Article

Rational Design, Synthesis, Separation, and Characterization of New Spiroxindoles Combined with Benzimidazole Scaffold as an MDM2 Inhibitor

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Abstract: Rational design for a new spiroxindoles, combined with a benzimidazole scaffold to identify a new murine double minute two (MDM2) inhibitor was synthesized and characterized. The desired spiroxindoles were achieved *via* a [3+2] cycloaddition reaction approach which afforded the cycloadducts with four asymmetric centers separated in an excellent regioselective and diastereoselective compound. The separated spiroxindoles were subjected to a set of biochemical assays including an NCI cell panel assay, MTT assay, and MDM2 binding analysis by a microscale thermophoresis assay. The anticancer reactivity for the tested compounds showed IC_{50} (μM) in the range between 3.797–6.879 μM, and compound **7d** with $IC_{50} = 3.797 \pm 0.205$ μM was the most active candidate between the series. The results showed promising results that identified that compound **7a** could be inhibited the MDM2 with $K_D = 2.38$ μM. Compound **7a** developed a network of interactions with the MDM2 receptor studied in silico by molecular docking.

Keywords: spiroxindole; benzimidazole; MDM2

1. Introduction

The MDM2–p53 protein–protein interaction inhibitor is a hot research topic and has been gaining a lot of attention recently [1–4]. The inhibition of the interaction between the two proteins, p53, and MDM2, leads to reactivation of the p53 which has many functionalities, including DNA repairing, apoptosis, cell cycle arrest, senescence, metabolic alteration, and tumor suppresser [5,6]. The mutant p53 protein has been found in approximately 50% of human cancer cells [7,8]. The dislocation between the MDM2 protein and p53 protein is a challenge and is important to the development of a new chemotherapeutic agent.

Based on the literature survey, it has been reported so far that more than 20 chemotypes of molecules have been identified as MDM2–p53 inhibitors such as spirooxindoles [9], nutlins [10], isoquinoline-1-one [11], chalcone [12], pyrrolin-2-one [13], piperidine [14], morpholinone [15], imidazolyl indole [16], benzodiazpinedione [17], diketopiperazines [18], chromenotriazolopyrimidines [19], and other pharmacophores. For this, protein–protein interaction (PPI) inhibitors have progressed into clinical trials including spirooxindoles such as APG-115 [20], SAR405838 [21], and other pharmacophores such as RG7388 [22], HDM201 [23], RG7112 [24], and AMG-232 [25]. Inhibiting the p53–MDM2 interaction is a promising strategy for cancer treatment, as it can help to restore normal cell growth and death.

Protein–protein interaction inhibitors (PPIs) are typically small molecules that are designed to bind to the p53 and MDM2 proteins and prevent them from interacting. Several PPIs have been developed and are currently in clinical trials for a variety of cancers. Common side effects including fatigue, nausea, and anemia were observed.

In between the small molecules reported as promising lead compounds for cancer research are the spirooxindoles. This scaffold is able to activate the p53 and bind with the MDM2 domain [26–47]. Spirotryprostatin B is an inspired natural product that exhibits anticancer reactivity [33] (Figure 1). Gollner A. et al. reported a novel chemically stable spiro [3*H*-indole-3, 2′-pyrrolidin]-2 (1*H*)-one lead compound and orally active inhibitors of the MDM2–p53 interaction [34]. Benzimidazole scaffold was introduced to many compounds which showed high efficacy against MDM2, MDMX, and NF-kB inhibitors [40–46]. Our research group has engaged in this research program for a couple of years and has been successful in designing and developing several molecules towards PPI [35–39]. Among the discovered molecules, a new spiroxindole [48], as a rigid structure with a combination of benzimidazole scaffold, has been discovered as a novel MDM2 protein inhibitor with dual effects of antimetastatic efficacy. Based on these findings, we have rationally designed and synthesized a new spirooxindoles-based benzimidazole unit as an MDM2 inhibitor.
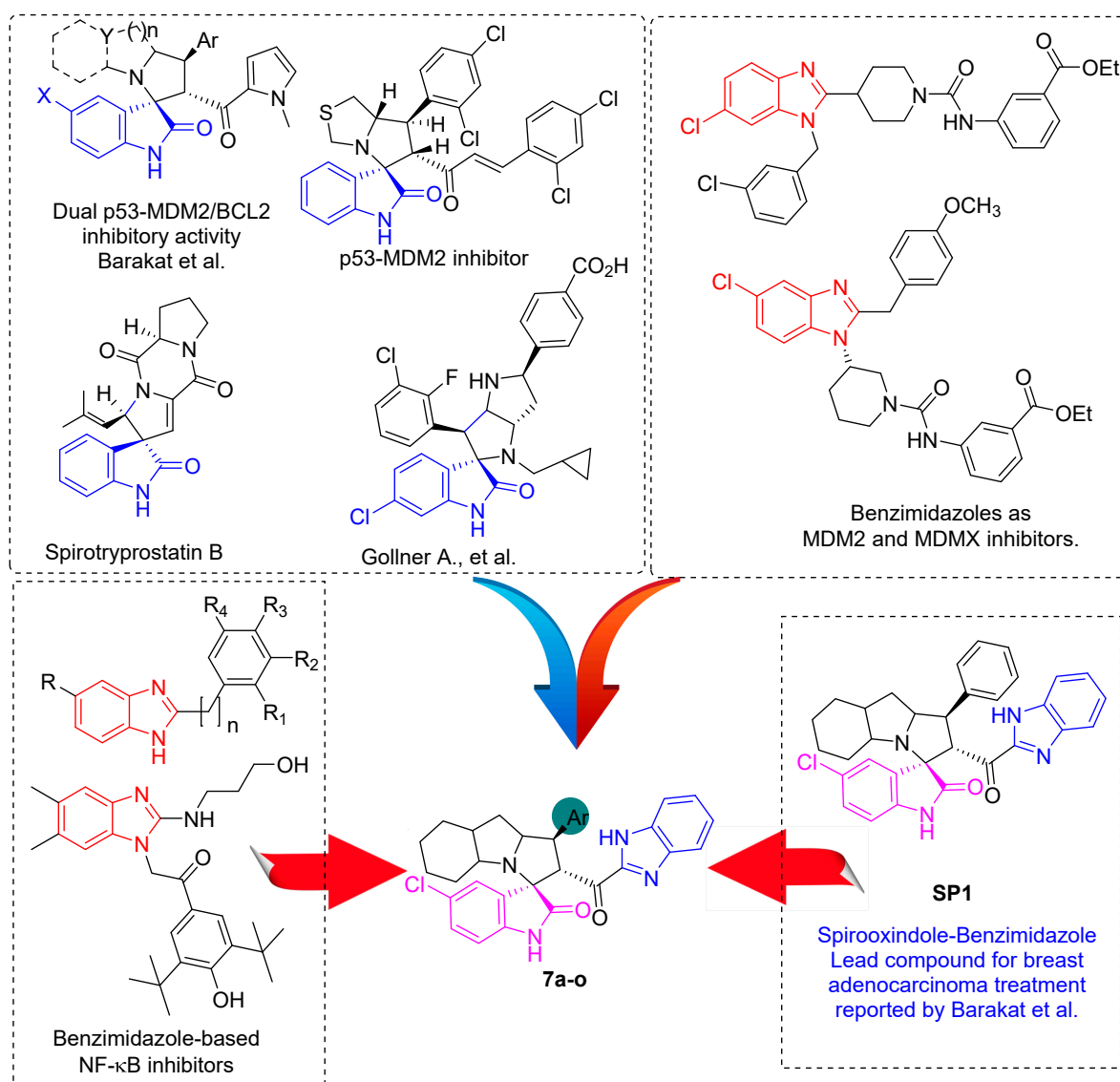
Figure 1. Reported spirooxindoles and benzimidazoles with anticancer activity and our rationally designed compound **7a-o** [34,36].

2. Materials and Methods

2.1. General

"All chemicals were purchased from Aldrich, Sigma-Aldrich and Fluka, which were used without further purification unless otherwise stated. All melting points were measured using a Gallenkamp melting point apparatus in open glass capillaries and were uncorrected. Crude products were purified by column chromatography on silica gel of 100–200 mesh. IR spectra were measured as KBr pellets using a Nicolet 6700 FT-IR spectrophotometer. The NMR spectra were recorded using a Varian Mercury Jeol-400 NMR spectrometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectroscopy were performed in either deuterated dimethylsulfoxide (DMSO-d_6) or deuterated chloroform (CDCl$_3$). Chemical shifts (δ) are reported in terms of ppm and coupling constants J are given in Hz. Elemental analysis was carried out using an Elmer 2400 Elemental Analyzer in CHN mode".

2.2. Synthesis of Spirooxindole Analogues (7a-o) General Procedure)

Chalcone derivative **4a-o** (0.5 mmol), octahydroindole-2-carboxylic acid **6** (84.62 mg, 0.5 mmol), and 5-chlorisatin **5** (90.79 mg, 0.5 mmol) were mixed in 20 mL MeOH then, heated up at 60–65 °C for 2–3 h. After the reaction was completed, as monitored by TLC, the crude material was subjected to column chromatography using ethylacetate/n-hexane (2: 6), yielding spiro compounds in pure form.

2′-(1*H*-Benzo[*d*]imidazole-2-carbonyl)-5-chloro-1′-(4-chlorophenyl)-1′,2′,4a′,5′,6′,7′,8′,8a′, 9′,9a′-decahydrospiro[indoline-3,3′-pyrrolo [1,2-*a*]indol]-2-one (7a).

Pale yellow solid; yield (80%); m.p.:176–178 °C; IR (KBr, cm^{-1}): 3434 (NH), 3277 (NH), 3093 (CH), 2929 (CH),1729 (CO), 1682 (CO); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 12.98 (1H, s, NH), 10.17 (1H, s, NH), 7.73 (1H, s, Ph-H) 7.45 (2H, d, *J* = 8.0 Hz, Ph-H), 7.42–7.26 (6H, m, Ph-H), 7.08 (1H, d, *J* = 8.0 Hz, Ph-H), 6.44 (1H, d, *J* = 8.0 Hz, Ph-H), 5.29 (1H, d, *J* = 11.9 Hz, C<u>H</u>CO), 4.00 (2H, m, C<u>H</u>N, C<u>H</u>Ph), 3.23 (1H, d, *J* = 3.7 Hz), 2.16–2.08 (1H, m), 2.06–1.98 (1H, m), 1.55–0.70 (10H, m, aliphatic CH); Anal. for C$_{32}$H$_{28}$Cl$_2$N$_4$O$_2$; calcd: C, 67.25; H, 4.94; N, 9.80 Exper.: C, 66.89; H, 5.03; N, 10.04.

2′-(1*H*-Benzo[*d*]imidazole-2-carbonyl)-5-chloro-1′-(4-(trifluoromethyl)phenyl)-1′,2′,4a′, 5′,6′,7′,8′,8a′,9′,9a′-decahydrospiro[indoline-3,3′-pyrrolo[1,2-*a*]indol]-2-one (7b).

Pale yellow solid; yield (32%); m.p.:125–127 °C; IR (KBr, cm^{-1}): 3429 (NH), 3282 (NH), 3099 (CH), 2927 (CH),1724 (CO), 1686 (CO); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 12.98 (1H, s, NH), 10.20 (1H, s, NH), 7.75 (1H, d, *J* = 8 Hz, Ph-H), 7.68 (4H, m, Ph-H), 7.48–7.20 (4H, m, Ph-H), 7.09 (1H, d, *J* = 8.8 Hz, Ph-H), 6.43 (1H, d, *J* = 8.3 Hz, Ph-H), 5.34 (1H, d, *J* = 11.7 Hz, C<u>H</u>CO), 4.11 (1H, m, C<u>H</u>N), 3.46 (1H, m, C<u>H</u>Ph), 2.10 (2 H, d, *J* = 12.9Hz), 1.58–0.67 (10 H, m, aliphatic C-H); Anal. for C$_{33}$H$_{28}$ClF$_3$N$_4$O$_2$; calcd: C, 65.51; H, 4.66; N, 9.26 Exper.: C, 66.09; H, 4.77; N, 9.14.

2′-(1*H*-Benzo[*d*]imidazole-2-carbonyl)-5-chloro-1′-(*p*-tolyl)-1′,2′,4a′,5′,6′,7′,8′,8a′,9′, 9a′-decahydrospiro[indoline-3,3′-pyrrolo[1,2-*a*]indol]-2-one (7c).

Yellow solid; yield (41%); m.p.:163–165 °C; IR (KBr, cm^{-1}): 3649 (NH), 3277 (NH), 3087(CH), 2926 (CH),1727 (CO), 1690 (CO); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 12.98 (1H, s, NH), 10.18 (1H, s, NH), 7.76 (1H, d, *J* = 8.0 Hz, Ph-H), 7.40 (1H, d, *J* = 2.2 Hz, Ph-H), 7.35–7.26 (5H, m, Ph-H), 7.10–7.06 (3H, d, *J* = 8.0 Hz, Ph-H), 6.44 (1H, d, *J* = 8 Hz, Ph-H), 5.34 (1H, d, *J* = 12.4 Hz, C<u>H</u>CO), 4.11–4.00 (1H, m, C<u>H</u>N), 3.96–3.86 (1H, m, C<u>H</u>Ph), 2.20 (3H, s, CH$_3$), 2.11 (2 H, d, *J* = 8.0Hz), 1.56–0.72 (10H, m, aliphatic C-H); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ = 189.91, 180.05, 148.02, 143.07, 141.62, 136.60, 136.47, 135.18, 129.69, 127.85, 126.33, 125.17, 123.63, 111.02, 71.74, 63.94, 57.24, 52.79, 36.80, 28.19, 25.07; Anal. for C$_{33}$H$_{31}$ClN$_4$O$_2$; calcd: C, 71.92; H, 5.67; N, 10.17 Exper.: C, 71.59; H, 5.71; N, 10.44.

2′-(1*H*-Benzo[*d*]imidazole-2-carbonyl)-5-chloro-1′-(thiophen-2-yl)-1′,2′,4a′,5′,6′,7′,8′,8a′, 9′,9a′-decahydrospiro[indoline-3,3′-pyrrolo[1,2-*a*]indol]-2-one (7d).

Pale yellow solid; yield (48%); m.p.:130–132 °C; IR (KBr, cm^{-1}): 3624 (NH), 3258 (NH), 3091(CH), 2927 (CH),1728 (CO), 1689 (CO); ^{1}H-NMR (CDCl$_3$, 400 MHz): δ 10.18 (1H, s, NH), 8.64 (1H, s, NH), 7.84 (1H, d, *J* = 8.0 Hz, Ph-H), 7.34 (1H, d, *J* = 8.0 Hz, thio-H), 7.31–7.20 (4H, m, Ph-H), 6.99 (1H, d, *J* = 3.5 Hz, thio-H), 6.95 (1H, dd, *J* = 8.2, 1.7 Hz, thio-H), 6.90–6.85 (1H, m, Ph-H), 6.39 (1H, d, *J* = 8.6 Hz, Ph-H), 5.30 (1H, d, *J* = 12.0 Hz, C<u>H</u>CO), 4.53–4.34(1H, m, C<u>H</u>N), 4.12 (1H, t, *J* = 11.0 Hz, C<u>H</u>Ph), 3.20 (1H, d, *J* = 4.0 Hz), 2.16 (1H, d, *J* = 5.0Hz), 1.83–0.82 (10 H, m, aliphatic C-H); ^{13}C-NMR (CDCl$_3$, 100 MHz): δ = 189.65, 181.65, 146.83, 143.16, 141.99, 139.94, 133.80, 129.28, 126.77, 125.88, 123.80, 111.02, 72.12, 71.33, 65.76, 57.93, 48.77, 37.52, 28.38, 27.86, 19.70; Anal. for C$_{30}$H$_{27}$ClN$_4$O$_2$S; calcd: C, 66.35; H, 5.01; N, 10.32 Exper.: C, 66.49; H, 5.20; N, 10.14.

2′-(1*H*-Benzo[*d*]imidazole-2-carbonyl)-5-chloro-1′-(4-fluorophenyl)-1′,2′,4a′,5′,6′,7′,8′, 8a′,9′,9a′-decahydrospiro[indoline-3,3′-pyrrolo[1,2-*a*]indol]-2-one (7e).

Pale yellow solid; yield (35%); m.p.:148–150 °C; IR (KBr, cm^{-1}): 3431 (NH), 3268 (NH), 3096 (CH), 2960 (CH),1732 (CO), 1684 (CO); ^{1}H-NMR (CDCl$_3$, 400 MHz): δ 10.02 (1H, s, NH), 8.55 (1H, s, NH), 7.82 (1H, d, *J* = 8.3 Hz, Ph-H), 7.40 (2 H, dd, *J* = 8.5, 5.4 Hz), 7.35–7.12 (5H, m, Ph-H), 6.94 (3H, m, Ph-H), 6.36 (1H, d, *J* = 8 Hz, Ph-H), 5.32 (1H, d, *J* = 12.4 Hz, C<u>H</u>CO), 4.31 (1H, q, *J* = 7.4 Hz, C<u>H</u>N), 3.79 (1H, t, *J* = 12.4 Hz, C<u>H</u>Ph), 3.19–0.87 (12 H, m, aliphatic C-H); ^{13}C-NMR (CDCl$_3$, 100 MHz): δ = 189.71, 181.92, 163.19, 160.76, 146.85, 143.08, 139.91, 134.65, 134.62, 133.69, 129.56, 129.49, 129.30, 127.35, 126.82, 126.64, 126.09, 123.68, 122.61, 115.63, 115.42, 112.08, 110.91, 72.01, 71.26, 65.28, 57.78, 53.18, 41.88, 37.45, 29.79, 28.43, 27.82, 24.79, 19.75; Anal. for C$_{32}$H$_{28}$ClFN$_4$O$_2$; calcd: C, 69.25; H, 5.08; N, 10.09 Exper.: C, 69.49; H, 5.18; N, 10.44.

2′-(1*H*-Benzo[*d*]imidazole-2-carbonyl)-5-chloro-1′-(2,4-dichlorophenyl)-1′,2′,4a′,5′,6′,7′, 8′,8a′,9′,9a′-decahydrospiro[indoline-3,3′-pyrrolo[1,2-*a*]indol]-2-one (7f).

Pale yellow solid; yield (48%); m.p.:138–140 °C; IR (KBr, cm^{-1}): 3436 (NH), 3281 (NH), 3090 (CH), 2925 (CH),1730 (CO), 1685 (CO); ^{1}H-NMR (CDCl$_3$, 400 MHz): δ 10.14 (1H, s, NH), 8.40 (1H, s, NH), 7.84 (1H, d, *J* = 8.0 Hz, Ph-H), 7.55 (1H, d, *J* = 8.7 Hz, Ph-H), 7.37 (1H, s, Ph-H), 7.35–7.14 (5H, m, Ph-H), 6.95 (1H, d, *J* = 8.0 Hz, Ph-H), 6.37 (1H, d, *J* = 8.0 Hz, Ph-H), 5.39 (1H, d, *J* = 12.3 Hz, C<u>H</u>CO), 4.47 (1H, t, *J* = 11.0 Hz, C<u>H</u>Ph), 4.20 (1H, q, *J* = 8.5 Hz, C<u>H</u>N), 3.20 (1 H, d, *J* = 4.4 Hz), 2.14–0.89 (12 H, m, aliphatic C-H); ^{13}C-NMR (CDCl$_3$, 100 MHz): δ = 189.44, 181.66, 146.72, 143.03, 139.94, 135.42, 135.35, 133.76, 133.00, 129.56, 126.98, 125.98, 110.98, 71.87, 48.25, 27.66, 19.79; Anal. for C$_{32}$H$_{27}$Cl$_3$N$_4$O$_2$; calcd: C, 63.43; H, 4.49; N, 9.25 Exper.: C, 63.59; H, 5.04; N, 9.04.

2′-(1*H*-Benzo[*d*]imidazole-2-carbonyl)-5-chloro-1′-(3-hydroxyphenyl)-1′,2′,4a′,5′,6′,7′,8′, 8a′,9′,9a′-decahydrospiro[indoline-3,3′-pyrrolo[1,2-*a*]indol]-2-one (7g).

Pale yellow solid; yield (62%); m.p.:186–188 °C; IR (KBr, cm^{-1}): 3626 (NH), 3257 (OH), 2931 (CH), 1726 (CO), 1688 (CO); ^{1}H-NMR (CDCl$_3$, 400 MHz): δ 9.00 (1H, s, NH), 8.25 (1H, s, NH), 7.54 (1H, d, *J* = 4.0 Hz, Ph-H), 7.46 (1H, s, OH), 7.22 (1H, d, *J* = 8.0 Hz, Ph-H), 7.08–7.04 (3H, m, Ph-H), 6.89–6.72 (5H, m, Ph-H), 6.23 (1H, d, *J* = 8.0 Hz, Ph-H), 5.31 (1H, d, *J* = 12.5 Hz, C<u>H</u>CO), 4.49–4.38 (1H, m, C<u>H</u>N), 3.75–3.65 (1H, m, C<u>H</u>Ph), 3.14 (1H, d, *J* = 4 Hz), 2.16–0.81 (12 H, m, aliphatic C-H); Anal. for C$_{32}$H$_{29}$ClN$_4$O$_3$; calcd: C, 69.49; H, 5.29; N, 10.13 Exper.: C, 69.69; H, 5.14; N, 9.94.

2′-(1*H*-Benzo[*d*]imidazole-2-carbonyl)-5-chloro-1′-(3,4,5-trimethoxyphenyl)-1′,2′,4a′,5′, 6′,7′,8′,8a′,9′,9a′-decahydrospiro[indoline-3,3′-pyrrolo[1,2-*a*]indol]-2-one (7h).

Pale yellow solid; yield (88%); m.p.:160–162 °C; IR (KBr, cm^{-1}): 3430 (NH), 3269 (NH), 3093 (CH), 2996 (CH),1722 (CO), 1683 (CO); ^{1}H-NMR (CDCl$_3$, 400 MHz): δ 10.15 (1H, s, NH), 8.500 (1H, s, NH), 7.81 (1H, d, *J* = 8.0 Hz, Ph-H), 7.37–7.19 (4H, m, Ph-H), 6.97 (1H, d, *J* = 8.4 Hz, Ph-H), 6.66 (2H, s, Ph-H), 6.38 (1H, d, *J* = 8.5 Hz, Ph-H), 5.38 (1H, d, *J* = 12.3 Hz, C<u>H</u>CO), 4.33 (1H, m, C<u>H</u>Ph), 3.78 (6H, s, OCH$_3$), 3.73 (3H, s, OCH$_3$), 3.19 (1H, m, C<u>H</u>N), 2.02–0.78 (12 H, m, aliphatic C-H); ^{13}C-NMR (CDCl$_3$, 100 MHz): δ = 189.75, 181.68, 153.30, 146.99, 143.14, 139.85, 136.95, 134.53, 133.80, 133.31, 126.78, 126.18, 123.39, 122.48, 110.86,

104.89, 72.25, 71.07, 60.86, 56.33, 56.18, 54.56, 41.84, 37.58, 28.44, 27.83, 24.76.; Anal. for $C_{35}H_{35}ClN_4O_5$; calcd: C, 67.03; H, 5.63; N, 8.93 Exper.: C, 67.59; H, 5.34; N, 9.05.

2′-(1*H*-Benzo[*d*]imidazole-2-carbonyl)-5-chloro-1′-(2-hydroxyphenyl)-1′,2′,4a′,5′,6′,7′,8′, 8a′,9′,9a′-decahydrospiro[indoline-3,3′-pyrrolo[1,2-*a*]indol]-2-one (7i).

Yellow solid; yield (82%); m.p.:128–130 °C; IR (KBr, cm^{-1}): 3311 (OH), 3063 (CH), 2925 (CH),1716 (CO), 1667 (CO); ^{1}H-NMR (CDCl$_3$, 400 MHz): δ 10.57 (1H, s, NH), 8.93 (1H, s, NH), 7.80 (1H, d, *J* = 8.0 Hz, Ph-H), 7.49 (1H, d, *J* = 8.0 Hz, Ph-H), 7.29 (2H, d, *J* = 7.3 Hz, Ph-H), 7.23 (1H, s, OH), 7.08 (2H, t, *J* = 7.7 Hz, Ph-H), 6.97 (2H, d, *J* = 8 Hz, Ph-H), 6.87 (2H, d, *J* = 8 Hz, Ph-H), 6.45 (1H, d, *J* = 8 Hz, Ph-H), 5.07 (1H, dd, *J* = 11.7, 6.6 Hz, C*H*CO), 4.54 (1H, q, *J* = 7.3 Hz, C*H*N), 4.43 (1H, t, *J* = 11.0 Hz, C*H*Ph), 3.22 (1H, d, *J* = 4.4 Hz), 2.16–0.83 (12 H, m, aliphatic C-H); ^{13}C-NMR (CDCl$_3$, 100 MHz): δ = 191.67, 181.97, 154.84, 146.69, 142.54, 139.78, 133.34, 129.39, 128.16, 127.44, 126.93, 125.55, 125.42, 124.12, 122.07, 121.41, 118.44, 112.22, 111.31, 85.63, 85.25, 83.06, 73.14, 57.83, 46.34, 41.88, 40.99, 37.30, 28.55, 28.44, 27.76, 24.73, 23.97; Anal. for $C_{32}H_{29}ClN_4O_3$; calcd: C, 69.49; H, 5.29; N, 10.13 Exper.: C, 69.55; H, 5.16; N, 10.34.

2′-(1*H*-Benzo[*d*]imidazole-2-carbonyl)-5-chloro-1′-(4-(dimethylamino)phenyl)-1′,2′,4a′, 5′,6′,7′,8′,8a′,9′,9a′-decahydrospiro[indoline-3,3′-pyrrolo[1,2-*a*]indol]-2-one (7j).

Orange solid; yield (45%); m.p.:140–142 °C; IR (KBr, cm^{-1}): 3434 (NH), 3275 (NH), 3094 (CH), 2929 (CH),1729 (CO), 1682 (CO); ^{1}H-NMR (CDCl$_3$, 400 MHz): δ 9.81 (1H, s, NH), 8.01 (1H, s, NH), 7.78 (1H, d, *J* = 8.0 Hz, Ph-H), 7.31 (5H, m, Ph-H), 7.17 (1H, d, *J* = 2.2 Hz, Ph-H), 6.94 (1H, dd, *J* = 8.3, 2.3 Hz, Ph-H), 6.65 (2H, d, *J* = 8.8 Hz, Ph-H), 6.31 (1H, d, *J* = 8.6 Hz, Ph-H), 5.32 (1H, d, *J* = 12.4 Hz, C*H*CO), 4.38–4.27 (1H, m, C*H*N), 4.10 (1H, t, *J* = 7.3 Hz, C*H*Ph), 3.19 (1H, d, *J* = 3.8 Hz), 2.87 (6H, s, NCH$_3$), 2.07–0.83 (12 H, m, aliphatic C-H); ^{13}C-NMR (CDCl$_3$, 100 MHz): δ = 189.78, 181.66, 149.77, 147.05, 139.62, 133.54, 129.10, 128.85, 128.72, 127.56, 126.77, 126.36, 112.88, 110.59, 72.04, 71.13, 65.09, 57.80, 53.18, 40.70, 31.67, 28.50, 27.81, 24.84, 22.74, 19.86; Anal. for $C_{34}H_{34}ClN_5O_2$; calcd: C, 70.39; H, 5.91; N, 12.07 Exper.: C, 70.65; H, 6.06; N, 11.94.

2′-(1*H*-Benzo[*d*]imidazole-2-carbonyl)-1′-(4-bromophenyl)-5-chloro-1′,2′,4a′,5′,6′,7′,8′, 8a′,9′,9a′-decahydrospiro[indoline-3,3′-pyrrolo[1,2-*a*]indol]-2-one (7k).

Yellow solid; yield (72%); m.p.:159–161 °C; IR (KBr, cm^{-1}): 3625 (NH), 3422 (NH), 3088 (CH), 2913 (CH),1725 (CO), 1682 (CO); ^{1}H-NMR (CDCl$_3$, 400 MHz): δ 10.08 (1H, s, NH), 8.46 (1H, s, NH), 7.80 (1H, d, *J* = 8.2 Hz, Ph-H), 7.36 (2H, d, *J* = 8.6 Hz, Ph-H), 7.31–7.16 (7H, m, Ph-H), 6.99–6.93 (1H, m, Ph-H), 6.34 (1H, d, *J* = 8.2 Hz, Ph-H), 5.30 (1H, d, *J* = 12.3 Hz, C*H*CO), 4.36–4.24 (1H, m, C*H*N), 3.75 (1H, t, *J* = 12.4 Hz, C*H*Ph), 3.19 (1H, d, *J* = 3.9 Hz), 2.22–0.82 (12 H, m, aliphatic C-H); ^{13}C-NMR (CDCl$_3$, 100 MHz): δ = 189.58, 181.78, 146.77, 143.06, 139.92, 138.08, 133.72, 131.76, 129.87, 129.35, 127.32, 126.82, 126.65, 126.03, 122.61, 120.92, 112.11, 110.94, 71.97, 71.19, 65.20, 57.78, 53.43, 41.86, 37.41, 28.43, 27.81, 24.78, 19.75; Anal. for $C_{32}H_{28}BrClN_4O_2$; calcd: C, 62.40; H, 4.58; N, 9.10 Exper.: C, 62.60; H, 4.34; N, 9.03.

2′-(1*H*-Benzo[*d*]imidazole-2-carbonyl)-5-chloro-1′-(3-fluorophenyl)-1′,2′,4a′,5′,6′,7′,8′, 8a′,9′,9a′-decahydrospiro[indoline-3,3′-pyrrolo[1,2- *a*]indol]-2-one (7l).

Pale yellow solid; yield (42%); m.p.:143–145 °C; IR (KBr, cm^{-1}): 3625 (NH), 3422 (NH), 3088 (CH), 2913 (CH),1725 (CO), 1682 (CO); ^{1}H-NMR (CDCl$_3$, 400 MHz): δ 9.51 (1H, s, NH), 7.86 (1H, d, *J* = 8.0 Hz, Ph-H), 7.42 (1H, s, NH), 7.32 (2H, d, *J* = 4 Hz, Ph-H), 7.29–7.27 (2H, m, Ph-H), 7.23–7.13 (3H, m, Ph-H), 6.96 (1H, dd, *J* = 8.5, 2.2 Hz, Ph-H), 6.91–6.85 (1H, m, Ph-H), 6.32 (1H, d, *J* = 8.4 Hz, Ph-H), 5.31 (1H, d, *J* = 12.0 Hz, C*H*CO), 4.41–4.30 (1H, m, C*H*N), 3.79 (1H, t, *J* = 12.4 Hz, C*H*Ph), 3.22 (1H, d, *J* = 4.3 Hz, C*H*Ph), 2.17–0.88 (12 H, m, aliphatic C-H); Anal. for $C_{32}H_{28}ClFN_4O_2$; calcd: C, 69.25; H, 5.08; N, 10.09 Exper.: C, 69.40; H, 4.94; N, 10.13.

2′-(1*H*-Benzo[*d*]imidazole-2-carbonyl)-5-chloro-1′-(furan-2-yl)-1′,2′,4a′,5′,6′,7′,8′,8a′,9′, 9a′-decahydrospiro[indoline-3,3′-pyrrolo[1,2- *a*]indol]-2-one (7m).

Pale yellow solid; yield (62%); m.p.:165–167 °C; IR (KBr, cm^{-1}): 3435 (NH), 3256 (NH), 3090 (CH), 2928 (CH),1729 (CO), 1687 (CO); ^{1}H-NMR (CDCl$_3$, 400 MHz): δ 10.31 (1H, s, NH), 8.77 (1H, s, NH), 7.83 (1H, d, J = 8 Hz, Ph-H), 7.36 (1H, d, J = 8 Hz, Ph-H), 7.28 (1H, t, J = 7.5 Ph-H), 7.22 (2H, d, J = 7.9 Hz, Ar), 7.11 (1H, s, Ph-H), 6.93 (1H, d, J = 8.7 Hz, Ph-H), 6.39 (1H, d, J = 8.7 Hz, Ph-H), 6.20 (1H, t, J = 1.6 Hz, fur-H), 6.12 (1H, d, J = 3.6 Hz, fur-H), 5.37 (1H, d, J = 12.3 Hz, C$\underline{H}$CO), 4.39 (1H, q, J = 8.3 Hz, C$\underline{H}$N), 3.97 (1H, t, J = 11.4 Hz, C$\underline{H}$Ph), 3.17 (1H, d, J = 4.4 Hz), 2.16–0.85 (12 H, m, aliphatic C-H); ^{13}C-NMR (CDCl$_3$, 100 MHz): δ = 189.68, 181.80, 153.04, 146.83, 143.19, 141.75, 141.71, 141.67, 139.98, 133.89, 129.32, 127.40, 126.74, 126.63, 125.97, 123.69, 122.69, 112.20, 111.05, 110.27, 106.01, 71.94, 68.30, 62.85, 57.68, 46.89, 41.94, 37.74, 28.39, 27.79, 24.80, 19.69; Anal. for C$_{30}$H$_{27}$ClN$_4$O$_3$; calcd: C, 68.37; H, 5.16; N, 10.63 Exper.: C, 68.60; H, 4.94; N, 10.33.

2'-(1*H*-Benzo[*d*]imidazole-2-carbonyl)-5-chloro-1'-(4-nitrophenyl)-1',2',4a',5',6',7',8',8a', 9',9a'-decahydrospiro[indoline-3,3'-pyrrolo[1,2-*a*]indol]-2-one (7n).

Pale yellow solid; yield (46%); m.p.:165–167 °C; IR (KBr, cm^{-1}): 3437 (NH), 3255 (NH), 3094 (CH), 2929 (CH),1728 (CO), 1686 (CO); ^{1}H-NMR (CDCl$_3$, 400 MHz): δ 9.81 (1H, s, NH), 8.12 (1H, s, NH), 8.12 (2H, d, J = 8.6 Hz, Ph-H), 7.80 (1H, d, J = 8.0 Hz, Ph-H), 7.63 (2H, d, J = 8.6 Hz, Ph-H), 7.33–7.23 (3H, m, Ph-H), 7.14 (1H, s, Ph-H), 6.97 (1H, dd, J = 8.3, 1.8 Hz, Ph-H), 6.36 (1H, d, J = 8.6 Hz, Ph-H), 5.35 (1H, d, J = 11.9 Hz, C$\underline{H}$CO), 4.41–4.35 (1H, m, C$\underline{H}$N), 3.90 (1H, t, J = 12 Hz, C$\underline{H}$Ph), 3.23 (1 H, d, J = 3.9 Hz), 2.03- 0.84 (12 H, m, aliphatic C-H); ^{13}C-NMR (CDCl$_3$, 100 MHz): δ = 189.08, 181.27, 147.16, 146.89, 146.55, 143.00, 139.84, 133.60, 129.53, 129.10, 129.04, 126.98, 126.86, 125.72, 123.92, 122.54, 112.06, 110.85, 71.90, 71.25, 65.46, 57.83, 53.73, 41.86, 37.38, 31.67, 28.38, 27.80, 24.73, 23.95; Anal. for C$_{32}$H$_{28}$ClN$_5$O$_4$; calcd: C, 66.03; H, 4.85; N, 12.03 Exper.: C, 66.10; H, 4.74; N, 12.11.

2'-(1*H*-benzo[*d*]imidazole-2-carbonyl)-5-chloro-1'-(3-nitrophenyl)-1',2',4a',5',6',7',8',8a', 9',9a'-decahydrospiro[indoline-3,3'-pyrrolo[1,2-*a*]indol]-2-one (7o).

Pale yellow solid; yield (52%); m.p.:135–137 °C; IR (KBr, cm^{-1}): 3438 (NH), 3257 (NH), 3092 (CH), 2930 (CH),1729 (CO), 1688 (CO); ^{1}H-NMR (CDCl$_3$, 400 MHz): δ 9.55 (1H, s, NH), 8.36 (1H, s, NH), 8.06 (1H, dd, J = 8.1, 2.3 Hz, Ph-H), 7.86 (2H, d, J = 8.2 Hz, Ph-H), 7.57 (1H, s, Ph-H), 7.47 (1H, t, J = 8 Hz, Ph-H), 7.31 (3H, d, J = 4.0 Hz, Ph-H), 7.14 (1H, d, J = 2.1 Hz, Ph-H), 6.98 (1H, dd, J = 8.3, 2.4 Hz, Ph-H), 6.33 (1H, d, J = 8.0 Hz, Ph-H), 5.31 (1H, d, J = 12.0 Hz, C$\underline{H}$CO), 4.46–4.36 (1H, m, C$\underline{H}$N), 3.92 (1H, t, J = 12.0 Hz, C$\underline{H}$Ph), 3.24 (1H, d, J = 4.3 Hz), 2.19–0.81 (12 H, m, aliphatic C-H); Anal. for C$_{32}$H$_{28}$ClN$_5$O$_4$; calcd: C, 66.03; H, 4.85; N, 12.03 Exper.: C, 66.09; H, 4.73; N, 12.10.

2.3. NCI Screening

The compounds have been processed according to the standard method NCI-60 Human Tumor Cell Lines Screen for the organic compound at the development therapeutic program (DTP) (see Supplementary Materials, Table S2; Figures S1 and S2).

2.4. Anticancer Activity Protocol

The anticancer activity protocol was carried out according to the method reported in [48]. "The cytotoxicity of tested compounds was investigated on a human normal lung fibroblast (Wi-38) cell line, triple-negative breast (MDA-MB 231) cells, and prostate cancer (PC3) cells. These cells were cultured in DMEM containing 10% fetal bovine serum. After cell seeding (10,000, 4000, and 5000 cells, respectively, per well) in 96-well cell culture plates and incubating for 24 h in a 5% CO$_2$ incubator, serial dilutions (2,4,6,8, and 10 μM) of the tested compounds were added. Following 48 h in a 5% CO$_2$ incubator, 20 μL of MTT (5 mg/mL) was added and incubated for 4 h, then this solution was removed and 100 μL of DMSO was added. The absorbance was measured at 590 nm (BMG LabTech, Ortenberg, Germany). The half-inhibitory growth concentration (IC$_{50}$) was calculated by GraphPad Prism software" [48].

2.5. MDM2 Binding Analysis by Microscale Thermophoresis (MST) Assay

The full protocol has been provided in SI and the binding curve is shown in the Supplementary Material (Figures S3–S6).

2.6. Methodology for Molecular Docking

Two-dimensional structures of compounds **7a, 7g, 7h**, and **7k** were drawn via the builder and subjected to preliminary structure preparation, namely energy minimization with the force field MMFF94x and the subsequent application of partial charges. The X-Ray crystal structure of MDM2 with PDB ID: 5LAZ, having a cocrystallized ligand with structural similarity to the studied compounds, was retrieved from the RCSB Protein Data Bank for the docking studies. Structure preparation of the protein was brought about by energy minimization with force field Amber10:EHT and partial charges were then applied to the protein. The cocrystallized ligand (6ST), after the necessary structure preparations, was used as a reference compound for validation of the results in docking studies of the aforementioned compounds [34]. Benchmarking of the docking protocols was performed well before the docking studies. Redocking of the 6ST ligand was brought about to observe the deviation of the ligand conformation from the original one (SI Figure S7). All the operations were performed in the molecular operating environment (MOE 2019.01) [49], which was chosen based on the RMSD value (0.14 Å) between the coordinates of the cognate ligand and the simulated pose. Induced fit docking was directed to the ligand atoms, brought about by placement of the ligand into the binding site of MDM2 utilizing the triangle matcher algorithm followed by determining the scores of the generated fifteen conformations through the London dG scoring function. Finally, five top-scored conformations were retained and evaluated by the GBVI/WSA dG scoring function. Thereafter, the same protocols were followed for the docking simulation of the studied compounds. The interaction patterns of the ligands with binding site residues were analyzed by the Protein-Ligand Interaction Profiler [50].

2.7. Statistical Analysis

The data are expressed as mean $\pm$ standard error of the mean (SEM) and values were considered significantly different at $p < 0.05$, using one-way analysis of variance (ANOVA) and Tukey's test (SPSS software version 16).

3. Results and Discussion

3.1. Chemistry

Based on the recently published idea of spiroxindole having the benzimidazole nucleus and showing promising results against cancer cell lines and an antimetastatic effect. To study the cytotoxicity and structure reactivity relationship, a new library **7a-o** has been synthesized and characterized (Scheme 1). Different electronic effects on the aromatic ring, including electron-donating and electron-withdrawing effects, also achieved a heterocycle aromatic ring and were explored. The synthetic methodology was carried out based on a multicomponent one-pot reaction *via* the [3+2] cycloaddition reaction approach [48]. The desired dipolarphiles were synthesized from orthophenylene diamine in a consequential step. Mixing the chalcones **4a-o** with the 5-chloroisatin, **5** and the key amino acid **6** in methanol under reflux for 2–3 h, afforded the final compounds in a high chemical yield and a regio- and diastero-selective manner. The stereochemistry for the final cycloadduct is matched with the previous lead compound published by our research group [48].
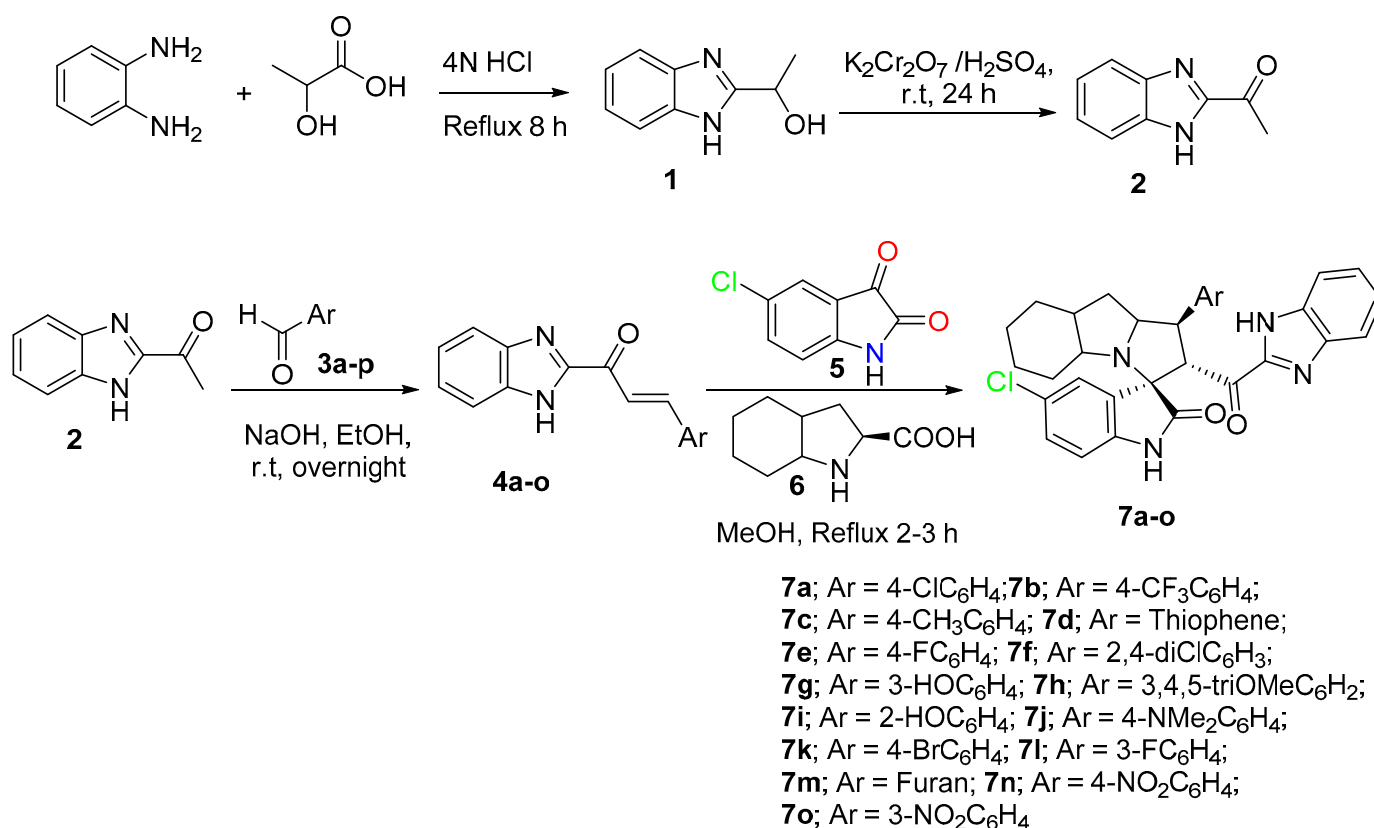
Scheme 1. Synthetic methodology for the desired spirooxindole derivative **7a-o**.

7a; Ar = 4-ClC$_6$H$_4$; **7b**; Ar = 4-CF$_3$C$_6$H$_4$;
7c; Ar = 4-CH$_3$C$_6$H$_4$; **7d**; Ar = Thiophene;
7e; Ar = 4-FC$_6$H$_4$; **7f**; Ar = 2,4-diClC$_6$H$_3$;
7g; Ar = 3-HOC$_6$H$_4$; **7h**; Ar = 3,4,5-triOMeC$_6$H$_2$;
7i; Ar = 2-HOC$_6$H$_4$; **7j**; Ar = 4-NMe$_2$C$_6$H$_4$;
7k; Ar = 4-BrC$_6$H$_4$; **7l**; Ar = 3-FC$_6$H$_4$;
7m; Ar = Furan; **7n**; Ar = 4-NO$_2$C$_6$H$_4$;
7o; Ar = 3-NO$_2$C$_6$H$_4$

3.2. In Vitro Anti-Cancer Activity Assays

3.2.1. NCI Screening (Development Therapeutic Program, DTP)

The successfully synthesized spirooxindoles (**7a-o**) were submitted for NCI for screening against 60 various cancer cell lines, classified into nine subpanels: breast, kidney, melanoma colon, prostate, CNS, ovary, melanoma, leukemia, and lung cancers. The initial single dose assay for the assessed spirooxindoles were tested at a 10 μM and the results were then expressed as a percentage of growth inhibition (GI%) (Table S2). As observed, the synthesized compounds inhibited the growth of the NCI cell-line panel according to the following order: breast > renal > leukemia cancer cell lines > other tested cancer cell lines (Table S2). The initial results afforded the most active compound, **7g**, which entered the five dose assays, and the results are shown in Supporting Materials (Figures S1 and S2).

3.2.2. MTT Assay

In order to determine the IC$_{50}$ (μM) of the synthesized spirooxindoles, **7a-o** were subjected to an MTT assay in vitro against the two-cancer cell line MDA-MB 231 and PC3 cells, and the data reported in Table 1. For the breast cancer line (MDA-MB 231) the compounds are shown in IC$_{50}$ (μM) in the range between 3.797–6.879 μM; the most active candidate between the series was compound **7d** with IC$_{50}$ = 3.797 ± 0.205 μM, the chemical structure compromises a thiophene ring. On the other hand, the least reactivity was compound **7n** with IC$_{50}$ = 6.879 ± 0.308 μM. All other compounds are shown in the range of 4 μM reactivity. In the case of prostate cancer (PC3), the reactivity of the synthesized compounds exhibited the range of IC$_{50}$ = 4.252 to 7.567 μM. In the case of compound **7a** with the Cl-atom in the fourth position showed IC$_{50}$ = 4.763 ± 0.069 and 4.574 ± 0.011 μM, for both tested two-cancer cells MDA-MB 231, and PC3, respectively. The reactivity slightly improved compared with compound 7a when the CF$_3$-group was introduced into the aromatic ring, as indicated in compound **7b** which showed IC$_{50}$ = 4.284 ± 0.007 and 4.404 ± 0.008 μM; 7e contain the *p*-fluoro atom on the aromatic ring compared

with **7l** having the *m*-fluoro atom on the aromatic, where no differences were observed in the cytotoxicity. In the isosteric analog of the compound **7d**, which replaced the thiophene with furan heterocycle, as shown in compound **7m**, the cytotoxicity dropped to IC_{50} = 6.039 ± 0.111 and 5.098 ± 0.119 μM with less than 1.6 and 1.18 times, compared to the compound **7d**. Introducing the electron-withdrawing effect of the NO_2 group either in the *para-* or *meta*-position, we observed that NO_2 in the *meta* (compound **7o**) was more active than the *para*-position (compound **7n**) with 1.66 and 1.77 folds. The existence of electron donating groups such as methyl group (compound **7c**); hydroxyl group (compound **7g** or compound **7c**); trimethoxy groups (compound **7h**); and dimethyl amine (compound **7j**) did not alter the reactivity.

Table 1. The estimated IC_{50} (μM) of **7a-o** on Wi-38 viability, the growth of MDA-MB 231, and PC3 cells.
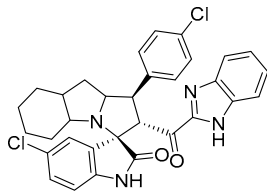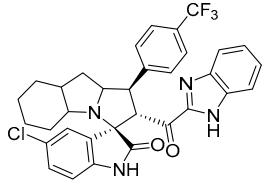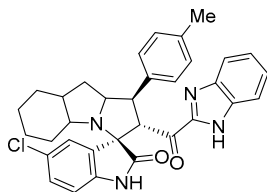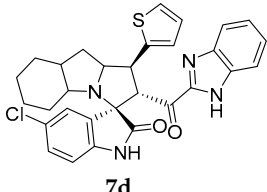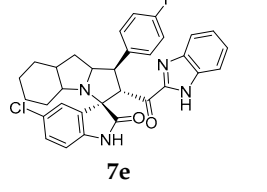
Chemical Structure	Wi-38	MDA-MB 231	PC3
	IC_{50} (μM)		
7a	5.178 ± 0.333	4.763 ± 0.069	4.574 ± 0.011
7b	5.157 ± 0.039	4.284 ± 0.007	4.404 ± 0.008
7c	4.466 ± 0.088	4.221 ± 0.070	4.504 ± 0.059
7d	4.325 ± 0.062	3.797 ± 0.205	4.314 ± 0.036
7e	4.024 ± 0.011	4.143 ± 0.164	4.252 ± 0.128

Table 1. *Cont.*
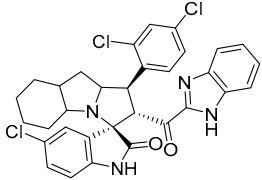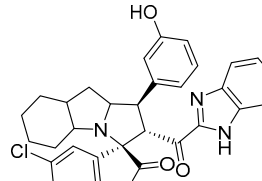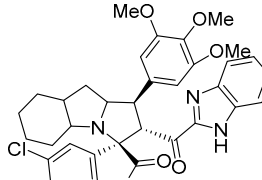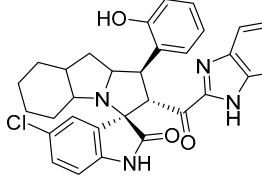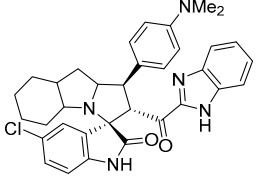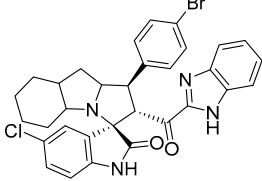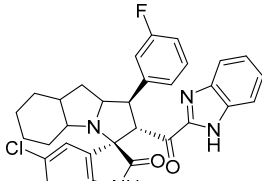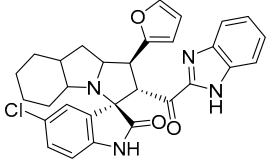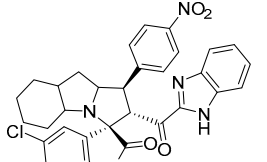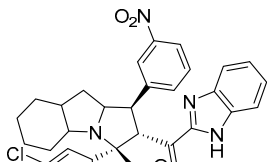
Chemical Structure	Wi-38	MDA-MB 231	PC3
	IC$_{50}$ (μM)		
7f	5.084 ± 0.152	4.274 ± 0.167	4.388 ± 0.008
7g	5.120 ± 0.115	4.329 ± 0.163	4.294 ± 0.023
7h	4.938 ± 0.161	4.261 ± 0.095	4.241 ± 0.045
7i	4.854 ± 0.009	4.371 ± 0.174	4.495 ± 0.034
7j	4.267 ± 0.153	4.261 ± 0.079	4.420 ± 0.008
7k	4.316 ± 0.007	4.479 ± 0.313	4.539 ± 0.094
7l	4.401 ± 0.092	4.156 ± 0.135	4.360 ± 0.208

Table 1. *Cont.*

Chemical Structure	Wi-38	MDA-MB 231	PC3
	IC$_{50}$ (µM)		
7m	4.698 ± 0.129	6.039 ± 0.111	5.098 ± 0.119
7n	3.959 ± 0.046	6.879 ± 0.308	7.567 ± 0.709
7o	5.187 ± 0.089	4.138 ± 0.186	4.275 ± 0.005

All values are presented as mean ± SEM.

3.2.3. Microscale Thermophoresis Assay (MST) for MDM2 Binding Detection

Compounds **7a**, **7g**, **7h**, and **7k** shown very good anti-cancer potential as well as high safety profiles as obtained by the MTT assay. Accordingly, the compound **7a, 7g, 7h**, and **7k** were then tested for MDM2 binding analysis. The tested compound was incubated with a fluorescently labeled MDM2 at increasing concentrations (0.763 nM to 25 µM; Solubility play a crucial role to reach maximum concentration). MST binding curves showed that spirooxindole-based benzimidazole **7a**, **7g**, and **7h** showed moderate binding affinity in the range of (K_D; 2.38–38 µM). Compound **7a** showed better binding reactivity compared to our previous spirooxindole-based benzimidazole, which has been shown (K_D; 7.94 µM) [48]. The **7k** did not show any binding detection (Table 2).

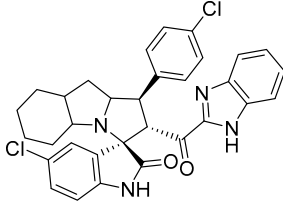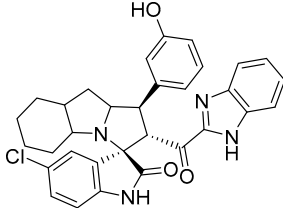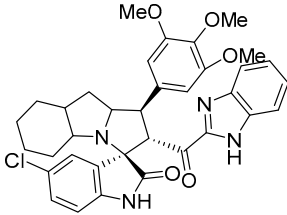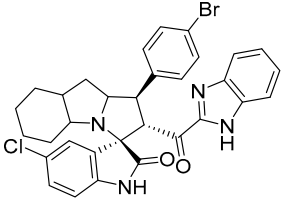**Table 2.** MST binding assay results of MDM2.

#	Code	Chemical Structures	KD (µM)
1	**7a**		2.38
2	**7g**		38

Table 2. *Cont.*

#	Code	Chemical Structures	KD (μM)
3	**7h**		10.6
4	**7k**		No binding detected

3.3. Molecular Docking of the Studied Compound

Results of the docking simulations of the spirooxindoles molecules were validated relative to the reference compound (6ST), which was firmly held into the binding site by a diverse network of interactions. The nitrogen of the imidazole ring of His96 and oxygen in the carboxylic acid moiety of Leu54 accepted hydrogens from the N-H of indolinone and the N-H of pyrrolidine rings, resulting in hydrogen bonds with 1.99 Å and 2.16 Å lengths, respectively. Additionally, His96 had π-stacking and halogen bonding with the chloroindolinone moiety. There were formed salt bridges by the carboxylate group of this compound with His73 and Lys94. This binding was further enhanced by hydrophobic interactions with seven amino acid residues.

Compound **7a** developed a network of interactions similar to that of the reference compound. A hydrogen bond was established with the ligand at a distance of 2.08 Å, where hydrogen was donated by the N-H of benzimidazole to Leu54. Furthermore, His96 formed π-stacking and halogen bonding with the chlorobenzene ring the same way it was created with the reference compound. Similarly, hydrophobic interactions formed by seven residues well accommodated this compound into the binding site. In contrast to **7a**, compound **7g** formed two hydrogen bonds. One of the hydrogen bonds between benzimidazole moiety and Val93 was of 3.02 Å length, and the other bond resulted between the amino group of Lys96, being the donor, and phenolic group of the ligand was accepting at 2.59 Å distance. His96 provided π-stacking with the aromatic rings of benzimidazole moiety. However, fewer hydrophobic interactions were observed for this compound as compared to **7a**. In the case of **7h**, only one hydrogen bond was formed, which was between the amino group of Lys94 and the central methoxy group of the trimethoxybenzene substituent in the compound. The benzimidazole of this compound was anchored by π-stacking, twice with His96 and once with Tyr100. Three hydrophobic interactions were also developed. For compound **7k**, the observed interactions included a halogen bond between its bromobenzene substituent and His96, and hydrophobic interactions with six residues of the MDM2 binding site.

The docked poses of the studied compounds are presented in Figure 2, and Table 3 enlists all the observed interactions, the interacting groups, and docking scores.
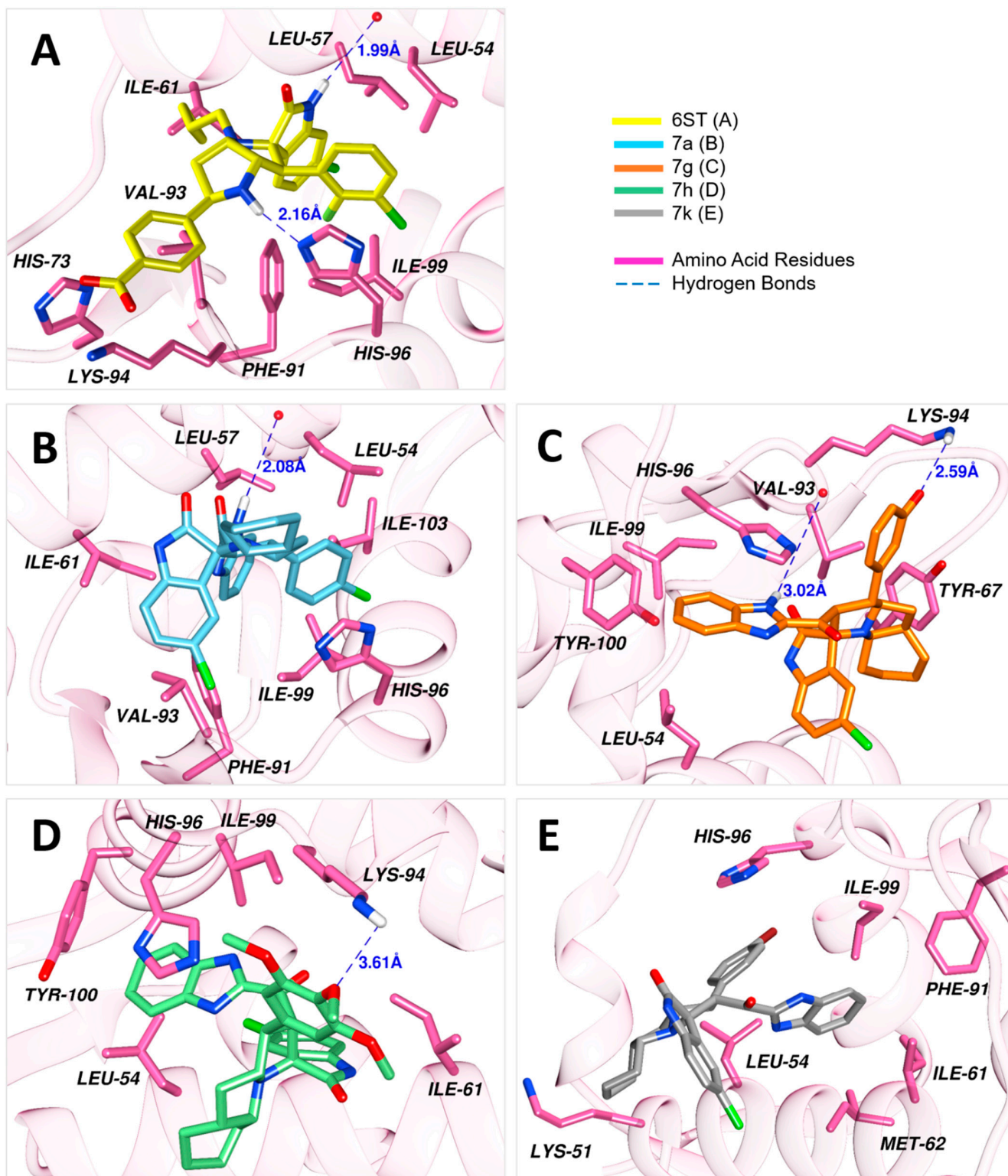
Figure 2. Docked conformations of the ligands into the binding site of MDM2 (PDB ID: 5LAZ).

Table 3. Docking scores and network of interactions formed between the ligands and residues of MDM2.

Cpd. ID	S (kcal/mol)	Hydrogen Bonding			Hydrophobic Interactions	Other Interactions
		Residues	Ligand Residues	Distance (Å)		
7a	−7.05	Leu54	N-H of benzmidazole	2.08	Leu54, Leu57, Ile61, Phe91, Val93, Ile99, Ile103	π-stacking, Halogen bonding with His96
7g	−7.09	Val93	N-H of benzmidazole	3.02	Leu54, Tyr67, Ile99, Tye100	π-stacking with His96
		Lys94	O of phenol ring	2.59		
7h	−7.43	Lys94	O of methoxy	3.29	Leu54, Ile61, Ile99	π-stacking with His96, Tyr100
7k	−7.29	-	-	-	Lys51, Leu54, Leu57, Ile61, Phe91, Ile99	halogen bonding with His96
6ST	−8.97	Leu54	N-H of indolinone	1.99	Leu54, Leu57, Ile61, Phe91, Val93, Lys94, Ile99	π-stacking, Halogen bonding with His96; salt bridge with His73, Lys94
		His96	N-H of pyrrolidine	2.16		

4. Conclusions

New spiroxindoles, combined with a benzimidazole scaffold, were synthesized, characterized, and identified as an MDM2 inhibitor. The requisite spiroxindoles were successfully achieved *via* the [3+2] cycloaddition reaction approach, which separated in an excellent regioselective and diastereoselective manner. The separated spirooxindoles showed promising results against cancer cells including MDA-MB 231 and PC3 in microscale reactivity. The anticancer reactivity for compound **7d** showed potential activity with IC_{50} = 3.797±0.205 μM and was recognized as the most active candidate in the series. MDM2 binding analysis showed that compound **7a** could be inhibited by the MDM2 with K_D = 2.38 μM. This finding could be of possible use for cancer research development in the future.

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