

Benzotriazole: an overview on its versatile biological behaviour

Questa è la versione Pre print del seguente articolo:

*Original*

Benzotriazole: an overview on its versatile biological behaviour / Briguglio, Irene; Piras, Sandra; Corona, Paola; Gavini, Elisabetta; Nieddu, M; Boatto, Gianpiero; Carta, Antonio. - In: EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY. - ISSN 0223-5234. - 97:(2015), pp. 612-648. [10.1016/j.ejmech.2014.09.089]

*Availability:*

This version is available at: 11388/61218 since: 2022-05-30T12:17:12Z

*Publisher:*

*Published*

DOI:10.1016/j.ejmech.2014.09.089

*Terms of use:*

Chiunque può accedere liberamente al full text dei lavori resi disponibili come "Open Access".

*Publisher copyright*

note finali coverpage

(Article begins on next page)

# BENZOTRIAZOLE: AN OVERVIEW ON HIS VERSATILE BIOLOGICAL BEHAVIOURS

I. Briguglio, S. Piras, P. Corona, E. Gavini, M. Nieddu, G. Boatto, A. Carta\*

*Department of Chemistry and Pharmacy, University of Sassari, Via Muroni 23/A, 07100 Sassari,*

*Italy*

## ABSTRACT

Discovered in late 1960, azoles are a class of heterocyclic compounds that constitute the largest group of available antifungal drugs, and the imidazole ring is the synthetic component that confers activity to azoles. Triazoles are obtained by a slight modification of this ring and present similar or improved activity and less adverse effects. Consequently it is not too surprising that benzimidazole/benzotriazole containing molecules and benzimidazole/benzotriazole derivatives have been found to be biologically active. Since benzimidazole has been widely investigated, this review is focalized on define the place in biomedical research of benzotriazole derivatives, highlighting their versatile biological properties, the mode of action and Structure Activity Relationship (SAR) studies for a variety of antimicrobial, antiparasitic, and even antitumor, choleric, cholesterol-lowering agents.

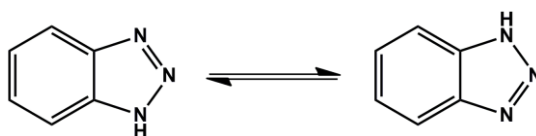
**Keywords:** 1H-benzo[d][1,2,3]triazole; antimicrobial; antiprotozoal; antiviral; antimycobacterial; anti-tubulin.

## Corresponding author:

\* A. Carta, phone +39 079 228722; fax +39 079 228720; e-mail [acarta@uniss.it](mailto:acarta@uniss.it)

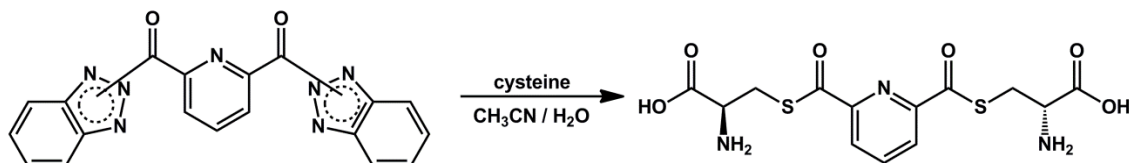
## INTRODUCTION

1H -benzo[d][1,2,3]triazole (Fig. 1), in this context more simply called benzotriazole (BT), may be considered for its several pharmacological activities as privileged structures, and potentially useful as scaffold for the design of new pharmacologically active compounds, undergoing rapid development in the synthesis of heterocycles.



**Fig. 1.** Chemical structure of 1H-benzo[d][1,2,3]triazole.

From a purely chemical point of view, the benzotriazole structure proved extremely versatile applicability, for instance being used as a intermediate [1-5] or as a good leaving group after reaction with a variety of carbonyl groups [6-9]. An example of his use as a reaction intermediate is given by the use that some researchers have done of the acylbenzotriazole methodology, taken from Katrizsky and collaborators. [10]. The *N*-acylbenzotriazole is an easy-to-handle acylating agent for advantageous N-, O-, C- and S-acylations, and dicarboxylic benzotriazole derivatives were used to obtain new peptidomimetic macrocycles, (Fig. 2), that showed antibacterial properties [11].



**Fig. 2.** BT as leaving group.

The benzotriazole, in fact, can be considered a good leaving group, but also acts as an electron-donor and a precursor of radicals or carbanions. It is easily insertable into other chemical structures through a series of reaction, such as condensation, addition reactions and benzotriazolyl-alkylation [12-14]. Some authors have also reported the synthesis of stable nitrenium ions using benzotriazole as synthon [15].

However, the main interest on BT is focused in the pharmaceutical field, as suitably substituted benzotriazole derivatives can boast the most diverse pharmaceutical properties, including plant-growth regulator [16-19], choleric [20], antibacterial [21], antiprotozoaric [22], antiviral [23] and antiproliferative [24] activities.

### BENZOTRIAZOLE AS ANTIMICROBIAL AND ANTIPROTOZOAL AGENT

Antimicrobial activity of benzotriazole derivatives has been extensively investigated since the late 1980s, and together with all azolic rings they have become one of the active highlights in recent years [25]. In the first part of the twentieth century the discovery and development of antibacterial drugs were major scientific achievements, even if no new drug class has been discovered in the past 20 years despite the investments in antimicrobial drug discovery [26]. Furthermore, antibiotics *resistance* also make infections more difficult to treat, highlighting the urgent need for new drugs [27]. For some decades Sparatore and co-workers studied and characterized various nitrogenous rings and reported that when benzotriazole is part of larger heterocyclic system bears biological activities, antibacterial in particular) [28-30]. In 1989 Sanna's research group also reported the relevance of the benzotriazole moiety in triazolo[4,5-*f*]-quinolinone carboxylic acids, closely related to oxolinic acid, showed encouraging *in vitro* antimicrobial activity against *E. coli*, with MIC values

included between 12.5 and 25 µg/ml. In fact a different annulations position of the triazole ring as in triazolo[4,5-*h*]-quinolinone carboxylic acids is not profitable, causing a partial or total antimicrobial activity loss [31, 32]. More compounds are reported by Al-Omran and co-workers, which synthesized a variety of benzotriazole derivatives incorporating thiophene, pyridine, thiadiazole or pyrazole moiety: screened for antimicrobial activity, most of tested samples resulted bactericidal [33].

Purohit and Srivatava synthesized and screened a series of chlorosubstituted, phenoxyacetyl and propionyl benzotriazoles: all compounds showed mild to moderate antibacterial and antifungal activity, thus demonstrating that even the simple benzotriazole derivatives possess antibacterial properties [34]. Similar activity is reported for a series of 1-(*N*-heteroyl/diphenyl amino acetyl/propionyl) benzotriazoles [35]. 1*H*-Benzotriazol-1-yl(2-hydroxy-5-[(*E*)phenyldiazenyl]phenyl)methanone derivatives, prepared by Jamkhandi and co-workers through diazonium coupling reaction, showed good antibacterial and antifungal activities, with remarkable zone of inhibition in comparison with standards drugs [36]. Same results were also reported for [(1*H*-benzotriazol-1-ylacetyl)amino]acetic acid derivatives [37], while 2-(1*H*-1,2,3-Benzotriazol-1-yl)-*N*-phenylacetamide derivatives displayed less antibacterial potency [38].

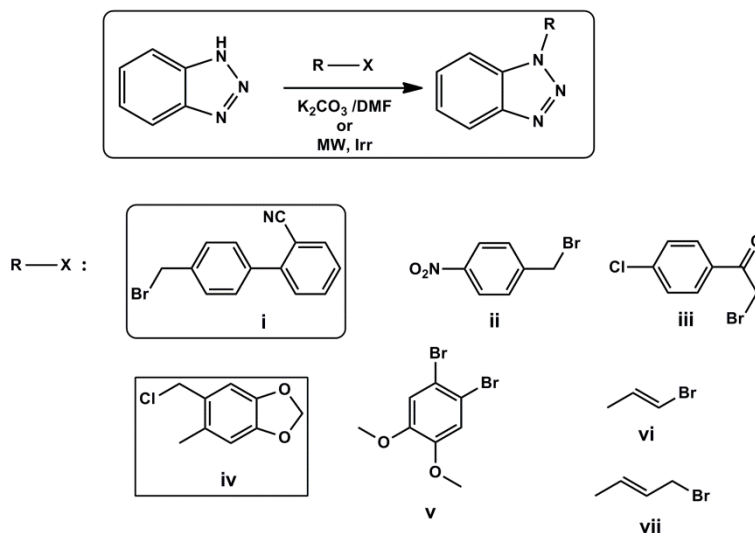
By Ochal group is also reported the *in vitro* antibacterial activity of 5-halogenomethylsulfonyl-benzotriazoles, all tested against a series of reference (including Gram-positive and Gram-negative bacteria) and clinical strains (including methicillin-resistant (MRSA) and methicillin-sensitive (MSSA) *S. aureus* strains, plus methicillin-resistant *S. epidermidis*). Compound with trifluoromethyl- substituent at C-2 position displayed significant antibacterial activities comparable with nitrofurantoin against some strains, being able to inhibit *Staphylococci* strains (MRSA) with MIC values 12.5-25 µg/mL [39].

Benzotriazole can also be used to modulate the biological activity of other heterocyclic nuclei, as for 9-substituted acridines reported by Singh *et al.* [40], or for levofloxacin derivatives, where 2-aminobenzotriazole is linked to the carboxyl moiety. In this second case, the modification led to a comparable antimicrobial activity against Gram-positive and Gram-negative bacteria in comparison with the levofloxacin nucleus [41]. Das *et al.* synthesized and evaluated as antimicrobial agents some novel oxazolidinone derivatives with benzotriazole as pendant, which relevance was confirmed after his replacement with benzimidazole, benzthiazole, or benzoxazole, modifications that provide less active or inactive the molecules. Effects of positional and geometrical isomerism on triazole moiety demonstrated that linearly attached benzotriazole derivatives showed more potency compared to angular ones *in vitro*, while, for angularly attached derivatives, *E*-isomer was found to be more potent than *Z*-isomer. Finally, thioacetamide analogue of linear compound gave a

lead compound possessing similar activity to linezolid *in vitro* [42]. A series of novel substituted and unsubstituted benzotriazolyl oxazolidinone derivatives was also evaluated against a panel of susceptible and resistant Gram-positive and Gram-negative bacteria, some of which resistant to methicillin and vancomycin, and some of them were found to be *in vitro* equipotent or more potent than linezolid [43]

Asati's group linked benzotriazole nucleus to 4-oxo-thiazolidines and their 5-arylidene derivatives, obtaining 5-arylidene-2-aryl-3-(benzotriazoloacetamidyl)-1,3-thiazolidin-4-ones. The derivatives were screened against *Bacillus subtilis*, *Salmonella typhimurium*, *Escherichia coli* and *Bacillus anthracis* at 50 and 100 ppm, and some of them resulted equipotent to streptomycin [44].

In 2006 Swamy *et al.* prepared a series of *N*-alkylated benzotriazole derivatives through a microwave-assisted synthesis [45], as reported in Fig. 3.



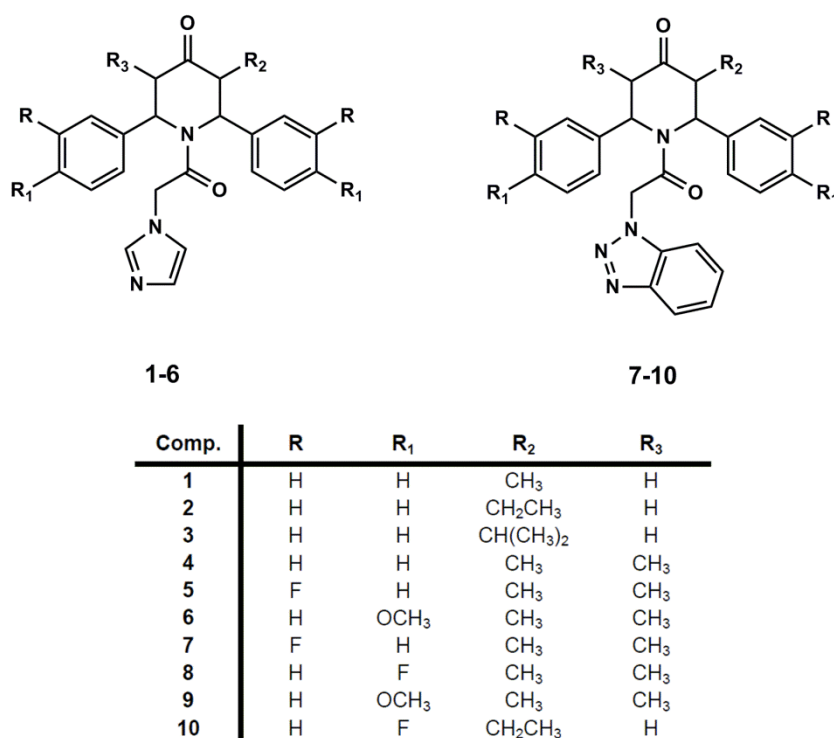
**Fig. 3.** Microwave-assisted synthesis of *N*-substituted benzotriazole derivatives.

The antibacterial activity of all compounds was tested against bacterial strains like *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, *Xanthomonas campestris* e *Xanthomonas orza*. Results highlight how 4'-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)-[1,1'-biphenyl]-2-carbonitrile (**i**) and 1-(6-methylbenzo[*d*] [1,3]dioxol-5-yl)methyl)-1*H*-benzo[*d*][1,2,3]triazole (**iv**) act as potent antimicrobial agents, behavior probably due to the presence of bulky hydrophobic groups (cyano-biphenyl and benzodioxole), while smaller derivatives bearing 5-dimethoxy-benzyl (**v**) and 1-butyl (**vii**) groups outcome equipotent to Streptomycin, used as reference drugs.

Finally, even if some derivatives demonstrate to be active on drug-resistant bacterial strains, all of them lack of selectivity, being active on both Gram-positive and Gram-negative bacteria, over on different fungal strains.

Papers by different authors report that imidazole and triazole nuclei are isosteric analogs,

synthetically relevant, associated to a variety of biological and pharmacologic activities [46]. In the same way the N substitution for CH, that transform benzimidazole in benzotriazole derivative, can be considered an example of a classical isosteric replacement [47]. Leading this idea, Ramachandran and colleagues synthesized and evaluated the antibacterial and antifungal activities against various pathogenic microbial strains of a series of new imidazole/benzotriazole substituted piperidin-4-one derivatives, as reported in Fig. 4 [48].

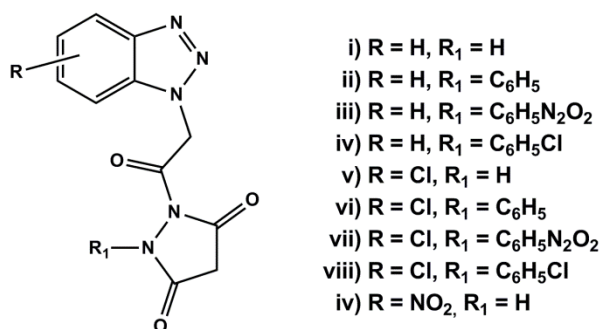


**Fig. 4.** Imidazole/benzotriazole substituted piperidin-4-one derivatives.

This study highlights how the presence of bulky substituents, such as the isopropyl group, at position C-3 of piperidinic cycle increases the antibacterial activity against *B. subtilis* for both imidazole (**3**) and benzotriazole (**10**) derivatives (MIC = 12.5 and 6.25 µg/ml, respectively), in comparison to reference drug Streptomycin. A similar behavior is not observed in antifungal test. Additionally, a methyl group at C-3 and C-5 along with *m*-fluorophenyl (**5**) and *p*-methoxyphenyl (**8**) at C-2 and C-6 exerted good growth inhibition toward *E. coli* (MIC = 6.25 µg/ml and 12.5 µg/ml, respectively). Surprisingly, the introduction of an isobutane group in C-3 and with a CH in 5 leads to a higher inhibitory activity against *B. subtilis* (MIC = 6.25 µg/ml) [39]. Most of the compounds demonstrate nonexistent or moderate growth inhibition activity on different fungal strains, exception for compounds **3** and **8** against *A. niger* (12.5 µg/mL), **5** against *Candida neoformans* (12.5 µg/mL), **6** against *Candida albicans* (6.25 µg/mL) and **8** against *Rhizopus sp.* (6.25 µg/mL).

Finally, Suma's research group investigated the benzotriazole antimicrobial proprieties introducing not only electron withdrawing substituent as Cl or NO<sub>2</sub> in the phenilic moiety, but also variously

substituted pyrazolidin-3,5-dione moieties linked to the heterocyclic nucleus [49]. All synthesized compounds were characterized and biologically evaluated by cup plate diffusion method. Between the examined derivatives, showed in Fig. 5, only compound **viii** resulted comparable to Ciprofloxacin on *Staphylococcus. Aureus*, while a limited activity is reported toward *C. Albicans*. Unfortunately, no SAR analysis is reported.

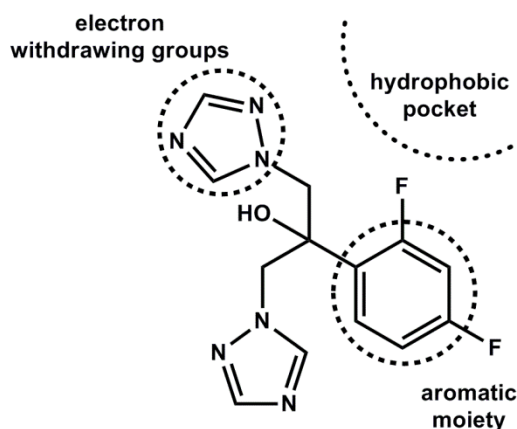


**Fig. 5.** 1-(2-(1H-benzo[d][1,2,3]triazol-1-yl)acetyl)-2-R<sub>1</sub>-pyrazolidine-3,5-dione derivatives.

Finally, little is known about the antiprotozoaric activity of benzotriazole derivatives. A simply substitution on BT generate 6-chloro-1H-benzotriazole, a compound endowed with micromolar activity against *Entamoeba histolytica*, demonstrating that this scaffold, as indazole nucleus, is more active than metronidazole, the drug most commonly used in the treatment of anaerobic protozoan parasitic infections [22]. Pagliero's works demonstrated that compounds possessing the heterocyclic nucleus 2-methyl-1,2,3,4-tetrahydroquinoline linked to a benzenesulfonyl moiety showed protozoan antiparasitic proprieties [50, 51]. On this basis, through the fragment-based drug design strategy, Becerra's group designed and prepared a library of *N*-benzenesulfonyl derivatives of BT, replacing the H at N<sub>1</sub> with substituted-benzenesulfonyl groups, all compounds prepared by sulfonylation, as reported by Katrinsky [52]. Researcher determined *in vitro* the biological activity of these compounds on the protozoan parasite *Trypanosoma cruzi*. Adding different concentrations of *N*-benzenesulfonylbenzotriazole to the growth medium on epimastigote and trypomastigote forms, they demonstrate that derivatives have an *in vitro* growth inhibitory dose-dependent activity against epimastigotes. Particularly, after incubation for 72 h the parasite number in epimastigotes form decrease of about 50% at 25 µg/mL and of 64% at 50 µg/mL. In the same conditions BT, used as reference compound, did not show anti-trypanosomal activity. On trypomastigotes, the infective form of the parasite, *N*-benzenesulfonylbenzotriazole appeared to be even more effective: at 50 µg/mL it was effective at an earlier time than on epimastigote forms, causing a percentages of trypomastigotes dead higher than 95%. BT was less effective, causing at the same concentration only 21% of dead parasites [53].

## BENZOTRIAZOLE AS ANTIMICOTIC AGENT

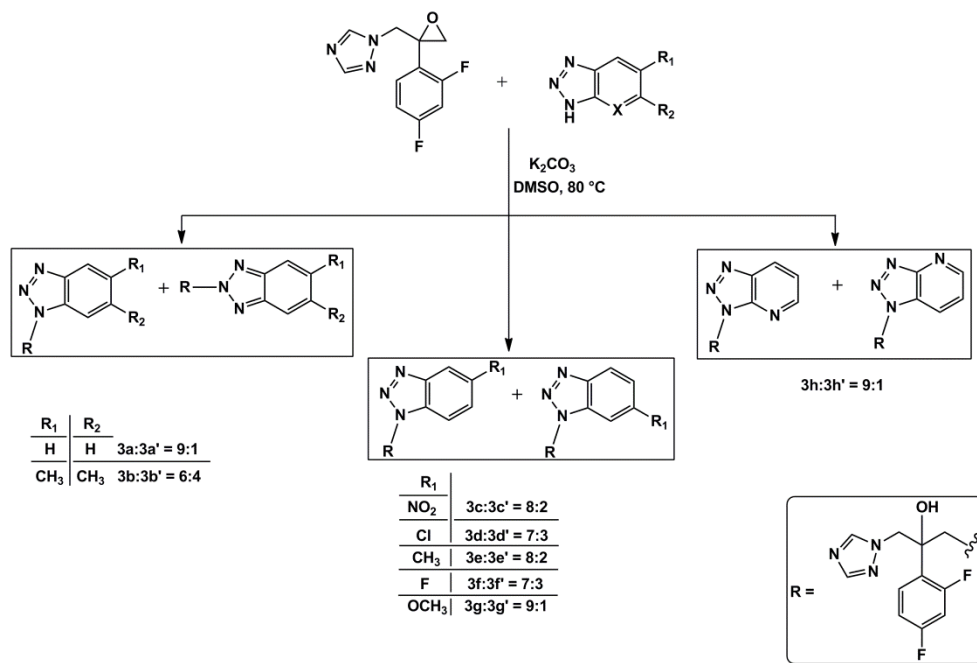
As previously reported, several authors reported the biological evaluation of imidazole derivatives and benzotriazole analogues as antibacterial and antimycotic agents, and for these derivatives, a selective action is not demonstrated [45, 48]. However, the structural model of the best-known antifungal drug, fluconazole, offers an interesting starting point for drug design studies on the triazolic system, which can then be replaced by a benzotriazole nucleus, in order to evaluate the effect of the bioisosteric replacement on the biological activity. Concerning the mechanism of action, it is known that antimycotic drugs, such as fluconazole [54], itraconazole [55], voriconazole [56], ravuconazole [57] and posaconazole [58] exert their pharmacological action by inhibiting the fungal 14  $\alpha$ -demethylase cytochrome P450, known as CYP51, an essential enzyme in the biosynthesis of sterols. In particular the CYP51 removes by oxidation the 14  $\alpha$ -methyl group of lanosterol, using oxygen and NADPH, transforming it into ergosterol, an essential component of the fungal cell membrane [59]. These drugs act by displacing lanosterol from CYP51 binding site, causing a block in the biosynthesis of ergosterol and an accumulation of 14  $\alpha$ -methylsterols [60]. For fluconazole and analogues, crucial interactions at the enzymatic active site are favored by these components: 1) the basic nitrogen atom in position 3 in the triazole moiety, forms a bond with the acid iron of the CYP51 heme prosthetic group, in a position normally occupied by the oxygen, 2) the presence of aromatic rings and 3) the molecular behavior almost non-polar [61], as described in Fig. 6.



**Fig. 6.** Schematic view of the key interaction between fluconazole and its receptor.

Now, fluconazole is effective against candidiasis after both *oral* and parenteral administration, but is ineffective against aspergillosis. An increasing incidence of infections caused by *Candida not albicans*, such as *C. glabrata* and *C. krusei* resistant to this drug was also observed [62]. These evidences have greatly stimulated the pharmaceutical research in the antimycotic field, leading to the synthesis of new derivatives having as central structure a benzotriazole group. These

compounds, in comparison with imidazole antifungal drugs, seems to possess a broader antifungal spectrum paired with a lower toxicity [63]. From this perspective, Patel's research group modified the 1,2,4-triazole ring of fluconazole to 5(6)-(un)substituted benzotriazole lead compounds (Fig. 7) [64].

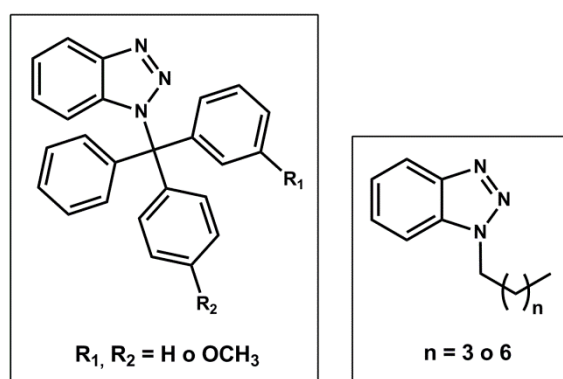


**Fig. 7.** Synthesis scheme of 5,6 substituted benzotriazoles derivatives endowed with antifungal activity.

Particularly, 5,6-dimethylbenzotriazol-2-yl, 5-chlorobenzotriazol-1-yl and 6-methylbenzotriazol-1-yl derivatives exhibited potent antifungal activity, with MICs values on *Candida spp.* ranging from 1.6 µg/ml to 25 µg/ml. Finally, the introduction on the benzotriazole ring of the small hydrophobic groups –Cl, –CH<sub>3</sub> and di–CH<sub>3</sub>, lead to compounds successful against both *Candida* and *Aspergillus spp.*, with 12.5-25 µg/ml MIC values on *A. niger*. Replacement of the benzotriazole ring with a triazolopyridinic nucleus generate a polar analogue less potent. Finally, the replacement of hydrogen in position 5 of the benzotriazole with electron-withdrawing groups such as -NO<sub>2</sub>, -Cl, -F, increases the antimycotic activity; on the contrary, the same substitution at position 6 of the ring leads to compounds definitely less potent.

XP-Glide docking method analysis demonstrated the relevance of hydrophobic substituents in the molecular interaction with the *Mycobacterium tuberculosis* CYP51 (MT-CYP51), selected model in the absence of crystal structure of fungal CYP51. Particularly, for S enantiomers a lower glide score (expressed in Kcal/mol) is reported; indeed, they have an antimycotic activity higher than the respective enantiomers R. Furthermore, to evaluate interaction differences at the binding site, CYP51 aminoacid sequences of *Mycobacterium Tuberculosis* and *Aspergillus niger* have been compared. The obtained results showed that antifungal potency differences among benzotriazole

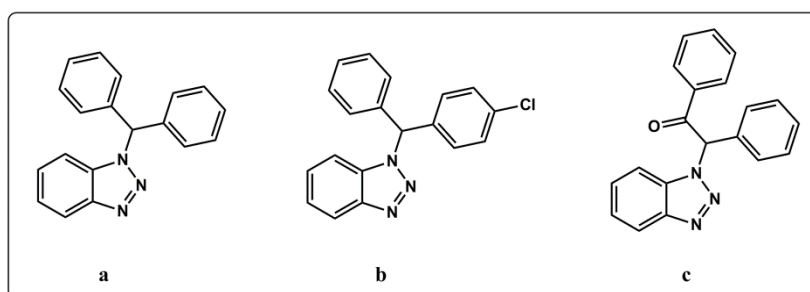
regioisomer couples depended on the interactions of substituents with the aminoacids contained by MT-CYP51 active site, but it is mainly due to the triazole heterocycles placement into it. Also Rezaei and colleagues synthesized a set of triazolic derivatives, which included benzotriazole compounds targeting the CYP51. Obtained compounds, reported in Fig. 8, had a molecular weight between 139 and 421 kDa and, as fluconazole, the molecular docking simulation was performed at the level of the CYP51 active site.



**Fig. 8.** Chemical structure of the 1,2,3-benzotriazole derivatives proposed by Rezaei *et al.*

Collected data showed values such as these obtained for fluconazole; in particular, lower FDE values, index of better drug-target interaction, were obtained with 1,2,4 triazole derivatives. However, there is no correlation between antifungal activity and FDE. These analysis underlined that the antimicrobial potency was related to the molecular penetration into fungi cell, and the presence of bulky substituents, as  $\text{OCH}_3$ , is believed to be responsible for the decrease of this activity [65, 66].

In following research, the team headed by Rezaei continued the investigation on CYP51 inhibitors synthesizing new 1,2,4-triazole, imidazole, benzimidazole and, interesting for us, benzotriazole derivatives (Fig. 9). Throughout drug-design studies, applying Autodock on MT-CYP51, the best *in silico* promising derivative were synthesized and their antimicotic activity was compared to that of fluconazole and itraconazole, used as reference drugs [66].

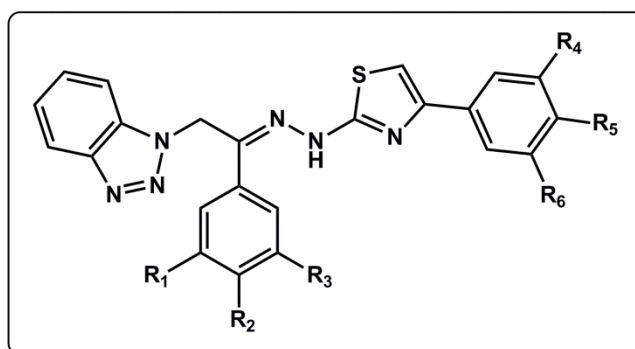


**Fig. 9.** Biphenyl and benzhydryl benzotriazole derivatives.

Unfortunately, obtained results from this studies showed that benzotriazole derivatives do not have antifungal activity against different *Candida* species. Exceptionally, these compounds possess

activity against the dermatophyte *Microsporum canis*. The only compounds active against all microbe species taken into account are derivatives of 1,2-diphenyl-2-(1*H*-1,2,4-triazol-1-yl)ethanone, 1-(diphenylethyl)-1*H*-imidazole and 1-[(4-chlorophenyl)((phenyl)methyl)]-1*H*-imidazole. This activity was probably due to their lower size, which allow a better penetration into the fungi cell, and the presence of the five-membered nitrogen heterocyclic ring. Finally, in the benzimidazole series, inserting two substituents –OCH<sub>3</sub> on phenyl *para* position induce total loss of antifungal activity; whereas, inserting an ethyl-piperazine reduce antifungal potency in the imidazole series.

Concerning the same azole antifungal model, a novel work was presented by Gaikwad and colleagues [67], wherein the benzotriazole core was modified by heterocyclic alkylation with complicated side chains bearing a substituted thiazolic ring (Fig. 10).



**Fig. 10.** Benzotriazol-1-yl-1-(phenyl)ethylidene)hydrazinyl)-4-phenylthiazole derivatives chemical structure.

Tested on a wide number of microorganisms, Gram-positive, Gram-negative and fungi, in both cases the best activities were observed for derivatives bearing electron withdrawing substituents as F, Cl, Br, and NO<sub>2</sub> on side phenyl groups, independently by their position on the ring. More precisely, these derivatives were able to inhibit the growing of Gram-positive and Gram-negative bacteria in equal way, as well as *C. Albicans* and *A. Niger* in a comparable way as Nistatine.

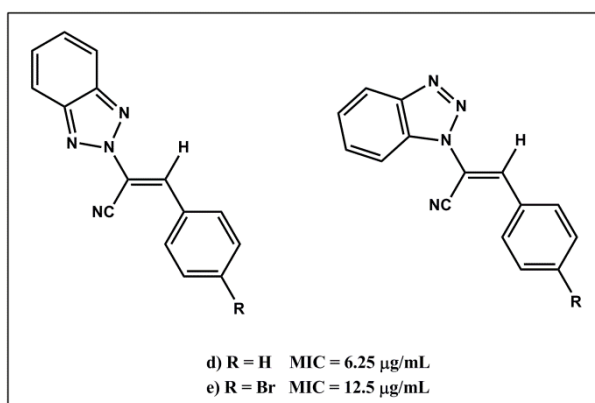
#### BENZOTRIAZOLE AS ANTIMYCOBACTERIAL AGENT

Despite the perception, nowadays Tuberculosis (TB) is still one of the most common infectious diseases, resulting in as many as one-third of world's population (~2 billion people) infected with *Mycobacterium tuberculosis* (*M. tb*) [68]. Every year about nine million new cases of tuberculosis occur and up to two million individuals die due to this disease [69]. Even more frightening is the rapid emergence of Multi-Drug-Resistant (MDR) [70] and Extensively-Drug-Resistant (XDR) strains [71] in all regions of the world, along with the dangerous synergy between the Human

Immunodeficiency Virus (HIV) and *M. tb* [72, 73]. TB remain a leading cause of death among HIV positive patients [74].

The treatment of active tuberculosis require a combination of several drugs, such as isoniazid, pyrazinamide, ethambutol, rifampicin and streptomycin [75], all used in various combinations as first-line therapy [76]. On the contrary quinolones are classified as second-line drugs [77], even if their use is recommended in managing MDR-TB [78]. However, due to outcoming of MDR and XDR TB strains, worldwide there is an urgent need for new drugs having a different mechanism of action from those mentioned above, also to short the duration of pharmaceutical therapy.

Various research groups focalized their attention on this field and a large amount of molecules have been tested for this purpose [79-82]. Since '90s Carta and co-workers focalized their attention on the bioisosteric modification of these heterocycles creating various benzotriazole derivatives. A first series of 3-aryl substituted-2-(1*H*(2*H*)benzotriazol-1(2)-yl)acrylonitriles [83] was prepared with the aim to characterize the best substituents connected with the aryl moiety selecting R within groups holding various electron-accepting or donor properties and lipophilic–hydrophilic balance. During the synthesis, the performed Knoevenagel condensation had as result in most cases the only *E*-isomer formation, while in a few cases a mixture of *E/Z*-isomers was observed. From this preliminary study 1-substituted benzotriazole derivatives resulted more active than the 2-benzotriazolyl isomers, while the unsubstituted phenyl moiety (**d**) outcome the best option, exhibiting the highest antimycobacterial activity *in vitro*, also against *Mycobacterium avium*. The only exception is represented by 4-bromophenyl derivative (**e**), even if the activity resulted lower than that of the unsubstituted terms, as depicted in Fig. 11.



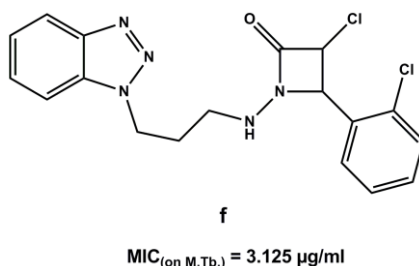
**Fig. 11.** 3-aryl substituted-2-[1*H*(2*H*)benzotriazol-1(2)-yl]acrylonitriles general formula and MIC values of the compounds **d** and **e**.

With the aim to improve the antimycobacterial activity, further modifications, such as the introduction in the phenyl ring C-4 of two or more electron-releasing groups, or the replacement of the phenyl ring with cyclohexyl or larger aromatic rings, were performed [84]. Unfortunately the

produced 3-aryl-, 3-cyclohexyl and 3-heteroaryl substituted-2-(1*H*(2*H*)-benzotriazol-1(2)-yl)prop-2-enitriles, prop-2-enamides and propenoic acids showed a strong reduction or a loss of the activity in spite of their increased lipophilic character. This indicates that the steric hindrance, as well as the substituents nature, may play a relevant role in determining the ability to inhibit the *M. tb* proliferation. Finally, in order to extend the SAR studies, various substituents were introduced alternatively at position 2', 3' and 4' in the phenyl moiety [85]. Both the previously synthesized and the new derivatives were evaluated for cytotoxicity against MT-4 cells. Unluckily, the relevant cytotoxicity showed by many derivatives induced to dispose of acrylonitriles as antitubercular and to evaluate them as antiproliferative agents, as above reported.

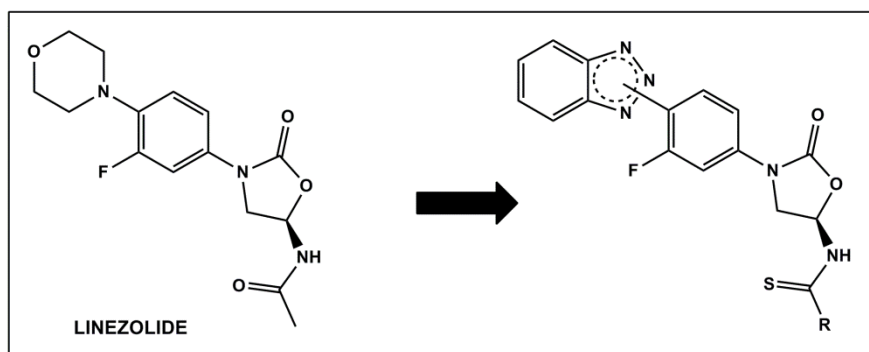
Nevertheless, benzotriazole remains an interesting heterocycle for its antimycobacterial properties, and various groups tried to combine chemical structures of well known antibacterial agents within benzotriazole, designing and synthesizing new molecular series with the aim to develop new antitubercular agents able to succeed toward *M. tb* sensitive and resistant strains.

Dubey *et al.* coupled the benzotriazole properties with the antimicrobial activities of the  $\beta$ -lactams 2-azetidinones [86, 87]. The 2-oxo-4-substituted aryl-azetidinone derivatives of benzotriazole were prepared by both conventional and microwave irradiation, this one outcoming the best performing synthetic way. Resulting no cytotoxicity for all of the prepared compounds, the derivatives were tested against *M. tb* and some other microorganisms, such as bacteria and fungi. The best product of this series was compound **f**, endowed with a promising activity against *M. tb* (MIC = 3.125  $\mu$ g/ml), but also active toward some bacteria (MIC = 0.1  $\mu$ g/ml on *Escherichia Coli*) and fungi (MIC = 0.5  $\mu$ g/ml on *Aspergillus niger*), (Fig. 12). For this class of compounds no selectivity of action is reported.



**Fig. 12.** 2-oxo-4-substituted aryl-azetidinone derivatives of benzotriazole.

Dixit *et al.* used as basis scaffold the Linezolid structure to create a new class of oxazolidinone derivatives [88] (Fig. 13), performing a bioisosteric substitution of the morpholinic moiety with the benzotriazole nucleus.



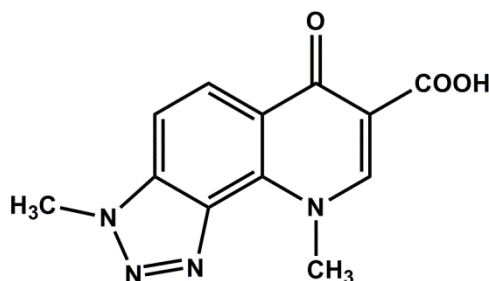
**Fig. 13.** Bioisosteric substitution on the structure of Linezolid.

Nine 1-[3-(4-benzotriazol-1/2-yl-3-fluoro-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-3-substituted-thiourea derivatives were synthesized starting from commercial benzotriazole and the antimycobacterial activity was determined against susceptible (sensitive strains; inhibited by the two front line anti-TB drugs such as isoniazid or rifampicin) and resistant strains (not inhibited by either isoniazid or rifampicin or both). The (R)-N-(3-(4-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-3-fluorophenyl)-2-oxooxazolidin-5-yl)cyclopropanecarbothioamide sort out to be the best derivative, endowed with an excellent in vitro profile against *M. Tb.* H37Rv (MIC = 0.06  $\mu$ M) and both drug sensitive and resistant clinical isolates (MIC = 0.125–1.0 and 1–2  $\mu$ M respectively). These MIC values were equivalent to linezolid and superior to isoniazid against all strains.

As observed until now, the applied strategies to design a new antitubercular agent normally scheduled the bioisosteric modification of a know structure to obtain new linear derivatives. In this perspective an exception is represented by a series of angular derivatives synthesized by Carta *et al.*, structurally related to quinolones, one of the most widely prescribed family of synthetic antimicrobial agents. In particular Fluoroquinolones, fluorine-containing derivatives of older quinolones such as nalidixic acid, are currently used as antitubercular agents in MDR-TB infection and, to a lesser extent, in the case of severe adverse reactions to the conventional antituberculous regimen [89]. However, they are still classified as second-line drugs, since their use in tuberculosis treatment remains controversial [77]. This behavior is justified by the fact that the emergence of fluoroquinolone resistance in MDR strains is possible [90], even if it does not appear to be related to poor bactericidal activity but to rapid emergence of resistance at the doses used clinically, as seen for ciprofloxacin [91].

Quinolones mechanism of action and pharmacokinetics are widely studied, and various review can be found in literature [92-95]. Aiming to exploit the pharmaceutical peculiarities of this antibiotic class, Carta *et al.* created a new series of [1,2,3]Triazolo[4,5-*h*] and [4,5-*f*]quinolones with the purpose to obtain new more potent and selective agents toward *M.tb* sensitive and resistant strains

[32, 96, 97]. As reported by Milata review [98], the Carta's group discovered that only few triazolo[4,5-*h*]quinolone carboxylic acids exhibited interesting low MIC<sub>90</sub> values (5.0-1.6 µg/mL), observing also that the activity was related to the length and position of the substituent at triazolo-nitrogen. A methyl in N-3 position has showed the higher activity. This first observation prompted the group to focalize the study designing and selectively synthesizing a series of 3-methyl-9-substituted-6-oxo-6,9-dihydro-3*H*-[1,2,3]-triazolo[4,5-*h*]quinolone-carboxylic acids, where a variety of substituents on the quinolone nitrogen were introduced to improve activity. All derivatives were tested against *M. tb* H37Rv and further 11 clinically isolated strains endowed with different drug resistance. 3,9-dimethyl-6-oxo-6,9-dihydro-3*H*-[1,2,3]triazolo[4,5-*h*]quinoline-7-carboxylic acid stood out as the most potent derivative, exhibiting MIC<sub>90</sub> = 0.5 µg/mL against all tested strains paired with no cytotoxicity.



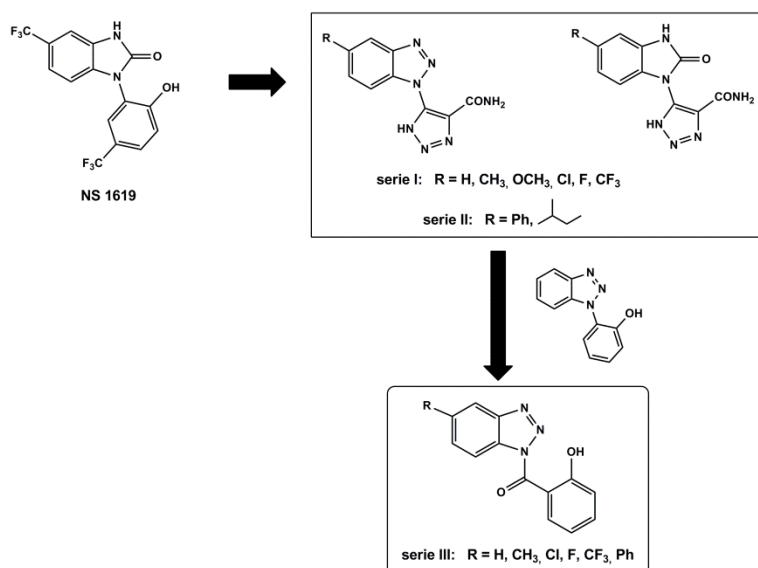
**Fig.14.** 3,9-dimethyl-6-oxo-6,9-dihydro-3*H*-[1,2,3]triazolo[4,5-*h*]quinoline-7-carboxylic acid.

The latter derivative was selected as lead compound for further examination. Human macrophages J774-A1 were infected with *M. tb* H37Rv strain and successively grown in the absence of antitubercular agent or in the presence of the lead compound at both the concentrations of 0.5 and 0.25 µg/mL. After 7 days the macrophages were lysed and the growth of mycobacterial culture was of 5000 and 8000 CFU/mL, respectively, while the untreated culture grew regularly. In addition, from supplementary analysis a good antimycobacterial activity emerged against several *M. avis paratuberculosis*, *M. smegmatis* and *M. bovis* strains [99]. Further modifications of the substituents in N-3 and N-9 positions of the ring system with bulking groups do not improved the researched activity, and comparing the new derivatives with the previous series, methyl group was confirmed as the most effective substituent at both positions [100]. Lastly, when MICs were determined against a panel of Gram-positive and Gram-negative bacteria and against *Candida sp.*, all compounds resulted inactive (MIC = 64-100 µg/mL). These actions suggest for this new class of quinolones a specific antimycobacterial potential due probably to a different mechanism of action toward quinolones, leading them to be good candidates for further developments [101].

#### **BENZOTRIAZOLE AS POTASSIUM CHANNEL ACTIVATOR**

The activators of the potassium channels represent an emerging class of drugs for the treatment of disorders of the nervous, respiratory and cardiovascular systems, and for this reason represent an interesting research field [102]. From literature, (1-(2'-hydroxy-5'-trifluoromethylphenyl)-5-trifluoromethyl- 2(3*H*)benzimidazolone), or NS1619, pop up as an activator of the calcium-dependent potassium channels. This molecule is able to determine *in vitro* not only cellular hyperpolarization through direct activation of BKCa, but also a Ca<sup>2+</sup> channel block: both of these effects contribute to its vasorelaxant properties [103].

Based on NS1619 properties, the Baragatti and Biagi research group conducted broad studies that primarily led to the design and synthesis of a series of new derivatives, including 5- substituted-carboxamido-triazolyl-benzotriazoles (proposed as bioisosteric substitution of benzimidazole with benzotriazole moiety), and the corresponding series of 5-substituted-carboxamido-triazolyl-benzimidazolones (Fig. 15, series I).



**Fig. 15.** Potential activators of potassium channel opener.

For all benzotriazole derivatives are reported vasorelaxing properties and potency parameters a lower than that of the reference compound NS 1619, being able to completely break down the contractile tone induced by administration of KCl 20 mM on rat aorta (efficacy 100%). In contrast, benzimidazolone compounds were found to be ineffective or inactive. Particularly, SAR studies shown that the absence of substituents at position 5 on the benzotriazole moiety leads to decrease in potency but not in efficacy, while only the presence of a methoxy group adversely affects both properties. The best results have being observed when the substituent is simple a methyl group. Experimental data seem to suggest therefore a possible correlation between the potency and the steric hindrance in this position. Finally, the benzotriazole derivatives lacking the carboxamido group in position 4 of the triazole ring are totally vasorelaxing powerless, and this demonstrate that the CONH<sub>2</sub> group represents an essential structural requirement to exert the vasorelaxing effect due

to the potassium channels BK activation [104].

For this reason, the analysis was carried out studying the effects of steric hindrance and lipophilicity caused by the substituent at position 5. The introduction of bulky and lipophilic groups, such as sec-butyl and phenyl, leads to derivatives retaining equivalent power, but not greater than derivatives bearing a methyl group in the same position (Fig. 15, series II). Alongside these derivatives was then prepared the 1-(2-hydroxyphenyl)-benzotriazole. This derivative showed good vasorelaxing activity, full efficacy and potency 10 times higher than the triazolyl benzotriazole derivative bearing an H atom at position 5. The activity of the latter compound is significantly reduced by high levels of KCl (60 mM), suggesting a possible pharmacodynamic profile similar to that operated by drugs known as activators of potassium channels [105]. Finally, another series of 5-substituted-1-(2-hydroxybenzoyl)-benzotriazoles, wherein the triazole group is replaced by a hydroxybenzoyl group, (Fig. 15, series III); thereby, thanks to the bridge determined by the carbonyl function, the new molecules were ensured by a greater degree of freedom and therefore flexibility between the two main parts. Biological analysis shows that all these derivatives show high vasorelaxing potency and efficacy. However, once again, the more potent derivative in the context of this set appears to be the one having, at 5-position of the benzotriazole moiety, the methyl group; this compound also has cardioprotective properties. In contrast, both the introduction of the 2-hydroxyphenyl group in position 1 of the benzotriazole ring and the replacement of the carbonyl bridge (an electron withdrawing group) with a methylene bridge causes a considerable decrease of the biological activity [106].

#### **BENZOTRIAZOLE AS TUBULIN POLYMERIZATION INHIBITOR**

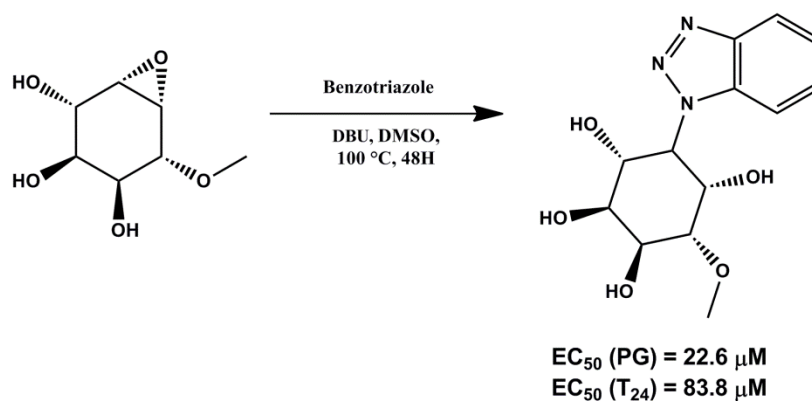
Cancer is actually the second leading cause of death worldwide after cardiovascular diseases, accounting for about 8 million deaths (around 13% of all deaths) [107, 108]. The origin of a tumor resides in a somatic cell that has undergone a series of genetic modifications, thus ceasing to respond to normal regulatory mechanisms that operate in a healthy organism. This cell then proliferates to form a clone of neoplastic cells, characterized by lack of control proliferation. At the same time the cell also loses control of cellular replicative potential, and becomes immortal. Loss is also the mechanism for density-dependent inhibition, which, in normal conditions, allows the cells to multiply up to a defined cell density, at which they become quiescent. The tumors can be distinguished in benign and malignant, depending on the biological and morphological characteristics that determine a greater or lesser aggressiveness. The most common cancers are lung cancer (1.4 million deaths), stomach cancer (740.000 deaths), liver cancer (700.000 deaths), colorectal cancer (610.000 deaths) and breast cancer (460.000 deaths) [109].

The therapeutic approach for the treatment of cancer diseases may be different, and includes:

surgical treatment, radiation therapy, immunotherapy, chemotherapy. Polychemotherapy therefore provides the use of virtually toxic drugs, which operates selectively in respect of cancerous cells, thus saving the healthy cells of the host [110]. Unfortunately, so far this selectivity is practically unattainable due to the difficulty in discriminating between tumor and healthy cells, these being very similar to each other. All this translates into an exposure to the toxic effects on the part of all healthy somatic cells, especially those in more rapid replication, such as those of the hair follicles, intestinal epithelium and bone marrow [111].

For this reason, the research for new molecules able to present a selective toxicity against specific types of cancer is still present. Nitrogen heterocycles, in particular, attracted the attention of researchers as possible isosteres of structural components of natural nucleotides [112-114], and also benzotriazole has been reported as possible antiproliferative agent [85, 115, 116].

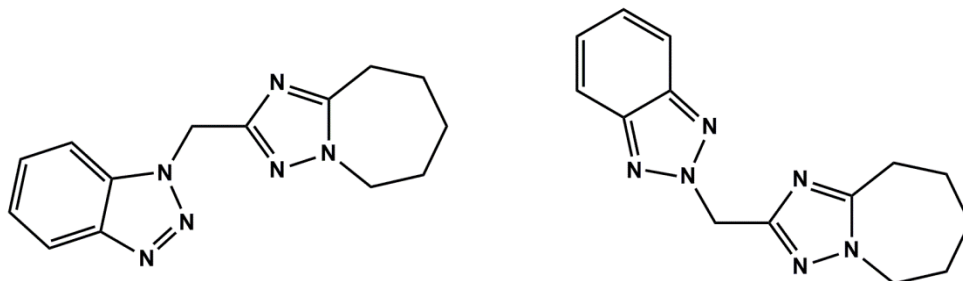
This principle of isosterism was followed by Zhan and Lou [117] in the preparation of new nucleoside analogues with antitumor activity. Previously it had been shown that the carbocyclic nucleosides (obtained by replacing oxygen with a sugar-CH<sub>2</sub> group) are more stable to the action of nucleoside phosphorylase and are also equipped with anti-tumor activity [118-120]. On this basis, Zhan and Lou decided to further modify the structure of the nucleoside analogues, replacing the heterocyclic base with various polinitrogen heterocycles. The different analogues of D-pinitol were tested on tumor cell lines derived from human lung and bladder, but only derivatives with substitution of the triazole and benzotriazole were active, albeit at micromolar level, as shown in Fig. 16.



**Fig.16.** 3'-O-Methyl-5'-deoxy-5'-(1,2,4-triazole-1-yl)-D-chiro-inositol.

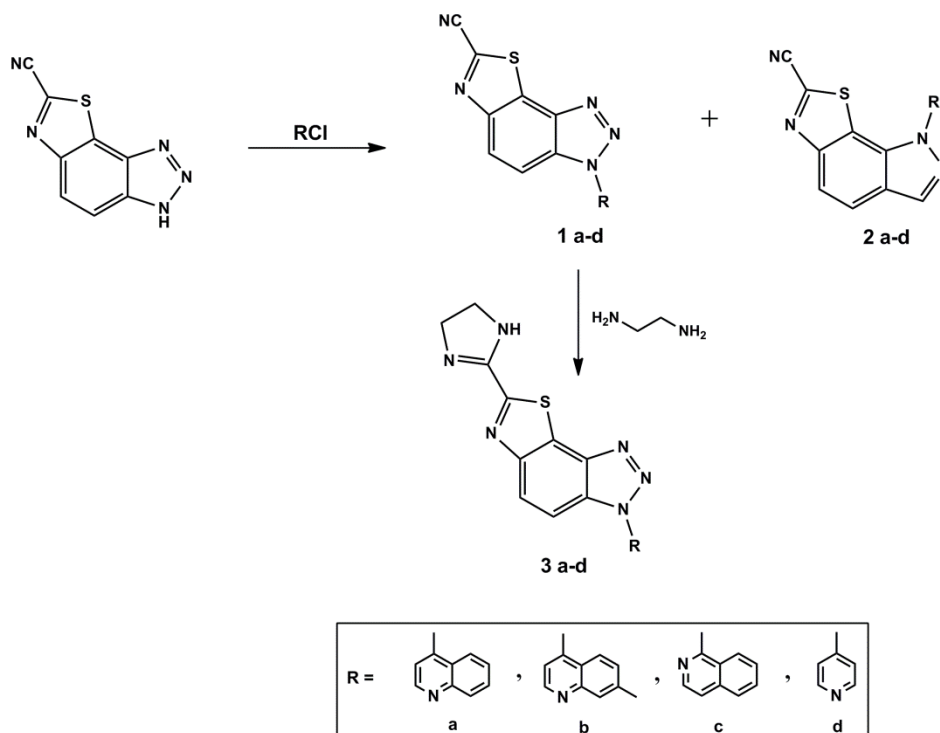
On the same principle worked Al-Soud's research group, combining the potential reported for several alkylated benzotriazoles with that of 1,2,4 triazole nuclei (Fig. 17). Starting from 1 and 2 (cyanomethyl)benzotriazole were prepared azepinic compounds, which, as result from biological evaluation, were found to be extremely promising. From the analyzes performed on several human tumor cell lines, the compound 2-((1*H*-benzo [*d*] [1,2,3] triazol-1-yl) methyl) -6,7,8,9-tetrahydro-

5*H*-[1,2,4] triazolo [1,5-*a*] azepine stands out for its micromolar activity against tumor cells derived from leukemic (CCRF-CEM and RPMI-8226,  $GI_{50} = 0.07$  mM), ovarian (OVCAR-3,  $GI_{50} = 0.02$  mM) and renal (CARKI-1,  $GI_{50} = 0.06$  mM), although not showing selective in its action. In contrast, the isomer of substitution at N-2 demonstrated no activity [121].



**Fig. 17.** Benzotriazol-1(2-yl)-tetrahydro-triazoloazepine.

The interest generated by the indoloquinolinic alkaloids, i.e. Cryptosanguinolentine [122, 123], has instead pushed the group coordinated by Beauchard to design and prepare a series of derivatives in which the thiazole ring system is fused in polycyclic systems. They prepared a series of new thiazole-benzotriazoles as synthesis intermediates, all of which are evaluated as potential anticancer agents [124]. Starting from 2-cyano thiazole-benzotriazoles [24], for substitution on the nitrogen *N*-1 and *N*-3 with quinoline and pyridine nuclei, were prepared compounds whose reactivity of the cyano group in the 2-position of the benzothiazole has been exploited in order to prepare imidazoline derivative, obtained by reaction with ethylenediamine, as shown in Fig. 18.

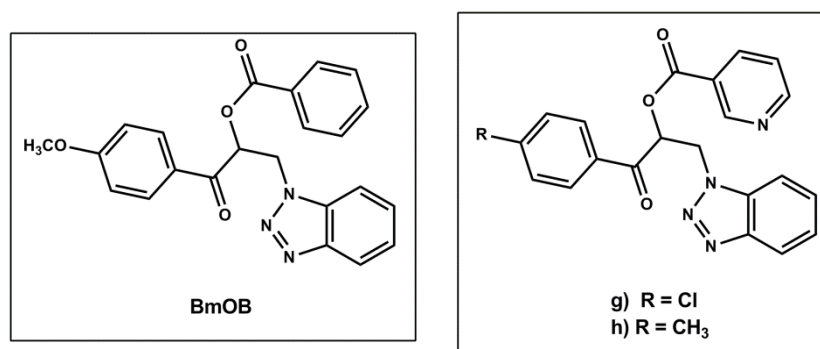


**Fig. 18.** Cyano thiazole-benzotriazoles derivatives.

All compounds of substitution at *N*-1, tested on two cellular lines derived from breast cancer, were found to be from moderately to very active (% inhibition between  $32.4 \pm 1.0$  for **1a** derivative, and  $97.4 \pm 1.0$  for **2b** on MDA-MB- 231, the most resistant cell line) with the exception of compounds **1c** and **3c**, inactive on both lines. Generally the introduction of an imidazolinic side chain determines an increase of the biological activity, except for the compound **3d**.

Finally, the group of Wan and co-workers has long been devoted to the evaluation of the benzotriazole derivatives antiproliferative activity [125-127]. 3-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-(4-methoxyphenyl)-1-oxopropan-2-benzoate (BmOB) emerged for its antiproliferative effect on cell lines of different tumor type. This molecule was therefore used in order to define a possible mechanism of action. The analyzes carried out on BEL-7402 hepatocellular carcinoma cells, the most susceptible to BmOB action ( $IC_{50} = 0.082 \pm 0.008$  mM ), show that this molecule can lead to cell death through induction of a collapse of mitochondrial membrane potential, determining both the production of reactive oxygen species and DNA fragmentation [128].

In later studies, exploiting the concept of bioisosterism, on known 3-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-2-bromo-1-phenylpropan-1-onic intermediates [129, 130], were introduced similar and bulky side chains by reaction with nicotinic or isonicotinic acid. The 3-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-(4-chlorophenyl)-1-oxopropan-2-nicotinate (A) and 3-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-oxo-1-p-tolilpropan-2-nicotinate (B), shown in Fig. 19, demonstrate to possess potent propagation inhibition activity in liver and galactophore cancer cells [131].

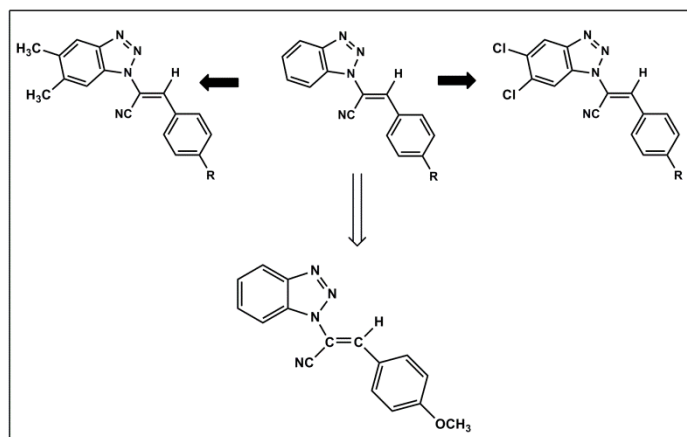


**Fig. 19.** Chemical structure of BmOB and nicotinic derivatives **g** and **h**.

Finally, as part of a wide study focused on benzotriazole derivatives that could act against *M. tb.*, Carta and coworkers discovered that compounds belonging to the class of 3-Aryl-2-[1*H*-benzotriazol-1-yl]acrylonitriles were able to inhibit cellular proliferation in a series of liquid and solid human tumors [10, 83, 84].

Principally, researchers identified (*E*)-2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-3-(4-methoxyphenyl) acrylonitrile as lead compound, shown in Fig. 20, found to be 5 to 100 times more potent than 6-mercaptopurine and comparable to etoposide on all cell lines [85, 115]. Similar derivatives

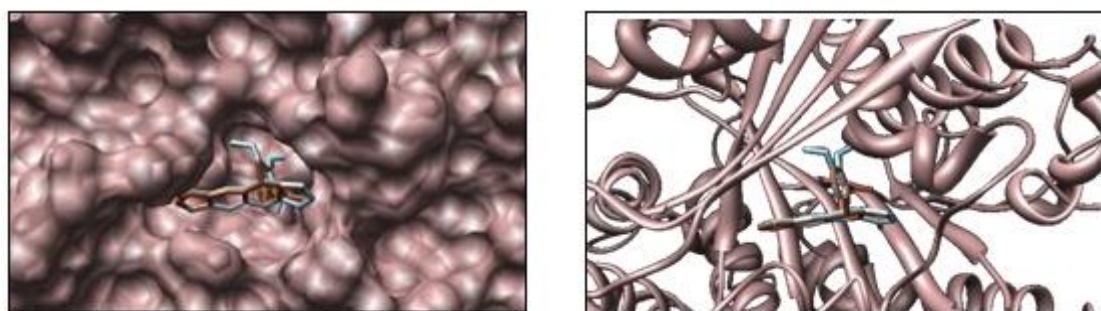
possessing in the benzotriazole positions 5 and 6 electron-donor substituents, such as methyl groups, results to be absolutely devoid of efficacy on the same tumor lines.



**Fig. 20.** Benzotriazol acrylonitriles: structure and lead compound of the series, (*E*)-2-(1*H*-benzo [*d*][1,2,3]triazol-1-yl)-3-(4-methoxyphenyl)acrylonitrile.

Therefore, in 2011, the group completed the SAR studies, designing and synthesizing molecules possessing at position 5 and 6 electron-withdrawing group, such as chlorine. However, the derivatives (*E*)-2-(5,6-dichloro-1*H*-benzo [*d*][1,2,3]triazol-1-yl)-3-(4-*R*-phenyl)acrylonitriles [115] resulted almost devoid of activity, demonstrating that the introduction of a chlorine atom in the benzotriazole moiety determines a considerable decrease of the antiproliferative activity for this class of derivatives. The cell cycle analysis also revealed that this series of compounds act at the G2/M phase of the cell cycle and this result can be explained by a possible interaction of the derivative with tubulin [132, 133].

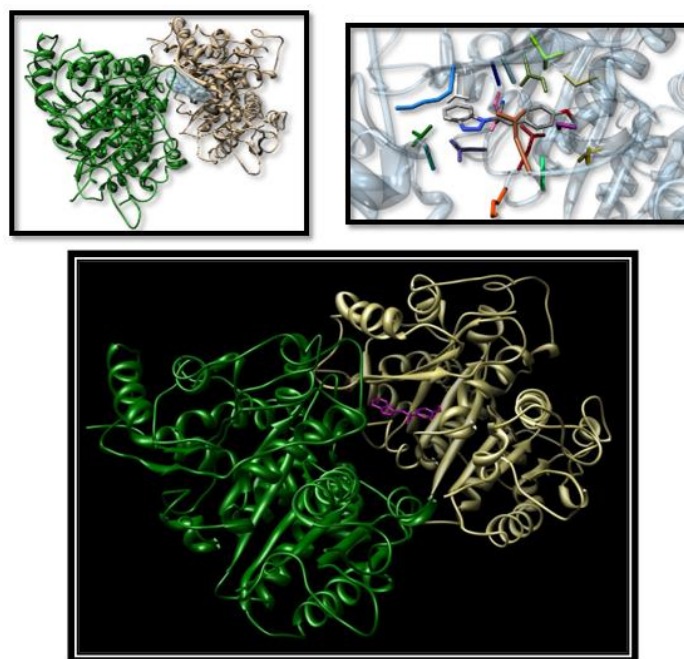
This result was further confirmed by [<sup>3</sup>H]Colchicine competition-binding scintillation proximity assay (SPA) [134], in which one of the derivatives of benzotriazole displaces strongly colchicine radio-labeled from its binding site on tubulin. It is also reported an extended molecular modeling study carried out using a model of tubulin obtained by homology from *Bos taurus*  $\beta$ -tubulin, which shows a 98% sequence homology with the human one, Fig. 21. This model has allowed to thoroughly study the binding site and the molecular interactions that exist between the *E*-3-aryl-2-(1*H*-benzotriazol-1-yl)acrylonitriles and the amino acidic residues of the binding pocket.



**Fig. 21.** Validation of the three-dimensional model. Comparison of co-crystallized podophyllotoxin

(brown) and subjected to docking (blue) in the allosteric binding site on human  $\beta$ -tubulin.

From this type of molecular analysis, and as shown in Fig. 22, it emerges that the benzotriazole ring interacts with tubulin mainly through Van der Waals interactions and that the compounds act by inserting the triazolic moiety in the colchicine binding site. Consequently, these compounds inhibit tubulin polymerization acting as microtubules destabilizing agents.



**Fig. 22.** Interaction of the derivative (*E*)-2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-3-(4-methoxyphenyl)acrylonitrile in the binding site of tubulin.

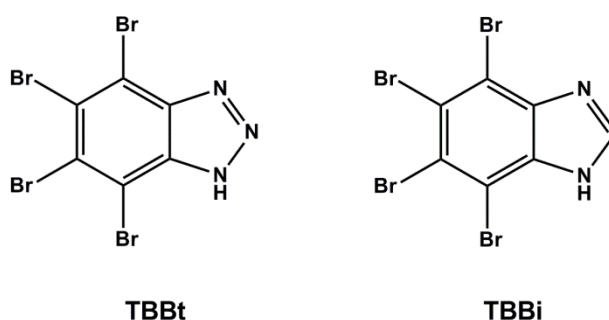
#### **BENZOTRIAZOLE AS PROTEIN KINASE CK2 INHIBITOR**

The Casein kinase 2 (CK2) is a pleiotropic protein highly conserved, a serine/threonine and tyrosine kinase constitutively active [135] involved in numerous metabolic processes. It is believed that the CK2 is involved in cell growth, both in healthy and cancerous cells [136], in the synthesis of tRNA and mRNA [137], also in apoptosis and in cell transformation. The CK2 has been described for the first time by Burnett and Kennedy [138] and different isoforms were identified [139, 140]. However, the CK2 conventionally exists either in form of tetrameric complexes, composed of two catalytic subunits ( $\alpha$  and  $\alpha'$ , or  $\alpha''$ ) [141-143] and two regulatory subunits ( $\beta$  and  $\beta'$ ), or as free subunits [144, 145]. There are also indications that tetrameric CK2 can be assembled into more complex structures. There is increasing evidence to suggest that different molecular forms of CK2 may have different cellular functions. For instance, some substrates of CK2 can only be phosphorylated by tetrameric CK2, while others are phosphorylated exclusively by the free catalytic subunits [146]. Also, have been identified proteins able to interact with CK2, and some of these

discriminate between tetrameric and CK2 as individual subunits [147].

Scientific literature demonstrate that the CK2 has antiapoptotic properties, being able to interfere with important component of cell survival pathways, especially by determining down-regulation of pro-apoptotic proteins such as caspases [148]. More papers shown that the evolution of neoplastic disease and the onset of cancer is directly proportional to the CK2 activity [149]. Furthermore, it was demonstrated that the CK2 shows oncogenic activity in transgenic mice and is frequently over-expressed in tumor and leukemia cells [150-152]. Finally, the high constitutive activity of CK2 is suspected to be behind its pathogenicity potential, since it is exploited by several viruses to exert the phosphorylation of proteins essential to their life cycle [153]. Furthermore, the catalytic subunit  $\alpha$  cooperates with other protooncogenes to promote cell transformation in different experimental models [150]. This makes CK2 an attractive target in the search for new antineoplastic and antiviral agents.

In recent years, potent and selective CK2 inhibitors have been developed; among these are the 4,5,6,7-tetrabromobenzimidazole (TBBi) and 4,5,6,7-tetrabromobenzotriazole (TBBt), shown in Fig. 23.



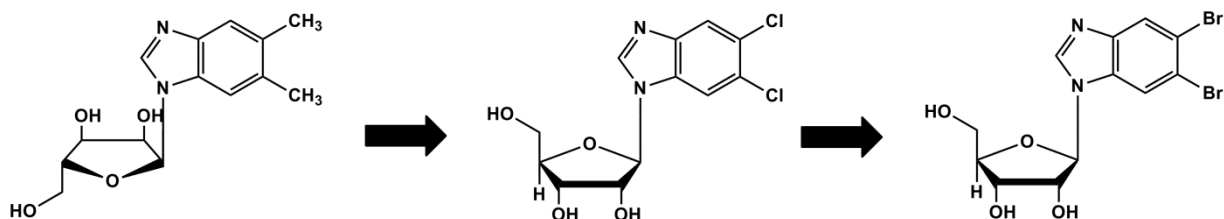
**Fig. 23.** 4,5,6,7-tetrabromobenzimidazole (TBBi) and 4,5,6,7-tetrabromobenzotriazole (TBBt) chemical structure.

Sarno and co-workers showed that TBBt is the most selective and specific inhibitor of CK2 among those so far analyzed. Among the 33 protein kinases tested by this research group, only three are inhibited by TBBt and their respective  $IC_{50}$  values appear to be about two orders of magnitude higher than the value calculated using the CK2. The three kinases inhibited by TBBt are CDK2 and GSK3L, belonging to the same protein kinase subfamily (named CMGC group), and PHK, belonging to the CaMK group. Moreover, it was shown that, within the same CK family, TBBt turns out to be more selective for CK2 ( $K_i = 0.4 \mu M$ ), compared with CK1 ( $K_i = 47 \mu M$ ) [154]. A third class of CK proteins, localized to the Golgi apparatus (G-CK), were instead totally refractory to TBBt [155]. All this suggests that TBBt possess a particular selectivity only towards CK2 as inhibitor agent. This property, combined with its ability to penetrate inside the cell and lack of

evident short term cytotoxicity, makes the TBBt a promising leads to design new compounds having a high therapeutic potential .

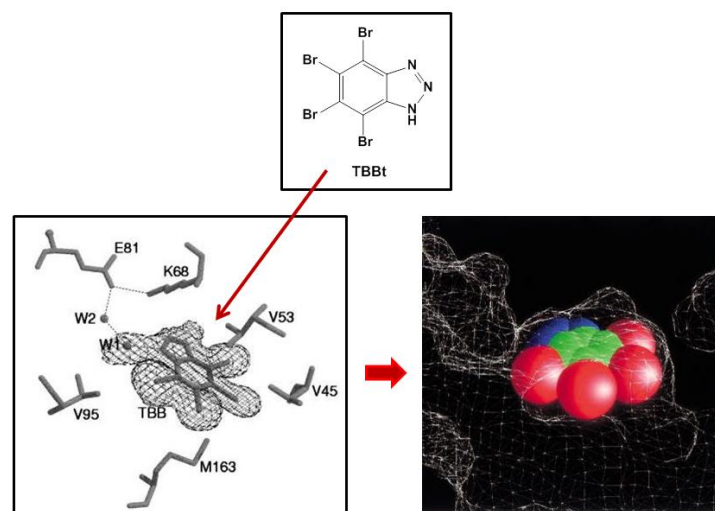
Zien research group compared the ability of action of TBBt and TBBi against CK2 purified holoenzymes, showing that the latter is not only far more effective in discriminating between different forms of CK2 present in yeast [156, 157], but is also dramatically more effective at inducing apoptosis and, to a lesser extent, necrosis, in transformed human cells [158]. Moreover, the solution of the crystal structure of the complex between CK2 and 4,5,6,7-tetrabromo-1*H*-benzimidazole [159] has allowed a comparison with the structures of the complexes with 4,5,6,7-tetrabromo-1*H*-benzotriazole [160] leading to the conclusion that despite their structural similarities they binds the biological target in a quite different manner.

The history of TBBt born in 1950, when it was demonstrate that the 1- $\alpha$ -ribofuranosido-5,6-dimethylbenzimidazole (Fig. 24) represents a key constituent for vitamin B<sub>12</sub> [161, 162]. This has subsequently stimulated the synthesis of a variety of structural analogues to evaluate their biological activity, particularly on cell proliferation. It was then discovered that the 5,6-dichloro-1-( $\beta$ -D-ribofuranosyl)benzimidazole (DRB) is able to inhibit the mRNA synthesis in eukaryotic cells, *in vivo* and *in vitro* [163]. Additionally 5,6-dibromo-1- $\beta$ -D-ribofuranosylbenzimidazole is a more potent inhibitor of *in vivo* and *in vitro* transcription, inhibiting at the same time purified CK2 activity at 6-10 times lower concentrations than DRB [164].



**Fig. 24.** 1- $\alpha$ -ribofuranosido-5,6-dimethylbenzimidazole derivatives.

All these works stimulated the study of other halogenated benzimidazole nucleoside analogues [146, 165] and led eventually to the synthesis of TBBt [166]. The specificity of inhibition of TBBt was then exploited extensively to understand the functionality of the CK2. The research group of Battistutta, for instance, studied the interactions between the TBBt and *Zea mays* protein kinase CK2 $\alpha$  catalytic subunit. Particularly, the enzyme-inhibitor complex crystal structure has been delineated, highlighting that the specificity and selectivity of TBBt for CK2 appears to be mainly dictated by the reduced size of the active site, a small hydrophobic pocket adjacent to the binding site of ATP/GTP, in which TBBt fit perfectly, as depicted in Fig. 25.



**Fig. 25.** TBBt inside CK2 $\alpha$  hydrophobic binding pocket. From: *Protein Sci.* 2001 November; 10(11): 2200–2206. © Copyright 2001 The Protein Society.

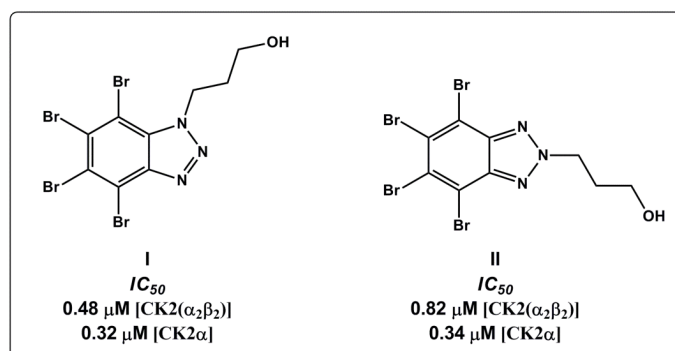
The main interactions between TBBt and CK2 are hydrophobic in nature and involve the four bromine atoms, whose size makes it the perfect insertion into the cavity and at the same time hinder the release of the inhibitor, once it has been trapped inside the pocket [160].

The inhibitory power decreases if the four bromine atoms of TBBt are replaced with smaller atoms, for instance with chlorine atoms, as previously observed by the Szyszka's research group [166]. It therefore seems that the TBBt lose its inhibitory potency against the majority of protein kinases, simply because the hydrophobic binding site in most other protein kinases is generally too large for making stable interactions with this inhibitor.

The relevance of the individual bromine atoms of 4,5,6,7-tetrabromobenzotriazole (TBBt) was then investigated by the group of Wasik, which synthesized all the possible isomers of analogs TBBt, mono-, four di-, and two tri-bromobenzotriazoles, evaluating the physico-chemical properties in an aqueous medium and confirming that hydrophobic and electrostatic interactions are predominant in halogenated benzotriazoles, determining a selective activity inhibition on protein kinase CK2 $\alpha$  [167].

In order to increase the TBBt and TBBi CK2 inhibitory activity, the research groups of Bretner and Najda-Bernatowicz designed new tetrabromo derivatives in order to evaluate the effect of alkyl substituents with different hydrophobic, steric and electrophilic features attached to TBBt and TBBi on their inhibitory potency. Their research program is based on the evidence that water molecules, necessary for enzymatic activity, are coordinated at the binding site on CK2 [159], molecules that could be displaced by similar substituents. New N-hydroxyalkyl derivatives were then tested on human CK2 holoenzyme ( $\alpha_2\beta_2$ ), demonstrating that the pharmacological activity depends on the length of the alkyl chain. Some of the N-hydroxyalkyl derivatives showed IC<sub>50</sub> values similar to that

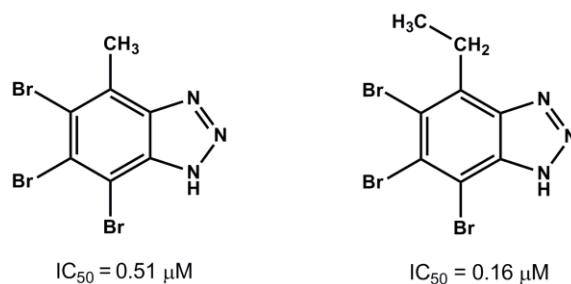
of the parent compounds, and best results were ensured by the propan-1-ol chain, with  $IC_{50}$  values of  $0.48 \mu\text{M}$  [168]. In 2009, the group investigated the effects of bulkier alkyl chains, with both hydrophobic and polar properties. Once again, the 3-(4,5,6,7-tetrabromo-1*H*-benzimidazol-1-yl)propan-1-ol (**I**) appears to be the best in the series, with small differences from the N2-substituted derivative (**II**) (Fig. 26). On the contrary, derivatives with shorter or longer chains see a drastic decrease in their activity. Additionally they demonstrated that the four bromine atoms in the benzene ring seem to be an essential requirement for biological activity. Indeed, the substitution of bromine with chlorine or  $\text{CH}_3$  groups leads to significantly lower inhibitory effect versus different forms of CK2, human and not. The relevance of the triazole system is finally put in evidence with its replacement with a phthalimide moiety, present in many biologically active compounds [169, 170], or phthalazine system. The derivatives thus obtained results in fact powerless [171].



**Fig. 26.** TBBt N-hydroxyalkyl derivatives.

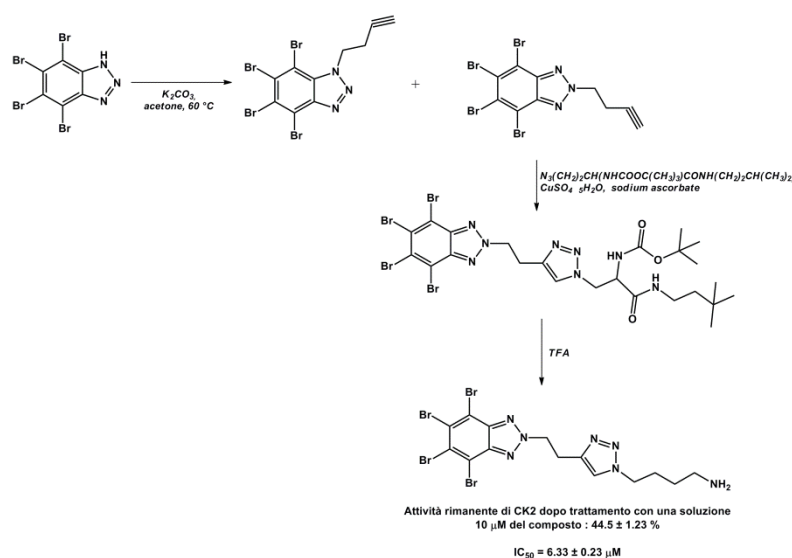
Other research groups continued to design new TBBt derivatives in consideration of the fact that the hypothesis concerning the small size of the TBBt binding site on CK2 has faded over the years [172], especially in the light of the biological successes reported by dimensionally bigger molecules [173, 174].

Were then conducted new researches introducing on the triazolic moiety even bulkier side chains. Was also evaluated a modification of the tetrabrominate benzene ring, replacing the two bromine atoms in 4 and 5 with aryl and alkyl substituents. Unfortunately it was not found any improvement in activity compared to TBBt inhibitory potency ( $IC_{50} = 0.46 \text{ mM}$ ), except for 5,6,7-tribromo-4-methyl-1*H*-benzotriazole ( $IC_{50} = 0.51 \mu\text{M}$ ) and 5,6,7-tribromo-4-ethyl-1*H*-benzotriazole ( $IC_{50} = 0.16 \mu\text{M}$ ), as shown in Fig. 27 [175].



**Fig. 27.** 5,6,7-tribromo-4-methyl-1*H*-benzotriazole and 5,6,7-tribromo-4-ethyl-1*H*-benzotriazole chemical structures.

The Swider group instead synthesized new benzotriazole derivatives, designed using a receptor-based design approach to interact simultaneously with the  $Mg^{2+}$ -chelating residues and the protein-substrate binding residues [176]. From the synthetic point of view, these compounds were obtained by Azide-alkyne Huisgen cycloaddition on tetrabromobenzotriazole derivatives. The result is a combination of the tetra-halogenated moiety, which nicely occupies the ATP-binding, with side chains able to bind simultaneously both the enzymatic active site and the basic residues that participate in protein substrate binding, like similar bisubstrate inhibitors of other kinases [177]. In this paper were reported the preliminary and non-optimal results for the most active compound, 4-(4-(2-(perbromo-2*H*-benzo[*d*][1,2,3]triazol-2-yl)ethyl)-1*H*-1,2,3-triazol-1-yl)butan-1-amine ( $IC_{50}$   $6.33 \pm 0.23$ ), which synthesis is reported in Fig. 28.



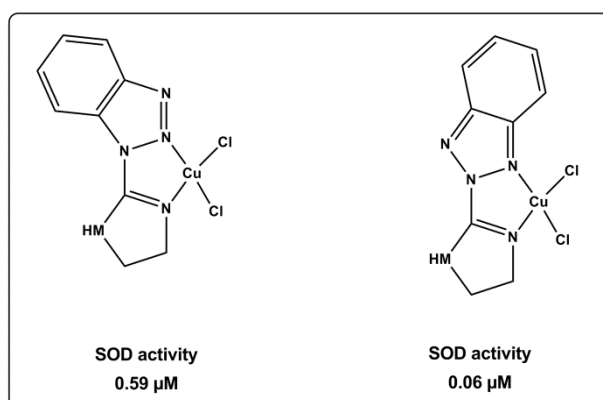
**Fig. 28.** 4-(4-(2-(perbromo-2*H*-benzo[*d*][1,2,3]triazol-2-yl)ethyl)-1*H*-1,2,3-triazol-1-yl)butan-1-amine synthesis.

Finally, it is important to report the new synthetic approach of Wawro and co-workers, which is based on two considerations: 1) none of the previously synthesized halogenated derivatives of TBBt had a *chiral* center, and 2) the presence of a stereocenter close to a polar group, i.e. an OH, could lead to a different inhibitory activity in the two enantiomers. Based on these considerations and

taking as reference the hydroxyalkyl derivatives synthesized by Nadja-Bernatowicz [171] and Makowska [175], the group has developed a new synthetic approach to isolate enantiopure hydroxyalkyl derivatives of 4,5,6,7-tetrabromo-1*H*-benzotriazole, obtaining optically active compounds endowed with enantioselectivity factor (*E*) >200 [178]. These molecules, potential CK2 inhibitors, might be used to synthesize new, optically pure TBBt derivatives.

### BENZOTRIAZOLE IN COORDINATION COMPOUNDS

As above, the benzotriazole coordination complexes showed antiproliferative activity [69]. A study on *N*-(4,5-dihydroimidazol-2-yl)azoles derivatives, involved in coordination complexes with transition metals, has highlighted their potential anticancer properties [179]. On this basis, the group of Saczewski has reported the synthesis and biological evaluation of some chelating bidentate benzotriazole Copper(II) Complexes [180]. Such complexes have been designed with the idea to create compounds endowed with potential copper-zinc-superoxide dismutase (Cu, Zn-SOD) mimicking activity. This activity in tumor cells, according to the theory of Oberley and Buettner, is less than that found in normal cells [181]. We therefore analyzed the [2-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-benzotriazole]-dichlorocopper(II) and [1-(4,5-dihydro-1*H*-imidazol-2-yl)-2*H*-benzotriazole]-dichlorocopper(II) complexes results possess the lower and the higher SOD activity respectively. Since these compounds differ only in the substitution position to the benzotriazole ring, it follows that the biological activity is exquisitely sensitive to the structure of the coordinating ligand (Fig. 29). The derivative of substitution in 2 showed a potent *in vitro* SOD activity, with 0.06  $\mu$ M IC<sub>50</sub> value, comparable with that reported in literature for other agents mimicking SOD. Evaluating the *in vitro* cytotoxicity on seven tumor lines, once again the derivative of substitution in 2 turns out to be the best one, recording an IC<sub>50</sub> values between 13 and 28  $\mu$ M.



**Fig. 29.** Chelating bidentate benzotriazole Copper (II) complexes.

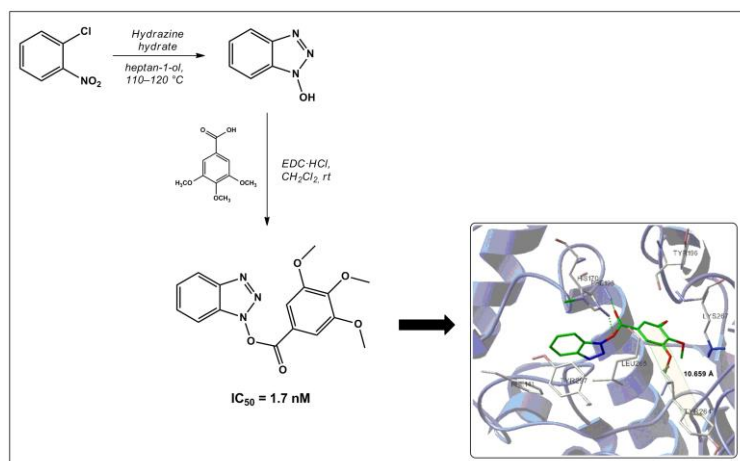
### BENZOTRIAZOLE AS HISTONE DEACETYLASE INHIBITOR

The histone deacetylase (HDAC) catalyzes the deacetylation of lysine residues, especially at the

histones H3 and H4 level [182]. This chemical modification is a key step in the regulation of genes expression that influence cell differentiation and proliferation processes [183]. An abnormal functioning of the HDACs has been linked to the carcinogenesis process [184]. It was found that many compounds able to inhibit HDACs can exert an antitumor effect *in vivo* [185].

There are eighteen humans HDAC and these enzymes are divided into four classes, according to their homologies to yeast's HDAC, to the subcellular localization and to the enzymatic activity. Class I includes HDAC1, 2, 3 and 8; class II is composed of six members, HDAC4, 7, 9 and 10; class III, also known as sirtuines, includes SIRT1-7, which are NAD(+)-dependent enzymes; class IV, which includes HDAC11, has the properties of both HDACs class I and class II [186]. HDAC inhibitors can be classified into five groups, according to their chemical structure: 1) hydroxamic acids, 2) cyclic tetrapeptides (apicidina); 3) short chain carboxylic acids, such as valproic acid; 4) benzamides 5) ketoacids [187].

However, in a recent paper by Fu and co-workers, even benzotriazole derivatives have been recognized as antiproliferative agents that exert their biological action through inhibition of histone deacetylase. In particular, the group synthesized various benzotriazole compounds bearing substituted benzoic acids, whose antitumor activity was evaluated on three different human tumor cell lines. All compounds showed antiproliferative activity and IC<sub>50</sub> values between 1.2 and 666 nM. Among these compounds 1*H*-Benzo[*d*][1,2,3]triazol-1-yl 3,4,5-trimethoxybenzoate stands out with an IC<sub>50</sub> value of 1.2-2.4 nM, very close to that of positive control, doxorubicin. The SAR analysis emphasized the relevance of the OCH<sub>3</sub> group for the antiproliferative activity: in fact derivatives bearing such substituents at benzoic ring position 3, 4 and 5 are the most powerful, while methoxyl substitution of benzotriazole group results in a drastic reduction of antiproliferative activity [188]. The HDAC inhibitory activity was determined using HeLa nuclear extract as the enzyme source, taking trichostatin (TSA) and suberoylanilide hydroxamic acid (HSA) as reference drugs. From the obtained results it is clear that the more potent derivative turns out to be once again trimethoxy derivative, in agreement with the result obtained for the antiproliferative activity. From this we can deduce that this activity is related to the ability of the compound to inhibit HDAC. This derivative was finally subjected to studies of molecular docking to simulate its interaction with the HDAC and to studies on the binding model based on the crystal structure of HDLP extracted from the HDLP/TSA complex using the AUTODOCK 4.0 software [189]. From these researches it appears that there are some kinds of hydrophobic interactions of the benzotriazole and phenyl moieties with Phe141, Tyr196, Leu265, Lys267, Tyr297 of the enzyme (Fig. 30).



**Fig. 30.** 1*H*-benzo[*d*][1,2,3]triazol-1-yl 3,4,5-trimethoxybenzoate synthesis and binding mode.

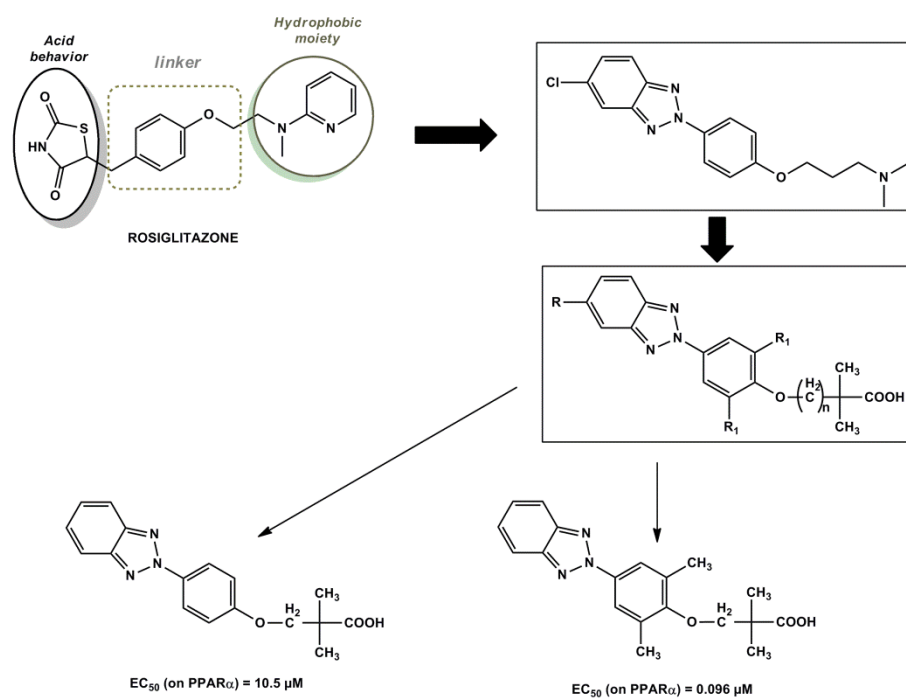
### BENZOTRIAZOLE ALKANOIC ACIDS DERIVATIVES AS PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARs) AGONIST.

The peroxisome proliferator-activated receptors (PPARs) belong to the superfamily of intracellular receptors regulate many biological processes, including energy metabolism (glucose and lipid metabolism), cell proliferation, skin development and inflammatory process [190, 191]. The PPAR $\gamma$  is a member of the second class of the steroid nuclear receptors superfamily and regulate gene transcription through the formation of functional heterodimers with retinoid X receptor (RXR) for 9-*cis*-retinoic acid. The PPAR/RXR complex modulates the expression of target genes by recognizing specific responsive sequences consist of a direct repeat (DR) of a hexanucleotide sequence [5'-TGA(A/C/T)CT] separated by a single nucleotide (DR1) [192].

The PPAR subfamily includes three isoforms:  $\alpha$ ,  $\beta/\delta$ , and  $\gamma$ . Encoded by different genes, only for the PPAR $\alpha$  and PPAR $\gamma$  specific ligands have been identified consisting of saturated and polyunsaturated long-chain fatty acids, eicosanoids, and *hypolipidemic* agents. In particular, among PPAR $\alpha$  agonists we can remember the fenofibrates, gemfibrozil, bezafibrate and clofibrate, all developed as drugs for the treatment of dyslipidemia, whereas pioglitazone and rosiglitazone are agonists of PPAR $\gamma$  and have been developed as drugs for the treatment of *type 2 diabetes mellitus* [193]. However, neither fibrates nor glitazones are able to simultaneously lowering triglycerides and glucose blood levels; therefore PPAR agonists research is thus ever timely, useful for the treatment of dyslipidemic patients suffering from type 2 diabetes [194, 195].

According to studies conducted by the Sparatore research group, many PPAR agonists possess three key regions: a) an acid head (a thiazolidinedione ring or an alkanoyl residue); b) a linker portion (a benzene ring bearing variously functionalized chain in the para position to the head); c) an hydrophobic tail (formed by a mono- or polycyclic aromatic or heteroaromatic moiety).

Thus, to develop new PPAR  $\alpha$ ,  $\beta/\delta$ , and  $\gamma$  activators, Sparatore *et al.* decided to stiffen the last two parts, suppressing the flexible chain linker between the aromatic rings. Following this way a series of [4-(2*H*-1,2,3-benzotriazol-2-yl)phenoxy]alkanoic acids was synthesized [196]. The 4-(2*H*-1,2,3-benzotriazol-2-yl)phenoxy moiety, which characterizes these compounds, is also present in the 5-chloro-2-(4-[3-(dimethylamino)propoxy]phenyl)-2*H*-1,2,3-benzotriazole, molecule previously recognized able to reduce significantly the level of cholesterol in hypercholesterolemic mice, measurement comparable to that of bezafibrate [197]. Undergoing biological tests, the study shows that the compounds 3-(4-(2*H*-benzo[*d*][1,2,3]triazol-2-yl)phenoxy)-2,2-dimethylpropanoic acid and 3-(4-(2*H*-benzo[*d*][1,2,3]triazol-2-yl)-2,6-dimethylphenoxy)-2,2-dimethylpropanoic acid, dual activators of PPAR  $\alpha/\delta$  and PPAR  $\alpha/\gamma$ , respectively, possess an effectiveness equal to that of the reference compound, the Wy-14643 [198], but differ strongly in potency, presenting respectively  $EC_{50}$  of 10.5  $\mu$ M and 96 nM ( $EC_{50}$  Wy-14643 = 9.3  $\mu$ M) (Fig. 31).



**Fig. 31.** Benzotriazol-2-yl-phenoxy-2,2-dimethylpropanoic acid derivatives.

Interestingly, the 5-chloro-2-(4-[3-(dimethylamino)propoxy]phenyl)-2*H*-1,2,3-benzotriazole is found to be inactive on all three receptors at concentration up to 100  $\mu$ M, demonstrating that probably the lipid-lowering effect of these molecule is due to a different mechanism of action from the activation of PPAR.

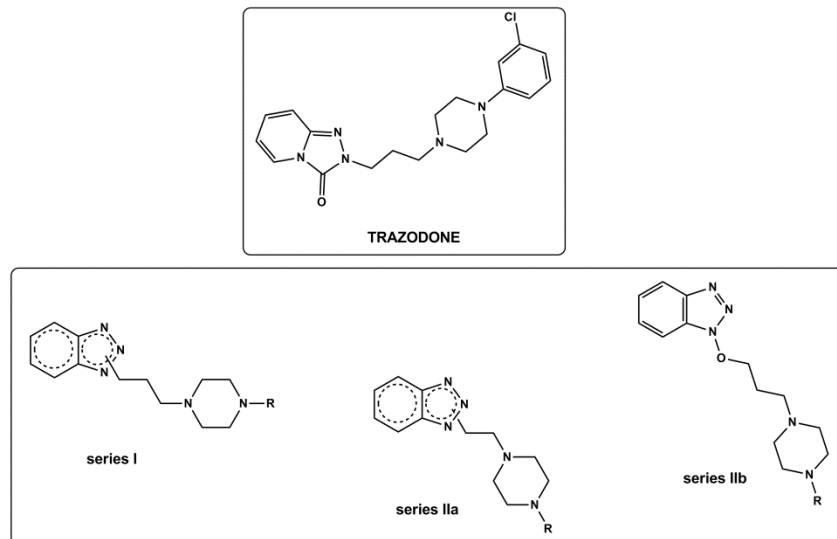
In summary, the obtained results show that the 4-(2*H*-1,2,3-benzotriazol-2-yl)phenoxy moiety that characterizes these compounds represents a new scaffold for the synthesis of potential PPAR agonists for the treatment of dyslipidemia associated with type 2 diabetes (NIDDM) or particular kinds of dyslipidemia not associated with diabetes.

## BENZOTRIAZOLE AS LIGAND FOR SEROTONIN AND DOPAMINE RECEPTORS

It is well known that the neurotransmitter 5-hydroxytryptamine (5-HT), more simply called serotonin, is implicated in numerous physiological functions, including control of appetite, regulating mood, voluntary movement, sleep body temperature, attention, memory and learning [199-201]. Alteration in the serotonergic system may then determine psychiatric disorders such as anxiety, depression and obsessive-compulsive disorder (OCD) [202]. For this reason, dopamine and serotonin agonists and antagonists have been used in recent years for the treatment of schizophrenia and Parkinson's diseases [203-205].

The Caliendo's research group reported a series of 1/2-[3-(4-(x)-1-piperazinyl)alkyl]-benzotriazoles derivatives, designed as structural analogues of trazodone, known psychoactive drug belonging to the piperazine and triazolopyridine class. The synthesized derivatives, as shown in Fig. 32, contain three major structures:

- 1) a N-4 substituted piperazine ring;
- 2) a cyclic system containing a substituted benzothiazole nucleus;
- 3) an alkyl or alkyloxy linker which acts as a *trait d'union* between the piperazinic and benzotriazole moiety.



**Fig. 32.** 1/2-[3-(4-(x)-1-piperazinyl)alkyl]-benzotriazoles derivatives.

For these derivatives were evaluated *in vitro* the anti-serotonin, anti-adrenergic and anti-histaminergic effect, but also *in vivo* the analgesic activity [206, 207]. Concerning the anti-serotonin activity, the results obtained show that the most powerful derivatives belong to the 1-(2-(4-(R)-1-piperazinyl)-ethyl)-benzotriazoles series, where R = Ph, 2-Cl-Ph, 3-Cl-Ph, 4-Cl-Ph, CH<sub>2</sub>-Ph, Me, CH<sub>2</sub>-CH<sub>2</sub>-OH. Derivatives bearing alkyl substituents on the 4-piperazine nitrogen showed no or

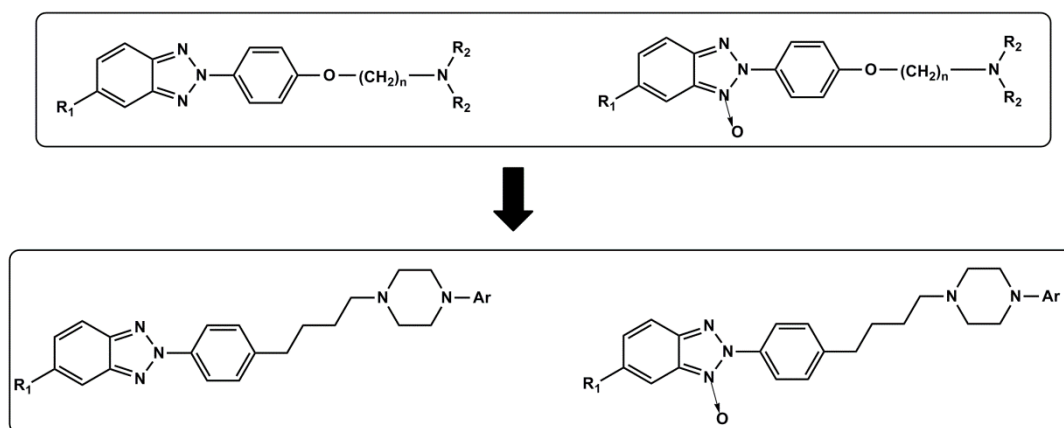
extremely low activity. Moreover, the introduction of a chlorine atom at the ortho and meta positions of the phenyl ring determines an increase of activity, while at the para position determine a loss of it by 10 to 100 times. Still, the introduction of a methylene or ethylene bridge between the aromatic ring and the piperazine nucleus leads to a decrease in activity due to a steric and electronic effect, rather than to a hydrophobic effect.

With regard to the anti-adrenergic activity, has been evaluated the *in vivo* ability to block the noradrenaline-induced contraction of rat vas deferens. The most active compounds were derivatives 1- and 2-[3-[4-(x)-1-piperazinyl]-propyl]-benzotriazoles (x = Ph, 4-Cl-Ph).

The anti-histamine effect was instead determined for compounds presenting simultaneously at the side chain an oxypropylene bridges and an aromatic moiety. The substitution of the aryl or benzyl group with a methyl or a  $\beta$ -hydroxy-ethyl lead to significant activity reduction.

In 1996, the Caliendo group, through binding assays with radiolabeled ligand assays, noted the affinity of previously synthesized derivatives for the following recombinant human receptor subtypes: 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>1D $\beta$</sub>  [208]. The experimental results shown that most of the compounds possess an high affinity for the 5-HT<sub>2A</sub> receptor, while none of them bear affinity for 5-HT<sub>1D $\beta$</sub>  receptor. The bond to the 5-HT<sub>2A</sub> receptor is favored by groups Ph, 3-Cl-Ph or 4-Cl-Ph on the N-4 piperazine ring. Instead, as regards to the 5-HT<sub>1A</sub> receptor, the potency increase significantly when a substituent 2-MeO-Ph is located at the N-4 piperazine ring. Then the receptors binding affinity is due to the double effect of the R substituent and the type of carbon linker. This means that there are different modes of interaction between ligand and receptor, as postulated by Ismaiel *et al.* [156] The research group of Caliendo has also compared the binding affinity and selectivity of the derivatives against different receptor isoforms, expressing it as 5-HT<sub>2A</sub>/5-HT<sub>2C</sub> ratio. The results of the experiments demonstrate that the best selectivity ratio is obtained when R is a Ph or 4-Cl-Ph group. On the contrary, when R is represented by a 2-MeOPh group is obtained a receptor ligand that acts as an antagonist toward the 5-HT<sub>1A</sub> receptor.

Other potential receptor ligands and trazodone like compounds are those reported by Sparatore *et al.*, whom analyzed for many years the benzotriazole pharmaceutical properties. They reported a series of 2-[4-[3-(4-aryl/heteroaryl-1-piperazinyl)propoxy]phenyl]- 2*H*-benzotriazoles and their N-oxides [209], which design is based on previous biological evidence reported for a series of 2-4-(dialkylaminoalkoxy)phenyl]benzotriazoles and corresponding N-oxides [197]. Fig. 33 shows the above series.



**Fig. 33.** Piperazinyl-phenyl-2*H*-benzotriazoles derivatives and their N-oxides.

Indeed, the aryl/heteroaryl moiety 3-trifluoromethylphenyl is a well known ligand for 5-HT<sub>1</sub> and 5-HT<sub>2</sub> receptors, but the affinity of these derivatives for dopamine and adrenergic  $\alpha_1$  receptors has not been excluded [210, 211]. The synthesized compounds were able to bind dopamine receptors D<sub>2</sub> and D<sub>3</sub> and serotonin receptors 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub>, but they present low affinity for subtypes 5-HT<sub>1B</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>3</sub>, and 5-HT<sub>4</sub>.

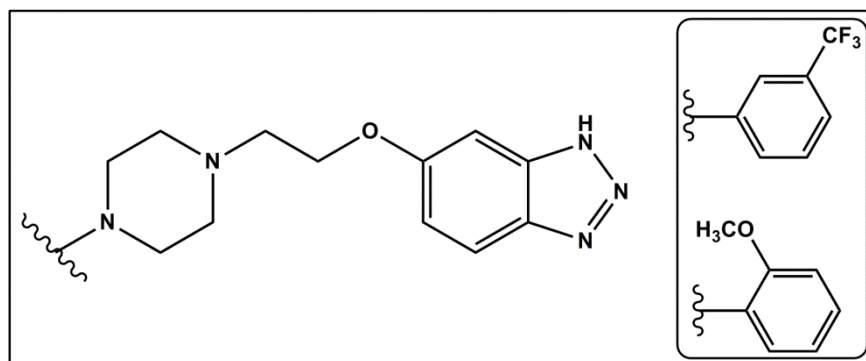
Also the next 2-(4-[ $\omega$ -[4-(2-Methoxyphenyl)-1-piperazinyl]alkoxy]phenyl)-2*H*-benzotriazoles and their N-Oxides shown an affinity for 5-HT<sub>1A</sub> and D<sub>3</sub> receptors, affinity that increases with the length of the aliphatic linker [212]. Even if the benzotriazole nucleus is identified as provider to the affinity towards the 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> receptors, the 4-[3-(benzotriazol-1-yl)propyl]-1-(2-methoxyphenyl)piperazine was instead identified as potent receptor antagonist for the pre- and post-synaptic 5-HT<sub>1A</sub> receptors [213].

Paluchowska and coworkers highlighted the relevance of the aliphatic linker length between methoxy phenyl piperazines moiety and the benzotriazole nucleus. They synthesized and evaluated a series of aryl piperazine in which the linker was composed of two to four methylene groups. Part of this set has proved to act as a ligand of the 5-HT<sub>1A</sub> receptor, with K<sub>i</sub> between 4 and 88 nM. The derivatives with bi-methylene bridges results *in vivo* inactive, while those with tetra-methylene bridges proved to operate as antagonists for postsynaptic 5-HT<sub>1A</sub> receptors [214].

More aryl/heteroaryl-piperazinyl alkyl benzotriazoles were prepared and evaluated, by modification of the benzotriazole moiety (introduction of substituents such as Cl and OCH<sub>3</sub>) or the aryl side chain (introduction of 2-pyrimidinyl or 3-trifluoromethylphenyl groups). These modifications have been conducted in an attempt to increase the affinity of the derivatives to the 5-HT<sub>1A</sub> receptors, and new compounds have in fact demonstrated moderate to good affinity towards serotonin receptor, but none or modest affinity for dopamine D<sub>2</sub> receptor [215].

Finally, demonstrating the significance of the structures previously reported, here is reported the

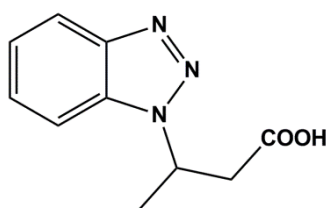
failure of the derivatives trifluoromethyl and methoxyphenyl piperazin-1-yl-ethoxy-1*H*-benzotriazoles, described in Fig. 34, that proved to be completely inactive as 5HT<sub>1A</sub> and D2 competitor [216].



**Fig. 34.** 6-(2-(4-Methylpiperazin-1-yl)ethoxy)-1*H*-benzo[*d*][1,2,3]triazole derivatives.

#### CHOLERETIC, CHOLESTEROL-LOWERING AND ANTI-INFLAMMATORY ACTIVITY OF BENZOTRIAZOLE

In 1964 Sparatore group described a series of benzotriazolylalkanoic acids, including the 3-(benzotriazol-1-yl)butanoic acid, which structure is detectable in Fig. 35, emerging for its strong choleretic activity [217, 218]. On this model was therefore carried out a series of structural changes in order to understand the meaning of modifications on the aromatic moiety of the heterocycle [219, 220]. Results demonstrate that the introduction of a methyl or a methoxy group at 6 position of the benzotriazole ring cause a decrease in biological activity compared to the 3-(benzotriazol-1-yl)butanoic acid, while the presence of a trifluoromethyl group determines its increase.



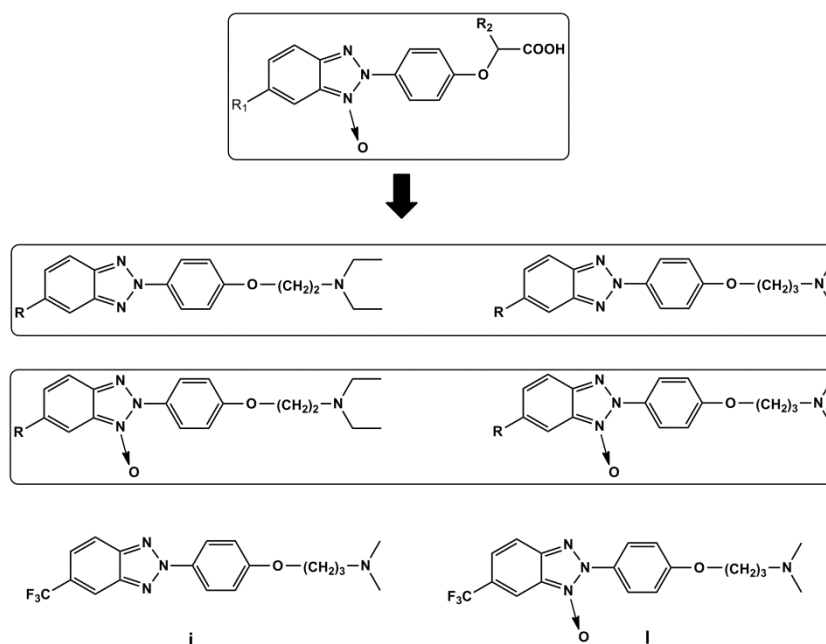
**Fig. 35.** 3-(Benzotriazol-1-yl)butanoic acid.

Also the modification of the side chain on the benzotriazole nitrogen N-1 has consequences: the activity is maintained *introducing unsaturation* into the N-alkyl chain, but is lost *shortening* it [221]. For some of these derivatives the antinociceptive and anti-inflammatory activity was also evaluated [222].

Researcher synthesized so many other alkanoyl acids, including a series of 4'-(benzotriazol-2-yl)phenylalkanoic and -phenoxyalkanoic acids their N-oxides with different biological properties, such as anti-inflammatory, diuretic or anti-hypertensive [223, 224].

Over the years additional modification have been done basing on Buu-Hoi studies [225], which determined, for some substituted benzimidazoles such as 2-[4-(2-diethylaminoethoxy)phenyl]benzimidazole, strongly depressive effects on the CNS and inhibitor activity against cholesterol biosynthesis.

Basing on this evidences a series of 2-[4-(dialkylaminoalkoxy)phenyl]benzotriazoles and their N-oxides has been synthesized [197], as described in Fig. 36:



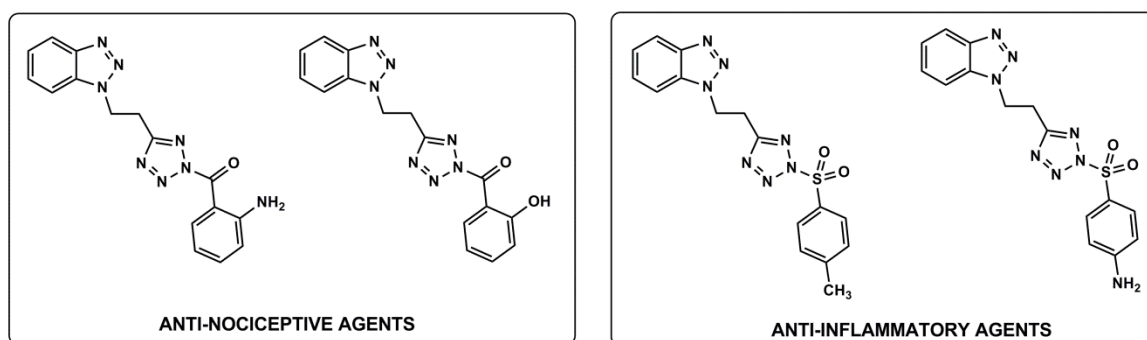
**Fig. 36.** Series of 4'-(benzotriazol-2-yl-phenyl)alkanoic and -phenoxyalkanoic acids and derivatives.

The pharmacological investigation demonstrated that compounds **i** and **I** showed different behavior:

- 1) to prevent the death by hypoxia in mice treated with potassium cyanide. This effect is normally exercised by calcium antagonists, such as cinnarizine and flunarizine. This effect may be related to antagonism versus leukotriene D4 receptor, effect exhibited by both derivatives;
- 2) the inhibition of thromboxane A2, exerted by compound **i**. The latter showed also anti-atherogenic properties.
- 3) the diuretic action exerted by the compound **I**.
- 4) strongly cholesterol-lowering activity of both derivatives.

Widely used to synthesize new compounds of pharmaceutical interest, benzotriazole, as mentioned so far, stands out for its multiple biological activities, including the anti-inflammatory, anesthetic and anticonvulsant. 1-lupinylbenzotriazoles have been tested for anti-inflammatory, diuretic, analgesic and anti-hypertensive activity, obtaining good results in one or more areas [226]. Instead, compounds *N*-[2-(tert-amino)ethyl]- and *N*-(lupinyl)-benzotriazol-1/2-yl acetamides, assessed as local anesthetics, have shown an activity comparable or higher to that of lidocaine [227].

The benzotriazole ring was also combined with tetrazole systems in order to enhance the antinociceptive and anti-inflammatory properties of both nuclei [228]. Among the reported in the scientific literature 1-[2-(1*H*-tetrazol-5-yl)ethyl]-1*H*-benzo[*d*][1,2,3]triazoles derivatives, the 5-(2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)ethyl)-1*H*-tetrazol-1-yl(4-aminophenyl)methanone and (2-hydroxyphenyl)methanone emerge for antinociceptive properties, being able to triple and quadruple, respectively, the reaction time in the hot plate test. Instead, the 1-(2-(1-tosyl-1*H*-tetrazol-5-yl) ethyl)-1*H*-benzo[*d*][1,2,3]triazole and 4,5-(2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)ethyl)-1*H*-tetrazol-1-yl)sulfonyl)benzenamine possess anti-inflammatory activity superior to that of the derivatives of the same series (Fig. 37).



**Fig. 37.** Tetrazolyl-benzotriazole derivatives endowed with anti-nociceptive and anti-inflammatory activity.

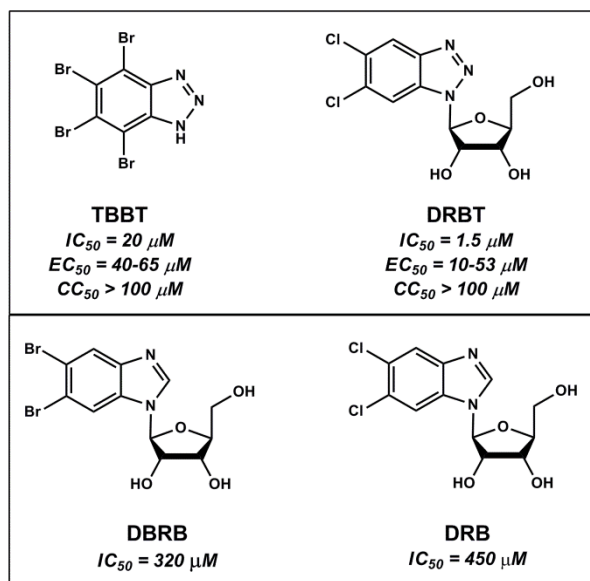
Among the new derivatives that can fit in the category of non-steroidal anti-inflammatory agents there is to count a number of compounds obtained by the combination of both the benzotriazole and benzofuranic nuclei [229]. These derivatives were evaluated concerning the antinociceptive and anti-inflammatory activities. The 3-acetyl-4-(1-benzotriazolyl)-5-(2-benzofuryl)-1-(*p*-chlorophenyl)pyrazole stands out for its analgesic action, and SAR studies show that the pyrazole nucleus is more effective than the 1,3,4-thiadiazole ring. The results obtained in several animal models suggest that these derivatives exert centrally and peripherally mediated antinociceptive properties. Almost all of the prepared compounds also expressed anti-inflammatory activity, with a maximum for the 2-(5-acetyl-3-phenyl-1,3,4-thiadiazol-2-ylidene)-1-(2-benzofuryl)-2-(1-benzotriazolyl)-ethanone. SAR analysis of 5-acetyl-1,3,4-thiadiazole derivatives demonstrates that such activity decreases with the modification on the phenyl side chain. Studies on the mechanism of action have finally shown that new compounds act through a selective inhibition of COX-2 [230].

#### BENZOTRIAZOLE AS ANTIVIRAL AGENT

Viruses are pathogens agents that can cause major diseases both in human and animals, determining life lost, economical loss and higher productivity costs. Bartorfd classification part them in DNA

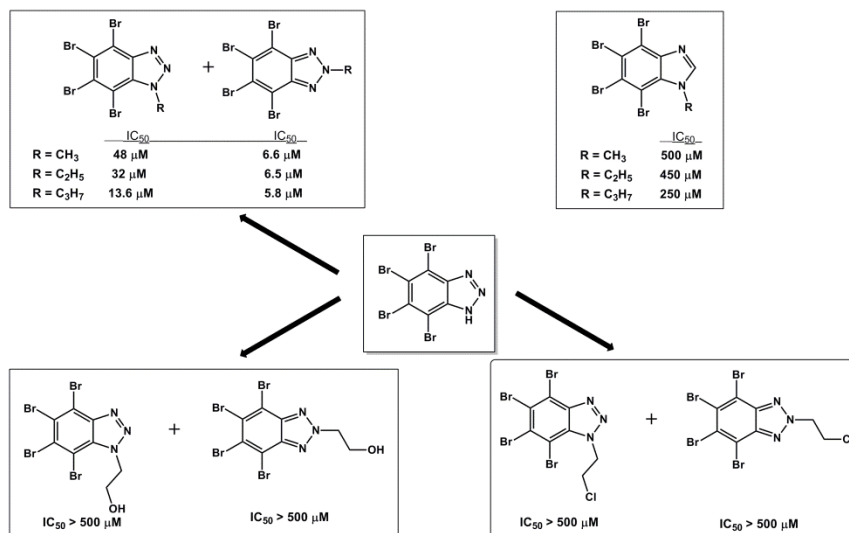
and RNA viruses, tabulation genomic based. Numerous targets can be used to develop antiviral agents, and a large part of them is represented by enzymes, such as polymerase, protease, and helicase. Nucleosides and non-nucleosides inhibitors are widely investigated to implement the pharmacological arsenal and to obtain more potent and selective antiviral agents. Resulting from the potent bioactivity of benzotriazole, several derivatives act as antiviral agents, objecting to a variety of targets and showing sometimes an interesting selectivity of action.

Starting from helicases, synthesis and biological activity of benzotriazole analogous as NTPase/helicase inhibitors has been extensively investigated [231]. As recently reported NTPase/helicase is a promising target and ssRNA<sup>+</sup> (positive sense single-stranded RNA) enzymes have been studied in detail [232], since ssRNA<sup>+</sup> viruses, belonging to families like Flaviviridae and Picornaviridae, continues to pose threats to public health [233, 234]. Benzotriazole helicase inhibitors represent an interesting class of drugs, and potent derivatives were identified during the course of random screening studies. Particularly, previously reported as CK2 inhibitor 4,5,6,7-tetrabromobenzotriazole (TBBt), and 5,6-dichloro-1-( $\beta$ -D-ribofuranosyl) benzotriazole (DRBT) displayed an antiviral activity, showing IC<sub>50</sub> values of 20 and 1.5  $\mu$ M, respectively [23]. DRBT and TBBt have been tested in four different HCV subgenomic replicon systems, resulting both able to inhibit HCV replication (EC<sub>50</sub> DRBT = 10–53  $\mu$ M, EC<sub>50</sub> TBBt = 40–65  $\mu$ M) in a comparable way to the inhibition reported in the enzymatic essays, showing a property that has been detected only for a handful group of HCV inhibitors. Neither they are cytotoxic at concentrations up to 100  $\mu$ M [235]. Furthermore, N-alkyl derivatives of TBBt showed good inhibitory activity against HCV, WNV, and JEV NTPase/helicases, and less cytotoxicity [236]. The relevance of the benzotriazole moiety was finally demonstrated by replacing it with a benzimidazole in the 5,6-dichloro and 5,6-dibromo-1-( $\beta$ -D-ribofuranosyl) benzimidazole (DRB and DBRB), much less potent in inhibit HCV helicases (Fig. 38).



**Fig. 38.** Structures and anti HCV (DNA substrate) activity of benzimidazole and benzotriazole derivatives.

To explain this finding, Bretner *et al.* synthesized and studied a new series of N-substituted 1H-benzimidazole and 1H-benzotriazole [231]. The starting compounds 1H-benzotriazole and 1H-benzimidazole showed a very low anti HCV-helicase activity on a DNA substrate ( $IC_{50}$  200  $\mu M$  and 500  $\mu M$ , respectively) and no activity ( $IC_{50} > 500 \mu M$ ) when measured either with an RNA substrate or against the enzymes of WNV, DENV, and JEV (family: Flaviviridae). On the contrary, the whole brominated 1H-benzotriazole revealed to be 9-10 fold more effective in inhibit the HCV helicase when determined with a RNA or DNA substrate (more potency in case of JEV helicase,  $IC_{50}$  20  $\mu M$ ). Moreover, the brominated 1H-benzimidazole resulted to be less effective than TBBT but more potent than the non-halogenated parent compounds against HCV helicase. To enhanced hydrophobicity, N-alkylated derivatives (substituted with methyl, ethyl and propyl moieties) were synthesized, as shown in Fig. 39.

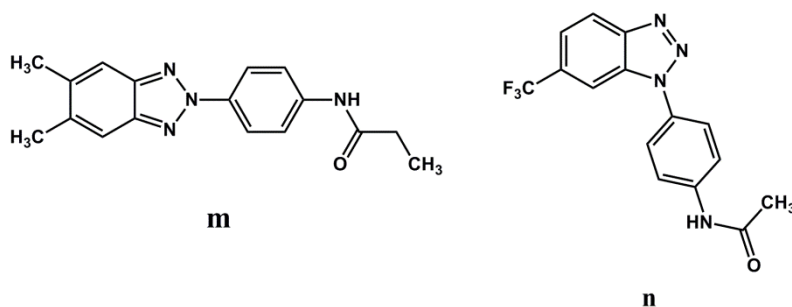


**Fig. 39.** Structures and anti-HCV helicase activities of 1*H*- tetrabromobenzotriazole and tetrabromobenzimidazole derivatives using a DNA substrate.

The comparison of 1- or 2-alkyl benzotriazoles effectiveness on the HCV-helicase using the DNA substrate demonstrated that the 2-alkylated derivatives resulted significantly more potent as helicase inhibitors, while in 1-alkylated benzotriazoles and benzimidazoles the aliphatic chain elongation determined an activity enhancement. However, in DNA substrate the benzimidazole derivatives inhibitory activity was very low (ranged between 250 and 500  $\mu\text{M}$ ), and no inhibitory activity was observed using the RNA substrate, as well as using other viral NTPase/helicases. This behavior suggests that these inhibitors do not act through a block on the enzymatic NTP binding sites, and that the occupation of an allosteric nucleoside binding site should be considered, as previously suggested by Porter [237].

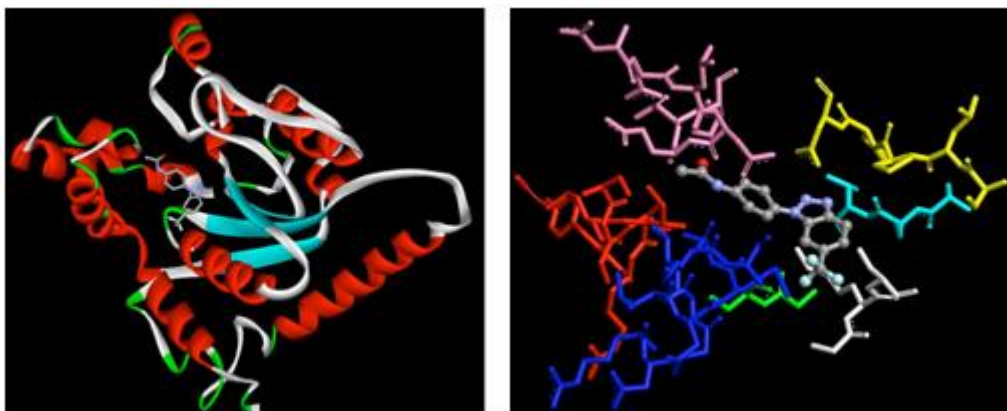
Investigating the role of hydrophobic N-alkyl substituents, the authors observed that replacement of the alkyl side-chain by a substituent endowed with higher hydrophilicity (such as in hydroxyethyl derivatives) or with higher hydrophobicity (such as in chloroethyl derivatives) severely decreases the activity of the TBBt derivatives, demonstrating that a small hydrophobic alkyl moiety (methyl or ethyl) at TBBt position 2 could play a crucial role in the inhibition of HCV NTPase/helicase. Finally, authors report that introduction of a ribofuranosyl ring in both benzotriazole and tetrabromobenzotriazole improves the water solubility but leads to a decrease of the inhibitory activity against HCV and all the enzymes tested [23].

More NS3-targeting benzotriazole were investigated by Carta and coworkers, that evaluated *in vitro* cytotoxicity and antiviral activity against a wide spectrum of ssRNA+ viruses of a series of *N*-[4-(1*H*(2*H*)-benzotriazol-1(2)-yl)phenyl]alkylcarboxamides [238]. From this study emerged that the Enteroviruses CVB-2 and Sb-1 (Picornaviridae family) were the only inhibited by title compounds. This is an interesting target, since actually no specific antiviral therapy is available for the treatment of Picornaviridae infections. Particularly, two of them emerged for their selectivity: *N*-(4-(5,6-dimethyl-2*H*-benzo[*d*][1,2,3]triazol-2-yl)phenyl)acetamide (**m**), which was the most active against CVB-2 ( $\text{EC}_{50}=10 \mu\text{M}$  and  $\text{CC}_{50}>100 \mu\text{M}$ ) and *N*-(4-(6-(trifluoromethyl)-1*H*-benzo[*d*][1,2,3]triazol-1-yl)phenyl)acetamide (**n**), which was the most active against Sb-1 ( $\text{EC}_{50} = 30 \mu\text{M}$  and  $\text{CC}_{50}=90 \mu\text{M}$ ) (Fig. 40).



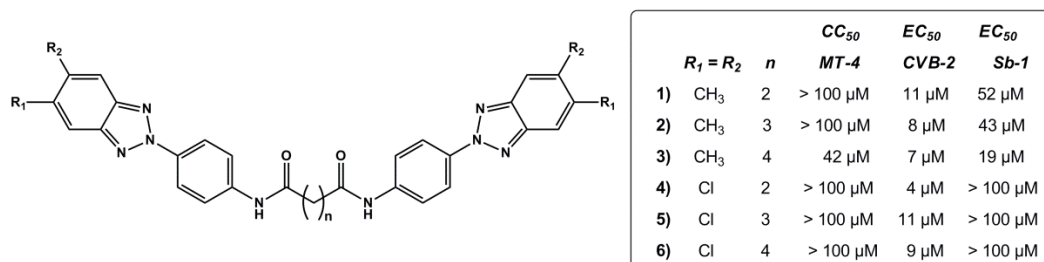
**Fig. 40.** N-[4-(1*H*(2*H*)-benzotriazol-1(2)-yl)phenyl]alkylcarboxamides derivatives.

SAR analysis suggest that the potency and selectivity of **m** is probably due to the small electron donor methyl group at positions 5 and 6, a phenyl group at position 2 and a propanoyl amide group at 4', while the selectivity of **n** is correlated to a CF<sub>3</sub> group at position 6, a phenyl at position 1 and an amide group at 4'. Title compounds were also evaluated *in silico* against the Polio virus (Sb-1) helicase, which 3D model was obtained by homology techniques. Molecular dynamics simulations showed that all inhibitors are able to rank binding affinities with a similar docking mode in the putative binding site, as depicted in Fig. 41.



**Fig. 41.** Cartoon of the binding mode of compound **n** in the putative binding site of the Polio (Sb-1) helicase and snapshot of the docked compound in the putative binding site.

Using these molecules as lead compounds and making use of Diana's patent concerning *N,N'*-bis[4-(2-benzimidazolyl)phenyl]alkyldicarboxamides [239], Carta *et al.* prepared a series of *N,N*-bis-[4-(1*H*(2*H*)-benzotriazol-1(2)-yl)phenyl]alkyldicarboxamides [240]. Evaluated in parallel cell-based assays for cytotoxicity and antiviral activity, none of the them resulted active against representative RNA viruses, while best activity is reported for (2*H*) analogues. Among them bis-5,6-dimethyl-derivatives (**1-3**) exhibited good activity against Enteroviruses (EC<sub>50</sub> = 7–11 μM against CVB-2, EC<sub>50</sub> = 19–52 μM against Sb-1), and some bis-5,6-dichloro-benzotriazol-2-yl derivatives (**4-6**) emerged for the interesting selective activity against CVB-2 (EC<sub>50</sub> = 4–11 μM) resulting to be completely inactive against all the other viruses screened, pairing this behavior with a good no cytotoxic profile (Fig. 42).



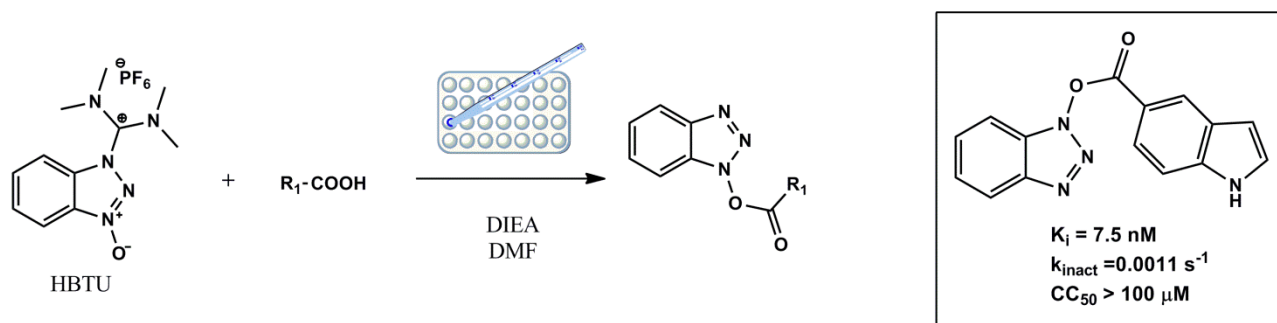
**Fig. 42.** *N,N*-bis[4-(2*H*)-benzotriazol-(2-yl)phenyl]alkylcarboxamides and their antiviral activities.

Finally, an extended binding mode study was performed on *Sb-1* helicase previously obtained through homology techniques. Furthermore, in the absence of a 3D model for the *CVB-2* helicase, the activity of **4-6** derivatives was explained adopting a 2D alignment analysis.

Benzotriazole derivatives were also found to be very interesting protease inhibitors toward a new breaking out human Coronavirus: the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) [241]. Spreading from southern China in late 2002, in 2003 SARS became epidemic, rapidly *diffused* from its origin to more than 25 countries. It affected almost 8000 patients and cause the death of about 10% of those infected, a high mortality rate [242].

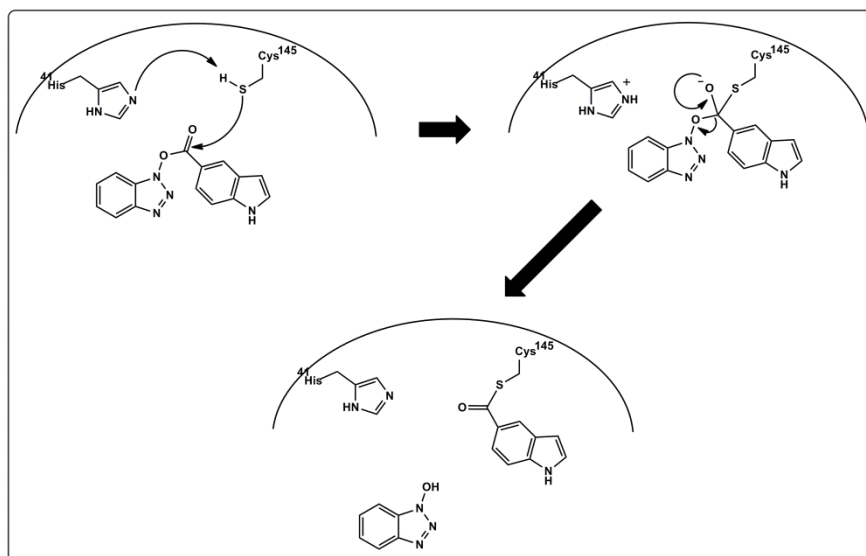
Since no effective anticoronaviral therapeutics are available, considering the lingering danger to human health represented by SARS-CoV as well as other coronaviruses, a search for helpful antivirals for the SARS-CoV is of current interest. The most important enzymatic target that the scientific community identified is represented by a protease, the main proteinase or  $M^{pro}$  (also called dimeric chymotrypsin-like protease or  $3CL^{pro}$ ) [243], that cleavage two polyproteins, pp1a and pp1ab [244, 245], to provide the functional proteins for viral propagation. Only few  $M^{pro}$  inhibitors have been reported, like aryl boronic acid [246], keto-glutamine analogues [247], phthalhydrazide ketones [248], an  $\alpha,\beta$ -epoxyketone [249], thiopurine analogues [250].

In 2006, Wu *et al.* reported a new class of stable benzotriazole esters, which appeared to act as  $3CL^{pro}$  irreversible inactivators, with inhibition constants in the nanomolar range [251]. All derivatives were obtained using the strategy of combinatorial reaction in microtiter plates followed by screening *in situ* [250-252]. Particularly, benzotriazole esters were prepared by condensation of 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) with 90 various carboxylic acids. Only the derivatives that resulted stable at pH 5.0–8.0 over 24 hr at room temperature were screened. All compound are not cytotoxic ( $CC_{50}$  on Vero E6 cells > 100  $\mu M$ ), and the 1*H*-indole-5-carboxylic acid benzotriazol-1-yl ester (Fig. 43) stood out, exhibiting a  $k_{inact}$  of 0.0011  $s^{-1}$  and a  $K_i$  of 7.5 nM.



**Fig. 43.** Synthesis of benzotriazole esters starting from HBTU and 90 acids in microtiter plates, and 1*H*-benzo[*d*][1,2,3]triazol-1-yl 1*H*-indole-5-carboxylate activity.

An extensive mode of action study suggest that these benzotriazole esters could determine inhibition of 3CLpro<sup>PRO</sup> *via* irreversible acylation of Cys145, as shown in Fig. 44. Particularly, Cys145 and His41 are a essential at the protease active site. They act as the principal catalytic residues, in which the cysteine thiol functions operate as nucleophile in the proteolytic process [252]. However, even though the registered  $K_i$  values are among the lowest for any inhibitor of the SARS-CoV 3CLpro<sup>PRO</sup> described so far, authors also reported that full inhibition of the enzyme could not be achieved.



**Fig. 44.** Proposed mechanism for inhibition of SARS-CoV 3CLpro<sup>PRO</sup> by acylation with benzotriazole esters by Wu *et al.*

Finally, additional benzotriazole esters proposed in the paper were developed to obtain more stable derivatives, in which ester oxygen is replaced by a carbon. Unfortunately they resulted weaker than the corresponding esters, and they bind non-covalently to the enzyme.

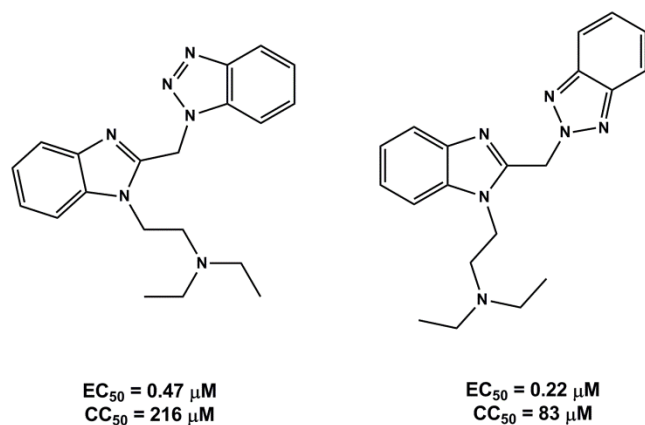
Verschueren *et al.* studied the binding mode of benzotriazole esters to the SARS-CoV main proteinase using two of such compounds, 1-(benzoyloxy)-benzotriazole (compound XP-27) and 1-(4-dimethylaminobenzoyloxy)-benzotriazole (compound XP-59) [253]. These derivatives are able

to inhibit the enzymatic activity at low micromolar concentrations (<5  $\mu\text{M}$  and 0.1  $\mu\text{M}$  for XP-27 and XP-59, respectively). Authors demonstrate that benzotriazole esters act as suicide inhibitors by covalently binding to the proteinase.

So far, benzotriazole derivatives were individuated as ssRNA+ viruses inhibitors. However, almost one Single-stranded negative strand RNA virus was identified as a target for compounds bearing a benzotriazole moiety: the Human Respiratory Syncytial Virus (RSV), classified in the genus Pneumovirus of the family Paramyxoviridae. RSV is the leading cause of acute respiratory tract infections in persons of all ages but can also cause serious lower respiratory tract infections in infants aged < 6 months, infants born before 35 weeks of gestation and infants and children with underlying lung disease or congenital heart disease [254], elderly and immunocompromised persons. Severe infection of the virus may result in bronchiolitis or pneumonia which may require hospitalization or result in death. The World Health Organization estimates that RSV causes 64 million infections and 160,000 deaths annually [74]. Currently only Ribavirin is approved for the treatment of this viral infection, as for some other emerging/neglected viral infections, and its efficacy has remained controversial. Therefore, it is still mandatory to identify effective therapeutic options that can treat RSV infections in the at-risk population.

In this perspective is collocated the paper by Yu et coworkers. In 2003 the group reported 1-[(dialkylamino)alkyl]-2-[(benzotriazol-1/2-yl)methyl] benzimidazoles as a new class of inhibitors able to protect HEP-2 human lung carcinoma cell line cells against *RSV cytopathic effects* [255]. Indeed, these compounds were protected by a Bristol-Myers Squibb Company patent [256], but a part of them were first disclosed by Pagani [217] and Paglietti [257], which found them to possess analgesic and antiarrhythmic activity. Lead compounds of this first series of new RSV-inhibitors are depicted in Fig. 45. Particularly, 2-(2-((2*H*-benzo[*d*][1,2,3]triazol-2-yl)methyl)-1*H*-benzo[*d*]imidazol-1-yl)-*N,N*-diethylethanamine demonstrate potent antiviral activity against both A and B subtypes of RSV with a good cytotoxicity profile paired with  $\text{EC}_{50}$  values an order of magnitude lower than that of ribavirin ( $\text{EC}_{50} = 2.7$ ,  $\text{CC}_{50} = 34 \mu\text{M}$ , data not shown) [258].

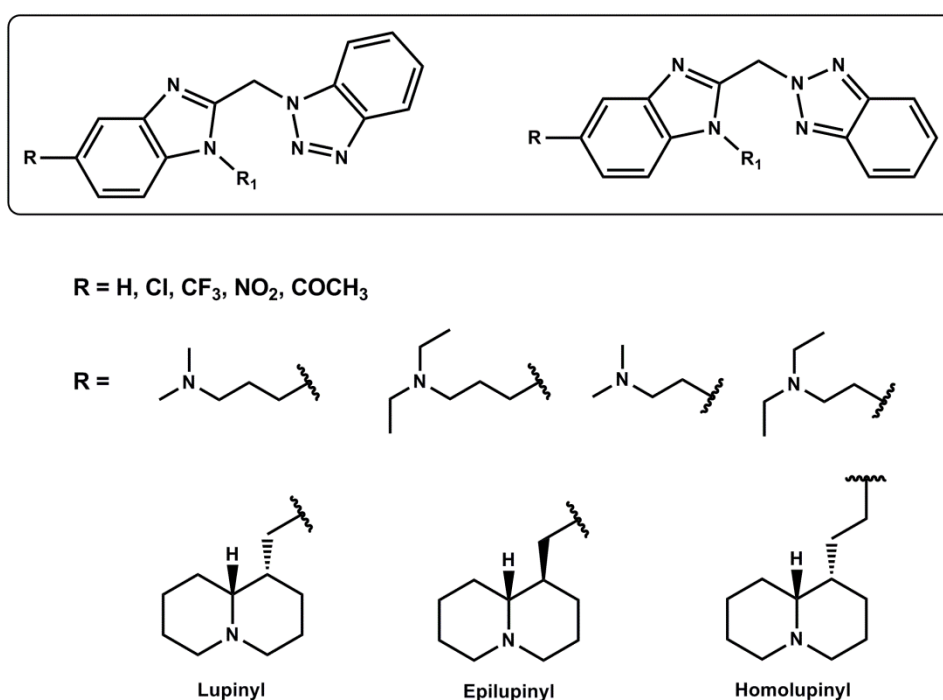
Analysis of structure-activity relationships centered to the variation of the dialkylaminoalkyl side chain determined a wide tolerance to structural variation, and both polar and lipophilic functionality at the chain terminus preserve the RSV inhibition. However, the only requirement is a minimum of two atoms of separation between terminus and the heterocyclic moiety. Authors also established that the topological relationship of benzotriazole respect the substituted benzoimidazole moiety is not critical since both isomers properties were essentially equal [255, 259].



**Fig. 45.** Lead compounds benzotriazole-derived that act as inhibitors of Respiratory Syncytial Virus fusion.

Unfortunately for our review, the broad survey designed to delineate the pharmacophore associated with leads compounds guided Yu *et al.* to replace the benzotriazole element with a benzimidazol-2-one moiety. This modification led to even more potent anti-RSV agents and to broad tolerance for functionality in this region of the pharmacophore [260].

However, Tonelli *et al.* extended the analysis on 1-Substituted-2-[(Benzotriazol-1/2-yl)methyl]benzimidazoles following the report from Bristol researchers. They evaluated the antiviral activity of related 1-substituted 2-[(benzotriazol-1/2-yl)methyl]benzimidazoles, firstly prepared by the group and not examined by Yu *et al.* In particular, some 5-substituted derivatives, as well as compounds bearing at position 1 the simple (dialkylamino)alkyl chains or the bulky, strongly basic and lipophilic (quinolizidinyl)alkyl nucleus (Lupinyl, Epilupinyl and Homolupinyl), were re- or synthesized. Derivatives are depicted in Fig. 46.



**Fig. 46.** 1-Substituted-2-[(Benzotriazol-1/2-yl)methyl]benzimidazoles proposed by Tonelli *et al.*

A total of forty-three 1-substituted 2-[(benzotriazol-1/2-yl)methyl]benzimidazoles have been tested for cytotoxicity and antiviral activity against a large panel of RNA and DNA viruses and, among them, thirty-nine compounds exhibited potent activity against RSV, in some cases with EC<sub>50</sub> values below 50 nM [261]. SAR studies suggested that the presence of substituents at position 5 of the benzimidazole ring leads to an increase in cytotoxicity, especially if compound bear the (quinolizidiny)alkyl nucleus. Toward antiviral activity, the most potent compounds present a Cl in position 5 paired with an R<sub>1</sub> equal to (CH<sub>2</sub>)<sub>2</sub>N(CH<sub>3</sub>)<sub>2</sub> or (CH<sub>2</sub>)<sub>3</sub>N(CH<sub>3</sub>)<sub>2</sub>, while the replacement of Cl with H, NO<sub>2</sub>, CF<sub>3</sub> or COCH<sub>3</sub> leads to lower potency. Surprisingly, the introduction at R<sub>1</sub> of bulkier group leads to a progressive increase of potency, as seen when (CH<sub>2</sub>)<sub>2</sub>N(CH<sub>3</sub>)<sub>2</sub> is replaced with (CH<sub>2</sub>)<sub>2</sub>N(CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub> or lupinyl group, and homolupinyl derivatives are the most potent, regardless of whether R is H or Cl. Finally, comparison of the two isomeric series, N(2)-substituted benzotriazoles were generally less active.

## REFERENCES

- [1] R. Kale, V. Prasad, P. Mohapatra, V. Tiwari, Recent developments in benzotriazole methodology for construction of pharmacologically important heterocyclic skeletons, *Monatshefte für Chemie - Chemical Monthly*, 141 (2010) 1159-1182.
- [2] A.R. Katritzky, S.A. Henderson, B. Yang, Applications of benzotriazole methodology in heterocycle ring synthesis and substituent introduction and modification, *J.Heterocyclic Chem.*, 35 (1998) 1123-1159.
- [3] A.R. Katritzky, X. Lan, J.Z. Yang, O.V. Denisko, Properties and Synthetic Utility of N-Substituted Benzotriazoles, *Chem. Rev.*, 98 (1998) 409-548.
- [4] A.R. Katritzky, L. Khelashvili, P.P. Mohapatra, P.J. Steel, Efficient N-arylation of substituted indoles with N-arylbzotriazoles, *Synthesis*, 23 (2007) 3673-3677.
- [5] A.R. Katritzky, P. Angrish, E. Todadze, Chiral Acylation with N-(Protected  $\alpha$ -Aminoacyl)benzotriazoles for Advantageous Syntheses of Peptides and Peptide Conjugates, *Synlett* (2009) 2392-2411.
- [6] A.R. Katritzky, B.V. Rogovoy, Benzotriazole: an ideal synthetic auxiliary, *Chemistry*, 9 (2003) 4586-4593.
- [7] X. Wang, Y. Liu, Y. Zhang, Elimination of benzotriazolyl group in N-( $\alpha$ -benzotriazol-1-ylalkyl)amides and N-( $\alpha$ -benzotriazol-1-ylalkyl)sulfonamides: their self-coupling and cross-coupling reactions with carbonyl compounds, *Tetrahedron*, 59 (2003) 8257-8263.
- [8] A.R. Katritzky, S. Rachwal, G.J. Hitchings, Benzotriazole: A novel synthetic auxiliary *Tetrahedron*, 47 (1991) 2683-2732.
- [9] A.R. Katritzky, K. Manju, S.K. Singh, N.K. Meher, Benzotriazole mediated amino-, amido-, alkoxy- and alkylthio-alkylation, *Tetrahedron*, 61 (2005) 2555-2581.
- [10] A.R. Katritzky, S.R. Tala, N.E. Abo-Dya, K. Gyanda, D. El-Gendy Bel, Z.K. Abdel-Samii, P.J.

Steel, Selective synthesis and structural elucidation of S-acyl- and N-acylcysteines, *J. Org. Chem.*, 74 (2009) 7165-7167.

[11] W.M. Berhanu, M.A. Ibrahim, G.G. Pillai, A.A. Oliferenko, L. Khelashvili, F. Jabeen, B. Mirza, F.L. Ansari, I. Ul-Haq, S.A. El-Feky, A.R. Katritzky, Similarity analysis, synthesis, and bioassay of antibacterial cyclic peptidomimetics, *J. Org. Chem.*, 8 (2012) 1146-1160.

[12] A.R. Katritzky, B.V. Rogovoy, C. Chassaing, V. Vvedensky, Di(benzotriazol-1-yl)methanimine: a new reagent for the synthesis of tri- and tetrasubstituted guanidines, *J. Org. Chem.*, 65 (2000) 8080-8082.

[13] A.R. Katritzky, V. Rogovoy, V.Y. Vvedensky, K. Kovalenko, P.J. Steel, V.I. Markov, B. Foroode, Synthesis of N,N-disubstituted 3-amino-1,2,4-triazoles, *Synthesis*, 6 (2001) 897-903.

[14] A.R. Katritzky, L. Xie, D. Toader, L. Serdyuk, General and Efficient Carbon Insertion Route to One-Carbon-Homologated alpha-Aryl, alpha-Alkenyl, alpha-Alkoxy, and alpha-Phenylthio Alkyl Ketones, *J. Am. Chem. Soc.*, 117 (1995) 12015-12016.

[15] G. Boche, P. Andrews, K. Harms, M. Marsch, K.S. Rangappa, M. Schimeczek, C. Willeke, Crystal and Electronic Structure of Stable Nitrenium Ions. A Comparison with Structurally Related Carbenes, *J. Am. Chem. Soc.*, 118 (1996) 4925-4930.

[16] D. Davis, Benzotriazole, a plant-growth regulator, *Science*, 120 (1954) 989.

[17] V. Picci, Effect of benzotriazole and of 2 benzotriazolylacetic acids on plant growth, *Farmaco Sci.*, 21 (1966) 172-177.

[18] F. Sparatore, M.I. La Rotonda, G. Paglietti, E. Ramundo, C. Silipo, A. Vittoria, Benzotriazole derivatives active on plant growth. I. Preparation, characterization, and correlation between physicochemical properties and structure, *Farmaco Sci.*, 33 (1978) 901-923.

[19] F. Sparatore, M.I. La Rotonda, E. Ramundo, C. Silipo, A. Vittoria, Effect of benzotriazole derivatives on plant growth. II, *Farmaco Sci.*, 33 (1978) 924-944.

[20] G. Paglietti, P. Sanna, A. Carta, F. Sparatore, I. Vazzana, A. Peana, M. Satta, Choleric activity of 3-[ring substituted benzotriazol-1(2)-yl]alkanoic and alkenoic acids., *Farmaco Sci.*, 40 (1994) 693-702.

[21] C.M. Jamkhandi, J.I. Disouza, Benzotriazole Derivatives As Antimicrobial Agents, *Asian J. Phar. Biol. Res.*, 2 (2012) 123-130.

[22] F. Lopez-Vallejo, R. Castillo, L. Yopez-Mulia, J.L. Medina-Franco, Benzotriazoles and indazoles are scaffolds with biological activity against *Entamoeba histolytica*, *J. Biomol. Screen.*, 16 (2011) 862-868.

[23] P. Borowski, J. Deinert, S. Schalinski, M. Bretner, K. Ginalski, T. Kulikowski, D. Shugar, Halogenated benzimidazoles and benzotriazoles as inhibitors of the NTPase/helicase activities of hepatitis C and related viruses, *Eur. J. Biochem.*, 270 (2003) 1645-1653.

[24] A. Beauchard, A. Jaunet, L. Murillo, B. Baldeyrou, A. Lansiaux, J.R. Cherouvrier, L. Domon, L. Picot, C. Bailly, T. Besson, V. Thiery, Synthesis and antitumoral activity of novel thiazolobenzotriazole, thiazoloindolo[3,2-c]quinoline and quinolinoquinoline derivatives, *Eur. J. Med. Chem.*, 44 (2009) 3858-3865.

[25] X.M. Peng, G.X. Cai, C.H. Zhou, Recent developments in azole compounds as antibacterial and antifungal agents, *Curr. Top. Med. Chem.*, 13 (2013) 1963-2010.

[26] I. Chopra, The 2012 Garrod Lecture: Discovery of antibacterial drugs in the 21st century, *Antimicrob. Chemother.*, 68 (2013) 496-505.

[27] H.W. Boucher, G.H. Talbot, J.S. Bradley, J.E. Edwards, D. Gilbert, L.B. Rice, M. Scheld, B. Spellberg, J. Bartlett, Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America, *Clin. Infect. Dis.*, 48 (2009) 1-12.

[28] F. Sparatore, F. Pagani, Aminoalkyl derivatives of benzotriazole, *Farmaco Sci.*, 17 (1962) 414-429.

[29] F. Sparatore, F. Pagani, Aminoalkyl Derivatives of Benzotriazole. II, *Farmaco Sci.*, 20 (1965) 248-258.

[30] F. Savelli, A. Pau, A. Boido, F. Sparatore, Aminoalkyl derivatives of benzotriazole and

naphthotriazole, *Boll. Chim. Farm.*, 127 (1988) 144-147.

[31] A. Nuvole, P. Sanna, G. Paglietti, C. Juliano, S. Zanetti, P. Cappuccinelli, 1,2,3-triazolo[4,5-f]quinolines. II. Preparation and antimicrobial evaluation of 6-ethyl-6,9-dihydro-1(2)(3)-R-1(2)(3)H-triazolo [4,5-f]quinolin-9-one-8-carboxylic acids as anti-infectives of the urinary tract (1), *Farmaco Sci.*, 44 (1989) 619-632.

[32] P. Sanna, A. Carta, G. Paglietti, S. Zanetti, G. Fadda, 1,2,3-Triazolo[4,5-h]quinolines. III. Preparation and antimicrobial evaluation of 4-ethyl-4,7-dihydro-1(2)H Triazolo [4,5-h]quinolin-7-one-6-carboxylic acids as anti-infectives of the urinary tract, *Farmaco Sci.* 47 (1992) 1001-1019.

[33] F. Al-Omran, A.A. El-Khair, R.M. Mohareb, Synthesis and biological effects of new derivatives of benzotriazole as antimicrobial and antifungal agents, *J. Heterocycl. Chem.*, 39 (2002) 877-883.

[34] M. Purohit, S.K. Srivastava, Studies in aryloxyated benzotriazoles., *Indian J. Pharm. Sci.*, 54 (1992) 25-27.

[35] R.K. Upadhyay, S.D. Srivastava, Synthesis And Studies On Biologically Active Mono And Biheterocycles, *Indian J. Pharm. Sci.*, 57 (1995) 12-18.

[36] C.M. Jamkhandi, J.I. Disouza, Synthesis and antimicrobial evaluation of 1H-Benzotriazol-1-yl {2-hydroxy-5-[(E)phenyldiazenyl]phenyl}methanone derivatives, *Int. J. Pharm. Pharm. Sci.*, 5 (2013) 225-228.

[37] C.M. Jamkhandi, J.I. Disouza, Synthesis and Antimicrobial Evaluation of [(1H-benzotriazol-1-ylacetyl) amino] Acetic Acid Derivatives, *Research J. Pharm. and Tech.*, 5 (2012) 1197-1200.

[38] C.M. Jamkhandi, J.I. Disouza, Synthesis and Antimicrobial Evaluation of 2-(1H-1,2,3-Benzotriazol-1-yl)-N-Phenylacetamide Derivatives, *Research J. Pharm. and Tech.*, 5 (2012) 1072-1075.

[39] Z. Ochal, M. Bretner, R. Wolinowska, S. Tyski, Synthesis and in vitro antibacterial activity of 5-Halogenomethylsulfonyl- benzimidazole and benzotriazole derivatives, *Med. Chem.*, 9 (2013) 1129-1136.

[40] N.P. Singh, R. Kumar, D.N. Prasad, S. Sharma, O. Silakari, Synthesis and Antibacterial Activity of Benzotriazole Substituted Acridines, *International Journal of Biological Chemistry*, 5 (2011) 193-199.

[41] K.Y. Saour, R.A. Atto, Synthesis of new levofloxacin derivatives and their biological activity, *Pharmacie Globale (IJCP)* 3(2012) 1-5.

[42] J. Das, C.V. Rao, T.V. Sastry, M. Roshaiyah, P.G. Sankar, A. Khadeer, M.S. Kumar, A. Mallik, N. Selvakumar, J. Iqbal, S. Trehan, Effects of positional and geometrical isomerism on the biological activity of some novel oxazolidinones, *Bioorg. Med. Chem. Lett.*, 15 (2005) 337-343.

[43] P.P. Dixit, P.S. Nair, V.J. Patil, S. Jain, S.K. Arora, N. Sinha, Synthesis and antibacterial activity of novel (un)substituted benzotriazolyl oxazolidinone derivatives, *Bioorg. Med. Chem. Lett.*, 15 (2005) 3002-3005.

[44] K.C. Asati, S.K. Srivastava, S.D. Srivastava, Synthesis of 5-arylidene-2-aryl-3-(benzotriazoloacetamidyl)-1,3-thiazolidin-4-ones as analgesic and antimicrobial agents, *Indian Journal of Chemistry*. 45B (2006) 526-531.

[45] S. Nanjunda Swamy, Basappa, G. Sarala, B.S. Priya, S.L. Gaonkar, J. Shashidhara Prasad, K.S. Rangappa, Microwave-assisted synthesis of N-alkylated benzotriazole derivatives: antimicrobial studies, *Bioorg. Med. Chem. Lett.*, 16 (2006) 999-1004.

[46] S. Demirayak, K. Benkli, K. Guven, Synthesis of some 3-arylamino-5-aryloxymethyl[1,2,4]triazole derivatives and their antimicrobial activity, *Pharm. Acta Helv.*, 72 (1998) 285-290.

[47] N.A. Meanwell, Synopsis of some recent tactical application of bioisosteres in drug design, *J. Med. Chem.*, 54 (2011) 2529-2591.

[48] R. Ramachandran, M. Rani, S. Senthana, Y.T. Jeong, S. Kabilan, Synthesis, spectral, crystal structure and in vitro antimicrobial evaluation of imidazole/benzotriazole substituted piperidin-4-one derivatives, *Eur. J. Med. Chem.*, 46 (2011) 1926-1934.

- [49] B.V. Suma, N.N. Natesh, C.H.S. Venkataramana, J. Jays, V. Madhavan, Synthesis and antibacterial of some new 1,2,3 Benzotriazoles derivatives containing Pyrazolidinedione moieties *Int. J. Pharm. Pharm. Sci.*, 4 (2012) 169-173.
- [50] R.J. Pagliero, S. Lusvardi, A.B. Pierini, R. Brun, M.R. Mazzieri, Synthesis, stereoelectronic characterization and antiparasitic activity of new 1-benzenesulfonyl-2-methyl-1,2,3,4-tetrahydroquinolines, *Bioorg. Med. Chem.*, 18 (2010) 142-150.
- [51] R.J. Pagliero, A.B. Pierini, R. Brun, M.R. Mazzieri, Design, Synthesis and 3-D Characterization of 1-Benzenesulfonyl-1,2,3,4- Tetrahydroquinolines as lead scaffold for antiparasitic drug, *Lett. Drug Des. Discov.*, 7 (2010) 461-470.
- [52] A.R. Katritzky, N. Shobana, J. Pernak, A.S. Afridi, W.-Q. Fan, Sulfonyl derivatives of benzotriazole: Part 1. A novel approach to the activation of carboxylic acids, *Tetrahedron*, 48 (1992) 7817-7822.
- [53] M.C. Becerra, N. Guinazu, L.Y. Hergert, A. Pellegrini, M.R. Mazzieri, S. Gea, I. Albesa, In vitro activity of N-benzenesulfonylbenzotriazole on *Trypanosoma cruzi* epimastigote and trypomastigote forms, *Exp. Parasitol.*, 131 (2012) 57-62.
- [54] J.H. Rex, M.G. Rinaldi, M.A. Pfaller, Resistance of *Candida* species to fluconazole, *Antimicrob. Agents Chemother.*, 39 (1995) 1-8.
- [55] P. Escribano, S. Recio, T. Pelaez, M. Gonzalez-Rivera, E. Bouza, J. Guinea, In vitro acquisition of secondary azole resistance in *Aspergillus fumigatus* isolates after prolonged exposure to itraconazole: presence of heteroresistant populations, *Antimicrob. Agents Chemother.*, 56 (2012) 174-178.
- [56] R.H. Rimawi, Y. Carter, T. Ware, J. Christie, D. Siraj, Use of Voriconazole for the Treatment of *Paecilomyces lilacinus* Cutaneous Infections: Case Presentation and Review of Published Literature, *Mycopathologia*, 175 (2013) 345-349
- [57] N.D. Gaikwad, S.V. Patil, V.D. Bobade, Hybrids of ravuconazole: synthesis and biological evaluation, *Eur. J. Med. Chem.*, 54 (2012) 295-302.
- [58] A. Cacciapuoti, D. Loebenberg, E. Corcoran, F. Menzel, Jr., E.L. Moss, Jr., C. Norris, M. Michalski, K. Raynor, J. Halpern, C. Mendrick, B. Arnold, B. Antonacci, R. Parmegiani, T. Yarosh-Tomaine, G.H. Miller, R.S. Hare, In vitro and in vivo activities of SCH 56592 (posaconazole), a new triazole antifungal agent, against *Aspergillus* and *Candida*, *Antimicrob. Agents Chemother.*, 44 (2000) 2017-2022.
- [59] J.M. Trzaskos, W.D. Bowen, A. Shafiee, R.T. Fischer, J.L. Gaylor, Cytochrome P-450-dependent oxidation of lanosterol in cholesterol biosynthesis. Microsomal electron transport and C-32 demethylation, *J. Biol. Chem.*, 259 (1984) 13402-13412.
- [60] P. Marichal, J. Gorrens, L. Laurijssens, K. Vermuyten, C. Van Hove, L. Le Jeune, P. Verhasselt, D. Sanglard, M. Borgers, F.C. Ramaekers, F. Odds, H. Vanden Bossche, Accumulation of 3-ketosteroids induced by itraconazole in azole-resistant clinical *Candida albicans* isolates, *Antimicrob. Agents Chemother.*, 43 (1999) 2663-2670.
- [61] H.B. John, M.B.J. John, Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, 11th ed. (2004) 217-281.
- [62] V. Krcmery, A.J. Barnes, Non-albicans *Candida* spp. causing fungaemia: pathogenicity and antifungal resistance, *J. Hosp. Infect.*, 50 (2002) 243-260.
- [63] R.A. Fromtling, Overview of medically important antifungal azole derivatives, *Clin. Microbiol. Rev.*, 1 (1988) 187-217.
- [64] P.D. Patel, M.R. Patel, B. Kocsis, E. Kocsis, S.M. Graham, A.R. Warren, S.M. Nicholson, B. Billack, F.R. Fronczek, T.T. Talele, Design, synthesis and determination of antifungal activity of 5(6)-substituted benzotriazoles, *Eur. J. Med. Chem.*, 45 (2010) 2214-2222.
- [65] Z. Rezaei, S. Khabnadideh, K. Pakshir, Z. Hossaini, F. Amiri, E. Assadpour, Design, synthesis, and antifungal activity of triazole and benzotriazole derivatives, *Eur. J. Med. Chem.*, 44 (2009) 3064-3067.
- [66] Z. Rezaei, S. Khabnadideh, K. Zomorodian, K. Pakshir, G. Kashi, N. Sanagoei, S. Gholami,

Design, synthesis and antifungal activity of some new imidazole and triazole derivatives, *Archiv. der Pharmazie*, 344 (2011) 658-665.

[67] N.D. Gaikwad, S.V. Patil, V.D. Bobade, Synthesis and biological evaluation of some novel thiazole substituted benzotriazole derivatives, *Bioorg. Med. Chem. Lett.*, 22 (2012) 3449-3454.

[68] 2010/2011 Tuberculosis global facts, in, World Health Organization, 2010.

[69] Global tuberculosis report, Geneva, 2013.

[70] Global Tuberculosis Control, Geneva: World Health Organization, (2011).

[71] Anti-tuberculosis drug resistance in the world: Report N° 4, World Health Organization, 2008.

[72] TB/HIV Facts 2009, Geneva: World Health Organization, (2009).

[73] M.S. Sanchez, J.O. Lloyd-Smith, W.M. Getz, Monitoring linked epidemics: the case of tuberculosis and HIV, *PloS one*, 5 (2010) e8796.

[74] P.L. Collins, B.S. Graham, Viral and host factors in human respiratory syncytial virus pathogenesis, *J. Virol.*, 82 (2008) 2040-2055.

[75] J.C. Garcia-Monco, Tuberculosis, *Handbook of clinical neurology*, 121 (2014) 1485-1499.

[76] Treatment of tuberculosis: guidelines, Geneva, 2009.

[77] A. Singh, Fluoroquinolones should not be the first-line antibiotics to treat community-acquired pneumonia in areas of tuberculosis endemicity, *Clin. Infect. Dis.*, 45 (2007) 134-135.

[78] G.B. Migliori, C. Lange, E. Girardi, R. Centis, G. Besozzi, K. Kliiman, L.R. Codecasa, A. Spanevello, D.M. Cirillo, S.T.S. Group, Fluoroquinolones: are they essential to treat multidrug-resistant tuberculosis?, *Eur. Respir. J.*, 31 (2008) 904-905.

[79] K. Manna, Y.K. Agrawal, Design, synthesis, and antitubercular evaluation of novel series of 3-benzofuran-5-aryl-1-pyrazolyl-pyridylmethanone and 3-benzofuran-5-aryl-1-pyrazolylcarbonyl-4-oxo-naphthyridin analogs, *Eur. J. Med. Chem.*, 45 (2010) 3831-3839.

[80] V.N. Telvekar, V.K. Bairwa, K. Satardekar, A. Bellubi, Novel 2-(2-(4-aryloxybenzylidene)hydrazinyl)benzothiazole derivatives as anti-tubercular agents, *Bioorg. Med. Chem. Lett.*, 22 (2012) 649-652.

[81] P.S. Mathapati, S.A. Durgad, K.C. Singh, S.D. Bhinge, Synthesis and Antimycobacterial Activity of Benzimidazole Derivatives, *Indian Drugs*, 49 (2012) 17-25.

[82] K. Kumar, D. Awasthi, S.Y. Lee, I. Zanardi, B. Ruzsicska, S. Knudson, P.J. Tonge, R.A. Slayden, I. Ojima, Novel trisubstituted benzimidazoles, targeting Mtb FtsZ, as a new class of antitubercular agents, *J. Med. Chem.*, 54 (2011) 374-381.

[83] P. Sanna, A. Carta, M.E. Nikookar, Synthesis and antitubercular activity of 3-aryl substituted-2-[1H(2H)benzotriazol-1(2)-yl]acrylonitriles, *Eur. J. Med. Chem.*, 35 (2000) 535-543.

[84] P. Sanna, A. Carta, L. Gherardini, M. Esmail, R. Nikookar, Synthesis and antimycobacterial activity of 3-aryl-, 3-cyclohexyl- and 3-heteroaryl- substituted-2-(1H(2H)-benzotriazol-1(2)-yl)prop-2-enitriles, prop-2-enamides and propenoic acids. II, *Farmaco Sci.*, 57 (2002) 79-87.

[85] A. Carta, P. Sanna, M. Palomba, L. Vargiu, M. La Colla, R. Loddo, Synthesis and antiproliferative activity of 3-aryl-2-(1H-benzotriazol-1-yl)acrylonitriles. Part III, *Eur. J. Med. Chem.*, 37 (2002) 891-900.

[86] A. Dubey, S.K. Srivastava, S.D. Srivastava, Conventional and microwave assisted synthesis of 2-oxo-4-substituted aryl-azetidine derivatives of benzotriazole: a new class of biological compounds, *Bioorg. Med. Chem. Lett.*, 21 (2011) 569-573.

[87] G.S. Singh, E. Mbukwa, T. Pheko, Synthesis and antimicrobial activity of new 2-azetidinones from N-(salicylidene)amines and 2-diazo-1,2-diarylethanones, *Arkivoc*, 9 (2007) 80-90.

[88] P.P. Dixit, V.J. Patil, P.S. Nair, S. Jain, N. Sinha, S.K. Arora, Synthesis of 1-[3-(4-benzotriazol-1/2-yl-3-fluoro-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-3-substituted-thiourea derivatives as antituberculosis agents, *Eur. J. Med. Chem.*, 41 (2006) 423-428.

[89] H.M. Blumberg, W.J. Burman, R.E. Chaisson, C.L. Daley, S.C. Etkind, L.N. Friedman, P. Fujiwara, M. Grzemska, P.C. Hopewell, M.D. Iseman, R.M. Jasmer, V. Koppaka, R.I. Menzies, R.J. O'Brien, R.R. Reves, L.B. Reichman, P.M. Simone, J.R. Starke, A.A. Vernon, C.f.D.C. American Thoracic Society, Prevention, S. the Infectious Diseases, American Thoracic Society/Centers for

Disease Control and Prevention/Infectious Diseases Society of America: treatment of tuberculosis, *Am. J. Respir. Crit. Care Med.*, 167 (2003) 603-662.

[90] K. Manika, I. Kioumis, The role of fluoroquinolones in the treatment of tuberculosis, *Pneumon*, 4 (2008) 395-401.

[91] E.R. Grimaldo, T.E. Tupasi, A.B. Rivera, M.I. Quelapio, R.C. Cardano, J.O. Derilo, V.A. Belen, Increased resistance to ciprofloxacin and ofloxacin in multidrug-resistant mycobacterium tuberculosis isolates from patients seen at a tertiary hospital in the Philippines, *J. Tuberc. Lung Dis.*, 5 (2001) 546-550.

[92] C. Mugnaini, S. Pasquini, F. Corelli, The 4-quinolone-3-carboxylic acid motif as a multivalent scaffold in medicinal chemistry, *Curr. Med. Chem.*, 16 (2009) 1746-1767.

[93] C.M. Oliphant, G.M. Green, Quinolones: a comprehensive review, *Am. Fam. Physician*, 65 (2002) 455-464.

[94] A. Carta, S. Piras, M. Palomba, D. Jabes, P. Molicotti, S. Zanetti, Anti-mycobacterial activity of quinolones. Triazoloquinolones a new class of potent anti mycobacterial agents., *Antiinfect. Agents Med. Chem.*, 7 (2008) 134-147.

[95] L.E. Ziganshina, S.B. Squire, Fluoroquinolones for treating tuberculosis, *Cochrane Database Syst Rev.*, (2008) CD004795.

[96] A. Nuvole, P. Sanna, G. Paglietti, C. Juliano, S. Zanetti, P. Capucinelli, 1,2,3,-Triazolo-[4,5 f]-quinolines II. Preparation and microbial evaluation of 6-ethyl-6,9-dihydro-1(2)(3)H-triazolo-[4,5 f]-quinolin-9-one-8-carboxylic acids as anti-infectives of urinary tract., *Farmaco Sci.*, 44 (1989) 619-632.

[97] A. Carta, M. Palomba, G. Paglietti, P. Molicotti, B. Paglietti, S. Cannas, S. Zanetti, [1,2,3]Triazolo[4,5-h]quinolones. A new class of potent antitubercular agents against multidrug resistant Mycobacterium tuberculosis strains, *Bioorg. Med. Chem. Lett.*, 17 (2007) 4791-4794.

[98] V. Milata, Tricyclic azoloquinolines *Adv. Heterocycl. Chem.*, (2001) 189-268.

[99] A. Carta, S. Zanetti, Triazolquinolonic derivatives with antimycobacterial activity, WO2008107781 A3, 2008.

[100] A. Carta, M. Palomba, I. Briguglio, P. Corona, S. Piras, D. Jabes, P. Gugliera, P. Molicotti, S. Zanetti, Synthesis and anti-mycobacterial activities of triazoloquinolones, *Eur. J. Med. Chem.*, 46 (2011) 320-326.

[101] I. Briguglio, S. Piras, P. Corona, M.A. Pirisi, D. Jabes, A. Carta, SAR and Anti-Mycobacterial Activity of Quinolones and Triazoloquinolones. An update., *Anti-Infective Agents*, 11 (2013) 75-89.

[102] D. Tricarico, A. Mele, S. Calzolaro, G. Cannone, G.M. Camerino, M.M. Dinardo, R. Latorre, D. Conte Camerino, Emerging role of calcium-activated potassium channel in the regulation of cell viability following potassium ions challenge in HEK293 cells and pharmacological modulation, *PloS one*, 8 (2013) e69551.

[103] M. Holland, P.D. Langton, N.B. Standen, J.P. Boyle, Effects of the BKCa channel activator, NS1619, on rat cerebral artery smooth muscle, *Br. J. Pharmacol.*, 117 (1996) 119-129.

[104] B. Baragatti, G. Biagi, V. Calderone, I. Giorgi, O. Livi, E. Martinotti, V. Scartoni, Triazolyl-benzimidazolones and triazolyl-benzotriazoles: new potential potassium channel activators. II, *Eur. J. Med. Chem.*, 35 (2000) 949-955.

[105] G. Biagi, V. Calderone, I. Giorgi, O. Livi, V. Scartoni, B. Baragatti, E. Martinotti, Some structural changes on triazolyl-benzotriazoles and triazolyl-benzimidazolones as potential potassium channel activators. III, *Farmaco Sci.*, 56 (2001) 841-849.

[106] G. Biagi, I. Giorgi, O. Livi, V. Scartoni, P.L. Barili, V. Calderone, E. Martinotti, New 5-substituted-1-(2-hydroxybenzoyl)-benzotriazoles, potassium channel activators. IV, *Farmaco Sci.*, 56 (2001) 827-834.

[107] A. Jemal, F. Bray, M.M. Center, J. Ferlay, E. Ward, D. Forman, Global cancer statistics, *Cancer J. Clin.*, 61 (2011) 69-90.

[108] Globocan 2012, Cancer Fact Sheets, [http://globocan.iarc.fr/Pages/fact\\_sheets\\_cancer.aspx](http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx).

[109] J. Ferlay, H.R. Shin, F. Bray, D. Forman, C. Mathers, D.M. Parkin, Globocan 2008: Cancer

Incidence and Mortality Worldwide, IARC CancerBase No. 10, (2010).

[110] E. Enger, F.C. Ross, D. Bailey, Concepts in Biology, 2007.

[111] K. Lotfi-Jam, M. Carey, M. Jefford, P. Schofield, C. Charleson, S. Aranda, Nonpharmacologic Strategies for Managing Common Chemotherapy Adverse Effects: A Systematic Review, *J. Clin. Oncology*, 26 (2008) 5618-5629.

[112] A.A. El Rashedy, H.Y. Aboul-Enein, Benzimidazole derivatives as potential anticancer agents., *Mini Rev. Med. Chem.*, 13 (2013) 399-407.

[113] A. Husain, M. Rashid, R. Mishra, S. Parveen, D.S. Shin, D. Kumar, Benzimidazole bearing oxadiazole and triazolo-thiadiazoles nucleus: design and synthesis as anticancer agents, *Bioorg. Med. Chem. Lett.*, 22 (2012) 5438-5444.

[114] J. Chen, S. Ahn, J. Wang, Y. Lu, J.T. Dalton, D.D. Miller, W. Li, Discovery of novel 2-aryl-4-benzoyl-imidazole (ABI-III) analogues targeting tubulin polymerization as antiproliferative agents, *J. Med. Chem.*, 55 (2012) 7285-7289.

[115] A. Carta, M. Palomba, G. Boatto, B. Busonera, M. Murreddu, R. Loddo, Synthesis and antiproliferative activity of 3-aryl-2-[1H(2H)-benzotriazol-1(2)-yl]acrylonitriles variously substituted: Part 4, *Farmaco Sci.*, 59 (2004) 637-644.

[116] S. Zanas, G.S. Papaefstathiou, C.P. Raptopoulou, K.T. Papazisis, V. Vala, D. Zambouli, A.H. Kortsaris, D.A. Kyriakidis, T.F. Zafiropoulos, Synthesis, Structure, and Antiproliferative Activity of Three Gallium(III) Azole Complexes, *Bioinorg. Chem. Appl.*, vol. 2010, Article ID 168030, 10 pages, 2010.

[117] T. Zhan, H. Lou, Synthesis ofazole nucleoside analogues of D-pinitol as potential antitumor agents, *Carbohydr. Res.*, 342 (2007) 865-869.

[118] J. Saunders, J.M. Cameron, Recent development in the design of antiviral agents, *Med. Res. Rev.*, 15 (1995) 497-531.

[119] L. Agrofoglio, E. Suhasa, A. Faresea, R. Condoma, S.R. Challandb, R.A. Earlc, R. Guedja, Synthesis of carbocyclic nucleosides, *Tetrahedron*, 50 (1994) 10611-10670.

[120] M.T. Crimmins, New developments in the enantioselective synthesis of cyclopentyl carbocyclic nucleosides, *Tetrahedron*, 54 (1998) 9229-9272.

[121] Y.A. Al-Soud, N.A. Al-Masoudi, R. Ferwanah Ael, Synthesis and properties of new substituted 1,2,4-triazoles: potential antitumor agents, *Bioorg. Med. Chem.*, 11 (2003) 1701-1708.

[122] C. Lamazzi, S. Leonce, B. Pfeiffer, P. Renard, G. Guillaumet, C.W. Rees, T. Besson, Expedient synthesis and cytotoxic activity of new cyanoindolo[3,2-c]quinolines and benzimidazo[1,2-c]quinazolines, *Bioorg. Med. Chem. Lett.*, 10 (2000) 2183-2185.

[123] L. He, H.X. Chang, T.C. Chou, N. Savaraj, C.C. Cheng, Design of antineoplastic agents based on the '2-phenylnaphthalene-type' structural pattern--synthesis and biological activity studies of 11H-indolo[3,2-c]quinoline derivatives, *Eur. J. Med. Chem.*, 38 (2003) 101-107.

[124] A. Beauchard, H. Chabanea, S. Sinbandhitb, P. Guenotb, V. Thiérya, T. Bessona, Synthesis of original thiazoloindolo[3,2-c]quinoline and novel 8-N-substituted-11H-indolo[3,2-c]quinoline derivatives from benzotriazoles. Part I, *Tetrahedron*, 62 (2006) 1895-1903.

[125] Wan J, C.L. Li, X.M. Li, S.S. Zhang, Synthesis, structure and biological activities of 2-(1H-1,2,3-benzotriazol-1-yl)-1-(4-methylphenyl)-2-(1H-1,2,4-triazol-1-yl)-1-ethanone, *Chem. Res. Chin. Univ.*, 22 (2006) 465-467.

[126] S.S. Zhang, J. Wan, X.M. Li, C.L. Li, L.Z. Xu, Syntheses, structure and biological activities of 2-(1H-benzo[1,2,3]triazole-1-yl)-1-(3-methyl-4-bromobiphenyl)-2-(1H-1,2,4-triazole-1-yl)ethanone, *Chem. Res. Chin. Univ.*, 23 (2007) 120-122.

[127] S.S. Zhang, J. Wan, C.L. Li, M. Li, B. Qu, Syntheses and biological activities of novel triazole compounds, *J. Heterocycl. Chem.*, 44 (2007) 75-78.

[128] S.S. Zhang, H.Q. Zhang, D. Li, L.H. Sun, C.P. Ma, W. Wang, J. Wan, B. Qu, A novel benzotriazole derivative inhibits proliferation of human hepatocarcinoma cells by increasing oxidative stress concomitant mitochondrial damage, *Eur. J. Pharmacol.*, 584 (2008) 144-152.

[129] R. Abonia, B. Insuastya, J. Quirogaa, M. Noguerasb, H. Meierc, Use of  $\beta$ -(N,N-

dialkylamino)propiophenones in the synthesis of nitrogenated heterocyclic compounds, *Mini Rev. Org. Chem.*, 1 (2004) 387-402.

[130] K.M. Kasiotis, S.A. Haroutounian, 2-Pyridin-2-yl-1H-indole derivatives: synthesis, estrogen receptor binding affinity, and photophysical properties, *Bioorg. Chem.*, 34 (2006) 1-14.

[131] J. Wan, X. Yan, C. Cuiping Ma, S. Bi, H.L. Zhu, Synthesis, structure characterization, and biological evaluation of some new 1,2,3-benzotriazole derivatives, *Med. Chem. Res.*, 19 (2010) 970-983.

[132] B.R. Hearn, S.J. Shaw, D.C. Myles, Microtubule targeting agents, *Comprehensive Medicinal Chemistry II* (Taylor J.B. and Triggle D.J. eds) (2007) 81-110.

[133] N. Mahindroo, J.P. Liou, J.Y. Chang, H.P. Hsieh, Antitubulin Agents for Treatment of Cancer-A Medicinal Chemistry Update, *Expert Opin. Ther. Pat.*, 16 (2006) 647-691.

[134] S.K. Tahir, P. Kovar, S.H. Rosenberg, S.C. Ng, Rapid colchicine competition-binding scintillation proximity assay using biotin-labeled tubulin, *BioTechniques*, 29 (2000) 156-160.

[135] D.W. Litchfield, Protein kinase CK2: structure, regulation and role in cellular decisions of life and death, *Biochem. J.*, 369 (2003) 1-15.

[136] K. Ahmed, D.A. Gerber, C. Cochet, Joining the cell survival squad: an emerging role for protein kinase CK2, *Trends Cell. Biol.*, 12 (2002) 226-230.

[137] A. Ghavidel, M.C. Schultz, TATA binding protein-associated CK2 transduces DNA damage signals to the RNA polymerase III transcriptional machinery, *Cell*, 106 (2001) 575-584.

[138] G. Burnett, E.P. Kennedy, The enzymatic phosphorylation of proteins, *J. Biol. Chem.*, 211 (1954) 969-980.

[139] C.V. Glover, A filamentous form of Drosophila casein kinase II, *J. Biol. Chem.*, 261 (1986) 14349-14354.

[140] E. Valero, S. De Bonis, O. Filhol, R.H. Wade, J. Langowski, E.M. Chambaz, C. Cochet, Quaternary structure of casein kinase 2. Characterization of multiple oligomeric states and relation with its catalytic activity, *J. Biol. Chem.*, 270 (1995) 8345-8352.

[141] W. Pyerin, K. Ackermann, Transcriptional coordination of the genes encoding catalytic (CK2alpha) and regulatory (CK2beta) subunits of human protein kinase CK2, *Mol. Cell. Biochem.*, 227 (2001) 45-57.

[142] W. Pyerin, K. Ackermann, The genes encoding human protein kinase CK2 and their functional links, *Prog. Nucleic Acid Res. Mol. Biol.* 74 (2003) 239-273.

[143] U. Wirkner, H. Voss, W. Ansorge, W. Pyerin, Genomic organization and promoter identification of the human protein kinase CK2 catalytic subunit alpha (CSNK2A1), *Genomics*, 48 (1998) 71-78.

[144] V. Martel, O. Filhol, A. Nueda, D. Gerber, M.J. Benitez, C. Cochet, Visualization and molecular analysis of nuclear import of protein kinase CK2 subunits in living cells, *Mol. Cell. Biochem.*, 227 (2001) 81-90.

[145] V. Martel, O. Filhol, A. Nueda, C. Cochet, Dynamic localization/association of protein kinase CK2 subunits in living cells: a role in its cellular regulation?, *Ann. N. Y. Acad. Sci.*, 973 (2002) 272-277.

[146] F. Meggio, D. Shugar, L.A. Pinna, Ribofuranosyl-benzimidazole derivatives as inhibitors of casein kinase-2 and casein kinase-1, *Eur. J. Biochem.*, 187 (1990) 89-94.

[147] A.C. Bibby, D.W. Litchfield, The multiple personalities of the regulatory subunit of protein kinase CK2: CK2 dependent and CK2 independent roles reveal a secret identity for CK2beta, *Int. J. Biol. Sci.*, 1 (2005) 67-79.

[148] L. Volodina Iu, A.A. Shtil, [Casein kinase 2, the versatile regulator of cell survival], *Mol. Biol. (Mosk)*, 46 (2012) 423-433.

[149] L.A. Pinna, F. Meggio, Protein kinase CK2 ("casein kinase-2") and its implication in cell division and proliferation, *Prog. Cell. Cycle Res.*, 3 (1997) 77-97.

[150] D.C. Seldin, P. Leder, Casein kinase II alpha transgene-induced murine lymphoma: relation to theileriosis in cattle, *Science*, 267 (1995) 894-897.

- [151] R.A. Faust, M. Gapany, P. Tristani, A. Davis, G.L. Adams, K. Ahmed, Elevated protein kinase CK2 activity in chromatin of head and neck tumors: association with malignant transformation, *Cancer Lett.*, 101 (1996) 31-35.
- [152] P. Channavajhala, D.C. Seldin, Functional interaction of protein kinase CK2 and c-Myc in lymphomagenesis, *Oncogene*, 21 (2002) 5280-5288.
- [153] B. Guerra, O.G. Issinger, Protein kinase CK2 and its role in cellular proliferation, development and pathology, *Electrophoresis*, 20 (1999) 391-408.
- [154] S. Sarno, H. Reddy, F. Meggio, M. Ruzzene, S.P. Davies, A. Donella-Deana, D. Shugar, L.A. Pinna, Selectivity of 4,5,6,7-tetrabromobenzotriazole, an ATP site-directed inhibitor of protein kinase CK2 ('casein kinase-2'), *FEBS letters*, 496 (2001) 44-48.
- [155] M. Lasa, O. Marin, L.A. Pinna, Rat liver Golgi apparatus contains a protein kinase similar to the casein kinase of lactating mammary gland, *Eur. J. Biochem.*, 243 (1997) 719-725.
- [156] P. Zień, M. Bretner, K. Zastapilo, R. Szyszka, D. Shugar, Selectivity of 4,5,6,7-tetrabromobenzimidazole as an ATP-competitive potent inhibitor of protein kinase CK2 from various sources, *Biochem. Biophys. Res. Commun.*, 306 (2003) 129-133.
- [157] P. Zien, O. Abramczyk, K. Domanska, M. Bretner, R. Szyszka, TBBz but not TBBt discriminates between two molecular forms of CK2 in vivo and its implications, *Biochem. Biophys. Res. Commun.*, 312 (2003) 623-628.
- [158] P. Zień, J.S. Duncan, J. Skierski, M. Bretner, D.W. Litchfield, D. Shugar, Tetrabromobenzotriazole (TBBt) and tetrabromobenzimidazole (TBBz) as selective inhibitors of protein kinase CK2: evaluation of their effects on cells and different molecular forms of human CK2, *Biochim. Biophys. Acta*, 1754 (2005) 271-280.
- [159] R. Battistutta, M. Mazzorana, L. Cendron, A. Bortolato, S. Sarno, Z. Kazimierczuk, G. Zanotti, S. Moro, L.A. Pinna, The ATP-binding site of protein kinase CK2 holds a positive electrostatic area and conserved water molecules, *Chembiochem*, 8 (2007) 1804-1809.
- [160] R. Battistutta, E. De Moliner, S. Sarno, G. Zanotti, L.A. Pinna, Structural features underlying selective inhibition of protein kinase CK2 by ATP site-directed tetrabromo-2-benzotriazole, *Protein Sci*, 10 (2001) 2200-2206.
- [161] N.G. Brink, K. Folkers, Vitamin B<sub>12</sub>. VI. 5,6-Dimethylbenzimidazole, a degradation product of vitamin B<sub>12</sub>, *J. Am. Chem. Soc.*, 71 (1949) 2951.
- [162] N.G. Brink, F.W. Holly, C.H. Shunk, E.W. Peel, J.J. Cahill, K. Folkers, Vitamin B<sub>12</sub>. IX. 1- $\alpha$ -ribofuranosido -5,6-dimethylbenzimidazole, a degradation product of vitamin B<sub>12</sub>, *J. Am. Chem. Soc.*, 72 (1950) 1866.
- [163] R. Zandomeni, R. Weinmann, Inhibitory effect of 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole on a protein kinase, *J. Biol. Chem*, 259 (1984) 14804-14811.
- [164] R. Zandomeni, M.C. Zandomeni, D. Shugar, R. Weinmann, Casein kinase type II is involved in the inhibition by 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole of specific RNA polymerase II transcription, *J. Biol. Chem*, 261 (1986) 3414-3419.
- [165] G. Dobrowolska, G. Muszynska, D. Shugar, Benzimidazole nucleoside analogues as inhibitors of plant (maize seedling) casein kinases, *Biochim. Biophys. Acta.*, 1080 (1991) 221-226.
- [166] R. Szyszka, N. Grankowski, K. Felczak, D. Shugar, Halogenated benzimidazoles and benzotriazoles as selective inhibitors of protein kinases CK I and CK II from *Saccharomyces cerevisiae* and other sources, *Biochem. Biophys. Res. Commun.*, 208 (1995) 418-424.
- [167] R. Wasik, P. Winska, J. Poznanski, D. Shugar, Synthesis and physico-chemical properties in aqueous medium of all possible isomeric bromo analogues of benzo-1H-triazole, potential inhibitors of protein kinases, *J. Phys. Chem. B*, 116 (2012) 7259-7268.
- [168] M. Bretner, A. Najda-Bernatowicz, M. Lebska, G. Muszynska, A. Kilanowicz, A. Sapota, New inhibitors of protein kinase CK2, analogues of benzimidazole and benzotriazole, *Mol. Cell. Biochem.*, 316 (2008) 87-89.
- [169] A. Orzeszko, W. Lasek, T. Switaj, M. Stoksik, B. Kaminska, Tumor necrosis factor-alpha production-regulating activity of phthalimide derivatives in genetically modified murine melanoma

cells B78H1, *Farmaco Sci.*, 58 (2003) 371-376.

[170] M.F. Brana, G. Dominguez, B. Saez, C. Romerdahl, S. Robinson, T. Barlozzari, Synthesis of antitumor dendritic imides, *Bioorg. Med. Chem. Lett.*, 11 (2001) 3027-3029.

[171] A. Najda-Bernatowicz, M. Lebska, A. Orzeszko, K. Kopanska, E. Krzywinska, G. Muszynska, M. Bretner, Synthesis of new analogs of benzotriazole, benzimidazole and phthalimide-potential inhibitors of human protein kinase CK2, *Bioorg. Med. Chem.*, 17 (2009) 1573-1578.

[172] M.A. Pagano, M. Andrzejewska, M. Ruzzene, S. Sarno, L. Cesaro, J. Bain, M. Elliott, F. Meggio, Z. Kazimierczuk, L.A. Pinna, Optimization of protein kinase CK2 inhibitors derived from 4,5,6,7-tetrabromobenzimidazole, *J. Med. Chem.*, 47 (2004) 6239-6247.

[173] F. Pierre, P.C. Chua, S.E. O'Brien, A. Siddiqui-Jain, P. Bourbon, M. Haddach, J. Michaux, J. Nagasawa, M.K. Schwaebe, E. Stefan, A. Vialettes, J.P. Whitten, T.K. Chen, L. Darjania, R. Stansfield, K. Anderes, J. Bliesath, D. Drygin, C. Ho, M. Omori, C. Proffitt, N. Streiner, K. Trent, W.G. Rice, D.M. Ryckman, Discovery and SAR of 5-(3-chlorophenylamino) benzo[c][2,6]naphthyridine-8-carboxylic acid (CX-4945), the first clinical stage inhibitor of protein kinase CK2 for the treatment of cancer, *J. Med. Chem.*, 54 (2011) 635-654.

[174] Z. Nie, C. Perretta, P. Erickson, S. Margosiak, R. Almassy, J. Lu, A. Averill, K.M. Yager, S. Chu, Structure-based design, synthesis, and study of pyrazolo[1,5-a][1,3,5]triazine derivatives as potent inhibitors of protein kinase CK2, *Bioorg. Med. Chem. Lett.*, 17 (2007) 4191-4195.

[175] M. Makowska, E. Lukowska-Chojnacka, P. Winska, A. Kus, A. Bilinska-Chomik, M. Bretner, Design and synthesis of CK2 inhibitors, *Mol. Cell. Biochem.*, 356 (2011) 91-96.

[176] R. Swider, M. Maslyk, S. Martin-Santamaria, A. Ramos, B. de Pascual-Teresa, Multisite-directed inhibitors of protein kinase CK2: new challenges, *Mol. Cell. Biochem.*, 356 (2011) 117-119.

[177] D. Lavogina, E. Enkvist, A. Uri, Bisubstrate inhibitors of protein kinases: from principle to practical applications, *Chem. Med. Chem.*, 5 (2010) 23-34.

[178] A.M. Wawroa, M. Wielechowska, M. Bretner, Synthesis of new optically pure tetrabromobenzotriazole derivatives via lipase-catalyzed transesterification, *J. Mol. Catal. B: Enzym.*, 87 (2013) 44-50.

[179] B.K. Keppler, Metal complexes in cancer chemotherapy, *Appl. Organomet. Chem.*, 8 (1994).

[180] F. Saczewski, E. Dziemidowicz-Borys, P.J. Bednarski, M. Gdaniec, Synthesis, crystal structure, cytotoxic and superoxide dismutase activities of copper(II) complexes of N-(4,5-dihydroimidazol-2-yl)azoles, *Archiv der Pharmazie*, 340 (2007) 333-338.

[181] L.W. Oberley, G.R. Buettner, Role of superoxide dismutase in cancer: a review., *Cancer Res.*, 39 (1979) 1141-1149.

[182] M. Grunstein, Histone acetylation in chromatin structure and transcription, *Nature*, 389 (1997) 349-352.

[183] J.R. Davie, Covalent modifications of histones: expression from chromatin templates, *Curr. Opin. Genet. Dev.*, 8 (1998) 173-178.

[184] A. Mai, S. Massa, R. Ragno, I. Cerbara, F. Jesacher, P. Loidl, G. Brosch, 3-(4-Aroyl-1-methyl-1H-2-pyrrolyl)-N-hydroxy-2-alkylamides as a new class of synthetic histone deacetylase inhibitors. 1. Design, synthesis, biological evaluation, and binding mode studies performed through three different docking procedures, *J. Med. Chem.*, 46 (2003) 512-524.

[185] R.W. Johnstone, Histone-deacetylase inhibitors: novel drugs for the treatment of cancer, *Nat. Rev. Drug. Discov.*, 1 (2002) 287-299.

[186] M. Dokmanovic, C. Clarke, P.A. Marks, Histone deacetylase inhibitors: overview and perspectives, *Mol. Cancer Res.*, 5 (2007) 981-989.

[187] D.C. Drummond, C.O. Noble, D.B. Kirpotin, Z. Guo, G.K. Scott, C.C. Benz, Clinical development of histone deacetylase inhibitors as anticancer agents, *Annu. Rev. Pharmacol. Toxicol.*, 45 (2005) 495-528.

[188] J. Fu, Y. Yang, X.W. Zhang, W.J. Mao, Z.M. Zhang, H.L. Zhu, Discovery of 1H-benzo[d][1,2,3]triazol-1-yl 3,4,5-trimethoxybenzoate as a potential antiproliferative agent by

- inhibiting histone deacetylase, *Bioorg. Med. Chem.*, 18 (2010) 8457-8462.
- [189] R. Huey, G.M. Morris, A.J. Olson, D.S. Goodsell, A semiempirical free energy force field with charge-based desolvation, *J. Comput. Chem.*, 28 (2007) 1145-1152.
- [190] T.M. Willson, P.J. Brown, D.D. Sternbach, B.R. Henke, The PPARs: from orphan receptors to drug discovery, *J. Med. Chem.*, 43 (2000) 527-550.
- [191] V.J. Ram, Therapeutic role of peroxisome proliferator-activated receptors in obesity, diabetes and inflammation, *Progress in drug research. Fortschritte der Arzneimittelforschung. Prog. Drug. Res.*, 60 (2003) 93-132.
- [192] K.L. Gearing, M. Göttlicher, M. Teboul, E. Widmark, J.A. Gustafsson, Interaction of the peroxisome-proliferator-activated receptor and retinoid X receptor, *Proc. Nati. Acad. Sci.*, (1993) 1440-1444.
- [193] M. Bortolini, M.B. Wright, M. Bopst, B. Balas, Examining the safety of PPAR agonists - current trends and future prospects, *Expert Opin. Drug Saf.*, 12 (2013) 65-79.
- [194] B.R. Henke, Peroxisome proliferator-activated receptor alpha/gamma dual agonists for the treatment of type 2 diabetes, *J. Med. Chem.*, 47 (2004) 4118-4127.
- [195] P. Sauerberg, P.S. Bury, J.P. Mogensen, H.J. Deussen, I. Pettersson, J. Fleckner, J. Nehlin, K.S. Frederiksen, T. Albrektsen, N. Din, L.A. Svensson, L. Ynddal, E.M. Wulff, L. Jeppesen, Large dimeric ligands with favorable pharmacokinetic properties and peroxisome proliferator-activated receptor agonist activity in vitro and in vivo, *J. Med. Chem.*, 46 (2003) 4883-4894.
- [196] A. Sparatore, C. Godio, E. Perrino, S. Romeo, B. Staels, J.C. Fruchart, M. Crestani, [4-(2H-1,2,3-benzotriazol-2-yl)phenoxy]alkanoic acids as agonists of peroxisome proliferator-activated receptors (PPARs), *Chem. Biodivers.*, 3 (2006) 385-395.
- [197] A. Sparatore, F. Sparatore, Synthesis and preliminary pharmacological investigation of some 2-[4-(dialkylaminoalkoxy)phenyl]benzotriazoles and their N-oxides, *Farmaco Sci.*, 53 (1998) 102-112.
- [198] A.A. Santilli, A.C. Scotese, R.M. Tomarelli, A potent antihypercholesterolemic agent: (4-chloro-6-(2,3-xylylidino)-2-pyrimidinylthio) acetic acid (Wy-14643), *Experientia*, 30 (1974) 1110-1111.
- [199] S. Kato, Role of serotonin 5-HT(3) receptors in intestinal inflammation, *Biol. Pharm. Bull.*, 36 (2013) 1406-1409.
- [200] E. Zifa, G. Fillion, 5-Hydroxytryptamine receptors, *Pharmacol. Rev.*, 44 (1992) 401-458.
- [201] P. Masek, A.C. Keene, Dopamine: on the threshold of sleep, *Curr. Biol.*, 22 (2012) R949-951.
- [202] J. Zohar, T.R. Insel, R.C. Zohar-Kadouch, J.L. Hill, D.L. Murphy, Serotonergic responsivity in obsessive-compulsive disorder. Effects of chronic clomipramine treatment, *Arch. Gen. Psychiatry*, 45 (1988) 167-172.
- [203] E. Shin, J. Garcia, C. Winkler, A. Bjorklund, M. Carta, Serotonergic and dopaminergic mechanisms in graft-induced dyskinesia in a rat model of Parkinson's disease, *Neurobiol. Dis.*, 47 (2012) 393-406.
- [204] R.A. Urbanek, H. Xiong, Y. Wu, W. Blackwell, G. Steelman, J. Rosamond, S.S. Wesolowski, J.B. Campbell, M. Zhang, B. Brockel, D.V. Widzowski, Synthesis and SAR of aminothiazole fused benzazepines as selective dopamine D(2) partial agonists, *Bioorg. Med. Chem. Lett.*, 23 (2013) 543-547.
- [205] R. Condray, J.K. Yao, Cognition, dopamine and bioactive lipids in schizophrenia, *Front Biosci (Schol Ed)*, 3 (2011) 298-330.
- [206] G. Caliendo, R. Di Carlo, R. Meli, E. Perissutti, V. Santagada, C. Silipo, A. Vittoria, Synthesis and trazodone-like pharmacological profiles of 1- and 2-[3-[4-(x)-1-piperazinyl]-propyl]-benzotriazoles, *Eur. J. Med. Chem.*, 28 (1993) 969-974.
- [207] G. Caliendo, R. Di Carlo, G. Greco, R. Meli, E. Novellino, E. Perissutti, S. V., Synthesis and biological activity of benzotriazole derivatives structurally related to trazadone, *Eur. J. Med. Chem.*, 30 (1995) 77-84.
- [208] G. Caliendo, G. Greco, P. Grieco, E. Novellino, E. Perissutti, V. Santagada, D. Barbarulo, E.

- Esposito, A. De Blasi, Structure—affinity relationship studies on benzotriazole derivatives binding to 5-HT receptor subtypes, *Eur. J. Med. Chem.*, 31 (1996) 207-213.
- [209] A. Sparatore, M. Goegan, A. Cagnotto, F. Sparatore, 2-[4-[3-(4-aryl/heteroaryl-1-piperazinyl)propoxy]phenyl]-2H-benzotriazoles and their N-oxides as ligands for serotonin and dopamine receptors, *Farmaco Sci.*, 54 (1999) 402-410.
- [210] R.A. Glennon, R. Westkaemper, P. Bartyzel, *Medicinal Chemistry of Serotonergic Agents, Serotonin Receptor Subtypes*. S. Peroutka. ed. Wiley-Liss, NY (1991) 19-64.
- [211] M.J. Mokrosz, M.H. Paluchowska, S. Charakchieva-Minol, A. Bien, Structure-activity relationship studies of CNS agents, Part 32: Effect of structural modifications in 1-arylpiperazine derivatives on alpha(1) -adrenoreceptor affinity, *Archiv der Pharmazie*, 330 (1997) 177-180.
- [212] A. Sparatore, F. Sparatore, 2-{4-[ω-[4-(2-Methoxyphenyl)-1-piperazinyl]alkoxy]phenyl}-2H-benzotriazoles and their N-Oxides as Ligands for some 5-Hydroxytryptamine, Dopamine and Adrenergic Receptor Subtypes, *Pharm. Pharmacol. Commun.*, 6 (2000) 421-425.
- [213] J.L. Mokrosz, M.H. Paluchowska, E. Chojnacka-Wojcik, M. Filip, S. Charakchieva-Minol, A. Deren-Wesolek, M.J. Mokrosz, Structure-activity relationship studies of central nervous system agents. 13. 4-[3-(Benzotriazol-1-yl)propyl]-1-(2-methoxyphenyl)piperazine, a new putative 5-HT1A receptor antagonist, and its analogs, *J. Med. Chem.*, 37 (1994) 2754-2760.
- [214] M.H. Paluchowska, A. Klodzinska, E. Tatarczynska, A. Szaro, E. Chojnacka-Wojcik, Effect of linking bridge modifications on the 5-HT1A receptor activity of some 4-(omega-benzotriazol-1-yl)alkyl-1-(2-methoxy-phenyl)piperazines, *Pol. J. Pharmacol.*, 50 (1998) 341-347.
- [215] A. Boido, C.C. Boido, F. Sparatore, Synthesis and pharmacological evaluation of aryl/heteroaryl piperazinyl alkyl benzotriazoles as ligands for some serotonin and dopamine receptor subtypes, *Farmaco Sci.*, 56 (2001) 263-275.
- [216] V. Sukalovic, D. Andric, G. Roglic, S. Kostic-Rajacic, A. Schratzenholz, V. Soskic, Synthesis, dopamine D2 receptor binding studies and docking analysis of 5-[3-(4-arylpiperazin-1-yl)propyl]-1H-benzimidazole, 5-[2-(4-arylpiperazin-1-yl)ethoxy]-1H-benzimidazole and their analogs, *Eur. J. Med. Chem.*, 40 (2005) 481-493.
- [217] F. Pagani, F. Sparatore, Benzotriazolyl-alkyl-benzimidazoles and their dialkyl-aminoalkyl derivatives., *Boll. Chim. Farm.*, 104 (1965) 427-431.
- [218] P. Preziosi, P. Manca, G. Paglietti, Choloretic activity of benzotriazolylalkanoic acids, *Farmaco Sci.*, 20 (1965) 238-247.
- [219] G. Paglietti, F. Sparatore, Preparation of variously substituted beta-benzotriazolylbutyric acids with potential choloretic activity., *Farmaco Sci.* , 27 (1972 ) 380-396.
- [220] G. Paglietti, F. Sparatore, Preparation of beta-benzimidazolyl- and indazolylbutyric acids as potential choloretic agents, *Farmaco Sci.*, 27 (1972 ) 471-479.
- [221] G. Paglietti, P. Sanna, A. Carta, F. Sparatore, I. Vazzana, A. Peana, M. Satta, Choloretic activity of 3-[ring substituted benzotriazol-1(2)-yl]alkanoic and alkenoic acids, *Farmaco Sci*, 40 (1994) 693-702.
- [222] A. Boido, I. Vazzana, F. Mattioli, F. Sparatore, Antiinflammatory and antinociceptive activities of some benzotriazolylalkanoic acids, *Farmaco Sci*, 58 (2003) 33-44.
- [223] F. Sparatore, A. Boido, B. Cappello, V. Diurno, M.T. Mazza, E. Piscopo, I. Vazzana, [4'-Benzotriazol-2-yl)-phenylalkanoic and -phenoxyalkanoic acids and corresponding N-oxides as potential anti-inflammatory agents], *Boll. Soc. Ital. Biol. Sper.*, 57 (1981) 871-876.
- [224] A. Boido, I. Vazzana, F. Sparatore, B. Cappello, V. Diurno, C. Matera, R. Marrazzo, S. Russo, M.L. Cenicola, E. Marmo, [Preparation and pharmacologic activity of N-oxides of 4'-(benzotriazol-2-yl)-phenylalkanoic and -phenoxyalkanoic acids], *Farmaco Sci*, 44 (1989) 257-277.
- [225] N.P. Buu-Houi, P. Jacquignon, J.P. Hoeffinger, The pharmacological activity of some 2-substituted benzimidazoles, *Arzneimittelforschung*, 13 (1963 ) 865-867.
- [226] A. Boido, I. Vazzana, F. Sparatore, M.L. Cenicola, D. Donnoli, E. Marmo, Preparation and pharmacological activity of some 1-lupinylbenzimidazoles and 1-lupinylbenzotriazoles, *Farmaco Sci*, 46 (1991) 775-788.

- [227] I. Vazzana, A. Boido, F. Sparatore, R. Di Carlo, G.M. Raso, M. Pacilio, Preparation and local anaesthetic activity of N-[2-(tert-amino)ethyl]- and N-(lupinyl)-benzotriazol-1/2-ylacetamides, *Farmaco Sci*, 52 (1997) 131-139.
- [228] A. Rajasekaran, K.A. Rajagopal, Synthesis of some novel triazole derivatives as anti-nociceptive and anti-inflammatory agents, *Acta Pharm.*, 59 (2009) 355-364.
- [229] L. Santana, M. Teijeira, E. Uriarte, C. Teran, B. Linares, R. Villar, R. Laguna, E. Cano, AM1 theoretical study, synthesis and biological evaluation of some benzofuran analogues of anti-inflammatory arylalkanoic acids, *Eur. J. Pharm. Sci.*, 7 (1999) 161-166.
- [230] K.M. Dawood, H. Abdel-Gawad, E.A. Rageb, M. Ellithey, H.A. Mohamed, Synthesis, anticonvulsant, and anti-inflammatory evaluation of some new benzotriazole and benzofuran-based heterocycles, *Bioorg. Med. Chem.*, 14 (2006) 3672-3680.
- [231] M. Bretner, A. Baier, K. Kopanska, A. Najda, A. Schoof, M. Reinholz, A. Lipniacki, A. Piasek, T. Kulikowski, P. Borowski, Synthesis and biological activity of 1H-benzotriazole and 1H-benzimidazole analogues--inhibitors of the NTPase/helicase of HCV and of some related Flaviviridae, *Antivir. Chem. Chemother.*, 16 (2005) 315-326.
- [232] I. Briguglio, S. Piras, P. Corona, A. Carta, Inhibition of RNA Helicases of ssRNA+ Virus Belonging to Flaviviridae, Coronaviridae and Picornaviridae Families, *International Journal of Medicinal Chemistry*, 2011 (2011).
- [233] Nonpolio Enterovirus and Human Parechovirus Surveillance - United States, 2006-2008, *Morbidity and Mortality Weekly Report*, 59 (2010) 1577-1580.
- [234] P.H. Hayashi, A.M. Di Bisceglie, The progression of hepatitis B- and C-infections to chronic liver disease and hepatocellular carcinoma: presentation, diagnosis, screening, prevention, and treatment of hepatocellular carcinoma, *Med. Clin. North Am.*, 89 (2005) 345-369.
- [235] J. Paeshuyse, I. Vliegen, L. Coelmont, P. Leyssen, O. Tabarrini, P. Herdewijn, H. Mittendorfer, J. Easmon, V. Cecchetti, R. Bartenschlager, G. Puerstinger, J. Neyts, Comparative in vitro anti-hepatitis C virus activities of a selected series of polymerase, protease, and helicase inhibitors, *Antimicrob. Agents Chemother.*, 52 (2008) 3433-3437.
- [236] M. Bretner, A. Najda, R. Podwinska, A. Baier, K. Paruch, A. Lipniacki, A. Piasek, P. Borowski, T. Kulikowski, Inhibitors of the NTPase/helicases of hepatitis C and related Flaviviridae viruses, *Acta Pol. Pharm.*, 61 Suppl (2004) 26-28.
- [237] D.J. Porter, A kinetic analysis of the oligonucleotide-modulated ATPase activity of the helicase domain of the NS3 protein from hepatitis C virus. The first cycle of interaction of ATP with the enzyme is unique, *J. Biol. Chem.*, 273 (1998) 14247-14253.
- [238] A. Carta, G. Loriga, S. Piras, G. Paglietti, M. Ferrone, M. Fermeiglia, S. Pricl, P. La Colla, B. Secci, G. Collu, R. Loddo, Synthesis and in vitro evaluation of the anti-viral activity of N-[4-(1H(2H)-benzotriazol-1(2)-yl)phenyl]alkylcarboxamides, *Med. Chem.*, 2 (2006) 577-589.
- [239] G.D. Diana, T.R. Bailey, US patent 5633388, (1997).
- [240] A. Carta, M. Loriga, S. Piras, G. Paglietti, M. Ferrone, M. Fermeiglia, S. Pricl, P. La Colla, G. Collu, T. Sanna, R. Loddo, Synthesis and anti-picornaviridae in vitro activity of a new class of helicase inhibitors the N,N'-bis[4-(1H(2H)-benzotriazol-1(2)-yl)phenyl] alkyldicarboxamides, *Med. Chem.*, 3 (2007) 520-532.
- [241] J.S.M. Peiris, S.T. Lai, L.L.M. Poon, Y. Guan, L.Y.C. Yam, W. Lim, J. Nicholls, W.K.S. Yee, M.T. Cheung, V.C. Cheng, e. al., Coronavirus as a possible cause of severe acute respiratory syndrome, *Lancet* 361 (2003) 1319-1325.
- [242] L.J. Stockman, R. Bellamy, P. Garner, SARS: Systematic Review of Treatment Effects, *PLoS medicine*, 3 (2006) e343.
- [243] K. Chou, D. Wei, W. Zhong, Binding mechanism of coronavirus main protease with ligands and its implication to drug design against SARS., *Biochem. Biophys. Res. Commun.*, 308 (2003) 148-151.
- [244] P.A. Rota, M.S. Oberste, S.S. Monroe, W.A. Nix, R. Campagnoli, J.P. Icenogle, S. Penaranda, B. Bankamp, K. Maher, M.H. Chen, S. Tong, A. Tamin, L. Lowe, M. Frace, J.L. DeRisi, Q. Chen,

D. Wang, D.D. Erdman, T.C. Peret, C. Burns, T.G. Ksiazek, P.E. Rollin, A. Sanchez, S. Liffick, B. Holloway, J. Limor, K. McCaustland, M. Olsen-Rasmussen, R. Fouchier, S. Gunther, A.D. Osterhaus, C. Drosten, M.A. Pallansch, L.J. Anderson, W.J. Bellini, Characterization of a novel coronavirus associated with severe acute respiratory syndrome, *Science*, 300 (2003) 1394-1399.

[245] M.A. Marra, S.J. Jones, C.R. Astell, R.A. Holt, A. Brooks-Wilson, Y.S. Butterfield, J. Khattra, J.K. Asano, S.A. Barber, S.Y. Chan, A. Cloutier, S.M. Coughlin, D. Freeman, N. Girn, O.L. Griffith, S.R. Leach, M. Mayo, H. McDonald, S.B. Montgomery, P.K. Pandoh, A.S. Petrescu, A.G. Robertson, J.E. Schein, A. Siddiqui, D.E. Smailus, J.M. Stott, G.S. Yang, F. Plummer, A. Andonov, H. Artsob, N. Bastien, K. Bernard, T.F. Booth, D. Bowness, M. Czub, M. Drebot, L. Fernando, R. Flick, M. Garbutt, M. Gray, A. Grolla, S. Jones, H. Feldmann, A. Meyers, A. Kabani, Y. Li, S. Normand, U. Stroher, G.A. Tipples, S. Tyler, R. Vogrig, D. Ward, B. Watson, R.C. Brunham, M. Kraiden, M. Petric, D.M. Skowronski, C. Upton, R.L. Roper, The Genome sequence of the SARS-associated coronavirus, *Science*, 300 (2003) 1399-1404.

[246] U. Bacha, J. Barrila, A. Velazquez-Campoy, S.A. Leavitt, E. Freire, Identification of novel inhibitors of the SARS coronavirus main protease 3CLpro, *Biochemistry*, 43 (2004) 4906-4912.

[247] R.P. Jain, H.I. Pettersson, J. Zhang, K.D. Aull, P.D. Fortin, C. Huitema, L.D. Eltis, J.C. Parrish, M.N. James, D.S. Wishart, J.C. Vederas, Synthesis and evaluation of keto-glutamine analogues as potent inhibitors of severe acute respiratory syndrome 3CLpro, *J. Med. Chem.*, 47 (2004) 6113-6116.

[248] J. Zhang, H.I. Pettersson, C. Huitema, C. Niu, J. Yin, M.N. James, L.D. Eltis, J.C. Vederas, Design, synthesis, and evaluation of inhibitors for severe acute respiratory syndrome 3C-like protease based on phthalhydrazide ketones or heteroaromatic esters, *J. Med. Chem.*, 50 (2007) 1850-1864.

[249] D.H. Goetz, Y. Choe, E. Hansell, Y.T. Chen, M. McDowell, C.B. Jonsson, W.R. Roush, J. McKerrow, C.S. Craik, Substrate specificity profiling and identification of a new class of inhibitor for the major protease of the SARS coronavirus, *Biochemistry*, 46 (2007) 8744-8752.

[250] C.Y. Chou, C.H. Chien, Y.S. Han, M.T. Prebanda, H.P. Hsieh, B. Turk, G.G. Chang, X. Chen, Thiopurine analogues inhibit papain-like protease of severe acute respiratory syndrome coronavirus, *Biochem. Pharmacol.*, 75 (2008) 1601-1609.

[251] C.Y. Wu, K.Y. King, C.J. Kuo, J.M. Fang, Y.T. Wu, M.Y. Ho, C.L. Liao, J.J. Shie, P.H. Liang, C.H. Wong, Stable benzotriazole esters as mechanism-based inactivators of the severe acute respiratory syndrome 3CL protease, *Chem. Biol.*, 13 (2006) 261-268.

[252] Y.F. Shan, S.F. Li, G.J. Xu, A novel auto-cleavage assay for studying mutational effects on the active site of severe acute respiratory syndrome coronavirus 3C-like protease, *Biochem. Biophys. Res. Commun.*, 324 (2004) 579-583.

[253] K.H. Verschueren, K. Pumpor, S. Anemuller, S. Chen, J.R. Mesters, R. Hilgenfeld, A structural view of the inactivation of the SARS coronavirus main proteinase by benzotriazole esters, *Chem. Biol.*, 15 (2008) 597-606.

[254] K. O'Connell, Use of palivizumab and infection control measures to control an outbreak of respiratory syncytial virus in a neonatal intensive care unit confirmed by real-time polymerase chain reaction, *J. Hosp. Infect.*, 77 (2011) 338-342.

[255] K.L. Yu, Y. Zhang, R.L. Civiello, K.F. Kadow, C. Cianci, M. Krystal, N.A. Meanwell, Fundamental structure-activity relationships associated with a new structural class of respiratory syncytial virus inhibitor, *Bioorg. Med. Chem. Lett.*, 13 (2003) 2141-2144.

[256] K.L. Yu, R.L. Civiello, M. Krystal, K.F. Kadow, N.A. Meanwell, Patent WO 00/04900, *Chem. Abstr.* 2000, 132 (2000).

[257] G. Paglietti, V. Boido, F. Sparatore, Substituted benzimidazoles antiviral agents, *Farmaco Sci.*, 30 (1975) 505-507.

[258] C. Cianci, K.L. Yu, K. Combrink, N. Sin, B. Pearce, A. Wang, R. Civiello, S. Voss, G. Luo, K. Kadow, E.V. Genovesi, B. Venables, H. Gulgeze, A. Trehan, J. James, L. Lamb, I. Medina, J. Roach, Z. Yang, L. Zadjura, R. Colonno, J. Clark, N. Meanwell, M. Krystal, Orally active fusion

- inhibitor of respiratory syncytial virus, *Antimicrob. Agents Chemother.*, 48 (2004) 413-422.
- [259] N.A. Meanwell, M. Krystal, Respiratory syncytial virus - The discovery and optimization of orally bioavailable fusion inhibitors, *Drugs Fut.*, 32 (2007) 441-455.
- [260] K.L. Yu, Y. Zhang, R.L. Civiello, A.K. Trehan, B.C. Pearce, Z. Yin, K.D. Combrink, H.B. Gulgeze, X.A. Wang, K.F. Kadow, C.W. Cianci, M. Krystal, N.A. Meanwell, Respiratory syncytial virus inhibitors. Part 2: Benzimidazol-2-one derivatives, *Bioorg. Med. Chem. Lett.*, 14 (2004) 1133-1137.
- [261] M. Tonelli, G. Paglietti, V. Boido, F. Sparatore, F. Marongiu, E. Marongiu, P. La Colla, R. Loddo, Antiviral activity of benzimidazole derivatives. I. Antiviral activity of 1-substituted-2-[(benzotriazol-1/2-yl)methyl]benzimidazoles, *Chem. Biodivers.*, 5 (2008) 2386-2401.