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Synthesis and Antimicrobial Activity of 2-amino-4-pyrazolyl-4H-1,3-oxazines

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Abstract: The article is devoted to synthesizing and investigating the antimicrobial action of a class of functionalized heterocyclic systems. 2-amino-4-pyrazolyl-4H-1,3-oxazines were obtained by the three-component reaction of pyrazole-4-carbaldehydes, phenylacetylene, and urea. Their regioselective condensation with 1-chloroalkyl isocyanates was used to anneal the 1,3,5-triazine ring. The bactericidal activity of the synthesized compounds against typical strains of bacteria *Staphylococcus aureus* and *Escherichia coli* was studied.

Keywords: pyrazole-4-carbaldehydes; 2-amino-4-pyrazolyl-4H-1,3-oxazines; condensation; antimicrobial activity; synthesis.

1. Introduction

Monocyclic 1,3-oxazines are basic systems for designing other types of heterocycles, as well as key intermediates in synthesizing acyclic carbonyl compounds [1,2]. Methods for obtaining poly-substituted non-functionalized 4H-1,3-oxazines are usually based on intermolecular cyclization of β-acylamino ketones [3-5] or cyclo-condensation of β-chloromethanes with nitriles [6-8]. Their 2-functionally substituted representatives, 2-amino derivatives in particular, due to the complexity of the synthesis, remained poorly studied for an extended period of time. The method of obtaining 2-amino-4,6-diaryl-1,3-oxazines suggested in 2005 by the reaction of aromatic aldehydes with mono-substituted acetylenes and urea [9] created the preconditions for a significant expansion of the scope of its application both in terms of the synthesis of previously unknown 4-substituted derivatives, as well as new condensations involving the amino group [10].

The analysis of literary sources convincingly proved the presence of powerful pharmacological potential in a series of hybrid compounds in which the oxazine fragment is connected at position 4 with a 3-aryl-substituted pyrazole ring. Among them, substances with pronounced antimicrobial [11-21], anti-inflammatory [22-25], and antidiabetic [26-30] effects were found. Every year, there is a tendency to expand the range of drugs available in the pharmaceutical market [31].

In a series of 4-heteryl-substituted 2-amino-1,3-benzoxazines [32], the ability to modulate the β-secretase activity of enzymes was found, which can be used in the creation of effective means for the treatment of Alzheimer's disease, metabolic disorders [33]. Considering

this fact, synthesizing a number of new 2-amino-1,3-oxazines with a pharmacophoric pyrazole fragment [34] in the 4th position of the ring and studying their certain chemical and biological properties seem reasonable.

2. Materials and Methods

2.1. Materials.

All chemicals were of analytical grade and used without further purification [35,36]. The solvents were purified according to standard procedures [37]. The initial Pyrazole-4-carbaldehyde 1 a-h was synthesized using the methods [38,39].

2.2. Microorganisms.

An experiment used a standardized suspension of testing strains American Type Culture Collection (ATCC) of the following microorganisms: *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 [40-44]. Cell concentration was 0.5 McFarland (used to compare the standard turbidity) [45, 46].

2.3. Chemistry.

IR spectra of the compounds were recorded in the KBr pellets by the device Bruker Vertes 70. The NMR ^{1}H spectra were recorded using the spectrometer Varian VXR-400 (at 399.97 MHz) in the DMSO-d 6 solutions using TMS as an internal standard. Chromatomass spectra were obtained using the device Agilent LC\MSD SL equipped with the column Zorbax SB-C18, 4.6x15 mm, 1.8 μm (PN 82(c)75-932) and DMSO as the solvent. The electrospraying technique ionized the samples under atmospheric pressure. Elemental analysis was performed in the analytical laboratory of the Institute of Organic Chemistry of NAS of Ukraine using the Perkin Elmer CHN Analyzer series 2400. Melting points were determined using a Kofler Heizbank apparatus and left uncorrected.

2.4. Antibacterial test.

New synthesized poly-substituted non-functionalized 4H-1,3-oxazines were evaluated for their antimicrobial activity against tested strains of gram-positive bacteria *Staphylococcus aureus* ATCC 25923 and gram-negative bacteria *Escherichia coli* ATCC 25922 by the value of the minimal bacteriostatic (MBSC) and minimal bactericidal (MBCC) concentrations.

The antimicrobial activity of compounds (II a-h) (Table 1) was determined by the micromethod, which involves the preparation of two-fold serial dilutions using a Takachi microtitrator in disposable polystyrene tablets.

Table 1. Antimicrobial activity of compounds (II a-h).

Compound	Test-cultures of microorganisms			
	***Staphylococcus aureus* ATCC 2592**		***Escherichia coli* ATCC 25922**	
	MBsC	**MBcC**	**MBsC**	**MBcC**
II a	62.5	>500	500	500
b	62.5	>500	500	500
c	62.5	>500	500	>500
d	62.5	>500	>500	>500
e	125	>500	>500	1.95
f	125	>500	>500	>500
g	125	>500	250	250
h	125	>500	500	500
Ethonium	7.8	31.2	125	250

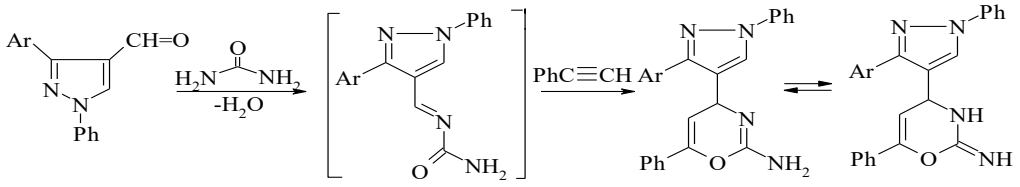
For broth microdilution, 0.05 ml of each dilution of microorganisms' culture was distributed over 96-well polystyrene microwell plates (1 ml of medium contained 10^5 CFU of bacteria) [47].

A 0.05 ml platinum basket was used to collect the testing sample matrix solution and added to the first well. The next samples were similarly introduced into the other wells of the first row. The dilutions in all wells from 1: 2 to 1: 256 were received. Similarly, an experiment with other testing cultures was conducted. The plates were then placed in a humid thermostat chamber at 37°C and incubated for 24 h. The results were assessed, taking into account the absence and presence of the growth of microorganisms. The minimum bacteriostatic concentration was considered the dilution of the sample at which the bacterial growth was inhibited. The experiment was performed three times.

The determination of minimal bactericidal concentration was as follows. Microorganisms were removed from wells with a liquid nutrient medium, where their growth was practically not observed. They were transplanted to a solid nutrient medium (MPA). Determination of MBCC was performed after culturing microorganisms at the optimum temperature and time. The minimum bactericidal concentrations were considered those at which the bacterial's vital activity was not restored, i.e., its growth was not observed on a solid nutrient medium.

3. Results and Discussion

10 h heating of pyrazole 4-carbaldehydes (I a-h) with phenylacetylene and urea in the ratio of 1:1:1.5 in boiling acetonitrile in the presence of the mixture of acetic and trifluoroacetic acids (3:1) appeared to lead to 2-amino-4 -pyrazolyl-4H-1,3-oxazines (II a-h) formation with the yield of 58-86%. It should be noted that, unlike 4-aryl analogs, the preparative isolation of compounds (II a-h) does not provoke any particular complications since they fall out of the reaction mixture in the form of a precipitate. Taking into account the results of the work [15], the stepwise scheme of the process involving the primary formation of N-alkylidenesureas (A), the subsequent cyclocondensation of which with phenylacetylene leads to the target products, seems to be the most reliable (II).



I a-h **A** **II a-h**

Figure 1. I, II: **(a)** Ar=Ph; **(b)** 4-FC_6H_4; **(c)** 4-ClC_6H_4; **(d)** 4-BrC_6H_4; **(e)** 4-MeC_6H_4; **(f)** 4-$MeOC_6H_4$; **(g)** 4-(1,4-benzodioxin-6-yl); **(h)** 4-(1-benzofuran-2-yl).

The individuality and composition of the synthesized compounds are consistent with the results of chromatography-mass spectrometry and elemental analysis, and their structure is confirmed by IR- and NMR ^1H spectra. In the latter, for the compounds (II a-g), doublets of protons H4 (5.52 - 5.57 ppm) and H5 [48] (6.40 – 6.44 ppm) of the oxazine cycle with the frequency of KCCB 2.8-3.2 Hz and singlets H^5 of the pyrazole nucleus at 8.87 are indicative of 8.95 ppm. For the compound (II h), there is a weak-field shift of the H^4 doublet signal to the region of 5.92 ppm, which is obviously due to the de-screening effect of the benzofuranyl substituent in the 3-position of pyrazole. In addition, it should be noted that the presence of

spatially hindered pyrazole fragments in position 4 of the oxazine cycle adds specificity to the nature of proton absorption of the amino group: it is prescribed by more than one signal with the intensity of 2H at 9.39 ppm [9], and two broad one-proton singlets in the ranges 9.11-9.51 ppm and 10.58 - 10.85 ppm, which is most likely due to the presence of amino-imine tautomerism in the DMSO solution.

2-Amino-1,3-oxazines (II a-h) belong to binucleophilic systems in which the presence of a ureide fragment can be successfully used for the further annulation of the 1,3,5-triazine ring. Previously, we have developed a convenient method for synthesizing a number of condensed 1,3,5-triazines, which is based on the reactions of 2-aminoazines with 1-chloroalkylisocyanates [49]. The possibilities of 2-amino-1,3-oxazines in this kind of transformation were studied on the examples of the interaction of the compounds (II b, d, e) with 1-aryl-1-chloro-2,2,2-trifluoroethylisocyanates (III a-c). As a result of the ambident nature of the compounds (II) and (III), the formation of both 2-trifluoromethyl-2-aryl[1,3]oxazino[3,2-a][1,3,5]triazin-4-ones (IV a-c), and 4-trifluoromethyl-2-aryl[1,3]oxazino[3,2-a][1,3,5]triazin-2-ones isomeric to them could be expected.

It was experimentally established that in the presence of triethylamine as a hydrogen chloride acceptor, the reaction proceeds selectively and leads to derivatives of a new heterocyclic system – [1,3]oxazino[3,2-a][1,3,5]-triazine-4 -on (IV a-c). This process course is definitely included in the scheme of primary carbamoylation of the more nucleophilic endocyclic nitrogen atom of oxazines (III) with subsequent intramolecular cyclization of the intermediate (B).

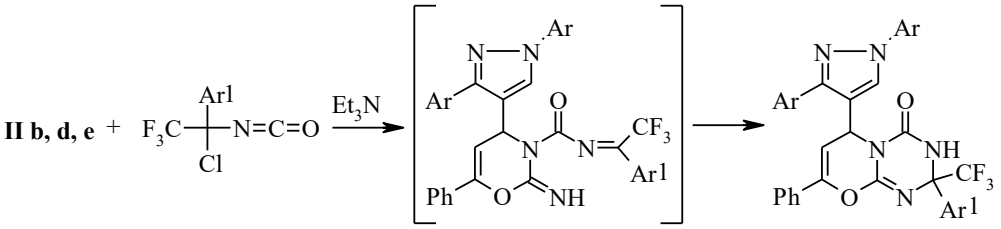


Figure 2. III: Ar^1=Ph (a), 4-FC_6H_4 (b), 4-$MeOC_6H_4$ (c); IV: Ar=4-FC_6H_4, Ar^1=4-$MeOC_6H_4$ (a); Ar=4-BrC_6H_4, Ar^1=Ph (b); Ar=4-MeC_6H_4, Ar^1=4- FC_6H_4 (c).

The structure of the compounds (IVa-c) was confirmed by a complex spectral examination, including the use of IR, NMR (^{1}H, ^{13}C, ^{19}F), and chromato-mass spectra. The ^{19}F NMR spectra, in which CF_3 groups are fixed in the interval -82 ÷ -83 ppm and strictly indicate their location in the structural fragment =N–C(CF_3)–NH, turned out to be the most convincing among them [50]. In the case of an alternative 4-trifluoromethyl-4-aryl-2-oxo structure, the signal of the specific group should be expected at -73 ÷ -75 ppm.

In addition to the considered chemical transformation, 2-amino-4-pyrazolyl-1,3-oxazines were tested for bacterial activity concerning the two types of microorganisms: gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli*. The determined indicators of MBsC and MBcC concentrations of the compounds (II a-h) are presented in Table 1 and demonstrate that they are characterized by pronounced antimicrobial activity against *Staphylococcus aureus* strains and are practically inactive against *Escherichia coli* strains.

The IR spectrum of the compounds in KBr was recorded using the example of UR-20 in KBr tablets. ^{1}H and ^{13}C NMR spectra of the compounds (II a-h) in DMSO-d_6, and the compounds (IV a-c) in $CDCl_3$, measured Bruker Avance DRX-500 (500.13, 125.75 MHz, respectively), internal standard – TMS. ^{19}F NMR of the compounds (IV a-c) in CDCl3 were

obtained on a Varian-Gemini spectrometer (188.14 MHz, internal standard – CCl_3F). Chromato-mass spectra were obtained using PE SCXAPI 150 EX, UV (250 nm), and ELSOJ detectors.

4-(3-Aryl-1-phenyl-1*H*-pyrazol-4-yl)-6-phenyl-4*H*-1,3-oxyzin-2-amines (II a-h). 3 ml of acetic and 1 ml of trifluoroacetic acid were added to the mixture of 2 mmol of aldehyde (I a-h), 0.20 g (2 mmol) of phenylacetylene, and 0.12 g (2 mmol) of urea in 10 ml of acetonitrile and boiled during 10 h. The reaction mixture was cooled, and the formed precipitate was filtered off and crystallized from 80% ethanol.

4-(3-phenyl-1-phenyl-1*H*-pyrazol-4-yl)-6-phenyl-4*H*-1,3-oxyzin-2-amines (2a). Yield 82 %; m. p. 247-249°C. IR (ν/cm-1): 3320 (NH). 1H NMR: δ = 5.56 д (1H, J 2.8 Hz, $H^4_{oxazine}$), 6.44 d (1H, *J* 2.8 Hz, $H^5_{oxazine}$), 7.34-8.02 m (15$H_{arom.}$), 8.94 s (1H, $H^5_{pyrazole}$), 9.15 m.s. (1H, NH), 10.79 br.s. (1H, NH).. LC-MS: m/z = 393 [M+1] (100%). Anal. Calcd. for $C_{25}H_{20}N_4O$, % : C 76.51; H 5.14; N 14.28. Found, % : C 76.75; H 5.24; N 14.07.

4-(3-phenyl(4-fluoro-1-phenyl-1*H*-pyrazol-4-yl)-6-phenyl-4*H*-1,3-oxyzin-2-amines (2b). Yield 58 %; m. p. 238-240°C. IR (ν/cm-1): 3325 (NH). 1H NMR: δ = 5.54 d (1H, J 3.2 Hz, $H^4_{oxazine}$), 6.42 d (1H, J 3.2 Hz, $H^5_{oxazine}$), 7.33-7.78 м (12$H_{arom.}$), 7.92 d (2$H_{arom.}$ *J* 8.0 Hz), 8.92 s (1H, $H^5_{pyrazole}$), 9.17 br.s. (1H, NH), 10.88 br.s. (1H, NH). LC-MS: m/z = 411 [M+1] (100%). Anal. Calcd. for $C_{25}H_{19}FN_4O$, % : C 73.16; H 4.67; N 13.65. Found, % : C 73.39; H 4.53; N 13.44.

4-(3-phenyl(4-chloro-1-phenyl-1*H*-pyrazol-4-yl)-6-phenyl-4*H*-1,3-oxyzin-2-amines (2c). Yield 86 %; m. p. 254-256°C. IR (ν/cm-1): 3325 (NH). 1H NMR: δ = 5.57 d (1H, *J* 2.8 Hz, $H^4_{oxazine}$), 6.42 d (1H, *J* 2.8 Hz, H5oxazine), 7.36-7.79 m (12$H_{arom.}$), 7.92 d (2$H_{arom.}$, *J* 7.6 Hz), 8.94 s (1H, $H^5_{pyrazole}$), 9.14 br.s.. (1H, NH), 10.85 br.s. (1H, NH). LC-MS: m/z = 427 [M+1] (100%). Anal. Calcd. for $C_{25}H_{19}ClN_4O$, % : C 70.34; H 4.49; N 13.12. Found, % : C 70.53; H 4.38; N 13.32.

4-(3-phenyl(4-bromo-1-phenyl-1*H*-pyrazol-4-yl)-6-phenyl-4*H*-1,3-oxyzin-2-amines (2d). Yield 69 %; m. p. 233-235°C. IR (ν/cm-1): 3325 (NH). 1H NMR: δ = 5.56 d (1H, *J* 3.2 Hz, $H^4_{oxazine}$), 6.43 d (1H, *J* 3.2 Hz, $H^5_{oxazine}$), 7.31-7.76 m (12$H_{arom.}$), 7.91 d (2$H_{arom.}$ *J* 8.2 Hz), 8.93 s (1H, $H^5_{pyrazole}$), 9.16 br.s. (1H, NH), 10.82 br.s. (1H, NH). LC-MS: m/z = 427 [M+1] (100%). Anal. Calcd. for $C_{25}H_{19}BrN_4O$, % : C 63.70; H 4.06; N 11.89. Found, % : C 63.42; H 4.16; N 11.71.

4-(3-phenyl(4-methyl-1-phenyl-1*H*-pyrazol-4-yl)-6-phenyl-4*H*-1,3-oxyzin-2-amines (2e). Yield 67 %; m. p. 224-236°C. IR (ν/cm-1): 3320 (NH). 1H NMR: δ = 2.38 s (3H, CH_3), 5.54 d (1H, *J* 2.8 Hz, $H^4_{oxazine}$), 6.41 d (1H, *J* 2.8 Hz, $H^5_{oxazine}$), 7.28-7.76 m (12$H_{arom.}$), 7.91 d (2$H_{arom.}$ *J* 7.6 Hz), 8.89 s (1H, $H^5_{pyrazole}$), 9.11 br.s. (1H, NH), 10.80 br.s. (1H, NH). LC-MS: m/z = 407 [M+1] (100%). Anal. Calcd. for $C_{26}H_{22}N_4O$, % : C 76.83; H 5.46; N 13.78. Found, % : C 76.56; H 5.58; N 13.99.

4-(3-phenyl(4-methoxy-1-phenyl-1*H*-pyrazol-4-yl)-6-phenyl-4*H*-1,3-oxyzin-2-amines (2f). Yield 63 %; m. p. 228-230°C. IR (ν/cm-1): 3320 (NH). 1H NMR: δ = 3.82 c (3H, CH_3O), 5.52 d (1H, $H^4_{oxazine}$, *J* 2.8 Hz), 6.42 d (1H, $H^5_{oxazine}$, *J* 2.8 Hz), 7.02 d (2$H_{arom.}$, J 8.0 Hz), 7.35 t (1$H_{arom.}$, *J* 7.8 Hz), 7.44-7.55 m (5$H_{arom.}$), 7.62 d (2$H_{arom.}$ *J* 8.4 Hz), 7.74 d (2$H_{arom.}$ J 7.4 Hz), 7.90 d (2$H_{arom.}$ *J* 8.0 Hz), 8.89 s (1H, $H^5_{pyrazole}$), 9.21 br.s. (1H, NH), 10.58 br.s. (1H, NH). LC-MS: m/z = 423 [M+1] (100%). Anal. Calcd. for $C_{26}H_{22}N_4O_2$, % : C 73.92; H 5.25; N 13.26. Found, % : C 73.64; H 5.35; N 13.02.

4-(3-phenyl(4-(1,4-benzodioxin-6-yl**)-1-phenyl-1*H*-pyrazol-4-yl)-6-phenyl-4*H*-1,3-oxyzin-2-amines (2g).** Yield 73 %; m. p. 226-238°C. IR (ν/cm-1): 3325 (NH). 1H NMR: δ =

4.28 s (4H, O(CH_2)$_2$O), 5.52 d (1H, $H^4_{oxazine}$, *J* 2.8 Hz), 6.41 d (1H, $H^5_{oxazine}$, *J* 2.8 Hz), 6.92 d (2$H_{arom.}$, *J* 8.0 Hz), 7.14-7.52 m (7$H_{arom.}$), 7.74 d (2$H_{arom.}$ *J* 7.8 Hz), 7.94 d (2$H_{arom.}$ *J* 7.6 Hz), 8.87 s (1H, $H^5_{pyrazole}$), 9.18 br.s. (1H, NH), 10.60 br.s. (1H, NH). LC-MS: m/z = 451 [M+1] (100%). Anal. Calcd. for $C_{27}H_{22}N_4O_3$, % : C 71.99; H 4.92; N 12.44. Found, % : C 71.78; H 5.03; N 12.23.

4-(3-phenyl(4-(11-benzofuran-2-yl)**)-1-phenyl-1*H*-pyrazol-4-yl)-6-phenyl-4*H*-1,3-oxyzin-2-amines (2h)**. Yield 84 %; m. p. 248-250°C. IR (ν/cm-1): 3330 (NH). 1H NMR: δ = 5.92 d (1H, $H^4_{oxazine}$, *J* 2.8 Hz), 6.40 d (1H, *J* 2.8 Hz, $H^5_{oxazine}$), 7.29-7.72 m (12$H_{arom.}$), 7.94 d (2$H_{arom.}$ *J* 7.6 Hz), 8.95 s (1H, $H^5_{pyrazole}$), 9.51 m (1H, NH), 10.83 m (1H, NH).). LC-MS: m/z = 433 [M+1] (100%). Anal. Calcd. for $C_{27}H_{20}N_4O_2$, % : C 74.99; H 4.66; N 12.95. Found, % : C 74.73; H 4.57; N 13.19.

6-(3-Aryl-1-phenyl-1H-pyrazol-4-yl)-2-aryl-8-phenyl-2-trifluoromethyl-2,3-dihydro-4*H*,6*H*-[1,3]oxazino[3,2-a][1,3,5]triazine-4-on (IV a-c). To the solution of 1 mmol of isocyanate (III a-b) in 10 ml of benzene, 1 mmol of 2-amino-1,3-oxazine (II b, d, e) was added, and then, with stirring, 0.14 ml (1 mmol) of triethylamine in 5 ml of benzene. The reaction mixture was stirred at room temperature for 1 h, boiled for 2 h, and filtered hot. The filtrate was evaporated, and the residue was purified by crystallization.

6-[3-(4-Fluorophenyl)-1-phenyl-1H-pyrazol-4-yl]-2-(4-methoxyphenyl)-8-phenyl-2-trifluoromethyl-2,3-dihydro-4*H*,6*H*- [1,3]oxazino[3,2-a][1,3,5]-triazin-4-on (IV a). Yield 75%, m. p.: > 250°C. IR (ν/cm^{-1}):1730 (C=O), 3255 (N–H). NMR spectra ^{1}H, δ, ppm: 3.51 s (3H, CH_3O), 5.58 d (1H, H^6, *J* 4.8 Hz), 6.25 d (1H, H^7, *J* 4.8 Hz), 6.77 d (2$H_{arom.}$, *J* 7.8 Hz), 7.21 t (2$H_{arom.}$, *J* 7.6 Hz), 7.23-7.69 m (15H, 14$H_{arom.}$+NH), 8.22 s (1H, $H^5_{pyrazole}$). NMR spectra ^{19}F, δ, ppm: -114.34 s (1F), - 83.44 s (3F). NMR spectra ^{13}C, *δ*, ppm: 44.89 (C^6), 55.02 (CH_3O), 74.54 q (C^2, $^2J_{C\text{-}F}$ 31.4 Hz), 98.78 (C^7), 113.81, 115.58, 118.62, 121.31, 124.88, 125.75, 128.49, 128.75, 129.25, 129.80, 130.14, 130.21, 130.62, 139.21, 146.46, 160.57 (C_{Ar}+$C_{pyrazole}$), 126.56 q (CF_3, *J* 256.2 Hz), 147.07 (C^8), 149.99 (C^{9a}), 151.75 (C^4), 162.85 (C_{Ar}–F, *J* 128.4 Hz). Found, %: C 65.97; H 4.08; N 11.18. $C_{36}H_{25}FN_5O_3$. Calculated, %: C 65.73; H 3.94; N 10.95.

6-[3-(4-Bromophenyl)-1-phenyl-1*H*-pyrazole-4-yl]-2,8-diphenyl-2-trifluoromethyl-2,3-dihydro-4*H*,6*H*-[1,3]oxazino[3,2-a]|1,3,5]-triazin-4-on (IV b). Yield 69%, m. p.: > 250°C. IR (ν/cm^{-1}): 1730 (C=O), 3255 (N–H). NMR spectra ^{1}H, δ, ppm: 5.58 d (1H, H^6, *J* = 5.0 Hz), 6.21 d (1H, H^7, J 5.0 Hz), 7.19-7.69 m (20H, 19$H_{arom.}$+NH), 8.16 s (1H, $H^5_{pyrazole}$). NMR spectra ^{19}F, *δ*, ppm: - 83.23. NMR spectra ^{13}C, *δ*, ppm: 44.73(C^6), 74.72 q (C^2, $^2J_{C\text{-}F}$ 30.0 Hz), 98.69 (C^7), 118.93, 119.40, 121.12, 121.98, 122.66, 124.24, 124.89, 126.72, 128.51, 128.56, 129.15, 129.75, 129.83, 130.57, 131.65, 137.77, 139.04, 139.20, 146.38 (C_{Ar}+$C_{pyrazole}$), 126.43 q (CF_3, J 258.6 Hz), 147.02 (C^8), 149.89 (C^{9a}), 151.54 (C^4). Found, %: C 60.64; H 3.52; N 10.62. $C_{34}H_{23}F_3BrN_5O_2$. Calculated, %: C 60.91; H 3.46; N 10.45.

6-[3-(4-Methylphenyl)-1-phenyl-1*H*-pyrazol-4-yl]-8-phenyl-2-trifluoromethyl-2-(4-fluorophenyl)-2,3-dihydro-4*H*,6*H*-[1,3]oxazino[3,2-a][1,3,5]-triazin-4-on (IV c). Yield 78%, m. p.: > 250°C. IR (ν/cm^{-1}): 1735 (C=O), 3250 (N–H). ^{1}H NMR: δ = 2.31 s (3H, CH_3), 5.50 d (1H, H^6, *J* 4.6 Hz), 6.24 d (1H, H^7, *J* 4.6 Hz), 6.89-7.63 m (19H, 18$H_{arom.}$+NH), 8.01 s (1H, $H^5_{pyrazole}$). NMR spectra ^{19}F, *δ*, ppm: - 82.01. NMR spectra ^{13}C, *δ*, ppm: 21.30 (CH_3), 44.99 (C^6), 74.58 q (C^2, $^2J_{C\text{-}F}$ 30.8 Hz), 39.00 (C^7), 115.52, 118.60, 121.13, 124.91, 125.53, 126.62, 128.12, 128.36, 128.84, 129.30, 129.44, 129.72, 130.68, 133.82, 133.84, 138.28, 139.25, 146.32 (C_{Ar}+$C_{pyrazole}$), 125.84 q (CF_3, *J* 252.6 Hz), 147.29 (C^8), 150.97 (C^{9a}), 151.51

(C^4), 163.38 d (C_{Ar}–F, ^1J 126.8 Hz). Found, %: C 67.23; H 3.92; N 11.07. $C_{35}H_{25}F_4N_5O_2$. Calculated, %: C 67.41; H 4.04; N 11.23.

4. Conclusions

New 2-amino-4-pyrazolyl-4*H*-1,3-oxazines were synthesized by a three-component reaction of pyrazole-4-carbaldehydes, phenylacetylene, and urea. Their regioselective condensation with 1-chloroalkylisocyanates was used to annulate the 1,3,5- triazine cycle. The antibacterial activity of the synthesized compounds against typical strains of bacteria *Staphylococcus aureus* and *Escherichia coli* was studied. 2-Amino-4-pyrazolyl-4*H*-1,3-oxazines obtained exhibit strong bactericidal activity against strains of *Staphylococcus aureus*.

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Conflicts of Interest

The authors declare no conflict of interest.

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