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Original article

Cytotoxicity and TOP1-targeting activity of 8- and 9-amino derivatives of 5-butyl- and 5-(2-*N*,*N*-dimethylamino)ethyl-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-ones

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ABSTRACT

Studies on substituted 5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-ones and 6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-ones have demonstrated that hydrophilic substituents at the 2-position of an ethyl group at the 5- and 6-positions, respectively, can enhance biological activity. The compatibility of such hydrophilic groups at other sites with either TOP1-targeting activity or potent cytotoxic activity has not been explored. The present study examines the influence on biological activity of either a 2-(*N*,*N*-dimethylamino)ethyl or a *N*,*N*-dimethylacetamide derivative of 8- or 9-amino-5*H*-dibenzo[*c*,*h*]naphthyridin-6-ones that have a 5-butyl- or 5-[2-(*N*,*N*-dimethylamino)ethyl]-substituent.

1. Introduction

Topoisomerases are ubiquitous enzymes that participate in processes such as DNA replication, repair, transcription, and recombination as well as chromosome condensation and segregation [1,2]. Topoisomerase I (TOP1) is the target of several antitumor agents based upon their ability to stabilize the enzyme–DNA cleavage complex, which results in DNA damage and ultimately cell death [3,4]. Camptothecin (CPT) was the first compound identified as a TOP1-targeting agent [5]. One of the factors that limited its development into the clinic was its poor water solubility. Extensive studies on several camptothecin analogs with improved physicochemical properties resulted in the clinical development of two TOP1-targeting agents, topotecan (Hycamtin®) and irinotecan (CPT-11/Camptosar®), Fig. 1. Both of these agents have incorporated within their structure the camptothecin ring system, which includes the presence of a δ-lactone. Hydrolysis of this lactone results in an inactive derivative that has high affinity for human serum albumin [6–8]. Considerable interest in non-camptothecin TOP1-targeting agents has developed in view of the instability of δ-lactone of camptothecin analogs together with reports which reveal that both topotecan and irinotecan are substrates for efflux transporters that have been associated with multidrug resistance [9–14].

Studies in our laboratory have demonstrated that benzo[*i*]phenanthridines and dibenzo[*c*,*h*]cinnolines can exert TOP1-targeting activity and cytotoxicity against several human tumor cell lines. Several 5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-ones, 6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-ones and 11*H*-isoquino[4,3-*c*]cinnolin-12-ones have been identified to possess exceptional TOP1-targeting activity and cytotoxicity [15–19]. The presence of a 2-(*N*,*N*-dimethylamino)ethyl substituent attached to the lactam nitrogen within the core structure of these various TOP1-targeting agents is associated with improved formulation properties and enhanced efficacy *in vivo*.

ARC-111 and its 5-butyl analog (Fig. 1) are known to be potent TOP1-targeting agents. The TOP1-targeting activity and cytotoxicity associated with amino and nitro substitents on both the A- and D-ring of 5*H*-dibenzo[*c*,*h*][1,6]naphthyridines-6-ones has been investigated [20–22]. The data from these studies indicate that the 8- and 9-nitro derivatives retain similar activity to that of 2,3-methylenedioxy-8,9-dimethoxy-5*H*-dibenzo[*c*,*h*]naphthyridin-6-ones. The present study examines the influence on biological activity of either a 2-(*N*,*N*-dimethylamino)ethyl or a *N*,*N*-dimethylacetamide derivative of 8- or 9-amino-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-ones that have a 5-butyl- or 5-[2-(*N*,*N*-dimethylamino)ethyl]-substituent.

Abbreviations: TOP1, topoisomerase I; CPT, camptothecin.

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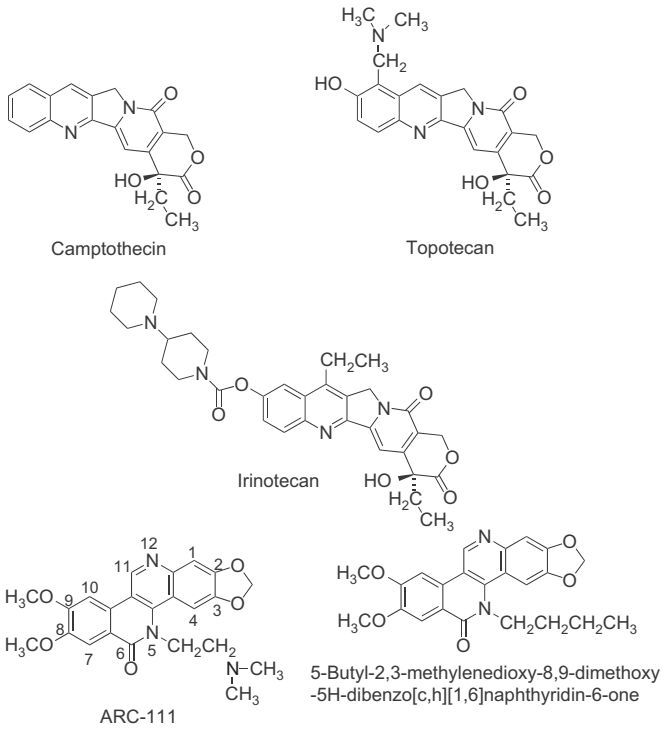


Fig. 1. Structure of camptothecin, topotecan, irinotecan, ARC-111 and its 5-butyl analog.

2. Chemistry

The synthetic methodology employed for the preparation of 5*H*-5-butyl-2,3-methylenedioxy-9-nitrodibenzo[*c*,*h*]1,6-naphthyridin-6-one **4** is outlined in Scheme 1. Oxidation of 2-bromo-4-nitrotoluene with $KMnO_4$ in the presence of aqueous pyridine provided the benzoic acid **1**. Treatment of **1** with thionyl chloride provided the acid chloride, which was reacted without purification with *N*-butyl-4-amino-6,7-methyenedioxyquinoline **2** to provide **3** in 96% overall yield. Under Heck cyclization conditions **3** was converted to **4** in 53% yield. Synthetic methods for the preparation of the 5-[2-(*N*,*N*-dimethylamino)ethyl]-2,3-methylenedioxy-9-nitrodibenzo[*c*,*h*]1,6-naphthyridine, **5**, as well as the 8-nitro 2,3-methylenedioxy-8,9-dimethoxy-5*H*-dibenzo[*c*,*h*]naphthyridin-6-ones, **6** and **7**, have been previously described [20]. Conversion of each of these nitro compounds to their amine derivatives **8**–**11** was accomplished with Raney-nickel in the presence of hydrazine, Scheme 2.

These aryl amines were converted into their *N*-(2-*N*,*N*-dimethylamino)ethyl derivatives by treatment with (dimethylamino)-acetaldehyde diethyl acetal in the presence of trifluoroacetic acid, followed by reduction with sodium cyanoborohydride to provide **12**–**15** (Scheme 3) in modest yields that ranged from 26 to 40%.

The 2-(*N*,*N*-dimethylamino)acetamide derivatives **16**–**19** were synthesized by reaction of the appropriate aryl amine with (dimethylamino)acetyl chloride hydrochloride in the presence of DMAP using DMF as solvent in yields of 61–65%, Scheme 3.

3. Results and discussion

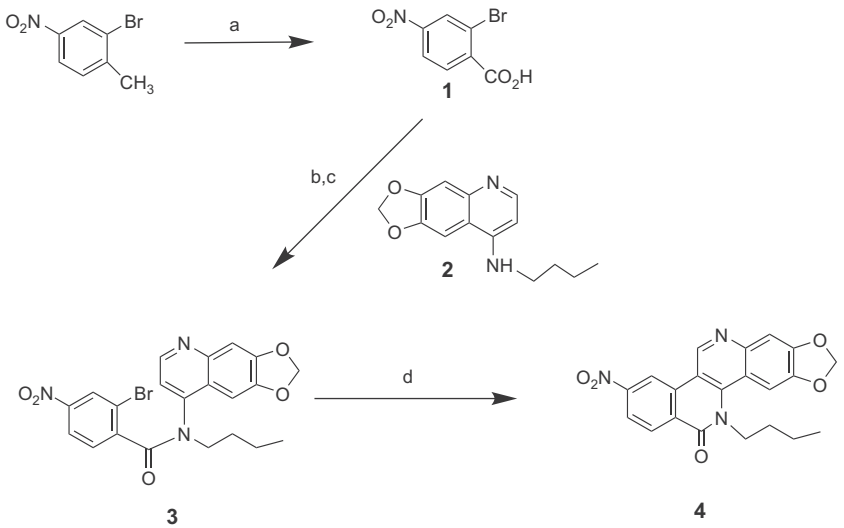
3.1. TOP1-targeting activity

The TOP1-targeting activities and cytotoxic activities in RPMI8402 and P388 cells are provided in Table 1. Only **5** and **7** had comparable activity to camptothecin. The greater TOP1-targeting activity and cytotoxicity observed with **5** and **7** relative to their *N*-butyl analogs could be associated with the poor solubility observed for both **4** and **6**. Similarly, the significantly lower activity of **8** and **10**, relative to **9** and **11** may be linked to differences in solubility. These data do indicate that **9** and **11** had decreased TOP1-targeting activity and cytotoxicity relative to their nitro precursors.

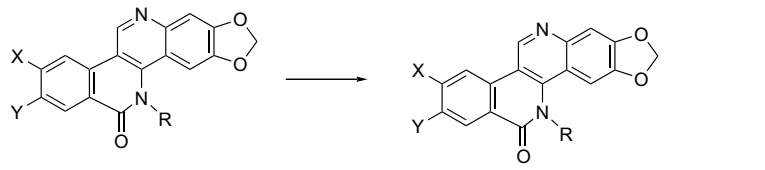
Derivatization of these aryl amines to increase their aqueous solubility failed to improve either their TOP1-targeting activity or cytotoxicity. All four of the *N*-(2-*N*,*N*-dimethylamino)ethyl derivatives **12**–**15** were significantly less active as TOP1-targeting agents than either their unsubstituted amines **9** and **11**, or each of their nitro precursor compounds, **4**–**7**. In a comparison of the relative activity of the 2-(*N*,*N*-dimethylamino)acetamide derivatives **16**–**19**, none of these derivatives exhibited significant TOP1-targeting activity.

3.2. Cytotoxicity

Consistent with the TOP1-targeting activity, **5** and **7** exhibited the more potent cytotoxic activity. Compounds **9** and **11** had moderate cytotoxic activity with IC_{50} values ranging from 28 to 90 nM in RPMI8402 and P388 cells. Despite having no significant TOP1-targeting activity, **15** exhibited moderate cytotoxic activity (40–120 nM) in RPMI8402 or P388 cells. None of the compounds



Scheme 1. Reagents and conditions: (a) $KMnO_4$, Pyr, and H_2O; (b) $SOCl_2$; (c) TEA, CH_2Cl_2, and **2**; (d) $P(tolyl)_3$, $Pd(OAc)_2$, Ag_2CO_3, DMF.



4 X= NO_2; Y = H; R = $CH_2CH_2CH_2CH_3$
5 X= NO_2; Y = H; R = $CH_2CH_2N(CH_3)_2$
6 X= H; Y = NO_2; R = $CH_2CH_2CH_2CH_3$
7 X= H; Y = NO_2; R = $CH_2CH_2N(CH_3)_2$

8 X= NH_2; Y = H; R = $CH_2CH_2CH_2CH_3$
9 X= NH_2; Y = H; R = $CH_2CH_2N(CH_3)_2$
10 X= H; Y = NH_2; R = $CH_2CH_2CH_2CH_3$
11 X= H; Y = NH_2; R = $CH_2CH_2N(CH_3)_2$

Scheme 2. Reagents and conditions: Raney-Nickel, NH_2NH_2 at rt in EtOH for 2 h.

evaluated were found to be substrates for MDR1 or BCRP based upon comparative cytotoxicity data (Table 2) performed in KB3-1, KBV-1 or KBH5.0 cells.

4. Conclusion

The data indicate that replacement of the 8,9-dimethoxy substituents 5-butyl or 5-[(2-*N*,*N*-dimethylamino)ethyl]-2,3-methylenedioxy-8,9-dimethoxydibenzo[*c*,*h*][1,6]naphthyridin-6-ones with a *N*,*N*-dimethylaminoethylamino or a *N*,*N*-dimethylaminoacetamido group at either the 8- or 9-position does improve water solubility, but results in a significant loss of TOP1-targeting activity and cytotoxic activity. None of these analogs have comparable TOP1-targeting activity or cytotoxicity to ARC-111 [15,18] or the nitro derivatives **5** and **7**. These data indicate that not only is activity not enhanced by such modifications in the case of the less polar and poorly soluble 5-butyl analog of ARC-111, but also activity is diminished in the case of those analogs that do possess a 5-[2-(*N*,*N*-dimethylamino)]ethyl substituent. It has been recently shown that replacement of the 8,9-dimethoxyl groups of ARC-111 with diethoxy substituents results in a dramatic loss of activity [23]. While these data suggest that steric tolerance may be limited at these sites, steric factors cannot explain the decreased activity of the unsubstituted amino derivatives **8**–**11** in the present study relative to their nitro precursors. The data on these derivatives suggest that an amino substituent at either the 8- or 9-position, at least in the absence of a neighboring methoxy group at the available 8- or 9-position, is associated with a decrease in topoisomerase I-targeting activity and cytotoxicity relative to its nitro precursor.

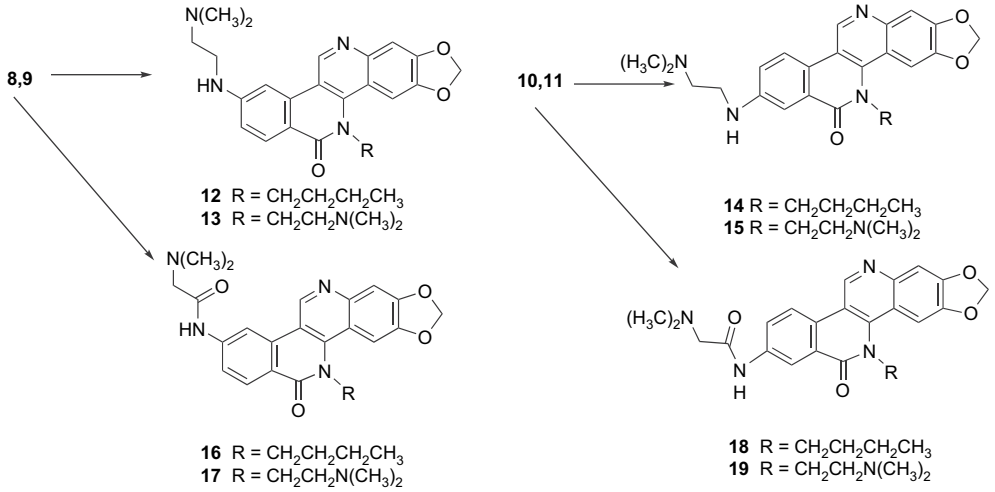
5. Experimental

5.1. Chemistry

Melting points were determined with a Meltemp capillary melting point apparatus. Column chromatography refers to flash chromatography conducted on SiliTech 32-63 μm, (ICN Biomedicals, Eschwege, Ger.) using the solvent systems indicated. Infrared spectral data were obtained using a Thermo-Nicolet Avatar 360 Fourier transform spectrometer and are reported in cm^{-1}. Proton (^1H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance were recorded on a Varian Gemini-200 Fourier Transform spectrometer. NMR spectra (200 MHz ^1H and 50 MHz ^{13}C) were recorded in the deuterated solvent with chemical shifts reported in δ units downfield from tetramethylsilane (TMS). All NMR analyses were performed using $CDCl_3$ unless otherwise noted. Coupling constants are reported in hertz (Hz). Mass spectra were obtained from Washington University Resource for Biomedical and Bio-organic Mass Spectrometry within the Department of Chemistry at Washington University, St. Louis, MO. All starting materials and reagents were purchased from Aldrich. Solvents were purchased from Fisher Scientific, and were A.C.S. grade or HPLC grade. Methylene chloride was freshly distilled from calcium hydride. All other solvents were used as provided without further purification. Compounds **1**, **2**, **5**–**7**, and **9**–**11** were prepared as previously described [20].

5.1.1. *2-Bromo*-N-*butyl*-N-*(6,7-methylenedioxyquinolin-4-yl)-4-nitrobenzamide* (***3***)

A solution of 2-bromo-4-nitrobenzoic acid (338 mg, 1.37 mmol) in thionyl chloride (16 mL) was refluxed for 4 h. It was concentrated under vacuum to a semi-solid, which was placed



12 R = $CH_2CH_2CH_2CH_3$
13 R = $CH_2CH_2N(CH_3)_2$

14 R = $CH_2CH_2CH_2CH_3$
15 R = $CH_2CH_2N(CH_3)_2$

16 R = $CH_2CH_2CH_2CH_3$
17 R = $CH_2CH_2N(CH_3)_2$

18 R = $CH_2CH_2CH_2CH_3$
19 R = $CH_2CH_2N(CH_3)_2$

Scheme 3. Preparation of *N*-(2-dimethylaminoethyl) and *N*-(2-dimethylaminoacetyl) derivatives of 5-substituted 8- and 9-amino-2,3-methylenedioxy-5*H*-dibenzo[*c*,*h*][1,6]-naphthyridin-6-ones.

Table 1
Relative TOP1-targeting activity and cytotoxicity of 5-substituted 8- or 9-amino-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one derivatives in human lymphoblastoma cells (RPMI8402) and a murine leukemia cell line (P388).

Compound	TOP1-mediated DNA cleavage[a]	Cytotoxicity IC_{50} (μM)	
		RPMI8402	P388
4	>10	0.48	0.70
5	0.1	0.002	0.005
6	>10	2.4	0.15
7	0.2	0.005	0.009
8	>20	3.5	6.0
9	0.5	0.033	0.028
10	>20	1.25	0.28
11	8.0	0.09	0.04
12	>20	1.8	1.8
13	>20	0.85	0.06
14	>20	1.57	0.78
15	>20	0.12	0.04
16	>20	0.3	0.60
17	>20	0.035	0.035
18	>20	0.33	0.25
19	>20	0.12	0.10
CPT	0.2	0.006	0.014
Topotecan	1.0	0.021	0.045

[a] Topoisomerase I cleavage values are reported as REC, Relative Effective Concentration, these are concentrations relative to topotecan, whose value is arbitrarily assumed as 1, that are able to produce 10% cleavage of the plasmid DNA in the presence of human topoisomerase I.

under vacuum for 2 h. It was then dissolved in 10 mL of dry CH_2Cl_2 and was added to a solution of **2** [20] (244 mg, 0.99 mmol), TEA (0.5 mL) and dry CH_2Cl_2 (25 mL). The reaction mixture was refluxed overnight. The mixture was allowed to cool, the organic layer was then washed with saturated $NaHCO_3$ solution, water, brine and then dried (anhyd. Na_2SO_4). Solvent was removed under vacuum to yield **3** as a sticky brown glue (430 mg) in 96% yield; IR (CH_2Cl_2) 1658 cm^{-1}; ^1H NMR δ 0.96 (t, 3H, $J = 7.2$), 1.36–1.55 (m, 2H), 1.71–1.84 (m, 2H), 3.38–3.35 (m, 1H), 4.42–4.54 (m, 1H), 6.20 (d, 2H, $J = 2.2$), 7.02 (d, 1H, $J = 8.4$), 7.26 (s, 1H), 7.34 (s, 1H), 7.69 (dd, 1H, $J = 8.4$, $J = 2.3$), 7.94 (d, 1H, $J = 6.1$), 8.18 (dd, 1H, $J = 2.2$), 8.52 (d, 1H, $J = 6$); ^{13}C NMR δ 12.8, 19.2, 28.9, 48.0, 96.7, 101.6, 105.2, 118.4, 119.8, 121.0, 121.6, 127.2, 128.1, 130.6, 142.6, 143.9, 146.9, 148.9, 150.8, 165.6; HRMS calcd for $C_{21}H_{18}BrN_3O_5Li$: 478.0590; found: 478.0582.

Table 2
Cytotoxicity of derivatives of 5-substituted 8- or 9-amino-5*H*-dibenzo[*c*,*h*][1,6]-naphthyridin-6-one in the epidermoid carcinoma cell line KB3-1 and its multidrug-resistant variants, KBV-1 and KBH5.0.

Compound	Cytotoxicity IC_{50} (μM)		
	KB3-1 (wt)	KBV-1 ($^+$MDR1)	KBH5.0 ($^+$BCRP)
4	0.31	0.85	0.36
5	0.005	0.004	0.005
6	0.40	0.30	0.50
7	0.026	0.025	0.025
8	5.8	2.5	3.5
9	0.13	0.13	0.15
10	3.2	2.5	3.5
11	0.23	0.28	0.33
12	3.0	4.5	3.5
13	0.12	0.55	0.32
14	1.7	3.2	1.7
15	0.05	0.41	0.3
16	0.9	2.0	1.9
17	0.036	0.19	0.12
18	1.5	2.6	1.6
19	0.18	0.6	0.24
Topotecan	0.04	0.44	0.44

KB3-1 cell line is the parent cell line. KBV-1 is a variant that overexpresses MDR1, *p*-glycoprotein and KBH5.0 is the variant that overexpresses the efflux transporter BCRP.

5.1.2. 5-Butyl-2,3-dimethoxy-9-nitro-5H-dibenzo[c,h][1,6]naphthyridin-6-one (**4**)

A mixture of **3** (430 mg, 0.91 mmol), $Pd(OAc)_2$ (41 mg, 0.18 mmol), Ag_2CO_3 (499 mg, 1.81 mmol), P(*o*-tolyl)$_3$ (107 mg, 0.35 mmol) were placed in a flask and kept under vacuum for 3 h. DMF (40 mL) was then added and the mixture was refluxed for 45 min. The reaction mixture was cooled to room temperature, filtered through Celite and then washed with 50 mL of 2:1 mixture of CH_3OH:$CHCl_3$. The solvent was removed under vacuum to provide a dark semi-solid, which was purified by flash chromatography eluting with chloroform to give 190 mg of **4** as a yellow solid in 53% yield; mp 220–221 °C; IR (KBr) 1657 cm^{-1}; ^1H NMR δ 1.18 (t, 3H, $J = 7.4$), 1.63 (m, 2H, $J = 7.5$), 2.28 (m, 2H, $J = 7.6$), 4.7 (t, 2H, $J = 7.5$), 6.41 (s, 2H), 7.72 (s, 1H), 7.79 (s, 1H), 8.57 (dd, 1H, $J = 10.8$, $J = 2.2$), 8.89 (d, 1H, $J = 10.8$), 9.42 (d, 1H, $J = 2.2$), 9.71 (s, 1H); ^{13}C NMR δ 13.5, 20.1, 30.7, 51.1, 101.2, 102.3, 107.2, 110.7, 114.8, 117.2, 121.3, 128.9, 130.3, 133.2, 142.4, 143.2, 148.2, 148.7, 150.8, 151.0, 163.2; HRMS calcd for $C_{21}H_{17}N_4O_5Li$: 398.1328; found: 398.1314.

5.1.3. 9-Amino-5-butyl-2,3-dimethoxy-5H-dibenzo[c,h][1,6]naphthyridin-6-one (**8**)

To a solution of **4** (127 mg, 0.35 mmol) in ethanol (33 mL) was successively added Raney-Nickel (~80 mg) and hydrazine hydrate (0.63 mL). The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was filtered through Celite and the filtrate was concentrated under vacuum to provide 85 mg of a light yellow solid in 73% yield; mp >300 °C; IR (KBr) 1659 cm^{-1}; ^1H NMR (CD_3OD) δ 0.99 (t, 3H, $J = 7.1$), 1.43 (m, 2H, $J = 7.2$), 2.09 (m, 2H, $J = 7.1$), 4.46 (t, 2H, $J = 7.5$), 6.22 (s, 2H), 7.52 (s, 1H), 7.59 (s, 1H), 8.38 (dd, 1H, $J = 8.8$, $J = 1.8$), 8.70 (d, 1H, $J = 8.8$), 9.22 (d, 1H, $J = 1.6$), 9.57 (s, 1H); ^{13}C NMR (CD_3OD) δ 13.7, 20.2, 31.0, 50.5, 101.8, 102.8, 103.2, 106.7, 112.0, 114.3, 114.8, 116.3, 130.4, 134.6, 142.2, 144.2, 147.9, 150.3, 154.3, 164.2; HRMS calcd for $C_{21}H_{19}N_3O_3Li$: 368.1586; found: 368.1588.

5.2. General method for the preparation of N-(2-dimethylaminoethyl) derivatives of 5-substituted 8- and 9-amino-2,3-methylenedioxy-5H-dibenzo[c,h][1,6]-naphthyridin-6-ones

5.2.1. 5-Butyl-9-(2-dimethylaminoethylamino)-2,3-methylenedioxy-5H-dibenzo[c,h][1,6]naphthyridin-6-one (**12**)

Trifluoroacetic acid (1 mL, 4.06 mmol) was added dropwise at 0 °C into a mixture of **8** (111 mg, 0.30 mmol) and (dimethylamino)acetaldehyde diethyl acetal (0.035 mL, 0.21 mmol). The reaction mixture was stirred for 5 min and sodium cyanoborohydride (36 mg, 0.57 mmol) was added batchwise (added 8 mg at a 4–5 min time interval) to the mixture. The reaction mixture was then heated at 50–60 °C for 2.5 h. A saturated solution of sodium bicarbonate (10 mL) was added and the mixture was extracted with chloroform (2 × 15 mL). The organic layers were washed with water (30 mL), brine (50 mL) and dried (anhyd. Na_2SO_4). The solvent was removed under vacuum and the crude product was chromatographed eluting with 2:98 CH_3OH:$CHCl_3$ to provide 34 mg of **12** as a light yellow solid in 26% yield; mp 175–176 °C; IR (KBr) 1652 cm^{-1}; ^1H NMR δ 0.94 (t, 3H, $J = 7.2$), 1.34 (m, 2H, $J = 7.7$), 2.05 (m, 2H, $J = 7.5$), 2.39 (s, 6H), 2.76 (t,2H, $J = 5.7$), 3.39 (t, 2H, $J = 5.2$), 4.40 (t, 2H, $J = 7.5$), 5.31 (s, 1H), 6.16 (s, 2H), 6.87 (dd, $J = 8.8$, $J = 2$), 7.28 (s, 1H), 7.41 (s, 1H), 7.54 (s, 1H), 8.26 (d, 1H, $J = 8.8$), 9.34 (s, 1H); ^{13}C NMR δ 13.7, 20.1, 29.7, 31.3, 39.9, 44.7, 45.4, 50.3, 57.4, 100.5, 101.0, 102.1, 106.8, 111.9, 114.9, 115.3, 115.8, 130.4, 134.5, 142.5, 143.9, 147.2, 149.9, 151.9, 164.5; HRMS calcd for $C_{25}H_{28}N_4O_3Li$: 439.2321; found: 439.2306.

5.2.2. 5-(2-Dimethylaminoethyl)-9-(2-dimethylethylamino)-2,3-methylenedioxy-5H-dibenzo[c,h][1,6]naphthyridin-6-one (**13**)

Trifluoroacetic acid (0.5 mL, 2.03 mmol) was added dropwise at 0 °C into a mixture of **9** [20] (60 mg, 0.15 mmol) and

dimethylamino acetaldehyde diethyl acetal (0.018 mL, 0.11 mmol). The reaction mixture was stirred for 5 min and sodium cyanoborohydride (19 mg, 0.31 mmol) was added batchwise to the mixture. The cooled reaction was worked up and chromatographed to provide 23 mg of **13** as a light yellow solid in 32% yield; mp 194–195 °C; IR (KBr) 1647 cm^{-1}; ^1H NMR δ 2.30 (s, 6H), 2.34 (s, 6H), 2.69 (t, 2H, $J = 5.8$), 2.95 (t, 2H, $J = 7.0$), 3.36 (t, 2H, $J = 5.0$), 4.58 (t, 2H, $J = 7.1$), 5.19 (s, 1H), 6.16 (s, 2H), 6.89 (dd, 1H, $J = 8.8$, $J = 2.2$), 7.29 (d, 1H, $J = 2.2$), 7.45 (s, 1H), 7.83 (s, 1H), 8.28 (d, 1H, $J = 8.8$), 9.37 (s, 1H); ^{13}C NMR δ 29.6, 40.1, 44.9, 45.4, 57.5, 57.6, 100.3, 101.0, 102.3, 106.2, 112.3, 114.9, 115.1, 115.3, 130.3, 134.5, 142.4, 143.5, 147.6, 150.3, 152.4, 164.8; HRMS calcd for $C_{25}H_{29}N_5O_3H$: 448.2349; found 448.2348.

5.2.3. 5-Butyl-8-[2-(dimethylamino)ethylamino]-2,3-methylenedioxy-5H-dibenzo[c,h][1,6]naphthyridin-6-one (**14**)

Trifluoroacetic acid (1 mL, 4.06 mmol) was added dropwise at 0 °C into a mixture of **10** (25 mg, 0.07 mmol) and (dimethylamino)acetaldehyde diethyl acetal (0.012 mL, 0.07 mmol). The reaction mixture was stirred for 5 min and sodium cyanoborohydride (12 mg, 0.19 mmol) was added batchwise (added 4 mg at a 4–5 min time interval) to the mixture. The cooled reaction was worked up and chromatographed to provide 12 mg of **14** as a light yellow solid in 40% yield; mp 218–220 °C; IR (KBr) 1649 cm^{-1}; ^1H NMR δ 0.91 (t, 2H, $J = 7.2$), 1.34 (m, 2H, $J = 7.6$), 2.00 (m, 2H, $J = 7.5$), 2.39 (s, 6H), 2.75 (t,2H, $J = 5.6$), 3.37 (t, 2H, $J = 5.8$), 4.42 (t, 2H, $J = 7.5$), 5.31 (s, 1H), 6.12 (s, 2H), 7.13(dd, $J = 8.8$, $J = 3$), 7.37(s, 1H), 7.48 (s, 1H), 7.50 (s, 1H), 8.13 (d, 1H, $J = 8.8$), 9.29 (s, 1H); ^{13}C NMR δ 13.6, 20.0, 31.0, 40.2, 50.5, 57.5, 100.4, 102.1, 106.4, 107.1, 112.8, 115.1, 121.2, 122.4, 122.6, 126.6, 139.2, 143.1, 145.9, 147.5, 148.5, 149.5, 168.8; HRMS calcd for $C_{25}H_{28}N_4O_3H$: 433.2161; found 433.2222.

5.2.4. 5-(2-(Dimethylamino)ethyl)-8-(2-(dimethylamino)ethylamino)-2,3-methylenedioxy-5H-dibenzo[c,h][1,6]naphthyridin-6-one (**15**)

Trifluoroacetic acid (1 mL, 4.06 mmol) was added dropwise at 0 °C into a mixture of **11** (120 mg, 0.316 mmol) and (dimethylamino)acetaldehyde diethyl acetal (0.036 mL, 0.21 mmol). The reaction mixture was stirred for 5 min and sodium cyanoborohydride (38 mg, 0.63 mmol) was added batchwise (added 10 mg at a 4–5 min time interval) to the mixture. The cooled reaction was worked up and chromatographed to provide 23 mg of **15** as a light yellow solid in 35% yield; mp 231–232 °C; IR (KBr) 1637 cm^{-1}; ^1H NMR δ 2.32 (s, 6H), 2.34 (s, 6H), 2.69 (t, 2H, $J = 5.6$), 2.97 (t, 2H, $J = 7.2$), 3.33 (t, 2H, $J = 5.5$), 4.63 (t, 2H, $J = 7$), 4.59 (s, 1H), 6.15 (s, 2H), 7.17 (dd, 1H, $J = 9$, $J = 3$), 7.44 (s, 1H), 7.56 (d, 1H, $J = 2.6$), 7.8 (s, 1H), 8.17 (d, 1H, $J = 8.8$), 9.39 (s, 1H); ^{13}C NMR δ 29.7, 40.78, 45.1, 49.0, 57.7, 100.9, 102.0, 107.1, 107.6, 112.8, 115.0, 121.1, 122.5, 122.6, 126.7, 139.0, 143.4, 146.5, 147.5, 148.7, 149.3, 164.8; HRMS calcd for $C_{25}H_{29}N_5O_3H$: 448.2349; found 448.2349.

5.3. General method for the preparation of N-(2-dimethylaminoacetyl) derivatives of 5-substituted 8- and 9-amino-2,3-methylenedioxy-5H-dibenzo[c,h][1,6]naphthyridin-6-ones

5.3.1. N-(5-Butyl-2,3-methylenedioxy-6-oxo-5H-dibenzo[c,h][1,6]naphthyridin-9-yl)-2-(dimethylamino)acetamide (**16**)

A mixture of (dimethylamino)acetyl chloride hydrochloride (17.8 mg, 0.12 mmol), **8** (40 mg, 0.11 mmol), DMAP (50 mg, 0.36 mmol), DCC (35.6 mg, 0.17 mmol) were placed in a flask. DMF (2.5 mL) was added and the reaction mixture was stirred overnight at 40 °C. After the reaction was complete, 10 mL chloroform was added. The organic layer was then washed with sodium bicarbonate (2 × 15 mL), water (20 mL) and brine (2 × 30 mL). The organic phase was dried (anhyd. Na_2SO_4) and concentrated under vacuum to provide a solid. This solid residue was subjected to column chromatography eluting with 5:95 CH_3OH:$CHCl_3$ to provide 30 mg of **16** as a yellow solid in 61% yield; mp 184–185 °C; IR (KBr) 1648 cm^{-1}, 3256 cm^{-1}; ^1H NMR δ 0.95 (t, 3H, $J = 7.3$), 1.38 (m, 2H, $J = 7.6$), 2.05 (m, 2H, $J = 7.8$), 2.45 (s, 6H), 3.18 (s, 2H), 4.44 (t, 2H, $J = 8$), 6.17 (s, 2H), 7.46 (s, 1H), 7.55 (s, 1H), 7.67 (dd, 1H, $J = 8.4$, $J = 1.8$), 8.46 (d, 1H, $J = 8.4$), 8,83 (d, 1H, $J = 2$), 9.45 (s, 1H), 9.55 (s, 1H); ^{13}C NMR δ 13.7, 20.1, 31.1, 46.2, 50.6, 63.8, 100.8, 102.2, 107.1, 110.1, 111.7, 114.7, 119.7, 121.3, 130.1, 113.9, 142.2, 144.1, 147.4, 147.9, 148.9, 150.1, 164.0, 169.6; HRMS calcd for $C_{25}H_{26}N_4O_4H$: 447.2032; found 447.2033.

5.3.2. 2-(Dimethylamino)-N-(5-(2-(dimethylamino)ethyl)-2,3-methylenedioxy-6-oxo-5H-5,6-dibenzo[c,h][1,6]naphthyridin-9-yl)acetamide (**17**)

A mixture of (dimethylamino)acetyl chloride hydrochloride (13 mg, 0.08 mmol), **9** (40 mg, 0.10 mmol), DMAP (42 mg, 0.30 mmol), DCC (34 mg, 0.17 mmol) was placed in a flask. DMF (2.5 mL) was added and the reaction mixture was stirred overnight at 40 °C. The cooled reaction was worked up and chromatographed to provide 32 mg of **17** as a yellow solid in 65% yield; mp 192–194 °C; IR (KBr) 1655 cm^{-1}, 3286 cm^{-1}; ^1H NMR δ 2.28 (s, 6H), 2.46 (s, 6H), 2.97 (t, 2H, $J = 7.0$), 3.18 (s, 2H), 4.59 (t, 2H, $J = 6.5$), 6.17 (s, 1H), 7.67 (dd, 1H, $J = 8$, $J = 1.8$), 7.83 (s, 1H), 8.44 (d, 1H, $J = 8.8$), 8.82 (d, 1H, $J = 1.8$), 9.45 (s, 1H), 9.54 (s, 1H), 9.54 (s, 1H); ^{13}C NMR δ 45.6, 46.1, 48.8, 63.7, 101.0, 102.3, 106.6, 110.2, 111.9, 114.7, 119.8, 121.0, 130.0, 133.9, 142.2, 142.3, 143.7, 147.5, 147.8, 150.4, 164.3, 169.8; HRMS calcd for $C_{25}H_{27}N_5O_4Li$: 468.2223; found 468.2227.

5.3.3. N-(5-Butyl-2,3-methylenedioxy-6-oxo-5H-dibenzo[c,h][1,6]naphthyridin-8-yl)-2-(dimethylamino)acetamide (**18**)

A mixture of (dimethylamino)acetyl chloride hydrochloride (12 mg, 0.08 mmol), **10** (24 mg, 0.07 mmol), DMAP (32 mg, 0.23 mmol) and DCC (23 mg, 0.10 mmol). DMF (2 mL) was added and the reaction mixture was stirred overnight at 40 °C. The cooled reaction was worked up and chromatographed to provide 19.4 mg of **18** as a yellow solid in 63% yield; mp 214–216 °C; IR (KBr) 1646 cm^{-1}, 3220 cm^{-1}; ^1H NMR δ 0.95 (t, 3H, $J = 6.7$), 1.36 (m, 2H, $J = 7.4$), 2.02 (m, 2H, $J = 7.2$), 2.45 (s, 6H), 3.17 (s, 2H), 4.48 (t, 2H, $J = 7.5$), 6.18 (s, 2H), 7.45 (s, 1H), 7.54 (s, 1H), 8.28 (d, 1H, $J = 2.2$), 8.33 (d, 1H, $J = 8.8$), 8.53 (dd, 1H, $J = 8.8$, $J = 2.2$), 9.43 (s, 1H), 9.56 (s, 1H); ^{13}C NMR δ 13.7, 20.1, 30.9, 46.1, 50.6, 63.6, 100.6, 102.2, 107.1, 111.8, 114.9, 117.5, 122.5, 125.1, 126.0, 128.4, 138.2, 140.9, 143.6, 147.4, 147.5, 149.9, 164.1, 169.2; HRMS calcd for $C_{25}H_{26}N_4O_4Li$: 453.2114; found 453.2103.

5.3.4. 5H-2-(Dimethylamino)-N-(5-(2-(dimethylamino)ethyl)-2,3-methylenedioxy-6-oxo-5H-dibenzo[c,h][1,6]naphthyridin-8-yl)acetamide (**19**)

A mixture of (dimethylamino)acetyl chloride hydrochloride (13 mg, 0.08 mmol), **11** (30 mg, 0.08 mmol), DMAP (32 mg, 0.26 mmol) and DCC (26 mg, 0.12 mmol). DMF (2 mL) was added and the reaction mixture was stirred overnight at 40 °C. The cooled reaction was worked up and chromatographed to provide 22 mg of **19** as a yellow solid in 61% yield; mp 199–201 °C; IR (KBr) 1655 cm^{-1}, 3290 cm^{-1}; ^1H NMR δ 2.28 (s, 6H), 2.44 (s, 6H), 2.91 (t, 2H, $J = 7.0$), 3.15 (s, 2H), 4.65 (t, 2H, $J = 7.2$), 6.17 (s, 1H), 7.46 (s, 1H), 7.82 (s, 1H), 8.28 (d, 1H, $J = 2.2$), 8.35 (d, 1H, $J = 8.8$), 8.54 (dd, 1H, $J = 8.8$, $J = 2.2$), 9.44 (s, 1H), 9.54 (s, 1H); ^{13}C NMR δ 45.7, 46.1, 49.1, 57.5, 63.6, 101.0, 102.2, 111.9, 114.9, 117.5, 122.5, 125.2, 125.9, 128.6, 138.2, 140.8, 143.6, 147.5, 150.0, 164.3, 169.2; HRMS calcd for $C_{25}H_{27}N_5O_4Li$: 468.2223; found: 468.2225.

5.4. Topoisomerase-mediated DNA cleavage assays

Human topoisomerase I was expressed in *Escherichia coli* and isolated as a recombinant fusion protein using a T7 expression system as described previously [24]. Plasmid YepG was also purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium isopycnic centrifugation method as described [25]. The 3′ endlabeling of the plasmid was accomplished by digestion with a restriction enzyme followed by end filling with Klenow polymerase as previously described [26]. The cleavage assays were performed as previously reported [27,28]. The drug and the DNA in the presence of topoisomerase I was incubated for 30 min at room temperature. The reactions were terminated by the addition of 5 μL of 5% SDS and 1 mg/mL protein kinase K with an additional 1 h of incubation at 37 °C. Samples were then alkali denatured by the addition of NaOH, EDTA, sucrose, and bromophenol blue to final concentrations of 75 mM, 2.5%, and 0.05 mg/mL, respectively, prior to loading onto a neutral agarose gel. After development of the gels, typically 24-h exposure was used to obtain autoradiograms outlining the extent of DNA fragmentation. Topoisomerase I-mediated DNA cleavage values are reported as Relative Effective Concentration (REC), which represents concentrations relative to camptothecin, whose value is arbitrarily assumed as 0.2, that are able to produce the same 10% cleavage on the plasmid DNA in the presence of human topoisomerase I.

5.5. Cytotoxicity assays

The cytotoxicity was determined using the MTT-microtiter plate tetrazolinium cytotoxicity assay (MTA). The human lymphoblast RPMI8402 was provided by Dr. Toshiwo Andoh (Aichi Cancer Center Research Institute, Nagoya, Japan) [29]. The P388 mouse leukemia cell line [30] was obtained from Michael R. Mattern and Randal K. Johnson (GlaxoSmithKline, King of Prussia, PA). The KB3-1 cell line and its multidrug-resistant (MDR1, p-glycoprotein) variant KBV-1 [31] was obtained from K.V. Chin (The Cancer Institute of New Jersey, New Brunswick, NJ). The KBH5.0 cell line as noted previously [17] was derived from KB3-1 by stepwise selection against Hoechst 33342. The cytotoxicity assay was performed using 96-well microtiter plates. Cells were grown in suspension at 37 °C in 5% CO_2 and maintained by regular passage in RPMI medium supplemented with 10% heat inactivated fetal bovine serum, L-glutamine (2 mM), penicillin (100 U/mL), and streptomycin (0.1 mg/mL). For determination of IC_{50}, cells were exposed continuously for four days to varying concentrations of drug, and MTT assays were performed at the end of the fourth day. Each assay was performed with a control that did not contain any drug. All assays were performed at least twice in six replicate wells.

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