Synthesis and Molecular Docking Studies of New Dispiropyrrolidines on West Nile Virus NS2B-NS3 Protease

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Abstract: *West Nile virus (WNV) is among the other four flavivirus genus, rapidly spreading worldwide. The number of cases increases globally as there are no clinically available approved drugs and vaccines against this disease. Based on our previous finding related to a flavivirus, a series of spiropyrrolidine derivatives were regioselectively synthesized via [3+2]-cycloaddition reaction of three components between isatins, sarcosine, and (E)-3,5-bis (arylidene)-4-piperidones. The yield of synthesized compounds was in a range between 81–95%. The structures of all the synthesized compounds were characterized using FT-IR, 1D- and 2D-NMR, and HRMS. Molecular docking studies of spiropyrrolidines on NS2B-NS3 protease were done to understand and explore the ligand-receptor interactions and hypothesize the drug's refinements. The inhibition of NS2B-NS3 protease has been considered a promising strategy because this enzyme is responsible for the viral replication process. Among them, compound 5c shows an excellent binding affinity with −7.71 kcal/mol free binding energy and an inhibition constant of 1.73 μM. It also showed the binding orientation into the active site of WNV NS2B-NS3 protease on Asn84, Tyr1161, Gly1151, and Gly1153.*

Keywords: *spiropyrrolidine; [3+2]-cycloaddition; molecular docking; West Nile virus; WNV NS2B-NS3 protease*

■ INTRODUCTION

The flaviviral disease is an infectious disease transmitted by mosquitoes. The members of the Flavivirus genus include dengue virus (DENV), West Nile virus (WNV), yellow fever virus (YFV), and Japanese encephalitis (JEV) [1-3]. The number of cases increases day by day worldwide [1-3]. Thus, there is a pressing need to develop newer agents against these viruses. Discovered in 1937 in the West Nile district of Uganda, WNV was first isolated from the blood of a woman with mild febrile infection [4-5]. Since then, primary and sporadic outbreaks have been commonly recorded in Africa, Middle East, and Europe in the 1960s [4-5]. Infections in humans are typically asymptomatic or cause a moderate flu-like disease called West Nile fever for a few days. Recent WNV infections have been associated with much

higher fatality rates, especially among the elderly [6-8].

WNV is transmitted to humans by the bites of infected mosquitoes of several species, including *Culex* sp., *Aedes* sp., and *Anopheles* sp. [9]. Symptomatic cases cause headaches, fever, drowsiness, lethargy, dizziness, nausea, and confusion, escalating to encephalitis, convulsions, seizures, and eventually death. Among all arthropod-borne Flaviviruses, WNV has the broadest geographic distribution and the most diverse vector and host variety, increasing its potential as a global health threat [9]. In 2020, there were 315 human cases of WNV infection in Mediterranean countries reported by the European Centre for Disease Prevention and Control (ECDC). Greece and Spain reported the highest cases with 143 and 77, respectively [10]. In WNV, an NS3 protease is associated with NS2B, forming a complex enzymatic NS2B-NS3, which is important for synthesizing the polyprotein precursor in viral replication [11-12]. Hence, NS2B-NS3 protease has been considered a favorable target to prevent virus infection and lead to flaviviral death [11,13-15]. Most of the research for flaviviral infections focuses on the inhibition of the NS2B-NS3 protease. In addition, the complexity of this enzyme is very challenging due to its shallow and open pocket active site [16].

Previous studies reported considerable biological activities of the dispiropyrrolidines on antimycobacterial [17-20], antifungal [21], antimicrobial [22-25], antitumor [26-28], anti-neoplastic [29], and antidiabetic activities [30-31]. No reported data explained the activity and interaction between dispiropyrrolidines and their roles as WNV NS2B-NS3 protease inhibitors. Previously, we reported the synthesis of new dibenzylidene-1-phenylethylpiperidine-4-ones and new dispiropyrrolidines to evaluate their application as antimycobacterial against *Mycobacterium tuberculosis* [17-19,32]. Based on our previous achievement and our continuation on this research domain, this manuscript focuses on synthesizing new dispiropyrrolidines as a WNV NS2B-NS3 protease inhibitor, which is the recent finding dibenzylidene-1-phenylethylpiperidine-4-ones are employed as dipolarophiles in [3+2]-cycloaddition reactions of azomethine ylide for the synthesis of new dispiropyrrolidines. The dispiropyrrolidines were investigated as potential WNV NS2B-NS3 protease inhibitors using a molecular docking approach. This information is very useful for predicting the binding behavior in the rational design of drugs and elucidating the fundamentals of biochemical processes.

■ EXPERIMENTAL SECTION

Materials

Unless otherwise noted, materials were purchased from Sigma-Aldrich Co., Acros Organics, QReC, and Merck Chemical Co., i.e., 1-phenylethyl-4-piperidone 98%, Benzaldehyde 99%, 4-Methoxybenzaldehyde 98%, 4-Methylbenzaldehyde 97%, 4-Methoxybenzaldehyde 97%, Isatin 98%, 5-Chloroisatin 95%, Sarcosine 98%, Acetone, AR Grade, Ethanol, AR Grade, Methanol, AR Grade, Hexane AR Grade and Methyl sulfoxide-d_6-deuteration degree for NMR. All chemicals and solvents were of reagent grade and were used without further purification. Column chromatography was performed using Merck silica gel (40–63 μm).

Instrumentation

Thin-layer chromatography (TLC) was performed on alumina plates pre-coated with silica gel (Merck silica gel, 60 F254), which were visualized by the quenching of UV fluorescence when applicable (λ_{max} = 254 nm and/or 366 nm) and/or by spraying with vanillin or anisaldehyde in acidic ethanol followed by heating with a heat gun. It was performed using a solvent system of benzene-methanol (8:2) and toluene-ethyl formate-formic acid (5:4:1) to check the reactions' completion. All reactions were carried out in heat-dried glassware under a dry nitrogen atmosphere unless otherwise stated. All liquids transfer was conducted using standard syringe or cannula techniques. All spectral data were obtained on the following instruments: Infrared spectra were recorded on a Perkin Elmer 2000 FTIR spectrometer at wavenumber from 4000-600 cm^{-1}. ^1H (500 MHz) and ^{13}C (125 MHz) Nuclear magnetic resonance spectra were obtained on Bruker AVN 500 MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) which were reported in units of ppm on the δ scale. NMR analyses were done using solvent DMSO-d_6 and TMS as the

internal standard. Data were analyzed via the TopSpin software package. The chemical shift was internally referenced to the solvent signals in DMSO-d_6 (^{1}H δ 2.50; ^{13}C δ 39.5). The coupling constants are given in Hz. The mass spectra were measured using the Waters Xevo QTOF MS system.

Procedure

Synthesis of (3E,5E)-3,5-bis(substitutedarylidene)-1-phenethyl piperidin-4-one (1a-c)

The procedure of preparation of dibenzylidene-1-phenylethylpiperidine-4-ones **1a-c** was reported previously by our group [32]. 1-phenylethyl-4-piperidone (1.0 equiv.) and appropriate aldehyde (2.0 equiv.) were dissolved together in ethanol (10 mL) and 30% sodium hydroxide (5 mL; prepared in ethanol). The reaction mixture was stirred for 2–6 h at room temperature until completion (TLC). The mixture was then poured into crushed ice. The precipitated solid was filtered, washed with water, and purified by recrystallization. The spectroscopic data were compared with the literature [32].

(3E,5E)-3,5-dibenzylidene-1-phenylethyl-4-piperidinone (1a). 1-Phenylethyl-4-piperidone (1.22 g, 6.0 mmol) and benzaldehyde (1.27 g, 12.0 mmol) were dissolved together in ethanol (10 mL) and 30% sodium hydroxide (5 mL; prepared in ethanol) according to the general procedure above. The precipitated solid was filtered, washed with water and purified by recrystallization to give a bright yellow solid (2.12 g, 94.6%), M.p. 167–170 °C. FTIR (ATR, cm^{-1}): 3026 (w, C–H), 1669 (s, C=O), 1608 (s, C=C), 1181 (m, C–N). ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H, ppm 2.70 (2H, t, *J* = 7.5 Hz, 8-CH_2), 2.81 (2H, t, *J* = 7.5 Hz, 7-CH_2), 3.88 (4H, s, 2-CH_2, 6-CH_2), 7.14-7.23 (5H, m, H-10, H-11, H-12, H-13, H-14), 7.42-7.51 (10H, m, H-17, H-18, H-19, H-20, H-21), 7.58 (2H, s, H-15). ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C, ppm 33.3, 54.5, 58.3, 126.3, 128.6, 129.0, 129.2, 129.7, 131.0, 134.3, 135.1, 135.3, 140.5, 187.4. HRMS (TOF-ES$^+$): m/z 380.2139 (MH^+ $C_{27}H_{26}NO^+$ requires 380.2009).

(3E,5E)-3,5-bis(4-methylbenzylidene)-1-phenylethyl-4-piperidinone (1b). 1-Phenylethyl-4-piperidone (1.22 g, 6.0 mmol) and 4-methylbenzaldehyde (1.44 g, 12.0 mmol) were dissolved together in ethanol (10 mL) and 30% sodium hydroxide (5 mL; prepared in ethanol) according to the general procedure above. The precipitated solid was filtered, washed with water and purified by recrystallization to give a yellow solid (2.19 g, 90.1%), M.p. 118–121 °C. FTIR (ATR, cm^{-1}): 3024 (w, C–H), 1668 (s, C=O), 1606 (m, C=C), 1174 (m, C–N). ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H, ppm 2.36 (6H, s, 22-CH_3), 2.71 (2H, t, *J* = 7.5 Hz, 8-CH_2), 2.81 (2H, t, *J* = 7.5 Hz, 7-CH_2), 3.86 (4H, s, 2-CH_2, 6-CH_2), 7.14-7.24 (5H, m, H-10, H-11, H-12, H-13, H-14), 7.29 (4H, d, *J* = 8.5 Hz, H-18, H-20), 7.40 (4H, d, *J* = 8.50 Hz, H-17, H-21,), 7.58 (2H, s, H-15). ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C, ppm 21.5, 33.3, 54.6, 58.4, 126.3, 128.7, 129.0, 129.9, 131.0, 132.4, 133.5, 135.2, 139.6, 140.5, 187.3. HRMS (TOF-ES$^+$): m/z 408.2324 (MH^+ $C_{29}H_{30}NO^+$ requires 408.2322).

(3E,5E)-3,5-bis(4-methoxybenzylidene)-1-phenylethyl-4-piperidinone (1c). 1-Phenylethyl-4-piperidone (0.61 g, 3.0 mmol) and 4-methoxybenzaldehyde (0.82 g, 6.0 mmol) were dissolved together in ethanol (5 mL) and 30% sodium hydroxide (3 mL; prepared in ethanol) according to the general procedure above. The precipitated solid was filtered, washed with water and purified by recrystallization to give a yellow solid (1.13 g, 85.4%), M.p. 143–146 °C, Lit. M.p. 147 °C. FTIR (ATR, cm^{-1}): 3023 (w, C–H), 1667 (s, C=O), 1596 (m, C=C), 1166 (m, C–N); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H, ppm 2.73 (2H, t, *J* = 7.5 Hz 8-CH_2,), 2.82 (2H, t, *J* = 7.5 Hz, 7-CH_2), 3.82 (6H, s, 22-OCH_3), 3.84 (4H, s, 2-CH_2, 6-CH_2), 7.04 (4H, d, *J* = 8.5 Hz, 18-CH, 20-CH,), 7.15-7.25 (5H, m, 10-CH, 11-CH, 12-CH, 13-CH, 14-CH), 7.45 (4H, d, *J* = 8.5 Hz, 17-CH, 21-CH), 7.56 (2H, s, H-15). ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C, ppm 33.3, 54.7, 55.8, 58.5, 113.7, 114.8, 126.3, 127.7, 128.7, 129.0, 132.2, 132.9, 134.9, 140.6, 160.5, 187.1. HRMS (TOF-ES$^+$): m/z 440.2399 (MH^+ $C_{29}H_{30}NO_3^+$ requires 440.2226).

Synthesis of spiropyrrolidines 5 and 6

3,5-Bis(substitutedarylidene)-1-phenethylpiperidin-4-one (**1**) (1.0 equiv.) was refluxed together with appropriate isatins **3** or **4** (1.0 equiv.) and sarcosine (**2**) (2.0 equiv.) in methanol (20 mL) for 4–7 h. After completing the reaction, the excess solvent in the mixture was removed under reduced pressure and cooled before being poured onto crushed ice. The

products were filtered, washed with water, and purified by recrystallization.

1-Methyl-4(phenyl)pyrrolo-(spiro[2.3'']oxindole)-spiro[3.3']-5'-(phenylmethylidene)-1'-phenylethyl-4'-piperidinone (5a). 3,5-Bis(substitutedarylidene)-1-phenethylpiperidin-4-one **1a** (0.10 g, 0.26 mmol) was refluxed together with appropriate isatin **3** (0.04 g, 0.26 mmol) and sarcosine (**2**) (0.05 g, 0.52 mmol) in methanol (20 mL) according to the general procedure above. Products obtained were filtered, washed with water and purified by recrystallization to give pale yellow solid (Yield = 0.13 g (89.6%), M.p. 117–120 °C. FTIR (ATR, cm^{-1}): *v* 3346 (w, N–H), 3027 (w, C–H), 1697 (s, C=O), 1599 (m, C=C), 1182 (m, C–N). ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H, ppm: 1.76 (1H, d, *J* = 12.5 Hz, 7'-CH_{2a}), 2.00 (s, 3H, *N*-CH_3), 2.34-2.45 (m, 4H, 2′-CH_2, 6′-CH_2), 3.06 (dd, 1H, *J* = 2.4, 2.5 Hz, 5-CH_{2a}), 3.20-3.25 (m, 2H, 7′-CH_{2b}, 8′-CH_{2a}), 3.36 (*overlap signal, 1H, 5-CH_{2b}), 3.82-3.86 (m, 1H, 8′-CH_{2a}), 4.64–4.68 (m, 1H, H-4), 6.65–7.33 (19H, m, ArH), 10.46 (s, 1H, NH). ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C, ppm: 32.0, 34.1, 45.2, 53.9, 56.2, 56.3, 59.1, 64.6, 75.2, 108.7, 120.7, 125.8, 126.75, 126.85, 126.91, 128.18, 128.20, 128.46, 128.47, 128.6, 128.9, 129.1, 129.1, 130.0, 132.9, 134.5, 136.6, 138.4, 140.0, 143.4, 176.6, 198.2. HRMS (TOF-ES$^+$): m/z 554.2899 (MH^+ $C_{37}H_{36}N_3O_2^+$ requires 554.2803).

1-Methyl-4(-4-methylphenyl)pyrrolo-(spiro[2.3'']oxindole)-spiro[3.3']-5'-(4–methylphenylmethylidene)-1'-phenylethyl-4'-piperidinone (5b). 3,5-Bis(substitutedarylidene)-1-phenethylpiperidin-4-one **1b** (0.10 g, 0.26 mmol) was refluxed together with appropriate isatin **3** (0.04 g, 0.26 mmol) and sarcosine (**2**) (0.05 g, 0.52 mmol) in methanol (20 mL) according to the general procedure above. Products obtained were filtered, washed with water and purified by recrystallization to give pale yellow solid (Yield = 0.13 g (88.7%), M.p. 192–195 °C. FTIR (ATR, cm^{-1}): ν 3397 (w, N–H), 3023 (w, C–H), 1694 (s, C=O, 1605 (m, C=C), 1177 (m, C–N). ^{1}H-NMR (500 MHz, DMSO-d_6) δ_H, ppm: 1.78 (d, 1H, *J* = 12.5 Hz, 7′-CH_{2a}), 1.98 (s, 3H, N-CH_3), 2.27 (d, 6H, *J* = 5.8 Hz, 2×CH_3), 2.33–2.46 (m, 4H, 2′-CH_2, 6′-CH_2), 3.05 (dd, 1H, *J* = 2.4, 2.5 Hz, 5-CH_{2a}), 3.17–3.22 (m, 2H, 7′-CH_{2b}, 8′-CH_{2a}), 3.26 (*overlap signal, 1H, 5-CH_{2b}), 3.78-3.82 (m, 1H, 8′-CH_{2b}), 4.58 (dd, 1H, *J* = 7.4, 7.5 Hz, H-4), 6.63–7.26 (18H, m, ArH), 10.36 (s, 1H, NH). ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C, ppm: 20.6, 20.9, 32.1, 34.1, 44.8, 54.0, 56.3, 56.4, 59.1, 64.5, 75.2, 108.6, 120.6, 125.8, 126.88, 126.93, 128.2, 128.5, 128.4, 128.8, 129.0, 129.1, 130.2, 131.7, 132.1, 135.3, 135.7, 136.6, 138.8, 140.1, 143.4, 176.6, 198.2. HRMS (TOF-ES$^+$): m/z 582.3081 (MH^+ $C_{39}H_{40}N_3O_2^+$ requires 582.3115, 604.2894 (MNa^+ $C_{39}H_{39}N_3NaO_2^+$ requires 604.2935).

1-Methyl-4(-4-methoxyphenyl)pyrrolo-(spiro[2.3'']oxindole)-spiro[3.3']-5'-(4-methoxyphenylmethylidene)-1'-phenylethyl-4'-piperidinone (5c). 3,5-Bis(substitutedarylidene)-1-phenethylpiperidin-4-one **1c** (0.10 g, 0.26 mmol) was refluxed together with appropriate isatin **3** (0.04 g, 0.26 mmol) and sarcosine (**2**) (0.05 g, 0.52 mmol) in methanol (20 mL) according to the general procedure above. Products obtained were filtered, washed with water and purified by recrystallization to give pale yellow solid (Yield = 0.13 g (88.9%), M.p. 130–133 °C. FTIR (ATR, cm^{-1}): 3289 (w, N–H), 2934 (w, C–H), 1706 (s, C=O), 1580 (m, C=C), 1247 (m, C–O), 1173 (m, C–N). ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H, ppm 1.76 (1H, d, *J* = 12.5 Hz, 7′-CH_{2a}), 1.97 (3H, s, N-CH_3), 2.31–2.46 (4H, m, 2′-CH_2, 6′-CH_2), 3.04 (1H, dd, *J* = 2.3, 2.4 Hz, 5-CH_{2a}), 3.16–3.19 (2H, m, 7'-CH_{2b}, 8'-CH_{2a}), 3.40 (1H, *overlap signal, 5-CH_{2b}), 3.72 (3H, s, OCH_3), 3.76 (1H, *overlap signal, 8′-CH_{2b}), 3.82 (3H, s, OCH_3), 4.57 (1H, dd, *J* = 7.5, 7.5 Hz, H-4), 6.64–7.26 (20H, m, ArH), 10.38 (1H, s, NH). ^{13}C-NMR (125.8 MHz, DMSO-d_6): δ_C, ppm 32.6, 34.5, 45.0, 54.7, 55.4, 55.7, 55.8, 56.7, 57.1, 59.8, 64.8, 75.9, 109.1, 121.1, 126.3, 127.4, 127.5, 127.6, 127.7, 128.7, 128.9, 129.0, 130.6, 130.8, 131.2, 132.2, 132.9, 132.9, 137.0, 140.6, 143.9, 177.2, 198.6. HRMS (TOF-ES$^+$): m/z 614.3027 (MH^+ $C_{39}H_{40}N_3O_4^+$ requires 614.3019).

1-Methyl-4(phenyl)pyrrolo-(spiro[2.3'']-5''-chlorooxindole)-spiro[3.3']-5'-(phenylmethylidene)-1'-phenylethyl-4'-piperidinone (6a). 3,5-Bis(substitutedarylidene)-1-phenethylpiperidin-4-one **1a** (0.10 g, 0.26 mmol) was refluxed together with appropriate 5-chloroisatin **4** (0.04 g, 0.26 mmol) and sarcosine (**2**) (0.05 g, 0.52 mmol) in

methanol (20 mL) according to the general procedure above. Products obtained were filtered, washed with water and purified by recrystallization to give pale yellow solid (Yield = 0.15 g (95.4%), M.p. 131–134 °C. FTIR (ATR, cm^{-1}): ν 3244 (w, N–H), 3027 (w, C–H), 1698 (s, C=O), 1615 (m, C=C), 1182 (m, C–N). ^{1}H-NMR (500 MHz, DMSO-d_6) δ_H, ppm: 1.74–1.77 (d, 1H, *J* = 12.6 Hz, 7′-CH_{2a}), 2.02 (s, 3H, N-CH_3), 2.37–2.44 (m, 4H, 2′-CH_2, 6′-CH_2), 3.09–3.12 (dd, 1H, *J* = 2.4, 2.5, Hz, 5-CH_{2a}), 3.22–3.25 (m, 2H, 7′-CH_{2b}, 8′-CH_{2a}), 3.37 (*overlap signal, 1H, 5-CH_{2b}), 3.80–3.84 (1H, m, 8′-CH_{2b}), 4.63–4.67 (m, 1H, H-4), 6.66–6.83 (1H, m, ArH), 7.11–7.37 (19H, m, ArH), 10.57 (s, 1H, NH). ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C, ppm: 32.0, 34.2, 45.1, 54.0, 56.3, 56.4, 59.1, 65.1, 75.2, 110.3, 125.0, 125.9, 126.8, 126.9, 128.27, 128.34, 128.5, 128.6, 128.7, 129.17, 129.23, 129.1, 130.1, 133.1, 134.3, 137.2, 138.1, 140.0, 142.4, 176.3, 198.2. HRMS (TOF-ES$^+$): m/z 588.2390 (MH^+ $C_{37}H_{35}{}^{35}ClN_3O_2^+$ requires 588.2413).

1-Methyl-4(-4-methylphenyl)pyrrolo-(spiro[2.3'']-5''-chlorooxindole)-spiro[3.3']-5'-(4-methylphenylmethylidene)-1'-phenylethyl-4'-piperidinone (6b). 3,5-Bis(substitutedarylidene)-1-phenethyl piperidin-4-one **1b** (0.10 g, 0.26 mmol) was refluxed together with appropriate 5-chloroisatin **4** (0.04 g, 0.26 mmol) and sarcosine (**2**) (0.05 g, 0.52 mmol) in methanol (20 mL) according to the general procedure above. Products obtained were filtered, washed with water and purified by recrystallization to give pale yellow solid (Yield = 0.13 g (84.4%), M.p. 186–189 °C. FTIR (ATR, cm^{-1}): ν 3180 (w, N–H), 3025 (w, C–H), 1691 (s, C=O), 1607 (m, C=C), 1182 (m, C–N). ^{1}H-NMR (500 MHz, DMSO-d_6) δ_H, ppm: 1.75 (d, 1H, *J* = 12.5 Hz, 7′-CH_{2a}), 2.01 (s, 3H, N-CH_3), 2.30 (d, 6H, *J* = 11.6 Hz, 2×CH_3), 2.36 (s, 2H, 6′-CH_2), 2.42–2.46 (m, 2H, 2′-CH_2), 3.08 (dd, 1H, *J* = 1.8, 1.9 Hz, 5-CH_{2a}), 3.17–3.22 (m, 2H, 7′-CH_{2b}, 8′-CH_{2b}), 3.39 (*overlap signal, 1H, 5-CH_{2b}), 3.76–3.80 (m, 1H, 8′-CH_{2b}), 4.60 (dd, 1H, *J* = 7.4, 7.4 Hz, H-4), 6.64–7.40 (19H, m, ArH), 10.54 (s, 1H, NH). ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C, ppm: 20.6, 20.9, 32.0, 34.1, 44.6, 54.0, 56.3, 56.4, 59.0, 64.9, 75.2, 110.1, 124.8, 125.8, 126.8, 128.2, 128.3, 128.4, 128.8, 128.9, 129.1, 129.2, 130.1, 131.5, 132.2, 135.0, 135.8, 137.1, 139.1, 139.9, 142.3, 176.2, 198.1. HRMS (TOF-ES$^+$): m/z 616.2735 (MH^+ $C_{39}H_{39}{}^{35}ClN_3O_2^+$ requires 616.2726), 638.2595 (MNa^+ $C_{39}H_{38}{}^{35}ClN_3O_2Na^+$ requires 638.2545).

1-Methyl-4(-4-methoxyphenyl)pyrrolo-(spiro[2.3'']-5''-chlorooxindole)-spiro[3.3']-5'-(-4-methoxyphenylmethylidene)-1'-phenylethyl-4'-piperidinone (6c). 3,5-Bis(substitutedarylidene)-1-phenethylpiperidin-4-one **1c** (0.10 g, 0.26 mmol) was refluxed together with appropriate 5-chloroisatin **4** (0.04 g, 0.26 mmol) and sarcosine (**2**) (0.05 g, 0.52 mmol) in methanol (20 mL) according to the general procedure above. Products obtained were filtered, washed with water and purified by recrystallization to give pale yellow solid (Yield = 0.11 g (81.3%), M.p. 187–190 °C. IR (ATR, cm^{-1}): 3297 (w, N–H), 2943 (w, C–H), 1709 (s, C=O), 1578 (m, C=C), 1250 (C–O), 1172 (C–N). ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H, ppm 1.76 (d, 1H, d, *J* = 12.5 Hz, 7′-CH_{2a}), 2.00 (s, 3H, N-CH_3), 2.37–2.43 (m, 4H, 2′-CH_2, 6′-CH_2), 3.10 (dd, 1H, *J* = 2.4, 2.5 Hz, 5-CH_{2a}), 3.16–3.21 (m, 2H, 7′-CH_{2b}, 8′-CH_{2a}), 3.38 (*overlap signal, 1H, 5-CH_{2b}), 3.73 (s, 3H, OCH_3), 3.75 (*overlap, 1H, 8′-CH_{2b}), 3.78 (s, 3H, OCH_3), 4.60 (dd, 1H, *J* = 7.65, 7.55 Hz, H-4), 6.65–7.27 (17H, m, ArH), 10.55 (s, 1H, NH). ^{13}C-NMR (125.8 MHz, DMSO-d_6): δ_C, ppm 32.5, 34.6, 44.8, 54.7, 55.4, 55.7, 56.8, 57.2, 59.7, 65.2, 75.8, 110.5, 124.8, 125.3, 126.3, 128.3, 128.4, 128.7, 128.8, 128.9, 129.7, 130.5, 130.6, 131.3, 132.7, 135.1, 135.9, 137.5, 139.0, 140.5, 142.8, 176.8, 198.5. HRMS (TOF-ES$^+$): m/z 670.2453 (MNa^+ $C_{39}H_{38}ClN_3O_4Na^+$ requires 670.2449).

Molecular docking studies

Autodock 4.2 software was used to dock the ligands to the catalytic triad of NS3-NS2B protease. The target protein of West Nile virus (PDB: 2YOL) structure with a resolution of 3.20 Å was retrieved from the RCSB Protein Data Bank. Water and chlorine atoms molecules were removed from the crystal structure. The native ligand, EBN in the 2YOL, was extracted and re-docked with a grid box in a dimension of 40 Å × 40 Å × 40 Å along the x, y, z coordinates to investigate the root mean square deviation (rmsd) between crystal geometry and the docked pose. The low rmsd value of 1.50–1.82 Å indicated that the docking was able to reproduce the native conformation.

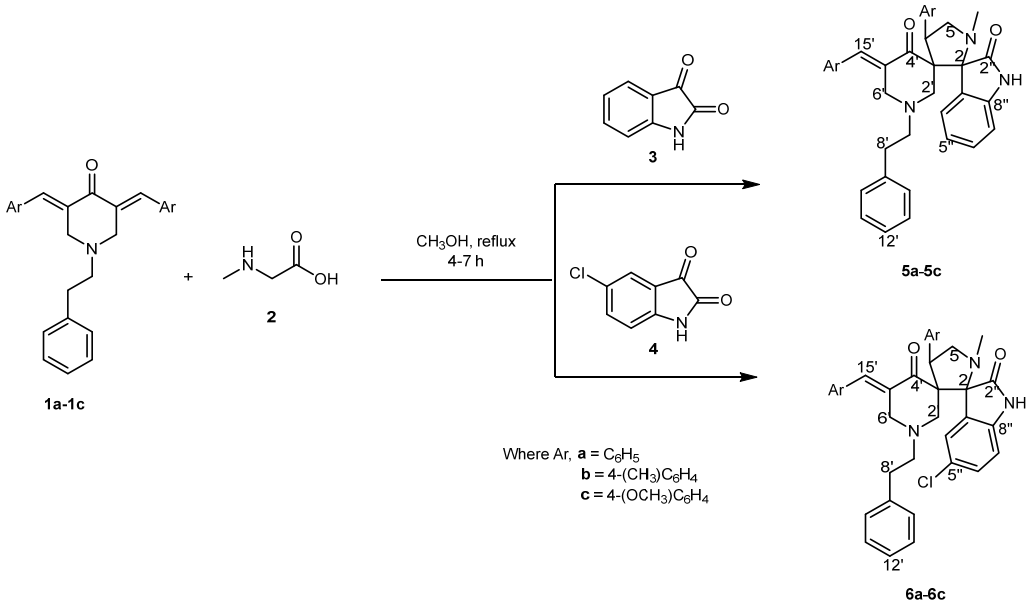
■ RESULTS AND DISCUSSION

Chemistry

In the first step, we prepared the starting material of a dipolarophile known as dibenzylidene-1-phenylethyl piperidine-4-ones (**1**) via Claisen-Schmidt condensation between 1-phenylethyl-4-piperidone with an appropriate aromatic aldehyde in dilute ethanolic sodium hydroxide at room temperature. The completion of the reaction was checked using thin-layer chromatography (TLC) [32]. Next, we examined the three-component reaction of **1** and sarcosine (**2**) and isatin analogs (**3** or **4**) in methanol under reflux conditions to give the spiropyrrolidines (**5** and **6**), according to the method developed in our laboratory [19]. The reaction of spiropyrrolidine was conducted using two different isatin types: isatin (**3**) and 5-chloroisatin (**4**). The compounds **5** and **6** described in this study are shown in Scheme 1. The yield of synthesized compounds was in a range between 81–95%.

The structures and regiochemistry of products **5**-**6** were characterized by IR, 1D- and 2D-NMR spectroscopy data, and HRMS analysis. Compound **6b** is taken as an example to describe the result of analyses done. The FT-IR spectrum of compound **6b** manifests an absorption band at 3180 cm^{-1} attributed to the N–H bending vibration, the aromatic band of C–H stretch at 3025 cm^{-1}, and C–N stretching occur at 1182 cm^{-1}. In addition, the aromatic C=C stretching band can be observed at 1606 cm^{-1}. The strong corresponding out of the plane C–H bending vibration band appears at 820 cm^{-1}, indicating a *para*-disubstituted ring in compound **6b**. The structure of dispiropyrrolidine **6b** is agreed with the 1D- and 2D-NMR spectroscopic data. The ^{1}H-NMR spectrum of **6b** displayed two singlets at δ_H 2.01, and 10.54 was corresponding to the *N*–CH_3 and N–H, while the doublet at δ_H 2.30 (J= 11.6 Hz) was corresponding to 29′-CH_3 and 28′-CH_3, respectively. The doublet at δ_H 1.75 (J = 12.5 Hz) and multiplet at δ_H 2.42–2.46 were assigned to 7′-CH_{2a} and 2′-CH_2, respectively. The proton signal of 5-CH_{2a} and H-4 appeared as doublets of a doublet at δ_H 3.08 (J = 1.8, 1.9 Hz) and 4.60 (J = 7.4, 7.4 Hz).

The ^{1}H-^{1}H-COSY spectrum of **6b** revealed a cross peak between two neighboring protons of H-7′ and H-8′, while H-4 showed a cross peak with its neighboring proton of 5-H_{2a}. Two multiplets at δ_H 3.17–3.22 and 3.76–3.80 were assigned to 7′-CH_{2b}/8′-CH_{2b} and 8′-CH_{2b}, respectively. The presence of pyrrolidine ring attached at C-3′ causes H-2′ to become more shielded than H-6′. Hence, the proton signal of H_a-2′ is located at more upfield than H_b-6′ in **6b**. The multiplet proton signals that appeared in the aromatic region at δ_H 6.64–7.40 were



Scheme 1. Reaction scheme of synthesis of spiropyrrolidines **5** and **6**

assigned to aromatic protons. The HSQC spectrum data of **6b** showed all direct correlations between protons with their respective carbons in molecule **6b**. The carbon signals of *N*–CH_3, C-2″, C-4′ and two methyl (–CH_3) substituent of C-28′ and C-29′ were assigned in ^{13}C-NMR at δ_C 34.6, 176.7 and 198.6, respectively. Two methyl (–CH_3) substituent of CH_3-28′ and CH_3-29′ showed a correlation with carbon signals at δ_C 21.38 (C-28′) and 21.12 (C-29′). The signal of H-29′ showed correlation with δ_C 20.6 (C-29′), H-28′ with δ_C 20.9 (C-28′), H-8′ with δ_C 32.0 (C-8′), H-4 with δ_C 44.6 (C-4), H_a-2′ and H_b-2′/H_a-6′ with δ_C 56.3 (C-2′), H_b-6′ and H_b-2′/H_a-6 with δ_C 56.4 (C-6′), and H-7′ with δ_C 59.0 (C-7′). The peak of C-4″ in **6b** shifted slightly to downfield due to chlorine atom (-Cl) attach at C-3″. The carbon signal at δ_C 54.0 was assigned to C-5 due to its correlation with two signals of proton H_a-5 and water signal. It was suggested H_b-5 overlapped with the water signal at δ_C 3.39. Based on the DEPT135 spectrum of **6b**, two quaternary carbons at δ_C 75.2 and 64.9 were assigned due to the spiro carbons C-2 and C-3, respectively. The carbon for the carbonyl group can be observed at a downfield of ^{13}C-NMR. The signals at δ_C 176.7 and 198.6 were due to the carbonyl group and the carbonyl group of oxindole, respectively.

The HMBC spectrum data (Fig. 1) showed correlations of H-4 with C-3, C-2', C-4', and C-aromatic. The proton of *N*–CH_3 with C-3, C-5, and C-4'. These cross-correlations of proton and carbon further confirming the position of C-4, C-5, and *N*–CH_3 and two spiro carbon, C-2, and C-3, in the same pyrrolidine ring of compound **6b**. In addition, H-15′ and H-28' correlation with C-17′/ C-21′ and C-18′/C-20′, while CH_2-6 with C-15′. Similar correlations were observed between H-4′ and H-29' with C-23′/C-27′ and C-24′/C-26′. These correlations confirmed the presence of the aromatic rings. The H-15′ was correlated with C-5′ and C-4', thus confirming the location of the aromatic ring with piperidinone. The H-2′/H-6′ show correlation with C-2, C4', C-3/C-5′, C-7′, and C-15′. The H-7′ show correlation with C-2′/C-6′, and C-9′. The H-8′ show correlation with C-7′, C-9′, and C-aromatic (C-10′ and C11'). The H-aromatic show correlation with C-9′ and C-aromatic. These correlations confirmed the aromatic ring with piperidinone ring of

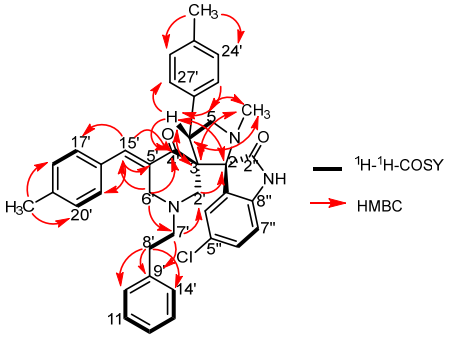


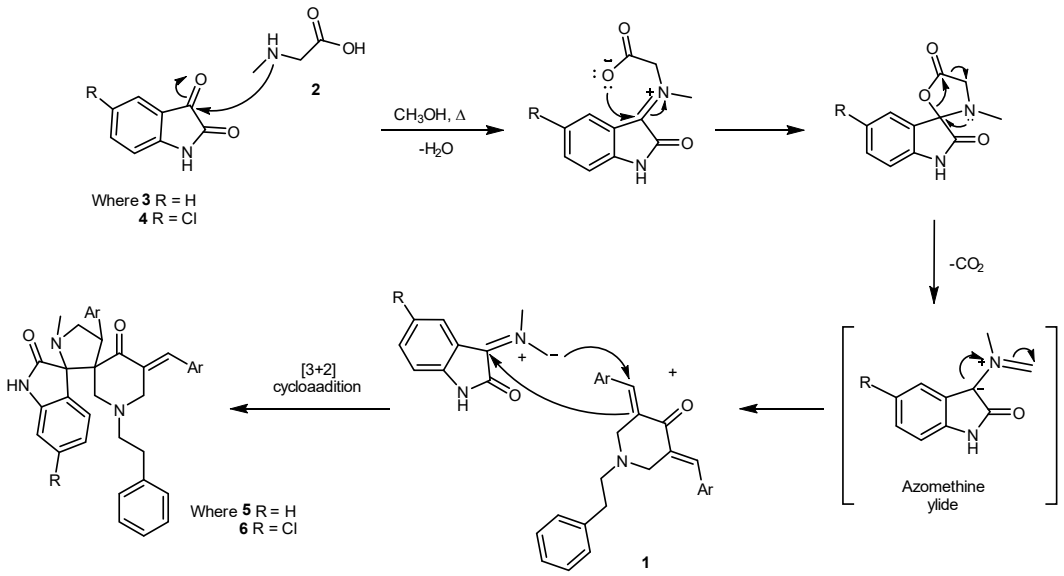
Fig 1. Selected ^{1}H-^{1}H COSY and HMBC correlations of **6b**

compound **6b**. The elucidated structure of the compound was further confirmed with mass spectroscopy analysis. The spectral of compounds obtained agreed with the proposed structure.

A proposed reaction mechanism for the formation of the spiro-pyrrolidine is shown in Scheme 2. The mechanism involves the formation of azomethine ylides, formed via decarboxylative condensation of isatin analogs (**3** or **4**) and sarcosine (**2**), which then undergo [2+3] cycloaddition to give the spiro-pyrrolidines **5** and **6**, respectively. Previous studies showed that spiropyrrolidine reactions are chemoselective and regioselective [26,33]. A dipole addition has occurred only to the available C=C bond and not at the C=O functional group of **1**. The nucleophilic carbon of azomethine ylide tends to attack the end of enone fragment of the exocyclic dipolarophiles substrate **1** to produce cycloadducts **5** and **6**, respectively. This reaction was conducted in methanol solvent. A previous study reported that methanol solvent has a high stabilization of polar transition state. Thus, it produces a good yield of spiro-pyrrolidine in a shorter reaction time [34]. The stereochemistry of spiropyrrolidines was compared with literature and confirmed with x-ray structure reported by Girgis et al. [28-29].

Molecular Docking Studies

The compounds **5** and **6** were constructed using Chemdraw Professional 15.1 and were converted from sdf to pdb format before starting the docking process. The native ligand, EBN in the active site of WNV NS2B-NS3 protease, was removed and re-dock (-8.20 kcal/mol)



Scheme 2. Proposed mechanism for the formation of spiropyrrolidines

with the synthesized compounds using Autodock 4.2. From Table 1, it was found that all the synthesized compounds docked well into the enzyme's active site. The low binding energy indicates the formation of a favorable stable enzyme-ligand complex with a low inhibitory constant, K_i value [35]. Hydrogen bonds were the primary interaction between compounds **5** and **6** with catalytic triad residue of WNV NS2B-NS3 serine protease (His51, Asp75, and Ser135) [36]. Compound **5c** was postulated from the docking results to form four hydrogen bonds, while the other compounds may have two or fewer hydrogen bonds.

Based on this result, Compound **5c** was deemed to have a good binding affinity with the enzyme with the free binding energy of -7.71 kcal/mol and estimated inhibition constant, K_i of 1.73 μM. Fig. 2 showed the binding orientation of compound **5c** into the active site of WNV NS2B-NS3 protease (PDB: 2YOL) with two hydrogen interactions (yellow dotted lines) formed between **5c** and

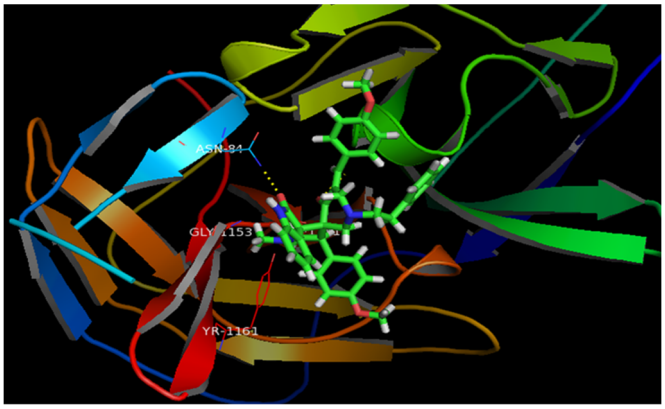


Fig 2. Docking pose of compound **5c** in the active site of WNV NS2B-NS3 protease (PDB code: 2YOL). Hydrogen bonds which are shown as yellow dotted lines were formed between **5c** and residues Asn84

Table 1. Binding energy and inhibitory constant of synthesized spiropyrrolidine compounds

Compd.	Ar	Binding energy (kcal/mol)	Estimated inhibition constant, K_i (μm)	RMSD (Å)
5a	Phenyl	-7.49	1.82	1.50
5b	4-Methylphenyl	-7.54	1.76	1.76
5c	4-Methoxyphenyl	-7.71	1.73	1.54
6a	Phenyl	-7.56	1.86	1.82
6b	4-Methylphenyl	-7.83	1.71	1.60
6c	4-Methoxyphenyl	-7.84	1.83	1.58
EBN	-	-8.20	0.98	-

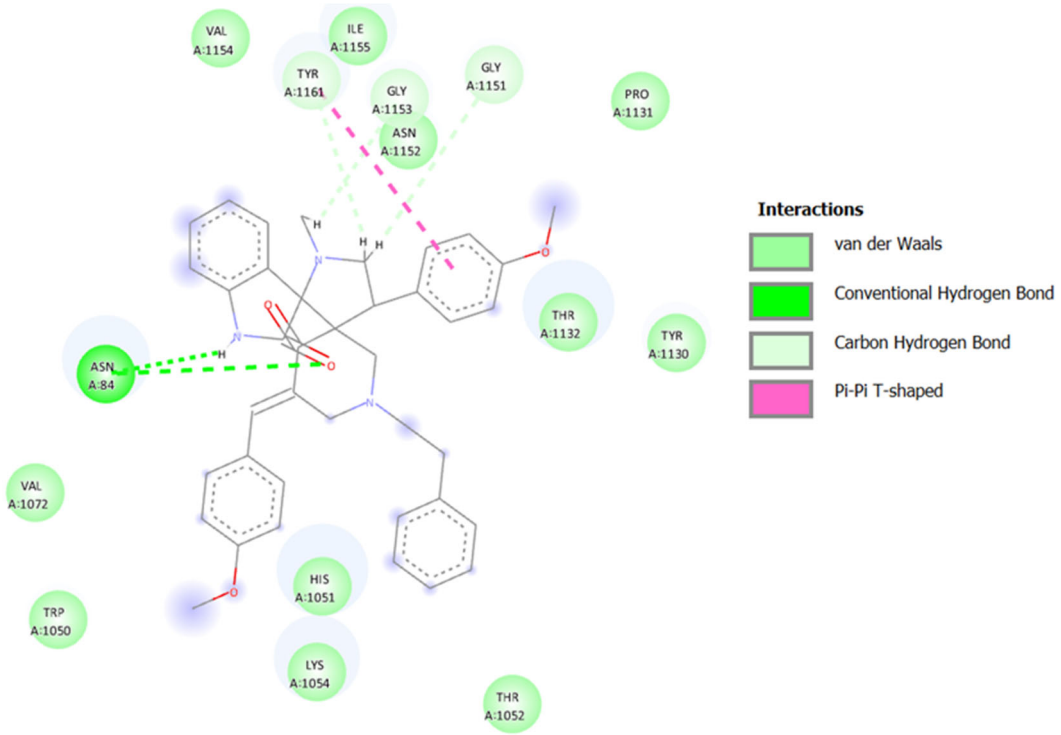


Fig 3. 2D interaction diagram of **5c** with the residues within 2 Å in the active pocket. Hydrogen bonding interactions were represented by green dashed lines

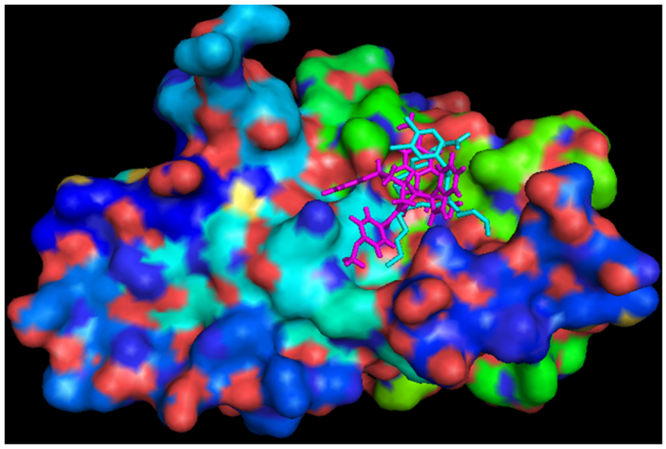


Fig 4. Comparison of docking pose of the native ligand (cyan) and **5c** (magenta) in the active site of WNV N2B-NS3 protease

residues Asn84 (2.139 Å). The distance of hydrogen bond interaction between hydrogen and heteroatom is within the range of 2.5–3.5 Å, with a bond angle at 109–110° [37]. π-π interactions between the Tyr1161 and terminal phenyl ring and two Van der Waals interactions with the Gly1151 and Gly1153 residues were also observed. The hydrogen bond formed between **5c** with the Asn84 through the C=O and -NH of the oxindole ring (Fig. 3). However, there was no interaction has been detected between compound **5c** with the catalytic triad of NS2B-NS3. It was observed that the complexes are exhibiting a similar type of interactions occupying the same active site pocket compared to the reference compound of the native ligand. Fig. 4 showed docking poses of native ligand and **5c**. The structure of synthesized compounds was more complex and bulkier, thus hindering them from embedding well into the enzyme's active site. As this is a preliminary prediction, further experimental studies are needed to validate the docking results.

■ CONCLUSION

The facile one-pot, three components [3+2]-cycloaddition protocol leading to the synthesis of new spiro-pyrrolidines has been described. In docking studies, compound **5c** shows a promising result as the inhibitor for WNV with four hydrogen bonding occur with active site residue, the free binding energy of -7.71 kcal/mol and estimated inhibition constant, Ki of 1.73 μm. Although the series can be useful for developing anti-

dengue agents, further study is required to understand the interaction, improvise, and validate the result.

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■ AUTHOR CONTRIBUTIONS

Conceptualization, HO, MAA, YKY, and MNA; methodology, NMY, MAA, YKY, HO, MNA, and VM; software, MZH, and EEK; validation, HO, and MNA; formal analysis, NMY, MZH, MSAG, and EEK; investigation, NMY, MSAG, MZH, EEK, and the US; resources, HO, and MNA; data curation, NMY, and MSAG; writing-original draft preparation, NMY, MSAG, MZH, and MNA; writing-review and editing, MNA, US, EEK, and MZH; visualization, NMY, and MNA; supervision, HO, and MNA; project administration, HO, and MNA; funding acquisition, HO, and MNA; All authors have read and agreed to the published version of the manuscript.

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