JOTCS
Journal of the Turkish Chemical Society
Section: A
Chemistry
TURKISH CHEMICAL SOCIETY

Synthesis of New *N*-Phthalimide Substituted Tricyclic Imide Containing Isoxazoline and Bispiro Functional Group as Possible Anti-cancer Agents

Omer Tahir Gunkara*, Nuket Ocal

Department of Chemistry, Yildiz Technical University, Davutpasa Campus Istanbul 34220, Turkey

Abstract: [3+2]-Cycloaddition reactions of *N*-phthalimide substituted tricyclic imide were studied to synthesize their isoxazoline and bispiro derivatives in excellent yields. All new synthesized compounds have been characterized by their FTIR, ^{1}H NMR, ^{13}C NMR, GC/MS and LC/MS (HRMS) analyses.

Keyword: [3+2]-Cycloaddition reaction, Azomethine ylide, Bispiromolecules, Isoxazolines, Tetracyclic imides.

Submitted: September 14, 2017. **Accepted:** October 30, 2017.

Cite this: Gunkara O, Ocal N. Synthesis of New N-Phthalimide Substituted Tricyclic Imide Containing Isoxazoline and Bispiro Functional Group as Possible Anti-cancer Agents. JOTCSA. 2018;5(1):73–84.

DOI: http://dx.doi.org/10.18596/jotcsa.338218.

***Corresponding author.** E-mail: gunkara@yildiz.edu.tr Tel +90-2123834193.

INTRODUCTION

In recent years, tricyclic imides have been found in many drug molecules. Ocal and co-workers (1-3) have also conducted various investigations on tandospirone which is an antidepressant in Asia and has tricyclic imide structure (4-6). Imides are also very useful intermediates in the synthesis of various natural products (7-12).

In the class of heterocyclic compounds which form the basis of the drug chemistry, isoxazolines have an important place in terms of biological activity due to the oxygen and nitrogen atoms in their structure, are commonly found in natural compounds and pharmaceuticals; they are also versatile synthetic blocks in organic synthesis (13-21).

The most important place in the formation of the isoxazoline, the five-membered ring at the stages of organic synthesis is [3 + 2]-cycloaddition reactions. In this study, 1,3-dipolar adducts were obtained with oximes to obtain the isoxazoline ring (22-24). On the other hand, the reaction of azomethine ylides as 1,3-dipoles (3, 25) with olefinic dipolarophiles forms highly substituted five-membered ring nitrogen heterocycles. This extremely versatile and atom-economical process has been applied toward the syntheses of substituted prolines, which can be used as new catalysts (26, 27) and served as important motifs in many biologically active molecules (28-30).

EXPERIMENTAL

Materials and Methods

All reagents and solvents were obtained from commercial suppliers and were used without further purification. The solvents were dried by standard procedures. Reactions were monitored using TLC. Visualizations of the chromatograms were performed with UV light, $KMnO_4$ or Vanillin stain. All melting points are uncorrected and were determined on a Gallenkamp digital thermometer. IR spectra were obtained with a Perkin Elmer Spectrum One FTIR Spectrometer and are reported in terms of the frequency of absorption (cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance III-500 MHz NMR spectrometer relative to tetramethylsilane, with coupling constant (J) values in Hertz (Hz). Peak multiplicities are designated by the following abbreviations: s, singlet; d, doublet; dd, double doublet; t, triplet; dt, double triplet; m, multiplet; br, broad. Mass spectra were measured on an Agilent 6890N/5973 GC/IMSD system. High-resolution mass spectra were acquired in the positive ion mode using an Agilent G6530B TOF/Qtof Mass spectrometer.

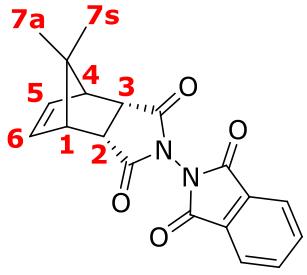
Synthesis and Characterization

Synthesis of Bicyclic Endic Anhydride **(1)** (31)

Bicyclic endic anhydride **1** was prepared with freshly distilled cyclopentadiene and maleic anhydride in ethyl acetate at 0 °C with the known procedure (31). White crystals, Yield 82%, m.p. 165-167 °C, R_f: 0.42 (20:1, diethyl ether/2-propanol), IR (ATR) 2982, 2956, 1840, 1764, 1666, 1450, 1333, 1228, 1194 cm^{-1}, ^1H NMR ($CDCl_3$, 500 MHz) δ 6.31 (2H, m, H-5, H-6), 3.58 (2H, m, H-2, H-3), 3.51 (2H, m, H-1, H-4), 1.78 (2H, m, H-7a, H-7s) ppm, ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.9 (C=Ox2), 136.1 (C-5, C-6), 53.3 (C-2, C-3), 47.7 (C-1, C-4), 40.7 (C-10) ppm, GC-MS (*m/z*) 164 (M^+), 121 (C_8H_8O), 92 (C_7H_8), 66 (C_5H_6).

Synthesis of N-aminobicyclo[2.2.1]hept-5-ene-2-endo,3-endodicarboximide **(2)** (32)

A solution of hydrazine hydrate (0.6 mL) was added drop wise to bicyclic endic anhydride **1** (1.25 g) in benzene under nitrogen atmosphere. The resulting mixture was stirred at room temperature for 4 hours. White solid was filtered and recrystallized with isopropyl alcohol (32). White crystals, Yield 65%, m.p. 145-147 °C, R_f: 0.57 (5:1, ethyl acetate/*n*-hexane), IR (ATR) 3337, 2971, 2953, 2879, 1764, 1691, 1458, 1397, 1207, 1195, 1133 cm^{-1}, GC-MS (m/z) 178 (M^+), 162 (M^+-NH_2), 112 ($C_4H_2O_2N_2$), 92 (C_7H_8), 66 (C_5H_6).



3

Figure 1: Numbering of title compounds.

Synthesis of N-(1H-isoindole-1,3-dionyl)bicyclo[2.2.1]hept-5-ene-2-endo,3-endodicarboximide **(3)** (32)

Compound **2** (0.890 g, 5 mmol) was dissolved in 10 mL of acetic acid. Phthalic anhydride (0.740 g, 5 mmol) was added to the solution. The resulting mixture was stirred at reflux overnight. After cooling room temperature, saturated $NaHSO_3$ solution (50 mL) was added, and the mixture was extracted with EtOAc. The aqueous layer was dried over $MgSO_4$, filtered, and concentrated. The product obtained as a brown solid (32). Brown solid, Yield 97%, m.p. 203-205 °C, R_f: 0.60 (1:1, ethyl acetate/*n*-hexane), IR (ATR) 2959, 2868, 1737, 1704, 1595, 1468, 1364, 1346, 1232, 1179, 1120 cm^{-1}, ^1H NMR ($CDCl_3$, 500 MHz) δ 7.88-7.93 (2H, m, Ar-*H*), 7.80-7.81 (2H, m, Ar-*H*), 6.33 (2H, brs, H-5, H-6), 3.51 (2H, brs, H-2, H-3), 3.46 (2H, brs, H-1, H-4), 1.82 (1H, d, *J* = 9.14 Hz, H-7s), 1.60 (1H, d, *J* = 9.14 Hz, H-7a) ppm, GC-MS (m/z) 308 (M^+), 242 (M^+-C_5H_6), 147 ($C_8H_4O_2N$), 104 (C_7H_4O).

General procedure for the synthesis of compounds **4-5**

A sealed tube containing ninhydrin (0.178 g, 1 mmol), *N*-methylglycine or *N*-benzylglycine (1 mmol) and compound **3** (0.308 g, 1 mmol) in EtOH/dioxane (1:1, 6 mL) was heated at 65 °C for 6 hours under nitrogen atmosphere. After completion of the reaction with TLC control, the organic phase was concentrated and the residue purified by chromatography on silica gel.

6-(1,3-Dioxoisoindoline-2-yl)-2-methyl-3,3a,4,4a,8,8a-hexahydro-2H-spiro[4,8-methanopyrole[3,4-f]isoindole-1,2'indene]-1',3',5,7-(6H,7aH)tetraone **(4)**

Yellow oil, Yield 52%, R_f: 0.10 (1:1, ethyl acetate/*n*-hexane), IR (ATR) 2959, 2868, 1737, 1704, 1595, 1468, 1364, 1346, 1232, 1179, 1120 cm^{-1}, ^1H NMR ($CDCl_3$, 500 MHz) δ 7.95-7.97 (2H, m, Ar-*H*), 7.90-7.91 (1H, m, Ar-*H*), 7.78 (2H, dt, *J* = 1.26 and 4.01 Hz, Ar-*H*), 7.74 (2H, dt, *J* = 1.26 and 4.01 Hz, Ar-*H*), 7.69 (1H, brs, Ar-*H*), 3.57 (1H, t, *J* = 9.45 Hz, *N*-C*H*$_2$), 3.20-3.23 (1H, m, H-3), 3.13-3.16 (1H, m, H-2), 2.92 (1H, dd, *J* = 1.57 and 9.45 Hz, H-5), 2.81-2.86 (3H, m, H-1, H-4, *N*-C*H*$_2$), 2.47 (1H, d, *J* = 7.56 Hz, H-6), 2.16 (3H, s, C*H*$_3$), 1.51-1.55 (2H, m, H-7a, H-7s) ppm, ^{13}C NMR ($CDCl_3$, 125 MHz) δ 202.75 (C=O), 200.8 (C=O), 174.6 (C=O), 172.7 (C=O), 162.9 (C=O), 162.5 (C=O), 152.2 (Ar-*C*), 141.8 (Ar-*C*), 139.7 (Ar-*C*), 136.0 (Ar-*C*H), 135.0 (Ar-*C*H), 130.2 (Ar-*C*), 129.8 (C), 124.8 (Ar-*C*H), 124.6 (Ar-*C*H), 124.4 (Ar-*C*H), 124.2 (Ar-*C*H), 123.6 (Ar-*C*H), 123.1 (Ar-*C*H), 50.5 (*C*H$_3$), 47.4 (*C*H$_2$), 45.3 (*C*H), 45.1 (*C*H), 42.7 (*C*H), 42.4 (*C*H), 42.1 (*C*H), 38.1 (*C*H), 29.7 (C-7) ppm, HRMS (ESI): calcd for [$C_{28}H_{21}N_3O_6$] ([M]$^+$): *m/z* 495.1430, found 496.1812 [M+H]$^+$.

2-Benzyl-6-(1,3-dioxoisoindoline-2-yl)-3,3a,4,4a,8,8a-hexahydro-2H-spiro[4,8-methanopyrole[3,4-f]isoindole-1,2'indene]-1',3',5,7-(6H,7aH)tetraone **(5)**

Yellow solid, Yield 32%, m.p. 266-268 °C, R_f: 0.48 (1:1, ethyl acetate/*n*-hexane), IR (ATR) 3066, 2954, 2921, 2850, 1737, 1702, 1593, 1468, 1455, 1346, 1288, 1179, 1156 cm^{-1}, ^1H NMR ($CDCl_3$, 500 MHz) δ 7.90-7.92 (2H, m, Ar-*H*), 7.83-7.87 (2H, m, Ar-*H*), 7.76-7.80 (2H, m, Ar-*H*), 7.73-7.75 (2H, m, Ar-*H*), 7.13-7.21 (5H, m, Ar-*H*), 3.50 (1H, d, *J* = 12.92 Hz, C*H*$_2$), 3.45 (1H, d, *J* = 9.45 Hz, C*H*$_2$), 3.39 (1H, d, *J* = 12.92 Hz, C*H*$_2$), 3.14-3.20 (2H, m, H-2, H-3), 2.97 (1H, d, *J* = 10.40 Hz, *N*-C*H*$_2$), 2.82 (1H, t, *J* = 8.19 Hz, H-5), 2.75-2.78 (2H, m, H-1, H-4), 2.70 (1H, d, *J* = 4.41 Hz, H-6), 2.48 (1H, d, *J* = 8.51 Hz, H-7s), 1.53 (1H, d, *J* = 13.55 Hz, H-7a) ppm, ^{13}C NMR ($CDCl_3$, 125 MHz) δ 202.8 (C=O), 200.4 (C=O), 172.7 (C=O), 172.6 (C=O), 162.9 (C=O), 162.5 (C=O), 142.0 (Ar-*C*), 139.5 (Ar-*C*), 138.6 (Ar-*C*), 135.0 (Ar-*C*H), 132.3 (Ar-*C*H), 130.1 (Ar-*C*), 129.7 (Ar-*C*), 128.8 (Ar-*C*H), 128.6 (Ar-*C*Hx2), 128.4 (Ar-*C*H), 128.0 (C), 128.0 (Ar-*C*H), 127.4 (Ar-*C*H), 127.2 (Ar-*C*Hx2), 127.0 (Ar-*C*H), 124.6 (Ar-*C*H), 123.3 (Ar-*C*H), 59.5 (*N*-*C*H$_2$), 49.7 (*C*H), 46.9 (*C*H), 42.5 (*C*H), 42.3 (*C*H), 39.0 (*C*H), 38.1 (*C*H$_2$), 31.3 (*C*H), 30.8 (C-7) ppm, HRMS (ESI): calcd for [$C_{34}H_{25}N_3O_6$] ([M]$^+$): *m/z* 571.1743, found 572.7224 [M+H]$^+$.

General procedure for the synthesis of oxime derivatives **6-9**

Oxime derivatives were prepared with aldehyde, hydroxylamine hydrochloride, and sodium carbonate in ethyl alcohol at room temperature with the known procedure (33-35). A solution of hydroxyl amine hydrochloride (0.417 g, 6 mmol) in 0.5 mL of water and a solution of Na_2CO_3 in 1.5 mL of water were added drop wise to the solution of an aldehyde (2 mmol) in 1.5 mL of ethyl alcohol, respectively. Resulting solid was filtered and purified by recrystallization from alcohol.

4-Chlorobenzaldehyde oxime **6** (33)

White solid, yield 100%, m.p. 92-94 ºC, R_f: 0.52 (1:2 ethyl acetate/*n*-hexane), FT-IR (ATR) 3301 (OH), 1589 (C=N), 1496 (C-N), 971 (C-H), 694 (C-Cl) cm^{-1}, GC-MS (EI), m/z (%): 155 (M+·, 99), 139 (100), 136 (82), 111 (73), 75 (70).

2,4-Dimethoxybenzaldehyde oxime **7** (34)

White solid, Yield 94%, m.p. 103-105 ºC, FT-IR (KBr pellet) 2944, 1610, 1504, 1466, 1414, 1270, 1206, 1112, 1026, 922, 832 cm^{-1}, ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.73 (1H, s, C*H*=N-OH), 7.0 (1H, d, *J* = 8.5 Hz, Ar-*H*), 6.03 (1H, s Ar-*H*), 6.06 (1H, d, *J* = 8.5 Hz, Ar-*H*), 3.6 (6H, s, 2xOCH_3) ppm, calcd for [$C_9H_{11}NO_3$] C 59.66 H 6.11 N 7.70%, found C 60.01 H 6.19%.

4-Methylbenzaldehyde oxime **8** (33)

White solid, yield 99%, m.p. 54-55 ºC, R_f: 0.52 (1:2 ethyl acetate/*n*-hexane), FT-IR (ATR) 3333 (OH), 2994 (CH3), 1572 (C=N), 1497 (C-N), 1028 (C-H) cm^{-1}, ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.01 (1H, s, C*H*=N-OH), 7.71 (2H, d, *J* = 7.6 Hz, Ar-*H*), 7.24 (2H, d, *J* = 7.6 Hz, Ar-*H*), 2.21 (3H, s, CH_3) ppm, GC-MS (EI), m/z (%): 135 (M+·, 96), 119 (100), 104 (76), 91 (83), 75 (61).

2-Thiophenealdehyde oxime **9** (35)

White solid, Yield 98%, m.p. 132-136 ºC. FT-IR (ATR) 3320 (OH), 1568 (C=N), 1468 (C-N), 1026 (C-H) cm^{-1}.

General procedure for the synthesis of compounds **10-13**

The compound **3** (0.154 g, 0.5 mmol) and oxime derivative (*p*-chlorobenzaldehyde oxime, 2,4-dimethoxybenzaldehyde oxime, *p*-methylbenzaldehyde oxime, or thiophene-2-carbaldehyde oxime, respectively) (0.5 mmol) was dissolved in 4 mL of dichloromethane. A solution of NaOCl (0.6 mL) was added drop wise to the reaction mixture at 0 °C. The resulting mixture was stirred at 0 °C overnight. After completion of the reaction, the mixture was extracted with dichloromethane (3x10 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated.

3-(4-Chlorophenyl)-6-(1,3-dioxoisoindolin-2-yl)-4,4a,8,8a-tetrahydro-3aH-4,8-methanoisoxazol[3,4-f]isoindole-5,7-dione **(10)**

White solid, Yield 97%, m.p. 317-319 °C (decomp.), R_f: 0.48 (1:1, ethyl acetate/*n*-hexane), IR (ATR) 3004, 2984, 2973, 2953, 1746, 1733, 1468, 1402, 1349, 1207, 1171, 1123 cm^{-1}, ^1H NMR ($CDCl_3$, 500 MHz) δ 7.90-7.92 (1H, m, Ar-*H*), 7.85-7.87 (1H, m, Ar-*H*), 7.78-7.80 (2H, m, Ar-*H*), 7.54 (2H, d, *J* = 8.51 Hz, Ar-*H*), 7.31 (2H, d, *J* = 8.51 Hz, Ar-*H*), 4.90 (1H, d, *J* = 8.19 Hz, H-5), 3.91 (1H, d, *J* = 8.19 Hz, H-6), 3.37-3.38 (2H, m, H-2, H-3), 3.21 (1H, brs, H-4), 3.02 (1H, brs, H-1), 1.86 (1H, d, *J* = 11.35 Hz, H-7s), 1.58 (1H, d, *J* = 11.35 Hz, H-7a) ppm, ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.1 (C=O), 171.1 (C=O), 162.9 (C=O), 162.7 (C=O), 155.5 (C), 136.3 (C), 135.4 (C), 130.1 (C), 129.5 (Ar-*C*), 129.4 (Ar-*C*), 129.2 (Ar-*C*), 128.9 (Ar-*C*), 128.2 (C), 126.6 (Ar-*C*), 124.8 (Ar-*C*), 124.5 (Ar-*C*), 123.7 (Ar-*C*), 83.5 (*C*H), 52.7 (*C*H), 51.9 (*C*H), 46.2 (*C*H), 44.9 (*C*H), 42.3 (*C*H), 42.0 (CH_2) ppm, HRMS (ESI): calcd for [$C_{24}H_{16}ClN_3O_5$] ([M]$^+$): *m/z* 461.0778, found 462.2232 [M+H]$^+$.

3-(2,4-Dimethoxyphenyl)-6-(1,3-dioxoisoindolin-2-yl)-4,4a,8,8a-tetrahydro-3aH-4,8-methanoisoxazol[3,4-f]isoindole-5,7-dione **(11)**

Yellow solid, Yield 65%, m.p. 214-216 °C, R_f: 0.50 (1:1, ethyl acetate/*n*-hexane), IR (ATR) 3064, 2923, 2850, 2838, 1783, 1738, 1465, 1422, 1344, 1290, 1217, 1177 cm^{-1}, ^1H NMR ($CDCl_3$, 500 MHz) δ 7.87-7.90 (2H, m, Ar-*H*), 7.77-7.79 (2H, m, Ar-*H*), 7.35 (1H, d, *J* = 3.15 Hz, Ar-*H*), 6.86 (1H, dd, *J* = 3.15 and 9.14 Hz, Ar-*H*), 6.80 (1H, d, *J* = 9.14 Hz, Ar-*H*), 4.84 (1H, d, *J* = 8.51 Hz, H-5), 4.31 (1H, d, *J* = 8.51 Hz, H-6), 3.74 (3H, s, OCH_3), 3.71 (3H, s, OCH_3), 3.30-3.32 (2H, m, H-2, H-3), 3.15 (1H, brs, H-4), 2.90 (1H, brs, H-1), 1.82 (1H, d, *J* = 11.35 Hz, H-7s), 1.50 (1H, d, *J* = 11.35 Hz, H-7a) ppm, ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.8 (C=O), 171.5 (C=O), 163.2 (C=O), 162.9 (C=O), 155.9 (C), 153.4 (Ar-*C*), 151.7 (Ar-*C*), 135.3 (Ar-*C*H), 135.2 (Ar-*C*H), 130.1 (Ar-*C*), 129.7 (Ar-*C*), 124.7 (Ar-*C*H), 124.4 (Ar-*C*H), 117.1 (Ar-*C*), 113.6 (Ar-*C*H), 113.0 (Ar-*C*H), 112.7 (Ar-*C*H), 82.8 (*C*H), 56.1 (O*C*H_3), 55.8 (O*C*H_3), 51.2 (*C*H), 46.2 (*C*H), 45.7 (*C*H), 44.6 (*C*H), 42.7 (CH_2), 43.1 (*C*H) ppm, HRMS (ESI): calcd for [$C_{26}H_{21}N_3O_7$] ([M]$^+$): *m/z* 587.1379, found 588.3660 [M+H]$^+$.

3-(4-Methylphenyl)-6-(1,3-dioxoisoindolin-2-yl)-4,4a,8,8a-tetrahydro-3aH-4,8-methanoisoxazol[3,4-f]isoindole-5,7-dione **(12)**

White solid, Yield 95%, m.p. 270-272 °C, R_f: 0.37 (1:1, ethyl acetate/*n*-hexane), IR (ATR) 3028, 2971, 1746, 1731, 1420, 1349, 1294, 1171, 1111, 724 cm^{-1}, ^1H NMR ($CDCl_3$, 500 MHz) δ 7.89-7.90 (1H, m, Ar-*H*), 7.85-7.86 (1H, m, Ar-*H*), 7.77-7.79 (2H, m, Ar-*H*), 7.49 (2H, d, *J* = 8.19 Hz, Ar-*H*), 7.13 (2H, d, *J* = 8.19 Hz, Ar-*H*), 4.88 (1H, d, *J* = 8.19 Hz, H-5), 3.93 (1H, d, *J* = 8.19 Hz, H-6), 3.35-3.37 (2H, m, H-2, H-3), 3.19 (1H, brs, H-4), 3.04 (1H, brs, H-1), 2.23 (3H, s, CH_3), 1.87 (1H, d, *J* = 11.35 Hz, H-7s), 1.55 (1H, d, *J* = 11.35 Hz, H-7a) ppm, ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.1 (C=O), 171.2 (C=O), 162.9 (C=O), 162.8 (C=O), 156.2 (C), 140.6 (Ar-*C*), 135.3 (Ar-*C*H), 129.9 (Ar-*C*H), 129.7 (Ar-*C*), 129.5 (Ar-*C*), 129.4 (Ar-*C*H), 129.3 (Ar-*C*H), 126.5

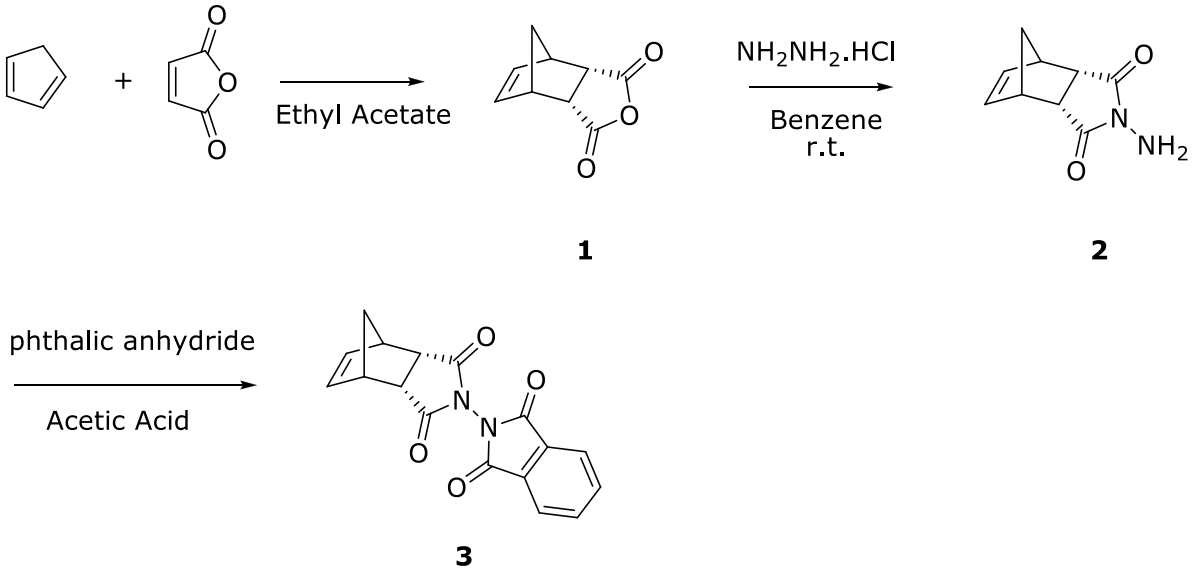
(Ar-*C*Hx2), 125.3 (Ar-*C*), 124.6 (Ar-*C*H), 124.4 (Ar-*C*H), 81.9 (*C*H), 52.2 (*C*H_2), 45.5 (*C*H), 43.1 (*C*H), 42.5 (*C*H), 36.0 (*C*H), 35.4 (*C*H), 21.3 (*C*H_3) ppm, HRMS (ESI): calcd for [$C_{25}H_{19}N_3O_5$] ([M]$^+$): *m/z* 441.1325, found 442.6204 [M+H]$^+$.

3-(Thiophen-2-yl)-6-(1,3-dioxoisoindolin-2-yl)-4,4a,8,8a-tetrahydro-3aH-4,8-methanoisoxazol[3,4-f]isoindole-5,7-dione **(13)**

Beige solid, Yield 85%, m.p. 280-282 °C (decomp.), R_f: 0.32 (1:1, ethyl acetate/*n*-hexane), IR (ATR) 3095, 2953, 2921, 2850, 1794, 1737, 1468, 1435, 1345, 1285, 1174, 1118, 700, 670 cm^{-1}, ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.90-7.92 (1H, m, Ar-*H*), 7.86-7.87 (1H, m, Ar-*H*), 7.78-7.80 (2H, m, Ar-*H*), 7.32 (1H, dd, *J* = 0.94 and 5.04 Hz, Ar-*H*), 7.17 (1H, dd, *J* = 0.94 and 3.78 Hz, Ar-*H*), 6.99 (1H, dd, *J* = 3.78 and 5.04 Hz, Ar-*H*), 4.91 (1H, d, *J* = 8.19 Hz, H-5), 3.91 (1H, d, *J* = 8.19 Hz, H-6), 3.37-3.39 (2H, m, H-2, H-3), 3.22 (1H, brs, H-4), 3.14 (1H, brs, H-1), 1.92 (1H, d, *J* = 11.35 Hz, H-7s), 1.60 (1H, d, *J* = 11.35 Hz, H-7a) ppm, ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.9 (C=O), 171.1 (C=O), 162.9 (C=O), 162.7 (C=O), 152.3 (C), 135.4 (Ar-*C*H), 135.3 (Ar-*C*H), 130.8 (Ar-*C*), 130.1 (Ar-*C*), 129.6 (Ar-*C*), 128.9 (Ar-*C*H), 128.4 (Ar-*C*H), 127.2 (Ar-*C*H), 124.7 (Ar-*C*H), 124.5 (Ar-*C*H), 83.4 (CH), 53.2 (*C*H_2), 46.3 (CH), 45.5 (CH), 43.0 (CH), 42.8 (CH), 45.2 (CH) ppm, HRMS (ESI): calcd for [$C_{22}H_{15}N_3O_5S$] ([M]$^+$): *m/z* 433.0732, found 434.0920 [M+H]$^+$.

RESULT AND DISCUSSION

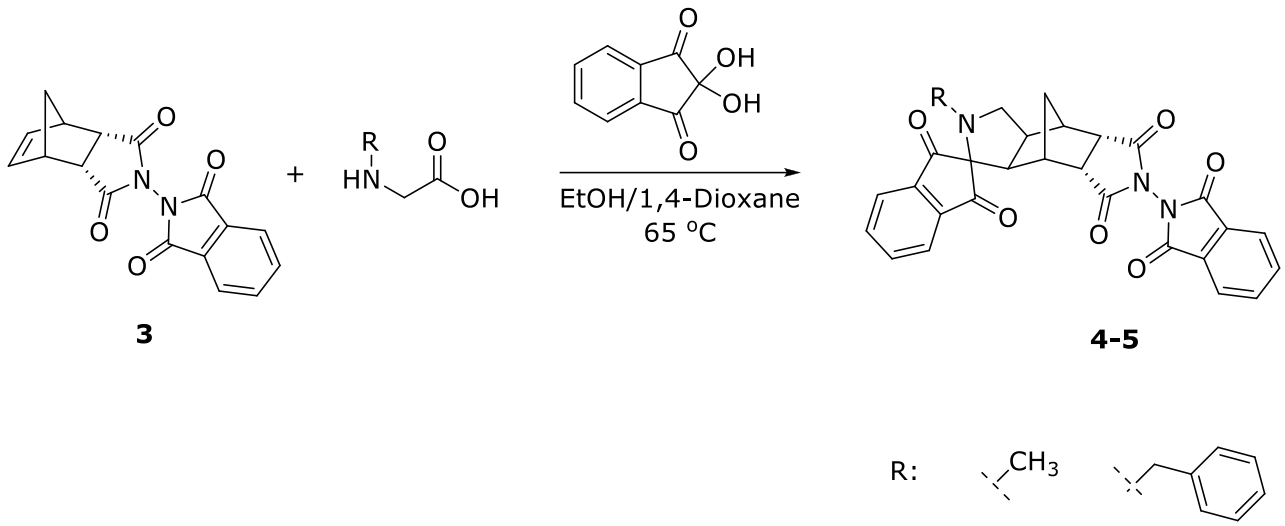
In this study, we synthesized bicyclic endic anhydride **1** by Yamamoto's procedure (31). Thereafter, compound **2** was synthesized from the reaction of hydrazonium chloride and bicyclic endic anhydride **1** in benzene at room temperature (32). We continued to prepare *N*-phthalimide substituted tricyclic imide **3** from compound **2** using Kas'yan procedure (32) (**Scheme 1**).



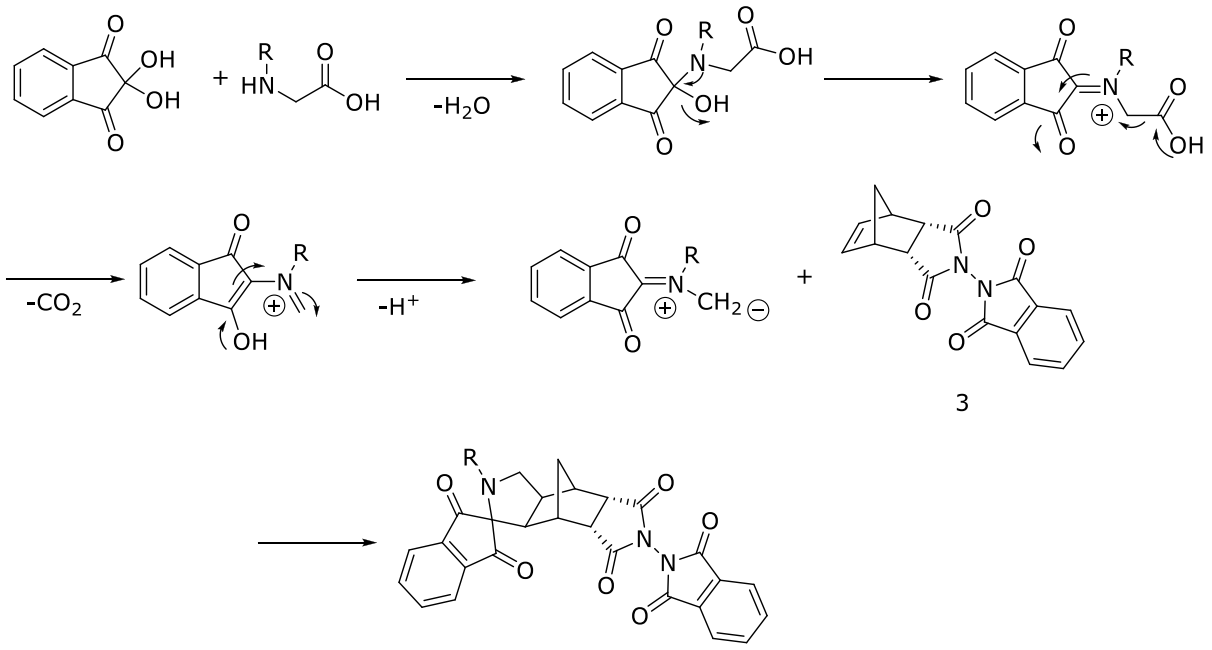
1 **2** **3**

Scheme 1: Preparation of Compounds **1-3.**

Our purpose is to prepare new *N*-phthalimide-substituted tricyclic imide with bispiro-functional group because of their importance in biological activity. In order to achieve this purpose, we designed and synthesized the new bispiro tricyclic imide derivatives **4** and **5** from compound **3** in ethanol/1,4-dioxane solvent system at 65 °C in good yields. (**Scheme 2**) In this reaction series, we used ninhydrin and *N*-methylglycine or *N*-benzylglycine to form azomethine ylide in the reaction flask (**Scheme 3**).

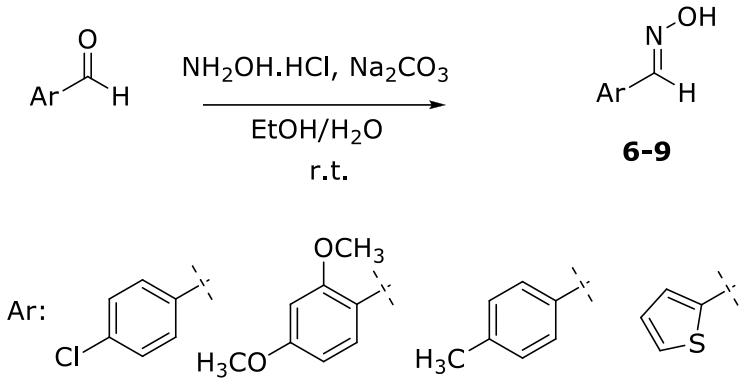


Scheme 2: Preparation of Compound **4-5.**



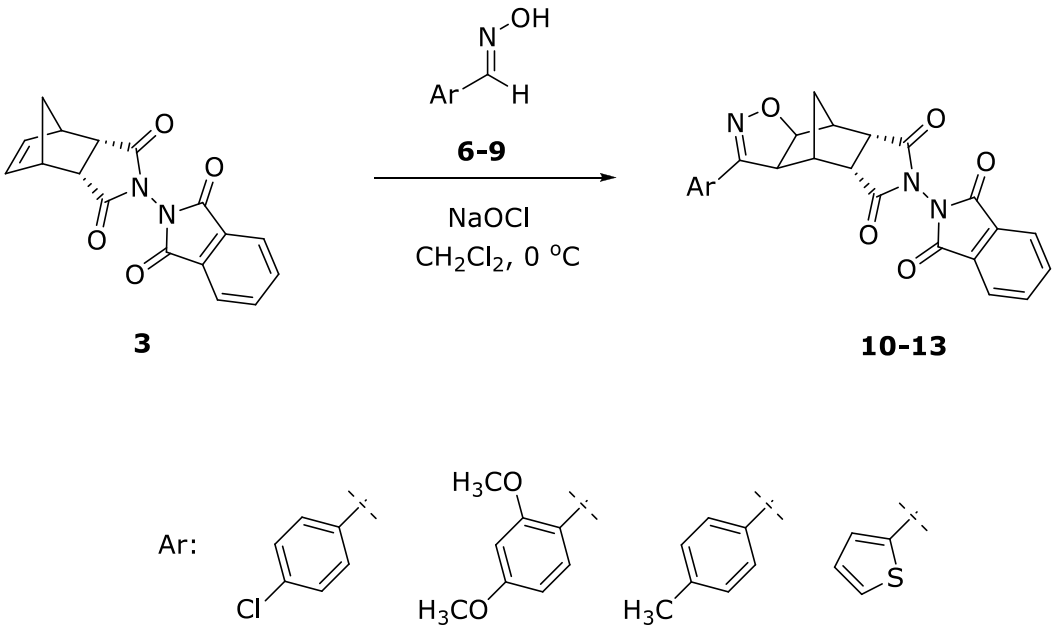
Scheme 3: Proposed mechanism of the reaction.

Our other goal is to prepare new *N*-phthalimide-substituted tricyclic imide with isoxazoline group. Because of our synthetic strategy, we firstly synthesized oxime derivatives **6**-**9** with known procedure in excellent yields (33-35) (**Scheme 4**).



Scheme 4: Synthesis of oxime derivatives **6-9**.

In the next step, we synthesized new tricyclic imide derivatives bearing isoxazoline ring **10-13** with 1,3-dipolar cycloaddition reaction using oxime derivatives **6**-**9** as starting materials in dichloromethane solvent system at 0 °C (**Scheme 5**).



Scheme 5: Synthesis of compounds **10-13.**

Overall, we designed and synthesized new bispiro functionalized tricyclic imide molecules and tricyclic imide molecules containing isoxazoline ring as possible anti-cancer agents. We characterized all of the new molecules with ^{1}H NMR, ^{13}C NMR and FTIR spectral data. The reaction of compound **3** with azomethine ylide obtained from ninhydrin and sarcosine gave the desired products; **4** and **5**. ^{1}H-NMR spectra of compound **3** showed two alkenic protons (H5 and H6) at 6.33 ppm as a singlet. The absent of alkenic protons in the ^{1}H and ^{13}C NMR spectrum of compound **4** confirmed the structure. ^{1}H NMR spectra of compound **4** showed *N*-CH_2 protons at 3.57 ppm and four aromatic protons at 7.95–7.97, 7.74 and 7.69 ppm as an additional. ^{13}C NMR spectra of compound **4** showed four carbonyl carbons with the addition of ninhydrin to the

structure. The ^{1}H and ^{13}C NMR spectrum of compound **5** showed the similar results and confirmed the structure of compound **5**. The ^{1}H and ^{13}C NMR spectrum of compound **10**-**13** proved the 1,3-dipolar cycloaddition reaction occurs. ^{1}H-NMR spectra of compound **10** showed protons (H5 and H6) at 4.90 and 3.91 ppm insead of alkenic protons and showed four additional aromatic protons at 7.54 and 7.31 ppm as expected. The ^{1}H and ^{13}C NMR spectrum of compounds **11-13** showed the similar results and confirmed their structures.

In addition to the ^{1}H-NMR, ^{13}C NMR and FTIR spectral data which were in agreement with the proposed structures, LC-MSMS (Qtof) results of all new compounds showed the expected accurate mass with hydrogen additions.

CONCLUSION

In conclusion, we synthesized tetracyclic systems that we hope will demonstrate new anti-cancer activity by applying azomethine ylide [3+2]-cycloaddition and 1,3-dipolar cycloaddition reactions to the *N*-phthalimide substituted tricyclic imide molecule. Further work toward exploring anticancer activity of all newly synthesized compounds with MTT essay will be forthcoming.

REFERENCES

1. Kulu I, Goksu G, Sucu BO, Kopruceli A, Ocal N, Kaufmann DE. Synthesis of New Aryl-substituted Tandospirone and Epiboxidine Analogues and Isoxazoline Derivatives. Org Prep Proced Int. 2013;45(1):44-56.

2. Gunkara OT, Sucu BO, Ocal N, Kaufmann DE. Synthesis of new aryl(hetaryl)-substituted tandospirone analogues with potential anxiolytic activity via reductive Heck type hydroarylations. Chem Pap. 2013;67(6):643-9.

3. Gunkara OT, Sucu BO, Guleli M, Ocal N. Synthesis of New Tandospirone Analogues Carrying 1-(3-(Trifluoromethyl)Phenyl)Piperazine. Synthetic Commun. 2014;44(11):1619-28.

4. Wieland S, Fischette CT, Lucki I. Effect of Chronic Treatments with Tandospirone and Imipramine on Serotonin-Mediated Behavioral-Responses and Monoamine Receptors. Neuropharmacology. 1993;32(6):561-73.

5. Trawinski J, Skibinski R. Photolytic and photocatalytic degradation of tandospirone: Determination of kinetics, identification of transformation products and in silico estimation of toxicity. Sci Total Environ. 2017;590:775-98.

6. Andoh T, Sakamoto A, Kuraishi Y. 5-HT1A receptor agonists, xaliproden and tandospirone, inhibit the increase in the number of cutaneous mast cells involved in the exacerbation of mechanical allodynia in oxaliplatin-treated mice. J Pharmacol Sci. 2016;131(4):284-7.

7. Mikolajczyk M, Kielbasinski P. Recent Developments in the Carbodiimide Chemistry. Tetrahedron. 1981;37(2):233-84.

8. Brana MF, Castellano JM, Roldan CM, Santos A, Vazquez D, Jimenez A. Synthesis and mode(s) of action of a new series of imide derivatives of 3-nitro-1,8 naphthalic acid. Cancer Chemother Pharmacol. 1980;4(1):61-6.

9. Walter WG. Antitumor imide derivatives of 7-oxabicyclo[2.2.1]heptane-2,3-dimethyl-2,3-dicarboxylic acid. J Pharm Sci. 1989;78(1):66-7.

10. Miyazaki H, Ogiku T, Sai H, Ohmizu H, Murakami J, Ohtani A. Design, synthesis, and evaluation of orally active inhibitors of plasminogen activator inhibitor-1 (PAI-1) production. Bioorg Med Chem Lett. 2008;18(24):6419-22.

11. Bojarski AJ, Mokrosz MJ, Duszynska B, Koziol A, Bugno R. New imide 5-HT1A receptor ligands - modification of terminal fragment geometry. Molecules. 2004;9(3):170-7.

12. Ando I, Ohtsuka T, Miki N, Takahashi T, Hayase Y, Hayashi Y. Synthesis and Biological-Activity of Cyclic Imide Derivatives and Related-Compounds. Agr Biol Chem Tokyo. 1989;53(7):2001-3.

13. Conti P, Dallanoce C, De Amici M, De Micheli C, Klotz KN. Synthesis of new Delta(2)-isoxazoline derivatives and their pharmacological characterization as beta-adrenergic receptor antagonists. Bioorgan Med Chem. 1998;6(4):401-8.

14. Kaur K, Kumar V, Sharma AK, Gupta GK. Isoxazoline containing natural products as anticancer agents: A review. Eur J Med Chem. 2014;77:121-33.

15. Ribeiro CJA, Amaral JD, Rodrigues CMP, Moreira R, Santos MMM. Synthesis and evaluation of spiroisoxazoline oxindoles as anticancer agents. Bioorgan Med Chem. 2014;22(1):577-84.

16. Kozikowski AP. The Isoxazoline Route to the Molecules of Nature. Accounts Chem Res. 1984;17(12):410-6.

17. Arai MA, Kuraishi M, Arai T, Sasai H. A new asymmetric Wacker-type cyclization and tandem cyclization promoted by Pd(II)-spiro bis(isoxazoline) catalyst. J Am Chem Soc. 2001;123(12):2907-8.

18. Studer A, Curran DP. A strategic alternative to solid phase synthesis: Preparation of a small isoxazoline library by ''fluorous synthesis''. Tetrahedron. 1997;53(19):6681-96.

19. Damkaci F, DeShong P. Stereoselective synthesis of alpha- and beta-glycosylamide derivatives from glycopyranosyl azides via isoxazoline intermediates. J Am Chem Soc. 2003;125(15):4408-9.

20. Fuller AA, Chen B, Minter AR, Mapp AK. Succinct synthesis of beta-amino acids via chiral isoxazolines. J Am Chem Soc. 2005;127(15):5376-83.

21. Yildirim A, Cetin M. Synthesis and evaluation of new long alkyl side chain acetamide, isoxazolidine and isoxazoline derivatives as corrosion inhibitors. Corros Sci. 2008;50(1):155-65.

22. Goksu G, Ocal N. 1,3-Dipolar Cycloaddition Reactions of N-Methyl-Substituted Tricyclic Imides. Acta Chim Slov. 2011;58(2):256-61.

23. Gul M, Ocal N. Heck-type hydroarylations and 1,3-dipolar cycloaddition reactions of new tricyclic hydrazones. Can J Chem. 2010;88(4):323-30.

24. Kulu I, Gul M, Gunkara OT, Ocal N. Cycloaddition Reactions of Some Tricyclic Imides. Mini-Rev Org Chem. 2013;10(4):409-18.

25. Gul M, Kulu I, Gunkara OT, Ocal N. Reductive Heck Reactions and [3+2] Cycloadditions of Unsaturated N,N'-Bistricyclic Imides. Acta Chim Slov. 2013;60(1):87-94.

26. Ahrendt KA, Borths CJ, MacMillan DWC. New strategies for organic catalysis: The first highly enantioselective organocatalytic Diels-Alder reaction. J Am Chem Soc. 2000;122(17):4243-4.

27. Jen WS, Wiener JJM, MacMillan DWC. New strategies for organic catalysis: The first enantioselective organocatalytic 1,3-dipolar cycloaddition. J Am Chem Soc. 2000;122(40):9874-5.

28. Obst U, Betschmann P, Lerner C, Seiler P, Diederich F, Gramlich V, et al. Synthesis of novel nonpeptidic thrombin inhibitors. Helv Chim Acta. 2000;83(5):855-909.

29. Sebahar PR, Williams RM. The asymmetric total synthesis of (+)- and (-)-spirotryprostatin B. J Am Chem Soc. 2000;122(23):5666-7.

30. Kaffy J, Pontikis R, Carrez D, Croisy A, Monneret C, Florent JC. Isoxazole-type derivatives related to combretastatin A-4, synthesis and biological evaluation. Bioorg Med Chem. 2006;14(12):4067-77.

31. Yamamoto Y, Yamamoto S, Yatagai H, Ishihara Y, Maruyama K. Lewis Acid Mediated Reactions of Organocopper Reagents - Entrainment in the Conjugate Addition to Alpha,Beta-Unsaturated Ketones, Esters, and Acids Via the Rcu.Bf3 System. J Org Chem. 1982;47(1):119-26.

32. Kas'yan LI, Tarabara IN, Bondarenko YS, Svyatenko LK, Bondarenko AV. Reactions of 4-amino-4-azatricyclo[5.2.1.0(2,6-endo)]dec-8-ene-3,5-dione with dicarboxylic acid anhydrides. Russ J Org Chem+. 2007;43(7):1014-26.

33. Galvis CEP, Kouznetsov VV. An unexpected formation of the novel 7-oxa-2-azabicyclo[2.2.1]hept-5-ene skeleton during the reaction of furfurylamine with maleimides and their bioprospection using a zebrafish embryo model. Org Biomol Chem. 2013;11(3):407-11.

34. Iqbal A, Mohammed AA, Ozair A, Amit N, Subhash CT, Surrinder K. Molecular modelling and snake venom phospholipase A2 inhibition by phenolic compounds: Structure-activity relationship. Tetrahedron. 2016;114:209-19.

35. Jung JY, Jung SH, Koh HY, Pae AN, Park WK, Kong JY. Synthesis of piperazinylalkylisoxazoline analogues and their binding affinities for dopamine receptor subtypes. B Korean Chem Soc. 2006;27(11):1861-4.