

Study of 1,2,4-triazole-3(5)-thiol Behavior in Reactions with 1-phenyl-1H-pyrrole-2,5-dione Derivatives and 3-bromodihydrofuran-2(3H)-one and Antimicrobial Activity of Products †

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† Presented at the 24th International Electronic Conference on Synthetic Organic Chemistry, 15 November–15 December 2020; Available online: <https://ecsoc-24.sciforum.net/>.

Received: date; Accepted: date; Published: date

Abstract: In present work we report our studies of the interaction of 1-phenyl-1H-pyrrole-2,5-dione derivatives (N-arylmaleimides) and 3-bromodihydrofuran-2(3H)-one (α -bromo- γ -butyrolactone) as possible $[C_2]^{2+}$ synthons with 1,2,4-triazole-3(5)-thiole targeting on synthesis of novel 5-substituted thiazolo[3,2-b][1,2,4]triazole-6-ones. According to obtained results was establish that in above-mentioned interactions (conditions: convenient heating, range of solvents/reaction time) the thiol-ene click (for N-arylmaleimides) and S_N (for α -bromo- γ -butyrolactone) processes take place, but not [2+3]-cyclocondensation reaction. The structure of compounds was studied using 1H , ^{13}C NMR spectroscopy, LC-MS spectrometry and X-ray analysis. The prescreening of antimicrobial activity for synthesized compounds was performed against Gram-positive and Gram-negative bacteria, as well as yeasts.

Keywords: 1,2,4-triazole-3(5)-thiol; N-arylmaleimides; α -bromo- γ -butyrolactone; thiol-ene click; S_N ; [2+3]-cyclocondensation; antimicrobial/antifungal activity

1. Introduction

The thiazolo [3,2-b][1,2,4]triazole-6-one represent a bicyclic heteroatom-rich scaffold which recently, has considerably attracted the attention of medicinal chemists due to their diverse biological activities [1–3]. The [2+3]-cyclocondensation reaction of substituted 1,2,4-triazole-3(5)-thioles with equivalents of electrophilic synthon $[C_2]^{2+}$ is key synthetic approach for 5-substituted thiazolo [3,2-b][1,2,4]triazole-6-ones [4]. The chloroacetic acid as $[C_2]^{2+}$ synthon is studied well (mostly in

multicomponent reaction with aromatic/heteroaromatic aldehydes) [5–7] in interaction with substituted 1,2,4-triazole-3(5)-thioles meanwhile data about other ones are limited. Taking account that thiazole moiety in thiazolo[3,2-b][1,2,4]triazole-6-one core is structure equivalent of 4-thiazolidone ring and accordingly to retrosynthetic methodology it was logical to study the utilization of electrophilic synthon $[C_2]^{2+}$ equivalents which normally used in 4-thiazolidone one synthesis. On the other hand, the 1,2,4-triazole-3(5)-thiol could be considered as a cyclic equivalent of thiosemicarbazide or formyl aldehyde thiosemicarbazone that allows expecting the formation of 4-thiazolidone core in the reaction process.

The wide range of 1,3-S,N-binucleophiles and electrophilic synthon $[C_2]^{2+}$ equivalents are used in this type reaction provide to excellent structure diversity of target 4-thiazolidones. The recyclization of substituted 1-phenyl-1H-pyrrole-2,5-diones (N-arylmaleimides) in interaction with S,N-binucleophiles is widely used as straightforward and convenient approach for synthesis of 2-(4-oxothiazolidin-5-yl)-N-R-phenylacetamides as well as respective annelated 4-thiazolidinone derivatives [8–12]. Some protocols describe utilization of 3-bromodihydrofuran-2(3H)-one (α -bromo- γ -butyrolactone) as $[C_2]^{2+}$ synthons for synthesis of substituted 4-thiazolidinone derivatives in [2+3]-cyclocondensation reactions with row of S,N-binucleophiles. The thioamides [13], mono- and disubstituted thioureas [14], thiosemicarbazides and thiocarbohydrazides [15] react with α -bromo- γ -butyrolactone by Hantzsch type reaction under mild conditions provides to appropriate 5-hydroxyethyl-4-thiazolidinone derivatives.

In our early study [5] we have made an attempt to study the interaction of the 1,2,4-triazole-3(5)-thiol with N-arylmaleimides (convenient heating, acetic acid medium). We have described products of interaction of 1,2,4-triazole-3(5)-thiol with N-arylmaleimides as bicyclic functionalized derivatives of thiazolo[3,2-b][1,2,4]triazol-6-one [5]. Our conclusions have been made based on the spectral properties of compounds. The singlet signals in 1H NMR spectra at $\delta = 13.80 - 14.30$ ppm have played a key role in the structure determination of reaction products and have being interpreted as signals of $-NH-$ amide-group protons.

The similar structure determination of products is reported in later protocols [16,17] describing the interaction of some substituted 1,2,4-triazole-3(5)-thiols and N-arylmaleimides. The conclusions about products structure in works [16,17] were also performed based only on spectral data and obtaining of thiazolo[3,2-b][1,2,4]triazol-6-one system were argued using an interpretation of singlet signals in 1H NMR at $\delta = 13.80 - 14.30$ ppm which were considered as signals of NH protons in amide group of appropriate 2-(6-oxo-5,6-dihydrothiazolo[3,2-b][1,2,4]triazol-5-yl)-N-phenylacetamides. However, it should be noted that the similar singlet in a similar magnetic field can belong to signal of NH proton of 1,2,4-triazole ring. In work [18] we hypothesized and reported that interaction of some 1,2,4-triazole-3(5)-thiols with N-arylmaleimides does not lead to [2+3]-cyclocondensation products.

As a part of our ongoing interest to chemistry and pharmacology of azoles [19–22], in present paper we tried to study this issue more deeply using wide range of solvents/reaction conditions and clarify the structure of products in interactions of 1,2,4-triazole-3(5)-thiol with N-arylmaleimides using X-ray technique. Also, we report about early undescribed interaction between α -bromo- γ -butyrolactone and 1,2,4-triazole-3(5)-thiol and characterization of product. The paper also presents the screening results of antimicrobial activity for synthesized compounds against Gram-positive and Gram-negative bacteria, as well as yeasts.

2. Materials and Methods

2.1.1. General Experimental Methods

Melting points were measured in open capillary tubes on a BÜCHI B-545 melting point apparatus (BÜCHI Labortechnik AG, Flawil, Switzerland) and are uncorrected. The elemental analyses (C, H, N) were performed using the Perkin-Elmer 2400 CHN analyzer (PerkinElmer, Waltham, MA, USA) and were within $\pm 0.4\%$ of the theoretical values. The 500 MHz- 1H and 126 MHz- ^{13}C spectra were recorded on Bruker AVANCE-500 spectrometer (Bruker, Bremen, Germany). All

spectra were recorded at room temperature except where indicated otherwise and were referenced internally to solvent reference frequencies. Chemical shifts (δ) are quoted in ppm and coupling constants (J) are reported in Hz. LC–MS spectra were obtained on a Finnigan MAT INCOS-50 (Thermo Finnigan LLC, San Jose, CA, USA). Solvents and reagents that are commercially available were used without further purification.

2.1.2. The Synthesis of Compound 1 was Performed Using Protocol Described in [23].

2.1.3. The Synthesis and Spectral Properties of Compound 2a-f was Early Described in [5].

2.1.4. The Synthesis of 3-((1H-1,2,4-triazol-3-yl)thio)dihydrofuran-2(3H)-one (3).

A mixture of 1H-1,2,4-triazole-5-thiol (**1**) (10,0 mmol), 3-bromodihydrofuran-2(3H)-one (α -bromo- γ -butyrolactone) (10,0 mmol) and anhydrous sodium acetate (10,0 mmol, medium - 10 mL of acetic acid) or triethylamine (10,0 mmol, medium-10 mL of ethanol) refluxed for 30 min to 24 h. After cooling to the room temperature and evaporated the resulting masse were treated with diethyl ether and recrystallized from mixture acetonitrile–water.

Beige prisms, yield 45%, mp 67–70 °C. ¹H NMR (500 MHz, DMSO-d₆, δ): 14.20 (br.s, 1H, NH, 1,2,4-triazole), 8.53 (s, 1H, 1,2,4-triazole), 4.53 (t, J = 9.4 Hz, 1H, CH), 4.36 (td, J = 8.8, 3.2 Hz, 1H, CH₂), 4.28 (td, J = 8.8, 7.1 Hz, CH₂), 2.71 (dddd, J = 12.5, 9.2, 7.1, 3.2 Hz, 1H, CH₂), 2.43 (dq, J = 12.6, 9.0 Hz, 1H, CH₂). ¹³C NMR (126 MHz, DMSO-d₆, δ): 174.82 (C=O), 155.96 (1,2,4-triazol), 146.10 (1,2,4-triazol), 66.80 (CH₂-O), 42.20 (CH), 29.27(CH₂). LCMS (ESI+) m/z 186 (M+H)⁺. Calc. for C₆H₇N₃O₂S: C 38.91%, H 3.81%, N 22.69%. Found: C 39.00%, H 3.90%, N 22.90%.

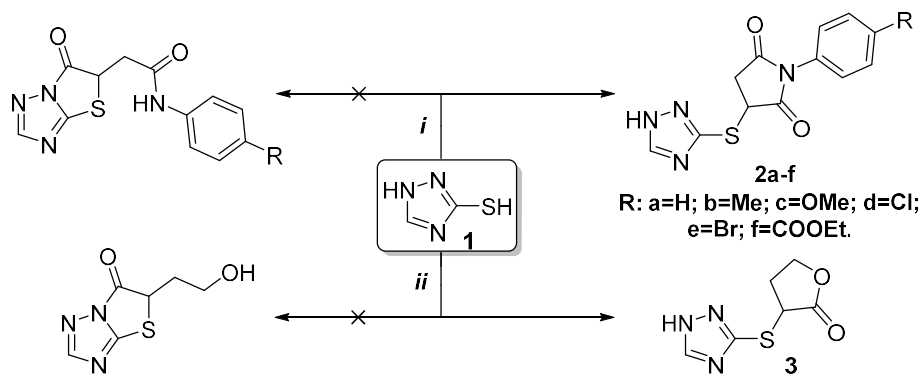
2.2. Antimicrobial Activity

The antimicrobial activity of the compounds 2a, 2d, 2e was determined by the agar diffusion method [24,25]. Nutrient agar (0,5% peptone, 0,3% beef extract, 1,5% agar, 0,5% sodium chloride, distilled water, pH ~ 6.8) was used as a nutrient medium. The test cultures suspensions (in concentration 1×10⁷ CFU/mL), standardized previously by the optical standard of turbidity, were uniformly sown in Petri dishes with the nutrient agar. Aliquots (20 μ L) of 0.1% tested derivatives (concentration 1000 μ g/mL) in EtOH/DMSO/water (2:1:1) were placed into wells (diameter of 4.0 \pm 0.1 mm) in agar in Petri plates with test microorganisms. The antimicrobial activity was evaluated by measuring the diameter of zone inhibition of microbial growth. The plates were incubated for 24 h at 37 °C. The inhibition zone appeared after 24 h and was measured in mm around the well in each plate. Digital images of culture growth on dishes were obtained and processed with a computer program UTHSCSA ImageTool 2.0 (The University of Texas Health Science Center in San Antonio, © 1995–1996) for calculation of growth inhibition zone diameters. Each experiment was performed in triplicate. Results were expressed as the means \pm S.D. The experiments were carried out on microorganism strains, which were isolated in the laboratory of the microbiology research of the Department of Microbiology, Virology and Immunology of the Ivano-Frankivsk National Medical University from ambulatory patients. The following bacterial strains were used: methicillin-sensitive *Staphylococcus aureus* ATCC 25,923 (MSSA); methicillin-resistant *Staphylococcus aureus* (MRSA), clinical isolate; methicillin-resistant *Staphylococcus epidermidis* (MRSE), clinical isolate; *Escherichia coli* ATCC 25922; yeasts *Candida albicans* (clinical isolate). Staphylococci of clinic origin were identified using chemical micro-tests “STAPHYtest 16” (Lachema, Czech Republic). Fungi culture was identified on the basis of 40 biochemical tests using the VITEK 2 system with the VITEK 2 YST ID card (bioMerieux, France). The sensitivity of strains to antibiotics was determined by disc diffusion and serial dilutions methods.

3. Results and Discussion

3.1. Chemical Synthesis and Structure Determination

According to our objectives we revised our previous protocol and studied interaction of 1,2,4-triazole-3(5)-thiol (1.0 eq) with series of N-arylmaleimides (1.0 eq) using convenient heating and wide range of solvents: polar protic (acetic acid, ethanol), polar aprotic (acetone, acetonitrile), non-polar (benzene, toluene) (Scheme 1). The reflux of reaction mixtures was performed in time range from 30 min to 24 h and was monitored by TLC. The spots of starting reagents and only of one new product in each reaction were observed in TLC within all the time. The increase of reaction time duration doesn't lead to a significant yield increase even after 24 h reflux as well as to formation of new products. The addition of the anhydrous AcONa in the reaction mixture didn't lead to obtain of new products too.



Scheme 1. Synthesis of non-condensed 3-(1H-[1,2,4]triazol-3-ylsulfanyl)-derivatives 2a-f, 3. Reagents and conditions: (i) 1,2,4-triazole-3(5)-thiol (1) (1.0 eq); N-arylmaleimides (1.0 eq.); studied solvents: acetic acid, ethanol, acetone, acetonitrile, benzene, toluene; without or in presence of AcONa anhydrous (1.0 eq.); reflux from 30 min to 24 h; (ii) 1,2,4-triazole-3(5)-thiol (1) (1.0 eq); α -bromo- γ -butyrolactone (1.0 eq.); studied solvents: acetic acid, ethanol; in presence of AcONa anhydrous (1.0 eq) or triethylamine (1.0 eq); reflux from 30 min to 24 h.

Since the application of spectral methods to analyze the structure of products is not completely convincing the X-ray crystallographic analysis was used for structure determination of compounds 2b and 2e. The ORTEP drawing and atomic numbering are shown in Figure 1.

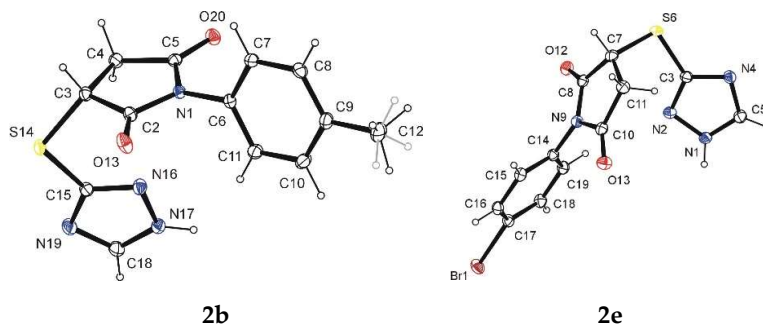


Figure 1. The molecular structures of 2b and 2e.

The reaction between 1,2,4-triazole-3(5)-thiol (1.0 eq) and α -bromo- γ -butyrolactone (1.0 eq) were studied in glacial acetic acid and ethanol medium. Such bases as anhydrous sodium acetate (1.0 eq) and triethylamine (1.0 eq) were used for reaction. The process was monitored by TLC. It should be noted that nucleophilic substitution is very fast process in mentioned conditions and S_N product's (3, Scheme 1) spot had been observed in TLC already after 10–15 min of reflux. The extending of reflux even to 24 h didn't lead to formation of new products accordingly to TLC and finally LCMS, NMR data. In the ^1H NMR spectrum of 3 the broad signal at 14.20 ppm belongs to the NH proton of 1,2,4-triazole ring and indicates the absence of bicyclic annelated product (Figure 2).

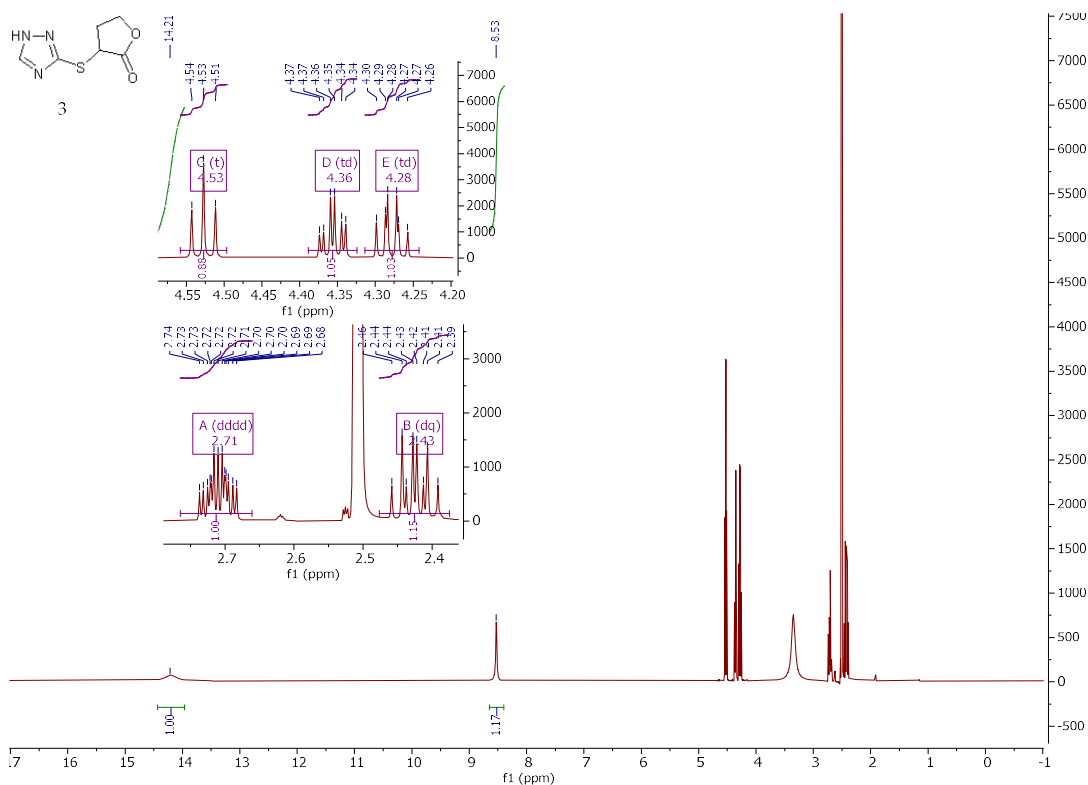


Figure 2. ^1H NMR spectrum of 3.

The signals of protons of furan-2(3H)-one moiety give a characteristic pattern for cyclic lactone ring [26] with triplet at 4.53 ($J = 9.4$ Hz); triplets of doublets at 4.36 ($J = 8.8, 3.2$ Hz), and 4.28 ($J = 8.8, 7.1$ Hz); doublet of doublet of doublet of doublets at 2.71 ($J = 12.5, 9.2, 7.1, 3.2$ Hz), and doublet of quartet at 2.43 ($J = 12.6, 9.0$ Hz) (Figure 2).

3.2. Antimicrobial Activity

The compounds 2a, 2d, 2e were pre-screened for their potential antimicrobial activities against Gram-positive bacterium (*Staphylococcus aureus*, *S. aureus*; *Staphylococcus epidermidis*, *S. epidermidis*), Gram-negative bacterium (*Escherichia coli*, *E. coli*) and yeasts (*Candida albicans*, *C. albicans*). The antimicrobial activity was evaluated in terms of zone of growth inhibition and values were compared to standard reference antimicrobial agents [24,25]. The tested compounds were found highly active against methicillin-resistant strains of *S. aureus* (MRSA) and *S. epidermidis* (MRSE), as well as *C. albicans* (Table 1).

Table 1. In vitro antimicrobial activity of compounds 2a, 2d, 2e. Zone of growth inhibition (in mm, at conc. 200 $\mu\text{g}/\text{mL}$ after 24 h, $M \pm S(\sigma)$).

Compound, Reference Drugs	Zone of inhibition				
	<i>S. aureus</i> ATCC 25,923 MSSA	<i>S. aureus</i> MRSA ^a	<i>S. epidermidis</i> MRSE ^a	<i>E. coli</i> ATCC 25922	<i>C. albicans</i> ^a
2a	7,40 \pm 0,40	8,55 \pm 0,24	8,55 \pm 0,24	-	11,03 \pm 0,61
2d	9,13 \pm 0,43	7,89 \pm 0,30	9,83 \pm 0,20	-	12,58 \pm 0,75
2e	14,06 \pm 0,51	17,25 \pm 0,38	15,50 \pm 0,50	8,00 \pm 0,48	12,75 \pm 0,45
Ampicillin, 10,0 $\mu\text{g}/\text{ml}$	23,58 \pm 0,63	7,69 \pm 0,28	7,88 \pm 0,34	16,80 \pm 0,40	-
Fluconazole, 10,0 $\mu\text{g}/\text{ml}$	-	-	-	-	14,71 \pm 0,39

^a—clinical isolate; “-” — compounds didn’t tested in experiment.

The antibacterial screening results indicated that nature of substituent in synthesized derivatives of 1-(phenyl)-3-(2H-[1,2,4]triazol-3-ylsulfanyl)-pyrrolidine-2,5-dione has impact on activity level and antibacterial/antifungal properties increase as follows: 4-H > 4-Cl > 4Br.

4. Conclusions

In present report the interaction of 1,2,4-triazole-3(5)-thiol with such electrophilic synthons [C₂]²⁺ equivalents as N-arylmaleimides and α -bromo- γ -butyrolactone have been studied. The X-ray analysis and spectral methods were used for products structure determination. According to dates was establish that in mentioned interactions the thiol-ene click process and nucleophilic substitution take place, but not [2+3]-cyclocondensation reaction as could be expected. The obtained results of pharmacological screening suggest to coming design and synthesis of new 1,2,4-triazol/pyrrolidine-2,5-dione hybrids as potential molecules with promising antimicrobial properties.

Author Contributions: Conceptualization, S.H. and R.L.; methodology, S.H. and R.L.; synthesis of compounds, S.H. and R.L.; pharmacological screening, H.D., O.A. and R.K.; spectral study, S.H. and N.S.; X-ray study, A.G. writing-original draft preparation, S.H.; writing-review and editing, S.H., R.K., A.G. and R.L. All authors have read and agreed to the published version of the manuscript.

Funding: This work was partially supported by COST Action NutRedOx-CA16112 “Personalized Nutrition in aging society: redox control of major age-related diseases” (for S.H.).

Acknowledgments: This research was supported by the Danylo Halytsky Lviv National Medical University, which is gratefully acknowledged.

Conflicts of Interest: The authors declare no conflict of interest.

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