Improved synthesis of the PAR-1 thrombin receptor antagonist RWJ-58259

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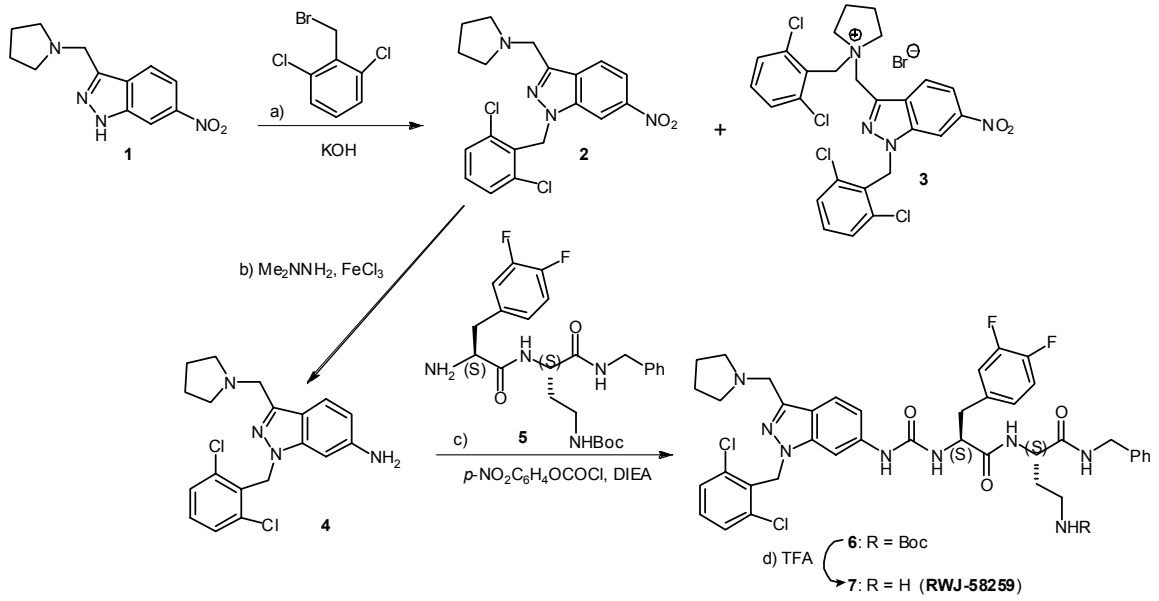
Abstract
A significant improvement on the synthesis of the PAR-1 antagonist RWJ-58259 is described, which involves a base-related two-fold yield increase in the indazole *N*-alkylation, and an easier purification and a nine-fold yield increase in the urea formation, by using triphosgene/propylene oxide as urea coupling traceless reagents.

Keywords: Peptidomimetics, heterocycles, alkylation, ureas, protease-activated receptor antagonists

Introduction

In addition to the key role of thrombin in blood coagulation processes, this serine protease mediates multiple cellular responses, such as platelet aggregation and cell proliferation in fibroblasts, neurons, and endothelial, smooth muscle, and tumor cells.[1] These thrombin effects on cells are mediated by activation of the denominated protease-activated receptors (PARs). Among these, PAR-1 is the principal thrombin-activated receptor involved in platelet aggregation and in endothelial and tumor cell proliferation. Human PAR-1 is activated by the thrombin-mediated cleavage of the N-terminal extracellular domain at the Arg^{41}/Ser^{42} peptide bond, unveiling the recognition sequence SFLLRN, which acts as a tethered activation ligand. It has been proposed that a PAR-1 antagonist could have potential for treating thrombosis, atherosclerosis, inflammation and cancer metastasis, without altering thrombin's role in hemostasis.[1] The first potent and in vivo effective PAR-1 antagonists were SFLLRN-based peptidomimetics, which provided the first in vivo proof-of-concept of the therapeutic potential of PAR-1 antagonists.[1a,2] Among these, the indazole-derived urea RWJ-58259 (Scheme 1, **7**) reached advanced preclinical studies in different animal models[3,4] and, in spite of its low oral bioavailability,[3] it is considered an standard reference in pharmacological studies on PAR-1 receptors.[4,5]

In the context of a research project on novel peptidomimetic PAR-1 antagonists, we needed a reference antagonist as standard for the biological evaluation of new compounds. Up to now, no PAR-1 antagonist is commercially available and we could not get a sample from their respective developing companies. So, we decided to prepare RWJ-58259 following the reported methodology.[2a,6] As shown in Scheme 1, this methodology involved a convergent solution-phase formation of the urea derivative **6** from the two corresponding moieties: the 6-amino-indazole fragment **4** and the dipeptide fragment **5**. However, when we tried to reproduce the patented procedure[6] we obtained very low yields both in the step of the *N*-alkylation of the indazole (a) and in the formation of the urea (c), which made it impractical to get the final compound RWJ-58259 (**7**) in a reasonable yield for our studies. Consequently, we undertook and describe herein the optimization of these two steps.



Scheme 1. Patented synthesis of RWJ-58259.[6]

Results and Discussion

N-Alkylation of the indazole derivative 1

The alkylation of indazole **1** to the 1-(2,6-dichlorobenzyl) derivative **2** has been described in 33% yield using one equiv of 2,6-dichlorobenzyl bromide and KOH as base.[2a,6] When we first attempted to reproduce this alkylation, compound **2** was obtained in a similar low yield (34%) and the TLC and HPLC (Novapak C_{18}) analyses of the crude reaction mixture showed the presence of a peak (30%) with the same R_f and t_R of the starting material **1**. Trying to increase the yield of **2**, we repeated the reaction using a 30% excess of 2,6-dichlorobenzyl bromide, but, contrary to what we expected, the yield of **2** decreased and we could only isolate the bromide of

the product of dialkylation (in the indazole and in the pyrrolidine) **3**, which coeluted with the starting indazole **1** on TLC and on the HPLC Novapak C_{18} column. In view of this result, we evaluated by HPLC the influence of different bases (KOH, Cs_2CO_3, TEA, NaH) on the mono/dialkylation rate. As shown in Table 1, the best yield of the monoalkylation product **2** was obtained with Cs_2CO_3 (entry 2), although, the reaction was slower and required 24 h. TEA (entry 3) not only produced higher ratio of the dialkylation product **3**, as also happened with NaH, but led to a more complex reaction mixture. Taking into consideration these results, the alkylation was carried out with one equiv of 2,6-dichlorobenzyl bromide in the presence of one equiv of Cs_2CO_3. Under these conditions, the monoalkylated indazole **2** was chromatographically isolated in a 66% yield (double compared to the described procedure[2a,6]).

Table 1. Influence of the base on the *N*-alkylation of **1**

			Yield (%)[a]	
Entry	Base	t (h)	**2**	**3**
1	KOH	4	57 (33)[b]	42
2	Cs_2CO_3	24	80 (66)[c]	13
3	TEA	4	20	45
4	NaH	4	39	60

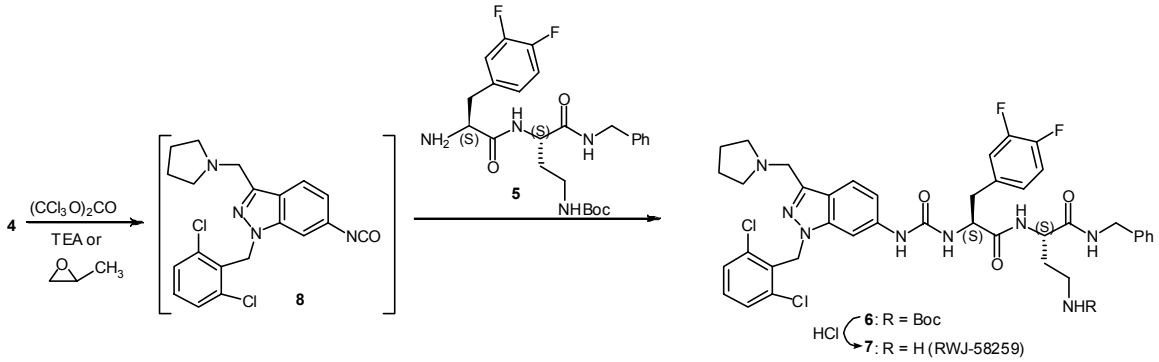
[a]Determined by HPLC (Novapak C_{18}, 4 μM, 3.9×150 mm). [b]Described yield of isolated compound. [c]Yield of isolated compound.

Urea formation from the indazole and dipeptide units 4 and 5

This coupling was described in 26% yield, by using 4-nitrophenyl chloroformate as urea-coupling reagent in the presence of diisopropylethylamine at -20 ºC.[2a,6] When we applied this methodology, the urea **6** was obtained in a yield lower than 10%, what made it impractical for our objective and which moved us to study alternative procedures for its improvement. It is worth mentioning that, as the urea **6** and the 6-aminoindazole **4** coeluted on TLC and on our initially used HPLC column (Novapak C_{18}, 4 μM, 3.9×150 mm), HPLC-MS (Xbride C_{18}, 3.5 μM, 2.1×100 mm column) was used to analyze the results of this study. First, we observed that the low yield of the described methodology was not significantly affected by the reaction temperature (-40, -20 or 0 ºC). Then, we decided to evaluate a methodology that had been successfully used in our laboratory for the synthesis of asymmetric ureas,[7] which involved the *in situ* formation of the isocyanate of one of the two amino moieties, by reaction with bis(trichloromethyl)carbonate (triphosgene) in the presence of Et_3N as HCl acceptor, followed by reaction with the other amine. When this methodology was applied to the formation of the 6-aminoindazole-derived isocyanate **8** (Scheme 2), previously to the reaction with 0.5 equiv of the dipeptide **5**, the urea **6** precipitated from the reaction medium in a 50 % yield, but, the ^{1}H NMR

spectrum of this precipitate showed that $Et_3N{\cdot}HCl$ had coprecipitated along with urea **6**. Then, we tried to remove the ammonium salt by washing the precipitate with H_2O and several organic solvents, but all attempts were unsuccessful.

In view of the significant improvement in the yield of **6** achieved using triphosgene, and to avoid the formation of the ammonium chloride, we studied the methodology using a traceless HCl-acceptor, such as propylene oxide. This replacement gave an excellent result, as the urea **6** was isolated by precipitation from the reaction medium in 91% yield and in a HPLC purity higher than 95%. The excess of the starting 6-aminoindazole **4** was recovered from the filtrate of the reaction mixture.



Scheme 2. Improved formation and *N*-Boc deprotection of urea **6**.

Trying to optimize the reaction efficacy, the influence of the molar ratio **4**/**5** (1/0.5, 1/0.7 and 1/0.9) was also studied. The results showed that while the reaction yield was not significantly affected, when the molar proportion of the dipeptide **5** was higher than 0.5, this compound co-precipitated with the urea **6**, and the purification of the reaction mixture required chromatography.

The final *N*-Boc-deprotection was carried out quantitatively by treatment with a 3 N solution of HCl in MeOH. This last step did not require chromatographic purification, as in the previously described deprotection using TFA/anisole.[2a,6] The hydrochloride of RWJ-58259 (**7**) was obtained in 91% overall yield (from **5**, nine-fold higher than by the described methodology) and with higher than 95% HPLC purity.

Conclusions

In summary, we have developed a significant improvement of the described synthesis of the PAR-1 antagonist RWJ-58259 at laboratory scale (0.1-1 g) required to obtain enough quantity to be used as a standard reference in our pharmacological studies. First, replacement of KOH by Cs_2CO_3 has allowed to double the yield of the *N*-alkylation of the indazole derivative **1**. Second, by using triphosgene in the presence of propylene oxide as traceless reagents for the urea

formation we have achieved a nine-fold increase in the yield of this key step, with respect to that obtained using 4-nitrophenyl chloroformate and diisopropylethylamine. Finally, the *N*-Boc deprotection by 3N HCl solution in MeOH allowed the quantitative isolation of the corresponding hydrochloride, avoiding a chromatographic purification in this step.

Experimental Section

General Procedures. All reagents were of commercial quality. Solvents were dried and purified by standard methods. Analytical TLC was performed on aluminum sheets coated with a 0.2 mm layer of silica gel 60 F_{254}. Silica gel 60 (230-400 mesh) was used for flash chromatography. Analytical RP-HPLC was performed on a Novapak C_{18} (3.9×150 mm, 4μm) column, with a flow rate of 1mL/min, and using a tunable UV detector set at 214 nm. Mixtures of CH_3CN (solvent A) and 0.05 % TFA in H_2O (solvent B) were used as mobile phases. HPLC-EMS was performed on an XBride C_{18} (2.1×100 mm, 3.5 μm) column at 30 ºC, with a flow rate of 0.25 mL/min. 5-80% Gradients of CH_3CN with 0.08% of formic acid (solvent A) in 0.1% of formic acid in H_2O (solvent B) were used as mobile phases. ^{1}H NMR spectra were recorded at 300, 400, or 500 MHz, using TMS as reference, and ^{13}C NMR spectra were recorded at 100, or 125 MHz. ESI-MS spectra were performed, in positive mode, using MeOH as solvent.

N-Alkylation of the indazole 1. Synthesis of 1-(2,6-dichlorobenzyl)-6-nitro-3-(pyrrolidin-1-ylmethyl)-1*H*-indazole (2) and 1-(2,6-dichlorobenzyl)-1-[1-(2,6-dichlorobenzyl)-6-nitro-1*H*-indazol-3-ylmethyl]pyrrolidinium bromide (3). 2,6-dichlorobenzyl bromide (1.438 g, 6 mmol) was added to a solution of the indazole **1**[2a] (1.478 mg, 6 mmol) in THF (80 mL) under argon, followed by portionwise addition of the base (KOH, Cs_2CO_3, TEA, or NaH) (6 mmol) over 20 min. After the reaction time indicated in Table 1, the reaction mixture was evaporated under reduced pressure. The residue was partitioned between EtOAc (200 mL) and H_2O (50 mL). The organic phase was successively washed with H_2O (2×50 mL) and brine (50 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography, using 0-5% gradient of MeOH in CH_2Cl_2 as eluant to obtain **2** and **3** as a brown solids in the yields indicated in Table 1.

1-(2,6-Dichlorobenzyl)-6-nitro-3-(pyrrolidin-1-ylmethyl)-1*H*-indazole (2)[2a]. Mp 138-140ºC; HPLC-EMS [XBridge C_{18}, 5-80% gradient of solvent A in solvent B in 15 min] t_R 8.81; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.70, 2.52 (2s, 8H, pyrrolidine), 3.94 (s, 2H, CH_2-pyrrolidine), 5.79 (s, 2H, CH_2-diClPh), 7.15-7.23 and 7.30-7.37 (2m, 3H, diClPh), 7.90 [s, 2H, 4-H and 5-H (indazole)], 8.25 [s, 1H, 7-H (indazole)]; ES-MS m/z 405.2 (100%) [M+1]+.

1-(2,6-Dichlorobenzyl)-1-[1-(2,6-dichlorobenzyl)-6-nitro-1*H*-indazol-3-ylmethyl]-pyrrolidinium bromide (3). Mp 210 ºC (dec); HPLC-EMS [XBridge C_{18}, gradient 5-80% A, 15 min] t_R 14.8; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.90-2.05, 2.12-2.20, 3.10-3.20, 3.70-3.78 [4m, 8H, CH_2 (pyrrolidine)], 4.86 (s, 2H, CH_2-pyrrolidine), 5.20 (s, 2H, pyrrolidine-CH_2-diClPh), 6.12 (s, 2H,

CH_2-diClPh), 7.42-7.59 (m, 6H, diClPh), 8.17 [dd, 1H, J = 1.5 and 9.0 Hz, 5-H (indazole)], 8.41 [d, 1H, J = 9.0 Hz, 4-H (indazole)], 9.12 [d, 1H, J = 1.5 Hz, 7-H (indazole)]; ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.2 [CH_2 (pyrrolidine)], 48.8 (CH_2-diClPh), 54.1 (pyrrolidine-CH_2-diClPh), 56.7 (CH_2-pyrrolidine), 59.4 [CH_2 (pyrrolidine)], 108.7 [C_7 (indazole)], 117.5 [C_5 (indazole)], 122.1 [C_4 (indazole)], 127.5 [C_{3a} (indazole)], 129.4-140.4 (Ph), 139.0 [C_3 (indazole)], 140.1 [C_{7a} (indazole)], 147.4 [C_6 (indazole)]; ES-MS *m/z* 566.4 (100%) $[M+1]^+$; Anal. Calc. for $C_{26}H_{23}BrCl_4N_4O_2$: C 48.40, H 3.59, N 8,86; Found C 48.69, H 3.73, N 9.03.

Reduction of indazole 2. Synthesis of 6-amino-1-(2,6-dichlorobenzyl)-3-(pyrrolidin-1-ylmethyl)-1*H*-indazole (4).[2a] Ferric chloride hexahydrate (245 mg, 0.91 mmol) and activated charcoal (1.920 g) were added to a solution of indazole **2** (1.600 g, 3.95 mmol) in MeOH (100 mL). Afterwards, dimethylhydrazine (6.0 mL, 79 mmol) was added and the reaction was refluxed for 2 h, cooled to rt and filtered through celite, which was washed several times with CH_2Cl_2/MeOH (4:1, 3×30 mL). The combined filtrates were evaporated under reduced pressure and the residue was purified by flash chromatography using 1-8% gradient of MeOH in CH_2Cl_2 with 2% of NH_4OH as eluant to obtain the 6-amino-indazole **4** (889.4 mg, 60%). ES-MS *m/z* 376.3 (100%) $[M+1]^+$.

Synthesis of urea 6. Bis(trichloromethyl)carbonate (196 mg, 0.66 mmol) was added to a 0 ºC cooled solution of the 6-amino-indazole **4** (751 mg, 2 mmol) in dry THF (5 mL). Then, propylene oxide (285 μL, 4 mmol) was added dropwise throughout 5 min, continuing the stirring at 0 ºC for 5 additional min. Afterwards, a solution of the dipeptide **5**[2a,6] (491 mg, 1 mmol) in dry THF (5 mL) was added at room temperature, and the reaction mixture was stirred for 1 h. Then, the reaction mixture was diluted with EtOAc (100 mL), successively washed with H_2O (2×25 mL) and brine (25 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was treated with cold CH_2Cl_2 (50 mL). The precipitate was filtered and washed with cold CH_2Cl_2 (50 mL) to give the urea **6** as a yellow amorphous solid (812 mg, 91 %) in 95.67 % HPLC purity. HPLC [XBridge C_{18}, 5-80% gradient of solvent A in solvent B in 15 min] t_R 11.26; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.34 (s, 9H, Boc), 1.68-1.84 [m, 6H, 3- and 4-H (pyrrolidine) and β-H (diamino-butyric acid)], 2.85 [dd, 1H, J = 8.0 and 13.5 Hz, β-H (diF-Phe)], 2.82-3.29 [m, 6H, 2- and 5-H (pyrrolidine) and γ-H (diaminobutyric)], 3.06 [dd, 1H, J = 4.0 and 13.5 Hz, β-H (diFPhe)], 4.28-4.35 [m, 3H, CH_2-Ph and α-H (diaminobutiric)], 4.58-4.63 [m, 3H, CH_2-pyrrolidine and α-H (diFPhe)], 5.63 (s, 2H, CH_2-diClPh), 6.54 [d, 1H, J = 8.0 Hz, NH (diFPhe)], 6.78 (t, 1H, J = 5.5 Hz, NH-Boc), 7.01 [d, 1H, J = 9.0 Hz, 5-H (indazole)], 7.03-7.06 [m, 1H, 2-H (diFPhe)], 7.21-7.32 [m, 7H, Ph, 5-H and 6-H (diFPhe)], 7.43 [t, 1H, J = 8.0 Hz, 4-H (diClPh)], 7.54 [d, 2H, J = 8.0 Hz, 3-H and 5-H (diClPh)], 7.79[d, 1H, J = 9.0 Hz, 4-H (indazole)], 7.99 [s, 1H, 7-H (indazole)], 8.47-8.52 [m, 2H, NH-Bn and α-NH (diaminobutyric)], 9.15 (s, 1H, NH-indazole); ES-MS m/z 891.5 (100%) $[M+1]^+$.

***N*-Boc-Deprotection of urea 6. Synthesis of RWJ-58259 (7)[2a].** The *N*-Boc-protected urea **6** (713 mg, 0.8 mmol) was dissolved in 3N solution of HCl in MeOH (50 mL). After 2 h of stirring at room temperature, the solution was evaporated to dryness and the residue was dissolved in H_2O (20 mL) and lyophilized to afford the hydrochloride of RWJ-58259 (**7**) as a pale yellow solid (633 mg, 100%) in 95.05% HPLC purity. HPLC [XBridge C_{18}, 5-80% gradient of solvent A in solvent B in 15 min] t_R 11.26; ES-MS *m/z* 791.2 (38%) $[M+1]^+$.

Supplementary Material Avalaible

HPLC-EMS and ^{1}H NMR spectra of ureas **6** and **7.**

Acknowledgements

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