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# Molecular features of the YAP inhibitor, Verteporfin - synthesis of hexasubstituted dipyrrens as potential inhibitors of YAP/TAZ, the downstream effectors of the Hippo pathway

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**Abstract:** Porphyrin derivatives, and in particular Verteporfin (VP), a photosensitizer initially designed for cancer therapy, have been identified as inhibitors of YAP-TEAD interaction and transcriptional activity. We herein report the efficient convergent synthesis of the dipyrren western half-part of protoporphyrin IX dimethyl ester (PPIX-DME) where the sensitive vinyl group was created at the final stage by a dehydroiodation reaction. Two other dipyrren derivatives were synthesized including dipyrren **19** containing two vinyl groups. We found that VP and dipyrren **19** showed a significant inhibitory effect on TEAD transcriptional activity in MDA-MB-231 cells whereas other compounds did not show significant changes. In addition, we observed a marked decrease in both YAP and TAZ levels following VP treatment whereas dipyrren **19** treatment primarily reduced the level of YAP and the receptor kinase Axl, a downstream target of YAP. Altogether, our data suggests that in function of their chemical structure, porphyrin- and dipyrren-related derivatives can directly target YAP and/or TAZ proteins and inhibits TEAD transcriptional activity.

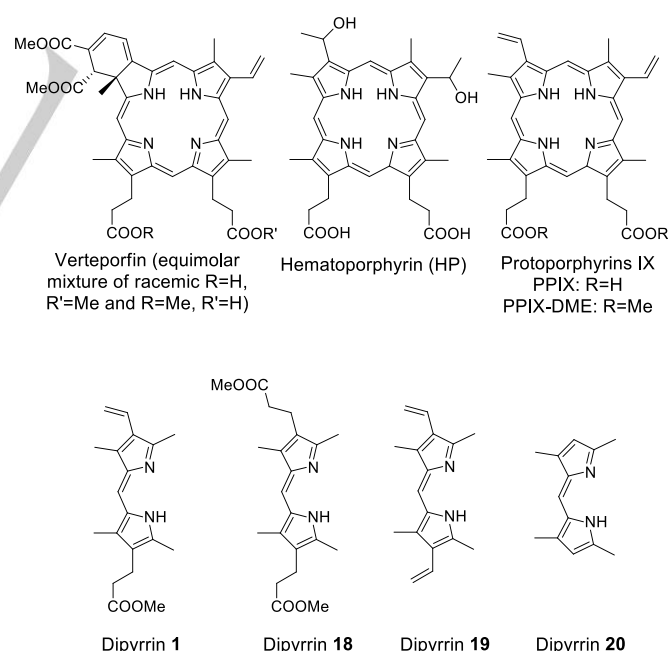
## Introduction

YAP(TAZ), the downstream effectors of the Hippo pathway, interact with TEAD to regulate stem cell proliferation, tissue growth and organ size [4]. Oncogenic YAP/TAZ are overexpressed in many cancers [5-8] causing overgrowth phenotypes and metastasis. Liu-Chittenden and colleagues [9] were the first to demonstrate to feasibility to disrupt YAP/TEAD complex as a pertinent therapeutic strategy in cancer therapy and identified three members of the porphyrin family, namely VP, protoporphyrin IX (PPIX) and hematoporphyrin (HP), as inhibitors of YAP/TEAD-dependent transcription. VP was found to be the most effective compound to inhibit the complex between YAP and TEAD2 and was used VP for *in vivo* validation. Indeed, VP treatment efficiently suppressed liver overgrowth induced by YAP overexpression in mice models.

Verteporfin, is a photosensitizer initially designed for cancer therapy, which presents high cytotoxicity for tumor cells only under light stimulation [1]. Rapidly VP was repositioned in the field of ophthalmology and photodynamic therapy for the treatment of age-related macular degeneration [2] (VP was FDA-approved in 2000 and marketed by Novartis under the trade name: visudyne®).

VP was recently reported to exert different biological properties without light activation [3 and references cited therein]. However, the use of VP as an inhibitor of YAP-induced overgrowth presents several drawbacks: (i) it is a photosensitizer and therefore is highly phototoxic; (ii) it has been identified as an inhibitor of autophagy via a low-level production of singlet oxygen [10]; (iii) it induces the oligomerization of up to 250 proteins including 80 mitochondrial proteins [11]; (iv) it has also been reported that VP up-regulates 14-3-3 $\sigma$  (a protein responsible for the cytoplasmic retention of YAP)[12].

So, several questions therefore arise on how VP interacts with YAP.



**Figure 1.** Structures of VP, HP, PPIX and related dipyrrens

In this paper, we first focused our attention on the structural features that confer to VP its ability to control the YAP/TEAD interaction and decrease the induction of the main target genes. For this purpose, we first compared the biological properties of

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VP, PPIX and its dimethyl ester (Figure 1) in MDA-MB-231 breast cancer cell line. In a second round, we synthesized the hexasubstituted dipyrin **1** corresponding to the western part of PPIX dimethyl ester (PPIX-DME).

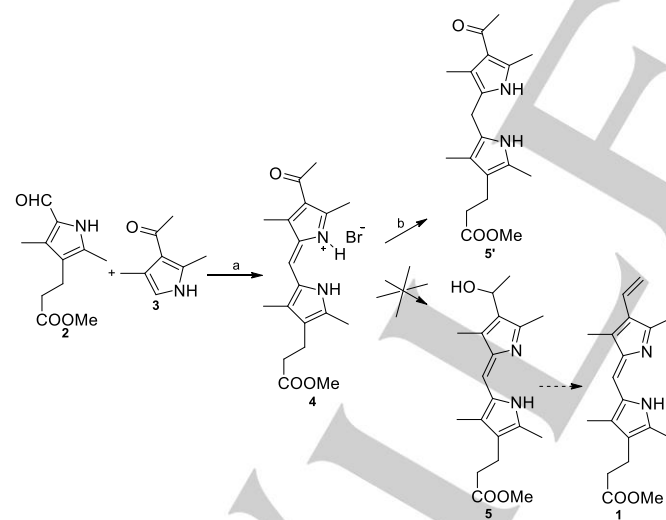
We report herein the first convergent synthesis of this dipyrin derivative starting from the known 2-formylpyrrole **2** (Scheme 1) [13]. Additionally, we synthesized two other symmetric hexasubstituted dipyrins **18** and **19** and report their cellular activities on the YAP(TAZ)/TEAD complexes. Chemistry of dipyrins [14] is directly connected to the synthesis of porphyrins and the access to dipyrinato complexes possessing remarkable optical properties, however nothing is known about the biological properties of this class of molecules.

The biological evaluation has been made in two distinct steps: firstly, as a reference, we measured the effect of the inhibition of expression YAP and/or TAZ by siRNA in MDA-MB-231 cells on the expression of some relevant target genes (Cyr61, CTGF and AXL). In a second time, we measured the biological effect of VP and related protoporphyrins and dipyrins on by using two different methods: 1- measurement of TEAD transcriptional activity using 8xGTIC-luciferase reporter assay [15], 2- evaluation of the protein levels of Cyr61, CTGF and AXL.

## Results and Discussion

## Chemistry

Our first strategy to obtain the dipyrin **1** (Scheme 1) was inspired from the total synthesis of hematoporphyrin published by Martin *et al.* [16].

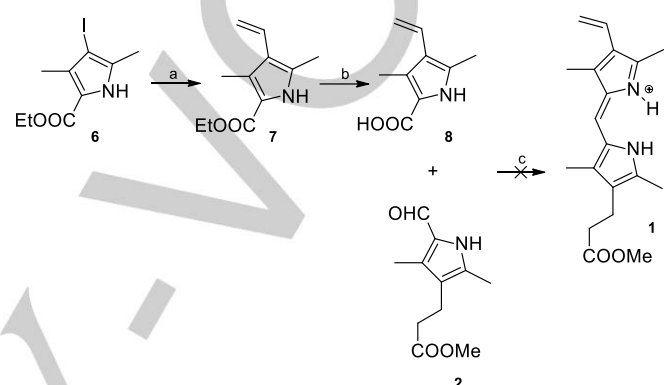


**Scheme 1.** (a) HBr 33% in AcOH (8 eq.), MeOH, rt, 1 h, 89%; (b) CeCl<sub>3</sub>·7H<sub>2</sub>O (1 eq.), NaBH<sub>4</sub> (2 eq.), dioxane/propan-2-ol, 100 °C, 20 h, 75%.

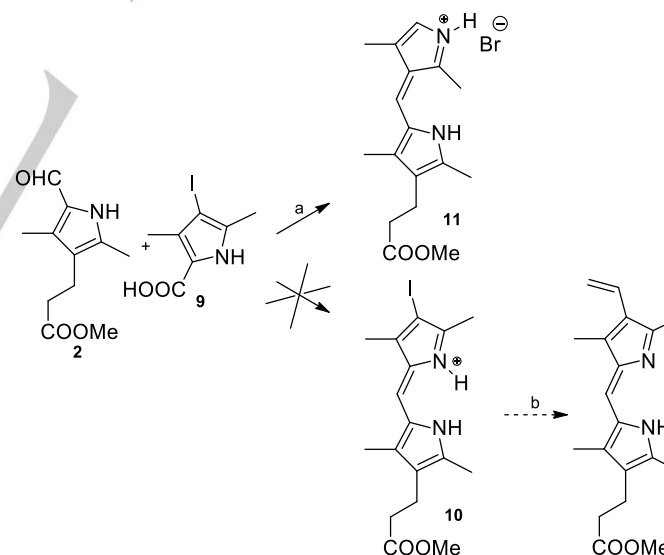
The condensation of the two pyrrolo units **2** [17] and **3** [18] using HBr in acetic acid gave the dipyrin **4** with high yield (89%) but the reduction of the acetyl group in presence of NaBH<sub>4</sub> [15] alone or with CeCl<sub>3</sub> [19] yielded the dipyrromethane **5'** instead of the expected alcohol **5**.

In a second strategy (Scheme 2), we planned to obtain the target compound **1** through the similar condensation of the pyrrolo units **2** and **8**. Firstly the vinylpyrrole **8** was obtained via the Stille vinylation [20] of the iodopyrrole **6**. Unfortunately, vinylpyrrole **8** was found to be unstable under the acidic conditions required for the dipyrin synthesis.

The third strategy (Scheme 3) consisted in the ultimate Stille vinylation of the iododipyrin **10** which could be issued from the condensation of the formylpyrrole **2** and the iodopyrrole **9** [21, 22]. Unfortunately, the condensation of the two pyrrolo units **2** and **7** yielded the unexpected diiododipyrin **11**.



**Scheme 2.** (a) Vinyltributyltin (2 eq.), Pd(PPh<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub> (0.05 eq.), toluene, 110 °C, 2 h, 48%; (b) LiOH (8 eq.), EtOH/H<sub>2</sub>O, 90 °C, 8 h, 32%; (c) HBr 33% in AcOH (8 eq.), MeOH, rt, 1h.

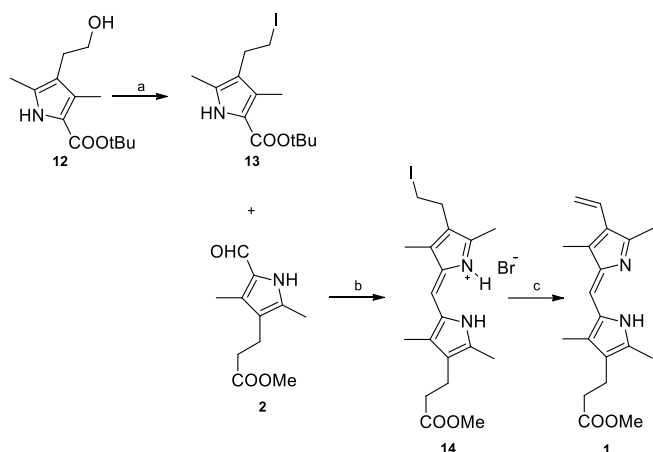


**Scheme 3.** (a) HBr 33% in AcOH (8 eq.), MeOH, rt, 1 h, 75%; (b) Vinyltributyltin (2 eq.), Pd(PPh<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub> (0.05 eq.), toluene, 110 °C, 2 h.

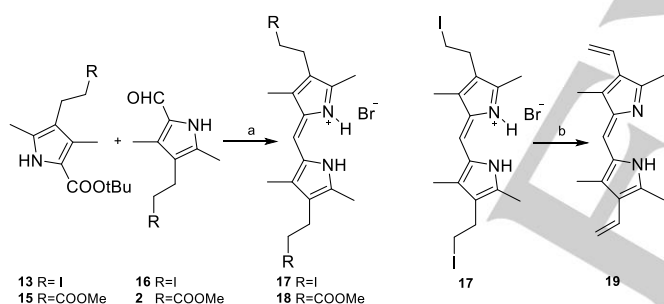
Our fourth strategy (Scheme 4) was based on the condensation of the formylpyrrole **2** and the iodoethylpyrrole **13** (synthesized from the known hydroxyethylpyrrole **12** [23]). Base-induced dehydroiodation of the yielded iodoethylpyrrole **14** gave successfully the target dipyrin **1** with a moderate yield (47%). Symmetric tetramethyldi(3-methoxy-3-oxopropyl)dipyrin **18** and di(2-iodoethyl)tetramethyldipyrin **17** were also synthesized using

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the same strategy than for the synthesis of **14** (Scheme 5). **18** was obtained with the same yield as recently reported by Lund and Thompson, who synthesized symmetric dipyrin hydrobromides from 2-formylpyrroles [24]. The double dehydroiodation of di(2-iodoethyl)tetramethyldipyrin **17** using DBU gave tetramethyldivinylidipyrin **19** with a modest 36% yield. (Scheme 5).



**Scheme 4.** (a) PPh<sub>3</sub> (2 eq.), Imidazole (2 eq.), I<sub>2</sub> (2 eq.), DCM, rt, 20 h, 83%; (b) i) TFA (44 eq.), rt, 30 min ii) HBr 33% in AcOH (8 eq.), MeOH, rt, 1 h, 63%; (c) DBU (2.5 eq.), DCM, rt, 20 h, 47%.



**Scheme 5.** (a) HBr 33% in AcOH (8 eq.), MeOH, rt, 1 h, 65% for **17** and 68% for **18**; (b) DBU (4 eq.), DCM, rt, 20 h, 36%.

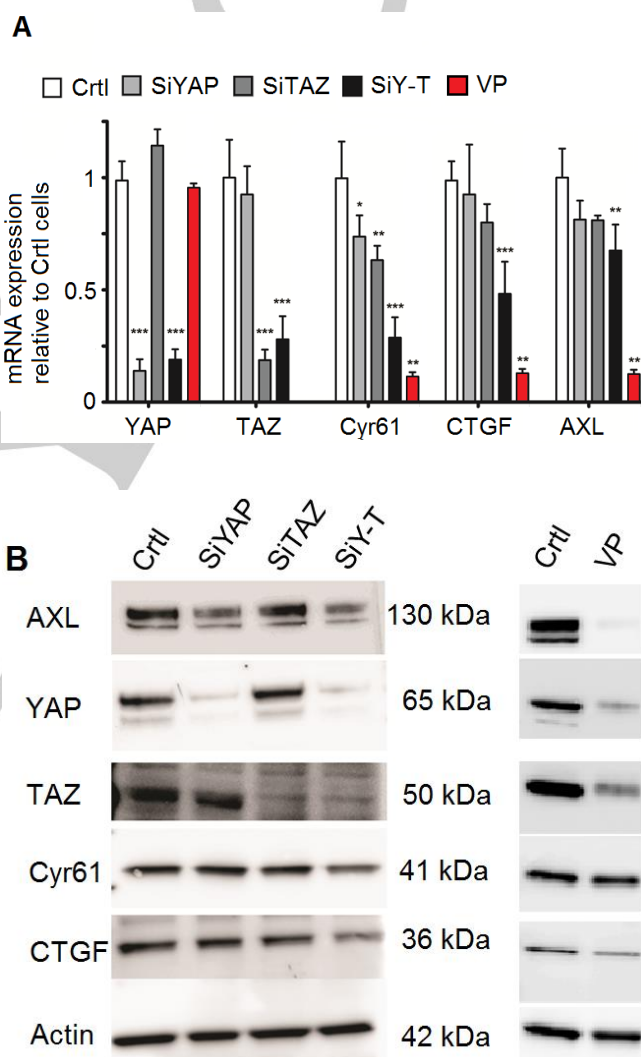
## Biology

YAP and TAZ are transcriptional coactivators that function as effectors of the Hippo pathway. To maintain Hippo pathway homeostasis, TAZ accumulation can be negatively regulated by YAP abundance [25]. More recently, Moroishi *et al.* [26] reported a reciprocal negative regulation between these two coactivators in MCF10A cells, through TEAD-dependent transcription. Submitted to oxidative stress, differences were found between YAP and TAZ. TAZ was less sensitive to oxidative stress than YAP [27]. For these reasons, we focused our attention on the effects of VP, PPIX and dipyrins on the two Hippo pathway effectors (YAP and TAZ) and on the YAP(TAZ)/TEAD gene targets (Axl, Cyr61 and CTGF).

Depending on the cell lines, the Hippo pathway effectors can be differently expressed. This has been clearly shown on different colon cancer cell lines by some of us [15] that HT-29 cells expressed mainly YAP whereas HCT-116 or RKO cells

expressed both YAP and TAZ. Moreover, the relative TEAD activity is also cell line-dependent. We selected MDA-MB-231 breast cancer cell line because it similarly expressed YAP and TAZ co-activators, presented a high TEAD transcriptional activity and yielded a high expression of gene targets (Axl, Cyr61 and CTGF).

We first analyzed the expression of mRNA (by RT-qPCR) and proteins (Western Blot) of YAP, TAZ, AXL, Cyr61 and CTGF in the presence of siYAP, siTAZ or both (Si Y-T). Knockdown of either YAP or TAZ decreased the expression of gene targets (Figure 2) but did not show any selectivity of one co-activator. Dual YAP/TAZ knockdown significantly decreased the protein levels of Cyr61, CTGF and Axl.



**Figure 2.** Effects of VP (10  $\mu$ M), YAP, TAZ or YAP/TAZ-silencing on mRNA expression (A) or protein production (B) of Cyr61, CTGF or Axl in MDA-MB-231 cells. For the mRNA expression, results are the average of three independent experiments. Data are presented as mean SEM (n = 3, \*p < 0.1, \*\* p < 0.01, \*\*\* p < 0.001). For protein production, data are representative of 3 independent experiments.

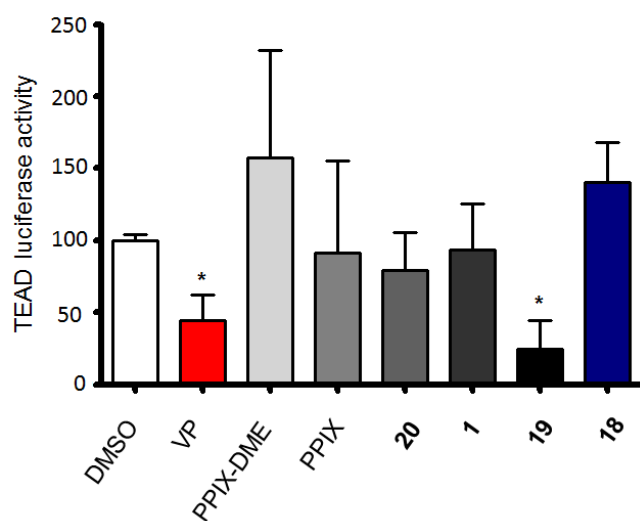
As previously reported [10], VP is a photoactivable molecule and exposure to light must always be stringently avoided during cell treatment. However, even in the absence of light, we observed a decrease in YAP and TAZ levels in western blots. While the RNA

expression of YAP is not affected (Figure 2A), its protein production decreases by 74%. This already reported proteotoxicity [11] was also observed TAZ (Figure 2B) even if TAZ could be considered to be less sensitive to oxidative stress conditions [27]. The reduction of YAP expression was previously reported in hepatocellular carcinoma cells treated by a 1,2-dithiole-3-thione derivative [28], known to generate *in vitro* superoxide anion [29] and hydrogen peroxide [30].

VP (10  $\mu$ M) efficiently decreased the mRNA expression of the downstream gene targets (Figure 3A). At the protein levels, AXL, and CTGF appeared mostly affected (Figure 3B). CTGF was already found to be more sensitive than Cyr61 in ovarian cancer cells treated by VP [31]. Inversely, VP affects more efficiently Cyr61 than CTGF in retinoblastoma cells [32]. It may be suggested that VP could also affect Axl, and CTGF levels through its proteotoxic properties.

Using the TEAD luciferase assay, we measured the ability of VP and related compounds to inhibit the TEAD activity (Figure 3). We found that VP at a 10  $\mu$ M concentration inhibited this activity by 50%. Tested protoporphyrins and dipyrriins did not show significant effect except dipyrriin **19**.

Conversely to the results obtained in HEK293 cells [9], we did not observe a significant decrease of the TEAD luciferase activity following PPIX (or PPIX-DME) treatment.

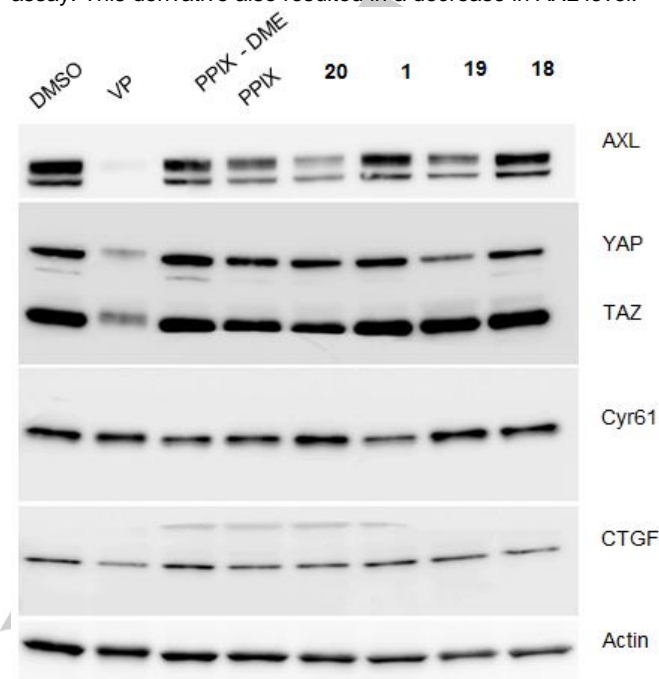


**Figure 3.** Inhibition of YAP(TAZ)/TEAD interaction by VP, protoporphyrins and dipyrriins. MDA-MB-231 cells expressing 8xGTIIIC luciferase were incubated for 48h with tested drugs (10  $\mu$ M). Data are representative of three independent experiments in duplicate (Data are presented as mean SEM (n = 6, \*p < 0.1).

We then analyzed the effect of PPIX, PPIX-DME and dipyrriin derivatives on the expression of YAP, TAZ and their target genes. Conversely to VP, PPIX, PPIX-DME did not induce a dramatic decrease in YAP and TAZ (Figure 4). This may be related to the expected weaker proteotoxicity of both compounds, which possess a significantly lowest singlet oxygen quantum yield than VP [3].

Dipyrriin **1**, the western part of protoporphyrin IX dimethyl ester (PPIX-DME) did not show any significant changes. But interestingly, symmetric dipyrriin **19** containing two vinyl groups significantly decreased the level of YAP whereas TAZ level

remained at a similar level. This result is well connected to the fact that dipyrriin **19** is the only dipyrriin derivative able to induce a decrease in TEAD transcriptional activity in the TEAD luciferase assay. This derivative also resulted in a decrease in AXL level.



**Figure 4.** Effects of porphyrins (left) and dipyrriins (right) on YAP, TAZ, Cyr61, CTGF and Axl levels. MDA-MB-231 cells were incubated in the presence of the drug (10  $\mu$ M) for 48h. Actin was used as loading control. Data are representative of 3 independent experiments.

## Conclusion

We successfully synthesized the dipyrriin western part of protoporphyrin IX, dipyrriin **1**, from the known tert-butyl 4-(2-hydroxyethyl)-3,5-dimethyl-1H-pyrrole-2-carboxylate. The sensitive vinyl group was created at the final stage by a dehydroiodation reaction. We submitted dipyrriin **1** to Diels-Alder reaction conditions but failed to obtain the western part of VP due to the instability of **1** to prolonged heat at toluene boiling point. This dipyrriin **1** could be useful to study other biological properties. Using the same strategy, the divinyl-dipyrriin **19** was synthesized. Independently to its mechanism of action, it has been well-established in several animal models that VP administration allows to control efficiently the organ growth via the Hippo pathway. VP has been firstly reported as a YAP/TEAD complex inhibitor whereas its ability to oligomerize several cellular proteins could correspond to a low protein selectivity [11].

We observed that VP significantly inhibits the TEAD luciferase activity in agreement to previously reported data [9]. However, we also observed that VP decreases the expression of YAP and TAZ, and such effect must result in a decrease in YAP/TEAD co-transcriptional activity. It has been reported that VP can induce the formation of high molecular weight oligomers through a cross-linking of proteins potentiated by oxidative stress [10]. The decrease in YAP and TAZ here observed after VP treatment might result from oligomerization of these proteins. In addition, we identified here a compound, dipyrriin **19**, able to decrease YAP level and TEAD transcriptional activity. It may be suggested that

such effect of dipyrin **19** can be due the addition of two vinyl groups to the dipyrin skeleton resulting in proteotoxicity.

Altogether, these data suggest that in function of their chemical structure, porphyrin- and dipyrin-related derivatives can directly target YAP and/or TAZ proteins and inhibits TEAD transcriptional activity.

VP, which is mainly localized in mitochondria, might also affected the YAP and TAZ expression via the mitochondrial fusion/Hippo pathway cross-talking [33]. This link between Hippo pathway and mitochondria fusion could be of significance to cancer biology [34].

## Experimental Section

### Biology

#### Human breast cancer cell line

MDA-MB-231 cell line was purchased from the American Type Culture Collection and cultivated in DMEM media containing 10% of heat inactivated FBS, L-glutamine and penicillin/streptomycin.

#### Western blotting

Total extracts of cells were obtained with a RIPA based buffer containing protease and phosphatase inhibitors (Roche). Western blot were carried out using 20 µg of protein lysates with the NuPage Electrophoresis and Iblot transfer systems (Life Technologies). β-Actin was used as loading control for total extracts.

#### TEAD transcriptional activity

Cells at 50% confluence were transfected with the TEAD luciferase reporter plasmid 8XGTIC-Luciferase (Addgene reference 34615) vs a control luciferase plasmid. After 48 hours post transfection, cells are lysed in Reporter Lysis Buffer (Promega) and luciferase activity was measured on the Mithras LB940 plate reader and normalized to protein concentration. mRNA expression

mRNAs were extracted from cultured cells or human tumors with the NucleoSpin kit (Macherey-Nagel). Retrotranscription was done on 1 µg of mRNA accordingly to the Advantage RT-for-PCR Kit protocol (Clontech). PCR was performed using SsoFast™ Evagreen Supermix kit following the manufacturer's protocol using the CFX96 real time PCR system (Bio-Rad). To monitor any change in mRNA expression, we used the ΔΔCt method between one condition and a control condition after normalization with the housekeeper gene *RPLP0*. Each sample was done in triplicate.

### Chemistry

Chemicals and solvents were purchased from various suppliers (Sigma-Aldrich, Alfa Aesar, Fisher, VWR) and used without purification.

The reaction monitoring was performed by thin layer chromatography (TLC) on Macherey-Nagel Alugram® Sil 60/UV254 (thickness 0.2 mm). TLC were revealed by UV (λ = 254 nm) and/or the appropriate stain.

Purification of the compounds was carried out by column chromatography (flash or manual). Manual chromatography was performed using Macherey-Nagel silica gel (0.04-0.063 mm of particule size). Flash chromatography was performed on a Reveleris® Flash Chromatography System using Macherey-Nagel Chromabond flash RS columns.

NMR spectra were recorded on a Bruker DRX 300 spectrometer (operating at 300 MHz for <sup>1</sup>H and 75 MHz for <sup>13</sup>C). Chemical shifts are expressed in ppm relative to tetramethylsilane (TMS) or to residual proton signal in deuterated solvents. Chemical shifts are reported as position (δ in ppm), multiplicity (s = singulet, d = doublet, t = triplet, q = quartet, m = massif, br = broad), coupling constant (J in Hz), relative integral and assignment. The attributions of protons and carbons were achieved by analysis of 1D and 2D experiments (<sup>1</sup>H, <sup>13</sup>C, COSY, HSQC and HMBC).

LC-MS were performed on a Varian triple quadrupole 1200W mass spectrometer equipped with a non-polar C18 TSK-gel Super ODS (4.6 x 50 mm) column, using electrospray ionisation and a UV detector (diode array). Elution was performed at a flow rate of 2 mL/min with water-formic acid (pH = 3.8) as eluent A and ACN-formic acid (pH = 3.8) as eluent B, employing a 0.25 min plateau with 0 % B and a linear gradient from 0 % B to 98 % B in 3.25 min, followed by a 0.5 min plateau with 98 % B. Then, column re-equilibration was performed for 1 min. The injection duty cycle was 5 min, taking into account the column equilibration time.

The melting point analyses were performed on Barnstead Electrothermel Melting Point Series IA9200.

The UV-vis spectra were recorded using a NanoDrop spectrometer (ThermoFisherScientific). Characteristic peaks are given in nm and classified according to their relative intensity (s strong, m medium, w weak).

#### (Z)-4-Acetyl-2-((4-(3-methoxy-3-oxopropyl)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-3,5-dimethyl-2H-pyrrolium bromide (**4**)

A solution of 33% HBr in AcOH (2.7 eq., 497 mg, 0.3 mL, 2.9 mmol) was added dropwise to a solution of methyl 3-(5-formyl-2,4-dimethyl-1H-pyrrol-3-yl)propanoate [17] (1 eq., 228 mg, 1.1 mmol) and 3-acetyl-2,4-dimethylpyrrole [18] (1 eq., 150 mg, 1.1 mmol) in MeOH (3 mL) at room temperature under nitrogen atmosphere. The mixture was stirred at room temperature for 4 h and the product was collected by filtration. The residue was used without further purification in the next step.

The title compound (400 mg, 0.97 mmol, 89%) was obtained as an orange solid.

<sup>1</sup>H NMR (300 MHz), δ (ppm, CDCl<sub>3</sub>): 2.37 (s, 3 H, CH<sub>3</sub>), 2.48-2.53 (m, 5 H, CH<sub>2</sub> + CH<sub>3</sub>), 2.59 (s, 3 H, CH<sub>3</sub>), 2.77-2.82 (m, 5 H, CH<sub>2</sub> + CH<sub>3</sub>), 2.98 (s, 3 H, CH<sub>3</sub>), 3.68 (s, 3 H, OCH<sub>3</sub>), 7.26 (s, 1 H, CH), 13.22 (s, 1 H, NH), 13.69 (s, 1 H, NH).

<sup>13</sup>C NMR (75 MHz), δ (ppm, CDCl<sub>3</sub>): 10.4 (CH<sub>3</sub>), 12.7 (CH<sub>3</sub>), 13.5 (CH<sub>3</sub>), 15.8 (CH<sub>3</sub>), 19.2 (CH<sub>2</sub>), 31.6 (CH<sub>3</sub>), 33.4 (CH<sub>2</sub>), 51.9 (OCH<sub>3</sub>), 120.6 (CH), 126.8, 127.8, 129.4, 132.7, 143.4, 144.8, 154.3, 160.5, 172.5 (CO), 203.5 (CO).

LC-MS (ESI) m/z Calculated: 408.10, Found: 329.16 [M-HBr+H]<sup>+</sup>; t<sub>R</sub> = 2.15

#### Methyl 3-(5-((4-acetyl-3,5-dimethyl-1H-pyrrol-2-yl)methyl)-2,4-dimethyl-1H-pyrrol-3-yl)propanoate (**5'**)

Dipyrin **4** (1 eq., 0.19 g, 0.46 mmol) was dissolved in a mixture of DCM (28 mL) and MeOH (7 mL). Sodium borohydride (5.0 eq., 88 mg, 2.3 mmol) was added in one portion and the mixture was stirred at room temperature for 2 h. Then the solution was washed with saturated aqueous bicarbonate solution and brine, dried over sodium sulfate and concentrated under vacuum. The residue was purified by flash chromatography (DCM/MeOH, 95:5 (v/v), NH<sub>3</sub>g sat). The title compound (115 mg, 0.35 mmol, 75%) was obtained as an orange solid.

<sup>1</sup>H NMR (300 MHz), δ (ppm, CDCl<sub>3</sub>): 1.98 (s, 3 H, CH<sub>3</sub>), 2.11 (s, 3 H, CH<sub>3</sub>), 2.26 (s, 3 H, CH<sub>3</sub>), 2.41 (s, 3 H, CH<sub>3</sub>), 2.43 (s, 3 H, CH<sub>3</sub>), 2.46 (t, J = 8.4 Hz, 2 H, CH<sub>2</sub>), 2.70 (t, J = 8.4 Hz, 2 H, CH<sub>2</sub>), 3.68 (s, 3 H, OCH<sub>3</sub>), 3.75 (s, 2 H, CH<sub>2</sub>), 8.05 (bs, 1 H, NH), 8.95 (bs, 1 H, NH).

<sup>13</sup>C NMR (75 MHz), δ (ppm, CDCl<sub>3</sub>): 9.5 (CH<sub>3</sub>), 11.3 (CH<sub>3</sub>), 12.3 (CH<sub>3</sub>), 15.5 (CH<sub>3</sub>), 20.6 (CH<sub>2</sub>), 22.0 (CH<sub>2</sub>), 31.2 (CH<sub>3</sub>), 35.9 (CH<sub>2</sub>), 51.9 (OCH<sub>3</sub>), 113.8, 115.7, 117.0, 121.6, 122.5, 122.8, 125.3, 135.0, 174.5 (CO), 196.5 (CO).

#### Ethyl 4-iodo-3,5-dimethyl-1H-pyrrole-2-carboxylate (**6**)

To a solution of N-chlorosuccinimide (1.2 eq., 0.96 g, 7.2 mmol) in DMF (20 mL) was added NaI (1.2 eq., 1.08 g, 7.2 mmol) in small portions. The resulting brown solution was stirred at room temperature for 1 h before the slow addition at 0 °C of ethyl 3,5-dimethyl-1H-pyrrole-2-carboxylate (1 eq., 1 g, 6.0 mmol) in DMF (20 mL). The reaction was stirred at room temperature for 1 h. Aqueous 10% sodium thiosulfate solution (20 mL) was added and the mixture was stirred for 1 h. The solid was collected by filtration. The residue was used without further purification in the next step.

The title compound (1.61 g, 5.5 mmol, 92%) was obtained as a white solid. <sup>1</sup>H NMR (300 MHz), δ (ppm, CDCl<sub>3</sub>): 1.36 (t, J = 1.7 Hz, 3 H, CH<sub>3</sub>), 2.29 (s, 3 H, CH<sub>3</sub>), 2.29 (s, 3 H, CH<sub>3</sub>), 4.33 (q, J = 1.7 Hz, 2 H, CH<sub>2</sub>), 8.96 (s, 1 H, NH).

<sup>13</sup>C NMR (75 MHz), δ (ppm, CDCl<sub>3</sub>): 14.3 (CH<sub>3</sub>), 14.5 (CH<sub>3</sub>), 14.7 (CH<sub>3</sub>), 60.3 (CH<sub>2</sub>), 72.0 (C), 118.3 (C), 130.7 (C), 134.6 (C), 161.4 (CO).

LC-MS (ESI) m/z Calculated: 292.99, Found: 291.99 [M-H]<sup>-</sup>; t<sub>R</sub> = 3.14 min

#### Ethyl 3,5-dimethyl-4-vinyl-1H-pyrrole-2-carboxylate (**7**)

A mixture of ethyl 4-iodo-3,5-dimethyl-1H-pyrrole-2-carboxylate (1 eq., 1.00 g, 3.4 mmol), vinyltributyltin (2 eq., 2.16 g, 2.0 mL, 6.8 mmol) and bis(triphenylphosphine)palladium(II) dichloride (5 %, 0.12 g, 0.17 mmol) in toluene (50 mL) was refluxed for 2 h under a nitrogen atmosphere. During the course of the reaction, the color changed from yellow to black as Pd<sup>0</sup> was formed. The reaction was cooled to room temperature and DCM (50 mL) was added. The resulting solution was washed with saturated aqueous NaCl (50 mL) and water (50 mL). The organic phase was separated, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum. The residue was purified by flash chromatography (DCM/MeOH, 95:5 (v/v)).

The title compound (317 mg, 1.6 mmol, 48%) was obtained as a dark brown solid.

<sup>1</sup>H NMR (300 MHz), δ (ppm, CDCl<sub>3</sub>): 1.36 (t, J = 7.1 Hz, 3 H, CH<sub>3</sub>), 2.32 (s, 3 H, CH<sub>3</sub>), 2.39 (s, 3 H, CH<sub>3</sub>), 4.31 (q, J = 7.1 Hz, 2 H, CH<sub>2</sub>), 5.17 (dd, J = 1.7 Hz, J<sub>cis</sub> = 11.6 Hz, 1 H, CH<sub>2</sub>), 5.28 (dd, J = 1.7 Hz, J<sub>trans</sub> = 17.9 Hz, 1 H, CH<sub>2</sub>), 6.59 (dd, J<sub>trans</sub> = 17.9 Hz, J<sub>cis</sub> = 11.6 Hz, 1 H, CH), 8.62 (s, 1 H, NH).

<sup>13</sup>C NMR (75 MHz),  $\delta$  (ppm, CDCl<sub>3</sub>): 11.2 (CH<sub>3</sub>), 12.8 (CH<sub>3</sub>), 14.5 (CH<sub>3</sub>), 59.8 (CH<sub>2</sub>), 112.7 (CH<sub>2</sub>), 125.3 (CH), 128.2 (C), 129.0 (C), 133.6 (C), 139.3 (C), 161.6 (CO).

#### 3,5-Dimethyl-4-vinyl-1H-pyrrole-2-carboxylic acid (**8**)

Ethyl 4-ethenyl-3,5-dimethyl-1H-pyrrole-2-carboxylate (1 eq., 158 mg, 0.82 mmol) and LiOH (8 eq., 156 mg, 6.5 mmol) were dissolved in EtOH (2 mL) and H<sub>2</sub>O (2 mL). The solution was heated at 90 °C for 1 h. The solvent was evaporated under vacuum. The residue was taken up in EtOAc (5 mL) and water (5 mL). The aqueous layer was acidified until pH 5.0 with H<sub>3</sub>BO<sub>3</sub> (pH 5.1) or a few drops of concentrate H<sub>2</sub>SO<sub>4</sub> before being extracted with EtOAc. Organics layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum. The residue was used without further purification in the next step.

The title compound (43 mg, 0.3 mmol, 32%) was obtained as a dark brown solid.

<sup>1</sup>H NMR (300 MHz),  $\delta$  (ppm, Acetone-d<sub>6</sub>): 2.32 (s, 3 H, CH<sub>3</sub>), 2.36 (s, 3 H, CH<sub>3</sub>), 5.07 (dd,  $J = 1.8$  Hz,  $J_{cis} = 11.6$  Hz, 1 H, CH<sub>2</sub>), 5.25 (dd,  $J = 1.8$  Hz,  $J_{trans} = 17.9$  Hz, 1 H, CH<sub>2</sub>), 6.61 (dd,  $J_{trans} = 17.9$  Hz,  $J_{cis} = 11.6$  Hz, 1 H, CH), 10.24 (s, 1 H, NH).

<sup>13</sup>C NMR (75 MHz),  $\delta$  (ppm, CDCl<sub>3</sub>): 10.5 (CH<sub>3</sub>), 11.5 (CH<sub>3</sub>), 111.0 (CH<sub>2</sub>), 117.7 (C), 126.0 (C), 129.4 (CH), 131.2 (C), 132.6 (C), 164.1 (CO).

#### 4-iodo-3,5-dimethyl-1H-pyrrole-2-carboxylic acid (**9**)

Ethyl 4-iodo-3,5-dimethyl-1H-pyrrole-2-carboxylate (1 eq., 500 mg, 1.7 mmol) and LiOH (8 eq., 326 mg, 13.6 mmol) were dissolved in EtOH (5 mL) and H<sub>2</sub>O (5 mL). The solution was heated at 90 °C for 1 h. The solvent was evaporated under vacuum. The residue was taken up in EtOAc (50 mL) and water (50 mL). The aqueous layer was acidified to pH 5.0 with H<sub>3</sub>BO<sub>3</sub> (pH 5.1) or few drops of concentrate H<sub>2</sub>SO<sub>4</sub> before being extracted with EtOAc. Organics layers were dried over sodium sulfate and concentrated under vacuum. The residue was purified by flash chromatography (DCM/MeOH, 95:5 (v/v)).

The title compound (399 mg, 1.5 mmol, 88%) was obtained as a dark pink solid.

<sup>1</sup>H NMR (300 MHz),  $\delta$  (ppm, Acetone-d<sub>6</sub>): 2.25 (s, 3 H, CH<sub>3</sub>), 2.27 (s, 3 H, CH<sub>3</sub>), 10.82 (s, 1 H, OH).

<sup>13</sup>C NMR (75 MHz),  $\delta$  (ppm, CDCl<sub>3</sub>): 13.1 (CH<sub>3</sub>), 13.7 (CH<sub>3</sub>), 70.8 (C), 129.7 (C), 134.3 (C), 134.3 (C), 161.0 (CO).

LC-MS (ESI) *m/z* Calculated: 264.96, Found: 263.92 [M-H]<sup>-</sup>; *t<sub>R</sub>* = 2.50 min

#### (Z)-3-((4-(3-methoxy-3-oxopropyl))-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2,4-dimethyl-3H-pyrrol-1-ium bromide (**11**)

A solution of 48% HBr in water (2.7 eq., 0.18 mL, 1.6 mmol) was added dropwise to a solution of pyrrole **2** (1 eq., 0.12 g, 0.6 mmol) and pyrrole **9** (1 eq., 0.16 g, 0.6 mmol) in methanol (1.5 mL). The mixture was stirred for 1 h at room temperature and concentrated under vacuum. The residue was purified by flash chromatography (DCM/MeOH, 95:5 (v/v)).

The title compound (65 mg, 0.2 mmol, 30%) was obtained as a dark brown solid.

<sup>1</sup>H NMR (300 MHz),  $\delta$  (ppm, CDCl<sub>3</sub>): 2.25 (s, 3 H, CH<sub>3</sub>), 2.29 (s, 3 H, CH<sub>3</sub>), 2.38 (t,  $J = 7.3$  Hz, 2 H, CH<sub>2</sub>), 2.58 (bs, 6 H, 2 CH<sub>3</sub>), 2.68 (t,  $J = 7.3$  Hz, 2 H, CH<sub>2</sub>), 3.60 (s, 3 H, OCH<sub>3</sub>), 6.08 (s, 1 H, CH), 7.01 (s, 1 H, CH), 13.00 (bs, 1 H, NH).

<sup>13</sup>C NMR (75 MHz),  $\delta$  (ppm, CDCl<sub>3</sub>): 10.2 (CH<sub>3</sub>), 12.1 (CH<sub>3</sub>), 12.8 (CH<sub>3</sub>), 14.4 (CH<sub>3</sub>), 14.2 (CH<sub>2</sub>), 33.7 (CH<sub>2</sub>), 51.8 (OCH<sub>3</sub>), 117.2 (CH), 119.6 (CH), 126.2 (C), 126.7 (C), 127.0 (C), 142.7 (C), 145.6 (C), 154.5 (C), 154.7 (C), 172.6 (CO).

#### *Tert*-butyl 4-(2-iodoethyl)-3,5-dimethyl-1H-pyrrole-2-carboxylate (**13**)

*Tert*-butyl 4-(2-hydroxyethyl)-3,5-dimethyl-1H-pyrrole-2-carboxylate (1 eq., 100 mg, 0.42 mmol), imidazole (2 eq., 56.9 mg, 0.84 mmol) and triphenylphosphine (2 eq., 219 mg, 0.84 mmol) were dissolved in DCM (5 mL) under a nitrogen atmosphere at room temperature. After complete dissolution, iodine (2 eq., 212 mg, 0.84 mmol) was added portionwise at 0 °C. The mixture was stirred overnight at room temperature. The reaction was quenched with aqueous 10% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution (10 mL). The aqueous layer was extracted with DCM (3 x 10 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum. The residue was purified by flash chromatography (Cyclohexane/EtOAc, 7:3 (v/v)).

The title compound (138 mg, 0.4 mmol, 95%) was obtained as a white solid.

<sup>1</sup>H NMR (300 MHz),  $\delta$  (ppm, CDCl<sub>3</sub>): 1.56 (s, 9 H, 3 CH<sub>3</sub>), 2.22 (s, 3 H, CH<sub>3</sub>), 2.24 (s, 3 H, CH<sub>3</sub>), 2.95 (t,  $J = 7.9$  Hz, 2 H, CH<sub>2</sub>), 3.14 (t,  $J = 8.3$  Hz, 2 H, CH<sub>2</sub>), 8.64 (s, 1 H, NH).

<sup>13</sup>C NMR (75 MHz),  $\delta$  (ppm, CDCl<sub>3</sub>): 5.6 (CH<sub>2</sub>), 10.6 (CH<sub>3</sub>), 11.6 (CH<sub>3</sub>), 28.5 (3 CH<sub>3</sub>), 29.3 (CH<sub>2</sub>), 80.4 (C), 118.5 (C), 121.0 (C), 125.7 (C), 129.1 (C), 161.1 (CO).

LC-MS (ESI) *m/z* Calculated: 349.05, Found: 295.07 [M-tBu+H]<sup>+</sup>, *t<sub>R</sub>* = 3.39 min

#### (Z)-4-(2-Iodoethyl)-2-((4-(3-methoxy-3-oxopropyl))-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-3,5-dimethyl-2H-pyrrol-ium bromide (**14**)

TFA (44.4 eq., 1.15 mL, 25.5 mmol) was added dropwise to a solution of *tert*-butyl 4-(2-iodoethyl)-3,5-dimethyl-1H-pyrrole-2-carboxylate (1 eq., 200 mg, 0.57 mmol) at room temperature under a nitrogen atmosphere. The solution was stirred for 30 min. Then, a solution of methyl 3-(5-formyl-2,4-dimethyl-1H-pyrrol-3-yl)propanoate (1 eq., 119 mg, 0.57 mmol) in MeOH (2.3 mL) was added dropwise, followed by 33% HBr solution in AcOH (8.99 eq., 1263 mg, 0.86 mL, 5.1 mmol). The mixture was stirred at room temperature for 1 h and the product was collected by filtration. The residue was used without further purification in the next step.

The title compound (200 mg, 0.38 mmol, 67%) was obtained as an orange solid.

<sup>1</sup>H NMR (300 MHz),  $\delta$  (ppm, CDCl<sub>3</sub>): 2.31 (s, 6 H, 2 CH<sub>3</sub>), 2.47 (t,  $J = 7.4$  Hz, 2 H, CH<sub>2</sub>), 2.71 (s, 6 H, 2 CH<sub>3</sub>), 2.76 (t,  $J = 7.3$  Hz, 2 H, CH<sub>2</sub>), 3.02 (t,  $J = 6.9$  Hz, 2 H, CH<sub>2</sub>), 3.19 (t,  $J = 6.9$  Hz, 2 H, CH<sub>2</sub>), 3.67 (s, 3 H, CH<sub>3</sub>), 7.08 (s, 1 H, CH), 13.11 (s, 1 H, NH).

<sup>13</sup>C NMR (75 MHz),  $\delta$  (ppm, CDCl<sub>3</sub>): 3.4 (CH<sub>2</sub>), 10.2 (CH<sub>3</sub>), 10.4 (CH<sub>3</sub>), 13.0 (2 CH<sub>3</sub>), 19.3 (CH<sub>2</sub>), 28.3 (CH<sub>2</sub>), 33.7 (CH<sub>2</sub>), 51.8 (OCH<sub>3</sub>), 119.4 (CH), 126.0 (C), 126.4 (C), 127.3 (C), 127.4 (C), 142.1 (C), 142.9 (C), 153.4 (C), 155.0 (C), 172.6 (CO).

LC-MS (ESI) *m/z* Calculated: 520.02, Found: 441.03 [M-HBr+H]<sup>+</sup>, *t<sub>R</sub>* = 2.73 min

#### (Z)-Methyl 3-(5-((3,5-dimethyl-4-vinyl-2H-pyrrol-2-ylidene)methyl)-2,4-dimethyl-1H-pyrrol-3-yl)propanoate (**1**)

(Z)-4-(2-Iodoethyl)-2-((4-(3-methoxy-3-oxopropyl))-3,5-dimethyl-1H-pyrrol-2-yl)methylidene)-3,5-dimethyl-2H-pyrrol-1-ium bromide (1 eq., 510 mg, 0.98 mmol) was dissolved in dry DCM (10.9 mL) under nitrogen atmosphere. DBU (2.5 eq., 0.36 mL, 2.4 mmol) was added dropwise at room temperature and the mixture was heated at 50 °C. After complete reaction, the solvent was evaporated under vacuum and the residue was purified by flash column chromatography with DCM/MeOH (95:5).

The title compound (143 mg, 0.45 mmol, 47%) was obtained as a dark brown solid.

UV-vis (Acetonitrile)  $\lambda_{max}$  (nm) 490 (s), 370 (w), 291 (w), 224 (m).

<sup>1</sup>H NMR (300 MHz),  $\delta$  (ppm, CDCl<sub>3</sub>): 2.32 (s, 3 H, CH<sub>3</sub>), 2.37 (s, 3 H, CH<sub>3</sub>), 2.47 (t,  $J = 1.5$  Hz, 2 H, CH<sub>2</sub>), 2.72 (s, 3 H, CH<sub>3</sub>), 2.76 (t,  $J = 1.5$  Hz, 2 H, CH<sub>2</sub>), 2.80 (s, 3 H, CH<sub>3</sub>), 5.40 (dd,  $J = 1.1$  Hz,  $J_{cis} = 7.7$  Hz, 1 H, CH<sub>2</sub>), 5.45 (dd,  $J = 1.1$  Hz,  $J_{trans} = 14.1$  Hz, 1 H, CH<sub>2</sub>), 6.54 (dd,  $J_{cis} = 11.6$  Hz,  $J_{trans} = 17.9$  Hz, 1 H, CH), 7.12 (s, 1 H, CH), 12.92 (s, 1 H, NH).

<sup>13</sup>C NMR (75 MHz),  $\delta$  (ppm, CDCl<sub>3</sub>): 10.3 (CH<sub>3</sub>), 11.0 (CH<sub>3</sub>), 13.0 (CH<sub>3</sub>), 14.0 (CH<sub>3</sub>), 19.3 (CH<sub>2</sub>), 33.7 (CH<sub>2</sub>), 51.8 (OCH<sub>3</sub>), 117.4 (CH<sub>2</sub>), 119.4 (CH), 125.8 (C), 126.1 (C), 126.5 (C), 126.7 (C), 127.4 (CH), 141.0 (C), 142.9 (C), 154.0 (C), 155.1 (C), 172.6 (CO).

LC-MS (ESI) *m/z* Calculated: 312.18, Found: 313.14 [M+H]<sup>+</sup>, *t<sub>R</sub>* = 2.65 min

#### 4-(2-Iodoethyl)-3,5-dimethyl-1H-pyrrole-2-carbaldehyde (**16**)

TFA (9 eq., 0.59 mL, 7.7 mmol) was added dropwise to a solution of *tert*-butyl 4-(2-iodoethyl)-3,5-dimethyl-1H-pyrrole-2-carboxylate (1 eq., 0.3 g, 0.9 mmol) in DCM (4 mL) at 0 °C under a nitrogen atmosphere. Trimethyl orthoformate (5 eq., 0.47 mL, 4.3 mmol) was added dropwise and the reaction was stirred under N<sub>2</sub> for 20 min at 0 °C. The solution was warmed to room temperature and stirred for 30 min. The mixture was neutralized carefully with aqueous 10% NaHCO<sub>3</sub> solution and extracted with DCM (3 x 20 mL). The combined organic layers dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum. The residue was purified by flash column chromatography with Cyclohexane/EtOAc (8:2).

The title compound (150 mg, 0.5 mmol, 63 %) was obtained as light brown solid.

<sup>1</sup>H NMR (300 MHz),  $\delta$  (ppm, CDCl<sub>3</sub>): 2.29 (s, 3 H, 3 CH<sub>3</sub>), 2.30 (s, 3 H, CH<sub>3</sub>), 2.97 (t,  $J = 7.9$  Hz, 2 H, CH<sub>2</sub>), 3.17 (t,  $J = 7.4$  Hz, 2 H, CH<sub>2</sub>), 9.47 (s, 1 H, CHO), 10.25 (s, 1 H, NH).

<sup>13</sup>C NMR (75 MHz),  $\delta$  (ppm, CDCl<sub>3</sub>): 4.9 (CH<sub>2</sub>), 9.07 (CH<sub>3</sub>), 11.8 (CH<sub>3</sub>), 28.7 (CH<sub>2</sub>), 117.6 (Cquat), 122.0 (Cquat), 128.0 (Cquat), 139.5 (Cquat), 176.3 (CHO).

#### (Z)-4-(2-Iodoethyl)-2-((4-(2-iodoethyl))-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-3,5-dimethyl-2H-pyrrol-ium bromide (**17**)

TFA (27 eq., 1.1 mL, 14.5 mmol) was added dropwise to a solution of *tert*-butyl 4-(2-iodoethyl)-3,5-dimethyl-1H-pyrrole-2-carboxylate (1 eq., 0.19 g, 0.5 mmol) at room temperature under a nitrogen atmosphere. The solution was stirred for 30 min. Then a solution of 4-(2-iodoethyl)-3,5-dimethyl-1H-pyrrole-2-carbaldehyde (1 eq., 0.15 g, 0.5 mmol) in MeOH (2 mL) was added dropwise, followed by 33% HBr in AcOH (8.6 eq., 0.81 mL, 4.6 mmol). The mixture was stirred at room temperature for 1 h and the product was collected by filtration. The residue was used without further purification in the next step.

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The title compound (0.2 g, 0.3 mmol, 65%) was obtained as a dark red solid.

<sup>1</sup>H NMR (300 MHz),  $\delta$  (ppm, CDCl<sub>3</sub>): 2.31 (s, 3 H, 3 CH<sub>3</sub>), 2.70 (s, 3 H, CH<sub>3</sub>), 3.02 (t,  $J = 7.1$  Hz, 2 H, CH<sub>2</sub>), 3.19 (t,  $J = 7.4$  Hz, 2 H, CH<sub>2</sub>), 7.10 (s, 1 H, CH), 13.12 (s, 1 H, NH).

<sup>13</sup>C NMR (75 MHz),  $\delta$  (ppm, CDCl<sub>3</sub>): 3.3 (2 CH<sub>2</sub>), 10.5 (2 CH<sub>3</sub>), 13.1 (2 CH<sub>3</sub>), 28.3 (2 CH<sub>2</sub>), 119.7 (CH), 126.3 (2 C), 127.7 (2 C), 142.6 (2 C), 154.3 (2 C).

**(Z)-2-((3,5-Dimethyl-4-vinyl-2H-pyrrol-2-ylidene)methyl)-3,5-dimethyl-4-vinyl-1H-pyrrole (19)**

(Z)-4-(2-Iodoethyl)-2-[[4-(2-iodoethyl)-3,5-dimethyl-1H-pyrrol-2-yl]methylidene]-3,5-dimethyl-2H-pyrrol-1-ium bromide (1 eq., 170 mg, 0.3 mmol) was dissolved in dry DCM (3 mL) under a nitrogen atmosphere. DBU (4 eq., 0.172 mL, 1.2 mmol) was added dropwise to the solution at room temperature and the mixture was heated at 50 °C. After complete reaction, the solvent was evaporated under vacuum and the solid residue was purified by flash column chromatography with DCM/MeOH (95:5).

The title compound (26 mg, 0.1 mmol, 36%) was obtained as a brown solid.

UV-vis (Acetonitrile)  $\lambda_{\text{max}}$  (nm) 506 (s), 366 (s), 292 (s), 230 (s).

<sup>1</sup>H NMR (300 MHz),  $\delta$  (ppm, CDCl<sub>3</sub>): 2.40 (s, 6 H, 2 CH<sub>3</sub>), 2.83 (s, 6 H, 2 CH<sub>3</sub>), 5.36-5.49 (m, 4 H, 2 CH<sub>2</sub>), 6.53 (dd,  $J_{\text{cis}} = 11.9$  Hz,  $J_{\text{trans}} = 17.6$  Hz, 2 H, 2 CH) 7.19 (s, 1 H, CH), 12.66 (s, 1 H, NH).

<sup>13</sup>C NMR (75 MHz),  $\delta$  (ppm, CDCl<sub>3</sub>): 11.1 (2 CH<sub>3</sub>), 14.2 (2 CH<sub>3</sub>), 117.8 (2 CH<sub>2</sub>), 119.7 (CH), 126.5 (2 CH), 137.5 (2 C), 141.8 (2 C), 154.0 (2 C), 154.9 (2 C).

LC-MS (ESI) m/z Calculated: 252.16, Found: 253.14 [M+H]<sup>+</sup>, t<sub>R</sub> = 3.31 min

**(Z)-4-(3-Methoxy-3-oxopropyl)-2-((4-(3-methoxy-3-oxopropyl)-3,5-dimethyl-1H-pyrrol-2-yl)methylene)-3,5-dimethyl-2H-pyrrolium bromide (18)**

TFA (44 eq., 1.56 mL, 21.1 mmol) was added dropwise to a solution of 4-(3-methoxy-3-oxopropyl)-3,5-dimethyl-1H-pyrrole-2-carboxylic acid (1 eq., 110 mg, 0.50 mmol) at room temperature under a nitrogen atmosphere. The solution was stirred for 30 min. Then, a solution of methyl 3-(5-formyl-2,4-dimethyl-1H-pyrrol-3-yl)propanoate (1 eq., 100 mg, 0.50 mmol) in MeOH (2.0 mL) was added dropwise, followed by 33% HBr solution in AcOH (8.6 eq., 1.13 g, 0.81 mL, 4.6 mmol). The mixture was stirred at room temperature for 1 h and the product was collected by filtration. The residue was used without further purification in the next step.

The title compound (192 mg, 0.40 mmol, 63%) was obtained as a brown solid.

UV-vis (Acetonitrile)  $\lambda_{\text{max}}$  (nm) 480 (s), 392 (w), 226 (m).

<sup>1</sup>H NMR (300 MHz),  $\delta$  (ppm, CDCl<sub>3</sub>): 2.29 (s, 6 H, 2 CH<sub>3</sub>), 2.47 (t,  $J = 7.5$  Hz, 2 H, CH<sub>2</sub>), 2.69 (s, 6 H, 2 CH<sub>3</sub>), 2.76 (t,  $J = 7.5$  Hz, 2 H, CH<sub>2</sub>), 3.69 (s, 6 H, 2 OCH<sub>3</sub>), 7.05 (s, 1 H, CH), 13.05 (bs, 1 H, NH).

<sup>13</sup>C NMR (75 MHz),  $\delta$  (ppm, CDCl<sub>3</sub>): 10.2 (2 CH<sub>3</sub>), 12.9 (2 CH<sub>3</sub>), 19.3 (2 CH<sub>2</sub>), 33.8 (2 CH<sub>2</sub>), 51.8 (2 OCH<sub>3</sub>), 119.2 (CH), 126.1 (2 C), 126.9 (2 C), 143.2 (2 C), 154.2 (2 C), 172.7 (2 CO).

LC-MS (ESI) m/z Calculated: 452.13, Found: 373.19 [M-HBr+H]<sup>+</sup>, t<sub>R</sub> = 2.52 min.

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**Keywords:** Dipyrrins • verteporfin • non-photoinduced therapy • Hippo pathway • cancer

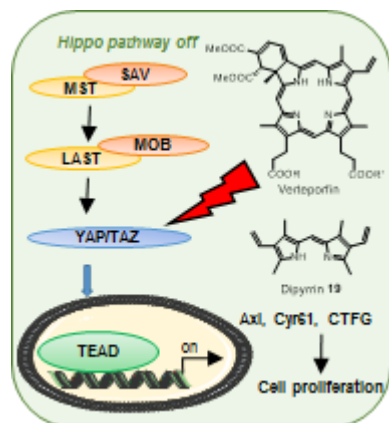
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We herein report the efficient convergent synthesis of the hexasubstituted dipyrrens structurally related to verteporfin where the sensitive vinyl group was created at the final stage by a dehydroiodation reaction. VP showed a direct effect on YAP and TAZ levels whereas dipyrin **19** showed a direct effect only on YAP level. Altogether, these data suggests that dipyrren-related compounds may directly affect the expression of YAP and/or TAZ.