



Regioselective synthesis and biological evaluation of bis(indolyl)methane derivatized 1,4-disubstituted 1,2,3-bistriazoles as anti-infective agents

M. Damodiran, D. Muralidharan, Paramasivan T. Perumal *

Organic Chemistry Division, Central Leather Research Institute, Adyar, Chennai 600 020, India

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ABSTRACT

The regioselective synthesis of 1,4-disubstituted 1,2,3-bistriazoles from a variety of *N*-propargyl bis(indolyl)methanes with sodium azide using CuI as the catalyst in polyethyleneglycol-400 is reported. This process is of considerable synthetic advantages in terms of high atom economy, low environmental impact, mild reaction condition and good yields. The synthesized compounds have also been screened for their biological activity.

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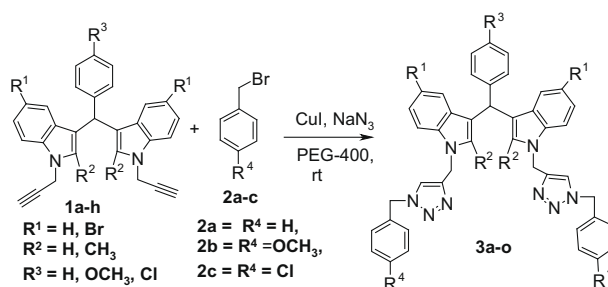
'Click chemistry' has emerged as a fast and efficient approach for synthesis of novel heterocyclic compounds.¹ The Huisgen 1,3-dipolar cycloaddition of azides and alkynes resulting in 1,2,3-triazoles is one of the most powerful click reactions.² 1,2,3-Triazole synthesis has been intensively studied, and triazoles are widely used in pharmaceuticals, agrochemicals, dyes, photographic materials, and in corrosion inhibitory materials.³ In addition, they possess anti-HIV,⁴ antimicrobial activities⁵ and selective β_3 adrenergic receptor agonism.⁶ In the absence of a transition-metal catalyst, these reactions are not regioselective, relatively slow, and require high temperatures to reach acceptable yields. In early 2002, Meldal and co-workers reported that the use of catalytic amounts of copper(I), which can bind to terminal alkynes, leads to fast, highly efficient, and regioselective azide–alkyne cycloadditions at room temperature in organic medium.⁷ Recently, Sharpless and co-workers have reported a high yielding synthesis of triazoles using a Cu(I) catalyst with an excellent 1,4-regioselectivity.⁸ The resulting 'clicked' products can even be obtained via in situ generation of the corresponding organic azides from organic halides–NaN₃ in the presence of an alkyne and a copper catalyst, avoiding the need to handle organic azides.⁹

Nitrogen heterocycles have received special attention in pharmaceutical chemistry due to their diverse medicinal potential.¹⁰ The availability of therapeutically important drugs such as itavastatin, cerivastatin, streptonigrin, sumatriptan, avitriptan, almotriptan, sumatriptan, pravodoline, remosetron, terconazole, itraconazole, fluconazole and voriconazole¹¹ are a few examples

which contain nitrogen heterocyclic nucleus. Since bis(indolyl)methanes possess a wide range of biological activities, their synthesis has received a paramount interest.^{12,13}

Recently, polyethylene glycols (PEG) as a reaction medium has received considerable attention in synthetic organic chemistry and emerged as alternative green reaction media with unique properties such as thermal stability, commercial availability, non-volatility, recyclable, non-halogenated, easily degradable, and possess low toxicity.¹⁴ A few reports are available on PEG-400¹⁵ for the synthesis of triazoles with limited and narrow application scope for different substrates.

As part of our ongoing program directed towards the development of new methodologies for the synthesis and biological evaluation of diverse heterocyclic compounds,¹⁶ herein we disclose the synthesis of *N*-propargylated bis(indolyl)methanes followed by 1,3-dipolar cycloaddition with alkyl azide to form 1,2,3-triazole



Scheme 1.

* Corresponding author. Tel.: +91 44 24913289; fax: +91 44 24911589.

E-mail address: ptperumal@gmail.com (P.T. Perumal).

in PEG-400 using 10 mol % of CuI. Bis(indolyl)methanes were prepared from *N*-propargylindoles¹⁷ with aldehydes as per the earlier reported procedure.¹³

To begin our study, we carried out the reaction of alkyne **1a**, with 2.4 equiv of sodium azide, 2.6 equiv of triethylamine and 2.2 equiv of benzyl bromide (**2a**) in the presence of 10 mol % CuI in *t*BuOH. However, the reaction did not proceed at all. Our attempts to carry out the reactions with different solvents such as H₂O, CH₃CN, EtOH or mixture of solvents such as CH₃CN/H₂O (1:1v/v) did not yield fruitful results. However, the use of a mixture of EtOH/H₂O (1:1v/v) at room temperature drove the reaction to form the desired bis-triazole product (**3a**) in 40% yield in 8 h. Surprisingly, when the same reaction was carried out in PEG-400 the desired product (**3a**) was obtained in 80% yield at 6 h.¹⁸ This may be due to the hydrophilic and hydrophobic character of the PEG-400. The reaction took place regioselectively at the alkynes moiety to produce the corresponding 1,4-disubstituted-1,2,3-triazole. The generality of this solvent system (PEG-400) was tested with various alkynes (**1a–1h**) and benzyl bromides (**2a–c**). Under this condition, bistriazoles (**3a–3o**) were obtained in good to excellent yields (Scheme 1, Table 1, entries 1–15).

The results revealed that the reaction was highly dependent on the nature of substituents on the aromatic ring of the alkynes (**1a–1h**). An evident electronic effect was observed when we compared the yields of the products (**3a–3o**). The yield of the products decreased when electron-donating groups were present on the phenyl ring of bis(indolyl)methanes (OMe at R³ in entries, 4–6 and CH₃ at R² in entries 11–15). Indole substrates bearing an electron-withdrawing group, the Br group at R¹ position (entries 8–10) and Cl group at R³ position (entry 7) afforded higher yields. In compounds **3j** and **3n**, the presence of both electron-withdrawing and electron-donating groups competes resulting in more or less yield. The yields were dramatically decreased when electron-donating groups present in benzyl bromide and the reaction times were found to be longer (entries 3 and 6). The structure of the obtained 1,4-disubstituted 1,2,3-triazoles is in good agreement with those described in the previous reports on the synthesis of substituted triazoles via three component coupling.¹⁹

All compounds were characterized by ¹H, NMR, ¹³C NMR, IR spectroscopy and mass spectroscopy. The ¹H NMR spectra of compounds (**3a–3o**) in CDCl₃ consist of a characteristic singlet due to the triazole proton in the region of 7.02–7.25 ppm. Another characteristic feature of the ¹H NMR spectra is the appearance of AB quartet at δ_H 5.26–5.31 ppm, which corresponds to four methylene protons of triazolyl-CH₂-phenyl and appearance of singlet at δ_H 5.30–5.42 ppm, which corresponds to another four methylene protons of indolyl-CH₂-triazolyl protons. These observations confirm the formation of bis(indolyl)methane derivatised 1,4-disubstituted 1,2,3-triazole.

All the newly synthesized compounds (**3a–o**) were screened for their in vitro antibacterial and antifungal activities. The in vitro antibacterial activities of the synthesized compounds were evaluated against Gram-positive bacteria namely *Staphylococcus aureus* (ATCC 25923) and Gram-negative bacteria namely *Escherichia coli* (ATCC 25922) by disc diffusion method²⁰ using a concentration of 100 μ g/mL. Muller Hinton Agar was employed as culture media and DMSO was used as solvent control for antibacterial activity.²¹ The diameter of zone of inhibition was measured in mm. All the compounds have shown almost comparable activity when compared to the standard drug Ciprofloxacin. Out of 15 synthesized compounds, **3a**, **3g**, **3j**, **3k** and **3n** showed maximum activity (20 mm inhibition) against *S. aureus*. The compounds are not biologically active against Gram-negative bacteria *E. coli*.

The in vitro antifungal activities of the synthesized compounds were evaluated against *Candida albicans* (ATCC 10231) by diffusion

method²⁰ at a concentration of 100 μ g/mL.²² Out of 15 synthesized compounds **3b**, **3e**, **3h**, **3i**, and **3m** have shown maximum activity

Table 1

Synthesis of 1,2,3-triazole derivatised bis(indolyl)methanes using PEG-400 as solvent

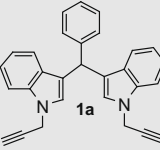
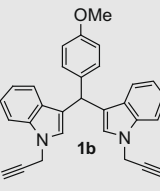
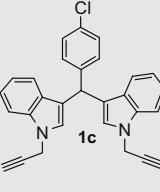
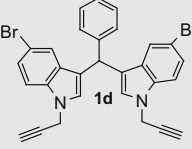
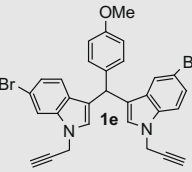
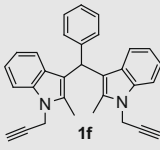
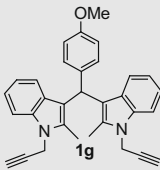
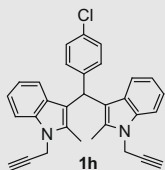
Entry	Alkyne (1)	R ⁴	Product (3)	Time (h)	Yield ^a (%)
1		H	3a	6	80
2	1a	Cl	3b	6	82
3	1a	OMe	3c	24	27
4		H	3d	6	75
5	1b	Cl	3e	6	78
6	1b	OMe	3f	24	32
7		Cl	3g	8	90
8		H	3h	6	85
9	1d	Cl	3i	6	90
10		H	3j	6	85
11		H	3k	6	72
12	1f	Cl	3l	6	78
13		Cl	3m	6	70

Table 1 continued

Entry	Alkyne (1)	R ⁴	Product (3)	Time (h)	Yield ^a (%)
14		H	3n	8	75
15	1h	Cl	3o	8	75

^a Isolated yields.

Table 2

The antibacterial and antifungal screening data

S. No	Compound	Zone of inhibition (in mm)	
		Antibacterial activity <i>S. aureus</i>	Antifungal activity <i>C. albicans</i>
1	3a	20	16
2	3b	17	20
3	3c	18	16
4	3d	19	17
5	3e	16	21
6	3f	18	16
7	3g	20	16
8	3h	16	21
9	3i	16	21
10	3j	20	16
11	3k	20	16
12	3l	17	19
13	3m	16	20
14	3n	20	18
15	3o	19	18
16	Ciprofloxacin	22	—
17	Ketoconazole	—	24

against *C. albicans*. The antibacterial and antifungal screening data are recorded in Table 2.

In conclusion a safe and efficient method for the generation of 1,4-disubstituted 1,2,3-bis-triazole in a complete regioselective manner has been developed. This method avoids isolation and handling of potentially unstable organic azide and provides triazole product in pure form. The operational simplicity of this method and the high yields of the product make it attractive for the synthesis of this class of potential biologically active molecules.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.131.

References and notes

- (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004; (b) Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128; (c) Moses, J. E.; Moorhouse, A. D. *Chem. Soc. Rev.* **2007**, *36*, 1249; (d) Hein, C. D.; Liu, X.-M.; Wang, D. *Pharm. Res.* **2008**, *25*, 2216; (e) Tron, G. C.; Pirali, T.; Billington, R. A.; Canonico, P. L.; Sorba, G.; Genazzani, A. A. *Med. Res. Rev.* **2008**, *28*, 278.
- (a) Huisgen, R. In *1,3-Dipolar Cycloaddition Chemistry*; Padwa, A., Ed.; Wiley: New York, 1984; p 1; (b) Ming, X.; Leonard, P.; Heindl, D.; Seela, F. *Nucleic Acids Symp. Ser.* **2008**, *52*, 471; (c) Zeng, Q.; Li, T.; Cash, B.; Li, S.; Xie, F.; Wang, Q. *Chem. Commun.* **2007**, 1453; (d) Munteanu, M.; Choi, S. W.; Helmut Ritter, H. *Macromolecules* **2008**, *41*, 9619.
- For reviews see: (a) Meldal, M.; Tornøe, C. W. *Chem. Rev.* **2008**, *108*, 2952; (b) Nandivada, H.; Jiang, X.; Lahann, J. *Adv. Mater.* **2007**, *19*, 2197; (c) Fan, W. Q.; Katrisky, A. R. In *Comprehensive Heterocyclic Chemistry II*; Katrisky, A. R., Rees, C. W., Scriven, C. W. V., Eds.; Elsevier Science: Oxford, 1996; Vol. 4, p 1; (d) Dehne, H. In *Methoden der organischen Chemie (Houben-Weyl)*; Schumann, E., Ed.; Thieme: Stuttgart, 1994; Vol. E8d, p 305.
- (a) Alvarez, S.; San, F.; Aquaro, S.; De, C.; Perno, C. F.; Karlsson, A.; Balzarini, J.; Camarasa, M. J. *J. Med. Chem.* **1994**, *37*, 4185; (b) Velazquez, S.; Alvarez, R.; Perez, C.; Gago, F.; De, C.; Balzarini, J.; Camarasa, M. J. *Antiviral Chem. Chemother.* **1998**, *9*, 481.
- 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.
- Brockunier, L. L.; Parmee, E. R.; Ok, H. O.; Candelore, M. R.; Cascieri, M. A.; Colwell, L. F.; Deng, L.; Feeney, W. P.; Forest, M. J.; Hom, G. J.; MacIntyre, D. E.; Tota, L.; Wyvratt, M. J.; Fisher, M. H.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2111.
- (a) Chandrasekhar, S.; Prakash, S. J.; Jagadeshwar, V.; Narsihmulu, Ch. *Tetrahedron Lett.* **2000**, *42*, 5561; (b) Chandrasekhar, S.; Narsihmulu, Ch.; Jagadeshwar, V. *Synlett* **2001**, *5*, 771; (c) Christensen, C.; Tornøe, C. W.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057; (d) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.
- (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596; (b) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057; (c) Wang, Q.; Chan, T. R.; Hilgraf, R.; Fokin, V. V.; Sharpless, K. B.; Finn, M. G. *J. Am. Chem. Soc.* **2003**, *125*, 3192; (d) Lober, S.; Rodriguez-Loaiza, P.; Gmeiner, P. *Org. Lett.* **2003**, *5*, 1753.
- Scriven, E. F. V.; Turnbull, K. *Chem. Rev.* **1988**, *88*, 297.
- (a) Larhed, M.; Hallberg, A. *Drug Discovery Today* **2001**, *6*, 406; (b) Suzuki, M.; Kato, N.; Motomu Kanai, M.; Shibasaki, M. *Org. Lett.* **2005**, *7*, 2527; (c) Sheng, C.; Zhang, W.; Ji, H.; Zhang, M.; Song, Y.; Xu, H.; Zhu, J.; Miao, Z.; Jiang, Q.; Yao, J.; Zhou, Y.; Zhu, J.; Jianguo, L. *J. Med. Chem.* **2006**, *49*, 2512.
- (a) Sanati, H.; Belenger, P.; Fratti, R.; Ghannoum, M. *Antimicrob. Agents Chemother.* **1997**, *41*, 2492; (b) Johnson, L. B.; Kauffman, S. L.; Ivanov, D. S.; Danchev, N. D. *Biometals* **2002**, *15*, 79; (f) Grandberg, I. I. *Chem. Heterocycl. Compd. (Engl. Transl.)* **1974**, *10*, 101; (g) Brodfuehrer, P. R.; Chen, B.-C.; Sattelberg, T. R.; Smith, P. R.; Reddy, J. P.; Stark, D. R.; Quinlan, S. L.; Reid, J. G.; Thottathil, J. K.; Wang, S. J. *J. Org. Chem.* **1997**, *62*, 9192; (h) Haubrich, D. R.; Ward, S. J.; Baizman, E.; Bell, M. R.; Bradford, J.; Ferrari, R.; Miller, M.; Perrone, M.; Pierson, A. K.; Saelens, J. K.; Luttinger, D. J. *Pharmacol. Exp. Ther.* **1990**, *255*, 511; (i) Ogata, A.; Yamada, Y.; Sugiura, M.; Takayanagi, R.; Sawada, Y.; Iga, T. *J. Pharm. Soc. Jpn.* **2001**, *121*, 793.
- (a) Praveen, C.; Sagayaraj, Y. W.; Perumal, P. T. *Tetrahedron Lett.* **2009**, *50*, 644; (b) Porter, J. K.; Bacon, C. W.; Robins, J. D.; Himmelsbach, D. S.; Higman, H. C. *J. Agric. Food Chem.* **1977**, *25*, 88; (c) Li, J.-T.; Dai, H.-G.; Xu, W.-Z.; Li, T.-S. *Ultrason. Sonochem.* **2006**, *13*, 24; (d) Bifulco, G.; Bruno, I.; Riccio, R.; Lavayre, J.; Bourdy, G. *J. Nat. Prod.* **1995**, *58*, 1254; (e) Bell, R.; Carmell, S.; Sar, N. J. *Nat. Prod.* **1994**, *57*, 1587; (f) Zhang, Z.-H.; Yin, L.; Wang, Y.-M. *Synthesis* **2005**, 1949; (g) Wang, L.; Han, J.; Tian, H.; Sheng, J.; Fan, Z.; Tang, X. *Synlett* **2005**, 337; (h) Mo, L.-P.; Ma, Z.-C.; Zhang, Z.-H. *Synth. Commun.* **2005**, *35*, 1997; (i) Bandgar, B. P.; Shaikh, K. A. *Tetrahedron Lett.* **2003**, *44*, 1959; (j) Singh, P. R.; Singh, D. U.; Samant, S. D. *Synth. Commun.* **2005**, *35*, 2133.
- Nagarajan, R.; Perumal, P. T. *Chem. Lett.* **2004**, *33*, 288.
- (a) Chen, J.; Spear, S. K.; Huddleston, J. G.; Rogers, R. D. *Green Chem.* **2005**, *7*, 64; (b) Jorapur, Y. R.; Rajagopal, G.; Saikia, P. J.; Pal, R. R. *Tetrahedron Lett.* **2008**, *49*, 1495; (c) Chandrasekhar, S.; Prakash, S. J.; Rao, C. L. *J. Org. Chem.* **2006**, *71*, 2196; (d) Heldebrandt, D. J.; Jessop, P. G. *J. Am. Chem. Soc.* **2003**, *125*, 5600; (e) Haimov, A.; Neumann, R. *Chem. Commun.* **2002**, 876; (f) Chandrasekar, S.; Narsihmulu, C.; Shameem, S. S.; Reddy, N. R. *Green Chem.* **2003**, *5*, 1716.
- (a) Sreedhar, B.; Reddy, P. S.; Kumar, N. S. *Tetrahedron Lett.* **2006**, *47*, 3055; (b) Kumaraswamy, G.; Ankamma, K.; Pichaiiah, A. *J. Org. Chem.* **2007**, *72*, 9822.
- (a) Damodiran, M.; Kumar, R. S.; Sivakumar, P. M.; Doble, M.; Perumal, P. T. *J. Chem. Sci.* **2009**, *121*, 65; (b) Sujatha, K.; Shanmugam, P.; Perumal, P. T.; Muralidharan, D.; Rajendran, M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4893; (c) Sridhar, R.; Perumal, P. T.; Etti, S.; Shanmugam, G.; Ponnuswamy, M. N.; Prabavathy, V. R.; Mathivanan, N. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 6035; (d) Sivaprasad, G.; Perumal, P. T.; Prabavathy, V. R.; Mathivanan, N. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6302; (e) Magesh, C. J.; Makesh, S. V.; Perumal, P. T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2035.
- To a mixture of indole (8.5 mmol), propargyl bromide (12.8 mmol) and 50 mol % Tetrabutylammonium bromide in 12 mL of toluene at room temperature, 12 mL of 50% NaOH was added dropwise. Stirring was continued until completion of the reaction as evidenced by TLC analysis. The products were separated by column chromatography using silica gel with petroleum ether and ethyl acetate as the eluents and characterized by ¹H, NMR, ¹³C NMR, IR spectroscopy and mass spectroscopy.
- A typical experimental procedure for synthesis of 1,4-disubstituted 1,2,3-bis-triazole for compound **3a**. N-propargylbis(indolyl)methane **1a** (0.501 mmol, 200 mg), sodium azide **2a** (1.204 mmol, 78 mg) and benzylbromide (1.104 mmol,

0.131 mL) were suspended in polyethylene glycol-400 (5 mL). To this copper iodide (10 mol %) was added and the reaction mixture was stirred for 6 h. After completion of the reaction (monitored by TLC), the product was extracted with EtOAc (3 × 15 mL) and the organic extract was dried. The crude product was subjected to column chromatography to yield the desired product that was characterized by ¹H NMR, ¹³C NMR, IR spectroscopy, mass spectroscopy and elemental analysis. Pink solid; mp 55–57 °C; *R*_f 0.46 (60% EtOAc/petroleum ether); IR (KBr): 2929, 1610, 1462, 1330 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 5.27 (AB quartet, 4H, *J* = 11.6 Hz, –triazolyl-CH₂-Ar), 5.38 (s, 4H, –indolyl-CH₂-triazolyl), 5.81 (s, 1H, –CH), 6.59 (s, 2H, –Indolyl-H), 6.92 (t, 2H, *J* = 6.9 Hz, –Ar-H), 7.04 (s, 2H, –triazolyl-H), 7.11–7.23 (m, 9H, –Ar-H), 7.28–7.33 (m, 12H, –Ar-H); ¹³C NMR (125 MHz, CDCl₃): 40.1, 42.0, 54.2, 109.6, 119.1 (2C), 120.0, 121.7, 121.8, 126.2, 127.2, 127.9 (3C), 128.0, 128.2, 128.3, 128.6, 128.7, 128.9, 129.0 (3C), 129.1 (3C), 131.2, 134.5, 136.6, 136.8, 143.7, 145.2; MS (EI) *m/z* 665 [M⁺+H⁺]; Anal. Calcd for C₄₃H₃₆N₈: C, 77.69; H, 5.46; N, 16.85. Found: C, 77.77; H, 5.43; N, 16.79. **Spectral data for compound 3b**: Pink solid; mp 95–97 °C; *R*_f 0.48 (60% EtOAc/petroleum ether); IR (KBr): 2918, 1610, 1330, 1462 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 5.28 (AB quartet, 4H, *J* = 10.4 Hz, –triazolyl-CH₂-Ar), 5.35 (s, 4H, –indolyl-CH₂-triazolyl), 5.81 (s, 1H, –CH), 6.59 (s, 2H, –indolyl-H), 6.92 (t, 2H, *J* = 7.6 Hz, –Ar-H), 7.04 (s, 2H, –triazolyl-H), 7.09 (d, 4H, *J* = 8.4 Hz, –Ar-H), 7.13 (t, 2H, *J* = 7.6 Hz, –Ar-H), 7.19–7.23 (m, 3H, –Ar-H), 7.26–7.35 (m, 10H, –Ar-H); ¹³C NMR (125 MHz, CDCl₃): 40.0, 42.0, 53.3, 109.5, 119.1 (2C), 120.0, 121.5, 121.8, 122.9, 127.7, 128.2, 128.3, 128.5, 128.7, 129.2 (3C), 129.3 (3C), 129.4, 129.5, 129.6, 132.9, 134.7, 136.6, 136.8, 143.6, 145.4; MS (EI) *m/z* 733 [M⁺+H⁺], 735 [M⁺+H⁺], 737 [M⁺+H⁺]; Anal. Calcd for C₄₃H₃₄Cl₂N₈: C, 70.39; H, 4.67; N, 15.27. Found: C, 70.51; H, 4.64; N, 15.24.

19. (a) Sreedhar, B.; Reddy, P. S.; Krishna, V. R. *Tetrahedron Lett.* **2007**, 48, 5831; (b) Chandrasekhar, S.; Basu, D.; Rambabu, Ch. *Tetrahedron Lett.* **2006**, 47, 3059; (c) Appukkuttan, P.; Dehaen, W.; Fokin, V. V.; Eycken, E. V. *Org. Lett.* **2004**, 6, 4223.
20. Bauer, A. W.; Kirby, W. M.; Sherris, J. C.; Turck, M. *Am. J. Clin. Pathol.* **1966**, 45, 493.
21. **Experimental for antibacterial activity**: 1000 mL of media was prepared using agar (2%) with beef extract (30 g), casein hydrolysate (17.5 g), soluble starch (1.5 g) and sodium hydroxide (5 g) in distilled water. The pH of the media was set to 7.4 ± 0.2 at 25 °C. The standard drug ciprofloxacin disc was placed on the media and the Whatmann No. 2 filter disc (5 mm diameter) were cut and fitted in to vials plugged with cotton. These vials were kept in hot air oven at 160 °C for 30 min for sterilization. The synthesized compounds are dissolved in DMSO solution at 100 µg/10 µL. With the help of micro pipette the test solution was added over the paper disc and dried. After drying, discs were placed over solid agar media and kept in the refrigerator for 1 h to facilitate uniform diffusion of the drug and later kept in the incubator for a period of 24 h at 37 °C. Observations were made for the zone of inhibition around the synthesized compounds and compared with standard. Each experiment was performed for three times. Observations were made as an average of three works performed.
22. **Experimental for antifungal activity**: The media was prepared using agar with glucose (<10 g), peptone (10 g) and sabouroud dextrose agar (15 g) in distilled water. The pH of the media was set to 5.5 ± 0.2 at 25 °C. The same procedure employed for the evaluation of antibacterial activities was followed for antifungal evaluation also.