Synthesis of New TGX-221 Analogs

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Received March 31, 2014

TGX-221 is a potent phosphoinositide 3-kinase (PI3K)\textsubscript{β} inhibitor that has great therapeutic potential to treat prostate cancer. Chemical modification of TGX-221 at positions 2 and 9 was made. Five new TGX-221 analogs with different heterocyclic substituents of morpholine, 1-methylpiperazine, aniline, and thiazole-2-amine at positions 2 and 9 were synthesized. Parallel synthetic methods were employed in \textit{S}\textsubscript{N}2 replacement reactions at positions 2 and 9 of TGX-221.

Key words: TGX-221, Heterocyclic Substituent, Parallel Synthesis, \textit{S}\textsubscript{N}2 Reaction, Microwave Reaction

Introduction

TGX-221 \{7-methyl-2-morpholino-9-(1-(phenylamino)ethyl)-4\textsubscript{H}-pyrido[1,2-\textit{a}]pyrimidin-4-one\} is a potent phosphoinositide 3-kinase (PI3K)\textsubscript{β} inhibitor [1 – 4]. Its key chemical features include a pyridopyrimidinone nucleus (I), a morpholinyl substituent at position 2 (II) and a 1-phenylaminoethyl moiety at position 9 (III) as shown in Fig. 1 [2]. Following on earlier reports of the PI3K\textsubscript{β}-inhibiting ability of TGX-221, structural modifications on TGX-221 were carried out. On the core structure of TGX-221, replacement of the pyridopyrimidinone nucleus was made [5 – 8]. Two families of imidazopyrimidinones and triazolopyrimidinones were generated [5, 6]. Both series of the compounds were found to display high clearance in metabolism [7, 8]. Therefore two series of pyrazolopyrimidines and thiazolopyrimidinones were synthesized in an attempt to improve clearance [7, 8]. On the side fragment of TGX-221, a hydroxyethyl moiety was added to the aniline group at position 9 to produce analogs [9, 10].

In this paper chemical modifications of TGX-221 at positions 2 and 9 were undertaken. The group at position 2 was replaced by 1-methylpiperazine and morpholine. The moiety at position 9 was substituted with thiazole-2-amine, 1-methylpiperazine and aniline. Five new TGX-221 analogs were synthesized. Parallel synthetic methods were employed in \textit{S}\textsubscript{N}2 replacement reactions.

Results and Discussion

The synthesis of TGX-221 analogs was initiated from 2-amino-3-bromo-5-methylpyridine and malonyl dichloride to generate the scaffold of 9-bromo-2-hydroxy-7-methyl-4\textsubscript{H}-pyrido[1,2-\textit{a}]pyrimidin-4-one (I). The hydroxy group at position 2 of compound I was substituted with morpholine or 1-methylpiperazine. Subsequent replacement at position 9 of the resulting compounds was made with 1-methyl-
piperazine, aniline or thiazole-2-amine. The synthetic procedures are illustrated in Scheme 1.

Compound 1 was formed through a nucleophilic cyclization reaction by treatment of malonyl dichloride with a solution of 2-amino-3-bromo-5-methylpyridine in dichloromethane (CH₂Cl₂). The hydroxyl group in compound 1 was substituted with morpholine and 1-methylpiperazine through parallel synthesis to produce compounds 2 and 5, respectively. The bromo group in compound 2 was transformed to an acetyl group to afford compound 3 through an intramolecular Heck reaction. Using the same method compound 6 was generated from compound 5. The ketone group in compound 3 was reduced to the secondary alcohol by sodium borohydride (NaBH₄) to afford compound 4. Compound 7 was obtained in the same manner from compound 6.

Parallel synthesis was employed for the Sₙ₂ reaction to replace the secondary alcohol in compounds 4 and 7 with thiazole-2-amine, 1-methylpiperazine and aniline to yield five compounds of TGX-221 analogs, as shown in Scheme 1. Analog TGX-221a and TGX-221b were generated from compound 4; analogs TGX-221c, TGX-221d and TGX-221e were synthesized from compound 7. The new TGX-221 analogs were characterized by ¹H and ¹³C NMR spectra and high-resolution mass spectrometry.

A series of pyridopyrimidinone was reported in the patent by Jackson and co-workers [1]. In their patent, pyridyl and morpholino groups were used as substituents at position 2 of the pyridopyrimidinone nucleus [1]. Some substituents, such as benzyl, 4-hydroxyphenylamino, pyridin-4-yl-ethyl and thioephene-2-yl-methyl were employed at position 9 [1]. The hydroxylethyl moiety was added to the aniline group at position 9 of TGX-221 [9, 10]. In our work, modifications of TGX-221 at positions 2 and 9 were undertaken. All analogs contain amino-substituted heterocyclic moieties as substituents. 1-Methylpiperazine and morpholine rings were used as substituents at position 2. Thiazole-2-amine, 1-methylpiperazine and aniline were selected as substituents at position 9. Combination of the substituents generated five new TGX-221 analogs. Four analogs with the 1-methylpiperazine group are particularly attractive, since 1-methylpiperazine possesses a protonatable nitrogen atom which will enable the formation of more water-soluble acid addition salts, such as hydrochlorides.

We developed a synthetic procedure for TGX-221 analogs which is different from the previous synthetic method for a series of pyridopyrimidinone in Jackson’s patent [1]. In our work, a microwave synthesizer was used to speed up the Sₙ₂ replacement reactions from compound 1 to compounds 2 and 5. We employed two reaction steps to achieve the formation of the final compounds via compounds 3 and 6, which made methylation and Sₙ₂ replacement reactions to proceed easily. Our synthesis is facile, proceeds from commercially available materials, is easily scaled up, and very manageable in relation to the synthesis of analogs. Morpholine and 1-methylpiperazine groups at position 2 are installed by a nucleophilic displacement reaction. 1-Methylpiperazine, aniline and thiazole-2-amine moieties at position 9 are installed by a Sₙ₂ reaction.

Conclusion

Chemical modification of TGX-221 was carried out. 9-Bromo-2-hydroxy-7-methyl-4H-pyrido[1,2-a]-pyrimidin-4-one was synthesized as the scaffold. Five new TGX-221 analogs were synthesized. Parallel synthetic methods were developed for Sₙ₂ replacement of the hydroxyl group at positions 2 and 9 of the scaffold.

Experimental Section

Reactions that required an inert atmosphere were carried out under argon with flame-dried glassware. Column chromatography was carried out by employing silica gel (230 – 400 mesh). Thin-layer chromatography (TLC) was performed on a silica gel w/uv254 uniplate™. Parallel synthesis was conducted on Mettler Toledo MiniBlock and MiniBlock XT. Anhydrous organic solvents were purchased. Melting points were determined using a Barnstead International MET-TEMP® Melting Point Apparatus, Model 1001D-120VAC. IR spectra were measured with a Perkin Elmer™ Spectrum One FT-IR spectrometer. ¹H and ¹³C NMR spectra were recorded on a 400 MHz spectrometer (400 and 100 MHz, respectively), or a 500 MHz spectrometer (500 and 125.5 MHz, respectively). Abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiple. High-resolution mass spectra (HRMS) were obtained on a double-focusing mass spectrometer.

Procedures for the synthesis of intermediates 1–7

Compounds 1, 2, and 3 were synthesized following the procedures given in refs. [1, 2].

9-Bromo-2-hydroxy-7-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (1)

To a solution of 2-amino-3-bromo-5-methylpyridine (2.25 g, 12 mmol) in CH$_2$Cl$_2$ (25 mL) was added malonyl dichloride (1.25 mL, 12.5 mmol) at 0°C. The mixture was stirred at room temperature for 48 h. The yellow solid was collected by filtration, washed with CH$_2$Cl$_2$ (3 × 25 mL), and dried under reduced pressure. Compound 1 was obtained as a yellow solid with a yield of 88% (2.75 g). M. p. 209 – 211°C. – $^1$H NMR (D$_6$DMSO, 400 MHz): δ = 8.74 (s, 1H, 8-CH), 8.29 (s, 1H, 6-CH), 5.55 (s, 1H, 3-CH), 2.35 (s, 3H, 7-CH$_3$) ppm. – HRMS (ESI): m/z = 254.9793 (calcd. 254.9769 for C$_9$H$_8$BrN$_2$O$_2$, [M+H]$^+$).

9-Bromo-7-methyl-2-morpholino-4H-pyrido[1,2-a]pyrimidin-4-one (2)

To a suspension of compound 1 (1.275 g, 5 mmol) in CH$_2$Cl$_2$ (30 mL) were added triethylamine (1.4 mL, 10 mmol) and methanesulfonyl chloride (0.54 mL, 7 mmol) at 0°C. The mixture was stirred at room temperature for 30 min. Morpholine (1.25 mL, 12.5 mmol) was added, and the mixture was heated in a microwave synthesizer at 90°C for 45 min. The mixture was diluted with water (30 mL) and extracted with CH$_2$Cl$_2$ (3 × 100 mL). The organic layer was washed with water and dried over Na$_2$SO$_4$. After concentration under reduced pressure, the dark-yellow residue was purified through a silica flash column using EtOAc/hexane.
2:1 as an eluent to give compound 2 as a pale-yellow solid with a yield of 45% (0.65 g). M. p. 198 – 199 °C. 1H NMR (CDCl3, 400 MHz): δ = 8.69 (s, 1H, 8-CH), 7.85 (s, 1H, 6-CH), 5.59 (s, 1H, 3-CH), 3.82 (m, 4H, 2 O-CH2), 3.75 (m, 4H, 2 N-CH2), 2.33 (s, 3H, 7-CH3) ppm. – HRMS (ESI): m/z = 324.0348 (calcd. 324.0348 for C13H13BrN3O2, [M+H]+).

9-Acetyl-7-methyl-2-morpholino-4H-pyrido[1,2-a]pyrimidin-4-one (3)

Compound 2 (650 mg, 2 mmol) in DMF (10 mL) was mixed with N,N-diisopropylethylamine (1.5 mL), butyl vinyl ether (1.6 mL) and dichloro-1,1-bis(diphenylphosphino)ferrocene palladium(II) (70 mg, 0.066 mmol) at room temperature under argon for 30 min until a homogeneous solution was formed. The solution was heated to 120 °C for 16 h. After cooling, the solution was poured into 100 mL of 1 M HCl aqueous solution at 0 °C. The mixture was stirred at room temperature overnight and extracted with CH2Cl2 (2 × 100 mL). The combined organic phases were washed with water and dried over Na2SO4. Removal of the solvent under reduced pressure followed by purification of the resulting residue through a silica flash column using EtOAc/hexanes 3:1 as an eluent afforded compound 3 as a yellow solid with a yield of 60% (389 mg). M. p. 207 – 208 °C. 1H NMR (CDCl3, 400 MHz): δ = 8.88 (s, 1H, 8-CH), 7.86 (s, 1H, 6-CH), 5.65 (s, 1H, 3-CH), 3.84 – 3.79 (m, 4H, 2 O-CH2), 3.67 – 3.62 (m, 4H, 2 N-CH2), 2.75 (s, 3H, CH3), 2.35 (s, 3H, 7-CH3) ppm. 13C NMR (CDCl3, 100 MHz): δ = 199.17 (11-C=O), 160.17 (4-C=O), 158.21 (2-C), 147.30 (10-C), 141.07 (8-C), 133.29 (6-C), 128.40 (9-C), 121.98 (7-C), 81.37 (3-C), 66.56 (O-CH2), 44.55 (N-CH2), 31.35 (CH3), 17.87 (7-CH3) ppm. – HRMS (ESI): m/z = 288.1347 (calcd. 288.1348 for C14H13NO4, [M+H]+).

9-(1-Hydroxyethyl)-7-methyl-2-morpholino-4H-pyrido[1,2-a]pyrimidin-4-one (4)

Sodium borohydride (52.2 mg, 1.38 mmol) was added to a suspension of compound 3 (198 mg, 0.69 mmol) in CH2Cl2 (5 mL) and methanol (10 mL) at 0 °C. The reaction mixture was stirred for 2 h at room temperature. Water was added and the mixture extracted with chloroform (3 × 30 mL). The organic layer was washed with water and dried over Na2SO4. After concentration under reduced pressure, the residue was crystallized in EtOAc/hexane (1:1) to obtain compound 4 as a colorless solid with a yield of 75% (150 mg). M. p. 218 – 219 °C. 1H NMR (CDCl3, 400 MHz): δ = 8.57 (s, 1H, 8-CH), 7.51 (d, J = 2 Hz, 1H, 6-CH), 5.58 (s, 1H, 3-CH), 5.22 (q, J = 6 Hz, 1H, O-CH), 4.66 (s, 1H, OH), 3.80 (m, 4H, 2 O-CH2), 3.59 (m, 4H, 2 N-CH2), 2.29 (s, 3H, 7-CH3), 1.57 (d, J = 6 Hz, 3H, CH3) ppm. 13C NMR (CDCl3, 100 MHz): δ = 160.21 (4-C=O), 158.69 (2-C), 147.45 (10-C), 137.22 (8-C), 135.47 (6-C), 129.77 (9-C), 117.80 (7-C), 81.39 (3-C), 66.57 (O-CH2), 49.23 (O-CH), 44.63 (N-CH2), 22.11 (CH3), 18.29 (7-CH3) ppm. – HRMS (ESI): m/z = 301.1654 (calcd. 301.1664 for C16H21N4O2, [M+H]+).
9-(1-Hydroxyethyl)-7-methyl-2-(4-methylpiperyazin-1-yl)-4H-pyrindof[1,2-a]pyrimidin-4-one (7)

Sodium borohydride (100 mg, 2.6 mmol) was added to a suspension of compound 6 (200 mg, 0.66 mmol) in CH₂Cl₂ (5 mL) and methanol (10 mL) at 0 °C. The reaction mixture was stirred for 3 h at room temperature. Water was added and extracted with chloroform (3 × 30 mL). The organic layer was washed with water and dried over Na₂SO₄. After concentration under reduced pressure, the residue was purified by column chromatography with 2% MeOH/CHCl₃ as an eluent to obtain compound 7 as a colorless solid with a yield of 78% (156 mg). M. p. 215–216 °C. IR: ν = 3423 (NH), 2925 (CH₃), 1667 (C=O), 1643 (N=C) cm⁻¹. ¹H NMR (CDCl₃, 400 MHz): δ = 8.69 (s, 1H, 8-CH), 7.58 (s, 1H, 6-CH), 7.12 (d, J = 4.6 Hz, 1H, thiazole-CH), 6.75 (d, J = 7.3 Hz, 1H, NH), 6.48, (d, J = 4.6 Hz, 1H, thiazole-CH), 5.66 (s, 1H, 3-CH), 5.27 (m, 1H, N-CH), 3.82 (t, J = 4.0 Hz, 4H, 2 O-CH₂), 3.66 (t, J = 4.0 Hz, 4H, 2 N-CH₂), 2.31 (s, 3H, CH₃), 1.69 (d, J = 6.8 Hz, 3H, 7-CH₃) ppm. ¹³C NMR (CDCl₃, 100 MHz): δ = 169.31 (thiazole-2-C), 160.13 (4-C=O), 158.87 (2-C), 147.47 (10-C), 139.02 (thiazole-5-C), 135.97 (thiazole-4-C), 135.39 (8-C), 124.38 (6-C), 122.24 (9-C), 107.06 (7-C), 81.49 (3-C), 66.52 (O-CH₂), 52.62 (N-CH₂), 44.67 (N-CH₂), 21.33 (CH₃), 18.21 (7-CH₃) ppm. – HRMS (+)ESI: m/z = 372.1442 (calcd. 372.1494 for C₁₉H₂₂N₄O₄·[M+H]^+).

General procedure for the parallel synthesis of TGX-221a-e

Parallel synthesis was carried out in five reactors on a Mettler Toledo MiniBlock for the S₂ reaction to replace the secondary alcohol in compounds 4 and 7 with thiazole-2-amine, 1-methylpiperyazine and aniline. Triethylamine and methanesulfonyl chloride were added to the solution of compounds 4 and 7 in CH₂Cl₂ at 0 °C. The mixtures were stirred at room temperature for 1 h. After the methylation reactions were completed, thiazole-2-amine, 1-methylpiperyazine and aniline were added to the obtained solutions of methylated compounds 4 and 7, respectively. After the mixtures were refluxed for 24 h, the solutions were diluted with CH₂Cl₂ (30 mL). The organic layer was washed with water and dried over Na₂SO₄. Removal of the solvent under reduced pressure followed by purification of the resulting residue through a silica flash column using MeOH/CH₂Cl₂ as an eluent produced the five analogs TGX-221a-e.

7-Methyl-2-morpholin-9-(1-thiazol-2-ylamino)ethyl)-4H-pyrindof[1,2-a]pyrimidin-4-one (TGX-221a)

Compound 4 (35 mg, 0.121 mmol), triethylamine (0.47 mL, 3.63 mmol), methanesulfonyl chloride (0.05 mL, 0.609 mmol), thiazole-2-amine (242 mg, 2.42 mmol), and CH₂Cl₂ (5 mL). TGX-221a: a pale-yellow solid. Yield: 67% (22 mg). M. p. 212–213 °C. IR: ν = 3274 (NH), 2925 (CH₃), 1728 (C=O), 1667 (C=O), 1641 (N=C) cm⁻¹. ¹H NMR (CDCl₃, 400 MHz): δ = 8.69 (s, 1H, 8-CH), 7.58 (s, 1H, 6-CH), 7.12 (d, J = 4.6 Hz, 1H, thiazole-CH), 6.75 (d, J = 7.3 Hz, 1H, NH), 6.48, (d, J = 4.6 Hz, 1H, thiazole-CH), 5.66 (s, 1H, 3-CH), 5.27 (m, 1H, N-CH), 3.82 (t, J = 4.0 Hz, 4H, 2 O-CH₂), 3.66 (t, J = 4.0 Hz, 4H, 2 N-CH₂), 2.31 (s, 3H, CH₃), 1.69 (d, J = 6.8 Hz, 3H, 7-CH₃) ppm. ¹³C NMR (CDCl₃, 100 MHz): δ = 169.31 (thiazole-2-C), 160.13 (4-C=O), 158.87 (2-C), 147.47 (10-C), 139.02 (thiazole-5-C), 135.97 (thiazole-4-C), 135.39 (8-C), 124.38 (6-C), 122.24 (9-C), 107.06 (7-C), 81.49 (3-C), 66.52 (O-CH₂), 52.62 (N-CH₂), 44.67 (N-CH₂), 21.33 (CH₃), 18.21 (7-CH₃) ppm. – HRMS (+)-ESI: m/z = 372.1442 (calcd. 372.1494 for C₁₉H₂₂N₄O₄·[M+H]^+).

Compound 7 (150 mg, 0.5 mmol), triethylamine (0.4 mmol), methanesulfonyl chloride (0.2 mL, 2.2 mmol), aniline (1.0 mL, 10 mmol), and CH₂Cl₂ (20 mL). TGX-221c: a pale-yellow solid. Yield: 75% (120 mg). M. p. 214–215 °C. IR: ν = 3269 (NH), 2925 (CH₃), 1728 (C=O), 1665 (C=O), 1645 (N=C) cm⁻¹. ¹H NMR (CDCl₃, 400 MHz): δ = 8.67 (s, 1H, 8-CH), 7.60 (s, 1H, 6-CH), 7.15 (d, J = 6.6 Hz, 2H, aniline-CH), 6.68 (d, J = 7.3 Hz, 1H, aniline-CH), 6.48, (d, J = 6.6 Hz, 2H, aniline-CH), 5.68 (s, 1H, 3-CH), 5.15 (m, 1H, 11-CH), 3.81 (m, 4H, 2 N-CH₂), 2.72 (m, 4H, 2 N-CH₂), 2.41 (s, 3H, N-CH₃), 2.28 (s, 3H, 7-CH₃), 1.59 (d, J = 6.0 Hz, 3H, CH₃) ppm. ¹³C NMR (CDCl₃, 100 MHz): δ = 159.88 (4-C=O), 159.09 (2-C), 147.45 (10-C), 146.77 (Ar-C), 137.13 (8-C), 135.33 (Ar-
C), 129.25 (Ar-C), 123.79 (6-C), 122.20 (9-C), 117.69 (7-C), 113.24 (Ar-C), 81.42 (3-C), 54.66 (N-CH), 49.28 (N-CH), 46.10 (N-CH), 44.22 (N-CH), 22.12 (CH3), 18.28 (7-CH3) ppm. – HRMS ((+)-ESI): m/z = 378.1951 (caled. 378.2224 for C22H28N2O, [M+H]+).

7-Methyl-2-(4-methylpiperazin-1-yl)-9-(1-(thiazol-2-ylamino)ethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (TGX-221d)

Compound 7 (150 mg, 0.5 mmol), triethylamine (0.4 mL, 3.0 mmol), methanesulfonyl chloride (0.2 mL, 2.2 mmol), 2-aminooxazole (960 mg, 9.6 mmol), and CH2Cl2 (20 mL).


7-Methyl-2-(4-methylpiperazin-1-yl)-9-(1-(4-methylpiperazin-1-yl)ethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (TGX-221e)

Compound 7 (80 mg, 0.27 mmol), triethylamine (1.0 mL, 8.1 mmol), methanesulfonyl chloride (0.1 mL, 1.35 mmol), 1-methylpiperazine (0.54 mL, 5.4 mmol), and CH2Cl2 (10 mL).

TGX-221e: a pale-yellow solid. Yield: 55% (42 mg). M. p. 217 – 219 °C. – IR: ν = 1728 (C=O), 2930 (CH3), 1668 (C=O), 1640 (N=O) cm−1. – 1H NMR (CDCl3, 400 MHz): δ = 8.67 (s, 1H, 8-CH), 7.73 (s, 1H, 6-CH), 5.67 (s, 1H, 3-CH), 4.39 (m, 1H, 11-CH), 3.75 (m, 4H, N-CH2), 2.96 (s, 3H, N-CH3), 2.52 (s, 3H, N-CH3), 2.37 (m, 1H, N-CH2), 2.31 (s, 3H, 7-CH3), 1.65 (m, 3H, CH3), ppm. – 13C NMR (CDCl3, 100 MHz): δ = 160.22 (4-C=O), 159.13 (2-C), 147.45 (10-C), 135.33 (8-C), 122.27 (6-C), 117.60 (9-C), 113.28 (7-C), 81.32 (3-C), 56.67 (N-CH2), 49.23 (N-CH3), 46.18 (N-CH3), 44.69 (N-CH3), 42.23 (N-CH3), 22.11 (CH3), 18.30 (7-CH3) ppm. – HRMS ((+)-ESI): m/z = 385.2558 (caled. 385.2716 for C21H13N3O, [M+H]+).