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REVIEW

Fused bicyclic 1,2,4-triazoles with one extra sulfur atom: Synthesis, properties, and biological activity

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Abstract

Fused heterocyclic systems with 1,2,4-triazole scaffold arouse great interest from researchers in the branch of heterocyclic and medical chemistry because of their wide biological activities as they are considered as fungicidal, antimicrobial, analgesic, bronchodilators, antioxidant, anti-inflammatory agents, and they are G-quadruple stabilizers; the molecular docking data for coumarin-containing 1,2,4-triazoles indicate their ability to act as a urease inhibitor. Therefore, the present review aims to investigate new trends in the chemistry of heterocycles incorporating thiazolo[3,2-b][1,2,4]triazoles, thiazolo[2,3-c][1,2,4]triazoles, thiazino[5,1-b][1,2,4]triazoles, thiazino[3,4-b][1,2,4]triazoles, triazolothiazepines, and their biological characteristics. The main sections discuss: (a) the synthetic routes to the production of substituted fused heterocyclic systems, which include condensation reactions, multiple bond annulation, and the reactions of electrophilic heterocyclization. (b) Description of chemical and biological characteristics of these fused heterocycles.

1 | INTRODUCTION

The chemistry of triazoles includes two representatives—1,2,3- and 1,2,4-triazoles. Both systems are known since early 1885 and their increasing significance is proved by the impressive number of studies. For example, while considering review articles from the last decade, we have found a lot of studies dedicated to 1,2,3,-triazole-containing compounds that are valuable in medicinal chemistry.[1–3] Thus, 1,2,3-triazole-containing hybrids are known as antimalarial agents,[4] α-glucosidases inhibitors,[5] exhibit therapeutic action,[6] displayed variable anti-bacterial activity.[7] Except of these, the class of 1,2,3-triazoles is known as functional materials,[8] organometallic complexes.[9,10]

1,2,4-Triazoles are attractive compounds because of their pharmacological application[11] and biological significance.[12–15] Numerous derivatives of 1,2,4-triazole are used as organic corrosion inhibitors.[16] 1,2,4-Triazole-3-thiones, which are the starting material in a lot of

Abbreviations: $(PPh_3)_2PdCl_2$, Bis(*triphenylphosphine*)palladium chloride; 2-PrONa, sodium propan-*2*-olate; Ac_2O, acetic anhydride; AcOH, acetic acid; Alk, alkyl; Ar, aryl; BnNH, benzylamine; CH_2Cl_2, dichloromethane; $CHCl_3$, chloroform; $Cu(OAc)_2$, copper (II) acetate; CuI, copper (I) iodide; CyNH, cyclohexylamine; DMAD, dimethyl acetylenedicarboxylate; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; Et, ethyl; Et_3N, triethylamine; *EtOH*, e*thanol*; H_2SO_4, sulfuric acid; HPA, *h*ypophosphorous acid; *Ht*, h*eteryl*; K_2CO_3, potassium carbonate; $K_3(CN)_6$, potassium ferricyanide; KOH, potassium hydroxide; LC50, the lethal concentration for 50% of the animals tested; LD50, the lethal dose for 50% of the animals tested; LDA, lithium diisopropylamide; Me, methyl; MeCN, acetonitrile; MeOH, methanol; MW, microwave; Na_2CO_3, sodium carbonate; Na_2SO_3, *s*odium sulphite; $NaHCO_3$, sodium bicarbonate; NaOAC, Sodium acetate; *NaOH*, s*odium hydroxide*; NH_3, ammonia; NH_4Cl, ammonium chloride; $Pd(OAc)_2$, palladium (II) acetate; Ph, phenyl; $POCl_3$, phosphoryl chloride; PPA, polyphosphoric acid; Pr, propyl; *p*TSA, *p*-Toluenesulfonic acid; Py, pyridine; *t*-BuOH, *tert*-butyl alcohol; *t*-BuOK, potassium *tert*-butoxide; TEA, triethylamine; THAC, tetrahexylammonium chloride; THF, tetrahydrofuran.

Dedicated to the 75th anniversary of the Uzhhorod University.

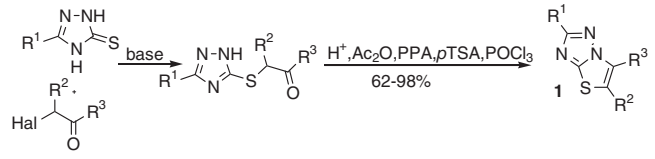
reactions for obtaining fused heterocycles are described in the current review, and have also found application in the composition of drugs and bioactive materials.[17]

Fused heteroaromatic compounds with 1,2,4-triazole scaffold have recently occupied a notable place in the work of chemists, thanks to their application as valuable catalysts in synthesis or as analytical ligands in heavy metal determination, as well as due to the wide spectrum of biological activity. Considering the importance of this class of compounds, many original research studies are reported in the literature. The application of 1,2,4-triazolo [1,5-a]pyrimidines in drug design has been covered and extensively discussed in various remarkable reviews.[18,19] The synthesis of thiazolo[3,2-b][1,2,4]triazole system and its medicinal value was shortly covered in microreview.[20] In the present study, based on our best knowledge, there are no review articles dedicated to thiazolo[2,3-c][1,2,4] triazoles, thiazino[5,1-b][1,2,4]triazoles, thiazino[3,4-b] [1,2,4]triazoles, and triazolothiazepine systems.

The goal of this survey is to chart the progress in the synthesis of fused bicyclic 1,2,4-triazoles with one extra sulfur atom and it will focus on the synthesis, chemical, and biological properties of thiazolo[3,2-b][1,2,4]triazoles, thiazolo[2,3-c][1,2,4]triazoles, thiazino[5,1-b][1,2,4]triazoles, thiazino[3,4-b][1,2,4]triazoles, and triazolothiazepines.

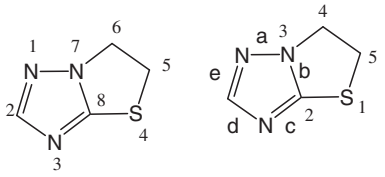
2 | SYNTHESIS OF THIAZOLO [3,2-B][1,2,4]TRIAZOLE DERIVATIVES

The system of thiazolo[3,2-b][1,2,4]triazole was first reported by Dymek et al[21] in the late 1960s by the reaction illustrated in Scheme 1. During the next decade, researchers were actively involved in the investigation of new methods for obtaining thiazolotriazoles.[22–25]



R^1 = H, Ar, Het; R^2 = H, Alk, C(O)CH_3; R^3 = Ar, Me, CH_2CO_2Et

SCHEME 1 Synthesis of thiazolo[3,2-b][1,2,4]triazoles **1**



thiazolo[3,2-b][1,2,4]triazole

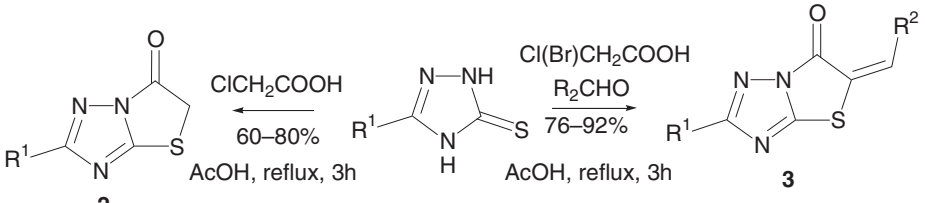
The condensation reactions of triazolethiones with α-halomethylcarbonyl compounds are the main methods for the synthesis of thiazolo[3,2-b][1,2,4]triazoles **1**. This reaction was carried out with acetone at room temperature in the presence of a base (NaOAc, Na_2CO_3). The obtained thioesters can be converted to the corresponding thiazolo [3,2-b][1,2,4]triazoles **1** under the action of sulfuric acid,[26–30] acetic anhydride in acidic medium,[21,31–33] PPA,[34] *p*TSA,[35] or $POCl_3$.[36] Synthesis of thiazolo[3,2-b] [1,2,4]triazoles **1** proceeds through oxidative alkylation by acid condensation and MW radiation (Scheme 1).[37,38]

2-Chloroacetic acid is also used as an alkylating agent in reactions with 1,2,4-triazole-3-thiones.[39,40] As a consequence, thiazolo[3,2-b][1,2,4]triazolones **2** were obtained. If (hetero)arylcarbaldehydes were added to the reaction mixture, various thiazolo[3,2-b][1,2,4]triazole-6 (5*H*)-ones **3** with various 5-(het)arylidene substituents were formed (Scheme 2).[41–46]

According to El-Sherief et al,[47] 5-phenyl-1,-2,4-triazole-3-thiol was treated with various cyano-compounds that contain the active methylene group in the boiling acetic acid in the presence of sulfuric acid. As a result, the corresponding 5-amino-2-phenyl-[1,3] thiazolo[3,2-b][1,2,4]triazoles **4** were obtained in moderate yields (Scheme 3).

Reaction of mercaptotriazoles with various *N*-arylmaleimides in acetic acid medium gives *N*-aryl-2-(6-oxo-5,6-dihydro[1,3]thiazolo[3,2-b][1,2,4]triazole-5-yl)acetimides **5** (Scheme 4).[48,49]

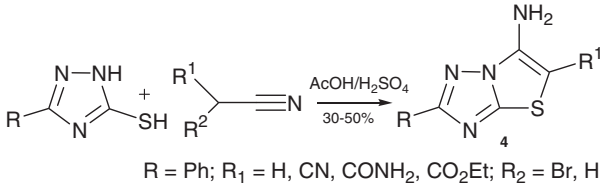
The next study describes the interaction of 1,2,4-triazole-3-thiones with bromomalononitrile in boiling *t*-butanol, containing potassium *t*-butoxide. This cyclocondensation reaction leads to the formation of 6-aminothiazolo[3,2-b] [1,2,4]triazole-5-carbonitrile **6** (Scheme 5).[50]



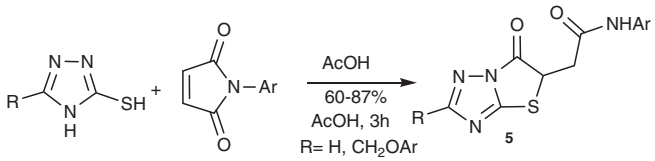
R_1 = Alk, Ar; R_2 = Ar, Het

SCHEME 2 Synthesis of thiazolo [3,2-b][1,2,4]triazolones **2**,**3**

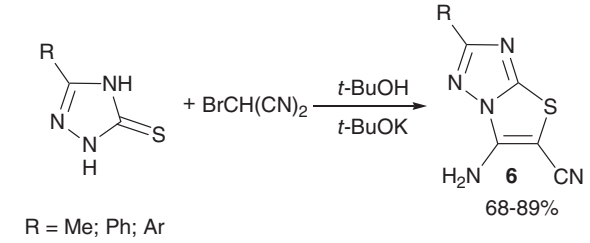
The non-selective method for obtaining 5,6-dihydro-[1,3]thiazolo[3,2-b][1,2,4]triazolium salts was developed by Il'inykh et al[51] via iodination of 3-alkenylsulfanyl-4*H*-1,-2,4-triazoles **7** in methylene chloride, ether, or chloroform at room temperature. The above electrophilic heterocyclization led to the formation of the mixture of thiazolotriazoles **8** and 5,6-dihydro-[1,2,4]triazolo[5,1-b][1,3]thiazinium salts **9** (Scheme 6). At the same time, Usenko et al[52] noted that the replacement of the solvent for the reaction with glacial acetic acid in the case of the allylthioether of 4,5-diaryl-substituted analogue leads to an increase of regioselectivity of halogenation with a dominant formation of salt **8**.



SCHEME 3 Synthesis of 5-amino-2-phenyl-[1,3]thiazolo [3,2-b][1,2,4]triazoles **4**



SCHEME 4 Synthesis of *N*-aryl-2-(6-oxo-5,6-dihydro-[1,3] thiazolo[3,2-b][1,2,4]triazole-5-yl)acetimides **5**

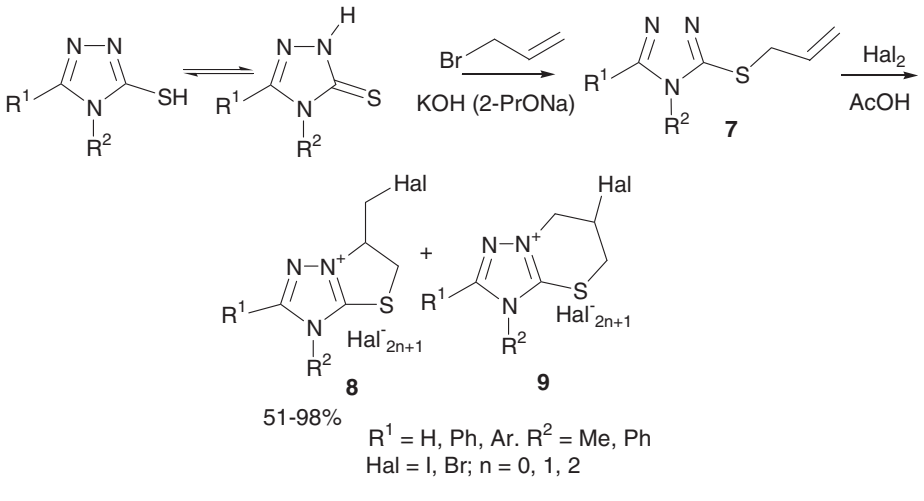


SCHEME 5 Synthesis of 6-aminothiazolo[3,2-b][1,2,4] triazole-5-carbonitrile **6**

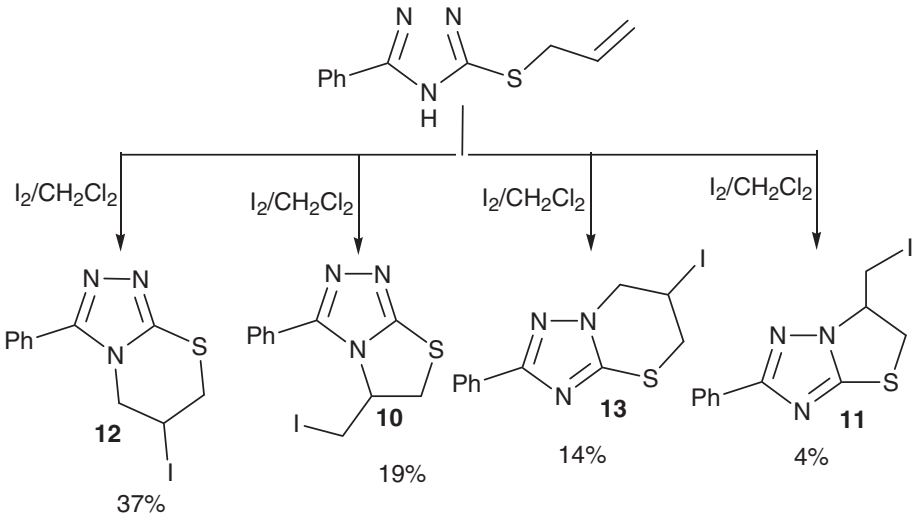
The similar electrophilic iodocyclization of alkenylthio-derivative of 4-unsubstituted 1,2,4-triazole was investigated in dichloromethane at room temperature. As a result, the low selectivity was observed again with the formation of mixture of four products: triazolothiazoles **10**, **11** and thiazinotriazoles **12**, **13** (Scheme 7).[53]

Erkhitueva et al[54] indicated that the reaction of dialkyl-1-chloroacetylene-2-phosphonate with 4-amino-5-methyl-1*H*-1,2,4-triazole-3-thiol proceeds easily under mild conditions, with high selectivity, for the production of 6-(dialkoxyphosphoryl)-3*H*-thiazolo[3,2-b][1,2,4]triazole-7-chloro chlorides **14**. The prolonged exposure of the resulting intermediate in water or heating in a polar solvent at 50°C to 70°C led to the cleavage of one alkyl group, dialkoxyphosphoryl fragment, with the formation of zwitterionic structure **15** (Scheme 8). In the 1H NMR spectra, the singlet at 7.96 to 8.31 ppm was interpreted as the chemical shift of the signal of hydrogen atom at the fifth position. In the 13C NMR spectra, for these systems, there are following characteristic signals: 111.49 to 111.56 ppm for C-5, 130.61 to 131.14 ppm for C-6, 157.92 to 158.41 ppm for C-2 and 163.87 to 164.27 ppm for C-8. ESI-MS spectra include [M + H] signal in the range of 234 to 263 (m/z), characteristic signals in the IR spectra are as follow: the signal of C═N bond is at 1557 to 1603 cm^{-1}, the signal of C—H bond is at 2848 to 3087 cm^{-1}.

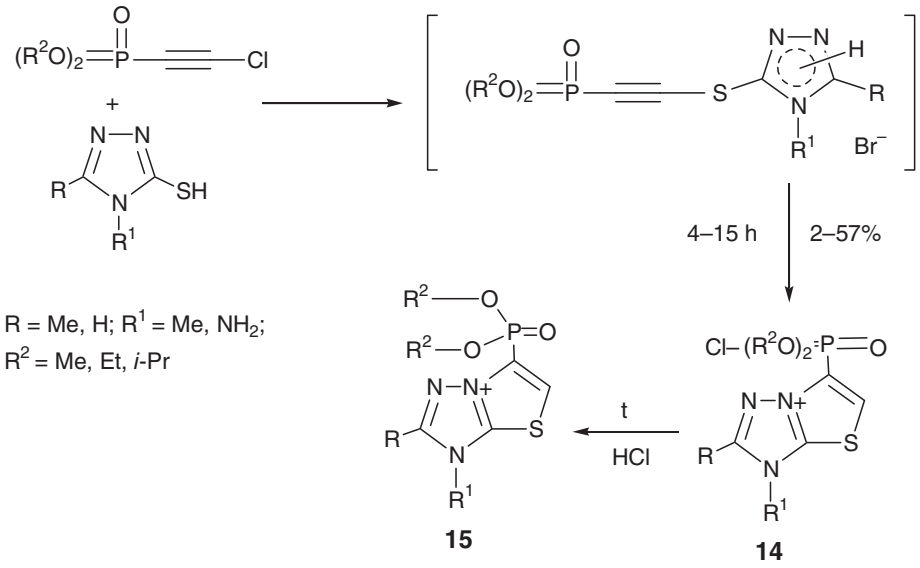
The action of bromine, iodine, iodine bromide, and tetrahalides of selenium and tellurium on 4,5-disubstituted 1,2,4-triazole thioethers in the acetic acid medium under conditions of excess of electrophilic reagent, strong dilution of solutions and room temperature lead to selective annulation of the thiazole ring to 1,2,4-triazole scaffold



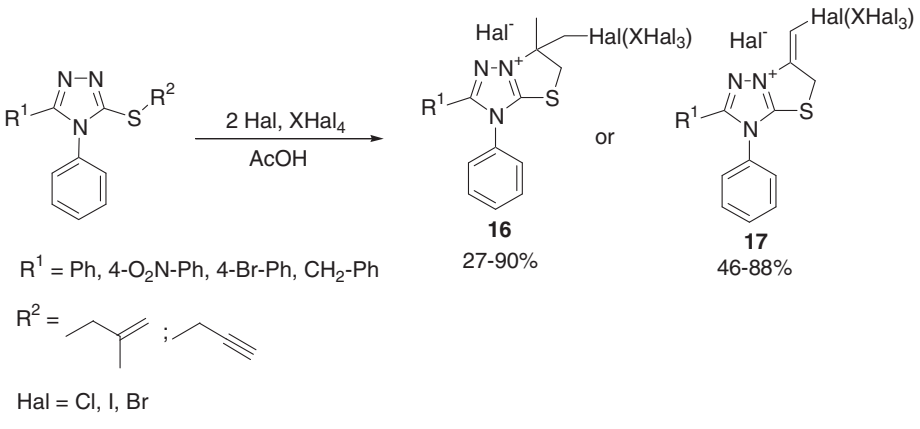
SCHEME 6 Synthesis of 5,6-dihydro-[1,3]thiazolo[3,2-b][1,2,4] triazolium halides **8**



SCHEME 7 Synthesis of triazolothiazoles **10**, **11** and thiazinotriazoles **12**, **13**



SCHEME 8 Synthesis of 6-(dialkoxyphosphoryl)-3*H*-thiazolo [3,2-b][1,2,4]triazole-7-chloro chlorides **14** and zwitterionic saslt **15**



SCHEME 9 Synthesis of thiazolo [3,2-b][1,2,4]triazole-7-ium salts **16**, **17**

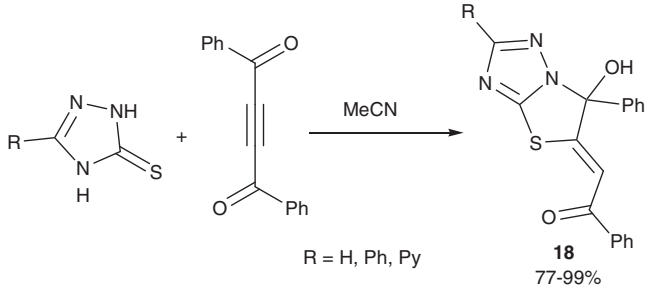
by obtaining saturated thiazolo[3,2-b][1,2,4]triazole-7-ium salts **16**, in the case of methallylthioether, or unsaturated thiazolo[3,2-b][1,2,4]triazole-7-ium salts **17**, in the case of propargylthioether (Scheme 9).[55,56] Two Hydrogen atoms at the fifth position of compounds **11**, **16**, and **17** were found in 1H NMR spectra at 3.95 to 5.10 ppm. In

the 13C NMR spectra, there were signals found at 156.75 to 157.55 ppm for C-2, 159.20 to 162.20 ppm for C-8, and 35.60 to 42.30 ppm for C-5. The XRD study for structure **17** showed the following characteristics: M = 611.00, monoclinic, a = 12.1614(12) Å, b = 7.2386(4) Å, c = 24.0202(17) Å, β = 102.896(8)°, U = 2061.2(3) Å3, T = 293.0, space group $P2\bar{1}$ n (no. 14), Z = 4, μ(Mo Kα) = 7.917, 7981 reflections measured, 4369 unique (Rint = 0.0621), which were used in all calculations.

Shah et al[57] obtained functionalized thiazolo[3,2-b] triazoles **18** via reaction of dibenzoylacetylene and 1,2,4-triazole-3-thione with high yields and without the usage of a catalyst (Scheme 10).

The interaction of 3,5-dibromo-1-(thiiran-2-ylmethyl)-1,2,4-triazole and secondary amines lead to the disclosure of a thiirane cycle with the formation of 1-(3-amino-2-mercaptopropyl)-3,5-dibromo-1,2,4-triazole. Further cyclization of the intermediate, under reflux, in ethanol for 3 hours leads to the formation of 5,6-dihydrothiazolo [3,2-b][1,2,4]triazoles **19**. 2-bromo-5-piperidin-1-ylmethyl-5,6-dihydrothiazolo[3,2-b][1,2,4]triazole can also be obtained with 83% yield in KOH medium by the reaction of 3,5-dibromo-1,2,4-triazole with 1-(thiiran-2-ylmethyl) with piperidine (Scheme 11).[58]

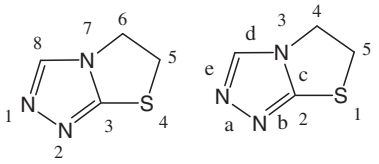
Aryl substituted thiazolo[3,2-b][1,2,4]triazoles **20** were obtained by the action of 4-nitro-1-iodobenzene and triethylamine on 3-mercaptopropargyl-1,2,4-triazoles in the presence of Palladium and Copper salts. Later, the mechanism of this reaction was confirmed by theoretical calculations (Scheme 12).[59,60]



SCHEME 10 Synthesis of thiazolo[3,2-b]triazoles **18**

3 | SYNTHESIS OF THIAZOLO [2,3-C][1,2,4]TRIAZOLE DERIVATIVES

The synthesis of thiazolo[2,3-c][1,2,4,]triazole ring was first reported by Potts et al in 1971.[22] Further investigation of this system increased the interest of a lot of researchers[61–65] and as a consequence, new modified methods of obtaining were developed.



thiazolo[2,3-c][1,2,4]triazole

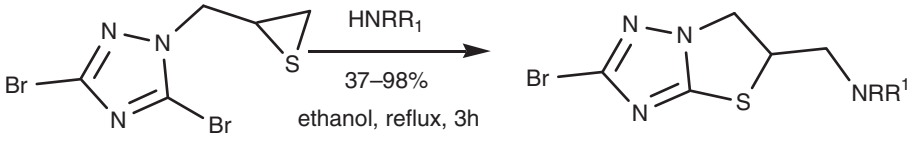
Cyclization of 2-thiazolidinone hydrazone derivatives led to the formation of thiazolo[2,3-c][1,2,4]triazoles **21** derivatives with extremely low yields (6%-8%) and the starting materials were recovered from a mixture (Scheme 13).[66]

Thiazolo[2,3-c][1,2,4]triazole derivatives **22** were obtained from ketones via cyclization reaction with $POCl_3$ in anhydrous xylene (Scheme 14).[67] The singlet at 7.90 to 7.45 ppm for proton at the sixth position of thiazole moiety was found in the NMR spectra of compounds **22**. The mass spectrometry gives results with the molecular ion as the base peak; the signal deriving from the loss of a N_2 molecule [M + −28], whose peak is relivable for the beginning of fragmentation.

Another reaction for the preparation of thiazolo[2,3-c] [1,2,4]triazole derivatives **23** from ketone is described by Sasaki et al,[68] but under other conditions: the action of ammonium acetate in boiling ethanol (Scheme 15).

The preparation of thiazolo[2,3-c][1,2,4]triazoles **24** from 5-substituted 3-mercaptotriazoles via boiling with 1,2-dichloroethane in the presence of sodium bicarbonate as a catalyst is presented by Wang et al[69] (Scheme 16).

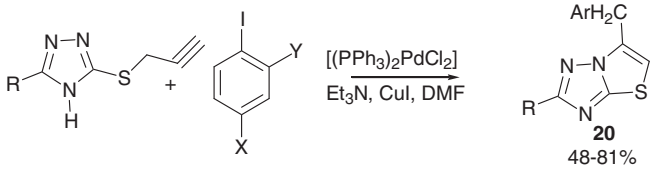
Thiazolo[3,4-b][1,2,4]triazolium salts **25** were obtained by the action of bromine and iodine via the reaction of electrophilic heterocyclization of 3-mercapto-4-alkenyl-1,2,4-triazoles.[70–72] The reaction was carried out in a medium of glacial acetic acid, chloroform, acetonitrile, or ethanol (Scheme 17).



19

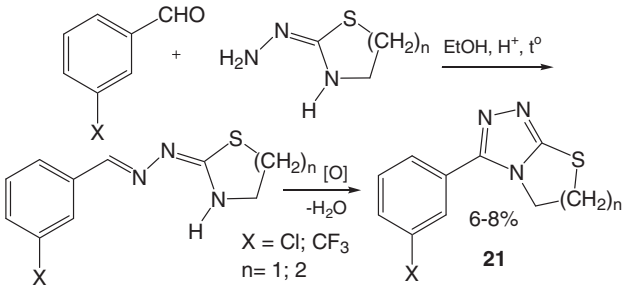
NRR^1 = = BnNH, CyNH, (Et)N, piperidino, azepan-1-yl, PhNH, 4-$EtO_2CC_6H_4NH$

SCHEME 11 Synthesis of thiazolo [3,2-b][1,2,4]triazoles **19**

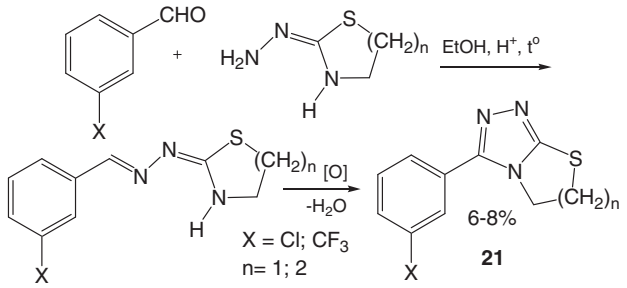


R = CH_3, Ph; X = NO_2, CN, H, Cl; Y = H, NO_2, Cl, CN

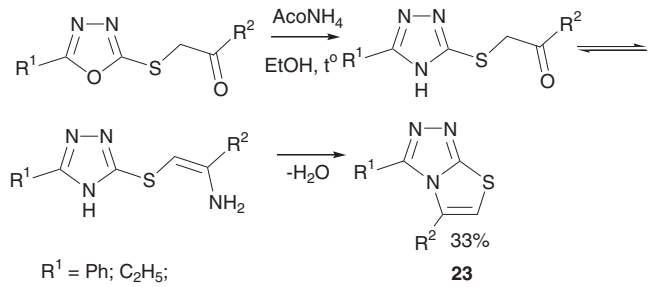
SCHEME 12 Synthesis of thiazolo[3,2-b][1,2,4]triazoles **20**



SCHEME 13 Synthesis of thiazolo[2,3-c][1,2,4]triazoles **21**

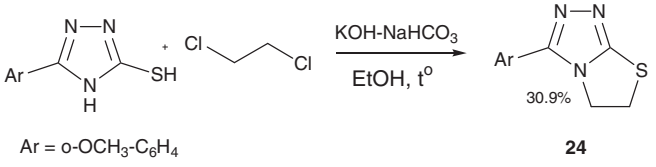


SCHEME 14 Synthesis of thiazolo[2,3-c][1,2,4]triazoles **22**

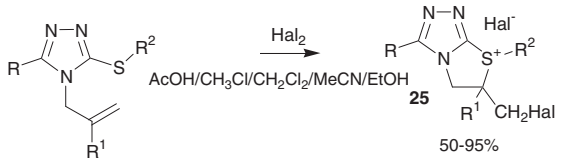


SCHEME 15 Synthesis of thiazolo[2,3-c][1,2,4]triazoles **23**

The investigation of bromination of 4-methallyl-5-methallylamino-1,2,4-triazole-3-thione under mild reaction conditions was reported.[73] As a result, thiazolo [2,3-c][1,2,4]triazol-2-ium bromide **26** was obtained. Further bromination of the exocyclic methallyamino fragment does not lead to cyclization—addition of bromine to the double bond took place (Scheme 18). 1H NMR spectrum of compound **26** contains the following signals

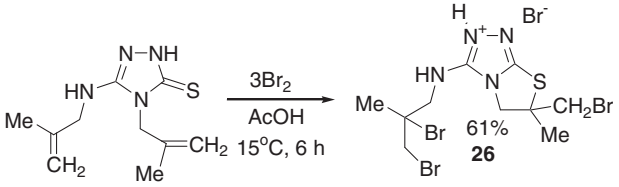


SCHEME 16 Synthesis of thiazolo[2,3-c][1,2,4]triazoles **24**

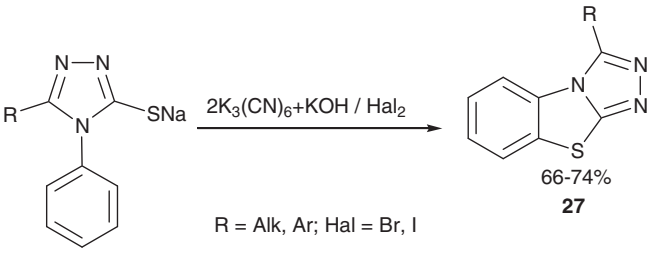


R = 4-O_2N-Ph; Pr, *i*-Bu; Ph; 4-Br-Ph; 2-Br-Ph; 2-MeOPh; N-Allyl ; R^1 = H; CH_3; R^2 = H, Alk, Ar; Hal = Br, I

SCHEME 17 Synthesis of thiazolo[3,4-b][1,2,4]triazolium salts **25**



SCHEME 18 Synthesis of thiazolo[2,3-c][1,2,4]triazol-2-ium bromide **26**

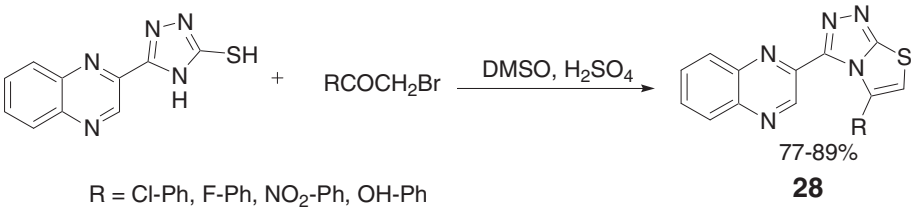


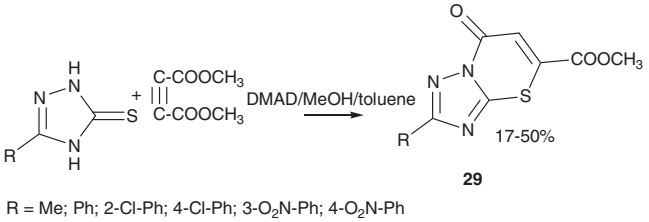
SCHEME 19 Synthesis of 1,2,4-triazolo[3,4-b] benzothiazoles **27**

that belong to thiazolo[2,3-c][1,2,4]triazole moiety: doublet and multiplet in the range 4.09 to 4.50 ppm as Hydrogen signals of CH_2N fragment. 13C NMR spectrum includes the signal of C-6 atom at 53.0 ppm, signal of CH_2N fragment at 67.1 ppm, C-8 atom at 149.9 ppm, and C-3 at 153.5 ppm. Mass spectrum of salt **26** has two signals of molecular ion at 302 $[M(^{79}Br) + H\text{-}HBr]^+$ (100) and 304 $[M(^{81}Br) + H\text{-}HBr]^+$.

The oxidation of sodium salts of 3-mercapto-4-phenyl-1,2,4-triazoles under the action of halogens or potassium hexacyanoferrate was investigated. The reaction was carried out in an alkaline medium and at room temperature. As a result, 3-substituted-1,2,4-triazolo[3,4-b] benzothiazoles **27** were obtained (Scheme 19).[74]

SCHEME 20 Synthesis of thiazolo [2,3-c][1,2,4]triazoles **28**



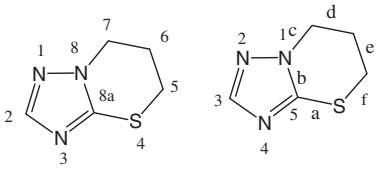


SCHEME 21 Synthesis of [1,2,4]triazolo[5,1-b][1,3]thiazin-7-ones **29**

Almandil et al[75] described the interaction between 5-(quinoxalin-3-yl)-4*H*-1,2,4-triazol-3-thiol and phenacyl bromide derivatives, which resulted in obtaining the target fused compounds **28** (Scheme 20).

4 | SYNTHESIS OF [1,2,4] TRIAZOLO[5,1-B]-[1,3]THIAZINE DERIVATIVES

System of [1,2,4]triazolo[5,1-b][1,3]thiazine was firstly described by Upadhyaya et al[24] in 1978 via the synthetic approach illustrated in Scheme 21. In 1980, Heindel et al and Clayton et al became the next research groups who reported about the methods of production of this fused heterocycle.[76,77]



The most well-known methods for the synthesis of [1,2,4] triazolo[5,1-b][1,3]thiazines are the reactions with acetylenedicarboxylic acid, cyclocondensation with 3-R-propiolic acid derivatives, cyclization with 2-R-propenic acids, and their esters. Thus, Xie et al[78] obtained the fused products of 4,5-dihydro-1*H*-1,2,4-triazole-5-thione with DMAD in methanol, which were identified as 5-methoxycarbonyl-2-phenyl-7*H*-[1,2,4]triazolo[5,1-b] [1,3]thiazin-7-ones **29**, while other researchers[79–81] considered that these compounds have the structure of 7-methoxycarbonyl-3-phenyl[1,2,4]triazolo[3,4-b][1,3] thiazin-5-one. Condensation of 1,2,4-triazole-5-thione with DMAD in toluene gave 5-carbomethoxy-7*H*-1, 2,4-triazolo[3,2-b][1,3]thiazin-7-ones **29** with a yield of 17% to 50% (Scheme 21).

It was shown that the reaction of 3-R-1,2,4-triazoline-5-thione and 3-trifluoroacetyl(perfluorohexanoyl)thiosemicarbazide with methyl 3-phenylpropiolate in acetic acid (ethanol) leads to the formation of a mixture of two isomeric bicyclic compounds—3-R-7-phenyl-5*H*-[1,2,4] triazolo[3,4-b][1,3]thiazin-5-ones **30** and 2-R-5-phenyl [1,2,4]triazolo[5,1-b][1,3]thiazin-7-ones **31**—with a total yield of 61% to 68% in a 10:1 ratio (Scheme 22).[82]

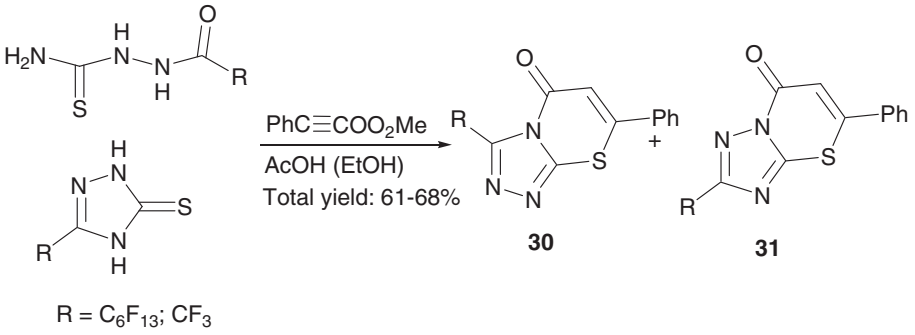
The reaction of acryloyl chloride with 3-R-4, 5-dihydro-1*H*-1,2,4-triazole-5-thione provides a preparative production of 2-R-5-R2-5,6-dihydro-7*H*-[1,2,4] triazolo[5,1-b][1,3]thiazin-7-ones **32** (Scheme 23).[83]

2-R-5-aryl-5,6-dihydro-7*H*-[1,2,4]triazolo[5,1-b][1,3] thiazin-7-ones **33** were obtained by the action of cinnamoyl chlorides on mercaptotriazoles.[84–86] However, the amide bond in the formed condensed compounds is weak and it can be easily hydrolyzed with water to the corresponding 3-aryl-3-(5-thio-1*H*-1,2,4-triazol-5-yl)propionic acids **34** (Scheme 24).[86] In IR spectra of compounds **33**, the characteristic absorption band of C═N group is at 1590 to 1610 cm^{-1}. In the NMR spectra, there are signals of two H^6 atoms at 3.36 to 3.46 ppm and 3.78 to 3.99 ppm as multiplets, multiplet of H^5 at 5.41 to 5.76 ppm and singlet of H^2 at 8.06 to 8.31 ppm.

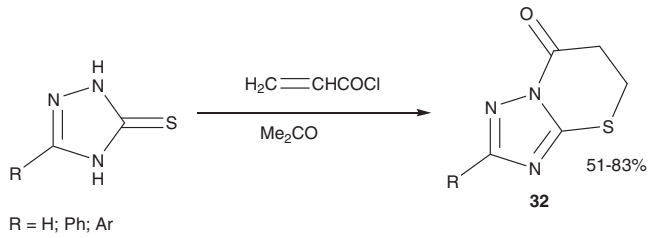
Triazolothiazinones **35** were obtained due to the heterocyclization reaction upon conversion with 3,3-dichloro-2-phenylacryloyl chloride. Two different isomers are formed in equal amounts, depending on which nitrogen atom in the triazole cycle undergoes the cyclization (Scheme 25).[87]

Condensation of equimolar amounts of triazoles and propiolic acid yields adducts identified as S-substituted acrylic acids in high yields (70%-80%). Physical and spectroscopic data of these compounds confirmed the formation of adducts. Regioselective cyclization of propenylmercapto heterocyclic compounds to fused triazoles **36** occurs in the presence of conc. H_2SO_4 for 2 hours at 50°C with yields of 68% to 80% (Scheme 26).[88]

Electrophilic heterocyclization of 4,5-disubstituted thioethers of 1,2,4-triazole leads to the formation of the target thiazinotriazolium salts **37**.[55,89] The action of



SCHEME 22 Synthesis of 3-R-7-phenyl-5*H*-[1,2,4]triazolo[3,4-b][1,3]thiazin-5-ones **30** and 2-R-5-phenyl[1,2,4]triazolo[5,1-b][1,3]thiazin-7-ones **31**

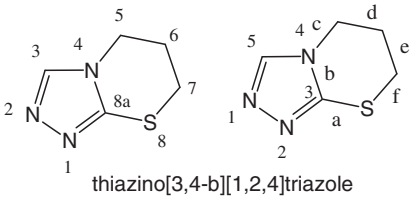


SCHEME 23 Synthesis of [1,2,4]triazolo[5,1-b][1,3]thiazin-7-ones **32**

selenium and tellurium tetrahalides on cinnamyl thioethers does not lead to annulation of the additional cycle, instead molecular complexes **38** were formed (Scheme 27).[55] In the 13C NMR spectra of system **37**, the signal of C-5 atom is at 40 to 42 ppm. In the XDR investigation for salt **37** can be observed in the presence of Cg1-Cg1 and Cg2-Cg2 interactions that are characterized with the lengths of 4.08 and 3.87 Å, respectively.

5 | SYNTHESIS OF [1,2,4] TRIAZOLO[3,4-B]-[1,3]THIAZINE DERIVATIVES

[1,2,4]Triazolo[3,4-b][1,3]thiazine system was first synthesized by Dhaka et al in 1974.[90] This fused compound has not become popular among scientists, so the next study is dated by the year 1994.[91]

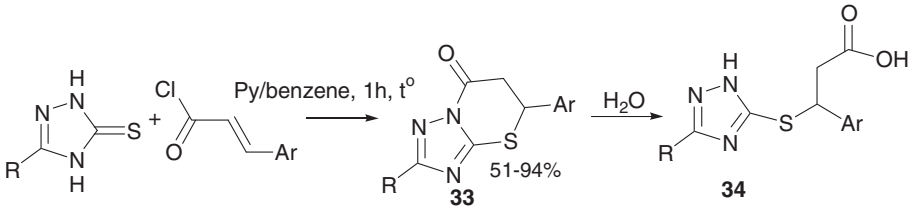


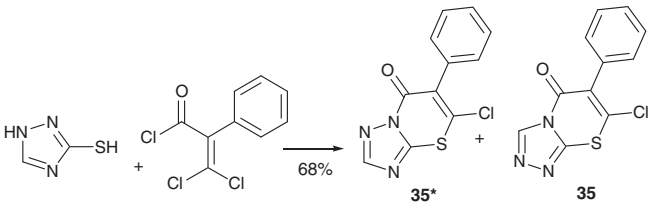
N-Substituted-hydrazinocarbothioamides react with diethyl maleate through the initial stage of S-acylation and subsequent cyclization conjugation to unsaturated ether. Subsequent cyclization results to the formation of a triazole ring further undergoes oxidation with air leading to obtain [1,2,4]triazolo[3,4-b][1,3]thiazine-5-carboxylate 39 (Scheme 28).[92] The H-6 proton of compounds 39 in the 1H NMR spectra appears at 6.82 ppm carbon signals in the 13C NMR spectra, representing the triazolothiazine skeleton at chemical shifts 184.0 ppm (C-7) 158.0 ppm (C-3) 150.8 ppm (C-8a) 131.0 ppm (C-5) and 124.2 ppm (CH-6)

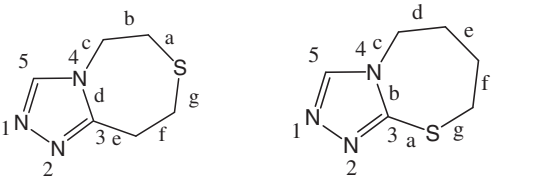
[1,2,4]Triazolo[3,4-b][1,3]thiazine **40** were obtained via the reaction of 5-substituted-1,2,4-triazole-3-thione with epichlorohydrine or with 1,3-dichloro-2 -propanol (Scheme 29).[91]

Thiazino[3,4-b][1,2,4]triazolium salts **41** were obtained by the interaction of 1-(perfluoroheptanoyl) thiosemicarbazide and 5-(perfluorohexyl)triazole-3-thiol with methyl phenylpropanoate in boiling acetic acid or ethanol or through halogenation of unsaturated thio-derivatives of 5-trifluoromethyl-1,2,4-triazole-3-thiol (Scheme 30).[83,93] In the 1H NMR spectrum of triazolothiazines 41, the SCH_2 protons of the thiazine ring were revealed as nonequivalent one-proton doublets of doublets at 3.61 to 3.67 ppm. The mass-spectrum of 41 showed an expected molecular ion peak at m/z = 335, as well as two ion peaks at m/z = 207. These two peaks seemed to be attributed to two isomers formed as a result of the removal of HI from thiazine. Compound **41**, according to its XRD study, is crystallized in the centrosymmetric space group of the monoclinic system. In particular S(1)-C(1) bond (1.804(4) *Å*) bearing aliphatic carbon atom is more than S(1)-C(7) bond (1.736(4) Å) bearing sp^2-hybridised carbon atom. The conformation of tetrahydrothiazine ring can be described as a "pseudo-twist." Atoms S(1), C(7), N(3), and C(3) are located in the plane (deviations <0.011 Å) and atoms C(1) and C(2) are deviated out of the plane by +0.218 and −0.550 Å, respectively. Molecular packing (symmetry: 1 + xy, 1 + z) is characterized by the presence of short intermolecular contacts, which are less by ∼0.15 Å than the sum of van der Waals radii.

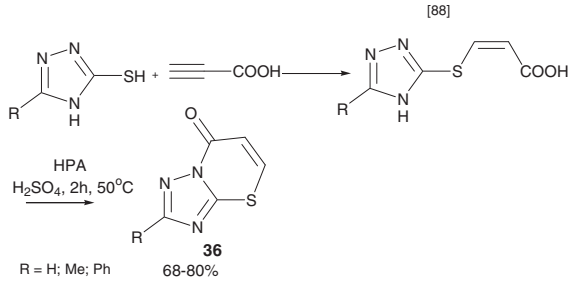
SCHEME 24 Synthesis of [1,2,4] triazolo[5,1-b][1,3]thiazin-7-ones **33**







SCHEME 25 Synthesis of [1,2,4]triazolo[3,4-b][1,3]thiazin-5-ones **35** and [1,2,4]triazolo[5,1-b][1,3]thiazin-7-ones **35***



SCHEME 26 Synthesis of [1,2,4]triazolo[5,1-b][1,3]thiazin-7-ones **36**

The following method for the preparation of [1,2,4] triazolo[3,4-b][1,3]thiazines was presented by the interaction of cinnamoyl-thiosemicarbazide and sodium ethoxide, which led to the formation of fused products **42** (Scheme 31).[94]

The iodination of 4-methallyl-5-methallylamino-1,-2,4-triazole-3-thione goes with unexpected annulation via the sulfur atom of six-membered thiazine cycle to form triazolo[3,4-b][1,3]thiazin-2-ium iodide **43** (Scheme 32).[73]

6 | SYNTHESIS OF TRIAZOLOTHIAZEPINE DERIVATIVES

Triazoothiazepines were first mentioned in 1983 by Polivka et al,[95] and all the known investigations dedicated to these systems are described below.

Dibenzo[b,f]-1,2,4-triazolo[4,3-d][1,4]thiazepines **44** can be easily obtained via interaction of the dibenzo[b,f]-1,4-thiazepine hydrazines with triethylorthoformiate in the presence of sulfuric acid in ethyl alcohol under heating of the reaction mixture (Scheme 33).[95]

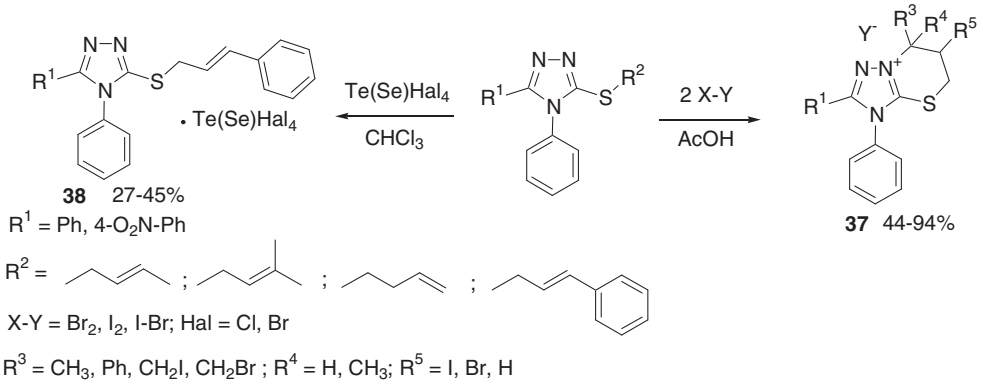
The reaction of 4,6-dichloro-2-methylthiopyrimidine-5-carbaldehyde with 3-substituted 4-amino-1,2,4-triazole-5-thiones at 50°C to 60°C in DMF gave 7-chloro-9-methyl-pyrimido[5,4-f][1,2,4]triazole[3,4-b][1,3,4]thiadiazepines **45**, which were introduced as a new heterocyclic system. Reacting 4,6-dichloro-2-methylthiopyrimidine-5-carbaldehyde with 4-amino-1,2,4-triazole-5-thione at room temperature gave 7-chloro-9-methylthio-5,6-dihydropyrimido[5,4-f][1,2,4] triazolo[3,4-b][1,3,4]-thiadiazepin-6-ol **46** (Scheme 34).[96]

The reaction of 4-anilino-5-phenyl-4*H*-1,2,4-triazole-3-thiol with β-ketoesters yielded triazolothiazepines **47**. The reaction mixture was heated for 1 hour and treated with ethanol (Scheme 35).[97]

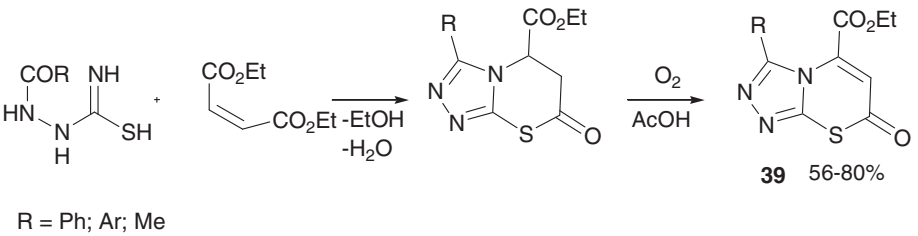
An efficient method for the synthesis of new 1,5-benzothiazepine derivatives by the reaction of 1,3-diaride-2-propenones with 2-amino-thiophenol in the presence of H-zeolite was described by Farghaly.[98] The reaction was carried out using acid catalysis without solvent. 1,3-dipolar cycloaddition of hydrazonoyl chlorides to the C,N bond of 1,5-benzothiazepines in water in the presence of Na_2CO_3/(TEA)THAC as the main catalyst lead to the formation of 1,2,4-triazolo[3,4-d][1,5]benzothiazepines **48** (Scheme 36).[98]

7 | CHEMICAL PROPERTIES OF BICYCLIC 1,2,4-TRIAZOLES WITH ONE EXTRA SULFUR ATOM

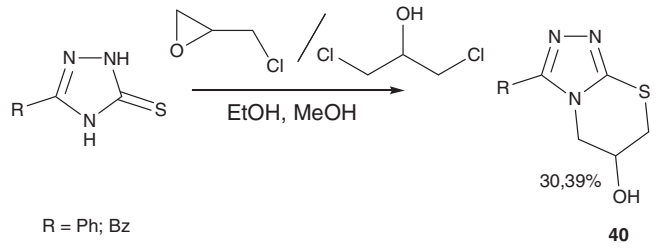
C—H Functionalization of the annulated thiazole cycle is the most investigated chemical transformation of



SCHEME 27 Synthesis of thiazinotriazolium salts **37**



SCHEME 28 Synthesis of [1,2,4]triazolo[3,4-b][1,3]thiazines **39**



SCHEME 29 Synthesis of [1,2,4]triazolo[3,4-b][1,3] thiazines **40**

condensed thiazolo[3,2-b][1,2,4]triazoles. Effective arylation by Pd-, Cs-, Ru-, Cu of thiazolo[3,2-b][1,2,4] triazoles with aryl halides[99–103] or sodium salt of arylsulfonic acid[104] was carried out under different conditions. These techniques have been successfully applied to the synthesis of 5-substituted thiazolo[3,2-b][1,2,4] triazoles **49** in high yields (Scheme 37).

The method for direct regioselective phosphonation of thiazolo[3,2-b][1,2,4]triazoles with dialkylphosphites using an Ag catalyst was developed by obtaining the derivatives **50** (Scheme 38).[105]

The efficient and regioselective oxidation reaction using a palladium catalyst for cross-linking between 2,5-unsubstituted thiazolo[3,2-b][1,2,4]triazoles and alkenes has been developed and provides easy access to functionalization of 5-alkenylsubstituted thiazolo[3,2-b] [1,2,4]triazoles **51** (Scheme 39).[106]

For obtaining a variety of 5-sulfonylated thiazolo [3,2-b][1,2,4]triazoles **52**, the simple and regioselective strategy was developed by direct sulfonylation with thiols in the presence of a CuI catalyst (Scheme 40).[107]

The synthesis of 2-substituted thiazolo[3,2-b][1,2,4] triazoles **53** was carried out without the usage of copper. The palladium-catalyzed Sonogashira reaction of 6-(iodomethyl)-2-methyl-thiazolo[3,2-b][1,2,4]triazoles with terminal alkynes was provided in DMF at 70°C (Scheme 41).[108]

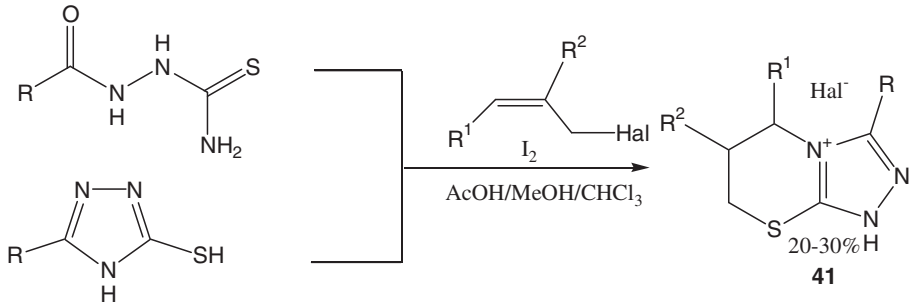
The reaction between C-5 lithium thiazolo[3,2-b] [1,2,4]triazoles and enantiomerically pure (S,S)-*N*-tert-butanesulfinyl-3,3,3-trifluoroacetaldimines leads to the formation of thiazolo[3,2-b][1,2,4]triazoles **54** with chiral (trifluoro)-ethylamine at position 5 (Scheme 42).[109,110]

Functionalization of the salts under the influence of nucleophiles and temperature was investigated.[70] Thermal cleavage was carried out by heating of the corresponding salts in ethanol for 2 hours. As a result, the products **55** were obtained (Scheme 43).

The interaction between salts **16** and O-nucleophiles (water solution of NaOH, Na_2CO_3, Na_2SO_3) at room temperature was studied (Scheme 44). The cleavage of thiazoline moiety with the formation of poly-functional symmetric triazoles **56** was observed in all cases, the usage of more concentrated solutions of reagents and longer reaction time leads to the formation of elimination product **57**.[111]

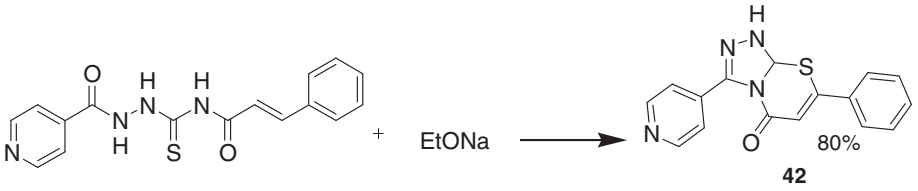
The heating of the thiazolotriazolium salt in ethanol leads to the destruction of thiazole ring with the formation of dibromide **58** (Scheme 45).[111]

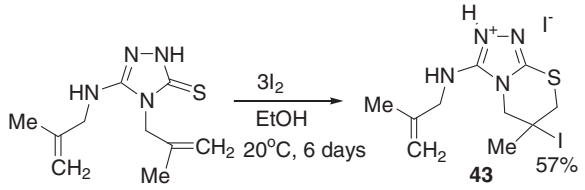
SCHEME 30 Synthesis of thiazinotriazolium salts **41**



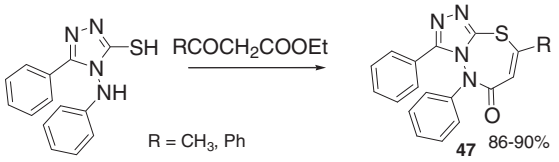
R = C_6F_{13}; CF_3; R^1 = Ph, Me, CH_2Hal; R^2 = Me,H, Hal; Hal = Br, I

SCHEME 31 Synthesis of [1,2,4] triazolo[3,4-b][1,3]thiazines **42**

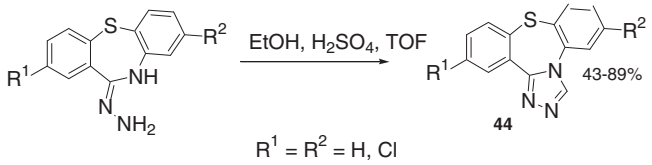




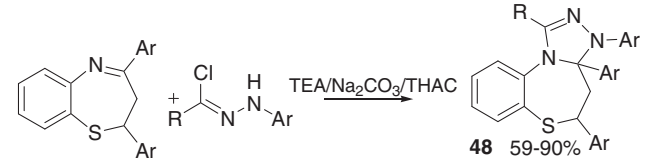
SCHEME 32 Synthesis of thiazinotriazolium salts **43**



SCHEME 35 Synthesis of triazolothiazepines **47**

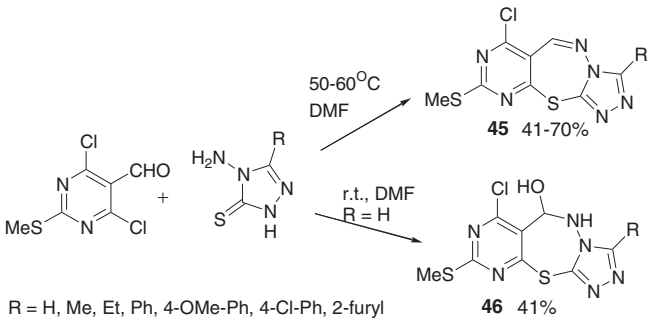


SCHEME 33 Synthesis of 1,2,4-triazolo[4,3-d][1,4] thiazepines **44**

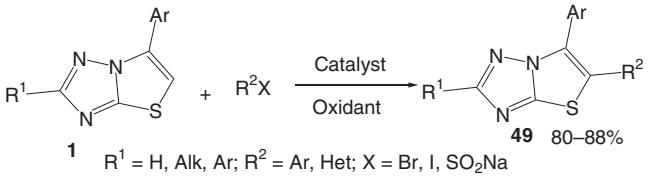


R= Ph, COOEt; Ar = Ph, 4-F-Ph, 4-Cl-Ph, 4-NO_2-Ph, 4-Br-Ph

SCHEME 36 Synthesis of 1,2,4-triazolo[3,4-d][1,5]-benzothiazepines **48**



SCHEME 34 Synthesis of triazolothiazepines **45**, **46**

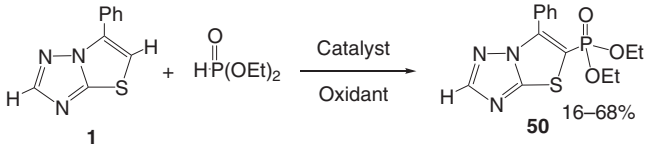


SCHEME 37 5-C Arylation of thiazolo[3,2-b][1,2,4]triazoles **1**

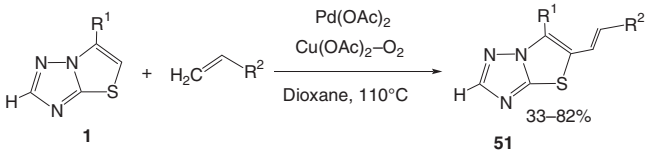
The elimination of hydrobromic acid from thiazolotriazolium moiety was performed in an acetic acid medium with sodium acetate. Moreover, a multiple bond is formed near the thiazoline cycle, and the

bromomethyl group of pyrimidine cycle is inactive under descriptive conditions (Scheme 46).[72]

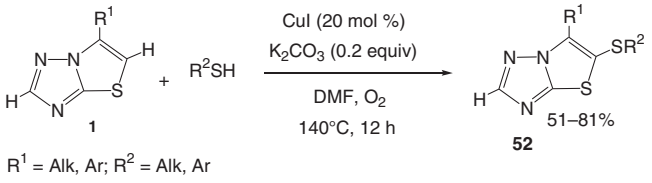
The elimination of iodine was developed under the action of alkaline alcohol solution. As a result—in the case of triazolothiazoles one product **59**, **60** was obtained and in the case of triazolothiazines—two isomers **61-64** were isolated (Scheme 47).[53]



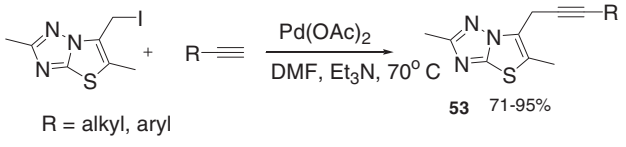
SCHEME 38 5-C Phosphonation of thiazolo[3,2-b][1,2,4] triazoles **1**



SCHEME 39 5-C Alkenylation of thiazolo[3,2-b][1,2,4] triazoles **1**



SCHEME 40 5-C Sulfonylation of thiazolo[3,2-b][1,2,4] triazoles **1**



SCHEME 41 Synthesis of thiazolo[3,2-b][1,2,4]triazoles **53**

8 | BIOLOGICAL ACTIVITY OF FUSED TRIAZOLES

The development of effective routes for obtaining new fused triazoles is relevant because of their wide range of bioactivity and as a consequence—great perspective for usage. Obtained compounds exhibit fungicidal,[26,37,39,46–48] antimicrobial,[26,37–39,46–48,56] analgesic,[27,34,42–44] antinociceptive,[28] ulcerogenic,[28,43] antioxidant,[27,39] anti-inflammatory,[27–29,34,42–44] and anticancer[41] activity. Thiazolo[3,2-b][1,2,4]triazoles have the ability to act as a urease inhibitor,[36] stabilizers of G-quadruplex[32] and may be used as cationic surfactants.[112]

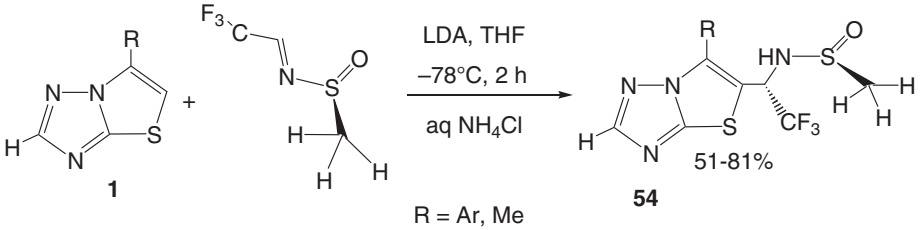
On the other hand, the biological evaluations have not been performed for other classes of condensed triazoles. For example, there is no data on the bioactivity of triazolothiazines and triazolothiazepine. Thus, if taken into account the excellent properties of triazolothiazoles, other representatives need further investigations.

8.1 | Fungicidal activity

The antifungal activity of structure **1** was tested against yeast strains (*Candida albicans*, *Candida parapsilosis*, *Candida glabrata* and *Candida tropicalis*). The results of the antifungal screening indicated insignificant behaviors between these classes of the tested compounds. The best antifungal activity against *C. glabrata fungus* and *C. albicans* was MIC = 64 μg/mL.[26]

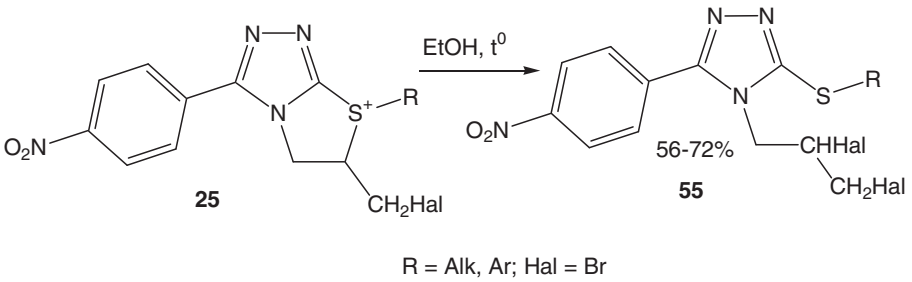
Compounds of **1** have been evaluated for their antifungal activities against bacterial species including four standard fungi (*Candida albicans*, *Candida tropicalis*, *Aspergillus niger*, and *Aspergillus flavus*). Some representatives showed good activity with the minimal inhibitory concentration in the range of 100 to 200 mg/mL.[37]

The antifungal activity of structure **4** was investigated.[47] For antifungals, *Candida albicans*, *Geotrichum candidum*, *Aspergillus flavus*, *Trichophyton rubrum*, *Scopulariopsis bervicaulisand Fusarium oxysporum* were used. All the thiazolo[3,2-b][1,2,4]triazole derivatives exhibited high activity against *Geotrichum candidum*. The inhibition zone is about 11 to 12 mm in each case.

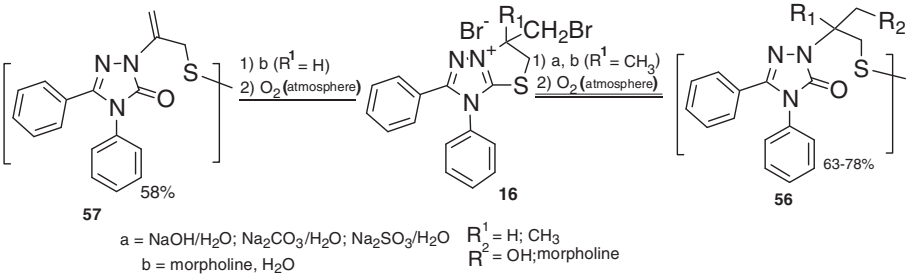


SCHEME 42 Synthesis of thiazolo [3,2-b][1,2,4]triazolyl trifluoroacetaldimines **54**

SCHEME 43 Thermal cleavage of salts **25**



SCHEME 44 The action of nucleophiles on salt **16**



N-aryl-2-(6-oxo-5,6-dihydro[1,3]thiazolo[3,2-b][1,2,4] triazole-5-yl)acetimides **5** were screened for their antifungal activity against *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium marneffei* and *Trichophyton mentagrophytes*.[48] According to the results, all tested compounds are active against each bacteria, inhibition zone was in the frame of 11 to 21 mm and only a few results illustrated low activity—less than 10 mm.

All tested compounds **3** showed antifungal effect with MIC in the range of 3.67 to $34.6*10^{-2}$ μmol/mL and MFC in the range of 7.35 to $39.6*10^{-2}$ μmol/mL.[46]

Compounds of **2** were tested for their antifungal activity against *Aspergillus oryzae* and *Aspergillus niger*. Results showed moderate activity with the inhibition zone in the range of 9 to 13 mm.[39]

8.2 | Antimicrobial activity

The antimicrobial activity of the investigated compounds **1** was tested against some reference bacterial belonging to the following species: Gram-positive strains (*Staphylococcus aureus* and *Bacillus cereus*), Gram-negative strains (*Escherichia coli*, *Enterobacter cloacae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*), using the broth microdilution method. The investigation showed good activity according to the measured minimum inhibitory concentration (MIC) in the range of 8 to 32 μg/mL.[26]

For the investigation of antimicrobial activity of the derivatives of **1**, Gram-positive (*Staphylococcus aureus*, *Bacillus pumilis*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria were taken.[37] The results revealed that all the synthesized compounds displayed variable inhibitory effects on the growth of the tested bacteria, with moderate to high activities.

The antibacterial activity of the compounds of **1** was determined against Gram-negative bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Salmonella tuhii*, and one Gram-positive bacteria, *Staphylococcus aureus*.[38] All the compounds were found to show moderate to strong activity against the Gram-negative bacteria. Against Gram-positive, there was moderate activity of some representatives.

The newly synthesized compounds of **5** were screened for their antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Measured inhibition zone is between 11 and 30 mm in the case of each tested compound.[48]

For antibacterial studies of structure **4** following microorganisms were employed: *Serratia marcescens*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*.[47] Tested thiazolotriazoles exhibited high activity against *Serratia marcescens* and *Escherichia coli* was indicated by measured inhibition zone.

Compounds **17** exhibited moderate activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*, slight activity against *Escherichia coli* and *Candida albicans* that were reported in the study.[56]

Series of novel 5-benzylideno-2-adamantylthiazol [3,2-b][1,2,4]triazol-6(5*H*)ones **3** were synthesized and evaluated in vitro for their antimicrobial properties

against Gram-positive and Gram-negative bacteria. Almost all the tested compounds showed antibacterial activity but on different levels and this activity was even better than that of Streptomycin against *L. monocytogenes*, and *E. coli*.[46]

The results of the antimicrobial activity evaluations reveals that substances **2** exhibited maximum inhibitory activity against *Pseudomonas aeruginosa* with the inhibition zone at 6 to 13 mm.[39]

8.3 | Analgesic activity

The analgesic activity of the compounds was studied using the acetic acid-induced writhing test in mice and expressed as "mean increase in latency after drug administration ± SEM" relative to the control and percentage inhibition in the writhing reflex. Compounds of **1** attracted attention with higher analgesic activity than aspirin with percentage inhibition values of 62.8% to 89.7% at 100 mg/kg dose level.[27]

In the study,[34] analgesic activity was carried out by acetic acid-induced writhing method in albino mice. Compounds of **1** showed analgesic activity ranging from 26.90% to 56.76% protection, which was compared with that of standard drug ibuprofen (56.20%).

Acetic acid writhing test was performed on mice, and mefenamic acid was used as the standard drug. Test compounds were injected ip to the animals at a dose of 30 mg/kg as suspension in saline and Tween 80 (4% wt/vol) 30 minutes after the animals were injected ip with acetic acid (0.6%, 0.1 mL/10 g). All the compounds of **3** induced significant reduction in the writhing response in comparison with the control, and all of them were more effective than mefenamic acid. The best compound **9d** was 2.4 times more effective than mefenamic acid.[42]

For the investigation of analgesic activity of structure **3** in the study[43] two types of tests were performed: hot plate and tail-flick tests. Oral administration of all synthesized derivatives produced markedly increased latency of the tail-flick response compared with vehicle control at the 50 mg/kg dose level. All synthesized derivatives of compounds of **3** produced significant inhibition of the hot plate paw-licking response compared with the vehicle control at a dose of 50 mg/kg.

When performing the tail-flick test, all the tested condensed derivatives of **3** exhibited moderate to high analgesic activity compared with the control group in the tail-flick test. The analgesic activity of the compounds was in the range of 11% to 52% at the 50 mg/kg dose. Compounds with favorable analgesic properties in the tail-flick test showed also favorable analgesic profiles in the hot plate. At the dose of 50 mg/kg, compounds of **3** were found to be more efficacious than naproxen and indomethacin.[44]

8.4 | Antioxidant activity

Compounds of **1** were also analyzed for their antioxidant properties by determining the lipid peroxidation level. The lipid peroxidation is measured as nmol of TBARS/g wet weight of tissue. The obtained lipid peroxidation values revealed that the effect of compounds of **1** was appreciably less than aspirin. Only one representative, exhibited both noteworthy anti-inflammatory and analgesic activities, was found relatively safer for ulcerogenic risk than the other compounds.[27]

Thiazolo[3,2,-b][1,2,4]triazoles derivative **2** showed high DPPH scavenging activity with percent inhibition of 78.85 at a concentration of 75 μg/mL. This increased activity may be due to the existence of the CH2CO group. The presence of cyano group was inadequate for the antioxidant activity.[39]

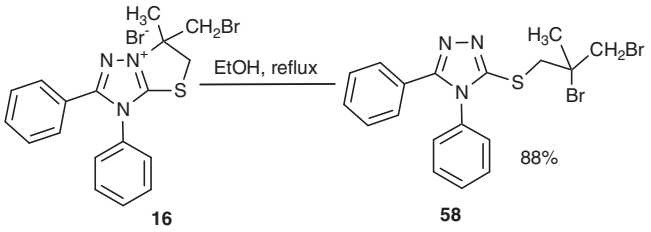
8.5 | Anti-inflammatory activity

The anti-inflammatory activity of the derivatives of **1** was assessed from their ability to inhibit the paw edema induced by carrageenan in mice, and activity was expressed as "mean increase in paw volume ± SEM," in terms of mm and percentage inhibition in paw volume by different doses of the compounds. Compounds of **1** possessed moderate-to-good anti-inflammatory activity at 100 mg/kg dose/p.o. in any of the measurement intervals.[27]

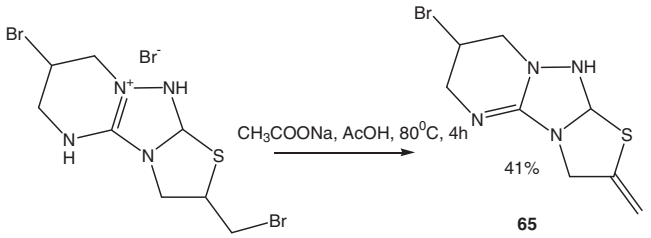
All the synthesized compounds of **1** and diclofenac, as reference drug, were tested for their in vivo anti-inflammatory activity by modified carrageenan-induced rat paw oedema model. The results of anti-inflammatory activities against carrageenan-induced paw oedema showed that compounds of **1** reduced carrageenan-induced paw oedema when administered with a dose of 50 mg/kg bw, p.o., compared with the negative control group. Diclofenac (20 mg/kg bw, p.o.), a nonselective NSAID used as reference drug, significantly reduced paw volumes at all periods of time, compared with the negative control group.[28]

The anti-inflammatory activity of **1** was evaluated by using the carrageenan-induced rat paw oedema assay, according to the method of Winter, modified by introducing a Ugo Basile plethysmometer. The reference drug used was diclofenac. The anti-inflammatory activity was evaluated hourly, from 1 to 4 hours after the

administration of carrageenan. The results showed that pyridin-3/4-yl-1,2,4-triazol S-alkylated derivatives proved to have better anti-inflammatory activity than diclofenac



SCHEME 45 Thermal cleavage of salt **16**

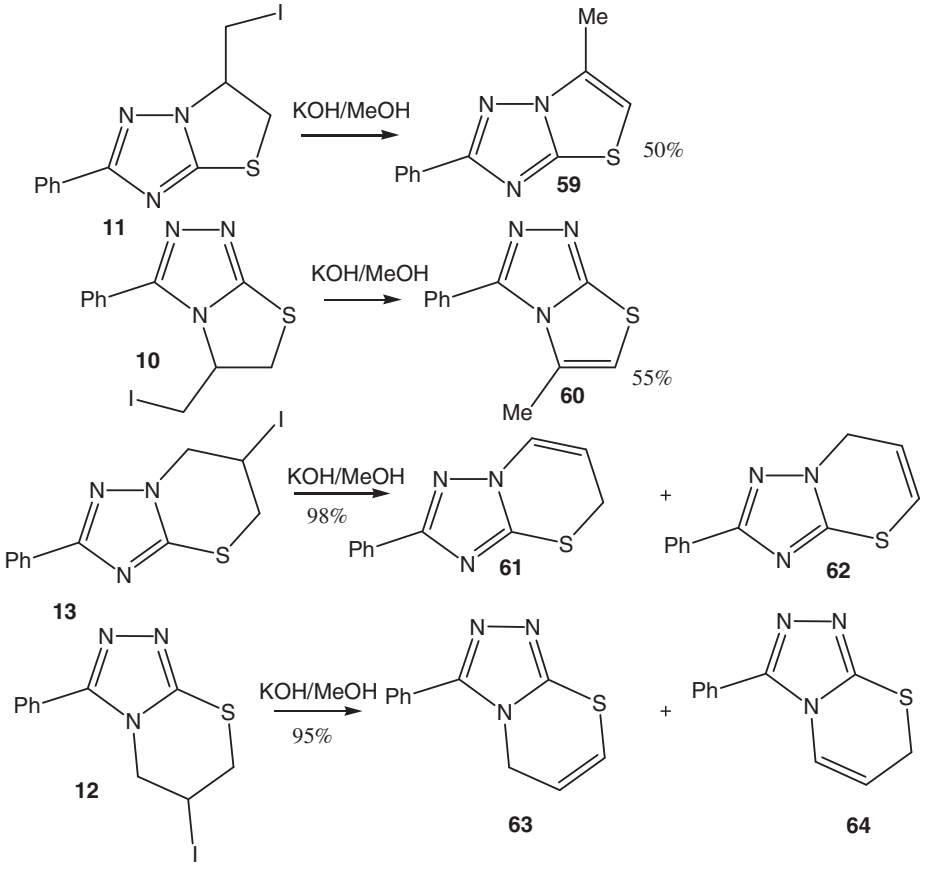


SCHEME 46 Hydrobromic acid elimination

after 1 hour from the induction of the inflammation, but the activity decreased considerably after 2, 3, and 4 hours. The thiazolo[3,2-b][1,2,4]triazole derivatives of **1** presented a higher anti-inflammatory activity than diclofenac after 1 and 2 hours (% oedema inhibition: 50.28%-63.56%). Several decrease in the anti-inflammatory activity was observed after 3 hours. The activity increased back to more than 44.99% oedema inhibition after 4 hours.[29]

The synthesized compounds were evaluated for their anti-inflammatory activity following carrageenan-induced rat paw edema method in the study.[12] The compounds of **1** showed anti-inflammatory activity ranging from 47.67% to 90.83% inhibition at 4 hours of carrageenan injection, which was compared with the standard drug, ibuprofen (93.57%). Among the screened compounds, only three representatives showed highest anti-inflammatory activity with 90.83%, 85.81%, and 88.40% protection, respectively. Rest of the compounds were moderate in their action.[34]

Thiazolo[3,2-b][1,2,4]triazole-6(5*H*)-ones **3** with 2-phenoxyphenyl and different aryliden and heteroaryliden moieties were synthesized and their anti-inflammatory activities were screened in vivo by writhing and carrageenan-induced rat paw edema tests. Results revealed that most of them were more potent analgesic



SCHEME 47 Dehydrohalogenation of fused triazoles **10**-**13**

agents than mefenamic acid and most of the final products showed significant anti-inflammatory activity in comparison to control, and, in some cases, the pharmacological properties of the compounds of **3** were comparable or better than indomethacin and mefenamic acid as the reference drugs.[42]

The results of the anti-edematous effect of orally administered test compounds of **3** on carrageenan-induced paw edema in mice at 50 and 100 mg/kg doses. In the oral administration of a 50 mg/kg dose, almost all compounds showed moderate to high anti-inflammatory activity, ranging from 30% to 56%, while ibuprofen, indomethacin, and oxycodone references showed 55%, 53%, and 1% of anti-inflammatory activity, respectively.[43]

Compounds of **3** were tested for their in vivo anti-inflammatory activity by modified carrageenan-induced paw edema model. All of the compounds tested showed marked inhibition of carrageenan-induced paw edema as a measure of anti-inflammatory activity. Following oral administration of a 50 mg/kg dose, compounds of 37% to 51% produced significant inhibition of edema. Under the same conditions, naproxen and indomethacin, which are well known nonselective COX inhibitors, showed 49% and 51% of anti-inflammatory activity, respectively. When a 2-fold dose (100 mg/kg) was administered, the anti-inflammatory properties of the compounds of **3** were observed to be favorable.[44]

8.6 | Antinociceptive activity

The antinociceptive activity of the compounds of **1** in a model of inflammatory pain was evaluated by Randall-Selitto test. The oral administration of the tested compounds, in doses of 50 mg/kg bw, produced a good increase of nociceptive threshold when compared with the negative control group. Some compounds showed a significant increase of nociceptive threshold when compared with diclofenac, as a reference drug.[28]

8.7 | Ulcerogenic activity

The compounds of **1** were evaluated for their ulcerogenic activity after a single oral administration of 50 mg/kg bw in rats. The gastric mucosa of rats was examined for irritations, ulcerations, and microhemorrhages by a magnifier. The evaluation of gastric mucosal lesions was according to their number and size, on a scale from 0.5 to 3.0 points. A significant reduction in ulcerogenic activity with the stomach mucosal ulceration score between 0.08 ± 0.08 and 0.50 ± 0.12 was observed for the tested compounds. However, none of the synthesized compounds presented significant ulceration when compared with the diclofenac group.[28]

The stomachs of mice were examined for lesions in gastric mucosa by using a dissecting microscope. The quantification of gastric mucosal lesions was scored according to their number and size on a scale from 0 up to 7 points. In spite of the high gastric ulcer incidence in reference compounds, ibuprofen (50 and 100 mg/kg doses) and indomethacin (10 and 50 mg/kg doses), all of the test compounds of **3** were generally found safe from the viewpoint of ulcer induction at both 50 and 100 mg/kg dose levels. Particularly, the ulcerogenic effect of the compounds of **3** was appreciably lesser than ibuprofen ($P < .01$) and indomethacin in both doses.[43]

8.8 | Anticancer activity

Primary anticancer assay was performed in the 3-cell line panel, consisting of NCI-H460 (lung), MCF7 (breast), and SF-268 (CNS), in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda. Tested compounds were added to the culture at a single concentration (10^{-4} M) and the cultures were incubated for 48 hours. Compounds of **2** were consequently selected for in vitro testing against the full panel of nearly 60 cell lines. The synthesized *N*-(R-phenyl)-(6-oxo-5,6-dihydro[1,3]thiazol[3,2-b][1,2,4]triazol-5-yl) acetamides displayed moderate activity in the in vitro screen on renal cancer, leukemia, colon cancer, breast cancer, and melanoma cell lines but low activity on CNS cancer, ovarian cancer, and prostate cancer cell lines. It is noteworthy that there is observed selective influence of compounds on some cancer cell lines, depending on the nature of the substituent R in the phenylacetamide fragment. The unsubstituted derivative was highly active on a non-small cell lung cancer (NCI-H23, log GI_{50} value −6.94), colon cancer (SW-620, log GI_{50} value −5.49) and breast cancer (BT-549, log GI_{50} value −6.47). And also the compounds of **2** with R = Br,COOEt were highly active on breast cancer (HS 578 T, log GI_{50} value −5.14) and leukemia (RPMI-8226, log GI_{50} value −5.34) cells, respectively. 5-Ylidene-[1,3]thiazolo[3,2-b][1,2,4]triazol-6-ones have shown more potent anticancer activity than amides. Therefore, the compound with Ar = *p*-Cl-C_6H_4 was highly active on all cell lines, especially on leukemia (for all lines log GI_{50} value less than −5.00), and exchange of the chlorine atom in 5-arylidene moiety for fluorine gives the decrease of activity against most of the cancer cell lines, besides leukemia (HL-60 [TB], log GI_{50} value −5.19).[41]

8.9 | G-quadruplex stabilizers

Compounds of **1** were evaluated for their ability to bind and stabilize a telomeric G-quadruplex structure by a fluorescence resonance energy transfer (FRET) assay using the 21-mer d[(GGGTTA)3GGG] oligonucleotide end-labeled with a fluorescent donor-acceptor pair (F21T). The change in F21T emission in the presence of the tested compounds (3 lM) was monitored as a function of temperature whether it be in Na + (NaCl 100 mM) or K+ (KCl 10 mM; LiCl 90 mM) conditions. The resulting DTm values provide an indication of the stability of a ligand-quadruplex complex and are summarized for Na + and K+. The results indicate that the compounds of **1** induce a significant stabilization of the telomeric G-quadruplex (DTm >1 C) in both Na + and K+ conditions.[32]

8.10 | Urease inhibitors

The hybrid skeleton incorporating coumarin and thiazolotriazole heterocycles of **1** were probed for their urease inhibition potential. The molecular docking analysis revealed several important binding interactions between the leading compound and amino acid residues in the active site. The amino functionality present at the *meta*-position of the aryl ring interacts with the sulfhydryl group of Cys322 (2.11 Å) and carbonyl oxygen of Ala366 (2.75 Å). Other notable interactions stabilizing the chromenone moiety include Ala279, Thr301, Pro303, Thr304, His315, and Met367. A comprehensive survey of substituents present at various positions was also considered and their influence on the anti-urease potential was comprehended with the binding interactions. The structurally diversified hybrid motifs were identified as a new class of urease inhibitors.[36]

8.11 | Toxicity

Predicted LD_{50} (acute oral rat toxicity) and LC_{50} (fathead minnow after 96 hours exposure) values indicate that toxicity of tested heterocyclic compounds of **16** is close to the toxicity of common popular cationic surfactants.[112] The presence of alkyl chains (pentadecyl or hexyl) and positively charged triazolium ring correspond structural requirements to cationic surfactants—the presence of "non-polar tail" and "polar head," respectively.

9 | CONCLUSIONS

Analysis of the investigations on the synthesis of condensed bicyclic 1,2,4-triazoles with one extra sulfur atom shows that the study of these systems has been investigated in many directions and is promising in view of the bioactivity of their individual derivatives. The most effective and universal synthetic approaches to obtaining titled fused triazoles were described: condensation reactions and annulation of multiple bonds. It has been noticed that there is a lack of data on efficient and easy-to-use methods for producing fused triazoles with a charged heteroatom, especially the regioselectivity of their preparation. The chemical properties of unsaturated and partially saturated heterocycle annulated to triazole ring have been described on the thiazolotriazole system: it was shown the high reactivity of 5-C-H center in thiazolo[3,2-b][1,2,4]triazoles; also considered their thermal stability and interaction with nucleophiles. Because of the growing interest of researchers to fused triazoles, their valuable characteristics on bioactivity and increasing of branches of usage of these systems ask for further investigation.

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REFERENCES

[1] K. Bozorov, J. Zhao, H. A. Aisa, *Bioorg. Med. Chem.* **2019**, *27*, 3511.
[2] E. Bonandi, M. S. Christodoulou, G. Fumagalli, D. Perdiccia, G. Rastelli, D. Passarella, *Drug Discov. Today* **2017**, *22*, 1572.
[3] J. S. S. Neto, G. Zeni, *Coord. Chem. Rev.* **2020**, *409*, 213217.
[4] S. Mahonar, S. I. Khan, D. S. Rawat, *Chem. Biol. Drug Des.* **2011**, *78*, 124.
[5] S. B. Ferreira, A. C. R. Sodero, M. F. S. Cardoso, E. S. Lima, C. R. Kaiser, F. P. Silva, V. F. Ferreira, *J. Med. Chem.* **2010**, *53*, 2364.
[6] V. F. Ferreira, D. R. da Rocha, F. C. da Silva, P. G. Ferreira, N. A. Boechat, J. L. Magalhaes, *Expert Opin. Ther. Pat.* **2013**, *23*, 319.
[7] B. Zhang, *Eur. J. Med. Chem.* **2019**, *168*, 357.
[8] J. Huo, H. Hu, M. Zhang, X. Hu, M. Chen, D. Chen, J. Liu, G. Xiao, Y. Wang, Z. Wen, *RSC Adv.* **2017**, *7*, 2281.
[9] P. I. P. Elliot, *Organomet. Chem.* **2014**, *39*, 1.
[10] D. Huang, P. Zhao, D. Astruc, *Coord. Chem. Rev.* **2014**, *272*, 145.
[11] A. Al-Masoudi, Y. A. Al-Soud, N. J. Al-Salihi, N. A. Al-Masoudi, *Chem. Heterocycl. Compd.* **2006**, *42*, 1377.
[12] N. Singhal, P. K. Sharma, R. Dudhe, N. Kumar, *J. Chem. Pharm. Res.* **2011**, *3*, 126.
[13] M. S. Saini, J. Dwivedi, *IJPSR* **2013**, *4*, 2866.
[14] R. Sathyanarayana, B. Poojary, *J. Chin. Chem. Soc.* **2020**, *67*, 459.
[15] M.-X. Song, X.-Q. Deng, *J. Enzyme Inhib. Med. Chem.* **2018**, *33*, 453.
[16] N. P. Swathi, V. D. P. Alva, S. Samshuddin, *J. Bio Tribo Corros.* **2017**, *3*, 42.
[17] S. G. Küçükgüzel, P. Çıkla-Süzgün, *Eur. J. Med. Chem.* **2015**, *97*, 830.

[18] K. Oukoloff, B. Lucero, K. R. Francisco, K. R. Brunden, C. Ballatore, *Eur. J. Med. Chem.* **2019**, *165*, 332.

[19] P. K. Singh, S. Choudhary, A. Kashyap, H. Verma, S. Kapil, M. Kumar, M. Arora, O. Silakari, *Bioorg. Chem.* **2019**, *88*, 102919.

[20] N. Korol, M. Slivka, *Chem. Heterocycl. Compd.* **2017**, *53*, 852.

[21] V. W. Dymek, B. Janik, A. Cygankiewicz, H. Gawron, *Acta Pol. Pharm.* **1967**, *24*, 97.

[22] K. T. Potts, S. Husain, *J. Org. Chem.* **1971**, *36*, 10.

[23] K. Pilgram, G. E. Pollard, *J. Heterocyclic Chem.* **1976**, *13*, 1225.

[24] V. P. Upadhyaya, T. G. Surendra Nath, V. R. Srinivasan, *Synthesis* **1978**, *4*, 288.

[25] A. Mignot, H. Moskowitz, M. Miocque, *Synthesis* **1979**, *1*, 52.

[26] S. Barbuceanu, C. Draghici, F. Barbuceanu, G. Bancescu, G. Saramet, *Chem. Pharm. Bull.* **2015**, *63*, 694.

[27] B. Tozkoparan, S. Aytaç, S. Gürsoy, G. Aktay, *Med. Chem. Res.* **2010**, *21*, 192.

[28] A. Cristina, *Farmacia* **2018**, *66*, 883.

[29] A. Toma, C. Mogoşan, L. Vlase, D. Leonte, V. Zaharia, *Med. Chem. Res.* **2017**, *26*, 2602.

[30] A. Cristina, D. Leonte, L. Vlase, L. C. Bencze, S. Imre, V. Zaharia, *Farmacia* **2018**, *66*, 88.

[31] V. Zaharia, A. Silvestru, N. Palibroda, C. Mogosan, *Farmacia* **2011**, *59*, 624.

[32] J. El Bakali, F. Klupsch, A. Guédin, B. Brassart, G. Fontaine, A. Farce, P. Roussel, R. Houssin, J. Bernier, P. Chavatte, J. Mergny, J. Riou, J. Hénichart, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3434.

[33] S. Holota, Y. Shylych, H. Derkach, O. Karpenko, A. Gzella, R. Lesyk, *Molbank* **2018**, *2048*, M1022.

[34] M. A. Naseer, A. Husain, *J. Drug Deliv. Ther.* **2019**, *9*, 236.

[35] A. Yengoyan, V. Pivazyan, E. Chazaryan, R. Hakobyan, *Russ. J. Gen. Chem.* **2019**, *89*, 32.

[36] I. Khan, A. Khan, S. Ahsan Halim, A. Saeed, S. Mehsud, R. Csuk, A. Al-Harrasi, A. Ibrar, *Int. J. Biol. Macromol.* **2020**, *142*, 345.

[37] P. Kumar, A. Kumar, J. Makrandi, *J. Heterocyclic Chem.* **2013**, *50*, 1223.

[38] J. Komal, J. K. Makrandi, *Indian J. Chem.* **2012**, *51B*, 1511.

[39] A. Saundane, Y. Manjunatha, *Arab. J. Chem.* **2016**, *9*, S501.

[40] N. Seelam, S. S. P. Shrivastava, S. Gupta, J. Saudi, *Chem. Soc.* **2016**, *20*, 411.

[41] R. Lesyk, O. Vladzimirska, S. Holota, L. Zaprutko, A. Gzella, *Eur. J. Med. Chem.* **2007**, *42*, 641.

[42] M. Assarzadeh, A. Almasirad, A. Shafiee, M. Koopaei, M. Abdollahi, *Med. Chem. Res.* **2013**, *23*, 948.

[43] A. Uzgören-Baran, B. Tel, D. Sarıgöl, E. Öztürk, I. Kazkayası, G. Okay, M. Ertan, B. Tozkoparan, *Eur. J. Med. Chem.* **2012**, *57*, 398.

[44] D. Sarigol, A. Uzgoren-Baran, B. Tel, E. Somuncuoglu, I. Kazkayasi, K. Ozadali-Sari, O. Unsal-Tan, G. Okay, M. Ertan, B. Tozkoparan, *Bioorg. Med. Chem.* **2015**, *23*, 2518.

[45] A. Rzhevskii, N. Gerasimova, E. Alov, O. Kozlova, A. Danilova, S. Khapova, K. Suponitskii, *Russ. Chem. Bull.* **2012**, *61*, 2133.

[46] C. Tratat, M. Haroun, A. Paparisva, A. Geronikaki, C. Kamoutsis, A. Ciric, J. Glamoclija, M. Sokovic, C. Fotakis, P. Zoumpoulakis, S. S. Bhunia, A. K. Saxena, *Arab. J. Chem.* **2018**, *11*, 573.

[47] H. El-Sherief, Z. Hozien, A. El-Mahdy, A. Sarhan, *Arkivoc* **2011**, *2011*, 71.

[48] P. L. Lobo, B. Poojary, M. Kumsi, N. S. Kumari, *Naturforsch* **2010**, *65B*, 617.

[49] M. Kumsi, B. Poojary, P. Lobo, J. Fernandes, C. Chikkanna, *Z. Naturforsch. B* **2010**, *65*, 1353.

[50] A. Davoodnia, S. Ameli, N. Tavakoli, *Asian J. Chem.* **2011**, *23*, 3707.

[51] E. Il'inykh, D. Kim, *Chem. Heterocycl. Compd.* **2011**, *47*, 636.

[52] R. M. Usenko, M. V. Slivka, V. G. Lendel, *Chem. Heterocycl. Compd.* **2011**, *47*, 1029.

[53] V. Shmygarev, D. Kim, *Chem. Heterocycl. Compd.* **2004**, *40*, 1207.

[54] E. Erkhitueva, A. Dogadina, A. Khramchikhin, B. Ionin, *Tetrahedron Lett.* **2012**, *53*, 4304.

[55] M. Slivka, N. Korol, I. Rusyn, V. Lendel, *Heterocycl. Commun.* **2015**, *21*, 397.

[56] M. Slivka, N. Korol, V. Pantyo, V. Baumer, V. Lendel, *Heterocycl. Commun.* **2017**, *23*, 109.

[57] T. Shah, Z. Ahmad, N. Mir, M. Muneer, N. Rath, M. Ahmad, *RSC Adv.* **2015**, *130*, 107931.

[58] E. Klen, F. Khaliullin, G. Iskhakova, *Russ. J. Org. Chem.* **2005**, *41*, 1847.

[59] M. Heravi, A. Kivanloo, M. Rahimzadeh, M. Bakavoli, M. Ghassemzadeh, B. Neumüller, *Tetrahedron Lett.* **2005**, *46*, 1607.

[60] T. Hosseinnejad, M. Heravi, R. Firouzi, *J. Mol. Model.* **2012**, *19*, 951.

[61] M. M. Tsyhyka, S. M. Khrypak, I. V. Smolanka, *Chem. Heterocycl. Compd.* **1974**, *10*, 1425.

[62] M. M. Tsyhyka, S. M. Khrypak, I. V. Smolanka, *Chem. Heterocycl. Compd.* **1976**, *8*, 841.

[63] L. G. Payne, M. T. Wu, A. A. Patchett, *Heterocycles* **1979**, *12*, 1171.

[64] K. H. Ongania, *Chem. Ber.* **1981**, *114*, 1200.

[65] H. S. El-Kashef, K. U. Sadek, M. H. Elnagd, *J. Chem. Eng. Data* **1982**, *27*, 103.

[66] A. Ali, J. Wilkie, K. Winzenberg, *Aust. J. Chem.* **1997**, *50*, 911.

[67] R. Pignatello, S. Mazzone, A. Panico, G. Mazzone, G. Pennisi, R. Castana, M. Matera, G. Blandino, *Eur. J. Med. Chem.* **1991**, *26*, 929.

[68] T. Sasaki, E. Ito, I. Shimizu, *J. Org. Chem.* **1982**, *47*, 2757.

[69] Z. Wang, H. Shi, *Synth. Commun.* **2001**, *31*, 2841.

[70] M. V. Slivka, S. M. Khripak, V. M. Britcun, V. I. Staninetc, *Russ. J. Org. Chem.* **2000**, *36*, 1064.

[71] T. Kochikyan, M. Samvelyan, A. Petrosyan, P. Langer, *Russ. J. Org. Chem.* **2015**, *51*, 1469.

[72] M. Fizer, M. Slivka, E. Rusanov, A. Turov, V. Lendel, *J. Heterocyclic Chem.* **2015**, *52*, 949.

[73] M. Fizer, M. Slivka, V. Lendel, *Chem. Heterocycl. Compd.* **2019**, *55*, 478.

[74] S. Khrypak, V. Stanynets, M. Slivka, Y. Zborovskyi, *Ukr. Chem. J.* **2001**, *66*, 108.

[75] N. B. Almandil, M. Taha, R. K. Farooq, A. Alhibshi, M. Ibrahim, E. H. Anouar, M. Gollapalli, F. Rahim, M. Nawaz, S. A. Ali Shah, Q. U. Ahmed, Z. A. Zakaria, *Molecules* **2019**, *24*, 1002.

[76] J. P. Clayton, P. J. O'Hanlon, *J. Chem. Soc. Perkin Trans.* **1980**, *1*, 1352.

[77] N. D. Heindel, J. R. Reid, *J. Org. Chem.* **1980**, *45*, 2479.
[78] C. Xie, H. Mei, L. Wu, J. Han, V. Soloshonok, Y. Pan, *J. Fluor. Chem.* **2014**, *165*, 67.
[79] G. Giammona, M. Neri, B. Carlisi, A. Palazzo, C. La Rosa, *J. Heterocyclic Chem.* **1991**, *28*, 325.
[80] M. Heravi, Y. Beheshtiha, H. Oskooie, N. Nami, *Indian J. Heterocyclic Chem.* **1999**, *8*, 245.
[81] S. Akrami, B. Karami, M. Farahi, *Mol. Divers.* **2019**, *1*, 1.
[82] B. Tozkoparan, G. Aktay, E. Yeşilada, *II Farmaco* **2002**, *57*, 145.
[83] M. Danilkina, S. Vershilov, M. Ganina, L. Mikhailov, B. Ivin, *Russ. J. Gen. Chem.* **2004**, *74*, 472.
[84] V. Britsun, A. Esipenko, A. Kudryavtsev, M. Lozinskii, *Russ. J. Org. Chem.* **2004**, *40*, 232.
[85] V. Britsun, V. Pirozhenko, M. Lozinskii, *Russ. J. Org. Chem.* **2001**, *37*, 1056.
[86] V. Britsun, M. Lozinskii, *Chem. Heterocycl. Compd.* **2004**, *40*, 1092.
[87] C. Lamberth, F. Querniard, *Tetrahedron Lett.* **2008**, *49*, 2286.
[88] R. Motamedi, M. Heravi, Z. Nazari, F. Bamoharram, *Phosphorus Sulfur Silicon Relat. Elem.* **2010**, *185*, 1672.
[89] N. Korol, M. Slivka, M. Fizer, V. Baumer, V. Lendel, *Monatsh. Chem.* **2020**, *151*, 191.
[90] K. S. Dhaka, J. Mohan, V. K. Chadha, H. K. Pujari, *Indian J. Chem.* **1974**, *12*, 485.
[91] M. Główka, H. Foks, W. Rudnicka, *J. Chem. Crystallogr.* **1994**, *24*, 527.
[92] A. Aly, A. Hassan, Y. Ibrahim, M. Abdel-Aziz, *J. Heterocyclic Chem.* **2009**, *46*, 687.
[93] E. Il'inykh, D. Kim, M. Kodess, E. Matochkina, P. Slepukhin, *J. Fluor. Chem.* **2013**, *149*, 24.
[94] M. E. Farhan, M. G. Assy, *Egypt. J. Chem.* **2019**, *62*, 171.
[95] Z. Polivka, J. Holubek, E. Svatek, A. Dlabac, D. Pucek, Z. Sedivy, M. Protiva, *Collect. Czechoslov. Chem. Commun.* **1983**, *48*, 1465.
[96] A. Brukstus, I. Susvilo, S. Tumkevicius, *Heterocycl. Commun.* **2003**, *9*, 77.
[97] A. E.-A. A. G. Ghattas, H. M. Moustafa, E. A. A. Hassanein, B. R. M. Hussein, *Phosphorus Sulfur Silicon Relat. Elem.* **2012**, *187*, 1469.
[98] T. A. Farghaly, H. M. E. Hassaneen, *Arab. J. Chem.* **2014**, *10*, S3255.
[99] E. Klen, F. Khaliullin, *Russ. J. Org. Chem.* **2011**, *47*, 236.
[100] S. El Kazzouli, J. Koubachi, N. El Brahmi, G. Guillaumet, *RSC Adv.* **2015**, *5*(20), 15292.
[101] M. Le Meur, S. Bourg, S. Massip, M. Marchivie, C. Jarry, G. Guillaumet, S. Routier, *Eur. J. Org. Chem.* **2014**, *2014*, 3704.
[102] H. Zhang, *J. Organomet. Chem.* **2014**, *756*, 47.
[103] S. Wang, W. Liu, H. Zhan, H. Zhang, Y. Liang, Y. Tian, *RSC Adv.* **2013**, *46*, 23972.
[104] Z. Xie, X. Zhu, Y. Guan, D. Zhu, H. Hu, C. Lin, Y. Pan, J. Jiang, L. Wang, *Org. Biomol. Chem.* **2013**, *11*, 1390.
[105] W. Liu, S. Wang, J. Lin, Y. Jiang, Q. Zhang, Y. Zhong, *Synlett* **2013**, *25*, 586.
[106] S. Wang, W. Liu, Z. Li, Y. Huang, S. Li, A. Wang, *Synlett* **2015**, *26*, 2561.
[107] W. Liu, S. Wang, H. Zhan, J. Lin, P. He, Y. Jiang, *Tetrahedron Lett.* **2014**, *55*, 3549.
[108] M. Bakherad, A. K. Ghalenoei, A. Keivanloo, *Supramol. Chem.* **2019**, *4*, 9238.
[109] W. Liu, S. Wang, Z. Cai, S. Li, J. Liu, A. Wang, *Synlett* **2016**, *27*, 2264.
[110] H. Mei, Y. Xiong, C. Xie, V. Soloshonok, J. Han, Y. Pan, *Org. Biomol. Chem.* **2014**, *12*(13), 2108.
[111] M. Slivka, N. Korol, M. Fizer, V. Baumer, V. Lendel, *Heterocycl. Commun.* **2018**, *24*, 197.
[112] M. Fizer, M. Slivka, R. Mariychuk, O. Fizer, N. Korol, A. Kryvoviaz, V. Lendel, *ICTEP* **2019**, *2019*, 87.

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