Synthesis of Tetrasubstituted Benzotriazepinone Turn Mimics

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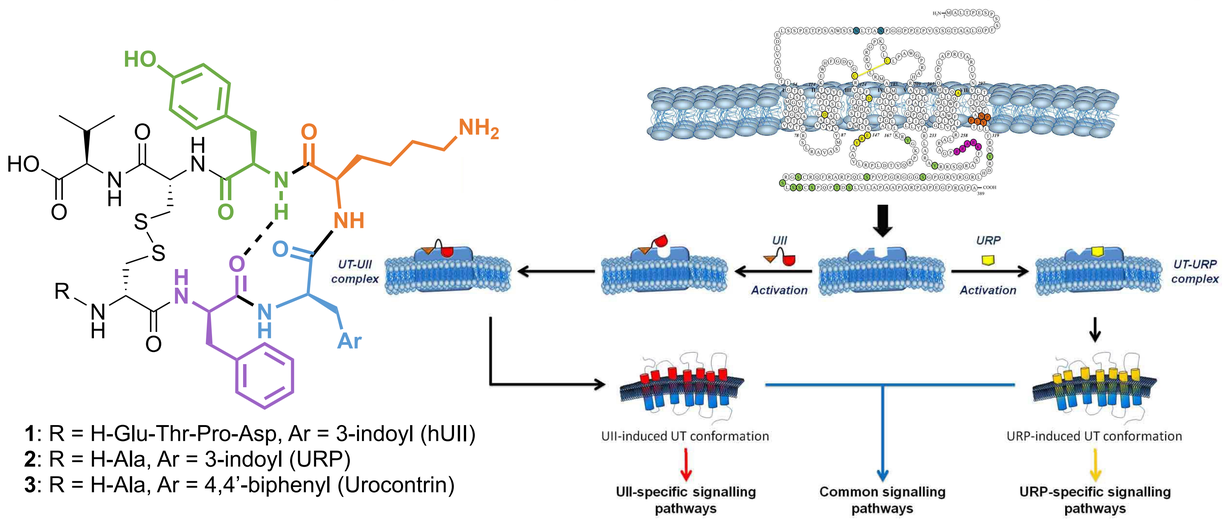
ABSTRACT

Benzotriazepines are a class of heterocycles that have exhibited binding affinity and activity at various receptors [1]. 1,3,5,8-Tetrasubstituted 1,3,4-benzotriazepin-2-ones have been shown to mimic both type I and I' β-turn conformations due in part to nitrogen pyramidalization and dynamic chirality [2]. Moreover, biological assessment of tetrasubstituted benzotriazepinones designed to mimic the peptide allosteric modulator Urocontrin have demonstrated ability to selectively enhance or diminish the activity of one of the two endogenous urotensin II receptor ligands without influence on the activity of its counterpart [3]. Our presentation focuses on the development of a modular strategy to prepare tetrasubstituted benzotriazepinones. Notably, photoreactive components are being introduced on the heterocycle structure with the goal of creating photoaffinity probes to label the binding site of the urotensin II receptor.

INTRODUCTION

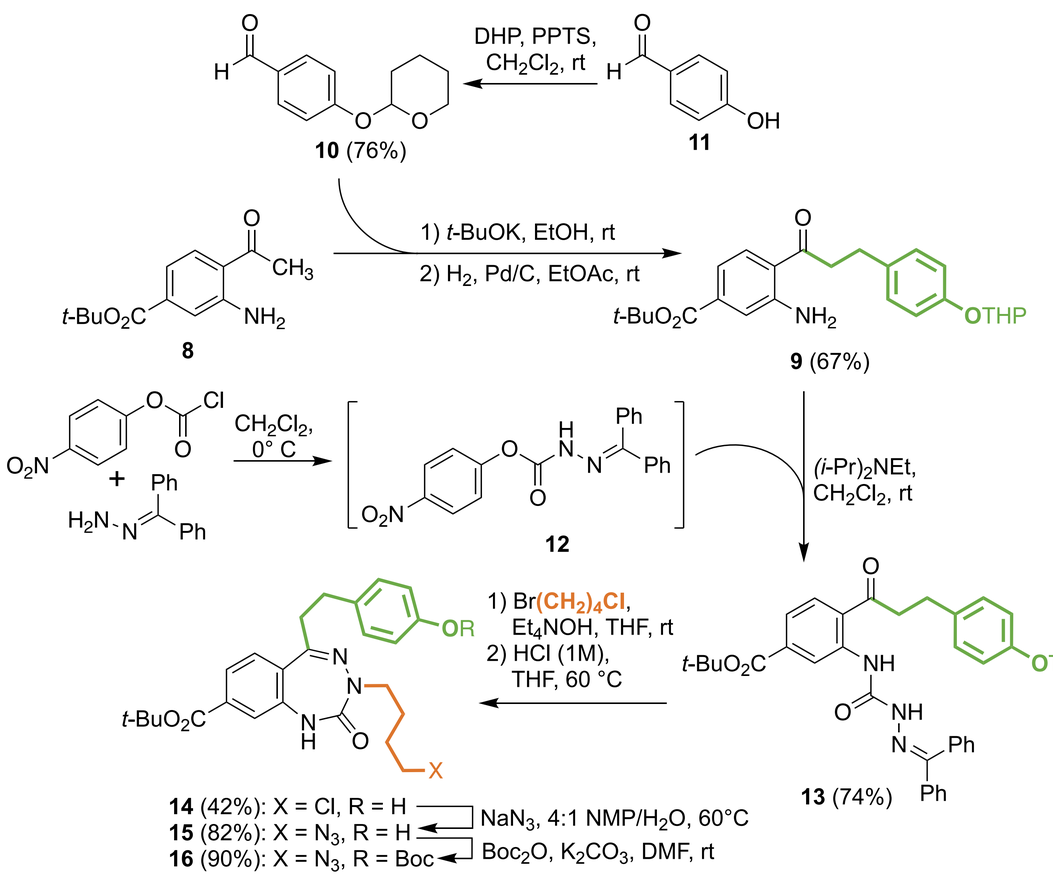
The urotensin receptor (UT) is a G protein-coupled receptor (GPCR) that binds two endogenous peptide ligands: urotensin II (UII) and urotensin II-related peptide (URP) [5]. The triad (UT, UII and URP) plays an important role in normal cardiovascular function. Notably, UII is among the most potent vasoconstrictors [4]. Understanding of the distinct signaling pathways that UII and URP induce on binding is critical for advancing therapy targeting UT [5]. Notably, the peptide urocontrin exhibits allosteric modulation of UT reducing UII-mediated vasoconstriction without influencing URP-induced activity [4]. Tetrasubstituted benzotriazepinones were designed to mimic the Phe-Bip-Lys-Tyr tetrapeptide of urocontrin and the β-turn conformation adopted by UII and URP [3,6]. Such benzotriazepinones can respectively and selectively positively and negatively modulate the activity of one of the endogenous ligands (UII or URP) without effect on the counterpart. Optimization of the synthesis is pursued to diversify the benzotriazepinones to create other GPCR ligands and to introduce photoreactive groups for making photoaffinity labeling probes to study UT binding.



1: R = H-Glu-Thr-Pro-Asp, Ar = 3-indoyl (hUII)
2: R = H-Ala, Ar = 3-indoyl (URP)
3: R = H-Ala, Ar = 4,4'-biphenyl (Urocontrin)

BENZOTRIAZEPINONE RING SYNTHESIS

Aminobenzoate **8** was prepared from *p*-ethyl benzoic acid by nitration, benzylic oxidation, Steglich esterification and nitro group reduction. Dihydrochalcone **9** was obtained in 67% yield by olefin reduction of a chalcone prepared by aldol condensation between THP-protected aldehyde **10** and aminobenzoate **8**. Activated carbazate **12** was generated in situ from benzophenone hydrazone and p-nitrophenyl chloroformate and reacted with dihydrochalcone **9** to yield semicarbazone **13** in 74% yield. Selective alkylation of **13** followed by intramolecular cyclization afforded chloride **14** in 42% yield. Chloride displacement with sodium azide led to azide **15** in 82% yield. Phenol protection gave carbonate **16** in 90 % yield.



CONCLUSION

Tetrasubstituted benzotriazepinones have been synthesized by aza-amino acid chemistry. Optimization enables late-stage peptide coupling to introduce a variety of amines. A library of UT modulators and probes for photoaffinity labeling are now being pursued to enhance modulator potency and selectivity and to identify the ligand-receptor binding site.

ACKNOWLEDGMENT

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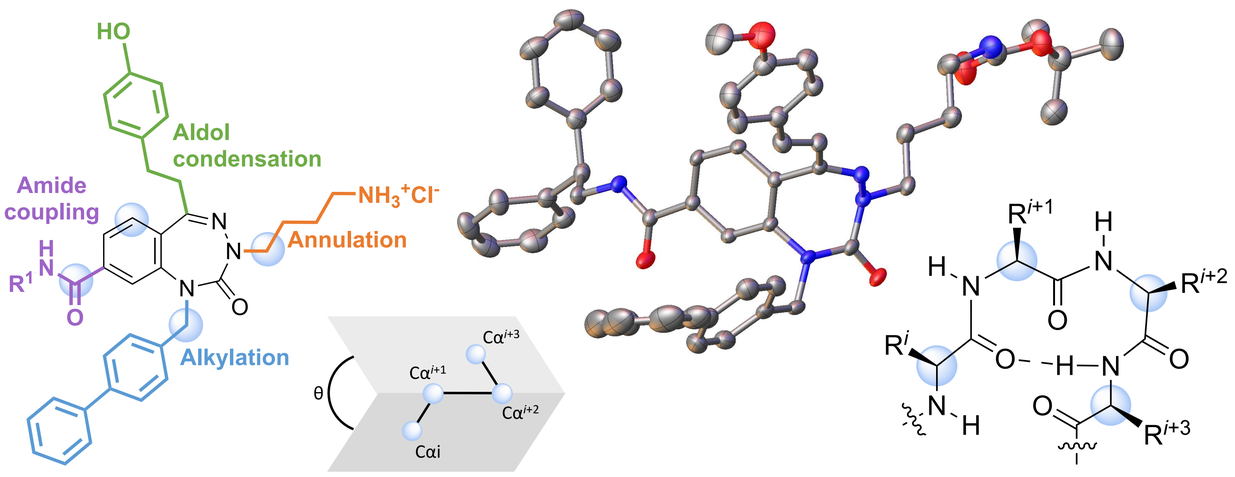
BENZOTRIAZEPINONE MODULATORS OF UT

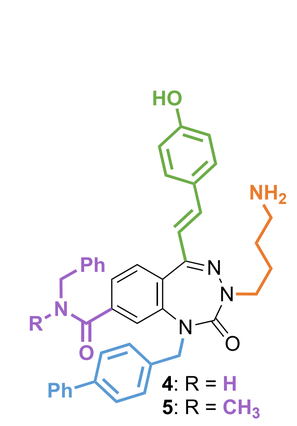


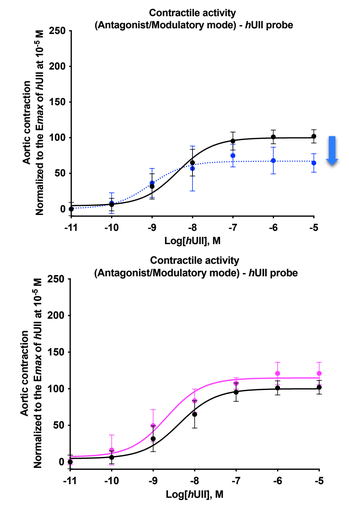
Tetrasubstituted benzotriazepinone synthesis has been achieved by a modular strategy featuring aldol condensation, triazepinone annulation, alkylation, and amide coupling [2]. To facilitate benzotriazepinone diversification, a more optimal synthesis is under study to enable *N*1-alkylation prior to amide coupling.

The β-turn secondary structure is often adopted by peptide GPCR ligands. Crystallographic studies revealed benzotriazepinone mimicry of both type I and I' β-turn conformers due to nitrogen pyramidalization and dynamic chirality [2].

Studies of vasoconstrictive effects *ex vivo* have shown that without exhibiting activity on the endogenous ligand counterpart, different benzotriazepinone analogs are positive or negative allosteric modulators of UII or URP [3].

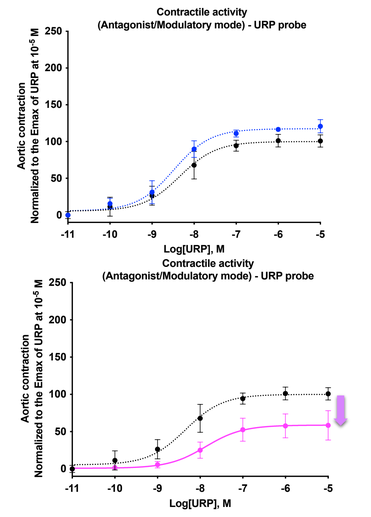
Selective Negative Allosteric Modulation of hUII and URP



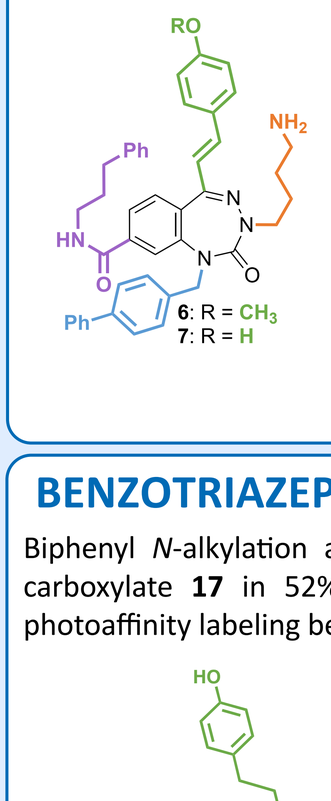


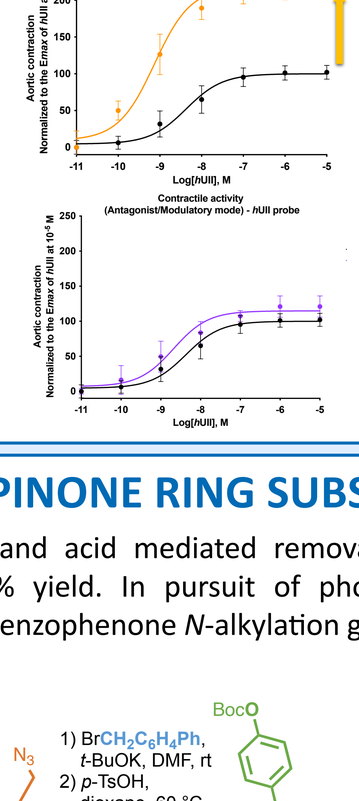
4: Selective negative allosteric modulator of hUII

5: Selective negative allosteric modulator of URP



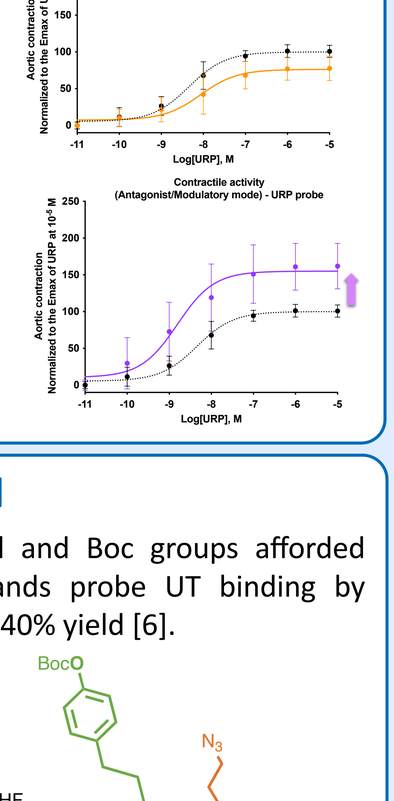
Selective Positive Allosteric Modulation of hUII and URP





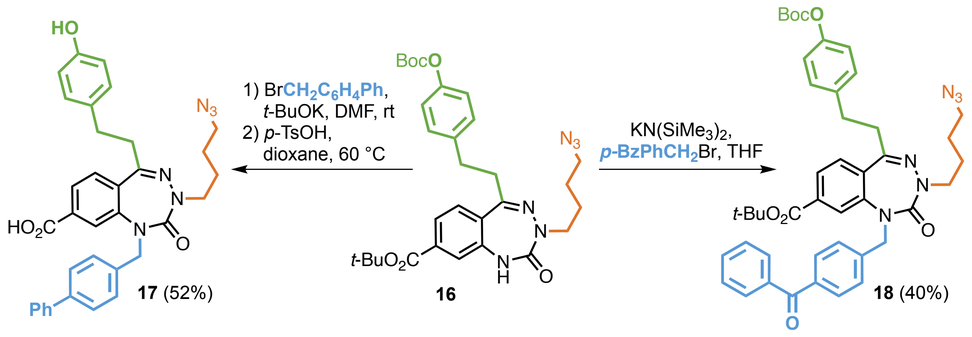
6: Selective positive allosteric modulator of hUII

7: Selective positive allosteric modulator of URP



BENZOTRIAZEPINONE RING SUBSTITUTION

Biphenyl *N*-alkylation and acid mediated removal of *tert*-butyl and Boc groups afforded carboxylate **17** in 52% yield. In pursuit of photoreactive ligands probe UT binding by photoaffinity labeling benzophenone *N*-alkylation gave ester **18** in 40% yield [6].



REFERENCES

[1] Harvey, S.-M.; Wei, X.; Truong, D.; Lubell, W. D. *Can. J. Chem.* **2024**, *102* (8), 469–498.
[2] Wei, X.; Douchez, A.; Lubell, W. D. *J Org Chem* **2023**, *88* (7), 4633–4648.
[3] Wei, X.; Diarra, S.; Douchez, A.; Cunico Dallagnol, J. C.; Hébert, T. E.; Chatenet, D.; Lubell, W. D. *J. Med. Chem.* ***2023***, *66* (20), 14241–14262.
[4] Chatenet, D.; Nguyen, T. T. M.; Letourneau, M.; Fournier, A. *Front. Endocrinol.* **2013**, *3* (174), 1–13.
[5] Haensele, E.; Mele, N.; Miljak, M.; Read, C. M.; Whitley, D. C.; Banting, L.; Delépée, C.; Sopkova-de Oliveira Santos, J.; Lepailleur, A.; Bureau, R.; Essex, J. W.; Clark, T. *J. Chem. Inf. Model.* **2017**, *57*, 298–310.
[6] Wei, X.; Harvey, S.-M.; Iaculli, D.; Ballet, S.; Lubell, W. D. *Proc. 37th Eur. Pept. Symp.*, Florence, Italy, 2024.