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Bioorganic & Medicinal Chemistry 12 (2004) 1845–1852
Bioorganic & Medicinal Chemistry

Synthesis, antimicrobial activity and conformational analysis of novel substituted pyridines: BF_3-promoted reaction of hydrazine with 2-alkoxy pyridines

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Received 4 December 2003; revised 22 January 2004; accepted 27 January 2004

Abstract—Some new 2-alkoxy-3-cyano-4,6-diarylpyridines **3**,**4** were synthesized by condensation of different α,β-unsaturated ketones **1** with malononitrile **2**, followed by cyclization in sodium alkoxide/alcohol system. Lewis acid-catalyzed reaction of **4** with hydrazine afforded the corresponding 1*H*-pyrazolo[3,4-*b*]pyridines **5**. The potency of the results as antimicrobial agents has been evaluated. The structure of the newly prepared compounds was assessed by microanalysis, IR and NMR spectra. Molecular mechanics (MM2) and semiemperical (AM1) molecular orbital calculations have been performed for the most biologically active compounds **5b** and **c** to get insight into their molecular structures and to learn more about their stable molecular conformations.

1. Introduction

The rising prevalence of multi-drug resistant Gram-positive and Gram-negative bacteria continues to provide impetus for the search and discovery of novel antimicrobial agents active against these pathogens. During the last two decades, a large number of substituted pyridines have been claimed to have several biological activities.[1–9] The antifungal and antibacterial properties of these compounds have opened up the possibility of their potential use as a novel class of totally synthetic antimicrobial agents active against pathogenic bacteria including *Pesudomonas aeruginosa* IFO 3448, *Escherechia coli* IFO 3301, *Staphylococcus aureus* IFO 3060, *Bacillus subtilis* IFO 3007 and *Candida albicans* IFO 0583.[3–9] Moreover, 1*H*-pyrazolo[3,4-*b*]pyridines comprise a very interesting class of compounds because of their significant and versatile biological and pharmacological activities, such as antimicrobial, antimalarial, antiviral and antiproliferative.[9–16]

Keywords: Pyridine; 1*H*-pyrazolo[3,4-*b*]pyridines; Antimicrobial; Conformational analysis; Molecular mechanics; Semiemperical molecular orbital.
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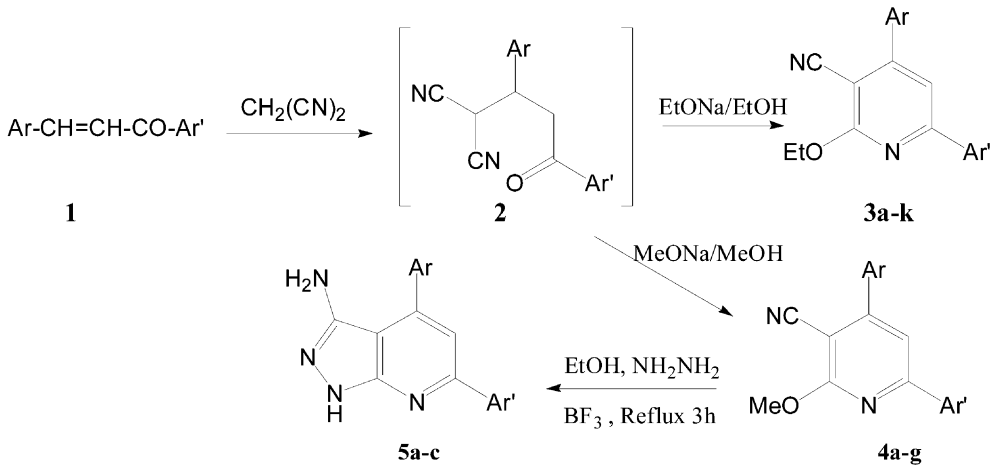
doi:10.1016/j.bmc.2004.01.040

2. Results and discussion

2.1. Chemistry

In the view of those reports, new compounds containing 2-alkoxy pyridine moieties and their fused 1*H*-pyrazolo[3,4-*b*]pyridines, have been designed to be tested as antimicrobial agents. It was reported that the reaction of chalcones with malononitrile and ethyl cyanoacetate in the presence of ammonium acetate and absolute ethanol afforded cyanopyridines in low yield.[17,18] Similarily, the preparation of 2-alkoxy cyanopyridines in good yields was reported via Michael addition of malononitrile to the ∝,β-unsaturated ketones.[19,20] In the present work ∝,β-unsaturated ketones **1** were condensed with malononitrile in either sodium methoxide/methanol or sodium ethoxide/ethanol to yield the corresponding cyanopyridines **3**,**4** in a good yield. It was observed that the increase in the carbon number of alkoxyl group caused much prolongation time of the reaction and relatively low yield (Scheme 1). The reaction proceeds through Michael addition of the ∝, β-unsaturated ketones to the malononitrile to afford adduct **2** which undergoes a nucleophilic attack by alkoxide anion followed by cyclization. Subsequent dehydration of the cyclized product leads to the 2-alkoxy cyanopyridines **3** or **4**. The structure was assigned on



Scheme 1. Synthesis of novel pyridines **3**,**4** and 1*H*-pyrazolo[3,4-*b*]pyridines **5**.

the basis of analytical and spectral data. The IR spectra showed a peak at 2250 cm^{-1} due to the cyano stretching frequency. In the ^1H NMR spectrum of the 2-ethoxy pyridines **3**, the ether group gave rise to a quartet around 4.62 ppm and a triplet at 1.44 ppm. The aromatic protons appear as a multiplet at 7.20–8.20 ppm. On the other hand, the 2-methoxypyridines **4** gave a singlet at 4.0 ppm characteristic for the methoxyl group and the aromatic protons appeared at 7.0–8.10 ppm.

It was reported that the 4-methoxyl group in pyrido[3,2-*d*]pyrimidines and 6,7-dimethoxyl group in 2-chloro-6,7-dimethoxyquinoline-3-carboxylic acid were readily susceptible to nucleophilic displacement by hydrazine.[21] Moreover, 2-methylthio-3-cyanopyridines have easily been converted to the corresponding 1*H*-pyrazolo[3,4-*b*]pyridines by refluxing with hydrazine hydrate.[9] Trials to apply the same idea failed in replacing the 2-alkoxyl group in compounds **3** or **4** using anhydrous hydrazine, with or without solvent, even with refluxing periods up to 72 h.[21] It is noteworthy, the usage of Lewis acid (1.0 equiv), $BF_3{\cdot}Et_2O$,[22] in refluxing ethanol under anhydrous condition yielded compounds **5a–c** with very good yields and short reaction time. IR spectra of each **5a–c** were found free from the bands of the nitrile function and instead the bands of the newly born NH_2 group were detected in each case. In addition no methoxyl proton signals were revealed in the ^1H NMR spectra of **5a–c**. On the above backgrounds, compounds **5a–c** were then formulated as the 1*H*-pyrazolo[3,4-*b*]pyridine derivatives. The role of Lewis acid could be understood on the basis of the coordination of Lewis acid with methoxyl group followed by the ease removal of methoxyl moiety (Scheme 1).[23]

Table 1. Antimicrobial screening results of the tested compounds at 1 mg/mL and their CLogP[a]

No.	Ar	Ar′	CLogP	*P. aeruginosa*	*E. coli*	*S aureus*	*B. subtilis*	*C. albicans*
3a	2-C_4H_3O	3-C_5H_4N	2.99	8	8	na	na	na
3b	2-C_4H_3S	4-NO_2-C_6H_4	4.66	9	na	10	na	na
3c	4-Cl-C_6H_4	3-C_5H_4N	4.31	na	na	na	na	8
3d	C_6H_5	3-C_5H_4N	3.60	na	na	na	na	9
3e	C_6H_5	4-NO_2-C_6H_4	4.77	na	10	na	na	na
3f	4-CH_3O-C_6H_5	3-C_5H_4N	3.61	na	15	na	na	14
3g	CH = CH-C_6H_5	3-C_5H_4N	4.40	na	9	na	na	na
3h	4-CH_3-C_6H_4	4-NO_2-C_6H_4	5.27	na	na	na	na	na
3i	4-CH_3O-C_6H_4	4-NO_2-C_6H_4	4.79	14	12	na	na	na
3j	3,4,5-$(CH_3O)_3$-C_6H_2	3,4-$(Cl)_2$-C_6H_3	5.75	na	18	na	14	na
3k	3,4,5-$(CH_3O)_3$-C_6H_2	4-Br-C_6H_4	5.00	na	17	na	na	13
4a	2-C_4H_3S	4-NO_2-C_6H_4	5.19	na	9	12	na	na
4b	C_6H_5	4-NO_2-C_6H_4	5.30	na	8	na	na	na
4c	4-CH_3O-C_6H_4	3-C_5H_4N	4.14	na	8	na	na	na
4d	CH = CH-C_6H_4	3-C_5H_4N	4.93	na	8	na	na	9
4e	4-CH_3O-C_6H_4	4-NO_2-C_6H_4	5.31	na	14	na	na	15
4f	3,4,5-$(CH_3O)_3$-C_6H_2	3,4-$(Cl)_2$-C_6H_3	6.28	na	18	na	na	8
4g	3,4,5-$(CH_3O)_3$-C_6H_2	4-Br-C_6H_4	5.53	na	18	na	na	9
5a	4-CH_3O-C_6H_4	4-NO_2-C_6H_4	4.33	na	14	na	na	8
5b	3,4,5-$(CH_3O)_3$-C_6H_2	3,4-$(Cl)_2$-C_6H_3	5.29	na	22	na	na	18
5c	3,4,5-$(CH_3O)_3$-C_6H_2	4-Br-C_6H_4	4.55	na	21	na	na	18

[a] Ref. 26. Strong activity (>15 mm), moderate activity (11–15 mm), weak activity (7–10 mm), na; no activity (inhibition zone < 7 mm), solvent: DMSO (6 mm).

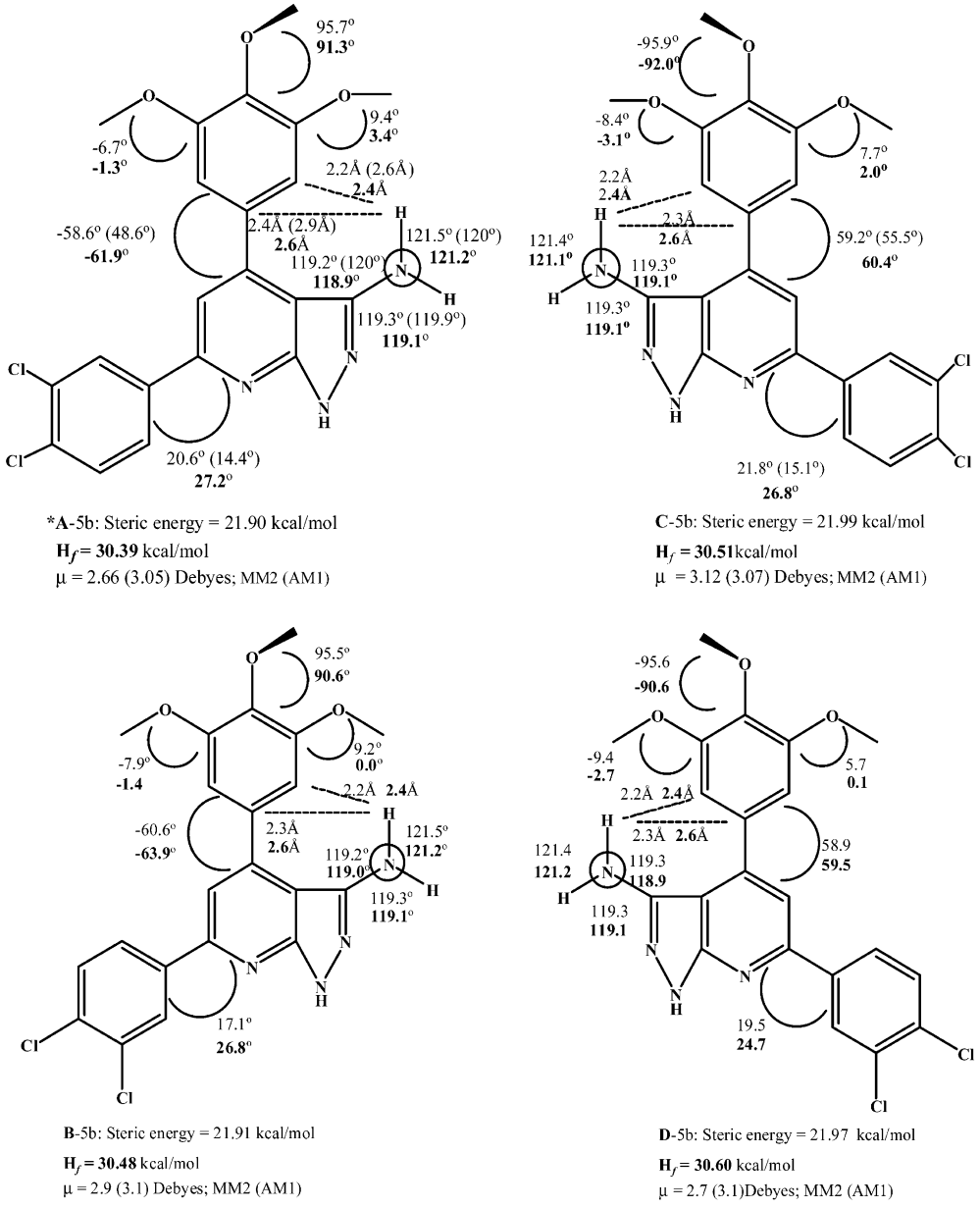
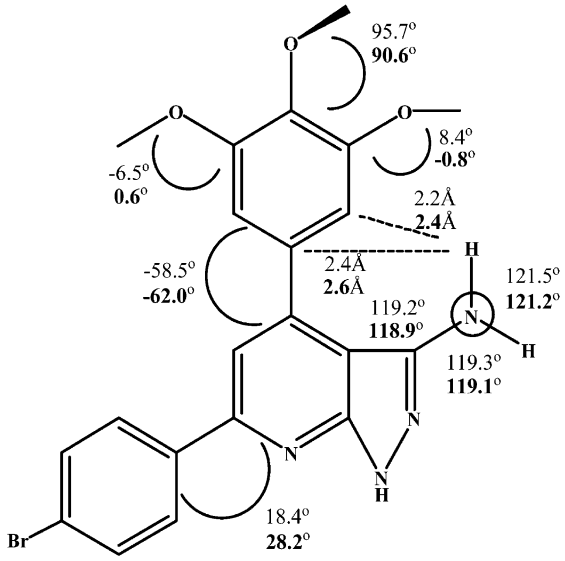


Figure 1. Four isomeric conformations of **5b** as minimized by MM2 and AM1 and their atomic coordinates and dipole moments. *Results in plain

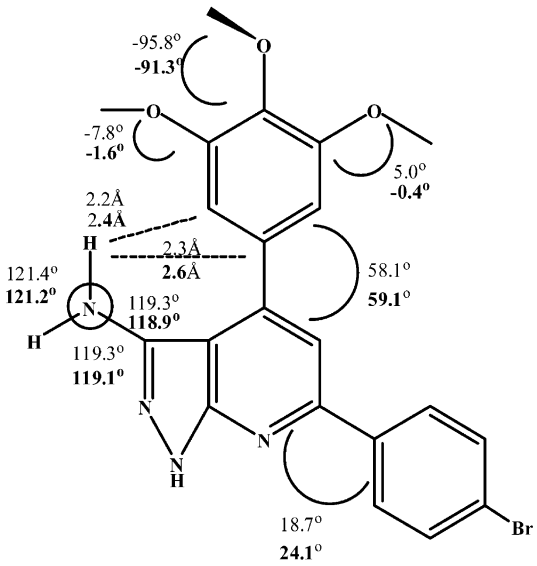
3. Antimicrobial

The antimicrobial screening of all the synthesized compounds was done using cup diffusion technique.[24] This screening was performed against the Gram-negative *Pesudomonas aeruginosa* and *Escherechia coli* and the Gram-positive *Staphylococcus aureus* and *Bacillus subtilis*, in addition to the pathogenic fungi *Candida albicans*. From the inhibition zone diameter data analysis, only compounds **3j**, **k**, **4f**, **g**, **5b** and **c** exhibited considerable antimicrobial activity against *E. coli* and *C. albicans* (Table 1). The maximum antimicrobial activity was observed with compound **5b**, which proved to possess remarkable activity against *E. coli* and *C. albicans*. Minimum inhibitory concentration (MIC)[25] was determined for each of the active compounds along with cefotaxime, gentamicin, streptomycin, clotrimazole and nystatin as standard controls; results are shown in (Table 2). Amongst all the compounds tested, **5b** and **5c**, demonstrated the most potent antimicrobial activity against both *E. coli* and *C. albicans*.

Attempts were made to correlate the antimicrobial activities of these compounds to the calculated log of partition coeffecient (ClogP).[26] However, no direct correlation could be established between the Clog P and antibacterial activity in series **3**, such as the Clog P values of compounds **3h** (5.27) and **k** (5.00), that were almost similar (Table 1). However, both of these compounds **3h** and **k** demonstrated different activity regarding with their inhibition zone diameter of 17 mm for **3k**, against *E. coli*, and **3h** was void of antimicrobial activity. Moreover, good correlation can be observed in series **4** and **5** as indicated by their MIC values (Table 2), such as the the Clog P values of compounds **4f** (6.28) and **g** (5.53) with MIC values of 2.6 and 2.8 μg/mL



A-5c: Steric energy = 19.84 kcal/mol; MM2
H_f = 47.57 kcal/mol; AM1
μ = 0.5 (2.2) Debyes; MM2 (AM1)



C-5c: Steric energy =19.93 kcal/mol; MM2
H_f =47.90 kcal/mol; AM1
μ = 0.5 (2.3) Debyes; MM2 (AM1)

Figure 2. Two isomeric conformations of **5c** as minimized by MM2 and AM1 and their atomic coordinates and dipole moments.

against *E. coli* respectively. In addition, compounds **5b** and **5c** demonstrated MIC values of 1.6 and 1.8 μg/mL against *E. coli* with Clog P values of 5.29 and 4.55 respectively (Table 2). It becomes apparent that the criteria relating to favorable Clog P value range may partially be the sole predicting factor for antimicrobial activity; since most of the compounds reported in the present study showed ClogP values (4.0–6.3). This further indicated significance of the trimethoxyl group present in compounds **3j**, **k**, **4f**, **g**, **5b** and **c** for antimicrobial activity compared to the others. Previous studies[21] have revealed that compound containing Ar = 3,4,5-$(MeO)_3$-C_6H_2; Ar′ = C_6H_5 was less active than other compound containing halgen atom (Ar′ = *p*-Cl-C_6H_4). It is noteworthy that the antimicrobial activity was reinforced by the presence of one or two halogen atoms on the phenyl ring (Ar′). However, other structural requirements including the presence of the 4-nitro, 4-methyl and 4-methoxyl groups on the phenyl ring, or replacement of phenyl ring with thiophene, furan or even pyridine nucleus did not improve the activity.

3.1. Conformational analysis of 1*H*-pyrazolo[3,4-*b*]pyridines 5b and 5c

An attempt to gain a better insight on the molecular structures of the most active compounds **5b** and **c**, conformational analysis of the target compounds has been performed by the use of MM2[27] forcefield as implemented in Chem3D.[28] The starting atomic coordinate of the target compounds were obtained from the X-ray data of the structurally related molecule; 3-amino-1-methyl-4,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine **6**.[29] Full geometry optimization has been carried out with semi-emperical AM1[30] as implemented in Hyperchem 5.1[31] running on PC. Calculation of the isopotential molecular surface was performed with hyperchem 5.1. The results show that the occurance of four isomeric conformations for **5b**, each pair **A**-5b, **C**-5b and **B**-5b, **D**-5b (Fig. 1) have approximate similar heat of formation and atomic coordinate as calculated by AM1 depending on the rotation of both aryl groups regarding the plane of fused heterocycle. Similarly, conformational analysis of compound **5c** afforded two isomeric conformations **A**-5c and **B**-5c (Fig. 2) with approximately identical atomic coordinates. Due to low barrier of rotations which were lower than 1 kcal/mol, all possible isomers of compound **5b** or **5c** can be existed. As clear from the calculation on **5b** and **5c**, the trimethoxyl groups; whose rotations are highly free, were arranged in spatial manner in which the terminal dimethoxyl groups arranged itself approximately coplanar and parallel with aryl edges. On the contrary, the middle methoxyl group was arranged itself perpendicular to the plane of the phenyl ring and syn-position with amino group. MM2 calculation showed plannar structure of the NH_2 group (360°) which is in agreement with the crystal data of com-

Table 2. Antibacterial and antimycotic activities in terms of MIC (μg/mL) after 48 h

Compd	Clog P[a]	*E. coli*	*C. albicans*
3j	5.75	2.0	4.0
3k	5.00	2.3	2.2
4f	6.28	2.6	2.1
4g	5.53	2.8	3.3
5b	5.29	**1.6**	**1.8**
5c	4.55	**1.8**	**1.9**
Cefotaxime		1.5	
Gentamicin		2.4	
Streptomycin		2.6	
Clotrimazole			1.2
Nystatin			2.0

[a] Table 1.

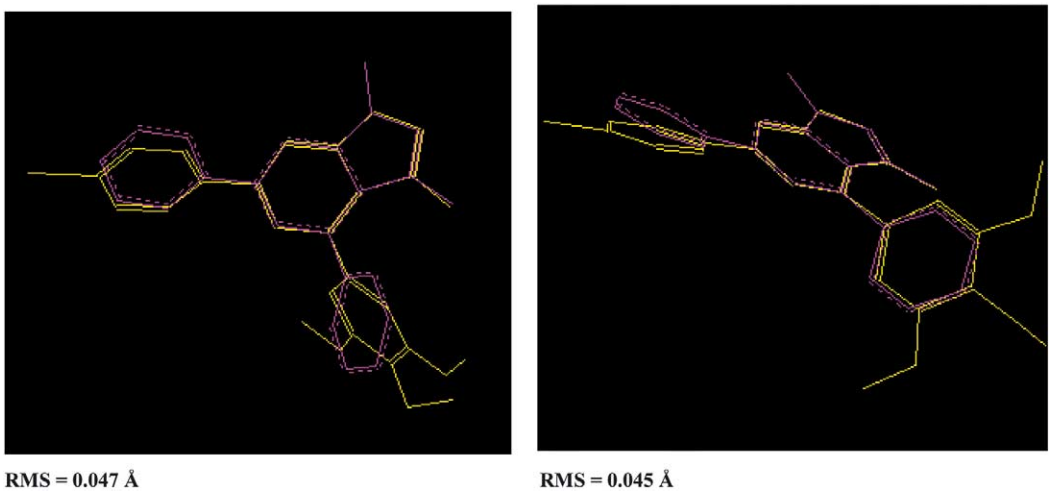


Figure 3. Superimposition of low energy conformers **A** (left; yellow) and **B** (right; yellow) of **5c** respectively and X-ray structure of **6** (violet), and the corresponding root means squares (RMS) values showing the close match of the bicyclic core and slight differences in aryl ring geometry.

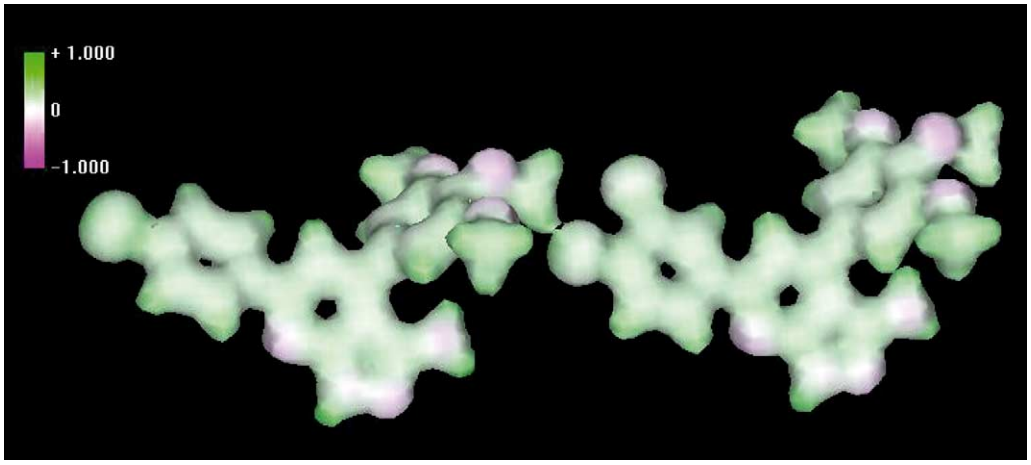


Figure 4. Electrostatic potential isosurface of the lowest energy conformers **A**-5b (left) and **A**-5c (right) for compounds **5b** and **5c** respectively, negative region colors pink and positive region colors green.

pound **6**, while tend to be pyramidal with semiemperical AM1 which can be avoided by repeating the semiemperical AM1 calculation under restraint of the NH_2 group to plannar form. Moreover, strong NH/π[32] interaction with MM2 (2.2 Å) or AM1 calculations (2.5 Å) compared with crystal data (2.8 Å) may come from the electron-donating effect of the three methoxyl groups which increase the electon density over the 4-aryl group. These NH/π interaction may be the reason for the existence of the plannar amino group. Nevertheless, hyperconjugation of the lone pair of amino group with the conjugated fused heterocycle may be another factor fixing this NH_2 group in plannar form rather than the most stable pyramidal conformation. These two factors may be strong enough to compensate the steric repulsion. It is noteworthy to say that the MM2 calculation gives good results compared with other forcefield such as MM+ in which the angel ∠HNH was overestimated 130° while underestimated 111° with AM1 semiemperical molecular orbital (nonrestraint results). Moreover, the torsional angle between fused heterocycle and 6-aryl group was 0° as calculated by MM+, while it was 17° as calculated by MM2 which is in a good agreement with the X-ray data and deviated by +8° from MM2 results as calculated by AM1. Similarly the torsional angle beween the pyridine ring and 4-aryl group has the same pattern of agreement between MM2 and X-ray data. The conformers **A**-5c and **B**-5c for **5c** resulting form computational chemistry analysis as a representative example and the X-ray structure of **6** were superimposed in order to reveal the similarities and differences in structure (Fig. 3). The strategy of overlay fit was match 1*H*-pyrazolo[3,4-*b*]pyridine rings and examine any spatial differences between the atoms of the aryl groups. The results show that atoms of the aryl groups ocupy slight different spatial position relative to the plane of 1*H*-pyrazolo[3,4-*b*]pyridines with RMS values 0.045 Å and 0.047 Å respectively. In an attempt to understand the enhanced antimicrobial activity of **5b** and **c**, electrostatic isopotential isosurface has been carried out for the lowest energy conformers **A**-5b and **A**-5c respectively, to examine the similarity in electronic and conformational properties. Figure 4 presents the electorstatic potentials (ESP) mapped on the isosurface of the most biologically active **5b** and **c**. Pink colors indicate negative ESP regions and green colors indicate

positive ESP regions. Comparison of the ESP of **5b** with **c** shows that increased negative charge regions located on the trimethoxyl groups and heterocyclic nitrogen atoms indicating structural similarity and so on similar biological activity as evident from the experimental data.

4. Conclusion

We successfully prepared new derivatives of 2-alkoxypyridines which could not be converted to 1*H*-pyrazolo[3,4-*b*]pyridines under normal condition, while the latter compound can easily be synthesized under mild condition catalyzed by Lewis acid. The novel compounds have evaluated for their antimicrobial activity in which compounds containing 1*H*-pyrazolo[3,4-*b*]pyridines pharmacophore were more active than the parent pyridine derivatives. Conformational analysis of the most active molecules **5b** and **c** were performed using MM2 calculations and fully optimized with semi-emperical AM1 molecular orbital calculations. These MM2 calculations give the most consistent results with experimental data for **6** and six-membered nitrogen heterocycles as reported.[27a]

5. Experimental

Melting points were recorded on a Fisher–Johns apparatus (°C, uncorrected). IR spectra (KBr) were recorded on Hewlett Packard Laser Jet 6L spectrometer (ν in cm^{-1}) and ^1H NMR spectra on Varian EM-390 (90 MHz) spectrometer using TMS as internal standard (chemical shift, δ ppm). Microanalytical data (C,H,N) agreed with the proposed structures within ±0.4% of the theoretical values. The following standard organisms used in the antimicrobial screening were obtained from IFO (Institute Fermentation of Osaka); *P. aeruginosa* IFO 3448, *E. coli* IFO 3301, *S. aureus* IFO 3060, *B. subtilis* IFO 3007 and *C. albicans* IFO 0583.

5.1. General method for preparation of 2-alkoxy-3-cyano-4,6-diaryl pyridines 3a–k and 4a–g

Compound **1** (0.015 mole) were added during stirring to a freshly prepared sodium alkoxide solution (0.014 mole of sodium in 100 mL of each of absolute methanol or ethanol, respectively). Malononitrile (1.3 gm, 0.02 mole) was then added with continuous stirring at room temperature until the precipitate was separated out. The solid separated was collected by filteration and recrystallized from suitable solvent. IR (KBr pellet, cm^{-1}): ν 1140–1155 (CO), 1430–1440, 1500, 1550, 1580 (C=C, pyridine nucleus), 2230–2250 (C=N) and 2940 (CH). **3a**: (75%), mp 247–249 °C (ethanol). ^1H NMR (DMSO-d_6, 90 MHz): δ 1.55 (t, 3H, CH_2–CH_3), 4.60 (q, 2H, CH_2–CH_3), 7.0–8.10 (m, 8H, ArH). Anal. $C_{17}H_{13}N_3O_2$: calcd 70.09, 4.50, 14.42, found 69.95, 4.61, 14.40. **3b**: (65%), mp 190–192 °C (methanol). ^1H NMR (DMSO-d_6, 90 MHz): δ 1.45 (t, 3H,CH_2–CH_3). 4.65 (q, 2H, CH_2–CH_3), 7.05–8.25 (m, 8H, ArH). Anal. $C_{18}H_{13}N_3O_3S$: calcd 61.53, 3.73, 11.96, found 61.63, 3.60, 11.91. **3c**: (60%), mp 149–150 °C (ethanol). ^1H NMR (DMSO-d_6, 90 MHz): δ 1.43 (t, 3H,CH_2–CH_3). 4.61 (q, 2H, CH_2–CH_3), 7.11–8.41 (m, 9H, ArH). Anal. $C_{19}H_{14}ClN_3O$: calcd 67.96, 4.20, 12.51, found 67.83, 4.33, 12.65. **3d**: (55%), mp >300 °C (acetic acid). ^1H NMR (DMSO-d_6, 90 MHz): δ 1.39 (t, 3H,CH_2–CH_3). 4.01 (q, 2H, CH_2–CH_3), 7.20–8.51 (m, 10H, ArH). Anal. $C_{19}H_{15}N_3O$: calcd 75.73, 5.02, 13.94, found 75.67, 4.94, 13.90. **3e**: (70%), mp 168–169 °C (ethanol). ^1H NMR (DMSO-d_6, 90 MHz): δ 1.39 (t, 3H,CH_2–CH_3). 4.11 (q, 2H, CH_2–CH_3), 7.24–8.26 (m, 10H, ArH). Anal. $C_{20}H_{15}N_3O_3$: calcd 69.56, 4.38, 12.17, found 69.60, 4.51, 12.05. **3f**: (63%), mp 150–152 °C (ethanol). ^1H NMR (DMSO-d_6, 90 MHz): δ 1.50 (t, 3H, CH_2–CH_3), 3.86 (s, 3H, OCH_3), 4.63 (q, 2H, CH_2–CH_3), 6.99–8.0 (m, 9H, ArH). Anal. $C_{20}H_{17}N_3O_2$: calcd 72.49, 5.17, 12.68, found 72.60, 5.29, 12.41. **3g**: (61%), mp 250–251 °C (acetic acid). ^1H NMR (DMSO-d_6, 90 MHz): δ 1.52 (t, 3H, CH_2–CH_3), 4.53 (q, 2H, CH_2–CH_3), 6.98–8.21 (m, 12H, styryl-CH and ArH). Anal. $C_{21}H_{17}N_3O$: calcd 77.04, 5.23, 12.84, found 76.98, 5.33, 12.93. **3h**: (64%), mp 145–146 °C (acetic acid). ^1H NMR (DMSO-d_6, 90 MHz): δ 1.42 (t, 3H, CH_2–CH_3), 2.46 (s, 3H, CH_3), 4.44 (q, 2H, CH_2–CH_3), 7.11–8.01 (m, 9H, ArH). Anal. $C_{21}H_{17}N_3O_3$: calcd 70.18, 4.77, 11.69, found 70.21, 4.75, 11.66. **3i**: (71%), mp 168–169 °C (ethanol). ^1H NMR (DMSO-d_6, 90 MHz): δ 1.44 (t, 3H, CH_2–CH_3), 3.86 (s, 3H, OCH_3), 4.62 (q, 2H, CH_2–CH_3), 6.99–8.0 (m, 9H, ArH). Anal. $C_{21}H_{17}N_3O_4$: calcd 67.19, 4.56, 11.19, found 67.30, 4.70, 11.00. **3j**: (66%), mp 193–195 °C (ethanol). ^1H NMR (DMSO-d_6, 90 MHz): δ 1.45 (t, 3H, CH_2–CH_3). 3.50 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.65 (q, 2H, CH_2–CH_3), 6.60–8.1 (m, 6H, ArH). Anal. $C_{23}H_{20}Cl_2N_2O_4$: calcd 60.14, 4.39, 6.10, found 60.00, 4.50, 6.21. **3k**: (85%), mp >300 °C (acetic acid). ^1H NMR (DMSO-d_6, 90 MHz): δ 1.46 (t, 3H, CH_2–CH_3). 3.48 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.36 (q, 2H, CH_2–CH_3), 6.63–7.78 (m, 7H, ArH). Anal. $C_{23}H_{21}BrN_2O_4$: calcd 58.86, 4.51, 5.97, found 58.79, 4.57, 5.88. **4a**: (62%), mp 128–129 °C (methanol). ^1H NMR (DMSO-d_6, 90 MHz): δ 3.95 (s, 3H, OCH_3), 7.10–8.20 (m, 8H, ArH). Anal. $C_{17}H_{11}N_3O_3S$: calcd 60.52, 3.29, 12.46, found 60.38, 3.37, 12.40. **4b**: (80%), mp 230–232 °C (methanol). ^1H NMR (DMSO-d_6, 90 MHz): δ 4.18 (s, 3H, OCH_3), 7.30–8.20 (m, 10H, ArH). Anal. $C_{19}H_{13}N_3O_3$: calcd 68.88, 3.95, 12.68, found 68.72, 3.81, 12.47. **4c**: (65%), mp 145–146 °C (acetic acid). ^1H NMR (DMSO-d_6, 90 MHz): δ 3.86 (s, 3H, OCH_3), 4.17 (s, 3H, OCH_3), 6.95–8.10 (m, 9H, ArH). Anal. $C_{19}H_{15}N_3O_2$: calcd 71.91, 4.76, 13.24, found 71.77, 4.66, 13.30. **4d**: (62%), mp 238–239 °C (methanol). ^1H NMR (DMSO-d_6, 90 MHz): δ 3.97 (s, 3H, OCH_3), 6.99–8.01 (m, 12H, styryl-CH and ArH). Anal. $C_{20}H_{15}N_3O$: calcd 76.66, 4.82, 13.41, found 76.57, 4.93, 13.33. **4e**: (70%), mp 140–141 °C (methanol). ^1H NMR (DMSO-d_6, 90 MHz): δ 3.88 (s, 3H, OCH_3), 4.21 (s, 3H, OCH_3), 6.99–8.20 (m, 9H, ArH). Anal. $C_{20}H_{15}N_3O_4$: calcd 66.48, 4.18, 11.63, found 66.60, 4.07, 11.82. **4f**: (81%), mp 210–212 °C (ethanol). ^1H NMR (DMSO-d_6, 90 MHz): δ 3.28 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.21(s, 3H, OCH_3), 7.0–7.9 (m, 6H, ArH). Anal. $C_{22}H_{18}Cl_2N_2O_4$: calcd 59.34, 4.07, 6.29, found 59.50,

4.10, 6.37. **4g**: (85%), mp >300 °C (acetic acid). ^{1}H NMR (DMSO-d_6, 90 MHz): δ 3.35 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 4.15 (s, 3H, OCH_3), 6.80–8.10 (m, 7H, ArH). Anal. $C_{22}H_{19}BrN_2O_4$: calcd 58.04, 4.21, 6.15, found 58.10, 4.07, 6.30.

5.2. Synthesis of 1*H*-pyrazolo [3,4-*b*]pyridines 5a–c

To a stirred solution of **4i–k** (0.1 mol) and $BF_3{\cdot}OEt_2$ (0.1 mol) in absolute ethanol (10 mL) under nitrogen atmosphere, solution of anhydrous hydrazine (0.2 mole) in absolute ethanol (5 mL) was slowly added over 10 min at 0 °C and then the whole mixture was heated under reflux for 2–3 h. The solid products obtained from hot solution or after cooling were filtered off and recrystallized from the proper solvent. **5a**: (76%), mp 160–162 °C (ethanol). IR (KBr pellet, cm^{-1}): ν 3480–3300 (NH_2), 3200 (NH), 1630 (C=N), 1610 (C=C). ^{1}H NMR (DMSO-d_6, 90 MHz): δ 3.81 (s, 3H, OCH_3), 5.00 (s, br, 2H, NH_2, D_2O-exchangeable), 7.20–8.20 (m, 9H, ArH), 11.90 (s, br, 1H, NH, D_2O-exchangeable). Anal. $C_{19}H_{15}N_5O_3$: calcd 63.15, 4.18, 19.38, found 62.99, 4.10, 19.49. **5b**: (68%), mp 179–180 °C (ethanol). IR (KBr pellet, cm^{-1}): ν 3450–3350 (NH_2), 3250 (NH), 1630 (C=N), 1600 (C=C). ^{1}H NMR (DMSO-d_6, 90 MHz): δ 3.30 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 5.20 (s,br, 2H, NH_2, D_2O-exchangeable), 7.40–8.0 (m, 6H, ArH), 12.00 (s, br, 1H, NH, D_2O-exchangeable). Anal. $C_{21}H_{18}Cl_2N_4O_3$: calcd 56.64, 4.07, 12.58, found 56.56, 4.14, 12.59. **5c**: (70%), mp 192–193 °C (ethanol). IR (KBr pellet, cm^{-1}): ν 3480–3350 (NH_2), 3200 (NH), 1630 (C=N), 1600 (C=C). ^{1}H NMR (DMSO-d_6, 90 MHz): δ 3.45 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 5.10 (s, br, 2H, NH_2, D_2O-exchangeable), 7.20–8.10 (m, 7H, ArH), 11.80 (s, br, 1H, NH, D_2O-exchangeable). Anal. $C_{21}H_{19}BrN_4O_3$: calcd 55.40, 4.21, 12.31, found 55.46, 4.19, 12.43.

5.3. Antimicrobial testing

Inhibition zone measurements: the tested compounds were dissolved in dimethylsulfoxide at a concentration of 1 mg/mL. The suitable medium (nutrient agar for bacteria and Sabouraud agar for fungi) was inoculated with the test organisms. A volume of the solution of each the test compounds equivalent to 100 μg was placed separately in cups, cut in the agar. The plates were incubated at 37 °C for 18–24 h for bacteria and 48 h for *C. albicans*, and the resulting inhibition zones were measured (Table 1). Dimethylsulfoxide, which exhibited no antimicrobial activity against the test organisms, was used as a negative control. Minimal inhibitory concentration (MIC) was determined using the broth dilution technique.[25] Cefotaxime, gentamicin, streptomycin, clotrimazole and nystatin were used during the test procedure as reference antibiotics.

Acknowledgements

We are indebted to Prof. M.A. Moustafa (Department of Medicinal Chemistry, Faculty of Pharmacy, Mansoura University, Egypt) for valuable comments. The authors would also like to thank staff members (Department of Microbiology, Faculty of Pharmacy, Mansoura University, Egypt) for providing the antibacterial and antifungal data.

References and notes

1. Attia, A.; Michael, M. *Pharmazie* **1982**, *37*, 551.
2. Patel, P.; Koregaokar, S.; Shah, M.; Pareckh, H. *Il Farmaco* **1996**, 51.
3. Pourrias, B.; Dorme, N.; Pouvet, P.; Raynaud, G. *Therapie* **1971**, *26*, 859.
4. Yamada, K.; Takeda, M.; wakuua, L.; Itoh, N.; Ohtsuka, H.; Tsunashima, A.; Wakuua, T. I. *Chem. Pharm. Bull.* **1982**, *30*, 3197.
5. Vìra, K.; Martin, S.; Karel, W.; Milan, P.; Jarmila, K. *Il Farmaco* **1999**, *54*, 666 and references therein.
6. Massimo, P.; Marco, T.; Daniela, U.; Giuseppe, P.; Pietro, B.; Fiorenzo, P.; Fabrizio, Z. *Il Farmaco* **2000**, *55*, 669.
7. Borgna, P.; Pregnolato, M.; Gamba, I. A.; Mellerio, G. *J. Heterocyclic Chem.* **1993**, *30*, 1079.
8. Maria, G. M.; Valeria, F.; Daniele, Z.; Luciano, V.; Elena, B. *Il Farmaco* **2001**, *56*, 587 and references therein.
9. Sanna, M. E.; Wahid, M. B.; Laila, I. I. *Egypt J. Pharm. Sci.* **1998**, *39*, 197.
10. Carla, M. S. M.; Carlos, M. R. S. A.; Carlos, R. R.; Eliezer, J. B. *Journal of Molecular Structure (Theochem)* **2002**, *579*, 31.
11. Poreba, K.; Opolski, A.; Wietrzyk, J.; Kowalska, M. *Arch Pharm (Weinheim)* **2001**, *334*, 219.
12. Poreba, K.; Opolski, A.; Wietrzyk, J. *Acta Pol. Pharm.* **2002**, *59*, 215.
13. Bernardino, A. M. R.; Ferreira, V. F.; Fontoura, G. A. T.; Frugulhetti, I. C. P. P.; Lee, M. M.; Romeiro, G. A.; souza, M. C. B.; Sá, P. M. *J. Braz. Chem. Soc.* **1996**, *7*, 273.
14. Kuo, S. C.; Huang, L. J.; Nakamura, H. *J. Med. Chem.* **1984**, *27*, 539.
15. Arita, M.; Saito, T.; Sato, H.; Uehata, M.; Okuda, H. JP App. 91/255, 689; *Chem. Abstr.* **1993**, *119*, 95006u.
16. Sato, Y.; Shimogi, Y.; Kumakura, S.; Takagi, H. Ja pan, Kokai 75,151,896; Appl. 74,61,149; *Chem. Abstr.* **1976**, *84*, 164771.
17. Sakurai, A.; Midoikawa, N. *Bull. Chem. Soc. Japan* **1967**, *40*, 1680.
18. Sakurai, A.; Midoikawa, N. *Bull. Chem.Soc. Japan* **1968**, *41*, 430.
19. Al-Arab, M. M. *J. Heterocycl. Chem.* **1989**, *26*, 1665.
20. Al-Arab, M. M.; Tabba, H. D.; Abu-Yousef, L. A.; Marilyn, M. O. *Tetrahedron* **1988**, *44*, 7293.
21. Moustafa, M. A.; El-Emam, A. A.; Mashaley, M. M. *Chin .Pharm. J.* **1992**, *44*, 37 and references therein.
22. More details about the effect of Lewis acids on the displacement of 2-alkoxypyridines with hydrazines and other nucleophiles, mechanistic study will be the subject of separate paper.
23. Abdel-Aziz, A. A.-M.; Matsunaga, H.; Kunieda, T. *Tetrahedron Lett.* **2001**, *42*, 6565 and references cited therein.
24. Jain, S. R.; Kar, A. *Planta Med.* **1971**, *20*, 118.
25. Jones, R. N.; Barry, A. L.; Gavan, T. L., Washington IIJA 1985, *Manual of Clinical Microbiology* 4th ed.; Lennette, E. H., Ballows, A., Hausler, W. J., Jr., Shadomy, H. J., Eds.; American Society for microbiology, Washington DC, pp 972–977.

26. Calculated logP (ClogP) values were computed by using the CS ChemDraw Ultra ver.7.0, November 27, 2001 by CambridgeSoft.Com, Cambridge, MA, USA.
27. (a) Profeta, S.; Allinger, N. L. *J. Am. Chem. Soc*. **1985**, *107*, 1907. (b) Allinger, N. L. *J. Am. Chem. Soc*. **1977**, *99*, 8127.
28. CS Chem 3-D ultra™, Version 7.0 CambridgeSoft Corporation (2001).
29. Alvarez-Larena, A.; Piniella, J. F. *Victory, P, Borrell, J. I. and Vidal-Ferran, A., Zeitschrift für Kristallographie* **1994**, *209*, 773.
30. Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc*. **1985**, *107*, 3902.
31. Hyperchem version 5.1Hypercube, Inc.
32. Umezawa, Y.; Tsuboyama, S.; Takahashi, H.; Uzawa, J.; Nishio, M. *Tetrahedron* **1999**, *55*, 10047.