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Research Paper

A Facile Synthesis and Cytotoxicity of a Novel Porphyrin-Cryptolepine Conjugate

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Abstract: A novel porphyrin–cryptolepine conjugate **7** with a 1,2,3-triazole linker was synthesized by the cycloaddition reaction of propargyl porphyrin **2** and azido cryptolepine **6** under click conditions. The conjugate **7** exhibited enhanced photocytotoxicity against A549 cells with IC_{50} values of 2.5 µM and 7.9 µM in the visible and UV light, respectively.

Introduction

There has been immense interest to develop synthesis of porphyrins as drugs and photosensitizers due to their tendency to accumulate in neoplastic tissue at higher concentrations than in neighbouring normal tissues [1]. Porphyrins were also explored for their photochemical nuclease activities and tight interaction with DNA in different types of binding modes such as intercalation, outside binding in the groove and with self-stacking along DNA surface [2]. Photodynamic therapy (PDT) is used in the treatment of cancer, psoriasis, age-related macular degeneration, and also in the inactivation of microorganisms and viruses [3]. This therapy requires combination of a photosensitizer, oxygen and light in which the cell destruction occurs mainly due to the disruption of the cellular, mitochondrial, or nuclear membranes by cytotoxic agents [4]. The photofrin is an oligomeric hemato-porphyrin mixture which is used in the treatment of variety of cancers but suffers from dosage and selectivity problems.

Attachment of moieties with already established pharmacological effects to porphyrin may influence the efficacy and specificity of porphyrin hybrid. Porphyrins conjugated with different heterocycles such as acridine [5], phenyl piperazine [6] and peptides [7] have been synthesized and evaluated for their biological and physical properties. Most of the drugs available in the market consist of nitrogen containing heterocycles which are widely used in the pharmaceutical industries. But there are issues of side effects and selectivity from the drug to the patient's body. Also selective localization of the drug in the

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cancerous tissues over normal tissues plays a vital role in minimizing side effects and improving efficacy [8, 9]. The conjugation of potent drug molecules with porphyrins can help in improving the selectivity due to specific binding mode of porphyrins. In view of the problems associated with the existing photosensitizers there is lot of scope for the development of new chemical entities that are selective to malignant tissue over normal tissue.

On the other hand, Cryptolepine is a naturally occurring indoloquinoline alkaloid extracted from *Cryptolepis Sanguinolenta* and it has been used for the treatment of hypertension, hyperglycemia [10], malaria [11] and cancer [12]. Cryptolepine contains two fused hetero cyclic rings (indolo[3,2-b]quinoline) with a planar geometry, has shown DNA intercalation between nonalternating cytosine-cytosine sites [13]. Recently, it has been disclosed that p53 associated molecular events led to alteration of cell cycle and cell death in human lung adeno carcinoma A549 cells [14]. The anti inflammatory activity of cryptolepine by inhibiting DNA of activated NF-κB has recently been reported [15]. In view of immense biological significance of porphyrins and cryptolepine it would be interesting to investigate the anticancer activity of their conjugate. In this communication we have reported synthesis and preliminary cytotoxicity of novel porphyrin-cryptolepine conjugate **7**.

Materials and Methods

All chemicals were procured from Sigma-Aldrich, India and Spectrochem Pvt Ltd., and were of analytical grade. IR spectra of the compounds were recorded on a FT-IR Shimadzu spectrophotometer using KBr discs. ^{1}H NMR spectra were recorded in DMSO-d_6 and $CDCl_3$ on a Bruker-avance 400 MHz instrument using TMS as an internal standard. The UV–visible spectroscopy was carried out on Hitachi U-2900 spectrophotometer and fluorescence spectra were recorded on Horiba Jobin Yvon Fluoro max-4-scanning fluorimeter. Quartz cuvettes were used with 1-cm path length.

Chemistry

5-(4-Propargyloxyphenyl)-10,15,20–triphenylporphyrinato zinc(II) (2):

(a) Preparation of 5-(4-hydroxyphenyl)-10,l5,20-triphenylporphyrin: Acetoxy porphyrin **1** (0.5 g, 0.89 mmol) was dissolved in methanol (25 mL) and 2M potassium hydroxide (45 mL) and the reaction mixture was refluxed for 5 h. The contents were cooled to RT and solvent was evaporated in vacuo. The residue obtained was taken into water (5 mL) and acidified (pH ~ 4) with 2N HCl. The solid obtained was filtered, washed with water and dried.

(b) To a suspension of 5-(4-hydroxy-phenyl)-10,l5,20-triphenyl-porphyrin (0.25 g, 0.4 mmol) in dry acetone (20 mL) was added fused potassium carbonate (0.065g, 0.47 mmol) and propargyl bromide (80% in toluene, 0.17 mL, 1.18 mmol) at 0 °C over a period of 10 min and the reaction mixture was warmed to room temperature and then heated at 60 ºC for 3 h. The reaction contents were cooled to room temperature, filtered, and washed with dichloromethane. The combined organic phase was dried over anhydrous sodium sulphate and evaporated under vacuum to obtain the desired propargyloxy porphyrin as purple crystals (0.17 g, yield 65 %). IR (KBr) : 3219, 2965, 2935, 2873, 2115 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.88 - 8.64 (m, 8H), 8.22 - 8.20 (m, 6H), 8.16 (d, *J* = 7.6 Hz, 2H), 7.78 – 7.75 (m, 9H), 7.38 (d, *J* = 7.6 Hz, 2H), 4.99 (s, 2H), 2.7 (s, 1H), -2.78 (s, 2H). ESI-MS: Observed M^+: 668.3; calculated $[C_{47}H_{32}N_4O]^+$: 668.2. To a stirred solution of above synthesized propargyloxy porphyrin (0.2 g, 0.3 mmol) in chloroform-methanol (1:1, 10 mL) was added $Zn(OAc)_2$ (0.069 g, 0.31 mmol) and heated under reflux for 2 h.

ISSN: 2249 –4820

Chemistry & Biology Interface, 2012, 2, 2, 114-121

After completion of reaction, the solvent was evaporated and diluted with chloroform (20 mL) and water (5 mL). The organic phases were collected, washed with 10% $NaHCO_3$, water and dried over anhydrous sodium sulphate. Solvent was evaporated under vacuum to afford compound **2** (0.17 g, yield 95%) as purple solid. IR (KBr): 2123, 1604, 1230 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 8.97 – 8.94 (m, 8H), 8.22 (d, *J* = 8 Hz, 6H), 8.14 (d, *J* = 8.4 Hz, 2H), 7.75 – 7.73 (m, 9H), 7.35 (d, *J* = 8.8 Hz, 2H), 4.96 (s, 2H), 2.68 (s, 1H).

3-Acetoxy-1-acetylindole (4): Anthranilic acid **3** (5 g, 34.6 mmol) was dissolved in 30 mL of aqueous sodium hydroxide (1.45 g, 0.25 mol). To this solution, KI (2.1 g, 12 mol) followed by 2-chloroacetic acid (6.52 g, 69.3 mmol) dissolved in 20 mL of 1M NaOH were added. The mixture was heated at reflux for 4 h. On cooling, the precipitated solid was filtered off and washed well with water to afford 1-carboxymethylanthranilic acid (5.02 g, yield 71%). mp: 217-219 °C (lit.[12] mp: 218-219 °C). 1-Carboxymethyl anthranilic acid (5g, 24.7 mmol) was taken in acetic anhydride (50 mL) and added anhydrous sodium acetate (1.8 g, 53.6 mmol). The mixture was stirred at 60 °C for 5 h, cooled to room temperature and removed the excess of sodium acetate by filtration. The resultant solution was distilled in vacuo. The residue thus obtained was taken into ethyl acetate (50 mL) and a saturated solution of sodium bicarbonate (30 mL) was added. The layers were separated and the aqueous layer was extracted with ethyl acetate (2 × 50 mL). The combined organic extract was washed with a saturated sodium bi- carbonate solution (2 × 50 mL), dried over anhydrous sodium sulphate and then evaporated to get compound **4** as viscous oil (2.4 g, yield 61%).

Indoloquinoline (5): A solution of isatin (1.42 g, 9.21 mmol) and potassium hydroxide (13.5 g) dissolved in water (50 mL) was added to 3-acetoxy-1-acetylindole **4** (2.1 g, 9.21 mmol) in water (50 mL) and stirred under nitrogen at room temperature. The reaction mixture was refluxed for 4 h and then cooled to 70 °C, air was bubbled into the mixture for 20 min at the same temperature. The mixture was filtered through hot celite bed and washed well with hot water. The filtrate was then acidified (pH ~1) with concentrated hydrochloric acid. The solid thus obtained was filtered and washed with water, dried and used for the next step without further purification. The obtained solid (1.6 g, 6.2 mmol) was taken in diphenyl ether (30 mL) and stirred at 250 °C for 6 h. The reaction mixture then cooled to room temperature, hexane was added and kept under refrigeration overnight. The solid obtained was filtered and. dried to give indoloquinoline **5** (1.3 g, yield 88%). mp: 248-251 °C (lit.[15] mp: 251-252 °C).

5-Azidoethyl-10H-indolo[3,2-b]quinolinium bromide (6): A mixture of indoloquinoline **5** (1.3 g, 5.96 mmol), dibromoethane (2 mL, 23.15 mmol) and dimethylformamide (0.5 mL) was allowed to stir under reflux for 24 h. After cooling the reaction contents to room temperature, methanol was added into it and precipitated with diethyl ether to afford ethyl indoloquinolium bromide as a brownish yellow solid (0.8 g, yield 44%). To a solution of ethyl indoloquinolium bromide (0.8 g, 2.46 mmol) in ethanol-water (10 mL, 1:1) was added sodium azide (0.48 g, 7.48 mmol) and stirred at 60 °C for 48 h. The solvent was evaporated under vacuo and the residual mass was taken into methanol. The excess sodium azide was precipitated, filtered and washed with methanol. The 2-azidoethyl indoloquinolium bromide **6** was purified by precipitation with diethyl ether. (0.41 g, yield 71%). mp: > 250 °C; IR (KBr): 3313, 2125, 1620 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 9.25 (s, 1H), 8.77 (d, *J* = 9.2 Hz, 1H), 8.59 (d, *J* = 8 Hz, 1H), 8.53 (d, *J*

ISSN: 2249 –4820

Chemistry & Biology Interface, 2012, 2, 2, 114-121

= 8.4 Hz, 1H), 7.87 – 7.82 (m, 5H), 7.42 (s, 1H), 5.81 (t, *J* = 6 Hz, 2H), 4.36 (t, *J* = 6 Hz, 2H). ESI MS: Observed M^+: 288.1, Calculated M^+: 288.1.

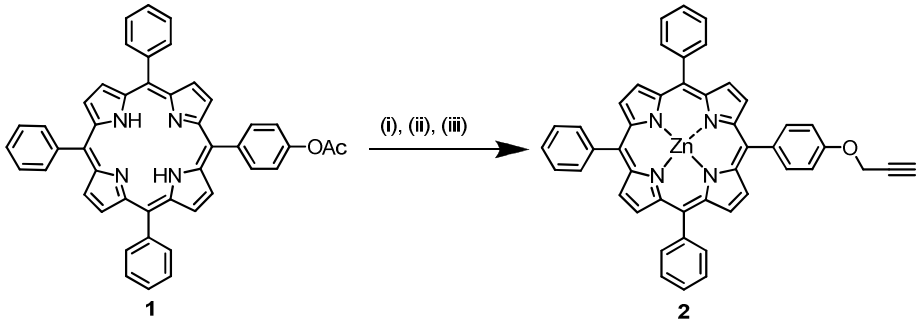
Preparation of porphyrin-cryptolepine conjugate (7): To a stirred solution of metallated porphyrin **2** (0.08 g, 0.2 mmol) in DMF: water (1:1, 20 mL) was added copper sulphate (0.051 g, 0.2 mmol), sodium ascorbate (0.069 g, 0.41 mmol) and azide **6** (0.097 g, 0.41 mmol). The mixture was allowed to warm at 50 °C and continued stirring till the completion of the reaction (7 days). The reaction contents were diluted with chloroform (50 mL) and 20 % ammonia solution (15 mL) was added. The resulting solution was filtered over thin celite bed and washed thoroughly with chloroform. The combined organic layer was washed with water (5 × 15 mL), dried over anhydrous sodium sulphate and solvent was evaporated under reduced pressure. The crude residue was purified by column chromatography using chloroform: methanol (8:2) as eluent to afford pure conjugate **7** (0.043 g, yield 59%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.89 – 8.83 (m, 8H,), 8.65 (s, 1H), 8.38 (d, *J* = 8 Hz, 2H), 8.21 – 8.19 (m, 6H), 8.13 (d, *J* = 7.2 Hz, 2H), 7.99 (s, 1H), 7.89 (d, *J* = 7.4 Hz, 2H), 7.77 – 7.73 (m, 9H), 7.61 (d, *J* = 7.2 Hz, 2H), 7.30 (d, *J* = 8 Hz, 4H), 5.49 (s, 2H), 5.31 (s, 2H), 3.55 (d, *J* = 7.2 Hz, 2H). ESI-MS: Observed M^+:1018.2, Calculated $[C_{64}H_{44}N_9OZn]^+$:1018.2.

Results and Discussion

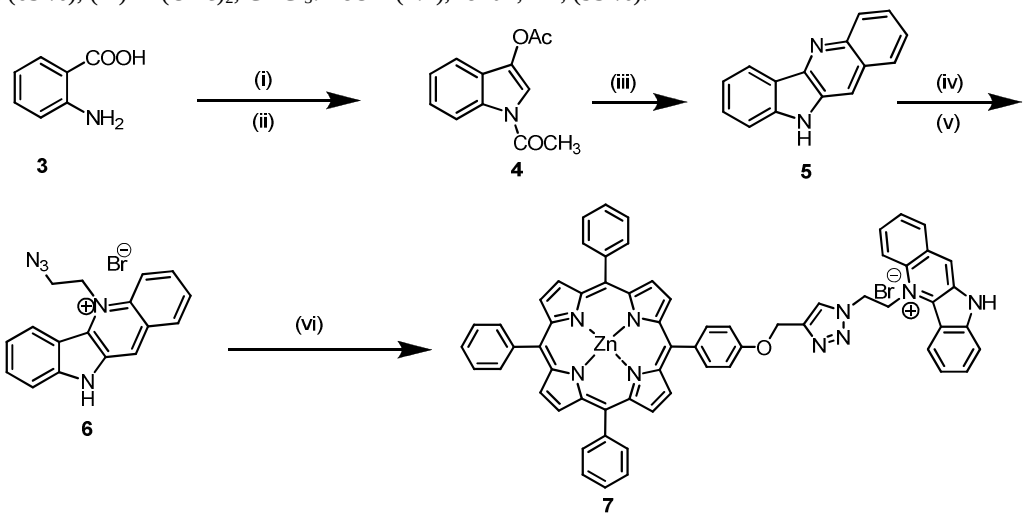
The acetoxyporphyrin **1** was prepared from the reaction of 4-acetoxy benzaldehyde (1equiv), benzaldehyde (3 equiv) and pyrrole (4 equiv) in refluxing propionic acid followed by oxidation with DDQ as described in Adler-Longo's method [16]. Treatment of **1** with potassium hydroxide and followed by reaction with propargyl bromide in presence of potassium carbonate produced propargyloxytriphenylporphyrin which upon metallation with zinc acetate led to the formation of porphyrin **2** (Scheme 1). The 2-azidoethyl indoloquinolium bromide **6** was prepared from anthranilic acid as outlined in scheme 2. The reaction of **3** with 2-chloroacetic acid in presence of sodium hydroxide and potassium iodide resulting in carboxymethyl anthranilic acid which upon treatment with acetic anhydride and anhydrous sodium acetate led to the formation of 3-acetoxy-1-acetylindole **4**. Reaction of **4** with isatin and potassium hydroxide produced indoloquinoline carboxylic acid which upon decarboxylation in diphenyl ether resulted in indoloquinoline **5.** Alkylation of **5** with dibromoethane followed by treatment with sodium azide in ethanol-water gave 2-azidoethyl indoloquinolium bromide **6** in 71% yield. The reaction of propargyloxyporphyrin **2** with **6** was initially carried out under click chemistry conditions in *tert*-butanol/water and acetonitrile/water, but it was observed that the progress of the reaction was sluggish. This may be due to the poor solubility of the propargyloxy porphyrin in these solvents. The reaction was monitored from room temperature to 80 °C which also did not improve the yield of the product significantly even after several days (10 days). Finally, the reaction was performed using sodium ascorbate and copper sulphate in DMF/H_2O (1:1) at 50 °C to afford porphyrin-cryptolepine conjugate **7** in 59 % yield (Scheme 2). Reaction at elevated temperature led to poor yield of the conjugate **7**. The structure of **7** was confirmed by ^{1}H NMR and ESI-MS mass spectrometry. The expected molecular ion peak was observed at *m/z* 1018.2 which is in agreement with calculated *m/z* for $[C_{64}H_{44}N_9OZn]^+$ 1018.2.

ISSN: 2249 –4820

Chemistry & Biology Interface, 2012, 2, 2, 114-121



Scheme 1. (i) KOH, MeOH:H_2O (1:1), reflux, 5 h, (90 %); (ii) Propargyl bromide, K_2CO_3, Acetone, 0-60°C, 3 h, (65 %); (iii) $Zn(OAc)_2$, $CHCl_3$:MeOH (1:1), reflux, 2 h, (95 %).



Scheme 2: (i) 2-Chloroacetic acid, KI, NaOH, H_2O, 4 h (71 %); (ii) Ac_2O, NaOAc, 60 °C, 5 h, (61 %); (iii) (a) Isatin, KOH, N_2, reflux; (b) Ph_2O, 250 °C, (88 %); (iv) Dibromoethane, DMF, 24 h (44 %); (v) NaN_3, EtOH, 60 °C, 48 h (71 %); (vi) **2**, $CuSO_4.5H_2O$, sodium ascorbate, DMF/H_2O, 50 °C, 7 days (59 %).

The ^{1}H NMR spectrum of conjugate **7** exhibited a characteristic multiplet at δ 8.83–8.89 corresponding to the eight β–pyrrolic protons. The C_5-H of the triazole appeared as a singlet at δ 7.99 and the protons of indoloquinoline moiety are in agreement with the literature [15].

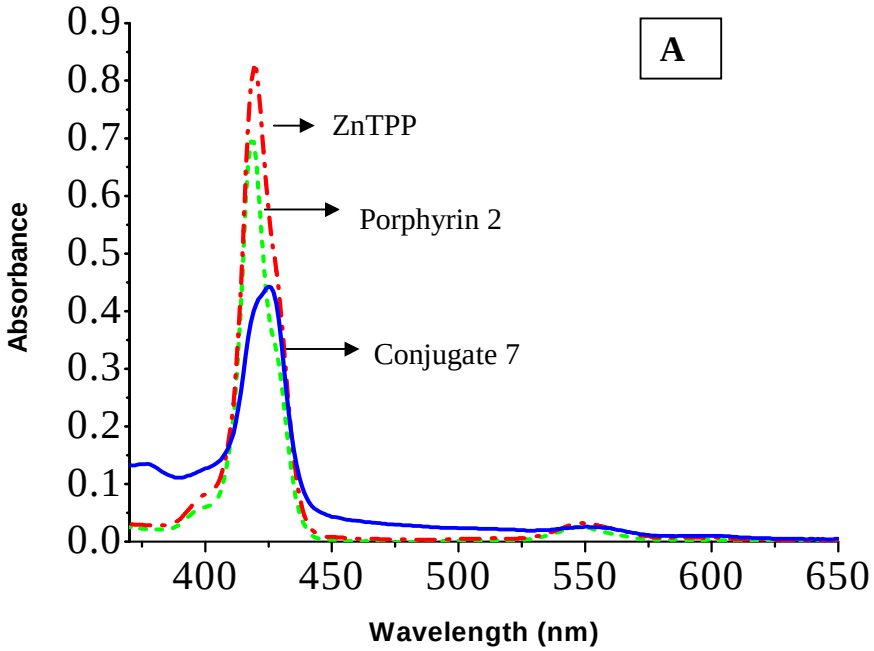
Absorption and Fluorescence spectra

The UV-vis spectrum of conjugate **7** is shown in figure 1. The absorption studies were carried out using tetraphenyl-porphyrinato zinc (II) (ZnTPP) as a reference for obtaining the quantum yield. The quantum yield of ZnTPP in chloroform was taken as 0.03 [17]. A 10^{-6} M solution of all the compounds was prepared and their UV-visible and fluorescence spectra were recorded. It is clearly evident from the spectra (Figure 1) that there is a decrease in the intensity and a red shift of 7 nm in soret band (425 nm) of conjugate **7** with respect to ZnTPP and compound **2** (Table 1). No particular change was observed in UV-vis spectra of compound **2,** though an extended conjugation is present, which suggests that the electronic structure of the compound is not distorted. The gradual decrease in absorbance for conjugate **7** indicates that the conjugation of cryptolepine to the porphyrin has shown decrease in fluorescence intensity. The same trend was observed in case of fluorescence spectra. This was in accordance with the obtained low fluorescence quantum yield as given in the table 1. The preliminary luminescence measurement reveals lower emission from the porphyrin moiety in

ISSN: 2249 –4820

Chemistry & Biology Interface, 2012, 2, 2, 114-121

conjugate **7** indicating a decrease in the fluorescence by the cryptolepine subunit and thus, quantum yield was seen to be drastically reduced in comparison with ZnTPP (Table 1). This decrease of fluorescence in conjugate **7** can be attributed to the occurrence of a photon induced electron transfer (PET) from the porphyrin singlet state to the appended cryptolepine. This particular property on porphyrin was in well acceptance with the reported literature [18]. The fluorescence quenching can be enlightened by different mechanisms such as electron transfer or energy transfer from the porphyrin to the cryptolepine subunit which does not show any fluorescent property.



ISSN: 2249 –4820

Chemistry & Biology Interface, 2012, 2, 2, 114-121

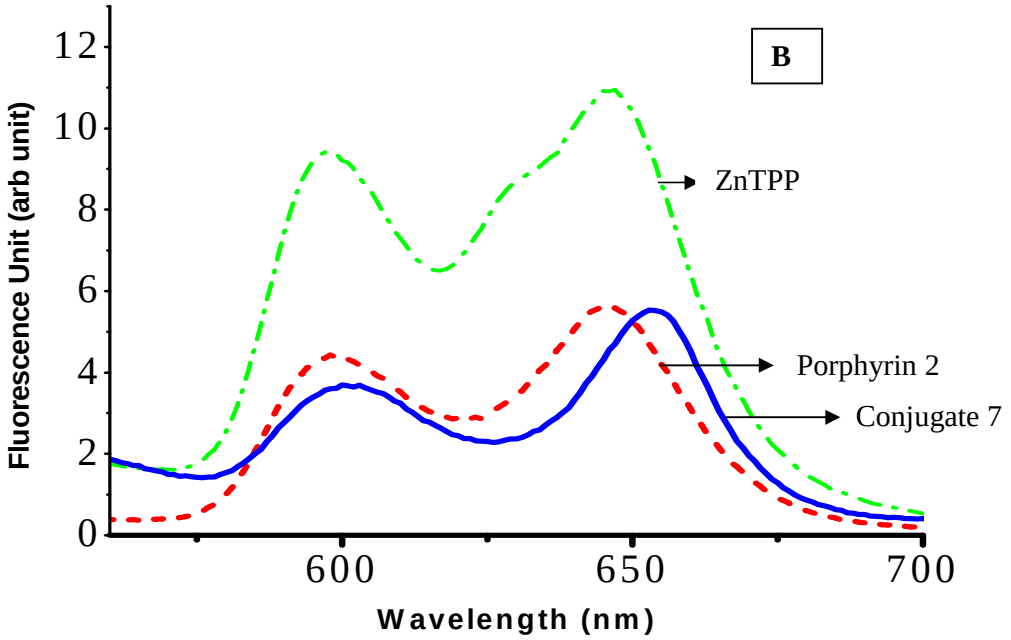


Figure 1. (**A**) UV-visible and (**B**) fluorescence spectra of ZnTPP, Propargyloxyporphyrin **2** and Porphyrin-cryptolepine conjugate **7**.

Table 1. Quantum yield of the porphyrin-cryptolepine conjugate **7**

Compound	Soret λ_{max} (nm)	Q bands λ_{max} (nm)	Emission λ_{max} (nm)	Quantum Yield[a]
Propargyloxy porphyrin **2**	419	548, 601	597, 646	0.011
Porphyrin-cryptolepine conjugate **7**	425	552, 602	600, 653	0.023
ZnTPP	418	548, 601	597, 646	0.03

[a]Excitation wavelength 418 nm

Cytotoxicity Studies

The cytotoxicity of **7** against A549 (a human epithelial cell line derived from a lung carcinoma) was determined by means of the WST assay. The cytotoxicity results (Table 2) are expressed as the concentration of the drugs that inhibit 50% of cell proliferation (IC_{50}). Interestingly, conjugate **7** showed potent toxicity with an IC_{50} value of 2.5 µM under visible light and 7.9 µM in the UV light exposure;

Table 2. Photocytotoxicity of the porphyrin-cryptolepine conjugate **7**

Compound	IC_{50} (µM)		
	UV Light	Visible	Dark
Propargyloxy porphyrin **2**	35.1	17.9	ND
Porphyrin- cryptolepine conjugate **7**	7.9	2.5	33.6
ZnTPP	28.3	6.3	ND

ISSN: 2249 –4820

Chemistry & Biology Interface, 2012, 2, 2, 114-121

whereas the conjugate **7** is relatively less toxic (IC_{50} > 33 µM) in the dark. The higher phototoxicity of conjugate **7** than **2** might be due to efficient sensitization of oxygen to form ROS by photo-excited porphyrin moiety, because excited state of conjugate **7** is stable enough to react with oxygen. This enhanced photocytotoxicity relative to the dark controls is an essential property for a photochemotherapeutic agent. More importantly, the conjugate **7** exhibited higher photocytotoxicity under visible light than ZnTPP (IC_{50}= 6.3 µM) or cryptolepine (IC_{50} =17.9 µM).

Conclusion

We have synthesized a novel porphyrin-cryptolepine conjugate **7** using click chemistry protocol and studied its photophysical properties and cytotoxicity on A549 lung carcinoma cell line under UV irradiation, visible light and in dark. Our preliminary findings showed that the conjugate **7** is photocytotoxic under the influence of light. Further structure-activity relationship studies by varying linker length are in progress.

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