Photoactivation of Boron-Caged Prodrug via Phenyl Radical under Hypoxia

2024.05.02 Literature Seminar

B6 Mizuki Sawada

Contents

1. Introduction
   Boron-caged prodrugs with anticancer activity

2. Main Paper

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Article

Photoactivation of Boronic Acid Prodrugs via a Phenyl Radical Mechanism: Iridium(III) Anticancer Complex as an Example

Moyi Liu, Yunli Luo, Junyu Yan, Xiaolin Xiong, Xiwen Xing,* Jong Seung Kim,* and Taotao Zou*

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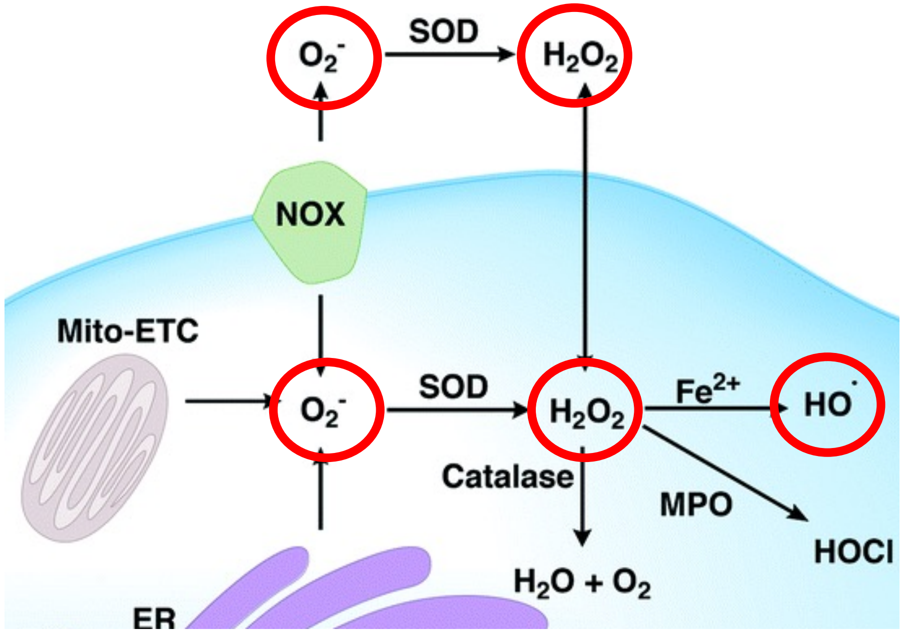
Cancer

Cancer = serious disease
About 10 million people were died of cancer in 2020 all over the world. [1)]

The features of cancer

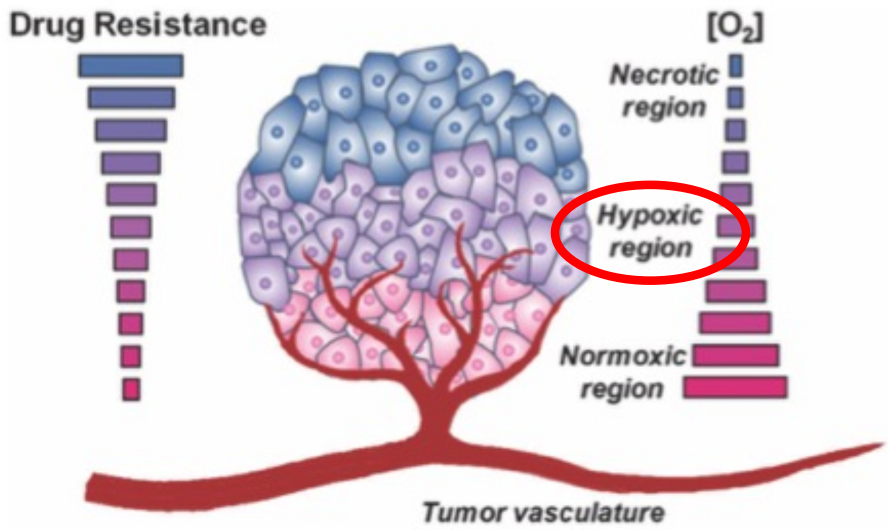
(1) Increased level of ROS [2)]



reactive oxygen species (ROS):
$O_2^{-\bullet}$, H_2O_2, OH•, $^1O_2$

H_2O_2: ~0.5 nmol/10^4 cells/h in cancer cells [3)]

(2) Hypoxic (low O_2 level) [4)]



in cancer cell: 0.02–2% O_2
in normal cell: 2–9% O_2

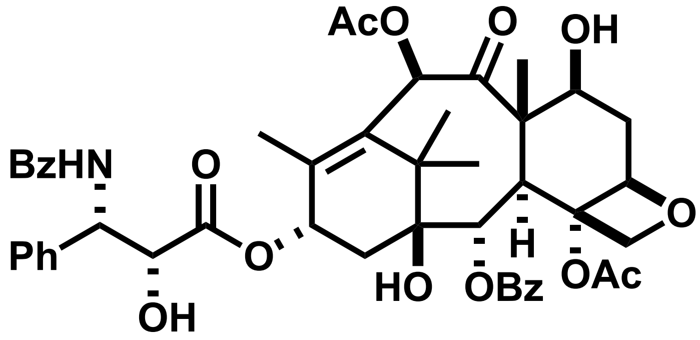
1) https://japan-who.or.jp/factsheets/factsheets_type/cancer/ 2) Cadahía, J. P.; Previtail, V.; Troelsen, N. S.; Clausen, M. H. MedChemComm **2019**, *10*, 1531. 3) Szatrowski, T. P.; Nathan, C. F.; *Cancer Res*. **1991**, *51*, 794. *4*) Sharma, A.; Arambula, J. F.; Koo, S.; Kumar, R.; Singh, H.; Sessler, J. L.; Kim, J. S. *Chem. Soc. Rev.* **2019**, *48*, 771.

Anticancer Agents and Prodrugs

Chemotherapy with anticancer agents is one of the major treatments of cancer.
However, anticancer drugs are sometimes toxic to normal cells and cause side effects

to decrease side effects…

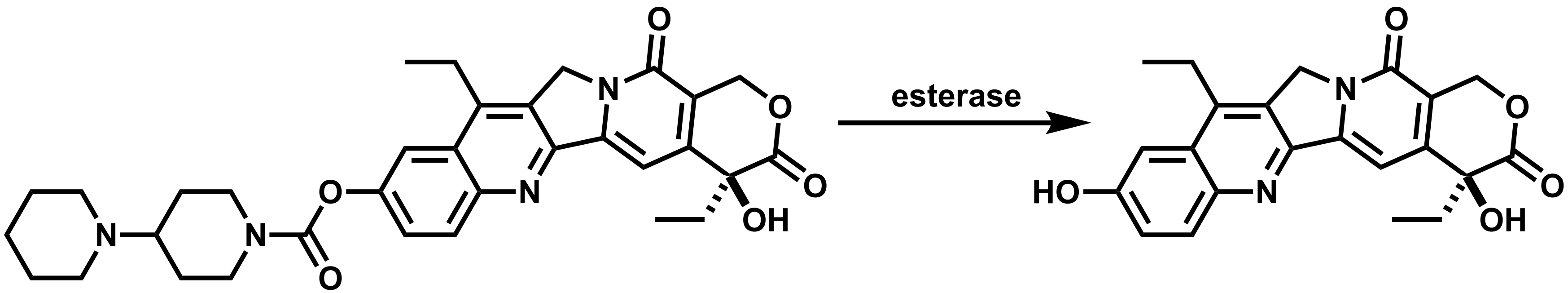
An example of anticancer agent



taxol

Prodrug: a pharmacologically inactive derivative converted into an active parent drug in vivo.

An example of clinically approved anticancer prodrug



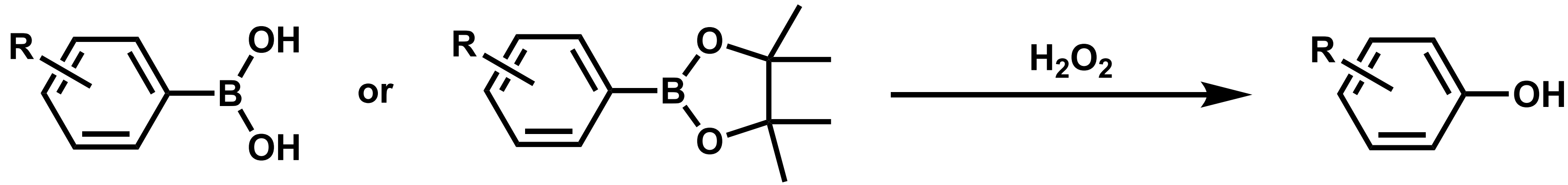
irinotecan (prodrug)
1000 times weaker activity than SN-38

SN-38 (parent drug)

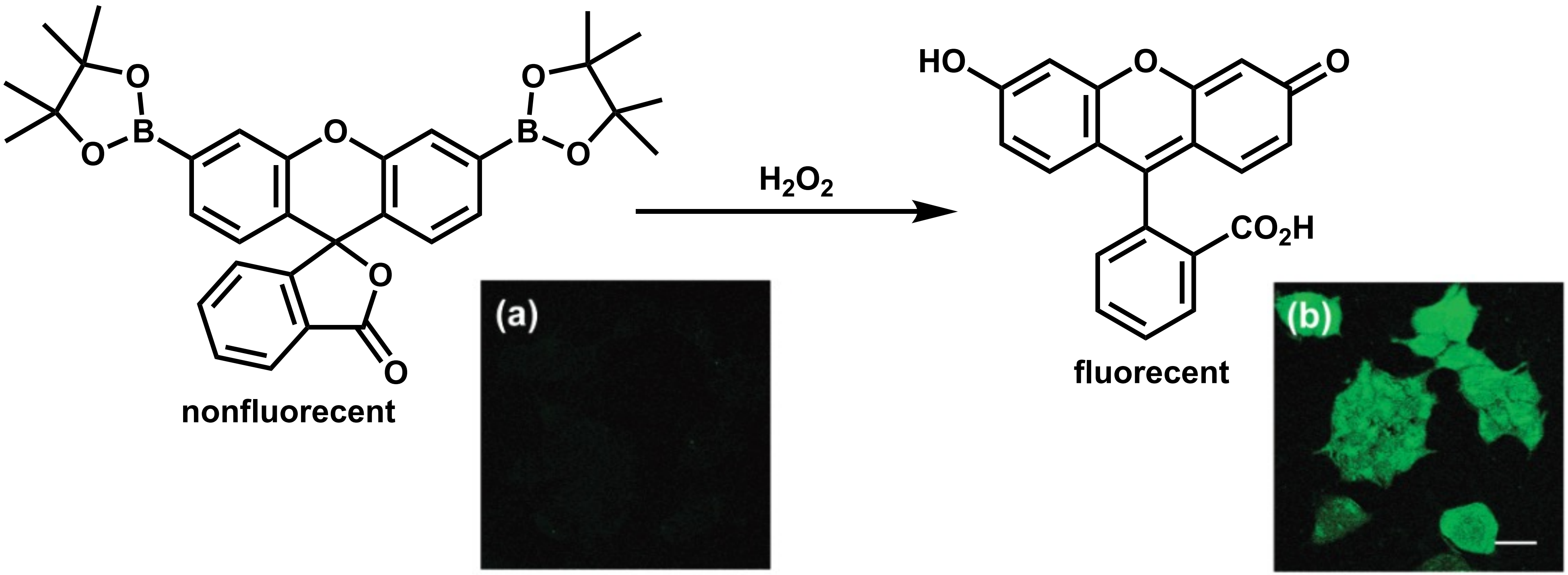
Prodrugs **selectively activated in tumor tissue** are effective to reduce side effects.

Boronic Acids or Esters and H_2O_2

Aryl boronic acids and esters are oxidized to phenol by H_2O_2.

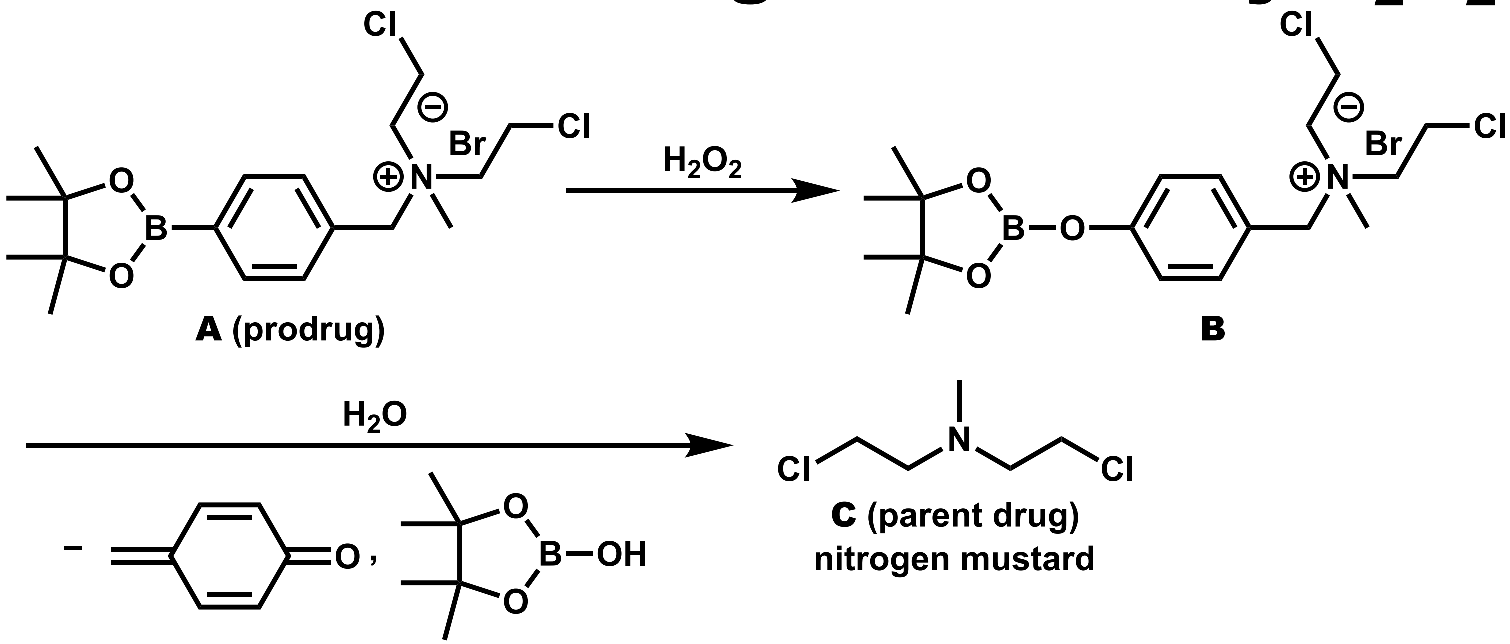


An example of application of this reactivity: H_2O_2-activated fluorescent probe [1)]

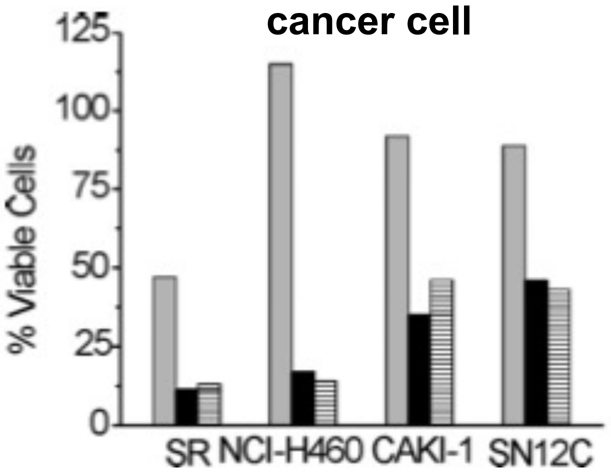


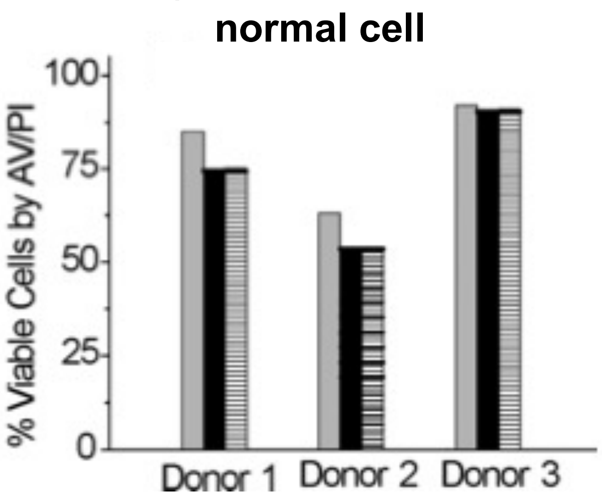
1) Miller, E. W.; Albers, A. E.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 16652.

First Example of Boronic Ester Anticancer Prodrug Activated by H_2O_2



Cytotoxicity (black bar = A)



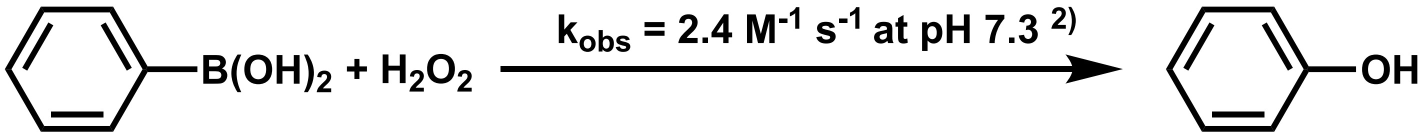


Compound A selectively inhibited the growth of cancer cells.

1) Kuang, Y.; Balakrishnan, K.; Gandhi, V.; Peng, X. *J. Am. Chem. Soc.* **2011**, *133*, 19278.

Limitation of Activation of Prodrugs by H_2O_2

[prodrug][H_2O_2] is expected to be less that 10^{-10} M^2. [1)]
-> Very fast reaction is suitable for efficient activation.



The reaction between phenyl boronic acid and H_2O_2 is relatively slow.

Spontaneously generated H_2O_2 is not enough to uncage boronic acid/ester prodrugs.

Strategy for overcoming this limitation [1)]
conjugation of ROS amplifiers, directing to lysosomes (higher H_2O_2 level than cytoplasm)…

Development of universal approach to efficiently activating boron-caged prodrug is needed.

1) Daum, S.; Reshetnikov, M. S. V.; Sisa, M.; Dumych, T.; Lootsik, M. D.; Bilyy, R.; Bila, E.; Janko, C.; Alexiou, C.; Herrmann, M.; Sellner, L.; Mokhir, A. *Angew. Chem., Int. Ed*. **2017**, *56*, 15545. 2) Graham, B. J.; Windsor, I. W.; Gold. B.; Raines, R. *Proc. Natl. Acad. Sci. USA.* **2021**, *118*, e2013691118.

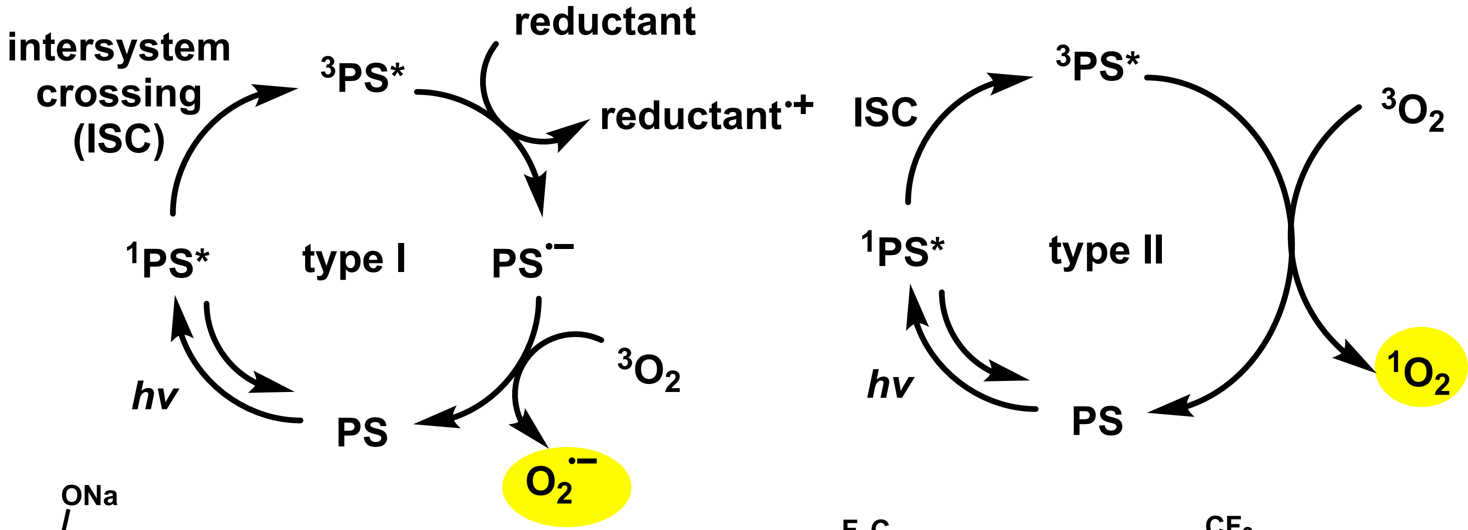
Generation of ROS by Photoactivation

Photodynamic therapy (PDT)

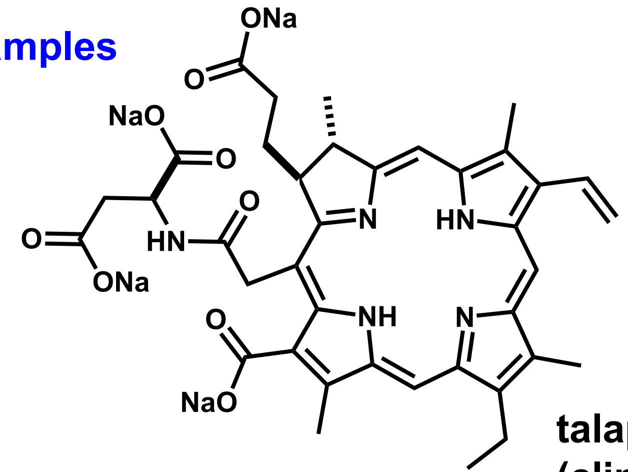
photosensitizer (PS) + O_2 + *hv* ⟶ reactive oxygen species (ROS)
harmless by itself — damaging to cell

The cytotoxicity can be spatiotemporally controlled by photoirradiation only to diseased region.

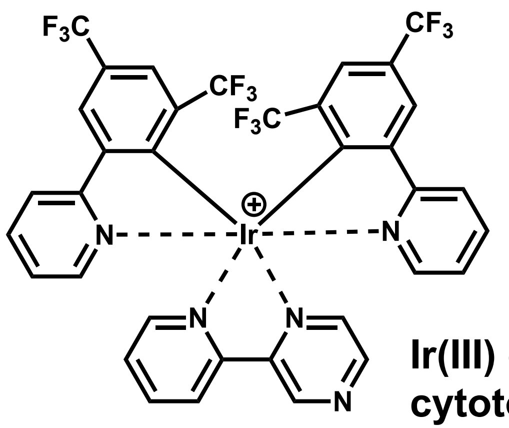
Mechanism [1]



Examples



talaporfin sodium (clinically approved)

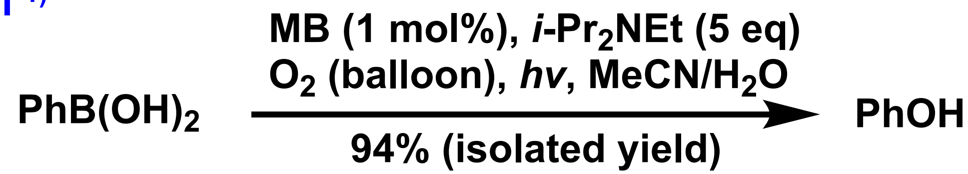


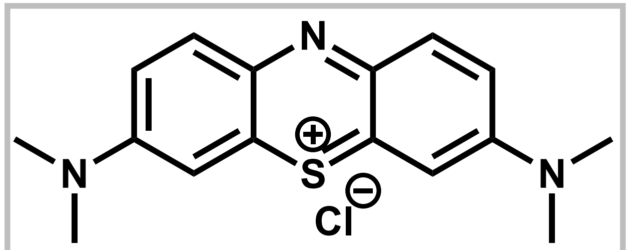
Ir(III) complex cytotoxicity toward cancer cells [2]

1) Sharman, W. M.; Allen, C. M.; Lier, J. E. *Drug Discov. Today.* **1999**, *4*, 507. 2) Bevernaegie, R.; Doix, B.; Bastein, E.; Diman, A.; Decottignies, A.; Feron, O.; Elias, B. *J. Am. Chem. Soc.* **2019**, *141*, 18486.

Photo-uncaging of Boronic Acids and Esters

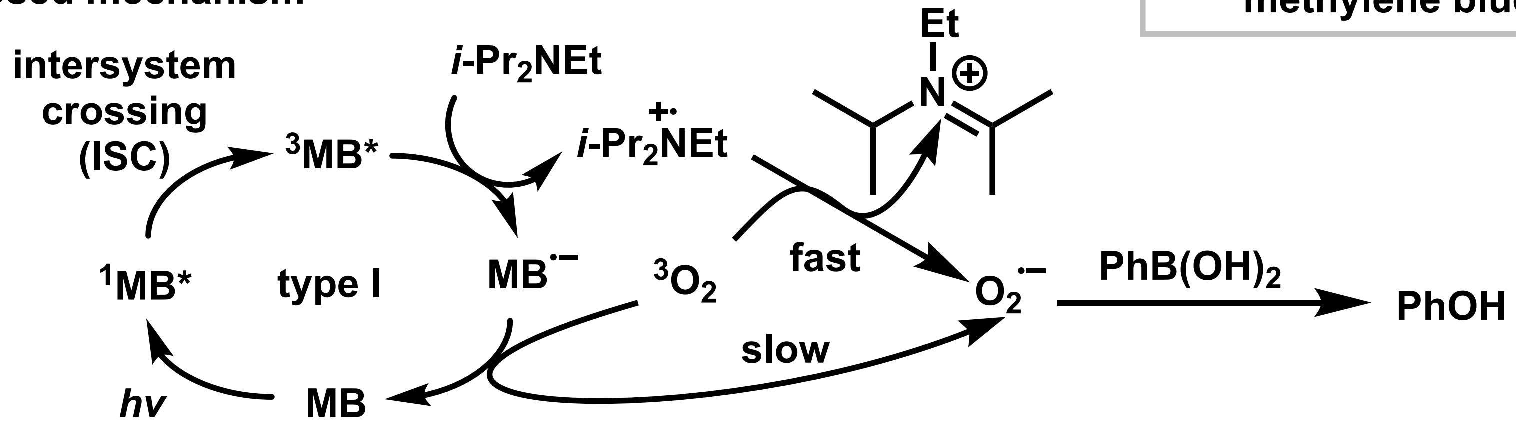
example 1 [1)]



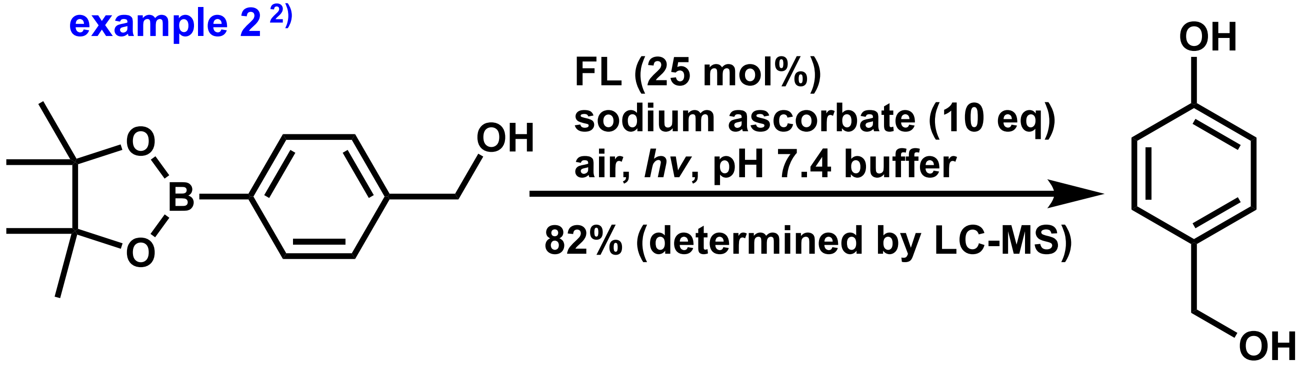


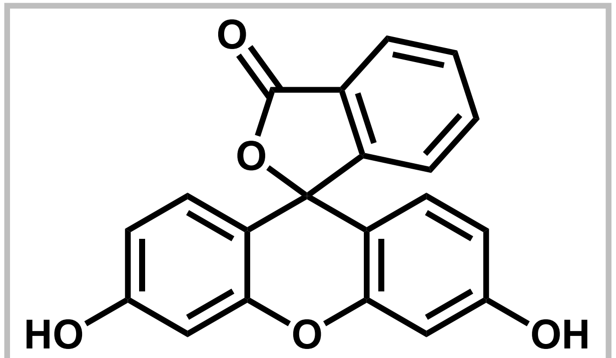
methylene blue (MB)

proposed mechanism



example 2 [2)]





fluorescein (FL)

Photo-uncaging of boronic acids and esters in hypoxic condition has not been reported.

1) Pitre, S. P.; McTiernan, C. D.; Ismaili, H.; Scaiano, J. C. *J. Am. Chem. Soc.* **2013**, *135*, 13286. 2) Wang, H.; Li, W.-G.; Zeng, K.; Wu, Y.-J.; Zhang, Y.; Xu, T.-L.; Chen, Y. *Angew. Chem. Int. Ed.* **2019**, *58*, 561.

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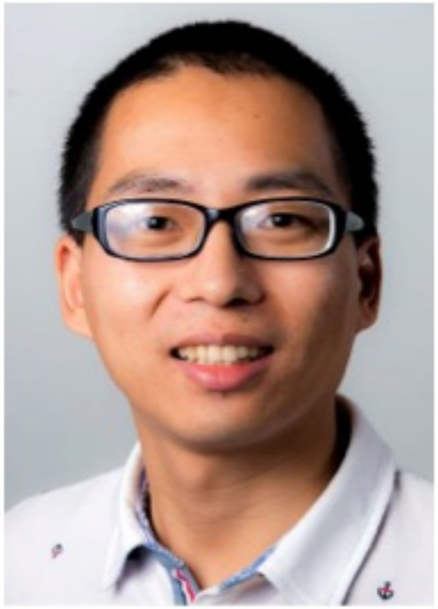
Prof. Taotao Zou



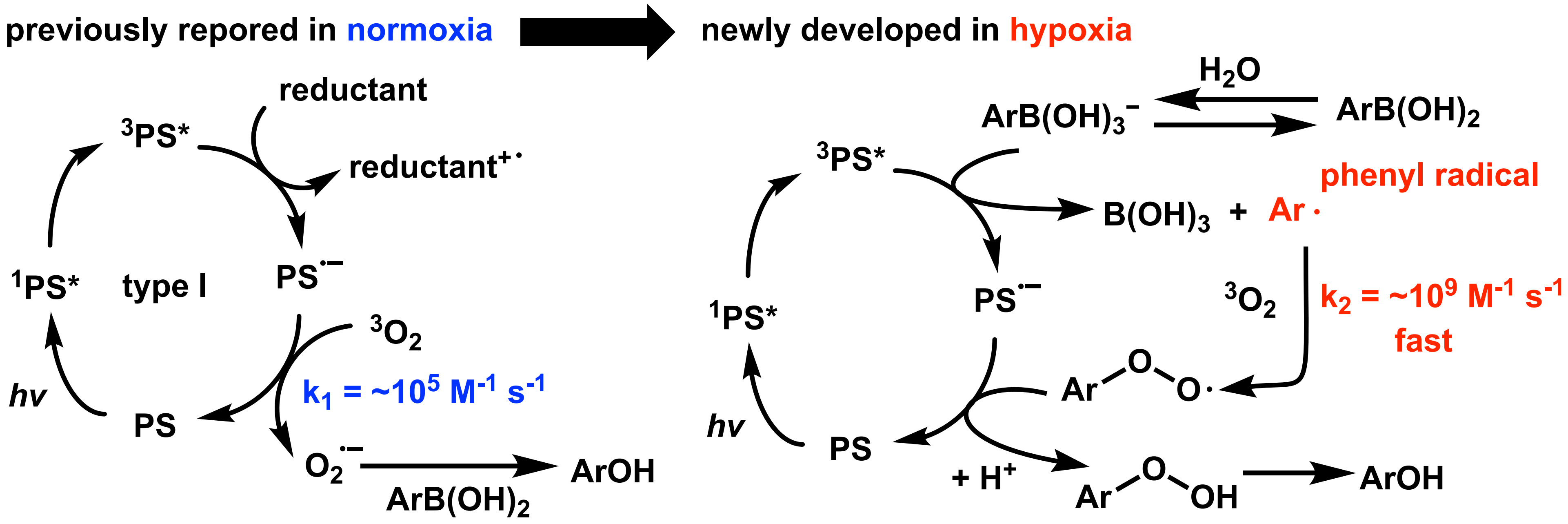
Career

2010	**Bachelor @ Wuhan University (Prof. Chuluo Yang)**
2015	**Ph.D. @ The University of Hong Kong (Prof. Chi-Ming Che)**
2015.04-2015.10	**Postdoctoral fellow @ University of Warwick (Prof. Peter J. Sadler)**
2015.03-2017.04	**Postdoctoral fellow @ The University of Hong Kong (Prof. Chi-Ming Che)**
2017.04-2018.02	**Research Associate @ The Scripps Research Institute (Prof. Xiang-Lei Yang and Prof. Paul Schimmel)**
2018.03-2018.07	**Assistant Professor @ The Chinese University of Hong Kong**
2018.07-	**Professor @ Sun Yat-Sen University**

Research topic: inorganic chemical biology, medicinal inorganic chemistry

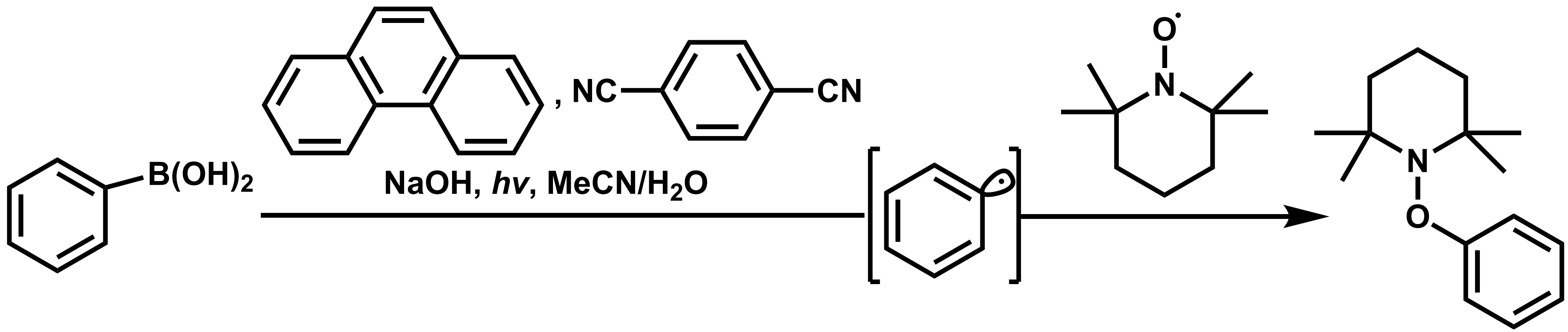
1) https://ttzouchem.wixsite.com/zoulab

Concept



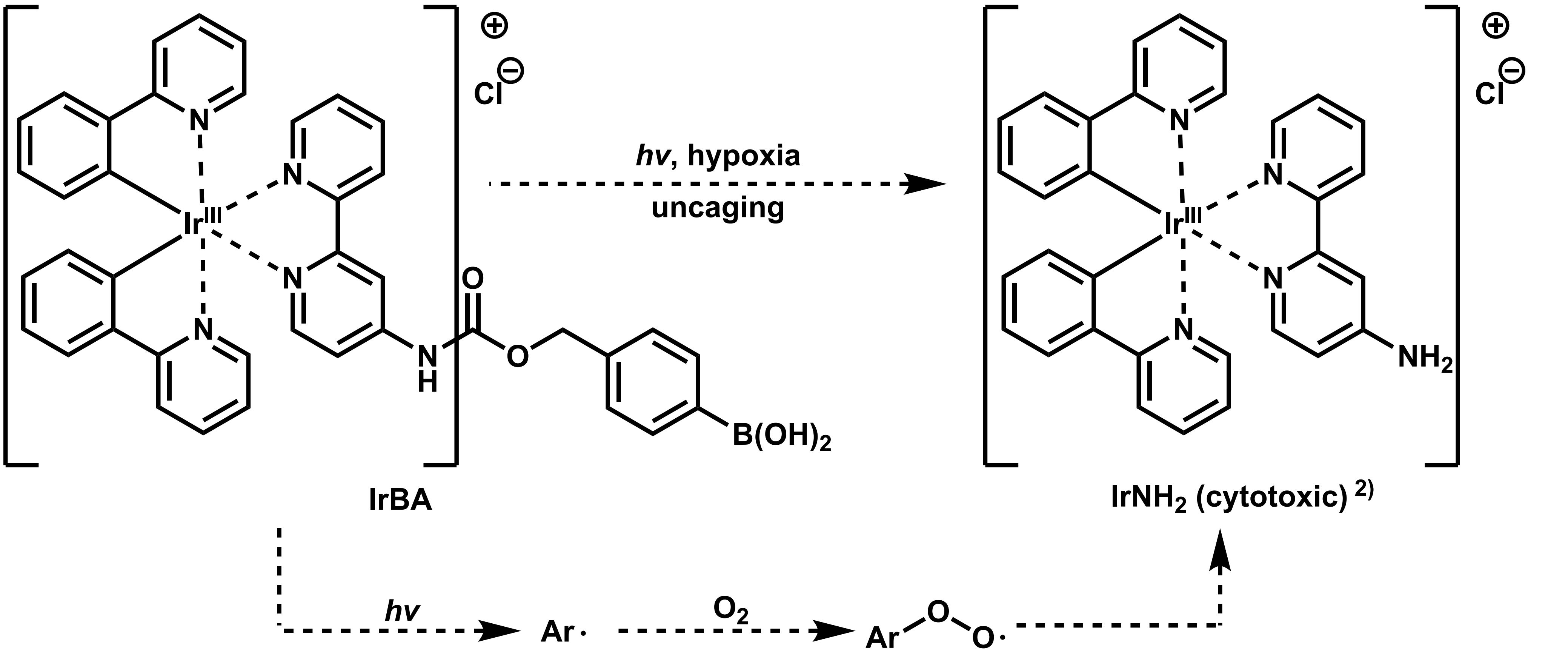
The use of highly reactive phenyl radical was planned to efficient uncage under hypoxia.

Boronate anion was photocatalytically converted into phenyl radical. [1)]



1) Iwata, Y.; Tanaka, Y. Kubosaki, S. Morita, T.; Yoshimi, Y. *Chem. Commun.* **2018**, *54*, 1257.

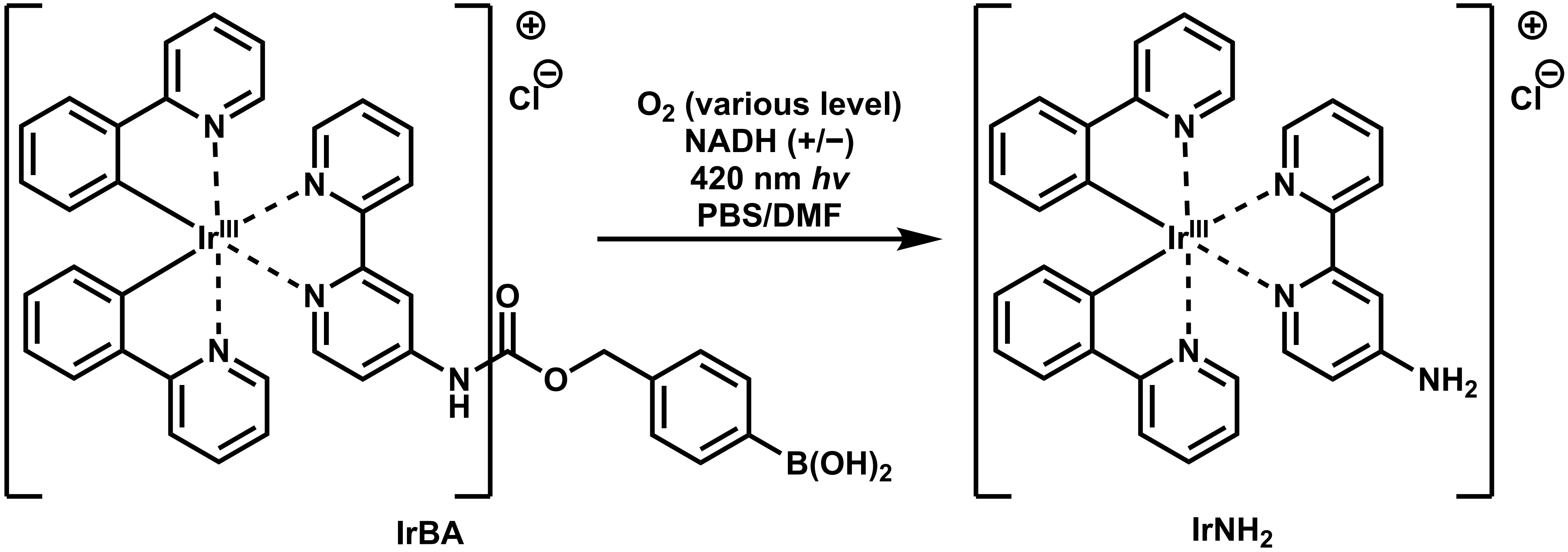
IrBA as Proof of Concept



Ir(III) complex has
photocatalytic activity [1] = work as a photosensitizer
cytotoxicity [2] = work as an anticancer drug

1) Bevernaegie, R.; Doix, B.; Bastein, E.; Diman, A.; Decottignies, A.; Feron, O.; Elias, B. *J. Am. Chem. Soc.* **2019**, *141*, 18486. 2) Kuang, S.; Liao, X.; Zhang, X.; Rees, T. W.; Guan, R.; Xiong, K.; Chen, Y.; Ji, L.; Chao, H. *Angew. Chem. Int. Ed.* **2019**, *59*, 3315.

Photoreactivity of IrBA

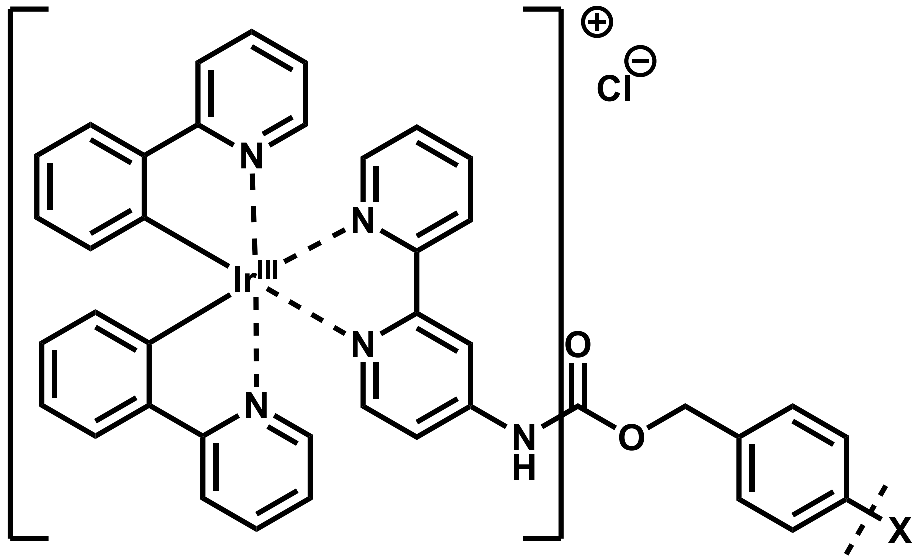


entry	O_2 level	NADH	time required for full conversion of IrBA to $IrNH_2$
1	21%	+	< 10 min
2	21%	−	15 min
3	1%	−	15 min
4	0.1%	−	30 min
5	0.02%	−	30 min
6	0% (degassed)	−	no reaction (reaction time is not reported)

IrBA was successfully converted to $IrNH_2$ without external reductant and under hypoxic condition.

Analysis of Reaction Intermediate (1): Boronate Anion

Redox potential (vs Ag/AgCl)
[p-MeOCH$_2$PhB(OH)$_2$]$^{\bullet+}$/[p-MeOCH$_2$PhB(OH)$_2$] = + 2.23 V
[p-MeOCH$_2$PhB(OH)$_3$]$^{\bullet}$/[p-MeOCH$_2$PhB(OH)$_3$]$^{-}$ = + 0.85 V
[Ir^{III}806]*/[Ir^{II}806] = + 1.02 V



X = H: Ir806, X = B(OH)$_2$: IrBA

Only boronate anion can be oxidized by [Ir^{III}806]*.

$$ArB(OH)_2 \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} ArB(OH)_3^-$$

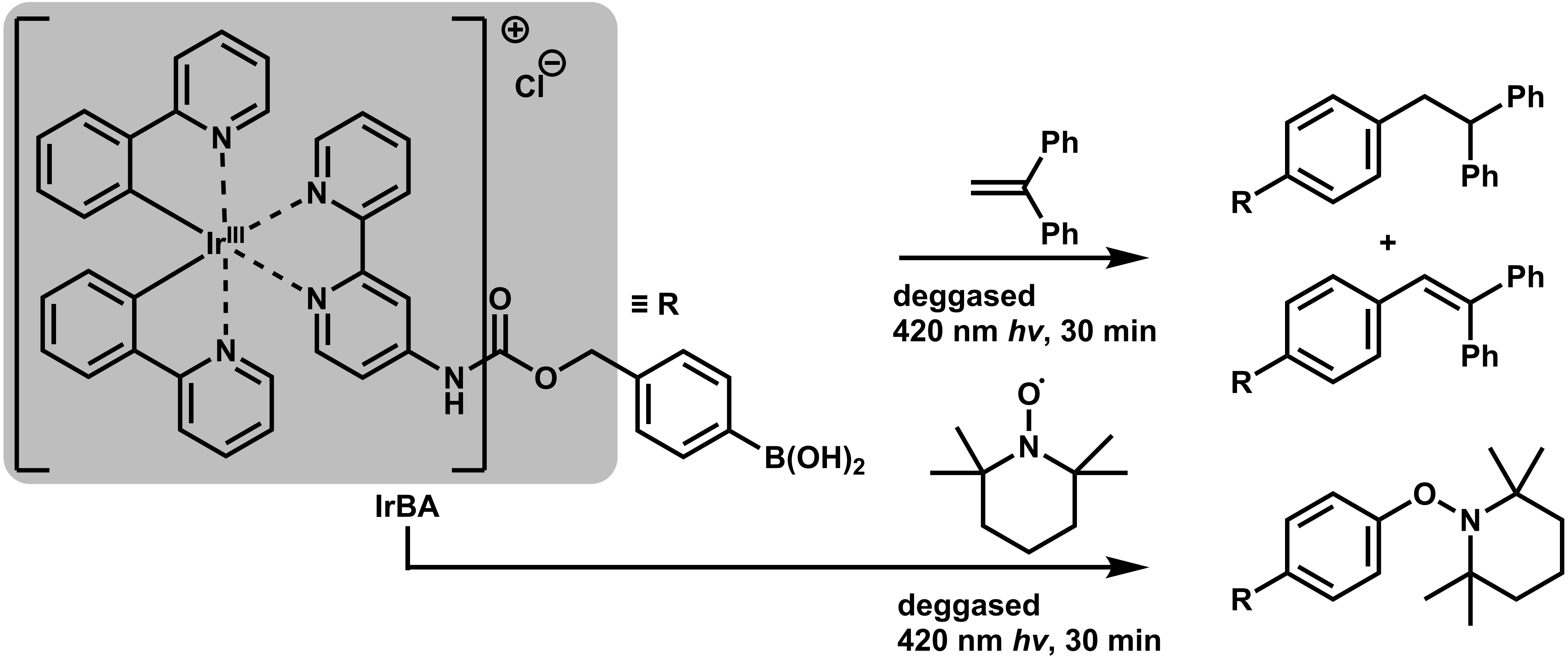
Reaction rate under various pH conditions

$$\text{IrBA} \xrightarrow[T\ \text{min}]{1\%\ O_2,\ 420\ \text{nm}\ h\nu,\ \text{buffer}} \text{IrNH}_2$$

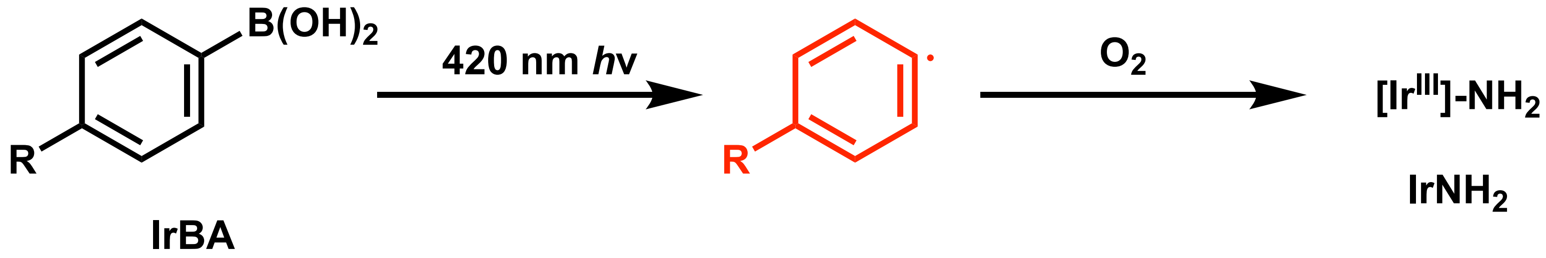
entry	pH	T	yield
1	4.5	30	31%
2	6.0	30	60%
3	8.0	< 10	> 99%

Boronate anion seems to be involved in photoreaction.

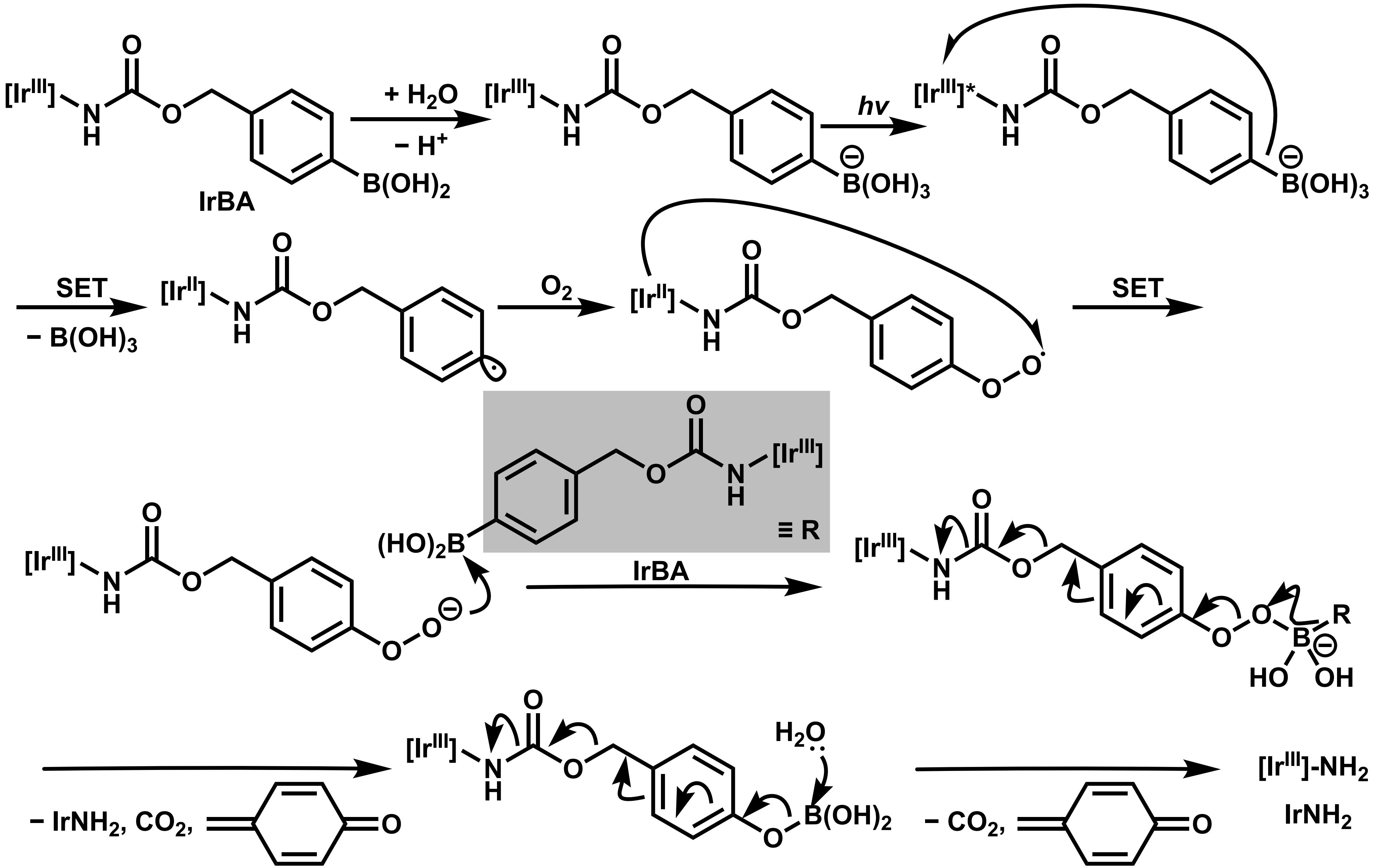
Analysis of Reaction Intermediate (2): Phenyl Radical



These results indicate the generation of phenyl radical intermediate by photoactivation of IrBA.

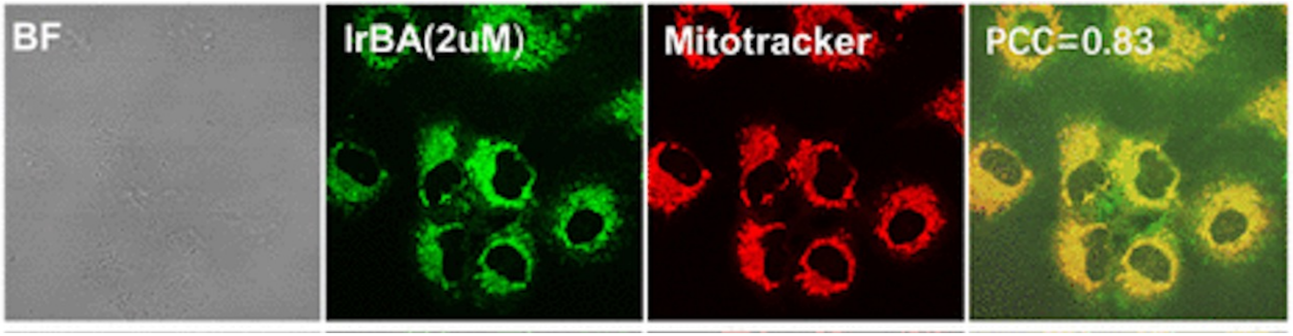


Proposed Reaction Mechanism



Formation of Phenyl Radical in Cell

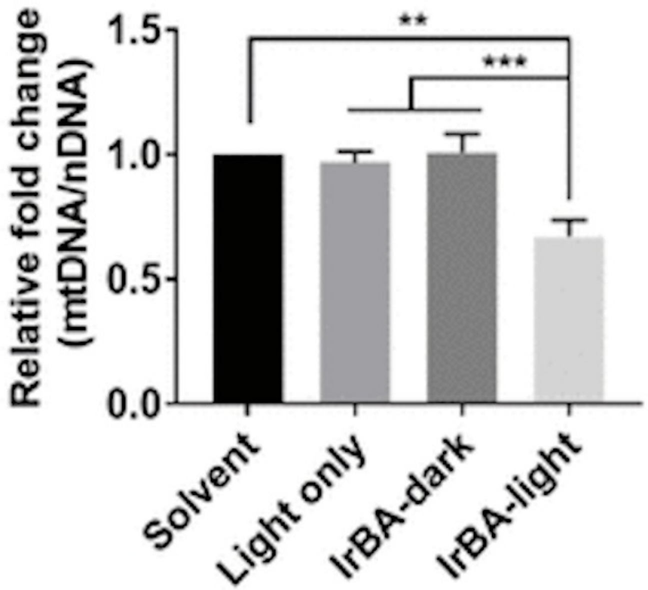
IrBA was accumulated in mitochondria in A549 cell (human non-small-cell lung cancer cell).



Phenyl radical is known to damage DNA.

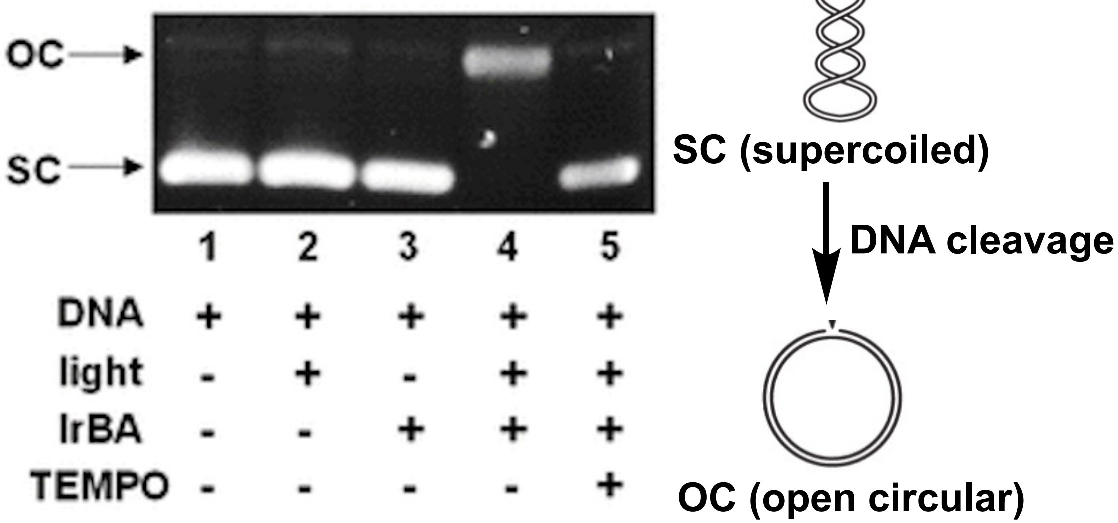
mtDNA damage by IrBA + *hv* in live cell

O_2: < 0.1%, *hv*: 420 nm



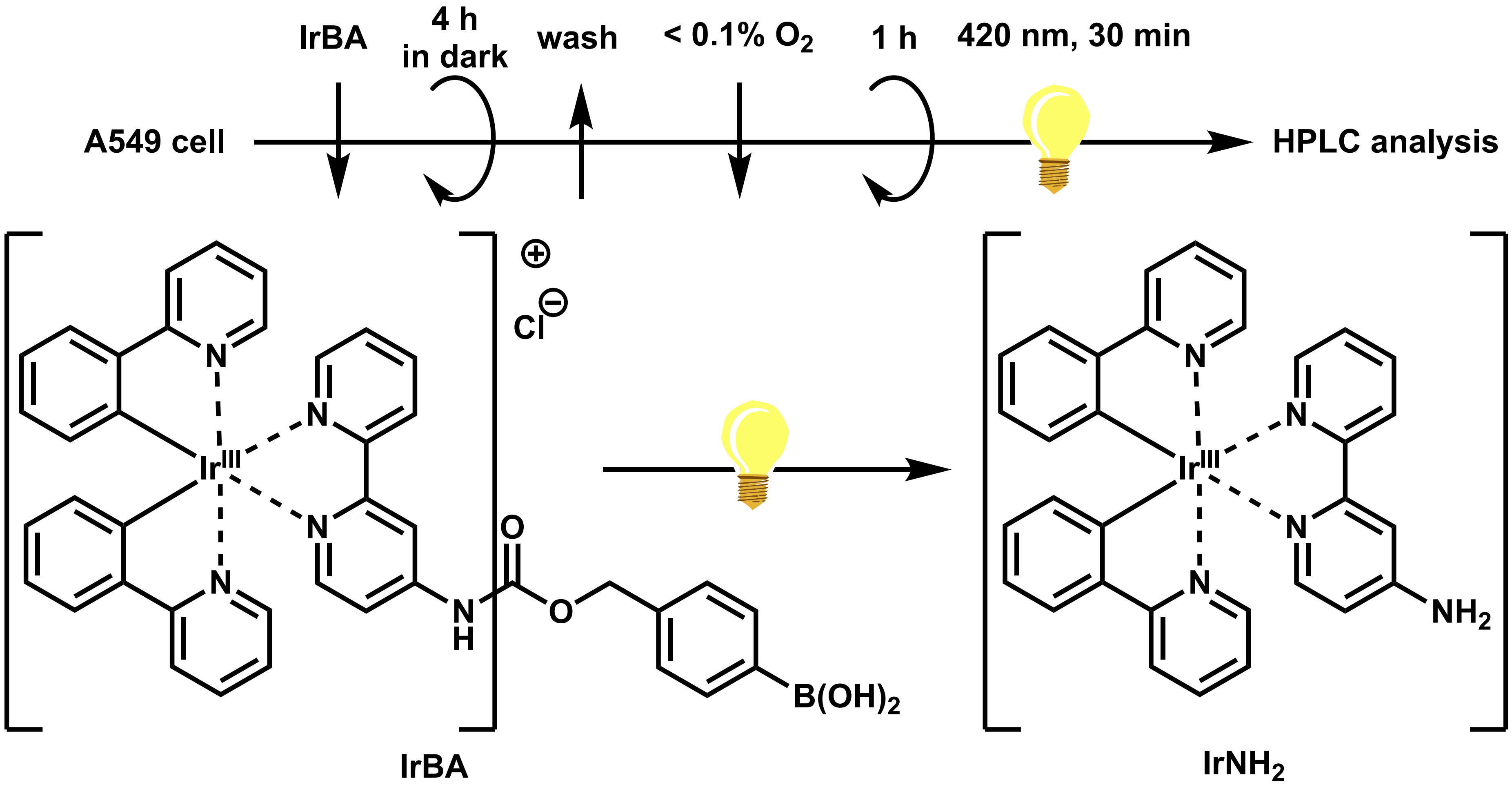
Inhibition of DNA damaging activity by TEMPO

O_2: < 0.1%, *hv*: 420 nm



These results suggest that phenyl radical is generated under cellular environment.

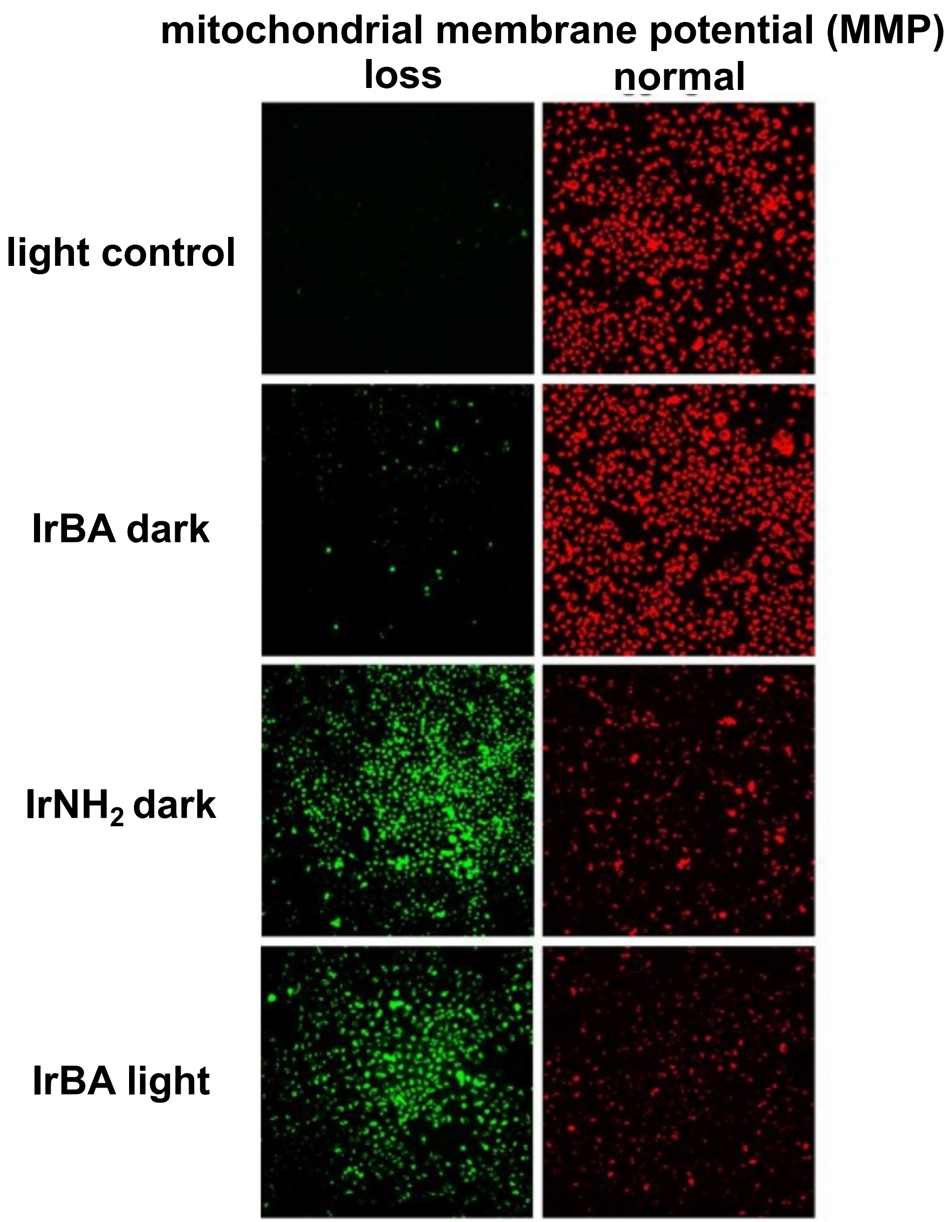
Release of $IrNH_2$ in Cell under Hypoxia (1)

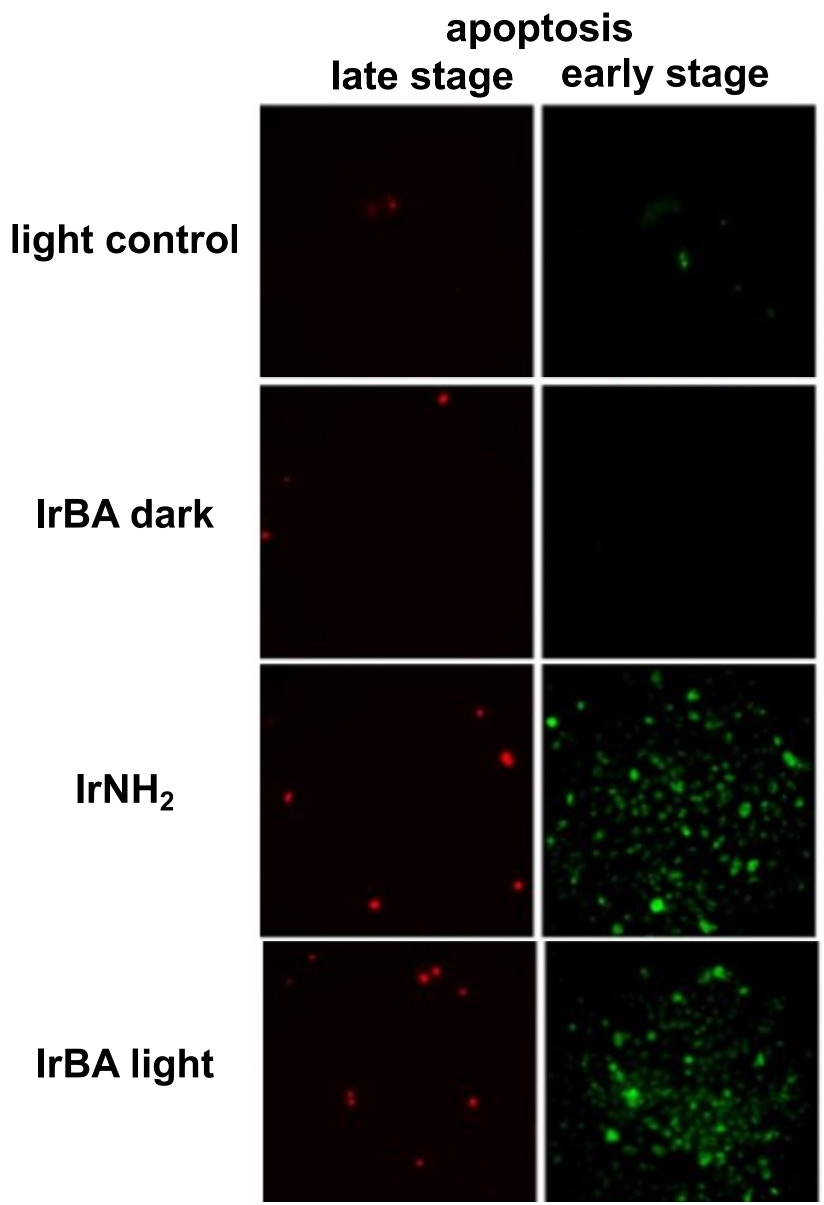


light irradiation	conversion rate
+	> 95%
−	< 10%

IrBA was successfully converted into $IrNH_2$ by light irradiation in cancer cell under hypoxia.

Release of $IrNH_2$ in Cell under Hypoxia (2)





IrBA was photochemically activated to induce MMP loss and apoptosis under hypoxia.

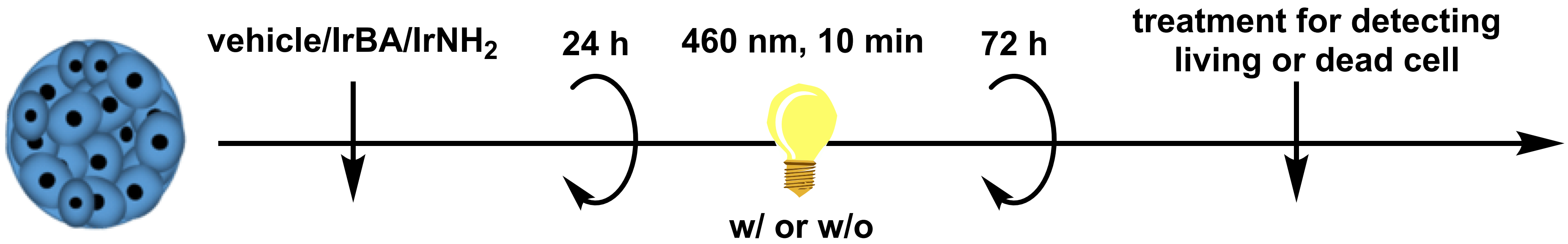
Cytotoxicity against Cancer Cells

cell	compound	IC_{50} (μM) under hypoxia (O_2 < 0.1%)		
		dark	light	PI
A549	IrBA	> 150	4.4 ± 0.3	> 34
	$IrNH_2$	9.6 ± 0.6	2 ± 0.4	4.5
MCF-7	IrBA	> 200	6.0 ± 1.4	> 33
	$IrNH_2$	10.2 ±1.4	2.7 ± 0.2	4
A375	IrBA	75.6 ± 4.2	2.0 ± 0.1	38
	$IrNH_2$	4.6 ± 0.4	0.7 ± 0.2	6.5

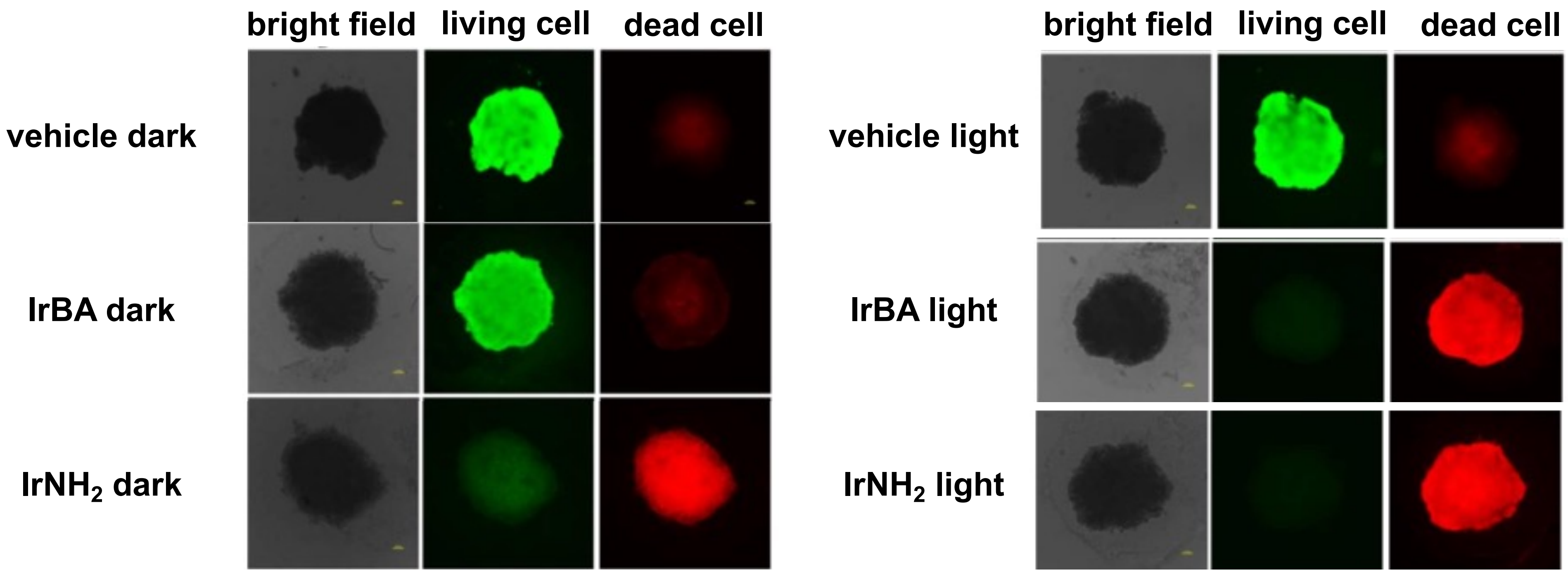
photo index (PI) = $IC_{50, dark}/IC_{50, light}$
MCF-7: human breast adenocarcinoma cell, A375: human melanoma cell

Photoactivated IrBA showed cytotoxicity compared to $IrNH_2$ under dark.
Since PI value of IrBA was more than 30, cytotoxicity of IrBA can be controlled by light irradiation.

Cytotoxicity against 3D Tumor Model

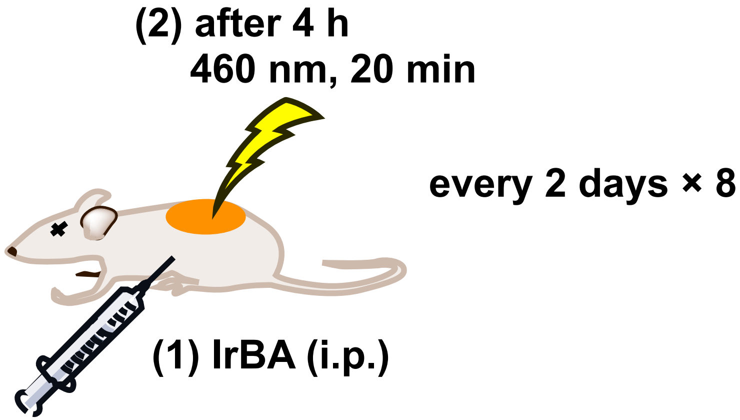


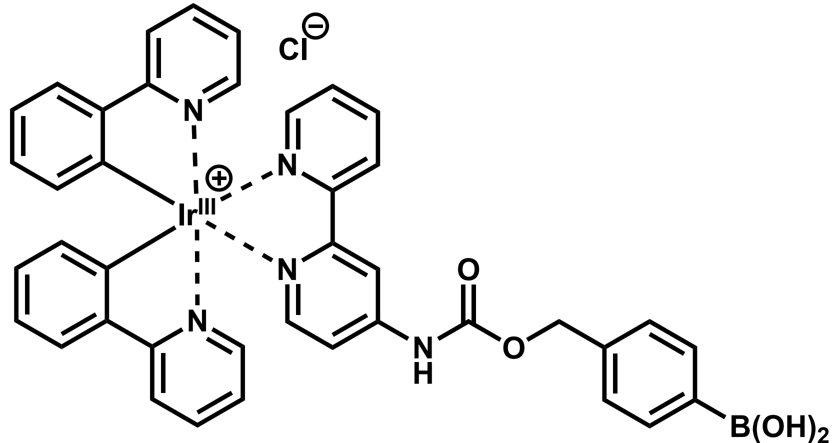
A549 tumor spheroid
3D tumor model with hypoxia resion



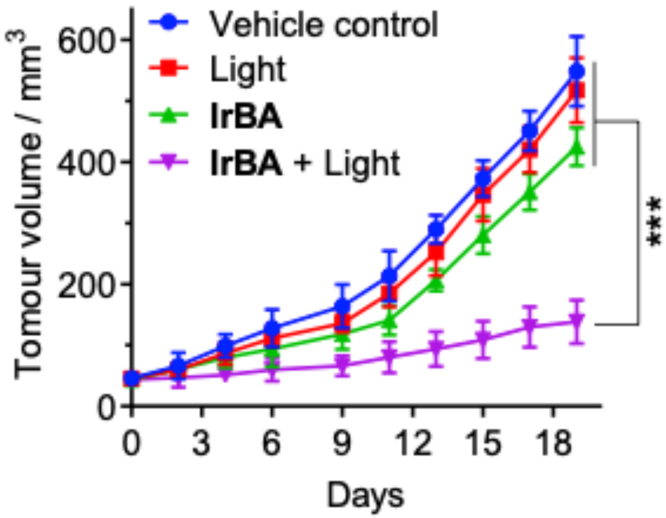
IrBA was activated by photoirradiation to show cytotoxicity against 3D tumor model including hypoxia region.

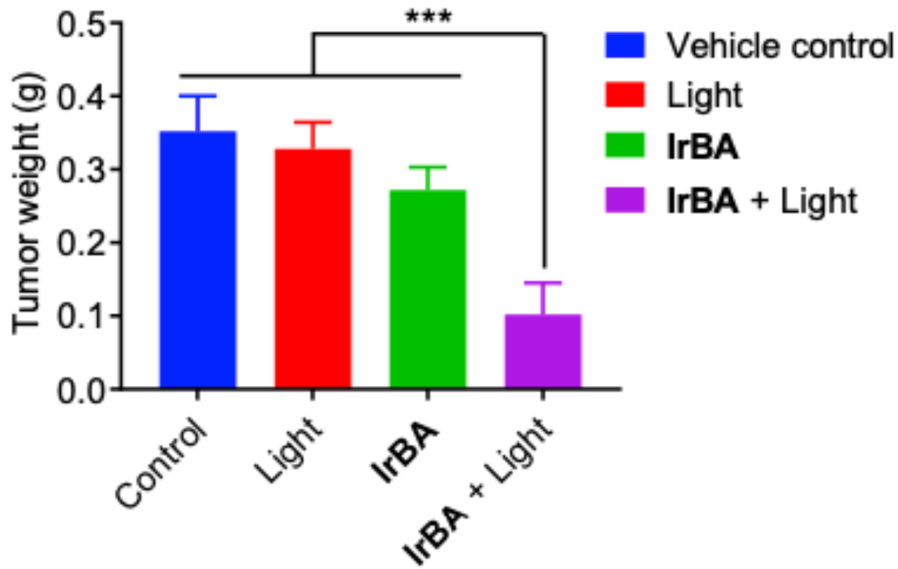
Antitumor Activity *in vivo*





IrBA



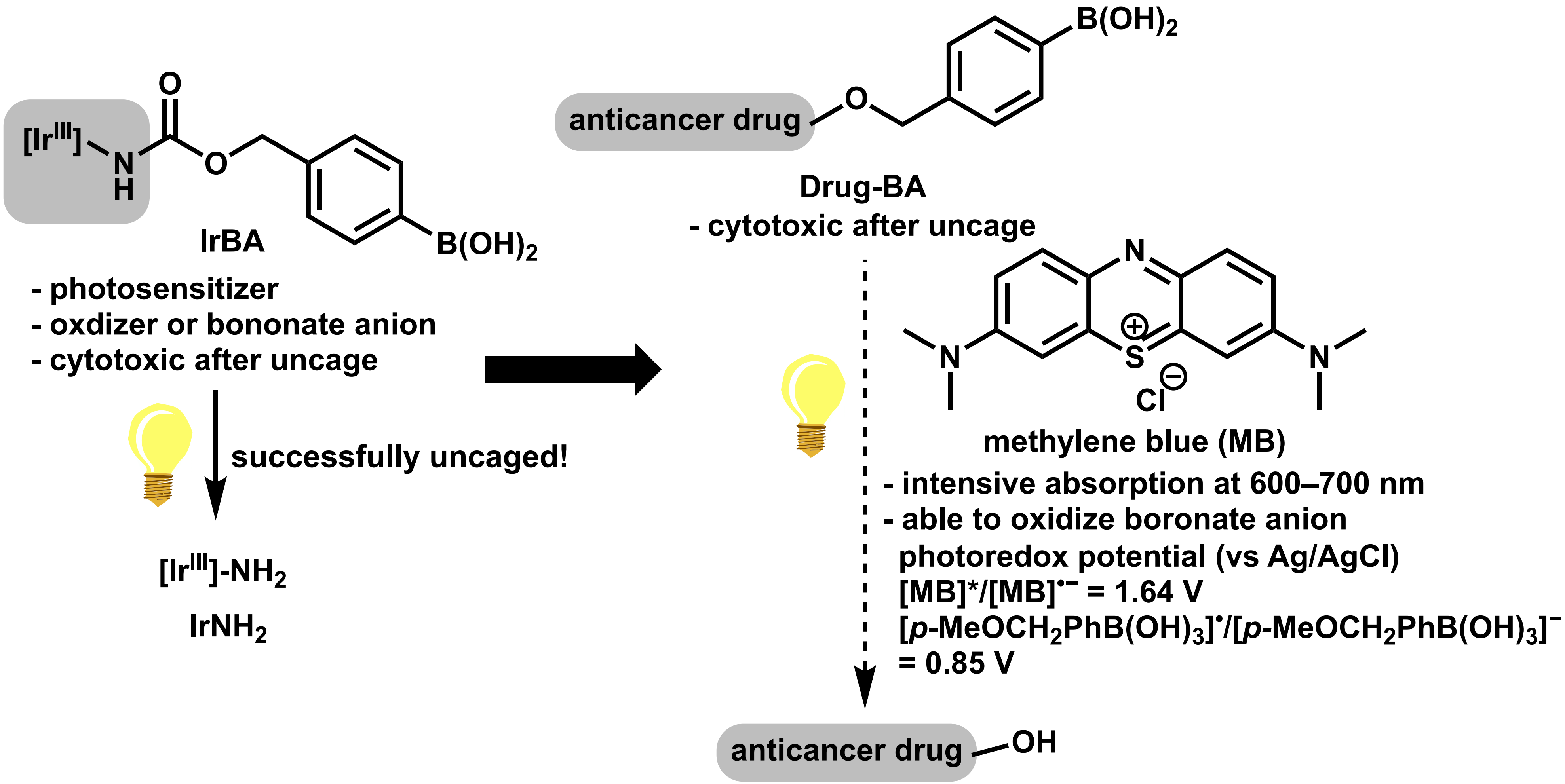


- Combination of IrBA and light irradiation could suppress tumor growth in mice.
- IrBA without light showed low tumor inhibiting activity.

-> Intratumonal ROS was insufficient to activate IrBA.

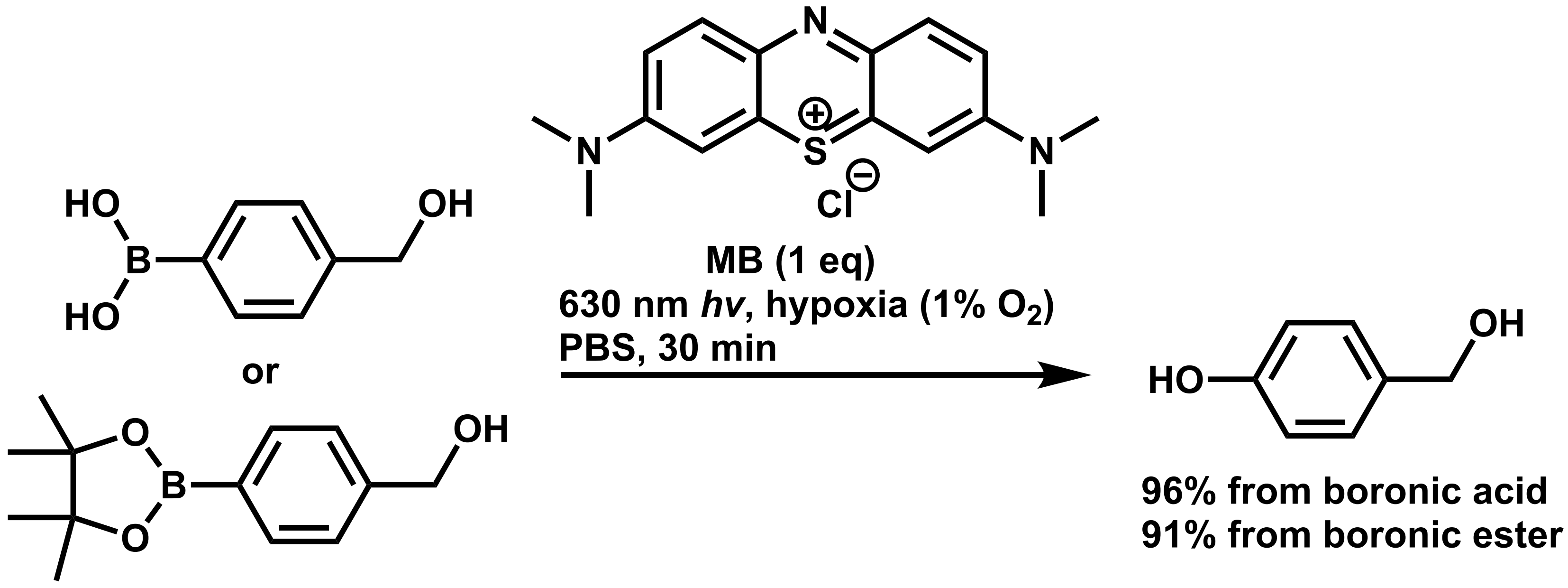
- No mouse death or mouse body weight less was observed.

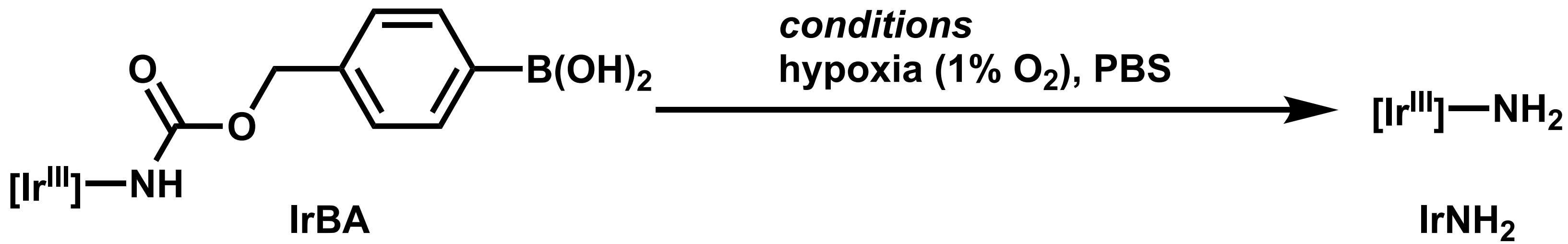
Photoactivation by Extramolecular photosensitizer



Extramolecular photosensitizer, MB, was used to show the generality of this strategy;
this uncaging strategy can be applied for activation of boron-caged prodrugs without photosensitivity.

Photoactivation by Methylene Blue (1)





conditions	result
MB (1 eq), 630 nm *hv*, 30 min	IrBA: full conversion within 30 min, $IrNH_2$: 92%
H_2O_2 (10 eq)	IrBA: full conversion afer 3 h

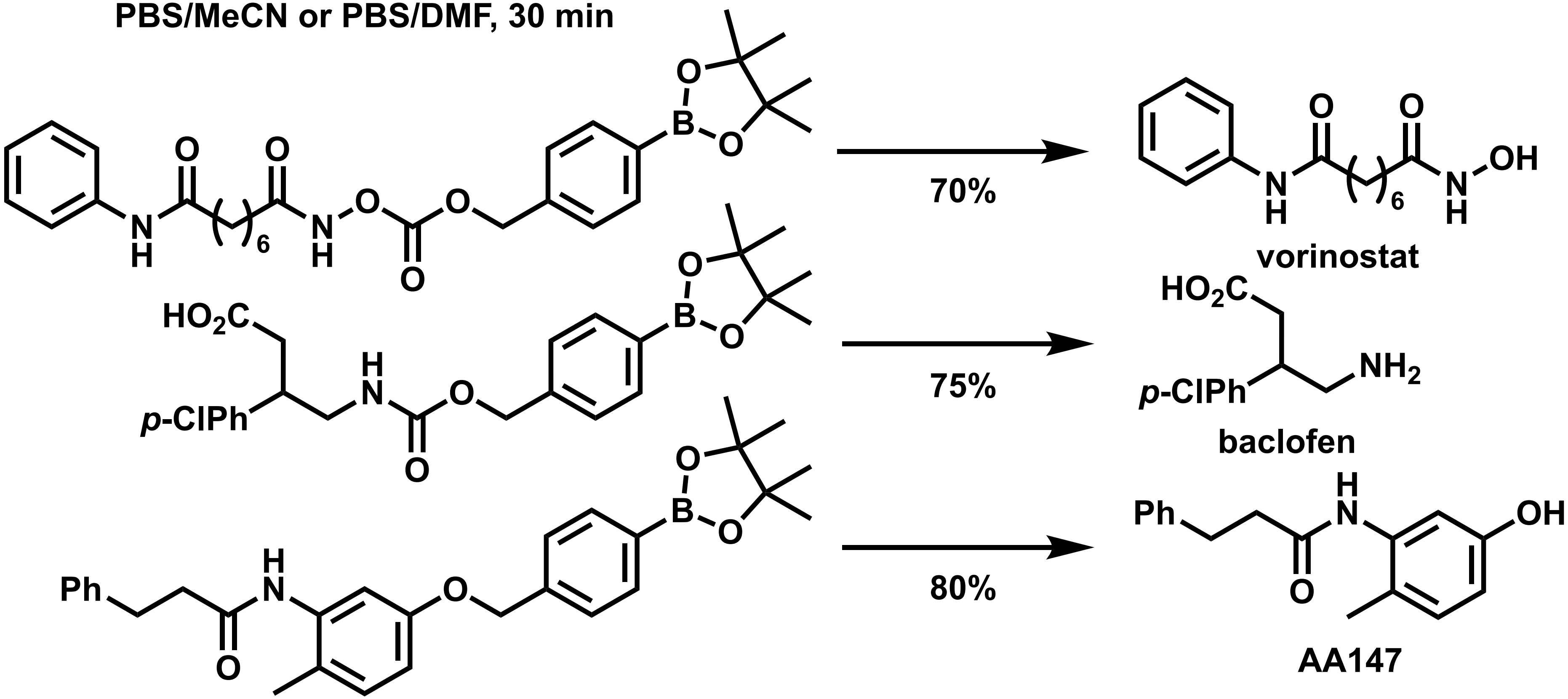
Yield was determined by HPLC analysis.

Boronic acids and esters were successfully uncaged by MB and 630 nm *hv* under hypoxia.

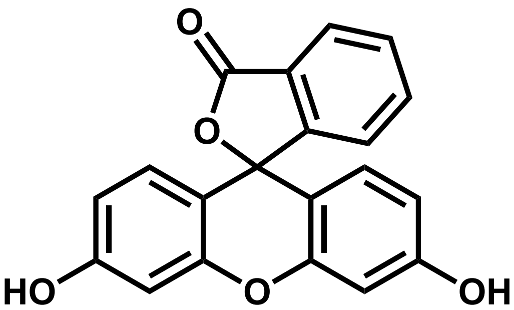
Photoactivation by Methylene Blue (2)

Condition: MB (1 eq), 630 nm *hv*, hypoxia (1% O_2)
PBS/MeCN or PBS/DMF, 30 min

Yield was determined by HPLC analysis.



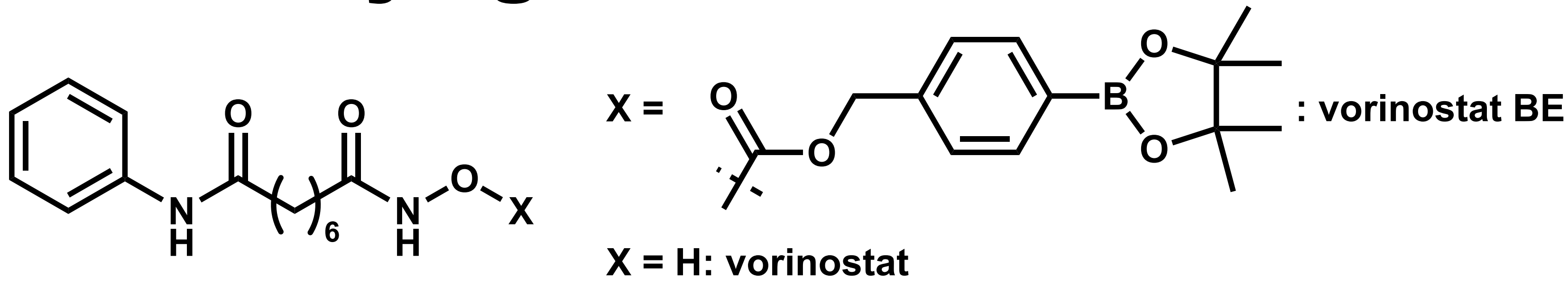
Three prodrugs were uncaged by MB and 630 nm *hv* under hypoxia.


fluorescein (FL)

redox potential (vs Ag/AgCl)
$[FL]^*/[FL]^{\bullet-}$ = + 0.81 V, $[FL]^{\bullet+}/[FL]^*$ = − 1.03 V
[*p*-$MeOCH_2PhB(OH)_3]^{\bullet}$/[*p*-$MeOCH_2PhB(OH)_3]^-$ = + 0.85 V
$O_2/[O_2]^{\bullet-}$ = − 0.83 V

"No obvious reaction" was occurred with FL and *hv*.
-> ruling out the involvement of $O_2^{\bullet-}$

Cytotoxicity against Cancer Cells with MB

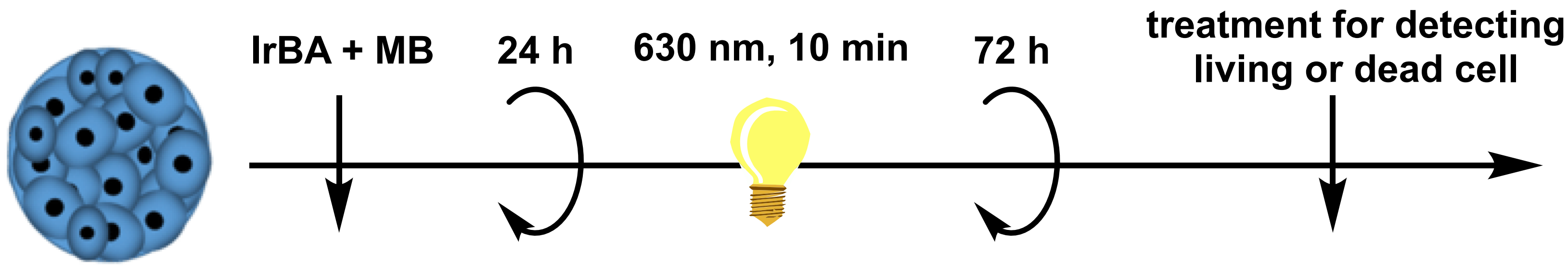


cell	compound	IC$_{50}$ (μM) under hypoxia (O_2 < 0.1%) with MB (2 μM)		
		dark	light	PI
A549	IrBA	> 150	9.3 ± 2.1	> 16
	vorinostat BE	> 200	23.6 ± 2.3	> 8.5
	vorinostat	15.7 ± 2.6	not reported	—
MCF-7	IrBA	> 200	13.6 ± 1.6	> 15
	vorinostat BE	> 200	40.6 ± 1.9	> 4.9
	vorinostat	35.5 ± 2.5	not reported	—
A375	IrBA	70.2 ± 3.4	3.6 ± 0.5	20

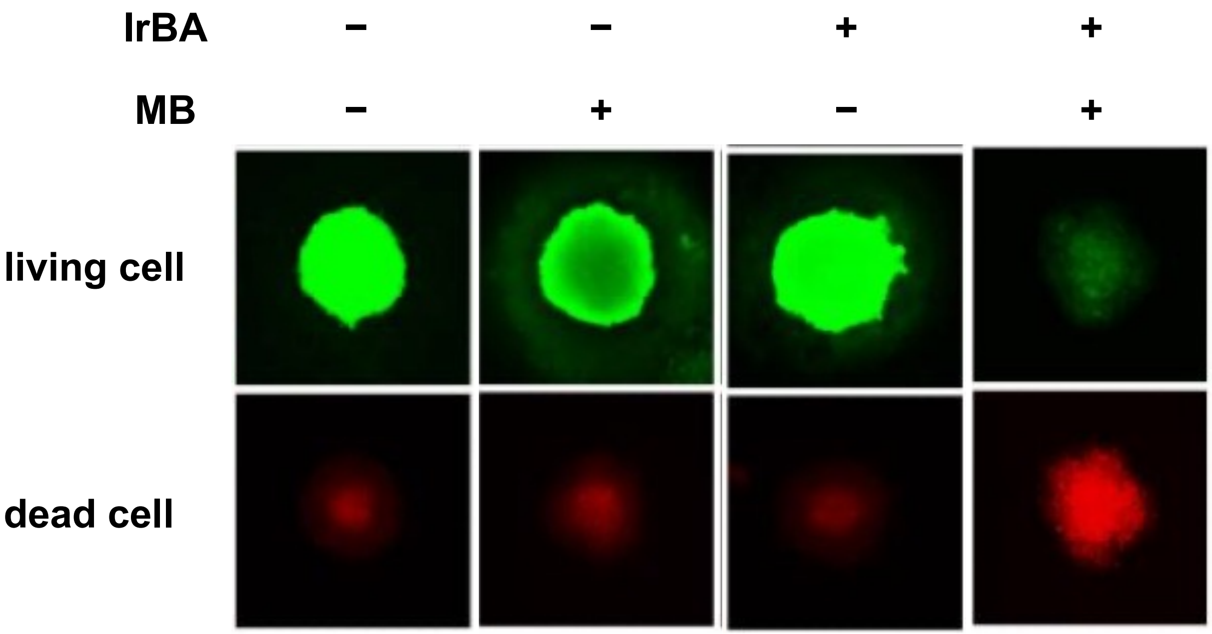
More than 90% cell survived when treated with MB (2 μM) and 630 nm light irradiation.

IrBA and vorinostat BE activated by the combination of MB and 630 nm *hν* showed cytotoxicity against cancer cells under hypoxia.

Cytotoxicity against 3D Tumor Model with MB

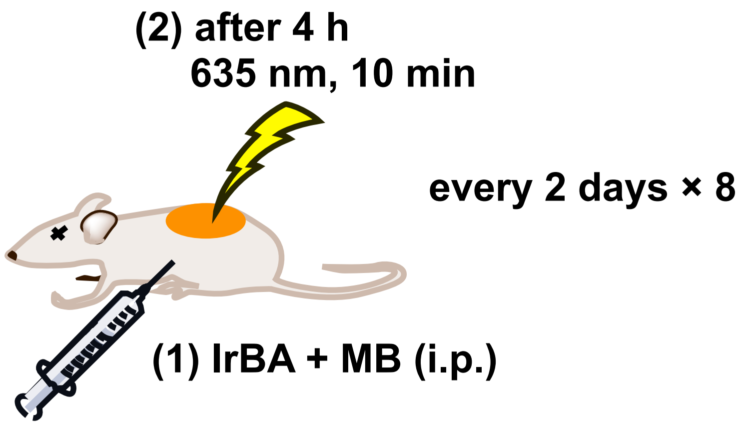


A549 tumor spheroid
3D tumor model with hypoxia resion

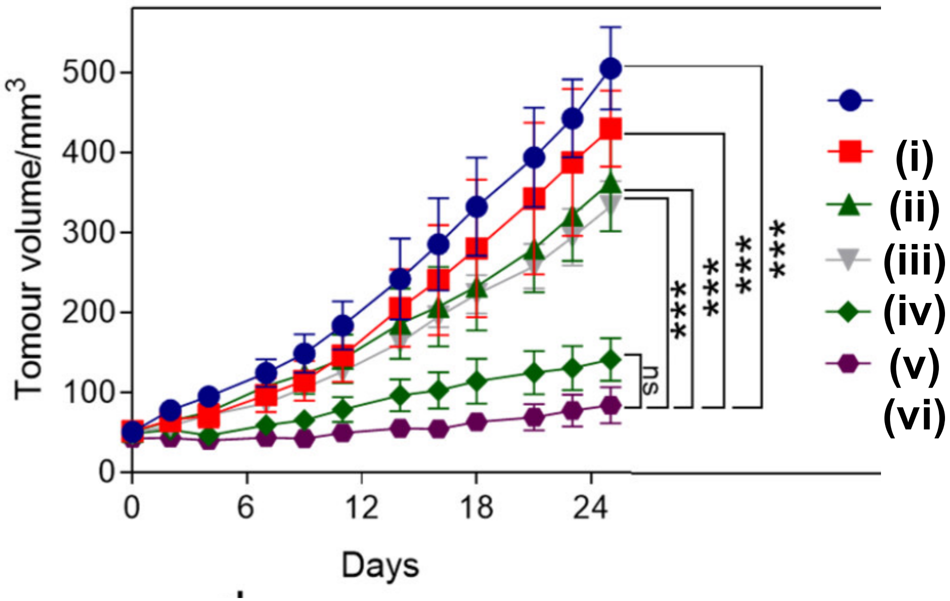


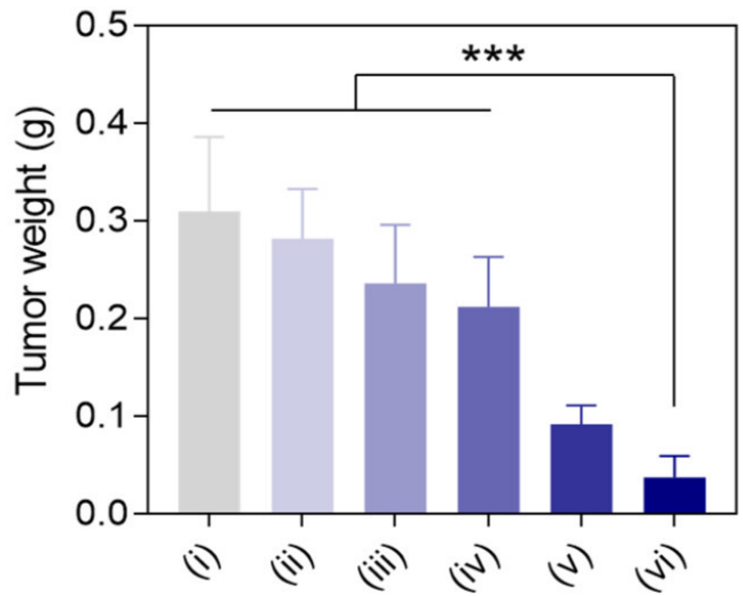
Only the combination of IrBA and MB with 630 nm *hv* showed cytotoxicity against tumor spheroid.

Antitumor Activity *in vivo* with MB



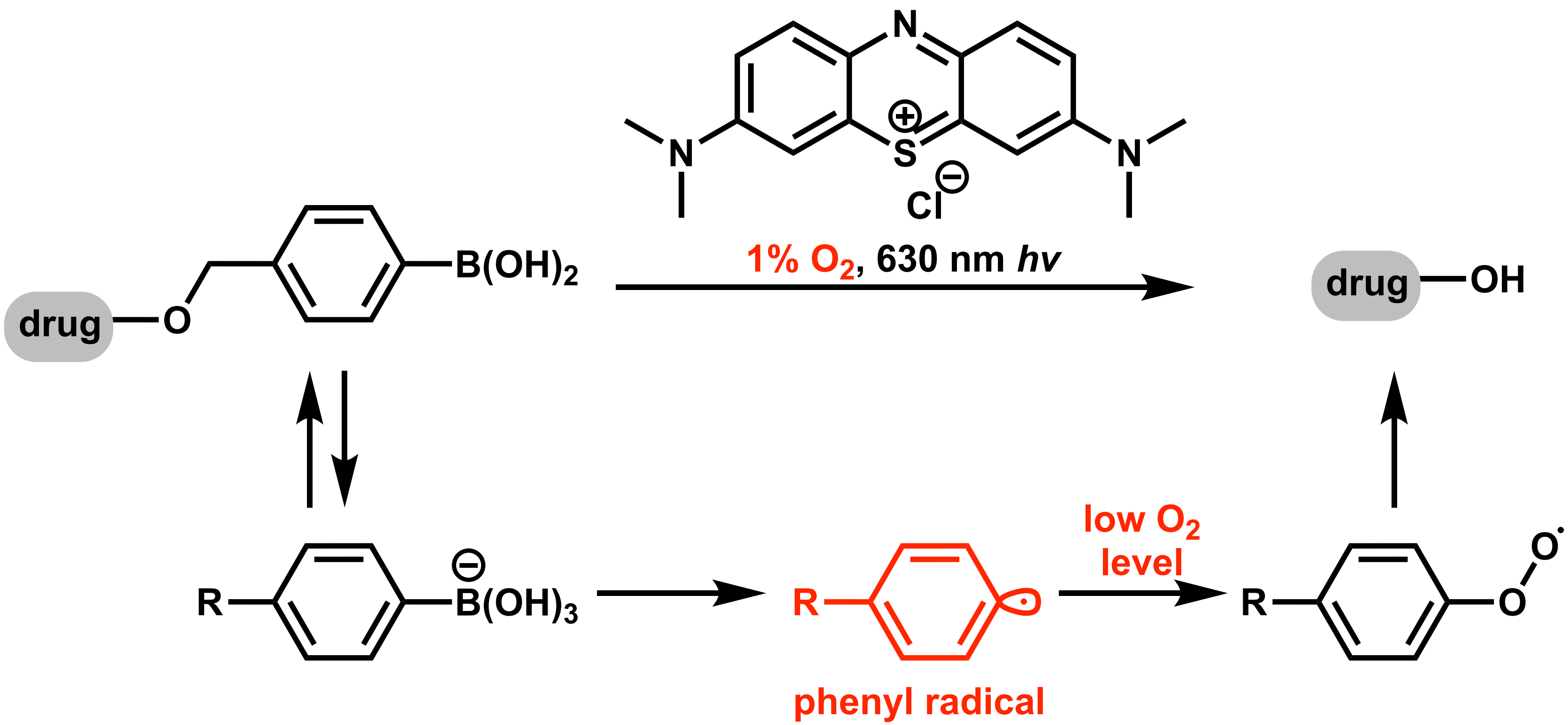
(i) vehicle control
(ii) IrBA + MB, dark
(iii) IrBA + 635 nm *hv*
(iv) MB + 635 nm *hv*
(v) $IrNH_2$, dark
(vi) IrBA + MB + 635 nm *hv*





- The combination of IrBA, MB and photoirradiation showed significant tumor growth inhibition activity compared to its parent drug $IrNH_2$.
- No mouse death and body weight loss occurred.

Summary



Boronic acids/esters prodrug can be photochemically uncaged under hypoxia via phenyl radical.

Boron-caged Ir(III) complex showed

- good PI value (= $IC_{50,\ dark}/IC_{50,\ light}$) against cancer cells under hypoxia
- good tumor growth inhibition activity and low side effects *in vivo*

by combination with methylene blue and light irradiation.

This activation approach would be effective for cancer treatment.