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Click chemistry based rapid one-pot synthesis and evaluation for protease inhibition of new tetracyclic triazole fused benzodiazepine derivatives

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ABSTRACT

We describe herein a one-pot synthesis of novel tetracyclic scaffolds that incorporate a fusion of a proline, 1,2,3-triazole ring with [1,4]-benzodiazepin-8(4*H*)-one ring systems following click chemistry. The expected peptide bond formation followed by in situ 1,3-dipolar cycloaddition in absence of any catalyst led to the formation of new triazole fused benzodiazepine derivatives.

The development of new therapeutic agents, as well as the identification of molecular probes for the study of the chemical/biological interfaces, is one of the major goals in biomedical research. In this context, the availability of libraries of small molecules, which depend heavily on the availability of synthetic transformations that allow the rapid assembly of complex molecular frameworks providing maximum diversity and covering as much chemical space as possible, is seen as the only means which guarantees potential modulation of the many biological targets that are ultimately being unveiled by genomics.[1] The design of such libraries may involve novel scaffolds to which diverse side chains are attached, the trajectories of which seek to explore three-dimensional space. An important overlapping principle is the concept of privileged structures, recently updated by Patchett et al.[2] One of the privileged structure 1,4-benzodiazepine derivative display tranquilizing, muscular relaxant, anti-convulsant and sedative effects.[3,4] The use of this class of compounds with therapeutic purposes is not only limited to anxiety and stress conditions, seemingly minor changes in their structures can produce a host of different biological activities, such as central nervous system receptors,[5] G-proteins coupled receptors[6] and central nervous system diseases.[7] It has also found applications for the synthesis of peptidomimetics,[8] peptide antagonists,[9] inhibitors of DNA interactions,[10] antiviral or antimalarial activity,[11] and many other potentially active molecules.[12] Currently there is a considerable interest in the discovery and development of small molecules such as pyrrolo[2,1-*c*][1,4]-benzodiazepines (PBD's) that have to be used as potential antitumor and gene targeted drugs.[13] Anthramycin (**1**), DC-81 (**2**) and Neothramycin (**3**) are well-known and promising members of the PBD class (Fig. 1).[14] A number of pyrrolo[2,1-*c*][1,4]-benzo-diazepin-5-ones constitute a new class of anthramycin antibiotics, a typical example of which is abbeymycin (**4**). Recently, the tetracyclic compound **5**, referred to as bretazenil,[15] has emerged due to its potential usage against neurodegenerative diseases.

In the context of our ongoing effort directed towards the synthesis of 1,4-benzodiazepine derivatives of pharmacological interest, we turned our attention to new classes of tetracyclic compounds, namely benzo[*e*]pyrrolo[1,2-*a*][1,2,3]triazolo[5,1-*c*][1,4]diaze-pin-8(4*H*)-one (**6**), 5-(benzyloxy)-benzo[*e*]pyrrolo[1,2-*a*][1,2,3]triazolo[5,1-*c*][1,4]diazepine-8(4*H*)-one (**7**) and their derivatives (Fig. 2). In developing a strategy toward the synthesis of these compounds, we perceived the usefulness of intramolecular azide-alkyne 1,3-dipolar cycloaddition in order to construct simultaneously the seven-membered heterocycle, the triazole and pyrazole and report

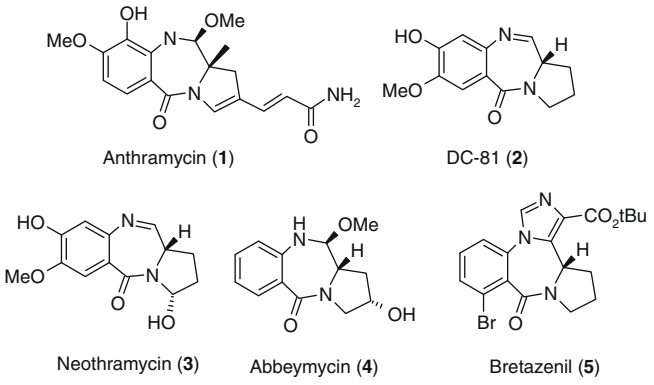

Figure 1. Structures of promising members of PBD class.

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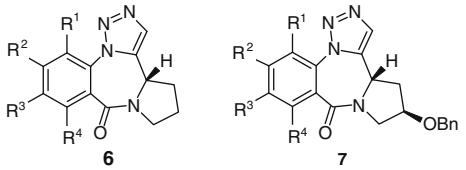
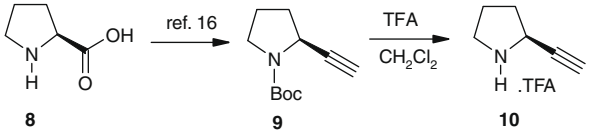


Figure 2. Structures of new tetracyclic triazole fused benzodiazepines.



Scheme 1. Synthesis of alkyne **10**.

herein the facile assembly of a library of triazole fused tetracyclic compounds.

Naturally occurring L-proline (**8**) was converted to alkyne **9** in 80% overall yield following literature known procedure (Scheme 1).[16] Boc deprotection of alkyne **18** was carried out with TFA in CH_2Cl_2 to afford the TFA salt of amine **10**.

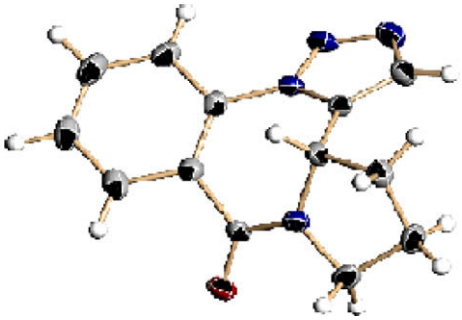


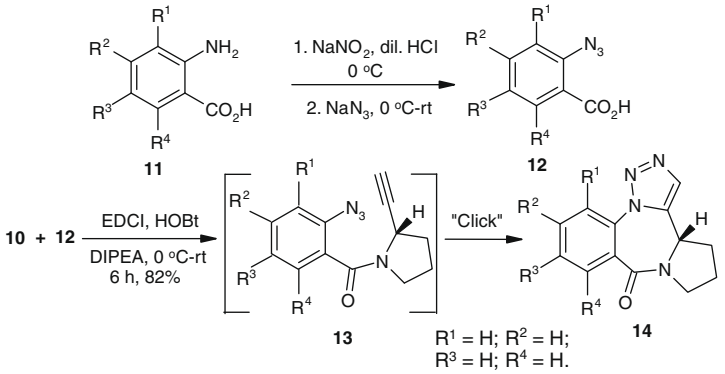
Figure 3. ORTEP diagram of compound **14**.

The corresponding aromatic azido acid **12** was prepared from anthranilic acid (**11**) by diazotization reaction using $NaNO_2$ and dil. HCl in Et_2O at 0 °C followed by treatment with NaN_3 at the same temperature in good yield (Scheme 2).[17]

Coupling between aromatic azido acid **12** and TFA salt of amine **10** was carried out in the presence of EDCI, HOBT and DIPEA in dry DMF expecting to isolate azido-alkyne **13**. To our pleasant surprise, the isolated product was resultant of a further 1,3-dipolar cycloaddition reaction to afford very interesting tetracyclic compound **14** in 82% yield (Scheme 2),[18] which was fully characterized by spectral and analytical data.[19] In addition, the

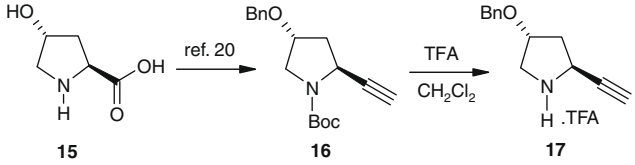
Table 1
One-pot amide coupling and intramolecular 1,3-dipolar cycloaddition reaction under catalyst free condition

S.No.	Acid	Amine (10)	Product (14)	Time (h)	Yield (%)
2	R^1 = H; R^2 = Cl; R^3 = H; R^4 = H.		**14a**	08	87
3	R^1 = H; R^2 = H; R^3 = Br; R^4 = H.		**14b**	07	92
4	R^1 = H; R^2 = H; R^3 = H; R^4 = Me.		**14c**	08	91
5	R^1 = H; R^2 = H; R^3 = OMe; R^4 = OMe.		**14d**	06	82
6	R^1 = H; R^2 = I; R^3 = H; R^4 = I.		**14e**	07	87
7	R^1 = H; R^2 = Br; R^3 = H; R^4 = Me.		**14f**	06	88
8	R^1 = H; R^2 = H; R^3 = H; R^4 = F.		**14g**	06	92
9	R^1 = H; R^2 = H; R^3 = H; R^4 = NO_2.		**14h**	08	91
10	R^1 = H; R^2 = H; R^3 = H; R^4 = OMe.		**14i**	07	82



Scheme 2. Synthesis of **14**.

structure of **14** was unambiguously assigned by its single crystal X-ray diffraction studies (Fig. 3).[20] This result encouraged us to verify other aromatic azido acid derivatives under identical reaction conditions. As exemplified in Table 1, the reaction proceeded smoothly to completion and the corresponding benzo[*e*]pyrrolo[1,2-*a*][1,2,3]triazolo[5,1-*c*][1,4]diazepin-8(4*H*)-one products were obtained in 6 to 8 h with excellent yields and high purity.



Scheme 3. Synthesis of alkyne **10**.

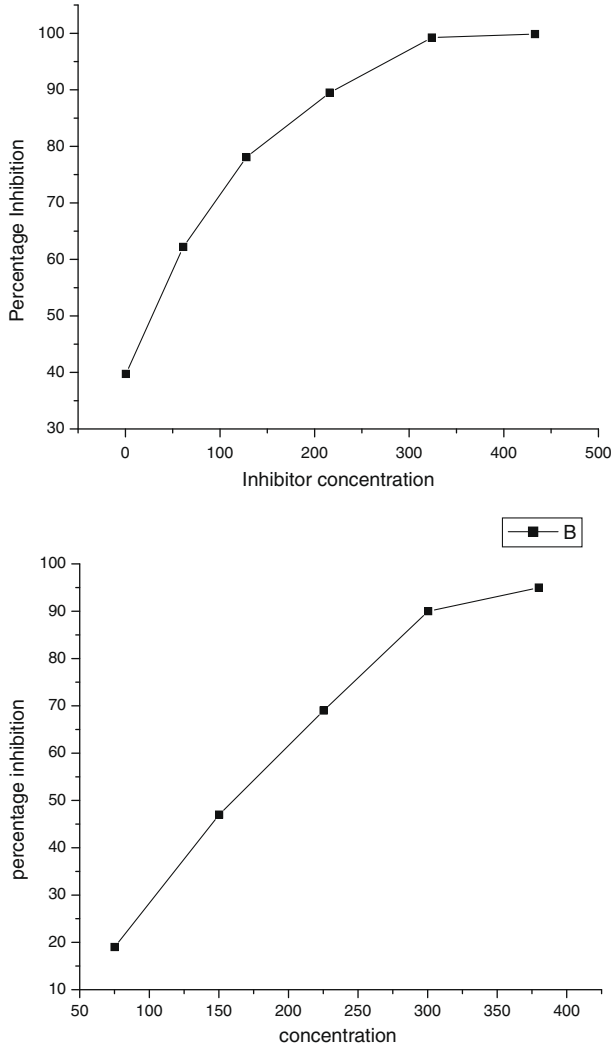


Figure 4. IC_{50} plot against trypsin for **14h** and papain for **14f**.

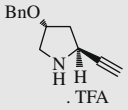
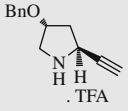
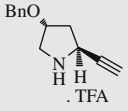
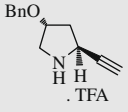
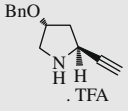
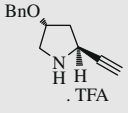
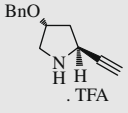
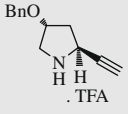
We extended our studies further to *trans*-4-hydroxy-L-proline (**15**) which was converted into alkyne **17** in 91% yield (Scheme 3).[21] In the ^1H NMR spectrum, the characteristic acetylinic proton was observed at δ 2.26 ppm. Alkyne **17** was subjected to Boc-deprotection with TFA in CH_2Cl_2 at 0 °C. After drying the reaction mixture, the crude material was coupled with azido acid **12** by treatment with EDCI, HOBT and DIPEA in DMF to afford azido-alkyne **18** followed by an in situ 1,3-dipolar cycloaddition to yield 1,2,3-triazole fused tetracyclic **19**[22] in 8 h with 84% yield over two steps (Scheme 4). The ^1H NMR spectrum showed a singlet resonance at δ 7.61 ppm and a multiplet at δ 4.36 ppm corresponding to olefinic proton and methine proton attached to the olefin, respectively. Debenzylation of compound **19** was carried out by using H_2/Pd-C in EtOAc to afford **20** in 95% yield.

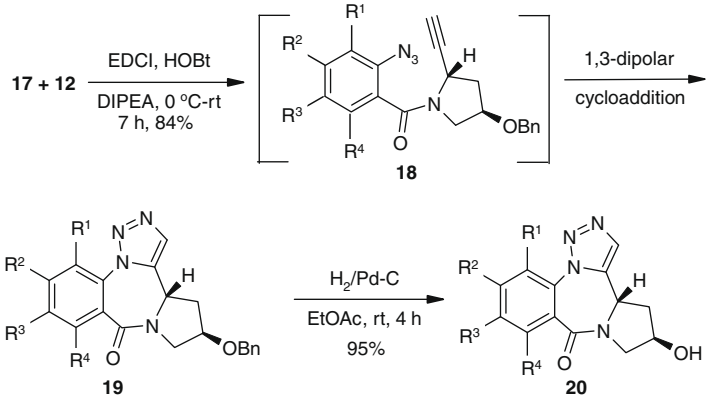
These results were then extended to other aromatic azido acid derivatives under identical reaction conditions. As exemplified in Table 2, the reaction proceeded smoothly to completion and the corresponding 5-(benzyloxy)-benzo[*e*]-pyrrolo[1,2-*a*][1,2,3]-triazolo[5,1*c*][1,4]diazepine-8(4*H*)-one products (**19a**–**h**) were obtained in 7–10 h with excellent yields and high purity (Table 2).

These Triazole compounds have been analysed for their efficacy as enzymatic protease inhibitors like serine protease, cysteine protease and aspartase protease.[23]

The inhibitors were screened at different level of concentrations. Initially the inhibition at concentration level of 1 μM was determined. The compounds showing no inhibition or inhibition less than 50% were not investigated further. Those showing activities more than 50% were taken and the screening was done at lower level of concentration (Fig. 4). Thus, optimum range of concentration was found where compound showed activity in the range of 50%. Several assays in a varying range of concentration at that level were performed to determine the IC_{50} (the concentration of inhibitor at which it shows 50% inhibition of enzyme)[24] and later the experiment was repeated with a different concentration of substrate. The results are summarized in the tabular format. None of the compound showed aspartic protease inhibition activity (Table 3).

Table 2
One-pot amide coupling and intramolecular 1,3-dipolar cycloaddition reaction under catalyst free condition

S.No.	Acid	Amine (17)	Product (19)	Time (h)	Yield (%)
2	R^1 = H; R^2 = Cl; R^3 = H; R^4 = H.		**19a**	07	84
3	R^1 = H; R^2 = H; R^3 = NO_2; R^4 = H.		**19b**	10	90
4	R^1 = H; R^2 = H; R^3 = Br; R^4 = H.		**19c**	10	85
5	R^1 = H; R^2 = H; R^3 = H; R^4 = Me.		**19d**	09	83
6	R^1 = H; R^2 = OMe; R^3 = OMe; R^4 = H.		**19e**	08	80
7	R^1 = I; R^2 = H; R^3 = I; R^4 = H.		**19f**	09	86
8	R^1 = Br; R^2 = H; R^3 = Me; R^4 = H.		**19g**	07	89
9	R^1 = H; R^2 = H; R^3 = F; R^4 = H.		**19h**	09	87



Scheme 4. Synthesis of alkyne **19**.

In conclusion, we have achieved the first regioselective one-pot synthesis of several new chiral tetracyclic triazole derivatives by peptide bond formation and an in situ intramolecular 1,3-dipolar cycloaddition reaction between easily affordable azides and alkynes with excellent yield and high purity. The synthesized compounds were evaluated against protease inhibitors and some of them showed weak to good serine protease inhibition activity. Even though the inhibition levels are only at μM levels, we believe, these novel and unprecedented class of molecules are certain to find applications in biology.

Table 3
Evaluation against protease inhibitors

Compds	Enzyme (serine/cysteine protease) (SP/CP)	IC_{50}[b] (μM)
14h	SP	108.2
	CP	na[a]
14b	SP	290.2
	CP	na
19d	SP	535.8
	CP	na
19c	SP	206.4
	CP	na
14f	SP	674.3
	CP	na
19f	SP	195.6
	CP	na

[a] Values are means of three experiments (na = not active).
[b] IC_{50} compliment inhibition.

Acknowledgement

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19. *Analytical and spectral data of compound* **14**: mp = 195–196 °C; $[\alpha]_D^{25}$ = +220.9 (*c* 1.6, $CHCl_3$); IR ($CHCl_3$) 3436, 2976, 1636, 1473, 1411, 1244 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.14 (quin, 2H, *J* = 6.8 Hz), 2.51–2.62 (m, 2H), 3.71–3.86 (m, 2H), 4.76 (t, 1H, *J* = 5.8 Hz), 7.56 (dt, 1H, *J* = 1.4, 7.7 Hz), 7.64 (s, 1H), 7.69 (dt, 1H, *J* = 1.6, 7.5 Hz), 8.00 (dd, 1H, *J* = 1.3, 8.0 Hz), 8.12 (dd, 1H, *J* = 1.6, 7.7 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.5, 29.2, 47.5, 49.4, 122.8, 127.1, 128.6, 128.8, 131.6, 132.6, 132.9, 138.8, 163.8; ESI-MS (*m/z*) 241 $[M+H]^+$, 263 $[M+Na]^+$; Anal. Calcd for $C_{13}H_{12}N_4O$: C, 64.99; H, 5.03; N, 23.32. Found: C, 65.12; H, 5.16; N, 23.24.
20. Crystallographic data for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 727708 for **14**. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44-0-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].
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22. *Analytical and spectral data of compound* **19**: mp = 73–75 °C $[\alpha]_D^{25}$ = +147.5 (*c* 1.4, $CHCl_3$); IR ($CHCl_3$) 3436, 2925, 1637, 1471, 1409, 1085 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.49–2.62 (ddd, 1H, *J* = 4.6, 8.4, 13.2 Hz), 2.79 (ddt, 1H, *J* = 2.4, 7.4, 13.2 Hz), 3.68 (dd, 1H, *J* = 4.1, 13.1 Hz), 4.19 (dt, 1H, *J* = 2.0, 13.1 Hz), 4.36 (m, 1H), 4.60 (ABq, 2H, *J* = 11.8 Hz), 4.94 (t, 1H, *J* = 7.9 Hz), 7.30–7.38 (m, 5H), 7.53 (dt, 1H, *J* = 1.8, 7.6 Hz), 7.61 (s, 1H), 7.70 (dt, 1H, *J* = 1.8, 7.6 Hz), 8.02 (dd, 1H, *J* = 1.4, 8.0 Hz), 8.1 (dd, 1H, *J* = 1.7, 8.0 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 35.5, 48.0, 52.1, 71.0, 74.8, 122.7, 126.4, 127.5, 1279, 128.4, 128.8, 129.5, 131.9, 132.6, 132.8, 137.2, 138.2, 164.3; ESI-MS (*m/z*) 347 $[M+H]^+$, 369 $[M+Na]^+$; Anal. Calcd for $C_{20}H_{18}N_4O_2$: C, 69.35; H, 5.24; N, 16.17. Found: C, 69.22; H, 5.28; N, 16.04.
23. Kindly see the Supplementary data.
24. (a) Thornberry, N.; Bull, H. G.; Calaycay, J. R.; Chapman, K. T.; Howard, A. D. *Nature* **1992**, *356*, 768; (b) Henkart, P. A. *Immunity* **1996**, *4*, 194; (c) Rawal, G. K.; Kumar, A.; Tawar, U.; Vankar, Y. D. *Org. Lett.* **2007**, *9*, 5171; (d) Pandey, G.; Dumbre, S. G.; Khan, M. I.; Shabab, M. *J. Org. Chem.* **2006**, *71*, 8481.