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SYNTHESIS OF NOVEL 1-(3-PHENYLBENZO[*C*]ISOXAZOL-5-YL)-*1H*-1,2,3-TRIAZOLE-4-CARBOXAMIDES AND THEIR ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES

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Abstract

Novel 1-(3-arylbenzo[*c*]isoxazol-5-yl)-*1H*-1,2,3-triazole-4-carboxamides were designed, synthesizing and evaluated for antimicrobial activity toward five key ESKAPE pathogenic bacteria, one Gram-positive bacteria methicillin-resistant *Staphylococcus aureus* (ATCC 43300), four Gram-negative bacteria, *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 700603), *Acinetobacter baumannii* (ATCC 19606), and *Pseudomonas aeruginosa* (ATCC 27853) and antifungal activity towards two pathogenic fungal strains *Candida albicans* (ATCC 90028) and *Cryptococcus neoformans* var. *Grubii* (H99; ATCC 208821). The target compounds were obtained in a convenient synthetic path including consequent Dimroth cyclocondensation of 4-nitrophenyl azide with β-ketoesters, vicarious nucleophilic substitution in nitroaryl fragments and amidation of 1,2,3-triazole-4-carboxylic acid motif. In this way, a mini combinatorial library of 24 compounds was obtained with good overall yields. Five compounds, 7a, 7b, 7i, 7t and 7u, reduced the growth of microorganisms by approximately 20 %. Compounds 7b, 7i, and 7u demonstrated the inhibitory activity towards *Staphylococcus aureus*. In contrary 7a and 7t towards *Cryptococcus neoformans*. The data obtained will be used for further design and scaffold optimization.
Keywords: azide; benzo[*c*]isoxazoles; *1H*-1,2,3-triazole-4-carboxamides; antimicrobial.

СИНТЕЗ НОВИХ 1-(3-ФЕНІЛБЕНЗО[*C*]ІЗОКСАЗОЛ-5-ІЛ)-1Н-1,2,3-ТРИАЗОЛ-4-КАРБОКСАМІДІВ ТА ЇХ АНТИБАКТЕРІАЛЬНА ТА ПРОТИГРИБКОВА АКТИВНІСТЬ

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Анотація

Нові 1-(3-арилбензо[*c*]ізоксазол-5-іл)-*1H*-1,2,3-триазол-4-карбоксаміди були розроблені, синтезовані та оцінені на антимікробну активність щодо п'яти ключових патогенних бактерій ESKAPE, однієї грампозитивної бактерії, стійкої до метициліну, *Staphylococcus aureus* (ATCC 43300), чотирьох грамнегативних бактерій, *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 700603), *Acinetobacter baumannii* (ATCC 19606) і *Pseudomonas aeruginosa* (ATCC 27853) та протигрибкову активність щодо двох патогенних штамів грибів *Candida albicans* (ATCC 90028) і *Cryptococcus neoformans* var. *Grubii* (H99; ATCC 208821). Цільові сполуки були отримані зручним синтетичним шляхом, з послідовними стадіями циклоконденсації Дімрота 4-нітрофенілазиду з β-кетоестерами, вікаріозного нуклеофільного заміщення в нітроарильних фрагментах та амідування мотиву 1,2,3-триазол-4-карбонової кислоти. Таким чином була отримана міні-комбінаторна бібліотека з 24 сполук із хорошими загальними виходами. П'ять сполук, 7a, 7b, 7i, 7t та 7u, зменшили зростання мікроорганізмів приблизно на 20 %. Сполуки 7b, 7i та 7u продемонстрували інгібіторну активність щодо *Staphylococcus aureus*, на противагу 7a і 7t до *Cryptococcus neoformans*. Отримані дані будуть використані для подальшого дизайну та оптимізації скафолдів.
Ключові слова: азид; бензо[c]ізоксазоли; *1H*-1,2,3-триазол-4-карбоксаміди; протимікробна дія.

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Introduction

Infectious diseases have become a significant challenge to the global health system, especially in the conditions of the pandemic and the growing number of military conflicts, which have led to the deaths of a large number of people. Deterioration of socio-economic conditions, accompanied by the growth of the disease's causes, unavailability and high cost of medicines, also lead to psychological disorders and a reduction of the standard of living as a whole [1–3]. A wide variety of pathogens and the emergence of new multidrug-resistant pathogenic strains complicate the treatment and prevention of infectious diseases. Invasive fungal infections represent a global problem, resulting in 1.7 million deaths every year. They are common to immunocompromised patients, as reflected in their chemotherapy, acquired immune deficiency syndrome, and organ transplantation [4–6]. The recent annual incidence of invasive aspergillosis, candidiasis, and mucormycosis is over 300,000, 750,000, and 10,000 cases, respectively [7]. Therefore, to address the above problems, there is an urgent need to develop new anti-bacterial and anti-fungal drugs.

One of the mechanisms responsible for the formation of acquired antibiotic resistance is the bacterial DNA damage response mechanism known as the SOS response induced by many antibiotics [8; 9]. Scaffolds containing 1,2,3-triazole-4-carboxamide motif, for example 5-amino-*N*-(3,4-dimethylphenyl)-1-(2-((4-ethoxy-phenyl)amino)-2-oxoethyl)-*1H*-1,2,3-triazole-4-carboxamide **A** (GSK1010702A) (Fig. 1), have demonstrated good efficiency in inhibiting SOS-dependent response [10]. In this regard, studies of *1H*-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)-*1H*-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*) [11]. 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) [12]. 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 to develop novel pesticides [13]. In our previous work [14] A series of *N*-substituted 1-aryl-5-substituted-*1H*-1,2,3-triazole-4-carboxamides were designed, synthesized and evaluated for their antimicrobial potential toward the seven pathogens (*Escherichia coli, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa, Staphylococcus aureus, Cryptococcus neoformans var. grubii* and *Candida albicans*) and several compounds against Methicillin-resistant *S. aureus*. and pathogenic yeast *C. albicans* were discovered (fig. 1).

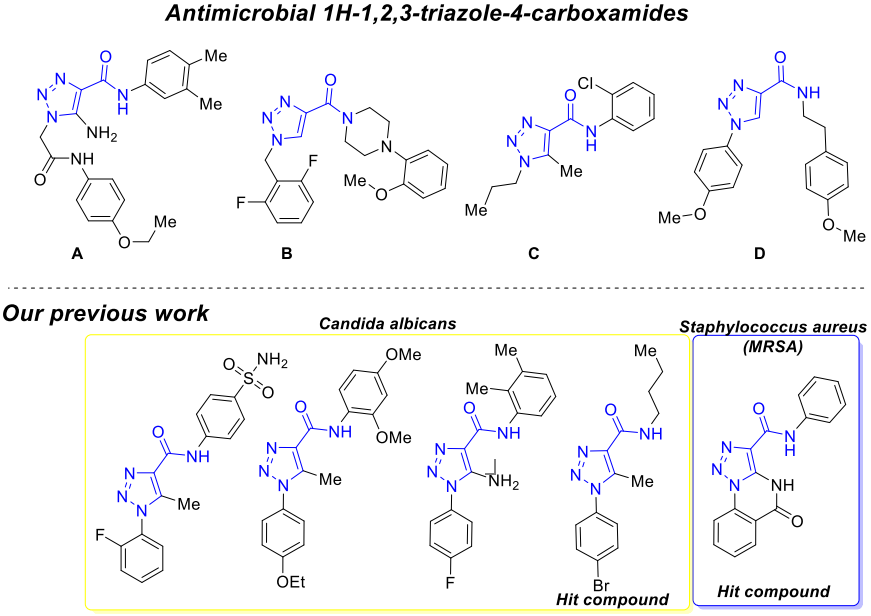


Fig. 1. Antimicrobial 1-aryl-1H-1,2,3-triazole-4-carboxamides

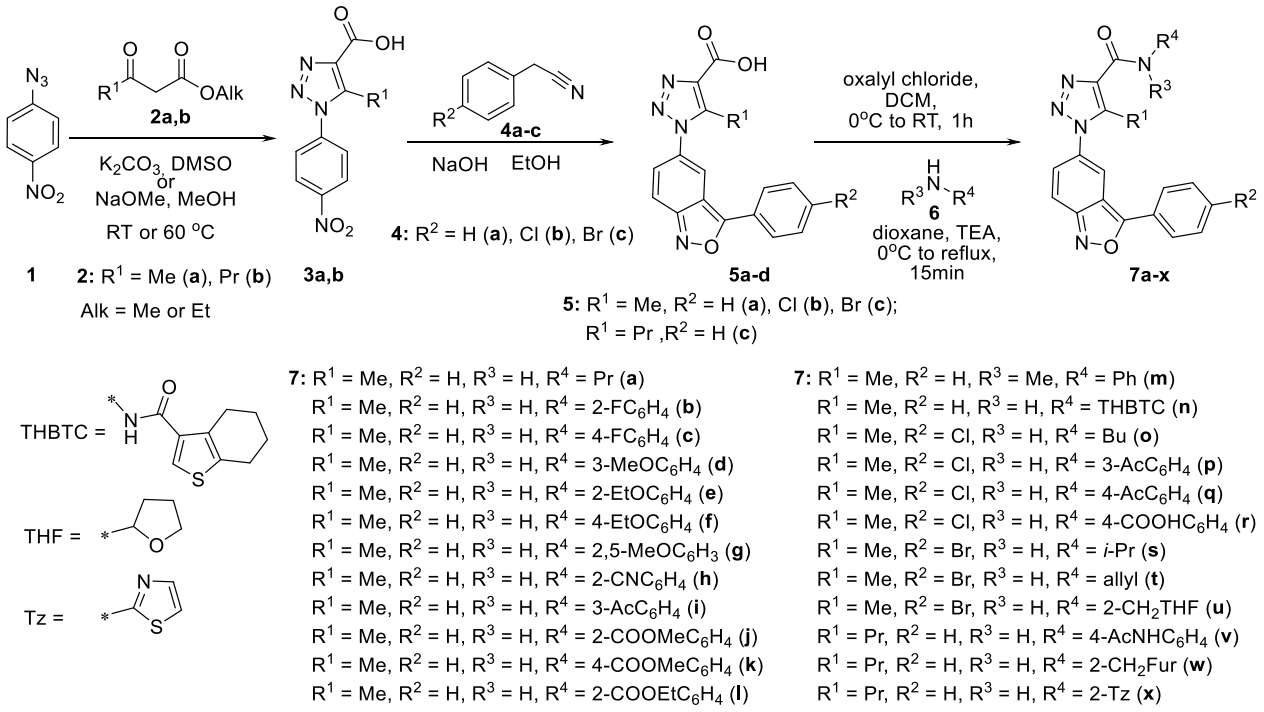
In addition, it should be noted that the compounds containing the 1,2,3-triazole-4-carboxamide moiety are already used as drugs (for example, Rufinamide or Carboxyamido-triazole) and are promising antiproliferative agents for anticancer studies. [15–24]

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

Results and discussion

Chemistry. The compounds presented in the article were obtained in a convenient synthetic path (Scheme 1) starting from organic azides **1**. At the first stage, the Dimroth method was used for preparation 1-(4-nitrophenyl)-5-R^1-*1H*-1,2,3-triazole-4-carboxylic acids **3** via the base-catalyzed cyclocondensation of 4-nitrophenyl azide **1** with *β*-ketoesters **2**. It is worth mentioning that the diversity of *1H*-1,2,3-triazole 4-carboxylic acids with various substituents in positions 1 and 5 of the 1,2,3-triazole ring can be prepared by the Dimroth method due to the synthetic and commercial availability of azides and β-ketoesters [25]. The reaction can be carried out both in strong base (NaOMe, *t*-BuOK) and mild base (K_2CO_3, organic bases) conditions, depending on the electronic and steric effects of the substituents, ensuring high product yields [26–30]. For example, when bulky substituents (such as diethyl acetal) in ketoester or the ortho position to the azido group, the yields are much higher in mild conditions [31]. In addition, under mild conditions, the ester group can be replaced by a phosphate group [32], which opens up additional opportunities to design compounds to study biological activity.

The second stage was a unique vicarious nucleophilic substitution in nitroaryl fragments. By the reaction of 1-(4-nitrophenyl)-5-R^1-*1H*-1,2,3-triazole-4-carboxylic acids **3** with arylacetonitriles **4** in the alcoholic medium in the presence of excess alkali, the 2,1-benzisoxazoles **5** were prepared. A high reactivity of nitroarenes **3** activated electron-deficient azole ring should be emphasised. [33]. The 1,2,3-triazole-4-carboxylic acids are versatile building blocks for synthesising combinatorial libraries of their amides via rapid chloroanhydride formation following reaction with amines. At the amidation stage, the yields were 81-94%, allowing the quick preparation of a library of such compounds without chromatographic purification. The structure and purity of the compounds are proved by ^{1}H and LCMS methods.



THBTC =

THF =

Tz =

7: R^1 = Me, R^2 = H, R^3 = H, R^4 = Pr (**a**)
R^1 = Me, R^2 = H, R^3 = H, R^4 = 2-FC_6H_4 (**b**)
R^1 = Me, R^2 = H, R^3 = H, R^4 = 4-FC_6H_4 (**c**)
R^1 = Me, R^2 = H, R^3 = H, R^4 = 3-$MeOC_6H_4$ (**d**)
R^1 = Me, R^2 = H, R^3 = H, R^4 = 2-$EtOC_6H_4$ (**e**)
R^1 = Me, R^2 = H, R^3 = H, R^4 = 4-$EtOC_6H_4$ (**f**)
R^1 = Me, R^2 = H, R^3 = H, R^4 = 2,5-$MeOC_6H_3$ (**g**)
R^1 = Me, R^2 = H, R^3 = H, R^4 = 2-CNC_6H_4 (**h**)
R^1 = Me, R^2 = H, R^3 = H, R^4 = 3-AcC_6H_4 (**i**)
R^1 = Me, R^2 = H, R^3 = H, R^4 = 2-$COOMeC_6H_4$ (**j**)
R^1 = Me, R^2 = H, R^3 = H, R^4 = 4-$COOMeC_6H_4$ (**k**)
R^1 = Me, R^2 = H, R^3 = H, R^4 = 2-$COOEtC_6H_4$ (**l**)

7: R^1 = Me, R^2 = H, R^3 = Me, R^4 = Ph (**m**)
R^1 = Me, R^2 = H, R^3 = H, R^4 = THBTC (**n**)
R^1 = Me, R^2 = Cl, R^3 = H, R^4 = Bu (**o**)
R^1 = Me, R^2 = Cl, R^3 = H, R^4 = 3-AcC_6H_4 (**p**)
R^1 = Me, R^2 = Cl, R^3 = H, R^4 = 4-AcC_6H_4 (**q**)
R^1 = Me, R^2 = Cl, R^3 = H, R^4 = 4-$COOHC_6H_4$ (**r**)
R^1 = Me, R^2 = Br, R^3 = H, R^4 = *i*-Pr (**s**)
R^1 = Me, R^2 = Br, R^3 = H, R^4 = allyl (**t**)
R^1 = Me, R^2 = Br, R^3 = H, R^4 = 2-CH_2THF (**u**)
R^1 = Pr, R^2 = H, R^3 = H, R^4 = 4-$AcNHC_6H_4$ (**v**)
R^1 = Pr, R^2 = H, R^3 = H, R^4 = 2-CH_2Fur (**w**)
R^1 = Pr, R^2 = H, R^3 = H, R^4 = 2-Tz (**x**)

Scheme 1. Synthetic routes to the target compounds

Biological activity. Antimicrobial screening. The results of the preliminary screening (two parallel trials) of the newly synthesized compounds in concentration 32 μg/mL (100–200 μM) on seven pathogens (*S. aureus, E. coli, K. pneumoniae, P. aeruginosa, A. baumannii, C. albicans* and *C. neoformans*) are presented in Table 1.

Table 1.

Preliminary screening of selected cage amides and imides

	The percentage of growth inhibition (GI) in concentration 32 μg/mL, %[a]						
	Bacteria					**Fungal**	
N	*S. aureus ATCC 43300*	*E. coli ATCC 25922*	*K. pneumoniae ATCC 700603*	*A. baumannii ATCC 19606*	*P. aeruginosa ATCC 27853*	*C. albicans ATCC 90028*	*C. neoformans ATCC 208821*
7a	−7.80 ± 5.09	−7.65 ± 3.75	−6.35 ± 9.55	−5.55 ± 6.15	5.10 ± 1.84	5.25 ± 0.35	**23.60 ± 2.40**
7b	**18.05 ± 1.20**	2.80 ± 2.83	−0.30 ± 6.08	5.65 ± 4.03	6.15 ± 2.47	15.60 ± 1.98	7.25 ± 12.80
7c	10.25 ± 8.41	4.00 ± 2.69	−1.95 ± 11.10	1.85 ± 8.27	−0.50 ± 3.11	0.95 ± 1.06	−14.75 ± 5.73
7d	−6.50 ± 7.64	2.00 ± 0.99	2.75 ± 8.56	−0.70 ± 6.51	5.05 ± 4.17	5.50 ± 1.98	12.15 ± 0.78
7e	14.05 ± 0.78	2.05 ± 2.62	4.65 ± 5.44	**17.00 ± 16.69**	−2.10 ± 1.27	5.25 ± 2.62	−0.65 ± 11.81
7f	−2.95 ± 1.20	−2.20 ± 6.93	−2.40 ± 0.71	-4.55 ± 2.05	−1.60 ± 3.54	4.10 ± 4.67	−8.20 ± 12.45
7g	−11.10 ± 3.82	3.30 ± 0.14	8.65 ± 1.48	−1.90 ± 11.60	6.40 ± 0.14	4.45 ± 0.07	10.55 ± 6.43
7h	13.20 ± 2.83	5.50 ± 5.80	1.80 ± 9.76	3.90 ± 6.79	7.05 ± 0.07	5.30 ± 3.96	−3.95 ± 11.81
7i	**19.30 ± 0.57**	6.50 ± 5.94	7.20 ± 2.55	10.35 ± 7.00	1.35 ± 0.49	9.70 ± 5.23	−22.30 ± 3.39
7j	7.25 ± 1.20	−2.95 ± 7.14	−0.55 ± 1.06	−0.80 ± 3.11	1.30 ± 3.54	2.65 ± 1.06	1.95 ± 7.57
7k	7.55 ± 5.16	4.10 ± 3.82	7.55 ± 2.05	−0.95 ± 2.33	5.35 ± 1.91	0.95 ± 2.19	7.00 ± 15.98
7l	−1.80 ± 0.85	5.95 ± 4.31	7.80 ± 2.26	−0.35 ± 0.07	1.35 ± 0.92	3.45 ± 0.07	4.20 ± 9.62
7m	15.95 ± 12.66	−1.25 ± 2.90	2.60 ± 0.42	−6.45 ± 1.34	−2.55 ± 2.47	5.55 ± 3.04	−63.10 ± 17.54
7n	7.70 ± 4.53	−3.50 ± 1.27	0.65 ± 0.92	−10.45± 13.79	−2.15 ± 2.05	0.05 ± 2.33	−26.95 ± 2.19
7o	−4.00 ± 5.09	−3.60 ± 0.57	−2.05 ± 9.83	-5.20 ± 8.34	−2.15 ± 0.21	1.60 ± 1.13	−2.00 ± 2.69
7p	−4.30 ± 3.25	−4.10 ± 0.71	3.60 ± 0.85	−6.70 ± 2.83	−1.95 ± 2.05	3.20 ± 3.11	−2.55 ± 4.03
7q	9.70 ± 12.16	3.60 ± 1.27	7.60 ± 0.85	8.05 ± 0.21	−1.80 ± 2.83	2.20 ± 0.99	−2.45 ± 1.77
7r	3.15 ± 6.29	8.25 ± 1.06	**10.70 ± 7.21**	3.90 ± 6.08	−3.75 ± 1.34	3.05 ± 0.35	2.05 ± 3.89
7s	−9.35 ± 7.00	3.60 ± 1.70	2.60 ± 6.51	−8.45 ± 13.08	−3.25 ± 2.76	1.00 ± 1.70	4.05 ± 0.78
7t	−11.15± 10.68	**10.25 ± 1.20**	0.05 ± 8.98	0.35 ± 6.43	2.30 ± 4.67	5.75 ± 0.64	**19.35 ± 2.05**
7u	**18.75 ± 0.64**	4.10 ± 2.12	8.50 ± 3.68	14.55 ± 15.49	−0.30 ± 4.81	6.45 ± 0.64	−5.10 ± 7.50
7v	14.30 ± 0.14	0.00 ± 3.25	5.10 ± 1.41	6.00 ± 1.41	−1.70 ± 2.40	9.15 ± 0.49	−41.55 ± 20.01
7w	5.70 ± 1.56	−2.70 ± 7.92	2.75 ± 1.91	-0.30 ± 8.91	−5.05 ± 4.88	4.05 ± 8.56	−1.35 ± 2.19
7x	−2.85 ± 1.91	0.80 ± 0.42	0.65 ± 0.92	7.45 ± 3.04	7.15 ± 1.91	3.80 ± 0.14	7.95 ± 4.31

Comment: Data are presented as M ± SD calculated from two parallel trials [a].

According to the results, most tested compounds did not show activity against resistant strains. However, we identified five structures (compounds **7a**, **7b**, **7i**, **7t** and **7u**, Fig. 2) that reduced the growth of microorganisms by approximately 20 % and can be taken as a basis. Thus, compounds **7b**, **7i** and **7u** inhibited the growth of methicillin-resistant *Staphylococcus aureus* (ATCC 43300) with a GI of 18.05 ± 1.20, 19.30 ± 0.57, and 18.75 ± 0.64%, respectively. The common motifs present in these derivatives should be noted. In particular, these compounds contain an isostructural bridge C2 and C4 connecting the amide nitrogen with fluorine or oxygen, respectively. Its importance in binding to the biotarget can be assumed. On the other hand, compounds **7a** and **7i** were active against parasitic fungi of the strain *Cryptococcus neoformans* var. *grubii* (ATCC 208821), which are causative agents of cryptococcosis. compounds **7a** and **7i** inhibited fungal growth with a GI of 23.60 ± 2.40 and 19.35 ± 2.05, respectively. The isostructural feature of these compounds was the C3 fragment – propyl and allyl, respectively. It is important that the aryl substituent in isoxazole had no appreciable effect on the activity. Instead, increasing the substituent from methyl to propyl in the 5-position of the triazole led to a loss of activity, which correlates with our previous studies. [14]

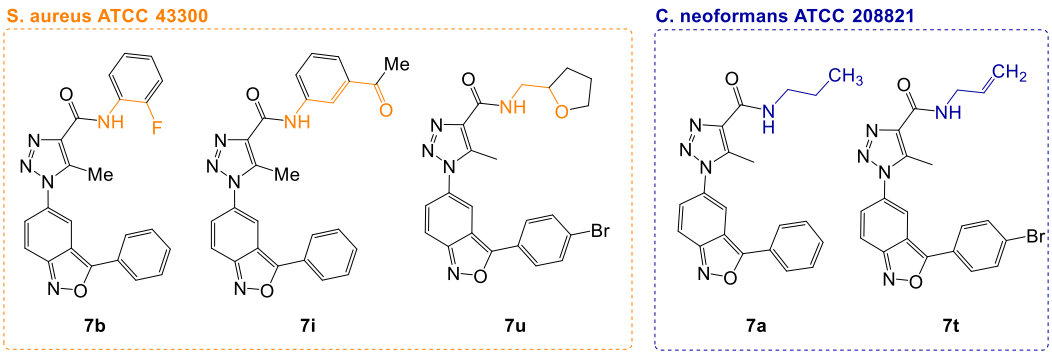


Fig. 2. Most potent compounds

Experimental Section

All chemicals used were of laboratory grade and used without further purification. ^{1}H NMR spectra were recorded on Varian Unity Plus 400 (400 MHz) spectrometers in DMSO-d_6 solutions using TMS or the deuterated solvent as internal reference. 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. Melting points were determined on a Boetius melting point apparatus. The starting *1H*-1,2,3-triazole 4-carboxylic acids 3 were synthesized according to previously described synthetic procedures [33].

General procedure for synthesis of compounds ***7***

The appropriate 1,2,3-triazole-4-carboxylic acids **3** (1 mmol, 1 eq.) was dissolved in dry DCM (30 mL) and cooled in an ice bath. Oxalyl chloride (173 μL, 2 mmol, 2 eq.) was added, followed by 1 drop of the DMF, and the reaction was stirred for 1 h at room temperature. Evaporation of the volatiles under reduced pressure afforded crude acid chloride, which was immediately carried onto the next step. The acid chloride was added to the solution of appropriate amine **6** (1 mmol, 1 eq.) and TEA (140 μL, 1 mmol, 1 eq.) in dioxane (10 mL) under ice bath cooling. The was left for 1 h at room temperature, then heated to reflux and reflux for 1 min. Then was mixture cooled to room temperature and diluted with water (50 mL). Crude product 5 was collected by filtration and recrystallized from ethanol with a small addition of the DMF. Finally, the compounds **7** were dried at 60°C under vacuum to yield pure products.

5-Methyl-1-(3-phenylbenzo[c]isoxazol-5-yl)-N-propyl-1H-1,2,3-triazole-4-carboxamide ***7a***

Yield: 83% as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35 (s, 1H, H^{BIX}-4), 8.28 (t, *J* = 5.7 Hz, 1H, NH), 7.97 (d, *J* = 8.6 Hz, 1H, H^{BIX}-7), 7.84 (d, *J* = 9.4 Hz, 1H, H^{BIX}-6), 7.64 – 7.48 (m, 5H, H^{Ph}), 3.36 – 3.20 (m, 2H, CH_2), 2.61 (s, 3H, CH_3), 1.71 – 1.50 (m, 2H, CH_2), 0.95 (t, *J* = 7.4 Hz, 3H, CH_3); MS (m/z, ES-API) 362 (M^++1); Anal. calcd for $C_{20}H_{19}N_5O_2$: C 66.47; H 5.30; N 19.38; Found: C 66.43; H 5.21; N 19.70.

N-(2-Fluorophenyl)-5-methyl-1-(3-phenylbenzo[c]isoxazol-5-yl)-1H-1,2,3-triazole-4-carboxamide ***7b***

Yield: 88% as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.74 (d, *J* = 1.9 Hz, 1H, NH), 8.44 (d, *J* = 0.9 Hz, 1H, H^{BIX}-4), 8.18 – 8.11 (m, 2H), 8.11 – 8.01 (m, 1H), 7.86 (dd, *J* = 9.4, 0.9 Hz, 1H, H^{BIX}-6), 7.65 – 7.52 (m, 4H), 7.26 – 7.12 (m, 3H), 2.69 (s, 3H, CH_3); MS (m/z, ES-API) 414 (M^++1); Anal. calcd for $C_{23}H_{16}FN_5O_2$: C 66.82; H 3.90; N 16.94; Found: C 67.00; H 3.99; N 16.78.

N-(4-Fluorophenyl)-5-methyl-1-(3-phenylbenzo[c]isoxazol-5-yl)-1H-1,2,3-triazole-4-carboxamide ***7c***

Yield: 91% as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.49 (s, 1H, NH), 8.45 (s, 1H, H^{BIX}-4), 8.16 (d, *J* = 7.4 Hz, 2H, $H^{arom.}$), 8.04 – 7.77 (m, 3H, $H^{arom.}$), 7.72 – 7.48 (m, 4H, $H^{arom.}$), 7.16 – 6.94 (m, 2H, $H^{arom.}$), 2.71 (s, 3H, CH_3); MS (m/z, ES-API) 414 (M^++1); Anal. calcd for $C_{23}H_{16}FN_5O_2$: C 66.82; H 3.90; N 16.94; Found: C 66.71; H 4.03; N 17.04.

N-(3-methoxyphenyl)-5-methyl-1-(3-phenylbenzo[c]isoxazol-5-yl)-1H-1,2,3-triazole-4-carboxamide ***7d***

Yield: 8 5% as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.29 (s, 1H, NH), 8.45 (s, 1H, H^{BIX}-4), 8.16 (d, *J* = 6.7 Hz, 2H), 7.89 (d, *J* = 9.4 Hz, 1H, H^{BIX}-6), 7.64 – 7.56 (m, 4H, $H^{arom.}$), 7.53 (dd, *J* = 9.3, 1.4 Hz, 1H, -7), 7.47 (d, *J* = 8.2 Hz, 1H, $H^{arom.}$), 7.19 (t, *J* = 8.1 Hz, 1H, $H^{arom.}$), 6.62 (dd, *J* = 8.2, 2.4 Hz, 1H, $H^{arom.}$), 3.80 (s, 3H, CH_3O), 2.71 (s, 3H, CH_3); MS (m/z, ES-API) 426 (M^++1); Anal. calcd for $C_{24}H_{19}N_5O_3$: C 67.76; H 4.50; N 16.46; Found: C 67.71; H 4.44; N 16.57.

N-(2-ethoxyphenyl)-5-methyl-1-(3-phenylbenzo[c]isoxazol-5-yl)-1H-1,2,3-triazole-4-carboxamide ***7e***

Yield: 82 % as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.62 (s, 1H, NH), 8.38 (d, *J* = 7.4 Hz, 1H, $H^{arom.}$), 8.40 (s, 1H, H^{BIX}-4), 7.90 (d, *J* = 8.6 Hz, 1H, H^{BIX}-6), 7.76 (d, *J* = 9.4 Hz, 1H, H^{BIX}-7), 7.64 – 7.48 (m, 5H, H^{Ph}), 7.07 – 7.00 (m, 2H, $H^{arom.}$), 6.96 – 6.91 (m, 1H, $H^{arom.}$), 4.21 (q, *J* = 7.0 Hz, 2H, CH_2O), 2.63 (s, 3H, CH_3), 1.52 (t, *J* = 7.0 Hz, 3H, CH_3); MS (m/z, ES-API) 440 (M^++1); Anal. calcd for $C_{25}H_{21}N_5O_3$: C 68.33; H 4.82; N 15.94; Found: C 68.43; H 4.90; N 15.81.

N-(4-ethoxyphenyl)-5-methyl-1-(3-phenylbenzo[c]isoxazol-5-yl)-1H-1,2,3-triazole-4-carboxamide ***7f***

Yield: 92% as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.19 (s, 1H, NH), 8.43 (s, 1H, H^{BIX}-4), 8.19 – 8.11 (m, 2H, $H^{arom.}$), 7.86 (d, *J* = 9.4 Hz, 1H, H^{BIX}-6), 7.74 (d, *J* = 9.0 Hz, 2H, $H^{arom.}$), 7.65 – 7.52 (m, 4H, H^{Ph}), 6.81 (d, *J* = 9.0 Hz, 2H, $H^{arom.}$), 4.00 (q, *J* = 7.0 Hz, 2H, CH_2O), 2.68 (s, 3H, CH_3), 1.37 (t, *J* = 7.0 Hz, 3H, CH_3); MS (m/z, ES-API) 440 (M^++1); Anal. calcd for $C_{25}H_{21}N_5O_3$: C 68.33; H 4.82; N 15.94; Found: C 68.21; H 4.88; N 15.99.

N-(2,5-Dimethoxyphenyl)-5-methyl-1-(3-phenylbenzo[c]isoxazol-5-yl)-1H-1,2,3-triazole-4-carboxamide ***7g***

Yield: 86 % as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.54 (s, 1H, NH), 8.37 (s, 1H, H^{BIX}-4), 8.06 (s, 1H, $H^{arom.}$), 7.97 (d, *J* = 8.6 Hz, 1H, H^{BIX}-7), 7.84 (d, *J* = 9.4 Hz, 1H, H^{BIX}-6), 7.65 – 7.48 (m, 5H, H^{Ph}), 6.93 (d, *J* = 9.1 Hz, 1H, $H^{arom.}$), 6.57 (d, *J* = 9.1 Hz, 1H, $H^{arom.}$), 3.93 (s, 3H, CH_3O), 3.76 (s, 3H, CH_3O), 2.64 (s, 3H, CH_3); MS (m/z, ES-API) 456 (M^++1); Anal. calcd for $C_{25}H_{21}N_5O_4$: C 65.93; H 4.65; N 15.38; Found: C 65.99; H 4.78; N 15.25.

N-(2-Cyanophenyl)-5-methyl-1-(3-phenylbenzo[c]isoxazol-5-yl)-1H-1,2,3-triazole-4-carboxamide ***7h***

Yield: 82 % as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.51 (s, 1H, NH), 8.48 (s, 1H, H^{BIX}-4), 8.19 – 8.12 (m, 2H, $H^{arom.}$), 7.93 (d, *J* = 8.2 Hz, 1H, H^{BIX}-7), 7.87 (d, *J* = 9.4 Hz, 1H, H^{BIX}-6), 7.77 (d, *J* = 7.8 Hz, 1H, $H^{arom.}$), 7.69 (t, *J* = 7.9 Hz, 1H, $H^{arom.}$), 7.65 – 7.52 (m, 4H, H^{Ph}), 7.35 (t, *J* = 7.6 Hz, 1H, $H^{arom.}$), 2.69 (s, 3H, CH_3); MS (m/z, ES-API) 421 (M^++1); Anal. calcd for $C_{24}H_{16}N_6O_2$: C 68.56; H 3.84; N 19.99; Found: C 68.49; H 3.73; N 20.04.

N-(3-Acetylphenyl)-5-methyl-1-(3-phenylbenzo[c]isoxazol-5-yl)-1H-1,2,3-triazole-4-carboxamide ***7i***

Yield: 90 % as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (s, 1H, NH), 8.55 (t, *J* = 1.9 Hz, 1H, $H^{arom.}$), 8.45 (d, *J* = 1.0 Hz, 1H, H^{BIX}-4), 8.19 – 8.10 (m, 2H, $H^{arom.}$), 7.87 (dd, *J* = 9.4, 0.9 Hz, 1H, H^{BIX}-6), 7.64 (d, *J* = 8.4 Hz, 2H, $H^{arom.}$), 7.61 – 7.52 (m, 4H, H^{Ph}), 7.43 (t, *J* = 7.9 Hz, 1H, $H^{arom.}$), 2.70 (s, 3H, CH_3), 2.58 (s, 3H, CH_3); MS (m/z, ES-API) 438 (M^++1); Anal. calcd for $C_{25}H_{19}N_5O_3$: C 68.64; H 4.38; N 16.01; Found: C 68.79; H 4.34; N 15.88.

Methyl 2-(5-methyl-1-(3-phenylbenzo[c]isoxazol-5-yl)-1H-1,2,3-triazole-4-carboxamido)benzoate ***7j***

Yield: 82 % as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.38 (s, 1H, NH), 8.83 (dd, *J* = 8.5, 1.2 Hz, 1H, $H^{arom.}$), 8.46 (d, *J* = 1.0 Hz, 1H, H^{BIX}-4), 8.19 – 8.12 (m, 2H, $H^{arom.}$), 8.07 (dd, *J* = 8.0, 1.7 Hz, 1H, $H^{arom.}$), 7.87 (dd, *J* = 9.4, 0.9 Hz, 1H, H^{BIX}-6), 7.67 – 7.52 (m, 5H, H^{Ph}), 7.17 (t, *J* = 7.7 Hz, 1H, $H^{arom.}$), 3.98 (s, 3H, CH_3O), 2.71 (s, 3H, CH_3); MS (m/z, ES-API) 454 (M^++1); Anal. calcd for $C_{25}H_{19}N_5O_4$: C 66.22; H 4.22; N 15.44; Found: C 66.09; H 4.34; N 15.47.

Methyl 4-(5-methyl-1-(3-phenylbenzo[c]isoxazol-5-yl)-1H-1,2,3-triazole-4-carboxamido)benzoate ***7k***

Yield: 93 % as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.71 (s, 1H, NH), 8.44 (s, 1H, H^{BIX}-4), 8.19–8.12 (m, 2H, $H^{arom.}$), 8.04 (d, *J* = 8.8 Hz, 2H, $H^{arom.}$), 7.92 (d, *J* = 8.8 Hz, 2H, $H^{arom.}$), 7.87 (dd, *J* = 9.4, 0.9 Hz, 1H, H^{BIX}-6), 7.65 – 7.52 (m, 4H, H^{Ph}), 3.09 (s, 3H, CH_3O), 2.69 (s, 3H, CH_3); MS (m/z, ES-API) 454 (M^++1); Anal. calcd for $C_{25}H_{19}N_5O_4$: C 66.22; H 4.22; N 15.44; Found: C 66.17; H 4.15; N 15.51.

Ethyl 2-(5-methyl-1-(3-phenylbenzo[c]isoxazol-5-yl)-1H-1,2,3-triazole-4-carboxamido)benzoate ***7l***

Yield: 84% as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.37 (s, 1H, NH), 8.82 (dd, *J* = 8.5, 1.1 Hz, 1H, $H^{arom.}$), 8.45 (s, 1H, H^{BIX}-4), 8.19 – 8.11 (m, 2H, $H^{arom.}$), 8.07 (dd, *J* = 8.0, 1.7 Hz, 1H, $H^{arom.}$), 7.87 (dd, *J* = 9.4, 0.9 Hz, 1H, H^{BIX}-6), 7.66 – 7.52 (m, 5H, H^{Ph}), 7.21 – 7.12 (m, 1H, $H^{arom.}$), 4.44 (q, *J* = 7.1 Hz, 2H, CH_2O), 2.71 (s, 3H, CH_3), 1.44 (t, *J* = 7.1 Hz, 3H, CH_3); MS (m/z, ES-API) 468 (M^++1); Anal. calcd for $C_{26}H_{21}N_5O_4$: C 66.80; H 4.53; N 14.98; Found: C 66.59; H 4.38; N 15.09.

N,5-Dimethyl-N-phenyl-1-(3-phenylbenzo[c]isoxazol-5-yl)-1H-1,2,3-triazole-4-carboxamide ***7m***

Yield: 86 % as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (s, 1H, H^{BIX}-4), 8.18 – 8.09 (m, 2H, $H^{arom.}$), 7.81 (d, *J* = 9.4 Hz, 1H, H^{BIX}-6), 7.62 – 7.51 (m, 3H, H^{Ph}), 7.45 (d, *J* = 9.9 Hz, 1H, $H^{arom.}$), 7.34 (t, *J* = 7.7 Hz, 2H, $H^{arom.}$), 7.27 – 7.19 (m, 3H, $H^{arom.}$), 3.50 (s, 3H, CH_3), 2.49 (s, 3H, CH_3); MS (m/z, ES-API) 410 (M^++1); Anal. calcd for $C_{24}H_{19}N_5O_2$: C 70.40; H 4.68; N 17.10; Found: C 70.34; H 4.75; N 17.17.

5-Methyl-1-(3-phenylbenzo[c]isoxazol-5-yl)-N'-(4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonyl)-1H-1,2,3-triazole-4-carbohydrazide ***7n***

Yield: 92 % as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.24 (s, 1H, NH), 10.03 (s, 1H, NH), 8.43 (s, 1H, H^{BIX}-4), 8.15 (d, *J* = 7.9 Hz, 2H, $H^{arom.}$), 7.89 – 7.83 (m, 2H, $H^{arom.}$), 7.64 – 7.52 (m, 4H, $H^{arom.}$), 2.85 – 2.72 (m, 4H, 2xCH_2), 2.64 (s, 3H, CH_3), 1.89 – 1.69 (m, 4H, 2xCH_2); MS (m/z, ES-API) 499 (M^++1); Anal. calcd for $C_{26}H_{22}N_6O_3S$: C 62.64; H 4.45; N 16.86; Found: C 62.77; H 4.49; N 16.94.

N-Butyl-1-(3-(4-chlorophenyl)benzo[c]isoxazol-5-yl)-5-methyl-1H-1,2,3-triazole-4-carboxamide ***7o***

Yield: 84 % as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.39 (s, 1H, H^{BIX}-4), 8.33 (t, *J* = 6.0 Hz, 1H, NH), 8.17 (d, *J* = 8.6 Hz, 2H, H^{ArCl}), 7.86 (d, *J* = 9.4 Hz, 1H, H^{BIX}-7), 7.60 (d, *J* = 8.4 Hz, 2H, H^{ArCl}), 7.55 (dd, *J* = 9.4, 1.7 Hz, 1H, H^{BIX}-6), 3.29 (dd, *J* = 13.3, 6.8 Hz, 2H, CH_2N), 2.62 (s, 3H, CH_3), 1.61 – 1.49 (m, 2H, CH_2), 1.43 – 1.31 (m, 2H, CH_2), 0.95 (t, *J* = 7.3 Hz, 3H, CH_3); MS (m/z, ES-API) 410 (M^++1); Anal. calcd for $C_{21}H_{20}ClN_5O_2$: C 61.54; H 4.92; N 17.09; Found: C 61.43; H 4.99; N 17.00.

N-(3-Acetylphenyl)-1-(3-(4-chlorophenyl)benzo[c]isoxazol-5-yl)-5-methyl-1H-1,2,3-triazole-4-carboxamide ***7p***

Yield: 87 % as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.64 (s, 1H, NH), 8.56 (t, *J* = 1.9 Hz, 1H, $H^{arom.}$), 8.48 (s, 1H, H^{BIX}-4), 8.20 (d, *J* = 8.5 Hz, 2H, H^{ArCl}), 8.15 (d, *J* = 7.9 Hz, 1H, H^{BIX}-7), 7.89 (d, *J* = 9.5 Hz, 1H, H^{BIX}-6), 7.68 – 7.57 (m, 4H, $H^{arom.}$), 7.44 (t, *J* = 7.9 Hz, 1H, $H^{arom.}$), 2.71 (s, 3H, CH_3), 2.59 (s, 3H, CH_3); MS (m/z, ES-API) 472 (M^++1); Anal. calcd for $C_{25}H_{18}ClN_5O_3$: C 63.63; H 3.84; N 14.84; Found: C 63.79; H 3.71; N 14.79.

N-(4-Acetylphenyl)-1-(3-(4-chlorophenyl)benzo[c]isoxazol-5-yl)-5-methyl-1H-1,2,3-triazole-4-carboxamide ***7q***

Yield: 94 % as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.66 (s, 1H, NH), 8.48 (s, 1H, H^{BIX}-4), 8.20 (d, *J* = 8.5 Hz, 2H, H^{ArCl}), 8.15 (d, *J* = 7.9 Hz, 1H, H^{BIX}-7), 8.05 (d, *J* = 8.7 Hz, 2H, $H^{arom.}$), 7.91 (d, *J* = 8.7 Hz, 2H, $H^{arom.}$), 7.89 (d, *J* = 9.5 Hz, 1H, H^{BIX}-6), 7.62 (d, *J* = 8.4 Hz, 2H, H^{ArCl}), 2.59 (s, 3H, CH_3), 2.54 (s, 3H); MS (m/z, ES-API) 472 (M^++1); Anal. calcd for $C_{25}H_{18}ClN_5O_3$: C 63.63; H 3.84; N 14.84; Found: C 63.58; H 3.90; N 14.89.

4-(1-(3-(4-Chlorophenyl)benzo[c]isoxazol-5-yl)-5-methyl-1H-1,2,3-triazole-4-carboxamido)benzoic acid ***7r***

Yield: 90 % as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.72 (s, 1H, NH), 8.47 (s, 1H, H^{BIX}-4), 8.19 (d, *J* = 8.4 Hz, 2H, H^{ArCl}), 8.04 (d, *J* = 8.6 Hz, 2H, $H^{arom.}$), 7.92 (d, *J* = 8.6 Hz, 2H, $H^{arom.}$), 7.88 (d, *J* = 9.5 Hz, 1H, H^{BIX}-7), 7.63 – 7.57 (m, 3H, $H^{arom.}$), 2.69 (s, 3H, CH_3); MS (m/z, ES-API) 474 (M^++1); Anal. calcd for $C_{24}H_{16}ClN_5O_4$: C 60.83; H 3.40; N 14.78; Found: C 60.71; H 3.51; N 14.72.

1-(3-(4-Bromophenyl)benzo[c]isoxazol-5-yl)-N-isopropyl-5-methyl-1H-1,2,3-triazole-4-carboxamide ***7s***

Yield: 92% as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.39 (s, 1H, H^{BIX}-4), 8.11 (d, *J* = 8.6 Hz, 2H, H^{ArBr}), 8.03 (d, *J* = 8.3 Hz, 1H, NH), 7.87 (d, *J* = 9.4 Hz, 1H, H^{BIX}-7), 7.76 (d, *J* = 8.6 Hz, 2H, H^{ArBr}), 7.56 (dd, *J* = 9.5, 1.7 Hz, 1H, H^{BIX}-6), 4.26 – 4.10 (m, 1H), 2.63 (s, 3H, CH_3), 1.24 (d, *J* = 6.2 Hz, 6H); MS (m/z, ES-API) 440, 442 (M^++1); Anal. calcd for $C_{20}H_{18}BrN_5O_2$: C 54.56; H 4.12; N 15.91; Found: C 54.45; H 4.03; N 15.99.

N-Allyl-1-(3-(4-bromophenyl)benzo[c]isoxazol-5-yl)-5-methyl-1H-1,2,3-triazole-4-carboxamide ***7t***

Yield: 81 % as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (t, *J* = 5.7 Hz, 1H, NH), 8.39 (s, 1H, H^{BIX}-4), 8.11 (d, *J* = 8.6 Hz, 2H, H^{ArBr}), 7.87 (d, *J* = 9.4 Hz, 1H, H^{BIX}-7), 7.76 (d, *J* = 8.6 Hz, 2H, H^{ArBr}), 7.56 (dd, *J* = 9.5, 1.7 Hz, 1H, H^{BIX}-6), 5.90 (ddd, *J* = 15.6, 10.4, 5.3 Hz, 1H, CH=), 5.19 (d, *J* = 17.2 Hz, 1H, CH_2=), 5.09 (d, *J* = 8.8 Hz, 1H, CH_2=), 3.93 (t, *J* = 5.7 Hz, 2H, CH_2N), 2.63 (s, 3H, CH_3); MS (m/z, ES-API) 438, 440 (M^++1); Anal. calcd for $C_{20}H_{16}BrN_5O_2$: C 54.81; H 3.68; N 15.98; Found: C 54.89; H 3.59; N 15.93.

1-(3-(4-Bromophenyl)benzo[c]isoxazol-5-yl)-5-methyl-N-((tetrahydrofuran-2-yl)methyl)-1H-1,2,3-triazole-4-carboxamide ***7u***

Yield: 85 % as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.39 (s, 1H, H^{BIX}-4), 8.18 (t, *J* = 6.0 Hz, 1H, NH), 8.12 (d, *J* = 8.6 Hz, 2H, H^{ArBr}), 7.87 (d, *J* = 9.4 Hz, 1H, H^{BIX}-7), 7.76 (d, *J* = 8.6 Hz, 2H, H^{ArBr}), 7.56 (dd, *J* = 9.5, 1.7 Hz, 1H, H^{BIX}-6), 4.09 – 3.95 (m, 1H, CHO), 3.89 – 3.78 (m, 1H, CHO), 3.72 – 3.60 (m, 1H, CHO), 3.44 – 3.29 (m, 2H, CH_2N), 2.62 (s, 3H, CH_3), 2.01 – 1.81 (m, 3H, CH), 1.71 – 1.58 (m, 1H, CH); MS (m/z, ES-API) 482, 484 (M^++1); Anal. calcd for $C_{22}H_{20}BrN_5O_3$: C 54.78; H 4.18; N 14.52; Found: C 54.73; H 4.29; N 14.42.

N-(4-Acetamidophenyl)-1-(3-phenylbenzo[c]isoxazol-5-yl)-5-propyl-1H-1,2,3-triazole-4-carboxamide ***7v***

Yield: 89 % as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.12 (s, 1H, NH), 9.72 (s, 1H, NH), 8.36 (s, 1H, H^{BIX}-4), 8.14 (d, *J* = 6.8 Hz, 2H, H^{Ph}), 7.86 (d, *J* = 9.4 Hz, 1H, H^{BIX}-7), 7.71 (d, *J* = 8.6 Hz, 2H, $H^{arom.}$), 7.67 – 7.55 (m, 3H, H^{Ph}), 7.50 (d, *J* = 8.6 Hz, 2H, $H^{arom.}$), 7.48 (d, *J* = 9.2 Hz, 1H, H^{BIX}-6), 3.07 – 2.99 (m, 2H, CH_2), 2.02 (s, 3H, CH_3CO), 1.62 – 1.45 (m, 2H, CH_2), 0.83 (t, *J* = 7.3 Hz, 3H, CH_3); MS (m/z, ES-API) 481 (M^++1); Anal. calcd for $C_{27}H_{24}N_6O_3$: C 67.49; H 5.03; N 17.49; Found: C 67.63; H 5.14; N 17.37.

N-(Furan-2-ylmethyl)-1-(3-phenylbenzo[c]isoxazol-5-yl)-5-propyl-1H-1,2,3-triazole-4-carboxamide ***7w***

Yield:83 % as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.79 (t, *J* = 6.0 Hz, 1H, NH), 8.34 (s, 1H, H^{BIX}-4), 8.17 (d, *J* = 7.8 Hz, 2H, H^{Ph}), 7.86 (d, *J* = 9.4 Hz, 1H, H^{BIX}-7), 7.65 – 7.54 (m, 3H, H^{Ph}), 7.49 (d, *J* = 9.2 Hz, 1H, H^{BIX}-6), 7.43 (d, *J* = 0.8 Hz, 1H, H^{Fur}-5), 6.32 (dd, *J* = 2.9, 1.9 Hz, 1H, H^{Fur}-4), 6.23 (d, *J* = 3.0 Hz, 1H, H^{Fur}-3), 4.47 (d, *J* = 6.0 Hz, 2H, CH_2N), 3.02 – 2.90 (m, 2H, CH_2), 1.57 – 1.38 (m, 2H, CH_2), 0.82 (t, *J* = 7.3 Hz, 3H, CH_3); MS (m/z, ES-API) 428 (M^++1); Anal. calcd for $C_{24}H_{21}N_5O_3$: C 67.44; H 4.95; N 16.38; Found: C 67.34; H 4.87; N 16.21.

1-(3-Phenylbenzo[c]isoxazol-5-yl)-5-propyl-N-(thiazol-2-yl)-1H-1,2,3-triazole-4-carboxamide ***7x***

Yield: 86 % as a white solid; mp >250 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.05 (s,

1H, NH), 8.34 (s, 1H, H^{BIX}-4), 8.17 (d, *J* = 7.8 Hz, 2H, H_{Ph}), 7.86 (d, *J* = 9.4 Hz, 1H, H^{BIX}-7), 7.63 – 7.49 (m, 5H, $H^{arom.}$), 7.15 (d, *J* = 3.5 Hz, 1H, H^{Tz}), 3.08 – 2.98 (m, 2H, CH_2), 1.65 – 1.48 (m, 2H, CH_2), 0.87 (t, *J* = 7.4 Hz, 3H, CH_3); MS (m/z, ES-API) 431 (M^++1); Anal. calcd for $C_{22}H_{18}N_6O_2S$: C 61.38; H 4.21; N 19.52; Found: C 61.25; H 4.44; N 19.72.

Antimicrobial assays via CO-ADD [34]

The compounds have been investigated for activity towards one Gram-positive bacteria (*S. aureus* ATCC 43300 MRSA), four Gram-negative bacteria (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 700603, *A. baumannii* ATCC 19606), and two yeasts (*C. albicans* ATCC 90028 and *C. neoformans* H99 ATCC 208821), and this research was performed by the Community for Open Antimicrobial Drug Discovery (CO-ADD).

Initially, the tests were carried out at a single compound concentration of 32 μg/mL in duplicate, to identify any active compound. All substances were dissolved in DMSO to form a stock concentration of 10 mg/mL. Aliquots were diluted in water and 5 μL were dispensed into empty 384-well plates in duplicate for each strain and cell-assayed. As soon as cells were added to the plates, this gave a final compound concentration of 32 μg/mL, a maximum DMSO concentration of 0.3 %.

All bacteria were overnight cultured in cation-adjusted Q14 Mueller–Hinton broth (CAMHB) at 37 °C. The resultant mid-log phase cultures were added to each well of the compound containing plates (384-well nonbinding surface plates-Corning 3640), giving a cell density of 5 × 10^5 CFU/mL (colony-forming units/mL). All plates were covered and incubated at 37 °C for 18 h without shaking. Inhibition of bacterial growth was determined measuring 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 measuring absorbance at 530 nm, while the growth inhibition of *C. neoformans* was determined 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. Percentage growth inhibition of an individual sample is calculated based on Negative controls (media only) and Positive Controls (bacterial/fungal media without inhibitors). Negative inhibition values indicate that the growth rate (defined in OD = 600 nm) 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.

Conclusion

In summary, we designed and synthesis the serial of 1-(3-phenylbenzo[*c*]isoxazol-5-yl)-*1H*-1,2,3-triazole-4-carboxamides and tested for antimicrobial activity toward seven drug-resistant bacteria and fungal infections strains. It was found that most tested compounds did not show activity against resistant strains; however, five compounds, **7a**, **7b**, **7i**, **7t** and **7u**, reduced the growth of microorganisms by approximately 20%. The obtained data will be used for further design and scaffold optimization *1H*-1,2,3-triazole-4-carboxamides for antimicrobial and anticancer studies.

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