

N-oxide immune agonists activation by radiotherapy

Technical Field

The present disclosure pertains to the biomedical field, and particularly relates to a method for activating N-oxide TLR7/8 agonists with radiation, and a method of treating or suppressing cancers, reducing its severity, lowering its risk or inhibiting its metastasis in an individual. The method comprises administering to the individual a therapeutically effective amount of an N-oxide TLR7/8 agonist and radiating the individual with a therapeutically effective amount of radiotherapy.

Background of the Invention

Therapeutic activation of myeloid cells to stimulate adaptive immunity in tumours and generate potent cytotoxic T lymphocyte (CTL) responses against tumour neoantigens is crucial in anti-tumour immunotherapy. Among immunomodulatory molecules, toll-like receptor (TLR) 7/8 agonists are potent activators of myeloid cells and lead to the direct killing of tumour cells via enhanced infiltration of CTLs. However, patients receiving these agonists upon systemic administration experienced uncontrollable immune-related adverse events (irAEs) including fatal cytokine storms, ultimately leading to premature discontinuation of clinical trials. Though efforts have been made to develop TLR7/8 agonists for reducing irAEs, ideal one that can circumvent severe side effects upon systemic exposure and enable spatiotemporally activation in a tumour-selective manner is still a pressing need and full of challenges.

Over 50% of cancer patients take radiotherapy. Radiotherapy-activated prodrug (RAP) strategy is promisingly suited to solving the clinical dilemma of TLR7/8 agonists, since clinically relevant ionizing radiation (X-ray, γ -ray, etc.) is gradually being developed as a precise perturbing tool in living bodies, due to the advantages of tumour targeting and deep tissue penetration (up to 15 cm). Further, radiotherapy also has attracted considerable interest as a potential immune-stimulation and combination partner for synergy with tumour immunotherapy, however, the abscopal effect of radiotherapy is an infrequent event in the clinic. Consequently, radiotherapy-activated

tumour-selective immunotherapy is expected to reduce the irAEs and achieve better therapeutic efficacy, even promoting local irradiation for the treatment of distant tumour. However, the concept of radiotherapy-activated agonist has not yet been proposed.

For decades, a large number of work has focused on the development of synthetic TLR7/8 agonists imidazoquinoline (IMQ) derivatives. Especially the success of Imiquimod (R837), approved by Food and Drug Administration (FDA), led to the development of more potent IMQs such as Resiquimod (R848) which was on the National Cancer Institute's list with the highest potential for treating cancer. Previous structure-activity relationship studies that nitrogen atom on quinoline and C4 amine group synergistically form stable hydrogen bonds with Asp543 upon R848 binding to the interface of TLR8 dimer, which plays an essential role in receptor activation. Interestingly, we found insertion of a single oxygen atom on the quinoline group can block the immuno-stimulatory activity of R848. In addition, the inserted oxygen atom can be effectively eliminated via X-ray irradiation to release parent R848.

Summary of the Invention

Optimizing anti-tumor immunotherapy is critical, necessitating the selective activation of myeloid cells while avoiding immune-related adverse events (irAEs). Toll-like receptor (TLR) 7/8 agonists effectively activate myeloid cells but trigger uncontrollable irAEs upon systemic use, impeding clinical progress. Clinically relevant ionizing radiation, such as X-rays and γ -rays, is precise and deeply penetrating, holding promise for tumor-selective activation. The present disclosure explored the insertion of a single oxygen atom into TLR7/8 agonists, blocking their immuno-stimulatory activity, which can be selectively reversed by X-ray irradiation. This approach offers a solution for reducing irAEs, enhancing radio-immunotherapy, and promoting the coveted abscopal effect, revolutionizing cancer therapy.

In one aspect, the disclosure provides a N-oxide compound of Formula I. The N-oxide compound can be reduced to a corresponding non-N-oxide form compound after irradiated with the radiation.

In another aspect, the disclosure provides a composition comprising a N-oxide compound with Formula I.

In another aspect, the disclosure provides a preparation of a non-N-oxide compound of Formula II *in vivo*. The non-N-oxide compound of Formula II is afforded by reducing the N-oxide compound of Formula I after irradiated with the radiation *in vivo*.

In another aspect, the disclosure provides a method for activating N-oxide compound comprising imidazoquinoline group with radiation. The N-oxide compound can be reduced to a corresponding imidazoquinoline after irradiated with the radiation.

In another aspect, the disclosure provides a method of radio-immunotherapy. The method comprises administering to an individual a therapeutically effective amount of an N-oxide TLR7/8 agonist, and radiating the individual with a therapeutically effective amount of radiation.

In another aspect, the disclosure provides a use of an N-oxide TLR7/8 agonist for preparing a medicament for radio-immunotherapy in an individual, and the individual is also radiated with radiation.

Brief Description of the Drawings

Figure 1 shows, **a,b**, serum IFN- γ measurements taken at 4 h postinjection of R848 and NO-1 (30 $\mu\text{mol/kg}$ (**a**), 60 $\mu\text{mol/kg}$ (**b**), respectively). $n = 3$ C57BL/6J mice for each group tested. **c,d**, Serum TNF- α measurements taken at 4 h postinjection of R848 and NO-1 (30 $\mu\text{mol/kg}$ (**c**), 60 $\mu\text{mol/kg}$ (**d**), respectively). $n = 3$ C57BL/6J mice for each group tested. **e**, Weight change measurements for C57BL/6J mice ($n = 6$ mice each group) following intravenous administration of 30 $\mu\text{mol/kg}$ of R848 and NO-1, respectively. **f**, Concentration-dependent TLR7/8 activation curves of R848 and NO-1 in RAW-Blue report cells after 24 h incubation ($n = 6$ independent samples for each group tested).

Figure 2 shows, **a,b**, X-ray-induced R848 releasing from NO-1 (10 μM) in PBS determined by UPLC-MS. UPLC-MS spectra (selected-ion monitoring signal of $[\text{R848}+\text{H}]^+$, $m/z = 315$) of generated R848 (**a**). Positive ion mode mass spectrum is shown for R848, retention time = 2.18 min in **a** (**b**).

Figure 3 shows, **a**, Schematic illustration of the experimental design: In **b-h**, NO-1 (10 μM) was incubated with living RAW 264.7 and bone marrow-derived DCs (BMDCs) for 24 h and irradiated by X-ray (10 Gy), then

the markers expression, released cytokines, and T cell priming were measured. Cells incubated with R848 (1 μ M) were set as positive control. **b,c**, CD80/86 expression in **(b)** RAW 264.7 and **(c)** BMDCs. **d,e**, Secreted TNF- α **(d)** and MCP-1 **(e)** releasing from BMDCs were analyzed by CBA. **f-h**, NO-1 treated BMDCs were incubated with 10 μ g/mL OVA peptides for 24 h, then co-cultured with OT I CD8⁺ T cells for 3 days. Proliferation **(f,g)** and IFN- γ production **(h)** of OT I CD8⁺ T cells were analyzed. Data are presented as mean values $\pm$ s.d., $n = 3$ independent samples for each group tested, two-tailed unpaired Student's *t*-test.

Figure 4 shows, **a**, Treatment scheme. In **b-g**, C57BL/6J mice were implanted subcutaneously with MC38 cells followed by intravenous (iv) injection of NO-1 (30 μ mol/kg) and radiotherapy (6 Gy each treatment). **b**, Time-dependent accumulation of NO-1 in blood and tumour tissues detected by UPLC-MS ($n = 3$ mice). **c-e**, Average tumour growth **(c)**, weight change curves **(d)**, and survival curves of mice until reaching euthanasia criteria **(e)** were shown. **f**, Tumour volume of an individual mouse. **g**, Photographs of representative tumours on day 25.

Figure 5 shows, Treatment scheme. C57BL/6J mice ($n = 6$ mice per group) were implanted subcutaneously with MC38 cells on day 0 and day 2, followed by intratumoural (it) injection of NO-1 (3 μ mol/kg) and radiotherapy (6 Gy each treatment). **b**, Average volume of primary and distant tumours. Data are presented as mean values $\pm$ s.d.

Figure 6 shows, Concentration-dependent TLR7/8 activation curves of agonists and corresponding *N*-oxide prodrugs in RAW-Blue report cells after 24 h incubation ($n = 6$ independent samples for each group tested).

Figure 7 shows, Schematic illustration of deoxygen of *N*-oxide agonists induced by e^-_{aq} and activation yield of agonists from corresponding *N*-oxide agonists (10 μ M) in PBS after 60 Gy radiation. Data are presented as mean values $\pm$ s.d., $n = 3$ independent samples for each group tested.

Figure 8 shows, MC38 tumor-bearing mice treated with different dose (20 μ g/mice and 100 μ g/mice) on day 10, 13 and 16. Body weight **(a)** and tumor growth was measured and shown ($n = 6$) **(b)**. Serum was collected 24 h after the first injection. Cytokines in serum were analyzed by cytometric bead assay **(c, d)**. **e, f** MC38 tumor-bearing mice were i.v. treated with NO-10 on day 10,

13 and 16. 1 h later, mice were irradiated by 6 Gy or not. Tumor growth was measured as indicated (e) and survival curve was shown in (f) (n = 6).

Figure 9 shows, C57BL/6J mice (n = 6) were inoculated with 2.5×10^5 B16F10 cells. After one week, 2.5×10^6 B16F10 cells were intravenously injected. Tumor-bearing mice were i.v. treated with NO-10 and/or radiation on day 8, 11 and 14. The local tumor volume was measured (a). On day 19, the mice were sacrificed, and the colony number on the lung was counted (b, c).

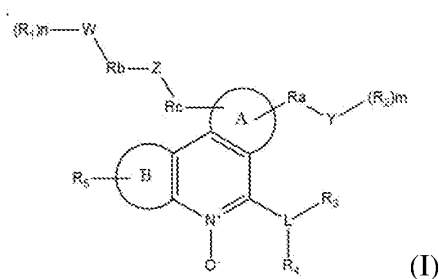
Figure 10 shows, d-g Balb/c mice (n = 8) were inoculated with 2×10^5 4T1-luc2 cells. Tumor-bearing mice were i.v. treated with NO-10 and/or radiation on day 10, 13 and 17. The metastasis of lung was measured by the IVIS Spectrum (a, b). Survival curve was shown in (c).

Detailed Description of the Invention

(I) Compound

In one aspect, the disclosure provides a N-oxide compound of Formula I. The N-oxide compound can be reduced to a corresponding non-N-oxide form compound after irradiated with the radiation.

In some embodiments, the present disclosure provides the compound of Formula I:



Wherein,

ring A and is a 5-10 membered aryl or heteroaryl containing one to four heteroatoms independently selected from nitrogen, oxygen, or sulfur;

ring B is a 5-10 membered aryl or heteroaryl containing one to four heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Y, Z and W are independently selected from the group consisting of carbon, nitrogen, oxygen or sulfur, optionally Y, Z, W are independently absent;

R_a, R_b and R_c is independently selected from the group consisting of a

branched or non-branched, substituted or non-substituted, saturated or unsaturated aliphatic hydrocarbon chain having 1-10 carbon atoms in its backbone chain;

each R₁ is independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, halo, haloalkyl, haloalkoxy, alkoxy, hydroxy, cyano, cyanoalkyl, optionally substituted carboxy, optionally substituted acylamino, aminocarbonyl, hydroxyalkyl, hydroxyalkynyl, alkoxyalkyl, aminoalkyl, aminocarbonylalkyl, sulfonylalkyl, aminosulfonylalkyl, optionally substituted alkoxyalkyl, optionally substituted cycloalkyl, optionally substituted cyclyl alkyl, optionally substituted phenyl, optionally substituted heteroaryl, optionally substituted heterocyclyl, optionally substituted bicyclic heterocyclyl, optionally substituted bridged optionally substituted heterocyclyl, optionally substituted spiral, optionally substituted heterospiral, or optionally substituted spiroheterocyclyl, or two R₁ combine to form a optionally substituted cyclo alkyl, heterocyclo alkyl, aryl or heteroaryl;

each R₂ is independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, halo, haloalkyl, haloalkoxy, alkoxy, hydroxy, cyano, cyanoalkyl, optionally substituted carboxy, acylamino, aminocarbonyl, hydroxyalkyl, hydroxyalkynyl, alkoxyalkyl, aminoalkyl, aminocarbonylalkyl, sulfonylalkyl, aminosulfonylalkyl, optionally substituted alkoxyalkyl, optionally substituted cycloalkyl, optionally substituted cyclyl alkyl, optionally substituted phenyl, optionally substituted heteroaryl, optionally substituted heterocyclyl, optionally substituted bicyclic heterocyclyl, optionally substituted bridged optionally substituted heterocyclyl, optionally substituted spiral, optionally substituted heterospiral, or optionally substituted spiroheterocyclyl, or two R₂ combine to form a optionally substituted cyclo alkyl, heterocyclo alkyl, aryl or heteroaryl;

R₃, R₄ and R₅ are independently selected from the group consisting of hydrogen, alkyl, halo, haloalkyl, haloalkoxy, alkoxy, hydroxy, cyano, cyanoalkyl, carboxy, alkoxycarbonyl, acylamino, aminocarbonyl, hydroxyalkyl, hydroxyalkynyl, alkoxyalkyl, aminoalkyl, aminocarbonylalkyl, sulfonylalkyl, aminosulfonylalkyl, optionally substituted cycloalkyl, optionally substituted cyclyl alkyl, optionally substituted phenyl, optionally substituted heteroaryl, optionally substituted heterocyclyl, optionally substituted bicyclic heterocyclyl, optionally substituted bridged optionally substituted heterocyclyl, or optionally substituted spiroheterocyclyl, or R₃ and R₄ combine to form a optionally substituted C3-10 heterocyclo alkyl;

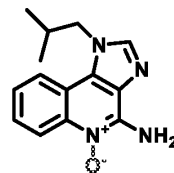
wherein the optionally substituent of R₁, R₂, R₃, R₄, R₅, R_a, R_b and R_c is independently selected from optionally substituted alkyl, cycloalkyl,

cycloalkoxy, halo, haloalkyl, haloalkoxy, alkoxy, alkoxyalkyl, hydroxy, carboxyl;

n is 0, 1, 2 or 3;

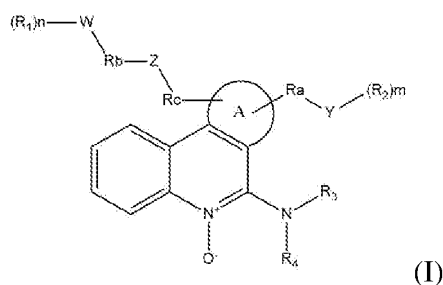
m is 0, 1, 2 or 3;

or a pharmaceutically acceptable salt thereof;



provided that the compound of formula I is not

In some embodiments, the present disclosure provides the compound of Formula I:



Wherein,

ring A is a 5-10 membered aryl or heteroaryl containing one to four heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Y, Z and W are independently selected from the group consisting of carbon, nitrogen, oxygen or sulfur, optionally Y, Z, W are independently absent;

Ra, Rb and Rc is independently selected from the group consisting of a branched or non-branched, substituted or non-substituted, saturated or unsaturated aliphatic hydrocarbon chain having 1-10 carbon atoms in its backbone chain;

each R₁ is independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, halo, haloalkyl, haloalkoxy, alkoxy, hydroxy, cyano, cyanoalkyl, optionally substituted carboxy, optionally substituted acylamino, aminocarbonyl, hydroxyalkyl, hydroxyalkynyl, alkoxyalkyl, aminoalkyl, aminocarbonylalkyl, sulfonylalkyl, aminosulfonylalkyl, optionally substituted alkoxyalkyl, optionally substituted cycloalkyl, optionally substituted cyclyl

alkyl, optionally substituted phenyl, optionally substituted heteroaryl, optionally substituted heterocyclyl, optionally substituted bicyclic heterocyclyl, optionally substituted bridged optionally substituted heterocyclyl, optionally substituted spiral, optionally substituted heterospiral, or optionally substituted spiroheterocyclyl, or two R₁ combine to form a optionally substituted cyclo alkyl, heterocyclo alkyl, aryl or heteroaryl;

each R₂ is independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, halo, haloalkyl, haloalkoxy, alkoxy, hydroxy, cyano, cyanoalkyl, optionally substituted carboxy, acylamino, aminocarbonyl, hydroxyalkyl, hydroxyalkynyl, alkoxyalkyl, aminoalkyl, aminocarbonylalkyl, sulfonylalkyl, aminosulfonylalkyl, optionally substituted alkoxyalkyl, optionally substituted cycloalkyl, optionally substituted cyclyl alkyl, optionally substituted phenyl, optionally substituted heteroaryl, optionally substituted heterocyclyl, optionally substituted bicyclic heterocyclyl, optionally substituted bridged optionally substituted heterocyclyl, optionally substituted spiral, optionally substituted heterospiral, or optionally substituted spiroheterocyclyl, or two R₂ combine to form a optionally substituted cyclo alkyl, heterocyclo alkyl, aryl or heteroaryl;

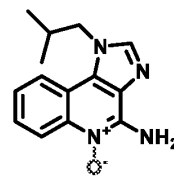
R₃ and R₄ are independently selected from the group consisting of hydrogen, alkyl, halo, haloalkyl, haloalkoxy, alkoxy, hydroxy, cyano, cyanoalkyl, carboxy, alkoxy carbonyl, acylamino, aminocarbonyl, hydroxyalkyl, hydroxyalkynyl, alkoxyalkyl, aminoalkyl, aminocarbonylalkyl, sulfonylalkyl, aminosulfonylalkyl, optionally substituted cycloalkyl, optionally substituted cyclyl alkyl, optionally substituted phenyl, optionally substituted heteroaryl, optionally substituted heterocyclyl, optionally substituted bicyclic heterocyclyl, optionally substituted bridged optionally substituted heterocyclyl, or optionally substituted spiroheterocyclyl, or R₃ and R₄ combine to form a optionally substituted C3-10 heterocyclo alkyl;

wherein the optionally substituent of R₁, R₂, R₃, R₄, R_a, R_b and R_c is independently selected from optionally substituted alkyl, cycloalkyl, cycloalkoxy, halo, haloalkyl, haloalkoxy, alkoxy, alkoxyalkyl, hydroxy, carboxyl;

n is 0, 1, 2 or 3;

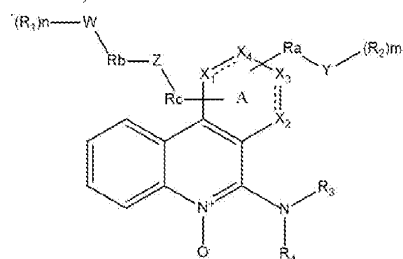
m is 0, 1, 2 or 3;

or a pharmaceutically acceptable salt thereof;

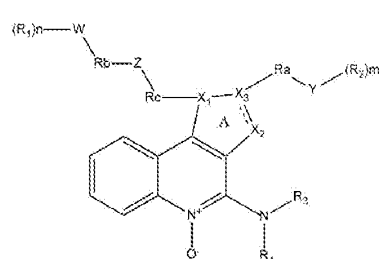


provided that the compound of formula I is not

In some embodiments, the Formula I is selected from Formula Ia and Formula Ib,



(Ia)



(Ib)

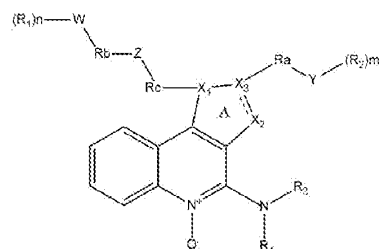
Wherein,

the X_1 , X_2 , X_3 , X_4 are independently selected from carbon, nitrogen, oxygen, or sulfur;

the $\sim$ is single bond or double bond;

or a pharmaceutically acceptable salt thereof.

In some embodiments, the Formula I is Formula Ib:



(Ib)

Wherein,

X_1 , X_2 , X_3 are independently selected from the group consisting of carbon, nitrogen, oxygen or sulfur, optionally Y, Z, W are independently absent;

Ra, Rb and Rc is independently selected from the group consisting of a branched or non-branched, substituted or non-substituted, saturated or unsaturated aliphatic hydrocarbon chain having 1-10 carbon atoms in its backbone chain;

each R_1 is independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, halo, haloalkyl, haloalkoxy, alkoxy, hydroxy, cyano,

cyanoalkyl, optionally substituted carboxy, optionally substituted acylamino, aminocarbonyl, hydroxyalkyl, hydroxyalkynyl, alkoxyalkyl, aminoalkyl, aminocarbonylalkyl, sulfonylalkyl, aminosulfonylalkyl, optionally substituted alkoxyalkyl, optionally substituted cycloalkyl, optionally substituted cyclyl alkyl, optionally substituted phenyl, optionally substituted heteroaryl, optionally substituted heterocyclyl, optionally substituted bicyclic heterocyclyl, optionally substituted bridged optionally substituted heterocyclyl, optionally substituted spiral, optionally substituted heterospiral, or optionally substituted spiroheterocyclyl, or two R₁ combine to form a optionally substituted cyclo alkyl, heterocyclo alkyl, aryl or heteroaryl;

each R₂ is independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, halo, haloalkyl, haloalkoxy, alkoxy, hydroxy, cyano, cyanoalkyl, optionally substituted carboxy, acylamino, aminocarbonyl, hydroxyalkyl, hydroxyalkynyl, alkoxyalkyl, aminoalkyl, aminocarbonylalkyl, sulfonylalkyl, aminosulfonylalkyl, optionally substituted alkoxyalkyl, optionally substituted cycloalkyl, optionally substituted cyclyl alkyl, optionally substituted phenyl, optionally substituted heteroaryl, optionally substituted heterocyclyl, optionally substituted bicyclic heterocyclyl, optionally substituted bridged optionally substituted heterocyclyl, optionally substituted spiral, optionally substituted heterospiral, or optionally substituted spiroheterocyclyl, or two R₂ combine to form a optionally substituted cyclo alkyl, heterocyclo alkyl, aryl or heteroaryl;

R₃ and R₄ are independently selected from the group consisting of hydrogen, alkyl, halo, haloalkyl, haloalkoxy, alkoxy, hydroxy, cyano, cyanoalkyl, carboxy, alkoxy carbonyl, acylamino, aminocarbonyl, hydroxyalkyl, hydroxyalkynyl, alkoxyalkyl, aminoalkyl, aminocarbonylalkyl, sulfonylalkyl, aminosulfonylalkyl, optionally substituted cycloalkyl, optionally substituted cyclyl alkyl, optionally substituted phenyl, optionally substituted heteroaryl, optionally substituted heterocyclyl, optionally substituted bicyclic heterocyclyl, optionally substituted bridged optionally substituted heterocyclyl, or optionally substituted spiroheterocyclyl, or R₃ and R₄ combine to form a optionally substituted C3-10 heterocyclo alkyl;

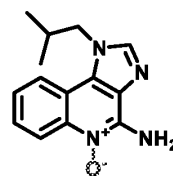
wherein the optionally substituent of R₁, R₂, R₃, R₄, R_a, R_b and R_c is independently selected from optionally substituted alkyl, cycloalkyl,

cycloalkoxy, halo, haloalkyl, haloalkoxy, alkoxy, alkoxyalkyl, hydroxy, carboxyl;

n is 0, 1, 2 or 3;

m is 0, 1, 2 or 3;

or a pharmaceutically acceptable salt thereof;



provided that the compound of formula I is not

In some embodiments, the compound Formula I, Formula Ia or Formula Ib, wherein,

the heteroatoms of ring A, X₁, X₂, X₃, X₄ is independently selected from the group consisting of carbon, nitrogen;

Y is absent or independently selected from the group consisting of oxygen or nitrogen;

Z is absent or independently selected from the group consisting of oxygen or nitrogen;

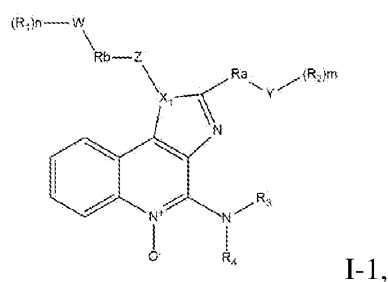
W is absent or nitrogen;

R_a is optionally substituted C₁₋₁₀alkylene;

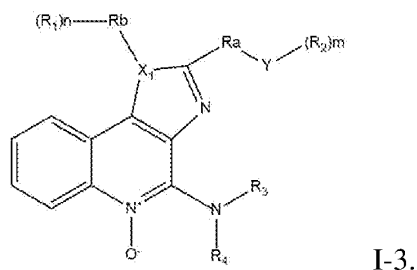
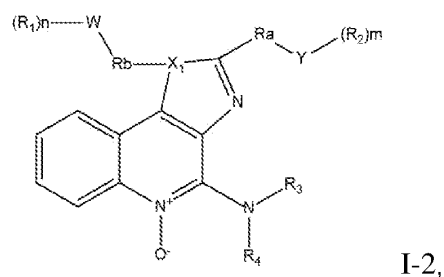
R_b is independently selected from the group consisting of a optionally substituted -C₁₋₁₀ alkylene-, optionally substituted C₆₋₁₀aryl-C₁₋₁₀alkylene- or optionally substituted -C₁₋₁₀alkylene -C₆₋₁₀aryl-C₁₋₁₀alkylene-;

R_c is optionally substituted -C₁₋₁₀ alkylene-.

In some embodiments, Formula I is Formula I-1, Formula I-2 or Formula I-3:



I-1,

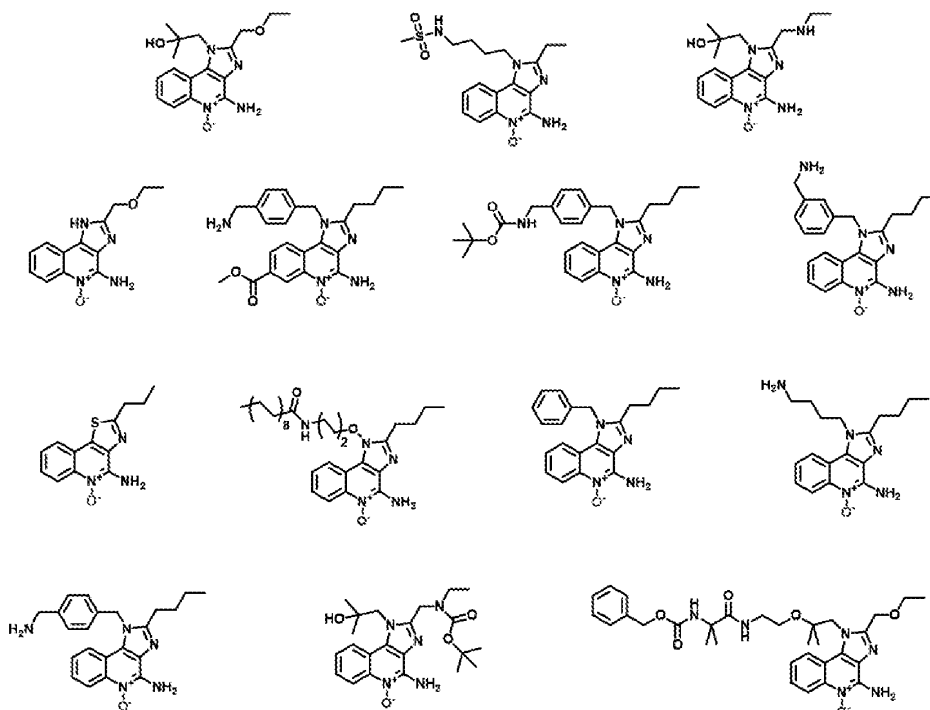


In some embodiments, each R_2 is independently selected from the group consisting of hydrogen, alkyl, optionally substituted carboxy; preferably, each R_2 is independently selected from the group consisting of hydrogen, C_{1-10} alkyl, C_{1-10} alkyl-O-(CO)-.

In some embodiments, each R_1 is independently selected from the group consisting of hydrogen, hydroxy, C_{1-10} alkyl-, C_{1-10} alkyl-(CO)-, C_{1-10} alkyl-O-(CO)-, C_{1-10} alkyl-SO₂-, C_{1-10} alkyl-NH- C_{1-10} alkyl-(CO)-, C_{1-10} alkyl-O-(CO)-NH- C_{1-10} alkyl-(CO)- or C_{6-10} aryl- C_{1-10} alkyl-O-(CO)-NH- C_{1-10} alkyl-(CO)-.

In some embodiments, the R_3 and R_4 are independently selected from the group consisting of hydrogen, C_{1-10} alkyl, -F, -Cl, -Br, -I, halo C_{1-10} alkyl, halo C_{1-10} alkoxy, C_{1-10} alkoxy, hydroxy, cyano, cyano C_{1-10} alkyl.

In some embodiments, the compound Formula I, wherein the compound is selected from



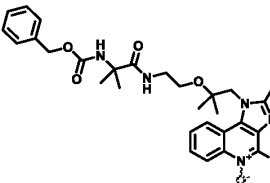
or a pharmaceutically acceptable salt thereof.

In some embodiments, the compound Formula I is selected from Table 1 or a pharmaceutically acceptable salt thereof.

Table 1

Compound No.	Structure	Chemical Name	LC-MS (ES+) m/z
NO-1		4-amino-2-(ethoxymethyl)-1-(2-hydroxy-2-methylpropyl)-1H-imidazo[4,5-c]quinoline 5-oxide	330.17
NO-2		4-amino-2-ethyl-1-(4-(methylsulfonyl)butyl)-1H-imidazo[4,5-c]quinoline 5-oxide	377.15
NO-3		4-amino-2-((ethylamino)methyl)-1-(2-hydroxy-2-methylpropyl)-1H-imidazo[4,5-c]quinoline 5-oxide	329.19

NO-4		4-amino-2-(ethoxymethyl)-1H-imidazo[4,5-c]quinoline 5-oxide	258.11
NO-5		4-amino-2-propylthiazolo[4,5-c]quinoline 5-oxide	259.08
NO-6		4-amino-1-benzyl-2-butyl-1H-imidazo[4,5-c]quinoline 5-oxide	346.18
NO-7		4-amino-1-(4-aminobutyl)-2-butyl-1H-imidazo[4,5-c]quinoline 5-oxide	327.201
NO-8		4-amino-1-(4-(aminomethyl)benzyl)-2-butyl-7-(methoxycarbonyl)-1H-imidazo[4,5-c]quinoline 5-oxide	433.21
NO-9		4-amino-1-(3-(aminomethyl)benzyl)-2-butyl-1H-imidazo[4,5-c]quinoline 5-oxide	375.21
NO-10		4-amino-1-(4-(aminomethyl)benzyl)-2-butyl-1H-imidazo[4,5-c]quinoline 5-oxide	375.21

NO-11		4-amino-2-(ethoxymethyl)-1-(5,5,11,11-tetramethyl-3,6-dioxo-1-phenyl-2,10-dioxa-4,7-diazadodecan-12-yl)-1H-imidazo[4,5-c]quinoline 5-oxide	592.30
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(II) Pharmaceutical Composition

In another aspect, the present disclosure provides a pharmaceutical composition comprising a compound of formula I or a pharmaceutically acceptable salt. The composition further comprising at least one pharmaceutically acceptable diluent, excipient or inert carrier.

In some embodiments, said N-oxide compound of Formula I is selected from any one of above defined Formula I, Formula Ia, Formula Ib, Formula I-1, Formula I-2 and Formula I-3.

In one particular variation, the composition is a solid formulation adapted for oral administration. In another particular variation, the composition is a liquid formulation adapted for oral administration. In yet another particular variation, the composition is a tablet. In still another particular variation, the composition is a liquid formulation adapted for parenteral administration.

The present disclosure also provides a pharmaceutical composition comprising a compound according to any one of the above embodiments and variations, wherein the composition is adapted for administration by a route selected from the group consisting of orally, parenterally, intraperitoneally, intravenously, intraarterially, transdermally, sublingually, intramuscularly, rectally, transbuccally, intranasally, liposomally, via inhalation, vaginally, intraocularly, via local delivery (for example by catheter or stent), subcutaneously, intraadiposally, intraarticularly, and intrathecally.

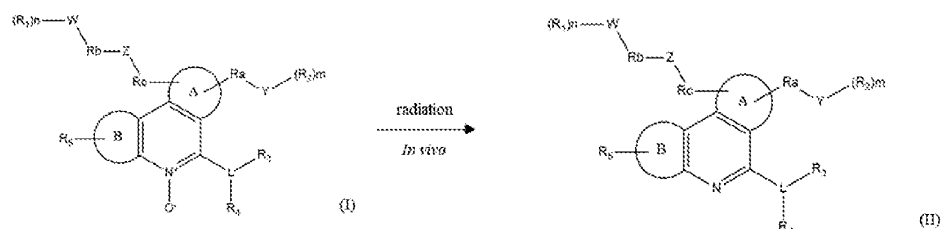
The present disclosure also provides a combination comprising a therapeutically effective amount of compound formula I or a pharmaceutically acceptable salt thereof and an additional therapeutically active agent.

(III) Preparation of a non-N-oxide compound

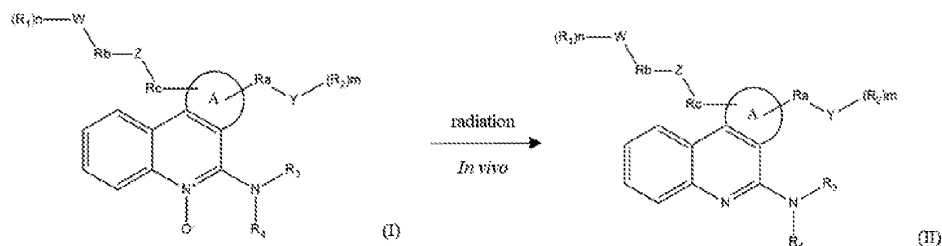
In one aspect, the present disclosure provides a method for activating N-oxide compound I with radiation. Said N-oxide compound comprises imidazoquinoline group, and the N-oxide compound is reduced to a

corresponding imidazoquinoline after irradiated with the radiation.

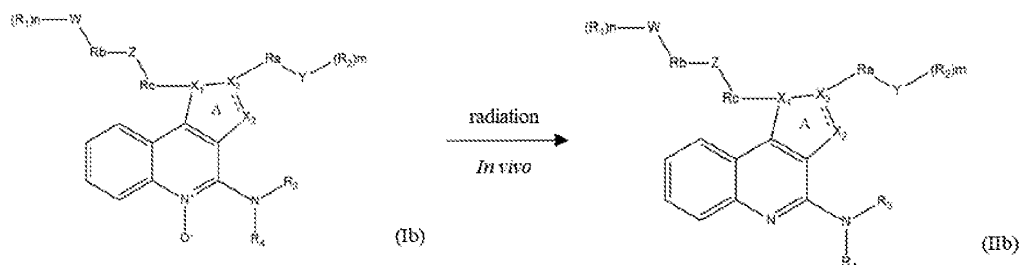
In another aspect, the disclosure provides a preparation of a non-N-oxide compound of Formula II in vivo. Said non-N-oxide compound of Formula II is afforded by reducing the N-oxide compound of Formula I after irradiated with the radiation in vivo.



In some embodiments, said N-oxide compound of Formula I is selected from any one of above defined Formula I, Formula Ia, Formula Ib, Formula I-1, Formula I-2 and Formula I-3.



In some embodiments, Said non-N-oxide compound of Formula IIb is afforded by reducing the N-oxide compound of Formula Ib after irradiated with the radiation in vivo.



(IV) Method of treatment

The present disclosure also provides a method for treating cancer, comprising administering said N-oxide compound of Formula I or a pharmaceutically acceptable salt thereof to the subject in need thereof and said subject is given radiation after administering said N-oxide compound.

The present disclosure also provides a N-oxide compound of Formula I or a pharmaceutically acceptable salt thereof, for use as a medicament for treating cancer in the subject in need thereof, and said subject is given radiation after administering said N-oxide compound.

In some embodiments, said N-oxide compound of Formula I is selected from any one of above defined Formula I, Formula Ia, Formula Ib, Formula I-1, Formula I-2 and Formula I-3.

In some embodiments, the subject is given radiation after administering the compound, preferably 20 mins, 1 hour, 2 hours, 4 hours after administering the compound.

In some embodiments, the cancer is solid cancer or non-solid tumors.

In some embodiments, the cancer is selected from the group consisting of colon cancer, small cell lung cancer, Hodgkin's lymphoma, malignant lymphoma, melanoma, nasopharyngeal cancer, head and neck cancer, skin cancer, esophageal cancer, lung cancer, liver cancer, prostate cancer, cervical cancer, thyroid cancer, and breast cancer. In some embodiments, the cancer is primary cancer and/or secondary cancer (metastasis).

In some embodiments, the radiation after administering said N-oxide compound is a radiotherapy. The combination of radiotherapy and chemotherapy with said N-oxide compound of Formula I provides a synergistic therapeutic effect compared to administration of the radiotherapy, the N-oxide compound of Formula I or the non-N-oxide compound of Formula II alone. In some embodiments, the combination of radiotherapy and chemotherapy with the N-oxide compound of Formula I is a radiotherapy-activated tumor-selective immunotherapy, which is expected to reduce the irAEs and achieve better therapeutic efficacy, even promoting local irradiation for the treatment of distant tumour.

In some embodiments, the compound is administrated in an amount of from about 0.005 mg/kg to about 100 mg/kg, such as an amount of about 0.005,

0.05, 0.5, 5, 10, 20, 30, 40, 50, 100 mg/kg.

In some embodiments, the compound and the radiation are administered once every two days, once every three days, once every four days, once every five days, once every seven days, once every ten days, once every two weeks, once every three weeks, once every four weeks, and the compound and radiation are administered continuously for at least 3 rounds, at least 4 rounds, at least 5 rounds, at least 6 rounds, at least 7 rounds, at least 8 rounds, at least 9 rounds, at least 10 rounds.

In some embodiments, the compound is administered orally, parenterally, intraperitoneally, intravenously, intraarterially, transdermally, sublingually, intramuscularly, rectally, transbuccally, intranasally, liposomally, via inhalation, vaginally, intraocularly, via local delivery, subcutaneously, intraadiposally, intraarticularly, intrathecal, intratumorally.

In some embodiments, the radiation is X-ray or gamma ray.

In some embodiments, the intensity of the X-ray or gamma ray is 60 Gy or lower, 50 Gy or lower, 40 Gy or lower, 30 Gy or lower, 20 Gy or lower, 10 Gy or lower, 8 Gy or lower, 6 Gy or lower, 4 Gy or lower.

(V) Articles of Manufacture

In another aspect the present disclosure also provides a use of said N-oxide compound of Formula I or a pharmaceutically acceptable salt thereof in the manufacture of a medicament in the treatment of cancer in the subject in need thereof. The subject is given radiation after administering said N-oxide compound.

In some embodiments, the cancer is solid cancer or non-solid tumors. In some embodiments, the cancer is selected from the group consisting of colon cancer, small cell lung cancer, Hodgkin's lymphoma, malignant lymphoma, melanoma, nasopharyngeal cancer, head and neck cancer, skin cancer, esophageal cancer, lung cancer, liver cancer, prostate cancer, cervical cancer, thyroid cancer, and breast cancer. In some embodiments, the cancer is primary cancer and/or secondary cancer (metastasis).

(VI) General definitions

Unless defined otherwise, all technical and scientific terms used herein

have the same meaning as is commonly understood by one of skill in the art to which the claimed subject matter belongs.

Unless the context requires otherwise, throughout the present disclosure and claims, the word “comprise” and variations thereof, such as, “comprises” and “comprising” are to be construed in an open, inclusive sense, that is as “including, but not limited to”.

“Aliphatic hydrocarbon chain” refers to a straight or branched chain hydrocarbon radical containing hydrogen and carbon atoms that are usually linked together in chains via single, double or triple bonds. The aliphatic hydrocarbon chain has 1-10, 1-8, 1-5 or 1-3 carbon atoms in its backbone chain and can be interrupted by one or more heteroatoms independently selected from nitrogen, oxygen, or sulfur.

“Alkyl” refers to a straight or branched chain hydrocarbon radical consisting of carbon and hydrogen atoms, which is saturated, having from one to ten carbon atoms (C₁₋₁₀ alkyl), or one to eight carbon atoms (C₁₋₈ alkyl), or one to six carbon atoms (C₁₋₆ alkyl), or one to four carbon atoms (C₁₋₄ alkyl), or one to three carbon atoms (C₁₋₃ alkyl), and which is attached to the rest of the molecule by a single bond, *e.g.*, methyl, ethyl, *n*-propyl, 1-methylethyl (*iso*-propyl), *n*-butyl, 1-methylpropyl (*sec*-butyl), 2-methylpropyl (*iso*-butyl), 1,1-dimethylethyl (*t*-butyl), *n*-pentyl, hexyl, 3-methylhexyl, 2-methylhexyl, and the like.

“Alkylene” refers to a straight or branched chain hydrocarbon radical having from 1 to 10 carbon atoms, optionally substituted with substituents selected from the group which includes lower alkyl, lower alkoxy, lower alkylsulfanyl, lower alkylsulfenyl, lower alkylsulfonyl, oxo, hydroxy, mercapto, amino optionally substituted by alkyl, carboxy, carbamoyl optionally substituted by alkyl, aminosulfonyl optionally substituted by alkyl, nitro, cyano, halogen and lower perfluoroalkyl, multiple degrees of substitution being allowed. Examples of “alkylene” as used herein include, but are not limited to, methylene, ethylene, *n*-propylene, *n*-butylene.

“Alkenyl” refers to a hydrocarbon group selected from linear and branched hydrocarbon groups comprising at least one C=C double bond and from 2 to 8, further such as from 2 to 6, carbon atoms. Examples of the alkenyl group, *e.g.*, C₂₋₆ alkenyl, include without limitation to ethenyl or vinyl,

prop-1-enyl, prop-2-enyl, 2-methylprop-1-enyl, but-1-enyl, but-2-enyl, but-3-enyl, buta-1, 3-dienyl, 2-methylbuta-1, 3-dienyl, hex-1-enyl, hex-2-enyl, hex-3-enyl, hex-4-enyl, and hexa-1, 3-dienyl groups.

"Alkynyl" refers to a hydrocarbon group selected from linear and branched hydrocarbon group, comprising at least one C≡C triple bond and from 2 to 8, further such as from 2 to 6, carbon atoms. Examples of the alkynyl group, e.g., C 2-6 alkynyl, include without limitation to ethynyl, 1-propynyl, 2-propynyl (propargyl), 1-butyne, 2-butyne, and 3-butyne groups.

"Alkoxy" means an oxygen moiety having a further alkyl substituent. The alkoxy groups of the present disclosure can be optionally substituted C₁-C₈ alkoxy.

"Cycloalkyl" refers to a hydrocarbon group selected from saturated cyclic hydrocarbon groups, comprising monocyclic and polycyclic (e.g., bicyclic and tricyclic) groups including fused, bridged or spiro cycloalkyl. For example, the cycloalkyl group may comprise from 3 to 8. Examples of cycloalkyl include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl.

The term "heterocyclyl" or "heterocycloalkyl" means a non-aromatic carbocyclic system containing 1 or 2 heteroatoms selected independently from N, O or S and having 1 or 2 rings wherein such rings may be fused, wherein fused is defined above. Heterocyclyl also includes bicyclic structures that may be Bridged-ring or Spiro-ring in nature with each individual ring within the bicycle varying from 3-6 atoms, and containing 0, 1 or 2 N atoms. The term "heterocyclyl" includes cyclic esters (i.e., lactones) and cyclic amides (i.e., lactams) and also specifically includes, but is not limited to, epoxidyl, oxetanyl, tetrahydrofuranyl, tetrahydropyranyl (i.e., oxanyl), pyranyl, dioxanyl, aziridinyl, azetidiny, pyrrolidinyl, 2,5-dihydro-1H-pyrrolyl, oxazolidinyl, thiazolidinyl, piperidinyl, morpholinyl, piperazinyl, thiomorpholinyl, 1,3-oxazinanyl, 1,3-thiazinanyl.

The term "aryl" used alone or in combination with other terms refers to 6 to 10 membered carbocyclic aromatic rings, e.g., phenyl or naphthyl.

The term "heteroaryl" refers to 6 to 10 membered monocyclic rings or bicyclic rings comprising at least one heteroatom, for example, from 1 to 3 heteroatoms selected from N, O, and S. Examples of C₆₋₁₀ heteroaryl includes pyridyl, cinnoliny, pyrazinyl, pyrimidinyl, imidazolyl, imidazopyridinyl,

isoxazolyl, oxazolyl, thiazolyl, isothiazolyl, thiadiazolyl, tetrazolyl, thienyl, triazinyl, benzothienyl, furyl or furanyl, benzofuryl, benzoimidazolyl, indolyl, isoindolyl, oxadiazolyl, phthalazinyl, pyrazinyl, pyridazinyl, pyrrolyl, triazolyl, quinolinyl, isoquinolinyl, pyrazolyl, pyrrolopyridinyl, pyrazolopyridinyl, benzoxazolyl, pteridiny, purinyl, 1-oxa-2,3-diazolyl, 1-oxa-2,4-diazolyl, 1-oxa-2,5-diazolyl, 1-oxa-3,4-diazolyl, 1-thia-2,3-diazolyl, 1-thia-2,4-diazolyl, 1-thia-2,5-diazolyl, 1-thia-3,4-diazolyl, furazanyl, benzofurazanyl, benzothiophenyl, benzothiazolyl, benzoxazolyl, quinazolinyl, quinoxalyl, naphthyridinyl, furopyridinyl, benzothiazolyl, and indazolyl.

“Halo” or “halogen” refers to bromo, chloro, fluoro or iodo.

“Haloalkyl” refers to an alkyl group, as defined above, that is substituted by one or more halo radicals, as defined above. Examples of cycloalkyl includes C₁-C₈ haloalkyl, *e.g.*, trifluoromethyl, difluoromethyl, trichloromethyl, 2,2,2-trifluoroethyl, 1,2-difluoroethyl, 3-bromo-2-fluoropropyl, 1,2-dibromoethyl, and the like.

“Acylamino” refers to any radical (as acetamido) formed by removal of one hydrogen atom from nitrogen in an organic acid amide. Examples of acylamino includes R-CONH-R, C₁₋₁₀alkyl-CONH-, -CONH-C₁₋₁₀alkyl, C₁₋₈alkyl-CONH-, -CONH-C₁₋₈alkyl, C₁₋₆alkyl-CONH-, -CONH-C₁₋₆alkyl, C₁₋₃alkyl-CONH-, -CONH-C₁₋₃alkyl, and the like.

“Sulfonylalkyl” refers to an alkyl substituent which is further substituted with one or more sulfonyl groups. Examples of sulfonylalkyl includes C₁₋₁₀alkyl-SO₂-, C₁₋₈alkyl-SO₂-, C₁₋₆alkyl-SO₂-, C₁₋₃alkyl-SO₂-.

The term “pharmaceutically acceptable” as used herein refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase “effective amount” means an amount of a compound or composition which is sufficient enough to significantly and positively modify the symptoms and/or conditions to be treated (*e.g.*, provide a positive clinical response). The effective amount of an active ingredient for use in a pharmaceutical composition will vary with the particular condition being

treated, the severity of the condition, the duration of the treatment, the nature of concurrent therapy, the particular active ingredient(s) being employed, the particular pharmaceutically-acceptable excipient(s)/carrier(s) utilized, and like factors within the knowledge and expertise of the attending physician.

The term "treating", as used herein, unless otherwise indicated, means reversing, alleviating, inhibiting the progress of, delaying the progression of, delaying the onset of, or preventing the disorder or condition to which such term applies, or one or more symptoms of such disorder or condition. The term "treatment", as used herein, unless otherwise indicated, refers to the act of treating as "treating" is defined immediately above. The term "treating" also includes adjuvant and neo-adjuvant treatment of a subject. For the avoidance of doubt, reference herein to "treatment" includes reference to curative, palliative and prophylactic treatment, and to the administration of a medicament for use in such treatment.

The compounds of Formula I may form stable pharmaceutically acceptable acid or base salts, and in such cases administration of a compound as a salt may be appropriate.

The salts may be formed by conventional means, such as by reacting the free base form of the product with one or more equivalents of the appropriate acid in a solvent or medium in which the salt is insoluble, or in a solvent such as water, which is removed in vacuo or by freeze drying or by exchanging the anions of an existing salt for another anion on a suitable ion-exchange resin.

The compounds of Formula I may have more than one chiral center, and it is to be understood that the application encompasses all individual stereoisomers, enantiomers and diastereoisomers and mixtures thereof. Thus, it is to be understood that, insofar as the compounds of Formula I can exist in optically active or racemic forms by virtue of one or more asymmetric carbon atoms, the application includes in its definition any such optically active or racemic form which possesses the above-mentioned activity. The present application encompasses all such stereoisomers having activity as herein defined.

It is also to be understood that certain compounds of Formula I, and pharmaceutically salts thereof, can exist in solvated as well as un-solvated forms such as, for example, hydrated and anhydrous forms. It is to be

understood that the compounds herein encompass all such solvated forms. For the sake of clarity, this includes both solvated (e.g., hydrated) forms of the free form of the compound, as well as solvated (e.g., hydrated) forms of the salt of the compound.

Formula I as described herein is intended to encompass all isotopes of its constituent atoms. For example, H (or hydrogen) includes any isotopic form of hydrogen including ^1H , ^2H (D), and ^3H (T); C includes any isotopic form of carbon including ^{12}C , ^{13}C , and ^{14}C ; O includes any isotopic form of oxygen including ^{16}O , ^{17}O and ^{18}O ; N includes any isotopic form of nitrogen including ^{13}N , ^{14}N and ^{15}N . It is to be understood that the present application encompasses all such isotopic forms.

The compounds of Formula I, or pharmaceutically acceptable salts thereof, will normally be administered via the oral route in the form of pharmaceutical preparations comprising the active ingredient or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt, in a pharmaceutically acceptable dosage form. Depending upon the disorder and patient to be treated, the compositions may be administered at varying doses.

The term "synergistic" as used herein refers to a therapeutic combination which is more effective than the additive effects of the two or more single therapy. The combination therapy may provide "synergy" and prove "synergistic", i.e., the effect achieved when the therapies used together is greater than the sum of the effects that results from using the therapies separately. In some embodiments, the irAEs is reduced when the therapies used together. Synergy may be evaluated by the tumor growth inhibition. Specifically, the tumor volume growth trend is suppressed, and preferably the tumor volume is superior shrunk, or the tumor is complete regression.

Examples

In order to make the objects and technical solutions of the present invention clearer, the present invention will be further described below in conjunction with specific example. It should be understood that the examples are not intended to limit the scope of the invention. Further, specific experimental methods not mentioned in the following examples were carried out in accordance with a conventional experimental method.

I. Materials and Synthesis

The compounds of the present invention can be prepared according to the procedures of the following Examples, using appropriate materials. The compounds illustrated in the examples are not, however, to be construed as forming the only genus that is considered as the invention.

Abbreviations

Throughout the Examples section, the following abbreviations are used to indicate various reagents, substituents and solvents.

1-isobutylimidazo[4,5-c]quinolin-4-amine(Imiquimod,R837)
 1-[4-amino-2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]-2-methyl-propan-2-ol (R848),
 N-[4-(4-amino-2-ethyl-imidazo[4,5-c]quinolin-1-yl)butyl]methanesulfonamide (852A)
 1-[4-amino-2-(ethylaminomethyl)imidazo[4,5-c]quinolin-1-yl]-2-methyl-propan-2-ol(Gardiquimod), 2-propylthiazolo[4,5-c]quinolin-4-amine (CL075)
 1-benzyl-2-butyl-imidazo[4,5-c]quinolin-4-amine(BBIQ)
 1-(4-aminobutyl)-2-butyl-imidazo[4,5-c]quinolin-4-amine(T785)
 2-(ethoxymethyl)-1H-imidazo[4,5-c]quinolin-4-amine (CL097)
 1-[[3-(aminomethyl)phenyl]methyl]-2-butyl-imidazo[4,5-c]quinolin-4-amine(IM Q3)
 1-[[4-(aminomethyl)phenyl]methyl]-2-butyl-imidazo[4,5-c]quinolin-4-amine(IM Q4)
 BenzylN-[2-[2-[2-[4-amino-2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]-1,1-dimethyl -ethoxy]ethylamino]-1,1-dimethyl-2-oxo-ethyl]carbamate (IMQ6)
 m-Chloroperbenzoic acid (m-CPBA)
 Phosphate-buffered saline (PBS)
 Fetal bovine serum (FBS), penicillin
 Cell Counting Kit-8 (CCK-8)

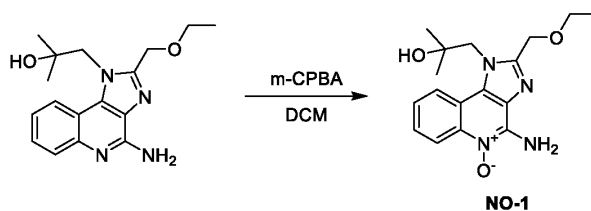
Instrumentation

X-Ray irradiation was delivered by an X-ray generator (RS2000 Pro 225, 225 kV, 17.7 mA, Rad Source Technologies, Inc.). Nuclear magnetic resonance (NMR)

spectra were recorded on Bruker AVANCE 400 MHz spectrometer. Signals are presented as parts per million (ppm), and multiplicity is presented as single (s), broad (b), doublet (d), triplet (t), quartet (q), or multiplet (m). Ultra-performance liquid chromatography (UPLC-MS) was performed on ACQUITY UPLC H-Class PLUS instrument equipped with Waters PDA eλ Detector and a Waters SQ Detector 2. prep-HPLC (neutral condition, column: Waters Xbridge Prep OBD C18). High-resolution mass spectroscopy was performed on a Bruker Fourier Transform Ion Cyclotron Resonance Mass Spectrometer. Fluorescence spectra were measured on an F-7000 spectrophotometer (Hitachi, Japan). Flow cytometry was performed on BD FACSLyric™ Clinical. In vivo optical images were taken on IVIS Lumina III in vivo imaging system. The absorbance of cells was measured on TECAN Infinite E Plex.

Example 1

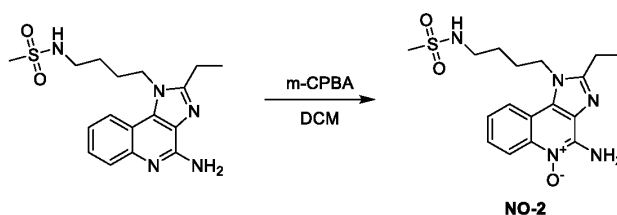
4-amino-2-(ethoxymethyl)-1-(2-hydroxy-2-methylpropyl)-1H-imidazo[4,5-c]quinoline 5-oxide (Compound NO-1)



Five reactions were carried out in parallel. To a solution of 1-[4-amino-2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]-2-methyl-propan-2-ol (100 mg, 318.09 μmol, 1 eq) in DCM (2 mL) was added m-CPBA (96.87 mg, 477.13 μmol, 85% purity, 1.5 eq) at 0°C. The mixture was stirred at 20°C for 12 h. Five reactions were combined. The reaction was added into saturated sodium thiosulfate aqueous solution (1 mL), then concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC to give NO-1 (76.4 mg, 228.88 μmol, 14.39% yield). ¹H NMR (400 MHz, DMSO-d₆) δ = 8.58 - 8.51 (m, 1H), 8.46 (d, J = 8.2 Hz, 1H), 7.64 (t, J = 7.8 Hz, 1H), 7.57 (br d, J = 6.2 Hz, 1H), 7.45 (t, J = 7.2 Hz, 1H), 4.91 (s, 2H), 4.71 (br s, 2H), 3.54 (q, J = 7.0 Hz, 2H), 1.38 - 1.01 (m, 9H).

Example 2

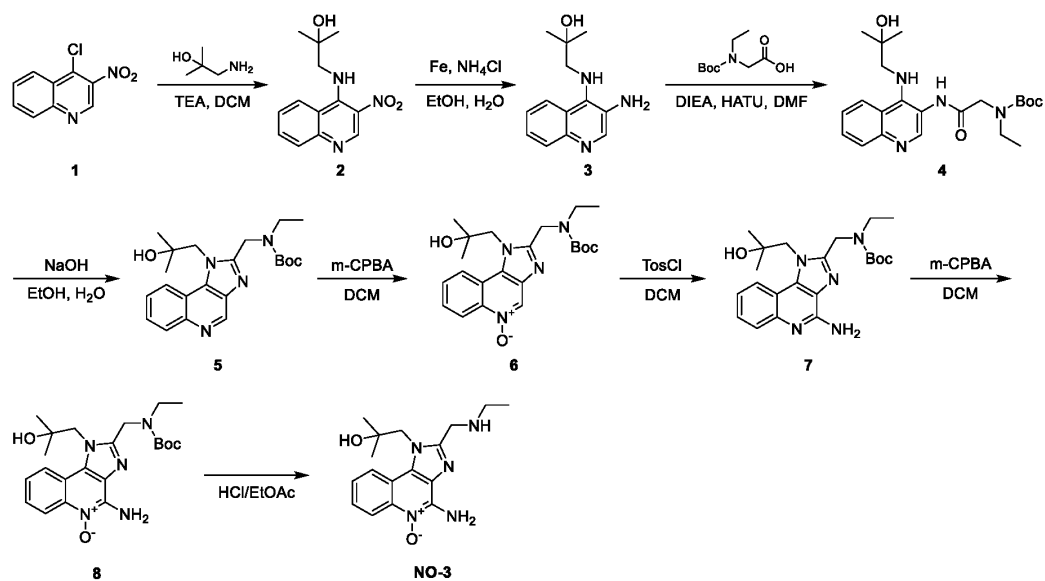
4-amino-2-ethyl-1-(4-(methylsulfonamido)butyl)-1H-imidazo[4,5-c]quinoline 5-oxide

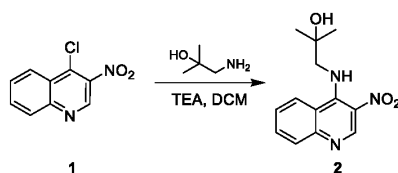
(Compound NO-2)

To a solution of N-[4-(4-amino-2-ethyl-imidazo[4,5-c]quinolin-1-yl)butyl]methanesulfonamide (180 mg, 497.98 μ mol, 1 eq) in DCM (3 mL) was added m-CPBA (151.65 mg, 746.97 μ mol, 85% purity, 1.5 eq) at 0°C. The mixture was stirred at 20°C for 12 h. The reaction was added saturated sodium thiosulfate aqueous solution (0.5 mL), then filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC to give NO-2 (11.3 mg, 28.02 μ mol, 5.63% yield). ¹H NMR (400 MHz, METHANOL-d₄) δ = 8.52 (d, J = 8.6 Hz, 1H), 8.27 (d, J = 8.4 Hz, 1H), 7.75 (t, J = 7.7 Hz, 1H), 7.66 - 7.59 (m, 1H), 4.65 - 4.61 (m, 2H), 3.15 (t, J = 6.6 Hz, 2H), 3.06 (q, J = 7.4 Hz, 2H), 2.91 (s, 3H), 2.07 - 1.98 (m, 2H), 1.82 - 1.72 (m, 2H), 1.51 (t, J = 7.4 Hz, 3H).

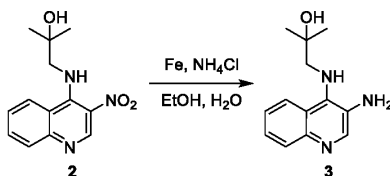
Example 3

4-amino-2-((ethylamino)methyl)-1-(2-hydroxy-2-methylpropyl)-1H-imidazo[4,5-c]quinoline 5-oxide (Compound NO-3)

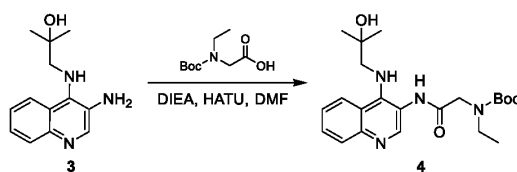


Step 1: 2-methyl-1-[(3-nitro-4-quinolyl)amino]propan-2-ol

To a solution of 4-chloro-3-nitroquinoline (5 g, 23.97 mmol, 1 eq) and TEA (1.60 g, 15.81 mmol, 2.20 mL, 0.66 eq) in DCM (80 mL) was added 1-amino-2-methylpropan-2-ol (2.33 g, 26.18 mmol, 1.09 eq), then the mixture was stirred at 20°C for 2 h. The reaction was concentrated under reduced pressure to give 2-methyl-1-[(3-nitro-4-quinolyl)amino]propan-2-ol (6.2 g, crude).

Step 2: 1-[(3-amino-4-quinolyl)amino]-2-methylpropan-2-ol

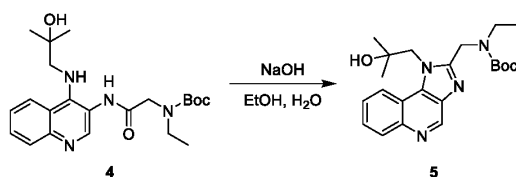
To a solution of 2-methyl-1-[(3-nitro-4-quinolyl)amino]propan-2-ol (6.2 g, 23.73 mmol, 1 eq) and NH₄Cl (12.69 g, 237.30 mmol, 10 eq) in EtOH (60 mL) and H₂O (6 mL) was added Fe (6.63 g, 118.65 mmol, 5 eq). The mixture was stirred at 80 °C for 3 h. The reaction was filtered and concentrated under reduced pressure, then added water (200 mL), then extracted with ethyl acetate (3*200 mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give 1-[(3-amino-4-quinolyl)amino]-2-methylpropan-2-ol (5.5 g, crude).

Step 3: tert-butyl N-[2-[(3-amino-4-quinolyl)-(2-hydroxy-2-methyl-propyl)amino]-2-oxo-ethyl]-N-ethyl-carbamate

To a solution of 1-[(3-amino-4-quinolyl)amino]-2-methylpropan-2-ol (4.4 g, 19.02 mmol, 1 eq) and 2-tert-butoxycarbonyl(ethyl)aminoacetic acid (4.64 g, 22.83 mmol, 1.2 eq) in DMF (100 mL) was added DIEA (4.92 g, 38.05 mmol, 6.63 mL, 2 eq) and HATU (7.96 g, 20.93 mmol, 1.1 eq), then the mixture was stirred at 20°C for

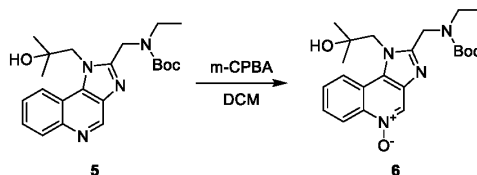
12 h. The reaction was added into water (100 mL), then extracted with ethyl acetate (3*100 mL). The combined organic layers were washed with brine (3*150 mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give tert-butyl N-[2-[(3-amino-4-quinolyl)-(2-hydroxy-2-methyl-propyl)amino]-2-oxo-ethyl]-N-ethyl l-carbamate (7.9 g, crude).

Step 4: tert-butyl N-ethyl-N-[[1-(2-hydroxy-2-methyl-propyl)imidazo[4,5-c]quinolin-2-yl]methyl]carbamate



To a solution of tert-butyl N-[2-[(3-amino-4-quinolyl)-(2-hydroxy-2-methyl-propyl)amino]-2-oxo-ethyl]-N-ethyl l-carbamate (7.90 g, 18.97 mmol, 1 eq) in EtOH (100 mL) was added a solution of NaOH (2.66 g, 66.38 mmol, 3.5 eq) in H₂O (10 mL), then the mixture was stirred at 80°C for 2 h. The reaction was added into saturated sodium thiosulfate aqueous solution (100 mL), then extracted with dichloromethane (3*100 mL). The combined organic layers were washed with saturated sodium bicarbonate solution (3*150 mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give a residue. The crude product (1.8 g scale) was combined to purify. The residue was purified by column chromatography to give tert-butyl N-ethyl-N-[[1-(2-hydroxy-2-methyl-propyl)imidazo[4,5-c]quinolin-2-yl]methyl]carbamate (6.4 g, crude).

Step 5: tert-butyl N-ethyl-N-[[1-(2-hydroxy-2-methyl-propyl)-5-oxido-imidazo[4,5-c]quinolin-5-ium-2-yl]methyl]carbamate

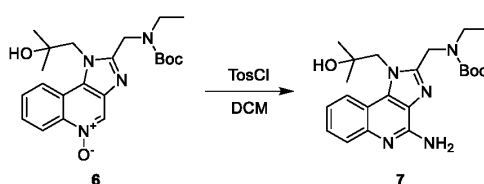


To a solution of tert-butyl N-ethyl-N-[[1-(2-hydroxy-2-methyl-propyl)imidazo[4,5-c]quinolin-2-yl]methyl]carbamate (6.4 g, 16.06 mmol, 1 eq) in DCM (100 mL) was added m-CPBA (4.89 g, 24.09 mmol, 85% purity, 1.5 eq) in portions at 0°C, then the mixture was stirred at 20°C for

12 h. The reaction was added into saturated sodium thiosulfate aqueous solution (100 mL), then extracted with dichloromethane (3*100 mL). The combined organic layers were washed with saturated sodium bicarbonate solution (3*150 mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography to give tert-butyl

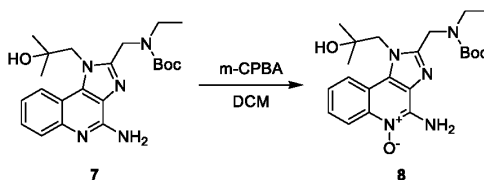
N-ethyl-N-[[1-(2-hydroxy-2-methyl-propyl)-5-oxido-imidazo[4,5-c]quinolin-5-ium-2-yl]methyl]carbamate (4.15 g, 10.01 mmol, 62.34% yield).

Step 6: tert-butyl N-[[4-amino-1-(2-hydroxy-2-methyl-propyl)imidazo[4,5-c]quinolin-2-yl]methyl]-N-ethyl-carbamate



Eight reactions were carried out in parallel. To a solution of tert-butyl N-ethyl-N-[[1-(2-hydroxy-2-methyl-propyl)-5-oxido-imidazo[4,5-c]quinolin-5-ium-2-yl]methyl]carbamate (500 mg, 1.21 mmol, 1 eq) in DCM (15 mL) and NH₃.H₂O (5 mL) was added TosCl (459.95 mg, 2.41 mmol, 2 eq), then the mixture was stirred at 20°C for 12 h. Eight reactions were combined. The reaction was added water (50 mL), then extracted with dichloromethane (3*50 mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography to give tert-butyl N-[[4-amino-1-(2-hydroxy-2-methyl-propyl)imidazo[4,5-c]quinolin-2-yl]methyl]-N-ethyl-carbamate (3.77 g, 9.12 mmol, 94.47% yield).

Step 7: tert-butyl N-[[4-amino-1-(2-hydroxy-2-methyl-propyl)-5-oxido-imidazo[4,5-c]quinolin-5-ium-2-yl]methyl]-N-ethyl-carbamate

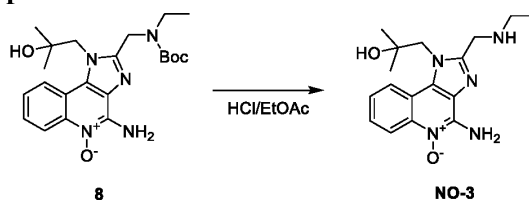


Four reactions were carried out in parallel. To a solution of tert-butyl N-[[4-amino-1-(2-hydroxy-2-methyl-propyl)imidazo[4,5-c]quinolin-2-yl]methyl]-N-ethyl-carbamate (250 mg, 604.58 μmol, 1 eq) in DCM (3 mL) was added m-CPBA (135.01 mg, 665.04 μmol, 85% purity, 1.1 eq), then the mixture was stirred at 0°C for

1 h. The crude product (100 mg scale) was combined to work up. Four reactions were combined. The reaction was added into saturated sodium thiosulfate aqueous solution (20 mL), then extracted with ethyl acetate (3*20 mL). The combined organic layers were washed with saturated sodium bicarbonate solution (3*20 mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography to give

tert-butyl N-[[4-amino-1-(2-hydroxy-2-methyl-propyl)-5-oxido-imidazo[4,5-c]quinolin-5-ium-2-yl]methyl]-N-ethyl-carbamate (275 mg, 640.26 μ mol, 26.48% yield).

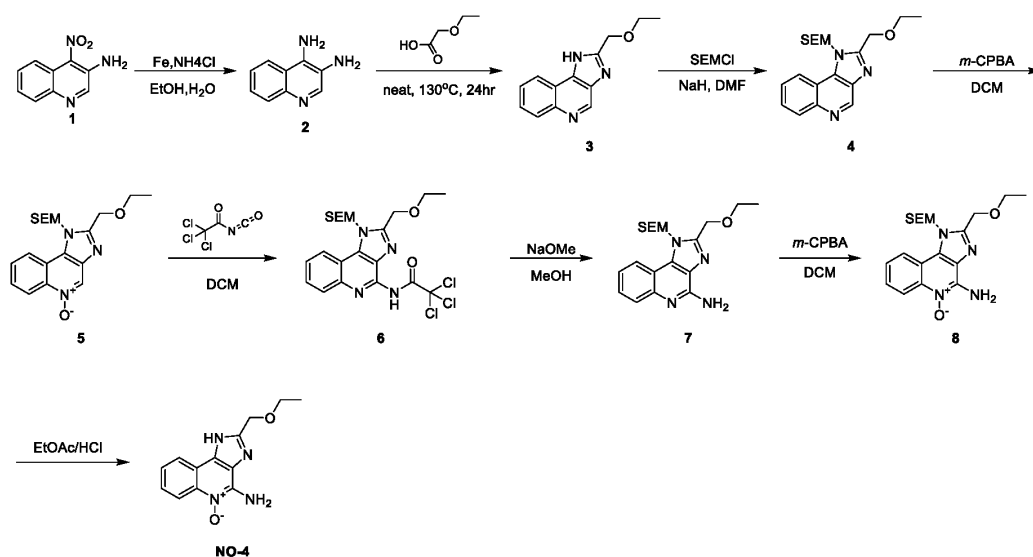
Step 8: 1-[4-amino-2-(ethylaminomethyl)-5-oxido-imidazo[4,5-c]quinolin-5-ium-1-yl]-2-methyl-propan-2-ol



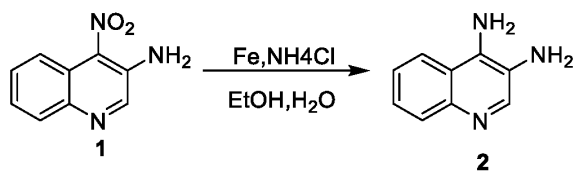
A solution of tert-butyl N-[[4-amino-1-(2-hydroxy-2-methyl-propyl)-5-oxido-imidazo[4,5-c]quinolin-5-ium-2-yl]methyl]-N-ethyl-carbamate (275 mg, 640.26 μ mol, 1 eq) in HCl/EtOAc (5 mL) and was stirred at 20°C for 1 h. The reaction was concentrated under reduced pressure to give a residue. The residue was purified by *prep*-HPLC to give NO-3 (103.1 mg, 272.26 μ mol, 42.52% yield, HCl). ^1H NMR (400 MHz, METHANOL- d_4) δ = 8.55 (d, J = 8.1 Hz, 1H), 8.22 - 8.16 (m, 1H), 7.87 (t, J = 7.7 Hz, 1H), 7.74 - 7.65 (m, 1H), 4.84 (br s, 4H), 3.38 (q, J = 7.3 Hz, 2H), 1.46 (t, J = 7.3 Hz, 3H), 1.32 (br s, 6H).

Example 4

4-amino-2-(ethoxymethyl)-1H-imidazo[4,5-c]quinoline 5-oxide (Compound NO-4)

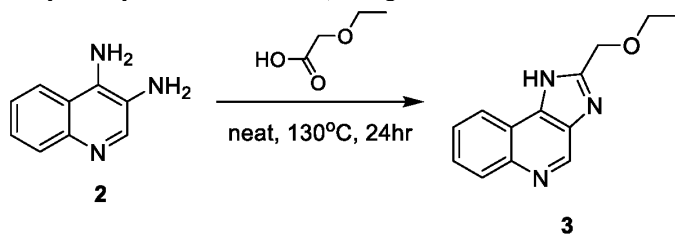


Step 1: quinoline-3,4-diamine



To a solution of 4-nitroquinolin-3-amine (5 g, 26.43 mmol, 1 eq) in EtOH (20 mL), H₂O (2 mL) was added Fe (7.38g, 132.16 mmol, 5 eq), NH₄Cl (14.14 g, 264.31 mmol, 10 eq), then the mixture was stirred at 80°C for 3 h. LCMS showed starting material was complete mostly and the desired ms was detected. The reaction mixture was filtered and the filtrate concentrated to give the crude product. The reaction mixture was added to water (50 mL), extracted with EtOAc (200 mL*3). The combined organic layers were washed with 200 mL of brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure to give quinoline-3,4-diamine (4.9 g, crude).

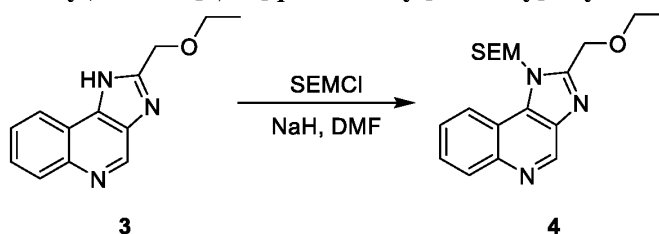
Step 2: 2-(ethoxymethyl)-1H-imidazo[4,5-c]quinoline



To a solution of quinoline-3,4-diamine (4.9 g, 30.78 mmol, 1 eq) in 2-ethoxyacetic acid (23.55 g, 226.24 mmol, 21.37mL, 7.35 eq). The mixture was stirred at 140°C for 12 h. LCMS showed starting material was complete mostly and the desired ms was detected. The reaction mixture was quenched by sat. NaHCO₃ aq.(200 mL), extracted with EtOAc (500 mL*3). The organic layer was dried over Na₂SO₄, filtered and the filtrate concentrated to give the crude product. The residue was purified by column chromatography (Plate 1,SiO₂, petroleum ether:ethyl acetate=0:1) to give 2-(ethoxymethyl)-1H-imidazo[4,5-c]quinoline (5.1 g, crude) was obtained.

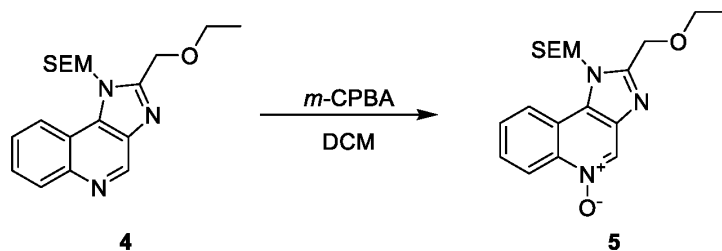
Step _____ **3:**

2-[[2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]methoxy]ethyl-trimethylsilane



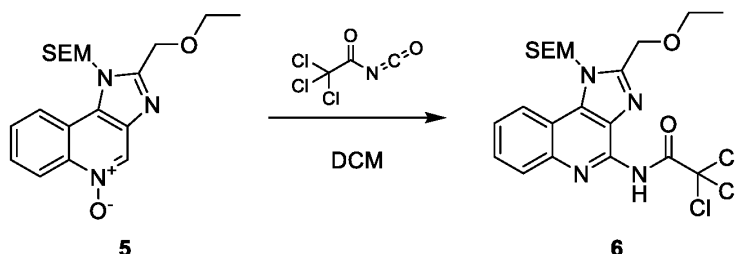
To a solution of 2-(ethoxymethyl)-1H-imidazo[4,5-c]quinoline (4.6 g, 20.24 mmol, 1 eq)in DMF (50 mL)was added NaH (971.48 mg, 24.29 mmol, 60% purity, 1.2 eq) at 0°C for 0.5h. 2-(chloromethoxy)ethyl-trimethyl-silane (3.71 g,22.27 mmol, 3.94 mL, 1.1 eq) was added. The mixture was stirred at 25°C for 0.5 h. LCMS showed starting was consumed completely and the desired ms was detected. The reaction was concentrated under reduced pressure, then added into water (100 mL), then extracted with ethyl acetate (3*300 mL). The combined organic layers were washed with brine (3*500mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, petroleum ether/ethyl acetate = 1/0 to 1/1) to give 2-[[2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]methoxy]ethyl-trimethyl-silane (6.4 g, crude).

Step 4: 2-[[2-(ethoxymethyl)-5-oxido-imidazo[4,5-c]quinolin-5-ium-1yl]methoxy]ethyltrimethyl-silane



To a solution of 2-[[2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]methoxy]ethyl-trimethyl-silane (6.4 g, 17.90 mmol, 1 eq) in DCM (70 mL) was added *m*-CPBA (5.45 g, 26.85 mmol, 85% purity, 1.5 eq) at 0°C, then the mixture was stirred at 20°C for 9 h. LCMS showed starting was consumed completely and the desired ms was detected. The reaction was added saturated sodium thiosulfate aqueous solution (100 mL), then extracted with ethyl acetate (3*300 mL). The combined organic layers were washed with saturated sodium bicarbonate solution (3*300 mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, ethyl acetate:Methanol=5:1) to give 2-[[2-(ethoxymethyl)-5-oxido-imidazo[4,5-c]quinolin-5-ium-1-yl]methoxy]ethyl-trimethyl-silane (8 g, crude) was obtained.

Step 5: 2,2,2-trichloro-N-[2-(ethoxymethyl)-1-(2-trimethylsilylethoxymethyl)imidazo[4,5-c]quinolin-4-yl]acetamide



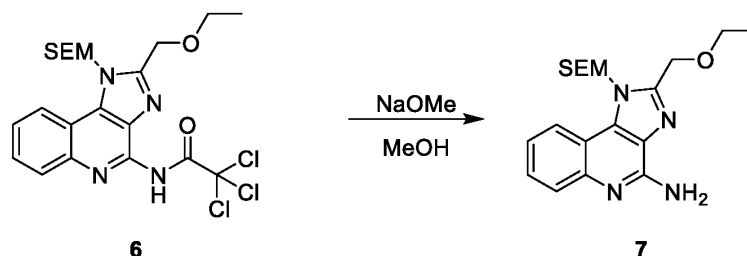
To a mixture of 2-[[2-(ethoxymethyl)-5-oxido-imidazo[4,5-c]quinolin-5-ium-1-yl]methoxy]ethyl-trimethyl-silane (2 g, 5.35 mmol, 1 eq) in DCM (30 mL) was added 2,2,2-trichloroacetyl isocyanate (1.21 g, 6.43 mmol, 762.29 μL , 1.2 eq) at 0°C, then the mixture was stirred at 25°C for 2 hrs. LCMS showed the starting material was complete mostly and the desired ms was detected. The 3 batches of the reaction mixture were mixed together for work up. The reaction mixture was filtered and the filtrate concentrated to give 2,2,2-trichloro-N-[2-(ethoxymethyl)-1-(2-trimethyl

ylsilylethoxymethyl)imidazo[4,5-c]quinolin-4-yl]acetamide (8 g, crude).

Step 6:

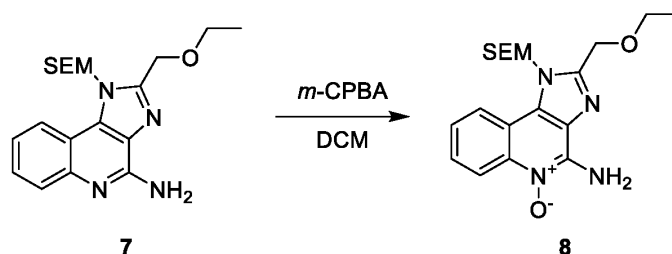
2-(ethoxymethyl)-1-(2-trimethylsilylethoxymethyl)imidazo[4,5-c]quinolin-4-amine

e



To a mixture of 2,2,2-trichloro-N-[2-(ethoxymethyl)-1-(2-trimethylsilylethoxymethyl)imidazo[4,5-c]quinolin-4-yl]acetamide (4 g, 7.72 mmol, 1 eq) in MeOH (60 mL) was added sodium methanolate (2.09 g, 11.59 mmol, 30% purity, 1.5 eq) at 25°C, then the mixture was stirred at 25°C for 1 hr. LCMS showed the starting material was remained and desired ms was detected. Then the mixture was stirred at 25°C for 12 hrs. LCMS showed the starting material was complete mostly and the desired ms was detected. The reaction mixture was filtered and the filtrate concentrated to give the crude product. The crude product was added to water (30 mL), extracted with EtOAc (100 mL*3). The combined organic layers were washed with 200mL of brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The crude product was purified by column chromatography on silica gel (Petroleum ether: Ethyl acetate = 1:0-1:1) to give 2-(ethoxymethyl)-1-(2-trimethylsilylethoxymethyl)imidazo[4,5-c]quinolin-4-amine (3.4 g, crude).

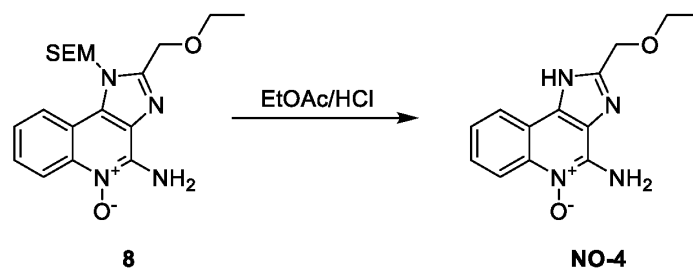
Step 7: 2-(ethoxymethyl)-5-oxido-1-(2-trimethylsilylethoxymethyl)imidazo[4,5-c]quinolin-5-ium-4-amine



To a mixture of

2-(ethoxymethyl)-1-(2-trimethylsilylethoxymethyl)imidazo[4,5-c]quinolin-4-amine (2.5 g, 6.71 mmol, 1 eq) in DCM (30 mL) was added m-CPBA (1.36 g, 6.71 mmol, 85% purity, 1 eq) at 0°C, then the mixture was stirred at 0°C for 0.5 hr. LCMS showed about 38% of the starting material was remained and the desired ms(21%) was detected. The reaction mixture was quenched by sat. Na₂S₂O₃ aq.(50mL), extracted with EtOAc (150 mL*3). The combined organic layers were washed with 200 mL of sat. NaHCO₃ aq., dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography on silica gel(Petroleum ether : Ethyl acetate = 0:1 to Ethyl acetate : Methanol=1:1) to give 2-(ethoxymethyl)-5-oxido-1-(2-trimethylsilylethoxymethyl)imidazo[4,5-c]quinolin-5-ium-4-amine (1.8 g, crude).

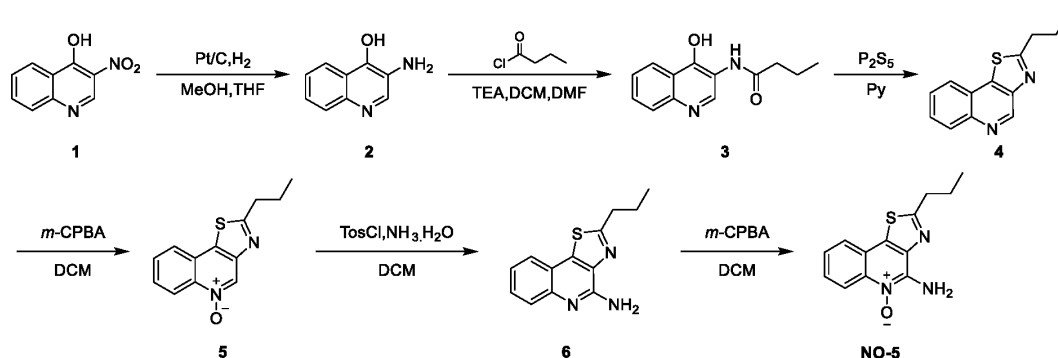
Step 8: 2-(ethoxymethyl)-5-oxido-1H-imidazo[4,5-c]quinolin-5-ium-4-amine



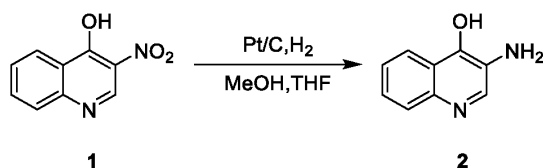
A mixture of 2-(ethoxymethyl)-5-oxido-1-(2-trimethylsilylethoxymethyl)imidazo[4,5-c]quinolin-5-ium-4-amine (500 mg, 1.29 mmol, 1 eq) in EtOAc/HCl (5 mL) at 25°C, then the mixture was stirred at 25°C for 2 hrs. LCMS showed the starting material was complete mostly and the desired ms was detected. The mixture was filtered and the filter cake was dried in vacuum. To give NO-4 (57.2 mg, crude, HCl). ¹H NMR (400 MHz, METHANOL-d₄) δ = 8.28 (d, J = 8.0 Hz, 1H), 8.11 (d, J = 8.6 Hz, 1H), 7.87 - 7.81 (m, 1H), 7.68 - 7.63 (m, 1H), 4.83 (s, 2H), 3.72 (q, J = 7.0 Hz, 2H), 1.31 (t, J = 7.0 Hz, 3H).

Example 5

4-amino-2-propylthiazolo[4,5-c]quinoline 5-oxide (Compound NO-5)

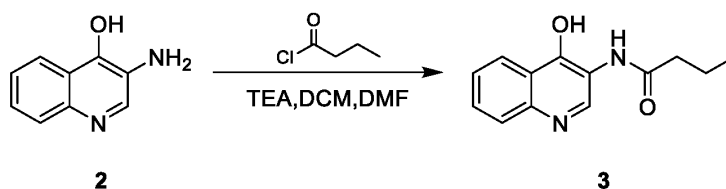


Step 1: Synthesis of 3-aminoquinolin-4-ol



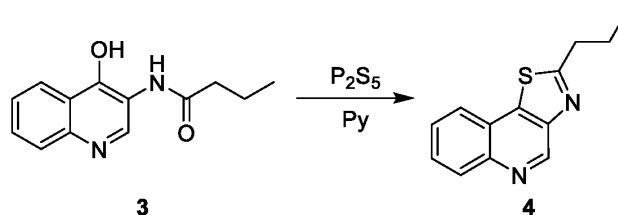
To a solution of 3-nitroquinolin-4-ol (25 g, 131.47 mmol, 1 eq) in MeOH (250 mL) and THF (250 mL) was added TEA (40 g, 395.30 mmol, 55.02 mL, 3.01 eq) and Pt/C (2.5 g, 1% purity). It was stirred at 60°C for 3.3 min under H₂(1 MPa)(Flow Chemistry). It was filtered and the filtrate was concentrated under reduced pressure to give 3-aminoquinolin-4-ol (21 g, crude).

Step 2: Synthesis of N-(4-hydroxy-3-quinolyl)butanamide



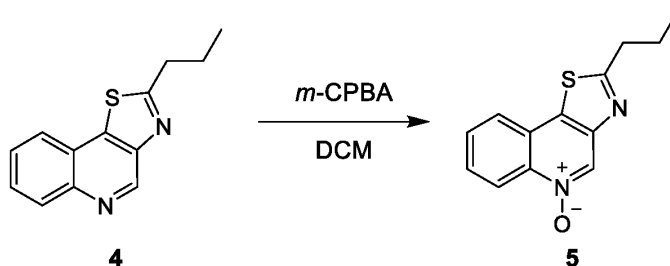
To a solution of 3-aminoquinolin-4-ol (5 g, 31.22 mmol, 1 eq) in DCM (500 mL) and DMF (50 mL) was added TEA (3.16 g, 31.22 mmol, 4.34 mL, 1 eq), then it was added dropwise butanoyl chloride (4.99 g, 46.82 mmol, 4.90 mL, 1.5 eq) at 0°C. It was stirred at 0°C for 1.5 h. It was concentrated under reduced pressure and then purified by flash chromatography (silica gel, Dichloromethane : Methanol= 1/0 to 20/1) to give N-(4-hydroxy-3-quinolyl)butanamide (5 g, crude).

Step 3: Synthesis of 2-propylthiazolo[4,5-c]quinoline



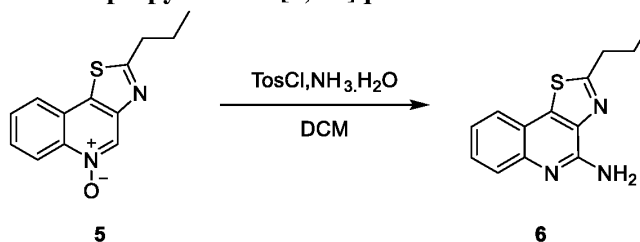
To a solution of N-(4-hydroxy-3-quinolyl)butanamide (7 g, 30.40 mmol, 1 eq) in Py (350 mL) was added P_2S_5 (6.76 g, 30.40 mmol, 3.23 mL, 1 eq). It was stirred at 120°C for 3 h. It was concentrated under reduced pressure, then added into water (500 mL), then extracted with ethyl acetate (3 × 300 mL). The combined organic layers were dried over anhydrous sodium sulfate. It was filtered and the filtrate was concentrated under reduced pressure and then purified by flash chromatography (silica gel, petroleum ether/ethyl acetate = 1/0 to 3/1) to give 2-propylthiazolo[4,5-c]quinoline (7 g, crude).

Step 4: Synthesis of 2-propylthiazolo[4,5-c]quinoline



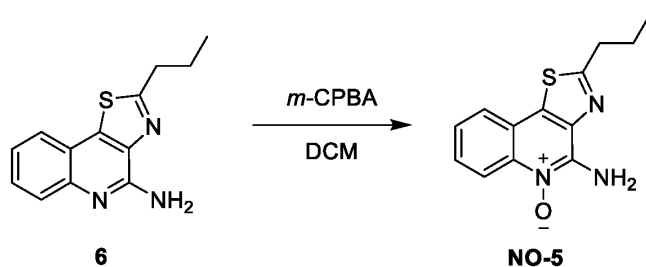
To a solution of N-(4-hydroxy-3-quinolyl)butanamide (7 g, 30.40 mmol, 1 eq) in Py (350 mL) was added P_2S_5 (6.76 g, 30.40 mmol, 3.23 mL, 1 eq). It was stirred at 120°C for 3 h. It was concentrated under reduced pressure, then added into water (500 mL), then extracted with ethyl acetate (3*300 mL). The combined organic layers were dried over anhydrous sodium sulfate. It was filtered and the filtrate was concentrated under reduced pressure and then purified by flash chromatography (silica gel, petroleum ether/ethyl acetate = 1/0 to 3/1) to give 2-propylthiazolo[4,5-c]quinoline (7 g, crude)

Step 5: Synthesis of 2-propylthiazolo[4,5-c]quinolin-4-amine



To a solution of 5-oxido-2-propyl-thiazolo[4,5-c]quinolin-5-ium (0.3 g, 1.23 mmol, 1 eq) in DCM (5 mL) was added TosCl (468.21 mg, 2.46 mmol, 2 eq) and NH₃.H₂O (5 mL). It was stirred at 20°C for 1 h. The organic layers were concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (neutral condition, column: Waters Xbridge BEH C18 100*30mm*10 um; mobile phase: [H₂O (10 mM NH₄HCO₃)-ACN];gradient:43%-73% B over 8.0 min) to give 2-propylthiazolo[4,5-c]quinolin-4-amine (20.5 mg, 82.26 μmol, 6.70% yield, 97.644% purity).

Step 6: Synthesis of 5-oxido-2-propyl-thiazolo[4,5-c]quinolin-5-ium-4-amine

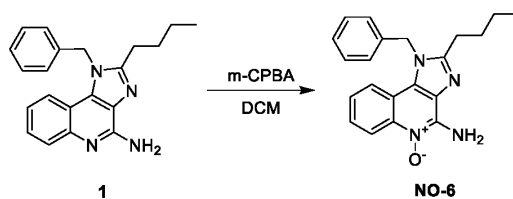


To a solution of 2-propylthiazolo[4,5-c]quinolin-4-amine (1 g, 4.11 mmol, 1 eq) in DCM (10 mL) was slowly added m-CPBA (1.50 g, 7.40 mmol, 85% purity, 1.8 eq) at 20°C. It was stirred at 20°C for 2 h. The reaction mixture was added into a mixture of sat. Na₂SO₃ (20 mL) and sat. NaHCO₃ (20 mL), then extracted with DCM (3 × 20 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (neutral condition, column: Waters Xbridge BEH C18 250*50mm*10 um; mobile phase: [H₂O (10 mM NH₄HCO₃)-ACN];gradient:25%-65% B over 10.0 min) to give 5-oxido-2-propyl-thiazolo[4,5-c]quinolin-5-ium-4-amine (62.5 mg, 239.90 μmol, 5.84% yield). LCMS m/z 260.0 [M+H]⁺ 1H NMR (400 MHz, DMSO-d₆) δ = 8.45 (d, J = 8.4 Hz, 1H), 7.99 (d, J = 7.6 Hz, 1H), 7.74 (br t, J = 7.3 Hz, 3H), 7.51 (t, J = 7.3 Hz, 1H), 3.19 (t, J = 7.5 Hz, 2H), 1.93 - 1.85 (m, 2H), 1.03 (t, J = 7.3 Hz, 3H).

Example 6

4-amino-1-benzyl-2-butyl-1H-imidazo[4,5-c]quinoline 5-oxide (Compound NO-6)

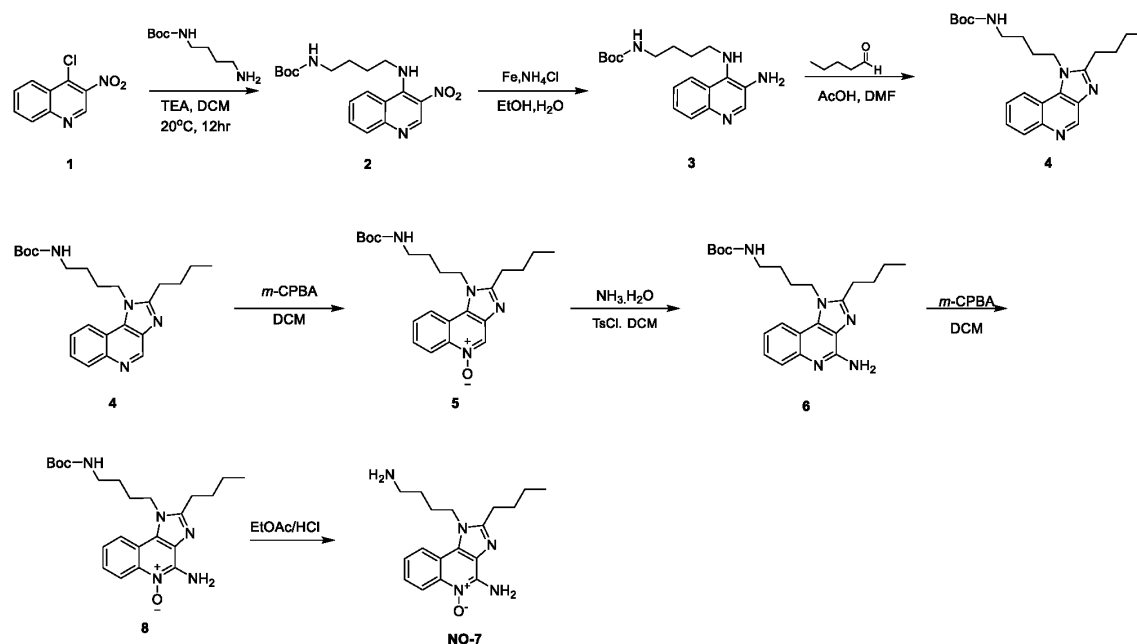
Step 1: 1-benzyl-2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-4-amine

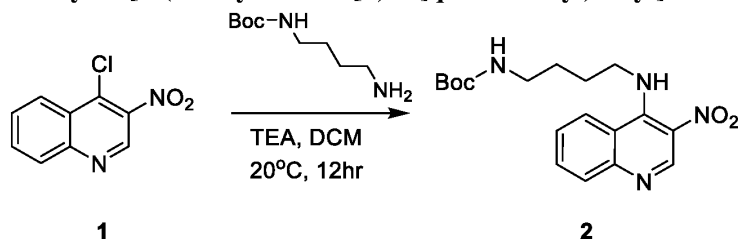


Four reactions were carried out in parallel. To a solution of 1-benzyl-2-butyl-imidazo[4,5-c]quinolin-4-amine (110 mg, 332.90 μmol , 1 eq) in DCM (3 mL) was added m-CPBA (101.38 mg, 499.36 μmol , 85% purity, 1.5 eq) at 0°C. The mixture was stirred at 20°C for 12 h. Four reactions were combined. The reaction was added into saturated sodium thiosulfate aqueous solution (1 mL), then concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC to give NO-6 (53.3 mg, 150.30 μmol , 11.29% yield) was obtained. ^1H NMR (400 MHz, DMSO- d_6) δ = 8.50 (d, J = 8.6 Hz, 1H), 7.92 (d, J = 8.3 Hz, 1H), 7.72 - 7.48 (m, 3H), 7.34 - 7.23 (m, 4H), 7.04 (br d, J = 7.5 Hz, 2H), 5.92 (s, 2H), 2.94 (br t, J = 7.6 Hz, 2H), 1.71 (quin, J = 7.6 Hz, 2H), 1.37 (sxt, J = 7.4 Hz, 2H), 0.85 (t, J = 7.3 Hz, 3H)

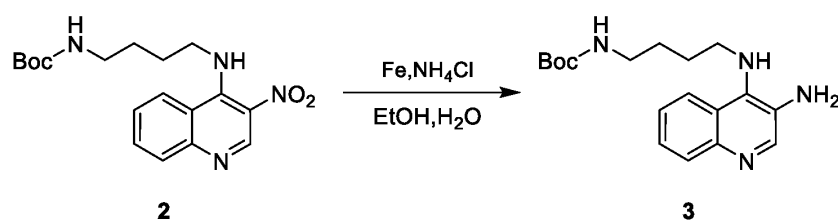
Example 7

4-amino-1-(4-aminobutyl)-2-butyl-1H-imidazo[4,5-c]quinoline 5-oxide
(Compound NO-7)



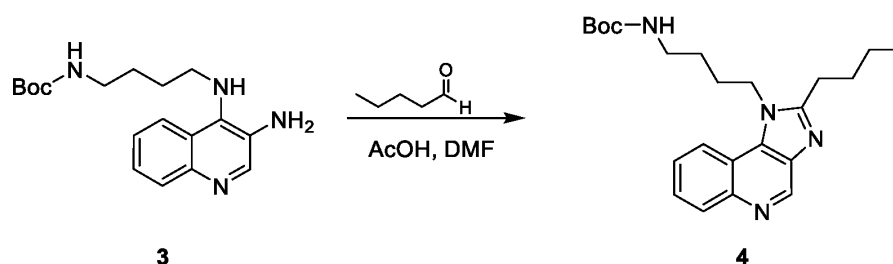
Step 1: tert-butyl N-[4-(2-butylimidazo[4,5-c]quinolin-1-yl)butyl]carbamate

To a solution of 4-chloro-3-nitro-quinoline (10 g, 47.94 mmol, 1 eq) in DCM (200 mL) was added TEA (3.20 g, 31.64 mmol, 4.40 mL, 0.66 eq.) and tert-butyl N-(4-aminobutyl)carbamate (9.93 g, 52.73 mmol, 1.1 eq). The mixture was stirred at 25°C for 2hr. LCMS showed starting material remained and the desired ms was detected. Then the mixture was stirred at 25°C for 12 hr. LCMS showed starting material was complete mostly and the desired ms was detected. The reaction was filtered and the filtrate concentrated under reduced pressure to give tert-butyl N-[4-[(3-nitro-4-quinolyl)amino]butyl]carbamate (17 g, crude) was obtained.

Step 2: tert-butyl N-[4-[(3-amino-4 quinolyl)amino]butyl]carbamate

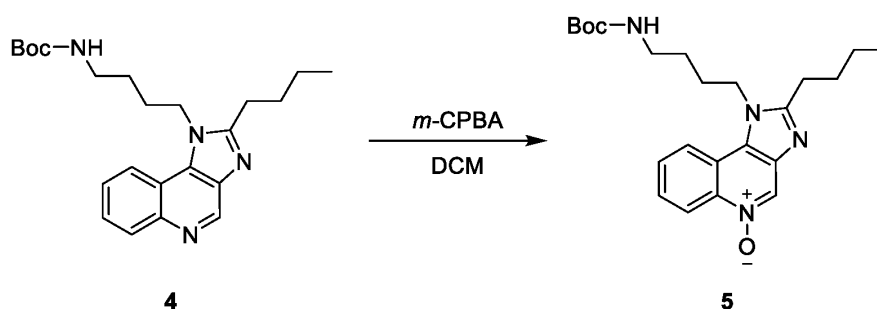
To a solution of tert-butyl N-[4-[(3-nitro-4-quinolyl)amino]butyl]carbamate (7 g, 19.42 mmol, 1 eq) in EtOH (500mL), H₂O (50 mL) was added Fe (54.23 g, 97.11 mmol, 10% purity, 5 eq) and NH₄Cl (10.39 g, 194.23 mmol, 10 eq). The mixture was stirred at 80°C for 12 hr. LCMS showed the reaction was complete and the desired ms was detected. The reaction mixture was filtered and the filtrate concentrated under reduced pressure to give a residue. The residue was added to water (200 mL), extracted with EtOAc (500 mL*3). The combined organic layers were washed with 1000 mL of brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a crude product. The crude product was purified by column chromatography on silica gel (SiO₂, Plate1, Petroleum ether: Ethyl acetate= 0:1) to give tert-butyl N-[4-[(3-amino-4 quinolyl)amino]butyl]carbamate (6.4 g, crude) was obtained.

Step 3: **tert-butyl N-[4-(2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-1-yl)butyl]carbamate**



To a solution of tert-butyl N-[4-[(3-amino-4-quinolyl)amino]butyl]carbamate (6.4 g, 19.37 mmol, 1 eq) and pentanal (2.00 g, 23.24 mmol, 2.47 mL, 1.2 eq) in DMF (100 mL) was added AcOH (116.32 mg, 1.94 mmol, 110.88 μ L, 0.1eq). The mixture was stirred at 85°C for 12 h. LCMS showed starting material was complete mostly and the desired ms was detected. The reaction was concentrated under reduced pressure, then added into water (150 mL), then extracted with ethyl acetate (3*300 mL). The combined organic layers were washed with brine (3*500 mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (Plate 1, SiO₂, petroleum ether/ethyl acetate = 0/1) to give tert-butyl N-[4-(2-butylimidazo[4,5-c]quinolin-1-yl)butyl]carbamate (5.8 g, crude) was obtained.

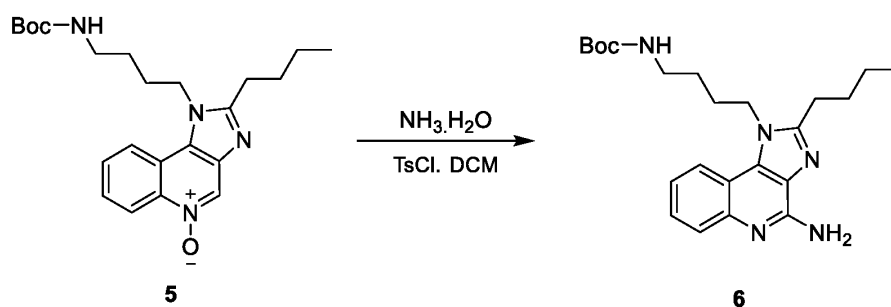
Step 4: tert-butyl
N-[4-(2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-1yl)butyl]carbamate



To a solution of tert-butyl N-[4-(2-butylimidazo[4,5-c]quinolin-1-yl)butyl]carbamate (5.8 g, 14.63 mmol, 1 eq) in DCM (100 mL) was added m-CPBA (4.45 g, 21.94 mmol, 85% purity, 1.5 eq) at 0°C. The mixture was stirred at 25°C for 12 h. LCMS showed starting material remained and the desired ms was detected. The reaction was added saturated sodium thiosulfate aqueous solution (200 mL), then extracted with ethyl acetate (3*600 mL).

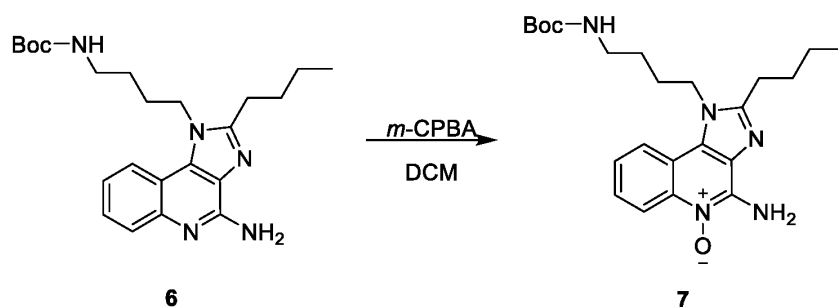
The combined organic layers were washed with saturated sodium bicarbonate solution(3*500mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give the residue. The residue was purified by column chromatography(SiO₂,ethyl acetate :Methanol=5:1) to give tert-butyl N-[4-(2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-1-yl)butyl]carbamate (1.98 g, crude).

Step _____ 5: **tert-butyl**
N-[4-(4-amino-2-butyl-imidazo[4,5-c]quinolin-1-yl)butyl]carbamate



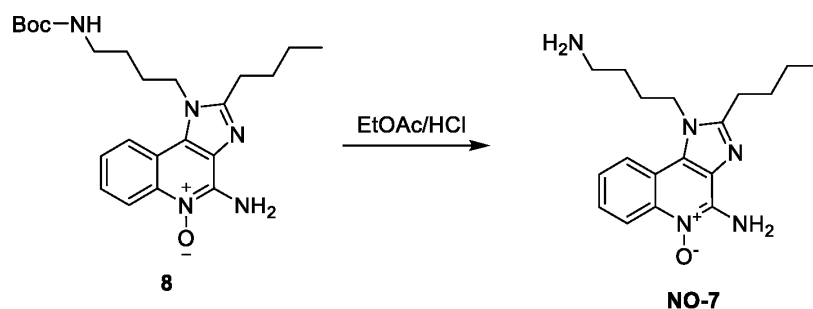
To a mixture of tert-butyl N-[4-(2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-1-yl)butyl]carbamate (300 mg, 727.23 μmol , 1 eq) in DCM (6 mL) was added TosCl (277.29 mg, 1.45 mmol, 2 eq), NH₃.H₂O (2 mL) at 25°C, then the mixture was stirred at 25°C for 10 hrs. LCMS showed about 24% of the starting material was remained and the desired ms(13%) was detected. The 6 batches of the reaction mixture were mixed together for work up. The reaction mixture was added to water (50 mL), extracted with EtOAc (200 mL*3). The combined organic layers were washed with 300 mL of brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The crude product was purified by prep-TLC (Petroleum ether: Ethyl acetate = 0:1) to give tert-butyl N-[4-(4-amino-2-butyl-imidazo[4,5-c]quinolin-1-yl)butyl]carbamate (800 mg, crude).

Step _____ 6: **tert-butyl**
N-[4-(4-amino-2-butyl-imidazo[4,5-c]quinolin-1-yl)butyl]carbamate



To a mixture of tert-butyl N-[4-(4-amino-2-butyl-imidazo[4,5-c]quinolin-1-yl)butyl]carbamate (500 mg, 1.21 mmol, 1 eq) in DCM (6 mL) was added m-CPBA (246.66 mg, 1.21 mmol, 85% purity, 1 eq) at 0°C, then the mixture was stirred at 0°C for 0.5 hr. LCMS showed about 27.4% of the starting material was remained and the desired ms(6.6%) was detected. The reaction mixture was quenched by sat. Na₂S₂O₃ aq.(10mL), extracted with EtOAc (50 mL*3). The combined organic layers were washed with 50mL of sat. NaHCO₃ aq., dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography on silica gel(Petroleum ether: Ethyl acetate = 0:1 to Ethyl acetate: Methanol=1:1) to give tert-butyl N-[4-(4-amino-2-butyl-imidazo[4,5-c]quinolin-1-yl)butyl]carbamate (176 mg, crude).

Step 7: 1-(4-aminobutyl)-2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-4-amine

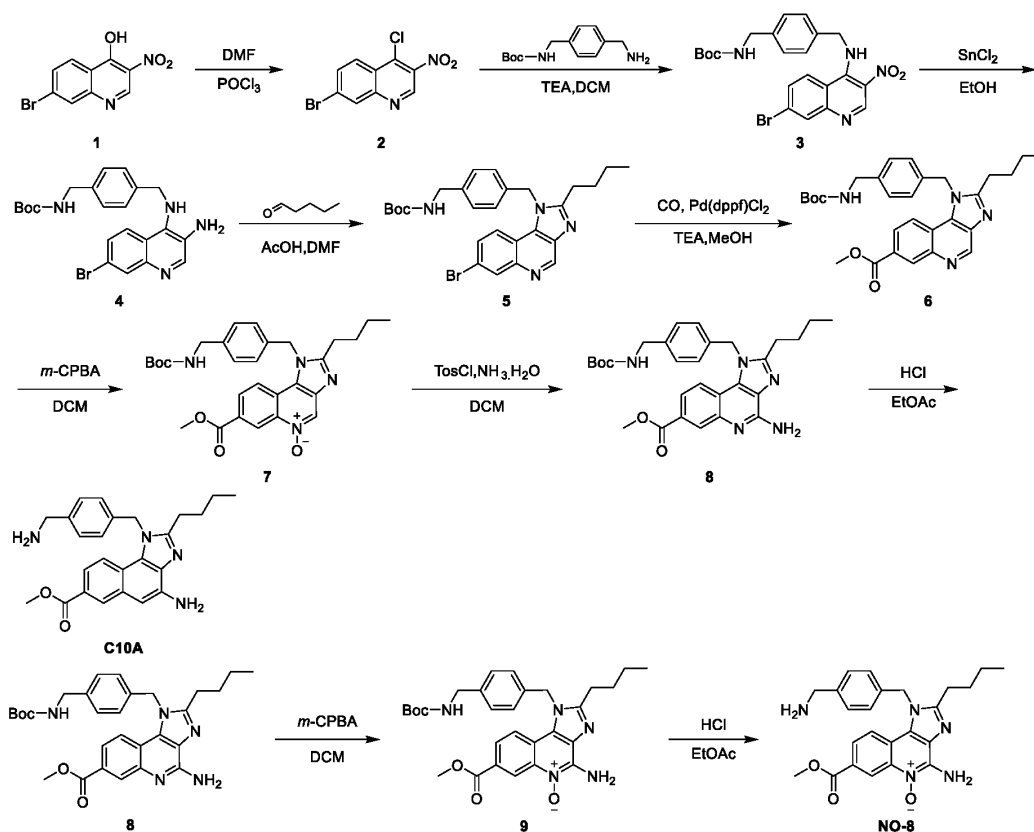


A mixture of tert-butyl N-[4-(4-amino-2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-1-yl)butyl]carbamate (300 mg, 701.69 μmol, 1 eq) in EtOAc/HCl (2 mL) at 25°C, then the mixture was stirred at 25°C for 0.5 hr. LCMS showed the starting material was complete mostly and the desired ms was detected. The reaction mixture was concentrated to give the crude product. The crude product was purified by prep-HPLC (HCl condition; column: Phenomenex luna C18 100*40mm*5 μm; mobile phase: [H₂O (0.04%

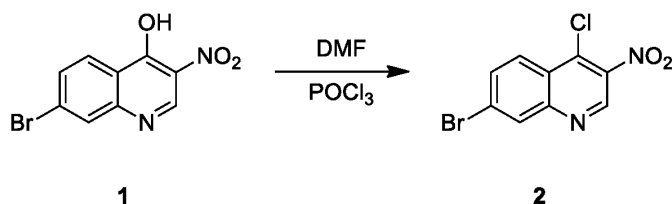
HCl)-ACN]; gradient: 1%-40% B over 8.0 min) to give NO-7 (67.3 mg, 297.91 μ mol, 42.46% yield). ^1H NMR (400 MHz, DMSO- d_6) δ = 9.39 (br s, 1H), 9.15 (br s, 1H), 8.27 (d, J = 8.1 Hz, 1H), 8.18 (br s, 2H), 8.10 (dd, J = 0.8, 8.6 Hz, 1H), 7.83 - 7.76 (m, 1H), 7.68 - 7.62 (m, 1H), 4.62 (br t, J = 7.2 Hz, 2H), 3.06 - 2.93 (m, 2H), 2.87 - 2.75 (m, 2H), 1.94 - 1.70 (m, 6H), 1.47 (qd, J = 7.4, 14.9 Hz, 2H), 0.96 (t, J = 7.4 Hz, 3H).

Example 8

4-amino-1-(4-(aminomethyl)benzyl)-2-butyl-7-(methoxycarbonyl)-1H-imidazo[4,5-c]
quinoline 5-oxide (Compound NO-8)

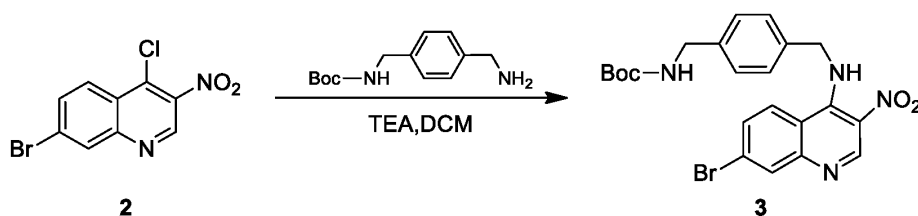


Step 1: Synthesis of methyl 7-bromo-4-chloro-3-nitro-quinoline



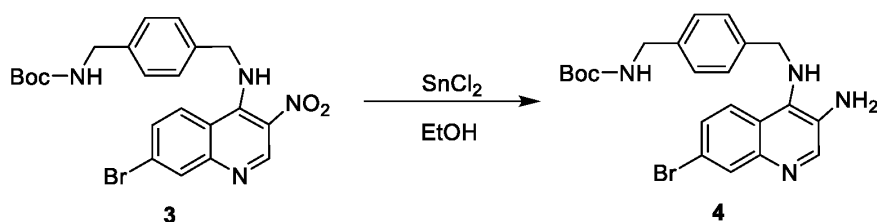
To a solution of 7-bromo-3-nitro-quinolin-4-ol (5 g, 18.58 mmol, 1 eq) in POCl₃ (15 mL) was added DMF (712.50 mg, 9.75 mmol, 0.75 mL, 5.25e-1 eq). It was stirred at 85°C for 12 h. It was filtered to give 7-bromo-4-chloro-3-nitro-quinoline (4 g, crude).

Step 2: Synthesis of tert-butyl N-[[4-[(7-bromo-3-nitro-4-quinolyl)amino]methyl]phenyl]methyl]carbamate



To a solution of 7-bromo-4-chloro-3-nitro-quinoline (3.98 g, 13.84 mmol, 1 eq) in DCM (80 mL) was added TEA (1.40 g, 13.84 mmol, 1.93 mL, 1 eq) and tert-butyl N-[[4-(aminomethyl)phenyl]methyl]carbamate (3.57 g, 15.09 mmol, 1.09 eq) at 0°C. Then it was stirred at 25 °C for 12 h. It was concentrated under reduced pressure to give a residue. The residue was purified by flash chromatography (silica gel, petroleum ether/ethyl acetate = 1/0 to 0/1) to give tert-butyl N-[[4-[(7-bromo-3-nitro-4-quinolyl)amino]methyl]phenyl]methyl]carbamate (3.5 g, 7.18 mmol, 51.88% yield).

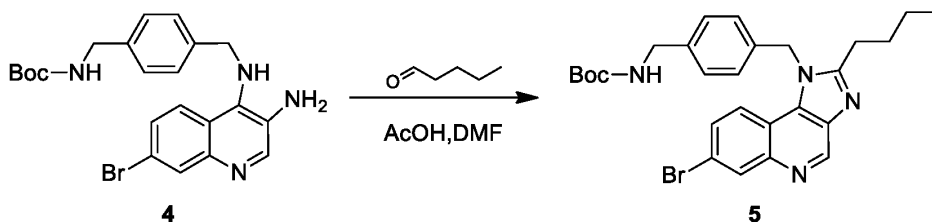
Step 3: Synthesis of tert-butyl N-[[4-[(3-amino-7-bromo-4-quinolyl)amino]methyl]phenyl]methyl]carbamate



To a solution of tert-butyl N-[[4-[(7-bromo-3-nitro-4-quinolyl)amino]methyl]phenyl]methyl]carbamate (2.9 g, 5.95 mmol, 1 eq) in EtOH (72 mL) was added SnCl₂ (3.72 g, 19.64 mmol, 3.3 eq). It

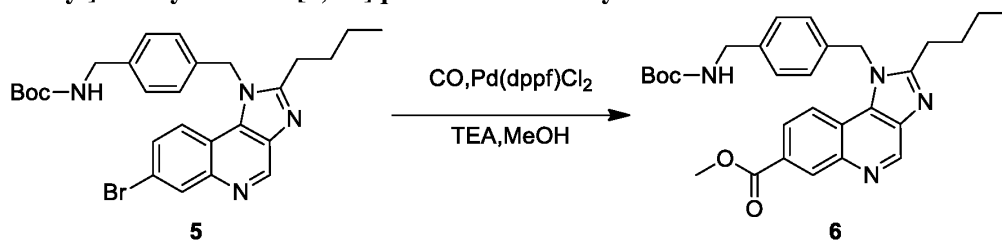
was stirred at 65 °C for 3 h. The reaction mixture was concentrated under reduced pressure, then added into saturated sodium bicarbonate solution (50 mL), then extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine (3 × 50 mL) and dried over anhydrous sodium sulfate. It was filtered and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by flash chromatography (silica gel, petroleum ether/ethyl acetate = 1/0 to 1/1) to give tert-butyl N-[[4-[(3-amino-7-bromo-4-quinolyl)amino]methyl]phenyl]methyl]carbamate (3.1 g, crude).

Step 4: Synthesis of tert-butyl N-[[4-[(7-bromo-2-butyl-imidazo[4,5-c]quinolin-1-yl)methyl]phenyl]methyl]carbamate



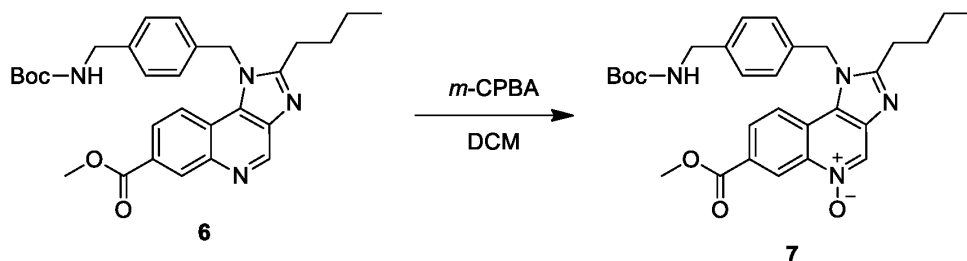
To a solution of pentanal (564.97 mg, 6.56 mmol, 697.49 μ L, 1.2 eq), tert-butyl N-[[4-[(3-amino-7-bromo-4-quinolyl)amino]methyl]phenyl]methyl]carbamate (2.5 g, 5.47 mmol, 1 eq) in DMF (25 mL) was added AcOH (32.83 mg, 546.61 μ mol, 31.29 μ L, 0.1 eq), then it was stirred at 85°C for 15 h. The reaction mixture was added into saturated sodium bicarbonate solution (50 mL), then extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with brine (3 × 20 mL) and dried over anhydrous sodium sulfate. It was filtered and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by flash chromatography (silica gel, petroleum ether/ethyl acetate = 1/0 to 1/1) to give tert-butyl N-[[4-[(7-bromo-2-butyl-imidazo[4,5-c]quinolin-1-yl)methyl]phenyl]methyl]carbamate (3.5 g, crude).

Step 5: Synthesis of methyl 1-[[4-[(tert-butoxycarbonylamino)methyl]phenyl]methyl]-2-butyl-imidazo[4,5-c]quinoline-7-carboxylate



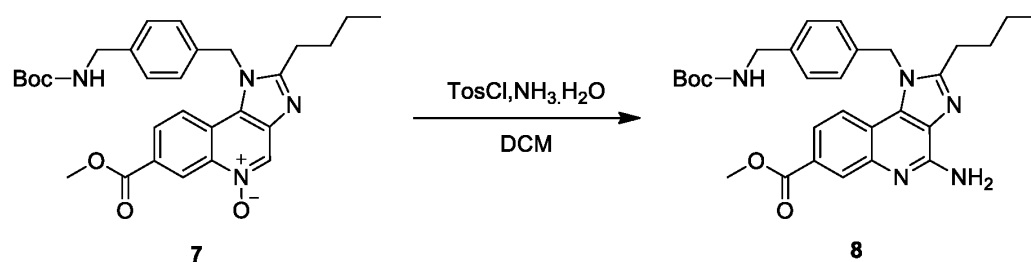
To a solution of tert-butyl N-[[4-[(7-bromo-2-butyl-imidazo[4,5-c]quinolin-1-yl)methyl]phenyl]methyl]carbamate (1.9 g, 3.63 mmol, 1 eq), TEA (1.84 g, 18.15 mmol, 2.53 mL, 5 eq) in MeOH (200 mL) was added Pd(dppf)Cl₂ (265.59 mg, 362.97 μmol, 0.1 eq). The suspension was degassed under vacuum and purged with CO (50 PSI) several times. It was stirred at 80 °C for 12 h under CO (50 PSI). It was concentrated under reduced pressure to give a residue. The residue was purified by flash chromatography (silica gel, petroleum ether/ethyl acetate =1:1) to give methyl 1-[[4-[(tert-butoxycarbonylamino)methyl]phenyl]methyl]-2-butyl-imidazo[4,5-c]quinoline-7-carboxylate (2 g, crude).

Step 6: Synthesis of methyl 1-[[4-[(tert-butoxycarbonylamino)methyl]phenyl]methyl]-2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-7-carboxylate



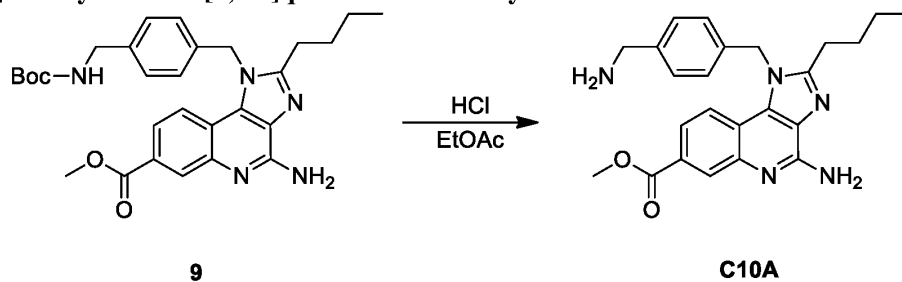
To a solution of methyl 1-[[4-[(tert-butoxycarbonylamino)methyl]phenyl]methyl]-2-butyl-imidazo[4,5-c]quinoline-7-carboxylate (2 g, 3.98 mmol, 1 eq) in DCM (40 mL) was added m-CPBA (1.21 g, 5.97 mmol, 85% purity, 1.5 eq) at 0 °C. Then it was stirred at 25 °C for 2 h. The reaction mixture was slowly added to 50 mL of sat. Na₂SO₃, washed with 30 mL of sat. NaHCO₃, then extracted with DCM (3 × 40 mL). The combined organic layers were washed with brine (3 × 20 mL) and dried over anhydrous sodium sulfate. It was filtered and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by flash chromatography (silica gel, petroleum ether/ethyl acetate = 3/1 to 0/1) to give methyl 1-[[4-[(tert-butoxycarbonylamino)methyl]phenyl]methyl]-2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-7-carboxylate (1.26 g, 2.43 mmol, 53.09% yield).

Step 7: Synthesis of methyl 4-amino-1-[[4-[(tert-butoxycarbonylamino)methyl]phenyl]methyl]-2-butyl-imidazo[4,5-c]quinoline-7-carboxylate



To a solution of methyl 1-[[4-[(tert-butoxycarbonylamino)methyl]phenyl]methyl]-2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-7-carboxylate (300 mg, 578.48 μmol , 1 eq) in DCM (5 mL) and $\text{NH}_3 \cdot \text{H}_2\text{O}$ (5 mL) was added TosCl (220.57 mg, 1.16 mmol, 2 eq). Then it was stirred at 20 °C for 1 h. The 4 batches of the reaction mixture were mixed together for work up. It was extracted with DCM (3 $\times$ 50 mL). The combined organic layers were washed with brine (3 $\times$ 50 mL) and dried over anhydrous sodium sulfate. It was filtered and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by flash chromatography (silica gel, petroleum ether/ethyl acetate = 1/0 to 1/1) to give methyl 4-amino-1-[[4-[(tert-butoxycarbonylamino)methyl]phenyl]methyl]-2-butyl-imidazo[4,5-c]quinoline-7-carboxylate (1.4 g, crude).

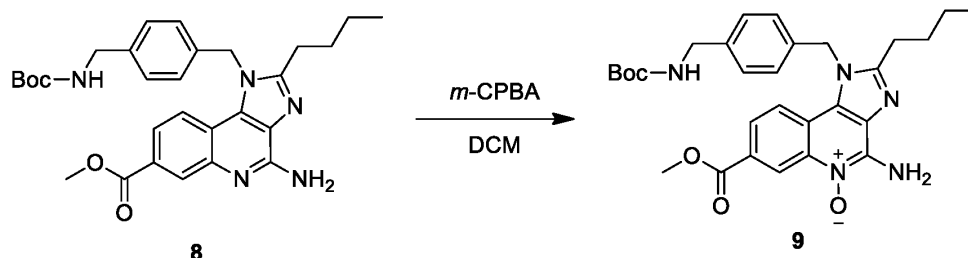
Step 8: Synthesis of methyl methyl 4-amino-1-[[4-(aminomethyl)phenyl]methyl]-2-butyl-imidazo[4,5-c]quinoline-7-carboxylate



A solution of methyl 4-amino-1-[[4-[(tert-butoxycarbonylamino)methyl]phenyl]methyl]-2-butyl-imidazo[4,5-c]quinoline-7-carboxylate (80 mg, 154.55 μmol , 1 eq) in HCl/EtOAc (3 mL) was stirred at 20 °C for 1 h. It was filtered to give a residue. The residue was purified by prep-HPLC (HCl condition, column: Phenomenex luna C18 100*40mm*5 μm ; mobile phase: [H_2O (0.04% HCl)- ACN]; gradient: 1%-45% B over 8.0 min) to give methyl

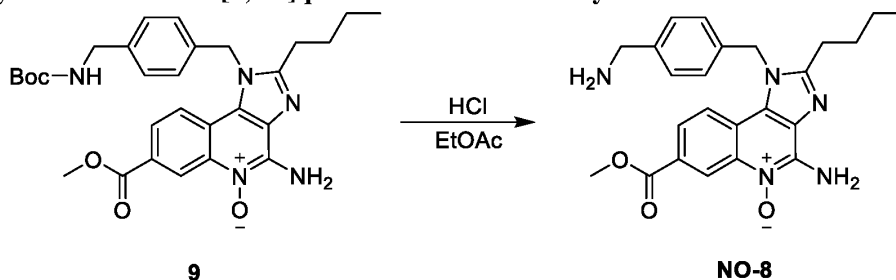
4-amino-1-[[4-(aminomethyl)phenyl]methyl]-2-butyl-imidazo[4,5-c]quinoline-7-carboxylate (17.2 mg, 37.58 μ mol, 24.31% yield, 99.183% purity, HCl).

Step 9: Synthesis of methyl 4-amino-1-[[4-[(tert-butoxycarbonylamino)methyl]phenyl]methyl]-2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-7-carboxylate



To a solution of methyl 4-amino-1-[[4-[(tert-butoxycarbonylamino)methyl]phenyl]methyl]-2-butyl-imidazo[4,5-c]quinoline-7-carboxylate (100 mg, 193.19 μ mol, 1 eq) in DCM (5 mL) was added m-CPBA (58.83 mg, 289.79 μ mol, 85% purity, 1.5 eq) at 0 °C. Then it was stirred at 25 °C for 1 h. The 10 batches of the reaction mixture were mixed together for work up. The reaction mixture was slowly added to 50 mL of sat. Na₂SO₃, washed with 50 mL of sat. NaHCO₃, then extracted with DCM (3 $\times$ 50 mL). The combined organic layers were washed with brine (3 $\times$ 50 mL) and dried over anhydrous sodium sulfate. It was filtered and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by flash chromatography (silica gel, petroleum ether/ethyl acetate = 3/1 to 0/1) to give methyl 4-amino-1-[[4-[(tert-butoxycarbonylamino)methyl]phenyl]methyl]-2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-7-carboxylate (350 mg, 655.90 μ mol, 33.95% yield).

Step 10: Synthesis of methyl 4-amino-1-[[4-(aminomethyl)phenyl]methyl]-2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-7-carboxylate



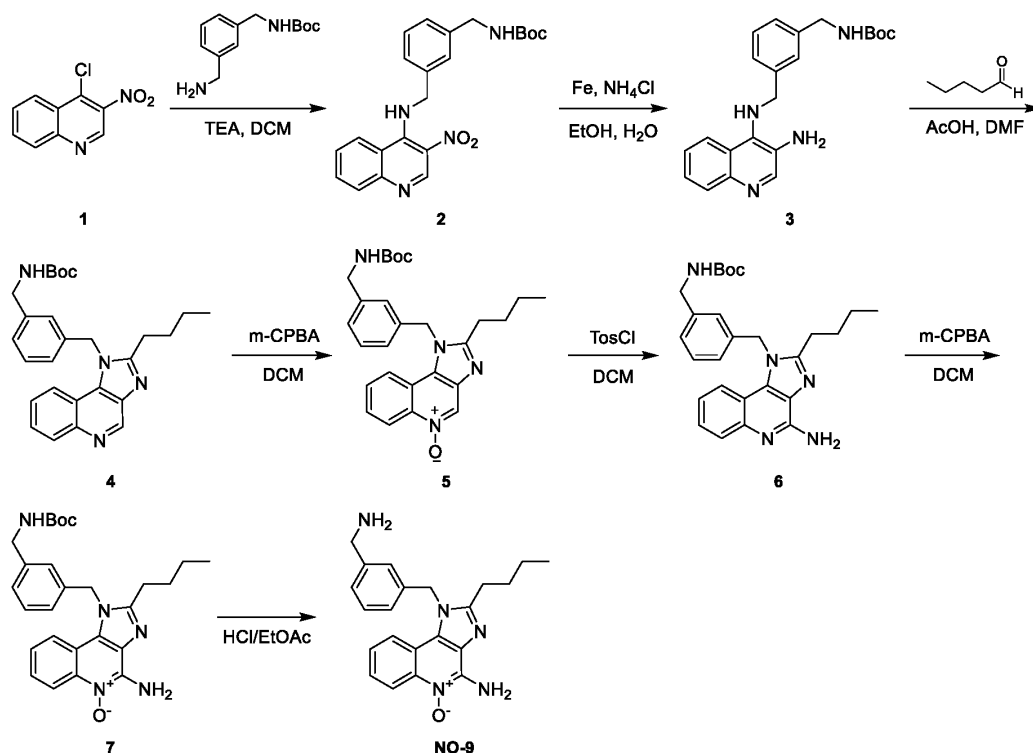
A solution of methyl 4-amino-1-[[4-[(tert-butoxycarbonylamino)methyl]phenyl]methyl]-2-butyl-5-oxido-i

midazo[4,5-c]quinolin-5-ium-7-carboxylate (0.3 g, 562.20 μmol , 1 eq) in HCl/EtOAc (3 mL) was stirred at 20 °C for 1 h. It was filtered to give a residue. The residue was purified by prep-HPLC (HCl condition, column: Phenomenex luna C18 100*40 mm*5 μm ; mobile phase: [H₂O(0.04% HCl)-ACN]; gradient: 1%-45% B over 8.0 min,) to give NO-8 (80.4 mg, 164.55 μmol , 29.27% yield, HCl). LCMS m/z 434.2 [M+H]⁺ 1H NMR (400 MHz, METHANOL- d_4) δ = 8.70 (d, J = 1.1 Hz, 1H), 8.06 (d, J = 8.6 Hz, 1H), 7.95 (dd, J = 1.1, 8.5 Hz, 1H), 7.48 (d, J = 8.1 Hz, 2H), 7.20 (d, J = 8.0 Hz, 2H), 6.03 (s, 2H), 4.09 (s, 2H), 3.97 (s, 3H), 3.03 (t, J = 7.6 Hz, 2H), 1.87 (quin, J = 7.6 Hz, 2H), 1.48 (sxt, J = 7.5 Hz, 2H), 0.95 (t, J = 7.4 Hz, 3H).

Example 9

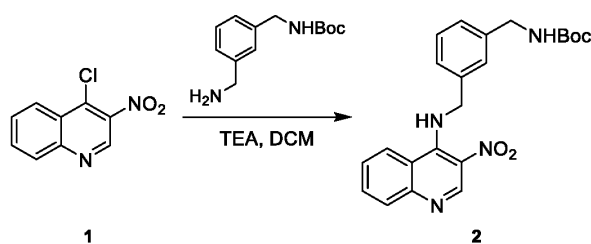
4-amino-1-(3-(aminomethyl)benzyl)-2-butyl-1H-imidazo[4,5-c]quinoline 5-oxide

(Compound NO-9)



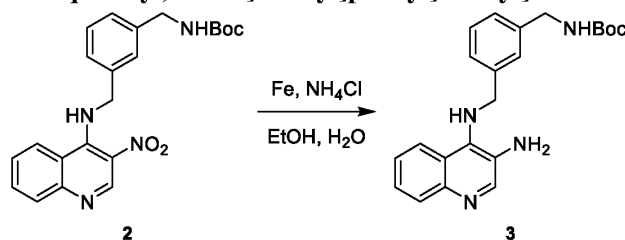
Step 1: tert-butyl

N-[[3-[[[(3-nitro-4-quinolyl)amino]methyl]phenyl]methyl]carbamate



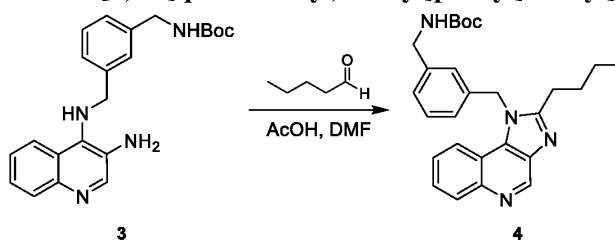
To a solution of 4-chloro-3-nitro-quinoline (10 g, 47.94 mmol, 1 eq) and TEA (3.20 g, 31.64 mmol, 4.40 mL, 0.66 eq) in DCM (150 mL) was added tert-butyl N-[[3-(aminomethyl)phenyl]methyl]carbamate (11.33 g, 47.94 mmol, 1 eq) at 0°C. The mixture was stirred at 20°C for 12 h. The reaction mixture was filtered and concentrated under reduced pressure to give tert-butyl N-[[3-[[[(3-nitro-4-quinolyl)amino]methyl]phenyl]methyl]carbamate (39 g, crude) was obtained.

Step _____ 2: tert-butyl
N-[[3-[[[(3-amino-4-quinolyl)amino]methyl]phenyl]methyl]carbamate



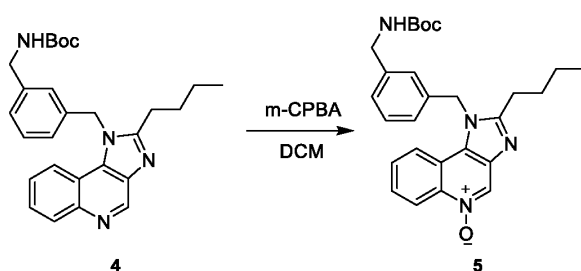
To a solution of tert-butyl N-[[3-[[[(3-nitro-4-quinolyl)amino]methyl]phenyl]methyl]carbamate (13 g, 31.83 mmol, 1 eq) in EtOH (150 mL) and H₂O (30 mL) was added Fe (8.89 g, 159.14 mmol, 5 eq) and NH₄Cl (17.03 g, 318.28 mmol, 10 eq). The mixture was stirred at 80 °C for 6 h. The reaction mixture was filtered and concentrated under reduced pressure to give tert-butyl N-[[3-[[[(3-amino-4-quinolyl)amino]methyl]phenyl]methyl]carbamate (41 g, crude) was obtained.

Step _____ 3: tert-butyl
N-[[3-[(2-butylimidazo[4,5-c]quinolin-1-yl)methyl]phenyl]methyl]carbamate



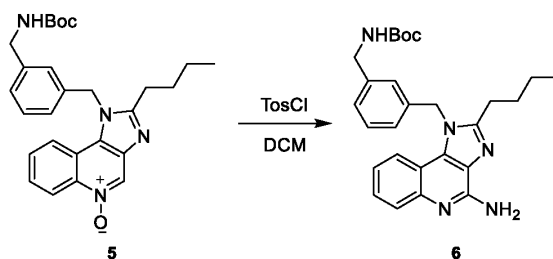
To a solution of tert-butyl N-[[3-[[3-(3-amino-4-quinolyl)amino]methyl]phenyl]methyl]carbamate (20 g, 52.84 mmol, 1 eq) and pentanal (5.46 g, 63.41 mmol, 6.74 mL, 1.2 eq) in DMF (200 mL) was added AcOH (317.34 mg, 5.28 mmol, 302.52 μ L, 0.1 eq). The mixture was stirred at 80°C for 12 h. Then the mixture was stirred at 95°C for another 12 h. The reaction was concentrated under reduced pressure, then added into water (500 mL), then extracted with ethyl acetate (3*500 mL). The combined organic layers were washed with brine (3*500 mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography to give tert-butyl N-[[3-[(2-butylimidazo[4,5-c]quinolin-1-yl)methyl]phenyl]methyl]carbamate (20 g, 44.99 mmol, 42.57% yield) was obtained.

Step 4: tert-butyl N-[[3-[(2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-1-yl)methyl]phenyl]methyl]carbamate



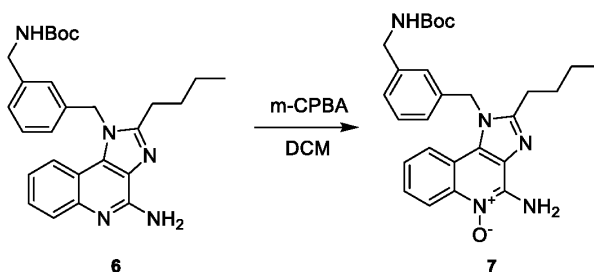
To a solution of tert-butyl N-[[3-[(2-butylimidazo[4,5-c]quinolin-1-yl)methyl]phenyl]methyl]carbamate (10 g, 22.49 mmol, 1 eq) in DCM (150 mL) was added m-CPBA (6.85 g, 33.74 mmol, 85% purity, 1.5 eq) at 0°C, then the mixture was stirred at 20°C for 12 h. The reaction was added into saturated sodium thiosulfate aqueous solution (200 mL), then extracted with dichloromethane (5*200 mL). The combined organic layers were washed with saturated sodium bicarbonate solution (3*300 mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography to give tert-butyl N-[[3-[(2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-1-yl)methyl]phenyl]methyl]carbamate (9.5 g, crude).

Step 5: tert-butyl N-[[3-[(4-amino-2-butyl-imidazo[4,5-c]quinolin-1-yl)methyl]phenyl]methyl]carbamate



Six reactions were carried out in parallel. To a solution of tert-butyl N-[[3-[(2-butyl-5-oxidoimidazo[4,5-c]quinolin-5-ium-1-yl)methyl]phenyl]methyl]carbamate (500 mg, 1.09 mmol, 1 eq) and $\text{NH}_3 \cdot \text{H}_2\text{O}$ (3 mL) in DCM (9 mL) was added TosCl (413.94 mg, 2.17 mmol, 2 eq), then the mixture was stirred at 20°C for 12 h. Six reactions were combined. The reaction was added into water (50 mL), then extracted with dichloromethane (3*50 mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give a residue. The crude product (800 mg scale) was combined to purification. The residue was purified by column chromatography to give tert-butyl N-[[3-[(4-amino-2-butylimidazo[4,5-c]quinolin-1-yl)methyl]phenyl]methyl]carbamate (2.8 g, 6.09 mmol, 73.65% yield).

Step 6: tert-butyl N-[[3-[(4-amino-2-butyl-5-oxidoimidazo[4,5-c]quinolin-5-ium-1-yl)methyl]phenyl]methyl]carbamate

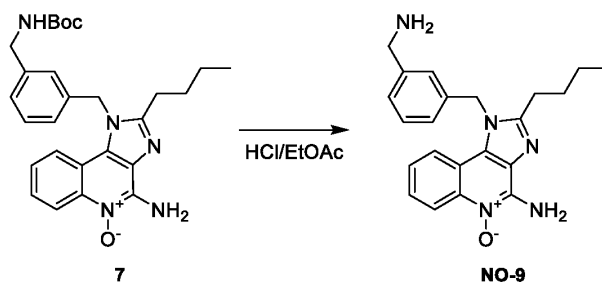


Four reactions were carried out in parallel. To a solution of tert-butyl N-[[3-[(4-amino-2-butylimidazo[4,5-c]quinolin-1-yl)methyl]phenyl]methyl]carbamate (250 mg, 543.97 μmol , 1 eq) in DCM (3 mL) was added m-CPBA (121.48 mg, 598.37 μmol , 85% purity, 1.1 eq) at 0°C, then the mixture was stirred at 0°C for 1 h. Four reactions were combined. The reaction was added into saturated sodium thiosulfate aqueous solution (20 mL), then extracted with dichloromethane (3*20 mL). The combined organic layers were washed with saturated sodium bicarbonate solution (3*20 mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column

chromatography to give tert-butyl N-[[3-[(4-amino-2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-1-yl)methyl]phenyl]methyl]carbamate (340 mg, 714.91 μ mol, 32.86% yield).

Step **7:**

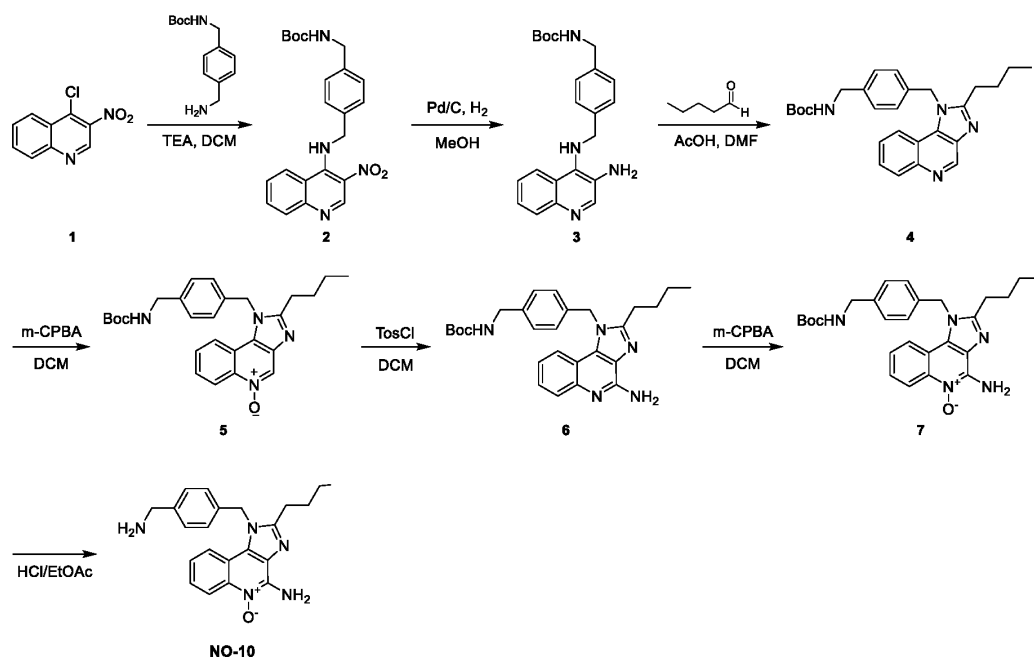
1-[[3-(aminomethyl)phenyl]methyl]-2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-4-amine



A solution of tert-butyl N-[[3-[(4-amino-2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-1-yl)methyl]phenyl]methyl]carbamate (340 mg, 714.91 μ mol, 1 eq) in HCl/EtOAc (5 mL) and was stirred at 20°C for 1 h. The reaction was concentrated under reduced pressure to give a residue. The crude product (50 mg scale) was combined to purity. The residue was purified by prep-HPLC to give NO-9 (204.1 mg, 480.25 μ mol, 58.41% yield, HCl). ¹H NMR (400 MHz, DMSO-d₆) δ = 9.48 (br s, 1H), 9.19 (br s, 1H), 8.53 (br s, 3H), 8.06 (d, J = 8.1 Hz, 1H), 7.96 (d, J = 8.0 Hz, 1H), 7.76 - 7.65 (m, 1H), 7.51 - 7.37 (m, 3H), 7.25 (s, 1H), 7.19 - 7.09 (m, 1H), 5.95 (s, 2H), 3.93 (br d, J = 5.0 Hz, 2H), 2.98 (t, J = 7.7 Hz, 2H), 1.77 (quin, J = 7.6 Hz, 2H), 1.41 (sxt, J = 7.4 Hz, 2H), 0.89 (t, J = 7.3 Hz, 3H).

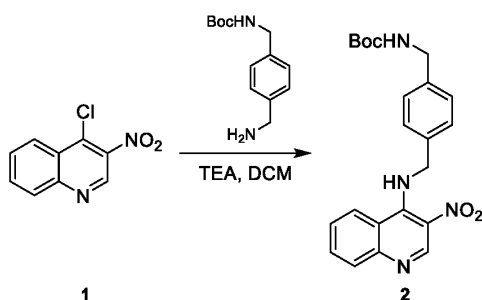
Example 10

4-amino-1-(4-(aminomethyl)benzyl)-2-butyl-1H-imidazo[4,5-c]quinoline 5-oxide
(Compound NO-10)

**Step 1:**

tert-butyl

N-[[4-[(3-nitro-4-quinolyl)amino]methyl]phenyl]methyl]carbamate

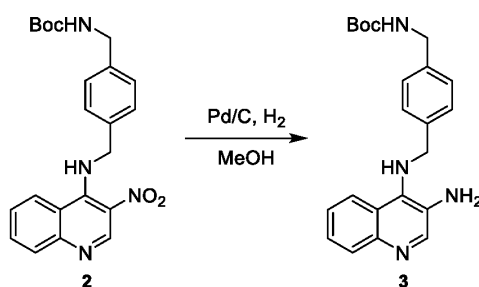


Two reactions were carried out in parallel. To a solution of 4-chloro-3-nitroquinoline (10 g, 47.94 mmol, 1 eq) and TEA (3.20 g, 31.64 mmol, 4.40 mL, 0.66 eq) in DCM (200 mL) was added *tert*-butyl N-[4-(aminomethyl)phenyl]methylcarbamate (11.33 g, 47.94 mmol, 1 eq), then the mixture was stirred at 20°C for 12 h. The reaction was filtered and concentrated under reduced pressure to give *tert*-butyl N-[[4-[(3-nitro-4-quinolyl)amino]methyl]phenyl]methyl]carbamate (40 g, crude).

Step 2:

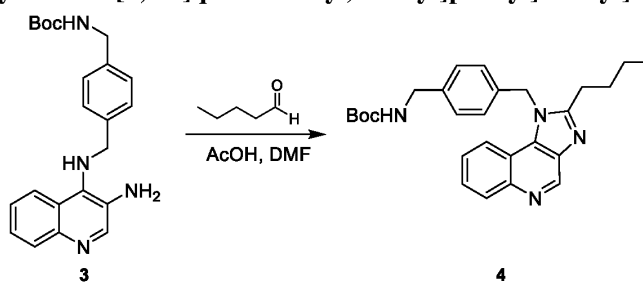
tert-butyl

N-[[4-[(3-amino-4-quinolyl)amino]methyl]phenyl]methyl]carbamate



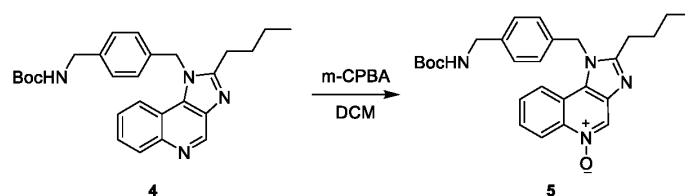
Two reactions were carried out in parallel. To a solution of tert-butyl N-[[4-[(3-nitro-4-quinolyl)amino]methyl]phenyl]methyl]carbamate (20 g, 48.97 mmol, 1 eq) in MeOH (400 mL) was added Pd/C (2 g, 10% purity) under N₂. The mixture was stirred under H₂ (15 Psi) at 20 °C for 3 h. Two reactions were combined. The reaction was filtered and concentrated under reduced pressure to give tert-butyl N-[[4-[(3-amino-4-quinolyl)amino]methyl]phenyl]methyl]carbamate (37 g, crude).

Step 3: **tert-butyl N-[[4-[(2-butylimidazo[4,5-c]quinolin-1-yl)methyl]phenyl]methyl]carbamate**



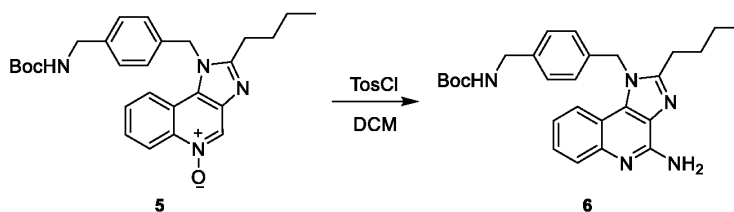
Two reactions were carried out in parallel. To a solution of tert-butyl N-[[4-[(3-amino-4-quinolyl)amino]methyl]phenyl]methyl]carbamate (18.5 g, 48.88 mmol, 1 eq) and pentanal (4.63 g, 53.77 mmol, 5.72 mL, 1.1 eq) in DMF (200 mL) was added AcOH (293.54 mg, 4.89 mmol, 279.83 μ L, 0.1 eq), then the mixture was stirred at 100°C for 24 h. The reaction was added into water (800 mL), then extracted with ethyl acetate (5*500 mL). The combined organic layers were washed with brine (3*500 mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography to give tert-butyl N-[[4-[(2-butylimidazo[4,5-c]quinolin-1-yl)methyl]phenyl]methyl]carbamate (14 g, 31.49 mmol, 32.21% yield).

Step 4: **tert-butyl N-[[4-[(2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-1-yl)methyl]phenyl]methyl]carbamate**



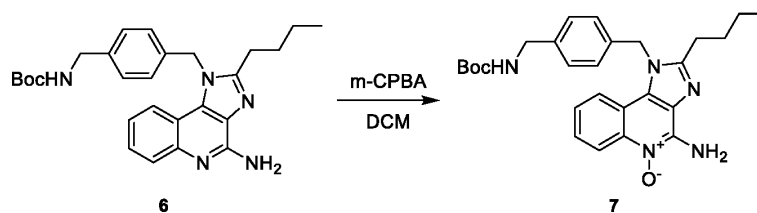
To a solution of tert-butyl N-[[4-[(2-butylimidazo[4,5-c]quinolin-1-yl)methyl]phenyl]methyl]carbamate (14 g, 31.49 mmol, 1 eq) in DCM (200 mL) was added m-CPBA (9.59 g, 47.24 mmol, 85% purity, 1.5 eq) in portions at 0°C, then the mixture was stirred at 20°C for 3 h. The reaction was added into saturated sodium thiosulfate aqueous solution (200 mL), then extracted with dichloromethane (3*200 mL). The combined organic layers were washed with saturated sodium bicarbonate solution (3*300 mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography to give tert-butyl N-[[4-[(2-butyl-5-oxidoimidazo[4,5-c]quinolin-5-ium-1-yl)methyl]phenyl]methyl]carbamate (9.88 g, 21.45 mmol, 68.12% yield).

Step 5: tert-butyl N-[[4-[(4-amino-2-butyl-imidazo[4,5-c]quinolin-1-yl)methyl]phenyl]methyl]carbamate



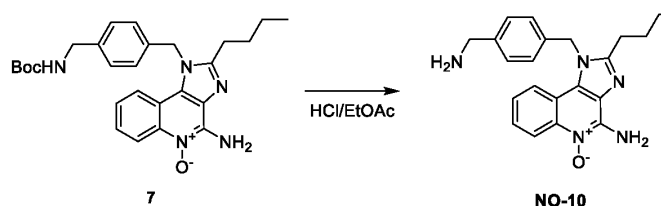
Six reactions were carried out in parallel. To a solution of tert-butyl N-[[4-[(2-butyl-5-oxidoimidazo[4,5-c]quinolin-5-ium-1-yl)methyl]phenyl]methyl]carbamate (500 mg, 1.09 mmol, 1 eq) in DCM (9 mL) and NH₃.H₂O (3 mL) was added TosCl (413.94 mg, 2.17 mmol, 2 eq), then the mixture was stirred at 20°C for 12 h. Six reactions were combined. The reaction was added water (50 mL), then extracted with dichloromethane (3*50 mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give a residue. The crude product (380 mg scale) was combined to purification. The residue was purified by column chromatography to give tert-butyl N-[[4-[(4-amino-2-butyl-imidazo[4,5-c]quinolin-1-yl)methyl]phenyl]methyl]carbamate (3 g, 6.53 mmol, 88.92% yield).

Step 6: tert-butyl N-[[4-[(4-amino-2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-1-yl)methyl]phenyl]methyl]carbamate



Four reactions were carried out in parallel. To a solution of tert-butyl N-[[4-[(4-amino-2-butyl-5-imidazo[4,5-c]quinolin-1-yl)methyl]phenyl]methyl]carbamate (250 mg, 543.97 μmol , 1 eq) in DCM (3 mL) was added m-CPBA (121.48 mg, 598.37 μmol , 85% purity, 1.1 eq) at 0°C, then the mixture was stirred at 0°C for 1 h. Four reactions were combined. The reaction was added into saturated sodium thiosulfate aqueous solution (20 mL), then extracted with dichloromethane (3*20 mL). The combined organic layers were washed with saturated sodium bicarbonate solution (3*20 mL) and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography to give tert-butyl N-[[4-[(4-amino-2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-1-yl)methyl]phenyl]methyl]carbamate (270 mg, 567.73 μmol , 26.09% yield).

Step 7:
1-[[4-(aminomethyl)phenyl]methyl]-2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-4-amine

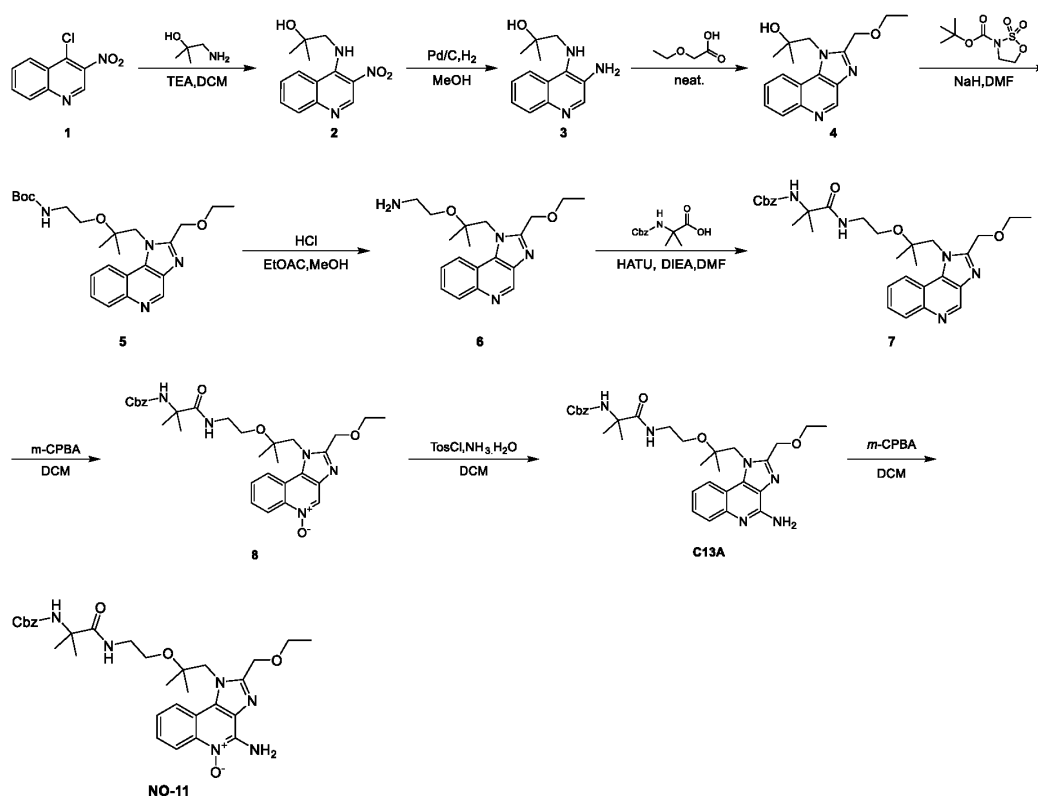


A solution of tert-butyl N-[[4-[(4-amino-2-butyl-5-oxido-imidazo[4,5-c]quinolin-5-ium-1-yl)methyl]phenyl]methyl]carbamate (270 mg, 567.73 μmol , 1 eq) in HCl/EtOAc (3 mL) was added and stirred at 20°C for 1 h. The reaction was concentrated under reduced pressure to give a residue. The crude product (55 mg scale) was combined to purity. The residue was purified by prep-HPLC to give NO-10 (173.9 mg, 421.42 μmol , 62.91% yield, HCl). ¹H NMR (400 MHz, DMSO-d₆) δ = 9.60 - 9.06 (m, 2H), 8.48 (br s, 3H), 8.06 (d, J = 8.5 Hz, 1H), 7.98 (d, J = 8.2 Hz, 1H), 7.68 (t, J = 7.9 Hz, 1H), 7.47 (d, J = 8.1 Hz, 2H),

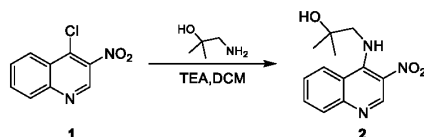
7.40 (t, $J = 7.7$ Hz, 1H), 7.11 (d, $J = 8.1$ Hz, 2H), 5.99 (s, 2H), 3.95 (br s, 2H), 2.97 (t, $J = 7.7$ Hz, 2H), 1.74 (quin, $J = 7.6$ Hz, 2H), 1.39 (sxt, $J = 7.4$ Hz, 2H), 0.88 (t, $J = 7.3$ Hz, 3H).

Example 11

4-amino-2-(ethoxymethyl)-1-(5,5,11,11-tetramethyl-3,6-dioxo-1-phenyl-2,10-dioxo-4,7-diazadodecan-12-yl)-1H-imidazo[4,5-c]quinoline 5-oxide
(Compound NO-11)



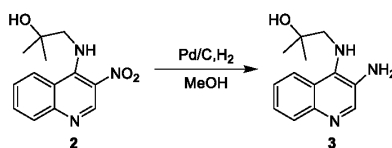
Step 1: Synthesis of 2-methyl-1-[(3-nitro-4-quinolyl)amino]propan-2-ol



To a solution of 4-chloro-3-nitroquinoline (5 g, 23.97 mmol, 1 eq) in DCM (75 mL) was added TEA (1.60 g, 15.81 mmol, 2.20 mL, 0.66 eq). Then it was added dropwise 1-amino-2-methyl-propan-2-ol (2.33 g, 26.13 mmol, 1.09 eq) at 0°C. It was stirred at 20°C for 2 h. The reaction mixture was filtered to give a yellow solid.

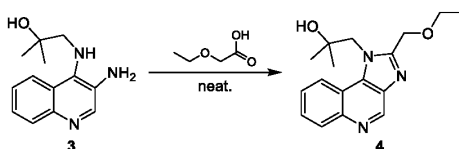
2-methyl-1-[(3-nitro-4-quinolyl)amino]propan-2-ol (5.3 g, crude) was obtained.

Step 2: Synthesis of 1-[(3-amino-4-quinolyl)amino]-2-methyl-propan-2-ol



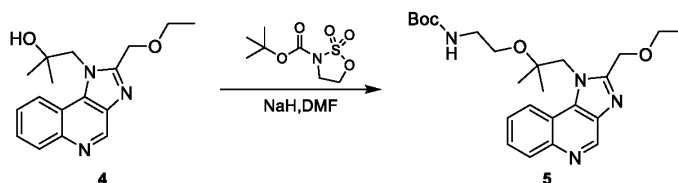
To a solution of 2-methyl-1-[(3-nitro-4-quinolyl)amino]propan-2-ol (4.3 g, 16.46 mmol, 1 eq) in MeOH (90 mL) was added Pd/C (0.43 g, 10% purity). The suspension was degassed under vacuum and purged with H₂ (15PSI) several times. It was stirred at 20°C for 5 h under H₂(15PSI). The reaction mixture was filtered and the filtrate was concentrated under reduced pressure to give 1-[(3-amino-4-quinolyl)amino]-2-methyl-propan-2-ol (3.8 g, crude).

Step 3: Synthesis of 1-[2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]-2-methyl-propan-2-ol



To a solution of 1-[(3-amino-4-quinolyl)amino]-2-methyl-propan-2-ol (3.8 g, 16.43 mmol, 1 eq) in 2-ethoxyacetic acid (12.51 g, 120.21 mmol, 11.36 mL, 7.32 eq) was stirred at 120°C for 12 h. The reaction mixture was slowly added to saturated sodium bicarbonate solution (200 mL), then extracted with ethyl acetate (3 × 200 mL). The combined organic layers were washed with brine (3 × 100 mL) and dried over anhydrous sodium sulfate. It was filtered and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by flash chromatography (silica gel, petroleum ether/ethyl acetate = 1/0 to 1/1) to give 1-[2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]-2-methyl-propan-2-ol (4.9 g, 16.37 mmol, 99.63% yield).

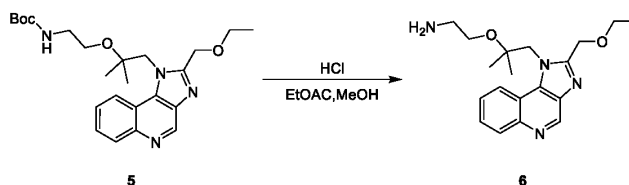
Step 4: Synthesis of tert-butyl N-[2-[2-[2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]-1,1-dimethyl-ethoxy]ethyl]carbamate



To a solution of

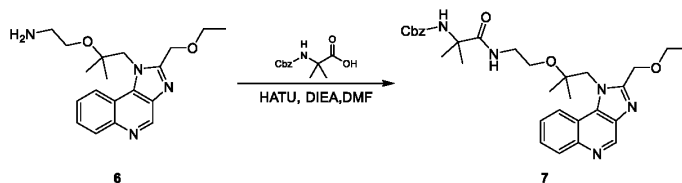
1-[2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]-2-methyl-propan-2-ol (4.9 g, 16.37 mmol, 1 eq) in DMF (50 mL) was added NaH (785.66 mg, 19.64 mmol, 60% purity, 1.2 eq) at 0°C. It was stirred at 0°C for 1 h and then it was stirred at 20°C for 0.5 h to give a yellow solution. To this solution was added tert-butyl 2,2-dioxathiazolidine-3-carboxylate (3.65 g, 16.37 mmol, 1 eq) at 0°C and the result solution was stirred at 20°C for 12 h. It was added to 100 ml of sat. NH₄Cl, then extracted with ethyl acetate (3 × 500 mL). The combined organic layers were washed with brine (3 × 500 mL) and dried over anhydrous sodium sulfate. It was filtered and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (neutral condition, column: Phenomenex luna c18 250 mm*100 mm*10 um; mobile phase: [H₂O(0.2%FA)-ACN]; gradient:10%-37% B over 20.0 min) to give tert-butyl N-[2-[2-[2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]-1,1-dimethyl-ethoxy]ethyl]carbamate (5 g, 11.30 mmol, 69.03% yield).

Step 5: Synthesis of 2-[2-[2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]-1,1-dimethyl-ethoxy]ethanamine



To a solution of tert-butyl N-[2-[2-[2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]-1,1-dimethyl-ethoxy]ethyl]carbamate (2 g, 4.52 mmol, 1 eq) in HCl/EtOAc (20 mL, 4M) was added MeOH (5 mL). It was stirred at 20°C for 1 h. It was concentrated under reduced pressure to give 2-[2-[2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]-1,1-dimethyl-ethoxy]ethanamine (1.7 g, crude).

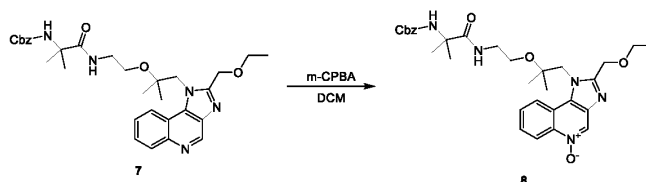
Step 6: Synthesis of 2-[2-[2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]-1,1-dimethyl-ethoxy]ethanamine



To a solution of 2-(benzyloxycarbonylamino)-2-methyl-propanoic acid (1.18 g, 4.96 mmol, 1 eq),

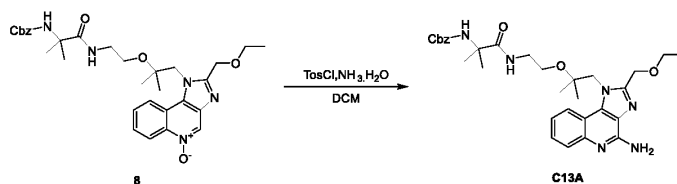
2-[2-[2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]-1,1-dimethyl-ethoxy]ethanamine (1.7 g, 4.96 mmol, 1 eq), DIEA (1.92 g, 14.89 mmol, 2.59 mL, 3 eq) in DMF (35 mL) was added HATU (2.27 g, 5.96 mmol, 1.2 eq). It was stirred at 20°C for 12 h. The reaction mixture was added into water (100 mL), then extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine (3 × 50 mL) and dried over anhydrous sodium sulfate. It was filtered and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by flash chromatography (silica gel, petroleum ether/ethyl acetate = 3/1 to 0/1) to give benzyl N-[2-[2-[2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]-1,1-dimethyl-ethoxy]ethyl amino]-1,1-dimethyl-2-oxo-ethyl]carbamate (3 g, crude).

Step 7: Synthesis of benzyl N-[2-[2-[2-(ethoxymethyl)-5-oxido-imidazo[4,5-c]quinolin-5-ium-1-yl]-1,1-dimethyl-ethoxy]ethylamino]-1,1-dimethyl-2-oxo-ethyl]carbamate



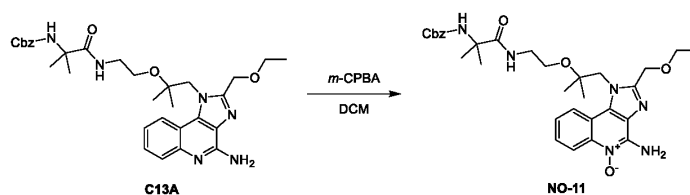
To a solution of benzyl N-[2-[2-[2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]-1,1-dimethyl-ethoxy]ethyl amino]-1,1-dimethyl-2-oxo-ethyl]carbamate (3 g, 5.34 mmol, 1 eq) in DCM (30 mL) was slowly added m-CPBA (1.41 g, 6.94 mmol, 85% purity, 1.3 eq) at 0°C. It was stirred at 20°C for 1 h. The reaction mixture was added into sat. Na₂SO₃ (30 mL), then extracted with DCM (3 × 30 mL). The combined organic layers were washed with brine (3 × 50 mL) and dried over anhydrous sodium sulfate. It was filtered and the filtrate was concentrated under reduced pressure to give benzyl N-[2-[2-[2-(ethoxymethyl)-5-oxido-imidazo[4,5-c]quinolin-5-ium-1-yl]-1,1-dimethyl-ethoxy]ethylamino]-1,1-dimethyl-2-oxo-ethyl]carbamate (3 g, crude).

Step 8: Synthesis of benzyl N-[2-[2-[2-[4-amino-2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]-1,1-dimethyl-ethoxy]ethylamino]-1,1-dimethyl-2-oxo-ethyl]carbamate



To a solution of benzyl N-[2-[2-[2-(ethoxymethyl)-5-oxido-imidazo[4,5-c]quinolin-5-ium-1-yl]-1,1-dimethyl-ethoxy]ethylamino]-1,1-dimethyl-2-oxo-ethyl]carbamate (0.5 g, 865.55 μmol , 1 eq) in DCM (8 mL) was added TosCl (330.03 mg, 1.73 mmol, 2 eq) and $\text{NH}_3\cdot\text{H}_2\text{O}$ (8 mL). It was stirred at 20°C for 1 h. The organic layers were concentrated under reduced pressure to give a residue. The residue was purified by flash chromatography (silica gel, petroleum ether/ethyl acetate = 1/0 to 0/1) to give a brown gum. The brown gum was purified by prep-HPLC (neutral condition, column: Waters Xbridge BEH C18 100*30 mm*10 μm ; mobile phase: $[\text{H}_2\text{O}(10\text{mM NH}_4\text{HCO}_3)\text{-ACN}]$; gradient: 30%-60% B over 8.0 min) to give benzyl N-[2-[2-[2-[4-amino-2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]-1,1-dimethyl-ethoxy]ethylamino]-1,1-dimethyl-2-oxo-ethyl]carbamate (18.8 mg, 32.60 μmol , 18.80% yield, 100.0% purity).

Step 9: Synthesis of benzyl N-[2-[2-[2-[4-amino-2-(ethoxymethyl)-5-oxido-imidazo[4,5-c]quinolin-5-ium-1-yl]-1,1-dimethyl-ethoxy]ethylamino]-1,1-dimethyl-2-oxo-ethyl]carbamate



To a solution of benzyl N-[2-[2-[2-[4-amino-2-(ethoxymethyl)imidazo[4,5-c]quinolin-1-yl]-1,1-dimethyl-ethoxy]ethylamino]-1,1-dimethyl-2-oxo-ethyl]carbamate (0.1 g, 173.40 μmol , 1 eq) in DCM (5 mL) was slowly added m-CPBA (52.81 mg, 260.11 μmol , 85% purity, 1.5 eq) at 0°C. It was stirred at 0°C for 0.5 h. The 6 batches of the reaction mixture were mixed together for work up. The reaction mixture was slowly added to 20 mL of sat. Na_2SO_3 , washed with 10 mL of sat. NaHCO_3 , and then extracted with ethyl acetate (3 $\times$ 20 mL). The combined organic layers were washed with brine (3 $\times$ 25 mL) and dried over anhydrous sodium sulfate. It was filtered and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (neutral condition, column: Waters Xbridge Prep OBD C18 150*40 mm*10 μm ; mobile phase: $[\text{H}_2\text{O}(10\text{mM NH}_4\text{HCO}_3)\text{-ACN}]$; gradient: 25%-45% B over 8.0 min) to give crude product 1. The crude product 1 was re-purified by prep-HPLC (FA condition, column: Phenomenex luna C18 100*40 mm*5 μm ; mobile phase:

[H₂O(0.2% FA)-ACN];gradient:20%-50% B over 8.0 min) to give **NO-11** (26.1 mg, 44.04 μ mol, 4.23% yield). **LCMS** m/z 593.3 [M+H]⁺ **¹H NMR** (400 MHz, DMSO-d₆) δ = 8.55 (d, J = 8.6 Hz, 1H), 8.49 (br d, J = 8.3 Hz, 1H), 8.35 (s, 1H), 7.69 - 7.49 (m, 3H), 7.47 (br t, J = 7.5 Hz, 1H), 7.38 - 7.26 (m, 6H), 7.05 (br s, 1H), 4.96 (s, 2H), 3.54 (q, J = 6.9 Hz, 2H), 3.48 - 3.22 (m, 4H), 3.13 (br s, 2H), 2.88 (br d, J = 3.7 Hz, 2H), 1.21 (s, 6H), 1.14 (br t, J = 6.9 Hz, 9H)

II. Biological evaluation

Cell Culture.

MC38, B16F10, and RAW 264.7 were purchased from Procell. RAW-Blue reporter cells and QUANTI-Blue solution were purchased from Invivogen. MC38, B16, RAW 264.7, RAW-Blue reporter cells were cultured in DMEM medium. The media were supplemented with 10% (v/v) fetal bovine serum (FBS), penicillin (100 units/mL) and streptomycin (100 μ g/mL). All these cells were cultured in a 5% CO₂ incubator at 37 °C and the medium was replaced every 2-3 days. After growing to 80% confluence, the cells were treated with trypsin or cell scraper and then seeded on dishes or 96-well plates overnight for further experiments.

RAW-Blue reporter cells were plated in a 96-well plate at a density of 5×10^4 cells/well. The cells were treated with a series of concentrations of agonists for 24 h. Then, 20 μ L of supernatant from each well was extracted and mixed with 180 μ L of QUANTI-Blue solution. The absorbance was detected via a microplate reader.

BMDCs were generated by culturing bone-marrow cells in the presence of rmGM-CSF (20 ng/ml, Biolegend) for 6 days followed by stimulation with NO-1 without or with radiation 10 Gy for 24 h. After incubation, cells were sorted, and surface markers CD80/86 were analyzed on flow cytometry (BD Bioscience). Supernatant was collected and measured by CBA (BD).

OT I CD8⁺ T cells were isolated from spleens using magnetic beads (Stem cell). T cells were loaded with 2.5 μ M CFSE, then cocultured with antigen and agonist treated DCs at 8:1. After 72 h incubation, T cells were analyzed after staining with anti-mouse CD8 α -APC and anti-mouse CD3-BV421 by flow cytometry to assess CFSE dilution. Supernatant was collected and IFN γ was measured by CBA.

Animal Model.

MC38 and B16 (5×10^5) cells were injected subcutaneously into the right flank of 6-8 weeks old C57BL/6J mice. After tumour was established, mice were randomized into treatment groups with PBS or 30 $\mu\text{mol/kg}$ NO-1 (intravenous injection) every three day and/or radiation (6 Gy, 3 times in total). For abscopal model, MC38 (5×10^5) cells were injected subcutaneously into the right flank. 2 days later, MC38 (2.5×10^5) cells were injected subcutaneously into the left flank, and then intratumoural treatment of 3 $\mu\text{mol/kg}$ NO-1 and radiation only focused on right tumour. Tumour volumes were measured twice a week and calculated by $\text{length} \times \text{width} \times \text{height} / 2$.

Activating N-oxide compound with radiation

X-Ray irradiation was delivered by an X-ray generator (RS2000 Pro 225, 225 kV, 17.7 mA; Rad Source Technologies, Inc.). Test tubes were X-ray-irradiated with total doses of 0~60 Gy at the rate of 5 Gy/min. Cells were X-ray-irradiated with total doses of 10 Gy at the rate of 1.6 Gy/min. Local irradiation of the implanted tumours was conducted using a customized mouse jig. Tumours were locally irradiated at a dose of 6 Gy at a dose rate of 1 Gy/min with other parts of the body shielded with 5-mm thick lead.

For agonists activation by radiotherapy in living bodies, MC38 and B16 cells (5×10^5) were injected subcutaneously into the right flank of 6 to 8 weeks old C57BL/6J mice. After tumour was established, mice were randomized into treatment groups with PBS or 30 $\mu\text{mol/kg}$ NO-1 every three day and/or radiation (6 Gy, 3 times in total). For abscopal model, MC38 (5×10^5) cells were injected subcutaneously into the right flank, 2 days later, MC38 (2.5×10^5) cells were injected subcutaneously into the left flank, and then intratumoural treatment of 3 $\mu\text{mol/kg}$ NO-1 and radiation only focused on right tumor.

Biodistribution analysis.

MC38 tumour-bearing mice were injected with NO-1 (30 $\mu\text{mol/kg}$) via the intravenous route. The mice were then sacrificed at preset time points post-injection and tumour tissues were collected, wet weighed, and the concentration of NO-1 was measured by UPLC.

Statistical Analysis.

Statistical analyses were performed using GraphPad Prism 6. Statistical

comparisons were analyzed using two-way ANOVA.

Example 12: Study of the immune stimulation of R848 and NO-1

Serum IFN- γ measurements taken at 4 h postinjection of R848 and NO-1 (30 $\mu\text{mol/kg}$ (a), 60 $\mu\text{mol/kg}$ (b), respectively). $n = 3$ C57BL/6J mice for each group tested. The test result showed in Figure 1a and Figure 1b. Serum TNF- α measurements taken at 4 h postinjection of R848 and NO-1 (30 $\mu\text{mol/kg}$ (c), 60 $\mu\text{mol/kg}$ (d), respectively). $n = 3$ C57BL/6J mice for each group tested. The test result showed in Figure 1c and Figure 1d. Weight change measurements taken for C57BL/6J mice ($n = 6$ mice each group) following intravenous administration of 30 $\mu\text{mol/kg}$ of R848 and NO-1 respectively. The test result showed in Figure 1e and Figure 1f. Concentration-dependent TLR7/8 activation curves of R848 and NO-1 were studied in RAW-Blue report cells after 24 h incubation ($n = 6$ independent samples for each group tested). The test results were showed in Figure 1f.

Example 13: Study of radiation induced reduction of NO-1 in aqueous solution and in biological environment

X-ray-induced R848 releasing from NO-1 (10 μM) in PBS determined by UPLC-MS. UPLC-MS spectra (selected-ion monitoring signal of $[\text{R848}+\text{H}]^+$, $m/z = 315$) of generated R848. Positive ion mode mass spectrum is shown for R848, retention time = 2.18 min. The test results were showed in Figure 2a and Figure 2b.

Example 14: Study of immune stimulation of R848 and NO-1 after radiation in reporter cell-line

Figure 3a shows Schematic illustration of the experimental design: In Figure 3b-3h, NO-1 (10 μM) was incubated with living RAW 264.7 and bone marrow-derived DCs (BMDCs) for 24 h and irradiated by X-ray (10 Gy), then the markers expression, released cytokines, and T cell priming were measured. Cells incubated with R848 (1 μM) were set as positive control. In Figure 3b-3c, CD80/86 expression in (3b) RAW 264.7 and (3c) BMDCs. In Figure 3d-3e, Secreted TNF- α (3d) and MCP-1 (3e) releasing from BMDCs were analyzed by CBA. In Figure 3f-3h, NO-1 treated BMDCs were incubated with 10 $\mu\text{g/mL}$ OVA peptides for 24 h, then co-cultured with OT I CD8 $^+$ T cells for 3 days.

Proliferation (3f, 3g) and IFN- γ production (3h) of OT I CD8⁺ T cells were analyzed. Data are presented as mean values $\pm$ s.d., n = 3 independent samples for each group tested, two-tailed unpaired Student's t-test.

Example 15: Radiotherapy activated NO-1 that rejected tumor growth in MC38 cells by intravenous (iv) injection

Figure 4a shows the treatment scheme. In Figure 4b-4g, C57BL/6J mice were implanted subcutaneously with MC38 cells followed by intravenous (iv) injection of NO-1 (30 μ mol/kg) and radiotherapy (6 Gy each treatment). In Figure 4b, the time-dependent accumulation of NO-1 in blood was shown and the tumour tissues were detected by UPLC-MS (n = 3 mice). In Figure 4c-4e, average tumour growth (4c), weight change curves (4d), and survival curves of mice until reaching euthanasia criteria (4e) were shown. In Figure 4f, tumour volume of an individual mouse was shown. In Figure 4g, Photographs of representative tumours on day 25 were shown.

Example 16: Radiotherapy activated NO-1 that rejected tumor growth in MC38 cells by intratumoural (it) injection

Figure 5a shows the treatment scheme. C57BL/6J mice (n = 6 mice per group) were implanted subcutaneously with MC38 cells on day 0 and day 2, followed by intratumoural (it) injection of NO-1 (3 μ mol/kg) and radiotherapy (6 Gy each treatment). In Figure 5b, average volume of primary and distant tumours were shown. Data are presented as mean values $\pm$ s.d.

Example 17: Study of the TLR7/8 activation of agonists and corresponding N-oxide prodrugs in RAW-Blue report cells

The Concentration-dependent TLR7/8 activation curves of agonists and corresponding N-oxide prodrugs in RAW-Blue report cells after 24 h incubation were shown in Figure 6 (n = 6 independent samples for each group tested). The comparison of EC₅₀ between N-oxides and corresponding deoxygenated structures were summarized in Table 2.

Table 2

Compound	EC ₅₀	Compound	EC ₅₀
NO-1	>100 μ M	R848	~0.2 μ M
NO-2	>500 μ M	852A	~10 μ M
NO-3	>500 μ M	Gardiquimod	~10 μ M
NO-4	>>100 μ M	CL097	~0.3 μ M
NO-5	>1 mM	CL075	~10 μ M
NO-6	~100 μ M	BBIQ	~0.03 μ M
NO-7	~100 μ M	T785	~2 μ M
NO-8	~10 μ M	IMQ1	~0.1 μ M
NO-9	~10 μ M	IMQ2	~0.1 μ M
NO-10	~1 μ M	IMQ3	~0.05 μ M
NO-11	~100 μ M	IMQ4	~1 μ M

Example 18: Study of activation yield of agonists from corresponding N-oxide agonists after radiation

Figure 7 shows the schematic illustration of deoxygen of N-oxide agonists induced by e-aq. and activation yield of agonists from corresponding N-oxide agonists (10 μ M) in PBS after 60 Gy radiation. Data are presented as mean values $\pm$ s.d., n = 3 independent samples for each group tested.

Example 19: Radiotherapy activated NO-10 that rejected tumor growth in MC38 cells by intravenous (iv) injection

Figure 8 shows MC38 tumor-bearing mice treated with different dose (20 μ g/mice and 100 μ g/mice) on day 10, 13 and 16. Body weight (a) and tumor growth was measured and shown (n = 6) (b). Serum was collected 24 h after the first injection. Cytokines in serum were analyzed by cytometric bead assay (c, d). e, f MC38 tumor-bearing mice were i.v. treated with NO-10 on day 10, 13 and 16. 1 h later, mice were irradiated by 6 Gy or not. Tumor growth was measured as indicated (e) and survival curve was shown in (f) (n = 6).

Example 20: Radiotherapy activated NO-10 that rejected metastasis of lung tumor growth in B16F10 cells by intravenous (iv) injection

Figure 9 shows: C57BL/6J mice (n = 6) were inoculated with 2.5×10^5

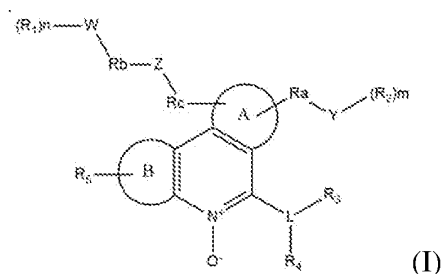
B16F10 cells. After one week, 2.5×10^6 B16F10 cells were intravenously injected. Tumor-bearing mice were i.v. treated with NO-10 and/or radiation on day 8, 11 and 14. The local tumor volume was measured (a). On day 19, the mice were sacrificed, and the colony number on the lung was counted (b, c).

Example 21: Radiotherapy activated NO-10 that rejected metastasis of lung tumor growth in 4T1 cells by intravenous (iv) injection

Figure 10 shows: d-g Balb/c mice ($n = 8$) were inoculated with 2×10^5 4T1-luc2 cells. Tumor-bearing mice were i.v. treated with NO-10 and/or radiation on day 10, 13 and 17. The metastasis of lung was measured by the IVIS Spectrum (a, b). Survival curve was shown in (c).

Claims

1. A compound of Formula I:



Wherein,

ring A and is a 5-10 membered aryl or heteroaryl containing one to four heteroatoms independently selected from nitrogen, oxygen, or sulfur;

ring B is a 5-10 membered aryl or heteroaryl containing one to four heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Y, Z and W are independently selected from the group consisting of carbon, nitrogen, oxygen or sulfur, optionally Y, Z, W are independently absent;

Ra, Rb and Rc is independently selected from the group consisting of a branched or non-branched, substituted or non-substituted, saturated or unsaturated aliphatic hydrocarbon chain having 1-10 carbon atoms in its backbone chain;

each R₁ is independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, halo, haloalkyl, haloalkoxy, alkoxy, hydroxy, cyano, cyanoalkyl, optionally substituted carboxy, optionally substituted acylamino, aminocarbonyl, hydroxyalkyl, hydroxyalkynyl, alkoxyalkyl, aminoalkyl, aminocarbonylalkyl, sulfonylalkyl, aminosulfonylalkyl, optionally substituted alkoxyalkyl, optionally substituted cycloalkyl, optionally substituted cyclyl alkyl, optionally substituted phenyl, optionally substituted heteroaryl, optionally substituted heterocyclyl, optionally substituted bicyclic heterocyclyl, optionally substituted bridged optionally substituted heterocyclyl, optionally substituted spiral, optionally substituted heterospiral, or optionally substituted spiroheterocyclyl, or two R₁ combine to form a

optionally substituted cyclo alkyl, heterocyclo alkyl, aryl or heteroaryl;

each R₂ is independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, halo, haloalkyl, haloalkoxy, alkoxy, hydroxy, cyano, cyanoalkyl, optionally substituted carboxy, acylamino, aminocarbonyl, hydroxyalkyl, hydroxyalkynyl, alkoxyalkyl, aminoalkyl, aminocarbonylalkyl, sulfonylalkyl, aminosulfonylalkyl, optionally substituted alkoxyalkyl, optionally substituted cycloalkyl, optionally substituted cyclyl alkyl, optionally substituted phenyl, optionally substituted heteroaryl, optionally substituted heterocyclyl, optionally substituted bicyclic heterocyclyl, optionally substituted bridged optionally substituted heterocyclyl, optionally substituted spiral, optionally substituted heterospiral, or optionally substituted spiroheterocyclyl, or two R₂ combine to form a optionally substituted cyclo alkyl, heterocyclo alkyl, aryl or heteroaryl;

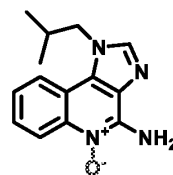
R₃, R₄ and R₅ are independently selected from the group consisting of hydrogen, alkyl, halo, haloalkyl, haloalkoxy, alkoxy, hydroxy, cyano, cyanoalkyl, carboxy, alkoxy carbonyl, acylamino, aminocarbonyl, hydroxyalkyl, hydroxyalkynyl, alkoxyalkyl, aminoalkyl, aminocarbonylalkyl, sulfonylalkyl, aminosulfonylalkyl, optionally substituted cycloalkyl, optionally substituted cyclyl alkyl, optionally substituted phenyl, optionally substituted heteroaryl, optionally substituted heterocyclyl, optionally substituted bicyclic heterocyclyl, optionally substituted bridged optionally substituted heterocyclyl, or optionally substituted spiroheterocyclyl, or R₃ and R₄ combine to form a optionally substituted C3-10 heterocyclo alkyl;

wherein the optionally substituent of R₁, R₂, R₃, R₄, R₅, R_a, R_b and R_c is independently selected from optionally substituted alkyl, cycloalkyl, cycloalkoxy, halo, haloalkyl, haloalkoxy, alkoxy, alkoxyalkyl, hydroxy, carboxyl;

n is 0, 1, 2 or 3;

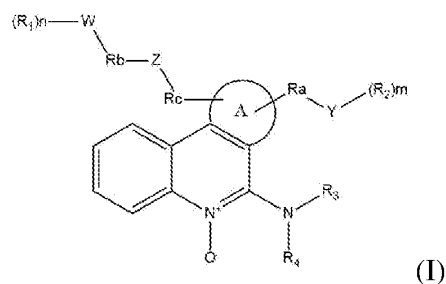
m is 0, 1, 2 or 3;

or a pharmaceutically acceptable salt thereof;



provided that the compound of formula I is not

2. The compound according to claim 1, wherein the Formula I is



Wherein,

ring A is a 5-10 membered aryl or heteroaryl containing one to four heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Y, Z and W are independently selected from the group consisting of carbon, nitrogen, oxygen or sulfur, optionally Y, Z, W are independently absent;

Ra, Rb and Rc is independently selected from the group consisting of a branched or non-branched, substituted or non-substituted, saturated or unsaturated aliphatic hydrocarbon chain having 1-10 carbon atoms in its backbone chain;

each R₁ is independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, halo, haloalkyl, haloalkoxy, alkoxy, hydroxy, cyano, cyanoalkyl, optionally substituted carboxy, optionally substituted acylamino, aminocarbonyl, hydroxyalkyl, hydroxyalkynyl, alkoxyalkyl, aminoalkyl, aminocarbonylalkyl, sulfonylalkyl, aminosulfonylalkyl, optionally substituted alkoxyalkyl, optionally substituted cycloalkyl, optionally substituted cyclyl alkyl, optionally substituted phenyl, optionally substituted heteroaryl, optionally substituted heterocyclyl, optionally substituted bicyclic heterocyclyl, optionally substituted bridged optionally substituted heterocyclyl, optionally substituted spiral, optionally substituted heterospiral, or optionally substituted spiroheterocyclyl, or two R₁ combine to form a

optionally substituted cyclo alkyl, heterocyclo alkyl, aryl or heteroaryl;

each R₂ is independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, halo, haloalkyl, haloalkoxy, alkoxy, hydroxy, cyano, cyanoalkyl, optionally substituted carboxy, acylamino, aminocarbonyl, hydroxyalkyl, hydroxyalkynyl, alkoxyalkyl, aminoalkyl, aminocarbonylalkyl, sulfonylalkyl, aminosulfonylalkyl, optionally substituted alkoxyalkyl, optionally substituted cycloalkyl, optionally substituted cyclyl alkyl, optionally substituted phenyl, optionally substituted heteroaryl, optionally substituted heterocyclyl, optionally substituted bicyclic heterocyclyl, optionally substituted bridged optionally substituted heterocyclyl, optionally substituted spiral, optionally substituted heterospiral, or optionally substituted spiroheterocyclyl, or two R₂ combine to form a optionally substituted cyclo alkyl, heterocyclo alkyl, aryl or heteroaryl;

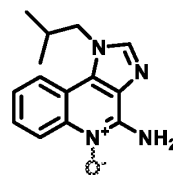
R₃ and R₄ are independently selected from the group consisting of hydrogen, alkyl, halo, haloalkyl, haloalkoxy, alkoxy, hydroxy, cyano, cyanoalkyl, carboxy, alkoxy carbonyl, acylamino, aminocarbonyl, hydroxyalkyl, hydroxyalkynyl, alkoxyalkyl, aminoalkyl, aminocarbonylalkyl, sulfonylalkyl, aminosulfonylalkyl, optionally substituted cycloalkyl, optionally substituted cyclyl alkyl, optionally substituted phenyl, optionally substituted heteroaryl, optionally substituted heterocyclyl, optionally substituted bicyclic heterocyclyl, optionally substituted bridged optionally substituted heterocyclyl, or optionally substituted spiroheterocyclyl, or R₃ and R₄ combine to form a optionally substituted C3-10 heterocyclo alkyl;

wherein the optionally substituent of R₁, R₂, R₃, R₄, R_a, R_b and R_c is independently selected from optionally substituted alkyl, cycloalkyl, cycloalkoxy, halo, haloalkyl, haloalkoxy, alkoxy, alkoxyalkyl, hydroxy, carboxyl;

n is 0, 1, 2 or 3;

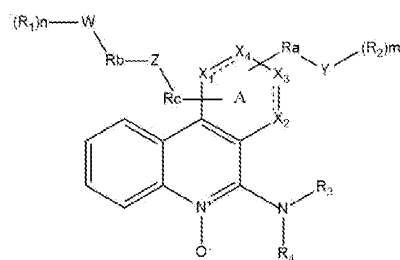
m is 0, 1, 2 or 3;

or a pharmaceutically acceptable salt thereof;

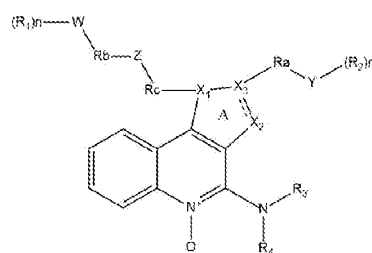


provided that the compound of formula I is not

3. The compound according to claim 2, wherein the Formula I is selected from Formula Ia and Formula Ib,



(Ia)



(Ib)

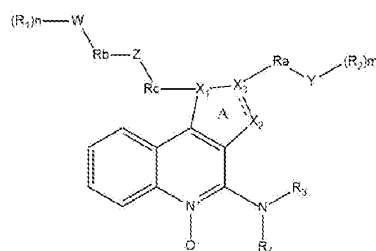
wherein

the X_1 , X_2 , X_3 , X_4 are independently selected from carbon, nitrogen, oxygen, or sulfur;

the $\sim$ is single bond or double bond;

or a pharmaceutically acceptable salt thereof.

4. The compound according to claim 3, wherein Formula I is Formula Ib :



(Ib)

wherein,

X_1 , X_2 , X_3 , are independently selected from the group consisting of carbon, nitrogen, oxygen or sulfur, optionally Y, Z, W are independently absent;

Ra, Rb and Rc is independently selected from the group consisting of a branched or non-branched, substituted or non-substituted, saturated or

unsaturated aliphatic hydrocarbon chain having 1-10 carbon atoms in its backbone chain;

each R₁ is independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, halo, haloalkyl, haloalkoxy, alkoxy, hydroxy, cyano, cyanoalkyl, optionally substituted carboxy, optionally substituted acylamino, aminocarbonyl, hydroxyalkyl, hydroxyalkynyl, alkoxyalkyl, aminoalkyl, aminocarbonylalkyl, sulfonylalkyl, aminosulfonylalkyl, optionally substituted alkoxyalkyl, optionally substituted cycloalkyl, optionally substituted cyclyl alkyl, optionally substituted phenyl, optionally substituted heteroaryl, optionally substituted heterocyclyl, optionally substituted bicyclic heterocyclyl, optionally substituted bridged optionally substituted heterocyclyl, optionally substituted spiral, optionally substituted heterospiral, or optionally substituted spiroheterocyclyl, or two R₁ combine to form a optionally substituted cyclo alkyl, heterocyclo alkyl, aryl or heteroaryl;

each R₂ is independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, halo, haloalkyl, haloalkoxy, alkoxy, hydroxy, cyano, cyanoalkyl, optionally substituted carboxy, acylamino, aminocarbonyl, hydroxyalkyl, hydroxyalkynyl, alkoxyalkyl, aminoalkyl, aminocarbonylalkyl, sulfonylalkyl, aminosulfonylalkyl, optionally substituted alkoxyalkyl, optionally substituted cycloalkyl, optionally substituted cyclyl alkyl, optionally substituted phenyl, optionally substituted heteroaryl, optionally substituted heterocyclyl, optionally substituted bicyclic heterocyclyl, optionally substituted bridged optionally substituted heterocyclyl, optionally substituted spiral, optionally substituted heterospiral, or optionally substituted spiroheterocyclyl, or two R₂ combine to form a optionally substituted cyclo alkyl, heterocyclo alkyl, aryl or heteroaryl;

R₃ and R₄ are independently selected from the group consisting of hydrogen, alkyl, halo, haloalkyl, haloalkoxy, alkoxy, hydroxy, cyano, cyanoalkyl, carboxy, alkoxycarbonyl, acylamino, aminocarbonyl, hydroxyalkyl, hydroxyalkynyl, alkoxyalkyl, aminoalkyl, aminocarbonylalkyl, sulfonylalkyl, aminosulfonylalkyl, optionally substituted cycloalkyl, optionally substituted cyclyl alkyl, optionally substituted phenyl, optionally substituted heteroaryl, optionally substituted heterocyclyl, optionally

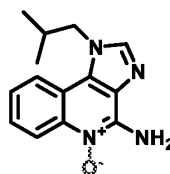
substituted bicyclic heterocyclyl, optionally substituted bridged optionally substituted heterocyclyl, or optionally substituted spiroheterocyclyl, or R_3 and R_4 combine to form a optionally substituted C3-10 heterocyclo alkyl;

wherein the optionally substituent of R_1 , R_2 , R_3 , R_4 , R_a , R_b and R_c is independently selected from optionally substituted alkyl, cycloalkyl, cycloalkoxy, halo, haloalkyl, haloalkoxy, alkoxy, alkoxyalkyl, hydroxy, carboxyl;

n is 0, 1, 2 or 3;

m is 0, 1, 2 or 3;

or a pharmaceutically acceptable salt thereof;



the compound of formula I is not

5. The compound according to any one of claim 1 to 4, wherein

The heteroatoms of ring A, X_1 , X_2 , X_3 , X_4 is independently selected from the group consisting of carbon, nitrogen;

Y is absent or independently selected from the group consisting of oxygen or nitrogen;

Z is absent or independently selected from the group consisting of oxygen or nitrogen;

W is absent or nitrogen;

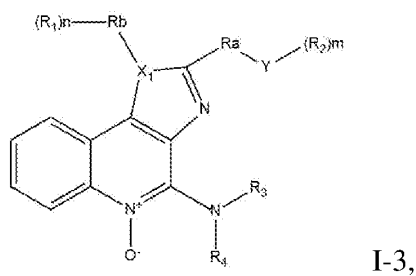
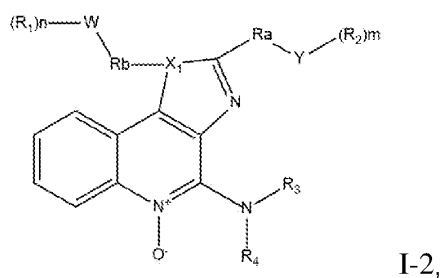
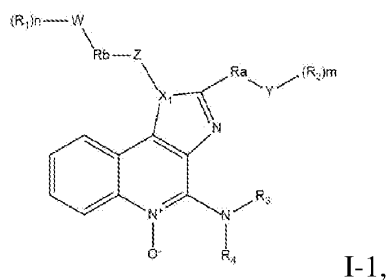
R_a is optionally substituted C_{1-10} alkylene;

R_b is independently selected from the group consisting of a optionally substituted $-C_{1-10}$ alkylene-, optionally substituted C_{6-10} aryl- C_{1-10} alkylene- or optionally substituted $-C_{1-10}$ alkylene- C_{6-10} aryl- C_{1-10} alkylene-;

R_c is optionally substituted $-C_{1-10}$ alkylene-.

6. The compound according to according to any one of claims 1 to 5, wherein

Formula I is Formula I-1, Formula I-2 or Formula I-3:

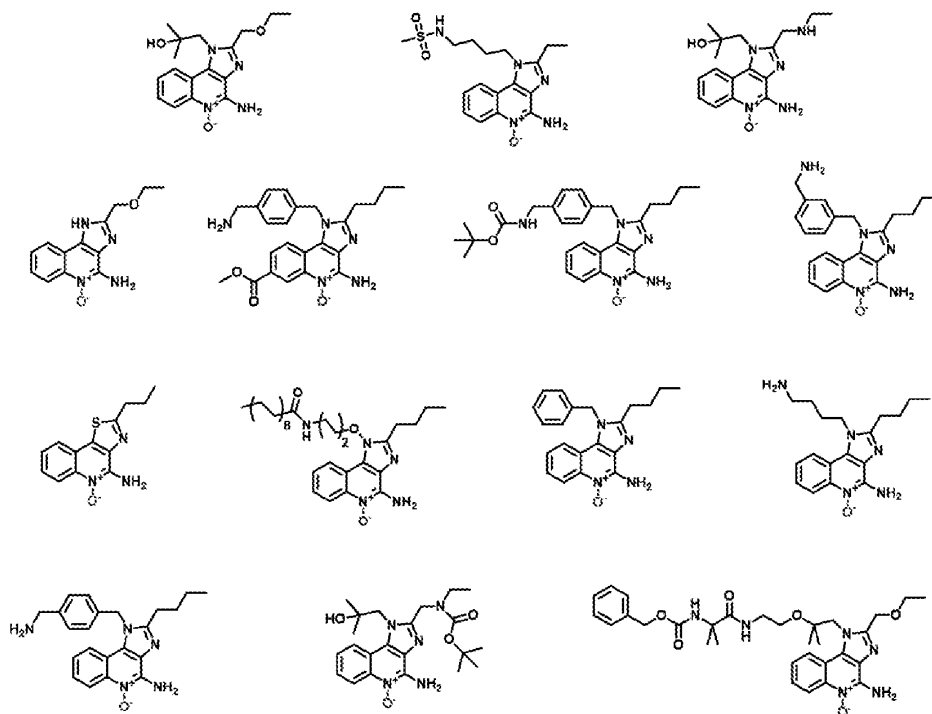


or a pharmaceutically acceptable salt thereof.

7. The compound according to any one of claims 1 to 6, wherein each R₂ is independently selected from the group consisting of hydrogen, alkyl, optionally substituted carboxy; preferably, each R₂ is independently selected from the group consisting of hydrogen, C₁₋₁₀alkyl, C₁₋₁₀alkyl-O-(CO)-.
8. The compound according to any one of claims 1 to 7, wherein each R₁ is independently selected from the group consisting of hydrogen, hydroxy, C₁₋₁₀alkyl-, C₁₋₁₀alkyl-(CO)-, C₁₋₁₀alkyl-O-(CO)-, C₁₋₁₀alkyl-SO₂-, C₁₋₁₀alkyl-NH-C₁₋₁₀alkyl-(CO)-, C₁₋₁₀alkyl-O-(CO)-NH-C₁₋₁₀alkyl-(CO)- or C₆₋₁₀aryl-C₁₋₁₀alkyl-O-(CO)-NH-C₁₋₁₀alkyl-(CO)-.
9. The compound according to any one of claims 1 to 8 wherein R₃ and R₄ are independently selected from the group consisting of hydrogen, C₁₋₁₀ alkyl, -F,

-Cl, -Br, -I, haloC₁₋₁₀alkyl, haloC₁₋₁₀alkoxy, C₁₋₁₀alkoxy, hydroxy, cyano, cyanoC₁₋₁₀alkyl.

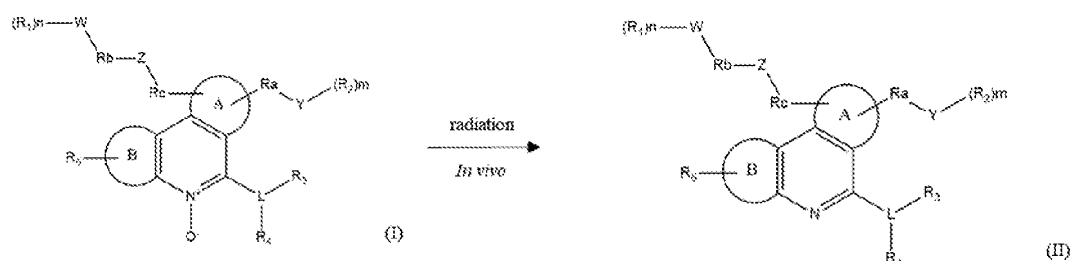
10. The compound according to claim 1, wherein the compound is selected from



or a pharmaceutically acceptable salt thereof.

11. A pharmaceutical composition comprising a compound or a pharmaceutically acceptable salt thereof according to any of claims 1 to 10, and at least one pharmaceutically acceptable diluent, excipient or inert carrier.

12. A preparation method of a non-N-oxide compound of Formula II in vivo, wherein the compound of Formula I according to any one of claims 1-10 or a pharmaceutically acceptable salt thereof is reduced to the non-N-oxide compound by irradiation in the subject in need thereof,



13. Use of a compound according to any one of claims 1-10 or a pharmaceutically acceptable salt thereof in the manufacture of a medicament in the treatment of cancer in the subject in need thereof; or

a method for treating cancer, comprising administering therapeutically effective amounts of a compound according to any one of claims 1-10 or a pharmaceutically acceptable salt thereof to the subject in need thereof; or

the compound according to any one of claims 1-10 or a pharmaceutically acceptable salt thereof, for use as a medicament for treating cancer in the subject in need thereof.

14. The use, method or compound according to claim 13, wherein the subject is given radiation after administering the compound, preferably 20 mins, 1 hour, 2 hours, 4 hours after administering the compound.

15. The use, method or compounds according to claim 13 or 14, wherein the cancer is solid cancer or non-solid tumors, preferably the cancer is selected from the group consisting of colon cancer, small cell lung cancer, Hodgkin's lymphoma, malignant lymphoma, melanoma, nasopharyngeal cancer, head and neck cancer, skin cancer, esophageal cancer, lung cancer, liver cancer, prostate cancer, cervical cancer, thyroid cancer and breast cancer, preferably the cancer is primary cancer and/or secondary cancer.

16. The use, method or compounds according to any one of claims 13 to 15, wherein the compound is administrated in an amount of from about 0.005 mg/kg to about 100 mg/kg, such as an amount of about 0.005, 0.05, 0.5, 5, 10, 20, 30, 40, 50, 100 mg/kg.

17. The use, method or compounds according to any one of claims 13 to 16,

wherein the compound and the radiation are administered once every two days, once every three days, once every four days, once every five days, once every seven days, once every ten days, once every two weeks, once every three weeks, once every four weeks, and the compound and radiation are administered continuously for at least 3 rounds, at least 4 rounds, at least 5 rounds, at least 6 rounds, at least 7 rounds, at least 8 rounds, at least 9 rounds, at least 10 rounds.

18. The use, method or compounds according to any one of claims 13 to 17, wherein the compound is administrated orally, parenterally, intraperitoneally, intravenously, intraarterially, transdermally, sublingually, intramuscularly, rectally, transbuccally, intranasally, liposomally, via inhalation, vaginally, intraocularly, via local delivery, subcutaneously, intraadiposally, intraarticularly, intrathecal, intratumorally.
19. The use, method or compounds according to any one of claims 13 to 18, wherein the radiation is X-ray or gamma ray.
20. The use, method or compounds according to claims 19, wherein the intensity of the X-ray or gamma ray is 60 Gy or lower, 50 Gy or lower, 40 Gy or lower, 30 Gy or lower, 20 Gy or lower, 10 Gy or lower, 8 Gy or lower, 6 Gy or lower, 4 Gy or lower.