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Synthesis, Biological Evaluation and 2D-QSAR Studies of Novel 6-Oxo-Pyridine-3-Carboxamide Derivatives as Antimicrobial and Antifungal Agents

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Abstract

In this study twenty six novel derivatives of 6-oxo-pyridine-3-carboxamide were synthesized and evaluated as antibacterial and antifungal agents. Synthesis of the 2-amino-4-methyl-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide **1** was carried out by reacting equimolar quantities of acetoacetanilide and cyanoacetanilide in ethanol using triethylamine as a catalyst. The results of the *in vitro* antimicrobial evaluation showed that, the 6-oxo-N,1-diphenyl-5-(p-tolyldiazenyl)-1,6-dihydropyridine-3-carboxamide, **5c** displayed broad-spectrum antibacterial activity which was equipotent to both Ampicillin and Gentamicin against the tested bacteria. Moreover, compounds **3a, 5c** and **9b** were equipotent to the reference drug, Amphotericin B, against *Aspergillus fumigatus* (MIC = 1.95µg/ml). 2D QSAR studies were carried out in order to correlate the observed activity to the binding mode of these compounds, in addition to their molecular properties that might be controlling their activities.

Keywords: synthesis, antimicrobial activity, 6-oxo-pyridine-3-carboxamide; antifungal, 2D-QSAR

1. Introduction

In the past few decades, drug-resistant human pathogenic microbes have been developed (Bax, Mullan & Verhuef, 2000; Cohen, 1992; Finch, Greenwood, Norrby & Whitley, 2003; Neu, 1998 and Struelens, 1992). The widespread and misuse of antibiotics in humans and animals (Ritter & Wong, 2001; Alekshun & Levy, 2007) may directly contribute to this resistance, which in fact, represents a major medical challenge (Setti, Quattrocchio & Micetich, 1997). Despite the development of several new antibacterial agents, their clinical value is limited in the treatment of an increasing array of life threatening systemic infections. Thus, the development of potent and effective antimicrobial agents is very important to overcome the emerging multi-drug resistance strains of bacteria and fungi. The 2-pyridone has proven to be effective antibacterial and antibacterial agents (Saiki et al, 1999; Li, Mitscher & Shen, 2000; Gupta & Plott, 2004; Khokhani, Khatri, & Patel, 2013; Tipparaju et al, 2008 and Vyas et al, 2008) the clinical candidate, CG400549 (Yum et al, 2007 & Park et al, 2007) and the promising lead compound, PT171 (Schiebel et al, 2014) were rationally designed as broad spectrum antibacterial agents, figure 1. Moreover, the 2-pyridone CG400549 (Figure 1) was identified as a potent antibacterial against multidrug-resistant staphylococci strains (Kim et al, 2005 & Gerusz, 2010).

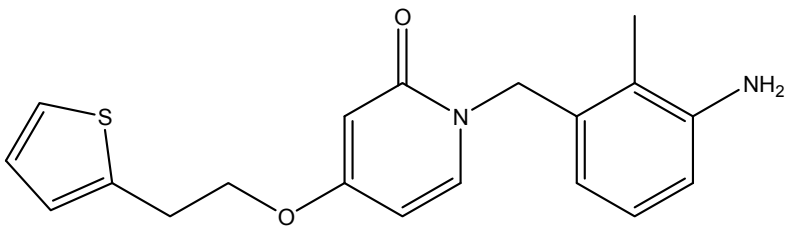


Figure 1. The 2-pyridone based clinical candidate **CG400549.**

Based on these considerations, we report herein the synthesis, characterization, antibacterial, antifungal and 2D QSAR studies of a novel series of **2-amino-4-methyl-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide**, aiming to obtain new potent antibacterial and antifungal agents. A 2D QSAR study was performed on this novel series, whereas,

the main objective of this study was to develop a relationship between the biological activity of the compounds and their structural or molecular descriptors including, physico-chemical, electronic, geometrical, topological or thermodynamic parameters. Thereby, predicting the activity of novel molecules prior to their synthesis.

2. Materials and Methods

2.1 Experimental

Melting points were measured with a Stuart melting point apparatus and were uncorrected. The NMR spectra were recorded by Varian Gemini-300BB 300 MHz FT-NMR spectrometers (Varian Inc., Palo Alto, CA). ^{1}H and ^{13}C spectra were run at 300 and 75 MHz, respectively, in deuterated dimethylsulphoxide (DMSO-d_6). Chemical shifts (δ_H) are reported relative to TMS as internal standard. All coupling constant (J) values are given in hertz. Chemical shifts (δ_C) are reported relative to DMSO-d_6 as internal standards. The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet. IR spectra were recorded with a Bruker FT-IR spectrophotometer. Electron impact mass spectra were measured on a Varian MAT 311-A (70 e.v.). Reaction courses and product mixtures were routinely monitored by thin layer chromatography (TLC) on silica gel precoated F_{254} Merck plates. Unless otherwise noted, all solvents and reagents were commercially available and used without further purification.

Synthesis of 2-amino-4-methyl-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (1)

Equimolar amounts of acetoacetanilide (0.012 mol, 2.2 gm) and cyanoacetanilide (0.012 mol, 2 gm) were dissolved in ethanol (10ml) containing a catalytic amount of triethylamine. The mixture was refluxed for 8 h. The precipitated solid obtained after cooling, was filtered off, washed with ethanol, dried and recrystallized out from ethanol.

Yield (45%), m.p. = 180-3 ^{o}C. (EtOH); ν (cm^{-1}) (KBr) 3274, 3211 (NH, NH_2), 1668, 1617 (CO). ^{1}HNMR (DMSO-d_6-D_2O): δ 2.20 (s, 3H, CH_3); 3.87 (s, 2H, NH_2; D_2O exchangeable); 7.09-7.11 (t, 2H, 2ph-H_4); 7.30-7.35 (m, 5H, 2ph-$H_{3,5}$ + pyridone-H); 7.52 (d, 4H, 2ph-$H_{2,6}$); 10.23 (s, 1H, NH; D_2O exchangeable).^{13}C-NMR δ, ppm (DMSO-d6): 26.65, 115.83, 119.22, 123.85, 128.84, 138.30, 160.91. Anal. Calcd. for $C_{19}H_{17}N_3O_2$; M.wt: 320; C, 71.46; H, 5.37; N, 13.16; (%); Found: C, 71.69; H, 5.42; N, 13.39; (%). MS: m/z (%): 320 (M^{+1}, 19.06), 52 (100).

Synthesis of 2-(benzylideneamino)-4-methyl-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (2a-f)

A mixture of 2-amino-1,6-dihydro-4-methyl-6-oxo-N,1-diphenylpyridine-3-carboxamide **1** and the appropriate substituted aromatic aldehyde (0.0015 mol), glacial acetic acid and ethanol was refluxed for (8 h 2a-b/ 18 h 2c-f). The precipitated solid formed after cooling was filtered off, washed and recrystallized from ethanol.

2-((4-Chlorobenzylidene)amino)-4-methyl-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (2a). Yield (67.9%), m.p. = 208-10 ^{o}C; IR (KBr) ν cm^{-1}: 3321 (NH), 1675, 1594 (CO). ^{1}HNMR (DMSO-d_6-D_2O): δ 2.08 (s, 3H, CH_3), 7.12-7.70 (m, 13H, Ar-H + pyridone-H), 7.98 (d, 2H, 4-Cl-ph-$H_{2,6}$), 8.27 (s,1H , N=C<u>H</u>), 10.37 (s, 1H, NH; D_2O exchangeable). Anal. Calcd. for $C_{26}H_{20}ClN_3O_2$; Mwt: 441; C, 70.67; H, 4.56; N, 9.51; (%); Found: C, 70.82; H, 4.61; N, 9.68 (%). MS m/z (%): 443 (M+2, 2.46), 441(M^+, 1.28), 57 (100).

2-((4-Fluorobenzylidene)amino)-4-methyl-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (2b). Yield (45%), m.p. = 208-11 ^{o}C; IR (KBr) ν cm^{-1}) 3315 (NH), 1674, 1592 (CO).^{1}H-NMR (DMSO-d_6-D_2O): δ 2.09 (s, 3H, CH_3), 7.12 –7.68 (m, 13H, Ar-H + pyridone-H), 8.05 (d, 2H, 4-F-ph-$H_{2,6}$), 8.28 (s, 1H, N=C<u>H</u>), 10.35 (s, 1H, NH; D_2O exchangeable). Anal. Calcd. for $C_{26}H_{20}FN_3O_2$; Mwt: 427; C, 73.40; H, 4.74; N, 9.88; (%); Found: C, 73.54; H, 4.82; N, 9.94 (%). MS: m/z (%): 443 [$(M+H_2O)^+$, 11.40], 174 (100).

2-((4-Methoxybenzylidene)amino)-4-methyl-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (2c). Yield (64.23 %), m.p. = 150-2 ^{o}C. IR (KBr) ν cm^{-1}: 3317 (NH), 1674, 1591 (CO). ^{1}HNMR (DMSO-d_6-D_2O): δ 2.09 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 7.01-7.39 (m, 11H, ph-H + pyridone-H), 7.65 (d, 2H, 4-OCH_3-ph-$H_{3,5}$, J = 9 Hz), 8.0 (d, 2H, 4-OCH_3-ph-$H_{2,6}$, J = 9 Hz), 8.20 (s, 1H, N=C<u>H</u>), 10.21 (s, 1H, NH; exchangeable with D_2O). Anal. Calcd. for $C_{27}H_{23}N_3O_3$; Mwt: 437; C, 74.12; H, 5.30; N, 9.60; (%). Found: C, 74.23; H, 5.36; N, 9.71 (%). MS: m/z (%): 437 (M^+, 3.64), 53 (100).

4-Methyl-6-oxo-N,1-diphenyl-2-((3-phenylallylidene)amino)-1,6-dihydropyridine-3-carboxamide (2d). Yield (39.78%), m.p. = 196-8 ^{o}C. IR (KBr) ν cm^{-1}: 3346 (NH), 1681, 1602 (CO). ^{1}HNMR (DMSO-d_6-D_2O): δ 2.50 (s, 3H, CH_3), 7.13 - 7.73 (m, 18H, Ar-H + pyridone-H and C<u>H</u>=C<u>H</u>), 8.10 (d, 1H, N=C<u>H</u>), 10.26 (s, 1H, NH; exchangeable with D_2O). Anal. Calcd. for $C_{28}H_{23}N_3O_2$; Mwt: 433; C, 77.58; H, 5.35; N, 9.69; (%). Found: C, 77.64; H, 5.38; N, 9.82 (%). MS: m/z (%): 433 (M^+, 1.79), 116 (100).

4-Methyl-2-((4-nitrobenzylidene)amino)-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (2e). Yield (36.69%), m.p. = 208-10 ^{o}C. IR (KBr) ν cm^{-1}: 3324 (NH), 1675, 1599 (CO), 1524, 1341 (NO_2). ^{1}HNMR (DMSO-d_6-D_2O): δ 2.48 (s,3H,CH_3), 7.14-7.19 (t, 2H, 2ph-H_4), 7.35-7.40 (m, 5H, 2ph-$H_{3,5}$ + pyridone-H), 7.60 (d, 4H, 2ph-$H_{2,6}$), 8.10 (d, 2H, 4-NO_2-ph-$H_{2,6}$, J = 8.4 Hz), 8.31 (s, 1H, N=C<u>H</u>), 8.35 (d, 2H, 4-NO_2-ph-$H_{3,5}$, J = 8.4 Hz), 10.52

(s,1H, NH; exchangeable with D_2O). Anal. Calcd. for $C_{26}H_{20}N_4O_4$; Mwt: 452; C, 69.02; H, 4.46; N, 12.38; (%). Found: C, 69.09; H, 4.51; N, 12.47 (%). MS: m/z (%): 452 (M^+, 1.54), 261 (100).

4-Methyl-2-((4-methylbenzylidene)amino)-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (2f). Yield (45.46%), m.p. = 170-2 ^oC. IR (KBr) ν cm^{-1}: 3337 (NH), 1681, 1588 (CO). ^{1}HNMR (DMSO-d_6-D_2O): δ 2.15 (s, 3H, CH_3), 2.40 (s, 3H, ph-C<u>H</u>$_3$), 7.14- 7.16 (m, 2H, 2 ph-H_4), 7.37- 7.43 (m, 9H, ph-H + pyridone-H), 7.65 (d, 2H, 4-methyl-ph-$H_{3,5}$, *J* = 9 Hz), 7.89 (d, 2H, 4-methyl-ph-$H_{2,6}$, *J* = 9 Hz), 8.23 (s, 1H, N=C<u>H</u>) ,10.35(s, 1H, NH; exchangeable with D_2O). Anal. Calcd. for $C_{27}H_{23}N_3O_2$; Mwt: 421; C, 76.94; H, 5.50; N, 9.97; (%). Found: C, 77.08; H, 5.57; N, 10.08 (%). MS: m/z (%): 421(M^+, 0.63), 261 (100).

Synthesis of 2-benzamido-4-methyl-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (3a-c).

A mixture of 2-amino-1,6-dihydro-4-methyl-6-oxo-N,1-diphenylpyridine-3-carboxamide **1** and substituted benzoyl chloride (0.0015 mol) was refluxed for 10 h. in pyridine. The reaction mixture was poured on ice-water then acidified with HCl. The solid precipitate was filtered off, washed with water, dried and recrystallized from the appropriate solvent.

2-Benzamido-4-methyl-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (3a).

Yield (75.4%), m.p. = 160-2 ^oC. IR (KBr) ν cm^{-1}: 3269, 3208 (2NH), 1666, 1613 (CO). ^{1}HNMR (DMSO-d_6-D_2O): δ 3.36 (s, 3H, CH_3), 7.06-7.11 (m, Ar-H + pyridone-H), 7.30-7.35 (m, Ar-H), 7.55 (d, 4H, 2ph-$H_{2,6}$), 7.76 (d, 2H, phenylcarboxamide-$H_{2,6}$), 10.28 (s, 2H, 2NH; exchangeable with D_2O). Anal. Calcd. for $C_{26}H_{21}N_3O_3$; Mwt: 423; C, 73.74; H, 5.00, N, 9.92; (%). Found: C, 73.97; H, 5.12; N, 10.06 (%). MS m/z (%): 425 (M^++2, 0.09), 64 (100).

2-(4-Chlorobenzamido)-4-methyl-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (3b). Yield (79.57%); m.p. = 164-6 ^oC. IR (KBr) ν cm^{-1}: 3269, 3208 (2NH), 1665, 1612 (CO). ^{1}HNMR (DMSO-d_6-D_2O) ppm: δ 3.38 (s, 3H, CH_3), 7.07-7.55 (m, 11H, ph-H and pyridone-H), 7.59 (d, 2H, 4-Cl-ph-$H_{3,5}$, *J* = 8.1Hz), 7.97 (d, 2H,4-Cl-ph-$H_{2,6}$, *J* = 8.1Hz), 10.27 (s , 2H, 2NH; exchangeable with D_2O). Anal. Calcd. for $C_{26}H_{20}ClN_3O_3$; Mwt: 459; C, 68.20; H, 4.40; N, 9.18; (%). Found: C, 68.32; H, 4.45; N, 9.37 (%). MS m/z (%): 458 [$(M^{+2}-1)^+$, 20.64], 456 [$(M-1)^+$, 24.78], 411(100).

4-Methyl-2-(4-nitrobenzamido)-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (3c). Yield (54.5%), m.p. = 155-7 ^oC. IR (KBr) ν cm^{-1}: 3270, 3208 (2NH), 1664, 1603 (CO), 1558, 1346 (NO_2).^{1}HNMR (DMSO-d_6-D_2O): δ 3.34 (s, 3H, CH_3), 7.09-7.27 (m, 2H, 2ph-H_4), 7.30-7.33 (m ,5H, 2ph-$H_{3,5}$ + pyridone-H), 7.52 (d, 4H, 2ph-$H_{2,6}$), 8.17 (d, 2H, 4-NO_2-ph-$H_{2,6}$, *J* = 8.7Hz) , 8.36 (d, 2H, 4-NO_2-ph-$H_{3,5}$, *J* = 8.7Hz), 10.28(s ,2H ,2NH; exchangeable with D_2O). Anal. Calcd. for $C_{26}H_{20}N_4O_5$; Mwt: 466; C, 66.66; H, 4.30; N, 11.96; (%). Found: C, 66.81; H, 4.36; N, 12.08 (%). MS m/z (%): 464 [$(M-2H_2)^+$, 0.82], 92 (100).

Synthesis of 2-(alkylamino)-4-methyl-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (4a-b).

Compound **1** (0.0025 mol, 0.79 gm) was dissolved in the least amount of DMF then added to a stirred solution of NaOH (0.01mol , 0.4 gm) in DMF (10 ml). Dimethylsulphate or diethylsulphate (0.00275 mol) was added drop-wise. The mixture was stirred for 3 h after which the reaction mixture was poured on ice-water and acidified with HCl. The solid precipitate was filtered off, washed with water, dried and recrystallized from the appropriate solvent.

4-Methyl-2-(methylamino)-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (4a). Yield (28.73%), m.p. = 138-40 ^oC. IR (KBr) ν cm^{-1}: 3293 (2NH), 1689, 1600 (CO). ^{1}HNMR (DMSO-d_6-D_2O): δ 2.40 (s, 3H, CH_3), 3.75 (s, 3H, N-CH_3), 5.12 (s, 1H, NH; exchangeable with D_2O), 7.12 -7.64 (m, 11H, Ar-H + pyridone-H), 9.71 (s, 1H, NH; exchangeable with D_2O). Anal. Calcd. for $C_{20}H_{19}N_3O_2$; Mwt: 333; C, 72.05; H, 5.74; N, 12.60; (%). Found: C, 72.19; H, 5.81; N, 12.78 (%). MS m/z (%): 333 [M^+, 45.71], 286 (100).

2-(Ethylamino)-4-methyl-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (4b). Yield (36.77 %), m.p. = 150-2 ^oC. IR (KBr) ν cm^{-1}: 3299, 3191(2NH), 1651, 1617 (CO). ^{1}HNMR (DMSO-d_6-D_2O): δ 1.05 (m, 3H, NCH_2-C<u>H</u>$_3$), 1.23 (s, 3H, CH_3), 3.2 (m, 2H, CH_2), 7.04 -7.64 (m, 11H, Ar-H and pyridone -H), 9.72 (s, 1H, NH; exchangeable with D_2O), 11.36 (s, 1H, NH; exchangeable with D_2O). Anal. Calcd. for $C_{21}H_{21}N_3O_2$; Mwt: 347; C, 72.60; H, 6.09; N, 12.10; (%). Found: C, 72.68; H, 6.17; N, 12.22 (%). MS m/z (%): 347 (M^+, 2.27), 93 (100).

Synthesis of 2-amino-4-methyl-6-oxo-N,1-diphenyl-5-((4-substitutedphenyl)diazenyl)-1,6-dihydropyridine-3-carboxamide (5a-d).

A mixture of compound **1** (0.0015mol, 0.5gm) and sodium acetate trihydrate (0.004 mol, 0.64g) in ethanol (50 ml) was stirred at 0^o C. A cold solution of the appropriate diazonium chloride was added drop-wise over a period of 20 min. [prepared by addition of sodium nitrite (0.12 g, 0.001 mol) to appropriate aromatic amine (aniline, p-nitroaniline, p-toluidine and p-anisidine) (0.0015mol) in HCl at 0-5 ^oC over a period of 30 min]. After complete addition, the reaction mixture was stirred for further 2hr. The resulting solid was filtered off, and recrystallized from appropriate

solvent.

2-Amino-4-methyl-6-oxo-N,1-diphenyl-5-(phenyldiazenyl)-1,6-dihydropyridine-3-carboxamide(5a). Yield = (53%), mp = 189-92 ^{o}C. IR (KBr) ν cm^{-1}: 3324, 3237, 3140 (NH, NH_2), 1651, 1598 (CO). ^{1}H-NMR (DMSO-d_6-D_2O): δ 2.49 (s, 3H, CH3), 3.89 (s, 2H, NH_2; exchangeable with D_2O), 7.09 - 7.73 (m, 15H, Ar-H), 9.91(s, 1H, NH; exchangeable with D_2O). Anal. Calcd. for $C_{25}H_{21}N_5O_2$; M.wt: 423; C, 70.91; H, 5.00; N, 16.54; (%); Found: C, 71.08; H, 5.08; N, 16.72 (%). MS m/z (%): 425 [$(M+2)^+$, 1.10], 78 (100).

2-Amino-4-methyl-5-((4-nitrophenyl)diazenyl)-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (5b). Yield = (28.6%), mp = 164-6 ^{o}C. IR (KBr) ν cm^{-1}: 3267, 3206, 3142 (NH, NH_2), 1662, 1599 (CO), 1554, 1339 (NO_2). ^{1}H-NMR (DMSO-d_6-D_2O): δ 2.53 (s, 3H, CH3), 3.30 (s, 2H, NH_2; exchangeable with D_2O), 7.06 – 7.55 (m,10H, Ar-H), 7.68 (d, 2H, 4-NO_2-ph-$H_{2,6}$, J = 9 Hz), 8.25 (d, 2H, , 4-NO_2-ph-$H_{3,5}$, J = 9 Hz), 10.25(s, 1H, NH; exchangeable with D_2O). Anal. Calcd. for $C_{25}H_{20}N_6O_4$; M.wt: 468; C, 64.10; H, 4.30; N, 17.94; (%); Found: C, 64.24; H, 4.36; N, 18.09 (%). MS m/z (%): 466 [$(M-2)^+$, 10.7], 103 (100).

2-Amino-4-methyl-6-oxo-N,1-diphenyl-5-(p-tolyldiazenyl)-1,6-dihydropyridine-3-carboxamide(5c). Yield = (21.8%), mp = 151-3 ^{o}C. IR (KBr) ν cm^{-1}: 3343, 3188 (NH, NH_2), 1643, 1596 (CO). ^{1}H-NMR (DMSO-d_6-D_2O): δ 2.48 (s, 6H, $2CH_3$), 3.89 (s, 2H, NH_2; exchangeable with D_2O), 7.06 - 7.55 (m,10H, Ar-H), 7.60 (d, 2H, 4-CH_3-ph-$H_{3,5}$, J = 9 Hz), 7.68 (d, 2H, 4-CH_3-ph-$H_{2,6}$, J = 9 Hz), 9.87 (s,1H, NH; exchangeable with D_2O). Anal. Calcd. for $C_{26}H_{23}N_5O_2$; M.wt: 433; C, 71.38; H, 5.30; N, 16.01; (%); Found: C, 71.53; H, 5.37; N, 16.32 (%). MS m/z (%): 429 [$(M-4H_2)^+$, 61.25], 93 (100).

2-Amino-5-((4-methoxyphenyl)diazenyl)-4-methyl-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide(5d). Yield = (61.9%), mp = 152-154 ^{o}C. IR (KBr) ν cm^{-1}: 3346, 3224, 3127 (NH, NH_2), 1646, 1597 (CO). ^{1}H-NMR (DMSO-d_6-D_2O): δ 2.50 (s, 3H, CH3), 3.76 (s, 3H, OCH_3), 6.96 (d, 2H, 4-OCH_3-ph-$H_{3,5}$), 7.08 - 7.71(m, 12H, Ar-H and 4-OCH_3-ph-$H_{2,6}$), 9.84 (s, H, NH; exchangeable with D_2O) . ^{13}C-NMR (DMSO-d_6): δ 10.56, 55.31, 106.38, 111.57, 114.35, 117.68, 120.88, 123.82, 128.50, 135.57, 138.08, 156.43, and 159.75. Anal. Calcd. for $C_{26}H_{23}N_5O_3$; M.wt: 453; C, 68.86; H, 5.11; N, 15.44; (%); Found: C, 69.02; H, 5.19; N, 15.69 (%). MS m/z (%): 452 [$(M-1)^+$, 0.02], 335 (100).

Synthesis of 4-methyl-6-oxo-N,1-diphenyl-2-(3-phenylureido)-1,6-dihydropyridine-3-carboxamide (6).

A mixture of compound **1** and phenyl isocyanate (0.0015mol) was fused together for 3 h. The reaction mixture was poured on ice-water. The orange precipitate formed was filtered off, washed with petroleum ether then diethyl ether.

Yield = (94.6 %), mp = 158-60 ^{o}C. IR (KBr) ν cm^{-1}: 3325, 3286, 3193 (3NH), 1709, 1650, 1596 (CO). ^{1}H-NMR (DMSO-d_6-D_2O): δ 2.38 (s, 3H, CH3), 6.93-6.98 (t, 3H, 3 ph-H_4), 7.24-7.29 (t, 7H, 3ph-$H_{3,5}$ + pyridone-H), 7.45 (d, 6H, 3ph-$H_{2,6}$), 8.62 (s, 2H, NH; exchangeable with D_2O), 9.69 (s, 1H, amide-NH; D_2O exchangeable). Anal. Calcd. for $C_{26}H_{22}N_4O_3$; M.wt: 438; C, 71.22; H, 5.06; N, 12.78; (%). Found: C, 71.34; H, 5.13; N, 12.95 (%). MS m/z (%0: 438 [M^+, 6.71], 93 (100).

Synthesis of 2-amino-6-oxo-N,1-diphenyl-4-(4- Substituted styryl)-1,6-dihydropyridine-3-carboxamide (7a-c).

A mixture of equimolar amounts of compound **1,** the appropriate aromatic aldehyde (0.0015mol) in ethanol (10ml) and a catalytic amount of piperidine was refluxed for 20 h. The precipitated solid formed after cooling was filtered off, washed and recrystallized from ethanol.

2-Amino-4-(4-chlorostyryl)-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (**7a)**. Yield = (54.9 %), mp = 288-90 ^{o}C. IR (KBr) ν cm^{-1}: 3296, 3224 (NH, NH_2), 1638, 1593(CO).^{1}H-NMR (DMSO-d_6-D_2O): δ 6.93 (d, 1H, C<u>H</u>=CH-4-Cl-ph, J =7.8Hz), 7.12 – 7.39 (m, 15H, Ar-H + pyridone-H), 7.66 (d, 1H, CH=C<u>H</u>-4-Cl-ph, J = 7.8 Hz), 9.02 (s, 1H, NH; exchangeable with D_2O). Anal. Calcd. for $C_{26}H_{20}ClN_3O_2$; M.wt: 441: C, 70.67; H, 4.56; N, 9.51; (%). Found: C, 70.89; H, 4.62; N, 9.67 (%). MS m/z (%): 443 [$(M+2)^+$, 1.79], 441(M^+, 7.25), 94 (100).

2-Amino-4-(4-fluorostyryl)-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (7b). Yield = (25.5 %), mp = 278-80 ^{o}C. IR (KBr) ν cm^{-1}: 3268, 3230, 3192 (NH, NH_2), 1640, 1594 (CO). ^{1}H-NMR (DMSO-d_6-D_2O): δ6.91 – 7.45 (m, 16H, Ar-H and C<u>H</u>=CH-4-F-ph + pyridone-H), 7.63 (d, 1H, CH=C<u>H</u>-4-F-ph), 9.03 (s, 1H, NH; exchangeable with D_2O). Anal. Calcd. for $C_{26}H_{20}FN_3O_2$; M.wt: 425; C, 73.40; H, 4.74; N, 9.88; (%). Found: C, 73.59; H, 4.78; N, 9.95 (%). MS m/z (%): 425 (M^+, 18.86), 359 (100).

2-Amino-4-(4-nitrostyryl)-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (7c). Yield = (66%), mp = 118-20 ^{o}C. IR (KBr) ν cm^{-1}: 3349, 3264 (NH, NH_2), 1655, 1624 (CO), 1526, 1350 (NO_2). ^{1}H-NMR (DMSO-d_6-D_2O): δ ppm: 6.99-7.60 (m, 13H, Ar-H, pyridone -H + olefinic protons), 7.68 (d, 2H, 4-NO_2-ph-$H_{2,6}$, J = 8.4 Hz), 8.27 (d, 2H, 4-NO_2-ph-$H_{3,5}$, J = 8.4 Hz), 9.11(s, 1H, NH; exchangeable with D_2O), 10.13 (s, 2H, NH2; exchangeable with D_2O). Anal. Calcd. for $C_{26}H_{20}N_4O_4$; M.wt: 452; C, 69.02; H, 4.46; N, 12.38; (%). Found: C, 69.23; H, 4.51; N, 12.46 (%). MS m/z (%): 452 (M^+, 10.67), 93 (100).

Synthesis of 2-(((dimethylamino)methylene)amino)-4-methyl-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (8).

A mixture of equimolar amounts of compound **1**and DMF-DMA (0.0015 mol) in xylene (5ml) was refluxed for 4 h. The precipitated solid formed after cooling was filtered off and washed with diethyl ether.

Yield = (88.7%), mp = 140-2 ^{o}C. IR (KBr) ν cm^{-1}: 3327 (NH), 1669, 1623 (CO). ^{1}H-NMR (DMSO-d_6-D_2O): δ 2.49 (s, 3H, CH_3), 3.17, 3.24 (2s, 6H, $N(CH_3)_2$), 7.01 (t, 2H, 2ph-H_4), 7.26 (m, 5H, 2ph-$H_{3,5}$ + pyridone -H), 7.57 (d, 4H, 2ph-$H_{2,6}$), 7.81(s, 1H, N=CH), 9.01 (s, 1H, NH; exchangeable with D_2O). Anal. Calcd for $C_{22}H_{22}N_4O_2$; Mwt: 374; C, 70.57; H, 5.92; N, 14.96; (%). Found: C, 70.74; H, 5.95; N, 15.21 (%). MS m/z (%): 374 (M^+, 0.09), 124 (100).

Synthesis of compounds 9a-c

A solution of compound **8** (0.0015 mol, 0.5gm) in ethanol (10 ml) was refluxed for 20 h. with an equimolar amount of the appropriate secondary amine. The precipitated solid formed after cooling was filtered off, washed and recrystallized from ethanol.

4-Methyl-2-((morpholinomethylene)amino)-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (9a). Yield = (61%), mp > 300 ^{o}C. IR (KBr) ν cm^{-1}: 3332 (NH), 1681, 1635 (CO). ^{1}H-NMR (DMSO-d_6-D_2O): δ 2.44 (s, 3H, CH3), 2.55 (m, 4H, morpholine-$H_{2,6}$), 3.35(m, 4H, morpholine-$H_{3,5}$), 7.07-7.62 (m, 11H, Ar-H + pyridone-H), 8.68 (s, 1H, N=CH), 9.91 (s, 1H, NH; exchangeable with D_2O). Anal. Calcd. for $C_{24}H_{24}N_4O_3$; M.wt: 416; C, 69.21; H, 5.81; N, 13.45; (%). Found: C, 69.29; H, 5.89; N, 13.62 (%). MS m/z (%): 416 (M^+, 1.01), 93 (100).

4-Methyl-6-oxo-N,1-diphenyl-2-((piperazin-1-ylmethylene)amino)-1,6-dihydropyridine-3-carboxamide (9b). Yield = (36%), mp > 300 ^{o}C. IR (KBr) ν cm^{-1}: 3359, 3332 (2NH), 1681, 1635 (CO). ^{1}H-NMR (DMSO-d_6-D_2O): δ 2.37 (s, 3H, CH_3), 3.45 (m, 8H, piperazine-H), 4.36 (s, 1H, NH; exchangeable with D_2O), 7.06-7.11 (t, 2H, 2ph-H_4), 7.31 (s, 1H, pyridone-H), 7.33-7.38 (m, 4H, 2ph-$H_{3,5}$), 7.56-7.62 (d, 4H, 2ph-$H_{2,6}$), 8.68 (s,1H, N=CH), 9.94 (s, 1H, NH; exchangeable with D_2O). Anal. Calcd. for $C_{24}H_{25}N_5O_2$; M.wt: 415; C, 69.38; H, 6.06; N, 16.86; (%). Found: C, 69.61; H, 6.12; N, 17.04 (%). MS m/z (%): 415 (M^+, 1.40), 93 (100).

4-Methyl-6-oxo-N,1-diphenyl-2-((piperidin-1-ylmethylene)amino)-1,6-dihydropyridine-3-carboxamide (9c). Yield = (22.5%), mp = 90-2 ^{o}C. IR (KBr) ν cm^{-1}: 3328 (NH), 1654, 1620 (CO). ^{1}H-NMR (DMSO-d_6-D_2O): δ 1.55 (m, 2H, piperidine-H_4), 2.39 (s, 3H, CH_3), 2.99 (m, 4H, piperidine-$H_{3,5}$), 3.48 (m, 4H, piperidine-$H_{2,6}$), 6.90-7.62 (m,11H, Ar-H + pyridone-H), 8.15(s, 1H, N=CH), 9.02 (s, 1H, NH; exchangeable with D_2O). Anal. Calcd. for $C_{25}H_{26}N_4O_2$; M.wt: 414; C, 72.44; H, 6.32; N, 13.52; (%). Found: C, 72.68; H, 6.39; N, 13.78 (%). MS m/z (%): 413[$(M-1)^+$, 5.44], 64 (100).

Synthesis of compounds 10a, b.

A solution of compound **8** (0.0015 mol, 0.5gm) in ethanol (10ml) was refluxed for 20 h with the appropriate substituted secondary amine. The precipitated solid formed after cooling was filtered off, washed and recrystallized ethanol.

4-Methyl-6-oxo-N,1-diphenyl-2-(((4-phenylpiperazin-1-yl)methylene)amino)-1,6-dihydropyridine-3-carboxamide (10a). Yield = (38%), mp = 185-7 ^{o}C. IR (KBr) ν cm^{-1}: 3371 (NH), 1670, 1619 (CO). ^{1}H-NMR (DMSO-d_6-D_2O): δ ppm: 2.50 (s, 3H, CH3), 3.15 (m, 4H, piperazine-$H_{2,6}$), 3.42 (m, 4H, piperazine-$H_{3,5}$), 6.80-7.58 (m,16H , Ar-H + pyridone-H), 7.93 (s, 1H, N=CH), 9.12 (s, 1H, NH; exchangeable with D_2O). Anal. Calcd. for $C_{30}H_{29}N_5O_2$; M.wt: 491; C, 73.30; H, 5.95; N, 14.25; (%). Found: C, 73.47; H, 6.02; N, 14.37 (%). MS m/z (%): 493 [$(M+2)^+$, 2.56], 104 (100).

4-Methyl-2-(((4-methylpiperazin-1-yl)methylene)amino)-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide (10b). Yield = (31.3%), mp > 300 ^{o}C. IR (KBr) ν cm^{-1}: 3332 (NH), 1681, 1635 (CO). ^{1}H-NMR (DMSO-d_6-D_2O): δ 2.22 (s, 3H, CH3), 2.44 (s, 3H, N-CH_3), 3.15 (m, 4H, piperazine-$H_{3,5}$), 3.42 (m, 4H, piperazine-$H_{2,6}$), 7.07-7.12 (t, 2H, 2ph-H_4), 7.31 (s,1H, pyridone-H), 7.34-7.38 (t, 4H, 2ph-$H_{3,5}$), 7.60 (d, 4H, 2ph-$H_{2,6}$), 8.69(s, 1H, N=CH), 9.91 (s, 1H, NH; exchangeable with D_2O).^{13}C-NMR (DMSO-d_6): δ 35.91, 65.98, 66.06, 85.02, 120.89, 126.31, 128.50, 130.30, 133.87, 138.51, 146.14, 157.14, and 164.80. Anal. Calcd. for $C_{25}H_{27}N_5O_2$; M.wt: 429; C, 69.91; H, 6.34; N, 16.31; (%). Found: C, 70.08; H, 6.38; N, 16.58 (%). MS m/z (%): 429 (M^+, 1.38), 93 (100).

2.2 Antimicrobial Screening

Methodology of the antimicrobial screening

The newly synthesized compounds were tested for their antibacterial and antifungal activities according to The Clinical and Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical Laboratory Standards (NCCLS), based on the brothmicrodilution reference method according to the National Committee for Clinical Laboratory Standards (2002).

These assays were performed at the Regional Center for Mycology and Bio-technology, Antimicrobial unit test organisms, Cairo, Egypt.

Microorganism's strains and preparation of inoculum

A. fumigatus (RCMB 02568), C. albicans (RCMB 05036), S. pneumoniae (RCMB 010010), B. subtilis (RCMB 010067), P. aeruginosa (RCMB 010049), E. coli (RCMB 010058) and S.Typhimurium (RCMB 010315) Strains were used in this study. The microbial suspension equivalent to the turbidity of 0.5 McFarland (10^8 CFU/ml i.e colony forming unit per millilitre) standard was prepared from a fresh subculture of tested bacteria in a Mueller Hinton broth (MHB) and tested with fungi in a Sabouraud dextrose broth (SDB) then this suspension was diluted to 10^6 CFU/ml using MHB for bacteria and Sabouraud dextrose Broth (SDB) for tested fungi. The adjusted microbial inoculum (100 µl) was added to each well of a sterile 96-well flat-bottomed microtiter plate containing the tested concentration of tested samples (100 µl/well). Three wells containing a microbial suspension with no sample using DMSO employed for dissolving the tested compound (growth control) and two wells containing only media (background control) were included in this plate. Optical densities were measured after 24 hours at 37°C for bacteria and after 48 hours at 28°C for fungi using a multi-detection microplate reader at The Regional Center for Mycology and Biotechnology (Sun Rise–Tecan, USA) at 600 nm. Ampicillin, Gentamicin and Amphotericin B were used as standards for Gram positive bacteria, Gram negative bacteria and fungi, respectively. The percentage of microbial inhibitory was calculated using the Microsoft Excel®.

Microbial viability % was calculated according to the following equation:

$$\text{Percentage of viability} = [1-(ODt/ODc)] \times 100\%$$

Where, ODt is the mean optical density of wells treated with the tested compound and ODc is the mean optical density of untreated cells, while Inhibitory % = (100 – viability) %.

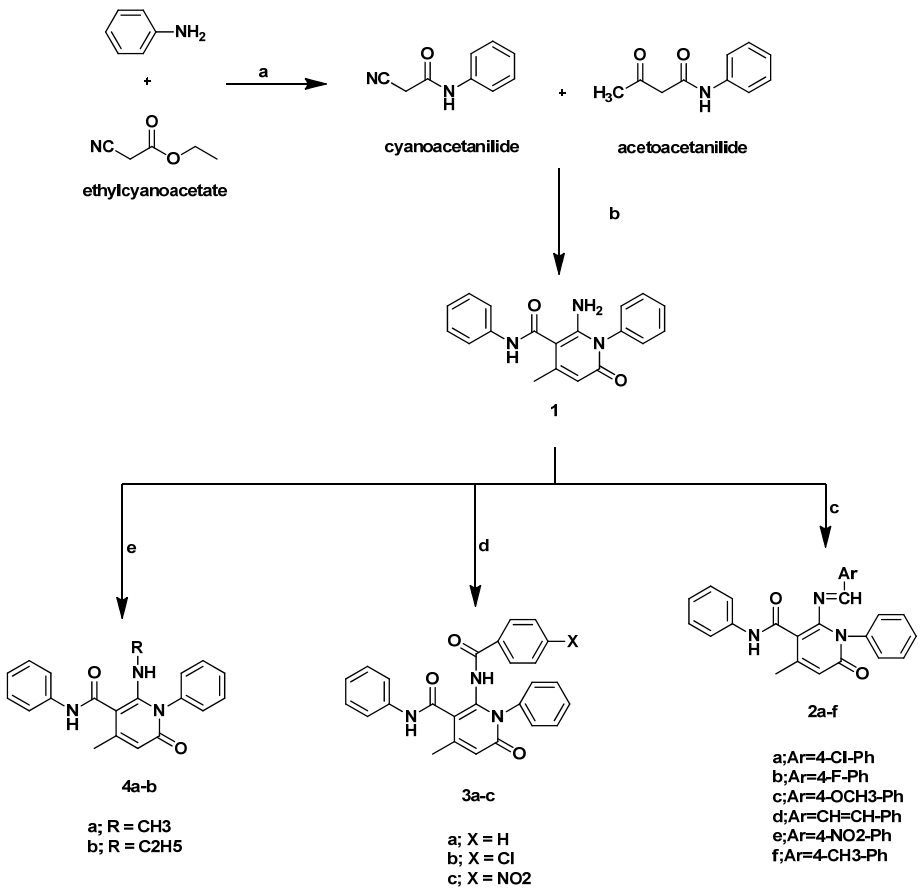
Minimum Inhibitory Concentration (MIC) & Inhibitory concentration50 (IC_{50}) measurement

For the determination of MIC of tested samples microdilution test was performed in 96-well plates. Two-fold dilutions of each compound were prepared in the test wells, the final drug concentrations being (125–0.004) µg/mL, control wells were prepared with culture medium only and microbial suspension only. The plates were sealed and incubated for 24 hours at 37°C for bacteria and for 48 hours at 28°C for fungi, after each incubation time. MIC was detected as the lowest sample concentration that prevented microbial growth. Each MIC was determined three times.The test compounds were also compared using the IC_{50} value, i.e., the concentration of the compound leading to 50% microbial death that was estimated from graphical plots.

3. Results and Discussion

3.1 Chemistry

In the present work, synthesis of the novel twenty six 2-pyridone derivatives bearing different aryl and heteroaryl rings are depicted in (Schemes 1-3). Cyanoacetamides are highly reactive compounds that have been extensively utilized in building different organic heterocycles. Thus, the known intermediate cyanoacetanilide was obtained through the fusion of aniline with slight excess of ethyl cyanoacetate (Fadda, Bondock, Rabie & Etman, 2008). Synthesis of the 2-amino-4-methyl-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide **1** was carried out by reacting equimolar quantities of acetoacetanilide and cyanoacetanilide in ethanol using triethylamine as a catalyst (Fadda, Bondock, Rabie & Etman, 2008; Ammar et al, 2005; Mohamed, Awad, El-Hallouty & El-Araby, 2012).

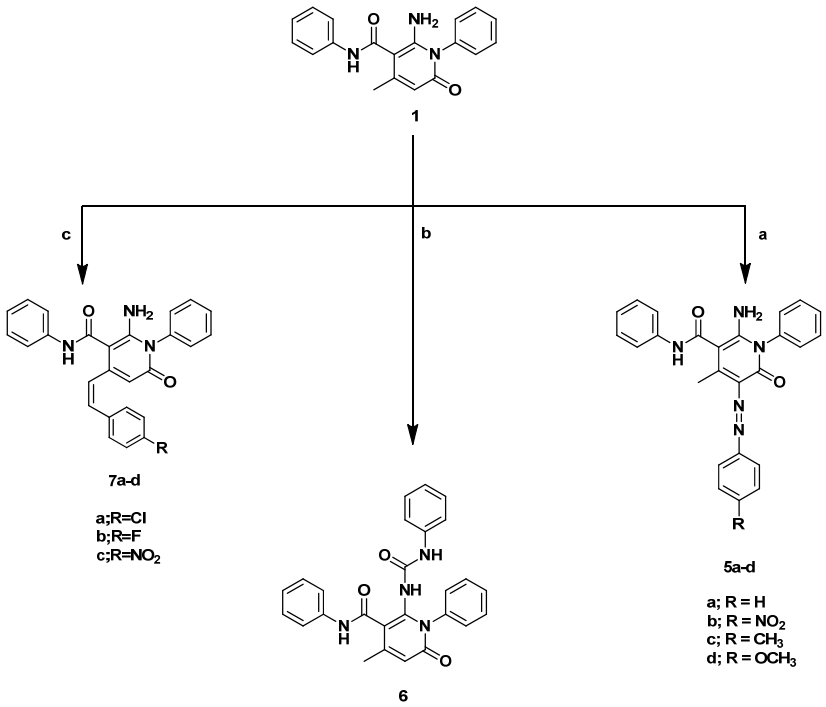


Scheme 1.

Reagents and conditions: a) fusion. b) ethanol, TEA, reflux. c) substituted aromatic aldehydes , glacial acetic acid, ethanol, reflux. d) 4-substituted benzoyl chloride, pyridine, reflux. e) DMS/DES, DMF, NaOH, stirring.

^{1}HNMR spectrum revealed the existence of a singlet at δ 2.20 ppm corresponding to CH_3 group and two D_2O exchangeable singlets at δ 3.87 and 10.23 ppm corresponding to NH, NH_2 groups. **^{13}CNMR** spectrum revealed the existence of CH_3 group at δ 26.65 ppm and 2 carbonyl groups at δ 160.91 ppm and was taken as an evidence for the formation of the pyridone ring. In order to examine the effect of incorporation of arylideneamino moiety, compounds **2a-f** were synthesized via refluxing compound **1** with substituted aromatic aldehydes, using absolute ethanol as a solvent and in the presence of catalytic amount of acetic acid (Parmar et al, 2011).

IR spectra of these derivatives exhibited a sharp band around 3321 cm^{-1} corresponding to NH group along with the disappearance of NH_2 bands. Moreover, **^{1}HNMR** spectra for this group exhibited a singlet corresponding to the imine proton (CH=N) at the range δ 8.10 to 8.31 ppm denoting the formation of the schiff's base. Compounds **3a-c** were successfully prepared by refluxing equimolar quantities of **1**and substituted benzoyl chloride in pyridine. **IR** spectra for this series were in agreement with the predicted structures as they showed the disappearance of NH_2 bands. In addition,**^{1}HNMR** spectra of **3b** and **3c,** as representatives of this group,displayed two doublets corresponding to the para-substituted systems at δ 7.59,7.97 ppm and at δ 8.17,8.36 ppm with *J* constant = 8.1 and 8.7 Hz respectively. Alkylation of the amino group was achieved by the reaction of compound **1** with dimethylsulfate or diethylsulfate in DMF / NaOH to afford compounds **4a,b**. **^{1}HNMR** spectrum of compound **4a** showed a singlet at δ 3.75 ppm assigned to the methyl group, as for compound **4b**, the triplet quartet pattern of the N-ethyl group appeared at δ 1.05 and 3.20 ppm.



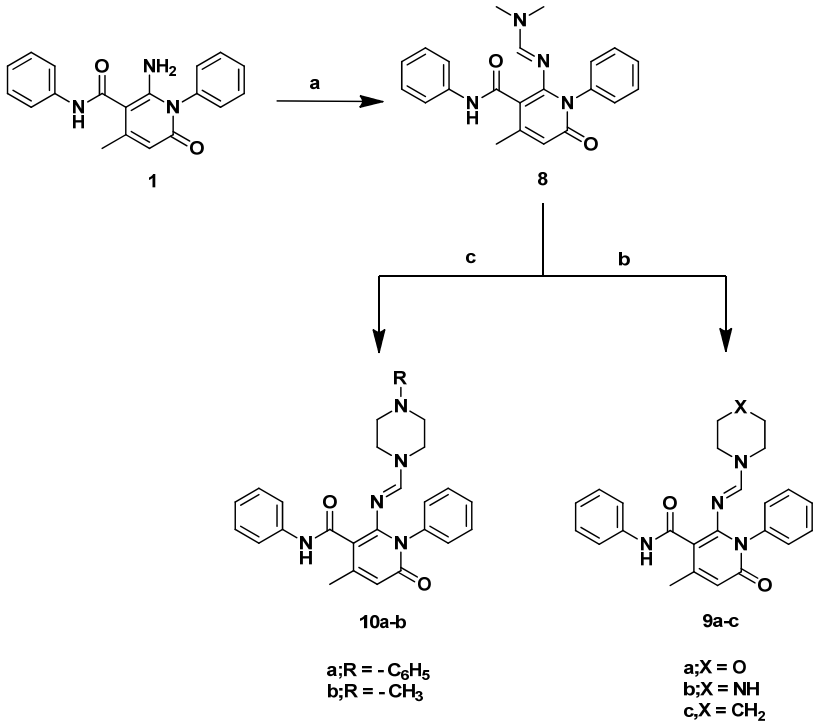
Scheme 2.

Reagents and conditions: a) ArN_2Cl, ethanol, Na acetate, 0 ^{o} C, stirring. b) PhNCO, fusion. c) substituted aromatic aldehydes, ethanol, piperidine, reflux.

Scheme 2, describes the schematic steps carried out in the synthesis of derivatives **5a-d**, **6**, **7a-d**. Compounds **5a-d** were prepared by hybridization of the aryldiazenyl moiety with the pyridone ring through a hydrazine linker. Thus, diazodization of aromatic amines with hydrochloric acid and sodium nitrite gave the diazonium salts, which were subsequently coupled with compound **1** in ethanolic sodium acetate to afford the target compounds (Hartz et al., 2010 & Badea, Sxofei, Venterb & Bercean, 2007).

The **IR** spectra of **5a-d** showed the presence of characteristic absorption bands of NH, NH_2. ^{1}**HNMR** spectra of **5b** and **5c** as representative examples of these series revealed the existence of two doublets representing AB system at δ 7.68, 8.25 ppm and at δ 7.60,7.68 ppm respectively with *J* constant = 9 Hz for both of them, while ^{1}**HNMR** spectrum of **5d** showed a singlet at δ 3.76 ppm representing OCH_3 protons. Moreover, ^{13}**CNMR** spectrum of **5d** illustrated a characteristic signal at 55.31 ppm corresponding to carbon of OCH_3 group, in addition to a characteristic signal at 159.00 ppm assigned to carbon of carbonyl amide and another one at 156.00 ppm assigned to carbon of pyridone carbonyl. The title compound **6** was prepared through the fusion of compound **1** with slight excess of phenylisocyanate. The spectral data of compound **6** was in agreement with the assigned structure. **IR** spectrum displayed three bands at 3325, 3286, 3193 cm^{-1} corresponding to 3NH groups. Compounds **7a-c** were prepared by refluxing compound **1** with an equimolar amount of substituted aromatic aldehydes in the presence of piperidine using absolute ethanol as a solvent (Ammar et al., 2004; Abdelghani, Shehab, El-Mobayed & Abdel Hamid, 2012 and El-Sharkawy & Ibrahim, 2013).

IR spectra of these derivatives showed the usual bands of NH, NH_2 at 3349, 3264 cm^{-1} and carbonyl groups at 1655, 1624 cm^{-1}. ^{1}**HNMR** spectrum of **7c** as a representative example of this group displayed two doublets representing AB system of the incorporated aldehyde at δ 7.68 and 8.27 ppm with *J* constant = 8.4 Hz.



Scheme 3.

Reagents and conditions: a) DMF-DMA, dry xylene, reflux . b) secondary amines: (morpholine, piperazine and piperidine), ethanol, reflux . c) phenyl piperazine, methyl piperazine, ethanol, reflux.

The synthetic route followed for the preparation of compounds **9a-c** and **10a,b** is described in Scheme 3. Condensation of the amino group with DMF-DMA is one of the synthetic approaches to the synthesis of enamines. Thus, compound **8** was successfully prepared by refluxing compound **1** with DMF-DMA in dry xylene (Elneairy, Gad-Elkareem & Taha, 2007). **IR** spectrum revealed sharp band at 3327cm^{-1} corresponding to NH group, in addition to the disappearance of NH_2 bands. **^{1}HNMR** displayed two singlets at δ 3.17 and 3.24 ppm representing the 6 protons of dimethylamino group, in addition to the singlet at δ 7.81 ppm which is attributed to the imine proton. Furthermore, this derivative was used as an intermediate in the preparation of compounds **9a-c** and **10a,b**. Where, compounds **9a-c** and **10a,b** were successfully prepared according to the literature procedures via the reaction of the enamine **8** with an equimolar amount of appropriate secondary amines for **9a-c** and substituted secondary amines for **10a,b** in ethanol (Shawali, 2012 & Al-Zaydia, Al-Shamarya & Elnagdi, 2006).

The above mentioned structures were confirmed based on elemental and spectral data. Their structures were established by **^{1}HNMR** spectra which displayed the disappearance of the characteristic signals of dimethylamino group, along with the appearance of a singlet at δ 7.93-8.69 ppm for the imine proton. Further confirmation was obtained by the **MS** spectra, which were consistent with their molecular weights. In addition, **^{13}CNMR** spectrum of **10b** showed a characteristic signal at 35.91 ppm assigned to carbon of $\underline{C}H_3$-N and at 65.98, 66.26 ppm corresponding to the four piperazine carbons, in addition to a characteristic signal at 157.14ppm assigned to imine carbon N=$\underline{C}$H-N and 2 carbonyl groups at δ 164.80 ppm.

3.2 Anti-microbial Activity

Antibacterial and antifungal activities of the newly synthesized compounds were performed at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. Initially, target compounds **1, 2a-f, 3a-c, 4a,b, 5a-d, 6, 7a-c, 8, 9a-c, 10a,b**andreference drugs were evaluated *in-vitro* for their antimicrobial and antifungal activities, using the broth microdilution reference method and minimum inhibitory concentration (MIC). The compounds were tested against two fungal strains (*Aspergillus fumigates* and *Candida albicans*), two Gram-positive bacteria (*Bacillus subtillus* and *Streptococcus pneumonia*) and two Gram-negative bacteria *(Escherichia coli and Salmonella Typhimurium*).

3.2.1 Anti-fungal Activity

*Aspergillus fumigatus*is largely responsible for increased the incidence of invasive aspergillosis (IA) in immunocompromised patients (Moore, Walls & Denning, 2001). Thus, the activity of the novel compounds were

presented in Table 1 and Table 2 which revealed that compounds **3a**, **5c** and **9b** were equipotent to the reference drug, Amphotericin B, against *Aspergillus fumigatus* (MIC = 1.95µg/ml), additionally, compound **3a** was also equipotent to the reference drug against *Candida albicans*. Good activity was also observed for compounds **2d**, **3b**, **5b** and **9a**, against *Aspergillus fumigatus* (MIC = 3.9 µg/ml)

Table 1. The *in-vitro* antimicrobial activities, means of inhibitory % ± Standard deviation produced on a range of clinically pathogenic microorganisms using (125 µg) concentration of tested samples.

Test Organism / Comp. No.	Fungi		Gm +ve Bacteria		Gm -ve Bacteria	
	Aspergillus fumigatus	*Candida albicans*	*Streptococcus pneumonia*	*Bacillis subtilis*	*Escherichia coli*	*Salmonella Typhimurium*
1	16.15 ± 0.25	13.24 ± 0.25	14.38 ± 0.58	15.15 ± 0.58	14.16 ± 0.44	22.3 ± 0.58
2a	44.33 ± 0.63	32.25 ± 0.44	56.25 ± 0.25	67.25 ± 0.37	20.44 ± 0.37	32.15 ± 0.63
2b	73.25 ± 0.58	60.44 ± 0.63	61.23 ± 0.25	71.24 ± 0.25	42.51 ± 0.63	51.24 ± 0.58
2c	26.43 ± 0.44	20.63 ± 0.63	28.14 ± 0.37	35.3 ± 0.25	19.25 ± 0.37	29.8 ± 0.58
2d	80.63 ± 0.58	72.41 ± 0.37	69.21 ± 0.44	81.32 ± 0.58	62.14 ± 0.44	72.1 ± 0.58
2e	NA	NA	NA	NA	NA	NA
2f	NA	NA	NA	NA	NA	NA
3a	86.24 ± 0.28	90.24 ± 0.21	72.32 ± 0.12	91.85 ± 0.33	66.34 ± 0.19	74.32 ± 0.58
3b	76.25 ± 0.24	84.32 ± 0.42	71.21 ± 0.39	86.24 ± 0.58	71.24 ± 0.44	83.24 ± 0.63
3c	72.61 ± 0.24	80.23 ± 0.42	69.25 ± 0.44	84.32 ± 0.58	68.32 ± 0.44	74.32 ± 0.58
4a	NA	NA	NA	NA	NA	NA
4b	NA	NA	NA	NA	NA	NA
Ampicillin	—	—	86.32 ± 0.58	99.62 ± 0.63	—	—
Gentamicine	—	—	—	—	75.42 ± 0.58	86.32 ± 0.63
Amphotericin B	90.31 ± 0.58	95.21 ± 0.44	—	—	—	—

Table 2. Antimicrobial activity as MICs (µg/ml) of tested samples against tested organisms

Test Organism / Comp. No.	Fungi		Gm +ve Bacteria		Gm -ve Bacteria	
	Aspergillus fumigatus	*Candida albicans*	*Streptococcus pneumonia*	*Bacillis subtilis*	*Escherichia coli*	*Salmonella Typhimurium*
1	125	125	125	125	125	125
2b	7.81	15.63	15.63	7.81	31.25	31.25
2d	3.9	7.81	7.81	3.9	15.63	7.81
3a	1.95	1.95	7.81	1.95	7.81	7.81
3b	3.9	3.9	7.81	1.95	7.81	3.9
3c	7.81	3.9	7.81	3.9	7.81	7.81
5a	15.63	31.25	7.81	3.9	31.25	7.81
5b	3.9	15.63	1.95	1.95	15.63	3.9
5c	1.95	7.81	1.95	0.98	7.81	1.95
5d	62.5	31.25	31.25	15.63	31.25	15.63
9a	3.9	3.9	3.9	0.98	15.63	3.9
9b	1.95	7.81	7.81	3.9	31.25	7.81
Ampicillin	—	—	1.95	0.98	—	—
Gentamicine	—	—	—	—	7.81	1.95
Amphotericin B	1.95	1.95	—	—	—	—

3.2.2 Anti-bacterial Activity

The obtained results against Gram-positive and Gram-negative bacteria showed that all the tested compounds exhibited superior activity to the starting 2-amino-4-methyl-6-oxo-N,1-diphenyl-1,6-dihydropyridine-3-carboxamide **1**. Interestingly, compound **5c** displayed broad-spectrum antibacterial activity equipotent to both Ampicillin and Gentamicin against the tested bacteria. This result shows that the introduction of the aryldiazenyl moiety in position 5 greatly enhanced the antibacterial activity. Moreover, in this series the 4-methylphenyl showed better activity than the nitro- and methoxyhpenyl counterparts with the exception of **5b** which was equipotent to Ampicillin against the gram-positive bacteria, *Streptococcus pneumonia*. Additionally, the incorporation of morpholinomethylene to the 2-amino group resulted in an improved activity against *Bacillus subtillus* as demonstrated by compound **9a,** MIC value = 0.98 µg/mL, equivalent to Ampicillin. Interestingly, the 2-benzamido derivatives **3a-c** exhibited good activity against *Escherichia coli* with same potency as Gentamicin, 7.81 µg/mL, these results show that acylation of the amino group directs the activity against this bacterial strain, it also shows that the activity is not affected by the substituents on the phenyl ring. All the tested compounds were inactive against the gram-negative bacteria, *Pseudomonas aeruginosa.* Moreover, compounds **2e,f**, **4a,b** and **6** were devoid of any antifungal or antibacterial activities.This may suggest that

reactions which transform the amino group to alkylamino or urea moieties were not beneficial for the activity contrary to the benzamido-substitution as compounds **3a,b.** Furthermore, with the exception of compounds **2d** and **9a**, the potency was greatly reduced in the enamine series such as compounds **2a-f, 9a-c** and **10a,b**.

3.3 2D QSAR Study

3.3.1 Development of QSAR Models

QSAR analyses for antibacterial and antifungal activities of the prepared derivatives(**1, 2b,d, 3a-c, 5a-d and 9a,b**) were performed in order to correlate these activities with thestructural features of the synthesized compounds, and to identify the positive and negative structural features within them.

The analysis was run by means of the DS 2.5 software (Discovery Studio 2.5, Accelrys, Co., Ltd., San Diego, CA, USA). A set of the newly synthesized 2-pyridones, twelve compounds, was used as a trainingset with their measured pMIC against *Bacillis subtilis*, *Aspergillus fumigates* and *Escherichia coli* for QSAR modeling. Compounds (**1**, **5c** and **5d**) were adopted as an external test subset for validating the QSAR models. "Calculate Molecular Properties" module was used for calculatingdifferent molecular properties for the training set compounds. 2D Descriptors involved: AlogP, molecular properties, molecular property counts, surface area and volume and topological descriptors, while the 3D descriptors involved: Dipole, jurs descriptors, principle moments of inertia, shadow indicesand surface area and volume. Genetic function approximation (GFA) was utilized to search for the best possible QSAR regression equation capable of correlating the variations in the biological activities ofthe training set compounds with variations in the generated descriptors, *i.e.*, multiple linear regressionmodeling (MLR). QSAR model was validated employing leave one-out cross-validation by settingthe folds to a number much larger than the number of samples, r^2 (squared correlation coefficient value) and r^2 prediction (predictive squared correlation coefficient value), residuals between the predicted andexperimental activity of the test set and training set.

3.3.2 QSAR Study Results

Equation (1).Represents the best performing QSAR model for the activity against *Aspergillus fumigates*;

−logMIC = 1.3196 + 0.3312 **Num_RotatableBonds** - 5.8276 **Shadow_YZfrac** (1)

Equation (2).Represents the best performing QSAR model for the activity against *Bacillus subtillus*;

−logMIC = 4.3021 - 1.6266e-003 **PMI_Z** - 5.1754 **Shadow_XYfrac** (2)

Equation (3).Represents the best performing QSAR model for the *Escherichia coli*;

−logMIC= -1.2398 - 7.1752e-002 **ES_Sum_dsN** - 8.1325e-004 **Jurs_WNSA_2** (3)

According to equations (1)–(3), the QSAR models were represented graphically by scattering plotsof the experimental *versus* the predicted bioactivity values −logMIC for the training set compounds asshown in Figures 2-4. The method used to build the model was Least-Squares, r^2 = 0.75, 0.818 and0.818, respectively, r^2 (adj) = 0.667, 0.766 and 0.766, respectively, r^2 (pred) = 0.404, 0.672 and 0.635,respectively, Least-Squared error = 0.019, 0.022 and 0.0113, respectively, where r^2 (adj) is r^2adjustedfor the number of terms in the model; r^2 (pred) is the prediction *c*, equivalent to q^2 from a leave-1-outcross validation.

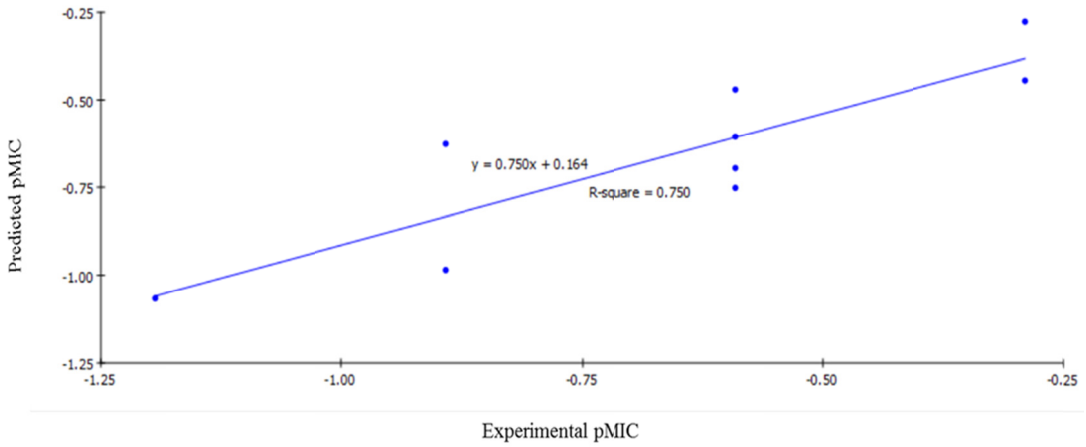


Figure 2. Predicted versus experimental pMIC of the tested compounds against ***Aspergillus fumigates*** according to equation (1), r^2 = 0.750.

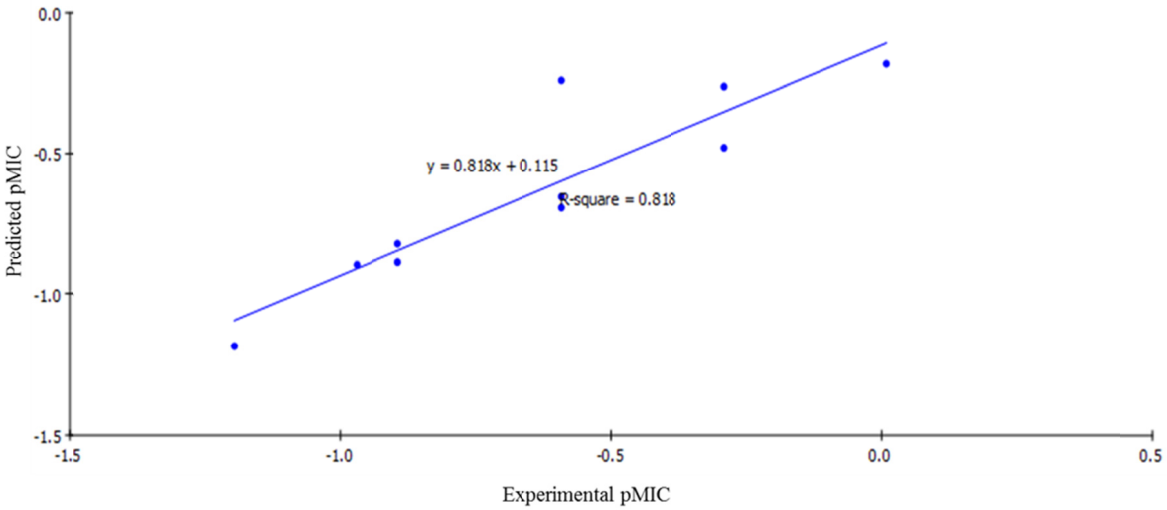


Figure 3. Predicted versus experimental pMIC of the tested compounds against Bacillus subtillus according to equation (2), r2 = 0.818.

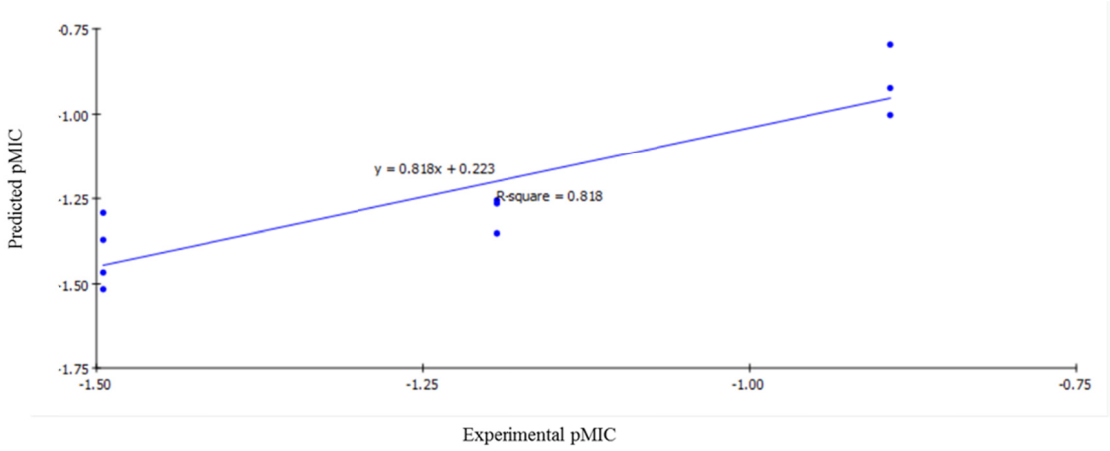


Figure 4. Predicted versus experimental PMIC of the tested compounds against Escherichia coli according to equation (3), r2 = 0.818.

In conclusion, equation (1) suggested that the antifungal activity of the synthesized compounds is mainly affected by molecular property counts (Num_RotatableBonds) and shadow index, **Shadow_YZfrac**. Rotatable bonds, are defined as single bonds between heavy atoms that are both not in a ring and not terminal, i.e., connected to a heavy atom that is attatched to only hydrogens. While shadow indices are a set of geometric descriptors to characterize the shape of the molecules (Rohrbaug, 1987). The descriptors are calculated by projecting the model surface on three mutually perpendicular planes: *xy*, *yz*, and *xz*. These descriptors depend not only on conformation, but also on the orientation of the model. To calculate them, the models are first rotated to align the principal moments of inertia with the *x*-, *y*-, and *z*-axes. Shadow_YZ is area of the molecular shadow in the *yz* plane. On the other hand, equation (2) indicated that the principal moments of inertia-Z component (PMI_Z) and the shadow index (Shadow_XYfrac) were the principle descriptors contributing negatively to the activity of the compounds against *Bacillus subtillus*. Whereas, the principal moments of inertia calculates the principal moments of inertia about the principal axes of a molecule according to certain rules: the moments of inertia are computed for a series of straight lines through the center of mass (Hill, 1960). The moments of inertia are given by: distances are established along each line proportional to the reciprocal of the square root of *I* on either side of the center of mass. The locus of these distances forms an ellipsoidal surface. The principal moments are associated with the principal axes of the ellipsoid. If all three moments are equal, the molecule is considered to be a symmetrical top. If no moments are equal, the molecule is considered to be an unsymmetrical top. Finally, equation (3) shows that the activity against *Escherichia coli*might be affected by the E-state sum for nitrogen atom (ES_Sum_dsN) and Jurs descriptor (Jurs_WNSA_2). The estate keys calculate the sums of electrotopological state (E-state) values and/or the counts of each atom type (Hall & kier, 2000) & Hall, Mohney & kier, 1991). ES_Sum_dsN calculates the E-state count for nitrogen. Moreover, Jurs descriptors are those ones that combine shape and electronic

information to characterize molecules (Rohrbaugh, 1987).The descriptors are calculated by mapping atomic partial charges on solvent-accessible surface areas of individual atoms. Jurs_WNSA_2 is surface-weighted charged partial surface areas, it is calculates by multiplying a set of six descriptors (partial positive surface area, partial negative surface area, total charge weighted negative surface area, total charge weighted positive surface area, atomic charge weighted positive surface area, atomic charge weighted negative surface area) the total molecular solvent-accessible surface area and dividing by 1000.

3.3.3 QSAR Validation

Robustness of the established QSAR models (1, 2 and 3) was verified by using; Leave-one-out (LOO) internal validation (r^2 = 0.75, 0.818 and 0.818, respectively). Cross-validation was also employed where q^2, which is equivalent to r^2 (pred), was 0.404, 0.672 and 0.635, respectively. In addition, validation was employed by measuring the residuals between the experimental and the predicted activities of the training set (Table 3).

Table 3. Experimental activities of the synthesized derivatives against the predicted activities according to equations 1, 2 and 3.

Comp	*Aspergillus fumigatus*			*Bacillus subtillus*			*Escherichia coli*		
	Experimental Activity (pMIC)	Predicted Activity (pMIC)	Residuals	Experimental Activity (pMIC)	Predicted Activity (pMIC)	Residuals	Experimental Activity (pMIC)	Predicted Activity (pMIC)	Residuals
2b	-0.892095	-0.695721	0.0930167	-0.892651	-0.885362	-0.00728928	-1.49485	-1.29206	-0.202789
2d	-0.591065	-0.695721	0.104656	-0.892651	-0.820696	-0.071955	-1.19396	-1.26466	0.0707032
3a	-0.290035	-0.444128	0.154093	-0.290035	-0.261903	-0.0281316	-0.892651	-1.00249	0.109841
3b	-0.591065	-0.47012	-0.120945	-0.290035	-0.47968	0.189645	-0.892651	-0.923027	0.0303755
3c	-0.892095	-0.626313	-0.265782	-0.591065	-0.692944	0.101879	-0.892651	-0.796406	-0.096245
5a	-1.19396	-1.06652	-0.127436	-0.591065	-0.653966	0.0629012	-1.49485	-1.51574	0.0208936
5b	-0.591065	-0.752191	0.161126	-0.969416	-0.894512	-0.074904	-1.19396	-1.25509	0.0611264
5c	---------	-------	------	0.00877392	-0.180768	0.189541	-------	------	--------
5d	--------	-------	------	---------	-------	--------	-1.49485	-1.46673	-0.0281184
9a	-0.591065	-0.605672	0.0146066	0.00877392	-0.180768	0.189541	-1.19396	-1.3522	0.158237
9b	-0.290035	-0.276699	-0.0133358	-0.591065	-0.240204	-0.350861	-1.49485	-1.37083	-0.124024

Moreover, the experimental and expected activities as well as the residuals of the compounds, used as statistical outliers in building the three models, are presented in (Table 4). Interestingly, the predicted activities by the generated QSAR models were very close to those observed experimentally, indicating that these models could be applied for further prediction of more effective hits having the same skeletal framework.

Table 4. Experimental activities of compounds 1, 5c and 5d, used as statistical outliers against the predicted activities according to equations 1, 2 and 3.

Comp	*Aspergillus fumigatus*			*Bacillus subtillus*			*Escherichia coli*		
	Experimental Activity (pMIC)	Predicted Activity (pMIC)	Residuals	Experimental Activity (pMIC)	Predicted Activity (pMIC)	Residuals	Experimental Activity (pMIC)	Predicted Activity (pMIC)	Residuals
1	-2.09691	-2.09691	-4.0496e-12	-2.09691	-2.09691	-3.59579e-12	-2.09691	-2.09691	-4.9378e-12
5c	-0.290035	-0.290035	1.03528e-11	--------	----------	----------	-0.290035	-0.290035	3.60278e-12
5d	-1.79588	-1.79588	-7.1334e-12	-0.290035	-0.261903	-0.0281316	----------	----------	---------

4. Conclusions

In the present work twenty six novel derivatives of 6-oxo-pyridine-3-carboxamide were synthesized and evaluated as antibacterial and antifungal agents, using Amphotericin B, Ampicillin and Gentamicin as reference drugs. Amongst these novel compound, the 5-(p-tolyldiazenyl)-1,6-dihydropyridine-3-carboxamide derivative, **5c,** displayed broad-spectrum antibacterial activity equipotent to both Ampicillin and Gentamicin against the tested bacteria. It also showed antifungal activity comparable to Amphotericin B. The 2D QSAR models generated by Discovery studio 2.5 software, showed some important geometric and molecular descriptors that might be controlling the activities of these novel compounds.These results suggest that the novel pyridone derivatives could be further investigated for their potential antifungal and antibacterial activities.

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