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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NEW TRIAZINO-, TRIAZOLO-, AND PYRAZOLOPYRIDAZINE DERIVATIVES

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The reaction of 3-hydrazino-4,5,6-triphenylpyridazine with phenacyl bromide afforded triazolopyridazine, while the reaction with different aldehydes gave the corresponding 3-arylidene hydrazinopyridazine derivatives which, on reaction with Br_2/Na_2CO_3, gave the corresponding triazinopyridazines. Also, fusion with ethyl acetoacetate, ethyl benzoylacetate, diethyl malonate, or ethyl phenylacetoacetate gave the corresponding pyrazolo- and triazinopyridazine derivatives. The antimicrobial activity of some of the new compounds has been discussed.

Keywords: diethyl malonate, ethyl acetoacetate, ethylbenzoylacetate, ethyl cyanoacetate, hydrazinopyridazine, phenacyl bromide, pyrazolopyridazine, triazinopyridazine, thiazolopyridazine.

Recently, it has been reported that some pyridazine derivatives display antihypertensive, anticancer, and anti-HIV activities [1-3]. In addition, it is well documented that substituted pyridazines possess antimicrobial, antifungal, and antiviral activities [4-6]. On the other hand, triazoles, triazines, and pyrazoles possess antimicrobial and antifungal activities [7-9]. On the basis of these findings and in continuation of our studies in this field, the present investigation deals with the synthesis of some triazolo-, triazino-, and pyrazolopyridazine derivatives likely possessing antimicrobial activity.

In the previous studies [10, 11] we have reported the synthesis of 4,5,6-triphenylpyridazin-3(2H)-one **1** *via* the reaction of benzylmonohydrazone with ethyl phenylacetate in the presence of sodium ethoxide/ethanol solution. The reaction of compound **1** with $POCl_3/PCl_5$ mixture gave 3-chloro-4,5,6-triphenylpyridazine **2** which, on reaction with hydrazine hydrate in boiling ethanol, yielded 3-hydrazino-4,5,6-triphenylpyridazine (**3**) as a starting material of the present work.

The reaction of compound **3** with phenacyl bromide in refluxing dry dioxane gave 3,7,8,9-tetraphenyl-4H-pyridazino[6,1-*c*][1,2,4]triazine (**4**). The structure of compound **4** was confirmed from its analytical and spectral data. The IR spectrum of compound **4** showed absorption bands at 1645 (C=N) and 1516 cm^{-1} (C=C); it also revealed the absence of the band of NH and NH_2 groups. The ^1H NMR spectrum showed signals at δ 2.7 (2H, s, CH_2) and 7.1-7.6 ppm (20H, m, aromatic protons).

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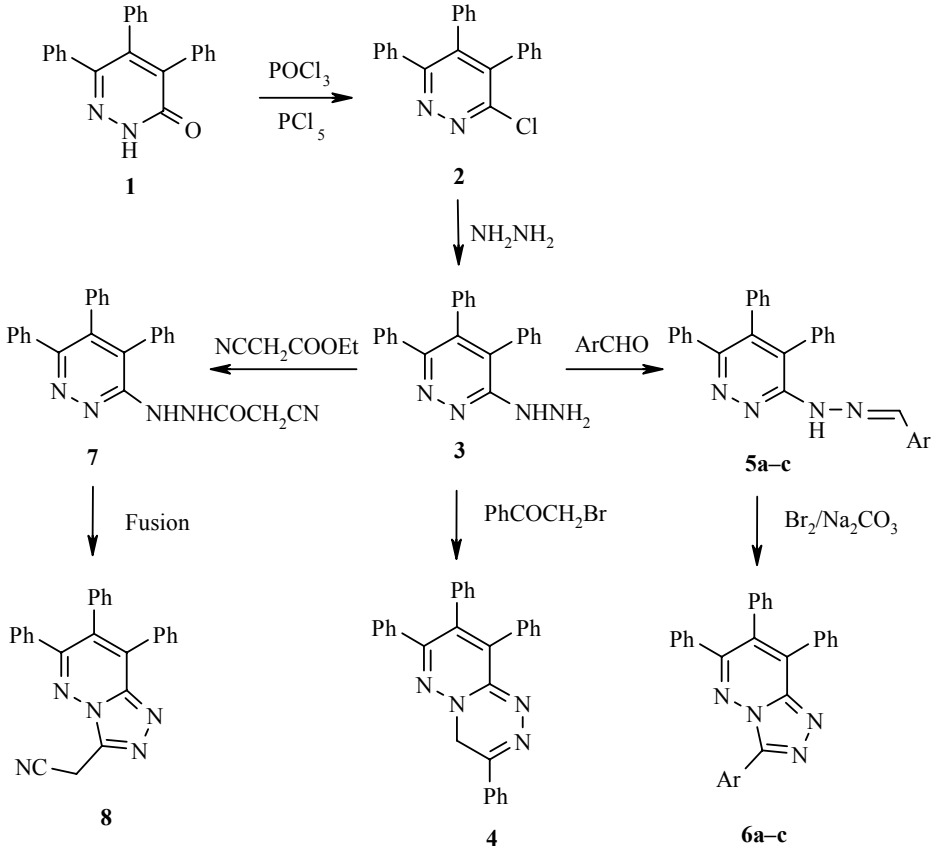
Condensation of 3-hydrazinopyridazine **3** with different aldehydes [12] such as benzaldehyde, *p*-chlorobenzaldehyde, and anisaldehyde in boiling ethanol afforded the corresponding arylidene (4,5,6-triphenylpyridazin-3-yl)hydrazones **5a-c**, which, on oxidative cyclization using bromine/sodium carbonate, gave 3-substituted 6,7,8-triphenyl[1,2,4]triazolo[4,3-*b*]pyridazines **6a–c**. The IR spectra of compounds **5a–c** showed absorption bands at 3349-3339 (NH), 1663-1646 (C=N), and 1520 (C=C) in addition to 1030 cm^{-1} (C–O–C) for compound **5c**. The ^1H NMR spectrum of compound **5c** showed signals at δ 3.7 (3H, s, CH_3), 4.2 (1H, s, NH), and 7.2-7.6 (19H, m, aromatic protons). The IR spectra of compounds **6a–c** showed absorption bands at 1640-1645 (C=N) and 1510-1516 cm^{-1} (C=C), while the ^1H NMR spectrum of compound **6c** showed signals at δ 3.7 (3H, s, CH_3) and 6.8-7.5 ppm (19H, m, aromatic protons).

The reaction of 3-hydrazinopyridazine **3** with ethyl cyanoacetate in boiling ethanol yielded N-(4,5,6-triphenylpyridazin-3-yl)-2-cyanoacetohydrazide (**7**), which cyclized on heating in an oil bath to give 3-cyanomethyl-6,7,8-triphenyl[1,2,4]triazolo[3,4-*b*]pyridazine (**8**). The IR spectrum of compound **7** revealed absorption bands at 3313 (NH), 2263 (CN), 1689 (CO), 1645 (C=N), and 1519 cm^{-1} (C=C), while the ^1H NMR spectrum showed signals at δ 3.3 (2H, s, CH_2), 4.0 (1H, s, NH), 7.2-7.7 (15H, m, aromatic protons), and 8.0 ppm (1H, s, NHCO). IR spectrum of compound **8** showed absorption bands at 2263 (CN), 1645 (C=N), and 1519 cm^{-1} (C=C). The ^1H NMR spectrum of compound **8** showed signals at δ 3.7 (2H, s, CH_2) and 7.1-7.6 ppm (15H, m, aromatic protons) (Scheme 1).

Scheme 1

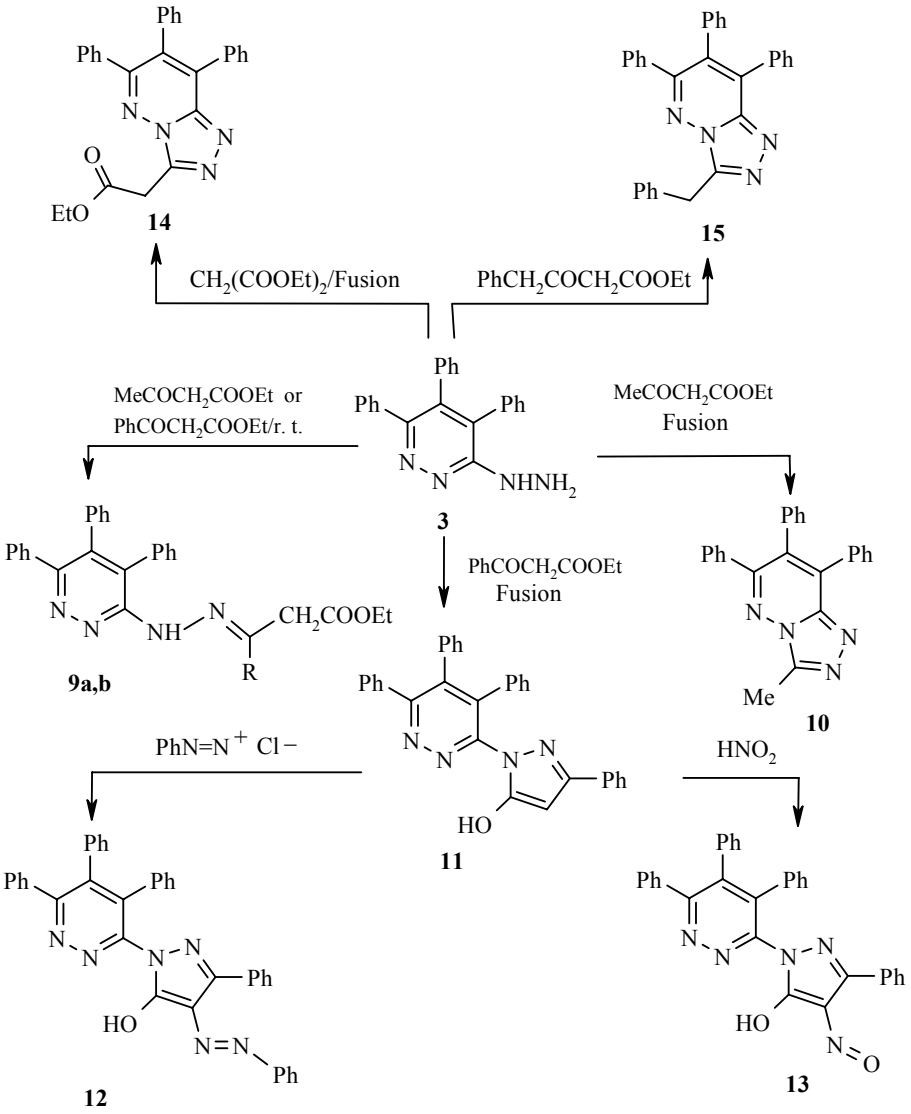


a Ar = Ph, **b** Ar = *p*-ClC_6H_4, **c** Ar = *p*-$MeOC_6H_4$

The reaction of 3-hydrazinopyridazine **3** with each of ethyl acetoacetate and ethyl benzoylacetate [13] in MeOH/$CHCl_3$ at room temperature afforded ethyl N-(4,5,6-triphenylpyridazin-3-yl)-3-hydrazonobutanoate (**9a**) and ethyl N-(4,5,6-triphenylpyridazin-3-yl)-3-hydrazono-3-phenylpropanoate (**9b**).

The IR spectra of compounds **9a**,**b** showed absorption bands at 3449-3339 (NH), 1712-1710 (C=O), 1643-1626 (C=N), 1580 (C=C), and 1080-1056 cm^{-1} (C–O–C). The ^1H NMR spectrum of compound **9a** showed signals at δ 1.4 (2H, t, $CH_2C\underline{H}_3$), 2.3 (3H, s, $N{=}CCH_3$), 3.4 (2H, s, CH_2CO), 3.7 (1H, s, =CH), 4.5 (3H, q, $C\underline{H}_2CH_3$), 7.1-7.5 (15H, m, aromatic protons), and 8.5 ppm (1H, s, NH). Fusion of compound **3** with ethyl acetoacetate in an oil bath afforded the corresponding 3-methyl-6,7,8-triphenyl[1,2,4]triazolo[4,3-*b*]pyridazine (**10**).

Scheme 2



9 a R = Me, **b** R = Ph

The IR spectrum of compound **10** showed absorption bands at 1645 (C=N) and 1516 cm^{-1} (C=C), whereas the ^1H NMR spectrum showed signals at δ 2.3 (3H, s, CH_3) and 7.2-7.5 (15H, m, aromatic protons). In the present investigation compound **10** was also prepared by refluxing compound **9a** in acetic anhydride. Fusion of compound **3** with ethyl benzoylacetate in an oil bath gave the corresponding 3-phenyl-1-(4,5,6-triphenylpyridazin-3-yl)-1H-pyrazol-5-ol **11**. The IR spectrum of compound **11** revealed absorption bands at 3440 (br. OH enolic), 1681 (C=O), 1664 (C=N), and 1513 cm^{-1} (C=C), while the ^1H NMR spectrum showed

TABLE 1. Characterization and Physical Data

Compound	Empirical formula	Found, % / Calculated, %/ C	H	N	mp, °C*	Yield, %
1	$C_{22}H_{16}N_2O$	81.46 / 81.33	4.97 / 4.91	8.64 / 8.58	280	75
2	$C_{22}H_{15}ClN_2$	77.08 / 77.04	4.41 / 4.93	8.17 / 8.13	138	72
3	$C_{22}H_{18}N_4$	78.08 / 78.00	5.36 / 5.30	16.56 / 16.50	227	82
4	$C_{30}H_{22}N_4$	82.17 / 82.11	5.06 / 5.03	12.78 / 12.72	243	76
5a	$C_{29}H_{22}N_4$	81.66 / 81.61	5.20 / 5.10	13.14 / 13.09	212	79
5b	$C_{29}H_{21}ClN_4$	75.56 / 75.49	4.59 / 4.51	12.15 / 12.08	197	69
5c	$C_{30}H_{24}N_4O$	78.92 / 78.88	5.30 / 5.27	12.27 / 12.19	185	79
6a	$C_{29}H_{20}N_4$	82.05 / 82.00	4.75 / 4.69	13.20 / 13.00	135	88
6b	$C_{29}H_{19}ClN_4$	75.89 / 75.85	4.17 / 4.13	12.21 / 12.11	155	66
6c	$C_{30}H_{22}N_4O$	79.27 / 79.19	4.88 / 4.85	12.33 / 12.29	190	60
7	$C_{25}H_{19}N_5O$	74.06 / 74.02	4.72 / 4.70	17.27 / 17.22	157	79
8	$C_{25}H_{17}N_5$	77.50 / 77.47	4.42 / 4.40	18.08 / 18.02	220	75
9a	$C_{28}H_{26}N_4O_2$	74.66 / 74.62	5.77 / 5.73	12.44 / 12.40	145	70
9b	$C_{33}H_{28}N_4O_2$	77.34 / 77.30	5.46 / 5.42	10.93 / 10.90	230	69
10	$C_{24}H_{18}N_4$	79.54 / 79.51	5.01 / 4.98	15.46 / 15.41	254	76
11	$C_{31}H_{22}N_4O$	79.81 / 79.78	4.75 / 4.70	12.01 / 11.98	275	78
12	$C_{37}H_{26}N_6O$	77.88 / 77.82	4.59 / 4.56	14.73 / 14.70	238	73
13	$C_{31}H_{21}N_5O_2$	75.14 / 75.10	4.27 / 4.22	14.13 / 14.09	143	78
14	$C_{27}H_{22}N_4O_2$	74.64 / 74.60	5.10 / 5.07	12.89 / 12.85	135	88
15	$C_{30}H_{22}N_4$	82.17 / 82.12	5.06 / 5.04	12.78 / 12.74	232	77

***** Solvent: benzene (compounds **1**, **6b,c**, **11**, **12**), acetic acid (compound **2**), ethanol (compounds **3-5a-c**, **6a**, **7-9a**,**b, 13-15**), petroleum ether (60-80°C) (compound **10**).

singlets at δ 3.7 (4-CH_2), 6.0 (4-CH), 8.8 (OH), and a multiplet at 7.2-7.8 ppm (aromatic protons). The spectral data of compound **11** showed that it exists in both keto-enol forms. For further evidence about the structure of compound **11**, the coupling of compound **11** with benzene diazonium chloride produced 4-phenylazopyrazol-5-ol derivative **12**, while the reaction of compound **11** with nitrous acid gave 4-nitroso-3-phenylpyrazol-5-ol derivative (**13**). The IR spectra of compounds **12** and **13** revealed absorption bands at 3527-3501 (OH, enolic), 1666-1643 (C=N), 1590-1563 (C=C), and 1520 cm^{-1} (N=N in compound **12**).

The treatment of 3-hydrazino-4,5,6-triphenylpyridazine **3** with diethyl malonate and ethyl phenylacetoacetate by fusion in an oil bath gave the corresponding ethyl (6,7,8-triphenyl[1,2,4]triazolo[3,4-*b*]pyridazin-3-yl)acetate (**14**) and 3-benzyl-6,7,8-triphenyl[1,2,4]triazolo[3,4-*b*]pyridazine (**15**) (Scheme 2).

TABLE 2. Antimicrobial activity

Compound	*Candida albicans*	*Staphylococcus aureus*	*Escherichia coli*
4	+	++	++
5a	++	+	+++
6b	+	+++	++
7	+++	++	+
9a	++	+	+
10	+++	++	++
12	+	++	+++
13	+	+	++
15	+++	++	+

The structure of compounds **14** and **15** was confirmed by their correct analytical and spectral data. The IR spectrum of compound **14** showed absorption bands at 1715 (C=O, ester), 1610 (C=N), 1512 (C=C), and 1080-1056 cm^{-1} (C–O–C), while the ^1H NMR spectrum showed signals at δ 1.3 (3H, t, $CH_2C\underline{H}_3$), 4.2 (2H, q, $C\underline{H}_2CH_3$), 4.7 (2H, s, CH_2), and 7.2-7.8 ppm (15H, m, aromatic protons).

The results of the antimicrobial studies presented in Table 2 revealed that most of the newly synthesized compounds showed antimicrobial activity against the fungus *Candida albicans* and the gram-negative bacteria *Escherichia coli*. However, they were less active against the gram-positive bacteria *Straphylococcus aureus*. The most active compounds against *C. albicans* were compounds **7**, **10**, and **15.**

EXPERIMENTAL

All melting points were uncorrected. The IR spectra were measured on KBr on a Bruker FT-IR ISS 25 spectrophotometer (ν_{max}, cm^{-1}). The ^1H NMR spectra (DMSO-d_6 and $CDCl_3$) were measured on a Bruker Avance 300 MHz spectrometer using TMS as an internal reference.

3-Hydrazino-4,5,6-triphenylpyridazine (3). To a solution of 3-chloro-4,5,6-triphenylpyridazine **2** (0.01 mol) in *n*-butanol (10 ml), hydrazine hydrate (3 ml) was added. The reaction mixture was refluxed for 12 h. The precipitated product was filtered off, dried, and recrystallized from ethanol.

3,7,8,9-Tetraphenyl-4H-pyridazino[6,1-c][1,2,4]triazine (4). To a solution of compound **3** (0.01 mol) in dry dioxane (20 ml), phenacyl bromide (0.01 mol) was added. The reaction mixture was heated under reflux, a reddish precipitate was formed during the first 5 min, the reflux was continued for 1 h, then the reaction mixture was cooled. The crystalline product obtained was filtered off, dried, and crystallized from ethanol.

4-Substituted Benzaldehyde (4,5,6-Triphenylpyridazin-3-yl)hydrazones 5a-c. To a solution of compound **3** (0.01 mol) in ethanol (30 ml), the appropriate aromatic aldehyde was added. The reaction mixture was heated under reflux for 5 h, and then cooled to room temperature. The hydrazones precipitated were filtered off, dried, and crystallized from the proper solvents (Table 1).

3-Substituted 6,7,8-Triphenyl[1,2,4]triazolo[4,3-b]pyridazines 6a-c. To a solution of each of compounds **5a-c** (0.01 mol) and anhydrous Na_2CO_3 in $CHCl_3$ (20 ml), bromine (0.5 ml) was added dropwise at room temperature with continuous stirring for 3 h. The reaction mixture was left to stand overnight, then the solvent was evaporated under vacuum. The residue was dried and crystallized from the proper solvents (Table 1).

N-(4,5,6-Triphenylpyridazin-3-yl)-2-cyanoacetohydrazide (7). A mixture of compound **3** (0.01 mol) and ethyl cyanoacetate (0.01 mol) in ethanol (30 ml) was refluxed for 5 h. The reaction mixture was cooled, and the solid precipitated was filtered off, dried, and crystallized from ethanol.

3-Cyanomethyl-6,7,8-triphenyl[1,2,4]triazolo[3,4-b]pyridazine (8). A mixture of compound **3** (0.01 mol) and ethyl cyanoacetate (0.01 mol) was heated on an oil bath at 160-170°C for 1 h, then cooled, the residue was triturated with ethanol, and the solid precipitated was filtered off, dried, and crystallized from ethanol.

Ethyl N-(4,5,6-triphenylpyridazin-3-yl)-3-hydrazonobutanoate (9a) and Ethyl N-(4,5,6-Triphenyl-pyridazin-3-yl)-3-hydrazono-3-phenylpropanoate (9b). To the solution of compound **3** (0.01 mol) in MeOH/$CHCl_3$ mixture (20 ml) (1:1), each of ethyl acetoacetate or ethyl benzoylacetate was added. The reaction mixture was left at room temperature for 24 h, then the solvent was evaporated and the residue was triturated with petroleum ether at 60-80°C; the solid precipitated was filtered off, dried, and crystallized from ethanol.

3-Methyl-6,7,8-triphenyl[1,2,4]triazolo[4,3-b]pyridazine (10). A mixture of compound **3** (0.01 mol) and ethyl acetoacetate (0.01 mol) was heated on an oil bath at 160-170°C for 1 h, then cooled, the residue was triturated with petroleum ether at 60-80°C, and the solid precipitated was filtered off, dried, and crystallized.

3-Phenyl-1-(4,5,6-triphenylpyridazin-3-yl)-1H-pyrazol-5-ol (11). A mixture of compound **3** (0.01 mol) and ethyl benzoylacetate (0.01 mol) was heated on an oil bath at 160-170°C for 1 h, then cooled, the residue was triturated with petroleum ether at 60-80°C, and the solid precipitated was filtered off, dried, and crystallized from benzene.

4-Phenylazopyrazol-5-ol Derivative (12). To an ice cooled solution of compound **11** (0.46 g, 0.01 mol) in ethanol (15 ml) containing NaOH (1 g) and sodium acetate (2 g), the benzene diazonium salt was added. The cooling was continued for 1 h with stirring; the mixture was left to stand in a refrigerator overnight, then neutralized carefully by dropwise addition of HCl. The formed orange precipitate was filtered off, dried, and crystallized from benzene.

4-Nitroso-3-phenylpyrazol-5-ol Derivative 13. To an ice cooled solution of compound **11** (0.46 g, 0.01 mol) in ethanol containing acetic acid (3 ml), a cold solution of $NaNO_2$ (0.1 g) in H_2O (1 ml) was added. The mixture was left at room temperature overnight. The obtained orange precipitate was filtered off, dried, and crystallized from ethanol.

Ethyl (6,7,8-triphenyl[1,2,4]triazolo[3,4-b]pyridazin-3-yl)acetate (14). A mixture of compound **3** (0.01 mol) and diethyl malonate (0.01 mol) was heated on an oil bath at 160-170°C for 1 h, then cooled, the residue was triturated with ethanol, and the solid precipitated was filtered off, dried, and crystallized.

3-Benzyl-6,7,8-triphenyl[1,2,4]triazolo[3,4-b]pyridazine (15). A mixture of compound **3** (0.01 mol) and ethyl phenylacetoacetate (0.01 mol) was heated on an oil bath at 160-170°C for 1 h, then cooled, the residue was triturated with ethanol, and the solid precipitated was filtered off, dried, and crystallized.

Method for Testing of Biological Properties.

The biological activity of the tested compounds has been evaluated using the filter paper disk method after dissolving the substances in ethanol. The inhibition zones of microbial growth surrounding the paper disk were measured in millimeters at the end of incubation period [14] (18-24 h at 27^oC).

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