

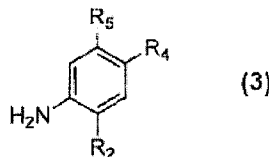
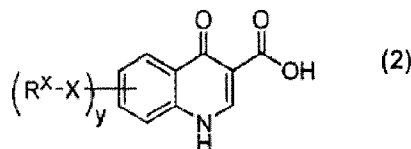
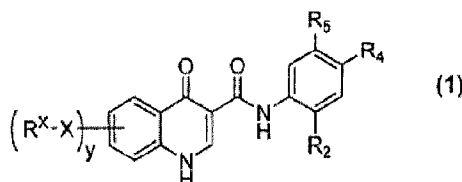


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(54) **Titre : PROCÉDE POUR PRÉPARER DES MODULATEURS DE RÉGULATEUR DE CONDUCTANCE TRANSMÉBRANAIRE DE MUCOVISCIDOSE**

(54) **Title: PROCESS FOR MAKING MODULATORS OF CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR**



(57) **Abrégé/Abstract:**

The invention provides a process for the preparation of a compound of Formula 1;

(see formula 1)

comprising coupling a carboxylic acid of Formula 2;

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(57) **Abrégé(suite)/Abstract(continued)**:

(see formula 2)

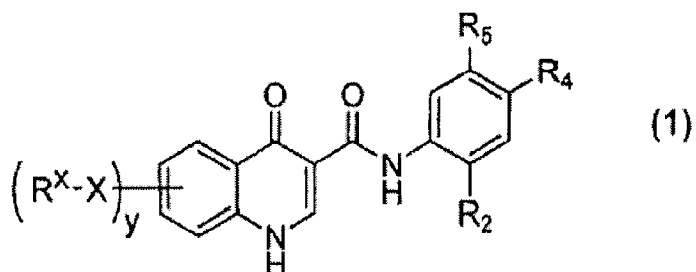
with an aniline of Formula 3;

(see formula 3)

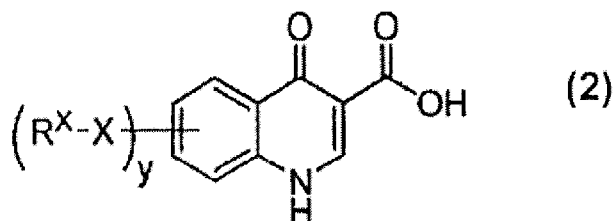
in the presence of a coupling agent.

ABSTRACT

The invention provides a process for the preparation of a compound of Formula 1;

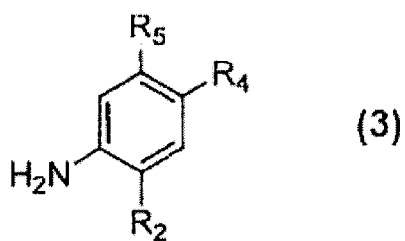


comprising coupling a carboxylic acid of Formula 2;



5

with an aniline of Formula 3;



in the presence of a coupling agent.

**PROCESS FOR MAKING MODULATORS OF CYSTIC FIBROSIS TRANSMEMBRANE
CONDUCTANCE REGULATOR**

CLAIM OF PRIORITY

[0001] This application is a divisional of Canadian patent application number 2756031, filed on March 19, 2010, and claims priority to three U.S. Patent Applications having Serial Nos. 61/162,148, filed on March 20, 2009; 61/246,303, filed on September 28, 2009; and 61/248,565, filed on October 5, 2009.

TECHNICAL FIELD OF THE INVENTION

[0002] The present invention relates to a process for making modulators of cystic fibrosis transmembrane conductance regulator ("CFTR").

BACKGROUND OF THE INVENTION

[0003] Cystic fibrosis (CF) is a recessive genetic disease that affects approximately 30,000 children and adults in the United States and approximately 30,000 children and adults in Europe. Despite progress in the treatment of CF, there is no cure.

[0004] CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene that encodes an epithelial chloride ion channel responsible for aiding in the regulation of salt and water absorption and secretion in various tissues. Small molecule drugs, known as potentiators that increase the probability of CFTR channel opening represent one potential therapeutic strategy to treat CF.

[0005] Specifically, CFTR is a cAMP/ATP-mediated anion channel that is expressed in a variety of cells types, including absorptive and secretory epithelia cells, where it regulates anion flux across the membrane, as well as the activity of other ion channels and proteins. In epithelia cells, normal functioning of CFTR is critical for the maintenance of electrolyte transport throughout the body, including respiratory and digestive tissue. CFTR is composed of approximately 1480 amino acids that encode a protein made up of a tandem repeat of transmembrane domains, each containing six transmembrane helices and a nucleotide binding domain. The two transmembrane domains are linked by a large, polar, regulatory (R)-domain with multiple phosphorylation sites that regulate channel activity and cellular trafficking.

[0006] The gene encoding CFTR has been identified and sequenced (See Gregory, R. J. et al. (1990) Nature 347:382-386; Rich, D. P. et al. (1990) Nature 347:358-362), (Riordan, J. R. et al. (1989) Science 245:1066-1073). A defect in this gene causes mutations in CFTR resulting in cystic fibrosis ("CF"), the most common fatal genetic disease in humans. Cystic fibrosis affects approximately one in every 2,500 infants in the United States. Within the

general United States population, up to 10 million people carry a single copy of the defective gene without apparent ill effects. In contrast, individuals with two copies of the CF associated gene suffer from the debilitating and fatal effects of CF, including chronic lung disease.

[0007] In patients with CF, mutations in CFTR endogenously expressed in respiratory epithelia leads to reduced apical anion secretion causing an imbalance in ion and fluid transport. The resulting decrease in anion transport contributes to enhanced mucus accumulation in the lung and the accompanying microbial infections that ultimately cause death in CF patients. In addition to respiratory disease, CF patients typically suffer from gastrointestinal problems and pancreatic insufficiency that, if left untreated, results in death. In addition, the majority of males with cystic fibrosis are infertile and fertility is decreased among females with cystic fibrosis. In contrast to the severe effects of two copies of the CF associated gene, individuals with a single copy of the CF associated gene exhibit increased resistance to cholera and to dehydration resulting from diarrhea – perhaps explaining the relatively high frequency of the CF gene within the population.

[0008] Sequence analysis of the CFTR gene of CF chromosomes has revealed a variety of disease causing mutations (Cutting, G. R. et al. (1990) *Nature* 346:366-369; Dean, M. et al. (1990) *Cell* 61:863:870; and Kerem, B-S. et al. (1989) *Science* 245:1073-1080; Kerem, B-S et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:8447-8451). To date, greater than 1000 disease causing mutations in the CF gene have been identified (<http://www.genet.sickkids.on.ca/cftr/app>). The most prevalent mutation is a deletion of phenylalanine at position 508 of the CFTR amino acid sequence, and is commonly referred to as $\Delta F508$ -CFTR. This mutation occurs in approximately 70% of the cases of cystic fibrosis and is associated with a severe disease.

[0009] The deletion of residue 508 in $\Delta F508$ -CFTR prevents the nascent protein from folding correctly. This results in the inability of the mutant protein to exit the ER, and traffic to the plasma membrane. As a result, the number of channels present in the membrane is far less than observed in cells expressing wild-type CFTR. In addition to impaired trafficking, the mutation results in defective channel gating. Together, the reduced number of channels in the membrane and the defective gating lead to reduced anion transport across epithelia leading to defective ion and fluid transport. (Quinton, P. M. (1990), *FASEB J.* 4: 2709-2727). Studies have shown, however, that the reduced numbers of $\Delta F508$ -CFTR in the membrane are functional, albeit less than wild-type CFTR. (Dalemans et al. (1991), *Nature Lond.* 354: 526-528; Denning et al., supra; Pasyk and Foskett (1995), *J. Cell. Biochem.* 270:

12347-50). In addition to $\Delta F508$ -CFTR, other disease causing mutations in CFTR that result in defective trafficking, synthesis, and/or channel gating could be up- or down-regulated to alter anion secretion and modify disease progression and/or severity.

[0010] Although CFTR transports a variety of molecules in addition to anions, it is clear that this role (the transport of anions) represents one element in an important mechanism of transporting ions and water across the epithelium. The other elements include the epithelial Na^+ channel, ENaC, $\text{Na}^+/\text{2Cl}^-/\text{K}^+$ co-transporter, Na^+/K^+ -ATPase pump and the basolateral membrane K^+ channels, that are responsible for the uptake of chloride into the cell.

[0011] These elements work together to achieve directional transport across the epithelium via their selective expression and localization within the cell. Chloride absorption takes place by the coordinated activity of ENaC and CFTR present on the apical membrane and the Na^+/K^+ -ATPase pump and Cl^- ion channels expressed on the basolateral surface of the cell.

Secondary active transport of chloride from the luminal side leads to the accumulation of intracellular chloride, which can then passively leave the cell via Cl^- channels, resulting in a vectorial transport. Arrangement of $\text{Na}^+/\text{2Cl}^-/\text{K}^+$ co-transporter, Na^+/K^+ -ATPase pump and the basolateral membrane K^+ channels on the basolateral surface and CFTR on the luminal side coordinate the secretion of chloride via CFTR on the luminal side. Because water is probably never actively transported itself, its flow across epithelia depends on tiny transepithelial osmotic gradients generated by the bulk flow of sodium and chloride.

[0012] As discussed above, it is believed that the deletion of residue 508 in $\Delta F508$ -CFTR prevents the nascent protein from folding correctly, resulting in the inability of this mutant protein to exit the ER, and traffic to the plasma membrane. As a result, insufficient amounts of the mature protein are present at the plasma membrane and chloride transport within epithelial tissues is significantly reduced. In fact, this cellular phenomenon of defective ER processing of ABC transporters by the ER machinery has been shown to be the underlying basis not only for CF disease, but for a wide range of other isolated and inherited diseases.

[0013] Accordingly, there is a need for modulators of CFTR activity, and compositions thereof, which can be used to modulate the activity of the CFTR in the cell membrane of a mammal.

[0014] There is a need for methods of treating diseases caused by mutation in CFTR using such modulators of CFTR activity.

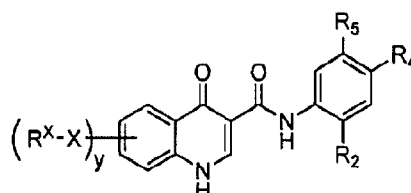
[0015] There is a need for methods of modulating CFTR activity in an *ex vivo* cell membrane of a mammal.

[0016] There is also a need for processes for the preparation of compounds which modulate CFTR activity.

SUMMARY OF THE INVENTION

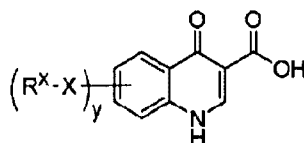
[0017] In general, the invention provides processes for the preparation of compounds useful as modulators of CFTR.

[0018] In one aspect, the invention provides a process for the preparation of a compound of Formula 1,



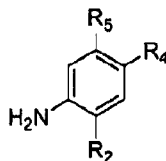
Formula 1

comprising coupling a carboxylic acid of Formula 2



Formula 2

with an aniline of Formula 3



Formula 3

in the presence of a coupling agent selected from the group consisting of 2-chloro-1,3-dimethyl-2-imidazolium tetrafluoroborate, HBTU, HCTU, 2-chloro-4,6-dimethoxy-1,3,5-triazine, HATU, HOBT/EDC, and T3P®.

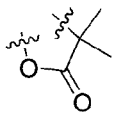
Each R_2 and R_4 is independently selected from hydrogen, CN, CF_3 , halo, C_{1-6} straight or branched alkyl, 3-12 membered cycloaliphatic, phenyl, C_{5-10} heteroaryl or C_{3-7} heterocyclic, wherein said heteroaryl or heterocyclic has up to 3 heteroatoms selected from O, S, or N, and each C_{1-6} straight or branched alkyl, 3-12 membered cycloaliphatic, phenyl, C_{5-10} heteroaryl or C_{3-7} heterocyclic is independently and optionally substituted with up to three substituents selected from $-OR'$, $-CF_3$, $-OCF_3$, SR' , $S(O)R'$, SO_2R' , $-SCF_3$, halo, CN,

—COOR', —COR—, —O(CH₂)₂N(R')(R'), —O(CH₂)N(R')(R'), —CON(R')(R'),
 —(CH₂)₂OR', —(CH₂)OR', CH₂CN, optionally substituted phenyl or phenoxy, —N(R')(R'),
 —NR'C(O)OR', —NR'C(O)R', —(CH₂)₂N(R')(R'), or —(CH₂)N(R')(R').

Each R₅ is independently selected from hydrogen, —OH, NH₂, CN, CHF₂, NHR',
 N(R')₂, —NHC(O)R', NHC(O)OR', NHSO₂R', —OR', OC(O)OR', OC(O)NHR', OC(O)NR'₂,
 CH₂OH, CH₂N(R')₂, C(O)OR', SO₂NHR', SO₂N(R')₂, or CH₂NHC(O)OR'.

Or R₄ and R₅ are taken together form a 5-7 membered ring containing 0-3 three
 heteroatoms selected from N, O, or S, wherein said ring is optionally substituted with up to
 three R₃ substituents.

In some embodiments, R₅ is —OC(O)OR', —OC(O)NHR', —OC(O)N(R')₂, wherein
 R' is not hydrogen, or



R₄ and R₅ are taken together to form the moiety

Each X is independently a bond or is an optionally substituted C₁₋₆ alkylidene chain
 wherein up to two methylene units of X are optionally and independently replaced by
 —CO—, —CS—, —COCO—, —CONR'—, —CONR'NR'—, —CO₂—, —OCO—,
 —NR'CO₂—, —O—, —NR'CONR'—, —OCONR'—, —NR'NR', —NR'NR'CO—,
 —NR'CO—, —S—, —SO—, —SO₂—, —NR'—, —SO₂NR'—, NR'SO₂—, or —
 NR'SO₂NR'—. Each R^X is independently R', halo, NO₂, CN, CF₃, or OCF₃.

y is an integer from 0-4.

Each R' is independently selected from hydrogen or an optionally substituted group
 selected from a C₁₋₈ aliphatic group, a 3-8-membered saturated, partially unsaturated, or fully
 unsaturated monocyclic ring having 0-3 heteroatoms independently selected from nitrogen,
 oxygen, or sulfur, or an 8-12 membered saturated, partially unsaturated, or fully unsaturated
 bicyclic ring system having 0-5 heteroatoms independently selected from nitrogen, oxygen, or
 sulfur; or two occurrences of R' are taken together with the atom(s) to which they are bound to
 form an optionally substituted 3-12 membered saturated, partially unsaturated, or fully
 unsaturated monocyclic or bicyclic ring having 0-4 heteroatoms independently selected from
 N, O, or S.

Each R_3 is independently $-C_1-C_3$ alkyl, C_1-C_3 perhaloalkyl, $-O(C_1-C_3$ alkyl), $-CF_3$, $-OCF_3$, $-SCF_3$, $-F$, $-Cl$, $-Br$, $-COOR'$, $-COR'$, $-O(CH_2)_2N(R')(R')$, $-O(CH_2)N(R')(R')$, $-CON(R')(R')$, $-(CH_2)_2OR'$, $-(CH_2)OR'$, optionally substituted monocyclic or bicyclic aromatic ring, optionally substituted arylsulfone, optionally substituted 5-membered heteroaryl ring, $-N(R')(R')$, $-(CH_2)_2N(R')(R')$, or $-(CH_2)N(R')(R')$.

[0019] Embodiments of this aspect include one or more of the following features. R_5 is independently $-OC(O)OR'$, $-OC(O)NHR'$, or $-OC(O)N(R')_2$, and R' is not hydrogen; at least one of R_4 or R_2 is independently a C_{1-6} straight or branched alkyl which is substituted

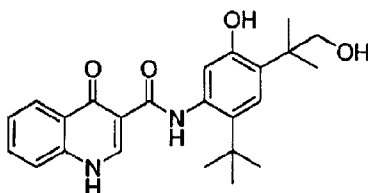
with $-\text{COOR}'$ or $-\text{CON}(\text{R}')(\text{R}')$, and R' is not hydrogen. The process further comprises cleaving the $-\text{OC}(\text{O})\text{OR}'$, $-\text{OC}(\text{O})\text{NHR}'$, or $-\text{OC}(\text{O})\text{N}(\text{R}')_2$ group to form $-\text{OH}$. The process further comprises hydrolyzing each $-\text{COOR}'$, or $-\text{CON}(\text{R}')_2$ group to form $-\text{COOH}$. The hydrolysis is performed by treating a compound of Formula 1 with an alcoholic solvent in the presence of base such as NaOH, KOH or sodium methoxide. The alcoholic solvent used in the hydrolysis is methanol. The coupling a compound of Formula 2 and a compound of Formula 3 to produce a compound of Formula 1 is performed in the presence of a base such as K_2CO_3 , Et_3N , NMM, pyridine or DIEA. The coupling a compound of Formula 2 and a compound of Formula 3 to produce a compound of Formula 1 is performed in the presence of a solvent such as EtOAc, IPAc, THF, MEK, NMP, acetonitrile, DMF, or 2-methyltetrahydrofuran. The coupling a compound of Formula 2 and a compound of Formula 3 to produce a compound of Formula 1 is performed at a reaction temperature which is maintained between about 10°C and 78°C such as between about 20°C and 30°C , between about 40°C and 50°C , and between about 42°C and 53°C . The coupling reaction is stirred for at least 2 hours such as for at least 70 hours or for at least 3 days.

[0020] In some embodiments, R_5 is independently $-\text{OC}(\text{O})\text{OR}'$, $-\text{OC}(\text{O})\text{NHR}'$, or $-\text{OC}(\text{O})\text{N}(\text{R}')_2$, and R' is not hydrogen; and each of R_2 and R_4 is independently selected from hydrogen, CF_3 , $\text{C}_1\text{-C}_6$ straight or branched alkyl, 3-12 membered cycloaliphatic or phenyl.

[0021] In some further embodiments, R_5 is independently $-\text{OC}(\text{O})\text{OR}'$, and R' is not hydrogen; and each of R_2 and R_4 is independently $\text{C}_1\text{-C}_6$ straight or branched alkyl or 3-12 membered cycloaliphatic.

[0022] In some embodiments, R_2 and R_4 are *t*-butyl.

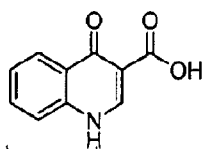
[0023] In another aspect, the invention provides a process for the preparation of compound 27



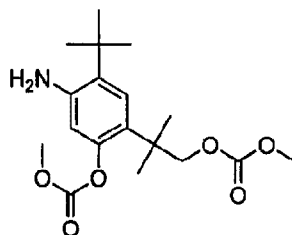
Compound 27

comprising:

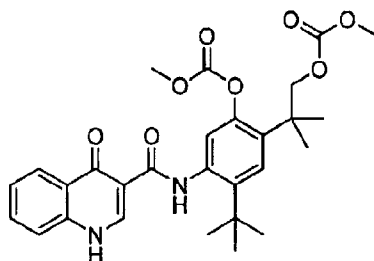
(a) coupling compound 26

**Compound 26**

with compound 13

**Compound 13**

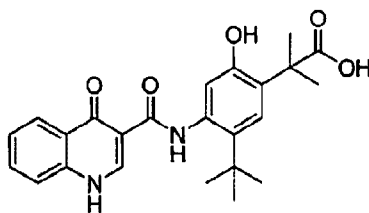
in the presence of EDCI, HOBT and DIEA using DMF as the solvent, wherein the reaction temperature is maintained between about 20 °C and 30 °C, and the reaction is allowed proceed for at least 70 hours, to produce compound 14

**Compound 14**

; and

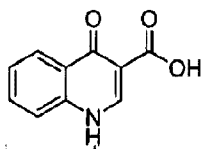
(b) treating compound 14 with KOH in methanol.

[0024] In still another aspect, the invention provides a process for the preparation of compound 28

**Compound 28**

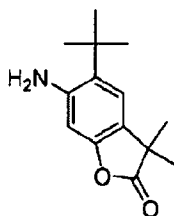
comprising:

(a) coupling compound 26



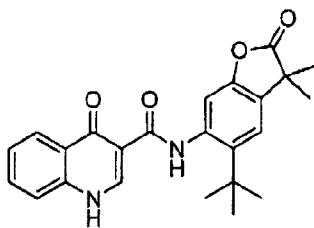
Compound 26

with compound 20



Compound 20

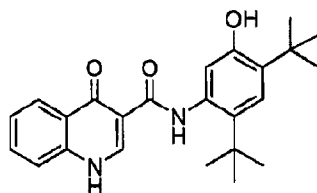
in the presence of HATU and DIEA using acetonitrile as the solvent, wherein the reaction temperature is maintained between about 40 °C and 50 °C, and wherein the reaction is allowed proceed for at least 3 days, to produce compound 21



Compound 21 ; and

(b) treating compound 21 with NaOH in methanol.

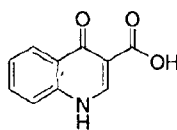
[0025] In yet another aspect, the invention provides a process for the preparation of compound 34



Compound 34

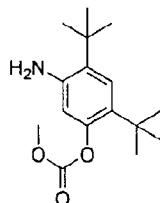
comprising:

(a) coupling compound 26



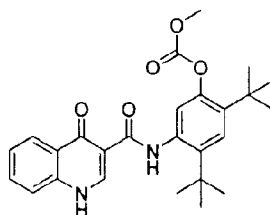
Compound 26

with compound 32



Compound 32

in the presence of T3P® and pyridine using 2-methyl tetrahydrofuran as the solvent, wherein the reaction temperature is maintained between about 42 °C and 53 °C, and wherein the reaction is allowed proceed for at least 2 hours, to produce compound 33



Compound 33 ;

b) treating compound 33 with NaOMe/MeOH in 2-methyl tetrahydrofuran, followed by a quench step.

[0026] In one embodiment, the method further includes the step of forming a slurry of compound 34 in isopropyl acetate or in a mixture of acetonitrile and water, wherein the solid form of compound 34 is converted to Compound 34, which may be isolated.

[0027] Embodiments of the foregoing aspect include one or more of the following features. The process further comprises dissolving Compound 34 in a biphasic solution of 2-methyltetrahydrofuran and 0.1N HCl, which is stirred. The process further comprises separating the organic phase from the biphasic solution. The process further comprises filtering and removing solid matter from the organic phase. The process further comprises reducing the volume of the organic phase by approximately 50% using distillation. The process further comprises performing thrice the procedure of: adding MeOAc, EtOAc, IPAc, *t*-BuOAc, tetrahydrofuran (THF), Et₂O or methyl-*t*-butyl ether (MTBE) to the organic phase until the volume of the organic phase increases by 100% and reducing the volume of the

organic phase by 50% using distillation. The process further comprises adding MeOAc, EtOAc, IPAc, *t*-BuOAc, tetrahydrofuran (THF), Et₂O or methyl-*t*-butyl ether (MTBE) to the organic phase until the volume of the organic phase increases by 100%. The process further comprises heating the organic phase to reflux temperature, and maintaining said reflux temperature for a time at least about 5 hours. The process further comprises cooling the organic phase to a temperature between -5 °C and 5 °C over a time period of 4.5 hours to 5.5 hours.

[0028] In still another aspect, the invention provides compounds produced by any process described herein.

[0029] In a further aspect, the invention provides a pharmaceutical composition comprising a compound produced by any process described herein.

[0030] In still a further aspect, the invention provides a method of modulating CFTR activity in a biological sample comprising the step of contacting said biological sample with a compound produced by any process described herein.

[0031] In another aspect, the invention also provides a method of treating or lessening the severity of a disease in a patient comprising administering to said patient one of the compositions as defined herein, and said disease is selected from cystic fibrosis, asthma, smoke induced COPD, chronic bronchitis, rhinosinusitis, constipation, pancreatitis, pancreatic insufficiency, male infertility caused by congenital bilateral absence of the vas deferens (CBAVD), mild pulmonary disease, idiopathic pancreatitis, allergic bronchopulmonary aspergillosis (ABPA), liver disease, hereditary emphysema, hereditary hemochromatosis, coagulation-fibrinolysis deficiencies, such as protein C deficiency, Type 1 hereditary angioedema, lipid processing deficiencies, such as familial hypercholesterolemia, Type 1 chylomicronemia, abetalipoproteinemia, lysosomal storage diseases, such as I-cell disease/pseudo-Hurler, mucopolysaccharidoses, Sandhof/Tay-Sachs, Crigler-Najjar type II, polyendocrinopathy/hyperinsulemia, Diabetes mellitus, Laron dwarfism, myeloperoxidase deficiency, primary hypoparathyroidism, melanoma, glycanosis CDG type 1, congenital hyperthyroidism, osteogenesis imperfecta, hereditary hypofibrinogenemia, ACT deficiency, Diabetes insipidus (DI), neurophyseal DI, neprogenic DI, Charcot-Marie Tooth syndrome, Perlizaeus-Merzbacher disease, neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, progressive supranuclear palsy, Pick's disease, several polyglutamine neurological disorders such as Huntington's, spinocerebellar ataxia type I, spinal and bulbar muscular atrophy, dentatorubal pallidoluisian, and myotonic dystrophy, as well as spongiform encephalopathies, such as hereditary Creutzfeldt-Jakob

disease (due to prion protein processing defect), Fabry disease, Strausler-Scheinker syndrome, COPD, dry-eye disease, or Sjogren's disease, Osteoporosis, Osteopenia, bone healing and bone growth (including bone repair, bone regeneration, reducing bone resorption and increasing bone deposition), Gorham's Syndrome, chloride channelopathies such as myotonia congenita (Thomson and Becker forms), Bartter's syndrome type III, Dent's disease, hyperekplexia, epilepsy, hyperekplexia, lysosomal storage disease, Angelman syndrome, and Primary Ciliary Dyskinesia (PCD), a term for inherited disorders of the structure and/or function of cilia, including PCD with situs inversus (also known as Kartagener syndrome), PCD without situs inversus and ciliary aplasia.

[0032] In certain embodiments, the disease is cystic fibrosis.

[0033] In another aspect, the invention provides a kit for use in measuring the activity of CFTR or a fragment thereof in a biological sample in vitro or in vivo, comprising:

- i. a composition comprising a compound produced by any process described herein; and
- ii. instructions for:
 - a. contacting the composition with the biological sample; and
 - b. measuring the activity of said CFTR or a fragment thereof.

[0034] In certain embodiments, the kit further comprises instructions for:

- i. contacting an additional compound with the biological sample;
- ii. measuring the activity of said CFTR or a fragment thereof in the presence of said additional compound; and
- iii. comparing the activity of the CFTR in the presence of the additional compound with the density of the CFTR in the presence of a composition of Formula 1.

[0035] Advantageously, the invention provides processes for the synthesis of compounds useful as modulators of CFTR in higher yield and in higher purity relative to known processes.

DETAILED DESCRIPTION

[0036] I. DEFINITIONS

[0037] As used herein, the following definitions shall apply unless otherwise indicated.

[0038] The term "ABC-transporter" as used herein means an ABC-transporter protein or a fragment thereof comprising at least one binding domain, wherein said protein or fragment thereof is present in vivo or in vitro. The term "binding domain" as used herein means a domain on the ABC-transporter that can bind to a modulator. See, e.g., Hwang, T. C. et al., J. Gen. Physiol. (1998): 111(3), 477-90.

[0039] The term "CFTR" as used herein means cystic fibrosis transmembrane conductance regulator or a mutation thereof capable of regulator activity, including, but not limited to, ΔF508 CFTR and G551D CFTR (see, e.g., <http://www.genet.sickkids.on.ca/cftr/>, for CFTR mutations).

[0040] The term "modulating" as used herein means increasing or decreasing by a measurable amount.

[0041] For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed. Additionally, general principles of organic chemistry are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 1999, and "March's Advanced Organic Chemistry", 5th Ed., Ed.: Smith, M. B. and March, J., John Wiley & Sons, New York: 2001.

[0042] As described herein, compounds of the invention may optionally be substituted with one or more substituents, such as are illustrated generally above, or as exemplified by particular classes, subclasses, and species of the invention. It will be appreciated that the phrase "optionally substituted" is used interchangeably with the phrase "substituted or unsubstituted." In general, the term "substituted", whether preceded by the term "optionally" or not, refers to the replacement of hydrogen radicals in a given structure with the radical of a specified substituent.

[0043] Unless otherwise indicated, an optionally substituted group may have a substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. Combinations of substituents envisioned by this invention are preferably those that result in the formation of stable or chemically feasible compounds.

[0044] The term "stable", as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and preferably their recovery, purification, and use for one or more of the purposes disclosed herein. In some embodiments, a stable compound or chemically feasible compound is one that is not substantially altered when kept at a temperature of 40 °C or less, in the absence of moisture or other chemically reactive conditions, for at least a week.

[0045] The term "aliphatic" or "aliphatic group", as used herein, means a straight-chain (i.e., unbranched) or branched, substituted or unsubstituted hydrocarbon chain that is completely saturated or that contains one or more units of unsaturation, or a monocyclic hydrocarbon or

bicyclic hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic (also referred to herein as "carbocycle", "cycloaliphatic", or "cycloalkyl"), that has a single point of attachment to the rest of the molecule. Unless otherwise specified, aliphatic groups contain 1-20 aliphatic carbon atoms. In some embodiments, aliphatic groups contain 1-10 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-8 aliphatic carbon atoms. In still other embodiments, aliphatic groups contain 1-6 aliphatic carbon atoms, and in yet other embodiments aliphatic groups contain 1-4 aliphatic carbon atoms. In some embodiments, "cycloaliphatic" (or "carbocycle" or "cycloalkyl") refers to a monocyclic C₃₋₈ hydrocarbon or bicyclic or tricyclic C₈₋₁₄ hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic, that has a single point of attachment to the rest of the molecule wherein any individual ring in said bicyclic ring system has 3-7 members. Suitable aliphatic groups include, but are not limited to, linear or branched, substituted or unsubstituted alkyl, alkenyl, alkynyl groups and hybrids thereof such as (cycloalkyl)alkyl, (cycloalkenyl)alkyl or (cycloalkyl)alkenyl. Suitable cycloaliphatic groups include cycloalkyl, bicyclic cycloalkyl (e.g., decalin), bridged bicycloalkyl such as norbornyl or [2.2.2]bicyclo-octyl, or bridged tricyclic such as adamantyl.

[0046] The term "heteroaliphatic", as used herein, means aliphatic groups wherein one or two carbon atoms are independently replaced by one or more of oxygen, sulfur, nitrogen, phosphorus, or silicon. Heteroaliphatic groups may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and include "heterocycle", "heterocyclyl", "heterocycloaliphatic", or "heterocyclic" groups.

[0047] The term "heterocycle", "heterocyclyl", "heterocycloaliphatic", or "heterocyclic" as used herein means non-aromatic, monocyclic, bicyclic, or tricyclic ring systems in which one or more ring members is an independently selected heteroatom. In some embodiments, the "heterocycle", "heterocyclyl", "heterocycloaliphatic", or "heterocyclic" group has three to fourteen ring members in which one or more ring members is a heteroatom independently selected from oxygen, sulfur, nitrogen, or phosphorus, and each ring in the system contains 3 to 7 ring members.

[0048] The term "heteroatom" means one or more of oxygen, sulfur, nitrogen, phosphorus, or silicon (including, any oxidized form of nitrogen, sulfur, phosphorus, or silicon; the quaternized form of any basic nitrogen or; a substitutable nitrogen of a heterocyclic ring, for example N (as in 3,4-dihydro-2H-pyrrolyl), NH (as in pyrrolidinyl) or NR⁺ (as in N-substituted pyrrolidinyl)).

[0049] The term "unsaturated", as used herein, means that a moiety has one or more units of unsaturation.

[0050] The term "alkoxy", or "thioalkyl", as used herein, refers to an alkyl group, as previously defined, attached to the principal carbon chain through an oxygen ("alkoxy") or sulfur ("thioalkyl") atom.

[0051] The terms "haloaliphatic" and "haloalkoxy" means aliphatic or alkoxy, as the case may be, substituted with one or more halo atoms. The term "halogen" or "halo" means F, Cl, Br, or I. Examples of haloaliphatic include —CHF_2 , $\text{—CH}_2\text{F}$, —CF_3 , $\text{—CF}_2\text{—}$, or perhaloalkyl, such as, $\text{—CF}_2\text{CF}_3$.

[0052] The term "aryl" used alone or as part of a larger moiety as in "aralkyl", "aralkoxy", or "aryloxyalkyl", refers to monocyclic, bicyclic, and tricyclic ring systems having a total of five to fourteen ring members, wherein at least one ring in the system is aromatic and wherein each ring in the system contains 3 to 7 ring members. The term "aryl" may be used interchangeably with the term "aryl ring". The term "aryl" also refers to heteroaryl ring systems as defined herein below.

[0053] The term "heteroaryl", used alone or as part of a larger moiety as in "heteroaralkyl" or "heteroarylalkoxy", refers to monocyclic, bicyclic, and tricyclic ring systems having a total of five to fourteen ring members, wherein at least one ring in the system is aromatic, at least one ring in the system contains one or more heteroatoms, and wherein each ring in the system contains 3 to 7 ring members. The term "heteroaryl" may be used interchangeably with the term "heteroaryl ring" or the term "heteroaromatic".

[0054] An aryl (including aralkyl, aralkoxy, aryloxyalkyl and the like) or heteroaryl (including heteroaralkyl and heteroarylalkoxy and the like) group may contain one or more substituents. Suitable substituents on the unsaturated carbon atom of an aryl or heteroaryl group are selected from halo; —R° ; —OR° ; —SR° ; 1,2-methylene-dioxy; 1,2-ethylenedioxy; phenyl (Ph) optionally substituted with R° ; —O(Ph) optionally substituted with R° ; $\text{—(CH}_2\text{)}_{1-2}\text{(Ph)}$, optionally substituted with R° ; —CH=CH(Ph) , optionally substituted with R° ; —NO_2 ; —CN ; $\text{—N(R}^\circ\text{)}_2$; $\text{—NR}^\circ\text{C(O)R}^\circ$; $\text{—NR}^\circ\text{C(O)N(R}^\circ\text{)}_2$; $\text{—NR}^\circ\text{CO}_2\text{R}^\circ$; $\text{—NR}^\circ\text{NR}^\circ\text{C(O)R}^\circ$; $\text{—NR}^\circ\text{NR}^\circ\text{C(O)N(R}^\circ\text{)}_2$; $\text{—NR}^\circ\text{NR}^\circ\text{CO}_2\text{R}^\circ$; —C(O)C(O)R° ; $\text{—C(O)CH}_2\text{C(O)R}^\circ$; $\text{—CO}_2\text{R}^\circ$; —C(O)R° ; $\text{—C(O)N(R}^\circ\text{)}_2$; $\text{—OC(O)N(R}^\circ\text{)}_2$; $\text{—S(O)}_2\text{R}^\circ$; $\text{—SO}_2\text{N(R}^\circ\text{)}_2$; —S(O)R° ; $\text{—NR}^\circ\text{SO}_2\text{N(R}^\circ\text{)}_2$; $\text{—NR}^\circ\text{SO}_2\text{R}^\circ$; $\text{—C(=S)N(R}^\circ\text{)}_2$; $\text{—C(=NH)—N(R}^\circ\text{)}_2$; or $\text{—(CH}_2\text{)}_{0-2}\text{NHC(O)R}^\circ$ wherein each independent occurrence of R° is selected from hydrogen, optionally substituted C_{1-6} aliphatic, an

unsubstituted 5-6 membered heteroaryl or heterocyclic ring, phenyl, —O(Ph), or —CH₂(Ph), or, notwithstanding the definition above, two independent occurrences of R^o, on the same substituent or different substituents, taken together with the atom(s) to which each R^o group is bound, form a 3-8-membered cycloalkyl, heterocyclyl, aryl, or heteroaryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Optional substituents on the aliphatic group of R^o are selected from NH₂, NH(C₁₋₄aliphatic), N(C₁₋₄aliphatic)₂, halo, C₁₋₄ aliphatic, OH, O(C₁₋₄ aliphatic), NO₂, CN, CO₂H, CO₂(C₁₋₄ aliphatic), O(halo C₁₋₄ aliphatic), or haloC₁₋₄ aliphatic, wherein each of the foregoing C₁₋₄ aliphatic groups of R^o is unsubstituted.

[0055] An aliphatic or heteroaliphatic group, or a non-aromatic heterocyclic ring may contain one or more substituents. Suitable substituents on the saturated carbon of an aliphatic or heteroaliphatic group, or of a non-aromatic heterocyclic ring are selected from those listed above for the unsaturated carbon of an aryl or heteroaryl group and additionally include the following: =O, =S, =NNHR*, =NN(R*)₂, =NNHC(O)R*, =NNHCO₂(alkyl), =NNHSO₂(alkyl), or =NR*, where each R* is independently selected from hydrogen or an optionally substituted C₁₋₆ aliphatic. Optional substituents on the aliphatic group of R* are selected from NH₂,

NH(C₁₋₄ aliphatic), N(C₁₋₄ aliphatic)₂, halo, C₁₋₄ aliphatic, OH, O(C₁₋₄ aliphatic), NO₂, CN, CO₂H,

CO₂(C₁₋₄ aliphatic), O(halo C₁₋₄ aliphatic), or halo(C₁₋₄ aliphatic), wherein each of the foregoing C₁₋₄ aliphatic groups of R* is unsubstituted.

[0056] Optional substituents on the nitrogen of a non-aromatic heterocyclic ring are selected from —R⁺, —N(R⁺)₂, —C(O)R⁺, —CO₂R⁺, —C(O)C(O)R⁺, —C(O)CH₂C(O)R⁺, —SO₂R⁺, —SO₂N(R⁺)₂, —C(=S)N(R⁺)₂, —C(=NH)—N(R⁺)₂, or —NR⁺SO₂R⁺; wherein R⁺ is hydrogen, an optionally substituted C₁₋₆ aliphatic, optionally substituted phenyl, optionally substituted

—O(Ph), optionally substituted —CH₂(Ph), optionally substituted —(CH₂)₁₋₂(Ph); optionally substituted —CH=CH(Ph); or an unsubstituted 5-6 membered heteroaryl or heterocyclic ring having one to four heteroatoms independently selected from oxygen, nitrogen, or sulfur, or, notwithstanding the definition above, two independent occurrences of R⁺, on the same substituent or different substituents, taken together with the atom(s) to which each R⁺ group is bound, form a 3-8-membered cycloalkyl, heterocyclyl, aryl, or heteroaryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Optional substituents on the aliphatic group or the phenyl ring of R⁺ are selected from NH₂, NH(C₁₋₄ aliphatic),

$N(C_{1-4} \text{ aliphatic})_2$, halo, C_{1-4} aliphatic, OH, $O(C_{1-4} \text{ aliphatic})$, NO_2 , CN, CO_2H , $CO_2(C_{1-4} \text{ aliphatic})$, $O(\text{halo } C_{1-4} \text{ aliphatic})$, or $\text{halo}(C_{1-4} \text{ aliphatic})$, wherein each of the foregoing C_{1-4} aliphatic groups of R^+ is unsubstituted.

[0057] The term "alkylidene chain" refers to a straight or branched carbon chain that may be fully saturated or have one or more units of unsaturation and has two points of attachment to the rest of the molecule. The term "spirocycloalkylidene" refers to a carbocyclic ring that may be fully saturated or have one or more units of unsaturation and has two points of attachment from the same ring carbon atom to the rest of the molecule.

[0058] The term "slurry," as used herein, is defined as a mixture comprising a solid and a liquid, wherein the solid is, at most, partially soluble in the liquid. The term "slurrying" or "slurried," as used herein (example, "the solid product was *slurried* for 24 hours"), is defined as the act of creating a slurry, and stirring said slurry for a length of time.

[0059] The term "protecting group" (PG) as used herein, represents those groups intended to protect a functional group, such as, for example, an alcohol, amine, carboxyl, carbonyl, etc., against undesirable reactions during synthetic procedures. Commonly used protecting groups are disclosed in Greene and Wuts, *Protective Groups in Organic Synthesis, 3rd Edition* (John Wiley & Sons, New York, 1999).

Examples of nitrogen protecting groups include acyl, aroyl, or carbamyl groups such as formyl, acetyl, propionyl, pivaloyl, *t*-butylacetyl, 2-chloroacetyl, 2-bromoacetyl, trifluoroacetyl, trichloroacetyl, phthalyl, *o*-nitrophenoxycetyl, α -chlorobutyryl, benzoyl, 4-chlorobenzoyl, 4-bromobenzoyl, 4-nitrobenzoyl and chiral auxiliaries such as protected or unprotected D, L or D, L-amino acids such as alanine, leucine, phenylalanine and the like; sulfonyl groups such as benzenesulfonyl, *p*-toluenesulfonyl and the like; carbamate groups such as benzyloxycarbonyl, *p*-chlorobenzyloxycarbonyl, *p*-methoxybenzyloxycarbonyl, *p*-nitrobenzyloxycarbonyl, 2-nitrobenzyloxycarbonyl, *p*-bromobenzyloxycarbonyl, 3,4-dimethoxybenzyloxycarbonyl, 3,5-dimethoxybenzyloxycarbonyl, 2,4-dimethoxybenzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, 2-nitro-4,5-dimethoxybenzyloxycarbonyl, 3,4,5-trimethoxybenzyloxycarbonyl, 1-(*p*-biphenyl)-1-methylethoxycarbonyl, α,α -dimethyl-3,5-dimethoxybenzyloxycarbonyl, benzhydryloxycarbonyl, *t*-butyloxycarbonyl, diisopropylmethoxycarbonyl, isopropylloxycarbonyl, ethoxycarbonyl, methoxycarbonyl, allyloxycarbonyl, 2,2,2-trichloroethoxycarbonyl, phenoxycarbonyl, 4-nitrophenoxycarbonyl, fluorenyl-9-methoxycarbonyl, cyclopentylloxycarbonyl, adamantylloxycarbonyl, cyclohexylloxycarbonyl, phenylthiocarbonyl and the like, arylalkyl groups such as benzyl,

triphenylmethyl, benzyloxymethyl and the like and silyl groups such as trimethylsilyl and the like. Another exemplary *N*-protecting group is *tert*-butyloxycarbonyl (Boc).

[0060] Examples of useful protecting groups for acids are substituted alkyl esters such as 9-fluorenylmethyl, methoxymethyl, methylthiomethyl, tetrahydropyranyl, tetrahydrofuranyl, methoxyethoxymethyl, 2-(trimethylsilyl)ethoxymethyl, benzyloxymethyl, pivaloyloxymethyl, phenylacetoxymethyl, triisopropylsilylmethyl, cyanomethyl, acetol, phenacyl, substituted phenacyl esters, 2,2,2-trichloroethyl, 2-haloethyl, ω -chloroalkyl, 2-(trimethylsilyl)ethyl, 2-methylthioethyl, *t*-butyl, 3-methyl-3-pentyl, dicyclopropylmethyl, cyclopentyl, cyclohexyl, allyl, methallyl, cinnamyl, phenyl, silyl esters, benzyl and substituted benzyl esters, 2,6-dialkylphenyl esters such as pentafluorophenyl, 2,6-dialkylpyphenyl. Other protecting groups for acids are methyl or ethyl esters.

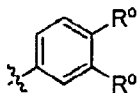
[0061] Methods of adding (a process generally referred to as "protection") and removing (process generally referred to as "deprotection") such amine and acid protecting groups are well-known in the art and available, for example in P.J.Kocienski, *Protecting Groups*, Thieme, 1994, and in Green and Wuts, *Protective Groups in Organic Synthesis, 3rd Edition* (John Wiley & Sons, New York, 1999).

[0062] Examples of suitable solvents that may be used in this invention are, but not limited to water, methanol, dichloromethane (DCM), acetonitrile, dimethylformamide (DMF), methyl acetate (MeOAc), ethyl acetate (EtOAc), isopropyl acetate (IPAc), *t*-butyl acetate (*t*-BuOAc), isopropyl alcohol (IPA), tetrahydrofuran (THF), methyl ethyl ketone (MEK), *t*-butanol, diethyl ether (Et₂O), methyl-*t*-butyl ether (MTBE), 1,4-dioxane and *N*-methyl pyrrolidone (NMP).

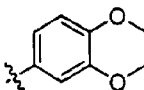
[0063] Examples of suitable coupling agents that may be used in this invention are, but not limited to 1-(3-(dimethylamino)propyl)-3-ethyl-carbodiimide hydrochloride (EDCI), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 1-hydroxybenzotriazole (HOBT), 2-(1H-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate (HATU), 2-chloro-1,3-dimethyl-2-imidazolium tetrafluoroborate, 1-H-benzotriazolium-1-[bis(dimethylamino)methylene]-5-chlorohexafluorophosphate (HCTU), 2-chloro-4,6-dimethoxy-1,3,5-triazine, and 2-propane phosphonic anhydride (T3P®).

[0064] Examples of suitable bases that may be used in this invention are, but not limited to potassium carbonate (K₂CO₃), *N*-methylmorpholine (NMM), triethylamine (Et₃N; TEA), diisopropyl-ethyl amine (*i*-Pr₂EtN; DIEA), pyridine, potassium hydroxide (KOH), sodium hydroxide (NaOH), and sodium methoxide (NaOMe; NaOCH₃).

[0065] In some embodiments, two independent occurrences of R^o , as depicted in the structure below, are taken together with the atom(s) to which they are attached to form a 3-8-membered cycloalkyl, heterocyclyl, aryl, or heteroaryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Exemplary rings that are formed when two independent occurrences of R^o are taken together with the atom(s) to which they are attached include, but are not limited to the following: a) two independent occurrences of R^o that are bound to the same atom and are taken together with that atom to form a ring, for example, $N(R^o)_2$, where both occurrences of R^o are taken together with the nitrogen atom to form a piperidin-1-yl, piperazin-1-yl, or morpholin-4-yl group; and b) two independent occurrences of R^o that are bound to different atoms and are taken together with both of those atoms to form a ring, for example where a phenyl group is substituted with two occurrences of OR^o



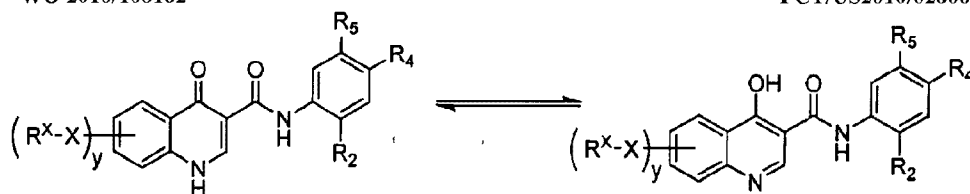
[0066] these two occurrences of R^o are taken together with the oxygen atoms to which they are bound to form a fused 6-membered oxygen containing ring:



[0067] It will be appreciated that a variety of other rings can be formed when two independent occurrences of R^o are taken together with the atom(s) to which each variable is bound and that the examples detailed above are not intended to be limiting.

[0068] Ring substituents on, for example, mono and poly aryl, aliphatic, heteroaliphatic ring systems can be attached on any ring position for which it is chemically feasible to attach a substituent.

[0069] Unless otherwise stated, structures depicted herein are also meant to include all isomeric (e.g., enantiomeric, diastereomeric, and geometric (or conformational)) forms of the structure; for example, the R and S configurations for each asymmetric center, (Z) and (E) double bond isomers, and (Z) and (E) conformational isomers. Therefore, single stereochemical isomers as well as enantiomeric, diastereomeric, and geometric (or conformational) mixtures of the present compounds are within the scope of the invention. Unless otherwise stated, all tautomeric forms of the compounds of the invention are within the scope of the invention. That is when R^X-X- in a compound of Formula 1 is hydrogen, said compound of Formula 1 may exist as a tautomer:



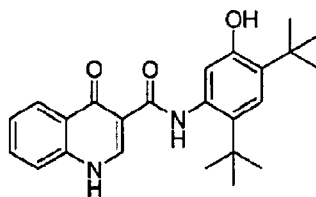
Tautomers of Formula 1

[0070] Additionally, unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of hydrogen by deuterium or tritium, or the replacement of a carbon by a ^{13}C or ^{14}C are within the scope of this invention. Such compounds are useful, for example, as analytical tools, probes in biological assays or as therapeutic agents.

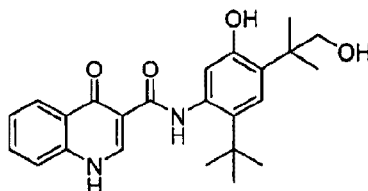
[0071] II. PROCESSES OF THE INVENTION

[0072] In general, the invention provides processes for the synthesis of compounds useful as modulators of CFTR.

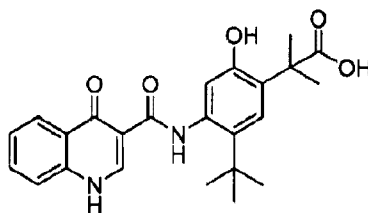
[0073] In some embodiments, the invention provides a process for the preparation of a compound having the structure



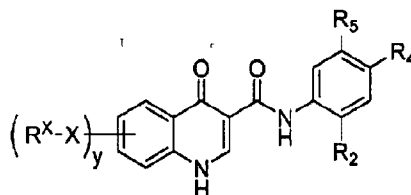
[0074] In some embodiments, the invention provides a process for the preparation of a compound having the structure



[0075] In some embodiments, the invention provides a process for the preparation of a compound having the structure

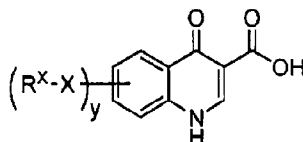


[0076] In one aspect, the invention provides a process for the preparation of a compound of Formula 1,



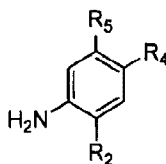
Formula 1

comprising coupling a carboxylic acid of Formula 2



Formula 2

with an aniline of Formula 3



Formula 3

in the presence of a coupling agent selected from the group consisting of 2-chloro-1,3-dimethyl-2-imidazolium tetrafluoroborate, HBTU, HCTU, 2-chloro-4,6-dimethoxy-1,3,5-triazine, HATU, HOBT/EDC, and T3P®.

Each R_2 and R_4 is independently selected from hydrogen, CN, CF_3 , halo, C_{1-6} straight or branched alkyl, 3-12 membered cycloaliphatic, phenyl, C_{5-10} heteroaryl or C_{3-7} heterocyclic, wherein said heteroaryl or heterocyclic has up to 3 heteroatoms selected from O, S, or N, and each C_{1-6} straight or branched alkyl, 3-12 membered cycloaliphatic, phenyl, C_{5-10} heteroaryl or C_{3-7} heterocyclic is independently and optionally substituted with up to three substituents selected from $-OR'$, $-CF_3$, $-OCF_3$, SR' , $S(O)R'$, SO_2R' , $-SCF_3$, halo, CN,

$-COOR'$, $-COR-$, $-O(CH_2)_2N(R')(R')$, $-O(CH_2)N(R')(R')$, $-CON(R')(R')$, $-(CH_2)_2OR'$, $-(CH_2)OR'$, CH_2CN , optionally substituted phenyl or phenoxy, $-N(R')(R')$, $-NR'C(O)OR'$, $-NR'C(O)R'$, $-(CH_2)_2N(R')(R')$, or $-(CH_2)N(R')(R')$.

Each R_5 is independently selected from hydrogen, $-OH$, NH_2 , CN, CHF_2 , NHR' , $N(R')_2$,

—NHC(O)R', NHC(O)OR', NHSO₂R', —OR', OC(O)OR', OC(O)NHR', OC(O)NR'₂,
CH₂OH, CH₂N(R')₂, C(O)OR', SO₂NHR', SO₂N(R')₂, or CH₂NHC(O)OR'.

Or, R₄ and R₅ are taken together form a 5-7 membered ring containing 0-3 three heteroatoms selected from N, O, or S, wherein said ring is optionally substituted with up to three R₃ substituents.

Each X is independently a bond or is an optionally substituted C₁₋₆ alkylidene chain wherein up to two methylene units of X are optionally and independently replaced by —CO—, —CS—, —COCO—, —CONR'—, —CONR'NR'—, —CO₂—, —OCO—, —NR'CO₂—, —O—, —NR'CONR'—, —OCONR'—, —NR'NR', —NR'NR'CO—, —NR'CO—, —S—, —SO—, —SO₂—, —NR'—, —SO₂NR'—, NR'SO₂—, or —NR'SO₂NR'—.

Each R^X is independently R', halo, NO₂, CN, CF₃, or OCF₃. y is an integer from 0-4. Each R' is independently selected from hydrogen or an optionally substituted group selected from a C₁₋₈ aliphatic group, a 3-8-membered saturated, partially unsaturated, or fully unsaturated monocyclic ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-12 membered saturated, partially unsaturated, or fully unsaturated bicyclic ring system having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; or two occurrences of R' are taken together with the atom(s) to which they are bound to form an optionally substituted 3-12 membered saturated, partially unsaturated, or fully unsaturated monocyclic or bicyclic ring having 0-4 heteroatoms independently selected from N, O, or S.

Each R₃ is independently —C₁₋₃ alkyl, C₁₋₃ perhaloalkyl, —O(C₁₋₃ alkyl), —CF₃, —OCF₃, —SCF₃, —F, —Cl, —Br, or —COOR', —COR', —O(CH₂)₂N(R')(R'), —O(CH₂)N(R')(R'), —CON(R')(R'), —(CH₂)₂OR', —(CH₂)OR', optionally substituted monocyclic or bicyclic aromatic ring, optionally substituted arylsulfone, optionally substituted

5-membered heteroaryl ring, —N(R')(R'), —(CH₂)₂N(R')(R'), or —(CH₂)N(R')(R').

[0077] In one embodiment, R₅ is independently —OC(O)OR', —OC(O)NHR', or —OC(O)N(R')₂, and R' is not hydrogen. In certain instances R₅ is —OC(O)OR' and R' is not hydrogen. In other instances, R₅ is —OC(O)NHR' and R' is not hydrogen. In still other instances, R₅ is —OC(O)N(R')₂ and R' is not hydrogen.

[0078] In one embodiment, the process further comprises cleaving the —OC(O)OR', —OC(O)NHR', or —OC(O)N(R')₂ R₅ group to form —OH. The cleavage is performed by treating a compound of Formula 1 containing the —OC(O)OR',

—OC(O)NHR', or —OC(O)N(R')₂ R₅ group with an alcoholic solvent in the presence of base such as NaOH, KOH or sodium methoxide. The alcoholic solvent used in the cleavage reaction is methanol, ethanol, isopropyl alcohol or *t*-butanol.

[0079] In another embodiment, at least one of R₄ or R₂ is independently a C₁-C₆ straight or branched alkyl which is substituted with —COOR' or —CON(R')₂, and R' is not hydrogen. In certain instances, one of R₄ or R₂ is —COOR' and R' is not hydrogen. In other instances, one of R₄ or R₂ is —CON(R')₂ and R' is not hydrogen.

[0080] In one embodiment, the process further comprises hydrolyzing the —COOR' or —CON(R')₂ on at least one of R₄ and R₂. The hydrolysis is performed by treating a compound of Formula 1 containing the —COOR' or —CON(R')₂ group on at least one of R₄ and R₂ with an alcoholic solvent in the presence of base such as NaOH, KOH or sodium methoxide. The alcoholic solvent used in the hydrolysis is methanol, ethanol, isopropyl alcohol or *t*-butanol.

[0081] In another embodiment, at least one of R₄ or R₂ is independently a C₁₋₆ straight or branched alkyl which is substituted with —COOR' or —CON(R')₂ and R₅ is independently —OC(O)OR', —OC(O)NHR', or —OC(O)N(R')₂, and each R' is not hydrogen.

[0082] In one embodiment, the process further comprises hydrolyzing the —COOR' or —CON(R')₂ on at least one of R₄ and R₂ and cleaving the —OC(O)OR', —OC(O)NHR', or —OC(O)N(R')₂ R₅ group. The hydrolysis/cleavage reaction is performed by treating a compound of Formula 1 containing the —COOR' or —CON(R')₂ group on at least one of R₄ and R₂ and —OC(O)OR', —OC(O)NHR', or —OC(O)N(R')₂ R₅ group with an alcoholic solvent in the presence of base such as NaOH, KOH or sodium methoxide. The alcoholic solvent used in the hydrolysis/cleavage reaction is methanol, ethanol, isopropyl alcohol or *t*-butanol.

[0083] In another embodiment, the coupling of the carboxylic acid of Formula 2 and the aniline of Formula 3 is performed in the presence of a base such as K₂CO₃, Et₃N, N-methylmorpholine (NMM), pyridine or DIEA.

[0084] In another embodiment, the coupling of the carboxylic acid of Formula 2 and the aniline of Formula 3 is performed in the presence of pyridine or DIEA.

[0085] In yet another embodiment, the coupling of the carboxylic acid of Formula 2 and the aniline of Formula 3 is performed in the presence of a solvent such as EtOAc, IPAc, THF, MEK, NMP, acetonitrile, DMF, or 2-methyltetrahydrofuran.

[0086] In further embodiments, the coupling of the carboxylic acid of Formula 2 and the aniline of Formula 3 is performed at a reaction temperature which is maintained between 10

°C and 78 °C such as between about 20 °C and 30 °C, between about 40 °C and 50 °C, and between about 42 °C and 53 °C.

[0087] In still further embodiments, the coupling reaction is stirred for at least 2 hours such as for at least 8 hours, for at least 70 hours or for at least 3 days.

[0088] In another embodiment, y is 0.

[0089] In still other embodiments, R_2 is *tert*-butyl.

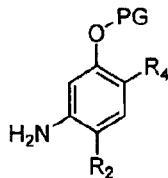
[0090] In some embodiments, R_5 is independently $—OC(O)OR'$, $—OC(O)NHR'$, or $—OC(O)N(R')_2$, and R' is not hydrogen; and each of R_2 and R_4 is independently selected from hydrogen, CF_3 , C_1 - C_6 straight or branched alkyl, 3-12 membered cycloaliphatic or phenyl.

[0091] In some embodiments, R_5 is independently $—OC(O)OR'$, $—OC(O)NHR'$, or $—OC(O)N(R')_2$, and R' is not hydrogen; and each of R_2 and R_4 is independently selected from C_1 - C_6 straight or branched alkyl.

[0092] In some embodiments, R_5 is independently $—OC(O)OR'$, $—OC(O)NHR'$, or $—OC(O)N(R')_2$, and R' is not hydrogen; and each of R_2 and R_4 is independently selected from methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *t*-butyl, *n*-pentyl, or *n*-hexyl.

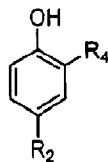
[0093] In some embodiments, R_2 and R_4 are *t*-butyl.

[0094] In one embodiment, the invention provides a process for the preparation of a compound of Formula 5



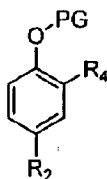
Formula 5

by reacting a compound of Formula 6



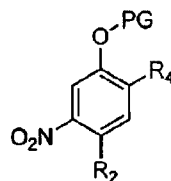
Formula 6

with a reagent capable of causing a protecting group to be attached to the phenolic oxygen of a compound of Formula 6 in the presence of a solvent, thereby producing a compound of Formula 7



Formula 7

which is nitrated to form a compound of Formula 8



Formula 8

which is then reduced to give a compound of Formula 5, wherein PG is a protecting group and R₄ and R₅ are defined as above.

[0095] In one embodiment, the solvent used in the conversion of compound of Formula 6 to a compound of Formula 7 is diethyl ether, or methylene chloride.

[0096] In another embodiment, the solvent used in the protection reaction is methylene chloride.

[0097] In a further embodiment, PG is propoxy formyl, methanesulfonyl, 4-nitro-benzoyl, ethoxy formyl, butoxy formyl, *t*-butoxy formyl, *i*-propoxy formyl or methoxy formyl.

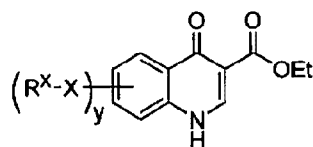
[0098] In another embodiment, PG is methoxy formyl.

[0099] In another embodiment, a compound of Formula 7 is nitrated using a mixture of sulfuric acid, nitric acid and methylene chloride.

[00100] In one embodiment, the nitro compound of Formula 8 is purified by crystallization.

[00101] In a further embodiment, the nitro compound of Formula 8 is purified by crystallization using hexane.

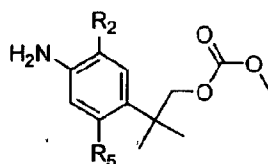
[00102] In another embodiment, the process further comprises the step of contacting a compound of Formula 4



Formula 4

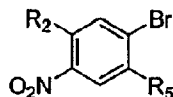
with an aqueous acid to produce a compound of Formula 2.

[00103] In one embodiment, the compound of Formula 3 is a compound of Formula 40



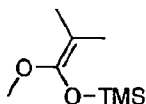
Formula 40

[00104] In another embodiment, the process further comprises the step of contacting a compound of Formula 41



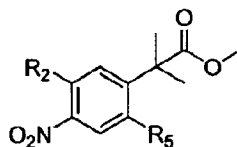
Formula 41

with methyl trimethylsilyl dimethylketene acetal (MTDA)



MTDA

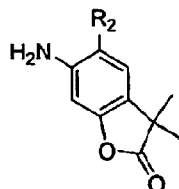
to produce a compound of Formula 42



Formula 42

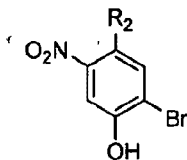
[00105] In a further embodiment, the process comprises the step of reducing a compound of Formula 42 to produce a compound of Formula 40.

[00106] In one embodiment, the compound of Formula 3 is a compound of Formula 43



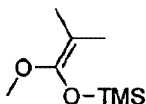
Formula 43

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[00107] In a further embodiment, the process comprises the step of contacting a compound of Formula 44



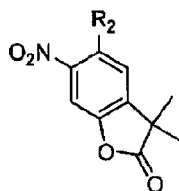
Formula 44

with methyl trimethylsilyl dimethylketene acetal (MTDA)



MTDA

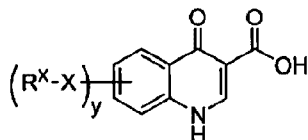
to produce a compound of Formula 45



Formula 45

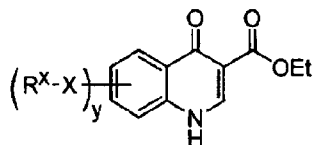
[00108] In a further embodiment, the process comprises the step of reducing a compound of Formula 45 to produce a compound of Formula 43.

[00109] In another aspect, the invention provides a process for the preparation of a compound of Formula 2



Formula 2

comprising contacting a compound of Formula 4



Formula 4

with an aqueous acid, wherein

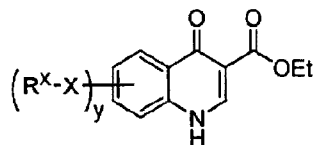
each X is independently a bond or is an optionally substituted C₁₋₆ alkylidene chain wherein up to two methylene units of X are optionally and independently replaced by —CO—, —CS—, —COCO—, —CONR'—, —CONR'NR'—, —CO₂—, —OCO—, —NR'CO₂—, —O—, —NR'CONR'—, —OCONR'—, —NR'NR', —NR'NR'CO—, —NR'CO—, —S—, —SO—, —SO₂—, —NR'—, —SO₂NR'—, NR'SO₂—, or —NR'SO₂NR'—;

each R^X is independently R', halo, NO₂, CN, CF₃, or OCF₃;

y is an integer from 0-4; and

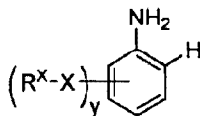
each R' is independently selected from hydrogen or an optionally substituted group selected from a C₁₋₈ aliphatic group, a 3-8-membered saturated, partially unsaturated, or fully unsaturated monocyclic ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-12 membered saturated, partially unsaturated, or fully unsaturated bicyclic ring system having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; or two occurrences of R' are taken together with the atom(s) to which they are bound to form an optionally substituted 3-12 membered saturated, partially unsaturated, or fully unsaturated monocyclic or bicyclic ring having 0-4 heteroatoms independently selected from N, O, or S.

[00110] In one embodiment of this aspect, the compound of Formula 4



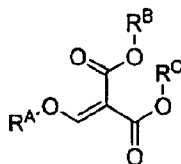
Formula 4

was prepared by contacting a compound of Formula 50



Formula 50

with a compound of Formula 51

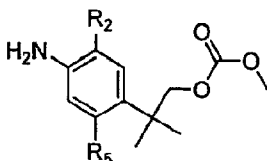


Formula 51

[00111] In one embodiment of this aspect, the compound of Formula 50 and the compound of Formula 50 are reacted at a temperature from about 100 °C to about 300 °C. In another embodiment, the compound of Formula 50 and the compound of Formula 50 are reacted at a temperature of about 100 °C. In another embodiment, the compound of Formula 50 and the compound of Formula 50 are reacted at a temperature of about 250 °C. In one further embodiment, the compound of Formula 50 and the compound of Formula 50 are reacted at a temperature of about 100 °C, and then at a temperature of about 250 °C.

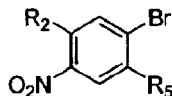
[00112] In one further embodiment of this aspect, y is 0.

[00113] In another aspect, the invention provides a process for the preparation of a compound of Formula 40



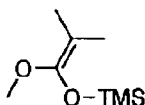
Formula 40

comprising the step of contacting a compound of Formula 41



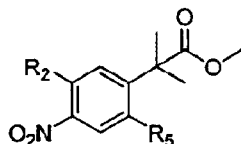
Formula 41

with methyl trimethylsilyl dimethylketene acetal (MTDA)



MTDA

to produce a compound of Formula 42



Formula 42, wherein

each R₂ is independently selected from hydrogen, CN, CF₃, halo, C₁₋₅ straight or branched alkyl, 3-12 membered cycloaliphatic, phenyl, C₅₋₁₀ heteroaryl or C₃₋₇ heterocyclic, wherein said heteroaryl or heterocyclic has up to 3 heteroatoms selected from O, S, or N, and each C₁.

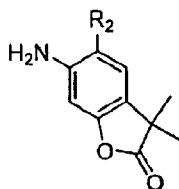
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6 straight or branched alkyl, 3-12 membered cycloaliphatic, phenyl, C₅₋₁₀ heteroaryl or C₃₋₇
heterocyclic is independently and optionally substituted with up to three substituents selected
from —OR', —CF₃, —OCF₃, SR', S(O)R', SO₂R', —SCF₃, halo, CN, —COOR',
—COR—, —O(CH₂)₂N(R')(R'), —O(CH₂)N(R')(R'), —CON(R')(R'), —(CH₂)₂OR',
—(CH₂)OR', CH₂CN, optionally substituted phenyl or phenoxy, —N(R')(R'), —
NR'C(O)OR', —NR'C(O)R', —(CH₂)₂N(R')(R'), or —(CH₂)N(R')(R');

each R₅ is independently selected from hydrogen, —OH, NH₂, CN, CHF₂, NHR',
N(R')₂,
—NHC(O)R', NHC(O)OR', NHSO₂R', —OR', OC(O)OR', OC(O)NHR', OC(O)NR'₂,
CH₂OH, CH₂N(R')₂, C(O)OR', SO₂NHR', SO₂N(R')₂, or CH₂NHC(O)OR'; and

each R' is independently selected from hydrogen or an optionally substituted group
selected from a C₁₋₈ aliphatic group, a 3-8-membered saturated, partially unsaturated, or fully
unsaturated monocyclic ring having 0-3 heteroatoms independently selected from nitrogen,
oxygen, or sulfur, or an 8-12 membered saturated, partially unsaturated, or fully unsaturated
bicyclic ring system having 0-5 heteroatoms independently selected from nitrogen, oxygen,
or sulfur; or two occurrences of R' are taken together with the atom(s) to which they are
bound to form an optionally substituted 3-12 membered saturated, partially unsaturated, or
fully unsaturated monocyclic or bicyclic ring having 0-4 heteroatoms independently selected
from N, O, or S.

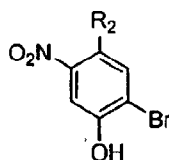
[00114] In one embodiment of this aspect, the process comprises the step of reducing a
compound of Formula 42 to produce a compound of Formula 40.

[00115] In another aspect, the invention provides a process for the preparation of a
compound of Formula 43



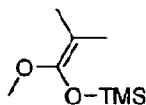
Formula 43

comprising the step of contacting a compound having the Formula 44



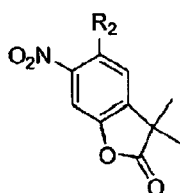
Formula 44

with methyl trimethylsilyl dimethylketene acetal (MTDA)



MTDA

to produce a compound of Formula 45



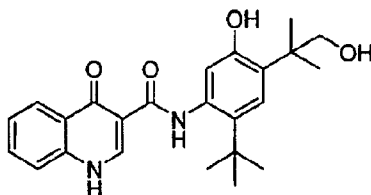
Formula 45, wherein

each R_2 is independently selected from hydrogen, CN, CF_3 , halo, C_{1-6} straight or branched alkyl, 3-12 membered cycloaliphatic, phenyl, C_{5-10} heteroaryl or C_{3-7} heterocyclic, wherein said heteroaryl or heterocyclic has up to 3 heteroatoms selected from O, S, or N, and each C_{1-6} straight or branched alkyl, 3-12 membered cycloaliphatic, phenyl, C_{5-10} heteroaryl or C_{3-7} heterocyclic is independently and optionally substituted with up to three substituents selected from $-OR'$, $-CF_3$, $-OCF_3$, SR' , $S(O)R'$, SO_2R' , $-SCF_3$, halo, CN, $-COOR'$, $-COR-$, $-O(CH_2)_2N(R')(R')$, $-O(CH_2)N(R')(R')$, $-CON(R')(R')$, $-(CH_2)_2OR'$, $-(CH_2)OR'$, CH_2CN , optionally substituted phenyl or phenoxy, $-N(R')(R')$, $-NR'C(O)OR'$, $-NR'C(O)R'$, $-(CH_2)_2N(R')(R')$, or $-(CH_2)N(R')(R')$; and

each R' is independently selected from hydrogen or an optionally substituted group selected from a C_{1-8} aliphatic group, a 3-8-membered saturated, partially unsaturated, or fully unsaturated monocyclic ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-12 membered saturated, partially unsaturated, or fully unsaturated bicyclic ring system having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur; or two occurrences of R' are taken together with the atom(s) to which they are bound to form an optionally substituted 3-12 membered saturated, partially unsaturated, or fully unsaturated monocyclic or bicyclic ring having 0-4 heteroatoms independently selected from N, O, or S.

[00116] In one embodiment of this aspect, the process comprises the step of reducing a compound of Formula 45 to produce a compound of Formula 43.

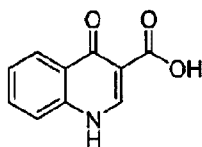
[00117] In some specific embodiments, a process for the preparation of compound 27



Compound 27

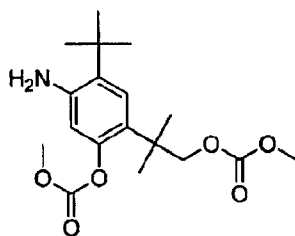
comprises:

(a) reacting compound 26



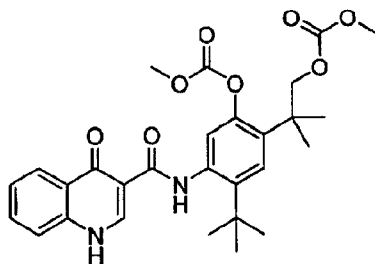
Compound 26

with compound 13



Compound 13

in the presence of EDCI, HOBT and DIEA using DMF as the solvent, wherein the reaction temperature is maintained between about 20 °C and 30 °C, and the reaction is allowed proceed for at least 70 hours, to produce compound 14

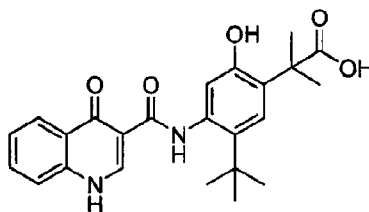


Compound 14

; and

(b) treating compound **14** with KOH in methanol.

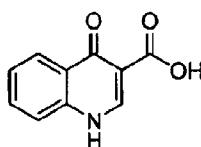
[00118] In another specific embodiment, a process for the preparation of compound **28**



Compound 28

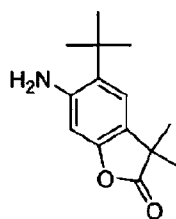
comprises:

(a) reacting compound **26**



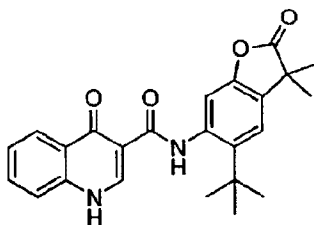
Compound 26

with compound **20**



Compound 20

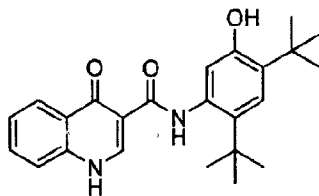
in the presence of HATU and DIEA using acetonitrile as the solvent, wherein the reaction temperature is maintained between about 40 °C and 50 °C, and wherein the reaction is allowed proceed for at least 3 days, to produce compound **21**



Compound 21 ; and

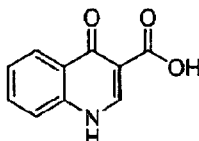
(b) treating compound **21** with NaOH in methanol.

[00119] In yet another specific embodiment, a process for the preparation of compound **34**

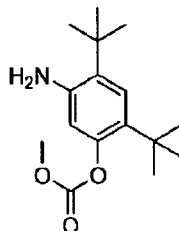
**Compound 34**

comprises:

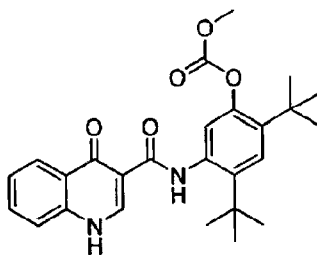
(a) reacting compound 26

**Compound 26**

with compound 32

**Compound 32**

in the presence of T3P® and pyridine using 2-methyl tetrahydrofuran as the solvent, wherein the reaction temperature is maintained between about 42 °C and about 3 °C, and wherein the reaction is allowed proceed for at least 2 hours, to produce compound 33

**Compound 33**

; and

(b) treating compound 33 with NaOMe/MeOH in 2-methyl tetrahydrofuran.

[00120] In another embodiment, the method also includes the step of forming a slurry of compound 34 in a mixture of acetonitrile and water, wherein the solid form of compound 34 is converted to Compound 34.

[00121] In one embodiment, the ratio of acetonitrile to water is about 9:1 in the slurry.

[00122] In another embodiment, the slurry is heated to a temperature between about 73 °C and 83 °C.

[00123] In another embodiment, compound 34 is in the slurry for at least about 3 hours.

[00124] In a further embodiment, the process includes quenching the reaction mixture with 1N HCl; adding 0.1N HCl to the mixture, thereby creating a biphasic mixture; agitating the biphasic mixture; separating the organic phase from said biphasic mixture; filtering and removing solid matter from said organic phase; reducing the volume of the organic phase by approximately 50% using distillation; performing thrice the steps of: adding acetonitrile to the organic phase until the volume of said organic phase increases by 100% and reducing the volume of the organic phase by approximately 50%; increasing the volume of the organic phase by approximately 100% by adding acetonitrile and then adding water, to form a slurry wherein the final solvent ratio is 9:1 acetonitrile/water; heating said slurry to a temperature between about 73 °C and 83 °C; stirring said slurry for at least 5 hours; and cooling said slurry to a temperature between about -5 °C and 5 °C.

[00125] In an alternative embodiment, the process includes quenching the reaction mixture with 1.2 N HCl; thereby creating a biphasic mixture; agitating said biphasic mixture; separating the organic phase from said biphasic mixture; adding 0.1N HCl to the organic layer thereby creating a biphasic mixture; agitating said biphasic mixture; separating the organic phase; filtering and removing solid matter from said organic phase; reducing the volume of the organic phase by approximately 50% using distillation; performing thrice the steps of: adding acetonitrile to the organic phase until the volume of said organic phase increases by 100% and reducing the volume of the organic phase by approximately 50%; increasing the volume of the organic phase by approximately 100% by adding acetonitrile and then adding water, to form a slurry wherein the final solvent ratio is 9:1 acetonitrile/water; heating said slurry to a temperature between about 73 °C and 83 °C; stirring said slurry for at least 5 hours; and cooling said slurry to a temperature between about 20 °C and 25 °C; filtering and removing solid matter from said slurry; washing the solid matter with acetonitrile having a temperature of between about 20 °C and 25 °C four times; and drying the solid material under vacuum at a temperature of from 45 °C to about 55 °C.

[00126] In one embodiment, the volume of 1N HCl used to quench the reaction is equal to 25% of the total volume of the original reaction mixture; the volume of 0.1N HCl added to the reaction mixture is equal to 25% of the total volume of the original reaction mixture; and the distillation steps are performed at reduced pressure wherein the temperature outside the reaction vessel is less than about 45 °C and the temperature of the reaction mixture is more

0 °C.

[00127] In a further embodiment, the process includes forming a slurry of compound 34 in isopropyl acetate.

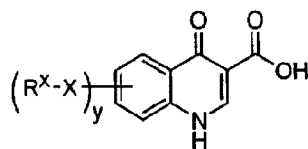
[00128] In one embodiment, the slurry is heated to reflux temperature.

[00129] In another embodiment, compound 34 is in the slurry for at least about 3 hours.

[00130] In certain embodiments, the process for the preparation of Compound 34 further comprises dissolving compound 34 in 2-methyltetrahydrofuran; adding 0.1N HCl to the solution, to creating a biphasic solution, which is stirred. In another embodiment, the process further comprises separating the organic phase from the biphasic solution. In another embodiment, the process further comprises filtering and removing solid matter from the organic phase. In another embodiment, the process further comprises reducing the volume of the organic phase by approximately 50% using distillation. In another embodiment, the process further comprises performing thrice the procedure of: adding MeOAc, EtOAc, IPAc, *t*-BuOAc, tetrahydrofuran (THF), Et₂O or methyl-*t*-butyl ether (MTBE) to the organic phase until the volume of the organic phase increases by 100% and reducing the volume of the organic phase by 50% using distillation. In another embodiment, the process further comprises adding MeOAc, EtOAc, IPAc, *t*-BuOAc, tetrahydrofuran (THF), Et₂O or methyl-*t*-butyl ether (MTBE) to the organic phase until the volume of the organic phase increases by 100%. In another embodiment, the process further comprises heating the organic phase to reflux temperature, and maintaining said reflux temperature for a time at least about 5 hours. In another embodiment, the process further comprises cooling the organic phase to a temperature between about -5 °C and about 5 °C over a time period of 4.5 hours to 5.5 hours.

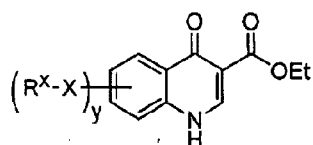
[00131] In another embodiment, the process for the preparation of Compound 34 further comprises crystallizing Compound 34, comprising seeding a saturated reaction mixture comprising Compound 34 in solution with at least one crystal of substantially pure Compound 34.

[00132] In another embodiment, the invention provides a process for the preparation of a compound of Formula 2



Formula 2

comprising hydrolyzing a compound of Formula 4

**Formula 4**

[00133] In a further embodiment, the compound of Formula 4 is hydrolyzed using a hydrolyzing agent in the presence of a solvent.

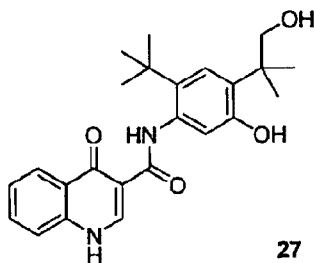
[00134] In some further embodiments, the hydrolyzing agent is HCl, H₂SO₄, H₃PO₄, Na₂CO₃, LiOH, KOH, or NaOH.

[00135] In some embodiments, the solvent used in the hydrolysis is H₂O, methanol, ethanol, isopropanol or *t*-butanol.

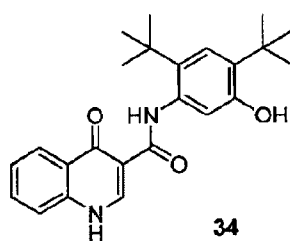
[00136] In still other embodiments, the invention provides a compound produced by any process described herein.

[00137] In a further embodiment, the invention provides a pharmaceutical composition comprising a compound produced by any process described herein.

[00138] In one aspect, the invention provides a process for the preparation of Compound 27

**27**

comprising contacting Compound 34

**34**

with a biological composition.

[00139] In one embodiment of this aspect, the biological composition includes a biological organism selected from the group consisting of fungi, bacteria and archaea.

[00140] In one embodiment, the biological composition is fungi. In a further embodiment, the fungi is a single cell fungi. In another embodiment, the fungi is a multicell fungi.

[00141] In a further embodiment, the fungi is a multicell fungi selected from the group consisting of *Absidia*, *Aspergillus*, *Beauveria*, *Botrytis*, *Cunninghamella*, *Cyathus*,

Gliocladium, Mortierella, Mucor, Phanerochaete, Stemphylium, Syncephalastrum and *Verticillium*.

[00142] In a further embodiment, the fungi is a multicell fungi selected from the group consisting of *Absidia pseudocylindrospora, Aspergillus alliaceus, Aspergillus ochraceus, Beauveria bassiana, Cunninghamella blakesleeana, Cunninghamella echinulata, Mortierella isabellina, Mucor plumbeus, Phanerochaete chrysosporium, Syncephalastrum racemosum* and *Verticillium theobromae*.

[00143] In another embodiment, the fungi is a single cell fungi selected from the group consisting of *Candida, Debaryomyces, Geotrichum, Pichia, Rhodotorula, Saccharomyces, Sporobolomyces, Williopsis* and *Yarrowia*.

[00144] In further embodiment, the fungi is a single cell fungi selected from the group consisting of *Candida paripsilosis, Debaryomyces hansenii, Geotrichum candidum, Pichia methanolica, Pichia subpellicosa, Rhodotorula glutinis, Rhodotorula mucaliginosa, Saccharomyces cerevisiae, Sporobolomyces salmonicolor, Williopsis saturnis* and *Yarrowia lipolytica*.

[00145] In another embodiment, the biological organism is an archaea. In a further embodiment, the archaea is *Pyrococcus*. In still a further embodiment, the archaea is *Pyrococcus furiosus*.

[00146] In another embodiment, the biological organism is a bacteria.

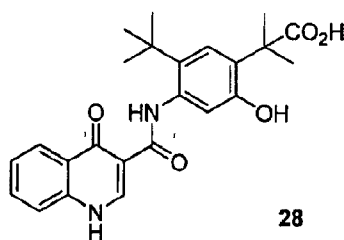
[00147] In a further embodiment, the bacteria is selected from the group consisting of *Lactobacillus, Pseudomonas, Rhodococcus* and *Streptomyces*.

[00148] In a further embodiment, the bacteria is selected from the group consisting of *Lactobacillus reuterii, Pseudomonas methanolica, Rhodococcus erythropolis, Streptomyces griseus, Streptomyces griseolus, Streptomyces platensis* and *Streptomyces rimosus*.

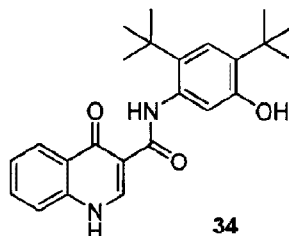
[00149] In still a further embodiment, the biological composition includes *Streptomyces rimosus*, or a fragment thereof.

[00150] In one embodiment of this aspect, the biological composition includes a solvent. In a further embodiment, the solvent includes water. In still a further embodiment, the solvent is a buffer. In still a further embodiment, the solvent is a potassium phosphate buffer having a pH of about 7.

[00151] In one aspect, the invention provides a process for the preparation of Compound 28:



comprising reacting Compound 34



with a biological composition.

[00152] In one embodiment of this aspect, the biological composition includes a biological organism selected from the group consisting of fungi, bacteria and archaea.

[00153] In one embodiment, the biological composition is fungi. In a further embodiment, the fungi is a single cell fungi. In another embodiment, the fungi is a multicell fungi.

[00154] In a further embodiment, the fungi is a multicell fungi selected from the group consisting of *Absidia*, *Aspergillus*, *Beauveria*, *Botrytis*, *Cunninghamella*, *Cyathus*, *Gliocladium*, *Mortierella*, *Mucor*, *Phanerochaete*, *Stemphylium*, *Syncephalastrum* and *Verticillium*.

[00155] In a further embodiment, the fungi is a multicell fungi selected from the group consisting of *Absidia pseudocylindrospora*, *Aspergillus alliaceus*, *Aspergillus ochraceus*, *Beauveria bassiana*, *Cunninghamella blakesleeana*, *Cunninghamella echinulata*, *Mortierella isabellina*, *Mucor plumbeus*, *Phanerochaete chrysosporium*, *Syncephalastrum racemosum* and *Verticillium theobromae*.

[00156] In another embodiment, the fungi is a single cell fungi selected from the group consisting of *Candida*, *Debaryomyces*, *Geotrichum*, *Pichia*, *Rhodotorula*, *Saccharomyces*, *Sporobolomyces*, *Williopsis* and *Yarrowia*.

[00157] In further embodiment, the fungi is a single cell fungi selected from the group consisting of *Candida parapsilosis*, *Debaryomyces hansenii*, *Geotrichum candidum*, *Pichia methanolica*, *Pichia subpellicosa*, *Rhodotorula glutinis*, *Rhodotorula mucaliginosa*, *Saccharomyces cerevisiae*, *Sporobolomyces salmonicolor*, *Williopsis saturnis* and *Yarrowia lipolytica*.

[00158] In another embodiment, the biological organism is an archaea. In a further embodiment, the archaea is *Pyrococcus*. In still a further embodiment, the archaea is *Pyrococcus furiosus*.

[00159] In another embodiment, the biological organism is a bacteria.

[00160] In a further embodiment, the bacteria is selected from the group consisting of *Lactobacillus*, *Pseudomonas*, *Rhodococcus* and *Streptomyces*.

[00161] In a further embodiment, the bacteria is selected from the group consisting of *Lactobacillus reuterii*, *Pseudomonas methanolica*, *Rhodococcus erythropolis*, *Streptomyces griseus*, *Streptomyces griseolus*, *Streptomyces platensis* and *Streptomyces rimosus*.

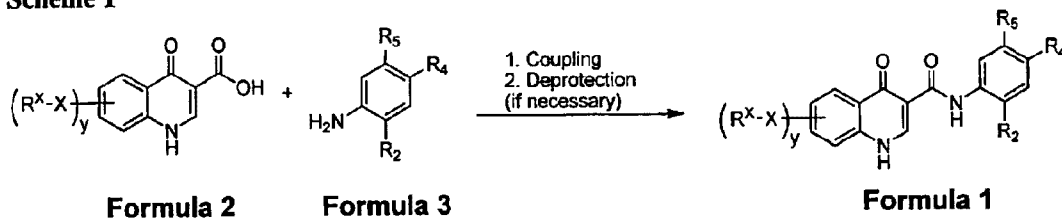
[00162] In one embodiment of this aspect, the biological composition includes *Streptomyces rimosus*, or a fragment thereof.

[00163] In one embodiment of this aspect, the biological composition includes a solvent. In a further embodiment, the solvent includes water. In still a further embodiment, the solvent is a buffer. In still a further embodiment, the solvent is a potassium phosphate buffer having a pH of about 7.

[00164] III. GENERAL SYNTHESIS

[00165] Compounds of Formula 1 can be synthesized according to Scheme 1.

Scheme 1

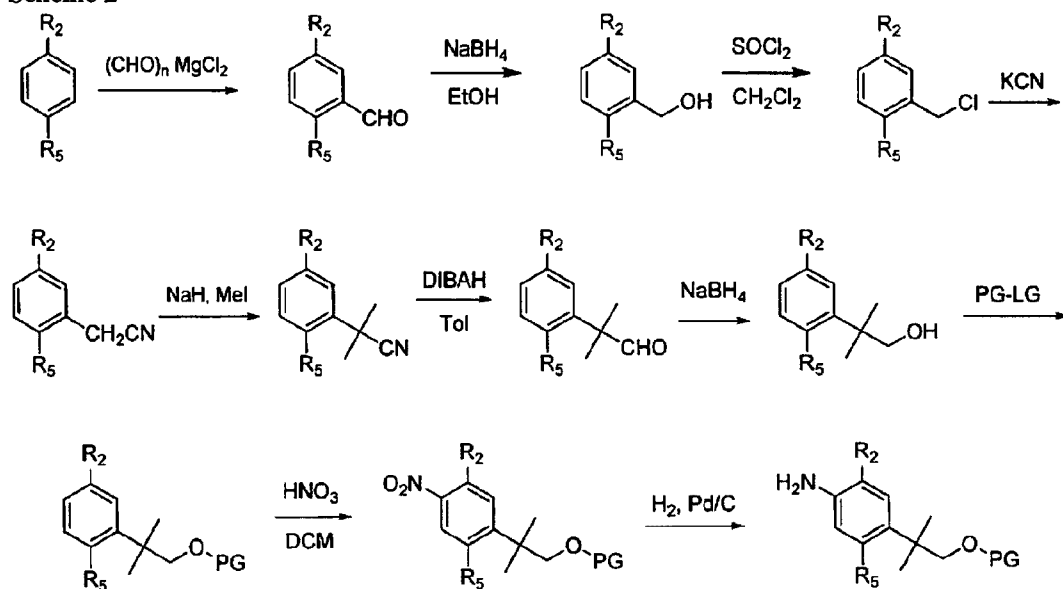


[00166] In Scheme 1, anilines of Formula 3, wherein R_2 , R_4 and R_5 are optionally and independently substituted with functional groups defined above, and wherein those functional groups optionally and independently bear protecting groups thereon, are reacted with carboxylic acid intermediates of Formula 2 under coupling conditions. Derivatives of Formula 1 that bear one or more protecting groups can then be deprotected to provide unprotected derivatives of Formula 1.

[00167] The coupling reaction described in Scheme 1 can be achieved by dissolving the reactants in a suitable solvent, treating the resulting solution with a suitable coupling reagent optionally in the presence of a suitable base.

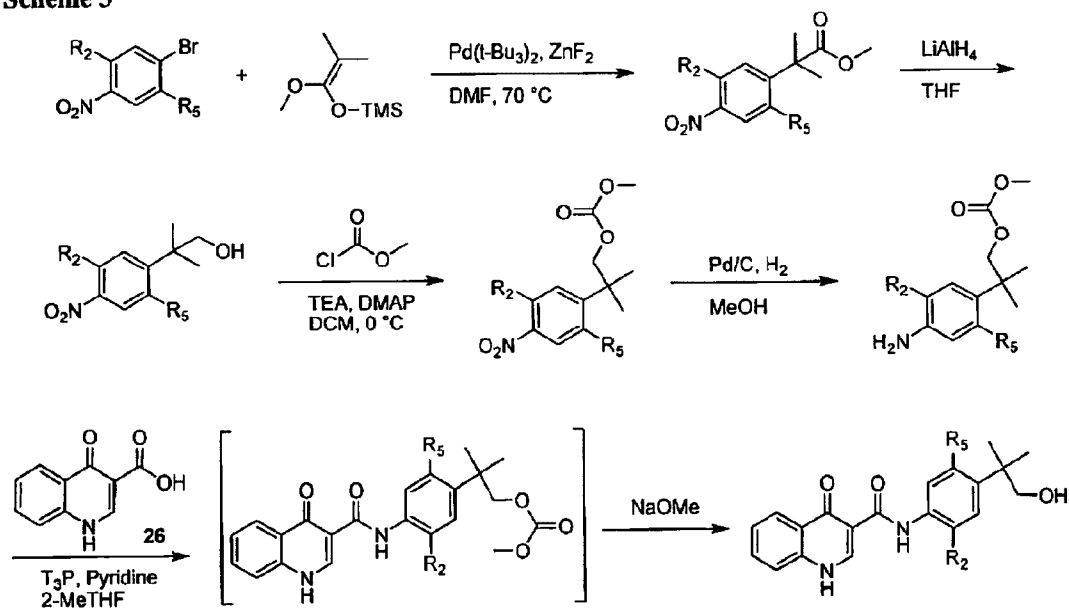
[00168] Anilines of Formula 3, wherein R_4 is a protected 1-hydroxy-2-methylpropan-2-yl can be synthesized according to Scheme 2.

Scheme 2



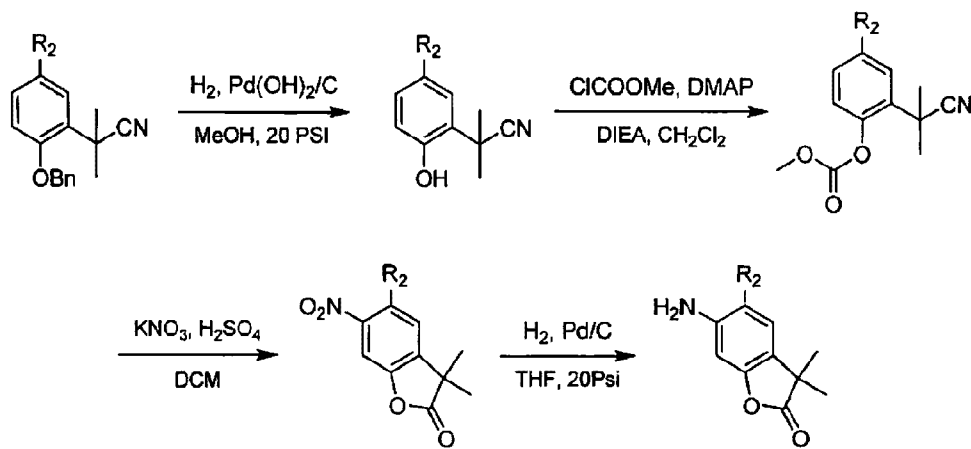
[00169] Alternatively, anilines of Formula 3, wherein R_4 is a protected 1-hydroxy-2-methylpropan-2-yl can be synthesized according to Scheme 3.

Scheme 3

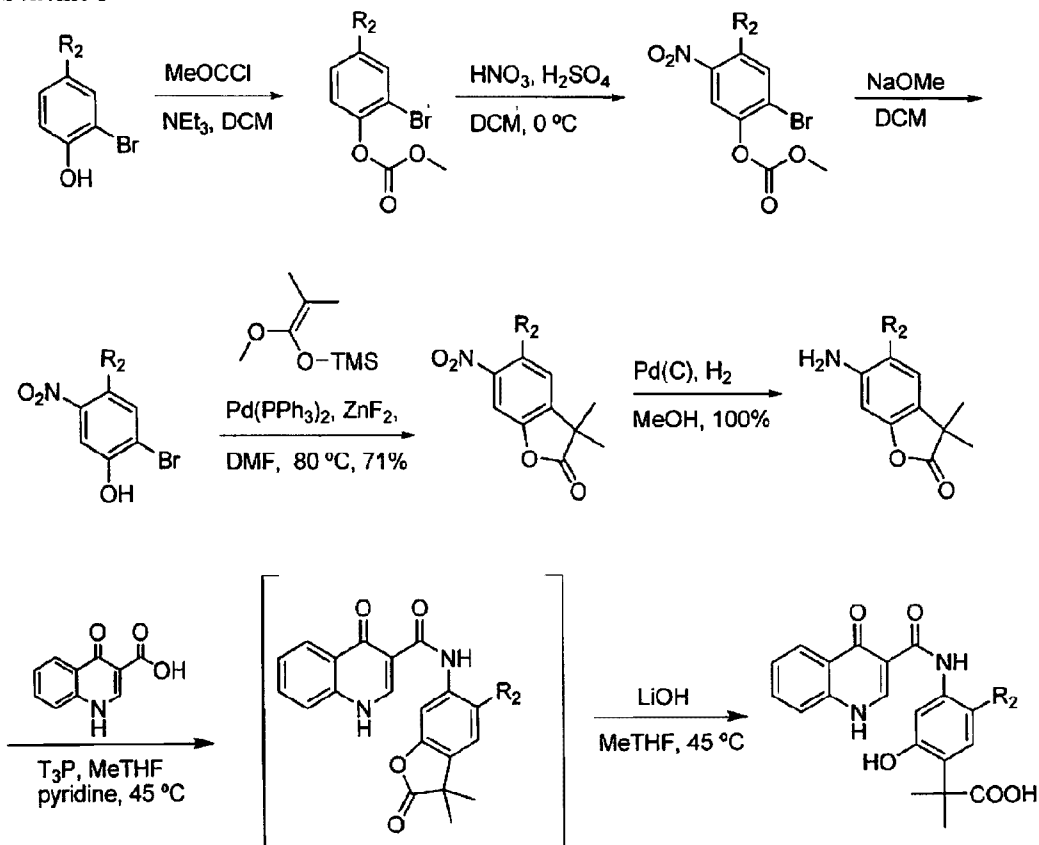


[00170] Anilines of Formula 3, wherein R₄ and R₅ together with the phenyl ring to which they are attached form a 3,3-dimethylbenzofuran-2(3H)-one, can be synthesized according to Scheme 4.

Scheme 4

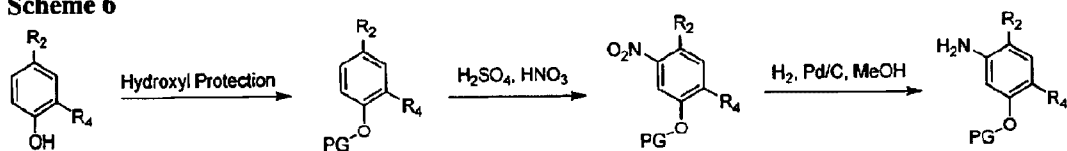


[00171] Alternatively, anilines of Formula 3, wherein R₄ and R₅ together with the phenyl ring to which they are attached form a 3,3-dimethylbenzofuran-2(3H)-one, can be synthesized according to Scheme 5.

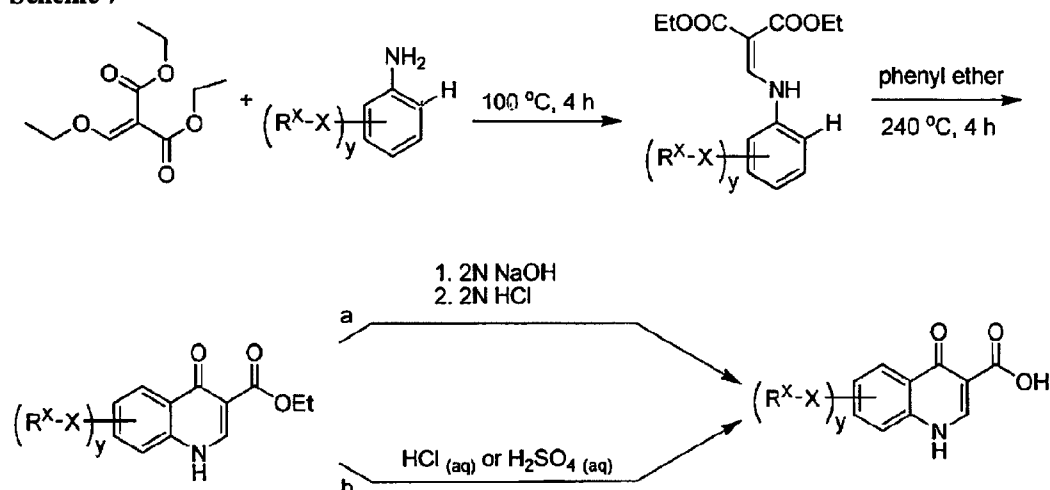


[00172] Anilines of Formula 3, wherein R_5 is a protected hydroxyl, can be synthesized according to Scheme 6.

Scheme 6



[00173] Dihydroquinoline carboxylic acids of Formula 2 can be synthesized according to Scheme 7, wherein the aniline derivative undergoes conjugate addition to $\text{EtOCH}=\text{C}(\text{COOEt})_2$, followed by thermal rearrangement and hydrolysis.



[00174] IV. USES AND METHODS OF USE

[00175] Pharmaceutically acceptable compositions

[00176] In one aspect of the present invention, pharmaceutically acceptable compositions are provided, wherein these compositions comprise any of the compounds as described herein, and optionally comprise a pharmaceutically acceptable carrier, adjuvant or vehicle. In certain embodiments, these compositions optionally further comprise one or more additional therapeutic agents.

[00177] It will also be appreciated that certain of the compounds of present invention can exist in free form for treatment, or where appropriate, as a pharmaceutically acceptable derivative or a prodrug thereof. According to the present invention, a pharmaceutically acceptable derivative or a prodrug includes, but is not limited to, pharmaceutically acceptable salts, esters, salts of such esters, or any other adduct or derivative which upon administration to a patient in need thereof is capable of providing, directly or indirectly, a compound as otherwise described herein, or a metabolite or residue thereof.

[00178] As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. A "pharmaceutically acceptable salt" means any non-toxic salt or salt of an ester of a compound of this invention that, upon administration to a recipient, is capable of providing, either directly or indirectly, a compound of this invention or an inhibitorily active metabolite or residue thereof.

[00179] Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, *et al.* describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 1977, 66, 1-19. Pharmaceutically acceptable salts of the compounds of this invention include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, edisylate (ethanedisulfonate), ethanesulfonate, formate, fumarate, glucoheptonate, glyccrophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and $N^+(C_{1-4}alkyl)_4$ salts. This invention also envisions the quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. Water or oil-soluble or dispersible products may be obtained by such quaternization. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

[00180] As described above, the pharmaceutically acceptable compositions of the present invention additionally comprise a pharmaceutically acceptable carrier, adjuvant, or vehicle, which, as used herein, includes any and all solvents, diluents, or other liquid vehicle, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's *Pharmaceutical Sciences*, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980) discloses various carriers used in formulating pharmaceutically acceptable compositions and known techniques for the

preparation thereof. Except insofar as any conventional carrier medium is incompatible with the compounds of the invention, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutically acceptable composition, its use is contemplated to be within the scope of this invention. Some examples of materials which can serve as pharmaceutically acceptable carriers include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, or potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, wool fat, sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil and soybean oil; glycols; such a propylene glycol or polyethylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator.

[00181] Uses of Compounds and Pharmaceutically Acceptable Compositions

[00182] In yet another aspect, the present invention provides a method of treating, or lessening the severity of a condition, disease, or disorder implicated by CFTR mutation. In certain embodiments, the present invention provides a method of treating a condition, disease, or disorder implicated by a deficiency of the CFTR activity, the method comprising administering a composition comprising a compound of Formula 1 to a subject, preferably a mammal, in need thereof.

[00183] In another aspect, the invention also provides a method of treating or lessening the severity of a disease in a patient comprising administering to said patient one of the compositions as defined herein, and said disease is selected from cystic fibrosis, asthma, smoke induced COPD, chronic bronchitis, rhinosinusitis, constipation, pancreatitis,

pancreatic insufficiency, male infertility caused by congenital bilateral absence of the vas deferens (CBAVD), mild pulmonary disease, idiopathic pancreatitis, allergic bronchopulmonary aspergillosis (ABPA), liver disease, hereditary emphysema, hereditary hemochromatosis, coagulation-fibrinolysis deficiencies, such as protein C deficiency, Type 1 hereditary angioedema, lipid processing deficiencies, such as familial hypercholesterolemia, Type 1 chylomicronemia, abetalipoproteinemia, lysosomal storage diseases, such as I-cell disease/pseudo-Hurler, mucopolysaccharidoses, Sandhof/Tay-Sachs, Crigler-Najjar type II, polyendocrinopathy/hyperinsulemia, Diabetes mellitus, Laron dwarfism, myeloperoxidase deficiency, primary hypoparathyroidism, melanoma, glycanosis CDG type 1, congenital hyperthyroidism, osteogenesis imperfecta, hereditary hypofibrinogenemia, ACT deficiency, Diabetes insipidus (DI), neurophyseal DI, neprogenic DI, Charcot-Marie Tooth syndrome, Perlizaeus-Merzbacher disease, neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, progressive supranuclear palsy, Pick's disease, several polyglutamine neurological disorders such as Huntington's, spinocerebellar ataxia type I, spinal and bulbar muscular atrophy, dentatorubal pallidolusian, and myotonic dystrophy, as well as spongiform encephalopathies, such as hereditary Creutzfeldt-Jakob disease (due to prion protein processing defect), Fabry disease, Straussler-Scheinker syndrome, COPD, dry-eye disease, or Sjogren's disease, Osteoporosis, Osteopenia, bone healing and bone growth (including bone repair, bone regeneration, reducing bone resorption and increasing bone deposition), Gorham's Syndrome, chloride channelopathies such as myotonia congenita (Thomson and Becker forms), Bartter's syndrome type III, Dent's disease, hyperekplexia, epilepsy, hyperekplexia, lysosomal storage disease, Angelman syndrome, and Primary Ciliary Dyskinesia (PCD), a term for inherited disorders of the structure and/or function of cilia, including PCD with situs inversus (also known as Kartagener syndrome), PCD without situs inversus and ciliary aplasia.

[00184] In some embodiments, the method includes treating or lessening the severity of cystic fibrosis in a patient comprising administering to said patient one of the compositions as defined herein. In certain embodiments, the patient possesses mutant forms of human CFTR. In other embodiments, the patient possesses one or more of the following mutations $\Delta F508$, R117H, and G551D of human CFTR. In one embodiment, the method includes treating or lessening the severity of cystic fibrosis in a patient possessing the $\Delta F508$ mutation of human CFTR comprising administering to said patient one of the compositions as defined herein. In one embodiment, the method includes treating or lessening the severity of cystic fibrosis in a patient possessing the G551D mutation of human CFTR comprising administering to said

patient one of the compositions as defined herein. In one embodiment, the method includes treating or lessening the severity of cystic fibrosis in a patient possessing the $\Delta F508$ mutation of human CFTR on at least one allele comprising administering to said patient one of the compositions as defined herein. In one embodiment, the method includes treating or lessening the severity of cystic fibrosis in a patient possessing the $\Delta F508$ mutation of human CFTR on both alleles comprising administering to said patient one of the compositions as defined herein. In one embodiment, the method includes treating or lessening the severity of cystic fibrosis in a patient possessing the G551D mutation of human CFTR on at least one allele comprising administering to said patient one of the compositions as defined herein. In one embodiment, the method includes treating or lessening the severity of cystic fibrosis in a patient possessing the G551D mutation of human CFTR on both alleles comprising administering to said patient one of the compositions as defined herein.

[00185] In some embodiments, the method includes lessening the severity of cystic fibrosis in a patient comprising administering to said patient one of the compositions as defined herein. In certain embodiments, the patient possesses mutant forms of human CFTR. In other embodiments, the patient possesses one or more of the following mutations $\Delta F508$, R117H, and G551D of human CFTR. In one embodiment, the method includes lessening the severity of cystic fibrosis in a patient possessing the $\Delta F508$ mutation of human CFTR comprising administering to said patient one of the compositions as defined herein. In one embodiment, the method includes lessening the severity of cystic fibrosis in a patient possessing the G551D mutation of human CFTR comprising administering to said patient one of the compositions as defined herein. In one embodiment, the method includes lessening the severity of cystic fibrosis in a patient possessing the $\Delta F508$ mutation of human CFTR on at least one allele comprising administering to said patient one of the compositions as defined herein. In one embodiment, the method includes lessening the severity of cystic fibrosis in a patient possessing the $\Delta F508$ mutation of human CFTR on both alleles comprising administering to said patient one of the compositions as defined herein. In one embodiment, the method includes lessening the severity of cystic fibrosis in a patient possessing the G551D mutation of human CFTR on at least one allele comprising administering to said patient one of the compositions as defined herein. In one embodiment, the method includes lessening the severity of cystic fibrosis in a patient possessing the G551D mutation of human CFTR on both alleles comprising administering to said patient one of the compositions as defined herein.

[00186] In some aspects, the invention provides a method of treating or lessening the severity of Osteoporosis in a patient comprising administering to said patient compound of Formula 1 or a pharmaceutically acceptable salt thereof.

[00187] In some embodiments, the method of treating or lessening the severity of Osteoporosis in a patient comprises administering to said patient substantially amorphous compound of Formula 1 or a pharmaceutically acceptable salt thereof.

[00188] In still other embodiments, the method of treating or lessening the severity of Osteoporosis in a patient comprises administering to said patient amorphous compound of Formula 1 or a pharmaceutically acceptable salt thereof.

[00189] In certain embodiments, the method of treating or lessening the severity of Osteoporosis in a patient comprises administering to said patient a pharmaceutical composition as described herein.

[00190] In some aspects, the invention provides a method of treating or lessening the severity of Osteopenia in a patient comprising administering to said patient compound of Formula 1 or a pharmaceutically acceptable salt thereof.

[00191] In some embodiments, the method of treating or lessening the severity of Osteopenia in a patient comprises administering to said patient substantially amorphous compound of Formula 1 or a pharmaceutically acceptable salt thereof.

[00192] In still other embodiments, the method of treating or lessening the severity of Osteopenia in a patient comprises administering to said patient amorphous compound of Formula 1.

[00193] In certain embodiments, the method of treating or lessening the severity of Osteopenia in a patient comprises administering to said patient a pharmaceutical composition as described herein.

[00194] In some aspects, the invention provides a method of bone healing and/or bone repair in a patient comprising administering to said patient compound of Formula 1 or a pharmaceutically acceptable salt thereof.

[00195] In some embodiments, the method of bone healing and/or bone repair in a patient comprises administering to said patient substantially amorphous compound of Formula 1 or a pharmaceutically acceptable salt thereof.

[00196] In still other embodiments, the method of bone healing and/or bone repair in a patient comprises administering to said patient amorphous compound of Formula 1 or a pharmaceutically acceptable salt thereof.

In certain embodiments, the method of bone healing and/or bone repair in a patient comprises administering to said patient a pharmaceutical composition as described herein.

[00198] In some aspects, the invention provides a method of reducing bone resorption in a patient comprising administering to said patient compound of Formula 1 or a pharmaceutically acceptable salt thereof.

[00199] In some embodiments, the method of reducing bone resorption in a patient comprises administering to said patient substantially amorphous compound of Formula 1 or a pharmaceutically acceptable salt thereof.

[00200] In still other embodiments, the method of reducing bone resorption in a patient comprises administering to said patient amorphous compound of Formula 1 or a pharmaceutically acceptable salt thereof.

[00201] In some aspects, the invention provides a method of increasing bone deposition in a patient comprising administering to said patient compound of Formula 1 or a pharmaceutically acceptable salt thereof.

[00202] In some embodiments, the method of increasing bone deposition in a patient comprises administering to said patient substantially amorphous compound of Formula 1 or a pharmaceutically acceptable salt thereof.

[00203] In still other embodiments, the method of increasing bone deposition in a patient comprises administering to said patient amorphous compound of Formula 1 or a pharmaceutically acceptable salt thereof.

[00204] In certain embodiments, the method of increasing bone deposition in a patient comprises administering to said patient a pharmaceutical composition as described herein.

[00205] In some aspects, the invention provides a method of treating or lessening the severity of COPD in a patient comprising administering to said patient compound of Formula 1 or a pharmaceutically acceptable salt thereof.

[00206] In some embodiments, the method of treating or lessening the severity of COPD in a patient comprises administering to said patient substantially amorphous compound of Formula 1 or a pharmaceutically acceptable salt thereof.

[00207] In still other embodiments, the method of treating or lessening the severity of COPD in a patient comprises administering to said patient amorphous compound of Formula 1 or a pharmaceutically acceptable salt thereof.

[00208] In certain embodiments, the method of treating or lessening the severity of COPD in a patient comprises administering to said patient a pharmaceutical composition as described herein.

- [00209] In some aspects, the invention provides a method of treating or lessening the severity of smoke induced COPD in a patient comprising administering to said patient compound of Formula 1 or a pharmaceutically acceptable salt thereof.
- [00210] In some embodiments, the method of treating or lessening the severity of smoke induced COPD in a patient comprises administering to said patient substantially amorphous compound of Formula 1 or a pharmaceutically acceptable salt thereof.
- [00211] In still other embodiments, the method of treating or lessening the severity of smoke induced COPD in a patient comprises administering to said patient amorphous compound of Formula 1 or a pharmaceutically acceptable salt thereof.
- [00212] In certain embodiments, the method of treating or lessening the severity of smoke induced COPD in a patient comprises administering to said patient a pharmaceutical composition as described herein.
- [00213] In some aspects, the invention provides a method of treating or lessening the severity of chronic bronchitis in a patient comprising administering to said patient compound of Formula 1 or a pharmaceutically acceptable salt thereof.
- [00214] In some embodiments, the method of treating or lessening the severity of chronic bronchitis in a patient comprises administering to said patient substantially amorphous compound of Formula 1 or a pharmaceutically acceptable salt thereof.
- [00215] In still other embodiments, the method of treating or lessening the severity of chronic bronchitis in a patient comprises administering to said patient amorphous compound of Formula 1 or a pharmaceutically acceptable salt thereof.
- [00216] In certain embodiments, the method of treating or lessening the severity of chronic bronchitis in a patient comprises administering to said patient a pharmaceutical composition as described herein.
- [00217] According to an alternative embodiment, the present invention provides a method of treating cystic fibrosis comprising the step of administering to said mammal an effective amount of a composition comprising a compound of the present invention.
- [00218] According to the invention an "effective amount" of the compound or pharmaceutically acceptable composition is that amount effective for treating or lessening the severity of one or more of the diseases, disorders or conditions as recited above.
- [00219] Another aspect of the present invention provides a method of administering a pharmaceutical composition by orally administering to a patient at least once per day the composition comprising a compound of Formula 1. In one embodiment, the method comprises administering a pharmaceutical composition comprising a compound of Formula 1

every 24 hours. In another embodiment, the method comprises administering a pharmaceutical composition comprising a compound of Formula 1 every 12 hours. In a further embodiment, the method comprises administering a pharmaceutical composition comprising a compound of Formula 1 three times per day. In still a further embodiment, the method comprises administering a pharmaceutical composition comprising a compound of Formula 1 every 4 hours.

[00220] The compounds and compositions, according to the method of the present invention, may be administered using any amount and any route of administration effective for treating or lessening the severity of one or more of the diseases, disorders or conditions as recited above.

[00221] In certain embodiments, the compounds and compositions of the present invention are useful for treating or lessening the severity of cystic fibrosis in patients who exhibit residual CFTR activity in the apical membrane of respiratory and non-respiratory epithelia. The presence of residual CFTR activity at the epithelial surface can be readily detected using methods known in the art, e.g., standard electrophysiological, biochemical, or histochemical techniques. Such methods identify CFTR activity using *in vivo* or *ex vivo* electrophysiological techniques, measurement of sweat or salivary Cl⁻ concentrations, or *ex vivo* biochemical or histochemical techniques to monitor cell surface density. Using such methods, residual CFTR activity can be readily detected in patients heterozygous or homozygous for a variety of different mutations, including patients homozygous or heterozygous for the most common mutation, ΔF508.

[00222] In another embodiment, the compounds and compositions of the present invention are useful for treating or lessening the severity of cystic fibrosis in patients who have residual CFTR activity induced or augmented using pharmacological methods or gene therapy. Such methods increase the amount of CFTR present at the cell surface, thereby inducing a hitherto absent CFTR activity in a patient or augmenting the existing level of residual CFTR activity in a patient.

[00223] In one embodiment, the compounds and compositions of the present invention are useful for treating or lessening the severity of cystic fibrosis in patients within certain genotypes exhibiting residual CFTR activity, e.g., class III mutations (impaired regulation or gating), class IV mutations (altered conductance), or class V mutations (reduced synthesis) (Lee R. Choo-Kang, Pamela L., Zeitlin, *Type I, II, III, IV, and V cystic fibrosis Transmembrane Conductance Regulator Defects and Opportunities of Therapy*, Current Opinion in Pulmonary Medicine 6:521 – 529, 2000). Other patient genotypes that exhibit

residual CFTR activity include patients homozygous for one of these classes or heterozygous with any other class of mutations, including class I mutations, class II mutations, or a mutation that lacks classification.

[00224] In one embodiment, the compounds and compositions of the present invention are useful for treating or lessening the severity of cystic fibrosis in patients within certain clinical phenotypes, e.g., a moderate to mild clinical phenotype that typically correlates with the amount of residual CFTR activity in the apical membrane of epithelia. Such phenotypes include patients exhibiting pancreatic insufficiency or patients diagnosed with idiopathic pancreatitis and congenital bilateral absence of the vas deferens, or mild lung disease.

[00225] The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the infection, the particular agent, its mode of administration, and the like. The compounds of the invention are preferably formulated in dosage unit form for ease of administration and uniformity of dosage. The expression "dosage unit form" as used herein refers to a physically discrete unit of agent appropriate for the patient to be treated. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific effective dose level for any particular patient or organism will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed, and like factors well known in the medical arts. The term "patient", as used herein, means an animal, preferably a mammal, and most preferably a human.

[00226] The pharmaceutically acceptable compositions of this invention can be administered to humans and other animals orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or patch), buccally, as an oral or nasal spray, or the like, depending on the severity of the infection being treated. In certain embodiments, the compounds of the invention may be administered orally or parenterally at dosage levels of about 0.01 mg/kg to about 50 mg/kg and preferably from about 0.5 mg/kg to about 25 mg/kg, of subject body weight per day, one or more times a day, to obtain the desired therapeutic effect.

[00227] Liquid dosage forms for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

[00228] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

[00229] The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[00230] In order to prolong the effect of a compound of the present invention, it is often desirable to slow the absorption of the compound from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the compound then depends upon its rate of dissolution that, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered compound form is accomplished by dissolving or suspending the compound in an oil vehicle. Injectable depot forms are made by forming microcapsule matrices of the compound in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of compound to

polymer and the nature of the particular polymer employed, the rate of compound release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the compound in liposomes or microemulsions that are compatible with body tissues.

[00231] Compositions for rectal or vaginal administration are preferably suppositories which can be prepared by mixing the compounds of this invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

[00232] Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

[00233] Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

[00234] The active compounds can also be in microencapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active compound may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., tableting lubricants and other tableting aids such as magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes.

[00235] Dosage forms for topical or transdermal administration of a compound of this invention include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. The active component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required. Ophthalmic formulation, eardrops, and eye drops are also contemplated as being within the scope of this invention. Additionally, the present invention contemplates the use of transdermal patches, which have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms are prepared by dissolving or dispensing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix or gel.

[00236] The activity of a compound utilized in this invention as a modulator of CFTR may be assayed according to methods described generally in the art and in the Examples herein.

[00237] It will also be appreciated that the compounds and pharmaceutically acceptable compositions of the present invention can be employed in combination therapies, that is, the compounds and pharmaceutically acceptable compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, an

inventive compound may be administered concurrently with another agent used to treat the same disorder), or they may achieve different effects (e.g., control of any adverse effects).

As used herein, additional therapeutic agents that are normally administered to treat or prevent a particular disease, or condition, are known as "appropriate for the disease, or condition, being treated."

[00238] In one embodiment, the additional agent is selected from a mucolytic agent, bronchodilator, an anti-biotic, an anti-infective agent, an anti-inflammatory agent, a CFTR modulator other than a compound of the present invention, or a nutritional agent.

[00239] In one embodiment, the additional agent is an antibiotic. Exemplary antibiotics useful herein include tobramycin, including tobramycin inhaled powder (TIP), azithromycin, aztreonam, including the aerosolized form of aztreonam, amikacin, including liposomal formulations thereof, ciprofloxacin, including formulations thereof suitable for administration by inhalation, levofloxacin, including aerosolized formulations thereof, and combinations of two antibiotics, e.g., fosfomycin and tobramycin.

[00240] In another embodiment, the additional agent is a mucolyte. Exemplary mucolytes useful herein includes Pulmozyme®.

[00241] In another embodiment, the additional agent is a bronchodilator. Exemplary bronchodilators include albuterol, metaprotenerol sulfate, pirbuterol acetate, salmeterol, or tetrabuline sulfate.

[00242] In another embodiment, the additional agent is effective in restoring lung airway surface liquid. Such agents improve the movement of salt in and out of cells, allowing mucus in the lung airway to be more hydrated and, therefore, cleared more easily. Exemplary such agents include hypertonic saline, denufosol tetrasodium ([[(3S, 5R)-5-(4-amino-2-oxopyrimidin-1-yl)-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl] [[[2R,3S,4R,5R)-5-(2,4-dioxypyrimidin-1-yl)-3, 4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]oxy-hydroxyphosphoryl] hydrogen phosphate), or bronchitol (inhaled formulation of mannitol).

[00243] In another embodiment, the additional agent is an anti-inflammatory agent, i.e., an agent that can reduce the inflammation in the lungs. Exemplary such agents useful herein include ibuprofen, docosahexanoic acid (DHA), sildenafil, inhaled glutathione, pioglitazone, hydroxychloroquine, or simvastatin.

[00244] In another embodiment, the additional agent is a CFTR modulator other than compound 1, i.e., an agent that has the effect of modulating CFTR activity. Exemplary such agents include ataluren ("PTC124®"; 3-[5-(2-fluorophenyl)-1,2,4-oxadiazol-3-yl]benzoic

acid), sinapultide, lancovutide, depelestat (a human recombinant neutrophil elastase inhibitor), cobiprostone (7-((2R, 4aR, 5R, 7aR)-2-((3S)-1,1-difluoro-3-methylpentyl)-2-hydroxy-6-oxooctahydrocyclopenta[b]pyran-5-yl)heptanoic acid), or (3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid. In another embodiment, the additional agent is (3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid.

[00245] In another embodiment, the additional agent is a nutritional agent. Exemplary such agents include pancrelipase (pancreatic enzyme replacement), including Pancrease®, Pancrecarb®, Ultrase®, or Creon®, Liprotomase® (formerly Trizyte®), Aquadeks®, or glutathione inhalation. In one embodiment, the additional nutritional agent is pancrelipase.

[00246] The amount of additional therapeutic agent present in the compositions of this invention will be no more than the amount that would normally be administered in a composition comprising that therapeutic agent as the only active agent. Preferably the amount of additional therapeutic agent in the presently disclosed compositions will range from about 50% to 100% of the amount normally present in a composition comprising that agent as the only therapeutically active agent.

[00247] The compounds of this invention or pharmaceutically acceptable compositions thereof may also be incorporated into compositions for coating an implantable medical device, such as prostheses, artificial valves, vascular grafts, stents and catheters.

Accordingly, the present invention, in another aspect, includes a composition for coating an implantable device comprising a compound of the present invention as described generally above, and in classes and subclasses herein, and a carrier suitable for coating said implantable device. In still another aspect, the present invention includes an implantable device coated with a composition comprising a compound of the present invention as described generally above, and in classes and subclasses herein, and a carrier suitable for coating said implantable device. Suitable coatings and the general preparation of coated implantable devices are described in US Patents 6,099,562; 5,886,026; and 5,304,121. The coatings are typically biocompatible polymeric materials such as a hydrogel polymer, polymethyldisiloxane, polycaprolactone, polyethylene glycol, polylactic acid, ethylene vinyl acetate, and mixtures thereof. The coatings may optionally be further covered by a suitable topcoat of fluorosilicone, polysaccharides, polyethylene glycol, phospholipids or combinations thereof to impart controlled release characteristics in the composition.

[00248] Another aspect of the invention relates to modulating CFTR activity in a biological sample or a patient (e.g., *in vitro* or *in vivo*), which method comprises administering to the patient, or contacting said biological sample with a compound of Formula 1 or a composition comprising said compound. The term "biological sample", as used herein, includes, without limitation, cell cultures or extracts thereof; biopsied material obtained from a mammal or extracts thereof; and blood, saliva, urine, feces, semen, tears, or other body fluids or extracts thereof.

[00249] Modulation of CFTR in a biological sample is useful for a variety of purposes that are known to one of skill in the art. Examples of such purposes include, but are not limited to, the study of CFTR in biological and pathological phenomena; and the comparative evaluation of new modulators of CFTR.

[00250] In yet another embodiment, a method of modulating activity of an anion channel *in vitro* or *in vivo*, is provided comprising the step of contacting said channel with a compound of Formula 1. In embodiments, the anion channel is a chloride channel or a bicarbonate channel. In other embodiments, the anion channel is a chloride channel.

[00251] According to an alternative embodiment, the present invention provides a method of increasing the number of functional CFTR in a membrane of a cell, comprising the step of contacting said cell with a compound of Formula 1.

[00252] According to another embodiment, the activity of the CFTR is measured by measuring the transmembrane voltage potential. Means for measuring the voltage potential across a membrane in the biological sample may employ any of the known methods in the art, such as optical membrane potential assay or other electrophysiological methods.

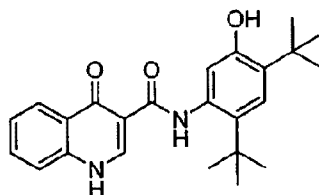
[00253] The optical membrane potential assay utilizes voltage-sensitive FRET sensors described by Gonzalez and Tsien (*See*, Gonzalez, J. E. and R. Y. Tsien (1995) "Voltage sensing by fluorescence resonance energy transfer in single cells." *Biophys J* 69(4): 1272-80, and Gonzalez, J. E. and R. Y. Tsien (1997); "Improved indicators of cell membrane potential that use fluorescence resonance energy transfer" *Chem Biol* 4(4): 269-77) in combination with instrumentation for measuring fluorescence changes such as the Voltage/Ion Probe Reader (VIPR) (*See*, Gonzalez, J. E., K. Oades, et al. (1999) "Cell-based assays and instrumentation for screening ion-channel targets" *Drug Discov Today* 4(9): 431-439).

[00254] These voltage sensitive assays are based on the change in fluorescence resonant energy transfer (FRET) between the membrane-soluble, voltage-sensitive dye, DiSBAC₂(3), and a fluorescent phospholipid, CC2-DMPE, which is attached to the outer leaflet of the plasma membrane and acts as a FRET donor. Changes in membrane potential (V_m) cause the

negatively charged DiSBAC₂(3) to redistribute across the plasma membrane and the amount of energy transfer from CC2-DMPE changes accordingly. The changes in fluorescence emission can be monitored using VIPR™ II, which is an integrated liquid handler and fluorescent detector designed to conduct cell-based screens in 96- or 384-well microtiter plates.

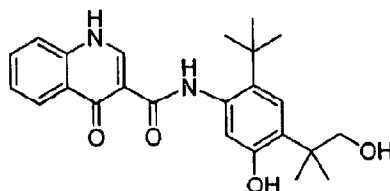
[00255] In one embodiment, the present invention provides a method of modulating CFTR activity in a biological sample comprising the step of contacting said biological sample with a compound of Formula 1, or a pharmaceutically acceptable salt thereof, wherein R₁, R₂, R₃, R₄ and Y are defined as above.

[00256] In one embodiment, the present invention provides a method of modulating CFTR activity in a biological sample comprising the step of contacting said biological sample with a compound, produced via the processes described herein, of the structure:



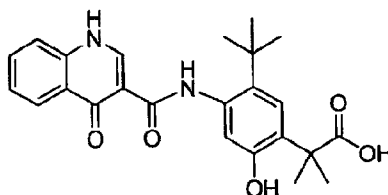
or a pharmaceutically acceptable salt thereof.

[00257] In one embodiment, the present invention provides a method of modulating CFTR activity in a biological sample comprising the step of contacting said biological sample with a compound, produced via the processes described herein, of the structure:



or a pharmaceutically acceptable salt thereof.

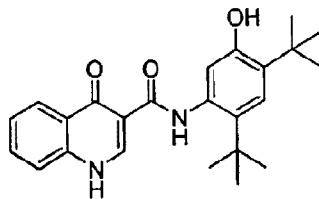
[00258] In one embodiment, the present invention provides a method of modulating CFTR activity in a biological sample comprising the step of contacting said biological sample with a compound, produced via the processes described herein, of the structure:



or a pharmaceutically acceptable salt thereof.

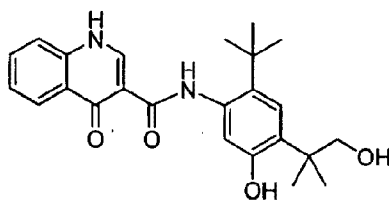
[00259] In one embodiment, the present invention provides a method of treating or lessening the severity of a disease in a patient comprising administering to said patient an effective amount of a compound of Formula 1, or a pharmaceutically acceptable salt thereof, wherein R₁, R₂, R₃, R₄ and Y are defined as above, and said disease is selected from cystic fibrosis, asthma, smoke induced COPD, chronic bronchitis, rhinosinusitis, constipation, pancreatitis, pancreatic insufficiency, male infertility caused by congenital bilateral absence of the vas deferens (CBAVD), mild pulmonary disease, idiopathic pancreatitis, allergic bronchopulmonary aspergillosis (ABPA), liver disease, hereditary emphysema, hereditary hemochromatosis, coagulation-fibrinolysis deficiencies, such as protein C deficiency, Type I hereditary angioedema, lipid processing deficiencies, such as familial hypercholesterolemia, Type I chylomicronemia, abetalipoproteinemia, lysosomal storage diseases, such as I-cell disease/pseudo-Hurler, mucopolysaccharidoses, Sandhof/Tay-Sachs, Crigler-Najjar type II, polyendocrinopathy/hyperinsulemia, Diabetes mellitus, Laron dwarfism, myeloperoxidase deficiency, primary hypoparathyroidism, melanoma, glycanosis CDG type 1, congenital hyperthyroidism, osteogenesis imperfecta, hereditary hypofibrinogenemia, ACT deficiency, Diabetes insipidus (DI), neurophyseal DI, neprogenic DI, Charcot-Marie Tooth syndrome, Perlizaeus-Merzbacher disease, neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, progressive supranuclear palsy, Pick's disease, several polyglutamine neurological disorders such as Huntington, spinocerebellar ataxia type I, spinal and bulbar muscular atrophy, dentatorubal pallidolusian, and myotonic dystrophy, as well as spongiform encephalopathies, such as hereditary Creutzfeldt-Jakob disease (due to prion protein processing defect), Fabry disease, Straussler-Scheinker syndrome, COPD, dry-eye disease, or Sjogren's disease.

[00260] In one embodiment, the method includes treating or lessening the severity of a disease in a patient by administering to said patient an effective amount of a compound, produced via the processes described herein, having the structure:



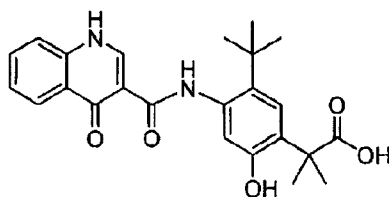
or a pharmaceutically acceptable salt thereof.

[00261] In one embodiment, the method includes treating or lessening the severity of a disease in a patient by administering to said patient an effective amount of a compound, produced via the processes described herein, having the structure:



or a pharmaceutically acceptable salt thereof.

[00262] In another embodiment, the method includes treating or lessening the severity of a disease in a patient by administering to said patient an effective amount of a compound, produced via the processes described herein, having the structure:



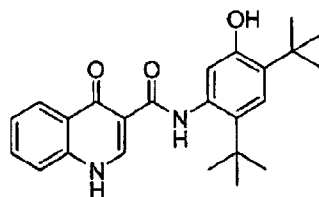
or a pharmaceutically acceptable salt thereof.

[00263] In another aspect the present invention provides a kit for use in measuring the activity of CFTR or a fragment thereof in a biological sample *in vitro* or *in vivo* comprising (i) a composition comprising a compound of Formula 1 or any of the above embodiments; and (ii) instructions for a) contacting the composition with the biological sample and b) measuring activity of said CFTR or a fragment thereof.

[00264] In one embodiment, the kit further comprises instructions for a) contacting an additional composition with the biological sample; b) measuring the activity of said CFTR or a fragment thereof in the presence of said additional compound, and c) comparing the activity of the CFTR in the presence of the additional compound with the density of the CFTR in the presence of a composition of Formula 1.

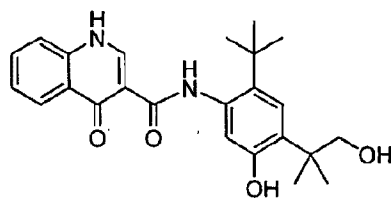
[00265] In embodiments, the kit is used to measure the density of CFTR.

[00266] In one embodiment, the kit includes a composition comprising a compound, produced via the processes described herein, having the structure:



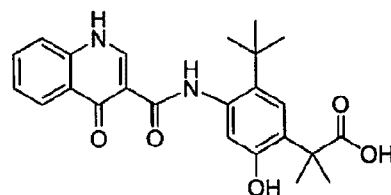
or a pharmaceutically acceptable salt thereof.

[00267] In one embodiment, the kit includes a composition comprising a compound, produced via the processes described herein, having the structure:



or a pharmaceutically acceptable salt thereof.

[00268] In some embodiments, the kit includes a composition comprising a compound, produced via the processes described herein, having the structure:

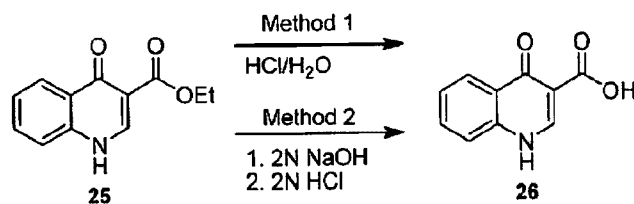
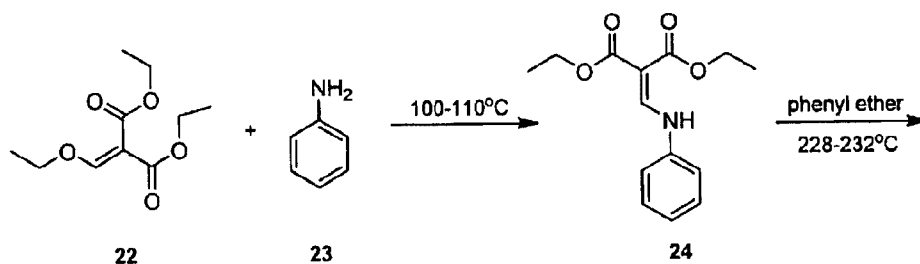


or a pharmaceutically acceptable salt thereof.

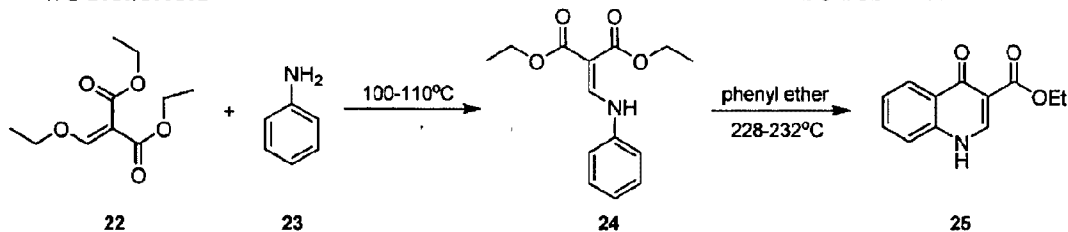
[00269] In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting this invention in any manner.

[00270] V. EXAMPLES

[00271] Preparation 1: Total Synthesis of 4-oxo-1,4-dihydroquinoline-3-carboxylic acid
(26)

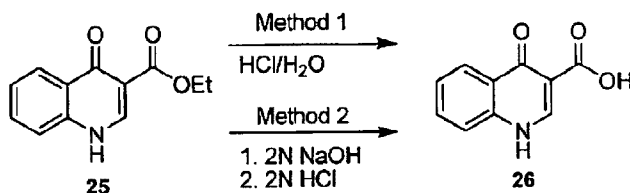


[00272] Procedure for the preparation of ethyl 4-oxo-1,4-dihydroquinoline-3-carboxylate
(25)



[00273] Compound **23** (4.77 g, 47.7 mmol) was added dropwise to compound **22** (10 g, 46.3 mmol) with subsurface N_2 flow to drive out ethanol below $30\text{ }^{\circ}\text{C}$ for 0.5 hours. The solution was then heated to $100\text{-}110\text{ }^{\circ}\text{C}$ and stirred for 2.5 hours. After cooling the mixture to below $60\text{ }^{\circ}\text{C}$, diphenyl ether was added. The resulting solution was added dropwise to diphenyl ether that had been heated to $228\text{-}232\text{ }^{\circ}\text{C}$ for 1.5 hours with subsurface N_2 flow to drive out ethanol. The mixture was stirred at $228\text{-}232\text{ }^{\circ}\text{C}$ for another 2 hours, cooled to below $100\text{ }^{\circ}\text{C}$ and then heptane was added to precipitate the product. The resulting slurry was stirred at $30\text{ }^{\circ}\text{C}$ for 0.5 hours. The solids were then filtrated, and the cake was washed with heptane and dried *in vacuo* to give compound **25** as brown solid. $^1\text{H NMR}$ (DMSO-d_6 ; 400 MHz) δ 12.25 (s), δ 8.49 (d), δ 8.10 (m), δ 7.64 (m), δ 7.55 (m), δ 7.34 (m), δ 4.16 (q), δ 1.23 (t).

[00274] *Procedure for the preparation of 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (26)*



Method 1

[00275] Compound **25** (1.0 eq) was suspended in a solution of HCl (10.0 eq) and H_2O (11.6 vol). The slurry was heated to $85\text{--}90\text{ }^{\circ}\text{C}$, although alternative temperatures are also suitable for this hydrolysis step. For example, the hydrolysis can alternatively be performed at a temperature of from about 75 to about $100\text{ }^{\circ}\text{C}$. In some instances, the hydrolysis is performed at a temperature of from about 80 to about $95\text{ }^{\circ}\text{C}$. In others, the hydrolysis step is performed at a temperature of from about 82 to about $93\text{ }^{\circ}\text{C}$ (e.g., from about 82.5 to about $92.5\text{ }^{\circ}\text{C}$ or from about 86 to about $89\text{ }^{\circ}\text{C}$). After stirring at $85\text{--}90\text{ }^{\circ}\text{C}$ for approximately 6.5 hours, the reaction was sampled for reaction completion. Stirring may be performed under any of the temperatures suited for the hydrolysis. The solution was then cooled to $20\text{--}25\text{ }^{\circ}\text{C}$

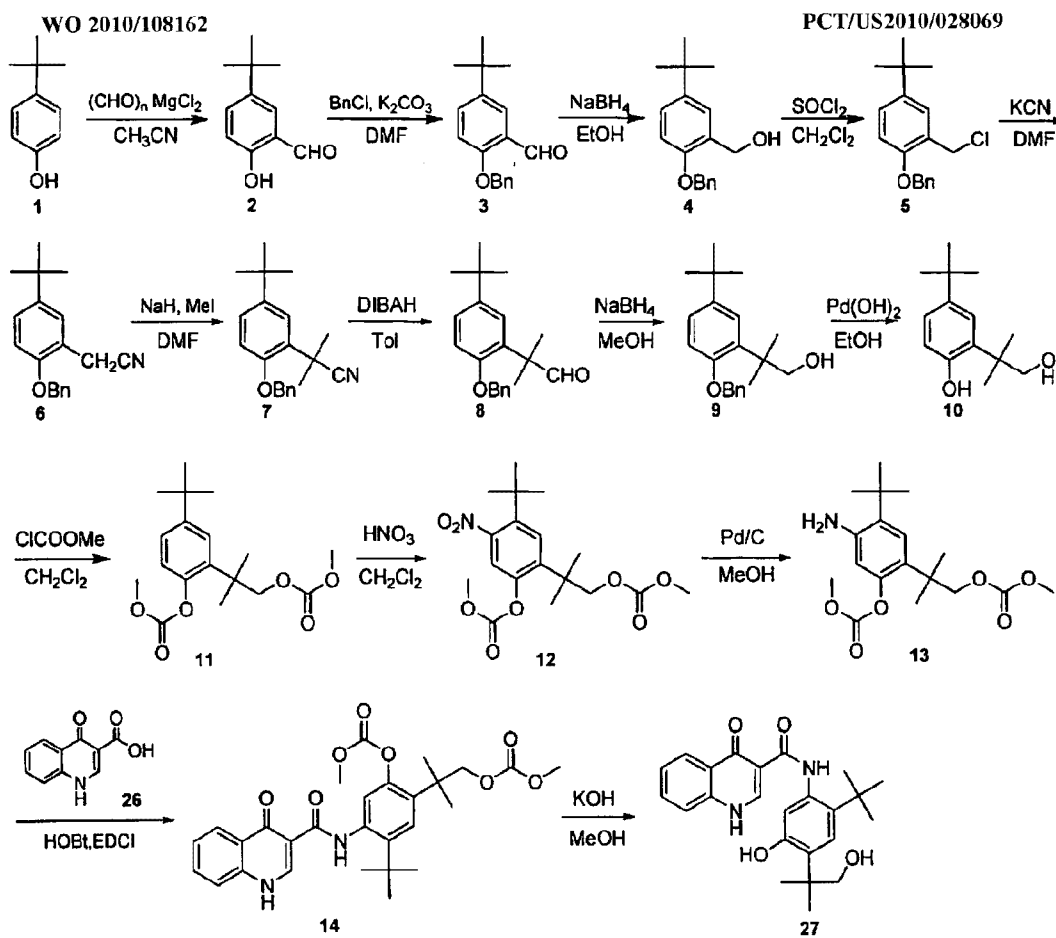
and filtered. The reactor/cake was rinsed with H₂O (2 vol x 2). The cake was then washed with 2 vol H₂O until the pH \geq 3.0. The cake was then dried under vacuum at 60 °C to give compound **26**.

Method 2

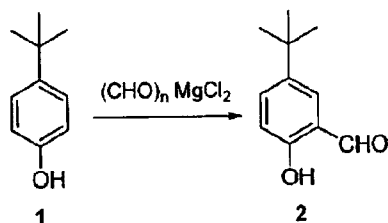
[00276] Compound **25** (11.3 g, 52 mmol) was added to a mixture of 10% NaOH (aq) (10 mL) and ethanol (100 mL). The solution was heated to reflux for 16 hours, cooled to 20-25 °C and then the pH was adjusted to 2-3 with 8% HCl. The mixture was then stirred for 0.5 hours and filtered. The cake was washed with water (50 mL) and then dried *in vacuo* to give compound **26** as a brown solid. ¹H NMR (DMSO-d₆; 400 MHz) δ 15.33 (s), δ 13.39 (s), δ 8.87 (s), δ 8.26 (m), δ 7.87 (m), δ 7.80 (m), δ 7.56 (m).

[00277] Example 1: Total synthesis of N-(2-tert-butyl-5-hydroxy-4-(1-hydroxy-2-methylpropan-2-yl)phenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (27)

[00278] The overall scheme of the synthesis of compound **27** is shown below, followed by the procedure for the synthesis of each synthetic intermediate.



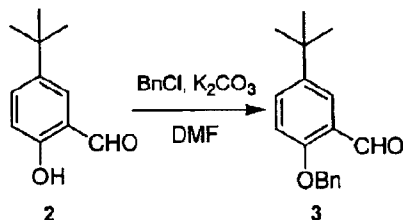
[00279] Procedure for the preparation of 2-hydroxy-5-tert-butylbenzaldehyde (2)



[00280] To a stirred solution of compound **1** (700 g, 4.66 mol) in CH₃CN (7.0 L) was added MgCl₂ (887 g, 9.32 mol), Para-Formaldehyde (1190 g) and TEA (2.5L, 17.9 mol) under N₂. The mixture was heated to reflux for 5 hours. After cooling to room temperature, 2 L ice water was added to the mixture, followed by 6 L of 3 M HCl (aq). The suspension was left stirring until the solution became clear. The organic layer was separated and the aqueous layer was extracted with MTBE (3 L×3). The organic layers were combined and concentrated to dryness. The residue was dissolved in MTBE (4000 mL), washed with water (1000 mL×2) and brine (1000mL), dried over anhydrous Na₂SO₄, filtered, then concentrated

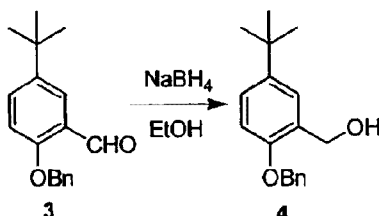
to give compound **2** as a light-yellow solid which was used in the next reaction without further drying or purification. $^1\text{H NMR}$ (CDCl_3 ; 400 MHz) δ 10.86 (s), δ 9.89 (s), δ 7.59 (m), δ 7.51 (d), δ 6.94 (d), δ 10.61 (s).

[00281] Procedure for the preparation of 2-(benzyloxy)-5-tert-butylbenzaldehyde (3)



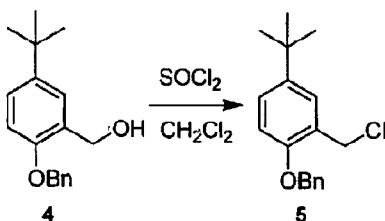
[00282] To a stirred solution of compound **2** (614.5 g, 3.33 mol) in DMF (3.5 L) was added K_2CO_3 (953 g, 6.90 mol) and benzyl chloride (480 g, 3.80 mol). The mixture was heated to 90 °C and left stirring for 3 hours. The suspension was cooled to room temperature, then MTBE (2 L) was added, followed by water (12 L). The mixture was then stirred for 10 minutes and the aqueous layer was separated and extracted with MTBE (2 L \times 3). The organic layers were combined and washed with water (2 L \times 2) and brine (1.5 L \times 1) and concentrated to give compound **3** as a light-yellow solid. $^1\text{H NMR}$ (DMSO-d_6 ; 400 MHz) δ 10.42 (s), δ 7.71 (m), δ 7.51 (m), δ 7.43 (m), δ 7.35 (m), δ 7.24 (m), δ 5.27 (s), δ 1.26 (s).

[00283] Procedure for the preparation of 2-(benzyloxy)-5-tert-butylbenzyl alcohol (4)



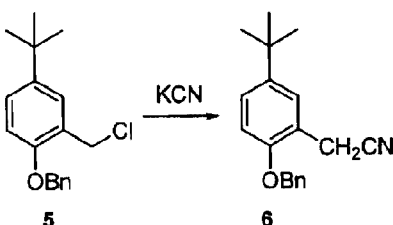
[00284] To a stirred suspension of compound **3** (974 g, 3.63 mol) in MeOH (4000 mL) was slowly added NaBH_4 (121 g, 3.20 mol) at 0-20 °C. The solution was left stirring at 15 °C for 3 hours, and then cooled to 0 °C. 2N HCl (aq) (1300mL) was added dropwise at below 20 °C. The solution was then filtered and evaporated to dryness, and the residue was dissolved in MTBE (5L). The solution was then washed with water (2 L \times 2) and brine (1.5 L \times 1). Evaporation of the solvent gave compound **4** as a light-yellow solid which was used in the next reaction without further purification. $^1\text{H NMR}$ (DMSO-d_6 ; 400 MHz) δ 7.40 (m), δ 7.32 (m), δ 7.17 (m), δ 6.91 (m), δ 5.09 (s), δ 5.00 (t), δ 4.56 (d), δ 1.26 (s).

[00285] Procedure for the preparation of 2-(benzyloxy)-5-tert-butylbenzyl chloride (5)



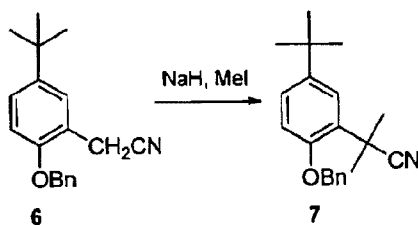
[00286] To a stirred solution of compound 4 (963 g, 3.56 mol) in anhydrous DCM (2000 mL) was added slowly SOCl_2 (535 g, 4.5 mol) at 0 °C. The mixture was stirred at 20 °C for 2 hours, then concentrated *in vacuo* to give compound 5 as an oil, which was used in the next reaction without further drying or purification.

[00287] *Procedure for the preparation of 2-(benzyloxy)-5-tert-butylbenzyl nitrile (6)*



[00288] To a stirred solution of compound 5 (1045 g, 3.54 mol) in anhydrous DMF (1000 mL) was added KCN (733 g, 11.3 mol). The mixture was stirred at 35 °C for 24 hours, then poured into water (10 L). Ethyl acetate (4 L) was added and the mixture was stirred for 30 minutes. The organic layer was then separated and the aqueous layer was extracted with ethyl acetate (3000 mL \times 2). The organic layers were combined and washed with water (4 L \times 2) and brine (3 L \times 1), then concentrated *in vacuo* to give compound 6 as a yellow solid. ^1H NMR (DMSO- d_6 ; 400 MHz) δ 7.51 (m), δ 7.37 (m), 7.02 (d), δ 5.17 (s), δ 3.88 (s), 1.26 (s).

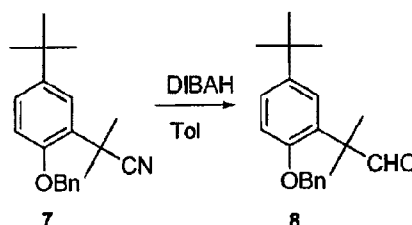
[00289] *Procedure for the preparation of 2-(2-(benzyloxy)-5-tert-butylphenyl)-2-methylpropanenitrile (7)*



[00290] To a stirred suspension of NaH (86 g, 2.15 mol, 60% in mineral oil) in DMF (1000 mL) was added dropwise a solution of compound 6 (100.0 g, 0.358 mol) in DMF (500 mL) at 20 °C. After stirring for 30 minutes, MeI (205 g, 1.44 mol) in DMF (500 mL) was added dropwise at below 30 °C during a period of 2 hours. The suspension was stirred for 1.5 hours

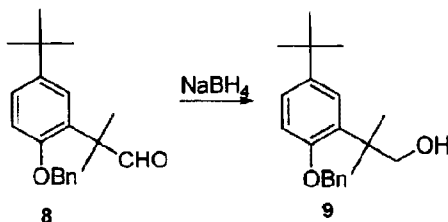
at 25-30 °C, then ice (100 g) was added slowly until no gas was generated. The pH was adjusted to approximately 7 by the slow addition of 2N HCl. The mixture was diluted with water (4 L) and MTBE (2 L). The organic layer was separated and the aqueous layer was extracted with MTBE (500 mL×2). The combined organic layers were washed with water and brine, dried over Na₂SO₄, filtered, and then concentrated *in vacuo* to give compound **7** as a white solid. ¹H NMR (DMSO-d₆; 400 MHz) δ 7.56 (m), δ 7.40 (m), δ 7.34 (m), δ 7.10 (d), δ 5.21 (s), δ 1.73 (s), δ 1.27 (s).

[00291] Procedure for the preparation of 2-(2-(benzyloxy)-5-tert-butylphenyl)-2-methylpropanal (8)



[00292] To a stirred solution of compound **7** (20 g, 0.065 mol) in toluene (300 mL), was added drop wise DIBALH (80 mL, 1 M in toluene) at about -60 to -50 °C. After stirring for 2 hours, 6 N HCl (300 mL) was added to the reaction mixture and stirring was continued for 30 minutes. The organic layer was then separated, washed with 2 N HCl followed by a NaHCO₃ solution, then a brine solution, dried over Na₂SO₄ and concentrated *in vacuo* to afford the compound **8** as an oil. The product was used in the next reaction without further purification. ¹H NMR (CDCl₃; 400 MHz) δ 9.61 (s), δ 7.36 (m), δ 7.25 (m), δ 6.87 (m), δ 5.06 (m), δ 1.43 (s), δ 1.33 (s).

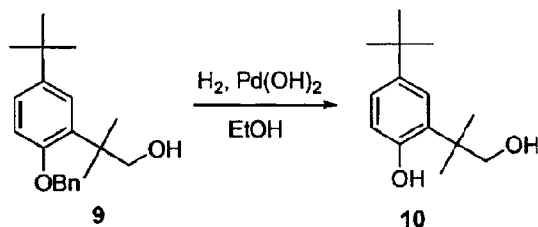
[00293] Procedure for the preparation of 2-(2-(benzyloxy)-5-tert-butylphenyl)-2-methylpropan-1-ol (9)



[00294] To a stirred solution of compound **8** (9.21 g, 0.030 mol) in MeOH (150 mL) was added slowly NaBH₄ (2.3 g, 0.061 mol) at 0 °C. After the mixture was stirred at 20 °C for 3 hours, 12 mL of 6 N HCl was added, and the mixture was stirred for an additional 30 minutes. The solution was then concentrated to about one-quarter of the original volume and extracted with EtOAc. The organic layer was separated and washed with water and brine,

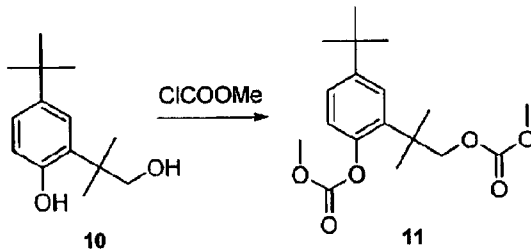
dried with Na_2SO_4 , filtered, and then concentrated *in vacuo* to afford compound **9** as a white solid. ^1H NMR (DMSO- d_6 ; 400 MHz) δ 7.47 (m), δ 7.42 (m), δ 7.34 (m), δ 7.28 (m), δ 7.16 (m), δ 6.94 (m), δ 5.08 (s), δ 4.45 (t), δ 3.64 (d), δ 1.28 (s), δ 1.25 (s).

[00295] Procedure for the preparation of 2-(2-hydroxy-5-tert-butylphenyl)-2-methylpropan-1-ol (10)



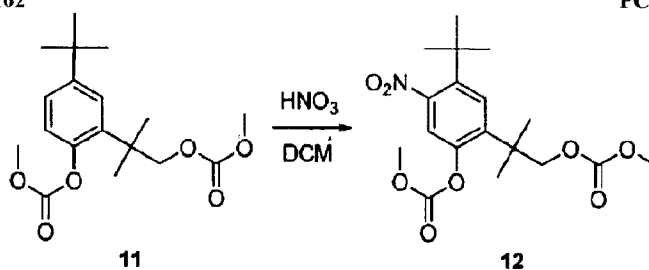
[00296] $\text{Pd}(\text{OH})_2$ (1 g) and compound **9** (9.26 g, 0.030 mol) in MeOH (200 mL) were stirred under hydrogen at 20-30 psi pressure for 16-18 hours. The mixture was then filtered through Celite®, and the filtrate was concentrated to give compound **10** as a white solid. ^1H NMR (DMSO- d_6 ; 400 MHz) δ 9.16 (s), δ 7.16 (d), δ 7.00 (m), δ 6.65 (m), δ 4.71 (t), δ 3.62 (d), δ 1.27 (s), δ 1.22 (s).

[00297] Procedure for the preparation of 1-((methylcarboxy)oxy)-2-(1-((methylcarboxy)oxy)-2-methylpropan-2-yl)-4-tert-butyl benzene (11)



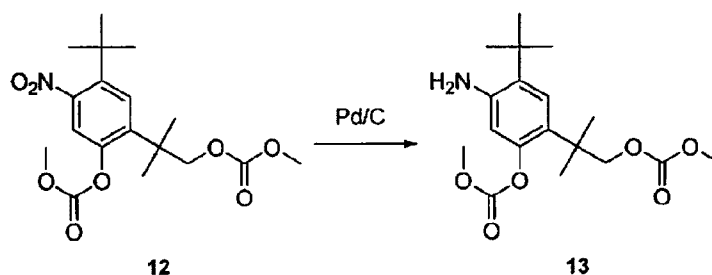
[00298] To a stirred solution of compound **10** (23.2 g, 0.10 mol), DMAP (1.44 g) and DIEA (72.8 g, 0.56 mol) in anhydrous DCM (720 mL) was added dropwise methyl chloroformate (43.5 g, 0.46 mol) in DCM (160 mL) at 0 °C. After the mixture was stirred at 20 °C for 16 hours, it was washed with water, 1 N HCl and brine, dried with MgSO_4 and concentrated *in vacuo*. The residue was purified using column chromatography on silica gel (1:20 EtOAc:Petroleum ether) to give compound **11** as a white solid. ^1H NMR (DMSO- d_6 ; 400 MHz) δ 7.32 (m), δ 7.10 (d), δ 4.26 (s), δ 3.84 (s), δ 3.64 (s), δ 1.31 (s), δ 1.28 (s).

[00299] Procedure for preparation of 1-((methylcarboxy)oxy)-2-(1-((methylcarboxy)oxy)-2-methylpropan-2-yl)-4-tert-butyl-5-nitro benzene (12)



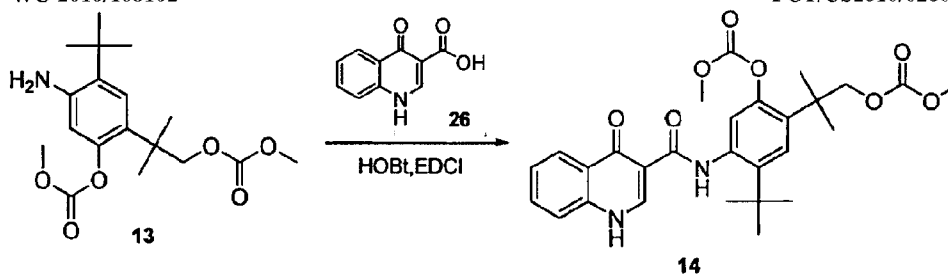
[00300] To a stirred solution of compound **11** (32 g, 0.095 mol) in DCM (550 mL) was added dropwise 98% H₂SO₄ (43 g, 0.43 mol) at 0 °C. After stirring for 20 minutes at 0 °C, 65% HNO₃ (16.2 g, 0.17 mol) was added to the mixture dropwise at 0 °C. The mixture was then stirred at 1-10 °C for 4 hours and then ice-water (200 mL) was added. The aqueous layer was separated and extracted with DCM (200 mL × 3) and the combined organic layers were washed with water (aq), NaHCO₃ and brine, then dried with MgSO₄ and concentrated *in vacuo*. The residue was purified via column chromatography on silica gel (1:20 EtOAc:Petroleum ether) to afford crude compound **12** as an oil.

[00301] *Procedure for the preparation of 2-tert-butyl-5-((methylcarboxy)oxy)-4-(1-((methylcarboxy)oxy)-2-methylpropan-2-yl)aniline (13)*



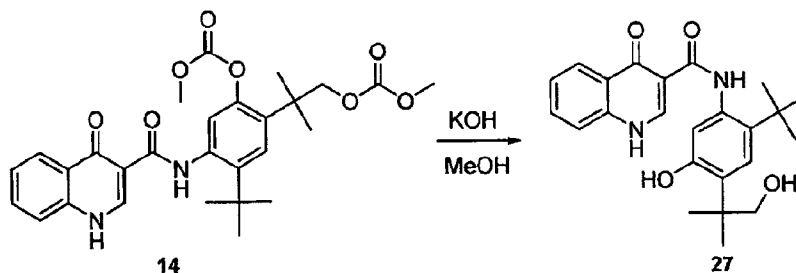
[00302] Pd/C (2.6 g) and compound **12** (14 g, crude) were stirred in MeOH (420 mL) at room temperature under hydrogen at 20-30 psi pressure for 16-18 hours. Then the mixture was filtered with kieselguhr®, and the filtrate was concentrated *in vacuo*. The residue was purified via column chromatography on silica gel (1:10 EtOAc:Petroleum ether) to give compound **13** as a gray solid. ¹H NMR (CDCl₃; 400 MHz) δ 7.26 (s), δ 7.19 (s), δ 4.26 (s), δ 3.89 (s), δ 3.74 (s), δ 1.40 (s), δ 1.35 (s).

[00303] *Procedure for the preparation of N-(2-tert-butyl-5-((methylcarboxy)oxy)-4-(1-((methylcarboxy)oxy)-2-methylpropan-2-yl)phenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (14)*

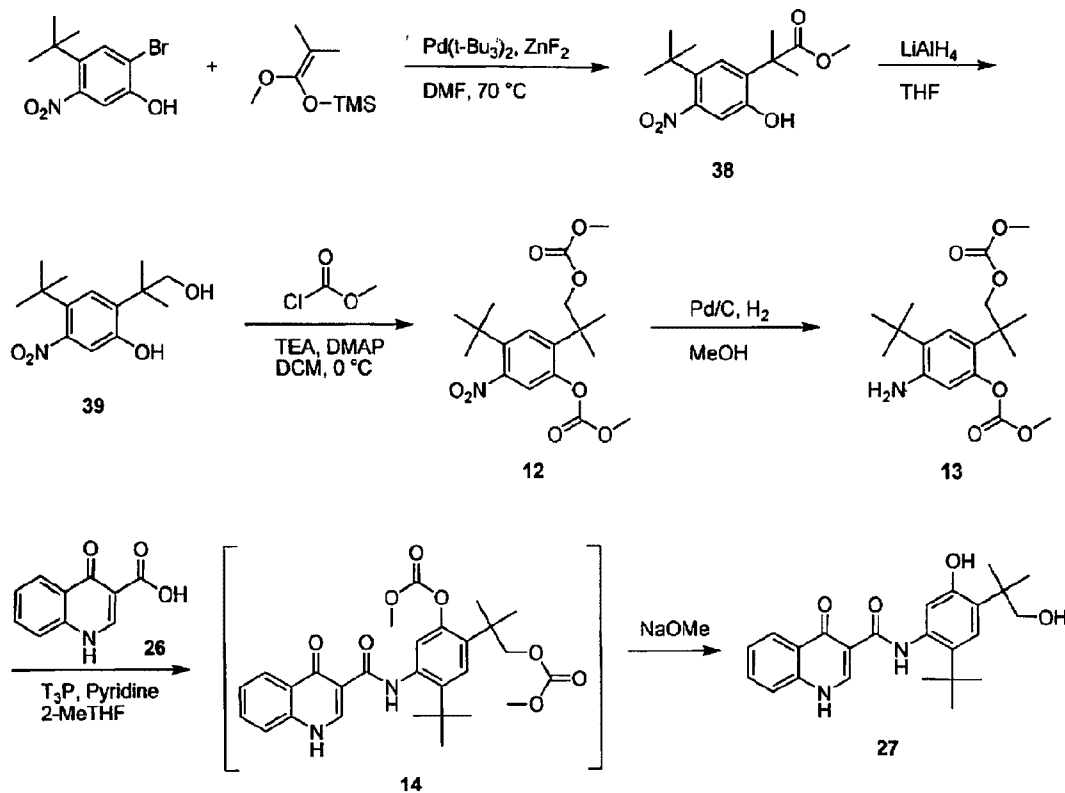
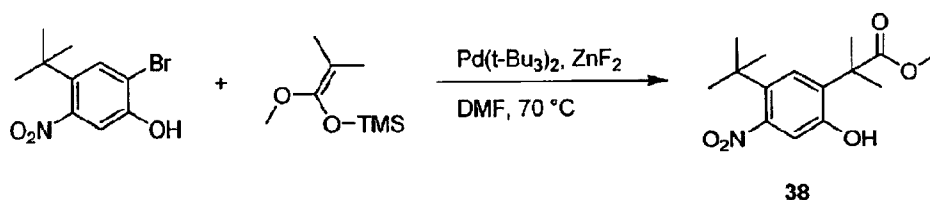


[00304] To a stirred solution of compound 26 (5.0 g, 0.026 mol) in anhydrous DMF (120 mL) was added EDCI (5.6 g, 0.029 mol), HOBT (3.8 g, 0.028 mol) and DIEA (6.6 g, 0.051 mol) at 0 °C. After stirring for 1 hour, the mixture was added dropwise a solution of compound 13 (3.0 g, 0.008 mol) in DCM (30 ml) at 0 °C. The mixture was stirred at 25 °C for 72 hours, and then was concentrated *in vacuo*. The residue was dissolved in EtOAc (225 mL) and washed with water (120 mL×1), 1N HCl (120 mL) and brine, dried with Na₂SO₄ and concentrated *in vacuo*. The residue was purified via column chromatography on silica gel (1:1 EtOAc:Petroleum ether) to give compound 14 as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 12.34 (s, 1H), 11.58 (s, 1H), 9.07 (s, 1H), 8.42 (d, 1H), 7.66 (s, 1H), 7.51 (s, 1H), 7.47 (s, 1H), 7.39 (s, 1H), 6.72 (s, 1H), 4.34 (s, 2H), 3.82 (s, 3H), 3.74 (s, 3H), 1.41 (s, 9H), 1.40 (s, 6H).

[00305] *Procedure for the preparation of N-(2-tert-butyl-5-hydroxy-4-(1-hydroxy-2-methylpropan-2-yl)phenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (27)*



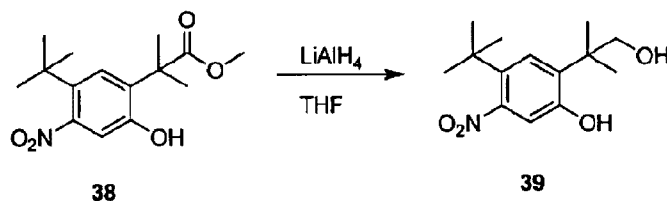
[00306] To a stirred solution of KOH (1.2 g, 0.02 mol) in MeOH (80 mL) was added compound 14 (1.9 g, 0.0036 mol) at 0 °C. After stirring for 2-3 hours at 5-15 °C, the mixture was concentrated to dryness. The residue was then triturated in water (10 mL), filtered, washed with DCM and dried *in vacuo* for 24 hours to give compound 27 as a white solid. ¹H NMR (DMSO-d₆; 400 MHz) δ 12.77 (s), δ 8.86 (s), δ 8.20 (d), δ 7.55 (d), δ 7.42 (t), δ 7.16 (q), δ 7.02 (s), δ 6.85 (m), δ 3.55 (s), δ 1.55 (s), δ 1.35 (s), δ 1.27 (s). MS Found (M + H) 409.2

[00307] Example 2: Alternative Total Synthesis of N-(2-tert-butyl-5-hydroxy-4-(1-**hydroxy-2-methylpropan-2-yl)phenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (27)****[00308] Procedure for the preparation of methyl 2-(5-tert-butyl-2-hydroxy-4-nitrophenyl)-2-methylpropanoate (38):**

[00309] A mixture of 2-bromo-4-tert-butyl-5-nitrophenol (15.00 g, 54.72 mmol), bis(tri-tert-butylphosphine)palladium(0) (1.422 g, 2.783 mmol), zinc fluoride (2.82 g, 27.27 mmol), methyl trimethylsilyl dimethylketene acetal (MTDA) (19.35 g, 111.0 mmol), and dimethylformamide (150 mL) was heated at 70°C for 18 h. The mixture was cooled to room temperature and diluted with water. After stirring for one hour, the aqueous phase was extracted with MTBE. The organic layer was dried *in vacuo* to afford the crude product as a brown solid. Purification of the product was accomplished by trituration in n-heptane. ^1H -

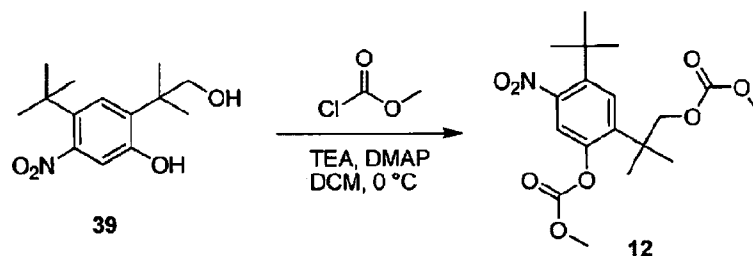
NMR (400MHZ, DMSO-*d*6) δ 10.38 (s, 1H); 7.37 (s, 1H); 6.79 (s, 1H); 3.54 (s, 3H); 1.45 (s, 6H); 1.32 (s, 9H)

[00310] Procedure for the preparation of 4-tert-butyl-2-(1-hydroxy-2-methylpropan-2-yl)-5-nitrophenol (39):



[00311] A 1M solution of lithium aluminum hydride in THF (11.80 mL, 11.80 mmol) was added to a solution of methyl 2-(5-tert-butyl-2-hydroxy-4-nitrophenyl)-2-methylpropanoate (5.36 g, 18.15 mmol) in THF (50 mL). The mixture was stirred at ambient temperature for 3 h, and then diluted with methanol. The mixture was acidified with 1N HCl (pH 1-2) and the aqueous phase was extracted with MTBE. The organic phase was dried *in vacuo* to afford 4-tert-butyl-2-(1-hydroxy-2-methylpropan-2-yl)-5-nitrophenol which was used without further purification in the next step. ¹H-NMR (400MHZ, DMSO-*d*6) δ 10.12 (s, 1H); 7.37 (s, 1H); 6.80 (s, 1H); 4.77 (s, 1H); 3.69-3.65 (m, 2H); 1.30 (s, 9H); 1.29 (s, 6H)

[00312] Procedure for the preparation of 4-tert-butyl-2-(2-methoxycarbonyloxy-1,1-dimethyl-ethyl)-5-nitro-phenyl] methyl carbonate (12)

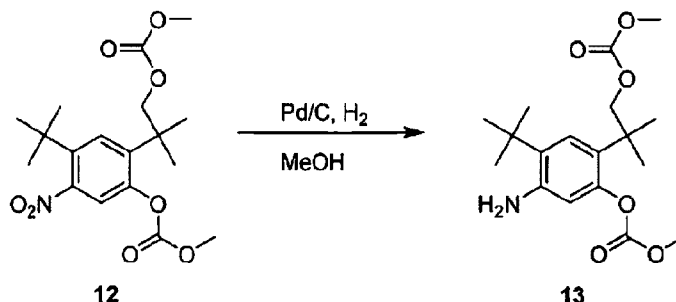


[00313] To a solution of 4-tert-butyl-2-(1-hydroxy-2-methylpropan-2-yl)-5-nitrophenol (1.92 g, 7.18 mmol), triethylamine (1.745 g, 17.24 mmol), and dimethylaminopyridine (87.74 mg, 0.718 mmol) in dichloromethane (30 mL) at 0 °C was slowly charged methylchloroformate (2.376 g, 25.14 mmol), keeping the temperature below 5 °C. After the addition, the mixture was allowed to warm to ambient temperature and was stirred until HPLC showed complete conversion of the starting material (2-8 h). The reaction mixture was diluted with water and acidified with 1N HCl (pH 1-2). The aqueous phase was extracted with DCM and the combined organics dried *in vacuo*. The crude amber semi-solid was re-crystallized from methanol and dichloromethane to give the title compound as a yellow

WO 2010/108162
 crystalline solid. ¹H-NMR (400MHZ, DMSO-*d*₆) δ 7.67 (s, 1H); 7.52 (s, 1H); 4.30 (s, 2H);
 3.86 (s, 3H); 3.64 (s, 3H); 1.35 (s, 9H); 1.35 (s, 6H)

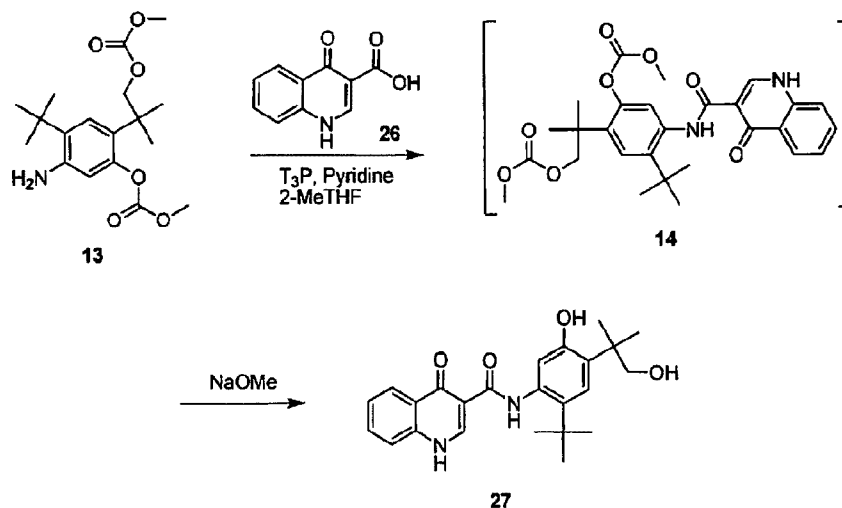
PCT/US2010/028069

[00314] Procedure for the preparation of 5-amino-4-tert-butyl-2-(2-methoxycarbonyloxy-1,1-dimethyl-ethyl)phenyl] methyl carbonate (13):



[00315] A mixture of [4-tert-butyl-2-(2-methoxycarbonyloxy-1,1-dimethyl-ethyl)-5-nitrophenyl] methyl carbonate (1.27 g, 3.313 mmol) and Pd/C (75 mg, 0.035 mmol) in methanol (50 mL) was purged with nitrogen. After purging the flask with hydrogen, the mixture was hydrogenated for 18 hours at ambient temperature and pressure. The solution was filtered through Celite® and dried *in vacuo* to obtain the product as a solid. ¹H-NMR (400MHZ, DMSO-*d*₆) δ 6.99 (s, 1H); 6.39 (s, 1H); 4.92(s, 2H); 4.13 (s, 2H); 3.82 (s, 3H); 3.65 (s, 3H); 1.32 (s, 9H); 1.23 (s, 6H)

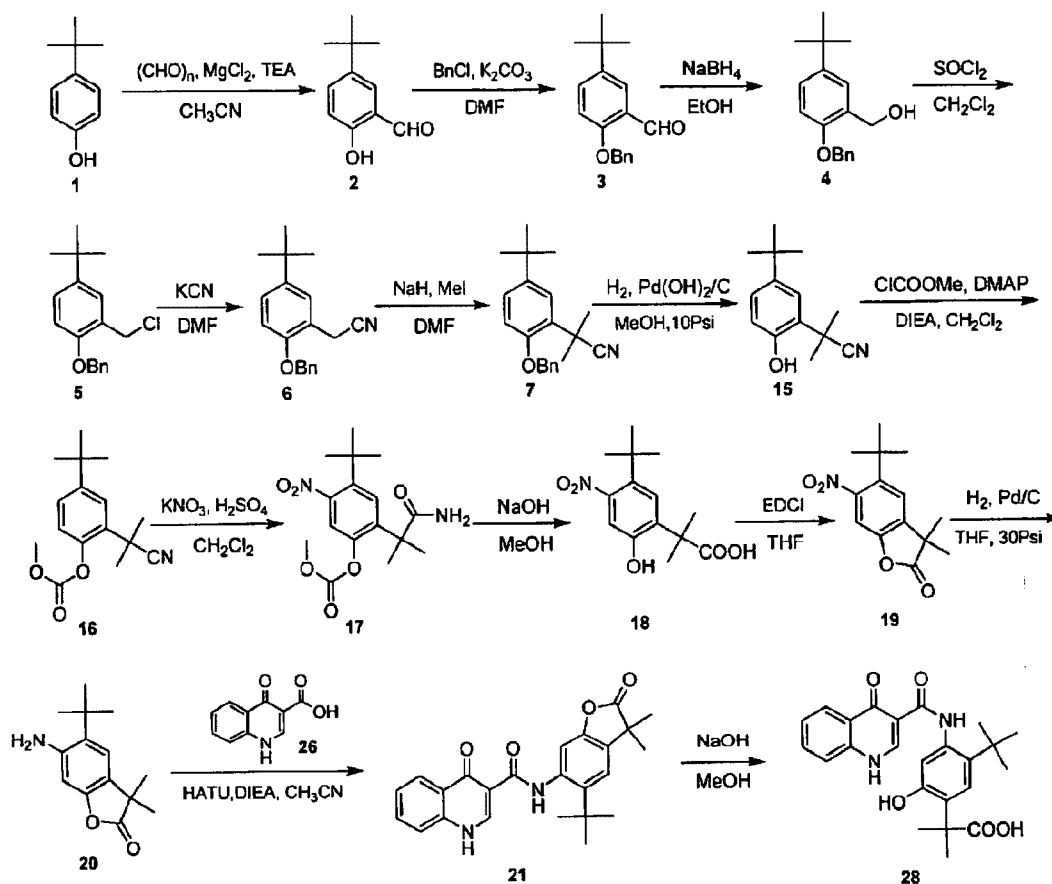
[00316] Procedure for the preparation of N-(2-tert-butyl-5-hydroxy-4-(1-hydroxy-2-methylpropan-2-yl)phenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (27):



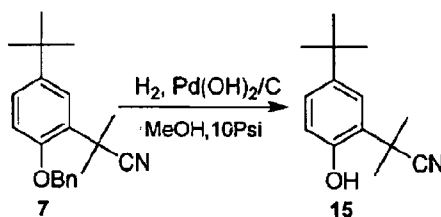
[00317] To a mixture of [5-amino-4-tert-butyl-2-(2-methoxycarbonyloxy-1,1-dimethyl-ethyl)phenyl] methyl carbonate (103 mg, 0.29 mmol), 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (50 mg, 0.26 mmol), and pyridine (42 mg, 0.53 mmol) in 2-MeTHF (3.0 mL) was charged T3P as a 50 wt% solution in 2-MeTHF (286 mg, 0.45 mmol). The mixture was

heated to 50 °C for 18 h. After cooling to ambient temperature, the mixture was diluted with water. The organic phase was separated and again washed with water. Sodium methoxide (39 mg, 0.72 mmol) was charged to the organic phase and the solution stirred for 2 hours. The reaction was quenched with 1N HCl, and after separating the phases, the organic phase was washed with 0.1N HCl. The organic phase was then dried *in vacuo* to yield Compound 27 as a solid. The ¹H-NMR spectrum was consistent with that reported above.

[00318] Example 3: Total Synthesis of 2-(5-tert-butyl-2-hydroxy-4-(4-oxo-1,4-dihydroquinoline-3-carboxamido)phenyl)-2-methylpropanoic acid (28):

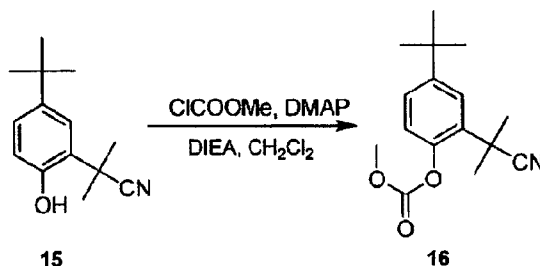


[00319] Procedure for the preparation of 2-(5-tert-butyl-2-hydroxyphenyl)-2-methylpropanenitrile (15)



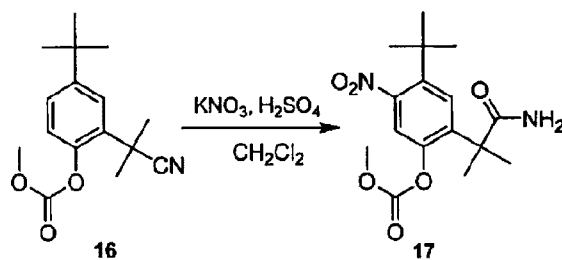
[00320] Pd(OH)₂/C (2.0 g) and compound 7 (20.0 g, 0.104 mol) were stirred in MeOH (150 mL) at room temperature under hydrogen at 10 psi pressure for 16-18 hours. The mixture was then filtered through a pad of Celite®, and the filtrate was concentrated to give compound 15, which was used in the next reaction without further purification. ¹H NMR (DMSO-d₆; 400 MHz) δ 9.83 (s), δ 7.24 (s), δ 7.18 (m), δ 6.80 (m), δ 1.71 (s), δ 1.24 (s).

[00321] *Procedure for the preparation of 4-tert-butyl-2-(2-cyanopropan-2-yl)phenyl methyl carbonate (16)*



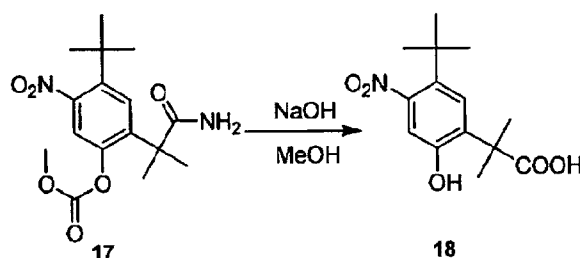
[00322] To a stirred mixture of compound 15 (126.6 g, 0.564 mol), DMAP (6.0 g) and DIEA (188 g, 1.46 mol) in anhydrous DCM (1500 mL) was added dropwise methyl chloroformate (110 g, 1.17 mol) in anhydrous DCM (300 mL) at 0 °C within 2 hours. After stirring for 12 hours at 0 °C, ice-water (1.5 L) was added and the mixture was stirred at 0 °C for 30 minutes. The organic layer was separated and washed with 1 N HCl, water, and brine. The DCM solution was dried over MgSO₄ and concentrated *in vacuo* to give compound 16 as a yellow solid. ¹H NMR (DMSO-d₆; 400 MHz) δ 7.47 (m), δ 7.39 (d), δ 7.24 (d), δ 3.84 (s), δ 1.71 (s), δ 1.30 (s).

[00323] *Procedure for the preparation of 2-(1-amino-2-methyl-1-oxopropan-2-yl)-4-tert-butyl-5-nitrophenyl methyl carbonate (17)*



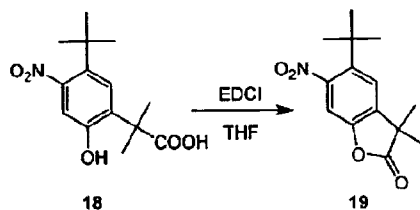
[00324] To a stirred mixture of compound **16** (10.0 g, 36.3 mmol) and KNO_3 (5.51 g, 54.5 mmol) in DCM (1000 mL) was added dropwise 98% H_2SO_4 (145.4 g, 1.45 mol) at 0 °C. The mixture was stirred at 30 °C for 4 days. The H_2SO_4 layer was then separated and poured into ice-water (50 g) and then extracted with DCM (100 mL \times 3). The combined organic layers were washed with water, aqueous NaHCO_3 solution and brine, then dried over MgSO_4 and concentrated *in vacuo*. The residue was purified via column chromatography on silica gel (Petroleum ether/EtOAc 20:1 \rightarrow 10:1 \rightarrow 5:1 \rightarrow 3:1) to give compound **17** as a yellow solid. ^1H NMR (CDCl_3 ; 400 MHz) δ 8.05 (s), δ 7.74 (s), δ 7.61 (s), δ 7.32 (s), δ 5.32 (s), δ 3.91 (s), δ 3.92 (s), δ 1.62 (s), δ 1.59 (s), δ 1.42 (s), δ 1.38 (s).

[00325] *Procedure for the preparation of 2-(5-tert-butyl-2-hydroxy-4-nitrophenyl)-2-methylpropanoic acid (18)*



[00326] To a mixture of compound **17** (7.3 g, 21.6 mmol) in methanol (180 mL) was added water (18 mL) and NaOH (8.64 g, 216 mmol). The solution was heated and maintained at reflux for 3 days. The solvent was evaporated *in vacuo* and the residue was dissolved in 140 mL of water. Then the solution was acidified to pH 2 by the addition of 2N HCl . The aqueous phase was extracted with ethyl acetate (100 mL \times 3), and the combined organic phases were washed with water and brine, dried over anhydrous Na_2SO_4 and then concentrated to give compound **18** as a yellow solid, which was used in the next reaction without further purification.

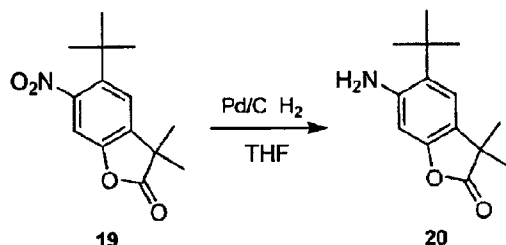
[00327] *Procedure for the preparation of 5-tert-butyl-3,3-dimethyl-6-nitrobenzofuran-2(3H)-one (19)*



[00328] To a solution of compound **18** (7.10 g, 25.2 mmol) in 710 mL of anhydrous THF was added EDCI (14.5 g, 75.6 mmol). The resulting suspension was left stirring at 30 °C overnight. The precipitate was filtered and thoroughly washed with DCM. The filtrate was

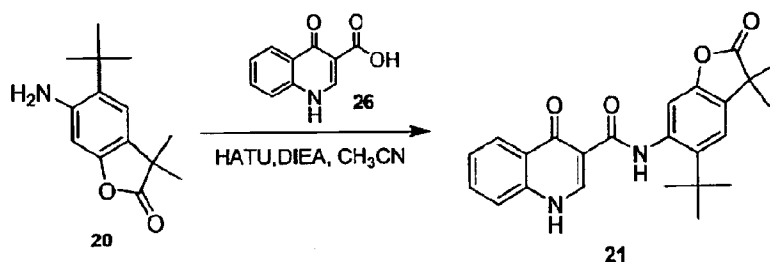
concentrated to dryness and the residue was dissolved in DCM (100 mL). The solution was washed with water (50 mL×2) and brine (50 mL×1). The DCM layer was then dried over anhydrous Na₂SO₄ and concentrated to give the crude product, which was purified via column chromatography on silica gel (Petroleum ether/EtOAc 200:1→100:1→50:1) to give compound **19** as a white solid. ¹H NMR (CDCl₃; 400 MHz) δ 7.36 (s), δ 7.10 (s), δ 1.53 (s), δ 1.41 (s).

[00329] Procedure for the preparation of 6-amino-5-tert-butyl-3,3-dimethylbenzofuran-2(3H)-one (20)



[00330] Pd/C (1.50 g) and compound **19** (3.00 g, 1.14 mmol) were suspended in THF (1500 mL) at 25 °C under hydrogen at 30 psi for 4 hours. The mixture was then filtered through a pad of Celite®, and the filtrate was concentrated *in vacuo* to give compound **20** as a white solid. ¹H NMR (DMSO-d₆; 400 MHz) δ 7.05 (s), δ 6.49 (s), δ 5.01 (s), δ 1.35 (s), δ 1.33 (s).

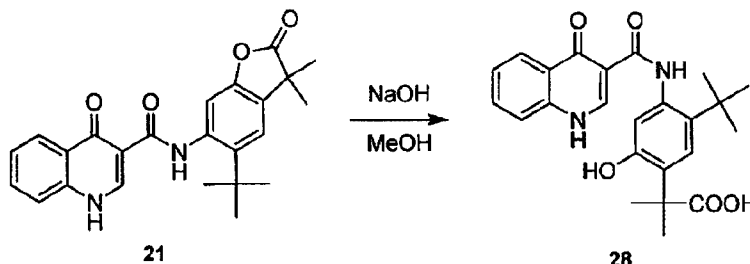
[00331] Procedure for the preparation of N-(5-tert-butyl-3,3-dimethyl-2-oxo-2,3-dihydrobenzofuran-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (21)



[00332] A suspension of HATU (17.6 g, 46.3 mol) and compound **26** (8.36 g, 44.2 mmol) in anhydrous acetonitrile (1 L) was stirred at room temperature for 1 hour. Compound **20** (3.40 g, 14.6 mmol) was added to the suspension, and then DIEA (11.5 g, 89.0 mmol) was added dropwise. The mixture was stirred at 45 °C for 4 days. The resulting precipitate was filtered and thoroughly washed with DCM. The filtrate was concentrated to dryness and the residue was dissolved in DCM (200 mL) and washed with 1N HCl (200 mL×2) followed by 5% aqueous NaHCO₃ (200 mL×3) and then brine (200 mL×1). The mixture was then dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified via column chromatography on silica gel (CH₂Cl₂/MeOH 100:1→50:1) to give compound **21** as a light

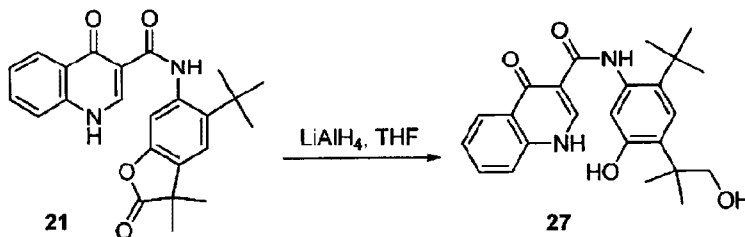
yellow solid. $^1\text{H-NMR}$ (400MHz, DMSO- d_6) δ 12.96 (d J 6.4 Hz, 1H); 12.1 (s, 1H); 8.9 (d, J 6.4Hz, 1H); 8.33 (d, J 8Hz, 1H); 7.84-7.75 (m, 2H); 7.55-7.48 (m, 3H); 1.47 (s, 6H); 1.45 (s, 9H).

[00333] Procedure for the preparation of 2-(5-tert-butyl-2-hydroxy-4-(4-oxo-1,4-dihydroquinoline-3-carboxamido)phenyl)-2-methylpropanoic acid (28)



[00334] To a stirred solution of compound **21** (0.9 g, 2.45 mmol) in MeOH (50 mL) was added NaOH (1.5 g, 37.5 mmol) at 0 °C. After stirring for 16 hours at 40 °C, the solvent was evaporated *in vacuo*, then the residue was dissolved in H₂O (50 ml). The precipitate was filtered and the filtrate was washed with DCM (100 mL×1) and ethyl acetate (100 mL×1). The aqueous layer was acidified with 2N HCl to pH 1-2. The precipitate was filtered and washed with H₂O (80 mL) and heptane (50 mL). It was dried *in vacuo* to give compound **28** as a white solid. $^1\text{H NMR}$ (DMSO- d_6 ; 400 MHz) δ 12.85 (s), δ 11.84 (s), δ 11.77 (s), δ 9.39 (s), δ 8.86 (s), δ 8.33 (s), δ 7.79 (m), δ 7.52 (m), δ 7.18 (s), δ 7.09 (s), δ 1.44 (s), δ 1.40 (s). MS found (M + H) 423.08

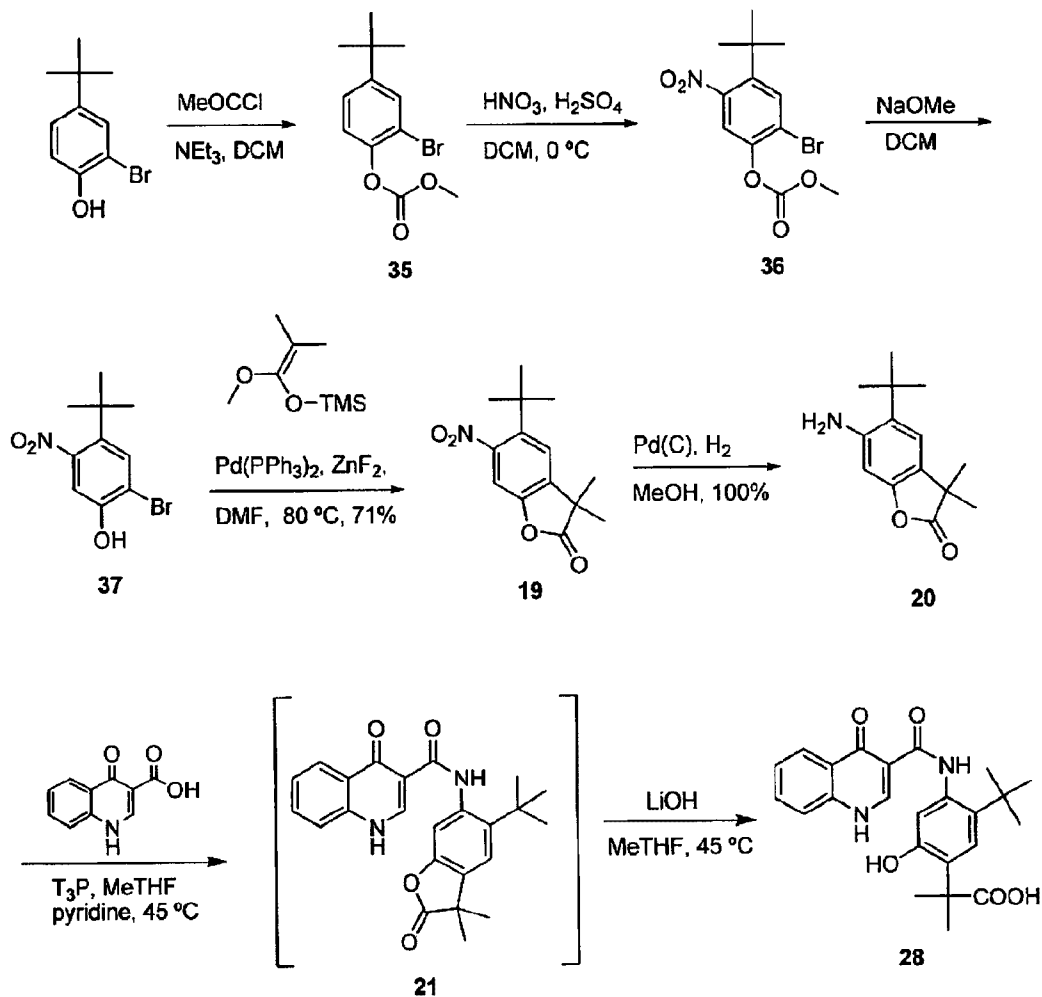
[00335] Example 4: Second alternative Synthesis of N-(2-tert-butyl-5-hydroxy-4-(1-hydroxy-2-methylpropan-2-yl)phenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (27)



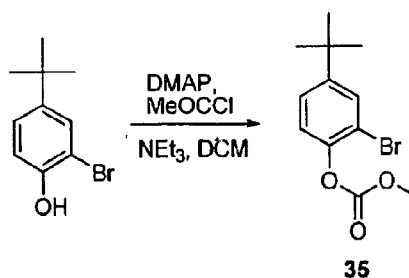
[00336] A 3-neck 50mL round bottom flask was equipped with magnetic stirrer, nitrogen bubbler and thermocouple. Compound **21** (514mg, 1.27mmol) and 2-MeTHF (4mL) are charged to the flask. The reaction mixture was stirred at room temperature. Lithium aluminum hydride (204 mg, 6.6 mmol) was added as solid until 100% conversion is achieved, which was monitored using HPLC. Potassium sodium 2,3-dihydroxybutanedioate tetrahydrate salt (50 mL of a 400 g/L solution) and MTBE (50mL) were added to the reaction

mixture. The resulting solution was stirred for 15 minutes and then let sit for 15min. The organic layer was separated and the pH of the aqueous layer was adjusted to a pH of about 6-7 by adding Tartaric acid. The aqueous layer was extracted with MTBE. The organic layer was concentrated and dried under high vacuum to provide the title compound as an off-white powder. The ¹H-NMR spectrum was consistent with that reported above.

[00337] **Example 5: Alternative Total synthesis of 2-(5-tert-butyl-2-hydroxy-4-(4-oxo-1,4-dihydroquinoline-3-carboxamido)phenyl)-2-methylpropanoic acid (28):**

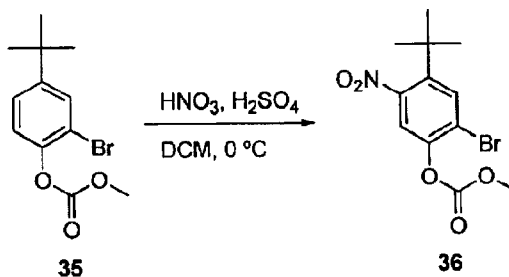


[00338] **Procedure for the preparation of Carbonic acid 2-bromide, 4-tertbutyl phenyl ester methyl ester (35)**



[00339] A 3-neck 2L round bottom flask was equipped with mechanical stirrer, nitrogen bubbler and thermocouple. 2-Bromo-4-tert-butyl phenol (50g, 211.7mmol) was added followed by DCM (1.75L), DMAP (1.29g, 10.58mmol) and Et₃N (44.3mL, 317.6mmol). The reaction mixture was cooled down to 0 °C. Methyl chloroformate (19.62mL, 254mmol) was added drop-wise to the reaction mixture. The mixture was allowed to warm to room temperature while stirring overnight. When the reaction was complete, the mixture was filtered via sintered funnel. The filtrate was transferred into 1L separatory funnel. To quench, 1N HCl (300mL) was added to filtrate and the organic layer was separated. The organic layer was then washed with a mixture of 291 mL saturated NaHCO₃ and 100 mL water. The layers were separated, and the aqueous layer was determined to have a pH of about 8. The organic layer was concentrated and dried under high vacuum for about 16 hours to give the title compound as a clear yellow oil, which was used in the next step without further purification. ¹H-NMR (400MHz, DMSO-*d*₆) 7.66 (d, *J* 2.0 Hz, 1H), 7.46 (dd, *J* 8.4, 2.0 Hz, 1H), 7.32 (d, *J* 8.4 Hz, 1H), 3.86 (s, 3H), 1.28 (s, 9H)

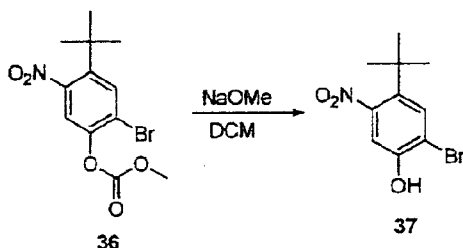
[00340] *Procedure for the preparation of (2-bromo-4-tert-butyl-5-nitro-phenyl) methyl carbonate (36)*



[00341] A 3-neck 2L round bottom flask was equipped with mechanical stirrer, nitrogen bubbler and thermocouple. Compound **35** (176g, 612.9mmol) and concentrated sulfuric acid (264mL) were charged to the flask. The reaction mixture was cooled to -5 °C - 0 °C. Nitric acid (28.6mL, 612.9mmol) was added drop-wise and the reaction mixture was stirred at 0 °C for 2 hours. When complete, water (264mL) was added followed by MTBE (264mL). The

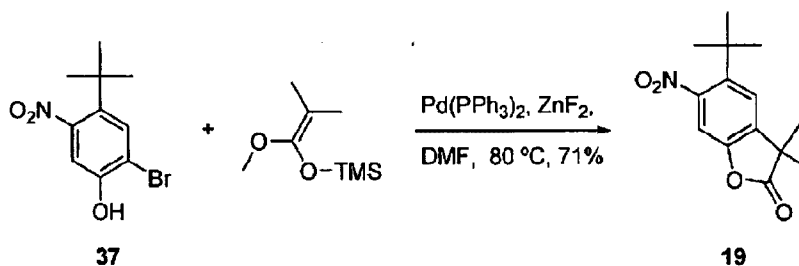
solution was stirred for 15 minutes, then let stand for 15 minutes. The organic layer was separated, concentrated and dried under high vacuum to give the title compound as a dark brown oil, which was used in the next step without further purification. $^1\text{H-NMR}$ (400MHz, DMSO-*d*6) 7.96 (s, 1H), 7.92 (s, 1H), 3.89 (s, 3H), 1.34 (s, 9H)

[00342] *Procedure for the preparation of 2-bromo-4-tert-butyl-5-nitro-phenol (37)*



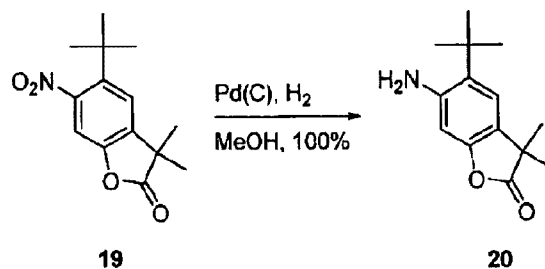
[00343] (2-Bromo-4-tert-butyl-5-nitro-phenyl)methyl carbonate (72.9 g, 219.5 mmol) was charged to a reactor and DCM (291.6 mL) was added. The yellow reaction solution was cooled using an ice bath. Sodium methoxide (67.04 g, 69.11 mL of 5.4 M, 373.2 mmol) was added portion-wise at 2.2 - 6.9 °C. After complete addition, the reaction was slowly warmed to ambient temperature. When complete, the reaction was cooled to 0 °C and quenched with 1M HCl (373.2 mL, 373.2 mmol). The biphasic mixture was stirred for 20 min and transferred to a separatory funnel. The organic layer was separated and washed with water (300 mL) followed by brine (300ml). The organic layer was concentrated and the crude product dried under high vacuum. The product was further purified using Supercritical Fluid Chromatography (SFC) separation on a Berger MultiGram III (Mettler Toledo AutoChem, Newark DE). The method conditions were 20% methanol at 250mL/min on a PPU column (30*150) from Princeton Chromatography, 100 bar, 35C, 220nm. An injection of 3.5mL of a 55-70mg/mL solution was injected. The data was collected using SFC ProNTTM software. The purified product received from SFC purification was a methanol solvate. To remove the methanol, an azeotropic distillation was performed. The dark yellow solid, 2-bromo, 4-tertbutyl, 5-nitro phenol methanol solvate, (111.3g, .59.9mmol) was charged to a 1L round bottom flask, followed by heptane (500mL). The slurry is heated to 64 °C to obtain a clear solution. The solvent was distilled under reduced pressure (649 mbar) for 30 minutes and then stripped to dryness. This procedure was repeated three times until no MeOH was detected by $^1\text{H-NMR}$. The product was dried under high vacuum for 16 hours to give the product as a dark yellow semi solid. $^1\text{H-NMR}$ (400MHz, DMSO-*d*6) δ 11.2 (bs, OH), 7.69 (s, 1H); 7.03 (s, 1H); 1.30 (s, 9H)

[00344] Procedure for the preparation of 5-tert-butyl-3,3-dimethyl-6-nitrobenzofuran-2(3H)-one (19)



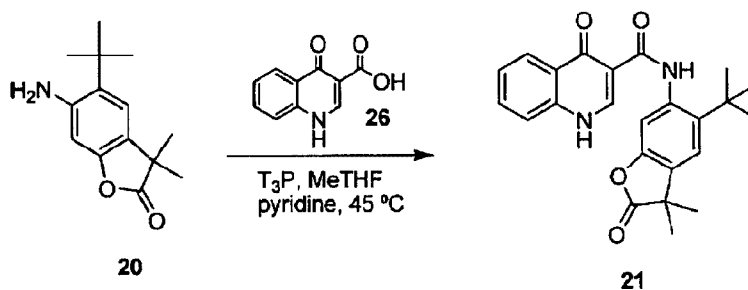
[00345] Difluorozinc (6.093 g, 58.92 mmol) was added to a round bottomed flask, which was flushed with nitrogen. Pd(*t*Bu₃P)₂ (2 g, 3.835 mmol) was then added under nitrogen stream. 2-Bromo-4-tert-butyl-5-nitro-phenol (16.15 g, 58.92 mmol) dissolved in DMF (80.75 mL) was then added to the flask. The reaction mixture was an orange suspension. (1-Methoxy-2-methyl-prop-1-en-1-yl)trimethylsilane (21.61 g, 25.13 mL, 117.8 mmol) was added to the mixture and the resulting mixture was heated to 80 °C and stirred for 16 h. When complete, the reaction mixture was cooled to ambient temperature and filtered through Celite®. The filter cake was washed with MTBE (536.0 mL) and water (893.3 mL) was added to the filtrate. The mixture was stirred for 15 min and settled for another 15 min. The layers were separated and 0.5M HCl (500 mL, 250.0 mmol) was added to the organic phase. The layers were separated and the organic layer was washed with water (500 mL). The layers were separated and the organic layer was washed with NaCl (500 mL; 8 wt%). The organic layer was separated and the solvent removed *in vacuo*. The crude product was obtained as a brown crystalline solid and was then purified through a silica plug, using hexane:MTBE 20:1-10:1 as an eluent. The fractions containing product were combined and the solvent removed *in vacuo* to give the pure product as a white crystalline solid. ¹H-NMR (400MHZ, DMSO-*d*₆) δ 7.80 (s, 1H); 7.62 (s, 1H); 1.49 (s, 6H); 1.34 (s, 9H)

[00346] Procedure for the preparation of 6-amino-5-tert-butyl-3,3-dimethylbenzofuran-2(3H)-one (20)



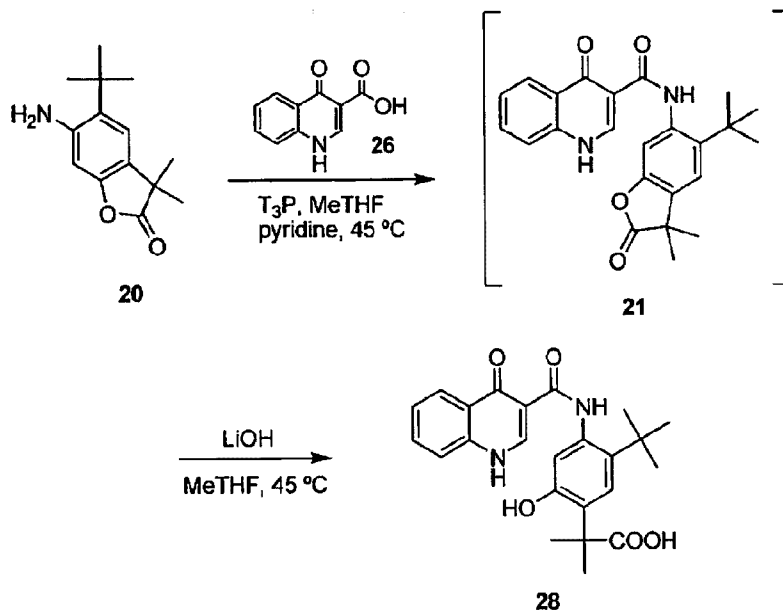
[00347] Palladium on carbon (wet; 5 wt%) was placed into a round bottomed flask under nitrogen flow. 5-tert-butyl-3,3-dimethyl-6-nitro-benzofuran-2-one (4.7 g, 17.85 mmol) was then added to the vessel. Methanol (120 mL) was then carefully charged to the vessel under nitrogen atmosphere. The vessel was then purged with N₂, evacuated, then charged with hydrogen gas. The vessel was evacuated and re-charged with hydrogen gas, and then a continuous hydrogen gas stream was introduced. After completion, the reaction was filtered through Celite® and the cake was washed with MeOH (300ml). The solvent was removed *in vacuo* and the product dried under high vacuum to give a white crystalline solid. ¹H-NMR (400MHZ, DMSO-*d*₆) δ 7.05 (s, 1H); 6.48 (s, 1H); 5.02 (s, 2H, NH₂); 1.34 (s, 6H); 1.30 (s, 9H)

[00348] *Procedure for the preparation of N-(5-tert-butyl-3,3-dimethyl-2-oxo-2,3-dihydrobenzofuran-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (21)*



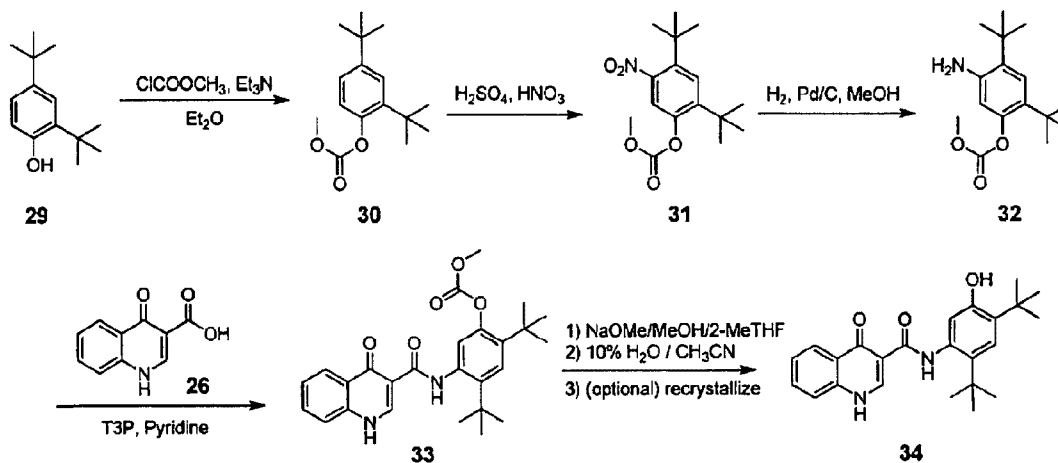
[00349] A reaction vessel was charged with compound **26** (2.926 g, 15.43 mmol), Compound **20** (4.32 g, 18.52 mmol), 2-MeTHF (35.99 mL), and subsequently 50% T₃P in 2-MeTHF (13.36 g, 21.00 mmol). Pyridine (2.441 g, 2.496 mL, 30.86 mmol) was added and the suspension heated at 47.5 °C ±5 °C for 18 h. After completion, the reaction was cooled to ambient temperature and 2-MeTHF (36) and water (30 ml) were added. The layers were split and the organic layer was washed with 10 wt% citric acid solution (30ml), water (30 ml) and twice with NaHCO₃ (20 ml). The organic layer was washed with brine (50 ml), separated and the solvent removed *in vacuo*. The crude product was dissolved in MTBE (100ml) and hexane (200ml) was added as an anti-solvent. A solid precipitated and the resulting slurry was stirred for two hours. The solid was collected by suction filtration and the cake was washed with hexane. The resulting product was dried in a vacuum oven at 55 °C with nitrogen bleed to give the title compound as a beige solid. ¹H-NMR (400MHZ, DMSO-*d*₆) δ 12.96 (d, *J* 6.4 Hz, 1H); 12.1 (s, 1H); 8.9 (d, *J* 6.4Hz, 1H); 8.33 (d, *J* 8Hz, 1H); 7.84-7.75 (m, 2H); 7.55-7.48 (m, 3H); 1.47 (s, 6H); 1.45 (s, 9H).

[00350] Procedure for the preparation of 2-(5-tert-butyl-2-hydroxy-4-(4-oxo-1,4-dihydroquinoline-3-carboxamido)phenyl)-2-methylpropanoic acid (28)

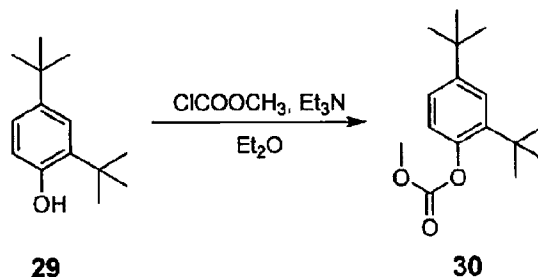


[00351] Compound 26 (81.30 mg, 0.4288 mmol) and Compound 20 (110 mg, 0.4715 mmol) were charged to a round bottomed flask. 2-MeTHF (1 mL) followed by 50% T₃P in 2-MeTHF (371.4 mg, 0.5836 mmol) and pyridine (67.84 mg, 69.37 μ L, 0.8576 mmol) in 2-MeTHF were then added. The suspension was heated at 47.5 \pm 5 $^\circ$ C overnight. After completion, the reaction was cooled to ambient temperature. 2-MeTHF (1.014 mL) and water (811.2 μ L) were added. The layers were separated and the organic layer was washed with water (811.2 μ L) and twice with NaHCO₃ (2 ml). The organic layer was transferred into a round bottomed flask. LiOH (38.6 mg, 0.9 mmol) dissolved in water (2 mL) was added and the reaction was heated to 45 $^\circ$ C. After completion, the layers were separated and the organic layer was discarded. The aqueous layer was cooled with an ice bath and hydrochloric acid (10.72 mL of 1.0 M, 10.72 mmol) was added to the solution until the pH reached a pH of about 3-4. The aqueous layer was extracted twice with 2-MeTHF (5ml), and the organic layers were combined and washed with brine (5ml). The organic layer was separated and the solvent removed *in vacuo*. The resulting solid was dried in a vacuum oven with nitrogen bleed at 50 $^\circ$ C to give the title compound. ¹H-NMR (400MHZ, DMSO-*d*₆) δ 12.89 (d, *J* 6.8 Hz, 1H); 11.84 (s, 1H); 11.74 (s, 1H); 9.36 (s, 1H); 8.87-8.61 (d, *J* 6.4 Hz, 1H); 8.34-8.32 (d, *J* 9.1 Hz 1H); 7.83-7.745 (m, 2H); 7.17-7.09 (m, 1H); 7.17 (s, 1H); 7.09 (s, 1H); 1.43 (s, 6H);

[00352] **Example 6: Total synthesis of *N*-(2,4-di-*tert*-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (34)**



[00353] **Procedure for the preparation of 2,4-di-*tert*-butylphenyl methyl carbonate (30)**



Method 1

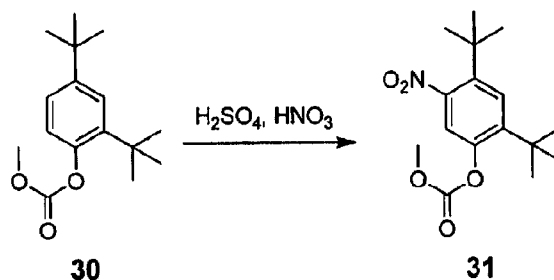
[00354] To a solution of 2,4-di-*tert*-butyl phenol, **29**, (10 g, 48.5mmol) in diethyl ether (100 mL) and triethylamine (10.1 mL, 72.8 mmol), was added methyl chloroformate (7.46 mL, 97 mmol) dropwise at 0 °C. The mixture was then allowed to warm to room temperature and stir for an additional 2 hours. An additional 5 mL triethylamine and 3.7 mL methyl chloroformate was then added and the reaction stirred overnight. The reaction was then filtered, the filtrate was cooled to 0 °C, and an additional 5 mL triethylamine and 3.7 mL methyl chloroformate was then added and the reaction was allowed to warm to room temperature and then stir for an addition 1 hours. At this stage, the reaction was almost complete and was worked up by filtering, then washing with water (2x), followed by brine. The solution was then concentrated to produce a yellow oil and purified using column chromatography to give compound **30**. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.35 (d, *J* = 2.4 Hz,

1H), 7.29 (dd, $J = 8.4, 2.4$ Hz, 1H), 7.06 (d, $J = 8.4$ Hz, 1H), 3.85 (s, 3H), 1.30 (s, 9H), 1.29 (s, 9H).

Method 2

[00355] To a reactor vessel charged with 4-dimethylaminopyridine (DMAP, 3.16 g, 25.7 mmol) and 2,4-di-*tert*-butyl phenol (compound **29**, 103.5 g, 501.6 mmol) was added methylene chloride (415 g, 313 mL) and the solution was agitated until all solids dissolved. Triethylamine (76 g, 751 mmol) was then added and the solution was cooled to 0 – 5 °C. Methyl chloroformate (52 g, 550.3 mmol) was then added dropwise over 2.5 – 4 hours, while keeping the solution temperature between 0 – 5 °C. The reaction mixture was then slowly heated to 23 – 28 °C and stirred for 20 hours. The reaction was then cooled to 10 – 15 °C and charged with 150 mL water. The mixture was stirred at 15 – 20 °C for 35 – 45 minutes and the aqueous layer was then separated and extracted with 150 mL methylene chloride. The organic layers were combined and neutralized with 2.5% HCl (aq) at a temperature of 5 – 20 °C to give a final pH of 5 – 6. The organic layer was then washed with water and concentrated *in vacuo* at a temperature below 20 °C to 150 mL to give compound **30** in methylene chloride.

[00356] *Procedure for the preparation of 5-nitro-2,4-di-*tert*-butylphenyl methyl carbonate (31)*



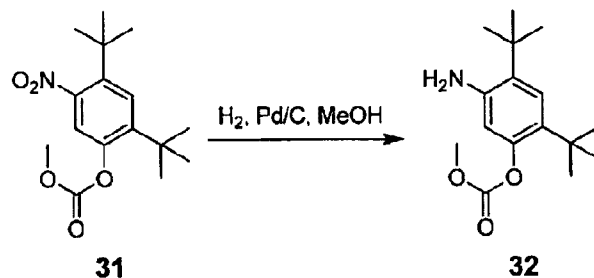
Method 1

[00357] To a stirred solution of compound **30** (6.77g, 25.6 mmol) was added 6 mL of a 1:1 mixture of sulfuric acid and nitric acid at 0 °C dropwise. The mixture was allowed to warm to room temperature and stirred for 1 hour. The product was purified using liquid chromatography (ISCO, 120 g, 0-7% EtOAc/Hexanes, 38 min) producing about an 8:1 – 10:1 mixture of regioisomers of compound **31** as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.63 (s, 1H), 7.56 (s, 1H), 3.87 (s, 3H), 1.36 (s, 9H), 1.32 (s, 9H). HPLC ret. time 3.92 min 10-99% CH₃CN, 5 min run; ESI-MS 310 m/z (MH)⁺.

Method 2

[00358] To compound **30** (100g, 378 mmol) was added DCM (540 g, 408 mL). The mixture was stirred until all solids dissolved, and then cooled to $-5 - 0$ °C. Concentrated sulfuric acid (163 g) was then added dropwise, while maintaining the initial temperature of the reaction, and the mixture was stirred for 4.5 hours. Nitric acid (62 g) was then added dropwise over 2-4 hours while maintaining the initial temperature of the reaction, and was then stirred at this temperature for an additional 4.5 hours. The reaction mixture was then slowly added to cold water, maintaining a temperature below 5 °C. The quenched reaction was then heated to 25 °C and the aqueous layer was removed and extracted with methylene chloride. The combined organic layers were washed with water, dried using Na_2SO_4 , and concentrated to 124 – 155 mL. Hexane (48 g) was added and the resulting mixture was again concentrated to 124 – 155 mL. More hexane (160 g) was subsequently added to the mixture. The mixture was then stirred at $23 - 27$ °C for 15.5 hours, and was then filtered. To the filter cake was added hexane (115 g), the resulting mixture was heated to reflux and stirred for 2 – 2.5 hours. The mixture was then cooled to $3 - 7$ °C, stirred for an additional 1 – 1.5 hours, and filtered to give compound **31** as a pale yellow solid.

[00359] *Procedure for the preparation of 5-amino-2,4-di-tert-butylphenyl methyl carbonate (32)*

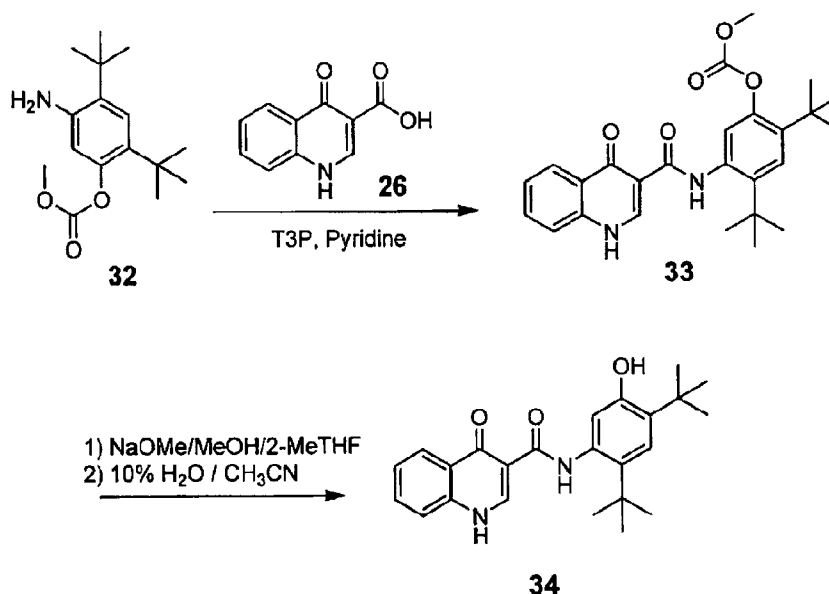


[00360] 2,4-Di-tert-butyl-5-nitrophenyl methyl carbonate (1.00 eq) was charged to a suitable hydrogenation reactor, followed by 5% Pd/C (2.50 wt% dry basis, Johnson-Matthey Type 37). MeOH (15.0 vol) was charged to the reactor, and the system was closed. The system was purged with N_2 (g), and was then pressurized to 2.0 Bar with H_2 (g). The reaction was performed at a reaction temperature of 25 °C ± 5 °C. When complete, the reaction was filtered, and the reactor/cake was washed with MeOH (4.00 vol). The resulting filtrate was distilled under vacuum at no more than 50 °C to 8.00 vol. Water (2.00 vol) was added at 45 °C ± 5 °C. The resultant slurry was cooled to 0 °C ± 5 °C. The slurry was held at 0 °C ± 5 °C for no less than 1 hour, and filtered. The cake was washed once with 0 °C ± 5 °C MeOH/ H_2O (8:2) (2.00 vol). The cake was dried under vacuum (-0.90 bar and -0.86 bar) at

35 °C – 40 °C to give compound **32**. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.05 (s, 1H), 6.39 (s, 1H), 4.80 (s, 2H), 3.82 (s, 3H), 1.33 (s, 9H), 1.23 (s, 9H).

[00361] Once the reaction was complete, the resulting mixture was diluted with from about 5 to 10 volumes of MeOH (e.g., from about 6 to about 9 volumes of MeOH, from about 7 to about 8.5 volumes of MeOH, from about 7.5 to about 8 volumes of MeOH, or about 7.7 volumes of MeOH), heated to a temperature of about 35 ± 5 °C, filtered, washed, and dried, as described above.

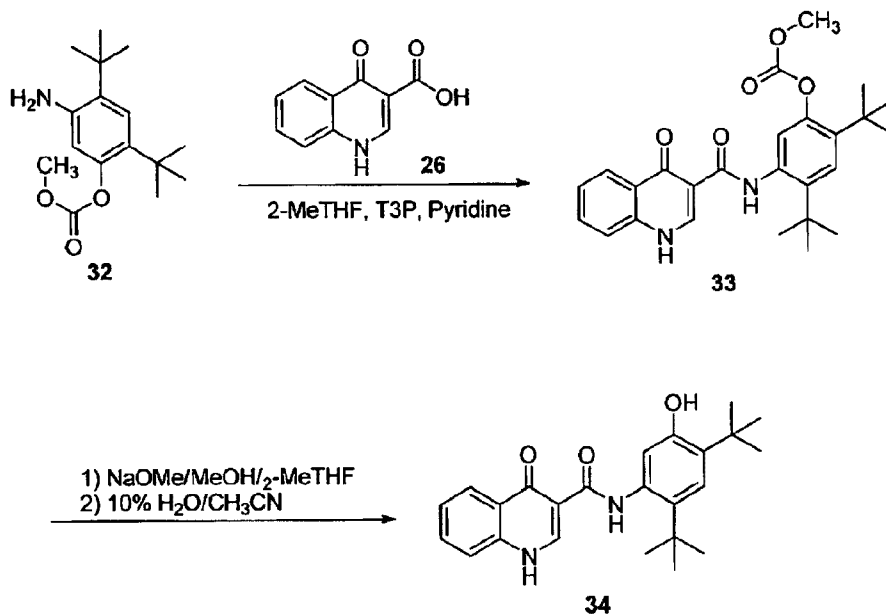
[00362] *Preparation of N-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (34).*



[00363] 4-Oxo-1,4-dihydroquinoline-3-carboxylic acid, **26**, (1.0 eq) and 5-amino-2,4-di-tert-butylphenyl methyl carbonate, **32**, (1.1 eq) were charged to a reactor. 2-MeTHF (4.0 vol, relative to the acid) was added followed by T3P[®] 50% solution in 2-MeTHF (1.7 eq). The T3P charged vessel was washed with 2-MeTHF (0.6 vol). Pyridine (2.0 eq) was then added, and the resulting suspension was heated to 47.5 +/- 5.0 °C and held at this temperature for 8 hours. A sample was taken and checked for completion by HPLC. Once complete, the resulting mixture was cooled to 25.0 °C +/- 2.5 °C. 2-MeTHF was added (12.5 vol) to dilute the mixture. The reaction mixture was washed with water (10.0 vol) 2 times. 2-MeTHF was added to bring the total volume of reaction to 40.0 vol (~16.5 vol charged). To this solution was added NaOMe/MeOH (1.7 equiv) to perform the methanolysis. The reaction was stirred for no less than 1.0 hour, and checked for completion by HPLC. Once complete, the reaction was quenched with 1 N HCl (10.0 vol), and washed with 0.1 N HCl (10.0 vol). The organic

solution was polish filtered to remove any particulates and placed in a second reactor. The filtered solution was concentrated at no more than 35 °C (jacket temperature) and no less than 8.0 °C (internal reaction temperature) under reduced pressure to 20 vol. CH₃CN was added to 40 vol and the solution concentrated at no more than 35 °C (jacket temperature) and no less than 8.0 °C (internal reaction temperature) to 20 vol. The addition of CH₃CN and concentration cycle was repeated 2 more times for a total of 3 additions of CH₃CN and 4 concentrations to 20 vol. After the final concentration to 20 vol, 16.0 vol of CH₃CN was added followed by 4.0 vol of H₂O to make a final concentration of 40 vol of 10% H₂O/CH₃CN relative to the starting acid. This slurry was heated to 78.0 °C +/- 5.0 °C (reflux). The slurry was then stirred for no less than 5 hours. The slurry was cooled to 0.0 °C +/- 5 °C over 5 hours, and filtered. The cake was washed with 0.0 °C +/- 5.0 °C CH₃CN (5 vol) 4 times. The resulting solid (compound **34**) was dried in a vacuum oven at 50.0 °C +/- 5.0 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.8 (s, 1H), 11.8 (s, 1H), 9.2 (s, 1H), 8.9 (s, 1H), 8.3 (s, 1H), 7.2 (s, 1H), 7.9 (t, 1H), 7.8 (d, 1H), 7.5 (t, 1H), 7.1 (s, 1H), 1.4 (s, 9H), 1.4 (s, 9H).

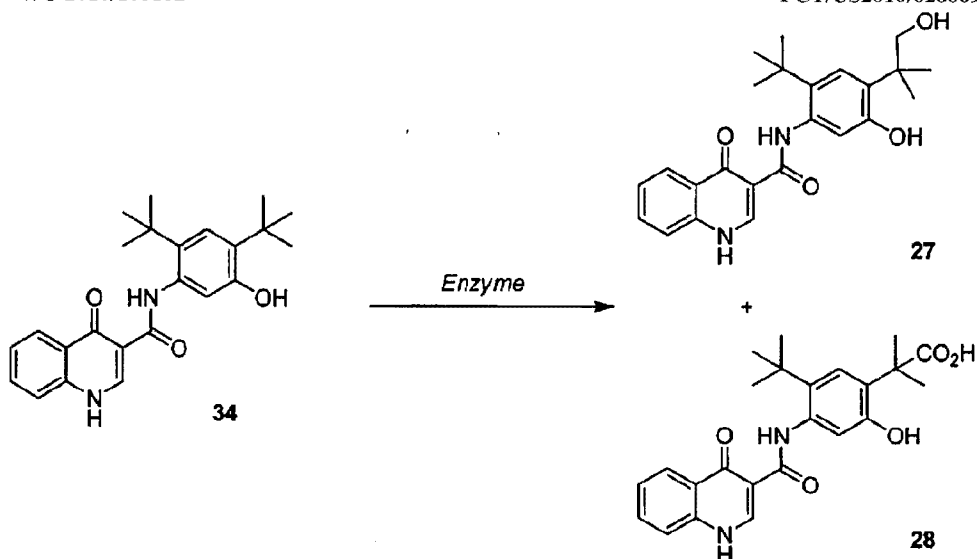
[00364] Alternative Preparation of *N*-(2,4-di-*tert*-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (34**).**



[00365] 4-Oxo-1,4-dihydroquinoline-3-carboxylic acid, **26**, (1.0 eq) and 5-amino-2,4-di-*tert*-butylphenyl methyl carbonate, **32**, (1.1 eq) were charged to a reactor. 2-MeTHF (4.0 vol, relative to the acid) was added followed by T3P[®] 50% solution in 2-MeTHF (1.7 eq). The T3P charged vessel was washed with 2-MeTHF (0.6 vol). Pyridine (2.0 eq) was then added,

and the resulting suspension was heated to 47.5 +/- 5.0 °C and held at this temperature for 8 hours. A sample was taken and checked for completion by HPLC. Once complete, the resulting mixture was cooled to 20 °C +/- 5 °C. 2-MeTHF was added (12.5 vol) to dilute the mixture. The reaction mixture was washed with water (10.0 vol) 2 times and 2-MeTHF (16.5 vol) was charged to the reactor. This solution was charged with 30% w/w NaOMe/MeOH (1.7 equiv) to perform the methanolysis. The reaction was stirred at 25.0 °C +/- 5.0 °C for no less than 1.0 hour, and checked for completion by HPLC. Once complete, the reaction was quenched with 1.2 N HCl/H₂O (10.0 vol), and washed with 0.1 N HCl/H₂O (10.0 vol). The organic solution was polish filtered to remove any particulates and placed in a second reactor. [00366] The filtered solution was concentrated at no more than 35 °C (jacket temperature) and no less than 8.0 °C (internal reaction temperature) under reduced pressure to 20 vol. CH₃CN was added to 40 vol and the solution concentrated at no more than 35 °C (jacket temperature) and no less than 8.0 °C (internal reaction temperature) to 20 vol. The addition of CH₃CN and concentration cycle was repeated 2 more times for a total of 3 additions of CH₃CN and 4 concentrations to 20 vol. After the final concentration to 20 vol, 16.0 vol of CH₃CN was charged followed by 4.0 vol of H₂O to make a final concentration of 40 vol of 10% H₂O/CH₃CN relative to the starting acid. This slurry was heated to 78.0 °C +/- 5.0 °C (reflux). The slurry was then stirred for no less than 5 hours. The slurry was cooled to 20 to 25 °C over 5 hours, and filtered. The cake was washed with CH₃CN (5 vol) heated to 20 to 25 °C 4 times. The resulting solid (compound 34) was dried in a vacuum oven at 50.0 °C +/- 5.0 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.8 (s, 1H), 11.8 (s, 1H), 9.2 (s, 1H), 8.9 (s, 1H), 8.3 (s, 1H), 7.2 (s, 1H), 7.9 (t, 1H), 7.8 (d, 1H), 7.5 (t, 1H), 7.1 (s, 1H), 1.4 (s, 9H), 1.4 (s, 9H).

[00367] Example 7: Procedure for the biosynthesis of *N*-(2-*tert*-butyl-5-hydroxy-4-(1-hydroxy-2-methylpropan-2-yl)phenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (27) and 2-(5-*tert*-butyl-2-hydroxy-4-(4-oxo-1,4-dihydroquinoline-3-carboxamido)phenyl)-2-methylpropanoic acid (28)



[00368] *Streptomyces rimosus* (DSM 40260) was purchased from DSMZ as frozen culture. This culture was used to inoculate agar slants, which were maintained and stored at 4 °C. Yeast extract-malt extract-peptone (YMP) media containing yeast extract (4 g/L), malt extract (10 g/L) and soya flour (5 g/L) was prepared and sterilized at 130 °C for 60 minutes. Five flasks containing 1 L of YMP media were directly inoculated with *S. rimosus* from the agar slants. The culture was allowed to grow for 2 – 3 days at 30 °C with gentle agitation of approximately 100 rpm. Under these conditions, two growth types have been observed, either a cloudy solution or spherical particulates which aggregate at the bottom of the flask. The latter growth type has been shown to result in higher conversions to Compound 27. The cells were then spun down, harvested and resuspended in two flasks containing 1 L of 0.1 M potassium phosphate buffer, pH 7.0. 5.0 g of Compound 34 in 50 mL N,N-dimethylformamide (DMF) were added to the flasks. The reactions proceeded for 24 hours at 30 °C with gentle agitation of about 100 rpm at which point conversions of 7.59% Compound 27 and 1.17% Compound 28 were indicated by HPLC.

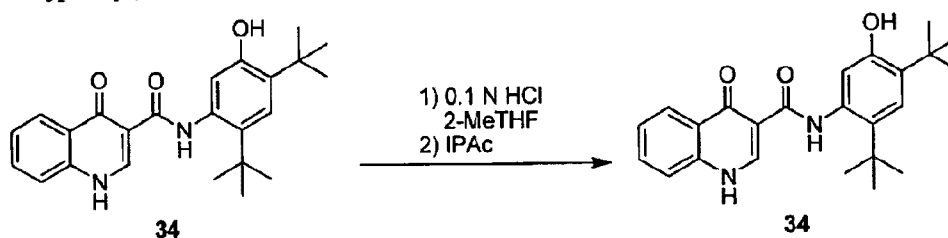
[00369] Both flasks were combined, centrifuged at 3500 rpm for 10 minutes, and re-suspended in 500 mL of methanol. This suspension was stirred vigorously for 30 minutes and then spun down again at 6000 rpm for 10 minutes. The organic layer was collected and the process was repeated two times. The methanol extracts were concentrated *in vacuo* to yield 2.50 g, 1.57 g and 1.11 g of solid material, respectively. The solids from these extracts were shown to contain 74.78 – 91.96% Compound 34, 7.66 – 19.73% Compound 27 and 0.39 – 5.49% Compound 28. In an effort to cull off a portion of Compound 34 from the bio-oxidation products, the solids from the first two extractions were combined, suspended in 250

mL methanol, agitated vigorously for 1 hour and vacuum filtered. While Compounds **27** and **28** were enriched in the filtrate (22.09 and 6.14%, respectively), the solids still also contained Compound **27** (8.96%) and Compound **28** (0.50%).

[00370] The methanol filtrate containing approximately 2.2 g of dissolved solids was adsorbed onto 4.5 g of silica and purified by flash chromatography using a gradient of 100% dichloromethane to 88:12 dichloromethane/methanol. Fractions containing Compound **27** were concentrated *in vacuo* and further dried via freeze-drying to obtain 130 mg of Compound **27** (98.5% purity by HPLC). A fraction containing impure Compound **28** was also concentrated *in vacuo* to yield less than 10 mg of solid.

[00371] The cell pellet was re-suspended in 500 mL methanol and homogenized in a BeadBeater to break apart the cells and recover any remaining product. The organic layer was obtained by centrifuging the homogenized suspension at 6000 rpm for 10 minutes. This was added to the solid obtained from the third extraction and the filtered solids from the slurry enrichment of the first two extractions and slurried at reflux overnight. The slurry was then cooled and suction filtered to obtain 1.99 g of solid. The solid was re-dissolved in 300 mL methanol which was then adsorbed onto approximately 5 g of silica and purified by flash chromatography using a gradient of 100% dichloromethane to 94:6 dichloromethane/methanol to provide 820 mg of solid containing Compound **34** and Compound **27** as well as other impurities. This was re-columned using a more gradual solvent gradient (100% DCM up to a mixture of 6% MeOH/94% DCM) to obtain an additional 89 mg of Compound **27**. The ¹H-NMR spectrum was consistent with that reported above.

[00372] Example 8: Procedure for the recrystallization of N-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (34)



[00373] Compound **34** (1.0 eq) was charged to a reactor. 2-MeTHF (20.0 vol) was added followed by 0.1N HCl (5.0 vol). The biphasic solution was stirred and separated and the top organic phase was washed twice more with 0.1N HCl (5.0 vol). The organic solution was polish filtered to remove any particulates and placed in a second reactor. The filtered

solution was concentrated at no more than 35 °C (jacket temperature) and no more than 8.0 °C (internal reaction temperature) under reduced pressure to 10 vol. Isopropyl acetate (IPAc) (10 vol) was added and the solution concentrated at no more than 35 °C (jacket temperature) and no more than 8.0 °C (internal reaction temperature) to 10 vol. The addition of IPAc and concentration was repeated 2 more times for a total of 3 additions of IPAc and 4 concentrations to 10 vol. After the final concentration, 10 vol of IPAc was charged and the slurry was heated to reflux and maintained at this temperature for 5 hours. The slurry was cooled to 0.0 °C +/- 5 °C over 5 hours and filtered. The cake was washed with IPAc (5 vol) once. The resulting solid was dried in a vacuum oven at 50.0 °C +/- 5.0 °C.

[00374] Example 9: General Procedure to Test Solubility at pH 7.4

[00375] A high throughput shake flask assay was used to determine solubility of compounds in pH 7.4 buffer. To calculate the concentration of compounds in solution, two conditions per compound were run: 300 uM in 100% DMSO and 200 uM in pH 7.4 phosphate buffer with 2% DMSO present. Each sample was left to shake overnight then injected onto HPLC-UV to determine peak area using the following conditions: Phenomenex 00A-4251-B0 – 30x2.00 mm Luna 3u C18(2) 100A column; 0.8 mL/min flow rate; 20 uL injection volume; HPLC grade water with 0.1% formic acid and HPLC grade acetonitrile with 0.1% formic acid mobile phases; peak area determined at 254 nm. Solubility in uM was calculated using the following equation: $\text{conc.} = (\text{peak area pH 7.4}) / (\text{peak area 300uM DMSO standard condition}) \times 300 \text{ uM concentration of standard condition}$. Peaks of interest were identified in buffer conditions based on retention time (RT) of the largest area peak in the 300 uM DMSO standard condition.

[00376] VI. ACTIVITY ASSAYS

[00377] Example 10: General Procedure for Activity Assays

[00378] **Assays for Detecting and Measuring Δ F508-CFTR Potentiation Properties of Compounds**

[00379] Membrane potential optical methods for assaying Δ F508-CFTR modulation properties of compounds

[00380] The assay utilizes fluorescent voltage sensing dyes to measure changes in membrane potential using a fluorescent plate reader (e.g., FLIPR III, Molecular Devices, Inc.) as a readout for increase in functional Δ F508-CFTR in NIH 3T3 cells. The driving force for the response is the creation of a chloride ion gradient in conjunction with channel

activation by a single liquid addition step after the cells have previously been treated with compounds and subsequently loaded with a voltage sensing dye.

[00381] Identification of Potentiator Compounds

[00382] To identify potentiators of $\Delta F508$ -CFTR, a double-addition HTS assay format was developed. This HTS assay utilizes fluorescent voltage sensing dyes to measure changes in membrane potential on the FLIPR III as a measurement for increase in gating (conductance) of $\Delta F508$ CFTR in temperature-corrected $\Delta F508$ CFTR NIH 3T3 cells. The driving force for the response is a Cl^- ion gradient in conjunction with channel activation with forskolin in a single liquid addition step using a fluorescent plate reader such as FLIPR III after the cells have previously been treated with potentiator compounds (or DMSO vehicle control) and subsequently loaded with a redistribution dye.

[00383] Solutions

Bath Solution #1: (in mM) NaCl 160, KCl 4.5, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, pH 7.4 with NaOH.

Chloride-free bath solution: Chloride salts in Bath Solution #1 are substituted with gluconate salts.

[00384] Cell Culture NIH3T3 mouse fibroblasts stably expressing $\Delta F508$ -CFTR are used for optical measurements of membrane potential. The cells are maintained at 37 °C in 5% CO_2 and 90% humidity in Dulbecco's modified Eagle's medium supplemented with 2 mM glutamine, 10% fetal bovine serum, 1 X NEAA, β -ME, 1 X pen/strep, and 25 mM HEPES in 175 cm^2 culture flasks. For all optical assays, the cells were seeded at ~20,000/well in 384-well matrigel-coated plates and cultured for 2 hours at 37 °C before culturing at 27 °C for 24 hours for the potentiator assay. For the correction assays, the cells are cultured at 27 °C or 37 °C with and without compounds for 16 – 24 hours. Electrophysiological Assays for assaying $\Delta F508$ -CFTR modulation properties of compounds.

[00387] 1. Ussing Chamber Assay

[00388] Ussing chamber experiments were performed on polarized airway epithelial cells expressing $\Delta F508$ -CFTR to further characterize the $\Delta F508$ -CFTR modulators identified in the optical assays. Non-CF and CF airway epithelia were isolated from bronchial tissue, cultured as previously described (Galiotta, L.J.V., Lantero, S., Gazzolo, A., Sacco, O., Romano, L., Rossi, G.A., & Zegarra-Moran, O. (1998) *In Vitro Cell. Dev. Biol.* **34**, 478-481), and plated onto Costar® Snapwell™ filters that were pre-coated with NIH3T3-conditioned media. After four days the apical media was removed and the cells were grown at an air liquid interface for >14 days prior to use. This resulted in a monolayer of fully differentiated

columnar cells that were ciliated, features that are characteristic of airway epithelia. Non-CF HBE were isolated from non-smokers that did not have any known lung disease. CF-HBE were isolated from patients homozygous for $\Delta F508$ -CFTR.

[00389] HBE grown on Costar® Snapwell™ cell culture inserts were mounted in an Ussing chamber (Physiologic Instruments, Inc., San Diego, CA), and the transepithelial resistance and short-circuit current in the presence of a basolateral to apical Cl^- gradient (I_{sc}) were measured using a voltage-clamp system (Department of Bioengineering, University of Iowa, IA). Briefly, HBE were examined under voltage-clamp recording conditions ($V_{\text{hold}} = 0 \text{ mV}$) at 37 °C. The basolateral solution contained (in mM) 145 NaCl, 0.83 K_2HPO_4 , 3.3 KH_2PO_4 , 1.2 MgCl_2 , 1.2 CaCl_2 , 10 Glucose, 10 HEPES (pH adjusted to 7.35 with NaOH) and the apical solution contained (in mM) 145 NaGluconate, 1.2 MgCl_2 , 1.2 CaCl_2 , 10 glucose, 10 HEPES (pH adjusted to 7.35 with NaOH).

[00390] Identification of Potentiator Compounds

[00391] Typical protocol utilized a basolateral to apical membrane Cl^- concentration gradient. To set up this gradient, normal ringers was used on the basolateral membrane, whereas apical NaCl was replaced by equimolar sodium gluconate (titrated to pH 7.4 with NaOH) to give a large Cl^- concentration gradient across the epithelium. Forskolin (10 μM) and all test compounds were added to the apical side of the cell culture inserts. The efficacy of the putative $\Delta F508$ -CFTR potentiators was compared to that of the known potentiator, genistein.

[00392] 2. Patch-clamp Recordings

[00393] Total Cl^- current in $\Delta F508$ -NIH3T3 cells was monitored using the perforated-patch recording configuration as previously described (Rae, J., Cooper, K., Gates, P., & Watsky, M. (1991) *J. Neurosci. Methods* 37, 15-26). Voltage-clamp recordings were performed at 22 °C using an Axopatch 200B patch-clamp amplifier (Axon Instruments Inc., Foster City, CA). The pipette solution contained (in mM) 150 *N*-methyl-D-glucamine (NMDG)-Cl, 2 MgCl_2 , 2 CaCl_2 , 10 EGTA, 10 HEPES, and 240 $\mu\text{g/ml}$ amphotericin-B (pH adjusted to 7.35 with HCl). The extracellular medium contained (in mM) 150 NMDG-Cl, 2 MgCl_2 , 2 CaCl_2 , 10 HEPES (pH adjusted to 7.35 with HCl). Pulse generation, data acquisition, and analysis were performed using a PC equipped with a Digidata 1320 A/D interface in conjunction with Clampex 8 (Axon Instruments Inc.). To activate $\Delta F508$ -CFTR, 10 μM forskolin and 20 μM genistein were added to the bath and the current-voltage relation was monitored every 30 sec.

[00394] Identification of Potentiator Compounds

[00395] The ability of $\Delta F508$ -CFTR potentiators to increase the macroscopic $\Delta F508$ -CFTR Cl^- current ($I_{\Delta F508}$) in NIH3T3 cells stably expressing $\Delta F508$ -CFTR was also investigated using perforated-patch-recording techniques. The potentiators identified from the optical assays evoked a dose-dependent increase in $I_{\Delta F508}$ with similar potency and efficacy observed in the optical assays. In all cells examined, the reversal potential before and during potentiator application was around -30 mV, which is the calculated E_{Cl} (-28 mV).

[00396] Cell Culture

[00397] NIH3T3 mouse fibroblasts stably expressing $\Delta F508$ -CFTR are used for whole-cell recordings. The cells are maintained at 37 °C in 5% CO_2 and 90% humidity in Dulbecco's modified Eagle's medium supplemented with 2 mM glutamine, 10% fetal bovine serum, 1 X NEAA, β -ME, 1 X pen/strep, and 25 mM HEPES in 175 cm^2 culture flasks. For whole-cell recordings, 2,500 - 5,000 cells were seeded on poly-L-lysine-coated glass coverslips and cultured for 24 - 48 hrs at 27 °C before use to test the activity of potentiators; and incubated with or without the correction compound at 37 °C for measuring the activity of correctors.

[00398] 3. Single-channel recordings

[00399] Gating activity of wt-CFTR and temperature-corrected $\Delta F508$ -CFTR expressed in NIH3T3 cells was observed using excised inside-out membrane patch recordings as previously described (Dalemans, W., Barbry, P., Champigny, G., Jallat, S., Dott, K., Dreyer, D., Crystal, R.G., Pavirani, A., Lecocq, J-P., Lazdunski, M. (1991) *Nature* 354, 526 - 528) using an Axopatch 200B patch-clamp amplifier (Axon Instruments Inc.). The pipette contained (in mM): 150 NMDG, 150 aspartic acid, 5 $CaCl_2$, 2 $MgCl_2$, and 10 HEPES (pH adjusted to 7.35 with Tris base). The bath contained (in mM): 150 NMDG-Cl, 2 $MgCl_2$, 5 EGTA, 10 TES, and 14 Tris base (pH adjusted to 7.35 with HCl). After excision, both wt- and $\Delta F508$ -CFTR were activated by adding 1 mM Mg-ATP, 75 nM of the catalytic subunit of cAMP-dependent protein kinase (PKA; Promega Corp. Madison, WI), and 10 mM NaF to inhibit protein phosphatases, which prevented current rundown. The pipette potential was maintained at 80 mV. Channel activity was analyzed from membrane patches containing ≤ 2 active channels. The maximum number of simultaneous openings determined the number of active channels during the course of an experiment. To determine the single-channel current amplitude, the data recorded from 120 sec of $\Delta F508$ -CFTR activity was filtered "off-line" at 100 Hz and then used to construct all-point amplitude histograms that were fitted with multigaussian functions using Bio-Patch Analysis software (Bio-Logic Comp. France). The total microscopic current and open probability (P_o) were determined from 120 sec of channel activity. The P_o was determined using the Bio-Patch software or from the relationship $P_o =$

$I/i(N)$, where I = mean current, i = single-channel current amplitude, and N = number of active channels in patch.

[00400] Cell Culture

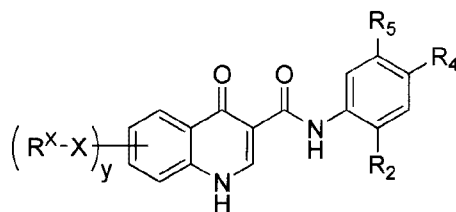
- 5 **[00401]** NIH3T3 mouse fibroblasts stably expressing $\Delta F508$ -CFTR are used for excised-membrane patch-clamp recordings. The cells are maintained at 37 °C in 5% CO₂ and 90% humidity in Dulbecco's modified Eagle's medium supplemented with 2 mM glutamine, 10% fetal bovine serum, 1 X NEAA, β -ME, 1 X pen/strep, and 25 mM HEPES in 175 cm² culture flasks. For single channel recordings, 2,500 - 5,000 cells were seeded on poly-L-lysine-coated glass coverslips and cultured for 24 - 48 hrs at 27 °C before use.
- 10 **[00402]** Compounds of Formula 1 are useful as modulators of ATP binding cassette transporters.

OTHER EMBODIMENTS

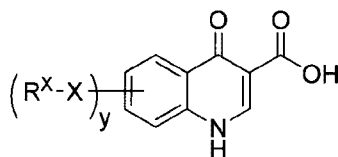
- 15 **[00403]** Should the meaning of the terms in any of the patents or publications referenced herein conflict with the meaning of the terms used in this disclosure, the meaning of the terms in this disclosure are intended to be controlling. Furthermore, the foregoing discussion discloses and describes merely exemplary embodiments of the present invention. One skilled in the art will readily recognize from such discussion and from the accompanying drawings and claims, that various changes, modifications and variations can be made therein without departing from the spirit and scope of the invention as defined in the following claims.

CLAIMS:

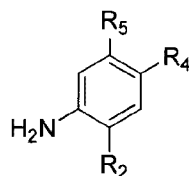
1. A process for the preparation of a compound of Formula 1,

**Formula 1**

comprising coupling a carboxylic acid of Formula 2

**Formula 2**

with an aniline of Formula 3

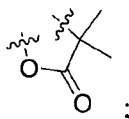
**Formula 3**

in the presence of a coupling agent selected from the group consisting of 2-chloro-1,3-dimethyl-2-imidazolium tetrafluoroborate, HBTU, HCTU, 2-chloro-4,6-dimethoxy-1,3,5-triazine, HATU, HOBT/EDC, and propane phosphonic anhydride; wherein

each R_2 and R_4 is independently selected from the group consisting of hydrogen, CN, CF_3 , halo, C_{1-6} straight or branched alkyl, 3-12 membered cycloaliphatic, phenyl, C_{5-10} heteroaryl and C_{3-7} heterocyclic, wherein said heteroaryl or heterocyclic has up to 3 heteroatoms selected

from the group consisting of O, S, and N, and each C₁₋₆ straight or branched alkyl, 3-12 membered cycloaliphatic, phenyl, C₅₋₁₀ heteroaryl or C₃₋₇ heterocyclic is independently and optionally substituted with up to three substituents selected from the group consisting of —OR', —CF₃, —OCF₃, SR', S(O)R', SO₂R', —SCF₃, halo, CN, —COOR', —COR'—, —O(CH₂)₂N(R')(R'), —O(CH₂)N(R')(R'), —CON(R')(R'), —(CH₂)₂OR', —(CH₂)OR', CH₂CN, optionally substituted phenyl or phenoxy, —N(R')(R'), —NR'C(O)OR', —NR'C(O)R', —(CH₂)₂N(R')(R'), and —(CH₂)N(R')(R');

R₅ is —OC(O)OR', —OC(O)NHR', —OC(O)N(R')₂, wherein R' is not hydrogen, or



R₄ and R₅ are taken together to form the moiety ;

each X is independently a bond or is an optionally substituted C₁₋₆ alkylidene chain

wherein up to two methylene units of X are optionally and independently replaced by —CO—, —CS—, —COCO—, —CONR'—, —CONR'NR'—, —CO₂—, —OCO—, —NR'CO₂—, —O—, —NR'CONR'—, —OCONR'—, —NR'NR', —NR'NR'CO—, —NR'CO—, —S—, —SO—, —SO₂—, —NR'—, —SO₂NR'—, NR'SO₂—, or —NR'SO₂NR'—;

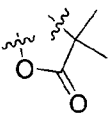
each R^X is independently R', halo, NO₂, CN, CF₃, or OCF₃;

y is an integer from 0-4; and

each R' is independently hydrogen or an optionally substituted group selected from the group consisting of: a C₁₋₈ aliphatic group; a 3-8-membered saturated, partially unsaturated, or fully unsaturated monocyclic ring having 0-3 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur; and an 8-12 membered saturated, partially unsaturated, or fully unsaturated bicyclic ring system having 0-5 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur; or two occurrences of R' are taken together with the atom(s) to which they are bound to form an optionally substituted

3-12 membered saturated, partially unsaturated, or fully unsaturated monocyclic or bicyclic ring having 0-4 heteroatoms independently selected from the group consisting of N, O, and S.

2. The process of claim 1, further comprising cleaving the $\text{—OC(O)OR}'$,

$\text{—OC(O)NHR}'$, , or —OC(O)N(R)'_2 to provide —OH as the R_5 substituent.

3. The process of claim 2, wherein the cleavage is performed by treating a compound of Formula 1 with an alcoholic solvent in the presence of NaOH, KOH or sodium methoxide.

4. The process of claim 3, wherein the alcoholic solvent is methanol.

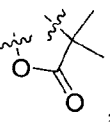
5. The process of claim 1, wherein at least one of R_4 or R_2 is independently a C_{1-6} straight or branched alkyl which is substituted with $\text{—COOR}'$ or —CON(R)'_2 , wherein R' is not hydrogen.

6. The process of claim 5, further comprising hydrolyzing each $\text{—COOR}'$, or —CON(R)'_2 to form —COOH .

7. The process of claim 6, wherein the hydrolysis is performed by treating a compound of Formula 1 with an alcoholic solvent in the presence of NaOH, KOH or sodium methoxide.

8. The process of claim 7, wherein the alcoholic solvent is methanol.

9. The process of claim 5, further comprising cleaving the $\text{—OC(O)OR}'$,

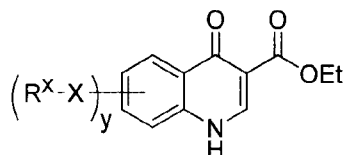
$\text{—OC(O)NHR}'$, , or —OC(O)N(R)'_2 to provide —OH as the R_5 substituent.

10. The process of claim 9, wherein the cleavage is performed by treating a compound of Formula 1 with an alcoholic solvent in the presence of NaOH, KOH or sodium methoxide.

11. The process of claim 10, wherein the alcoholic solvent is methanol.

12. The process of any one of claims 1-11, wherein the coupling is performed in the presence of a base.
13. The process of claim 12, wherein the base is K_2CO_3 , Et_3N , NMM, pyridine or DIEA.
14. The process of any one of claims 1-13, wherein the coupling is performed in the presence of a solvent.
15. The process of claim 14, wherein the solvent is acetonitrile.
16. The process of claim 14, wherein the solvent is DMF.
17. The process of claim 14, wherein the solvent is 2-methyltetrahydrofuran.
18. The process of any one of claims 1-17, wherein the coupling is performed at a reaction temperature which is maintained between about 10 °C and 78 °C.
19. The process of claim 18, wherein the coupling is performed at a reaction temperature which is maintained between about 20 °C and 30 °C.
20. The process of claim 18, wherein the coupling is performed at a reaction temperature which is maintained between about 40 °C and 50 °C.
21. The process of claim 18, wherein the coupling is performed at a reaction temperature which is maintained between about 42 °C and 53 °C.
22. The process of any one of claims 1-21, wherein the coupling reaction is stirred for at least 2 hours.
23. The process of claim 22, wherein the coupling reaction is stirred for at least 70 hours.
24. The process of claim 22, wherein the coupling reaction is stirred for at least 3 days.
25. The process of any one of claims 1-24, wherein y is 0.
26. The process of any one of claims 1-25, wherein R_2 is *tert*-butyl.

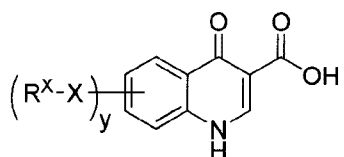
27. The process according to any one of claims 1-26, further comprising the step of contacting a compound of Formula 4



Formula 4

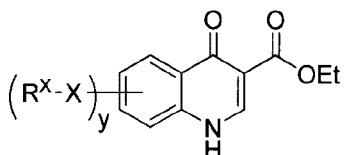
with an aqueous acid to produce a compound of Formula 2.

28. The process for the preparation of a compound of Formula 2



Formula 2

comprising contacting a compound of Formula 4



Formula 4

with an aqueous acid, wherein

each X is independently a bond or is an optionally substituted C₁₋₆ alkylidene chain

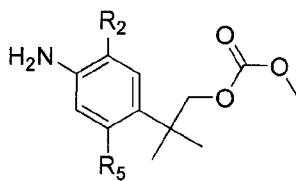
wherein up to two methylene units of X are optionally and independently replaced by —CO—, —CS—, —COCO—, —CONR'—, —CONR'NR'—, —CO₂—, —OCO—, —NR'CO₂—, —O—, —NR'CONR'—, —OCONR'—, —NR'NR', —NR'NR'CO—, —NR'CO—, —S—, —SO—, —SO₂—, —NR'—, —SO₂NR'—, NR'SO₂—, or —NR'SO₂NR'—;

each R^X is independently R' , halo, NO_2 , CN , CF_3 , or OCF_3 ;

y is an integer from 0-4; and

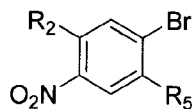
each R' is independently hydrogen or an optionally substituted group selected from the group consisting of: a C_{1-8} aliphatic group; a 3-8-membered saturated, partially unsaturated, or fully unsaturated monocyclic ring having 0-3 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur; or an 8-12 membered saturated, partially unsaturated, or fully unsaturated bicyclic ring system having 0-5 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur; or two occurrences of R' are taken together with the atom(s) to which they are bound to form an optionally substituted 3-12 membered saturated, partially unsaturated, or fully unsaturated monocyclic or bicyclic ring having 0-4 heteroatoms independently selected from the group consisting of N, O, and S.

29. The process of any one of claims 1-27, wherein the compound of Formula 3 is a compound of Formula 40



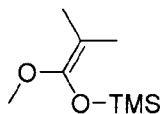
Formula 40

30. The process of claim 29 further comprising the step of contacting a compound of Formula 41

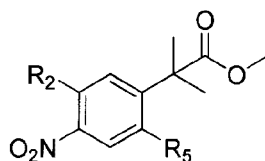


Formula 41

with methyl trimethylsilyl dimethylketene acetal (MTDA)

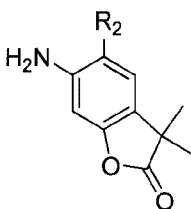
**MTDA**

to produce a compound of Formula 42

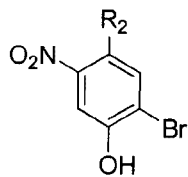
**Formula 42**

31. The process of claim 30 further comprising the step of reducing a compound of Formula 42 to produce a compound of Formula 40.

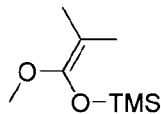
32. The process of any one of claims 1-27, wherein the compound of Formula 3 is a compound of Formula 43

**Formula 43**

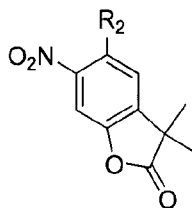
33. The process of claim 32, comprising the step of contacting a compound of Formula 44

**Formula 44**

with methyl trimethylsilyl dimethylketene acetal (MTDA)

**MTDA**

to produce a compound of Formula 45

**Formula 45**

34. The process of claim 33 further comprising the step of reducing a compound of Formula 45 to produce a compound of Formula 43.

