Process Development of a Platelet Aggregation Inhibitor

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Abstract:

A practical and efficient method for N-amination of piperazine via a nitrosoamine, suitable for a large scale synthesis, is described. This method involved the temporary transformation of an in situ prepared aminopiperazine to a hydrazone, allowing efficient separation of zinc salt byproducts from the system. Acylation and deprotection with hydroxylamine directly afforded FR062732 in satisfactory quality for pharmacological evaluation. These methods solved the operational problems usually inherent in zinc reduction of nitrosoamines.

Results and Discussion

Classical nonsteroidal antiinflammatory drugs (NSAIDs) such as indomethacin and aspirin have been reported to have a marked inhibitory action on platelet aggregation.¹ Tiaramide (2, Figure 1), developed at Fujisawa Pharmaceutical Co. Ltd., is also a widely used NSAID, used for treatment of inflammatory diseases.² This drug has also been shown to have potent effects on platelet aggregation.³ This discovery prompted efforts to find novel, selective platelet aggregation inhibitors. During investigations into Tiaramide derivatives, it was found that platelet aggregation inhibitory activity mainly depends on the piperazine moiety, leading to the discovery of 3-[(4-amino-1-piperazinyl)carbonylmethyl]-5-chloro-2(3H)-benzothiazolone hydrochloride (FR062732, 1, Figure 1), which was a crucial lead showing good activity.4

The original method for the preparation of 1 in gram quantities, shown in Scheme 1, was used to support the initial pharmacological screening.⁴ The synthesis started with hydrolysis of 3-(methoxycarbonylmethyl)-5-chloro-2(3H)benzothiazolone (3), an intermediate in the Tiaramide production process, and involved coupling with piperazine in the presence of TiCl₄ to give 5, which was then converted to 6 with isoamyl nitrite. 6 was then reduced with zinc powder to afford the desired product 1. At this stage, several severe complications were encountered. The major drawback to this method was that zinc salt byproducts contaminated the final product. Tedious and multistep purification procedures, including recrystallization and/or column chromatography, were required in order to meet acceptable limits of amounts of residual metal (<1 ppm), resulting in a



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Figure 1. Structures of Tiaramide and FR062732.

Scheme 1. Original route to FR062732 $(1)^a$



^a Reagents and conditions: (1) NaOH, H₂O-MeOH; (2) piperazine, TiCl₄, THF; (3) (CH₃)₂CHCH₂CH₂ONO, CH₂Cl₂; (4) (a) Zn, AcOH, (b) HCl.

significantly reduced yield. Additionally, since nitroso derivatives usually have carcinogenic activity, isolation of 6 was problematic from the viewpoint of personal hazards, especially on a large scale.

In accordance with project progress, we were faced with having to supply 1 in large quantities for toxicological and biological evaluation. To develop a more practical and efficient synthesis of 1, we investigated several synthetic methods. 1 may be prepared most readily by the amination of 5 with NH₂Cl or NH₂OSO₃H. However, these reagents are unavailable in large quantities, are difficult to handle in pilot plants with ordinary equipment, and cannot be stored for long periods of time. Furthermore, since these methods produce several byproducts, unacceptably low yields (\sim 50%) were obtained, and this strategy was not pursued further.

We thus turned our attention to the preparation of aminopiperazine in situ and coupling with 4 (Scheme 2). To our knowledge, practical methods suitable for a large Scheme 2. New synthetic route to FR062732 $(1)^a$



^{*a*} Reagents and conditions: (1) (a) NaNO₂, AcOH, H₂O; (b) zinc powder; (c) PhCHO; (2) Et₃N, CH₂Cl₂; (3) NH₂OH·HCl, HCl, CH₃CN-H₂O.

scale synthesis are not known in the literature. Aminopiperazine may be prepared by nitrosation of piperazine followed by reduction with reagents such as Na,⁵ LiAlH₄,^{5,6} and Zn.⁷ Despite the usefulness of reactions with Na or LiAlH₄ on a laboratory scale, it was decided to investigate alternative methods owing to the pyrophoric hazards associated with these reagents. Thus, we selected zinc powder as reagent in the presence of acetic acid. However, this method also had serious shortcomings in the scale-up procedures that needed to be solved before the required results could be realized. One problem is that nitrosopiperazine, which possesses marked carcinogenic activity,8 should be immediately reduced with zinc powder without isolation. As a result, the products are usually a mixture of piperazine, monoaminopiperazine, and diaminopiperazine. Another problem is that, even when nitrosopiperazine was purified by extraction and/or column chromatography, the reduction with zinc powder also involved deamination as a side reaction, resulting in contamination by piperazine in the product. A more fundamental problem is that piperazine derivatives are strong bases and the zinc salt byproducts, precipitated after adjusting the aqueous layer to pH 7.0, could not be separated by filtration without significant product loss. During our investigation, the most practical solution to these problems was the temporary transformation of the aminopiperazine to a hydrazone, followed by extraction into an organic phase.

Nitrosation of piperazine was conducted with isoamyl nitrite, but NaNO₂ in the presence of acetic acid was more efficient for a large scale synthesis.⁹ Nitrosopiperazine was immediately reduced with zinc powder in the presence of acetic acid, which was already present from the nitrosation

step, affording a mixture of piperazine, monoaminopiperazine, and diaminopiperazine. Simple addition of benzaldehyde to the reaction mixture led to the formation of the corresponding hydrazones. Whilst toluene had poor extraction efficacy and CH₂Cl₂ extracted both **7** and the dihydrazone, ethyl acetate was able to selectively extract **7**. After addition of aqueous NH₄Cl, followed by adjusting the pH to 9.5 with aqueous NaOH, the layers were separated. In this sequence, piperazine was completely removed in the aqueous layer. Quantitative HPLC analysis showed that 2 molar equiv of piperazine was converted to **7** in 65% yield. Since piperazine is inexpensive (\$2.50/lb in bulk) and also available in large quantities, excess use does not significantly increase the cost.¹⁰

Hydrolysis of **3** smoothly proceeded in the presence of excess NaOH in a mixture of water and MeOH. After the reaction was judged complete by HPLC analysis, water was added to the reaction mixture in order to improve the filtration procedure, followed by hydrochloric acid. Activation of 4 was conducted with excess amounts of $SOCl_2$ (5 equiv), requiring removal of the remaining SOCl₂ by distillation; however, the Vilsmier method (SOCl₂ (1.02) equiv), DMF, CH₂Cl₂) was more suitable for a large scale synthesis. The subsequent acylation proceeded quantitatively in the presence of an excess amount of 7 on a laboratory scale; however, this reaction did not give reproducible yields in the pilot plant campaign. A \sim 13 °C exotherm (from 0 to 13 °C) was seen, and the yield was only 68%. Further studies indicated that the yield is impacted by reaction temperature, since the acyl chloride of 4 partially decomposes at >10 $^{\circ}$ C in basic solution to give unknown byproducts. Pure 8 was isolated by precipitation from ethyl acetate and MeOH, with a small amount of dihydrazone remaining in the mother liquor.

In early studies, the deprotection of 8 was conducted in the presence of a large excess of hydrogen chloride. However, the reaction did not proceed smoothly, and hydrolysis of 8 to 4 was predominant and yielded several unknown byproducts under higher temperature conditions. As a result, both methods resulted in 1 in low yield (<10%). Since 8 and 1 are in an equilibrium state, removal of benzaldehyde from the system by converting it to an oxime with hydroxylamine was investigated. Analysis of the isolated 1 following deprotection of 8 with 5 molar equiv of hydroxylammonium chloride revealed a small amount (1-3%) of the starting material. However, HPLC analysis of the wet cake immediately after filtration confirmed that the product did not contain any starting material. The reason for the contamination is not known, although it is possible that small amounts of benzaldehyde in the filtrate reacted with 1 after removal of excess hydrogen chloride. The problem was easily solved by using a large excess of hydroxylammonium chloride (10 equiv) to ensure complete removal of the benzaldehyde. The wet cake was triturated to remove excess hydroxylammonium chloride prior to the product being dried under reduced pressure. The isolated

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yield of **1** was about 62% from **8**, regardless of the scale, mainly due to significant loss in the mother liquor (19%) and partitioning in the aqueous layer (5%), and contained less than 0.4% total impurities and no zinc salt byproducts. Several alternative isolation methods were investigated with the aim of decreasing the mechanical loss, but these only resulted in product of unsatisfactory quality and requiring further purification procedures. Therefore, the methods described were the best at this point when process economics and operation were considered.

Conclusion

In conclusion, we have established a practical and facile synthesis of 3-[(4-amino-1-piperazinyl)carbonylmethyl]-5-chloro-2(3H)-benzothiazolone hydrochloride (FR062732, 1). The conversion of a hydrazine to a hydrazone resolved several operational problems and allowed the development of a workable process. The procedures described in this paper should be useful for the efficient synthesis of hydrazines on a large scale. The use of an optimum amount of hydroxylammonium chloride as a scavenger for benzalde-hyde resulted in a significant improvement in yield for the deprotection of hydrazone, and these methods were used to produce approximately 14 kg of 1.

Experimental Section

Melting points were measured on a Thomas-Hoover apparatus and are uncorrected. IR spectra were recorded on a HORIBA FT-210 spectrometer. NMR spectra were measured on a Bruker AC200P (¹H, 200 MHz). Chemical shifts are given in parts per million, and tetramethylsilane was used as the internal standard. Mass spectra were measured on a Hitachi model M-80 mass spectrometer using EI for ionization. Elemental analyses were carried out on a Perkin-Elmer 2400 CHN elemental analyzer. HPLC analyses were performed using a YMC GEL ODS 120-Å S-7 column and a 0.1% TFA in CH₃CN and water mobile phase. The amount of zinc salt byproducts was measured on a Hitachi Zeemann type atomic absorption spectrophotometer 180-80. Reagents and solvents were used as obtained from commercial suppliers without further purification.

3-Carboxymethyl-5-chloro-2(3H)-benzothiazolone (4). To a mixture of 3-(methoxycarbonylmethyl)-5-chloro-2(3H)benzothiazolone (3) (200 g, 0.776 mol) in MeOH (500 mL) was added dropwise 8% NaOH in water (500 mL, 1 mol). The reaction mixture was stirred at 25-30 °C over 3 h, followed by addition of water (5.6 L). After the reaction mixture was cooled to 10 °C, 36% HCl in water (107 mL) was added. On complete addition, the resulting mixture was stirred at ambient temperature for 1 h and the precipitate filtered off and washed with water (133 mL). Drying under reduced pressure afforded 4 (182 g, 96% yield) as a white solid: mp 242–243 °C; ¹H NMR (200 MHz, DMSO- d_6) δ 4.77 (s, 2H), 7.27 (dd, 1H, J = 8.4, 2.0 Hz), 7.57 (d, 1H, J = 2.0 Hz), 7.71 (d, 1H, J = 8.4 Hz), 13.34 (brs, 1H); IR (KBr) 1745, 1687, 1678, 1590, 1575, 1474 cm⁻¹; MS (EI) m/z 244 (M + H)⁺, 170, 75. Anal. Calcd for C₉H₆- ClNO₃S: C, 44.36; H, 2.48; N, 5.75. Found: C, 44.33; H, 2.40; N, 5.68.

3-[(4-Benzylideneamino-1-piperazinyl)carbonylmethyl]-5-chloro-2(3H)-benzothiazolone (8). Piperazine (86.14 g, 1 mol) was dissolved in a mixture of acetic acid (300 g, 5 mol) and water (430 mL). To this solution was added dropwise a solution of sodium nitrite (69 g, 1 mol) in water (172 mL) at 10 °C, and the reaction mixture was stirred for 1 h at ambient temperature. Zinc powder (172 g, 2.63 mol) was then added to the solution in portions, maintaining the temperature below 40 °C, and the reaction mixture was stirred for 1 h. The precipitate was filtered off, and the cake of zinc salts was washed with water (172 mL). Benzaldehyde (106 g, 1 mol) in 424 mL of ethanol was added to the combined solutions of the filtrate and washings, and after the mixture was stirred for 1 h at room temperature, ethyl acetate (1034 mL) and ammonium chloride (281 g, 5.25 mol) were added, and the pH was adjusted to 9.5 with 24% sodium hydroxide in water. The layers were separated, and the aqueous layer was re-extracted with ethyl acetate (690 mL). The insoluble materials in the combined organic layer were filtered off. The filtrate was dried over magnesium sulfate, and, after filtration, the magnesium sulfate was washed with methylene chloride (300 mL). To the combined filtrate was added triethylamine (126.5 g, 1.25 mol), followed by cooling below 0 °C. In another vessel, thionyl chloride (61 g, 0.51 mol) was added to 4 (122 g, 0.5 mol) in a mixture of methylene chloride (610 mL) and dimethylformamide (61 mL). The reaction mixture was refluxed for 1 h and then cooled to 20 °C. This solution was added dropwise to the previous solution, maintaining the temperature below 0 °C, and the reaction was stirred for 1 h at ambient temperature. The reaction mixture was concentrated to ~910 mL, and methanol (1.2 L) was added. After the mixture was stirred for 0.5 h at 0 °C, the precipitate was filtered off, washed with methanol (366 mL), and dried to afford 8 (189 g, 91% yield) as a white solid: mp 213-215 °C; ¹H NMR (200 MHz, DMSO- d_6) δ 3.15–3.29 (m, 4H), 3.65–3.76 (m, 4H), 5.01 (s, 2H), 7.23-7.74 (m, 9H); IR (KBr) 1695, 1683, 1647, 1588, 1570, 1472 cm⁻¹; MS (EI) m/z 415 (M + H)⁺. Anal. Calcd for C₂₀H₁₉ClN₄O₂S: C, 57.90; H, 4.62; N, 13.50. Found C, 57.76; H, 4.56; N, 13.25.

3-[(4-Amino-1-piperazinyl)carbonylmethyl]-5-chloro-2(*3H*)-benzothiazolone hydrochloride (1, FR062732). To a solution of hydroxylamonium chloride (168 g, 2.42 mol) in a mixture of acetonitrile (2.0 L) and 17.5% hydrochloric acid (1.0 L) was added 8 (100 g, 241 mmol). The reaction mixture was stirred for 4 h at 47–50 °C and then cooled to 0 °C. The precipitate was filtered off and washed successively with water (200 mL) and methylene chloride (500 mL). Trituration of the crude cake with water (200 mL) afforded **1** (54.3 g, 62% yield) as a white solid. The product was confirmed as containing no zinc salt byproducts with the atomic absorption spectrophotometer: mp 261–263 °C; ¹H NMR (200 MHz, D₂O) δ 3.13–3.28 (m, 4H), 3.78–3.87 (m, 4H), 4.98 (s, 2H), 7.16 (d, 1H, J = 1.9 Hz), 7.27 (dd, 1H, J = 8.4, 1.9 Hz), 7.53 (d, 1H, J = 8.4 Hz); IR (KBr) 1677, 1667, 1642, 1591, 1533, 1480, 1467 cm⁻¹; MS (EI) m/z 327, 306, 73. Anal. Calcd for C₁₃H₁₆Cl₂N₄O₂S: C, 42.98; H, 4.44; N, 15.42. Found C, 43.03; H, 4.43; N, 15.36.

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