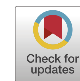
Bioorg. Med. Chem. Lett. 57 (2022) 128517

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl



Synthesis of hybrid phosphorated indenoquinolines and biological evaluation as topoisomerase I inhibitors and antiproliferative agents

María Fuertes [a], Asier Selas [a], Angela Trejo [a], Birgitta R. Knudsen [b], Francisco Palacios [a], Concepción Alonso [a,*]

[a] *Departamento de Química Orgánica I, Facultad de Farmacia and Centro de Investigación Lascaray (Lascaray Research Center). Universidad del País Vasco/Euskal Herriko Unibertsitatea (UPV/EHU). Paseo de la Universidad 7, 01006 Vitoria-Gasteiz, Spain*

[b] *Department of Molecular Biology and Genetics and Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Aarhus 8000, Denmark*

ARTICLE INFO

Keywords:
Indenoquinolinyl phosphonates
Topoisomerase I
Enzyme inhibition
Antiproliferative effect

ABSTRACT

This work describes the first synthesis of diethyl 6,6a,7,11b-tetrahydro-5*H*-indeno[2,1-*c*]quinolinylphosphonates **5**, diethyl 7*H*-indeno[2,1-*c*]quinolinylphosphonates **6** and diethyl 7-oxo-7*H*-indeno[2,1-*c*]quinolinylphosphonates **7**, which were prepared in good to high overall yields. The synthetic route involves a multicomponent reaction of 2-phosphonateaniline, aldehydes and indene as olefin and allows the selective generation of three stereogenic centres in a short, efficient and reliable manner. The selective dehydrogenation of 1,2,3,4-tetrahydroindenoquinolines leads to the formation of corresponding indenoquinolines, and subsequent oxidation of methylene group of the indenoquinolines allows the access to indenoquinolinones.

Introduction

For the design of new small molecules that inhibit the uncontrolled proliferation of cancer cells, human topoisomerase (TopI) has become an important target[1]. This ubiquitous enzyme reduces superhelical stress, as well as other topological consequences generated in the separation of DNA strands in metabolic processes such as replication, transcription and recombination. [2–4] In cancer cells, compared to non-cancerous cells, higher TopI activity is observed, leading to a higher rate of replication. In this regard, inhibitors of this enzyme have been and are being developed for application in cancer treatment. Among the TopI inhibitors are the so-called poisons, which act on the catalytic cycle of TopI by stabilizing the transient covalent phosphotyrosine bond (Tyr723) that forms between the enzyme and the DNA and thus inhibiting the moment of religation, creating a stable DNA-enzyme-drug complex, which causes cell death by apoptosis. Other types of TopI inhibitors, such as suppressors, prevent the formation of the enzyme-DNA complex by inhibiting DNA binding to the enzyme and/or breaking one of the two DNA strands. [5] The most representative TopI poisons are camptothecin (CPT, Fig. 1) and its derivatives (CPTs), which have been widely used as anticancer drugs for the past 20 years, but with certain limitations. [6]

The structural analysis of camptothecin (Fig. 1) shows several fused heterocycles, one of them containing a lactone function. Furthermore, according to the proposed mechanism of TopI inhibition, the presence of flat or *quasi* flat fused heterocycles seems to be relevant in the efficacy of the inhibitory activity. [7] Nitrogenated compounds such as quinolines show, in fact, an interesting biological activity with this target. [8–9] Among the diverse strategies for the preparation of quinoline derivatives, the Povarov reaction, [10–11] both in stepwise and multicomponent version, presents a great versatility.

Previously, our group designed the synthesis of heterocyclic compounds hybridized with phosphorus groups (phosphine, phosphine oxide and phosphine sulfide). [12–13] Moreover, some derivatives with phosphonate groups have been reported to have interesting biological activity as antituberculous[14] or antileishamniasic [15] agents. In this work, the importance of the phosphonate group when preparing TopI inhibitors has also been considered, since these substituents may affect the reactivity of heterocycles and regulate important biological functions[16,17]. The development of new strategies for the preparation of aminophosphonates[18] or azaheterocycles[19] with phosphonate groups involves the incorporation of organophosphorus functionalities into

Abbreviations: CCK8, cell counting kit; CPT, camptothecin; DDQ, dichloro-5,6-dicyanobenzoquinone; HDAr, hetero-Diels-Alder reaction; MCR, multicomponent reaction; SDS, sodium dodecyl sulfate; TOPO1, topoisomerase I; TLC, thin layer chromatography.

* Corresponding author.
E-mail address: concepcion.alonso@ehu.eus (C. Alonso).

https://doi.org/10.1016/j.bmcl.2021.128517
Received 7 September 2021; Received in revised form 14 December 2021; Accepted 15 December 2021
Available online 21 December 2021
0960-894X/© 2021 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

simple synthons.

With these considerations in mind, this work consists of finding a synergy between all these concepts and preparing fused heterocyclic derivatives between quinolines and indenes or indenones, in addition to incorporating a phosphonate group that could modulate the biological activity. Nowadays, it is observed that the structural conjunction in molecular hybrids can give rise to potent biologically active molecules.[20] Comparison of CPT and phosphorated indenoquinoline structures **I**, **II** and **III** (Fig. 1) shows that they would be totally superimposable with the phosphonate group occupying the place of the lactone. This fact motivated us to prepare and study the biological activity of these new phosphorus heterocyclic compounds, which represents an interesting challenge in organic chemistry, due to the potential interest of these molecules not only in synthetic but also in medicinal chemistry.

Results and discussion

Chemistry. The hetero-Diels–Alder reactions between indene **4** and 2-(diethylphosphonate)aldimines **3**, prepared *in situ* by reaction of diethyl (2-aminophenyl)phosphonate **1** and aldehydes **2** in the presence of 1 equivalent of $BF_3{\cdot}Et_2O$ gave in a regio- and stereospecific way the tetracyclic *endo*-6,6a,7,11b-tetrahydro-5*H*-indeno[2,1-*c*]quinolin-4-ylphosphonates **5** with good yields (Scheme 1, Chart 1).

Alternatively, a three-component synthetic protocol was carried out by reacting diethyl (2-aminophenyl)phosphonate **1a**, aromatic aldehydes **2** and indene **4** in the presence of 1 equivalent of $BF_3{\cdot}Et_2O$ in refluxing chloroform to obtain also the corresponding *endo*-6,6a,7,11b-tetrahydro-5*H*-indeno[2,1-*c*]quinolin-2-ylphosphonates **5a-j** with good yields, in a regio- and diastereoselective fashion (Scheme 1, Chart 1). In some cases, these tetrahydroindenoquinolines **5** were isolated along with different amounts of corresponding dehydrogenated derivatives **6** (Scheme 1, Chart 3).

The structure of *endo*-6,6a,7,11b-tetrahydro-5*H*-indeno[2,1-*c*]quinolin-4-yl)phosphonates **5** was assigned on the basis of the 1D and 2D spectroscopy. Thus, the ^{1}H NMR spectrum of compound **5a** (R = C_6H_5) showed one singlet at δ_H = 4.71 ppm corresponding to a proton at 11b position, one doublet at δ_H = 4.45 ppm with coupling constant of $^3J_{HH}$ = 6.6 Hz corresponding to the proton at 6 position, one multiplet at 3.01–3.11 ppm corresponding to the protons at 6a position and to one of the methylenic protons of CH_2 group, and another double doublet at δ_H = 2.25 ppm with coupling constants of $^2J_{HH}$ = 12.6 Hz and $^3J_{HH}$ = 7.5 Hz corresponding to the other proton of the methylenic group. Moreover, in the ^{13}C NMR spectrum of compound **5a** the most characteristic signals corresponding to carbons at positions 11b, 6a and 6 appear at δ_C = 46.0, 47.6 y 56.2 ppm respectively and also that one corresponding to CH_2 group at δ_C = 31.1 ppm. In the ^{31}P NMR spectrum one signal is observed at δ_P = 22.6 ppm. In addition, the presence of a hydrogen bridge bonding between the hydrogen atom of the amino group and the oxygen atom of phosphonate group has been confirmed by IR spectroscopy (see Fig. S1 in the Experimental Section) where a signal corresponding to a frequency associated with an amino group at 3409 cm^{-1} does not change with sample dilution (0.5 M, 0.25 M, 0.12 M in $CHCl_3$). Formation of derivatives **5** can be explained through a regio- and stereoselective [4 + 2]-cycloaddition reaction between aldimines **3**, obtained from aldehydes **2** and amine **1**, and olefin **4**, affording the corresponding cycloadduct followed by a prototropic tautomerization to yield heterocycles **5** (Scheme 1).

The scope of the reaction was then expanded to the preparation of quinolinyl derivatives **5** with ethyl phosphonate group at 2-position of the quinolinyl ring by employing diethyl (4-aminophenyl)phosphonate **1b**. The corresponding derivatives **5k-o** (Chart 2) were obtained by a multicomponent reaction between aniline **1b**, aldehydes **2** and indene **4** in the presence of 1 equivalent of $BF_3{\cdot}Et_2O$ (Scheme 1, Route B).

Afterwards, the dehydrogenation of diethyl 6,6a,7,11b-tetrahydro-5*H*-indeno[2,1-*c*]quinolinylphosphonates **5** was studied. First attempts by treatment with 2 equivalents of DDQ in toluene under microwave irradiation for 1 h produced the corresponding diethyl 7*H*-indeno[2,1-*c*]quinolinylphosphonates **6** in low yields (Scheme 1, Chart 3). The formation of dehydrogenated compounds **6** was determined by ^{1}H NMR spectroscopy where upfield signals corresponding to the protons of tetrahydroindenoquinoline ring of starting compounds **5** disappeared and only aromatic signals for derivatives **6** were observed.

Finally, in order of increasing the diversity of these polycyclic heterocycles, methylene carbonylation of 7*H*-indenoquinolines **6** was subjected to investigation using mild oxidation conditions such as selenium oxide in dioxane at reflux or manganese(III) acetate under microwave irradiation generating the corresponding diethyl 7-oxo-7*H*-indeno[2,1-*c*]quinolinylphosphonates **7** in good yields (Chart 4).

In summary, by this methodology a wide range of 7*H*-indenoquinolines **5** and **6** and indenoquinolin-7-ones **7** with electron-donating and electron-withdrawing substituents has been obtained. In this sense, hybrid tetracyclic compounds with different substituents, among them fluorinated or phosphorated ones, are prepared which may be interesting substrates from a biological point of view. As far as we know, this strategy represents the first example for the preparation of tetrahydroindenoquinolines **5**, indenoquinolines **6** and 7-oxoindenoquinolines **7** containing a phosphonate group. The biological behavior of all these new compounds as Topoisomerase I inhibitors and as antiproliferative agents was studied.

Inhibition of Topoisomerase I. We first investigated if the new synthesized derivatives, namely diethyl 6,6a,7,11b-tetrahydro-5*H*-indeno[2,1-*c*]quinolinylphosphonates **5**, diethyl 7*H*-indeno[2,1-*c*]quinolinylphosphonates **6** and diethyl 7-oxo-7*H*-indeno[2,1-*c*]quinolinylphosphonates **7**, could act as inhibitors of human topoisomerase type IB (TopI). The modulation in the activity of this enzyme was evaluated through the assessment of conventional supercoiled DNA relaxation assay, by quantifying the transformation of supercoiled DNA substrate into the relaxed form in the presence of these compounds. For this purpose, a conventional supercoiled plasmid relaxation assay was used. In these experiments compound samples were mixed with enzyme followed by addition of supercoiled plasmid DNA substrate, continued incubation for increasing time periods (15 s, 1 min and 3 min) and subsequent addition of SDS to stop the enzymatic reaction. The DNA relaxation products were then resolved by electrophoresis in 1% agarose gel and visualized by gel red staining. Camptothecin was used as a positive control (Fig. 2).

After performing the enzymatic experiment in the presence of the corresponding compounds, the bands representing relaxed DNA and those representing supercoiled DNA were quantified for each of the enzymatic reactions. In this sense, in the presence of DMSO (Fig. 2, lanes

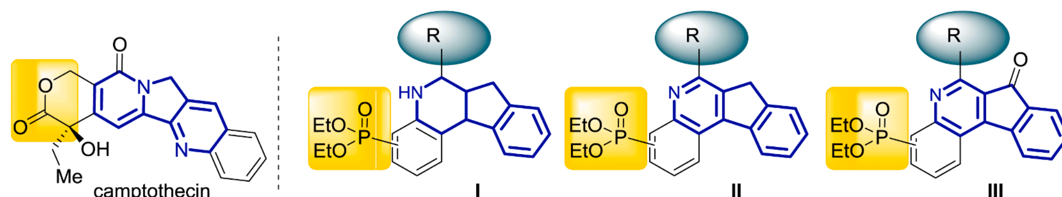
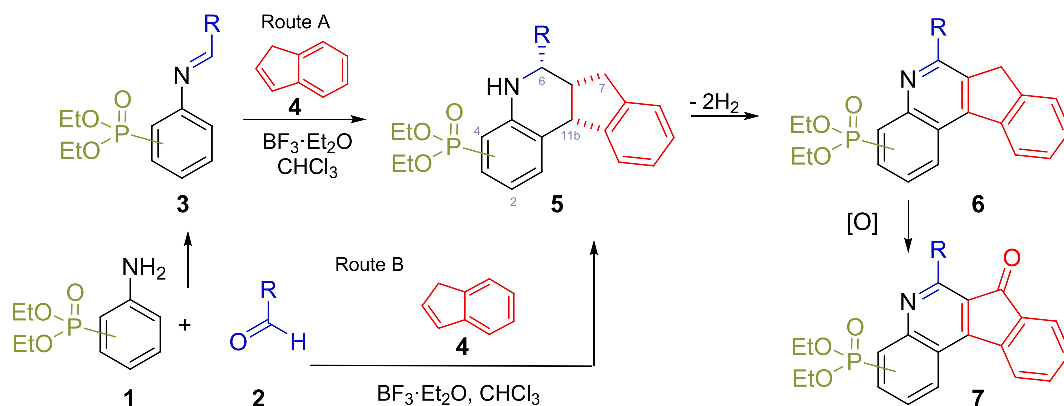


Fig. 1. Structures of camptothecin and novel synthesized antiproliferative tetrahydroindenoquinolinylphosphonates **I**, indenoquinolinylphosphonates **II** and 7-oxoindenoquinolinylphosphonates **III**.



Scheme 1. Syntheses of tetrahydroindenoquinolinylphosphonates **5**, indenoquinolinylphosphonates **6** and 7-oxoindenoquinolinylphosphonates **7**.

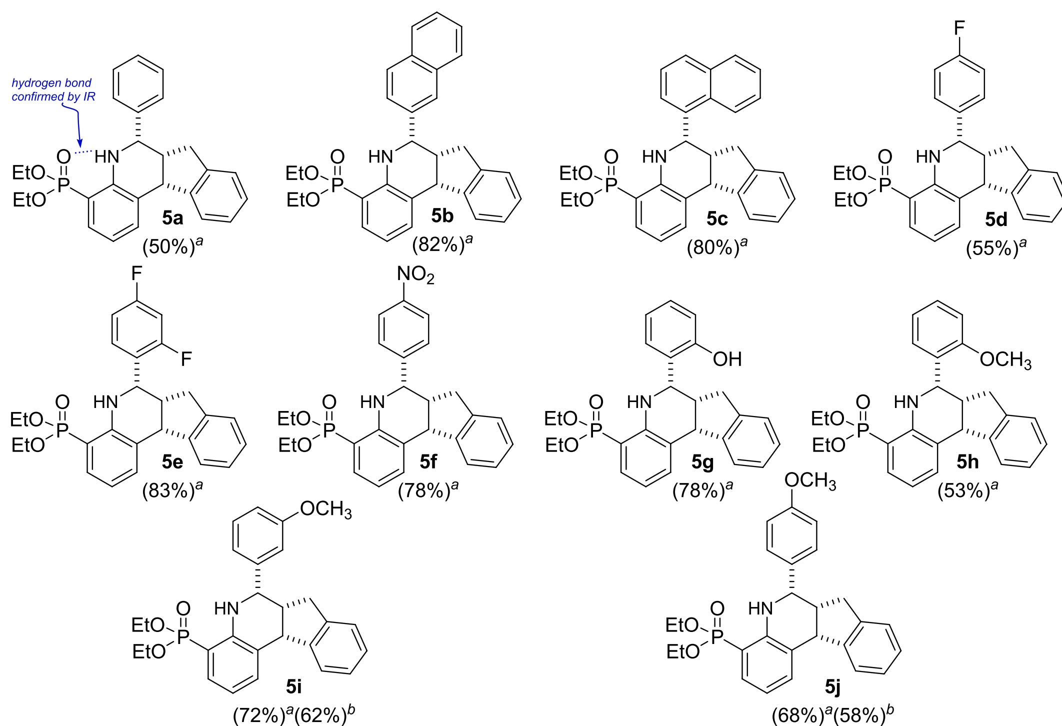


Chart 1. Structures of diethyl *endo*-6,6a,7,11b-tetrahydro-5*H*-indeno[2,1-*c*]quinolin-2-ylphosphonates **5a-j**. [a]Isolated yield obtained by Route A. [b]Isolated yield obtained by Route B.

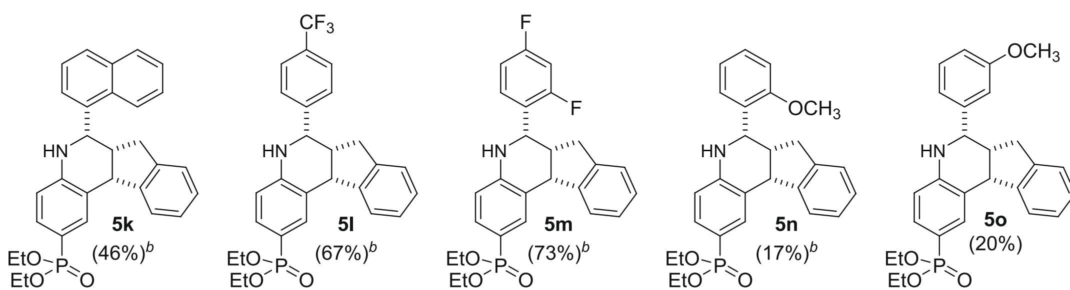


Chart 2. Structures of diethyl 6,6a,7,11b-tetrahydroindenoquinolin-2-ylphosphonate **5k-o**. [a]Isolated yield obtained by Route A. [b]Isolated yield obtained by Route B.

1–3) TopI relaxes supercoiled DNA. On the other hand, camptothecin (CPT), which is used as a positive control, inhibited the relaxation, as indicated by the increased intensity of the band corresponding to the supercoiled DNA (Fig. 2, lanes 4–5), and a decrease of camptothecin activity was observed after 3 min of incubation as indicated in Fig. 2 (lane 6). Fig. 2 also illustrates the TopI inhibitory activity of the new derivatives **5e**, **5h** and **7f**. For example, all three compounds show inhibition of TopI after 15 s of reaction of the mixture TopI, DNA and corresponding compound (lanes 7, 10 and 13). In addition, compounds **7f** and **5e** maintain their inhibition after 1 min of enzymatic reaction

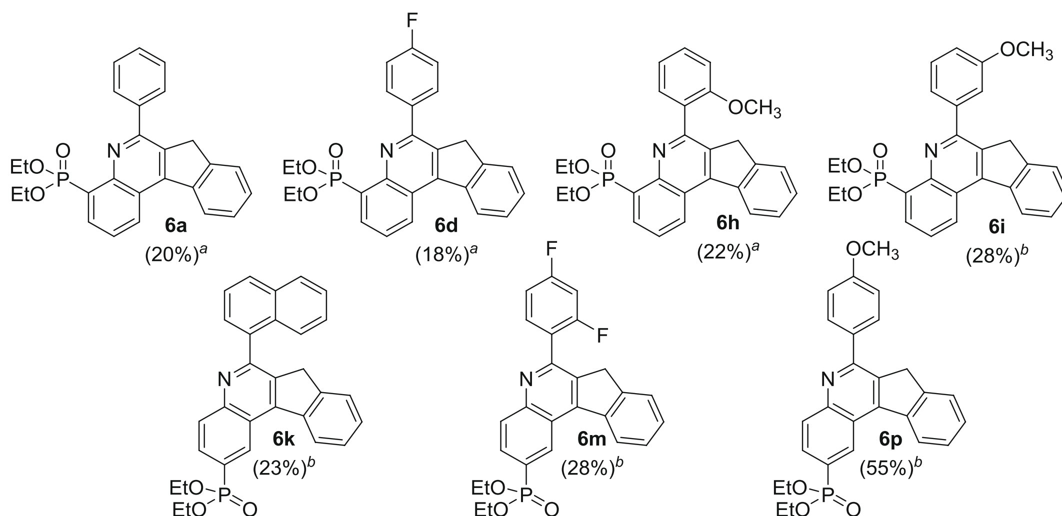


Chart 3. Structures of indenoquinolinylphosphonates **6**. [a]Isolated yield obtained by Povarov reaction of anilines **1**, aldehydes **2** and indene **4** and subsequent aromatization. [b]Isolated yield obtained by the dehydrogenation of compounds **5** with DDQ.

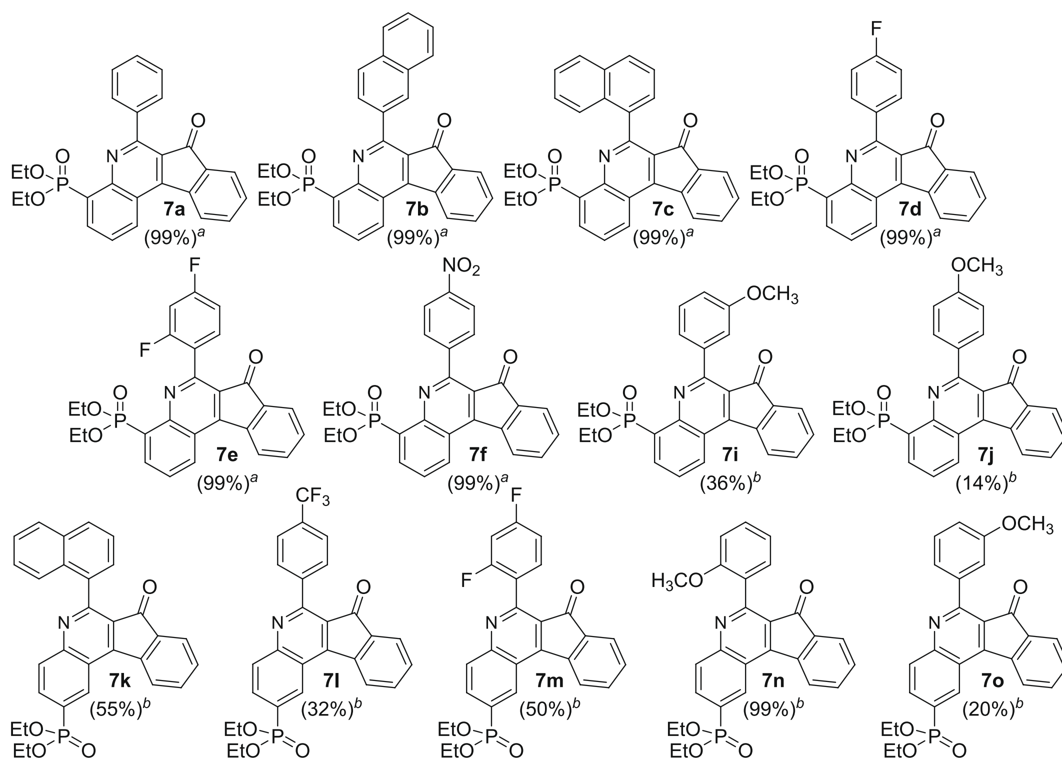


Chart 4. Structures of 7-oxoindenoquinolinylphosphonates **7**. [a]Isolated yield obtained by oxidation with SeO_2. [b]Isolated yield obtained by oxidation with $Mn(OAc)_3$.

(lanes 11 and 14), while compound **5h** presented no inhibition after 1 min reaction (lane 8). As with the natural inhibitor, camptothecin, the inhibition of all compounds **5** stopped after 3 min of enzymatic reaction (lanes 9, 12 and 15). Likewise, the inhibition of all new derivatives was tested in the presence of the purified enzyme and expressed in semi-quantitative fashion relative to the TopI inhibitory activity of camptothecin (Table 1). In these relaxation experiments, for tetrahy droindenoquinolinylphosphonates **5**, indenoquinolinylphosphonates **6** and 7-oxo- indenoquinolinylphosphonates **7**, in a similar way than camptothecin, the highest potency was observed at the beginning of the enzymatic reaction (15 s, 1 min) but this inhibitory activity decreased after 3 min in all cases. According to the data on Table 1 several results should be highlighted for the new synthesized compounds **5**, **6** and **7** compared with the natural inhibitor camptothecin. At short times of the enzymatic reaction, similar inhibition of TopI activity to CPT is observed for some tetrahydroindenoquinolinylphosphonates **5**, such as derivatives **5a** (R = C_6H_5, Table 1, entry 2), **5e** (R = 2,4-F_2-C_6H_3, Table 1, entry 6), **5f** (R = 4-NO_2-C_6H_4, Table 1, entry 7) and **5i** (R = 3-MeO-C_6H_4, Table 1, entry 10). In more aromatized derivatives, indenoquinolinylphosphonates **6**, similar behavior is also observed, as for example in compounds **6a** (R = C_6H_5, Table 1, entry 17), **6d** (R = 4-F-C_6H_4, Table 1, entry 18) and **6i** (R = 3-MeO-C_6H_4, Table 1, entry 20). Regarding the diethyl 7-oxo-7*H*-indeno[2,1-*c*]quinolinylphosphonates **7**, some of them also presented similar inhibition of TopI enzymatic activity to CPT, such as derivative **7b** (R = 2-naphthyl, Table 1, entry 25), derivative **7c** (R = 1-naphthyl, Table 1 , entry 26) and derivative **7f**

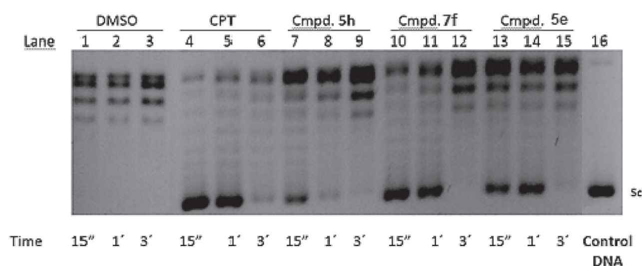


Fig. 2. Inhibition of Top1 activity along the time (15″, 1′ and 3′) by compounds **5h, 7f, 5e** and camptothecin at 100 μM: lanes 1–3, DNA + Top1 + DMSO; lanes 4–6, DNA + Top1 + camptothecin; lanes 7–9, DNA + Top1 + **5h**; lanes 10–12, DNA + Top1 + **7f**; lanes 13–15, DNA + Top1 + **5e**; lane 16, control DNA. Reaction samples were preincubated during 15 min at 37 °C before adding the supercoiled DNA substrate and, separated by electrophoresis on a 1% agarose gel, and then stained with gel red, and photographed under UV light as described in the Top1 mediated DNA relaxation assay.

(R = 4-NO_2-C_6H_4, Table 1, entry 29).

In Vitro Cytotoxicity. The cytotoxic effects of the newly synthesized diethyl tetrahydro-5*H*-indeno[2,1-*c*]quinolinylphosphonates **5**, diethyl 7*H*-indeno[2,1-*c*]quinolinylphosphonates **6** and diethyl 7-oxo-7*H*-indeno[2,1-*c*]quinolinphosphonates **7** were evaluated. The assay was performed using a carcinomic human alveolar basal epithelial cell line (A549), human ovarian carcinoma cell line (SKOV3) and human embryonic kidney cell line (HEK293). In order to analyze the selective cytotoxicity facing exclusively cancerous cell lines, cytotoxicity against non-cancerous cell lines (MRC-5, lung fibroblast cells) was also evaluated. Cell counting kit (CCK-8) assay was employed to assess growth inhibition and, cell proliferation inhibitory activities of compounds that are listed in Table 2 as IC_{50} values. The tested compounds displayed a broad spectrum of antiproliferative activity against the cancer cell lines tested in culture.

From the obtained results in the cytotoxicity assays, several characteristics of the newly synthesized compounds can be highlighted. The first point to emphasize could be the different biological response shown against the three cancerous cell lines. It is noteworthy that all the compounds are more cytotoxic against A549 cell line than against the other two cell lines (SKOV3 and HEK293). Moreover, in this A549 cell line, it is surprising how cytotoxicity varies depending on the position of the phosphonate group, substitution at position 4 (4-PO$(OEt)_2$) or substitution at position 2 (2-PO$(OEt)_2$). In general, higher cytotoxicity is observed in compounds **5**, **6** and **7** with the phosphonate group in position 4, and lower cytotoxicity is observed in derivatives with the phosphonate group in position 2. For example, compound **5c** (R = 1-naphthyl and 4-(PO$(OEt)_2$) showed an IC_{50} value in the nanomolar range (IC_{50} = 0.99 ± 0.16 μM, Table 2, entry 4) while compound **5k** (R = 1-naphthyl and 2-(PO$(OEt)_2$) exhibited an IC_{50} > 50 μM (Table 2, entry 12). Similarly occurs with the fluorinated derivatives, when compound **5e** (R = 2,4-F_2-C_6H_3 and 4-(PO$(OEt)_2$) showed a value of IC_{50} = 1.87 ± 0.38 μM (Table 2, entry 6), while the value of derivative **5m** (R = 2,4-F_2-C_6H_3 and 2-(PO$(OEt)_2$) is 22.20 ± 0.95 μM (Table 2, entry 14). Respect to the more aromatized derivatives **6**, this behavior can also be observed since the IC_{50} values for those compounds with a phosphonate group at position 4 are between 0.73 and 4.28 μM, while those substituted at position 2 present IC_{50}values between 17.97 and>50 μM. Likewise, the same response is observed for derivatives **7** with carbonyl group and phosphonate in position 4, which present better cytotoxicity values (IC_{50} values lower than 3 μM) than derivatives **7** with phosphonate in position 2. For example, derivative **7a** (R = C_6H_5), the most cytotoxic one among all tested compounds in this paper, showed an IC_{50} = 190 nM; derivatives **7b**, **7d** and **7j** with IC_{50} = 1.05, 1.72 and 1.51 μM respectively. In short, we can say that for tested compounds **5**, **6** and **7** the IC_{50} values for 4-phosphonates are less than 8 μM, except some punctual deviations, whereas for 2-phosphonates they

Table 1
TopI Inhibitory Activity of tetrahydroindenoquinolinylphosphonates **5**, indenoquinolinylphosphonates **6** and 7-oxoindenoquinolinylphosphonates **7**.

Entry	Compound	PO$(OEt)_2$	R	% Inhibition[a] 15s	1 min	3 min
1	camptothecin			80	76	3
2	**5a**	4-(PO$(OEt)_2$)	C_6H_5	48	38	1
3	**5b**	4-(PO$(OEt)_2$)	2-naphthyl	63	26	2
4	**5c**	4-(PO$(OEt)_2$)	1-naphthyl	35	34	3
5	**5d**	4-(PO$(OEt)_2$)	4-F-C_6H_4	67	13	1
6	**5e**	4-(PO$(OEt)_2$)	2,4-F_2-C_6H_3	67	60	3
7	**5f**	4-(PO$(OEt)_2$)	4-NO_2-C_6H_4	52	43	4
8	**5g**	4-(PO$(OEt)_2$)	2-HO-C_6H_4	58	33	3
9	**5h**	4-(PO$(OEt)_2$)	2-MeO-C_6H_4	62	23	5
10	**5i**	4-(PO$(OEt)_2$)	3-MeO-C_6H_4	61	58	5
11	**5j**	4-(PO$(OEt)_2$)	4-MeO-C_6H_4	6	5	2
12	**5k**	2-(PO$(OEt)_2$)	1-naphthyl	14	7	1
13	**5l**	2-(PO$(OEt)_2$)	4-CF_3-C_6H_4	18	10	1
14	**5m**	2-(PO$(OEt)_2$)	2,4-F_2-C_6H_3	19	4	0
15	**5n**	2-(PO$(OEt)_2$)	2-MeO-C_6H_4	12	6	1
16	**5o**	2-(PO$(OEt)_2$)	3-MeO-C_6H_4	1	1	1
17	**6a**	4-(PO$(OEt)_2$)	C_6H_5	67	65	4
18	**6d**	4-(PO$(OEt)_2$)	4-F-C_6H_4	68	66	6
19	**6h**	4-(PO$(OEt)_2$)	2-MeO-C_6H_4	39	16	1
20	**6i**	4-(PO$(OEt)_2$)	3-MeO-C_6H_4	80	57	9
21	**6k**	2-(PO$(OEt)_2$)	1-naphthyl	19	6	1
22	**6m**	2-(PO$(OEt)_2$)	2,4-F_2-C_6H_3	25	5	1
23	**6p**	2-(PO$(OEt)_2$)	4-MeO-C_6H_4	8	8	1
24	**7a**	4-(PO$(OEt)_2$)	C_6H_5	36	11	1
25	**7b**	4-(PO$(OEt)_2$)	2-naphthyl	61	42	3
26	**7c**	4-(PO$(OEt)_2$)	1-naphthyl	66	51	5
27	**7d**	4-(PO$(OEt)_2$)	4-F-C_6H_4	46	24	0
28	**7e**	4-(PO$(OEt)_2$)	2,4-F_2-C_6H_3	80	1	1
29	**7f**	4-(PO$(OEt)_2$)	4-NO_2-C_6H_4	81	70	2
30	**7i**	4-(PO$(OEt)_2$)	3-MeO-C_6H_4	34	30	3
31	**7j**	4-(PO$(OEt)_2$)	4-MeO-C_6H_4	18	13	2
32	**7k**	2-(PO$(OEt)_2$)	1-naphthyl	18	6	1
33	**7l**	2-(PO$(OEt)_2$)	4-CF_3-C_6H_4	10	10	1
34	**7m**	2-(PO$(OEt)_2$)	2,4-F_2-C_6H_3	19	6	1
35	**7n**	2-(PO$(OEt)_2$)	2-MeO-C_6H_4	15	8	2
36	**7o**	2-(PO$(OEt)_2$)	3-MeO-C_6H_4	1	1	1

Table 2
Antiproliferative activity of 5,6,6a,11b-tetrahydro-7*H*-indenoquinolines **5**, 7*H*-indenoquinolines **6** and indenoquinolin-7-ones **7**.

Entry	Comp.	$PO(OEt)_2$	R	Cytotoxicity IC_{50} (μM)[a] lungA549	ovarianSKOV3	kidneyHEK293	MRC-5
1	camptothecin			$(1.0 \pm 0.06)\cdot10^{-3}$	$(5.5 \pm 0.01)\cdot10^{-3}$	$(5.6 \pm 2.04)\cdot10^{-3}$	0.8 ± 0.099[21]
2	**5a**	4-(PO(OEt)$_2$)	C_6H_5	4.64 ± 0.20	17.68 ± 1.02	>50	16.65 ± 3.77
3	**5b**	4-(PO(OEt)$_2$)	2-naphthyl	3.76 ± 0.24	>50	>50	>50
4	**5c**	4-(PO(OEt)$_2$)	1-naphthyl	0.99 ± 0.16	27.09 ± 3.55	27.85 ± 5.45	>50
5	**5d**	4-(PO(OEt)$_2$)	4-F-C_6H_4	4.47 ± 0.30	40.97 ± 4.93	>50	>50
6	**5e**	4-(PO(OEt)$_2$)	2,4-F_2-C_6H_3	1.87 ± 0.38	>50	13.34 ± 3.36	>50
7	**5f**	4-(PO(OEt)$_2$)	4-NO_2-C_6H_4	7.28 ± 0.62	>50	>50	>50
8	**5g**	4-(PO(OEt)$_2$)	2-HO-C_6H_4	10.20 ± 1.93	24.08 ± 3.32	13.51 ± 2.56	>50
9	**5h**	4-(PO(OEt)$_2$)	2-MeO-C_6H_4	2.80 ± 1.68	15.43 ± 2.50	>50	>50
10	**5i**	4-(PO(OEt)$_2$)	3-MeO-C_6H_4	8.71 ± 0.50	20.95 ± 1.88	>50	>50
11	**5j**	4-(PO(OEt)$_2$)	4-MeO-C_6H_4	2.10 ± 0.30	12.62 ± 0.73	>50	>50
12	**5k**	2-(PO(OEt)$_2$)	1-naphthyl	>50	16.93 ± 4.59	>50	>50
13	**5l**	2-(PO(OEt)$_2$)	4-CF_3-C_6H_4	2.91 ± 1.08	45.53 ± 7.01	12.51 ± 1.82	>50
14	**5m**	2-(PO(OEt)$_2$)	2,4-F_2-C_6H_3	22.20 ± 0.95	18.78 ± 6.77	>50	>50
15	**5n**	2-(PO(OEt)$_2$)	2-MeO-C_6H_4	>50	NT	35.30 ± 2.40	>50
16	**5o**	2-(PO(OEt)$_2$)	3-MeO-C_6H_4	2.07 ± 0.28	>50	28.60 ± 0.50	>50
17	**6a**	4-(PO(OEt)$_2$)	C_6H_5	4.28 ± 1.08	>50	>50	>50
18	**6d**	4-(PO(OEt)$_2$)	4-F-C_6H_4	2.43 ± 0.49	17.76 ± 6.61	>50	>50
19	**6h**	4-(PO(OEt)$_2$)	2-MeO-C_6H_4	4.66 ± 0.26	14.12 ± 0.59	>50	>50
20	**6i**	4-(PO(OEt)$_2$)	3-MeO-C_6H_4	0.73 ± 0.09	9.79 ± 0.83	11.80 ± 0.97	>50
21	**6k**	2-(PO(OEt)$_2$)	1-naphthyl	17.97 ± 0.52	25.20 ± 10.60	>50	>50
22	**6m**	2-(PO(OEt)$_2$)	2,4-F_2-C_6H_3	27.10 ± 3.96	18.80 ± 5.46	>50	>50
23	**6p**	2-(PO(OEt)$_2$)	4-MeO-C_6H_4	>50	>50	>50	>50
24	**7a**	4-(PO(OEt)$_2$)	C_6H_5	0.19 ± 0.07	2.88 ± 0.52	>50	47.02 ± 5.83
25	**7b**	4-(PO(OEt)$_2$)	2-naphthyl	1.05 ± 0.20	>50	>50	31.89 ± 3.60
26	**7c**	4-(PO(OEt)$_2$)	1-naphthyl	5.57 ± 1.39	>50	30.51 ± 8.61	>50
27	**7d**	4-(PO(OEt)$_2$)	4-F-C_6H_4	1.72 ± 0.39	>50	>50	>50
28	**7e**	4-(PO(OEt)$_2$)	2,4-F_2-C_6H_3	21.20 ± 3.47	19.61 ± 1.09	>50	>50
29	**7f**	4-(PO(OEt)$_2$)	4-NO_2-C_6H_4	6.59 ± 0.90	>50	>50	>50
30	**7i**	4-(PO(OEt)$_2$)	3-MeO-C_6H_4	2.58 ± 0.25	26.82 ± 3.36	39.46 ± 5.98	39.68 ± 7.37
31	**7j**	4-(PO(OEt)$_2$)	4-MeO-C_6H_4	1.51 ± 0.19	>50	>50	>50
32	**7k**	2-(PO(OEt)$_2$)	1-naphthyl	17.20 ± 2.24	23.30 ± 3.30	>50	>50
33	**7l**	2-(PO(OEt)$_2$)	4-CF_3-C_6H_4	>50	>50	>50	>50
34	**7m**	2-(PO(OEt)$_2$)	2,4-F_2-C_6H_3	> 50	> 50	>50	>50
35	**7n**	2-(PO(OEt)$_2$)	2-MeO-C_6H_4	29.20 ± 5.78	NT	>50	>50
36	**7o**	2-(PO(OEt)$_2$)	3-MeO-C_6H_4	4.41 ± 1.74	>50	>50	>50

[a] The cytotoxicity IC_{50} values listed are the concentrations corresponding to 50% growth inhibition. Cytotoxicity values of NT indicate compounds that were not tested.

are higher than 15 μM.

An analogous behavior with respect to the SKOV3 cell line cannot be systematized. If we analyze the cytotoxicity data obtained for SKOV3 cell line, there is no clear pattern of response. Whether the phosphonate group is in position 4 or in position 2, varied cytotoxicity is observed. The derivative **7a** (R = C_6H_5) could be selected as the most cytotoxic one in this SKOV3 cell line, with $IC_{50} = 2.88 \pm 0.52$ μM (Table 2, entry 24). And respect the HEK293 cell line, most of the tested compounds presented high IC_{50} values, higher than 50 μM, except for compounds **5e** (R = 2,4-F_2-C_6H_3), **5g** (R = 2-HO-C_6H_4), **5l** (R = 3-CF_3-C_6H_4) and **6i** (R = 3-MeO-C_6H_4) whose IC_{50} values range from 11 to 13 μM (Table 2, entries 6, 8, 13 and 20, respectively).

Finally, another very interesting information from these new derivatives **5**, **6** and **7** is their response against the non-cancerous cell line, MRC5. In general, high IC_{50} values are observed, most of them > 30 μM, with the exception of compound **5a** (R = C_6H_5) with an IC_{50} of 16.65 ± 3.77 nM. These results support the higher cytotoxicity against cancer cells than against non-cancerous cells.

Conclusions

To conclude, in the present work a new family of compounds, indenoquinolinylphosphonates **5** and **6**, may be easily prepared by a stepwise Povarov protocol or a multicomponent approach. Due to the presence of a methylene group in their structure, carbonyl derivatives can also be prepared by mild oxidation conditions.

These molecules, featuring several fused heterocycles in their structure, were designed in order to test their biological activity. Some of the compounds were found to exhibit TopI inhibition, similar to the natural inhibitor camptothecin. These results indicate that the hybrid indenoquinolinylphosphonates **5**, **6** and 7-oxoindenoquinolinylphosphonates **7** may be further developed to act as a class of the potential inhibitors of the TopI activity.

Regarding the cytotoxicity of these new compounds, the preliminary studies suggest that some of the compounds possess interesting antiproliferative activity against the growth of human tumour cell lines, while they show little cytotoxicity against non-cancerous cells. The performed experiments indicate that some compounds show good cytotoxicity (in the micromolar range) while no correlation with TopI inhibition is maintained, that it may be due to another mechanism in which other biological target is involved. In these sense, taking into account the potential value of these molecules, further evaluation is deserved.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Financial support from the *Ministerio de Ciencia, Innovación y Universidades (MCIU), Agencia Estatal de Investigación (AEI) y Fondo Europeo de Desarrollo Regional (FEDER;* RTI2018-101818-B-I00, UE) and by *Gobierno Vasco, Universidad del País Vasco* (GV, IT 992-16; UPV) is

gratefully acknowledged. Technical and human support provided by IZO-SGI, SGIker (UPV/EHU, MICINN, GV/EJ, ERDF and ESF) is gratefully acknowledged. AS thanks the Basque Government for a formation contract.

Appendix A. Supplementary data

Supplementary data (including supplementary tables, experimental protocols, and compound characterization data) to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128517.

References

[1] Selas A, Martin-Encinas E, Fuertes M, et al. A patent review of topoisomerase I inhibitors (2016–present). *Expert Opin Ther Pat*. 2021;31(6):473–508. https://doi.org/10.1080/13543776.2021.1879051.

[2] Pommier Y, Marchand C. Interfacial inhibitors: targeting macromolecular complexes. *Nat Rev Drug Discov*. 2012;11(1):25–36. https://doi.org/10.1038/nrd3404.

[3] Pommier Y. Topoisomerase I inhibitors: camptothecins and beyond. *Nat Rev Cancer*. 2006;6(10):789–802. https://doi.org/10.1038/nrc1977.

[4] Champoux JJ. DNA Topoisomerases: structure, function, and mechanism. *Annu Rev Biochem*. 2001;70(1):369–413. https://doi.org/10.1146/annurev.biochem.70.1.369.

[5] Wang JC. DNA Topoisomerases. *Annu Rev Biochem*. 1996;65(1):635–692. https://doi.org/10.1146/annurev.bi.65.070196.003223.

[6] Thomas A, Pommier Y. Targeting Topoisomerase I in the Era of Precision Medicine. *Clin Cancer Res*. 2019;25(22):6581–6589. https://doi.org/10.1158/1078-0432.CCR-19-1089.

[7] a) Alonso C, Fuertes M, González M, Rubiales G, Palacios F. Synthesis and biological evaluation of indeno[1,5]naphthyridines as topoisomerase I (TopI) inhibitors with antiproliferative activity. Eur J Med Chem; 2016;115:179–190. https://doi.org/10.1016/j.ejmech.2016.03.031.
b) Palacios F, Alonso C, Fuertes M, Ezpeleta JM, Rubiales G. Glyoxalate-derived aldimines in cycloaddition reactions with olefins. *Eur J Org Chem* 2011;4318–4326. https://doi.org/10.1002/ejoc.201100395.
c) Palacios F, Alonso C, Arrieta A, Cossío FP, Ezpeleta JM, Fuertes M, Rubiales G. Lewis acid activated aza-Diels–Alder reaction of N-(3-Pyridyl)aldimines: an experimental and computational study. *Eur J Org Chem* 2010; 2091–2099. https://doi.org/10.1002/ejoc.200901325.

[8] Pommier Y. *DNA Topoisomerases and Cancer*. New York: Springer; 2012.

[9] Pommier Y, Marchand C. Interfacial inhibitors: targeting macromolecular complexes. *Nat Rev Drug Discov*. 2012;11:25–36. https://doi.org/10.1038/nrd3404.

[10] [10a] For reviews see: Elliott MC, Jones DH. Multicomponent Reactions with [4+2] Cycloadditions as the key step. In: Müller TJJ, ed. *Multicomponent Reactions*. Volume 2. New York, NY: Thieme; 2014:243–286.[10b] Voskressensky LG, Festa AA. [4+2] Cycloaddition. In: Müller TJJ, ed. *Multicomponent Reactions*. 2. New York, NY: Thieme; 2014:303–343.[10c] Du H, Ding K. Diels-Alder and Hetero-Diels-Alder Reactions. In: Dalko PI, ed. *Comprehensive Enantioselective Organocatalysis: Catalysts, Reactions, and Applications*. Germany: Wiley-VCH; 2013:1131–1162.

[11] [11a] Vicente-Garcia E, Ramon R, Lavilla R. New heterocyclic inputs for the Povarov multicomponent reaction. *Synthesis*. 2011:2237–2246. https://doi.org/10.1055/s-0030-1260062.[11b] Kouznetsov VV. Recent synthetic developments in a powerful imino Diels-Alder reaction (Povarov reaction): application to the synthesis of N-polyheterocycles and related alkaloids. *Tetrahedron*. 2009;65:2721–2750. https://doi.org/10.1016/j.tet.2008.12.059.[11c] Povarov LS. α,β-Unsaturated ethers and their analogues in reactions of diene synthesis. *Russ Chem Rev*. 1967;36:656–670. https://doi.org/10.1070/RC1967v036n09ABEH001680.

[12] Alonso C, Fuertes M, Martin-Encinas E, et al. Novel topoisomerase I inhibitors. Syntheses and biological evaluation of phosphorus substituted quinoline derivates with antiproliferative activity. *Eur J Med Chem*. 2018;149:225–237.

[13] Tejería A, Pérez-Pertejo Y, Reguera RM, et al. Antileishmanial activity of new hybrid tetrahydroquinoline and quinoline derivatives with phosphorus substituents. *Eur J Med Chem*. 2019;162:18–31.

[14] Bhagat S, Supriya M, Pathak S, Sriram D, Chakraborti AK. α-Sulfonamidophosphonatesasnewanti-mycobacterialchemotypes:Design, developmentofsyntheticmethodology, andbiologicalevaluation. *Bioorg Chem*. 2019;82:246–252.

[15] Bhagat S, Shah P, Garg SK, et al. α-Aminophosphonates as novel anti-leishmanial chemotypes: synthesis, biological evaluation, and CoMFA studies. *Med Chem Commun*. 2014;5:665–670.

[16] Engel R. *Handbook of Organophosphorus Chemistry*. New York, NY: M Dekker Inc; 1992.

[17] [17a] Chen L, Liu X-Y, Zou Y-X. Recent advances in the construction of phosphorus-substituted heterocycles 2009-2019. *Adv Synth Catal*. 2020;362(9):1724–1818. https://doi.org/10.1002/adsc.201901540.[17b] Rodriguez JB, Gallo-Rodriguez C. The role of the phosphorus atom in drug design. *ChemMedChem*. 2019;14(2):190–216. https://doi.org/10.1002/cmdc.201800693.[17] Kafarski P, Lejezak B. Biological activity of aminophosphonic acids. *Phosphorus Sulfur Silicon Relat Elem*. 1991;63:193–215. https://doi.org/10.1080/10426509108029443.

[18] a) Maestro A, del Corte X, Lopez-Frances A, Martinez de Marigorta E, Palacios F, Vicario J. Asymmetric synthesis of tetrasubstituted α-aminophosphonic acid derivatives. Molecules 2021; 26: 3202.
b) Palacios F, Alonso C, de los Santos JM. Synthesis of beta-aminophosphonates and –phosphinates. *Chem Rev*. 2005;105:899–931. Doi: 10.1021/cr040672y.

[19] [19a] For excellent reviews see: Van der Jeught K, Stevens CV. Direct phosphonylation of aromatic azaheterocycles *Chem Rev*. 2009;109:2672–2702. https://doi.org/10.1021/cr800315j.[19b] Moneen K, Laureyn I, Stevens CV. Synthetic methods for azaheterocyclic phosphonates and their biological activity. *Chem Rev*. 2004;104:6177–6215. https://doi.org/10.1021/cr030451c.

[20] [20a] Berube G. An overview of molecular hybrids in drug discovery. *Expert Opin Drug Discov*. 2016;11:281–305. https://doi.org/10.1517/17460441.2016.1135125.[20b] Fortin S, Berube G. Advances in the development of hybrid anticancer drugs. *Expert Opin Drug Discov*. 2013;8:1029–1047. https://doi.org/10.1517/17460441.2013.798296.

[21] Jones DC, Hallyburton I, Stojanovski L, Read KD, Frearson JA, Fairlamb AH. Identification of a k-opioid agonist as a potent and selective lead for drug development against human African trypanosomiasis. *Biochem Pharm*. 2010;80:1478–1486.