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Synthesis and Biological Evaluation of Some New Substituted Fused Pyrazole Ring Systems as Possible Anticancer and Antimicrobial agents

Hassan M. Faidallah, Sherif A.F. Rostom,[1] and **Mohammed S. Al-Saadi**[2]

Department of Chemistry, Faculty of Science,
[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy,*
[2]*Division of Medicinal Chemistry, Faculty of Medicine,*
King Abdulaziz University, Jeddah, Saudi Arabia

Abstract. This research work describes the synthesis and biological properties of some novel polysubstituted fused heterocyclic ring systems namely; pyrano[4,3-c] pyrazoles and pyrazolo[4,3-c]pyridines. Such targeted compounds where designed so as to hybridize the pyrazole ring with the pyrone and/or pyridine moieties, respectively, hoping to obtain synergistic anticancer and/or antimicrobial activities. The chemistry of the reactions employed in the synthesis of the target compounds together with their chemical behaviour, are discussed and the structures of the newly synthesized compounds were confirmed by the IR and ^{1}H-NMR spectral data. All the synthesized compounds showed weak anticancer activity according to the protocol of the National Cancer Institute (NCI), Maryland, USA. Additionally, they showed weak antimicrobial activity against some bacteria and fungi.

Key Words: Synthesis; Fused Pyrazoles; Antimicrobial; Anticancer.

Introduction

Among the wide variety of heterocycles that have been explored for developing potential pharmacologically active compounds, pyrazoles fused with different heterocycles that are known to contribute to various chemotherapeutic effects have emerged as antimicrobial,[1,2] antifungal,[3] and antiviral agents.[4] In addition, some fused pyrazole derivatives were reported to induce various antileukemic,[5] antitumor[6,7] and antiproliferative[8,9] activities. On the other hand, investigations in the chemistry and biology of 2-pyrones have become

highly intensified with the recognition that they constitute an essential pharmacophore in many naturally occurring and biologically active agents.[10] Some pyrones were reported to possess distinctive chemotherapeutic potentials including cytotoxicity against some human cancer cell lines.[11,12] Furthermore, a wide range of chemotherapeutic activities have been ascribed to pyridine derivatives including antimicrobial,[13,14] and anticancer activities.[15-17] Motivated by these facts, we were interested to synthesize and investigate the in vitro anticancer, antibacterial and antifungal activities of some novel polysubstituted fused heterocyclic ring systems namely; pyrano[4,3-c]pyrazoles and pyrazolo[4,3-c] pyridines. Such targeted compounds where designed so as to hybridize the pyrazole ring with the pyrone and/or pyridine moieties, respectively, hoping to obtain synergistic anticancer and/or antimicrobial activities. The structures of the newly synthesized compounds were confirmed with elementary microanalyses and substantiated with IR and ^{1}H-NMR data. The target compounds have been subjected to the National Cancer Institute NCI in vitro disease-oriented human cells screening panel assay, Maryland, USA, to screen their anticancer activity. In addition, the in vitro antibacterial and antifungal activities of the target compounds were also tested.

Experimental

Melting points were determined in open glass capillaries on a Gallenkamp melting point apparatus and were uncorrected. The infrared (IR) spectra were recorded on Shimadzu FT-IR 8400S infrared spectrophotometer using the KBr plate technique. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian EM 360 spectrometer using tetramethylsilane as the internal standard and DMSO-d_6 as the solvent (Chemical shifts in δ, ppm). Splitting patterns were designated as follows: S: Singlet; D: Doublet; M: Multiplet. Elemental analyses were performed at the Microanalytical Unit, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia, and the found values were within ±0.4% of the theoretical values. Follow up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminum sheets (Type 60 F254, Merck) and the spots were detected by exposure to UV-lamp at λ 254 nm.

5-Amino-3,6-Dimethyl-2H-Pyrazolo[4,3-C] Pyridine-4 (5H)one (2)

A solution of dehydroacetic acid (1.68 g, 0.01 mol) in ethanol (20 mL) was refluxed with hydrazine hydrate (1.1 mL, 0.022 mol) for 2 h. The reaction mixture was concentrated to half its volume and allowed to cool. The solid product separated was filtered, washed with cold ethanol and recrystallized from ethanol. Physicochemical and analytical data are recorded in Table 1. ^{1}H-NMR and IR spectra are shown in Table 2. ^{13}C-NMR spectrum is recorded in Table 3.

Table 1. Physicochemical and analytical data for compounds 2-7.

Cpd No.	R	Yield (%)	M.p. (^{O}C)	Mol. Form. (M. Wt.)		Analysis*			
						C	H	N	S
2	-	72	225-7	$C_8H_{10}N_4O$ (178.19)	C F	53.92 54.11	5.66 5.35	31.44 31.07	- -
3a	C_6H_5	68	248-9	$C_{15}H_{14}N_4O$ (266.30)	C F	67.65 67.23	5.30 5.12	21.04 20.89	- -
3b	4-CH_3-C_6H_4	70	230-2	$C_{16}H_{16}N_4O$ (280.32)	C F	68.55 98.72	5.75 5.90	19.99 20.16	- -
3c	4-OCH_3-C_6H_4	72	227-9	$C_{16}H_{16}N_4O_2$ (296.32)	C F	64.85 65.02	5.44 5.27	18.91 18.66	- -
3d	4-Cl-C_6H_4	76	242-4	$C_{15}H_{13}ClN_4O$ (300.74)	C F	59.91 60.18	4.36 4.25	18.63 18.43	- -
3e	2-thienyl	70	232-4	$C_{13}H_{12}N_4OS$ (272.33)	C F	57.34 57.45	4.44 4.29	20.57 20.23	11.77 11.58
4a	Cyclo-C_6H_{11}	69	182-4	$C_{22}H_{32}N_6O_3$ (428.53)	C F	61.66 61.81	7.53 7.32	19.61 19.74	- -
4b	C_6H_5	72	248-9	$C_{22}H_{20}N_6O_3$ (416.43)	C F	63.45 63.29	4.84 4.98	20.18 19.91	- -
4c	4-Cl-C_6H_4	78	229-31	$C_{22}H_{18}Cl_2N_6O_3$ (485.32)	C F	54.45 54.39	3.74 3.91	17.32 17.25	- -
5a	CH_3	75	235-7	$C_{12}H_{16}N_6OS_2$ (324.43)	C F	44.43 44.11	4.97 5.06	25.90 26.04	19.77 19.46
5b	n-C_4H_9	72	249-50	$C_{18}H_{28}N_6OS_2$ (408.58)	C F	52.91 53.12	6.91 6.79	20.57 20.33	15.70 15.61
5c	Cyclo-C_6H_{11}	78	242-4	$C_{22}H_{32}N_6OS_2$ (460.66)	C F	57.36 57.19	7.00 7.23	18.24 18.07	13.92 14.05
5d	CH_2-C_6H_5	71	192-4	$C_{24}H_{24}N_6OS_2$ (476.62)	C F	60.48 60.19	5.08 5.16	17.63 17.42	13.46 13.61
5e	C_6H_5	76	247-8	$C_{22}H_{20}N_6OS_2$ (448.56)	C F	58.91 59.13	4.49 4.27	18.74 18.61	14.30 14.54
5f	4-CH_3-C_6H_4	77	255-7	$C_{24}H_{24}N_6OS_2$ (476.62)	C F	60.48 60.29	5.08 5.16	17.63 17.49	13.46 13.31
5g	4-F-C_6H_4	78	236-8	$C_{22}H_{18}F_2N_6OS_2$ (484.54)	C F	54.53 54.39	3.74 3.88	17.34 17.26	13.24 13.07
6a	C_6H_5	79	195-7	$C_{14}H_{12}N_2O_2$ (240.26)	C F	69.99 70.11	5.03 4.87	11.66 11.52	- -
6b	4-Cl-C_6H_4	72	218-9	$C_{14}H_{11}ClN_2O_2$ (274.70)	C F	61.21 61.35	4.04 3.92	10.20 10.31	- -
6c	4-F-C_6H_4	70	212-4	$C_{14}H_{11}FN_2O_2$ (258.25)	C F	65.11 64.93	4.29 4.41	10.85 10.47	- -
6d	4-NO_2-C_6H_4	74	216-8	$C_{14}H_{11}N_3O_4$ (285.25)	C F	58.95 58.72	3.89 4.08	14.73 14.56	- -
7a	C_6H_5	65	233-5	$C_{14}H_{14}N_4O$ (254.29)	C F	66.13 66.27	5.55 5.39	22.03 21.91	- -
7b	4-Cl-C_6H_4	63	250-2	$C_{14}H_{13}ClN_4O$ (288.73)	C F	58.24 58.37	4.54 4.36	19.40 19.19	- -
7c	4-F-C_6H_4	60	216-8	$C_{14}H_{13}FN_4O$ (272.28)	C F	61.76 61.58	4.81 5.03	20.58 20.42	- -

***** The found values (F) are within ±0.4% of the calculated (C) values.

Table 2. ^{1}H-NMR (δ-ppm) and IR (Cm^{-1}) spectra of some 2-11 derivatives.

Cpd	CH_3 (S, 3H)	H-6 (S, 1H)	ArH & NH_2 (m)	Others	IR (Cm^{-1})
2	2.18, 2.51	6.56		12.02(S,1H,NH)	1667(CO); 3300-3370(NH_2 & NH)
3a	2.15, 2.58	6.38	7.10-7.62 (5H)	11.52(S,1H,NH); 8.12(S,1H,CH=)	1650(CO)
3b	2.17, 2.58	6.40	7.15-7.52 (4H)	2.34(S,3H,CH_3); 11.68(S,1H,NH); 8.12(S,1H,CH=)	1652(CO)
3c	2.18, 2.60	6.45	7.12-7.54 (4H)	2.32(S,3H,CH_3); 8.21(S,1H,CH=); 11.25(S,1H,NH)	1650 (CO)
3e	2.18, 2.54	6.39	7.00-7.25 (3H)	11.82(S,1H,NH); 8.20(S,1H,CH=)	1654(CO)
4a	2.08, 2.64	6.40		1.70-2.65 (m,11H,cyclohex); 8.62 (S,1H,NH); 9.96(S,1H,NH), 10.01(S,1H,NH)	1655(CO); 1662(CO); 1667(CO); 3310-3365(NH)
4b	2.19, 2.56	6.28	7.02-7.62 (10H)	8.62(S,1H,NH); 9.80(S,1H,NH); 10.12(S,1H,NH)	1658(CO); 1662(CO); 1658(CO); 3300-3370(NH)
4c	2.25, 2.50	6.35	6.92-7.55 (8H)	8.72(S,1H,NH); 9.60(S,1H,NH) 10.12(S,1H,NH)	1658(CO); 1660(CO); 1665(CO); 3310-3395(NH)
5a	2.15, 2.56	6.35		2.35(S,3H,CH_3); 2.40(S,3H,CH_3); 8.40(S,1H,NH), 9.05(S,1H,NH); 10.25(S,1H,NH)	1652(CO); 1150(CS); 1154(CS); 3300-3368(NH
5d	2.17, 2.55	6.42	7.00-7.85 (10H)	4.26(d,2H,CH_2); 4.31(d,2H,CH_2); 8.52(m,1H,NH); 9.35(m,1H,NH); 10.02(S,1H,NH)	1650(CO); 1150(CS); 1158(CS); 3322-3368(NH)
5e	2.18, 2.56	6.45	7.33-7.62 (10H)	8.52(S,1H,NH); 9.35(S,1H,NH) 10.12(S,1H,NH)	1662(CO); 1140(CS); 1152(CS); 3300-3362(NH)
5f	2.20, 2.52	6.28	7.15-7.56 (8H)	2.29(S,3H,CH_3); 2.33(S,3H,CH_3); 8.42(S,1H,NH); 9.05(S,1H,NH), 10.21(S,1H,NH)	1658(CO); 1148(CS); 1150(CS); 3310-3380(NH)
6a	2.32, 2.58	6.45	7.30-7.62 (5H)		1720(CO)
6b	2.41, 2.64	6.28	7.20-7.72 (4H)		1735(CO)
6c	2.15, 2.50	6.30	7.10-7.82		1738(CO)
6d	2.13, 2.47	6.42	7.01-7.95 (4H)		1736(CO)
7a	2.07, 2.53	6.62	7.09-7.52 (5H)		1665(CO); 3380-3420(NH_2)
7b	1.96, 2.45	6.52	6.82-7.47		1658(CO); 3350-

Cpd	CH_3 (S, 3H)	H-6 (S, 1H)	ArH & NH_2 (m)	Others	IR (Cm^{-1})
			(4H)		3390(NH_2)
7c	1.98, 2.42	6.55	6.80-7.40 (4H)		1655(CO); 3320-3350(NH_2)
8a	2.10, 2.39	6.49	7.01-7.52 (10H)	8.11(S, 1H, CH=)	1655(CO)
8b	2.15, 2.35	6.12	7.12-7.60 (9H)	2.35(S, 1H, CH_3); 8.10(S, 1H, CH=)	1650(CO)
8c	2.16, 2.54	6.45	6.80-7.52 (9H)	3.85(S,3H,OCH_3); 8.14(S,1H,NH)	1658(CO)
8d	2.18, 2.55	6.55	6.82-7.60 (9H)	8.14(S,1H,NH)	1648(CO)
8e	2.14, 2.40	6.23	7.00-7.72 (8H)	8.05(S, 1H, CH=)	1652(CO)
8f	2.15, 2.54	6.51	6.92-7.65 (9H)	8.14(S,1H,CH=)	1652(CO)
8g	2.16, 2.56	6.45	6.89-7.42 (8H)	8.15(S,1H,CH=)	1649(CO)
8i	2.15, 2.54	6.48	6.88-7.51 (9H)	8.16(S,1H,CH=)	1652(CO)
8j	2.18, 2.60	6.42	6.82-7.48 (8H)	8.18(S,1H,CH=)	1654(CO)
8k	2.19, 2.58	6.50	6.85-7.32 (7H)	8.15(S,1H,CH=)	1656(CO)
9a	2.18, 2.56	6.45	7.30-7.67 (10H)	8.28(S,1H,NH)	1658; 1175, 1342(SO_2N); 3280-3300(NH)
9b	2.15, 2.54	6.43	7.10-7.58 (9H)	2.35(S,3H,CH_3); 8.35(S,1H,NH)	1656(CO); 1182, 1350(SO_2N); 3300-3310(NH)
9c	2.16, 2.55	6.40	6.92-7.61 (9H)	8.32(S,1H,NH)	1652(CO); 1173, 1345(SO_2N); 3350-3380(NH)
9d	2.15, 2.54	6.42	6.95-7.52 (9H)	8.25(S,1H,NH)	1658(CO); 1185, 1338(SO_2N); 3270-3300(NH)
9e	2.17, 2.52	6.40	6.89-7.45	2.36(S,3H,CH_3), 8.30(S,1H,NH)	1654(CO); 1175, 1400(SO_2N);3290-3310(NH)
10a	2.15, 2.43	6.50	7.04-7.48	1.63-2.20(m,11H,cyclo); 8.50(S,1H,NH); 9.68(S,1H,NH)	1648(CO); 1652(CO); 3330-3380(NH)
10b	2.01, 2.41	6.52	7.22-7.48 (10H)	8.35(S,1H,NH); 9.85(S,1H,NH)	1650(CO); 1645(CO); 3310-3360(NH)
10d	2.18, 2.49	6.45	6.90-7.36 (4H)	1.65-2.32(m,1H,cyclohex); 8.60(S,1H,NH); 10.12(S,1H,NH)	1658(CO); 1660(CO); 3310-3380(NH)
10e	2.18, 2.52	6.38	6.98-7.52 (9H)	8.62(S,1H,NH); 10.02(S,1H,NH)	1656(CO); 1650(CO); 3335-3365(NH)
10f	2.19, 2.54	6.40	6.87-7.42 (8H)	8.58(S,1H,NH); 9.95(S,1H,NH)	1658(CO); 1665(CO); 3300-3380(NH)
10g	2.16, 2.51	6.42	6.85-7.56	8.58(S,1H,NH);	1665(CO);

Cpd	CH_3 (S, 3H)	H-6 (S, 1H)	ArH & NH_2 (m)	Others	IR (Cm^{-1})
			(9H)	9.98(S,1H,NH)	1652(CO); 3320-3375(NH)
11a	2.18, 2.52	6.48	7.12-7.58 (5H)	2.50(S,3H,CH_3); 8.70(S,1H,NH) 9.88(S,1H,NH)	1658(CO); 1152(CS); 3300-3335(NH)
11c	2.14, 2.56	6.45	7.02-7.61 (10H)	8.75(S,1H,NH); 10.02(S,1H,NH)	1652(CO); 1145(CS); 3290-3340(NH)
11d	2.22, 2.51	6.41	7.09-7.52 (9H)	2.40(S,3H,CH_3); 8.45(S,1H,NH); 9.80(S,1H,NH)	1660(CO); 1158(CS); 3320-3365(NH)
11f	2.17, 2.61	6.38	6.92-7.60 (9H)	8.70(S,1H,NH); 10.05(S,1H,NH)	1662(CO); 1150(CS); 3320-3380
11g	2.15, 2.54	6.42	6.90-7.52 (9H)	2.38(S,3H,CH_3); 8.62(S,1H,NH); 10.10(S,1H,NH)	1655(CO); 1152(CS); 3310-3380(NH)
11i	2.10, 2.58	6.32	6.89-7.55 (9H)	8.45(S,1H,NH), 9.20(S,1H,NH)	1660(CO); 1155(CS); 3290-3352(NH)

Table 3. ^{13}C-NMR (δ-ppm) spectra of some 2-11 derivatives.

Cpd	CH_3	C_2	C_3	C_4	C_5	C_6	C_7	Ar C & others
2	11.52, 16.75	155.66	111.80	145.12	136.11	94.42	135.71	
4b	12.05, 17.00	155.62, 158.98, 159.2	128.80	148.20	128.72	94.51	136.98	120.22, 121.32, 125.95, 126.33, 128.52, 129.60, 138.70, 139.72
5a	12.12, 17.12, 33.20, 33.45	155.89	127.98	146.25	129.05	100.25	136.22	185.85(CS), 186.22(CS)
5d	12.02, 17.11	155.02	128.70	146.23	128.89	98.25	136.27	120.23, 121.91, 123.02, 127.98, 129.25, 132.23, 136.12, 139.52; 64.23(CH_2), 63.98(CH_2), 184.12(CS), 185.12(CS)
5f	11.58, 16.78, 21.78	154.80	128.20	147.20	129.12	97.25	135.12	120.12, 121.12, 124.58, 127.50, 129.23, 130.98, 134.52, 138.89; 183.12(CS), 185.42(CS)
6a	11.82, 17.2	154.96	128.90	149.10	129.05	94.41	135.70	120.20, 126.33, 129.40, 139.72
6b	11.95, 20.20	152.20	118.52	149.25	129.05	102.22	142.15	121.60, 129.52, 131.88, 137.85
6c	12.02, 20.25	156.4	128.35	149.52	130.26	96.48	145.89	116.12, 121.82, 135.35, 160.22
6d	11.82,	155.6	126.25	147.11	128.25	94.45	139.46	121.11, 121.78,

Cpd	CH_3	C_2	C_3	C_4	C_5	C_6	C_7	Ar C & others
	18.25							145.82, 145.95
7a	11.56, 16.72	155.66	111.82	145.56	136.20	94.45	135.78	121.52, 127.02, 128.98, 138.35
7b	11.89, 19.82	154.20	120.25	148.52	130.12	101.52	143.52	122.50, 129.82, 132.11, 138.23
8a	11.78, 17.52	154.62	128.90	149.11	129.52	94.56	135.79	120.32, 127.20, 129.82, 139.52, 125.15(CH=)
8b	11.72, 16.95	156.29	120.22	145.52	131.56	94.65	140.01	119.25, 120.82, 125.98, 126.1, 128.82, 137.65, 139.25, 140.23, 55.98(OCH_3), 162.2(CH=)
8e	12.01, 17.98	154.82	127.98	148.20	131.23	96.12	138.11	125.80, 127.11, 127.45, 144.20, 125.10(CH=)
8f	12.01, 20.12	153.45	119.02	148.56	129.12	100.52	143.12	120.2, 121.66, 127.51, 129.82, 131.82, 136.72, 137.89, 138.98, 126.20(CH=)
9a	11.62, 16.82	155.82	113.42	145.23	129.05	94.65	135.86	120.42, 121.23, 122.25, 128.11, 129.32, 130.65, 138.52, 139.56
9b	11.78, 17.02, 21.52	154.90	112.52	146.25	128.12	95.75	136.92	121.50, 125.82, 127.23, 129.45, 132.33, 135.12, 136.12, 138.22
10a	11.98, 17.12	154.69, 159.25	128.72	149.22	129.12	94.68	135.82	121.25, 126.78, 129.25, 138.75; 23.05, 29.12, 34.55, 49.56 (cyclohexyl)
10b	11.80, 16.85	155.22, 157.12	122.58	144.98	132.15	98.50	139.15	120.98, 122.24, 127.12, 128.29, 129.35, 136.12, 138.25, 139.20
11c	11.62, 16.82	155.25	129.82	148.12	128.98	95.50	136.01	120.32, 122.24, 127.89, 128.15, 129.50, 130.76, 139.25, 140.12, 185.12(CS)
11d	11.88, 17.05, 22.25	154.99	128.25	148.92	128.67	94.25	135.95	120.98, 128.12, 133.25, 138.12, 181.5(CS)
11f	11.80, 16.56	156.42	120.80	147.98	129.23	98.67	136.12	120.75, 121.6, 126.30, 129.4, 129.80, 131.22, 137.80, 139.78; 185(CS)
11g	11.78, 16.98, 31.52	156.42	119.75	148.20	129.10	95.12	136.70	121.26, 123.50, 127.33, 129.12, 131.23, 135.25, 138.25, 139.98; 184(CS)

5-(Arylideneamino)-3,6-Dimethyl-2H-Pyrazolo[4,3-C]Pyridin-4(5H)-Ones (3)

A mixture of the pyrazolopyridine **2** (0.3g, 0.002 mol) and the appropriate aldehyde (0.002 mol) in benzene (10 mL) was heated on a boiling water bath for 4-6 h. Excess solvent was removed under reduced pressure, and the remaining residue was treated with methanol, filtered and recrystallized from ethanol. Physicochemical and analytical data are recorded in Table 1. ^{1}H-NMR and IR spectra are shown in Table 2. ^{13}C-NMR spectra are recorded in Table 3.

General Procedure for the Preparation of the N-Substituted 3,6-Dimethyl-5-(3-Substituted ureido)-4-Oxo-4,5-Dihydropyrazolo[4,3-C]Pyridine-1-Carboxylic Acid Amides (4) and N-Substituted 3,6-Dimethyl-5-(3-Substituted thioureido)-4-Oxo-4,5-Dihydropyrazolo[4,3-C]-Pyridine-1-Carbothioamides (5)

To a solution of **2** (0.3g, 0.002 mol) in pyridine (10mL) was added the appropriate isocyanate or isothiocyanate (0.0042 mol), and the reaction mixture was heated under reflux for 5-8 h. After cooling to room temperature, the reaction mixture was poured on crushed ice and the separated solid product was filtered, washed thoroughly with water, dried and crystallized from acetic acid containing few drops of water. Physicochemical and analytical data are recorded in Table 1. ^{1}H-NMR and IR spectra are shown in Table 2. ^{13}C-NMR spectra are recorded in Table 3.

3,6-Dimethyl-1-Substituted-Pyrano[4,3-C]Pyrazol-4(5H)-Ones (6)

A solution of dehydroacetic acid **1** (1.68 g, 0.01 mol) in ethanol (20 mL) was heated under reflux with the appropriate arylhydrazine (0.011 mol) for 3-4 h. The reaction mixture was concentrated to half its volume and allowed to cool. The solid product separated was filtered, washed with ethanol and recrystallized from ethanol. Physicochemical and analytical data are recorded in Table 1. ^{1}H-NMR and IR spectra are shown in Table 2. ^{13}C-NMR spectra are recorded in Table 3.

5-Amino-3,6-Dimethyl-1-Substituted-1H-Pyrazolo[4,3-C]Pyridin-4(1H)-Ones (7)

A solution of the appropriate **6** derivative (0.01 mol) in ethanol (20 mL) was refluxed with hydrazine hydrate (1.1 mL, 0.022 mol) for 2-4 h. The reaction mixture was concentrated to half its volume and allowed to cool. The solid product separated was filtered, washed with cold ethanol and recrystallized from ethanol. Physicochemical and analytical data are recorded in Table 1. ^{1}H-NMR and IR spectra are shown in Table 2. ^{13}C-NMR spectra are recorded in Table 3.

5- (Arylidenamino)-3,6-Dimethyl-1-Substituted-1H-Pyrazolo[4,3-C]Pyridin-4(5H)-Ones (8)

A mixture of the appropriate **7** derivative (0.001 mol) and the appropriate aldehyde (0.001 mol) in benzene (10 mL) was heated on a boiling water bath for 5-8 h. Excess solvent was removed under reduced pressure, and the remaining residue was treated with methanol, filtered and recrystallized from ethanol. Physicochemical and analytical data are recorded in Table 4. ^{1}H-NMR and IR spectra are shown in Table 2. ^{13}C-NMR spectra are recorded in Table 3.

Table 4. Physicochemical and analytical data for compounds 8-11.

Cpd No.	R	R_1 or X	Yield (%)	M.p. (^{O}C)	Mol. Form. (M. Wt.)		Analysis*			
							C	H	N	S
8a	C_6H_5	C_6H_5	70	238-40	$C_{21}H_{18}N_4O$	C	73.67	5.30	16.36	-
					(342.39)	F	73.53	5.52	16.21	-
8b	C_6H_5	4-CH_3-C_6H_4	72	225-7	$C_{22}H_{20}N_4O$	C	74.14	5.66	15.72	-
					(356.42)	F	73.88	5.79	14.82	-
8c	C_6H_5	4-OCH_3-C_6H_4	68	227-9	$C_{22}H_{20}N_4O_2$	C	70.95	5.41	15.04	-
					(372.42)	F	71.13	5.24	14.82	-
8d	C_6H_5	4-Cl-C_6H_4	72	240-2	$C_{21}H_{17}ClN_4O$	C	66.93	4.55	14.87	-
					(376.84)	F	67.14	4.39	14.58	-
8e	C_6H_5	2-thienyl	74	242-4	$C_{19}H_{16}N_4OS$	C	65.50	4.63	16.08	9.20
					(348.42)	F	65.37	4.85	15.93	9.04
8f	4-Cl-C_6H_4	C_6H_5	76	198-9	$C_{21}H_{17}ClN_4O$	C	66.93	4.55	14.87	-
					(376.84)	F	66.81	4.72	14.64	-
8g	4-Cl-C_6H_4	4-Cl-C_6H_4	78	214-6	$C_{21}H_{16}Cl_2N_4O$	C	61.33	3.92	13.62	-
					(411.28)	F	61.21	4.17	13.49	-
8h	4-Cl-C_6H_4	2-thienyl	72	222-4	$C_{19}H_{15}ClN_4OS$	C	59.60	3.95	14.63	8.37
					(382.87)	F	59.34	4.11	14.44	8.16
8i	4-F-C_6H_4	C_6H_5	69	220-2	$C_{21}H_{17}FN_4O$	C	69.99	4.75	15.55	-
					(360.38)	F	70.08	4.49	15.36	-
8j	4-F-C_6H_4	4-Cl-C_6H_4	66	216-8	$C_{21}H_{16}ClFN_4O$	C	63.88	4.08	14.19	-
					(394.83)	F	63.97	3.87	13.93	-
8k	4-F-C_6H_4	2-thienyl	64	207-9	$C_{19}H_{15}FN_4OS$	C	62.28	4.13	15.29	8.75
					(366.41)	F	62.39	3.92	15.41	8.58
9a	C_6H_5	H	76	176-8	$C_{20}H_{18}N_4O_3S$	C	60.90	4.60	14.20	8.13
					(394.45)	F	60.73	4.71	14.12	8.01
9b	C_6H_5	CH_3	78	158-60	$C_{21}H_{20}N_4O_3S$	C	61.75	4.94	13.72	7.85
					(408.47)	F	61.69	5.07	13.55	7.67
9c	4-Cl-C_6H_4	H	77	134-6	$C_{20}H_{17}ClN_4O_3S$	C	56.01	4.00	13.06	7.48
					(428.89)	F	55.82	4.13	12.91	7.56
9d	4-F-C_6H_4	H	76	136-8	$C_{20}H_{17}FN_4O_3S$	C	58.24	4.15	13.58	7.77
					(412.44)	F	58.37	3.92	13.41	7.85
9e	4-F-C_6H_4	CH_3	78	162-4	$C_{21}H_{19}FN_4O_3S$	C	59.14	4.49	13.14	7.52
					(426.46)	F	59.22	4.56	12.93	7.40
10a	C_6H_5	Cyclo-C_6H_{11}	68	167-9	$C_{21}H_{25}N_5O_2$	C	66.47	6.64	18.46	-
					(379.46)	F	66.31	6.82	18.29	-
10b	C_6H_5	C_6H_5	70	203-5	$C_{21}H_{19}N_5O_2$	C	67.55	5.13	18.76	-
					(373.41)	F	67.68	5.01	18.43	-
10c	C_6H_5	4-Cl-C_6H_4	68	212-14	$C_{21}H_{18}ClN_5O_2$	C	61.84	4.45	17.17	-
					(379.46)	F	62.07	4.29	16.95	-
10d	4-Cl-C_6H_4	Cyclo-C_6H_{11}	70	262-4	$C_{21}H_{24}ClN_5O_2$	C	60.94	5.84	16.92	-
					(413.90)	F	60.82	6.01	17.08	-
10e	4-Cl-	C_6H_5	72	247-9	$C_{21}H_{18}ClN_5O_2$	C	61.84	4.45	17.17	-

Cpd No.	R	R_1 or X	Yield (%)	M.p. (^{O}C)	Mol. Form. (M. Wt.)		Analysis*			
							C	H	N	S
	C_6H_4				(379.46)	F	61.69	4.62	16.93	-
10f	4-Cl-C_6H_4	4-Cl-C_6H_4	65	258-9	$C_{21}H_{17}Cl_2N_5O_2$ (442.30)	C F	57.03 56.86	3.87 4.08	15.83 15.94	- -
10g	4-F-C_6H_4	C_6H_5	66	216-8	$C_{21}H_{18}FN_5O_2$ (391.40)	C F	64.44 64.26	4.64 4.75	17.89 17.63	- -
10h	4-F-C_6H_4	4-Cl-C_6H_4	68	248-50	$C_{21}H_{17}ClFN_5O_2$ (425.84)	C F	59.23 59.11	4.02 3.94	16.45 16.33	- -
11a	C_6H_5	CH_3	69	258-60	$C_{16}H_{17}N_5OS$ (327.40)	C F	58.70 58.53	5.23 5.47	21.39 21.14	9.79 9.51
11b	C_6H_5	n-C_4H_9	66	248-9	$C_{19}H_{23}N_5OS$ (369.48)	C F	61.76 61.59	6.27 6.36	18.95 19.06	8.68 8.41
11c	C_6H_5	C_6H_5	72	252-4	$C_{21}H_{19}N_5OS$ (389.47)	C F	64.76 64.53	4.92 5.08	17.98 18.11	8.23 8.07
11d	C_6H_5	4-CH_3-C_6H_4	70	260-2	$C_{22}H_{21}N_5OS$ (403.50)	C F	65.49 65.26	5.25 5.43	17.36 17.18	7.95 7.69
11e	C_6H_5	4-F-C_6H_4	69	214-6	$C_{21}H_{18}FN_5OS$ (407.46)	C F	61.90 61.79	4.45 4.61	17.19 16.94	7.87 7.58
11f	4-Cl-C_6H_4	C_6H_5	70	264-6	$C_{21}H_{18}ClN_5OS$ (423.92)	C F	59.50 59.61	4.28 4.14	16.52 16.37	7.56 7.44
11g	4-Cl-C_6H_4	4-CH_3-C_6H_4	72	241-3	$C_{22}H_{20}ClN_5OS$ (437.95)	C F	60.34 60.19	4.60 4.74	15.99 16.05	7.32 7.21
11h	4-Cl-C_6H_4	4-F-C_6H_4	68	256-7	$C_{21}H_{17}ClFN_5OS$ (441.91)	C F	57.08 56.93	3.88 4.07	15.85 15.63	7.26 7.18
11i	4-F-C_6H_4	C_6H_5	67	252-4	$C_{21}H_{18}FN_5OS$ (407.46)	C F	61.90 62.12	4.45 4.26	17.19 17.03	7.87 7.62

***** The found values (F) are within ±0.4% of the calculated (C) values

5-*(N-Substituted Benzenesulfonylamino)-3,6-Ddimethyl-1-Substituted-1H-Pyrazolo[4,3-C] Pyridin-4(5H)-Ones (9)*

To a solution of the appropriate **7** derivative (0.001 mol) in pyridine (10 mL) was added the appropriate benzenesulfonyl chloride derivative (0.0011 mol), and the mixture was heated under reflux for 3-4 h. After cooling to room temperature, the reaction mixture was poured on crushed ice and the separated solid product was filtered, washed thoroughly with water, dried and crystallized from a mixture of ethanol and benzene (3:1). Physicochemical and analytical data are recorded in Table 4. ^{1}H-NMR and IR spectra are shown in Table 2. ^{13}C-NMR spectra are recorded in Table 3.

General Procedure for the Preparation of the N_1-Substituted N_3-(3,6-Dimethyl-4-Oxo-1-Substituted-1H-Pyrazolo[4,3-C]Pyridin-5(4H)-yl)ureas (10) and N_1-Substituted N_3-(3,6-Dimethyl-4-Oxo-1-Substituted-1H-Pyrazolo[4,3-C]Pyridin-5(4H)-yl)thioureas (11)

To a solution of the appropriate **7** derivative (0.001 mol) in pyridine (10 mL) was added the appropriate isocyanate or isothiocyanate (0.0011 mol), and the reaction mixture was heated under reflux for 5-8 h. After cooling to room temperature, the reaction mixture was poured on crushed ice and the separated

solid product was filtered, washed thoroughly with water, dried and crystallized from acetic acid containing few drops of water. Physicochemical and analytical data are recorded in Table 4. ^{1}H-NMR and IR spectra are shown in Table 2. ^{13}C-NMR spectra are recorded in Table 3.

Results and Discussion

The starting compound in this research work is dehydroacetic acid; 3-acetyl-6-methyl-3*H*-pyran-2,4-dione **1;** was purchased from ACROS Organics (Cat. No.11194). In scheme 1, when dehydroacetic acid **1** reacted with hydrazine hydrate, the 5-amino-3,6-dimethyl-1*H*-pyrazolo[4,3-*c*]pyridin-4(5*H*)-one **2** was obtained and utilized as the key intermediate in this part. Its IR spectrum showed an absorption band at1667 cm^{-1} due to the carbonyl group and a broad band at 3300-3370 cm^{-1} due to the (NH_2 & NH) absorptions. The ^{1}H- NMR spectrum (δ-ppm) of **2** showed the characteristic two singlets at 2.18, 2.51 corresponding to the methyl groups and a singlet at 6.56 due to C_7-H. Its ^{13}C-NMR spectrum (δ-ppm) showed a characteristic singlet at 145.12 corresponding to the carbonyl group. Condensing **2** with the appropriate aldehyde gave rise to the corresponding 5-(arylideneamino)-3,6-dimethyl-1*H*-pyrazolo[4,3-*c*]pyridin-4(5*H*)-ones **3**. Their ^{1}H-NMR spectra (δ-ppm) are characterized by the presence of singlets at 8.12 to 8.21 due to the C*H*=N protons. On the other hand, reacting **2** with an excess of the appropriate isocyanate in pyridine as alkaline medium, afforded the corresponding N-substituted 3,6-dimethyl-5-(3-substituted ureido)-4-oxo-4,5-dihydropyrazolo[4,3-*c*]pyridine-1-carboxylic acid amides **4.** Their IR spectra revealed three characteristic absorption bands at the range of 1652-1667 cm^{-1} due to the three carbonyl groups. Their ^{1}H-NMR spectra (δ-ppm) revealed the new NH protons at their expected ranges in addition to other signals assigned for the ureido nitrogens and the respective substituents. Moreover, condensing **2** with an excess of the appropriate isothiocyanate in pyridine as alkaline medium, afforded the corresponding N-substituted 3,6-dimethyl-5-(3-substituted thioureido)-4-oxo-4,5-dihydro-pyrazolo[4,3-*c*]pyridine-1-carbothioamides **5**. The IR spectra of these compounds revealed beside the characteristic absorption bands corresponding to the carbonyl group, a characteristic C=S band at 1140-1158 cm^{-1}. Their ^{1}H-NMR spectra (δ-ppm) revealed the new NH protons at their expected ranges in addition to other signals assigned for the thioureido nitrogens and the respective substituents. Their ^{13}C-NMR spectra (δ-ppm) showed characteristic singlets at 183.12 to 186.22 corresponding to the C=S.

On the other hand, reaction of dehydroacetic acid **1** with the appropriate substituted hydrazine derivative to obtain the 3,6-dimethyl-1-substituted-pyrano[4,3-*c*]pyrazol-4(5*H*)-ones **6.** Their IR spectra showed absorption bands

at 1720-1738 cm^{-1} due to the carbonyl groups. Reacting compounds **6** with hydrazine hydrate resulted in the formation of the key intermediates 5-amino-3,6-dimethyl-1-substituted-1*H*-pyrazolo[4,3-*c*]pyridin-4(5*H*)-ones **7**. At this stage, condensing **7** with the appropriate aldehyde gave rise to the corresponding 5-(arylidenamino)-3,6-dimethyl-1-substituted-1*H*-pyrazolo[4,3-*c*]pyridin-4(5*H*)-ones **8**. Their ^{1}H-NMR spectra (δ-ppm) are characterized by the presence of singlets at 8.05 to 8.18 due to the C*H*=N protons. Moreover, reacting **7** with benzenesulfonyl chloride or p-toluenesulfonyl chloride in the presence of pyridine led to the formation of the N-substituted benzenesulfonyl derivatives **9**. Their IR spectra showed absorption bands at 1173-1185 and 1338-1345 cm^{-1} due to the SO_2N groups. Their ^{1}H-NMR spectra (δ-ppm) revealed the new NH protons at 8.25-8.35, in addition to other signals assigned for the aromatic substituents. Finally, condensing **7** with different isocyanates and isothiocyanates in the presence of pyridine afforded the corresponding substituted ureido and thioureido derivatives **10** and **11**, respectively. The IR spectra of these compounds revealed beside the characteristic absorption bands corresponding to the ester group, a characteristic C=S band at 1148-1155 cm^{-1}. Their ^{1}H-NMR spectra (δ-ppm) revealed the new NH protons at their expected ranges in addition to other signals assigned for the thioureido nitrogens and the respective substituents. Their ^{13}C-NMR spectra (δ-ppm) showed characteristic singlets at 181.5 to 185.12 corresponding to the C=S.

Biological Testings

After confirming the structure of the newly synthesized compounds using different spectral and analytical data, they were sent consequently to the Department of Health and Human Services, National Cancer Institute (NCI), Bethesda, Maryland, U.S.A to evaluate their *in vitro* antitumor activity. Out of the newly synthesized derivatives, 12 compounds namely; **3d,e, 4c, 5g, 6c, 7c, 8d,h, 9d, 10h** and **11f,h**; have been selected by the NCI to be evaluated for their preliminary antitumor activity. Unfortunately, most of them showed weak anticancer activity. In addition, the in vitro antibacterial and antifungal activities of the target compounds were also tested using the Agar-diffusion method. Test organisms utilized were *Staphylococcus aureus* as an example of Gram positive bacteria, *Escherichia coli* and as an example of Gram negative bacteria and *Candida albicans* as a representative of fungi. None of the tested compounds was able to exert significant antibacterial or antifungal activities.

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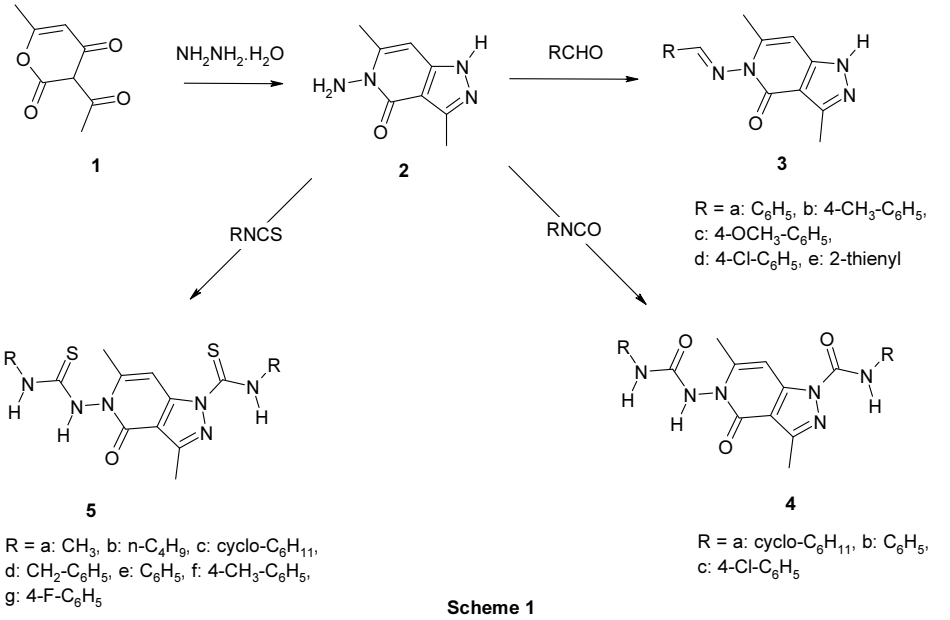
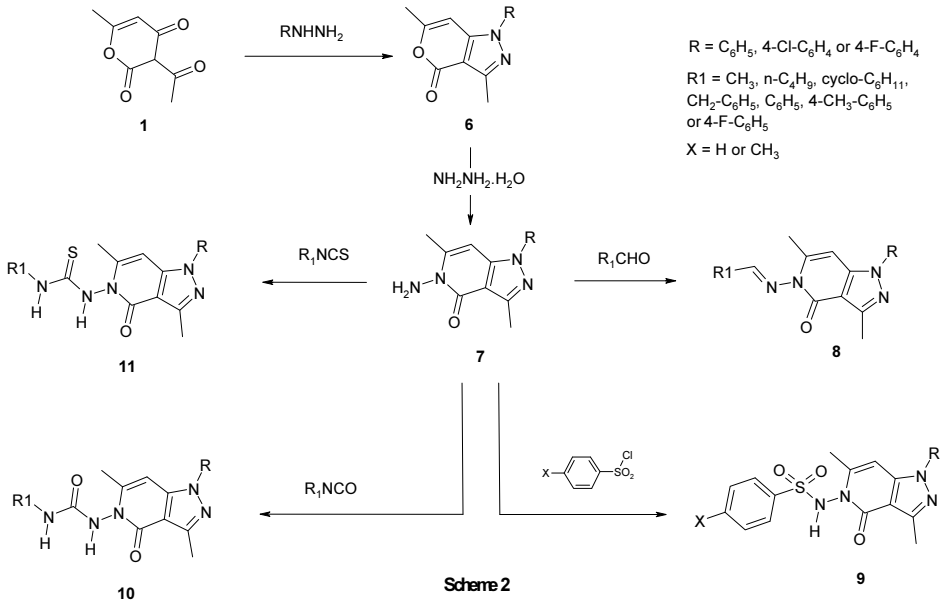
members of the Department of Health and Human Services, National Cancer Institute (NCI), Bethesda, Maryland, U.S.A. for carrying out the anticancer screening of the newly synthesized compounds.



Scheme 1



Scheme 2

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تشييد وتقييم الفاعلية البيولوجية لبعض حلقات البيرازول المدمجة والمستبدلة كمضادات للسرطان والميكروبات

حسن مصطفى فيض الله، شريف أحمد رستم[١]، محمد صادر الصاعدى[٢]

كلية العلوم، قسم الكيمياء، كلية العلوم

و [١] قسم الكيمياء الصيدلية، كلية الصيدلة

و [٢] شعبة الكيمياء الطبية، كلية الطب

جامعة الملك عبد العزيز – جدة – المملكة العربية السعودية

المستخلص. لقد ثبت في التراث العلمي أن لنواة البيرازول وبعض الحلقات المدمجة المشتقة منه دورا بارزا في تصميم وتشييد العديد من المركبات الكيميائية التي يمكن أن تستخدم كمضادات الميكروبات والفيروسات والسرطان. كذلك، تمتاز المركبات التي تحتوي في تركيبها البنائي على أنوية البيرون والبيريدين بتعدد أنشطتها البيولوجية، وأهمها نشاطها المعروف كمضادات للعديد من الميكروبات والخلايا السرطانية. بناء على ما تقدم، فقد تم التخطيط في هذا البحث لتشييد مجموعات متنوعة من المركبات العضوية تحتوى كلها بصفة أساسية على نواة البيرازول مدمجة مع حلقة البيرون أوالبيريدين، ومحملة بمجموعات وظيفية مختلفة مثل الأمين واليوريا والثيويوريا ومشتقاتها، بحيث تتشابه بنائيا مع بعض مضادات السرطان والميكروبات المعروفة. وبعد إثبات التركيب البنائى للمركبات المشيدة حديثا باستخدام التحاليل الدقيقة والأطياف المختلفة تم تقديم المركبات المشيدة إلى المعهد القومي للسرطان في ولاية مريلاند بالولايات المتحدة الأمريكية لاختبار فعاليتها المضادة لنمو ما يقرب من ستين نوعًا من الخلايا السرطانية، بالإضافة إلى إجراء مسح ميكروبيولوجى أولي لهذه المركبات لاختبار فعاليتها المضادة للعديد من أنواع البكتيريا والفطريات. هذا وقد أظهرت النتائج عدم تمتع المركبات المشيدة بفاعلية مضادة لنمو الخلايا السرطانية أو الميكروبات.