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SYNTHESIS OF NEW B*IS*-IMIDAZOLYLPYRIDINES AS ANTCANCER AGENTS

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ABSTRACT

The reaction of two equivalents of 5-acetylimidazole with one equivalent of aldehyde in acetic acid and ammonium acetate yielded 2,6-(bis-imidazol-5-yl)pyridine derivatives in a multicomponent reactions. The structures of all the new compounds were elucidated on the basis of elemental analysis and spectral data. The anticancer activities of the synthesized compounds were screened for their activity against human breast cell line (MCF-7) comparable to doxorubicin and the results showed that most of such compounds exhibit considerable activities.

Keywords: 5-Acetylimidazole, Multicomponent reactions, Anticancer activity.

INTRODUCTION

The pyridine nucleus is a key constituent, present in a range of bioactive compounds, occurring both synthetically and naturally with wide range of biological applications.[1-4] Among the successful examples as drug candidates possessing pyridine nucleus are streptonigrin, streptonigrone and lavendamycin which are described in the literature as anticancer drugs, and cerivastatin is reported as the HMG-CoA enzyme inhibitor.[5-7] Substituted pyridines are used as leukotriene B-4 antagonists.[8] In particular 2,2`-pyridines and its derivatives have been invoked as functional modules within the domain of supramolecular chemistry, coordination chemistry and material science.[9-11] Multi-component reactions (MCRs) are powerful tools in modern medicinal chemistry, enabling straightforward access to large libraries of structurally related drug-like compounds and thereby facilitating lead generation. Hence, combined with the use of combinatorial chemistry and high-through put parallel synthesis, such reactions have constituted an increasingly valuable approach to drug discovery efforts in recent years.[12, 13]

In view of these observations and in continuation of our previous work [14-23] we report herein the synthesis of some new derivatives of pyridines in multi-component reaction and preliminarily evaluate their anticancer properties with aiming to get better anticancer drugs without side effects.

Experimental

Melting points were measured on Electrothermal IA 9000 series digital melting point apparatus. The IR spectra were recorded in potassium bromide discs on a Pye Unicam SP 3300 and Shimadzu FT IR 8101 PC infrared spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in deuterated dimethyl sulfoxide (DMSO-*d*6) using a Varian Gemini 300 NMR spectrometer (300 MHz for ^{1}H NMR). Mass spectra were recorded on a Shimadzu GCMS-QP1000 EX mass spectrometer at 70 eV. Elemental analysis was carried out at the Microanalytical Centre of Cairo University, Giza, Egypt. All reactions were followed by TLC (Silica gel, Merck). Antitumor activity was evaluated by the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

General procedure for the synthesis of 5,5'-(4-substitutedpyridine-2,6-diyl)bis(4-methyl-1-phenyl-1*H*-imidazole-2-thiol) (3a-l)

To a solution of **1** (0.464 g, 2 mmol) and the appropriate aldehyde **2a-l** (1 mmol) in acetic acid (20 mL) containing excess ammonium acetate (0.616 g, 8 mmol) was refluxed for 6-10h (monitored by TLC). The reaction mixture was left to cool and the solid product formed upon pouring onto ice/water was collected by filtration, washed with water, dried and recrystallized from EtOH to give the corresponding pyridine derivatives **3a-l**.

5,5'-(4-Phenylpyridine-2,6-diyl)bis(4-methyl-1-phenyl-1*H*-imidazole-2-thiol) (3a). Yield 72%; yellow solid; mp 96-98 ^{o}C; ^{1}H NMR (DMSO-d_6): δ 2.41 (s, 6H, 2CH$_3$), 7.02-7.63 (m, 15H, ArH), 8.27 (s, 2H, pyridine-H3, H5), 10.68 (s, 2H, 2SH); IR (KBr): ν_{max} 1610 (C=N), 3037 (CH) cm^{-1}; MS m/z (%): 531(M^{+}, 19), 320(63), 243(64), 103(61), 77(100). Anal.Calcd for $C_{31}H_{25}N_5S_2$ (531.69): C, 70.03; H, 4.74; N, 13.17. Found C, 70.17; H, 4.65; N, 13.00%.

5,5'-(4-(p-Tolyl)pyridine-2,6-diyl)bis(4-methyl-1-phenyl-1*H*-imidazole-2-thiol) (3b). Yield 76%; yellow solid; mp 82-84 ^{o}C; ^{1}H NMR (DMSO-d_6): δ 2.24 (s, 3H, CH$_3$), 2.35 (s, 6H, 2CH$_3$), 6.75-7.75 (m, 14H, ArH), 8.26 (s, 2H, pyridine-H3, H5), 10.93 (s, 2H, 2SH); IR (KBr): ν_{max} 1610(C=N), 3040(CH), cm^{-1}; MS m/z (%): 545(M^{+}, 18), 300(47), 258(46),

105(39), 77(100). Anal. Calcd for $C_{32}H_{27}N_5S_2$ (545.72): C, 70.43; H, 4.99; N, 12.83. Found C, 70.22; H, 4.76; N, 12.69%.

5,5'-(4-(4-Methoxyphenyl)pyridine-2,6-diyl)bis(4-methyl-1-phenyl-1*H*-imidazole-2-thiol) (3c). Yield 73%; yellow solid; mp 92-94 ^oC; ^1H NMR (DMSO-d_6): δ 2.33 (s, 6H, $2CH_3$), 3.81 (s, 3H, OCH_3), 6.75-7.75 (m, 14H, ArH), 8.26 (s, 2H, pyridine-H3, H5), 10.92 (s, 2H, 2SH); IR (KBr): ν_{max} 1626 (C=N), 3030 (CH) cm^{-1}; MS m/z (%): 562(M^++1, 20), 561(M^+, 31), 350(27), 258(73), 133(43), 77(100). Anal. Calcd for $C_{32}H_{27}N_5OS_2$ (561.72): C, 68.42; H, 4.84; N, 12.47. Found C, 68.31; H, 4.76; N, 12.29%.

5,5'-(4-(4-Chlorophenyl)pyridine-2,6-diyl)bis(4-methyl-1-phenyl-1*H*-imidazole-2-thiol) (3d). Yield 76%; yellow solid; mp 117-119 ^oC; ^1H NMR (DMSO-d_6): δ 2.41 (s, 6H, $2CH_3$), 7.02-7.60 (m, 14H, ArH), 8.34 (s, 2H, pyridine-H3, H5), 10.69 (s, 2H, 2SH); IR (KBr): ν_{max} 1613 (C=N), 3037 (CH) cm^{-1}; MS m/z (%): 566(M^+, 18), 500(58), 232(34), 217(73), 189(50), 104(43), 77(100). Anal. Calcd for $C_{31}H_{24}ClN_5S_2$ (566.14): C, 65.77; H, 4.27; N, 12.37. Found C, 65.70; H, 4.06; N, 12.21%.

5,5'-(4-(4-Bromophenyl)pyridine-2,6-diyl)bis(4-methyl-1-phenyl-1*H*-imidazole-2-thiol) (3e). Yield 78%; yellow solid; mp 106-108 ^oC; ^1H NMR (DMSO-d_6): δ 2.39 (s, 6H, $2CH_3$), 6.98-7.78 (m, 14H, ArH), 8.28 (s, 2H, pyridine-H3, H5), 10.93 (s, 2H, 2SH); IR (KBr): ν_{max} 1608 (C=N), 3034 (CH) cm^{-1}; MS m/z (%): 610(M^+, 32), 300(43), 217(60), 112(45), 77(100). Anal. Calcd for $C_{31}H_{24}BrN_5S_2$ (610.59): C, 60.98; H, 3.96; N, 11.47. Found C, 60.79; H, 3.91; N, 11.25%.

2-(2,6-Bis(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)pyridin-4-yl)phenol (3f). Yield 72%; yellow solid; mp 92-94 ^oC; ^1H NMR (DMSO-d_6): δ 2.41 (s, 6H, $2CH_3$), 5.61 (s, 1H, OH), 6.78-7.67 (m, 14H, ArH), 8.20 (s, 2H, pyridine-H3, H5), 10.88 (s, 2H, 2SH); IR (KBr): ν_{max} 1607 (C=N), 3031 (CH), 3374 (OH) cm^{-1}; MS m/z (%): 548(M^+ +1, 35), 547(M^+, 45), 440(52), 232(68), 217(45), 77(100). Anal. Calcd for $C_{31}H_{25}N_5OS_2$ (547.69): C, 67.98; H, 4.60; N, 12.79. Found C, 67.79; H, 4.49; N, 12.64%.

5,5'-(4-(2,4-Dimethylphenyl)pyridine-2,6-diyl)bis(4-methyl-1-phenyl-1*H*-imidazole-2-thiol) (3g). Yield 70%; yellow solid; mp 80-82 ^oC; ^1H NMR (DMSO-d_6): δ 2.20 (s, 1H, CH_3), 2.41 (s, 6H, $2CH_3$), 2.93 (s, 1H, CH_3), 6.73-7.69 (m, 13H, ArH), 8.23 (s, 2H, pyridine-H3, H5), 10.67 (s, 2H, 2SH); IR (KBr): ν_{max} 1609 (C=N), 3023 (CH) cm^{-1}; MS m/z (%):

560(M^+ +1, 23), 559(M^+, 35), 445(63), 217(100), 104(78), 77(69). Anal. Calcd for $C_{33}H_{29}N_5S_2$ (559.75): C, 70.81; H, 5.22; N, 12.51. Found C, 70.48; H, 5.14; N, 12.30%.

5,5'-(4-(2,4-Dichlorophenyl)pyridine-2,6-diyl)bis(4-methyl-1-phenyl-1*H*-imidazole-2-thiol) (3h).

Yield 73%; yellow solid; mp 141-143 ^oC; ^1H NMR (DMSO-d_6): δ 2.41 (s, 6H, $2CH_3$), 6.88-7.79 (m, 13H, ArH), 8.37 (s, 2H, pyridine-H3, H5), 10.84 (s, 2H, 2SH); IR (KBr): ν_{max} 1609 (C=N), 3039 (CH) cm^{-1}; MS m/z (%): 600(M^+, 28), 403 (52), 217(48), 105(62), 77(100). Anal. Calcd for $C_{31}H_{23}Cl_2N_5S_2$ (600.58): C, 61.99; H, 3.86; N, 11.66. Found C, 61.80; H, 3.76; N, 11.43%.

5,5'-(4-(2,6-Dichlorophenyl)pyridine-2,6-diyl)bis(4-methyl-1-phenyl-1*H*-imidazole-2-thiol) (3i). Yield 76%; yellow solid; mp 122-124 ^oC; ^1H NMR (DMSO-d_6): δ 2.41 (s, 6H, $2CH_3$), 6.83-7.92 (m, 13H, ArH), 8.29 (s, 2H, pyridine-H3, H5), 10.74 (s, 2H, 2SH); IR (KBr): ν_{max} 1609 (C=N), 3063 (CH) cm^{-1}; MS m/z (%): 600(M^+, 14), 332 (43), 217(100), 105(39), 77(86). Anal. Calcd for $C_{31}H_{23}Cl_2N_5S_2$ (600.58): C, 61.99; H, 3.86; N, 11.66. Found C, 61.87; H, 3.64; N, 11.52%.

5,5'-(4-(Thiophen-2-yl)pyridine-2,6-diyl)bis(4-methyl-1-phenyl-1*H*-imidazole-2-thiol) (3j). Yield 69%; yellow solid; mp 127-129 ^oC; ^1H NMR (DMSO-d_6): δ 2.41 (s, 6H, $2CH_3$), 6.78 -7.90 (m, 13H, ArH), 8.20 (s, 2H, pyridine-H3, H5), 10.76 (s, 2H, 2SH); IR (KBr): ν_{max} 1609 (C=N), 3033 (CH) cm^{-1}; MS m/z (%): 538(M^+ +1, 13), 537(M^+, 41), 353 (38), 217(92), 104(53), 77(100). Anal. Calcd for $C_{29}H_{23}N_5S_3$ (537.72): C, 64.78; H, 4.31; N, 13.02. Found C, 64.82; H, 4.16; N, 12.87%.

5,5'-(4-(Furan-2-yl)pyridine-2,6-diyl)bis(4-methyl-1-phenyl-1*H*-imidazole-2-thiol) (3k). Yield 70%; yellow solid; mp 116-118 ^oC; ^1H NMR (DMSO-d_6): δ 2.38 (s, 6H, $2CH_3$), 2.93 (s, 1H, CH_3), 6.77-7.63 (m, 13H, ArH), 8.25 (s, 2H, pyridine-H3, H5), 10.72 (s, 2H, 2SH); IR (KBr): ν_{max} 1602 (C=N), 3039 (CH) cm^{-1}; MS m/z (%): 521(M^+, 35), 337(48), 217(85), 104(82), 77(100). Anal. Calcd for $C_{29}H_{23}N_5OS_2$ (521.66): C, 66.77; H, 4.44; N, 13.43. Found C, 66.64; H, 4.29; N, 13.21%.

5,5'-(4-(1,3-Diphenyl-1*H*-pyrazol-4-yl)pyridine-2,6-diyl)bis(4-methyl-1-phenyl-1*H*-imidazole-2-thiol) (3l). Yield 70%; yellow solid; mp 163-165 ^oC; ^1H NMR (DMSO-d_6): δ 2.38 (s, 6H, $2CH_3$), 7.13-7.87 (m, 20H, ArH), 8.14 (s, 1H, pyrazole-H5), 8.29 (s, 2H,

pyridine-H3, H5), 10.83 (s, 2H, 2SH); IR (KBr): ν_{max} 1609 (C=N) cm^{-1}; MS m/z (%): 673(M^+, 19), 445 (51), 217(100), 105(69), 77(89). Anal. Calcd for $C_{40}H_{31}N_7S_2$ (673.85): C, 71.30; H, 4.64; N, 14.55. Found C, 71.16; H, 4.60; N, 14.32%.

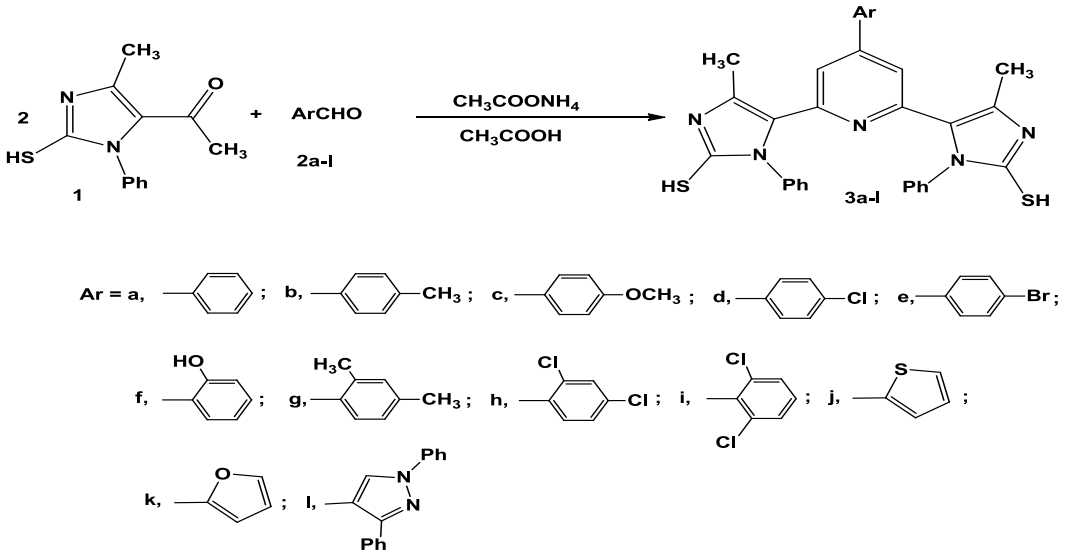
Cytotoxic Activity

Potential cytotoxicity of the compounds was tested using the method of Skehan *et al.*[24] using Sulfo-Rhodamine-B stain (SRB). Cells were plated in 96-multiwill plates (10^4 cells/well) for 24 h before treatment with the tested compound to allow attachment of cell to the wall of the plate. Different concentrations of the compound under test (0, 1.56, 3.125, 6.25, 12.5, 25, and 50 µg/mL) were added to the cell monolayer in triplicate wells individual dose, monolayer cells were incubated with the compounds for 48 h at 37 °C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained with SRB stain, excess stain was washed with acetic acid and attached stain was recovered with *tris*-EDTA buffer, color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted. The response parameter calculated was the IC50 value, which corresponds to the compound concentration causing 50% mortality in net cells.

RESULTS AND DISCUSSION

The required 5-acetyl-2-mercapto-4-methyl-1-phenyl-1*H*-imidazole (**1)** was prepared following the literature method.[25]

A convenient one-pot, three-component synthesis of 2,6-bis (imidazol-5-yl)-4-aryl pyridine derivatives (**3a–l**) by Chichibabin reaction has been reported. These compounds were synthesized by the reaction of two equivalents of 5-acetylimidazole **1** with one equivalent of substituted aromatic aldehydes and ammonium acetate under acidic conditions (scheme 1). The structure of the products was established based on their elemental and spectral data. Structure of compound **3a** was inferred from its spectral data. For example, the mass spectrum gave a strong peak at m/z = 531 corresponding to its molecular weight. The ^{1}HNMR spectrum showed singlet signal at δ = 8.27 ppm corresponds to two protons of the pyridine ring, also, A mutiplet signal at δ = 7.02-7.63 ppm assignable for 15 aromatic protons. The IR spectrum of compounds **3a-l** revealed the disappearance of the absorption band of the carbonyl group (See experimental section).

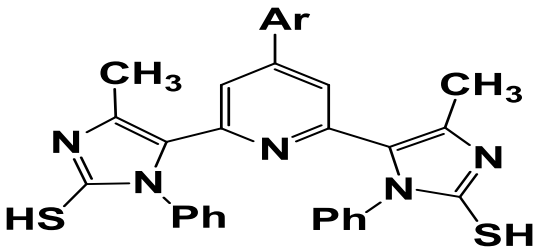


Scheme 1. Synthesis of pyridine derivatives 3a-l

Anti-cancer Activity: The cytotoxicity of synthesized products was evaluated against human breast cell line (MCF-7) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and doxorubicin was used as a reference drug (IC_{50} value of doxorubicin = 0.42 ± 0.03 µg/mL). Data generated were used to plot a dose response curve of which the concentration of test compounds required to kill 50% of cell population (IC_{50}) was determined. Cytotoxic activity was expressed as the mean IC_{50} of three independent experiments. The results are represented in Tables 1. The results indicated that:

The order of activity was **3l > 3c > 3k > 3b > 3g > 3e > 3d > 3j > 3a > 3h > 3i > 3f** which is in accordance with the order of breast carcinoma cells inhibitory activity (Table 1).

Table 1. IC50 values of tested compounds ± standard deviation against (MCF-7)



3a-l

Compound No.	Ar	IC50
3a	C_6H_5	11.4 ± 0.16
3b	4-$CH_3C_6H_4$	1.3 ± 0.21
3c	4-$OCH_3C_6H_4$	0.89 ± 0.09
3d	4-ClC_6H_4	5.3 ± 0.15
3e	4-BrC_6H_4	5.0 ± 0.13

3f	2-ClC_6H_4	34.3 ± 0.05
3g	2, 4-$DiCH_3C_6H_3$	1.6 ± 0.07
3h	2, 4-$DiClC_6H_3$	12.7 ± 0.24
3i	2, 6-$DiClC_6H_3$	16.5 ± 0.08
3j	2-Furyl	5.4 ± 0.23
3k	2-Thienyl	1.2 ± 0.12
3l	4-Pyrazolyl	0.64 ± 0.14
Doxorubicin	-	0.42 ± 0.03

CONCLUSIONS

A synthesis of some new bis- imidazolylpyridine derivatives from 5-acetylimidazole in multi-component reaction was established. Moreover, some of the newly synthesized products were tested as antitumor agents and the results obtained were promising.

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