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Design, Synthesis and *in vitro* anti-tuberculosis activity of Benzo[6,7]cyclohepta[1,2-*b*]pyridine-1,2,3-triazole derivatives.

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ABSTRACT

A series of novel benzo[6,7]cyclohepta[1,2-*b*]pyridine-1,2,3-triazole hybrids (**7a-j** & **8a-j**) have been designed and synthesized in excellent yields by Huisgen's [3+2] cyclo addition reaction of 3-(azidomethyl)-2-methyl-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*b*]pyridine (**5**) with various alkynes **6** in presence of copper sulphate and sodium ascorbate and their structures were confirmed by IR, ^{1}H NMR, ^{13}C NMR and HRMS. The newly synthesized compounds **7a-j** & **8a-j** were evaluated for their *in vitro* anti-mycobacterial activity against *Mycobacterium tuberculosis* H37Rv (ATCC 27294). Among the compounds tested, the compounds **7i** and **8g** displayed most potent activity with MIC value of 1.56 µg/mL with low cytotoxicity.

Tuberculosis (TB) is a potential and chronic infectious disease caused by *Mycobacterium tuberculosis*.[1-4] About one-third of the current world population have TB in its latent form.[5] In 2015, there were approximately 10.4 million cases of active TB and this disease caused approximately 1.8 million deaths (World Health Organization 2017).[6] Additionally, the resurgence of its new virulent forms like multidrug-resistant (MDR-TB) and extremely drug resistant (XDR-TB) has become a major threat to mankind.[7,8] The worsening situation necessitated an urgent need for discovery of modern curative drugs active to all forms of TB.[9,10] All these facts prompted various research groups across the globe to revisit some of the natural and synthetic bioactive compounds for the development of new anti-tubercular drugs with novel mechanism of action to achieve effective TB control.[11-13]

A large number of heterocyclic compounds containing pyridine rings were associated with diverse pharmacological properties such as antimicrobial,[14,15] anticancer,[16] anticonvulsant,[17] antiviral,[18] anti-HIV,[19] antifungal and anti-mycobacterial activities.[20] The condensed derivatives of pyridines play significant role in bioactive molecules, especially in the form of benzocyclohepta[1,2-*b*]pyridines which are structural analogues of benzosuberone. The benzocyclohepta[1,2-*b*]pyridine is an important core biologically active compound with diverse biological activities, such as antihistamine as well as antitumor and anti-inflammatory activities[21-26] **Fig.1(i)**. It is a highly potent pharmacophore with wide use for drug molecular design.

In the recent years, the nitrogen containing heterocyclic compounds have become potential targets for drug discovery [27,28] 1,2,3-triazoles have been studied for over a century as an important class of heterocyclic compounds and still continue to attract considerable attention due to their synthetic and effective biological properties like anti-tubercular, anti-HIV, anti-fungal, anti-HSV, anti-platelet, anti-microbial, and anti-inflammatory activities.[29,30] The 1,2,3-triazole scaffold is being frequently used as a pharmacophore for the modification of known pharmaceuticals. Currently, there is a significant need to identify new scaffolds as anti-tubercular agents that can form an effective anti-tubercular therapy.[31,32] *N*-acylhydrazones (NAH)[33-37] and several 1,2,3-triazole analogues[38-40] have been reported to possess anti-tubercular activity **(Fig.1 (ii))**.

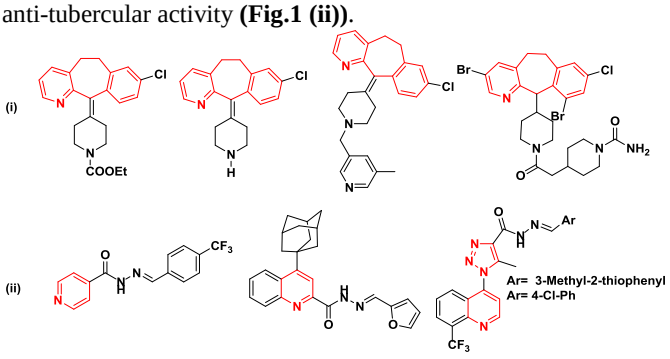


Figure 1: (i) Representative examples of biological active benzo[5,6]cyclohepta[1,2-*b*]pyridine derivatives and (ii) Pyridine substituted tuberculostatic agents.

Based on the biological significance of triazole and benzocyclohepta[1,2-*b*]pyridine moieties we have made an attempt to design amalgamation of three biologically versatile heterocyclic scaffolds like benzosuberone, pyridine and 1,2,3-triazole in a single molecular platform and synthesized new molecules with better anti-TB properties as depicted in **Fig. 2.** In

continuation to our ongoing research programme,[41-48] we designed and synthesized a series of novel benzo[6,7]cyclohepta[1,2-b]pyridine-1,2,3-triazole hybrids (**7a-j** & **8a-j**) in excellent yields by employing copper-catalyzed click chemistry,[49,50] and further explored their anti-mycobacterial activity against *Mycobacterium tuberculosis* H37Rv. Compounds **7i** and **8g** exhibited very promising activity with MIC value of 1.56 µg/mL.

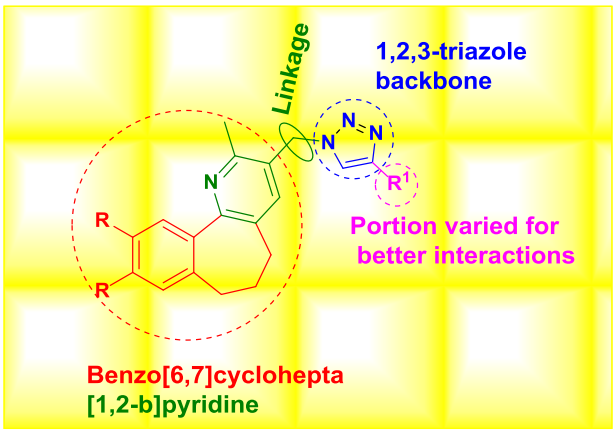
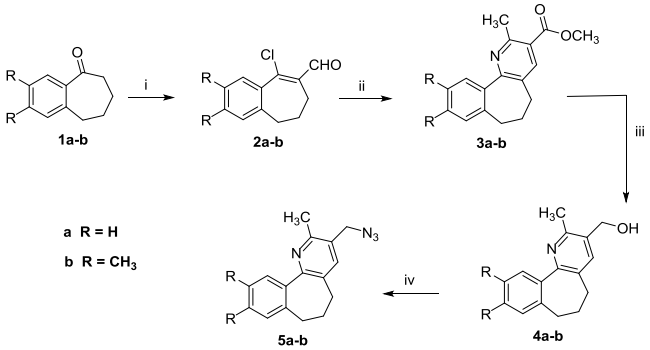


Figure 2: Design strategy for new benzo[6,7]cyclohepta[1,2-*b*]pyridine -1,2,3-triazole hybrids.

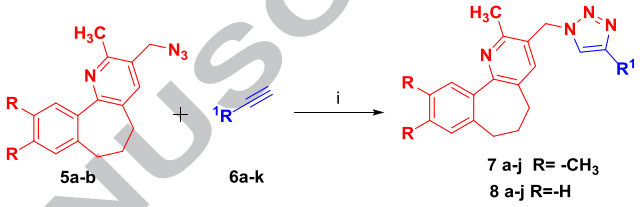
To begin with, substituted benzosuberone (**1**), the required starting compounds were synthesized by using Friedel-Crafts acylation of aromatic hydrocarbons with glutaric anhydride furnishing arylbutyric acid which on Clemmensen reduction followed by cyclization with excess polyphosphoric acid gave substituted benzosuberones (**1a-b**). This substituted benzosuberones undergo Vilsmeier-Haack-Arnold reaction by treating with $POCl_3$, dimethyl formamide at 0 °C to 60 °C for 4 h to afford the (*Z*)-9-chloro-6,7-dihydro-5*H*-benzo [7] annulene-8-carbaldehyde (**2a-b**). The key intermediate benzo[6,7]cyclohepta[1,2-*b*]pyridine derivative **3a-b** was accomplished by Hantzch-type reaction *via* Michael addition by treating with *β*-chloroacroleins, ethyl acetoacetate and NH_4OAc in presence of ethanol at reflux temperature for 8 h [51]. The ester group of compound **3a-b** was reduced by using lithium aluminum hydride at 0 °C in dry THF under nitrogen atmosphere to afford the corresponding alcohols **4a-b**. The corresponding alcoholic group was protected with TsCl and the subsequently corresponding tosyl derivative was then reacted with sodium azide in DMF at 80 °C for 12 h to afford its azides **5a-b** in good yield (**Scheme 1**) and confirmed by absorption of a strong band at 2104 cm^{-1}, attributing to the stretch vibrations of the N_3 bond of the azide group.

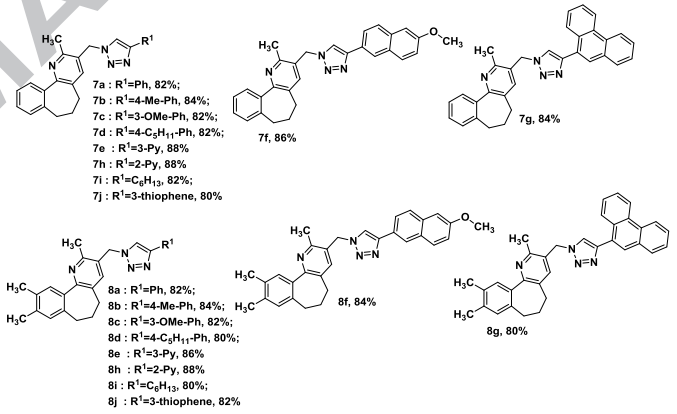


Reagents and conditions: i) $POCl_3$, DMF, 0 °C - 80 °C; ii) Methyl acetoacetate (MAA), NH_4OAc, EtOH, reflux, 8 h; iii) $LiAlH_4$, THF, 0 °C; iv) a) Ts-Cl, dry DCM, DMAP, Et_3N, 2 h, rt; b) NaN_3, DMF, 80 °C, 12 h.

Scheme 1: Synthesis of 3-(azidomethyl)-2-methyl-6,7-dihydro-5*H*-benzo [6,7] cyclohepta[1,2-*b*] pyridine.

To construct the desired analogues, 3-(azidomethyl)-2-methyl-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*b*]pyridine (**5a**) was further reacted with various aromatic and aliphatic alkynes **6a-k** using click chemistry (**Scheme 2**). For example, compound **5a** was reacted with alkyne **6a** in presence of $CuSO_4$-$5H_2O$ and sodium ascorbate in THF and water (1:1, v/v) to give 2-methyl-3-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*b*]pyridine (**7a**) with 82% yield. Under similar conditions, compounds **7a-j** was synthesized in high yields (80-88%) and fully characterized based on the ^1H and ^{13}C NMR, IR and Mass spectral data.





Reagents and conditions: i) $CuSO_4$-$5H_2O$, Sodium ascorbate, THF:H_2O (1:1), RT, 5-6 h.

Scheme 2: Synthesis of novel 2-methyl-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*b*]pyridine triazole derivatives **7a-j** & **8a-j.**

The formation of compound **7a** was evident from the ESI-MS spectrum with the appearance of $[M+H]^+$ peak at *m/z* 367, while the FT-IR spectrum revealed the absence of stretching vibrations of the azide group (2104 cm^{-1}) and the presence of an absorption band at 1734 cm^{-1} indicated the formation of triazole ring in **7a**. A singlet observed in the ^1H NMR spectrum at δ 7.69 ppm confirmed the presence of the triazolyl hydrogen, supported by the signals in the ^{13}C NMR spectrum at δ 119.4 ppm. The proposed structure was substantiated by its mass spectra, which exhibited molecular ion peak at *m/z* 367 $[M+H]^+$.

All the synthesized compounds were screened for their *in vitro* anti-tubercular activity against *M. tuberculosis* H37Rv (ATCC 27294) by using agar dilution method. The MIC was determined for each compound which was measured as the minimum concentration of compound required to completely inhibit the

bacterial growth. The MIC values (µg/mL) of all the synthesized compounds (**7a-j** & **8a-j**) and three standard anti-tubercular drugs (Isoniazid, Rifampicin, Ethambutol) determined in triplicate at pH 7.40 are presented in **Fig. 3**. Almost all synthesized compounds showed anti-tubercular activity against MTB with MICs ranged between **1.56** to **25** µg/mL. Among the tested compounds **7i** and **8g** showed a potent anti-tubercular activity with MIC value of **1.56** µg/mL, two compounds **7e** and **8e** inhibited MTB with MIC value of **6.25** µg/mL and seven compounds **7b, 7d, 7h, 7j, 8b, 8i,** and **8j** inhibited MTB with MIC at **12.5** µg/mL. When compared with the first-line anti-tubercular drug, Ethambutol (MIC **3.13** µg/mL) two compounds **7i** and **8g** were found to be more active (**1.56** µg/mL) as compared to the standard drug Ethambutol. All the other compounds showed moderate activity when compared with other anti-TB drugs, isoniazid (MIC **0.1** µg/mL) and rifampicin (MIC **0.2** µg/mL).

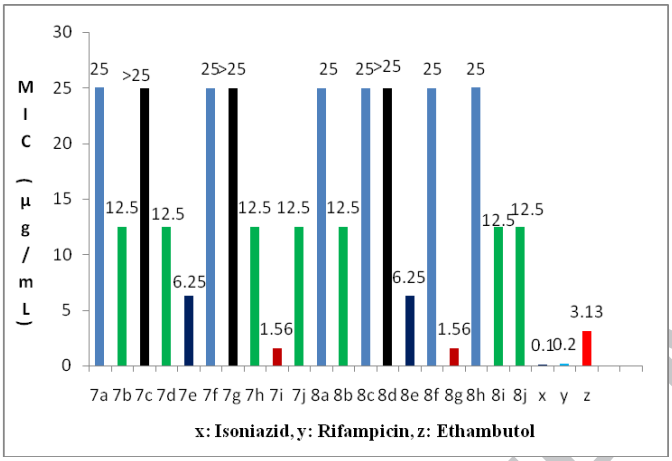


Figure 3: Anti-tubercular evaluation of novel analogues **7a-j** & **8a-j** against *M. tuberculosis* $H_{37}RV$.

The safety profile of anti-tubercular active benzo[6,7]cyclohepta[1,2-*b*]pyridine-1,2,3-triazole derivatives with MIC ≤12.5 µg/mL were assessed by testing the *in vitro* cytotoxicity against Human Embryonic Kidney (HEK-293T) cells using 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. **Fig. 4**. describes the percentage Cell Viability of twelve analogues at 50 µg/mL concentration. The results clearly indicated that the potent analogues **7i** and **8g** did not exhibit significant changes in viability (cytotoxicity) as compared to vehicle (DMSO) treatment and can form as good leads suitable for further mechanistic evaluation.

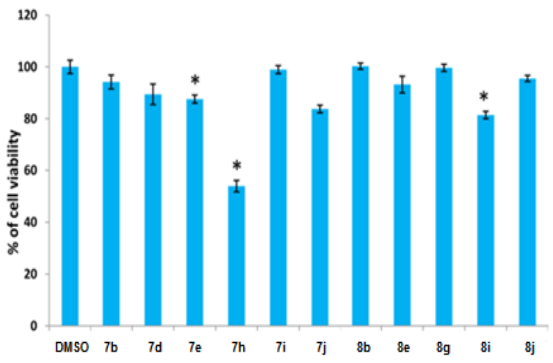


Figure 4: Percentage cell viability of benzo[6,7]cyclohepta[1,2-*b*]pyridine-1,2,3-triazole derivatives on HEK-293T cells at a concentration of 50µg/mL.

In conclusion, we developed a novel series of benzo[6,7]cyclohepta[1,2-*b*]pyridine-1,2,3-triazole hybrids bearing interesting bioactive triazole scaffold using click chemistry and evaluated their *in vitro* anti-mycobacterial activity against *Mycobacterium tuberculosis* H37Rv (ATCC 27294). Among the compounds tested, the compounds **7i** and **8g** displayed most potent activity against *M. tuberculosis* H37Rv with MIC value of 1.56 µg/mL with low cytotoxicity. These findings suggest that these lead compounds would be worth investigating in the near future for the mechanistic aspects.

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References:

1. Russell, D. G.; Barry, C. E.; Flynn, J. L.; Science. **2010**, *328* , 852.
2. Russell, D. G. *Nat. Rev. Microbiol*. **2007**, *5*, 39.
3. Kaufmann, S. H. E.; McMichael, A. J. *Nat. Med*. **2005**, *11*, 578.
4. Manabe1, Y. C.; Bishai1, W. R. *Nat. Med*. **2000**, *6*, 1237.
5. Koul, A.; Arnoult, E.; Lounis, N.; Guillemont, J.; Andries, K. Nature, **2011**, *469*, 483–490.
6. World Health Organization (2017b) Tuberculosis. Fact sheet Reviewed October 2017. http://www.who.int/mediacentre/factsheets/fs104/en/ (Retrieved 17 October 2017).
7. Prabowo, S. A.; Gröschel, M. I.; Schmidt, E .D.; Skrahina, A.; Mihaescu, T.; Hastürk, S.; Mitrofanov, R.; Pimkina, E.; Visontai, I.; De Jong, B.; Stanford, J. L.; Cardona, P. J.; Kaufmann, S. H.; van der Werf, T. S.; Med. Microbiol. Immunol. **2013**, *202* 95.
8. Falzon, D.; WHO guidelines for the programmatic management of drugresistant tuberculosis: 2011 update, Eur. Respir. J. **2011**, *38*, 516.
9. Zumla, A.; Nahid, P.; Cole, S. T.; Nat. Rev. Drug Discov. **2013**, *12*, 388.
10. Zumla, A.; Kim, P.; Maeurer, M.; Schito, M.; Lancet Infect Dis. **2013** *13*, 285.
11. Palomino,J. C.; Martin, A.; Antimicrob. J.; Chemother. **2013**, *68*, 275.
12. Nguyen, L.; Jacobs, M. R.; Expert Rev. Anti Infect. Ther. **2012**, *10*, 959.
13. Nzila, A.; Ma, Z.; Chibale, K.; Future Med. Chem. **2011**, *3*, 1413.
14. Patel, N. B.; Agravat, S. N.; Shaikh, F. M.; Med. Chem. Res. **2011,** *20*, 1033.
15. Patel, N. B.; Agravat, S. N.; Chem. Heterocycl. Compd. **2009**, *45*, 1343.
16. Srivastava, A.; Pandeya, S. N.; Int. J. Curr. Pharm. Rev. Res. **2011**, *4*, 5.
17. Paronikyan, E. G.; Noravyan, A. S.; Dzhagatspany, I. A.; Nazaryan, I. M.; Paronikyan, R. G.; Pharm. Chem. J. **2002**, *36*, 465.
18. Bernardino, A. M. R.; De Azevedo, A. R.; Pinheiro, L. C. D.; Borges, J. C.; Carvalho, V. L.; Miranda, M. D.; De Meneses, M. D. F.; Nascimento, M.; Ferreira, D.; Rebello, M. A.; Med. Chem. Res. **2007**, *16*, 352.

19. Tucker, T. J.; Sisko, J. T.; Tynebor, R. M.; Williams, T. M.; Felock, P. J.; Flynn, J. A.; Lai, M.; Liang, Y.; McGaughey, G.; Liu, M.; J. Med. Chem. **2008**, *51*, 6503.
20. Mamolo, M. G.; Zampieri, D.; Falagiani, V.;. Vio, L.; Fermeglia, M.; Ferrone, M.; Pricl, S.; Banfi, E.; Scialino, G.; ARKIVOC. **2004**, *5*, 231.
21. Wang, Y.; Wang, J.; Lin, Y.; Si-Ma, L. F.; Wang, D. H.; Chen, L. G.; Liu, D. K.; Bioorg. Med. Chem. Lett. **2011** *21*, 4454.
22. Liu, G. Z.; Xu, H. W.; Chen, G. W.; Wang, P.; Wang, Y. N.; Liu, H. M.; Yu, D. Q.; Bioorg. Med. Chem. **2010**, *18* 1626.
23. Soule, B. P.; Simone, N. L.; DeGraff, W. G.; Choudhuri, R.; Cook, J. A.; Mitchell, J. B.; Radiat. Oncol. **2010** *5*, 8.
24. Chen, J. S.; Lin, S. Y.; Tso, W. L.; Yeh, G. C.; Lee, W. S.; Tseng, H.; Chen, L. C.; Ho, Y.S.; Mol. Carcinog. **2006**, *45*, 461.
25. Gelfand, E. W.; Appajosyula, S.; Meeves, S.; Curr. Med. Res. Opin. **2004**, *20*, 73.
26. Agrawal, D. K.; Clin. Exp. Allergy. **2004**, *34*, 1342.
27. Moorthouse, A. D.; Moses, J. E.; Chem. Med. Chem. **2008** *3*, 715.
28. Hou, J. L.; Liu, X. F.; Shen, J.; Zhao, G. L.; Wang, P. G.; Expert. Opin. Drug Discov. **2012**, *7*,489.
29. Zumla, A.; Kim, P.; Maeurer, M.; Schito, M.; Lancet Infect Dis. **2013**, *13*, 285.
30. Palomino, J. C.; Martin, A.; J. Antimicrob. Chemother. **2013,** *68*, 275.
31. Duncan, K., Barry, C. E.; III Curr.Opin. Microbiol. **2004**, *7*, 460.
32. Lamichhane, G.; Trends Mol. Med. **2011**, *17*, 25.
33. Costa, M. S.; Boechat, N.; Rangel, E. A.; Silva, F. C.; Souza, A. M. T.; Rodrigues, C. R.; Castro, H. C.; Junior, I. N.; Lourenço, M. C. S.; Wardell, S. M. S. V.; Ferreira, V. F.; Bioorg. Med. Chem. **2006**, *14*, 8644.
34. Maccari, R.; Ottaná, R.; Vigorita, M. G.; Bioorg. Med. Chem. Lett. **2005**, *15*, 2509.
35. Kamal, A.; Ahmed, S. K.; Reddy, K. S.; Khan, M. N. A.; Shetty, R. V. C. R. N. C.; Siddhardha, B.; Murthy, U. S. N.; Khan, I. A.; Kumar, M.; Sharma, S.; Ram, A. B.; Bioorg. Med. Chem. Lett. **2007**, *17*, 5419.
36. Nayyar, A.; Monga, V.; Malde, A.; Coutinho, E.; Jaina, R.; Bioorg. Med. Chem. **2007**, *15*, 626.
37. Holla, B. S.; Mahalinga, M.; Karthikeyan, M. S.; Poojary, B.; Akberali, P. M.; Kumari, N. S.; Eur. J. Med. Chem. **2005,** *40*, 1173.
38. Gill, C.; Jadhav, G.; Shaikh, M.; Kale, R.; Ghawalkar, A.; Nagargoje, D.; Shiradkar, M.; Bioorg. Med. Chem. Lett. **2008**, *18*, 6244.
39. Singh, B. K.; Yadav, A. K.; Kumar, B.; Gaikwad, A.; Sinha, S. K.; Chaturvedic, V.; Tripathia, R. P.; Carbohydr. Res. **2008**, *343*,1153.
40. Gallardo, H.; Conte, G.; Bryk, F.; Lourenço, M. C. S.; Costa, M. S.; Ferreira, V. F. J.; Braz. Chem. Soc. **2007** *18*,1285.
41. Nagarapu, L.; Gaikwad, H. K.; Sirikonda, K.; Mateti, J.; Bantu, R.; Raghu, P. S.; Mahduri, K. M.; Kalivendi, S. V.; Eur. J. Med. Chem. **2010**, *45*, 4720.
42. Nagarapu, L.; Paparaju, V.; Satyenar, A.; Biorg. Med. Chem. Lett. **2008**, *18*, 2351.
43. Nagarapu. L.; Satyendar, A.; Rajashaker, B.; Srinivas, K.; Ruparani, P.; Radhika, K.; Shubhashini, G.; Bioorg. Med. Chem. Lett. **2008**, *18*, 1167.
44. Nagarapu, L.; Vanaparthi, S.; Bantu, R.; Kumar, C. G.; Eur. J. Med. Chem. **2013**, *69*,817.
45. Nagarapu, L.; Yadagiri, B.; Bantu, R.; Kumar, C. G.; Pombala, S.; Nanubolu, J.; Eur. J. Med. Chem. **2014**, *71*, 91.
46. Yadagiri, B.; Holagunda, U. D.; Bantu, R.; Nagarapu, L.; Kumar, C. G.; Pombala, S.; Sridhar, B.; Eur. J. Med. Chem. **2014**, *79*, 260.
47. Nagarapu, L.; Vulupala, H. R.; Bantu, R.; Sajja, Y.; Nanubolu, J. B.; Tetrahedron Assymetry. **2014**, 578.
48. Sajja, Y.; Vulupala, H. R.; Bantu, R.; Nagarapu, L.; Bioorg. Med. Chem. Lett. **2016**, *26*, 858;
49. Bollu, R.; Palem, J. D.; Bantu, R.; Nagarapu, L.; Eur. J. Med. Chem. **2015**, *89*, 138.
50. Sajja, Y.; Vanguru, S.; Jilla, L.; Vulupala, H. R.; Bantu, R.; Nagarapu, L.; Bioorg. Med. Chem. Lett. **2016**, *26*, 4292.

Supplementary Information

Experimental section and Copies of the ^{1}H, ^{13}C NMR and ESI-MS spectra for some of the important compounds.

Graphical Abstract

Design, Synthesis and *in vitro* Anti-tuberculosis activity of Benzo[6,7]cyclohepta[1,2-*b*]pyridine-1,2,3-triazole Derivatives.

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Yasodakrishna Sajja, Sowmya Vanguru, Hanmanth Reddy Vulupala, Lingaiah Nagarapu[a*], Perumal Yogeeswari[b], Dharmarajan Sriram[b] .

Benzo[6,7]cyclohepta[1,2-*b*]pyridine -1,2,3- triazole hybrids (**7a-j and 8a-j**) were synthesised and screened against *Mycobacterium tuberculosis* H37Rv. Preliminary results were promising with MIC values in the range **1.56-25** µg/mL.