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Synthesis, Characterization and Biological Evaluation of Novel Methoxy Benzaldehyde Substituted Derivatives of Pyrazolopyrimidine-4-hydrazide

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Several novel pyrrolopyrimidine derivatives of methoxybenzaldehydes with biologically active pyrrole moieties were synthesized to investigate their antibacterial, antifungal and cytotoxic activities. Elemental analysis, UV-spectra, FT-IR, ^{1}H NMR and ^{13}C NMR spectra, were used to characterize the synthesized compounds. The *in vitro* antifungal and antibacterial activities were tested for all the synthesized compounds. Compared to the reference drugs *e.g*. fluconazole and streptomycin, most of the compounds tested positive for antifungal and antibacterial activity. All tested compounds demonstrated superior cytotoxic activity to the reference drug doxorubicin, with IC_{50} values ranging from 6.52 to 7.77 mM and comparable activity with IC_{50} values of 8.21 and 8.27 mM, respectively. *In vitro* antibacterial and antifungal activities revealed that the compouds **b** and **e** were more effective than the standard drugs streptomycin and fluconazole, respectively.

Keywords: Methoxybenzaldehydes, 4-Aminopyrrolo[2,3-*d*]pyrimidine, Dimethoxyaldehydes, 4-Hydroxy-3-methoxybenzaldehyde.

INTRODUCTION

The first synthesis approach for pyrrolopyrimidines, which used monocyclic pyrimidines as the main substrate, was published in 1974. Development of these compounds started early in the 1970s [1]. Later, various synthesis methods were developed using pyrrole derivatives and changes to the bicyclic pyrimidines' skeletons. At the same time, more straightforward methods using substituted pyrimidines were prepared, utilizing highly reactive reagents like acetals of acid amides and aminals [2]. From various perspectives, forming new fused heterocyclic compounds is an important task for medicinal chemists. Pyrrole and pyrrolopyrimidine, for example, have been studied for antimicrobial [3,4], analgesic [5,6], anti-inflammatory [7,8], antiviral [9,10] and anticancer activities. Pyrrolo[2,3-*d*]pyrimidines have recently attracted chemical and biological attention due to their useful properties as antimetabolites in purine biochemical reactions [10]. Due to their wide pharmacological profile, fused pyrimidines are one of the fascinating classes of heterocycles thoroughly investigated by medicinal chemists [11]. The presence of fused pyrimidines in various physiological molecules may be one of the causes for such intriguing pharmacological potential.

Several fused pyrimidine molecules such as oxazine, purine, xanthine, pteridine, triazole pyrimidine, quinazoline, pyrrolopyrimidine, pyridopyrimidine, pyrimidoazepine, fluoropyrimidine and thiazolopyrimidine are well-established as antibacterial, anticancer, antifungal and anti-inflammatory agents [12-15]. When purine derivatives, such as 6-mercaptopurine, were discovered to be effective anticancer agents, the first study on pyrrolopyrimidines was initiated. An extensive study on the pharmacological spectrum of purines and their analogues, in particular deaza analogues, pyrrolo[2,3-*d*]pyrimidines and pyrrolo[3,2-*d*]pyrimidines, was conducted after initial studies [16]. Natural product-based nucleoside antibiotics, including tubercidin, toyocamycin and sanguivomycin, were also isolated and identified [17,18]. Interestingly, pyrrolo[2-3-*d*]pyrimidine nucleus was the common component in all of these natural product based antibiotics.

Researchers later turned their attention to the second isomer due to discovering several biological activities, including anticancer potential of substituted 4-aminopyrrolo[3,2-*d*]pyrimidines. Several mechanisms, including antifolate inhibitors of dihydrofolate reductases [16,19], are also involved in their cytotoxic activities. Furthermore, pyrroles have good antibacterial

activity *in vivo* and *in vitro* mechanisms of action [20-23]. This study reports the synthesis and biological evaluation of novel pyrrolopyrrolopyrimidine derivatives carrying a biologically active pyrrole moiety as we are interested in developing novel antifungal and antibacterial agents. Furthermore, the newly synthesized compounds' cytotoxic activities were also achieved on the active site of the mechanism of action for their cytotoxic activity.

EXPERIMENTAL

The S.D. Fine Chem Company supplied the entire set of chemicals used in this research. On a Lab Junction melting point/boiling point apparatus, melting points (ºC, incorrect) were measured in open capillaries. For thin-layer chromatography, precoated silica gel plates were used. A pet. ether/ethyl acetate (9:1) combination was used as the developing solvent system. Using a BRUKER FT-IR spectrophotometer, infrared spectra in KBr discs were captured. On a Bruker 400 MHz spectrometer, NMR spectra (in DMSO-d_6) were measured using TMS as an internal reference. At room temperature, methanol solvent was used to capture UV spectra on a JASCO V650 spectrophotometer. The Carlo Erba 1108 Elemental Analyzer was used to conduct elemental analysis.

Methoxybenzaldehyde derivatives of 4-hydrazinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (a-e): A mixture of 4-hydrazinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (1.49 g, 0.01 mol) and substituted methoxybenzaldehyde (0.01 mol) was refluxed in ethanol (20 mL) in the presence of catalytic amount of conc. HCl for 3-7 h. The solid obtained was filtered off and recrystallized from ethanol to yield compounds **a-g** (**Scheme-I**).

4-[(2*Z*)-2-(2-Methoxybenzylidene)hydrazinyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (a): Yield: 84.67%, m.p.: 186 ºC, IR (KBr, ν_{max}, cm^{-1}): 3400 (-NH- arom.), 3122 (-NH- aliph.), 2860 (-OCH_3), 3082 (-CH=), 1641 (>C=NN-), 1546 (>C=C<), 1305 (-C-O), 1033 (-N-N-), 646 (disub-benzene ring). ^{1}H NMR (DMSO-d_6) δ ppm: 14.25 (s, 1H, -NH- aliph.), 12.97 (s, 1H, NH, arom.), 8.73 (s, 1H, -CH=), 8.46 (s, 1H, pyrimidine-H), 7.09-8.01 (6H, 7.09 (ddd, *J* = 8.25, 2.47, 1.70 Hz), 7.16 (d, *J* = 3.86 Hz), 7.51 (ddd, *J* = 2.47, 1.60, 0.54 Hz), 7.57 (ddd, *J* = 8.51, 1.54, 0.53 Hz), 7.75 (d, *J* = 3.86 Hz), 8.01 (ddd, *J* = 7.87, 1.34, 0.53 Hz), 3.87 (s, 3H, -OCH_3). ^{13}C NMR (DMSO-d_6) δ ppm: 55.91 (-OCH_3), 142.57 (-CH=), 150.18 (C2), 100.10 (C3), 148.84 (C4), 114.83 (C5), 125.90 (C6), 103.22 (C2), 114.98 (C3), 126.26 (C4). Anal. calcd. (found) % for $C_{14}H_{13}N_5O$ (*m.w.* 267.29): C, 62.91 (62.88); H, 4.90 (4.87); N, 26.20 (26.24); O, 5.99 (5.91).

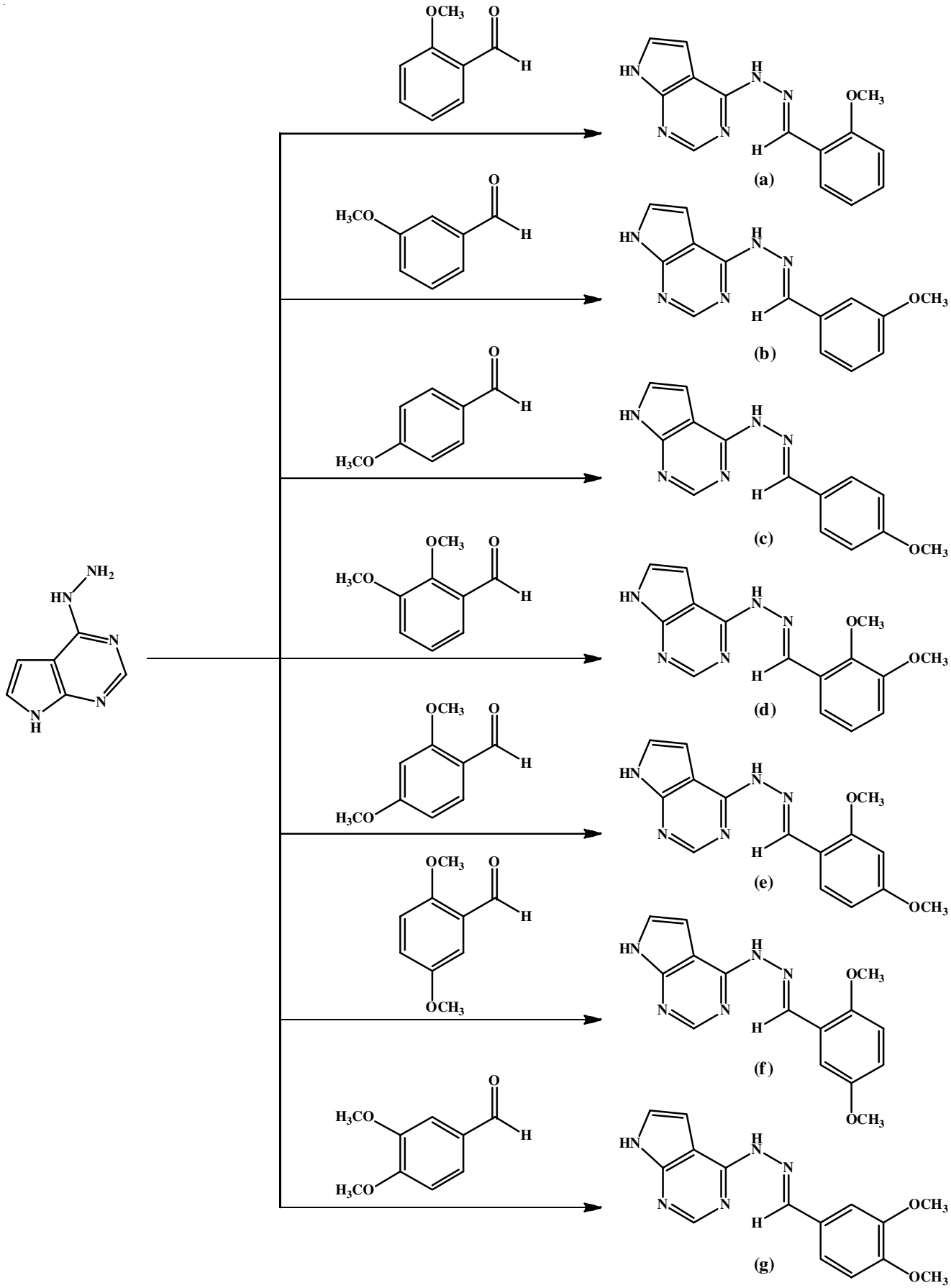
4-[(2*Z*)-2-(3-Methoxybenzylidene)hydrazinyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (b): Yield: 82.75%, m.p.: 188 ºC, IR (KBr, ν_{max}, cm^{-1}): 3424 (-NH- arom.), 3121 (-NH- aliph.), 2840 (-OCH_3), 3077 (-CH=), 1642 (>C=NN-), 1595 (>C=C<), 1326 (-C-O), 1033 (-N-N-), 872 (disub-benzene ring). ^{1}H NMR (DMSO-d_6) δ ppm: 14.31 (s, 1H, -NH- aliph.), 12.98 (s, 1H, NH, arom.), 8.75 (s, 1H, -CH=), 8.46 (s, 1H, pyrimidine-H), 7.06-8.00 (6H, 7.06 (1H, d, *J* = 3.89 Hz), 7.10 (1H, ddd, *J* = 8.20, 2.49, 1.71 Hz), 7.27 (1H, ddd, *J* = 2.49, 1.60, 0.55 Hz), 7.28 (1H, ddd, *J* = 8.22, 7.73, 0.59 Hz), 8.00 (1H, dt, *J* = 7.77, 1.66 Hz), 4.05 (s, 3H, -OCH_3). ^{13}C NMR (DMSO-d_6) δ ppm: 55.91 (-OCH_3), 142.56 (-CH=), 150.14 (C2), 100.06 (C3), 148.87 (C4), 114.82 (C5), 125.97 (C6), 103.16 (C2), 126.22 (C4). Anal. calcd. (found) % for $C_{14}H_{13}N_5O$ (*m.w.* 267.29): C, 62.91 (62.37); H, 4.90 (4.89); N, 26.20 (26.17); O, 5.99 (5.94).

4-[(2*Z*)-2-(4-Methoxybenzylidene)hydrazinyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (c): Yield: 85.69%, m.p.: 189 ºC, IR (KBr, ν_{max}, cm^{-1}): 3431 (-NH- arom.), 3118 (-NH- aliph.), 3078 (-OCH_3), 3098 (-CH=), 644 (>C=NN-), 1584 (>C=C<), 1265 (-C-O), 1020 (-N-N-), 733 (disub-benzene ring). ^{1}H NMR (DMSO-d_6) δ ppm: 14.39 (s, 1H, -NH- aliph.), 13.03 (s, 1H, NH, arom.), 8.78 (s, 1H, -CH=), 8.51 (s, 1H, pyrimidine-H), 7.12-7.72 (6H, 7.12 (1H, d, *J* = 3.75 Hz), 7.24 (2H, ddd, *J* = 8.81, 1.34, 0.48 Hz), 7.46 (2H, ddd, *J* = 8.80, 1.80, 0.47 Hz), 7.72 (1H, d, *J* = 3.88 Hz), 3.19 (s, 3H, -OCH_3). ^{13}C NMR (DMSO-d_6) δ ppm: 55.88 (-OCH_3), 142.52 (-CH=), 150.18 (C2), 100.04 (C3), 148.32 (C4), 114.73 (C5), 125.96 (C6), 103.14 (C2), 126.17 (C4). Anal. calcd. (found) % for $C_{14}H_{13}N_5O$ (*m.w.* 267.29): C, 62.91 (62.66); H, 4.90 (4.88); N, 26.20 (26.19); O, 5.99 (5.98).

4-[(2*Z*)-2-(2,3-Dimethoxybenzylidene)hydrazinyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (d): Yield: 80.53%, m.p.: 196 ºC, IR (KBr, ν_{max}, cm^{-1}): 3424 (-NH- arom.), 3128 (-NH- aliph.), 3083 (-OCH_3), 3005 (-CH=), 1668 (>C=NN-), 1589 (>C=C<), 1378 (-C-O), 1025 (-N-N-), 768 (trisub-benzene ring). ^{1}H NMR (DMSO-d_6) δ ppm: 14.25 (s, 1H, -NH- aliph.), 12.97 (s, 1H, NH, arom.), 8.73 (s, 1H, -CH=), 8.46 (s, 1H, pyrimidine-H), 7.09-8.01 (5H, m, *J* = 3.25 Hz), 7.09 (1H, dd, *J* = 8.75, 2.44 Hz), 7.12 (1H, d, *J* = 3.81 Hz), 7.16 (1H, dd, *J* = 8.20, 2.47 Hz), 7.17 (1H, dd, *J* = 8.61 Hz), 8.01 (1H, d, *J* = 3.78 Hz), 3.87 (s, 3H, -OCH_3). ^{13}C NMR (DMSO-d_6) δ ppm: 55.91 (-OCH_3), 142.57 (-CH=), 150.18 (C2), 100.10 (C3), 148.84 (C4), 114.83 (C5), 125.90 (C6), 103.22 (C2), 114.98 (C3), 126.26 (C4). Anal. calcd. (found) % for $C_{15}H_{15}N_5O_2$ (*m.w.* 297.31): C, 60.60 (59.99); H, 5.09 (4.99); N, 23.56 (23.51); O, 10.76 (10.71).

4-[(2*Z*)-2-(2,4-Dimethoxybenzylidene)hydrazinyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (e): Yield: 79.29%, m.p.: 197 ºC, IR (KBr, ν_{max}, cm^{-1}): 3425 (-NH- arom.), 3117 (-NH- aliph.), 3094 (-OCH_3), 3006 (-CH=), 1632 (>C=NN-), 1587 (>C=C<), 1349 (-C-O), 1025 (-N-N-), 887 (trisub-benzene ring). ^{1}H NMR (DMSO-d_6) δ ppm: 13.82 (s, 1H, -NH- aliph.), 12.94 (s, 1H, NH, arom.), 8.58 (s, 1H, -CH=), 8.46 (s, 1H, pyrimidine-H), 7.08-7.45 (5H, m, *J* = 3.19 Hz), 7.08 (1H, dd, *J* = 8.79, 2.64 Hz), 7.09 (1H, dd, *J* = 2.68, 0.45 Hz), 7.11 (1H, d, *J* = 3.89 Hz), 7.19 (1H, d, *J* = 3.81 Hz), 7.45 (1H, dd, *J* = 8.78, 0.44 Hz), 3.91 (s, 6H, -OCH_3). ^{13}C NMR (DMSO-d_6) δ ppm: 56.62, 56.22 (-OCH_3), 142.69 (-CH=), 100.21 (C3), 110.10 (C4), 112.44 (C5), 123.70 (C6), 150.56 (C2), 103.11 (C3), 152.25 (C4). Anal. calcd. (found) % for $C_{15}H_{15}N_5O_2$ (*m.w.* 297.31): C, 60.60 (59.88); H, 5.09 (4.87); N, 23.56 (22.24); O, 10.76 (9.91).

4-[(2*Z*)-2-(2,5-Dimethoxybenzylidene)hydrazinyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (f): Yield: 86.70 %, m.p.: 198 ºC, IR (KBr, ν_{max}, cm^{-1}): 3433 (-NH- arom.), 3123 (-NH- aliph.), 3082 (-OCH_3), 2832 (-CH=), 1633 (>C=NN-), 1589 (>C=C<), 1264 (-C-O), 1037 (-N-N-), 763 (trisub-benzene ring). ^{1}H NMR (DMSO-d_6) δ: 13.93 (s, 1H, -NH- aliph.), 12.98 (s, 1H, NH, arom.), 8.99 (s, 1H, -CH=), 8.45 (s, 1H, pyrimidine-H), 7.04-7.92 (5H, m, *J* = 3.29 Hz), 7.04 (1H, d, *J* = 3.84 Hz), 7.19



Scheme-I: Synthetic pathways for compounds **a-g**

(1H, dd, *J* = 8.61, 0.48 Hz), 7.20 (1H, dd, *J* = 8.60, 2.87 Hz), 7.27 (1H, dd, *J* = 2.91, 0.49 Hz), 7.92 (1H, d, *J* = 3.88 Hz), 3.89 (s, 6H, -OCH_3). ^{13}C NMR (DMSO-d_6) δ ppm: 56.45, 56.80 (-OCH_3), 142.67 (-CH=), 149.05 (C2), 100.16 (C3), 113.76 (C4), 153.26 (C5), 122.13 (C6), 103.12 (C2), 111.61 (C3), 152.25 (C4). Anal. calcd. (found) % for $C_{15}H_{15}N_5O_2$ (*m.w.* 297.31): C, 60.60 (60.41); H, 5.09 (5.08); N, 23.56 (23.44); O, 10.76 (10.69).

4-[(2*Z*)-2-(3,4-Dimethoxybenzylidene)hydrazinyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (g): Yield: 81.14%, m. p. 196 ºC, IR (KBr, ν_{max}, cm^{-1}): 3411 (-NH- arom.), 3122 (-NH- aliph.), 3099 (-CH=), 3012 (-OCH_3), 1582 (>C=C<), 1633 (>C=NN-), 1308/1284 (-C-O), 1023 (-N-N-), 867 (trisub-benzene ring). ^{1}H NMR (DMSO-d_6) δ ppm: 14.14 (s, 1H, -NH- aliph.), 12.92 (s, 1H, NH, arom.), 8.65 (s, 1H, -CH=), 8.44 (s, 1H, pyrimidine-H), 7.64-7.75 (5H, m, *J* = 3.06 Hz), 7.64 (1H, d, *J* = 3.84 Hz), 7.72 (1H, dd, *J* = 1.91, 0.47 Hz), 7.66 (1H, dd, *J* = 1.90, 0.47 Hz), 7.67 (1H, dd, *J* = 8.41, 1.94 Hz), 7.75 (1H, d, *J* = 3.78 Hz), 3.90 (s, 6H, -OCH_3). ^{13}C NMR (DMSO-d_6) δ ppm: 56.09, 56.56 (-OCH_3), 142.61 (-CH=), 100.06 (C2), 149.66 (C3), 152.07 (C4), 111.74 (C5), 123.61 (C6), 103.19 (C2), 110.01 (C3), 152.25 (C4). Anal. calcd. (found) % for $C_{15}H_{15}N_5O_2$ (*m.w.* 297.31): C, 60.60 (60.49); H, 5.09 (5.06); N, 23.56 (23.51); O, 10.76 (10.72).

Biological screening

Antibacterial screening: The antimicrobial activity of the synthesized compounds was assessed using two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative bacteria. (*Escherichia coli* and *Pseudomonas aeruginosa*). For antibacterial analysis, Muller-Hilton agar medium was autoclaved at 15 lbs/in^2 for 15 min [24]. The antimicrobial activity of the synthesized compounds was evaluated using the disc diffusion technique. The size of the inoculum was modified to roughly 10^8 cfu/mL to assess antimicrobial activity by suspending the culture in sterile distilled water. The microbial strain cultures were swabbed into Petri dishes containing 20 mL of Muller Hilton agar medium and left for 15 min to enable culture absorption. The wells (6 mm in diameter) were made using a clean borer and 100 L of compound in DMSO was added to the pre-inoculated plates. At 37 ºC, each dish was incubated for 24 h. DMSO was used as a negative control and streptomycin was used as a positive control.

Antifungal activity: The compounds were also tested against two fungi (*Candida albicans* and *S. cerevisiae*) using the cup-and-plate method [25]. Using a micropipette, the test solution was injected into the 5 mm diameter and 1 mm-thick disc. The plates were then kept at 37 ºC for 72 h. The diameter of the inhibition was determined after 36 h of incubation at 37 ºC. MIC was used in diagnostic labs to verify microorganism resistance to antimicrobial agents and check the effectiveness of new antimicrobial agents.

***In vitro* cytotoxicity:** In a brine shrimp bioassay, the cytotoxicity of the synthesized compounds was assessed [26]. In a divided tank with artificial seawater (38 g NaCl/1000 mL tap water), shrimp eggs were put in one half while the other half was covered. The shrimp needed 48 h to hatch and transform into nauplii. For a bioassay, the recently hatching crustaceans were taken out. The sample containers were filled with dried complexes at different concentrations (2.5, 5, 7.5, 10 and 12.5 mg/mL). To evaluate the potential cytotoxicity of the compounds, DMSO was dissolved in them. Each test container was filled with 10 live shrimp using a Pasteur pipette. A control group was added to verify the test method and the inferences based on the test agent's cytotoxic activity. The tubes were inspected under a microscope after 24 h and observations and the quantity of surviving nauplii in each vial were noted. Five copies of each experiment were made and then run three more times. Abbott's formula [27] was used to correct the statistics when there were control deaths:

$$\text{Deaths (\%)} = \frac{\text{Test} - \text{Control}}{\text{Control}} \times 100$$

RESULTS AND DISCUSSION

This study investigated the reaction of substituted methoxy benzaldehyde with 4-hydrazinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine to obtain methoxy benzaldehyde substituted derivatives of pyrazolopyrimidine-4-hydrazide in a good to moderate yields.

By comparing the FTIR spectra of the synthesized molecules with those of free 4-hydrazinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine, it was possible to analyze the bonding of 4-hydrazinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine to substituted methoxy benzaldehydes. The development of all synthesized compounds is confirmed by the lack of stretching vibrations caused by aldehyde (CHO) and amino (NH_2) moieties. Instead, a strong new band formed at region 1538-1512 cm^{-1}, corresponding to the azomethine (HC=NN-) group [28]. A broadband suggests the presence of the synthesized compounds in the 3433-3274 cm^{-1}, which is attributed to the aromatic (NH) [29,30]. A peak at 3064-3122 cm^{-1} can be ascribed to the aromatic -OCH_3 group. All prepared compounds are observed aldehydic (-CH=) bands in the 2836-2718 cm^{-1} range. The infrared spectra of compounds **a-g** exhibit sharp peaks at 1589-1582 and 1462-1421 cm^{-1}, assoc-iated with the >C=C< group of an aromatic ring, whereas the strong bands at 1335-1316, 739-722 and 691-654 cm^{-1} are ascribed to the aromatic (C–N), di/trisubstituted benzene ring and monosubstituted benzene ring, respectively.

The pyrrolyl ring's aromatic -NH- moiety is responsible for the broad singlet signals observed at δ 12.919-13.029 ppm in the ^{1}H NMR spectra of all the synthesized compounds. The aliphatic -NH- singlet peak appeared at δ 13.931-14.387 ppm and the aldehydic -CH= group of all the synthesized compounds is assigned to another singlet peak at δ 8.653-8.995 ppm range. Moreover, ^{1}H NMR spectra show no broad singlet signature at 9.84 ppm (2H), which corresponds to -NH_2 of 4-hydrazinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine, proving that Schiff base was successful in replacing the amino group [31]. For molecules **a-g**, the ^{1}H NMR spectra revealed a singlet for the pyrimidine proton at δ 8.444-8.513 ppm. The singlet signals in all the synthesized compounds are in between δ 3.190 and 4.050 ppm. The ^{1}H NMR bands are consistent with the reported works [31,32].

TABLE-1
BIOLOGICAL ACTIVITY DATA OF METHOXY BENZALDEHYDE SUBSTITUTED DERIVATIVES OF PYRAZOLO PYRIMIDINE-4-HYDRAZIDE (**a-g**)

Compound	Zone of inhibition (mm)						Cytotoxicity LD_{50} (M)
	Antibacterial activity				Antifungal activity		
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*S. cerevisiae*	
HPPHoMB **(a)**	17.00	20.90	25.00	19.09	29.54	9.80	$> 4.45 \times 10^{-4}$
HPPHmMB **(b)**	18.44	20.30	20.56	19.56	30.87	25.21	$> 5.19 \times 10^{-4}$
HPPHpMB **(c)**	20.91	20.21	20.18	23.99	33.78	29.35	$> 3.89 \times 10^{-4}$
HPPH23DMB **(d)**	25.00	21.00	20.99	20.98	34.57	23.67	$> 4.49 \times 10^{-4}$
HPPH24DMB **(e)**	21.88	20.56	22.60	20.76	29.47	25.63	$> 7.31 \times 10^{-4}$
HPPH25DMB **(f)**	19.96	20.63	24.23	19.00	20.98	22.90	$> 4.01 \times 10^{-4}$
HPPH34DMB **(g)**	21.31	20.45	23.90	19.57	29.60	23.78	$> 6.99 \times 10^{-4}$
Ciprofloxacin	22.00	20.00	22.00	19.50	–	–	–
Fluconazole	–	–	–	–	15.9	11.30	–

The presence of a singlet was also observed in the ^{13}C NMR spectra of compounds **a** through **g** at δ 55.45-56.80 and δ 14.26-142.61 ppm, which are attributed to the -OCH_3 and -CH= groups, respectively. In the synthesized compounds, aromatic carbon is present at δ 149.05-150.18 (C2), 100.04-100.16 (C3), 148.32-148.87 (C4), 114.73-114.83 (C5) and 122.13-125.97 (C6) ppm and also pyrrolopyrimine carbon observed at δ 103.12-103.22 (C2), 110.01-11.98 (C3) and 126.17-126.22 (C4).

Antibacterial activity: The reference drug in present study was ciprofloxacin, a wide-spectrum antibacterial with a MIC of 10 g/mL against the bacterial species. The inhibition zones for *Staphylococcus aureus* (MCC 2010), *Pseudomonas aeruginosa* (MCC 2080), *Bacillus subtilis* (MCC 2010) and *Escherichia coli* (MCC 2412) were 17-25 mm, 20-21 mm and 18-26 mm, respectively (Table-1). The antibacterial findings demonstrated that the tested compounds were active against all the bacteria tested, with MICs ranging from 8 to 32 g/mL.

Considering each bacterial species, for *S. aureus*, the most active compound is compound **d** (25 mm) which was found to be more active than the reference drug, while, compound **g** was less active for *P. aeruginosa*. For *E. coli*, the most active compounds are **a**, **b** and **c**. In contrast, for *K. pneumonia*, the most active compounds are **c**, **e** and **g**. The antimicrobial results is likely a consequence of the easier penetration into cells of microorganisms with fewer lipophilic cell walls. This is presumably because the molecule can pass through the lipid cell membrane of Gram-negative bacteria thanks to the lipophilic alkyl chain. The results showed that as the length of the carbon chain increases, the antibacterial activity decreases. This might result from the carbon chain's bulkiness, which prevents the molecule from passing through the bacteria's cell membrane [29].

Antifungal activity: The reference drug used in this study was fluconazole with MIC 50 mg/mL against the tested fungal species; the inhibition zones were 16-25 mm for *Candida albicans* and 19-26 mm for *Saccharomyces cerevisiae*, respectively. From Table-1, all compounds tested showed high fungicidal potential with MIC of 54 mg/mL against *Candida albicans* and *Saccharomyces cerevisiae,* which is more potent than the reference drug.

***In vitro* cytotoxicity:** All the synthesized compounds had cytotoxic activity against *Artemia salina*, according to the data in Table-1, with LD_{50} values varying from 2.178 to 8.439 × 10^{-4} M/mL.

Conclusion

A novel substituted methoxy benzaldehydes derivatives of pyrazolopyrimidine-4-hydrazide (**a-g**), were successfully synthesized and characterized using the elemental, FT-IR, UV-vis, NMR spectral studies. The synthesized compounds displayed exceptional antimicrobial activity. On the sensitive cell lines, all synthesized compounds also exhibit a substantial cytotoxicity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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