Synthesis and anticancer activity of novel 5-(indole-2-yl)-3-substituted 1,2,4-oxadiazoles

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ABSTRACT: A new series of 5-(indole-2-yl)-3-substituted 1,2,4-oxadiazoles were synthesized and evaluated for their anticancer activities. Structures of the compounds were confirmed by spectroscopic methods. Structural modifications were done to improve the antiproliferative activity of compound 1. The results indicated that a benzylxoyl substituent on the C-4 position was better than a methyl substituent on the C-6 position of the indole component. Compounds 10a, 10b, 10g, 10i, 10l, 10n, 10o, and 10p were found to be more active than lead compound 1 in the PC-3 cell line. These compounds may serve as lead candidates in the development of novel chemotherapeutics for cancer treatment.

Keywords: Indole, 1,2,4-oxadiazole, anticancer

1. Introduction

Cancer has become the second largest cause of death in many countries (1). Inhibition of apoptotic pathways is known as an important hallmark for cancer (2,3). In cancer cells, blockage of apoptosis could lead to excessive cell proliferation as well as resistance to cancer treatment. Apoptosis is caused by the activation of intracellular caspases. There are two main pathways triggering activation of caspases, referred to as the intrinsic and extrinsic pathways. Numerous cellular targets of these two pathways have been identified, such as caspases (caspase-2, -3, -6~10, and -12), B-cell lymphoma-2 (Bcl-2) family proteins (Bcl-2, Bcl-XL, Bcl-W, Bcl-B, Mcl-1, and Bfl-1), and tumor necrosis factor (TNF) family death receptors (4,5). It has been recently reported that several anticancer agents exhibited apoptosis-inducing ability, such as imatinib, sorafenib, and lapatinib (6-10). Therefore, the identification of apoptosis inducers becomes an attractive approach for discovery and development of potential anticancer agents. Pro-apoptotic agents affected more than one target, making optimizations through structure-activity relationships (SAR) studies difficult (2,11,12). For multiple actions of pro-apoptotic agents, screening of these agents was often based on cell line evaluation (11,12).

In recent years, many compounds with diverse structures were identified as pro-apoptotic agents (3,10-21). Compound 1 (Table 1) which might activate caspase-3/7 was reported as a novel 5-(indole-2-yl)-3-substituted 1,2,4-oxadiazole as a pro-apoptotic agent with anticancer activity at micromole concentrations (12). The effects of substitution on the C-6 position of the indole ring system have been studied (12). In order to study the SAR on other positions of the indole ring system, a series of 5-(indole-2-yl)-3-substituted 1,2,4-oxadiazoles were designed and synthesized. Their anticancer activities were tested in this paper.

2. Materials and Methods

2.1. Chemicals

The synthesis of intermediates 5b, 8, and 9 are shown in Scheme 1. Starting with 5-substituted salicylaldehyde (2), 5-substituted-2-benzylxoybenzaldehydes (3) were prepared by benzyl chloride in DMF catalyzed by K2CO3 (22,23). Intermediates 4 were prepared by a condensation reaction between 3 and methyl 2-azidoacetate. Heating of intermediates 4 in p-xylene to reflux for 1 h generated compounds 5 (22). Compound 6 was easily prepared from 5a by a hydrolysis reaction. Compound 6 reacted with sodium methoxide in DMF/CH3OH catalyzed by cuprous iodide afforded compound 7. Compound 8 was synthesized by the methylate reaction from 7 using dimethyl sulfate. The preparation of compound 9 was a methylate reaction from 5a.

The synthesis of 5-(indole-2-yl)-3-substituted 1,2,4-oxadiazoles are shown in Scheme 2. The targeting compounds were synthesized using a one-pot reaction between amidoximes (11a-11d) and substituted indoles (5b, 8, 9, 12, and 13) under microwave conditions. Compounds 12 and 13 were commercially available.
Table 1. The cells growth inhibitory activities of target compounds

<table>
<thead>
<tr>
<th>Compounds</th>
<th>R^1</th>
<th>R^2</th>
<th>R^3</th>
<th>IC_{50} (μM)</th>
<th>PC-3</th>
<th>MCF-7</th>
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<td>4-OCH_2Ph-7-OCH_3</td>
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<td>Ph</td>
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Scheme 1. Reagents and conditions. (a) K_2CO_3, DMF, N_2, 50°C, 10 h; (b) methyl 2-azidoacetate, CH_3ONa, 50% THF/CH_3OH, N_2, −17~0°C, 4 h; (c) p-xylene, reflux, 1 h; (d) KOH, THF/H_2O, reflux, 4 h; (e) NaH, CH_3OH, DMF, CuI, 120°C, 4 h; (f) (CH_3O)_2SO_2, DMF, NaH, 0°C ~ room temperature.

Scheme 2. Reagents and conditions (DMF, Cs_2CO_3, microwave 800 W, 150°C, 10 min).
2.2. Cell lines

Human prostate cancer cells (PC-3) and human breast adenocarcinoma cells (MCF-7) were maintained in RPMI 1640 medium (HyClone®, Thermo Fisher Scientific Inc., Waltham, MA, USA). The medium was supplemented with 10% fetal bovine serum (FBS) (Gibco®, Invitrogen, Carlsbad, CA, USA), 100 μg/mL penicillin and 100 μg/mL streptomycin. Cells were cultured in a humidified atmosphere of 5% CO₂ at 37°C.

2.3. MTT assay

Cells were seeded into 96-well culture plates at a density of 5 × 10⁴ cells per well and cultured for 12 h. Thereafter the cells were treated with various concentrations of tested compounds and incubated for 72 h. Five mg/mL MTT solution was added to each well and the cells were incubated at 37°C for 4 h. The resulting crystals were extracted with dimethyl sulfoxide (DMSO) for 15 min. The optical density (OD) was measured using a plate microreader (Bio-Rad 680, Bio-Rad Co., Hercules, USA).

3. Results and Discussion

The in vitro antitumour effect of compounds 10a–10p was assessed against prostate cancer cells (PC-3) and human breast adenocarcinoma cells (MCF-7). Antiproliferative data was compared with the previously reported pro-apoptotic compound 1 as control. All inhibition results are shown in Table 1. The PC-3 cell line was more responsive to 5-(indole-2-yl)-3-substituted 1,2,4-oxadiazoles than the MCF-7 cell line.

First we studied the single substituted indoles (R¹, compounds 10j–10p). When comparing 10n to 10k, 10o to 10l, we found that compounds (10n and 10o) with a benzyloxyl group on the C-4 position showed more potent inhibitory activities than compounds (10k and 10l) with a methyl group on the C-6 position (IC₅₀ values of 21.41 and 10.52 μM for 10n and 10o, > 80 and 16.65 μM for 10k and 10l). This result showed that introduction of a benzyloxyl group on the C-4 position of the indole ring was better than a methyl group on the C-6 position.

Keeping the benzyloxyl group on the C-4 position, we introduced different substitutions on the N-1 and/or C-7 position of the indole ring leading to compounds 10a–10l. Compound 10b showed the highest inhibitory activity among these compounds (IC₅₀ = 8.96 μM), and was up to six times more potent than lead compound 1. Introduction of a chlorine on the 4-position of benzene ring (R²) in compound 10b led to compound 10c, and the activity was decreased dramatically (IC₅₀ > 50 μM). Besides, compounds 10a, 10g, 10i, 10l, 10n, 10o, and 10p were found to be more active than lead compound 1 in the PC-3 cell line. They showed no activities against the MCF-7 cell line (IC₅₀ > 80 μM).

4. Conclusion

In conclusion, a new series of 5-((indole-2-yl)-3)-substituted 1,2,4-oxadiazoles were synthesized with cell inhibitory activities. Several compounds showed more potent activities than lead compound 1. Compounds with benzyloxyl substituent on the C-4 position had greater antiproliferative activity than those with methyl on the C-6 position of the indole component. Compounds with both C-4 benzyloxyl and C-7 methylloxyl substituents were more efficient than those with C-4 benzyloxyl and C-7 bromine. We successfully identified compound 10b as the most active compound. Further studies based on this structure will be continued.

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References

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Appendix

The proton nuclear magnetic resonance (1H-NMR and 13C-NMR) spectra were recorded with a Bruker Avance DRX600 instrument with tetramethylsilane (TMS) as the internal standard at 600 MHz. The chemical shifts (δ) were reported in parts per million (ppm) and were relative to the central peak of the solvent, which was DMSO-d6 or CDCl3. Mass spectra (MS) were measured with an API 4000 and the high resolution mass spectra data were obtained using an Accela UPLC-LTQ Orbitrap mass spectrometer. All melting points were determined in a BUCHI capillary melting point apparatus and are uncorrected. Microwave syntheses were carried out in an XH-100A Xiang Hu instrument with focused microwave heating (microwave power supply 0-1,000 W, open vessel mode). Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Column chromatography was carried out with silica gel using the solvents indicated. Thin-layer chromatography (TLC) was performed on silica gel GF254 plates (layer thickness, 0.2 mm), and compounds were visualized using UV light. Petroleum ether used for TLC and column chromatography had a boiling range of 60-90°C.

Synthesis of compounds 3a and 3b

To a mixture of substituted salicylaldehyde (2) (0.1 mol) and K2CO3 (20.7 g, 0.15 mol) in DMF (100 mL), benzyl chloride (19.0 g, 0.15 mol) was added dropwise. Then the mixture was heated to 60°C for 8 h. After being allowed to cool to r.t., the mixture was poured into ice-water (1,000 mL). The precipitate was filtered, washed several times with water, and further purified by recrystallization in ethanol to afford 3.

2-(Benzylxoxy)-5-bromobenzaldehyde (3a) Yield 26.8 g (92%); colorless crystal; mp 72.4-73.6°C. 1H-NMR (600 MHz, DMSO-d6): δ = 5.30 (s, 2H), 7.32 (d, J = 8.4 Hz, 1H), 7.35 (t, J = 7.2 Hz, 1H), 7.42 (t, J = 7.8 Hz, 2H), 7.52 (d, J = 7.2 Hz, 2H), 7.76 (d, J = 2.4 Hz, 1H), 8.1 (dd, J = 9.0 Hz and J = 3.0 Hz, 1H), 10.33 (s, 1H). MS (ESI): m/z = 291.4 [M + H]+.

2-(Benzylxoxy)-5-chlorobenzaldehyde (3b) Yield 22.2 g (90%); colorless crystal; mp 81.0-81.9°C. 1H-NMR (600 MHz, DMSO-d6): δ = 5.31 (s, 2H), 7.35 (t, J = 7.2 Hz, 1H), 7.38 (d, J = 8.4 Hz, 1H), 7.42 (t, J = 7.2 Hz, 2H), 7.51 (d, J = 7.2 Hz, 2H), 7.65 (d, J = 2.4 Hz, 1H), 7.71 (dd, J = 8.4 Hz and J = 2.4 Hz, 1H), 10.35 (s, 1H). MS (ESI): m/z = 247.4 [M + H]+.

Synthesis of compounds 4a and 4b

To a cooled (−20°C) solution of 3 (0.05 mol) and methyl 2-azidoacetate (23.0 g, 0.2 mol) in anhydrous CH3OH (75 mL) and THF (45 mL) under a N2 atmosphere, was added a solution of CH3ONa (10.8 g,
0.2 mol) in anhydrous CH$_2$OH (30 mL) dropwise over 1 h, maintaining the temperature below −17°C. After 4 h, the mixture was placed in an ice-bath overnight. Cooled CH$_2$OH (50 mL) was added into the mixture and then the precipitate was filtered, and washed twice with CH$_2$OH to get compounds 4.

(Z)-Methyl 2-azido-3-(2-(benzyloxy)-5-bromophenyl)acrylate (4a) Yellow solid, yield 70%, mp 88.9-90.1°C. $^1$H-NMR (600 MHz, DMSO-d$_6$): $\delta$ = 8.25 (s, 1H), 7.51 (dd, $J$ = 9.0 Hz and $J$ = 2.4 Hz, 1H), 7.39-7.43 (m, 4H), 7.33-7.36 (m, 1H), 7.20 (s, 1H), 7.11 (d, $J$ = 9.0 Hz, 1H), 5.21 (s, 2H), 3.84 (s, 3H). MS (ESI): $m/z$ = 388.2 [M + H]$^+$.

(Z)-Methyl 2-azido-3-(2-(benzyloxy)-5-chlorophenyl)acrylate (4b) Yellow solid, yield 70%, mp 89.2-91.2°C. $^1$H-NMR (600 MHz, DMSO-d$_6$): $\delta$ = 8.23 (s, 1H), 7.52 (dd, $J$ = 9.0 Hz and $J$ = 2.4 Hz, 1H), 7.36-7.45 (m, 4H), 7.33-7.36 (m, 1H), 7.22 (s, 1H), 7.15 (d, $J$ = 9.0 Hz, 1H), 5.18 (s, 2H), 3.86 (s, 3H). MS (ESI): $m/z$ = 344.1 [M + H]$^+$.

Synthesis of compounds 5a and 5b
Comounds 4 were suspended in p-xylene (400 mL). The mixture was heated to 150°C for 4 h. After being allowed to cool to r.t., the crude product precipitated was collected by filtration, and further purified by recrystallization in ethyl acetate/petroleum ether to afford 5.

Methyl 4-(benzoyl氧)-7-bromo-1H-indole-2-carboxylate (5a) Yield 68%; colorless crystal; mp 148.7-150.4°C. $^1$H-NMR (600 MHz, DMSO-d$_6$): $\delta$ = 3.87 (s, 3H), 5.25 (s, 2H), 6.65 (d, $J$ = 7.8 Hz, 1H), 7.27 (s, 1H), 7.34 (t, $J$ = 7.8 Hz, 1H), 7.38-7.42 (m, 3H), 7.51 (d, $J$ = 7.2 Hz, 2H), 11.98 (s, 1H). MS (ESI): $m/z$ = 360.3 [M + H]$^+$.

Methyl 4-(benzoyl氧)-7-chloro-1H-indole-2-carboxylate (5b) Yield 65%; colorless crystal; mp 179.9-180.9°C. $^1$H-NMR (600 MHz, DMSO-d$_6$): $\delta$ = 3.87 (s, 3H), 5.25 (s, 2H), 6.67 (d, $J$ = 7.8 Hz, 1H), 7.23-7.26 (m, 2H), 7.34 (t, $J$ = 7.8 Hz, 1H), 7.41 (t, $J$ = 7.8 Hz, 2H), 11.21 (s, 1H). MS (ESI): $m/z$ = 316.3 [M + H]$^+$.

4-(Benzyloxy)-7-bromo-1H-indole-2-carboxylic acid (6)
A solution of 5a (3.6 g, 10 mmol), KOH (2.24 g, 40 mmol) in THF (40 mL) and water (15 mL) was heated to reflux for 4 h. After cooling to r.t., the THF was evaporated under reduced pressure. The mixture was poured into water and acidified with aq HCl (6N) to pH 1-2. The precipitate was filtered, washed several times with water, and further purified by recrystallization in ethyl acetate/petroleum ether to afford 6. Yield 3.29 g (95%); white powder; mp 248.6-249.8°C. $^1$H-NMR (600 MHz, DMSO-d$_6$): $\delta$ = 5.25 (s, 2H), 6.63 (d, $J$ = 7.8 Hz, 1H), 7.20 (d, $J$ = 2.4 Hz, 1H), 7.34 (t, $J$ = 7.8 Hz, 1H), 7.36 (d, $J$ = 8.4 Hz, 1H), 7.41 (t, $J$ = 7.8 Hz, 2H), 7.51 (d, $J$ = 7.8 Hz, 2H), 11.75 (s, 1H), 13.05 (br s, 1H). MS (ESI): $m/z$ = 346.3 [M + H]$^+$.

4-(Benzyloxy)-7-methoxy-1H-indole-2-carboxylic acid (7)
A mixture of 6 (3.46 g, 10 mmol), CuI (1.9 g, 10 mmol), CH$_3$ONa (3.8 g, 70 mmol) in anhydrous CH$_2$OH (20 mL) and DMF (40 mL) under N$_2$ atmosphere was heated and refluxed for 5 h. After cooling to r.t., the mixture was poured into water (500 mL) and acidified with aq HCl (6N) to pH 1-2. The precipitate was filtered, washed several times with water, and further purified by recrystallization in ethyl acetate/petroleum ether to afford 7. Yield 80%, mp 235.2-236.5°C. $^1$H-NMR (600 MHz, DMSO-d$_6$): $\delta$ = 3.85 (s, 3H), 5.23 (s, 2H), 6.59 (d, $J$ = 7.8 Hz, 1H), 7.22 (d, $J$ = 2.4 Hz, 1H), 7.34 (t, $J$ = 7.8 Hz, 1H), 7.38 (d, $J$ = 8.4 Hz, 1H), 7.42 (t, $J$ = 7.8 Hz, 2H), 7.50 (d, $J$ = 7.8 Hz, 2H), 7.85 (d, $J$ = 6.6 Hz, 2H). MS (ESI): $m/z$ = 298.4 [M + H]$^+$.

Methyl 4-(benzoyl氧)-7-methoxy-1-methyl-1H-indole-2-carboxylate (8)
Compound 7 was dissolved in anhydrous DMF. NaH (0.36 g, 15 mmol) was added to the solution at 0°C followed by dimethyl sulfate (1.89 g, 15 mmol). The mixture was stirred at r.t. for 4 h, and then was poured into ice-cold water. The precipitate was filtered, washed several times with water, and further purified by recrystallization in ethyl acetate/petroleum ether to afford 8. Yield 2.09 g (64%); colorless crystal; mp 100.1-101.8°C. $^1$H-NMR (600 MHz, DMSO-d$_6$): $\delta$ = 3.82 (s, 3H), 3.85 (s, 3H), 4.26 (s, 3H), 5.16 (s, 2H), 6.51 (d, $J$ = 7.8 Hz, 1H), 6.69 (d, $J$ = 9.0 Hz, 1H), 7.21 (s, 1H), 7.33 (t, $J$ = 7.8 Hz, 1H), 7.40 (t, $J$ = 7.8 Hz, 2H), 7.49 (d, $J$ = 7.2 Hz, 2H). MS (ESI): $m/z$ = 326.3 [M + H]$^+$.

Methyl 4-(benzoyl氧)-7-bromo-1-methyl-1H-indole-2-carboxylate (9)
The procedure was the same as compound 7 to 8. Yield 3.39 g (91%); colorless crystal; mp 108.6-109.9°C. $^1$H-NMR (600 MHz, DMSO-d$_6$): $\delta$ = 3.84 (s, 3H), 4.35 (s, 3H), 5.24 (s, 2H), 6.65 (d, $J$ = 8.4 Hz, 1H), 7.27 (s, 1H), 7.35 (t, $J$ = 7.2 Hz, 1H), 7.41 (t, $J$ = 7.2 Hz, 2H), 7.44 (d, $J$ = 9.0 Hz, 1H), 7.50 (d, $J$ = 6.6 Hz, 2H). MS (ESI): $m/z$ = 374.3 [M + H]$^+$.

General procedure for synthesis of 10a–10p under microwave irradiation
Substituted indole-based carboxylic acid esters (5b, 8, 9, 12, and 13) (1 mmol), amidoximes (2 mmol) and cesium carbonate (0.65 g, 2 mmol) in DMF (5 mL) were placed in a 10 mL flask followed by microwave
irradiation (300 W, 150°C) for the desired time. After cooling to r.t., the mixture was poured into 100 mL cold water, and then extracted with ethyl acetate. The combined organic solvent was dried over Na$_2$SO$_4$, filtered, and concentrated in vacuum. The crude product was purified by silica-gel chromatography with petroleum ether-ethyl acetate (15:1) to give the desired compounds 10a–10p.

5-(4-Benzoyloxy)-7-methoxy-1-methyl-1H-indol-2-yl)-3-phenyl-1,2,4-oxadiazole (10a) Yield 87%; white powder, mp 145.8-147.6°C. $^1$H-NMR (600 MHz, DMSO-d$_6$): $\delta$ = 2.44 (s, 3H), 3.87 (s, 3H), 4.38 (s, 3H), 5.20 (s, 2H), 6.56 (d, $J$ = 8.4 Hz, 1H), 6.73 (d, $J$ = 8.4 Hz, 1H), 7.34 (t, $J$ = 7.8 Hz, 1H), 7.38 (s, 1H), 7.41 (t, $J$ = 7.8 Hz, 2H). HRMS-ESI: m/z [M + H]$^+$ calcd for C$_{25}$H$_{24}$N$_3$O$_3$: 412.1656; found 412.1654.

3-Methyl-5-(6-methyl-1H-indol-2-yl)-1,2,4-oxadiazole (10b) Yield 81%, white acicular crystal; mp 179.3-180.7°C. $^1$H-NMR (600 MHz, DMSO-d$_6$): $\delta$ = 3.89 (s, 3H), 4.48 (s, 3H), 5.22 (s, 2H), 6.59 (d, $J$ = 8.4 Hz, 1H), 6.76 (d, $J$ = 8.4 Hz, 1H), 7.34 (t, $J$ = 7.8 Hz, 1H), 7.42 (t, $J$ = 7.8 Hz, 2H), 7.48 (s, 1H), 7.53 (d, $J$ = 7.8 Hz, 2H), 7.61-7.65 (m, 3H), 8.13 (d, $J$ = 6.6 Hz, 2H). HRMS-ESI: m/z [M + H]$^+$ calcd for C$_{25}$H$_{23}$N$_3$O$_3$: 412.1656; found 412.1654.

5-(4-Benzoyloxy)-7-methoxy-1-methyl-1H-indol-2-yl)-3-phenyl-1,2,4-oxadiazole (10b) Yield 81%; white powder, mp 195.0-196.4°C. $^1$H-NMR (600 MHz, DMSO-d$_6$): $\delta$ = 5.30 (s, 2H), 6.74, (d, $J$ = 7.8 Hz, 1H), 7.32 (d, $J$ = 7.8 Hz, 1H), 7.36 (t, $J$ = 7.8 Hz, 1H), 7.43 (t, $J$ = 7.8 Hz, 2H), 7.54-7.56 (m, 3H), 7.70 (d, $J$ = 8.4 Hz, 2H), 8.12 (d, $J$ = 8.4 Hz, 2H), 12.82 (s, 1H). HRMS-ESI: m/z [M + H]$^+$ calcd for C$_{24}$H$_{20}$ClN$_3$O$_3$: 402.1004; found 402.1003.

5-(4-Benzoyloxy)-7-chloro-1H-indol-2-yl)-3-(4-chlorophenyl)-1,2,4-oxadiazole (10i) Yield 60%; white powder; mp 229.4-230.4°C. $^1$H-NMR (600 MHz, DMSO-d$_6$): $\delta$ = 5.29 (s, 2H), 6.74 (d, $J$ = 7.8 Hz, 1H), 7.32 (d, $J$ = 8.4 Hz, 1H), 7.36 (t, $J$ = 7.8 Hz, 1H), 7.43 (t, $J$ = 7.8 Hz, 2H), 7.54-7.56 (m, 3H), 7.70 (d, $J$ = 8.4 Hz, 2H), 12.83 (s, 1H). HRMS-ESI: m/z [M + H]$^+$ calcd for C$_{24}$H$_{19}$ClN$_3$O$_3$: 436.0614; found 436.0609.

3-Methyl-5-(6-methyl-1H-indol-2-yl)-1,2,4-oxadiazole (10j) Yield 89%; white acicular crystal; mp 147.5-149.4°C. $^1$H-NMR (600 MHz, DMSO-d$_6$): $\delta$ = 2.41 (s, 2H), 2.42 (s, 3H), 6.96 (dd, $J$ = 8.4 Hz and 1.8 Hz, 1H), 7.26 (s, 1H), 7.30 (d, $J$ = 1.8 Hz, 1H), 7.58 (d, $J$ = 8.4 Hz, 1H), 12.23 (s, 1H). HRMS-ESI: m/z [M + H]$^+$ calcd for C$_{16}$H$_{14}$N$_3$O: 234.0975; found 234.0973.

5-(6-Methyl-1H-indol-2-yl)-3-phenyl-1,2,4-oxadiazole (10k) Yield 68%; white powder; mp 215.0-216.8°C. $^1$H-NMR (600 MHz, DMSO-d$_6$): $\delta$ = 2.43 (s, 3H), 6.98 (dd, $J$ = 8.4 Hz and 1.7 Hz, 1H), 7.32 (s, 1H), 7.42 (dd, $J$ = 2.4 Hz and 0.6 Hz, 1H), 7.60-7.65 (m, 4H), 8.10-8.12 (m, 2H), 12.33 (s, 1H). HRMS-ESI: m/z [M + H]$^+$ calcd for C$_{24}$H$_{20}$N$_3$O$_3$: 310.0742; found 310.0743.

5-(4-Benzoyloxy)-7-chloro-1H-indol-2-yl)-3-methyl-1,2,4-oxadiazole (10m) Yield 71%; white powder; mp 170.9-172.5°C. $^1$H-NMR (600 MHz, DMSO-d$_6$): $\delta$ = 2.42 (s, 3H), 5.27 (s, 2H), 6.68 (d, $J$ = 7.8 Hz, 1H), 7.07 (d, $J$ = 7.8 Hz, 1H), 7.20 (t, $J$ = 7.8 Hz, 1H), 7.34 (t, $J$ = 7.8 Hz, 2H), 7.41 (t, $J$ = 7.8 Hz, 2H), 7.53 (d, $J$ = 7.8 Hz, 2H).
5-(4-(Benzyloxy)-1H-indol-2-yl)-3-phenyl-1,2,4-oxadiazole (10n) Yield 73%; white powder; mp 177.2-179.1°C. 1H-NMR (600 MHz, DMSO-d6): δ = 5.29 (s, 2H), 6.71 (d, J = 7.8 Hz, 1H), 7.12 (d, J = 7.8 Hz, 1H), 7.24 (t, J = 7.8 Hz, 1H), 7.34 (t, J = 7.8 Hz, 1H), 7.43 (t, J = 7.8 Hz, 2H), 7.46 (d, J = 1.8 Hz, 1H), 7.55 (d, J = 7.8 Hz, 1H), 7.60-7.65 (m, 3H), 8.11 (dd, J = 7.8 Hz and 1.8 Hz, 2H), 12.42 (s, 1H). HRMS-ESI: m/z [M + H] calcd for C18H16N3O2: 306.1237; found 306.1239.

5-(4-(Benzyloxy)-1H-indol-2-yl)-3-(4-chlorophenyl)-1,2,4-oxadiazole (10o) Yield 82%; white powder; mp 205.3-207.1°C. 1H-NMR (600 MHz, DMSO-d6): δ = 5.28 (s, 2H), 6.71 (d, J = 7.8 Hz, 1H), 7.11 (d, J = 8.4 Hz, 1H), 7.24 (t, J = 7.8 Hz, 1H), 7.36 (t, J = 7.8 Hz, 1H), 7.43 (t, J = 7.8 Hz, 2H), 7.46 (d, J = 1.8 Hz, 1H), 7.55 (d, J = 8.4 Hz, 2H), 7.70 (d, J = 8.4 Hz, 2H), 8.11 (d, J = 8.4 Hz, 2H), 12.54 (s, 1H). HRMS-ESI: m/z [M + H] calcd for C23H17ClN3O2: 402.1004; found 402.1001.

5-(6-Methyl-1H-indol-2-yl)-3-(trifluoromethyl)-1,2,4-oxadiazole (10p) Yield 85%; white acicular crystal; mp 158.5-160.4°C. 1H-NMR (600 MHz, DMSO-d6): δ = 2.41 (s, 2H), 2.42 (s, 3H), 6.96 (dd, J = 8.4 Hz and 1.8 Hz, 1H), 7.26 (s, 1H), 7.30 (d, J = 1.8 Hz, 1H), 7.58 (d, J = 8.4 Hz, 1H), 12.23 (s, 1H). HRMS-ESI: m/z [M + H] calcd for C12H8F3N3O: 267.2066; found 267.2066; 267.2071.