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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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Abstract: The modification of heterocyclic systems still remains one of the most promising areas in heterocyclic chemistry. Benzodiazepines (BZDs), representing a diverse class of heterocyclic molecules, have piqued the 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 the rings of different sizes and bearing various hetero-atoms 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 4*H*-[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 has 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 synthesized 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 in lesser extent may act as both substrates and inducers of cytochrome P-450 (CYP) super-family enzymes.

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

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1. Introduction

The structural modifications of heterocyclic systems continues to be one of the most promising areas in heterocyclic chemistry ([1], and refs. therein). Amid 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], and refs. therein). Albeit BZD-derived medications by some experts have been viewed as having only limited benefits, they are nonetheless broadly supplied to patients worldwide [1,2]. Some of them displayed relatively high antineoplastic activity against a variety of tumour cell lines ([1], and refs. therein). 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], and refs. therein). Nonetheless, only non-condensed monocyclic 1,3,5,2-oxodiazaphosphol-2-oxides have been described by far ([5], and refs. therein).

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-2H-1,5-benzodiazepines), including benzodiazepine and oxodiazaphospholo fragments (Figure 1). In an effort to reveal the likely reaction pathways leading to their formation, the semiempirical tight-binding computational technique GFN2-xTB was employed. The predictive profile of their biological/drug-like features was analyzed, suggesting that these substances may have a high potential for use as antineoplastic agents.

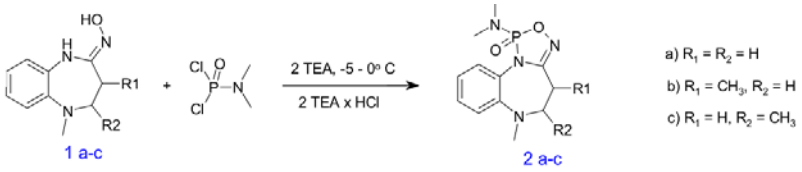

Figure 1. The concise scheme of synthesis of 4*H*-[1,3,5,2]oxadiazaphospholo[3,4a][1,5]benzodiazepin-1-amine-1-oxides (**2a–c**).

2. Materials and Methods

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 a Elemental Analyzer CE—440. IR spectrum (4000–400 cm^{-1}) was recorded on a PERKIN Elmer 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_3PO4 (85%) as an external standard (^{31}P). The reactions were monitored by thin layer chromatography (TLC), using Silica gel 60 F254 aluminum 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 visualisation.

Computational details. Reaction pathway-related calculations were performed with xTB program (v. 6.6.1) using extended semi-empirical tight-binding technique GFN2-xTB [6]. The implicit linearized Poisson-Boltzmann (ALPB) model was utilized to simulate solvation in ether. A "tight" criterion was used for all geometry optimizations. xtB uses metadynamics-based RMSD-Push/Pull path finder to estimate reaction paths and activation energies. The following parameters were employed as input for transition state search:nrun = 6 (refinement steps), npoint = 100 (maximum number of points to optimize along the pathway), alp = 0.4 (metadynamics alpha parameter). Molecular indices were obtained by using of the graph-convolution neural network program (https://xundrug.cn/molgpka). The Prediction of Activity Spectra for Substances (PASS) programme was used to assess projected biological and/or drug-like activities, expressing 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 [6].

3. Results and Discussion

3.1. General Synthetic Procedure

Utilising our previously reported approach [7], 5-methyl-1,3,4,5-tetrahydro-2H-1,5-benzodiazepin-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, II) for 2 h. The mixture was then agitated for 5 h at this temperature, followed by 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. Finaly, the solution was evaporated 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, ^{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}; ^{1}H NMR ($CDCl_3$) δ: 2.52 (1H, ddd, J = 7.2 Hz, 11.1 Hz, 14.5 Hz, OCH_2); 2.66 (6H, d, J_{P-H}=10.9 Hz, CH_3NCH_3); 2.70 (1H, ddd, J = 21 Hz, 6.7 Hz, 14.4 Hz, OCH_2); 2.83 (3H, s, CH_3N); 3.19 (1H, ddd, J = 2.1 Hz, 7.2 Hz, 10.2 Hz, CH_2N); 3.47 (1H, ddd, J = 6.7 Hz, 10.4 Hz, 10.9 Hz CH_2N);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 Plausable 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.

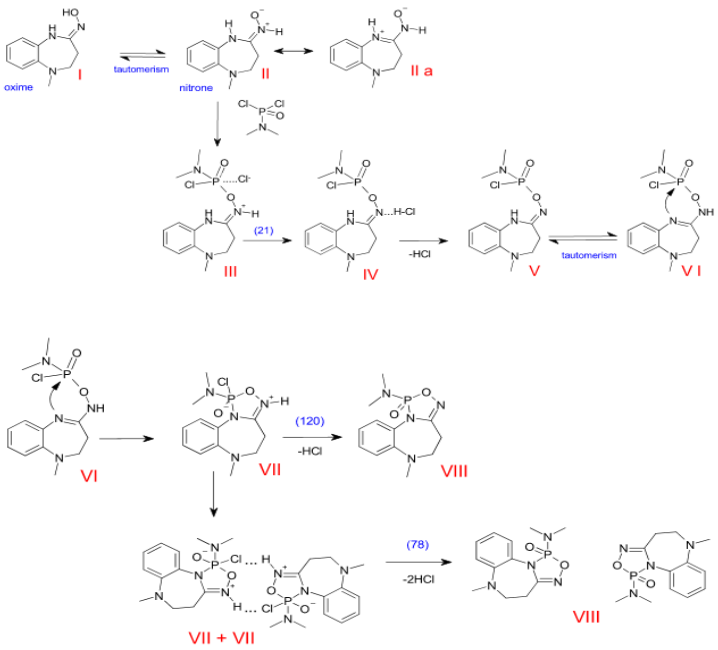

Figure 2. The most plausible reaction pathways for producing 4*H*-[1,3,5,2]oxadiazaphospholo[3,4-a][1,5]benzodiazepin-1-amine-1-oxide (**2a**, VIII) computed by means of tight-binding GFN2-xTB method. Activation energies are provided in kJ/mol between the brackets.

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 3) is taken into account. Nitrone has been shown to engage in nucleophilic addition reactions and forms more easily through a bimolecular reaction mechanism ([8], and refs. therein). As depicted in Figure 3, tautomer II, which has the highest local nucleophilic potency on oxygen atom of 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 cyclization process between N and P may happen not directly in V, but through its tautomer VI. The benzodiazepine nitrogen can perform nucleophilic attack on phosphorus, and with the proper rotamer, so that P and N face each other, the cyclization 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 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 less in that case. As a result, the latter reaction should be more consistent.

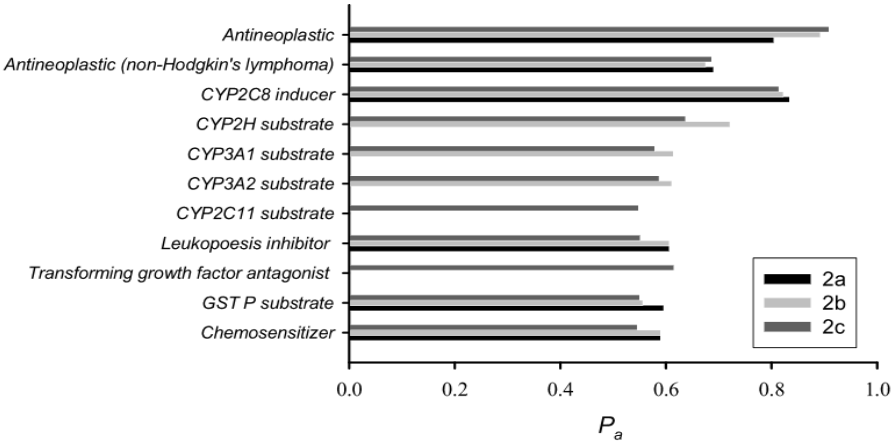

Figure 3. Projected activities of 4H-[1,3,5,2]oxadiazaphospholo[3,4-a][1,5]benzodiazepin-1-amine-1-oxides in terms of active index (Pa) values.

3.3. Prediction of Biological/Drug-like Activity

The compounds perfectly comply with Lipinski's rule, viz. molecular weight (MW) of 280–294, octanol/water partition coefficient (Log P) of 2.35–2.74, H-bond donor (HBD) and H-bond acceptor (HBA) counts of 0 and 6, respectively, indicating a good bioavailability, and the topological polar surface areas (TPSA) are around 50 Å^2, implying that they may have a good intestinal absorption ([9], and refs. therein). Figure 3 displays the prediction profile of biological/drug-like activities of the compounds established by PASS analysis. According to PASS, these compounds may have substantial antineoplastic potency, with Pa and Pi of 0.834–0.910 and 0.001–0.005, respectively, and may also have antineoplastic (non-Hodgkin's lymphoma) activity, albeit at much lesser 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. At much lesser extent, they may also act as Leukopoesis inhibitors and glutathione transferase substrates. The addition a methyl group to the heterocyclic system should significantly boost the anticancer activity of the compounds.

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 to 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 their research.

References

1. Arora, N.; Dhiman, P.; Kumar, S.; Singh, G.; Monga, V. Recent advances in synthesis and medicinal chemistry of benzodiazepines. *Bioorg. Chem*. **2020**, *97*, 103668. https://doi.org/10.1016/j.bioorg. 2020.103668.
2. Dubovsky, S.L.; Marshall, D. Benzodiazepines remain important therapeutic options in psychiatric practice. *Psychother. Psychosom.* **2022**, *91*, 307–334. https://doi.org/10.1159/000524400.
3. Kosychova, L.; Karalius, A.; Staniulytė, Z.;Sirutkaitis, R.A.; Palaima, A.; Laurynėnas, A.; Anusevičius, Ž. New 1-(3-Nitrophenyl)-5,6-dihydro-4*H*-[1,2,4]triazolo [4,3-*a*][1,5]benzodiazepines: Synthesis and Computational Study. *Molecules* **2015**, *20*, 5392–5408;. https://doi.org/10.3390/molecules20045392.
4. Zalán, Z.; Martinek, T.; Lazar, L.; Sillanpää, R.; Fulop, F. Synthesis and conformational analysis of tetrahydroisoquinoline- and piperidine-fused 1,3,4,2-oxadiazaphosphinanes, new ring systems. *Tetrahedron* **2006**, *62*, 2883–2891. https://doi.org/10.1016/j.tet.2006.01.018.

5. Dürüst, Y.; Gözlükaya, Ö.; İvgen, H. Synthesis of some 1,3,5,2-oxadiazaphosphol-2-oxides,3*H*-1,2,3,5-oxathiadiazol-2-oxides, and 1,3,2-oxazaphospholidn-2-oxides (sulfides). *Phosphorus Sulfur Silicon* **2014**, *189*, 215–225.
6. Bannwarth, C.; Ehlert, S.; Grimme, S. GFN2-xTB—An Accurate and Broadly Parametrized Self-Consistent Tight-Binding Quantum Chemical Method with Multipole Electrostatics and Density-Dependent Dispersion Contributions. *J. Chem. Theory Comput.* **2019**, *15*, 1652–1671. https://doi.org/10.1021/acs.jctc.8b01176.
7. Rudika, A.V.; Dmitrieva, A.V.; Lagunina, A.A.; Filimonova, D.A.; Poroikova, V.V. PASS-based prediction of metabolites detection in biological systems. *SAR QSAR Environ. Res.* **2019**, *30*, 751–758. https://doi.org/10.1080/1062936X.2019.1665099.
8. Kosychova, L.; Stumbrevičiūtė, Z.; Plečkaitienė, L.; Staniulyte, Z.; Puodžiūnaitė, B.D. Convenient synthesis of novel tetrahydro-1,5-benzodiazepine amide oximes. *Chemija* **2006**, *17*, 21–25.
9. Bannwarth**, C.;** Ehlert, S.; Grimme, S. GFN2-xTB—An Accurate and Broadly Parametrized Self-Consistent Tight-Binding Quantum Chemical Method with Multipole Electrostatics and Density-Dependent Dispersion Contributions. *J. Chem. Theory Comput.* **2019**, *15*, 1652–1671. https://doi.org/10.1021/acs.jctc.8b01176.
10. Roca-Lopez, D.; Daru, A.; Tejero, T.; Merino, P. Revisiting oxime–nitrone tautomerism. Evidence of nitrone tautomer participation in oxime nucleophilic addition reactions. *RSC Adv*. **2016**, *6*, 22161–22173. https://doi.org/10.1039/c6ra02638a.
11. da Silva, M.M.; Comin, M.; Duarte, T.S.; 2, Mary Ann Foglio, M.A.; de Carvalho, J.E.; do Carmo Vieira, M.; Formagio, A.S.M. Synthesis, antiproliferative activity and molecular properties predictions of galloyl derivatives. *Molecules* **2015**, *20*, 5360–5373;. https://doi.org/10.3390/molecules20045360.