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Palladium(II) pincer complexes of *N*-(thiophosphorylalkyl)picolinamides: effect of the length of the P^V-pendant arm on the cytotoxic activity

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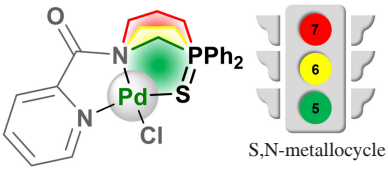
A convenient synthetic route to β- and γ-thiophosphorylated alkylamines is suggested based on the nucleophilic substitution between the ω-bromoalkylamines and Ph_2PK followed by the addition of elemental sulfur. These functionalized phosphine sulfides are shown to serve as useful synthons for the new non-classical picolinamide-based pincer ligands. The cyclopalladated derivatives of the latter exhibit promising cytotoxic properties, which strongly depend on the length of the thiophosphoryl pendant arm.

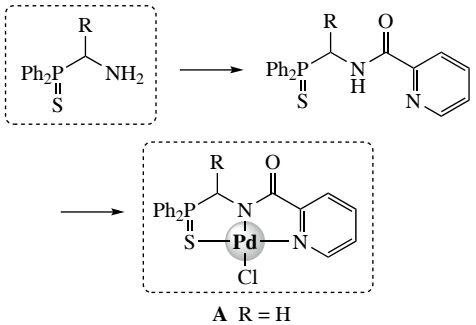


*Effect of the length of the **P=S** arm on the cytotoxic activity*

Keywords: organothiophosphorus compounds, functionalized amides, picolinamides, pincer ligands, palladium, cytotoxicity.

Organothiophosphorus compounds have a long history of studies owing to a broad spectrum of biological activity, which predetermined their extensive application in pharmaceutical and agricultural industry.[1,2] Currently, their utility in materials science has also been recognized.[3] Functionalized thiophosphoryl derivatives bearing additional amino, cyano, nitro and other groups are of particular interest owing to their diverse reactivity patterns and complexing ability.[4–10] Recently, we have shown that α-thiophosphorylated alkylamines **A**, **B** could be used as building blocks for the production of Pd^{II} pincer complexes with high anticancer potential (Scheme 1).[11] In continuation of these studies, herein, we present the synthesis of β- and γ-thiophosphorylalkylamines and non-classical amide pincer ligands based on them, as well as the preparation and biological evaluation of their cyclopalladated derivatives.

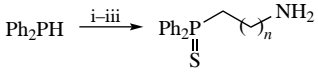
The target thiophosphoryl-substituted amines were readily obtained by the addition of elemental sulfur to the corresponding phosphine derivatives, synthesized, in turn, by the reactions of



A R = H
B R = Ar

Scheme 1

2-bromoethyl- and 3-bromopropylamine hydrobromides with *in situ* generated Ph_2PK according to the slightly modified literature method.[12] Unlike the analogous reaction with 2-chloroethylamine hydrochloride,[13] the nucleophilic substitution of the bromine-containing substrates proceeded under mild reaction conditions and provided gram-scale synthesis of amines **1**, **2** (Scheme 2). The latter were isolated in moderate yields after a simple purification procedure.

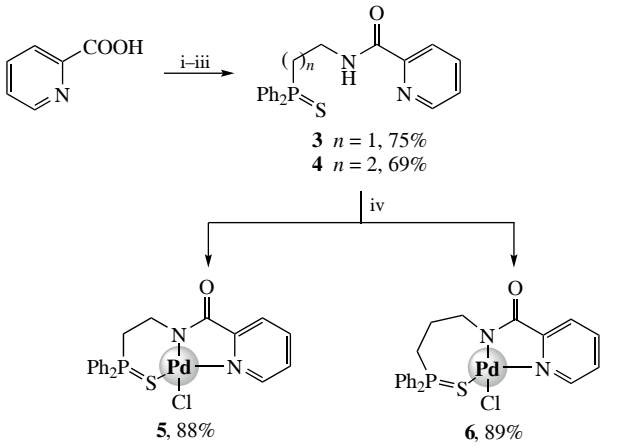


1 n = 1, 43%
2 n = 2, 50%

Scheme 2 *Reagents and conditions*: i, Bu^tOK (2.0–2.5 equiv.), THF, room temperature, 1 h; ii, $Br(CH_2)_nNH_2 \cdot HBr$, room temperature, 0.5 h (for n = 1) or 1 day (for n = 2); iii, S_8, room temperature, 1 day.

The key thiophosphorylated amines smoothly reacted with picolinic acid activated through the formation of a mixed anhydride under the action of isobutyl chloroformate, affording functionalized amides **3**, **4** in good yields (Scheme 3). The resulting picolinamides, as well as their predecessors derived from α-thiophosphorylated alkylamines (see Scheme 1),[11] readily underwent direct cyclopalladation upon tretment with $(PhCN)_2PdCl_2$ under mild reaction conditions (see Scheme 3). Palladium(II) pincer complexes **5**, **6** were isolated in high yields after the chromatographic purification.

The occurrence of metalation of the central secondary amide unit in both palladocycles was unambiguously supported by the lack of the NH proton singlet in the ^1H NMR spectra as well as the absence of amide II and amide A bands in their IR spectra (see Online Supplementary Materials). The coordination of the



Scheme 3 *Reagents and conditions*: i, Et_3N, CH_2Cl_2, –5 °C, 30 min; ii, $Bu^iOC(O)Cl$, –5 °C, 30 min; iii, –5 °C, amine **1**, 0.5 h (for **3**) or **2**, 1 h (for **4**), then room temperature, 1 day; iv, $(PhCN)_2PdCl_2$, Et_3N, CH_2Cl_2, room temperature, 1 day.

pyridine unit was indirectly indicated by the strong downfield shifts of the CH proton signal located at the *ortho*-position relative to the heteroatom ($\Delta\delta_H \sim 0.7$ ppm) and *ipso*-carbon nucleus connected with the amide moiety ($\Delta\delta_C \sim 5.1$ ppm). The coordination of the thiophosphoryl group was evident from a considerable shift of the P=S bond stretches towards lower frequencies, which reached up to 62 cm^{-1}. Interestingly, the phosphorus resonances of the resulting complexes appeared to be close to those of the free ligands ($\Delta\delta_P = 0.2$–2.3 ppm). Last but not least, the X-ray crystallographic study unequivocally confirmed the realization of the monoanionic tridentate pincer-type coordination of the deprotonated picolinamide ligand even in the case of the longer chain derivative **6** (Figure 1).[†]

To evaluate the potential of compounds **5** and **6** as anticancer agents and identify the key structural features responsible for

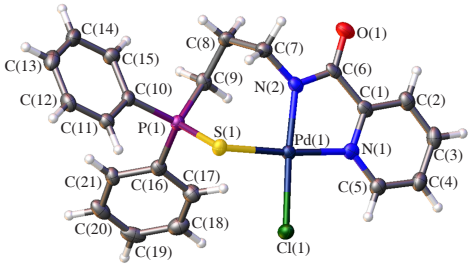


Figure 1 General view of complex **6** in representation of atoms as thermal ellipsoids ($p = 50\%$).

[†] *Crystal data for* **6**. $C_{21}H_{20}ClN_2OPPdS$ ($M = 521.27$), orthorhombic, space group *Pna*21, $a = 12.995(3)$, $b = 15.600(4)$ and $c = 10.069(2)$ Å, $\alpha = \beta = \gamma = 90°$, $V = 2041.2(8)$ Å^3, $Z = 4$, $T = 100$ K, μ(Mo-Kα) = 12.36 cm^{-1}, $d_{calc} = 1.696$ g cm^{-1}. Total of 14385 reflections were measured, and 4344 independent reflections ($R_{int} = 0.0994$) were used in the further refinement. The refinement converged to $wR_2 = 0.0724$ and GOF = 0.965 for all independent reflections [$R_1 = 0.0442$ was calculated against F for 3678 observed reflections with $I > 2\sigma(I)$].

Single crystals of compound **6** were obtained by slow evaporation of its solution in CH_2Cl_2. The data were collected with a Bruker APEXII Quazar CCD diffractometer using graphite monochromated Mo-Kα radiation ($\lambda = 0.71073$ Å). Using Olex2,[14] the structures were solved with the ShelXT[15] structure solution program using Intrinsic Phasing and refined with the XL[16] refinement package using Least Squares minimization against F^2_{hkl} in anisotropic approximation for non-hydrogen atoms. The positions of hydrogen atoms were calculated, and they were refined in the isotropic approximation within the riding model.

CCDC 2447592 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* https://www.ccdc.cam.ac.uk.

Table 1 Cytotoxicity of the thiophosphoryl-functionalized Pd^{II} pincer complexes against selected human cell lines.

Cell lines	$IC_{50} \pm SD$,[a] μM			
	A	**5**	**6**	Cisplatin
Solid cancer cell lines				
HCT116	12.0±3.5	26.0±3.2	>50	18.0±2.0
MCF7	27.5±4.5	40.0±2.0	>50	25.0±4.0
PC3	16.5±3.5	23.0±3.2	>50	16.0±3.0
Hematopoietic cancer cell lines				
K562	10.6±1.4	7.5±2.2	14.8±0.8	15.5±0.5
K562/iS9	16.5±1.5	9.9±1.6	23.5±2.5	16.0±2.0
AMO1	3.5±0.7	4.7±1.2	16.8±1.3	3.2±0.6
H9	2.2±1.2	3.0±0.8	13.2±1.6	3.0±1.0
MOLT4	13.2 ±0.4	8.5±1.5	23.0±2.5	6.5±1.0
Non-cancerous cell lines				
HEK293	15.5±1.5	30.7±1.9	>50	12.5±1.5
HBL100	33.0±8.2	35.0±1.0	>50	14.6±3.6
HBL100/dox	42.5±2.5	34.5±4.5	>50	23.6±3.6

[a] SD is the standard deviation of the value.

their activity, their cytotoxicities against several solid and hematopoietic human cancer cell lines as well as non-cancerous cells were determined by the conventional colorimetric MTT assay. The resulting values of half-maximal inhibitory concentrations measured after 48 h exposure to the compounds under consideration and the corresponding data for cisplatin used as a positive control are summarized in Table 1. For comparison, the values of IC_{50} for their α-thiophosphorylated analog (compound **A**, see Scheme 1) are also presented.

As can be seen, S,N,N-complex **5** derived from the β-thiophosphorylated derivative demonstrated moderate cytotoxicity against the tested solid cancer cell lines, being inferior to its α-thiophosphorylated analog **A**. Also for the latter, a comparable level of sensitivity was observed in the case of colon (HCT116) and prostate (PC3) cancer cells, while breast cancer cells (MCF7) appeared to be more resistant. At the same time, complex **6** with fused 5- and 7-membered metallocycles did not exhibit anticancer properties against the epithelial cell cultures even at a fairly high concentration (50 μM). These results are in good agreement with our hypothesis about the dependence of the biological activity of Pd^{II} pincer complexes on their kinetic stability: the elongation of the phosphorus coordination arm may serve as a factor contributing to an increase in the lability of the system, leading to the lower stability of the resulting complex in biological media.

Compound **5** did not surpass cisplatin in the activity against the solid cancer cell lines. However, its important advantage over this clinically used drug appeared to be the lower toxicity towards pseudonormal cells (HEK293, HBL100). This difference becomes especially obvious when comparing the data obtained on hematopoietic cancer cells, for which the selectivity indices of compound **5** exceeded 3.5 and reached up to 10.2. Acute lymphoblastic leukemia (H9) and multiple plasmocytoma (AMO1) cell lines were found to be the most sensitive ones to the complexes obtained both in this and previous study,[11] with IC_{50} values falling in the low micromolar range. As for the activity of complex **6** against hematopoietic cancer cells, it again appeared to be inferior to its shorter chain counterparts. However, its low toxicity on non-cancerous cells (>50 μM) deserves special mention.

An important finding of this study is the comparable levels of activities of β-thiophosphorylated derivative **5** against the pairs of parent and doxorubicin-resistant pseudonormal mammary

epithelial cell lines (HBL100 and HBL100/dox) and chronic myelogenous leukemia cells K562 and K562/iS9, which suggests the prospects of overcoming drug resistance. It is also noteworthy that free ligands **3** and **4** were non-toxic to the studied cell lines even at a concentration of 60 μM. Therefore, the cytotoxic properties of their cyclopalladated complexes were mainly determined by the coordination with Pd^{II} ions. Furthermore, neither the previously reported α-thiophosphorylated cyclopalladated derivatives (**A**, **B**), nor the newly obtained longer chain complex (**5**) exhibited affinity to adenosine monophosphate according to the results of the ^{31}P NMR spectroscopic studies (see Online Supplementary Materials), which may indicate their low binding ability with DNA, often serving as the main target for metal-based chemotherapeutics.

Hence, β- and γ-thiophosphorylated alkylamines readily available by the facile approach can serve as useful building blocks for non-classical pincer-type ligands. The biological profile of the cyclopalladated derivatives of the thiophosphoryl-functionalized picolinamides was shown to strongly depend on the length of the phosphorus pendant arm.

This paper is dedicated to the centenary of the birth of Tatyana A. Mastryukova, the corresponding member of the Russian Academy of Sciences and the former head of the Group of Organothiophosphorus Compounds of INEOS RAS.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.71267/mencom.7815.

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