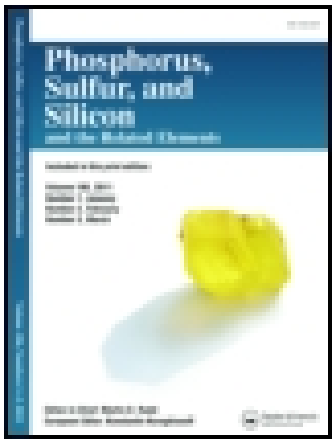

Phosphorus, Sulfur, and Silicon and the Related Elements

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# 3,5-Bis(arylidene)-4-piperidones Modified by Bisphosphonate Groups as Novel Anticancer Agents

Mikhail V. Makarov[a], Ekaterina Yu. Rybalkina[b] & Valery K. Brel[a]

[a] A. N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, Vavilova str., 28, Moscow, 119991, Russian Federation

[b] Institute of Carcinogenesis, N. N. Blokhin Russian Cancer Research Center, Russian Academy of Medical Sciences, Kashirskoe sh. 24, Moscow, 115478, Russian Federation

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[a] *A. N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, Vavilova str., 28, Moscow, 119991, Russian Federation*

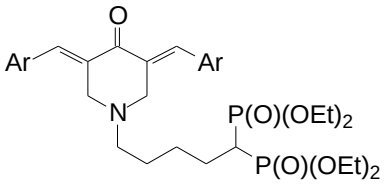
[b] *Institute of Carcinogenesis, N. N. Blokhin Russian Cancer Research Center, Russian Academy of Medical Sciences, Kashirskoe sh. 24, Moscow, 115478, Russian Federation*

*e-mail: mmak78@yandex.ru*

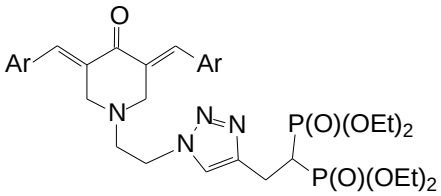


**Abstract.** Synthetic approaches for conjugating 3,5-bis(arylidene)-4-piperidones with bisphosphonate moiety were elaborated. These approaches are based either on reaction of Grignard reagent containing dioxolane protected 4-piperidone with tetraethyl ethylidenbisphosphonate followed by crotonic condensation with aromatic aldehydes or on Cu(I) catalyzed 1,3-cycloaddition of tetraethyl but-3-yne-1,1-diylbisphosphonate to *N*-(2-azidoethyl)-3,5-bis(arylidene)-4-piperidones resulting in corresponding 1,2,3-triazole derivatives. Cytotoxic activity of the synthesized conjugates was dependent on the length of linker connecting piperidone nitrogen atom and bisphosphonate residue. Triazole derivatives of 3,5-bis(arylidene)-4-piperidone series displayed moderate *in vitro* inhibitory properties towards HCT116 and MCF7 human cancer cell lines with $IC_{50}$ values in the range of 5.0–7.5 μM, whereas conjugates with butylene linker between piperidone nitrogen atom and bisphosphonate moiety were significantly less active.




**2a**




**3a,c**

Ar = 4-$O_2NC_6H_4$ (**a**), 4-$NCC_6H_4$ (**c**)

$IC_{50}$ = 40 μM (MCF7 breast cancer)

$IC_{50}$ = 5.0 - 7.0 μM (MCF7 breast cancer)



**Key words:** 3,5-bis(arylidene)-4-piperidones, aminobisphosphonates, bisphosphonates, anticancer properties

**Shortened title of the article:** Bisphosphonates of bis(arylidene)piperidone series

Bisphosphonates (BPs) occupy a prominent position among biologically active organophosphorus compounds due to high efficacy of some their representatives in treatment of osteoporosis and bone metastases.[1] They are usually classified in two types: non-nitrogen BPs (first generation as exemplified by etidronate in Fig. 1) and nitrogen-containing BPs or amino-BPs (second and third generations as exemplified by aledronate and risedronate, respectively, in Fig. 1). Compounds belonging to the second generation contain nitrogen atom in a short alkyl chain attached to the carbon atom of bisphosphonate (P–C–P) moiety. In the case of the third generation BPs, nitrogen atom is a part of a heterocyclic system connected with the BP moiety through a short methylene linker. The third generation amino-BPs demonstrate the highest antiresorptive activity that is several thousand times higher than that of the first generation BPs, and some of the former, for example, risedronate and zoledronate, find wide application in clinical practice. Structure-activity relationship studies revealed that the proximity of nitrogen atom to the P–C–P moiety in the structure of amino-BPs plays a crucial role for the biological activity of these compounds. Indeed, in the case of the most active representatives the P–C–P residue and nitrogen atom are spaced from each other by only one to three carbon atoms.[1]

Moreover, due to high affinity of BPs to osseous tissue, these compounds may be used for targeted delivery of other pharmacophores to the affected bone. The idea of conjugation of BP moieties with various anticancer drugs was implemented in a number of publications describing BP conjugates of gemcitabine[2], methotrexate[3], doxorubicin[4], and nucleoside antimetabolites[5]. Further examples of pharmacologically active compounds conjugated with BPs are provided elsewhere.[6]

In our research we are trying to modify the structure of anticancer active 3,5-bis(arylidene)-4-piperidones using organophosphorus groups, including BP residues, to improve their pharmacological properties and to enhance cytotoxic activity. Recently, we have shown that *NH*-3,5-bis(arylidene)-4-piperidones may be easily reacted with tetraethyl ethylidenbisphosphonate in an aza-Michael type addition reaction resulting in a series of corresponding β-amino-BPs **1**.[7] Some of these β-amino-BPs showed high in vitro cytotoxicity (with inhibition concentration $IC_{50}$ values in the range of 0.5–4 μM) towards human cancer cell lines CaoV3 (ovarian cancer), A549 (lung cancer), PC3 (prostate cancer) and KB3-1 (oral epidermoid cancer). That study demonstrated that combining in one molecule two pharmacophores – 3,5-bis(arylidene)-4-piperidone moiety and BP group – may be an effective approach to increase the cytotoxicity because, in some instances, BP-modified piperidones were more active than the parent *NH*-compounds.

The aim of this study was to evaluate the influence of type and length of a linker connecting the BP moiety with the piperidone scaffold on the cytotoxic activity of 3,5-bis(arylidene)-4-piperidones. In this regard, synthetic approaches to novel bisphosphonates **2a,b** and **3a-d** containing 3,5-bis(arylidene)-4-piperidone pharmacophore were elaborated (Scheme 1) and in vitro cytotoxicity of these compounds towards some human cancer cell lines was evaluated and compared with that of compounds **1**.

Detailed synthetic procedures for preparing compounds **2a,b** and **3a-d** have been described in our recent publications;[8] therefore, only short description of their synthesis will be provided now. Commercially available 1,4-dioxa-8-azaspiro[4.5]decane (**4**) was used as a starting compound and was converted to chlorides **5** and **6** using alkylation with 2-chlroethanol

or 3-chloropropanol followed by reaction of resulting alcohols with thionyl chloride.[9] In the first approach, chloride **6** was treated with a sodium hydrogen carbonate aqueous solution to remove intramolecular HCl which resulted in corresponding free base subsequently converted to a Grignard reagent; the latter was reacted with tetraethyl ethylidenbisphosphonate resulting in aminobisphosphonate **7** isolated in the yield of 55% after column chromatography. Crotonic condensation of compound **7** with aromatic aldehydes under acidic conditions provided desired piperidones **2a,b** in the yields of ca. 30% due to partial hydrolysis of ethoxy groups at phosphorus atoms.

In the second approach, chloride **5** was reacted with sodium azide in water to afford corresponding azide from which dioxolane protection was removed to liberate ketone function. Resulting compound **8** was reacted with aromatic aldehydes in the presence of lithium perchlorate and diethylamine according to known procedure[10] to give *N*-(2-azidoethyl)-3,5-bis(arylidene)-4-piperidones **9a-d** in the yields of 31–56%. The final step in the synthesis of compounds **3a-d** was copper(I) catalyzed 1,3-cycloaddition reaction between azides **9a-d** and terminal acetylene containing BP moiety. This reaction afforded desired conjugates **3a-d** in regioselective manner (only 1,4-regioisomers of 1,2,3-triazoles were formed) in good yields (57–89%).

The cytotoxic activity of BPs **2a,b** was tested in vitro against following human cancer cell lines: ovarian (Scov3, Caov3), lung (A549) and breast (MCF7) carcinoma using MTT method and compared with that of analogues **1a,b** containing shorter alkylene chain. The results are summarized in Table 1 showing the corresponding $IC_{50}$ values ($IC_{50}$ is the concentration of

compound required to inhibit the growth of the cells by 50%). Anticancer antibiotic Doxorubicin was used as a positive control.

Two basic conclusions may be drawn form the data in Table 1. First, BPs **2a** and **1a** containing electron-withdrawing nitro substituents in aromatic rings have higher anticancer activity as compared to analogues **2a** and **1a**, respectively, having electron-releasing dimethylamino groups. Second, as it follows from comparison of activity of compounds **2a** and **1a**, bisphosphonate **2a** having longer alkylene chain of four carbon atoms between piperidone and BP moieties revealed significantly lower activity than counterpart **1a** with one $CH_2$ unit between those moieties.

[Insert Table 1]

The cytotoxic activity of triazoles **3a-d** was tested in vitro against human colon cancer (HCT116) and human breast cancer (MCF7) cell lines as well as normal human embryonic fibroblasts (HEF) using MTT method. Cytotoxic activity of nitro derivative **3a** was also compared with the activity of analogues **1a** and **2a** containing different linkers between piperidone and bisphosphonate moieties. The results are summarized in Table 2, with anticancer agent Doxorubicin being used again as a positive control.

As is seen from the data in Table 2, compounds **3a,c** with triazole linker between BP and piperidone moieties and having electron-withdrawing substituents demonstrate moderate activity towards used cell lines. At the same time, pyridine derivative **3d** and bisphosphonate **3b** with electron-releasing dimethylamino groups were non-active. Among nitro-derivatives **1a**, **2a** and **3a**, compound **1a** with shortest alkylene chain is the most active towards breast cancer cell line.

Therefore, we believe that further studies may include synthesis of 3,5-bis(arylidene)-4-piperidones with BP moiety attached directly to piperidone nitrogen atom.

[Insert Table 2]

In conclusion, this study has demonstrated the suitable approaches to the modification of 3,5-bis(arylidene)-4-piperidone structure with BP moiety using different linkers connecting these two pharmacologically active units. The cytotoxicity screening revealed that the highest anticancer activity is revealed by BP conjugates of 3,5-bis(arylidene)-4-piperidones having the shortest linker between BP moiety and piperidone nitrogen atom. Comparison of $IC_{50}$ values for triazoles **3a,c** (in the range of 5.0–7.5 μM) with those for related BP **1a** (in the range of 2.6–5.2 μM) showed that 1,2,3-triazole ring did not have strong negative impact on the antitumor activity of compounds and, therefore, the approach based on conjugation of 3,5-bis(arylidene)-4-piperidone pharmacophore with other biologically active moieties using 1,2,3-triazole linker may be effectively used for the synthesis of novel cytostatics.

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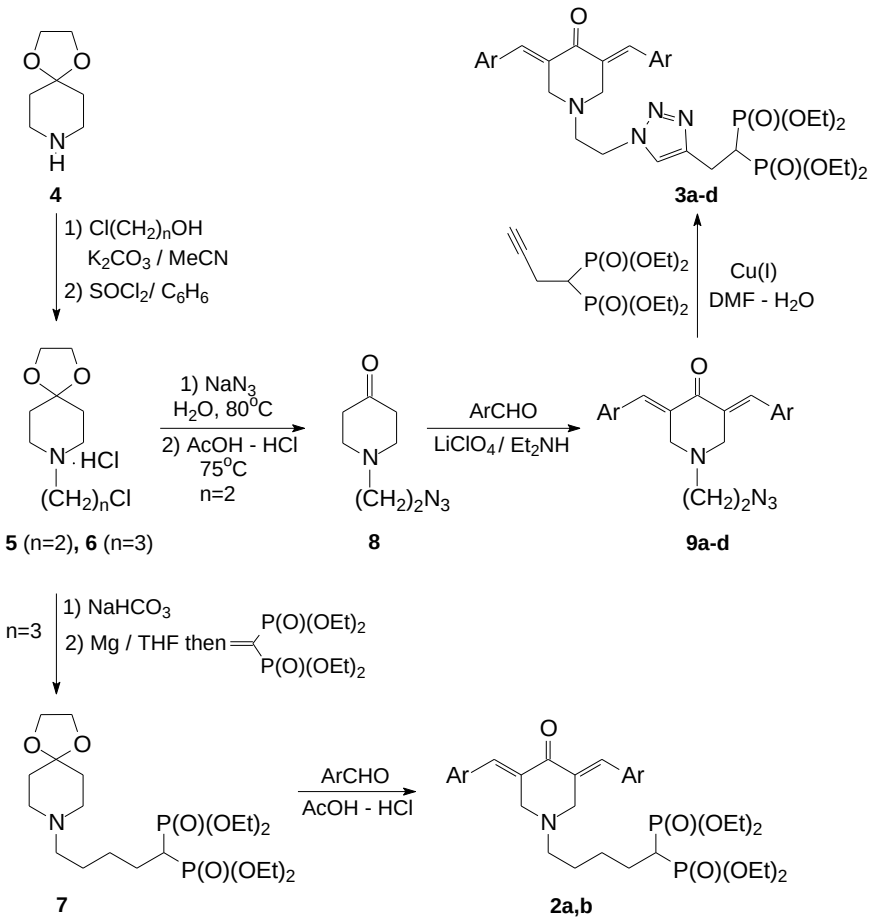


**Scheme 1.** Synthesis of aminobisphosphonates containing 3,5-bis(arylidene)-4-piperidone pharmacophore

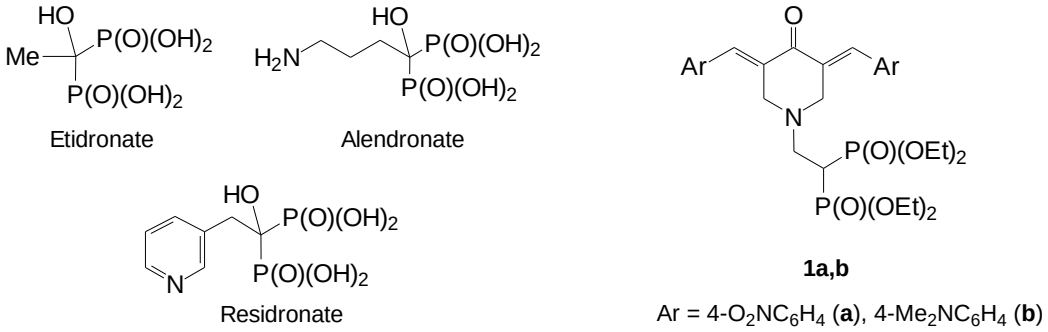


**Fig. 1.** Structures of known aminobisphosphonates and compounds of series **1**

**Table 1.** In vitro cytotoxicity of BPs **2a,b** and **1a,b** towards human carcinoma cell lines

| Compound | Cell lines/$IC_{50}$, μM | | | |
|---|---|---|---|---|
| | Scov3 | Caov3 | A549 | MCF7 |
| **2a** | 38±10 | 31±4 | 58±2 | 40±5 |
| **2b** | IC50 > 70 μM for all cell lines | | | |
| **1a** | 4.0±0.8 | 3.6±1.0 | 5.2±0.6 | 2.6±0.5 |
| **1b** | 56±7 | 25±11 | 70±10 | 32±5 |
| Doxorubicin | 0.8±0.15 | 0.38±0.12 | 0.34±0.12 | 0.27±0.07 |

**Table 2.** In vitro cytotoxicity of BPs **3a-d** towards HCT116, MCF7, and HEF cells

| Compound | Cell lines/$IC_{50}$, μM | | |
|---|---|---|---|
| | HCT116 | MCF7 | HEF |
| **3a** | 7.5±0.5 | 5.0±0.4 | 6.0±0.4 |
| **1a** | n/d | 2.6±0.5 | n/d |
| **2a** | n/d | 40±5 | n/d |
| **3c** | 7.5±0.6 | 7.0±0.5 | 5.0±0.3 |
| **3b** | >20 for all cell lines | | |
| **3d** | | | |
| Doxorubicin | 1.6±0.3 | 0.3±0.1 | 2.1±0.4 |