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Synthesis and Bioactivity of 4,10-Dimethyl-pyridino[2,3-*h*]quinolin-2(1*H*)-one-9-carboxylic Acid and Its Esters

Qian Zhang,[a] Ying Chen,[a] Yun Qing Zheng,[a] Peng Xia,[a,*] Yi Xia,[b] Zheng Yu Yang,[b] Kenneth F. Bastow,[b] Susan L. Morris-Natschke[b] and Kuo-Hsiung Lee[b,*]

[a]*Department of Medicinal Chemistry, School of Pharmacy, Fudan University, Shanghai 200032, China*
[b]*Natural Products Laboratory, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, USA*

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Abstract—4,10-Dimethyl-pyridino[2,3-*h*]quinolin-2(1*H*)-one-9-carboxylic acid (**1**) was synthesized by a new approach via the key intermediate 7-[1-aza-2-(dimethylamino)vinyl]-4-methylquinolin-2(1*H*)-one (**4**). Compound **1** and its esters were evaluated in cytotoxicity and anti-HIV assays. The 9-carboxyl (1s)-endo-(−)-borneol ester (**9**) showed marginal cytotoxic activity in CAK1-1, HOS, KB, and HCT-8 cells.

Introduction

As recently reported, 4-methylhydro-2*H*-pyrano[6,5-*h*]-chromen-2-ones and 4-methylhydro-2*H*-pyrano[6,5-*h*]-quinolin-2-ones show interesting bioactivities with di-*O*-(*S*)-camphanoyl(+)-*cis*-khellactone (DCK) derivatives[1] displaying potent anti-HIV activity and 4,8,8-trimethyl-hydro-2*H*-pyrano[6,5-*h*]quinolin-2-one displaying significant cytotoxic activity.[2] These findings prompted us to synthesize two series of diaza three-ring heterocyclic analogues, trihydropyridino[2,3-*h*]quinolin-2-ones and hydropyridino[2,3-*h*]quinolin-2-ones (Scheme 1).

To our knowledge, the following three synthetic approaches to the above diaza three-ring heterocyclic analogues have been reported. (1) The first route uses 1,3-phenylenediamine as the starting material and simultaneously constructs the A and C rings.[3] Although this route is short, the structures of the A and C rings must be identical (Scheme 2). (2) The second route uses 5-aminoquinoline as starting material and then constructs the A ring.[4] However, although the C ring is already established in the starting material and the A ring formation is highly regioselective, preparation of the 5-aminoquinoline is tedious (Scheme 3). (3) The third route uses 7-aminoquinoline as starting material and subsequently constructs the C ring.[5] The key point is regioselective formation of the C ring (Scheme 4).

Chemistry

We have now developed a different synthetic approach starting from 7-amino-4-methylquinolin-2(1*H*)-one (**3**), which is readily available by heating 1,3-phenylenediamine with ethyl acetoacetate. Highly regioselective C ring closure via the intermediate 7-[1-aza-2-(dimethylamino)vinyl]-4-methylquinolin-2(1*H*)-one (**4**) led to the target hydropyridino[2,3-*h*]quinolin-2-ones (Scheme 5).

7-Amino-4-methylquinolin-2(1*H*)-one (**3**) was obtained by reacting 1,3-phenylenediamine with ethyl acetoacetate using a reported method.[6] We then tried to construct the C ring by intermolecular electrophilic substitution of **3** with β-substituted acid chloride, anhydride or methylketone reagents at position-8 followed by ring closure through formation of the N–C bond or alternatively by first establishing the N–C bond followed by ring closure through an intramolecular electrophic substitution. However, numerous reactions failed, most likely because of low nucleophilic reactivity of the B ring. However, when **3** was reacted with the strongly electrophilic Vilsmeier Reagent (DMF and $POCl_3$), an unexpected product was identified as: 7-[1-aza-2-(dimethylamino)vinyl]-4-methylquinolin-2(1*H*)-one (**4**). This compound exhibited different ^{1}H NMR behavior in

*Corresponding authors. Tel.: +86-21-6404-1900x2563; e-mail: pxia@shmu.edu.cn (P. Xia); tel.: +1-919-962-0066; e-mail: khlee@unc.edu (K.-H. Lee).

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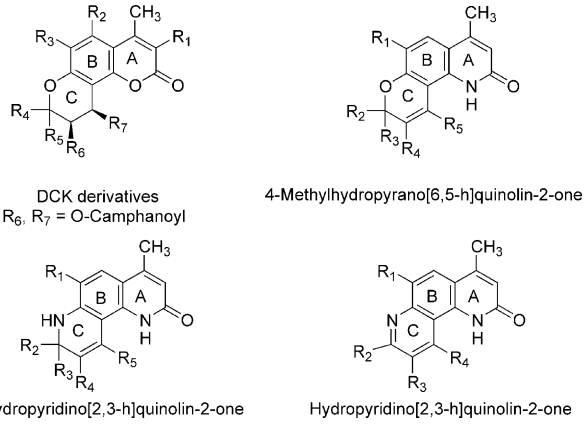
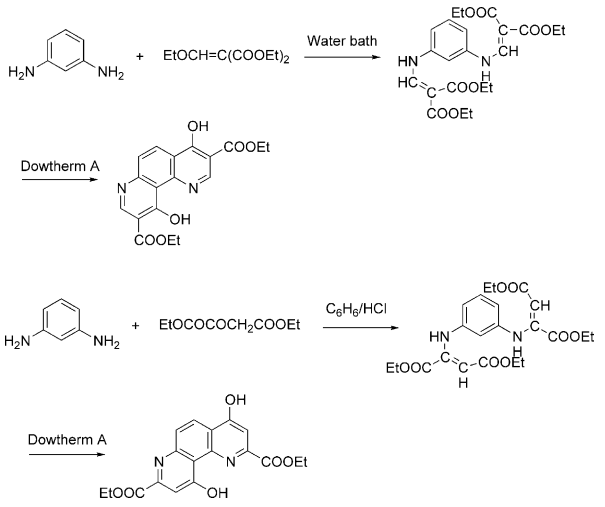
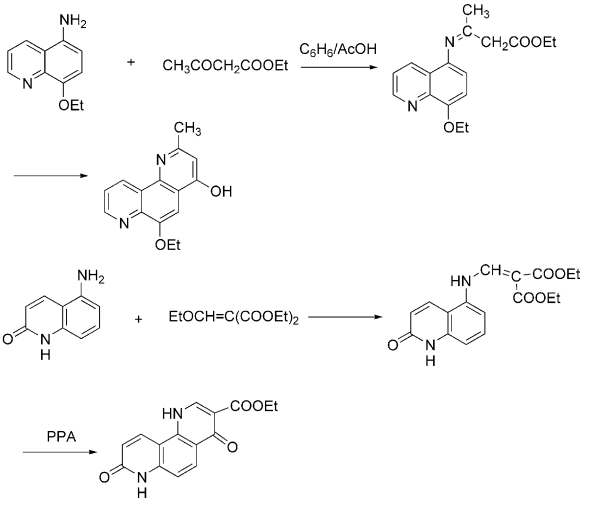
various solvents, which suggested a partial double-bond nature of the C–N single-bond in the –N=CH–N(CH_3)$_2$ group, presumably due to hindered rotation around the C–N σ-bond. Accordingly, a rotation energy barrier of $\Delta G = 66.5$ KJ/mol[7] was calculated via ^{1}H NMR (DMSO-d_6) at different temperatures.



Scheme 1.



Scheme 2.

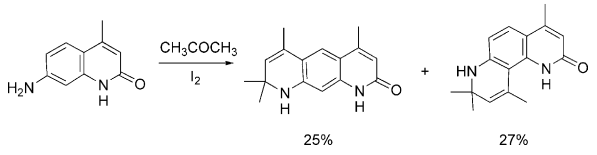


Scheme 3.

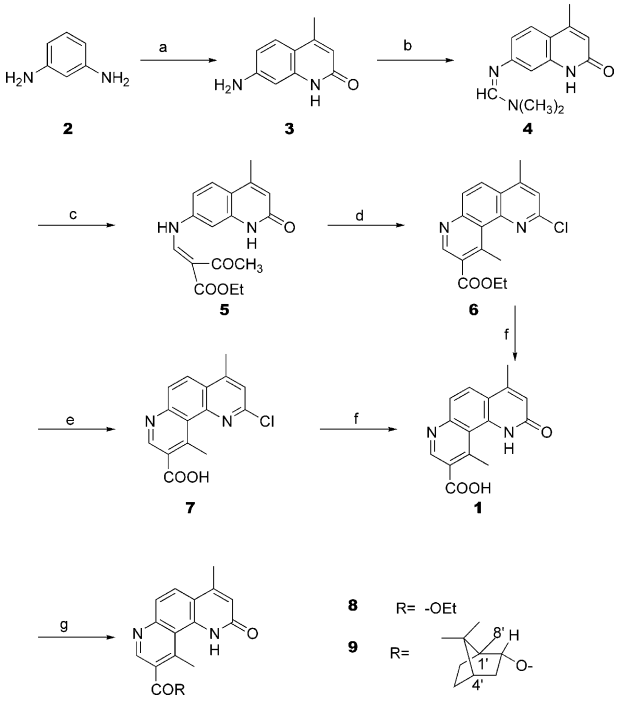
Because the –N=CH–N(CH_3)$_2$ group could enhance the nucleophilic reactivity of the B-ring via an electron donating effect and also act as a nucleophile acceptor at the –N=C– carbon atom, we turned our attention to using **4** as an intermediate to form the C ring. Initially, we tried reacting **4** with ethyl acetoacetate and obtained 7-[*N*-(2′-acetyl-2′-ethoxycarbonyl-vinyl)]amino-4-methyl-quinolin-2(1*H*)-one (**5**), which was conveniently converted to 2-chloro-4,10-dimethylpyridino[2,3-*h*]quinoline-9-carboxyl ethyl ester (**6**) by heating with $POCl_3$. The C ring closure was highly regioselective at position-8 and no linear tri-ring product was isolated; however, the amide group in **5** was also converted to a chloro imine moiety in **6**. Hydrolysis of **6** with concd HCl gave the 2-chloro-9-carboxylic acid (**7**), which was further hydrolyzed with aq NaOH (13%, g/mL) to yield **1**. Additionally, treating **6** with aq NaOH (13%, g/mL) resulted in complete hydrolysis of both the 9-ester and chloro imine to directly produce 4,10-dimethyl-pyridino[2,3-*h*]quinolin-2(1*H*)-one-9-carboxylic acid (**1**).

Biological Results

Two esters of **1**, 9-carboxyl ethyl ester (**8**) and 9-carboxyl (1s)-endo-(−)-borneol ester (**9**), were also synthesized for bioactivity screening. Cytotoxic evaluation of



Scheme 4.



Scheme 5. (a) CH_3COCH_2COOEt, reflux; (b) DMF/$POCl_3$; (c) CH_3COCH_2COOEt/Et_3N; (d) $POCl_3$, reflux; (e) HCl, reflux; (f) NaOH, reflux; (g) $SOCl_2$, alcohol.

Table 1. ED_{50} values in cytotoxicity assay (μg/mL)[8]

Compd	Cell lines[a]							
	KB	A549	HCT-8	CAKI-1	MCF-7	HOS	1A9	U-87-MG
1	12	20	24	8	25	11	NA[b]	18
Salt of 1	16	20	NA[b]	11	25	9	NA[b]	18
8	15	30	NA[b]	11	25	9	NA[b]	13
9	5	41	6	4.3	28	4.8	35	31

[a]Human tumor cell lines include KB, nasopharyngeal; A549 lung; HCT-8, ileocecal; CAKI, kidney; MCF-7, breast; HOS, bone; 1A9, ovarian; U87-MG, gliobastoma.
[b]NA, not active.

1, **8**, **9** and the sodium salt of **1** (Table 1) showed that **9** was marginally active against CAK1-1, HOS, KB, and HCT-8 cells with EC_{50} values of 4.3, 4.8, 5.0, and 6.0 μg/mL, respectively. None of the compounds showed significant anti-HIV activity.

Experimental

7-Amino-4-methylquinolin-2(1*H*)-one (3). 1,3-Phenylenediamine (**2**) (2.00 g, 18.49 mmol) and ethyl acetoacetate (2.36 mL, 18.51 mmol) were refluxed for 48 h. The mixture solidified gradually. MeOH (5 mL) was added to loosen the solid and filtration gave crude **3** (3.00 g). Crystallization using MeOH gave colorless needle crystals (1.60 g, 49.69%). Mp 268–271 °C (ref. 271–272 °C).

7-[1-Aza-2-(dimethylamino)vinyl]-4-methylquinolin-2(1*H*)-one (4). $POCl_3$ (0.45 mL, 4.60 mmol) was added dropwise to a mixture of 0.80 g (4.59 mmol) of 3 and 8 mL DMF at −5 to 0 °C. After stirring for 30 min at 0 °C, 20% Na_2CO_3 was added until the pH = 8. The precipitate was filtered and washed with ice water to give crude **4** in quantitative yield. Crystallization from $CHCl_3$ gave colorless needle crystals. Mp 221–222 °C; MS (*m*/*z*, %): 229 (M^+, 100), 214 (M^+-Me, 20.18), 44 (NMe_2^+, 16.49); ^1H NMR ($CDCl_3$) δ 10.45 (s, 1H, N–H), 7.62 (s, 1H, N=C–H), 7.54 (d, J = 8.60 Hz, 1H, 5-H), 6.89 (dd, J = 8.60 Hz, J = 2.04 Hz, 1H, 6-H), 6.76 (d, J = 2.04 Hz, 1H, 8-H), 6.39 (s, 1H, 3-H), 3.06 (s, 6H, NMe_2), 2.45 (s, 3H, 4-Me). ^1H NMR (DMSO-d_6) δ 11.30 (s, 1H, N–H), 7.80 (s, 1H, N=C–H), 7.50 (d, J = 8.67 Hz, 1H, 5-H), 6.82 (dd, J = 8.67 Hz, J = 1.90 Hz, 1H, 6-H), 6.75 (d, J = 1.90 Hz, 1H, 8-H), 6.18 (s, 1H, 3-H), 3.04 and 2.94 (2s, 6H, NMe_2), 2.53 (s, 3H, 4-Me).

7-[*N*-(2′-Acetyl-2′-ethoxycarbonyl)vinyl]amino-4-methylquinolin-2(1*H*)-one (5). Compound **4** (8.20 g, 35.76 mmol), triethylamine (9 mL) and ethyl acetoacetate (75 mL) were refluxed for 72 h and the mixture solidified gradually. The solid was filtered and washed with water and diethyl ether successively. Column chromatography (Silica H, EtOAc/MeOH = 15:1) gave a white solid (**5**), mp 272 °C (carbonization); MS *m*/*z* (%): 314 (M^+, 59.77); ^1H NMR ($CDCl_3$–CD_3OD, 5:1) δ 8.38 (s, 1H, HC=C), 7.55 (d, J = 8.79 Hz, 1H, 5-H), 6.96–6.91 (m, 2H, 6- and 8-H), 6.27 (s, 1H, 3-H), 4.07 (q, 2H, –OC**H**$_2$CH$_3$), 2.37 (s, 1H, CH_3CO–), 2.30 (s, 3H, 4-Me), 1.18 (t, 3H, –OCH$_2$C**H**$_3$).

2-Chloro-4,10-dimethylpyridino[2,3-*h*]quinoline-9-carboxyl ethyl ester (6). Compound **5** was poorly soluble and crude **5** was used directly in the preparation of **6**. Crude **5** (4.50 g) was added to $POCl_3$ (30 mL) and refluxed for 2 h. The mixture was poured into 200 mL ice water. The pH was adjusted to 8 with NaOH (10%) and the solution extracted with $CHCl_3$ (100 mL × 3). The extract was passed through a silica H column and concentrated to give a brown solid (**6**). Crude **6** was recrystallized from EtOAc to give colorless floccular crystals (1.50 g). The yield from **4** to **6** was 16.80%. Mp 190–192 °C; MS *m*/*z* (%): 314 (M^+, 36.96), 316 (^{37}Cl, 9.92), 313 (M^+-1, 100), 315 (^{37}Cl, 38.09), 269 ($M^+-OCH_2CH_3$, 79.29), 241 ($M^+-COOEt$, 59.39); ^1H NMR ($CDCl_3$) δ 9.90 (s, 1H, 8-H), 8.19 (d, J = 9.34 Hz, 1H, 6-H), 8.05 (d, J = 9.34 Hz, 1H, 5-H), 7.45 (s, 1H, 3-H), 4.50 (q, 2H, –OC**H**$_2$CH$_3$), 3.05 (s, 3H, 10-Me), 2.77 (s, 3H, 4-Me), 1.50 (t, 3H, –OCH$_2$C**H**$_3$). Anal. for $C_{17}H_{15}ClN_2O_2$: calcd C 64.87, H 4.80, N 8.90, Cl 11.26; found C 64.88, H 4.72, N 8.85, Cl 11.44.

2-Chloro-4,10-dimethylpyridino[2,3-*h*]quinoline-9-carboxylic acid (7). HCl (concd 5 mL) was added to a solution of **6** (0.25 g, 0.79 mmol) and dioxane (5 mL) and refluxed for 7 h. The mixture gradually produced a white solid. The mixture then was poured into 20 mL ice water, filtered and washed with water until pH = 7 to give crude **7** (0.16 g, 70.28%). The product was precipitated from an alkaline (NaOH) solution by addition of HCl to give pure **7** as a white solid. Mp 264–266 °C (carbonization); MS *m*/*z* (%): 285 (M^+-1, 100), 287 (^{37}Cl, 38.60), 241 (M^+-COOH, 58.95); ^1H NMR (DMSO-d_6) δ 9.79 (s, 1H, 8-H), 8.49 (d, J = 9.07 Hz, 1H, 6-H), 8.20 (d, J = 9.07 Hz, 1H, 5-H), 7.79 (s, 1H, 3-H), 4.0–4.4 (br, COOH), 3.07 (s, 3H, 10-Me), 2.81 (s, 3H, 4-Me); IR (KBr) ν cm^{-1}: 1715 (C=O), 1640, 1610, 1590, 1560 (C=C, C=N), 610 (C–Cl), 3000–2600 (CH, OH).

4,10-Dimethylpyridino[2,3-*h*]quinolin-2(1*H*)-one-9-carboxylic acid (1). Compound **6** (0.08 g, 0.25 mmol) and NaOH (13%, 15 mL) were mixed and refluxed for 19 h with gradual dissolution of the solid. The mixture was filtered and extracted with $CHCl_3$. Then HCl (concd) was added until pH = 2 and a white solid (**1**) was obtained in quantitative yield. Mp >310 °C; MS *m*/*z* (%): 268 (M^+, 100), 224 (M^+-COO^-, 23.36); ^1H NMR (DMSO-d_6) δ 9.95 (s, 1H, 8-H), 8.18 (d, J = 7.83 Hz, 1H, 5-H), 7.79 (d, J = 7.83 Hz, 1H, 6-H), 6.62 (s, 1H, 3-H), 3.30 (s, 3H, 10-Me), 2.50 (s, 3H, 4-Me); IR (KBr) ν cm^{-1} 1640–1690 (C=O), 1625, 1590, 1545, 1470 (C=C, C=N), 3500–2800 (NH, OH); ^{13}C NMR (D_2O) δ 177.2 (COOH), 165.9 (2-C), 160.4 (4-C), 153.5 (8-CH), 148.0 (6a-C), 135.4 (4b-C), 135.3 (10-C), 131.5 (5-CH), 127.8 (9-C), 123.7 (6-CH), 121.0 (4a-C), 118.0 (10a-C), 116.6 (3-CH), 25.6 (4-CH_3), 21.2 (10-CH_3).

4,10-Dimethylpyridino[2,3-*h*]quinolin-2(1*H*)-one-9-carboxyl ethyl ester (8). $SOCl_2$ (10 mL) was added to **1** (0.10 g, 0.37 mmol). After refluxing 12 h, the $SOCl_2$ was

removed by vacuum distillation. Then 15 mL EtOH was added to the residue and reflux continued for 5 h. A yellow solid was obtained after removing solvent. Pure **8** was obtained by column chromatography (silica H, $CHCl_3$/MeOH = 30:1). Mp 264–266 °C; MS *m*/*z* (%): 296 (M^+, 100), 251 (M^+-OEt, 38.11), 223 ($M^+-COOEt$, 27.69), 268 (M^+-CO, 18.22); ^1H NMR ($CDCl_3$) δ 9.72 (s, 1H, 8-H), 8.00 (d, $J = 9.31$ Hz, 1H, 5-H), 7.85 (d, $J = 9.31$ Hz, 1H, 6-H), 6.67 (s, 1H, 3-H), 4.53 (q, 2H, –$\mathbf{OCH_2}CH_3$), 3.05 (s, 3H, 10-Me), 2.62 (s, 3H, 4-Me), 1.50 (t, 3H, –$OCH_2\mathbf{CH_3}$); ^{13}C NMR ($CDCl_3$) δ 165.9 (COO–), 163.9 (2-C), 160.2 (4-C), 149.8 (8-CH), 135.6 (6a-C), 134.4 (4b-C), 127.4 (10-C), 124.3 (5-CH), 123.3 (9-C), 123.2 (6-CH), 120.9 (4a-C), 116.7 (10a-C), 115.4 (3-CH), 61.7 (–$\mathbf{OCH_2}CH_3$), 25.5 (4-CH_3), 19.8 (10-CH_3), 14.4 (–$OCH_2\mathbf{CH_3}$).

4,10-Dimethylpyridino[2,3-*h*]quinolin-2(1*H*)-one-9-carboxyl (1s)-endo-(−)-borneol ester (9). $SOCl_2$ (10 mL) was added to **1** (0.10 g, 0.37 mmol). After refluxing 12 h, the $SOCl_2$ was removed by vacuum distillation. Then a solution of (1s)-endo-(−)-borneol (0.10 g, 0.65 mmol) and DMAP (0.05 g, 0.37 mmol) in $CHCl_3$ (15 mL) was added. After refluxing for 30 min, the solvent was removed by distillation and the residue was washed with water and diethyl ether. The crude **9** was purified via column chromatography (Silica H, $CHCl_3$/MeOH = 40:1) to give pure **9**. Mp. > 360 °C (dec.); MS *m*/*z* (%): 209 (100.00), 296 (84.73), 267 ($M^+-C_{10}H_{17}O$, 46.69), 294 (46.69), 341 (44.35), 208 (37.50), 295 (36.31), 153 (8.77, $C_{10}H_{17}O^+$), 223 ($M-C_{10}H_{17}OOC$–, 15.10); ^1H NMR ($CDCl_3$) δ 13.52 (s, 1H, N–H), 10.02 (s, 1H, 8-H), 8.01 (d, $J = 9.12$ Hz, 1H, 5-H), 7.91 (d, $J = 9.12$ Hz, 1H, 6-H), 6.77 (s, 1H, 3-H), 4,09 (m, 1H, borneol C**H**OH), 4.43, 4.03, 3.65 (s×3, 3H×3, CH_3 in borneol), 2.60, 2.57 (s×2, 3H×2, 4- and 10-Me), 2.18–0.88 (m, 7H, the other H in borneol); ^{13}C NMR (DMSO-d_6) δ 162.5 (COO–), 162.1 (2-C), 161.0 (4-C), 150.8 (8-CH), 148.5 (6a-C), 131.6 (4b-C), 131.4 (10-C), 130.2 (5-CH), 129.6 (9-C), 125.9 (6-CH), 122.4 (4a-C), 122.2 (10a-C), 120.5 (3-CH), 79.1 (2′-CH), 63.1 (1′-C), 63.0 (7′-C), 54.3 (4′-CH), 31.2 (4-CH_3), 30.6 (3′,6′-CH_2), 28.9 (5′-CH_2), 18.9 (9′ or 10′-CH_3), 18.6 (9′ or 10′-CH_3), 15.9 (10-CH_3), 15.9 (8′-CH_3).

Bioactivity evaluation

Cytotoxicity assay. In vitro cytotoxicity assay was carried out according to the procedures described in Rubinstein et al.[8] All stock cultures were grown in T-25 flasks (5 mL of RPMI-1640 medium supplemented with 25 mM HEPES, 0.25% sodium bicarbonate, 10% fetal bovine serum, and 100 μg/mL kanamycin). Freshly trypsinized cell suspensions were seeded in 96-well microtitre plates at densities of 1500–7500 cells per well with test compounds from DMSO-diluted stock. After 3 days in culture, cells attached to the plastic substractum were fixed with cold 50% trichloroacetic acid and then stained with 0.4% sulforhodamine B (SRB). The absorbancy at 562 nm was measured using a microplate reader after solubilizing the bound dye. The ED_{50} is the concentration of test compound that reduced cell growth by 50% over a 3-day assay period.

Anti-HIV assay. The anti-HIV assay was carried out according to the procedures described in our previous papers.[1]

Acknowledgements

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