European Journal of Medicinal Chemistry 56 (2012) 127–133

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and biological activity of obatoclax derivatives as novel and potent SHP-1 agonists

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ARTICLE INFO

Article history:
Received 6 December 2011
Received in revised form
15 August 2012
Accepted 17 August 2012
Available online 27 August 2012

Keywords:
Obatoclax
STAT3
SHP-1
Cytotoxicity

ABSTRACT

Obatoclax is a linear oligopyrrole compound which antagonizes the antiapoptotic effects of the Bcl-2 family. Herein we describe the synthesis of obatoclax derivatives by replacement of the pyrrole and indole ring of obatoclax with thiophene, furan and thiazolidinedione. The in vitro cytotoxicity of the newly synthesized compounds is evaluated against hepatocellular carcinoma cells. Pyrrole and indole substituents of obatoclax analogues exhibited potent inhibition of cell growth. Among the tested compounds, **5d** and **5e** were active at 6.3 and 13.2 μM against PLC5 cells. Further assays confirmed a correlation between cell death, and p-STAT3 inhibition and SHP-1 activation by these analogues.

1. Introduction

Hepatocellular carcinoma is a major global health problem. Advanced or recurrent HCC is resistant to chemotherapy and has a poor prognosis. Despite increased attention being placed on target therapy for HCC, sorafenib, a multi-kinase inhibitor, is currently the only by FDA-approved anti-HCC targeted drug [1–3]. The discovery of new agents with tolerable toxicity may provide new target therapies for the treatment of advanced HCC.

Obatoclax is an oligopyrrole compound that antagonizes the anti-apoptotic function of Bcl-2, Bcl-xL and Mcl-1 [4]. Obatoclax binds to the BH3 domain of Bcl-2 and disrupts its interaction with proapoptotic proteins, such as Bax and Bak. In addition to Bcl-2 antagonism, obatoclax has been reported to synergize with clinical drugs by regulating several other biological and pharmacological effects [5–8]. For example, the combination of obatoclax and molecular targeted drugs such as lapatinib (dual inhibitors of EGFR and HER-2/neu), gifitinib (inhibitor of EGFR), bortezomib (inhibitor of proteasome), and entinostat (inhibitor of histone deacetylase) showed synergistic repression of cell growth in MCF-7 human breast cancer cells and the tamoxifen-resistant variant (MTR-3). In addition, obatoclax also synergizes chemotherapy agents such as cisplatin, Ara C and topotecan in cancer cell lines [9,10].

For the current project, we started with typical pyrrole-based Mcl-1 inhibitors containing an indole moiety. Aiming to expand the structural diversity of the pyrrole substituents, we added various pyrrole with indole ring. We found that Mcl-1 expression level decrease and the p-STAT3, the upstream regulator of Mcl-1, show the same reduction in HCC. Therefore, our hypothesis is that indole–pyrrole ring can be a scaffold for STAT3 inhibitor. We designed and synthesized novel oligopyrrole derivatives with the aim of examining their ability to induce growth inhibition in HCC cells. We further investigated the structure-activity relationship (SAR) and found that these agents mediate apoptosis in association with p-STAT3 downregulation in HCC cells. Western blot analyses of downstream signaling in the human HCC cell-line PLC-5 are also presented.

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http://dx.doi.org/10.1016/j.ejmech.2012.08.024

2. Chemistry

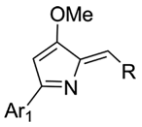
The synthesis of conjugated heterocyclic obatoclax derivatives (Fig. 1) was initiated by converting the readily accessible 2-carbaldehyde heterocycle (**1**) to the corresponding condensation product (**3**) with 4-methoxy-1H-pyrrol-2(5H)-one (**2**). The carbonyl groups of the corresponding products were then smoothly converted to trifluoromethanesulfonate (**4**) by using triflate anhydride. Use of the Suzuki coupling reaction permitted the position of trifluoromethanesulfonate with the boron acid and $Pd(PPh_3)_4$ to obtain compound **5** [11–13]. Using methodologies outlined in the literature, the key intermediates **6** for the aryl-4-methoxy-5-carboxaldehyde (**7**) were generated from the coupling reaction of compound **2**, N,N-diethylformamide and phosphoryl tribromide [14]. The condensation of aldehyde (**7**) and appropriate substrates under the acidic conditions resulted in the final products (**8**).

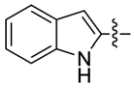
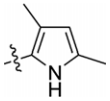
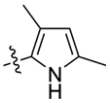
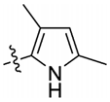
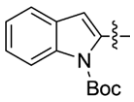
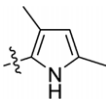
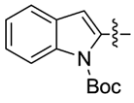
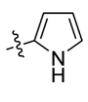
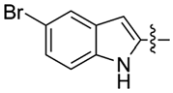
3. Biological activity

All the newly synthesized obatoclax analogues were assessed by MTT assay for in vitro antitumor activity against HCC cancer cells (PCL5). The compound concentrations causing 50% cell growth inhibition (IC_{50} values) are summarized in Tables 1–3. IC_{50} values were determined by interpolation from dose–response curves.

A set of analogues **8a**–**8c** (Table 1) were generated in which a Boc-indole, thiophen and furan replaced the indole ring on the left-hand side of the core moiety. In vitro analysis of the activity of these compounds against PLC5 cells demonstrated significantly diminished activity ($IC_{50} > 40$ μM) relative to that of obatoclax ($IC_{50} = 13.2$ μM). In addition, the removal of the dimethyl group of pyrrole in obatoclax did not increase the potency of the resulting compounds against PLC5 cells (compounds **5a**–**5c**). The presence of a bromide substituent in the indole ring, however, to led to an increase in activity (**5d**). These results suggest that the left-hand side indole ring of obatoclax is important for antitumor activity. We next tested the hypothesis that the pyrrole on the right-hand side of obatoclax might induce potent antitumor activity with the heteroaryl ring. Subsequent analogues were thus synthesized to obtain a SAR with the replacement of the right-hand side pyrrole of obatoclax with thiophene, furan, and indole groups. These compounds were screened for cell viability in PLC5 cells and the

Table 1
Chemical structure of compounds **5** and **8** and IC_{50} of growth inhibition in PLC-5 cells.



CPD	Ar_1	R	Cell death MTT assay (μM)
Obatoclax			12.1 ± 2.5
8a			>40
8b			>40
8c			28.4 ± 1.2
5a			>40
5b			>40
5c			>40
5d			6.3 ± 2.1

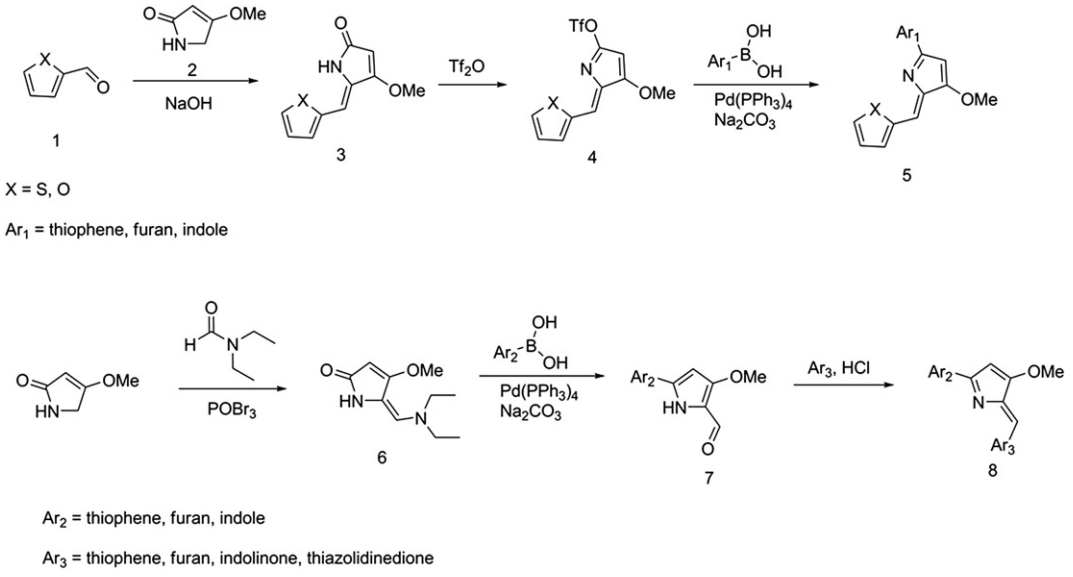
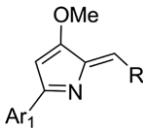
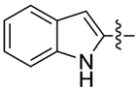
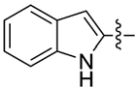
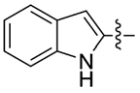
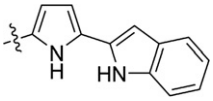


Fig. 1. General synthetic procedure for obatoclax derivatives.

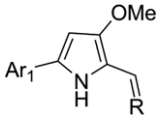
Table 2
Chemical structure of compounds **5** and **8** and IC_{50} of growth inhibition in PLC-5 cells.

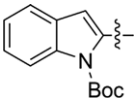
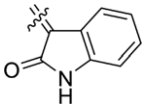
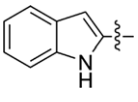
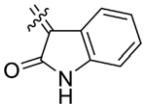
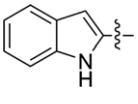
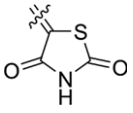
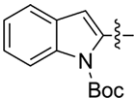
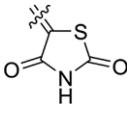
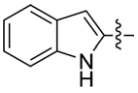


CPD	Ar_1	R	Cell death MTT assay (μM)
5e			13.2 ± 4.7
5f			>40
5g			25.9 ± 3.4
8d			15.9 ± 3.6

results are demonstrated at Table 2. The pyrrole ring of compound **5e** exhibited equal potency to the dimethyl pyrrole of obatoclax. Compound **5f**, with thiophene introduced to replace dimethyl pyrrole, led to a reduction in the antitumor effect. Interestingly, the introduction of thiophene rings on both sides of the obatoclax moiety resulted in better growth inhibition than a single thiophene

Table 3
Chemical structure of compounds **8** and IC_{50} of growth inhibition in PLC-5 cells.



CPD	Ar_1	R	Cell death MTT assay (μM)
8e			>40
8f			35.4 ± 4.6
8g			27.5 ± 4.2
8h			>40
8i			>40

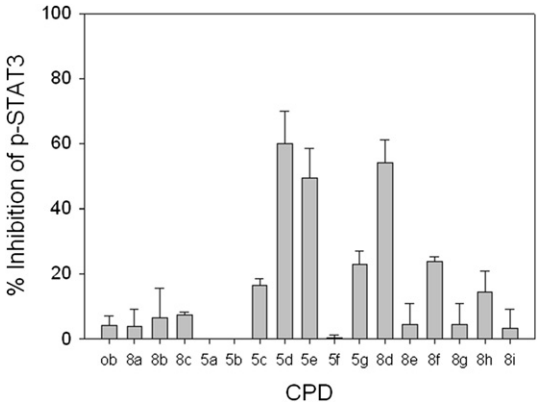


Fig. 2. ELISA analysis of the inhibitory effects of obatoclax derivatives versus obatoclax, each at 10 μM, on IL-6 stimulated p-STAT3 in PLC5 cells.

on either the right-hand or the left-hand side. Analogues **8e**–**8i**, which contained indolin and thiazolidinedione on the right-hand side of obatoclax were also tested for cell viability. Among these analogues, compounds **8f** and **8g** showed a moderate effect but with lower potency than obatoclax.

4. Mechanistic study of obatoclax analogues in PLC5 cells

Originally, obatoclax was shown to inhibit the protein–protein interactions of the Bcl-2 family. The analogues synthesized here showed more potent cytotoxicity than obatoclax. In addition, the expression level of Mcl-1 was repressed by the new compounds. We, therefore, analyzed the upstream regulator STAT3 activity with a p-STAT3 ELISA kit. The result showed that potency of p-STAT3 inhibition by the new analogues correlated with cell death (Fig. 2). Therefore, we applied four agents, **5a**, **5d**, **5e** and **8d**, to PLC5 cells and studied the effect of the upstream and downstream signals of STAT3. As shown in Fig. 3, **5d**, **5e** and **8d** resulted in a high degree inhibition of p-STAT3 and subsequent repression of downstream targets such as Mcl-1, survivin and cyclin D1. On the other hand, **5a**,

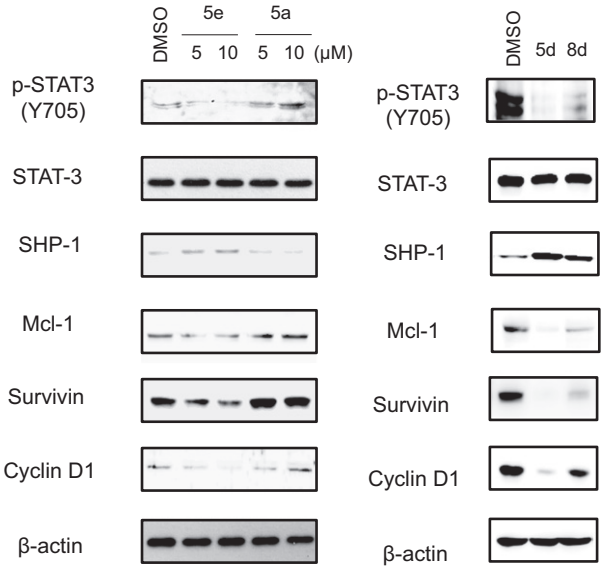


Fig. 3. Obatoclax derivatives downregulate p-STAT3 and its downstream signaling pathway. Cells were treated with **5a**, **5d**, **5e** and **8d** at 10 μM for 12 h. p-STAT3, STAT3, SHP-1, survivin, cyclin D1 and β-actin were examined by western blot.

which had no effect on cell toxicity, demonstrated no appreciable effects on regulating p-STAT3 and its downstream targets. We then further explored the expression level of SHP-1, a protein tyrosine phosphatase that acts as negative regulator of STAT3. Compound **5d**, **5e** and **8d** significantly increased the level of SHP-1 in PLC5 cells, but **5a** had no effect on SHP-1. We further confirmed that down-regulated p-STAT3 in PLC5 cells resulted from phosphatase activation (Fig. 4).

5. Discussion

Highly phosphorylated STAT3 has been linked to cancer incidence. Several factors have been reported to activate STAT3. For example, IL-6 induces Jak2 phosphorylation through its receptor and activates STAT3. In addition, growth factors, such as EGF and VEGF are able to activate STAT3. STAT3 activity is also regulated through negative feedback by SHP-1. The literature shows that loss-of-function of SHP-1 leads to STAT3 activation contributing to tumor formation [15–17]. In this study, we generated a series of obatoclax analogues that exhibit anticancer activity against PLC5 cells. Our data have several implications: (1) The mechanism of apoptosis induction driven by obatoclax analogues occurred, at least in part, through downregulation of p-STAT3. Obatoclax is known to induce apoptosis through targeting the Bcl-2 family; however, in addition to new analogues, **5d**, **5e** and **8d** inhibited phosphorylation of STAT3; (2) Compounds **5d**, **5e** and **8d** acted as SHP-1 agonists which enhanced SHP-1 phosphatase activity and further dephosphorylated p-STAT3. At present, kinase and growth factor inhibitors are common strategies for anticancer therapy whereas upregulation of negative regulators of STAT3 have seldom been considered. The enhancement of SHP-1 expression antagonizing the function of STAT3 may provide a new direction for HCC treatment; (3) We prepared a set of obatoclax analogues via two synthetic pathways. These standardized procedures used commercially available starting materials and reagents to generate large amounts of obatoclax analogues that can be used in future in vivo studies. Our current findings suggest that the indole ring on the left-hand side of the obatoclax analogue is important for biological activity. Replacement with thiophene and furan resulted in loss of anticancer activity and SHP-1 expression. On the other hand, dimethyl pyrrole and pyrrole on the right-hand side of the obatoclax moiety exhibited greater activity than thiophene, indolin and furan.

In conclusion, the obatoclax analogues synthesized in this study exhibited anti-HCC through a novel mechanism that directly enhanced SHP-1 expression level and consequently suppressed p-STAT3. This indicates that targeting STAT3 is a promising strategy for HCC and could be applied to other unregulated STAT3-driven cancers. Further studies of the detailed mechanism by which obatoclax analogues enhance SHP-1 activity may lead to new targets for cancer therapy. These agents may provide structure activity relationships for compound modification of SHP-1 agonists.

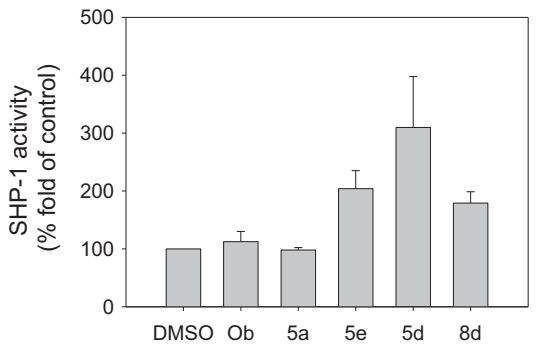


Fig. 4. SHP-1 activity in obatoclax, **5a**, **5d**, **5e** and **8d**-treated PLC5 cells. Cells were treated with obatoclax, **5a**, **5d**, **5e** and **8d** at 10 μM for 6 h and then cell lysates were analyzed by phosphatase activity assay.

6. Experimental section

6.1. Materials

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Bruker DPX400 (400 MHz) instruments. Chemical shifts are reported as ppm. Peak multiplicities are expressed as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; ddd, doublet of doublet of doublets; dt, doublet of triplet; brs, broad singlet; m, multiplet. Coupling constants (*J* values) are given in hertz (Hz). Reaction progress was determined by thin layer chromatography (TLC) analysis on silica gel 60 F254 plates (Merck). Chromatographic purification was carried out on silica gel 60 (0.063–0.200 mm or 0.040–0.063 mm, Merck) basic silica gel. Commercial reagents and solvents were used without additional purification. Abbreviations are used as follows: $CDCl_3$, deuterated chloroform; DMSO-*d*6, dimethyl sulfoxide-*d*6; EtOAc, ethyl acetate; DMF, *N*,*N*-dimethylformamide; MeOH, methanol; THF, tetrahydrofuran; EtOH, ethanol; DMSO, dimethyl sulfoxide; DCM, dichloromethane. High resolution mass spectra were recorded on a Finnigan MAT 95S mass spectrometer.

6.2. Chemical synthesis

6.2.1. General procedure for the synthesis of compound *5*

$PdCl_2$ (0.1 equiv, 59%) and PPh_3 (0.45 equiv) were added to a nitrogen passed solution of toluene (1 mL). The mixture became bright yellow and was stirred at 70 °C for 20 min under nitrogen. The $Pd(PPh_3)_4$ in toluene suspension was transferred into a solution of 1.0 equiv triflate (compound **4**) and 1.2 equiv aryl boronic acid (compound **5** or commercially available) in 10% water/dioxane (5 mL) purged with nitrogen. One equiv solid sodium carbonate was added. The reaction mixture was stirred at 100 °C for 90 min, then poured into 10 mL water and extracted with ethyl acetate (20 mL) three times. The organic layer was collected, washed with brine, dried over $MgSO_4$ and concentrated. The residue was then chromatographed by silica gel with the eluent ethyl acetate:hexane (1:20 to 1:5). This procedure afforded the expected coupling product in 81%–94% yield.

*6.2.1.1. 2-((5-(furan-2-yl)-3-methoxy-2H-pyrrol-2-ylidene)methyl)-1H-pyrrole (**5a**).* ^{1}H NMR (400 MHz, $CDCl_3$):δ 7.56 (d, J = 2.0 Hz, 1H), 7.13 (d, J = 2.4 Hz, 1H), 7.00 (d, J = 3.2 Hz, 1H), 6.92 (s, 1H), 6.61 (d, J = 3.6 Hz, 1H), 6.54 (dd, J = 3.6 Hz, J = 2.0 Hz, 1H), 6.26 (dd, J = 3.6 Hz, J = 2.4 Hz, 1H), 5.96 (s, 1H), 3.88 (s, 3H); LCMS(ESI): *m*/*z* 241.2 (100, M + H^+); HRMS calculated for $C_{14}H_{12}N_2O_2$ (M + H^+): 241.0972. Found 241.0964 (M + H^+).

*6.2.1.2. 2-((3-methoxy-5(-thiophen-2-yl)-2H-pyrrol-2-ylidene) methyl)-1H-pyrrole (**5b**).* ^{1}H NMR (400 MHz, $CDCl_3$):δ 7.50 (d, J = 3.6 Hz, 1H), 7.41 (d, J = 4.8 Hz, 1H), 7.14 (d, J = 2.0 Hz, 1H), 7.10 (dd, J = 5.2 Hz, J = 4.0 Hz, 1H), 6.90 (s, 1H), 6.61 (d, J = 3.6 Hz, 1H), 6.27 (t, J = 3.6 Hz, J = 2.4 Hz, 1H), 5.95 (s, 1H), 3.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 58.4, 95.2, 110.9, 117.4, 118.8, 126.1 127.7, 127.9, 128.1, 128.4, 130.7, 140.3, 161.5, 168.6; LCMS(ESI): *m*/*z* 257.2 (100, M + H^+); HRMS calculated for $C_{14}H_{12}N_2OS$ (M + H^+): 257.0743. Found 257.0731.

*6.2.1.3. tert-butyl-2-(2-((1H-pyrrol-2-yl)methylene)-3-methoxy-2H-pyrrol-5-yl)-1H-indol-1-carboxylate (**5c**).* ^{1}H NMR (400 MHz, $CDCl_3$):δ 8.12 (d, J = 8.0 Hz, 1H), 7.59 (d, J = 7.6 Hz, 1H), 7.36 (t,

J = 8.0 Hz, 1H), 7.25 (t, J = 7.6 Hz, 1H), 7.08 (d, J = 2.4 Hz, 1H), 7.01 (s, 1H), 6.98 (s, 1H), 6.64 (d, J = 3.6 Hz, 1H), 6.26 (dd, J = 3.6 Hz, J = 2.4 Hz, 1H), 5.82 (s, 1H), 3.88 (s, 3H), 1.51 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.5 (3C), 58.1, 83.7, 98.0, 110.7, 112.0, 114.4, 118.4, 118.7 121.0, 122.8, 125.2, 126.0, 128.6, 130.3, 136.0, 138.11, 141.5, 150.0, 161.1, 167.1; LCMS(ESI): m/z 388.4 (100, M − H$^+$). HRMS calculated for $C_{23}H_{23}N_3O_3$ (M + H$^+$): 389.1739. Found: 389.1744.

*6.2.1.4. 2-(2-((1H-pyrrol-2-yl)methylene)-3-methoxy-2H-pyrrol-5-yl)-5-bromo-1H-indol (**5d**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 (d, J = 1.6 Hz, 1H), 7.15 (dd, J = 8.8 Hz, J = 2.0 Hz, 1H), 7.07 (s, 1H), 6.88 (s, 1H), 6.83 (d, J = 8.4 Hz, 1H), 6.73 (s, 1H), 6.60 (d, J = 3.6 Hz, 1H), 6.22 (s, 1H), 6.12 (t, J = 3.2 Hz, 1H), 4.04 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 59.0, 96.2, 105.6, 111.6, 113.0, 113.4, 119.2, 121.4 123.8, 127.1 127.7, 129.6, 130.3 134.4, 136.5, 140.2, 160.8, 169.5; LCMS(ESI): m/z 368.2 (100, M + H$^+$); HRMS calculated for $C_{18}H_{14}BrN_3O$ (M + H$^+$): 368.0393. Found 368.0345.

*6.2.1.5. 2-(2-((1H-pyrrol-2-yl)methylene)-3-methoxy-2H-pyrrol-5-yl)-1H-indol (**5e**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.54 (d, J = 8.0 Hz, 1H), 7.10 (t, J = 7.2 Hz, 1H), 7.04–6.97 (m, 4H), 6.78 (s, 1H), 6.58 (d, J = 3.6 Hz, 1H), 6.23 (s, 1H), 6.12 (dd, J = 3.6 Hz, J = 2.4 Hz, 1H), 4.03 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 58.6, 95.8, 106.4, 111.1, 111.4, 118.0, 120.0, 120.4, 121.3, 124.1, 127.0, 128.4, 129.5, 133.0, 137.8, 140.1, 160.7, 168.8; LCMS(ESI): m/z 290.3 (100, M + H$^+$); HRMS calculated for $C_{18}H_{15}N_3O$ (M + H$^+$): 290.1288. Found 290.1281.

*6.2.1.6. 2-(3-methoxy-2-(thiophen-2-ylmethylene)-2H-pyrrol-5-yl)-1H-indol (**5f**).* 1H NMR (400 MHz, $CDCl_3$):δ 9.32 (bs, 1H), 7.64 (d, J = 8.0 Hz, 1H), 7.61 (d, J = 5.2 Hz, 1H), 7.47 (d, J = 4.0 Hz, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.26 (t, J = 7.2 Hz, 1H), 7.23 (s, 1H), 7.12–7.07 (m, 2H), 7.00 (s, 1H), 6.05 (s, 1H), 3.94 (s, 3H); LCMS(ESI): m/z 307.3 (100, M + H$^+$); HRMS calculated for $C_{18}H_{14}N_2OS$ (M + H$^+$): 307.0900. Found: 307.0895.

*6.2.1.7. 3-ethoxy-5-(thiophen-2-yl)-2-(thiophen-2-ylmethylene)-2H-pyrrole (**5g**).* ^{1}H NMR (400 MHz, $CDCl_3$):δ 7.59 (d, J = 5.2 Hz, 1H), 7.55 (d, J = 4.0 Hz, 1H), 7.47 (d, J = 5.2 Hz, 1H), 7.45 (d, J = 3.6 Hz, 1H), 7.20 (s, 1H), 7.11 (dd, J = 5.2 Hz, J = 4.0 Hz, 1H), 7.05 (dd, J = 5.2 Hz, J = 3.6 Hz, 1H), 5.96 (s, 1H), 3.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 58.5, 96.5, 120.8, 127.2, 128.0, 128.6, 129.7, 133.8, 134.3, 139.2, 140.6, 145.6, 164.4, 169.1; LCMS(ESI): m/z 274.2(100, M + H$^+$). HRMS calculated for $C_{14}H_{11}NOS_2$ (M + H$^+$): 273.0282. Found: 273.0281.

6.2.2. Preparation of compound 7

$PdCl_2$ (0.1 equiv, 59%) and PPh_3 (0.45 equiv) was added to a degassed solution of toluene (1 mL). The mixture became bright yellow and was stirred at 70 °C for 20 min under nitrogen. The freshly prepared $Pd(PPh_3)_4$ in toluene suspension was transferred into a solution of 1.0 equiv bromo pyrrole enamine (compound **6**) and 1.5 equiv aryl boronic acid in 10% water/dioxane (5 mL) purged with nitrogen. Three-equiv sodium carbonate was added. The reaction mixture was stirred at 100 °C for 90 min and then poured onto 50 mL ice-water. The pH of the mixture was adjusted to 7.0 using 2N HCl (5 mL) and stirred for 20 min. The slurry was extracted with ethyl acetate (20 mL) twice. The precipitate was recovered by filtration, washed with water and collected from acetone. The solid was recrystallized by 1:2 chloroform:ether (5 mL). This procedure afforded the expected coupling product in 71% to quantitative yield.

*6.2.2.1. 5-(Furan-2-yl)-3-methoxy-1H-pyrrole-2-carbaldehyde (**7a**).* ^{1}H NMR (400 MHz, $CDCl_3$):δ 10.03 (bs, 1H), 9.50 (s, 1H), 7.43 (d, J = 1.6 Hz, 1H), 6.82 (d, J = 3.6 Hz, 1H), 6.47 (dd, J = 3.6 Hz, J = 1.6 Hz, 1H), 6.10 (d, J = 2.4 Hz, 1H), 3.89 (s, 3H).

6.2.2.2. 3-Methoxy-5-(thiophen-2-yl)-1H-pyrrole-2-carbaldehyde (7b). ^{1}H NMR (400 MHz, $CDCl_3$):δ 9.46 (s, 1H), 7.32 (d, J = 5.2 Hz, 1H), 7.30 (d, J = 3.6 Hz, 1H), 7.07 (dd, J = 5.2 Hz, J = 3.6 Hz, 1H), 6.06 (s, 1H), 3.89 (s, 3H).

*6.2.2.3. 5-(1H-Indol-2-yl)-3-methoxy-1H-pyrrole-2-carbaldehyde (**7c**).* ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.84 (bs, 1H), 11.43 (bs, 1H), 9.42 (s, 1H), 7.53 (d, J = 8.0 Hz, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.12 (t, J = 7.6 Hz, 1H), 7.10 (s, 1H), 7.00 (t, J = 7.6 Hz, 1H), 6.55 (d, J = 2.8 Hz, 1H), 3.88 (s, 3H).

*6.2.2.4. tert-Butyl-2-(5-formyl-4-methoxy-1H-pyrrol-2-yl)-1H-indole-1-carboxylate (**7d**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.98 (bs, 1H), 9.57 (s, 1H), 8.08 (d, J = 8.0 Hz, 1H), 7.54 (d, J = 8.0 Hz, 1H), 7.33 (t, J = 7.6 Hz, 1H), 7.25 (t, J = 7.6 Hz, 1H), 6.89 (s, 1H), 6.13 (d, J = 2.8 Hz, 1H), 3.90 (s, 3H), 1.59 (s, 9H).

*6.2.3. General procedure for the synthesis of compound **8a**–**8b***

1.2 equiv 2,4-dimethyl pyrrole was added to a solution of 1.0 equiv aldehydes (compound **7**) in 4 mL methanol. The mixture was stirred and 1N methanolic HCl was added. The reaction was stirred continuously overnight at room temperature under nitrogen. Methanol was removed under rotary evaporation. The residue was then purified by a flash column. This procedure afforded the expected coupling product in 81% to quantitative yield.

*6.2.3.1. 2-((3,5-Dimethyl-2H-pyrrol-2-ylidene)methyl)-5-(furan-2-yl)-3-methoxy-1H-pyrrole (**8a**).* ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.67 (d, J = 1.6 Hz, 1H), 7.03 (d, J = 3.2 Hz, 1H), 6.92 (s, 1H), 6.60 (dd, J = 3.2 Hz, J = 1.6 Hz, 1H), 6.10 (s, 1H), 5.89 (s, 1H), 3.90 (s, 3H), 2.35 (s, 3H), 2.19 (s, 3H); LCMS(ESI): m/z 269.6 (100, M + H$^+$); HRMS calculated for $C_{16}H_{16}N_2O_2$ (M + H$^+$): 269.1285. Found 269.1284 (M + H$^+$).

*6.2.3.2. 2-((3,5-Dimethyl-2H-pyrrol-2-ylidene)methyl)-3-methoxy-5-(thiophen-2-yl)-1H-pyrrole (**8b**).* ^{1}H NMR (400 MHz, $CDCl_3$):δ 7.44 (d, J = 3.6 Hz, 1H), 7.35 (d, J = 5.2 Hz, 1H), 7.08 (dd, J = 5.2 Hz, J = 3.6 Hz, 1H), 6.88 (s, 1H), 5.96 (s, 1H), 5.83 (s, 1H), 3.89 (s, 3H), 2.35 (s, 3H), 2.20 (s, 3H); LCMS(ESI): m/z 285.3 (100, M + H$^+$); HRMS calculated for $C_{16}H_{16}N_2OS$ (M + H$^+$): 285.1056. Found: 285.1063.

*6.2.4. General procedure for the synthesis of compound obatoclax, **8c** and **8d***

2,4-dimethyl pyrrole or indolyl-pyrrole (1.2 equiv) was added to a solution of 1.0 equiv aldehydes (**7d**) in 4 mL methanol. The mixture was stirred and 1N methanolic TFA was added. The reaction was stirred continuously overnight at room temperature under nitrogen. Methanol was removed under rotary evaporation. The residue was then purified by a flash column. This procedure afforded the expected coupling product in 70% to quantitative yield.

*6.2.4.1. 2-(5-((3,5-Dimethyl-2H-pyrrol-2-ylidene)methyl)-4-methoxy-1H-pyrrol-2-yl)-1H-indole (**obatoclax**).* ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.56 (d, J = 8.0 Hz, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.15 (t, J = 7.6 Hz, 1H), 7.00 (t, J = 7.6 Hz, 1H), 6.96 (s, 1H), 6.90 (s, 1H), 6.25 (s, 1H), 5.87 (s, 1H), 3.95 (s, 3H), 2.38 (s, 3H), 2.20 (s, 3H); LCMS(ESI): m/z 318.2 (100, M + H$^+$).

6.2.4.2. tert-Butyl-2-(5-((3,5-Dimethyl-2H-pyrrol-2-ylidene)methyl) 4-methoxy-1H-pyrrol-2-yl)-1H-indole-1-carboxylate (8c). ^{1}H NMR (400 MHz, $CDCl_3$):δ 8.10 (d, J = 8.0 Hz, 1H), 7.56 (d, J = 8.0 Hz, 1H), 7.32 (t, J = 7.6 Hz, 1H), 7.22 (t, J = 7.6 Hz, 1H), 6.95 (s, 1H), 6.93 (s, 1H), 5.83 (s, 1H), 5.82 (s, 1H), 3.88 (s, 3H), 2.30 (s, 3H), 2.21 (s, 3H), 1.47 (s, 9H); DIP(EI-70ev): m/z 417.3 (53, M), 360.1 (89), 317.2 (100),

286.2 (84); HRMS calculated for $C_{25}H_{27}N_3O_3$ 417.2052. Found: 417.2048 (M).

*6.2.4.3. 2-(5-((5-(1H-Pyrrol-2-yl)-2H-pyrrol-2-ylidene)methyl)-4-methoxy-1H-pyrrol-2-yl)-1H-indole (**8d**).* ^{1}H NMR (400 MHz, MeOD-d_4): δ 8.81 (bs, 1H), 7.63 (s, 1H), 7.60 (d, J = 8.0 Hz, 1H), 7.44 (d, J = 8.4 Hz, 1H), 7.40–7.38 (m, 1H), 7.25 (m, 2H), 7.18 (t, J = 7.6 Hz, 1H), 7.13 (s, 1H), 7.05 (m, 2H), 6.63 (d, J = 3.6 Hz, 1H), 6.35 (m, 2H), 3.99 (s, 3H); LCMS(ESI): m/z 405.3 (100, M + H^+); HRMS calculated for $C_{26}H_{20}N_4O$ (M + H^+): 405.1710. Found: 405.1723.

*6.2.5. General procedure for the synthesis of compound **8e–8h***

Piperidine (0.15 equiv) and benzoic acid (0.13 equiv) was added to a mixture of 1.0 equiv aldehydes (compound **7c** or **7d**) and 1.0 equiv oxindole or thiazolidinedione in 3 mL toluene. The mixture was reacted in a closed system in a microwave reactor. The reaction condition was set at 50 W, 150 °C holding for 25 min, and stirring at high speed. After cooling to room temperature, the reaction mixture was poured into 10 mL water to quench the reaction. The organic layer was extracted with 20 mL ethyl acetate, brined, dried over anhydrous $MgSO_4$, filtered and concentrated under rotary evaporation. The orange solid was recrystallized with 1:10 ethyl acetate:-hexane (2 mL). The residue was then purified by a flash column. This procedure afforded the expected coupling product in 70%–85% yield.

6.2.5.1. tert-Butyl-2-(4-methoxy-5-((2-oxoindolin-3-ylidene) methyl)-1H-pyrrol-2-yl)-1H-indole-1-carboxylate (8e). ^{1}H NMR (400 MHz, $CDCl_3$):δ 13.40 (bs 1H), 9.01 (bs, 1H), 8.22 (d, J = 8.0 Hz, 1H), 7.57 (s, 1H), 7.56 (d, J = 6.8 Hz, 1H), 7.49 (d, J = 6.8 Hz, 1H), 7.36 (t, J = 8.0 Hz, 1H), 7.26 (t, J = 8.0 Hz, 1H), 7.07–7.00 (m, 2H), 6.87 (s, 1H), 6.86 (d, J = 8.0 Hz, 1H), 6.14 (d, J = 2.8 Hz, 1H), 3.95 (s, 3H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.8, 58.1, 84.2, 96.1, 109.3, 111.5, 111.9, 115.6, 117.6, 118.1, 120.8, 121.2, 121.5, 123.2, 125.1, 125.5, 126.3, 128.9, 129.9 131.7, 137.1, 137.8, 149.1, 155.2, 169.8; LCMS(ESI): m/z 478.0 (100, M + Na^+); HRMS calculated for $C_{27}H_{25}N_3O_4$ (M − H^+): 454.1772. Found: 454.1779.

6.2.5.2. 3-((5-(1H-Indol-2-yl)-3-methoxy-1H-pyrrol-2-yl)methylene)indolin-2-one (8f). ^{1}H NMR (400 MHz, DMSO-d_6):δ 13.94 (bs 1H), 11.72 (bs, 1H), 10.84 (bs, 1H), 7.59 (d, J = 7.6 Hz, 2H), 7.49 (s, 1H), 7.41 (d, J = 8.0 Hz, 1H), 7.14 (t, J = 8.0 Hz, 1H), 7.09 (t, J = 7.6 Hz, 1H), 7.03 (t, J = 7.6 Hz, 1H), 6.97 (t, J = 7.6 Hz, 1H), 6.91 (d, J = 7.6 Hz, 1H), 6.79 (d, J = 1.6 Hz, 1H), 6.65 (d, J = 2.8 Hz, 1H), 3.95 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 57.5, 92.9, 97.9, 108.8, 110.7, 111.5, 116.9, 117.1, 119.0, 119.2, 119.7, 120.4, 121.8, 125.0, 125.0, 127.7, 128.8, 129.5, 136.7, 137.5, 154.9, 168.8; LCMS(ESI): m/z 354.4 (100, M − H^+); HRMS calculated for $C_{22}H_{17}N_3O_2$ (M + H): 356.1394. Found: 356.1410.

6.2.5.3. 5-((5-(1H-Indol-2-yl)-3-methoxy-1H-pyrrol-2-yl)methylene)thiazolidine-2,4-dione (8g). ^{1}H NMR (400 MHz, DMSO-d_6):δ 7.56 (d, J = 7.2 Hz, 1H), 7.39 (d, J = 8.0 Hz, 1H), 7.12 (t, J = 8.0 Hz, 1H), 7.01 (t, J = 7.2 Hz, 2H), 6.72 (s, 1H), 6.59 (d, J = 2.4 Hz, 1H), 3.87 (s, 3H); LCMS(ESI): m/z 338.5 (100, M − H^+).

6.2.5.4. tert-Butyl-2-(5-((2,4-dioxothiazolidin-5-ylidene)methyl)-4-methoxy-1H-pyrrol-2-yl)-1H-indole-1-carboxylate (8h). ^{1}H NMR (400 MHz, $CDCl_3$):δ 12.15 (bs, 1H), 8.12 (d, J = 8.8 Hz, 1H), 7.55 (d, J = 7.6 Hz, 1H), 7.32 (t, J = 8.4 Hz, 1H), 7.22 (t, J = 7.6 Hz, 1H), 6.92 (s, 1H), 6.83 (s, 1H), 6.11 (d, J = 2.4 Hz, 1H), 3.88 (s, 3H), 1.53 (s, 9H); LCMS(ESI): m/z 438.0 (100, M − H^+); HRMS calculated for $C_{22}H_{21}N_3O_5S$ (M + H): 438.1129. Found: 438.1137.

6.2.5.5. 2-(5-(1,3-dithian-2-yl)-4-methoxy-1H-pyrrol-2-yl)-1H-indole (8i). A mixture of 1.0 equiv indolyl methoxypyrrole aldehyde (compound **7c**) and 1.0 equiv propanedithiol in 2 mL dichloromethane was stirred under nitrogen, followed by addition of 0.1 equiv para-toluenesulfonic acid. The solution was stirred for 2 h at room temperature under nitrogen. The mixture was diluted with ethyl acetate, washed with three 20 mL portions each of saturated Na_2CO_3, and brined. Purification by flash chromatography using an eluent ethyl acetate:hexane 1:20 to 1:5 yielded dithiane. The yield was 31%.

^{1}H NMR (400 MHz, $CDCl_3$):δ 8.33 (bs, 1H), 8.20 (bs, 1H), 7.55 (d, J = 7.6 Hz, 1H), 7.32 (d, J = 8.0 Hz, 1H), 7.14 (t, J = 8.0 Hz, 1H), 7.08 (t, J = 7.6 Hz, 1H), 6.52 (d, J = 2.0 Hz, 1H), 6.11 (d, J = 2.8 Hz, 1H), 5.55 (s, 1H), 3.81 (s, 3H), 3.12–3.05 (t, J = 13.2 Hz, 2H), 2.92–2.87 (m, 2H), 0.91–0.82 (m, 2H); LCMS(ESI): m/z 329.5 (100, M − H^+). HRMS calculated for $C_{25}H_{25}NOS_2$ (M + H^+): 273.0282. Found: 273.0281.

6.3. Biological assays

6.3.1. Cell culture

PLC5 cells were purchased from ATCC and maintained in DMEM supplemented with 10% FBS, 100 units/mL penicillin G, 100 μg/mL streptomycin sulfate and 25 μg/mL amphotericin B in a 37 °C humidified incubator in an atmosphere of 5% CO_2 in air.

6.3.2. Western blot

PLC5 cells were treated with compounds **5a**, **5e** and **8d** at 10 μM for 12 h. Cell lysates were analyzed by western blot. p-STAT3, STAT3, SHP-1, Mcl-1, Survivin, Cyclin D1, PARP and actin antibodies were purchased from Cell Signaling.

6.3.3. SHP-1 phosphatase activity

A RediPlate 96 EnzChek® Tyrosine Phosphatase Assay Kit (R-22067) was used for SHP-1 activity assay (Molecular Probes, Carlsbad, CA). The method was as described previously [18].

6.3.4. STAT3 activity assay

PLC5 cells were pretreated with the indicated compounds in 10 μM for 24 h and then stimulated with IL-6 (10 ng/mL) for 30 min. The PathScan Phospho-Stat3 (Tyr705) Sandwich ELISA Kit was purchased from Cell Signaling, Danvers, MA.

Acknowledgments

We thank the National Science Council, Taiwan (NSC98-2320-B-010-005-My3 and NSC-100-2325-B-010-007) and National Yang Ming University, Taiwan for financial support. C.W.S. also thanks the National Research Institute of Chinese Medicine for NMR support.

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