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POLYFUNCTIONAL PYRAZOLES. 9*. SYNTHESIS OF 1-ALKYL(ARYL)-3-[4-(HYDROXYMETHYL)-1*H*-PYRAZOL-3-YL]UREAS

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We have developed an effective method for the preparation of 1-alkyl(aryl)-3-[4-(hydroxymethyl)-1H-pyrazol-3-yl]ureas based on the interaction of 4-hydroxymethylpyrazole-3-carbonyl azides with primary aliphatic and aromatic amines under the conditions of Curtius reaction. In the absence of amines in the reaction mixture, in situ generated 4-hydroxymethyl-3-isocyanatopyrazoles underwent intramolecular cyclization to pyrazolo[3,4-d][1,3]oxazin-6(4H)-ones. The latter showed a tendency to form 1-alkyl(aryl)-3-[4-(hydroxymethyl)-1H-pyrazol-3-yl]ureas in the presence of amines.

Keywords: 4-hydroxymethylpyrazole-3-carbonyl azides, (pyrazol-3-yl)ureas, pyrazolo[3,4-*d*][1,3]oxazines, intramolecular cyclization.

Functionalized pyrazole derivatives containing a hydroxymethyl or ureide group are compounds of synthetic and biological importance. For example, 4-(hydroxymethyl)pyrazoles present interest as modulators of AMPA receptor [2], and are also used as building blocks for the design of nonsteroidal anti-inflammatory drugs [3] and antineoplastic agents [4]. At the same time, 3-ureidopyrazoles are characterized by antitumor [5], anti-inflammatory properties [6], and inhibitory properties against some types of kinases [7, 8]. Besides that, such compounds serve as precursors to pharmacologically valuable pyrazolo[3,4-*d*]pyrimidines [9, 10]. Taking into account these data, we decided to combine the indicated fragments into new structures of pyrazole type, which could serve as potential biologically active compounds.

Among 3-ureido-substituted pyrazoles, examples are currently known that are additionally functionalized at the ring position 4 with ethoxycarbonyl [7, 9, 10] or carboxy groups [8], and are obtained by adding alkyl- or arylisocyanates to the respective 4-aminopyrazoles. This approach to the synthesis of 4-hydroxymethyl analogs appears less appropriate, because 3-amino-4-hydroxymethylpyrazoles are unknown in the literature, and even if such compounds were available, selectivity problems would arise when adding isocyanates to amino group.

*For Communication 8, see [1].

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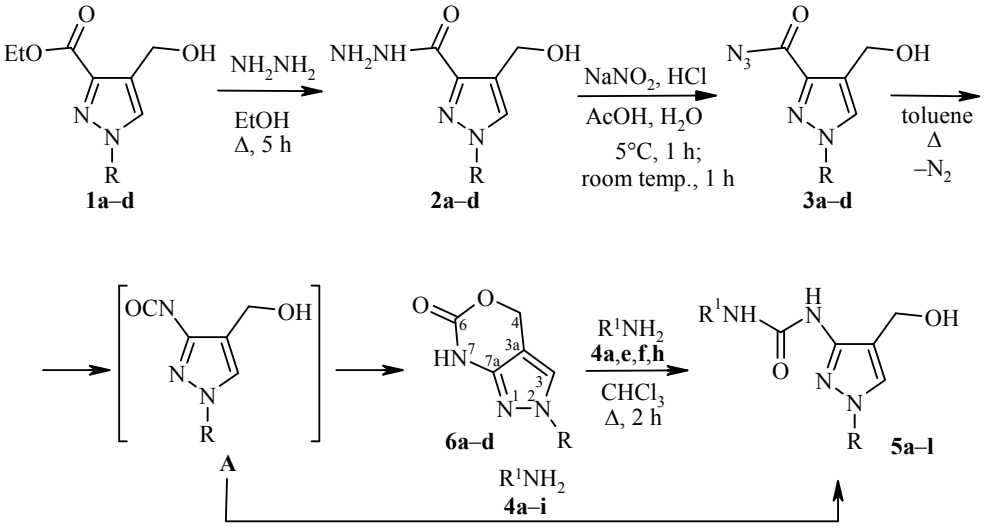
Due to this reason, to achieve our goals we explored an effective synthetic route that relied on simple transformations of previously described [11] ethyl 4-hydroxymethylpyrazole-3-carboxylates **1a-d**. Hydrazinolysis of these starting materials in refluxing ethanol gave the hydrazides **2a-d**, the interaction of which with sodium nitrite in a mixture of hydrochloric and acetic acids led to the pyrazoyl azides **3a-d**, compounds that are stable at room temperature. According to ^{1}H NMR data, the obtained samples had an assay of 90-93%, and were used in further transformations without additional purification.

We established that heating of acyl azides **3a-d** for 2 h in refluxing toluene with alkyl(aryl)amines **4a-i** led to the formation of 1-substituted 3-[(4-hydroxymethyl)-1*H*-pyrazol-3-yl]ureas **5a-l** in 69-83% yields (method A). This reaction involved initial thermal Curtius rearrangement of pyrazoyl azides [12] to 3-isocyanatopyrazoles **A**, which immediately reacted with the respective amines. Such an approach was recently used [13] for the preparation of 3-ureidopyrazoles, which did not contain nucleophilic functional groups at position 4 of the azole ring. The presence of the latter may have a certain impact on the reactivity of *in situ* generated isocyanates. Thus, the absence of amines in the reaction mixture enables intramolecular cyclization involving isocyanate and hydroxymethyl groups, leading to the formation of 1-substituted pyrazolo[3,4-*d*][1,3]oxazin-6(4*H*)-ones **6a-d**, examples of a little known [14, 15] bisheterocyclic system. Compounds **6a-d** are characterized by relative lability of the oxazine ring and may undergo ring opening upon interaction with the amines **4a**,**e**,**f**,**h** in refluxing chloroform, forming the ureas **5a**,**g**,**j**,**k** (method B). This fact points to the formation of target compounds **5** under the reaction conditions both through direct interaction of amines **4** with isocyanates **A**, as well as with oxazines **6**. In general, the developed method allows to use a wide range of amines, which is important for systematic building of focussed libraries of target compounds useful for biological screening.



1–**3a**, **5a**,**b**, **6a** R = Me; **1**–**3b**, **5c**–**i**, **6b** R = Ph; **1**–**3c**, **5j**,**k**, **6c** R = 4-ClC_6H_4; **1**–**3d**, **5l**, **6d** R = 4-MeC_6H_4;
4a, **5a**,**c** R^1 = $HO(CH_2)_2$; **4b**, **5d** R^1 = $Me_2N(CH_2)_2$; **4c**, **5b**,**e** R^1 = $Me_2N(CH_2)_3$;
4d, **5f** R^1 = 4-$ClC_6H_4CH_2$; **4e**, **5j**,**l** R^1 = 4-$MeC_6H_4CH_2$; **4f**, **5g** R^1 = 4-$MeOC_6H_4CH_2$
4g, **5h** R^1 = 4-$MeOC_6H_4(CH_2)_2$; **4h**, **5k** R^1 = 4-MeC_6H_5; **4i**, **5i** R^1=4-$MeOC_6H_4$

The structure and composition of the intermediate hydrazides **2a-d**, pyrazolooxazines **6a-d**, and also the target products **5a-l** were reliably confirmed by elemental analysis, chromato-mass spectrometry, IR and NMR spectroscopy (Tables 1-4).

The bactericidal properties of compounds **5a-h** were studied by double serial dilutions on test microorganisms *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *B. subtilis* (ATCC 8236F800), and *C. albicans* (ATCC 885-653), and moderate antimicrobial activity was found (Table 5).

TABLE 1. Physicochemical Characteristics of the Synthesized Compounds **2a-d**, **5a-l**, **6a-d**

Compound	Empirical formula	Found, % / Calculated, %			Mp, °C	Yield, % (method)
		C	H	N		
2a	$C_6H_{10}N_4O_2$	42.64 / 42.35	6.08 / 5.92	32.70 / 32.94	159-160	84
2b	$C_{11}H_{12}N_4O_2$	56.58 / 56.89	5.34 / 5.21	24.31 / 24.12	94-95	93
2c	$C_{11}H_{11}ClN_4O_2$	49.31 / 49.54	4.29 / 4.16	21.17 / 21.01	161-162	90
2d	$C_{12}H_{14}N_4O_2$	58.75 / 58.53	5.52 / 5.73	23.01 / 22.75	130-131	89
5a	$C_8H_{14}N_4O_3$	42.03 / 44.85	6.74 / 6.59	26.17 / 26.15	139-140	60 (A) 68 (B)
5b	$C_{11}H_{21}N_5O_2$	51.47 / 51.75	8.03 / 8.29	27.31 / 27.43	84-85	79
5c	$C_{13}H_{16}N_4O_3$	56.70 / 56.51	5.96 / 5.84	20.42 / 20.28	203-205	70
5d	$C_{15}H_{21}N_5O_2$	59.17 / 59.39	7.14 / 6.98	23.18 / 23.09	184-185	75
5e	$C_{16}H_{23}N_5O_2$	60.79 / 60.55	7.41 / 7.30	22.30 / 22.07	140-141	83
5f	$C_{18}H_{17}ClN_4O_2$	60.84 / 60.59	4.95 / 4.80	15.48 / 15.70	179-180	73
5g	$C_{19}H_{20}N_4O_3$	64.99 / 64.74	5.83 / 5.72	16.01 / 15.90	189-190	81 (A) 83 (B)
5h	$C_{20}H_{22}N_4O_3$	65.81 / 65.56	5.78 / 6.05	15.42 / 15.29	179-180	69
5i	$C_{18}H_{18}N_4O_3$	64.20 / 63.89	5.32 / 5.36	16.47 / 16.56	184-185	72
5j	$C_{19}H_{19}ClN_4O_2$	61.41 / 61.54	5.32 / 5.16	14.97 / 15.11	193-194	71 (A) 79 (B)
5k	$C_{18}H_{17}ClN_4O_2$	60.41 / 60.59	4.71 / 4.80	15.89 / 15.70	201-203	69 (A) 76 (B)
5l	$C_{20}H_{22}N_4O_2$	68.31 / 68.55	6.50 / 6.33	16.11 / 15.99	174-175	72
6a	$C_6H_7N_3O_2$	46.81 / 47.06	4.69 / 4.61	27.58 / 27.44	213-215	63
6b	$C_{11}H_9N_3O_2$	61.64 / 61.39	4.11 / 4.22	19.33 / 19.52	184-185	59
6c	$C_{11}H_8ClN_3O_2$	53.21 / 52.92	3.27 / 3.23	16.92 / 16.83	218-220	67
6d	$C_{12}H_{11}N_3O_2$	62.59 / 62.87	4.99 / 4.84	18.28 / 18.33	201-202	62

Thus, we have established that ethyl 4-hydroxymethylpyrazole-3-carboxylates may act as effective substrates in the synthesis of promising biologically active 1-alkyl(aryl)-3-[4-(hydroxymethyl)-1*H*-pyrazol-3-yl]ureas with the reaction proceeding through *in situ* generated 4-hydroxymethyl-3-isocyanatopyrazoles as intermediates.

EXPERIMENTAL

IR spectra were recorded on a UR-20 spectrometer in KBr pellets. ^1H and ^{13}C NMR spectra were acquired on a Bruker Avance DRX-500 instrument (500 and 125 MHz, respectively) in DMSO-d_6, with TMS as internal standard. Mass spectra were recorded on an Agilent LC/MSD SL system; Zorbax SB-C18 column, 4.6 × 15 mm, 1.8 μm (PN 82(c)75-932); solvents: A – MeCN–H_2O (95:5), 0.1% trifluoroacetic acid, B – 0.1% aqueous trifluoroacetic acid; flow rate 3 ml/min; injection volume – 1 μl; UV detectors: 215, 254, 285 nm;

TABLE 2. ^{1}H NMR Spectra of Compounds **2a-d**, **5a-l**, **6a-d**

Com-pound	Chemical shifts, δ, ppm (*J*, Hz)
2a	3.83 (3H, s, CH_3); 4.41 (2H, br. s, NH_2); 4.53 (2H, s, CH_2); 5.20 (1H, br. s, OH); 7.65 (1H, s, H-5); 9.33 (1H, br. s, NH)
2b	4.52 (2H, br. s, NH_2); 4.64 (2H, s, CH_2); 5.24-5.28 (1H, m, OH); 7.33 (1H, t, *J* = 6.8, H Ph); 7.51 (2H, t, *J* = 7.2, H Ph); 7.91 (2H, d, *J* = 7.6, H Ph); 8.44 (1H, s, H-5); 9.64 (1H, br. s, NH)
2c	4.49 (2H, s, NH_2); 4.63 (2H, d, *J* = 4.8, CH_2); 5.28 (1H, t, *J* = 4.6, OH); 7.57 (2H, d, *J* = 8.4, H Ar); 7.96 (2H, d, *J* = 8.4, H Ar); 8.46 (1H, s, H-5); 9.69 (1H, s, NH)
2d	2.33 (3H, s, CH_3); 4.48 (2H, s, NH_2); 4.62 (2H, d, *J* = 5.2, CH_2); 5.27 (1H, t, *J* = 5.2, OH); 7.30 (2H, d, *J* = 8.0, H Ar); 7.79 (2H, d, *J* = 8.0, H Ar); 8.38 (1H, s, H-5); 9.60 (1H, s, NH)
5a	3.17-3.23 (2H, m, CH_2); 3.40-3.45 (2H, m, CH_2); 3.68 (3H, s, CH_3); 4.22 (2H, d, *J* = 5.2, CH_2); 4.80 (2H, br. s, OH); 7.36 (1H, br. s, NH); 7.46 (1H, s, NH); 8.18 (1H, s, H-5)
5b	1.52-1.57 (2H, m, CH_2); 2.11 (6H, s, $2CH_3$); 2.21 (2H, t, *J* = 6.8, CH_2); 3.13 (2H, q, *J* = 6.4, CH_2); 3.68 (3H, s, CH_3); 4.20 (2H, s, CH_2); 4.80 (1H, br. s, OH); 7.35 (1H, br. s, NH); 7.40 (1H, s, NH); 8.11 (1H, s, H-5)
5c	3.22-3.29 (2H, m, CH_2); 3.50-3.56 (2H, m, CH_2); 4.39 (2H, d, *J* = 5.2, CH_2); 4.82-4.85 (1H, m, OH); 4.99 (1H, t, *J* = 5.2, OH); 7.22 (1H, t, *J* = 7.6, H Ph); 7.44 (2H, t, *J* = 8.2, H Ar); 7.74 (2H, d, *J* = 8.0, H Ph); 8.02 (1H, br. s, NH); 8.32 (1H, s, H-5); 8.54 (1H, s, NH)
5d	2.23 (6H, s, $2CH_3$); 2.40-2.45 (2H, m, CH_2); 3.29-3.34 (2H, m, CH_2); 4.39 (2H, d, *J* = 5.4, CH_2); 4.99 (1H, t, *J* = 5.4, OH); 7.20-7.47 (3H, m, H Ph); 7.79 (2H, d, *J* = 7.0, H Ar); 8.23 (1H, br. s, NH); 8.33 (1H, s, H-5); 8.56 (1H, s, NH)
5e	1.58-1.65 (2H, m, CH_2); 2.10 (6H, s, $2CH_3$); 2.27 (2H, t, *J* = 6.8, CH_2); 3.22 (2H, q, *J* = 6.8, CH_2); 4.37 (2H, s, CH_2); 5.00 (1H, br. s, OH); 7.22 (1H, t, *J* = 7.6, H Ph); 7.45 (2H, t, *J* = 7.8, H Ph); 7.54 (1H, br. s, NH); 7.72 (2H, d, *J* = 8.0, H Ph); 8.32 (1H, s, H-5); 8.49 (1H, br. s, NH)
5f	4.38 (2H, s, CH_2); 4.42 (2H, d, *J* = 4.4, CH_2); 5.00 (1H, t, *J* = 4.4, OH); 7.22-7.47 (7H, m, H Ar); 7.70 (2H, d, *J* = 8.0, H Ar); 8.04 (1H, br. s, NH); 8.32 (1H, s, H-5); 8.64 (1H, s, NH)
5g	3.73 (3H, s, CH_3O); 4.32 (2H, d, *J* = 7.6, CH_2); 4.98 (2H, d, *J* = 5.2, CH_2); 4.99 (1H, t, *J* = 5.2, OH); 6.91 (2H, d, *J* = 8.4, H Ar); 7.26 (1H, t, *J* = 7.6, H Ar); 7.30 (2H, d, *J* = 8.0, H Ar); 7.42 (2H, t, *J* = 7.8, H Ar); 7.66 (2H, d, *J* = 8.4, H Ar); 8.02 (1H, br. s, NH); 8.32 (1H, s, H-5); 8.61 (1H, s, NH)
5h	2.73 (2H, d, *J* = 6.0, CH_2); 3.45 (2H, q, *J* = 6.0, CH_2); 3.67 (3H, s, CH_3O); 4.36 (2H, d, *J* = 5.6, CH_2); 4.99 (1H, t, *J* = 5.6, OH); 6.84 (2H, d, *J* = 8.0, H Ar); 7.17-7.28 (3H, m, H Ar); 7.41-7.56 (5H, m, H Ar, NH); 8.28 (1H, s, H-5); 8.52 (1H, s, NH)
5i	3.70 (3H, s, CH_3O); 4.42 (2H, d, *J* = 5.0, CH_2); 4.96 (1H, t, *J* = 5.0, OH); 6.86 (2H, d, *J* = 8.0, H Ar); 7.25 (1H, t, *J* = 7.6, H Ar); 7.29 (2H, d, *J* = 8.0, H Ar); 7.40 (2H, t, *J* = 7.8, H Ar); 7.60 (2H, d, *J* = 8.2, H Ar); 8.31 (1H, s, H-5); 8.57 (1H, s, NH); 9.32 (1H, s, NH)
5j	2.26 (3H, s, CH_3); 4.40 (2H, d, *J* = 6.0, CH_2); 4.64 (2H, d, *J* = 5.6, CH_2); 5.23 (1H, t, *J* = 5.6, OH); 7.11 (2H, d, *J* = 8.0, H Ar); 7.22 (2H, d, *J* = 8.0, H Ar); 7.58 (2H, d, *J* = 8.4, H Ar); 7.96 (2H, d, *J* = 8.4, H Ar); 8.36 (1H, s, H-5); 8.96 (1H, t, *J* = 6.0, NH); 9.30 (1H, s, NH)
5k	2.46 (3H, s, CH_3); 4.40 (2H, d, *J* = 4.8, CH_2); 4.98 (1H, t, *J* = 4.8, OH); 7.09 (2H, d, *J* = 8.0, H Ar); 7.36 (2H, d, *J* = 8.0, H Ar); 7.53 (2H, d, *J* = 8.4, H Ar); 8.00 (2H, d, *J* = 8.4, H Ar); 8.38 (1H, s, H-5); 8.74 (1H, s, NH); 9.36 (1H, s, NH)
5l	2.26 (3H, s, CH_3); 2.34 (3H, s, CH_3); 4.40 (2H, d, *J* = 6.0, CH_2); 4.64 (2H, d, *J* = 5.2, CH_2); 5.22 (1H, t, *J* = 6.0, OH); 7.12 (2H, d, *J* = 8.0, H Ar); 7.21 (2H, d, *J* = 8.0, H Ar); 7.30 (2H, d, *J* = 8.0, H Ar); 7.80 (2H, d, *J* = 8.0, H Ar); 8.39 (1H, s, H-5); 8.90 (1H, t, *J* = 6.0, NH)
6a	3.68 (3H, s, CH_3); 5.24 (2H, s, CH_2); 7.41 (1H, s, H-3); 10.34 (1H, s, NH)
6b	5.38 (2H, s, CH_2); 7.22 (1H, t, *J* = 8.8, H Ph); 7.46 (2H, t, *J* = 8.6, H Ph); 7.67 (2H, d, *J* = 8.8, H Ph); 8.48 (1H, s, H-3); 10.72 (1H, s, NH)
6c	5.38 (2H, s, CH_2); 7.49 (2H, d, *J* = 7.5, H Ar); 7.69 (2H, d, *J* = 7.5, H Ar); 8.26 (1H, s, H-3); 10.77 (1H, s, NH)
6d	2.30 (3H, s, CH_3); 5.37 (2H, s, CH_2); 7.25 (2H, d, *J* = 7.8, H Ar); 7.56 (2H, d, *J* = 7.8, H Ar); 8.18 (1H, s, H-3); 10.69 (1H, s, NH)

TABLE 3. IR and Mass Spectra of Compounds **2a-d**, **5a-l**, **6a-d**

Compound	IR spectrum, ν, cm^{-1}			Mass spectrum, *m*/*z* $[M+H]^+$	Compound	IR spectrum, ν, cm^{-1}			Mass spectrum, *m*/*z* $[M+H]^+$
	C=O	NH	OH			C=O	NH	OH	
2a	1680	3340	3420	171	**5g**	1685	3275	3460	353
2b	1680	3315	3415	233	**5h**	1695	3280	3480	367
2c	1685	3340	3425	267	**5i**	1675	3285	3470	339
2d	1685	3340	3420	247	**5j**	1685	3270	3470	371
5a	1695	3280	3478	215	**5k**	1675	3285	3475	357
5b	1690	3280	3460	256	**5l**	1685	3280	3480	351
5c	1695	3265	3480	277	**6a**	1735	3230		154
5d	1695	3270	3475	304	**6b**	1730	3240		216
5e	1690	3270	3465	318	**6c**	1735	3235		250
5f	1685	3265	3480	357	**6d**	1730	3235		230

TABLE 4. ^{13}C NMR Spectra of Compounds **6a-d**

Compound	Chemical shifts, δ, ppm					
	C-3	C-3a	C-4	C-6	C-7a	R
6a	121.7	101.2	63.8	150.3	147.0	39.2
6b	122.5	100.4	64.3	150.4	148.1	119.8; 128.2; 129.7; 139.6
6c	122.9	100.9	64.1	150.4	148.4	118.7; 129.4; 129.8; 138.4
6d	122.6	100.4	64.2	150.5	148.2	23.3; 119.2; 127.3; 130.3; 137.6

TABLE 5. The Minimum Bacteriostatic (MIC), Bactericidal (MBC), Fungostatic (MIC), and Fungicidal (MFC) Concentrations (μg/ml)

Compound	Test microorganism							
	S. aureus ATCC 25923		*E. coli* ATCC 25922		*B. subtilis* ATCC 8236F800		*C. albicans* ATCC 885-653	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
1, **6 a**,**b**, **5a–h**	250	250	250	250	250	250	250	250
2a	250	250	125	250	125	250	125	250
2b	250	250	125	250	250	250	250	250
Furacilin	1.95	7.8	3.9	7.8	1.95	7.8	1.95	3.9

CI at atmospheric pressure, *m*/*z* scanning range 80-1000. Elemental analysis was performed on a Perkin Elmer CHN Analyzer at the analytical laboratory of the Institute of Organic Chemistry, National Academy of Sciences of Ukraine. Melting points were determined on a Kofler hot bench and were not corrected.

Compounds 1a-d were synthesized according to a published method [11].

Compound 1a. Yield 72%. Mp 59-60°C (EtOH). IR spectrum, ν, cm^{-1}: 1720 (C=O), 3460 (OH). ^1H NMR spectrum, δ, ppm (*J*, Hz): 1.27 (3H, t, *J* = 7.2, CH_3); 3.87 (3H, s, OCH_3); 4.23 (2H, q, *J* = 7.2, OCH_2); 4.57 (2H, d, *J* = 6.4, CH_2); 4.98 (1H, t, *J* = 6.4, OH); 7.98 (1H, s, H-5). Found, %: C 52.36; H 6.67; N 15.51. $C_8H_{12}N_2O_3$. Calculated, %: C 52.17; H 6.57; N 15.21.

Preparation of 4-(Hydroxymethyl)-1-methyl(aryl)-1*H*-pyrazole-3-carbohydrazides 2a-d (General Method). A solution of ester **1a-d** (0.02 mol) in EtOH (30 ml) was treated with 99% $NH_2NH_2 \cdot H_2O$ (1 g, 0.02 mol). The mixture was refluxed for 5 h, cooled, the solvent was removed, the residue was crystallized from EtOH.

Preparation of 4-(Hydroxymethyl)-1-methyl(aryl)-1*H*-pyrazole-3-carbonyl Azides 3a-d (General Method). A solution of $NaNO_2$ (1.38 g, 0.02 mol) in H_2O (10 ml) was added with stirring and cooling (5°C) to a solution of hydrazide **2a-d** (0.015 mol) in AcOH (25 ml) and conc. HCl (1.5 ml). The reaction mixture was stirred for 1 h at this temperature, then for 1 h at room temperature, poured into ice water (100 ml), and maintained for 1 h at 5°C. The precipitate was filtered off, washed with ice water (2×30 ml), and dried in vacuum desiccator over P_2O_5. Yield 68-75%. The products were used without additional purification for the synthesis of compounds **5a-l** and **6a-d**.

Preparation of 2-Methyl(aryl)-2,7-dihydropyrazolo[3,4-*d*][1,3]oxazin-6(4*H*)-ones 6a-d (General Method). A solution of azide **3a-d** (0.011 mol calculated for 90% assay) in anhydrous toluene (30 ml) was refluxed for 3 h; solvent was removed by distillation to 1/4 of the starting volume and the residue was cooled to room temperature. The precipitate formed was filtered off, washed with 1:1 mixture of toluene–hexane (20 ml), dried, and crystallized from toluene.

Preparation of 1-Alkyl(aryl)-3-[4-(hydroxymethyl)-1*H*-pyrazol-3-yl]ureas 5a-l (General Method). A. Amine **4a-i** (0.01 mol) in toluene (5 ml) was added to a solution of azide **3a-d** (0.01 mol calculated for 90% assay) in anhydrous toluene (25 ml) and the mixture was refluxed for 1 h. The solvent was removed by distillation, the residue was crystallized from a 2:1 mixture of EtOH–AcOH.

B. Amine **4a**,**e**,**f**,**h** (0.005 mol) in $CHCl_3$ (5 ml) was added to a solution of pyrazolooxazinone **6a-d** (0.005 mol) in $CHCl_3$ (10 ml) and the mixture was refluxed for 2 h. The solvent was removed by evaporation, the residue was crystallized from a 2:1 mixture of EtOH–AcOH.

Antimicrobial activity against standard lines of *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *B. subtilis* (ATCC 8236F800), and *C. albicans* (ATCC 885-653) was determined in liquid broth with four-hour inoculation of test culture determined to contain 1-2 million cells per 1 ml according to calibration curve. The results were collected after 24 h incubation at 37°C, or 48 h at 28°C in the case of fungal cultures. The MIC values were established as the highest dilution that suppressed the growth and multiplication of microorganisms. The MBC and MFC values were established by inoculating bacteriological droplets from test tubes free of visually observed microbial growth on Petri dish sectors containing beef peptone agar (for bacteria) or Saburo medium (for fungi). Bacteria were incubated for 2 days at 37°C, fungi – for 4 days at 28°C. The sector containing the minimum concentration that lacked microbial growth was interpreted as containing the minimum bactericidal concentration [16].

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