A scalable and facile process for the preparation of *N*-(pyridin-4-yl) piperazine-1-carboxamide hydrochloride

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A scalable and facile synthetic process for *N*-(pyridin-4-yl)piperazine-1-carboxamide hydrochloride, a novel Rho kinase inhibitor with an unsymmetrical urea structure currently under investigation for the treatment of central nervous system disorders, was established. After optimisation of the reaction conditions, *N*-(pyridin-4-yl)piperazine-1-carboxamide hydrochloride was synthesised from 4-aminopyridine and *N,N'*-carbonyldiimidazole through acylation, deprotection and salt formation. This new procedure affords the product in 53% overall yield with high purity and it can be easily scaled up for production.

Keywords: *N*-(pyridin-4-yl)piperazine-1-carboxamide hydrochloride, Rho kinase inhibitor, asymmetric urea, *N,N'*-carbonyldiimidazole

Multiple sclerosis (MS), a chronic inflammatory demyelinating disease of the central nervous system (CNS),[1–3] is characterised by the destruction of myelin and axons leading to progressive disability.[4] Some reports have demonstrated that intervention in the CNS by Rho kinase inhibitors can improve clinical symptoms and pathological changes.[5] Rho kinases, which play important roles in a series of cellular activities, are serine/threonine kinases and are downstream substrates of the Rho protein.[6–8] A comparative study showed that the efficacy of Rho kinase inhibitors reduced leukocyte infiltration in damaged areas of the CNS was better than a conventional dose of methylprednisolone.[9] Research on experimental autoimmune encephalomyelitis (EAE) and phagocytic cell culture confirmed that Rho kinase inhibitors could inhibit leukocyte migration, infiltration and phagocytosis, which significantly reduced demyelination.[10] Rho kinase activation contributes to neurite retraction,[11] while Rho kinase inhibitors enhance axonal regeneration.[12]

Rho kinase inhibitors fall into four structural categories: 4-aminopyridine, 1*H*-indazole, isoquinoline and phthalimide derivatives. Since the discovery[13] of the novel 4-aminopyridine Rho kinase inhibitor *N*-(pyridin-4-yl)piperazine-1-carboxamide hydrochloride (**1**) and its potent action[14] as a potential agent for neurological disorders, multikilogram quantities of compound **1** were required to support further pharmacological profiling and clinical trials. X. Wang *et al*.[13] first synthesised *N*-(pyridin-4-yl)piperazine-1-carboxamide (**5**), however, the process was found unsuitable for large-scale manufacture as it required column chromatography and suffered from prolonged reaction times and harsh reaction conditions *etc*. The promise shown by *N*-(pyridin-4-yl)piperazine-1-carboxamide hydrochloride (**1**) prompted the search for a better synthetic route that is efficient, simple and robust to meet the needs for future clinical trials.

Results and discussion

Although studies of 4-aminopyridine derivatives containing a urea unit as Rho kinase inhibitors are of importance, the synthesis of this class of compounds is seldom reported. In 2009, Sebti and Hamilton[15] successfully prepared 4-aminopyridine derivatives as Rho kinase inhibitors from 4-isocyanatopyridine, but no experimental details were mentioned, nor was the synthesis of the isocyanate disclosed. Because this isocyanate was commercially unavailable, we first tried to prepare it from 4-aminopyridine and triphosgene[16–20] and found it was unstable and difficult to separate from the reaction mixture. Hence, we attempted to carry on the next step without separation. Unfortunately, many impurities were detected in the reaction mixture and optimisation of the reaction conditions did not provide promising results. Consequently, it was difficult to obtain the pure compound by a simple separation. These problems may possibly be attributed to the high reactivity of the isocyanate, which led to the generation of impurities. We therefore theorised that use of substrates with controllable reactivity may work better. Based on this consideration, we decided to use the approach of X. Wang *et al*.,[13] in which the isocyanate group was replaced by a carbamate function[21] (Scheme 1) and the reaction was achieved in a stepwise manner.

Ethyl chloroformate was used to form the intermediate carbamate **4** that subsequently reacted with piperazine to give compound **5**. Subsequent salt formation by treatment with 36% hydrochloric acid gave target compound **1**. It was found that the nucleophilic substitution of ethyl chloroformate with 4-aminopyridine proceeds effectively to produce compound **4** in 89% yield, but the second step does not work well and so we attempted to improve it further. Catalysts, such as $AlMe_3$ and 1-methylpyrrolidine were examined for this reaction and the results obtained showed that 1-methylpyrrolidine exhibits better catalytic performance, while no reaction is observed without a catalyst. Polar aprotic solvents were preferable, so DMF, DMSO, DCM, THF and 1,4-dioxane were evaluated. All the reactions were carried out at the boiling point of the solvent to enhance the reaction. The results indicated that 1,4-dioxane was the best choice not only in terms of yield but also in post-processing. 1,4-Dioxane was found to be a better solvent than the higher boiling DMF and DMSO. This may possibly be due to volatilisation of the catalyst. We obtained the pure target compound **5** successfully *via* this method and prepared sufficient product for evaluation, but this synthetic route is only suitable for small scale production. Scaling up the production only produced a yield of 38%, because, in the second step, a reaction time of 24 h was needed even under optimised conditions. Furthermore, pure product could only be obtained by two chromatographic separations.

A sealed reaction vessel was considered to raise the temperature without volatilisation of the catalyst and an autoclave was used to perform this reaction at 160 °C for 6 h with a pressure of 1 MPa (Scheme 1). After this time, analysis of the reaction mixture showed the reaction was complete, no starting material was detected and the yield increased from 38% to 50%. However, a crystalline by-product had precipitated from the reaction solution. Analysis of this solid revealed it to be the bis-substituted piperazine **2**.

Two methods were investigated to decrease the formation of the bis-substituted by-product. Thus, when *N*-Boc-piperazine, in

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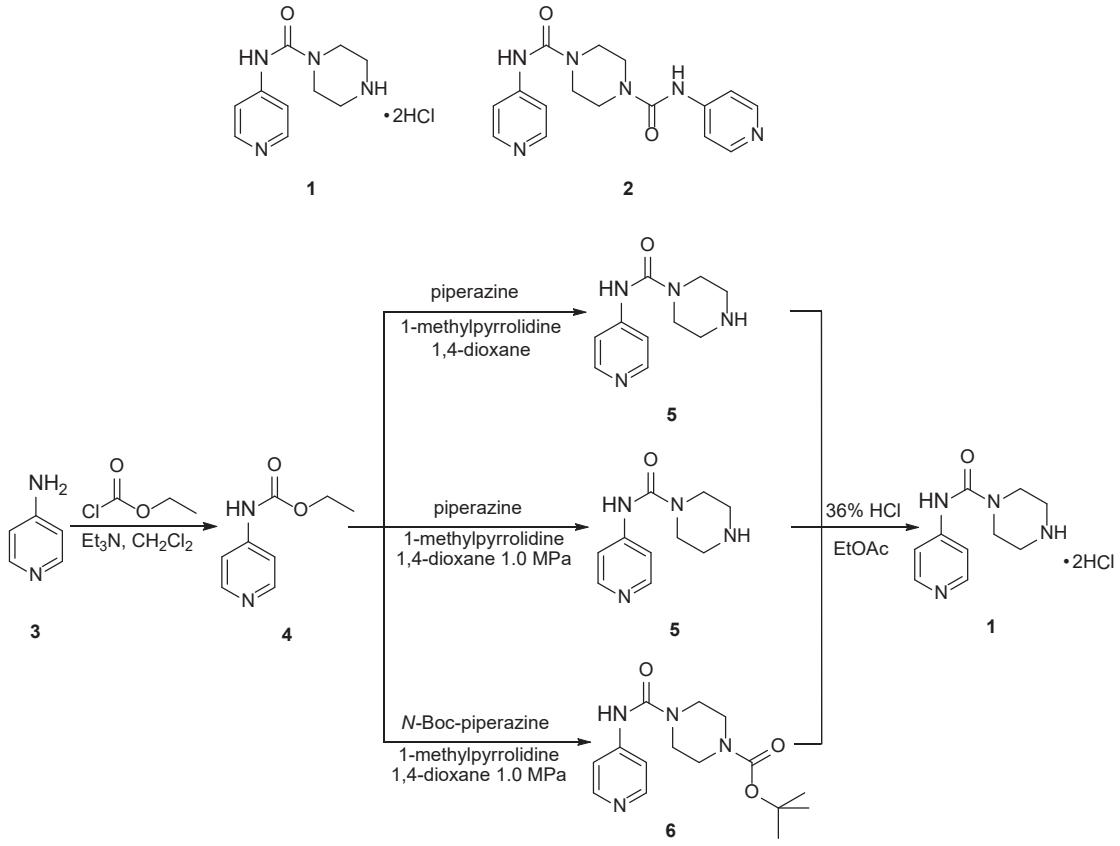
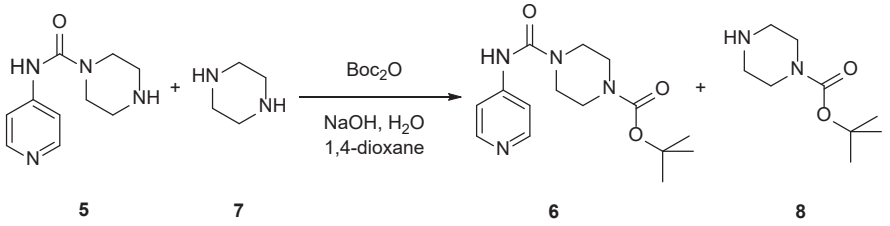


Scheme 1 Synthesis of compound **1** from ethyl chloroformate and 4-aminopyridine.



Scheme 2 Reaction of crude product with *t*-butyloxycarbonyl anhydride.

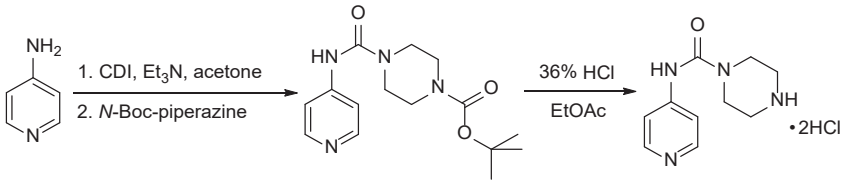
which one active site of the piperazine is blocked, was used as the nucleophilic reagent, substitution on nitrogen by the Boc group led to lower reactivity and conversion to the product was poor (Scheme 1). The amount of piperazine was increased in an attempt to avoid by-products. It was found that none of the piperazine **2** was formed with a 5:1 molar ratio of piperazine to compound **4.**

The next step was to remove the large amount of excess piperazine to meet the demands for pharmaceutical evaluation. We found that upon acidification of the reaction mixture with HCl gas, most of the piperazine hydrochloride precipitated from the methanol solution while target compound **1** remained in solution, but further recrystallisation gave little improvement. Other solvents such as acetonitrile, ethanol, isopropyl alcohol, acetone, 1,4-dioxane, ethyl acetate and methylene chloride were selected to recrystallise the crude product, but compound **1** and piperazine hydrochloride exhibited similar solubility in all solvents, so it was difficult to remove all of the piperazine.

Based on consideration of the structures of compound **5** and piperazine, we assumed the derivatisation of these two compounds may change their solubility in certain solutions. So, compound **6** and *N*-Boc-piperazine were dissolved in DMF, toluene, acetonitrile, acetone, 1,4-dioxane, ethyl acetate, DCM and THF. As a result, compound **6** and *N*-Boc-piperazine could be effectively separated in acetone and 1,4-dioxane. Hence, the reaction mixture was reacted with *t*-butyloxycarbonyl anhydride (Scheme 2). This was followed by recrystallisation from acetone to obtain the pure compound **6**. Deprotection of compound **6** by hydrogen chloride formed the target compound **1** in high purity. Thus we have developed a route to prepare the target compound on a large scale.

Although we obtained the product in large scale with high purity, there were also some drawbacks such as pressure and the protection–deprotection reaction *etc.* Moreover, the reagent ethyl chloroformate is highly toxic and difficult to store and transport. Further study is needed to develop a more convenient way to solve these problems. Thus, *N,N'*-carbonyldiimidazole (CDI) was chosen to effect *N*-acylation of 4-aminopyridine. CDI is comparatively stable, as available as ethyl chloroformate and also can be used as the reagent to prepare unsymmetric ureas.[22–24] The newly designed route is shown in Scheme 3.

A number of solvents such as DMSO, acetonitrile, ethyl acetate, DCM, toluene, THF, 1,4-dioxane and acetone were



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Scheme 3 Synthesis of compound **1** from CDI and 4-aminopyridine.

examined, of which acetone showed particular promise, while the reaction did not proceed in DMSO or in acetonitrile. Although the reaction proceeded when ethyl acetate, DCM, toluene, THF and 1,4-dioxane were used as solvents, it was necessary to increase the amount of CDI. This route with mild conditions can operate at room temperature without the need to separate any of the intermediates. The use of *N*-Boc-piperazine not only reduced the incidence of impurities, but also effectively avoided column chromatography, because in the system only compound **6** precipitated from the acetone solution. Compound **6** was recrystallised from acetone–water (5:1 v/v). In the last step, in order to effectively save resources and time, 36% hydrochloric acid was added in excess to give the hydrochloride directly. The reaction cycle was shortened to 5 h and the whole yield was raised to 53%.

Ultimately, through the optimisation of the process, a scalable and facile process for the synthesis of the novel Rho kinase inhibitor *N*-(pyridin-4-yl)piperazine-1-carboxamide hydrochloride, an unsymmetrical urea derivative, was established in overall yield of 53.8%. The use of an autoclave can shorten the time from 24 h to 5 h, however, the process of removing the impurities was cumbersome. The use of *N*-Boc-piperazine and CDI not only reduced the incidence of impurities, but also effectively avoided column chromatography. The reaction operated with acetone as solvent at room temperature furnishing a pilot-scale synthesis of 3 kg quantities of high quality active pharmaceutical ingredient, which provided convenience for future clinical trials.

Experimental

Solvents and reagents were obtained from commercial sources and used without further purification unless otherwise noted. Solvents were dried and purified according to standard procedures before use. The course of the reactions was monitored by TLC (silica gel GF254s). Flash chromatography was performed using 200–300 mesh silica gel. ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$, MeOD and DMSO-d_6 solutions and recorded on INOVA 600 MHz and Bruker 400 MHz spectrometers with TMS as an internal standard. HRMS were recorded with a Bruker MicrOTOF-QII spectrometer. IR spectra were obtained using KBr disks on the FTIR Bruker Tensor 27.

Ethyl pyridin-4-ylcarbamate (**4**)

Triethylamine (1295.23 g, 12.75 mol) and ethyl chloroformate (1153.12 g, 10.63 mol) were added to a solution of 4-aminopyridine (1000.06 g, 10.62 mol) in anhydrous DCM (1.2 L) at –8 °C over 2 h. The resulting mixture was stirred at –8 °C for 3 h. The reaction mixture was adjusted to pH 5–6 by 5% hydrochloric acid. Then the aqueous phase was extracted with DCM (3 × 1 L). The DCM phases were combined, dried over $MgSO_4$ and concentrated to afford **4** as: White solid; yield 1574.64 g (89%); m.p. 126.7–127.4 °**C** (lit.[25] 127–128 °**C**); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.72 (s, 1H), 8.46 (d, *J* = 5.1 Hz, 2H), 7.45 (d, *J* = 5.1 Hz, 2H), 4.25 (q, *J* = 6.9 Hz, 2H), 1.31 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 153.4, 150.1, 146.3, 112.7, 61.6, 14.4; HRMS (ESI) for $C_8H_{10}N_2O_2$: $[M + H]^+$: calcd: 167.0821; found: 167.0821.

Synthesis of N-*(pyridin-4-yl) piperazine-1-carboxamide hydrochloride* (**1**) *from* **4** *and piperazine*

Compound **4** (100.00 g, 621.20 mmol) was added to a solution of piperazine (66.26 g, 745.44 mmol) in 1,4-dioxane (300 mL) with 1-methylpyrrolidine (52.85 g, 621.20 mmol) as catalyst. The mixture was refluxed for 24 h and then concentrated. The residue was purified by column chromatography (ethyl acetate–methanol 5:1, v/v) to afford compound **5** (47.14 g, 38% yield). The product **5** was dissolved in ethyl acetate (400 mL) and converted to the hydrochloride **1** by introduction of 36% hydrochloric acid (60 mL). The mixture was filtered off and dried to obtain compound **1**: Yield 59.78 g (94%); m.p. 196.8–197.4 °C; ^{1}H NMR (600 MHz, MeOD): δ 8.54 (d, *J* = 6.8 Hz, 2H), 8.11 (d, *J* = 6.7 Hz, 2H), 3.95 (d, *J* = 4.5 Hz, 4H), 3.38–3.36 (m, 4H); ^{13}C NMR (150 MHz, MeOD): δ 157.3, 154.5, 142.3, 115.4, 44.4, 42.7; HRMS (ESI) for $C_{10}H_{14}N_4O$: $[M + H]^+$: calcd: 207.1246; found: 207.1249.

Synthesis of N-*(pyridin-4-yl)piperazine-1-carboxamide hydrochloride* (**1**) *from* **4** *and piperazine in an autoclave*

Compound **4** (96.60 g, 581.44 mmol) was added to a solution of piperazine (258.42 g, 2907.17 mmol) in 1,4-dioxane (400 mL) with 1-methylpyrrolidine (49.47 g, 581.44 mmol) as catalyst. The mixture was placed in an autoclave for 6 h with a pressure of 1 MPa, temperature of 160 °C and a rotation speed of 385 r min^{-1} and then concentrated. The residue was purified by column chromatography (ethyl acetate–methanol 5:1, v/v) to afford compound **5** (61.65 g, 50% yield). The product **5** was converted to the hydrochloride **1** and precipitated from the ethyl acetate (500 mL) after 36% hydrochloric acid (75 mL) was added dropwise. The mixture was filtered off and dried to obtain compound **1** (93% yield).

Synthesis of N^1,N^4*-di(pyridin-4-yl)piperazine-1,4-dicarboxamide* (**2**) *from* **4** *and piperazine*

Compound **4** (96.00 g, 577.83 mmol) was added to a solution of piperazine (59.65 g, 693.39 mmol) in 1,4-dioxane (400 mL) with 1-methylpyrrolidine (49.47 g, 581.44 mmol) as catalyst. The mixture was placed in an autoclave for 6 h with a pressure of 1 MPa, temperature of 160 °C and a rotation speed of 385 r min^{-1}. The solid that precipitated from the solution was purified by recrystallisation from methanol to give compound **2**: Yield 35.92 g (19%); m.p. 220.1–221.6 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.04 (s, 2H), 8.32 (d, *J* = 4.5 Hz, 4H), 7.49 (d, *J* = 4.8 Hz, 4H), 3.53 (s, 8H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 154.1, 149.8, 147.4, 113.0, 43.6; HRMS (ESI) for $C_{16}H_{18}N_6O_2$: $[M + H]^+$: calcd: 327.1569; found: 327.1570.

Synthesis of N-*(pyridin-4-yl)piperazine-1-carboxamide hydrochloride* (**1**) *from* **4** *and N-Boc-piperazine in an autoclave*

Compound **4** (100.38 g, 604.19 mmol) was added to a solution of *N*-Boc-piperazine (154.10 g, 725.03 mmol) in 1,4-dioxane (400 mL) with 1-methylpyrrolidine (51.41 g, 604.19 mmol) as catalyst. The mixture was placed in an autoclave for 6 h with a pressure of 1 MPa, temperature of 160 °C, rotation speed of 385 r min^{-1} and then the reaction mixture was concentrated. The residue was purified by column chromatography (dichloromethane–methanol 10:1, v/v). The residue was slurried with acetone and water (1:5, v/v) and then the mixture was filtered off and dried to afford compound **6** (31.62 g, 17% yield). The solid product **6** was dissolved in ethyl acetate (350 mL) and then 36% hydrochloric acid (30 mL) was added and the mixture stirred

for 30 min. The product **6** was converted to the hydrochloride **1** in 91% yield.

Synthesis of tert-*butyl 4-(pyridin-4-ylcarbamoyl)piperazine-1-carboxylate* (**6**) *from compound* **5**

Sodium hydroxide (2.34 g, 58.41 mmol) was added to a mixture of compounds **5** and **7** (4.01 g, 19.47 mmol) in water–1,4-dioxane (3:1 v/v, 80 mL) at room temperature. Then, a solution of *t*-butyloxycarbonyl anhydride (8.51 g, 38.94 mmol) in water–1,4-dioxane (3:1 v/v, 40 mL) was added dropwise at room temperature under stirring and the resulting mixture was stirred for 1 h. The reaction mixture was filtered and the filter cake was slurried with water–1,4-dioxane (3:1 v/v, 40 mL) to give compound **6**: White solid; yield 5.42 g; m.p. 200.8–201.4 °C; ^{1}H NMR (400 MHz, MeOD): δ 8.31 (d, *J* = 6.4 Hz, 2H), 7.52 (d, *J* = 6.4 Hz, 2H), 3.53 (dd, *J* = 19.5, 5.5 Hz, 8H), 1.50 (s, 9H); ^{13}C NMR (100 MHz, MeOD): δ 155.0, 154.9, 148.9, 148.4, 113.6, 80.3, 43.7, 27.3; HRMS (ESI) for $C_{15}H_{22}N_4O_3$: $[M + H]^+$: calcd: 307.1770; found: 307.1770.

Synthesis of compound **1** *from* N,N′*-carbonyldiimidazole (CDI) and* N-*Boc-piperazine*

4-Aminopyridine (2000.15 g, 21.25 mol) was added to a solution of CDI (3445.60 g, 21.25 mol) in acetone (4.0 L) with triethylamine (2590.48 g, 25.50 mol) as base. The mixture was stirred for 4 h and then *N*-Boc-piperazine (4516.56 g, 21.25 mol) was added and the mixture stirred for 2.5 h. After filtration, the filter cake was slurried with acetone and water (1:5, v/v), and then compound **6** was filtered off and dried (3838.60 g, 59% yield). The solid product **6** was dissolved in ethyl acetate (10 × 4 L) and then 36% hydrochloric acid (10 × 350 mL) was added and the mixture stirred for 30 min. The product, the hydrochloride **1**, was obtained after filtration: m.p. 197.1–197.7 °C; yield 91%.

Electronic Supplementary Information

The ESI (^{1}H and ^{13}C NMR spectra of compounds **1**, **2**, **4** and **6**) is available through:
stl.publisher.ingentaconnect.com/content/stl/jcr/supp-data

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