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A new and efficient synthetic route for the anxiolytic agent CL285032

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ABSTRACT

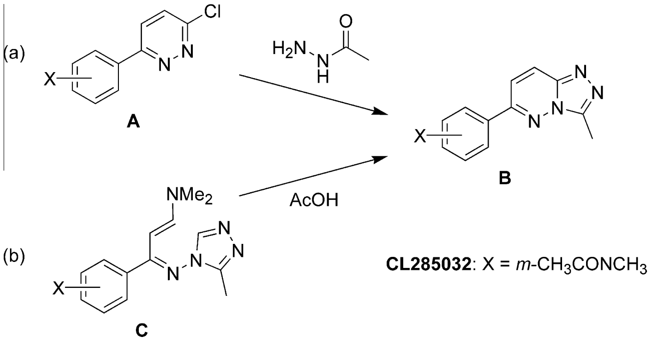
CL285032 is an anxiolytic compound currently under investigation as a possible treatment for canine noise phobia associated anxiety. A robust scale-up and manufacturing process is essential for the development and marketability of the drug. The current synthetic route, although reliable, requires seven steps and has a low overall yield (18%), leaving opportunity for improvement. We are presenting an efficient alternative approach toward the synthesis of CL285032 and the results thereof.

Noise and thunderstorm phobias are among the most commonly recognized disorders associated with panic or phobic responses in companion animals such as dogs, cats or horses. Thunderstorms, fireworks, gunfire, car backfire, etc. frequently induce undesirable nonspecific clinical symptoms in companion animals, particularly dogs, such as salivating, defecating, urinating, destroying, escaping, hiding, trembling, vocalizing and the like. Known treatments for general anxiety behavior in companion animals generally involve either a long period of onset, that is, 3–4 weeks, or if quick-acting, cause sedation and/or ataxia. However, most companion animal owners and veterinarians would prefer to treat their animals suffering from noise phobia with a drug which does not promote sedation or ataxia and which is effective within an hour or two of administration.[1]

Advanced investigations have shown that CL285032 possesses central nervous system activity at nontoxic doses and as such is useful as an anxiolytic agent. It produced certain responses in standard tests with laboratory animals which are known to correlate well with relief of anxiety in man, dogs, cats and horses, being particularly effective in dogs.[1] Therefore CL285032 was advanced for development as an anxiolytic agent and provides a therapeutically effective method for the treatment of noise phobia in a companion animal without undesirable sedative side effect. A robust scale-up and manufacturing process is essential for the development and marketability of the drug. The current synthetic route, although reliable, requires seven steps and has a low overall yield (18%), leaving opportunity for improvement. We are presenting two alternative approaches toward the synthesis of CL285032 and the results thereof.

The reported synthesis of CL285032 and congeners,[2,3] involves a key substitution/cyclization synthetic step of the chloro-pyridazine compounds of type **A** into [1,2,4]triazolo[4,3-*b*]pyridazines of type **B** (Scheme 1 a). The method includes various substituents **X**, for example, H, *p*-F, *m*-CF_3, *p*-OCH_3, etc., except for the one of interest for the synthesis of CL285032, the N-methylated acetamide. The only nitrogen containing functionality featured in the early reports was a *m*-NO_2 group, which for the purpose of CL285032 synthesis was being reduced, then alkylated and acylated respectively, each additional step adding to the overall decrease of the yield.

Later on, another method emerged in which the key step is an intramolecular pyridazine ring closure, from an aza-diene intermediate of the type **C** (Scheme 1 b), that has the prerequisite triazole



Scheme 1. Approaches to [1,2,4]triazolo[4,3-*b*]pyridazines.

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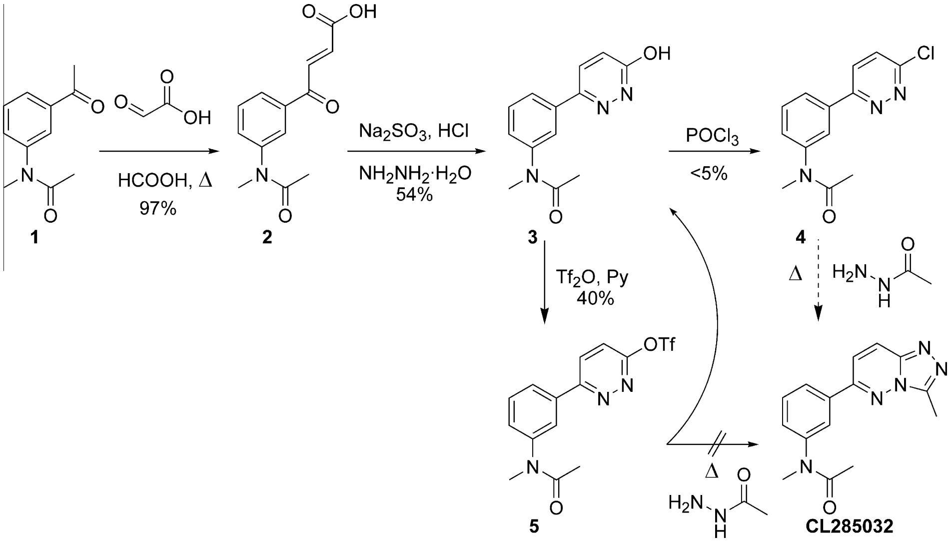
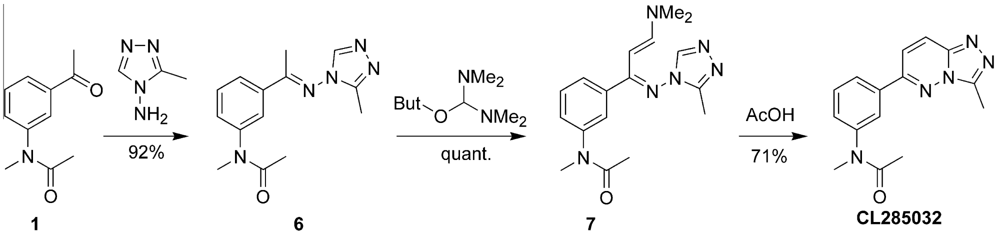


Scheme 2. Initial approach toward CL285032.



Scheme 3. Improved synthesis of CL285032.

ring already in place.[4] This method has eventually employed an acetamide substituent **X** throughout the synthetic sequence, however, instead of a methyl group, a *tert*-butyl group was the N-substituent.[5]

Our initial attempt at an improved synthesis of CL285032 concentrated on the first method (Scheme 1 a) and aimed at having the required N-methylated acetamide in place from the very beginning while maintaining the established four initial steps of the original sequence that affords the [1,2,4]triazolo[4,3-*b*]pyridazine.[3]

To this end, commercially available 3′-acetyl-*N*-methylacetanilide **1** was reacted with glyoxylic acid in an acid mediated aldol condensation (Scheme 2). The resulting α,β-unsaturated acid **2** was cyclized with hydrazine under acidic conditions to afford the desired hydroxy-pyridazine **3** in fair yield. From this point onward the anticipated sequence of reactions became, somehow expectedly, more difficult. Attempts to prepare chloro-pyridazine **4** have ultimately failed, although milligram amounts of material could be isolated for characterization purposes. We can only speculate that the amide reacts with POCl$_3$ to afford Vilsmeier-Haack like reactive species which then capture the electron-rich hydroxy-pyridazine ring. Circumventing this problem by preparing triflate **5** did not provide the desired outcome as well. The attempted substitution/condensation sequence in presence of acetyl hydrazine did not succeed, as the triflate hydrolyzed back to hydroxy-pyridazine **3,** despite our best efforts to keep the reaction mixture moisture-free.[7]

Consequently we undertook an alternative, more conservative approach to access the [1,2,4]triazolo[4,3-*b*]pyridazine,[4,5] by installing the triazole ring into the molecular framework before the pyridazine ring closure, which was designed as the last step (Scheme 3).

Condensation of commercially available **1** with 3-methyl-4*H*-1,2,4-triazole-4-amine,[6] afforded *N*-triazolo-imine **6** in near quantitative yield. Subsequent treatment with *tert*-butoxy bis(dimethylamino)methane affords the dimethylamino-aza-diene **7** in quantitative yield, which under standard acidic conditions generates the [1,2,4]triazolo[4,3-*b*]pyridazine ring system of CL285032 in 71% yield.[7]

Building on previous strategies for synthesizing CL285032 and related triazolo-pyridazines, our efforts delivered an improved synthetic sequence leading to CL285032. The overall yield was elevated from the previously moderate 18% to a much improved 65% and the number of synthetic steps of the process was cut in less than half. Our strategy is straightforward and amenable to direct scale-up of this valuable therapeutic agent.

Acknowledgments

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7. *Experimental*: Unless otherwise indicated, all commercial reagents were used as received without further purification. Commercially available anhydrous grade

solvents were used. Reactions were monitored by thin layer chromatography (TLC) using E. Merck pre-coated silica-gel plates. Visualization was accomplished with UV light, followed by fixation with alcoholic phosphomolybdic acid solution or aqueous potassium permanganate solution. Reaction progress and purity of intermediates was also assessed by LC–MS analysis using an Agilent Technologies 1200 Series LC system coupled to a 6130 Quadrupole MS. Chromatographic separation was performed using the indicated solvent system on a Teledyne Isco CombiFlash R_f system. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a 400 MHz Bruker Avance III spectrometer at ambient temperature. Chemical shifts (δ, ppm) are reported relative to tetramethylsilane internal standard or residual proton signal of the deuterated solvent used.

(E)-4-(3-(N-Methylacetamido)phenyl)-4-oxobut-2-enoic acid **2**: To a solution of **1** (2 g, 10.46 mmol) in anhydrous formic acid (2 mL) glyoxylic acid monohydrate (1.01 g, 10.98 mmol) was added portion wise. The mixture was warmed up to 110 °C and refluxed overnight. After cooling to room temperature the reaction mixture was injected on top of a silica gel chromatographic column and separated using CH_2Cl_2/MeOH (0–50%). Evaporation of solvent in vacuo afforded 2.52 g (97%) of **3** as a light-yellow solid: ^{1}H NMR ($CDCl_3$, δ, ppm): 8.08–7.98 (m, 1H), 7.94 (d, *J* = 15.6 Hz, 1H), 7.90–7.80 (m, 1H), 7.61 (q, *J* = 8 Hz, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 6.93 (d, *J* = 15.6 Hz, 1H), 3.32 (s, 3H), 1.93 (s, 3H). LC–MS (ESI): 248.1.

N-(3-(6-Hydroxypyridazin-3-yl)phenyl)-N-methylacetamide **3**: To a solution of **2** (2.52 g, 10.2 mmol) in 15 mL water at room temperature Na_2SO_3 (1.41 g, 11.2 mmol) was added portion wise and the reaction mixture was warmed up with stirring to 90 °C. After 1 h LC–MS analysis shows complete consumption of the starting material. The reaction flask was allowed to cool for 10 min then HCl 6 N (2.2 mL, 13.46 mmol) was added drop wise. After stirring for another 10 min the reaction mixture was re-heated to 90 °C and hydrazine hydrate (1.1 mL, 22.42 mmol) was added drop wise. Stirring was continued for another 2 h at 90 °C. The reaction mixture was cooled to room temperature, diluted with brine and extracted with CH_2Cl_2. Combined organic phases were dried over $MgSO_4$. Evaporation of volatiles in vacuo afforded 1.34 g (54%) of crude product as a light-yellow solid, suitable for the next synthetic step: ^{1}H NMR ($CDCl_3$, δ, ppm): 12.38 (s, 1H), 7.78 (d, *J* = 10 Hz, 1H), 7.77–7.69 (m, 2H), 7.53 (t, *J* = 7.8 Hz, 1H), 7.31–7.26 (m, 1H), 7.12 (d, *J* = 10 Hz), 3.31 (s, 3H), 1.92 (s, 3H). LC–MS (ESI): 244.1.

N-(3-(6-Chloropyridazin-3-yl)phenyl)-N-methylacetamide **4**: To a refluxing solution of **3** (0.1 g, 0.41 mmol) in $CHCl_3$ (3 mL) freshly distilled $POCl_3$ (0.11 mL, 1.23 mmol) was added drop wise. After 3 h reflux the reaction mixture was cooled to room temperature and carefully quenched with excess MeOH (safety warning: HCl evolution). The mixture was transferred to silica gel and the volatiles were removed in vacuo. Flash chromatography using EtOAc/Hexanes (0–100%) afforded approximately 5 mg (<5%) of **4** as a yellow oil: ^{1}H NMR ($CDCl_3$, δ, ppm): 7.09–7.96 (m, 2H), 7.85 (d, *J* = 8.8 Hz, 1H), 7.62 (d, *J* = 8.8 Hz, 1H), 7.60–7.57 (m, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 3.34 (s, 3H), 1.94 (s, 3H). LC–MS (ESI): 262.1.

6-(3-(N-Methylacetamido)phenyl)pyridazin-3-yl trifluoromethanesulfonate **5**: A solution of **3** (0.31 g, 1.27 mmol) in CH_2Cl_2 (5 mL) was cooled at 0 °C and pyridine (0.52 mL, 6.37 mmol) was added drop wise, followed by Tf_2O (0.43 mL, 2.55 mmol). After stirring for 1 h at 0 °C the reaction mixture was diluted with CH_2Cl_2 and quenched with excess ice cold water. Extraction with CH_2Cl_2 was followed by drying the combined organic fractions over $MgSO_4$. After removal of volatiles in vacuo the resulting residue was transferred to silica gel. Flash chromatography using EtOAc/Hexanes (60–100%) afforded **5** (0.19 g, 40%) as a light-yellow solid: ^{1}H NMR ($CDCl_3$, δ, ppm): 8.10 (d, *J* = 9.2 Hz, 1H), 8.02 (t, *J* = 0.8 Hz, 1H), 7.99 (d, *J* = 7.6 Hz, 1H), 7.62 (t, *J* = 7.8 Hz, 1H), 7.54 (d, *J* = 9.2 Hz, 1H), 7.40 (d, *J* = 7.6 Hz, 1H), 3.34 (s, 3H), 1.94 (s, 3H). LC–MS (ESI): 376.1.

(E)-N-Methyl-N-(3-(1-((3-methyl-4H-1,2,4-triazol-4-yl)imino)ethyl)phenyl)acetamide **6**: To a solution of the starting material **1** (0.65 g, 3.40 mmol) in toluene (40 mL) 3-methyl-4*H*-1,2,4-triazol-4-amine (0.33 g, 3.36 mmol) was added and the reaction mixture was refluxed overnight (22 h) with water removal via a Dean-Stark trap. After cooling to room temperature the reaction mixture was transferred to silica gel and the volatiles were removed in vacuo. Flash chromatography using MeOH/CH_2Cl_2 (0–10%) afforded **7** (0.85 g, 92%) as a light-yellow solid: ^{1}H NMR ($CDCl_3$, δ, ppm): 8.63 (s, 1H), 7.95 (br s, 2H), 7.61–7.59 (m, 2H), 3.20 (s, 3H), 2.37 (s, 3H), 2.32 (s, 3H), 1.82 (s, 3H). LC–MS (ESI): 272.2.

N-(3-((1E,2E)-3-(Dimethylamino)-1-((3-methyl-4H-1,2,4-triazol-4-yl)imino)allyl)phenyl)-N-methylacetamide **7**: To a solution of **6** (0.77 g, 2.84 mmol) in THF (5 mL) *tert*-butoxy bis(dimethylamino)methane (1.19 mL, 5.68 mmol) was added drop wise. After stirring at room temperature for 4.5 h the reaction mixture was transferred to silica gel and the volatiles were removed in vacuo. Flash chromatography using MeOH/CH_2Cl_2 (0–15%) afforded **8** (0.95 g, quantitative) as a light-yellow solid: ^{1}H NMR ($CDCl_3$, δ, ppm): 8.39 (s, 1H), 7.55–7.47 (m, 4H), 6.88 (d, *J* = 12.4 Hz, 1H), 4.52 (d, *J* = 12.8 Hz, 1H), 3.20 (s, 3H), 2.95 (s, 3H), 2.67 (s, 3H), 2.22 (s, 3H), 1.84 (s, 3H). LC–MS (ESI): 327.2.

CL285032**:** A solution of **7** (0.2 g, 0.61 mmol) in AcOH (3 mL) was refluxed (bath temperature 140 °C) overnight (20 h). After cooling to room temperature the reaction mixture was transferred to silica gel and the volatiles were removed in vacuo. Flash chromatography using MeOH/CH_2Cl_2 (0–15%) afforded CL285032 (0.122 g, 71%) as a light-yellow solid: ^{1}H NMR ($CDCl_3$, δ, ppm): 8.42 (d, *J* = 9.6 Hz, 1H), 8.11 (br s, 2H), 7.98 (d, *J* = 9.6 Hz, 1H), 7.66 (t, *J* = 7.8 Hz, 1H), 7.57 (d, *J* = 6.8 Hz, 1H), 3.24 (s, 3H), 2.78 (s, 3H), 1.85 (s, 3H). LC–MS (ESI): 282.1.