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PII: S0223-5234(19)30560-4

DOI: https://doi.org/10.1016/j.ejmech.2019.06.032

Reference: EJMECH 11435

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 13 May 2019

Revised Date: 12 June 2019

Accepted Date: 12 June 2019

Please cite this article as: E. Martín-Encinas, G. Rubiales, B.R. Knudssen, F. Palacios, Concepció. Alonso, Straightforward synthesis and biological evaluation as topoisomerase I inhibitors and antiproliferative agents of hybrid Chromeno[4,3-*b*][1,5]Naphthyridines and Chromeno[4,3-*b*][1,5]Naphthyridin-6-ones, *European Journal of Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.ejmech.2019.06.032.

Graphical Abstract

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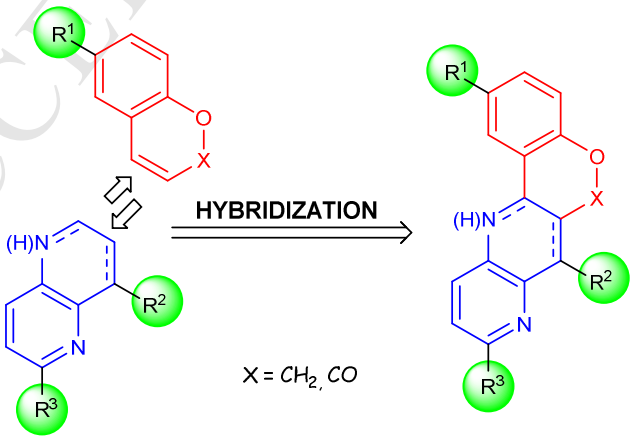

Straightforward Synthesis and Biological Evaluation as Topoisomerase I Inhibitors and Antiproliferative Agents of Hybrid Chromeno[4,3-*b*][1,5]Naphthyridines and Chromeno[4,3-*b*][1,5]Naphthyridin-6-ones.

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Dedicated to Professor Pablo Espinet on the occasion of his 70th anniversary

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Keywords: Chromeno[4,3-*b*][1,5]naphthyridines, chromeno[4,3-*b*][1,5]naphthyridin-6-ones, Topoisomerase I, Enzyme inhibition, Antiproliferative effect

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

ABSTRACT. This work describes the synthesis of hybrid tetrahydro-1,5-naphthyridine and 1,5-naphthyridine derivatives fused with heterocycles such as chromenes and chromen-2-ones or coumarins, which were synthesized in good to high general yields. The synthetic route involves an intramolecular [4+2]-cycloaddition reaction of functionalized aldimines obtained by the condensation of 3-aminopyridine and aldehydes containing a double or triple carbon-carbon bond in orto position and allows the selective generation of three stereogenic centers in a short, efficient and reliable synthesis. The subsequent dehydrogenation of the fused tetrahydrochromeno[4,3-*b*][1,5]naphthyridines and/or tetrahydrochromeno[4,3-*b*][1,5]naphthyridin-6-ones leads to the formation of the corresponding tetracyclic chromeno[4,3-*b*][1,5]naphthyridine derivatives and/or chromeno[4,3-*b*][1,5]naphthyridin-6-ones in quantitative yields. Some of the prepared products showed activity as inhibitors of Topoisomerase I (TopI). Additionally, the cytotoxic behavior of these compounds has been studied in cell lines derived from human lung adenocarcinoma (A549) and human ovarian carcinoma (SKOV03), and on non-cancerous lung fibroblasts cell line (MRC5) where, on the last ones, the absence of cytotoxicity was observed. 7-Phenyl-6*H*-6a,7,12,12a-tetrahydrochromeno[4,3-*b*][1,5]naphthyridine **5a** showed excellent cytotoxic activity with a IC_{50} value of 1.03±0.30 μM against the A549 cell line and a IC_{50} value of 1.75±0.20 μM against the SKOV03 cell line. The obtained results point to these compounds as good antiproliferative candidates.

1. Introduction

The development of antiproliferative compounds remains as one of the main attention focus in terms of the development of research activity. In recent years, different drugs have been developed based on the inhibition of the enzyme topoisomerase I (TopI), since it has been observed that it is overexpressed in tumor cells. 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].

Among the most representative TopI inhibitor drugs there is camptothecin **I** (CPT, Figure 1) and its derivatives (CPTs) which are currently used in the systemic treatment of colon, ovarian and small-cell lung cancers [2]. However, when being used in cancer therapy, these derivatives carry certain drawbacks, such as lactone instability in blood plasma and cancer cell resistance [3].

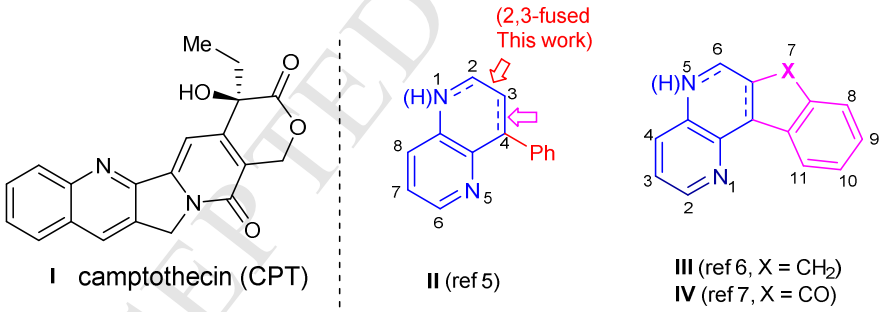


Figure 1. Structures of TopI inhibitors: camptothecin (CPT) **I**, 1,5-naphthyridines **II**, indeno[2,1-*c*][1,5]naphthyridines **III** and indeno[2,1-*c*][1,5]naphthyridin-7-ones **IV** with antiproliferative activity.

Among other features, camptothecin (CPT) and its derivatives show angularly fused pentacyclic heterocycles in their structural analysis. Moreover, with regard to the proposed inhibition mechanism of TopI, the presence of polycyclic heterocycles seems to be relevant in the effectiveness of the antiproliferative activity, probably due to an improvement in π-π*

stacking interactions with the DNA base pairs [1]. Regarding polycyclic heterocycles 1,5-naphthyridine derivatives **II** (Figure 1) belong to heterocycles identified as suitable candidates in medicinal chemistry [4]. Recently, our group has reported that dinitrogenated heterocycles such as 1,5-naphthyridines **II** (Figure 1) showed good inhibitory activity against *Leishmanial* TopI (LTopI) enzyme [5]. Moreover, 1,5-naphthyridines may be fused through the 3 and 4 position of the heterocycle with a carbocycle structure (indene) and the corresponding angularly fused bioactive molecules such as 7*H*-indeno[2,1-*c*][1,5]naphthyridines **III** (X = CH_2, Figure 1) prepared by our group showed good TopI inhibition with antileishmanial and antiproliferative activity [6]. What is more, after observing the behavior of these 1,5-naphthyridines when participating in the human TopI enzymatic process, 6-(naphthalen-2-yl)-7*H*-indeno[2,1-*c*][1,5]naphthyridin-7-one **IV** (X = CO, Figure 1) was used as a nanosensor and showed that it inhibited human topoisomerase I activity by blocking enzyme–DNA dissociation in a post-ligation step of catalysis [7].

2*H*-Chromenes **V** (X = CH_2, Figure 2) and coumarins or 2*H*-chromen-2-ones **VI** (X = CO, Figure 2) are heterocyclic derivatives with a benzene fused to a pyran [8]. They are present in a wide number of natural products and both heterocycles constitute an important moiety in bioactive heterocycles with pharmacological properties [9], including anticancer drugs [9,10]. Therefore, these scaffolds are very interesting substrates in the search of new drug candidates.

The low toxicity of the natural products containing chromene and their broad pharmacological properties are attractive feature for medicinal chemists and a source of inspiration for the design of novel therapeutic agents [9]. Moreover, naphthyridines fused with chromenes or chromenones present diverse biological activities as antifungal, antimicrobial and antibacterial [11]. Likewise,

angularly fused tricyclic chromeno[4,3-b]pyridines showed TopI inhibitory activity and cytotoxicity [12].

The hybrid anticancer drug approach is an innovative synthetic strategy for the discovery of new biologically active hybrid molecules [13]. Recently, the molecular hybrid approach has resulted in several novel chemical entities with improved anticancer activity and selectivity with reduced side effects[14].

With these considerations in mind, we believed that the development of new hybrid molecules [15] with an angularly fused polycyclic heterocyclic skeleton, containing a 1,5-naphthyridine fragment **VII** (Figure 2), and incorporating either a chromene structure (**V**, X = CH_2, Figure 2) or a coumarin heterocycle (**VI**, X = CO, Figure 2) fused through the position 2 and 3 of the naphthyridine ring (Figure 1) may be privileged scaffolds for the search of novel pharmaceuticals [16]. The new chromeno[4,3-*b*][1,5]naphthyridines **VIII** (Figure 2) and chromeno[4,3-*b*][1,5]naphthyridin-6-ones **IX** (Figure 2) may improve the antiproliferative cytotoxic properties with respect to other biologically active structures, and this strategy may be complementary to our previous studies [6,7] with angularly tetracyclic 1,5-naphthyridines **III** and **IV** fused with indenes through the positions 3 and 4 of the naphthyridine ring (*vide supra*, Figure 1).

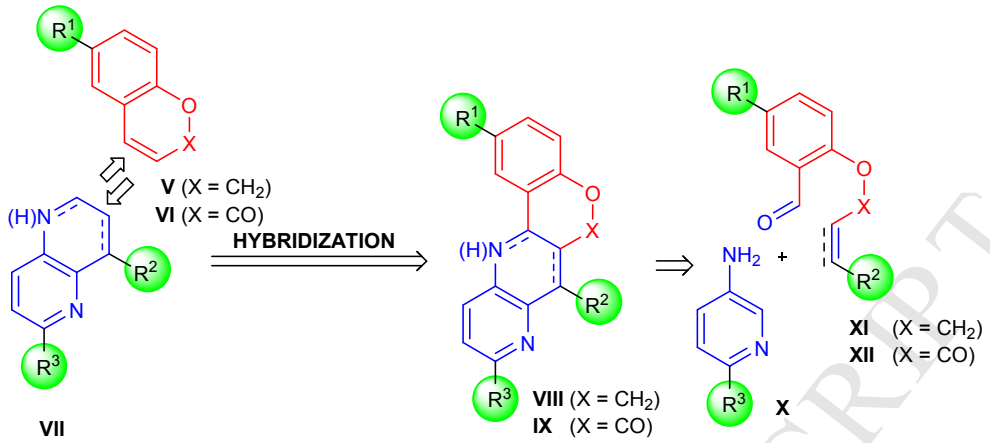


Figure 2. Hybridization of chromene **V** and/or chromen-2-one **VI** with 1,5-naphthyridines **VII** for the design of new bioactive chromeno[4,3-*b*][1,5]naphthyridines **VIII** (X = CH_2) and chromeno[4,3-*b*][1,5]naphthyridin-6-ones **IX** (X = CO).

In this sense, for the synthesis of the goal angularly fused new chromeno[4,3-*b*][1,5]naphthyridines **VIII** (Figure 2) and chromeno[4,3-*b*][1,5]naphthyridin-6-ones **IX** (Figure 2), the hetero-Diels-Alder reaction (HDAr) [17] is a highly atom-economic tool, which presents interesting applications [18]. In fact, among them the Povarov reaction may represent a direct route to naphthyridine derivatives [19]. Furthermore, in the search of a methodology for the preparation of molecules with higher molecular diversity, intramolecular Povarov reaction could be considered as an important strategy for the synthesis of several ring-fused derivatives by a single operation [20].

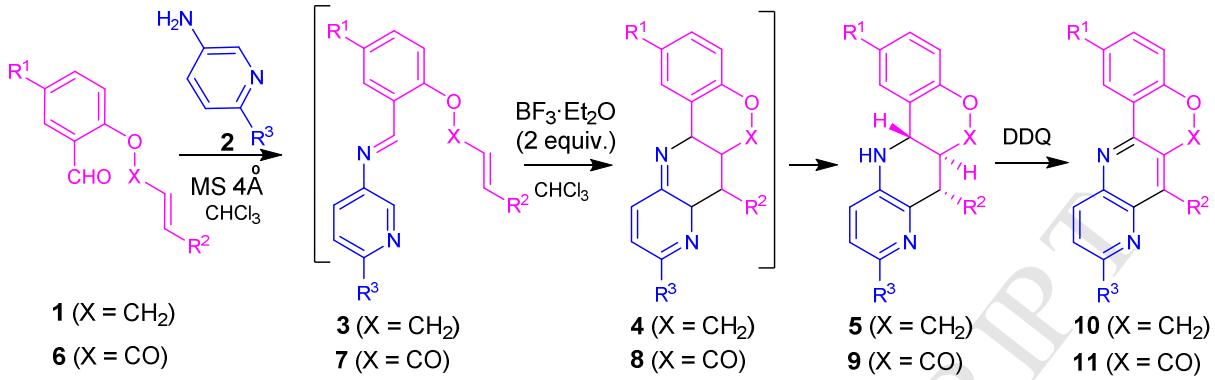
Bearing all this in mind, in this work we report the straightforward synthesis of chromeno[4,3-*b*][1,5]naphthyidirines **VIII** (X = CH_2, Figure 2) and chromeno[4,3-*b*][1,5]naphthyridin-6-ones **IX** (X = CO, Figure 2) by intramolecular Povarov reaction from 3-aminopyridine **X** (Figure 2) and functionalized aldehydes **XI** (X = CH_2, Figure 2) and **XII** (X = CO, Figure 2). The preparation of these new families of compounds 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. Chemistry

The hybrid chromeno[4,3-*b*][1,5]naphthyridines **VIII** (X = CH_2, Figure 2) and chromeno[4,3-*b*][1,5]naphthyridin-6-ones **IX** (X = CO, Figure 2) were synthesized by an intramolecular Povarov [4+2]-cycloaddition reaction. First, we started with the preparation of functionalized aldehydes **1** (Scheme 1, X = CH_2) which tailored a double bound in their structure as indicated (see Supporting Material). Then, aldimines **3** (X = CH_2), prepared *in situ* by condensation reaction of 3-aminopyridine **2** and previously prepared functionalized aldehydes **1**, cyclized intramolecularly in refluxing chloroform and in the presence of $BF_3 \cdot Et_2O$ (Scheme 1). Afterwards, the corresponding *endo*-6a,7,12,12a-tetrahydro-6*H*-chromeno[4,3-*b*][1,5]naphthyridines **5** (X = CH_2) were selectively obtained with good yields (56-87%, Scheme 1, Chart 1) in a regio- and stereospecific way. The structure of *endo*-6a,7,12,12a-tetrahydro-6*H*-chromeno[4,3-*b*][1,5]naphthyridines **5** (X = CH_2) was assigned by means of NMR experiments and confirmed by HRMS (see Experimental Section).

Formation of tetrahydrochromeno[4,3-*b*][1,5]naphthyridine derivatives **5** may be explained by a regio- and stereospecific intramolecular [4+2]-cycloaddition reaction between aldimines **3** to give intermediates **4** followed by prototropic tautomerization.



Scheme 1. Syntheses of chromeno[4,3-*b*][1,5]naphthyridine derivatives **5**/**10** and chromeno[4,3-*b*][1,5]naphthyridin-6-one derivatives **9**/**11**.

However, when imine **3f** (R^1 = H, R^2 = C_6H_5), obtained from the corresponding aldehyde **1** and 6-bromo-3-aminopyridine **2c** (R^3 = Br), was heated in chloroform in the presence of $BF_3 \cdot Et_2O$ the tetrahydro-1,5-naphthyridine **5** was not isolated and the chromeno[4,3-*b*][1,5]naphthyridine **10f** was directly obtained instead. The formation of compound **10f** could be explained by intramolecular Povarov reaction and subsequent dehydrogenation under the reaction conditions (Chart 2, Experimental Section).

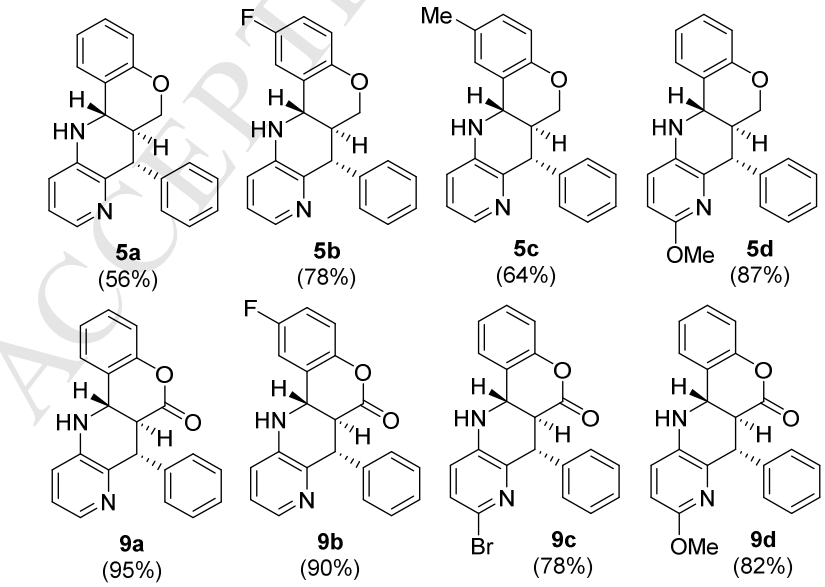


Chart 1. Structures of *endo*-6a,7,12,12a-tetrahydro-6*H*-chromeno[4,3-*b*][1,5]naphthyridine derivatives **5** and *endo*-6a,7,12,12a-tetrahydro-6*H*-chromeno[4,3-*b*][1,5]naphthyridin-6-one derivatives **9** obtained by intramolecular Povarov reaction.

In order to increase the molecular diversity, the methodology was extended to the preparation of angularly fused tetracyclic derivatives **9** (X = CO), in which the chromene scaffold was substituted by a coumarin (chromen-2-one).

As before, functionalized aldehydes **6** (Scheme 1, X = CO) were prepared as indicated (see Supporting Material) followed by the condensation reaction of 3-aminopyridine **2** to give aldimines **7** (X = CO). These imines **7** cyclized intramolecularly in refluxing chloroform in the presence of 2 equivalents of Lewis acid as $BF_3 \cdot Et_2O$ (Scheme 1) to afford *endo*-6a,7,12,12a-tetrahydro-6*H*-chromeno[4,3-*b*][1,5]naphthyridin-6-ones **9** (X = CO) with good yields (78-95%, Scheme 1, Chart 1) in a regio- and stereospecific way. The formation of these polycyclic compounds **9** may be explained, as before, by a regio- and stereospecific intramolecular [4+2]-cycloaddition reaction of aldimines **7** (X = CO) to give intermediates **8** (X = CO) followed by prototropic tautomerization.

The methodology tolerates electron-releasing and electron-withdrawing substituents in the aromatic aldehydes, even fluorinated ones that allow the preparation of fluoro containing compounds, interesting substrates from a biological point of view [21]. As far as we know, this strategy represents the first example for the preparation of 6a,7,12,12a-tetrahydro-6*H*-chromeno[4,3-*b*][1,5]naphthyridines **5** (X = CH_2, Scheme 1) and 6a,7,12,12a-tetrahydro-6*H*-chromeno[4,3-*b*][1,5]naphthyridin-6-ones **9** (X = CO, Scheme 1).

Subsequent dehydrogenation of tetrahydro-6*H*-chromeno[4,3-*b*][1,5]naphthyridines **5** (X = CH_2) and tetrahydro-6*H*-chromeno[4,3-*b*][1,5]naphthyridin-6-ones **9** (X = CO) under the reaction conditions would afford chromeno[4,3-*b*][1,5]naphthyridines **10** (X = CH_2) and chromeno[4,3-*b*][1,5]naphthyridin-6-ones **11** (X = CO), respectively, in quantitative yields (Scheme 1, Chart 2). This dehydrogenation reaction was performed with 1 equivalent of DDQ in toluene at 40 ºC under microwave irradiation for 2 hours as previously reported [6b].

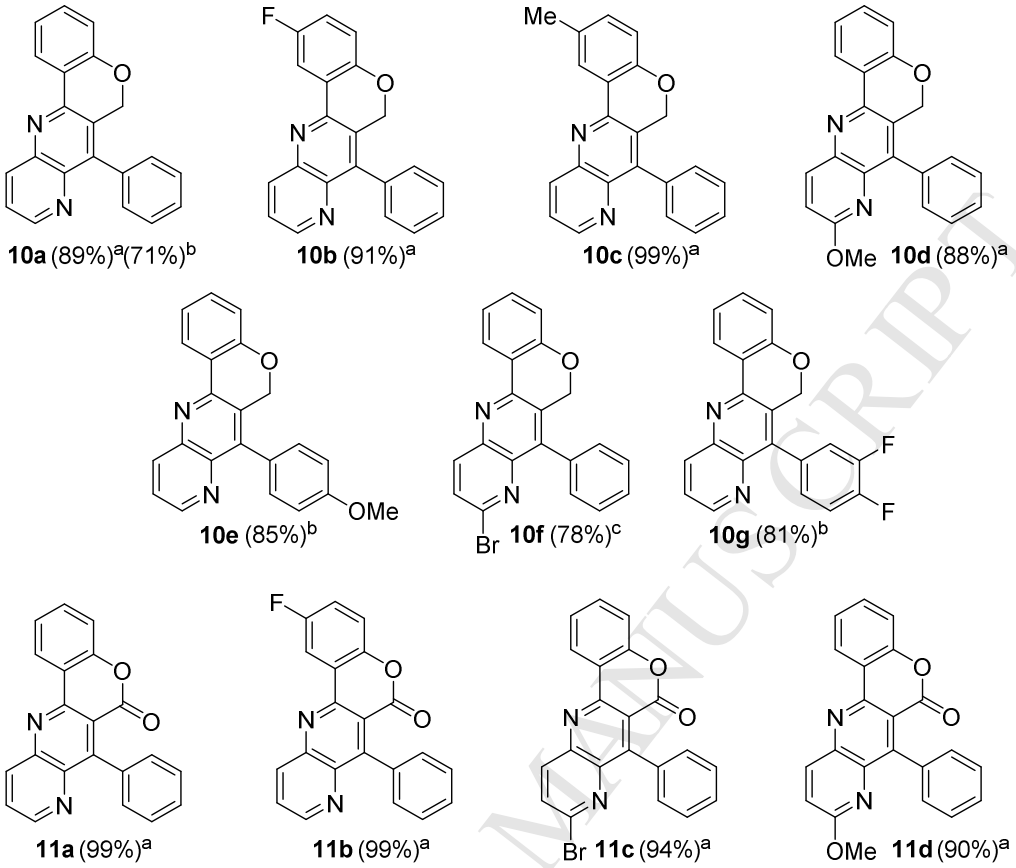
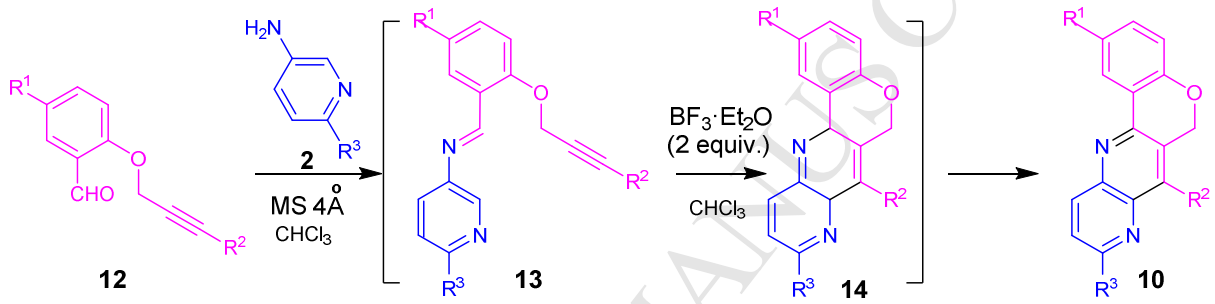


Chart 2. Structures of chromeno[4,3-*b*][1,5]naphthyridines **10** and chromeno[4,3-*b*][1,5]naphthyridin-6-ones **11** obtained by dehydrogenation reaction with DDQ[a] or by intramolecular Povarov reaction of propargyl aldimines **13** in the presence of $BF_3 \cdot Et_2O$[b]. [c]Chromeno[4,3-*b*][1,5]naphthyridine **10f** obtained by intramolecular Povarov reaction and subsequent dehydrogenation under the reaction conditions.

As we previously reported, if acetylenes are used as dienophiles instead of olefins when reacting with aldimines derived from 3-aminopyridines, the corresponding 1,5-naphthyridine compounds may be obtained [22]. For these reasons, we decided to study the synthesis of chromeno[4,3-*b*][1,5]naphthyridines **10** in a direct way by means of the intramolecular Povarov reaction by using aldehydes **12** (Scheme 2) containing an alkyne group in the *orto* position. It is noteworthy, that with this strategy, from a preparative point of view, the aromatic 1,5-naphthyridine core may be directly obtained.

The formation of chromeno[4,3-*b*][1,5]naphthyridines **10** can be performed by initial condensation reaction between 3-aminopyridine **2** and functionalized aldehydes **12**. Subsequent intramolecular [4+2]-cycloaddition reaction in refluxing chloroform, in the presence of 2 equivalents of $BF_3 \cdot Et_2O$, would give intermediates **14** followed by dehydrogenation under the reaction conditions to afford chromeno[4,3-*b*][1,5]naphthyridines **10** with good yields (Scheme 2, Chart 2)[23].



Scheme 2. Syntheses of 6*H*-chromeno[4,3-*b*][1,5]naphthyridines **10** by intramolecular Povarov reaction with acetylenic aldehydes.

The methodology described in this work represents an easy, efficient and straightforward strategy for the preparation of angularly hybrid tetrahydro-1,5-naphthyridine and 1,5-naphthyridine derivatives fused with heterocycles such as chromenes and chromenones (coumarins) and tolerates electron-donating and electron-withdrawing of substituents either in the starting aldehyde and in the initial amine. Next, the biological behavior of compounds prepared as TopI inhibitors and as antiproliferative agents has been studied.

3. Biological results and discussion

3.1. Inhibition of Topoisomerase I. The inhibitory effect of the synthesized derivatives on Topoisomerase type I (TopI) was investigated. A conventional supercoiled plasmid relaxation assay was used to determine if the newly angularly fused tetrahydro derivatives **5** and **9**, as well as more unsaturated compounds **10** and **11** inhibit human TopI activity by converting supercoiled plasmid DNA to relaxed DNA (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, 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 (CPT) was used as a positive control (Figure 3).

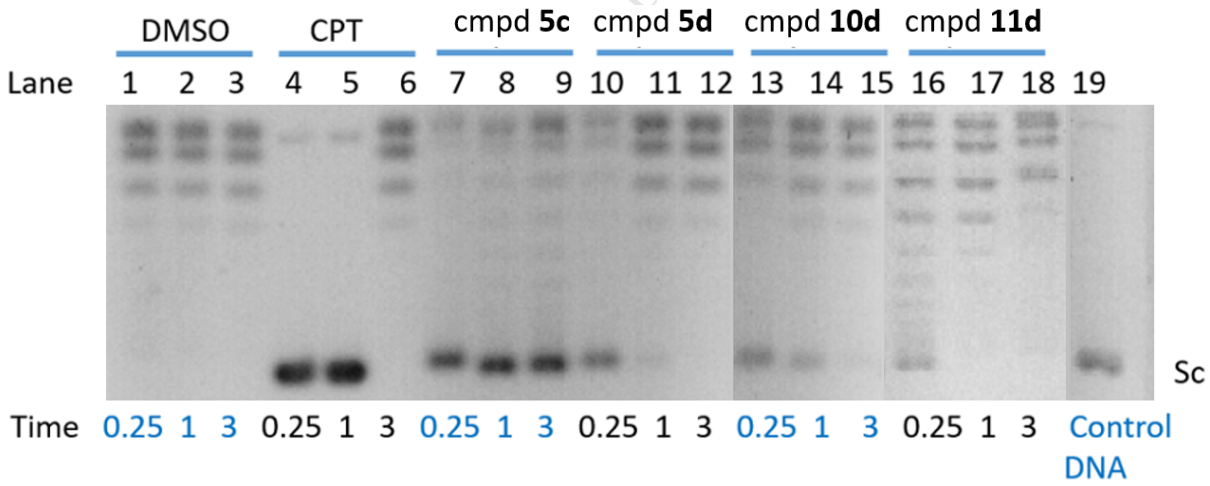


Figure 3. Inhibition of TopI activity along the time (15", 1' and 3') by compounds **5c**, **5d**, **10d** and **11d** and camptothecin at 100 μM: lanes 1-3, DNA+TopI+DMSO; lanes 4-6, DNA+TopI+camptothecin 100μM; lanes 7-9, DNA+TopI+**5c** 100μM; lanes 10-12, DNA+TopI+**5d** 100μM; lanes 13-15, DNA+TopI+**10d** 100μM; lanes 16-18, DNA+TopI+**11d** 100μM; lane 19, 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 TopI mediated DNA relaxation assay.

TopI inhibitory activity of new compounds was tested by detecting the conversion of supercoiled DNA (Sc, Figure 3) to its relaxed form in the presence of the purified enzyme and expressed in quantitative fashion relative to the TopI inhibitory activity of camptothecin (Table 1). As shown in Figure 3, TopI relaxes supercoiled DNA even in the presence of DMSO (Figure 3, lanes 1-3), and camptothecin (CPT) inhibits the relaxation, as indicated by the increased intensity of the band corresponding to the supercoiled DNA (Figure 3, lanes 4-6).

Based on the results of the relaxation assays, the tetrahydro-6*H*-chromeno[4,3-*b*][1,5]naphthyridines **5**, and the tetrahydro-6*H*-chromeno[4,3-*b*][1,5]naphthyridin-6-ones **9** showed low-moderate inhibitory activity, and only present remarkably activity, compound **5c** (R^1 = Me) containing a methyl group in the chromeno moiety and **5d** (R^3 = OMe) containing a methoxy group in the naphthyridine moiety (Table 1, entries 4 and 5), which inhibited the TopI relaxation similar to camptothecin (CPT) when added at the same concentration and in the same time intervals. With respect to 6*H*-chromeno[4,3-*b*][1,5]naphthyridines **10** and 6*H*-chromeno[4,3-*b*][1,5]naphthyridin-6-ones **11**, the inhibitory activity is low-moderate. However, notably inhibitory activity could be observed for compounds **10d** (R^3 = OMe) and **10g** (R^2 = 3,4-$F_2C_6H_3$) (Table 1, entries 13 and 16), which at short times inhibited TopI activity almost completely and the effect was released at longer time intervals. Also, 6*H*-chromeno[4,3-*b*][1,5]naphthyridin-6-one **11d** (R^3 = OMe) exhibited inhibitory activity at short time and a minor effect at a later time (Table 1, entry 20). In general, a greater effect is observed in those derivatives with a methoxy group in their structure as in compounds **5d**, **10d** and **11d** (Charts 1 and 2). In addition, the derivative with a double substitution of fluorine **10g** (R^2 = 3,4-$F_2C_6H_3$) showed good activity.

Table 1. TopI inhibitory activity of compounds **5**, **9**, **10**, **11** and **CPT**.

Entry	Compound	R^1	R^2	R^3	% Inhibition[a]		
					15 seconds	1 minute	3 minutes
1	Camptothecin (CPT)				80	76	3
2	**5a**	H	C_6H_5	H	5	4	1
3	**5b**	F	C_6H_5	H	2	1	1
4	**5c**	Me	C_6H_5	H	70	69	19
5	**5d**	H	C_6H_5	OMe	81	26	12
6	**9a**	H	C_6H_5	H	5	7	5
7	**9b**	F	C_6H_5	H	30	25	2
8	**9c**	H	C_6H_5	Br	4	4	4
9	**9d**	H	C_6H_5	OMe	4	4	4
10	**10a**	H	C_6H_5	H	30	14	2
11	**10b**	F	C_6H_5	H	2	2	2
12	**10c**	Me	C_6H_5	H	2	2	2
13	**10d**	H	C_6H_5	OMe	86	16	15
14	**10e**	H	4-$MeOC_6H_4$	H	29	30	29
15	**10f**	H	C_6H_5	Br	4	4	4
16	**10g**	H	3,4-$F_2C_6H_3$	H	84	6	6
17	**11a**	H	C_6H_5	H	25	2	2
18	**11b**	F	C_6H_5	H	26	2	2
19	**11c**	H	C_6H_5	Br	4	4	4
20	**11d**	H	C_6H_5	OMe	72	12	12

[a] The activity of the compounds inhibiting TopI relaxation was expressed quantitatively by comparison with the inhibitory activity observed for camptothecin.

3.2. In Vitro Cytotoxicity. The cytotoxicity of the new angularly fused tetracyclic hybrid molecules **5**, **9**, **10** and **11** was investigated *in vitro* by testing the antiproliferative activities against two human cancer cell lines: A549 (carcinomic human alveolar basal epithelial cell) and

SKOV3 (human ovarian carcinoma). The cell counting kit (CCK8) assay was employed to assess growth inhibition and, the cell proliferation inhibitory activities of the compounds are listed in Table 2 as IC_{50} values.

Table 2. Antiproliferative activity of compounds **5**, **9**, **10**, **11** and **CPT**.

Entry	Comp.	R^1	R^2	R^3	Cytotoxicity IC_{50} (μM)[a]		
					lung A549	ovarian SKOV03	lung MRC-5
1	Camptothecin (CPT)				$(1.0\pm0.06)\cdot10^{-3}$	$(5.5\pm0.01)\cdot10^{-3}$	-
2	**5a**	H	C_6H_5	H	1.03±0.30	1.75±0.20	>50
3	**5b**	F	C_6H_5	H	11.303±1.23	>50	>50
4	**5c**	Me	C_6H_5	H	7.25±0.27	12.69±0.80	>50
5	**5d**	H	C_6H_5	OMe	5.43±2.18	>50	>50
6	**9a**	H	C_6H_5	H	25.42±1.82	>50	>50
7	**9b**	F	C_6H_5	H	>50	>50	>50
8	**9c**	H	C_6H_5	Br	>50	>50	>50
9	**9d**	H	C_6H_5	OMe	>50	>50	>50
10	**10a**	H	C_6H_5	H	>50	>50	>50
11	**10b**	F	C_6H_5	H	>50	>50	>50
12	**10c**	Me	C_6H_5	H	>50	>50	>50
13	**10d**	H	C_6H_5	OMe	11.74±2.38	>50	>50
14	**10e**	H	4-$MeOC_6H_4$	H	17.89±2.00	>50	>50
15	**10f**	H	C_6H_5	Br	>50	>50	>50
16	**10g**	H	3,4-$F_2C_6H_3$	H	>50	>50	>50
17	**11a**	H	C_6H_5	H	9.03±1.47	>50	>50
18	**11b**	F	C_6H_5	H	>50	>50	>50
19	**11c**	H	C_6H_5	Br	29.00±3.15	>50	>50
20	**11d**	H	C_6H_5	OMe	>50	>50	>50

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

The most cytotoxic derivative in both cancerous cell lines resulted to be the tetrahydro-6*H*-chromeno[4,3-*b*][1,5]naphthyridine **5a** (R^1 = H, R^2 = C_6H_5, R^3 = H) with a IC_{50} value of 1.03±0.30 μM against the A549 cell line and a IC_{50} value of 1.75±0.20 μM against the SKOV03 cell line (Table 2, entry 2). Some other tetrahydro derivatives **5**, such as **5c** (R^1 = Me, R^2 = C_6H_5, R^3 = H) and **5d** (R^1 = H, R^2 = C_6H_5, R^3 = OMe), showed IC_{50} values under 10 μM against the A549 cell line, with values of IC_{50} = 7.25±0.27 μM and IC_{50} = 5.43±2.18 μM, respectively (Table 2, entries 4 and 5). Regarding the 6*H*-chromeno[4,3-*b*][1,5]naphthyridines **10** and 6*H*-chromeno[4,3-*b*][1,5]naphthyridin-6-ones **11**, the derivative **11a** presented as low cytotoxicity as 9.03±1.47 μM against the A549 cell line (Table 2, entry 17).

According to the data presented in Table 2, in general when cytotoxicity is observed this is higher against the human lung adenocarcinoma cell line (A549) than in the human ovarian carcinoma cell line (SKOV3). Moreover, MRC-5 non-malignant lung fibroblasts were tested for studying selective toxicity [24] and in contrast, none of the synthesized 6*H*-chromeno[4,3-*b*][1,5]naphthyridines **5** nor **10** as well as 6*H*-chromeno[4,3-*b*][1,5]naphthyridin-6-ones **9** nor **11** compounds exhibited any toxicity toward MRC-5 cells (Table 2). Topoisomerase inhibitors present limited tumor selectivity [25]. However, in our case a very high selectivity has been observed against the human lung adenocarcinoma cell line (A549).

4. Computational analysis

Taking into account that theoretical calculations allowed the estimation of Molecular Electrostatic Potential Surface (MEPS), HOMO-LUMO energy gap and related parameters, which depicted the potential kinetic stability and reactivity of the target compounds [26], theoretical studies using Density Functional Theory (DFT) [27] involving the well-known Becke

three-parameter Lee-Yang-Parr function (B3LYP) [28] and 6-311G (d, p) level of theory for the synthesized compounds were carried out.

4.1. Stereoelectronic properties. The molecular DFT-based parameters such as electronic chemical potential (μ), chemical hardness (η), global electrophilicity (ω), maximum number of accepted electrons (ΔNmax) and Free energy in gas and in aqueous medium for compounds **5**,**9**-**11** are reported in Table 3.

Thus, tetrahydro-6*H*-chromeno[4,3-*b*][1,5]naphthyridines **5** and tetrahydro-6*H*-chromeno[4,3-*b*][1,5]naphthyridin-6-ones **9** are the most stable (Table 3), with the highest gap values from 4.77 to 5.09, higher hardness and higher chemical potential than the rest of compounds, and also are the least electrophilic. In this group, compounds **5** (Chart 1), which experimentally have shown higher cytotoxicity and better inhibition of TopI than **9** (Tables 1 and 2), present the highest chemical potentials (which would imply greater reactivity), are the least electrophilic and have the lowest dipole moment. Among the tetrahydro derivatives **5** the most cytotoxic ones are compounds **5a**, **5c** and **5d** (Table 2) and precisely these are the least electrophilic and have the highest values of chemical potential (Table 3, entries 1, 3 and 4).

With respect to more unsaturated compounds, **10** and **11**, they seem to be more reactive than compounds **5** and **9** of the former group presenting gap values of 3.73 to 4.00 (Table 3) with lower chemical potential and higher electrophilicity. In this case, within the group of 1,5-naphthyridines fused with chromene **10**, the compound with the highest cytotoxicity and which best inhibits TopI is **10d**, showing, among derivatives **10** and **11**, the highest chemical potential value and the lowest electrophilicity (Table 3, entry 12). Likewise, in the case of 1,5-naphthyridines fused with coumarin **11**, the compound **11d**, with good inhibitory activity, has the

highest values of chemical potential, dipole moment and is the least electrophilic (Table 3, entry 19).

The computational results obtained seem to indicate that there is a direct relationship between the low electrophilicity and the high chemical potential, with the biological activity within each type of structure.

Table 3. Calculated energies and molecular proprieties computed at B3LYP/6-311G** basis set level of theory for compounds **5,9-11** and **CPT**.

Entry	Compound	ΔG (g) (in a.u.)	ΔG (aq) (in a.u.)	E_{HOMO} (in a.u.)	E_{LUMO} (in a.u.)	Gap (-eV)	η (in a.u.)	μ (in a.u.)	ω (eV)	ΔNmax (in a.u.)	Dipole moment (debye)
1	**5a**	-995.74250	-995.75452	-0.21492	-0.03051	5.02	0.18441	-0.12272	0.040830	0.665447	1.570
2	**5b**	-1095.01472	-1095.026986	-0.21899	-0.0338	5.04	0.18519	-0.12640	0.043133	0.682515	0.727
3	**5c**	-1035.04548	-1035.05751	-0.20689	-0.0297	5.00	0.18377	-0.12159	0.040221	0.661615	2.598
4	**5d**	-1110.27061	-1110.28600	-0.21347	-0.03153	4.77	0.17536	-0.11921	0.040520	0.679802	1.940
5	**9a**	-1069.81223	-1069.82773	-0.22859	-0.04141	5.09	0.18718	-0.13500	0.048683	0.721231	5.002
6	**9b**	-1169.08411	-1169.09976	-0.23261	-0.04623	5.07	0.18638	-0.13942	0.052146	0.748042	3.499
7	**9c**	-3643.37008	-3643.38682	-0.23311	-0.05151	4.94	0.18160	-0.14231	0.055760	0.783645	5.981
8	**9d**	-1184.34120	-1184.35985	-0.22049	-0.03925	4.93	0.18124	-0.12987	0.046530	0.716564	5.735
9	**10a**	-993.387886	-993.39773	-0.22573	-0.07899	3.99	0.14674	-0.15236	0.079098	1.038299	1.010
10	**10b**	-1092.66012	-1092.66998	-0.22698	-0.08396	3.89	0.14302	-0.15547	0.084502	1.087051	2.346
11	**10c**	-1032.69077	-1032.70067	-0.21986	-0.07777	3.87	0.14209	-0.14882	0.077929	1.047329	0.890
12	**10d**	-1107.91838	-1107.93199	-0.21730	-0.07398	3.90	0.14332	-0.14564	0.073999	1.016188	2.593
13	**10e**	-1107.91245	-1107.92398	-0.22387	-0.07687	4.00	0.14700	-0.15037	0.076909	1.022925	1.276
14	**10f**	-3566.94648	-3566.95652	-0.22978	-0.08653	3.90	0.14325	-0.15816	0.087305	1.104049	1.724
15	**10g**	-1191.92762	-1191.93771	-0.23071	-0.08523	3.96	0.14548	-0.15797	0.085766	1.085854	3.142
16	**11a**	-1067.46187	-1067.47581	-0.24152	-0.09887	3.88	0.14265	-0.17020	0.101529	1.193095	3.918
17	**11b**	-1166.73367	-1166.74754	-0.2442	-0.10293	3.84	0.14127	-0.17357	0.106621	1.228605	2.967
18	**11c**	-3641.01952	-3641.03346	-0.24531	-0.10606	3.79	0.13925	-0.17569	0.110827	1.261652	3.822
19	**11d**	-1181.99254	-1182.01049	-0.23195	-0.09475	3.73	0.13720	-0.16335	0.097242	1.190598	5.792
20	**CPT**	-1182.22076	-1182.237563	-0.22711	-0.0929	3.65	0.13426	-0.15998	0.095314	1.191569	6.419

Abbreviations: ΔG (g): Free energy in gas phase[a]; ΔG (aq): Free energy in aqueous medium[b];Gap: E_{HOMO} - $E_{LUMO;}$ η: Hardness[c]; μ:Chemical Potentials[c]; ω:Global Electrophilicities[c] and Δ*N*max: Maximun Number of Accepted Electrons[c].

[a] Computed a B3LYP/6-311G**+ΔZPVE level; [b] Computed a B3LYP(PCM)/6-311G**+ΔZPVE level using water as solvent; [c]Computed at the B3LYP/6-311G** level of theory according to the approach and equations described previously [19].

4.2. Molecular Electrostatic Potential Surface (MEPS) analysis. Figure 4 shows the MEPS [26] of 1,5-naphthyridines fused with chromene **5** and **10** and Figure 5 shows the MEPS of 1,5-naphthyridines fused with chromenone **9** and **11** which were calculated using DFT [27] with the standard basis set B3LYP / 6-311G (d, p) level of theory. From these calculations, it can be seen in Figure 4 that compounds **5** and **10** have a local negative electrostatic potential close to the nitrogen at position 8 of the tetracyclic ring system. Furthermore, in the case of compounds **5d** and **10d** (Chart 1, Figure 4) due to the presence of the methoxy group (OCH_3), a local negative electrostatic potential appears in the oxygen at position 9 where the methoxy group is located, greater than in the other member compounds of this group. Noteworthy, these compounds **5d** and **10d** are cytotoxic and present the highest values of TopI inhibition (Table 1 and Table 2, entries 5 and 13). Considering the limits of the electrostatic potentials calculated for these two molecules, **5d** and **10d** (Figure 4), the ranges are much wider than for the rest of compounds **5** and **10**, which may suggest that the electrostatic interactions with the target are an important factor. On the other hand, the presence of a fluorine substituent (F) at position 2 of compound **5b** (with the minor cytotoxicity of this group) modifies the local positive electrostatic potential that appears in this position for **5a**, **5c** and **5d**. A particular electrostatic potential map is also observed for compound **10g**, with two fluorine atoms in its structure (Chart 2, Figure 4). In this case, the positioning of local positive electrostatic potential has been modified, being situated over the hydrogens of aromatic ring at position 7 of the tetracyclic system. By chance, this fluorinated chromeno[4,3-*b*][1,5]naphthyridine **10g** showed a good inhibition of TopI at short reaction time (Table 1, entry 16) which is consistent with the biological role of fluorine derivatives [21].

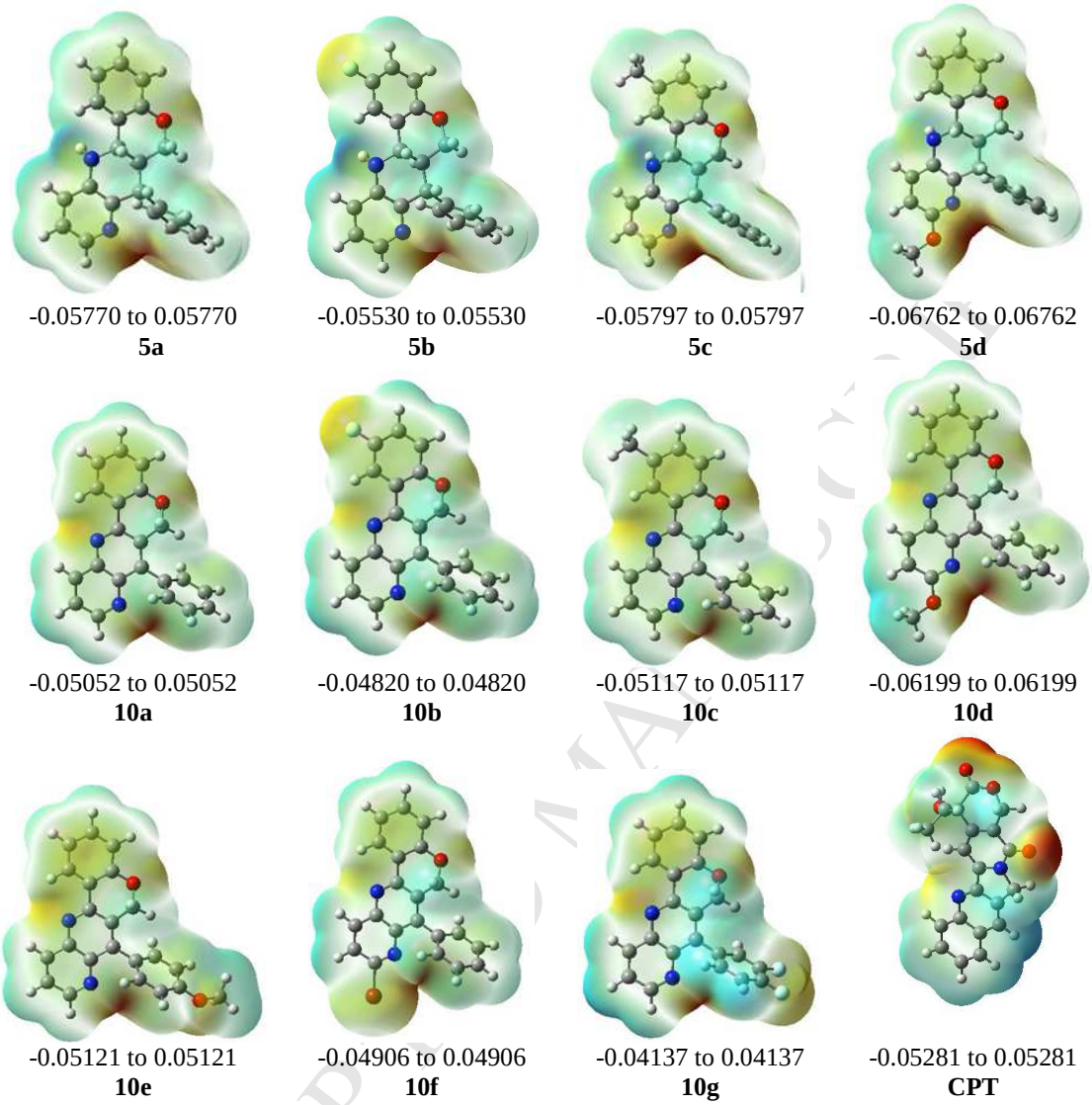


Figure 4. MEP surfaces mapped from total electron density for compounds **5**, **10** and **CPT**. Electrostatic potentials are displayed on a 0.002 a.u. isodensity surface. The limits of electrostatic potentials for each molecule are under surfaces. Potential increases in the following order: red (most negative)/orange/yellow/green/blue (most positive).

As observed in Figure 5, the most negative electrostatic potentials for compounds **9** and **11** are located over the oxygen of carbonyl group and on the nitrogen at position 8 of the tetracyclic systems. On the other hand, the tetrahydro derivatives **9a-d**, which showed low inhibitory

activity of TopI, presented the most positive local electrostatic potential over the hydrogen of the NH at position 12.

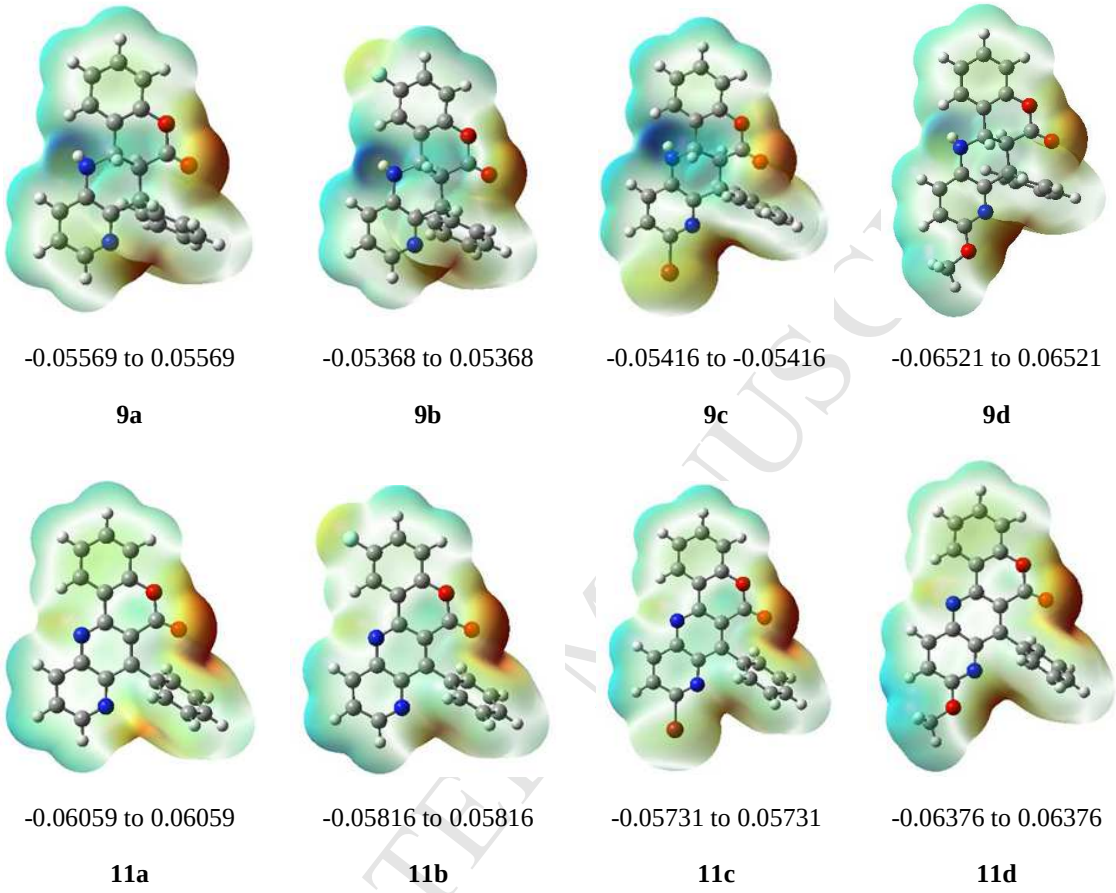


Figure 5. MEP surfaces mapped from total electron density for compounds **9** and **11**. Electrostatic potentials are displayed on a 0.002 a.u. isodensity surface. The limits of electrostatic potentials for each molecule are under surfaces. Potential increases in the following order: red (most negative)/orange/yellow/green/blue (most positive).

A very different electrostatic potential is obtained through these theoretical calculations for the more unsaturated compounds **11**. In this case, a small negative local electrostatic potential appears on the nitrogen at the same position 12 for compounds **11a-d**. In addition, the most biologically active compound in this group, the derivative **11d** (Table 1, entry 20), shows a local positive electrostatic potential over the hydrogens at positions 10 and 11 and over the hydrogens

of the methoxy group (OCH_3) sited at position 9 (Figure 5). Conversely, for compound **11c**, with the lowest value of TopI inhibiton, the presence of bromine atom at the 9 position of the tetracyclic system makes that a small negative electrostatic potential appears at that position.

4.3. Docking studies. Among all the compounds synthesized in this work, a molecular docking study of those who showed significant inhibition of TopI was carried out to investigate its plausible binding pattern and its interaction with the key amino acids and DNA nucleobases in the active site of the TopI. The model was derived by docking of compounds **5c**,**d**, **10d,g** and **11d**, in the camptothecin binding site of the camptothecin−TopI−DNA ternary complex (PDB ID: 1T8I) [29].

The most important evaluation criterion was the observation of whether the ligands were located between C112-TGP11 and A113-T10 nucleobases, where the DNA rupture site is located, avoiding the re-ligation of such bases, according to the concept of interfacial inhibition proposed by Pommier [1]. The formation of hydrogen bonds with important residues [30] and the existence of hydrophobic interactions with TopI residues and DNA were also taken into account.

Likewise, based on the above interactions, the obtained values from the gscore parameters indicate the virtual affinity of the ligands to the complex, and gemodel, which is the theoretical value of the interaction energy of the ligand with the TopI/DNA complex, were considered (see Table 4).

Table 4. gscore and gemodel values for compounds **5c,d**, **10d,g**, **11d** and **CPT** TopI inhibitors.

Entry	Compound	gscore (kcal/mol)	gemodel (kcal/mol)
1	**5c**	- 5.354	- 50.613
2	**5d**	- 4.934	- 63.067
3	**10d**	- 5.473	- 67.251

4	**10g**	- 5.125	- 62.562
5	**11d**	-5.413	- 69.372
6	**CPT**	-8.074	- 91.172

The studied compounds have a weak affinity to the receptor with a low score and interaction energy values, which is consistent with experimental results. The different orientation modes and the interactions of each compound with nucleobases and various amino acid residues can be seen in Figure S1 and S2 of the supporting information. And according to the observed docking results, the structural part corresponding to chromene or coumarin (chromenone) participates in interactions with the active site oriented towards the minor grove, establishing π-π stacking interactions with nucleobases and suggesting that those heterocycles may play an important role in the interaction with the target.

4.4. In silico ADME/T analysis. The synthesized compounds were submitted to *in silico* pharmacokinetic properties prediction by using the graphical interface Maestro [31] using QikProp module of Schrödinger software. The results are included in Table S1 of Supporting Material. Thus, estimated number of hydrogen bonds that would be donated and accepted by the solute to the water molecules in an aqueous solution are in the range of 0.0-1.0 and the 2.75-4.50 respectively. Predicted octanol/water partition coefficient value are in the range of 3.72-5.24, indicating that the compounds present an high value of log P. Number of metabolic reactions of compounds **5** and **9** is high (in the range of 7 to 9), while for the compounds **10** and **11** is in the range of 1 to 3. All compounds have % Human Oral Absortion of 100%. Prediction of binding to human serum albumin for the compounds are in the range 0.27-9.67 and predicted brain/blood partition coefficient are in the range of -0.067 to 0.586. Number of violations of Lipinsky's rule

of five is 0-1. Thus, it was observed that most of the compounds were found to be within the limit of approved drug parameter range.

5. Conclusions

In summary, the preparation of hybrid compounds derived from tetrahydro-1,5-naphthyridines and heterocycles such as chromenes and chromen-2-ones (coumarins) is reported by a regio- and stereospecific intramolecular [4+2]-cycloaddition reaction. Subsequent dehydrogenation of 1,5-naphthyridine core allowed the isolation of corresponding 6*H*-chromeno[4,3-*b*][1,5]naphthyridines **10** and 6*H*-chromeno[4,3-*b*][1,5] naphthyridin-6-ones **11**. Some of these compounds showed selective inhibitory effects on human TopI mediated relaxation assays compatible to those observed for the natural inhibitor CPT. All the prepared derivatives have been further subjected to evaluation of their cytotoxicity against carcinomic human alveolar basal epithelial cell line (A549) and human ovarian carcinoma cell line (SKOV3). The most cytotoxic derivative in both cancerous cell lines resulted to be the *endo* 7-phenyl-6a,7,12,12a-tetrahydro-6*H*-chromeno[4,3-*b*][1,5]naphthyridine **5a**. However, in general, these preliminary studies revealed that some of newly synthesized compounds, such as **5c**, **5d** and **11a** exhibited significant cytotoxic effect against A549 cell line selectively. Moreover, when cytotoxicity was tested against non-malignant pulmonary fibroblasts (MRC-5), none of the compounds presented any activity.

The physicochemical properties of these hybrid compounds have been evaluated, and could be considered as candidates for drugs with promising pharmacotherapeutic profiles similar to those of currently used drugs. In addition, docking experiments inform us about the possible mode of

binding of these compounds and according to these estudies the structural part corresponding to chromene or coumarin seems to play an important role in the interaction with the site active. The interesting biochemical and biological features found for these compounds provide a promising basis for further development of biologically active naphthyridine derivatives.

6. Experimental protocols

6.1 Chemistry

6.1.1. 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). 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 deuterated 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.

6.1.2. 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, 5μm, 0.4

mm×25 mm). 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 **5**, **9**, **10** and **11**) is >95%, which meets the purity requirement by the Journal.

6.1.3. **Synthesis of aldimines 3, 7 and 13**

6.1.3.1 **General procedure.** To a solution of the corresponding aldehyde (1 mmol) in $CHCl_3$ (20 mL) was added the corresponding 3-aminopyridine (1 mmol,) in presence of molecular sieve (4Å) and two drops of $BF_3 \cdot Et_2O$. The mixture was refluxed for 16h and was checked by ^1H NMR and/or ^{19}F NMR spectroscopy. Due to the instability of the aldimines, they were used without purification for the following reactions.

6.1.3.1.1 ***(E)-1-(2-(Cinnamyloxy)phenyl)-N-(pyridin-3-yl)methanimine*** **(3a)** The general procedure was followed using aldehyde **1a** (1 mmol, 0.238 g) and 3-aminopyridine (1 mmol, 0.094 g). ^1H RMN (300 MHz, $CDCl_3$): δ = 4.71 (dd, $^3J_{HH}$ = 5.8 Hz, $^2J_{HH}$ = 1.4 Hz, 2 H, CH_2), 6.34 (dt,$^3J_{HH}$ = 15.8 Hz, $^3J_{HH}$ = 5.8 Hz, $^3J_{HH}$ = 5.8 Hz, 1H, =CH), 6.65 (d, $^3J_{HH}$ = 15.8 Hz, 1H,=CH), 6.92-7.01 (m, 2 H, H_{arom}), 7.17-7.46 (m, 8H, H_{arom}), 8.11 (dd, $^3J_{HH}$ = 7.9 Hz, $^4J_{HH}$ = 1.6 Hz, 1H, H_{arom}), 8.38 (dd, $^3J_{HH}$ = 4.7 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, H_{arom}), 8.43 (d, $^4J_{HH}$ = 2 Hz, 1H, H_{arom}), 8.90 (s, 1H, HC=N) ppm.

6.1.3.1.2 ***(E)-1-(2-(Cinnamyloxy)-5-fluorophenyl)-N-(pyridin-3-yl)methanimine*** **(3b)** The general procedure was followed using aldehyde **1b** (1 mmol, 0.256 g) and 3-aminopyridine (1 mmol, 0.094 g). ^1H RMN (300 MHz, $CDCl_3$): δ = 4.67 (d, Hz, $^3J_{HH}$ = 5.5 Hz, 2 H, CH_2), 6.30 (dt,$^3J_{HH}$ = 15.8 Hz, $^3J_{HH}$ = 5.8 Hz, 1H, =CH), 6.63 (d, $^3J_{HH}$ = 15.8 Hz, 1H, =CH), 6.85-7.46 (m, 9 H, H_{arom}), 7.78 (dd, $^3J_{HH}$ = 8.9 Hz, $^4J_{HH}$ = 3.0 Hz, 1 H, H_{arom}), 8.37 (d, $^3J_{HH}$ = 5.0 Hz, 1 H, H_{arom}), 8.41 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, H_{arom}) 8.83 (s, $^4J_{HH}$ = 1.6 Hz, HC=N) ppm.

6.1.3.1.3 ***(7E)-N-(2-(Cinnamyloxy)-5-methylbenzylidene)pyridin-3-amineamine*** **(3c)** The general procedure was followed using aldehyde **1c** (1 mmol, 0.252 g) and 3-aminopyridine (1 mmol, 0.094 g). ^1H RMN (300 MHz, $CDCl_3$): δ = 2.28 (s, 3 H, CH_3), 4.70 (dd, Hz, $^2J_{HH}$ = 6.0

Hz, $^{3}J_{HH}$ = 1.6 Hz, 2 H, CH_2), 6.35 (dt,$^{3}J_{HH}$ = 16.0 Hz, $^{3}J_{HH}$ = 6.0 Hz, 1 H, =CH), 6.65 (d, $^{3}J_{HH}$ = 16.0 Hz, 1 H, =CH), 6.85 (d, $^{3}J_{HH}$ = 8.5 Hz, 1 H, H_{arom}), 7.17-7.46 (m, 8 H, H_{arom}), 7.90 (d, $^{3}J_{HH}$ = 2.0 Hz, 1 H, H_{arom}), 8.39 (dd, $^{3}J_{HH}$ = 4.5 Hz, $^{4}J_{HH}$ = 1.6 Hz, 1 H, H_{arom}), 8.43 (d, $^{4}J_{HH}$ = 2.1 Hz, 1 H, H_{arom}), 8.89 (s, 1H, HC=N) ppm.

6.1.3.1.4 ***(E)-1-(2-(Cinnamyloxy)-5-methylphenyl)-N-(pyridin-3-yl)methanimine*** **(3d)** The general procedure was followed using aldehyde **1a** (1 mmol, 0.238 g) and 6-methoxypyridin-3-amine (1 mmol, 0.124 g). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 3.96 (s, 3H, CH_3), 4.79 (dd, $^{3}J_{HH}$ = 6.0 Hz, $^{2}J_{HH}$ = 1.5 Hz, 2 H, CH_2), 6.43 (dt,$^{3}J_{HH}$ = 16.0 Hz, $^{3}J_{HH}$ = 6.0 Hz, $^{3}J_{HH}$ = 6.0 Hz, 1H, =CH), 6.77 (d, $^{3}J_{HH}$ = 16.0 Hz, 1H,=CH), 6.99-7.02 (m, 2 H, H_{arom}), 7.16-7.45 (m, 8H, H_{arom}), 8.12 (dd, $^{3}J_{HH}$ = 2.7 Hz, $^{4}J_{HH}$ = 0.6 Hz, 1H, H_{arom}), 8.17 (dd, $^{3}J_{HH}$ = 7.7 Hz, $^{4}J_{HH}$ = 1.8 Hz, 1H, H_{arom}), 9.01 (s, 1H, HC=N) ppm.

6.1.3.1.5 ***2-((E)-(Pyridin-3-ylimino)methyl)phenyl cinnamate*** **(7a)** The general procedure was followed using aldehyde **6a** (1 mmol, 0.252 g) and 3-aminopyridine (1 mmol, 0.094 g). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 6.60 (d, Hz, $^{3}J_{HH}$ = 15.8 Hz, 1 H, =CH), 7.16-7.52 (m, 10 H, H_{arom}), 7.84 (d, $^{3}J_{HH}$ = 15.8 Hz, 1 H, =CH), 8.12 (dd, $^{3}J_{HH}$ = 8.0 Hz, $^{4}J_{HH}$ = 1.8 Hz, 1 H, H_{arom}), 8.37-8.39 (m, 1 H, H_{arom}), 8.51 (s, 1H, HC=N) ppm.

6.1.3.1.6 ***4-Fluoro-2-((E)-(pyridin-3-ylimino)methyl)phenyl cinnamate*** **(7b)** The general procedure was followed using aldehyde **6b** (1 mmol, 0.270 g) and 3-aminopyridine (1 mmol, 0.094 g). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 6.58 (d, $^{3}J_{HH}$ = 16.0 Hz, 1H, =CH), 7.12-7.51 (m, 9H, H_{arom}), 7.82-7.88 (m, 2 H, =CH y H_{arom}), 8.37-8.48 (m, 3H, 2 H_{arom} y HC=N) ppm.

6.1.3.1.7 ***2-((E)-((6-Bromopyridin-3-yl)imino)methyl)phenyl cinnamate*** **(7c)** The general procedure was followed using aldehyde **6a** (1 mmol, 0.252 g) and 6-bromopyridin-3-amine (1 mmol, 0.171 g). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 6.72 (d, Hz, $^{3}J_{HH}$ = 16.0 Hz, 1 H, =CH), 7.23-7.28 (m, 1 H, H_{arom}),7.32-7.48 (m, 8H, H_{arom}), 7.53-7.63 (m, 4H, H_{arom}), 7.92 (d, $^{3}J_{HH}$ = 16.0 Hz, 1 H, =CH), 8.16-8.21 (m, 2 H, H_{arom}), 8.56 (s, 1H, HC=N) ppm.

6.1.3.1.8 ***2-((E)-((6-Methoxypyridin-3-yl)imino)methyl)phenyl cinnamate*** **(7d)** The general procedure was followed using aldehyde **6a** (1 mmol, 0.252 g) and 6-methoxypyridin-3-amine (1 mmol, 0.124 g). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 3.93 (s, 3H, CH_3), 6.72 (d, Hz, $^{3}J_{HH}$ = 16.0 Hz,

1 H, =CH), 7.34-7.64 (m, 10 H, H_{arom}), 7.93 (d, $^3J_{HH}$ = 16.0 Hz, 1 H, =CH), 8.07 (d, $^3J_{HH}$ = 2.9 Hz, 1 H, H_{arom}), 8.20 (d, $^3J_{HH}$ = 8.0 Hz $^3J_{HH}$ = 2.8 Hz, 1 H, H_{arom}), 8.62 (s, 1H, HC=N) ppm.

6.1.3.1.9 ***(E)-1-(2-((3-Phenylprop-2-yn-1-yl)oxy)phenyl)-N-(pyridin-3-yl)methanimine*** **(13a)** The general procedure was followed using aldehyde **12a** (1 mmol, 0.236 g) and 3-aminopyridine (1 mmol, 0.094 g). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 5.03 (s, 2H, CH_2), 7.10-7.54 (m, 10 H, $H_{arom.}$), 8.19 (dd, $^3J_{HH}$ = 8.0 Hz, $^4J_{HH}$ = 2.0 Hz, 1 H, H_{arom}), 8.47 (dd, $^3J_{HH}$ = 4.7 Hz, $^4J_{HH}$ = 1.5 Hz, 1 H, H_{arom}), 8.50 (d, $^3J_{HH}$ = 2.5 Hz, 1 H, H_{arom}), 8.98 (s, 1H, HC=N) ppm.

6.1.3.1.10 ***(E)-1-(2-((3-(4-Methoxyphenyl)prop-2-yn-1-yl)oxy)phenyl)-N-(pyridin-3-yl)methanimine*** **(13b)** The general procedure was followed using aldehyde **12c** (1 mmol, 0.266 g) and 3-aminopyridine (1 mmol, 0.094 g). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 3.72 (s, 3 H, OCH_3), 4.97 (d, $^3J_{HH}$ = 8.0 Hz, 2H, CH_2), 6.76 (dd, $^3J_{HH}$ = 9.0 Hz, $^3J_{HH}$ = 5 Hz, 2 H, $H_{arom.}$), 6.99-7.31 (m, 7 H, $H_{arom.}$), 7.66 (dd, $^3J_{HH}$ = 9 Hz, $^4J_{HH}$ = 2 Hz, 1 H, $H_{arom.}$), 8.19 (dd, $^3J_{HH}$ = 7.5 Hz, $^4J_{HH}$ = 2 Hz, 1 H, $H_{arom.}$),8.43 (dd, $^3J_{HH}$ = 4.9 Hz, $^4J_{HH}$ = 1.7 Hz, 1 H, $H_{arom.}$), 9.49 (s, 1H, HC=N) ppm.

6.1.3.1.11 ***(E)-1-(2-((3-(3,4-Difluorophenyl)prop-2-yn-1-yl)oxy)phenyl)-N-(pyridin-3-yl)methanimine*** **(13c)** The general procedure was followed using aldehyde **12d** (1 mmol, 0.272 g) and 3-aminopyridine (1 mmol, 0.094 g). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 4.92 (s, 2 H, CH_2), 6.92-7.25 (m, 6 H, $H_{arom.}$), 7.37-7.48 (m, 2 H, $H_{arom.}$), 8.11 (dd, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 1.7 Hz, 1 H, $H_{arom.}$), 8.39 (dd, $^3J_{HH}$ = 5.2 Hz, $^4J_{HH}$ = 1.5 Hz, 1 H, $H_{arom.}$), 8.42 (d, $^3J_{HH}$ = 2.2 Hz, 1 H, $H_{arom.}$), 8.88 (s, 1H, HC=N) ppm.

6.1.4. **Synthesis of *6a,7,12,12a*-tetrahydro-6*H*-chromeno[4,3-*b*][1,5]naphthyridines 5, *6a,7,12,12a*-tetrahydro-6*H*-chromeno[4,3-*b*][1,5]naphthyridin-6-ones 9 derivatives and 6*H*-chromeno[4,3-*b*][1,5]naphthyridine 10f.**

6.1.4.1 **General procedure.** To a solution of the corresponding aldimine (1 mmol) generated *in situ* in $CHCl_3$ (20 mL) was added $BF_3{\cdot}Et_2O$ (2 mmol, 0.25 mL) and was refluxed for 16 h. 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 solvent was removed under vacuum affording an oil that was purified by silica gel flash column

chromatography using a elution of 20-80% ethyl acetate - hexane to afford products **5** or **9** or **10f**.

6.1.4.1.1 ***7-Phenyl-6a,7,12,12a-tetrahydro-6H-chromeno[4,3-b][1,5]naphthyridine*** **(5a).** The general procedure was followed using aldimine **3a** affording 0.175 g (56 %) of a white solid identified as **5a**, mp 164-165ºC (ethyl acetate/hexane). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 2.56 (dddd, $^{3}J_{HH}$ = 12.0 Hz, $^{3}J_{HH}$ = 11.0 Hz, $^{3}J_{HH}$ = 10.0 Hz, $^{3}J_{HH}$ = 3.7 Hz, 1 H, CH), 3.97 (dd, $^{3}J_{HH}$ = 11.0 Hz, $^{2}J_{HH}$ = 11.0 Hz, 1 H, CH_2), 3.98 (d, $^{3}J_{HH}$ = 4.0 Hz, 1 H, CH), 4.12 (dd, $^{2}J_{HH}$ = 11.0 Hz, $^{3}J_{HH}$ = 3.7 Hz, 1 H, CH_2), 4.40 (s, 1 H, NH), 4.51 (d, $^{3}J_{HH}$ = 10.0 Hz, 1 H, CH), 6.87 (dd, $^{3}J_{HH}$ = 8.0 Hz, $^{3}J_{HH}$ = 1.5 Hz, 1 H, H_{arom}), 6.98-7.37 (m, 10 H, H_{arom}), 8.03 (dd, $^{3}J_{HH}$ = 4.5 Hz, $^{3}J_{HH}$ = 4.5 Hz, 1 H, H_{arom}) ppm. ^{13}C {^{1}H} RMN (75 MHz, $CDCl_3$): δ = 41.9 (CH), 49.7 (CH), 51.8 (CH), 67.4 (CH_2), 117.2 (CH), 120.8 (CH), 121.9 (C), 122.2 (CH), 122.9 (CH), 124.7 (CH), 126.9 (CH), 128.7 (2 CH), 128.9 (3 CH), 140.9 (CH), 140.8 (C), 142.3 (C), 145.3 (C), 154.1 (C) ppm. HRMS (EI) calculated for $C_{21}H_{18}N_2O$ $[M]^+$ 314.1419; found 314.1428. Purity 96.00 % (EtOH/Heptane = 10/90, Rt = 6.50 min).

6.1.4.1.2 ***2-Fluoro-7-phenyl-6a,7,12,12a-tetrahydro-6H-chromeno[4,3-b][1,5]naphthyridine*** **(5b).** The general procedure was followed using aldimine **3b**, affording 0.258 g (78 %) of a white solid identified as **5b** mp 198-199 ºC (ethyl acetate/hexane). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 2.50 (dddd, $^{3}J_{HH}$ = 11.7 Hz, $^{3}J_{HH}$ = 11.2 Hz, $^{3}J_{HH}$ = 10.7 Hz, $^{3}J_{HH}$ = 3.7 Hz, 1 H, CH), 3.91 (dd, $^{3}J_{HH}$ = 11.2 Hz, $^{2}J_{HH}$ = 11.2 Hz,1 H, CH_2), 3.97 (d, $^{3}J_{HH}$ = 11.7 Hz, 1 H, CH), 4.10 (dd, $^{2}J_{HH}$ = 11.2 Hz, $^{3}J_{HH}$ = 3.7 Hz, 1 H, CH_2), 4.22 (s, 1H, NH), 4.46 (d, $^{3}J_{HH}$ = 10.7 Hz, 1 H, CH), 6.78 (dd, $^{3}J_{HH}$ = 8.6 Hz, $^{3}J_{HH}$ = 4.7 Hz, 1 H, H_{arom}), 6.88-6.94 (m, 1 H, H_{arom}), 6.98-7.13 (m, 4 H, H_{arom}), 7.23-7.36 (m, 3 H, H_{arom}), 8.02 (dd, $^{3}J_{HH}$ = 4.5 Hz, $^{4}J_{HH}$ = 1.6 Hz, 1 H, H_{arom}) ppm. ^{13}C {^{1}H} RMN (75 MHz, $CDCl_3$): δ = 41.8 (CH), 49.7 (CH), 51.9 (CH), 67.6 (CH_2), 111.3 (d, $^{2}J_{C-F}$ = 24.0 Hz, CH), 115.8 (d, $^{2}J_{C-F}$ = 24.0 Hz, CH), 118.3 (d, $^{3}J_{C-F}$ = 8.0 Hz, CH), 122.4 (CH), 123.0 (d, $^{3}J_{C-F}$ = 6.0 Hz, C), 123.3 (CH), 127.1 (CH), 128.9 (2 CH), 129.1 (2 CH), 140.7 (C), 141.2 (CH), 142.2 (C), 145.5 (C), 150.3 (C), 157.2 (d, $^{1}J_{C-F}$ = 239.4 Hz, CF) ppm. ^{19}F RMN (282 MHz, $CDCl_3$): δ = -123.3 to -123.4 (m) ppm. HRMS (EI) calculated for $C_{21}H_{17}FN_2O$ $[M]^+$ 332.1325; found 332.1314. Purity 95.20 % (EtOH/Heptane = 10/90, Rt = 4.60 min).

6.1.4.1.3 ***2-Methyl-7-phenyl-6a,7,12,12a-tetrahydro-6H-chromeno[4,3-b][1,5]naphthyridine*** **(5c).** The general procedure was followed using aldimine **3c**, affording 0.208 g (64 %) of a white

solid identified as **5c** mp 139-140 ºC (ethyl acetate/hexane). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 2.51 (s, 3H, CH_3) 2.68 (dddd, $^{3}J_{HH}$ = 11.7 Hz, $^{3}J_{HH}$ = 11.0 Hz, $^{3}J_{HH}$ = 10.2 Hz, $^{3}J_{HH}$ = 3.6 Hz, 1H, CH), 4.08 (t, $^{3}J_{HH}$ = 11.0 Hz, $^{2}J_{HH}$ = 11.0 Hz, 1 H, CH_2), 4.15 (d, $^{3}J_{HH}$ = 11.7 Hz, 1 H, CH), 4.26 (dd, $^{3}J_{HH}$ = 11.0 Hz, $^{3}J_{HH}$ = 3.6 Hz, 1H, CH_2), 4.55 (s, 1H, NH), 4.63 (d, $^{3}J_{HH}$ = 10.2 Hz, 1H, CH), 6.92 (d, $^{3}J_{HH}$ = 8.0 Hz, 1 H, H_{arom}), 7.15-7.29 (m, 6 H, H_{arom}), 7.43-7.52 (m, 3 H, H_{arom}), 8.17 (dd, $^{3}J_{HH}$ = 4.5 Hz, $^{3}J_{HH}$ = 1.5 Hz, 1 H, H_{arom}) ppm.^{13}C {^{1}H} RMN (75 MHz, $CDCl_3$): δ = 20.8 (CH_3), 42.2 (CH), 49.8 (CH), 51.9 (CH), 67.4 (CH_2), 117.1 (CH), 121.6 (C), 122.3 (CH), 122.9 (CH), 125.1 (CH), 127.0 (CH), 128.8 (2 CH), 129.1 (2 CH), 129.7 (CH), 130.1 (C), 140.8 (CH), 141.0 (C), 142.4 (C), 145.5 (C), 152.0 (C), ppm. HRMS (EI) calculated for $C_{22}H_{20}N_2O$ $[M]^+$ 328.1576; found 328.1577. Purity 97.00 % (EtOH/Heptane = 10/90, Rt = 6.10 min).

6.1.4.1.4 ***9-Methoxy-7-phenyl-6a,7,12,12a-tetrahydro-6H-chromeno[4,3-b][1,5]naphthyridine*** **(5d).** The general procedure was followed using aldimine **3d**, affording 0.300 g (87 %) of a white solid identified as **5d** mp 214-215 ºC (ethyl acetate/hexane). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 2.37-2.47 (m, 1H, CH), 3.42 (s, 3H, OCH_3), 3.79 (d, $^{3}J_{HH}$ = 12.0 Hz, 1 H, CH), 3.85 (t, $^{3}J_{HH}$ = 11.0 Hz, $^{2}J_{HH}$ = 11.0 Hz, 1 H, CH_2), 4.10 (dd, $^{3}J_{HH}$ = 10.7 Hz, $^{3}J_{HH}$ = 3.5 Hz, 1H, CH_2), 4.33 (d, $^{3}J_{HH}$ = 10.7 Hz, 1H, CH), 6.43 (dd, $^{3}J_{HH}$ = 8.6 Hz, $^{4}J_{HH}$ = 09 Hz, 1 H, H_{arom}), 6.76 (dd, $^{3}J_{HH}$ = 8.0 Hz, $^{4}J_{HH}$ = 09 Hz, 1 H, H_{arom}), 6.90-6.93 (m, 1 H, H_{arom}), 7.01-7.07 (m, 3H, H_{arom}), 7.12-7.24 (m, 6 H, H_{arom}), 7.31 (d, $^{3}J_{HH}$ = 4.0 Hz,1 H, H_{arom}).^{13}C {^{1}H} RMN (75 MHz, $CDCl_3$): δ = 41. 6 (CH), 49.4 (CH), 52.5 (CH), 53.2 (CH_3), 67.7 (CH_2), 109.4 (CH), 117.2 (CH), 120.9 (CH), 122.6 (C), 125.3 (CH), 126.7 (CH), 128.3 (CH), 129.0 (2 CH), 129.2 (CH), 129.3 (2 CH), 134.7 (C), 141.7 (C), 142.3 (C), 154.4 (C), 157.7 (C), ppm. HRMS (EI) calculated for $C_{22}H_{20}N_2O_2$ $[M]^+$ 344.1525; found 344.1252. Purity 95.00 % (EtOH/Heptane = 10/90, Rt = 5.33 min).

6.1.4.1.5 ***7-Phenyl-6a,7,12,12a-tetrahydro-6H-chromeno[4,3-b][1,5]naphthyridin-6-one*** **(9a).** The general procedure was followed using aldimine **7a**, affording 0.311 g (95 %) of a white solid identified as **9a** mp 225-226 ºC (ethyl acetate/hexane). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 3.26 (dd, $^{3}J_{HH}$ = 13.0 Hz, $^{3}J_{HH}$ = 10.0 Hz, 1 H, CH), 3.77 (s, 1 H, NH), 4.90 (d, $^{3}J_{HH}$ = 13.0 Hz, 1 H, CH), 4.27 (d, $^{3}J_{HH}$ = 10.0 Hz, 1 H, CH), 6.93-7.43 (m, 16 H, H_{arom}), 8.26 (d, $^{3}J_{HH}$ = 4.5 Hz, 1H, H_{arom}) ppm. ^{13}C {^{1}H} RMN (75 MHz, $CDCl_3$): δ = 46.2 (CH), 49.5 (CH), 49.7 (CH), 117.4 (CH), 122.2 (CH), 123.4 (CH), 124.1 (C), 124.8 (CH), 126.8 (CH), 128.0 (CH), 128.6 (2 CH), 129.4 (2 CH), 129.7 (CH), 139.2 (C), 142.3 (CH), 144.4 (C), 146.2 (C), 150.0 (C), 168.0 (COO)

ppm. HRMS (EI) calculated for $C_{21}H_{16}N_2O_2$ $[M]^+$ 328.1212; found 328.1217. Purity 98.10 % (EtOH/Heptane = 10/90, Rt = 5.40 min).

6.1.4.1.6 ***2-Fluoro-7-phenyl-6a,7,12,12a-tetrahydro-6H-chromeno[4,3-b][1,5]naphthyridin-6-one (*****9b).** The general procedure was followed using aldimine **7b**, affording 0.311 g (90 %) of a white solid identified as **9b** mp 291-292 ºC (ethyl acetate/hexane). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 3.26 (dd, $^3J_{HH}$ = 13.0 Hz, $^3J_{HH}$ = 10.0 Hz, 1H, CH), 4.38 (s, 1H, NH), 4.56 (d, $^3J_{HH}$ = 13.0 Hz, 1H, CH), 4.87 (d, $^3J_{HH}$ = 10.0 Hz, 1H, CH), 7.00-7.27 (m, 10 H, H_{arom}), 8.10 (dd, $^3J_{HH}$ = 4.5 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, H_{arom}), ppm. ^{13}C {^{1}H} RMN (75 MHz, $CDCl_3$): δ = 46.2 (CH), 49.0 (CH), 49.5 (CH), 110.7 (d, $^2J_{CF}$ = 25.6 Hz, CH), 116.3 (d, $^2J_{CF}$ = 23.0 Hz, CH), 118.7 (d, $^3J_{CF}$ = 8.5 Hz, CH), 122.3 (CH), 123.6 (CH), 126.1 (C), 126.8 (CH), 128.7 (2 CH and C), 129.4 (2 CH), 139.0 (C), 142.7 (CH), 144.3 (C), 145.9 (C), 146.2 (C), 159.5 (d, $^1J_{CF}$ = 247.0 Hz,CF), 167.7 (COO) ppm. **^{19}F-RMN** (282 MHz, $CDCl_3$): δ = -117.2 to -117.3 (m) ppm. HRMS (EI) calculated for $C_{21}H_{15}FN_2O_2$ $[M]^+$ 346.1118; found 3469.1222. Purity 98.30 % (EtOH/Heptane = 10/90, Rt = 4.50 min).

6.1.4.1.7 ***9-Bromo-7-phenyl-6a,7,12,12a-tetrahydro-6H-chromeno[4,3-b][1,5]naphthyridin-6-one (*****9c).** The general procedure was followed using aldimine **7c**, affording 0.362 g (78 %) of a white solid identified as **9c** mp 287-288 ºC (ethyl acetate/hexane). ^{1}H RMN (300 MHz, DMSO-d_6): δ = 3.40 (dd, $^3J_{HH}$ = 12.5 Hz, $^3J_{HH}$ = 10.2 Hz, 1H, CH), 4.61 (d, $^3J_{HH}$ = 10.2 Hz, 1H, CH), 4.78 (d, $^3J_{HH}$ = 12.5 Hz, 1H, CH), 7.11-7.81 (m, 10 H, H_{arom} and NH), 7.80 (d, $^3J_{HH}$ = 7.2 Hz, 1H, CH_{arom}) ppm. ^{13}C {^{1}H} RMN (75 MHz, DMSO-d_6): δ = 45.7 (CH), 46.8 (CH), 48.3 (CH), 116.3 (CH), 124.3 (CH) 124.5 (C), 124.9 (CH), 126.1 (CH), 126.1 (CH), 126.2 (CH), 127.9 (C), 128.0 (2CH), 129.1 (CH), 129.4 (2CH), 140.4(C), 143.9 (C), 145.5 (C), 149.4 (C), 167.7 (COO) ppm. HRMS (EI) calculated for $C_{21}H_{15}BrN_2O_2$ $[M]^+$ 406.0317; found 406.0313. Purity 95.00 % (EtOH/Heptane = 10/90, Rt = 4.50 min).

6.1.4.1.8 ***9-Methoxy-7-phenyl-6a,7,12,12a-tetrahydro-6H-chromeno[4,3-b][1,5]naphthyridin-6-one (*****9d).** The general procedure was followed using aldimine **5d**, affording 0.295 g (82 %) of a white solid identified as **9d** mp 172-173 ºC (ethyl acetate/hexane). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 3.25 (dd, $^3J_{HH}$ = 13.0 Hz, $^3J_{HH}$ = 10.0 Hz, 1H, CH), 3.57 (s, 3H, OCH_3), 4.06 (s, 1H, NH), 4.50 (d, $^3J_{HH}$ = 13.0 Hz, 1H, CH), 4.76 (d, $^3J_{HH}$ = 10.0 Hz, 1H, CH), 6.50 (d, $^3J_{HH}$ = 8.6 Hz, 1H, CH_{arom}), 7.08-7.48 (m, 10 H, H_{arom}) ppm. ^{13}C {^{1}H} RMN (75 MHz, $CDCl_3$): δ = 46.1 (CH),

49.2 (CH), 50.3 (CH), 53.3 (OCH_3), 109.5 (CH), 117.21 (CH) 123.7 (CH), 124.6 (C), 124.8 (CH), 126.4 (C), 128.2 (CH), 128.3 (2 CH), 129.0 (CH), 129.1 (CH), 129.3 (2CH), 129.6 (CH), 142.5 (C), 144.4 (C), 150.1 (C), 158.4 (C), 168.4 (COO) ppm. HRMS (EI) calculated for $C_{22}H_{18}N_2O_3$ $[M]^+$ 358.1317; found 358.1317. Purity 96.10 % (EtOH/Heptane = 10/90, Rt = 4.00 min).

6.1.4.1.9 ***9-Bromo-7-phenyl-6H-chromeno[4,3-b][1,5]naphthyridine (*10f).** The general procedure was followed using aldimine **3f**, affording 0.295 g (82 %) of a white solid identified as **10f** mp 219-220 ºC (ethyl acetate/hexane). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 5.24 (s, 2 H, CH_2), 7.00 (d, $^3J_{HH}$ = 8.2 Hz, 1 H, H_{arom}), 7.17-7.56 (m, 7 H, H_{arom}), 7.69 (d, $^3J_{HH}$ = 8.0 Hz, 1 H, H_{arom}), 8.247 (d, $^3J_{HH}$ = 8.1 Hz, 1 H, H_{arom}), 8.46 (d, $^3J_{HH}$ = 8.8 Hz, 1 H, H_{arom}) ppm. ^{13}C {^{1}H} RMN (75 MHz, $CDCl_3$): δ = 66.9 (CH_2), 117.5 (CH), 122.9 (CH), 123.1 (C), 126.0 (CH), 127.2 (C), 128.4 (2CH, C), 128.9 (CH), 129.4 (CH), 130.3 (2CH), 132.7 (CH), 133.1 (C), 139.66 (CH), 143.0 (2C), 144.0 (CH), 150.2 (C), 157.5 (C) ppm. HRMS (EI) calculated for $C_{21}H_{13}BrN_2O$ $[M]^+$ 388.0211; found 388.0221. Purity 98.60 % (EtOH/Heptane = 10/90, Rt = 3.90 min).

6.1.5. **Synthesis of chromeno[4,3-*b*][1,5]naphthyridines 10 and chromeno[4,3-*b*][1,5]naphthyridin-6-ones 11**

6.1.5.1 **General procedure A.** To a solution of the corresponding compound **5** or **9** (1 mmol) in toluene (15 mL) DDQ (1 mmol, 0.227 g) was added and the mixture was stirred for 2 h ant 40 °C under microwaves radiation. The reaction mixture was filtered off on celite 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 **10** or **11**.

General procedure B: To a solution of the corresponding aldimine **13** (1 mmol) in $CHCl_3$ (20 mL) was added $BF_3 \cdot Et_2O$ (2 mmol, 0.25 mL) and the mixture was refluxed for 12 h. The reaction mixture 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 **10**.

6.1.5.1.1 ***7-Phenyl-6H-chromeno[4,3-b][1,5]naphthyridine (*10a).** The general procedure **A** was followed using **5a**, affording 0.275 g (89 %) of a white solid identified as **10a** mp 209-210 ºC (ethyl acetate/hexane). When the general procedure **B** was followed using aldimine **13a**

compound **10a** (0.222 g, 71%) was obtanined. ^{1}H RMN (300 MHz, $CDCl_3$): δ = 5.22 (s, 2 H, CH_2), 7.01 (dd, $^{3}J_{HH}$ = 8.0 Hz, $^{4}J_{HH}$ = 1.0 Hz, 1 H, H_{arom}), 7.19-61 (m, 8 H, H_{arom}), 8.45 (dd, $^{3}J_{HH}$ = 8.5 Hz, $^{4}J_{HH}$ = 1.7 Hz, 1 H, H_{arom}), 8.50 (dd, $^{3}J_{HH}$ = 8.0 Hz, $^{4}J_{HH}$ = 1.6 Hz, 1 H, H_{arom}), 8.88 (dd, $^{3}J_{HH}$ = 4.2 Hz, $^{4}J_{HH}$ = 1.8 Hz, 1 H, H_{arom}) ppm. ^{13}C {^{1}H} RMN (75 MHz, $CDCl_3$): δ = 66.9 (CH_2), 117.4 (CH), 122.8 (CH), 123.2 (C), 124.2 (CH), 126.0 (CH), 126.5 (C), 128.5 (2 CH), 128.8 (CH), 129.9 (2 CH), 132.5 (CH), 134.1 (C), 137.3 (CH), 142.2 (C), 143.7 (C), 144.6 (C), 149.6 (C), 150.5 (CH), 157.4 (C) ppm. HRMS (EI) calculated for $C_{21}H_{14}N_2O_2$ $[M]^+$ 310.1106; found 310.1111. Purity 99.99 % (EtOH/Heptane = 10/90, Rt = 5.40 min).

6.1.5.1.2 ***2-Fluoro-7-phenyl-6H-chromeno[4,3-b][1,5]naphthyridine (*****10b*****).*** The general procedure **A** was followed using **5b**, affording 0.298 g (91 %) of a white solid identified as **10b** mp 236-237 ºC (ethyl acetate/hexane). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 5.20 (s, 2 H, CH_2), 6.96 (dd, $^{3}J_{HH}$ = 8.8 Hz, $^{4}J_{HH}$ = 3.4 Hz, 1 H, H_{arom}), 7.09 (dd, $^{3}J_{HH}$ = 8.0 Hz, $^{3}J_{HH}$ = 3.0 Hz, 1 H, H_{arom}), 7.37-7.39 (m, 2 H, H_{arom}), 7.52-7.63 (m, 4 H, H_{arom}), 8.18 (dd, $^{3}J_{HH}$ = 9.2 Hz, $^{4}J_{HH}$ = 3.1 Hz, 1 H, H_{arom}), 8.45 (dd, $^{3}J_{HH}$ = 8.5 Hz, $^{4}J_{HH}$ = 1.7 Hz, 1 H, H_{arom}), 8.90 (dd, $^{3}J_{HH}$ = 4.1 Hz, $^{4}J_{HH}$ = 1.7 Hz, 1 H, H_{arom}) ppm.^{13}C {^{1}H} RMN (75 MHz, $CDCl_3$): δ = 67.0 (CH_2), 111.7 (d, $^{2}J_{CF}$ = 24.2 Hz, CH), 118.7 (d, $^{3}J_{CF}$ = 7.6 Hz, CH), 119.2 (d, $^{2}J_{CF}$ = 24.0 Hz, CH), 124.4 (CH), 126.2 (C), 128.6 (2 CH), 128.9 (CH), 129.9 (2 CH), 134.0 (C), 137.4 (CH), 142.4 (C), 143.8 (C), 144.9 (C), 150.9 (CH), 153.5 (C), 157.3 (C), 158.4 (d, $^{1}J_{CF}$ = 241.3 Hz, CF) ppm. ^{19}F RMN (282 MHz, $CDCl_3$): -124 to -123.9 (m) ppm. HRMS (EI) calculated for $C_{21}H_{13}FN_2O$ $[M]^+$ 328.1012; found 238.1014. Purity 95.00 % (EtOH/Heptane = 10/90, Rt = 4.70 min).

6.1.5.1.3 ***2-Methyl-7-phenyl-6H-chromeno[4,3-b][1,5]naphthyridine (*****10c*****).*** The general procedure **A** was followed using **5c**, affording 0.324 g (99 %) of a white solid identified as **10c** mp 160-161 ºC (ethyl acetate/hexane). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 2.44 (s, 3 H, CH_3), 5.18 (s, 2 H, CH_2), 6.91 (d, $^{3}J_{HH}$ = 8.5 Hz, 1 H, H_{arom}), 7.21 (ddd, $^{3}J_{HH}$ = 8.4, Hz, $^{4}J_{HH}$ = 2.3 Hz, $^{4}J_{HH}$ = 0.8 Hz, 1 H, H_{arom}), 7.36-7.40 (m, 2 H, CH), 7.49-7.62 (m, 4 H, H_{arom}), 8.30 (d, $^{4}J_{HH}$ = 1.9 Hz, 1 H, H_{arom}), 8.45 (dd, $^{3}J_{HH}$ = 8.5 Hz, $^{4}J_{HH}$ = 1.7 Hz, 1 H, H_{arom}), 8.88 (dd, $^{3}J_{HH}$ = 4.0 Hz, $^{3}J_{HH}$ = 1.6 Hz, 1 H, H_{arom}) ppm. ^{13}C {^{1}H} RMN (75 MHz, $CDCl_3$): δ = 21.0 (CH_3), 63.9 (CH_2), 117.2 (CH), 122.8 (C), 124.2 (CH), 125.9 (CH), 126.8 (C), 128.5 (2 CH), 128.7 (CH), 129.9 (2 CH), 132.2 (C), 133.4 (CH), 134.2 (C), 137.2 (CH), 142.4 (C), 143.8 (C), 144.7 (C), 150.0 (C), 150.4 (CH),

155.5 (C) ppm. HRMS (EI) calculated for $C_{22}H_{16}N_2O$ $[M]^+$ 324.1263; found 324.1268. Purity 95.00 % (EtOH/Heptane = 10/90, Rt = 4.50 min).

6.1.5.1.4 ***9-Methoxy-7-phenyl-6H-chromeno[4,3-b][1,5]naphthyridine (*10d).** The general procedure **A** was followed using **5d**, affording 0.299 g (88 %) of a white solid identified as **10d** mp 219-220 ºC (ethyl acetate/hexane). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 3.91 (s, 3 H, OCH_3), 5.28 (s, 2 H, CH_2), 6.99-7.38 (m, 9 H, H_{arom}), 8.27 (bs, 1 H, H_{arom}), 8.45 (bs, 1 H, H_{arom}) ppm. ^{13}C {^{1}H} RMN (75 MHz, $CDCl_3$): δ = 55.4 (CH_3), 67.1 (CH_2), 113.3 (2 CH), 116.1 (CH), 117.2 (CH), 122.7 (C), 125.6 (CH), 126.1 (C), 126.2 (C), 131.5 (CH), 131.8 (2 CH), 139.9 (C), 140.3 (CH), 141.6 (C), 142.4 (C), 146.9 (C), 156.9 (C), 159.6 (CH), 161.7 (C) ppm. HRMS (EI) calculated for $C_{22}H_{16}N_2O_2$ $[M]^+$ 340.1212; found 340.1219. Purity 95.50 % (EtOH/Heptane = 10/90, Rt = 5.30 min).

6.1.5.1.5 ***7-(4-Methoxyphenyl)-6H-chromeno[4,3-b][1,5]naphthyridine (*10e).** The general **B** procedure was followed using aldimine **13b** affording 0.299 g (88 %) of a white solid identified as **10e** mp 222-223 ºC (ethyl acetate/hexane. ^{1}H RMN (300 MHz, $CDCl_3$): δ = 3.82 (s, 3 H, OCH_3), 5.18 (s, 2 H, CH_2), 6.93 (d, $^3J_{HH}$ = 9.0 Hz, 1 H, H_{arom}), 7.00 (d, $^3J_{HH}$ = 8.6 Hz, 1 H, H_{arom}), 7.06-7.19 (m, 1 H, H_{arom}), 7.28 (d, $^3J_{HH}$ = 8.6 Hz, 2 H, H_{arom}), 7.28-7.35 (m, 2 H, H_{arom}), 7.50 (dd, $^3J_{HH}$ = 8.5 Hz, $^3J_{HH}$ = 4.0 Hz, 1 H, H_{arom}), 8.35 (dd, $^3J_{HH}$ = 8.7 Hz, $^3J_{HH}$ = 1.5 Hz, 1 H, H_{arom}), 8.41 (dd, $^3J_{HH}$ = 7.7 Hz, 1 H, H_{arom}), 8.81 (dd, $^3J_{HH}$ = 4.0 Hz, $^3J_{HH}$ = 1.6 Hz, 1 H, H_{arom}) ppm. ^{13}C {^{1}H} RMN (75 MHz, $CDCl_3$): δ = 55.5 (OCH_3), 67.0 (CH_2), 114.0 (2 CH), 117.4 (CH), 122.6 (CH), 123.3 (C), 124.2 (CH), 126.0 (CH), 126.6 (C), 131.4 (2 CH), 132.4 (CH), 137.4 (CH), 142.6 (C), 143.9 (C), 144.5 (C), 149.8 (C), 150.4 (CH), 157.5 (C), 159.9 (C) ppm. HRMS (EI) calculated for $C_{16}H_{14}O_2$ $[M]^+$ 340.1212; found 340.1212. Purity 98.90 % (EtOH/Heptane = 10/90, Rt = 3.90 min).

6.1.5.1.6 ***7-(3,4-Difluorophenyl)-6H-chromeno[4,3-b][1,5]naphthyridine (*10g).** The general procedure **B** was followed using tetrahydronaphthyridine **13c**, affording 0.281 g (81 %) of a white solid identified as **10h** mp 176-177 ºC (ethyl acetate/hexane. ^{1}H RMN (300 MHz, $CDCl_3$): δ = 5.18 (s, 2H, CH_2), 7.00 (ddd, $^3J_{HH}$ = 8.2 Hz, $^3J_{HH}$ = 1.1 Hz, $^4J_{HH}$ = 0.4Hz 1 H, H_{arom}), 7.07-7.11 (m, 1H, H_{arom}), 7.16-7.21 (m, 1H, H_{arom}), 7.22-7.28 (m, 1H, H_{arom}), 7.31-7.42 (m, 2H, H_{arom}), 7.60 (dd, $^3J_{HH}$ = 8.0 Hz, $^3J_{HH}$ = 4.0 Hz, 1 H, H_{arom}), 8.42 (dd, $^3J_{HH}$ = 8.5 Hz, $^3J_{HH}$ = 1.8 Hz, 1 H, H_{arom}), 8.47 (ddd, $^3J_{HH}$ = 8.0 Hz, $^3J_{HH}$ = 1.7 Hz, $^4J_{HH}$ = 0.4 Hz, 1 H, H_{arom}), 8.86 (dd, $^3J_{HH}$ =

4.0 Hz, $^{3}J_{HH}$ = 1.7 Hz, 1 H, H_{arom}) ppm. ^{13}C {^{1}H} RMN (75 MHz, $CDCl_3$): δ = 66.6 (CH_2), 116.4 (CH), 117.6 (d, $^{2}J_{CF}$ = 18.0 Hz, CH), 119.5 (d, $^{2}J_{CF}$ = 18.0 Hz, CH), 122.8 (CH), 122.9 (C), 124.5 (CH), 126.0 (CH), 126.3 (dd, $^{3}J_{CF}$ = 6.5 Hz, $^{4}J_{CF}$ = 3.8 Hz, CH), 126.5 (C), 130.7 (dd, $^{3}J_{CF}$ = 6.4 Hz, $^{4}J_{CF}$ = 4.2 Hz, C), 132.6 (CH), 137.4 (CH), 141.8 (C), 142.3 (C), 143.8 (CH), 150.5 (dd, $^{1}J_{CF}$ = 248.0 Hz, $^{2}J_{CF}$ = 11.0 Hz, CF), 151.0 (dd, $^{1}J_{CF}$ = 256.0 Hz, $^{2}J_{CF}$ = 20.0 Hz, CF), 149.7 9 (C), 150.6 (C), 157.35 (C) ppm. ^{19}F RMN (282 MHz, $CDCl_3$): δ = -137.8 to -137.5 (m), -137.4 to -137.2 (m) ppm. HRMS (EI) calculated for $C_{21}H_{12}F_2N_2O$ $[M]^+$ 346.0918; found 346.0921 Purity 99.80 % (EtOH/Heptane = 10/90, Rt = 4.80 min).

6.1.5.1.7 ***7-Phenyl-6H-chromeno[4,3-b][1,5]naphthyridin-6-one (*****11a).** The general procedure **A** was followed using **9a**, affording 0.324 g (99 %) of a white solid identified as **11a** mp 302-303 ºC (ethyl acetate/hexane). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 7.35-7.63 (m, 8H, H_{arom}), 7.75 (dd, $^{3}J_{HH}$ = 8.6 Hz, $^{3}J_{HH}$ = 4.0 Hz, 1 H, H_{arom}), 8.52 (d, $^{3}J_{HH}$ = 8.7 Hz, 1 H, H_{arom}), 8.84 (d, $^{3}J_{HH}$ = 7.8 Hz, 1 H, H_{arom}), 9.03 (d, $^{3}J_{HH}$ = 3.8 Hz, 1 H, H_{arom}) ppm.^{13}C {^{1}H} RMN (75 MHz, $CDCl_3$): δ = 116.1 (C), 117.2 (CH), 119.5 (C), 119.6 (C), 124.8 (CH), 125.9 (CH), 126.7 (CH), 128.0 (2 CH), 128.5 (CH), 128.5 (2 CH), 132.9 (CH), 136.0 (C), 137.2 (CH), 142.3 (C), 147.0 (C), 150.9 (C), 152.1 (CH), 152.9 (C), 156.2 (C), 159.1 (COO) ppm. HRMS (EI) calculated for $C_{22}H_{12}N_2O_2$ $[M]^+$ 324.0899; found 324.0999. Purity 95.00 % (EtOH/Heptane = 10/90, Rt = 5.00 min).

6.1.5.1.8 ***2-Fluoro-7-phenyl-6H-chromeno[4,3-b][1,5]naphthyridin-6-one (*****11b).** The general procedure **A** was followed using **9b**, affording 0.342 g (99 %) of a white solid identified as **11b** mp 275-276 ºC (ethyl acetate/hexane). ^{1}H RMN (300 MHz, $CDCl_3$): δ = 7.25-7.29 (m, 4H, H_{arom}), 7.49-7.51 (m, 3 H, H_{arom}), 7.71 (dd, $^{3}J_{HH}$ = 8.6 Hz, $^{4}J_{HH}$ = 4.1 Hz, 1 H, H_{arom}), 8.43-8-49 (m, 2 H, H_{arom}), 8.98 (dd, $^{3}J_{HH}$ = 4.0 Hz, $^{3}J_{HH}$ = 1.9 Hz, 1H, $CH_{arom.}$) ppm. ^{13}C {^{1}H} RMN (75 MHz, $CDCl_3$): δ = 111.5 (CH), 111.7 (d, $^{2}J_{CF}$ = 24.0 Hz, CH), 118.8 (CH), 118.9 (d, $^{2}J_{CF}$ = 24.0 Hz, CH), 120.2 (C), 127.0 (CH), 127.3 (C), 127.4 (2CH), 128.6 (2CH), 135.9 (C), 137.8 (CH), 139.3 (C), 142.7 (C), 147.1 (C), 150.2 (C), 152.7 (CH), 153.2 (C), 158.6 (d, $^{1}J_{CF}$ = 242.0 Hz, CF), 159.3 (COO) ppm. ^{19}F RMN (282 MHz, $CDCl_3$): -117.4 a -117.3 (m) ppm. HRMS (EI) calculated for $C_{16}H_{14}O_2$ $[M]^+$ 342.0805; found 342.089. Purity 96.00 % (EtOH/Heptane = 10/90, Rt = 6.10 min).

6.1.5.1.9 ***9-Bromo-7-phenyl-6H-chromeno[4,3-b][1,5]naphthyridin-6-one (*****11c).** The general procedure **A** was followed using **9c**, affording 0.380 g (94 %) of a white solid identified as **11c**

mp 215-216 ºC (ethyl acetate/hexane. ^{1}H RMN (300 MHz, DMSO-d6): 6.81 (d, $^{4}J_{HH}$ = 16.0 Hz, 1 H, H_{arom}), 7.40-7.66 (m, 8H, H_{arom}), 8.10 (dd, $^{3}J_{HH}$ = 9.0 Hz, $^{4}J_{HH}$ = 3.0 Hz, 1 H, H_{arom}), 8.8 6(d, $^{4}J_{HH}$ = 3.7 Hz, 1 H, H_{arom}) ppm. ^{13}C {^{1}H} RMN (75 MHz, $CDCl_3$): δ = 117.9 (CH), 118.8 (CH), 122.8 (CH), 123.5 (CH), 125.1 (C), 125.6 (2 CH), 125.9 (2 CH), 126.3 (CH), 126.9 (C), 127.0 (CH), 128.6 (C), 130.1 (C), 134.3 (C), 137.9 (CH), 138.2 (C), 143.6 (C), 150.5 (C), 150.7 (C), 160.2 (COO) ppm. HRMS (EI) calculated for $C_{21}H_{11}BrN_2O_2$ $[M]^+$ 402.0004; found 402.0012. Purity 98.90 % (EtOH/Heptane = 10/90, Rt = 4.80 min).

6.1.5.1.10 ***9-Methoxy-7-phenyl-6H-chromeno[4,3-b][1,5]naphthyridin-6-one*** **(11d).** The general procedure **A** was followed using **9d**, affording 0.322 g (90 %) of a white solid identified as **11d** mp 200-201 ºC (ethyl acetate/hexane. ^{1}H RMN (300 MHz, $CDCl_3$): δ = 3.75 (s, 3H, OCH_3), 7.33-7.71 (m, 9H, H_{arom}), 8.33 (d, $^{3}J_{HH}$ = 9.0Hz, 1 H, H_{arom}), 8.79 (ddd, $^{3}J_{HH}$ = 7.0 Hz, $^{3}J_{HH}$ = 1.7 Hz, $^{4}J_{HH}$ = 0.4 Hz, 1H, $CH_{arom.}$) ppm. ^{13}C {^{1}H} RMN (75 MHz, $CDCl_3$): δ = 53.99 (OCH_3), 117.1 (CH), 119.5 (C), 119.8 (CH), 120.1 (CH), 124.7 (CH), 125.4 (CH), 127.6 (2CH), 127.9 (CH), 128.3 (C), 128.9 (2CH), 129.1 (CH), 130.5 (C), 132.1 (CH), 136.3 (C), 139.9 (CH), 140.2 (C), 145.6 (C), 148.6 (C), 152.5 (C), 152.7 (C), 159.6 (COO) ppm. HRMS (EI) calculated for $C_{22}H_{14}N_2O_3$ $[M]^+$ 354.1004; found 352.1007. Purity 95.50 % (EtOH/Heptane = 10/90, Rt = 5.00 min).

6.2 Biology

6.2.1. 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.

6.2.2. Expression and purification of Human Topoisomerase IB.

The yeast *Saccaromyces 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 [28]. 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).

6.2.3. 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 **5**, **9**, **10** and **11** 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.

6.2.4. 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.

6.3 Computational methodology

6.3.1. Molecular modeling

All calculations included in this paper were carried out with Gaussian 16 program [32] within the density functional theory (DFT) framework [27] using the B3LYP [28], along with the standard 6-311G** basis set. All minima were fully characterized by harmonic frequency analysis [33]. The solvent effect in DFT calculations was evaluated by means of the Polarizable Continuum Model (PCM) [34] using water as solvent. The pKa values were studied to determine the

dominant species (ionization states) at physiological pH (pH = 7.4) using Epik [35] and these were the species used in each case. After a conformational search with MacroModel [36] the most stable conformations were chosen and optimized at the B3LYP/6-311G** + ΔZPVE level of theory and also were computed at the B3LYP(PCM)/6-311G** + ZPVE level using water as solvent. Among them, the most stable of each compound was chosen to calculate the molecular DFT-based parameters, molecular electrostatic potential energetics and docking studies. The obtained results for the molecular electrostatic potential surfaces were generated using GaussView Rev 5.0.9 [37].

6.3.2. Docking studies

First, we proceeded with the choice of the most suitable TopIB/DNA complex for the docking in the Protein Data Bank (PDB). The X-ray structure code 1T8I [29] (3.00 Å resolution) was chosen, a TopIB of human origin covalently bounded to DNA and containing the anti-cancer agent CPT as a ligand. Maestro [31] graphic interface was used, and the Glide 6.9 application [38] in XP mode (extra-precision) [39] was chosen for the docking. The grid was set up in a box of 20 x 20 x 20 Å, centered in the geometric center of CPT. The DNA-binding region in the active site was selected as the target for the screening. The TopIB/DNA complex was prepared by reconstructing the phosphoester bond to nucleobase C12 in the 1T8I structure, and the 5'-SH of nucleobase G11 of the cleaved strand was converted to a 5'-OH by changing the sulfur atom by an oxygen. The hydrogen atoms were added. The binding orders and the protonation states of waste and DNA were corrected. The complex was optimized and minimized using the Protein

Preparation Wizard panel of Schrödinger Suites 2015.1 [40]. Likewise, the structures of the different ligands to be interacted with protein and the ligand initially present in the complex, CPT, were prepared as previously indicated and used for the different docking processes.

6.3.3. Predictive druggability

The predictive druggability of the phosphorus substituted quinoline derivatives were assessed using the physicochemical properties of lipophilic, electronic and structural profiles in order to support the understanding of their antileishmanial-mechanism of action and the potential toxicity effects.

The compounds were also studied by applying the Rule of Five Lipinski, through the use of online free web cheminformatics software SwissAdme, where the following values were obtained: number of rotatable bonds (nRotB), molecular weight, empirical molecular structure and number of hydrogen acceptor groups (HBA) and hydrogen bond donors (HBD).

Acknowledgements

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.

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Straightforward Synthesis and Biological Evaluation as Topoisomerase I Inhibitors and Antiproliferative Agents of Hybrid Chromeno[4,3-b][1,5]Naphthyridines and Chromeno[4,3-b][1,5]Naphthyridin-6-ones

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HIGHLIGHTS.

1. Hybrid chromeno[4,3-*b*][1,5]naphthyridine derivatives have been prepared by Intramolecular Povarov Reaction.
2. Compounds show inhibitory effects against TopI mediated relaxation.
3. Some of prepared compounds present significant antiproliferative against cancer cell lines.
4. Theoretical calculations have been also performed for estimation of MEPS, HOMO-LUMO energy gap and related parameters.