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Pharmacological profile of condensed heterocyclic compounds based on functionally substituted [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles: A review

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A B S T R A C T

Triazolo[3,4-*b*][1,3,4]thiadiazole molecules are found to be important tools in modern bioorganic and medicinal chemistry. This condensed system successfully combines two pharmacologically significant five-membered heterocycles – 1,2,4-triazole and 1,3,4-thiadiazole, which causes much more interest in the enhanced activity profile of its analogs than their parent separate constituents. It's considered that the triazoles fused to thiadiazoles exhibit various therapeutically important properties, probably due to the existence of N-C-S fragments in their structures. In this review, we presented the summarized literature data about the diversity of pharmacological effects of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole based compounds as promising objects for the rational design of drug-like molecules.

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1. Introduction

Heterocyclic compounds played a vital role in the metabolism of all living cells also they are frequently found in a wide variety of physiologically important biomolecules such as enzyme, vitamins, natural products and biological active compounds.[1-2] Large numbers of heterocycles belong to five or six membered cyclic molecules having one to three heteroatoms in their structures. They exhibit a broad spectrum of chemical and pharmacological properties that make them highly valuable as a key structural motif for synthetic, pharmaceutical and agrochemical fields.[3-8]

The majority of biologically active pharmaceuticals and agrochemicals belong to heterocycles. An important place among sulfur- and nitrogen-containing heterocycles is occupied by thia(dia)zole based compounds considering their pharmacological efficiency, including condensed ones. They are very interesting research objects in current organic and medical chemistry due to their diverse range of biological activity, as evidenced by numerous recent publications.[9-16]

Triazolo[3,4-*b*]thiadiazoles, being a condensed thia/aza-containing bicyclic system, are of current interest due to their wide spectrum of pharmacological properties including anticancer,[17] antibacterial,[18,19] antifungal,[20] anti-inflammatory,[21,22] anti-diabetic,[22] antioxidant,[23] antitubercular,[23] anticonvulsant,[24] analgesic,[25] antidepressant,[26] lipid peroxidation[27] action etc. Currently, triazolo[3,4-*b*][1,3,4]thiadiazole derivatives are the preferred structural moieties for the design and development of more potent and specific biologically active agents for various biological targets. In particular, a new class of SIRT1 inhibitors based on the 3-(furan-2-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole scaffold was discovered and described herein.[28] As stated, SIRT1 belongs to a NAD^+-dependent deacetylase, which involved in multiple diseases such as type 2 diabetes and cancer due to the deacetylation of a broad range of substrates. Llona-Minguez et al.[29] described the investigation of 3,6-disubstituted triazolo[3,4-*b*]thiadiazoles as potent dCTP pyrophosphatase 1 inhibitors. Thus, the dCTPase catalyzes the hydrolysis of canonical and noncanonical deoxynucleoside triphosphates to the corresponding

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deoxynucleoside monophosphates and diphosphate thus is involved in the regulation of the cellular dNTP pool and has been linked to cancer progression.

The first structure-based approach to the rational design of dual JCV and BKV helicase inhibitors among triazolo[3,4-*b*]thiadiazole based molecules has been reported by Bonafoux et al.[30] Thus, the effectiveness and possible use of triazolo-thiadiazoles in the treatment of asymptomatic persistent infections caused by human polyomaviruses JC (JCV) and BK (BKV) has been demonstrated. Using the virtual screening and optimization of lead-compound structure Zhang et al.[31] carried out a rational search of potent inhibitors of sortase A, which is a transpeptidase that anchors surface proteins in the develop of *Staphylococcus aureus*, and sortase mutants are unable to cause bacteremia or sepsis. In addition, triazolo[3,4-*b*][1,3,4]thiadiazole were investigated as ligands for the creation of metal-organic coordination polymers with Cu (II) and Cd (II) owning fluorescent properties and can be used as luminescent materials for their potential applications, such as light-emitting materials (LEDs).[32] Furthermore, triazolo[3,4-*b*]thiadiazoles are known as inhibitors of human fatty acid synthase (HFAS) – a multifunctional enzyme that is essential role for the endogenous synthesis of long chain fatty acid from its precursor acetyl Co-A and malonyl CoA.[33]

This review will attempt to present the literature data focusing on the diversity of pharmacological properties of triazolo[3,4-*b*][1,3,4]thiadiazole based functionalized compounds, which is a continuation of our numerous review researches on synthetic approaches for obtaining and discourse of pharmacological significance of (non)condensed 1,3,4-thiadiazole containing heterocycles.[34-36]

2. Pharmacological potential of triazolo[3,4-*b*][1,3,4]thiadiazole based heterocyclic compounds

The class of heterocycles known as triazolo[3,4-b][1,3,4]thiadiazoles has garnered significant interest in medicinal chemistry owing to their diverse range of biological activities (**Fig. 1**). There are ample evidence to support extensive synthetic capabilities and significant biological importance of this heterocyclic system that definitely proves them as a prospective building block for drug discovery. All of the above can be considered as a background for further in-depth studies in the areas of chemistry and pharmacology of the mentioned heterocyclic systems with possible applications in medicine.

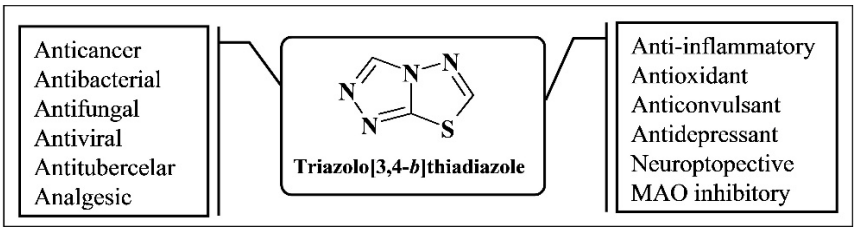


Fig. 1. Diversity of pharmacological profile of triazolo[3,4-*b*][1,3,4]thiadiazole based compounds.

In most cases, 4-amino-4*H*-1,2,4-triazole-3-thiols serve as versatile synthons for constructing of fused triazolo[3,4-b][1,3,4]thiadiazole systems.[37] The most common options used for this purpose are shown in Figure 2. Using these approaches, numerous different triazolo-thiadiazoles can be obtained, which depend on the chemical structure of the cyclizing reagent as well as from the nature of the substituent in position 5 of the starting 4-amino-1,2,4-triazole-3-thiol. In general, these synthetic protocols form the basis for the preparation of the triazolo[3,4-b]thiadiazole based compounds discussed below.

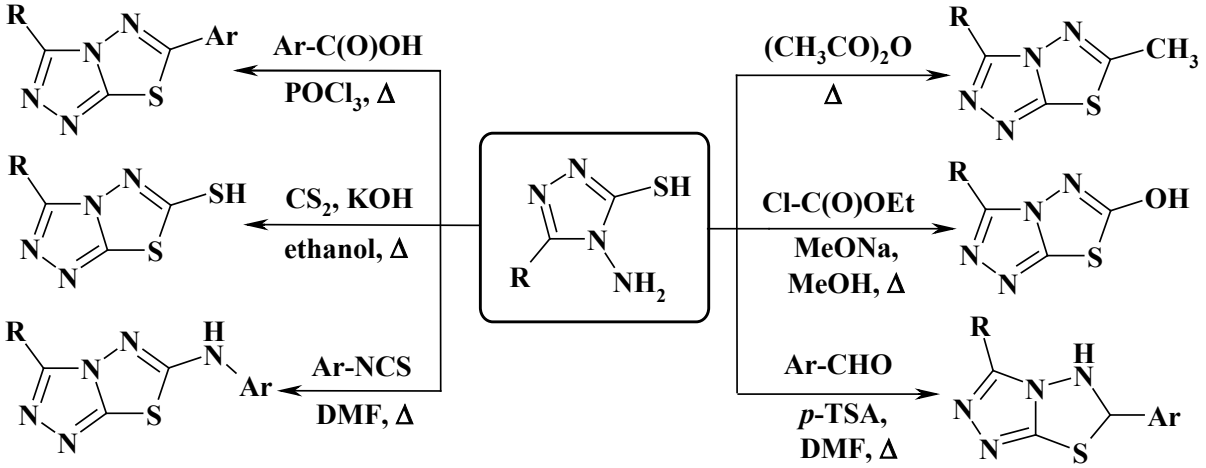


Fig. 2. Possible directions for the utilization of 5-substituted 4-amino-4*H*-1,2,4-triazole-3-thiols in the synthesis of functionalized [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives.

2.1. Anticancer activity of [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles

Antiproliferative activity of 3,6-diaryl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles was evaluated against three human cancer cell lines (gastric adenocarcinoma SGC-4901, lung adenocarcinoma A549 and fibrosarcoma HT-1080) using MTT assay.[38] As a result, 3,4,5-trimethoxy-substituted compound **I** (Fig. 3) exhibited the most excellent antiproliferative activity against all cancer cell lines with a range of IC_{50} values of 0.022-0.029 μM. To explore the relationship between the antiproliferative activity of **I** and tubulin, its effect on tubulin polymerization was evaluated, which showed that compound **I** also processes excellent antitubulin activity (IC_{50} = 0.77 μM). Furthermore, the cell cycle studies displayed that **I** significantly induced SGC-7901cells arrest in G2/M phase with a time-dependent correlation.

An antitumor activity investigation for 3-(2-naphthyloxy)methyl substituted 6-[3-(4-chlorophenyl)-1*H*-pyrazol-4-yl][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole **II** (Fig. 3) on hepatocellular carcinoma cell line HepG2 showed that tested compound exhibited a dose-dependent cytotoxic effect with very low IC_{50} value of 0.8 lg/ml in 24 h when compared with standard drug doxorubicin (IC_{50} = 19 lg/ml). Treatment of HepG2 cells with compound **II** showed a dose-dependent decreased cell division in [^{3}H] thymidine incorporation assay and increase in the subG1 population in flow cytometric analysis.[39]

The results of the anticancer activity evaluation of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives displayed their potent antitumor properties *in vitro* against a panel of cancer cells and *in vivo* efficacy in HT-29 human colon tumor xenograft in CB17 severe combined immunodeficient (SCID) mice.[40] Among all the tested compounds, **IIIa** (**Fig. 3**) is the most potent antitumor agent since it induced the most significant cytostatic and cytotoxic activities against all cancer cell lines, whereas **IIIb** induced cytotoxic effects only in ovarian and prostate cancer cells. Preliminary mechanistic studies showed that **IIIa** and **IIIb** exhibit time- and concentration-dependent inhibition of Akt Ser-473 phosphorylation, while *in silico* studies indicated that tested KA25 and KA39 bind well to the ATP binding site in Akt1 and Akt2.

The antiproliferative activity screening of 3,6-disubstituted [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives against SMMC-7721, HeLa, A549, and L929 cell lines *via* the CCK-8 assay displayed their stronger antitumor effects than reference drug 5-fluorouracil.[41] Thus, the most active compound with n-butyldisulfanyl- (**IVa-b**) and 2-butyldisulfanyl- (**IVc**) (Fig. 3) showed significant antitumor activities in inhibiting SMMC-7721cell proliferation with IC_{50} values of 1.64, 1.74 and 1.61 μM, respectively. Also, compounds **IVb** and **IVc** showed highly effective biological activity versus HeLa cells with IC_{50} values of 2.23 and 2.84 μM, respectively. Furthermore, the tested compounds exhibited weaker cytotoxic effects than 5-fluorouracil on the normal cell line L929.

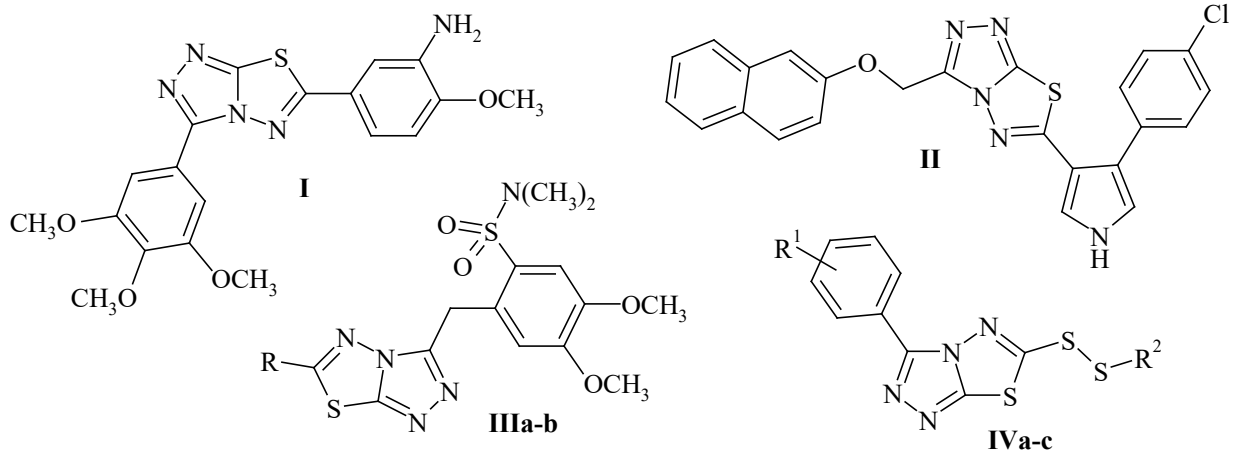


Fig. 3. Triazolo[3,4-*b*][1,3,4]thiadiazole derivatives with promising antitumor activity.

2.1.1. Impact of [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles on different antiproliferative biotargets

Depending on the realization pathway of their antitumor effect, heterocyclic compounds containing [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole scaffold are marked by considerable diversity of their impact on antiproliferative biotargets. For example, methoxyquinoline substituted triazolo[3,4-*b*][1,3,4]thiadiazoles were designed and evaluated as novel c-Met (*mesenchymal-epithelial transition factor*) inhibitors using hot-SpotSM kinase assay method and crizotinib as positive control.[42] The most effective and selective c-Met inhibitor **V** (Fig. 4) containing *N*-methyl pyrazole moiety displayed the highest inhibitory potency with an IC_{50} value of 2.02 nM, whereas for comparison drug crizotinib IC_{50} value was 8.29 nM.

Furthermore, compound V exhibited significant antiproliferative properties against various cancer cell lines (SNU-5, MKN45, and EBC-1) and exhibited a highly selective inhibition of c-Met, with a magnitude exceeding 2500-fold for 16 tyrosine kinases.

A group of 3-(indol-3-yl) substituted 6-aryl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles were tested to inhibit the growth of the Bcl-2-expressing human cancer cell lines (MDA-MB-231, HeLa and KG1a) compared with natural product *gossypol* as a positive control. As a result, the most potent 6-(2,4-dimethoxyphenyl) (**VIa)** and 6-(2,4-dimethoxyphenyl) (**VIb)** substituted analogues (**Fig. 4**) showed selective IC_{50} values of 0.31-0.7 μM (**VIa**) and 0.35-1.42 μM (**VIb**) against Bcl-2-expressing cell lines without inhibiting the Bcl-2-negative cell line (Jurkat). The observations of structure-activity relationships reveal that the dimethoxyphenyl substitution was the most favourable for potent activity.[43]

Evaluation of *in vitro* anti-proliferative activity for novel triazolo[3,4-*b*]thiadiazole derivatives using the MTT assay in three human colorectal cancer cell lines (DLD-1, HT-29, and LoVo) allowed to identify the most potent compound **VII** (Fig. 4), which showed promising anticancer activity against all cancer cell lines, demonstrating significant cytostatic and cytotoxic effects ($P < 0.001$).[44] It was established that compound **VII** exhibited inhibitory effect on topoisomerase IIα phosphorylation with values of TGI and IC_{50} concentrations of 5.5 μM and 10.5 μM, respectively, in human colorectal cancer LoVo cell line. I*n silico* studies revealed that compound **VII** may act directly through binding to the ATPase domain of topIIα through hydrogen bonds with only one residue, Ser149, indicating a highly specific way of interaction.

For 3,6-diaryl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles determination of their ability to inhibit the enzymatic activity of recombinant heparanase was carried out using a colorimetric assay based on the cleavage of the synthetic heparin pentasaccharide *fondaparinux*.[45] According to the obtained results, two highly active compounds **VIIIa** and **VIIIb** (Fig. 4) having 4-iodophenol group possessing a good heparanase inhibitory activity with IC_{50} values of 3 and 3.1 μg/mL, respectively, were identified. The release of radioactive heparan sulfate (HS) degradation fragments from an insoluble extracellular matrix (ECM) demonstrated that studied compounds inhibited (50-70%) heparanase activity at 25 μg/mL, but there was little or no inhibition at 5 μg/mL.

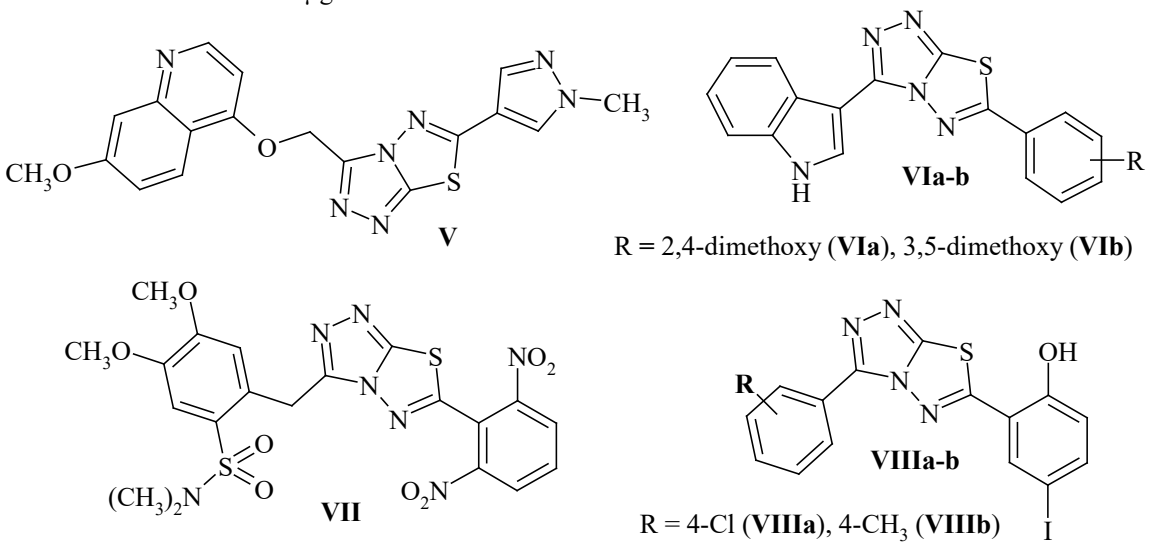


Fig. 4. Possible mechanisms of realization of the antitumor activity of triazolo[3,4-*b*][1,3,4]thiadiazole based compounds and their impact on anticancer targets.

2.2. Antibacterial and antifungal activity of [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles

Also, many works are devoted to highlighting the antibacterial and antifungal potential of fused triazolo[3,4-*b*]thiadiazole derivatives.[46-49] Thus, certain new 6-aryl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles were synthesized and screened for their *in vitro* antibacterial and antifungal activities against pathogenic microorganisms using twofold serial dilution method.[50] Among others, compounds **IXa** and **IXb** (Fig. 5) exhibited the highest antibacterial activity against all strains of both Gram positive (*Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus*) and Gram negative (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*) bacteria. In particular, the most sensitive to the action of the compound **IXa** were strains of *S. aureus*, *E. coli* and *P. aeruginosa* (IC_{50} = 0.78 μg/ml), which was comparable to gentamicin. Instead, compound **IXb** showed a significant inhibitory effect on *S. aureus*, *S. epidermidis*, *B. cereus* and *E. coli* with a range of IC_{50} values of 0.78-1.56 μg/ml. Furthermore, compound **IXa** exhibited the highest activity against both the strains of *Candida albicans* (IC_{50} values = 1.56 μg/ml) and *Aspergillus niger* (IC_{50} values = 3.125 μg/ml).

The antimicrobial activity of 6-arylamino-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles was studied against drug-sensitive bacteria (Gram-positive as well as Gram-negative ones) and towards the methicillin-resistant *S. aureus*.[51] 3-(3-Chlorophenyl) derivatives **Xa-b** (**Fig. 5**) turned out to be the most active in relation to the methicillin-sensitive strains of *S.*

aureus ATCC 25923 and *S. aureus* ATCC 6538. Especially stands out their influence on *S. aureus* ATCC 6538 (MIC for both compounds = 0.49 mg/mL), which was twice as high as the activity of cefuroxime (MIC = 0.98 mg/mL) and similar to the activity of vancomycin (MIC = 0.49 mg/mL). In relation to *S. epidermidis* ATCC 12228 strain, compounds **Xa** and **Xb** were characterised by activity similar to than vancomycin (MIC = 0.98 mg/mL). All the tested compounds were characterised by very high activity (higher than the activity of ampicillin and cefuroxime) against *Bacillus subtilis* ATCC 6633 and *Bacillus cereus* ATCC 10876.

A series of novel 6-sulfonyl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives were designed and synthesized in order to study their antibacterial activity against two strains namely *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*).[52] Among all tested compounds **XIa** and **XIb** (**Fig. 5**) showed the higher activity with the EC_{50} values of 0.61 and 0.59 mg/L against *Xanthomonas oryzae* pv. *oryzae*, respectively, and the EC_{50} values of 1.71 and 1.63 mg/L against *Xanthomonas oryzae* pv. *oryzicola*. Meanwhile, compound **XIb** exhibits good *in vivo* protective and curative activities against bacterial leaf blight (BLB), caused by *Xoc* and *Xoo*, with inhibition rates of 49.42% and 47.94%, respectively. Also, compound **XIb** showed good *in vivo* antibacterial activities against bacterial leaf streak (BLS) with protective and curative values of 49.65% and 40.67%, respectively.

Antifungal activity evaluation showed that 3-(4-methyl-1,2,3-thiadiazolyl)-6-trichloromethyl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (YZK-C22) **XII** (**Fig. 5**) inhibited the growth of *B. cinerea* and *S. turcica* with an EC_{50} of 18.9 and 5.7 μg/mL, respectively.[53] Considering that except a good inhibitory effect on fungi, **XII** was inactive against bacteria, the pyruvate kinase (PK) sequence in different species of bacteria and fungi was analysed. The PK sequence was 66% homologous across different species of fungi, but only 34% homologous across different species of bacteria. Therefore, the enzymatic activity of pyruvate kinase was tested in *B. cinerea* after treatment with **XII** (YZK-C22) at 0.5, 10 and 20 μg/mL. As a result, an obvious concentration-dependent relationship between **XII** (YZK-C22) and pyruvate kinase activity was observed. Thus, the enzymatic activity was 13.17 U/mg in the vehicle-treated group and 5.06 U/mg in the **XII** (YZK-C22)-treated group (20 μg/mL), which indicates that the tested compound may act as an inhibitor of pyruvate kinase.

As a result of antibacterial and antifungal activity investigation, the most promising compounds among triazolo[3,4-*b*]thiadiazoles containing sulfonamide moiety **XIIIa-d** (**Fig. 5**) were identified, which can replace those of the reference drugs by effectiveness indicators.[54] The most potent antibacterial activity was achieved for the derivative **XIIId** with minimal inhibitory concentration (MIC) in the range of 5-20 μg/mL against a panel of three Gram-positive (*L. monocytogenes*, *B. cereus* (clinical isolate), *S. aureus*) and three Gram-negative (*E. coli*, *P. aeruginosa*, *S Typhimurium*) bacteria strains. Also, compounds **XIIIa**, **XIIIb** and **XIIIc** displayed excellent antifungal activities with values of MIC at 2-10 μg/mL against all six fungal species (*A. niger*, *A. fumigatus*, *A. versicolor*, *P. funiculosum*, *T. viride*, *P. verrucosum var. cyclopium* (food isolate)). The results of docking indicated that compound **XIIId** exerts its antimicrobial properties through the binding of *E. coli* MurB enzyme, specifically by fitting into the binding center of the enzyme through the formation of an H-bond with Ser228. Instead, 14α-lanosterol demethylase (CYP51) of *C. albicans* is predicted to be a possible mechanism of the antifungal activity for the compound **XIIIb**.

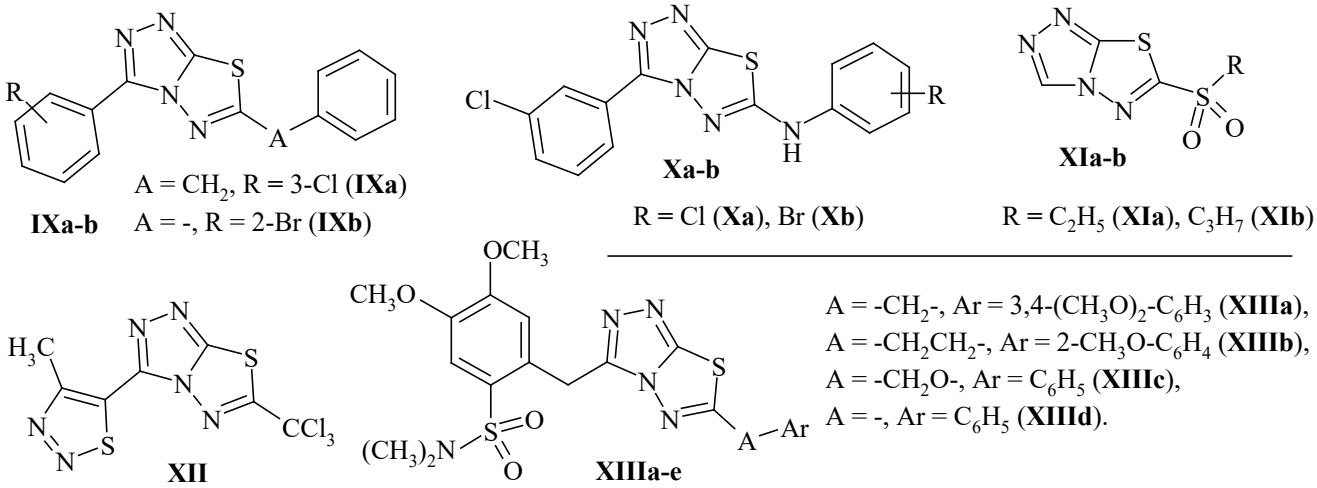
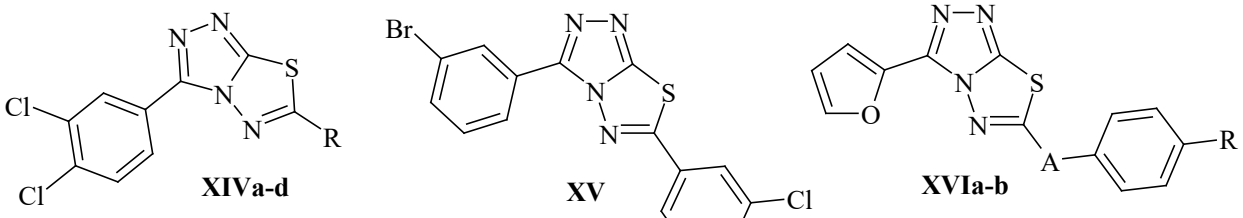


Fig. 5. Antibacterial and antifungal potential of triazolo[3,4-*b*][1,3,4]thiadiazole derivatives.

2.3. Antiviral activity of [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles

Antiviral activity evaluation of 3-aryl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles were performed *in-vitro* against HIV-1 (strain IIIB) and HIV-2 (strain ROD) viruses in human T-lymphocyte (MT-4) cells using the MT-4/MTT assay.[55] As result, the most active compounds **XIVa** and **XV** (**Fig. 6**) with EC_{50} values of 2.11 μg/mL and 4.12 μg/mL, respectively, were identified. In addition, derivatives **XIVb-d** exhibited an inhibitory effect against Coxsackie virus B (CVB-2) with the range of EC_{50} = 12÷22 μg/mL and tolerable toxicometric parameters (CC_{50} > 100 μg/mL).

A group of 6-aryl(oxy)alkyl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles containing thiophene were evaluated for their antiviral activity against the replication of HIV-1 and HIV-2 in MT-4 cells using an MTT assay.[56] Consequently, two compounds namely **XVIa-b** (**Fig. 6**) were found to be the most effective inhibitors of HIV-1 and 2 replication in cell cultures with EC_{50} > 1.51 and > 0.89 μM, respectively, but no selectivity was witnessed (SI < 1). The initial SAR analysis revealed that the introduction of halogen atom or hydroxyl groups in 4 positions of the phenyl ring considerably increased the anti-HIV activity. The molecular docking studies of **XVIa** and **XVIb** as HIV-1 reverse transcriptase (HIV-1 RT) inhibitors were performed, which showed that **XVIb** interacted with several amino acids in the reverse transcriptase (RT) binding site of HIV-1.



XIVa-d

R = pyrazin-2-yl (**XIVa**), thiophen-2-yl (**XIVb**), 3,4-Cl_2-C_6H_3 (**XIVc**), 4-NH_2-C_6H_4 (**XIVd**)

XV

XVIa-b

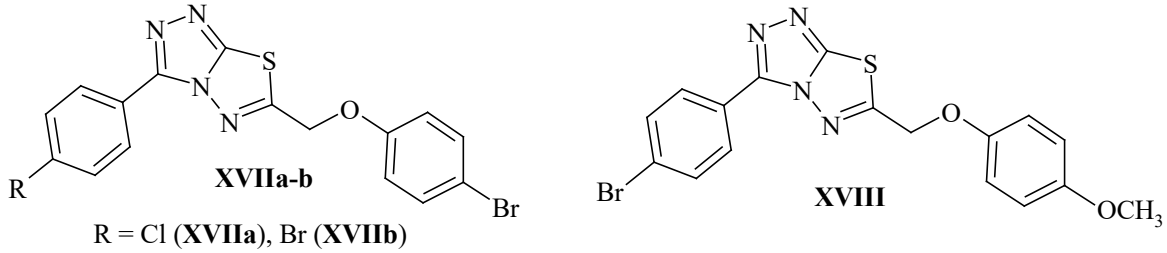
A = -CH_2-, R = F (**XVIa**),
A = -CH_2O-, R = OH (**XVIb**).

Fig. 6. Effective antiviral agents based on fused triazolo[3,4-*b*][1,3,4]thiadiazole system.

2.4. Antitubercular activity of [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles

Evaluation of antitubercular activity for 3,6-disubstituted 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazoles against *Mycobacterium tuberculosis* (*Mtb*) H37Rv strain, MDRTB (multidrug-resistant strains) and RDRTB (rifampin resistant strains) using the BacT/ALERT 3D liquid culture technology was performed and described herein.[57] It was observed that different electron-withdrawing groups such as halogen atoms, attached at para position of the phenyl ring at the 3 position as well as the phenoxymethyl group at the 6 position exhibits a remarkable enhancement on antitubercular potency, leading to the discovery of two highly active compounds **XVIIa-b** (**Fig. 7**) with MIC-H37Rv values of 0.5 μg/mL. Also, compounds **XVIIa** and **XVIIb** bearing 4-bromophenoxymethyl moiety exhibit good activity against multidrug-resistant strain (MIC-MDRTB = 4.0 μg/mL), which are approximately 4-fold more potent than rifampin (MIC-MDRTB = 16.0 μg/mL), the standard first-line drug used for the treatment of multidrug-resistant forms of tuberculosis. Moreover, compounds **XVIIa** and **XVIIb** possessed good to excellent inhibitory action against rifampin resistant strains (MIC-RDRTB = 1.0 μg/mL and 0.5 μg/mL, respectively), which are approximately comparable to isoniazid (MIC-RDRTB = 1.0 μg/mL) and 16-32-fold more potent than rifampin (MIC-RDRTB = 16.0 μg/mL). In addition, the results of *in vitro* inhibitory activity investigation for compounds showing the most effective inhibition confirmed that *Mycobacterium tuberculosis* shikimate dehydrogenase is the target of these compounds.

Based on the results of previous studies,[57] a lead compound **XVIII** (IMB-SD62) (**Fig. 7**) showing the stronger antitubercular activity (MIC against H37RV = 2.0 μg/mL, MIC against rifampin resistant strains = 4.0 μg/mL) and lower cytotoxicity (IC_{50} for Vero and HepG2 respectively were 26.91 and 21.02 μg/mL) was found. The next stage, which consisted in the comprehensively evaluation of the *in vivo* therapeutic efficacy of **XVIII** (IMB-SD62), was carried out by the murine model of acute infection using oral isoniazid as a positive control.[58] Compound **XVIII** (IMB-SD62) showed antitubercular activity to a certain extent at the dose of 50mg/kg, with the viable bacterial counts in the lung decreasing 1.7 lg units, whereas isoniazid reduced the viable bacterial counts in the lung 5.1 lg units at the dose of 25 mg/kg. The results of pharmacokinetics study in rats indicated that the oral bioavailability of **XVIII** (IMB-SD62) was 14% and the half-time was 1.05 h, instead of the distribution of **XVIII** (IMB-SD62) in tissues occurred mainly through the liver and lungs. Whereas, *in vitro* metabolism study suggested that the metabolic ways of **XVIII** (IMB-SD62) were dealkylated, oxidized and demethylated.



XVIIa-b

R = Cl (**XVIIa**), Br (**XVIIb**)

XVIII

Fig. 7. Triazolo[3,4-*b*][1,3,4]thiadiazole derivatives with the most promising antitubercular potential.

2.5. Antioxidant activity of [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles

Free radical scavenging activity of 6-phenyl-3-(4-pyridyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole **XIX** (Fig. 8) was determined by measuring the change in the absorbance of DPPH (1,1-di-phenyl-2-picrylhydrazylradical) solution at 517

nm spectrophotometrically.[59] The resulting compound **XIX** showed the scavenging activity between 40.7% and 52.1% at the investigated concentration of 62.5 μM and 125 μM, respectively. It's worth noting that the scavenging activity is growing with increasing sample concentration in the range tested, which indicates the obvious antioxidant potency of the title compound.

A group of 6-(2-aryl-2-aminoethyl) substituted triazolo[3,4-*b*][1,3,4]thiadiazoles were synthesized and screened for their antioxidant activity by DPPH, nitric oxide and hydrogen peroxide radical scavenging methods.[60] Among the synthesized compounds, **XXa-b** and **XXIa-b** (**Fig. 8**) were found to have moderate antioxidant activities with the range of IC_{50} values = 16.83÷17.53 μg/mL when compared with the standard ascorbic acid (IC_{50} = 15.57 ± 0.01 μg/mL). Also, compounds **XXa** and **XXIa** displayed greater NO radical scavenging activity with the IC_{50} of 16.81 ± 0.04 μg/mL and 16.99 ± 0.08 μg/mL, respectively, compared with ascorbic acid (IC_{50} = 16.61 ± 0.02 μg/mL), while compounds **XXb** and **XXIb** (IC_{50} = 17.26 ± 0.02 μg/mL and 17.33 ± 0.06 μg/mL, respectively) were moderately active. Furthermore, the good scavenging effect by H_2O_2 method was detected for compounds **XXa-b** and **XXIa-b** with the range of IC_{50} values = 16.82÷17.32 μg/mL, whereas ascorbic acid showed antioxidant activity at the level of IC_{50} = 16.38 ± 0.02 μg/mL. Structure activity relationship study allowed to reveal that the presence of electron-donating substituent on the phenyl ring at the position 3 of triazolo[3,4-*b*]thiadiazole system enhances the free radical scavenging effect, whereas electron-withdrawing group decreases the activity.

Determination of free radical scavenging activity for 3,6-disubstituted [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives was performed using the DPPH method.[61] The obtained results allowed to establish the excellent antioxidant activity for the compound **XXIIa** (**Fig. 8**) with IC_{50} value = 8.1 ± 0.325 μg/mL, which was better than the standard ascorbic acid (IC_{50} value 36.3 ± 0.21 μg/mL). Whereas, some compounds namely **XXIIb**, **XXIII** and **XXIV** showed moderate radical scavenging effect having IC_{50} value of 43.4 ± 0.338 μg/mL, 40.5 ± 0.274 μg/mL and 47.6 ± 0.431 μg/mL, respectively. Thus, as follows from the results the presence of mono- and di-chloro substituted aromatic rings at both 3 and 6 positions of triazolo[3,4-*b*]thiadiazole core have a decisive impact on the realization of antioxidant efficiency.

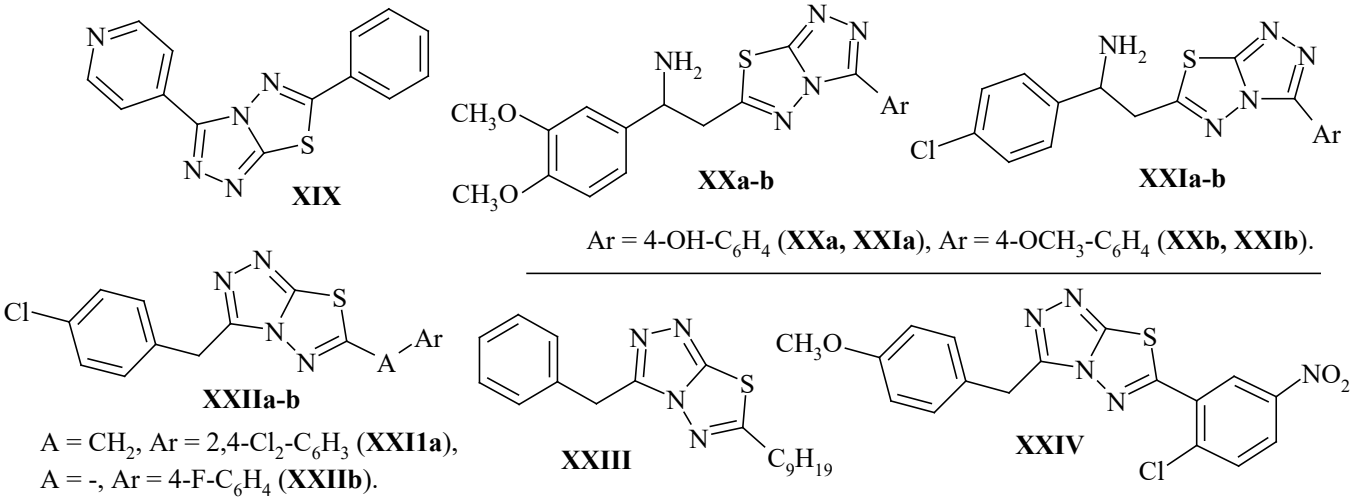


Fig. 8. Free radical scavenging action of 3,6-disubstituted triazolo[3,4-*b*][1,3,4]thiadiazoles.

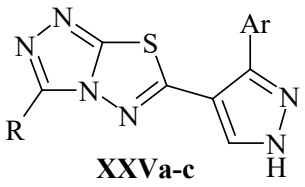
2.6. Anti-inflammatory and analgesic activities of [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles

A group of pyrazolyl substituted [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles were screened for their anti-inflammatory activity using carrageenan-induced rat paw edema model by employing 1% carrageenan solution as the phlogistic agent.[62] Among the screened compounds, **XXVa** (**Fig. 9**) showed significant anti-inflammatory activity with inhibition percentage of 64.7%, compared to the standard drug Diclofenac, which showed the inhibition percentage at 80.4%. Instead, compounds **XXVb** and **XXVc** have showed poor anti-inflammatory activity with the same for both inhibition percentage of 56.9%. To determine the best *in silico* conformation for compounds **XXVa-c** the comparative and automated molecular docking studies considering COX-2 as the target receptor were performed. *In-silico* studies revealed that all three synthesized molecules exhibited well established bonds with one or more amino acids in the receptor active pocket with good binding energy toward the target protein.

Anti-inflammatory activity evaluation of 3-(3-bromo-4-fluorophenyl) substituted [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles by carrageenan-induced rat paw oedema method was completed at a 20 mg/kg oral dose compared with the standard drug ibuprofen at the same dose.[63] Also, screening of analgesic activity was performed after oral administration of test compounds at a dose of 20 mg/kg by acetic acid-induced writhing method. The biological results indicate that compound **XXVIa** (Fig. 9), which has a fluorine atom at the *para*-position of the aryl ring, showed the significant activity (51.86 ± 4.10%) compared to ibuprofen (60.06 ± 1.66%), whereas chlorine substituted analogue **XXVIb** exhibited 46.61 ± 3.29% inhibition. Also, compounds **XXVIa** and **XXVIb** possessed moderate analgesic activity (48.18 ±

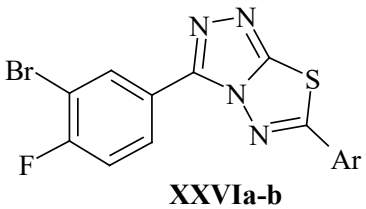
9.11% and 44.48 ± 5.7%, respectively) and appreciably less ulcerogenic effect (0.763 and 0.788) compared with the standard drug ibuprofen (60.48 ± 3.12% and 1.738, respectively). Thereafter, the introduction of halogen atoms in the both aryl rings at positions 3 and 6 of triazolo[3,4-*b*]thiadiazole scaffold was turned out to be favorable for enhancing both anti-inlammatory as well as analgesic activities.

The synthesis, conformational and quantitative analysis of 3-(4-fluorophenyl)-6-(2-fluorophenyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole **XXVII** (**Fig. 9**) as a promising bioactive agent was performed by Al-Wahaibi et al.[64] In particular, the potential anti-inflammatory activity of the compound **XXVII** was studied by *in silico* molecular docking against different biotargets including two cyclooxygenases (*Ovis aries* COX-1, pdbid: 1EQG and *Homo sapiens* COX-2, pdbid: 5IKR). To compare the binding affinity of **XXVII**, as standard inhibitors ibuprofen (COX-1) and mefenamic acid (COX-2) were used. The flexible ligand docking analysis revealed that the title compound makes two important stacking interactions with Tyr 385 and Trp 387 residues through its 2F-phenyl ring. It was also shown that the residue Tyr 355 is forming a stacking interaction with the triazole ring in the case of COX-1, which indicated that compound **XXVII** is slightly more selective towards COX-1.



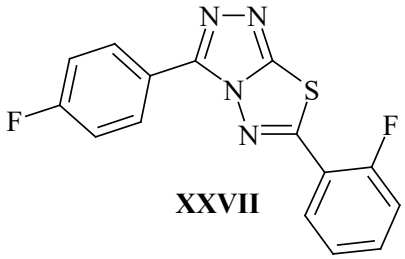
XXVa-c

R = C_3H_7, Ar = 4-Cl-C_6H_4 (**XXVa**),
R = C_2H_5, Ar = 4-Cl-C_6H_4 (**XXVb**),
R = C_3H_7, Ar = C_6H_5 (**XXVc**).



XXVIa-b

Ar = 4-F-C_6H_4 (**XXVIa**),
4-Cl-C_6H_4 (**XXVIb**).

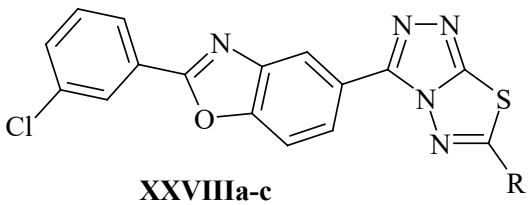


XXVII

Fig. 9. Triazolo[3,4-*b*][1,3,4]thiadiazoles with significant anti-inflammatory/analgesic potential.

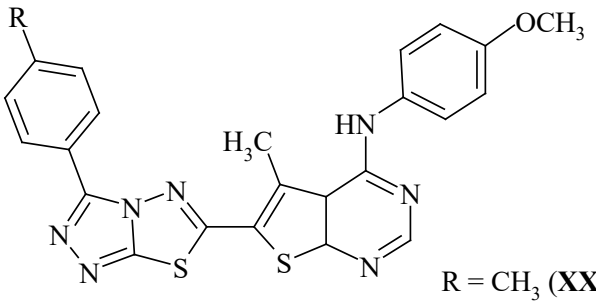
2.7. Anticonvulsant and neuroprotective activities of [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles

The anticonvulsant activity screening of benzoxazole substituted triazolo[3,4-*b*][1,3,4]thiadiazoles was performed on Swiss albino mice (20-25 g) using the Maximal Electroshock Seizure (MES) and scPTZ seizure test systems.[65] The obtained data indicated that the most active compounds **XXVIIIa**, **XXVIIIc** and **XXVIIId** (Fig. 10) possessed an expressive anticonvulsant effect against the MES method for 0.5 h time intervals at a dosage of 30 mg/kg, which was practically comparable to standard drugs – phenytoin and carbamazepine. Instead, compound **XXVIIIb** was found to be slightly less active, showing the resulting effect during the specified time period at 100 mg/kg. In the scPTZ examination, compound **XXVIIIa** showed 100% safety at a dosage of 300 mg/kg for 0.5 h and has rapid beginning however for short term of activity. Also, compounds **XXVIIIc** and **XXVIIId** displayed required effect in the same dose after 4 hrs expanded time of activity, whereas compound **XXVIIIb** established activity at the higher dose (300 mg/kg) on both time intervals. Additionally, neurotoxicity screening to measure the undesired impacts of the synthesized compounds like sedation and ataxia was carried out using rotarod test. As a result, compounds **XXVIIIc** and **XXVIIId** showed no any mortality at the higher dose (300 mg/kg). Compound **XXVIIIb** demonstrated lethality after 0.5 h and doesn't indicate harmfulness after 4 hrs, whereas compound **XXVIIIa** exhibited delayed toxicity simply after 4 h is practically identical to the standard drug carbamazepine (300 mg/kg).



XXVIIIa-c

R = 4-CHO-C_6H_4-OCH_2- (**XXVIIIa**),
4-CHO-C_6H_4 (**XXVIIIb**), C_5H_4N (**XXVIIIc**),
3,4,6-$(OH)_3$-C_6H_2 (**XXVIIId**).



XXIXa-b

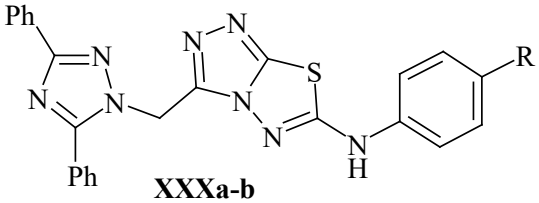
R = CH_3 (**XXIXa**),
NO_2 (**XXIXb**)

Fig. 10. Triazolo[3,4-*b*][1,3,4]thiadiazoles which exhibit anticonvulsant and neuroprotective activity.

Neuroprotective action of various [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles incorporated thieno[2,3-*d*]pyrimidine was evaluated by treating with PC12 cells followed by the assessment of cell viability using the MTT assay.[66] Initially, different concentrations were examined to find those that resulted in 100% cell viability – Maximum NonToxic Concentration

(MNTC) and 50% toxicity (CC_{50}) – a concentration of compounds required to show 50% cell apoptosis on PC12 cells. Based on these parameters, further the ability of tested thienopyrimidine-triazolothiadiazoles to protect against H_2O_2 induced cell death was evaluated. The results were shown as effective concentrations (EC_{50}) – a concentration of compounds required to induce up to 75% protection of PC12 cells from H_2O_2 induced cell death. Among all tested compounds, derivatives from the 4-methylphenyl **XXIXa** and 4-nitrophenyl **XXIXa** groups (**Fig. 10**) showed good neuroprotective activity at EC_{50} values of 10.44 and 14.12 μg/mL, respectively.

A group of 3,5-diphenyl-1,2,4-triazole substituted [1,2,4]riazolo[3,4-*b*][1,3,4]thiadiazoles was screened for its inhibitory activity against hMAO-A and hMAO-B by using *in vitro* Amplex Red reagent based fluorometric method.[67] The most active compounds containing NH-*para*-fluorophenyl (**XXXa**) and NH-*para*-(trifluoromethyl)phenyl (**XXXb**) (**Fig. 11**) substituents at the 6-position exhibited the highest inhibitory activity against hMAO-B with IC_{50} values of 2.51 ± 0.338 and 2.81 ± 0.155 μM, respectively, which was more than 25-fold selective towards the inhibition of hMAO-B. According to these findings, it turns out that fused triazolo[3,4-*b*]thiadiazoles can be an effective scaffold for the development of novel potential drug-candidates in the treatment of neurodegenerative diseases.



XXXa-b

R = F (**XXXa**), CF_3 (**XXXb**).

Fig. 11. Monoamine oxidase inhibitory activities of triazolo[3,4-*b*][1,3,4]thiadiazole derivatives.

3. Conclusions

Fused heterosystems bearing a 1,2,4-triazole and 1,3,4-thiadiazole moiety represent an interesting class of compounds possessing a wide spectrum of biological activities. The triazolo-thiadiazole system can be considered as a cyclic analogue of thiosemicarbazide and biguanide which exhibit diverse biological activity. Thus, there are numerous research papers focusing on the main chemical features of triazolo[3,4-*b*][1,3,4]thiadiazoles and their significant pharmacological potential that definitely proves the mentioned bicyclic structure as a prospective building block for drug discovery.

In the present review we highlighted the recent developments in the fast growing research area of chemistry and pharmacology of fused triazolo[3,4-*b*][1,3,4]thiadiazole system. The broad pharmacological profile and widespread use in organic and medicinal chemistry of this class compounds are evidenced by the numerous examples cited here. Thus, the current prospects and challenges of condensed triazolo[3,4-*b*]thiadiazole derivatives application as well as their high pharmacological potential found a strong basis for the directed synthesis and systematic research of these class compounds. Finally, we hope that all of the above will lead the scientists and researchers for rational design and development of novel, target oriented, and efficient triazolo[3,4-*b*]thiadiazole based therapeutic products.

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