Pyrazolo[1,4]diazepines as non-peptidic probes of the oxytocin and vasopressin receptors

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ABSTRACT
An improved synthesis of differently substituted pyrazolo[1,4]diazepine compounds is reported. In addition, we have used this methodology to obtain non-peptidic compounds to probe the oxytocin and vasopressin receptors.

The neuropeptide oxytocin has been implicated in multiple central nervous system functions in mammalian species. Increased levels have been reported to improve trust, alleviate symptoms related to autism and social phobias, and reduce social anxiety. 1 Drug discovery programs have developed novel non-peptidic oxytocin receptor agonists with greater affinity for the receptor and increased half-life and blood brain barrier permeability. Two of the first-generation non-peptide agonists reported were WAY 267,464 (1) and compound 2 (Fig. 1). However, further studies with WAY 267,464 have shown that it is not a selective oxytocin receptor (OTR) agonist as it exhibits only weak in vitro affinity for the receptor. 4 In contrast, strong vasopressin 1A receptor (V1A R) affinity was observed. 4 Recent in vivo results suggest that V1A R antagonism may be causing the observed biological effects rather than OTR agonism, or potentially a combination of both. 5 To fully investigate the role of OTR or V1A R in specific diseases non-peptidic molecules are needed to probe these systems.

A key feature of compounds 1 and 2 is the pyrazolo[1,4]diazepine head group 3, a five-step synthesis of which has been previously reported. 3,6 We recently reported an improved synthesis of this head group and used the new methodology to synthesize heteroatom analogues of the benzodiazepine center. 7 Herein, we report the exploration of this methodology for its applicability toward the synthesis of new analogues, namely appending different functional groups within and around the aromatic moiety. In addition, we also report a modified synthesis of compounds 1 and 2 with methods that are operationally simpler and applicable to gram scale. Greater availability of compounds 1, 2 and pyrazolo[1,4]diazepine analogues allows for further testing to better understand the role
of oxytocin and vasopressin systems in specific diseases and should aid the development of targeted therapies.

Our revised method for the synthesis of compound 3 involves a base-promoted nucleophilic aromatic substitution on pyrazole 4 with 2-nitroaniline (5) to afford compound 6, which can then undergo a one-pot tandem reductive cyclization to provide the head group 3 (Scheme 1). This procedure is applicable to the synthesis of compound 3 on a large scale (0.1 mol) and respectable yields (57% from pyrazole 4).

Due to the large number of commercially available 2-nitroanilines with additional substituents we thought it appropriate to elaborate further upon this methodology to determine the scope of the reactions reported. As part of this investigation, a variety of compound 3 analogues would also be produced that could then be elaborated to provide analogues of compounds 1 and 2.

The most common substitution pattern for additionally functionalized 2-nitroanilines that are commercially available is para to the amine moiety. Accordingly, the initial attempts to expand upon our synthetic methodology were to react pyrazole 4 with the 2-nitroanilines 7a–e, which possess an assortment of functional groups (Scheme 2). The initial nucleophilic aromatic substitution with an extra methyl substitution proceeded well to afford the substituted pyrazole 8a (R = Me) (69%). Substituting the aromatic ring with electron- withdrawing (R = COOMe) or electron-donating (R = OMe) groups did not impede the reaction though a decrease in yield (61% vs 42%) was noted for the latter case. Halide substitution (R = F or Cl) was also tolerated in the reaction with similar yields (59% and 58%, respectively).

With the substituted pyrazoles 8a–e in hand, we turned our attention to the tandem reductive cyclization. Using the palladium-catalyzed reducing conditions outlined previously the benzodiazepines 9a–d were all obtained in yields ≥ 90%. The conversion of compound 8e into 9e (R = Cl) under these conditions proved to be somewhat problematic due to the propensity of the chloride to cleave under the reaction conditions to instead afford benzodiazepine 3. This problem could be circumvented by using a Zn/HCl reducing system to thereby afford compound 9e, albeit in a reduced yield of 62%.

With these promising results we next tested the applicability of these reaction conditions with 2-methyl-6-nitroaniline (10) (Scheme 3). The steric bulk of the methyl substitution adjacent to the nucleophilic nitrogen was shown to have some impediment to the reaction, which required a longer reaction time (18 h vs 1 h) in addition to a reduced yield (44%) of substituted pyrazole 11 being obtained. The 1H and 13C NMR spectra indicated that compound 11 was synthesized as a mixture of atropisomers in a 1:1.5 ratio. The lack of free rotation did not appear to influence greatly the subsequent reductive cyclization with benzodiazepine 12 isolated in 82% yield.

The final variation to test this methodology was to attempt the reactions using 1,2-aminonitropyridines (Scheme 4). Reactions employing both 3-nitropyridin-2-amine (13a) and 2-nitropyridin-3-amine (13b) proceeded well in the first instance to provide substituted pyrazoles 14a (45%) and 14b (51%), respectively. However, when compound 14a was subjected to the tandem reductive cyclization, neither benzodiazepine 15a, nor any other discernable products were obtained despite complete consumption of the starting material. There was a potential for multiple side reactions to occur if the pyridine nitrogen reacted with the aldehyde group before reduction. However, a single product was seen via TLC analysis of the reaction mixture that was no longer present subsequent to work-up, suggesting instability of the desired product. Support for this hypothesis was provided when compound 14b was subjected to the same reaction conditions providing the benzodiazepine 15b in 42% yield. Despite the low yield, enough compound was obtained for characterization, however upon standing, both at room temperature and at 4 °C, the compound decomposed into indiscernible products. This suggests that while pyridine analogues can be synthesized they are unstable under ambient conditions.

The synthesis of WAY 267,464 (1) from benzodiazepine 3 (Scheme 5) initially followed the method of Hibert et al., whereby amide formation with acid chloride 16 gave compound 17 (76%).
Despite many attempts at different amide forming reactions with the parent carboxylic acid, the only conditions from which a product could be obtained were through the corresponding acid chloride. The reduction of the aromatic nitrile moiety as previously reported using a CoCl₂/NaBH₄ system, while successful on a large scale, resulted in reduced yields (60%) and required a laborious and time-consuming work-up procedure. In an attempt to circumvent this issue catalytic hydrogenation methods were explored as an alternative. An atmosphere of hydrogen with Raney cobalt has previously been shown to reduce selectively aliphatic nitriles into the corresponding amine, however, for the current aromatic nitrile no reduction took place. The more active Raney nickel had greater success, though for complete conversion in a reasonable timeframe (18 h) the reaction did require elevated temperatures. The significant benefit of this approach was that filtering the reaction mixture and subsequent concentration of the solution afforded the target amine (95%) without further work-up or purification required. Subjecting amine to urea formation with piperazine (available in two steps from commercially available material) in the presence of CDI (carbonyldiimidazole), using conditions slightly modified from those previously reported, gave the target WAY 267,464 (1) in 51% yield. Overall, this highly valuable drug target could be obtained in five steps and 21% yield from a commercially available starting material using this newly described procedure. By substituting the secondary amine with pyrrolidine, compound could also be obtained from primary amine using a similar urea-forming reaction to that of compound (Scheme 5). A five-step synthesis of pyrrolidine has already been reported, but herein we report a new shorter three-step method. The commercially available Cbz protected L-proline was first converted to a secondary amine using a modified reaction sequence.  

**Scheme 3.** Synthesis of benzodiazepine. Reagents and conditions: (i) 10 (3.0 equiv), KOH (2.0 equiv), DMF, 100 °C, 18 h; (ii) Pd/C (5 mol %), H₂ (1 atm), MeOH, rt, 18 h.

**Scheme 4.** Efforts toward pyridine-based benzodiazepines. Reagents and conditions: (i) 13a,b (3.0 equiv), KOH (2.0 equiv), DMF, 100 °C, 1 h; (ii) Pd/C (5 mol %), H₂ (1 atm), MeOH, rt, 18 h.

**Scheme 5.** Revised synthesis of WAY 267,464 (1). Reagents and conditions: (i) 16 (1.3 equiv), Et₃N (2.0 equiv), CH₂Cl₂, rt, 18 h; (ii) Raney Ni (1.0 equiv), H₂ (1 atm), MeOH (saturated with NH₃), 65 °C, 18 h; (iii) 19 (1.2 equiv), CDI (1.2 equiv), i-Pr₂EtN (2.4 equiv), DMF, rt, 18 h.

**Scheme 6.** Revised synthesis of amine and the synthesis of compound. Reagents and conditions: (i) (COCl)₂ (1.1 equiv), DMF (cat.), then 22 (1.1 equiv), Et₂N (2.0 equiv), CH₂Cl₂, rt, 16 h; (ii) Lawesson's reagent (1.5 equiv), chlorobenzene, 150 °C (μw), 2 h; (iii) HBr (33 wt % in AcOH), rt, 3 h; (iv) 18 (1.0 equiv), 20 (1.2 equiv), CDI (1.2 equiv), i-Pr₂EtN (2.4 equiv), DMF, rt, 18 h.
into the corresponding acid chloride and then, without isolation, treated with amine 22 to afford amide 23 as a mixture of rotamers and conformers (72%). Microwave irradiation of a solution of amide 23 and Lawesson’s reagent allowed for the fast conversion of the amide moiety into the corresponding thio derivative contained within compound 24 (58%), that also appeared as a mixture of rotamers and conformers in the NMR spectra. Cbz deprotection proved to be more challenging than expected with all hydrogenolysis methods failing to cleave the protecting group. Utilizing the previously reported procedure of HBr in acetic acid afforded the free secondary amine 20 (86%), however, no characterization data had been reported to allow for comparison. Finally, with compound 20 in hand, it could then be reacted with amine 18 in the presence of CDI to afford non-peptidic compound 2 in 55% yield.

In conclusion, we have expanded upon our methodology for the synthesis of pyrazolo[1,4]diazepine moieties to incorporate various functionalities about the aromatic ring. Attempts to incorporate a nitrogen atom into the ring were thwarted by the instability of the target compounds. Taking the parent benzodiazepine 3, we were able to improve upon the synthesis of compounds 1 and 2 in terms of practicality and step economy. With this improved methodology we aim to utilize the substituted benzodiazepine derivatives to synthesize analogues of compounds 1 and 2 and test them for activity toward OTR and V1aR. Efforts in this direction are ongoing and will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.06.022.

References and notes

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