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Novel ionic liquids incorporated pyridazinone-vanillyl motifs: Synthesis, characterization, pharmacological survey and molecular docking

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ABSTRACT

Inspired by an urgent unmet medical need for development of potent and broad-spectrum antibiotics, we apply herein diverse strategies to obtain novel pyridazinone-vanillyl conjugates having a N(2)-arm of arylpropanamides (**8a**–**c**) or vanillyl ionic liquids (Val-ILs) (**9a**–**d**) motifs. These new pyridazinone-based antibiotic candidates display remarkable and broad-spectrum antimicrobial efficacy. Combined analysis of pharmacological results coupled with the *in Silico* derived parameters demonstrated the importance of the chemical nature of the arm in tuning the antimicrobial potency for the target compounds. For instance, **9b** (with Val-IL arm) (MIC/MBC = 1.98/2.18 μg/mL) is about 7-fold more potent than **8a** (with neutral arm) (MIC/MBC = 13.50/14.12 μg/mL) as Anti-*P. aeruginosa* agent. The molecular docking study revealed that compound **8a** was found to the most effective in binding to the active site of *E. coli* FabH (PDB code 1HNJ) with H-bonding, π-stacking and hydrophobic groove interactions having minimum binding energy $\Delta G_b = -14.00$ kcal/mol.

1. Introduction

Microbial infection is a serious problem worldwide and considered as one of the most important key health challenge which can be devastating due to its pathological impacts on clinical and community environment [1,2]. The antibiotics are pivotal to tackle the above challenge and indispensable for the sustainability of a healthy community free from microbial infections, however, overusing of these antibiotics for prolonged time leads to adaptation of bacteria against antibiotics and multidrug-resistant (MDR) [3,4]. Thus, it becomes imperative to explore novel antimicrobial drugs time-to-time to fight the bacterial infection and to negate this drug resistance. However, there are major challenges should be addressed for discovery and development of new antimicrobial drug such as steps of synthesis protocol, cost, and potential side effects.

Recently, the pyridazinone derivatives gain tremendous attention in the field of medicinal chemistry, because of their wide-range pharmacological activities including antibacterial [5], anticancer [6], antiHIV [7], antihypertensive [8,9], antidepressant [10], anticonvulsant [11], antithrombotic [12], cardiotonic [13] and diuretics [14]. Interestingly that some pyridazinone-based drugs such as minaprine [15], emorfazone [16], azanrinone [17], indolidan [18], bemoradan [19], primobendan [20] and levosimendan [21] are already sold in the clinical markets.

The amazing features of ionic liquids (ILs) such as low melting point, negligible vapor pressure, nonflammability, excellent mechanical and thermochemical stability, wide electrochemical avenue and supreme dual solubility in both organic and aqueous solvents [22] put them in the forefront of interest many researchers. Moreover, the amphiphilic nature of ILs may play a crucial role in controlling the pharmacokinetic properties, stability, delivery options, polymorphism of pharmacological agent, or even tuning pharmaceutical cocktails [23].

Inspired with the aforementioned remarks and in resumption of our ongoing programs directed toward the development of novel ILs-based potent and therapeutic agents [24,25] we report herein a synthesis protocol and *in vitro* antimicrobial evaluation of new vanillyl ILs-based pyridazinone derivatives with emphasize to develop a novel therapeutic strategy to combat antibiotic resistance for sustainable antimicrobial activity. Moreover, the docking simulations of the most potent compounds **8a** and **9b** with *E. coli* FabH (PDB code 1HNJ) were carried out to minimize the gap between the theoretical and actual view, also to understanding the interaction of the target compounds with *E. coli* FabH protein. The selection of FabH as antibacterial target was attributed to

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that the fatty acid biosynthesis (FAB) act as the crucial metabolic process essential for the cell viability and the growth of bacterial strains [26]. Moreover, the initiation of this process is strongly correlated to the regulatory role for β-Ketoacyl-acyl carrier protein synthase III (FabH) [27] which is also important for initiating the FA elongation cycles and involved the feedback regulation of the biosynthesis pathway [28]. The structures of FabH proteins are highly conserved at the sequence in the gram-positive/-negative bacteria while no significantly homologous proteins are found in humans. Noteworthy, the active sites belong to various bacterial FabH molecules are basically invariant [29]. Consequently, FabH has confirmed to be a key target for the design of new antibiotics. Thus, a pharmacological agent which has the ability to inhibit the FabH enzymatic activity could be act as a promising candidate for selective, non-toxic, and broad-spectrum antibacterial agent.

2. Experimental section

2.1. Materials

See Supplementary data.

2.2. Instrumentation

See Supplementary data.

2.3. Synthesis

2.3.1. Synthesis of key starting materials (Val-ILs (**2a–d**) and pyridazinone 4)

See Supplementary data.

2.3.2. Synthesis of 4-(4-hydroxy-3-methoxybenzyl)-6-(4-methoxy-3-methylphenyl) pyridazin-3-one (**5**)

To a solution of KOH in absolute EtOH (25 mL, 5% w/v), compound **4** (0.9 g, 4.17 mmol) and vanillin (0.6 g, 4.17 mmol) were added. The mixture was refluxed under stirring for 2 h. After cooling, the mixture was concentrated *in vacuo*, diluted with cold water (25 mL), and acidified with 2 N HCl to pH = 2. After 1 h stirring in an ice-bath, compound **5** was completely precipitated, filtered off from the acidic solutions and recrystallized from ethanol. It was obtained as a dirty white solid (0.96 g, 65%); mp 198–199 °C; FT-IR (KBr) ν (cm^{-1}): 3408 (m, br), 3292 (m, br), 1661 (*vs*, sh). ^{1}H NMR (300 MHz, DMSO d_6): δ (ppm) 10.92 (s, 1H), 9.01 (s, 1H), 7.79–7.66 (m, 3H), 7.02–6.88 (m, 4H), 4.11 (s, 3H), 3.87 (s, 3H), 3.68 (s, 2H), 2.18 (s, 3H). ^{13}C NMR (75 MHz, DMSO d_6): δ (ppm) 165.50, 161.44, 155.42, 148.97, 145.80, 144.32, 133.68, 131.08, 128.32, 125.40, 122.59, 122.35, 119.84, 117.61, 116.28, 114.54, 58.02, 56.34, 43.03 and 20.57. EI-MS *m/z*: $[M]^+$ Calcd for $C_{20}H_{20}N_2O_4$ 352.38; Found 352.40. Anal. Calcd for $C_{20}H_{20}N_2O_4$ (M = 352.38 g/mol): C, 68.17; H, 5.72; N 7.95; Found: C, 67.98; H, 5.83; N 7.92.

2.3.3. Synthesis of methyl 3-(5-(4-hydroxy-3-methoxybenzyl)-3-(4-methoxy-3-methylphenyl)-6-oxopyridazin-1-yl)propanoate (**6**)

A mixture of the compound **5** (1.12 g, 2.68 mmol), K_2CO_3 (0.74 g, 5.36 mmol), and methyl bromopropionate (0.73 g, 4.02 mmol) in CH_3CN (20 mL) was refluxed under stirring for 3 h. The mixture was then concentrated *in vacuo*, diluted with cold water, and extracted with CH_2Cl_2 (3 × 25 mL). The solvent was evaporated *in vacuo*, and ester **6** was purified by column chromatography using cyclohexane/ethyl acetate 2:1 as an eluent. It was obtained as faint yellow crystals (0.68 g, 58%); mp 131–132 °C. FT-IR (KBr) ν (cm^{-1}): 3412 (m, br), 1740 (*vs*, sh) 1674 (s, sh); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 11.40 (s, 1H), 7.68–7.54 (m, 3H), 7.05–6.85 (m, 4H), 4.05 (s, 3H), 3.89 (s, 3H), 3.71 (s, 3H), 3.57 (s, 2H), 3.38 (t, J = 8.4 Hz, 2H), 2.60 (t, J = 8.3 Hz, 2H), 2.30 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 175.38, 163.98, 158.76, 155.61, 148.51, 142.87, 141.36, 131.51, 130.08, 127.98, 125.85, 124.63, 122.88, 120.06, 116.69, 115.50, 114.39, 58.28, 56.41, 51.12, 46.98, 42.03, 32.91 and 20.55. EI-MS *m/z*: $[M]^+$ Calcd for $C_{24}H_{26}N_2O_6$ 438.47; Found 438.30. Anal. Calcd for (M = 438.47 g/mol): C, 65.74; H, 5.98; N, 6.39; Found: C, 65.59; H, 6.01; N, 6.33.

2.3.4. Synthesis of 3-(5-(4-hydroxy-3-methoxybenzyl)-3-(4-methoxy-3-methylphenyl)-6-oxopyrid-azin-1(6H)-yl)propanoic acid (**7a**)

A suspension of the ester **6** (1.16 g, 2.66 mmol) in 6 N NaOH (20 mL) was stirred at 60 °C for 1 h. The mixture was diluted with cold water and acidified with 6 N HCl with stirring in an ice-bath, the acid **7a** was isolated, filtered off and then recrystallized from ethanol. It was obtained as white crystals (0.78 g, 69%); mp 185–186 °C. FT-IR (KBr) ν (cm^{-1}): 3396 (m, br), 1698 (*vs*, sh), 1676 (s, sh). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 11.79 (s, 1H), 10.88 (s, 1H), 7.72–7.59 (m, 2H), 7.11–6.98 (m, 3H), 6.84–6.72 (m, 2H), 3.99 (s, 3H), 3.87 (s, 3H), 3.63 (s, 2H), 3.21 (t, J = 8.1 Hz, 2H), 2.75 (t, J = 8.1 Hz, 2H), 2.28 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 175.68, 162.63, 158.81, 156.13, 148.11, 142.98, 141.46, 131.12, 130.03, 128.06, 125.82, 124.56, 122.71, 120.05, 116.69, 115.63, 114.42, 57.39, 56.70, 48.12, 43.22, 33.44 and 20.01. EI-MS *m/z*: $[M]^+$ Calcd for $C_{23}H_{24}N_2O_6$ 424.16; Found 424.10. Anal. Calcd for $C_{23}H_{24}N_2O_6$ (M = 424.16 g/mol): C, 65.08; H, 5.70; N, 6.60; Found: C, 65.01; H, 5.78; N, 6.49.

2.3.5. General procedure for aminolysis of pyridazinone propanoic acid (**7a**) with aromatic amines: synthesis of amides (**8a–c**)

To a cooled (−5 °C) and stirred solution of pyridazinone propanoic acid (**7a**) (0.254 g, 0.60 mmol) in anhydrous tetrahydrofuran (6 mL), Et_3N (2.10 mmol) was added. After 30 min, the mixture was allowed to warm up to 0 °C and ethyl chloroformate (0.66 mmol) was added. After 1 h, the commercially available substituted arylamine (1.20 mmol) was added. The reaction was stirred at room temperature for 12 h, then the mixture was concentrated *in vacuo*, diluted with cold water (20–30 mL) and then extracted with CH_2Cl_2 (3 × 15 mL). The solvent was evaporated to obtain final compounds (**8a–c**) which were purified by column chromatography using cyclohexane/ethyl acetate 2:1 and *n*-hexane/ethyl acetate 3:2 as eluents. Samples of the isolated products were characterized as follow;

2.3.5.1. 3-(5-(4-Hydroxy-3-methoxybenzyl)-3-(4-methoxy-3-methylphenyl)-6-oxopyridazin-1(6H)-yl)-N-(4-(trifluoromethyl)phenyl)propanamide (**8a**)

Obtained as a yellow powder (0.20 g, 58%); mp 239–241 °C. FT-IR (KBr) ν (cm^{-1}): 3298 (m, br), 1689 (*vs*, sh), 1676 (s, sh). ^{1}H NMR (300 MHz, DMSO d_6): δ (ppm) 11.06 (s, 1H), 8.99 (s, 1H), 7.79–7.71 (m, 3H), 7.67 (dt, J = 4.1, 2.4 Hz, 2H), 7.47–7.33 (m, 4H), 6.97 (dd, J = 8.4, 1.6 Hz, 2H), 4.02 (s, 3H), 3.92 (s, 3H), 3.69 (s, 2H), 3.49 (t, J = 8.3 Hz, 2H), 2.59 (t, J = 8.3 Hz, 2H), 2.18 (s, 3H). ^{13}C NMR (75 MHz, DMSO d_6): δ (ppm) 176.34, 161.70, 160.30, 156.03, 151.15, 148.69, 146.11, 142.64, 138.47, 136.61, 132.55, 132.12, 130.72, 128.83, 127.77, 127.47, 126.35, 124.31, 121.97, 118.90, 116.71, 115.46, 113.17, 111.45, 59.88, 57.28, 48.02, 43.14, 31.45 and 20.51. EI-MS *m/z*: $[M]^+$ Calcd for $C_{30}H_{28}F_3N_3O_5$ 567.56; Found 567.50. Anal. Calcd for $C_{30}H_{28}F_3N_3O_5$ (M = 567.56 g/mol): C, 63.49; H, 4.97; N, 7.40; Found: C, 63.38; H, 4.98; N, 7.36.

2.3.5.2. 3-(5-(4-Hydroxy-3-methoxybenzyl)-3-(4-methoxy-3-methylphenyl)-6-oxopyridazin-1(6H)-yl)-N-(4-methoxyphenyl)propanamide (**8b**)

Obtained as a faint yellow solid (0.24 g, 62%); mp: 218–219 °C. FT-IR (KBr) ν (cm^{-1}): 3210 (m, br), 1686 (*vs*, sh), 1666 (s, sh). ^{1}H NMR (300 MHz, DMSO d_6): δ (ppm) 11.08 (s, 1H), 8.99 (s, 1H), 7.79–7.66 (m, 4H), 7.47–7.33 (m, 5H), 6.97–6.85 (m, 2H), 4.23 (s, 3H), 4.02 (s, 3H), 3.92 (s, 3H), 3.76 (s, 2H), 3.49 (t, J = 8.1 Hz, 2H), 2.59 (t, J = 8.1 Hz, 2H), 2.18 (s, 3H). ^{13}C NMR (75 MHz, DMSO d_6): δ (ppm) 176.98, 161.64, 161.05, 158.24, 151.83, 148.09, 145.51, 141.42, 139.03, 136.96, 136.44, 134.53, 131.28, 129.15, 127.82, 127.50, 126.09, 123.25, 121.92, 120.13, 118.11, 117.21, 112.78, 111.77, 64.08, 60.12, 58.34, 49.15, 42.47, 31.71 and 19.27. EI-MS *m/z*: