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Simple Isoquinoline and Benzylisoquinoline Alkaloids as Potential Antimicrobial, Antimalarial, Cytotoxic, and Anti-HIV Agents

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Abstract—Twenty-six simple isoquinolines and 21 benzylisoquinolines were tested for antimicrobial, antimalarial, cytotoxic, and anti-HIV activities. Some simple isoquinoline alkaloids were significantly active in each assay, and may be useful as lead compounds for developing potential chemotherapeutic agents. These compounds include **13** (antimicrobial), **25**, **26**, and **42** (antimalarial), **13** and **25** (cytotoxic), and **28** and **29** (anti-HIV). A quaternary nitrogen atom of isoquinolium or dihydroisoquinolinium type may contribute to enhanced potency in the first three types of activities. In contrast, anti-HIV activity was found with tetrahydroisoquinoline and 6,7-dihydroxyisoquinolium salts.

Introduction

Several plant and mammalian species contain simple 1,2,3,4-tetrahydroisoquinolines, such as salsolinol and its *O*- and *N*-methyl analogues, and 1-benzyltetrahydroisoquinolines.[1,2] The latter compounds are related structurally to reticuline, which is an intermediate in biosynthesis of morphine in the opium poppy and also may play a role in the biosynthesis of mammalian morphines.[2,3]

We have previously described the antimalarial activity of structurally related protoberberine alkaloids[4,5] and were interested in further examining simple isoquinoline and benzylisoquinoline alkaloids and their aromatized derivatives for antimalarial activity. Also, prior literature reports have described the neurotoxicity of the tetrahydroisoquinoline salsolinol[6] and antitumor structure–activity relationship (SAR) patterns[7] of isoquinoline derivatives, and numerous reports have described the antimicrobial activity of isoquinoline alkaloids,[8–10] including benzyltetrahydroisoquinolines. Based on these reports, we investigated SARs based on different alkyl substituents at the C-1 and N-2 positions and on the 6,7-OH groups as well as the oxidation state of the pyridine ring.

In addition, prompted by a report of the anti-HIV activity of michellamine B, a naphthylisoquinoline alkaloid dimer from *Ancistrocladus korupenis*,[11] we investigated and describe herein the anti-HIV activities of tetrahydroisoquinolines, 3,4-dihydroisoquinolines, isoquinolines, 1- and 3-benzylisoquinolines, and corresponding quaternary salts.

Our focus in this study was to identify lead compounds and find preliminary SAR trends. Analogue synthesis based on these initial results and mechanism of action studies will be presented in future papers.

Chemistry

1-Methyl-6,7-dihydroxytetrahydroisoquinoline, salsolinol (1), was prepared previously.[12] 1-Ethyl-, 1-propyl-, 1-isopropyl-, and 1-pentyl-6,7-dihydroxytetrahydro-

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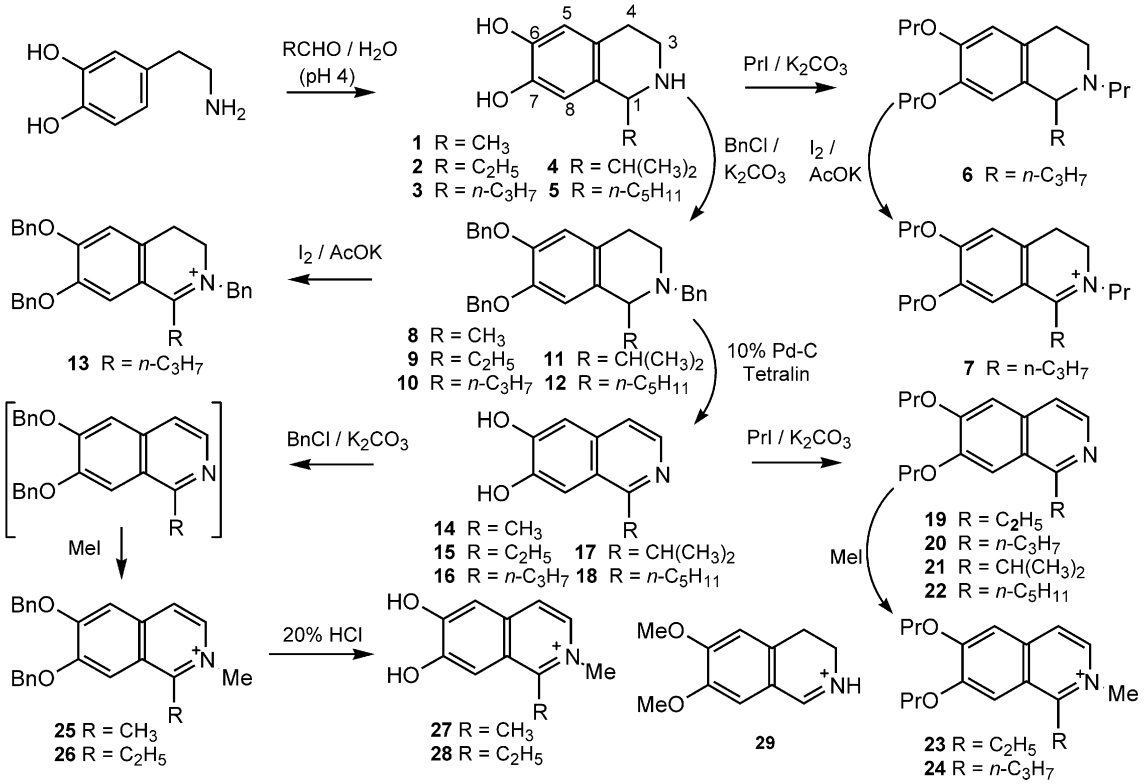
isoquinolines (**2**–**5**) were prepared by condensation of dopamine with an appropriate aldehyde (Scheme 1). Treating **3** with propyl iodide gave the *O*,*N*-tripropyl derivative (**6**), which was converted to its 3,4-dihydro quaternary salt (**7**) by oxidation using iodine. Similarly, treating **1**–**5** with benzyl chloride produced *O*,*N*-tribenzyl derivatives **8**–**12**. Oxidation of **10** by iodine gave *O*,*N*-tribenzyl-1-propyl-3,4-dihydroisoquinoline (**13**). Dehydrogenation of **8**–**12** with Pd-C in refluxing tetralin gave the 6,7-dihydroxy compounds **14**–**18**. Compounds **15**–**18** were *O*-propylated to give **19**–**22**. *N*-Methylation of **19** and **20** forded the isoquinolinium salts **23** and **24**, respectively. The 6,7-dihydroxy isoquinolines **14** and **15** were *O*-benzylated, then *N*-methylated to produce the corresponding salts **25** and **26**, respectively, which were debenzylated by reflux in 20% HCl to give the 6,7-dihydroxy salts (**27** and **28**, respectively). 6,7-Dimethoxy-3,4-dihydroisoquinoline (**29**) was prepared according to literature methods.[13] Papaverine (**30**) was commercially available, and its analogues (**31**–**39** and **41**) were prepared by previously reported methods.[14–23] 1-Benzyl- (**30**–**38**) and 3-benzyl- (**39**) isoquinolines were *N*-methylated to give **40** and **42**–**50**. The structures of **2**–**29** (Scheme 1) and **42**–**50** (Chart 1) were confirmed by ^{1}H NMR data (Tables 1 and 2) , including NOESY, and LSIMS and HRLSIMS data (Table 3).

Results and Discussion

Antimicrobial activity

6,7-Dihydroxytetrahydroisoquinolines, their *N*- and *O*-alkyl or -benzyl derivatives, and their 3,4-dihydro and dehydro analogues were tested against two Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) bacteria, two Gram-negative (*Salmonella enteritidis*, *Escherichia coli*), and one fungus (*Candida albicans*) by the liquid dilution method. Papaverine (**30**) and its analogues (**31**–**50**) also were tested. The minimum inhibitory concentrations (MIC) are presented in Table 4

All tetrahydroisoquinolines (**1**–**6**, **8**, **10**–**12**) and isoquinolines (**14**–**16**, **18**, **20**–**22**) displayed low activity (>500 μg/ml) against all tested strains. Oxidation of 6,7-dipropyloxy-*N*-propyl tetrahydroisoquinoline (**6**) to its dihydroisoquinolinium salt (**7**) increased activity slightly in some strains. While *N*-methylation of 6,7-dihydroxyisoquinolines **14** and **15** to the corresponding dihydroxyisoquinolinium salts **27** and **28** did not affect activity, *N*-methylation of 6,7-dipropyloxyisoquinolines **19** and **20** to the isoquinolinium derivatives **23** and **24** increased activity slightly. Thus, from these data, both *N*-quaternization/alkylation and *O*-alkylation appear to be important to enhanced activity. Accordingly, compounds **13**, **25**, and **26**, which contain both a *N*-alkylated quaternary nitrogen of the dihydroisoquinolinium or isoquinolinium-type and *O*-benzyl groups on the 6,7-hydroxy groups, displayed significant antimicrobial activity. Activity of the latter two 6,7-dibenzyloxy-*N*-methyl salts increased as the C-1 alkyl chain lengthened by one carbon (compare **26** with **25**). Among the tested simple isoquinolines, 6,7-dibenzyloxy-*N*-benzyl-1-propyl-dihydroisoquinolinium chloride (**13**) exhibited the highest activity against all strains tested, and its activity was higher than that of kanamycin sulfate. *O*,*N*-Benzylation was more effective than *O*,*N*-propylation (compare **13** with **7** and **25**, **26** with **23**, **24**). Compound **13**



Scheme 1.

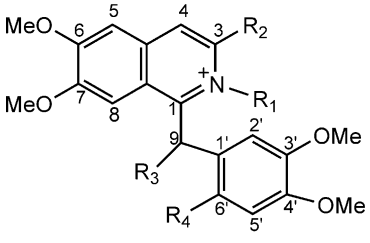
appears to be a useful new lead for further development of antimicrobial agents.

Papaverine (**30**) and related compounds (**31**–**50**) were tested against two Gram-positive, two Gram-negative, and one fungal microorganisms. Benzylisoquinolines (**30**–**39**) with a hydrogen substituent on the nitrogen atom showed weak activity against all test strains (Table 4). Among *N*-methylated benzylisoquinolinium salts (**40**–**50**), only **48** and **49** displayed slightly increased activity against *S. aureus*, *B. subtilis*, and *S. enteritidis*. Thus, activity increased when the four methoxy groups of *N*-methylpapaverine (**40**) were replaced by ethoxy groups (**48**).

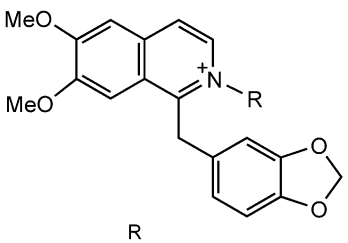
Antimalarial activity

Sixteen simple isoquinolines (**1**–**5**, **7**, **13**–**16**, and **23**–**28**) and 12 benzylisoquinolines (**30**, **40**–**50**) were tested in vitro against human malaria *Plasmodium falciparum* FCR-3. The antimalarial activity of each compound was determined as percentage reduction. The compound concentration required to inhibit cell growth by 50% was expressed as EC_{50}. To evaluate compounds' toxicity for mammalian cells, the concentration causing a 50% growth reduction (IC_{50}) of mouse mammary FM3A cells, a model of the host, was determined. The IC_{50}/EC_{50} ratios for the compounds were calculated as selectivity indexes, and these ratios were used as an evaluation of antimalarial activity. The results are presented in Table 5. Among the simple isoquinolines, 6,7-dihydroxytetrahydroisoquinolines (**1**–**5**) with varying C-1 alkyl side chains inhibited the growth of *P. falciparum* by only 0–31%. The corresponding aromatic isoquinolines (**14**–**16**) and *N*-methylated derivatives (**27** and **28**) showed slight inhibitory activity (EC_{50} ranged from 1.8E-05 to 5.9E-06) and low selectivity indexes (0.7–1.3). As found with antimicrobial activity, the *O*,*N*-tribenzyl-dihydroisoquinolinium salt (**13**) displayed more inhibitory activity than the *O*,*N*-tripropyl salt (**7**); however, both selectivity indexes were low. 6,7-Dipropyloxy-*N*-methyl isoquinolinium salts (**23** and **24**) inhibited *P. falciparum* with EC_{50} values on the order of 10^{-7} molar, but again, their selectivity indexes were low. However, the 6,7-dibenzyloxy-*N*-methyl salts (**25** and **26**) were potent antimalarial agents with higher selectivity indexes compared with the other test compounds. In conclusion, a quaternary nitrogen atom, especially in an isoquinolinium rather than a dihydroisoquinolinium ion, contributed to increased antimalarial activity. Benzylation of the C-6 and C-7 hydroxyl groups increased activity significantly (compare **25** and **26** with **27** and **28**, respectively), and to a larger extent than propylation of these groups (compare **26** and **13** with **23** and **7**, respectively). Among the tested simple isoquinolines, 6,7-dibenzyloxy-*N*-methyl isoquinolinium salts (**25** and **26**) showed the most potent antimalarial activity.

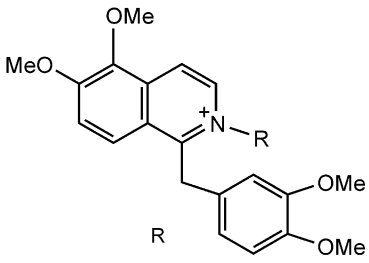
Among the tested benzylisoquinolines, **30**, **40**, **41**, **43**–**48** and **50** displayed slight inhibitory activity and their selectivity indexes were low. Only compounds **42** and **49** inhibited *P. falciparum* with EC_{50} values in the order of 10^{-7} molar and their selectivity indexes increased compared with other tested compounds. Introducing a methyl group into the C-3 or C-9 position increased activity (compare **42** and **49** with **40** and **48**, respectively), while



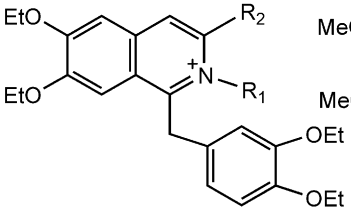
	R_1	R_2	R_3	R_4
30	H	H	H	H
31	H	H	Me	H
32	H	H	$=CH_2$	H
33	H	H	H	CH_2OH
34	H	Me	H	Ac
40	Me	H	H	H
41	Me	H	H	Ac
42	Me	H	Me	H
43	Me	H	$=CH_2$	H
44	Me	H	H	CH_2OH
45	Me	Me	H	Ac



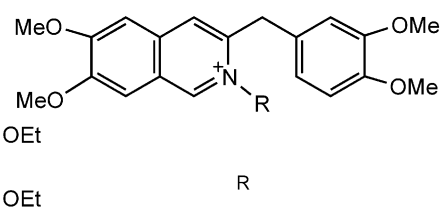
	R
35	H
46	Me



	R
36	H
47	Me



	R_1	R_2
37	H	H
38	H	Me
48	Me	H
49	Me	Me



	R
39	H
50	Me

Chart 1.

Table 1. [1]H NMR[a] data of simple isoquinolines **2**–**29**

Compd	1-H	3-H	4-H	5-H	6-H
2[b]	4.29 dd 1H (J=8, 4.5)	3.51 ddd 1H (J=12, 7, 6) 3.31 ddd 1H (J=12, 7.5, 6)	2.99 ddd 1H (J=17.5, 7.5, 6) 2.90 ddd 1H (J=17.5, 7, 6)	6.67 s 1H	6.61 s 1H
3[b]	4.34 dd 1H (J=8, 5)	3.50 ddd 1H (J=12, 7, 6) 3.31 ddd 1H (J=12.5, 7.5, 6)	2.98 ddd 1H (J=17.5, 7.5, 6) 2.90 ddd 1H(J=17.5, 7.5, 6)	6.65 s 1H	6.61 s 1H
4[b]	4.29 d 1H (J=5)	3.53 ddd 1H (J=12.5, 6, 4.5) 3.25 ddd 1H (J=12.5, 10, 5.5)	2.98 ddd 1H (J=17.5, 7, 6) 2.85 ddd 1H (J=17.5, 5.5, 4.5)	6.66 s 1H	6.62 s 1H
5[b]	4.33 dd 1H (J=8, 5)	3.49 ddd 1H (J=12.5, 7, 6) 3.30 ddd 1H (J=12.5, 7.5, 6)	2.98 ddd 1H (J=17, 7.5, 6) 2.90 ddd 1H (J=17, 7, 6)	6.65 s 1H	6.61 s 1H
6[c]	4.07 dd 1H (J=9, 4)	3.70 m 1H 3.51 m 1H	2.97[d] m 2H	6.66 s 1H	6.51 s 1H
7[c]		4.01 t 2H (J=7.5)	3.08 t 2H (J=7.5)	6.81 s 1H	7.19 s 1H
8[c]	4.28 q 1H (J=6.5)	3.61 m 1H 3.37 m 1H	3.04 m 1H 2.89 dd 1H (J=17.5, 5.5)	6.75 s 1H	6.53 s 1H
10[c]	3.95 dd 1H (J=10, 4)	3.66 m 1H 3.46 m 1H	3.04 m 1H 2.93 dd 1H (J=18, 6.5)	6.79 s 1H	6.42 s 1H
11[b]	3.79 d 1H (J=8)	3.81 m 1H 3.45 m 1H	3.16 m 1H 3.07 m 1H	6.61 s 1H	7.00 s 1H
12[c]	3.93 dd 1H (J=9, 3.5)	3.67 m 1H 3.04 m 1H	3.46 m 1H 2.93 dd 1H (J=18, 7)	6.79 s 1H	6.46 s 1H
13[c]		4.20 t 2H (J=7.5)	3.25 t 2H (J=7.5)	6.88 s 1H	7.24 s 1H
14[b]		8.03 d 1H (J=6.8)	7.88 d 1H (J=6.8)	7.63 s 1H	7.37 s 1H
15[b]		8.05 d 1H (J=6.8)	7.90 d 1H (J=6.8)	7.69 s 1H	7.40 s 1H
16[b]		8.05 d 1H (J=6.5)	7.90 d 1H (J=6.5)	7.68 s 1H	7.89 s 1H
18[b]		8.03 d 1H (J=6.5)	7.86 d 1H (J=6.5)	7.65 s 1H	7.37 s 1H
20[c]		8.36 d 1H (J=6.5)	7.74 d 1H (J=6.5)	7.21 s 1H	7.38 s 1H
21[c]		8.65 d 1H (J=6.5)	7.84 d 1H (J=6.5)	7.27 s 1H	7.49 s 1H
22[c]		8.33 d 1H (J=6.5)	7.73 d 1H (J=6.5)	7.20 s 1H	7.38 s 1H
23[c]		8.44 d 1H (J=6.5)	7.90 d 1H (J=6.5)	7.25 s 1H	7.40 s 1H
24[c]		8.57 d 1H (J=6.5)	7.92 d 1H (J=6.5)	7.25 s 1H	7.37 s 1H
25[b]		8.30 d 1H (J=6.8)	8.00 d 1H (J=6.8)	7.69 s 1H	7.88 s 1H
26[b]		8.27 d 1H (J=6.8)	7.80 d 1H (J=6.8)	7.69 s 1H	7.77 s 1H
27[b]		8.17 d 1H (J=6.8)	7.85 d 1H (J=6.8)	7.72 s 1H	7.34 s 1H
28[b]		8.15 d 1H (J=7.3)	7.86 d 1H (J=7.3)	7.71 s 1H	7.36 s 1H
29[b]	8.73 d 1H (J=3.5)	3.91 t 2H (J=7.5)	3.16 t 2H (J=7.5)	7.10 s 1H	7.38 d 1 H (J=3.5)

(*continued*)

Table 1 (*continued*)

Compd	6,7-*O*-Alkyl substituent	C-1 Substituent	N-2 Substituent
2[b]		1.11 t 3H ($J=7.5$); 1.92 dq 1H ($J=8$, 7.5); 2.11 qd 1H ($J=7.5$, 4.5)	
3[b]		1.05 t 3H ($J=7.5$); 1.53 m 2H; 1.86 m 1H; 2.01 m 1H	
4[b]		0.92 and 1.45 d each 3H ($J=7$); 2.45 m 1H	
5[b]		0.95 t 3H ($J=7$); 1.40 m 4H; 1.50 m 2H; 1.86 and 2.10 m each 1H	
6[c]	1.05[e] and 1.06[e] t 3H ($J=7.5$); 1.84[d] m 4H; 3.93 m 4H	0.93[e] t 3H ($J=7$); 1.48 and 1.69 m each 1H; 2.17 m 2H	0.96 t 3H ($J=7$); 1.84[d] m 2H; 2.97[d] m 2H
7[c]	1.06[e] and 1.07[e] t 3H ($J=7.5$); 1.90 m 4H; 3.97 and 3.98 t 2H ($J=6.5$)	1.08[e] t 3H ($J=7.5$); 1.73 m 2H; 3.04 m 2H	1.12[e] t 3H ($J=7.5$); 1.85[e] m 2H; 4.09 t 2H ($J=6.5$)
8[c]	5.14 and 5.16 s each 2H; 7.3–7.5[d] m 10H	1.57 d 3H ($J=6.5$)	4.27 and 4.22 d each 1H ($J=12.5$); 7.3–7.5[d] m
10[c]	5.15 and 5.22 d each 1H ($J=13$); 5.19 s 2H 7.3–7.5[d] m 10H	0.75 t 3H ($J=7$); 1.02 and 1.17 and 1.52 and 2.01 m each 1H	4.23 and 4.14 d each 1H ($J=13$); 7.3–7.5[d] m
11[b]	5.14 and 5.16 d each 1H ($J=12.5$); 5.18 s 2H; 7.26 –7.41[d] m 10H	0.63 and 0.93 d each 3H ($J=7$); 2.03 m 1H	4.40 and 4.13 d each 1H ($J=13$); 7.44–7.52 m 5H
12[c]	5.15 and 5.19 d each 1H ($J=12.5$); 5.18 s 2H; 7.3–7.5[d] m 10H	0.80 t 3H ($J=7$); 1.04–1.28 m 6H; 1.55 and 2.07 m each 1H	4.23 and 4.13 d each 1H ($J=13$); 7.3–7.5[d] m 5H
13[c]	5.27 and 5.31 s each 2H; 7.3–7.5[d] m 10H	0.96 t 3H ($J=7$); 1.54 m 2H; 3.02 s 3H	5.45 s 2H; 7.3–7.5[d] m 5H
14[b]		3.02 s 3H	
15[b]		1.49 t 3H ($J=7.5$); 3.40 q 2H ($J=7.5$)	
16[b]		1.03 t 3H ($J=7.5$); 1.91 m 2H; 3.35 t 2H ($J=7.5$)	
18[b]		0.94 t 3H ($J=7$); 1.43 m 4H; 1.85 m 2H; 3.33 m 2H	
20[c]	1.13 and 1.14 t each 3H ($J=7.5$); 1.98[d] m 4H; 4.16 and 4.20 t each 2H ($J=6.5$)	1.06 t 3H ($J=7.5$); 1.98[d] m 2H; 3.47 t 2H ($J=7.5$)	
21[c]	1.13 and 1.14 t each 3H ($J=7.5$); 1.99 m 4H; 4.17 and 4.22 t each 2H ($J=6.5$)	1.78 d 6H ($J=7$); 4.07 m 1H	
12[c]	1.13 and 1.14 t each 3H ($J=7.5$); 1.98 and 1.99 m each 2H; 4.14 and 4.20 t each 2H ($J=6.5$)	0.93 t 3H ($J=7.5$); 1.18 and 1.37 and 1.43 m each 2H; 3.46 t 2H ($J=8$)	
23[c]	1.12 and 1.13 t each 3H ($J=7.5$); 1.98 m 4H; 4.16 and 4.21 t each 2H ($J=6.5$)	1.48 t 3H ($J=7.5$); 3.51 q 2H ($J=7.5$)	4.46 s 3H
24[c]	1.12 and 1.14 t each 3H ($J=7.5$); 1.98 m 4H; 4.15 and 4.21 t each 2H ($J=6.5$)	1.21 t 3H; 1.84 m 2 H; 3.43 t 2H ($J=8$)	4.48 s 3H
25[b]	5.43 s 4H; 7.32–7.42 m 6H; 7.52–7.56 m 4H	3.10 s 3H	4.33 s 3H
26[b]	5.44 and 5.47 s each 2H; 7.31–7.43 m 6H; 7.52–7.56 m 4H	1.33 t 3H ($J=7.5$); 3.54 q 2H ($J=7.5$)	4.36 s 3H
27[b]		3.05 s 3H	4.30 s 3H
28[b]		1.44 t 3H ($J=7.5$); 3.49 q 2H ($J=7.5$)	4.34 s 3H
29[b]	3.89 and 3.99 s each 3H		

[a]Coupling constants (Hz in parentheses).
[b]The solvent was CD_3OD.
[c]The solvent was $CDCl_3$.
[d]Overlap with other protons.
[e]Assignments may be interchanged.

Table 2. [1]H NMR[a] Data of benzylisoquinolines **31**–**50**

Compd	2-Me	3-H or 1-H or 3-Me	4-H	5-H or 7-H	8-H	9-H	2′-H	5′-H	6′-H,–CH_2OH or–COMe
31	8.43 d 1H (J=5.5)	7.41 d 1H (J=5.5)	7.03 s 1H (J=5.5)	7.36 s 1H	4.84 q 1H	6.81 d 1H (J=6.5)	6.76 d 1H (J=3.0)	6.86 dd 1H (J=8.0)	(J=8.0, 3.0)
32	8.45 d 1H (J=5.5)	7.50 d 1H (J=5.5)	7.09[b] s 1H (J=5.5)	7.25[b] s 1H			6.97 brs1H		6.76 brs 2H
33		8.20 d 1H (J=5.5)	7.37 d 1H (J=5.5)	7.09 s 1H	7.67 s 1H	4.57 s 2H	6.76 s 1H	6.94 s 1H	4.74[e] s 2H
34		2.64[c] s 3H	7.26 s 1H	6.95 s 1H	7.55 s 1H	4.94 s 2H	6.76 s1H	7.22 s 1H	2.65[f] s 3H
35		8.36 d 1H (J=6.0)	7.42 d 1H (J=6.0)	7.05 s 1H	7.31 s 1H	4.50 s 2H	6.71 d 1H (J=1.5)	6.71 d 1H (J=8.0)	6.77 dd 1H (J=8.0, 1.5)
36		8.28 d 1H (J=7.0)	8.22 d 1H (J=7.0)	7.70[g] d 1H (J=9.5)	8.42 d 1H (J=9.5)	4.98 s 2H	6.73 brd 1H (J=1.0)	6.72 d 1H (J=8.0)	6.95 dd 1H (J=8.0, 1.0)
37		8.27 d 1H (J=6.0)	7.75 d 1H (J=6.0)	7.18 s 1H	7.50 s 1H	4.89 s 2H	7.11 d 1H (J=2.0)	6.76 d 1H (J=8.0)	6.83 dd 1H (J=8.0, 2.0)
38		2.65[c] s 3H	7.23 s 1H	6.93 s 1H	7.26 s 1H	4.47 s 2H	6.81 d 1H (J=2.0)	6.74 d 1H (J=8.5)	6.72 dd 1H (J=8.5, 2.0)
39		9.25[J] s 1H	7.48[b] s 1H	7.10 s 1H	7.46[b] s 1H	4.52 s 2H	7.08 d 1H (J=2.0)	6.86 d 1H (J=8.0)	6.94 dd 1H (J=8.0, 2.0)
40	4.65 s 3H	9.05 d 1H (J=7.0)	8.19 d 1H (J=7.0)	7.50[b] s 1H	7.53[b] s 1H	5.07 s 2H	6.88 d 1H (J=2.0)	6.71 d 1H (J=8.5)	6.27 dd 1H (J=8.5, 2.0)
41	4.64 s 3H	9.23 d 1H (J=7.0)	8.21d 1H (J=7.0)	7.44[b] s 1H	7.49[b] s 1H	5.36 s 2H	6.46 s 1H	7.37[b] s 1H	2.58[f] s 3H
42	4.84 s 3H	9.45 d 1H (J=7.0)	8.20 d 1H (J=7.0)	7.41 s 1H	7.28 s 1H	5.38 q 1H (J=7.5)	6.66 d 1H (J=1.5)	6.87 d 1H (J=8.5)	6.81 dd 1H (J=8.5, 1.5)
43	4.42 s 3H	9.53 d 1H	8.45 d 1H (J=7.0)	7.68 s 1H (J=7.0)	7.30 s 1H		7.00 d 1H (J=2.0)	6.75 d 1H (J=8.5)	6.31 dd 1H (J=8.5, 2.0)
44	4.50 s 3H	8.62 d 1H	8.11 d 1H	7.57[b] s 1H (J=7.0)	7.46[b] s 1H (J=7.0)	4.85 s 2H	6.97 s1H	5.72 s 1H	5.24[e] s 2H
45	4.39 s 3H	3.03[c] s 3H	8.16 s 1H	7.46 s 1H	7.33 s 1H	5.42 s 2H	6.64 s1H	7.35 s 1H	2.56[f] s 3H
46	4.62 s 3H	9.05 d 1H (J=6.5)	8.18 d 1H (J=6.5)	7.51[b] s 1H	7.48[b] s 1H	5.02 s 2H	6.53 d 1H (J=2.0)	6.71 d 1H (J=8.0)	6.46 dd 1H (J=8.0, 2.0)
47	4.67 s 3H	8.85 d 1H (J=7.0)	8.33 d 1H (J=7.0)	7.71[g] d 1H (J=9.0)	8.32 d 1H (J=9.0)	5.18 s 2H	6.93 brd 1H (J=2.0)	6.68 d 1H (J=8.5)	6.22 dd 1H (J=8.5, 2.0)
48	4.61 s 3H	9.00 d 1H (J=7.0)	8.09 d 1H (J=7.0)	7.39[b] s 1H	7.44[b] s 1H	4.96 s 2H	6.78 d 1H (J=2.0)	6.72 d 1H (J=8.5)	6.29 dd 1H (J=8.5, 2.0)
49	4.39 s 3H	2.86[c] s 3H	7.91 s 1H	7.28 s 1H	7.41 s 1H	5.13 s 2H	6.90 d 1H (J=2.0)	6.72 d 1H (J=8.5)	6.33 dd 1H (J=8.5, 2.0)
50	4.61 s 3H	10.92[J] s 1H	7.50 s 1H	7.09s 1H	8.00 s 1H	4.38 s 2H	6.79 d 1H (J=2.0)	6.88 d 1H (J=8.5)	6.70 dd 1H (J=8.5, 2.0)

(*continued*)

Table 2 (*continued*)

Compd	–OMe or–OEt at			OMe or OEt or OCH_2O at			Me or=CH_2 at
	C-6	and	C-7 or C-5	C-3′	and	C-4′	C-9
31	3.99 s 3H		3.76 s 3H	3.81 s 3H		3.88 s 3H	1.82[c] d 3H ($J=6.5$)
32	4.03 s 3H		3.85 s 3H	3.78 s 3H		3.80 s 3H	5.99[d] s 1H 5.50[d] s 1H
33	4.05 s 3H		4.11 s 3H	3.73 s 3H		3.87 s 3H	
34	3.98 s 3H		3.88 s 3H	3.64 s 3H		3.89 s 3H	
35	4.00 s 3H		3.91 s 3H		5.88 s 2H		
36	4.13 s 3H		4.03[h] s 3H	3.90 s 3H		3.78 s 3H	
37	4.30 q[i] 2H,1.57 t[i] 3H		4.16 q[i] 2H, 1.51 t[i]3H	4.01 q[i] 2H, 1.39 t[i] 3H		4.06 q[i] 2H, 1.43 t[i] 3H	
38	4.19 q[i] 2H,1.52 t[i] 3H		4.04 q[i] 2H, 1.42 t[i]3H	3.97 q[i] 2H, 1.36 t[i] 3H		4.01 q[i] 2H, 1.39 t[i] 3H	
39	4.08 s 3H		4.08 s 3H	3.88 s 3H		3.90 s 3H	
40	4.15 s 3H		4.01 s 3H	3.82[b] s 3H		3.84[b] s 3H	
41	4.13 s 3H		3.84 s 3H	3.70 s 3H		3.94 s 3H	
42	4.10 s 3H		3.65 s 3H	3.77 s 3H		3.87 s 3H	1.20[c] d 3H ($J=7.5$)
43	4.19 s 3H		3.91 s 3H	3.88 s 3H		3.90 s 3H	6.46[d] s 1H 5.61[d] s 1H
44	4.12 s 3H		3.96 s 3H	3.41 s 3H		3.85 s 3H	
45	4.13 s 3H		3.83 s 3H	3.77 s 3H		3.94 s 3H	
46	4.15 s 3H		4.01 s 3H		5.93 s 2H		
47	4.15 s 3H		4.07[h] s 3H	3.85 s 3H		3.81 s 3H	
48	4.36 q[i] 2H,1.60 t[i] 3H		4.17 q[i] 2H, 1.51 t[i]3H	4.02 q[i] 2H, 1.41 t[i] 3H		4.01 q[i] 2H, 1.42 t[i] 3H	
49	4.36 q[i] 2H,1.59 t[i] 3H		4.17 q[i] 2H, 1.51 t[i]3H	4.04 q[i] 2H, 1.41 t[i] 3H		4.02 q[i] 2H, 1.42 t[i] 3H	
50	4.08[b] s 3H		4.09[b] s 3H	3.87 s 3H		3.91 s 3H	

[a]Coupling constants (Hz in parentheses), the solvent was $CDCl_3$.
[b]Assignments may be interchanged.
[c]Me.
[d]=CH_2.
[e]CH_2OH.
[f]COMe.
[g]7-H.
[h]C-5.
[i]Coupling constant ($J=7.0$ Hz)1-H.

introducing a substituent such as acetyl or hydroxymethyl into the C-6′ position decreased activity (compare **41** or **44** with **40**).

Cytotoxicity evaluation

Fourteen simple isoquinolines (**1**–**5**, **13**–**16**, **23**, **25**, **27**–**29**) and 21 benzylisoquinolines (**30**–**50**) were assayed for in vitro cytotoxicity against six human tumor cell lines, including lung carcinoma (A-549), ileocecal carcinoma (HCT-8), ovarian cancer (1A9), breast cancer (MCF-7), glioblastoma (U-87 MG), and epidermoid carcinoma (KB). The cytotoxicity data are given as an ED_{50} value for each cell line, the concentration of compound that causes a 50% reduction in adsorbance at 562 nm relative to untreated cells using the SRB assay, and are shown in Table 6.

All tetrahydroisoquinolines (**1**–**5**) tested were inactive in all cell lines. 6,7-Dihydroxyisoquinolines and 6,7-dihydroxyisoquinolium salts were either inactive (**16** and **28**) or showed marginal activity only in the HCT-8 cell line (**14**, **15**, **27**).

6,7-Dimethoxydihydroisoquinolium chloride (**29**) was inactive, while 1-ethyl-6,7-dipropyloxy-*N*-methylisoquinolinium chloride (**23**) was active against KB and IA9 cells. The 6,7,*N*-tribenzylated dihydroisoquinolinium salt (**13**) was active against five cell lines and had an ED_{50} of 4.4 μg/mL in U-87 MG cells. 6,7-Dibenzyloxy-1-methyl-*N*-methyl isoquinolinium chloride **25** showed broad spectrum activity against all six cell lines tested, with ED_{50} values < 1.0 μg/mL in five cell lines and > 1 μg/mL in HCT-8 cells. Generally, the trends found above in the antimicrobial and antimalarial assays were also present in the cytotoxicity assays. *N*-alkylation to give a dihydroisoquinolium- or isoquinolium-type salt combined with *O*-alkylation of the 6,7-hydroxy groups led to the most potent compounds. Benzylation of the C-6 and C-7 hydroxy groups significantly increased cytotoxicity (compare **25** with **27**), whereas propylation had a weaker effect (compare **23** with **28**).

Table 3. Physical, mass spectral, and HPLC analysis data of isoquinolines and benzylisoquinolines

Compd	Mp (°C) (dec)	Recryst solvent	Yield	Formula	LSIMS *m/z* $[M-Cl]^+$ $[M-CF3COO]+$	HR-LSIMS		HPLC (C_R, min)[a]	
						Calcd	Found	I[b,c,d]	II[b,c,d]
2	213–219	MeOH	92	$C_{11}H_{16}NO_2$	194	194.1199	194.1180	6.8[b]	3.5[b]
3	223–233	MeOH	85	$C_{12}H_{18}NO_2$	208	208.1343	208.1337	13.5[b]	7.1[b]
4	215–225	MeOH	90	$C_{12}H_{18}NO_2$	208	208.1337	208.1337	11.5[b]	5.9[b]
5	164–170	Et_2O	41	$C_{14}H_{22}NO_2$[e]	236	236.1641	236.1649	36.4[b]	26.9[b]
6	Amorph		69	$C_{21}H_{35}NO_2$[f]	334	333.2627	333.2666	23.1[c]	17.4[c]
7	Amorph		17	$C_{21}H_{34}NO_2$[e]	332	332.2603	332.2588	21.3[c]	16.5[c]
8	Amorph		95	$C_{31}H_{32}NO_2$[e]	450	450.2454	450.2431	29.7[c]	19.9[c]
10	Amorph		85	$C_{33}H_{36}NO_2$[e]	478	478.2731	478.2744	32.5[c]	20.8[c]
11	Amorph		70	$C_{33}H_{36}NO_2$[e]	478	478.2756	478.2744	26.2[c]	20.1[c]
12	Amorph		38	$C_{35}H_{40}NO_2$[e]	506	506.3056	506.3057	34.0[c]	22.8[c]
13	179–181	MeOH–C_6H_6	41	$C_{33}H_{34}NO_2$[e]	476	476.2590	476.2587	23.2[c]	20.2[c]
14	268–284	MeOH–$(CH_3)_2CO$	59	$C_{10}H_{10}NO_2$	176	176.0732	176.0711	12.3[b]	7.2[b]
15	206–217	MeOH–$(CH_3)_2CO$	45[f]	$C_{11}H_{12}NO_2$	190	190.0880	190.0867	18.9[b]	11.8[b]
16	194–206	MeOH–$(CH_3)_2CO$	33	$C_{12}H_{14}NO_2$	204	204.1024	204.1024	29.0[b]	19.8[b]
18	Amorph		10	$C_{14}H_{18}NO_2$[e]	232	232.1324	232.1337	25.6[c]	10.6[c]
20	101–102	Et_2O–$(CH_3)_2CO$	58	$C_{18}H_{26}NO_2$[e]	288	288.1985	288.1962	27.2[c]	16.8[c]
21	127–128	MeOH	48	$C_{18}H_{26}NO_2$[e]	288	288.1976	288.1962	14.4[c]	16.2[c]
22	Amorph		40	$C_{20}H_{30}NO_2$[e]	316	316.2298	316.2275	30.0[c]	19.7[c]
23	73–76	EtOH	54[g]	$C_{18}H_{26}NO_2$[e]	288	288.1960	288.1962	17.3[c]	14.6[c]
24	85–87	Et_2O–$(CH_3)_2CO$	29	$C_{19}H_{28}NO_2$[e]	302	302.2143	302.2118	18.7[c]	16.0[c]
25	220–229	MeOH–$(CH_3)_2CO$	88	$C_{25}H_{24}NO_2$[e]	370	370.1808	370.1805	20.2[c]	16.9[c]
26	187–200	MeOH–$(CH_3)_2CO$	22[h]	$C_{26}H_{26}NO_2$[e]	384	384.1990	384.1962	20.7[c]	17.7[c]
27	254–264	$(CH_3)_2CO$	85	$C_{11}H_{12}NO_2$	190	190.0879	190.0867	13.7[b]	9.2[b]
28	196–211	$(CH_3)_2CO$	78	$C_{12}H_{14}NO_2$	204	204.1039	204.1023	20.1[b]	14.4[b]
29	200–201			$C_{11}H_{14}NO_2$	192	192.0974	192.0999	21.9[b]	12.7[b]
42	149–155	EtOAc–$(CH_3)_2CO$	85	$C_{22}H_{26}NO_4$	368	368.1868	368.1860	10.0[d]	8.5[d]
43	86–100	MeOH–$(CH_3)_2CO$	99	$C_{22}H_{24}NO_4$	366	366.1717	366.1703	11.1[d]	9.6[d]
44	169–177	$(CH_3)_2CO$	70	$C_{22}H_{26}NO_5$	384	384.1815	384.1809	8.4[d]	7.6[d]
45	193–198	MeOH–$(CH_3)_2CO$	93	$C_{24}H_{28}NO_5$	410	410.1975	410.1965	11.0[d]	9.5[d]
46	184–190	MeOH–$(CH_3)_2CO$	98	$C_{20}H_{20}NO_4$	338	338.1375	338.1391	11.4[d]	9.8[d]
47	114–119	$(CH_3)_2CO$	80	$C_{21}H_{24}NO_4$	354	354.1733	354.1704	10.5[d]	8.8[d]
48	205–210	EtOAc	76	$C_{25}H_{32}NO_4$	410	410.2360	410.2330	16.9[d]	14.8[d]
49	204–212	MeOH–$(CH_3)_2CO$	55	$C_{26}H_{34}NO_4$	424	424.2489	424.2485	17.9[d]	15.2[d]
50	144–146	MeOH–$(CH_3)_2CO$	70	$C_{21}H_{24}NO_4$	354	354.1710	354.1704	10.9[d]	9.3[d]

[a]I: 0.1 M NH_4OAc/MeOH (0.05% TFA) A/B, II; H_2O/MeOH (0.05%TFA) A/B.
[b]Initial A/B 95/5, 30 min 80/20.
[c]Initial A/B 80/20, 30 min 0/100.
[d]Initial A/B 75/25, 10 min 50/50, 20 min 80/20.
[e]When the compound was purified by preparative HPLC, the anion was CF_3COO^-.
[f]Free base.
[g]Yield from **2**.
[h]Yield from **15**.

Table 4. Antibacterial and antifungal activities of 1-alkyl substituted isoquinolines and 1- or 3-benzylisoquinolines

Compd	MIC (μg/mL)[a]				
	S. aureus	*B. subtilis*	*S. enteritidis*	*E. coli* (IFO 026)	*C. albicans* (IFO 1061)
1–5	> 500	> 500	′00	> 500	> 500
6	> 500	> 500	> 500	> 500	> 500
7	250	250	250	> 500	> 500
8, **10–11**	> 500	> 500	> 500	> 500	> 500
13	3.9	3.9	3.9	15.6	3.9
14–16, **18**	> 500	> 500	> 500	> 500	> 500
20–22	> 500	> 500	> 500	> 500	> 500
23	250	500	250	> 500	> 500
24	250	250	125	> 500	> 500
25	31.3	62.5	31.3	250	250
26	15.6	31.3	15.6	125	250
27, **28**	> 500	> 500	> 500	> 500	> 500
30–39	> 500	> 500	> 500	> 500	> 500
40–47, **50**	> 500	> 500	> 500	> 500	> 500
48	125	31.3	125	> 500	> 500
49	125	31.3	62.5	> 500	> 500
KA[b]	31.3	3.9	62.5	31.3	> 2000

[a]This value was defined as the lowest concentration of the test substance that did not induce growth in comparison with a blank experiment.

Table 5. In vitro antimalarial activity of 1-alkyl substituted isoquinolines and 1- or 3-benzylisoquinolines

Compd	50% Inhibitory concentration (mol)[a]		Selectivity index[b] IC_{50}/EC_{50}
	Plasmodium falciparum FCR-3 EC_{50} (growth%)	Mouse mammary cells FM3A IC_{50} (growth%)	
1	(81.5)	> 5.2E-5 (96.2)	
2	(69.2)	> 4.8E-05 (88.9)	
3	(76.7)	> 8.2E-05 (88.2)	
4	(100)	< 8.7E-05 (81.6)	
5	(81.5)	> 3.8E-05 (79.8)	> 4
7	8.2E-06	3.5E-06	0.4
13	4.0E-07	3.0E-07	1
14	1.3E-05	1.7E-05	1.3
15	1.7E-06	1.6E-05	0.9
16	1.8E-05	1.2E-05	0.7
23	4.6E-07	2.1E-06	5
24	4.6E-07	2.4E-06	5
25	3.0E-07	> 1.3E-05 (74)	> 43
26	1.7E-07	5.0E-06	29
27	7.7E-06	7.2E-06	0.9
28	5.9E-06	5.0E-06	0.8
30	8.5E-06	1.0E-05	1.2
40	5.8E-06	> 1.0E-05 (100)	> 2
41	8.2E-06	> 1.0E-05 (100)	> 1
42	3.1E-07	> 1.0E-05 (100)	> 32
43	4.0E-06	> 1.0E-05 (96)	> 3
44	9.2E-06	> 1.0E-05 (100)	> 1
45	8.3E-06	> 1.0E-05 (100)	> 1
46	4.10E-06	> 1.0E-05 (100)	> 2
47	5.0E-06	> 1.0E-05 (100)	> 2
48	3.6E-06	> 1.0E-05 (100)	> 3
49	5.8E-07	> 1.0E-05 (100)	> 17
50	4.0E-06	> 1.0E-05 (100)	> 3
Quinine	1.1E-07	> 1.0E-04	910

[a]The 50% inhibitory concentration was defined by comparison with drug-free controls incubated under same conditions.
[b]In vitro selectivity index was estimated from the ratio (IC_{50}/EC_{50}) of the drug concentrations necessary to inhibit the growth rate of cells to 50% of the growth value between the malaria parasites and mouse mammary FM3A cells which served as a model host.

Table 6. In vitro cytotoxicity of simple isoquinolines and 1- or 3-benzylisoquinolines against various human tumor Cell lines[a]

Compd	ED_{50} (μg/mL)[b]					
	KB	A-549	HCT-8	1A9	MCF-7	U-87 MG
1–	>4	>4	>4	>4	>4	> 4
13	1.1	2.7	> 1 (45)	0.5	1.8	4.4
14	5.3	10.0	3.8	4.2	10.0	>20 (46)
15	9.0	8.5	4.2	4.8	10.0	>20 (42)
16	>4	>4	>4	>4	>4	>4
23	3.1	6.5	16.0	0.8	10.0	18.5
25	0.4	0.3	>1 (45)	0.1	1.0	0.5
27	8.3	18.5	3.4	8.9	10.0	> 20 (47)
28, **29**	>4	>4	>4	>4	>4	>4
30–**39**	>4	>4	>4	>4	>4	>4
40–**50**	>4	>4	>4	>4	>4	>4
Colchicine	0.002	0.002	0.016	—	>0.4	—

[a]KB: epidermoid carcinoma of the nasopharynx, A-594: lung carcinoma; HCT-8: ileocecal carcinoma; 1A9: ovarian cancer; MCF-7: breast cancer; U-87 MG: glioblastoma.
[b]Cytotoxicity as ED_{50} for each cell line, the concentration of compound that causes a 50% reduction in adsorbance at 562 nm relative to untreated cells using the SRB assay.[27] Pure compound is considered to be significantly active when its ED_{50} < 4.0 μg/mL. Percent inhibition of compound with less than 50% inhibition at the highest concentration tested is given as the bracketed value.

The human tumor cells HCT-8 or IA9 appear to be the most sensitive in detecting active compounds of the simple isoquinolines among the six cell lines tested in this screening study. Human KB cells also are fairly sensitive.

All tested benzylisoquinolines (**30**–**39**) and their *N*-methylated quaternary salts (**40**–**50**) displayed low cytotoxicity (ED_{50} >4.00 μg/mL).

Anti-HIV activity

Fourteen simple isoquinolines (**1**–**5**, **13**–**16**, **23**, **25**, **27**–**29**) and 17 benzylisoquinolines (**31**–**33, 35**–**39, 42**–**50**) were tested against HIV-1 replication in H9 lymphocytes. The data are shown in Table 7. Compounds **1**, **28**, and **29** were most potent with ED_{50} values of 0.117 μg/mL (**1**) and <0.10 μg/mL (**28**, **29**). Among the 6,7-dihydroxy-tetrahydroisoquinolines, anti-HIV activity, but not cytotoxicity, decreased as the C-1 alkyl chain lengthened. The potent 1-methyl derivative (**1**) had an ED_{50} value of 0.117 μg/mL and an IC_{50} value of 21.2 μg/mL giving a calculated therapeutic index (TI) [defined as toxicity (IC_{50}) divided by anti-HIV activity (EC_{50})] of 181, while the 1-pentyl derivative (**5**) was inactive. The 6,7-dihydroxy isoquinolines (**14**–**16**) also were inactive, as was the *O*,*N*-tribenzyl dihydroisoquinolinium salt **13**. Isoquinolinium salts bearing an ethyl group at C-1 (**23** and **28**) were active with EC_{50} values of 4.98 and <0.10 μg/mL, respectively. They inhibited uninfected H9 cell growth with IC_{50} values of 5.40 and 2.07 μg/mL, respectively, giving TI values of 1.08 and 20.7, respectively. However, interestingly, similar isoquinolinium salts having a methyl group at C-1 (**25** and **27**) showed no anti-HIV activity. Among all tested compounds, **28** and **29** were most potent with an EC_{50} of < 0.10 μg/mL. The latter compound was most selective with an IC_{50} of 23.6 μg/mL and a TI of 236. These results suggested that the bulkiness of the chain at C-1 modulated the activity. Interestingly, results in the anti-HIV assay did not parallel those in the antimicrobial, antimalarial, and cytotoxicity assays. The most potent compounds (**13**, **25**) in the latter assays were inactive against HIV replication.

Table 7. Anti-HIV activity of simple isoquinolines and 1- or 3-benzylisoquinolines

Compd	EC_{50} (μg/mL)[a]	IC_{50} (μg/mL)[b]	TI (IC_{50}/EC_{50})[c]
1	0.117	21.2	181
2	1.52	20.9	13.7
3	2.88	21.8	7.57
4	3.21	22.8	7.11
5	NS[d]	21.6	—
13[e,f]	NS	0.197	—
14[e,f]	NS	2.22	—
15	NS	2.08	—
16	NS	2.23	—
23	4.98	5.40	1.08
25[e]	NS	0.171	—
27[e]	NS	2.15	—
28	<0.10	2.07	20.7
29	<0.10	23.6	236
31	NS	11.6	—
32[e]	NS	1.85	—
33	NS	18.8	—
35	NS	21.6	—
36	NS	10.2	—
37[e]	8.04	8.48	1.05
38[e,f]	82.8	>100[g]	> 1.21
39	NS	10.1	—
42	NS	>100[g]	—
43	NS	17.4	—
44	NS	>100[g]	—
45[e]	NS	20.6	—
46	NS	22.4	—
47	NS	>100[g]	—
48[e]	NS	17.1	—
49[e]	NS	13.0	—
50	NS	>100[g]	—
AZT[h]	0.013	500	38,462

[a]The agent concentration that inhibited viral replication in H9 cell by 50%.
[b]The agent concentration that inhibited H9 cell growth by 50%.
[c]In vitro therapeutic index (TI) ratio: $IC_{50}/EC50$.
[d]NS = no suppression.
[e]Crystals were seen at 100 μg/mL concentration by light microscopy for these agents.
[f]Crystals were also seen at 10 μg/mL concentration by light microscopy for these agents.
[g]Because all agents were dissolved in DMSO and their stock concentrations were 10 mg/mL, a minimum of a 1:100 dilution was needed to negate the effects of DMSO on this assay; thus, the greatest agent concentration possible was 100 μg/mL.
[h]Azidothymidine.

Among the 17 tertiary and quaternary benzylisoquinolines, only tertiary benzylisoquinolines **37** and **38** showed weak anti-HIV activity.

Conclusions

Regarding SARs, some common features were found among antimicrobial, antimalarial, and antitumor activities of the simple isoquinolines. A quaternary nitrogen atom of isoquinolinium or dihydroisoquinolinium type

contributed to increased potency in all three types of activities. Benzylation of the C-6 and C-7 hydroxy groups also enhanced activity, while propylation increased activity to a lesser extent. In contrast, anti-HIV activity was found with tetrahydroisoquinolines and 6,7-dihydroxy-isoquinolinium salts.

With these present studies, we have identified new biologically active compounds of the isoquinoline type. The compounds to be considered as lead structures for developing potential chemotherapeutic agents include **13** (antimicrobial), **25**, **26**, and **42** (antimalarial), **13** and **25** (cytotoxic), and **28** and **29** (anti-HIV). Further experiments are under development to explore the cellular processes targeted by these compounds and to better understand the differences between SAR of the anti-HIV and antimicrobial/antimalarial/cytotoxic activities.

Experimental

General methods

Melting points were determined on a Yanako Micromelting Point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian VXR-500 (500 MHz) using TMS as an internal standard and CD_3OD or $CDCl_3$ as solvent. Mass spectra were determined on a Hitachi M-4100 instrument. The secondary ion mass spectra (LSIMS) were measured using glycerol as matrix. HPLC and preparative HPLC analyses were performed on a Hitachi M-6200 intelligent pump (1 mL/min) and Hitachi M-6250 intelligent pump (6 mL/min), respectively, with a Hitachi L-4000 UV detector (280 nm). HPLC analyses were also performed on a Jasco HPLC system, which employed an 880-PU intelligent pump, 875-UV intelligent UV/VIS detector, 807-IT integrator, 802-SC system controller, and 860-CO column oven (40 °C). Cosmosil 5C_{18}-AR reversed-phase column of analytical (4.6 i.d.×150 mm) and preparative (20 i.d.×250 mm) columns were used for HPLC. Analyses with a Hitachi HPLC system were made using two solvent systems, I: (A) 0.1 M NH_4OAc (0.05%TFA)/(B) MeOH (0.05% TFA); II (A) H_2O (0.05% TFA)/(B) MeOH (0.05% TFA), under the following gradient conditions: A/B, initial (75/25), 10 min (50/50), 20 min (20/80). Preparative HPLC analyses were performed using a solvent system II under the following gradient condition: A/B 50/50 to 0/100, 30 min. HPLC Analysis with A Jasco HPLC system were carried out using solvent system, I: (A) 0.1M NH_4OAc (0.05%TFA)/(B) MeOH (0.05% TFA); II (A) H_2O (0.05% TFA)/(B) MeOH (0.05% TFA), under the following gradient conditions: A/B 95/5 to 80/20, 30 min or 80/20 to 0/100, 30 min. Preparative TLC was carried out on silica gel plates (Merck, 60F-254). All final compounds were homogeneous by HPLC analysis; elemental analysis data are found in Table 8 for selected compounds.

Salsolinol (**1**) was prepared previously. Isoquinoline **29** was synthesized in our laboratory.[13] Commercially available papaverine **30** (Sigma) was *N*-methylated with CH_3I to give *N*-methylpapaverine **40**. Benzylisoquinolines, **31**,[14] **32**,[15] **33**,[16] **34**,[17] **35**,[18] **36**,[19] **37**,[20] **38**,[21] **39**,[22] and **41**[23] were prepared according to literature procedures.

General procedure for the preparations of 6,7-dihydroxy-1-alkyl-1,2,3,4-tetrahydroisoquinolines (2–5). To a solution (pH 4) of dopamine HCl (1 g) and a few drops of concentrated HCl in H_2O (5 mL) was added an appropriate aldehyde (1.2 equivalent weight). The mixture was allowed to stand at room temperature for 2–5 days then evaporated to dryness. The crystalline products except for **5** were recrystallized from MeOH to give **2**–**4**. Crude **5** was purified by preparative HPLC to give pure **5**. For ^{1}H NMR and physical, LSIMS, HRLSIMS, and HPLC data, see Tables 1 and 3.

1,2-Propyl-6,7-dipropyloxy-1,2,3,4-tetrahydroisoquinoline (6). To a solution of **3** (500 mg) in MeOH (10 mL) and EtOH (20 mL) was added K_2CO_3 (1.5 g) and the mixture was stirred at room temperature for 30 min. Propyl iodide (1 mL) was added to the mixture, and the solution was refluxed for 17 h. Water (20 mL) was added, and the reaction mixture was concentrated under reduced pressure. The aqueous solution was extracted with $CHCl_3$. The combined organic layers were dried (Na_2SO_4) and evaporated to afford the crude product (650 mg, 81% yield), which was purified by preparative HPLC to give **6** (550 mg) as oil. For ^{1}H NMR and Physical, LSIMS, HRLSIMS, and HPLC data, see Tables 1 and 3.

1,2-Propyl-6,7-dipropyloxy-3,4-dihydroisoquinolinium salt (7). Iodine (500 mg) in EtOH (15 mL) was added dropwise to a stirred solution of **6** (200 mg) and CH_3COOK (250 mg) of EtOH (20 mL) for 20 min. Water (20 mL) was added to the reaction mixture and it was concentrated and extracted with $CHCl_3$. The organic layer was washed with 5% sodium bisulphite, dried (Na_2SO_4), and evaporated in vacuo. The residue was purified by preparative HPLC to give **7** (34 mg) as oil. For ^{1}H NMR and physical LSIMS, HRLSIMS, and HPLC data, see Tables 1 and 3.

***O*,*N*-Tribenzyl-1-alkyl-1,2,3,4-tetrahydroisoquinolines (8–12).** To a solution of **1** (1 g, 5.6 mM) in MeOH (20

Table 8. Elemental analysis of isoquinolines and benzylisoquinolines

Compd	Formula	Calcd			Found		
		C	H	N	C	H	N
2	$C_{11}H_{16}ClNO_2$	57.37	6.88	6.05	57.52	7.02	6.10
3	$C_{12}H_{18}ClNO_2$	58.84	7.53	5.83	59.13	7.44	5.75
4	$C_{16}H_{18}ClNO_2$	58.91	7.50	5.66	59.13	7.44	5.75
5	$C_{16}H_{22}F_3NO_4$	54.65	6.22	4.02	55.01	6.35	4.01
15	$C_{11}H_{12}ClNO_2 \cdot 1.5H_2O$	52.18	5.51	5.51	52.28	5.38	5.54
16	$C_{12}H_{14}ClNO_2 \cdot 0.5H_2O$	58.01	5.78	5.71	57.88	6.07	5.63
27	$C_{11}H_{12}ClNO_2 \cdot H_2O$	54.14	5.70	5.69	54.21	5.79	5.75
29	$C_{11}H_{14}ClNO_2 \cdot 3H_2O$	47.00	6.94	4.87	46.89	7.15	4.97
43	$C_{22}H_{24}ClNO_4 \cdot 2H_2O$	60.22	6.32	3.09	60.33	6.44	3.20
45	$C_{24}H_{28}ClNO_5 \cdot 2H_2O$	59.82	6.55	2.81	59.80	6.69	2.91
47	$C_{21}H_{24}ClNO_4 \cdot 1.5H_2O$	60.91	6.05	3.40	60.50	6.04	3.38
48	$C_{25}H_{32}ClNO_4 \cdot 2.5H_2O$	61.44	7.13	2.85	61.78	7.15	2.88
49	$C_{26}H_{34}ClNO_4 \cdot H_2O$	65.65	7.43	3.02	65.60	7.41	2.94
50	$C_{21}H_{24}ClNO_4 \cdot 1.5H_2O$	60.54	6.13	3.37	60.50	6.16	3.36

mL) and EtOH (40 mL) was added K_2CO_3 (2.2g, 16 mM) and the mixture was stirred at room temperature for 30 min. To the mixture was added $C_6H_5CH_2Cl$ (2.0 g, 16 mM) and it was refluxed for 14 h. Water (40 mL) was added to the reaction mixture and it was concentrated under reduced pressure. The aqueous layer was extracted with $CHCl_3$. The combined organic layer was dried (Na_2SO_4) and concentrated to give the crude product (2.0 g, 97% yield) which was purified by preparative HPLC to give **8** (1.55 g). Compounds **9**–**12** were prepared from **2**–**5** by a similar procedure as for the synthesis of **8**. Compound **9** was used in subsequent reactions without purification. For ^1H NMR and Physical, LSIMS, HRLSIMS, and HPLC data, see Tables 1 and 3.

***O*,*N*-Tribenzyl-1-propyl-3,4-dihydroisoquinolinium salt (13).** Iodine (2.3 g) in EtOH (20 mL) was added dropwise to a stirred solution of **10** (2.3 g) and CH_3COOK (1 g) in EtOH (30 mL) for 30 min. Water (50 mL) was added to the reaction mixture then it was concentrated and extracted with $CHCl_3$. The organic layer was washed with 5% sodium bisulphite, dried (Na_2SO_4), and evaporated in vacuo. The crystalline product was recrystallized from MeOH–C_6H_6 to give **13** (1.117g). For ^1H NMR and Physical, LSIMS, HRLSIMS, and HPLC data, see Tables 1 and 3.

1-Alkyl-6,7-dihydroxyisoquinolines (14–18). Crude *O*,*N*-tribenzyl derivative **9** (1.84 g) was heated at reflux with 10% palladium on charcoal (500 mg) in tetraline (60 mL) for 30 min. After cooling, the mixture was filtered through a small pad of Celite, and the residue was washed with $CHCl_3$–MeOH. The combined filtrate was concentrated and extracted with 10% HCl several times. The aqueous layer was washed with Et_2O, filtered, and evaporated to dryness to give the crystalline product, which was recrystallized from MeOH–acetone to afford **15** (397 mg). Compounds **14**, **16**–**18** were prepared from **8** and **10**–**12** in a similar manner. Preparative HPLC was used for purification of **18**. For ^1H NMR and Physical, LSIMS, HRLSIMS, and HPLC data, see Tables 1 and 3.

1-Alkyl-6,7-dipropyloxyisoquinolines (19–22). To a solution of **17** (560 mg) in MeOH (10 mL) and EtOH (20 mL) was added K_2CO_3 (1.5 g) and the mixture was stirred at room temperature for 30 min. Propyl iodide (1 mL) was added to the mixture then it was refluxed for 17 h. After water (20 mL) was added, the reaction mixture was concentrated under reduced pressure. The aqueous solution was extracted with $CHCl_3$. The combined organic solution was dried (Na_2SO_4) and evaporated to afford the crude product, which was purified by preparative HPLC to give **21** (464 mg) as oil. Compounds **19**, **20**, and **22** were prepared by a similar procedure. For ^1H NMR and Physical, LSIMS, HRLSIMS, and HPLC data, see Tables 1 and 3.

1-Ethyl-6,7-dipropyloxy-*N*-methylisoquinolinium chloride (23). Crude 1-ethyl-6,7-dipropyloxyisoquinoline (**19**) prepared from **15** (967 mg) and 5 mL of CH_3I in MeOH (50 mL) were placed in a glass-stoppered bottle and heated for 15 h at 100–110 °C in a oil bath. The solvent was evaporated to dryness and the resulting crystals were recrystallized from EtOH to give the iodide (755 mg), which was converted to the chloride **23** by treatment with AgCl in MeOH. For ^1H NMR and Physical, LSIMS, HRLSIMS, and HPLC data, see Tables 1 and 3.

1-Propyl-6,7-dipropyloxy-*N*-methylisoquinolinium salt (24). This was prepared in a similar manner from **20**. For ^1H NMR and Physical, LSIMS, HRLSIMS, and HPLC data, see Tables 1 and 3.

1-Methyl-6,7-dibenzyloxy-*N*-methylisoquinolinium chloride (25). A solution of **14** (545 mg), K_2CO_3 (2g), and $C_6H_5CH_2Cl$ (1 mL) in MeOH (10 mL) and EtOH (50 mL) was refluxed for 5 h. Water was added to the mixture then it was concentrated. The aqueous solution was extracted with $CHCl_3$. The $CHCl_3$ solution was dried (Na_2SO_4) and evaporated to dryness. The residue was purified by a preparative TLC (benzene/Et_2O 4:1) to give 1-methyl-6,7-dibenzyloxyisoquinoline (320 mg, yield 32%), which was dissolved in acetone (10 mL). CH_3I (1 mL) was added to the solution, and the mixture was allowed to stand at room temperature for 1 h. The resulting iodide was filtered and recrystallized from MeOH–acetone to give the pure iodide (358 mg), which was converted by treatment with AgCl in MeOH to the chloride **25**. For ^1H NMR and Physical, LSIMS, HRLSIMS, and HPLC data, see Tables 1 and 3.

1-Ethyl-6,7-dibenzyloxy-*N*-methylisoquinolinium chloride (26). This was prepared in a similar manner from **15**. For ^1H NMR and Physical, LSIMS, HRLSIMS, and HPLC data, see Tables 1 and 3.

1-Methyl-6,7-dihydroxyisoquinolinium chloride (27). The chloride **25** (246 mg) was dissolved in 20% HCl (10 mL) and the mixture was refluxed for 5 h. The aqueous solution was washed with Et_2O and evaporated to dryness. The crystalline residue was recrystallized from acetone to give **27** (94 mg). For ^1H NMR and Physical, LSIMS, HRLSIMS, and HPLC data, see Tables 1 and 3.

1-Ethyl-6,7-dihydroxyisoquinolinium chloride (28). This was prepared in similar fashion from **26**. For ^1H NMR and Physical, LSIMS, HRLSIMS, and HPLC data, see Tables 1 and 3.

1- and 3-Benzyl-*N*-methylisoquinolinium chlorides (42–49 and 50) [*N*-Methylations of 1-benzylisoquinolines (31–38) and 3-benzylisoquinolines (39)]. To a solution of **31** (1 g) in $CHCl_3$ (2 mL) and MeOH (4 mL) was added CH_3I (2 mL). After standing for 3 days at room temperature, the mixture was evaporated to dryness. The resulting iodide was converted by treatment with AgCl in MeOH to the chloride, which was recrystallized from AcOEt–acetone to give 42 (877 mg). Compounds **32**–**39** were dissolved in MeOH–acetone or MeOH–$CHCl_3$ and *N*-methylated by a similar procedure as in the synthesis of **42** to yield **43**–**50**, respectively. For ^1H NMR and Physical, LSIMS, HRLSIMS, and HPLC data, see Tables 2 and 3.

Antimicrobial assay

Antibacterial activity against *S. aureus, B. subtilis,* and *S. enteritidis*, isolated from hospitalized patients, and *E. coli* (IFO 026) and antifungal activity against *C. albicans* (IFO 1061) were determined by means of the minimum inhibitory concentration (MIC) using the 2-fold serial broth dilution test in liquid nutrient medium and 24-well microplates. MIC was defined as the lowest concentration of the test substance that did not induce visible growth in comparison with a blank experiment. The compounds were dissolved in water–1% DMF. Dilutions with the test medium furnished concentrations from 1.0 to 500 μg/mL. Blanks were prepared in water–1% DMF only. Kanamycin sulfate was used as standard. Each well of 24-well plates contained an appropriate growth medium with a different concentration of the respective berberine derivatives. The 24-well plate was incubated at 37 °C for 24 h for bacteria and at 25 °C for 48 h for the fungus. Bacteria tested were preliminarily cultivated in 3% nutrient broth ('Nissui', Japan) at 37 °C, while *C. albicans* was cultivated in 3% malt extract powder ('Oriental', Japan) at 25 °C. All experiments were run in duplicate or triplicate. For measuring the growth of cells, a constant amount of sample (200 μL) was transferred to a 96-well test plate from individual wells (1 mL) of a 24-well plate. After incubation, the microbial growth was examined by measuring the optical density at 655 nm with a Model 450 Microplate Reader (Bio-Rad).

In vitro antimalarial screening

Parasites. In all studies described in this report, *P. falciparum* strain FCR-3 (ATCC 30932) was used.[24,25] Human serum and erythrocytes were obtained from healthy donors, stored at 4 °C and used within 10–14 days from donation. Parasites were cultured in 10% heat inactivated A^+ human erythrocytes and suspended at a 5% hematocrit in RPMI 1640 medium (Gibco, NY, USA) which contained 50 mg of gentamicin per liter and 10% group A^+ human serum and was buffered with 25 mM *N*-2-hydroxyethyl- piperazine-*N*′-2-ethansulfonic acid (HEPES, pH 7.4) and 25 mM $NaHCO_3$.[19,20] Cultures were maintained at 37 °C in a gas mixture of 5% oxygen–5% carbon dioxide–90% nitrogen.[26]

Drug testing. The following procedure was used for routine assay of antimalarial activity. Various concentrations of compounds, suspended in 10 μL of distilled water were added to individual wells of a 24-well plate. Erythrocytes with 0.3% parasitemia were added to each well in 990 μL of culture medium to give a final hematocrit of 3%. The plates were incubated at 37 °C for 72 h under 5% O_2–5% CO_2–90% N_2. Parasite morphology in drug-treated culture after 72 h was measured by staining with Giemsa, and the number of parasitized red blood cells per 10,000 erythrocytes was counted and growth rates were calculated. All compounds were run in duplicate at each concentration. Drug-free control cultures were run simultaneously. All data points represent means of at least two experimental tests. The 50% inhibitory concentration (ECI_{50}) is defined by comparison with drug-free controls incubated under the same conditions.[27,28]

Mammalian cells. A wild-type mouse FM3A cell line (subclone F-28-7) was supplied by the Health Sciences Research Resources Bank (Osaka, Japan). FM3A cells were maintained in suspension culture at 37 °C in a 5% CO_2 atmosphere in plastic bottles containing ES medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 2% heat-inactivated fetal bovine serum (Gibco, NY, USA).[29,30]

Toxicity to mammalian cells. Cell lines grew with a doubling time of about 12 h. Before being exposed to drugs, cells were seeded at a 990 μL of density of 5×10^4 cells/mL and various concentrations of compounds dispensed in 10 μL of distilled water were added to individual wells of a 24-well plate. The plates were incubated at 37 °C in a 5% CO_2 atmosphere for 48 h. Cell numbers were measured using a blood cell counter CC-108 (Toa Medical Electric Co., Japan). All data points represent means of at least two experimental tests. The 50% inhibitory concentration (IC_{50}) is defined by comparison with that of drug-free controls incubated under the same conditions. Cell growth inhibition is the index of cytotoxicity including cytostatic activity of the test compounds.

Selective toxicity. The selective toxicity was estimated from the ratio (IC_{50}/EC_{50}) of the drug concentrations necessary to inhibit the growth rate of cells to 50% of the growth value between the malaria parasites and mouse mammary FM3A cells, which served as a model host.[31]

In vitro cytotoxicity assay. Cytotoxicity was evaluated using standard HTCL assay. The assay was carried out according to standard SRB assay procedure described in Rubinstein et al.[32] Samples were prescreened first against KB at, 40, 4, and 0.4 μg/mL for a two day exposure period. Active compounds that inhibited KB cell growth by 40% relative to control at 4 μg/mL, were re-tested in a dose–response study against HTCL panel. Drug stock solutions were prepared in DMSO, and the final solvent concentration was 2% DMSO (v/v), a concentration without effect on cell replication. The human tumor cell line panel constituted of epiderimoid carcinoma of the nasopharynx (KB), lung carcinoma (A-549), ileocecal carcinoma (HCT-8), renal cancer (CAKI-1), ovarian cancer (1A9), breast cancer (MCF-7), and glioblastoma (U-87 MG). Cells were cultured at 37 °C in RPMI-1640 with 100 μg/mL kanamycin and 10% (v/v) fetal bovine serum in a humidified atmosphere containing 5% CO_2. Initial seeding densities varied among the cell lines to ensure a final absorbance reading in control cultures in the range 1–2.5 A_{562} units. Drug exposure was for 3 days, and the ED_{50} value, the drug concentration that reduced the absorbance by 50%, was interpolated from dose–response data. Each test was performed in triplicate, and absorbance readings varied no more than 5%. ED_{50} values determined in independent tests varied no more than 30%.

Anti-HIV assay

The T cell line, H9, was maintained in continuous culture with complete medium [RPMI 1640 with 10% fetal calf serum (FCS) supplemented with L-glutamine] at 5% CO_2 and 37 °C. Aliquots of this cell line were used in experiments only when in log-phase of growth. Test samples were first dissolved in DMSO. The following are the final drug concentrations routinely used for screening: 100, 20, 4, and 0.8 μg/mL. There were crystals present at the 100 μg/mL concentration of **12**, **13**, **22**, **24**, **29**, **34**, **35**, **42**, and **46**. Crystals were also seen at 10 μg/mL concentration of **12**, **13**, and **35**. For active agents, additional dilutions are prepared for subsequent testing so that an accurate EC_{50} value (see definition below) could be achieved. As the test samples were being prepared, an aliquot of H9 cells was infected with HIV-1 (IIIB isolate), while another aliquot was mock-infected with complete medium. The mock-infected sample was used for toxicity determinations (IC_{50}, see definition below). The stock virus used for these studies typically had a $TCID_{50}$ value of 10^4 Infectious Units (IU)/mL. The appropriate amount of virus for a multiplicity of infection between 0.1 and 0.01 IU/cell was added to the first aliquot of cells. The other aliquot of cells received only culture medium and was then incubated under identical conditions to the HIV-infected cells. After a 4-h incubation at 37 °C and 5% CO_2, both cell populations were washed three times with fresh medium and then added to the appropriate wells of a 24-well plate containing the various concentrations of the test drug or culture medium (positive infected control/negative-control drug). In addition, AZT was also assayed during each experiment as a positive-control drug. The plates were incubated at 37 °C and 5% CO_2 for 4 days. Cell-free supernatants were collected on day 4 and tested by an in-house p24 antigen ELISA assay; p24 antigen is a core protein of HIV and, therefore, is an indirect measure of virus present in the supernatants. Toxicity was determined by performing cell counts by a coulter counter on the mock-infected cells, which had either received culture medium (no toxicity) or test sample or AZT. If a test sample had suppressive capability and was not toxic, its effects were reported in the following terms: IC_{50}, the concentration of test sample that was toxic to 50% of the mock-infected cells; EC_{50}, the concentration of the test sample that was able to suppress HIV replication by 50%; and therapeutic index (TI), the ratio of IC_{50} to EC_{50}.

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