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Theoretical Insights Elucidate Novel Active Phosphonate Esters—Cephalosporin Antibiotics' Intermediate

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Abstract

Theoretical insights elucidate a series of active phosphonate esters application in preparation of Cephalosporin antibiotics' intermediate. The B3LYP/6-311+G(d,p) method was employed to obtain the stable equilibrium geometries including comparing to the AE-active ester. It was found that the Ethyl-aminothiazoly Loximate (AT) molecule fragment is almost planar sheet, but it is almost perpendicular to the plane of phosphoryl ester. Moreover, the calculated Mulliken atomic charge distribution and frontier molecular orbital analysis of these esters showed that the amino N atom connected to the Thiazole ring of the AT had the maximum negative charge, which suggested that this area had high molecular activity. The value of $\Delta E_{L\text{-}H}$ was energy gap between E_{HOMO} and E_{LUMO} and indicated that compound 6a had high reaction activity. The theory calculation results can explain the reaction mechanism well and predict that the novel active phosphonate ester has a hopeful application prospect in preparation of Cephalosporin antibiotics' intermediate.

Keywords

Active Phosphonate Ester, Activity, Density Functional Theory, Molecular Orbital, Cephalosporin Antibiotics' Intermediate

1. Introduction

Cephalosporins, which contain thiazolidine-β-lactam rings, are isolated from fungi *Cephalosporium* analogous to *Penicillium*. Natural cephalosporins comprise cephalosporin C, N, and P [1]-[4]. There are many relevant antibiotics, including cefotaxime, ceftriaxone, and ceftazidime, among others [5] [6]. The typical core structure of cephalosporins is shown in **Figure 1**. The routine synthetic procedure was first with 7-

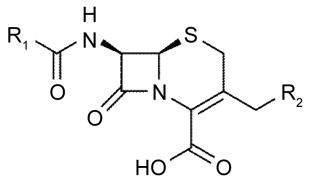


Figure 1. Structure of cephalosporin.

aminocephalosporanic acid (7-ACA) as raw material. To increase the yields of the antibiotics synthesis by elevating the activation efficiency of acylation of the amino group in 7-ACA, various types of acylating agents have been developed, of which carboxylic thiol esters, pyridinecarboxylic acid esters, and carboxylic trinitrophenyl esters are the most representative reagents [7]. Currently, third-generation cephalosporins are normally synthesized by using 2-(2-amino-4-thiazolyl)-2-methoxyiminoacetic thiobenzothiazole ester (popular name: AE-active ester; **Figure 2**) derived from 2-(2-aminothiazole-4-yl)-2-methoxyiminoacetic acid (ATMA) and 2,2'-dibenzothiazolyl disulfide (popular name: accelerator DM) (**Figure 2**) [8] [9]. In this reaction route, the raw material DM is often excessive and remains in the preparation of AE-active ester and is toxic for animals, which is stipulated the residual amount in US Food and Drug Administration and some European countries in cephalosporins. Hence, developing novel active esters without using accelerator DM is imperative [10]-[12]. Phosphphate is found to synthesis active phosphonate esters that are beneficial to improving the security of administering cephalosporin drugs [13] [14]. Our research group has reported the detail synthetic route in the previous articles [15] [16]. It is shown that the novel phosphonate esters (**Figure 3**) have high activity and are superior to AE-active ester, which are commonly applied in modifying second-generation and third-generation cephalosorins [17].

According to a series of experimental synthesized active phosphonate esters, the yield of ceftriaxone with different active ester increases compared with commonly used benzothiazole AE-active ester [15]. Among these esters, 2-(2-aminothiazol-5-yl)-2-(methoxyimino)acetic(O,O-bis(4-nitrophenyl)phosphorothioic)anh-ydride (6a) is most active in the synthesis of ceftriaxone. In order to elucidate novel active phosphonate esters, a quantum chemistry calculation study was used. This paper is focused on the theoretical elucidation active phosphonate esters of Cephalosporin antibiotics' intermediate. We mainly study the properties of the compounds 6a, 6b, 1a and 1b comparing to the AE-active ester that is now accounting for 80% of these commercially available reagents.

2. Experimental

The calculation procedures are as follows. The computational accuracy, feasibility and economical computational time are considered when choosing the computational levels and basis sets. The geometrical parameters are optimized at the B3LYP level with a standard 6-311+G(d, p) [18] [19] basis set. The B3LYP/6-311+G(d,p) is proved to be an outstanding method for prediction of thermochemical kinetics and vibrational frequencies

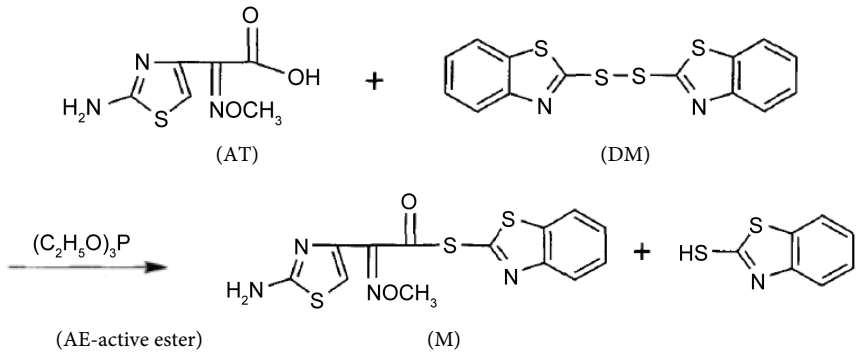
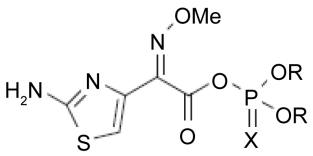


Figure 2. Synthetic route of AE-active ester.



Active phosphonate ester

1a: R=Me; X=S	1b: R=Me; X=O
2a: R=Et; X=S	2b: R=Et; X=O
3a: R=CCH_3; X=S	3b: R=CCh_3; X=O
4a: R=Ph; X=S	4b: R=Ph; X=O
5a: R=4Me-C_6H_4; X=S	5b: R=4Me-C_6H_4; X=O
6a: R=4NO_2-C_6H_4; X=S	6b: R=4NO_2-C_6H_4; X=O

Figure 3. Structures of active phosphonate esters.

of Organic Compounds. All structures of active phosphonate esters have been located on the potential energy surface (PES) by performing full geometry optimization without any symmetry restriction, and their natures including local minima have been identified by performing frequency calculations at the same level, from which the zero point energies (ZPEs) have also been derived. All of the quantum chemical calculations were performed in the framework of DFT using Gaussian by the Gaussian 03 program [20].

3. Results and Discussion

3.1. Molecular Geometry

Through a multi-step series of simulation optimization, stable equilibrium geometries of the active ester (1a, 1b, 6a, 6b) were obtained and shown in **Figure 4**. The ethyl-aminothiazolyloximate (AT) molecular fragment was almost planar sheet, with the plane of phosphoryl ester having the dihedral angle C11-O15-P17-O21 67˚. In addition, the steric structures of phosphoryl ester with sulfur and oxygen in P-S and P-O bonds are similar with the same R substituent. Moreover, with the change of R substituent, the ethyl-aminothiazolyloximate (AT) molecular fragment is almost no change. When R is a 4-NO_2-C_6H_4 substituent, two substituent benzene rings were almost vertical due to steric effect.

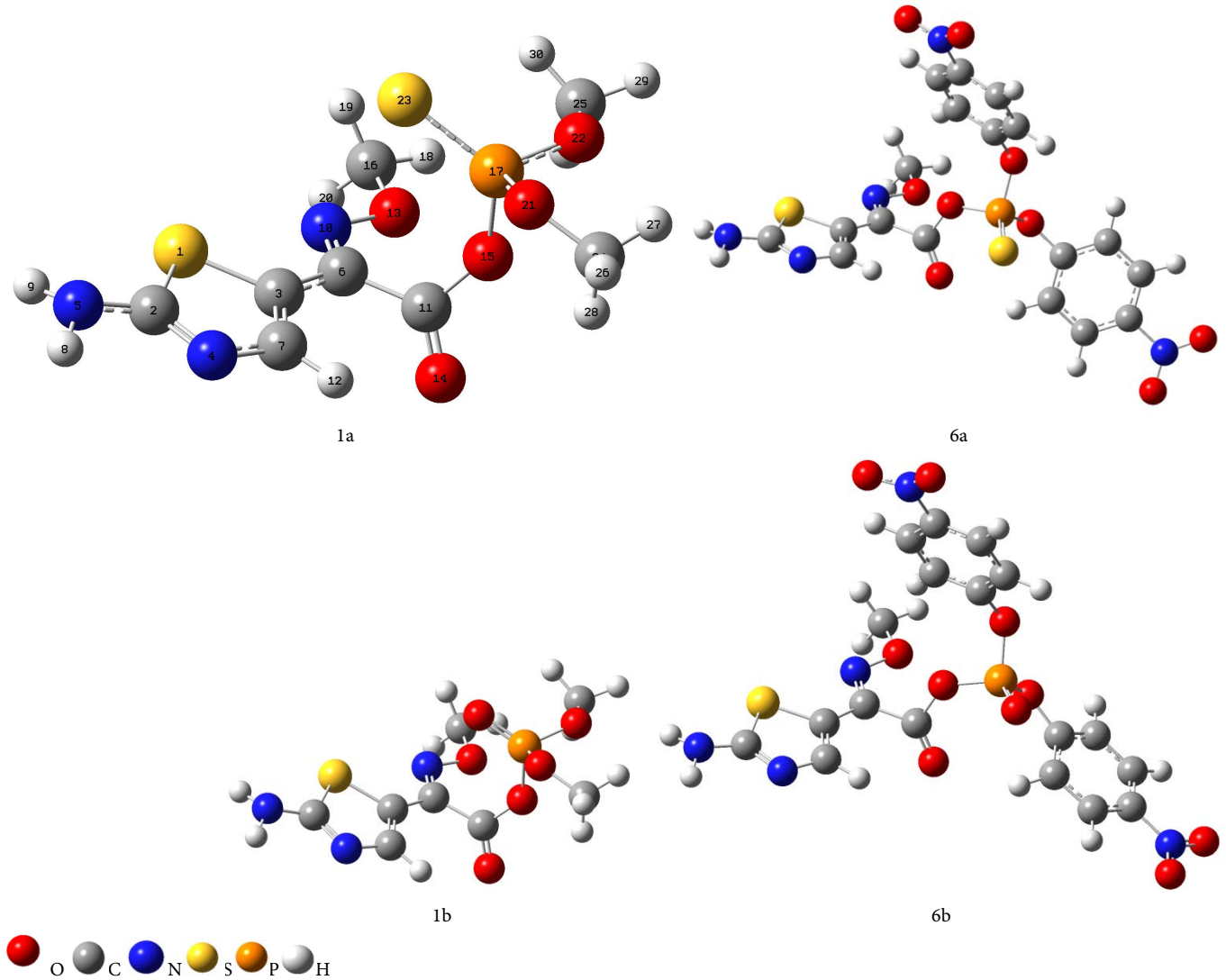


Figure 4. Optimized structures of active phosphonate ester at the B3LYP/6-311+G(d,p) level.

In order to compare the structural difference, AE-active ester was also optimized at the B3LYP/6-311+G(d,p) level and the molecular geometry was shown in **Figure 5**. AE-active ester is obtained by the thio-esterification condensation reaction of AT and DM (see **Figure 2**). The equilibrium molecular geometry is the vertical connection between AT molecular fragment and M (2-mercapto benzothiazole) molecular fragment. The dihedral angle of C6-C9-S13-C14 is 87˚, which is larger than that in the active phosphonate ester. At the connection place, the carboxyl C atom of AT connected with the thiol group S atom of M.

3.2. Charge Distribution

Table 1 gives the calculated mulliken atomic charge distribution of the active phosphonate ester core. All the N and O atoms have negative charges concentrated in AT molecular fragments. Moreover, the amino N (8) atom connected to the thiazole ring of AT

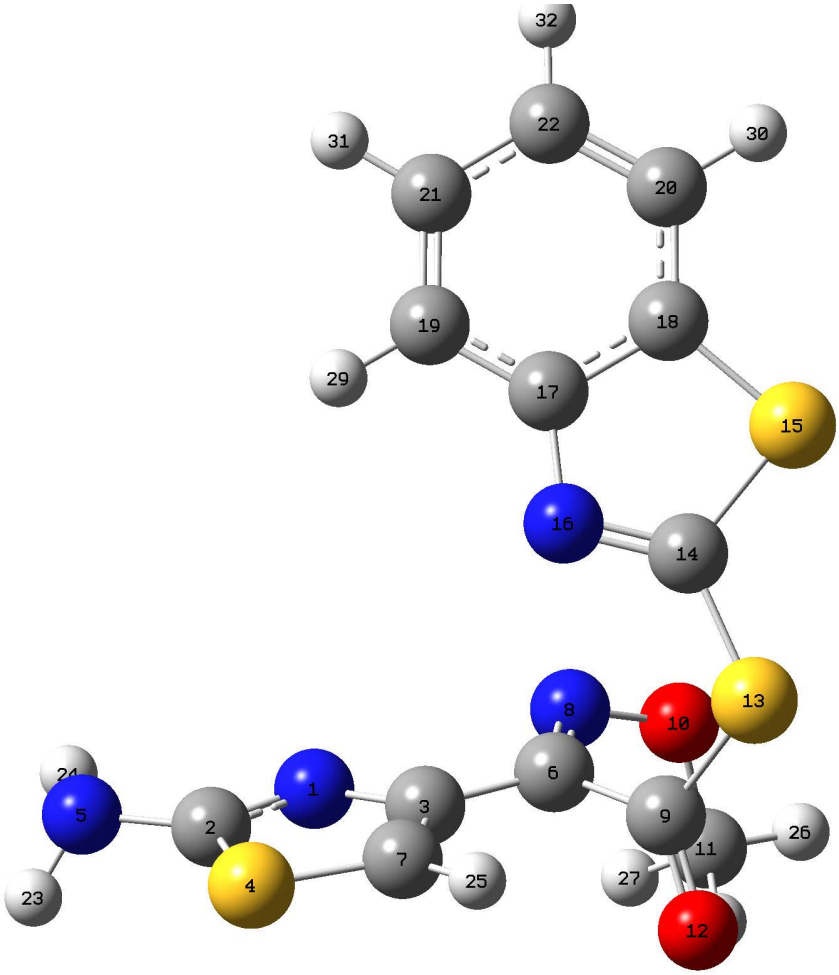


Figure 5. Optimized structures of AE-active ester at the B3LYP/6-311+G(d,p) level.

had the maximum negative charge. Experimentally synthesis of ceftriaxone using the phosphonate active esters with 7-ACA, the C11-O15 bond is broken. Seen from **Table 1**, it is found that the charge on C16 atom has largest negative charge, −1.461412 for compound 6a and −1.127062 for compound 6b. This results agree with experimental phenomenon that the element X connects to the phosphorus is S, activity of phosphonate ester is high.

For AE-active ester, the calculated Mulliken atomic charges were listed in **Table 2**. Similar to active phosphonate ester, the N and O atoms have negative charge in the AT the molecular fragment. Linked to O, and N atoms such as C atoms C2, C6, C17 distribute the main positive charge, and the C atoms are connected to the H atoms mainly distribute negative charge. When to synthesize cephalosporin antibiotics with AE-active ester, the ester bond C9-S13 was broken and the M molecular fragment is removed, and then connected with the β-lactam in the 7-ACA molecule. The C atom accepts the electrons offered by –NH 2 as electron acceptor. Seen from **Table 2**, it is found that the charge on C9 atom is −0.36768 and smaller than that on C16 atom of compound 6a. Hence, the high activity of this active ester can be attributed to the following reasons.

Table 1. Calculated Mulliken atomic charges of active phosphonate ester for compounds 1a, 1b, 6a and 6b, respectively.

atom	1a	1b	6a	6b
S1	−0.046261	−0.088404	−0.098049	−0.089855
C2	−0.182908	−0.128987	−0.276021	−0.280297
C3	0.918978	0.711214	1.007407	0.931253
N4	−0.080687	−0.090859	−0.088637	−0.083803
N5	−0.300217	−0.303431	−0.289096	−0.288201
C6	0.108411	−0.058120	0.222995	0.299641
C7	−0.422135	−0.130257	−0.271779	−0.353063
N10	−0.345016	−0.259018	−0.245743	−0.283129
C11	−0.375505	−0.512285	−1.461412	−1.127062
O13	0.117513	0.048156	0.115018	0.106771
O14	−0.140705	−0.139254	0.054591	0.024536
O15	−0.140772	−0.173887	0.266653	0.218750
C16	−0.244536	−0.231232	−0.229991	−0.229614
P17	−0.310921	0.166952	−0.117455	−0.045287
O21	−0.040990	−0.163310	0.074834	−0.066869
O22	−0.169908	−0.250323	−0.000435	−0.046130
S23	0.042225	0.031299	0.130594	0.032494
C24	−0.339169	−0.297619	0.177783	−0.239864
C25	−0.212809	−0.294822	0.074142	−0.405757

Table 2. Calculated Mulliken atomic charges of AE-active ester.

atom	charge	atom	charge	atom	charge
N1	0.52138	C9	−0.36768	C17	0.11831
C2	0.25767	O10	−0.40446	C18	−0.24109
C3	0.08776	C11	−0.31759	C19	−0.22306
S4	0.38427	O12	−0.50846	C20	−0.24186
N5	−0.85201	S13	0.37478	C21	−0.24382
C6	0.11512	C14	−0.1338	C22	0.36768
C7	−0.43885	S15	0.44851		
N8	−0.10935	N16	−0.4584		

1) Being electronegative, sulfur and oxygen in P-S and P-O bonds are prone to enhancing the electron-withdrawing ability and nucleophilicity of phosphorus-containing groups, thus facilitating the acylation of 7-amino. 2) Nitrophenyl groups are more subject to being removed than other alkyl groups due to large steric space, thereby in-

creasing the synthesis efficiency of ceftriaxone.

3.3. Frontier Molecular Orbital Analysis

According to the molecular orbital theory, the highest occupied orbital (HOMO) and the lowest empty orbital (LUMO) can effectively predict the biological activity site and provide important information for exploring reaction mechanisms. The energy of HOMO, E_{HOMO}, associates with molecular ionization potential, can be used as a measure of providing electronic ability of the molecular. The energy of LUMO, E_{LUMO}, stands for acceptor ability of the molecular. **Figure 6** shows HOMO and LUMO of compounds 1a, 6a and AT-active ester. The frontier molecular orbital analysis shows that the main active part centralizes at the AT molecular fragment, especially at the amido connected with the thiazole ring and it is easy to react while acting with other biomolecule. Therefore, novel active phosphonate ester 6a still maintains the biological activity site.

Table 3 shows the energy of molecular frontier orbital in novel active phosphonate ester and AE-active ester. The value of $\Delta E_{L\text{-}H}$ is energy gap between E_{HOMO} and E_{LUMO}.

The smaller value corresponds to high reaction activity. Seen the calculated values, the $\Delta E_{L\text{-}H}$ of compound 6a is smallest. The theory calculation can explain the reaction mechanism well.

4. Conclusion

Density functional theory B3LYP/6-311+G(d,p) method was employed to theoretically elucidate a series of active phosphonate esters application in preparation of Cephalosporin antibiotic's intermediate. First, the stable equilibrium geometries including comparing to the AE-active ester were obtained. It was found that the Ethyl-aminothiazoly Loximate (AT) molecule fragment is almost planar sheet, but it is almost perpendicular to the plane of phosphoryl ester. Moreover, the calculated Mulliken atomic charge distribution and frontier molecular orbital analysis of these esters showed that the amino N atom connected to the Thiazole ring of the AT had the maximum negative charge, which suggested that this area had high molecular activity. The high activity of 6a active ester can be attributed to the two aspects including large negative charge and steric space. Energy gap between E_{HOMO} and E_{LUMO}, $\Delta E_{L\text{-}H}$, indicated also that compound 6a has high reaction activity. The theory calculation results can explain the experimental phenomenon and predict that the novel active phosphonate ester has a hopeful application prospect in preparation of Cephalosporin antibiotics' intermediate.

Table 3. Molecular frontier orbital energy (Hartree).

	1a	1b	6a	6b	AE- active ester
E_{HOMO}	−0.21868	−0.21749	−0.23256	−0.23145	−0.21414
E_{LUMO}	−0.06676	−0.06226	−0.11373	−0.11158	−0.06424
$\Delta E_{L\text{-}H}$	0.15192	0.15523	0.11883	0.11987	0.1505

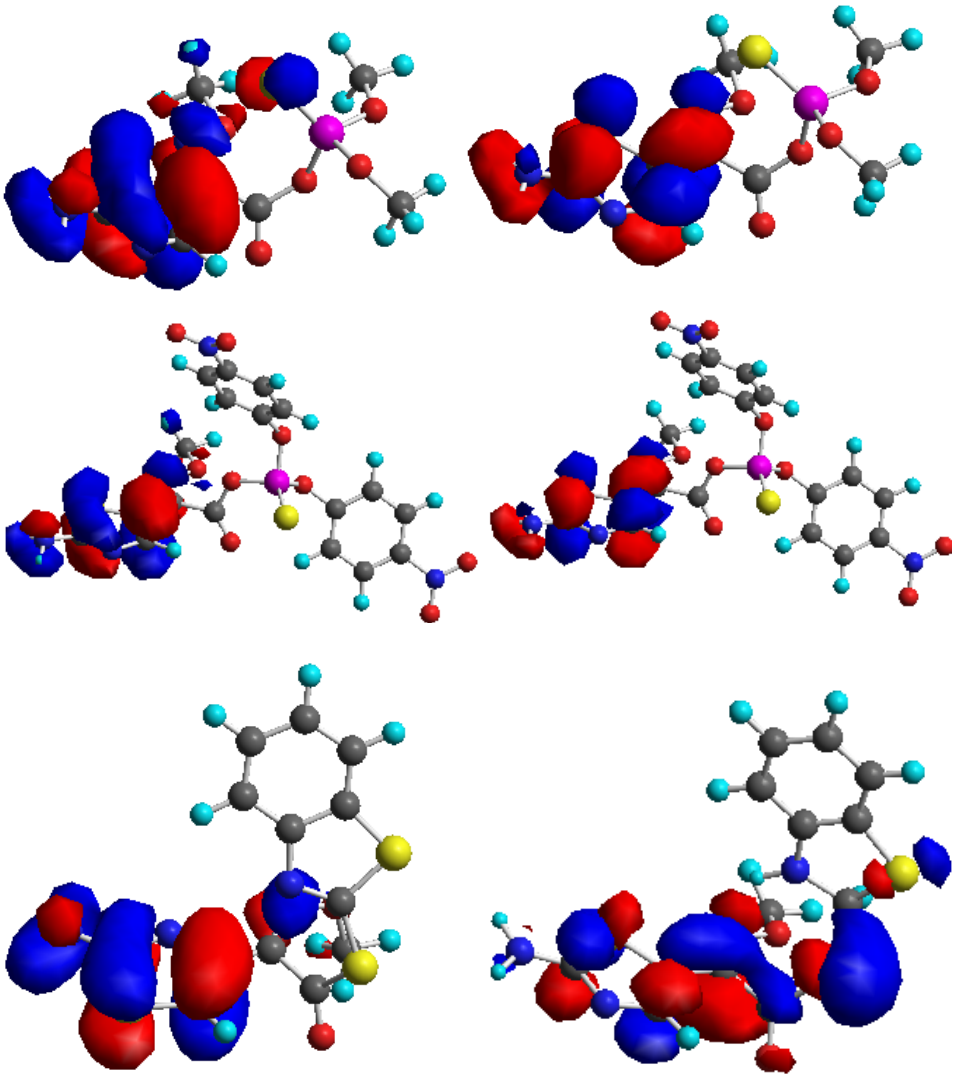


Figure 6. HOMO (right) and LUMO (left) of phosphonate active ester for 1a (Top), 6a (middle) and AE-active ester (bottom).

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