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Primary discovery of 1-aryl-5-substituted-1*H*-1,2,3-triazole-4-carboxamides as promising antimicrobial agents

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ABSTRACT

Three series of novel 1*H*-1,2,3-triazole-4-carboxamides: 1-aryl-5-alkyl/aryl-1*H*-1,2,3-triazole-4-carboxamides, 1-aryl-5-amino-1*H*-1,2,3-triazole-4-carboxamides and 1,2,3-triazolo[1,5-*a*]quinazoline-3-carboxamides were synthesized via base-mediated click azide reactions. Compounds were evaluated for their antimicrobial activities against primary pathogens: Gram-positive and Gram-negative bacterial strains *Escherichia coli, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa, Staphylococcus aureus*, as well as fungal strain *Cryptococcus neoformans var. grubii* and *Candida albicans*. Compounds exhibiting moderate to good activities were selected for SAR analysis. Several 5-methyl-1*H*-1,2,3-triazole-4-carboxamides **4d, 4l, 4r,** showed potent antibacterial effect against *S. aureus.* On the contrary, 5-amino-1*H*-1,2,3-triazole-4-carboxamide **8b** and [1,2,3]triazolo[1,5-*a*]quinazoline-3-carboxamide **9a** were active against pathogenic yeast *C. albicans.* Thus, compound **4l** under 1 μM demonstrated 50% growth inhibition against *S. aureus.* At the same concentration, the compound **9a** killed approx. 40% of *C. albicans* cells. In general, these compounds demonstrated selective action and no significant impact on the viability of human keratinocytes of HaCaT line.

1. Introduction

The increasing resistance of pathogenic microorganisms to available antibiotics is a serious problem and the discovery of new therapeutic agents and their targets is a big challenge [1, 2]. It is worth noting that the formation of resistance to antibiotics is the result of natural selection of the microorganisms. Two main strategies for elaboration of new antibacterial agents exist: 1) development and implementation of antibiotics that differ in structure and mechanism of action [3, 4]; 2) defining of the mechanisms of formation and realization of the already existing drugs and their further modification in order to slow down or stop drug resistance [5]. One of the mechanisms responsible for the formation of the acquired antibiotic resistance is the bacterial DNA damage response mechanism known as the SOS response induced by many antibiotics [6, 7]. The SOS system is widespread among microorganisms and involves the action of many genes (more than 40 genes in *Escherichia coli*). Thus, inhibition of the SOS mechanism is a potential way of blocking the formation of antibiotic resistance.

Scaffolds containing 1,2,3-triazole-4-carboxamide moiety, for example 5-amino-*N*-(3,4-dimethylphenyl)-1-(2-((4-ethoxyphenyl)amino)-2-oxoethyl)-1*H*-1,2,3-triazole-4-carboxamide **A** (GSK1010702A) (Fig. 1), have demonstrated good efficiency in inhibiting SOS-dependent response [8]. In this regard, studies of 1*H*-1,2,3-triazole-4-carboxamides appear to be promising for screening their antimicrobial activity (Fig. 1). Recently, a series of novel 1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxamides bearing the piperazine motif (Fig. 1, **B**) were evaluated for their antimicrobial activity and found to be efficient against Gram-positive, Gram-negative bacterial strains (*Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Streptococcus pyogenes, Klebsiella pneumonia, Streptococcus aureus, Klebsiella terrigena*), as well as fungal strains (*Candida albicans, Trichoderma viride, Aspergillus flavus, and Aspergillus*) [9]. Triazole-4-carboxamide **C** analogue of N-coumaroyl-tyramine (Fig. 1) was a potent inhibitor of biofilm formation by Gram-negative strain (*Pseudoalteromonas ulvae* TC14) with EC_{50} close to ampicillin (EC_{50} = 11 μM) and without toxic effect on bacterial growth even at high concen-

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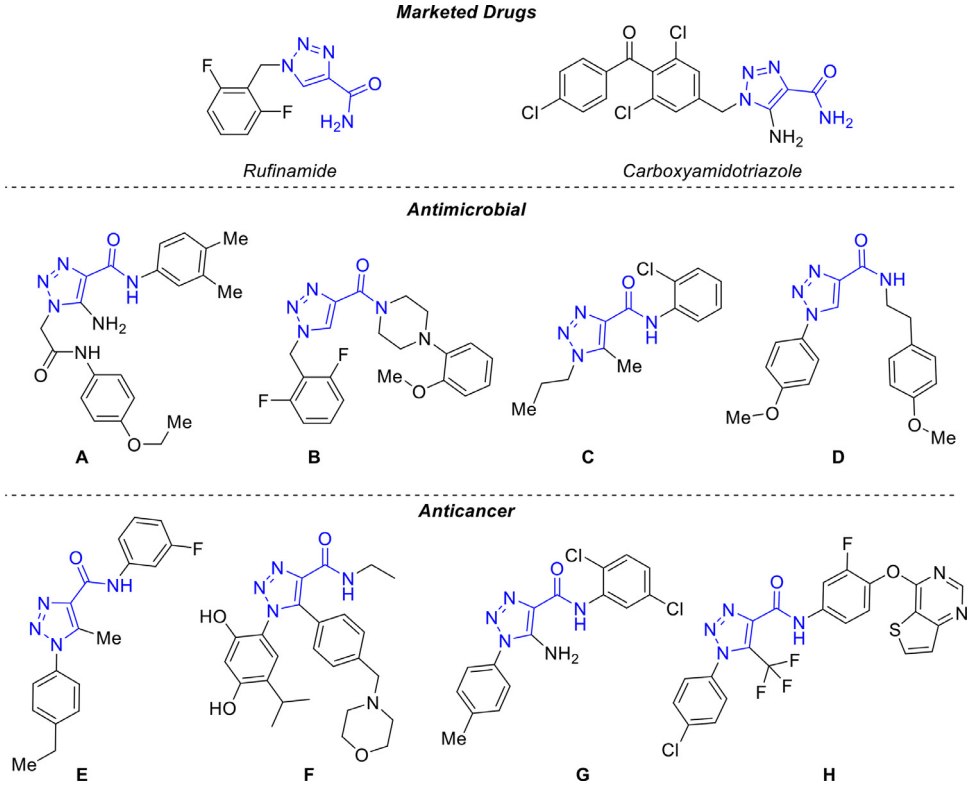


Fig. 1. Biologically active 1-aryl-1H-1,2,3-triazole-4-carboxamides.

trations (100 μM) [10]. The 1,2,3-triazole-4-carboxyl amide **D** (Fig. 1) acting as an inhibitor of succinate dehydrogenase enzyme possessed good fungicidal activity, especially towards *Sclerotinia sclerotiorum.* Potentially, it can be used for the development of novel pesticides [11]. Noteworthy, the compounds containing the 1,2,3-triazole-4-carboxamide moiety are already used as drugs or are currently under the preclinical studies. Two compounds are available on the market. For example, the well-known drug *Rufinamide* has been used since 2008 for treatment of Lenox-Gastaut syndrome (a form of epilepsy) [12]. *Carboxyamidotriazole* in the form of the orotic acid salt was shown to be an effective blocker of calcium channels and demonstrated high activity against chronic myelocytic leukaemia inhibiting the growth of cell lines LAMA84R and K562R [13]. Other compounds structurally related to *Carboxyamidotriazole* were evaluated as calcium-activated potassium channel activators [14]. The 1,2,3-triazole-4-carboxamides are promising antiproliferative agents for anticancer studies. For instance, the 1-benzyl-*N*-(2-(phenylamino)pyridine-3-yl)-1*H*-1,2,3-triazole-4-carboxamides have shown cytotoxicity against lung cancer A549 cell line by the inhibiting of tubulin polymerization [15]. 1,2,3-Triazole-4-carboxamides containing podophyllotoxin were screened for DNA topoisomerase-IIα inhibitory activity [16]. The dissymmetric bistriazoles were tested *in vitro* for their cytotoxic activity using B16 melanoma cells and showed activity at nanomolar level ($<$ 1 μM) [17, 18]. High activity compounds was found in 1-aryl-5-substituted-1*H*-1,2,3-triazole-4-carboxamides. For example, 1-aryl-5-methyl-1,2,3-triazole-4-carboxamide **E** (Fig. 1) was an inhibitor of ER stress-induced CHOP-luciferase [19]. The compound **F** (Fig. 1) exhibited inhibition of heat shock protein 90 [20]. Several 5-amino-1H-1,2,3-triazole-4-carboxamides structurally close to 5-methyl-1H-1,2,3-triazole-4-carboxamides were also acting as potent antiproliferative agents. In particular, the compound **G** was active toward CNS SNB-75 and thieno[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine-3-carboxamide suppressed melanoma cell line SK-MEL-5 [21, 22]. The 5-(trifluoromethyl)-1H-1,2,3-triazole-4-carboxamide **H** and related compounds (Fig. 1) were discovered as c-Met-targeting and apoptosis-inducing agents for various tumour cell lines (MCF-7, HepG2, A549, H460, HT-29, MKN-45 and U87MG) with 3–5-fold higher activity than the positive control drug foretinib [23, 24]. As an example of agents with other bioactivities, 1,2,3-triazole-4-carboxamides were shown to be the inhibitors of the Wnt/β-catenin signalling pathway [25], possessing promising anti-leishmanial activity [26] and exhibiting virus-inhibitory activities [27].

Therefore, the compounds with a 1,2,3-triazole-4-carboxamide pharmacophore moiety possess considerable potential for the discovery of the biological activity, in particular, the antimicrobial one.

2. Results and discussion

2.1. Chemistry

In this work, we demonstrated the antimicrobial activity of the selected 1-aryl-1*H*-1,2,3-triazole-4-carboxamides possessing the highest score. Using the developed combinatorial methods for the synthesis of triazoles [28], a library of 360 compounds was obtained and screened for primary pathogens: *Escherichia coli (E. coli, ATCC 25922), Klebsiella pneumonia (K. pneumoniae, ATCC 700603), Acinetobacter baumannii (A. baumannii, ATCC 19606), Pseudomonas aeruginosa (P. aeruginosa, ATCC 27853), Staphylococcus aureus (S. aureus, ATCC 43300), Cryptococcus neoformans var. grubii (C. neoformans, H99; ATCC 208821)* and *Candida albicans (C. albicans, ATCC 90028).* These microorganisms are among the pathogens listed by the World Health Organization with growing multidrug resistance. According to the screening results, the most active compounds were selected to create a structure–activity relationship (SAR) and further modifications of 1-aryl-5-substituted-1*H*-1,2,3-triazole-4-carboxamide scaffolds.

In Scheme 1, the preparation of the target compounds **4a-t, 8a-e, 9a-g** is described. A convenient synthetic strategy for the creation of combinatorial libraries of 1-aryl-5-methyl-*N*-R^2-1*H*-1,2,3-triazole-4-carboxamides involves cascade reactions of azides **1** with diketene **2** and amines **3** via acylation and cyclocondensation in one pot (Scheme 1, route A) [28]. By this multicomponent reaction, 1,2,3-triazole-4-carboxamides **4a-e** were prepared in a short time with high yields and purity. It should be noted that the reaction of diketene **2** with strongly nucleophilic amines (such as 3-aminomethyl-pyridine) and highly reactive azides underwent at room temperature, and the reaction yield increased with increasing the amine basicity. The reaction time correlates with the reactivity of azides and lasts on average about 30 min. For the synthesis of other 5-alkyl-1*H*-1,2,3-triazole-4-carboxamides **4** and 5-methyl-1*H*-1,2,3-triazole-4-carboxamides **4k-s** from low nucleophilic amines, the two-step procedure was used (Scheme 1, route B). The organic azides were condensed with β-ketoesters **5** to afford the intermediate 1,2,3-triazole-4-carboxylates, which were hydrolyzed without purification with NaOH to give the corresponding carboxylic acids **3** [29, 30, 31, 32]. It is noteworthy that the base solvent system for the Dimroth reaction should be selected depending on the structure of azides as well as β-ketoesters [29]. Treatment of acids with $SOCl_2$ gave the corresponding acid chlorides, which were aminolyzed to afford amides **4f-t**. It is well known that derivatives of cyanoacetic acid are highly reactive in the reaction with azides providing the diversity of 5-amino-1*H*-1,2,3-triazoles in a short time at room temperature [33, 34, 35, 36]. We used the reaction of azides with cyanoacetamides **7** to obtain the target 5-amino-1*H*-1,2,3-triazole-4-carboxamides **8** (Scheme 1, route C). The domino-reaction of 2-azidobenzoates **1r-t** [37] with cyanoacetamides led to [1,2,3]triazolo[1,5-*a*]quinazoline **9** allowing to "fix" aryl rotation. Compounds **8** and **9** were formed and sedimented rapidly after mixing the reagents with yields close to quantitative. The structures of all title compounds **4, 8, 9** were confirmed by ^{1}H NMR, ^{13}C NMR and LCMS. Amides **4, 8, 9** were formed with high purity and with no by-products observed. Additionally, the structural features of 5-cyclopropyl-1,2,3-triazolyl-4-carboxamide, in which perpendicular or bisected form of cyclopropyl could exist, has been recently studied by the X-ray crystallographic analysis [38].

2.2. Biological activity and structure-activity relationships

2.2.1. Primary antimicrobial screening

According to the results of the preliminary screening of the newly synthesized 1,2,3-triazolyl-4-carboxamides **4, 8** and **9** (at a concentration of 32 μg/mL (approx. 100 μM)) on seven pathogens (*S. aureus* (ATCC 43300), *E. coli* (ATCC 25922), *K. pneumoniae* (ATCC 700603), *P. aeruginosa* (ATCC 27853), *A. baumannii* (ATCC 19606), *C. albicans* (ATCC 90028) and *C. neoformans* (H99; ATCC 208821)), 33 compounds were selected. In Table 1, the results of two parallel trials are presented.

Based on the GI average values of parallel trials, one can see that most of the tested compounds frequently inhibited the growth of fungus *C. albicans* and *C. neoformans* and Gram-negative bacterium *A. baumannii* (Fig. 3). In addition, there was a compound highly active toward *S. aureus*.

The highest values of the antimicrobial activity were found toward the strain of diploid fungus *Candida albicans*. In particular, 1-(4-bromophenyl)-*N*-butyl-5-methyl-1*H*-1,2,3-triazole-4-carboxamide **4d** inhibited strain growth by 63% (GI, % = 63.7 and 67.6) showing good reproducibility (abs Z-Score 12.8; 15.1) (Fig. 4). This compound was selected as a title structure for the analysis of the structure–activity relationships, and several determinants were identified. It should be noted that an important factor is the length of the carbon framework in the amide moiety (Fig. 4, note A). In case of amides with fewer than **4** atoms, the activity was significantly reduced. This is evidenced by the activity data of structurally related compounds **4a** and **4b** in which, in contrast to **4d**, instead of butyl substituent there is propyl one (Fig. 4, note A). The second important factor was the increase in the activity of *Candida albicans* with a decrease in the substituent at position 5 of the triazole ring. For example, *N*-butyl-5-propyl-1*H*-1,2,3-triazole-4-carboxamide **4j** had significantly lower activity than 5-methyl-1*H*-1,2,3-triazole **4d** (GI, % 21.2; 27.4). The 5-isopropyl triazoles showed lower activity (compounds **4f** and **4g**) (Fig. 4, note B). This can be explained by the fact that, the triazole fragment becomes more like an amide bond and by the fact that the bulky substituent removes the aryl and triazole ring from the coplanar position increasing the angle between them [38].

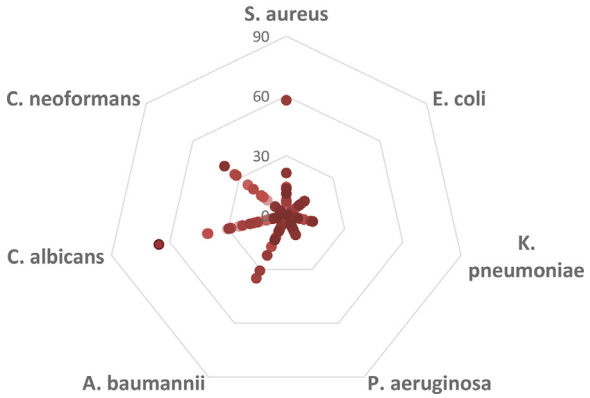


Fig. 3. Distribution diagram of the average percentage inhibition of the compounds **4, 8, 9** toward different pathogens.

The variation of the substituent at the para-position of aryl (Br on Et in **4a** and **4b**, respectively) was not noticeable in the activity (Fig. 4, note C). A replacement of the aryl moiety in the alkyl substituent (compound **4e**) in the amide moiety or complete replacement with aryl substituent (compounds **4k, 4l, 4m** and **4q**) leads to a decrease in the activity, which in some compounds remains sufficient for further research for its improvement. It is necessary to distinguish compounds that have a substituent in the para-position of the aryl amide moiety. Thus, the compounds **4l, 4m** and **4r** were slightly more active than *N*-phenyl triazole **4k**. The compounds **4l** and **4m** with *N*-(2,4-dimethoxyphenyl) substituent were the most active among the aryl structures, however, parallel trials of the compound **4m** were poorly correlated. In the series of *Sulfanilamide* derivatives obtained by acylation of *Sulfanilamide* with triazole carboxylic acids **3**, only 1-(2-fluorophenyl)-5-methyl-1*H*-1,2,3-triazole-4-carboxamide **4r** was detected close to partially active compounds against *C. albicans*. In addition, increase in activity was observed in *Sulfanilamide* series from phenyl to 2-fluorophenyl substituent in position 1 of the triazole ring H<4-Me<3-Me<3-MeO<2-F (GI, % = 1.1; 5.0 (H, **4n**); 2.1; 4.2 (4-Me, **4o**); 11.8; 8.8 (3-Me; **4p**) 21.8, 8.1 (3-MeO, **4q**), 40.4, 41.8 (2-F, **4r**)).

The compounds **4** were mainly low active toward the strain of *Cryptococcus neoformans var. grubii*. The compound **4p** with the meta-tolyl substituent and the sulfanilamide moiety, as well as two compounds **4s, t** (Fig. 4), containing bulky substituents disrupting the coplanarity of the aryl and triazole rings and obviously limiting the free rotation of the two rings relative to each other, were the only ones to show insignificant activity. In particular, (2,6-dimethylphenyl)-1,2,3-triazole-4-carboxamide **4s** and 5-cyclopropyl-1,2,3-triazole-4-carboxamide **4t** inhibited growth of *C. neoformans* by 28.6; 37.7 and 17.7; 24.7%, respectively. It is worth noting that the cyclopropyl substituent in bisector form rotates the

Table 1
Preliminary screening of 1-aryl-*N*-R^2-1*H*-1,2,3-triazole-4-carboxamides **4, 7**

Compnd	*S. aureus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*A. baumannii*	*C. albicans*	*C. neoformans*
4a	13.3; 14.9[a]	-0.6; 2.7	10.7; 15.9	-1.8; -2.8	11.9; 7.8	14.1; 16.2	-19.9; -68.4
4b	-10.8; -22.3	2.6; 3.0	1.6; 7.3	0.5; 7.7	-3.6; 11.3	13.2; 18.7	2.6; 7.3
4c	-4.0; 5.4	-0.0; -6.6	-2.0; 4.6	-2.4; 2.2	2.0; 6.0	17.9; **31.9**[b]	-29.7; -35.4
4d	-19.3; -8.7	-2.9; -6.8	-10.4; 0.6	-2.4; 0.3	-0.5; -7.3	**63.7; 67.6**	-29.4; -34.5
4e	-11.1; -13.6	-3.1; 1.3	-1.5; -4.3	9.8; 9.9	-5.2; 13.1	**24.1; 37.0**	10.1; 4.9
4f	7.1; 8.4	-0.2; -2.4	0.3; 10.2	0.9; 2.7	11.9; 2.2	15.9; **22.9**	-19.4; -9.8
4g	17.3; 3.2	-3.3; -5.9	-0.1; 0.2	-1.3; 6.1	-3.2; -5.8	11.6; **26.1**	-20.4; -5.2
4h	6.2; 7.8	-9.5; 0.7	-4.2; 1.5	-0.9; -0.9	-5.8; -8.6	9.0; 9.3	-15.1; -65.1
4i	4.7; 4.7	-11.7; 1.0	-0.9; -7.5	-0.3; -1.6	-11.3; -9.0	10.9; 15.8	-8.3; -8.7
4j	-16.8; -6.2	12.5; 9.3	3.0; 3.4	4.7; 6.5	-1.1; 18.1	**21.2; 27.4**	20.2; 4.4
4k	-8.2; -8.7	-2.8; 4.3	-4.0; 6.0	2.3; 3.2	-0.9; 4.3	**24.8; 32.8**	-34.3; -36.8
4l	-0.5; 12.0	-1.2; 5.8	-13.3; 6.2	-0.6; 2.2	-2.9; -7.6	**35.9; 45.9**	-4.7; -9.5
4m	-2.4; 0.1	-3.1; 1.2	-12.5; 3.9	-2.5; -4.5	-0.8; -2.0	**2.6; 54.4**	-4.7; -6.8
4n	2.4; 6.4	-1.3; -3.8	5.7; 6.1	-0.4; 1.9	-0.1; 8.4	1.1; 5.0	-4.4; 3.6
4o	-2.5; -7.2	-12.3; -7.7	1.5; 5.8	0.2; 2.2	0.2; 1.1	2.1; 4.2	1.7; 5.6
4p	-3.3; 6.8	-0.5; -1.3	-5.8; 1.3	0.7; 1.7	3.4; 9.5	11.8; 8.8	24.5; 25.0
4q	-17.8; -9.1	-12.0; -8.0	-5.2; 1.1	5.0; 8.9	2.8; 4.7	21.8; 8.1	20.9; 8.7
4r	-2.8; -5.2	4.0; 6.8	1.6; 13.2	4.5; 9.2	-5.2; -7.6	**40.4; 41.8**	15.1; 17.2
4s	-14.4; -2.6	9.3; 9.6	-0.0; -5.1	7.5; 8.2	-1.3; -8.0	5.9; 7.8	**28.6; 37.7**
4t	1.9; 11.5	10.9; 7.4	1.4; 4.9	1.8; 3.4	-3.6; 2.7	16.0; 17.2	**17.7; 24.7**
4u	12.6; 15.9	-1.3; 1.0	12.6; 5.5	-1.7; -1.9	12.2; **22.4**	3.6; 4.7	-4.0; -7.2
8a	-3.3; 3.8	-6.5; 1.5	-3.0; -8.6	0.7; 1.0	-4.1; -7.3	**24.9; 32.2**	**21.6; 42.7**
8b	-3.4; -6.4	-0.4; -5.5	-2.1; 1.5	-1.4; -5.4	-5.3; 3.0	**39.1; 41.8**	-0.6; -4.7
8c	-5.9; 0.9	6.9; 8.5	1.2; 6.5	14.1; 7.4	19.6; 5.0	**19.5; 25.6**	-9.4; 17.0
8d	7.6; 7.6	3.3; 3.7	-3.7; 7.5	1.9; 7.9	**28.5; 41.3**	10.5; 19.1	-24.2; -59.0
8e	6.8; 7.6	-4.0; 10.1	0.5; 9.0	2.5; 4.9	**23.0; 38.5**	15.6; 21.8	-78.2; -79.4
9a	**52.6; 63.1**	-1.9; -2.4	15.5; 9.7	11.9; 9.2	15.2; 29.2	**27.3; 32.0**	-22.4; -3.3
9b	-10.5; -19.4	-16.5; -9.9	-12.7; -5.8	-4.3; -6.7	-16.4; -7.2	6.9; 8.0	-1.0; -6.2
9c	**19.1; 23.5**	0.1; 1.7	-1.2; 10.3	10.8; 4.9	1.2; 9.4	5.8; 6.0	12.3; 2.2
9d	-23.7; -29.4	13.4; 9.8	-4.2; 7.7	8.2; 8.7	12.3; 4.0	6.2; 6.7	**36.7; 42.7**
9e	21.1; 5.7	7.9; 8.5	11.6; 15.5	10.0; 3.0	14.3; 3.2	4.2; 7.8	-0.2; 8.2
9f	15.4; 6.6	-0.8; 1.5	4.5; 7.1	2.9; 6.9	10.5; 16.7	1.9; 7.4	-2.3; -4.1
9g	-3.5; -6.2	-10.3; -5.8	-7.6; 7.5	-0.1; -5.2	-3.5; -6.7	4.4; 6.4	1.2; 3.9

[a] Percentage growth inhibition (GI, %).
[b] Highest percentage of antibacterial/antifungal growth inhibition is highlighted in bold.

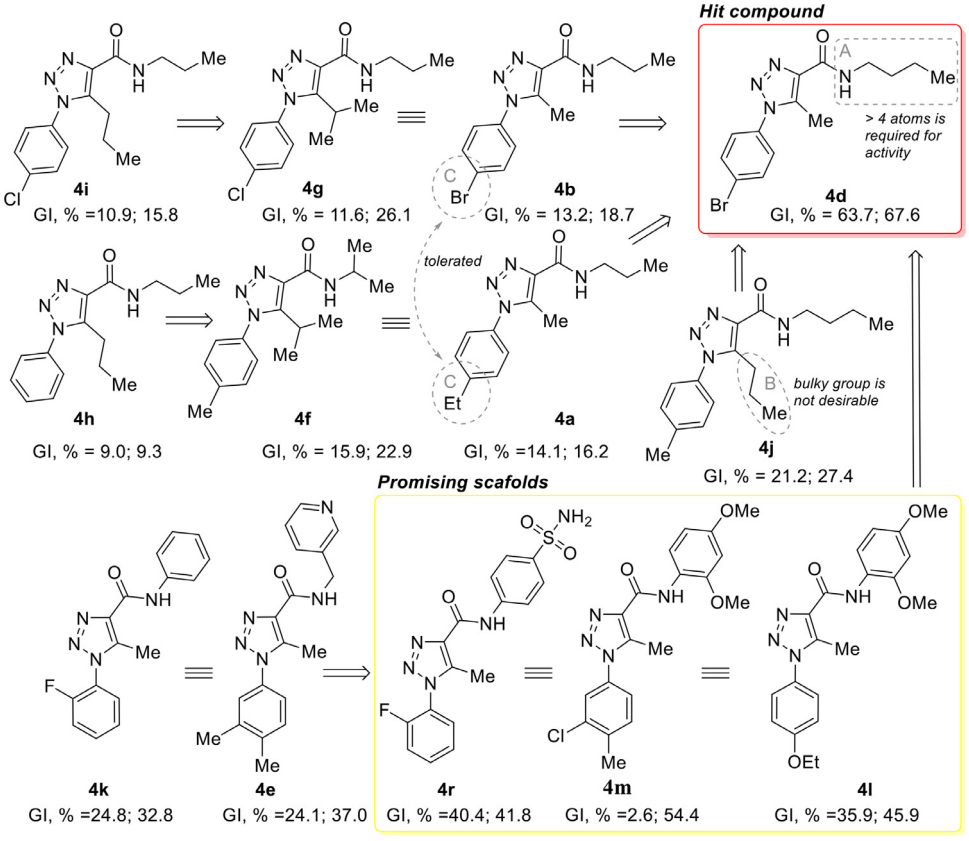


Fig. 4. SAR of 5-alkyl-1*H*-1,2,3-triazole-4-carboxamides **4** against *Candida albicans*.

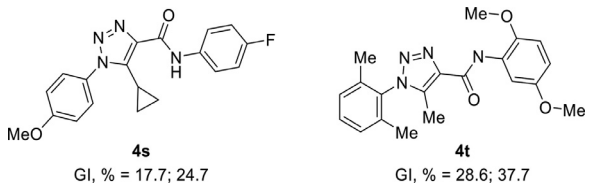


Fig. 5. 5-Alkyl-1H-1,2,3-triazole-4-carboxamides most active against *C. neoformans*.

aryl substituent by almost 90 degrees relative to the triazole plane [38] (Fig. 5).

A replacement of the alkyl moiety with an amino group did not enhance the activity leaving the value of antifungal activity against the strain of *Candida albicans* at the same level (compounds **8a-b, e**, Fig. 6). The compound **8b** was the most active one with reducing growth by 40%. Interestingly, the compound **7a** also showed activity toward the strain of *C. neoformans*. On the other hand, two compounds with substituents in the meta-position inhibited the growth of Gram-negative bacterium acinetobacteria *A. baumannii* (compounds **8e, d** Fig. 6). It should be noted that 5-alkyl triazoles were inactive to *A. bedaumannii* and only one compound, in particular, *N*-(thiazol-2-yl)-1*H*-1,2,3-triazole-4-carboxamide **4u** (GI, % 12.2; 22.4), was low active.

When the rotation of the triazole and aryl rings was "fixed" via condensation of the amino group to the [1,2,3]triazolo[1,5-a]quinazoline-3-carboxamide system **9**, the activity also changed dramatically. Representatives of this class were mainly active against *Staphylococcus aureus*, and can be considered as a potential scaffold for further antibacterial trials. Most of the tested compounds showed moderate activity, but it was found that 5-oxo-*N*-phenyl-4,5-dihydro-[1,2,3]triazolo[1,5-*a*]quinazoline-3-carboxamide **9a** inhibited more than 50% growth of *S. aureus* (Fig. 7). No stable structure–activity correlation was found but the introduction of the substituent in [1,2,3]triazolo[1,5-*a*]quinazoline core led to the loss of activity. Particular emphasis must be placed on the fact that the compound **9d** showed activity against *C. neoformans* (GI, % = 36.7; 42.7).

2.2.2. MIC

The five most active compounds (**4d, 4l, 4r, 8b, 9a**) were further studied at 1 μM concentrations. The compound **4l** demonstrated high antibacterial effect (approximately 50% of growth inhibition under 1 μM of compound) against *S. aureus*, but not so prominent antibacterial effect against *P. aeruginosa* in comparison to other studied compounds (Fig. 8). Furthermore, we observed that the compound **9a** killed nearly 40% of *C. albicans* cells (Fig. 9). Other studied compounds showed slight growth inhibition effect. We assumed that the main effect of those compounds was caused by the DMSO solvent. Based on the obtained results, we can conclude that the proposed chemical structure of compound **9a** can be used for development of potential antifungal agents.

Thus, the compounds **4l, 9a** show high enough antimicrobial effects allowing continuing research on their possible applications in medicine.

2.2.3. Cytotoxicity

The cytotoxicity of the five most active compounds (**4d, 4l, 4r, 8b, 9a**) was evaluated toward human keratinocytes of HaCaT cell line and the results are summarized in Fig. 10. Two compounds (**4l, 4r**) displayed tolerance to keratinocytes even at a high concentration of 50 μM. The compound **4l** inhibited the growth of HaCaT cells by 25.5%, and the compound **4r** – by 17.4%. The compound **8a** reached the IC_{50} at 50 μM for HaCaT cells that is 5-fold higher than the antimicrobial action concentration. Compounds **8b** and **4d** at a concentration of 50 μM inhibited the HaCaT cell growth by approximately of 45%.

3. Conclusion

A series of *N*-substituted 1-aryl-5-substituted-1*H*-1,2,3-triazole-4-carboxamides were designed, synthesized and evaluated for their antimicrobial potential toward the selected pathogens: *Escherichia coli, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa, Staphylococcus aureus, Cryptococcus neoformans var. grubii* and *Candida albicans*. Several 5-methyl-1*H*-1,2,3-triazole-4-carboxamides **4d, 4l, 4r,** showed potent antibacterial effect against *S. aureus.* On the contrary, 5-amino-1*H*-1,2,3-triazole-4-carboxamide **8b** and [1,2,3]triazolo[1,5-a]quinazoline-3-carboxamide **9a** were active against pathogenic yeast *C. albicans.* Thus, the compound **4l** demonstrated 50% growth inhibition under 1 μM toward *S. aureus.* At the same concentration, the compound **9a** killed approximately 40% of *C. albicans* cells. In general, these compounds demonstrated selective action and no impact on the viability of human keratinocytes of HaCaT line. Taking into account that the methods for preparing such compounds are highly variable and meet the requirements of click chemistry, and the compounds **4** and **9** themselves are highly active, 1*H*-1,2,3-triazole-4-carboxamides scaffolds are valuable scaffolds for the discovery of selective antimicrobial agents based on their molecules.

4. Experiment

4.1. Materials and instrumentation

All chemicals used were of laboratory grade and used without further purification. Starting azides **1** were prepared from corresponding amines via diazotization by treatment with sodium nitrite in hydrochloric and following reaction with sodium azide via described procedure [37, 39]. The β-ketoesters **5** and 1*H*-1,2,3-triazole-4-carboxylic acids were reported in our previous articles [29]. Purity of compounds was monitored by TLC on silica F254 coated aluminum plates (Merck) as adsorbent and U.V. light and iodine as visualizing agents. ^{1}H and ^{13}C NMR spectra were recorded on Varian Unity Plus 400 (400 and 101 MHz, respectively) and Bruker 170 Avance 500 (500 and 126 MHz, respectively) spectrometers in DMSO-d_6 solutions using TMS or the deuterated solvent as internal reference. Melting points of compounds were determined in open capillary tubes in a silicon oil bath using a Boetius melting point apparatus and are uncorrected. Mass spectral analyses were performed using an Agilent 1100 series LC/MSD with API-ES/APCI mode (200 eV). Elemental analyses were accomplished using a Carlo Erba 1106 instrument. The absorbance was measured using a Tecan M1000 Pro monochromator and Biotek Synergy HTX plate readers.

4.2. Synthesis of title compounds ***4, 7, 8***

4.2.1. General procedure for the synthesis of 1-aryl-5-methyl-1H-1,2,3-triazole-4-carboxyamide ***4a-e***

An appropriate amine **3** (10.0 mmol), arylazide **1** (10.0 mmol), and triethylamine 1.4 mL were added to the solution of diketene (10.0 mmol) in dry acetonitrile (20 mL). The mixture was heated under reflux during 30 min. Then, it was cooled to room temperature, and the solid started to sediment. The product was filtered and washed with methanol to give triazole **3** as a white solid. When the amide was well soluble in acetonitrile, water was added dropwise until the solid started to appear. The compounds were found to be of sufficient purity without further purification.

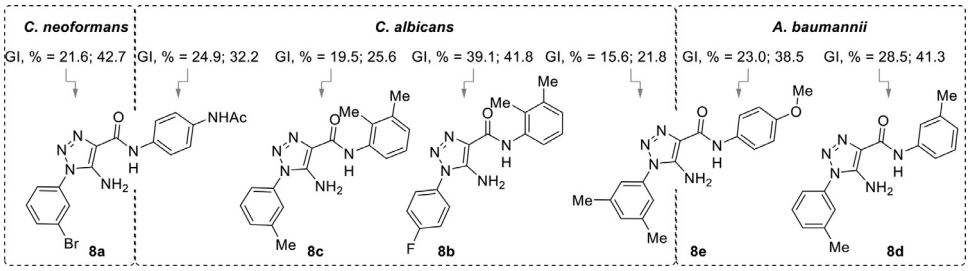


Fig. 6. The most active 5-amino-1H-1,2,3-triazole-4-carboxamides.

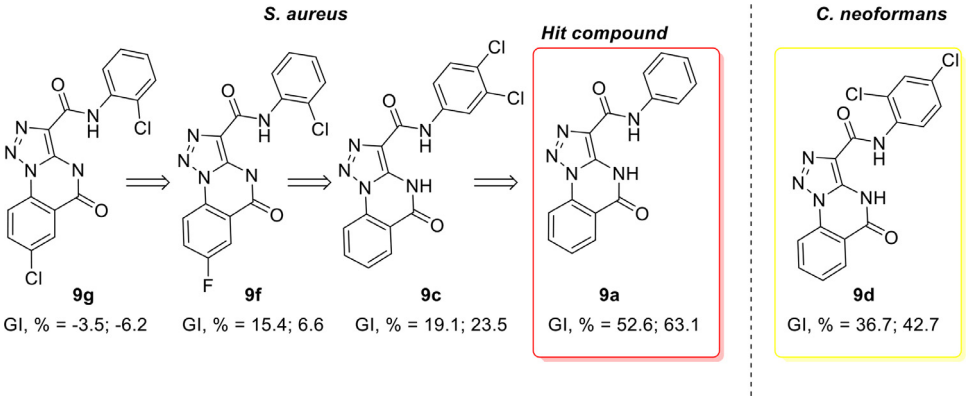


Fig. 7. The most active [1,2,3]triazolo[1,5-a]quinazoline-3-carboxamides.

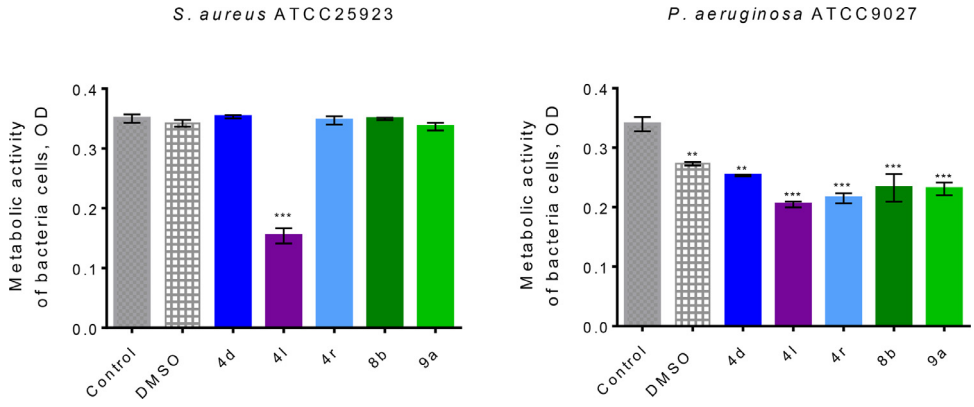


Fig. 8. Antibacterial effect of the tested compounds (1 μM per sample) toward *S. aureus* and *P. aeruginosa*. ** – $p \leq 0.01$, *** – $p \leq 0.001$ (difference compared with the not treated control cells).

*4.2.1.1. 1-(4-Ethylphenyl)-5-methyl-N-propyl-1H-1,2,3-triazole-4-carboxamide (**4a**).* White solid; Yield 95%; m.p. 114–115 °C (ethanol); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 8.55 (t, J = 6.3 Hz, 1H, NH), 7.52 (d, J = 7.7 Hz, 2H, H_{Ar}-2,6), 7.46 (d, J = 7.9 Hz, 2H, H_{Ar}-3,5), 3.31 – 3.15 (m, 2H, CH_2N), 2.72 (q, J = 7.4 Hz, 2H, CH_2), 2.50 (s, 3H, CH_3), 1.63 – 1.48 (m, 2H, CH_2), 1.23 (t, J = 7.4 Hz, 3H, CH_3), 0.88 (t, J = 7.1 Hz, 3H, CH_3); ^{13}C NMR (126 MHz, DMSO-d_6): δ 160.69 (CO), 145.78 (C_{Ar}-4), 138.22 (C_{Tr}-5), 136.30 (C_{Tr}-4), 133.12 (C_{Ar}-1), 128.88 (2xCH_{Ar}-3,5), 125.23 (2xCH_{Ar}-2,6), 40.02 (CH_2N), 27.79 (CH_2), 22.51 (CH_2), 15.36 (CH_3), 11.33 (CH_3), 9.23 (CH_3). MS (m/z): 273 (M^++1); anal. calcd. for $C_{15}H_{20}N_4O$: C, 66.15; H, 7.40; N, 20.57; found: C, 66.23; H, 7.43; N, 20.50.

*4.2.1.2. 1-(4-Bromophenyl)-5-methyl-N-propyl-1H-1,2,3-triazole-4-carboxamide (**4b**).* White solid; Yield 96%; m.p. 124–125 °C (ethanol); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 8.56 (t, J = 5.8 Hz, 1H, NH), 7.84 (d, J = 8.6 Hz, 2H, H_{Ar}-2,6), 7.60 (d, J = 8.6 Hz, 2H, H_{Ar}-3,5), 3.22 (q, J = 6.6 Hz, 2H, CH_2N), 2.52 (s, 3H, CH_3), 1.63 – 1.44 (m, 2H, CH_2), 0.87 (t, J = 7.4 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6): δ 161.07 (CO), 138.89 (C_{Tr}-5), 137.08 (C_{Tr}-4), 135.14 (C_{Ar}-1), 133.15 (2xCH_{Ar}-3,5), 127.90 (2xCH_{Ar}-2,6), 123.61 (C_{Ar}-4), 40.55 (CH_2N), 23.00 (CH_2), 11.84 (CH_3), 9.71 (CH_3). MS (m/z): 323, 325 (M^++1); anal. calcd. for $C_{13}H_{15}BrN_4O$: C, 48.31; H, 4.68; N, 17.34; found: C, 48.27; H, 4.75; N, 17.31.

*4.2.1.3. 1-(3-Chloro-2-methylphenyl)-5-methyl-N-propyl-1H-1,2,3-triazole-4-carboxamide (**4c**).* White solid; Yield 91%; m.p. 114–115 °C (ethanol); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 8.59 (t, J = 5.7 Hz, 1H, NH), 7.81 – 7.67 (m, 1H, H_{Ar}), 7.51 – 7.46 (m, 2H, H_{Ar}), 3.23 (q, J = 6.7 Hz, 2H, CH_2N), 2.33 (s, 3H, CH_3), 1.95 (s, 3H, CH_3), 1.61 – 1.46 (m, 2H, CH_2), 0.88 (t, J = 7.4 Hz, 3H, CH_3); ^{13}C NMR (126 MHz, DMSO-d_6): δ 161.04 (CO), 138.44 (C_{Tr}-5), 137.94 (C_{Tr}-4), 135.95 (C_{Ar}-2), 135.18 (C_{Ar}-1), 134.11 (C_{Ar}-3), 131.91 (CH_{Ar}-4), 128.59 (CH_{Ar}-5), 127.17 (CH_{Ar}-6), 40.58 (CH_2N), 23.00 (CH_2), 15.12 (CH_3), 11.87 (CH_3), 9.13 (CH_3).

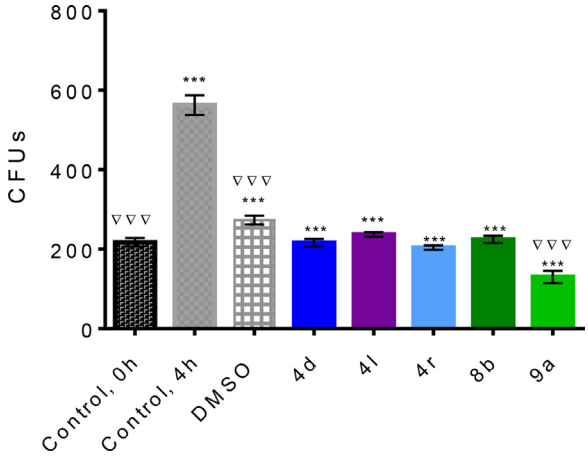


Fig. 9. Antifungal effects of the tested compounds (1 μM per sample) towards *C. albicans* ATCC 885653. C0 – background (basic number) Colony Formation Units (CFU), C4 – CFU number after 4 h of incubation. ▽▽▽ – $p \leq 0.001$ (difference compared with not treated control cells of C0), *** – $p \leq 0.001$ (difference compared with the not treated control cells of C4).

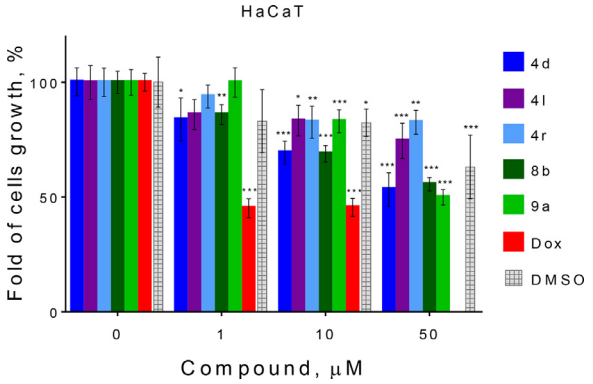
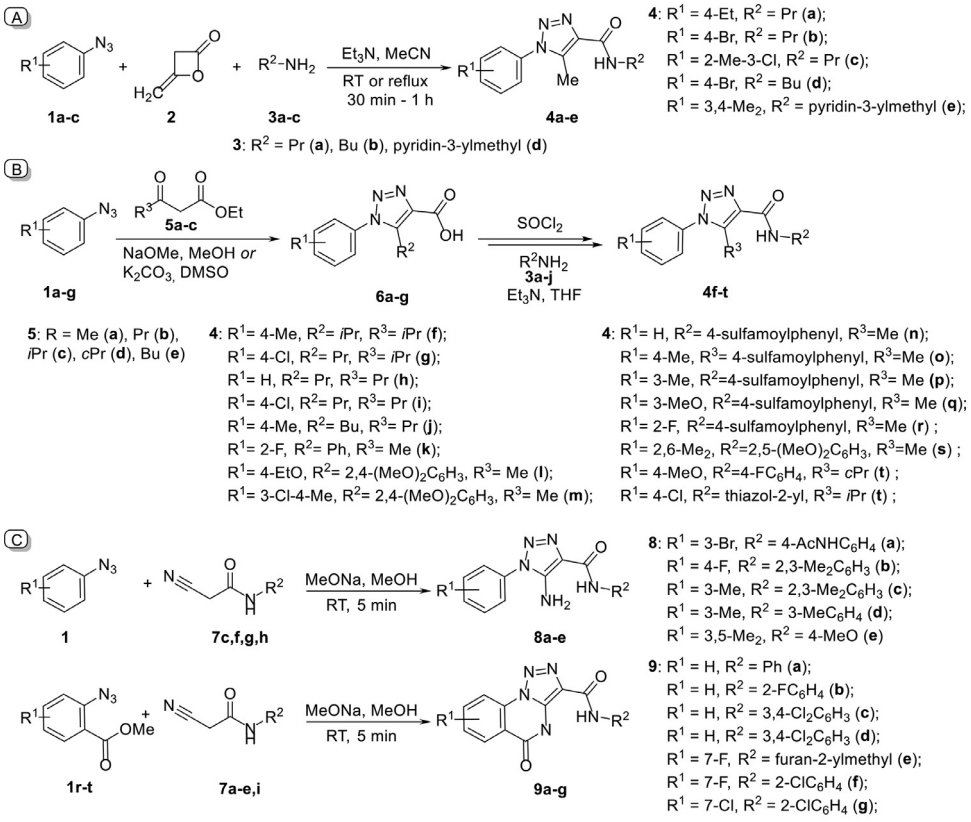


Fig. 10. Cytotoxicity of triazole-4-carboxamides **4d, 4l, 4r, 8b, 9a** towards human keratinocytes of HaCaT line. After a total experimental time (72 h), cell vitality was detected by the MTT assay. * – $p \leq 0.05$; ** – $p \leq 0.01$; *** – $p \leq 0.001$ (difference compared with the not treated control cells).

MS (m/z): 293 (M^++1); anal. calcd. for $C_{14}H_{17}ClN_4O$: C, 57.44; H, 5.85; N, 19.14; found: C, 57.49; H, 5.79; N, 19.21.

*4.2.1.4. 1-(4-Bromophenyl)-N-butyl-5-methyl-1H-1,2,3-triazole-4-carboxamide (**4d**).* White solid; Yield 97%; m.p. 144–145 °C (ethanol); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.55 (t, J = 5.6 Hz, 1H, NH), 7.84 (d, J = 8.5 Hz, 2H, H_{Ar}-2,6), 7.60 (d, J = 8.5 Hz, 2H, H_{Ar}-3,5), 3.31 – 3.20 (m, 2H, CH_2N), 2.52 (s, 3H, CH_3), 1.58 – 1.42 (m, 2H, CH_2), 1.35 – 1.23 (m, 2H, CH_2), 0.89 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (126 MHz, DMSO-d_6): δ 161.03 (CO), 138.89 (C_{Tr}-5), 137.07 (C_{Tr}-4), 135.13 (C_{Ar}-1), 133.15 (2xCH_{Ar}-3,5), 127.90 (2xCH_{Ar}-2,6), 123.60 (C_{Ar}-4), 38.43 (CH_2N), 31.85 (CH_2), 20.07 (CH_2), 14.19 (CH_3), 9.71 (CH_3). MS (m/z): 337, 339 (M^++1); anal. calcd. for $C_{14}H_{17}BrN_4O$: C, 49.77; H, 5.21; N, 16.71; found: C, 49.87; H, 5.20; N, 16.61.



Scheme 1. Synthetic routes to the target 1H-1,2,3-triazole-4-carboxamides.

*4.2.1.5. 1-(3,4-Dimethylphenyl)-5-methyl-*N*-(pyridin-3-ylmethyl)-1*H*-1,2,3-triazole-4-carboxamide (**4e**).* White solid; Yield 95%; m.p. 157–158 °C (ethanol); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.29 – 9.23 (m, 1H), 8.56 (d, J = 2.7 Hz, 1H, H_{Py}), 8.44 (t, J = 4.0 Hz, 1H, H_{Py}), 7.77 – 7.71 (m, 1H), 7.48 – 7.17 (m, 4H, $H_{Arom.}$), 4.50 – 4.46 (m, 2H, CH_2), 2.48 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 2.29 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6): δ 161.48 (CO), 149.41 (CH_{Py}-2), 148.51 (CH_{Py}-6), 138.98 (C_{Tr}-5), 138.55 (C_{Ar}-3), 138.32 (C_{Tr}-4), 137.17 (C_{Ar}-4), 135.71 (C_{Ar}-1), 135.66 (CH_{Py}-4), 133.54 (C_{Py}-3), 130.81 (CH_{Ar}-5), 126.52 (CH_{Ar}-2), 123.91 (CH_{Ar}-6), 123.01 (CH_{Py}-5), 40.13 (CH_2N), 19.72 (CH_3), 19.58 (CH_3), 9.76 (CH_3). MS (m/z): 322 (M^++1); anal. calcd. for $C_{18}H_{19}N_5O$: C, 67.27; H, 5.96; N, 21.79; found: C, 67.32; H, 5.91; N, 21.71.

*4.2.2. General procedure for the synthesis of 1-aryl-5-substituted-1H-1,2,3-triazole-4-carboxyamide **4f-t***

The solution of the appropriate 1,2,3-triazole-4-carboxylic acid **6** (2.0 mmol) in $SOCl_2$ (2 mL) was heated under reflux for 1h. Then, the mixture was evaporated to dryness to give a residue, which was washed, and hexane dissolved in anhydrous methylene dichloride (10 mL). This solution was added in a dropwise manner to a solution of organic amine **3** (2.0 mmol) and TEA (0.5 mL, 3.5 mmol) in anhydrous methylene dichloride (10 mL), and the resulting mixture was heated under reflux for 30 min. The reaction mixture was cooled to ambient and washed sequentially with 0.5 N HCl (10 mL), 0.5 M Na_2CO_3 (10 mL) and water (10 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness to give the desired product as a solid, which found to be of sufficient purity without further purification.

4.2.2.1. N,*5-Diisopropyl-1-*p*-tolyl-1*H*-1,2,3-triazole-4-carboxamide (**4f**).* White solid; Yield 89%; m.p. 102–103 °C (ethanol); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.27 (d, J = 8.1 Hz, 1H, NH), 7.44 (d, J = 8.0 Hz, 2H, H_{Ar}-2,6), 7.39 (d, J = 8.0 Hz, 2H, H_{Ar}-3,5), 4.21 – 4.08 (m, 1H, CHN), 3.17 (hept, J = 7.0 Hz, 1H, CH), 2.43 (s, 3H, CH_3), 1.26 (d, J = 7.0 Hz, 6H, CH_3), 1.19 (d, J = 6.5 Hz, 6H, CH_3); ^{13}C NMR (126 MHz, DMSO-d_6): δ 159.67 (CO), 144.92 (C_{Ar}-4), 140.27 (C_{Tr}-5), 137.95 (C_{Tr}-4), 133.35 (C_{Ar}-1), 130.01 (2xCH_{Ar}-3,5), 126.42 (2xCH_{Ar}-2,6), 40.23 (CH_2N), 24.18 (CH), 22.22 (2xCH_3), 20.75 (CH_3), 19.99 (2xCH_3). MS (m/z): 287 (M^++1); anal. calcd. for $C_{16}H_{22}N_4O$: C, 67.11; H, 7.74; N, 19.56; found: C, 67.05; H, 7.78; N, 19.49.

*4.2.2.2. 1-(4-Chlorophenyl)-5-isopropyl-*N*-propyl-1*H*-1,2,3-triazole-4-carboxamide (**4g**).* White solid; Yield 90%; m.p. 87–88 °C (ethanol); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.58 (t, J = 5.4 Hz, 1H, NH), 7.72 (d, J = 8.5 Hz, 2H, H_{Ar}-2,6), 7.60 (d, J = 8.5 Hz, 2H, H_{Ar}-3,5), 3.24 (q, J = 6.6 Hz, 2H, CH_2N), 3.16 (hept, J = 7.0 Hz, 1H, CH), 1.61 – 1.48 (m, 2H, CH_2), 1.27 (d, J = 7.0 Hz, 6H, CH_3), 0.88 (t, J = 7.4 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6): δ 160.89 (CO), 145.56 (C_{Tr}-5), 138.60 (C_{Tr}-4), 135.67 (C_{Ar}-1), 135.14 (C_{Ar}-4), 130.22 (2xCH_{Ar}-3,5), 129.08 (2xCH_{Ar}-2,6), 40.70 (CHN), 24.71 (CH_2), 23.00 (CH_2), 20.48 (2xCH_3), 11.84 (CH_3). MS (m/z): 307 (M^++1); anal. calcd. for $C_{15}H_{19}ClN_4O$: C, 58.72; H, 6.24; N, 18.26; found: C, 58.77; H, 6.34; N, 18.21.

*4.2.2.3. 1-Phenyl-*N*,5-dipropyl-1*H*-1,2,3-triazole-4-carboxamide (**4h**).* White solid; Yield 97%; m.p. 49–50 °C (ethanol); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.56 (t, J = 5.8 Hz, 1H, NH), 7.67 – 7.61 (m, 3H, H_{Ph}), 7.60 – 7.55 (m, 2H, H_{Ph}), 3.23 (q, J = 6.6 Hz, 2H, CH_2N), 2.99 – 2.86 (m, 2H, CH_2), 1.63 – 1.47 (m, 2H, CH_2), 1.45 – 1.32 (m, 2H, CH_2), 0.87 (t, J = 7.4 Hz, 3H, CH_3), 0.70 (t, J = 7.4 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6): δ 161.01 (CO), 140.66 (C_{Tr}-5), 138.72 (C_{Tr}-4), 136.06 (C_{Ph}-1), 130.68 (CH_{Ph}-4), 130.23 (2xCH_{Ar}-3,5), 126.36 (2xCH_{Ar}-2,6), 40.55 (CH_2N), 24.70 (CH_2), 23.02 (CH_2), 21.76 (CH_2), 13.93 (CH_3), 11.83 (CH_3). MS (m/z): 273 (M^++1); anal. calcd. for $C_{15}H_{20}N_4O$: C, 66.15; H, 7.40; N, 20.57; found: C, 66.23; H, 7.43; N, 20.50.

*4.2.2.4. 1-(4-Chlorophenyl)-N,5-dipropyl-1H-1,2,3-triazole-4-carboxamide (**4i**).* White solid; Yield 93%; m.p. 88–89 °C (ethanol); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.58 (t, J = 5.3 Hz, 1H, NH), 7.71 (d, J = 7.5 Hz, 2H), 7.65 (d, J = 7.5 Hz, 2H), 3.28 – 3.16 (m, 2H, CH_2N), 3.00 – 2.87 (m, 2H), 1.60 – 1.48 (m, 2H), 1.45 – 1.33 (m, 2H), 0.87 (t, J = 7.5 Hz, 3H, CH_3), 0.71 (s, 3H, CH_3); ^{13}C NMR (126 MHz, DMSO-d_6): δ 160.40 (CO), 140.35 (C_{Tr}-5), 138.28 (C_{Tr}-4), 134.85 (C_{Ar}-1), 134.37 (C_{Ar}-4), 129.81 (2xCH_{Ar}-3,5), 127.71 (2xCH_{Ar}-2,6), 40.05 (CH_2N), 24.15 (CH_2), 22.50 (CH_2), 21.27 (CH_2), 13.42 (CH_3), 11.31 (CH_3). MS (m/z): 307 (M^++1); anal. calcd. for $C_{15}H_{19}ClN_4O$: C, 58.73; H, 6.24; N, 18.26; found: C, 58.81; H, 6.29; N, 18.17.

4.2.2.5. N*-Butyl-5-propyl-1-p-tolyl-1*H*-1,2,3-triazole-4-carboxamide (**4j**).* White solid; Yield 92%; m.p. 72–73 °C (ethanol); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.64 – 8.45 (m, 1H, NH), 7.54 – 7.34 (m, 4H, H_{Ar}), 3.30 – 3.22 (m, 2H, CH_2), 2.96 – 2.84 (m, 2H, CH_2), 2.41 (d, J = 3.1 Hz, 3H, CH_3), 1.56 – 1.46 (m, 2H, CH_2), 1.44 – 1.35 (m, 2H, CH_2), 1.34 – 1.26 (m, 2H, CH_2), 0.94 – 0.85 (m, 3H, CH_3), 0.74 – 0.64 (m, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6): δ 161.00 (CO), 140.61 (C_{Ar}-4), 140.46 (C_{Tr}-5), 138.66 (C_{Tr}-4), 133.60 (C_{Ar}-1), 130.62 (2xCH_{Ar}-3,5), 126.14 (2xCH_{Ar}-2,6), 38.44 (CH_2N), 31.87 (CH_2), 24.70 (CH_2), 21.78 (CH_2), 21.23 (CH_2), 20.09 (CH_3), 14.17 (CH_3), 13.93 (CH_3). MS (m/z): 301 (M^++1); anal. calcd. for $C_{17}H_{24}N_4O$: C, 67.97; H, 8.05; N, 18.65; found: C, 67.90; H, 8.11; N, 18.74.

*4.2.2.6. 1-(2-Fluorophenyl)-5-methyl-*N*-phenyl-1*H*-1,2,3-triazole-4-carboxamide (**4k**).* White solid; Yield 94%; m.p. 114–115 °C (ethanol); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.54 (s, 1H, NH), 7.86 (d, J = 7.7 Hz, 2H, H_{ArN}-2,6), 7.79 – 7.70 (m, 2H, H_{Ar}), 7.62 (t, J = 8.9 Hz, 1H, H_{Ar}-4), 7.51 (t, J = 7.6 Hz, 1H, H_{Ar}-5), 7.34 (t, J = 7.9 Hz, 2H, H_{ArN}-3,5), 7.10 (t, J = 7.4 Hz, 1H, H_{ArN}-3,5), 2.48 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 159.72 (CO), 156.35 (d, $^1J_{C\text{-}F}$ = 251.7 Hz, C_{Ar}-2), 139.53 (C_{Tr}-5), 139.04 (C_{Tr}-4), 138.51 (C_{Ph}-1), 133.63 (d, $^3J_{C\text{-}F}$ = 7.9 Hz, CH_{Ar}-4), 129.59 (CH_{Ph}-4), 129.07 (2xCH_{Ph}-3,5), 126.20 (d, $^3J_{C\text{-}F}$ = 3.8 Hz, CH_{Ar}-6), 124.26 (CH_{Ar}-5), 123.16 (d, $^2J_{C\text{-}F}$ = 12.1 Hz, C_{Ar}-1), 120.97 (2xCH_{Ph}-2,6), 117.58 (d, $^2J_{C\text{-}F}$ = 19.1 Hz, C_{Ar}-3), 11.06 (CH_3). MS (m/z): 297 (M^++1); anal. calcd. for $C_{16}H_{13}FN_4O$: C, 64.86; H, 4.42; N, 18.91; found: C, 64.94; H, 4.47; N, 18.94.

4.2.2.7. N*-(2,4-Dimethoxyphenyl)-1-(4-ethoxyphenyl)-5-methyl-1*H*-1,2,3-triazole-4-carboxamide (**4l**).* White solid; Yield 92%; m.p. 174-175 °C (ethanol); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.39 (s, 1H, NH), 8.10 (d, J = 7.4 Hz, 1H, H_{ArN}-6), 7.53 (d, J = 8.5 Hz, 2H, H_{Ar}-2,6), 7.15 (d, J = 8.3 Hz, 2H, H_{Ar}-3,5), 6.70 (s, 1H, H_{ArN}-3), 6.55 (d, J = 8.4 Hz, 1H, H_{ArN}-5), 4.19 – 4.08 (m, 2H, CH_2O), 3.91 (s, 3H, CH_3O), 3.77 (s, 3H, CH_3O), 2.52 (s, 3H, CH_3), 1.37 (t, J = 5.9 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6): δ 160.01 (C_{Ar}-4), 158.81 (CO), 157.03 (C_{ArN}-4), 150.57 (C_{ArN}-2), 138.22 (C_{Tr}-5), 137.82 (C_{Tr}-4), 128.35 (C_{Ar}-1), 127.42 (2xCH_{Ph}-2,6), 121.40 (CH_{ArN}-6), 120.66 (C_{ArN}-1), 115.63 (2xCH_{Ar}-3,5), 104.73 (CH_{ArN}-5), 99.37 (CH_{ArN}-3), 64.12 (CH_2O), 56.57 (CH_3O), 55.85 (CH_3O), 15.02 (CH_3), 9.77 (CH_3). MS (m/z): 383 (M^++1); anal. calcd. for $C_{20}H_{22}N_4O_4$: C, 62.82; H, 5.80; N, 14.65; found: C, 62.79; H, 5.91; N, 14.71.

*4.2.2.8. 1-(3-Chloro-4-methylphenyl)-*N*-(2,4-dimethoxyphenyl)-5-methyl-1*H*-1,2,3-triazole-4-carboxamide (**4m**).* White solid; Yield 91%; m.p. 193-194 °C (ethanol); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.37 (s, 1H, NH), 8.11 (d, J = 8.6 Hz, 1H, H_{ArN}-6), 7.77 (s, 1H, H_{Ar}-2), 7.63 (d, J = 7.9 Hz, 1H, H_{Ar}-6),

7.54 (d, J = 7.6 Hz, 1H, H_{Ar}-5), 6.71 (s, 1H, H_{ArN}-3), 6.56 (d, J = 8.3 Hz, 1H, H_{ArN}-5), 3.92 (s, 3H, CH_3O), 3.79 (s, 3H, CH_3O), 2.58 (s, 3H, CH_3), 2.46 (s, 3H, CH_3); ^{13}C NMR (126 MHz, DMSO-d_6): δ 158.17 (CO), 156.61 (C_{ArN}-4), 150.17 (C_{ArN}-2), 137.90 (C_{Tr}-5), 137.90 (C_{Ar}-1), 137.54 (C_{Tr}-4), 134.03 (C_{Ar}-4), 133.88 (C_{Ar}-3), 132.04 (CH_{Ar}-5), 125.66 (CH_{Ar}-2), 124.18 (CH_{Ar}-6), 121.05 (CH_{ArN}-6), 120.07 (C_{ArN}-1), 104.26 (CH_{ArN}-5), 98.88 (CH_{ArN}-3), 56.07 (CH_3O), 55.35 (CH_3O), 19.38 (CH_3), 9.24 (CH_3). MS (m/z): 387 (M^++1); anal. calcd. for $C_{19}H_{19}ClN_4O_3$: C, 58.99; H, 4.95; N, 14.48; found: C, 58.91; H, 4.93; N, 14.41.

*4.2.2.9. 5-Methyl-1-phenyl-*N*-(4-sulfamoylphenyl)-1*H*-1,2,3-triazole-4-carboxamide (**4n**).* White solid; Yield 97%; m.p. 308-309 °C (ethanol-DMF); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.80 (s, 1H, NH), 8.05 (d, J = 8.7 Hz, 2H, H_{Ar}-3,5), 7.80 (d, J = 8.6 Hz, 2H, H_{Ar}-2,6), 7.71 – 7.58 (m, 5H, H_{Ph}), 7.24 (s, 2H, NH_2), 2.58 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6): δ 160.35 (CO), 142.11 (C_{ArN}-1), 139.27 (C_{ArN}-4), 138.66 (C_{Tr}-5), 138.43 (C_{Tr}-4), 135.69 (C_{Ph}-1), 130.64 (CH_{Ph}-4), 130.24 (2xCH_{Ph}-3,5), 126.94 (2xCH_{ArN}-3,5), 125.97 (2xCH_{Ph}-2,6), 120.48 (2xCH_{ArN}-2,6), 10.00 (CH_3). MS (m/z): 358 (M^++1); anal. calcd. for $C_{16}H_{15}N_5O_3S$: C, 53.77; H, 4.23; N, 19.60; found: C, 53.84; H, 4.23; N, 19.60.

*4.2.2.10. 5-Methyl-*N*-(4-sulfamoylphenyl)-1-p-tolyl-1*H*-1,2,3-triazole-4-carboxamide (**4o**).* White solid; Yield 95%; m.p. 294-295 °C (ethanol-DMF); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.84 (s, 1H, NH), 8.06 (d, J = 8.4 Hz, 2H, H_{Ar}-3,5), 7.80 (d, J = 8.4 Hz, 2H, H_{Ar}-2,6), 7.54 (d, J = 7.7 Hz, 2H, H_{Tol}-2,6), 7.46 (d, J = 7.6 Hz, 2H, H_{Tol}-3,5), 7.28 (s, 2H, NH_2), 2.57 (s, 3H, CH_3), 2.43 (s, 3H, CH_3); ^{13}C NMR (126 MHz, DMSO-d_6): δ 159.86 (CO), 141.61 (C_{ArN}-1), 139.95 (C_{ArN}-4), 138.74 (C_{Tr}-5), 138.08 (C_{Tr}-4), 137.84 (C_{Tol}-4), 132.73 (C_{Tol}-1), 130.10 (2xCH_{Tol}-3,5), 126.42 (2xCH_{ArN}-3,5), 125.25 (2xCH_{Tol}-2,6), 119.95 (2xCH_{ArN}-2,6), 20.74 (CH_3), 9.46 (CH_3). MS (m/z): 372 (M^++1); anal. calcd. for $C_{17}H_{17}N_5O_3S$: C, 54.97; H, 4.61; N, 18.86; found: C, 54.90; H, 4.73; N, 18.93.

*4.2.2.11. 5-Methyl-*N*-(4-sulfamoylphenyl)-1-m-tolyl-1*H*-1,2,3-triazole-4-carboxamide (**4p**).* White solid; Yield 91%; m.p. 253-254 °C (ethanol-DMF); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.86 (s, 1H, NH), 8.06 (d, J = 8.6 Hz, 2H, H_{Ar}-3,5), 7.80 (d, J = 8.5 Hz, 2H, H_{Ar}-2,6), 7.53 (t, J = 7.5 Hz, 1H, H_{Tol}-3), 7.50 – 7.42 (m, 3H, H_{Tol}), 7.29 (s, 2H, NH_2), 2.58 (s, 3H, CH_3), 2.43 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6): δ 160.35 (CO), 142.12 (C_{ArN}-1), 140.13 (CH_{Tol}-2), 139.26 (C_{ArN}-4), 138.57 (C_{Tr}-5), 138.37 (C_{Tr}-4), 135.62 (C_{Tol}-3), 131.22 (C_{Tol}-1), 129.98 (CH_{Tol}-5), 126.94 (2xCH_{ArN}-3,5), 126.31 (CH_{Tol}-4), 122.98 (CH_{Tol}-6), 120.47 (2xCH_{ArN}-2,6), 21.25 (CH_3), 10.00 (CH_3). MS (m/z): 372 (M^++1); anal. calcd. for $C_{17}H_{17}N_5O_3S$: C, 54.97; H, 4.61; N, 18.86; found: C, 54.95; H, 4.55; N, 18.81.

*4.2.2.12. 1-(3-Methoxyphenyl)-5-methyl-*N*-(4-sulfamoylphenyl)-1*H*-1,2,3-triazole-4-carboxamide (**4q**).* White solid; Yield 87%; m.p. 234-235 °C (ethanol-DMF); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.85 (s, 1H, NH), 8.05 (d, J = 8.8 Hz, 2H, H_{ArN}-3,5), 7.80 (d, J = 8.7 Hz, 2H, H_{ArN}-2,6), 7.56 (t, J = 8.1 Hz, 1H, H_{Ar}-5), 7.28 (s, 2H, NH_2), 7.26 – 7.18 (m, 3H, H_{Ar}-5), 3.84 (s, 3H, CH_3O), 2.59 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6): δ 160.43 (C_{Ar}-3), 160.34 (CO), 142.11 (C_{ArN}-1), 139.27 (C_{ArN}-4), 138.74 (C_{Tr}-5), 138.36 (C_{Tr}-4), 136.68 (C_{Ar}-1), 131.09 (CH_{Ar}-3), 126.94 (2xCH_{ArN}-3,5), 120.48 (2xCH_{ArN}-2,6), 118.07 (CH_{Ar}-6), 116.40 (CH_{Ar}-4), 111.74 (CH_{Ar}-2), 56.19 (CH_3O), 10.01 (CH_3). MS (m/z): 388 (M^++1); anal. calcd. for $C_{17}H_{17}N_5O_4S$: C, 52.70; H, 4.42; N, 18.08; found: C, 52.77; H, 4.48; N, 18.25.

*4.2.2.13. 1-(2-Fluorophenyl)-5-methyl-*N*-(4-sulfamoylphenyl)-1*H*-1,2,3-triazole-4-carboxamide (**4r**).* White solid; Yield 88%; m.p. 283-284 °C (ethanol-DMF); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.91 (s, 1H, NH), 8.06 (d, J = 8.6 Hz, 2H, H_{Ar}-3,5), 7.81 (d, J = 8.6 Hz, 2H, H_{Ar}-2,6), 7.79 – 7.72 (m, 2H, H_{Ar}-4,6), 7.63 (t, J = 9.0 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H, H_{Ar}-5), 7.29 (s, 2H, NH_2), 2.49 (s, 3H, CH_3); ^{13}C NMR (126 MHz, DMSO-d_6): δ 159.57 (CO), 155.83 (d, $^1J_{C\text{-}F}$ = 251.7 Hz, C_{Ar}-2), 141.53 (C_{ArN}-1), 139.54 (C_{ArN}-4), 138.84 (C_{Tr}-5), 137.68 (C_{Tr}-4), 133.19 (d, $^3J_{C\text{-}F}$ = 7.6 Hz), 129.07 (CH_{Ar}-5), 126.44 (2xCH_{ArN}-3,5), 125.71 (d, $^3J_{C\text{-}F}$ = 3.6 Hz, CH_{Ar}-4), 122.56 (d, $^2J_{C\text{-}F}$ = 12.5 Hz, C_{Ar}-1), 120.03 (2xCH_{ArN}-2,6), 117.09 (d, $^2J_{C\text{-}F}$ = 18.9 Hz, CH_{Ar}-3), 8.82 (CH_3). MS (m/z): 376 (M^++1); anal. calcd. for $C_{16}H_{14}FN_5O_3S$: C, 51.19; H, 3.76; N, 18.66; found: C, 51.05; H, 3.71; N, 18.75.

4.2.2.14. N*-(2,5-Dimethoxyphenyl)-1-(2,6-dimethylphenyl)-5-methyl-1*H*-1,2,3-triazole-4-carboxamide (**4s**).* White solid; Yield 93%; m.p. 144-145 °C (ethanol); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.62 (s, 1H, NH), 8.03 (s, 1H, H_{ArN}-2), 7.47 (t, J = 7.2 Hz, 1H, H_{Ar}-4), 7.35 (d, J = 7.2 Hz, 2H, H_{Ar}-3,5), 7.05 (d, J = 8.6 Hz, 1H, H_{ArN}-4), 6.68 (d, J = 8.6 Hz, 1H, H_{ArN}-5), 3.90 (s, 3H, CH_3O), 3.74 (s, 3H, CH_3O), 2.35 (s, 3H, CH_3), 1.91 (s, 6H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6): δ 158.40 (CO), 153.13 (C_{ArN}-3), 142.48 (C_{ArN}-6), 138.23 (C_{Tr}-5), 137.62 (C_{Tr}-4), 135.48 (2xC_{Ar}-2,6), 133.14 (C_{Ar}-1), 130.75 (CH_{Ar}-4), 128.66 (2xCH_{Ar}-3,5), 127.48 (C_{ArN}-1), 111.59 (CH_{ArN}-5), 107.86 (CH_{ArN}-4), 106.14 (CH_{ArN}-2), 56.42 (CH_3O), 55.37 (CH_3O), 16.67 (2xCH_3), 8.30 (CH_3). MS (m/z): 367 (M^++1); anal. calcd. for $C_{20}H_{22}N_4O_3$: C, 65.56; H, 6.05; N, 15.29; found: C, 65.63; H, 6.00; N, 15.35.

*4.2.2.15. 5-Cyclopropyl-*N*-(4-fluorophenyl)-1-(4-methoxyphenyl)-1*H*-1,2,3-triazole-4-carboxamide (**4t**).* White solid; Yield 96%; m.p. 156-157 °C (ethanol); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.51 (s, 1H, NH), 7.87 (dd, J = 7.9, 3.7 Hz, 2H, H_{ArN}-2,6), 7.59 (d, J = 7.9 Hz, 2H, H_{Ar}-2,6), 7.24 – 7.13 (m, 4H, $H_{Arom.}$), 3.86 (s, 3H, CH_3O), 2.11 – 1.99 (m, 1H, CH), 0.98 – 0.77 (m, J = 9.5 Hz, 4H, CH_2). ^{13}C NMR (101 MHz, DMSO-d_6): δ 160.64 (CO), 159.51 (C_{Ar}-4), 158.76 (d, $^1J_{C\text{-}F}$ = 240.1 Hz, C_{ArN}-4), 142.13 (C_{Tr}-5), 138.99 (C_{Tr}-4), 135.63 (C_{ArN}-1), 129.15 (C_{Ar}-1), 127.82 (2xCH_{Ar}-2,6), 122.58 (d, $^3J_{C\text{-}F}$ = 7.7 Hz, 2xCH_{ArN}-2,6), 115.61 (d, $^2J_{C\text{-}F}$ = 22.2 Hz, 2xCH_{ArN}-3,5), 115.03 (2xCH_{Ar}-3,5), 56.09 (CH_3O), 8.10 (2xCH_2), 5.77 (CH). MS (m/z): 353 (M^++1); anal. calcd. for $C_{19}H_{17}FN_4O_2$: C, 64.76; H, 4.86; N, 15.90; found: C, 64.71; H, 4.97; N, 15.97.

*4.2.2.16. 1-(4-Chlorophenyl)-5-isopropyl-*N*-(thiazol-2-yl)-1*H*-1,2,3-triazole-4-carboxamide (**4u**).* White solid; Yield 83%; m.p. 226-227 °C (ethanol-DMF); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.36 (s, 1H, NH), 7.74 (d, J = 8.7 Hz, 2H, H_{Ar}-2,6), 7.66 (d, J = 8.6 Hz, 2H, H_{Ar}-3,5), 7.57 (d, J = 3.5 Hz, 1H, $H_{Thiazole}$-4), 7.31 (d, J = 3.5 Hz, 1H, $H_{Thiazole}$-5), 3.21 (hept., J = 7.0 Hz, 1H), 1.31 (d, J = 7.0 Hz, 6H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 159.52 (CO), 157.96 ($C_{Thiazol}$-1), 147.72 ($CH_{Thiazol}$-4), 138.35 (C_{Tr}-5), 136.85 (C_{Tr}-4), 135.95 (C_{Ar}-1), 134.81 (C_{Ar}-4), 130.33 (2xCH_{Ar}-3,5), 129.11 (2xCH_{Ar}-2,6), 114.47 ($CH_{Thiazol}$-5), 24.88 (CH), 20.36 (CH_3).. MS (m/z): 348 (M^++1); anal. calcd. for $C_{15}H_{14}ClN_5OS$: C, 51.80; H, 4.06; N, 20.13; found: C, 51.74; H, 4.14; N, 20.21.

*4.2.3. General procedure for the synthesis of 1H-1,2,3-triazol-5-amines **8** and [1,2,3]triazolo[1,5-a]quinazolin-5(4H)-ones **9***

To the solution of sodium methoxide (108 mg, 2.0 mmol) in dry methanol (5 mL), an appropriate substituted cyanoacetamide **7** (2.0 mmol) was added. To this solution, substituted methyl 2-azidobenzoate **1r-t** (10.0 mmol) in dry methanol (2 mL) was added dropwise, and the solid started to precipitate. The mixture was

stirred for 1 h. The resulting suspension was filtered and the solid product was washed with water and methanol to give the corresponding 1H-1,2,3-triazol-5-amines **8** and [1,2,3]triazolo[1,5-*a*]quinazolin-5(4*H*)-ones **9**.

4.2.3.1. N-*(4-Acetamidophenyl)-5-amino-1-(3-bromophenyl)-1*H-*1,2,3-triazole-4-carboxamide (**8a**).* White solid; Yield 98%; m.p. 235-236 °C (ethanol-DMF); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.15 (s, 1H, NH), 9.88 (s, 1H, NH), 7.94 – 7.42 (m, 8H, $H_{Arom.}$), 6.68 (s, 2H, NH_2), 2.03 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6): δ 167.93 (CO), 160.43(CO), 145.29 (C_{Tr}-5), 135.97 (C_{Tr}-4), 134.85 (C_{ArN}-4), 134.03 (C_{ArN}-1), 132.04 (C_{Ar}-1), 131.59 (CH_{Ar}-5), 127.02 (CH_{Ar}-4), 123.50 (CH_{Ar}-6), 122.12 (CH_{Ar}-2), 121.48(C_{Ar}-3), 120.50 (2xCH_{ArN}-2,6), 119.17 (2xCH_{ArN}-3,5), 23.88 (CH_3). MS (m/z): 415, 417 (M^++1); anal. calcd. for $C_{17}H_{15}BrN_6O_2$: C, 49.17; H, 3.64; N, 20.24; found: C, 49.09; H, 3.71; N, 20.21.

*4.2.3.2. 5-Amino-*N*-(2,3-dimethylphenyl)-1-(4-fluorophenyl)-1*H*-1,2,3-triazole-4-carboxamide (**8b**).* White solid; Yield 97%; m.p. 209-210 °C (ethanol); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 9.69 (s, 1H, NH), 7.67 (dd, J = 8.9, 4.9 Hz, 2H, H_{Ar}-2,6), 7.47 (t, J = 8.8 Hz, 2H, H_{Ar}-3,5), 7.28 (d, J = 7.5 Hz, 1H, H_{ArN}-6), 7.08 (t, J = 7.6 Hz, 1H, H_{ArN}-5), 7.03 (d, J = 7.1 Hz, 1H, H_{ArN}-4), 6.48 (s, 2H, NH_2), 2.27 (s, 3H, CH_3), 2.14 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.57 (d, $^1J_{C\text{-}F}$ = 246.1 Hz), 161.27 (CO), 145.72 (C_{Tr}-5), 137.27 (C_{ArN}-1), 136.25 (C_{Tr}-4), 131.99 (C_{ArN}-3), 131.59 (d, $^4J_{C\text{-}F}$ = 2.5 Hz, C_{Ar}-1), 127.58 (d, $^3J_{C\text{-}F}$ = 9.1 Hz, 2xCH_{Ar}-2,6), 127.43 (CH_{ArN}-4), 125.63 (CH_{ArN}-5), 124.26 (CH_{ArN}-6), 121.93 (C_{ArN}-2), 117.15 (d, $^2J_{C\text{-}F}$ = 23.1 Hz, 2xCH_{Ar}-3,5), 20.67 (CH_3), 14.52 (CH_3). MS (m/z): 326 (M^++1); anal. calcd. for $C_{17}H_{16}FN_5O$: C, 62.76; H, 4.96; N, 21.53; found: C, 62.77; H, 4.90; N, 21.57.

*4.2.3.3. 5-Amino-*N*-(2,3-dimethylphenyl)-1-*m*-tolyl-1*H*-1,2,3-triazole-4-carboxamide (**8c**).* White solid; Yield 96%; m.p. 134-135 °C (ethanol); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 9.68 (s, 1H, NH), 7.51 (t, J = 7.6 Hz, 1H, H_{Ar}-5), 7.46 – 7.35 (m, 3H, H_{Ar}), 7.30 (d, J = 7.4 Hz, 1H, H_{ArN}-6), 7.09 (t, J = 7.5 Hz, 1H, H_{ArN}-5), 7.04 (d, J = 7.1 Hz, 1H, H_{ArN}-4), 6.45 (s, 2H, NH_2), 2.43 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.15 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6): δ 160.83 (CO), 144.88 (C_{Tr}-5), 139.50 (C_{ArN}-1), 136.75 (C_{Tr}-4), 135.74 (C_{Tol}-3), 134.67, 131.46 (C_{ArN}-3), 129.73 (C_{Tol}-1), 129.55 (CH_{Tol}-5), 126.91(CH_{ArN}-4), 125.12 (CH_{ArN}-5), 124.55 (CH_{ArN}-6), 123.73 (CH_{Tol}-4), 121.52 (C_{ArN}-2), 121.20 (CH_{Tol}-6), 20.85 (CH_3), 20.17 (CH_3), 14.02 (CH_3). MS (m/z): 322 (M^++1); anal. calcd. for $C_{18}H_{19}N_5O$: C, 67.27; H, 5.96; N, 21.79; found: C, 67.33; H, 5.99; N, 21.72.

*4.2.3.4. 5-Amino-*N*,1-di-m-tolyl-1*H*-1,2,3-triazole-4-carboxamide (**8d**).* White solid; Yield 96%; m.p. 153-155 °C (ethanol); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 9.81 (s, 1H, NH), 7.68 (s, 1H, H_{ArN}-2), 7.59 (d, J = 8.2 Hz, 1H, H_{ArN}-6), 7.47 (t, J = 7.7 Hz, 1H, H_{Ar}-5), 7.44 – 7.39 (m, 2H, H_{Ar}), 7.32 (d, J = 7.5 Hz, 1H, H_{Ar}-4), 7.14 (t, J = 7.8 Hz, 1H, H_{ArN}-5), 6.83 (d, J = 7.5 Hz, 1H, H_{ArN}-4), 6.44 (s, 2H, NH_2), 2.47 (s, 3H, CH_3), 2.34 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6): δ 160.58, 144.85, 140.28, 138.12, 136.47, 134.27, 130.43, 129.80, 128.59, 128.35, 127.27, 123.88, 122.51, 120.61, 120.54, 21.21, 20.26. MS (m/z): 308 (M^++1); anal. calcd. for $C_{17}H_{17}N_5O$: C, 66.43; H, 5.58; N, 22.79; found: C, 66.55; H, 5.70; N, 22.71.

*4.2.3.5. 5-Amino-1-(3,5-dimethylphenyl)-*N*-(4-methoxyphenyl)-1*H*-1,2,3-triazole-4-carboxamide (**8e**).* White solid; Yield 96%; m.p. 163-164 °C (ethanol); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.09 (s, 1H, NH), 7.74 (d, J = 8.9 Hz, 2H, H_{ArN}-2,6), 7.21 (s, 2H, H_{Ar}-2,6), 7.17 (s, 1H, H_{Ar}-4), 6.89 (d, J = 8.9 Hz, 2H, H_{ArN}-3,5), 6.49 (s, 2H, NH_2), 3.73 (s, 3H, CH_3O), 2.37 (s, 6H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6): δ 161.01 (CO), 155.73 (C_{ArN}-4), 145.37 (C_{Tr}-5), 139.71 (2xC_{Ar}-3,5), 135.04 (C_{Tr}-4), 132.46 (CH_{Ar}-4), 130.90 (C_{Ar}-1), 122.14 (2xCH_{Ar}-2,6), 122.09 (2xCH_{ArN}-2,6), 122.02 (C_{ArN}-1), 114.14 (2xCH_{ArN}-3,5), 55.63 (CH_3), 21.26 (2xCH_3). MS (m/z): 338 (M^++1); anal. calcd. for $C_{18}H_{19}N_5O_2$: C, 64.08; H, 5.68; N, 20.76; found: C, 64.14; H, 5.78; N, 20.71.

*4.2.3.6. 5-Oxo-*N*-phenyl-4,5-dihydro-[1,2,3]triazolo[1,5-*a*]quinazoline-3-carboxamide (**9a**).* White solid; Yield 97%; m.p. >250 °C (DMF); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 11.78 (br.s, 1H, NH), 10.43 (s, 1H, NH), 8.38 (d, J = 8.1 Hz, 1H, H_{quin}-6), 8.29 (d, J = 7.6 Hz, 1H, H_{quin}-9), 8.01 (t, J = 7.6 Hz, 1H, H_{quin}-8), 7.88 (d, J = 8.4 Hz, 2H, H_{Ph}-2,6), 7.71 (t, J = 7.6 Hz, 1H, H_{quin}-7), 7.31 (t, J = 7.7 Hz, 2H, H_{Ph}-3,5), 7.06 (t, J = 7.1 Hz, 1H, H_{Ph}-4). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.24 (CONH), 159.48 (CONH), 149.72 (C_{quin}-3a), 139.27 (C_{quin}-9a), 138.15 (C_{Ph}-1), 134.46 (CH_{quin}-8), 129.36 (2xCH_{Ph}-3,5), 129.17 (CH_{Ph}-4), 126.26 (CH_{quin}-7), 125.80 (CH_{quin}-6), 123.81 (C_{quin}-5a), 119.17 (2xCH_{Ph}-2,6), 117.32 (C_{quin}-3), 115.17 (CH_{quin}-9).MS (m/z): 306 (M^++1); anal. calcd. for $C_{16}H_{11}N_5O_2$: C, 62.95; H, 3.63; N, 22.94; found: C, 63.01; H, 3.74; N, 22.91.

*4.2.3.7. N-(2-Fluorophenyl)-5-oxo-4,5-dihydro-[1,2,3]triazolo[1,5-a]quinazoline-3-carboxamide (**9b**).* White solid; Yield 91%; m.p. >250°C (DMF); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 12.01 (br.s, 1H, NH), 9.79 (s, 1H, NH), 8.38 (d, J = 8.2 Hz, 1H, H_{quin}-6), 8.27 (d, J = 8.0 Hz, 1H, H_{quin}-9), 8.01 (t, J = 7.9 Hz, 1H, H_{quin}-8), 7.98 – 7.92 (m, 1H, H_{Ar}-3), 7.71 (t, J = 7.7 Hz, 1H, H_{quin}-7), 7.26 – 7.16 (m, 3H, H_{Ar}). ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.02 (CONH), 158.87 (CONH), 155.70 (d, $^1J_{C\text{-}F}$ = 246.4 Hz, C_{Ar}-2), 136.91 (C_{quin}-3a), 135.96 (CH_{quin}-8), 134.79 (C_{quin}-9a), 128.91 (CH_{quin}-7), 128.79 (CH_{Ar}-5), 127.05 (d, $^3J_{C\text{-}F}$ = 7.7 Hz, CH_{Ar}-4), 126.50 (CH_{quin}-6), 125.57 (d, $^2J_{C\text{-}F}$ = 11.4 Hz, C_{Ar}-1), 124.81 (d, $^3J_{C\text{-}F}$ = 3.0 Hz, CH_{Ar}-6), 123.89 (C_{quin}-5a), 117.59 (C_{quin}-3), 116.17 (d, $^2J_{C\text{-}F}$ = 19.6 Hz, CH_{Ar}-3), 115.70 (CH_{quin}-9). MS (m/z): 324 (M^++1); anal. calcd. for $C_{16}H_{10}FN_5O_2$: C, 59.44; H, 3.12; N, 21.66; found: C, 59.41; H, 3.19; N, 21.74.

4.2.3.8. N-*(3,4-dichlorophenyl)-5-oxo-4,5-dihydro-[1,2,3]triazolo[1,5-a]quinazoline-3-carboxamide (**9c**).* White solid; Yield 97%; m.p. >250°C (DMF); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 11.81 (br.s, 1H, NH), 10.79 (s, 1H, NH), 8.38 (d, J = 7.2 Hz, 1H, H_{quin}-6), 8.29 (d, J = 7.3 Hz, 1H, H_{quin}-9), 8.26 (s, 1H, H_{Ar}-2), 8.01 (t, J = 7.9 Hz, 1H, H_{quin}-8), 7.85 (d, J = 8.9 Hz, 1H, H_{Ar}-6), 7.71 (t, J = 7.5 Hz, 1H, H_{quin}-7), 7.45 (d, J = 8.7 Hz, 1H, H_{Ar}-5). ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.34 (CONH), 158.99 (CONH), 137.25 (C_{quin}-3a), 136.01 (CH_{quin}-8), 134.78 (C_{quin}-9a), 133.78 (C_{Ar}-1), 131.29 (C_{Ar}-3), 130.99 (CH_{Ar}-5), 128.96 (CH_{quin}-7), 128.86 (CH_{quin}-6), 125.57 (C_{Ar}-4), 124.02 (C_{quin}-5a), 121.84 (CH_{Ar}-2), 120.69 (CH_{Ar}-6), 117.63 (C_{quin}-3), 115.72 (CH_{quin}-9). MS (m/z): 374 (M^++1); anal. calcd. for $C_{16}H_9Cl_2N_5O_2$: C, 51.36; H, 2.42; N, 18.72; found: C, 51.44; H, 2.52; N, 18.71.

4.2.3.9. N-*(2,4-dichlorophenyl)-5-oxo-4,5-dihydro-[1,2,3]triazolo[1,5-a]quinazoline-3-carboxamide (**9d**).* White solid; Yield 95%; m.p. >250°C (DMF); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 12.19 (br.s, 1H, NH), 9.72 (s, 1H, NH), 8.40 (d, J = 8.2 Hz, 1H, H_{quin}-6), 8.29 (d, J = 8.0 Hz, 1H, H_{quin}-9), 8.25 (d, J = 8.9 Hz, 1H, H_{Ar}-2), 8.02 (t, J = 7.5 Hz, 1H, H_{quin}-8), 7.73 (t, J = 7.4 Hz, 1H, H_{quin}-7), 7.57 (d, J = 2.2 Hz, 1H, H_{Ar}-3), 7.40 (dd, J = 8.8, 2.1 Hz, 1H, H_{Ar}-5). MS (m/z): 374 (M^++1); anal. calcd. for $C_{16}H_9Cl_2N_5O_2$: C, 51.36; H, 2.42; N, 18.72; found: C, 51.40; H, 2.35; N, 18.75.

*4.2.3.10. 7-Fluoro-*N*-(furan-2-ylmethyl)-5-oxo-4,5-dihydro-[1,2,3]triazolo[1,5-a]quinazoline-3-carboxamide (**9e**).* White solid; Yield 89%; m.p. >250 °C (DMF); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.83 (br.s, 1H, NH), 8.92 (t, J = 5.9 Hz, 1H, NH), 8.41 (dd, J = 9.0, 4.2 Hz, 1H, H_{quin}-6), 7.91 (dd, J = 8.3, 2.7 Hz, 1H, H_{quin}-9), 7.81 (td, J = 8.8, 3.0 Hz, 1H, H_{quin}-8), 7.43 (d, J = 0.8 Hz, 1H, H_{Fur}-5), 6.32 (dd, J = 2.9, 1.9 Hz, 1H, H_{Fur}-4), 6.25 (d, J = 2.7 Hz, 1H, H_{Fur}-3), 4.50 (d, J = 5.9 Hz, 2H, CH_2N). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.25 (d, $^1J_{C\text{-}F}$ = 247.0 Hz, CF_{quin}-7), 160.21 (CONH), 158.23 (CONH), 152.88 (C_{Fur}-2), 142.36 (CH_{Fur}-5), 136.07 (C_{quin}-3a), 131.68 (C_{quin}-9a), 124.16 (C_{quin}-3), 123.63 (d, $^2J_{C\text{-}F}$ = 24.7 Hz, CH_{quin}-8), 119.77 (d, $^3J_{C\text{-}F}$ = 7.9 Hz, C_{quin}-5a), 118.59 (d, $^3J_{C\text{-}F}$ = 8.6 Hz, CH_{quin}-9), 114.55 (d, $^2J_{C\text{-}F}$ = 24.8 Hz, CH_{quin}-6), 110.92 (CH_{Fur}-4), 107.28 (CH_{Fur}-3), 35.65 (CH_2N). MS (m/z): 328 (M^++1); anal. calcd. for $C_{15}H_{10}FN_5O_2$: C, 55.05; H, 3.08; N, 21.40; found: C, 55.15; H, 3.17; N, 21.37.

*4.2.3.11. N-(2-Chlorophenyl)-7-fluoro-5-oxo-4,5-dihydro-[1,2,3]triazolo[1,5-a]quinazoline-3-carboxamide (**9f**).* White solid; Yield 94%; m.p. >250 °C (DMF); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.39 (br.s, 1H, NH), 9.63 (s, 1H, NH), 8.45 (dd, J = 8.9, 4.4 Hz, 1H, H_{quin}-6), 8.31 (dd, J = 8.1, 1.3 Hz, 1H, H_{Ar}-3), 7.94 (dd, J = 8.4, 2.9 Hz, 1H, H_{quin}-9), 7.84 (td, J = 8.5, 2.8 Hz, 1H, H_{quin}-8), 7.49 (dd, J = 8.0, 1.3 Hz, 1H, H_{Ar}-6), 7.36 (dt, J = 7.6, 1.3 Hz, 1H, H_{Ar}-5), 7.16 (dt, J = 7.7, 1.4 Hz, 1H, H_{Ar}-4). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.38 (d, $^1J_{C\text{-}F}$ = 248.4 Hz, CF_{quin}-7), 158.56 (2xCONH), 134.74 (C_{quin}-3a), 131.68 (C_{quin}-9a), 129.97 (C_{Ar}-1+CH_{Ar}-3), 128.21 (C_{Ar}-2+CH_{Ar}-5), 126.78 (CH_{Ar}-4), 125.16 (CH_{Ar}-6), 123.92 (C_{quin}-3), 123.70 (d, J = 24.5 Hz, CH_{quin}-8), 119.89 (d, $^3J_{C\text{-}F}$ = 7.3 Hz, C_{quin}-5a), 118.66 (d, $^3J_{C\text{-}F}$ = 8.3 Hz, CH_{quin}-9), 114.57 (d, $^2J_{C\text{-}F}$ = 24.7 Hz, CH_{quin}-6). MS (m/z): 358 (M^++1); anal. calcd. for $C_{16}H_9ClFN_5O_2$: C, 53.72; H, 2.54; N, 19.58; found: C, 53.70; H, 2.59; N, 19.51.

*4.2.3.12. 7-Chloro-*N*-(2-chlorophenyl)-5-oxo-4,5-dihydro-[1,2,3]triazolo[1,5-a]quinazoline-3-carboxamide (**9g**).* White solid; Yield 96%; m.p. >250°C (DMF); IR (KBr) ν_{max} 1670 (s, C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.46 (br.s, 1H, NH), 9.66 (s, 1H, NH), 8.41 (d, J = 8.6 Hz, 1H, H_{Ar}-3), 8.27 (d, J = 8.3 Hz, 1H, H_{quin}-8), 8.19 (s, 1H, H_{quin}-6), 8.02 (d, J = 9.0 Hz, 1H, H_{quin}-9), 7.50 (d, J = 8.1 Hz, 1H, $H^6{}_{Ar}$), 7.36 (t, J = 7.6 Hz, 1H, $H^5{}_{Ar}$), 7.17 (t, J = 7.6 Hz, 1H, $H^4{}_{Ar}$). MS (m/z): 374 (M^++1); anal. calcd. for $C_{16}H_9Cl_2N_5O_2$: C, 51.36; H, 2.42; N, 18.72; found: C, 51.45; H, 2.36; N, 18.81.

4.3. Biological screening

4.3.1. Primary screening data collection via CO-ADD [http://www.co-add.org/]

An inhibition of bacterial growth was determined via measuring the absorbance at 600 nm. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references. Growth inhibition of *C. albicans* was determined by measuring absorbance at 530 nm, while the growth inhibition of *C. neoformans* was determined by measuring the difference in absorbance between 600 and 570 nm, after the addition of resazurin (0.001% final concentration) and incubation at 35°C for additional 2 h. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references. Degree of growth inhibition of an individual sample was calculated based on negative controls (media only) and positive controls (bacterial/fungal media without inhibitors). Negative inhibition values indicate that the growth rate (or OD600) is higher compared to the negative control (Bacteria/fungi only, set to 0% inhibition). The growth rates for all bacteria and fungi have a variation of -/+ 10%, which is within the reported normal distribution of bacterial/fungal growth. Any significant variation (or outliers/hits) is identified by the modified Z-Score, and actives are selected by a combination of inhibition value and Z-Score.

4.3.2. Antimicrobial studies

Antibacterial activity was determined by using MTT test. Experiments were conducted at pH 7.2. Subsequent bacterial culture in the logarithmic phase of growth in Sabouraud medium, pH 7.2, was centrifuged for 10 min at 500 g, and sediment of bacteria was washed with sterile saline and resuspended in a small volume of sterile saline. A defined volume of this suspension was introduced into Sabouraud medium with pH 7.2 for obtaining OD 0.4-0.6 at 590 nm (optical path 1.0 cm). Then, 100 μL of each suspension were introduced into series of 1.5 mL Eppendorf tubes, and thereafter inoculation with 10, 5, and 2 μL of the tested sample solution was conducted. Each experiment was repeated in triplicate. Tubes were incubated for 4 h at 37 °C. Thereafter, 10 μL of MTT solution (5 mg/mL) was introduced, and incubation was continued for 1 h. Cells were harvested by centrifugation for 5 min at 1,500 g, the supernatant was discarded and small sediment was suspended in 1 mL of DMSO. After the incubation for 1 h at 37°C, the OD of liquid was measured at 580 nm on the spectrophotometer ULAB 102 UV (Ukraine). The effect of tested compound upon bacteria viability was compared with that in a control.

Antifungal activity was studied on *C. albicans* strains in the Sabouraud medium by using the Colony Forming Units (CFU) method. The quality of peptone as the component of the Sabouraud medium and control of pH are of great importance for the reproducibility of results. In the experiments, the enzymatic peptone (Kyiv, Ukraine) was used that provided a rapid growth of *C. albicans* and the appearance of distinct colonies after 24 h of incubation at 37 °C.

A suspension of *Candida sp* containing 10^7 cells/mL was prepared by suspending cells taken from the colonies grown on the Sabouraud agar, pH 5.8. Cells' number was counted in the hemocytometric chamber, since a size of *Candida* cells (2.5-4 μm) permitted doing that accurately. The tested compound solution in 10, 5 and 2,5 μL volume was introduced into 3 round bottom Eppendorf tubes, and thereafter, 100 μL of *Candida* cells suspension was added. Two control tubes were prepared: at the start (time 0) and at the end (4 h) of incubation. The tubes were incubated for 4 h at 37 °C (except control 0 kept at 4 °C). Then, 10 μL aliquote was withdrawn at the end of incubation from each tube after thorough mixing, diluted 10,000 fold with water and 0.2 mL of this dilution was distributed on the surface of Sabouraud agar medium, pH 5.8, in the Petri dish. They were incubated at 37 °C and after distinct formation of colonies (usually after 24 h) the image was scanned and colonies were counted using the Photoshop program. The number of colonies in control tube at 0 h time must be 200±50 per dish, in control after 4 h of incubation colonies number must be 1.5-2.5 fold higher. The experiment was abolished when the increase in a number of colonies in control after the incubation was less than 1.5 fold. The effect of tested compound upon the viability of *Candida* cells was expressed as a ratio of colony number.

The index lower than control (0-hour time) was considered as candidacidal effect, while higher than control (0-hour time) but less than in control after 4 h of incubation was classified as growth inhibition, and the index higher than in control after 4 h of incubation indicated a stimulation of growth [40].

4.3.3. Cell culture and cytotoxicity assay (MTT and Trypan Blue dye assays)

Human keratinocytes of HaCaT line were obtained from a Collection at the Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine (Kyiv, Ukraine). Cells were grown in the DMEM (Biowest, Nuaille, France) culture medium supplemented with 10% fetal bovine serum (Biowest, Nuaille, France) under standard conditions. *In vitro* evaluation of cytotoxic activity of the synthesized compounds compared with the doxorubicin used as a reference control, toward human keratinocytes of HaCaT line was measured by the MTT test [41]. Briefly, cells were seeded for 24 h in 96-well microtiter plates at a concentration of 5,000 cells/well (100 µL/well); after that, cells were incubated for 72 h with various additions of the synthesized compounds (0–50 µM). MTT, which is converted to dark blue, water-insoluble formazan by the mitochondrial dehydrogenases, was used to determine viable cells according to the Sigma-Aldrich protocol. Formazan was dissolved in the DMSO, and the results of the reaction were determined by an Absorbance Reader BioTek ELx800 (BioTek Instruments, Inc., Winooski, VT, USA). The IC_{50} of the tested compounds was calculated as a concentration of drug killing 50% of cells compared with the untreated culture.

4.3.4. Statistical Analysis

Z-Score analysis is done to investigate outliers or hits among the samples. The Z-Score is calculated based on the sample population using a modified Z-Score method, which accounts for possible skewed sample population. The modified method uses median and median average deviation (MAD) instead of average and Standard deviation (SD), and a scaling factor [42]: M(i) = 0.6745 *(x(i) - median(x))/MAD). All screening is performed as two replicas (n=2), with both replicas on different assay plates, but from single plating and performed in a single screening experiment (microbial incubation). Two values are used as quality controls for individual plates: Z-Factor= 1-[3*(SD(Negative controls) + SD (Positive Controls))/(average(Positive Controls)-average(Negative controls))].

Cytotoxicity data are presented as the mean (M) ± standard deviation (SD). Results were analysed and illustrated with GraphPad Prism (version 6; GraphPad Software, San Diego, CA, USA). Statistical analyses were performed using two-way ANOVA with Dunnett's multiple comparisons test (cells growth inhibition). A p-value of <0.05 was considered as statistically significant.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2021.131146.

References

[1] B. Li, T.J. Webster, Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections, J. Orthop. Res. 36 (2018) 22–32, doi:10.1002/jor.23656.
[2] E.D. Brown, G.D. Wright, Antibacterial drug discovery in the resistance era, Nature 529 (2016) 336–343, doi:10.1038/nature17042.
[3] L.L. Ling, T. Schneider, A.J. Peoples, A.L. Spoering, I. Engels, B.P. Conlon, A. Mueller, T.F. Schäberle, D.E. Hughes, S. Epstein, M. Jones, A new antibiotic kills pathogens without detectable resistance, Nature 517 (2015) 455–459, doi:10.1038/nature14098.
[4] M.N. Thaker, W. Wang, P. Spanogiannopoulos, N. Waglechner, A.M. King, R. Medina, G.D. Wright, Identifying producers of antibacterial compounds by screening for antibiotic resistance, Nat. Biotechnol. 31 (2013) 922–927, doi:10.1038/nbt.2685.
[5] M.A. Fischbach, C.T. Walsh, Antibiotics for emerging pathogens, Science 325 (2009) 1089–1093, doi:10.1126/science.1176667.
[6] C.Y. Mo, M.J. Culyba, T. Selwood, J.M. Kubiak, Z.M. Hostetler, A.J. Jurewicz, P.M. Keller, A.J. Pope, A. Quinn, J. Schneck, K.L. Widdowson, R.M. Kohli, Inhibitors of LexA autoproteolysis and the bacterial SOS response discovered by an academic-industry partnership, ACS Infect. Dis. 4 (2017) 349–359, doi:10.1021/acsinfecdis.7b00122.
[7] C.Y. Mo, S.A. Manning, M. Roggiani, M.J. Culyba, A.N. Samuels, P.D. Sniegowski, M. Goulian, R.M. Kohlib, Systematically altering bacterial SOS activity under stress reveals therapeutic strategies for potentiating antibiotics, mSphere 1 (2016) 1–15, doi:10.1128/mSphere.00163-16.
[8] C.Y. Mo, Make antibiotics great again: Combating drug resistance by targeting lexa, a regulator of bacterial evolution (2016). Publicly Accessible Penn Dissertations. 2489. https://repository.upenn.edu/edissertations/2489
[9] R.P. Jadhav, H.N. Raundal, A.A. Patil, V.D. Bobade, Synthesis and biological evaluation of a series of 1,4-disubstituted 1,2,3-triazole derivatives as possible antimicrobial agents, J. Saudi Chem. Soc. 21 (2017) 152–159, doi:10.1016/j.jscs.2015.03.003.
[10] C. Sall, M. Ayé, O. Bottzeck, A. Praud, Y. Blache, Towards smart biocide-free anti-biofilm strategies: Click-based synthesis of cinnamide analogues as anti-biofilm compounds against marine bacteria, BioOrg. Med. Chem. Lett. 28 (2018) 155–159, doi:10.1016/j.bmcl.2017.11.039.
[11] Z.-J. Wang, Y. Gao, Y.-L. Hou, C. Zhang, S.-J. Yu, Q. Bian, Z.-M. Li, W.-G. Zhao, Design, synthesis, and fungicidal evaluation of a series of novel 5-methyl-1H-1,2,3-trizole-4-carboxyl amide and ester analogues, Eur. J. Med. Chem. 86 (2014) 8794, doi:10.1016/j.ejmech.2014.08.029.
[12] J.W. Wheless, B. Vazquez, Rufinamide: a novel broad-spectrum antiepileptic drug, Epilepsy Curr 10 (2010) 1–6, doi:10.1111/j.1535-7511.2009.01336.x.
[13] C. Corrado, A.M. Flugy, S. Taverna, S. Raimondo, G. Guggino, R. Karmali, Giacomo De Leo, R. Alessandro, Carboxyamidotriazole-orotate inhibits the growth of imatinib-resistant chronic myeloid leukaemia cells and modulates exosomes-stimulated angiogenesis, PLoS One 7 (2012) 1–13, doi:10.1371/journal.pone.0042310.
[14] V. Calderone, F.L. Fiamingo, G. Amato, I. Giorgi, O. Livi, A. Martelli, E. Martinotti, 1,2,3-Triazol-carboxanilides and 1,2,3-triazol-(N-benzyl)-carboxamides as BK-potassium channel activators. XII, Eur. J. Med. Chem. 43 (2008) 2618–2626, doi:10.1016/j.ejmech.2008.02.032.
[15] B. Prasad, V. Lakshma, P.S. Srikanth, M.F. Baig, N.S. Reddy, K.S. Babu, A. Kamal, Synthesis and biological evaluation of 1-benzyl-N-(2-(phenylamino)pyridin3-yl)-1H-1,2,3-triazole-4-carboxamides as antimitotic agents, BioOrg. Chem. 83 (2019) 535–548, doi:10.1016/j.bioorg.2018.11.002.
[16] V.G. Reddy, S.R. Bonam, T.S. Reddy, R. Akunuri, V.G.M. Naidu, V.L. Nayak, S.K. Bhargava, H.S. Kumar, P. Srihari, A. Kamal, 4β-amidotriazole linked podophyllotoxin congeners: DNA topoisomerase-IIα inhibition and potential anticancer agents for prostate cancer, Eur. J. Med. Chem. 144 (2018) 595–611, doi:10.1016/j.ejmech.2017.12.050.
[17] H. Elamari, F. Meganem, J. Herscovici, C. Hirard, Chemoselective preparation of disymmetric bistriazoles from bisalkynes, Tetrahedron Lett 52 (2011) 558–560, doi:10.1016/j.tetlet.2010.11.141.
[18] H. Elamari, R. Slimi, G.G. Chabot, L. Quentin, D. Scherman, C. Girard, Synthesis and *in vitro* evaluation of potential anticancer activity of mono- and bis-

1,2,3-triazole derivatives of bis-alkynes, Eur. J. Med. Chem. 60 (2013) 360–364, doi:10.1016/j.ejmech.2012.12.025.
[19] H. Duan, D. Arora, Y. Li, H. Setiadi, D. Xu, H.Y. Lim, W. Wang, Identification of 1,2,3-triazole derivatives that protect pancreatic β cells against endoplasmic reticulum stress-mediated dysfunction and death through the inhibition of C/EBP-homologous protein expression, BioOrg. Med. Chem. 24 (2016) 2621–2630, doi:10.1016/j.bmc.2016.03.057.
[20] M. Taddei, S. Ferrini, L. Giannotti, M. Corsi, F. Manetti, G. Giannini, L. Vesci, F.M. Milazzo, D. Alloatti, M.B. Guglielmi, M. Castorina, Synthesis and evaluation of new Hsp90 inhibitors based on a 1,4,5-trisubstituted 1,2,3-triazole scaffold, J. Med. Chem. 57 (2014) 2258–2274, doi:10.1021/jm401536b.
[21] N. Pokhodylo, O. Shyyka, V. Matiychuk, Synthesis and anticancer activity evaluation of new 1,2,3-triazole4-carboxamide derivatives, Med. Chem. Res. 23 (2014) 2426–2438, doi:10.1007/s00044-013-0841-8.
[22] N.T. Pokhodylo, O.Ya. Shyyka, N.S. Finiuk, Anticancer activity evaluation of thieno[3,2-e][1,2,3]triazolo[1,5-a]pyrimidines and thieno[2,3-e][1,2,3]triazolo[1,5-a]pyrimidine derivatives, Biopolym. Cell. 35 (2019) 321–330 http://dx.doi.org/10.7124/bc.000A0F.
[23] L. Wang, S. Xu, X. Liu, X. Chen, H. Xiong, S. Hou, W. Zou, Q. Tang, P. Zheng, W. Zhu, Discovery of thinopyrimidine-triazole conjugates as c-Met targeting and apoptosis inducing agents, BioOrg. Chem. 77 (2018) 370–380, doi:10.1016/j.bioorg.2018.01.037.
[24] S. Zhou, H. Liao, M. Liu, G. Feng, B. Fu, R. Li, M. Cheng, Y. Zhao, P. Gong, Discovery and biological evaluation of novel 6,7-disubstituted-4-(2-fluorophenoxy) quinoline derivatives possessing 1,2,3-triazole-4-carboxamide moiety as c-Met kinase inhibitor, BioOrg. Med. Chem. 22 (2014) 6438–6452, doi:10.1016/j.bmc.2014.09.037.
[25] O.N. Obianom, Y. Ai, Y. Li, Wei Yang, D. Guo, H. Yang, S. Sakamuru, M. Xia, F. Xue, Y. Shu, Triazole-based inhibitors of the Wnt/β-catenin signaling pathway improve glucose and lipid metabolisms in diet-induced obese mice, J. Med. Chem. 62 (2019) 727–741, doi:10.1021/acs.jmedchem.8b01408.
[26] K. Maji, M. Abbasi, D. Podder, R. Datta, D. Haldar, Potential antileishmanial activity of a triazole-based hybrid peptide against leishmania major, Chemistry Select 3 (2018) 10220–10225, doi:10.1002/slct.201802002.
[27] A. Krajczyk, K. Kulinska, T. Kulinski, B.L. Hurst, C.W. Day, D.F. Smee, T. Ostrowski, P. Januszczyk, Joanna Zeidler, Antivirally active ribavirin analogues –4,5-disubstituted 1,2,3-triazole nucleosides: biological evaluation against certain respiratory viruses and computational modelling, Antivir. Chem. Chemother. 23 (2014) 161–171, doi:10.3851/IMP2564.
[28] N.T. Pokhodylo, V.S. Matiychuk, M.D. Obushak, One-pot multicomponent synthesis of 1-aryl-5-methyl-*N*-R^2-1*H*-1,2,3-triazole-4-carboxamides: an easy procedure for combinatorial chemistry, J. Comb. Chem. 11 (2009) 481–485, doi:10.1021/cc900012w.
[29] N.T. Pokhodylo, O.Ya. Shyyka, V.S. Matiychuk, M.D. Obushak, V.V. Pavlyuk, A novel base-solvent controlled chemoselective azide attack on an ester group versus keto in alkyl 3-substituted 3-oxopropanoates: mechanistic insights, ChemistrySelect 2 (2017) 5871–5876, doi:10.1002/slct.201700577.
[30] N.T. Pokhodylo, V.S. Matiychuk, M.D. Obushak, Synthesis of 1-(R–phenyl)-5-(R–methyl)-1H-1,2,3-triazole-4-carboxylic acids by One-Pot Tandem Reaction, Synth. Commun. 40 (2010) 1932–1938, doi:10.1080/00397910903174408.
[31] N.T. Pokhodylo, O.Y. Shyyka, R.D. Savka, M.D. Obushak, 2-Azido-1,3,4-thiadiazoles, 2-azido-1,3-thiazoles, and aryl azides in the synthesis of 1,2,3-triazole-4-carboxylic acids and their derivatives, Russ. J. Org. Chem. 54 (2018) 1090–1099, doi:10.1134/S1070428018070205.
[32] N.T. Pokhodylo, V.S. Matiychuk, M.D. Obushak, Synthesis of isothiocoumarin derivatives 46 (2010) 140–145 Chem. Heterocycl. Compd., doi:10.1007/s10593-010-0484-3.
[33] M.A. Tupychak, O.Ya. Shyyka, N.T. Pokhodylo, M.D. Obushak, Nitrileimines as an alternative to azides in base-mediated click [3+2]cycloaddition with methylene active nitriles, RSC Adv 10 (2020) 13696–13699, doi:10.1039/D0RA01417F.
[34] N.T. Pokhodylo, O.Ya. Shyyka, New cascade reaction of azides with malononitrile dimer to polyfunctional[1,2,3]triazolo[4,5-b]pyridine, Synth. Commun. 47 (2017) 1096–1101, doi:10.1080/00397911.2017.1313427.
[35] N.T. Pokhodylo, O.Ya. Shyyka, M.D. Obushak N.T. Pokhodylo, O.Ya. Shyyka, M.D. Obushak, Facile and efficient one-pot procedure for thieno[2,3-e][1,2,3]triazolo[1,5-a]pyrimidines preparation, Synth. Commun. 44 (2014) 1002–1006, doi:10.1080/00397911.2013.840729.
[36] N.T. Pokhodylo, O.Ya. Shyyka, R.D. Savka, M.D. Obushak, Novel selected tandem transformations of the amino and carbonyl/nitrile groups in the gewald thiophenes, Phosphorus Sulfur Silicon Relat. Elem. 185 (2010) 2092–2100, doi:10.1080/10426500903496739.
[37] N.T. Pokhodylo, V.S. Matiychuk, Synthesis of new 1,2,3-triazolo[1,5-a]quinazolinones, J. Heterocyclic Chem. 47 (2010) 415–420, doi:10.1002/jhet.321.
[38] N. Pokhodylo, Y. Slyvka, V. Pavlyuk, Synthesis, crystal structure and Hirshfeld surface analysis of N-(4-chlorophenyl)-5-cyclopropyl-1-(4-methoxyphenyl)-1H-1,2,3-triazole-4-carboxamide, Acta Cryst E76 (2020) 756–760, doi:10.1107/S2056989020005848.
[39] N.T. Pokhodylo, V.S. Matiichuk, N.D. Obushak, Synthesis and transformations of 1-(azidophenyl)-1H-tetrazoles, Russ. J. Org. Chem. 46 (4) (2010) 556–560, doi:10.1134/S1070428010040196.
[40] M. Lootsik, N. Manko, O. Gromyko, S. Tistechok, M. Lutsyk, R. Stoika, Honeybee chitosan-melanin complex: isolation and investigation of its antimicrobial activity, Ukr, Biochem. J. 92 (2020) 143–153, doi:10.15407/ubj92.06.143.
[41] X. Liu, Y.G. Zu, Y.J. Fu, L.P. Yao, C.B. Gu, W. Wang, T. Efferth, Antimicrobial activity and cytotoxicity towards cancer cells of Melaleuca alternifolia (tea tree) oil, Eur, Food Res. Technol. 229 (2009) 247–253, doi:10.1007/s00217-009-1057-5.
[42] B. Iglewicz, D.C. Hoaglin, Volume 16: How to detect and handle outliers. the asqc basic reference in quality control: Statistical Techniques, 1993.