European Journal of Medicinal Chemistry 48 (2012) 92–96

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis of pyrazolo[3,4-*b*]pyridines under microwave irradiation in multi-component reactions and their antitumor and antimicrobial activities – Part 1

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ARTICLE INFO

Article history:
Received 28 September 2011
Received in revised form
16 November 2011
Accepted 23 November 2011
Available online 1 December 2011

Keywords:
Pyrazolo[3,4-*b*]pyridine
Multi-component reactions
Microwave irradiation
Antimicrobial activities
Antitumor activities

ABSTRACT

An efficient one-pot synthesis in multi-component system (MRCs) for the preparation of pyrazolo[3,4-*b*] pyridine derivatives from the reaction of 5-amino-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazole with 4-anisaldehyde and *p*-substituted β-ketonitriles or with pyruvic acid and some aromatic aldehydes in acetic acid medium. The reactions were carried out by two different techniques, conventional heating and microwave irradiation. These compounds were screened for their antibacterial activity against Gram-positive bacteria (*Bacillus*), Gram-negative bacteria (*Escherichia coli, Enterobacter cloaca* and *serratia*) and antifungal activity against *Fusarium Oxysporum* and *Penicillium expansum*. Also, among the synthesized compounds **4a**–**f** tested for antitumor activity against liver cell line. Compounds 6-(4-Fluorophenyl)-4-(4-methoxyphenyl)-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**4e**) and 4-(4-Methoxyphenyl)-1-phenyl-3,6-di(pyridin-3-yl)-1H-pyrazolo[3,4-*b*] pyridine-5-carbonitrile (**4a**) showed the highest activity.

1. Introduction

Multi-component reactions (MCRs) are important in organic and medicinal chemistry [1–3]. This technique has been applied to develop the synthesis of organic compounds in a facile and benign way in comparison to multistage procedures. Microwave technique have made the organic synthesis more dynamic and effective than ever before due to the generally short reaction times, high purities and yields for the resulting products compared to conventional methods [4–6]. On the other hand, the pyrazole nucleus has long shown pharmacological interest as well as their antimicrobial [7], antifungal [8] and antitumor activities [9]. Pyrazolo[3,4-*b*]pyridine skeleton have proven to be interesting classes of heterocycles due to diverse biological properties including antitubercular, antibacterial and antioxidant activities [10–14]. Recently, many authors [15–17] synthesized pyrazolo[3,4-*b*]pyridine by novel methods. J.Quiroga and coworkers [18,19] have been prepared pyrazolo[3,4-*b*]pyridine by the reaction of5-amino-3-methyl/phenyl-1-phenyl/H-1*H*-pyrazole and chalcones of benzoyl acetonitrile/malononitrile with some aromatic aldehydes.

In this work we describe a novel synthesis of 6-substituted-4-(4-methoxyphenyl)-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazolo[3,4-*b*] pyridine-5-carbonitrile (**4**) and 4-substituted-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-6-carboxylic acid (**5**) in multi-component reactions by classical heating and microwave irridiation. A comparison of obtained results from both techniques were discussed.

2. Results and discussion

2.1. Chemistry

Our goal is the synthesis of 3-oxo-3-(pyridin-3-yl) propanenitrile (**2**) [20] in good yield by a modified method by using sodium hydride as basic catalyst instead of sodium ethoxide [21], as a key intermediate for the preparation of 5-amino-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazole (**3**). Compound (**3**) was synthesized in 75% yield based on a related literature procedure by methane sulphonic acid method [22] (Scheme 1).

The cyclocondensation in multi-component reactions of 5-amino-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazole (**3**), 4-anisaldehyde and some β-ketonitrile derivatives in boiling acetic acid in the presence of triethylamine as a catalyst gave 6-substituted-4-(4-methoxyphenyl)-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazolo[3,4-*b*] pyridine-5-carbonitrile(**4a**–**f**) in 63–81 % yields (Scheme 2).

Also, the reaction of compound (**3**) with some aromatic aldehydes and pyruvic acid in boiling acetic acid gave 4-substituted-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-6-carboxylic acid (**5a**–**g**) in 54–75% yields (Scheme 3).

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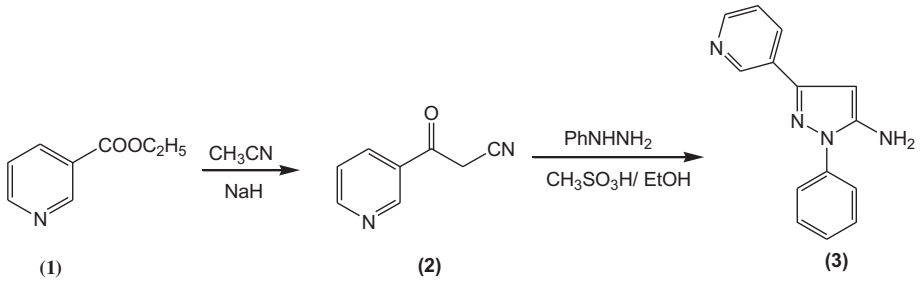
doi:10.1016/j.ejmech.2011.11.038



Scheme 1. 5-Amino-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazole (**3**).

The reactions described in Schemes 2 and 3 were also carried out under microwave conditions for a short reaction time to give products **4** and **5** in higher yields than those obtained by the conventional heating. The results are reported in (Table 1). Compounds **4** and **5** were found to be consistent in all respects with the ones produced by the traditional method.

It is worthy to note that the reactions carried out by microwave technology were done in few minutes (15 min) for compounds **4a**–**f** and in range (20–25 min) for compounds **5a**–**g** compared with classical heating methods. Also, the synthesized products were formed in pure form directly and in better yields (65–93%) (*cf.* Table 1).

3. Pharmacology

3.1. Antimicrobial evaluation

3.1.1. Tested organisms

The microorganisms used in this study included Gram-negative bacteria (*Escherichia coli, Enterobacter cloaca, serratia*), Gram-positive bacteria (*Bacillus)* in addition to some fungal species (*Fusarium oxysporum, Penicillium expansum*). The strains under study were obtained from the culture collection of Microbiology Unit, (Faculty of Science, Tanta University, Tanta, Egypt). Bacteria were cultured on nutrient agar and the fungus was cultured on sabaroud agar slopes.

3.1.2. Antibacterial screening

Antimicrobial activities of the synthesized compounds were tested *in vitro* against four different types of bacteria and two types of fungi by the cut-plug method according to Pridham et al. [23]. The assay plates were inoculated with the test bacteria by addition of one ml containing the diluted inoculums (10^7 CFU/ml) of each tested organism and spreaded on the corresponding media after solidification. It must be noted that, preliminary investigation had indicated that 10 mg/ml of the different compounds was the minimum inhibitory concentration (MIC) for the most tested bacteria. The wells were made then one mg of the synthesized compounds was dissolved in 100 ml DMSO and poured in the wells. The plates were incubated at 30 ± 1 °C for 24 h; thereafter the diameters of inhibition zones were evaluated.

3.1.3. Antibacterial activities

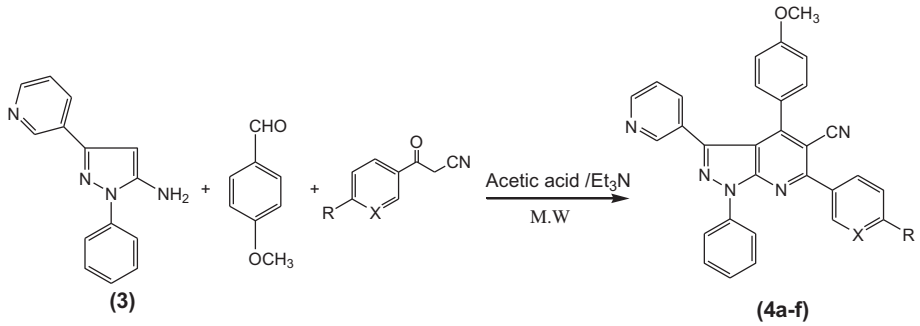
The results of the Antimicrobial activities are summarized in (Table 2). Among the compounds tested only **5a**, **5f**, **5d**, **5e** exhibited antibacterial activity of high order against all strains of the bacteria yeast used. Compounds **5b**, **4e**, **4d** exhibited nil to moderate activity against all strain of tested organisms. Compounds **4b**, **4c** exhibited inactivity against the tested organisms. For antifungal activity among the compounds tested only **4f**, **5a**, **5c**, **5d**, **5f**, **5g** exhibited marked activity against *Fusarium oxysporum* and *P. expansum*. Other compounds were inactive against the fungus-like yeast.

In general, most of the test compounds showed high activity against the gram-negative rather than the gram-positive bacteria and fungi. It would be also noticed that compounds **4a**–**f** (Scheme 2) exhibited higher antimicrobial activity than compounds **5a**–**g** (Scheme 3).

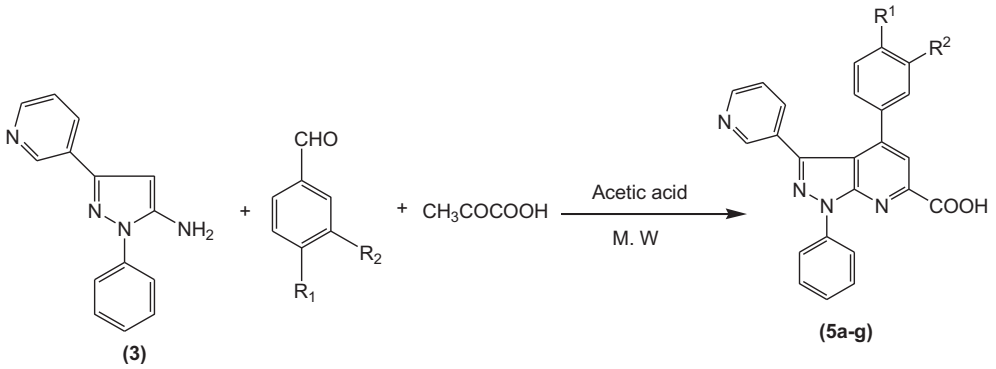
3.2. Antitumor evaluation

Compounds **4a**–**f** were tested for their antitumor activity against human liver cancer cell line (HEPG2) in the National Cancer Institute, Cairo University.

The screening involved calculation of the percentage growth of surviving fraction of the compound-treated cell lines compared by untreated control using Sulforhodamie B (SRB) colorimetric assay. Sulforhodamie B is a bright pink aminoxanthene anionic dye with two sulfonic acid groups that bind electrostatically to protein basic amino acid residues of trichloroacetic acid fixed cells under mild acidic conditions. Cultures fixed with trichloroacetic acid were stained for 30 min with 0.4% w/v Sulforhodamie B dissolved in 1% acetic acid, and proteinbound dye was extracted with 10 mM tris



Scheme 2. 6-Subsituted-4-(4-methoxyphenyl)-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**4a**–**f**).



Scheme 3. 4-Substituted-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-6-caboxylic acid (**5a**–**g**).

base for determination of optical density in a computer-interfaced, 96-well microtiter plate reader Skehan et al. [24].

The optical density measured is linear to the cell number of the surviving fraction. Therefore, the assay is a sensitive measure of compound-induced cytotoxicity with the best signal to noise ratio. The assay also, provides a colorimetric end point that is non-destructive, indefinitely stable and visible to naked eye.

Data were collected, revised and analyzed by SPSS statistical package version 11. Excel computer program was used to tabulate the results (Table 3). Probit regression analysis procedure had been introduced to select the best model that described the relationship between the probit (percentages of protection) as a dependant variable in order to be used for prediction of the concentration of the compound that caused inhibition of 50% (IC50) of cancer cells. The *in vitro* growth inhibition properties of each compound were described by IC50 and the degree of inhibition of cancer cell line was described by the equation:

$$\text{The probit (P)} = \text{intercept} + (\text{regression coefficient X Conc.})$$

Compounds **4a**–**f** show significant antitumor activity in which **4a** and **4e** were found to be the most effective ones (IC50 = 3.43–3.75 μg/ml).

4. Experimental

Melting points were measured on a Gallenkamp melting point apparatus. The infrared spectra were recorded on Perkin–Elmer1430 spectrophotometer using KBr disk technique. The ^{1}H NMR spectra were recorded in deuterated DMSO-d_6 on a Bruker AC spectrometer (300 MHz) using tetramethylsilane as an internal reference. Reactions under microwave irradiation were performed using Synthos 3000 dual magnetrons system in closed vessels under magnetic stirring and with maximum power of 1000 W. Reaction progress was monitored by thin layer chromatography (TLC) using benzene/acetone (2/1 by volume) as eluent. Mass spectra were recorded on a Finnigan MAT 8222 EX mass spectrometer at 70 eV. Microanalyses were carried out at Micro analytical center Cairo University.

The β-ketonitriles were prepared according literature procedures [25,26].

4.1. Synthesis of 5-amino-1-phenyl-3-(pyridin-3-yl)-1 H-pyrazole (*3*)

A mixture of β-ketonitrile (1.46 g, 10 mmol), ethanol (10 ml), phenylhydrazine (1.08 g, 10 mmol) and methanesulphonic acid (0.1 g, 1 mmol) was heated under reflux for 8 h. The solvent was removed under reduced pressure and the solid obtained was purified by washing with petroleum ether (40–60, 15 ml) to give faint brown crystals; yield, 75%. mp 210-212 °C; IR (KBr) ν_{max}/cm^{-1} = 1505 (C=N), 3419, 3287 (NH_2), 3063(Arom.–H). ^{1}HNMR (DMSO- d_6): δ_{ppm} = 5.63 (s, 2H, NH_2), 6.26 (s, 1H, CH $_{pyrazole}$), 7.33–7.74 (m, 5H, Ar-H), 8.12–9.00 (m, 4H, pyridine ring).

MS *m/z* (%): M^+ = 236 (20%), 103(22), 77(60), 51(100). Anal.; For $C_{14}H_{12}N_4$ (236.27) Calcd.: C 71.17; H 5.12; N 23.71%, Found: C 71.31; H 5.29; N 23.75%.

4.2. General procedure for the synthesis 6-substituted-4-(4-methoxyphenyl)-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-b] pyridine-5-carbonitrile (*4a*–*f*)

4.2.1. Method (A)

A mixture of 3 (1.18 g, 5 mmol), β-ketonitrile (5 mmol) and 4-anisaldehyde (0.68 g, 5 mmol) in acetic acid (20 ml) and in the presence of TEA (1 ml) was heated under reflux for 4 h. The reaction

Table 1
Comparison between reaction times and yield for compounds (**4a**–**f**), (**5a**–**g**) from conventional and microwave heating.

Compound	X	R	R_1	R_2	Conventional heating		Microwave irradiation	
					Yield (%)	Time (h)	Yield (%)	Time (min)
4a	N	H	—	—	81	4	93	15
4b	CH	H	—	—	78	4	86	15
4c	CH	Cl	—	—	72	4	88	15
4d	CH	CH_3	—	—	80	4	86	15
4e	CH	F	—	—	79	4	98	15
4f	CH	NO_2	—	—	63	4	89	15
5a	—	—	OCH_3	H	67	6	69	20
5b	—	—	H	H	54	6	65	20
5c	—	—	$N(C_2H_5)_2$	H	62	6	78	20
5d	—	—	OH	H	58	6	68	20
5e	—	—	Br	H	75	6	84	25
5f	—	—	OCH_3	OCH_3	71	6	81	25
5g	—	—	$N(CH_3)_2$	H	73	6	88	25

Table 2
Diameters of inhibition zones (mm) of newly synthesized compounds against different test bacteria on nutrient agar at 30 °C after 24 h by the cut-plug method.[a]

Compound	*Bacillus*	*Echerechia coli*	*Enterobacter cloaca*	*Serratia*	*Fusarium oxysporum*	*Penicillium expansum*
4a	0.7	-ve	-ve	-ve	-ve	-ve
4b	-ve	-ve	-ve	-ve	-ve	-ve
4c	-ve	-ve	-ve	-ve	-ve	-ve
4d	-ve	1.3	-ve	1.45	-ve	-ve
4e	-ve	-ve	-ve	1.05	-ve	-ve
4f	-ve	1.05	-ve	-ve	-ve	0.5
5a	1.15	2.2	2.5	1.6	0.3	-ve
5b	-ve	-ve	-ve	2.75	-ve	-ve
5c	-ve	-ve	-ve	-ve	0.4	-ve
5d	-ve	-ve	-ve	2.6	0.6	-ve
5e	-ve	-ve	-ve	3.05	-ve	-ve
5f	**1.2**	2	2.3	1.4	0.8	0.4
5g	-ve	-ve	0.2	0.3	0.6	-ve
Chloramphenicol	2.1	1.8	1.7	2.58	1.2	1

[a] The concentration used is 10 mg/ml. Control discs were performed in DMSO (dimethylsulphoxide) and no zones of inhibitions were observed. –ve = resistant.

mixture was poured into crushed ice (20 ml) and the solid obtained was collected by filtration, washed with water (3 × 10 ml) and recrystallized from ethanol to give pure **4**.

4.2.2. Method (B)

The procedure was similar to that described in Method A except that the mixture was capped in closed vessels and irradiated in a microwave oven at 150 °C for 15 min using Synthos 3000 (500 W). The reaction was worked and the solid obtained was recrystallized from ethanol to give **4**.

*4.2.3. 4-(4-Methoxyphenyl)-1-phenyl-3,6-di(pyridin-3-yl)-1 H-pyrazolo[3,4-b] pyridine-5-carbonitrile (**4a**)*

Faint green crystals; mp 231-233 °C; IR (KBr) ν_{max} /cm^{-1} = 1505 (C═N), 2218 (C≡N), 2923 (Aliph.–H), 3133(Arom.–H). ^{1}HNMR (DMSO- d_6): δ_{ppm} = 3.84 (s, 3H, OCH_3), 6.94–7.62 (m, 9H, Ar-H), 8.42–9.33 (m, 8H, pyridine rings). MS *m/z* (%): M^+ = 480 (100%), 479 (58), 450 (20), 449 (42), 240 (11), 77 (18), 51 (11). Anal.; For $C_{30}H_{20}N_6O$ (480.52) Calcd.: C 74.99; H 4.20; N 17.49%, Found: C 75.01; H 4.29; N 17.50%.

*4.2.4. 4-(4-Methoxyphenyl)-1,6-diphenyl-3-(pyridin-3-yl)-1 H-pyrazolo[3,4-b] pyridine-5-carbonitrile (**4b**)*

Buff crystals; mp 222–224 °C; IR (KBr) ν_{max}/cm^{-1} = 1501 (C═N), 2217 (C≡N), 2935 (Aliph.–H), 3128 (Arom.–H). ^{1}HNMR (DMSO- d_6): δ_{ppm} = 3.84 (s, 3H, OCH_3), 6.92–7.83 (m, 14H, Ar-H), 7.92–8.84 (m, 4H, pyridine ring). MS *m/z* (%): M^+ = 479(100%), 477(24), 448(22), 372(31), 357(26), 344(23), 282(30), 239(19), 224(22), 196(23), 121(24), 105(27), 77(65), 51(25). Anal.; For $C_{31}H_{21}N_5O$ (479.53) Calcd.: C 77.64; H 4.41; N 14.60%, Found: C 77.80; H 4.20; N 14. 65%.

*4.2.5. 6-(4-Chlorophenyl)-4-(4-methoxyphenyl)-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (**4c**)*

Yellowish green crystals; mp 262-264 °C; IR (KBr) ν_{max}/cm^{-1} = 1504 (C═N), 2214 (C≡N), 2963 (Aliph.–H), 3133(Arom.–H). ^{1}HNMR (DMSO- d_6): δ_{ppm} = 3.77 (s, 3H, OCH_3), 6.93–7.65 (m, 13H, Ar-H), 7.82–8.83 (m, 4H, pyridine ring). MS *m/z* (%): M^+ = 513(100%), M + 2 = 515(36%), 483(21), 455(63), 406(36), 369(24), 355(17), 241(16), 139(24), 121(67), 77(67), 51(29). Anal.; For $C_{31}H_{20}ClN_5O$ (513.98) Calcd.: C 72.44; H 3.92; Cl 6.90; N, 13.63%, Found: C 72.48; H 3.78; Cl 7.02; N 13.67%.

Table 3
Antitumor activity of compounds **4a**–**f** against liver cancer cell line.

Test compound	IC_{50} (μg/ml)	IC_{50} (nmol/ml)
4a	3.73	7.76
4b	-ve	-ve
4c	4	9.18
4d	4.34	10.6
4e	3.43	7.27
4f	13.5	29.8
DOX	3.73	

*4.2.6. 4-(4-Methoxyphenyl)-1-phenyl-3-(pyridin-3-yl)-6-p-tolyl-1H-pyrazolo[3,4-b] pyridine-5-carbonitrile (**4d**)*

Brownish crystals; mp 295-297 °C; IR (KBr) ν_{max}/cm^{-1} = 1504 (C═N), 2197 (C≡N), 2935 (Aliph.–H), 3182(Arom.–H). ^{1}HNMR (DMSO- d_6): δ_{ppm} = 2.40(s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 6.92–7.55 (m, 13H, Ar-H), 7.83–8.85 (m, 4H, pyridine ring). MS *m/z* (%): M^+ = 493(64%), 464(20), 417(23), 414(40), 384(21), 310(100), 247(26), 200(15), 186(13), 135(13), 121(25), 77(50), 64(20). Anal.; For $C_{32}H_{23}N_5O$ (493.56) Calcd.: C 77.87; H 4.70; N 14.19% Found: C 77.90; H 4.76; N 14.20%.

*4.2.7. 6-(4-Fluorophenyl)-4-(4-methoxyphenyl)-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (**4e**)*

Faint brown crystals; mp 265-267 °C; IR (KBr) ν_{max}/cm^{-1} = 1505 (C═N), 2213 (C≡N), 2964 (Aliph.–H), 3129 (Arom.–H). ^{1}HNMR (DMSO- d_6): δ_{ppm} = 3.78 (s, 3H, OCH_3), 6.92–7.65 (m, 13H, Ar-H), 7.82–8.84 (m, 4H, pyridine ring). MS *m/z* (%): M^+ = 496(100%), 466(24), 439(26), 389(26), 361(18), 248(18), 233(24), 196(24), 139(24), 77(60), 51(27).. Anal.; For $C_{31}H_{20}FN_5O$ (497.52) Calcd.: C 74.84; H 4.05; F 3.82; N 14.08%, Found: C 74.92; H 4.11; F 3.84; N 14.12%.

*4.2.8. 4-(4-Methoxyphenyl)-6-(4-nitrophenyl)-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo [3,4-b]pyridine-5-carbonitrile (**4f**)*

Brown crystals; mp 321–323 °C; IR (KBr) ν_{max}/cm^{-1} = 1507 (C═N), 2211 (C≡N), 2958 (Aliph.–H), 3178(Arom.–H). ^{1}HNMR (DMSO- d_6): δ_{ppm} = 3.78 (s, 3H, OCH_3), 6.90–7.81 (m, 13H, Ar-H), 7.92–8.94 (m, 4H, pyridine ring). MS *m/z* (%): M^+ = 524(64%), 511(45), 466(79), 441(81), 398(48), 373(58), 354(100), 310(53), 252(76), 247(53), 209(54), 194(57), 167(71), 89(73). Anal.; For $C_{31}H_{20}N_6O_3$ (524.53) Calcd.: C 70.98; H 3.84; N 16.02%, Found: C 71.02; H 3.92; N 16.04%.

*4.3. General procedure for the synthesis 4-substituted-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-b]pyridine-6-carboxylic acid (**5a**–**g**)*

4.3.1. Method (A)

A mixture of **3** (1.18 g, 5 mmol), pyruvic acid (0.44 g, 5 mmol) and aromatic aldehydes (5 mmol) in acetic acid (10 ml) was heated under reflux for 6 h. The reaction mixture was poured into crushed ice (20 ml) and the solid obtained was collected by filtration, washed with water (2 × 10 ml) and recrystallized from ethanol to give pure **5**.

4.3.2. Method (B)

The procedure was similar to that described in Method A except that the mixture was capped in closed vessels and irradiated in a microwave oven at 160 °C for 20 min using Synthos 3000 (500 W). The reaction was worked and the solid obtained was recrystallized from ethanol to give **5**.

*4.3.3. 4-(4-Methoxyphenyl)-1-phenyl-3-(pyridin-3-yl)-1 H-pyrazolo[3,4-b]pyridine-6-carboxylic acid (**5a**)*

Buff-green crystals; mp 233–235 °C; IR (KBr) ν_{max}/cm^{-1} = 1601(C=O), 3000 (Arom.–H), 3410 (OH). ^{1}HNMR (DMSO- d_6): δ_{ppm} = 3.92 (s, 3H, OCH_3), 7.02–7.81 (m, 9H, Ar-H), 8.13–9.14 (m, 5H, pyridine rings), 9.83 (s, 1H, COOH). MS *m/z* (%): M^+ = 422(48%), 405(15), 377(24), 354(18), 287(21), 247(14), 236(18), 121(33), 105(14), 91(20), 77(100), 65(13). Anal.; For $C_{25}H_{18}N_4O_3$ (422.44) Calcd.: C 71.08; H 4.29; N 13.26% Found: C 71.22; H 4.33; N 13.31%.

*4.3.4. 1,4-Diphenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-b] pyridine-6-carboxylic acid (**5b**)*

Brown crystals; mp 237–239 °C; IR (KBr) ν_{max}/cm^{-1} = 1591(C=O), 3058 (Arom.–H), 3376 (OH). ^{1}HNMR (DMSO- d_6): δ_{ppm} = 6.93–7.82 (m, 10H, Ar-H), 8.14–9.12 (m, 5H, pyridine rings), 9.92 (s, 1H, COOH). MS *m/z* (%): M^+ = 392(100%), 387(30), 348(73), 321(31), 268(34), 245(55), 219(32), 189(38), 160(49), 134(31), 98(52), 83(46). Anal.; For $C_{24}H_{16}N_4O_2$ (392.41) Calcd.: C 73.46; H 4.11; N 14.28% Found: C 73.52; H 4.14; N 14.31%.

*4.3.5. 4-(4-(Diethylamino)phenyl)-1-phenyl-3-(pyridin-3-yl)-1 H-pyrazolo[3,4-b]pyridine-6-carboxylic acid (**5c**)*

Yellowish brown crystals; mp 354–356 °C; IR (KBr) ν_{max}/cm^{-1} = 1597(C=O), 2968 (Aliph.–H), 3058 (Arom.–H), 3396 (OH). ^{1}HNMR (DMSO- d_6): δ_{ppm} = 1.73 (t, J = 6.9, 6H, $2CH_3$), 3.42 (q, J = 7.1, 4H, $2CH_2$), 6.92–7.83 (m, 9H, Ar-H), 8.15–9.13 (m, 5H, pyridine rings), 9.93 (s, 1H, COOH). MS *m/z* (%): M^+ = 463(54%), 433(51), 418(53), 391(94), 373(46), 310(80), 298(83), 285(100), 271(54), 153(71), 135(43), 106(77), 77(96), 50(33). Anal.; For $C_{26}H_{21}N_5O_2$ (463.53) Calcd.: C 72.55; H 5.44; N 15.11% Found: C 72.83; H 5.53; N 15.23%.

*4.3.6. 4-(4-Hydroxyphenyl)-1-phenyl-3-(pyridin-3-yl)-1 H-pyrazolo[3,4-b]pyridine-6-carboxylic acid (**5d**)*

Dark brown crystals; mp 320–322 °C; IR (KBr) ν_{max}/cm^{-1} = 1590 (C=O), 3063 (Arom.–H), 3390 (OH).^{1}HNMR (DMSO- d_6): δ_{ppm} = 5.42 (s, H, OH), 6.94–7.84 (m, 9H, Ar-H), 8.12–9.16 (m, 5H, pyridine rings), 9.90 (s, 1H, COOH). MS *m/z* (%): M^+ = 408(32%), 385(34), 363(33), 331(31), 298(25), 285(26), 244(22), 232(26), 196(23), 171(18), 137(23), 104(26), 77(100), 59(17). Anal.; For $C_{24}H_{16}N_4O_3$ (408.41) Calcd.: C 70.58; H 3.95; N 13.72% Found: C 70.76; H 3.90; N 13.55%.

*4.3.7. 4-(4-Bromophenyl)-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo [3,4-b]pyridine-6-carboxylic acid (**5e**)*

Brown crystals; mp 288–290 °C; IR (KBr) ν_{max}/cm^{-1} = 1587(C=O), 3058 (Arom.–H), 3386 (OH).^{1}HNMR (DMSO- d_6): δ_{ppm} = 6.93–7.82 (m, 9H, Ar-H), 8.14–9.12 (m, 5H, pyridine rings), 9.93 (s, 1H, COOH). MS *m/z* (%): M^+ = 472(83%), 426(58), 392(72), 361(55), 313(100), 294(60), 268(95), 259(42), 244(67), 199(50), 178(43), 147(62), 97(42), 80(64). Anal.; For $C_{24}H_{15}BrN_4O_2$ (471.31) Calcd.: C 61.16; H 3.21; Br 16.95; N, 11.89% Found: C 61.55; H 3.33; Br 17.05; N 12.01%.

*4.3.8. 4-(3,4-Dimethoxyphenyl)-1-phenyl-3-(pyridin-3-yl)-1 H-pyrazolo[3,4-b]pyridine-6-carboxylic acid (**5f**)*

Dark green crystals; mp 254–256 °C; IR (KBr) ν_{max}/cm^{-1} = 1591(C=O), 2853 (Aliph.–H), 3022 (Arom.–H), 3415 (OH). ^{1}HNMR (DMSO- d_6): δ_{ppm} = 3.82 (s, 6H, 2 OCH_3), 6.93–7.82 (m, 8H, Ar-H), 8.14–9.13 (m, 5H, pyridine rings), 9.90 (s, 1H, COOH).. MS *m/z* (%): M^+ = 452 (67%), 408(42), 391(42), 370(44), 320(40), 299(41), 280(63), 230(41), 203(53), 190(47), 153(61), 137(55), 81(100), 69(63). Anal.; For $C_{26}H_{20}N_4O_4$ (452.46) Calcd.: C 69.02; H 4.46; N 12.38% Found: C 69.22; H 4.66; N 12.49%.

*4.3.9. 4-(4-(Dimethylamino)phenyl)-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-b]pyridine-6-carboxylic acid (**5g**)*

Brown crystals; mp 318–320 °C; IR (KBr) ν_{max}/cm^{-1} = 1596 (C=O), 2919 (Aliph.–H), 3058 (Arom.–H), 3419 (OH). ^{1}HNMR (DMSO- d_6): δ_{ppm} = 2.44 (s, 6H, 2 CH_3), 6.90–7.88 (m, 9H, Ar-H), 8.12–9.15 (m, 5H, pyridine rings), 9.90 (s, 1H, COOH). MS *m/z* (%): M^+ = 435(72%), 391(97), 374(40), 347(44), 311(38), 286(51), 271(54), 243(36), 196(33), 181(35), 149(39), 105(55), 95(55), 77(100). Anal.; For $C_{28}H_{25}N_5O_2$ (435.48) Calcd.: C 71.71; H 4.86; N 16.08% Found: C 71.89; H 4.99; N 16.11%.

Acknowledgement

The authors express their deep thanks to Dr. M. E. H. Osman, Professor of plant physiology, Botany Department, Faculty of science, Tanta University, Tanta, for carrying out the biological activities at his research laboratory.

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