A convenient route to 5-aminopyrazole, bispyrazole and pyrazolo[1,5-α]pyrimidines incorporating antipyrine or furan moiety as potent antimicrobial agents

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ABSTRACT

Development of new antimicrobial agents is a good solution to overcome drug-resistance problems. In this context, (E)-3-(anthracen-9-yl)-2-cyano-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)acrylamide (3) was synthesized and allowed to react with hydrazines to afford 5-aminopyrazoles 4a,b. Also, an efficient three-component, two-step synthesis of 5-aminopyrazoles 7 was reported, via reaction of 2-cyano-N-(furan-2-ylmethyl)acetamide (2b), phenyl isothiocyanate and dimethylsulphate to produce the ketene N,S-acetal 6, which reacted with hydrazine hydrate to furnish 5-aminopyrazole 7. The latter was allowed to react with some electrophilic reagents and many 5-(benzylideneamino)pyrazole 8a,b, bispyrazole 9 and pyrazolo[1,5-α]pyrimidines 12, 17, 20, 22, 25, 26, 28 and 29 were obtained. The synthesized compounds were evaluated for their in vitro antibacterial and antifungal activities. Among the synthesized compounds, bispyrazole 9 showed equipotent to ampicillin and gentamycin against both of S. epidermidis (MIC 0.49 µ/mL), B. subtilis (MIC 0.24 µ/mL), P. vulgaris (MIC 0.98 µ/mL) and K. pneumonia (MIC 0.49 µ/mL), and displayed equipotent to amphotericin B versus A. clavatus (MIC 0.98 µ/mL). Azomethine derivatives 8a,b were equipotent to amphotericin B in inhibiting the growth of A. clavatus (MIC 0.98 µ/mL). Pyrazolo[1,5-α]pyrimidine 17 was equipotent to amphotericin B in inhibiting the growth of G. candidium (MIC 0.49 µ/mL). Structures of the new synthesized compounds were established by elemental analysis and spectral data.

Keywords: aminopyrazole; azomethine; bispyrazoles; pyrazolo[1,5-α]pyrimidines; antibacterial; antifungal.

INTRODUCTION

Several pyrazole derivatives received great attention due to their biological and pharmacological activities; not only as potential inhibitors of HIV-[1], pesticides [2], fungicides [3], antihypertensive agents [4], and anticancer activity [5], but they are also important and useful starting materials for the synthesis of other fused heterocyclic systems. The synthesis of pyrazolo[1,5-α]pyrimidine and their derivatives have attracted attention due to their interesting pharmacological properties[6-10]. Also, many biologically important derivatives of furan substituted at 2- and 5-positions are frequently observed in nature. These furan derivatives show broad-spectrum phytocidal, antibacterial and insecticidal activities [11,12] and exhibit pharmacological properties which include serving as antidepressant and anti-inflammatory agents [13,14]. Furthermore, Antipyrine derivatives are well known compounds used mainly as analgesic, antipyretic drugs, antitumoragen, antivirus, anticancer and radiosensitizing agent [15-17]. One of the best known antipyrine derivatives is 4-aminoantipyrine (4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one), which presents a structural similarity with metamizole, a well-known and very effective analgesic, anti-inflammatory agent [18]. In view of the above facts and in continuation of our research Program [19-26] directed towards the development of a new, simple and less toxic antimicrobial agents. It seems of considerable interest to synthesize newly pyrazole and pyrazolo[1,5-α]pyrimidine derivatives bearing antipyrine or furan moiety. Additionally, my
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Der Pharma Chemica, 2016, 8 (4):363-376

target is also to study the antimicrobial activities of the synthesized compounds, hoping to add some synergistic biological significance to the target molecules.

MATERIALS AND METHODS

All chemicals and solvents were commercially available and used without purification. Melting points were determined by open glass capillary method on a Cintex melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer spectrometer in KBr pellets. 1H NMR and 13C NMR spectra were obtained on a Varian Gemini 300 MHz spectrometer using TMS as internal standard; chemical shifts are reported as (ppm). Mass spectra were obtained on GCMS/QP 1000 Ex mass spectrometer at 70 ev. Elemental analyses were performed on a LECO-932 analyzer at the Department of Chemistry, Faculty of Science, Cairo University, Egypt. Microbiology screening was carried out in the Regional Center for Microbiology and Biotechnology (RCMB), Antimicrobial unit test organisms, Al-Azhar University, Cairo, Egypt.

Chemistry

Preparation of acrylamides 3 and 6

E-3-(anthracen-9-yl)-2-cyano-N(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)acrylamide (3)

A mixture of compound 2a (0.01 mol), anthracene-9-carbaldehyde (0.01 mol) and piperidine (0.5 ml) in ethanol (30 ml) was heated under reflux for 3 hrs; then it was poured into ice/water and acidified with 0.1 N HCl at pH 3-4. After the reaction was stirred at room temperature for 3 hrs, then a mixture of dimethylsulfate (0.01 mol) and stirred at room temperature for an additional 6 hrs. Then it was poured into ice/water and acidified with 0.1 N HCl at pH 3-4.; the resulting precipitate was filtered off, dried and recrystallized from ethanol as white crystals. Yield 70%; m.p 90-92 ºC; Anal. Calcd for C35H20N2O5S: C, 61.32; H, 4.82; N, 13.41. Found: C, 61.28; H, 4.88; N, 13.14.

Preparation of pyrazoles 4a, 4b, and 7: General procedure:

A mixture of acrylamide 3a or 6 (0.01 mol) and hydrazines (namely; hydrazine hydrate and / or phenyl hydrazine) (0.01 mol) in ethanol (30 ml) was heated under reflux for 3 hrs. Then it was poured into ice/water and acidified with 0.1 N HCl at pH 3-4.; the resulting precipitate was filtered off, dried and recrystallized from the proper solvent.

5-Amino-3-(anthracen-9-yl)-N(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-1H-pyrazole-4-carboxamide (4a)

Yield 55%; orange solid (MeOH); mp 268-270ºC; 1HNMR (300 MHz, DMSO-d6) δ = 2.30, 3.42 (2s, 6H, 2CH3), 7.01 (s, 2H, NH2; C6H5-NH-C6H5); C-H and NO2 exchanged with D2O; 7.35-9.18 (m, 14H, Ar-H), 9.44, 10.07 (2s, 2H, NH2); C-H and NO2 exchanged with D2O; IR (KBr, cm⁻1): 3427 (br, NH2), 3047 (arom.CH) and 1625 (C=O; amide); MS: m/z 488 (M⁺, 100%).

5-Amino-3-(anthracen-9-yl)-N(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-1-phenyl-1H-pyrazole-4-carboxamide (4b)

Yield 53%; orange solid (AcOH); mp 199-200 ºC; 1HNMR (300 MHz, DMSO-d6) δ = 2.31, 3.15 (2s, 6H, 2CH3), 6.70 (s, 2H, NH2; C6H5-NH-C6H5); C-H and NO2 exchanged with D2O; 7.35-9.18 (m, 19H, Ar-H), 10.66 (s, H, NH; exchangeable with D2O); IR (KBr, cm⁻1): 3430, 3300 (br, NH2-NH-C6H5), 3047 (arom.CH) and 1625 (C=O; amide); MS: m/z 564 (M⁺, 25.4%), 458 (100%).

5-Amino-N-(furan-2-ylmethyl)-3-(phenylamino)-1H-pyrazole-4-carboxamide (7)

Yield 75%; white solid (MeOH); mp 98-100 ºC; 1HNMR (300 MHz, DMSO-d6) δ = 4.41 (d, 2H, CH2-N, J = 6 Hz), 5.84 (s, 2H, NH2; exchangeable with D2O), 6.18 (d, 1H, furan-H3, J = 3 Hz), 6.36 (t, 1H, furan-H4, J = 3 Hz), 6.72-
was heated under reflux for 3 hrs; the solid product which produced by heating was filtered off, dried and recrystallized from the proper solvent.

5-(2-Chlorobenzylideneamino)-N-(furan-2-ylmethyl)-3-(phenylamino)-1H-pyrazole-4-carboxamide (8a)
Yield 55%; orange solid (Dioxane); mp 200-202 °C; 1HNMR (300 MHz, DMSO-d$_6$) δ = 4.41 (d, 2H, CH$_2$-N, J = 6 Hz), 6.19 (d, 1H, furan-H, J = 3 Hz), 6.36 (t, 1H, furan-H, J = 3 Hz), 6.44-8.09 (m, 10H, Ar-H + furan-H5), 8.64 (s, 1H, N=CH), 8.91 (t, 1H, O=C-NH-CH$_3$, J = 6 Hz), 8.97, 12.84 (2s, 2H, 2NH); IR (KBr, cm$^{-1}$): 3113 (NH), 3039 (arom.CH) and 1645 (C=O; amide); MS: m/z 483 (M$^+$, 30%), 387 (100%); Anal. Calcd for C$_9$H$_8$ClN$_2$O; C 65.85; H, 4.66; N, 20.17%.

Preparation of azomethines 8a and 9: General procedure:
A mixture of compound 7 (0.01 mol), aromatic aldehyde (namely: o-chlorobenzaldehyde, p-chlorobenzaldehyde, terephthalaldehyde) (0.01 mol) and piperidine (0.5ml) in ethanol (30 mL) was heated under reflux for 1 h; the solid product which produced by heating was collected and recrystallized from the proper solvent.

5-(4-Chlorobenzylideneamino)-N-(furan-2-ylmethyl)-3-(phenylamino)-1H-pyrazole-4-carboxamide (8b)
Yield 60%; yellow solid (AcOH); mp 158-160 °C; 1HNMR (300 MHz, DMSO-d$_6$) δ = 4.55 (d, 2H, CH$_2$-N, J = 3 Hz), 6.36 (d, 1H, furan-H, J = 3 Hz), 6.44 (t, 1H, furan-H, J = 3 Hz), 6.85-8.05 (m, 10H, Ar-H + furan-H5), 8.14 (s, 1H, N=CH), 8.93 (t, 1H, O=C-NH-CH$_3$, J = 3 Hz), 9.22, 13.00 (2s, 2H, 2NH); IR (KBr, cm$^{-1}$): 3105 (NH), 3119 (arom.CH) and 1642 (C=O; amide); MS: m/z 479.5 (M$^+$ (21.5%)), M$^{+}$ (10.5%)); 81(100%); Anal. Calcd for C$_9$H$_8$ClN$_2$O; C 62.93; H, 4.32; N, 16.68. Found: C, 62.89; H, 4.35; N, 16.72%.

Preparation of 12a and 12b: General procedure:
A mixture of 7(0.01 mol), α-cinnamonic acid 10 (0.01 mol) and piperidine (0.5ml) in ethanol (30 mL) was heated under reflux for 3 hrs; the solid product which produced by heating was filtered off and recrystallized from the proper solvent to give 12.

Preparation of 12a and 12b: General procedure:
A mixture of 7(0.01 mol), α-cinnamonic acid 10 (0.01 mol) and piperidine (0.5ml) in ethanol (30 mL) was heated under reflux for 3 hrs; the solid product which produced by heating was filtered off and recrystallized from the proper solvent to give 12.

7-Amino-5-(2-chlorophenyl)-6-cyano-N-(furan-2-ylmethyl)-2-(phenylamino)pyrazolo[1,5-a]pyrimidine-3-carboxamide (12a)
Yield 60%; white solid (Dioxane); mp 300-302 °C; 1HNMR (300 MHz, DMSO-d$_6$) δ = 4.54 (d, 2H, CH$_2$-N, J = 6 Hz), 6.25 (d, 1H, furan-H, J = 3 Hz), 6.35 (t, 1H, furan-H, J = 3 Hz), 6.96-7.88 (m, 10H, Ar-H + furan-H5), 8.10 (t, 1H, O=C-NH-CH$_3$, J = 6 Hz), 9.15 (s, 2H, NH$_2$), 9.54 (s, 1H, NH); IR (KBr, cm$^{-1}$): 3416, 3334, 3162 (NH$_2$/NH), 2216 (C=NH), and 1655 (C=O; amide); MS: m/z 483 (M$^+$, 20%), 387 (100%); Anal. Calcd for C$_9$H$_8$N$_3$O; C, 62.05; H, 3.75; N, 20.26. Found: C, 62.10; H, 3.72; N, 20.21%.

7-Amino-6-cyano-N-(furan-2-ylmethyl)-5-(4-methoxyphenyl)-2-(phenylamino)pyrazolo[1,5-a]pyrimidine-3-carboxamide (12b)
Yield 62%; white solid (Dioxane); mp 298-300 °C; 1HNMR (300 MHz, DMSO-d$_6$) δ = 3.86 (s, 3H, OCH$_3$), 4.58 (d, 2H, CH$_2$-N, J = 6 Hz), 6.33 (d, 1H, furan-H, J = 3 Hz), 6.43 (t, 1H, furan-H, J = 3 Hz), 6.97-7.86 (m, 10H, Ar-H + furan-H5), 8.32 (t, 1H, O=C-NH-CH$_3$, J = 6 Hz), 8.96 (s, 2H, NH$_2$), 9.52 (s, 1H, NH); 13C NMR (300 MHz, DMSO-d$_6$) δ = 35.24 (CH$_3$), 53.14 (OCH$_3$), 116.35, 137.50, 139.80, 142.50 (furan-C), 127.60, 128.65, 130.37, 132.15, 133.40 (Ar-C), 88.65 (pyrindine-C5), 149.56 (pyridine-C2), 160.59 (pyridine-C6), 161.35 (pyridine-C4), 106.90 (pyrazole-C3), 158.01 (pyrazole-C2), 152.80 (CN), 163.57 (C=O); IR (KBr, cm$^{-1}$): 3236-3270 (NH$_2$/NH), 2200 (C=NH), and 1660 (C=O; amide); MS: m/z 479 (M$^+$ (8.2%)), 386 (100%); Anal. Calcd for C$_9$H$_8$N$_3$O; C, 65.13; H, 4.41; N, 20.45. Found: C, 65.19; H, 4.36; N, 20.40 %.

7-Amino-5,6-dicyano-N-(furan-2-ylmethyl)-2-(phenylamino)pyrazolo[1,5-a]pyrimidine-3-carboxamide (17)
A mixture of compound 7 (0.01mol), tetracyanoethylene (15) (0.01 mol) and piperidine (0.5ml) in dioxane (30 mL) was heated under reflux for 3 hrs; the solid product which produced by heating was filtered off, dried and recrystallized from acetic acid as brown solid. Yield 45%; mp 285-287 °C; 1HNMR (300 MHz, DMSO-d$_6$) δ = 4.61 (d, 2H, CH$_2$-N, J = 6 Hz), 6.31 (d, 1H, furan-H, J = 3 Hz), 6.41 (t, 1H, furan-H4, J = 3 Hz), 6.97-7.86 (m, 6H, Ar-
H + furan-H5, 8.01 (t, 1H, O=C-NH-CH3, J = 6 Hz), 9.41 (br, 2H, NH3), 9.61 (s, 1H, NH); IR (KBr, ν, cm⁻¹): 3422, 3358 (NH2/NH), 2958 (aliph.CH), 2229(C=≡N), 1650 (C=O; amide); MS: m/z 398 (M⁺, 55.20%), 96 (100%); Anal. Calcd for C20H14N3O2. Calcd: C, 60.30; H, 3.54; N, 19.28. Found: C, 60.26; H, 3.49; N, 19.28.

[Caution: this reaction liberate HCN gas, which is very toxic]

6-Cyano-5-(cyanomethyl)-(N-(furan-2-ylmethyl)-7-imino-2-(phenylamino)-6,7-dihydropyrazolo[1,5-d]pyrimidin-3-carboxamide (20)
A mixture of 7 (0.01 mol), 1,1,3-tricyclo-2,2,2-aminopropene (18) (0.01 mol) and piperidine (0.5ml) in ethanol (30 ml) was heated under reflux for 3 hrs; the solid product which was produced by heating was filtered off, dried and recrystallized from dioxane as white solid. Yield 45%; mp 220-222 ºC; ¹H NMR (300 MHz, DMSO-d₆) δ = 2.20 (s, 2H, CH₂), 4.42 (d, 2H, CH₂-N, J = 6 Hz), 6.21 (d, 1H, furan-H3, J = 3 Hz), 6.37 (t, 1H, furan-H4, J = 3 Hz), 6.77-7.54 (m, 6H, Ar-H + furan-H5), 7.61 (s, 1H, pyrimidine-H), 8.55 (t, 1H, O=C-NH-CH₂, J = 6 Hz; exchangeable with D₂O), 10.16, 12.49 (2s, 2H, NH; exchangeable with D₂O); IR (KBr, ν, cm⁻¹): 3302 (NH), 2936 (aliph.CH), 2213(C=≡N) and 1676 (C=O; amide); MS: m/z 412 (M⁺, 10.50 %), 81 (100%); Anal. Calcd for C₃₂H₂₀N₁₀O₂. Calcd: C, 61.16; H, 3.91; N, 27.17. Found: C, 61.11; H, 3.88; N, 27.12%.

Ethyl 7-amino-3-([(furan-2-ylmethyl)carbamoyl]-2-(phenylamino)pyrazolo[1,5-d]pyrimidine-6-carboxylate (22)
A mixture of 7 (0.01 mol), ethyl 2-cyano-3-ethoxyacrylate (21) (0.01 mol) and piperidine (0.5ml) in ethanol (30 ml) was heated under reflux for 3 hrs; Then it was poured into ice/water and acidified with 0.1 N HCl at pH 3-4; the resulting precipitate was filtered off, dried and recrystallized from AcOH as white solid. Yield 42%; mp 189-190 ºC; ¹H NMR (300 MHz, DMSO-d₆) δ = 1.36 (t, 3H, CH₃), 4.35 (q, 2H, CH₂), 4.59 (d, 2H, CH₂-N, J = 6 Hz), 6.33 (d, 1H, furan-H3, J = 3 Hz), 6.40 (t, 1H, furan-H4, J = 3 Hz), 6.74-7.83 (m, 6H, Ar-H + furan-H5), 8.27 (t, 1H, O=C-NH-CH₂, J = 6 Hz; exchangeable with D₂O), 8.69 (s, 1H, pyrimidine-H), 9.57 (s, 2H, NH; exchangeable with D₂O); 11.06 (s, H, NH; exchangeable with D₂O); IR (KBr, ν, cm⁻¹): 3439, 3313 (NH₂/NH), 2926 (aliph.CH), 1691(C=O; ester) and 1650 (C=O; amide); MS: m/z 420 (M⁺, 39.40 %), 278 (100%); Anal. Calcd for C₃₂H₂₁N₁₀O₄. Calcd: C, 61.79; H, 4.79; N, 19.99. Found: C, 59.92; H, 4.83; N, 19.95%.

N-(furan-2-ylmethyl)-5-(4-methylphenyl)-7-phenyl-2-(phenylamino)pyrazolo[1,5-d]pyrimidine-3-carboxamide (25)
A mixture of 7 (0.01 mol), α,β-unsaturated carbonyl derivative 24 (0.01 mol) and piperidine (0.5ml) in ethanol (30 ml) was heated under reflux for 3 hrs; the solid product which produced by heating was filtered off, dried and recrystallized from dioxane as white solid. Yield 40%; mp 190-192 ºC; IR (KBr, ν, cm⁻¹): 3109 (NH), 2951 (aliph.CH) and 1655(C=O; amide); MS: m/z 515 (M⁺, 12.24%), 517 (M⁺, 100%); Anal. Calcd for C₃₃H₂₅N₁₀O₃. Calcd: C, 72.22; H, 4.89; N, 13.58. Found: C, 72.17; H, 4.84; N, 13.54%.

Preparation of compounds 26a,b, 28 and 29: General procedure
A mixture of compound 7 (0.01mol) and 1,3-dicarboxyl compound (namely; acetylaceton, benzoylaceton, ethyl acetoacetate and ethyl 2-methyl-3-oxobutanoate) (0.01 mol) in glacial acetic acid (10 ml) was heated under reflux for 3hrs, the solid product which produced by heating was collected and recrystallized from the proper solvent to give 26a,b, 28 and 29 respectively.

N-(furan-2-ylmethyl)-5,7-dimethyl-2-(phenylamino)pyrazolo[1,5-d]pyrimidine-3-carboxamide (26a)
Yield 60%; white solid (Dioxane); mp 200 ºC; ¹H NMR (300 MHz, DMSO-d₆) δ = 2.48, 2.68 (2s, 6H, 2CH₃), 4.59 (d, 2H, CH₂-N, J = 6 Hz), 6.31 (d, 1H, furan-H3, J = 3 Hz), 6.39 (t, 1H, furan-H4, J = 3 Hz), 6.92-7.70 (m, 6H, Ar-H + furan-H5), 7.58 (s, 1H, pyrimidine-H), 8.23 (t, 1H, O=C-NH-CH₂, J = 6 Hz; exchangeable with D₂O), 9.50 (s, 1H, NH; exchangeable with D₂O); ¹³C NMR (300 MHz, DMSO-d₆) δ = 17.11, 24.63 (2CH₃), 35.46 (2CH₂), 117.53, 121.32, 124.50, 129.53 (Ar-C), 109.47, 110.98, 140.70, 142.76 (furan-C), 86.51, 146.88 (pyrazole-C), 107.27, 156.52, 161.42, 164.38 (pyrimidine-C), 152.78 (C=O); IR (KBr, ν, cm⁻¹): 3307 (NH), 2917 (aliph.CH) and 1641(C=O; amide); MS: m/z 361(M⁺, 39.42%), 265 (100%); Anal. Calcd for C₂₈H₂₅N₁₀O₂. Calcd: C, 66.47; H, 5.30; N, 19.38. Found:C, 66.42; H, 5.25; N, 19.42%.

N-(furan-2-ylmethyl)-5-methyl-7-phenyl-2-(phenylamino)pyrazolo[1,5-d]pyrimidine-3-carboxamide (26b)
Yield 65%; white solid (Dioxane); mp 189-190 ºC; ¹H NMR (300 MHz, DMSO-d₆) δ = 2.49 (s, 3H, CH₃), 4.63 (d, 2H, CH₂-N, J = 6 Hz), 6.34 (d, 1H, furan-H3, J = 3 Hz), 6.43 (t, 1H, furan-H4, J = 3 Hz), 6.90-8.21 (m, 12H, Ar-H + furan-H5 + pyrimidine-H), 8.38 (t, 1H, O=C-NH-CH₂, J = 6 Hz; exchangeable with D₂O), 9.52 (s, 1H, NH; exchangeable with D₂O); IR (KBr, ν, cm⁻¹): 3101 (NH), 2920 (aliph.CH) and 1643 (C=O; amide); MS: m/z 423 (M⁺, 10.40 %), 327(100%); Anal. Calcd for C₂₃H₂₁N₁₀O₂. Calcd: C, 70.91; H, 5.00; N, 16.54. Found: C, 70.85; H, 4.98; N, 16.60%.
Antimicrobial screening

The disks of Whatman filter paper were prepared with standard size (6.0 mm diameter) and kept into 1.0 Oz screw capped wide mouthed containers for sterilization. These bottles were kept into hot air oven at a temperature of 150 °C. Then, the standard sterilizer filter paper disks impregnated with a solution of the test compound in DMF (100 µL, 5 mg/mL) were placed on nutrient agar plate seeded with the appropriate test organism in triplicates. Standard concentrations of 10^5 CFU/mL (Colony Forming Units/mL) and 10^7 CFU/mL were used for antibacterial and antifungal assay, respectively. Pyrex glass Petri dishes (9 cm in diameter) were used and two disks of filter paper were inoculated in each plate. The utilized test organisms were S. aureus, E. coli, and B. subtilis as examples of Gram-positive bacteria and P. aeruginosa, P. vulgaris and K. pneumonia as examples of Gram-negative bacteria. They were also evaluated for their in vitro antifungal potential against A. fumigatus, A. clavatus and G. candidum fungal strain. Ampicillin and gentamycin were used as standard antibacterial agents; while amphotericin B was used as standard antifungal agent. DMF alone was used as control at the same above-mentioned concentration and due to this, there was no visible change in bacterial growth. The plates were incubated at 37 °C for 24 hrs for bacteria and for 48 hrs at 25 °C for fungi. The mean zone of inhibition measured in mm ± standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms. Compounds that showed significant growth inhibition zones using the twofold serial dilution technique, were further evaluated for their minimal inhibitory concentrations (MICs).

Minimal inhibitory concentration (MIC) measurement

The micro dilution susceptibility test in Müller-Hinton Broth (Oxoid) and Subbouraud Liquid Medium (Oxoid) was used for the determination of antibacterial and antifungal activity, respectively. Stock solutions of the tested compounds, ampicillin, gentamycin, amphotericin B and sulfisoxazole were prepared in DMF at concentrations 1000 µg/mL. Each stock solution was diluted with standard method broth (Difco) to prepare serial twofold dilutions in the range of 500-0.007 µg/mL. 10 mL of the broth containing about 10^5 CFU/mL of test bacteria or 10^5 CFU/mL of the test fungus was added to each well of 96-well microtiter plate. The sealed microplates were incubated at 37 °C for 24 hrs for antibacterial activity and at 25 °C for 48 hrs for antifungal activity in a humid chamber. At the end of the incubation period, the minimal inhibitory concentrations (MIC) values were recorded as the lowest concentrations of the substance that had no visible turbidity. Control experiments with DMF and inoculated media were run parallel to the test compounds under the same conditions.

RESULTS AND DISCUSSION

Chemistry

The starting materials 2a,b were achieved in a good yield (90%) by the solvent free reaction of 4-aminoantipyrine and furan-2-ylmethanamine (1a,b) with ethyl cyanoacetate [27]. Knoevenagel condensation of compound 2a with anthracene-9-carboxaldehyde in refluxing ethanol containing a catalytic amount of piperidine produced a single stereoisomer, identified as (E)-3-(anthracen-9-yl)-2-cyano-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1Hpyrazol-4-yl)acrylamide (3). The E-configuration of compound 3 was assigned based on its 1H NMR spectrum which displayed a downfield singlet signal at δ 9.15 ppm due to the olefinic (CH) proton that agrees with the chemical shift of the olefinic proton of similar structure, (E)-N-(pyridin-2-yl)-2-cyano-3-phenylprop-2-enamide, confirmed by X-ray analysis [28]. Treatment of acrylamide 3 with hydrazine hydrate and/ or phenylhydrazine in
ethanol under reflux, furnished in each case a single product identified as 5-aminopyrazole derivatives 4a,b (scheme 1). $^1$H NMR spectrum of 4a in DMSO-$d_6$ displayed singlet signals at $\delta$ 2.30, 3.42 ppm characteristic of two methyl protons and three $D_2$O exchangeable signals at 7.01, 9.44 and 10.07 ppm corresponding to NH$_2$ and 2NH protons, respectively. Mass spectrum of compound 4b revealed a molecular ion peak at $m/z$ = 564 (M$,^+$, 25.4%) which is characteristic of the molecular formula C$_{35}$H$_{28}$N$_6$O$_2$. An efficient three-component, two-step “catch and release” synthesis of 5-aminopyrazoles was reported. Thus, treatment of 2-cyano-$N$-(furan-2-ylmeth-yl)acetamide (2b) with phenyl isothiocyanate in DMF in the presence of potassium hydroxide at room temperature gave the non-isolated adduct 5 which was then treated with dimethylsulfate to afford the 2-cyano-$N$-(furan-2-ylmethyl)-3-(methylthio)-3-(phenylamino)acrylamide (6). Reaction of ketene $N$, $S$-acetal 6 with hydrazine hydrate in refluxing ethanol furnished 5-aminopyrazole derivative 7 (scheme 1). The isolated product 7 was confirmed by analytical and spectral data. $^1$H NMR (DMSO-$d_6$) spectrum showed doublet and singlet signals at $\delta$ 4.41, 5.84 ppm characteristic for CH$_2$=NH and NH$_2$ protons in addition to, two singlet signals at $\delta$ 7.53, 11.06 ppm for 2 NH protons with triplet signals at $\delta$ 8.83 ppm characteristic of the amide group proton. $^{13}$C NMR (DMSO-$d_6$) of compound 7 revealed signals at $\delta$ 35.09 (CH$_2$), 106.40, 164.45, 164.47 (pyrazole carbons) in addition to, signal at 152.84 ppm (C=O).

To investigate the structure reactivity relationship with respect to antimicrobial properties, the reactivity of 5-amino-$N$-(furan-2-ylmethyl)-3-(phenylamino)-1H-pyrazole-4-carboxamide (7) toward some electrophilic reagents was investigated. Schiff base derivatives were reported to possess significant biological activities and new series

\[ \text{i = Ethyl cyanoacetate, ii = anthracene-9-carbaldehyde/EtOH/Pip.,} \]
\[ \text{iii = R-NHNH$_2$/EtOH, iv = Ph-NCS/KOH/DMF, v = (CH$_3$)$_2$SO$_4$} \]

Scheme 1. Synthetic route to 5-aminopyrazoles
have been tested for their antitumor, antimicrobial, and antiviral activities [29, 30]. In the light of these facts, 5-(benzylideneamino)pyrazole derivatives 8a,b were obtained by condensation of 5-aminopyrazole 7 with aromatic aldehydes in ethanol in the presence of piperidine under reflux. $^1$H NMR (DMSO-d$_6$) spectra of 8a,b showed singlet downfield CH=N signals at δ 8.14-8.64 ppm. Also, bis(azomethine)pyrazole derivative 9 was achieved by condensation of compound 7 with terephthalaldehyde (2 : 1 molar ratio) in refluxing ethanol in the presence of piperidine (scheme 2). A molecular ion peak at m/z = 692 (12.5%) was observed in the mass spectrum of compound 9 with a base peak at m/z 64, which is compatible with its molecular formula of C$_{18}$H$_{16}$N$_{10}$O$_3$.

![Scheme 2. Synthetic route to azomethine derivatives](image)

Scheme 2. Synthetic route to azomethine derivatives

5-Aminopyrazoles are versatile reagents and have been extensively used as synthetic starting materials for the synthesis of several polysubstituted fused pyrazoles of potential biological activity [31-34]. Thus, the reactivity of 5-aminopyrazole 7 towards some activated nitriles was investigated as an alternative route to obtain pyrazolo[1,5-$a$]pyrimidine derivatives. Reaction of 5-aminopyrazole 7 with $\alpha$-cinnaminitriles 10a,b in refluxing ethanol in the presence of a catalytic amount of piperidine yielded a single product for which structure 12 or 14 seemed possible. Structure of 12 appears more likely than 14 on the basis of single crystal X-ray structure analysis [35] and HMBC-$^1$N[36].$^1$H NMR spectrum of 12b in DMSO-d$_6$ revealed singlet signals at δ 8.36, 8.96 ppm characteristic of OCH$_3$ and amino group protons. $^{13}$C NMR spectrum of 12b in DMSO-d$_6$ revealed signals at δ = 53.14 (OCH$_3$), 88.65, 149.56, 160.59, 161.35 (pyrimidine-C), 106.90, 158.01 (pyrazole-C), 152.80 (CN), 163.57 (C=O) ppm. The formation of 12 is assumed to proceed via an initial Michael addition of the oxocyclic NH$_2$ in 7 to the activated double bond in 10 to yield the non-isolable Michael adduct 11, followed by intramolecular cyclization and aromatization by loss of hydrogen molecule. Similarly, pyrazolo[1,5-$a$]pyrimidine derivative 17 was obtained via the reaction of compound 7 with tetracyanoethylene (15) in refluxing dioxane in the presence of a catalytic amount of piperidine. Infrared spectrum of 17 showed the characteristic absorption bands at 3422, 3358, 2229, assignable to the reactivity of 5-aminopyrazole 7 with tetracyanoethylene (15) in refluxing ethanol in the presence of a catalytic amount of piperidine. The infrared spectrum of 17 showed the characteristic absorption bands at 3422, 3358, 2229, characteristic of the molecular formula C$_{30}$H$_{24}$N$_{10}$O$_3$ (Chart 1). Formation of pyrazolopyrimidines 17 takes place as depicted in scheme 3, via the formation of Michael adduct 16, followed by intramolecular cyclization with HCN elimination. Interaction of 5-aminopyrazole 7 with 1,1,3-tricyano-2-aminopropane (18) in ethanol in the presence of piperidine afforded two possible isomeric products 20A and 20B. Structure 20A appears more likely than 20B on the basis of spectral data. The infrared spectrum of the isolated product afforded intense absorption bands at 3302 (NH) and 2213 cm$^{-1}$ (C≡N). $^1$H NMR spectrum of 20A showed three D$_2$O exchangeable signals at δ 8.55, 10.16, 12.49 attributed to 3NH protons. In addition to, singlet signal at δ 7.61 ppm characteristic of pyrimidine-H proton. The reaction proceeds through the formation of Michael adduct 19 as intermediate, followed by the ammonia molecule elimination (scheme 3).
Scheme 3. Synthetic route to pyrazolo[1,5-a]pyrimidine

Chart 1: Fragmentation pattern of compound 17
Reaction of 5-aminopyrazole 7 with ethyl 2-cyano-3-ethoxyacrylate (21) in ethanolic piperidine solution, afforded pyrazolo[1,5-a]pyrimidine derivative 22 rather than 23 (Scheme 4). 

1HNMR spectrum of 22 revealed triplet and quartet signals at 1.36, 4.35 assignable for ester moiety with two singlet signals at, 8.69, 9.57 ppm due to CH-pyrimidine and amino protons. Furthermore, the reaction of 5-aminopyrazole 7 with α,β-unsaturated carbonyl derivative 24 in ethanolic piperidine solution under reflux, afforded pyrazolo[1,5-a]pyrimidine derivative 25. Its mass spectrum revealed a molecular ion peak at m/z = 515 (M+, 12.24%) and a base peak was observed in the spectrum at m/z = 517 (M+1), which is compatible with its molecular formula of C$_{31}$H$_{25}$N$_{5}$O$_{3}$.

The general literature procedure for the synthesis of pyrazolo[1,5-a]pyrimidines involves cyclocondensation of aminopyrazoles with reagents having 1,3-electrophilic centers such as β-diketones [37-40]. Thus, cyclocondensation of 5-aminopyrazole 7 with acetylacetone and / or benzoylacetone in boiling glacial acetic acid gave the pyrazolo[1,5-a]pyrimidine derivatives 26a and 26b - evidence for assigned structures being provided by analytical and spectral data. For example, 13C NMR (DMSO-d$_6$) of compound 26a revealed signals at δ 17.11, 24.63 (2CH$_3$), 107.27, 156.52, 161.42, 164.38 (pyrimidine carbons) in addition to, singlet signal at 152.78 ppm (C=O). The mass spectrum of compound 26a revealed a molecular ion peak at m/z = 361 (M+, 39.42%), and a base peak was observed in the spectrum at m/z = 265, which is compatible with its molecular formula of C$_{20}$H$_{19}$N$_{5}$O$_{2}$ (Chart 2).

Similarly, treatment of 5-aminopyrazole 7 with ethyl acetoacetate in boiling glacial acetic afforded two possible isomeric products 27 and 28. Structure 27 was excluded on the basis of spectral data and analogy with previous work [41-44]. H NMR spectrum of 28 in DMSO-d$_6$ displayed singlet signals at δ 2.35, 5.76 ppm characteristic of methyl protons and pyrimidine-H, in addition to, three D$_2$O exchangeable signals at δ 8.21, 9.33 and 11.57 ppm attributable to 2NH and OH protons. The mass spectrum of compound 28 revealed a molecular ion peak at m/z = 363 (M+, 35.3%), and a base peak was observed in the spectrum at m/z = 81, which is compatible with its molecular formula of C$_{19}$H$_{17}$N$_{5}$O$_{3}$. Finally, pyrazolo[1,5-a]pyrimidine derivative 29 could be achieved in excellent yield via cyclocondensation of compound 7 with ethyl 2-methyl-3-oxo-butanoate in glacial acetic acid under reflux condition (Scheme 5). The chemical structure of 29 was supported on the basis of elemental analysis and spectral data. Its H NMR spectrum in DMSO-d$_6$ displayed singlet signals at δ 1.98 and 2.37 ppm due to 2 CH$_3$ protons in addition to three D$_2$O exchangeable signals at δ 8.18, 9.43 and 11.30 assignable to 2NH and OH protons.

![Scheme 4. Synthetic route to pyrazolo[1,5-a]pyrimidine](image-url)
Antimicrobial evaluation

The antimicrobial screening and minimal inhibitory concentrations of the tested compounds were carried out at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. Eighteen of the newly synthesized target compounds were evaluated for their in vitro antibacterial activity against Staphylococcus aureus (RCMB 010027), Staphylococcus epidermidis (RCMB 010024) and Bacillus subtilis (RCMB 010063) as examples of Gram-positive bacteria and Pseudomonas aeruginosa (RCMB 010043), Proteus vulgaris (RCMB 010085) and Klebsiella pneumonia (RCMB 010093) as examples of Gram-negative bacteria. They were also evaluated for their
in vitro antifungal potential against Aspergillus fumigatus, Aspergillus clavatus and Geotrichum candidum fungal strain.

Agar-diffusion method [45] was used for the determination of the preliminary antibacterial and antifungal activity. Ampicillin, Gentamycin and Amphotericin B were used as reference drugs. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the disks in mm. The minimum inhibitory concentration (MIC) measurement was determined for compounds and showed significant growth inhibition zones using twofold serial dilution method [46]. The MIC (µg/mL) and inhibition zone diameters values are recorded in Table 1.

<table>
<thead>
<tr>
<th>Compounds no.</th>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>B. subtilis</td>
</tr>
<tr>
<td>3</td>
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<td>20.1</td>
<td>20.1</td>
</tr>
<tr>
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</tr>
<tr>
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<td>21.8(0.98)</td>
<td>22.3(0.98)</td>
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<tr>
<td>6</td>
<td>17.3</td>
<td>19.2</td>
<td>19.8</td>
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<tr>
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</tr>
<tr>
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<tr>
<td>8b</td>
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<td>22.4(0.98)</td>
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<td>9</td>
<td>22.3(0.98)</td>
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<tr>
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<td>25.4(0.49)</td>
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<tr>
<td>Amphotericin B</td>
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<td>NT</td>
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</tr>
</tbody>
</table>

*NA: no activity, *NT: not tested

Table 1: Antimicrobial inhibition zone in mm and minimal inhibitory concentrations (MIC, µg/mL, between brackets) of some new synthesized compounds

Antibacterial activity

Regarding the Antibacterial activities of acrylamides, acrylamide 3 was more potent than 6 against both Gram-positive and Gram-negative bacteria (fig.1). The pyrazoles 4b and 4a bearing antipyrene moiety displayed better antibacterial activity than pyrazole 7 bearing furan moiety when compared with ampicillin and gentamycin. Pyrazole 4b showed relatively good growth inhibitory profiles versus both of S. epidermidis (MIC 0.98µg/mL), P. vulgaris (MIC 1.95µg/mL) and K. pneumonia (MIC 0.98µg/mL), which was about 50% of the activity of ampicillin and gentamycin. When pyrazole 7 converted into Bis(azomethine)pyrazole derivative 9, it showed equal activity to ampicillin versus both of S. epidermidis (MIC 0.49µg/mL) and B. subtilis (MIC 0.24µg/mL), it also showed equipotent to gentamycin versus both of P. vulgaris (MIC 0.98µg/mL) and K. pneumonia (MIC 0.49µg/mL). Azomethine derivatives 8a displayed equipotent to ampicillin against B. subtilis (MIC 0.24µg/mL), but showed relatively good growth inhibitory profiles against both of S. epidermidis (MIC 0.98µg/mL) and K. pneumonia (MIC 0.98µg/mL), which was about 50% of the activity of ampicillin and gentamycin. Also, Azomethine 8b has more activities than 5-amino-pyrazole 7 when compared with ampicillin and gentamycin. Regarding the activity of pyrazolo[1,5-a]pyrimidines, the results revealed that, pyrazolo[1,5-a]pyrimidine 17 contain two cyano groups recorded higher activity than all other pyrazolo[1,5-a]pyrimidine derivatives when compared with ampicillin and gentamycin. Compounds 20, 22, 25, 26a, 28 and 29 showed relatively moderate growth inhibitory profiles versus both Gram-positive and Gram-negative bacteria. While, pyrazolo[1,5-a]pyrimidines 12b and 26b revealed weak activities when compared with ampicillin and gentamycin. Unfortunately, pyrazolo[1,5-a]pyrimidine 12a showed completely inactive toward Gram-positive and Gram-negative bacteria (fig.2). On the other hand, all the synthesized compounds showed were completely inactive toward P. aeruginosa (RCMB 010043) compared to gentamycin.
Antifungal activity

Both acrylamides 3 and 6 showed the same moderate activity when compared to amphotericine B in inhibiting the growth of *A. fumigatus*, *A. clavatus* and *G. candidium* fungal strain. Pyrazole 4b displayed *in vitro* antifungal activity equipotent to amphotericin B against both of *A. clavatus* (MIC 0.98 μg/mL), and *G. candidum* (MIC 0.49μg/mL), but revealed 50% of the activity of *A. fumigatus* (MIC 1.95μg/mL) when compared with amphotericin B.
Bis(azomethine)pyrazole 9 displayed equipotent to amphotericin B versus A. clavatus (MIC 0.98 µ/mL) and showed 50% of the activity of amphotericin B in inhibiting the growth of G. candidium (MIC 0.98 µ/mL). Azomethine derivatives 8a and 8b displayed 50% of the activity of amphotericin B versus the inhibiting of A. fumigatus (MIC 1.95 µ/mL) and G. candidium (MIC 0.98 µ/mL), but they were equipotent to amphotericin B against A. clavatus (MIC 0.98 µ/mL). Pyrazolo[1,5-a]pyrimidine 17 was equipotent to amphotericin B in inhibiting the growth of G. candidium (MIC 0.49 µ/mL). Other pyrazolo[1,5-a]pyrimidine derivatives 20, 22, 25, 26a, b, 28 and 29 displayed the moderate activity comparable to amphotericin B. Unfortunately, all the synthesized compounds 12a and 12b showed were completely inactive toward A. fumigatus, A. clavatus and G. candidium fungal strain (fig. 3).

CONCLUSION

The objective of this study was to synthesize and investigate the antimicrobial activities of some new 5-aminopyrazole and pyrazolo[1,5-a]pyrimidine derivatives. My aim has been verified by the synthesis of 5-aminopyrazole, 5-(benzylideneamino)pyrazole, bispyrazole and pyrazolo[1,5-a]pyrimidine derivatives bearing antipyrene or furan moiety. The obtained results clearly reveal that 5-aminopyrazoles 4b and 4a bearing antipyrene moiety display antimicrobial activity better than pyrazole 7 bearing furan moiety. Conversation of pyrazole 7 into 5-(benzylideneamino)pyrazole 8a,b and bispyrazole derivative 9 increase the potential activity. Some pyrazolo[1,5-a]pyrimidine derivatives record good potential activity.

REFERENCES