ISSN 0975-2366
DOI: https://doi.org/10.31838/ijpr/2021.13.02.218

Research Article

Synthesis and Evaluation of Antimicrobial and Antioxidant Activities of Some Phenylhydrazones Derived from 4-(4'-hydrazinylphenyl)-4*H*-1,2,4-Triazole

NARAYANAN RAVISANKAR[1*] AND K E POORNI[2]
[1]Department of Chemistry, School of Science and Humanities, Vel Tech Rangarajan Dr. Sagunthala R&D Institute of Science and Technology, Chennai, Tamilnadu, India.
[2]Department of Biochemistry, Vivekanandha College of Arts and Sciences for Women (Autonomous), Elayampalayam, Tiruchengode, Tamilnadu, India.
*Corresponding Author
Email ID: ravisankarnarayanan2473@gmail.com
Received: 09.11.20, Revised: 16.12.20, Accepted: 28.01.21

ABSTRACT
Hydrazones are the most widely used organic compounds. They are used as pigments, dyes, catalysts, intermediates in organic synthesis and polymer stabilizers. Hydrazones have been shown to exhibit a broad range of biological activities including antimicrobial, anti–hypertensive, anti–inflammatory, analgesics, anti–tubercular, anti–tumour, anti–material, antioxidant, antiviral and also used as quantitative analytical reagents, especially in colorimetric and fluorimetric determination of metal ions. The present work is based on the above hydrazones derivative form heterocyclic substituted phenylhydrazine. The structures of the synthesized compounds were characterized by elemental analysis and IR, ^{1}H and ^{13}C NMR, Mass spectral studies. All the newly synthesized compounds were screened to antimicrobial and antioxidant activity.

Keywords: Phenylhydrazones, Antimicrobial and Antioxidant.

INTRODUCTION

Hydrazones tend to form an important group of bioactive drug molecules [1-3], which due to their wide range of pharmacological properties has got the attention of medical chemists. Many researchers derived drug-based compounds capable of fighting pathogens with minimal toxicity and complete efficacy. Such various studies have successfully developed new biologically active compounds. In that order hydrazones are organic molecule that is used in the medicine field. Therefore several researchers have synthesis of these compounds and evaluated their biological functions. Hydrozones are obtained from hydrazine or substitute hydrazine with aldehydes and ketones. These are imine compounds with a carbon-nitrogen double bond. Hydrozones are widely used in pigments [4], dyes [5], intermediates in organic synthesis [6] and polymer stabilizers [7]. Hydrazones have been shown to exhibit a broad range of biological activities including antibacterial [8], antifungal [9], antiviral [10], antimicrobial [11], antioxidant [12], antiinflammatory [13], trypanocidal [14], toxoplasma gondii [15], antihypertensive [16], analgesic [17], antiplatelet [18], antidepressant [19], antitubercular [20], antimalarial [21], antimycobacterial [22], antiamoebic [23] and anticancer [24] activities. Hydrazones were also used as transportation devices in organic layer photoconductors as quantitative analytical reagents, particularly in the colorimetric and fluorimetric determination of metal ions [25, 26]. The present work is based on the above pharmaceutical importance of synthesis of heterocyclic phenylhydrazone derivative.

EXPERIMENTAL METHODS

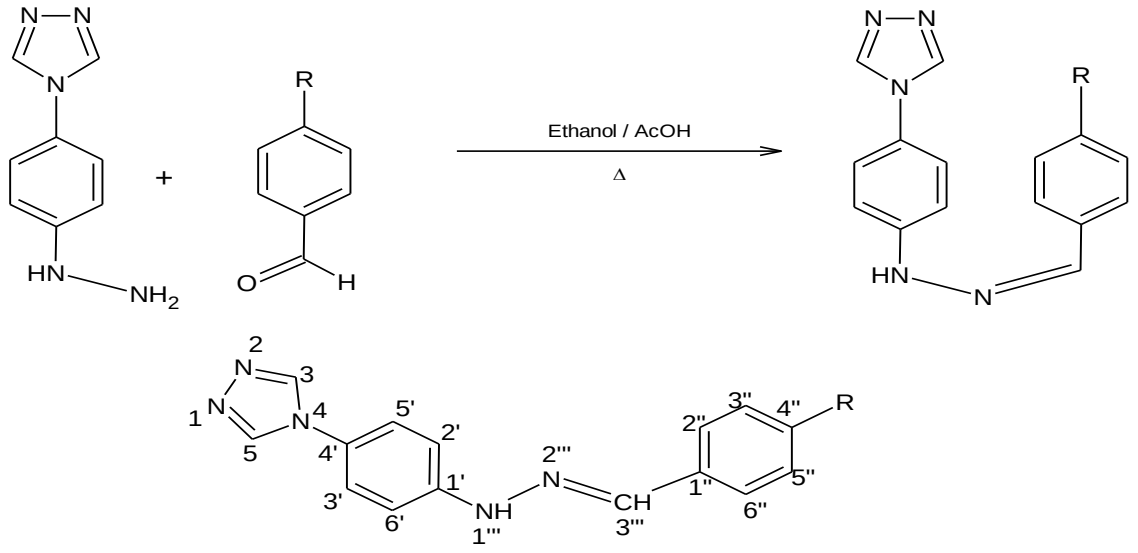
Materials

All chemicals were obtained in analytical quality and used without purification. The melting points were detected and not uncorrected. The purity of the compounds was confirmed by a thin layer chromatography using silica gel glass plates and suitable solvents. The IR spectra were recorded with the help of a *Shimadzu* spectrometer. The *Bruker* NMR Spectrometer was used to analysis the proton and carbon environments, using the TMS as the internal standard and the DMSO-d_6 for solvent. The Kirby-Bauer disc diffusion method was used to determine antimicrobial activity. Antioxidant activity was measured by DPPH radical method.

General procedure for synthesis of 1,2,4-triazole-4-substituted-phenylhydrazones

The reaction mixture of 4-(4'-hydrazinylphenyl)-4*H*-1,2,4-triazole (0.01mole) and 4-substituted benzaldehyde (0.01mole) was dissolved in 20 ml ethanol with add a few drops of acetic acid. This reaction mixture was heated under condensation conditions. The completion of reaction and formation of product was observed by TLC method. After that product was cooled under room temperature for 24 hours and was collected by filtration. The purity of the compounds was checked by recrystallization using chloroform-ethanol. All the compounds were synthesized (Scheme 1) using the same procedure.

Scheme 1: Synthesis of 1,2,4-Triazole-4-substitutedphenylhydrazones



PHYSICAL AND SPECTRAL CHARACTERIZATION OF SYNTHESIZED COMPOUND

a) 4-(4'-[2'''-Benzylidenehydrazinyl]phenyl)-4H-1,2,4-triazole (PH01)

m.p°c: 199^{0}C; IR (KBr, ν_{max}, cm^{-1}): 3265 (N-H str), 3064 (Aromatic C-H str), 2965 (Aliphatic C-H str),1631 (Aromatic C-C str), 1545 (C=N str) and 1505 (Aliphatic C-H str); ^{1}H NMR (DMSO-d_6 δ, ppm): 11.391 (s,1H, NH) , 9.593 (s, 1H, C3'''–H), 8.258 (s, 2H, C3&C5–H), 7.701 (d, 2H, C3'&C5' -H), 7.622 – 7.294 (m, 5H, C2'''–C6'''-H), and 7.118 (d, 2H, C2'&C6'-H); ^{13}C NMR (DMSO-d_6, δ, ppm): 181.44 (C3'''), 146.23 (C3&C5), 144.16 (C1'), 128.74 (C4''), 135.06(C1''), 131.85 (C4'), 127.03(C2''&C6''), 129.07(C3''&C5''), 124.61(C3'&C5') and 117.44(C2'&C6'); m/z 263 [M$^+$]; Anal. Found: C, 68.42; H, 4.98; N, 23.52; (%). Calc. for ($C_{15}H_{13}N_5$): C, 68.44; H, 4.92; N, 26.61.

b) 4-(4'-[2'''-(4''-Chlorobenzylidene)hydrazinyl]phenyl)-4H-1,2,4-triazole (PH02)

m.p°c: 221^{0}C; IR (KBr, ν_{max}, cm^{-1}): 3445 (N-H str), 2976 (Aromatic C-H str), 2976 (Aliphatic C-H str), 1639 (Aromatic C-C str), 1519 (C=N str) and 1456 (Aliphatic C-H str); ^{1}H NMR (DMSO-d_6 δ, ppm): 11.404 (s,1H, NH) , 9.594 (s, 1H, C3'''–H), 8.263 (s, 2H, C3&C5–H), 7.702 (d, 2H, C3''&C5'' -H), 7.560 (d, 2H, C3'&C5'-H), 7.124 (d, 2H, C2'&C6'-H) and 7.128 (d, 2H, C2''&C6''-H); ^{13}C NMR (DMSO-d_6, δ, ppm): 181.48 (C3'''), 146.25 (C3&C5), 144.16 (C1'), 135.13 (C4''), 134.12 (C1''), 131.85 (C4'), 130.06 (C2''&C6''), 129.41 (C3''&C5''), 124.64 (C3'&C5'), and 117.46 (C2'&C6'); m/z 297 [M$^+$]; Anal. Found: C, 60.51; H, 4.06; N, 23.52; (%). Calc. for ($C_{15}H_{12}ClN_5$): C, 60.51; H, 4.03; N, 23.53.

c) 4-(4'-[2'''-(4''-Bromobenzylidene)hydrazinyl]phenyl)-4H-1,2,4-triazole (PH03)

m.p°c: 203^{0}C; IR (KBr, ν_{max}, cm^{-1}): 3440 (N-H str), 3057 (Aromatic C-H str), 2969 (Aliphatic C-H str), 1592 (Aromatic C-C str), 1551 (C=N str) and 1495 (Aliphatic C-H str); ^{1}H NMR (DMSO-d_6 δ, ppm): 11.399 (s,1H, NH) , 9.498 (s, 1H, C3'''–H), 8.204 (s, 2H, C3&C5–H), 7.3 (d, 2H, C3''&C5'' -H), 7.497 (d, 2H, C2''&C6''-H), 7.62 (d, 2H, C3'&C5'-H), 7.097 (d, 2H, C2'&C6'-H); ^{13}C NMR (DMSO-d_6, δ, ppm): 181.58 (C3'''), 146.47 (C3&C5), 144.16 (C1'), 135.55 (C1''), 132.49 (C3''&C5''), 131.85 (C4'), 129.33 (C2''&C6''),

124.60 (C3'&C5'), 123.99 (C4'') and 117.44 (C2'&C6'); m/z 341 [M^+]; Anal. Found: C, 52.65; H, 3.53; N, 20.47; (%). Calc. for ($C_{15}H_{12}BrN_5$): C, 52.64; H, 3.51; N, 20.47.

d) 4-(4'-[2'''-(4''-Nitrobenzylidene)hydrazinyl]phenyl)-4H-1,2,4-triazole **(PH04)**

m.p°c: 223^{0}C; IR (KBr, ν_{max}, cm^{-1}): 3407 (N-H str), 3055 (Aromatic C-H str), 2945 (Aliphatic C-H str),1616 (Aromatic C-C str), 1543 (C=N str) and 1497 (Aliphatic C-H str); ^{1}H NMR (DMSO-d_6 δ, ppm): 11.412 (s,1H, NH) , 9.509 (s, 1H, C3'''–H), 8.261 (s, 2H, C3&C5–H), 8.122 (d, 2H, C3''&C5'' -H), 7.861 (d, 2H, C2''&C6''-H), 7.850 (d, 2H, C3' & C5'-H), 7.151 (d, 2H, C2' & C6'-H); ^{13}C NMR (DMSO-d_6, δ, ppm): 181.5 (C3'''), 149.8 (C4''), 144.4 (C3&C5), 143.1 (C1'), 139.3 (C1''), 131.8 (C4'), 127.95 (C2''&C6''), 124.6 (C3'&C5'), 124.4 (C3''&C5'') and 117.4 (C2'&C6'); m/z 308 [M^+]; Anal. Found: C, 58.44; H, 3.92; Cl, 13.91; N, 27.26; (%). Calc. for ($C_{15}H_{12}N_6O_2$): C, 58.44; H, 3.89; N, 27.27.

Antimicrobial activity

All synthesized compounds were screened for antibacterial and fungal activity by the Kirby-Bauer disc diffusion method [27]. Two gram positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus* and two gram negative bacteria such as *Salmonella typhi* and *Escherichia coli* were used. *Ciprofloxacin* was used as the reference antibacterial drug. For antifungal assay *Candida albicans* was used to test the activity of the compounds. *Fluconazole* was kept as the standard drug. The inhibition zone of synthesized compounds was compared with standard drugs. The results of the zone of inhibition for the antimicrobial activity of the synthesized compounds are given in Table 1 and Graph 1.

Antioxidant activity

Antioxidant activity was measured by DPPH radicals [28] using UV-Vis spectrophotometric methods. The principle of the DPPH method is based on colourizations. DPPH shows the colour change by accepting an electron or hydrogen ions and converted into a stable diamagnetic molecule. The DPPH in deep violet colour, shows absorption at 517nm due to unpaired electrons. After the completion of the reaction, solution turns yellow due to the formation of diphenylpicrylhydrazine. The solutions of synthesized hydrazones will be prepared in methanol at concentrations of between 20 to100 µg/ml. The 2ml test samples of synthesized hydrazones are blended with 2 ml of DPPH (0.1 mM) solution and were continuous stirring then incubated at 37°C for 30 min. The absorbance of test solutions and standards were measured by uv-vis spectrophotometer at 517 nm. AA and BHA were used as standards compounds in this method. The blank test (without samples) was run by equi-volume mixer (2ml) of DPPH and Methanol solution. The following formula was used to calculate the percentage of inhibition. Inhibition (%) = (blank OD– sample OD/blank OD)×100. The obtained results are illustrated in Table 2.

RESULT AND DISCUSSION

The synthesized compounds were confirmed by physical parameter of melting point and structure of the synthesized compound has been characterized based on IR, NMR, Mass spectral data and CHN analysis. The antimicrobial activities of synthesized compounds of zone of inhibitions are presented in the table 1. Antioxidant activity was measured by DPPH radicals method and results are illustrated in Table 2. The final compounds were purified by recrystallization with chloroform-ethanol. The compounds were confirmed on the basis of their physical and spectral data. The spectral study of compound PH01 is described as an example, the IR spectrum of the compound PH01 showed NH absorption band at 3265 cm^{-1}, aromatic C-H and aliphatic C- H stretching observed at 3064 and 2965 cm^{-1} respectively, an absorption band observed at 1545 cm^{-1} due to C=N stretching. ^{1}H NMR (DMSO –d_6) showed a singlet at δ 11.39 ppm assignable to NH proton. Two doublets at δ 7.70 ppm and δ 7.11 ppm each for two protons are assignable to H-3', H-5' and H-2', H-6' respectively. A singlet at δ 9.59 ppm for one proton is due to –CH=N (H-3''') and a singlet at δ 8.25 ppm for two protons is assigned to triazole ring (H-3 & H–5). The ^{13}C NMR spectrum of compound PH01, the signal of the triazole carbons of C3 & C5 are observed at δ146.23ppm and the carbon of C=N showed at δ181.44ppm. The aromatic carbons C1', C4'', C1'', C4', [C2''& C6''], [C3''& C5''], [C3' & C5'] and [C2'&C6'] appeared at δ 144.16, 128.74, 135.06, 131.85, 127.03, 129.07, 124.61and 117.44 ppm respectively. Mass spectrum showed a peak at m/z 263 [M^+]. Hence, the above spectral data are compatible with the structure of desired product, 4-(4'-[2'''-Benzylidenehydrazinyl]phenyl)-4*H*-1,2,4-triazole. All synthesized compounds were screened for *in vitro* antimicrobial activity by the Kirby-Bauer disc diffusion method. The inhibition zone was compared with standards. The results of the antibacterial and antifungal activity are given in table 1 and Graph 1. The newly synthesized compounds showed significant activity against selected bacteria. High antimicrobial activity was observed in the PH02 compared to other

compounds due to the chloro-substitution of the derivative. The results of antioxidant activity of synthesized compounds at different concentrations are shown in Table 2. The calculated IC_{50} values are given in table 2 and Graph 2. The most active compound among the synthesized compounds is PH04, which gave an IC_{50} value of 22.17μg/ml, while AA and BHA gave 7.36 and 5.38 μg/ml respectively.

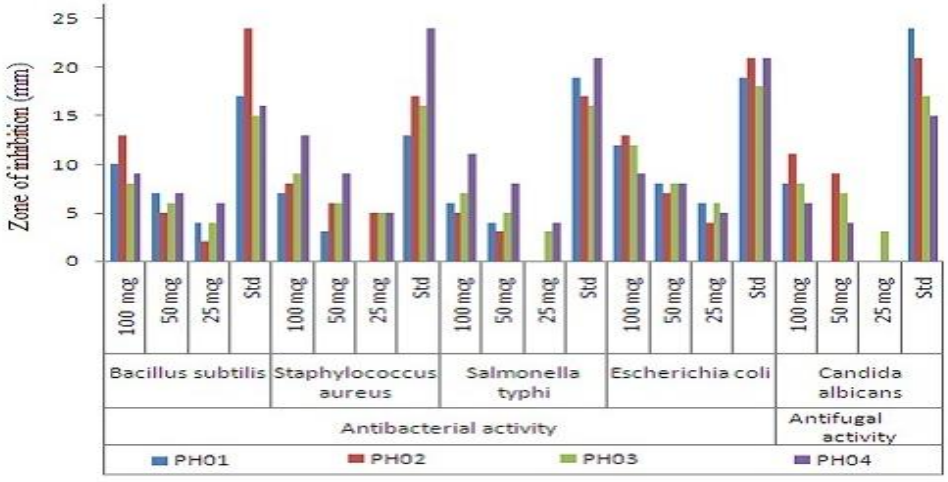
Table 1: Antimicrobial activity of the synthesized compounds

Sample code	Zone of inhibition (mm) of synthesized compounds																			
	Antibacterial activity																Antifugal activity			
	Bacillus subtilis				*Staphylococcus aureus*				*Salmonella typhi*				*Escherichia coli*				*Candida albicans*			
	100 mcg	50 mcg	25 mcg	Std	100 mcg	50 mcg	25 mcg	Std	100 mcg	50 mcg	25 mcg	Std	100 mcg	50 mcg	25 mcg	Std	100 mcg	50 mcg	25 mcg	Std
PH01	10	7	4	17	7	3	-	13	6	4	-	19	12	8	6	19	8	-	-	24
PH02	13	5	2	24	8	6	5	17	5	3	-	17	13	7	4	21	11	9	-	21
PH03	8	6	4	15	9	6	5	16	7	5	3	16	12	8	6	18	8	7	3	17
PH04	9	7	6	16	13	9	5	24	11	8	4	21	9	8	5	21	6	4	-	15

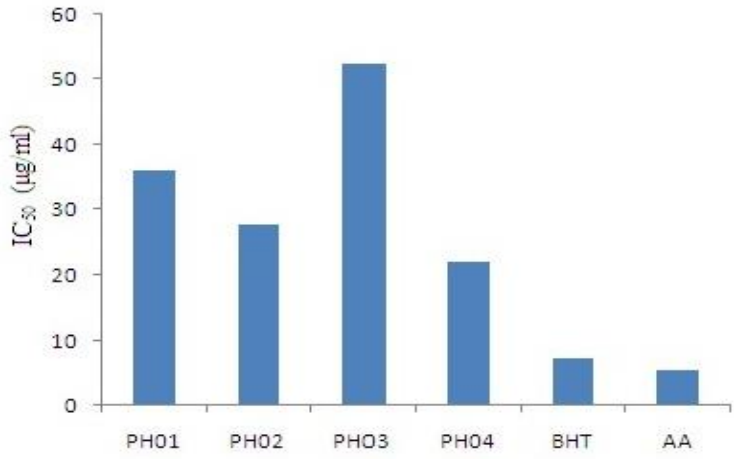
Table 2: Antioxidant activity of synthesized compounds

Compound	Concentration (μg/ml)					IC_{50} (μg/ml)*
	20	40	60	80	100	
PH01	75.34	71.29	69.84	65.49	59.23	35.94
PH02	77.35	74.76	69.47	66.15	59.47	27.69
PH03	80.17	77.6	73.47	68.92	64.19	52.56
PH04	79.58	72.48	67.25	55.8	48.86	22.17
BHT	94.01	81.73	71.47	64.45	57.45	7.36
AA	98.16	88.13	77.52	65.5	58.89	5.38
*Average of three independent determinations						

Graph 1: Antimicrobial activity of the synthesized compounds



Graph 2: Antioxidant activity of synthesized compounds



CONCLUSION

A serious of substituted phenyl hydrazones has been synthesized and the elemental and spectral analysis confirmed the structures of the compounds. Newly synthesized compounds exhibited significant antimicrobial activity against selected bacteria and fungi. Finally the compounds of 4-(4'-[2'''-(4''-Chlorobenzylidene)hydrazinyl]phenyl)-4*H*-1,2,4-triazole and 4-(4'-[2'''-(4''-Nitrobenzylidene)hydrazinyl]phenyl)-4*H*-1,2,4-triazole were observed to have good antimicrobial and high antioxidant activity.

REFERENCES

1. Seleem HS, El-Inany GA, El-Shetary BA, Mousa MA, The ligational behavior of a phenolic quinolyl hydrazone towards copper(II)- ions, BMC Chemistry, 2011; 5(1): 2.
2. Pham VH, Phan TPD, Phan DC, Vu BD, Synthesis and Bioactivity of Hydrazide-Hydrazones with the 1-Adamantyl-Carbonyl Moiety, Molecules, 2019; 24(21): 4000.
3. Ali MR, Marella A, Alam MT, Naz R, Akhter M, Shaquiquzzaman M, et al., Review of Biological activities of Hydrazones, Indonesian Journal of Pharmacy, 2012; 23(4): 193-202.
4. Shibata KY, Jin HM, Azo or Hydrazone Structure in Some Hydrogen-Bonded Azo Pigments, Journal of Imaging Science and Technology, 2011; 55 (3): 305081-305085.
5. Al-Sheikh M, Hanadi Y, Medrasi, Sadek KU, Mekheimer RA, Synthesis and Spectroscopic Properties of New Azo Dyes Derived from 3-Ethylthio-5-cyanomethyl-4-phenyl-1,2,4-triazole, Molecules, 2014; 19(3): 2993–3003.
6. Nataliya P, Belskaya, Dehaen W, Bakuleva VA, Synthesis and properties of hydrazones bearing amide, thioamide and amidine functions, ARKIVOC, 2010; (i):275-332.
7. Jeong MJ, Kim BJ, CHANG JY, Synthesis and Characterization of Soluble Main-Chain Hydrazone Polymers, Journal of Polymer Science Part A: Polymer Chemistry, 2002; 40:4493- 4497.
8. Govindasami T, Pandey A, Palanivelu N, Pandey A, Synthesis, Characterization and Antibacterial Activity of Biologically Important Vanillin Related Hydrazone Derivatives, International Journal of Organic Chemistry, 2011; 1:71-77.
9. Lonclea C, Brunela JM, Vidala N, Dherbomez M, Letourneuxa Y, Synthesis and antifungal activity of cholesterol-hydrazone derivatives, European Journal of Medicinal Chemistry, 2004; 39(12): 1067-1071.
10. Khattab RR, Hassan AA, Kutkat OM, Abuzeid KM, Hassan NA, Synthesis and Antiviral Activity of Novel Thieno[2,3-d]pyrimidine Hydrazones and Their C-Nucleosides, Russian Journal of General Chemistry, 2019; 89: 1707–1717.
11. Popiołek Ł, Biernasiuk A, Synthesis and investigation of antimicrobial activities of nitrofurazone analogues containing hydrazide-hydrazone moiety, Saudi Pharmaceutical Journal, 2017; 25(7), 1097-1102.
12. Giziroglu E, Sarikurkcu C, Sarac N, Synthesis and Characterization of Novel Hydrazone Based Antimutagenic and Antioxidative Agents, Journal of Applied Pharmaceutical Science, 2015; 5(3): 48-55.
13. Hernandez P, Cabrera M, Lavaggi M L et al., Discovery of new orally effective analgesic and anti-inflammatory hybrid furoxanyl N-acylhydrazone derivatives, Bioorganic & Medicinal Chemistry, 2012; 20(6): 2158–2171.
14. Glinma B, Gbaguidi FA, Kassehin UC, Kpoviessi DS, Houngbeme A, Houngue HD, et al., Synthesis and trypanocidal activity of salicylhydrazones and p-tosylhydrazones of S-(+)-carvone and arylketones on African trypanosomiasis, Journal of Applied Pharmaceutical Science, 2015; 5(06):001-007.

15. Sanford AG, Schulze TT, Potluri LP, Watson GF, Darner EB, Zach SJ, Derivatives of a benzoquinone acyl hydrazone with activity against Toxoplasma gondii, International Journal for Parasitology – Drugs and Drug Resistance, 2018; 8(3): 488-492.
16. Emilsson H, Selander H, Synthesis and antihypertensive activity of substituted (2,6-dichlorobenzylideneamino) guanidines and some hydrazone derivatives of C-cyanoformamidrazone, Acta pharmaceutica Suecica, 1988; 25(2): 75-86.
17. Lima PC, Lima LM, Da Silva KCM, et al., Synthesis and analgesic activity of novel N-acylarylhydrazones and isosters, derived from natural safrole, European Journal of Medicinal Chemistry, 2000; 35(2):187-203.
18. Tehrani KHME, Zadeh ME, Mashayekhi V, Hashemi M, Kobarfard F, Gharebaghi F, et al., Synthesis, Antiplatelet Activity and Cytotoxicity Assessment of Indole-Based Hydrazone, Derivatives, Iranian Journal of Pharmaceutical Research, 2015; 14(4): 1077-1086.
19. Mohareb RM, El-Sharkawy KA, Hussein MM, El-Sehrawi H. M. Synthesis of hydrazide-hydrazone derivatives and their evaluation of antidepressant, sedative and analgesic agents, Journal of Pharmaceutical Sciences and Research, 2010; 2(4), 185-196.
20. Joshi SD, Kumar D, Dixit SR, Tigadi N, More UA, Lherbet C, et al., Synthesis, characterization and antitubercular activities of novel pyrrolyl hydrazones and their Cu-complexes, European Journal of Medicinal Chemistry, 2016; 121(4): 21-39.
21. Sarkar S, Siddiqui AA, Saha SJ, De R, Mazumder S, Banerjee C, Mohd S, Antimalarial activity of small-molecule benzothiazole hydrazones, Antimicrobial Agents and Chemotherapy, 2016; 60: 4217-4228.
22. Rohane SH, Chauhan AJ, Fuloria NK, Fuloria S, Synthesis and in vitro antimycobacterial potential of novel hydrazones of eugenol, Arabian Journal of Chemistry, 2020; 13: 4495-4504.
23. F. Hayat, A. Salahuddin, J. Zargan, and A. Azam, "Synthesis, characterization, antiamoebic activity and cytotoxicity of novel 2-(quinolin-8-yloxy) acetohydrazones and their cyclized products (1,2,3-thiadiazole and 1,2,3-selenadiazole derivatives)," European Journal of Medicinal Chemistry, 2010; 45(12): 6127-6134.
24. Çkla P, Küçükgüzel ÛG, Küçükgüzel Ú, Rollas S, Clercq ED, Pannecouque C, et al., Synthesis and evaluation of antiviral, antitubercular and anticancer activities of some novel thioureas derived from 4-aminobenzohydrazide hydrazones, Marmara Pharmaceutical Journal, 2010; 14:13-20.
25. El-Sherif AA. Synthesis, spectroscopic characterization and biological activity on newly synthesized copper(II) and nickel(II) complexes incorporating bidentate oxygen–nitrogen hydrazone ligands, Inorganica Chimica Acta, 2009; 362: 4991–5000.
26. Gamil A, Al-Hazmi, El-Asmy AA, Synthesis, spectroscopy and thermal analysis of copper (II) hydrazone complexes, Journal of Coordination Chemistry, 2009; 62 (2): 337-345.
27. Hussain AZ., Meeran MN. Sankar A, Synthesis, characterization and antimicrobial activity of spiro-4-thiazolidione derivatives from 5-substituted indole-2,3-dione, Der Pharma Chemica, 2016; 8(2): 292–296.
28. Meeran MN, Hussain AZ, Synthesis, Characterization and DPPH Scavenging Assay of Isatin Related Spiroheterocyclic Compounds. Indian Journal of Pharmaceutical Sciences. 2017; 79(4): 641–645.