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Kilogram-Scale Synthesis of a Highly Selective α_1-Adrenoceptor Antagonist (DL-028A)

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Abstract:

This work presents an improved eight-step process, leading to kilogram quantities of high-quality DL-028A, an antihypertensive agent. The improvements include reducing the levels of toxic reagents and the removal of dangerous processes and waste gas treatment. Moreover, specification and impurity profiles were determined.

Introduction

Recent research in the field of α blockers has led to the discovery of several biologically specific α-adrenoceptor antagonists.[1−4] More recently, some α_1-antagonists are being investigated for treating dysuria secondary to benign prostatic hypertrophy.[5,6] DL-028, one newly developed α_1-antagonist,[7] is highly selective and with LDL-lowering properties.[8−10]

Furthermore, according to an in vivo antihypertensive experiment, the cardiotoxicity is greatly reduced by combination infusion with prazosin.[10] Consequently, DL-028 was selected as a leading molecule by the Development Center For Biotechnology (DCB) for preclinical study.

Chern et al. (1993) described the original synthesis of DL-028 for the first time.[10,11] The linear pathway employed a fused quinazoline **5** as the substrate that was coupled with the commercially available phenylpiperazine to generate the DL-028 skeleton (Figure 1). The same investigators later described an asymmetric synthesis of DL-028 in homochiral form,[12] in which some modification on the synthesis of early quinazoline intermediates had been reported. This work is concerned with part of their study and intends to extend the chemistry therein from bench scale to kilogram scale. However, the disclosed method required modifications to provide a scaleable process and to eliminate potentially hazardous reactions. This work concerns the preparation of kilogram quantities of DL-028 hydrochloride (i.e., DL-028A) to preclinical and toxicological specification, since a DL-028 salt had not been prepared and evaluated before.

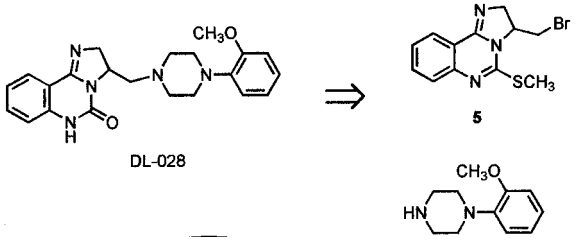
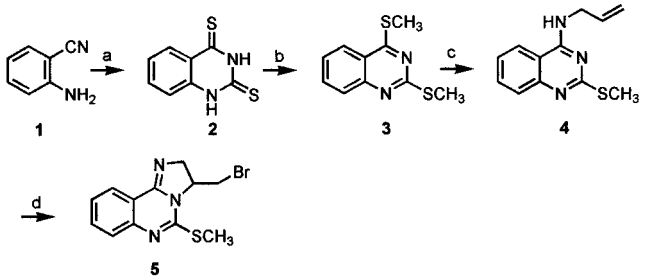


Figure 1.

Scheme 1. **Original method**[a] [10,11]



[a] a. CS_2/pyridine, reflux; b. CH_3I/NaOH, H_2O; c. allylamine/MeCN, autoclave, 120-130 °C; d. NBS/MeCN.

Results and Discussion

Reevaluation of the Original Process (Scheme 1).[10,11] The process reevaluation for the key intermediate **5** is described as follows (Scheme 1). In step one of the original process,[10,11] the quinazolinedithione **2** was formed by refluxing 2-aminobenzonitrile **1** with excess carbon disulfide in pyridine. However, carbon disulfide is a poisonous and highly flammable compound; moreover, the irritant pyridine is not amenable to scaling-up because of difficulties in workup. In step two of the original process, methylation of **2** required 2 mol equiv of methyl iodide, which is a volatile toxic and expensive agent. Consequently, this process is costly and inconvenient to perform. In step three of the original process, the substitution reaction was performed in an autoclave and required excessive amounts of highly volatile allylamine to displace the methylthio group regio-

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(7) Receptor binding studies revealed that the affinity constant (K_i) of DL-028 for α_1-adrenoceptor is more than 2000-fold greater than that for α_2 receptors.
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(11) Chern, J.-W.; Lu, G.-Y.; Lai, Y.-J.; Yen, M.-H.; Tao, P.-L. U.S. Patent 5,340,814, 1994.
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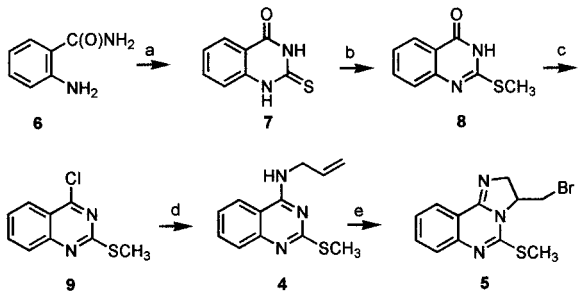
Scheme 2. **Modified method (for kilogram scale-up production)**[a]

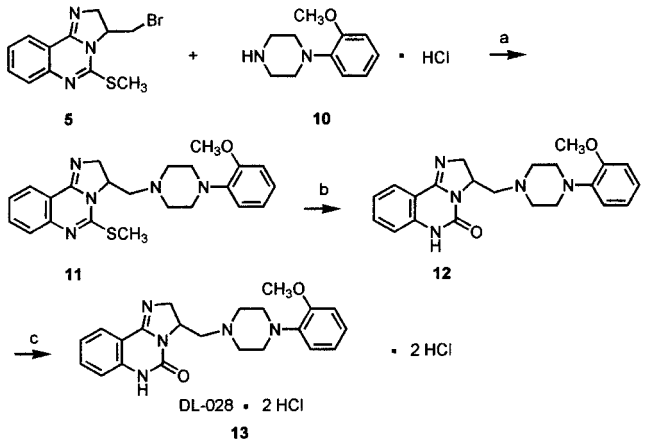


[a] a. CS_2, KOH/EtOH, reflux, 99.3%; b. $(CH_3)_2SO_4$/NaOH, H_2O, 82.5%; c. $POCl_3$/$ClCH_2CH_2Cl$, NEt_3; d. allylamine/THF, reflux, 50.9%, two steps (**8**-**4**); e. NBS/MeCN, 1.5 h, 82.0%.

selectively. The reaction is not suited for scaling-up because of serious air pollution and a potentially dangerous high-pressure operation. In step four of the original process, the bromination of **4** with concomitant dihydroimidazole ring formation was performed out in acetonitrile with *N*-bromosuccinimide. However, the bromide **5** is unstable under prolonged stirring (∼3 h), and several unidentified side products were observed; the extent of the reaction required frequent analysis, and the reaction period was minimized. Accordingly, a modified approach to these compounds was sought.

Kilogram Processes of the Intermediate 5 (Scheme 2). A modified reaction sequence was employed for kilogram-scale synthesis to circumvent problems associated with safety and cost and the concern of scale-up feasibility. The sequence employs anthranilamide **6**, which is much less expensive than 2-aminobenzonitrile **1**, as the starting material. Refluxing **6** with in situ generated potassium ethylxanthate (∼150 mol % vs **6**) in aqueous ethanol for a long period yielded 2-mercaptoquinazolin-2-one (**7**) in 99.3% isolated yield. Notably, excess potassium ethylxanthate is essential to drive the reaction to completion, otherwise, as shown by HPLC monitor, the reaction stops within a few hours and with low conversion. In the second step, the more nucleophilic quinazolin-2-thione group of **7** was selectively methylated with dimethyl sulfate in basic aqueous solution. After partial neutralization of the resulting solution with a limited amount of 3 N HCl (ca. 110 mol % vs **7**) during the workup, the unreacted **7** was left in the basic aqueous layer (pH = 9−10) in dissociated form, and the desired product **8** was selectively isolated by filtration of the resulting slurry, to yield **8** in 82.5% isolated yield. The unreacted dimethyl sulfate was quenched with ammonia water before disposal of wastewater. In the third step, **8** was heated with phosphoryl chloride in 1,2-dichloroethane that contained a stoichiometric amount of triethylamine to yield the chloride **9** as a lumpy solid, which was left in the reactor and used directly in the next step without filtration and purification. The fourth step was readily completed in refluxing THF with allylamine (∼500−700 mol % vs **9**) after a short period (1 h, 60 °C versus 48 h, 120−130 °C, autoclave, original process[10,11]) as the chloride **9** is more reactive to allylamine than is compound **3**. Product **4** is a fine granular crystalline solid of high quality, and the isolation and washing processes were much easier

Scheme 3. **Synthesis of DL-028A**[a]



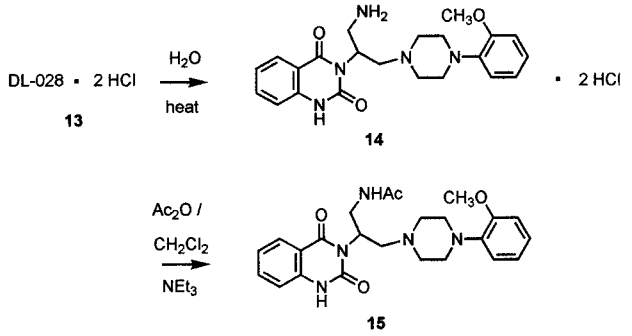
[a] a. $NaHCO_3$ (in portions)/MeCN, reflux, 66.0%; b. 1) NaOH/aqueous methanol, reflux; 2) aqueous sodium periodate, 35 °C, 81.0%; c. concd HCl/MeOH, 83.4%.

than those of original process.[10,11] The isolated yield of **4** is 50.9% (**8**-**4**, two steps). In the fifth step, bromination with concomitant cyclization was performed in the presence of *N*-bromosuccinimide (∼120 mol % vs **4**) in acetonitrile. The extent of reaction was checked by HPLC every 15 min. On completion of the reaction, the reaction was immediately cooled to avoid decomposition and to precipitate the brominated product **5**, which was obtained as a white powder in 82.0% isolated yield on filtration. In one laboratory-scale experiment, 0.5 mol equiv of 1,3-dibromo-5,5-dimethylhydantoin was employed as the bromination agent, yielding only 54.5%. This was not developed further even though the atom efficiency is higher than that of NBS, theoretically. Unlike the original method, the newly developed five-step sequence is safe, economical, and suited to scale-up, although one more step is required.

Kilogram Processes of DL-028A and Impurity Profile Determination (Scheme 3). Having solved the reagent issues and recovery problems for the key intermediate **5**, other scaling-up issues were encountered during subsequent manipulations, such as environmental concerns, noxious byproduct treatment, salt evaluation, and compound stability, among others. In the sixth step, the *N*-alkylation reaction was improved by the use of inexpensive piperazine hydrochloride **10** (∼190 mol % vs **5**) as the substitute for its free base (Scheme 3), and the acid scavenger sodium bicarbonate was added in two separate portions during refluxing to avoid the elimination of HBr from **5** by excessive amounts of base. After a long period of reflux, the cooled mixture was diluted with water and then cooled to −11 °C with stirring for 3 h to accelerate the precipitation of the desired product **11**, thereby eliminating the evaporation of the noxious solution.[13] The isolated yield was 66.0%. In the seventh step, **11** was hydrolyzed using excess aqueous sodium hydroxide in methanol. The noxious methanethiol byproduct in alkaline solution was destroyed by quenching with aqueous sodium periodate before workup by evaporation of methanol and subsequent manipulations. Sodium periodate (100 mol %)

(13) The noxious smell is attributed to methanethiol generated by partial hydrolysis of **11**.

Scheme 4. **Decompostion of DL-028A**



was initially added at 0 °C, and it digested the alkaline solution for a long period (∼12 h) at that temperature. However, a viscous and gel-like salt contaminated the partially crystallized product **12** (DL-028). The salt could not be removed from the crystalline solid by washing or recrystallization or both. Therefore, the generated methanethiol was quenched at room temperature using 100 mol % of aqueous sodium periodate, and the resulting mixture was digested for 12 h at 35 °C. Thereafter, the salt was easily removed by filtration, and no coprecipitated DL-028 was observed.[14] After evaporation of the pale odorous solvent, the oily crude product was triturated with ethyl acetate to give DL-028 free base as a fine powder. The isolated yield was 81.0%. DL-028 free base is water-insoluble and required conversion to an appropriate water-soluble salt before being submitted to in vivo safety evaluation. Many forms of the salt, such as citrate, hydrochloride, maleate, succinate, and methanesulfonate have been prepared. Of these, only the hydrochloride salt was sufficiently promising to produce good solubility in water (∼0.12 g/mL), showing superior crystallinity and high recovery during salt formation. Other salts, such as citrate or methanesulfonate, were not easily crystallized out from a variety of solvents and exhibited inferior crystallinity. The maleate was sparingly soluble in water, although the recovery of the salt from methanol was excellent (∼95%). Acetyl chloride was initially used to prepare methanolic hydrochloride and thus prepare kilogram quantities of DL-028 hydrochloride. However, the volume efficiency was low, and the crystallization rate was so high, because of the limited solubility of DL-028 hydrochloride in boiling methanol, that a highly uniform fine-powdered crystalline solid could not be obtained. Moreover, laborious multiple crystallization was needed. Surprisingly, during the period of recrystallization from boiling 6/1 (v/v) 2-propanol−water, the dihydroimidazole ring of DL-028 hydrochloride was easily hydrolyzed to form a ring-opened byproduct **14** (Scheme 4), which was a viscous polar oil, whose structure was confirmed by LC/MS spectrometry.[15] Further experimental results revealed that the hydrolysis was easily executed at room temperature even by trace amounts of moisture present in the solvent (e.g., MeOH or acetone), although the content of **14** was usually insignificant (<0.1%). During the course of the first kilogram run of DL-028A and, as a result of laborious recrystallization from boiling 6/1 (v/v) 2-propanol−water, only a 34.0% yield of DL-028A was obtained, approximately 40.0% of the expected yield. The labile property of the dihydroimidazole ring had also been verified by an accelerated degradation experiment, in which DL-028A was heated in boiling water (∼30 mL/g) for 16 h and then analyzed by HPLC, indicating that the resulting crude product consisted of unreacted DL-028 (15% by area) and the ring-opened product **14** (85% by area), besides which no other side products were detected. The structure of **14** was indirectly verified by NMR and mass spectra of an acylation derivative **15** (Scheme 4). A limited amount (∼600 mol %) of concentrated hydrochloric acid was used as a substitute for acetyl chloride, and the salt was formed at ca. 30 °C to prevent the hydrolysis of the dihydroimidazole ring of DL-028A and thus avoid problems associated with thermal instability of DL-028A with water, and volume efficiency. Under such conditions, the operation volume was greatly decreased, and the salt deposition rate was unexpectedly moderated to deposit DL-028A as a fine, white powder. The isolated yield was 83.4%. The overall yield of the eight-step sequence was 15.2%, which yield was consistent to within 5.0% deviation across four kilogram-scale runs (Table 1). The levels of **14** in the stored kilogram products were measured to be less than 0.1% and deemed acceptable for use in preclinical study.[16] Purity of a DL-028A reference standard was determined through a series of analyses, although it revealed a slightly hygroscopic property (see Experimental Section).

Table 1. **Batch yields of kilogram-synthesis of DL-028A**

batch no.	starting material wt. (anthranilamide) (kg)	final product wt. (DL-028A) (kg)	overall yield (eight steps) (%)
K001	1.00	0.668	19.6
K002	1.00	0.596	17.5
K003	2.00	1.038	15.2
K004	2.00	1.050	15.4

Conclusions

The described reaction sequence constitutes a convenient method for the kilogram-scale synthesis of DL-028 hydrochloride under normal conditions. The method also eliminates some commonly encountered problems associated with large-scale synthesis, such as hazardous reagents, dangerous operations, multiple crystallizations, and others. Incidentally, an in-process monitoring system and quality control for the intermediates and final product were established. To extend this work to larger scale (e.g., 100 kg), further studies, such as rescale ability, scaleable workups, and waste-treatment reevaluation, and so forth, are required. Results in this study will hopefully form the basis of a manufacturing process.

Experimental Section

Reagents and solvents were obtained from commercial sources and used as received. Proton and ^{13}C magnetic

(14) No oxidized DL-028 byproducts were detected by HPLC and MS, due to the stoichiometric amount of suspended sodium periodate used.

(15) LC/MS: PE API 300; mobile phase: $CH_3CN:H_2O$ = 9:1 (v/v) + 0.1% formic acid; flow rate: 5 μL/mL; direct inlet. Mass (*m*/*z*): 409.8 (M^+), 392.3 ($M^+ - NH_3$), 217.8 (M^+ − (2-methoxyphenyl)piperazinyl)), 188.8 (M^+− (2-methoxyphenyl)piperazinyl − H − C(O)).

(16) Owing to inferior operations in steps 7 and 8, the overall yield of the first kilogram run (zero batch) was only 4.58%.

resonance spectra were determined on a Bruker AC 500 MHz NMR. The molar equivalent of hydrochloride in DL-028A was determined using a Kyoto Electronics model AT-310 potentiometric automatic titrator. Melting points were generally measured in open capillary tubes using Buchi immersion apparatus and are uncorrected. The melting point of DL-028A was measured using a Stream model DSC92 differential scanning calorimeter. The water content of DL-028A was determined using a Dynamic TGA 2959 thermal gravimetric analyzer and a Kyoto Electronic MKC-210 Karl Fischer moisture titrator. HPLC analyses were performed on a Spectra-Physics HPLC system equipped with a Spectra-Physics Focus Photodiode array detector and TSP Chron-Quest integrator software. The HPLC method used for analysis of intermediates (**4**, **5**, **7**−**9**, and **11**) was performed using an Inertsil ODS-2 column (250 × 4.6 mm) and a acetonitrile/methanol/0.02 M phosphate buffer pH 4.5 (20/30/50, v/v/v) isocratic mobile phase. The highly polar intermediate **2** was determined using an alternative HPLC method with the same column as that used in the above and an acetonitrile/0.02 M phosphate buffer pH 4.5 (1/3, v/v) isocratic mobile phase. The HPLC method used for analysis of DL-028 (**12**) and DL-028A (**13**) was performed using a COSMOSIL column (150 × 4.6 mm) and a methanol/0.01M phosphate buffer pH 2.8 (30/70, v/v) isocratic mobile phase. Preparative TLC (PTLC) was performed with precoated silica gel plates, Merck 60 F_{254} (2.0 mm thickness). All the procedures described below are consecutive manipulations of a typical kilogram run (Batch K003, Table 1).

2-Mercaptoquinazoin-2-one (7). In a 100-L glass reactor were placed absolute ethanol (13.20 L) and potassium hydroxide (85% purity, 1.45 kg, 22.04 mol), and the mixture was heated to 25−30 °C to dissolve potassium hydroxide. Carbon disulfide (1.68 kg, 22.04 mol) was added, and the mixture was stirred at 25−30 °C for 0.5 h to produce a yellow xanthate salt suspension. Anthranilamide **6** (2.00 kg, 14.68 mol), 95% ethanol (15.20 L), and water (2.24 L) were then added to the mixture in succession. The progress of the reaction was monitored by HPLC every 1−2 h. After 21 h of reflux, HPLC revealed that the area % growth of **7** was less than 0.3%. Refluxing was discontinued, and the solution was cooled to 25 °C before being diluted with 20 L of water and then cooled to 3 °C. The resulting solution was carefully treated with 5 L of glacial acetic acid and stirred for 16 h at that temperature. The precipitate was filtered, washed with ice water, and dried in vacuo to give 2-mercaptoquinazoin-2-one (**7**) (2.60 kg, 99.3%) as an off-white powder, HPLC area 99.7%. An analytical sample was recrystallized from 95% ethanol to give a white powder: mp >295 °C; ^{1}H NMR (d_6-DMSO) δ 7.94 (d, J = 6.9 Hz, 1H), 7.73 (m, 1H), 7.37 (d, J = 8.2 Hz, 1H), 7.32 (m, 1H).; MS (EI) m/z 178 (M^+); Anal. Calcd for $C_8H_6N_2OS$: C, 59.04; H, 3.72; N, 17.23. Found C, 58.87; H, 3.56; N, 16.93.

2-Methylthioquinazolin-4(3*H*)-one (8). In a 100-L glass reactor were successively placed crude **7** (2.60 kg, 14.6 mol), water (38.0 L) and sodium hydroxide (93% purity, 1.00 kg, 23.25 mol). Heating to 25−30 °C dissolved the sodium hydroxide. Dimethyl sulfate (2.04 kg, 16.18 mol) was then added to the mixture at once. After 10 min of stirring the clear solution turned turbid., after a further 8 h of stirring, HPLC revealed that the area percent growth of **8** was less than 1.0%. The solution was carefully treated with 3 N hydrochloric acid (5.20 L, 15.6 mol) to pH = 10.2 to precipitate the desired product with the unreacted **7** that remained left in the mother liquid. The precipitate was collected and washed with water and then ethanol (3.0 L). The obtained solid was dried in vacuo to give 2-methylthioquinazolin-4(3*H*)-one (**8**) (2.31 kg, 82.5%) as an off-white powder, HPLC area 95.0%, mp 220−222 °C (lit.[18] mp 213-214 °C). An analytical sample was recrystallized from 95% ethanol: mp 224−225 °C; ^{1}H NMR ($CDCl_3$) δ 11.3 (br s, 1H, NH), 8.30 (dd, J = 4.0, 1.4 Hz, 1H), 7.65 (d, J = 7.9 Hz, 1H), 7.44 (m, 1H), 2.74 (s, 3H); MS (EI) m/z 192 (M^+); Anal. Calcd for $C_9H_8N_2OS$: C, 56.23; H, 4.19; N, 14.57. Found C, 56.02; H, 4.08; N, 14.23.

4-Chloro-2-methylthioquinazoline (9). In a 100-L glass reactor were successively placed 1,2-dichloroethane (23.0 L), 2-methylthioquinazolin-4(3*H*)-2-one (**8**) (2.31 kg, 12.03 mol), and triethylamine (1.70 L, 12.26 mol) before flushing with nitrogen. After the solution was cooled to −2 °C, phosphoryl chloride (4.6 L, 49.36 mol) was added at a rate that kept the solution below 3.0 °C. After this period, the mixture was refluxed for 2 h. After another 17 h of refluxing, HPLC revealed that the area % growth of **9** was less than 1.0%. The solution temperature was cooled to 3 °C, and then 26.8 L of water was added at a rate that kept the solution below 10.0 °C. The organic layer was separated and the water layer extracted twice with 1,2-dichloroethane (6 L × 2). The combined organic layers were washed twice with 8 L of water. The separated organic layer was added to the reactor and evaporated. The solidified residue was dried in vacuo to leave crude 4-chloro-2-methylthioquinazoline (**9**) as a lumpy crystal, which was employed as the starting material in the next step without purification: mp 104−105 °C (lit.[12] 107−108 °C). An analytical sample was recrystallized from isopropyl ether to give **9** as a white powder: mp 106−107 °C; ^{1}H NMR ($CDCl_3$) δ 8.16 (d, J = 8.2 Hz, 1H), 7.87 (d, J = 3.8 Hz, 2H), 7.56 (m, 1H), 2.68 (s, 3H); MS (EI) m/z 211 ($M^+ + 1$); Anal. Calcd for $C_9H_7ClN_2S$: C, 51.31; H, 3.35; N, 13.30. Found C, 51.13; H, 3.34; N, 13.25.

4-Allylamino-2-methylthioquinazoline (4). To the 100-L glass reactor that contained crude **9** (ca. 2.0 kg), as described in the above procedure, was added THF (24.2 L) before stirring to produce an homogeneous solution. To the mixture was added allylamine (6.70 L, 89.0 mol) before refluxing for 1 h. HPLC showed less than 1.0% unreacted **9**. After this period, the re-cooled mixture was evaporated in vacuo to remove THF. The residue was cooled to 0−5 °C, ice water (21.40 L) was added, and vigorous stirring was continued for 1 h at 5 °C. The precipitate was collected on a centrifuge filter, washed with a small amount of ice-cooled acetonitrile (3.00 L), and then dried in vacuo to give 4-allylamino-2-methylthioquinazoline (**4**) (1.41 kg, 50.9%, two steps) as a white powder: HPLC area 100.0%, mp 130−131 °C (lit.[10,11]

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(18) Hu, M.-K.; Liu, K.-C.; Hsu, L.-Y.; Shih, B.-J. *Chin. Pharm. J.* **1991**, *43*, 83.

133−135 °C). An analytical sample was recrystallized from 1/1 (v/v) ethyl acetate−hexane: mp 132−134 °C; ^{1}H NMR ($CDCl_3$) δ 7.66−7.72 (m, 3H), 7.33 (m, 1H), 6.04 (m, 1H), 5.92 (br s, 1H), 5.33 (d, J = 17.1 Hz, 1H), 5.23 (d, J = 10.2 Hz, 1H), 4.32 (m, 2H), 2.65 (s, 3H); MS (EI) m/z 231 (M^+), 205; Anal. Calcd for $C_{12}H_{13}N_3S$: C, 62.31; H, 5.66; N, 18.17. Found C, 62.17; H, 5.37; N, 18.05.

3-Bromomethyl-5-methylthio-2,3-dihydroimidazo[1,2-C]quinazoline (5). In a 100-L glass reactor were placed acetonitrile (16.3 L) and crude 4-allylamino-2-methylthioquinazoline (**4**) (1.41 kg, 6.10 mol). The mixture was stirred for 10 min. *N*-bromosuccinimide (1.29 kg, 7.24 mol) was added, and the reaction was stirred at room temperature. After 1 h of stirring, HPLC showed less than 1.0% unreacted **4**. The solution was cooled to 3 °C and stirred for 1 h at that temperature and then filtered; the precipitate was washed with a small amount of ice-cooled acetonitrile (3.0 L) and dried in vacuo to yield 3-bromomethyl-5-methylthio-2,3-dihydroimidazo[1,2-C]quinazoline (**5**) (1.55 kg, 82.0%) as a white powder: HPLC area 99.2%, mp 162−163 °C (lit.[11,12] 162−163 °C). An analytical sample was recrystallized from acetone: mp 161−162 °C; ^{1}H NMR ($CDCl_3$) δ 7.98 (d, J = 7.8 Hz, 1H), 7.55 (m, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.27 (m, 1H), 4.64 (m, 1H), 4.26 (m, 1H), 4.09 (dd, J = 7.5, 3.6 Hz, 1H), 3.75 (m, 1H), 3.62 (m, 1H), 2.67 (s, 3H); MS (EI) m/z 310 (M^+), 230, 174; Anal. Calcd for $C_{12}H_{12}N_3SBr$: C, 46.46; H, 3.90; N, 13.55. Found C, 46.22; H, 3.68; N, 13.26.

3-{4-[1-(2-Methoxyphenyl)piperazinyl]}methyl-5-methylthio-2,3-dihydroimidazo[1,2-C]quinazoline (11). In a 100-L glass reactor were successively placed 1-(2-methoxyphenyl)piperazine hydrochloride **10** (2.16 kg, 9.44 mol), sodium bicarbonate (1.19 kg, 14.20 mol), and acetonitrile (23.0 L) before stirring for 30 min. Then a solution **5** (1.55 kg, 5.00 mol) in acetonitrile (11.3 L) was added to the mixture. After 20 h of refluxing, the solution temperature was allowed to decline to 35 °C, and a second charge of sodium bicarbonate (1.26 kg, 15.04 mol) was added, with continued refluxing for 14 h. Then the reaction was monitored every 1 h. After another 5 h of refluxing, HPLC showed less than 1.0% unreacted **10**. The solution was cooled to 25 °C and the mixture diluted with water (32.0 L) and stirred vigorously. After the solution was cooled to −11 °C and stirred for 3 h, the precipitate was collected by filtration and dried in vacuo to yield **11** (1.39 kg, 66.0% yield) as a white powder: HPLC area 100.0%, mp 170−172 °C (lit.[10,11] 174−175 °C). An analytical sample was recrystallized from 2-butanone: mp 173−175 °C;[1] H NMR (d_6-DMSO) δ 7.84 (d, J = 7.8 Hz, 1H), 7.57 (m, 1H), 7.35 (d, J = 8.1 Hz, 1H), 7.28 (m, 1H), 6.94 (m, 2H), 6.88 (m, 2H), 4.55 (m, 1H), 4.07 (m, 1H), 3.93 (dd, J = 7.4, 3.2 Hz, 2H), 3.77 (s, 3H), 2.97 (m, 4H), 2.76 (m, 3H), 2.61 (s, 3H), 2.55 (m, 3H); MS (EI) m/z 422 ($M^+ + 1$), 374; Anal. Calcd for $C_{23}H_{27}N_5SO$: C, 65.53; H, 6.46; N, 16.61. Found C, 65.46; H, 6.38; N, 16.46.

3-{4-[1-(2-Methoxyphenyl)piperazinyl]}methyl-2,3-dihydroimidazo[1,2-C]quinazolin-5(6*H*)-one (12) (i.e., DL-028). In a 100-L glass reactor were successively placed compound **11** (1.39 kg, 3.30 mol) and methanol (27.8 L) before stirring for 30 min. To the mixture, a solution of sodium hydroxide (assay: 93%) (4.26 kg, 99.00 mol) in water (34.3 L) was added, and after 20 h of refluxing, HPLC revealed that the area % growth of **12** was less than 0.5%. The solution was cooled to 35 °C, and a solution of sodium periodate (0.71 kg, 3.31 mol) in water (6.3 L) was added and stirred at that temperature for 17 h to quench the methanethiol byproduct. The resulting mixture was filtered to remove viscous suspensions and washed with methanol, and the filtrate was concentrated under reduced pressure to remove methanol. The residue was diluted with dichloromethane (10.0 L) with stirring; then the organic layer was collected and successively washed with saturated brine (18.0 L) and finally with water. The separated organic layer was dried and evaporated, and the residual oil was diluted with ethyl acetate (9.4 L) and stirred vigorously at 15 °C for 16 h to allow the oily suspension to crystallize and thus produce a white powder. The precipitate was collected by filtration and dried in vacuo to yield **12** (i.e., **DL-028**) as a white powder (1.04 kg, 81.0% yield): HPLC area 98.7%, mp 210−211 °C (lit.[10,11] 211−212 °C). ^{1}H NMR (d_6-DMSO) δ 7.79 (d, J = 7.5 Hz, 1H), 7.49 (m, 1H), 7.08 (m, 2H), 6.94 (m, 2H), 6.87 (m, 2H), 4.48 (m, 1H), 4.04 (m, 1H), 3.90 (dd, J = 7.5, 5.0 Hz, 1H), 3.77 (s, 3H), 3.37 (m, 4H), 2.95 (m, 4H), 2.83 (m, 1H), 2.67 (m, 2H); MS (EI) m/z 391 (M^+), 376; Anal. Calcd for $C_{22}H_{25}N_5O_2$: C, 67.50; H, 6.44; N, 17.89. Found C, 67.45; H, 6.37; N, 17.77.

3-{4-[1-(2-Methoxymethyl)piperazinyl]}methyl-2,3-dihydroimidazo[1,2-C]quinazolin-5(6H)-one Dihydrochloride (13) (i.e., DL-028A). Into a 100-L glass reactor were placed **DL-028** (1.04 kg, 2.66 mol) and methanol (55.0 L), and the mixture was heated with stirring at 45 °C for 30 min to produce a solution. The solution was cooled to 30−32 °C, and concentrated hydrochloric acid (37%, 1.47 L, 14.9 mol) was added at a rate that kept the solution at 30−35 °C. After this period, the solution was cooled to room temperature over a 10-h period and stirred at a moderate rate for another 68 h, after which time, a large amount of white powder was deposited. The precipitate was filtered, washed with ice-cooled methanol, and dried in vacuo to yield **13** as a white powder (1.04 kg, 83.4% yield): HPLC area 100.0%, mp 254−255 °C dec; ^{1}H NMR (D_2O) δ 8.00 (d, J = 8.0 Hz, 1H), 7.92 (m, 1H), 7.46 (m, 1H), 7.38 (d, J = 8.5 Hz, 1H), 7.26 (m, 1H), 7.23 (m, 1H), 7.11 (d, J = 8.0 Hz, 1H), 7.04 (m, 1H), 5.48 (br s, 1H), 4.56 (t, J = 11.9 Hz, 1H), 4.11 (dd, J = 6.3, 6.3 Hz, 1H), 3.96 (m, 1H), 3.88 (s, 3H), 3.79 (br s, 2H), 3.68 (m, 1H), 3.60 (m, 8H).

Preparation and Purity Determination of DL-028A Reference Standard. *Preparation.* A set of 100 sample vials was prepared by dividing an appropriate amount of purified DL-028A, prepared by recrystallization of the product from the kilogram run from boiling 6/1 (v/v) 2-propanol/water; 50 mg of the purified DL-028A was transferred to each vial and placed into an oven in vacuo at 70 °C for 16 h.

Analytical Experiment and Purity Definition. Problems were experienced during measurement with the EA (elemental analyzer) because DL-028A absorbed moisture during the analytical process. Accordingly, these samples were treated

by the following procedure before analysis using the EA and TGA (thermal gravimetric analyzer). Vials from the above samples were placed into a desiccator at humidity RH 72% for 20 h, and vials were randomly selected for measurement with TGA and EA. The reported TGA and EA data are the mean measurements for the three random samples. The residual solvent (2-propanol) was not detected by standard gas chromatography analysis.[19] The acid−base molar ratio of DL-028A was determined to be 2.00 by a potentiometric automatic titrator. Thus, the water content of the reference standard can be given by the following formula:

$$(\text{w/w \%}) \text{ of } H_2O = \frac{18.0152x}{464.3936 + (18.0152x)}$$

in which DL-028A with H_2O is formulated as DL-028·2HCl·$x$$H_2O$, (w/w %) of H_2O is weight percentage of water in DL-028·2HCl·$x$$H_2O$. The purity of DL-028A (the reference standard) is shown by this formula:

$$\text{Purity of DL-028A} = 1 - \left(\frac{C_i - C_0}{C_i}\right)$$

in which C_0 is an observed value (C%) of DL-028·2HCl·$x$$H_2O$ determined by an EA, C_i is the theoretical value (C%) of DL-028·2HCl·$x$$H_2O$.

Analytical Data for DL-028·2HCl·xH_2O. The water content (i.e., (w/w %) of H_2O) was determined to be 6.7% by TGA or a Karl Fischer titrator; therefore

$$0.067 = \frac{18.0152x}{464.3936 + (18.0152x)}, \qquad x = 1.861$$

Elemental Analysis: Calcd for DL-028·2HCl·1.861-H_2O: C, 53.07; H, 6.22; N, 14.07. Found: C, 52.83; H, 5.99; N, 13.89. Therefore C_0 and C_i were determined as 53.07 and 52.83, respectively.

Purity Determination for DL-028A Reference Standard. According to the above purity definition, the purity of the DL-028A reference standard = 1 − ((53.07−52.83)/53.07) = 99.55%.

(19) USP organic volatile impurity (467 method V).

Hydrolysis of DL-028A: An Accelerated Preparation of the Ring-Opening Product 14 and Acylation Derivative 15 by Thermal Hydrolysis of DL-028A (13). A mixture of DL-028A (**13**) (1.0 g, 2.15 mmol) and water (30 mL) was heated under reflux for 16 h and then evaporated in vacuo to leave an oily residue. An aliquot was neutralized and subjected to LC/MS analysis.[15] The oily residue was dissolved in 1,2-dichloroethane (30 mL), cooled to 0 °C, and successively treated with triethylamine (0.84 g, 8.31 mmol) and acetic anhydride (0.32 g, 3.13 mmol). After stirring for 15 h at room temperature, the resulting solution was washed successively with water, 5% NaOH, and water. The separated organic layer was dried ($MgSO_4$) and evaporated to leave an oily residue (0.6 g). An aliquot of the crude product was purified on repeated PTLC using 2% methanol in ethyl acetate to isolate **15** from recovered DL-028 as a viscous oil (lower R_f portion). ^{1}H NMR ($CDCl_3$) δ 9.25 (br s, 1H, NHC(O)N, varies with concentration), 8.10 (br s, 1H), 7.56 (m, 1H), 7.20 (m, 1H), 7.05 (d, J = 8.0 Hz, 1H), 6.99 (m, 1H), 6.83−6.96 (m, 3H), 6.51 (br s, NHAc), 5.25, 5.37 (m, 1H, C(O)NCHC(O)),[20] 3.70, 3.85 (each m, 2H, CH_2NHAc), 3.84 (s, 3H, OCH_3), 3.04 (m, 6H, CH_2(piperazinyl) + piperazinyl H), 2.77 (m, 4H, piperazinyl H), 1.93 (br s, 3H, NHC(O)CH_3); ^{13}C NMR ($CDCl_3$) δ 170.8, 163.6, 152.3, 141.1, 139.3, 135.2, 133.5, 128.7, 128.0, 126.5, 123.2, 121.1, 118.3, 115.3, 111.3, 76.9, 60.1, 59.4, 59.3, 58.6, 54.5, 53.8, 50.8, 49.6. LC/MS: PE API 300; mobile phase: MeOH; flow rate: 25 μL/mL; direct infusion. Mass (m/z): 452.0 (M^++1), 392.0 (M^+− NHAc − H).

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(20) The unusual split low-field shifts of NCH are attributed to highly electron-withdrawing and enolisable neighboring groups, a related NCH (δ 5.08) appeared in a 3,4-dihydro-4-iminoquinazolinone derivative.[10]