A.W.; Formal analysis, A.J. and P.A.W.; Validation, H.S., A.J. and P.A.W.; Funding acquisition, H.S.; Writing—original draft preparation, H.S., A.J. and P.A.W.; Writing—review and editing, T.M.K.; Visualization, A.J. and P.A.W.; Supervision, H.S. All authors have read and agreed to the published version of the manuscript.

Funding: H.S. and T.M.K. thank the National Science Centre of Poland for supporting this work under grant no. UMO-2016/23/B/ST4/00082.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the article and in the associated Supplementary Materials.

Acknowledgments: The authors would like to thank the Wrocław Centre for Networking and Supercomputing and the Interdisciplinary Center for Mathematical and Computational Modeling (Warsaw, Poland) for providing computer time and facilities.

Conflicts of Interest: The authors declare no conflict of interest.

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