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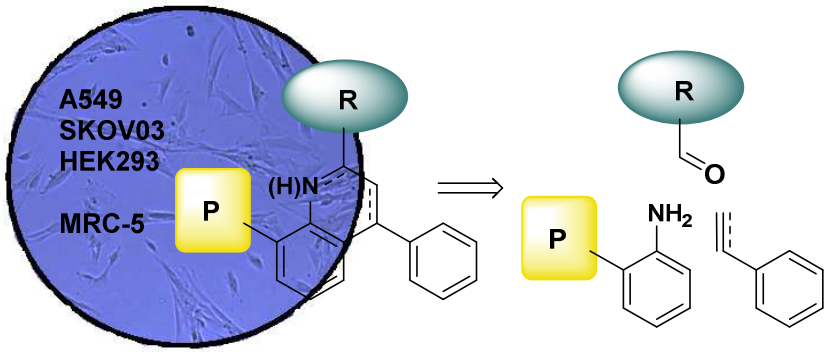

Novel Topoisomerase I Inhibitors. Syntheses and Biological Evaluation of Phosphorus Substituted Quinoline Derivates with Antiproliferative Activity.

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Keywords: Phosphorated Quinolines, Topoisomerase I, Enzyme inhibition, Antiproliferative effect

Abbreviations: CCK8, cell counting kit; CPT, camptothecin; CPTs, camptothecin derivatives; DDQ, dichloro-5,6-dicyanobenzoquinone; equiv, equivalents; HDAr, hetero-Diels-Alder reaction; MCR, multicomponent reaction; SDS, sodium dodecyl sulfate; Top1, topoisomerase I; TLC, thin layer chromatography.

ABSTRACT. This work describes the synthesis of 1,2,3,4-tetrahydroquinolinylphosphine oxides, phosphanes and phosphine sulfides as well as that of quinolinylphosphine oxides and phosphine sulfides, which were synthesized in good to high overall yield. The synthetic route involves a multicomponent reaction of (2-phosphine-oxide)-, 2-phosphine- or (2-phosphine-sulfide)-aniline, aldehydes and olefins and allows the selective generation of two stereogenic centres in a short, efficient and reliable synthesis. The selective dehydrogenation of 1,2,3,4-tetrahydroquinolinylphosphine oxides and phosphine sulfides leads to the formation of corresponding phosphorus substituted quinolines. Some of the products which were prepared showed excellent activity as topoisomerase I (Top1) inhibitors. In addition, prolonged effect of the most potent compounds is maintained with the same intensity even after three minutes of the beginning of the enzymatic reaction. The cytotoxic effect on cell lines derived from human lung adenocarcinoma (A549), human ovarian carcinoma (SKOV03) and human embryonic kidney (HEK293) was also screened. 1,2,3,4-Tetrahydroquinolinylphosphine oxide **6g** with an IC_{50} value of 0.25±0.03 μM showed excellent activity against the A549 cell line *in vitro*, while 1,2,3,4-tetrahydroquinolinylphosphane **9c** with an IC_{50} value of 0.08±0.01 μM and 1,2,3,4-tetrahydroquinolinylphosphine sulfide derivative **10f** with an IC_{50} value of 0.03±0.04 μM are more active against the A549 cell line. Moreover, selectivity towards cancer cell (A549) over non-malignant cells (MRC5) has been observed. According to their structure, they may be excellent antiproliferative candidates.

1. Introduction

Currently some cancer chemotherapies focus on inducing damage to the DNA of cancer cells. In this regard, it should be noted that human topoisomerase (hTop1) has become an important target

in the discovery of anti-cancer drugs. This ubiquitous nuclear 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 [1]. Increased Top1 activity is observed in cancer cells compared to that observed in healthy cells, which leads to a higher rate of replication. In this sense, Top1 inhibitors are usually the basis of some chemotherapeutic combinations in the treatment of a broad spectrum of tumors [2]. Among the most representative of Top1 inhibitor drugs there is camptothecin (CPT, Figure 1) and its derivatives (CPTs) which are currently used in the systemic treatment of colon, ovarian and small-cell lung cancers [1b, 3]. However, when being used in cancer therapy, these derivatives carry certain drawbacks, such as lactone instability in blood plasma and cancer cell resistance [4].

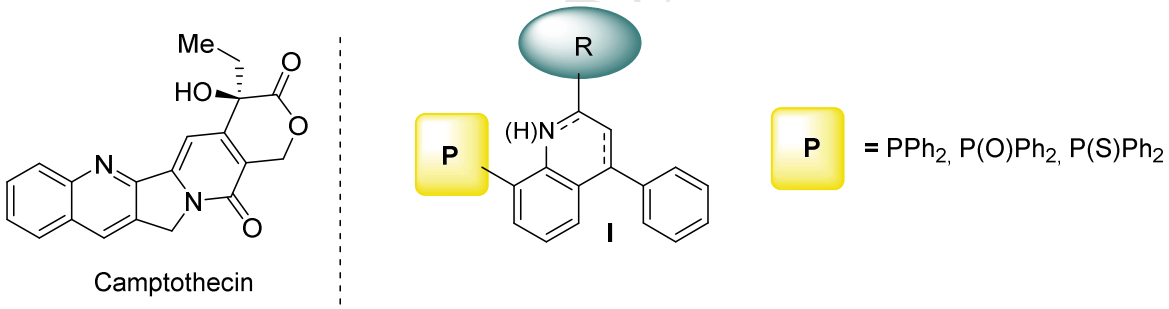


Figure 1. Structures of camptothecin and several novel synthesized antiproliferative *phosphorated (tetrahydro)quinolines I*.

Among other features, camptothecin and its derivatives show several fused heterocycles in their structural analysis. Moreover, with regard to the proposed inhibition mechanism of Top1, the presence of flat or *quasi*-flat polycyclic heterocycles seems to be relevant in the effectiveness of the antiproliferative activity [5]. Nitrogenated quinoline compounds show, in fact, an interesting biological activity with this target [6]. Recently [7a], fluorescein hydrazones [7b], indeno[1,2-

b]pyridines derivatives [7c,d], benzofuro[3,2-b]pyridines [7e], and benzimidazoles [7f] have been reported as Top1 inhibitors. In this sense, the Povarov reaction [8] is a highly atom-economic tool for the incorporation of nitrogen into chemical compounds, such as quinolines. Furthermore, in the search of a methodology for the preparation of molecules with high molecular diversity, multicomponent reactions (MCRs) represent a versatile alternative where lower costs, shorter reaction time and less energy are needed [9]. MCRs have been successfully applied in medicinal chemistry [10], and therefore the multicomponent version of the Povarov reaction may interestingly be considered as a powerful tool for the preparation of nitrogen derivatives with excellent regioselectivity.

Organophosphorus derivatives are interesting compounds from a biological point of view, since it is known that phosphorus substituents may affect the reactivity of heterocycles and regulate important biological functions [11]. In this context, antibiotics such as phosphinothricin, fosfomycin, fosfidomycin or dehydrophos [12a] have been described. Thus, from cyclophosphamide [12b] discovered in 1954 as an anticancer drug [12c] and used to treat leukemia and different cancers to brigatinib [12d], which contains a phosphine oxide substitute attached to an aryl group, recently approved in the USA for the treatment of metastatic non-small cell lung cancer (NSCLC) [12e], a large number of compounds containing phosphorus have been described [11]. The development of new strategies for the preparation of aminophosphonates [13], phosphinated [14] or phosphorylated azaheterocycles [15] implies the incorporation of organophosphorus functionalities in simple synthons. For example, the diphosphonylation of quinolines leads to tetrahydroquinolines [16]. Some phosphorated derivatives of fluoroquinolones (ciprofloxacin, norfloxacin, sparfloxacin) are characterized by a greater biological activity than the original drugs [17].

With these considerations in mind, we believe that the development of new hybrid molecules [18] incorporating a phosphine, phosphine sulfide or phosphine oxide group in the heterocyclic ring of a tetrahydroquinoline and/or a quinoline structure may be privileged scaffolds for pharmaceuticals and may improve the antiproliferative cytotoxic properties with respect to other biologically active structures. This fact could be due to an improvement in π-π* stacking interactions with the DNA base pairs [1].

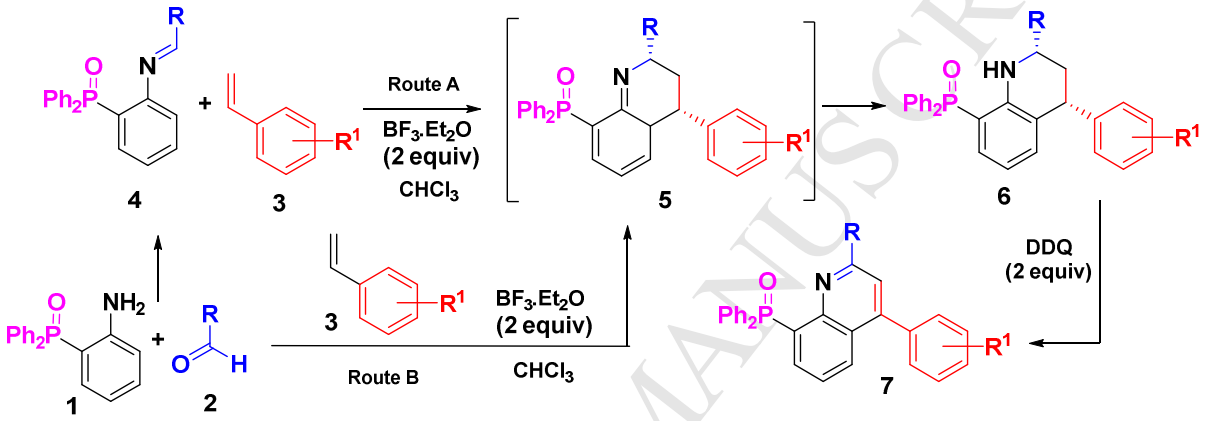
Bearing all this in mind, the development of highly selective methodologies to provide efficient and fast access to phosphorous heterocycles containing a skeleton of quinolines **I** (Figure 1) represents an interesting challenge in organic chemistry, due to the potential interest of these molecules not only in synthetic but also in medicinal chemistry.

2. Results and Discussion

2.1 Chemistry

The phosphine-oxide substituted quinoline derivatives were synthesized by a two-step Povarov type [4+2]-cycloaddition reaction. Therefore, 2-(diphenylphosphine oxide)aldimines **4**, prepared *in situ* by reaction of 2-(diphenylphosphine oxide)aniline **1** and aldehydes **2**, were reacted with styrenes **3** in the presence of 2 equivalents of $BF_3 \cdot Et_2O$ in refluxing chloroform (Scheme 1, Route A). After reaction completion, the corresponding *endo*-1,2,3,4-tetrahydroquinolinylphosphine oxides **6** were selectively obtained with good yields in a regio- and stereospecific way (Chart 1). The formation of (1,2,3,4-tetrahydroquinolin-8-yl)phosphine oxide **6** may be explained through a regio- and stereoselective [4+2]-cycloaddition reaction between aldimine **4**, obtained from amine **1**, aldehydes **2**, and olefins **3**, followed by a prototropic tautomerization of adduct **5**. Subsequent dehydrogenation of 1,2,3,4-

tetrahydroquinolines **6** with 2 equivalents of DDQ in $CHCl_3$ produced the corresponding quinolinylphosphine oxides **7** (Scheme 1, Chart 1). The formation of compounds **7** was determined by ^1H NMR spectroscopy where upfield signals corresponding to the protons of tetrahydroquinoline ring of starting compounds **6** disappeared and only aromatic signals were observed.



Scheme 1. Syntheses of 1,2,3,4-tetrahydroquinolinylphosphine oxides **6** and quinolinylphosphine oxides **7**.

Alternatively, same compounds **6** can be directly obtained by a multicomponent (MCR) protocol. In this sense, the three components (phosphine oxide amine **1**, aldehydes **2** and styrenes **3** were reacted in the presence of the Lewis acid ($BF_3 \cdot Et_2O$) and the corresponding *endo*-quinolinylphosphine oxide **6** were regio- and diastereoselectively obtained (Scheme 1, Route B, Chart 1).

The methodology tolerates a wide range of electron-releasing and electron-withdrawing aromatic aldehydes, even fluorinated ones which allow the preparation of fluoro containing compounds, interesting substrates from a biological point of view [19]. As far as we know, this strategy

represents the first example for the preparation of disubstituted 1,2,3,4-tetrahydroquinolines **6** and quinolines **7** containing a phosphine oxide group.

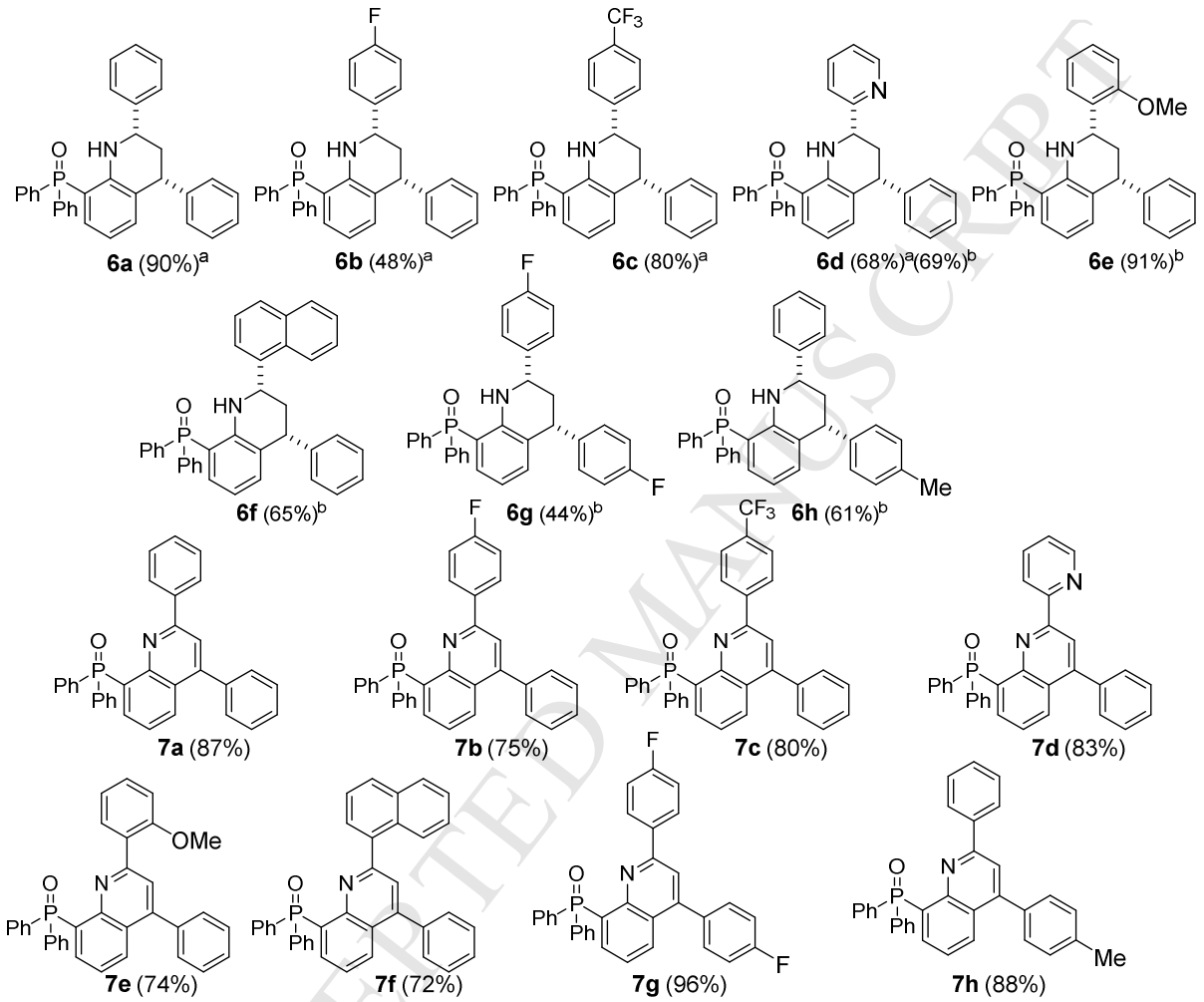
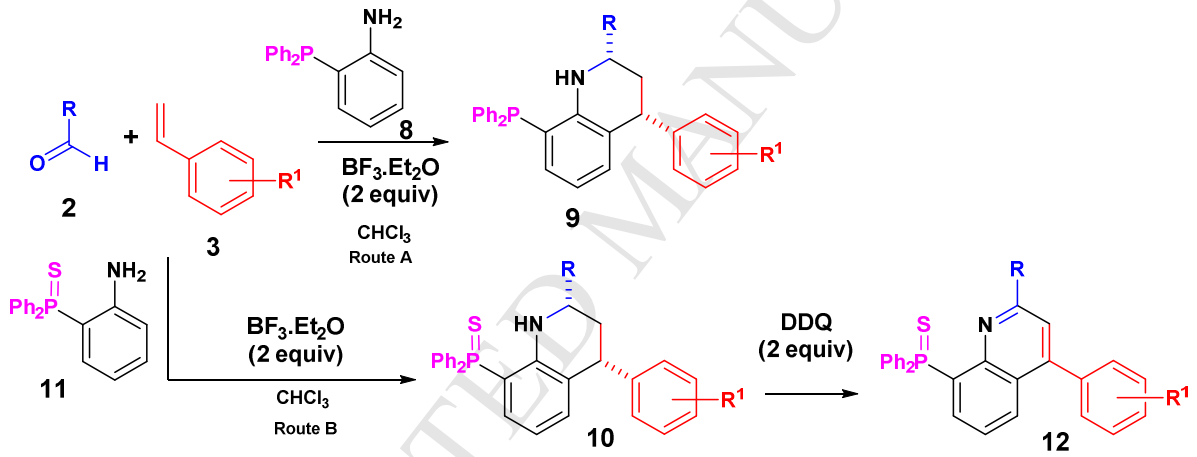


Chart 1. Structures of 1,2,3,4-tetrahydroquinolinylphosphine oxides **6** and quinolinylphosphine oxides **7** obtained ([a]Isolated yield obtained by Route A. [b]Isolated yield obtained by Route B).

Afterwards, with the aim of expanding the diversity in substitution of quinoline derivatives, the phosphino substituted quinolines were synthesized by multicomponent Povarov type [4+2]-cycloaddition reaction as shown in Scheme 2. First, the *endo*-1,2,3,4-tetrahydroquinolinylphosphanes **9** were regioselectively obtained by reaction between 2-(diphenylphosphino)aniline **8**, aldehydes **2a** (R = C_6H_5) and /or **2b** (R = 4-$CF_3C_6H_4$) and styrene

3a (R^1 = H) in the presence of 2 equivalents of $BF_3{\cdot}Et_2O$ in refluxing chloroform (Scheme 2, Route A) and led to the formation of the corresponding tetrahydroquinolines **9a** and **9b** (Chart 2) in a regioselective fashion.

In a similar way to that reported in the case of 1,2,3,4-tetrahydroquinolinylphosphine oxides **6** (Scheme 1, *vide supra*), the formation of 1,2,3,4-tetrahydroquinolinylphosphane **9** may be explained through a regio- and stereoselective [4+2]-cycloaddition reaction between aldimine, obtained from aldehyde **2** and amine **8**, and the corresponding olefin **3**, followed by a prototropic tautomerization.



Scheme 2. Syntheses of 1,2,3,4-tetrahydroquinolinylphosphanes **9**, 1,2,3,4-tetrahydroquinolinylphosphine sulfides **10** and quinolinylphosphine sulfides **12**.

In order to increase the diversity of the process, the reaction was extended to a substituted styrene containing an electron-withdrawing substituent (fluorine) such as *p*-fluorostyrene **3b** (R^1 = 4-F), since several recently approved drugs contain fluorine in their structure [19], and a substituted styrene containing an electron-donating group such as *p*-methylstyrene **3c** (R^1 = 4-Me). It is noteworthy that when substituted olefins on the aromatic ring are used, more stable 1,2,3,4-tetrahydroquinolinylphosphanes **9** could be obtained and isolated with better yields [20]. When using *p*-fluorostyrene **3b** and *p*-methylstyrene **3c** corresponding 1,2,3,4-

tetrahydroquinolinylphosphanes **9c-f** were obtained with good yields (Scheme 2, Route A, Chart 2).

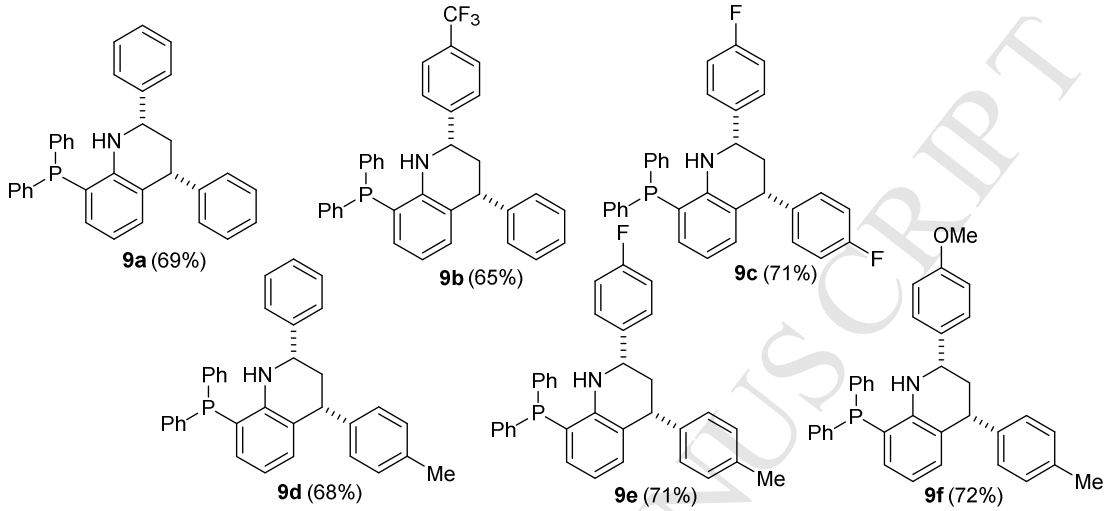


Chart 2. Structures of 1,2,3,4-tetrahydroquinolinylphosphanes **9** obtained by Povarov reaction.

Due to the instability of 1,2,3,4-tetrahydroquinolinylphosphanes **9** [21] and because phosphine sulfides constitute an important class of organophosphorus compounds with potential biological activity [11], we explored whether these 1,2,3,4-tetrahydroquinolinylphosphine sulfides **10** may be directly obtained by using a phosphine sulfide amine **11** through a multicomponent protocol. In this case, the three components (phosphine sulfide amine **11**, aldehydes **2** and olefins **3**, Scheme 2, Route B) were mixed in the presence of the Lewis acid ($BF_3 \cdot Et_2O$), and corresponding *endo*-1,2,3,4-tetrahydroquinolinylphosphine sulfides **10** were regioselectively obtained in refluxing chloroform during 48 hours.

As before, the formation of 1,2,3,4-tetrahydroquinolinylphosphine sulfides **10** may be explained through a regio- and stereoselective [4+2]-cycloaddition reaction between aldimines, initially generated by condensation reaction of phosphine sulfide amine **11** and aldehydes **2**, and olefins **3** followed by a prototropic tautomerization. Aromatization of 1,2,3,4-

tetrahydroquinolinylphosphine sulfides **10**, which appeared in some cases as a minor component in the sulfurization reaction of heterocycles **9**, was performed by treatment with 2 equivalents of DDQ in toluene under microwave irradiation for 1 hour. In this manner, the corresponding quinolinylphosphine sulfides **12** were obtained with good yields (Scheme 2, Chart 3).

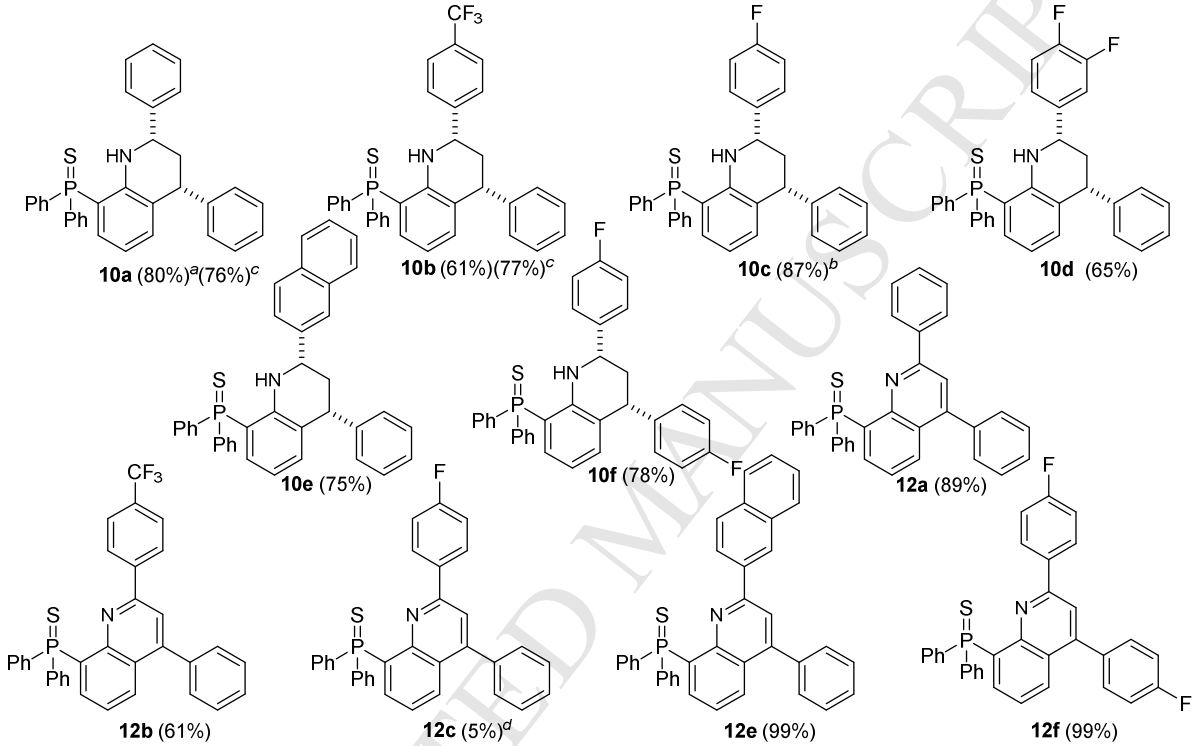


Chart 3. Structures of 1,2,3,4-tetrahydroquinolinylphosphine sulfides **10** and quinolinylphosphine sulfides **12** obtained ([a]a small amount (9%) of aromatized compound **12a** was also obtained; [b]a small amount (5%) of aromatized compound **12c** was also obtained; [c]isolated yields obtained from amine **11**; [d]obtained along with tetrahydroqueinoline **10c**).

The methodology represents an easy and efficient strategy for the preparation of functionalized quinoline derivatives containing phosphorus substituents and tolerates a wide range of aromatic aldehydes with electron-releasing and electron-withdrawing substituents. Regarding phosphorus substitution, a wide variety of derivatives, such as phosphine, phosphine oxide and phosphine sulfide derivatives, can be prepared. The biological behavior of prepared compounds as Top1 inhibitors and as antiproliferative agents was studied.

2.2 Biological results and discussion

2.2.1. Inhibition of topoisomerase I. The inhibitory effect of the synthesized derivatives on human Topoisomerase type I (hTop1) was investigated. A conventional supercoiled plasmid relaxation assay was used to determine if the newly synthesized phosphorous heterocycles, namely 1,2,3,4-tetrahydroquinolinylphosphine oxides **6** and quinolinylphosphine oxides **7**, 1,2,3,4-tetrahydroquinolinylphosphanes **9**, 1,2,3,4-tetrahydroquinolinylphosphine sulfides **10** and quinolinylphosphine sulfides **12** inhibit hTop1 activity and therefore the transformation of supercoiled plasmid DNA into the relaxed one (Table 1). In these experiments compound samples were mixed with enzyme followed by addition of supercoiled plasmid DNA substrate and continued incubation for increasing time periods (15 seconds, 45 seconds, 1 minute and 3 minutes). The reaction was terminated by the addition of SDS. DNA relaxation products were then resolved by gel electrophoresis in 1% agarose gel and visualized by gel red staining. Camptothecin was used as a positive control (Figure 2).

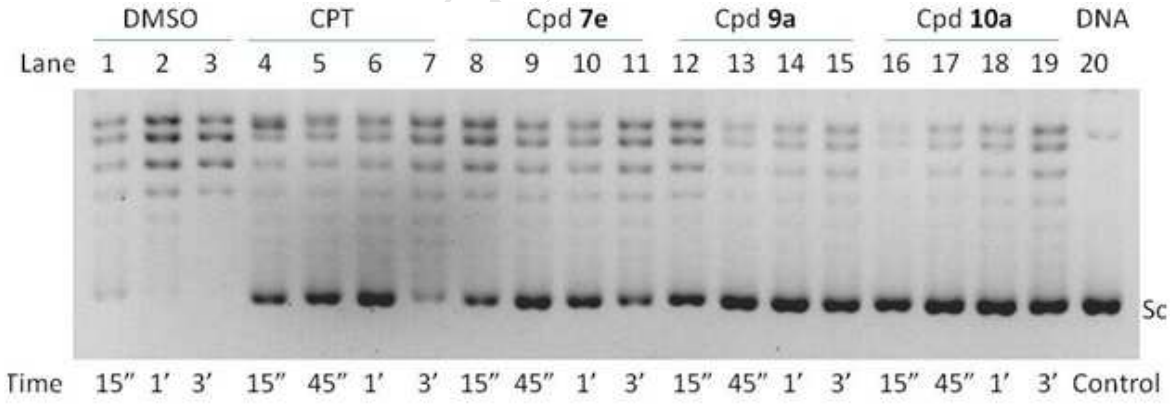


Figure 2. Inhibition of Top1 activity along the time (15", 45", 1' and 3') by compounds **7e**, **9a** and **10a** and camptothecin at 80 μM: lanes 1-3, DNA+Top1+DMSO; lanes 4-7, DNA+Top1+camptothecin 80μM; lanes 8-11, DNA+Top1+**7e** 80μM; lanes 12-15, DNA+Top1+**9a** 80μM; lanes 16-19, DNA+Top1+**10a** 80μM; lane 20, control DNA. Reaction samples were mixed with enzyme at 37 ºC 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; Sc, supercoiled DNA.

Table 1. Top1 inhibitory activity of quinolinylphosphine oxides **6** and **7**, quinolinylphosphanes **9** and quinolinylphosphine sulfides **10** and **12**.

Entry	Compound	R	R^1	Top1 Inhibition[a]		
				15 s	1 min	3 min
1		Camptothecin (CPT)		++	++	Θ
2	**6a**	Ph	H	++	++	Θ
3	**6b**	4-F-C_6H_4	H	++	++	Θ
4	**6c**	4-CF_3-C_6H_4	H	+	+	Θ
5	**6d**	2-pyridyl	H	+++	+++	+++
6	**6e**	2-MeO-C_6H_4	H	+++	+++	++
7	**6f**	1-naphthyl	H	++	++	+
8	**6g**	4-F-C_6H_4	4-F	++	++	+
9	**6h**	Ph	4-Me	+	+	Θ
10	**7a**	Ph	H	++	++	Θ
11	**7b**	4-F-C_6H_4	H	+	+	+
12	**7c**	4-CF_3-C_6H_4	H	+	+	Θ
13	**7d**	2-pyridyl	H	++	++	Θ
14	**7e**	2-MeO-C_6H_4	H	+++	+++	++
15	**7f**	1-naphthyl	H	+++	+++	++
16	**7g**	4-F-C_6H_4	4-F	++	++	Θ
17	**7h**	Ph	4-Me	Θ	Θ	Θ
18	**9a**	Ph	H	+++	+++	+++
19	**9b**	4-CF_3-C_6H_4	H	++	++	++
20	**9c**	4-F-C_6H_4	4-F	+++	+++	+++
21	**9d**	Ph	4-Me	+++	+++	+++
22	**9e**	4-F-C_6H_4	4-Me	+++	+++	+++
23	**9f**	4-MeO-C_6H_4	4-Me	++	++	++
24	**10a**	Ph	H	+++	+++	+++
25	**10b**	4-CF_3-C_6H_4	H	Θ	Θ	Θ
26	**10c**	4-F-C_6H_4	H	+	+	+
27	**10d**	3,4-F_2-C_6H_3	H	++	++	++
28	**10e**	2-naphthyl	H	+++	+++	+++
29	**10f**	4-F-C_6H_4	4-F	+++	+++	+++
30	**12a**	Ph	H	+++	++	Θ
31	**12b**	4-CF_3-C_6H_4	H	+++	+++	+++
32	**12f**	4-F-C_6H_4	4-F	+	+	+

[a] The activity of the compounds to inhibit Top1 relaxation was expressed semiquantitatively as follows: Θ, no activity; +, weak activity; ++ similar activity to camptothecin; +++ strong activity. See Supporting information for quantitative values of % inhibition (Table 1.SI).

Top1 inhibitory activity was tested by detecting the conversion of supercoiled DNA (Sc, Figure 2) to its relaxed form in the presence of the purified enzyme and expressed in semiquantitative fashion relative to the Top1 inhibitory activity of camptothecin (Table 1). As shown in Figure 2, Top1 relaxes supercoiled DNA even in the presence of DMSO (Figure 2, lanes 1-3). On the other hand, CPT inhibits the relaxation, as indicated by the increased intensity of the band corresponding to the supercoiled DNA (Figure 2, lanes 4-7). And on the basis of the results of the relaxation assays (Figure 2, lanes 4-19, Table 1), we conclude that some of the tested compounds inhibited Top1 relaxation more potently than camptothecin when added at the same concentration. For instance, phosphine oxide derivatives with aromatic or electron-donating substituents present higher inhibitory activity than CPT (Table 1), such as tetrahydroquinolines **6d** (R = 2-pyridyl, R^1 = H, entry 5) and **6e** (R = 2-$MeOC_6H_4$, R^1 = H, entry 6) or quinolines **7e** (R = 2-$MeOC_6H_4$, R^1 = H, entry 14) and **7f** (R = 2-naphthyl, R^1 = H, entry 15). However, in phosphine or phosphine sulfide derivatives electron-withdrawing substituents present the highest potency. Therefore, tetrahydroquinolinylphosphane **9a** (R = Ph, R^1 = H, entry 18), **9c** (R = 4-FC_6H_4, R^1 = 4-FC_6H_4, entry 20) and phosphine sulfides **10a** (R = Ph, R^1 = H, entry 24), **10e** (R = 2-naphthyl, R^1 = H, entry 28), **10f** (R = 4-FC_6H_4, R^1 = 4-FC_6H_4, entry 29), **12a** (R = Ph, R^1 = H, entry 30), **12b** (R = 4-$CF_3C_6H_4$, R^1 = H, entry 31) resulted to be more potent than CPT in inhibiting Top1 enzymatic activity. In addition, relaxation assays of compounds **6d**, **9a**, **9c**, **9d**, **10a**, **10e**, **10f** and **12b** after 3 minutes of enzymatic reaction have been performed at lower different concentrations as 60, 40, 20 and 10 μM (see Supporting Information, Table 2.SI). And it is noteworthy that some of those potent compounds, such as **6d**, **9d**, **9e**, **10e**, **10f** and **12b**, are able to inhibit Top1 activity even at such a low concentration as 20 and 10 at μM (Table 2.SI, entries 2, 5, 6, 8, 9, 10).

Remarkably, longer effect on those compounds presenting greater inhibition than that observed for the natural inhibitor, CPT, must be pointed out. It is well known that CPT binds reversibly to Top1 and that the cleavage complexes reverse within minutes as seen in lane 7 [6]. The quick detachment from the topoisomerase I-cleaved DNA complex represents one of the disadvantages of CPT based anticancer drugs which imposes long infusions [22]. As it can be observed in Table 1, the most potent compounds (Table 1, entries 5, 6, 14, 15, 18, 20, 21, 22, 24, 28, 29, 31) the potent effect on inhibiting Top1 activity is maintained with the same intensity even after 3 minutes the enzymatic reaction starts. In other words, these compounds show their ability to prevent relaxation of supercoiled DNA with high potency over prolonged period, representing an advantage with respect to CPT as it may reduce the amount and frequency of drug administration. And it is noteworthy that these compounds are not only more potent than CPT after 3 minutes of enzymatic reaction but are also able of inhibiting the action of the Top1 after that time even at low concentrations.

2.2.2. In Vitro Cytotoxicity. The cytotoxicity of the newly synthesized 1,2,3,4-tetrahydroquinolinylphosphine oxides **6** and quinolinylphosphine oxide derivatives **7**, 1,2,3,4-tetrahydroquinolinylphosphanes **9**, 1,2,3,4-tetrahydroquinolinylphosphine sulfides **10** and quinolinylphosphine sulfides **11** was evaluated *in vitro* by testing their antiproliferative activities against several human cancer cell lines: A549 (carcinomic human alveolar basal epithelial cell), SKOV03 (human ovarian carcinoma) and HEK293 (human embryonic kidney). Cell counting kit (CCK-8) assay was employed to assess growth inhibition and, cell proliferation inhibitory activities of the compounds are listed in Table 2 as IC_{50} values.

Table 2. Antiproliferative activity of quinolinylphosphine oxides **6** and **7,** quinolinylphosphanes **9**, quinolinylphosphine sulfides **10** and **12**.

Entry	Compound	R	R^1	Cytotoxicity IC_{50} (μM)[a]			
				lung A549	ovarian SKOV03	kidney HEK293	MRC-5
1		camptothecin		$(1.0\pm0.06)\cdot10^{-3}$	$(5.5\pm0.01)\cdot10^{-3}$	$(5.6\pm2.04)\cdot10^{-4}$	-
2	**6a**	Ph	H	1.72±0.20	>50	>50	>50
3	**6b**	4-F-C_6H_4	H	2.69±0.31	24.09±2.02	26.50±4.52	>50
4	**6c**	4-CF_3-C_6H_4	H	0.50±0.06	>50	28.00±2.02	>50
5	**6d**	2-pyridyl	H	3.80±1.21	19.07±3.56	14.83±2.56	>50
6	**6e**	2-MeO-C_6H_4	H	0.21±0.03	>50	16.26±1.11	>50
7	**6f**	1-naphthyl	H	3.57±0.31	>50	>50	>50
8	**6g**	4-F-C_6H_4	4-F	0.25±0.03	17.79±5.45	23.88±4.05	>50
9	**6h**	Ph	4-Me	1.20±0.12	>50	28.0±2.26	>50
10	**7a**	Ph	H	1.21±0.68	14.46±2.27	13.77±1,47	>50
11	**7b**	4-F-C_6H_4	H	2.26±0.21	8.16±1.99	17.17±2.98	>50
12	**7c**	4-CF_3-C_6H_4	H	1.03±0.16	15.19±0.81	19.37±1,08	>50
13	**7d**	2-pyridyl	H	2.93±0.27	17.31±1.33	12.01±0.98	>50
14	**7e**	2-MeO-C_6H_4	H	3.11±0.65	13.06±1.80	24.13±1.76	>50
15	**7f**	1-naphthyl	H	6.32±1.09	>50	54.37±6.46	>50
16	**7g**	4-F-C_6H_4	4-F	3.82±0.42	6.77±0.85	12.46±0.87	>50
17	**7h**	Ph	4-Me	7.65±0.90	17.38±1.58	8.97±0.54	>50
18	**9a**	Ph	H	0.6±2.1	>50	>50	>50
19	**9b**	4-CF_3-C_6H_4	H	16.17±4.9	>50	>50	>50
20	**9c**	4-F-C_6H_4	4-F	0.08±0.01	>50	>50	>50
21	**9d**	Ph	4-Me	0.11±0.01	>50	>50	>50
22	**9e**	4-F-C_6H_4	4-Me	0.25±0.12	>50	>50	>50
23	**9f**	4-MeO-C_6H_4	4-Me	2.48±0.96	>50	>50	>50
24	**10a**	Ph	H	0.16±0.01	>50	>50	>50
25	**10b**	4-CF_3-C_6H_4	H	0.22±0.01	>50	>50	>50
26	**10c**	4-F-C_6H_4	H	0.27±0.05	>50	>50	>50
27	**10d**	3,4-F_2-C_6H_3	H	0.08±0.03	>50	>50	>50
28	**10e**	2-naphthyl	H	0.27±0.05	>50	>50	>50
29	**10f**	4-F-C_6H_4	4-F	0.03±0.02	>50	>50	>50
30	**12a**	Ph	H	>50	>50	>50	>50
31	**12b**	4-CF_3-C_6H_4	H	>50	>50	>50	>50
32	**12f**	4-F-C_6H_4	4-F	>50	>50	>50	>50

[a]The cytotoxicity IC_{50} values listed are the concentrations corresponding to 50% growth inhibition.

The tested compounds displayed a broad spectrum of antiproliferative activity against the cancer cell lines tested in culture. We first focused on those compounds containing a phosphine oxide group such as 1,2,3,4-tetrahydroquinolinylphosphine oxides **6** and the aromatized quinolinylphosphine oxide derivatives **7** for our studies.

Afterwards, the corresponding 1,2,3,4-tetrahydroquinolinylphosphanes **9** were tested. And finally the family of compounds containing a phosphine sulfide group such as 1,2,3,4-tetrahydroquinolinylphosphine sulfides **10** and the aromatized quinolinylphosphine sulfides **12** derivatives were studied.

According to the data presented in Table 2, in general all these phosphorated derivatives present a higher selective cytotoxicity in the human lung adenocarcinoma cell line (A549) than in the other two cell lines, human ovarian carcinoma line (SKOV03) and human embryonic kidney cell line (HEK293). Moreover, MRC-5 non-malignant lung fibroblasts were tested for studying selective toxicity [23] and in contrast, none of the synthesized phosphorated compounds exhibited any toxicity toward MRC-5 cells (Table 2).

Concerning the new compounds containing a phosphine oxide group against the A549 cell line *in vitro*, 1,2,3,4-tetrahydroquinolinylphosphine oxides **6** with IC_{50} values between 0.21±0.03 and 3.57±0.31 μM are more active than the corresponding quinolinylphosphine oxides **7** with IC_{50} values between 1.03±0.16 and 7.65±0.90 μM, the most effective compounds being the derivative **6e** (R = 2-CH_3O-C_6H_4, R^1 = H) with an IC_{50} value of 0.21±0.03 μM, the compound **6g** (R = 4-FC_6H_4, R^1 = 4-F) with an IC_{50} value of 0.25±0.03 μM and the derivative **6c** (R = 4-$CF_3C_6H_4$, R^1 = H) with an IC_{50} value of 0.50±0.06 μM; while the quinoline **7c** (R = 4-$CF_3C_6H_4$, R^1 = H) with an IC_{50} value of 1.03±0.66 μM is the most active between quinolinylphosphine oxides **7**. However, against the SKOV03 and HEK293 cell lines *in vitro*, quinolines **7** are more active than

the corresponding 1,2,3,4-tetrahydroquinolines **6,** with the highest activity in quinolinylphosphine oxide **7g** (R = 4-FC_6H_4, R^1 = 4-F) with an IC_{50} value of 6.77±0.85 μM in the case of SKOV03 and in the derivative **7h** (R = C_6H_5, R^1 = 4-CH_3) with an IC_{50} value of 8.97±0.54 μM for HEK293 cell line, respectively.

Furthermore, very high selectivity was observed in the antiproliferative activity of the new compounds containing a phosphine **9** or a phosphine sulfide group **10** and **12** against the A549 *versus* SKOV03 and HEK293 cell lines, and it can even be observed that the fluorinated derivatives presented the highest cytotoxic effects [11]. Thus, 1,2,3,4-tetrahydroquinolinylphosphanes **9** showed IC_{50} values between 0.08±0.01 and 16.17±4.39 μM; the 1,2,3,4-tetrahydroquinolinylphosphane derivative **9c** (R = 4-FC_6H_4, R^1 = 4-F) with an IC_{50} value of 0.08±0.01 μM and compound **9d** (R = C_6H_5, R^1 = 4-CH_3) with an IC_{50} value of 0.11±0.01 μM being the most effective.

Finally, excellent results were observed for 1,2,3,4-tetrahydroquinolinylphosphine sulfides **10** most effectively being the better ones against the A549 cell line *in vitro* with IC_{50} values between 0.03±0.02 and 0.27±0.05 μM. Once again, the fluorinated derivatives were the most cytotoxic, such as tetrahydroquinoline **10f** (R = 4-FC_6H_4, R^1 = 4-F) with an IC_{50} value of 0.03±0.04 μM and the tetrahydroquinoline **10d** (R = 3,4-F_2-C_6H_4, R^1 = H) with an IC_{50} value of 0.08±0.03 μM. However, no cytotoxic effect was observed for aromatic quinolinylphosphine sulfides **12**.

3. Conclusions

In summary, the preparation of novel 1,2,3,4-tetrahydroquinolines and quinolines containing phosphorus substituents such as phosphine oxide, phosphine and phosphine sulfide by using the Povarov reaction and the development of a new family of TopI inhibitors are reported. The

synthesis of 1,2,3,4-tetrahydroquinolinylphosphine oxides **6** involves either the Aza-Diels-Alder reaction of functionalized imines **4**, obtained from phosphine oxide aniline **1** with aldehydes **2**, and styrenes **3** or by means of the multicomponent reaction of phosphino oxide aniline **1**, aldehydes **2** and styrenes **3** in a regio and stereoselective fashion. Subsequent aromatization with DDQ afforded the corresponding quinolinylphosphine oxides **7**. The synthesis of 1,2,3,4-tetrahydroquinolinylphosphanes **9** involves the multicomponent reaction of phosphinoaniline **8**, aldehydes **2** and styrenes **3**. 1,2,3,4-Tetrahydroquinolinylphosphine sulfides **10** can be obtained from anilines containing the phosphine sulfide group **11**, aldehydes **2** and styrenes **3**. Subsequent aromatization of 1,2,3,4-tetrahydroquinolines **10** with DDQ afforded the corresponding quinolinylphosphine sulfides **12**.

This strategy contributes to the possibility of introducing structural diversity at different positions of the resulting scaffold depending on the starting amines and commercial aromatic aldehydes and olefins, and allows the preparation of quinolines with a phosphorated substituent in its structure that could be interesting in terms of the biological activity since this fragment is present in compounds with very interesting biological properties.

Some of the newly synthesized compounds exhibited inhibitory effects against TopI mediated relaxation comparable to those observed for the natural inhibitor, camptothecin (CPT). To the best of our knowledge, this is the first time that phosphino, phosphino sulfide and quinolinylphosphine oxide derivatives have been tested as TopI inhibitors and may be considered as new families of TopI inhibitors. The therapeutic efficacy of all the prepared derivatives was evaluated against three different human cancer cell lines A549, SKOV03 and HEK293. The best results were observed in A549 cell lines and very good cytotoxic effects were observed for the 1,2,3,4-tetrahydroquinolinylphosphane derivative **9c** with an IC_{50} value of 0.08±0.01 μM as well

as for 1,2,3,4-tetrahydroquinolinylphosphine sulfide **10f** with an IC_{50} value of 0.03±0.04 μM and the 1,2,3,4-tetrahydroquinolinylphosphine sulfide **10d** with an IC_{50} value of 0.08±0.03 μM. These preliminary studies suggested that some of the newly synthesized compounds exhibited a significant antiproliferative activity by inhibiting the growth of human tumor cell lines.

4. Experimental protocols

4.1 Chemistry

4.1.1. General experimental information

All reagents from commercial suppliers were used without further purification. All solvents were freshly distilled before use from appropriate drying agents. All other reagents were recrystallized or distilled when necessary. Reactions were performed under a dry nitrogen atmosphere. Analytical TLCs were performed with silica gel 60 F_{254} plates. Visualization was accomplished by UV light. Column chromatography was carried out using silica gel 60 (230-400 mesh ASTM) or neutral alumina (70-290 mesh ASTM). Melting points were determined with an Electrothermal IA9100 Digital Melting Point Apparatus without correction. NMR spectra were obtained on a Bruker Avance 400 MHz and a Varian VXR 300 MHz spectrometers and recorded at 25 ºC. Chemical shifts for ^{1}H NMR spectra are reported in ppm downfield from TMS, chemical shifts for ^{13}C NMR spectra are recorded in ppm relative to internal chloroform (δ = 77.2 ppm for ^{13}C), chemical shifts for ^{19}F NMR are reported in ppm downfield from fluorotrichloromethane ($CFCl_3$). Coupling constants (*J*) are reported in Hertz. The terms m, s, d, t, q refer to multiplet, singlet, doublet, triplet, quartet. ^{13}C NMR, and ^{19}F NMR were broadband decoupled from hydrogen nuclei. High resolution mass spectra (HRMS) was measured by EI method with a Agilent LC-Q-TOF-MS 6520 spectrometer. Compounds **9a**,**b**, **9d-f**, **10a**,**b** and **12a**,**b** were prepared as previously described [20].

4.1.2. Compounds Purity Analysis

All synthesized compounds were analyzed by HPLC to determine their purity. The analyses were performed on Agilent 1260 infinity HPLC system (C-18 column, Hypersil, BDS, 5 μm, 0.4 mm×25 mm) at room temperature. All the tested compounds were dissolved in dichloromethane, and 5μL of the sample was loaded onto the column. Ethanol and heptane were used as mobile phase, and the flow rate was set at 1.0 mL/min. The maximal absorbance at the range of 190−400 nm was used as the detection wavelength. The purity of all the tested compounds (compounds **6**, **7**, **9**, **10** and **12**) is >95%, which meets the purity requirement by the Journal.

4.1.3.1. ***(2-Aminophenyl)diphenylphosphine oxide*** **(1)**. To a solution of *(2-nitrophenyl)diphenylphosphine oxide* [24] (10 mmol, 3.233 g) in methanol (15 mL), one spoon of RANEY® Nickel (approx. 5 g) was added. The reaction was stirred for 2 h a 80 psi with a H_2 containing balloon attached. The reaction mixture was filtered off through a pad of celite and evaporated to dryness to obtain 2.903 g of (2-aminophenyl)diphenylphosphine oxide **1** (99%) as a yellow solid; mp 167-168 ºC (methanol). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.87 (ws, 2 H, NH_2), 6.58-6.80 (m, 3 H), 7.22-7.27 (m 1 H), 7.46-7.67 (m, 10 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ : 111.7 (d, $^{1}J_{cp}$ = 105.8 Hz, C), 116.5 (d, $^{3}J_{cp}$ = 12.6 Hz, HC), 116.8 (d, $^{3}J_{cp}$ = 8.6 Hz, HC), 128.5-133.4 (m, 12 HC and 2 C), 152.6 (d, $^{2}J_{cp}$ = 4.5 Hz, C) ppm. ^{31}P NMR (120 MHz, $CDCl_3$): δ: 36.7 ppm. HRMS (EI): calculated for $C_{18}H_{16}NOP$ $[M]^+$ 293.0970; found 293.0984.

4.1.3.2. **General procedure for the preparation of aldimines (4).** To a solution of (2-aminophenyl)diphenylphosphine oxide **1** (10 mmol, 2.933 g) in $CHCl_3$ (30 mL) was added the corresponding aldehyde (10 mmol). The mixture was stirred under nitrogen at the opportune temperature until consumption of starting materials was checked by ^{1}H NMR and/or ^{31}P NMR and/or ^{19}F NMR spectroscopy. The reaction product is unstable during distillation and/or chromatography and was used without purification for the following reactions.

4.1.3.2.1. ***2-(Benzylideneaminophenyl)diphenylphosphine oxide*** (**4a**). The general procedure was followed using benzaldehyde (10 mmol, 1.016 mL) and heated to reflux for 24 h. ^{1}H NMR crude reaction mixture (300 MHz, $CDCl_3$) δ: 6.93-6.97(m, 1H), 7.22-7.61 (m, 14 H), 7.69-7.97 (m, 5 H), 8.03 (s, 1 H); ^{31}P NMR crude reaction mixture (120 MHz, $CDCl_3$) δ: 29.0 ppm.

4.1.3.2.2. ***2-(4-Fluorobenzylideneaminophenyl)diphenylphosphine oxide*** (**4b**). The general procedure was followed using 4-fluorobenzaldehyde (10 mmol, 1.079 mL) and heated to reflux

for 12 h. ^{1}H NMR crude reaction mixture (300 MHz, $CDCl_3$) δ: 6.66-6.74 (m, 1 H), 6.87-7.87 (m, 17 H), 8.01 (s, 1 H) ^{31}P NMR crude reaction mixture (120 MHz, $CDCl_3$) δ: 28.6 ppm; ^{19}F NMR crude reaction mixture (282 MHz, $CDCl_3$): δ = - 107.8 ppm.

4.1.3.2.3. ***2-(4-Trifluoromethylbenzylideneaminophenyl)diphenylphosphine oxide*** (**4c**). The general procedure was followed using 4-trifluoromethylbenzaldehyde (10 mmol, 1.339 mL) and heated to reflux for 12 h. ^{1}H NMR crude reaction mixture (300 MHz, $CDCl_3$) δ: 7.06-7.08 (m, 1 H), 7.15-7.96 (m, 17 H), 8.02 (s, 1 H) ppm; ^{31}P NMR crude reaction mixture (120 MHz, $CDCl_3$) δ: 28.6 ppm; ^{19}F NMR crude reaction mixture (282 MHz, $CDCl_3$): δ = - 63.3 ppm.

4.1.3.2.4. ***Diphenyl-2-(pyridin-2-ylmethylen)aminophenylphosphine oxide*** (**4d**). The general procedure was followed using 2-pyridinecarboxaldehyde (10 mmol, 0.9512 mL) and heated to reflux for 24 h. ^{1}H NMR crude reaction mixture (300 MHz, $CDCl_3$) δ: 6.58-7.91 (m, 17 H), 8.29 (s, 1 H), , 8.57 (d, $^{3}J_{HH}$ = 5.0 Hz, 1 H) ppm; ^{31}P NMR crude reaction mixture (120 MHz, $CDCl_3$) δ: 28.6 ppm.

4.1.3.3. **Synthesis of 1,2,3,4-tetrahydroquinolinylphosphine oxides 6 and/or quinolinylphosphine oxides 7 by Povarov reaction.**

4.1.3.3.1. **General procedure A. Reaction of imines 4 with styrenes 3**. Styrene **3** (12 mmol) and 1 equivalent of $BF_3{\cdot}Et_2O$ (10 mmol, 1.230 mL) were added to a solution of the *in situ* prepared aldimine **4** (10 mmol) in $CHCl_3$ (25 mL). The mixture was stirred at the appropriate temperature until TLC, ^{31}P NMR and ^{1}H NMR spectroscopy indicated the disappearance of aldimine. The reaction mixture was washed with 2M aqueous solution of NaOH (50 mL) and water (50 mL), extracted with dichloromethane (2 x 25 mL), and dried over anhydrous $MgSO_4$. The removal of the solvent under vacuum afforded and oil that was purified by silica gel flash column chromatography using a gradient elution of 10-70% ethyl acetate in hexane to afford products **6** and/or **7**.

4.1.3.3.2. **General procedure B. Multicomponent reaction (MCR).** A mixture of (2-Aminophenyl)diphenylphosphanoxide **1** (10 mmol, 2.933 g), freshly distilled aldehyde **2** (10 mmol) styrene **3** (12 mmol) and 1 equivalent of $BF_3{\cdot}Et_2O$ (10 mmol, 1.230 mL) in $CHCl_3$ (25 mL) and in the presence of molecular sieves (4Å) in $CHCl_3$ (25 mL), was stirred and heated at

reflux until TLC, ^{31}P NMR and ^{1}H NMR analysis indicated the consumption of starting materials. The molecular sieves were removed by filtration and the resulting solution was diluted with methylene chloride (50 mL), washed with a solution of NaOH 2M (50 mL) and water (50 mL), extracted with methylene chloride (2 x 20 mL), and dried over anhydrous $MgSO_4$. Removal of solvent under vacuum led to an solid that was purified by flash column chromatography on silica gel using a gradient of elution of 5-70% ethyl acetate in hexane to afford products **6** and/or **7**.

4.1.3.3.4. ***(2,4-Diphenyl-1,2,3,4-tetrahydroquinolin-8-yl)diphenylphosphine oxide*** (**6a**). The general procedure A was followed using aldimine **4a** (10 mmol) prepared *in situ* and styrene **3a** (12 mmol, 1.374 mL) for 2.5 h at room temperature, affording 4.334 g (90%) of a white solid identified as **6a**, mp 132-133 ºC (ethyl acetate/hexane). When the general procedure B was followed using benzaldehyde **2a** (10 mmol, 1.016 mL) and styrene **3a** (12 mmol, 1.374 mL) for 1 h at room temperature, 3.251 g (67%) of **6a** were obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.06 (ddd, $^{3}J_{HH}$ = 12.7 Hz, $^{3}J_{HH}$ = 11.7 Hz, $^{2}J_{HH}$ = 12.7 Hz, 1 H, CH_2) 2.28-2.31 (m, 1 H, CH_2), 4.29 (dd, $^{4}J_{HH}$ = 4.2 Hz, $^{3}J_{HH}$ = 12.5 Hz, 1 H, CH), 4.77 (dd, $^{4}J_{HH}$ = 3.2 Hz, $^{3}J_{HH}$ = 11.7 Hz, 1 H, CH), 6.33 (ddd, $^{3}J_{HH}$ = 7.6 Hz, $^{3}J_{HH}$ = 7.5 Hz, $^{4}J_{HP}$ = 2.9 Hz, 1 H), 6.63 (d, $^{3}J_{HH}$ = 7.6 Hz, 1 H), 6.66 (d, $^{3}J_{HH}$ = 7.5 Hz, 1 H), 7.08-7.86 (m, NH and 20 H) ppm; ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$) δ: 40.9 (CH_2), 44.7 (HC), 56.7 (HC), 110.3 (d, $^{1}J_{CP}$ = 105.6 Hz, C), 114.6 (d, $^{3}J_{CP}$ = 13.8 Hz, HC), 125.9-133.3 (m, 22 HC y 3 C), 143.8 (C), 144.1 (C), 150.0 (d, $^{2}J_{CP}$ = 4.6 Hz, C) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ: 36.4 ppm. HRMS (EI) calculated for $C_{33}H_{28}NOP$ $[M]^+$ 485.1909; found 485.1914. Purity 95.94% (EtOH/Heptane = 10/90, Rt = 4.848 min).

4.1.3.3.5. ***(2-(4-Fluorophenyl)-4-phenyl-1,2,3,4-tetrahydroquinolin-8-yl)diphenylphosphine oxide*** (**6b**). The general procedure A was followed using aldimine **4b** (10 mmol) prepared *in situ* and styrene **3a** (12 mmol, 1.374 mL) for 42 h. The mixture was refluxed affording compounds **6b** (2.417 g, 48%) as a yellow oil (R_f = 0.52, 50:50 hexane/EtOAc) and **7b** (0.400 g, 8%) as a yellow solid mp 219-220 ºC (ethyl acetate/hexane). When the general procedure B was followed using 4-fluorobenzaldehyde (10 mmol, 1.079 mL) and styrene **3a** (12 mmol, 1.374 mL) for 24 h heated to reflux only compound **7b** (3.444 g, 69%) was obtained. Data for **6b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.85 (ddd, $^{3}J_{HH}$ = 12.8 Hz, $^{3}J_{HH}$ = 11.4 Hz, $^{2}J_{HH}$ = 12.7 Hz, 1 H, CH_2), 2.08-2.12

(m, 1 H, CH_2), 4.11 (dd, $^3J_{HH}$ = 4.1 Hz, $^3J_{HH}$ = 12.8 Hz, 1 H, CH), 4.58 (dd, $^3J_{HH}$ = 2.3 Hz, $^3J_{HH}$ = 11.4 Hz, 1 H, CH), 6.23-6.29 (m, 1 H), 6.53 (d, $^3J_{HH}$ = 7.6 Hz, 1 H), 6.57 (d, $^3J_{HH}$ = 7.5 Hz, 1 H), 6.70-6.76 (m, 2H), 6.85-6.90 (m, 2 H), 7.08 (s, 1 H, NH), 7.11-7.23 (m, 5 H), 7.32-7.69 (m, 10 H) ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ: 41.1 (CH_2), 44.7 (CH), 56.2 (CH), 111.2 (d, $^1J_{CP}$ = 105.5 Hz, C), 114.8 (d, $^3J_{CP}$ = 13.8 Hz, HC), 115.3 (d, $^3J_{CF}$ = 21.3 Hz, 2 HC), 126.1 (d, $^3J_{CP}$ = 8.0 Hz, C),127.0 (HC), 128.7 (d, $^2J_{CP}$ = 4.9 Hz, HC), 128.8-132.0 (m, 13 HC), 132.3 (d, $^1J_{CP}$ = 104.8 Hz, C), 132.6 (2 HC), 132.9 (HC), 133.1 (d, $^1J_{CP}$ = 104.8 Hz, C), 133.6 (HC), 139.6 (d, $^4J_{CF}$ = 2.5 Hz, C), 144.1 (C), 149.9 (d, $^2J_{CP}$ = 3.5 Hz, C), 162.5 (d, $^1J_{CF}$ = 244.6 Hz, C) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ: 36.4 ppm; ^{19}F NMR (282 MHz, $CDCl_3$) δ: -116.0 to -115.9 (m) ppm. HRMS (EI): calculated for $C_{33}H_{27}FNOP$ $[M]^+$ 503.1814; found $C_{33}H_{23}FNOP$ $[-4H]^+$ 499.1500. Purity 97.83% (EtOH/Heptane = 10/90, Rt = 6.304 min). See data for compound **7b** (*vide infra)*.

4.1.3.3.6. ***(4-Phenyl-2-(4-trifluoromethylphenyl)-1,2,3,4-tetrahydroquinolin-8-yl) diphenylphosphine oxide*** (**6c**). The general procedure A was followed using compound **4c** (10 mmol) prepared *in situ* and styrene **3a** (12 mmol, 1.374 mL) for 1 h at room temperature, affording 4.425 g (80%) of **6c** as a white solid mp 199-201 ºC (ethyl acetate/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.97 (ddd, $^3J_{HH}$ = 12.0 Hz, $^3J_{HH}$ = 12.4 Hz, $^2J_{HH}$ = 12.5 Hz, 1 H, CH_2), 2.22-2.27 (m, 1 H, CH_2), 4.23 (dd, $^3J_{HH}$ = 12.4 Hz, $^3J_{HH}$ = 4.0 Hz, 1 H, CH), 4.78 (dd, $^3J_{HH}$ = 12.0 Hz, $^3J_{HH}$ = 2.7 Hz, 1 H, CH), 6.36-6.42 (m, 1 H), 6.64 (d, $^3J_{HH}$ = 7.6 Hz, 1 H), 6.69 (d, $^3J_{HH}$ = 7.5 Hz, 1 H), 7.11-7.79 (m, NH and 19 H) ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ: 40.8 (CH_2), 44.7 (HC), 56.5 (HC), 111.4 (d, $^1J_{CP}$ = 105.2 Hz, C), 115.3 (d, $^3J_{CP}$ = 13.6 Hz, HC), 124.3 (q, $^1J_{CF}$ = 272.1 Hz, CF_3), 125.6 (d, $^2J_{CP}$ = 3.5 Hz, HC), 126.1 (d, $^3J_{CP}$ = 8.1 Hz, C), 126.5-128.8 (m, 13 HC), 129.6 (q, $^2J_{CF}$ = 32.3 Hz, C), 131.9-132.4 (m, 6 HC and C) 132.9 (d, $^1J_{CP}$ = 105.2 Hz, C), 133.1 (HC), 143.9 (C), 148.1 (C), 149.7 (d, $^2J_{CP}$ = 4.5 Hz, C) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ: 36.5 ppm; ^{19}F NMR (282 MHz, $CDCl_3$) δ: - 62.8 ppm. HRMS (EI): calculated for $C_{34}H_{27}F_3NOP$ $[M]^+$ 553.1782; found 553.1802. Purity 97.84% (EtOH/Heptane = 10/90, Rt = 3.709 min).

4.1.3.3.7. ***(4-Phenyl-2-(pyridin-2-yl)-1,2,3,4-tetrahydroquinolin-8-yl)diphenylphosphine oxide*** (**6d**). The general procedure A was followed using aldimine **4d** (10 mmol) prepared *in situ* and styrene **3a** (12 mmol, 1.374 mL) for 6.5 h at room temperature affording 3.309 g (68%) of **7d** as

a yellow solid, mp 110-112 ºC (ethyl acetate/hexane). When the general procedure B was followed using 2-pyridinecarboxaldehyde (10 mmol, 0.951 mL) and styrene **3a** (12 mmol, 1.374 mL) for 24 h heated to reflux compound **6d** (3.357 g, 69%) was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.97 (ddd, $^{3}J_{HH}$ = 12.5 Hz, $^{3}J_{HH}$ = 11.4 Hz, $^{2}J_{HH}$ = 12.5 Hz, 1 H, CH_2), 2.45-2.52 (m, 1 H, CH_2), 4.27 (dd, $^{3}J_{HH}$ = 12.5 Hz, $^{3}J_{HH}$ = 4.6 Hz, 1 H, CH), 4.84 (dd, $^{3}J_{HH}$ = 11.3 Hz, $^{3}J_{HH}$ = 3.3 Hz, 1 H, CH), 6.38 (ddd $^{3}J_{HH}$ = 7.6 Hz, $^{3}J_{HH}$ = 7.6 Hz, $^{4}J_{HP}$ = 3.0 Hz, 1 H), 6.63-6.74 (m, 2 H), 6.96 (d, $^{3}J_{HH}$ = 7.9 Hz, 1 H), 7.17-7.81 (m, NH and 18 H), 8.45 (d, $^{3}J_{HH}$ = 7.4 Hz, 1 H) ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ: 39.1 (CH), 44.6 (CH_2), 57.9 (CH), 111.0 (d, $^{1}J_{CP}$ = 105.6 Hz, C), 115.1 (d, $^{3}J_{CP}$ = 13.9 Hz, HC), 119.6 (HC), 122.2 (HC), 126.2 (d, $^{3}J_{CP}$ = 7.9 Hz, C), 126.8 (HC), 128.2-132.1 (m, 15HC and C), 133.3 (d, $^{1}J_{CP}$ = 104.8 Hz, C), 133.4 (2HC), 137.2 (HC), 144.3 (C), 148.9 (HC), 149.9 (d, $^{2}J_{CP}$ = 4.5 Hz, C), 162.7 (C) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ: 37.0 ppm. HRMS (EI): calculated for $C_{32}H_{27}N_2OP$ $[M]^+$ 486.1861; found 486.1869. Purity 99.48% (EtOH/Heptane = 10/90, Rt = 6.045 min).

4.1.3.3.8. ***(2-(2-Methoxyphenyl)-4-phenyl-1,2,3,4-tetrahydroquinolin-8-yl)diphenylphosphine oxide*** (**6e**). The general procedure B was followed using o-anisaldehyde (10 mmol, 1.208 mL) and styrene **3a** (12 mmol, 1.374 mL) for 36 h heated to reflux affording compound **6e** (4.692 g, 91%) as a white solid, mp 233-235 ºC (ethyl acetate/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.75 (ddd, $^{3}J_{HH}$ = 12.5 Hz, $^{3}J_{HH}$ = 11.1 Hz, $^{2}J_{HH}$ = 12.5 Hz, 1 H, CH_2), 2.28-2.33 (m, 1 H, CH_2), 3.68 (s, 3 H, OCH_3), 4.15 (dd, $^{4}J_{HH}$ = 4.2 Hz, $^{3}J_{HH}$ = 12.5 Hz, 1 H, CH), 5.03 (dd, $^{4}J_{HH}$ = 3.1 Hz, $^{3}J_{HH}$ = 11.1 Hz, 1 H, CH), 6.25 (ddd $^{3}J_{HH}$ = 7.6 Hz, $^{3}J_{HH}$ = 7.4 Hz, $^{4}J_{HP}$ = 2.9 Hz, 1 H), 6.51-6.75 (m, 5 H), 6.89 (s, NH) 7.03-7.73 (m, 16 H) ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ: 38.0 (CH_2), 44.8 (HC), 50.0 (OCH_3), 55.4 (HC), 110.2 (HC) 110.9 (d, $^{1}J_{CP}$ = 105.6 Hz, C), 114.4 (d, $^{3}J_{CP}$ = 13.8 Hz, HC), 121.0 (HC), 125.8-133.8 (m, 19 HC and 4 C), 144.5 (C), 150.5 (d, $^{2}J_{CP}$ = 4.6 Hz, C), 156.5 (C) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ: 36.3 ppm. HRMS (EI): calculated for $C_{34}H_{30}NO_2P$ $[M]^+$ 515.2014; found 515.2022. Purity 98.19% (EtOH/Heptane = 10/90, Rt = 4.636 min).

4.1.3.3.9. ***(2-(Naphthalen-1-yl)-4-phenyl-1,2,3,4-tetrahydroquinolin-8-yl)diphenylphosphine oxide*** (**6f**). The general procedure B was followed using 1-naphthaldehyde (10 mmol, 1.358 mL) and and styrene **3a** (12 mmol, 1.374 mL) heated to reflux for 48 h affording compound **6f** (3.482 g, 65%) as a white solid, mp 267-269 ºC (ethyl acetate/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ:

2.11 (ddd, $^{3}J_{HH}$ = 12.7 Hz, $^{3}J_{HH}$ = 11.3 Hz, $^{2}J_{HH}$ = 12.5 Hz, 1 H, CH_2), 2.48-2.55 (m, 1 H, CH_2), 4.39 (dd, $^{3}J_{HH}$ = 12.7 Hz, $^{3}J_{HH}$ = 4.3 Hz, 1 H, CH), 5.56 (dd, $^{3}J_{HH}$ = 11.3 Hz, $^{3}J_{HH}$ =2.7 Hz, 1 H, CH), 6.40 (ddd $^{3}J_{HH}$ = 7.6 Hz, $^{3}J_{HH}$ = 7.5 Hz, $^{4}J_{HP}$ = 3.0 Hz, 1 H), 6.66-6.75 (m, 2 H), 7.16-7.99 (m, NH and 22 H) ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ: 39.3 (CH_2), 45.1 (HC), 52.9 (HC), 111.4 (d, $^{1}J_{CP}$ = 105.5 Hz,), 114.8 (d, $^{3}J_{CP}$ = 13.8 Hz, HC), 120,5 (C), 122.5 (HC), 122.8 (HC), 125.4 (HC), 125,9 (HC), 126.1 (HC), 126.3 (d, $^{3}J_{CP}$ = 7.8 Hz, C), 126.9 (HC), 127,8 (HC), 128.5-129.0 (m, 6 HC), 130.7 (C), 131.8-133.9 (m, 11 HC and 2 C $_{arom}$), 139.3 (C), 144.3 (C), 150.3 (d, $^{2}J_{CP}$ = 4.6 Hz, C) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ: 36.3 ppm. HRMS (EI): calculated for $C_{37}H_{30}NOP$ $[M]^+$ 535.2065; found 535.2076. Purity 98.32% (EtOH/Heptane = 10/90, Rt = 4.360 min).

4.1.3.3.10. ***(2,4-Bis(4-fluorophenyl)-1,2,3,4-tetrahydroquinolin-8-yl)diphenylphosphine oxide*** **(6g)**. The general procedure B was followed using 4-fluorobenzaldehyde (10 mmol, 1.079 mL) and 4-fluorostyrene **3b** (12 mmol, 1.437 mL), heated to reflux for 24 h affording compound **6g** (2.295 g, 44%) as a whit solid, mp 183-185 ºC (ethyl acetate/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.90 (ddd, $^{3}J_{HH}$ = 12.4 Hz, $^{3}J_{HH}$ = 11.3 Hz, $^{2}J_{HH}$ = 12.7 Hz, 1 H, CH_2), 2.14-2.20 (m, 1 H, CH_2), 4.22 (dd, $^{3}J_{HH}$ = 12.4 Hz, $^{3}J_{HH}$ = 4.3 Hz, 1 H, CH), 4.70 (dd, $^{3}J_{HH}$ = 11.3 Hz, $^{3}J_{HH}$ =3.2 Hz, 1 H, CH), 6.37 (ddd $^{3}J_{HH}$ = 7.6 Hz, $^{3}J_{HH}$ = 7.4 Hz, $^{4}J_{HP}$ = 3.0 Hz, 1 H), 6.62-7.19 (m, NH and 11 H), 7.44-7.78 (m, 9 H) ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ: 41.3 (CH_2), 44.0 (HC) 56.2 (HC), 111.3 (d, $^{1}J_{CP}$ = 105.5 Hz, C), 114.9 (d, $^{3}J_{CP}$ = 13.8 Hz, HC), 115.4 (d, $^{3}J_{CF}$ = 21.3 Hz, 2 HC), 115.6 (d, $^{3}J_{CF}$ = 21.3 Hz, 2 HC), 125.8 (d, $^{3}J_{CP}$ = 8.0 Hz, C), 127.6-133.6 (m, 16 HC and 2 C $_{arom}$), 139.6 (d, $^{4}J_{CF}$ = 3.1 Hz, C), 139.8 (d, $^{4}J_{CF}$ = 3.2 Hz, C), 149.9 (d, $^{2}J_{CP}$ = 4.6 Hz, C), 161.8 (d, $^{1}J_{CF}$ = 242.1 Hz, FC), 162.1 (d, $^{1}J_{CF}$ = 245.0 Hz, FC) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ: 36.3 ppm; ^{19}F NMR (282 MHz, $CDCl_3$) δ: - 116.6 to -116.4 (m), -116.0 to -115.9 (m) ppm. HRMS (EI): calculated for $C_{33}H_{26}F_2NOP$ $[M]^+$ 521.172; found 521.1727. Purity 98.95% (EtOH/Heptane = 10/90, Rt = 4.119min).

4.1.3.3.11. ***(2-Phenyl-4-(p-tolyl)-1,2,3,4-tetrahydroquinolin-8-yl)diphenylphosphine oxide*** (**6h**). The general procedure B was followed using benzaldehyde (10 mmol, 1.016 mL) and 1- methyl-4-vinylbenzene **3b** (12 mmol, 1.575 mL), heated to reflux for 48 h affording compound **6h** (3.045 g, 61%) as a light orange solid, mp 223-225 ºC (ethyl acetate/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.90-2.25 (m, 2 H, CH_2), 2.33 (s, 3 H CH_3), 4.19 (dd, $^{3}J_{HH}$ = 12.4 Hz, $^{3}J_{HH}$ = 4.0

Hz, 1 H, CH), 4.71 (dd, $^{3}J_{HH}$ = 11.4 Hz, $^{3}J_{HH}$ =3.4 Hz, 1 H, CH), 6.35 (ddd $^{3}J_{HH}$ = 7.8 Hz, $^{3}J_{HH}$ = 7.4 Hz, $^{4}J_{HP}$ = 2.8 Hz, 1 H), 6.57-6.68 (m, 2 H), 6.99-7.78 (m, NH and 19 H) ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ: 21.2 (CH_3), 41.0 (CH_2), 44.4 (HC) 57.0 (HC), 111.0 (d, $^{1}J_{CP}$ = 108.0 Hz, C), 114.8 (d, $^{3}J_{CP}$ = 11.7 Hz, HC), 126.1-133.6 (m, 21 HC and 3 C $_{arom}$), 136.4 (C), 141.2 (C), 143.9 (C), 150.0 (d, $^{2}J_{CP}$ = 3.6 Hz, C) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ: 36.4 ppm. HRMS (EI): calculated for $C_{34}H_{30}NOP$ $[M]^+$ 499.2065; found 499.2077. Purity 97.16% (EtOH/Heptane = 10/90, Rt = 4.326 min).

4.1.3.4. **General Procedure for the Preparation of quinolinylphosphine oxides 7 by Dehydrogenation with DDQ**: To a solution of the corresponding tetrahydroquinoline **6** (1 mmol) in $CHCl_3$ (7 mL) was added DDQ (0.545 g, 2 mmol), and the mixture was heated to reflux for 2 h. The removal of the solvent under vacuum afforded and oil, diethyl ether was added and the resulting solid was removed by filtration. The filtrate was removed under vacuum and the crude solid was purified by recrystallization on ether.

4.1.3.4.1. ***(2,4-Diphenylquinolin-8-yl)diphenylphosphine oxide*** (**7a**). The general procedure was employed with **6a** (0.485 g, 1 mmol) to afford **7a** (0.419 g, 87% yield) as a white solid; m.p. 178-180 °C (ethyl acetate/hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.16-7.57 (m, 17 H), 7.77 (s, 1 H), 7.91 (dd, $^{3}J_{HP}$ = 12.3 Hz, $^{3}J_{HH}$ = 7.8 Hz, 4 H), 8.09 (d, $^{3}J_{HH}$ = 8.4 Hz, 1 H), 8.61 (dd, $^{3}J_{HP}$ = 13.7 Hz, $^{3}J_{HH}$ = 6.9 Hz, 1 H) ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ: 119.2 (HC), 125.9 (C) 126.0 (d, $^{3}J_{CP}$ = 13.1 Hz, HC), 127.7-132.3 (m, 1 C and 21 HC), 134.0 (d, $^{1}J_{CP}$ = 108.8 Hz, 2 C), 137.9 (d, $^{3}J_{CP}$ = 7.0 Hz, HC), 138.0 (C), 138.5 (C), 148.1 (d, $^{2}J_{CP}$ = 5.6 Hz C), 149.6 (C), 155.8 (C) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ: 29.4 ppm. HRMS (EI): calculated for $C_{33}H_{24}NOP$ $[M]^+$ 481.1596; found 481.1589. Purity 95.74% (EtOH/Heptane = 10/90, Rt = 6.609 min).

4.1.3.4.2. ***(2-(4-Fluorophenyl)-4-phenylquinolin-8-yl)diphenylphosphine oxide*** (**7b**). The general procedure was employed with **6b** (0.504 g, 1 mmol) to afford **7b** (0.375 g, 75% yield) as a white solid; m.p. 219-220 °C (ethyl acetate/hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.84-6.88 (m, 2 H), 7.27-7.54 (m, 14 H), 7.65 (s, 1 H), 7.80-7.85 (m, 4 H), 8.02 (d, $^{3}J_{HH}$ = 8.4 Hz, 1 H), 8.46 (ddd, $^{3}J_{HP}$ = 13.8 Hz, $^{3}J_{HH}$ = 7.2 Hz, $^{4}J_{HH}$ = 2.0 Hz, 1 H) ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ: 115.4 (d, $^{2}J_{CF}$ = 22.1 Hz, 2 HC), 118.9 (HC), 125.8 (d, $^{3}J_{CP}$ = 7.6 Hz, C), 125.9 (d, $^{3}J_{CP}$ = 13.1 Hz, HC), 128.2-132.3 (m, 1 C and 18 HC), 134.1 (d, $^{1}J_{CP}$ = 108.2 Hz, 2 C), 134.7 (d, $^{4}J_{CF}$

= 2.6 Hz, C), 137.9 (C), 137.9 (d, $^{3}J_{CP}$ = 7.2 Hz, HC), 148.2 (d, $^{2}J_{CP}$ = 5.7 Hz, C), 149.8 (C), 154.8 (C), 163.8 (d, $^{1}J_{CF}$ = 249.9 Hz, C-F) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ:29.0 ppm; ^{19}F NMR (282 MHz, $CDCl_3$) δ: -112.17 to -112.05 (m) ppm. HRMS (EI): calculated for $C_{33}H_{23}FNOP$ $[M]^+$ 499.1501; found 499.1511. Purity 96.54% (EtOH/Heptane = 10/90, Rt = 6.374 min).

4.1.3.4.3. ***(4-Phenyl-2-(4-(trifluoromethyl)phenyl)quinolin-8-yl)diphenylphosphine oxide*** (**7c**). The general procedure was employed with **6c** (0.554 g, 1 mmol) to afford **7c** (0.439 g, 80% yield) as a yellow solid; m.p. 233-235 °C (ethyl acetate/hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.28-7.45 (m, 16 H), 7.72 (s, 1 H), 7.82 (dd, $^{3}J_{HP}$ = 12.2 Hz, $^{3}J_{HH}$ = 7.7 Hz, 4 H), 8.05 (d, $^{3}J_{HH}$ = 8.2 Hz, 1 H), 8.47 (dd, $^{3}J_{HP}$ = 13.8 Hz, $^{3}J_{HH}$ = 7.0 Hz, 1 H) ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ: 119.3 (HC), 124.2 (q, $^{1}J_{CF}$ = 272.4 Hz, CF_3), 125.6 (d, $^{4}J_{CP}$ = 3.6 Hz, HC), 126.3-132.6 (m, 20 HC and 3 C), 134.0 (d, $^{1}J_{CP}$ = 108.4 Hz, 2 C), 137.8 (C), 138.1 (d, $^{3}J_{CP}$ = 7.3 Hz, HC), 141.9 (C), 148.2 (d, $^{2}J_{CP}$ = 5.2 Hz, C), 150.2 (C), 154.3 (C) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ:28.0 ppm; ^{19}F NMR (282 MHz, $CDCl_3$) δ: - 62.9 ppm. HRMS (EI): calculated for $C_{34}H_{23}F_3NOP$ $[M]^+$ 549.1469; found 549.1471. Purity 96.62% (EtOH/Heptane = 10/90, Rt = 5.792 min).

4.1.3.4.4. ***(4-Phenyl-2-(pyridin-2-yl)quinolin-8-yl)diphenylphosphine oxide*** (**7d**). The general procedure was employed with **6d** (0.487 g, 1 mmol) to afford **7d** (0.401 g, 83% yield) as a yellow solid; m.p. 114–115 °C (ethyl acetate/hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.13-7.18 (m, 2 H), 7.28-7.55 (m, 14 H), 7.80-7.86 (m, 3 H), 8.09 (d, $^{3}J_{HH}$ = 8.4 Hz, 1 H), 8.34 (dd, $^{3}J_{HH}$ = 7.0 Hz, $^{3}J_{HH}$ = 7.0 Hz, 1 H), 8.43 (s, 1 H), 8.51 (d, $^{3}J_{HH}$ = 4.0 Hz, 1 H) ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ:119.5 (HC), 122.5 (HC), 124.3 (HC), 126.6 (d, $^{3}J_{CP}$ = 12.6 Hz, HC), 127.2 (d, $^{3}J_{CP}$ = 7.0 Hz, C), 128.4-132.1 (m, C and 16 HC), 134.1 (d, $^{1}J_{CP}$ = 108.3 Hz, 2C), 136.8 (HC), 137.7 (d, $^{3}J_{CP}$ = 8.6 Hz, HC), 138.0 (C), 148.1 (d, $^{2}J_{CP}$ = 5.5 Hz, C), 148.9 (HC), 149.9 (C), 154.9 (C), 155.5 (C) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ:29.1 ppm. HRMS (EI): calculated for $C_{32}H_{23}N_2OP$ $[M]^+$ 482.1548; found 482.1549. Purity 96.79% (EtOH/Heptane = 10/90, Rt = 8.604 min).

4.1.3.4.5. ***(2-(2-Methoxyphenyl)-4-phenylquinolin-8-yl)diphenylphosphine oxide*** (**7e**). The general procedure was employed with **6e** (0.516 g, 1 mmol) to afford **7e** (0.379 g, 74% yield) as

a white solid; m.p. 267-269 °C (ethyl acetate/hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.80 (s, 3 H, OCH_3), 6.52 (dd, *J* = 1.6 Hz, *J* = 7.6 Hz, 1 H), 6.69-6.75 (m, 1 H), 6.94 (d, $^{3}J_{HH}$ = 8.7 Hz, 1 H), 7.20-7.66 (m, 13 H), 7.89 (dd, $^{3}J_{HP}$ = 12.5 Hz, $^{3}J_{HH}$ = 7.0 Hz, 4 H), 7.96 (s, 1 H), 8.12 (d, $^{3}J_{HH}$ = 9.0 Hz, 1 H), 8.67 (ddd, $^{3}J_{HP}$ = 13.9 Hz, $^{3}J_{HH}$ = 7.1 Hz, $^{4}J_{HH}$ = 1.6 Hz, 1 H), ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ: 55.7 (OCH_3), 111.3 (HC), 120.9 (HC), 121.0-138.5 (m, 6 C, 21 HC), 147.7 (d, $^{2}J_{CP}$ = 5.6 Hz C), 148.1 (C), 155.5 (C), 157.5 (C) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ: 29.4 ppm;^{31}P NMR (120 MHz, $CDCl_3$) δ: 29.3 ppm. HRMS (EI): calculated for $C_{34}H_{26}NO_2P$ $[M]^+$ 511.1701; found 511.1719. Purity 96.29% (EtOH/Heptane = 10/90, Rt = 6.181 min).

4.1.3.4.6. ***(2-(Naphthalen-1-yl)-4-phenylquinolin-8-yl)diphenylphosphine oxide*** (**7f**). The general procedure was employed with **6f** (0.536 g, 1 mmol) to afford **7f** (0.383 g, 72% yield) as a white solid; m.p. 263-265 °C (ethyl acetate/hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.85-6.88 (m, 1H), 7.18-7.91 (m, 23 H), 8.23 (d, $^{3}J_{HH}$ = 8.7 Hz, 1 H), 8.80 (ddd, $^{3}J_{HP}$ = 13.7 Hz, $^{3}J_{HH}$ = 7.2 Hz, $^{4}J_{HH}$ = 1.5 Hz, 1 H), ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ: 123.9 (HC), 125.4 (HC), 125.7-133.2 (m, 4 C, 23 HC), 133.8 (C), 134.3 (d, $^{1}J_{CP}$ = 108.7 Hz, 2 C), 137.8 (d, $^{3}J_{CP}$ = 9.8 Hz, C), 138.0 (d, $^{3}J_{CP}$ = 6.7 Hz, HC), 148.3 (d, $^{2}J_{CP}$ = 6.2 Hz C), 149.0 (C), 158.1 (C) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ: 29.7 ppm. HRMS (EI): calculated for $C_{37}H_{26}NOP$ $[M]^+$ 531.1752; found 531.1763. Purity 99.34% (EtOH/Heptane = 10/90, Rt = 6.313 min).

4.1.3.4.7. ***(2,4-Bis(4-fluorophenyl)quinolin-8-yl)diphenylphosphine oxide*** (**7g**). The general procedure was employed with **6g** (0.522 g, 1 mmol) to afford **7g** (0.497 g, 96% yield) as a white solid; m.p. 258-260 °C (ethyl acetate/hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.93-6.99 (m, 2 H), 7.23-7.66 (m, 13 H), 7.72 (s, 1 H), 7.88-7.94 (m, 4 H), 8.07 (d, $^{3}J_{HH}$ = 8.4 Hz, 1 H), 8.55 (ddd, $^{3}J_{HP}$ = 13.9 Hz, $^{3}J_{HH}$ = 7.0 Hz, $^{4}J_{HH}$ = 1.3 Hz, 1 H) ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ:115.6 (d, $^{2}J_{CF}$ = 22.0 Hz, 2HC), 116.0 (d, $^{2}J_{CF}$ = 21.6 Hz, 2HC), 119.1(HC), 125.9 (d, $^{3}J_{CP}$ = 7.2 Hz, C), 126.2 (d, $^{3}J_{CP}$ = 12.9 Hz, HC), 128.2-132.3 (m, 15 HC), 134.0 (d, $^{4}J_{CF}$ = 3.2 Hz, C), 134.1 (d, $^{1}J_{CP}$ = 108.2 Hz, 2 C), 134.7 (d, $^{4}J_{CF}$ = 3.0 Hz, C), 138.1 (d, $^{3}J_{CP}$ = 7.2 Hz, HC), 148.2 (d, $^{2}J_{CP}$ = 5.6 Hz, C), 148.8 (2C), 154.8 (C), 163.7 (d, $^{1}J_{CF}$ = 248.9 Hz, C-F), 164.0 (d, $^{1}J_{CF}$ = 250.0 Hz, C-F) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ: 28.8 ppm. HRMS (EI): calculated for $C_{33}H_{22}F_2NOP$ $[M]^+$ 517.1407; found 517.1417. Purity 97.22% (EtOH/Heptane = 10/90, Rt = 6.489 min).

4.1.3.4.8. ***(2-Phenyl-4-(p-tolyl)quinolin-8-yl)diphenylphosphine oxide*** (**7h**). The general procedure was employed with **6h** (0.522 g, 1 mmol) to afford **7h** (0.497 g, 96% yield) as a white solid; m.p. 148-150 °C (ethyl acetate/hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.49 (s, 3 H, CH_3), 7.27-7.65 (m, 16 H), 7.78 (s, 1 H), 7.93 (dd, $^{3}J_{HP}$ = 12.3 Hz, $^{3}J_{HH}$ = 7.3 Hz, 4 H), 8.15 (d, $^{3}J_{HH}$ = 8.6 Hz, 1 H), 8.64 (dd, $^{3}J_{HP}$ = 13.7 Hz, $^{3}J_{HH}$ = 6.9 Hz, 1 H) ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ: 21.5 (CH_3), 119.3 (HC), 126.0 (d, $^{3}J_{CP}$ = 13.2 Hz, HC), 126.2 (d, $^{3}J_{CP}$ = 7.3 Hz, C), 127.8-138.8 (m, 6 C, 21 HC), 148.2 (d, $^{2}J_{CP}$ = 6 Hz, C), 149.7 (C), 155.8 (C) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ: 29.0 ppm. HRMS (EI): calculated for $C_{34}H_{26}NOP$ $[M]^+$ 495.1752; found 495.1760. Purity 96.24% (EtOH/Heptane = 10/90, Rt = 7.724 min).

4.1.3.5. **Preparation by Povarov reaction of *(2,4-Bis-(4-fluorophenyl)-1,2,3,4-tetrahydroquinolin-8-yl)diphenylphosphane*** (**9c**). A mixture of 2-(diphenylphosphino)aniline **8** (10 mmol, 2.773g) [18], freshly distilled, 4-fluorobenzaldehyde (10 mmol, 1.072 mL), 4-fluorostyrene (20 mmol, 2.385 mL) and three equivalents of $BF_3 \cdot Et_2O$ (30 mmol, 3.691 mL) in $CHCl_3$ (25 mL) in the presence of molecular sieves (4Å) was stirred and heated to reflux for 48 h. The molecular sieves were removed by filtration and the resulting solution was diluted with methylene chloride (50 ml), washed with a solution of NaOH 2M (50ml) and with water (50ml), extracted with methylene chloride (2x20ml), and dried ($MgSO_4$). Removal of solvent under vacuum led to an oil that was purified by flash column chromatography on silica gel using a gradient elution of 10 to 40% ethyl acetate in hexane to afford 3.586 g (71%) of **9c** as a white solid, mp 118.3-118.7 ºC (ethyl acetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.87 (ddd, $^{3}J_{HH}$ = 12.4 Hz, $^{2}J_{HH}$ = 12.4 Hz, $^{3}J_{HH}$ = 12.4 Hz, 1H, CH_2), 2.00-2.10 (m, 1H, CH_2), 3.69 (dd, $^{3}J_{HH}$ = 3.2 Hz, $^{3}J_{HH}$ = 12.4 Hz, 1H, HC), 3.97-4.03 (m, 1H, NH) 4.20 (dd, $^{3}J_{HH}$ = 4.2 Hz , $^{3}J_{HH}$ = 12.6 Hz ,1H, HC), 6.48-7.09. (m, 13 H, H), 7.41-7.84 (m, 8 H, H) ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ: 44.4 (CH), 60.8(CH_2) 61.5 (HC), 115.0-115.8 (m, 4 HC), 118.7 (d, $^{3}J_{CP}$ = 12.0 Hz, HC), 120.7 (d, $^{1}J_{CP}$ = 98 Hz, C), 128,4-138.8 (m, 16 HC and 5C $_{arom}$), 153.6 (d, $^{2}J_{CP}$ = 3.2 Hz, C), 162.2 (d, $^{1}J_{CF}$ = 244.9 Hz, FC), 162.7 (d, $^{1}J_{CF}$ = 245.2 Hz, FC) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ: 30.6 ppm; ^{19}F NMR (282 MHz, $CDCl_3$) δ: - 116.1 to -116.0 (m), -116.0 to -115.9 (m) ppm. HRMS (EI): calculated for $C_{33}H_{26}F_2NP$ [M]+ 505.1771; found 505.1743. Purity 98.64% (EtOH/Heptane = 10/90, Rt = 3.69 min).

4.1.3.6. **General procedure (Povarov reaction) for the preparation of (1,2,3,4-tetrahydroquinolin-8-yl)diphenylphosphine sulfides 10**.

A mixture of (2-aminophenyl)diphenylphosphine sulfide **11** (10 mmol, 3.094 g) [18], freshly distilled aldehyde **2** (10 mmol), styrene **3** (20 mmol) and three equivalents of $BF_3 \cdot Et_2O$ (30 mmol, 3.691 ml) in $CHCl_3$ (25 mL), in the presence of molecular sieves (4Å) was stirred and heated to reflux until TLC and ^1H and ^{31}P NMR analysis indicated the consumption of starting materials. The molecular sieves were removed by filtration and the resulting solution was diluted with methylene chloride (50 mL), washed with a solution of NaOH 2M (50 mL) and with water (50 mL), extracted with methylene chloride (2 x 20 mL), and dried ($MgSO_4$). Removal of solvent under vacuum led to an oil that was purified by flash column chromatography on silica gel using a gradient elution of 10 to 40% ethyl acetate in hexane to afford compounds **10**.

4.1.3.6.1. ***(2-(4-Fluorophenyl)-4-phenyl-1,2,3,4-tetrahydroquinolin-8-yl)diphenylphosphine sulfide*** (**10c**). The general procedure was followed using 4-fluorobenzaldehyde (10 mmol, 1.072 mL) and styrene (20 mmol, 2.292 mL) for 48 h affording compound **10c** (4.516 g, 87%) as a white solid, mp 173-175 ºC (ethyl acetate/hexane). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.9 (ddd, $^3J_{HH}$ = 12.4 Hz, $^3J_{HH}$ = 11.5 Hz, $^2J_{HH}$ = 11.5 Hz, 1 H, CH_2), 2.16-2.21 (m, 1 H, CH_2), 4.25 (dd, $^3J_{HH}$ = 12.4 Hz, $^3J_{HH}$ = 5.7, 1 H, HC), 4.70 (d, $^3J_{HH}$ = 11.5 Hz, 1 H, HC), 6.38 (ddd, $^3J_{HH}$ = 7.9 Hz, $^3J_{HH}$ = 7.8 Hz, $^4J_{HP}$ = 2.9 Hz, 1 H), 6.38 (ddd, $^3J_{HH}$ = 7.9 Hz, $^3J_{HH}$ = 7.8 Hz, $^4J_{HP}$ = 2.9 Hz, 1 H), 6.62-7.19 (m, NH and 12 H), 7.44-7.78 (m, 10 H) ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ: 41.4 (CH_2), 44.8 (CH), 56.2 (CH), 111.8 (d, $^1J_{CP}$ = 88.1 Hz, C), 115.3 (d, $^3J_{CF}$ = 21.3 Hz, 2HC), 115.6 (d, $^3J_{CP}$ = 13.5 Hz, HC), 123.9-133.2 (m, 19 HC and 3C), 139.4 (d, $^4J_{CF}$ = 2.9 Hz, C), 144.2 (C), 148.1 (d, $^2J_{CP}$ = 5.8 Hz, C), 161.9 (d, $^1J_{CF}$ = 245.0 Hz, C) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ: 40.2 ppm; ^{19}F NMR (282 MHz, $CDCl_3$) δ: -116.0 ppm. HRMS (EI): calculated for $C_{33}H_{27}FNPS$ [M]+ 519.1586; found 519.1583. Purity 99.62% (EtOH/Heptane = 10/90, Rt = 3.24 min).

4.1.3.6.2. ***2-(3,4-Difluorophenyl)-4-phenyl-1,2,3,4-tetrahydroquinolin-8-yl)diphenylphosphine sulfide*** (**10d**). The general procedure was followed using 3,4-difluorobenzaldehyde (10 mmol, 1.072 mL) and styrene (20 mmol, 2.292 mL) for 48 h affording compound **10d** (3.491 g, 65%) as

a white solid, mp 156-158 ºC (ethyl acetate/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.75 (ddd, $^{2}J_{HH}$ = 12.6 Hz, $^{3}J_{HH}$ = 12.3 Hz, $^{3}J_{HH}$ = 11.4 Hz, 1 H, CH_2), 2.02-2.08 (m, 1 H, CH_2), 4.11 (dd, $^{3}J_{HH}$ = 12.3 Hz, $^{3}J_{HH}$ = 4.5 Hz, 1 H, HC), 4.53 (dd, $^{3}J_{HH}$ = 11.3 Hz , $^{3}J_{HH}$ = 2.8 Hz ,1 H, HC), 6.27-7.84. (m, 22 H, NH and H) ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ:41.2 (CH_2), 44.6 (CH), 55.9 (CH), 112.3 (d, $^{1}J_{CP}$ = 87.8 Hz, C), 114.7 (d, $^{2}J_{CF}$ = 17.9 Hz, HC), 115.9 (d, $^{3}J_{CP}$ = 13.4 Hz, HC), 117.0 (d, $^{2}J_{CF}$ = 17.0 Hz, HC), 121.9 (dd, $^{3}J_{CF}$ = 6.5 Hz, $^{2}J_{CF}$ = 3.5 Hz, HC),125.3-133.2 (m, 17 HC and 2C), 140.8 (dd, $^{3}J_{CF}$ = 5.9 Hz, $^{4}J_{CF}$ = 3.6 Hz, C), 143.9 (C), 145.9 (C), 147.8 (d, $^{2}J_{CP}$ = 5.7 Hz, C), 149.6 (dd, $^{1}J_{CF}$ = 247.0 Hz, $^{2}J_{CF}$ = 12.8 Hz, C) 150.2 (dd, $^{1}J_{CF}$ = 248.0 Hz, $^{2}J_{CF}$ = 12.7 Hz, C)ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ:40.21 ppm; ^{19}F NMR (282 MHz, $CDCl_3$) δ: -140.5 to -140.3 (m), -137.8 to -137.6 (m) ppm. HRMS (EI): calculated for $C_{33}H_{26}F_2NPS$ [M]+ 537.1492; found 537.1495. Purity 98.39% (EtOH/Heptane = 10/90, Rt = 3.06 min).

4.1.3.6.3. ***(2-(Naphthalen-2-yl)-4-phenyl-1,2,3,4-tetrahydroquinolin-8-yl)diphenylphosphine sulfide***. (**10e**). The general procedure was followed using 2-naphtaldehyde (10 mmol, 1.072 mL) and styrene (20 mmol, 2.292 mL) for 48 h affording compound **10e** (4.133 g, 75%) as a white solid, mp 92-94 ºC (ethyl acetate/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.63 (bs, NH) 1.95 (ddd, $^{2}J_{HH}$ = 12.7 Hz, $^{3}J_{HH}$ = 12.5 Hz, $^{3}J_{HH}$ = 11.5 Hz, 1 H, CH_2), 2.19-2.26 (m, 1 H, CH_2), 4.24 (dd, $^{3}J_{HH}$ =4.6 Hz, $^{3}J_{HH}$ = 12.5 Hz, 1 H, HC), 4.79 (dd, $^{3}J_{HH}$ = 3.3 Hz, $^{3}J_{HH}$ = 11.5 Hz ,1 H, HC), 6.32 (ddd, $^{3}J_{HH}$ = 7.7 Hz, $^{3}J_{HH}$ = 7.5 Hz, $^{4}J_{HP}$ = 3.6 Hz, 1 H), 6.41-6.48 (m 1 H), 6.65 (d, $^{3}J_{HH}$ = 7.2 Hz, 1 H), 6.87 (dd, $^{3}J_{HH}$ = 7.0 Hz, $^{4}J_{HH}$ = 1.7 Hz), 6.93 (s 1 H), 7.10-7.89 (m, 20 H) ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ: 41.2 (CH_2), 45.1 (CH), 57.0 (CH), 111.6 (d, $^{1}J_{CP}$ = 87.9 Hz, C), 115.6 (d, $^{3}J_{CP}$ = 13.5 Hz, HC), 124.5-133.5 (m, 24 HC and 6 C), 141.0 144.4 (C), 148.4 (d, $^{2}J_{CP}$ = 5.7 Hz, C), ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ: 40.4 ppm; ppm. HRMS (EI): calculated for $C_{37}H_{30}NPS$ [M]+ 551.1837; found 551.1834. Purity 98.72% (EtOH/Heptane = 10/90, Rt = 3.27 min).

4.1.3.6.4. ***(2,4-Bis(4-fluorophenyl)-1,2,3,4-tetrahydroquinolin-8-yl)diphenylphosphine sulfide*** (**10f**). The general procedure was followed using 4-fluorobenzaldehyde (10 mmol, 1.072 mL) and 4-fluorostyrene (20 mmol, 2.385 mL) for 48 h affording compound **10f** (4.189 g, 78%) as a white solid, mp 114-115 ºC (ethyl acetate/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.90 (ddd, $^{2}J_{HH}$ = 12.9 Hz, $^{3}J_{HH}$ = 12.4 Hz, $^{3}J_{HH}$ = 11.4 Hz, 1 H, CH_2), 2.14-2.21 (m, 1 H, CH_2), 4.21 (dd, $^{3}J_{HH}$ = 12.4 Hz, $^{3}J_{HH}$ = 4.0 Hz, 1 H, CH), 4.69 (dd, $^{3}J_{HH}$ = 11.4 Hz, $^{3}J_{HH}$

=3.3 Hz, 1 H, CH), 6.37 (ddd, $^{3}J_{HH}$ = 7.6 Hz, $^{3}J_{HH}$ = 7.6 Hz, $^{4}J_{HP}$ = 2.9 Hz, 1 H), 6.61-7.19 (m, NH and 10 H), 7.45-7.78 (m, 10 H) ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ: 41.5 (CH_2), 44.1 (HC) 56.2 (HC), 111.9 (d, $^{1}J_{CP}$ = 105.5 Hz, C), 114.9-115.7 (m, 5 HC), 126.2-133.0 (m, 16 HC and 3 C $_{arom}$), 139.2 (d, $^{4}J_{CF}$ = 3.1 Hz, C), 139.9 (d, $^{4}J_{CF}$ = 3.2 Hz, C), 148.1 (d, $^{2}J_{CP}$ = 4.5 Hz, C), 161.8 (d, $^{1}J_{CF}$ = 249.6 Hz, FC), 162.0 (d, $^{1}J_{CF}$ = 245.4 Hz, FC) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ: 40.2 ppm; ^{19}F NMR (282 MHz, $CDCl_3$) δ: - 116.5 to -116.4 (m), -115.9 to -115.8 (m) ppm. HRMS (EI): calculated for $C_{33}H_{26}F_2NPS$ [M]+ 537.1492; found 537.1496. Purity 99.84% (EtOH/Heptane = 10/90, Rt = 3.20 min).

4.1.3.7. **General procedure for the preparation of quinolinylphosphine sulfides 12 by dehydrogenation with DDQ.**

To a solution of the corresponding tetrahydroquinoline **10** (1 mmol) in toluene (20 mL) was added DDQ (1.2 mmol, 0.3269 g) and the mixture was irradiated with microwave at 150 W at 40 ºC for 1 h. The solid formed was filtered off and the solvent of the resulting solution removed under vacuum leading to an oil that was purified by column chromatography on silica gel (ethyl acetate / hexane 1:20) to afford compounds **12**.

4.1.3.7.1. ***(2-(Naphthalen-2-yl)-4-phenylquinolin-8-yl)diphenylphosphine sulfide*** (**12e**). The general procedure was followed using tetrahydroquinoline **10e** (1 mmol, 0,551 g) affording compound **12e** (0.541 g, 99%) as a white solid, mp 98-99 ºC (ethyl acetate/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.34-7.63 (m, 15 H), 7.73 (d, $^{3}J_{HH}$ = 7.6 Hz, 1 H), 7.76-7.84 (m, 2 H), 7.95 (s, 1 H), 8.01-8.08 (m, 6 H),), 8.12-8.15 (m, 1 H), 8.68 (ddd, $^{3}J_{HH}$ = 8.5 Hz, $^{3}J_{HH}$ = 7.3 Hz, $^{4}J_{HP}$ = 1.3 Hz, 1 H), 1 H), ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ: 119.68 (HC), 125.2-140.0 (m, 6 C and 25 HC), 138.2 (C), 139.0 (d, $^{3}J_{CP}$ = 11.4 Hz, C), 147.7 (d, $^{2}J_{CP}$ = 4.2 Hz C), 149.8 (C), 155.5 (C) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ:29.7 ppm.HRMS (EI): calculated for $C_{37}H_{26}NPS$ [M]+ 547.1524; found 547.1519. Purity 97.00% (EtOH/Heptane = 10/90, Rt = 3.50 min).

4.1.3.7.2. ***(2,4-Bis(4-fluorophenyl)quinolin-8-yl)diphenylphosphine sulfide*** **(12f**). The general procedure was followed using tetrahydroquinoline **10f** (1 mmol, 0,537 g) affording compound **12f** (0.527 g, 99%) as a white solid, mp 115-116 ºC (ethyl acetate/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.843-6.89 (m, 3 H), 7.14-7.50 (m, 13 H), 7.63 (s, 1 H), 7.80-7.99 (m, 4 H), 8.38 (ddd, $^{3}J_{HH}$ = 8.4 Hz, $^{3}J_{HH}$ = 7.2 Hz, $^{4}J_{HP}$ = 1.4 Hz, 1 H), ppm; ^{13}C NMR {H} (75 MHz, $CDCl_3$) δ:

115.6 (d, $^{2}J_{CF}$ = 21.6 Hz, 2 HC), 115.9 (d, $^{2}J_{CF}$ = 21.6 Hz, 2 HC), 119.0 (HC), 126.0-134.8 (m, 5 C and 16 HC), 138.5 (d, $^{3}J_{CP}$ = 11.2 Hz, HC), 147.6 (d, $^{2}J_{CP}$ = 4.3 Hz, C), 148.8 (2 C), 154.5 (C), 163.2 (d, $^{1}J_{CF}$ = 249.0 Hz, C-F), 164.0 (d, $^{1}J_{CF}$ = 250.0 Hz, C-F) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ: 28.8 ppm. HRMS (EI): calculated for $C_{33}H_{22}F_2NPS$ [M]+ 533.1179; found 533.1183.Purity 97.58% (EtOH/Heptane = 10/90, Rt = 3.42 min).

4.2 Biology

4.2.1. Materials

Reagents and solvents were used as purchased without further purification. Camptothecin was purchased from Sigma-Aldrich. All stock solutions of the investigated compounds were prepared by dissolving the powered materials in appropriate amounts of DMSO. The final concentration of DMSO never exceeded 10% (v/v) in reactions. Under these conditions DMSO was also used in the controls and was not seen to affect TopI activity. The stock solution was stored at 5ºC until it was used.

4.2.2. Expression and purification of Human Topoisomerase IB.

The yeast *SacCyces cerevisiae* TopI null strain RS190, which was used for expression of recombinant human TopI was a kind gift from R. Sternglanz (State University of New York, Stony Brook, NY). Plasmid pHT143, for expression of recombinant TopI under the control of an inducible GAL promoter was described [25]. The plasmids pHT143 were transformed into the yeast *S. cerevisiae* strain RS190. The proteins were expressed and purified by affinity chromatography essentially as described [26]. The protein concentrations were estimated from Coomassie blue-stained SDS/polyacrylamide gels by comparison to serial dilutions of bovine serum albumin (BSA).

4.2.3. DNA relaxation assays.

TopI activity was assayed using a DNA relaxation assay by incubating 110 ng/μL of TopI with 0.5 μg of negatively supercoiled pUC18 in 20 μl of reaction buffer (20 mM Tris–HCl, 0.1 mM Na_2EDTA, 10 mM $MgCl_2$, 50 μg/mL acetylated BSA and 150 mM KCl, pH 7.5). The effect of the synthesized tetracyclic **6**, **7**, **9**, **10** and **12** derivatives on topoisomerase activity was measured by adding different concentrations of the compounds, at different time points as indicated in the text. Either relaxation was assayed without any preincubation or DNA or enzyme were preincubated with the drugs at 37 °C for 15 min, prior to the addition of the missing component i.e. DNA (in case of preincubation of drug and enzyme) or enzyme (in the case of preincubation of drug and DNA). The reactions were performed at 37°C stopped by the addition of 0.5% SDS after indicated time intervals. The samples were protease digested, electrophoresed in a horizontal 1% agarose gel in 1xTBE (50 mM Tris, 45 mM boric acid, 1 mM EDTA) at 25V during 18 hours. The gel was stained with gel red (BIOTIUM, 5 μg/mL), destained with water and photographed under UV illumination.

Since all drugs were dissolved in dimethyl sulfoxide (DMSO), a positive control sample containing the same DMSO concentration as the samples incubated with the drugs was included in all experiments. As a control for drug inhibition the well know TopI specific drug camptothecin was included.

4.2.4. Cytotoxicity assays.

Cells were cultured according to the supplier´s instructions. Cells were seeded in 96-well plates at a density of 2-4 x 10^3 cells per well and incubated overnight in 0.1 mL of media

supplied with 10% Fetal Bovine Serum (Lonza) in 5% CO_2 incubator at 37 ºC. On day 2, drugs were added and samples were incubated for 48 hours. After treatment, 10 µL of cell counting kit-8 was added into each well for additional 2 hours incubation at 37 ºC. The absorbance of each well was determined by an Automatic Elisa Reader System at 450 nm wavelength. Camptothecin was purchased from Sigma-Aldrich and used as positive control.

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List of Captions

Figure 1. Structures of camptothecin and several novel synthesized antiproliferative *phosphorated (tetrahydro)quinolines I.*

Scheme 1. Syntheses of 1,2,3,4-tetrahydroquinolinylphosphine oxides **6** and quinolinylphosphine oxides **7**.

Chart 1. Structures of 1,2,3,4-tetrahydroquinolinylphosphine oxides **6** and quinolinylphosphine oxides **7** obtained ([a]Isolated yield obtained by Route A. [b]Isolated yield obtained by Route B).

Scheme 2. Syntheses of 1,2,3,4-tetrahydroquinolinylphosphanes **9**, 1,2,3,4-tetrahydro-quinolinylphosphine sulfides **10** and quinolinylphosphine sulfides **12**.

Chart 2. Structures of 1,2,3,4-tetrahydroquinolinylphosphanes **9** obtained by Povarov reaction.

Chart 3. Structures of 1,2,3,4-tetrahydroquinolinylphosphine sulfides **10** and quinolinylphosphine sulfides **12** obtained (*[a]*a small amount (9%) of aromatized compound **12a** was also obtained; *[b]*a small amount (5%) of aromatized compound **12c** was also obtained; *[c]*isolated yields obtained from amine **11**; *[d]*obtained along with tetrahydroqueinoline **10c**).

Figure 2. Inhibition of Top1 activity along the time (15", 45", 1' and 3') by compounds **7e**, **9a** and **10a** and camptothecin at 80 μM: lanes 1-3, DNA+Top1+DMSO; lanes 4-7, DNA+Top1+camptothecin 80μM; lanes 8-11, DNA+Top1+**7e** 80μM; lanes 12-15, DNA+Top1+**9a** 80μM; lanes 16-19, DNA+Top1+**10a** 80μM; lane 20, control DNA. Reaction samples were mixed with enzyme at 37 ºC 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; Sc, supercoiled DNA.

Table 1. Top1 inhibitory activity of quinolinylphosphine oxides **6** and **7**, quinolinylphosphanes **9** and quinolinylphosphine sulfides **10** and **12**.

Table 2. Antiproliferative activity of quinolinylphosphine oxides **6** and **7,** quinolinylphosphanes **9**, quinolinylphosphine sulfides **10** and **12**.

Novel Topoisomerase I Inhibitors. Syntheses and Biological Evaluation of Phosphorus Substituted Quinoline Derivates with Antiproliferative Activity.

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HIGHLIGHTS.

1. Novel 1,2,3,4-tetrahydroquinolinyl- and quinolinylphosphine oxide, phosphane and phosphine sulfides were synthesized.
2. Prepared compounds show inhibitory effects against Top1 mediated relaxation comparable to those observed for the natural inhibitor, camptothecin (CPT).
3. Cytotoxicity of new compounds has been tested against several cell lines and some of them present excellent antiproliferative activity of A549 human lung cancer cell line.
4. Toxicity of all new compounds has been tested against healthy lung cell line MRC5.