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Cyclization and antimicrobial evolution of 1,2,4-triazoles by carbohydrazide

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Abstract: A sequence of dihydropyrimidine substituted 1,2,4-triazole derivatives was synthesized by cyclization of carbohydrazide by ammonium acetate and aryl aldehyde in acidic condition. The structures were accepted on the establishment of spectral tools and their concentration by elemental investigation. Every compound was preliminary considered for their *in vitro* antimicrobial behaviours against five bacterial strains *viz* [*Acinetobacter baumannii* (ATCC 19606), *Staphylococcus aureus* (MRSA; ATCC 43300), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 700603)] plus two fungi Strains *viz.* [*Cryptococcus neoformans var. grubii* (H99; ATCC 208821), *Candida albicans* (ATCC 90028)]. Out of fifteen compounds, four compounds *viz.* 3a, 3h, 3i and 3l indicated promising antifungal behaviours with no signs of human cells cytotoxic [Hk: Human Embryonic Kidney cells (ATCC CRL-1573)] and haemolytic activity [RBC (ARCBS 5400 00150)].

Keywords: 1,2,4-triazole, dihydropyrimidine, cytotoxicity, haemolysis, antimicrobial activity

Introduction

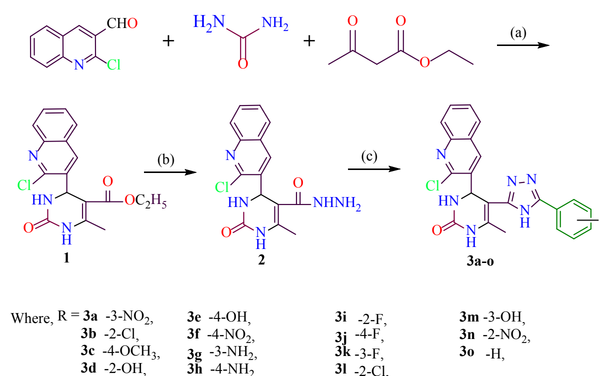
Extensive introduction of antibiotics in the 1940s, initiation with penicillin [1, 2] and streptomycin [3], transformed medicine, providing effective cures for the most prevalent diseases of the time. Resistance development limits the beneficial lifespan of antibiotics and results in the necessity for a constant introduction of new compounds [4, 5].

1,2,4-Triazole and its derivatives are an authoritative type of compounds which possess environmental [6], industrial [7, 8] and biological activities, including antimicrobial [9 - 12], antifungal [13], antitubercular [14], anticancer [15], anti-oxidant [16], anti-inflammatory [17], antiviral [18] and anticonvulsant [19]activities. An investigation of the recent literature exposed that very few published reports explain the route of cyclization of carbohydrazide to 1,2,4-triazole nucleus, (George *et al.*) [20] and (Piste *et al.*) [21]reported the synthesis of such compounds by the cyclization reaction of

an ammonium acetate under acidic condition and investigated the biological activity. Using similar methodology and different approach, we have synthesized DHPM substituted 1,2,4-triazole derivatives formed by the reaction of carbohydrazide of dihydropyrimidine and ammonium acetate under acidic condition. As outlined in **Scheme 1.** The derivatives prescribed herein were attained in good isolated yields, using simple methods which did not require further decontamination for the main products. In the extension of our previous work, we have developed ionic liquid such as Butylbenzimidazolium tetrafluoroborate. The ionic liquids have been used as a green solvent for the synthesis of dihydropyrimidine. We herein report the synthesis of fifteen novel dihydropyrimidine substituted 1,2,4-triazole derivatives furthermore their antimicrobial, haemolytic and cytotoxic behaviour were determined.

Results and discussion

New dihydropyrimidine substituted 1,2,4-triazole derivatives **(3a-o)** are prepared as per standard protocols with minor modifications as exhibited in **Scheme 1**. Biginelli reaction was transferred by solvent-free condition in presence of Ionic Liquid ([BBI][BF_4]). Hydrazinolysis of the ester in presence of hydrazine hydrate in 1,4-dioxane at 110 °C for 6 hours gave 4-(2-chloroquinolin-3-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide. Cyclization of carbohydrazide by ammonium acetate and aryl aldehydein acetic acid afford 4-(2-chloroquinolin-3-yl)-6-methyl-5-(5-(substituted phenyl)-4*H*-1,2,4-triazol-3-yl)-3,4-dihydropyrimidin-2(1*H*)-one, (3a-o) derivatives in 69-82% yield.



Reagents and conditions: (a) [BBI][BF_4], 80 °C, 24 h; (b) NH_2NH_2, 1,4-Dioxane, Conc. HCl, reflux, 6h; (c) Ar-CHO, CH_3COONH_4, CH_3COOH, reflux, 12 h.

Scheme 1. Synthetic track for the preparation of title compounds (3a-o).

Spectral techniques *viz.*^{1}H NMR, ^{13}C NMR, IR and Mass spectrometry were used for possible structure determination of newly synthesizeddihydropyrimidine substituted 1,2,4-triazole derivatives **(3a-o)**. As a representative example the ^{1}H NMR of 4-(2-chloroquinolin-3-yl)-6-methyl-5-(5-(3-nitrophenyl)-4*H*-1,2,4-triazol-3-yl)-3,4-dihydropyrimidin-2(1*H*)-one, (3a) is depicted here (**Figure 1**). The proton singlet at δ 2.37, 5.75, 8.28 and 9.38 ppm, due to presence of -CH_3 group, -C<u>H</u> of pyrimidine ring, -N<u>H</u>-C-Ph and –N<u>H</u>-C-CH_3, respectively. ten aromatic protons appeared as multiplet between δ 7.65-8.11 ppm. Appearance of characteristic peak in ^{13}C NMR spectra at δ 155.55, 49.08 and 16.30 ppm pointed out the presence of >C=O, -C<u>H</u> of pyrimidine ring, and -CH_3 group in the final compound respectively. The IR spectra of **3a** showed distinct stretching frequencies at 2885, 1620 cm^{-1} and 1024 cm^{-1} corresponding to -CH_3, >C=N and –N-N-, respectively. The absorption band at 1490 cm^{-1} showed the presence of –NO_2 stretching (**Figure 2**). Molecular ion peak at *m/z* 507.36 [M+2Na] in mass spectrometric data was in harmony with molecular weight of compound **3a**(**Figure 3**). Similarly, the structural confirmation of the remainingdihydropyrimidine substituted 1,2,4-triazole derivatives**(3a-o)** was carried out

on the basis of the above description.

Biological evaluation

Antimicrobial studies:[9, 22]

The biological evolution of synthesized compounds was assessed against varied bacterial and fungal strains by a conventional broth-dilution method. The active compounds were further screened for cytotoxicity against human embryonic kidney cell line, HEK293. The compounds were also screened for haemolysis of human blood cells. Fluconazole was used as a positive fungal inhibitor standard for fungi. Colistin was employed as positive bacterial inhibitor standards for Gram-negative and Vancomycin for Gram-positive bacteria. Melittin and tamoxifen were employed as a positive heamolytic and cytotoxicity standard, respectively. Each antibiotic standard was provided in 4 concentrations, with 2 above and 2 below its MIC value, and plated into the first 8 wells of column 23 of the 384-well NBS plates. Tamoxifen and melittin was used in 8 concentrations in 2-fold serial dilutions with 50 μg/mL highest concentration.

The quality control (QC) of the assays was determined by Z'-Factor, calculated from the Negative (media only) and Positive Controls (bacterial, fungal or cell culture without inhibitor), and the Standards. Plates with a Z'-Factor of ≥ 0.4 and Standards active at the highest and inactive at the lowest concentration, were accepted for further data analysis.

The results of these studies were represented in **Table 1**. Samples with inhibition value above 80% were classed as actives. Samples with inhibition values between 50 - 80% were classed as partial actives.In Cytotoxic and Haemolysis assay, samples were flagged as partial cytotoxic if $D_{Max} \geq 50\%$.

Single point bacterial inhibition assay

The primary bacteria panel, including *E. coli*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *S. aureus* were cultured in Cation-adjusted Muller–Hinton broth (CAMHB) at 37°C overnight. A sample of each culture was then diluted 40-fold in fresh MHB and incubated at 37°C for 1.5–3 h. The resultant mid-log phase cultures were diluted (CFU/mL measured by OD_{600}), then added to each well of the compound containing plates, giving a cell density of $5x10^5$ CFU/mL and a total volume of 50 μL. All the plates were covered and incubated at 37 °C for 18 h without shaking.

Fungi strains (*C. albicans* and *C. neoformans*) were cultured for 3 days on Yeast Extract-Peptone Dextrose (YPD) agar at 30 °C. A yeast suspension of $1x10^6$to $5x10^6$ CFU/mL (as determined by OD_{530}) was prepared from five colonies. The suspension was subsequently diluted and added to each well of the compound-containing plates giving a final cell density of fungi suspension of $2.5x10^3$ CFU/mL and a total volume of 50 μL. All plates were covered and incubated at 35 °C for 36 h without shaking.

Cytotoxicity Assay

HEK293 cells were counted manually in a Neubauer haemocytometer and then plated in the 384-well plates containing the compounds to give a density of 5000 cells/well in a final volume of 50 μL. DMEM supplemented with 10% FBS was used as growth media and the cells were incubated together with the compounds for 20 h at 37 °C in 5% CO_2.

Haemolysis Assay

Human whole blood was washed three times with 3 volumes of 0.9% NaCl and then resuspended in same to a concentration of $0.5x10^8$ cells/mL, as determined by manual

cell count in a Neubauer haemocytometer. The washed cells were then added to the 384-well compound-containing plates for a final volume of 50 µL. After a 10 min shake on a plate shaker the plates were then incubated for 1 h at 37 °C. After incubation, the plates were centrifuged at 1000g for 10 min to pellet cells and debris, 25 µL of the supernatant was then transferred to a polystyrene 384-well assay plate.

The results of the antimicrobial screening (at 32 µg/mL) revealed that generally nitrogen containing heterocycles shows antifungal activity. In which electron withdrawing group with *meta* directing compound **3a** had high activity against *Cryptococcus neoformans,* whereas its *ortho*-derivative **3n**, is partial active and *pera*-derivative **3f**, showed no comparable activity against any of the fungi. The halo derivatives **3i** and **3l** had high activity at **ortho**-position, while *pera* derivatives are slightly more active compare to *meta* derivatives which

Table 1. Percentage inhibition for compounds **3a-o** at 32µg/mL.

No.	-R	Antibacterial					Antifungal		Cytotoxicity	Haemolysis
		Sa	*Ec*	*Kp*	*Pa*	*Ab*	*Ca*	*Cn*	Hk, D_{Max}	RBC, D_{Max}
3a	-3-NO_2	-7.4; -8.7	-13.0; -4.0	1.3; 16.1	-4.2; 3.9	-9.6; 1.5	**28.8; 91.7**	**109.4; 117.8**	**25.6; 43.3**	**-1.4; -1.9**
3b	-4-Cl	-0.7; -1.7	-11.3; -8.0	15.9; 8.7	-0.7; 2.4	-6.6; 9.2	-1.2; 76.0	-86.5; -98.0	NT	NT
3c	-4-OCH_3	-2.9; 6.8	-8.1; -9.1	11.6; 12.7	5.3; 6.3	-7.3; 8.7	3.0; 4.1	25.9; 6.4	NT	NT
3d	-2-OH	-13.6; -5.8	-0.5; -3.3	16.8; 7.1	11.6; 12.7	6.2; 7.2	21.3; 21.3	-4.5; -4.8	NT	NT
3e	-4-OH	-2.3; 3.1	-2.8; -4.3	14.5; 15.2	5.0; 8.6	-0.8; 4.0	11.8; 14.0	-52.1; -86.5	NT	NT
3f	-4- NO_2	-3.7; 7.8	-2.5; -5.6	17.5; 18.0	4.2; 4.9	-3.9; 8.7	6.3; 8.3	-17.7; -60.2	NT	NT
3g	-3- NH_2	4.1; 8.0	-0.0; -7.7	13.5; 19.8	3.5; 9.8	-4.1; 10.7	0.6; 1.4	-101.5; -108.9	NT	NT
3h	-4- NH_2	6.6; 9.4	-8.0; 1.2	10.8; 20.6	10.3; 11.9	-4.7; 13.1	**16.0; 84.7**	-58.1; -66.0	**32.8; 7.9**	**-0.6; -1.7**
3i	-2-F	10.1; 12.1	-11.0; 3.6	14.3; 25.4	-2.3; 9.8	-6.4; 14.2	3.8; 4.5	**114.2; 121.3**	**31.9; 5.6**	**0.3; 3.4**
3j	-4-F	-15.2; -20.4	-13.8; -6.8	4.3; 7.2	-8.7; 4.7	-13.2; 4.8	24.1; 41.1	-111.0; -122.3	NT	NT
3k	-3-F	12.0; 8.1	-0.6; -7.5	13.1; 15.8	-0.1; 9.8	-4.8; 8.1	0.2; 9.9	-108.2; -114.0	NT	NT
3l	-2-Cl	10.5; 12.5	-6.3; 2.1	16.4; 17.0	1.7; 5.9	-3.9; 21.4	26.2; 33.0	**104.3; 111.4**	**31.4; 9.9**	**0.8; 4.4**
3m	-3-OH	11.8; 9.9	-3.0; -6.2	18.2; 20.8	11.4; 4.7	-4.0; 14.9	-0.2; -6.4	-68.0; -79.9	NT	NT
3n	-2-NO_2	30.4; 7.3	-7.0; -7.8	15.6; 16.5	7.8; 9.6	-3.5; 15.7	13.0; 6.3	24.6; 44.9	NT	NT
3o	-H	3.1; 3.7	-11.6; -9.6	13.6; 8.6	4.8; 6.6	-2.9; 22.0	-5.6; 2.6	-17.3; -17.4	NT	NT

Sa:*Staphylococcus aureus* (MRSA; ATCC 43300), *Ec*: *Escherichia coli* (ATCC 25922), *Kp*: *Klebsiella pneumoniae* (ATCC 700603), *Pa*:*Pseudomonas aeruginosa* (ATCC 27853),*Ab*: *Acinetobacter baumannii* (ATCC 19606), *Ca*: *Candida albicans* (ATCC 90028), *Cn*: *Cryptococcus neoformans* (ATCC 208821), Hk: Human Embryonic Kidney cells (ATCC CRL-1573), RBC: Human red blood cells (ARCBS 5400 00150), NT: Not Tested. Data with bold fonts are active compounds.

showed no comparable activity against any of the microbial strain. Furthermore, nitrogen containing compounds **3a** and **3h** exhibited substantial activity against *Candida albicans*. No other significant activity was observed for the other derivatives which were tested at same concentration intensities and with the uniform bacterial and fungal strains verified. Every compound which were tested verified to no noticeable haemolytic activity against human red blood cells and no cytotoxicity against the human embryonic kidney cell line, HK293.

Experimental

Material and methods:[9]

The starting ingredients were obtained from commercial chemical providers and used with or without purification as desired. TLC on silica gel plates (Merck, 60, F_{254}) was used for purity checking and reaction monitoring. Flash chromatography with silica gel (Merck, 230-400 mesh and 70-230 mesh ASTH) was useful when essential to separate and refine the reaction products. ^{1}H NMR spectra were recorded on a Bruker Advance II 400 MHz and ^{13}C NMR spectra on Varian Mercury-400, 100 MHz in DMSO as a solvent. Melting point was proven by an open capillary method on a 'Toshvin melting point' apparatus and are uncorrected. IR spectra was obtained from a Perkin-Elmer FT-IR spectrophotometer in KBr. Mass spectra were obtained from Shimadzu LC-MS 2010 spectrometer. Elemental analysis (C, H, N) was carried out by a Perkin-Elmer 2400 CHN analyser and found within ±0.4% of theoretical values.

Synthesis of ethyl 4-(2-chloroquinolin-3-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate, (1): [23]

A mixture of 2-chloroquinoline-3-carbaldehyde (0.1mol), ethyl acetoacetate (0.1 mol) and urea (0.1 mol) were heated at 80 °C using Ionic Liquid ([BBI][BF_4]) (10 ml) in a RBF. The progress of the reaction was monitored by TLC. After 24 hours. the reaction mixture was allowed to cool at room temperature. Thus, obtained pale yellow colored solid mass was filtered, washed with hot water, dried and refined by flash chromatography. Yield: 87.66%; m.p.: 292-294 °C

General procedure for synthesis of 4-(2-chloroquinolin-3-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide, (2):

In 1,4-dioxane (50 mL) solvent,compound **1** (0.05 mol) and hydrazine hydrate (85%) (0.05 mol) was added followed by the addition of a 2-3 drops of conc. HCl and stir for 6 hours with reflux. After cooling, the reaction mixture was poured into chilled water and lite yellow colored solid mass was filtered washed with hot water, dried and refined by flash chromatography to obtain compound **2**. Yield: 89.13%; m.p.: 301-303 °C

General procedure for synthesis of 4-(2-chloroquinolin-3-yl)-6-methyl-5-(5-aryl-4H-1,2,4-triazol-3-yl)-3,4-dihydropyrimidin-2(1H)-one, (3a–o):

To a solution of compound **2** (0.01 mol) and substituted aromatic aldehyde (0.01 mol) in acetic acid (50ml), a 0.2g of ammonium acetate was added and the mixture was refluxed for around 10-12 hour. The mother liquor on neutralization with ammonium hydroxide solution (15% solution of ammonia in water) gave a solid precipitate, which was filtered and refined by flash chromatography to afford pure product.

4-(2-chloroquinolin-3-yl)-6-methyl-5-(5-(3-nitrophenyl)-4H-1,2,4-triazol-3-yl)-3,4-dihydropyrimidin-2(1H)-one, (3a):

Dark yellow color powder, Yield 72%; mp 272-274 °C; IR (λ_{max}, cm^{-1}, KBr): 3205 (-N-H stretching), 3089 (-C-H- stretching, aromatic), 2958 (-C-H- stretching, H-C=C<), 2885 (-C-H stretching CH_3), 1697 (>C=O stretching), 1639 (>C=C< stretching), 1620 (>C=N- stretching), 1490 (-N=O stretching, $-NO_2$), 1301 (-C-N- stretching), 1024 (-N-N- stretching); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ = 9.38 (s, 1H, –N<u>H</u>-C-CH_3), 8.28 (s, 1H, -N<u>H</u>-C-Ph), 7.65-8.11 (m, 10H, Ar-H), 5.75 (s, 1H, CH), 2.37 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 155.55 (C, C-13), 151.41 (C, C-22), 150.86 (C, C-20), 149.29 (C, C-27), 148.36 (C, C-8), 145.19 (C, C-4), 135.86 (CH, C-10), 134.55 (CH, C-9), 132.40 (C, C-25), 132.35 (C, C-15), 131.79 (CH, C-30), 130.55 (CH, C-29), 130.10 (C, C-5), 130.01 (CH, C-2), 129.67 (CH, C-6), 127.52 (CH, C-1,3), 119.84 (CH, C-26, 28) 101.20 (C, C-16), 49.08 (CH, C-11), 16.30 (CH_3, C-19);TOF-MS (ESI+): m/z = 507.36 [M+2Na]; Anal. Calcd. For $C_{22}H_{16}ClN_7O_3$: C, 51.21; H, 3.49; N, 21.23. Found: C, 50.32; H, 3.16; N, 21.02%.

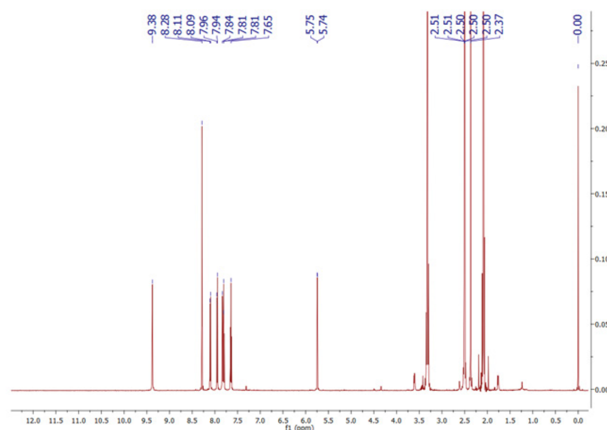


Figure 1.^{1}H NMR of the final compound, **3a**.

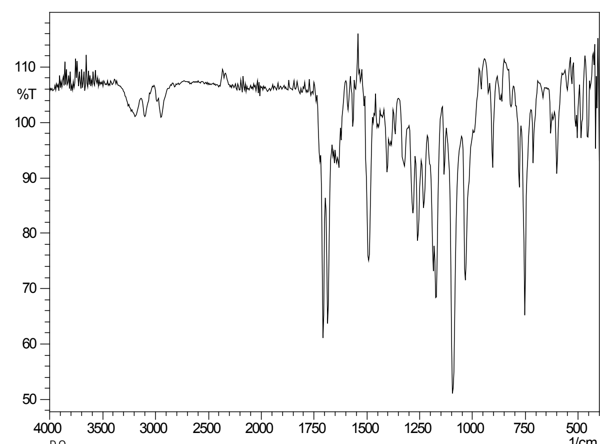


Figure 2. The IR spectrum of the final compound, **3a**.

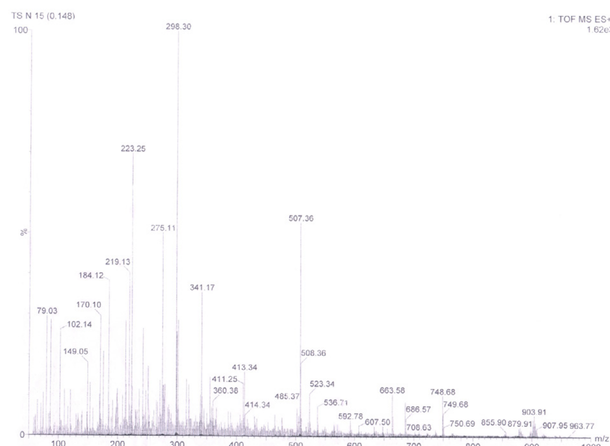


Figure 3. Mass spectra of the final compound, **3a**.

Conclusions

The cyclization of carbohydrazide to 1,2,4-triazole derivatives with dihydropyrimidine skeleton was synthesized and evaluated for their *in vitro* antimicrobial behaviours. Preliminary conclusions revealed that certain compounds displayed considerable antimicrobial activity. Compounds **3a**, **3h, 3i, 3l** and **3n** exhibited the maximum efficient inhibitory activity against *Cryptococcus neoformans* and *Candida albicans* fungi with no noticeable haemolytic activity against

human red blood cells and no cytotoxicity against the human embryonic kidney cell line, HK293. This determination might be beneficial to resist drug-resistant diseases.

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References

1. C.A. Kerantzas and W.R. Jacobs, mBio., 8(2), 2017, 1.
2. N. Kardos andA.L. Demain, Appl. Microbiol. Biotechnol, 92, 2011, 677. https://doi.org/10.1007/s00253-011-3587-6
3. H. Xiaoyan, H. Huimin, L. Liqin, S. Wenyu, L. Xiong, D. Jiandi, W. Yang and L. Xiaoquan, ACS Appl. Mater. Interfaces, 11(14) 2019, 13676.
4. B. Spellberg and D. Shlaes, Clin. Pharmacol. Ther., 96, 2014, 151. https://doi.org/10.1038/clpt.2014.106
5. K. Bush, Nature Rev. Microbiol., 9, 2011, 894. https://doi.org/10.1038/nrmicro2693
6. C. Kavakli, P.A. Kavakli and O. Güven, Rad. Phys. Chem., 94, 2014, 111. https://doi.org/10.1016/j.radphyschem.2013.07.018
7. F.A. Hassan andK.W. Younus, Res. J. Bio. Sci., 7(1), 2012, 48. https://doi.org/10.3923/rjbsci.2012.48.51
8. S. Sripriya1, C. Subha and A. Selvaraj, IOSR-JAC, 6(2), 2013, 25. https://doi.org/10.9790/5736-0622529
9. D.R. Godhani, V.B. Mulani, J.P. Mehta and N.B. Kukadiya, WSN, 100, 2018, 51.
10. D.R. Godhani, A.A. Jogel, A.M. Sanghani and J.P. Mehta, Indian J. of Chem., 54B, 2015, 556.
11. D.R. Godhani, D.B. Sanja, A.M. Sanghani and P.B. Dobariya, Indian J. Heterocycl. Chem., 22(3), 2013, 249.
12. D.R. Godhani, D.B. Sanja and A.M. Sanghani,J. Chem. Pharm. Res., 5(1), 2013, 240.
13. D.R. Godhani, D.B. Sanja, A.M. Sanghani and P.B. Dobariya, Indian J. Heterocycl. Chem., 20(4), 2011, 411.
14. D.R. Godhani, A.A. Jogel, A.M. Sanghani andJ.P. Mehta, Journal of Antimicrobials Photon., 130, 2015, 391.
15. Y.P. Hou,J. Sun, Z.H. Pang, P.C. Lv, D.D. Li, L. Yan, H.Z. Zhang, E.X. Zheng, J. Zhao andH.L. Zhu, Bioorg. Med. Chem., 19, 2011, 5948. https://doi.org/10.1016/j.bmc.2011.08.063
16. M. Hanif, M. Saleem, M.T. Hussain, N.H. Rama, S. Zaib, M.A. Aslam, P.G. Jones and J. Iqbal, J. Braz. Chem. Soc., 23(5), 2012, 854. https://doi.org/10.1590/S0103-50532012000500010
17. M.W. Akhter, M.Z. Hassan andM. Amir, Arab. J. Chem., 7(6), 2014, 955. https://doi.org/10.1016/j.arabjc.2014.05.036
18. V.K. Pandey, Z. Tusi, S. Tusi andM. Joshi, ISRN Org. Chem., 2012, 2012, 1. https://doi.org/10.5402/2012/760517
19. S. Jess, S. Kildea, A. Moody,G. Rennick, A.K. Murchie andL.R. Cooke,Pest. Mang. Sci., 70(11), 2014, 1646. https://doi.org/10.1002/ps.3801
20. S. George, R. Chakraborty, M. Parameswaran, A. Rajan and T.K. Ravi, J. Heterocyclic Chem., 52, 2015, 211. https://doi.org/10.1002/jhet.2029
21. P.B. Piste andS.P. Waghamale, Int. J. Pharm. Sci. Rev. Res., 22(2), 2013, 46.
22. M.A. Blaskovich, Z. Zuegg, A.G. Elliott andM.A. Cooper,ACS Infect. Dis., 1, 2015, 285. https://doi.org/10.1021/acsinfecdis.5b00044
23. V.H. Kariya,V.B. Mulani and M.M. Savant, Res. J. Chem. Sci., 7(2), 2017, 63.