An Efficient Synthesis of a New Class of DNA Intercalating Antitumor 7,10-Dihydroxy-6*H*-pyrazolo[4,5,1-*de*]acridin-6-ones

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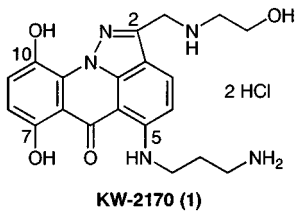
Abstract: An efficient synthesis of KW-2170 (**1**), which is a 7,10-dihydroxy-6*H*-pyrazolo[4,5,1-*de*]acridin-6-one derivative and a new class of DNA intercalating antitumor agents, is described. The selective monobromination of the methyl group before the cyclization to the pyrazoloacridone is easily carried out. In the improved process, the bromination of the corresponding methyl group is achieved prior to the hydroquinone formation, so the protection of the hydroxy groups is not necessary unlike the original method. In comparison with the original synthetic route of KW-2170 (**1**), the new route decreases the synthetic steps from 2-bromo-6-methoxybenzoic acid (**2**) from 15 to 13 and increases the overall yield from 2 to 12%.

Key words: antitumor agents, pyrazoloacridone, process improvement

6*H*-Pyrazolo[4,5,1-*de*]acridin-6-one (pyrazoloacridone) derivatives were synthesized as a new class of DNA intercalating antitumor agents.[1] In researching the structure–activity relationships, the 7,10-dihydroxy derivatives showed excellent antitumor activities.[2] The most promising compound, KW-2170 (**1**; Figure), has superior antiproliferactive activity in vitro against HeLa S_3 and excellent antitumor activities in vivo against the murine sarcoma 180 solid tumor, murine P388 leukemia and adriamycin-resistant murine P388 leukemia.[2,3] Thus, KW-2170 (**1**) was evaluated in pre-clinical studies and is now in phase I clinical trials.



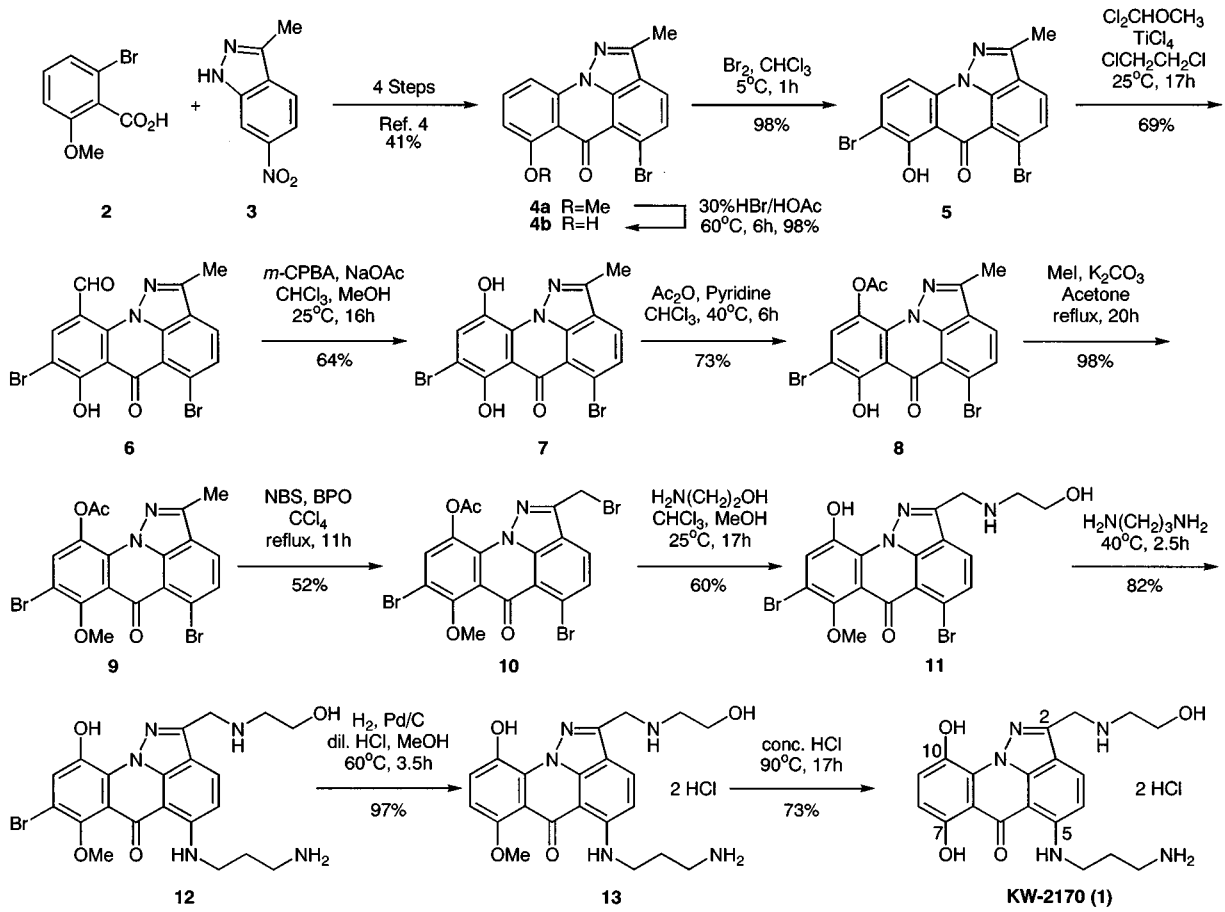
KW-2170 (1)

Figure

As these studies were proceeding, the development of a synthetic route of KW-2170 (**1**) that would be amenable to commercial production was required. The original synthetic route of KW-2170 (**1**) is depicted in Scheme 1.[2] This synthetic route was planned based on the synthetic method of the 7-hydroxy derivatives.[2,4] The synthetic route for introducing the 10-hydroxy moiety took priority over the development of the large scale synthesis. The important oxidation process to the quinone and the following reduction to the 7,10-dihydroxy compound were developed and the improved preparation of the key intermediate of the KW-2170 (**1**) was published.[5] However, there are remaining issues to be resolved for establishing the synthetic processes, and also the purification methods both of the intermediates and the final product should be improved. Herein, we describe the results of the development of the synthetic process and the accomplishment of a new efficient synthesis of KW-2170.

The substrate of the previously reported oxidation using the hypervalent iodine reagent was the 2-methyl compound **5**.[5] The resulting 7,10-hydroxy compound was expected to be easily converted to KW-2170 even on a practical synthetic scale. However, the selectivity of the monobromination at the 2-methyl group was low, because the dibromide was generated and also the unreacted starting material remained. Therefore, it was necessary to use chromatography for the isolation of the monobromide.

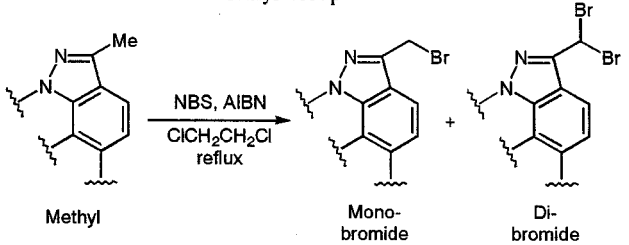
We first attempted the investigation of the selective monobromination with *N*-bromosuccinimide (NBS) at the 2-methyl group of various substrates, i.e., the original synthetic intermediate with protected C-7 and C-10 hydroxy groups **9**,[2] the 7-methoxypyrazoloacridone without the C-10 hydroxy group **4a**[4] and the ester of the indazole **14b** (Table). The ester **14b** was prepared from the corresponding sodium salt **14a**[4] in 87% yield (Scheme 2). In this study, 2,2'-azobisisobutyronitrile (AIBN) was tried as the radical initiator instead of the more explosive benzoyl peroxide (BPO). Among those compounds, it was found that the ester **14b** was the best substrate for bromination from the view point of the monobromination selectivity. The ratio of monobromide **15** in the reaction mixture was 76.0% (determined by HPLC analysis[6]). The monobrominated compound **15** was isolated from the reaction mixture with diisopropyl ether. This isolated **15** was suspended in methanol and the undissolved solid was collected to obtain the purified **15** in 65% yield (93% purity, determined by HPLC analysis[6]). The monobrominated ester was then cyclized to pyrazoloacridone with trifluoromethansulfonic acid at 100 °C in high yield. At this stage, the methyl ether at C-7 was partially cleaved and a mixture of the methyl ether **16a** and the phenol **16b** (47/53, determined by HPLC analysis[6]) was obtained. This mixture was treated with hydrogen bromide in acetic acid



Scheme 1

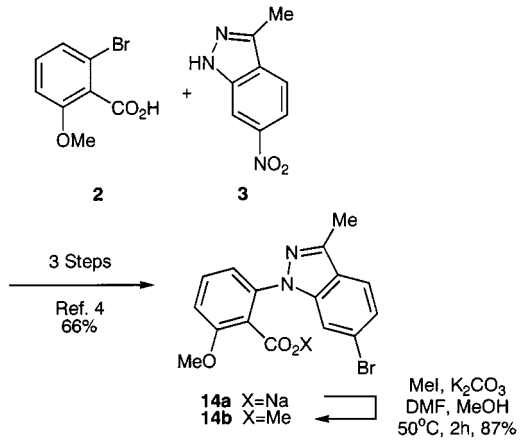
at 70 °C to accomplish the cleavage of the methyl ether. Preceding to the study of the oxidation, the protection at C-8 by bromination was carried out with bromine at 25 °C and gave the C-8 bromo derivative **17**. The overall yield of **17** from the C-2 monobrominated ester **15** was 80% and the purity of **17** was 96% based on an HPLC analysis[6] (Scheme 3).

Table Bromination at Methyl Group



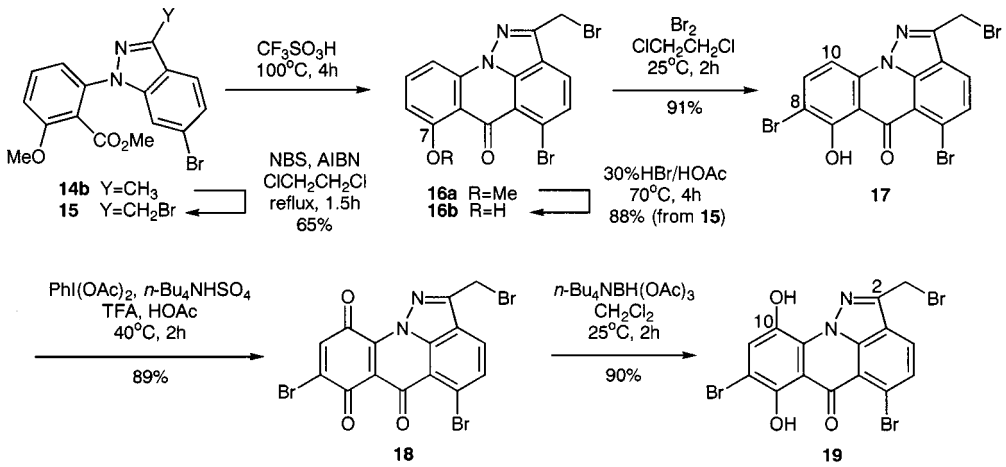
Entry	Substrate	Reaction Conditions			Ratios [a]		
		NBS (eq.)	AIBN (eq.)	Time (h)	Monobromide	Dibromide	Methyl
1	**9**	2.2	0.20	3.5	57.4	13.1	29.5
2	**4a**	1.1	0.05	1.0	67.6	8.0	24.4
3	**14b**	1.2	0.20	1.5	76.0	17.2	6.8

[a] Ratios were determined by HPLC analysis.[6]



Scheme 2

The introduction of the hydroxy group at C-10 of the 2-bromomethyl derivative **17** was accomplished by a two-step sequence involving the hypervalent iodine oxidation method followed by reduction. The reaction of **17** with [(diacetoxy)iodo]benzene in trifluoroacetic acid (TFA) and acetic acid (6:4) was heterogeneous, and the oxidation stopped. However, the reaction was promoted by the ad-



Scheme 3

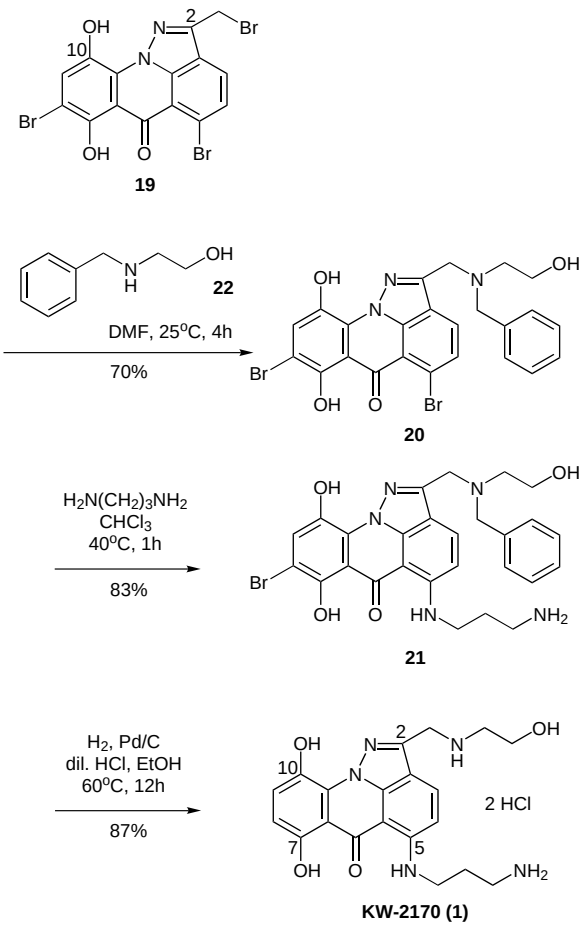
dition of tetra-*n*-butylammonium hydrogen sulfate (*n*-Bu_4NHSO_4) as the phase transfer catalyst and the quinone **18** was obtained in 89% yield. The quinone was incompletely converted to the hydroquinone with aqueous sodium hydrosulfite in the same way as already reported.[5,7] After the investigation of various reducing methods, the reduction was accomplished in dichloromethane using tetra-*n*-butylammonium triacetoxyborohydride (*n*-$Bu_4NBH(OAc)_3$)[8] which was prepared *in situ* from tetra-*n*-butylammonium borohydride (*n*-Bu_4NBH_4) and acetic acid. The hydroquinone **19** was obtained from the phenol **17** in 80% overall yield (Scheme 3). In the improved process, the bromination of the corresponding methyl group was achieved prior to the hydroquinone formation. Thus, there was no necessity for protection of the hydroxy groups unlike the original method in which those hydroxy groups should be protected by an acetyl or methyl group before reacting with NBS. So far, there were neither highly toxic intermediates nor reagents and the steps were carried out under common synthetic conditions. The improved synthetic route has been used for the practical preparation of a key intermediate **19** of KW-2170.

Although the two amino side chains at C-2 and C-5 could be introduced as in the original method,[1,2] the formation of the disubstituted amino compound should be avoided by the introduction of the C-2 amino side chain. The highly diluted conditions and the large excess amount of amine were adopted in the original method and it suffered from low productivity. Therefore, we attempted to use a secondary amine, 2-[(phenylmethyl)amino]ethanol (**22**), to generate the tertiary amine **20**. The benzyl group in the final product could be removed at the last step. The introduction of 2-[(phenylmethyl)amino]ethanol (**22**) was carried out at 25 °C in *N*,*N*-dimethylformamide and the concentration of the reaction increased from 3 g/L to 200 g/L. The amine **20** was crystallized from the reaction mixture with methanol. At this stage, the purity of **20** was 97% (determined by HPLC analysis[9]). To obtain the final product of KW-2170 in high purity, the compound **20** was purified by silica gel chromatography to over 99% based on HPLC analysis.[9] This procedure helped to avoid the HPLC purification of KW-2170 in the final process. The amino side chain at C-5 was introduced with propane-1,3-diamine at 40 °C in chloroform and the compound **21** was purified by recrystallization from a mixture of chloroform, methanol and acetonitrile. The final intermediate **21** was obtained from **19** for two steps with a 58% overall yield to keep the purity at 99% or more in the HPLC analysis[10] (Scheme 4).

The final step, the removal of *N*-benzyl and C-8 bromo groups, was accomplished by hydrogenolysis with 10% palladium on carbon in a mixture of dilute hydrochloric acid and ethanol. In the original route, the HPLC purification of the final product was indispensable for removing impurities which were formed at about 3% under the severe reaction conditions needed for the *O*-demethylation at C-7 (Scheme 1). In the improved route, however, the conditions were mild enough to keep the purity high (>99%),[11] so the final KW-2170 was obtained merely by recrystallization from dilute hydrochloric acid and ethanol.

In summary, we have established an efficient synthetic route to KW-2170 (**1**), a new DNA intercalating antitumor agent. In comparison with the original synthetic route,[2] the improved route showed that the synthetic steps from 2-bromo-6-methoxybenzoic acid (**2**) decreased from 15 to 13 and the overall yield was increased from 2 to 12%. In addition, the bulk substance of KW-2170 with a high purity (>99%) has been synthesized by an innovative methodology and provided for clinical trial tests.

All the reagents and solvents were commercially available and were used without purification. Mps were determined using a Mettler FP61 melting point instrument and are uncorrected. NMR spectra (internal standard TMS) were recorded on a Bruker AC300 (300 MHz) and JEOL LA300 (300 MHz) spectrometer. IR spectra were recorded using a Shimadzu FTIR-4300 spectrophotometer. Mass spectra were recorded on a Hitachi M-80B mass spectrometer. Ele-



Scheme 4

mental analyses were performed using a Yanaco MT-3 CHN apparatus. Sodium 2-(6-bromo-3-methyl-1*H*-indazol-1-yl)-6-methoxybenzoate (**14a**) was prepared from 2-bromo-6-methoxybenzoic acid (**2**)[4] and 3-methyl-6-nitroindazole (**3**),[12] according to the reported procedure.[4]

Methyl 2-(6-Bromo-3-methyl-1*H*-indazol-1-yl)-6-methoxybenzoate (14b)

A suspension of sodium 2-(6-bromo-3-methyl-1*H*-indazol-1-yl)-6-methoxybenzoate (**14a**,[4] 3,800 g, 9.91 mol) in MeOH (5.7 L) and DMF (5.7 L) was heated at 75 °C for 1 h and cooled to 30 °C. To this suspension was added K_2CO_3 (684 g, 9.91 mol) and MeI (3,085 mL, 49.6 mol) and the mixture was stirred at 50 °C for 2 h. After removing the solvent under reduced pressure, the mixture of MeOH (3.8 L) and H_2O (34.2 L) was added dropwise to the residue. The precipitate was filtered, washed with H_2O (8 L), and the filtered solid was dissolved in $CHCl_3$ (23 L) and dried ($MgSO_4$). The solution was concentrated under reduced pressure and the residue was crystallized from $CHCl_3$ (5.7 L) and MeOH (34.2 L) to give the methyl ester **14b** (3,231 g, 87%) as colorless solid; mp 164 °C.

^{1}H NMR ($CDCl_3$/TMS): δ = 2.58 (s, 3H), 3.66 (s, 3H), 3.91 (s, 3H), 6.99 (d, 1H, *J* = 8.4 Hz), 7.18 (d, 1H, *J* = 8.0 Hz), 7.29 (dd, 1H, *J* = 8.5, 1.5 Hz), 7.51 (t, 1H, *J* = 8.3 Hz), 7.54 (d, 1H, *J* = 8.4 Hz), 7.71 (d, 1H, *J* = 1.5Hz).

IR (KBr): ν = 1728, 1598, 1516, 1460, 1333, 1256, 1132, 1111, 1096, 1049, 980 cm^{-1}.

MS (EI): *m*/*z* (%) = 374/376 (M^+, 100/99), 343/345 (63/55).

Anal: calcd for $C_{17}H_{15}BrN_2O_3$: C, 54.42; H, 4.03; N, 7.47. Found: C, 54.28; H, 4.01; N, 7.19.

Methyl 2-(6-Bromo-3-bromomethyl-1*H*-indazol-1-yl)-6-methoxybenzoate (15)

To a solution of the methyl ester **14b** (2,934 g, 7.82 mol) in $ClCH_2CH_2Cl$ (58.7 L) was added NBS (1,670 g, 9.38 mol) and AIBN (257 g, 1.56 mol) at 25 °C. The mixture was refluxed for 1.5 h and the solution was concentrated under reduced pressure. To the residue was added diisopropyl ether (14.7 L) and the mixture was cooled to 5 °C, then the precipitated solid was filtered. To the filtered solid was added MeOH (30 L) and the suspension was cooled to 5 °C. The precipitate was filtered and washed with MeOH (4 L) to give the bromide **15** (2,319 g, 65%) as colorless solid; mp 133 °C.

^{1}H NMR ($CDCl_3$/TMS): δ = 3.64 (s, 3H), 3.93 (s, 3H), 4.81 (s, 2H), 7.05 (d, 1H, *J* = 8.4 Hz), 7.19 (d, 1H, *J* = 8.0 Hz), 7.40 (dd, 1H, *J* = 8.6, 1.5 Hz), 7.55 (t, 1H, *J* = 8.4 Hz), 7.73 (d, 1H, *J* = 8.6 Hz), 7.74 (d, 1H, *J* = 1.5 Hz).

IR (KBr): ν = 1724, 1609, 1587, 1472, 1285, 1121, 1059, 802 cm^{-1}.

MS (EI): *m*/*z* (%) = 452/454/456 (M^+, 5/9/5), 373/375 (100/91), 343/345 (39/38).

Anal: calcd for $C_{17}H_{14}Br_2N_2O_3$: C, 44.96; H, 3.11; N, 6.17. Found: C, 45.07; H, 3.16; N, 5.87.

5-Bromo-2-bromomethyl-7-hydroxy-6*H*-pyrazolo-[4,5,1-*de*]acridin-6-one (16b)

The ester **15** (2,273 g, 5.01 mol) was heated in CF_3SO_3H (45.5 L) at 100 °C for 4 h. The mixture was poured into MeOH (100 L) and cooled to 5 °C. Then, the precipitated solid was filtered. To the filtered solid was added MeOH (23 L) and the suspension was stirred at 5 °C. The precipitate was filtered to give 47/53 mixture (determined by HPLC analysis[6]) of 7-methoxy ether **16a** and 7-hydroxy derivative **16b** as yellow solid; 1,908 g. To the mixture of **16a** and **16b** (1,808 g) was added 30% HBr in HOAc (27.1 L) and the mixture was heated at 70 °C for 4 h. After cooling to 15 °C, to the mixture was added MeOH (54.3 L) dropwise and cooled to 5 °C. The precipitate was filtered and washed with MeOH (40.5 L) to give the phenol **16b** (1,710 g, 88% from the ester **15**) as yellow solid; mp 220 °C (dec).

^{1}H NMR ($CDCl_3$/TMS): δ = 4.90 (s, 2H), 6.90 (d, 1H, *J* = 8.1 Hz), 7.60 (d, 1H, *J* = 8.2 Hz), 7.67 (t, 1H, *J* = 8.1 Hz), 7.79 (d, 1H, *J* = 8.2 Hz), 8.04 (d, 1H, *J* = 8.2 Hz), 13.20 (s, 1H).

IR (KBr): ν = 1638, 1603, 1589, 1514, 1473, 1339, 1294, 1259, 1225, 1134, 1070, 812 cm^{-1}.

MS (EI): *m*/*z* (%) = 406/408/410 (M^+, 11/20/10), 327/329 (100/100).

Anal: calcd for $C_{15}H_8Br_2N_2O_2$: C, 44.15; H, 1.98; N, 6.87. Found: C, 44.21; H, 2.13; N, 6.68.

5,8-Dibromo-2-bromomethyl-7-hydroxy-6*H*-pyrazolo-[4,5,1-*de*]acridin-6-one (17)

To a solution of the phenol **16b** (1,613 g, 3.95 mol) in $ClCH_2CH_2Cl$ (341 L) was added Br_2 (647 g, 4.05 mol) in $ClCH_2CH_2Cl$ (4 L). The mixture was stirred at 25 °C for 2 h and the mixture was concentrated to 48 L under reduced pressure. After cooling, the precipitate was filtered and washed with *i*-PrOH (25 L) to give the bromide **17** (1,744 g, 91%) as brown solid; mp 262 °C (dec).

^{1}H NMR ($CDCl_3$/TMS): δ = 4.83 (s, 2H), 7.54 (d, 1H, *J* = 8.7 Hz), 7.79 (d, 1H, *J* = 8.3 Hz), 7.88 (d, 1H, *J* = 8.7 Hz), 8.03 (d, 1H, *J* = 8.3 Hz), 13.97 (s, 1H).

IR (KBr): ν = 1636, 1587, 1508, 1448, 1342, 1281, 1256, 1234, 1121, 1049, 814 cm^{-1}.

MS (EI): *m*/*z* (%) = 484/486/488/490 (M^+, 11/31/33/12), 405/407/409 (52/100/55).

Anal: calcd for $C_{15}H_7Br_3N_2O_2$: C, 37.00; H, 1.45; N, 5.75. Found: C, 37.13; H, 1.58; N, 5.56.

5,8-Dibromo-2-bromomethyl-6*H*-pyrazolo[4,5,1-*de*]acridine-6,7,10-trione (18)

To a suspension of the bromide **17** (300.0 g, 0.616 mol) in the mixture of HOAc (1.2 L) and TFA (1.8 L) was added tetra-*n*-butylammonium hydrogen sulfate (120.0 g, 40%wt) and [(diacetoxy)iodo]-benzene (597.0 g, 1.85 mol). The mixture was stirred at 40 °C for 2 h, then cooled to 25 °C. To the mixture was added MeOH (6.0 L) and cooled to 5 °C, then the precipitate was filtered and washed with MeOH (2.0 L) to give the quinone **18** (274.3 g, 89%) as brown solid; mp 205 °C (dec).

^{1}H NMR ($CDCl_3$/TMS): δ = 4.83 (s, 2H), 7.44 (s, 1H), 7.89 (d, 1H, *J* = 8.3 Hz), 8.03 (d, 1H, *J* = 8.3 Hz).

IR (KBr): ν = 1682, 1622, 1520, 1504, 1201, 853, 572 cm^{-1}.

MS (EI): *m*/*z* (%) = 500/502/504/506 (M^++2, 15/37/37/12), 421/423/425 (55/100/52).

Anal: calcd for $C_{15}H_5Br_3N_2O_3$: C, 35.97; H, 1.01; N, 5.59. Found: C, 36.03; H, 1.23; N, 5.35.

5,8-Dibromo-2-bromomethyl-7,10-dihydroxy-6*H*-pyrazolo-[4,5,1-*de*]acridin-6-one (19)

To a solution of tetra-*n*-butylammonium borohydride (168.9 g, 0.656 mol) in CH_2Cl_2 (1.4 L) was added HOAc (337 mL, 5.33 mol) under N_2 and the mixture was stirred at 25 °C for 30 min. To the suspension of the quinone **18** (274.0 g, 0.547 mol) in CH_2Cl_2 (1.4 L) was added the solution of the reducing agent under N_2 and the mixture was stirred at 25 °C for 2 h. To the mixture was added MeOH (3.3 L) dropwise and the suspension was cooled to 5 °C. The precipitate was filtered and washed with MeOH (1.4 L) to give the hydroquinone **19** (246.6 g, 90%) as dark red solid; mp 249 °C (dec).

^{1}H NMR ($CDCl_3$/TMS): δ = 4.85 (s, 2H), 7.63 (s, 1H), 7.83 (d, 1H, *J* = 8.4 Hz), 8.08 (d, 1H, *J* = 8.4 Hz), 9.40 (s, 1H), 13.35 (s, 1H).

IR (KBr): ν = 1638, 1605, 1590, 1512, 1290, 1245, 1210, 1130, 1095, 910 cm^{-1}.

MS (EI): *m*/*z* (%) = 500/502/504/506 (M^+, 14/38/38/13), 421/423/425 (73/100/48).

Anal: calcd for $C_{15}H_7Br_3N_2O_3$: C, 35.82; H, 1.40; N, 5.57. Found: C, 36.00; H, 1.56; N, 5.38.

2-{[(1-Benzyl-2-hydroxyethyl)amino]methyl}-5,8-dibromo-7,10-dihydroxy-6*H*-pyrazolo[4,5,1-*de*]acridin-6-one (20)

To a suspension of the hydroquinone **19** (246.0 g, 0.489 mol) in DMF (1.2 L) was 2-[(phenylmethyl)amino]ethanol (155.3 g, 1.03 mol) at 25 °C and the mixture was stirred at 25 °C for 4 h. After adding MeOH (1.2 L) to the mixture, a small amount of **20** (200 mg) was seeded. The mixture was stirred at 25 °C for 1 h, then MeOH (2.5 L) was added dropwise and the suspension was cooled to 5 °C. The precipitate was filtered to give the crude amine **20** (251.0 g). The crude compound (250.0 g) was purified by silica gel column chromatography (Wakogel-200,[13] 2,500 g, $CHCl_3$) and recrystallized from $CHCl_3$ (2.5 L) and MeOH (5.0 L) to give purified **20** (194.8 g, 70% from **19**) as dark red solid; mp 160 °C (dec).

^{1}H NMR ($CDCl_3$/TMS): δ = 2.10 (br s, 1H), 2.78 (t, 2H, *J* = 5.3Hz), 3.67 (t, 2H, *J* = 5.3Hz), 3.69 (s, 2H), 4.11 (s, 2H), 7.17–7.29 (m, 5H), 7.49 (s, 1H), 7.69 (d, 1H, *J* = 8.3 Hz), 7.86 (d, 1H, *J* = 8.3 Hz), 9.49 (s, 1H), 13.26 (s, 1H).

IR (KBr): ν = 1640, 1608, 1590, 1516, 1450, 1290, 1242, 1130, 1090, 810cm^{-1}.

MS (EI): *m*/*z* (%) = 571/573/575 (M^+, 17/19/8), 540/542/544 (9/17/9), 421/423/425 (57/100/53).

Anal: calcd for $C_{24}H_{19}Br_2N_3O_4$: C, 50.29; H, 3.34; N, 7.33. Found: C, 50.17; H, 3.34; N, 7.14.

5-[(3-Aminopropyl)amino]-2-{[(1-benzyl-2-hydroxyethyl)amino]methyl}-8-bromo-7,10-dihydroxy-6*H*-pyrazolo[4,5,1-*de*]acridin-6-one (21)

To a suspension of the amine **20** (97.0 g, 0.169 mol) in $CHCl_3$ (1.5 L) was added propane-1,3-diamine (705 mL, 8.45 mol) at 25 °C and the mixture was heated at 40 °C for 1 h. After cooling to 25 °C, *i*-PrOH (2.9 L) was added and the suspension was cooled to 5 °C. The precipitate was filtered to give the crude amine **21** (104.2 g). The crude compound **21** (104.0 g) was purified by recrystallizing from the mixture of $CHCl_3$ (4.7 L), MeOH (1.6 L) and MeCN (2.1 L) to give the amine **21** (79.2 g, 83% from **20**) as yellow solid; mp 182 °C (dec).

^{1}H NMR (DMSO-d_6/TMS): δ = 1.73 (m, 2H, *J* = 6.7 Hz), 2.57 (m, 2H, *J* = 6.3 Hz), 2.69 (t, 2H, *J* = 6.6 Hz), 3.53–3.59 (m, 4H), 3.67 (s, 2H), 4.10 (s, 2H), 6.94 (d, 1H, *J* = 9.2 Hz), 7.19–7.36 (m, 5H), 7.49 (s, 1H), 8.10 (d, 1H, *J* = 9.2 Hz).

IR (KBr): ν = 1658, 1600, 1580, 1520, 1402, 1290, 1250, 1062, 1030, 820 cm^{-1}.

MS (EI): *m*/*z* (%) = 565/567 (M^+, 27/18), 415/417 (52/56), 386/388 (37/33), 358/360 (57/54), 337 (100).

Anal: calcd for $C_{27}H_{28}BrN_5O_4$: C, 57.25; H, 4.98; N, 12.36. Found: C, 56.98; H, 4.91; N, 12.15.

5-[(3-Aminopropyl)amino]-7,10-dihydroxy-2-{[(2-hydroxyethyl)amino]methyl}-6*H*-pyrazolo[4,5,1-*de*]acridin-6-one Dihydrochloride (KW-2170, 1)

A mixture of the amine **21** (60.0 g, 0.106 mol) and 10% palladium on carbon (12.0 g, 20%wt) in a solution of 0.6 mol/L HCl (1.5 L) and EtOH (1.5 L) was stirred under hydrogen flow at 60 °C for 12 h. After the atmosphere of the reaction vessel was displaced with N_2, the mixture was filtered and the residue was washed with a mixture of H_2O and EtOH (1:1, 600 mL). To the filtrate was added conc. HCl (240 mL) and KW-2170 (**1**) (500 mg) as seed, and the mixture was stirred at 25 °C for 20 h and cooled to 5 °C. The precipitate was filtered to give the crude compound **1** (44.3 g). The crude compound **1** (42.0 g) was purified by recrystallization from the mixture of 1 mol/L HCl (420 mL) and EtOH (840 mL) to give the purified KW-2170 (**1**) (41.0 g, 87% from **21**) as a yellow crystalline solid; mp >300 °C.

^{1}H NMR (DMSO-d_6/TMS): δ = 2.01 (m, 2H), 2.94 (t, 2H, *J* = 6.8 Hz), 3.15 (t, 2H, *J* = 5.4 Hz), 3.70 (dd, 2H, *J* = 7.0, 13.4 Hz), 3.76 (dd, 2H, *J* = 7.1, 10.5 Hz), 4.75 (s, 2H), 5.29 (t, 1H, *J* = 4.8 Hz), 6.80 (d, 1H, *J* = 8.8 Hz), 7.17 (d, 1H, *J* = 9.2 Hz), 7.37 (d, 1H, *J* = 8.8 Hz), 8.05 (br s, 3H), 8.35 (d, 1H, *J* = 9.2 Hz), 9.33 (t, 1H, *J* = 6.0 Hz), 9.61 (br s, 2H), 9.70 (s, 1H), 13.02 (s, 1H).

^{13}C NMR (DMSO-d_6/TMS): δ = 26.7, 36.4, 39.5, 41.7, 49.0, 56.4, 99.2, 109.3, 110.1, 110.3, 112.0, 122.4, 123.6, 132.1, 137.4, 137.8, 141.8, 152.9, 154.8, 182.1.

IR (KBr): ν = 3404, 2928, 1659, 1626, 1582, 1520, 1286, 1234, 1170, 816 cm^{-1}.

SIMS: *m*/*z* = 398 (M^++1).

Anal: calcd for $C_{20}H_{23}N_5O_4 \cdot 2HCl$: C, 51.07; H, 5.36; N, 14.89. Found: C, 50.99; H, 5.38; N, 14.64.

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(6) HPLC analysis conditions; column: YMC pack AM-312 ODS 6 × 150 mm; mobile phase: MeOH/H_2O = 6/1, pH 2.7 adjusted with 85% phosphoric acid; flow rate: 1.0 mL/min; detection: UV 254 nm.

(7) In the similar method reported by Kato et al.,[5] the quinone **18** was reduced incompletely in MeCN and aqueous sodium hydrosulfite. On the other hand, the reduction reaction progressed in the two-phase system of aqueous sodium hydrosulfite and a large amount (300 v/w based on **18**) of $CHCl_3$. This method was too diluted and very inefficient for the production, so a new efficient reducing method was required.

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(9) HPLC analysis conditions; column: YMC pack AM-312 ODS 6 × 150 mm; mobile phase: MeCN/phosphate buffer (0.1 mol/L, pH 2.7) = 70/30 and sodium 1-octanesufonate (0.01 mol/L); flow rate: 1.0 mL/min; detection: UV 254 nm.

(10) HPLC analysis conditions; column: YMC pack AM-312 ODS 6 × 150 mm; mobile phase: MeCN/phosphate buffer (0.1 mol/L, pH 2.7) = 35/65 and sodium 1-octanesufonate (0.01 mol/L); flow rate: 1.0 mL/min; detection: UV 254 nm.

(11) HPLC analysis conditions; column: YMC pack AM-312 ODS 6 × 150 mm; mobile phase: MeCN/phosphate buffer (0.1 mol/L, pH 2.7) = 30/70 and sodium 1-octanesufonate (0.01 mol/L); flow rate: 1.0 mL/min; detection: UV 254 nm.

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