Tetrahedron Letters xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Synthesis of large Stokes shift and narrow emission indole–triazole–carboxamide peptidomimetics via MCR-click strategy

Rajeena Pathoor, D. Bahulayan *

Department of Chemistry, University of Calicut, Malappuram 673635, Kerala, India

ARTICLE INFO

Article history:
Received 7 March 2016
Revised 8 April 2016
Accepted 12 April 2016
Available online xxxx

Keywords:
Indole
Carboxamide
Stokes shift
Human African Trypanosomiasis
Click chemistry

ABSTRACT

A series of indole peptidomimetics with potential for the future emergence as efficient therapeutic agents for stage 2 Human African Trypanosomiasis (HAT) is described. The peptidomimetics are constructed based on a build-pair concept using green chemistry models like multicomponent coupling strategy and click chemistry. The photophysical properties of the molecules are promising and point to the added possibilities of these molecules for the development of optical imaging agents.

A newly emerging strategy for the diversity-oriented synthesis of functional organic molecules is the use of privileged scaffolds.[1] A privileged scaffold is a key structural entity present in a biologically active molecule from which the biological activity is mainly originating. Among the various privileged scaffolds, indoles, triazoles, and carboxamides are the three potential structural scaffolds frequently occurring in natural as well as synthetic therapeutics. For example, 3-substituted indole derivatives are reported to be useful for the development of enzyme inhibitors,[2] bioreceptormodulators,[3] cannabinoid receptors[4] etc. *N*-acyl or *N*-alkyl indole derivatives are also present in numerous biologically active molecules.[5] Carboxamides are another interesting moiety present in many drug molecules.[6] Telaprevir, used for the treatment of hepatitis C, bortezomib, a threonine protease inhibitor and praziquantel used for the treatment of schistosomiasis are some of the examples of this category. Similarly, triazole derivatives also possess valuable clinical profiles like anti-HIV,[7] anti-allergic,[8] antifungal,[9] or anti-viral properties.[10] Figure 1 shows selected drug molecules with one of these moieties as privileged scaffold.

Recently, indoline-2-carboxamide derivative A (Fig. 2a) was reported as a therapeutic agent for stage 1 Human African Trypanosomiasis (HAT).[11]

However, it fails to produce positive results for the treatment of stage 2 HAT due to lack of tolerability in biological environment. Stage 2 is more fatal because at this stage the parasites cross the blood–brain barrier and attack the central nervous system leading to medical conditions such as confusion, sleep disorders, coma or even death. No effective vaccine is available till date for the treatment of stage 2 HAT and therefore it is important to develop more bio-compatible molecules that can cross the blood–brain barrier to provide better bioavailability at the HAT infected sites.

The failure of A to produce better results for type 2 HAT may be the poor stability of the molecule due to the vulnerability of the indole amide moiety and the ether oxygen toward protease action. Similarly, lipophilicity affects both blood–brain barrier permeation and brain distribution. It has been recognized that lipophilic molecules have greater access to the brain than hydrophilic molecules. Lipophilicity could bring the inhibitor into closer proximity with a brain target and exists in a highly lipophilic environment.[12] One of the methods to improve the stability, lipophilicity and bioavailability of a peptide like drug molecule is the creation of its peptidomimetic version by substituting its protease unstable amide bonds with more stable isosteres.[13] Many functionalities like 1,2,3-triazole, —CH_2—O—, —CH_2—CO—, and —CH=CH— are identified as amide bond isosteres.[14] Among the various amide bond isosteres, 1,2,3-triazole is the more prominent one due to its close similarity with amide bonds in terms of physiochemical properties such as planarity, dipole moment, Cα distance, and number of H-bonding sites.[15] A 1,2,3-triazole can be easily linked between two subunits of a single drug via copper (I) catalyzed [3+2] azide-alkyne click cycloaddition (CuAAC)[16] which is now emerged as a waste-free and chemoselective ligation tool for the fragment coupling of small molecule segments to produce conformationally

* Corresponding author. Tel.: +91 9995538062; fax: +91 494 2400269.
E-mail address: bahulayan@yahoo.com (D. Bahulayan).

http://dx.doi.org/10.1016/j.tetlet.2016.04.040
0040-4039/

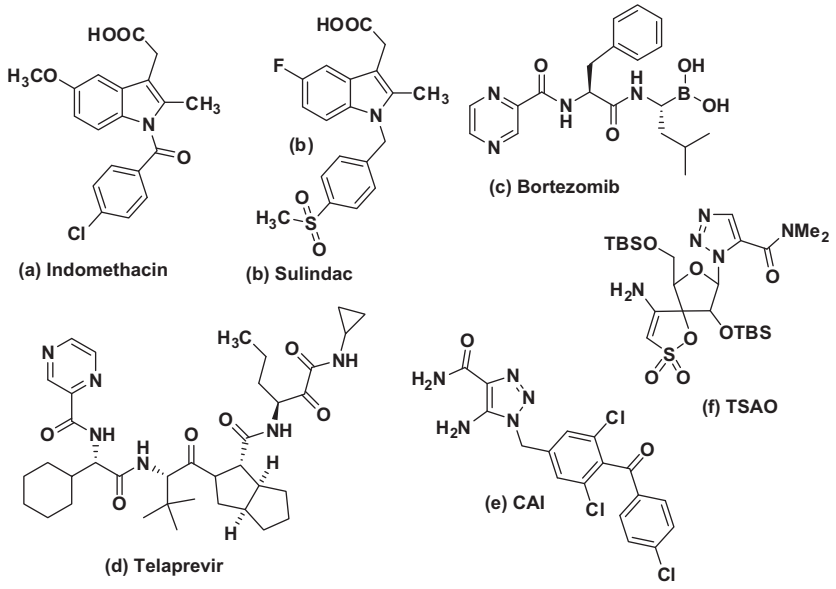


Figure 1. Representative examples of commercial drug molecules with: (a, b): indole as a structural part, (c, d): α-acylaminoamide as a core structure, (e, f): 1,2,3-triazole as core structure.

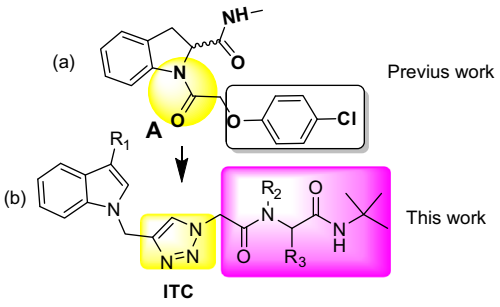


Figure 2. (a) Recently reported indoline-2-carboxamide derivative as potential curing agent for stage 1 Human African Trypanosomiasis (HAT). (b) General structure of the indole triazole carboxamide (ITC) obtained via isosteric substitution.

restricted drug-like molecules.[17] In continuation of our ongoing research on peptidomimetic small molecules,[18] we decided to investigate the possibility of designing new peptidomimetic versions of **A** via structural as well as isosteric modification to create indole–triazole–carboxamide derivatives (ITC) as shown in Figure 2b.

A build/pair strategy[19] based on multicomponent reactions (MCRs)[20] and click chemistry was used for the synthesis of the indole peptidomimetics ITC-1, ITC-2, and ITC-3 (Fig. 2) (Fig. 3).

The studies were started with the synthesis of the starting alkynes and azides. The alkynes **1a–c** were obtained via the base catalyzed propargylation of corresponding 3-substituted indole with propargyl bromide in DMF as shown in Scheme 1. The carboxamide fragments were synthesized in the form of azides (Fig. 4 and Table 1) via the post reaction modification of the corresponding carboxamide chlorides obtained from an Ugi 4 component reaction.[18]

The carboxamide scaffolds as shown in Figure 4 are selected due to their unique structural features comprising of at least four substitutable diversity points. Since large number of aldehydes, amines, carboxylic acids, and isonitriles are available, it is possible to construct large collections of carboxamides via solid phase or solution phase combinatorial synthesis.

As shown in Table 1, the carboxamide chloride intermediates were obtained by mixing equimolar amount of aldehyde, amine, chloroacetic acid, and *tert*-butylisonitrile in methanol at room temperature by following Ugi 4 component reaction protocol. These scaffolds were subsequently converted to the corresponding azides by treating with sodium azide in presence of potassium carbonate in DMF at room temperature (see Supplementary information for detailed procedure).

Having synthesized the indole alkynes and carboxamide azides, we proceed to the synthesis of indole peptidomimetics by CuAAC ligation between these two entities. Eight peptidomimetics were prepared from each category of alkynes with selected azides from Table 1. For example, for the synthesis of ICT-1 series, we reacted

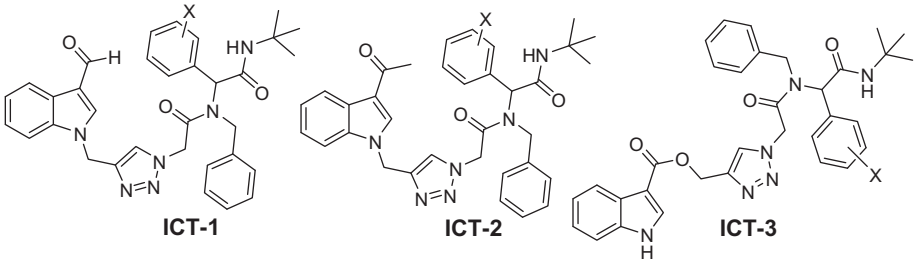
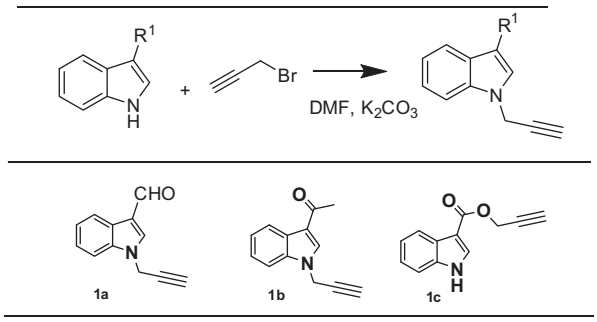


Figure 3. Representative structures of the indole peptidomimetics ICT-1, ICT-2 and ICT-3.



Scheme 1. List of alkyne functionalized indole scaffolds synthesized.

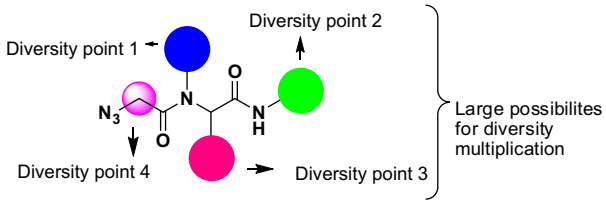


Figure 4. General structure of the carboxamide scaffolds in the azideform.

alkyne **1a** with azides **2c–f**, **2h**, **2j**, **2k,** and **2l** to obtain **3a–h**. In a representative reaction an equimolar mixture of alkyne functionalized indole and the carboxamideazide were mixed with 0.2 equiv of sodium ascorbate in a mixed solvent system containing *tert*-butanol, water, and DMSO (4:2:1) at room temperature for 48 h. The subsequent aqueous work-up followed by solvent wash afforded the peptidomimetics in pure form. The peptidomimetics and their starting scaffolds were thoroughly characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, and mass spectral studies (see Supplementary data for experimental and compound characterization). The structure of various peptidomimetics belong to the ICT-1 series are presented in Table 2. The synthesis of the peptidomimetics in the ICT-2 series and ICT-3 series were also done in the same way and the details are presented in Tables 3 and 4. Irrespective of the nature of the skeletal substitution patterns, all the peptidomimetics were formed in good to excellent yield with a maximum of 92% for **3a** (Table 2, entry 1).

We then moved on to the computation of the drug property descriptors of all the three series of peptidomimetics using molinspiration property calculation service.[21] Drug-likeness of the molecules mainly depends on their molecular size, lipophilicity, polarity (accessed by the polar surface area, tPSA) and the presence of optimal number of rotatable bonds.[22] Drug like molecules usually have log*P* values in between −0.4 and 5.6.[23] The calculated drug-property descriptors are presented in Table S1 in the Supplementary information. These peptidomimetics have log*P* values in between 4.25 and 5.28 and are in the preferred range of antipsychotic (CNS) drugs. The enhanced log*P* values of the new peptidomimetics compared to **A** point to the potential of these molecules for improved blood–brain barrier (BBB) permeation and brain distribution and the possibilities for further studies focused to stage 2 HAT inhibition.

It has been reported that indole or triazole based molecules are also useful for optical imaging of cellular targets.[24] Much attention has been given to the development of NIR dyes due to their good tissue permeability and low autofluoresence.[25] However, the use

Table 1
List of carboxamide azides **2a–l** synthesized for the ligation reaction with indole alkynes **1a–c**

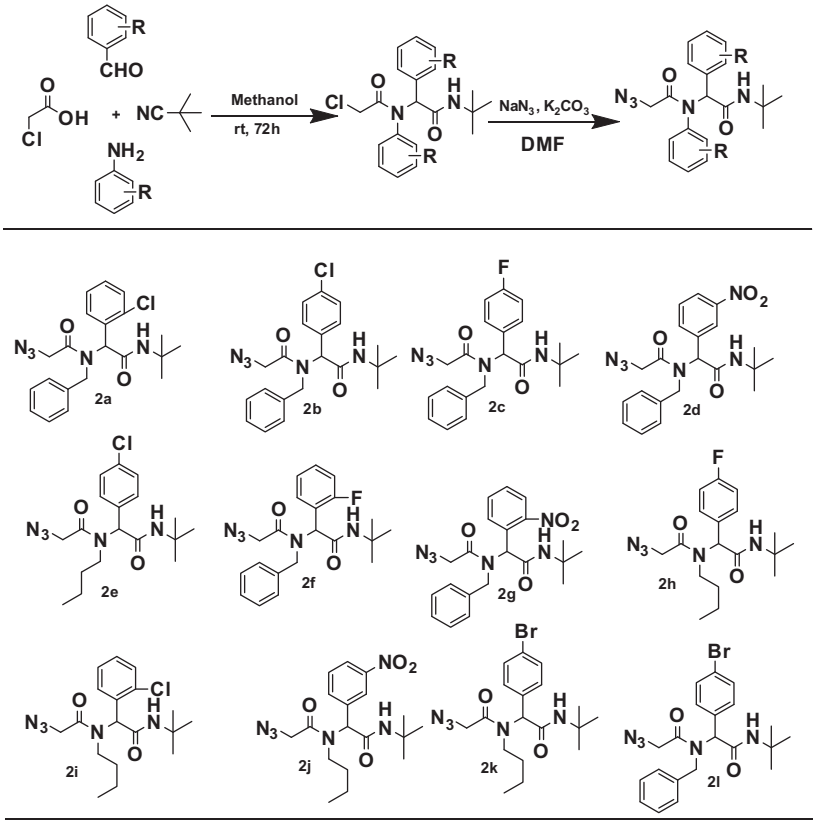
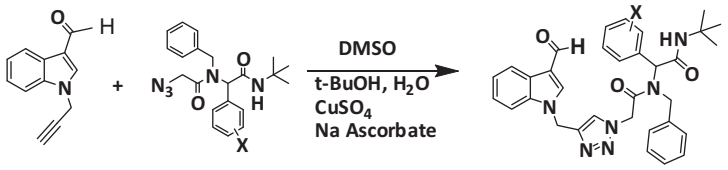


Table 2
List of ICT-1 series 1,4-substituted triazolyl-3-formyl indole scaffolds substituted with carboxamide residues



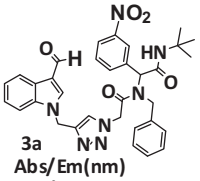
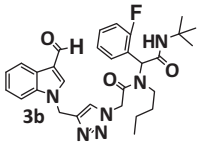
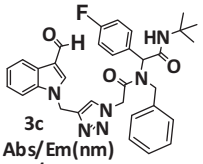
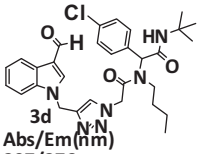
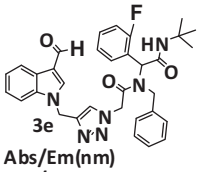
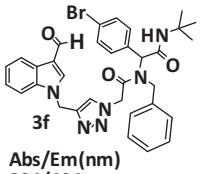
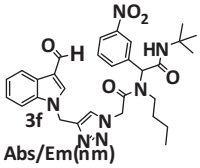
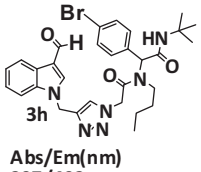
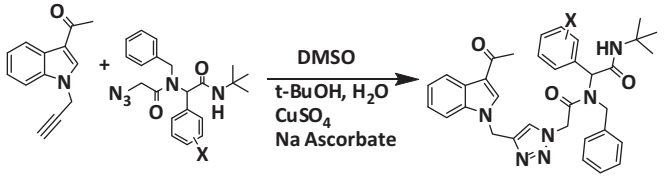
Entry	ICT-1 series	Yield (%)	Entry	ICT-1 series	Yield (%)
1	 3a Abs/Em(nm) 296/364	92	5	 3b Abs/Em(nm) 296/364	90
2	 3c Abs/Em(nm) 295/394	90	6	 3d Abs/Em(nm) 297/376	89
3	 3e Abs/Em(nm) 297/360	90	7	 3f Abs/Em(nm) 294/404	91
4	 3f Abs/Em(nm) 298/375	90	8	 3h Abs/Em(nm) 297/402	91

Table 3
List of ICT-2 series 1,4-substituted triazolyl-3-acetyl indole scaffolds substituted with carboxamide residues



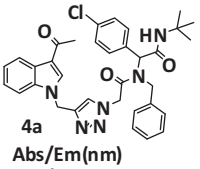
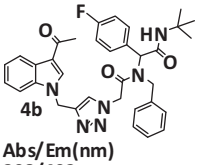
Entry	ICT-2 series	Yield (%)	Entry	ICT-2 series	Yield (%)
1	 4a Abs/Em(nm) 304/414	83	5	 4b Abs/Em(nm) 308/402	83

Table 3 (*continued*)

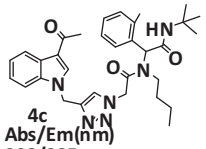
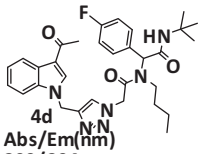
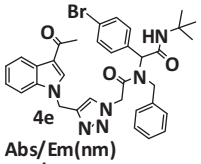
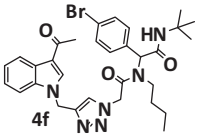
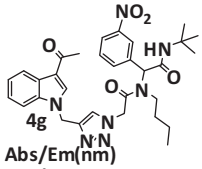
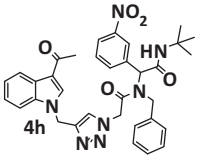
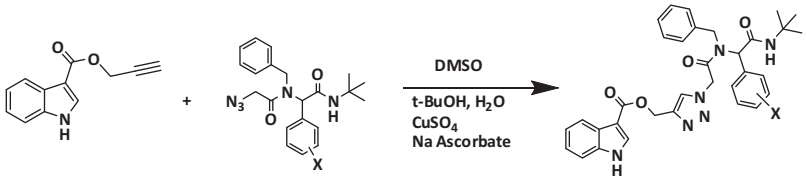
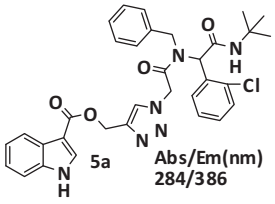
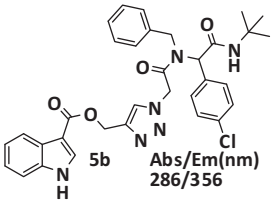
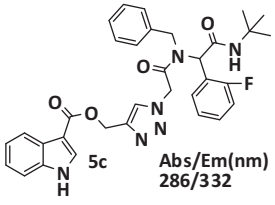
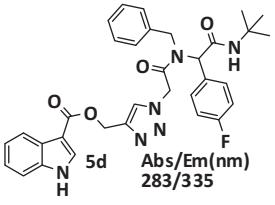
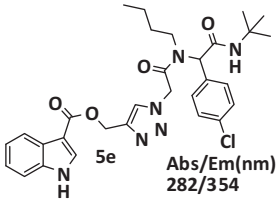
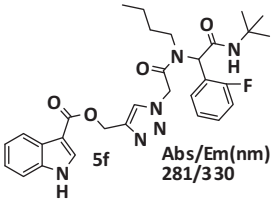
Entry	ICT-2 series	Yield (%)	Entry	ICT-2 series	Yield (%)
2	 4c Abs/Em(nm) 308/385	87	6	 4d Abs/Em(nm) 309/394	86
3	 4e Abs/Em(nm) 309/434	87	7	 4f Abs/Em(nm) 308/404	85
4	 4g Abs/Em(nm) 305/386	87	8	 4h Abs/Em(nm) 308/394	86

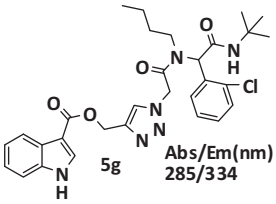
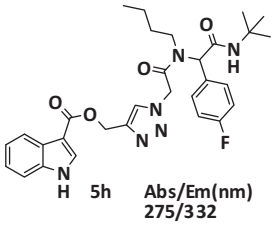
Table 4
List of ICT-3 series indole peptidomimetics



Entry	ICT-3 series	Yield (%)	Entry	ICT-3 series	Yield (%)
1	 5a Abs/Em(nm) 284/386	88	6	 5b Abs/Em(nm) 286/356	82
2	 5c Abs/Em(nm) 286/332	84	7	 5d Abs/Em(nm) 283/335	85
3	 5e Abs/Em(nm) 282/354	83	8	 5f Abs/Em(nm) 281/330	82

(*continued on next page*)

Table 4 (*continued*)

Entry	ICT-3 series	Yield (%)	Entry	ICT-3 series	Yield (%)
4	 5g Abs/Em(nm) 285/334	90	9	 5h Abs/Em(nm) 275/332	91

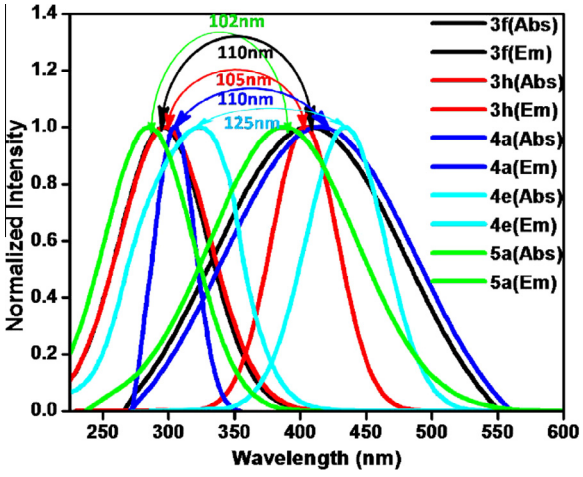


Figure 5. The normalized absorption and emission spectra of indole peptidomimetics **3h**, **4a**, **4e,** and **5h** in DMSO at room temperature (Stokes shift. 100 nm).

of these dyes can lead to tissue heating and it also requires the assistance of a night vision camera as the emitting light is not visible to naked eyes.[26] In this regard, lower wavelength emitting dyes with high quantum efficiency and less autofluorescence are expected to produce better results than NIR dyes for the naked eye detection of tumors and other malignant cell lines during in vivo imaging. Tryptophan is one such indole based probe used for the imaging of biological proteins.[27] Tryptophan absorbs light at 290 nm and emit light between 320 and 400 nm. However, the major drawback here also is the autofluorescence and the protease instability in biological conditions. One of the methods to overcome the issue of autofluorescence is the design of probes with high Stokes shift which is the asymmetry between the excitation and emission wavelengths.[28] Based on the stability providing structural features present in the new ICT series peptidomimetics and their promising drug property descriptor values, we decided to examine the photophysical properties our molecules to bring out their possibilities as optical imaging agents.

The absorption–emission properties all the peptidomimetics were evaluated in DMSO (50 ppm) at 0–10 pH to study the changes in Stokes shift with respect to the structural features of the molecules. The absorption and emission maxima values are documented under the respective structures given in Tables 2–4. Similar to tryptophan probes, the new peptidomimetics **3a**–**5h** showed absorption in the wavelength region between 275 and 309 nm and emits light in the blue region between 330 and 434 nm. Most of these compounds showed intense narrow blue emission with high Stokes shift ranging from 46 to 125 nm. Among the 24 peptidomimetics, **3h**, **4a**, **4e,** and **5h** showed Stokes shift values >100 nm from which **4e** showed the maximum Stokes shift of 125 nm. However, a generalization based on the structural features is inappropriate at this stage. Interestingly, the peptidomimetics were found to be intact to the changes in pH. This is important because the sensitivity toward pH and other reactive species like singlet oxygen affect the performance of the probe during in vivo imaging.[26] A normalized absorption–emission spectra of the peptidomimetics showing high Stokes shift is given in Figure 5.

In summary we have developed a convenient methodology for the synthesis of a series of indole based bifunctional peptidomimetics using a build-pair concept. The structural scaffolds are derived from readily available building blocks based on multicomponent coupling strategy and the fragments thus generated were assembled by copper (I) catalyzed [3+2] azide-alkyne cycloaddition.

The drug property descriptors of the molecules points to the possibilities of these new peptidomimetics as efficient therapeutic agents for stage 2-HAT infection whereas the photophysical properties indicates their potential as efficient fluorescent probes for optical imaging especially were tryptophan is used as probe. We hope this work will certainly open the way for an effective inhibitor for stage 2 Human African Trypanosomiasis (HAT).

Acknowledgments

R.P. thanks council of scientific and Industrial research (CSIR), New Delhi for senior research fellowship (SRF).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.04.040.

References and notes

1. (a) Welsch, M. E.; Snyder, S. A.; Stockwell, B. R. *Curr. Opin. Chem. Biol.* **2010**, *14*, 347; (b) Spring, D. R. *Org. Biomol. Chem.* **2003**, *1*, 3867; (c) Gao, Y.; Xu, Q.; Shi, M. *ACS Catal.* **2015**, *5*, 6608; (d) de Sa, A.; Fernando, R.; Barreiro, E. J.; Fraga, M.; Alberto, C. *Mini-Rev. Med. Chem.* **2009**, *9*, 782.
2. Hollis, H. D.; Showalter, et al. *J. Med. Chem.* **1997**, *40*, 413.
3. Leeson, P. D.; Iversen, L. L. *J. Med. Chem.* **1994**, *37*, 24.
4. D'Ambra, T. E.; Estep, K. G.; Bell, M. R.; Eissenstat, M. A.; Josef, K. A.; Ward, S. J.; Haycock, D. A.; Baizman, E. R.; Casiano, F. M.; Beglin, N. C.; Chippari, S. M.; Grego, J. D.; Kullnig, R. K.; Daley, G. T. *J. Med. Chem.* **1992**, *35*, 124.
5. (a) Lione, A.; Sciallia, A. R. *Reprod. Toxicol.* **1995**, *9*, 7; (b) Liu, B.; Barkely, M. D. *J. Phys. Chem. B* **2000**, *104*, 1837; (c) Sing, Anil K.; Hota, Prasanta K. *J. Photosci.* **2004**, *1*, 107.
6. Antonini, I.; Polucci, P.; Magnano, A.; Gatto, B.; Palumbo, M.; Menta, E.; Pescalli, N.; Martelli, S. *J. Med. Chem.* **2003**, *46*, 3109.
7. (a) Alvarez, R.; Velazquez, S.; San Felix, A.; Aquaro, S.; Clercq, E. D.; Perno, C.; Karlsson, A.; Balzarini, J.; Camarasa, M. J. *J. Med. Chem.* **1994**, *37*, 4185; (b) Buckle, D. R.; Outred, D. J.; Rockell, C. J. M.; Smith, H.; Spicer, B. A. *J. Med. Chem.* **1983**, *26*, 251; (c) Buckle, D. R.; Rockell, C. J. M.; Smith, H.; Spicer, B. A. *J. Med. Chem.* **1986**, *29*, 2269.
8. Genin, M. J.; Allwine, D. A.; Anderson, D. J.; Barbachyn, M. R.; Emmert, D. E.; Garmon, S. A.; Graber, D. R.; Grega, K. C.; Hester, J. B.; Hutchinson, D. K.; Morris,

J.; Reischer, R. J.; Ford, C. W.; Zurenko, G. E.; Hamel, J. C.; Schaadt, R. D.; Stapert, D.; Yagi, B. H. *J. Med. Chem.* **2000**, *43*, 953.
9. Ohwada, J.; Tsukazaki, M.; Hayase, T.; Oikawa, N.; Isshiki, Y.; Fukuda, H.; Mizuguchi, E.; Sakaitani, M.; Shiratori, Y.; Yamazaki, T.; Ichihara, S.; Umeda, I.; Shimma, N. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 191–196.
10. Alsoud, Y. A.; AlDweri, M. N.; Almasoudi, N. *IL FARMACO* **2004**, *59*, 775.
11. Cleghorn, L. A. T. et al *J. Med. Chem.* **2015**, *58*, 7695.
12. Kerns, E. H.; Di, L. *Drug-like Properties: Concepts, structure Design and Methods: From ADME to Toxicity Optimization*; Academic Press, 2008.
13. (a) Pedersen, D. S.; Abell, A. *Eur. J. Org. Chem.* **2011**, 2399; (b) Godballe, T.; Nilsson, L. L.; Petersen, P. D.; Jenssen, H. *Chem. Biol. Drug Des.* **2011**, *77*, 107.
14. Choudhary, A.; Raines, R. T. *ChemBioChem* **2011**, *12*, 1801.
15. Justin, M. H.; Kent, K. *Chem. Soc. Rev.* **2010**, *39*, 1325.
16. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004 (For more reviews, see Supplementary information).
17. Agalave, S. G.; Maujan, S. R.; Pore, V. S. *Chem. Asian J.* **2011**, *6*, 2696.
18. Pramitha, P.; Bahulayan, D. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2598 (for related work from our group, see Supplementary information).
19. (a) Luo, T.; Schreiber, S. L. *J. Am. Chem. Soc.* **2009**, *131*, 5667; (b) Beckmann, H. S. G.; Nie, F.; Hagerman, F. C. E.; Tan, Y. S.; Wilcke, D.; Spring, D. R. *Nat. Chem.* **2013**, *5*, 861; (c) Schreiber, S. L. *Science* **2000**, *287*, 1964.
20. (a)*Multicomponent Reactions*; Zhu, J., Bienayme, H., Eds.; Wiley-VCH: Weinheim, 2005; For reviews on MCRs, see (b) Dömling, A.; Ugi, I. *Angew. Chem., Int. Ed.* **2000**, *39*, 3168 (for more recent reviews on MCR, see Supplementary information).
21. (a) Zhang, M. Q. *Methods Mol. Biol.* **2012**, *803*, 297; (b) Verma, R. K.; Prajapati, V. K.; Verma, G. K.; Chakraborti, D.; Sundar, S.; Rai, M.; Dubey, V. K.; Singh, M. S. *ACS Med. Chem. Lett.* **2012**, *3*, 243; (c) Al-Hourani, B. J.; Sharma, S. K.; Suresh, M.; Wuest, F. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2235.
22. Giulio, V.; Alessandro, P. *Drug Discovery Today* **2008**, *13*, 285.
23. Arup, K. G.; Vellarkad, N. V.; John, J. W. *J. Comb. Chem.* **1999**, *1*, 55.
24. (a) Rathikrishnan, K. R.; Indirapriyadharshini, V. K.; Ramakrishna, S.; MuRugan, R. *Tetrahedron* **2011**, *67*, 4025–4030; (b) Gale, P. A. *Chem. Commun.* **2008**, 4525.
25. Luo, S.; Zhang, E.; Su, Y.; Cheng, T.; Shi, C. *Biomaterials* **2011**, *32*, 7127.
26. Kobayashi, H.; Ogawa, M.; Alford, R.; ChoykeP, L.; Urano, Y. *Chem. Rev.* **2010**, *110*, 2620.
27. Talukder, P.; Chen, S.; Liu, C. T.; Baldwin, E. A.; Benkovic, S. J.; Hechi, S. M. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 5924.
28. Noonberg, S. B.; Weiss, T. L.; Garovoy, M. R.; Hunt, C. A. *Antisense Res. Dev.* **1992**, *2*, 303.