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Synthesis, Characterization of analogues of carbamic acid tert-butyl ester and Evaluation of their Anti-Microbial Activity.

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ABSTRACT: We have synthesized new carbamic acid tert-butyl ester analogues **6 (a-l)** and these compounds **6 (a-l)** were assayed for their anti-microbial activity, against three representative Gram-positive bacteria *viz*. by disc diffusion method. The antifungal screening reveals that compounds **6d, 6g, 6j, 6k, and 6l** have exhibited promising activity among all synthesised compounds, remaining analogues shown moderate to good antifungal activity.

I. INTRODUCTION:

In recent decades, the frequency of microbial infection has skyrocketed to frightening levels all over the world due to the results of antimicrobial resistance. Consequently, it is crucial and much attention has been focused to address the problem of the multi drug resistance (MDR) Bacteria and fungi because of the ubiquitous use and misuse of classical antimicrobial agents with because of this situation, new classes of antimicrobial agents with novel mechanism has to be discovered to overcome Due to their many biological functions, organic molecules with heterocyclic ring structures continue to get a lot of attention. The 1,2,3 triazole moiety among all other five members heterocyclic system is an important heterocyclic with applications in pharmaceuticals, materials, chemical biology and agro chemicals.[2]

This scaffold is widely employed in various commercial medications as well as investigational therapeutic candidates that are used to address a variety of clinical conditions. Anti microbials drug potency can change depending on a number of conditions such as the concentration of the agent, the type of microorganisms, the mode of application and the presence of resistant mechanism. Furthermore, antimicrobial agents are useful tools for managing infections as well as ensuring safety, microorganisms could grow resistance to them as a consequence of misuse or overuse. This is why it's so important to use these agents carefully, adhere to dosage guidelines, and support behaviours that reduce the formation of resistance strains.

It is well known that triazole and thiadiazole rings are included in the composition of several drugs. [4-7]. The synthesis of novel derivatives of 1,2,4-triazole-3-thiones and 2-amino-1,3,4-thiadiazoles from these groups of heterocyclic compounds has been receiving a lot of attention due to various biological properties such as: antibacterial [8-13], antifungal [8,14,15], anti-tubercular [8,11,16], antiviral [9,10,16], antioxidant [17,18], antitumoral [19-21], anti-inflammatory [22-24], anticonvulsant [25-27].

The copper (I) -catalyzed 1,3-dipolar cyclo addition of azides and terminal alkynes is said to be the most notable reaction of the "click" chemistry [30]. It also serves as the easiest method for synthesizing 1,2,3-triazoles. This important family of compounds has been extensively used in material, combinatorial, and medicinal chemistry.[31] A number of methods explaining the in-situ production of azides have been devised in recent years.[32]

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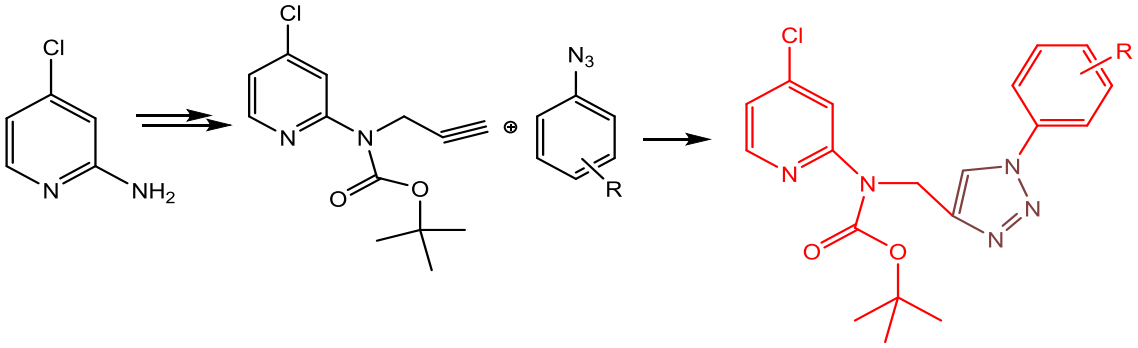

KEYWORDS: 4-chloropyridin-2-amine, Carbamic acid, triazoles, click reaction.

However, alkynes of low molecular weight are difficult to handle, and their usage under laboratory conditions is somewhat cumbersome due to their low boiling points [33]. It's important to note that the inappropriate or overuse of antimicrobial agents can lead to the development of antimicrobial resistance, where microorganisms become less responsive to the effects of the drugs. This is a growing concern in healthcare and agriculture, as it can limit the effectiveness of available treatments.

we need to study and develop new antimicrobial compounds to combat emerging microbial threats and to address the challenges posed by antimicrobial resistance. Natural products, synthetic compounds, and novel therapies are all areas of active exploration in the field of antimicrobial research. we are purposed to synthesize and investigate the antimicrobial activity of a new series from 1,2,3-triazole and carbamic acid having different moiety linked in 3 positions of triazole nucleus

II.RESULT AND DISCUSSION:

We chose to manufacture the carbamic acid tert-butyl ester (**6**) molecule because of its biological potential. We have used commercially accessible and inexpensive 4-chloropyridine-2amine (**1**) as a starting ingredient. Protection with Boc anhydride in presence of TEA (tri ethanolamine) and DMAP (di methyl amino pyridine) in dichloromethane (DCM) solvent at room temp to obtain carbamic acid derivative product **2** (tert-butyl (4-chloropyridin-2-yl) carbamate in 82% yield. Compound **2** which reacts with propargyl bromide (**3**), K_2CO_3 (2.5 eq), and DMF at room temperature to give **4** with 78 % yield.

Now DMF as solvent treatment of aromatic azides (**5**) and compound **4**, add CuSO4.7H2O and sodium ascorbate stir reaction mixture for about 16-18 hrs at room temperature, by click reaction. To obtain **6** with 86 % yield. it was a stable crystalline solid. Analytical data of final compound (**6**) matched with previously reported data and overall yield is 76-86%.

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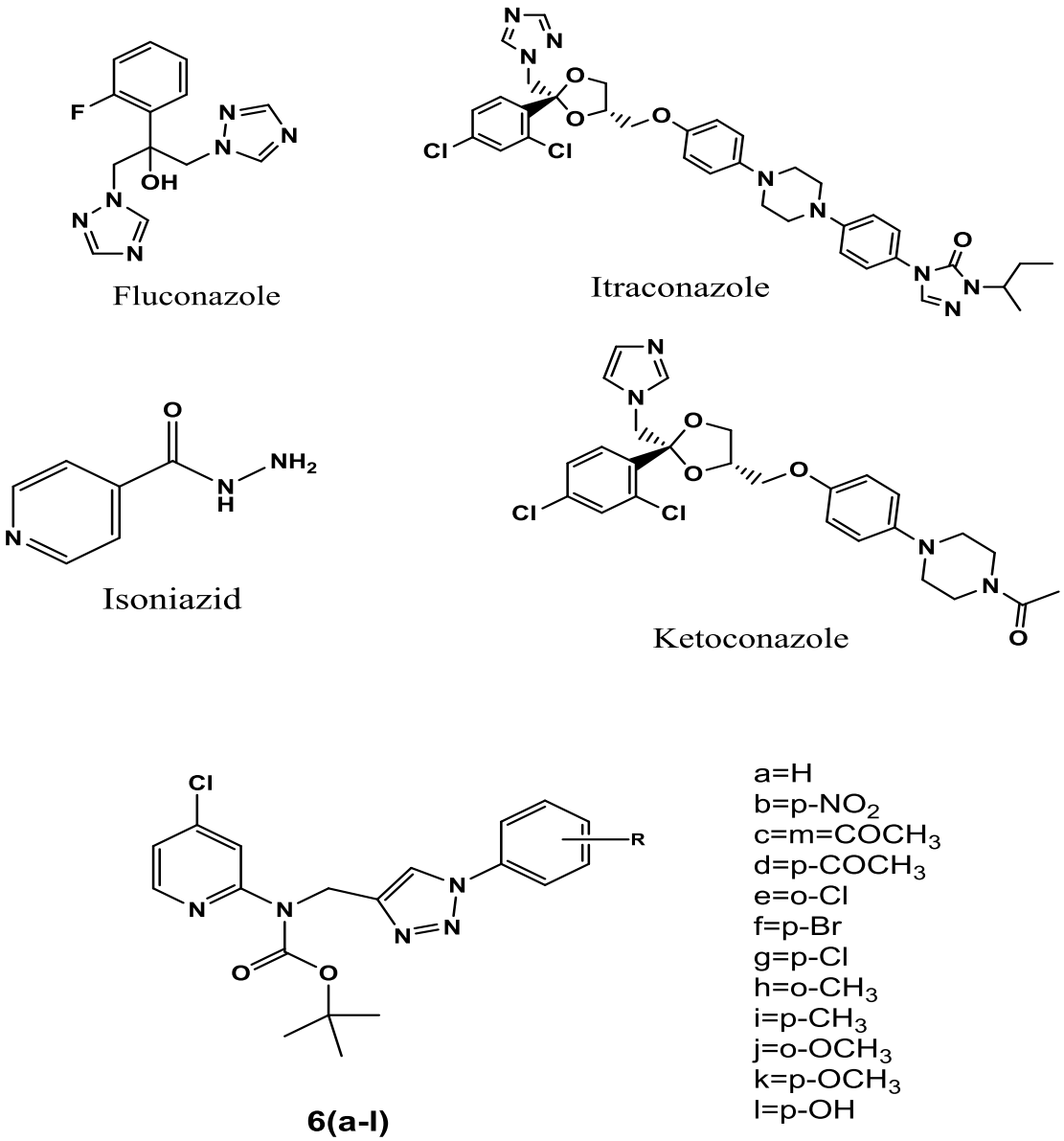

Figure 1. **Some of the existed well known antimicrobial compounds and our synthesized compounds**

III. Anti-Microbial Activity:

Antibacterial Activity of Compounds 6 (a-l):

All the newly synthesized compounds **6(a-l)** were assayed for their antibacterial activity against three representative Gram-positive bacteria *viz*. *Bacillus Subtilis* (MTCC 441), *Bacillus Sphaericus* (MTCC 11) and *Staphylococcus Aureus* (MTCC 96), and three Gram-negative bacteria *viz*. *Pseudomonas Aeruginosa* (MTCC 741), *Klobsinella PAerogenes* (MTCC 39) and *Chromobacterium Violaceum* (MTCC 2656) by disc diffusion method. For the antibacterial assay standard inoculums (1-2×10^7 c.f.u/mL 0.5 Mc Farland standards) were introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculums.

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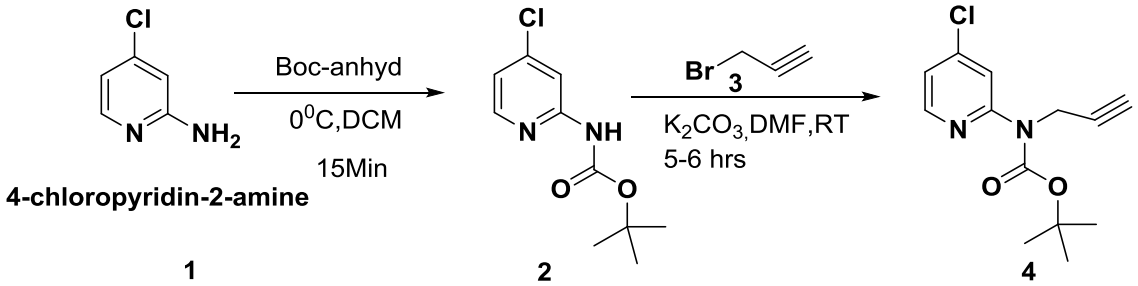
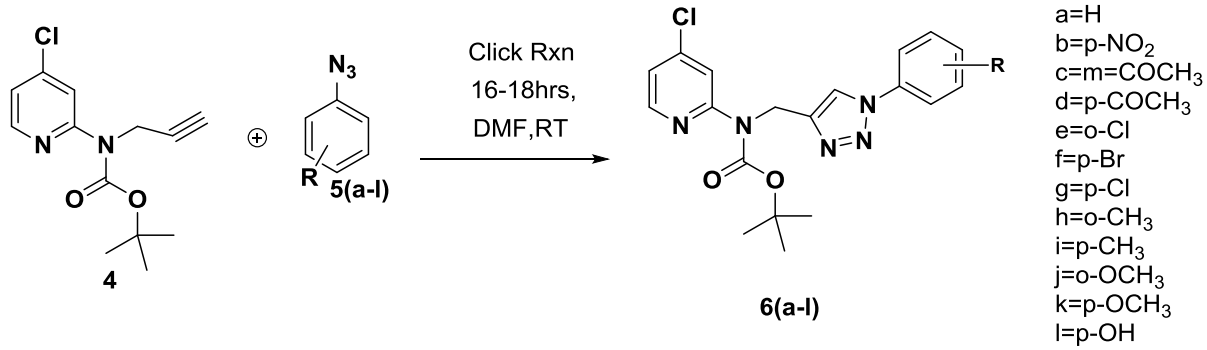


Scheme 1. Synthesis of compound (4)

The discs measuring 6.26 mm in diameter were prepared from Whatman no.1 filter paper and sterilized by dry heat at 140 ˚C for 1 h. The sterile discs previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. The plates were inverted and incubated for 24 h at 37 ˚C. The mean inhibition zones were measured and compared with the standard drug Streptomycin and the results are presented in **Table 1**. Amongst them **6d, 6e, 6g, 6j, 6k, and 6l** good activity (**Table 1**). The remaining compounds showed moderate to good activity.



Scheme 2. Synthesis of compound (6)

Antifungal Activity of Compounds 6(a-l): The compounds 6(a-l) were also screened for their antifungal activity against *Candida albicans* (ATCC 10231), *Aspergillus fumigatus* (HIC 6094), *Trichophyton rubrum* (IFO 9185), and *Trichophyton mentagrophytes* (IFO 40996) in dimethyl sulfoxide (DMSO) by **disc diffusion method.** Amphotericin B was used as a standard drug and the mean inhibition zone (MZI) data were measured and compared with controls, the MZI values of the compounds screened are given in **Table 2.**

The antifungal screening data showed appreciable activity of the test compounds. Among the screened compounds, compound It is interesting to note that the compounds **6d, 6e, 6g, 6k, and 6l** showed good antifungal activity towards *C.albicans* which is more than the activity of standard drug.

Table 1. Antibacterial Activity of Compounds 6(a-l)

Mean zone inhibition (MZI)[a] in 100 μg/mL						
Compound	***B. subtilis***	***B. sphaericus***	***S. aureus***	***P. aeruginosa***	***K. aerogenes***	***C. violaceum***
6a	19	16	20	14	15	18
6b	23	20	22	16	20	20
6c	22	20	26	16	22	22
6d	**34**	**24**	**31**	**18**	**28**	**26**
6e	**26**	**22**	**26**	**22**	**22**	**22**
6f	22	20	20	16	20	20
6g	**34**	**23**	**30**	**18**	**28**	**23**

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6h	21	22	26	16	22	23
6i	24	20	20	16	28	18
6j	**28**	**20**	**28**	**18**	**19**	**20**
6k	**32**	**24**	**29**	**18**	**28**	**23**
6l	**33**	**24**	**30**	**18**	**28**	**24**
Streptomycin	**33**	**28**	**34**	**25**	**28**	**28**

[a]Values are mean (n = 3).

Table 2. Antifungal Activity of Compounds 6(a-l):

Compound	Mean zone inhibition (MZI)[a] in 100 μg/mL			
	C. albicans	*A. fumigatus*	*T. rubrum*	*T. mentagropytes*
6a	16	17	14	16
6b	19	22	18	18
6c	15	19	16	17
6d	**22**	**27**	**22**	**25**
6e	**21**	**22**	**19**	**21**
6f	14	16	14	18
6g	**24**	**26**	**22**	**24**
6h	20	22	20	16
6i	19	16	12	20
6j	18	16	20	20
6k	**23**	**27**	**23**	**24**
6l	**24**	**26**	**22**	**24**
Amphotericin I	**28**	**30**	**26**	**28**

[a]Values are mean (n = 3).

IV.EXPERIMENTAL SECTION:

All reagents and solvents were obtained from commercial suppliers and were used without further purification. Analytical thin-layer chromatography (TLC) was performed on MERCK precoated silica gel 60-F254 (0.5 mm) aluminium plates. Visualization of the spots on TLC plates was achieved by UV light. ^{1}H and ^{13}C NMR spectra were recorded on Bruker 400 MHz, respectively by making a solution of samples in the DMSO using tetramethylsilane (TMS) as the internal standard. Chemical shifts for ^{1}H and ^{13}C are reported in parts per million (ppm) downfield from tetramethylsilane. Spin multiplicities are described as s (singlet), d (doublet), t (triplet), and m (multiplet). Wherever required, column chromatography was performed using silica gel (60-120). The reactions wherever anhydrous conditions required are carried out under nitrogen positive pressure using freshly distilled solvents. All evaporation of solvents was carried out under reduced pressure using rotary evaporator below 45 ^{o}C. Melting points were determined with an electrothermal digital melting point apparatus IA9100 and are uncorrected. The names of all the compounds given in the experimental section were taken from Chem Bio Draw Ultra, Version 12.0.

(4-Chloro-pyridin-2-yl)-(1-phenyl-1H-[1,2,3]triazol-4-ylmethyl)-carbamic acid tert-butyl ester.(6)

Arrange a clean and dry 50ml single neck RBF and charged compound-**4** and DMF as a solvent, add $CuSO_4.7H_2O$ (2 ml) Colour changed can be observed, add sodium Ascorbate ($Na_2S_2O_3$) (2ml) solid appearance is observed, than add different substituted aromatic azides (**5**) 1.2eq, stir reaction mixture for about 16-18 hours at room temperature, Check the TLC (50% EtOAc in pet ether) if starting material (SM) was completed than quinch with ice, and filter with reaction filtrate and wash with PET ether solid is formed substituted (4-

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Chloro-pyridin-2-yl)-(1-phenyl-1H-[1,2,3]triazol-4-ylmethyl)-carbamic acid tert-butyl ester (**6a-l**) (Yeild 76-86%). ^{1}H NMR (400 MHz, dmso) δ 8.850 (s, 1H), 8.442, 8.442, 8.420, 8.385-8.371(d,1H), 8.273-8.251(d,1H), 8.234, 8.212, 7.904 (s, 1H), 7.374 (d,1H), 7.352(d,1H), 7.309(d,1H), 7.299(d,1H), 5.285 (s, 2H), 1.437 (s, 9H),^{13}C NMR (400 MHz, dmso) δ 154.409, 152.769, 148.731, 146.576, 143.281, 140.807, 125.513, 121.414, 120.093, 119.691, 118.089, 81.869, 41.625, 27.682..IR: $\upsilon_{max.}$ 3128.07, 3078.29, 2977.54, 2931.25,1706.45, 1603.96,1578.43, 1556.30, 1501.10, 1461.59, 1384.98, 1364.99, 1319.56, 1274.81, 1240.92, 1149.03, 1103.46, 1043.73, 987.22, 943.02, 825.11, 759.45, 687.36, 619.50, 568.25, 540.66, 511.85 cm^{-1}; ESI-MS: *m/z* 385.13 $[M+H]^+$; HRMS: cacld. for $C_{19}H_{20}ClN_5O_2$, 385.85, found: 385.13.IR: $\upsilon_{max.}$ 3146.22, 2978.64, 2933.68, 2124.25, 2091.39, 1703.05, 1597.27, 1578.64, 1556.55, 1521.10, 1461.19, 1434.25, 1386.61, 1342.86, 1281.54, 1234.24, 1156.88, 1106.32, 1064.44, 1038.02, 1013.52, 987.46, 946.64, 848.76, 814.15, 775.85, 746.68, 714.62, 687.34, 628.66, 600.44, 563.51 cm^{-1}; ESI-MS: *m/z* 430.12 $[M+H]^+$; HRMS: cacld. for $C_{19}H_{19}ClN_6O_4$, 430.84, found: 430.12.

(4-Chloro-pyridin-2-yl)-carbamic acid tert-butyl ester (2)

Arrange a clean and 50ml single neck round bottom Flask (RBF). Charged 4-Chloro-pyridin-2-ylamine (1) and dry Chloro Méthane (DCM) as a solvent. Stirr the reaction mixture in cool condition at 0^{0}C. Add TEA of 1 équivalence, DMAP of 0.01 équivalence then add BoC unhydride of 1.2 equivalence dropwise. After addition of BoC anhydride stir the reaction mixture in room temperature for 30 Mints. Check the TLC (10% EtoAc in Pet Ether) if SM (Starting Material) was completed than quench with ice, liquid is formed, Extract with DCM. Distil the organic Layer, Solid is formed (**2**) (Yeild 82%).

^{1}HNMR (400 MHz, dmso) δ 1.35 (s, 9H), 8.3 (S, 1H, NH), 7.19 (d,1H, J=7.4 Hz), 8.18 (S, 1H), 8.41 (d, 1H, J=6.9Hz) IR: υ_{max} : 3340, 3121, 2945, 1786, 868, 676 cm^{-1} ESI-MS: *m/z* 228.07 $[M+H]^+$; HRMS: cacld. for $C_{10}H_{13}ClN_2O_2$, 228.68, found: 228.07. ^{13}C NMR (DMSO, 400 MHz) : 28.9, 54.5, 110.5, 115.6, 135.6, 148.9, 155.7, 178.5.

(4-Chloro-pyridin-2-yl)-prop-2-ynyl-carbamic acid tert-butyl ester (4)

Arrange a clean and dry 50ml single neck RBF and charged compound-**2** and DMF as a solvent, add K_2CO_3 (2.5 eq) as a base, and Propargyl Bromide (PBr) (1.2eq as a reactant stir the reaction mixture for about 4-5 hours in room temperature. Check the TLC (20% EtOAc) if starting material (SM) was completed than quinch with ice, liquid is formed extract with DCM, Distil the organic layer, liquid is formed (**4**). (Yield 78%).

^{1}H NMR (400 MHz, dmso) δ1.42 (s, 9H), 1.85 (s, 1H), 3.65 (s, 1H), 7.20 (d, 1H, J=7.6 Hz), 8.14 (s, 1H), 8.42 (d, 1H, 8.1 Hz). ESI-MS: *m/z* 252.07 $[M+H]^+$; HRMS: cacld. for $C_{12}H_{13}ClN_2O_2$, 252.70, found: 252.07. IR: $\upsilon_{max.}$ 3305, 3109, 2925, 2156, 1757, 852, 667 cm^{-1} ^{13}C NMR (DMSO, 400 MHz) : 26.5, 45.1, 58.2, 68.3, 69.4, 104.5, 114.5, 132.5, 150.9, 162.3, 181.5.

CONCLUSION: In this study we report the synthesis, characterization and antimicrobial activity evaluation of new compounds from 1,2,3-triazole class and their carbamic acid intermediates moiety. The target compounds from 1,2,3-triazole class and their carbamic acid intermediates were obtained from click reaction of aromatic azides and carbamic acid intermediates containing alkyne group. The most antibacterial activity was presented by 1,2,3-triazole against three representative Gram-positive bacteria *viz*. which shows good anti-microbial activity. Our synthetic route has advantages like commercially accessible inexpensive starting materials and reagents, standard normal reaction conditions, simple and broadly used reactions and satisfactory yields. Hence overall synthesis of compound 6 is achieved in 3 steps.

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REFERENCES:

[1]. a)Stefania-Felicia Barbuceanu a , Gabriel Saramet b,*, Gabriela Laura Almajan a , Constantin Draghici c , Florica Barbuceanu d , Gabriela Bancescu e

[2]. 1)a) S. Kantheti, R. Narayan, K. V. S. N. Raju, RSC Adv. 2015, 5, 3687-3708; b) J. Huo, H. Hu, M. Zhang, X. Hu, M. Chen, D. Chen, J. Liu, G. Xiao, Y. Wang, Z. Wen, RSC Adv. 2017, 7, 2281-2287; c) M. Kukwikila, N. Gale, A. H. El-Sagheer, T. Brown, A. Tavassoli, Nat. Chem. 2017, 9, 1089.

[3]. a) B. Zhang, Eur. J. Med. Chem. 2019, 168, 357-372; b) E. Bonandi, M. S. Christodoulou, G. Fumagalli, D. Perdicchia,

International Journal of Pharmaceutical research and Applications
Volume 8, Issue 5, Sep.-Oct. 2023, pp: 1704-1710 www.ijprajournal.com ISSN: 2456-4494

G. Rastelli, D. Passarella, Drug Discov. 2017, 22, 1572-1581.
[4]. F. Sabatelli, R. Patel, P.A. Mann, C.A. Mendrick, C.C. Norris, R. Hare, D. Loebenberg, T.A. Black, P.M. McNicholas, Antimicrob. Agents Chemother. 50 (2006) 2009e2015.
[5]. T. El-Sayed Ali, A.M. El-Kazak, Eur. J. Chem. 1 (2010) 6e11.
[6]. M. Serdar, N. Gümrükçüoglu, S¸ . Alpay Karao glu, N. Demirbas¸ , Turk. J. Chem. 31 (2007) 315e326.
[7]. N. Siddiqui, P. Ahuja, W. Ahsan, S.N. Pandeya, M.S. Alam, J. Chem. Pharm. Res. 1 (2009) 19e30.
[8]. N.U. Güzeldemirci, Ö. Küçükbasmaci, Eur. J. Med. Chem. 45 (2010) 63e68.
[9]. S¸ .G. Küçükgüzel, I. Küçükgüzel, E. Tatar, S. Rollas, F. S¸ ahin, M. Güllüce, E. De Clercq, L. Kabasakal, Eur. J. Med. Chem. 42 (2007) 893e901.
[10]. M.T. Abdel-Aal, W.A. El-Sayed, A.H. Abdel Aleem, E.S.H. El Ashry, Pharmazie 58 (2003) 788e792.
[11]. I. Küçükgüzel, S.G. Küçükgüzel, S. Rollas, M. Kiraz, Bioorg. Med. Chem. Lett. 11 (2001) 1703e1707.
[12]. A.A. Kadi, N.R. El-Brollosy, O.A. Al-Deeb, E.E. Habib, T.M. Ibrahim, A.A. ElEmam, Eur. J. Med. Chem. 42 (2007) 235e242.
[13]. T. Plech, M. Wujec, A. Siwek, U. Kosikowska, A. Malm, Eur. J. Med. Chem. 46 (2011) 241e248.
[14]. A.A. Siddiqui, A. Arora, N. Siddiqui, A. Misra, Indian J. Chem. 44B (2005) 838e841.
[15]. C. Camoutsis, A. Geronikaki, A. Ciric, M. Sokovic, P. Zoumpoulakis, M. Zervou, Chem. Pharm. Bull. 58 (2010) 160e167.
[16]. I. Küçükgüzel, E. Tatar, S¸ .G. Küçükgüzel, S. Rollas, E. De Clercq, Eur. J. Med. Chem. 43 (2008) 381e392.
[17]. I. Khan, S. Ali, S. Hameed, N.H. Rama, M.T. Hussain, A. Wadood, R. Uddin, Z. Ul-Haq, A. Khan, S. Ali, M.I. Choudhary, Eur. J. Med. Chem. 45 (2010) 5200e5207.
[18]. G. Ayhan-Kilcigil, C. Kus, T. Çoban, B. Can-Eke, M. Iscan, J. Enz. Inhib. Med. Chem. 19 (2004) 129e135.
[19]. A. Duran, H.N. Dogan, S. Rollas, Il Farmaco 57 (2002) 559e564.
[20]. W. Rzeski, J. Matysiak, M. Kandefer-Szerszen, Bioorg. Med. Chem. 15 (2007) 3201e3207.
[21]. A.T. Mavrova, D. Wesselinova, Y.A. Tsenov, P. Denkova, Eur. J. Med. Chem. 44 (2009) 63e69.
[22]. H. Kumar, S.A. Javed, S.A. Khan, M. Amir, Eur. J. Med. Chem. 43 (2008) 2688e2698.
[23]. M. Amir, K. Shikha, Eur. J. Med. Chem. 39 (2004) 535e545.
[24]. S.M. El-Moghazy, F.F. Barsoum, H.M. Abdel-Rahman, A.A. Marzouk, Med. Chem. Res. (2011). doi:10.1007/s00044-011-9691-4.
[25]. J.M. Kane, M.A. Staeger, C.R. Dalton, F.P. Miller, M.W. Dudley, A.M.L. Ogden, J.H. Kehne, H.J. Ketteler, T.C. McCloskey, Y. Senyah, P.A. Chmielewski, J.A. Miller, J. Med. Chem. 37 (1994) 125e132.
[26]. H.N. Dogan, A. Duran, S. Rollas, G. Sener, M.K. Uysal, D. Gülen, Bioorg. Med. Chem. 10 (2002) 2893e2898.
[27]. R. Sharma, G.P. Misra, J. Sainy, S.C. Chaturvedi, Med. Chem. Res. 20 (2011) 245e253.
[28]. (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. Angew. Chem., Int. Ed. 2002, 41, 2596–2599. (b) Tornøe, C. W.; Christensen, C.; Meldal, M. J. Org. Chem. 2002, 67, 3057–3064.
[29]. (a) Tam, A.; Arnold, U.; Soellner, M. B.; Raines, R. T. J. Am. Chem. Soc. 2007, 129, 12670–12671. (b) Boren, B. C.; Narayan, S.; Rasmussen, L. K.; Zhang, L.; Zhao, H.; Lin, Z.; Jia, G.; Fokin, V. V. J. Am. Chem. Soc. 2008, 130, 8923–8930. okin, V. V. J. Am. Chem. Soc. 2008, 130, 8923–8930.
[30]. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. Angew. Chem., Int. Ed. 2001, 40, 2004–2021.
[31]. (a) Holub, J. M.; Kirshenbaum, K. Chem. Soc. Rev. 2010, 39, 1325–1337. (b) Meldal, M.; Tornøe, C. W. Chem. Rev. 2008, 108, 2952–3015. (c) Spiteri, A. D.; Moses, J. E. ChemMedChem 2008, 3, 715–723. (d) Wu, P.; Fokin, V. V. Aldrichim. Acta 2007, 40, 7–17.
[32]. (a) Feldman, A. K.; Colasson, B.; Fokin, V. V. Org. Lett. 2004, 6, 3897–3899. (b) Chittaboina, S.; Xie, F.; Wang, Q. Tetrahedron Lett. 2005, 46, 2331–2336. (c) Ceylan, S.; Klande, T.; Vogt, C.; Friese, C.; Kirschning, A. Synlett 2010, 2009–2013.
[33]. “Click“approach using acetylene gas: Wu, L. Y.; Xie, Y. X.; Chen, Z. S.; Niu, Y. N.; Liang, Y. M. Synlett 2009, 1453–1456.