Journal of Medicinal Chemistry

Article

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Synthesis, Antitumor Activity, and Mechanism of Action of Benzo[*b*]chromeno[6,5-*g*][1,8]naphthyridin-7-one Analogs of Acronycine

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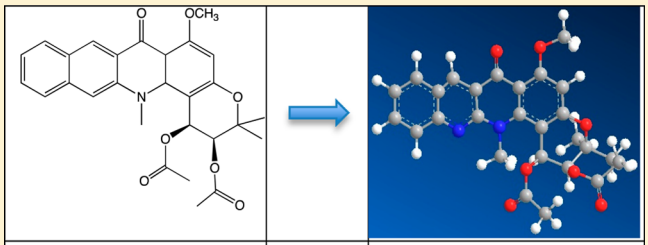
Supporting Information

ABSTRACT: A series of 6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]chromeno[6,5-*g*][1,8]naphthyridin-7-one (**4**), 13-aza derivatives of benzo[*b*]acronycine, the isomeric 5-methoxy-2,2,13-trimethyl-2,13-dihydro-6*H*-benzo[*b*]chromeno[7,6-*g*][1,8]-naphthyridin-6-one (**5**), and related *cis*-diols mono- and diesters were designed and synthesized. Their *in vitro* and *in vivo* biological activities were evaluated. As previously observed in the acronycine series, esters were the most potent derivatives exhibiting submicromolar activities; among them monoesters are particularly active. Racemic diacetate **21** showed a strong activity against KB-3-1 cell lines and was selected for *in vivo* evaluation and proved to be active, inhibiting tumor growth by more than 80%. After separation of the two enantiomers, compounds **21a** and **21b** were also evaluated against C38 colon adenocarcinoma; their activities were found to be significantly different.



3		**21**
KB-3-1: IC50 = 0.1 μM		KB-3-1: IC50 = 0.04 μM

INTRODUCTION

After the isolation of the pyranoacridone alkaloid acronycine (**1**) (Chart 1) from *Acronychia baueri* Schott (Rutaceae),[1] the interest in this compound was underlined by Svoboda et al.,[2] highlighting antitumor properties against a large panel of murine solid tumor models. However, subsequent clinical trials were hampered by the moderate potency and the very low solubility of **1** in aqueous solvents.[3] Consequently, the development of structural analogs with increased potency and/or better solubility in biocompatible solvents was highly desirable.

The isolation from New-Caledonian Sarcomelicope species of a natural unstable acronycine epoxide **2** permitted establishment of an hypothesis of bioactivation, *in vivo*, of the pyran 1,2-double bond into the corresponding oxirane. This led us to design structural analogs[4] with a double bond of the pyran ring functionalized. New compounds, with similar reactivity toward nucleophilic agents such as acronycine epoxide but with an improved chemical stability were obtained. Thus, diesters of *cis*- and *trans*-1,2-dihydroxy-1,2-dihydroacronycine[5] and *cis*-1,2-dihydroxy-1,2-dihydrobenzo[*b*]-acronycine[6] were shown to be very potent and are exemplified by (±)-*cis*-1,2-diacetoxy-1,2-dihydrobenzo[*b*]acronycine, which underwent phase I clinical trials under the code S23906-1 (**3**).[6b]

Similar activity observed with *cis*-1,2-dihydroxy-1,2-dihydrobenzo-[*b*]acronycine monoesters at position 2 could be rationalized on the basis of spontaneous transesterification into the isomeric *cis*-monoesters at position 1.[7]

The mechanism of action of **3** implies an unusual alkylation of the 2-amino group of DNA guanine residues, in the minor groove, by a carbocation. The latter results from the elimination of the ester leaving group at position 1 of the drug.[8] A marked destabilization of the double helix, with the formation of single-stranded DNA during the S-phase of the cell cycle, causes cell death by apoptosis.[9a,b] This atypical alkylating agent inhibits DNA synthesis, increases cyclin E1 and B1 protein levels, associated with CDk1 kinase activity, and suggests the induction of a mitotic catastrophe.[9c] Furthermore, Nucleotide Excision Repair (NER) proteins were shown to regulate sensitivity of the cells to **3**.[9d,e]

Received: June 18, 2014

   dx.doi.org/10.1021/jm500927d | *J. Med. Chem.* XXXX, XXX, XXX−XXX

Chart 1. Acronycine (1), Acronycine Epoxide (2), and (±)-*cis*-1,2-Diacetoxy-1,2-dihydrobenzo[*b*]acronycine (3)

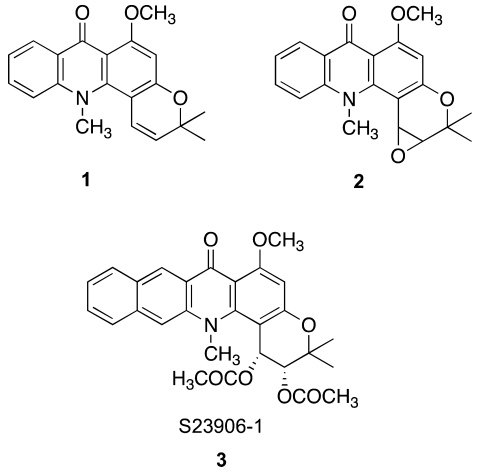
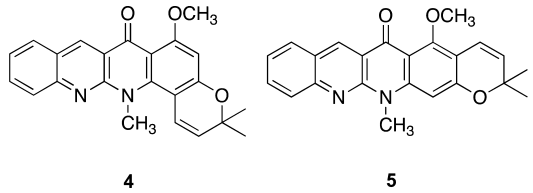


Chart 2. 6-Methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]chromeno[6,5-*g*][1,8]naphthyridin-7-one (4) and 5-Methoxy-2,2,13-trimethyl-2,13-dihydro-6*H*-benzo[*b*]chromeno[7,6-*g*][1,8]naphthyridin-6-one (5)



In a continuation of our studies on the structure−activity relationships in the acronycine series,[10] we describe here the synthesis and the biological properties of 6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]chromeno[6,5-*g*][1,8]-naphthyridin-7-one (**4**) (Chart 2), the isomeric 5-methoxy-2,2,13-trimethyl-2,13-dihydro-6*H*-benzo[*b*]chromeno[7,6-*g*]-[1,8]naphthyridin-6-one (**5**), and related *cis*-diols mono- and diesters. The aim of the present work is to determine the influence of the introduction of a nitrogen atom in the polyaromatic benzacridone core, while conserving unchanged the methoxydimethylchromene pharmacophore, which was previously shown to be indispensable to observe significant biological activity.[8b] Indeed, such derivatives should possess different pharmacokinetic and distribution properties together with a better solubility in biocompatible solvents than the parent compounds, in relation with the presence of the additional basic aromatic nitrogen atom.

CHEMISTRY

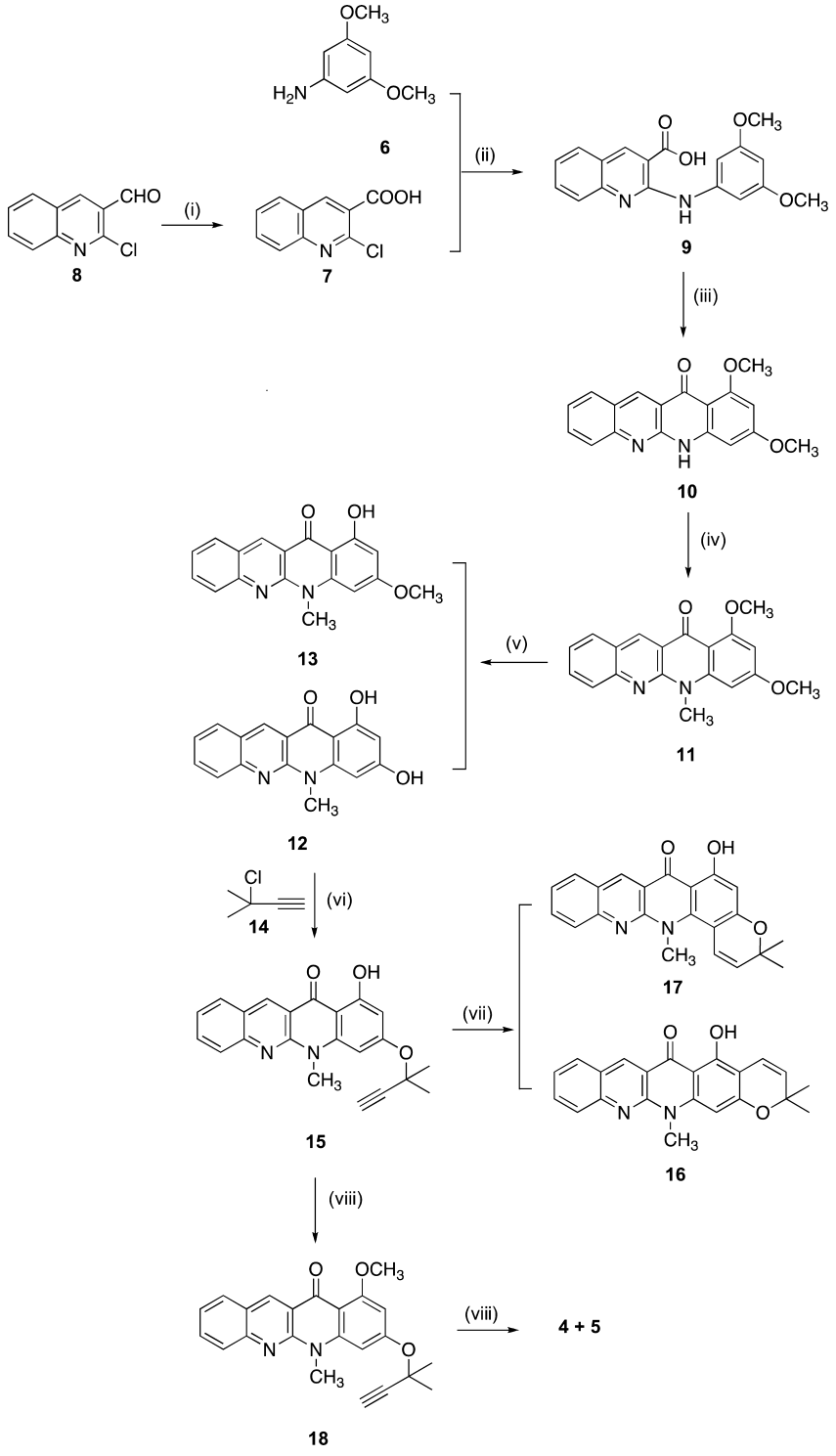
The construction of the required dibenzo[*b*,*g*][1,8]naphthyridin-11(6*H*)-one core was envisioned through Ullmann condensation[11] of a suitable halo-quinoline carboxylic acid with 3,5-dimethoxyaniline (**6**), followed by cyclization under acidic conditions (Scheme 1). Thus, reaction of **6** with 2-chloro-3-quinolinecarboxylic acid (**7**), prepared by oxidation of commercially available 2-chloro-3-quinolinecarbaldehyde (**8**), afforded the corresponding carboxylic diarylamine **9** in 48% yield. Cyclization of **9** to 8,10-dimethoxydibenzo[*b*,*g*][1,8]naphthyridin-11(6*H*)-one (**10**) was achieved in 91% yield by the use of polyphosphoric acid.[12] Subsequent methylation of **10** in acetone, using potassium hydroxide as base and methyl iodide as alkylating agent, gave 8,10-dimethoxy-6-methyldibenzo[*b*,*g*][1,8]naphthyridin-11(6*H*)-one (**11**) in 65% yield. Treatment of **11** with hydrogen bromide in acetic acid under reflux gave the required 8,10-dihydroxy-6-methyldibenzo[*b*,*g*][1,8]-naphthyridin-11(6*H*)-one (**12**) in 86% yield, accompanied by smaller amounts of 10-hydroxy-8-methoxy-6-methyldibenzo[*b*,*g*][1,8]-naphthyridin-11(6*H*)-one (**13**). Construction of the dimethylpyran ring onto the phenol at the 8-position of **12** was performed by a thermic rearrangement of the corresponding dimethylpropargyl ether.[6a,13] Treatment of **12** with 3-chloro-3-methylbut-1-yne (**14**)[14] at 65 °C in dimethylformamide in the presence of potassium carbonate and potassium iodide gave the desired 10-hydroxy-8-(1,1-dimethyl-propyn-1-oxy)-6-methyldibenzo[*b*,*g*][1,8]naphthyridin-11(6*H*)-one (**15**) isolated in 39% yield after purification by column chromatography, accompanied by 6% of the linearly fused 5-hydroxy-2,2,13-trimethyl-2,13-dihydro-6*H*-benzo[*b*]chromeno[7,6-*g*][1,8]-naphthyridin-6-one (**16**). When Claisen rearrangement was performed on compound **15**, by heating at 130 °C in dimethylformamide, the desired angular 6-hydroxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]chromeno[6,5-*g*][1,8]naphthyridin-7-one (**17**) was obtained in a moderate 32% yield, accompanied by its linear isomer **16**, isolated in 50% yield after column chromatography. Better results in terms of angular regioselectivity were obtained by thermal rearrangement of 10-methoxy-8-(1,1-dimethylpropyn-1-oxy)-6-methyldibenzo[*b*,*g*][1,8]naphthyridin-11(6*H*)-one (**18**), prepared in 79% yield by methylation of **15** with methyl iodide, in the presence of sodium hydride in dimethylformamide. Indeed, heating **18** in dimethylformamide at 130 °C gave the desired angular 6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]chromeno-[6,5-*g*][1,8]naphthyridin-7-one (**4**) in a satisfactory 93% yield, accompanied by only 6% of the linear 5-methoxy-2,2,13-trimethyl-2,13-dihydro-6*H*-benzo[*b*]chromeno[7,6-*g*][1,8]naphthyridin-6-one (**5**) (Scheme 1). A series of phase sensitive NOESY experiments permitted us to ascribe unambiguously the angular structures of **17** and **4**, and the linear structures of **16** and **5**.

Conversion of **4** and **5** into the corresponding (±)-*cis*-diols **19** and **20**, respectively, was conveniently obtained by catalytic osmium tetroxide oxidation, using *N*-methylmorpholine *N*-oxide to regenerate the oxidizing agent (Scheme 2).[6a,15] Treatment of diol **19** (Chart 3) with an excess of acylating reagent, acyl anhydride or acyl chloride, afforded the corresponding diesters exemplified by diacetate **21**, dicinnamate **22**, di-4-methoxycinnamate **23**, and di-4-trifluoromethylcinnamate **24**. Under controlled conditions, monoesters at the less hindered 2-position, **25**−**27**, were obtained. Treatment of monocinnamate **25**, mono-4-methoxycinnamate **26**, and mono-4-trifluoromethylcinnamate **27** with excess acetic anhydride led to the mixed esters **28**, **29**, and **30**, respectively. The reaction of diol **19** with *N*,*N*′-carbonyldiimidazole in 2-butanone under reflux afforded the cyclic carbonate **31**. In the linear 6*H*-benzo[*b*]-chromeno[7,6-*g*][1,8]naphthyridin-6-one series, diacetate **32** (Chart 4) and cyclic carbonate **33** were prepared similarly from diol **5**.

Finally, in order to better investigate the structure−activity relationships, the two enantiomeric (+)-(1*R*,2*R*)-1,2-diacetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]chromeno[6,5-*g*]-[1,8]naphthyridin-7-one (**21a**) and (−)-(1*S*,2*S*)-1,2-diacetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]-chromeno[6,5-*g*][1,8]naphthyridin-7-one (**21b**) were separated by preparative HPLC on a ChiralPak AS-H column, starting from racemic (±)-*cis*-diacetate **21**, since differences in terms of cytotoxicity have been recently observed between the two enantiomeric forms of the related *cis*-1,2-diacetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**3**).[16] The absolute configurations of **21a** and **21b** were deduced from their CD curves, in comparison with those obtained for the enantiomers of **3**.[16]

dx.doi.org/10.1021/jm500927d | *J. Med. Chem.* XXXX, XXX, XXX−XXX

Scheme 1. Synthesis of 6-Methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]chromeno[6,5-*g*][1,8]naphthyridin-7-one (4), the Isomeric 5-Methoxy-2,2,13-trimethyl-2,13-dihydro-6*H*-benzo[*b*]chromeno[7,6-*g*][1,8]naphthyridin-6-one (5)[a]



[a]Reagents and conditions: (i) Ag_2O, H_2O, 0 °C, 3 h (86%); (ii) $CH_3COOK/(CH_3COO)_2Cu.H_2O/Et_3N$, $(CH_3)_2CHOH$, 100 °C, 72 h (48%); (iii) PPA, 100 °C, 2 h (91%); (iv) CH_3I, KOH, $(CH_3)_2CO$, reflux, 24h (65%); (v) $HBr/H_2O/CH_3COOH$, reflux, 72 h; (vi) K_2CO_3/KI, DMF, 65 °C, 96 h; (vii) DMF, 130 °C, 24−48 h; (viii), NaH/MeI, DMF, 50 °C, 2 h (79%).

RESULTS AND DISCUSSION

All the new compounds were first evaluated *in vitro* for their cytotoxicity against two tumor cell lines, a murine leukemia cell line (L1210) and a human epidermoid carcinoma cell line (KB-3-1). The results (IC_{50}) are reported in Tables 1 and 2. As could be anticipated from results previously obtained in the isoacronycine series,[17] linear 5-methoxy-2,2,13-trimethyl-2,13-dihydro-6*H*-benzo-[*b*]chromeno[7,6-*g*][1,8]naphthyridin-6-one derivatives only displayed marginal cytotoxic activities against both cell lines. In contrast, the angular esters and diesters of *cis*-1,2-dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]chromeno[6,5-*g*]-

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Scheme 2. Synthesis of (±)-*cis*-1,2-Dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[*b*]chromeno[6,5-*g*]-[1,8]naphthyridin-7-one (19) and (±)-*cis*-3,4-Dihydroxy-5-methoxy-2,2,13-trimethly-2,3,4,13-tetrahydro-6H-benzo[*b*]chromeno[7,6-g][1,8]naphthyridin-6-one (20)

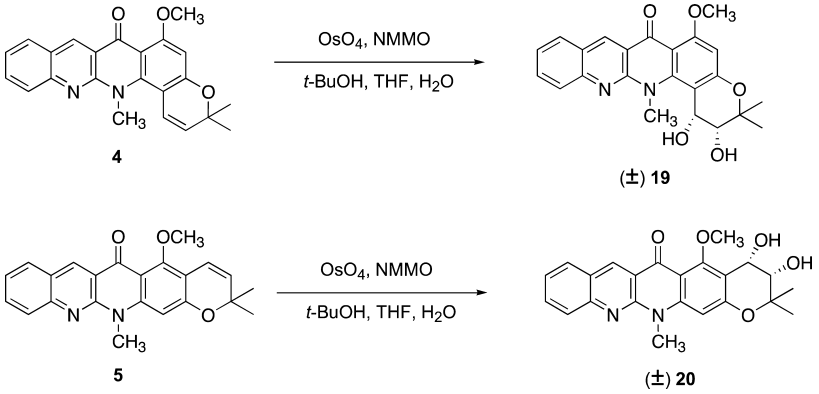
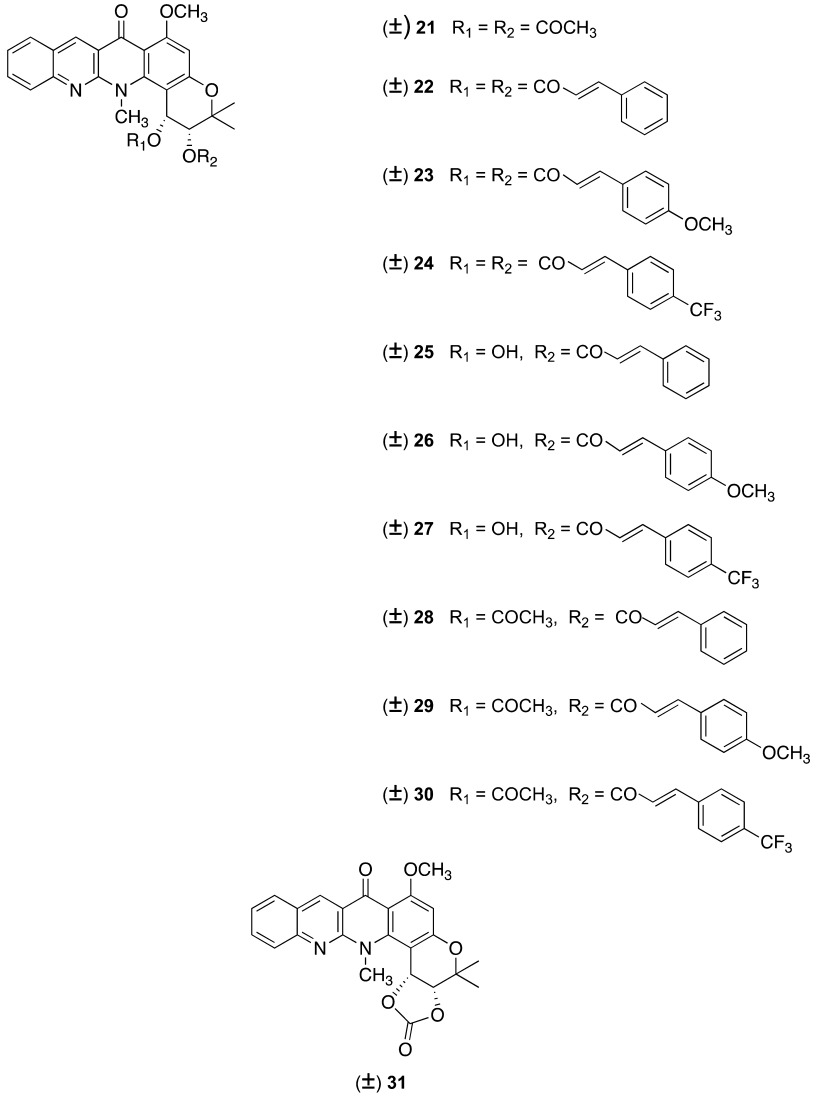


Chart 3. (±)-*cis*-1,2-Dihydroxy-6-methoxy-3,3,14-trimenthly-1,2,3,14-tetrahydro-7*H*-benzo[*b*]chromeno[6,5-*g*]-[1,8]naphthyridin-7-one Esters and Diesters



[1,8]naphthyridin-7-one exhibited antiproliferative activities (between 5 μM to 0.01 μM) within the same order of magnitude as *cis*-1,2-dihydroxy-1,2-dihydrobenzoacronycine diesters.

All compounds were founded significantly more potent on the solid tumor KB-3-1 cell line than on the L1210 leukemia (6- to 10-fold increase).

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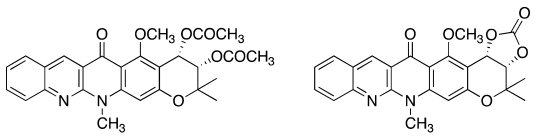
Chart 4. Compounds 32 and 33



(±) **32** (±) **33**

Table 1. Cytotoxicity of 5-Methoxy-2,2,13-trimethyl-2,13-dihydro-6*H*-benzo[*b*]chromeno[7,6-*g*][1,8]naphthyridin-6-one derivatives 5, 20, 32, and 33 in Comparison with 1 and 3

Compound	Cytotoxicity (L1210 cells, IC_{50}, μM)[a]	Cytotoxicity (KB-3-1 cells, IC_{50}, μM)[a]	L1210 cells % of cells in S phase (μM)[b]	*In vitro* DNA alkylation[c]
1	23	3.7		n.t.
3	0.7	0.1	73% 5 μM	++[c]
5	24	14	n.a.	n.t.
20	12.5	7	n.t.	n.t.
32	10.3	8.8	n.t.	0
33	20	12	68% 5 μM	++

[a]Inhibition of cell proliferation measured by the MTT assay (mean of at least 3 values obtained in separate experiments). [b]Highest percentage of L1210 cells arrested in the S phase after a 21 h exposure to the indicated concentration. Untreated control: 32% on average; n.a.: inactive; n.t.: not tested. [c]The capacity of the tested compounds to form complexes with purified DNA was investigated by a gel shift assay. Symbols ++ and + refer to strong and weak alkylation, respectively, whereas 0 means no DNA alkylation at all.

Table 2. Cytotoxicity of 6-Methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]chromeno[6,5-*g*][1,8]naphthyridin-7-one Derivatives 4, 19, and 21−31, in Comparison with 1 and 3

Compound	Cytotoxicity (L1210 cells, IC_{50}, μM)[a]	Cytotoxicity (KB-3-1 cells, IC_{50}, μM)[a]	L1210 cells % of cells in S phase (μM)[b]	*In vitro* DNA alkylation[c]
1	23	3.7		n.t.
3	0.7	0.1	73% 5 μM	++
4	11	3.7	n.a.	n.t.
19	5.1	2	n.t.	n.t.
21	0.20	0.037	68% 5 μM	++
21a	0.19	0.11		++
21b	0.21	0.035		++
22	5	0.54		n.t.
23	9.6	0.37	70% 5 μM	+
24	9.1	0.42	60% 10 μM	++
25	0.11	0.03	66% 10 μM	++
26	0.29	0.023	76% 5 μM	++
27	0.09	0.013	69% 5 μM	++
28	2.1	0.32	73% 5 μM	+
29	2.9	0.47	70% 5 μM	0
30	3.8	0.39	73% 2.5 μM	+
31	0.12	0.041	76% 5 μM	++

[a]Inhibition of cell proliferation measured by the MTT assay (mean of at least 3 values obtained in separate experiments). [b]Highest percentage of L1210 cells arrested in the S phase after a 21 h exposure to the indicated concentration. Untreated control: 32% on average; n.a.: inactive; n.t.: not tested. [c]Relative quantification of bound/free ratio from EMSA. ++ means strong bonding, equivalent to that of **3**; + corresponds to much lower bonding propensity that failed to reach a plateau even at 24 h; 0 relates to no bonding; n.t.: not tested.

Diacetate **21** and cyclic carbonate **31** displayed similar good cytotoxic activity (respectively 0.037 μM and 0.041 μM).

The cytotoxic activities of the two enantiomers of compound **21** were evaluated: the (+)-(*1R,2R*)-1,2-diacetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]chromeno-[6,5-*g*][1,8]naphthyridin-7-one (**21a**) (0.11 μM) was found less active than the corresponding (−)-(*1S,2S*)-1,2-diacetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]-chromeno[6,5-*g*][1,8]naphthyridin-7-one (**21b**) (0.035 μM) against the KB-3-1 cell line.

In the case of cinnamate esters, there is no influence of the substitution of the aromatic ring on the cinnamate moiety (no substitution, p-OCH_3 or p-CF_3). In contrast, some differences appeared between mono- and diesters. Diesters (**22**, **23**, **24** and **28**, **29**, **30**) are about 10-fold less active than the corresponding monoesters (**25**, **26**, **27**) with IC_{50} 0.4 versus 0.04 μM on average.

The perturbation of the cell cycle induced by the new derivatives was studied on the L1210 cell line. As previously observed in the benzo[*a*]acronycine[6] and benzo[*b*]acronycine series,[6] all active compounds induced accumulation in the S phase.

The compounds were also evaluated for their ability to form covalent complexes with DNA using the gel shift assay.[7] As shown in Figure 1, the various 13-aza derivatives were incubated at increasing concentrations (up to 50 μM) with the 117bp radiolabeled DNA fragment for 2 or 24 h (Figures 1A and 2B, respectively). A fixed concentration of **3** (50 μM) was used as a control. This result clearly identifies some compounds as efficient DNA binding agents. Monoesters **25** and **27** are strongly efficient even after a 2 h incubation period; as previously observed in the benzo[*b*]acronycine series, monocinnamoyl esters alkylate DNA faster than the corresponding diesters.[6d] Surprisingly, the *cis*-racemate diacetylated compound **21** was less potent than its pure *cis*-isomers **21a** and **21b** after either a 2 h or a 24 h reaction (parts A and B, respectively, of Figure 1).

Kinetic studies of the alkylation reaction were performed (Figure 2A), and the percent of maximum of band shifting was quantified for each compound relative to **3** (Figure 2B). The $T_{1/2}$ values corresponding to the time for which half of the maximum of the covalent reaction (gel shift of the radiolabeled DNA) was reached are deduced for Figure 2B and presented in Table 1. From comparison to **3**, the aza-derivative **21** presents huge differences in the alkylation kinetics since no plateau (maximum values) could be reached, even after a 24 h incubation time.

Similar difference (but to a much lesser extent) are obtained from comparison of the cinnamate derivative **27** in the 13-aza-series (120 min) to the corresponding compound in the benzo[*b*]acronycine series[6d] presenting a $T_{1/2}$ value of 60 min (personal data).

Replacement of the cinnamate **25** by a 4-trifluoromethylcinnamate **27** reduces the alkylation efficiencies, as evidenced from dose effects (Figure 1) and kinetic measurements (Figure 2 and Table 1). By using the two *cis*-pure enantiomers rather than the *cis*-racemate **21**, it is evidenced that the pure enantiomers are more reactive (intensity of the shift and speed of reaction) than the racemate. This could be attributed to molecular stacking of the two enantiomers present in the racemate aza-molecule in a head to tail orientation, affecting both compound solubility and alkylation potency, at least in the *in vitro* experiments. The more soluble pure enantiomers present much higher DNA alkylation potencies with $T_{1/2}$ values of 60 and 90 min for **21a** and **21b**, respectively (Table 3). Those $T_{1/2}$ values are lower than that obtained using the two pure enantiomers of the reference drug **3** (20−30 min).[16]

Finally, diacetate **21** and the two enantiomers **21a** and **21 b** were selected for an *in vivo* evaluation, comparatively with

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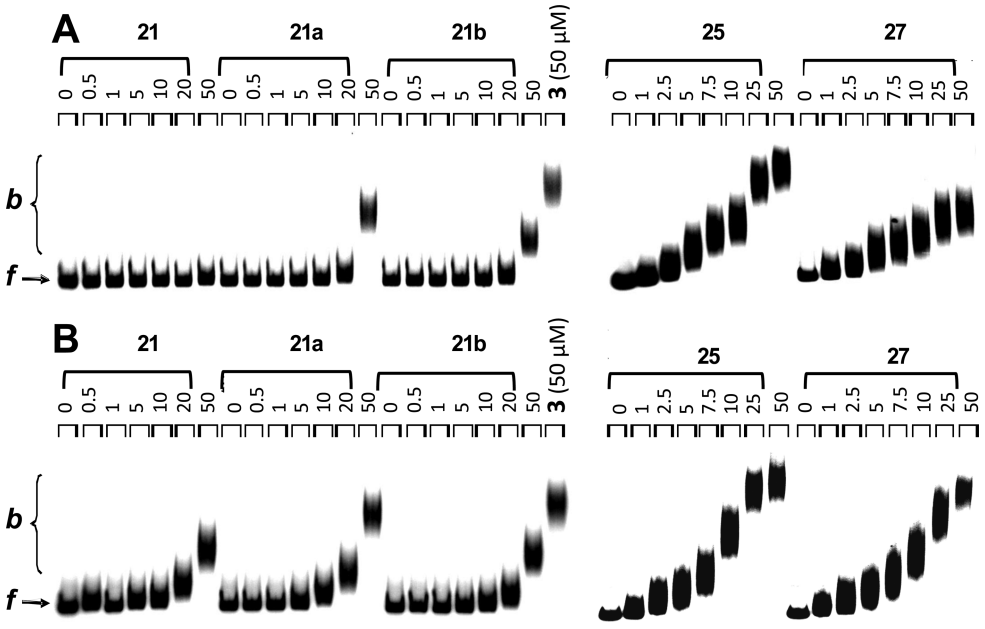

Figure 1. DNA bonding analysis using gel shift experiments. Increasing of fixed concentrations of the various compounds (μM) upon incubation for 2 h (panel **A**) or 24 h (panel **B**) with the 117-bp radiolabeled DNA fragment in 1 mM Na cacodylate buffer prior to being subjected to electrophoresis on a 10% native polyacrylamide gel. The lane "0" refers to the control DNA fragment alone. Bound and free DNA fragments are referred to as "*b*" and "*f*", respectively.

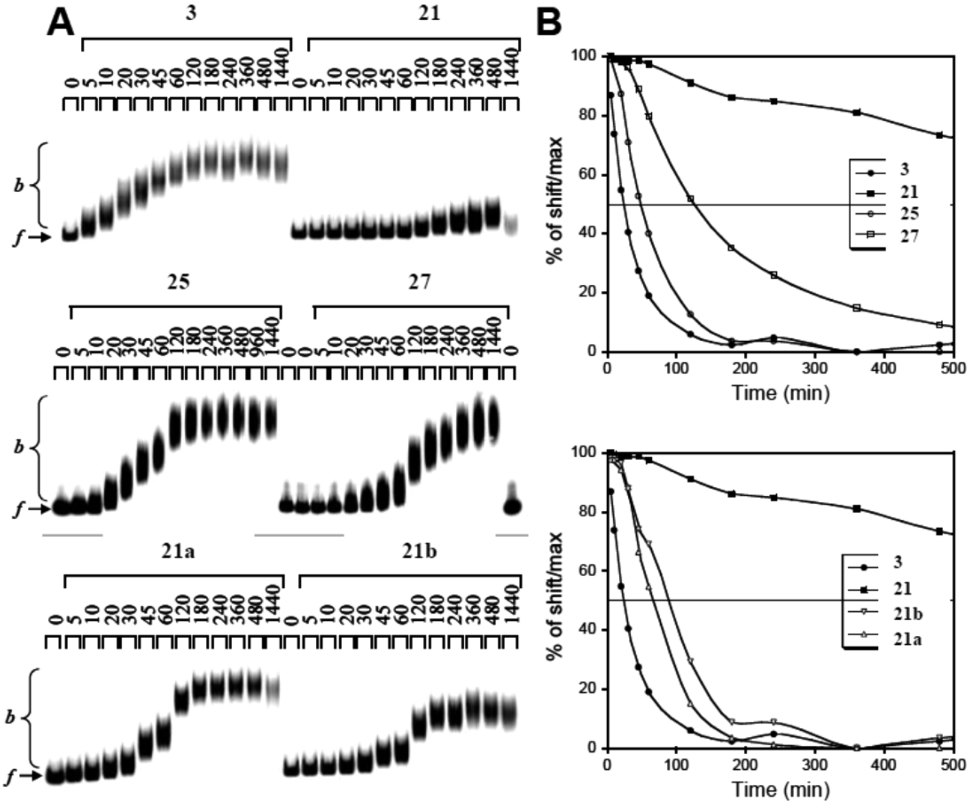

Figure 2. Kinetic measurements of DNA bonding using electrophoretic mobility shift assay. (A) Gel shift assays for kinetic measurements. The radiolabeled 117bp DNA fragment was incubated with 50 μM of the various indicated compounds for the appropriate time indicated on the top of the lanes (min). Bound and free DNA fragments are referred as "*b*" and "*f*", respectively. (B) Quantification of the retarded migration expressed as the percentage of the maximum of electrophoretic migration of the DNA alone (lanes "0" in panel A).

compound **3**, on an established C38 colon adenocarcinoma (sc implantation) in mice. Although less potent than compound **3** (Figure 3), compound **21** administered twice (day 12, day 24) by the iv route at the optimal dose of 4 mg/kg proved to be significantly active, inhibiting tumor growth by 95% and with four of seven tumor free mice at the end of the experiment.

At the dose of 4 mg/kg, the enantiomer **21b** was slightly less active than the racemate on the tumor growth, inducing two of seven cured mice (tumor free mice) at the end of the experiment.

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Table 3. Half Time and Plateau Values for Covalent Complex Formation[a]

Compounds	$T_{1/2}$ (min)	T_{max} (H)	Compounds	$T_{1/2}$ (min)	T_{max} (H)
3	30	2	**21**	nd	nd
25	45	3	**21a**	60	3
27	120	8	**21b**	90	3

[a]The $T_{1/2}$ values (expressed in minutes) correspond to the incubation time required to obtain half of the maximum of the covalent drug/DNA complex that could be obtained. The values (expressed in minutes) were deduced from the quantification plots (Figure 2B) of the kinetic gels (Figure 2A). Values could not be determined (nd) when a T_{max} is not reached.

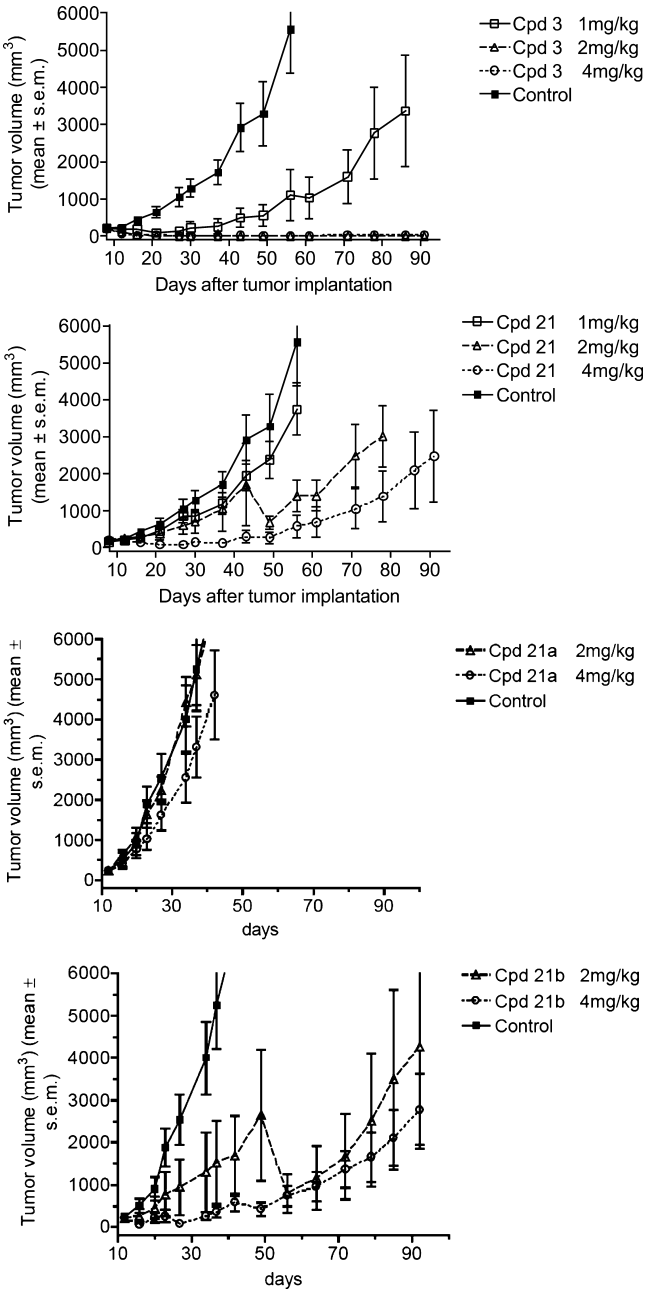

Figure 3. In *vivo* evaluation of **21a** and **21b** comparatively with compound **3** on C38 adenocarcinoma in mice.

In the same experiment, the enantiomer **21a** was inactive and did not induce any tumor growth inhibition (Figure 3B).

CONCLUSION

In conclusion, introduction of a basic nitrogen on the B ring of the benzo[*b*]acronycine led to the development of a new series of derivatives. As previously observed in the acronycine series, esters of the pyranediol were the most potent derivatives exhibiting submicromolar activities; among them monoesters are particularly active. Compound **21**, the corresponding aza derivative of **3**, was shown to be the most promising compound. Both enantiomeric compounds **21a** and **21b** were evaluated after separation on chiral chromatography. **21a** and **21b** presented some different properties, where **21b** was shown to be more cytotoxic on the KB-3-1 cell line but less effective in alkylating DNA. The good activity of compound **21b** was confirmed by *in vivo* evaluation.

EXPERIMENTAL SECTION

Chemistry. Melting points were determined on a hot stage Reichert microscope and are uncorrected. Mass spectra were recorded with ZQ 2000 Waters and Q-Tof1Micromass spectrometers using electrospray ionization (ESI-MS; V_c = 30 V), or with a Nermag R-10-10C spectrometer using desorption−chemical ionization (DCI-MS; reagent gas: NH_3). UV spectra (λ_{max} in nm) were recorded in spectroscopic grade MeOH on a Beckman Model 34 spectrophotometer. IR spectra (ν_{max} in cm^{-1}) were obtained from potassium bromide pellets or sodium chloride films on a PerkinElmer 257 instrument. ^1H NMR (δ [ppm], J [Hz]) spectra were run at 400 MHz and ^{13}C NMR spectra at 75 MHz, using Bruker AVANCE-400 and AC-300 spectrometers, respectively. When necessary, the structures of the novel compounds were ensured and the signals unambiguously assigned by 2D NMR techniques: $^1H-^1H$ COSY, $^1H-^1H$ NOESY, $^{13}C-^1H$ HMQC, and $^{13}C-^1H$ HMBC. These experiments were performed using standard Bruker microprograms. Circular dichroism (CD) spectra were recorded with a Jasco J-810 apparatus between 300 and 400 nm, at room temperature. The samples were prepared in methanol at 10−4 g/mL. Column chromatographies were carried out with silica gel 20−45 mm. Microanalyses were in agreement with calculated values ±0.4%, confirming a purity ≥95%.

Cell Culture and Cytotoxicity. L1210 and KB-3-1 cells were cultivated in RPMI 1640 or DMEM medium, respectively (Gibco), supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 mg/mL streptomycin, and 10 mM HEPES buffer (pH = 7.4). Cytotoxicity was measured by the microculture tetrazolium assay (MTA) as described.[18] Cells were exposed to graded concentrations of drug (nine serial dilutions in triplicate) for 4 doubling times (48 h for L1210 cells and 96 h for KB-3−-1 cells). Results are expressed as IC50, the concentration that reduced by 50% the optical density of treated cells with respect to the optical density of untreated controls.

For the cell cycle analysis, L1210 cells (5 × 105 cells/mL) were incubated for 21 h with various concentrations of drugs. Cells were then fixed by 70% ethanol (*v*/*v*), washed, and incubated in PBS containing 100 mg/mL RNase and 50 mg/mL propidium iodide for 30 min at 20 °C. For each sample, 10 000 cells were analyzed on an XLMCL flow cytometer (Beckman Coulter, France). Results are expressed as the % of cells in the S phase of the cell cycle.

Antitumor Activity. The antitumor activity of the diacetate **21** and the two enantiomers **21a** and **21b** was evaluated on murine colon C38 adenocarcinoma implanted in B6D2F1 (C57B1/6 x DBA2) mice. A C38 tumor fragment of approximately 50 mg was subcutaneously implanted into the dorsal flank of seven mice. The evaluated compounds were prepared in a mixture of 10% cremophor ELP, 10% ethanol and administrated intravenous at indicated doses in two administrations 10 days apart.

Mice bearing s.c. implanted tumors were treated when the tumors reached a volume about 150 to 220 mm^3. Tumors were measured twice a week and tumor volumes (V_t) were calculated using the following formula: length (mm) × width^2 (mm^2)/2. At the end of each experiment, tumor-free animals were defined as cured animals.

dx.doi.org/10.1021/jm500927d | *J. Med. Chem.* XXXX, XXX, XXX−XXX

DNA Restriction Fragments. The 117-bp DNA fragment was obtained as previously described[7] from the pBS plasmid digestion using *Eco*RI and PvuII restriction enzymes in their respective digestion buffers. The generated 117 bp DNA was then labeled at the *Eco*RI restriction site by incorporation of a-[^{32}P]-dATP (GEHealthcare) using AMV reverse transcriptase (Ozyme). The generated 117bp radiolabeled DNA fragment was purified by electrophoresis on a nondenaturing 10% (*w*/*v*) polyacrylamide gel. The portion of gel containing the 117bp DNA was cut out of the gel, crushed, and eluted overnight in 500 mM ammonium acetate, 10 mM magnesium acetate. After filtration to remove the polyacrylamide gel, the radiolabeled DNA was precipitated using cold ethanol, dried, and dissolved in MQ water.

Gel Shift Studies. The cross-linking reaction was conducted as previously described.[16] Briefly, it consists of incubating the drug at appropriate concentrations (increasing concentration or a fixed 50 μM concentration for kinetic studies) with the radiolabeled DNA in 1 mM Na cacodylate, pH 7.0, and incubation in the dark at room temperature during the period specified in the legend. After various incubation times from 5 min to 24 h, 5 mL of a 50% glycerol containing tracking dyes solution was added to each DNA sample. The covalent complex was evidenced as a shifted band resolved after electrophoresis on a nondenaturing 6% polyacrylamide gel in TBE buffer (89 mM boric acid, 2.5 mM Na_2EDTA, pH 8.3) for about 5 h at 300 V and room temperature. Gels were transferred to Whatman 3MM paper, dried under vacuum at 80 °C, and then analyzed on a Storm phosphorimaging system to evidence the shifted complex.

*2-Chloro-3-quinolinecarboxylic Acid (**7**).* A solution of sodium hydroxyde (16.8 g/L, 420 mmol) in water (80 mL) was added to a solution of silver nitrate (36.1 g, 200 mmol) in water (80 mL). 2-Chloro-3-quinolinecarbaldehyde (**8**) (20.0 g, 120 mmol) was added to the resulting silver hydroxide suspension, and the reaction mixture was stirred at 0 °C for 3 h and filtered. The filtrate was acidified to pH = 6 with conc. HCl aqueous solution, and the obtained precipitate was collected, washed with water, and dried. Purification by silica gel column chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH 99.9:0.1 to 95:5) gave 7 (18.7 g, 86%) as pale crystals: mp 263 °C (crystallized from MeOH/Me_2CO 1/1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.72 (dd, J = 8, 7 Hz, 1H, H-6), 7.91 (dd, J = 8, 7 Hz, 1H, H-7), 7.99 (d, J = 8 Hz, 1H, H-5), 8.16 (d, J = 8 Hz, 1H, H-8), 8.92 (s, 1H, H-4), 13.81 (br. s, 1H, D_2O exch., COOH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 126.6 (C-3), 126.9(C-4a), 128.7(C-5), 129.0(C-6), 130.0(C-8), 133.7(C-7), 142.2(C-4), 147.5(C-2), 148.4(C-8a), 166.7(COOH); DCI-MS m/z 208, 210 [MH]$^+$; IR (KBr) ν cm^{-1}: 3423 (br.), 3073, 2924, 2568, 1731, 1618, 1567, 1489, 1449, 1251, 1234, 1191, 1127, 1023, 899, 789, 767, 748; UV λ nm (MeOH) (log ε): 238 (4.48), 280 (3.52), 309 (3.32), 323 (3.34).

*2-(3,5-Dimethoxyphenylamino)-3-quinolinecarboxylic Acid (**9**).* A mixture of 3,5-dimethoxyaniline (**6**) (20.5 g, 130 mmol), **7** (21.3 g, 100 mmol), potassium acetate (23.2 g), and cupric acetate monohydrate (1.3 g) in triethylamine (17.1 mL) and 2-propanol (370 mL) was heated at 100 °C for 72 h. The reaction mixture was evaporated under reduced pressure, and the residue was partitioned between CH_2Cl_2 and 1 N aqueous HCl. The aqueous phase was extracted with CH_2Cl_2 (4 × 200 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. Silica gel column chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99.9:0.1 to 98:2) gave **9** (16 g, 48%) as an amorphous yellowish solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.78 (s, 6H, 2 OCH_3), 6.20 (t, J = 2 Hz, 1H, H-4′), 7.25 (m, 2H, H-2′, H-6′), 7.36 (ddd, J = 8, 7, 2 Hz, 1H, H-6), 7.69 (dd, J = 8, 2 Hz, 1H, H-5), 7.72 (ddd, J = 8, 7, 2 Hz, 1H, H-7), 7.93 (dd, J = 8, 2 Hz, 1H, H-8), 8.92 (s, 1H, H-4), 10.60 (s, 1H, D_2O exch., NH), 13.81 (br. s, 1H, D_2O exch., COOH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 56.1 (2C, 2 OCH_3), 95.6 (C-4′), 98.7(2C, C-2′, C-6′), 112.3(C-3), 123.3 (C-4a), 124.6 (C-6), 127.1 (C-5), 130.4 (C-8), 133.7 (C-7), 142.7 (C-1′), 143.7 (C-4), 149.6 (C-8a), 153.4 (C-2), 161.7 (2C, C-3′, C-5′), 169.8(COOH); DCI-MS m/z 325 [MH]$^+$, 347 [MNa]$^+$; IR (KBr) ν cm^{-1}: 3448 (br.), 1655, 1649, 1605, 1544, 1477, 1459, 1425, 1204, 1156, 1066; UV λ nm (MeOH) (log ε): 216 (4.61), 280 (4.44), 370 (3.84).

*8,10-Dimethoxydibenzo[b,g][1,8]naphthyridin-11(6H)-one (**10**).* A suspension of **9** (5.6 g, 17 mmol) in polyphosphoric acid (122 g) was heated at 100 °C for 2 h. The reaction mixture was slowly poured onto ice water (300 mL) and neutralized to pH = 7 by addition of a 30% aqueous NaOH solution. The obtained precipitate was collected, washed with water, and dried *in vacuum* over P_2O_5. Silica gel column chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99.9:0.1 to 98:2) gave **10** (4.84 g, 91%) as pale yellow crystals: mp 309 °C (crystallized from CH_2Cl_2/MeOH 1/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.77 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 6.18 (s, 1H, H-7), 6.25 (s, 1H, H-9), 7.49 (td, J = 8, 1 Hz, 1H, H-2), 7.81 (td, J = 8, 1 Hz, 1H, H-3), 7.97 (dd, J = 8, 1 Hz, 1H, H-4), 8.05 (dd, J = 8, 1 Hz, 1H, H-1), 9.26 (s, 1H, H-12), 9.93 (s, 1H, D_2O exch., NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 55.5 (C_8−OCH_3), 56.2 (C_{10}-OCH_3), 91.3 (C-7), 93.3 (C-9), 106.3 (C-10a), 118.9 (C-11a), 124.4 (C-2), 125.1 (C-12a), 126.2 (C-1), 130.0 (C-4), 132.5 (C-3), 139.2 (C-12), 145.5 (C-6a), 149.0 (C-4a), 149.5 (C-5a), 163.4 (C-10), 165.2 (C-8), 177.2 (C-11); DCI-MS m/z 307 [MH]$^+$; IR (KBr) ν cm^{-1}: 3448 (br.), 1624, 1618, 1271, 1170, 1098; UV λ nm (MeOH) (log ε): 224 (3.77), 278 (4.16), 338 (3.47).

*8,10-Dimethoxy-6-methyldibenzo[b,g][1,8]naphthyridin-11(6H)-one (**11**).* Potassium hydroxide pellets (3.9 g) were added to a solution of **10** (3.5 g, 15 mmol) in dry acetone (250 mL). The mixture refluxed for 15 min under argon and methyl iodide (30 mL, 480 mmol) was added. After 24 h of heating under reflux, the reaction mixture was evaporated under reduced pressure. Silica gel column chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99.9:0.1 to 99.5:0.5) afforded **11** (3.1 g, 65%) as yellow crystals: mp 335 °C (crystallized from CH_2Cl_2/MeOH 1/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.94 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 4.11 (s, 3H, NCH_3), 6.25 (s, 1H, H-9), 6.49 (s, 1H, H-7), 7.44 (ddd, J = 9, 7, 1 Hz, 1H, H-2), 7.75 (ddd, J = 9, 7, 1 Hz, 1H, H-3), 7.95 (dd, J = 9, 1 Hz, 1H, H-1), 7.98 (dd, J = 9, 1 Hz, 1H, H-4), 9.21 (s, 1H, H-12), 10.96 (br. s, 1H, D_2O exch., C_8−OH), 14.23 (s, 1H, D_2O exch., C_{10}−OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 31.5 (NCH_3), 55.4 (C_8−OCH_3), 56.3 (C_{10}-OCH_3), 91.4 (C-7), 92.0 (C-9), 107.1 (C-10a), 119.3 (C-11a), 124.3 (C-3), 124.5 (C-12a), 127.4 (C-1), 129.4 (C-4), 131.9 (C-2), 138.5 (C-12), 147.6 (C-6a), 149.1 (C-4a), 149.7 (C-5a), 163.8 (C-10), 164.9 (C-8), 177.2 (C-11); DCI-MS m/z 321 [MH]$^+$; IR (KBr) ν cm^{-1}: 1630, 1583, 1514, 1425, 1257, 1202, 1159, 1145; UV λ nm (MeOH) (log ε): 227 (4.36), 245 (4.24), 281 (4.59), 340 (4.16).

*8,10-Dihydroxy-6-methyldibenzo[b,g][1,8]naphthyridin-11(6H)-one (**12**) and 10-Hydroxy-8-methoxy-6-methyldibenzo[b,g][1,8]-naphthyridin-11(6H)-one (**13**).* Acetic acid (50 mL) was added to a solution of **11** (3.0 g, 9.4 mmol) in 48% hydrogen bromide aqueous solution (150 mL). The reaction mixture was stirred and refluxed for 3 days. The cooled mixture was poured onto ice water (500 mL). The precipitate was filtered, washed with water (4 × 50 mL), and dried *in vacuum* over P_2O_5. Column chromatography on silica gel (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99.9:0.1 to 98:2) gave successively **12** (2.35 g, 86%) and **13** (0.37 g, 13%) as yellow amorphous solids. Compound **12**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.03 (s, 3H, NCH_3), 6.10 (d, J = 2 Hz, 1H, H-9), 6.40 (d, J = 2 Hz, 1H, H-7), 7.51 (ddd, J = 8, 7, 1 Hz, 1H, H-2), 7.85 (ddd, J = 8, 7, 1 Hz, 1H, H-3), 7.92 (dd, J = 8, 1 Hz, 1H, H-1), 8.17 (dd, J = 8, 1 Hz, 1H, H-4), 9.21 (s, 1H, H-12), 10.96 (br. s, 1H, D_2O exch., C_8−OH), 14.23 (s, 1H, D_2O exch., C_{10}−OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 31.9 (N-CH_3), 93.5 (C-7), 97.0 (C-9), 103.6 (C-10a), 116.9 (C-11a), 124.8 (C-12a), 125.8 (C-2), 128.1(C-4), 130.7 (C-1), 134.2 (C-3), 138.7(C-12), 146.5 (C-6a), 149.8 (C-4a), 150.1 (C-12a), 166.3 (C-10), 167.1 (C-8), 181.4 (C-11); DCI-MS m/z 293 [MH]$^+$; IR (KBr) ν cm^{-1}: 3448 (br.), 3138 (br.), 1638, 1627, 1611, 1571, 1551, 1518, 1477, 1429, 1346, 1304, 1274, 1186, 1170, 1150, 809, 756, 727; UV λ nm (MeOH) (log ε): 225 (4.28), 281 (4.70), 355 (4.04).

Compound **13**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.93 (s, 3H, OCH_3), 4.06 (s, 3H, NCH_3), 6.29 (d, J = 1.5 Hz, 1H, H-9), 6.38 (d, J = 1.5 Hz, 1H, H-7), 7.46 (ddd, J = 9, 7, 1 Hz, 1H, H-2), 7.79 (ddd, J = 9, 7, 1 Hz, 1H, H-3), 7.95 (dd, J = 9, 1 Hz, 1H, H-1), 7.99 (dd, J = 9, 1 Hz, 1H, H-4), 9.19 (s, 1H, H-12), 14.25 (s, 1H, D_2O exch., C_{10}−OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 31.2 (N-CH_3), 55.7 (O-CH_3), 91.6 (C-7), 94.0 (C-9), 104.1 (C-10a), 116.6 (C-11a), 124.2 (C-12a), 124.8 (C-3), 127.7 (C-4), 129.4 (C-1), 132.8 (C-2), 138.0 (C-12), 145.5 (C-6a), 149.2 (C-4a), 149.9 (C-5a), 166.5 (C-10), 167.1 (C-8), 181.7 (C-11); DCI-MS m/z 307 [MH]$^+$; IR (KBr) ν cm^{-1}: 3448 (br.), 3138 (br.), 1638, 1605, 1585,

dx.doi.org/10.1021/jm500927d | J. Med. Chem. XXXX, XXX, XXX−XXX

1508, 1477, 1458, 1425, 1402, 1349, 1251, 1211, 1164, 1100, 1062, 813, 753; UV λ nm (MeOH) (log ε): 282 (4.70), 353 (4.13).

*Reaction of **12** with 3-Chloro-1-methylbut-1-yne (**14**).* A solution of **12** (2 g, 6.8 mmol) and 3-chloro-3-methylbut-1-yne (**14**) (5.0 g, 48 mmol) in dry *N*,*N*-dimethylformamide (200 mL) was stirred and heated at 65 °C for 4 days, under nitrogen, in the presence of anhydrous potassium carbonate (3.0 g, 23 mmol) and potassium iodide (3.0 g, 8 mmol). After addition of ice water (200 mL), the reaction mixture was extracted with CH_2Cl_2 (4 × 100 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. Purification by silica gel column chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99.9:0.1 to 99.6:0.4) successively afforded **15** (0.97 g, 39%) and **16** (0.15 g, 6%).

*10-Hydroxy-8-(1,1-dimethylpropyn-1-oxy)-6-methyldibenzo[b,g]-[1,8]naphthyridin-11(6H)-one (**15**).* Reddish crystals: mp 240 °C (crystallized from CH_2Cl_2/MeOH 1/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.80 (s, 6H, $C(CH_3)_2$), 2.75 (s, 1H, H-3′), 4.12 (s, 3H, NCH_3), 6.73 (d, J = 1.5 Hz, 1H, H-7), 6.79 (d, J = 1.5 Hz, 1H, H-9), 7.49 (ddd, J = 9, 7, 1 Hz, 1H, H-2), 7.83 (ddd, J = 9, 7, 1 Hz, 1H, H-3), 7.92 (dd, J = 9, 1 Hz, 1H, H-1), 7.99 (dd, J = 9, 1 Hz, 1H, H-4), 9.26 (s, 1H, H-12), 14.15 (s, 1H, D_2O exch., C_{10}−OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.7 (2C, $C(CH_3)_2$), 31.1 (N-CH_3), 72.7 (C-1′), 75.2 (C-3′), 85.0 (C-2′), 95.5 (C-7), 99.0 (C-9), 104.7 (C-10a), 115.6 (C-11a), 124.2 (C-12a), 124.8 (C-3), 127.7 (C-4), 129.4 (C-1), 132.8 (C-2), 138.0 (C-12), 145.1 (C-6a), 149.4(C-5a), 149.9 (C-4a), 163.7 (C-8), 165.5 (C-10), 181.9 (C-11); DCI-MS m/z 359 $[MH]^+$; IR (KBr) ν cm^{-1}: 3448 (br.), 3231, 1638, 1585, 1516, 1492, 1423, 1382, 1342, 1304, 1257, 1214, 1164, 1133, 1029, 824, 747, 727; UV λ nm (MeOH) (log ε): 283 (4.64), 345 (3.93).

*5-Hydroxy-2,2,13-trimethyl-2,13-dihydro-6H-benzo[b]chromeno-[7,6-g][1,8]naphthyridin-6-one (**16**).* Bright yellow crystals: mp 250 °C (crystallized from CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 6H, $C(CH_3)_2$), 4.03 (s, 3H, NCH_3), 5.51 (d, J = 10 Hz, 1H, H-3), 6.31 (s, 1H, H-14), 6.70 (d, J = 10 Hz, 1H, H-4), 7.39 (ddd, J = 9, 7, 1 Hz, 1H, H-9), 7.73 (ddd, J = 9, 7, 1 Hz, 1H, H-10), 7.88 (dd, J = 9, 1 Hz, 1H, H-8), 7.91 (dd, J = 9, 1 Hz, 1H, H-11), 9.14 (s, 1H, H-7), 14.49 (s, 1H, D_2O exch., C_5−OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.6 (2C, $C(CH_3)_2$), 31.1 (N-CH_3), 78.4 (C-2), 92.7 (C-14), 102.5 (C-4a), 103.9 (C-5a), 115.6 (C-4), 116.5 (C-6a), 124.2(C-7a), 124.8 (C-10), 126.6 (C-3), 127.7 (C-11), 129.3 (C-8), 132.7 (C-9), 137.8 (C-7), 145.1 (C-13a), 149.1 (C-12a), 149.8 (C-11a), 160.2(C-5), 161.2 (C-14a), 181.5 C-6); DCI-MS m/z 359 $[MH]^+$; IR (KBr) ν cm^{-1}: 3448 (br.), 1637, 1586, 1559, 1491, 1430, 1342, 1312, 1202, 1160, 1129, 811, 780, 763; UV λ nm (MeOH) (log ε): 250 (4.39), 312 (4.80), 364 (4.17).

*Thermal Rearrangement of 10-Hydroxy-8-(1,1-dimethylpropyn-1-oxy)-6-methyldibenzo[b,g][1,8]naphthyridin-11(6H)-one (**15**).* A solution of **15** (0.5 g, 1.4 mmol) in *N*,*N*-dimethylformamide (170 mL) was heated under nitrogen at 130 °C for 2 days. The solvent was evaporated under reduced pressure. Silica gel column chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99.9:0.1 to 99.6:0.4) gave successively **17** (0.16 g, 32%) and **16** (0.25 g, 50%). **6-Hydroxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]chromeno[6,5-*g*][1,8]naphthyridin-7-one (17)**: bright yellow crystals: mp 322 °C (crystallized from CH_2Cl_2/MeOH 1/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.56 (s, 6H, $C(CH_3)_2$), 4.10 (s, 3H, NCH_3), 5.59 (d, J = 10 Hz, 1H, H-2), 6.29 (s, 1H, H-5), 6.70 (d, J = 10 Hz, 1H, H-1), 7.50 (ddd, J = 9, 7, 1 Hz, 1H, H-10), 7.83 (ddd, J = 9, 7, 1 Hz, 1H, H-11), 8.00 (dd, J = 9, 1 Hz, 1H, H-9), 8.04 (dd, J = 9, 1 Hz, 1H, H-12), 9.18 (s, 1H, H-8), 14.32 (s, 1H, D_2O exch., C_6−OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.0 (2C, $C(CH_3)_2$), 41.7 (N-CH_3), 76.6 (C-3), 98.1 (C-5), 101.9 (C-14b), 105.9 (C-6a), 117.1 (C-7a), 121.6 (C-1), 123.5 (C-2), 124.5 (C-8a), 125.0 (C-11), 127.8 (C-12), 129.4 (C-9), 132.7 (C-10), 137.4 (C-8), 144.7 (C-14a), 150.0 (C-12a), 151.8 (C-13a), 162.6 (C-4a), 165.7 (C-6), 181.7 (C-7); DCI-MS m/z 359 $[MH]^+$; IR (KBr) ν cm^{-1}: 3448 (br.), 1625, 1578, 1560, 1501, 1405, 1375, 1338, 1313, 1277, 1174, 1156, 1137, 1092, 1025, 850, 825, 750; UV λ nm (MeOH) (log ε): 234 (4.12), 277 (4.40), 305 (4.43), 376 (3.70).

*10-Methoxy-8-(1,1-dimethylpropyn-1-oxy)-6-methyldibenzo-[b,g][1,8]naphthyridin-11(6H)-one (**18**).* Sodium hydride (0.41 g of 50% oil dispersion, 8.5 mmol) was added to a solution of **15** (0.1 g, 0.28 mmol) in dry *N*,*N*-dimethylformamide (20 mL), and the mixture was stirred under argon for 15 min. After addition of methyl iodide (1 mL, 16 mmol), the reaction mixture was stirred at 50 °C for 2 h and then poured carefully onto ice water. The precipitate was filtered and dried *in vacuum* over P_2O_5. Column chromatography on silica gel (solvent: CH_2Cl_2 then CH_2Cl_2/MeOH, 99.9:0.1 to 99:1) gave **18** (0.082 g, 79%) as yellow crystals: mp 223 °C (crystallized from CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.82 (s, 6H, $C(CH_3)_2$), 2.78 (s, 1H, H-3′), 4.07 (s, 3H, OCH_3), 4.12 (s, 3H, NCH_3), 6.64 (d, J = 2 Hz, 1H, H-9), 7.05 (d, J = 2 Hz, 1H, H-7), 7.42 (ddd, J = 9, 7, 1 Hz, 1H, H-2), 7.74 (ddd, J = 9, 7, 1 Hz, 1H, H-3), 7.96 (dd, J = 9, 1 Hz, 1H, H-1), 7.98 (dd, J = 9, 1 Hz, 1H, H-4), 9.22 (s, 1H, H-12); ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.7 (2C, $C(CH_3)_2$), 31.6 (N-CH_3), 56.3 (O-CH_3), 72.5 (C-1′), 75.0 (C-3′), 85.4 (C-2′), 96.2 (C-9), 97.1 (C-7), 107.6 (C-10a), 119.3 (C-11a), 124.3 (C-3), 124.5 (C-12a), 127.4 (C-1), 129.4 (C-4), 131.9 (C-2), 138.6 (C-12), 147.0 (C-6a), 149.2 (C-4a), 149.7 (C-5a), 161.5 (C-8), 163.3 (C-10), 177.4 (C-11); DCI-MS m/z 373 $[MH]^+$; IR (KBr) ν cm^{-1}: 1648, 1618, 1604, 1586, 1508, 1474, 1421, 1350, 1257, 1137, 1096, 1031; UV λ nm (MeOH) (log ε): 228 (4.36), 279 (4.74), 340 (4.11).

*Thermal Rearrangement of 10-Methoxy-8-(1,1-dimethylpropyn-1-oxy)-6-methyldibenzo[b,g][1,8]naphthyridin-11(6H)-one (**18**).* A solution of **15** (0.1 g, 0.27 mmol) in *N*,*N*-dimethylformamide (50 mL) was heated under nitrogen at 130 °C for 24 h. The reaction mixture was poured carefully onto ice water (100 mL) and extracted with CH_2Cl_2 (4 × 50 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. Purification by silica gel column chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99.9:0.1 to 99.5:0.5) gave successively **4** (0.93 g, 93%) and **5** (0.06 g, 6%).

*6-Methoxy-3,3,14-trimethyl-3,14-dihydro-7H-benzo[b]-chromeno[6,5-g][1,8]naphthyridin-7-one (**4**).* Yellow crystals: mp 275 °C (crystallized from CH_2Cl_2/MeOH 1/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.50 (s, 6H, $C(CH_3)_2$), 3.94 (s, 3H, OCH_3), 3.98 (s, 3H, NCH_3), 5.50 (d, J = 10 Hz, 1H, H-2), 6.27 (s, 1H, H-5), 6.60 (d, J = 10 Hz, 1H, H-1), 7.39 (ddd, J = 9, 7, 1 Hz, 1H, H-10), 7.71 (ddd, J = 9, 7, 1 Hz, 1H, H-11), 7.92 (dd, J = 9, 1 Hz, 1H, H-9), 7.95 (dd, J = 9, 1 Hz, 1H, H-12), 9.08 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.9 (2C, $C(CH_3)_2$), 42.4 (N-CH_3), 56.3 (O-CH_3), 76.6 (C-3), 94.4 (C-5), 103.6 (C-14b), 109.4 (C-6a), 120.0 (C-7a), 121.9 (C-1), 123.3 (C-2), 124.6 (C-11), 124.9 (C-8a), 127.5 (C-12), 129.4 (C-9), 131.9 (C-10), 137.9 (C-8), 147.1 (C-14a), 149.4 (C-12a), 152.4 (C-13a), 160.2 (C-4a), 163.3 (C-6), 177.8 (C-7); HRMS (ESI) calcd for $C_{23}H_{20}N_2O_3$ ($[MH]^+$) m/z: 373.1547, found: 373.1548; IR (KBr) ν cm^{-1}: 1645, 1621, 1587, 1561, 1493, 1395, 1344, 1203, 1134, 1120, 1093, 1032, 812, 758; UV λ nm (MeOH) (log ε): 236 (4.35), 276 (4.62), 299 (4.61), 368 (3.84); Anal. Calcd for $C_{23}H_{20}N_2O_3$: C, 74.18; H, 5.41; N, 7.52. Found: C, 74.05; H, 5.40; N, 7.50.

*5-Methoxy-2,2,13-trimethyl-2,13-dihydro-6H-benzo[b]-chromeno[7,6-g][1,8]naphthyridin-6-one (**5**).* Yellow crystals: mp 239 °C (crystallized from CH_2Cl_2/MeOH 1/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 6H, $C(CH_3)_2$), 3.97 (s, 3H, OCH_3), 4.05 (s, 3H, NCH_3), 5.62 (d, J = 10 Hz, 1H, H-3), 6.68 (s, 1H, H-14), 6.73 (d, J = 10 Hz, 1H, H-4), 7.36 (ddd, J = 9, 7, 1 Hz, 1H, H-9), 7.69 (ddd, J = 9, 7, 1 Hz, 1H, H-10), 7.91 (m, 2H, H-8, H-11), 9.16 (s, 1H, H-7); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.5 (2C, $C(CH_3)_2$), 31.5 (N-CH_3), 62.4 (O-CH_3), 77.9 (C-2), 98.1 (C-14), 109.9 (C-4a), 110.3 (C-5a), 116.2 (C-4), 118.8 (C-6a), 124.4 (2C, C-10, C-7a), 127.5 (C-11), 129.2 (C-3),129.3 (C-8), 132.1 (C-9), 138.5 (C-7), 146.6 (C-13a), 149.2 (C-11a), 149.5 (C-12a), 158.1 (C-5), 159.3 (C-14a), 177.0 (C-6); HRMS (ESI) calcd for $C_{23}H_{20}N_2O_3$ ($[MH]^+$) m/z: 373.1547, found: 373.1550; IR (KBr) ν cm^{-1}: 3052, 2968, 2920, 1650, 1631, 1608, 1589, 1551, 1486, 1425, 1343, 1121, 1091, 1021, 958, 821, 784, 754; UV λ nm (MeOH) (log ε): 242 (4.44), 301 (4.85), 356 (4.13); Anal. Calcd for $C_{23}H_{20}N_2O_3$: C, 74.18; H, 5.41; N, 7.52. Found: C, 73.95; H, 5.39; N, 7.49.

*(±)-cis-1,2-Dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetra-hydro-7H-benzo[b]chromeno[6,5-g][1,8]naphthyridin-7-one (**19**).* Compound **4** (0.500 g, 1.35 mmol) was added to a solution of osmium tetroxide (2.5% in 2-methyl-2-propanol) (3.9 mL) and *N*-methyl-morpholine *N*-oxide dihydrate (0.63 g, 4.6 mmol) in *t*-BuOH/THF/H_2O

dx.doi.org/10.1021/jm500927d | J. Med. Chem. XXXX, XXX, XXX−XXX

(10:3:1, v/v/v, 55 mL). The reaction mixture was stirred at room temperature for 3 days. After addition of saturated aqueous $NaHSO_3$ (55 mL), the mixture was stirred for 1 h and then extracted with CH_2Cl_2 (5 × 100 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. Silica gel column chromatography (solvent: CH_2Cl_2 then CH_2Cl_2/MeOH, 99.9:0.1 to 98:2) gave **19** (0.315 g, 58%) as pale yellow crystals crystals: mp 302 °C (crystallized from Me_2CO/MeOH 1/1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.40 (s, 3H, CCH_3), 1.43 (s, 3H, CCH_3), 3.68 (m, 1H, H-2), 3.83 (s, 3H, OCH_3), 3.99 (s, 3H, NCH_3), 4.68 (br. s., 1H, D_2O exch., OH), 5.10 (m, 2H, H-1, OH), 6.25 (s, 1H, H-5), 7.50 (t, J = 8 Hz, 1H, H-10), 7.83 (t, J = 8 Hz, 1H, H-11), 7.95 (d, J = 8 Hz, 1H, H-9), 8.18 (dd, J = 8 Hz, 1H, H-12), 9.01 (s, 1H, H-8); ^{13}C NMR (75 MHz, DMSO-d_6) δ 23.2 (C-CH_3), 26.1 (C-CH_3), 40.0 (N-CH_3), 56.9 (O-CH_3), 64.9 (C-1), 71.1 (C-2), 78.8 (C-3), 95.2 (C-5), 105.3 (C-14b), 110.7 (C-6a), 120.4 (C-7a), 125.1 (C-8a), 125.4 (C-11), 128.0 (C-9), 130.6 (C-12), 133.1 (C-10), 137.8 (C-8), 149.7 (C-12a), 150.8 (C-14a), 152.7 (C-13a), 161.1 (C-4a), 162.4 (C-6), 177.1 (C-7); DCI-MS m/z 407 $[MH]^+$; IR (KBr) ν cm^{-1}: 3422 (br.), 1635, 1618, 1585, 1505, 1397, 1351, 1212, 1140, 1117, 1097, 1037, 815; UV λ nm (MeOH) (log ε): 232 (4.35), 288 (4.73), 350 (4.06); Anal. Calcd for $C_{23}H_{22}N_2O_5$: C, 67.97; H, 5.46; N, 6.89. Found: C, 67.81; H, 5.45; N, 6.87.

*(±)-cis-3,4-Dihydroxy-5-methoxy-2,2,13-trimethyl-2,3,4,13-tetrahydro-6H-benzo[b]chromeno- [7,6-g][1,8]naphthyridin-6-one (**20**).* Compound **20** was synthesized from **5** (0.500 g, 1.35 mmol) according to the procedure described for the preparation of **19** from **4**, using osmium tetroxide and *N*-methylmorpholine *N*-oxide dihydrate in *t*-BuOH/THF/H_2O. Purification by silica gel column chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99.9:0.1 to 98:2) gave **20** (0.388 g, 71%) as a pale yellow amorphous product. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.41 (s, 6H, $C(CH_3)_2$), 3.63 (dd, J = 6, 5 Hz, 1H, H-3), 3.93 (s, 3H, OCH_3), 4.02 (s, 3H, NCH_3), 4.89 (dd, J = 5, 4 Hz, 1H, H-4), 5.05 (m, 2H, D_2O exch., OH-3, OH-4), 6.77 (s, 1H, H-14), 7.51 (td, J = 8, 1 Hz, 1H, H-9), 7.85 (td, J = 8, 1 Hz, 1H, H-10), 7.94 (dd, J = 8, 1 Hz, 1H, H-8), 8.17 (dd, J = 8, 1 Hz, 1H, H-11), 9.19 (s, 1H, H-7); ^{13}C NMR (75 MHz, DMSO-d_6) δ 22.6 (C-CH_3), 28.2 (C-CH_3), 32.3 (N-CH_3), 61.9 (C-4), 63.5 (O-CH_3), 72.7 (C-3), 80.2 (C-2), 99.0 (C-14), 110.5 (C-5a), 114.4 (C-4a), 119.3 (C-6a), 124.9 (C-7a), 125.5 (C-10), 127.9 (C-8), 130.6 (C-11), 133.6 (C-9), 139.2 (C-7), 147.0 (C-13a), 149.6 (C-11a), 150.3 (C-12a), 159.7 (C-14a), 163.9 (C-5), 176.6 (C-6); DCI-MS m/z 407 $[MH]^+$; IR (KBr) ν cm^{-1}: 3438, 2936, 1650, 1602, 1589, 1553, 1491, 1423, 1399, 1344, 1224, 1193, 1128, 1104, 1025, 953, 823, 758; UV λ nm (MeOH) (log ε): 228 (4.27), 289 (4.51), 340 (4.11); Anal. Calcd for $C_{23}H_{22}N_2O_5$: C, 67.97; H, 5.46; N, 6.89. Found: C, 67.72; H, 5.44; N, 6.86.

*(±)-cis-1,2-Diacetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[b]chromeno[6,5-g][1,8]naphthyridin-7-one (**21**).* An ice-cooled mixture of acetic anhydride (10 mL, 105 mmol) and dry pyridine (45 mL) was added to **19** (0.200 g, 0.5 mmol) and 4-dimethylaminopyridine (0.01 g). After stirring at room temperature for 24 h, the mixture was poured on cold water (30 mL). The precipitate was filtered, washed with water (3 × 15 mL), dried *in vacuum* over P_2O_5, and crystallized from CH_2Cl_2/cyclohexane (1/1) to afford **21** (0.223 g, 92%) as pale yellow needles: mp 267 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (s, 3H, CCH_3), 1.60 (s, 3H, CCH_3), 1.95 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 3.80 (s, 3H, OCH_3), 4.02 (s, 3H, NCH_3), 5.54 (d, J = 5 Hz, 1H, H-2), 6.33 (s, 1H, H-5), 6.55 (d, J = 5 Hz, 1H, H-1), 7.46 (t, J = 8 Hz, 1H, H-10), 7.76 (t, J = 8 Hz, 1H, H-11), 7.99 (m, 2H, H-9, H-12), 9.11 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.6 (CH_3CO), 21.0 (CH_3CO), 23.1 (C-CH_3), 24.8 (C-CH_3), 39.8 (N-CH_3), 56.3 (O-CH_3), 66.0 (C-1), 69.1 (C-2), 76.5 (C-3), 94.7 (C-5), 98.3 (C-14b), 110.9 (C-6a), 120.0 (C-7a), 124.6 (C-11), 124.9 (C-8a), 127.8 (C-12), 129.4 (C-9), 131.9 (C-10), 137.6 (C-8), 149.6 (C-12a), 149.9 (C-14a), 152.3 (C-13a), 160.6 (C-4a), 160.3 (C-6), 170.4 (CH_3CO), 170.9 (CH_3CO), 178.0 (C-7); HRMS (ESI) calcd for $C_{27}H_{26}N_2O_7$ ($[MH]^+$) m/z 491.1813, found: 491.1814; IR (KBr) ν cm^{-1}: 2985, 2361, 2344, 1752, 1645, 1619, 1590, 1502, 1459, 1397, 1374, 1350, 1235, 1214, 1159, 1090, 1030, 813, 759; UV λ nm (MeOH) (log ε): 231 (4.36), 286 (4.72), 343 (4.08); Anal. Calcd for $C_{27}H_{26}N_2O_7$: C, 66.11; H, 5.34; N, 5.71. Found: C, 65.95; H, 5.34; N, 5.71.

*(±)-cis-1,2-Dicinnamoyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[b]chromeno[6,5-g][1,8]naphthyridin-7-one (**22**).* Thionyl chloride (7.30 mL, 100 mmol), was added dropwise to a suspension of cinnamoic acid (1.48 g, 10 mmol) in anhydrous CH_2Cl_2 (40 mL). The reaction mixture was stirred under reflux for 3 h and filtered, and the solvent and excess thionyl chloride were removed under reduced pressure to give the crude cinnamoyl chloride as a whitish solid, which was immediately used without further purification in the following step. The crude cinnamoyl chloride (0.98 g, 5.9 mmol) was added to a solution of **19** (0.239 g, 0.59 mmol) in dry pyridine (10 mL) containing 4-dimethylaminopyridine (0.01 g). The reaction mixture was stirred at room temperature for 48 h and then evaporated under reduced pressure. Silica gel column chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH 99.9:0.1 to 99:1) afforded **22** as a yellow amorphous solid (0.133 g, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (s, 3H, CCH_3), 1.66 (s, 3H, CCH_3), 3.82 (s, 3H, OCH_3), 4.08 (s, 3H, NCH_3), 5.88 (d, J = 5 Hz, 1H, H-2), 6.26 (d, 1H, J = 16 Hz, 2−OCOC*H*=CH), 6.34 (d, 1H, J = 16 Hz, 1−OCOC*H*=CH), 6.41 (s, 1H, H-5), 6.67 (d, J = 5 Hz, 1H, H-1), 7.07 (m, 3H, H-3″, H-4″, H-5″), 7.24 (m, 2H, H-2″, H-6″), 7.31 (m, 3H, H-3′, H-4′, H-5′), 7.36 (m, 2H, H-2′, H-6′), 7.42 (t, J = 8 Hz, 1H, H-10), 7.45 (d, 1H, J = 16 Hz, 1−OCOCH=C*H*), 7.53 (d, 1H, J = 16 Hz, 2−OCOCH=C*H*), 7.63 (t, J = 8 Hz, 1H, H-11), 7.78 (d, 1H, J = 8 Hz, H-9), 7.97 (d, 1H, J = 8 Hz, H-12), 9.11 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.1 (C-CH_3), 25.0 (C-CH_3), 39.7 (N-CH_3), 56.4 (O-CH_3), 66.7 (C-1), 69.1 (C-2), 77.2 (C-3), 94.7 (C-5), 98.5 (C-14b), 110.9 (C-6a), 116.9 (2−OCO*C*H=CH), 117.0 (1−OCO*C*H=CH), 119.9 (C-7a), 124.6 (C-11), 124.9 (C-8a), 127.9 (C-9), 128.1 (2C, C3′, C5′), 128.2 (2C, C3″, C5″), 128.5 (2C, C2″, C6″), 128.7 (2C, C2′, C6′), 129.2 (C-12), 130.2 (C-4″), 130.4 (C-4′), 131.7 (C-10), 133.9 (C-1′), 134.0 (C-1″), 137.6 (C-8), 146.1 (1−OCOCH=*C*H), 146.2 (2−OCOCH=*C*H), 149.6 (C-12a), 150.0 (C-14a), 152.1 (C-13a), 160.8 (C-4a), 163.1 (C-6), 166.1 (2-OCOCH=CH), 166.7 (1-OCOCH=CH), 178.1 (C-7); HRMS (ESI) calcd for $C_{41}H_{34}N_2O_7$ ($[MH]^+$) m/z: 667.2439, found: 667.2440; IR (KBr) ν cm^{-1}: 2967, 2360, 1733, 1644, 1590, 1575, 1450, 1399, 1204, 1172, 1150, 1091, 979, 766; UV λ nm (MeOH) (log ε): 283 (4.95), 344 (4.14), 428 (3.89); Anal. Calcd for $C_{41}H_{34}N_2O_7$: C, 73.86; H, 5.14; N, 4.20. Found: C, 73.72; H, 5.13; N, 4.19.

*(±)-cis-6-Methoxy-1,2-di-(4-methoxycinnamoyloxy)-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[b]chromeno[6,5-g][1,8]-naphthyridin-7-one (**23**).* Compound **23** was synthesized from **19** (0.239 g, 0.59 mmol) according to the procedure described for the preparation of **22** from **19**, using 4-methoxycinnamoyl chloride (1.15 g, 5.9 mmol) prepared extemporaneously from 4-methoxycinnamoic acid. Silica gel column chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH 99.9:0.1 to 99:1) afforded **23** as a yellow amorphous solid (0.098 g, 22%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 (s, 3H, CCH_3), 1.69 (s, 3H, CCH_3), 3.73 (s, 3H, 4″-OCH_3), 3.78 (s, 3H, 4′-OCH_3), 3.86 (s, 3H, 6-OCH_3), 4.08 (s, 3H, NCH_3), 5.87 (d, J = 5 Hz, 1H, H-2), 6.14 (d, 1H, J = 16 Hz, 1−OCOC*H*=CH), 6.20 (d, 1H, J = 16 Hz, 2−OCOC*H*=CH), 6.41 (s, 1H, H-5), 6.58 (d, J = 9 Hz, 2H, H-3″, H-5″), 6.64 (d, J = 5 Hz, 1H, H-1), 6.80 (d, J = 9 Hz, 2H, H-3′, H-5′), 7.01 (d, J = 9 Hz, 2H, H-2″, H-6″), 7.33 (d, J = 9 Hz, 2H, H-2′, H-6′), 7.38 (d, 1H, J = 16 Hz, 1−OCOCH=C*H*), 7.44 (t, J = 8 Hz, 1H, H-10), 7.47 (d, 1H, J = 16 Hz, 2−OCOCH=C*H*), 7.62 (t, J = 8 Hz, 1H, H-11), 7.77 (d, 1H, J = 8 Hz, H-9), 7.97 (d, 1H, J = 8 Hz, H-12), 9.13 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.1 (C-CH_3), 25.0 (C-CH_3), 39.7 (N-CH_3), 55.3 (2C, 4′-O-CH_3, 4″-O-CH_3), 56.4 (6-O-CH_3), 66.6 (C-1), 68.8 (C-2), 77.5 (C-3), 94.7 (C-5), 98.7 (C-14b), 110.9 (C-6a), 113.9 (2C, C3″, C5″), 114.2 (2C, C3′, C5′), 114.5 (2C, 1−OCO*C*H=CH, 2−OCO*C*H=CH), 119.8 (C-7a), 124.5 (C-11), 124.8 (C-8a), 126.8 (2C, C-1′, C-1″), 127.9 (C-9), 129.2 (C-12), 129.9 (2C, C2″, C6″), 129.9 (2C, C2′, C6′), 131.7 (C-10), 137.6 (C-8), 145.8 (2C, 1−OCOCH=*C*H, 2−OCOCH=*C*H), 149.6 (C-12a), 150.0 (C-14a), 152.0 (C-13a), 160.8 (C-4a), 161.3 (C-4″), 161.5 (C-4′), 163.0 (C-6), 166.4 (1-OCOCH=CH), 167.0 (2-OCOCH=CH), 178.2 (C-7); HRMS (ESI) calcd for $C_{43}H_{38}N_2O_9$ ($[MH]^+$) m/z: 727.2650, found: 727.2650; IR (KBr) ν cm^{-1}: 2979, 2935, 2836, 2363, 1719, 1647, 1591, 1512, 1397, 1255, 1156, 1092, 1032, 827; UV λ nm (MeOH) (log ε): 288 (4.85),

dx.doi.org/10.1021/jm500927d | J. Med. Chem. XXXX, XXX, XXX−XXX

312 (4.65), 429 (3.87); Anal. Calcd for $C_{43}H_{38}N_2O_9$: C, 71.06; H, 5.27; N, 3.85. Found: C, 70.82; H, 5.26; N, 3.84.

*(±)-cis-6-Methoxy-1,2-di-(4-trifluoromethylcinnamoyloxy)-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[b]chromeno[6,5-g]-[1,8]naphthyridin-7-one (**24**).* Compound **24** was synthesized from **19** (239 mg, 0.59 mmol) according to the procedure described for the preparation of **22** from **19**, using 4-trifluoromethylcinnamoyl chloride (1.38 g, 5.9 mmol) prepared extemporaneously from 4-trifluoromethylcinnamoic acid. Silica gel column chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH 99.9:0.1 to 99:1) afforded **24** as a yellow amorphous solid (0.175 g, 37%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 (s, 3H, CCH_3), 1.69 (s, 3H, CCH_3), 3.81 (s, 3H, OCH_3), 4.08 (s, 3H, NCH_3), 5.91 (d, J = 5 Hz, 1H, H-2), 6.34 (d, 1H, J = 16 Hz, 1−OCOC*H*═CH), 6.41 (d, 1H, J = 16 Hz, 2−OCOC*H*═CH), 6.43 (s, 1H, H-5), 6.64 (d, J = 5 Hz, 1H, H-1), 7.06 (d, J = 9 Hz, 2H, H-3″, H-5″), 7.28 (d, J = 9 Hz, 2H, H-2″, H-6″), 7.36 (d, 1H, J = 16 Hz, 1−OCOCH═C*H*), 7.48 (m, 6H, H-2′, H-3′, H-5′, H-6′. H-10, 2−OCOCH═C*H*), 7.59 (t, J = 8 Hz, 1H, H-11), 7.68 (d, 1H, J = 8 Hz, H-9), 7.98 (d, 1H, J = 8 Hz, H-12), 9.11 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.9 (C-CH_3), 25.0 (C-CH_3), 39.7 (N-CH_3), 56.4 (O-CH_3), 67.1 (C-1), 69.2 (C-2), 77.1 (C-3), 94.7 (C-5), 98.1 (C-14b), 110.8 (C-6a), 119.4 (C-7a), 119.8 (1−OCOCH═CH), 120.4 (2−OCOCH═CH), 124.8 (C-11), 125.0 (C-8a), 125.5 (2C, C3″, C5″), 125.8 (2C, C3′, C5′), 127.7 (C-9), 128.1 (2C, C2″, C6″), 128.3 (2C, C2′, C6′), 129.3 (4″-CF_3), 129.4 (C-12), 129.8 (4′-CF_3), 131.9 (C-10), 132.0 (C-4″), 132.3 (C-4′), 137.0 (C-1″), 137.2 (C-1′), 137.7 (C-8), 144.2 (1−OCOCH═*C*H), 144.4 (2−OCOCH═*C*H), 149.5 (C-12a), 150.0 (C-14a), 152.1 (C-13a), 160.7 (C-4a), 163.2 (C-6), 165.6 (1-OCOCH═CH), 166.1 (2-OCOCH═CH), 178.1 (C-7); HRMS (ESI) calcd for $C_{43}H_{32}F_6N_2O_7$ ($[MH]^+$) m/z: 803.2186, found: 803.2186; IR (KBr) ν cm^{-1}: 2974, 2934, 2855, 2367, 1728, 1648, 1592, 1501, 1397, 1326, 1206, 1161, 1127, 1067, 1017, 832, 758; UV λ nm (MeOH) (log ε): 288 (4.63), 343 (4.05), 423 (3.80); Anal. Calcd for $C_{43}H_{32}F_6N_2O_7$: C, 64.34; H, 4.02; N, 3.49. Found: C, 64.12; H, 4.01; N, 3.49.

*(±)-cis-2-Cinnamoyloxy-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[b]chromeno[6,5-g][1,8]-naphthyridin-7-one (**25**).* Cinnamoyl chloride (0.186 g, 1.12 mmol), prepared extemporaneously from 4-methoxycinnamoic acid, was added to a solution of **19** (0.239 g, 0.59 mmol) in dry pyridine (10 mL). The reaction mixture was stirred at room temperature for 48 h and then evaporated under reduced pressure. Silica gel column chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99:1) afforded **25** as a yellow amorphous solid (0.196 g, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (s, 3H, CCH_3), 1.61 (s, 3H, CCH_3), 3.67 (br. d, J = 9 Hz, 1H, D_2O exch., OH-1), 3.88 (s, 3H, OCH_3), 4.04 (s, 3H, NCH_3), 5.50 (dd, J = 9, 5 Hz, 1H, H-1), 5.64 (d, J = 5 Hz, 1H, H-2), 6.17 (s, 1H, H-5), 6.58 (d, 1H, J = 16 Hz, OCOC*H*═CH), 7.25 (m, 3H, H-3′, H-4′, H-5′), 7.36 (m, 3H, H-2′, H-6′, H-10), 7.58 (t, J = 8 Hz, 1H, H-11), 7.77 (d, 1H, J = 16 Hz, OCOCH═C*H*), 7.78 (d, 1H, J = 8 Hz, H-9), 7.88 (d, 1H, J = 8 Hz, H-12), 8.85 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.6 (C-CH_3), 25.5 (C-CH_3), 39.3 (N-CH_3), 55.9 (O-CH_3), 63.8 (C-1), 72.3 (C-2), 77.2 (C-3), 94.1 (C-5), 103.1 (C-14b), 110.3 (C-6a), 117.1 (OCOCH═CH), 119.3 (C-7a), 124.3 (C-11), 124.5 (C-8a), 127.1 (C-9), 128.1 (2C, C3′, C5′), 128.8 (2C, C2′, C6′), 129.3 (C-12), 130.4 (C-4′), 131.7 (C-10), 134.1 (C-1′), 137.7 (C-8), 146.4 (OCOCH═*C*H), 149.1 (C-12a), 149.7 (C-14a), 151.7 (C-13a), 159.8 (C-4a), 162.5 (C-6), 167.1 (OCOCH═CH), 177.4 (C-7); HRMS (ESI) calcd for $C_{32}H_{28}N_2O_6$ ($[MH]^+$) m/z: 537.2020, found: 537.2021; IR (KBr) ν cm^{-1}: 3406 (br.), 2975, 2933, 2359, 1719, 1638, 1589, 1503, 1394, 1159, 1095, 1033, 818, 769; UV λ nm (MeOH) (log ε): 288 (4.76), 348 (4.03), 433 (3.79); Anal. Calcd for $C_{32}H_{28}N_2O_6$: C, 71.63; H, 5.26; N, 5.22. Found: C, 71.42; H, 5.25 N, 5.21.

*(±)-cis-1-Hydroxy-6-methoxy-2-(4-methoxycinnamoyloxy)-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[b]chromeno[6,5-g]-[1,8]naphthyridin-7-one (**26**).* Compound **26** was synthesized from **19** (0.307 g, 0.76 mmol) according to the procedure described for the preparation of **25** from **19**, using 4-methoxycinnamoyl chloride (0.282 g, 1.44 mmol) prepared extemporaneously from 4-methoxycinnamoic acid. Silica gel column chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH 99.9:0.1 to 99:1) afforded **26** as a yellow amorphous solid (0.194 g, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 (s, 3H, CCH_3), 1.58 (s, 3H, CCH_3), 3.38 (br. d, J = 9 Hz, 1H, D_2O exch., OH-1), 3.77 (s, 3H, 4′-OCH_3), 3.89 (s, 3H, 6-OCH_3), 4.05 (s, 3H, NCH_3), 5.49 (dd, J = 9, 5 Hz, 1H, H-1), 5.62 (d, J = 5 Hz, 1H, H-2), 6.18 (s, 1H, H-5), 6.40 (d, 1H, J = 16 Hz, OCOC*H*═CH), 6.76 (d, J = 9 Hz, 2H, H-3′, H-5′), 7.32 (d, J = 9 Hz, 2H, H-2′, H-6′), 7.38 (t, J = 8 Hz, 1H, H-10), 7.63 (t, J = 8 Hz, 1H, H-11), 7.69 (d, 1H, J = 16 Hz, OCOCH═C*H*), 7.84 (d, 1H, J = 8 Hz, H-9), 7.91 (d, 1H, J = 8 Hz, H-12), 8.90 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.6 (C-CH_3), 25.5 (C-CH_3), 39.4 (N-CH_3), 55.3 (4′-O-CH_3), 56,0 (6-O-CH_3), 63.8 (C-1), 72.1 (C-2), 77.1 (C-3), 94.2 (C-5), 103.1 (C-14b), 110.3 (C-6a), 114.3 (2C, C3′, C5′), 114.4 (OCOCH═CH), 119.4 (C-7a), 124.3 (C-11), 124.6 (C-8a), 126.8 (C-1′), 127.2 (C-9), 129.3 (C-12), 129,9 (2C, C2′, C6′), 131.7 (C-10), 137.7 (C-8), 146.1 (OCOCH═CH), 149.2 (C-12a), 149.7 (C-14a), 151.8 (C-13a), 159.8 (C-4a), 161.5 (C-4′), 162.5 (C-6), 167.1 (OCOCH═CH), 177.5 (C-7); HRMS (ESI) calcd for $C_{33}H_{30}N_2O_7$ ($[MH]^+$) m/z: 567.2126, found: 567.2127; IR (KBr) ν cm^{-1}: 3398 (br.), 2974, 2931, 2834, 1720, 1641, 1588, 1503, 1394, 1254, 1209, 1146, 1095, 1032, 819; UV λ nm (MeOH) (log ε): 288 (4.84), 434 (3.88); Anal. Calcd for $C_{33}H_{30}N_2O_7$: C, 69.95; H, 5.34; N, 4.94. Found: C, 69.80; H, 5.33; N, 4.93.

*(±)-cis-1-Hydroxy-6-methoxy-2-(4-trifluoromethylcinnamoyl-oxy)-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[b]chromeno-[6,5-g][1,8]naphthyridin-7-one (**27**).* Compound **27** was synthesized from **19** (0.431 g, 1.06 mmol) according to the procedure described for the preparation of **25** from **19**, using 4-trifluoromethylcinnamoyl chloride (0.471 g, 2.0 mmol) prepared extemporaneously from 4-trifluoromethylcinnamoic acid. Silica gel column chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH 99.9:0.1 to 99:1) afforded **27** as a yellow amorphous solid (0.243 g, 38%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54 (s, 3H, CCH_3), 1.61 (s, 3H, CCH_3), 3.63 (br. d, J = 9 Hz, 1H, D_2O exch., OH-1), 3.89 (s, 3H, OCH_3), 4.09 (s, 3H, NCH_3), 5.51 (dd, J = 9, 5 Hz, 1H, H-1), 5.63 (d, J = 5 Hz, 1H, H-2), 6.19 (s, 1H, H-5), 6.72 (d, 1H, J = 16 Hz, OCOC*H*═CH), 7.35 (t, J = 8 Hz, 1H, H-10), 7.46 (d, J = 8 Hz, 2H, H-3′, H-5′), 7.50 (d, J = 8 Hz, 2H, H-2′, H-6′), 7.56 (t, J = 8 Hz, 1H, H-11), 7.74 (d, 1H, J = 8 Hz, H-9), 7.82 (d, 1H, J = 16 Hz, OCOCH═C*H*), 7.85 (d, 1H, J = 8 Hz, H-12), 8.82 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.4 (C-CH_3), 25.6 (C-CH_3), 39.2 (N-CH_3), 55.6 (O-CH_3), 63.8 (C-1), 72.6 (C-2), 77.2 (C-3), 93.8 (C-5), 103.0 (C-14b), 110.0 (C-6a), 118.8 (C-7a), 120.2 (OCOCH═CH), 124.2 (C-8a), 124.3 (C-11), 125.7 (4′-CF_3), 125.8 (2C, C3′, C5′), 127.1 (C-9), 128.1 (2C, C2′, C6′), 129.2 (C-12), 131.4 (C-4′), 131.8 (C-10), 131.9 (C-1′), 137.7 (C-8), 144.2 (OCOCH═*C*H), 148.9 (C-12a), 149.8 (C-14a), 151.3 (C-13a), 159.9 (C-4a), 162.3 (C-6), 166.8 (OCOCH═CH), 177.2 (C-7); HRMS (ESI) calcd for $C_{33}H_{27}F_3N_2O_6$ ($[MH]^+$) m/z: 605.1894, found: 605.1894; IR (KBr) ν cm^{-1}: 3372 (br.), 2970, 2931, 2363, 1727, 1639, 1588, 1503, 1394, 1326, 1210, 1159, 1124, 1067, 1035, 834, 812, 747; UV λ nm (MeOH) (log ε): 288 (4.83), 348 (4.13), 432 (3.90); Anal. Calcd for $C_{33}H_{27}F_3N_2O_6$: C, 65.56; H, 4.50; N, 4.63. Found: C, 65.42; H, 4.50; N, 4.62.

*(±)-cis-1-Acetoxy-2-cinnamoyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[b]chromeno[6,5-g][1,8]naphthyridin-7-one (**28**).* Acetic anhydride (1.5 mL, 15.7 mmol) was added to an iced-cooled solution (0 °C) of monoester **25** (0.080 g, 0,15 mmol) and 4-dimethylaminopyridine (0.01 g) in dry pyridine (5 mL). After stirring at 25 °C for 5 h, the reaction mixture was poured on cold water (20 mL). The precipitate was filtered, washed with water (3 × 15 mL), and dried *in vacuum* over P_2O_5, to afford **28** as a yellow amorphous solid (0.072 g, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (s, 3H, CCH_3), 1.65 (s, 3H, CCH_3), 1.92 (s, 3H, CH_3CO), 3.81 (s, 3H, OCH_3), 4.06 (s, 3H, NCH_3), 5.73 (d, J = 5 Hz, 1H, H-2), 6.35 (d, 1H, J = 16 Hz, OCOC*H*═CH), 6.38 (s, 1H, H-5), 6.58 (d, J = 5 Hz, 1H, H-1), 7.34 (m, 3H, H-3′, H-4′, H-5′), 7.45 (m, 3H, H-2′, H-6′, H-10), 7.60 (d, 1H, J = 16 Hz, OCOCH═C*H*), 7.76 (t, J = 8 Hz, 1H, H-11), 7.99 (m, 2H, H-9, H-12), 9.11 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.0 (CH_3CO), 22.7 (C-CH_3), 25.0 (C-CH_3), 39.7 (N-CH_3), 56.4 (O-CH_3), 66.3 (C-1), 68.8 (C-2), 77.2 (C-3), 94.5 (C-5), 98.2 (C-14b), 110.9 (C-6a), 116.8 (OCOCH═CH), 120.0 (C-7a), 124.6 (C-11), 124.9 (C-8a), 127.8 (C-12), 128.3 (2C, C3′, C5′), 128.8 (2C, C2′, C6′), 129.4 (C-9), 130.6 (C-4′), 131,9 (C-10),

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133.9 (C-1′), 137.6 (C-8), 146,3 (OCOCH=*C*H), 149.6 (C-12a), 149.9 (C-14a), 152.2 (C-13a), 160.6 (C-4a), 163.0 (C-6), 166.3 (OCOCH=CH), 171.1 (CH_3CO), 178.1 (C-7); HRMS (ESI) calcd for $C_{34}H_{30}N_2O_7$ ($[MH]^+$) *m*/*z*: 579.2126, found: 579.2126; IR (KBr) ν cm^{-1}: 2976, 2360, 1746, 1717, 1641, 1589, 1500, 1399, 1217, 1153, 1092, 1035, 815, 768; UV λ nm (MeOH) (log ε): 288 (4.45), 343 (3.85), 427 (3.55); Anal. Calcd for $C_{34}H_{30}N_2O_7$: C, 70.58; H, 5.23; N, 4.84. Found: C, 70.43; H, 5.22; N, 4.83.

(±)-cis-1-Acetoxy-6-methoxy-2-(4-methoxycinnamoyloxy)-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[b]chromeno[6,5-g][1,8]naphthyridin-7-one (**29**). Acetic anhydride (1.5 mL, 15.7 mmol) was added to an iced-cooled solution (0 °C) of monoester **26** (0.131 g, 0,23 mmol) and 4-dimethylaminopyridine (0.01 g) in dry pyridine (5 mL). Following the procedure described for the preparation of **28** from **25**, the mixed ester **29** was obtained as a yellow amorphous solid (0.129 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.50 (s, 3H, CCH_3), 1.64 (s, 3H, CCH_3), 1.91 (s, 3H, CH_3CO), 3.79 (s, 6H, 6-OCH_3, 4′-OCH_3), 4.05 (s, 3H, NCH_3), 5.72 (d, *J* = 5 Hz, 1H, H-2), 6.22 (d, 1H, *J* = 16 Hz, OCOC*H*=CH), 6.38 (s, 1H, H-5), 6.57 (d, *J* = 5 Hz, 1H, H-1), 6.84 (d, *J* = 8 Hz, 2H, H-3′, H-5′), 7.39 (d, *J* = 8 Hz, 2H, H-2′, H-6′), 7.45 (t, *J* = 8 Hz, 1H, H-10), 7.55 (d, 1H, *J* = 16 Hz, OCOCH=*CH*), 7.76 (t, *J* = 8 Hz, 1H, H-11), 7.98 (m, 2H, H-9, H-12), 9.11 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.0 (CH_3CO), 22.7 (C-CH_3), 25.0 (C-CH_{3a}), 39.7 (N-CH_3), 55.4 (4′-O-CH_3), 56.4 (6-O-CH_3), 66.3 (C-1), 68.5 (C-2), 77.8 (C-3), 94.6 (C-5), 98.2 (C-14b), 110.9 (C-6a), 113.4 (OCO*C*H=CH), 114.3 (2C, C3′, C5′), 120.0 (C-7a), 124.6 (C-11), 124.9 (C-8a), 126.7 (C-1′), 127.8 (C-9), 129.4 (C-12), 130.0 (2C, C2′, C6′), 131.9 (C-10), 137.6 (C-8), 146.0 (OCOCH=*C*H), 149.6 (C-12a), 149.9 (C-14a), 152.2 (C-13a), 160.1 (C-4a), 161.6 (C-4′), 163.0 (C-6), 166.6 (OCOCH=CH), 171.1 (CH_3CO), 178.1 (C-7); HRMS (ESI) calcd for $C_{35}H_{32}N_2O_8$ ($[MH]^+$) *m*/*z*: 609.2231, found: 609.2232; IR (KBr) ν cm^{-1}: 2974, 1745, 1718, 1648, 1592, 1501, 1397, 1346, 1254, 1214, 1151, 1092, 1031, 815; UV λ nm (MeOH) (log ε): 288 (4.82), 314 (4.55), 427 (3.86); Anal. Calcd for $C_{35}H_{32}N_2O_8$: C, 69.07; H, 5.30; N, 4.60. Found: C, 68.98; H, 5.29; N, 4.59.

(±)-cis-1-Acetoxy-6-methoxy-2-(4-trifluoromethylcinnamoyloxy)-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[b]chromeno[6,5-g][1,8]naphthyridin-7-one (**30**). Acetic anhydride (1.5 mL, 15.7 mmol) was added to an iced-cooled solution (0 °C) of monoester **27** (0.072 g, 0.12 mmol) and 4-dimethylaminopyridine (0.01 g) in dry pyridine (5 mL). Following the procedure described for the preparation of **28** from **25**, the mixed ester **30** was obtained as a yellow amorphous solid (0.054 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (s, 3H, CCH_3), 1.64 (s, 3H, CCH_3), 1.92 (s, 3H, CH_3CO), 3.80 (s, 3H, OCH_3), 4.04 (s, 3H, NCH_3), 5.73 (d, *J* = 5 Hz, 1H, H-2), 6.39 (s, 1H, H-5), 6.43 (d, 1H, *J* = 16 Hz, OCOC*H*=CH), 6.58 (d, *J* = 5 Hz, 1H, H-1), 7.46 (t, *J* = 8 Hz, 1H, H-10), 7.56 (m, 5H, H-2′, H-3′, H-5′, H-6′, OCOCH=C*H*), 7.77 (t, *J* = 8 Hz, 1H, H-11), 7.98 (m, 2H, H-9, H-12), 9.11 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.0 (CH_3CO), 22.7 (C-CH_3), 25.0 (C-CH_{3a}), 39.7 (N-CH_3), 56.3 (O-CH_3), 66.2 (C-1), 69.2 (C-2), 77.2 (C-3), 94.5 (C-5), 98.1 (C-14b), 110.9 (C-6a), 119.5 (C-7a), 120.0 (OCOCH=CH), 124.7 (C-11), 124.9 (C-8a), 125.8 (4′-CF_3), 125.8 (2C, C3′, C5′), 127.8 (C-9), 128.4 (2C, C2′, C6′), 129.4 (C-12), 130.0 (C-4′), 131.8 (C-11), 131.9 (C-10), 137.6 (C-8), 144.3 (OCOCH=*C*H), 149.6 (C-12a), 150.0 (C-14a), 152.2 (C-13a), 160.6 (C-4a), 163.1 (C-6), 165.7 (OCOCH=CH), 171.0 (CH_3CO), 178.0 (C-7); HRMS (ESI) calcd for $C_{35}H_{29}F_3N_2O_7$ ($[MH]^+$) *m*/*z*: 647.2000, found: 647.1999; IR (KBr) ν cm^{-1}: 2971, 2931, 2362, 1751, 1736, 1647, 1592, 1502, 1399, 1324, 1214, 1161, 1094, 1067, 1033, 835; UV λ nm (MeOH) (log ε): 288 (4.63), 343 (4.03), 427 (3.77); Anal. Calcd for $C_{35}H_{29}F_3N_2O_7$: C, 65.01; H, 4.52; N, 4.33. Found: C, 64.82; H, 4.51; N, 4.32.

(±)-cis-1,2-Di-O-carbonyl-1,2-dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[b]chromeno[6,5-g][1,8]naphthyridin-7-one (**31**). *N*,*N*′-Carbonyldiimidazole (0.64 g, 3.92 mmol) was added to a solution of **19** (0.20 g, 0.49 mmol) in 2-butanone (90 mL). The reaction mixture was refluxed for 4 days under argon, and after cooling, 5% aqueous $NaHCO_3$ (15 mL) was added. The solution was extracted with ethyl acetate (3 × 30 mL), and the combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. Silica gel column chromatography (solvent: CH_2Cl_2 then CH_2Cl_2/MeOH, 99.9:0.1 to 98:2) gave **31** (0.078 g, 37%) as a yellowish amorphous solid. ^{1}H NMR (400 MHz, DMSO) δ 1.45 (s, 3H, CCH_3), 1.56 (s, 3H, CCH_3), 3.89 (s, 3H, OCH_3), 4.02 (s, 3H, NCH_3), 5.24 (d, *J* = 8 Hz, 1H, H-2), 6.46 (s, 1H, H-5), 6.78 (d, *J* = 8 Hz, 1H, H-1), 7.56 (ddd, *J* = 8, 7, 1 Hz, 1H, H-10), 7.88 (ddd, *J* = 8, 7, 1 Hz, 1H, H-11), 8.00 (dd, *J* = 8, 1 Hz, 1H, H-9), 8.22 (dd, *J* = 8, 1 Hz, 1H, H-12), 9.05 (s, 1H, H-8); ^{13}C NMR (75 MHz, DMSO) δ 22.0 (C-CH_3), 25.3 (C-CH_3), 42.0 (N-CH_3), 57.3 (O-CH_3), 71.7 (C-1), 76.4 (C-3), 79.4 (C-2), 96.5 (C-5), 99.8 (C-14b), 111.3 (C-6a), 120.6 (C-7a), 125.5 (C-8a), 126.0 (C-11), 128.2 (C-9), 130.7 (C-12), 133.6 (C-10), 138.2 (C-8), 149.6 (C-12), 150.2 (C-14a), 152.9 (C-13a), 154.5 (CO), 160.6 (C-4a), 164.0 (C-6), 177.3 (C-7); DCI-MS *m*/*z* 433 $[MH]^+$, 431, 389, 373; IR (KBr) ν cm^{-1}: 1810, 1640, 1619, 1588, 1572, 1501, 1459, 1399, 1345, 1213, 1199, 1172, 1158, 1097, 1032, 981, 814, 754; UV λ nm (MeOH) (log ε): 286 (4.56), 343 (3.98); Anal. Calcd for $C_{24}H_{20}N_2O_6$: C, 66.66; H, 4.66; N, 6.48. Found: C, 66.49; H, 4.65; N, 6.47.

(±)-cis-3,4-Diacetoxy-5-methoxy-2,2,13-trimethyl-2,3,4,13-tetrahydro-6H-benzo[b]chromeno[7,6-g][1,8]naphthyridin-6-one (**32**). Compound **32** was synthesized from **20** (0.200 g, 0.49 mmol) according to the procedure described for the preparation of **21** from **19**, using acetic anhydride (10 mL, 105 mmol) and 4-dimethylaminopyridine (0.01 g) in dry pyridine (40 mL). Crystallization from CH_2Cl_2 gave **32** (0.213 g, 88%) as pale yellow needles: mp 313 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 3H, CCH_3), 1.51 (s, 3H, CCH_3), 2.09 (s, 3H, CH_3CO), 2.11 (s, 3H, CH_3CO), 3.98 (s, 3H, OCH_3), 4.12 (s, 3H, NCH_3), 5.27 (d, *J* = 5 Hz, 1H, H-3), 6.48 (d, *J* = 5 Hz, 1H, H-4), 6.78 (s, 1H, H-14), 7.46 (ddd, *J* = 8, 7, 1 Hz, 1H, H-9), 7.77 (ddd, *J* = 8, 7, 1 Hz, 1H, H-10), 7.97 (m, 2H, H-8, H-11), 9.23 (s, 1H, H-7); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.7 ($COCH_3$), 20.9 ($COCH_3$), 21.9 (C-CH_3), 26.3 (C-CH_3), 31.6 (N-CH_3), 61.0 (C-4), 62.4 (O-CH_3), 71.5 (C-3), 77.3 (C-2), 98.4 (C-14), 107.8 (C-4a), 110.4 (C-5a), 118.7 (C-6a), 124.5 (C-7a), 124.6 (C-10), 127.5 (C-11), 129.4 (C-8), 132.4 (C-9), 138.7 (C-7), 147.4 (C-13a), 149.4 (C-11a), 158.9 (C-14a), 163.5 (C-5), 169.4 ($COCH_3$), 170.0 ($COCH_3$), 177.4 (C-6); HRMS (ESI) calcd for $C_{27}H_{26}N_2O_7$ ($[MH]^+$) *m*/*z*: 491.1813, found: 491.1814; IR (KBr) ν cm^{-1}: 2936, 1754, 1741, 1648, 1618, 1608, 1592, 1577, 1560, 1501, 1425, 1373,1344, 1241, 1196, 1153, 1127, 1100, 1049, 1026, 824, 760; UV λ nm (MeOH) (log ε): 228 (4.39), 289(4.83), 337 (4.18); Anal. Calcd for $C_{27}H_{26}N_2O_7$: C, 66.11; H, 5.34; N, 5.71. Found: C, 65.98; H, 5.33; N, 5.70.

(±)-cis-3,4-Di-O-carbonyl-3,4-dihydroxy-5-methoxy-2,2,13-trimethyl-2,3,4,13-tetrahydro-6H-benzo[b]chromeno[7,6-g][1,8]naphthyridin-6-one (**33**). Compound **33** was synthesized from **20** (0.200 g, 0.49 mmol) according to the procedure described for the preparation of **31** from **19**, using carbonyldiimidazole (0.64 g, 3.92 mmol) in 2-butanone (80 mL). Purification by silica gel column chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99.9:0.1 to 99.2:0.8) gave **33** (0.143 g, 67%) as pale yellow crystals: mp 272 °C (crystallized from CH_2Cl_2/MeOH 1/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (s, 3H, CCH_3), 1.59 (s, 3H, CCH_3), 4.05 (s, 3H, OCH_3), 4.10 (s, 3H, NCH_3), 4.73 (dd, *J* = 8 Hz, 1H, H-3), 6.04 (d, *J* = 8 Hz, 1H, H-4), 6.78 (s, 1H, H-14), 7.44 (ddd, *J* = 8, 7, 1 Hz, 1H, H-9), 7.75 (ddd, *J* = 8, 7, 1 Hz, 1H, H-10), 8.15 (m, 2H, H-8, H-11), 9.20 (s, 1H, H-7); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.5 (C-CH_3), 24.3 (C-CH_3), 31.7 (N-CH_3), 63.9 (O-CH_3), 68.6 (C-4), 76.2 (C-2), 78.1 (C-3), 99.4 (C-14), 106.0 (C-4a), 110.9 (C-5a), 118.7 (C-6a), 124.6 (C-7a), 124.9 (C-10), 127.6 (C-11), 129.4 (C-8), 132.6 (C-9), 138.8 (C-7), 148.1 (C-13a), 149.5 (2C, C-11a, C-12a), 153.9 (CO), 158.3 (C-14a), 164.1 (C-5), 177.0 (C-6); HRMS (ESI) calcd for $C_{24}H_{20}N_2O_6$ ($[MH]^+$) *m*/*z*: 433.1394, found: 433.1394; IR (KBr) ν cm^{-1}: 2940, 2363, 1794, 1649, 1619, 1607, 1577, 1498, 1425, 1345, 1206, 1177, 1158, 1100, 1080, 1029, 750; UV λ nm (MeOH) (log ε): 228 (4.39), 288 (4.78), 335 (4.20); Anal. Calcd for $C_{24}H_{20}N_2O_6$: C, 66.66; H, 4.66; N, 6.48. Found: C, 66.50; H, 4.66; N, 6.47.

(+)-(1R,2R)-1,2-Diacetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[b]chromeno[6,5-g][1,8]naphthyridin-7-one (**21a**) *and (−)-(1S,2S)-1,2-Diacetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[b]chromeno[6,5-g][1,8]naphthyridin-7-one* (**21b**). Chiral separation of the isomers was performed on Preparative pump K-1800 KNAUER using a Chiralpak AS-V (600 mm × 60 mm, 20 μm) column with CH_3OH−CH_3CN−DEA

dx.doi.org/10.1021/jm500927d | J. Med. Chem. XXXX, XXX, XXX−XXX

(diethylamine) 900-100-1 as the mobile phase and a flow rate of 50 mL/min at room temperature from the racemic compound **21** (269 mg). The desired compounds **21a** and **21 b** were isolated in quantitative yields. The absolute stereochemistry was deduced from the optical rotation sign and by observation of the curves of the Cotton effect by comparison with those of **3** enantiomers. For **3** enantiomers the absolute configuration of one of them was previously determined by RX diffraction of a brominated compound.[16]

21a[α]: D = +0.028° (CH_2Cl_2), negative Cotton effect *R,R*
21b[α]: D = −0.019° (CH_2Cl_2), positive Cotton effect *S,S*

■ ASSOCIATED CONTENT

Supporting Information

^1H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

M.-H.D.-C. thanks the IRCL and the Ligue Nationale contre le Cancer (Comité du Nord) for grants, and the IFR114-IMPRT for access to the Storm facilities.

■ DEDICATION

‖Dedicated to Professor François Tillequin.

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