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Development of a Practical Synthetic Route of a PDE V Inhibitor KF31327

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Abstract:

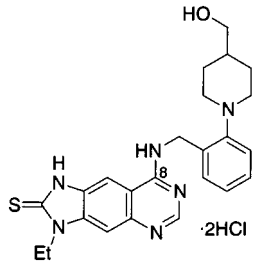
An efficient route suitable for a large-scale preparation of KF31327 (1), a potent phosphodiesterase V inhibitor, has been developed. We selected 7-chloro-2,4(1*H*,3*H*)-quinazolinedione (15) as a starting material, which gave the desired 6-nitro compound with good selectivity. In the chlorination of 7-ethylamino-6-nitro-2,4(1*H*,3*H*)-quinazolinedione (17), reaction conditions were optimized to minimize the amount of phosphorus oxychloride, and 2,4-dichloro-7-ethylamino-6-nitroquinazoline (14) was obtained in excellent yield. After the selective substitution at C4 position, the chloro substituent at C2 position was successfully removed by hydrogenation concomitant with the reduction of nitro group. The construction of the imidazothione ring was achieved by using phenyl isothiocyanate as a thiocarbonyl donor instead of extremely flammable carbon disulfide. Multikilograms of drug substance have been successfully prepared by these procedures.

Introduction

Phosphodiesterases (PDEs), which hydrolyze cyclic nucleotides such as cAMP and cGMP, have been classified into 11 isozyme families.[1] KF31327 (**1**) shows selective inhibitory activity against cGMP-specific phosphodiesterase, PDE V.[2] It is expected to be effective in treating or relieving cardiovascular diseases such as thrombosis, angina pectoris, hypertension, cardiac insufficiency, arteriosclerosis, etc. and in treating erectile dysfunction.

To support further pharmacological evaluation and clinical trials, a large-scale preparation of KF31327 was required. However, the original medicinal chemistry route had several drawbacks from the standpoint of the commercial production of KF31327, and thus it was necessary to develop an alternative synthetic route. We report on the process development for a kilogram-scale synthesis of KF31327.

The Medicinal Chemistry Route. The characteristic structure of KF31327 (**1**) consists of an imidazothione ring

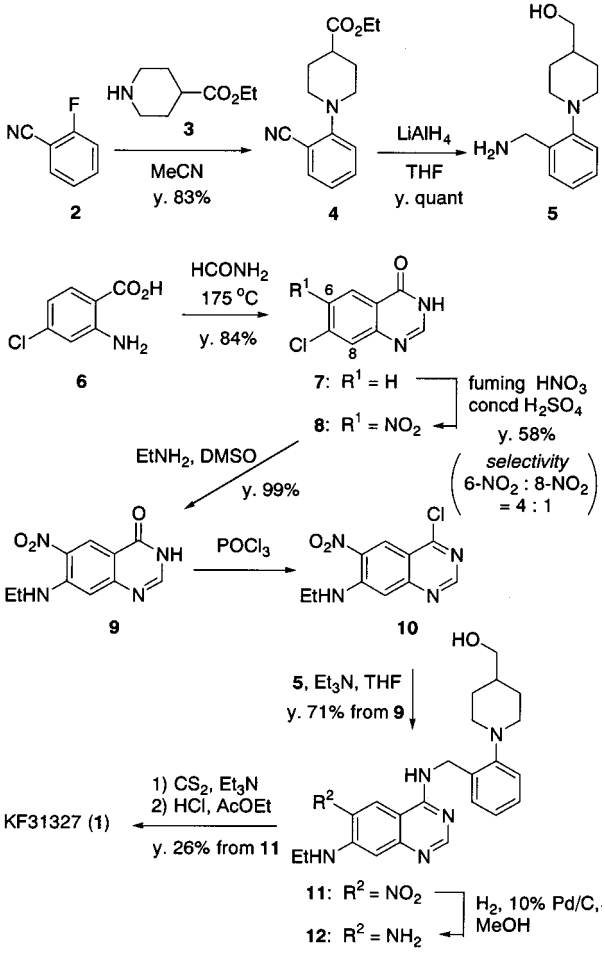


KF31327 (**1**)

attached to a quinazoline, substituted by benzylamine side chain at C8 position. Medicinal chemists developed the route of KF31327 as shown in Scheme 1.[2] While this route was used for the investigation of structure−activity relationships studies leading to the discovery of KF31327, it had several issues to be solved for a large-scale production as follows:

(1) The use of a flammable reagent, $LiAlH_4$, for the simultaneous reduction of ester and nitrile groups of **4**.

Scheme 1. **Medicinal chemistry route to KF31327**



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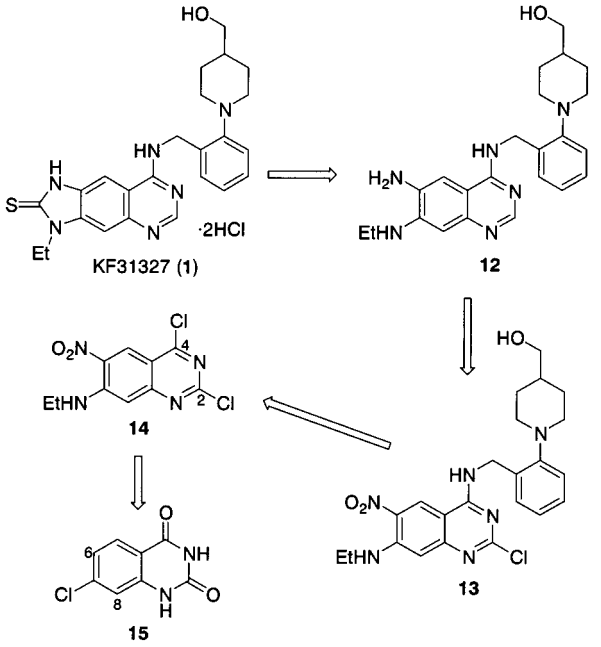
Scheme 2. **Retrosynthetic route to KF31327**



(2) A high temperature (175 °C) required to construct the quinazolone skeleton **7**.

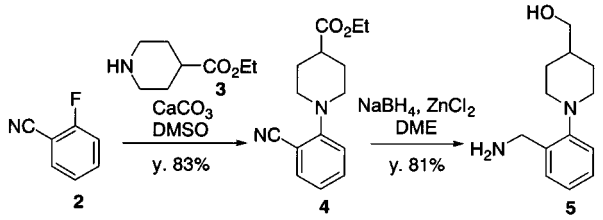
(3) Low yield and low selectivity in the nitration of **7**.

(4) The use of a large amount (22 mol equiv) of phosphorus oxychloride ($POCl_3$), which causes a problematic working-up procedure and environmental issues.

(5) The use of an extremely flammable reagent, carbon disulfide (CS_2), in the construction of the imidazothione ring of **1**.

Synthetic Strategy. The synthetic strategy of KF31327 is shown in Scheme 2. In the construction of the quinazoline skeleton, we selected commercially available 7-chloro-2,4-(1*H*,3*H*)-quinazolinedione (**15**) as the starting material. It is known that the nitration of **15** gave the desired 6-nitro-2,4-(1*H*,3*H*)-quinazolinedione with excellent selectivity and in high yield.[3] Moreover, it was reported that the treatment of 2,4(1*H*,3*H*)-quinazolinedione derivatives with $POCl_3$ in the presence of base gave 2,4-dichloroquinazoline derivatives in high yield,[4] and the C4 position on the quinazoline ring showed a higher reactivity than C2 position.[4d,5] After the selective substitution by a benzylamine derivative at C4 position, the removal of the 2-chloro group in the compound **13** would be achieved concomitant with the reduction of the nitro group.[6] In the preparation of the benzimidazole-2-thione derivatives such as KF31327 from the phenylenediamine derivative, isothiocyanate esters,[7] 1,1′-thiocarbonyl-diimidazole[8] or potassium ethylxanthate[9] would be practical reagents instead of CS_2.

Scheme 3. **Preparation of 5**



Results and Discussion

Preparation of Benzylamine 5. First, in the preparation of 2-[4-(ethoxycarbonyl)piperidino]benzonitrile (**4**) (Scheme 3), the condensation of 2-fluorobenzonitrile (**2**) and ethyl isonipecotate (**3**) was performed in DMSO in the presence of $Ca(OH)_2$ to scavenge the produced hydrogen fluoride, a toxic gas. However, it was found that $Ca(OH)_2$ caused hydrolysis of the ester group of the nitrile **4.** Thus, we selected $CaCO_3$, which was able to be an alternative of $Ca(OH)_2$ without hydrolysis. After the filtration of insoluble CaF_2, **4** was obtained in 83% yield by crystallization from aqueous MeOH. The powerful reducing agent $LiAlH_4$ has several drawbacks for scale-up. It is flammable, corrosive, and moisture-sensitive in nature, and the vigorous interaction with water compels careful decomposition treatment in the workup. To avoid the use of $LiAlH_4$ in the reduction of nitrile and ester groups of **4**, we investigated the alternative safer reducing reagents, $LiBH_4$[10] and $NaBH_4-ZnCl_2$.[11] Although the reaction with $LiBH_4$ was incomplete, the reduction with $NaBH_4-ZnCl_2$ proceeded smoothly. This system is easy to handle on large-scale and inexpensive. This reaction proceeded quantitatively to give 2-[4-(hydroxymethyl)piperidino]benzylamine (**5**) in 81% yield after crystallization from toluene.

Preparation of Quinazolinedione 17. The nitration of 4-chloro-2,4(1*H*,3*H*)-quinazolinedione (**15**) (Scheme 4) was carried out according to the known procedure[3] using fuming HNO_3 and concd H_2SO_4 to give a 10:1 mixture (based on HPLC analysis) of 7-chloro-6-nitro-2,4(1*H*,3*H*)-quinazolinedione (**16**) and the isomer, 7-chloro-8-nitro-2,4(1*H*,3*H*)-quinazolinedione. Furthermore, we found that the regio-

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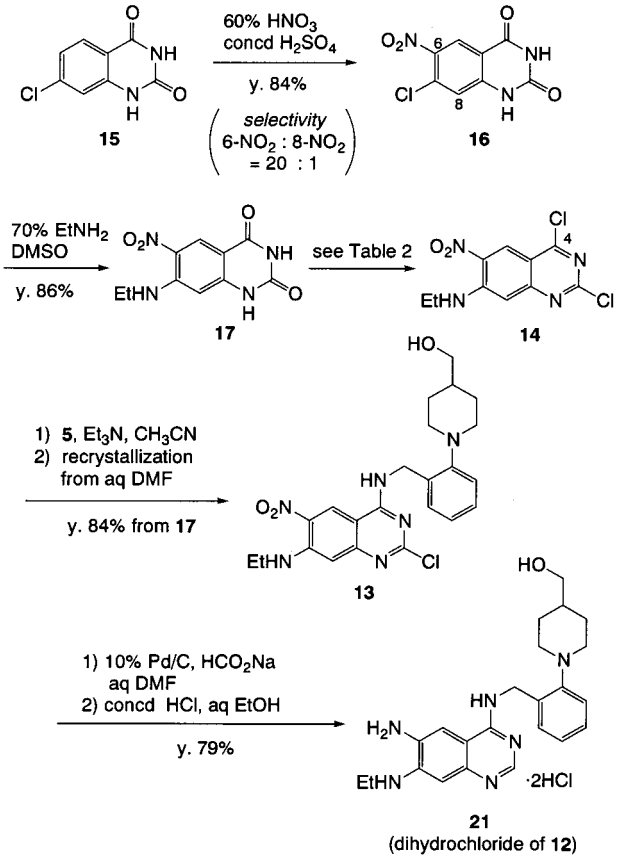
Scheme 4. **Improved synthesis to 21**



selectivity in the nitration was improved to 20:1 using 60% HNO_3 instead of fuming HNO_3. The compound **16** containing 5.0% of the isomer was isolated in 84% yield. Subsequent ethylamination at C7 position of **16** was performed by dropwise addition of 3.0 equiv of 70% aqueous ethylamine into the solution of **16** in DMSO at 80 °C. The direct crystallization from the reaction mixture by an addition of MeOH afforded the crude solids, and the subsequent purification by slurrying in MeOH gave almost pure 7-ethylamino-6-nitro-2,4(1*H*,3*H*)-quinazolinedione (**17**) in 86% yield. This solid contained 0.2% of 7-ethylamino-8-nitro-2,4(1*H*,3*H*)-quinazolinedione based on HPLC analysis.

Optimization of Chlorination. In the preparation of 2,4-dichloro-7-ethylamino-6-nitroquinazoline (**14**) from quinazolinedione **17**, we examined several chlorinating reagents,[12] such as PCl_3, PCl_5, and $POCl_3$. All attempts resulted in failure except that with $POCl_3$. In the preliminary studies,[13] **14** was obtained in 53% yield with 8.0 equiv of $POCl_3$ and 1.0 equiv of *i*-Pr_2NEt without solvent. We endeavored to improve the yield of **14** and to reduce the amount of $POCl_3$. The reaction using 1,2-dichloroethane as a solvent was investigated in detail. We found that subtle changes in the amount of *i*-Pr_2NEt remarkably affected the yield of **14**, and the graph showed a bell-shape curve as shown in Figure 1. There were

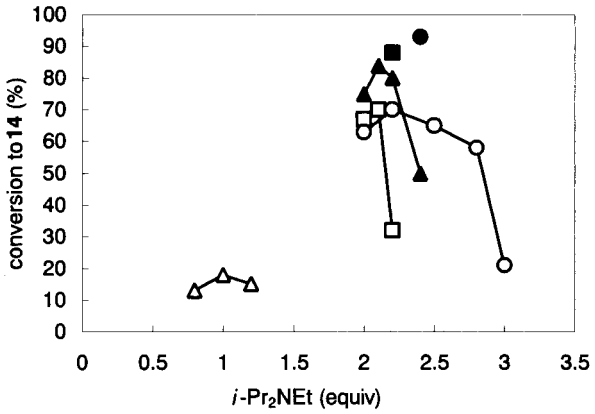


Figure 1. **Effect of equivalents of $POCl_3$ and *i*-Pr_2NEt in the chlorination reaction. All reactions were conducted on a 200-mg scale in $ClCH_2CH_2Cl$ (2 mL) at 60 °C for 1 or 2 h. Key to symbols depends on the equiv of $POCl_3$: (●) 8.0 equiv; (■) 5.0 equiv; (▲) 4.0 equiv; (○) 3.0 equiv; (□) 2.0 equiv; (△) 1.0 equiv. Conversion was determined by HPLC analysis.**

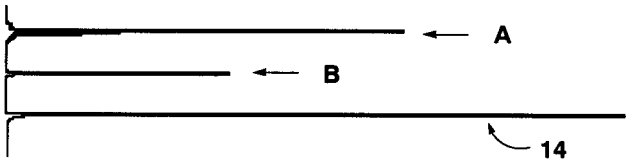


Figure 2. **HPLC analysis of the reaction mixture. Reaction was conducted on a 200-mg scale in the presence of 4.0 equiv of $POCl_3$ and 2.1 equiv of *i*-Pr_2NEt in $ClCH_2CH_2Cl$ (2 mL) at 60 °C for 1 h. t_R (min) A (4.5), B (13.5), 14 (23.0).**

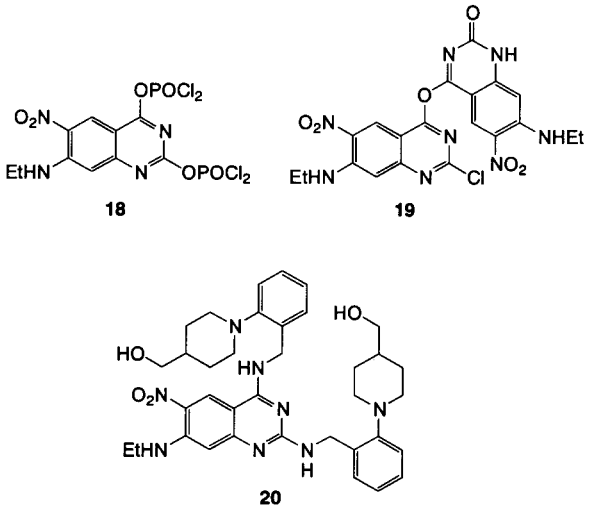


Figure 3. **Proposed structures of by-products.**

two observations concomitant with the lower yield. First, the starting material remained when the amount of base was less than the optimal point. Second, the peak area of unknown products (**A** and **B**) increased on HPLC analysis as shown in Figure 2 when the amount of base was more than the optimal point.

It was difficult to determine the structure of compound **A** because it was too unstable to isolate. We speculated that the compound **A** was the phosphoric ester intermediate **18** as shown in Figure 3, since the amount of **14** increased, concomitant with the disappearance of **A**, when chloride

(12) The treatment of Vilsmeier reagent ($SOCl_2$−DMF) with quinazolinedione yields *N*,*N*-dimethyl-2-[4-oxo-3(4*H*)-qunazolinyl]benzamide and *N*-formylanthranilic acid. Prashad, M.; Har, D.; Repic, O.; Blackloc, T. J. *Tetrahedron Lett.* **1997,** *38*, 1313−1316.

(13) *i*-Pr_2NEt showed the best results among bases examined such as *N*,*N*-dimethylaniline, *N*-methylmorpholine, *n*-Pr_3N, Et_3N, and 4-dimethylaminopyridine.

Table 1. **Effect of temperature in chlorination reaction**[a]

	conditions		product ratio (%)[b]		
entry	temp (°C)	time (h)	**14**	**18**	**17**
1	4	1	7	35	46
		3	66	10	14
		5	58	10	18
2	60	1	74	1	19
		3	88	1	0
		5	89	1	0
3	80	1	88	2	0
		3	88	1	0
		5	88	1	0

[a] Reactions were conducted on a 200-mg scale in the presence of 4.0 equiv of $POCl_3$ and 2.1 equiv of *i*-Pr_2NEt in $ClCH_2CH_2Cl$ (2 mL). [b] Ratios of the products were calculated from HPLC peak area of **14**, **18**, and **17**.

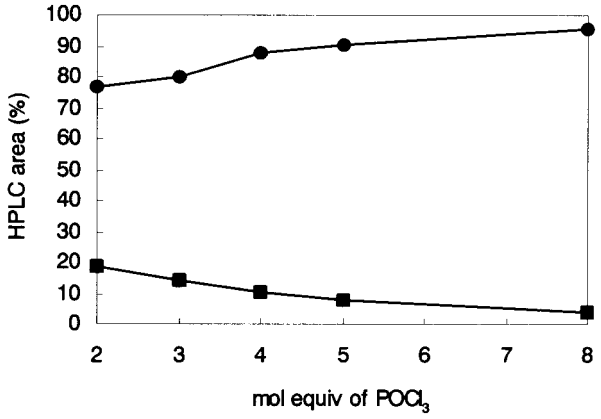


Figure 4. **Effect of the amount of $POCl_3$. The products' HPLC area % at the optimal condition on each equiv of *i*-Pr_2NEt shown in Figure 1 were plotted. Key to symbols: (●) 14; (■) 19.**

anion sources such as concentrated HCl or LiCl were added into the reaction mixture. Next, the effect of reaction temperature was investigated, and the results are shown in Table 1. The higher temperature accelerated the reaction and resulted in the higher conversion from **17** into **14** via **18**. We speculated that the lower yield of **14** at lower temperature was a result of incomplete reaction of the intermediate **18** with chloride anion. The product **B** was isolated and was assigned as a dimer **19** by ^1H NMR and mass spectra (Figure 3).[14] The conversion to **19** decreased concurrently with the increase of the amount of $POCl_3$ as shown in Figure 4.

In consideration of the above observations, we optimized the reaction conditions and selected a suitable solvent (Table 2). $POCl_3$ and 1,2-dichloroethane should be reduced or excluded from the standpoint of an environmentally benign process. Finally, we achieved the optimum conditions using 5.0 equiv of $POCl_3$, 2.2 equiv of *i*-Pr_2NEt, and toluene as a solvent to obtain **14** in 97% yield based on HPLC analysis (Table 2, entry 6). Furthermore, we developed robust work-up procedures. After the evaporation of excess $POCl_3$, the residue was dissolved in AcOEt and washed with a buffer,

(14) **19**: ^1H NMR ($CDCl_3$) δ 1.24 (t, $J = 7.1$ Hz, 3H), 1.29 (t, $J = 7.1$ Hz, 3H), 3.25−3.50 (m, 4H), 6.56 (s, 1H), 7.20 (s, 1H), 7.92 (t, $J = 5.5$ Hz, 1H), 8.62 (s, 1H), 9.05 (s, 1H); EIMS calcd for $C_{20}H_{17}{}^{35}ClN_8O_6$ *m/z* 500, found 500 $(M)^+$, 485 $(M - CH_3)^+$.

Table 2. **Optimization in the preparation of 14**[a]

	conditions					
entry	equiv of $POCl_3$	equiv of *i*-Pr_2NEt	solvent	concn (mol/L)	time (h)	yield (%)[c]
1	4.0	2.1	DCE[b]	0.3	1	84
2	5.0	2.1	DCE	0.3	7	88
3	5.0	2.2	DCE	0.3	7	88
4	5.0	2.2	DCE	0.7	5	92
5	5.0	2.4	DCE	0.7	7	89
6	5.0	2.2	toluene	0.7	2	97

[a] All reactions were conducted on a 200-mg scale at 80 °C. [b] 1,2-Dichloroethane. [c] Yields were determined by quantitative HPLC analysis.

2 mol/L K_2HPO_4, which was selected because an acidic work-up condition causes decomposition of **14**.[4d] The organic layer was used for the next reaction without isolation of **14**.

Preparation of the 6-Amino-7-ethylamino-quinazoline Derivative 21. The condensation of dichloroquinazoline **14** and benzylamine **5** proceeded with high selectivity at C4 position in the presence of Et_3N. The C2-substituted derivative was not detected, and the crude C4-substituted 2-chloroquinazoline **13** was obtained simply by filtration from the reaction mixture. The crude **13** was purified by recrystallization from aqueous DMF to remove a small amount of 2,4-disubstituted by-product **20**[15] (0.1% formation based on HPLC) and a large amount of triethylamine hydrochloride. The pure **13** was obtained as mono DMF solvate in 84% yield from **17**. This solvated form was clearly identified by thermal analysis and ^1H NMR studies.[16]

The simultaneous reduction of the nitro and chloro groups of **13** was accomplished by treatment with palladium on carbon and HCO_2Na in aqueous DMF at 80 °C. The 6-amino-7-ethylamino-quinazoline **12** was isolated as a dihydrochloride salt **21** by treatment with HCl in aqueous EtOH in 79% yield.

Imidazothione Construction to KF31327. Our attention then turned to implementing the construction of the imidazothione ring of KF31327. After an extensive investigation, we selected phenyl isothiocyanate (C_6H_5NCS)[7b−d] instead of extremely flammable carbon disufide (CS_2). The reaction conditions were investigated using the free base **12**, and the results are shown in Table 3. C_6H_5NCS reacted with **12** to give the imidazothione **23**, free base of KF31327, via unstable thiourea derivative **22**[17] as shown in pathway A (Scheme 5). However, the problem in this step was the

(15) **20**: ^1H NMR ($CDCl_3$) δ 1.34−2.17 (m, 15H), 2.73−2.80 (m, 4H), 3.16−3.32 (m, 6H), 3.54−3.56 (m, 4H), 4.83 (br s, 4H), 7.23−7.36 (m, 11H), 7.82 (t, $J = 4.8$ Hz, 1H), 8.50 (s, 1H); SIMS calcd for $C_{36}H_{46}N_8O_4$ *m/z* 655 $(M + H)^+$, found 655.

(16) Thermogravimetry and differential thermal analysis (TG-DTA) were performed using a MacSience TG-DTA 2000, and the heating rate was 10 °C/min from 25 to 300 °C. The DTA curve showed the endothermic peak at 115 °C. The TG curve indicated that 13% of the weight proportion, which corresponds to DMF portion in the 1:1 solvated form, diminished at 120 °C. Furthermore, ^1H NMR data and the elemental analysis indicated the solvated form. See the Experimental Section.

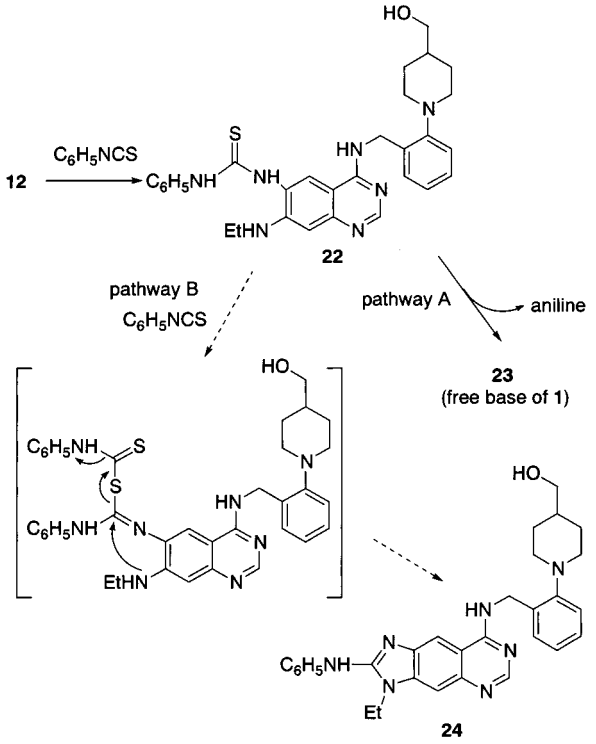
(17) **22**: ^1H NMR (DMSO-d_6) δ 1.21 (t, $J = 7.0$ Hz, 3H), 1.30−1.37 (m, 2H), 1.44−1.49 (m, 1H), 1.74−1.78 (m, 2H), 2.59−2.67 (m, 2H), 3.06−3.10 (m, 2H), 3.21 (dq, $J = 5.6$, 7.0 Hz, 2H), 3.30−3.32 (m, 2H), 4.47 (t, $J = 5.3$ Hz, 1H), 4.77 (d, $J = 5.6$ Hz, 2H), 4.56 (t, $J = 5.4$ Hz, 1H), 6.62 (s, 1H), 6.91−7.50 (m, 9H), 8.04 (s, 1H), 8.20−8.23 (m, 2H), 9.17 (s, 1H), 9.76 (s, 1H); SIMS calcd for $C_{30}H_{36}N_7OS$ *m/z* 542 $(M + H)^+$, found 542.

Table 3. **Optimization in the preparation of 23**[a]

	conditions				product ratio (%)[c]		
entry	solvent	temp (°C)	time (h)	method[b]	**23**	**24**	**12**
1	DMF	70	2	A	80	16	4
2	CH_3CN	70	6	A	63	23	14
3	EtOH	70	10	A	89	4	7
4	EtOH	78	10	A	92	3	5
5	MeOH	65	10	B	91	4	5
6	EtOH	78	10	B	94	3	3
7	*i*-PrOH	82	8	B	6	65	29
8	*n*-PrOH	97	4	B	96	2	2
9	*n*-PrOH	97	9	C	97	1	2

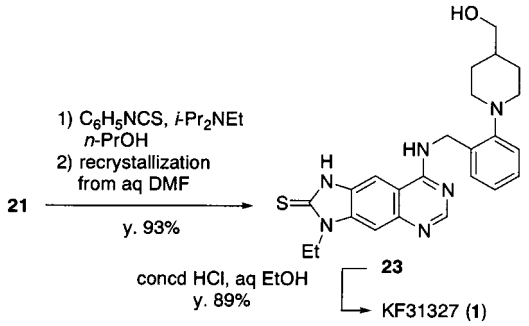
[a] All reactions were conducted on a 100-mg scale. [b] A: C_6H_5NCS (2.0 equiv) was added at room temperature in the presence of AcOH (1.0 equiv). B: C_6H_5NCS (2.0 equiv) was added at the reaction temperature. C: C_6H_5NCS was added portionwise in five times (0.4 equiv × 5) at the reaction temperature. [c] Ratios of the products were calculated from HPLC peak area of **12**, **23**, and **24**.

Scheme 5. **Reaction mechanism**



formation of an unexpected imidazole derivative **24**[18] derived from pathway B[19] as a major by-product (Scheme 5). Our investigation revealed that the formation of **24** was suppressed in alcohols rather than in other solvents, and a higher temperature resulted in better yield (Table 3). Although we found the advantageous effect of the addition of AcOH in the preliminary study, AcOH was not necessary when C_6H_5-NCS was added into a hot reaction mixture. The formation of **24** depended on the type of alcohols. *i*-PrOH showed a poor result because of poor solubility of the substrate. *n*-PrOH showed the best result of all examined alcohols under reflux conditions. To prevent the formation of **24**, we examined portionwise addition of C_6H_5NCS to keep the concentration of C_6H_5NCS low and obtained good results. Finally, the formation of **24** was suppressed to 1% and the yield of **23** was improved to 97% under the optimal condition (entry 9); portionwise addition of isothiocyanate into the reaction mixture under reflux in *n*-PrOH. This method is superior to the original method because of the easiness in handling of the reagent.

Scheme 6. **Imidazothione construction to KF31327**



The dihydrochloride salt **21**, as well as the free base **12**, was treated according to the conditions described above in the presence of *i*-Pr_2NEt (3.5 equiv) to give the free base **23**, which was isolated from the cooled reaction mixture only by a filtration. The crude **23**, contained small amount of **12**, **24**, and 1,3-diphenylthiourea which was produced by the side reaction of C_6H_5NCS with aniline, was recrystallized from DMF to give pure **23** as mono DMF solvate[20] in 93% yield. Then, the free base **23** was converted to the corresponding dihydrochloride salt, KF31327 (**1**), in 89% yield with 99.9% purity by treatment with concentrated HCl in aqueous EtOH (Scheme 6). The crystals were kept under 75% relative humidity at 25 °C, and the final water content of KF31327 (**1**) was 14% (wt %). Although KF31327 has a hygroscopic property at the initial period, the water content always remains constant (14%) after the treatment described above. As TG-DTA showed no evidence of the hydrated form of **1**, we speculate this water as resulting from physical adsorption.

Conclusions

We established an efficient process for a large-scale synthesis of KF31327 (**1**), which provided a higher overall yield than the original synthesis (41% from **15** vs 9% from **6**). A kilogram-scale synthesis of **1** was successfully achieved by using these procedures, and the drug substances were supplied for the pharmaceutical development.

Experimental Section

General Procedures. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC-300 or a JEOL LA300 spectrom-

(18) **24**: ^1H NMR (DMSO-d_6) δ 1.29−1.48 (m, 6H), 1.75−1.80 (m, 2H), 2.62−2.70 (m, 2H), 3.09−3.14 (m, 2H), 3.25−3.40 (m, 2H), 4.38 (q, J = 7.0 Hz, 2H), 4.48 (t, J = 5.2 Hz, 1H), 4.87 (d, J = 5.1 Hz, 2H), 6.92−7.04 (m, 2H), 7.10−7.18 (m, 3H), 7.33−7.38 (m, 2H), 7.56 (s, 1H), 7.93−7.96 (m, 2H), 8.25 (s, 1H), 8.31−8.34 (m, 1H), 9.19 (s, 1H); SIMS calcd for $C_{30}H_{34}N_7O$ m/z 508 $(M + H)^+$, found 508.

(19) A similar reaction mechanism with *N*,*N*′-dicyclohexylcarbodiimide instead of C_6H_5NCS was reported: (a) AboulWafa, O. M.; Omar, A. M. M. E. *Sulfur Lett.* **1992**, *14*, 181−188. (b) Ram, S.; Wise, D. S.; Townsend, L. B. *Org. Prep. Proced. Int.* **1985**, *17*, 215−218. (c) Omar, A. M. M. E.; Habib, N. S.; AboulWafa, O. M. *Synthesis* **1977**, 864−865.

(20) TG-DTA was performed on the condition described above. The DTA curve showed the endothermic peak at 228 °C. The TG curve indicated that 12% of the weight proportion, which corresponds to the DMF portion in the 1:1 solvated form, diminished at 120 °C. Furthermore, ^1H NMR data and the elemental analysis indicated the solvated form. See the Experimental Section.

eter, and signals are given in ppm using TMS as an internal standard. IR spectra were recorded on a Shimadzu FTIR-4300 spectrometer. MS spectra were measured on a Hitachi M-80B mass spectrometer using EI or SIMS for ionization. HRLCMS were recorded on a Micromass LCT mass spectrometer. Elemental analyses were performed using a Perkin-Elmer/2400 II CHN apparatus. Melting points were measured on Mettler FP 61 and are uncorrected. All reagents and solvents were of commercial quality.

HPLC Analyses. The HPLC data in Figures 1, 2, 4, and Table 1 were obtained under the following conditions: detector, ultraviolet absorption photometer (wavelength 254 nm); column, Inertsil ODS-2; mobile phase, a mixture of 0.01 mol/L KH_2PO_4 and 0.01 mol/L sodium 1-octanesulfonate: CH_3CN (1:1.5); flow rate, 1.0 mL/min; column temperature, 35 °C; t_R (min) **18** (4.5), **17** (5.2), **19** (13.5), **14** (23.0). The product ratio of **12**, **23**, and **24** in Table 2 was estimated under the following conditions: detector, ultraviolet absorption photometer (wavelength 254 nm); column, YMC ODS AM-312; mobile phase, a mixture of 0.01 mol/L KH_2PO_4 and 0.01 mol/L sodium 1-octanesulfonate:MeOH (1:2); flow rate, 1.0 mL/min; column temperature, 35 °C; t_R (min) **12** (9.0), **24** (12.0), **23** (14.0).

2-[4-(Ethoxycarbonyl)piperidino]benzonitrile (4). To a mixture of 2-fluorobenzonitirile (**2**) (200 g, 1.65 mol) and ethyl isonipecotate (**3**) (387 g, 2.47 mol) in DMSO (800 mL) was added $CaCO_3$ (165 g 1.65 mol). The mixture was then stirred at 120 °C for 7 h and cooled to room temperature. This mixture was filtered through a pad of Celite and subsequently washed with AcOEt (2000 mL). The filtrate was washed with H_2O (2000 mL) and concentrated under reduced pressure. The resulting residue was crystallized from a mixture of MeOH (1200 mL) and H_2O (360 mL), and the precipitated solids were collected, washed with H_2O (720 mL), and dried under vacuum to afford **4** as a colorless solid: 335 g (83%), mp 43−44 °C; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, J = 7.1 Hz, 3H, CH_2CH_3), 1.94−2.12 (m, 4H, 3′-H), 2.42−2.52 (m, 1H, 4′-H), 2.85−2.93 (m, 2H, 2′-H), 3.52−3.72 (m, 2H, 2′-H), 4.17 (q, J = 7.1 Hz, 2H, CH_2CH_3), 6.96−7.01 (m, 2H, 3-H and 5-H), 7.46 (ddd, J = 1.7, 7.4, 8.3 Hz, 1H, 4-H), 7.55 (ddd, J = 0.6, 1.7, 7.4 Hz, 1H, 6-H); ^{13}C NMR ($CDCl_3$) δ 14.16, 28.17, 40.58, 51.45, 60.43, 106.10, 118.34, 118.77, 121.51, 133.60 134.19, 156.12, 174.51; IR (KBr) 2230, 1730, 1595, 1490, 1445, 1310 cm^{-1}; EIMS m/z 258 $(M)^+$, 213 $(M - OCH_2CH_3)^+$; HRMS calcd for $C_{15}H_{19}N_2O_2$ m/z 259.1447 $(M + H)^+$, found 259.1438 (−0.9 mDa); Anal. Calcd for $C_{15}H_{18}N_2O_2$: C, 69.74; H, 7.02; N, 10.84. Found: C, 69.61; H, 7.04; N, 10.74.

2-[4-(Hydroxymethyl)piperidino]benzylamine (5). To a solution of **4** (100 g, 0.39 mol) in 1,2-dimethoxyethane (1500 mL) were added $NaBH_4$ (58.6 g, 1.55 mol) and $ZnCl_2$ (105 g, 0.78 mol), and the mixture was stirred for 7 h under reflux. After cooling to room temperature, to the reaction mixture were added 1,2-dimethoxyethane (215 mL), H_2O (85 mL), and saturated NH_4Cl (700 mL). Then the aqueous layer was separated, adjusted to pH 1 with 6 mol/L HCl, and stirred at room temperature for 2 h. Toluene (1000 mL) was added to the mixture, and the pH of the mixture was adjusted to 14 with 10 mol/L NaOH. The organic layer was separated and concentrated under reduced pressure. The resulting residue was crystallized from toluene (200 mL), and the precipitated crystals were collected, washed with cold toluene (200 mL), and dried under vacuum to afford **5** as a colorless solid: 69 g (81%), mp 94−95 °C; ^{1}H NMR ($CDCl_3$) δ 1.40−1.49 (m, 2H, 3′-H), 1.57−1.72 (m, 1H, 4′-H), 1.86 (br d, J = 12.3 Hz, 2H, 3′-H), 2.15 (br s, 3H, NH_2 and OH), 2.71 (ddd, J = 2.1, 11.8, 11.8 Hz, 2H, 2′-H), 3.11 (br d, J = 11.8 Hz, 2H, 2′-H), 3.54 (d, J = 6.3 Hz, 2H, CH_2O), 3.86 (s, 2H, CH_2NH_2), 7.07 (ddd, J = 1.3, 7.3, 7.7 Hz, 1H, 5-H), 7.13 (dd, J = 1.3, 7.7 Hz, 1H, 3-H), 7.23 (ddd, J = 1.7, 7.7, 7.7 Hz, 1H, 4-H), 7.26 (dd, J = 1.7, 7.3 Hz, 1H, 6-H); ^{13}C NMR ($CDCl_3$) δ 29.80, 38.45, 43.48, 53.20, 66.91, 120.42, 123.93, 127.67, 128.42, 138.02, 152.04; IR (KBr) 2900, 2800, 1600, 1490, 1450, 1380 cm^{-1}; EIMS m/z 220 $(M)^+$, 203 $(M - OH)^+$; HRMS calcd for $C_{13}H_{21}N_2O$ m/z 221.1654 $(M + H)^+$, found 221.1654 (0.0 mDa); Anal. Calcd for $C_{13}H_{20}N_2O$: C, 70.87; H, 9.15; N, 12.72. Found: C, 70.74; H, 9.26; N, 12.54.

7-Chloro-6-nitro-2,4(1*H*,3*H*)-quinazolinedione (16).[3] To a solution of **15** (205 g, 1.02 mol) in concentrated H_2SO_4 (1000 mL) was added dropwise 60% HNO_3 (78 mL, 0.51 mol) at 0 °C. The mixture was stirred for 2 h below 30 °C. Then the reaction mixture was poured dropwise into a mixture of MeOH (3300 mL) and H_2O (3300 mL) at 4 °C over 1 h, and stirred for 1 h at the same temperature. The precipitated solids were collected, washed with a cold mixture of MeOH (600 mL) and H_2O (600 mL), and dried under vacuum to afford a colorless solid (210 g). Then this solid was stirred in suspension in a mixture of MeOH (2500 mL) and H_2O (2250 mL), collected, washed with a cold mixture of MeOH (600 mL) and H_2O (600 mL), and dried under vacuum to afford **16** as a colorless solid: 208 g (84%), containing 5.0% of 7-chloro-8-nitro-2,4(1*H*,3*H*)-quinazolinedione based on HPLC analysis; ^{1}H NMR ($CDCl_3$) and IR (KBr) spectra agreed with those of a literature.[3]

7-Ethylamino-6-nitro-2,4(1*H*,3*H*)-quinazolinedione (17). To a solution of **16** (200 g, 0.88 mol) in DMSO (2000 mL) was added dropwise 70% aqueous $EtNH_2$ (194 mL, 2.40 mol) at 80 °C for 1 h, and the mixture was stirred at the same temperature for 3 h. After an addition of MeOH (2000 mL) at 30 °C, the mixture was stirred for 2 h at the same temperature. The precipitated crystals were collected and washed with MeOH (600 mL). The crystals were stirred in suspension in MeOH (1000 mL) at 30 °C for 3 h, collected, and washed with MeOH (200 mL), and then dried under vacuum to afford **17** as a bright yellow solid: 180 g (86%) (containing 0.2% of 7-ethylamino-8-nitro-2,4(1*H*,3*H*)-quinazolinedione based on HPLC analysis), mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 1.28 (t, J = 7.1 Hz, 3H, CH_2CH_3), 3.33 (dq, J = 5.5, 7.1 Hz, 2H, CH_2CH_3), 6.43 (s, 1H, 8-H), 8.33 (t, J = 5.5 Hz, 1H, NH), 8.59 (s, 1H, 5-H), 11.24 (br s, 2H, 1-H and 3-H); ^{13}C NMR (DMSO-d_6) δ 13.48, 37.46, 95.51, 103.86, 128.02, 128.17, 145.65, 147.97, 150.28, 161.42; IR (KBr) 3370, 3020, 1720, 1682, 1632, 1460, 1292 cm^{-1}; EIMS m/z 250 $(M)^+$, 235 $(M - CH_3)^+$, 190 $(M\text{-}CH_3, NO_2)^+$; HRMS calcd for $C_{10}H_{11}N_4O_4$ m/z 251.0780 $(M + H)^+$, found

251.0782 (0.2 mDa); Anal. Calcd for $C_{10}H_{10}N_4O_4$: C, 48.00; H, 4.03; N, 22.39. Found: C, 47.78; H, 3.98; N, 22.14.

2-Chloro-7-ethylamino-4-[[[2-[4-(hydroxymethyl)-1-piperidinyl]phenyl]methyl]amino]-6-nitroquinazoline (13). To a suspension of **17** (200 g, 0.80 mol) in toluene (600 mL) were added $POCl_3$ (374 mL, 4.00 mol) and *i*-Pr_2NEt (306 mL, 1.76 mol) at room temperature. The mixture was stirred at 80 °C for 2 h, and toluene (5000 mL) was added at 45 °C. The mixture was concentrated to 3000 mL under reduced pressure, and then AcOEt (1000 mL) was added. The solution was poured dropwise into 2 mol/L K_2HPO_4 (6000 mL) at 4 °C over 1 h. The resulting precipitates were filtered off through a pad of Celite and subsequently washed with AcOEt (1000 mL). The filtrate was separated, and the organic layer was concentrated to 3000 mL of the volume under reduced pressure. The solution contained 223 g of 2,4-dichloro-7-ethylamino-6-nitroquinazoline (**14**) (based on HPLC analysis; 97% yield) and was used for the following reaction without isolation. Compound **14** was isolated for analyses by purification using a silica gel column chromatography to afford **14** as a yellow solid: mp 136−138 °C; ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.2 Hz, 3H, CH_2CH_3), 3.43 (dq, J = 5.0, 7.2 Hz, 2H, CH_2CH_3), 7.10 (s, 1H, 8-H), 7.92 (br s, 1H, 7-NH), 9.10 (s, 1H, 5-H); IR (KBr) 3364, 1728, 1574, 1539, 1506, 1419, 1391, 1319, 1140, 856 cm^{-1}; EIMS calcd for $C_{10}H_8{}^{35}Cl_2N_4O_4$ *m*/*z* 286, found 286 $(M)^+$, 271 $(M - CH_3)^+$.

To the above solution of **14** were added CH_3CN (3000 mL), **5** (179 g, 0.81 mol), and Et_3N (108 mL, 0.81 mol) under ice cooling, and the mixture was stirred at the same temperature for 3 h. The precipitated crystals were collected, washed with cold CH_3CN (1000 mL), and dried under vacuum to afford the crude **13** as an orange-yellow solid (432 g). The solid was recrystallized from a mixture of DMF (3000 mL) and H_2O (430 mL) to afford mono DMF solvate of **13** as an orange-yellow solid: 315 g (84% from **17**), mp 197−200 °C; ^{1}H NMR ($CDCl_3$) δ 1.39 (t, J = 7.1 Hz, 3H, CH_2CH_3), 1.51−1.76 (m, 4H, 3″-H, 4″-H and OH), 1.95−2.00 (m, 2H, 3″-H), 2.77−2.85 (m, 2H, 2″-H), 2.89 (s, 3H, DMF), 2.97 (s, 3H, DMF), 3.31−3.40 (m, 4H, CH_2CH_3 and 2″-H), 3.55 (d, J = 5.6 Hz, 2H, CH_2O), 4.95 (d, J = 4.7 Hz, 2H, 4-NHCH_2), 6.91 (s, 1H, 8-H), 7.12 (ddd, J = 1.3, 7.3, 7.3 Hz, 1H, 5′-H), 7.25 (dd, J = 1.5, 7.3 Hz, 1H, 3′-H), 7.31−7.36 (m, 2H, 4′-H and 6′-H), 7.77 (t, J = 4.7 Hz, 1H, 7-NH), 8.01 (s, 1H, DMF), 8.64 (m, 2H, 5-H and 4-NH); ^{13}C NMR (DMSO-d_6) δ 13.53, 29.39, 37.29, 38.16 (2C), 52.82, 66.00, 103.26, 105.98, 119.82, 123.32, 125.14, 127.84, 128.06, 132.14, 132.53, 146.20, 152.02, 154.23, 160.97, 161.44; IR (KBr) 3400, 3277, 2933, 1629, 1569, 1411, 1342, 1290, 1026, 767 cm^{-1}; EIMS *m*/*z* 470 (M^+), 453 $(M - OH)^+$; HRMS calcd for $C_{23}H_{27}{}^{35}ClN_6O_3$ *m*/*z* 471.1920 $(M + H)^+$, found 471.1911 (−0.9 mDa); Anal. Calcd for $C_{23}H_{27}ClN_6O_3 \cdot C_3H_7NO$: C, 57.49; H, 6.30; N, 18.02. Found: C, 57.58; H, 6.33; N, 17.80.

6-Amino-7-ethylamino-4-[[[2-[4-(hydroxymethyl)-1-piperidinyl]phenyl]methyl]amino]quinazoline Dihydrochloride (21). To a solution of mono DMF solvate of **13** (200 g, 0.37 mol) in DMF (3000 mL) were added a suspension of 10% Pd/C (40 g, 50% wet with water) in H_2O (300 mL) and a solution of HCO_2Na (199 g, 2.93 mol) in H_2O (300 mL) at room temperature under atmosphere of nitrogen. After the mixture was stirred at 80 °C for 3 h, MeOH (2000 mL) was added at 30 °C, and the mixture was stirred for 2 h at the same temperature. The insoluble solids were filtered off and washed with MeOH (3000 mL). The combined filtrate was concentrated to 400 mL under reduced pressure. EtOH (4000 mL) was added to the residue, and then the mixture was stirred under ice cooling. The resulting solid was filtered off and washed with cold EtOH (1000 mL). To the combined filtrates were added concentrated HCl (94 mL, 1.11 mol) and H_2O (600 mL) at 50 °C, and the mixture was cooled under ice for 5 h. The precipitated crystals were filtered, washed with a cold mixture of EtOH (360 mL) and H_2O (40 mL), and dried under vacuum to afford **21** as a hygroscopic yellow solid: 141 g (79%), mp 220−222 °C; ^{1}H NMR (DMSO-d_6) δ 1.28 (t, J = 7.1 Hz, 3H, CH_2CH_3), 1.66−1.86 (m, 5H, 3″-H and 4″-H), 3.15−3.44 (m, 8H, 2″-H, CH_2O and CH_2CH_3), 4.14 (br s, 4H, OH, NH_2 and 7-NH), 4.97 (d, J = 5.4 Hz, 2H, 6-NH_2), 6.65 (s, 1H, 8-H), 7.21−7.45 (m, 5H, 3′-H, 4′-H, 5′-H, 6′-H and 5-H), 8.52 (s, 1H, 2-H), 9.68 (br s, 1H, 4-NH); ^{13}C NMR (CD_3OD) δ 13.91, 28.09, 36.40, 39.09, 41.20, 57.96, 66.04, 94.42, 103.58, 104.91, 121.59, 131.59, 131.70, 131.83, 133.31, 134.79, 139.10, 141.23, 147.01, 147.12, 158.37; IR (KBr) 3230, 1635, 1541, 1350, 1246, 766 cm^{-1}; SIMS *m*/*z* 407 $(M + H)^+$; HRMS calcd for $C_{23}H_{30}N_6O$ *m*/*z* 407.2568 $(M + H)^+$, found 407.2559 (−0.9 mDa).

3-Ethyl-1,3-dihydro-8-[[[2-[4-(hydroxymethyl)-1-piperidinyl]phenyl]methyl]amino]-2*H*-imidazo[4,5-*g*]quinazoline-2-thione (23). To a suspension of **21** (containing water and estimated as 92% net content based on HPLC analysis, 145 g, 0.28 mol) and *i*-Pr_2NEt (163 mL, 0.98 mol) in *n*-PrOH (2200 mL) was added phenyl isothiocyanate (16.2 mL, 0.14 mol) five times at 1-h intervals (total 81 mL, 0.70 mol) under reflux, and the mixture was stirred under reflux for 3 h (**Caution:** phenyl isothiocyanate is known as a mutagenicity suspect agent). The mixture was stirred under ice cooling for 5 h, and the precipitates were filtered, washed with cold *n*-PrOH (220 mL), and dried under vacuum to afford a slightly yellow solid (120 g). This solid (106 g) was dissolved in DMF (2500 mL), and H_2O (1500 mL) was added dropwise to this solution at 80 °C. After stirring under ice cooling for 6 h, the resulted precipitates were filtered, washed with a cold mixture of DMF (125 mL) and H_2O (75 mL), and dried under vacuum to afford mono DMF solvate of **23** as an off-white solid: 110 g (93%), mp 282−283 °C; ^{1}H NMR (DMSO-d_6) δ 1.25−1.38 (m, 5H, CH_2CH_3 and 3″-H), 1.40−1.60 (m, 1H, 4″-H), 1.80 (d, J = 11.1 Hz, 2H, 3″-H), 2.65 (t, J = 10.9 Hz, 2H, 2″-H), 2.72 (s, 3H, DMF), 2.88 (s, 3H, DMF), 3.10 (t, J = 11.5 Hz, 2H, 2″-H), 3.20−3.50 (m, 2H, OCH_2), 4.31 (q, J = 7.1 Hz, 2H, CH_2CH_3), 4.47 (t, J = 5.4 Hz, 1H, OH), 4.84 (d, J = 5.6 Hz, 2H, 8-NHCH_2), 6.95 (m, 1H, 5′-H), 7.10−7.20 (m, 3H, 3′, 4′ and 6′-H), 7.64 (s, 1H, 4-H), 7.94 (s, 1H, DMF), 8.10 (s, 1H, 9-H), 8.37 (s, 1H, 6-H), 8.70 (t, J = 5.6 Hz, 1H, 8-NH), 13.22 (br s, 1H, 1-H); ^{13}C NMR (DMSO-d_6) δ 12.52, 29.33, 38.13, 39.37 (2C),

52.59, 65.88, 101.19, 104.88, 110.97, 119.32, 122.92, 127.22, 127.30, 130.59, 133.44, 136.48, 145.74, 151.63, 153.50, 159.67, 171.94; IR (KBr) 3319, 2906, 1672, 1591, 1545, 1465, 1377, 1244, 1095, 900, 769, 547 cm^{-1}; SIMS *m*/*z* 449 $(M + H)^+$; HRMS calcd for $C_{24}H_{29}N_6OS$ *m*/*z* 449.2124 $(M + H)^+$, found 449.2132 (0.8 mDa); Anal. Calcd for $C_{24}H_{28}N_6OS \cdot C_3H_7NO$: C, 62.17; H, 6.76; N, 18.80. Found: C, 62.29; H, 6.65; N, 18.66.

3-Ethyl-1,3-dihydro-8-[[[2-[4-(hydroxymethyl)-1-piperidinyl]phenyl]methyl]amino]-2*H*-imidazo[4,5-*g*]quinazoline-2-thione Dihydrochloride (1, KF31327). To a suspension of mono DMF solvate of **23** (118 g, 0.23 mol) in EtOH (2240 mL) and H_2O (960 mL) was added concentrated HCl (48 mL, 0.57 mol) at 65 °C, and the mixture was stirred at the same temperature for 1 h. The solution was filtered through filter paper, which was washed with a hot mixture of EtOH (760 mL) and H_2O (40 mL). The filtrate was stirred under ice cooling, and the precipitated crystals were collected, washed with a cold mixture of EtOH (150 mL) and H_2O (50 mL), and dried under vacuum to afford **1** in 99.9% purity based on HPLC as a yellow solid: 117 g (89% yield based on quantitative HPLC analysis). The crystals were subsequently controlled to 14% of water content under 75% of relative humidity at 25 °C to afford 127 g of the product: mp 282−283 °C; ^{1}H NMR (DMSO-d_6) δ 1.28 (t, J = 7.1 Hz, 3H, CH_2CH_3), 1.65−1.86 (m, 5H, 3′-H_2 and 4″-H), 3.00−3.20 (m, 6H, 2′-H and OCH_2), 4.32 (q, J = 7.1 Hz, 2H, CH_2CH_3), 5.09 (d, J = 4.4 Hz, 2H, 8-$NHCH_2$), 7.20 (br s, 1H, 5′-H), 7.10−7.70 (m, 3H, 3′-H, 4′-H and 6′-H), 7.77 (s, 1H, 4-H), 8.41 (s, 1H, 9-H), 8.86 (s, 1H, 6-H), 10.81 (br s, 1H, 8-NH), 13.76 (s, 1H, 1-H); ^{13}C NMR (DMSO-d_6) δ 12.51 (CH_2CH_3), 27.24 (3″-C), 36.17 (4″-C), 38.66 (CH_2CH_3), 40.97 (8-$NHCH_2$), 55.04 (2″-C), 65.20 (OCH_2), 97.18 (4-C), 103.07 (9-C), 108.87 (8a−C), 120.93 (3′-C), 127.79 (5′-C), 129.08 (4′-C), 129.74 (6′-C), 131.40 (1′-C), 132.06 (9a−C), 134.08 (4a−C), 137.95 (3a-C), 143.40 (2′-C), 149.40 (6-C), 160.47 (8-C), 172.95 (2-C); IR (KBr) 3257, 1598, 1477, 1406, 1359, 1130, 777 cm^{-1}; SIMS *m*/*z* 449 $(M + H)^+$; HRMS calcd for $C_{24}H_{29}N_6OS$ *m*/*z* 449.2124 $(M + H)^+$, found 449.2131 (0.7 mDa).

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