chemistry proceedings

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Proceeding Paper

4*H*-[1,3,5,2]Oxadiazaphospholo[3,4-*a*][1,5]benzodiazepin-1-amine-1-oxides: Synthesis and Computational Studies †

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† Presented at the 27th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-27), 15–30 November 2023; Available online: https://ecsoc-27.sciforum.net/.

Abstract: The modification of heterocyclic systems remains one of the most promising areas in heterocyclic chemistry. Benzodiazepines (BZDs), representing a diverse class of heterocyclic molecules, have piqued interest due to their use as anticonvulsant/anti-inflammatory/analgesic/sedative/anti-depressive/hypnotic medications, as well as anti-inflammatory/anti-HIV drugs. Phosphorus heterocycle molecules fused with rings of different sizes and bearing various heteroatoms have also been attracting much interest. Phosphoramidate class compounds with an amino group linked directly to the phosphorus atom have gained considerable attention due to their wide range of biological activity and agricultural application. To date, however, only non-condensed monocyclic 1,3,5,2-oxodiazaphosphol-2-oxides have been described.Herein, we report the synthesis of previously undescribed *4H*-[1,3,5,2]oxadiazophospho[3,4-a][1,5]benzodiazepine-1-amino-1-oxides, comprising benzodiazepine and a fused five-member oxodiazophospholo cycle with four heteroatoms in the "*a*" position, which was made possible by phosphorylation of 1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin oximes with an equimolar amount of dimethylaminophosphoric acid dichloride. The chemical structures of the compounds were confirmed by IR, ^{1}H, ^{13}C, and ^{31}P NMR spectral analysis. A series of simulations were conducted by employing the semi-empirical, tight-binding computational technique GFN2-xTB to reveal the likely pathways leading to their formation. The synthesised compounds obeyed Lipinski's rule, implying a good bioavailability, and assessment of their projected drug-like abilities revealed that they may have a strong anti-neoplastic activity and, to a lesser extent, may act as both substrates and inducers of cytochrome P-450 (CYP) super-family enzymes.

Keywords: benzodiazepines; oxodiazaphosphol-2-oxides; tetrahydro-2*H*-1,5-benzodiazepin oximes; phosphorus heterocycles; GFN2-xTB calculation; drug-like activity; anti-neoplastic activity

Citation: Anusevičius, Ž.; Kosychova, L.; Kairys, V.; Krikštopaitis, K.; Šarlauskas, J. 4*H*-[1,3,5,2]Oxadiazaphospholo[3,4-*a*][1,5]benzodiazepin-1-amine-1-oxides: Synthesis and Computational Studies. *Chem. Proc.* **2023**, *14*, 22. https://doi.org/10.3390/ecsoc-27-16162

Published: 15 November 2023

1. Introduction

The structural modification of heterocyclic systems continues to be one of the most promising areas in heterocyclic chemistry [1]. Among heterocyclics, benzodiazepine class compounds (BZDs) have piqued the interest of pharmaceutical chemistry due to their usage as anticonvulsant/anti-inflammatory/analgesic/sedative/anti-depressive/hypnotic medicines, as well as anti-inflammatory/anti-HIV drugs [1,2]. Although BZD-derived medications have been viewed by some experts as having only limited benefits, they are nonetheless broadly supplied to patients worldwide [1,2]. Some of them displayed relatively high anti-neoplastic activity against a variety of tumour cell lines [1]. A number of the fused tri-cyclic nitro-substituted BZDs have been generated in our prior research, and the computational studies, by use of the conceptual density functional theory approach, provided a tentative description of the reaction processes [3].

Phosphoramidate-type compounds with an amino group directly linked to the phosphorus atom have garnered a lot of interest due to their broad-spectrum biological actions [4]. Nonetheless, only non-condensed, monocyclic 1,3,5,2-oxodiazaphosphol-2-oxides have been described so far [5].

Given the foregoing facts and our ongoing research into the synthesis of BZD-related compounds, we present here a brief report on the synthesis of novel, previously undescribed phosphorus heterocycles (5-substituted 1,3,5,2-tetrahydro-2*H*-1,5-benzodiazepines), including benzodiazepine and oxodiazaphospholo fragments (Figure 1). In an effort to reveal the likely reaction pathways leading to their formation, the semi-empirical tight-binding computational technique, GFN2-xTB, was employed. The predictive profile of their biological/drug-like features was analysed, suggesting that these substances may have a high potential for use as anti-neoplastic agents.

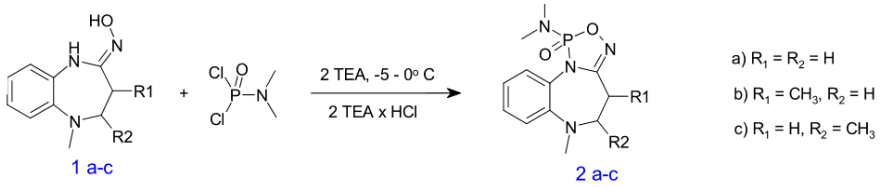

Figure 1. The scheme of synthesis of 4*H*-[1,3,5,2]oxadiazaphospholo[3,4a][1,5] benzodiaze-pin-amine-1-oxides (**2a**–**c**).

2. Materials and Methods

2.1. Reagents and Instrumentations

All reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Deuterated d-chloroform was obtained from Carl Roth GmbH (Karlsruhe, Germany). The melting points were measured using a Barnstead International MEL-TEMP apparatus. Elemental analyses were conducted on an Elemental Analyser CE—440. The IR spectrum (4000–400 cm^{-1}) was recorded on a PerkinElmer Spectrum GX FT-IR spectrometer in KBr pellets. ^{1}H, ^{13}C, and ^{31}P NMR spectra were measured on a Bruker Ascend 400 spectrometer at 302 K at 400, 100, and 162 MHz for ^{1}H, ^{13}C, and ^{31}P, respectively. NMR data were recorded in $CDCl_3$ and referenced to TMS as an internal standard (^{1}H and ^{13}C) and H_3PO_4 (85%) as an external standard (^{31}P). The reactions were monitored by thin-layer chromatography (TLC), using Silica gel 60 F254 aluminium plates (Merck, Darmstadt, Germany) in the following system: chloroform–ethyl acetate–methanol (*v*/*v*, 14:7:1.5). Iodine vapour and UV light (at 254 nm) were used for visualization.

2.2. Computational Details

Reaction-pathway-related calculations were performed with xTB program (v. 6.6.1) using the extended semi-empirical tight-binding technique, GFN2-xTB [6]. The implicit linearised Poisson–Boltzmann (ALPB) model was used to simulate solvation in ether. A "tight" criterion was used for all geometry optimisations. xTB uses the meta-dynamics-based RMSD-Push/Pull path finder to estimate reaction pathways and activation energies. The following parameters were employed as input for the transition state search:nrun = 6 (refinement steps), npoint = 100 (maximum number of points to optimize along the pathway), and alp = 0.4 (meta-dynamics alpha parameter). Molecular indices were obtained by using the graph-convolution neural network program [7]. The Prediction of Activity Spectra for Substances (PASS) programme was used to assess projected biological and/or drug-like activities, expressed in terms of active (Pa) and inactive (Pi) index values [6]. The possibility for experimentally defining activity is projected to be high when Pa > 0.7 [8].

3. Results and Discussion

3.1. General Synthetic Procedure

Using our previously reported approach [9], 5-methyl-1,3,4,5-tetrahydro-2*H*-1,5-benzo diazepin-2-one oximes (**1a**–**c**) were synthesised from thiolactams and thioethers by treatment with hydroxylamine in ethanol.To the mixture of **1a**–**c** (0.005 mol) in 20 mL of toluene containing 1.4 mL (0.011 mol) of TEA, a solution of 0.81 g (0.005 mol) of an equimolar amount of dimethylamidophosphorylic dichloride (Me_2NPOCl_2) in dry ether was carefully added dropwise at −268–273 K (−5–0 °C) with stirring for 2 h. The mixture was then agitated for 5 h at this temperature, followed by an additional 8 h at ambient temperature, and the precipitate formed was filtered out. The filtrate was evaporated to dryness without raising the temperature over 303 K (30 °C), and the resultant residue was extracted with 80 mL of ether and filtered. Finally, the solution was evaporated by applying a water pump with a capillary capacity of 20 mL and then frozen for an overnight period to produce the pure crystalline compounds **2a**–**c**.Their IR, ^{1}H, ^{13}C, and ^{31}P NMR spectra and the elemental analysis are reported below.

N,N,6-trimethyl-5,6-dihydro-4H-[1,3,5,2]oxadiazaphospholo[3,4-a][1,5]benzodiazepin-1-amine 1-oxide(**2a**). White crystals. Yield—17.2%, m.p =163–164 °C (ether); IR, ν: 1609.86 (C=N), 1254.07(P=O), 1004.56 (O-P=O) cm−1; 1H NMR (CDCl3)δ: 2.52 (1H, ddd, J = 7.2 Hz, 11.1 Hz, 14.5 Hz, OCH2); 2.66 (6H, d,JP-H = 10.9 Hz, CH3NCH3); 2.70 (1H, ddd, J = 21 Hz, 6.7 Hz, 14.4 Hz, OCH2); 2.83 (3H, s, CH3N); 3.19 (1H, ddd, J = 2.1 Hz, 7.2 Hz, 10.2 Hz, CH2N); 3.47 (1H, ddd, J = 6.7 Hz, 10.4 Hz, 10.9 Hz CH2N);7.11 (1H, dt, J = 1.4 Hz, 7.6 Hz, H-9); 7.14 (1H, m, H-7); 7.31 (1H, m, H-8); 7.52(1H, m, H-10) ppm; ^{13}C NMR ($CDCl_3$)δ: 24.90 (d, J_{CP} = 3.5 Hz, C-4); 36.25 (d, J_{CP} = 5.5 Hz, C′-1); 41.61(C-1);56,79 (C-5); 120.75 (C-7); 122.74 (C-8); 123.88 (C-10); 128.00 (C-9); 129.02 (d, J_{CP} = 6.7, C-10a); 142.27 (d, J_{CP} = 4.4 Hz, C-6a);159.96 (d, J_{CP} = 24.9 Hz, C-3a) ppm; ^{31}P NMR ($CDCl_3$) δ = 21.94 ppm.$C_{12}H_{17}N_4O_2P$(280,26), Rf = 0.85. Elemental Analysis, %: C, 51.43; H,6.11; N, 19.99; P, 11.05. Found: C, 51.29; H,6.14; N, 20.08; P, 11.09.

N,N,4,6-tetramethyl-5,6-dihydro-4H-[1,3,5,2]oxadiazaphospholo[3,4-a][1,5]benzodiazepin-1-amine 1-oxide(**2b**). White crystals. Yield—28.6%, m.p = 158–160 °C (ether); IR, ν:1602.37 (C=N), 1253.89(P=O), 1014.98 (O-P=O) cm^{-1};^{1}H NMR ($CDCl_3$)δ: 1.22 (3H, d, J = 6.4 Hz, CH_3), 2.61 (6H, d, $^{3}J_{P-H}$ = 10.9 Hz, $2CH_3$), 2.65–2.74 (1H, m, CH) [centras 2.69], 2.80 (3H, s, CH_3), 3.10–3.22 (2H, m, CH_2), 7.06–7.58 (4H, m, Ar) ppm;^{13}C NMR ($CDCl_3$)δ: 11.7(4-C); 31.3 (d,$^{3}J_{P-N-C}$ = 3.6 Hz, C-4); 36.3 (2C, ^{2}J = 5.3 Hz, 1-CH_3); 41.3 (6-CH_3); 65.1 (C-6); 120.2(C-7); 123.0(C-8); 123.8 (C-10); 127.9 (C-9); 128.7 (d, J = 6.5 Hz, C-10a); 143.5 (d, J = 4.9 Hz, C-6a); 162.3 (d, J = 23.6 Hz, C-3a) ppm;^{31}P NMR ($CDCl_3$) δ = 22.60 ppm.$C_{13}H_{19}N_4O_2P$(294,29), Rf = 0.77. Elemental Analysis, %: C, 53.06; H,6.51; N, 19.04; P, 10.52. Found: C, 52.87; H,6.54; N, 19.11; P, 10.58.

N,N,5,6-tetramethyl-5,6-dihydro-4H-[1,3,5,2]oxadiazaphospholo[3,4-a][1,5]benzodiazepin-1-amine 1-oxide(**2c**). White crystals. Yield—20.2%, m.p = 172–173 °C (ether); IR, ν: 1604.69 (C=N), 1263.03(P=O), 1009.25 (O-P=O) cm^{-1};^{1}H NMR ($CDCl_3$)δ:1.14 (3H, d, J = 7.0 Hz, CH_3), 2.78 (6H, d, $^{3}J_{P-H}$ = 10.8 Hz, $2CH_3$), 2.77–2.88 (1H, m, CH), 2.79 (3H, s, CH_3), 3.10 (1H, dd, ^{3}J = 8.4 Hz, ^{2}J =10.3 Hz, CH_2), 3.30 (1H, dd, ^{3}J = 7.1 Hz, ^{2}J = 10.3 Hz, CH_2), 7.03–7.31 (4H, m, Ar) ppm. ^{13}C NMR ($CDCl_3$)δ: 13.4 (5-CH_3); 31.0 (d, $^{3}J_{P-N-C}$ = 3.5 Hz, C-4); 36.1 (2C, d, ^{2}J = 5.3 Hz, 1-CH_3); 41.6 (6-CH_3); 64.3 (C-5); 120.4 (C-7); 121.6 (C-8); 123.4 (C-10); 127.9(C-9); 128.8 (d, J = 5.7 Hz, C-10a); 144.5 (d, J = 4.0 Hz, C-6a); 162.5 (d, J = 23.4 Hz, C-3a) ppm; ^{31}P NMR ($CDCl_3$) δ = 21.25 ppm. $C_{13}H_{19}N_4O_2P$(294.29), Rf = 0.75. Elemental Analysis, %: C, 53.06; H,6.51; N, 19.04; P, 10.52. Found: C, 53.01; H,6.49; N, 19.09; P, 10.46.

3.2. The Plausible Reaction Pathways

Using the GFN2-xTB approach, we computed various scenarios for the reaction (Figure 1). The simulated most plausible reaction pathways are depicted in Figure 2.

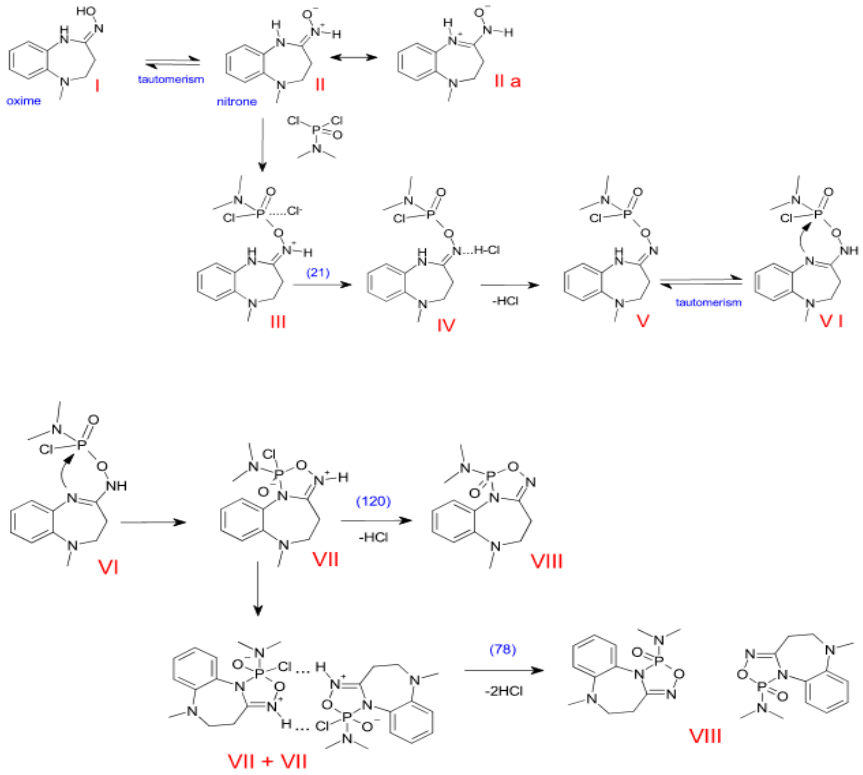

Figure 2. The most plausible reaction pathways for producing 4*H*-[1,3,5,2]oxadiazaphospholo[3,4-a][1,5]benzodiazepin-1-amine-1-oxide (**2a**, VIII), computed by means of the tight-binding GFN2-xTB method. Activation energies are provided in kJ/mol in brackets.

Provisional calculations showed that the interaction between oxime (I) and Me_2NPOCl_2 was not beneficial. The reaction was found to occur with the lowest energy barriers when the formation of the nitrone tautomer (II, Figure 2) was taken into account. Nitrone has been shown to engage in nucleophilic addition reactions and forms more easily through a bimolecular reaction mechanism [10]. As depicted in Figure 2, tautomer II, which has the highest local nucleophilic potency on the oxygen atom of the nitrone moiety, readily interacts with Me_2NPOCl_2 to produce III via a nucleophilic addition reaction, and the chloride ion has not yet left the molecule (due to resonance with IIIa). The cyclisation process between N and P may not happen directly in V, but instead through its tautomer VI. The benzodiazepine nitrogen can carry out a nucleophilic attack on phosphorus, and with the proper rotamer, so that P and N face each other, the cyclisation occurs spontaneously, yielding intermediate VII. Further studies revealed that removing HCl, resulting in the reaction product VIII, necessitates a rather high activation energy (120 kJ/mol) due to the presence of an oxygen atom in between, which interferes with the transition state. Alternately, the reaction VII→VIII can occur via a bimolecular mechanism with a markedly lower activation energy (78 kJ/mol) because the distance between H and Cl is much smaller in that case. As a result, the latter reaction should be more feasible.

3.3. Prediction of Biological/Drug-like Activity

The compounds perfectly complied with Lipinski's rule, viz. molecular weight (MW) of 280–294, octanol/water partition coefficient (Log P) of 2.35–2.74, and H-bond donor (HBD) and H-bond acceptor (HBA) counts are 0 and 6, respectively, indicating a good bioavailability, and the topological polar surface areas (TPSA) were around 50 $Å^2$, implying that they may have a good intestinal absorption [8,11]. Figure 3 displays the prediction profile of the biological/drug-like activities of the compounds established by PASS analysis. According to PASS, these compounds may have substantial anti-neoplastic potency, with Pa and Pi of 0.834–0.910 and 0.001–0.005, respectively, and may also have

anti-neoplastic (non-Hodgkin's lymphoma) activity, albeit at a much lower level (Pa of 0.690–0.675). Additionally, they may operate as inducers and substrates of cytochrome P-450 (CYP) super-family enzymes, such as CYP2C8 (Pa of 0.814–0.822), CYP2H (Pa of 0.721), and to a lesser extent, CYP3A1, CYP3A2, and CYP2C1, with Pa of 0.59–0.614. To an even lesser extent, they may also act as Leukopoesis inhibitors and glutathione transferase substrates. The addition of a methyl group to the heterocyclic system should significantly boost the anticancer activity of the compounds.

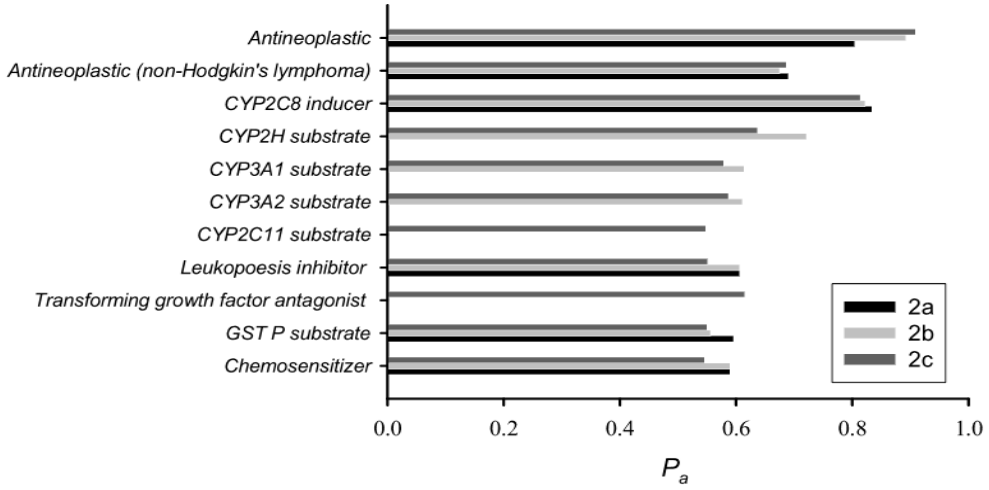

Figure 3. Projected activities of 4*H*-[1,3,5,2]oxadiazaphospholo[3,4-a][1,5]benzodiazepin-1-amine-1-oxides in terms of active index (Pa) values.

4. Conclusions

Novel phosphorus heterocycles, comprised of benzodiazepine and oxodiazaphospholo fragments, have been produced, and the plausible pathways leading to their production have been provided based on computational studies. In accordance with molecular indices and drug-like prediction studies, they appear to have good bioavailability, may have significant anti-neoplastic activity, and may, to a lesser extent, act as inducers or substrates of CYP enzymes. These preliminary results imply that the exploration of their anticancer activity should be the main field of interest for future research.

Author Contributions: Conceptualization, Ž.A. and L.K.; synthesis of compounds and spectral analysis, L.K., J.Š. and K.K.; computational research and data interpretation, V.K. and Ž.A.; manuscript writing, Ž.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The article supporting data can be requested from authors.

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

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