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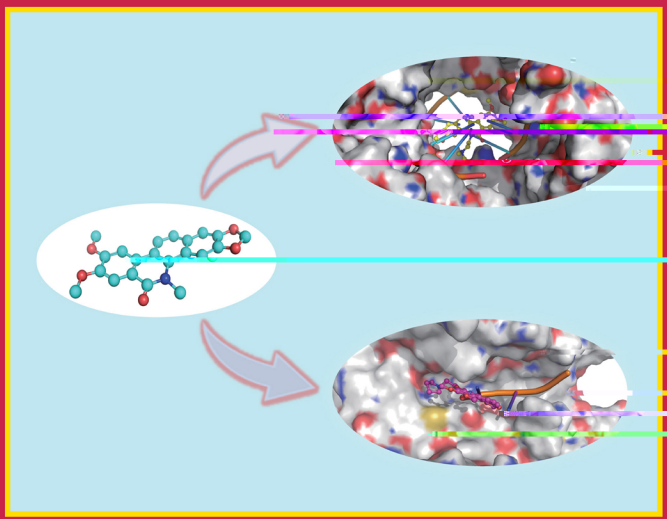
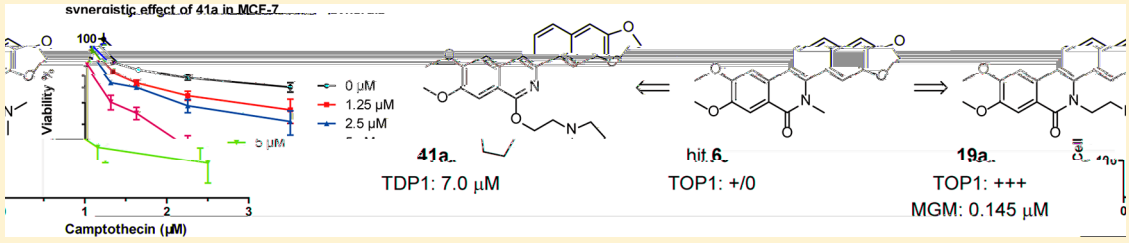


Discovery, Synthesis, and Evaluation of Oxynitidine Derivatives as Dual Inhibitors of DNA Topoisomerase IB (TOP1) and Tyrosyl-DNA Phosphodiesterase 1 (TDP1), and Potential Antitumor Agents

- , †,§ - , †,§ - , † , † , † - , †
, ‡ , ‡ , ‡ - , ‡ ,
, *,‡ - *,†

† , - , 510006, C
‡ B , C C R , C
, , B , 20892,

Supporting Information



ABSTRACT: – A 1 (1) A
B (1)– A . 1 1
. , ff
1 1 . , , -
, , 1 1
. A **19a** 1 (+++) 1 A
, . **19a**
ffi C 116 . **41a** 1 C_{50} 7 μ
ff C -7 . ff -
1 1 .

INTRODUCTION

B (1)
A
.[1−4] , 1
A
A (1A),
A 1– A
(1 , 1B). 1

5′- 1–
A „ 2.9(18.141933447( ) 07-1.1338)-062.4(.) 7.4999006.666152295

(3), A β (β), fi
A (1).[18,19] , 1-
3′-
1- A .
, 1 3′-
A ,[20–22] 5′-
-
A , 1
.[18,20,23,24]
1
(CA 1), 1 493
.

Table 1. TOP1 and TDP1 Inhibitions, Enhanced Melting Temperature with dsDNA and Cytotoxicity of the Indicated Compounds

GI_{50} (μM)						TOP1	TDP1	ΔT_m (°C)		
DU145	A549	Huh7	Cpd.	n	R	inhibition[a]	inhibition[b]	F10T	HCT116	CCRF-CEM
.021 ± 0.016	0.21 ± 0.069	0.099 ± 0.017	**1**	/[e]	/	++++	ND[f]	ND	0.009 ± 0.001	0.007 ± 0.003	0
[illegible]	[illegible]	[illegible]	**6**	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	OH	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.029 ± 0.003	0.018 ± 0.002	0.79 ± 0.11	0.12 ± 0.015	**19a**	2	NMe$_2$	+++	12%	0.5	0.076 ± 0.007	
[illegible]	[illegible]	[illegible]	[illegible]	**19b**	3	NMe$_2$	+	0	[illegible]	[illegible]	
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	NEt$_2$	[illegible]	[illegible]	[illegible]	[illegible]	
11.79 ± 1.14	15.24 ± 1.28	8.21 ± 1.99	36.72 ± 8.97	48.82 ± 45.58	**20b**	3	NEt$_2$	+	0	1.3	
[illegible]	[illegible]	[illegible]	20%	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	

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Table 1. continued

Cpd.	n	R	TOPO I inhibition[a]	TDP2 inhibition[b]	ΔTm (°C) F10T	IC_{50} (μM) HCT116	CCRF-CEM	DU145	A549	Huh7
3 ± 0.59	**41a**	2	0	92%	0.3	31.04 ± 6.08	2.63 ± 0.16	4.29 ± 1.31	1.53 ± 0.070	2.78
6.53 ± 3.16	**41b**	3	0	98%	0.4	21.88 ± 1.51	1.70 ± 0.010	2.04 ± 0.72	1.83 ± 0.45	8.04
13.21 ± 4.32	**42a**	2	0	0	0.2	35.49 ± 12.23	3.35 ± 0.25	44.70 ± 2.06	46.27 ± 32.96	
>100	**42b**	3	0	0	0.3	>100	>100	54.59 ± 37.50	3.58 ± 3.06	2.66 ± >100	>100	>100
	43a	2	0		0.1	84.98 ± 26.00	28.20 ± 1.30	74.99 ± 1.22	>100	>100
>100	>100	**43b**	3	0	0	0.1	>100	>100	>100	
7.60 ± 0.61	95.52 ± 2.20	**44a**	2	0	30%	0.4	53.31 ± 1.86	17.03 ± 2.92	29.13 ± 18.19	
37.57 ± 23.74	>100	**44b**	3	0	34%	0.7	38.91 ± 21.21	33.48 ± 23.20	11.85 ± 6.51	
± 0.81	38.88 ± 16.22	>100	5.39 ± 3.35	53.42 ± 32.83	**45a**	2	0	0	0.2	60.72
100	29.07 ± 1.03	58.68 ± 6.44	15.67 ± 9.47	58.56 ± 28.37	**45b**	3	0	0	0.2	>100
D	ND	ND	ND	ND	**49**	/	/	ND	ND	5.8	N

[a] 1 C 1 μ : 0, ; +, 20% 50% ; ++, 50% 75% ; +++, 75% 95% ; ++++, . [b] 1 fl 100 μ (DNA + compound)– (DNA). Every experiment was repeated at least twice independently. [d] $_{50}$ (±) fi 50% . [e] a

C 1 , 1 C_{50} , , [47] 1 , -3,4- , [48] , [49] - . [37,39,44] A 1 (C 00509), [50] . [51] , (7, 2) fi A 1 , 1 A 1. [52−56] **10** (2) . [45,46] , 1 C_{50} 1.52 μ . [52]

R

, (2), 7-
(**11**), (**8**), -
(**9**), 1
.[34−36]

RESULTS AND DISCUSSION

Hit Discovery for TOP1 Inhibitor.

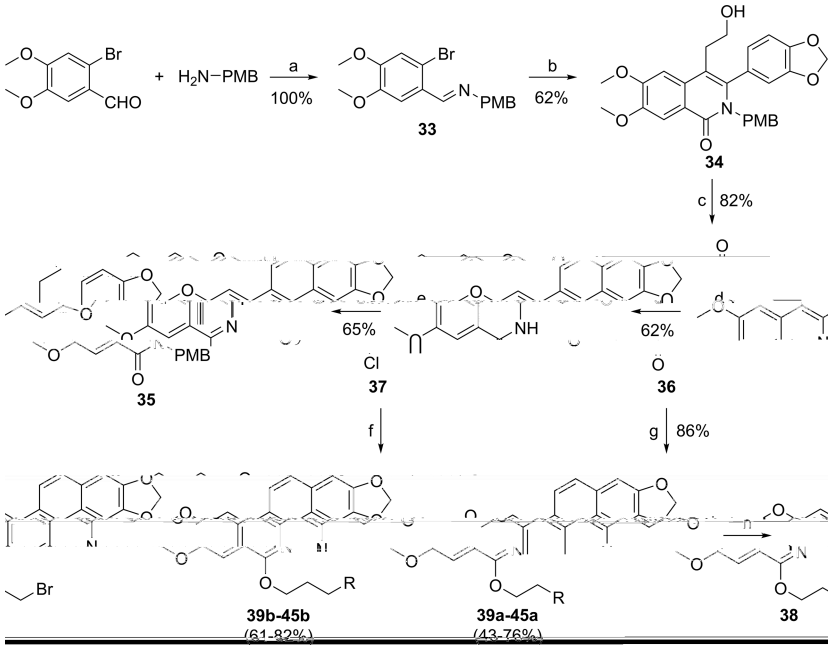
-
.[57,58] fi 1 , -
900
1- A .
1, -
(**6**, 1 ,
).
1
1 ,[59] , fi
1 .[60] B , 1- A
6
1 - (1 ,
).
6 ff
48 (2 ,).
, 17, 35, 79
6 **48** ,
1 ff
ff ff -
.[12]
-
A (A) 1.[61−63] B **6**
6- , 10
(5′-*FAM*- A A C A A - A A C A A-*TAMRA*-
3′)
(Δ*T*) **6**
A. A
A - ,
5−8 °C.[64]
6 Δ*T* (0.4 °C)
49 (2 ,)
Δ*T* 5.8 °C (1),[65] **6**
A .
ff

1 .

Chemistry.

,
1 .[66]
2- -4,5- 2-
3-
12a **12b** 100% .
(1),
12a **12b**
( ) **13a** **13b**,
fi **14**,
5- -
d 1,3 3- -1- . - ,[67]

Scheme 2. Synthesis of Compounds 39a/b–45a/b[a]



[a] : ( ) , ; ( ) ( ) 2, **14**, ( )2, (*o*-)3, C , 80 °C, ( ) C , 3 (C)6, , 2 , 80 °C; ( ) (C C)2, , A, C , −60 °C; ( ) C , A , ; ( ) C 3, , 100 °C; ( ) C 2C 2, , 70 °C; ( ) B (C 2)3B , , ; ( ) ( ), , 2C 3, , .

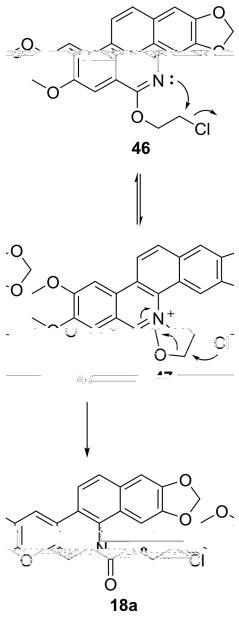


Figure 4. **46** **18a**.

, **39a–45a** 2- 6- 2, **36** fi C 3 **37**,[72] **37** 2- **39a–45a**. , , , , , , , 1 2 , C .

TOP1 Inhibition. A 1 1- 3′- [32] - - A .[73] 100, 10, 1, 0.1 *μ* , **1** **48** , - A 1 **1** 1 *μ* : 0, ; +, 20% 50% ; ++, 50% 75% ; +++, 75% 95% ; ++++, **1** (1). **19a/b–25a/b**, - 1 , **23b** 3- - 5- , **6** (+/0, 10% 1 **1**). B 5- 1 . - 1 +, **19a** (+++) **21a** (++), 1 , . - **17a** **17b** 1 +. C **19a** 1 +++. 5A **19a** **48** **1**. ,A C-111, 1 ,[41] ff **1** **48**,[74] , **19a** A C-111 , 1 ff A . , **26a/b–31a/b** **32a**, 1 (+/0 +) **6**.

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A , **32b** 3- 5- 1 (++). , 1 **40a** (+) **43a** (+/0), .

1– A . A - 1– A– (B 1 4).[75] C . A 5 , - ff **19a** A 1 +1 –1 fl A . A- B- **19a** (C A), C- - ( ). , **19a** A. A , (2.9 A) A 364, , **19a** 145- C0.1, 364 1. 145- C0.1 **19a** (4). A 722 (3.7 A) 718 (3.6 A), , 1 .

TDP1 Inhibition. B 1 1- A , 1 1 ,[54–56] 1 fl .[50] A fl - (5′-*FAM*- A A C AAAA AC -*BHQ*-3′) . 100 μ . , **19a**, **21b**, **22b**, **39a/b**, **40a/b**, **41a/b**, **42b**, **44a/b**, 1 12% 98% (1). 1 **19a** (+++) 1 (12% 100 μ). 1 100 μ . **39a/b**, **40a/b**, **41a/b**, **42b** 1 (>50%) C_{50} (2), 50% 1 . C **41a** C_{50} 7.0 ± 1.4 μ - (6A). 1 , **39a/b**, **40a/b**, **41a/b**, **42b**, - , - 5′-[32] - 14 3′- .[76]

Table 2. TDP1 In[illegible]bition of t[illegible]e Active Compounds

	C_{50} (μ)[a]	
	fl	-
39a	24 ± 0.80	16 ± 0.40
39b	18 ± 1.7	13 ± 4.0
40a	58 ± 20	40 ± 14
40b	15 ± 2.7	27 ± 5.2
41a	7.0 ± 1.4	8.2 ± 1.3
41b	20 ± 1.7	21 ± 1.2
42b	19 ± 4.8	20 ± 5.4

[a] C_{50} fi 50% . .

C **41a** 1 (C_{50} = 8.2 μ). 1 6C. **10** .[52] , **39a/b**, **40b**, **41a/b**, (111 μ) . - 1 , . 1 (265 495) (263 493) 1 - (B 1).[77] . - **41a** 6B. **41a** A 259 , $\pi-\pi$ 1 (6B), 1 (3 ,). 493 (3.3 A) A 283 (3.1 A), 1 . , 1,3- 538, 358 3.7 A, 1 .

Interaction with DNA.

A, 10 - . ΔT 1. fi 10 2 μ **27b** ΔT 1.9 °C. 1 **19a** ΔT

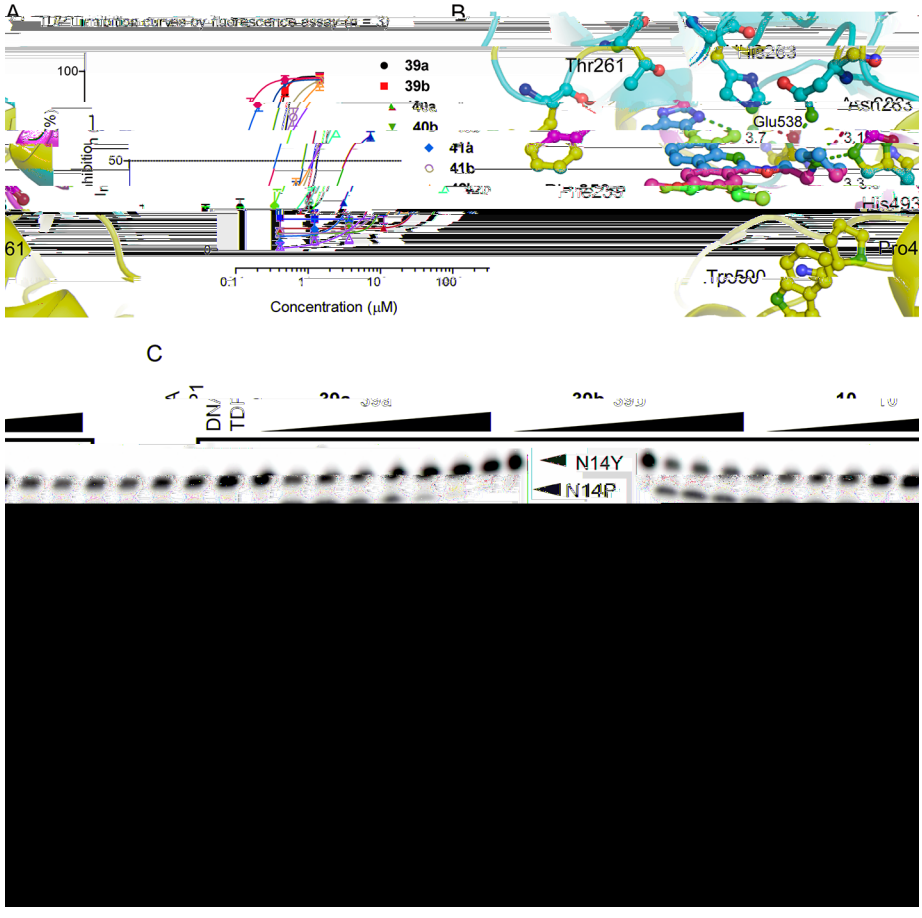

Figure 6. (A) 1 33.3, 100 μ . (B) **41a** ( ) . (C) 1 (B 1). A 1 . 1, A ; 2, A 1; 3–26, A, 1 . 0.05, 0.15, 0.46, 1.4, 4.1, 12.3, 37, 111 μ . 14 14 1, . fl . 0.41, 1.23, 3.70, 11.1,

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Table 3. Cytotoxicity of 19a against Individual NCI-60 Cell Lines

	$_{50}$ (μ)[a]		$_{50}$ (μ)
[b]	0.145	C 205	0.144
CC -C	0.144	CC-2998	0.63
-562	0.156	C 116	0.0855
-4	0.118	C -15	0.427
-8226	0.14	29	0.149
	0.0669	12	0.875
- A549/A CC	0.244	-620	0.345
	0.79	786-0	0.166
-62	0.18	A498	0.347
-92	0.558	AC	0.16
C - 226	0.512	CA -1	0.186
C - 23	0.201	393	0.516
C - 322	0.428	12C	0.258
C - 460	0.141	-10	0.722
C - 522	0.076	-31	0.157
C -268	0.283	C 7	0.118
-295	0.177	A- B-231/A CC	0.826
-539	0.186	578	1.86
B-19	0.233	B -549	0.291
B-75	0.239	-47	0.111
251	0.14	A- B-468	0.14
	0.112	1	0.312
A -3	0.284	CA -3	0.582
14	0.149	CA -4	0.557
A- B-435	0.395	CA -5	0.595
- -2	0.886	CA -8	0.554
- -28	0.861	C /A	0.29
- -5	0.181	- -3	0.24
ACC-257	0.528	C-3	0.317
ACC-62	0.0966	-145	0.215

[a] $_{50}$ fi 50% . 2 . [b] :

0.5 °C, A A . A A 1- ff , 1- A .[78] **19a**, 1- 1 .[79] A 5B, A (B) A B 322 A . , **19a** 1- ff 9 μ . B 322 A (5C)[79] fi **19a** ff . , 1- , fi 1 **19a** A 1 .

Cytotoxicity Assays. fi , (C 116), (CC -C), (-145), (A549), (7) . 72 fi - , 0.01 100 μ . A , . $_{50}$, fi 50% , 1.

C **6**, **17a/b** **19a/b−25a/b** fi , 1 . C **19a** 1 (+++) fi , $_{50}$ (0.076 μ C 116, 0.029 μ CC -C , 0.018 μ 145, 0.79 μ A549, 0.12 μ 7). A **20a** **22a** 5- 1 +, fi C 116 (0.21 μ **20a**, 0.16 μ **22a**), CC -C (0.18 μ **20a**, 0.32 μ **22a**), 145 (0.054 μ **20a**, 0.96 μ **22a**) . C **21a** 1 ++ CC -C (0.62 μ) 145 (0.19 μ) $_{50}$. C (**17a/b**) 5- , , (**19a/b**), (**20a/b**), (**21a/b**), (**22a/b**) , C 116 , (**23a/b**) 4- (**24a/b**) . C **23a/b** **24b** A549 7 , $_{50}$

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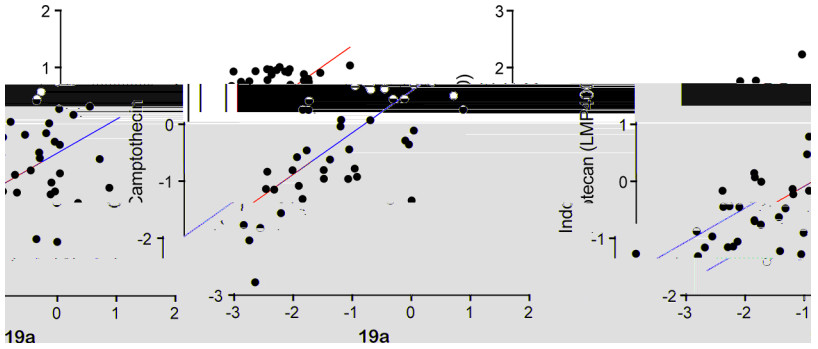

Figure 7. **19a** $_{50}$ C -60 . C -60. . *R*- 0.58 0.40 (400), . *P*- <0.0001 .

100 μ . 5-

C 116 , , **17a** **17b** **19−23a** **19−23b**, 4- **(24a/b)** **(25a/b)** .

A **(26a/b−32a/b)**, **26b** 5- C 116 ($_{50}$ = 0.27 μ), CC -C ($_{50}$ = 0.12 μ), 145 ($_{50}$ = 0.081 μ) . fi - , **26a**, **28b**, **29b**, **30a/b**, CC -C $_{50}$, - **28b** **29b**

C 116 , $_{50}$ 0.66 0.64 μ , . **32b** fi **6**, 1 ++. C - , 5- C 116 (**26a−30a** **26b−30b**). 4- **(31a/b)** **(32a/b)** . **(39a/b−45a/b)** 6- **(19a/b−25a/b)** 5- . A **39a/b** **41a/b** 1 100 μ , 1 , C_{50} , , CC -C , 145, A549, 7 , $_{50}$ 1.29 8.95 μ , . C **19a**, 1 , C (C , A) 60 (C -60).[80−82] A C , **19a** 48 B . C ($_{50}$ 3) . **19a** ( ) 0.145 μ , $_{50}$

C 116, C - 522, ACC-62 (<100).

B C -60 [82] (:// . . . / :// . . . /), **19a**. **19a** 1 . 7 **19a** 400. **19a** - - (4). C 116- 1

Table 4. Cytotoxicity of the 19a in Drug-Resistant Isogenic Human Cancer Cell Lines

	$_{50}$ ± (μ)[a]		[b]
	HCT116	**HCT116-siTOP1**	
19a	0.076 ± 0.010	0.45 ± 0.31	5.9
1	0.009 ± 0.001	0.075 ± 0.014	8.3
	DU-145	**DU145-RC0.1**	
19a	0.018 ± 0.002	2.38 ± 0.34	132
1	0.021 ± 0.016	4.73 ± 0.68	225
	MCF-7	**MCF-7/ADR**	
19a	0.34 ± 0.098	0.95 ± 0.35	2.8
	0.15 ± 0.003	11.67 ± 1.94	77.8
	HepG2	**HepG2/ADR**	
19a	0.30 ± 0.050	3.20 ± 0.40	10.7
	0.19 ± 0.048	9.04 ± 0.14	47.6

[a] $_{50}$ (±) fi 50% . . [b] R $_{50}$ $_{50}$.

C 116 1.[83] C C 116- 1 1 5.9- **19a**. A R A C 116, 8.3- **1**, [12,84] , 1 **19a**,

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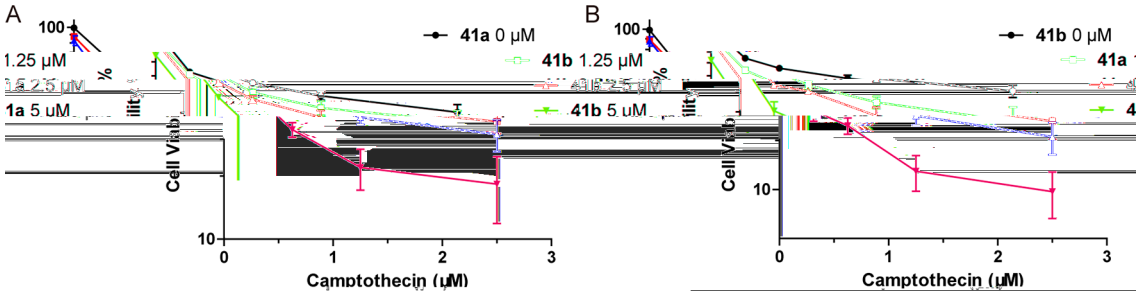

Figure 8. ff **41a** (A) **41b** (B) . C -7 96 .

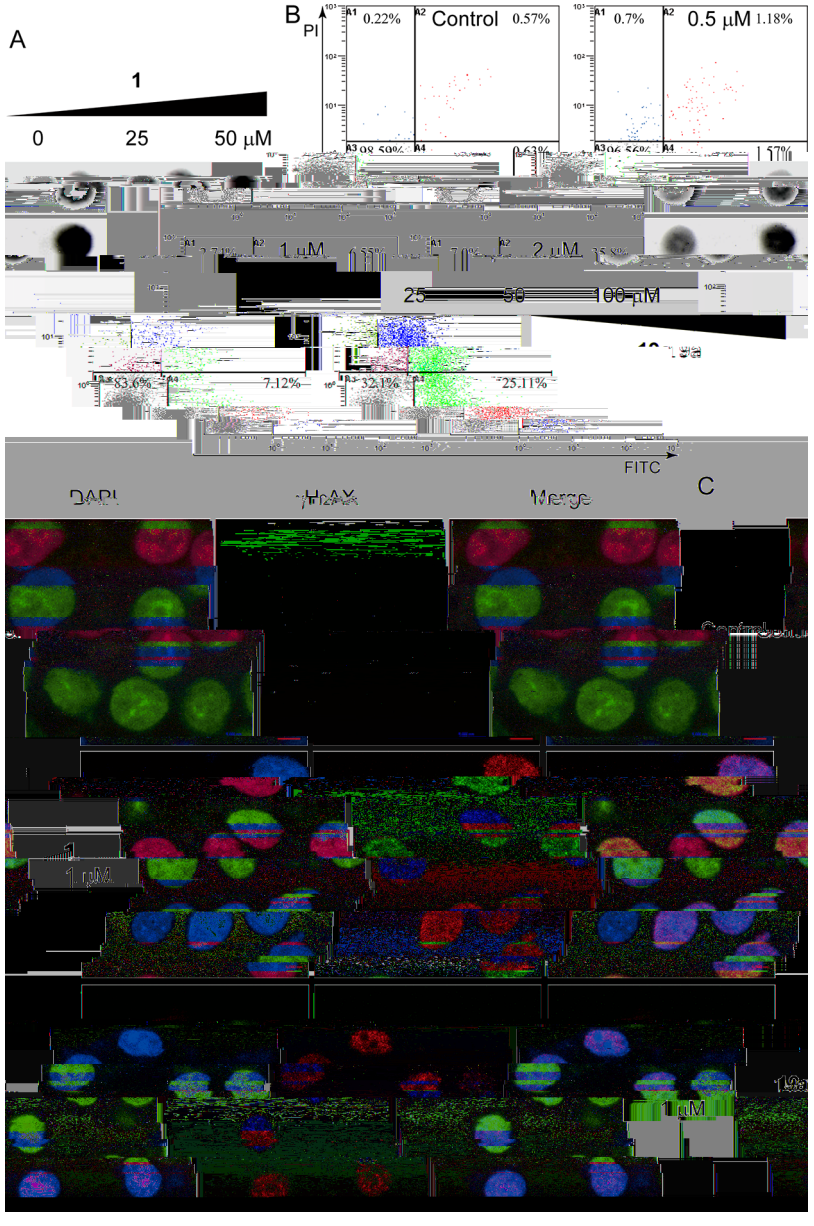

Figure 9. (A) 1–

- 145- C0.1 C0.1 **1** 364 1 - 364 -145.[85] 1 364 1 .[75,86] 145- C0.1

1 (225-) **19a** (132-),
(5)
364 **19a**.

EXPERIMENTAL SECTION

General Experiments.

A A , -A C ., A . ( )

fi . 4-(B *d* 1,3 -5-) -3- -1- (**14**) .[66] C

254 (C). A100 .

B A A C 400 .

A 6120 (C-) . -

A C - - . A C,

95%.

220 , 1.0 / fl , 50% 15% B ff (3) 50% 85% 35 .

A A C - . A A C - ,

C A .

General Procedure for Synthesis of Schiff's Base 12a and 12b. 6- (9.8 , 40) (3- , 42) (200) 12 .

(2 × 10) , 100%. ff' [1] fi .

*N-(2-Hydroxylethyl)-6-bromoveratraldimine (**12a**).* [1] (C C$_3$) δ 8.58 (, 1), 7.54 (, 1), 6.99 (, 1), 3.95–3.89 (, 8), 3.79 (, J = 4.7 , 2).

*N-(3-Hydroxylprapyl)-6-bromoveratraldimine (**12b**).* [1] (C C$_3$) δ 8.53 (, 1), 7.45 (, 1), 7.00 (, 1), 3.91–3.86 (, 8), 3.82 (, J = 6.2 , 2), 1.96 (, J = 6.0 , 2).

General Procedure for the Synthesis of 13a and 13b. , (60%, 6.69 , 167) (100) 0 °C 15 **12a** (**12b**, 33) (100). 0 °C 1 , (15 , 198) 1 . (20) 0 °C. . (100) (3 × 50). (4)

13a (**13b**), fi .

General Procedure for Synthesis of 15a and 15b. ,

General Procedure for Synthesis of 16a and 16b.

(C C)$_2$ (215 μ , 2.5) C $_2$C$_2$ (5), (365 μ , 5) −60 °C 15 . A **15a** (**15b**, 0.5) C $_2$C$_2$ (3) −60 °C 30 , $_3$ (1.5 , 10) . 2 . (1) 0 °C. C $_2$C$_2$ (3 × 20). (3 × 30) ($_4$) . fi .

*2-(3-(Benzo[d][1,3]dioxol-5-yl)-6,7-dimethoxy-2-(2-(methoxymethoxy)ethyl)-1-oxo-1,2-dihydroisoquinolin-4-yl)-acetaldehyde (**16a**).* , 85%. ^{1} (C C$_3$) δ 9.57 (, J = 1.8 , 1), 7.90 (, 1), 6.93 (, J = 7.8 , 1), 6.81−6.73 (, 3), 6.08 (, 2), 4.50 (, 2), 4.09 (, J = 6.8 , 2), 4.03 (, 3), 3.97 (, 3), 3.79−3.67 (, 2), 3.53−3.47 (, 2), 3.21 (, 3). ^{13}C (C C$_3$) δ 199.7, 161.6, 153.8, 149.3, 148.4, 148.2, 141.8, 131.6, 128.0, 123.7, 119.5, 110.2, 108.7, 108.3, 106.4, 103.4, 101.7, 96.2, 64.4, 56.2, 56.1, 55.1, 45.9, 44.6. - m/z: 455.2 + $^+$.

*2-(3-(Benzo[d][1,3]dioxol-5-yl)-6,7-dimethoxy-2-(3-(methoxymethoxy)propyl)-1-oxo-1,2-dihydroisoquinolin-4-yl)-acetaldehyde (**16b**).* , 87%. ^{1} (C C$_3$) δ 9.58 (, 1), 7.91 (, J = 2.4 , 1), 6.96−6.90 (, 1), 6.80−6.74 (, 3), 6.08 (, 2), 4.71−4.60 (, 2), 4.50 (, 2), 4.02 (, 3), 3.96 (, 3), 3.52−3.44 (, 4), 3.29 (, 3), 1.88−1.83 (, 2). ^{13}C (C C$_3$) δ 199.6, 161.4, 153.7, 149.3, 148.4, 148.3, 141.5, 131.4, 128.0, 123.3, 119.7, 109.8, 108.8, 108.4, 106.3, 103.4, 101.7, 96.1, 65.3, 56.2, 56.1, 55.1, 44.5, 44.3, 29.2. - m/z: 470.2 + $^+$.

General Procedure for the Synthesis of 17a and 17b.

16a (**16b**, 0.5) (0.4) (10) 50 - fl . fl . , fi . fi .

*12-(2-Hydroxyethyl)-2,3-dimethoxy-[1,3]dioxolo[4′,5′:4,5]benzo-[1,2-c]phenanthridin-13(12H)-one (**17a**).* , 97%, = 228.2−229.7 °C. ^{1} (C C$_3$) δ 8.00 (, J = 8.8 , 1), 7.91 (, 1), 7.59−7.57 (, 2), 7.25 (, 1), 7.19 (, 1), 6.11 (, 2), 5.08 (, J = 5.6 , 1), 4.54 (, J = 4.2 , 2), 4.37−4.33 (, 2), 4.12 (, 3), 4.06 (, 3). ^{13}C (C C$_3$) δ 165.8, 154.0, 149.8, 147.5, 147.5, 135.4, 131.9, 129.4, 123.8, 120.8, 118.7, 118.3, 117.3, 108.5, 105.0, 102.8, 102.0, 101.7, 64.0, 56.8, 56.3, 56.2. ( ) m/z: 394.1276 + $^+$, C$_{22}$ $_{20}$ $_6$ 394.1285. - m/z: 394.1 + $^+$.

*12-(3-Hydroxypropyl)-2,3-dimethoxy-[1,3]dioxolo[4′,5′:4,5]-benzo[1,2-c]phenanthridin-13(12H)-one (**17b**).* , 96%, = 263.4−264.2 °C. ^{1} (C C$_3$) δ 7.98 (, J = 8.8 , 1), 7.92 (, 1), 7.60 (, 1), 7.59−7.55 (, 2), 7.19 (, 1), 6.12 (, 2), 4.72 (, J = 6.6 , 2), 4.12 (, 3), 4.07 (, 3), 3.54 (, J = 6.6 , 2), 2.16−2.07 (, 2). ^{13}C (C C$_3$) δ 165.0, 153.7, 149.8, 147.6, 147.4, 135.1, 131.7, 129.0, 123.5, 121.1, 119.4, 118.4, 117.5, 108.9, 104.9, 102.8, 102.3, 101.6, 60.1, 56.2, 56.2, 48.3, 32.8. ( ) m/z: 408.1457 + $^+$, C$_{23}$ $_{22}$ $_6$ 408.1442. - m/z: 408.1 + $^+$.

General Procedure for the Synthesis of 18a and 18b.

17a (**17b**, 0.5) (10), C $_2$ (0.8) . 1 (5) 0 °C. fi . fi .

*12-(2-Chloroethyl)-2,3-dimethoxy-[1,3]dioxolo[4′,5′:4,5]benzo-[1,2-c]phenanthridin-13(12H)-one (**18a**).* , 94%, = 236.5−237.3 °C. ^{1} ( ) δ 8.83 (, J = 9.0 , 1), 8.31 (, 1), 8.26 (, 1), 8.16 (, J = 9.0 , 1), 7.72 (, 1), 7.68 (, 1), 6.33 (, 2), 5.76 (, J = 9.0 , 2), 5.35 (, J = 9.0 , 2), 4.20 (, 3), 4.04 (, 3). - m/z: 412.1 (100%), 414.0 (33%) + $^+$.

*12-(3-Chloropropyl)-2,3-dimethoxy-[1,3]dioxolo[4′,5′:4,5]benzo-[1,2-c]phenanthridin-13(12H)-one (**18b**).* , 96%, = 235.5−236.1 °C. ^{1} (C C$_3$) δ 7.97 (, J = 8.8 , 1), 7.89 (, 1), 7.59−7.55 (, 2), 7.51 (, 1), 7.19 (, 1), 6.12 (, 2), 4.69 (, J = 6.8 , 2), 4.11 (, 3), 4.06 (, 3), 3.39 (, J = 6.4 , 2), 2.43−2.33 (, 2). ( ) m/z: 426.1090 + $^+$, C$_{23}$ $_{21}$ $_5$C 426.1103. - m/z: 426.1 (100%), 428.1 (33%) + $^+$.

General Procedure for the Synthesis of 19a/b−25a/b.

18a (**18b**, 0.87), $_3$ (870 , 8.7), (, 8.7) (20) fl 3−6 , , . . fi .

*12-(2-(Dimethylamino)ethyl)-2,3-dimethoxy-[1,3]dioxolo-[4′,5′:4,5]benzo[1,2-c]phenanthridin-13(12H)-one (**19a**).* , 65%, = 136.4−138.0 °C. (B , $^{-1}$), 1632, 1611, 1590. ^{1} (C C$_3$) δ 7.97 (, J = 8.4 , 1), 7.91 (, 1), 7.66 (, 1), 7.57−7.54 (, 2), 7.18 (, 1), 6.10 (, 2), 4.66 (, J = 7.0 , 2), 4.10 (, 3), 4.05 (, 3), 2.78 (, J = 7.0 , 2), 2.21 (, 6). ^{13}C (C C$_3$) δ 164.7, 153.6, 149.7, 147.5, 147.3, 135.5, 131.7, 128.9, 123.3, 121.3, 119.6, 118.3, 117.3, 108.8, 104.8, 102.9, 102.3, 101.5, 57.6, 56.2, 56.1, 50.1, 45.5. ( ) m/z: 421.1761 + $^+$, C$_{24}$ $_{25}$ $_2$ $_5$ 421.1758.

*12-(3-(Dimethylamino)propyl)-2,3-dimethoxy-[1,3]dioxolo-[4′,5′:4,5]benzo[1,2-c]phenanthridin-13(12H)-one (**19b**).* , 70%, = 173.2−174.3 °C. (B , $^{-1}$), 1612 ( ), 1582. ^{1} (C C$_3$) δ 7.97 (, J = 8.4 , 1), 7.91 (, 1), 7.58−7.53 (, 2), 7.48 (, 1), 7.19 (, 1), 6.13 (, 2), 4.55 (, J = 7.2 , 2), 4.12 (, 3), 4.07 (, 3), 2.51 (, J = 7.2 , 2), 2.34 (, 6), 2.18 (, J = 7.2 , 2). ^{13}C (C C$_3$) δ 164.7, 108 149.8, 119.4,

. fi 2
- - - .

fi
. = C_{27} A^{32} 2 4, =
449.57, , *a* = 9.41991(12) Å, *b* = 10.11440(12) Å, *c* =
23.7285(4) Å, *β* = 91.6653(13)°, *V* = 2259.82(5) Å^3, = 103 ,
$P2_1/c$ (. 14), = 4, *μ* (C *α*) = 0.710 $^{-1}$, 10230
fl , 4283 (= 0.0458)
. fi (fl) = 0.0500, ^{2}(fl) =
0.1495. C **20b**
C C C
CC C 1579803. C
, , CC C, 12 ,
C CB2 1 , : + 44(0)1223−336033 - :
@ . . .

2,3-Dimethoxy-12-(2-(pyrrolidin-1-yl)ethyl)-[1,3]dioxolo-[4′,5′:4,5]benzo[1,2-c]phenanthridin-13(12H)-one (***21a***).
, 47%, = 209.2−210.3 °C. (B , $^{-1}$), 1639, 1610,
1594. ^{1} (C C $_3$) *δ* 7.93−7.88 (, 2), 7.65 (, 1), 7.52 (,
1), 7.49 (, *J* = 8.8 , 1), 7.12 (, 1), 6.08 (, 2), 4.66 (, *J* = 7.2
, 2), 4.08 (, 3), 4.05 (, 3), 2.91 (, *J* = 7.2 , 2), 2.52−2.42
(, 4), 1.73−1.61 (, 4). ^{13}C (C C $_3$) *δ* 164.7, 153.5, 149.6,
147.5, 147.3, 135.4, 131.6, 129.0, 123.2, 121.1, 119.6, 118.2, 117.3,
108.8, 104.7, 102.8, 102.6, 101.5, 56.2, 56.1, 54.3, 54.1, 51.0, 23.6.
( ) *m/z*: 447.1916 + $^+$, C_{26} $_{27}$ $_2$ $_5$
447.1914. - *m/z*: 447.2 + $^+$.

2,3-Dimethoxy-12-(3-(pyrrolidin-1-yl)propyl)-[1,3]dioxolo-[4′,5′:4,5]benzo[1,2-c]phenanthridin-13(12H)-one (***21b***).
, 65%, = 205.8−207.4 °C. (B , $^{-1}$), 1640, 1612,
1596. ^{1} (C C $_3$) *δ* 7.95 (, *J* = 8.8 , 1), 7.88 (, 1), 7.56
(, 1), 7.54 (, *J* = 8.8 , 1), 7.48 (, 1), 7.16 (, 1), 6.10 (,
2), 4.57 (, *J* = 7.2 , 2), 4.10 (, 3), 4.05 (, 3), 2.59 (, 4),
2.52 (, *J* = 7.2 , 2), 2.19 (, *J* = 7.2 , 2), 1.77 (, 4).
^{13}C (C C $_3$) *δ* 164.7, 153.6, 149.7, 147.6, 147.4, 135.3, 131.7,
129.0, 123.4, 121.2, 119.5, 118.3, 117.3, 108.8, 104.9, 102.9, 102.1,
101.6, 56.2, 56.2, 53.8, 53.3, 49.9, 27.4, 23.4. ( ) *m/z*:
461.2067 + $^+$, C_{27} $_{29}$ $_2$ $_5$ 461.2071.

2,3-Dimethoxy-12-(2-(piperidin-1-yl)ethyl)-[1,3]dioxolo-[4′,5′:4,5]benzo[1,2-c]phenanthridin-13(12H)-one (***22a***).
, 44%, = 178.3−180.1 °C. (B , $^{-1}$), 1642, 1612,
1596. ^{1} (C C $_3$) *δ* 7.98 (, *J* = 8.8 , 1), 7.93 (, 1), 7.74
(, 1), 7.58 (, 1), 7.56 (, *J* = 8.8 , 1), 7.19 (, 1), 6.12 (,
2), 4.69 (, *J* = 6.6 , 2), 4.12 (, 3), 4.07 (, 3), 2.69 (, *J* = 6.6
, 2), 2.34−2.23 (, 4), 1.40−1.29 (, 6). ^{13}C (C C $_3$)
δ 165.0, 153.5, 149.5, 147.5, 147.3, 135.6, 131.6, 129.1, 123.2, 121.2,
119.7, 118.2, 117.5, 108.9, 104.7, 102.8, 102.7, 101.5, 57.2, 56.2, 56.1,
54.6, 49.8, 25.8, 24.2. ( ) *m/z*: 461.2076 + $^+$,
C_{27} $_{29}$ $_2$ $_5$ 461.2071. - *m/z*: 461.2 + $^+$.

2,3-Dimethoxy-12-(3-(piperidin-1-yl)propyl)-[1,3]dioxolo-[4′,5′:4,5]benzo[1,2-c]phenanthridin-13(12H)-one (***22b***).
, 60%, = 110.6−112.3 °C. (B , $^{-1}$), 1639, 1612,
1592. ^{1} (C C $_3$) *δ* 7.98 (, *J* = 8.7 , 1), 7.91 (, 1), 7.60−
7.55 (, 2), 7.48 (, 1), 7.19 (, 1), 6.10 (, 2), 4.55 (, *J* = 7.2
, 2), 4.12 (, 3), 4.06 (, 3), 2.50 (, *J* = 7.2 , 2), 2.34−2.13
(, 6), 1.51−1.30 (, 6). ^{13}C (C C $_3$) *δ* 164.6, 153.7, 149.7,
147.6, 147.5, 135.1, 131.7, 128.9, 123.5, 121.0, 119.4, 118.3, 117.4,
108.7, 104.9, 102.9, 102.0, 101.6, 56.2, 56.1, 55.7, 53.9, 50.1, 25.4, 25.2,
23.9. ( ) *m/z*: 475.2227 + $^+$, C_{28} $_{31}$ $_2$ $_5$
475.2227.

2,3-Dimethoxy-12-(2-morpholinoethyl)-[1,3]dioxolo[4′,5′:4,5]-benzo[1,2-c]phenanthridin-13(12H)-one (***23a***). ,
48%, = 220.5−221.1 °C. (B , $^{-1}$), 1639, 1611, 1594. ^{1}
(C C $_3$) *δ* 7.93 (, *J* = 8.7 , 1), 7.88 (, 1), 7.64 (, 1),
7.54 (, 1), 7.51 (, *J* = 8.7 , 1), 7.14 (, 1), 6.09 (, 2), 4.66 (,
J = 6.2 , 2), 4.10 (, 3), 4.05 (, 3), 3.34 (, 4), 2.67 (, *J* =
6.2 , 2), 2.21 (, 4). ^{13}C (C C $_3$) *δ* 165.2, 153.5, 149.6,
147.5, 147.4, 135.6, 131.5, 129.0, 123.2, 121.2, 119.7, 118.3, 117.6,
108.9, 104.8, 102.8, 102.5, 101.6, 66.9, 56.6, 56.2, 56.2, 53.4, 49.3.
( ) *m/z*: 463.1875 + $^+$, C_{26} $_{27}$ $_2$ $_6$
463.1864. - *m/z*: 463.2 + $^+$.

2,3-Dimethoxy-12-(3-morpholinopropyl)-[1,3]dioxolo[4′,5′:4,5]-benzo[1,2-c]phenanthridin-13(12H)-one (***23b***

J= 407. 632 094504.0723 (C)-353.271(,-358.2703 9) 6.0299006.0299142.240 40 9009145.24C148.8,9-167.27037.8, 3 (δ 7.74 (, 1), 7.68 (, *J* = 8.8 , 1), 7.50 (, *J* = 8.8 , 1), 7.30 (, 1), 7.12 (, 1), 6.80 (, 1), 6.06 (, 2), 4.21 (, 2), 4.00 (, 3), 3.95 (, 3), 2.88 (, *J* = 6.4 , 2), 2.60 (, *J* = 6.4 , 2), 2.24 (, 6). ^{13}C (C C$_3$) δ 148.8, 148.7, 148.2, 147.6, 142.9, 131.0, 126.6, 125.5, 125.2, 124.9, 123.9, 119.9, 110.2, 106.4, 104.5, 101.2, 100.8, 58.4, 56.3, 56.2, 50.8, 50.0, 46.0. ( ) *m/z*: 407.1960 + $^+$, $C_{24\ 27\ 2\ 4}$ 407.1965. - *m/z*: 407.2 + $^+$.

m= ,-159. (,-235.8 (6),)-182.21212.6 7.12 (, 1), 6.80 (, 1), 6.06 (, 2), 4.21 (, 2), 4.003 992.2((

(, 1), 7.02 (, 1), 6.83 (, 1), 6.75 (, 1), 6.06 (, 2), 4.14 (, 2), 4.00 (, 3), 3.95 (, 3), 3.92 (, J = 7.2 , 2), 2.81 (, J = 7.2 , 2), 2.08 (, J = 7.2 , 2). [13]C (C C 3) δ 149.1, 148.8, 148.3, 147.7, 142.0, 137.0, 131.0, 129.6, 126.4, 125.4, 125.2, 125.0, 124.2, 120.0, 118.8, 109.9, 106.6, 104.6, 101.2, 100.3, 56.3, 56.2, 50.8, 49.6, 45.1, 30.0. ( ) m/z: 444.1920 + +, C26 26 3 4 444.1918.

*Synthesis of N-(4-Methoxybenzyl)-6-bromoveratraldimine (**33**).* A " ﬀ B **12a** **12b**", **33** 4- . [1] (C C 3) δ 8.64 (, J = 1.3 , 1), 7.59 (, 1), 7.27−7.24 (, 2), 7.00 (, 1), 6.92−6.86 (, 2), 4.77 (, 2), 3.91 (, 3), 3.90 (, 3), 3.81 (, 3).

*Synthesis of 3-(Benzo[d][1,3]dioxol-5-yl)-4-(2-hydroxyethyl)-6,7-dimethoxy-2-(4-methoxybenzyl)isoquinolin-1(2H)-one (**34**).* A - " **15a** **15b**", **34** **33** . , 62%. [1] (C C 3) δ 7.91 (, 1), 7.13 (, 1), 6.79−6.62 (, 5), 6.51−6.41 (, 2), 5.99 (, 1), 5.95 (, 1), 5.06 (, J = 14.4 , 1), 4.94 (, J = 14.1 , 1), 3.98 (, 3), 3.94 (, 3), 3.69 (, 3), 3.64 (, J = 6.8 , 2), 2.77−2.64 (, 2). [13]C (C C 3) δ 161.7, 158.5, 153.6, 149.1, 147.9, 147.6, 140.0, 131.9, 130.0, 128.0, 128.0, 123.8, 119.7, 113.6, 111.9, 110.4, 108.5, 108.1, 104.0, 101.4, 62.2, 56.1, 56.1, 55.2, 48.6, 32.0. ﬁ 2 .

*Synthesis of 2-(3-(Benzo[d][1,3]dioxol-5-yl)-6,7-dimethoxy-2-(4-methoxybenzyl)-1-oxo-1,2-dihydroisoquinolin-4-yl)acetaldehyde (**35**).* A " **16a** **16b**", **35** **34** . , 82%. [1] (C C 3) δ 9.56 (, J = 2.0 , 1), 7.98 (, 1), 6.87−6.72 (, 7), 6.52 (, 1), 6.05 (, 2), 5.19 (, J = 14.6 , 1), 5.06 (, J = 14.7 , 1), 4.05 (, 3), 3.99 (, 3), 3.76 (, 3), 3.50 (, J = 2.0 , 2). [13]C (C C 3) δ 199.6, 161.8, 158.6, 153.8, 149.4, 148.3, 148.0, 141.7, 131.6, 129.9, 128.1, 127.7, 123.7, 119.7, 113.7, 110.0, 108.8, 108.5, 106.6, 103.5, 101.6, 56.2, 56.1, 55.2, 48.8, 44.4. - m/z: 488.2 + +.

*Synthesis of 2,3-Dimethoxy-[1,3]dioxolo[4′,5′:4,5]benzo[1,2-c]phenanthridin-13(12H)-one (**36**).* **35** (270 , 0.5) (0.4) (4) 50 - ﬂ . ﬂ . , ﬁ . ﬁ **36**, 62%. (B , $^{-1}$), 1643 ( ), 1500. [1] ( ) δ 11.53 (, 1), 8.36 (, 1), 8.33 (, J 928.844.4.1), , 4111 8.8(, (2 4.145 46143 55.2,

*2,3-Dimethoxy-13-(2-(4-methylpiperazin-1-yl)ethoxy)-[1,3]-dioxolo[4′,5′:4,5]benzo[1,2-c]phenanthridine (**44a**).* , 74%, = 206.5−207.4 °C. (B, −1), 1622, 1596. 1 (C C3) δ 8.54 (, 1), 8.17 (, J = 8.8 , 1), 7.82 (, 1), 7.73−7.63 (, 2), 7.23 (, 1), 6.11 (, 2), 4.92 (, J = 6.4 , 2), 4.14 (, 3), 4.06 (, 3), 3.07 (, J = 6.4 , 2), 2.92−2.65 (, 4), 2.65−2.41 (, 4), 2.32 (, 3). 13C (C C3) δ 157.2, 152.7, 149.3, 148.1, 147.9, 138.9, 131.1, 130.0, 128.3, 123.7, 118.3, 117.5, 113.9, 104.5, 104.3, 102.3, 102.2, 101.3, 64.1, 57.2, 56.1, 55.3, 53.8, 46.2. ( ) *m/z*: 476.2179 + +, C27 30 3 5 476.2180.

*13-(2-(1H-Imidazol-1-yl)ethoxy)-2,3-dimethoxy-[1,3]dioxolo-[4′,5′:4,5]benzo[1,2-c]phenanthridine (**45a**).* , 56%, = 238.9−239.1 °C. (B, −1), 1621, 1597. 1 (C C3) δ 8.47 (, 1), 8.19 (, J = 8.8 , 1), 7.83 (, 1), 7.71 (, J = 8.8 , 1), 7.67 (, 1), 7.58 (, 1), 7.24 (, 1), 7.12−7.09 (, 2), 6.12 (, 2), 5.06 (, J = 5.2 , 2), 4.59 (, J = 5.2 , 2), 4.15 (, 3), 4.06 (, 3). 13C (C C3) δ 156.4, 153.1, 149.6, 148.2, 148.0, 138.5, 137.7, 131.3, 130.1, 129.8, 128.2, 124.2, 119.5, 118.4, 117.9, 113.6, 104.5, 104.1, 102.4, 101.9, 101.4, 64.9, 56.3, 56.2, 46.5. ( ) *m/z*: 444.1557 + +, C25 22 3 5 444.1554.

General Procedure for Synthesis of 39b−45b.

38 (221 , 0.47), 2C 3 (163 , 1.18), (20 , 0.118), (. 0.7) (20) , (100) (3 × 50). (4) . fi , .

*2,3-Dimethoxy-13-(3-(dimethylamino)propoxy)-[1,3]dioxolo-[4′,5′:4,5]benzo[1,2-c]phenanthridine (**39b**).* , 82%, = 148.6−150.4 °C. (B, −1), 1618, 1593. 1 (C C3) δ 8.56 (, 1), 8.18 (, J = 8.8 , 1), 7.83 (, 1), 7.72 (, 1), 7.68 (, J = 8.8 , 1), 6.11 (, 2), 4.82 (, J = 7.4 , 1), 4.14 (, 3), 4.07 (, 3), 2.60 (, J = 7.4 , 1), 2.32 (, 6), 2.20 (, J = 7.4 , 1). 13C (C C3) δ 157.5, 152.7, 149.3, 148.0, 147.8, 139.0, 131.1, 130.0, 128.3, 123.6, 118.4, 117.4, 114.0, 104.5, 104.4, 102.3, 102.2, 101.2, 64.5, 57.0, 56.2, 56.2, 45.7, 27.5. ( ) *m/z*: 435.1921 + +, C25 27 2 5 435.1914. - *m/z*: 435.3 + +.

*2,3-Dimethoxy-13-(3-diethylamino)propoxy)-[1,3]dioxolo-[4′,5′:4,5]benzo[1,2-c]phenanthridine (**40b**).* , 61%, = 146.2−147.8 °C. (B, −1), 1620, 1594. 1 ( ) δ 8.45 (, J = 9.0 , 1), 8.35 (, 1), 8.04 (, 1), 7.75 (, J = 9.0 , 1), 7.57 (, 1), 7.43 (, 1), 6.19 (, 2), 4.71 (, J = 6.4 , 2), 4.06 (, 3), 3.93 (, 3), 2.70 (, J = 6.8 , 2), 2.53 (, J = 7.2 , 4), 2.05 (, J = 6.8 , 2), 1.01 (, J = 7.2 , 6). 13C ( ) δ 157.3, 153.3, 149.7, 148.2, 148.0, 138.3, 131.0, 130.1, 127.7, 123.9, 119.6, 117.5, 113.4, 104.7, 104.0, 103.6, 101.8, 101.4, 64.5, 56.4, 55.9, 49.1, 47.0, 26.4, 12.2. ( ) *m/z*: 463.2233 + +, C27 31 2 5 463.2227. - *m/z*: 463.2 + +.

*2,3-Dimethoxy-13-(3-(pyrrolidin-1-yl)propoxy)-[1,3]dioxolo-[4′,5′:4,5]benzo[1,2-c]phenanthridine (**41b**).* , 80%, = 162.8−163.9 °C. (B, −1), 1619, 1594. 1 (C C3) δ 8.48 (, 1), 8.07 (, J = 8.8 , 1), 7.71 (, 1), 7.67−7.57 (, 2), 7.18 (, 1), 6.09 (, 2), 4.77 (, J = 6.8 , 2), 4.10 (, 3), 4.05 (, 3), 2.78 (, J = 6.8 , 2), 2.62 (, , 4), 2.24 (, J = 6.8 , 2), 1.83 (, , 4). 13C (C C3) δ 157.3, 152.5, 149.1, 147.9, 147.7, 138.8, 130.9, 129.9, 128.2, 123.5, 118.2, 117.2, 113.9, 104.3, 104.2, 102.1, 101.1, 64.5, 56.1, 56.0, 54.4, 53.7, 28.8, 23.5. ( ) *m/z*: 461.2082 + +, C27 29 2 5 461.2071. - *m/z*: 461.2 + +.

*2,3-Dimethoxy-13-(3-(piperidin-1-yl)propoxy)-[1,3]dioxolo-[4′,5′:4,5]benzo[1,2-c]phenanthridine (**42b**).* , , 81%, = 153.7−155.3 °C. (B, −1), 1618, 1593. 1 (C C3) δ 8.51 (, 1), 8.13 (, J = 8.8 , 1), 7.77 (, 1), 7.69−7.60 (, 2), 7.21 (, 1), 6.10 (, 2), 4.78 (, J = 6.2 , 2), 4.12 (, 3), 4.06 (, 3), 2.66 (, J = 7.6 , 2), 2.51 (, , 4), 2.24 (, J = 7.2 , 2), 1.72−1.58 (, 4), 1.55−1.41 (, 2). 13C (C C3) δ 157.4, 152.6, 149.2, 147.9, 147.7, 138.9, 130.9, 129.9, 128.2, 123.5, 118.3, 117.3, 113.9, 104.3, 104.3, 102.2, 102.1, 101.1, 64.6, 56.6, 56.1, 56.0, 54.7, 26.6, 25.9, 24.4. ( ) *m/z*: 475.2229 + +, C28 31 2 5 475.2227. - *m/z*: 475.2 + +.

*2,3-Dimethoxy-13-(3-morpholinopropoxy)-[1,3]dioxolo-[4′,5′:4,5]benzo[1,2-c]phenanthridine (**43b**).* , 65%, = 171.8−172.9 °C. (B, −1), 1618, 1593. 1 (C C3) δ 8.53 (, 1), 8.16 (, J = 9.2 , 1), 7.82 (, 1), 7.69 (, 1), 7.67 (, J = 8.8 , 1), 7.23 (, 1), 6.11 (, 2), 4.83 (, J = 6.6 , 2), 4.13 (, 3), 4.07 (, 3), 3.76 (, J = 4.8 , 4), 2.67 (, J = 7.2 , 2), 2.56−2.53 (, 4), 2.22 (, J = 7.2 , 2). 13C (C C3) δ 157.3, 152.6, 149.2, 147.9, 147.7, 138.8, 130.9, 129.9, 128.2, 123.5, 118.2, 117.3, 113.9, 104.3, 104.3, 102.2, 102.0, 101.1, 67.0, 64.3, 56.1, 56.1, 56.0, 53.9, 26.3. ( ) *m/z*: 477.2027 + +, C27 29 2 6 477.2020. - *m/z*: 477.2 + +.

*2,3-Dimethoxy-13-(3-(4-methylpiperazin-1-yl)propoxy)-[1,3]-dioxolo[4′,5′:4,5]benzo[1,2-c]phenanthridine (**44b**).* , 65%, = 142.5−143.8 °C. (B, −1), 1619, 1593. 1 (C C3) δ 8.55 (, 1), 8.18 (, J = 9.0 , 1), 7.83 (, 1), 7.70 (, 1), 7.68 (, J = 8.8 , 1), 7.23 (, 1), 6.11 (, 2), 4.83 (, J = 6.4 , 2), 4.14 (, 3), 4.07 (, 3), 2.70 (, J = 7.2 , 2), 2.67−2.41 (, 8), 2.32 (, 3), 2.22 (, J = 7.2 , 2). 13C (C C3) δ 157.3, 152.6, 149.2, 147.9, 147.7, 138.9, 131.0, 129.9, 128.2, 123.5, 118.3, 117.3, 114.0, 104.4, 104.2, 102.2, 102.1, 101.1, 64.5, 56.1, 56.0, 55.8, 55.2, 53.4, 46.0, 26.7. ( ) *m/z*: 490.2339 + +, C28 32 3 5 490.2336. - *m/z*: 490.23 + +.

*13-(3-(1H-Imidazol-1-yl)propoxy)-2,3-dimethoxy-[1,3]dioxolo-[4′,5′:4,5]benzo[1,2-c]phenanthridine (**45b**).* , 62%, = 217.4−218.5 °C. (B, −1), 1618, 1591. 1 (C C3) δ 8.47 (, 1), 8.19 (, J = 8.8 , 1), 7.84 (, 1), 7.70 (, J = 8.8 , 1), 7.64 (, 1), 7.60 (, 1), 7.25 (, 1), 7.12 (, 1), 7.03 (, 1), 6.13 (, 2), 4.83 (, J = 6.0 , 2), 4.28 (, J = 6.8 , 2), 4.16 (, 3), 4.11 (, 3), 2.53 (, J = 6.4 , 2). 13C (C C3) δ 156.8, 152.9, 149.4, 148.1, 147.8, 138.6, 137.2, 131.2, 130.0, 129.8, 128.1, 123.9, 118.9, 118.2, 117.5, 113.6, 104.3, 104.1, 102.4, 101.9, 101.2, 62.6, 56.1, 56.1, 44.3, 30.7. ( ) *m/z*: 458.1712 + +, C26 24 3 5 458.1710. - *m/z*: 458.2 + +.

TOP1-Mediated Cleavage Assay. A .[73] A 3′- [32] - 117- A [73] A 2 A 1 20 ff (10 - C 7.5, 50 C, 5 C 2, 0.1 A, 15 / B A) 25 °C 20 . (0.5% fi), (80% , 10 , 1 A, 0.1% , 0.1%). A 20% A . ( ). C fl 117- .

TOP1-Mediated Unwinding Assay. .[78] B fl , (20 μ) B, 322 A (0.5 μ), , 1 (10) ff (10 , 7.5, 0.1 A, 5 C 2, 50 C, 1 , 15 μ / B A). A 10 1. A 30 37 °C, 4 ff . 1% A ff 5 / . .

TDP1 Inhibition Assay. *A. Fluorescence Assay.* 1 fl .[50] B fl , *FAM* (fl , 6- fl) *BHQ* (B), 5′-*FAM*-A A C AAAA AC -*BHQ*-3′

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fl . 1 (20 μ / , 0.02 μ fi 1 (100) 10 - C , 7.5, 50 C , 1 A, 1) 384- ( ). (5 μ) 30 . , ( ) 485/ 510 - fl . (25 μ , 35) . fi ( ) (485/ 510). 1 fl .

B. Gel-Based Assay.[76] A 5′- 32 - - A 3′- (14) 1 10 1 15 ff 50 C , 7.5, 80 C , 2 A, 1 , 40 μ / B A, 0.01% -20. ff 1 99.5% (/) , 5 A, 0.01% (/) , 0.01% (/) . 16% A . ( ). A 9500 ( ), ( )

Melecular Modeling. - 1− A− (B 1 4) , , . fi . C C fi . , 94 fi 94 , - , 0.01 / . A . fi . 30 . - . - . A B fi . 1 (B 1) , , 1- , . A B 10 fi . 265, 495, 493. fi . - ( ) . A B fi . 0.05 / . A .

FRET Melting Assay. - C *FAM* (fl , 6- fl) *TAMRA* (fl , 6-), 10 (5′-*FAM*- (A A C A A- - A A C A A)-*TAM-ARA*-3′), B . , (fi 0.4 μ) (2 μ) - C ff (10 , 7.4) 60 C 37 °C 0.5 . C 2 - C 470 530 . 1 °C 37−99 °C, 30 .

Cell Culture and MTT Assay. - 1640 37 °C fi 5% C 2. A 3−6. , ( ) fi - 0.01 100 μ (0.001−10 μ) . A 72 37 °C, (50 μ , 1 /) B (B) (100). A 4 , 100 570 .[89] 50 ( ). , C -7 96 37 °C, .

Immunodetection of Cellular TOP1−DNA Complex. C 1− A .[90] B fl , C -116 1 , A (1) 25 °C 30 . (0.5 , 100%) , −20 °C. A (12000) 25 °C 10 75% . A (8 , 0.2). 7.2 (1). A , A . A (2 μ) 30 μ 2 4 ff (25 , 6.5) . 1 (A , 1:1000) 4 °C (C , 1:3000) 1 . C ( ).

γH2AX Detection. γ 2A .[74] B fl , C 116 (2 × 10^4 /) 3 37 °C. A , fi 4% / B 15 25 °C B ff . C 0.5% -100 B 0 °C 30 . 5% / B 37 °C 3 . fl , -γ 2A (139; . 9718, C) 37 °C . ff 488- (A21206,) 2.0 μ / 4′,6- -2- (A ,) 37 °C 2 . ff . 710 (,) .

Flow Cytometry. C 116 (3.0 × 10^5 /) 6- 24 . B , 1× ff , 5.0 μ C 10.0 μ (B , C) 15 . fl (B , AC C , A) 1 . .

Pharmacokinetic Study in Rat. (220−250 , $n = 3$) **19a** 10% 10% 15 ( ) (1 /) (5 /), . B (200 μ) : 0.083, 0.25, 0.5, 1, 2, 5, 7, 24 . (100 μ) 10 3000 −20 °C . C- - .

In Vivo Acute Toxicity. ,

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($n = 4$) ( ) .

.

19a

300, 240, 192, 154, 123 / , .

7 -

( ) . A

.

In Vivo Antitumor Activity. A /

A C -

-

C 116. 4–5

12–15 . C 116

C 116 (100 μ , 1×10^7)

.

80 ^{3}, ($n =$

6)

19a 10 5 / , .

.

(V)

: $V = ($

$\times$ $)^2/2$,

. A

,

. .

( ) : = (1 –

/

) × 100%.

Statistical Analysis. A ±

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(A A)

(, A), , .

■ ASSOCIATED CONTENT

Supporting Information

AC : 10.1021/ . -

.8 00639.

1- **6** ( )

- fi **20b** (C)

19a 1 (B)

41a 1 (B)

(C)

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§ .- . .- . . .

Notes

fi .

■ ACKNOWLEDGMENTS

C (. 81373257),

(. 2013010015609),

C (C C),

, B , , A (01 BC

006150-19).

■ ABBREVIATIONS USED

1, B; 1 , 1 ;

1, A- 1; A, -

A; - , - ; C ,

A; , fl ;

, ; B, *p*- ;

; , - ;

C, ; C,

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