The synthesis of functionalized 4,5-dihydroisoxazoles decorated with the dimethylphosphinoyl group

Abstract

Aim. To synthesize a hybrid molecular platform incorporating dimethylphosphinoyl and 4,5-dihydroisoxazole moieties suitable for the creation of focused combinatorial libraries of compounds.

Results and discussion. The base-promoted interaction of halogenoxides with dimethyl(vinyl)phosphine oxide under mild conditions allowed us to obtain 11 isoxazoline–dimethylphosphate oxide hybrids in moderate yields. The reaction was found to be regio- though non-stereoselective. Furoxans were identified as possible side products of the reaction.

Experimental part. The one-pot interaction with dimethyl(vinyl)phosphine oxide was used for the synthesis of the target compounds. Nitrile oxides were obtained in situ from the corresponding halogenoximes by base-promoted generation. The ADME parameters for a synthesized 5-P(O)Me₂-isoxazoline compared to its isosters with the same core structure were predicted using a SwissADME Web Tool. The compounds obtained were characterized by ¹H, ¹³C, ¹⁹F, ³¹P NMR spectroscopy and HPLC-MS spectrometry methods, as well as the elemental analysis.

Conclusions. A practical approach to the isoxazoline platform decorated with a 5-P(O)Me₂ “magic” group and containing 3-substituent with an easy-to-modify functionality has been developed. On example of the piperidine derivative, the effect of the dimethylphosphinoyl group on physicochemical properties and ADME parameters compared to its isosters has been determined.

Keywords: dimethylphosphine oxide; isoxazoline; dipolar cycloaddition; nitrile oxide; halogenoximes; selectivity; ADME profile
Introduction

Phosphorus along with carbon, oxygen, and nitrogen is an essential element undoubtedly required for the growth and development of all known forms of life. Either in the form of inorganic phosphates or being incorporated in the biomolecules (nucleotides, DNA, RNA, phospholipids, phosphorylated proteins, etc.) phosphorus is involved in multiple vital biochemical processes, like storing and transferring genetic information, transporting cellular energy, functioning the cell membrane, including several transport mechanisms and protection of the interior of all cells. Hence, no wonder that this element gained much attention from agriculture [1], biochemistry [2], and medicinal chemistry [3] researchers. Thus, organophosphates make up much part of insecticides and have been investigated for application in the veterinary field [4, 5]. The medical application of phosphorus-containing organic substances started with the release of menadiol sodium diphosphate by Roche in 1941 [6]. Further research has led to expansion of the nomenclature of “P”-containing functional groups embedded in drug molecules, and now it is possible to find pharmaceutical agents belonging to phosphoric amides [7], phosphonates [8], phosphinates [9], bisphosphonates [10], phosphoric anhydrides [11], etc., which have achieved commercial success [12].

Recently, excellent reviews highlighting and summarizing the phosphorus-containing drugs currently available at the market, their structural features, biological mechanism, as well as historical aspects of their development, have been published [13, 14].

Broadly, a phosphorus-containing functionality is added to the molecule to create selectivity- and bioavailability-enhanced pro-drugs and does not constitute a pharmacophoric part of the molecule [15]. This strategy is also frequently used to improve the aqueous solubility of sparingly soluble molecules and achieve a suitable pharmacokinetics profile by introducing a charged phosphate and related units [16]. A major obstacle associated with the latter approach is a low cell permeability and poor oral bioavailability of the charged molecules [17]. In view of this, it is surprising that their uncharged counterparts, namely phosphine oxides, have been largely neglected in drug discovery. It is quite strange as the phosphine oxide residue is usually easily formed and becomes (bio)chemically inert after its incorporation into the molecular structure. It also does not display much redox properties compared to carbonyl and alcohol functional groups. Furthermore, its capacity to act as an H-bond acceptor makes the phosphine oxide group an excellent alternative to existing common H-bond acceptors like carbonyls and sulfones [18].

A real breakthrough in understanding that a phosphine oxide fragment can be a game changer in creating innovative drugs have come with the accelerated FDA granted approval of brigatinib (Alunbrig®) for the treatment of metastatic non-small cell lung cancer [19]. A unique structural feature of brigatinib is the presence of the dimethyl phosphate oxide (DMPO) moiety, which solely causes the selectivity profile of brigatinib over anaplastic lymphoma kinase. It is amazing that such a simple group within a long thread-like brigatinib structure 70 times increases its potency compared to unsubstituted counterparts. Moreover, DMPO provides brigatinib with remarkable water solubility and reduced lipophilicity [20].

Later, after the release of brigatinib, a magnificent work by Finkbeiner et al. comprehensively showed that phosphine oxides (including DMPO) were outstanding polar structural elements deserving to become a routine part of medicinal chemistry toolkit [21]. In particular, the authors thoroughly investigated in vitro properties of a series of phosphorus(V)-containing compounds and came to conclusion that phosphine oxides gave high solubility and metabolic stability to organic substrates, but occasionally due to the worsened cell membrane permeability. All of the above has led to a revision of the generally accepted paradigm of medical chemistry, according to which phosphine oxides are considered an undesirable group. This change of views has
given rise to several clinical candidates containing the trialkylphosphine oxide group (Figure 1, A) and, in particular, the DMPO moiety (Figure 1, B) [13, 21]. Among them, Fosazepam deserves attention; it is a water-soluble analog of Diazepam and indicates the magic effect of the DMPO fragment on the physicochemical properties of organic molecules.

Turning to the synthetic side of the issue it should be emphasized that the ability to readily incorporate new functional groups into molecular frameworks is what determines how well these groups are adopted by medicinal chemistry. Taking into account the importance of the last statement, as well as the lack of methods suitable for introducing the DMPO fragment into low molecular-weight saturated heterocyclic amines, we have lately disclosed several scalable synthetic approaches to DMPO-containing azetidine, pyrrolidine, piperidine, and morpholine derivatives [22]. This work significantly contributed to medicinal chemistry and provided valuable sp³-enriched building blocks for the use in early drug discovery. Proceeding with the creation of MedChem relevant DMPO containing functionalized “bricks” with a high potential to generate lead-like compounds, in this report we describe an efficient strategy towards a hybrid molecular platform incorporating the Me₂P(O) and 4,5-dihydroisoxazole moiety along with a ready-to-modify functionality.

Interestingly, although the isoxazole ring is among the top heterocycles used in medicinal chemistry [23], its 4,5-dihydro analog is overlooked by major pharmaceutical players. The search over different databases returned no marketed drugs approved for the use in human medicine, and only 3 examples undergoing experimental clinical trials with 2 of them approved for veterinary use (Figure 2, A) [24–26]. On the other hand, naturally occurring 4,5-dihydroisoxazoles are a well-known class of heterocyclic compounds often with outstanding pharmacological properties (Figure 2, B) [27–29].

One should note that our research group has a profound experience in construction of polysubstituted isoxazoles and 4,5-dihydro analogs [30, 31]. We have devised new and optimized known methods towards the heterocyclic platforms, which rely on 1,3-dipolar addition of in situ generated nitrile oxides from halogenoximes to diverse unsaturated partners. It has been proven that the use of a component containing an alkene fragment is a simple and effective way to obtain isoxazolines [32]. It is worth mentioning that we have successfully exploited this way to reach diethyl phosphonates (Scheme 1, A) [33]. In this way, it becomes obvious that the target for working derivatives can be achieved by replacing diethyl phosphonate

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**Figure 1.** Recent examples (or investigational and approved drugs) of the use of phosphine oxides in medicine.
vinylphosphonate with dimethyl(vinyl)phosphine oxide in the last reaction. In turn, recently in our work, the procedure for preparing the latter on a scale of more than 100 g was disclosed [22]. Notably, the phosphine oxide was fruitfully examined in 1,3-dipolar addition with N-benzyl-1-methoxy-N-((trimethylsilyl)methyl)methanamine giving the corresponding pyrrolidine in a high yield (Scheme 1, B).

Thus, herein we discuss the interaction of dimethyl(vinyl)phosphine oxide with halogenoximes with an intention to assemble P(O)Me$_2$ decorated isoxazolines having an easy-to-modify randomization point (Scheme 1, C).

**Results and discussion**

According to the planned research strategy, we relied on the method for the synthesis of 3,5-disubstituted isoxazolines based on [3+2]-cycloaddition of nitrile oxides and dimethyl(vinyl) phosphine oxide (Scheme 2) [34]. The corresponding nitrile oxides 12a–k could be produced *in situ* from halogenoximes 4a–h, 6i, 8j and 11k, which, in turn, were obtained from the corresponding N-Boc-protected amino acids 1a–h (through their sequential reduction to alcohols 2 and generation of aldehydes 3) (Scheme 2, A), glyoxalic acid 5i (Scheme 2, B) [35], hemiacetal 7j.
Scheme 2. Synthesis of the starting halogen oximes

(Scheme 2, C) [36], or TIPS-protected acetylene 9k (Scheme 1, D) [37, 38] according to the procedures previously published. The choice of R-substituents in halogenoximes was justified by the utility of the [3+2]-adducts derived from them for subsequent synthetic transformations (e.g., the presence of protected NH and alkyne groups or bromine atom) and their attractiveness for medicinal chemistry in the case of the CF₃-substituent.

Having intermediate halogenoximes, we started studying their interaction with dimethyl(viny)phosphine oxide (13). For the reaction, we chose conditions that previously proved themselves as efficient and reliable ones for a wide range of substrates [30]. Precisely, the reactions were carried out using a 1-to-1 ratio of the starting materials in the presence of a 1.3-fold excess of a weakly basic sodium bicarbonate in the ethyl acetate medium at room temperature. Such gentle conditions allowed keeping intact all functional groups besides the reaction centers. The progress of the reaction was monitored by HPLC indicating that the reaction usually completed within 60 h. One should point out that application of insoluble base in this setup made it possible gradual generation of nitrile oxides, thus mostly avoiding dimerization of the latter with the formation of the corresponding furoxans (Scheme 3). However, the switch to diethyl vinylphosphonate restored the selectivity providing 5-phosphonate solely. According to all spectral data recorded, only one isomer was formed in the cycloaddition. Assignment of its structure to 5-dimethylphosphinoyl 14 was made based on 13C NMR spectral data (Figure 3, A). Thus, C-5 atom of the isoxazoline core was seen as a doublet with the ipso-coupling constant of ~81 Hz; C-4, in turn, was found as a doublet though with much lower J ~ 6 Hz. We did not detect any traces of another feasible isomer 15 neither in NMR nor in HPLC spectra of the crude products.

The regioselectivity of the process might be governed by steric and/or electronic factors (Figure 3, B). In particular, the transition state TS1 leading to the formation of 3,4-disubstituted isoxazolines 15 is unfavorable compared to TS1 (giving 3,5-disubstituted isomer) due to the steric repulsion between the R-group and the dimethylphosphinoyl fragment. In addition, information complying with the above-stated one can be derived from the analysis of the atomic charges over alkene carbons in compound 13. The values
retrieved from the Hückel molecular orbital theory calculations (ChemBio3D Ultra, ver. 14.0, https://www.cambridgesoft.com/Ensemble_for_Chemistry/details/Default.aspx?fid=13&pid=668) demonstrate a favored \( \text{C}\cdots\text{C} \) interaction (attraction of positive and negative charges) and less pronounced \( \text{C}\cdots\text{O} \) repulsion than in TS1 compared to TS2. Thus, the charge distribution also favors the product structures identical to those found experimentally.

Unlike the regioselectivity, the reaction was not stereoselective with reference to C-5 atom of isoxazolidine. This was judged from the formation of a diastereomic pair in the cases of chiral oximes 4b,d,f shown by NMR and HPLC data (Figure 3, C).

Further, we studied suitable ways to remove protecting Boc- (on the example of compound 14h) and TIPS-groups. Previously, the Boc-protection was washed out from similar isoxazoles with HCl dissolved in MeOH [34]. Surprisingly, our attempt to apply this method to 14h failed, and we obtained inseparable mixture of products. Changing the reaction setup to TFA/DCM enabled the
isolation of the desired amine in the form of a trifluoroacetate salt (Scheme 4). Unfortunately, all our attempts to deprotect alkyne 14k under standard conditions (TBAF, THF; HF, MeCN; Et₃N*3HF) did not result in a satisfactory result.

Currently, the above-mentioned unique properties of P(O)Me₂ the substituent are widely used by MedChem researchers, e.g. to dramatically increase solubility and decrease lipophilicity of organic compounds. For instance, this tactic was used to improve the solubility of the antihypertensive drug prazosin without affecting its biological profile [40]. Broadly, available chemical space of small molecular “bricks”, as well as powerful operational instruments allow altering the ADME properties of potential drug candidates to achieve necessary characteristics. This becomes possible with a right choice of pieces within complex thread-like molecules. Therefore, we decided to trace how some properties of the target compounds 14 changed with the replacement of the DMPO group with its isosters. The analysis was performed on the example of piperidine derivative 14c. As isosteric substituents, P-, C- and S-centered functionalities were selected (Figure 4).

The calculation was carried out with the aid of a SwissADME web tool [41, 42] and concerned two issues:

- prediction of the passive gastrointestinal absorption (HIA) and penetration of the blood-brain barrier (BBB) (BOILED-Egg method, Figure 4, A);
Conclusions

A practical approach to the isoxazoline platform decorated with a 5-P(O)Me₂ substituent and based on 1,3-dipolar addition between in situ generated nitrile oxides and dimethyl(vinyl)phosphine oxide has been developed. The base-promoted interaction of halogenoxides with dimethyl(vinyl)phosphine oxide under mild conditions has allowed us to obtain 11 isoxazoline–dimethylphosphine oxide hybrids in moderate yields. The reaction has been found to be regio-though non-stereo-selective. The approach to the Boc-deprotection has been also described. On the example of the piperidine derivative, the effect of the dimethylphosphinoyl group on physicochemical properties and ADME parameters compared to its isosters has been determined.

Experimental part

All starting compounds and solvents were obtained from commercial sources and used without further purification. Melting points were measured in open capillary tubes and given uncorrected. NMR experiments were performed on a Bruker 170 Avance 500 (at 500 MHz for ¹H NMR, 470 MHz for ¹³C NMR, 202 MHz for ³¹P NMR, and 126 MHz for ¹⁹F NMR) or a Varian Unity Plus 400 (at 400 MHz for ¹H NMR and 101 MHz for ¹³C NMR) spectrometers in the DMSO-d₆ or CDCl₃ solution. NMR chemical shifts were reported in ppm units using the δ scale and referenced using the solvent peaks at 7.26 and 77.1 ppm (CDCl₃) for ¹H and ¹³C nuclei, respectively, and 2.48 and 39.5 ppm (DMSO-d₆) for ¹H and ¹³C nuclei, respectively. For ³¹P NMR experiments hexafluorobenzene was used as an internal standard. The chemical shifts in ³¹P NMR studies were referenced to external 85% H₃PO₄. HPLC experiments were carried out on an Agilent LC/MSD SL 1100 instrument (atmospheric pressure electrospray ionization (ES-API)), GCMS spectra were taken on an Agilent 5890 Series II 5972 GCMS instrument (electron impact ionization (EI)). According to chromatographic studies, all compounds had purity of more than 95%. Elemental analyses were performed in the Analytical Laboratory of the Institute of Organic Chemistry of the National Academy of Sciences of Ukraine.

The general procedure for the synthesis of 3-(5-dimethylphosphoryl)-4,5-dihydroisoxazoles 14

To the solution of the corresponding halogeno-oxime 4 or 6 or 8 or 11 (5 mmol, 1.0 equiv) and dimethyl(vinyl)phosphine oxide (13, 0.52 g, 5 mmol, 1.0 equiv) in EtOAc (10 mL), NaHCO₃ (0.57 g, 6.5 mmol, 1.3 equiv) was added. The mixture was vigorously stirred at room temperature for 60 h. Then the inorganic precipitate was filtered off, the filtrate was dried over Na₂SO₄ and evaporated under reduced pressure. The residue was purified by the preparative high-performance liquid chromatography or flash column chromatography (details are given for each compound below) resulting in the target 4,5-dihydroisoxazoles 14.

tert-Butyl 3-(5-dimethylphosphoryl)-4,5-dihydroisoxazol-3-yl)azetidine-1-carboxylate (14a)

A colorless oil, solidified upon standing. HPLC purification: 9% water-acetonitrile 0.5–6.5 min; flow 30 mL min⁻¹ (loading pump 4 mL min⁻¹ acetonitrile); column SunFireC18 100×19 mm 5μm (R). Yield – 0.78 g (52%). Anal. Calcd for C₁₅H₂₃N₂O₆P, %: C 51.65; H 7.67; N 9.27. Found, %: C 51.82; H 7.74; N 9.11. ¹H NMR (400 MHz, CDCl₃), δ, ppm: 1.42 (9H, s, 3×CH₃); 1.46 (3H, d, J₁H_P = 12.7 Hz, CH₃); 1.61 (3H, d, J₂H_P = 12.8 Hz, CH₃); 3.31–3.45 (2H, m); 3.53 (1H, dq, J = 8.8, 4.4 Hz); 3.99 (2H, dd, J = 9.2, 4.9 Hz); 4.17 (2H, t, J = 8.7 Hz); 4.70–4.81 (1H, m). ¹³C NMR (126 MHz, CDCl₃), δ, ppm: 11.40 (d, J₁C_CP = 68 Hz, CH₃); 14.79 (d, J₂C_CP = 68 Hz, CH₃); 26.53; 28.24; 35.93; 52.09; 77.66; 79.95 (C(CH₃)₃); 155.91 (C=O); 158.30 (d, J₃C_P = 3.7 Hz, C-3 isoxazoline). ³¹P NMR (202 MHz, CDCl₃), δ, ppm: 43.38. LC-MS (ES-API), m/z: 325.2 [M+Na]+.
**tert-Butyl (2R)-2-(5-(dimethylphosphoryl)-4,5-dihydroisoxazol-3-yl)pyrrolidine-1-carboxylate (14b)**

A colorless oil. HPLC purification: 20–45% water-acetonitrile 2–7 min; flow 30 mL min⁻¹ (loading pump 4 mL min⁻¹ acetonitrile); column SunFireC18 100×19 mm 5 um (R). Yield – 0.91 g (58%). Anal. Calcd for C₁₅H₂₁N₅O₅P, %: C 53.16; H 7.66; N 10.14. Found, %: C 53.11; H 8.28; N 9.21. ¹H NMR (400 MHz, CDCl₃), δ, ppm: 1.34–1.70 (21H, m); 3.92–4.11 (1H, m); 4.13–4.30 (1H, m); 4.67–4.99 (2H, m). ¹³C and ³¹P NMR spectra are not informative due to hindered rotation (see Supporting information file). LC-MS (ES-API), m/z: 581.2 [M+H]⁺.

**tert-Butyl (2R)-2-(5-(dimethylphosphoryl)-4,5-dihydroisoxazol-3-yl)piperidine-1-carboxylate (14c)**

A yellow oil. Flash column chromatography (FCC) purification: CHCl₃:MeOH (10:1), 𝑅ₚ = 0.27. Yield – 1.1 g (67%). Anal. Calcd for C₁₅H₂₁N₅O₅P, %: C 54.54; H 8.24; N 8.48. Found, %: C 54.73; H 8.11; N 8.54. ¹H NMR (400 MHz, CDCl₃), δ, ppm: 1.38–1.47 (12H, m); 1.49–1.65 (5H, m); 1.83 (2H, d, J = 13.0 Hz); 2.56 (1H, tt, J = 11.1, 3.2 Hz); 2.79 (2H, t, J = 12.3 Hz); 3.24–3.37 (2H, m); 4.11 (2H, d, J = 13.5 Hz); 4.63–4.73 (1H, m). ¹³C NMR (126 MHz, CDCl₃), δ, ppm: 10.32 (d, ¹ JCP = 68 Hz, CH₃P); 13.10 (d, ¹ JCP = 68 Hz, CH₃P); 25.15 (d, ¹ JCP = 68 Hz, CH₃P); 31.50 (d, ¹ JCP = 68 Hz, CH₃P); 39.53 (d, ¹ JCP = 68 Hz, CH₃P); 44.31 (d, ¹ JCP = 68 Hz, CH₃P). ¹²C and ³¹P NMR (202 MHz, CDCl₃), δ, ppm: 44.13. LC-MS (ES-API), m/z: 331.1 [M+H]⁺.

**tert-Butyl (4S)-4-(5-(dimethylphosphoryl)-4,5-dihydroisoxazol-3-yl)-2,2-dimethyloxazolidine-3-carboxylate (14d)**

A white amorphous solid. HPLC purification: 9% water-acetonitrile 0.5–6.5 min; flow 30 mL min⁻¹ (loading pump 4 mL min⁻¹ acetonitrile); column SunFireC18 100×19 mm 5 um (R). Yield – 0.88 g (61%). Anal. Calcd for C₁₅H₂₁N₅O₅P, %: C 49.65; H 7.99; N 9.65. Found, %: C 49.58; H 7.95; N 9.78. ¹H NMR (400 MHz, CDCl₃), δ, ppm: 1.41–1.54 (15H, m); 1.60–1.69 (3H, m, CH₃P); 3.41 (2H, dd, J = 20.2, 11.7 Hz); 4.58 (1H, s); 4.71–4.91 (2H, m). ¹³C NMR (126 MHz, CDCl₃), δ, ppm: 10.41 (d, ¹ JCP = 68 Hz, CH₃P); 10.70 (d, ¹ JCP = 68 Hz, CH₃P); 13.98 (d, ¹ JCP = 68 Hz, CH₃P); 14.05 (d, ¹ JCP = 68 Hz, CH₃P); 18.09; 18.21; 27.78; 35.19; 35.80; 43.78; 43.98; 76.35 (d, ¹ JCP = 68 Hz, C-5 isoxazoline); 76.48 (d, ¹ JCP = 81 Hz, C-5 isoxazoline); 79.18 (C(CH₃)₃); 154.50; 154.63; 160.26; 160.57. ³¹P NMR (162 MHz, CDCl₃), δ, ppm: 43.71, 44.19. LC-MS (ES-API), m/z: 581.2 [M+DMSO+H]⁺.


