RECENT ADVANCES ON 1,2,4-OXADIAZOLES: FROM SYNTHESIS TO REACTIVITY AND PHARMACEUTICAL APPLICATIONS

DOI: http://dx.medra.org/10.17374/targets.2021.24.377

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Dedicated to Prof. Nicolò Vivona (1939-2020)

Abstract. *1,2,4-Oxadiazoles are aromatic heterocycles with many valuable applications and interesting reactivity features. In this review, some recent advances in this field are presented with a particular emphasis on relevant applications as drugs. In fact, the 1,2,4-oxadiazole ring is widely present in a large range of drugs, here presented accordingly to their biological activity.*

Contents

1. Introduction

Oxadiazoles are penta-atomic heterocycles containing two nitrogens and one oxygen. These atoms can have different distribution in the ring to generate 1,2,4-oxadiazole, 1,3,4-oxadiazole, 1,2,5-oxadiazole or 1,2,3-oxadiazole compounds. The majority of 1,2,4-oxadiazole compounds have the structure shown in Figure 1 in which both C(3) and C(5) positions are substituted. We focused our attention on recent advances in synthesis and reactivity of 1,2,4-oxadiazoles.[1] Considering the bio-isosterism of this heterocycle with esters and amides we discussed their new biological applications in medicinal chemistry. Applications in the field of material science are outside the scope of this review.

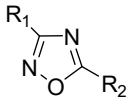
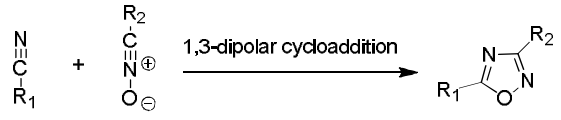


Figure 1. General structure of disubstituted 1,2,4-oxadiazole.

2. Synthesis of 1,2,4-oxadiazoles

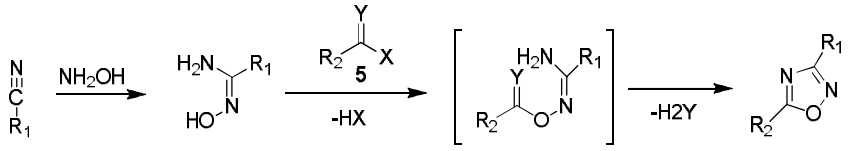
In 1884 Tiemann and Krüger synthetized for the first time the 1,2,4-oxadiazole nucleus.[2] In the classical approach of synthesis, disubstituted 1,2,4-oxadiazole is obtained toward a nitrile **1** nitrile oxide **2** 1,3-dipolar cycloaddition (Scheme 1). The most widely applied method to obtain these versatile heterocycles is the heterocyclization between the amidoxime **4** and the acid derivative **5** (Scheme 2). In some cases, the

O-acylamidoxime **6** could be isolated before the final cyclization step.[1] The R_1 substituent of original nitrile is in position C(5) when the oxadiazole ring is obtained by 1,3-dipolar cycloaddition. Conversely, from the same nitrile **1**, could be obtained the oxadiazole **7** with the R_1 group in C(3) position by means of the amidoxime route.[1]



1 **2** **3**

Scheme 1



1 **4** **6** **7**

Scheme 2

The 1,3-dipolar cycloaddition has been classified as a [3+2] reaction to indicate the number of components needed in precursors to form the final 1,2,4-oxadiazole. Instead, the amidoxime route can be considered a [4+1] approach. Four atoms derived from amidoxime **4** while an acid derivative **5** is the source of the other atom.

In the [4+1] synthetic route, an amidoxime is reacted with a carboxylic acid or its derivative and the reaction proceeds without the isolation of acylamidoxime intermediate. Carboxylic acids are activated in situ using various coupling reagents such as dicyclohexylcarbodimide (DCC),[3] 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)[4] or carbonyldiimidazole (CDI).[5] The reaction is carried out under various conditions, at room temperature or at high temperature, and using different solvents. Solvent-free reactions[6] and those microwave-induced were[7] found to be a valid alternative to conduct such reaction.

An alternative [4+1] route where the acid or its derivative has been replaced with another source of the C(5) substituent was proposed, that is a palladium-mediated carbonylative cyclization where the amidoxime reacts with aryl bromides to produce a 5-aryl-1,2,4-oxadiazoles.[8]

Also, nitriles are a source of C(5) substituent. They are activated in presence of $ZnCl_2$ to form an intermediate complex that cyclizes in the final 1,2,4-oxadiazole ring.[9] Recently, has been developed a new strategy in which amidoxime was not used as the four-atom component in [4+1] route. It has been replaced by an aminonitrones that reacted with an isocyanide to form 1,2,4-oxadiazolium salts.[10]

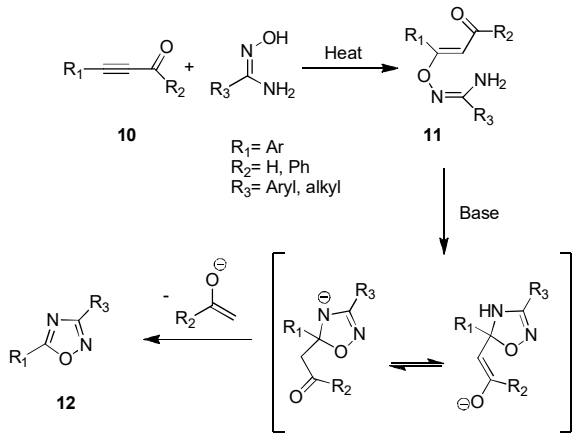
In the classical 1,3-dipolar cycloaddition, the nitrile or nitrile oxide have been variously substituted to synthesize different oxadiazole. For example, the reaction of cyanophosphonates with appropriates nitrile oxide generates 3-substituted-1,2,4-oxadiazol-5-yl-phosponates.[11]

1,2,4-Oxadiazoles could be synthetized starting from a five-atom component via cyclodehydration reaction [5+0]. In this approach the oxadiazole ring can be obtained melting or refluxing the isolated *O*-acylamidoxime precursor **6** in different reaction solvent such as toluene, pyridine or DMF. Catalytic agents have been used in cyclization of open-chain precursor to avoid too drastic reaction conditions and the formation of secondary products. Tetrabutylammonium hydroxide (TBAH) is an efficient catalyst for the synthesis of 3,5-di-substituted-1,2,4-oxadiazoles starting from *O*-acylamidoxime. It allows to use of mild reaction conditions as room temperature and THF solvent. Tetrabutylammonium fluoride (TBAF) also has been used as catalyst but, in large scale synthesis, fluoride can give corrosion of reactor vessel.[13] The superbase system MOH/DMSO (M=Li, Na, K) can be applied to produce a wide range of 1,2,4-oxadiazoles

with excellent yields and in short times.[14] The application of microwave energy has been reported as another alternative method to cyclize acylamidoxime derivatives.[15] The 1,2,4-oxadiazole ring was also synthetized by cyclization of other open chain precursors different than *O*-acylamidoxime derivatives. Acylguanidines **8** in presence of iodobenzene diacetate (IDB) can undergo oxidative cyclization to generate 1,2,4-oxadiazole **9** (Scheme 3).[16] The α-β-alkynic compounds **10** undergoes Micheal-like nucleophilic attack by amidoxime to give intermediate **11**, which, under basic condition, eliminates enolate ion to give 1,2,4-oxadiazole ring **12** (Scheme 4).[17]

8 **9**

Scheme 3



10 **11** **12**

Scheme 4

3. Reactivity of 1,2,4-oxadiazoles

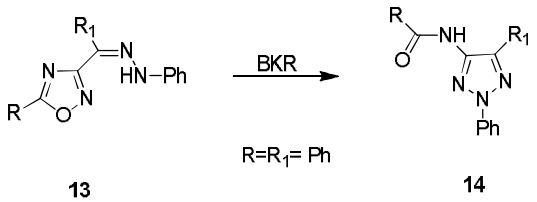
1,2,4-Oxadiazoles are multifunctional heterocyclic compounds, which can undergo different reactions.[18-20] The N(3) atom displays nucleophilic character while the carbon atoms have electrophilic properties. In addition, the 1,2,4-oxadiazole nucleus has a low level of aromaticity and the O-N bond can be reduced by opening the ring. These last two characteristics explain the numerous thermal or photochemical rearrangements to give other heterocyclic systems.

3.1. Thermal rearrangement reactions

The thermal Boulton-Katritzky rearrangement (BKR) is one of the most investigated transformation of 1,2,4-oxadiazoles (Scheme 5).[19] This reaction consists in an internal nucleophilic substitution. In this rearrangement, are involved the nucleophilic Z atom within the three atoms side chain and the N(2) electrophilic atom of the 1,2,4-oxadiazole ring. The high electrophilicity of N(2) is due to the polarized and easily cleavable O-N bond. When the Z atom attacks the N (2), oxygen acts as leaving group. The final result is the cleavage of instable O-N bound and the formation of more stable C-N, N-N or S-N bonds.

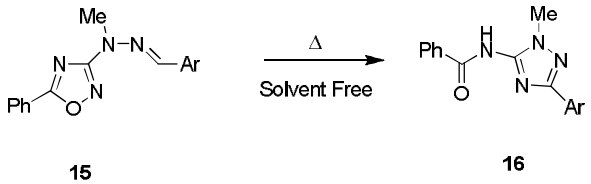
The BKR reaction can take place under different conditions and with the participation of various side-chains. 1,2,3-triazoles can be obtained when the hydrazone of the original 3-acyl-1,2,4-oxadiazole presents a CNN sequence in the side chain. In Scheme 6 is reported the BKR of the *Z*-phenylhydrazone of 3-benzoyl-5-phenyl-1,2,4-oxadiazole **13** into the corresponding 1,2,3-triazole **14**.[21]

Scheme 5. Boulton-Katritzky rearrangement of 1,2,4-oxadiazoles.



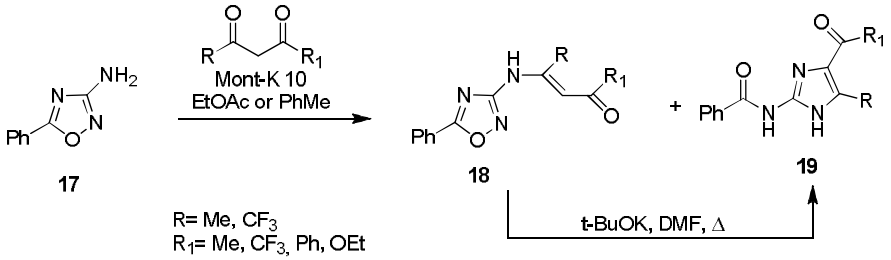
Scheme 6. Formation of 1,2,3-triazoles by BKR rearrangement involving CNN side-chain.

On the other hand, 1,2,4-triazoles **16** can be obtained starting from a *N*-1,2,4-oxadiazol-3-yl-hydrazones **15** having a NNC or NCN sequence in the side-chain.[22] In Scheme 7 is reported the first example of BKR in which a NNC side-chain is involved in the rearrangement to form 1,2,4-triazoles. In this case, the nucleophilic atom is the carbon (Z=C).



Scheme 7. Formation of 1,2,4-triazoles by BKR rearrangement involving NNC side-chain.

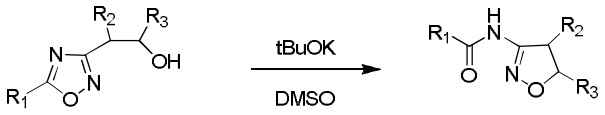
The BKR reaction has been also exploited for the preparation of imidazoles from 1,2,4-oxadiazoles with two different side-chain sequence NCC or CNC.[23,24] The *N*-(1,2,4-oxadiazol-3-yl)-β-enamino ketone **18** under basic conditions can give a BKR to generate trifluoromethylated 2-amino imidazoles **19**. The original amino-oxadiazole **17** reacts with fluorinated-β-dicarbonyl compounds under acid conditions, with Montmorillonite-K10 catalyst, to give fluorinated imidazoles (Scheme 8).



Scheme 8. Formation of fluorinated-imidazoles by BKR rearrangement.

Non-aromatic heterocycles as pyrazolines and isoxazolines can also be synthetized through BKR. The starting substrate are 1,2,4 oxadiazole derivatives substituted with a saturated side-chain. In particular, the CCO sequence is the key structural factor to produce isoxazolines (Scheme 9).[25] Mechanistic studies on BKR in the 1,2,4-oxadiazole series have evaluated the role of the reaction solvent, reaction conditions and the effect of the substituents.[21,26-28] These studies have been recently carried out in compartimentalized

micellar environment[29] and in ionic liquids.[30,31] In paarticular, ionic liquids have an important role to favor the BKR rearrangement.



20 **21**

Scheme 9. Formation of isoxazolines by BKR rearrangement.

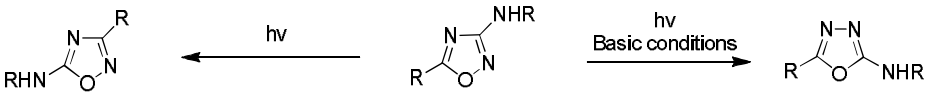
Among the thermal rearrangement, the Migration-Nucleophilic Attack-Cyclization (MNAC) is the mechanism under the base-mediated transformation of 3-acylamino-1,2,4-oxadiazoles **22** to the corresponding 2-acylamino-1,3,4-oxadiazole derivatives **23** (Scheme 10).[32]

22 **23**

Scheme 10. MNAC of 1,2,4-oxadiazoles to 1,3,4-oxadiazoles derivatives.

3.2. Photochemical rearrangements

In the last years, the photochemical reactions of 1,2,4-oxadiazoles have been object of numerous studies.[33] Generally, the labile O-N bound is involved in the photo-induced break to start intramolecular rearrangements.[34] In particular, the photo-induction generates a reactive open-chain intermediate having zwitteronic, bi-radical or nitrene like features. It can give different products depending on the reaction media and conditions. The formation of 1,3,4-oxadiazoles **25** by 3-amino-1,2,4-oxadiazoles **24** has been observed after irradiation under basic conditions. The process behind this rearrangement was named ring contraction-ring expansion route (RCE). The internal-cyclization isomerization route (ICI) is another photochemical rearrangement that allows to obtain the regioisomeric 1,2,4-oxadiazole **26** from the oxadiazole **24** (Scheme 11).



26 **24** **25**

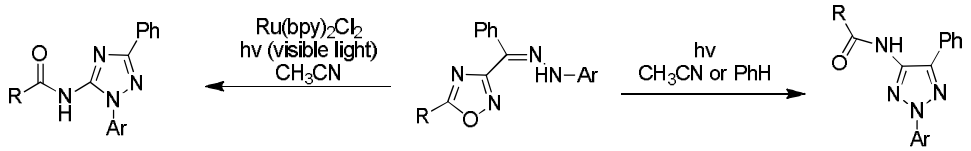
Scheme 11

1,2,3-Triazole **28** can be synthetized from the arylhydrazone **27** by photo induced BKR reaction.[34] The photo-rearrangement to the 1,2,4-triazole isomer **29** occurs when the oxadiazole **27** is irradiated with visible light in presence of ruthenium complex (Scheme 12).[35]

The photochemical reaction of 5-(pentafluorophenyl)-5-phenyl-1,2,4-oxadiazole **30** gave a quinazolinone derivative **31** through a ring opening-ring closure pattern (Scheme 13).[36]

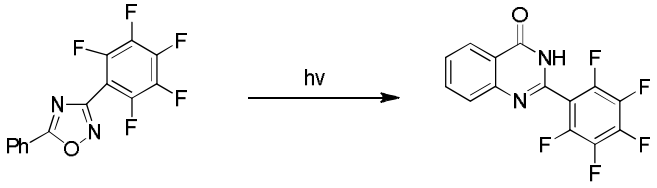
Irradiation of 3-alkenoxy-5-phenyl-1,2,4-oxadiazoles **32** gave oxazolines **35** in THF or oxazolines **36** in DCM.[37] In both cases the excitation causes the cleavage of O-N bond leading to the formation of nitrene intermediate **33**, which cyclizes to give the byciclic intermediate **34** The reduction of azirine ring in THF solvent leads to the opening of the ring and to the formation of oxazoline **35**. Conversely, chlorinated solvent

such as DCM produces acid hydrochloric under UV irradiation. Thus, HCl can mediate a nucleophilic ring cleavage of **34** to produce oxazolines **36** (Scheme 14).



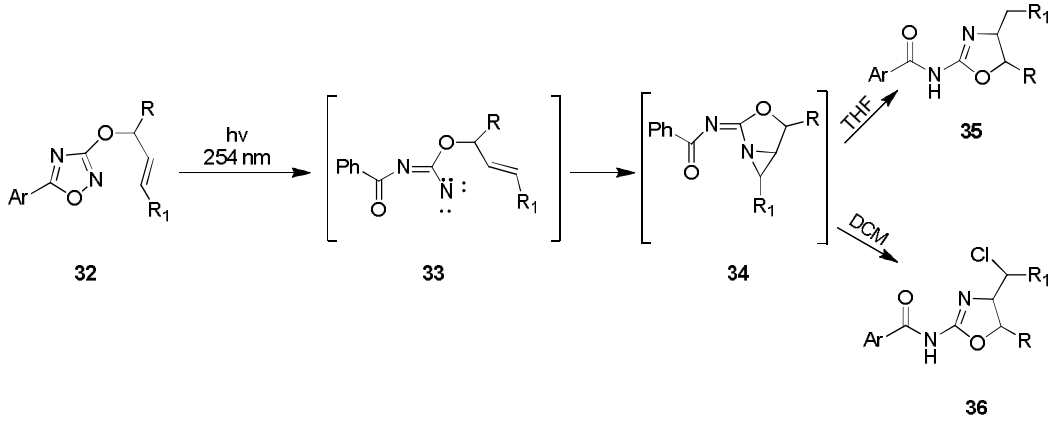
29 **27** **28**

Scheme 12



30 **31**

Scheme 13



32 **33** **34** **35** **36**

Scheme 14

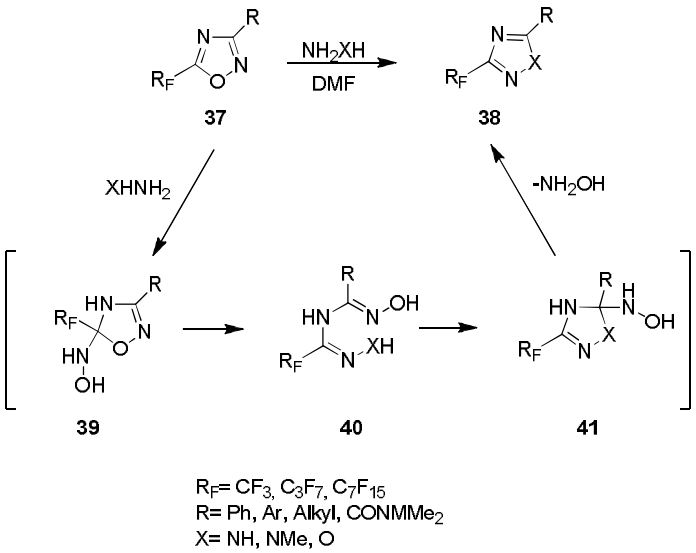
3.3. Nucleophilic aromatic substitutions (SNAr) and ANRORC rearrangements

Due the high electrophilicity of such heterocycle, nucleophilic attacks on oxadiazole ring are more common than electrophilic attacks.

The reaction of 3-chloro-5-phenyl-1,2,4-oxadiazole with methylhydrazine has been reported as a SNAr at C(3) position.[22] Other examples are the reactions of 5-aryl-3-chloro-1,2,4-oxadiazoles with allylamine[38] or allyl alcohols[39] to produce respectively 3-*N*-allylamino-1,2,4-oxadiazoles and *O*-allylether. Recently, the 5-trichloromethyl-1,2,4-oxadiazole-3-carboxylate was identified as a valid starting compound to obtain SNAr at C(5) position using several nucleophilic agents.[40] The nucleophilic substitution of C(5)-trichloromethyl group has also been used in the synthetic route of a new agonist of GPR119 receptor. In this case, the nucleophilic agent is the piperidin-4-yl-methanol.[41]

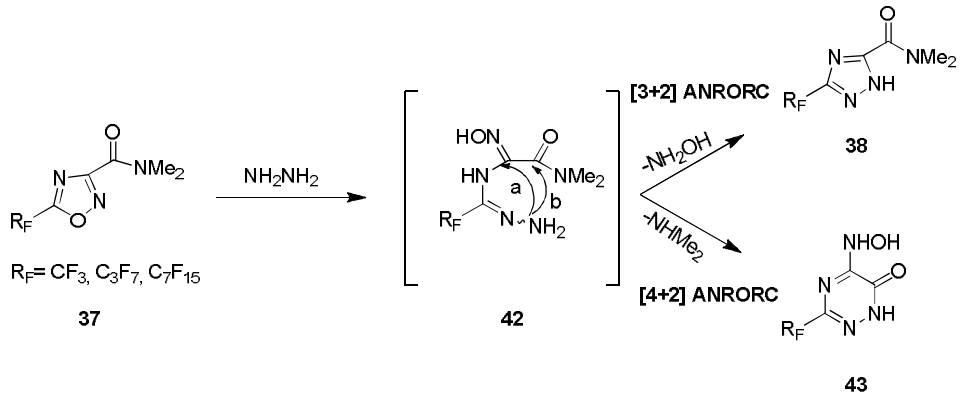
The ANRORC (Addition of Nucleophile Ring Opening and Ring Closure) rearrangement has been reported for five-membered heterocycles **37** presenting electron-withdrawing groups (EWG) like

perfluoroalkyl chains or polyfluoroaryl moieties. Indeed, this reaction involves the C(5) electrophilic position of the oxadiazole ring that is usually activated by the presence of electron-withdrawing chain. Bidentate nucleophiles as hydrazine/methylhydrazine[42] or hydroxylamine[43] attack the electrophilic C(5) position producing a ring-opening intermediate **40**. This intermediate cyclizes by attacking the electrophilic C(3) position of oxadiazole ring. Therefore, the 1,2,4-oxadiazole acts as 1,3-dielectrophiles and the ANRORC rearrangement leads to the formation of a five membered heterocycle **38** ([3+2] ANRORC) (Scheme 15). The final product is a triazole or a 1,2,4-oxadiazole regioisomer using respectively hydrazine or hydroxylamine as nucleophilic agents. When methylhydrazine was used the 3-perfluoroalkyl-1-methyl-1*H*-1,2,4-triazole **38** (X=NMe) was regio-selectively formed.[42]



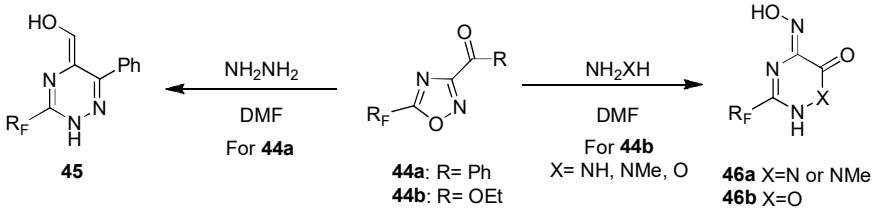
Scheme 15. ANRORC rearrangement.

1,2,4-Oxadiazoles **37** substituted with a carbonyl moiety at C(3) position can be considered as 1,4-dielectrophilic compounds and can also give a ring enlargement to a six-membered heterocycles **43** ([4+2] ANRORC) (Scheme 16).[44]



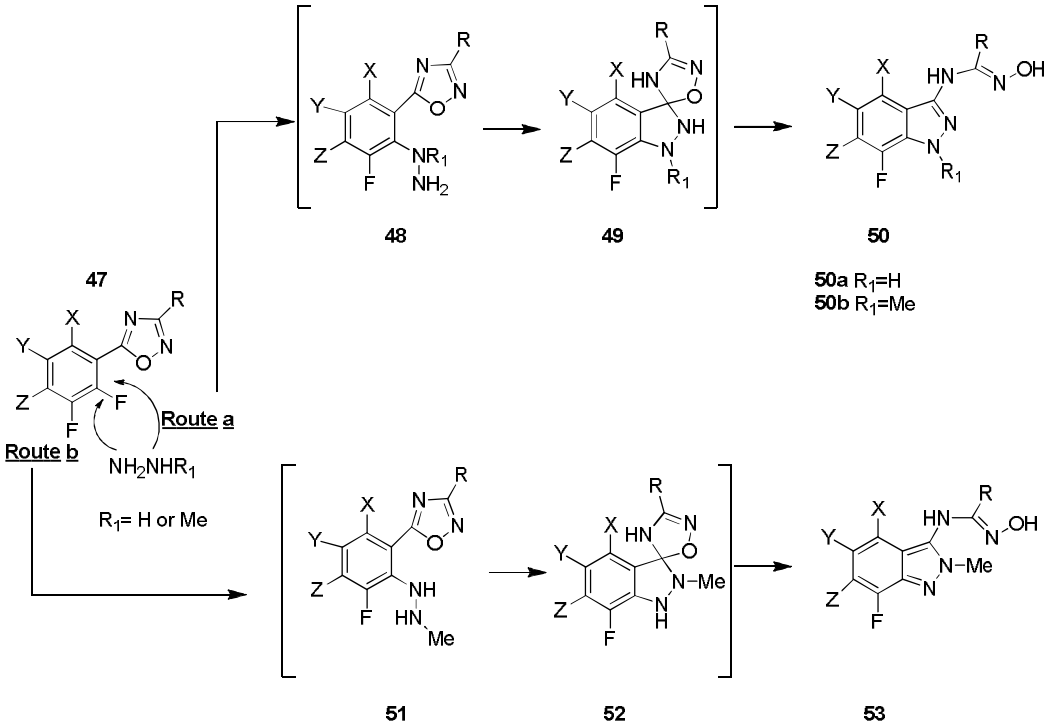
Scheme 16. [4+2] and [3+2] ANRORC rearrangements.

The 3-benzoyl-5-polyfluoroaryl-1,2,4-oxadiazole **44a** reacts only with hydrazine to produce triazines **45** by [4+2] ANRORC.[45] Conversely, the 3-ethoxycarbonyl-5-polyfluoroaryl-1,2,4-oxadiazole **44b** gave a [4+2] ANRORC with hydrazine/methylhydrazine[42] or hydroxylamine[46] to afford respectively the triazinone **46a** or the oxadiazine **46b** (Scheme 17).



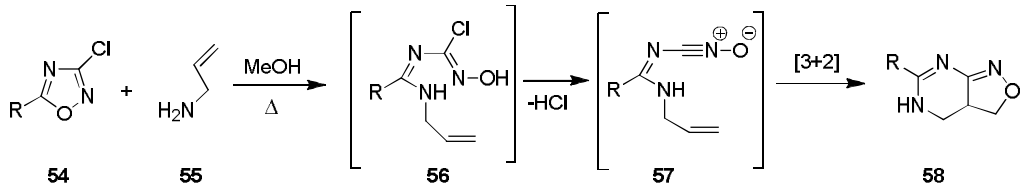
Scheme 17. [4+2] ANRORC of 3-carbonyl-1,2,4-oxadiazoles.

ANRORC-like rearrangements involving the side chain in C(5) have been proposed to explain the formation of the derivatives **50a**, **50b** and **53**, also computationally.[47] The SNAr of hydrazine or methylhydrazine at the orto position of the fluorinated oxadiazole **47** produces the intermediates **48** or **51**. Subsequently, the nucleophilic attack at the 1,2,4-oxadiazole C(5) position generates the closed intermediates **49** and **52**. In the final step, the opening of the ring gave the indazole compounds (Scheme 18). In the reaction with methylhydrazine, the regioselectivity depends on which of two nucleophilic nitrogens is involved (Route a or Route b in Scheme 18).



Scheme 18. ANRORC-like rearrangement of polyfluoroaryl-1,2,4-oxadiazoles.

It was reported that also 3-chloro-1,2,4-oxadiazoles, due to the electrophilic properties of C(5) position, can undergo the ANRORC-like rearrangement.[38] For example, the derivative **54** reacts at C(5) position with allylamine **55** to give the opening intermediate **56** which eliminates HCl to form the nitrile-oxide **57**. Finally, the heterocyclic system tetrahydro-isoxazolo-[3,4-d]-pyrimidine **58** is generated by [3+2] cycloaddition reaction (Scheme 19).



54 55 56 57 58

Scheme 19. ANRORC rearrangement of a 3-chloro-1,2,4-oxadiazole.

4. Biological properties of 1,2,4-oxadiazoles

The biological role of 1,2,4-oxadiazoles and its importance in drug design have been highlighted since the 1950s.[48] The 1,2,4-oxadiazole Oxolamine (Figure 2), reported in the 1960s, is the first drug placed on the market having cough suppressant activity.[49] In the last years, the medicinal chemistry has given great importance to the 1,2,4-oxadiazole nucleus as isoster of amide and ester groups. Indeed, it was considered as a pharmacophore component in the development of biological interesting compounds. The most recent biological applications of the 1,2,4-oxadiazoles will be discussed below.

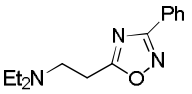


Figure 2. Oxolamine (brand name Parebron).

4.1. Antimicrobial agents

In recent years, the phenomenon of the multiple drug resistance of bacteria and fungi to existing therapeutic agents has represented a challenge in clinical practice.[50] To overcome this problem, medicinal chemistry carried out various studies to develop new antibacterial agents with a broad spectrum of action, high potency and without systemic side effects. 1,2,4-Oxadiazoles, together with other heterocyclic compounds containing nitrogen and oxygen atoms, have received particular attention as antibacterial agents (Figure 3).

Following the observation that quinoline-based oxadiazole and chromene based enones possess antibacterial activity, novel cromene-based oxadiazoles **59** were designed by a reaction between cromene-based amidoximes and carboxylic acids.[51] The synthesis of hybrid compounds **59** allows to combine two active entities (oxadiazole and chromene) to obtain a more potent final compound. Moreover, the chromene unit has a lipophilic nature important to cross the bacterial cell membrane. In vitro studies indicated that the compounds possess, similarly to gentamicin, a good antibacterial activity on *Escherichia coli* (MTCC614) and *Klebsiella pneumoniae* (MTCC4031). Molecular docking studies showed an interaction through hydrogen bond with the bacterial DNA gyrase of *E. Coli*. DNA gyrase and topoisomerase IV are enzymes important for replication of bacterial DNA and their ATPase domains are recognized as valid targets in antibiotic therapy. To date, no natural or synthetic compound is used as an inhibitor of their ATPase activity.

Compound **60** is a result of optimization studies of a pyrrolamide compound, a promising inhibitor of DNA gyrase with not good pharmacokinetic. Indeed, thiazole-piperidine motif was substituted by phenyl-oxadiazole scaffold to obtain a new inhibitor of ATPase activity of DNA gyrase and topoisomerase from *E. Coli*.[52]

To obtain a synergistic effect against bacterial species, the compound **61** was designed linking at C-3 position of the 1,2,4-oxadiazole ring a quinoline-morpholine moiety.[53] Indeed, morpholine scaffold is

present in several biological active compounds among these *Linezolid*, an approved antibiotic agent for Gram positive pathogens.[54] Similarly, some quinoline-derivatives possess antibacterial activity.[55] The evaluation of antibacterial and anti-fungal activity revealed that some of these derivatives possess good activity against gram positive or gram negative organisms (*Streptococcus pneumonia, Clostridium tetani, Escherichia coli, Vibrio cholerae*) while others have antifungal activity against *Candida albicans*.[56]

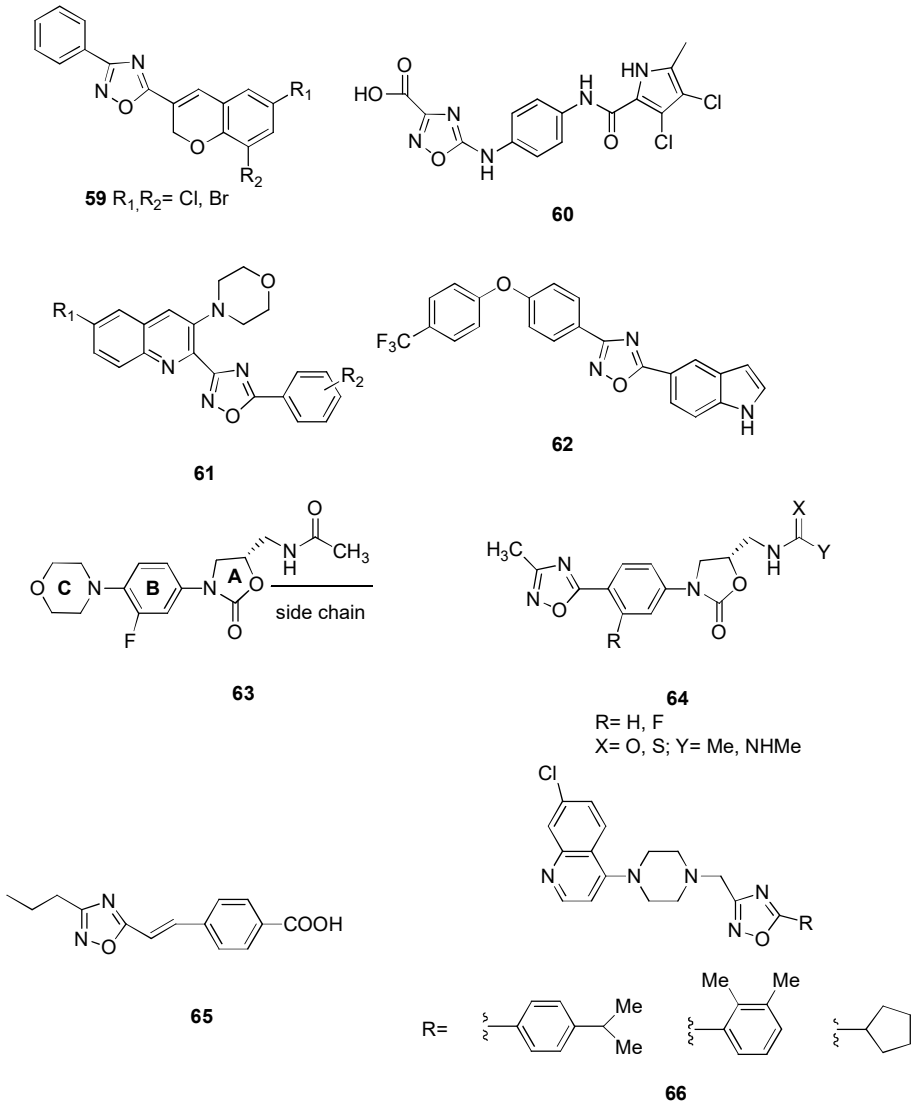


Figure 3. Antibacterial 1,2,4-oxadiazoles.

Staphylococcus Aureus is a pathogen linked to many infections but unfortunately have been reported numerous cases of antibiotic resistance to methicillin (MRSA-Methicillin Resistant Staphylococcus aureus). Docking studies made it possible to discover a new class of oxadiazole antibiotics.[57] They, as well as β-lactam antibiotics, are inhibitors of penicillin binding protein 2a of MRSA and therefore they are

bactericidal interfering with biosynthesis of cell wall. SAR study of a series of oxadiazole derivatives showed that compound **62** is the best antibiotic agent; its indole ring is important to circumvent toxicity and to exert the antibacterial activity. Moreover, **62** is active in mouse model and it is orally bioavailable exhibiting a long half-life time and a high volume of distribution.

In addition to MRSA, there are several infections caused by multi-resistant Gram positive bacterial as Vancomycin-Resistant Enterococci (VRE). *Linezolid* **63** is an approved antibiotic agent belonging to oxazolidinones compounds, a class of derivatives active against multidrug-resistant bacteria. To overcome the nascent resistance to *Linezolid*, the structure of this drug was modified to provide new analogues with improved activities. Potent anti-bacterial compounds were synthetized by replacing the morpholino moiety of Linezolid **63** with a 3-methyl-1,2,4-oxadiazol-5-yl group.[58] Instead, the substituent on the C(5) side-chain of oxazolidinone was replaced to obtain a series of new analogues **64**.[59] The replacement of the morpholine moiety with the oxadiazole ring gave a derivative active against *S. Aureus* both methicillin-susceptible and methicillin-resistant. In silico study showed that linezolid-like 1,2,4-oxadiazoles bind ribosomal subunit U2585 as well as the morpholinic ring. The presence at the C(5) side chain of acetamidomethyl or thioacetoamidomethyl groups is essential for the activity. Conversely, the fluorination of ring B have slight effects on antibacterial activity but reduce the cytotoxicity. Substitution of the oxazolidinone ring with a 1,2,4-oxadiazole produces low potency.[60]

The styryl oxadiazole derivative **65** displayed potent anti-tubercular properties (IC_{50}=0.045 μg/ml). Molecular docking studies revealed a good affinity of **65** for mycobacterial enoyl-ACP reductase an essential enzyme to the construction of mycobacterial cell wall.[61] Since the antitubercular activity of the quinoline and piperazine scaffold is known, 1,2,4-oxadiazole ring has been conjugate with these two structural motifs to design new potent antitubercular agents **66** (IC_{50}=0.25-0.50 μg/ml).[62]

4.2. Antitumor agents

Cancer is one of the leading causes of death worldwide and numerous efforts have been made to develop new chemotherapeutic agents able to act selectively on malignant cells saving health ones.[63] Several research groups highlighted the cytotoxic activity of 1,2,4-oxadiazoles against different cancer cell lines and some papers identified the mechanism underling this effect (Figure 4). Compounds with general structure **67** are analogues of Nortopsentin an alkaloid, isolated from a marine sponge *Spongsorites ruetzleri*, having significative antiproliferative activity.[64] The central imidazole ring of the lead compound Nortopsentin was replaced by 1,2,4-oxadiazole motif while one of lateral indoles was substituted by 7-azaindole known to enhance the cytotoxic activity of Nortopsentin derivatives. These compounds were pre-screened against HCT-116 (colon rectal carcinoma cell line) and lather against MCF-7 (human breast cancer), HeLa (cervix adenocarcinoma) and CaCo2 (colorectal carcinoma) cell lines showing a cytotoxicity at sub-micromolar concentrations. Compounds **67** cause cell cycle arrest in G0/G1 phase and cell death by apoptosis, as demonstrated by phosphatidylserine exposure. Quinoline derivatives with a fused-six members heterocyclic structure received the attention of chemists and biologists due to the broad spectrum of biological properties. Lenvanitib is an approved anticancer drug presenting a quinolone moiety. By conjugation of quinoline scaffold with 1,2,4-oxadiazole ring was obtained a library of 1,2,4-oxadiazole quinolone functionalized derivatives.

Compounds **68** are anticancer agent more potent than Etoposide against four cancer cell lines MCF-7 (breast), A549 (lung), DU-145 (prostate) and MDA MB231 (breast).[65]

1,2,4-Oxadiazoles **69** are potent Sirtuin-2 inhibitors. Sirtuins are a family of lysine deacylases that act on various proteins involved on several biological processes, including cell survival and cancer. The role of Sirt-2 in cancer progression is controversial but its pharmacological inhibition resulted in an anti-cancer effect. Compounds **69** were tested in Leukemia cell lines showing an induction of apoptosis and an antiproliferative effect at 10 or 25 μM.[66] DNA-Topoisomerases are the enzymes responsible of topological changes of the DNA conformation and they are one of the targets in anticancer therapy. These enzymes require Mg^{2+} ions and energy, derived from ATP hydrolysis, to cleave and manipulate DNA strands. The Topo II inhibitors are used in clinical practice but they have numerous side effects as cardiotoxicity and induction of secondary malignancies. Therefore, the development of new catalytic inhibitors binding ATP

site of TopoII is a valid strategy to overcome these problems. A new class of 3,5-substituted 1,2,4-oxadiazoles showed in docking simulations a good ability to bind the ATP site.[67]

Compound **70** binds isolated TopoII ATPase domain and was the best candidate in inhibition assays. Moreover, displayed cytotoxic activity towards MCF-7 cancer cells. The targeted therapy approach identified the hypoxic environment as a feature of solid tumors responsible of tumor progression. Carbonic anhydrase (CA) is an enzyme overexpresses in hypoxic tumors, as colorectal cancer, and its inhibitors are evaluated to develop new therapeutic strategies. Several research groups evaluated compounds with sulphonamide scaffold as anti-cancer agents and inhibitors of CA.

Compound **71**, obtained conjugating the sulphonamide moiety with 1,2,4-oxadiazole ring and thiophene group, exerts an inhibitory activity on CA from hypoxic cancer.[68] Moreover, it has an antiproliferative effect against HCT116 cells (colorectal cancer); it also induces apoptosis, caspase-3 activation, overexpression of ROS and inhibition of metastasis. The epidermal grow factor receptor (EGFR) and C-Met are two tyrosine kinase receptors implicated in oncogenesis and they are over-expressed in different type of cancer.

Compound **72** showed a capacity to suppress the expression of both receptors in tumors resistant to third-generation Tyrosin Kinase inhibitors. It also exhibits antiproliferative activity associated to the arrest of cellular cycle in G2/M phase.[69]

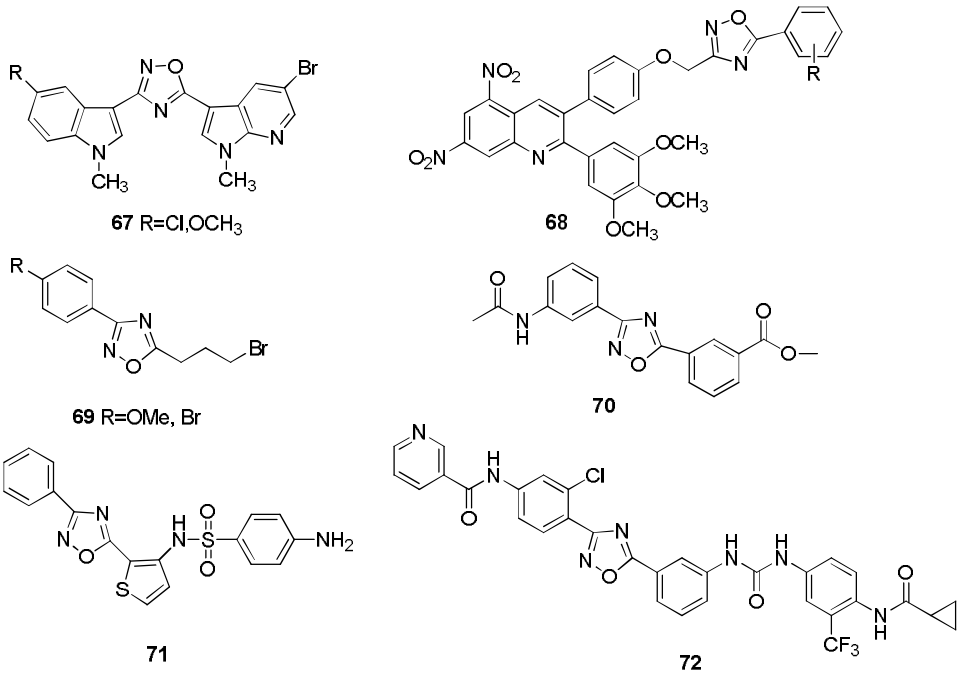


Figure 4. Antitumoral 1,2,4-oxadiazoles.

4.3. Anti-inflammatory and analgesic agents

Inflammation is a natural response of human organism to injury stimuli and infection. An excessive inflammation state represents a pathological factor in various chronic diseases such as diabetes, cancer, arthritis, atherosclerosis and also neurodegenerative disorders.[70-72] Inflammatory response is a multifactorial process involving numerous components of immune system, such as macrophage and monocyte cells as well as cytokines (TNFα, interleukins), nitric oxide or metalloproteinase.[73-76] Particularly, the activation of nuclear factor-kB (Nf-kB), in immune innate cells, mediates the expression of pro-inflammatory factors. Therefore, inhibitors of Nf-kB pathway can be considered as potential therapeutic agents in inflammation

disease. Several 1,2,4-oxadiazoles are anti-inflammatory compounds and inhibitors of NF-kB activity (Figure 5).[77]

Also, derivatives with indole moiety showed anti-inflammatory effects through inhibition of NF-kB. Instead, both these structural features have been considered for the synthesis of compound **73**.[78] It showed a good inhibitory activity of NF-kB and a reduction of nitric oxide release in LPS-stimulated cells. In chronic diseases mentioned above, the inflammatory process is accompanied by oxidative stress due to the production of reactive oxygen species as hydroxyl, peroxyl, superoxide radicals. Instead, a valid therapeutic approach consists in the use of compounds as polyphenols with anti-inflammatory and antioxidant properties. The polyphenol resveratrol is an antioxidant compound with a stilbene structure. It was demonstrated that the *E* configuration of ethylene bridge together with the hydroxy/methoxy substitution of aromatic ring are essential for the known properties of resveratrol. The *E* stilbene bridge has been replaced with 3,5-diphenyl-1,2,4-oxadiazoles to maintain the geometry of molecule similarly to those of *E* form.

In this way have been obtained a series of resveratrol analogues and the derivative **74** is a more powerful anti-inflammatory and antioxidant agent than resveratrol.[79] It possesses ROS scavenger ability, reduces LPS induced release of pro-inflammatory factors and exerts anti-inflammatory activity inhibiting NF-kB pathway. The non-steroidal drugs (NSAIDs) are the most used as anti-inflammatory and analgesic agents. These drugs inhibit the COX enzymes responsible of metabolic transformation of acid arachidonic to prostaglandins. The traditional NSAIDs have several side effects which include gastrointestinal toxicity due to the COX1 inhibition. COX1 is a constitutive form responsible of the production of prostaglandins that are essential for the integrity of gastric mucosa. Instead COX2 is an inducible form expressed in presence of inflammatory stimulus.[80] Therefore, the development of novel COX2 selective anti-inflammatory agents represents a research area of great interest. The bicyclic benzoxazoles ring was identified in different COX2 inhibitors.

The derivative **75**, with the benzoxazole linked to the oxadiazole, showed a good docking score with COX2 and was found to be a potent antioxidant compound.[81]

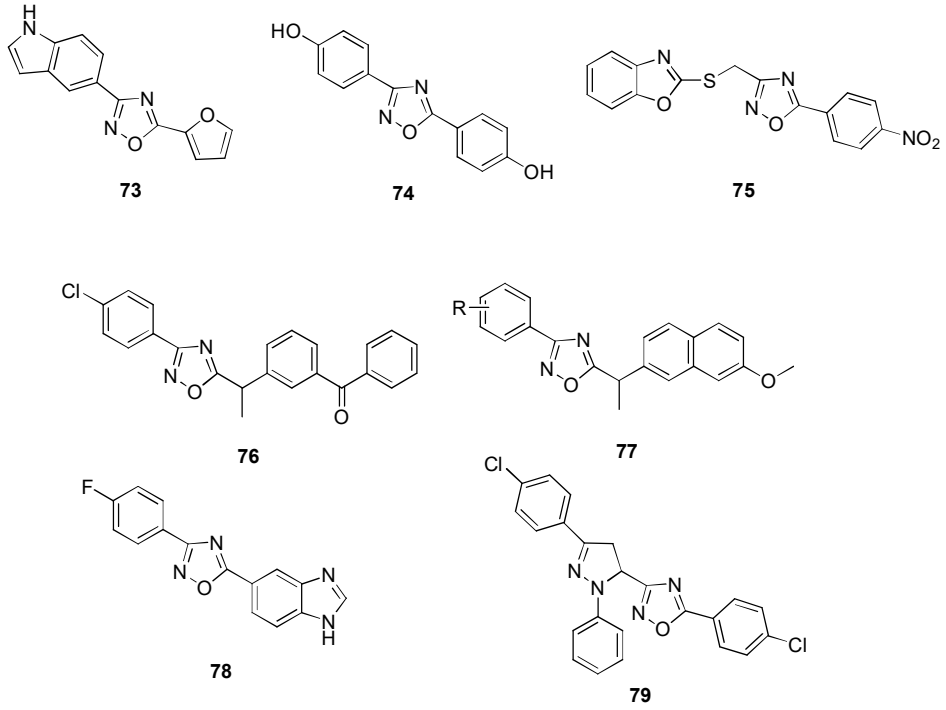


Figure 5. Anti-inflammatory and analgesic 1,2,4-oxadiazoles.

Among the selective COX2 inhibitors there are ketoprofen and naproxen analogues (compound **76** and **77** respectively) incorporating 1,2,4-oxadiazole ring. In these derivatives the carboxylate function of original drugs was modified to improve safety profile and reduce side effects. The compound **76** showed higher anti-inflammatory activity compared to ketoprofen. It has analgesic activity and selective activity toward COX2 with reduction of GI toxicity.[82] The naproxene analog **77** showed inhibitory activity against COX2 and lipoxygenase (15-LOX). Therefore, this compound interferes with the production of leukotrienes, other important mediators of inflammation process.[83]

Compound **78** was discovered as activator of Nuclear Factor erythroid 2 (Nrf2) a transcription factor normally bound to its inhibitor Keap 1. In presence of inflammatory and oxidant stimulus, Nrf2 detaches from Keap1, translocates to the nucleus and upregulates expression level of Nrf2 and protective gene against oxidative stress.[84] Also the pyrazoly-1,2,4-oxadiazole **79** was evaluated as anti-inflammatory compound and it showed a good activity compared to standard drug.[85]

4.4. Neuroprotective agents

Oxadiazole scaffold has also been identified in several compounds with neuroprotective activity (Figure 6). Among the neurodegenerative disorders, Alzheimer's disease is the most frequent form of dementia. The cognitive decline is accompanied by an increase accumulation of disordered and aggregated peptide Aβ and Tau in the brain. Currently, therapies can act only on symptomatic aspects but cannot blocks the progression of disease. Therefore, the development of novel drugs against amyloidosis is a present challenge for chemists.

The oxadiazole **80** is a curcumin-like derivative obtained through replacement of β-diketone group of curcumin with 1,2,4-oxadiazole ring. It was identified from a library of curcumin-like compounds able to bind Aβ-peptide in molecular docking simulation. Subsequently, biophysical techniques showed an interference of this compound on aggregation pattern of amyloid peptide.[86]

The 3-(4'-trifluoromethylphenyl)-5-(4'-methoxyphenyl)-1,2,4-oxadiazole **81** when is irradiated interfere with Aβ-fibrillation.[87] Moreover, the oxadiazole ring reduces the cytotoxicity of Aβ on LAN5 cells.

The glycogen synthase kinase (GSK-3β) is the enzyme involved in hyperphosphorylation of Tau. Hyperphosphorylated Tau detaches from microtubule and can aggregate to form neurofibrillary tangles (NFTs). Moreover, the activity of GSK-3β is also associated with the inflammation, oxidative stress and insulin resistance observed in the brain of Alzheimer's disease patients. Therefore, inhibitor compounds of GSK-3β are designed to become new candidates in Alzheimer's disease therapy. Oxadiazole and pyridine rings, known to have inhibitory activity on GSK-3β, were conjugated with a benzenesulfonamide motif presents in antioxidant and anti-inflammatory compounds. The obtained derivative **82** exerts a good inhibition of GSK3 β accompanied by an increase of the consumption of glucose in cells, an antioxidant and anti-inflammatory effect. In vivo experiments showed that it improves cognitive impairment in mouse model.[88]

Marine natural products attracted a growing interest from medicinal chemists for their broad spectrum of biological activity. Phidianidines represent a new class of biologically active 1,2,4-oxadiazoles alkaloids deriving from the marine mollusk *Phidiana militaris*. Considering their biological role, a series of phidianidine derivatives was synthetized. In particular, compounds **83** protected SHSY5Y cells from oxygen-glucose deprivation and oxidative stress, and exhibited in vitro neuroprotection against $A\beta_{25\text{-}35}$ toxicity.[89]

Coumarin/1,2,4-oxadiazole hybrids **84** have been evaluated for cholinesterase inhibitory activity. They exhibited inhibitory activity toward BChE over AChE and a neuroprotective effect against Aβ-neurotoxicity in SHSY5Y cells. Therefore, these compounds can be considered to treat cognitive problems associated to decline of cholinergic function.[90]

Parkinson's disease is the second common form of neurodegenerative diseases worldwide. Pathological features are the degeneration of dopaminergic neurons, neuroinflammation and oxidative stress. Oxadiazole **85** showed therapeutic effects in MPTP-induced Parkinson's disease mice.[91] It attenuated the induced dopaminergic neuronal loss and exerted an antioxidant effect in PC12 neurons. Moreover, it inhibited the inflammasome activity through activation of Nrf2-ARE signal and, as evidenced by pharmacokinetic studies, it was mostly distributed to brain tissue.

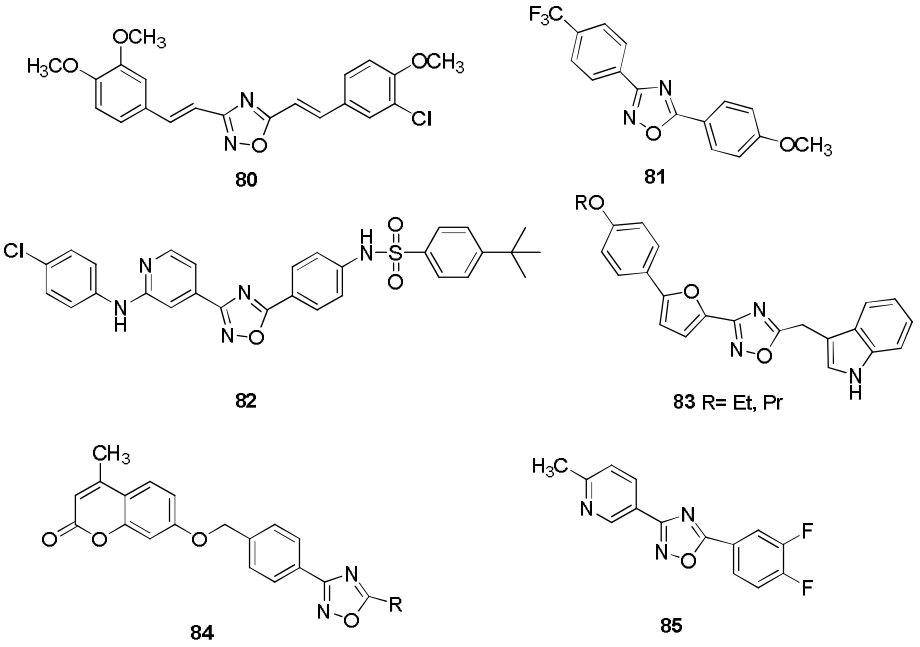


Figure 6. 1,2,4-Oxadiazoles as neuroprotective agents.

4.4. Antidiabetic agents

Diabetes is a multifactorial metabolic disorder characterized by failure of glucose homeostasis due to the deficit of insulin secretion or to the tissue resistance of its action. Diabetes of Type 2 represent the 90% of diabetic cases and it is often associated with an increased risk of developing cardiovascular diseases.[92] The therapeutic approach consists in the correct diet, physical exercise and in several cases in the administration of hypoglycemic drugs.[93] The currently employed oral drugs exhibit various side effects as gastrointestinal symptoms, hypoglycemia, edema or weight gain. Therefore, the development of new more potent drugs with few side effects represent an important research area. To control the postprandial hyperglycemia a valid therapeutic approach is the inhibition of gastrointestinal α-glucosidase or α-amylase enzymes. They are involved in the hydrolytic reaction of carbohydrates in monomeric units (α-glucose) that can be absorbed through the intestinal wall. Several natural or synthetic products have been reported as α-glucosidase inhibitors.[94] Among synthetic compounds, 1,2,4-oxadiazole ring were reported as hypoglycemic agents (Figure 7).[95]

The 3-morpholine/pyperidine-substituted 1,2,4-oxadiazoles **86** and **87** showed greater inhibition of α-glucosidase and α-amylase enzymes than acarbose as standard drug.[96] Peroxisome proliferator-activated receptors (PPARs α and γ) are nuclear receptors involved in glucose homeostasis. In particular, thiazolidinediones are a class of oral hypoglycemic drugs agonist of PPAR γ; they act by increasing the insulin sensitivity in muscles. The PPAR α receptor has a role in lipid metabolism and therefore in the reduction of diabetic complications, therefore can be evaluated as a therapeutic target in diabetes.

The 1,2,4-oxadiazoles **88** were designed and screened as dual agonist of PPARs receptors. These compounds possess a pharmacophore acid group indispensable to interact with the receptors, linked with a lipophilic part constituted by oxadiazole/aryl rings. In silico and in vitro studies evidenced that these molecules are more potent than pioglitazone for both PPARs. In vivo, they reduced glucose and cholesterol levels in diabetic rats.[97] Recently, the G-protein bile acid receptor 1 (GRBAR1) has been identified as a prominent target to treat metabolic and inflammatory disorders as type 2 diabetes. This receptor binds bile acid, released during digestion, and mediates multiple metabolic process. In intestinal enteroendocrine L

cells, it stimulates the expression of the incretin GLP1 and consequently an increase of insulin sensitivity and a reduction of glucagon release.

Oxadiazole **89** was discovered as a non-steroidal agonist of GPBAR1. It induces expression of pro-glucagon molecules and in molecular dynamics simulations it binds GRBAR1 similarly to bile acids.[98] Instead, the compound **90** was evaluated trough in vitro and in vivo assay as agonist of G-protein coupled receptor 119 (GPR119). This receptor, as well as GRBAR1, is highly expressed in intestinal L cells and pancreatic β-cells and mediates GLP1 and insulin secretion.[99] Protein tyrosine phosphatase 1B (PTP1B) is a negative regulator in insulin signal transduction. Therefore, inhibitor molecules could be used to treat type 2 diabetes.

Compounds **91**, derived from marine indole alkaloid Phidianidine, have been synthetized and they showed inhibitory potency and selectivity on PTP1B.[100]

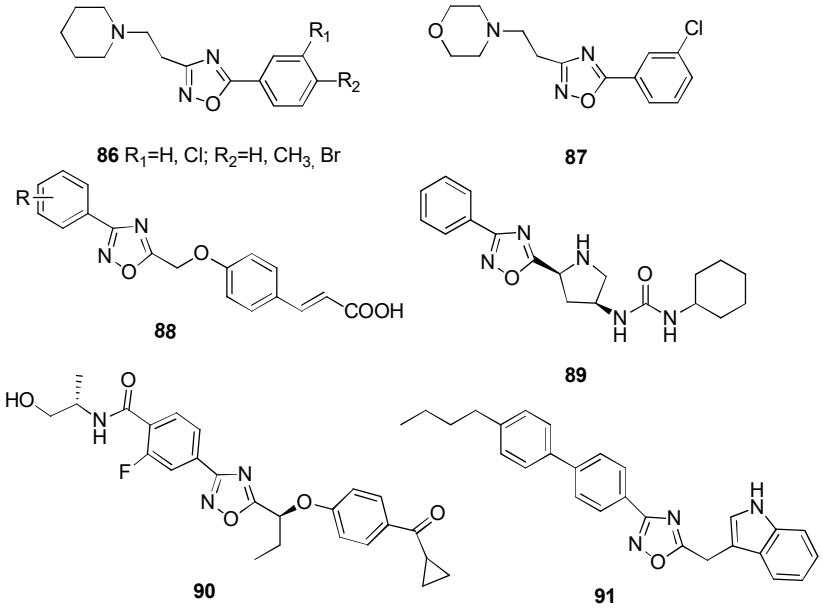


Figure 7. Antidiabetic 1,2,4-oxadiazoles.

4.5. Read-through promoters

Ataluren **92** (Figure 8), also known as PTC124, is a fluorinated 1,2,4-oxadiazole approved for the treatment of Duchenne muscular dystrophy, a genetic disorder due to nonsense mutations.[101] In this type of point mutations, a DNA base is substituted with another one to code for a premature termination codon (PTC) in the mRNA. Ribosomes read this codon as early termination signal and translates for a truncated and non-functional protein. Ataluren and other oxadiazole analogues can be considered as valid agents to treat genetic pathologies thanks to their ability to act in the translation phase. Translational readthrough drugs (TRIDS), allow to bypass the PTC sequence to obtain a full and functional protein. The nonsense mutation in the CF transmembrane regulator (CFTR gene) is the cause of cystic fibrosis.

New Ataluren derivatives **93** have been synthetized through the esterification of the carboxylic group and by varying the position and number of fluorine atoms on the aromatic ring. These compounds showed an improved readthrough activity than PTC124 and an increased expression of CFTR protein in IB3.1 cells.[102] Moreover, a series of new derivatives, without fluorine and carboxylic group, have been selected from a database using a ligand based virtual screening.

Compound **94** showed a readthrough activity comparable with Ataluren and it also increases the CFTR expression as evaluated by immunofluorescence and western blot assay.[103]

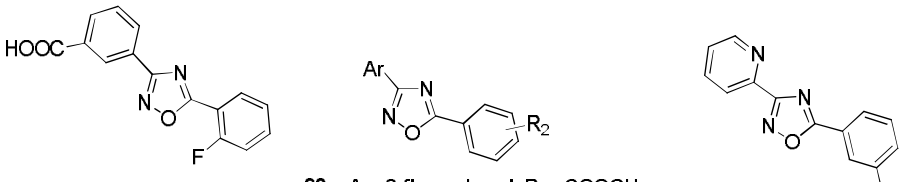


Figure 8. 1,2,4-Oxadiazoles with read-through promoting activity.

4.6. Miscellaneous properties

Several 1,2,4-oxadiazoles exhibit an antihypertensive activity. Some of them are under clinical evaluation or are already used in therapy (Figure 9).

Azilsartan **95** is a marketable antihypertensive agent acting as antagonist of Angiotensin II on Angiotensin I receptor. It was obtained by substitution of tetrazole ring of Candesartan with a 5-oxo-1,2,4-oxadiazole and it was approved since 2011 for the treatment of hypertension.[104]

Compound **96** is an analogue of azilsartan showing an indole substituted group in place of the diphenyl scaffold. The in vitro biological evaluation evidenced a high affinity of **96** for AT1 receptors. On spontaneously hypertensive rats, compound **96** induced a potent and long lasting anti-hypertensive effect accompanied by a good safety profile.[105] Currently SEW2871, an agonist of bioactive lipide-sphingosine-1-phospate (S1P), is an oxadiazole compound in clinical use. S1P has a pivotal role in immune cells trafficking. Moreover, it is involved in various pathological process related to the inflammation, atherosclerosis, diabetes, obesity, osteoporosis, tumor and Alzheimer's disease.[106] SEW2871 has been approved in the treatment of spontaneous autoimmune polyneuropathy, emphysema and diabetic nephrophaty.[107]

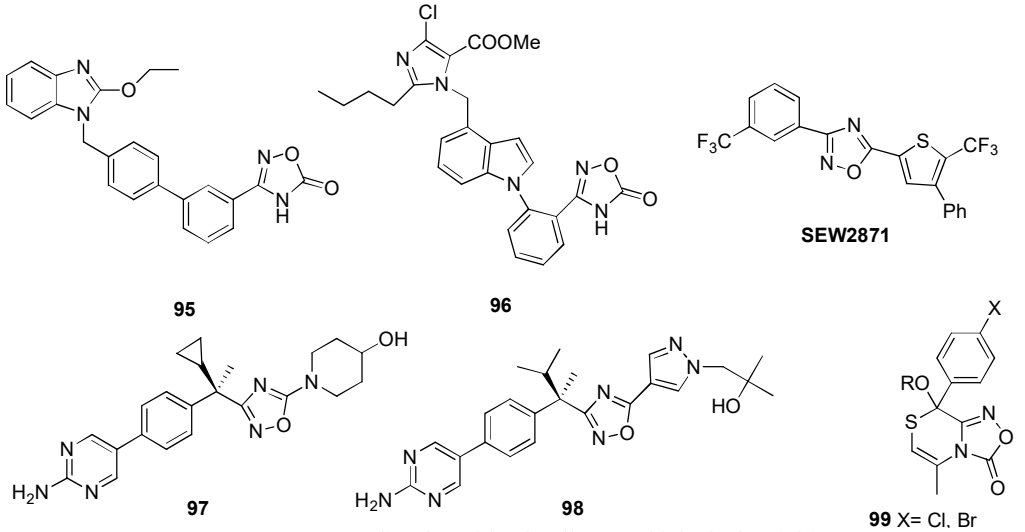


Figure 9. 1,2,4-Oxadiazoles with miscellaneous biological activities.

The SAR exploration of some oxadiazole derivatives revealed that compounds **97** and **98** are the best candidates as FLAP inhibitors. Subsequently at the inflammatory signal, arachidonic acid is released from cell membrane and transfers trough FLAP to 5-lipoxygenase to start leukotriene synthesis.[108] Therefore, the inhibition of FLAP activity can be considered a valid approach for the treatment of inflammation and

cardiovascular diseases. Moreover, these compounds showed good physicochemical properties and a great pharmacokinetic profile in rats.

Compounds **99** are diltiazem-like P-glycoprotein inhibitor recently proposed as inhibitors of drug resistance blocking ABC efflux pumps.[108]

5. Conclusion

Despite more than 130 years from its discovery, the 1,2,4-oxadiazole ring continues to attract the attention of researchers. The peculiar features and the many applications as drug gave a great importance to this pentatomic heterocycle. In the next future, more efficient synthetic procedures will be needed in order to support the increasing interest toward this ring into drug discovery programs. Moreover, the deep knowledge of the reactivity could improve the prediction of the metabolic fate of 1,2,4-oxadiazole-containing drugs.

Acknowledgement

University of Palermo is gratefully acknowledged for financial support.

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