Chemistry Africa (2019) 2:15–20
https://doi.org/10.1007/s42250-018-00034-x

SHORT COMMUNICATION

Synthesis, Cytotoxicity and Antimicrobial Evaluation of Some New 2-Aryl,5-Substituted 1,3,4-Oxadiazoles and 1,3,4-Thiadiazoles

Baby Ramana Mutchu[1] · Vijay Kotra[2] · Surendranatha Reddy Onteddu[1] · S. N. Murthy Boddapati[1] · Hari Babu Bollikolla[1]

Received: 11 September 2018 / Accepted: 1 December 2018 / Published online: 6 December 2018

Abstract

The synthesis of 2-(5-methyl-2-nitrophenyl)-5-(substituted)-1,3,4-oxadiazoles (**5a–e**) and thiadiazoles (**6a–e**) was carried out by refluxing of *N*-(5-methyl-2-nitrobenzoyl)-2-substituted carbohydrazide with phosphorous oxychloride and Lawesson's reagent respectively. The *N*-(5-methyl-2-nitrobenzoyl)-2-substituted carbohydrazides was synthesized by treating 2(5-methyl-2-nitrobenzohydrazide) with various aryl acids in the presence of HATU and DIPEA. Compounds **6a**, **5c** and **5d** exhibited significant cytotoxic properties when compared to standard podophyllotoxin. Further, the compounds were also studied for their antibacterial activities.

Keywords 1,3,4-Oxadiazoles · 1,3,4-Thiadiazoles · Cytotoxic activity · Antibacterial activity · Lawesson's reagent

1 Introduction

The oxadiazole nucleus has emerged as one of the potential pharmacophores in medicinal chemistry [1–4]. Substituted oxadiazoles and their analogs have also been used as precursors for synthesis of various biologically active molecules [5–7], and also showed various activities such as antibacterial [8–10], antimycobacterial [11], antifungal [12, 13], antiparasitic [14], anti-inflammatory [15], anti-allergy [16, 17], analgesic [18], anticonvulsant [19, 20], antihypoglycemic [21], insecticidal [22], anticancer properties [23, 24]. Moreover, the oxadiazoles have also been utilized as bioisosteres (surrogates) for carboxylic acids, esters and carboxamides [25]. The reviews [26–29] on 1,3,4-thiadiazoles and 2-amino-1,3,4-thiadiazoles clearly indicated that the 1,3,4-thiadiazoles nucleus is similarly important as 1,3,4-oxadiazoles. Some of the prominent clinical drugs having 1,3,4-oxadiazole and thiadiazole units are shown in Fig. 1. Among various diseases cancer is a major health problem worldwide today. Improvements in treatment and prevention have lead to a decrease in cancer deaths, but the number of new diagnoses continues to rise. Chemotherapy is one of the most commonly used treatment options for cancer. Thus, it is urgent to develop novel chemotherapeutic agents for the treatment of cancer.

The recent methodologies for preparation of 2,5-disubstituted-1,3,4-oxadiazoles include oxidative cyclization of aroyl/acyl hydrazones using catalytic Fe(III)/TEMPO [30], IBX/TEAB [31], catalytic $Cu(OTf)_2$ [32], and stoichiometric molecular I_2 [33]; cyclization of acylhydrazines using PPA or $BF_3{\cdot}OEt_2$ and others [34–36]. The one-pot methodologies include synthesis from aryltetrazoles using DTBP/DCE, by dehydrative cyclization of ethyl carbazate and *N*-acylbenzotriazoles using Ph_3P-I_2 [37], and three-component reaction of isocyaniminotriphenyl-phosphorane, aldehyde, and benzoic acid under mild conditions [38]. Moreover, the valuable 2-amino-5-substituted oxadiazoles were generated through I_2-mediated oxidative C–O/C–S bond formation of semicarbazones [39], eosin Y catalyzed oxidative heterocyclization of semicarbazones under visible-light photoredox catalysis using CBr_4 as a bromine source, oxidative annulation of *N*-acyl hydrazines [40], tosyl chloride/pyridine-mediated cyclization of thiosemicarbazides [41].

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s42250-018-00034-x) contains supplementary material, which is available to authorized users.

✉ Hari Babu Bollikolla
dr.b.haribabu@gmail.com

[1] Department of Chemistry, Acharya Nagarjuna University, Nagarjunanagar, Guntur, Andhra Pradesh, India

[2] Department of Pharmaceutical chemistry, Quest International University, Plaza The Teng, Perak Darul Ridzuan, 30250 Ipoh, Malaysia

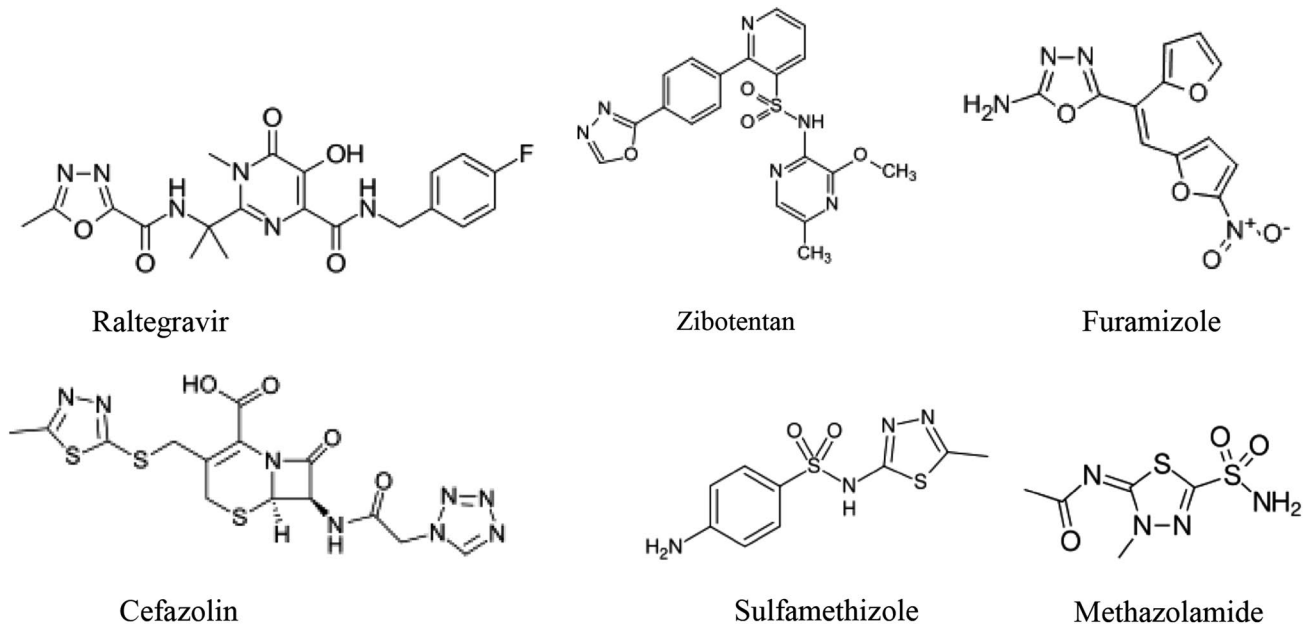


Fig. 1 Drugs with 1,3,4-oxadiazole and thiadiazole nucleus

Further, the methods which provide the 1,3,4-oxadiazole and thiadiazole nucleus from aryolthiosemicarbazide are oxidative desulfurization in the presence of iodobenzene/oxone [42] and EDC·HCl in DMSO or *p*-TsCl, triethylamine in *N*-methyl-2-pyrrolidone [43]. So, owing to the vast biological importance of 2,5-disubstituted-1,3,4-oxadiazoles [3, 9], 2,5-disubstituted-1,3,4-thiadiazoles [44–47], and in continuation to our research on oxadiazoles [48, 49], in the present investigation we extended our work with new aryl moiety at second position in oxadiazole nucleus to generate a new series of 2-aryl-5-substituted-1,3,4-oxadiazoles and thiadiazoles as given in Scheme 1 and studied their cytotoxic and antibacterial properties.

Synthesis of compounds **5**(**a–e**) and **6**(**a–e**): As shown in Scheme 1, initially the 5-methyl-2-nitro-benzoic acid (**1**) was treated with ethanol in presence of sulphuric acid to obtain the ester **2** which was then treated with hydrazine hydrate to get the acyl hydrazide moiety (**3**). The acyl hydrazide thus obtained was treated with various aromatic carboxylic acids (**a–e**) in presence of HATU and DIPEA in THF solvent at room temperature for 13 h to get the key intermediate *N*-(5-methyl-2-nitrobenzoyl)-2-substituted carbohydrazide

Scheme 1 Synthesis of 2-aryl-5-substituted-1,3,4-oxadiazoles (**5a–e**) and thiadiazoles (**6a–e**)

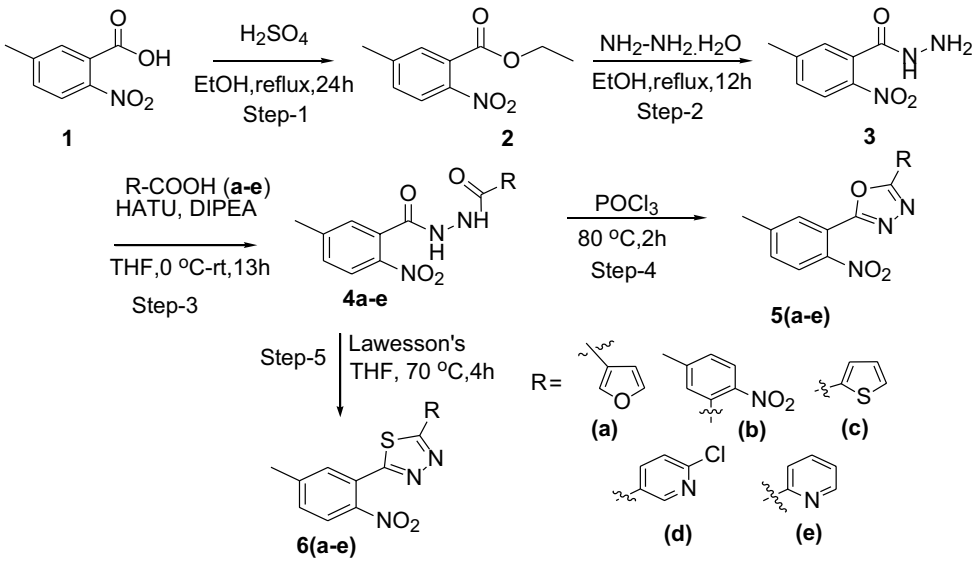


Table 1 Synthesis and cytotoxicity results of 2-aryl-5-substituted-1,3,4-oxadiazoles and thiadiazoles

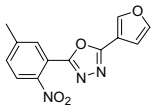
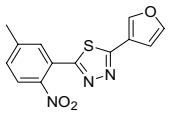
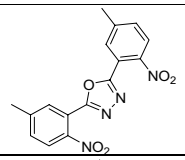
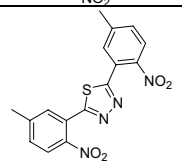
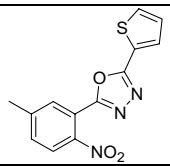
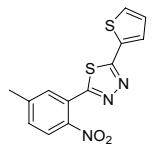
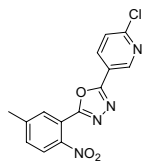
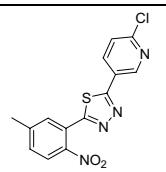
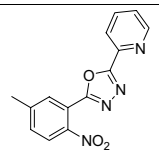
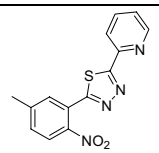
Compound No.	Compound Structure	Yield	M.P.	ED50 (µg/mL)
5a		85.30	123.5-125	>500
6a		78.78	152-154	19.45±0.52
5b		90.90	194.5-196	>500
6b		<10	--	--
5c		76.50	175.5-177	10.00±0.85
6c		74.00	158.5-160	>500
5d		90.40	184.5-186.5	19.29±0.34
6d		<10	--	--
5e		90.40	169-170.5	105.06±0.21
6e		<10	--	--
Std.	*Podophyllotoxin*	--	--	3.64±0.31

Table 2 Antibacterial activity of 2-aryl- 5-substituted-1,3,4-oxadiazoles and thiadiazoles

S. no.	Test organism	Diameter of zone of inhibition (mm)							Positive control (tetracycline)
		5a	**6a**	**5b**	**5c**	**6c**	**5d**	**5e**	
1.	*Bacillus megaterium* MTCC 428	5	5	3	4	4	4	3	7
2.	*Enterococcus faecalis* MTCC 439	5	4	4	3	3	5	3	7
3.	*Streptococcus mutans* MTCC 497	4	3	3	3	3	4	3	8
4.	*Escherichia coli* MTCC 1687	6	3	3	0	3	6	4	8
5.	*Proteus vulgaris* MTCC 426	4	4	3	3	5	4	3	7
6.	*Pseudomonas aeruginosa* MTCC 1688	5	3	0	4	3	3	3	7

(**4a–e**). Finally, the carbohydrazide was treated separately with $POCl_3$ and Lawesson's reagents to get the desired 2-aryl-5-substituted-1,3,4-oxadiazoles (**5a–e**) and thiadiazoles (**6a–e**) in good yields, respectively.

The isolated yields of the final compounds were about 75–91% (Table 1). The yields of 2-aryl-5-substituted-1,3,4-thiadiazoles (**6a–e**) were low compared to oxadiazoles (**5a–e**). The 1,3,4-oxadiazoles **5b**, **5d**, and **5e** with *o*-Nitro, *m*-methyl; 2-chloro-pyridine and pyridine substitutions respectively were obtained in greater than 90% yields. The compound **5a** with 2-furan substitution was obtained in 85% yield and the remaining compounds **6a** (thiadiazole with furan substitution), **5c** (oxadiazole with thiophene substitution) and **6c** (thiadiazole with thiophene substitution) were obtained about 75% yields. However, the thiadiazoles **6b**, **6d**, and **6e** could not proceeded with good yields (< 10%, TLC) even though the temperature and time are increased and no proper reason was assessed. The obtained 2-aryl-5-substituted-1,3,4-oxadiazoles and thiadiazoles were well characterized by the spectral data.

The cytotoxic activities of 2-(5-methyl-2-nitrophenyl)-5-(substituted)-1,3,4-oxadiazoles (**5a–e**) and thiadiazoles (**6a–e**) were tested using brine shrimp lethality bioassay according to literature protocol [50] and the obtained data was validated using Finney probed analysis software. The cytotoxicity properties of the compounds were compared with the standard podophyllotoxin and results of the activities were provided in Table 1. The standard podophyllotoxin has the ED_{50} of 3.64 µg/mL. The compounds **6a**, **5c** and **5d** exhibited significant cytotoxic properties (IC_{50} = 10–19.45 µg/mL) and **5e** exhibited less significant activity. But the cytotoxic property of the compounds **5a**, **5b**, and **6c** were not significant when compared to the standard drug. From the table it was observed that the 1,3,4-oxadiazole compound with thiophene substitution at 5th position (**5c**) or the 1,3,4-thiadiazole compound with furan substitution at 5th position (**6a**) were showed significant activity. Further, the symmetrical oxadiazole **5b** was found to be less cytotoxic than the unsymmetrical compounds (**6a**, **5c**, and **5d**).

Antibacterial activity of the synthesized 2-aryl-5-substituted-1,3,4-oxadiazoles and thiadiazoles was determined against Gram-positive bacteria (*Bacillus megaterium* MTCC 428, *Enterococcus faecalis* MTCC 439 and *Streptococcus mutans* MTCC 497) and Gram-negative bacteria (*Escherichia coli* MTCC 1687, *Proteus vulgaris* MTCC 426 and *Pseudomonas aeruginosa* MTCC 1688) in DMF by disc diffusion method on nutrient agar medium [51]. The results of antibacterial activity of 2-aryl-5-substituted-1,3,4-oxadiazoles (**5a**, **5b**, **5c**, **5d**, **5e**) and thiadiazoles (**6a**, **6c**) were presented in Table 2.

The compounds exhibited a wide range of antibacterial activity including both Gram positive and Gram-negative bacteria. Further, the compound **5a** was found be more sensitive towards most tested organisms followed by **5d**. The activity of compound **5d** may be because of presence of chlorine atom on the pyridine ring. Zavyalova et al. [52] reported that thienopyridine and other pyridine derivatives possessed good antimicrobial i.e. antibacterial activity against gram positive bacteria *S. aureus* and are also efficient against gram negative *E. coli, P. aeruginesa* and *P. vulgaris*. Some nitrophenoxymethyl-1,3,4-thiadiazole derivatives synthesized by Shah et al. [53] were also showed good antimicrobial activity. The introduction of the nitro group significantly increased the antibacterial activity against *S. aureus*, the studied compounds exhibiting comparable activity to standard drugs, chloramphenicol and ampicillin. In the present study, the newer analogues of 2-aryl-5-disubstituted-1,3,4-oxadiazoles and thiadiazoles were also exhibited similar pattern of antibacterial activity.

In summary, the newly synthesized 2-(5-methyl-2-nitrophenyl)-5-(substituted)-1,3,4-oxadiazoles (**5a–e**) and thiadiazoles (**6a–e**) were exhibited prominent biological activities including anticancer and antimicrobial properties. The potent compounds (**6a**, **5c** and **5d**) will be studied for LC50 using in vivo studies along with other sets of developed oxadiazoles. At this point, we could highlight the findings of this initial study and this will not only direct us to synthesize similar compounds with greater biological

activity, but also may have an influence on the synthesis of more compounds with broad range biological properties.

Acknowledgements The authors are highly thankful to Acharya Nagarjuna University, Guntur, Andhra Pradesh, India for constant encouragement, and Laila Impex R&D Centre, Vijayawada, Andhra Pradesh, India for biological studies.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

References

1. Kumar B, Kumar A, Beheraand AK, Raj V (2016) Latest update on pharmacological activities of 1,3,4-oxadiazole derivatives. J Cell Sci Ther 7:233–239
2. Singhai A, Gupta MK (2016) Oxadiazoles and their pharmacological potentials: a review. Curr Trends Tech Sci V(IV):647–654
3. Rajak H, Kharya MD, Mishra P (2009) Biologically active 2,5-disubstituted-1,3,4-oxadiazoles. Int J Pharm Sci Nanotech 2(1):390–406
4. Somani Rakesh R, Agrawal Anuj G, Kalantri Pushkar P, Gavarkar Pratibha S, De Clerq Erik (2011) Investigation of 1,3,4-oxadiazole scaffold as potentially active compounds. Int J Drug Des Discov 2(1):353–360
5. Boström J, Hogner A, Llinàs A, Wellner E, Plowright AT (2012) Oxadiazoles in medicinal chemistry. J Med Chem 55(5):1817–1830
6. Musmade Deepak S, Pattan Shashikant R, Yalgatti MS (2015) Oxadiazole a nucleus with versatile biological behavior. Int J Pharm Chem 5(1):11–20
7. Zarghi A, Hajimahdi Z (2013) Substituted oxadiazoles: a patent review (2010–2012). Expert Opin Ther Pat 23(9):1209–1232
8. Holla BS, Gonsalves R, Shenoy S (2000) Synthesis and antibacterial studies of a new series of 1,2-bis (1,3,4-oxadiazol-2-yl) ethanes and 1,2-bis (4-amino-1,2,4-triazol-3-yl) ethanes. Eur J Med Chem 35(2):267–271
9. Şahin G, Palaska E, Ekizoğlu M, Özalp M (2002) Synthesis and antimicrobial activity of some 1,3,4-oxadiazole derivatives. Il Farmaco 57(7):539–542
10. Shelke S, Mhaske G, Gadakh S, Gill C (2010) Green synthesis and biological evaluation of some novel azoles as antimicrobial agents. Bioorg Med Chem Lett 20(24):7200–7204
11. Macaev F, Rusu G, Pogrebnoi S, Gudima A, Stingaci E, Vlad L, Reynolds R (2005) Synthesis of novel 5-aryl-2-thio-1,3,4-oxadiazoles and the study of their structure-anti-mycobacterial activities. Bioorg Med Chem 13(16):4842–4850
12. Zou XJ, Lai LH, Jin GY, Zhang ZX (2002) Synthesis, fungicidal activity, and 3D-QSAR of pyridazinone-substituted 1,3,4-oxadiazoles and 1,3,4-thiadiazoles. J Agric Food Chem 50(13):3757–3760
13. Padmavathi V, Reddy GD, Reddy SN, Mahesh K (2011) Synthesis and biological activity of 2-(bis ((1,3,4-oxadiazolyl/1,3,4-thiadiazolyl) methylthio) methylene) malononitriles. Eur J Med Chem 46(4):1367–1373
14. Pitasse-Santos P, Sueth-Santiago V, Lima ME (2018) 1,2,4- and 1,3,4-Oxadiazoles as Scaffolds in the development of antiparasitic agents. J Braz Chem Soc 29(3):435–456
15. Palaska E, Şahin G, Kelicen P, Durlu NT, Altinok G (2002) Synthesis and anti-inflammatory activity of 1-acylthiosemicarbazides, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazole-3-thiones. Il Farmaco 57(2):101–107
16. Dinneswara Reddy G, Park SJ, Cho HM, Kim TJ, Lee ME (2012) Antiallergic activity profile in vitro RBL-2H3 and in vivo passive cutaneous anaphylaxis mouse model of new sila-substituted 1,3,4-oxadiazoles. J Med Chem 55(14):6438–6444
17. Dinneswara Reddy G, Park SJ, Lee MW, Kim TJ, Lee ME (2013) Syntheses and anti-allergic activity of 2-((bis (trimethylsilyl) methylthio/methylsulfonyl) methyl)-5-aryl-1,3,4-oxadiazoles. Eur J Med Chem 62:84–88
18. Amir M, Shikha K (2004) Synthesis and anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation activities of some new 2-[(2, 6-dichloroanilino) phenyl] acetic acid derivatives. Eur J Med Chem 39(6):535–545
19. Almasirad A, Tabatabai SA, Faizi M, Kebriaeezadeh A, Mehrabi N, Dalvandi A, Shafiee A (2004) Synthesis and anticonvulsant activity of new 2-substituted-5-[2-(2-fluorophenoxy) phenyl]-1,3,4-oxadiazoles and 1,2,4-triazoles. Bioorg Med Chem Lett 14(24):6057–6059
20. Almasirad A, Vousooghi N, Tabatabai SA, Kebriaeezadeh A, Shafiee A (2007) Synthesis, anticonvulsant and muscle relaxant activities of substituted 1,3,4-oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole. Acta Chim Slov 54(2):317–324
21. Husain MI, Kumar A, Srivastava RC (1986) Synthesis of *N*-(2-naphthyloxyacetyl) thiosemicarbazides and 2-arylamino-5-(2-naphthyloxymethyl)-1,3,4-thiadiazoles/oxadiazoles as oral hypoglycemic agents. Curr Sci 55:644–646
22. Zheng X, Li Z, Wang Y, Chen W, Huang Q, Liu C, Song G (2003) Syntheses and insecticidal activities of novel 2,5-disubstituted-1,3,4-oxadiazoles. J Fluorine Chem 123(2):163–169
23. Bhat KS, Karthikeyan MS, Holla BS, Shetty NS (2004) Synthesis of some new fluorine containing 1,3,4-oxadiazole derivatives as potential antibacterial and anticancer agents. Ind J Chem Sec B 43B:1765–1769
24. Agarwal M, Singh V, Sharma SK, Sharma P, Ansari MY, Jadav SS, Ahsan MJ (2016) Design and synthesis of new 2,5-disubstituted-1,3,4-oxadiazole analogues as anticancer agents. Med Chem Res 25(10):2289–2303
25. Jnyanaranjan P, Jagannath Patro V, Chandra Sekhar P, Mishra J (2011) Synthesis, characterization, antibacterial and analgesic evaluation of some 1,3,4-oxadiazole derivatives. Der Pharma Chem 3(2):485–490
26. Singh AK, Geeta M, Jyoti K (2011) Review on biological activities of 1,3,4-thiadiazole derivatives. J Appl Pharm Sci 1(5):44–49
27. Georgeta S, Oana S, Eugenia S, Sanda B (2018) 2-Amino-1,3,4-thiadiazole as a potential scaffold for promising antimicrobial agents. Drug Des Dev Ther 12:1545–1566
28. Joseph L, George M, Prabha M (2015) A review on various biological activities of 1,3,4-thiadiazole derivatives. J Pharm Chem Biol Sci 3(3):329–345
29. Jain AK, Sharma S, Vaidya A, Ravichandran V, Agrawal RK (2013) 1,3,4-Thiadiazole and its derivatives: a review on recent progress in biological activities. Chem Biol Drug Des 81(5):557–576
30. Zhang G, Yu Y, Zhao Y, Xie X, Ding C (2017) Iron(III)/TEMPO-catalyzed synthesis of 2,5-disubstituted 1,3,4-oxadiazoles by oxidative cyclization under mild conditions. Synlett 28:1373–1377
31. Kumar D, Pilania M, Arun V, Mishra B (2014) A facile and expeditious one-pot synthesis of α-Keto-1,3,4-oxadiazoles. Synlett 25:1137–1141
32. Guin S, Ghosh T, Rout SK, Banerjee A, Patel BK (2011) Cu(II) catalyzed imine C–H functionalization leading to synthesis of 2,5-substituted 1,3,4-oxadiazoles. Org Lett 13:5976–5979
33. Yu W, Huang G, Zhang Y, Liu H, Dong L, Yu X, Li Y, Chang J (2013) I_2-Mediated oxidative C–O bond formation for the

synthesis of 1,3,4-oxadiazoles from aldehydes and hydrazides. J Org Chem 78:10337–10343
34. Park Y-D, Kim J-J, Chung H-A, Kweon D-H, Cho S-D, Lee S-G, Yoon Y-J (2003) Facile synthesis of symmetric and unsymmetric 1,3,4-oxadiazoles using 2-acyl(or aroyl)pyridazin-3-ones. Synthesis 4:560–564
35. Taha M, Imran S, Wadood A, Rahim F, Saad SM, Khan KM, Nasir A (2016) Synthesis, molecular docking and α-glucosidase inhibition of 5-aryl-2-(6-nitrobenzofuran-2-yl-1,3,4-oxadiazoles. Bioorg Chem 66:117–123
36. Teimouri A, Salavati H, Chermahini AN (2014) Synthesis, characterization and application of various types of alumina and nano-g-alumina sulfuric acid for the synthesis of 2,5-disubstituted 1,3,4-oxadiazoles. Acta Chim Slov 6(1):51–58
37. Wet-osot S, Phakhodee W, Pattawarapan M (2017) Application of *N*-acylbenzotriazoles in the synthesis of 5-substituted 2-ethoxy-1,3,4-oxadiazoles as building blocks towards 3,5-disubstituted 1,3,4-oxadiazol-2(3*H*)-ones. J Org Chem 82:9923–9929
38. Adib M, Kesheh MR, Ansari S, Bijanzadeh HR (2009) Reaction between *N*-isocyaniminotriphenylphosphorane, aldehydes, and carboxylic acids: a one-pot and three-component synthesis of 2-aryl-5-hydroxyalkyl-1,3,4-oxadiazoles. Synlett 10:1575–1578
39. Niu P, Kang J, Tian X, Song L, Liu H, Wu J, Yu W, Chang J (2015) Synthesis of 2-amino-1,3,4-oxadiazoles and 2-amino-1,3,4-thiadiazoles via sequential condensation and I_2-mediated oxidative C–O/C–S bond formation. J Org Chem 80:1018–1024
40. Fang T, Tan Q, Ding Z, Liu B, Xu B (2014) Pd-catalyzed oxidative annulation of hydrazides with isocyanides: synthesis of 2-amino-1,3,4-oxadiazoles. Org Lett 16:2342–2345
41. Dolman SJ, Gosselin F, O'Shea PD, Davies IW (2006) Superior reactivity of thiosemicarbazides in the synthesis of 2-amino-1,3,4-oxadiazoles. J Org Chem 71:9548–9551
42. Patel KN, Jadhav NC, Jagadhane PB, Telvekar VN (2012) A novel strategy for the construction of azole heterocycles via an oxidative desulfurization approach using iodobenzene and oxone. Synlett 23:1970–1972
43. Yang S-J, Lee S-H, Kwak H-J, Gong Y-D (2013) Regioselective synthesis of 2-amino-substituted 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives via reagent-based cyclization of thiosemicarbazide intermediate. J Org Chem 78:438–444
44. Rezki N, Al-Yahyawi AM, Bardaweel SK, Al-Blewi FF, Aouad MR (2015) Synthesis of novel 2,5-disubstituted-1,3,4-thiadiazoles clubbed 1,2,4-triazole, 1,3,4-thiadiazole, 1,3,4-oxadiazole and/or schiff base as potential antimicrobial and antiproliferative agents. Molecules 20(9):16048–16067
45. Dogan HN, Duran A, Rollas S, Sener G, Uysal MK, Gülen D (2002) Synthesis of new 2,5-disubstituted-1,3,4-thiadiazoles and preliminary evaluation of anticonvulsant and antimicrobial activities. Bioorg Med Chem 10(9):2893–2898
46. AL-Bayati RI, Nief OA, Hameed AN (2015) Synthesis of 2,5-disubstituted-1,3,4-thiadiazole derivatives and evaluation of their antibacterial activity. Int J Appl Nat Sci 4(2):1–8
47. Mamolo MG, Vio L, Banfi E (1996) Synthesis and antimicrobial activity of some 2,5-disubstituted 1,3,4,-thiadiazole derivatives. Farmaco (Societa chimica italiana: 1989) 51(1):71–74
48. Siva Nagi Reddy M, Sailaja Kumari B, Hari Babu B (2012) Synthesis and cytotoxicity evaluation of some novel 3,5-disubstituted-1,2,4-oxadiazoles. Lett Drug Des Discov 9(10):942–946
49. Siva Nagi Reddy M, Sailaja Kumari B, Velupula G, Dinneswara Reddy G, Hari Babu B (2014) 10-Camphorsulfonic acid ((±)-CSA) catalyzed facile one-pot synthesis of a new class of 2,5-disubstituted 1,3,4-oxadiazoles. RSC Adv 4(102):58397–58403
50. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL (1982) Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med 45(5):31–34
51. Bauer AW, Kirby WMM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45(4_ts):493–496
52. Zav'yalova VK, Zubarev AA, Shestopalov AM (2009) Synthesis and reactions of 3-acetyl-6-methyl-2-(methylthio) pyridine. Russ Chem Bull 58(9):1939–1944
53. Shah H, Vashi S, Mehta S (1996) Synthesis of 2,5-disubstituted-1,3,4-oxadiazole, 1,5-disubstituted -2-mercapto-1,3,4-triazole and 2,5-disubstituted-1,3,4-thiadiazole as potential antimicrobial agents. Indian J Chem 35B:111–115