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Synthesis and Antimicrobial Activity of Novel 5-Amino-4-cyano-*1H*-pyrazole and Quinazolin-4(*3H*)-one Derivatives

Mohamed T. Abdel-Aal[1], Abdel-Aleem H. Abdel-Aleem[1], Laila I. Ibahim[2], and Ahmed L. Zein[2]

[1]Department of Chemistry, Faculty of Science, Menofeia University, Shebin-El-Koom 12345, Egypt and [2]National Organization of Drug Control and Research, Cairo 11978, Egypt

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New substituted aroylhydrazones (**4a-f**) were synthesized from the acid hydrazide (**3**) and the corresponding aldehyde or aldose. 5-Amino-4-cyano-*1H*-pyrazole derivatives (**6a-f**) were prepared by the reaction of the aroylhydrazones (**4a-f**) with malononitrile. The synthesized compounds were tested for antimicrobial activity against various bacteria and fungi and showed moderate to high inhibition activities. Compounds incorporating a sugar moiety as well as a pyrazolyl ring in their structure displayed the highest antimicrobial activity.

Key words: Aroylhydrazones, 5-Amino-4-cyanopyrazole, Quinazoline-4-one, Antimicrobial activity

INTRODUCTION

Pyrazole containing structural moieties are of biological interest because they are the key structures in various therapeutic compounds (Anzai et al., 2004; Haddad et al., 2004). These compounds are known to display antibacterial, antifungal (Tanitame et al., 2004), anti-inflammatory, analgesic, and antipyretic (Menozzi et al., 1992) activities. Pyrazole-containing compounds are also an essential structure in many chemotherapeutic agents with potential antimicrobial (Foks et al., 2005; Dardari et al., 2006; Gilbert et al., 2006), antiparasitic (Rathelot et al., 2002; Bernardino et al., 2006), antimalarial (Katiyar et al., 2005), and antiviral activities (Moukha-Chafiq et al., 2002; Allen et al., 2006). A previous investigation revealed that some 5-amino-4-cyanopyrazole derivatives have antibacterial activity (Spassova et al., 1980). For example, sulfaphenazole, which incorporates a pyrazolyl ring system, is a known potent antibacterial agent (Orth, 1968). Pyrazoles can be synthesized following various synthetic routes and acid hydrazides are either useful intermediates or precursors for their synthesis. Hydrazide and hydrazones are claimed to exhibit appreciable antimicrobial activity (Eisa et al., 1991; Gursoy et al., 1997; Rollas et al., 2002; Vicini et al., 2002). A number of hydrazones have been used in the treatment of several diseases such as tuberculosis, leprosy, and mental disorders and they are also used as herbicides, insecticides, rodenticides, and plant growth regulators (Alcock et al., 1972; Katyal and Dutta, 1975; Toth, 1981; El-Sayed et al., 2010).

Quinazolin-4(*3H*)-ones are another important class of compounds that have widespread and diverse pharmacological activities (Hori et al., 1990; Rewcastle et al., 1998). Quinazolin-4(*3H*)-one ring systems have demonstrated antimalarial, antifungal, antitumor, anticonvulsant, and antihypertensive activities (Vaidya et al., 1983; Witt and Bergman, 2003; Connolly et al., 2005). For example, albaconazole is one antifungal agent containing a quinazoline moiety in its structure. These compounds are also useful in the treatment of Parkinson's disease, depression, epilepsy, and diabetes (Parmer and Singh, 1979).

Nucleosides and their acyclic and *C*-nucleoside analogues possess a wide range of medicinal properties, including antibacterial, antiviral, and antitumor activities (Larsson et al., 1983; Remy and Secrist, 1985; Chu and Cutler, 1986; Holy, 1987; El Ashry and El Kilany, 1996, 1997a, 1997b; Franchetti et al., 1997; Hammerschmidt et al., 1997; Makara and Keseru,

Correspondence to: M. T. Abdel-Aal, Department of Chemistry, Faculty of Science, Menoufia University, Egypt
Tel: 2-048-2235690, Fax: 2-048-2235689
E-mail: mtaha2000_2000@yahoo.com

1997). The therapeutic importance of these ring systems has triggered a renewed interest in the development of new synthetic molecules formed by the combination of a pyrazole and a carbohydrate moiety. An interest in the attachment of carbohydrate moieties to heterocycles and the synthesis of nucleoside analogues has led us to develop new biologically active compounds (Abdel-Aal et al., 2003, 2006, 2008). Therefore, in the present work we aimed to synthesize new substituted pyrazole and pyrimidine derivatives as well as to evaluate their antimicrobial activity.

MATERIALS AND METHODS

Melting points were determined using a Kofler block instrument (BÜCHI Labortechnik AG). ^{1}H-NMR spectra were recorded with a Bruker AC 250 FT-NMR spectrometer (FT-NMR; IBM Instruments Co.) at 250 MHz with TMS as an internal standard. The microanalyses were performed at the Microanalytical Unit at Cairo University, Egypt, and were found to agree favorably with the calculated values. The antimicrobial activity was measured at the National Organization of Drug Control and Research, Cairo, Egypt. The starting compound 2-phenyl-1,3,4-benzoxazinone (**1**) was prepared from the reaction of anthranilic acid with benzoylchloride in the presence of dry pyridine according to a previously reported procedure (Sammour et al., 1971)

2-(Benzoylamino)-*N*-carbonyl-methylbenzoate (2)

A mixture of 2-phenyl-1,3,4-benzoxazinone (**1**) (4.5 g, 20 mmol) and of *p*-aminomethylbenzoate (3.2 g, 20 mmol) in glacial acetic acid (15 mL) was heated under reflux for 6 h, cooled, and then the precipitate was filtered and crystallized from glacial acetic acid to afford 5.61 g of **2** (75% yield) as a white powder, m.p. 220-221°C; IR (KBr) ν cm^{-1}: 1660 (C=O), 1720 (C=O), 3220 (NH); ^{1}H-NMR (DMSO-d_6): δ 3.80 (s, 3H, CH_3), 6.55 (m, 2H, Ar-H), 6.72 (m, 3H, Ar-H), 6.91 (d, 2H, *J* = 8.4 Hz, Ar-H), 6.98 (m, 2H, Ar-H), 7.15 (m, 2H, Ar-H), 7.48 (d, 2H, *J* = 8.4 Hz, Ar-H), 10.82 (s, 1H, NH), 11.35 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 167.54, 165.72, 164.71 (3CO), 143.15-120.01 (Ar-carbons), 51.86 (CH_3). Anal. calcd. for $C_{22}H_{18}N_2O_4$ (374.39): C, 70.58; H, 4.85; N, 7.48. Found: C, 70.27; H, 4.79; N, 7.39%.

2-(Benzoylamino)-*N*-carbonyl-benzoylhydrazide (3)

To a solution of 1.87 g of **2** (3.74 g 10 mmol) in ethanol (20 mL), 2 g of hydrazine hydrate (0.5 g, 10 mmol) was added. The reaction mixture was heated under reflux for 2 h. The solvent was removed under reduced pressure, and the solid residue that formed during evaporation was filtered off, washed with water, dried, and crystallized from ethanol to produce 2.92 g of **3** (78% yield) as white powder, m.p. 240-242°C; IR (KBr) ν cm^{-1}: 1718 (C=O), 1718 (C=O), 1764 (C=O), 3254 (NH); ^{1}H-NMR (DMSO-d_6): δ 4.3 (bs, 2H, NH_2), 6.92 (m, 3H, Ar-3H), 7.32 (d, 2H, Ar-2H), 7.43 (m, 2H, Ar-2H), 7.48 (d, 2H, *J* = 8.4 Hz, Ar-2H), 7.55 (m, 2H, Ar-2H), 7.92 (d, 2H, *J* = 8.4 Hz, Ar-2H), 10.1 (s, 1H, NH), 10.6 (s, 1H, NH), 11.3 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 167.44, 166.63, 164.63 (3CO), 141.05-120.05 (Ar-carbons). Anal. calcd. for $C_{21}H_{18}N_4O_3$ (374.39): C, 67.37; H, 4.85; N, 14.96. Found: C, 67.14; H, 4.78; N, 14.80%.

General procedure for the synthesis of aroylhydrazone derivatives (4a-d)

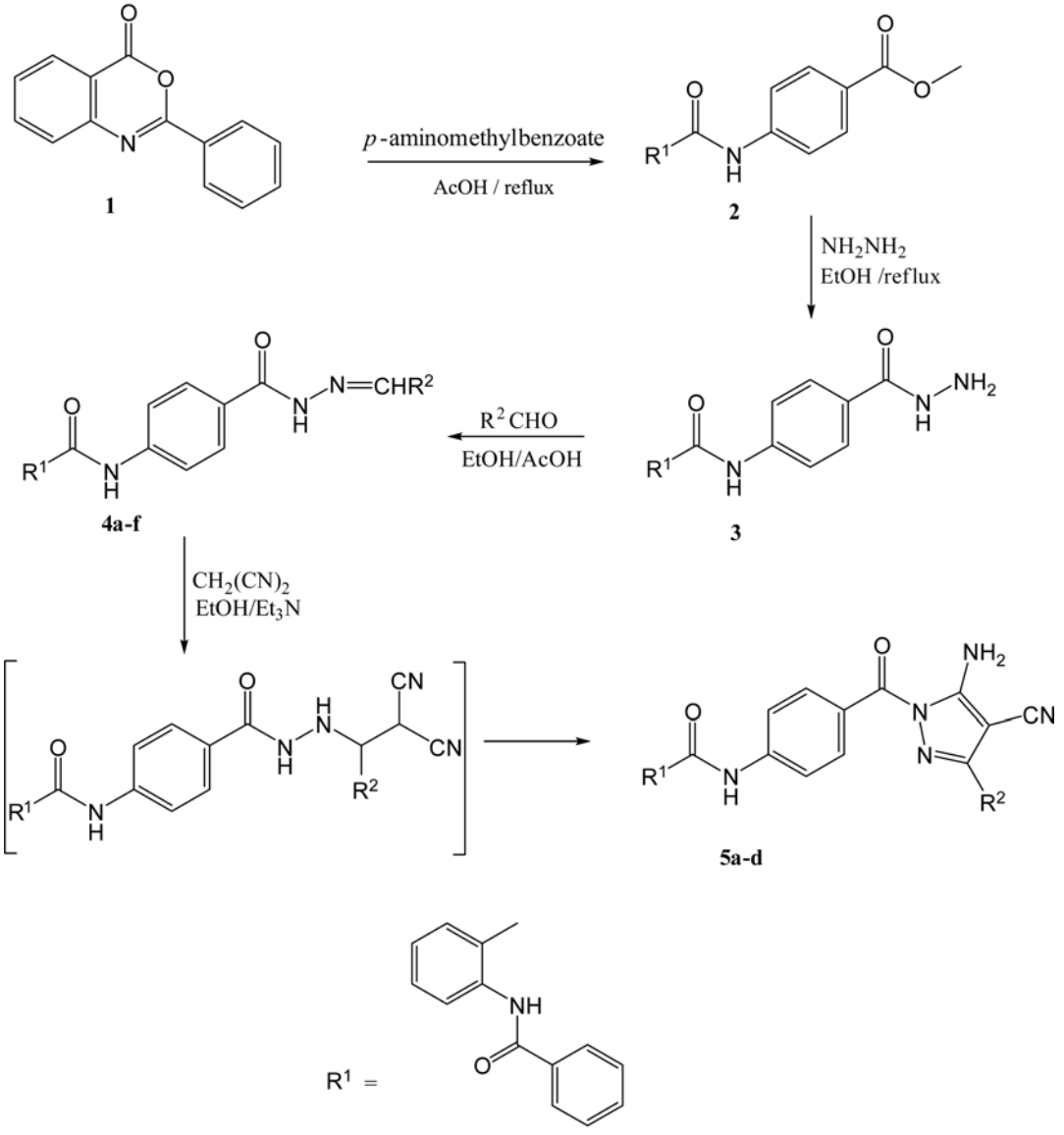
A mixture of hydrazino compound **3** (1.87 g, 5 mmol) and the corresponding aldehyde (thiophene-2-carboxaldehyde, furan-2-carboxaldehyde, D-glucose, D-xylose, D-galactose or D-arabinose) (5 mmol) in ethanol (20 mL) with a catalytic amount of glacial acetic acid was heated under reflux for 2 h. After concentration and cooling, the separated precipitate was filtered off, dried, and finally recrystallized from ethanol to afford the corresponding aroylhydrazone derivatives **4a-f** (77-80% yields)

2-(Benzoylamino)-*N*-[4-(2-(thiophen-2-ylmethylene) hydrazine)-carbonyl]phenylbenzamide (4a)

White powder (1.87 g, 80%), m.p. 250-252°C; IR (KBr) ν cm^{-1}: 1670 (C=O), 3250 (NH); ^{1}H-NMR (DMSO-d_6): δ 6.82 (m, 4H, Ar-H), 6.94 (d, 2H, *J* = 7.4 Hz, Ar-H), 7.05 (m, 4H, Ar-H), 7.36 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.45 (m, 3H, Ar-H), 7.98 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.64 (s, 1H, N=C*H*), 10.73 (s, NH), 11.42 (s, 1H, NH), 11.75 (s, NH); ^{13}C-NMR (DMSO-d_6): δ 167.50, 164.66, 162.34 (3C=O), 142.59 (C=N), 141.74-120.10 (Ar-carbons). Anal. calcd. for $C_{26}H_{20}N_4O_3S$ (468.53): C, 66.65; H, 4.30; N, 11.96. Found: C, 66.52; H, 4.22; N, 11.85%.

2-(Benzoylamino)-*N*-[4-[2-(furan-2-ylmethylene) hydrazino]-carbonyl]phenylbenzamide (4b)

White powder (1.74 g, 77%), m.p. 230-232°C; IR (KBr) ν cm^{-1}: 1659 (C=O), 1716 (C=O), 3285 (NH); ^{1}H-NMR (DMSO-d_6): δ 6.91 (m, 3H, Ar-H), 6.93 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.12 (m, 1H, Ar-H), 7.54 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.77 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.81 (m, 2H, Ar-H), 7.81 (m, 2H, Ar-H), 7.88 (d, 2H, *J* = 8.4 Hz, Ar-H), 8.05 (d, 2H, *J* = 8.4 Hz, Ar-H), 8.34 (s, 1H, N=C*H*), 10.55 (s, 1H, NH), 11.26 (s, 1H, NH), 11.62 (s, 1H, NH). Anal. calcd. for $C_{26}H_{20}N_4O_4$ (452.46): C, 69.02; H,



4	R^2
a	thiophen-2-yl
b	furan-2-yl
c	D-glucopentitolyl
d	D-xylotetrititolyl
e	D-galactopentitolyl
f	D-arabinotetritolyl

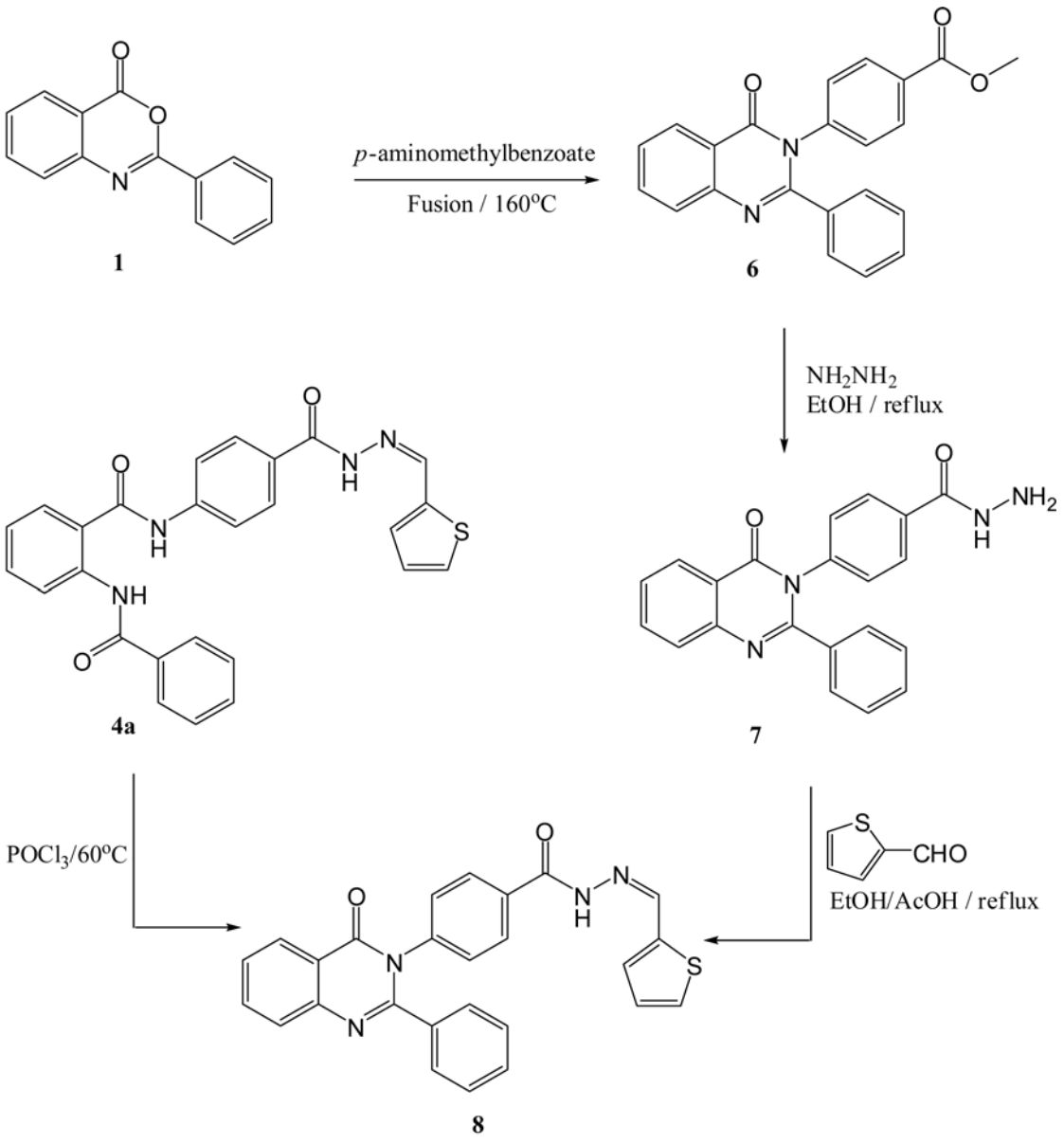
5	R^2
a	thiophen-2-yl
b	furan-2-yl
c	D-glucopentitolyl
d	D-xylotetrititolyl

Scheme 1

4.46; N, 12.38. Found: C, 70.14; H, 4.59; N, 12.21%.

D-Glucose-2-(benzoylamino)-*N*-phenylbenzamide-4-carbonyl-hydrazone (4c)

Yellow powder (2.09 g, 78%), m.p. 210-211°C; IR (KBr) ν cm^{-1}: 1960 (C=O), 3250 (NH), 3270 (OH); ^{1}H-NMR (DMSO-d_6): δ 3.41 (m, 2H, H-6, H-6), 3.63 (m, 1H, H-5), 3.75 (m, 2H, H-3,4), 3.86 (m, 1H, H-2), 4.27 (m, 2H, 2OH), 5.13 (d, 1H, J = 4.6 Hz, OH), 5.13 (t, 1H, J = 5.2 Hz, OH), 5.13 (m, 1H, OH), 6.95 (m, 3H, Ar-H), 7.49 (d, 2H, J = 8.4 Hz, Ar-H), 7.53 (d, 2H, J = 8.4 Hz, Ar-H), 7.85 (m, 2H, Ar-H), 7.88 (m, 2H, Ar-H), 7.89 (d, 1H, J = 8.2 Hz, H-1), 8.12 (d, 2H, J = 8.4 Hz, Ar-H), 10.52 (s, 1H, NH), 11.22 (s, 1H, NH), 11.65 (s,



Scheme 2

1H, NH). Anal. calcd. for $C_{27}H_{28}N_4O_8$ (536.53): C, 60.44; H, 5.26; N, 10.44. Found: C, 60.31; H, 5.18; N, 10.38%.

D-Xylose-2-(benzoylamino)-*N*-phenylbenzamide-4-carbonyl-hydrazone (4d)

White powder (1.95 g, 77%), m.p. 215-217°C; IR (KBr) ν cm^{-1}: 1690 (C=O), 3220 (NH), 3250 (OH); ^{1}H-NMR (DMSO-d_6): δ 3.44 (m, 2H, H-5, H-5), 3.75 (m, 2H, H-3,4), 3.83 (m, 1H, H-2), 4.25 (m, 1H, OH), 5.13 (d, 1H, J = 5.6 Hz, OH), 5.13 (t, 1H, J = 5.8 Hz, OH), 5.17 (m, 1H, OH), 6.55 (m, 3H, Ar-H), 6.88 (d, 1H, J = 7.8 Hz, H-1), 7.56 (d, 2H, Ar-H), 7.62 (d, 2H, J = 8.4 Hz, Ar-H), 7.71 (m, 2H, Ar-H), 7.82 (m, 2H, Ar-H), 8.43 (d, 2H, J = 8.4 Hz, Ar-H), 9.92 (s, 1H, NH), 10.63 (s, 1H, NH), 11.75 (s, 1H, NH). Anal. calcd. for $C_{26}H_{26}N_4O_7$ (506.18): C, 61.65; H, 5.17; N, 11.06. Found: C, 61.57; H, 5.19; N, 10.96%.

D-Galactose-2-(benzoylamino)-*N*-phenylbenzamide-4-carbonyl-hydrazone (4e)

White powder (2.12 g, 79%), m.p. 222-223°C; IR (KBr) ν cm^{-1}: 1690 (C=O), 3310 (NH), 3335 (NH); ^{1}H-NMR (DMSO-d_6): δ 3.45 (m, 2H, H-6, H-6), 3.68 (m, 1H, H-5), 3.71 (m, 2H, H-3,4), 3.82 (m, 1H, H-2), 4.21 (m, 2H, 2OH), 5.13 (d, 1H, J = 6.8 Hz, OH), 5.17 (t, 1H, J = 5.8 Hz, OH), 5.17 (m, OH), 6.78 (m, 3H, Ar-H), 6.85 (m, 2H, Ar-H), 7.51 (d, 2H, J = 8.4 Hz, Ar-H), 7.85 (m, 2H, Ar-H), 7.87 (m, 2H, Ar-H), 7.89 (d, 1H, J

= 8.5 Hz, H-1), 8.15 (d, 2H, *J* = 8.4 Hz, Ar-H), 10.52 (s, 1H, NH), 11.25 (s, 1H, NH), 11.66 (s, 1H, NH). Anal. calcd. for $C_{27}H_{28}N_4O_8$ (536.53): C, 60.44; H, 5.26; N, 10.44. Found: C, 60.29; H, 5.16; N, 10.36%.

D-Arabinose-2-(benzoylamino)-*N*-phenylbenzamide-4-carbonyl-hydrazone (4f)

White powder (1.97 g, 78%), m.p. 205-207°C; IR (KBr) ν cm^{-1}: 1690 (C=O), 3290 (NH), 3370 (NH); ^{1}H-NMR (DMSO-d_6): δ 3.39 (m, 2H, H-5, H-5), 3.88 (m, 2H, H-3,4), 3.91 (m, 1H, H-2), 4.35 (m, 1H, OH), 5.25 (d, 1H, *J* = 6.8 Hz, OH), 5.39 (t, 1H, *J* = 5.8 Hz, OH), 5.41 (m, 1H, OH), 6.95 (m, 3H, Ar-H), 7.14 (d, 1H, *J* = 8.2 Hz, H-1), 7.51 (m, 2H, Ar-H), 8.66 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.82 (m, 2H, Ar-H), 7.85 (m, 2H, Ar-H), 8.15 (d, 2H, *J* = 8.4 Hz, Ar-H), 9.96 (s, 1H, NH), 10.63 (s, 1H, NH), 11.75 (s, 1H, NH). Anal. calcd. for $C_{26}H_{26}N_4O_7$ (506.18): C, 61.65; H, 5.17; N, 11.06. Found: C, 61.54; H, 5.12; N, 10.95%.

General Procedure for the synthesis of substituted 5-amino-4-cyanopyrazole derivatives (5a-d)

To a solution of aroylhydrazone **4a-d** (1 mmol) and malononitrile (0.07 g, 1 mmol) in absolute ethanol (10 mL) a catalytic amount of triethylamine was added and then the mixture was heated under reflux for 6 h. The excess of solvent was removed by vacuum evaporation, and the residue was chromatographed on a silica gel using chloroform/methanol (90:10, v/v) as eluent to give 5-amino-4-cyanopyrazole derivatives **5a-d** in 72-78% yields.

2-(Benzoylamino)-*N*-[4-(5-amino-4-cyano-3-thiophen-2-yl-1*H*-pyrazol-1-yl)carbonylphenyl]benzamide (5a)

White powder (0.4 g, 75%), m.p. 265-266°C; IR (KBr) ν cm^{-1}: 1664 (C=O), 2220 (CN), 3430 (NH), 3457 (NH_2); ^{1}H-NMR (DMSO-d_6): δ 5.12 (s, 2H, NH_2), 6.72 (m, 3H, Ar-H), 6.95 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.38 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.44 (m, 3H, Ar-H), 7.49 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.86 (m, 2H, Ar-H), 7.88 (m, 2H, Ar-H), 8.05 (d, 2H, *J* = 8.4 Hz, Ar-H), 11.78 (s, 1H, NH). Anal. calcd. for $C_{29}H_{20}N_6O_3S$ (532.57): C, 65.40; H, 3.79; N, 15.78. Found: C, 65.22; H, 3.65; N, 15.59%.

2-(Benzoylamino)-*N*-[4-(5-amino-4-cyano-3-furan-2-yl-1*H*-pyrazol-1-yl)carbonylphenyl]benzamide (5b)

White powder (0.38 g, 74%), m.p. 245-246°C; IR (KBr) ν cm^{-1}: 1660 (C=O), 2260 (CN), 3280 (NH), 3390 (NH_2); ^{1}H-NMR (DMSO-d_6): δ 4.85 (s, 2H, NH_2), 6.9 (m, 3H, Ar-H), 6.97 (d, 1H, *J* = 8.2 Hz, Ar-H), 7.58 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.65 (m, 3H, Ar-H), 7.72 (m, 1H, Ar-H), 7.85 (m, 2H, Ar-H), 7.88 (m, 2H, Ar-H), 8.16 (d, 2H, *J* = 8.4 Hz, Ar-H), 10.67 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 118.45-148.32 (Ar-carbons), 167.24, 165.23, 164.53 (3C=O), 155.25 (CN). Analysis calcd. for $C_{29}H_{20}N_6O_4$ (516.51): C, 67.44; H, 3.90; N, 16.27. Found: C, 67.21; H, 3.79; N, 16.15%.

2-(Benzoylamino)-*N*-[4-(5-amino-4-cyano-3-glucosyl-1*H*-pyrazol-1-yl)carbonylphenyl]benzamide (5c)

White powder (0.47 g, 78%), m.p. 219-220°C; IR (KBr) ν cm^{-1}: 1690 (C=O), 2280 (CN), 3285 (NH), 3390 (OH); ^{1}H-NMR (DMSO-d_6): δ 3.48 (m, 2H, H-5, H-5), 3.55 (m, 1H, H-4), 3.72 (m, 2H, H-2,3), 3.89-4.12 (m, 2H, 2OH), 4.87 (s, 2H, NH_2), 5.13 (d, 1H, *J* = 5.4 OH), 5.13 (t, 1H, *J* = 5.6 Hz, OH), 5.18 (m, 1H, H-4), 5.89 (d, 1H, *J* = 7.5 Hz, H-1), 6.92 (m, 3H, Ar-H), 7.15 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.75 (m, 2H, Ar-H), 7.82 (m, 2H, Ar-H), 7.85 (m, 2H, Ar-H), 8.16 (d, 2H, *J* = 8.4 Hz, Ar-H), 10.88 (s, 1H, NH). Anal. calcd. for $C_{30}H_{28}N_6O_8$ (600.58): C, 60.00; H, 4.70; N, 13.00. Found: C, 60.21; H, 4.79; N, 12.89%.

2-(Benzoylamino)-*N*-[4-(5-amino-4-cyano-3-xylosyl-1*H*-pyrazol-1-yl)carbonylphenyl]benzamide (5d)

White powder (0.41 g, 72%) m.p. 220-221°C; IR (KBr) ν cm^{-1}: 1680 (C=O), 2250 (CN), 3370 (NH), 3420 (OH); ^{1}H-NMR (DMSO-d_6): δ 3.39 (m, 2H, H-4, H-4), 3.87 (m, 2H, H-2,3), 4.47 (m, 1H, OH), 4.85 (s, 2H, NH_2), 4.95 (m, 1H, H-1), 5.18 (d, 1H, *J* = 5.6 Hz, OH), 5.54 (t, 1H, *J* = 6.4 Hz, OH), 5.76 (m, 1H, OH), 6.93 (m, 3H, Ar-H), 7.76 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.80 (m, 2H, Ar-H), 7.91 (m, 2H, Ar-H), 8.56 (d, 2H, *J* = 8.4 Hz, Ar-H), 10.39 (s, 1H, NH). Anal. calcd. for $C_{29}H_{26}N_6O_7$ (570.55): C, 61.05; H, 4.59; N, 14.73. Found: C, 61.27; H, 4.71; N, 14.59%.

4-[4-Oxoquinazolin-2-phenyl-3(4*H*)-yl]-*N*-methylbenzoate (6)

A mixture of 2-phenyl-1,3,4-benzoxazinone (**1**) (2.23 g, 10 mmol) and *p*-aminomethylbenzoate (1.51 g, 10 mmol) was fused at 160°C for 1.5 h, then the temperature was elevated to 220°C until the evolution of water vapor ceased. The reaction mixture was left to cool, a few drops of methanol were added, and it was left to stand overnight. The separated solid was successively purified using column chromatography (methylene chloride/methanol) to afford 2.67 g of **6** (75%) as a white powder, m.p. 170-171°C; IR (KBr) ν cm^{-1}: 1675 (C=O), 1748 (C=O); ^{1}H-NMR (DMSO-d_6): δ 3.95 (s, 3H, CH_3), 6.66 (m, 2H, Ar-H), 6.78 (m, 3H, Ar-H), 7.80 (m, 2H, Ar-H), 6.92 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.18 (m, 2H,

Ar-H), 7.45 (d, 2H, *J* = 8.4 Hz, Ar-H). Anal. calcd. for $C_{22}H_{16}N_2O_3$ (356.37): C, 74.15; H, 4.53; N, 7.86. Found: C, 74.11; H, 4.48; N, 7.69%.

4-[4-Oxoquinazolin-2-phenyl-3(4*H*)-yl]-*N*-benzoylhydrazide (7)

Hydrazine hydrate (0.5 g, 10 mmol) was added to a solution of compound **6** (3.56 g, 10 mmol) in ethanol (20 mL). The reaction mixture was heated under reflux for 2 h. The solvent was evaporated under a vacuum; the resulting solid was filtered off, washed with water, and then recrystallized from ethanol to afford 2.67 g (75%) of hydrazide **7** as a white powder, m.p. 210-211ºC; IR (KBr) ν cm^{-1}: 1560 (C=O), 1720 (C=O), 3420 (NH); ^{1}H-NMR (DMSO-d_6): δ 3.95 (s, 3H, CH_3), 4.88 (s, 2H, NH_2), 6.66 (m, 2H, Ar-H), 6.81 (m, 3H, Ar-H), 6.95 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.15 (m, 2H, Ar-H), 7.19 (m, 2H, Ar-H), 7.56 (d, 2H, *J* = 8.4 Hz, Ar-H), 12.55 (s, 1H, NH). Anal. calcd. for $C_{21}H_{16}N_4O_2$: C, 70.77; H, 4.53; N, 15.72. Found: C, 70.57; H, 4.42; N, 15.40%.

4-[4-Oxoquinazolin-2-phenyl-3(4*H*)-yl]-*N*-[thiophen-2-ylmethylene]-benzohydrazone (8)

Method A

To a solution of hydrazide **7** (1.78 g, 5 mmol) and thiophene-2-carboxaldehyde (0.56 g, 5 mmol) in ethanol (20 mL) glacial acetic acid (1 mL) was added. The mixture was heated under reflux for 2 h and then concentrated under reduced pressure. The separated solid product was filtered off and crystallized from ethanol to give 1.57 g of **8** (70% yield)

Method B

Aroylhydrazone **4a** (2.34 g, 5 mmol) was added portion wise to phosphorous oxychloride (10 mL) at 0ºC with stirring for 1 h. The temperature was elevated gradually to 60ºC and kept at this temperature for 2 h. After cooling, the reaction mixture was poured onto crushed ice and the solid product was filtered off, washed with 5% NH_4OH and water, and then crystallized from ethanol to afford 1.53 g of **8** (68% yield) as a white powder, m.p. 223-225ºC; IR (KBr) ν cm^{-1}: 1684 (C=O), 3409 (NH); ^{1}H-NMR (DMSO-d_6): δ 3.95 (s, 3H, CH_3), 4.88 (s, 2H, NH_2), 6.66 (m, 2H, Ar-H), 6.81 (m, 3H, Ar-H), 6.95 (d, 2H, *J* = 8.4 Hz, Ar-H), 6.97 (d, 1H, *J* = 8.2 Hz, Ar-H), 7.31 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.45 (d, 1H, Ar-H), 7.49 (m, 3H, Ar-H), 7.56 (d, 2H, *J* = 8.4 Hz, Ar-H), 8.25 (s, 1H, N=C*H*), 12.5 (s, 1H, NH). Anal. calcd. for $C_{26}H_{18}N_4O_2S$ (450.51): C, 69.32; H, 4.03; N, 12.44. Found: C, 69.17; H, 3.91; N, 12.32%.

Antimicrobial activity

Growth media

Nutrient agar medium used in the evaluation of antibacterial activity consisted of (g/L): peptone 5.0 g, beef extract 3.0 g, sodium chloride 5.0 g, glucose 10.0 g, agar 18.0 g and water 1000 mL.

Sabouraud Dextrose agar medium used in the evaluation of antifungal activity consisted of (g/L): mycological peptone 10.0 g, glucose 40.0 g. Agar 15.09 and water 1000 mL with pH 5.0. Yeast extract medium used to measure anti-yeast activity consisted of (g/L): yeast extract 400 g, malt extract 10.0 g, glucose 4.0 g and water 1000 mL with pH 7.3. All media were autoclaved in 121ºC for 30 min under 1.56 atp.

The antimicrobial activities of the compounds were tested using the disc plate technique as described in British Pharmacopeia (1968). The specific media used for each test organism was melted, cooled to 45ºC, and then incubated with 1 mL of the bacteria, fungi. The flask was shaken well and the suspension was poured into a 10 cm Petri dish. Five 6 mm disks were dipped in different concentrations of compound solution (10, 100, 1000 μg), the solvent was allowed to evaporate, and the disks were placed upon the surface incubated with medium, which were kept in a refrigerator for 1 h to permit diffusion of the antimicrobial substance. The plates were incubated at 37ºC for 24 h for the bacteria and 28ºC for the fungi and yeast. The clear zone of inhibition was measured in mm, for each test the main values of inhibition zones were read in triplicate and the calculated results were classified as follows:

No clearing zone: inactive
Small clearing zone: slightly active 12 mm
Medium clearing zone: moderately active 18 mm
Large clearing zone: highly active 30 mm

RESULTS AND DISCUSSION

The reaction of the benzoxazinone derivative **1** with *p*-aminomethylbenzoate in acetic acid at reflux temperature did not give the expected oxaquinazoline derivative; instead 2-benzoylamino-*N*-carbonylmethylbenzoate (**2**) was formed. The IR spectrum showed two characteristic absorption bands in the carbonyl frequency region at 1660 and 1720 cm^{-1}, which correspond to the carbonyl amide and ester groups, respectively. The ^{1}H-NMR spectrum showed peaks corresponding to a methyl group, the NH groups, and the aromatic protons. The ^{13}C-NMR spectrum showed a methyl ester peak in addition to the aromatic carbons. Treatment of 2-benzoylamino-*N*-carbonylmethylbenzoate (**2**) with hydrazine hydrate in ethanol at reflux tem-

perature gave 2-benzoylamino-*N*-carbonylbenzoylhydrazide (**3**). The reaction of the benzoylhydrazide derivative (**3**) with thiophene-2-carboxaldehyde, furan-2-carboxaldehyde, D-glucose, D-arabinose, D-xylose, and D-galactose in ethanol and a catalytic amount of glacial acetic acid afforded the corresponding hydrazones (**4a-f**). The structures of the hydrazones (**4a-f**) were well characterized by the spectral data of IR, ^{1}H-NMR, ^{13}C-NMR, and elemental analysis. The assignment of NH and OH groups in these compounds was determined by D_2O exchange. The IR spectra of the sugar hydrazones (**4c-f**) showed that the hydroxyl groups of the sugar moiety were in the range of 3250-3420 cm^{-1}. The ^{1}H-NMR spectra of these compounds showed that the sugar protons were in the range of δ 3.45-5.65 and that H-1 was in the range of δ 7.65-8.11 ppm. In the reaction of the 5-amino-4-aroylhydrazone derivatives (**4a-f**) with malononitrile in ethanol the corresponding 5-amino-4-cyano-3-arylpyrazole derivatives (**5a-d**) were obtained after column chromatography purification. The IR spectra of these compounds showed a characteristic absorption band corresponding to the CN group as well as the carbonyl group band. The ^{1}H and ^{13}C spectra of these compounds were in full agreement with their assigned structures.

In order to obtain the desired quinazoline derivative from the reaction of the benzoxazinone derivative (**1**) with *p*-aminomethylbenzoate we modified the experimental conditions. Interestingly, the reaction of compound **1** with *p*-aminomethylbenzoate in an oil bath at 160ºC afforded 4-(4-oxoquinazolin-2-phenyl-3(*4H*)-yl)-*N*-methylbenzoate (**6**). The ^{1}H-NMR spectrum of this compound showed the protons of the methyl ester at δ 4.14 ppm and aromatic protons in the range of δ 6.15-7.15 ppm. Hydrazinolysis of the quinazoline methyl ester derivative (**7**) in refluxing ethanol gave the hydrazide compound **7**. The ^{1}H-NMR spectrum of this acid hydrazide showed the presence of a peak corresponding to the NH_2 at δ 4.7 ppm as well as aromatic protons in the range of δ 7.1-8.6 ppm. The reaction of the hydrazide (**7**) with thiophene-2-carboxaldehyde in ethanol in the presence of few drops of glacial acetic acid produced the hydrazone compound **8**. In addition, compound **8** was also successfully obtained by stirring compound **4a** with phosphorous oxychloride at 60ºC. The structure of the hydrazone derivative **8** synthesized by these two different methods was confirmed by IR, ^{1}H-NMR spectra, and elemental analysis, which were in agreement with the assigned structure.

Table I. Antibacterial activity of newly synthesized compounds

Compound	Gram negative bacteria						Gram positive bacteria					
	Salmonella			*Escherichia coli*			*Staphylococcus aureus*			*Bacillus subtilis*		
	C^c	C^b	C^a	C^c	C^b	C^a	C^c	C^b	C^a	C^c	C^b	C^a
2	+	+	-	+	+	-	-	-	-	-	-	-
3	++	+	+	++	+	+	++	+	-	++	+	-
4a	++	+	+	++	+	+	+	-	-	++	-	-
4b	++	+	-	++	-	-	++	+	+	++	+	+
4c	+	+	+	+++	++	+	+++	+++	++	+++	+++	++
4d	++	+	+	++	+	+	++	+	+	++	+	+
4e	++	+	+	++	+	+	++	+	+	++	+	+
4f	+	-	+	+	+	+	+	+	+	+	+	+
5a	+	-	+	+	-	-	+++	+	+	+++	+	+
5b	++	+	+	++	+	+	++	+	+	++	+	+
5c	++	+	-	++	+	-	++	-	-	++	-	-
5d	+	+	-	+++	++	-	+	-	-	++	-	-
5e	++	+	+	++	+	+	++	-	+	++	-	+
5f	+	+	-	+	+	-	+	-	-	+	-	-
6	++	+	+	++	+	+	++	+	+	++	+	+
7	-	+	+	+	+	+	+	-	-	+	-	-
8	++	+	-	++	+	+	++	+	+	++	+	+
Sulfaphenazole	+++	+++	++	+++	+++	+++	+++	+++	++	+++	+++	++

C^a Activity of synthesized compounds in concentration of 10 ppm.
C^b Activity of synthesized compounds in concentration of 100 ppm.
C^c Activity of synthesized compounds in concentration of 1000 ppm.
- No antimicrobial effect
+ Low antimicrobial effect (4 mm)
++ Moderate antimicrobial effect (8-10 mm)
+++ High antimicrobial effect (15-18 mm)

Antimicrobial activity

The newly synthesized compounds were tested for their antimicrobial action against Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), Gram negative bacteria (*Escherichia coli* and *Salmonella*), two fungal strains (*Aspergillus flavus* and *Aspergillus niger*), and one yeast strain (*Candida albicans*). Most of the synthesized products showed high bactericidal, fungicidal, and anti-yeast activity. The antibacterial and antifungal activities were studied using sulfaphenazole and albaconazole as positive controls, respectively.

The results of antimicrobial screening revealed that compound **4c** showed the highest activity against both types of bacteria (Table I and II). Compounds **3**, **4a**, **4c**, **4d**, and **4f** were found to be active against both Gram positive and Gram negative bacteria, whereas compound **5a** was only active against Gram positive bacteria and compound **2** was only active against Gram negative bacteria. Most of the synthesized compounds did not show activity against fungi or yeast except for compounds **5c**, **5d** and **8** that showed a wide spectrum of activity, especially at concentrations of 100 and 1000 ppm.

Table II. Antifungal activity of newly synthesized compounds

Compound	Fungi					
	Candida albicans		*Aspergillus niger*		*Aspergillus flavus*	
	C^b	C^a	C^b	C^a	C^b	C^a
2	-	-	-	-	-	-
3	-	-	-	-	-	-
4a	-	-	-	-	-	-
4b	-	-	-	-	-	-
4c	-	-	-	-	-	-
4d	-	-	-	-	-	-
4e	-	-	-	-	-	-
4f	-	-	-	-	-	-
5a	-	-	-	-	-	-
5b	-	-	-	-	-	-
5c	++	+	++	+	++	+
5d	++	+	++	+	++	+
5e	-	-	-	-	-	-
5f	-	-	-	-	-	-
6	-	-	-	-	-	-
7	-	-	-	-	-	-
8	++	+	++	+	++	+
Albaconazole	++	+++	+++	++	+++	++

C^a Activity of synthesized compounds in concentration of 100 ppm.
C^b Activity of synthesized compounds in concentration of 1000 ppm.
- No antimicrobial effect
+ Low antimicrobial effect (4 mm)
++ Moderate antimicrobial effect (8-10 mm)
+++ High antimicrobial effect (15-18 mm)

These results and the structure activity relationship suggest that the introduction of a sugar moiety as well as a pyrazolyl ring into the quinazolin-4(*3H*)-one structure enhances the antimicrobial activity against both Gram positive and Gram negative bacteria even at the lower concentrations. Both the acyclic nucleoside analogous **5c** and **5d** of the substituted pyrazole nucleobase displayed high inhibition activity against Gram negative bacteria (*Escherichia coli*). The most effective derivatives have the carbon ring substituted with a methyl or methoxy group. Consequently, it is expected that they could be the basis for further research.

In conclusion, new substituted aroylhydrazones, 5-amino-4-cyano-1*H*-pyrazole, and *N*-substituted quinazoline-4-one derivatives were synthesized and evaluated for their antimicrobial activity. Compounds incorporating a sugar moiety as well as a pyrazolyl ring in their structure displayed high antimicrobial activity against both Gram positive and Gram negative bacteria.

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