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Pharma Research

An International Peer Reviewed, Indexed Journal

10.62655/s-epub.2021.v13.i01.pp57-64

SYNTHESIS AND ANTIMICROBIAL EVALUATION OF NOVEL 1,3,4-OXADIAZOLE DERIVATIVES

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Article info

Received date:01-07-2020
Accepted date:15-11-2020
Published date:07-02-2021
ISSN;0975-8216

Keywords:

1,3,4-Oxadiazole, Antimicrobial activity, Heterocyclic synthesis, Minimum inhibitory concentration, Structure-activity

Abstract

Derivatives of 1,3,4-oxadiazole are frequently synthesised and screened for antimicrobial activity in the search for new agents against resistant pathogens. This review summarises published work on that topic. It outlines the properties of the ring as a bioisostere, the main synthetic routes and their characterisation, the diffusion and dilution methods used to measure activity, and the structural trends reported. Many series show activity against Gram-positive bacteria and some fungi, but potency is usually modest, series are small, methods are variably standardised and mechanisms are rarely proven. No compounds were prepared and no new data are reported.

1. INTRODUCTION

Antibiotic discovery has slowed to a trickle just as resistance has spread. Surveillance data collected by the World Health Organization (WHO) show resistant strains of common pathogens in every region, and economic analyses have pointed to a thin pipeline of new classes reaching patients [1, 2, 3]. Medicinal chemists therefore keep returning to small heterocyclic scaffolds that are easy to make, easy to decorate and often active against something. The 1,3,4-oxadiazole ring is one of the most visited of these. Papers describing the synthesis of a new series of oxadiazole derivatives, followed by a screen against a handful of bacteria and fungi, appear in large numbers every year.

This review looks at that body of work. It describes how 1,3,4-oxadiazole derivatives are made, how they are characterised, how their antimicrobial activity is tested and what the published results actually show. It is a narrative synthesis of the work of others. No compounds were prepared or tested for this

article, and no new data are presented. The emphasis is on what a reader needs to judge a typical synthesis-and-screening paper: whether the chemistry is sound, whether the assay is meaningful and whether the claimed activity matters in any practical sense [4, 5]. The subject sits within medicinal chemistry, microbiology and analytical science at once, and failures in any one of them can undo the others.

Fungal infections deserve a mention beside bacterial ones. Invasive candidiasis and aspergillosis cause considerable mortality in immunosuppressed patients, the available antifungal classes are few, and resistance to azoles has been reported [2, 3]. Many oxadiazole papers therefore include a fungal arm in the screen, which is sensible, although the same cautions about methods and controls apply and fungal susceptibility testing has its own standardised conditions that are not always followed. Azole antifungals and the echinocandins act on distinct targets, so a screen that uses only one reference drug captures only part of the possible picture.

Two ideas run through the discussion. First, activity in a screen is a starting point and not a result. A compound with a minimum inhibitory concentration (MIC) several-fold above that of the reference drug has told us little about future usefulness [6, 7]. Second, the chemistry of the oxadiazole ring is robust, so structural variety is cheap and a new series can be assembled in a few weeks, and the literature is full of near neighbours whose activities are hard to tell apart without careful, standardised testing [8, 9].

2. THE 1,3,4-OXADIAZOLE SCAFFOLD

The 1,3,4-oxadiazole is a five-membered aromatic ring containing one oxygen and two nitrogen atoms, with the nitrogens adjacent. It is flat, polar, and resistant to hydrolysis under physiological conditions, and it can accept hydrogen bonds through its nitrogens without donating any [8]. Compared with the isomeric 1,2,4-oxadiazole, the 1,3,4-isomer is generally more polar and less lipophilic, which tends to bring better aqueous solubility and lower metabolic clearance in matched pairs, a pattern documented in a systematic comparison of the two [8]. Medicinal chemists value the ring as a bioisostere. It can stand in for ester and amide groups, which are vulnerable to esterases and peptidases, while preserving a similar arrangement of hydrogen bond acceptors and overall geometry [8, 10]. The sulphur analogue, the 1,3,4-thiadiazole, behaves similarly and is often prepared alongside it, so many papers report both [11]. The ring also acts as a linker that holds two aromatic groups at a defined distance and angle, which explains its frequent appearance in hybrid molecules.

Tautomerism and acid-base behaviour affect how these molecules behave in assays. The 2-thiol derivatives exist mainly as thiones in the solid state and in polar solvents, with an acidic N–H that is partly ionised at physiological pH, a fact that changes lipophilicity, permeability and protein binding [8, 11]. Reported partition coefficients are often calculated for one tautomer only. In practice, measured distribution values at the assay pH are more relevant to the question of whether a compound can cross the bacterial envelope, and they are seldom provided. Several marketed or late-stage compounds contain the ring. The HIV integrase inhibitor raltegravir carries a methyl-substituted 1,3,4-oxadiazole carboxamide, and zibotentan, an endothelin receptor antagonist that reached clinical trials in cancer, has one too [8, 12]. Anticancer, anti-inflammatory, anticonvulsant and antidiabetic activities have been described for oxadiazole series, and reviews of anticancer derivatives illustrate how widely the scaffold has been explored [13]. This breadth is consistent with a privileged scaffold, but it is also a reminder that the ring alone does not confer selectivity.

3. SYNTHETIC ROUTES

Most routes begin with a carboxylic acid hydrazide, itself made by heating an ester with hydrazine hydrate. Condensation of the hydrazide with a second carboxylic acid in the presence of phosphorus oxychloride ($POCl_3$) gives the 2,5-disubstituted oxadiazole directly, through a diacylhydrazine that cyclodehydrates in the hot acidic medium [8, 9]. The method is old, general and tolerant of many substituents. It is also harsh, since phosphorus oxychloride is

corrosive and moisture-sensitive, the work-up involves quenching onto ice, and acid-labile groups may not survive.

A second common route reacts an acid hydrazide with carbon disulphide in basic alcoholic solution, followed by acidification, to give a 5-substituted 1,3,4-oxadiazole-2-thiol, usually drawn as the thione tautomer. The thiol can then be alkylated or acylated, or linked to other fragments through the sulphur or through a Mannich reaction [14, 15]. This is the route behind many of the antimicrobial series in the literature, because it is high-yielding and creates a convenient attachment point. The price is a sulphur-containing product that can complicate biological interpretation, as thiols and thiones are among the well-known classes of frequent hitters in screens [5].

Oxidative cyclisation of acylhydrazones, formed by condensing a hydrazide with an aldehyde, offers a milder alternative. Reagents such as iodine with potassium carbonate, chloramine-T and hypervalent iodine compounds have been used, and the aromatic aldehyde partner allows a wide range of substituents to be introduced [8, 16]. For 2-amino derivatives, cyclodesulphurisation of acylthiosemicarbazides provides an efficient route, and one study demonstrated superior reactivity of the thiosemicarbazide intermediates compared with the corresponding semicarbazides [16]. Side reactions are an under-reported feature of these syntheses. Acylthiosemicarbazides can cyclise under basic conditions to 1,2,4-triazole-3-thiones instead of oxadiazoles, and the outcome depends on the reagent and pH [16]. The two products are isomeric or nearly so and have very similar mass spectra, so the structure must be settled by spectroscopic detail or by crystallography. Reaction progress is generally followed by thin-layer chromatography, which shows consumption of starting material but not identity. A claim that a product is an oxadiazole should therefore be supported by evidence that distinguishes it from the alternatives [16, 17].

Microwave irradiation has been adopted widely to shorten reaction times from hours to minutes and, in some papers, to improve yields and reduce solvent use [18, 19]. The claim of improved yield should be read with care, because comparisons are often made with a conventional method that was not optimised. Green chemistry arguments, with water or ethanol as solvent and solid-supported reagents, are increasingly stated, though many procedures still depend on phosphorus oxychloride or large volumes of chlorinated solvents [19].

Scale and cost determine whether a synthetic route is useful beyond the first publication. Reactions that give a few hundred milligrams in a flask are not necessarily practical at gram scale, and reagents such as phosphorus oxychloride create waste streams that must be handled [18, 19]. Process considerations such as atom economy, number of isolation steps and avoidance of chromatography do not enter most screening papers, even though they affect the cost of any eventual drug. A route with fewer steps and benign solvents has real value when two series perform similarly.

Characterisation is expected to confirm structure and purity. Infrared (IR) spectra should show disappearance of the carbonyl and N–H bands of the hydrazide and the appearance of C=N and C–O–C stretches, proton and carbon nuclear magnetic resonance (NMR) spectra should account for every substituent, and mass spectra should give the molecular ion [17]. Elemental analysis within accepted limits and a melting point or chromatographic purity check complete the set. In the weaker papers, a handful of IR bands is offered as proof of structure, which cannot exclude an uncyclised intermediate.

Yields, reaction conditions and purification are reported inconsistently. Recrystallisation from ethanol is the usual purification, and chromatographic methods are used less often, which raises the question of residual starting materials or trace metals in biological samples [5]. A purity statement matters here, since an impurity present at a few per cent can produce activity that is then attributed to the main compound. Trace metals from catalysts and residual hydrazine, itself toxic to cells and microbes, are particular worries in biological samples.

4. ANTIMICROBIAL EVALUATION

Antimicrobial screening in these papers usually has two steps. A primary screen by agar diffusion, using paper discs in the manner introduced by Bauer and colleagues, or wells cut into seeded agar, gives a zone of inhibition that is easy to read [4, 20]. A second step determines the minimum inhibitory concentration by broth or agar dilution, which is the accepted quantitative measure [21, 22]. Diffusion zones depend on how far the compound moves through the agar, so a lipophilic compound can look inactive for reasons of diffusion alone, and zone sizes of different compounds are not comparable on a like-for-like basis.

The panel of organisms is typically modest: *Staphylococcus aureus* and *Bacillus subtilis* as Gram-positive representatives, *Escherichia coli* and *Pseudomonas aeruginosa* as Gram-negative ones, and *Candida albicans* and *Aspergillus niger* for fungi. Reference strains from culture collections are standard, while clinical isolates, especially resistant ones such as methicillin-resistant *S. aureus* (MRSA), are included less often [4, 23]. Since the clinical value of a new agent lies in activity against resistant organisms, the omission of such isolates limits what the screen can say.

Practical details of the assay deserve more attention than they usually get. Compounds of this class dissolve poorly in water, so stock solutions in dimethyl sulphoxide (DMSO) are standard, and the final DMSO concentration in the well must be low enough not to inhibit growth, generally at or below one per cent [21, 22]. Precipitation at higher concentrations can produce false end points. The inoculum must be prepared to a defined density, often a 0.5 McFarland standard, then diluted. Variation of even a few-fold in inoculum can shift an MIC by a dilution step, which is the same magnitude as the differences used to rank compounds in structure-activity tables.

Method standardisation determines whether numbers can be compared across laboratories. The Clinical and Laboratory Standards Institute (CLSI) specifies medium, inoculum density, incubation conditions and quality control strains for dilution tests, and many papers claim to follow it [21, 22]. Others use unspecified media, solvents such as dimethyl sulphoxide at undeclared concentrations, or visual end points without a growth indicator. Microplate methods with a tetrazolium dye make end points clearer, a refinement first popularised for plant extracts [24].

Controls are as important as the test compounds. A standard antibiotic such as ciprofloxacin and an antifungal such as fluconazole should be run in parallel, along with the solvent control and a sterility check. Authors should also state the concentration ranges, the number of replicates and how the MIC was read [21, 25]. Cytotoxicity testing against mammalian cells, for example by the MTT (tetrazolium) assay, shows whether the antimicrobial effect is selective or merely a general toxic one [26]. Few of the oxadiazole papers include this step, which leaves the selectivity question open. A selectivity index, the ratio of cytotoxic concentration to MIC, would make claims far easier to weigh.

How active is active? Reviewers of natural product and synthetic antimicrobial work have proposed thresholds for interpreting MIC values, in which compounds with MICs in the low micromolar or low microgram-per-millilitre range deserve interest, and values of a few hundred micrograms per millilitre are unlikely to lead anywhere [25]. Many oxadiazole papers describe compounds as "potent" or "excellent" on the basis of a modest MIC value that happens to be the lowest in the series. A conservative standard, which defines activity relative to the reference drug in the same experiment and also relative to an absolute clinically meaningful range, would prevent that kind of inflation [6, 25].

5. REPORTED ACTIVITY AND STRUCTURAL TRENDS

Published oxadiazole derivatives have shown activity against Gram-positive bacteria more often than against Gram-negative ones, and against fungi in a fair number of studies. Series carrying benzodioxane, biphenyl or thiazole fragments have been described as active against *S. aureus*, *B. subtilis*, *E. coli* and *C. albicans*, in some cases with MIC values close to those of reference drugs and in

many others well above them [14, 15, 27]. The findings are summarised in table 1, grouped by theme in fig. 1. Because test conditions differ from paper to paper, the table avoids numbers.

Table 1: Published features of the 1,3,4-oxadiazole scaffold, synthesis, testing and reported antimicrobial activity

Topic	Reported finding or feature	Ref.
1,3,4-Oxadiazole ring bioisostere	Replaces ester and amide groups; more polar and metabolically stable than the 1,2,4-isomer.	[8, 10]
Raltegravir marketed drug	HIV integrase inhibitor containing a methyl-1,3,4-oxadiazole carboxamide group.	[12]
Drug-likeness property rules	Lipinski and Veber limits used as a first filter; antibacterials often exceed them.	[28, 29, 30]
Hydrazide plus acid POCl3 route	Direct cyclodehydration to 2,5-disubstituted ring; harsh conditions.	[8, 9]
Hydrazide plus CS2 thiol route	Gives 2-thiol derivatives that are alkylated or linked to other fragments.	[14, 15]
Microwave irradiation reaction speed	Shorter reaction times; yield gains depend on how well the control was optimised.	[18, 19]
Disc and well diffusion primary screen	Quick, but zone size depends on diffusion; not quantitative.	[4, 20]
Broth dilution MIC	Quantitative standard; CLSI method specifies medium, inoculum and controls.	[21, 22]
Benzodioxane and biphenyl hybrids series	Activity against Gram-positive bacteria and Candida, from comparable to well below reference drugs.	[14, 27]
Thiazole-clubbed oxadiazoles series	Antibacterial and antifungal activity reported with some cytotoxicity data.	[15]
Halogen and nitro substituents SAR	Often linked to better activity, attributed to lipophilicity and penetration.	[9, 31]

The trends that authors draw are consistent enough to be worth stating, though none is strong. Electron-withdrawing groups such as chloro, fluoro and nitro on a pendant aryl ring are frequently linked to better antibacterial or antifungal activity, and it has been suggested that these substituents raise lipophilicity and help penetration of the cell envelope [9, 31]. Thioether-linked oxadiazoles are often more active than the corresponding free thiols, and hybrid structures joining the ring to another heterocycle recur among the most active compounds [14, 15].

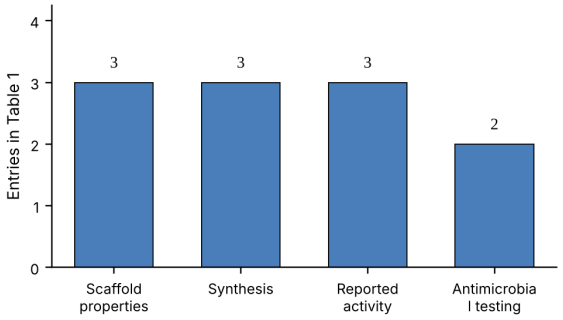


Fig. 1: Number of entries in table 1 by theme

Several features make the structure-activity relationship (SAR) claims weaker than they sound. Series are small, often ten to twenty compounds, and the differences between them in MIC are a single two-fold dilution, which is within the normal variation of the assay [21, 22]. Computed properties are offered to explain activity, but these are rarely tested statistically. Molecular docking into bacterial targets such as DNA gyrase or peptide deformylase is a popular supporting argument, yet docking scores are poor predictors of activity unless backed by enzymatic data [9, 31].

Heteroatom-rich hybrids deserve a separate comment. Linking the oxadiazole to a quinolone, azole or sulphonamide fragment is a logical way to combine pharmacophores, and some papers report additive or better activity than either component alone [15, 27]. Yet the improvement is often compared with the unsubstituted parent oxadiazole and not with the original drug the fragment came from. If the hybrid is less active than ciprofloxacin or fluconazole, the claim that it "improves" on its parts has to be qualified, and the extra molecular weight and complexity are a cost. Mechanism is seldom established. Proposed targets include DNA gyrase, enzymes of cell-wall synthesis and fungal sterol 14α-demethylase, usually on the basis of structural similarity to known inhibitors and docking, not on biochemical evidence [8, 9]. Resistance is a related

unknown. Resistance to established classes arises through target mutation, efflux and enzymatic inactivation, and new compounds must be tested for cross-resistance with them, but the screening papers rarely attempt it [6, 23].

Antitubercular and antifungal findings add a further dimension. Some oxadiazole series, including compounds designed with property prediction in mind, were screened against *Mycobacterium tuberculosis* and reported to have activity at concentrations that merit follow-up [9]. Antifungal activity is often reported against *Candida* species, and a few authors suggest the ring can mimic features of azole antifungals, although the evidence for a shared mechanism is thin [14, 31]. Slow-growing organisms need specialised containment and methods, which is why such work is usually done in collaboration with dedicated laboratories.

A number of papers also describe antitubercular, anticancer and anti-inflammatory screening of the same compounds, which makes their value hard to judge: broad activity of the kind described may indicate a nonspecific mechanism, such as membrane disruption or reactivity, and not a useful antimicrobial profile [5, 13]. Counter-screens against unrelated targets and mammalian cells help to separate the two cases, and a fair reading of such papers asks which controls were run before accepting a claim of broad-spectrum potential.

6. LIMITATIONS AND NEXT STEPS

The literature on antimicrobial oxadiazoles shares weaknesses that apply to screening studies of many scaffolds. Selection of compounds is driven by synthetic convenience, test organisms are limited, controls are inconsistently reported, and positive findings are far more likely to be published than negative ones [3, 7]. A reader should expect that many compounds tested without success never reached print, so the proportion of published compounds that look active is not a reliable estimate of the hit rate of the scaffold.

Cytotoxicity and selectivity belong at the centre of the next steps. A compound that kills bacteria at the same concentration at which it kills mammalian cells is a disinfectant, not a drug candidate. Standard colorimetric viability assays against a liver or kidney cell line, run in parallel with MIC testing, allow a selectivity index to be calculated, and several authors in the field suggest a ratio of at least ten as a minimum for further work [6, 26]. Haemolysis assays with red blood cells and early metabolic stability tests complete a minimal profile and cost little compared with the synthesis effort behind each series.

Activity at the MIC level shown in most papers, typically in the tens of micrograms per millilitre, is far from the single-digit values expected of leads. Moving from a hit to a lead requires potency against clinically relevant isolates, a defined mechanism, acceptable cytotoxicity, stability in plasma and microsomes, and in vivo efficacy in an infection model [6, 7, 30]. The antibacterial field has repeatedly found that compounds with attractive enzyme or whole-cell activity fail on permeability, efflux or toxicity [3]. Better practice is within reach. Authors can adopt standardised dilution methods and report quality control strain results, include resistant isolates and mammalian cell toxicity, state compound purity, and screen for assay interference [5, 22, 26]. Journals and reviewers could ask for these details routinely. Collaborations that link synthetic laboratories with microbiology groups equipped for resistance work would allow the best compounds from each series to be followed up, rather than abandoned after a first paper [6, 25].

Reproducibility and data sharing are the quiet requirements of a credible field. Raw MIC tables with replicate values, spectral data deposited as supplementary files and clear identification of the strain and medium batch would let others reproduce a result or recognise an artefact. Medicinal chemistry journals have moved towards requiring purity data for biologically tested compounds, and the same discipline would help antimicrobial papers [5, 21]. Open deposition of negative results, for instance in institutional repositories, would give a fairer picture of the hit rate of a scaffold than the published record allows.

7. CONCLUSION

The 1,3,4-oxadiazole ring is easy to make and a sensible isostere for labile ester and amide groups. Straightforward routes through hydrazides, carbon disulphide, acylhydrazones and thiosemicarbazides, now often accelerated by microwave heating, give wide structural variety [8, 16, 18]. That ease explains the volume of publications. Published antimicrobial screens report activity, mainly against Gram-positive bacteria and some fungi, and suggest that halogenated or nitro-substituted aryl groups and hybrid structures are favourable [14, 15, 31].

The evidence stops well short of a lead. Potencies are modest, comparisons rest on non-standard methods, resistant isolates and cytotoxicity are seldom examined, and mechanisms are inferred from docking rather than shown [9, 22, 26]. Studies that follow standardised protocols and test for selectivity and cross-resistance would be far more informative than additional unvalidated series [6, 21]. Until then the scaffold remains promising in principle and unproven in practice, and claims of potency should be read against the reference drug in the same experiment.

8. ABBREVIATIONS

CLSI-Clinical and Laboratory Standards Institute, DMSO-Dimethyl sulphoxide, IR-Infrared, MIC-Minimum inhibitory concentration, MTT-3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, NMR-Nuclear magnetic resonance, $POCl_3$-Phosphorus oxychloride, SAR-Structure-activity relationship, WHO-World Health Organization, MRSA-Methicillin-resistant *Staphylococcus aureus*

9. FUNDING

Nil

10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

12. REFERENCES

1. World Health Organization. Antimicrobial resistance: global report on surveillance. Geneva: WHO; 2014.
2. Laxminarayan R, Duse A, Wattal C, *et al.* Antibiotic resistance, the need for global solutions. Lancet Infect Dis 2013;13:1057–98.
3. Payne DJ, Gwynn MN, Holmes DJ, Pompliano DL. Drugs for bad bugs: confronting the challenges of antibacterial discovery. Nat Rev Drug Discov 2007;6:29–40.
4. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: a review. J Pharm Anal 2016;6:71–9.
5. Baell JB, Holloway GA. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. J Med Chem 2010;53:2719–40.
6. Brown ED, Wright GD. Antibacterial drug discovery in the resistance era. Nature 2016;529:336–43.
7. Silver LL. Challenges of antibacterial discovery. Clin Microbiol Rev 2011;24:71–109.
8. Boström J, Hogner A, Llinàs A, Wellner E, Plowright AT. Oxadiazoles in medicinal chemistry. J Med Chem 2012;55:1817–30.
9. Ahsan MJ, Samy JG, Khalilullah H, *et al.* Molecular properties prediction and synthesis of novel 1,3,4-oxadiazole analogues as potent antimicrobial and antitubercular agents. Bioorg Med Chem Lett 2011;21:7246–50.
10. Patani GA, LaVoie EJ. Bioisosterism: a rational approach in drug design. Chem Rev 1996;96:3147–76.
11. Hu Y, Li CY, Wang XM, Yang YH, Zhu HL. 1,3,4-Thiadiazole: synthesis, reactions, and applications in medicinal, agricultural, and materials chemistry. Chem Rev 2014;114:5572–610.
12. Summa V, Petrocchi A, Bonelli F, *et al.* Discovery of raltegravir, a potent, selective orally bioavailable HIV-integrase inhibitor for the treatment of HIV-AIDS infection. J Med Chem 2008;51:5843–55.
13. Glomb T, Szymankiewicz K, Swiatek P. Anti-cancer activity of derivatives of 1,3,4-oxadiazole. Molecules 2018;23:3361.
14. Khalilullah H, Khan S, Ahsan MJ, Ahmed B. Synthesis, characterization and antimicrobial activity of benzodioxane ring containing 1,3,4-oxadiazole derivatives. Bioorg Med Chem Lett 2012;22:2753–7.

15. Desai NC, Bhatt N, Somani H, Trivedi A. Synthesis, antimicrobial and cytotoxic activities of some novel thiazole clubbed 1,3,4-oxadiazoles. Eur J Med Chem 2013;67:54–9.
16. Dolman SJ, Gosselin F, O'Shea PD, Davies IW. Superior reactivity of thiosemicarbazides in the synthesis of 2-amino-1,3,4-oxadiazoles. J Org Chem 2006;71:9548–51.
17. Pavia DL, Lampman GM, Kriz GS, Vyvyan JR. Introduction to spectroscopy. 4th ed. Belmont: Brooks/Cole; 2008.
18. Kappe CO. Controlled microwave heating in modern organic synthesis. Angew Chem Int Ed 2004;43:6250–84.
19. Polshettiwar V, Varma RS. Microwave-assisted organic synthesis and transformations using benign reaction media. Acc Chem Res 2008;41:629–39.
20. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966;45:493–6.
21. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc 2008;3:163–75.
22. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, 9th ed. M07-A9. Wayne: CLSI; 2012.
23. Tenover FC. Mechanisms of antimicrobial resistance in bacteria. Am J Med 2006;119:S3–10.
24. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med 1998;64:711–3.
25. Cos P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: how to develop a stronger in vitro proof-of-concept. J Ethnopharmacol 2006;106:290–302.
26. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55–63.
27. Kumar H, Javed SA, Khan SA, Amir M. 1,3,4-Oxadiazole/thiadiazole and 1,2,4-triazole derivatives of biphenyl-4-yloxy acetic acid: synthesis and preliminary evaluation of biological properties. Eur J Med Chem 2008;43:2688–98.
28. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 1997;23:3–25.
29. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem 2002;45:2615–23.
30. Kerns EH, Di L. Drug-like properties: concepts, structure design and methods. Burlington: Academic Press; 2008.
31. Bakht MA, Yar MS, Abdel-Hamid SG, Al Qasoumi SI, Samad A. Molecular properties prediction, synthesis and antimicrobial activity of some newer oxadiazole derivatives. Eur J Med Chem 2010;45:5862–9.