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編號：030017

作品名稱

光動力化合物的合成及應用

得獎獎項

大會獎：三等獎

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關鍵字：光動力、DNA 裂解、Schiff base

作者簡介



我是黃薇臻，平時我最愛馳騁球場和閱讀英文小說，曾獲清華杯化學全國第一團體金牌、連續兩屆獲選進入化奧選訓營，並於 2011 年獲得第四十三屆國際化學奧林匹亞銅牌獎。步入科展之旅，使我一窺化學實驗與實際操作的奧妙，而奧林匹亞則提供我奠定化學理論的基礎。感謝學校老師、中興大學與青培計畫的教授和學長們的指導，也謝謝亭語及林謙兩位夥伴，伴我一起追尋夢想。



我是黃亭語，目前與薇臻和林謙就讀台中女中數理資優班三年級。曾有一段時間住在紐西蘭，促使我喜愛閱讀英文小說，擅長將英語應用在生活中，所以漸漸變成英文演講高手。上高中之後，因為參加青培、清大化學培育計畫，使我展開了科展漫長又收穫良多的旅途。雖然在高三要考學測及做科展，蠟燭兩頭燒，但因為老師們及家長的鼓勵，使我能夠堅持夢想，謝謝你們！



我是林謙，喜歡閱讀小說或聽音樂。在老師的帶領下，開始對化學產生興趣，不論是理論的理解或是實驗的操作，都深深吸引著我，曾經得到清華盃化學個人銀牌獎和化學學科能力競賽中區第二名。因為對化學的喜愛，讓我選擇做化學的專題研究。因為有了學校老師、實驗室教授、青培計畫的教授們及學長們的幫忙，在我感到困惑時，能給予我指引。謝謝父母的鼓勵和兩個搭檔，陪我一起追尋目標。

摘要

光動力療法為一個新興癌症治療方式，包含三大要件:光、光敏劑、氧氣。其概念為利用合成的錯合物嵌入細胞核 DNA，藉由特定光激發後，將能量傳遞給氧及水分子形成自由基，進而毒殺癌細胞，達到治療惡性腫瘤的目的。

本研究利用 NNO-Schiff base，成功合成出一系列的二價銅錯合物，針對不同的配位基及共配位基、濃度、光波長利用 DNA 裂解、細胞毒性測試，探討結構活性的關係與作用機制，以利研發相關藥物的研究，另外，錯化合物吸收波長可延伸至可見光—藍光，進而大大提升其應用的價值。

Abstract

Photodynamic therapy (PDT) is an effective tool for cancer treatment. It is a minimally invasive therapeutic modality and a complementary to chemotherapy and radiotherapy. PDT is a photochemical reaction which requires simultaneous presence of a photo-sensitizer(PS), light source and oxygen. Complexes are activated at a specific wavelength of light and in the presence of oxygen, produces reactive oxygen species (ROS) causing DNA cleavage and cell death.

In this research, a series of Cu(II) complexes supported on NNO-Schiff base ligands are synthesized. Concentration, structure, wavelengths of light (UV and visible) are key parameters in deciding about effectiveness and potential applications of PDT. Two mechanisms, singlet oxygen and hydroxyl radicals are verified. Moreover, significant loss of viability was detected under UVA in cellular experiments. Quinoline based complexes are more effective causing greater cleavage of visible light-irradiated DNA in comparison to amine based complexes.

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壹、研究動機

有一次看到有關光動力療法的專題報導，發現這是種新興療法。目前這種療法是被研究來治療癌症，我們對此非常有興趣，由於還有很大的研究空間，想進一步探討和研究。在讀過相關論文後，我們決定著手研究光動力的應用，合成各種錯合物進行 DNA 裂解實驗。

貳、研究目的

- (一) 合成一系列的二價銅錯合物
- (二) 探討不同波長下錯合物裂解 DNA 的效果
- (三) 找出效果較佳的共配位基及配位基組合
- (四) 探討錯化合物破壞 DNA 的作用途徑

參、研究設備及器材

(一) 藥品

藥品名稱	廠牌	處理方式
CDCl_3	Aldrich	直接取用
Ethanol	景明	直接取用
2-Hydroxy-1-naphthaldehyde	Aldrich	直接取用
Salicylaldehyde	Acros	直接取用
2-hydroxy-3-methoxybenzaldehyde	Alfa Aesar	直接取用
2-hydroxy-5-nitrobenzaldehyde	Alfa Aesar	直接取用
3-bromo-5-chloro-2-hydroxybenzaldehyde	Acros	直接取用
8-Aminoquinoline	Acros	直接取用
N,N-Dimethylethylenediamine	Alfa Aesar	直接取用
pUC19 DNA ^(註一)	Fermentas	直接取用
Sodium perchlorate	Acros	直接取用
Agarose	Invitrogen	直接取用
50mM tris-HCl buffer (pH, 7.2) containing 50mM NaCl	自行製備	直接取用
TAE buffer, (10x)	Invitrogen	直接取用
SYBR Safe in DMSO ^(註二)	Invitrogen	直接取用

(註一)：濃度 0.5 $\mu\text{g}/\mu\text{l}$. Storage Buffer：10 mM Tris-HCl (pH, 7.6) and 1 mM EDTA.保存於 $-20\text{ }^{\circ}\text{C}$ 。

(註二)：濃度 10,000x，保存於 $4\text{ }^{\circ}\text{C}$ 。

(二)研究設備

1. 紫外/可見光光譜儀(UV-Vis)：

吸收光譜的測量是利用型號 JASCO V-350 測得，所有化合物均已甲醇為溶劑，莫耳吸收光係數(ϵ :molar extinction coefficient)的測定是依據 Beer's Law:

$$A=\epsilon\times b\times c$$

其中 A:吸收度，b:光路徑長，c:化合物的濃度。在已知濃度的情況下，測得吸收度再計算其莫耳吸光係數。

2. 元素分析儀(Elemental Analyzer, EA)

元素分析資料是以 Heraeus CHN-O-S-Rapid Analyzer 儀器來分析化合物中之碳、氫、氮成分百分比。

3. 核磁共振儀(NMR)：

^1H NMR 是利用 Varian VXR-300 (300MHz)，Varian Mercury-400 (400MHz)，Varian Unity Inova 600 (600MHz)測量， ^{13}C NMR 以 Varian VXR-300 (300MHz) 測量，化學位移則分別以 CDCl_3 之 7.26ppm (^1H NMR) 及 77.00ppm (^{13}C NMR)。

分裂形式(splitting pattern)定義如下：

s，單峰；d，雙峰；t，三峰；q，四峰；m，多峰。

4. X-ray 晶體繞射儀(X-ray diffractometer)

晶體在氮氣、無水環境下，以 FOMBLIN[®] Y 沾取並以液態氮冷卻。晶體數據是晶體在 Mo/k 射線照射下，以 Oxford Diffraction Gemini S 之 X-光單晶繞射儀收集。空間群之判定以 Laue Symmetry 和 Systematic Absences 為依據，並由結構解析來判定。結構之確定為利用 Siemens SHELXTL PLUS 程式中之直接法(Direct Methods)來確認。所有的非氫原子皆是利用連續傅立葉圖(Successive Fourier Maps)來將之定域化(Localization)。而對於所有

非氫原子是使用非等熱向熱參數(Anisotropic Thermal Parameter)，而對所有的氫原子都是固定等向的熱參數 (Isotropic Parameter)，並以 Riding Model 加以修飾。

5. 電泳凝膠影像系統

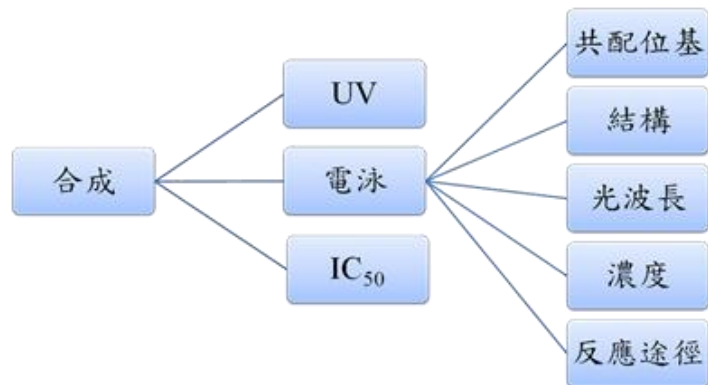
使用紫外光激發 DNA 螢光染劑藉以標示 DNA 位置，調整儀器曝光時間後以光學鏡頭存取畫面。使用儀器為 UVI doc。

6. ELISA Reader

使用微量滴定盤，於 ELISA reader 光度計測吸光值，將液體裝在 96 孔或 24 孔的微量滴定盤內，放進儀器內測量即可。使用儀器為 PerkinELmer 1420 Multilabel counter。

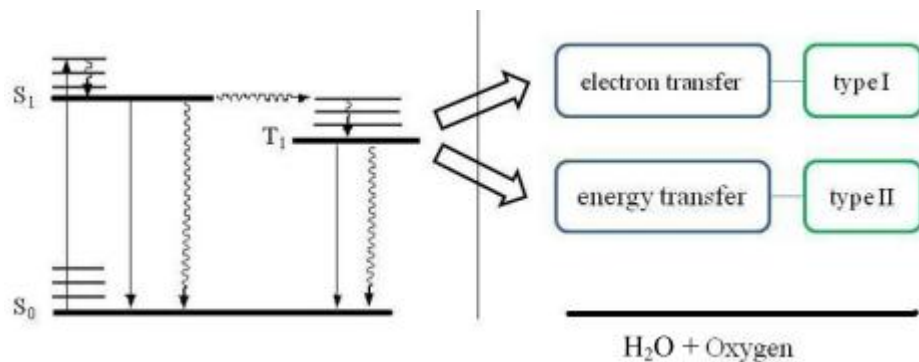
肆、研究過程及方法

(一)流程圖：



(二)原理：

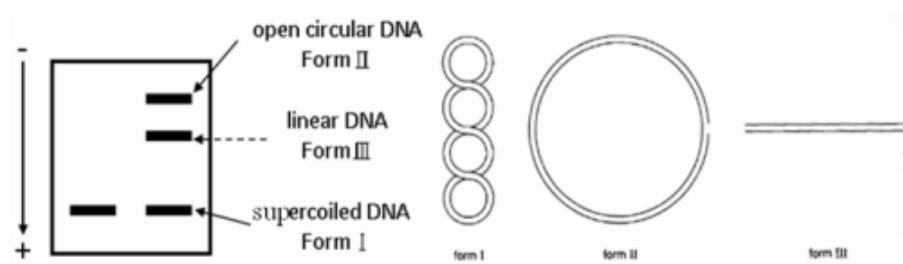
1.光動力原理



當光敏劑吸收了能量躍遷到激發態(S_1)，再經由 intersystem crossing 到達 triplet state 再由此激發態的化合物將能量傳遞給氧或水分子形成自由基或單態氧毒殺癌細胞。

2.質體 DNA 的移動速率

超螺旋雙股(supercoiled DNA)>直線型 DNA(linear DNA)>環型 DNA(open circular DNA)



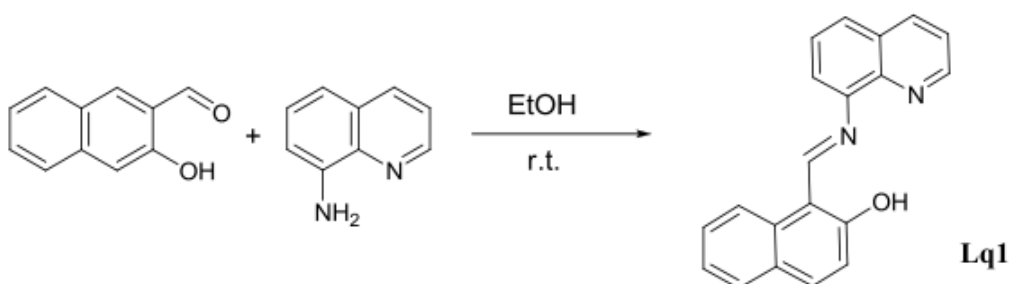
不同構形 DNA 在凝膠電泳上的相對位置

(三)方法

1.合成

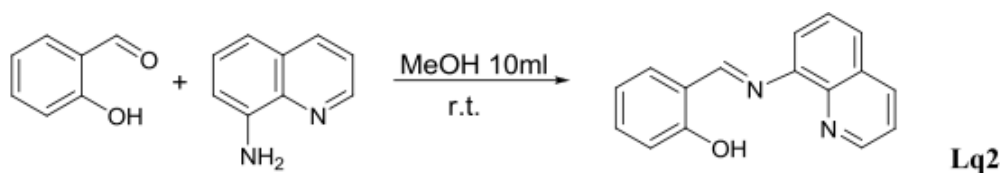
(1) Ligand 的合成

(a) Lq1



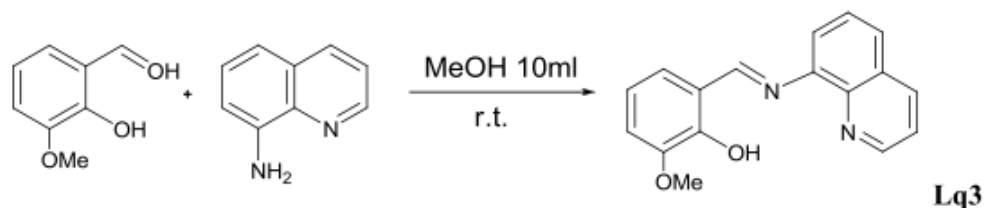
秤取 2-hydroxy-1-naphthaldehyde (0.860 克, 5.00 毫莫耳)和 quinolin-8-amine (0.72 克, 5.50 毫莫耳),置於 50 毫升圓底瓶中 20 毫升甲醇為溶劑,反應迴流 10 小時後將溶劑抽乾可得固體產物。(1.49g, 產率: 81%)

(b) Lq2



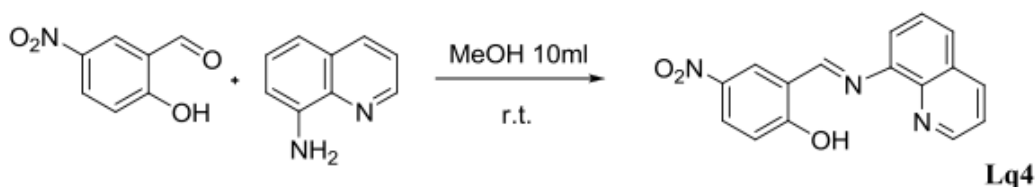
秤取 2-hydroxybenzaldehyde (0.610 克, 5.00 毫莫耳)和 quinolin-8-amine (0.793 克, 5.50 毫莫耳),置於 50 毫升圓底瓶中 20 毫升甲醇為溶劑,反應迴流 10 小時後將溶劑抽乾可得液體產物。(2.48g, 產率: 99%)

(c) **Lq3**



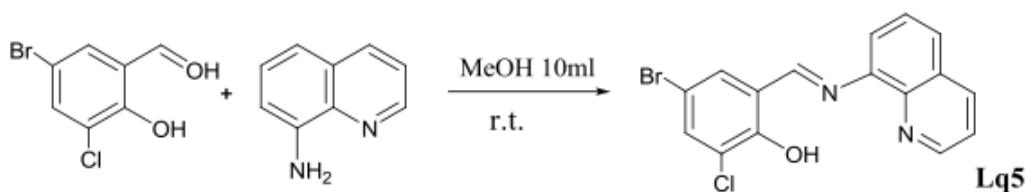
秤取 2-hydroxy-3-methoxybenzaldehyde (0.766 克, 5.00 毫莫耳)和 quinolin-8-amine(0.793 克, 5.50 毫莫耳),置於 50 毫升圓底瓶中 20 毫升甲醇為溶劑,反應迴流 10 小時後將溶劑抽乾可得固體產物。(1.39g, 產率: 63%)

(d) **Lq4**



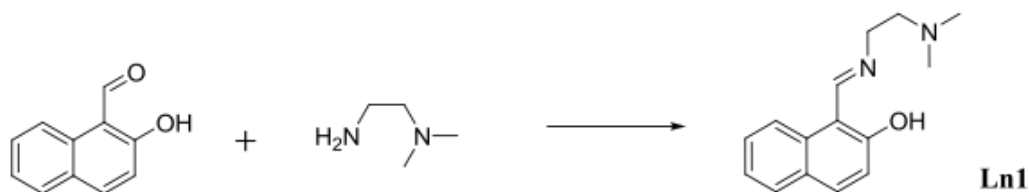
秤取 2-hydroxy-5-nitrobenzaldehyde (0.836 克, 5.00 毫莫耳)和 quinolin-8-amine (0.793 克, 5.50 毫莫耳),置於 50 毫升圓底瓶中 20 毫升甲醇為溶劑,反應迴流 10 小時後將溶劑抽乾可得固體產物。(1.47g, 產率: 89%)

(e) **Lq5**



秤取 5-bromo-3-chloro-2-hydroxybenzaldehyde (0.1182 克, 5.00 毫莫耳)和 quinolin-8-amine (0.793 克, 5.50 毫莫耳),置於 50 毫升圓底瓶中 20 毫升甲醇為溶劑,反應迴流 10 小時後將溶劑抽乾可得固體產物。(1.81g, 產率: 58%)

(f) **Ln1**



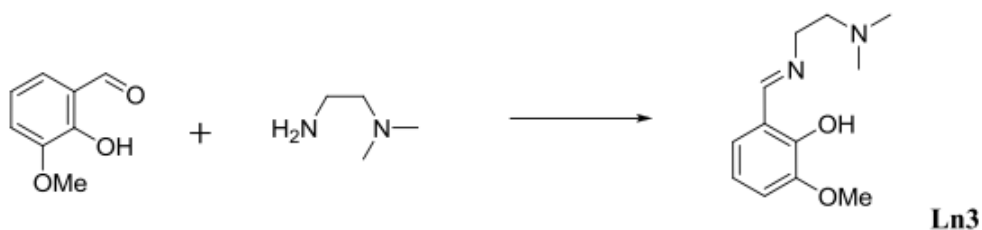
秤取 2-hydroxy-1-naphthaldehyde (0.860 克 , 5.00 毫莫耳)和 N,N-dimethylethane-1,2-diamine (0.485 克 , 5.50 毫莫耳),置於 50 毫升圓底瓶中 20 毫升甲醇為溶劑,反應迴流 10 小時後將溶劑抽乾可得固體產物。(1.21g, 產率: 78%)

(g) **Ln2**



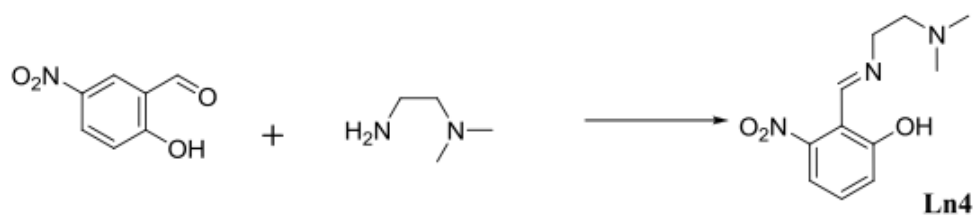
秤取 2-hydroxybenzaldehyde (0.610 克 , 5.00 毫莫耳)和 N,N-dimethylethane-1,2-diamine (0.485 克 , 5.50 毫莫耳),置於 50 毫升圓底瓶中 20 毫升甲醇為溶劑,反應迴流 10 小時後將溶劑抽乾可得液體產物。(0.96g, 產率: 66%)

(h) **Ln3**



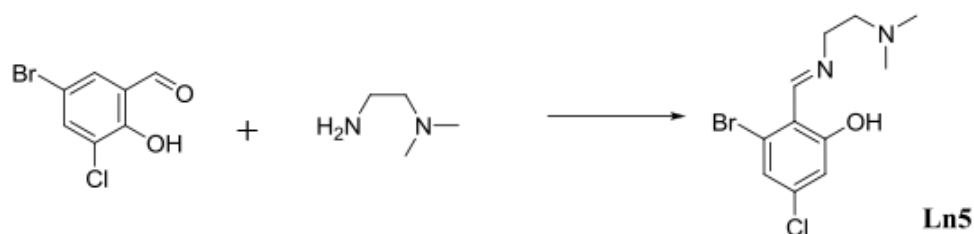
秤取 2-hydroxy-3-methoxybenzaldehyde (0.766 克 , 5.00 毫莫耳)和 N,N-dimethylethane-1,2-diamine (0.485 克 , 5.50 毫莫耳),置於 50 毫升圓底瓶中 20 毫升甲醇為溶劑,反應迴流 10 小時後將溶劑抽乾可得液體產物。(1.11g, 產率: 69%)

(i) **Ln4**



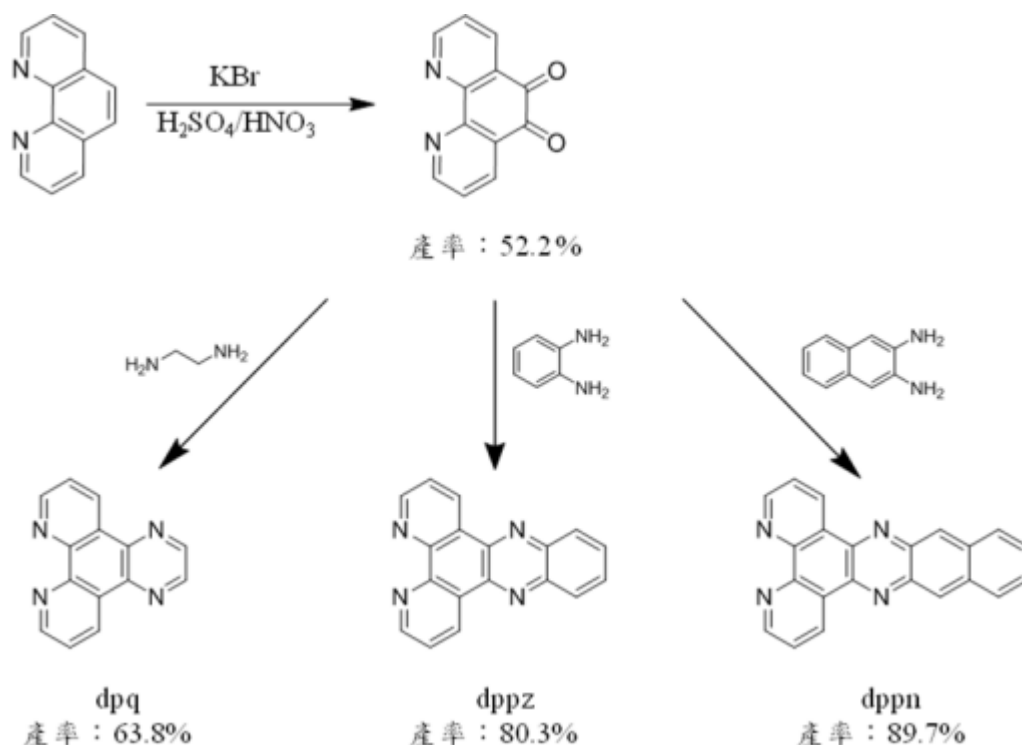
秤取 2-hydroxy-5-nitrobenzaldehyde (0.836 克 , 5.00 毫莫耳)和 N,N-dimethylethane-1,2-diamine (0.485 克 , 5.50 毫莫耳),置於 50 毫升圓底瓶中 20 毫升甲醇為溶劑,反應迴流 10 小時後將溶劑抽乾可得固體產物。(1.19g, 產率: 76%)

(j) **Ln5**



秤取 5-bromo-3-chloro-2-hydroxybenzaldehyde (1.182 克 , 5.00 毫莫耳)和 N,N-dimethylethane-1,2-diamine (0.485 克 , 5.50 毫莫耳),置於 50 毫升圓底瓶中 20 毫升甲醇為溶劑,反應迴流 10 小時後將溶劑抽乾可得固體產物。(1.53g, 產率: 63%)

(2) 共配位基的合成



(a) 1,10-phenanthroline-5,6-dione(phen)的合成：

秤取 1,10-phenanthroline (4.00 g, 22.2 mmol) 置於 100 ml 圓底瓶中，並加入 KBr (4.00 g, 33.6 mmol)，在冰浴下加入 20 ml 濃硫酸和 10 ml 硝酸，加熱迴流 3 小時，待冷卻後將酸液倒入 500 ml 去離子水中後，添加 NaOH 溶液並以廣用試紙調整至 pH 值至 6 再以 CH_2Cl_2 萃取有機層出來濃縮，熱乙醇做再結晶後可得黃色針狀固體(3.28 g，產率：66%)

(b) dibenzo[f,h]quinoxaline (dpq)的合成：

秤取 1,10-phenanthroline-5,6-dione (0.63 g, 3.00 mmol) 置於 50 ml 圓底瓶中，加入 ethane-1,2-diamine (0.18 g, 3.00 mmol)，取 30 ml 乙醇為溶劑，且以苯磺酸 (benzenesulphonic acid) 0.1 ml 當催化劑，加熱迴流，反應過程中會有白色粉末析出，持續反應 10 小時後冷卻靜置，將固體利用濾板過濾收集，並以少量乙醇清洗，在真空下抽乾可得到白色粉末 (0.44 g，產率：64%)。

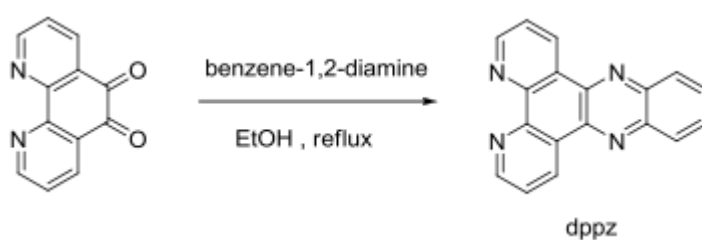
(c) **dipyrido[3,2-a:2',3'-c]phenazine (dppz)**的合成：

合成方法同 **dpq**, 1,10-phenanthroline-5,6-dione (0.63 g, 3.00 mmol) , benzene-1,2-diamine (0.32 g, 3.00 mmol) , 得到白色粉末(0.68 g , 產率：80%)。

(d) **benzo[i]dipyrido[3,2-a:2',3'-c]phenazine (dppn)**的合成：

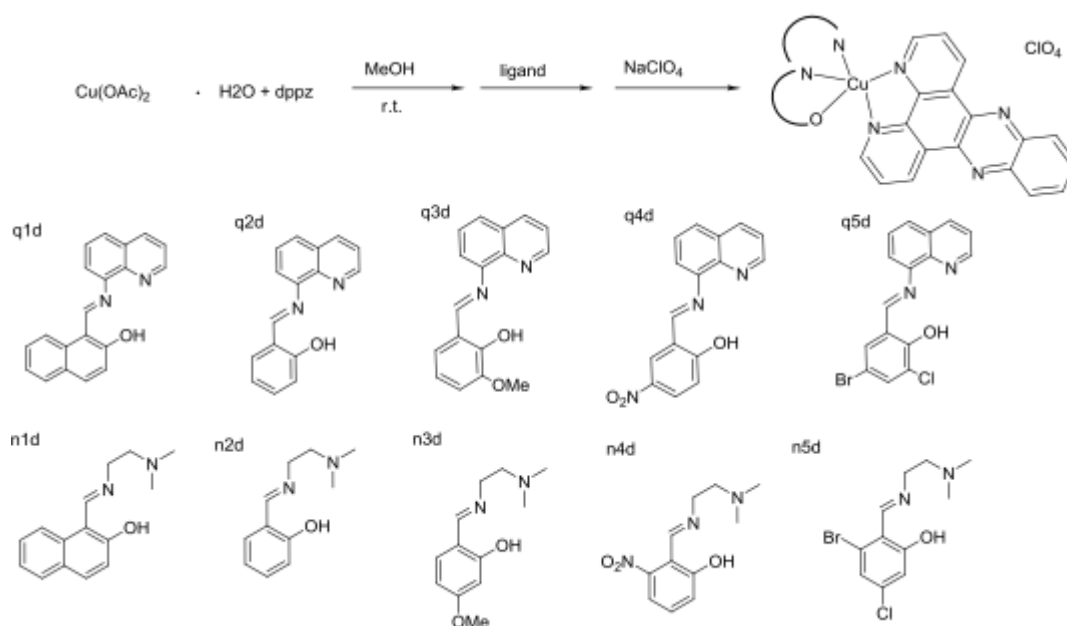
合成方法同 **dpq**, 1,10-phenanthroline-5,6-dione (0.63 g, 3.00 mmol) , naphthalene-2,3-diamine (0.47 g, 3.00 mmol) , 得到橘色粉末(0.90 g , 產率：90%)。

共配位基效果最佳者為 **dppz**：



爾後合成錯合物共配位基皆用 **dppz** 為代表。

(3) 錯合物 **q,n** 系列的合成



(a) **q1d** 的合成

秤取醋酸銅(0.14 g, 0.70 mmol)與 **dppz**(0.22 g, 0.77 mmol)置於 50 ml 圓底瓶中，以 20 ml 甲醇為溶劑，室溫下攪拌 1 小時，反應完後加入 **Lq1** (0.23g, 0.77 mmol)，並於室溫下攪拌 3 小時，再加入過氯酸鈉(0.094 g, 0.77 mmol)，即有固體沉澱，利用濾板過濾收集固體，並以冰甲醇清洗數次，最後於真空下抽乾而得到黃色固體。(0.32g，產率：68%)

(b) **q2d** 的合成

合成方法同 1，改加入 **Lq2** (0.19 g, 0.77 mmol)，可得深黃色固體。(0.35g，產率：73%)

(c) **q3d** 的合成

合成方法同 1，改加入 **Lq3**(0.21 g, 0.77 mmol)，可得褐色固體。(0.31g，產率：62%)

(d) **q4d** 的合成

合成方法同 1，改加入 **Lq4**(0.23 g, 0.77 mmol)，可得黃綠色固體。(0.38g，產率：56%)

(e) **q5d** 的合成

合成方法同 1，改加入 **Lq5**(0.28 g, 0.77 mmol)，可得深黃色固體。(0.35g，產率：71%)

(f) **n1d** 的合成

合成方法同 1，改加入 **Ln1**(0.19 g, 0.77 mmol)，可得深綠色固體。

(g) **n2d** 的合成

合成方法同 1，改加入 **Ln2**(0.15 g, 0.77 mmol)，可得草綠色固體。

(h) **n3d** 的合成

合成方法同 1，改加入 **Ln3**(0.17 g, 0.77 mmol)，可得深綠色固體。

(i) **n4d** 的合成

合成方法同 1，改加入 **Ln4**(0.18 g，0.77 mmol)，可得草綠色固體。

(j) **n5d** 的合成

合成方法同 1，改加入 **Ln5**(0.24 g，0.77 mmol)，可得草綠色固體。

2. 電泳

(1) DNA 電泳實驗前置工作

(a) tris-HCl buffer 的配置

秤取 tris (0.3 克)與 NaCl(0.146 克)，並以 50 毫升二次去離子水配置，再以 HCl 滴定至 pH 值為 7.2。

(b) TAE buffer 的配置

量取(10x)TAE buffer(50ml)，以 950ml 二次去離子水稀釋為(0.5x)後取用。

(c) 製備 1% Agarose gel

秤取 0.6g Agarose 加入至已稀釋的 60ml TAE(0.5x) buffer 中，加熱使其 Agarose 完全溶解以鋁箔紙包覆瓶子，再加入螢光染劑 SYBR Safe(4 μ L)，攪拌混合後，降溫至約 60 $^{\circ}$ C，倒入製膠膜具中並插上齒梳，避光下冷卻靜置成型。

(2) DNA 電泳實驗步驟

在暗室中將 DNA(1 μ L)，triz-HCL buffer(15.2 μ L)與 complex(40 μ M 於 DMF 溶液中)(1.8 μ L)混合與 eppendorf 中，在適當時間下施以不同光照波長，照光結束後置於 37 $^{\circ}$ C 恆溫槽中一小時。

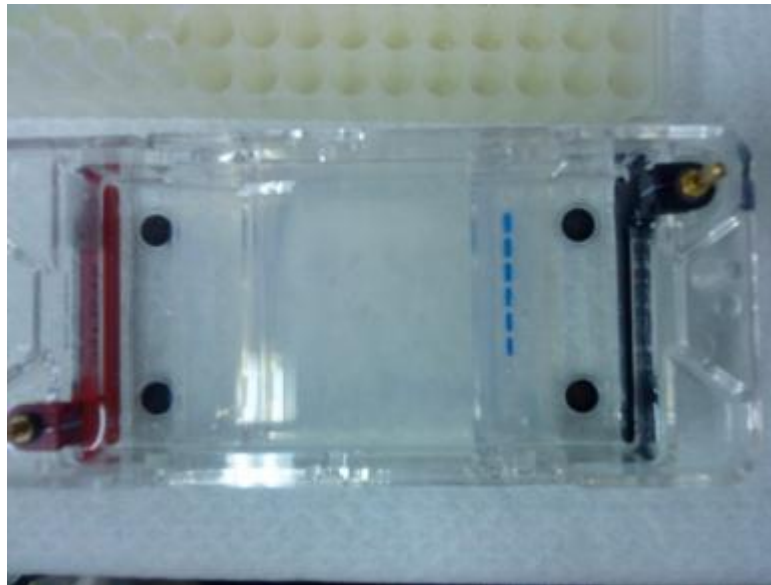
將製備好的洋菜凝膠至於電泳槽中，洋菜凝膠上裝載樣品井(loading well)需位於電泳槽負極的方向，加入 running buffer (0.5x TAE buffer)置剛好蓋過洋菜凝膠，即可 loading 樣品至 well 中。

填裝樣品完後，蓋上蓋子以及上層加蓋鋁箔紙避光，100V 電壓下進行電泳分析，約 30 分鐘後關閉電源，再以 UV 燈以及 photographed 觀察電泳膠。

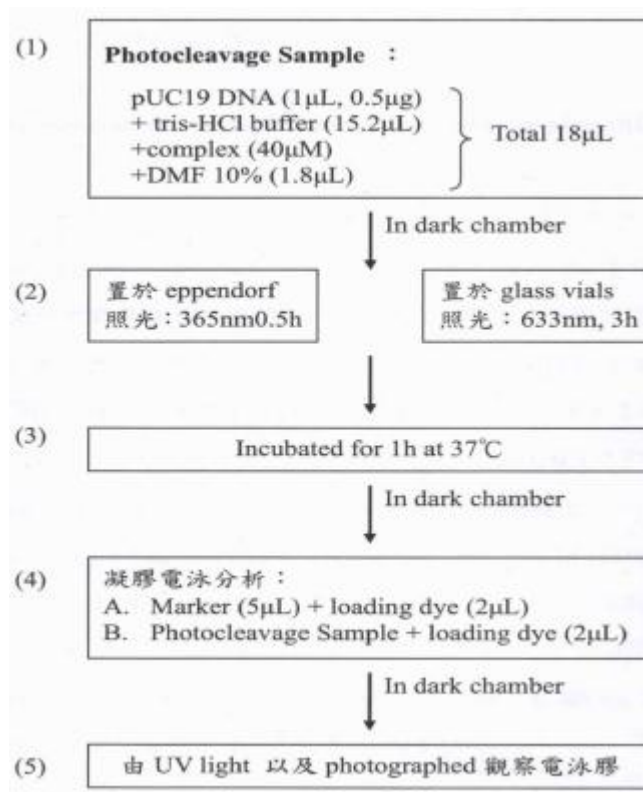


- (A) 圖為紫外光鍵結儀，實驗所使用的紫外光燈源為 365nm，8W，照光時間設定為 30min。
- (B) 將樣品注入在 eppendorf 上緣的蓋子，以利光源的照射。
- (C) 圖為 37°C 培養箱。
- (D) 圖為離心機，用途在於將 eppendorf 上緣的樣品離心至底部。

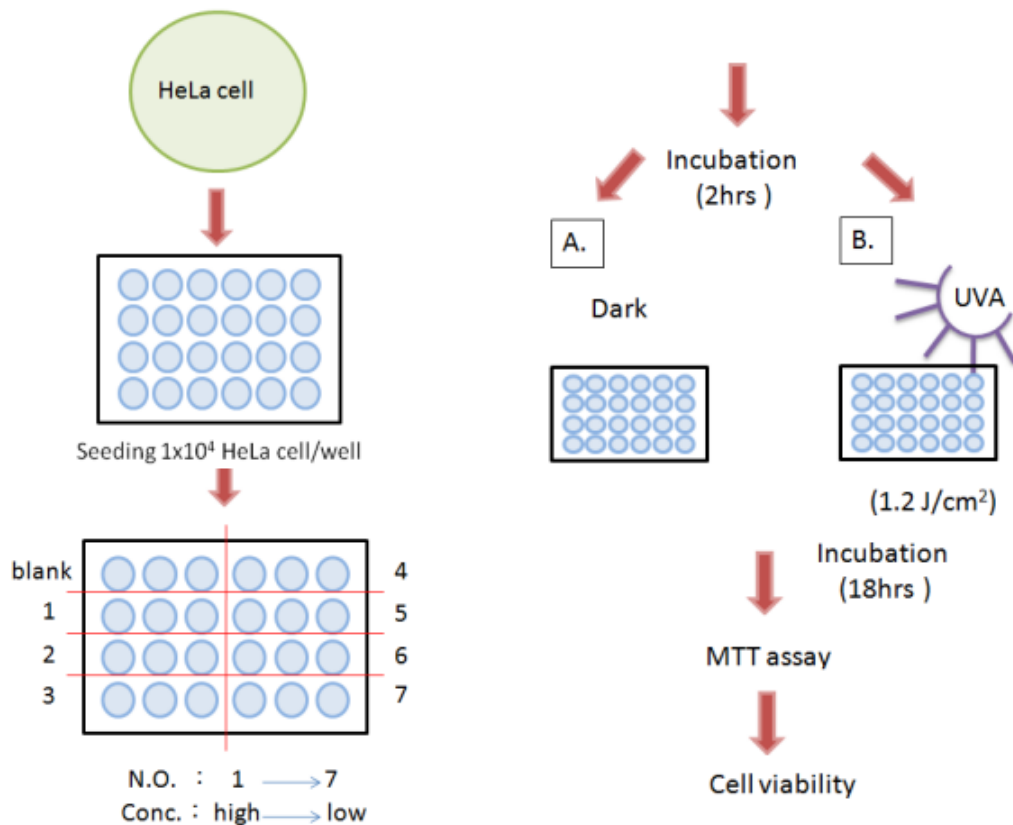
電泳



電泳流程



3.細胞實驗



(1)細胞株與細胞培養(Cell culture)

將 HeLa (人類子宮頸癌細胞)以無菌磷酸緩衝液 Phosphate- buffered saline(PBS)洗 2 次，之後加入 1X Trypsin-EDTA 溶液 1.5ml，置於 37°C 5%CO₂ 細胞培養箱作用 2 分鐘後，將貼在培養皿底的細胞切下，隨即加入 DMEM-F12 培養基稀釋，將懸浮細胞收集到無菌離心管中，以 1000rpm 離心 5 分鐘，加入適量 DMEM-F12 培養基均勻打散細胞後，種入 10 公分培養皿並置於 37°C 5%CO₂ 細胞培養箱，每二至三天進行培養。

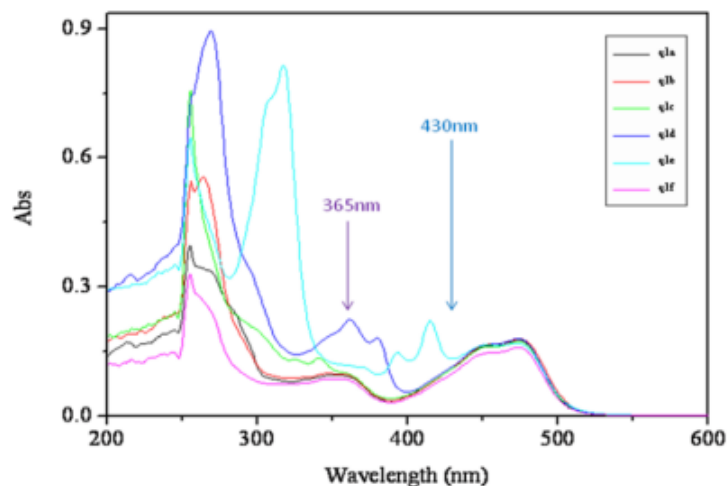
(2)細胞存活率的測定(MTT assay)

細胞存活率的測定是利用 MTT 在活細胞中會作用在粒線體酵素(琥珀酸去氫酶 dehydrogenase),而產生紫色的 formazan 之特性來測定細胞存活率，將 1×10^4 個 HeLa cell 置入 24 well 深孔盤中，按照濃度梯度 Lane1-Lane7 由

高到低加入錯合物處理 2 小時後，去上清液，加入 200 μ l PBS，隨後照光 (365nm)5min。去除 PBS，加入 500 μ l 培養基放置 24 小時，加入 50 μ g/ml MTT 於每一個 well 中，放置 37 $^{\circ}$ C 5%CO₂ 細胞培養箱作用 4 小時，以 1500rpm 離心五分鐘，隨後去除上清液，加入 200 μ l DMSO 於室溫下用 30 分鐘後，即可以 ELISA reader 於波長 490nm 讀取吸光值變化。

伍、研究結果與討論

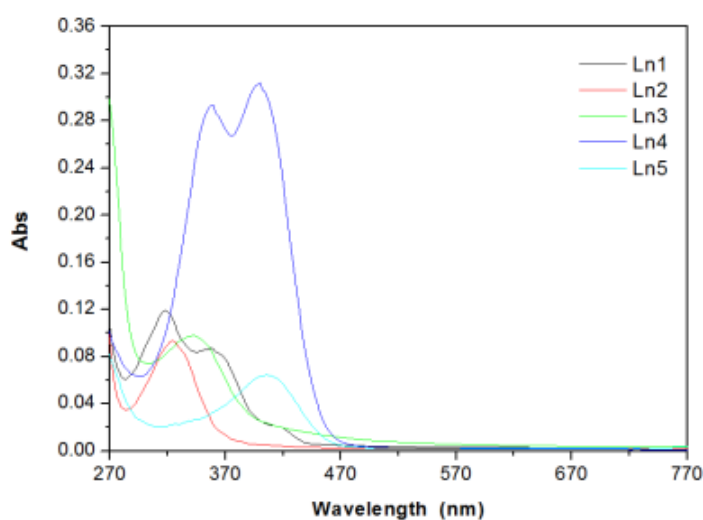
(一)錯合物 **q1a-q1f** 的吸收光譜(濃度：10 μ M)



錯合物 **q1a-q1f** 吸收光譜圖，溶劑為 DMF:H₂O (1:1)，濃度: 10 μ M

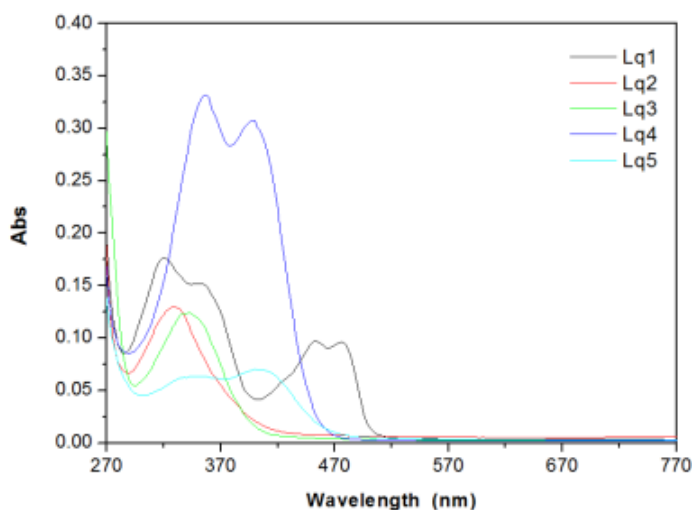
本圖中的紫色箭頭 365nm 代表所用紫外光燈源波長，藍色箭頭 430nm 代表所用藍光燈源波長，在 365nm **q1d** 吸收係數最大，因此我們可預期在紫外光下誘導的 DNA 光裂解錯合物 **q1d** 的效果會最好。而在 430nm 的地方，錯合物 **q1a-q1f** 都有不錯的吸收，代表在藍光的部分，也有其應用的潛力。

(二)Ln1-Ln5 的 UV 吸收光譜圖(濃度：20 μ M)



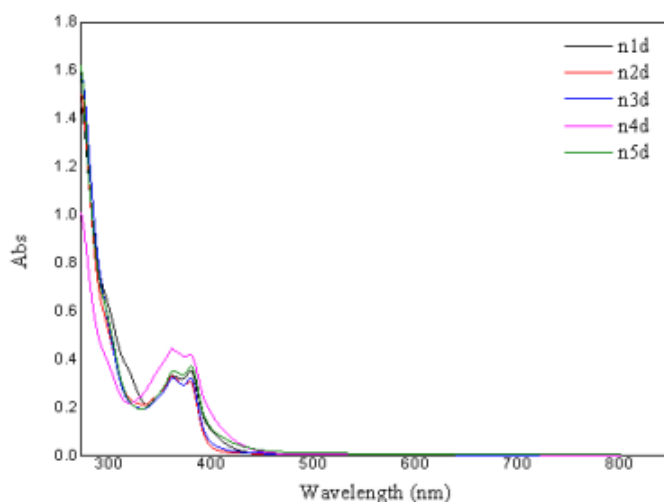
以配位基 **Ln1-Ln5** 配置成 DMF/H₂O(v/v)溶液所測量的吸收光譜，如上圖所示，配位基主要吸收為 UV 區，在 365nm 及 430nm **Ln4** 吸收係數皆最大，因此我們可預期在紫外光下誘導的 DNA 光裂解 **Ln4** 的效果會最好。

(三)Lq1-Lq5 的 UV 吸收圖(濃度：20μM)



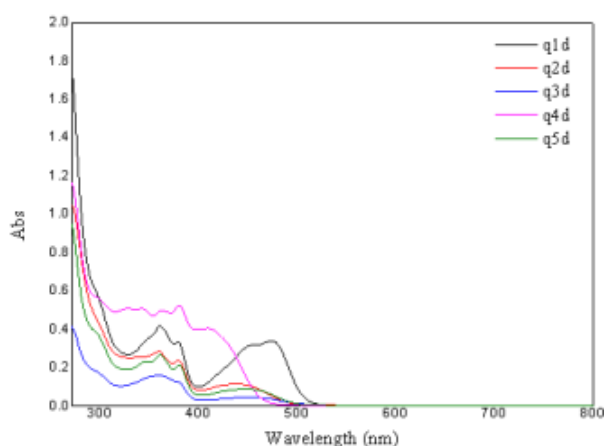
以配位基 **Lq1-Lq5** 配置成 DMF/H₂O(v/v)溶液所測量的吸收光譜，如上圖所示，配位基主要吸收為 UV 區，從此圖可得知 **Lq1** 跟 **Ln1** 有所差異，在 400-500nm 有一個不錯的吸收，原因推測為 quinoline 造成共軛延伸，但在 **Lq2-Lq5** 則沒有發現這樣的現象，而在 365nm **Lq4** 吸收係數最大，跟 **Ln4** 有相同的結果。

(四)n1d-n5d 的 UV 吸收圖(濃度：20μM)



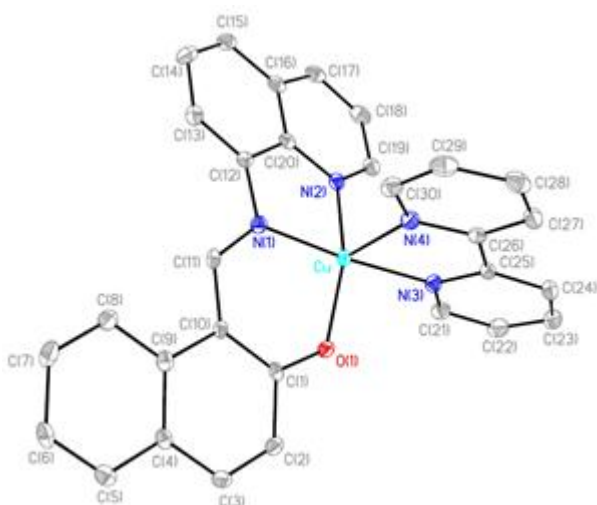
以配位基 **n1d-n5d** 配置成 DMF/H₂O(v/v)溶液所測量的吸收光譜，如上圖所示，錯合物主要吸收為 UV 區，**n1d-n5d** 的吸收光譜差異並不大，波峰都在 365nm，所以我們可以利用其在紫外光區較好的吸收對 DNA 進行光裂解。

(五)q1d-q5d 的 UV 吸收圖(濃度：20μM)



以配位基 **q1d-q5d** 配置成 DMF/H₂O(v/v)溶液所測量的吸收光譜，如上圖所示，錯合物主要吸收為 UV 區，而 **q1d** 的吸收較靠近長波長的區段，表示未來其在光動力的應用價值較高，另外，**q4d** 跟其他四個 complex 相比在 300-470nm 有一個較廣的吸收。

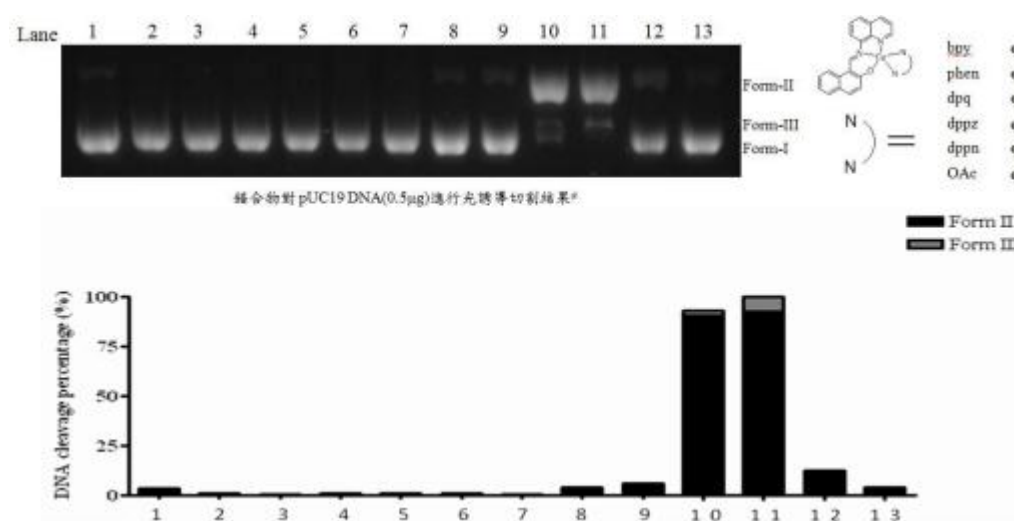
(六)晶體結構



Bond Length(Å)	
Cu O1	1.9283(15) Å
Cu N1	1.9410(17) Å
Cu N3	2.0149(17) Å
Cu N2	2.0147(18) Å
Cu N4	2.1904(16) Å
Bond Angle(°)	
O1 Cu N1	92.76(7) °
O1 Cu N3	91.32(7) °
N1 Cu N3	174.38(7) °
O1 Cu N2	155.21(6) °
N1 Cu N2	83.03(7) °
N3 Cu N2	94.84(7) °
O1 Cu N4	101.26(6) °
N1 Cu N4	97.16(6) °
N3 Cu N4	78.24(6) °
N2 Cu N4	103.50(7) °

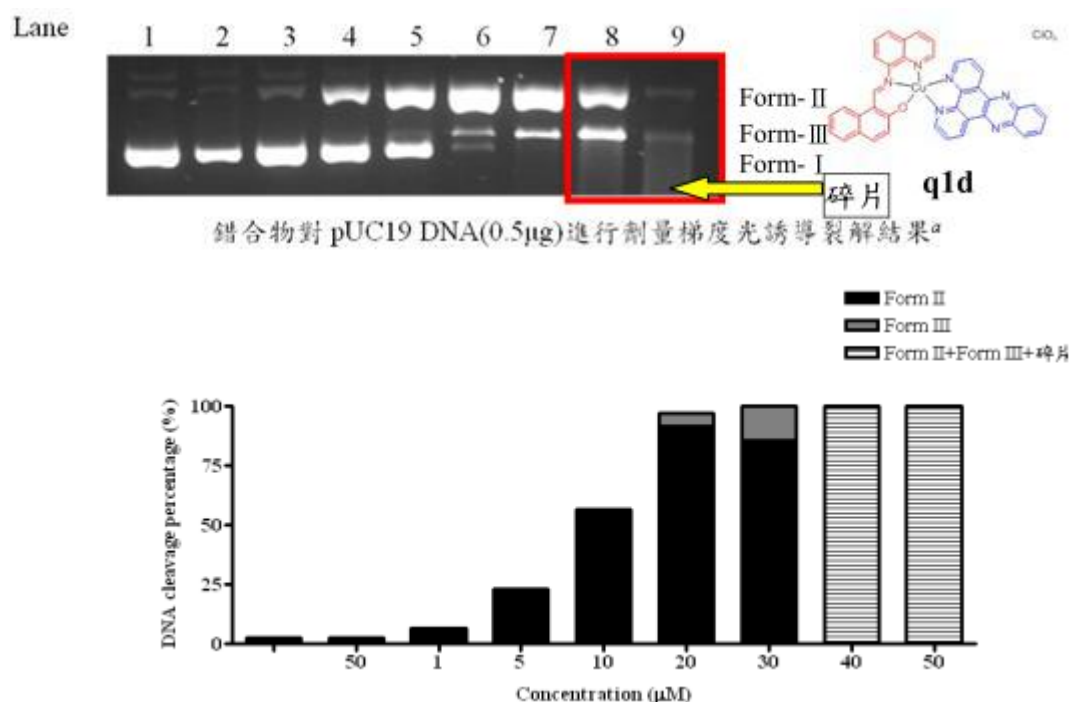
(七)電泳實驗

實驗一：不同配位基在照紫外光及暗室下對 DNA 裂解之影響



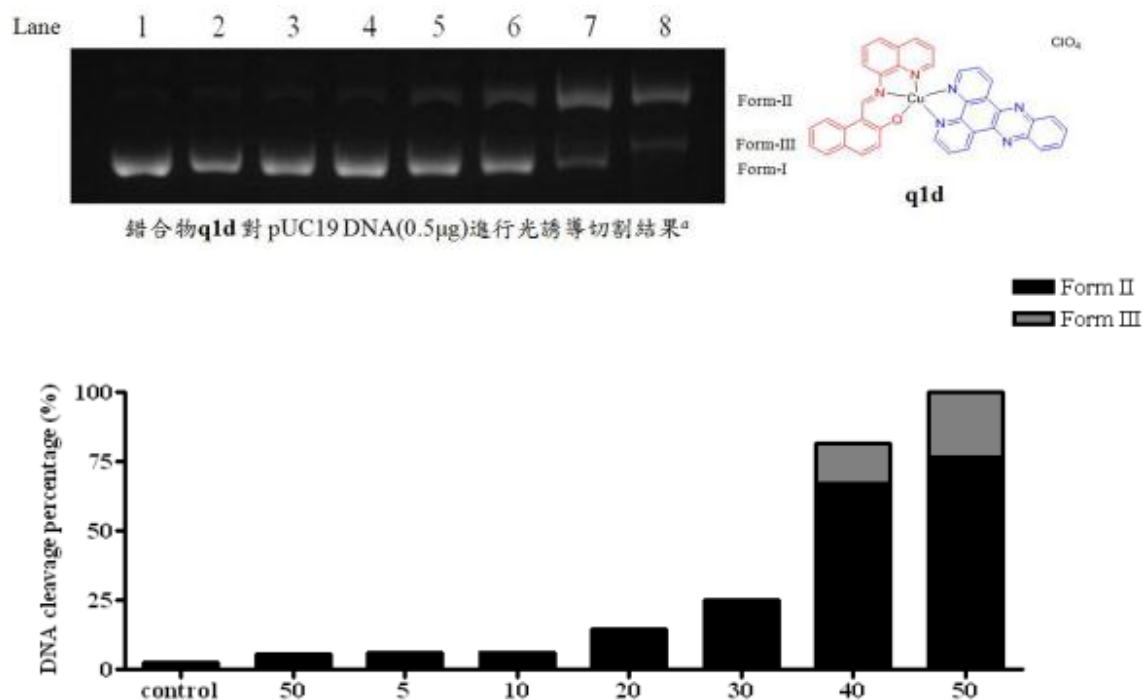
20 μ M 錯合物 **q1a-q1f** 在含 10%DMF 的 50 mM Tris-HCl buffer (pH, 7.2) 之中，對(SC) pUC19 DNA 光裂解實驗。如圖所示，在 Lane 2-7 避光條件下，**q1a-q1f** 對 DNA 不會造成任何破壞；然而，Lane 8-13 以紫外光鍵結儀(8W)照射 365 nm 紫外光 30 分鐘後，**q1a** 和 **q1b** 只有不到 10%的裂解，**q1c** 和 **q1d** 幾乎是 100%斷裂，甚至已經看到 10%的 Form III，但是 **q1e** 只有不到 15%的裂解，而 **q1f** 則是一如預期不具有活性。由文獻得知當錯合物共配位基苯環越長，其嵌入 DNA 的能力越好，因此從 **q1a-q1d** 我們觀察到這樣的趨勢，隨著雜環的延伸對 DNA 光裂解的效果就越好，然而 **q1e** 應具有最佳的嵌入效果，卻因為會放出螢光，使得吸收的能量以光能形式散失，導致活性較差。

實驗二：在紫外光下，錯合物 q1d 濃度對 DNA 裂解之影響



- 實驗結果顯示，在有照光的情況下(Lane3-9)，錯合物濃度愈高，裂解 DNA 的效果則越好，可以得知在此條件下，濃度 20μM 就足以造成 95% DNA 裂解，因此後續實驗在以紫外光為燈源時皆使用 20μM。
- Lane 2 dark 的對照組錯合物濃度為 50μM 的原因：之前的實驗結果顯示，錯合物 4 在不照光時沒有效果，於是我們直接選擇本實驗最高濃度做代表，如果 50μM 沒有效果，則無需再檢測其他較低濃度者。
- Lane 8 & Lane 9 兩者的電泳圖有明顯的差異，但 Form II & Form III 的百分比皆大於 99%，主要原因為濃度過高，造成 DNA 裂解成微小碎片。由於在電泳中，體積愈小穿過 gel 孔洞的速度愈快，因此，碎片跑的距離會較 Form I 遠，且因為碎片的大小不一而有拖尾的現象。故在 Lane 8 & Lane 9 中，兩者皆已出現拖尾，表示部分 DNA 已裂解成碎片，其中 Lane 9 幾乎完全變為碎片，其拖尾的部分已超出此圖的範圍，而在 Lane 8 中還保留部分 Form II & Form III 形式，但我們仍都用 >99% 以表示其裂解程度之高。

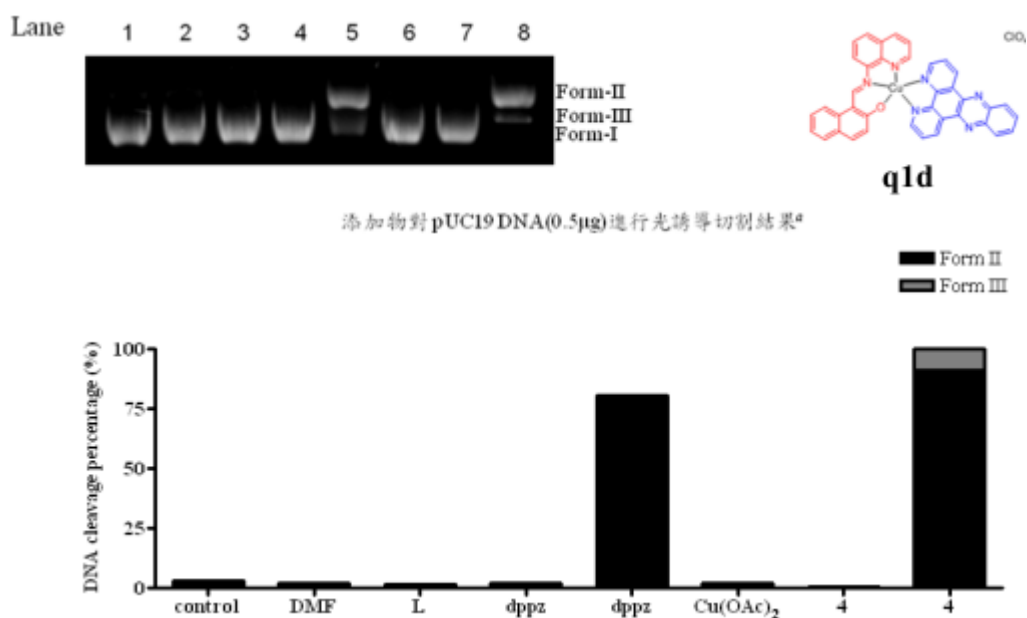
實驗三：錯合物 **q1d** 在藍光下對 DNA 裂解之影響



濃度 20 μM 的錯合物 **q1a-q1f** 在含有 10%DMF 的 50 mM Tris-HCl buffer (pH, 7.2)之中，以波長 430nm 的藍光 LED 為光源，對(SC) pUC19 DNA 進行光裂解實驗，考量到紫外光雖已有很多臨床的應用，將光源移往能量較弱的可見光區仍是較佳的選擇，除了對人體較為安全也可穿透至較深層的組織。此圖即為不同濃度的 **q1d** 在藍光下的電泳圖，其中 Lane2 為不照光，而 Lane3-9 為照光 60 分鐘。從實驗結果了解到光源改為藍光後，**q1d** 也有不錯的效果，而且濃度越高切斷 DNA 的效果越好。從先前結果得知，**dppz** 對 DNA 光裂解有較佳的效果，於是我們採用 **dppz** 做為共配位基進行以下的實驗。

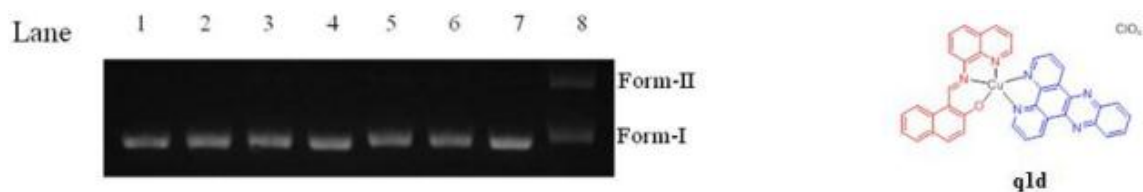
實驗四：錯合物 4(dppz)各個起始物在紫外光下對 DNA 裂解之影響

由於我們發現錯合物 **q1d** 有最好的效果，因此選擇其做為進一步實驗的對象。

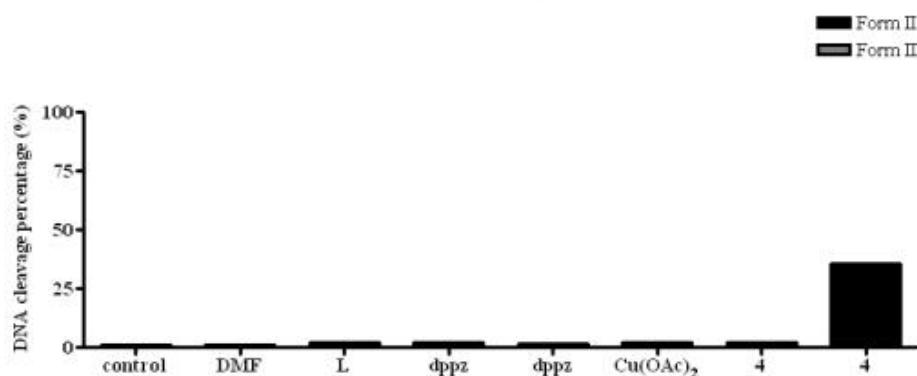


1. 由 Lane 2、3 和 6 得知，DMF、ligand 和金屬(Cu)單獨存在時，對 DNA 沒有影響。
2. 由 Lane 4-5 及 7-8 推測，單獨只有 dppz 存在時，照射紫外光就會對 DNA 造成裂解，原因是 dppz 在 365nm 下有吸收且其本身就具有嵌入 DNA 的能力，但仍以錯合物的效果較佳。

實驗五：錯合物 q1d(dppz)各個起始物在藍光下對 DNA 裂解之影響

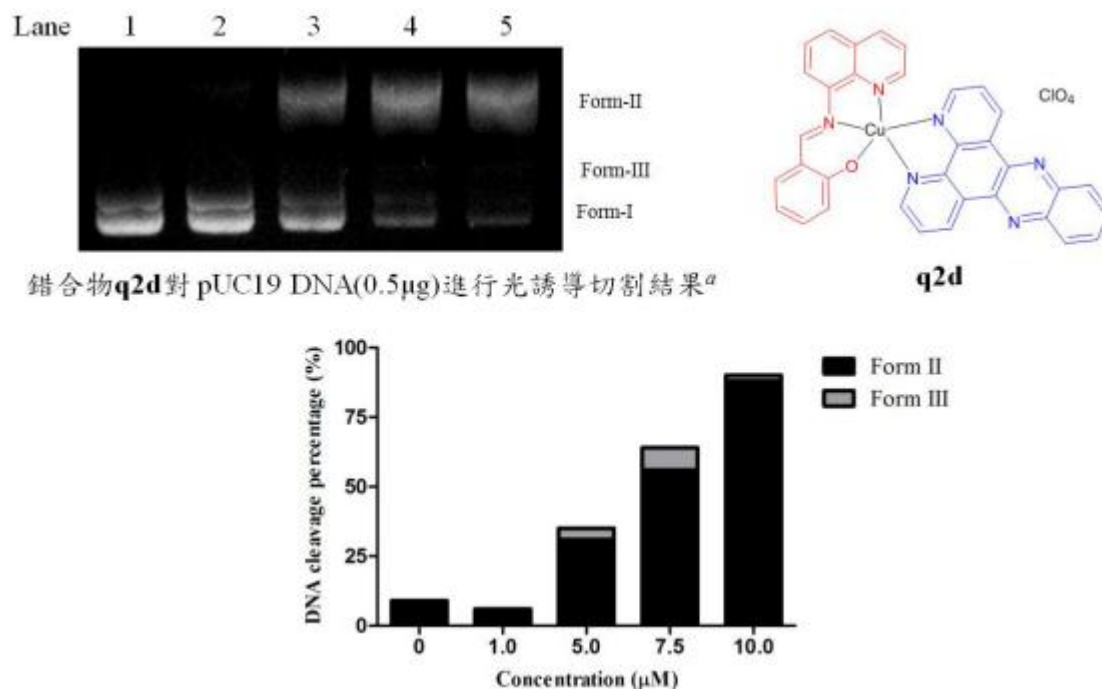


添加物對 pUC19 DNA(0.5μg)進行光誘導切割結果^a



1. 由表中得知，各起始物在藍光下皆無效果，在與上圖做個比較，可以發現 dppz 沒有活性，原因為 dppz 在 430nm 沒有吸收。
2. 由於錯合物在藍光的吸收較小，我們預期裂解的效果可能不如紫外光不明顯，故將錯合物的濃度從 20μM 調高至 40μM，但其裂解效果仍較紫外光差。

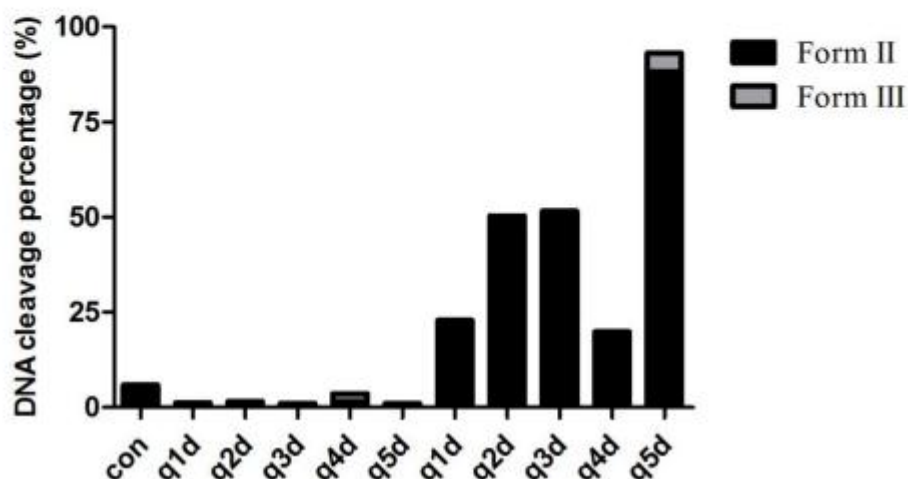
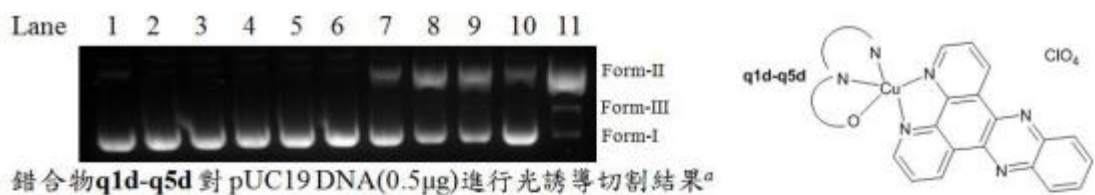
實驗六：q2d 在紫外光下濃度梯度對 DNA 裂解之影響



錯合物q2d對 pUC19 DNA(0.5μg)進行光誘導切割結果^a

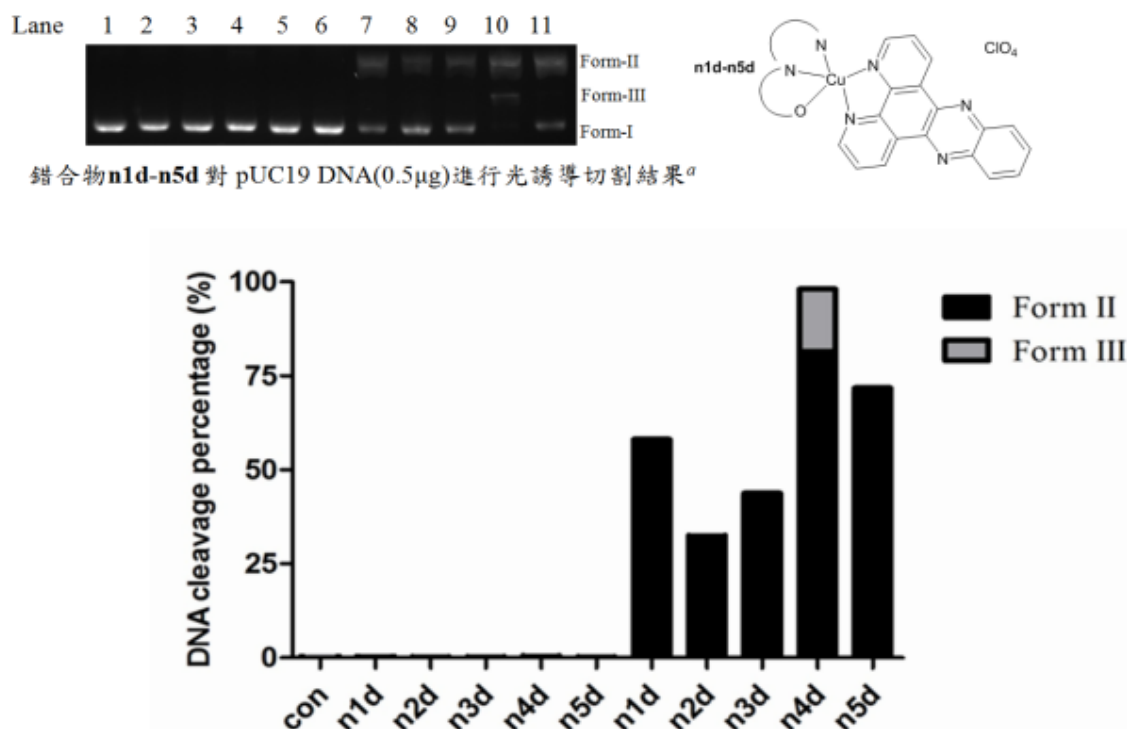
由於將 **q** 系列、**n** 系列錯合物以 20μM 進行 DNA 裂解實驗時，實驗會呈現過飽和的狀態，以至於無法分辨 **q** 系列和 **n** 系列的效果優劣。我們進行此次實驗找出最佳的濃度，使 DNA 裂解結果不會呈現過飽和的狀態，因此我們以不同濃度的 **q2d** 在含有 10%DMF 的 50 mM Tris-HCl/NaCl buffer(pH=7.2)之中，以波長 430nm 的藍光 LED 做為光源，進行 DNA 裂解的實驗。由實驗結果我們選擇以 7.5μM 進行以下的實驗。

實驗七：q1d-q5d 在紫外光下對 DNA 裂解之影響



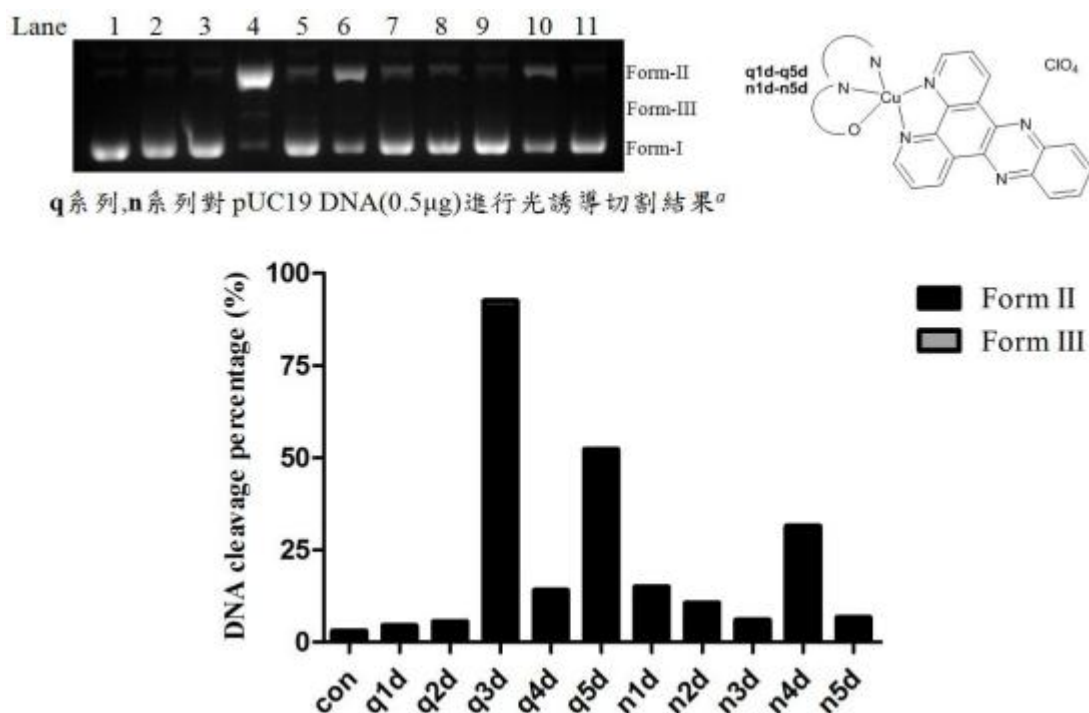
濃度 7.5 μM 的錯合物 **q1d-q5d** 在含 10%DMF 的 50 mM Tris-HCl buffer (pH, 7.2)之中，對(SC) pUC19 DNA 光裂解實驗，其中，Lane2-6 為不照光，Lane1,7-11 為以波長 365nm 的紫外光鍵結儀為光源，照光 30 分鐘。如圖所示，在避光條件下，**q1d-q5d** 對 DNA 不會造成任何破壞；然而，照射 365 nm 紫外光 30 分鐘後，**q1d** 到 **q5d** 都有不錯的效果。**q5d** 裂解 DNA 效果較好，雖然它有明顯的螢光，但其錯合物仍會裂解 DNA，因為主要與 DNA 作用的 dppz，而 dppz 不會放出螢光，因此 ligand 的螢光影響不大。

實驗八：n1d-n5d 在紫外光下對 DNA 裂解之影響



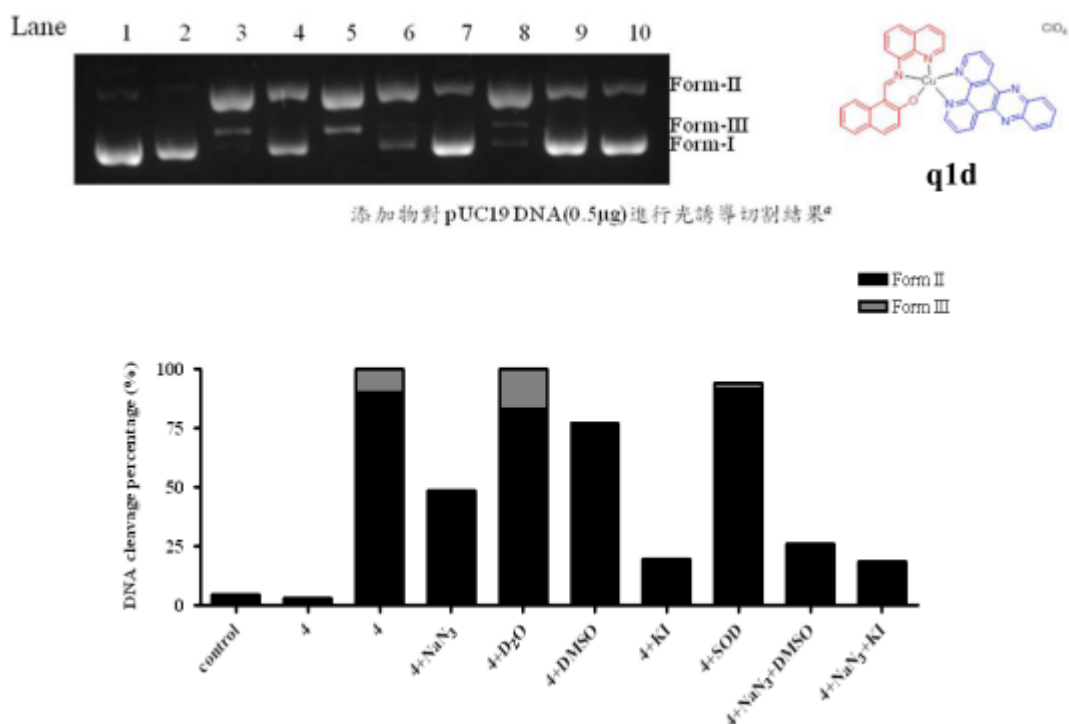
濃度 $7.5 \mu\text{M}$ 的錯合物 **n1d-n5d** 在含 10%DMF 的 50 mM Tris-HCl buffer (pH, 7.2) 之中，對(SC) pUC19 DNA 光裂解實驗，其中，Lane2-6 為不照光，Lane1,7-11 為以波長 365nm 的紫外光鍵結儀為光源，照光 30 分鐘。如圖所示，在避光條件下，**n1d-n5d** 對 DNA 不會造成任何破壞；然而，照射 365 nm 紫外光 30 分鐘後，**n1d** 到 **n5d** 都有不錯的效果。其中 **n4d** 裂解 DNA 效果較好，因為 **n4d** 接 NO_2 基為拉電子基。

實驗九：q 系列, n 系列在藍光下對 DNA 裂解之影響



將濃度 7.5 μM 的錯合物 **q** 系列和 **n** 系列在含 10%DMF 於 50 mM Tris-HCl buffer (pH, 7.2) 之中，對(SC) pUC19 DNA 光裂解實驗，並以波長 430nm 的藍光 LED 為光源。如圖所示，**q** 系列和 **n** 系列裂解 DNA 的效果較好者為 **q3d** 和 **n4d**。其中 **n4d** 裂解 DNA 效果較好，因為 **n4d** 接 NO₂ 基為拉電子基。整體來說，**q** 系列在照藍光下，裂解效果較 **n** 系列好，應用價值較高。

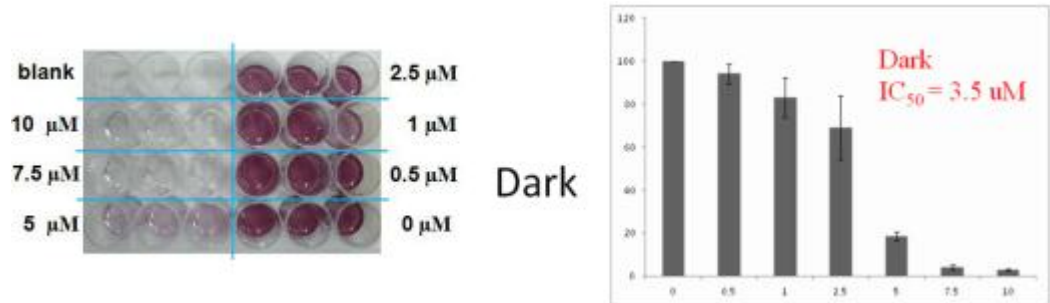
實驗十：探討錯合物裂解 DNA 之途徑



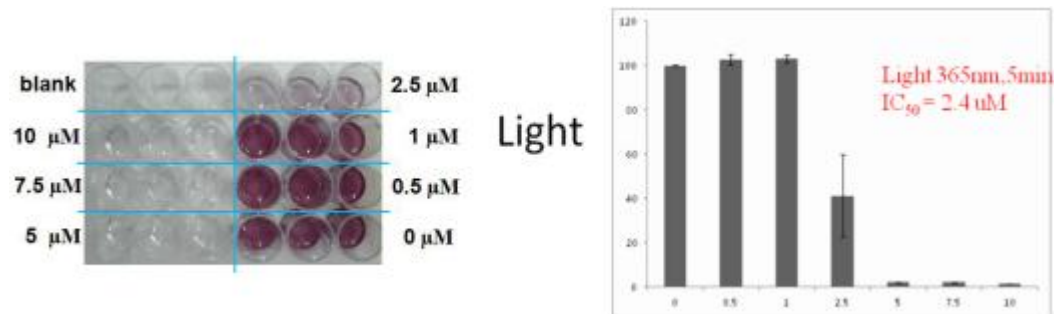
我們針對 **q1d** 加入不同的添加物探討其對 DNA 光裂解的影響，而這些添加物分別有不同的功能，NaN₃ 可以抑制單態氧的產生，D₂O 則是延長單態氧的生存時間，DMSO 與 KI 皆為抑制氫氧自由基的試劑，SOD 可以抑制超氧自由基；藉由圖表把所有的實驗組與 negative control(air)對照之後，可以發現 NaN₃ 與 D₂O 同時展現了抑制與加成的作用，DMSO 可抑制少許，KI 則大量抑制活性而 SOD 並沒有影響其表現，從這些結果可推敲出整個機制應同時包含了單態氧與氫氧自由基的產生所導致的 DNA 裂解，為了進一步確認，試著把單態氧與氫氧自由基的抑制劑同時加入並觀察其裂解程度，發現到兩種不同效果的試劑的確可以產生加成的抑制效果，而確認其活性物種為此二種。

實驗十一：q3d 細胞毒性測試

(一)



(二)



以濃度 0、0.5、1、2.5、5、7.5、10 μM 的 **q3d** 處理人類子宮頸癌 HeLa 細胞，利用 MTT assay 探討 HeLa 細胞存活率表現；其中(一)為 **q3d** 避光、(二)為 **q3d** 照 UVA (365nm)五分鐘，1.2J/cm²。結果可以經由 MTT assay 後發現細胞存活率會隨著錯合物濃度提升而降低。此外 從實驗結果得知，**q3d** 不照光時， IC_{50} 為 3.5 μM ，照光後， IC_{50} 為 2.4 μM ，由此發現 **q3d** 確實具有做為光動力治療藥物的潛力。

陸、結論

- (一) 利用 N,N,O-Schiff base 配位基與聯吡啶及其所衍生之共配位基，成功合成出一系列的二價銅錯合物。
- (二) 以波長 365nm 的紫外光燈源對錯合物 **q1a-q1f** 進行 DNA 光誘導裂解，優選後發現 **q1c** 及 **q1d** 有較好的表現，也就是當嵌入基為 **dpq** 及 **dppz** 時，活性較佳。
- (三) 使用 **dppz** 為共配位基並接上不同配位基的錯合物 **q1d-q5d** 和 **n1d-n5d** 在照射 430nm 的藍光下，我們發現 **q** 系列的整體 DNA 裂解效果比 **n** 系列佳，也就是 quinoline 是比 naphthalene 適合的配位基。
- (四) 整體來說，以 **q3d** 在藍光的照射下有最佳的 DNA 裂解效果。
- (五) 錯合物的作用機制應同時包含了單態氧與氫氧自由基的產生而導致 DNA 的裂解，了解此機構應有助於未來的應用。
- (六) 相對於先前僅能吸收紫外光波段的錯合物，此次合成的系列錯合物能在藍光波長區段有很好的吸收，已可延伸至可見光區，故在未來臨床上應用的價值也隨之提高。

柒、參考資料

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1. Introduction

When we were searching for topics to develop our interests, we came upon a clip that was about photodynamic therapy and we were fascinated about the idea of curing cancer with light exposure and complexes. After browsing some papers, we decided to bury ourselves in this study and discuss more ways of how to make complexes more effective to cleave DNA, even testing them on cancer cells to examine the effects.

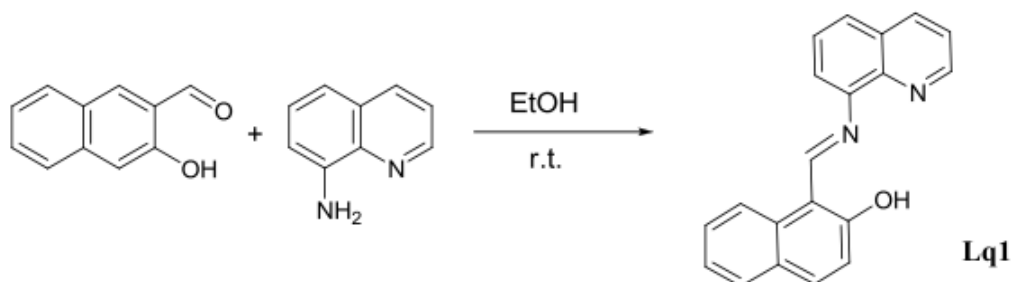
PDT is an effective way to kill cancer cells and is less invasive to the body compared to chemotherapy. We also focused on exposing the cells under visible light which does less harm than ultraviolet, which early papers use. We hope by doing our research we can bring more convenience to curing cancer and find out better ways of medication.

2. Procedures and Methods

I. Synthesis

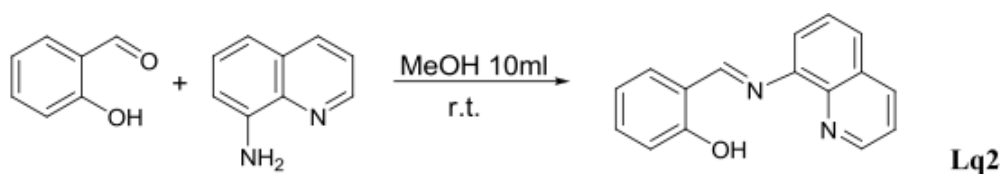
(1) Synthesis of ligands

a. Lq1



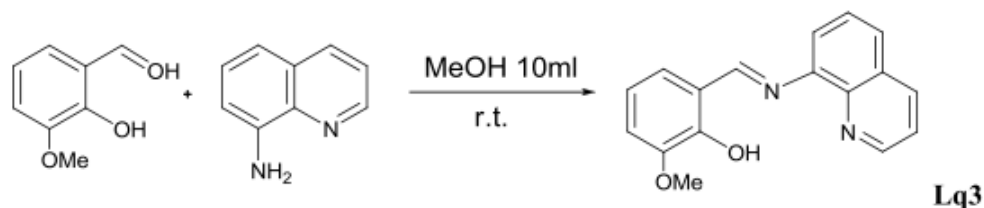
Weigh 2-hydroxy-1-naphthaldehyde (0.86 g, 5.00 mmol) and place it in a 100 ml round-bottomed flask. Add 8-aminoquinoline (0.72 g, 5.00 mmol). Use 40 ml ethanol as solvent, and benzenesulphonic acid 0.1 ml as catalyst. After constantly heating and refluxing it for 10 hours an orange solid precipitates, cool it and leave it for a while. Use a filter board to collect it, and use a little ethanol to wash it. After draining it in vacuity, we can attain orange powder (1.21 g, yield:81%).

b. Lq2



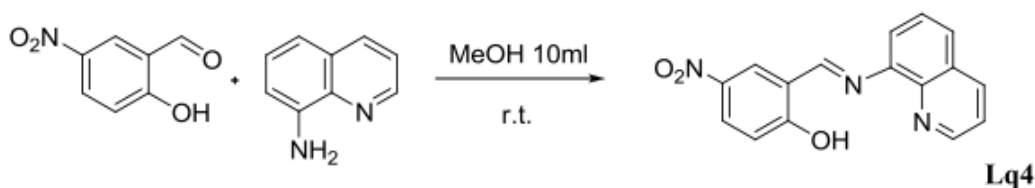
Weigh 2-hydroxybenzaldehyde (0.61 g, 5.00 mmol) and place it in a 50 ml round-bottomed flask. Add 8-aminoquinoline (0.793g, 5.50 mmol). Use 20 ml methanol as solvent, and benzenesulphonic acid 0.1 ml as catalyst. After constantly heating and refluxing it for 10 hours an orange solid precipitates, cool it and leave it for a while. Use a filter board to collect it, and use a little methanol to wash it. After draining it in vacuity, we can attain orange powder (0.893 g, yield:72%).

c. **Lq3**



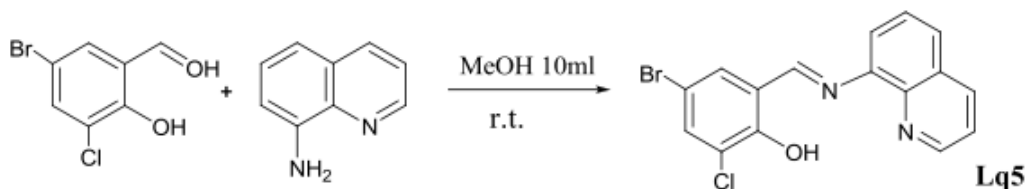
Weigh 2-hydroxy-3-methoxybenzaldehyde (0.766 g, 5.00 mmol) and place it in a 50 ml round-bottomed flask. Add 8-aminoquinoline (0.793 g, 5.50 mmol). Use 20 ml methanol as solvent, and benzenesulphonic acid 0.1 ml as catalyst. After constantly heating and refluxing it for 10 hours an orange solid precipitates, cool it and leave it for a while. Use a filter board to collect it, and use a little methanol to wash it. After draining it in vacuity, we can attain orange powder (0.877 g, yield:63%).

d. **Lq4**



Weigh 2-hydroxy-5-nitrobenzaldehyde (0.836 g, 5.00 mmol) and place it in a 50 ml round-bottomed flask. Add 8-aminoquinoline (0.793 g, 5.50 mmol). Use 20 ml methanol as solvent, and benzenesulphonic acid 0.1 ml as catalyst. After constantly heating and refluxing it for 10 hours an orange solid precipitates, cool it and leave it for a while. Use a filter board to collect it, and use a little methanol to wash it. After draining it in vacuity, we can attain orange powder (1.173 g, yield:80%).

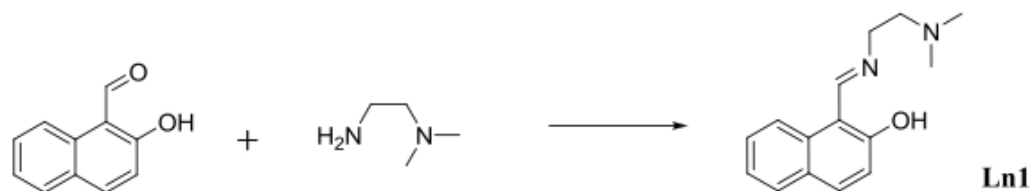
e. **Lq5**



Weigh 5-bromo-3-chloro-2-hydroxybenzaldehyde (1.182 g, 5.00 mmol) and place it in a 50 ml round-bottomed flask. Add 8-aminoquinoline (0.793 g, 5.50 mmol). Use 20 ml methanol as solvent, and benzenesulphonic acid 0.1

ml as catalyst. After constantly heating and refluxing it for 10 hours an orange solid precipitates, cool it and leave it for a while. Use a filter board to collect it, and use a little methanol to wash it. After draining it in vacuity, we can attain orange powder (1.049 g, yield:58%).

f. Ln1



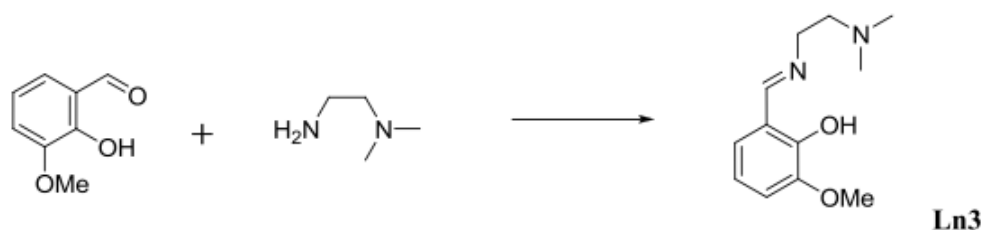
Weigh 2-hydroxy-1-naphthaldehyde(0.860 g, 5.00 mmol), N,N-dimethylethane-1,2-diamine (0.485g , 5.50mmol) and place it in a 50 ml round-bottomed flask. Use 20 ml methanol as solvent, and benzenesulphonic acid 0.1 ml as catalyst. After constantly heating and refluxing it for 10 hours an orange solid precipitates, cool it and leave it for a while. Use a filter board to collect it, and use a little methanol to wash it. After draining it in vacuity, we can attain orange powder (0.945 g, yield:78%).

g. Ln2



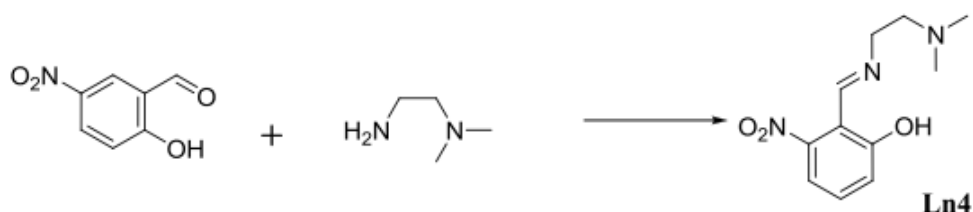
Weigh 2-hydroxybenzaldehyde (0.610g , 5.00mmol), N,N-dimethylethane-1,2-diamine (0.485g , 5.50mmol), and place it in a 50 ml round-bottomed flask. Use 20 ml methanol as solvent, and benzenesulphonic acid 0.1 ml as catalyst. After constantly heating and refluxing it for 10 hours an orange solid precipitates, cool it and leave it for a while. Use a filter board to collect it, and use a little methanol to wash it. After draining it in vacuity, we can attain orange powder (0.634 g, yield:66%)

h. Ln3



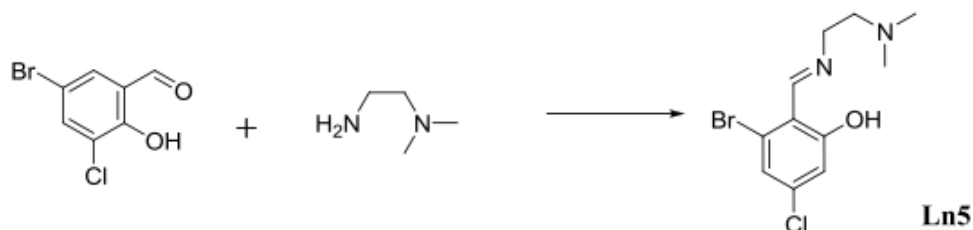
Weigh 2-hydroxy-3-methoxybenzaldehyde(0.766g , 5.00mmol), N,N-dimethylethane-1,2-diamine (0.485g , 5.50mmol), and place it in a 50 ml round-bottomed flask. Use 20 ml methanol as solvent, and benzenesulphonic acid 0.1 ml as catalyst. After constantly heating and refluxing it for 10 hours an orange solid precipitates, cool it and leave it for a while. Use a filter board to collect it, and use a little methanol to wash it. After draining it in vacuity, we can attain orange powder (0.767 g, yield:69%).

i. Ln4



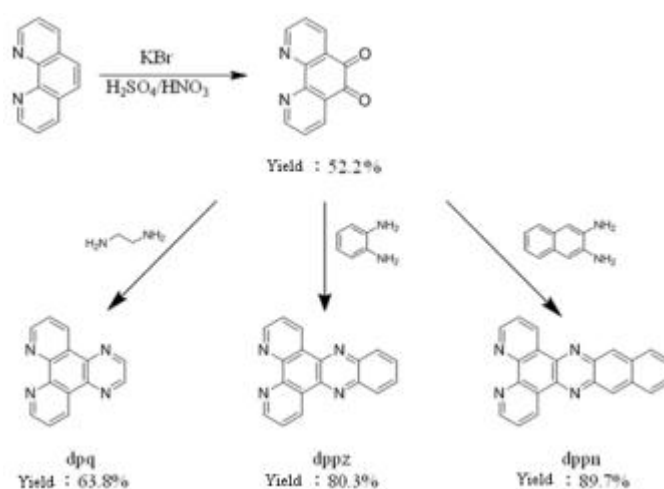
Weigh 2-hydroxy-5-nitrobenzaldehyde (0.836g , 5.00mmol), N,N-dimethylethane-1,2-diamine (0.485g , 5.50mmol), and place it in a 50 ml round-bottomed flask. Use 20 ml methanol as solvent, and benzenesulphonic acid 0.1 ml as catalyst. After constantly heating and refluxing it for 10 hours an orange solid precipitates, cool it and leave it for a while. Use a filter board to collect it, and use a little methanol to wash it. After draining it in vacuity, we can attain orange powder (0.901 g, yield:76%).

j. Ln5



Weigh 5-bromo-3-chloro-2-hydroxybenzaldehyde (1.182 g, 5.00 mmol), N,N-dimethylethane-1,2-diamine (0.485 g, 5.50 mmol), and place it in a 50 ml round-bottomed flask. Use 20 ml methanol as solvent, and benzenesulphonic acid 0.1 ml as catalyst. After constantly heating and refluxing it for 10 hours an orange solid precipitates, cool it and leave it for a while. Use a filter board to collect it, and use a little methanol to wash it. After draining it in vacuity, we can attain orange powder (0.962 g, yield:63%).

(2) Synthesis of Intercalator



(a) Synthesis of 1,10-phenanthroline-5,6-dione:

Weigh 1,10-phenanthroline (4.00 g, 22.2 mmol), place it in a 100 mL round-bottomed flask and add KBr (4.00 g, 33.6 mmol). While ice bathing add 20 ml dense sulfuric acid and 10 ml nitrate. Heat and reflux it for 3 hours. After it has cooled down, add it into 500 ml deionized water and add NaOH solution. Use an universal indicator to adjust the pH value to 6 and use CH_2Cl_2 to extract the organic layer to condense it. Add heated ethanol to recrystallize it, then we can attain a yellow acicular solid (3.28 g, yield:66%).

(b) Synthesis of dibenzo[f,h]quinoxaline (dpq):

Weigh 1,10-phenanthroline-5,6-dione (0.63 g, 3.00 mmol) and place it in a 50 ml round-bottomed flask. Add ethane-1,2-diamine (0.18 g, 3.00 mmol). Use 30 ml ethanol as solvent and benzenesulphonic acid 0.1 ml as catalyst. After constantly heating and refluxing it for 10 hours as a white solid

precipitates, cool it and leave it for a while. Use a filter board to collect it, and use a little ethanol to wash it. After draining it in vacuity, we can attain white powder (0.44 g, yield:64%).

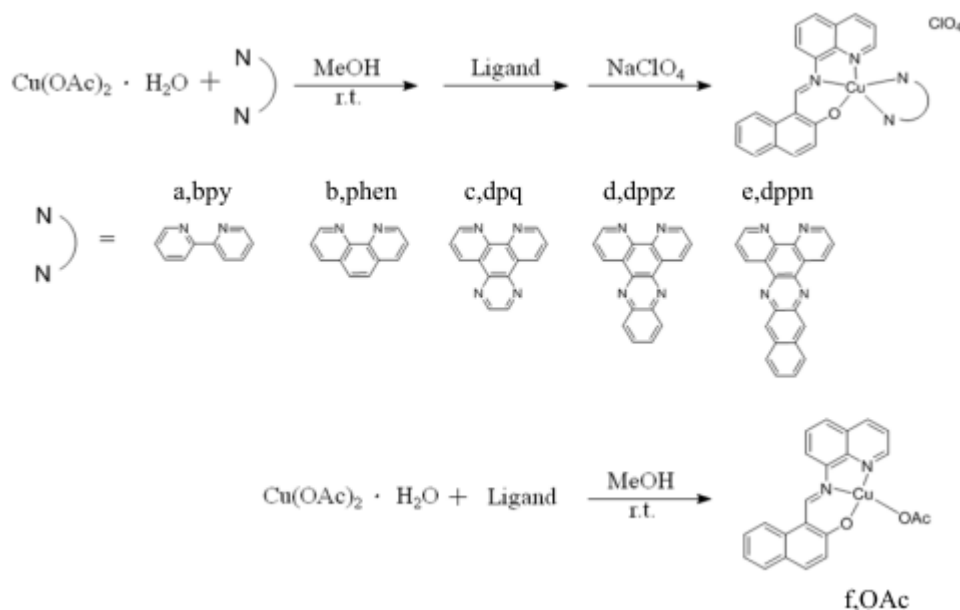
(c) Synthesis of dipyrido[3,2-a:2',3'-c]phenazine (dppz):

The method is same as **dpq**. 1,10-phenanthroline-5,6-dione (0.63 g, 3.00 mmol), benzene-1,2-diamine (0.32 g, 3.00 mmol), and we can attain white powder (0.68 g, yield:80%).

(d) Synthesis of benzo[i]dipyrido[3,2-a:2',3'-c]phenazine (dppn):

The method is same as **dpq**. 1,10-phenanthroline-5,6-dione (0.63 g, 3.00 mmol), naphthalene-2,3-diamine (0.47 g, 3.00 mmol), and we can attain orange powder(0.90 g, yield:90%).

(3) Synthesis of complexes **q1a-q1f**



(a) Synthesis of [Cu(L)bpy]ClO₄ (q1a):

Weigh copper acetate(0.14 g, 0.70 mmol)and bipyridine(0.12 g, 0.77 mmol), and place it in a 50 ml round-bottomed flask. Use 20 ml methanol as solvent, and stir it for 1 hour under room temperature. After it has finished reacting, add ligand (L)(0.23g, 0.77 mmol), and stir it for 3 hours under room

temperature. Add sodium perchlorate (0.094 g, 0.77 mmol), then we can soon attain solid precipitation. Use a filter board to collect the solid and wash it with cooled methanol several times. Last, drain it in vacuity. Then we can attain a brown solid(0.38g, yield: 89 %).

(b) Synthesis of [Cu(L)phen]ClO₄ (q1b):

The method is same as **q1a**, except changing bpy to 1,10-phenanthroline (0.14 g, 0.77 mmol), then we can attain a brown solid (0.41g, yield:91%).

(c) Synthesis of [Cu(L)dpq]ClO₄ (q1c):

The method is same as **q1a**, except changing bpy to 1,10-phenanthroline (0.18g, 0.77 mmol), then we can attain a brown solid (0.43g, yield:88%).

(d) Synthesis of [Cu(L)dppz]ClO₄ (q1d):

The method is same as **q1a**, except changing bpy to dppz (0.22 g, 0.77 mmol), then we can attain a brown solid (0.48g, yield:93%).

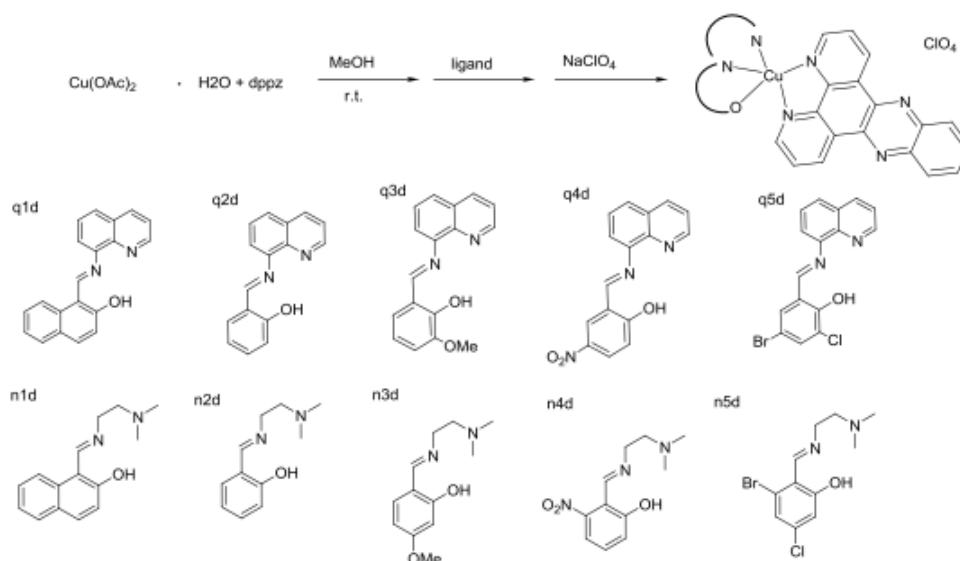
(e) Synthesis of [Cu(L)dppn]ClO₄ (q1e):

The method is same as **q1a**, except changing bpy to dppn (0.26 g, 0.77 mmol), then we can attain a dark brown solid (0.53g, yield:87%).

(f) Synthesis of [Cu(L)OAc] (q1f):

Weigh copper acetate(0.14 g, 0.70 mmol)and ligand (L)(0.23 g, 0.77 mmol), and place it in a 50 ml round-bottomed flask. Use 10 ml methanol as solvent, and stir it for 3 hours under room temperature. Use a filter board to collect the solid and wash it with cooled methanol several times. Last, drain it in vacuity. Then we can attain a brown solid(0.23 g, yield:78%).

q1d-q5d and n1d-n5d



(a) Synthesis of q1d

Weigh copper acetate(0.14 g, 0.70 mmol)and dppz(0.22 g, 0.77 mmol), and place it in a 50 ml round-bottomed flask. Use 20 ml methanol as solvent, and stir it for 1 hours under room temperature. Add **Lq1** (0.23g , 0.77 mmol) when the reaction is finished and stir it for3 hours under room temperature. Then add sodium perchlorate (0.094 g , 0.77 mmol), and solid can be attained immediately. Use a filter board to collect the solid and wash it with cooled methanol several times. Last, drain it in vacuity. Then we can attain a yellow solid.(0.32g, yield: 68%)

(b) Synthesis of q2d

The method is same as **q1d**, except changing **Lq1** to **Lq2**(0.19 g , 0.77 mmol), then a dark yellow solid is attained. (0.35g, yield: 73%)

(c) Synthesis of q3d

The method is same as **q1d**, except changing **Lq1** to **Lq3**(0.21 g , 0.77 mmol), then a brown solid is attained. (0.31g, yield: 62%)

(d) Synthesis of q4d

The method is same as **q1d**, except changing **Lq1** to **Lq4**(0.23 g , 0.77 mmol), then a yellow green solid is attained. (0.38g, yield: 56%)

(e) Synthesis of q5d

The method is same as **q1d**, except changing **Lq1** to **Lq5**(0.28 g , 0.77 mmol), then a dark yellow solid is attained. (0.35g, yield: 71%)

(f) Synthesis of n1d

The method is same as **q1d**, except changing **Lq1** to **Ln1**(0.19 g , 0.77 mmol), then a dark green solid is attained.

(g) Synthesis of n2d

The method is same as **q1d**, except changing **Lq1** to **Ln2**(0.15 g , 0.77 mmol), then a green solid is attained.

(h) Synthesis of n3d

The method is same as **q1d**, except changing **Lq1** to **Ln3**(0.17 g , 0.77 mmol), then a dark green solid is attained.

(i) Synthesis of n4d

The method is same as **q1d**, except changing **Lq1** to **Ln4**(0.18 g , 0.77 mmol), then a green solid is attained.

(j) Synthesis of n5d

The method is same as **q1d**, except changing **Lq1** to **Ln5**(0.24 g , 0.77 mmol), then a green solid is attained.

II. Electrophoresis

(1) Preparations for DNA electrophoresis

(a) Preparations for tris-HCl buffer

Weigh 0.3g tris and 0.146g NaCl, and use 50mL D.D.H₂O to prepare the solution. Then titrate it with HCl till pH=7.2.

(b) Preparations for TAE buffer

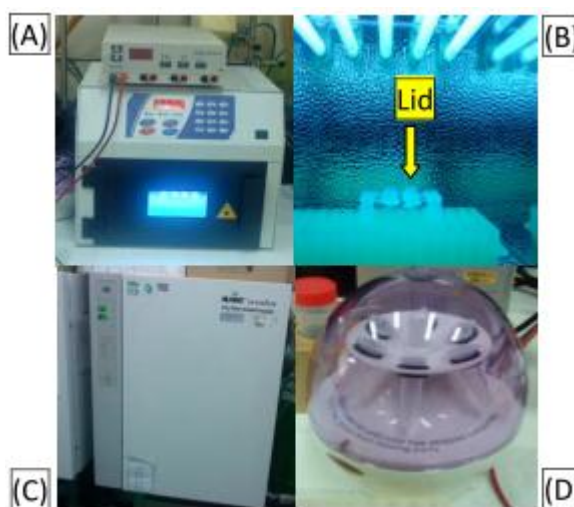
Measure 50mL (10x) TAE buffer (50mL), then dilute it with 950mL D.D.H₂O to 0.5x for use.

(c) Preparations for 1% Agarose gel

Weigh 0.6g Agarose, and add it into diluted 60ml TAE (0.5x) buffer. Heat the Agarose till it is fully dissolved. Then cover the rim with aluminum foil paper and add fluorescent dye SYBR Safe (4μL). Mix and cool it down to about 60°C, then pour it into the agarose gel model and insert the teeth comb. Leave it and wait until it cools down and takes shape.

(2) DNA electrophoresis procedure

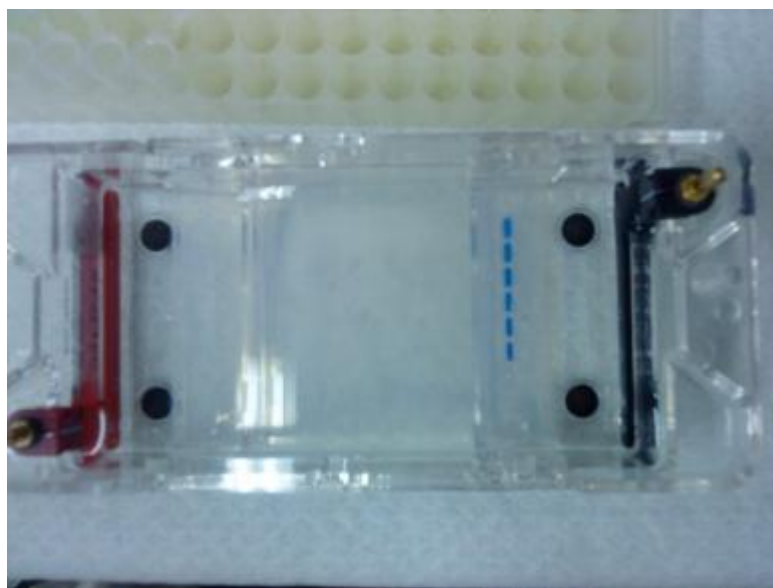
In a dark chamber, DNA(1μL), tris-HCL buffer(15.2μL) and complex(40μM in DMF solution)(1.8μL) are mixed in an eppendorf. Expose it with different wavelengths of lights. After the exposure, place it in an 37°C incubator for an hour.



- (A) This is a picture of a UV bio-link. The ultra violet light source we use is 365nm and the exposure time is 30 minutes.
- (B) Infuse the sample on the lid of the eppendorf to make the exposure more effective.
- (C) This is a picture of a 37°C incubator.
- (D) This is a picture of a centrifuge, and it can be used to descend the compound to the bottom of the eppendorf.

Place the prepared agarose gel into the electrophoresis tank, and the loading well should be close to the negative pole. Add running buffer (0.5x TAE buffer) till it covers the gel. Then we can load the sample into the well.

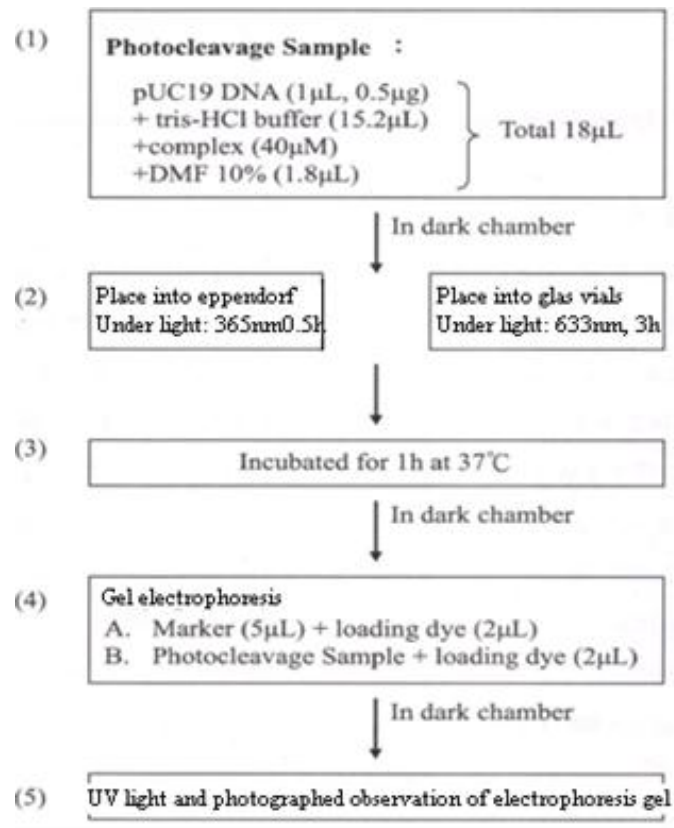
- (a) Loading of the Marker: Mix Marker (5 μ L) with loading dye (2 μ L) and then fill it in the well.
- (b) Loading of the sample: Mix the loading dye (2 μ L) with the sample, and fill the well with 10 μ L of the mixture.



This is a picture of an electrophoresis tank with wells already loaded

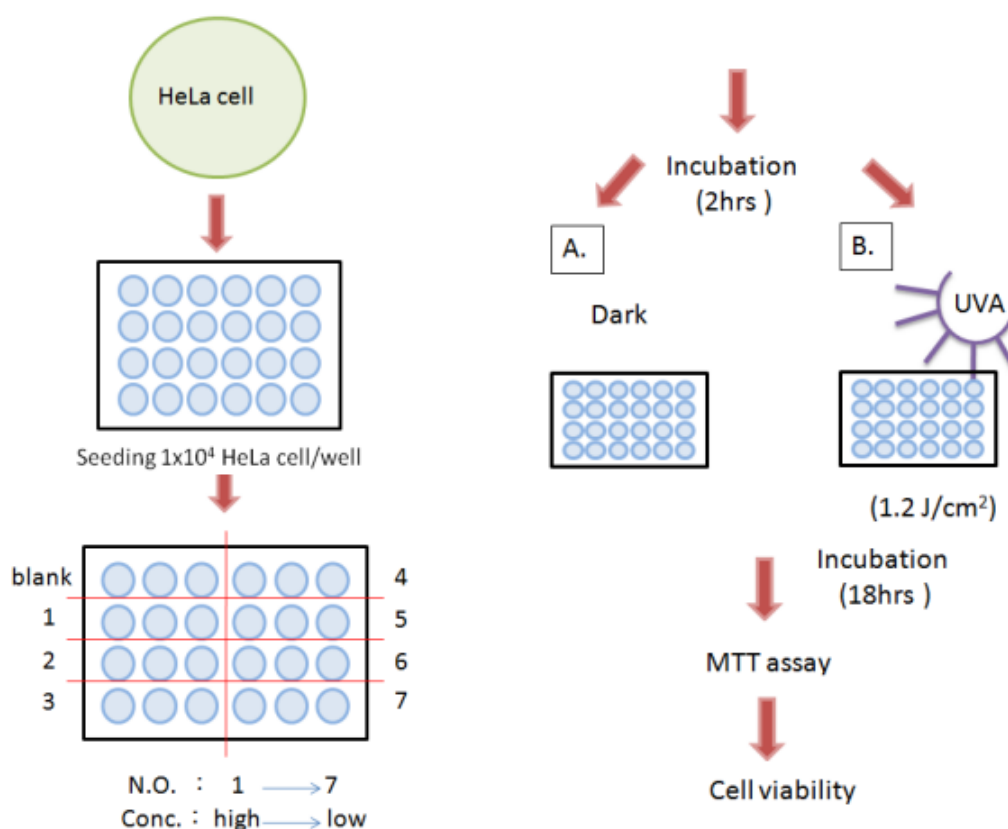
After loading the sample, cover the lid with a layer of aluminum foil paper to protect it from light. The electrophoresis is conducted at 100V. After 30 minutes, turn off the power and observe the electrophoresis gel by UV light and photograph.

The flow is presented in the following:



This is a picture of an electrophoresis gel image system

(3) Cell experiment



a. Cell culture

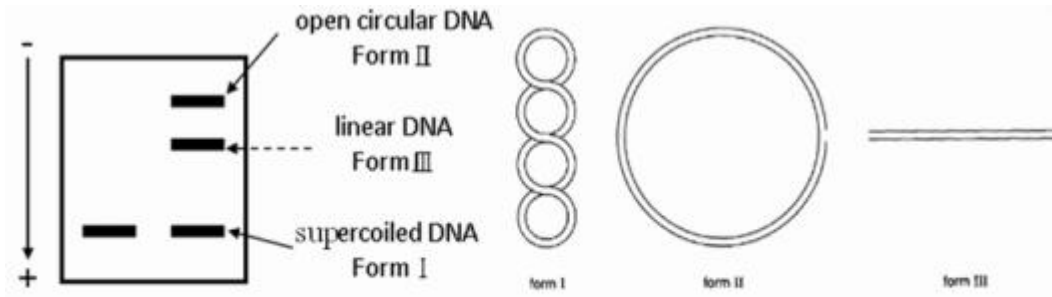
Human cervical cancer cells (HeLa) were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). Use phosphate-buffered saline (PBS) to wash HeLa cells twice, add in 1X Trypsin-EDTA 1.5ml and place it in an incubator (37°C 5% CO_2) for 2 min. Retrieve the cells stuck on the Petri dish and add in DMEM-F12 medium to dilute it. Collect the suspended cells onto an aseptic centrifuge tube and cells are centrifuged it for 5 minutes. Then, add in a suitable amount of DMEM-F12 medium and distribute the cells equally. Place it on a 10 cm Petri dish and put it in the incubator (37°C 5% CO_2). Culture it once in 2 or 3 days.

b. The measurement of MTT assay

The measurement of IC_{50} is done by MTT which reacts on the enzyme (dehydrogenase) of mitochondrium in a living cell and produces purple formazan which can be used to measure IC_{50} . Place 1×10^4 HeLa cells into a 24 well deep well plate from Lane1-Lane7 according to their density (high to low) , and add in the complexes to react for 2 hours. Eliminate the supernatant and add 200 μ l PBS. Expose it to 365nm light for 5 minutes. Eliminate PBS and add in 500 μ l medium and leave it for 24 hours. Then, add in 50 μ g/ml MTT in every well and place it in the incubator (37°C 5% CO₂) for 4 hours. Cells are centrifuged at 1500rpm for 5 minutes then eliminate the supernatant. Add in 200 μ l DMSO and leave it under room temperature for 30 minutes, and we can use the ELISA reader to read the change of extinction value by the wavelength of 490nm.

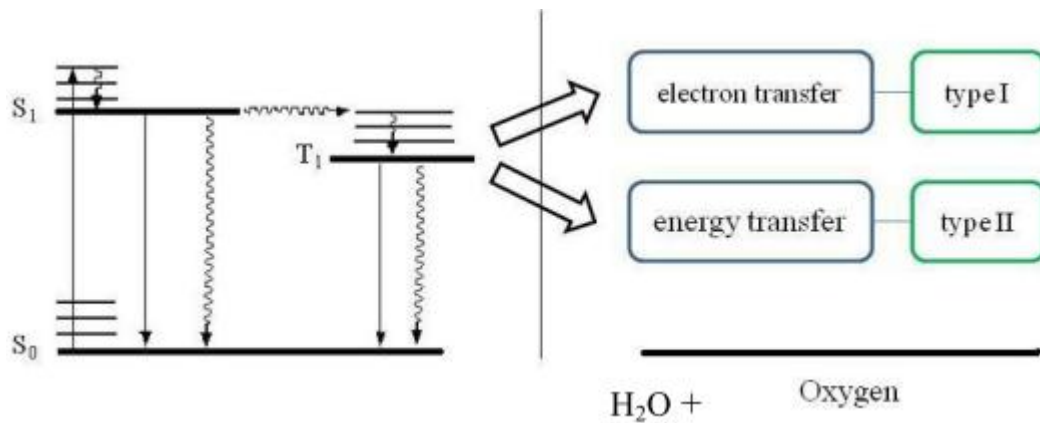
Comparison of the velocity of plasmid DNA

Supercoiled DNA > Linear DNA > Open circular DNA



Relative positions of different DNA forms on gel electrophoresis

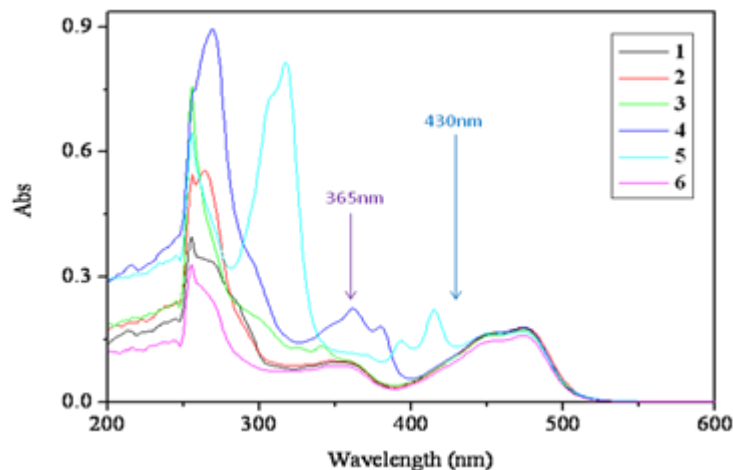
The mechanism of photodynamic therapy



When the photo-sensitizer absorbs energy, it excites to singlet state, and gets to triplet state through intersystem crossing. Then the excited compound transfers the energy to oxygen or water molecules and forms free radicals or singlet oxygen that can kill cancer cells

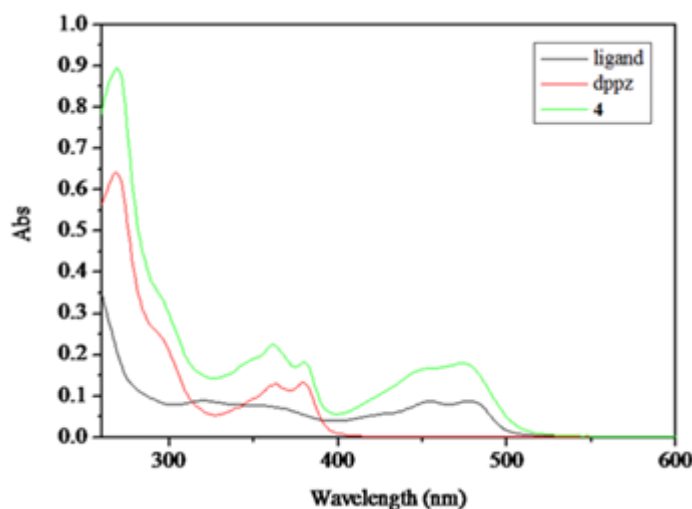
3. Results and Discussion

Experiment 1: Discussion of UV-Vis and fluorescence spectrum



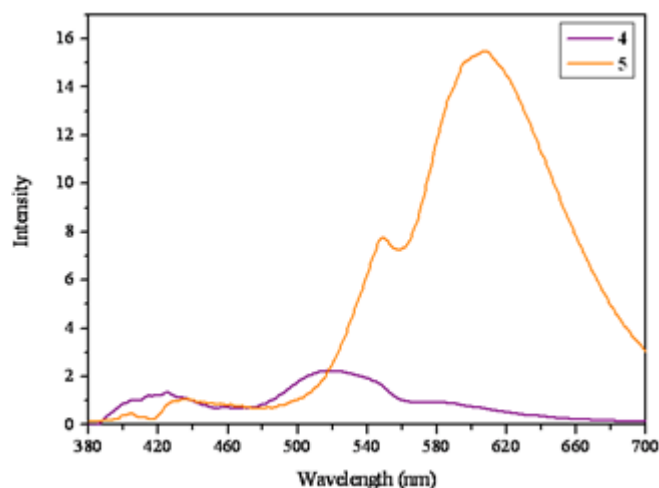
Absorption spectra of complexes 1 — 6, the solvent is DMF:H₂O(1:1), and the concentration: 10 μM.

The purple arrow in this picture represents the ultraviolet light source (365nm) we use, and the blue arrow represents the blue light source (430nm). Complex 4 has a maximum absorption coefficient at 365nm, so we can expect that complex 4 would be the most effective in inducing DNA cleavage under ultraviolet. Complex 1-6 all have pretty good absorptions at 430nm, this means that blue light can be applied as well.



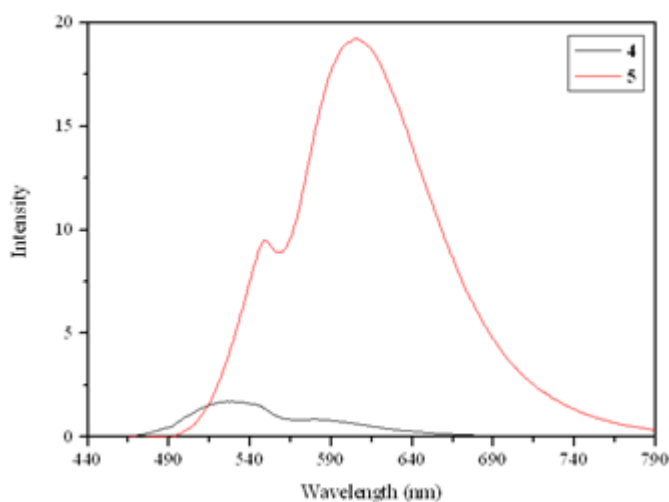
Absorption spectra of ligand, co-ligand and complex 4, the solvent is DMF:H₂O(1:1), and the concentration: 10 μM.

We can know from this chart that at 365nm the absorption of complex 4 mainly comes from co-ligand dppz and at 440-480nm, the absorption mainly comes from the ligand itself.



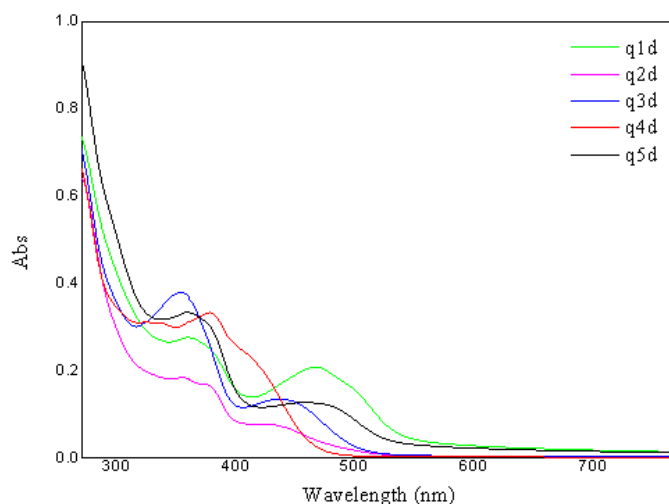
Fluorescence spectrum of complex q1d, q1e (conc: 20 μ M) in DMF:H₂O(1:1) and the excitation wavelength is 365nm.

We can know from this chart that at 365nm complex 5 mainly transfers the energy it absorbs into fluorescence because of its co-ligand dppn, so it does not transfer the energy to oxygen and water. Consequently, the result of DNA cleavage of dppn does not reach our expectations.



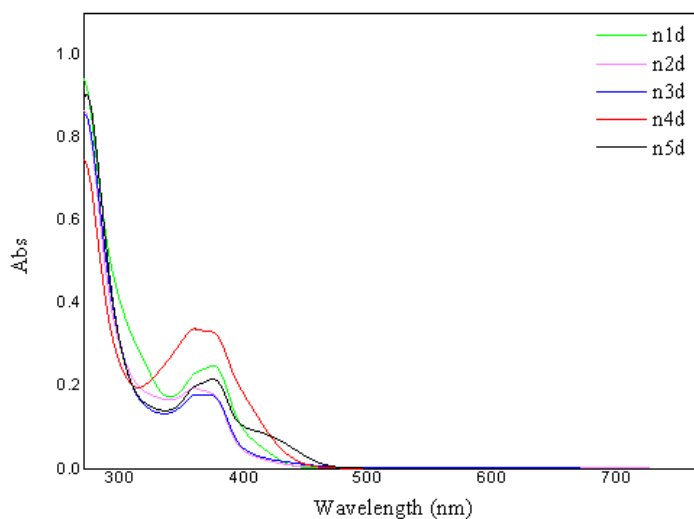
Fluorescence spectrums of **q1d**, **q1e**, the solvent: DMF:H₂O(1:1), concentration: 20 μ M, excitation wavelength : 430nm.

We can know from this chart that at 430nm complex 5 mainly transfers the energy it absorbs into fluorescence because of its co-ligand dppn, so it does not transfer the energy to oxygen and water. Consequently, the result of DNA cleavage of dppn does not reach our expectations.



Absorbing spectrum of complex q1d-q5d; solvent: 50mM Tris-HCl/NaCl buffer (pH, 7.2) with 10% DMF 20μM

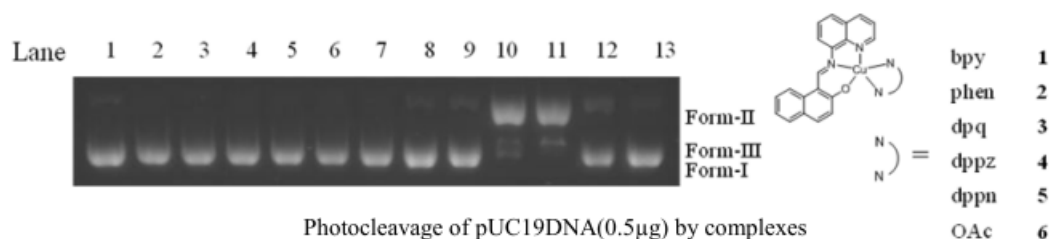
We can know from this chart that complexes mainly absorb in the area near UV wavelength, and the absorption of **q1d** is closer to the area of long wavelength. It indicates that the value of application of PDT can be enhanced. Also, compared with other complexes, q1d has a bigger range of absorption in 300-470nm.



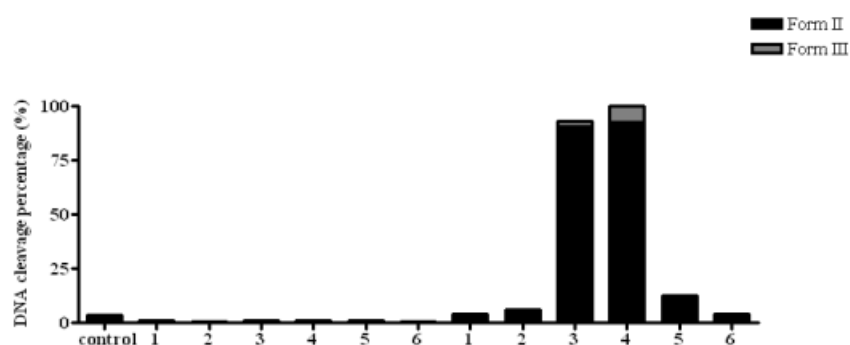
Absorbing spectrum of complex n1d-n5d; solvent: 50mM Tris-HCl/NaCl buffer (pH, 7.2) with 10% DMF 20μM

From this chart, it is known that complexes mainly absorb in the area near UV wavelength. The UV spectrum is similar for **n1d-n5d**, and its peak are near 365nm, so we UV is applied as the wavelength for DNA cleavage.

Experiment 2: Effects on DNA cleavage of different co-ligands in ultraviolet.

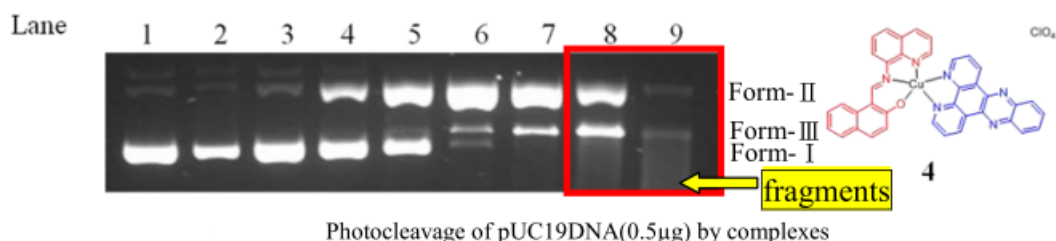


a The concentration of complexes are **20μM**, reacting in 50mM Tris-HCl/NaCl buffer (pH=7.2) and containing 10% DMF; *b* The light source is the UV crosslinker (8W); *c* The percentage is calculated by the software ONEDscan; *d* Without DNABand

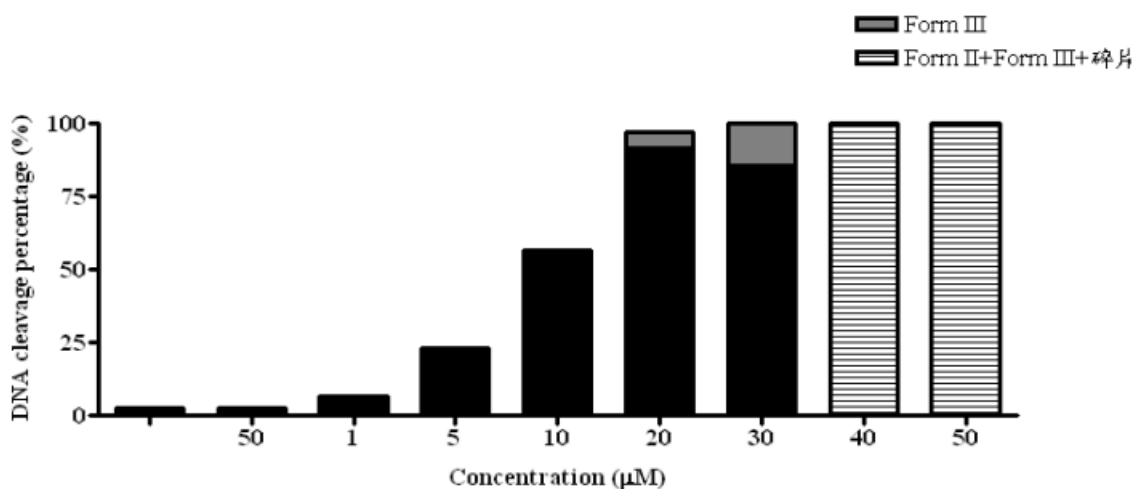


- (1) From Lane 1 we know that a 30-minute-exposure does not affect the DNA.
- (2) From Lane 2-7, complexes 1-6 are not active without exposure.
- (3) From Lane 8-13, after exposing complexes 1-6 in ultraviolet for 30 minutes, complexes 1, 2, 5, and 6 are not very effective. From early papers we can see that it is because bpy, phen, and OAc are not good at binding with DNA. Although dppn is better at binding with DNA, it transforms the energy into fluorescence which lowers its activity. Complexes 3 and 4 have more significant effects, especially complex 4, because it has maximum absorption coefficient at 365nm and dppz binds better with DNA.

Experiment 3: The effects of concentration toward the DNA cleavage of complex q1d in ultraviolet.



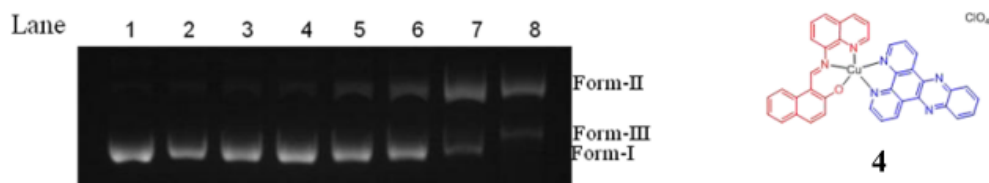
a The concentration of complexes are $20\mu\text{M}$, reacting in 50mM Tris-HCl/NaCl buffer (pH=7.2) and containing 10% DMF; *b* The light source is the UV crosslinker (8W); *c* The percentage is calculated by the software ONEDscan; *d* Without DNABand



- (1) The results indicate that under exposure (Lane 3-9), the higher the concentration is, the more effective the DNA cleavage is. So we can tell that under this condition, $20\mu\text{M}$ is enough for 95% of DNA cleavage. Thus, we use $20\mu\text{M}$ for each experiment that is in ultraviolet afterward.
- (2) The reason why we use $50\mu\text{M}$ of complex for Lane 2 dark is because earlier experiment results show that complex 4 is not active when there is no exposure. So we chose the highest concentration as a representative. If $50\mu\text{M}$ has no effect, we do not need to conduct experiments on lower concentrations.
- (3) Lane 8 & Lane 9 are quite different in electrophoresis, but it shows that the percentage of Form II & Form III are both $>99\%$. The main reason is because the concentration is too high, making DNA cleave into small

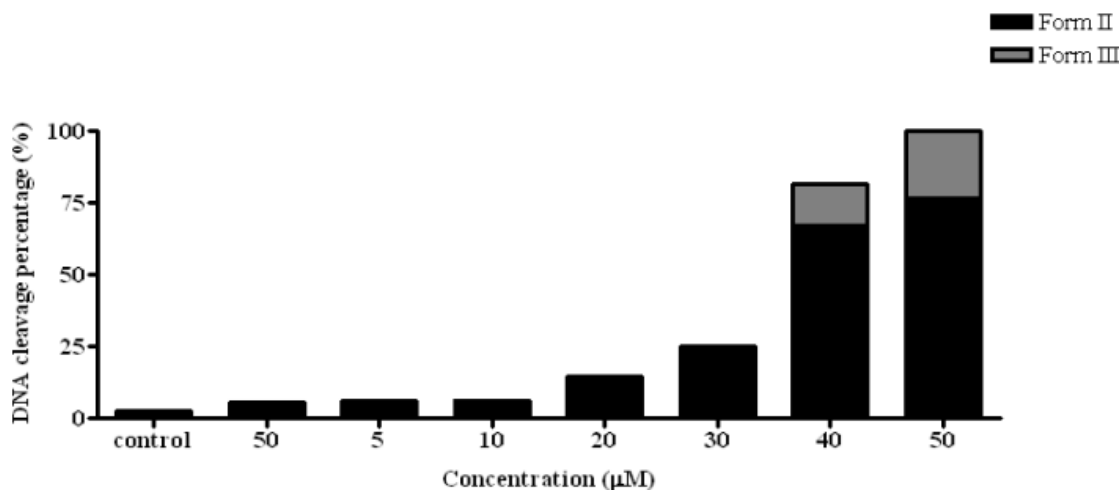
pieces. In electrophoresis, the smaller the DNA is, the higher the speed is through the holes of the gel. Therefore, fragments move faster than Form I, and leave a trail because its sizes are not the same. Since Lane 8 and 9 have both shown trails, it means that DNA has been cleaved into little pieces. Furthermore, the DNA of Lane 9 have nearly all become fragments, so that its trail has outrun this picture; whereas Lane 8 still preserves some of Form II & Form III. But we still use >99% for both lanes to indicate that it has been highly cleaved.

Experiment 4: The effects of concentration toward the DNA cleavage of complex q1d in blue light.



Photocleavage of pUC19DNA(0.5μg) by complexes

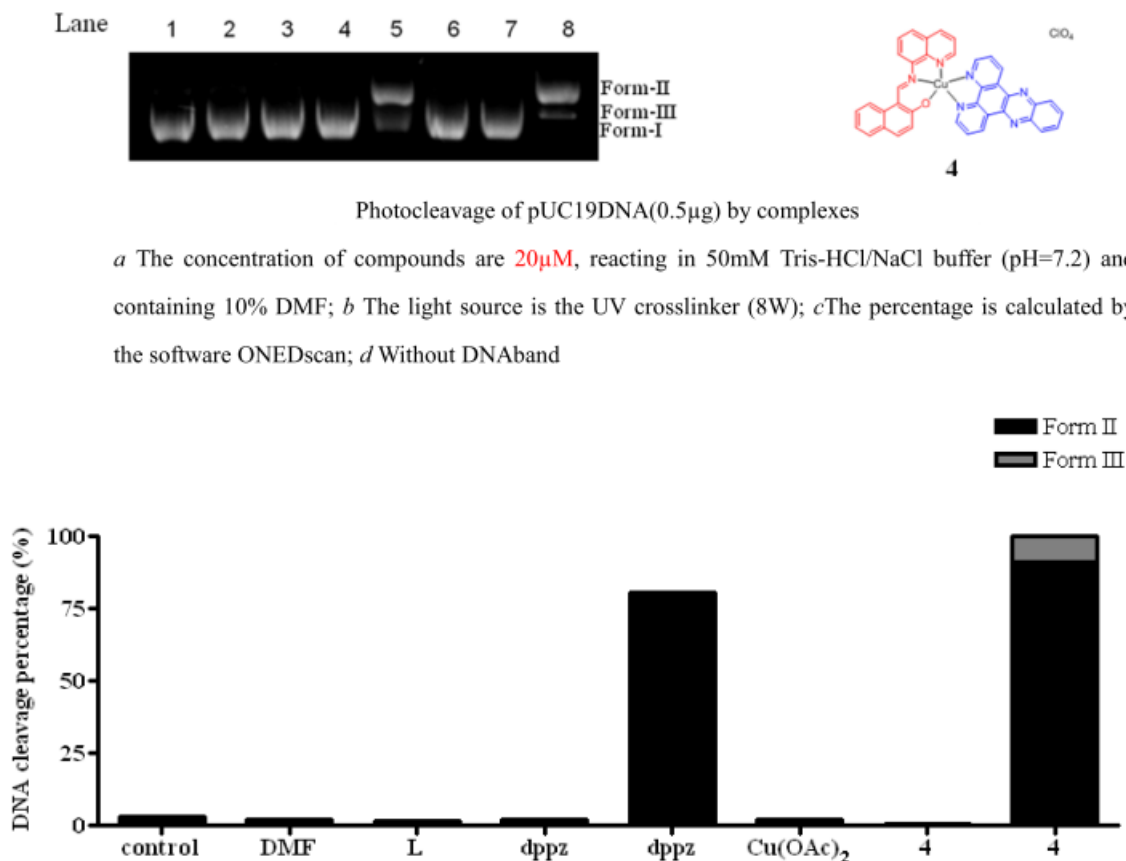
a The concentration of complexes are 20μM, reacting in 50mM Tris-HCl/NaCl buffer (pH=7.2) and containing 10% DMF; *b* The light source is the blue light LED; *c* The percentage is calculated by the software ONEDscan; *d* Without DNABand



As same as experiment 3, under exposure (Lane 3-8), the higher the concentration is, the better the DNA cleaves. But we can tell from the chart that blue light is not as effective as ultraviolet.

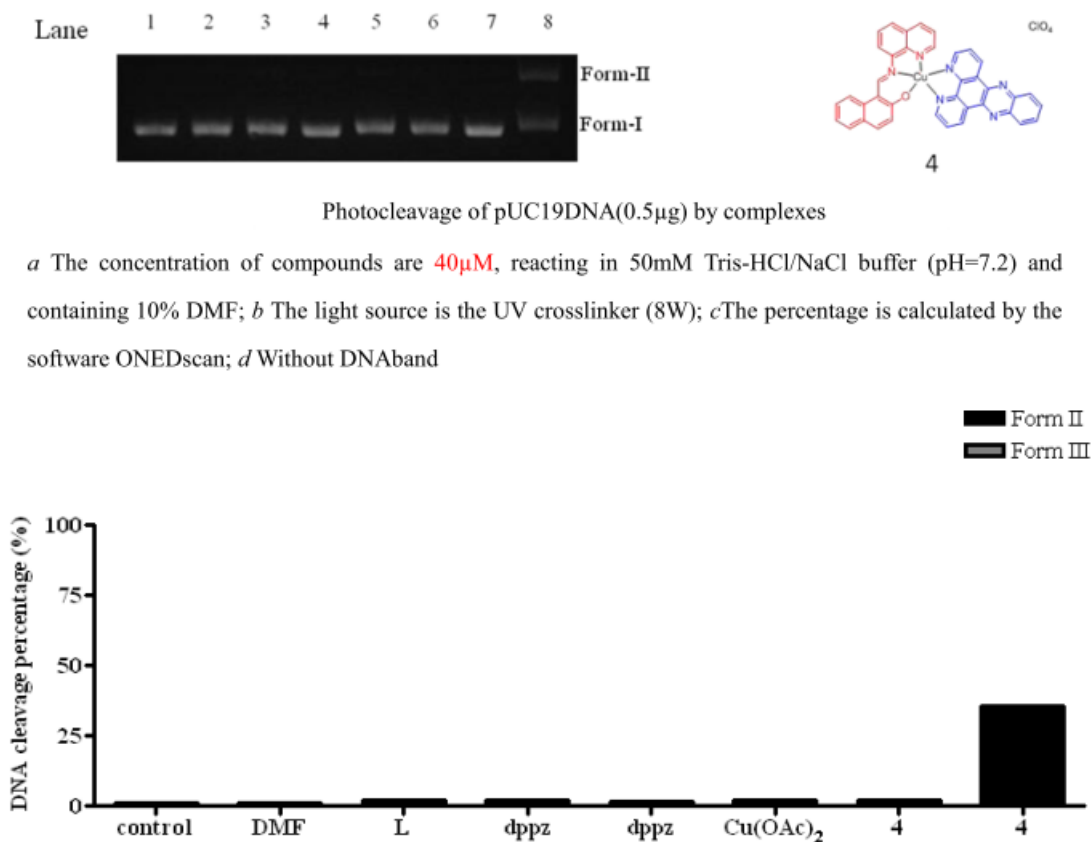
Experiment 5: Effects on DNA cleavage of reactants of complex **q1d** (dppz) in ultraviolet

Since we know that complex **q1d** is the most effective, so we chose it as the most suitable complex for further experiments.



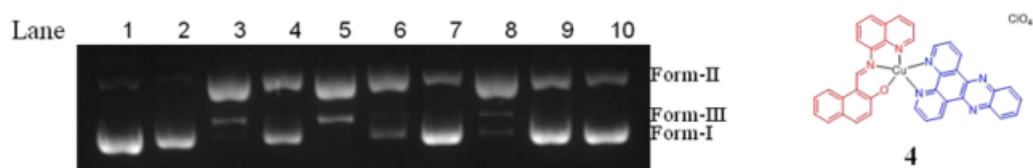
- (1) From Lane 2, 3, and 6 we can tell that when there is only DMF, ligand, or copper, it doesn't affect DNA.
- (2) From Lane 4-5 and 7-8 we can assume that if there is only dppz, it could cleave DNA in ultraviolet. The reason is at 365nm, dppz has a pretty good absorption and it can bind with DNA. However, the complex still cleaves better than dppz.

Experiment 6: Effects on DNA cleavage of reactants of complex q1d (dppz) in blue light

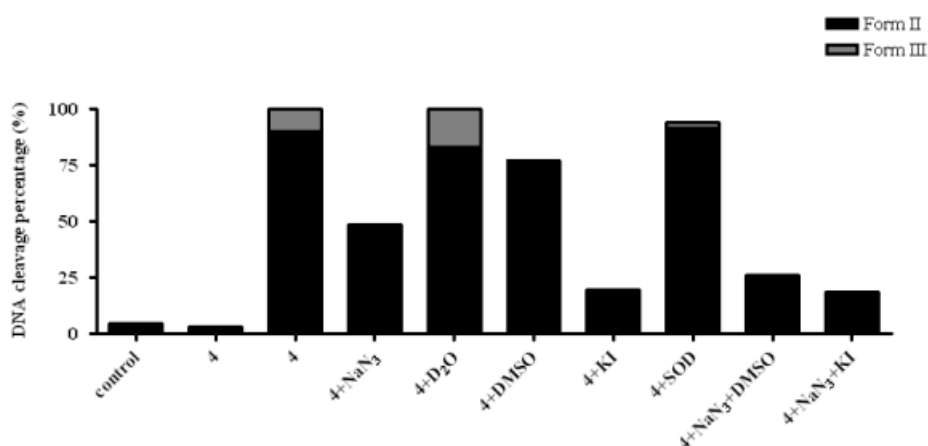


- (1) From the chart we can see that every reactant cleaves DNA in blue light. Compared with experiment 5, we found out that dppz is not activated because dppz does not absorb at 430nm.
- (2) Since the complex barely absorbs blue light, we can expect the effect of cleavage cannot be as significant as blue light. So we adjusted the concentration from 20μM to 40μM, but we found out that ultraviolet is still better than blue light.

Experiment 7: Discuss the mechanism of DNA cleavage of complex q1d



a The concentration of complex is **20μM**, reacting in 50mM Tris-HCl/NaCl buffer (pH=7.2) and containing 10% DMF; *b* The light source is the UV crosslinker (8W); *c* The percentage is calculated by the software ONEDscan; *d* Without DNAband



Note: NaN₃->inhibits singlet oxygen

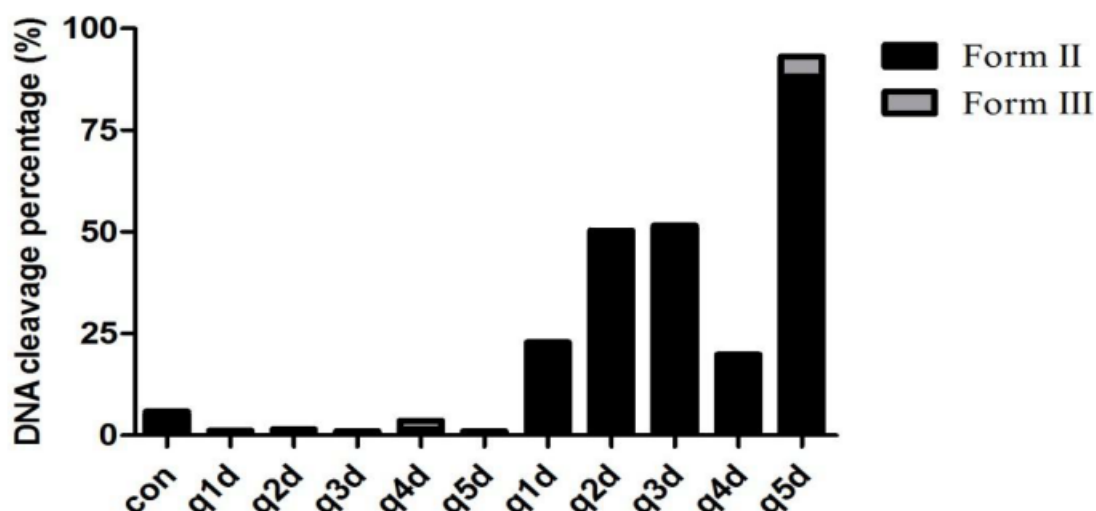
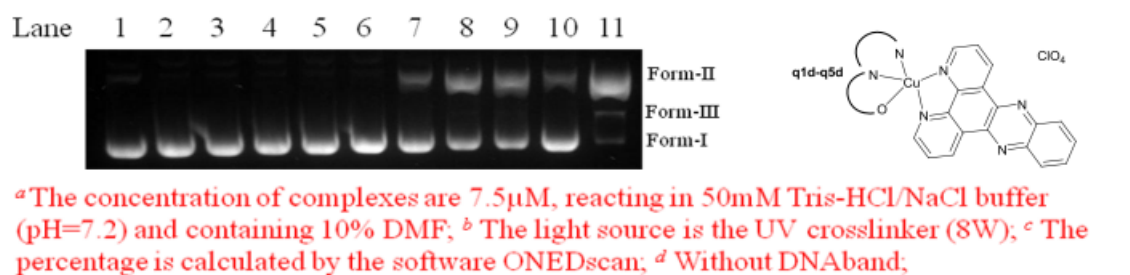
D₂O-> prolongs the life of singlet oxygen

DMSO&KI ->inhibit hydroxyl radical

SOD ->inhibits super oxide radical

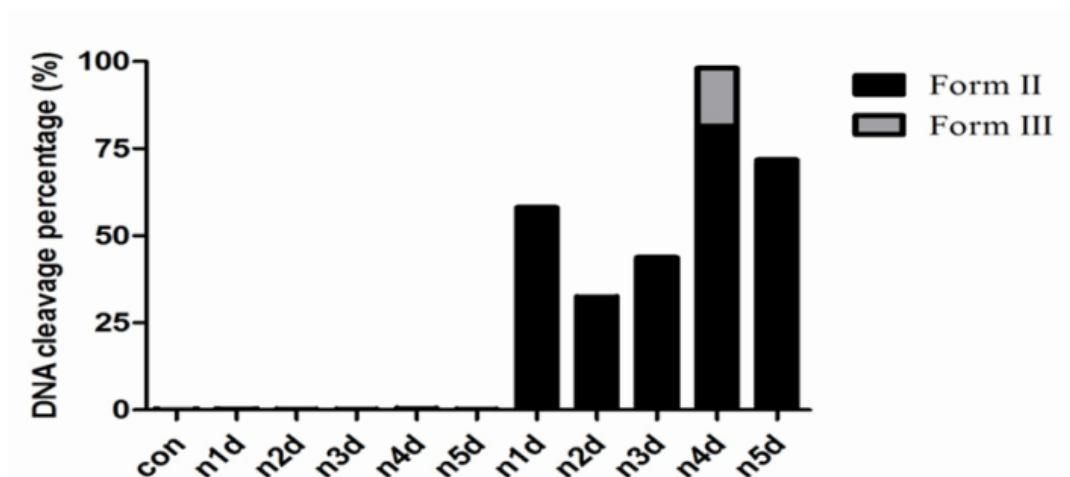
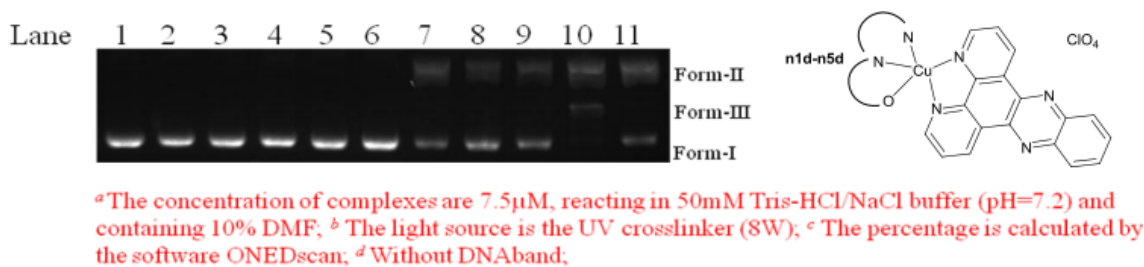
- (1) Comparing Lane 4, 6, and 7 respectively with Lane 2, we can tell that NaN₃, DMSO and KI can inhibit DNA cleavage.
- (2) The reason why we added two inhibitors in Lane 9 and 10 is to double check whether it has inhibited DNA cleavage respectively and examine if the effect can be multiplied. The chart shows that DMSO+NaN₃ has reached our expectations, so we assume that the mechanism should be able to produce singlet oxygen and hydroxyl radical.

Experiment 8: Discuss DNA cleavage of complex q1d-q5d in ultraviolet light



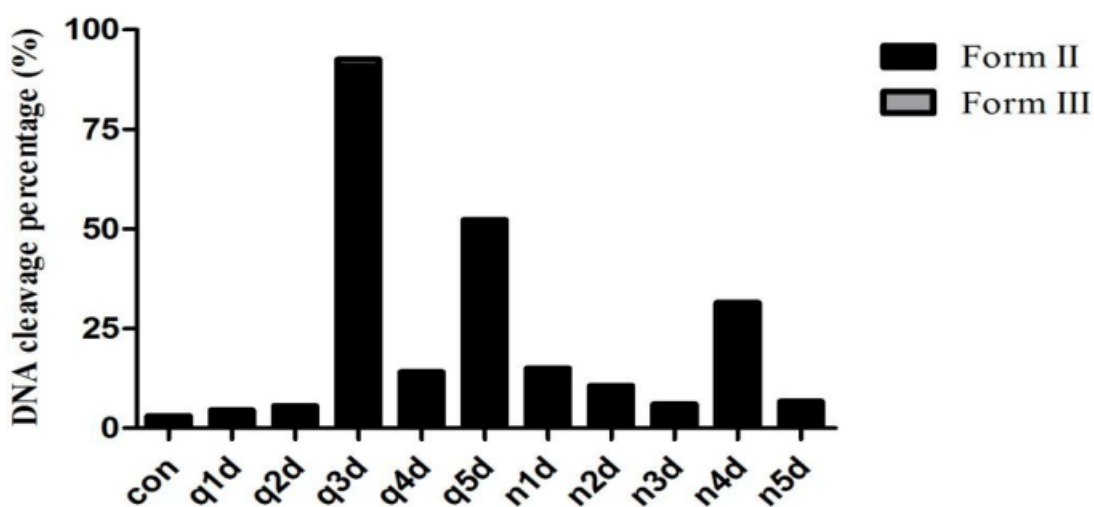
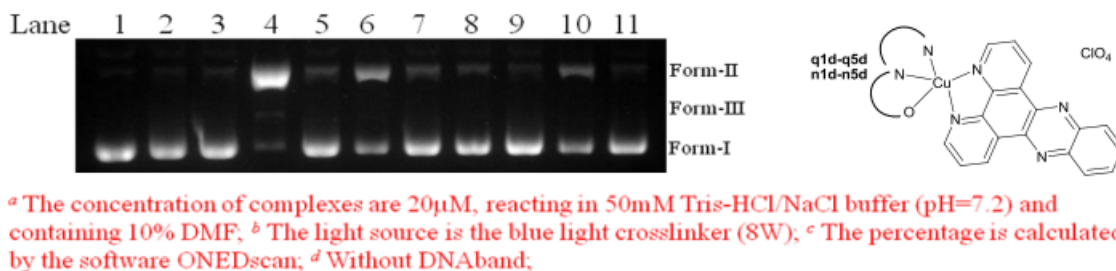
- (1) Lane2-6 are not exposed to light; meanwhile Lane1,7-11 are exposed to light for 30 minutes.
- (2) The q series cleave DNA more effectively when under a 30-minute exposure.
- (3) From the results we can find that q5d is better in DNA cleavage. Although it has obvious fluorescence, q5d can still cleave DNA. It is dppz (coligand) that binds with DNA; however, dppz doesn't emit fluorescence. Thus, the fluorescence emitted by the ligand doesn't affect the efficiency of DNA cleavage.

Experiment 9: Discuss DNA cleavage of complex n1d-n5d in ultraviolet light



- (1) Lane2-6 are not exposed to light; meanwhile Lane 1,7-11 are exposed to light for 30 minutes.
- (2) The n series cleave DNA more effectively when under a 30-minute exposure.
- (3) From the results we can tell that n4d is better in DNA cleavage because n4d is attached to a electron withdrawing group: NO₂.

Experiment 10: Discuss DNA cleavage of complex q1d-q5d and n1d-n5d in blue light



- (1) Lane1 is not exposed to light; meanwhile Lane2-11 are exposed to light for 30 minutes.
- (2) When Exposed to blue light, q3d and n4d have higher DNA cleavage.
- (3) From results, n4d is better in DNA cleavage because n4d is attached to electron withdraw-NO₂.
- (4) Overall, the q series is better than the n series when exposed to blue light. Consequently, the q series has higher value in application of visible light.

4. Conclusions

A series of copper(II) complexes supported on N,N,O-Schiff base have been successfully synthesized and characterized. Among **q1a-q1f**, their activities of photocleavage in (SC)pUC19 DNA show that **q1d(dppz)** is the most reactive species. Singlet oxygen and hydroxyl radicals have been verified as the mechanism of PDT-causing DNA cleavage. Moreover, complexes attached to electron withdrawing groups show higher photocleavage activity under UVA. In addition, the **q** series demonstrate higher activity toward DNA than the **n** series in visible light(430 nm) due to the benefit of more resonance on quinoline structure.

In cellular experiments, **q3d** is a potential candidate for PDT under UVA. However, the mechanism of cell death has to be established in the future. Overall, by extending the wavelength to visible light, we have enhanced the value of application for PDT.

5. References

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評語

1. 題目有相當的應用價值，研究亦有不錯的成果。
2. 建議加強相關現象之瞭解與解釋。