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(54) Title: FORMULATIONS OF 3-(6-(1-(2.2-DIFLUOROBENZO[D][1,3]DIOXOL-5-YL) CYCLOPROPANECARBOXAMIDO)-3-METHYLPYRIDIN-2-YL)BENZOIC ACID

(57) Abstract: A pharmaceutical composition comprising Compound 1, (3-(6-(1-(2,2- difluorobenzo [d] [1,3] dioxol-5 -yl) cyclopropanecarboxamido)-3 -methylpyridin-2-yl)benzoic acid), and at least one excipient selected from: a filler, a disintegrant, a surfactant, a binder, and a lubricant, the composition being suitable for oral administration to a patient in need thereof to treat a CFTR mediated disease such as Cystic Fibrosis. Processes of preparing pharmaceutical compositions comprising Compound 1 are also disclosed.

FORMULATIONS OF 3-(6-(1-(2,2-DIFLUOROBENZO[D][1,3]DIOXOL-5-YL) CYCLOPROPANECARBOXAMIDO)-3-METHYLPYRIDIN-2-YL)BENZOIC ACID

TECHNICAL FIELD OF INVENTION

[0001] The invention relates to pharmaceutical compositions comprising 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid (Compound 1), methods for manufacturing such compositions and methods for administering pharmaceutical compositions comprising same.

BACKGROUND

[0002] 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.

[0003] 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.

[0004] In patients with cystic fibrosis, mutations in CFTR endogenously expressed in respiratory epithelia lead to reduced apical anion secretion causing an imbalance in ion and fluid transport. The resulting decrease in anion transport contributes to enhance 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.

[0005] 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 as reported by the scientific and medical literature. The most prevalent mutation is a deletion of phenylalanine at position 508 of the CFTR amino acid sequence, and is commonly referred to as *F508del*-CFTR. This mutation occurs in approximately 70 percent of the cases of cystic fibrosis and is associated with a severe disease. Other mutations include the *R117H* and *G551D*.

[0006] The deletion of residue 508 in *F508del*-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 *F508del*-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 *F508del*-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.

[0007] 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, $Na^+/2Cl^-/K^+$ co-transporter, Na^+-K^+-ATPase pump and the basolateral membrane K^+ channels, that are responsible for the uptake of chloride into the cell.

[0008] 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- 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 $Na^+/2Cl^-/K^+$ 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.

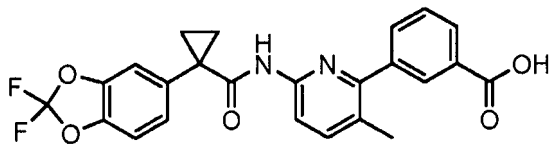
[0009] As discussed above, it is believed that the deletion of residue 508 in *F508del*-CFTR prevents the nascent protein from folding correctly, resulting in the inability of this mutant protein to exit the ER, and traffic to the plasma membrane. As a result, insufficient amounts of the mature protein are present at the plasma membrane and chloride transport within epithelial tissues is significantly reduced. In fact, this cellular phenomenon of defective endoplasmic reticulum (ER) processing of ATP-binding cassette (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. The two ways that the ER machinery can malfunction is either by loss of coupling to ER export of the proteins leading to degradation, or by the ER accumulation of these defective/misfolded proteins [Aridor M, *et al.*, Nature Med., 5(7), pp 745-751 (1999); Shastry, B.S., *et al.*, Neurochem. International, 43, pp 1-7 (2003); Rutishauser, J., *et al.*, Swiss Med Wkly, 132, pp 211-222 (2002); Morello, JP *et al.*, TIPS, 21, pp. 466- 469 (2000); Bross P., *et al.*, Human Mut., 14, pp. 186-198 (1999)].

[0010] Compound 1 in salt form is disclosed in International PCT Publication WO 2007056341 as a modulator of CFTR activity and thus as a useful treatment for CFTR-mediated diseases such as cystic fibrosis. Compound 1 Form I, which is substantially crystalline and salt-free, is disclosed in United States Published Patent Application US20090170905, filed December 4, 2008. Compound 1 Form II and Compound 1 HCl salt Form A are disclosed in United States Published Patent Application US20110263654, filed April 7, 2011. All applications are incorporated in their entirety by reference herein.

[0011] Compound 1, as part of a combination with ivacaftor (N-(5-hydroxy-2,4-ditert-butyl-phenyl)-4-oxo-1H-quinoline-3-carboxamide), has been granted a Breakthrough Therapy Designation from the Food and Drug Administration (FDA) for the treatment of cystic fibrosis, one of only two such grants at the time of the filing of this application (the other being for ivacaftor). This demonstrates a significant unmet need for the effective treatment of the cause of cystic fibrosis over symptomatic treatments. Additionally, a common challenge for drugs approved by the FDA is the occasional lack of drug availability for patients in need thereof. Accordingly, a significant unmet need exists for the presently disclosed Compound 1 formulations and processes for preparing them in a continuous and controlled manner.

SUMMARY

[0012] The invention relates to pharmaceutical compositions, pharmaceutical preparations, and solid dosage forms comprising 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid (Compound 1) which has the structure below:



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[0013] In one aspect, the invention provides a pharmaceutical composition comprising:

a. Compound 1;

b. a filler;

c. a disintegrant;

d. a surfactant;

e. a lubricant; and

f. a glidant or a binder.

[0014] In other embodiments, Compound 1 is in substantially one of its crystalline solid forms. In one embodiment, Compound 1 is in substantially crystalline Form I (Compound 1 Form I). In one embodiment, Compound 1 is in substantially crystalline Form II (Compound 1 Form II). In one embodiment, Compound 1 is in substantially crystalline HCl salt form (Compound 1 HCl Salt Form A). It is understood that the term "Compound 1," as used throughout, includes, amongst other forms, including non-crystalline forms, the following solid state forms: Compound 1 Form I, Compound 1 Form II, and/or Compound 1 HCl Salt Form A.

[0015] In some embodiments, the pharmaceutical composition comprises 25 mg to 400 mg. In some embodiments, the pharmaceutical composition comprises 25 mg of Compound 1. In some embodiments, the pharmaceutical composition comprises 50 mg of Compound 1. In some embodiments, the pharmaceutical composition comprises 100 mg of Compound 1. In some embodiments, the pharmaceutical composition comprises 125 mg of Compound 1. In some embodiments, the pharmaceutical composition comprises 150 mg of Compound 1. In some embodiments, the pharmaceutical composition comprises 200 mg of Compound 1. In some embodiments, the pharmaceutical composition comprises 250 mg of Compound 1. In some embodiments, the pharmaceutical composition comprises 300 mg of Compound 1. In some embodiments, the pharmaceutical composition comprises 400 mg of Compound 1.

[0016] In one aspect, the invention provides a pharmaceutical composition comprising the following components:

Roller Compaction Granule Blend	(%w/w)
Compound 1	20-40
Microcrystalline cellulose	30-50
Mannitol	10-30

Croscarmellose Sodium	1-5
Sodium Lauryl Sulfate	0.1-2
Colloidal Silica	0.1-1
Magnesium Stearate	1-3
Tablet Composition	(%w/w)
Roller Compaction Granule Blend	99-99.9
Magnesium Stearate	0.1-1

[0017] In one aspect, the invention provides a pharmaceutical composition comprising the following components:

High Shear Granule Blend	(%w/w)
Compound 1	60-70
Microcrystalline cellulose	5-15
Croscarmellose Sodium	1-5
Sodium Lauryl Sulfate	0.1-2
Polyvinylpyrrolidone	1-5
Tablet Composition	(%w/w)
High Shear Granule Blend	75-89
Microcrystalline cellulose	10-15
Croscarmellose Sodium	1-5
Magnesium Stearate	0.1-5

[0018] In another embodiment, the invention provides a pharmaceutical composition comprising the following components:

High Shear Granule Blend	(%w/w)
Compound 1 Form I	60-70
Microcrystalline cellulose	5-15
Croscarmellose Sodium	1-5
Polyvinylpyrrolidone	1-5
Sodium Lauryl Sulfate	0.1-2
Tablet Composition	**(%w/w)**
High Shear Granule Blend	78-89
Microcrystalline cellulose	10-15
Croscarmellose Sodium	1-5
Magnesium Stearate	0.1-2
Film Coated Tablet	**(%w/w)**
Core Tablet Composition	95-99
Film Coat	1-5
Wax	Trace

[0019] In another embodiment, the invention provides a pharmaceutical composition comprising the following components:

Roller Compaction Granule Blend	(%w/w)
Compound 1 Form I	30
Microcrystalline cellulose	42.3
Mannitol	21.2
Croscarmellose Sodium	3
Sodium Lauryl Sulfate	1

Colloidal Silica	0.5
Magnesium Stearate	2
Tablet Composition	(%w/w)
Roller Compaction Granule Blend	99.5
Magnesium Stearate	0.5

[0020] In another aspect, the invention provides a pharmaceutical composition comprising the following components:

High Shear Granule Blend	(%w/w)
Compound 1 Form I	40-80
Microcrystalline cellulose	20-40
Mannitol	10-15
Croscarmellose Sodium	1-5
Polyvinylpyrrolidone	1-10
Sodium Lauryl Sulfate	0.1-2
Tablet Composition	(%w/w)
High Shear Granule Blend	95-99
Croscarmellose Sodium	1-4
Magnesium Stearate	0.1-1

[0021] In another embodiment, the invention provides a pharmaceutical composition comprising the following components:

High Shear Granule Blend	(%w/w)
Compound 1 Form I	50

Microcrystalline cellulose	30
Mannitol	13
Croscarmellose Sodium	2
Polyvinylpyrrolidone	4
Sodium Lauryl Sulfate	1
Tablet Composition	(%w/w)
High Shear Granule Blend	97.5
Croscarmellose Sodium	2.0
Magnesium Stearate	0.5

[0022] In another embodiment, the invention provides a pharmaceutical composition comprising the following components:

High Shear Granule Blend	(%w/w)
Compound 1 Form I	60
Microcrystalline cellulose	20
Mannitol	13
Croscarmellose Sodium	2
Polyvinylpyrrolidone	4
Sodium Lauryl Sulfate	1
Tablet Composition	(%w/w)
High Shear Granule Blend	97.5
Croscarmellose Sodium	2.0
Magnesium Stearate	0.5

[0023] In another embodiment, the invention provides a pharmaceutical composition comprising the following components:

High Shear Granule Blend	(%w/w)
Compound 1 Form I	60
Microcrystalline cellulose	20
Mannitol	13
Croscarmellose Sodium	2
Polyvinylpyrrolidone	4
Sodium Lauryl Sulfate	1
Tablet Composition	**(%w/w)**
High Shear Granule Blend	83
Microcrystalline cellulose	14
Croscarmellose Sodium	2
Magnesium Stearate	1

[0024] In another embodiment, the invention provides a pharmaceutical composition comprising the following components:

Twin Screw Granule Blend	(%w/w)
Compound 1 Form I	60
Microcrystalline cellulose	20
Mannitol	13
Croscarmellose Sodium	2
Polyvinylpyrrolidone	4
Sodium Lauryl Sulfate	1

Tablet Composition	(%w/w)
Twin Screw Granule Blend	83
Microcrystalline cellulose	14
Croscarmellose Sodium	2
Magnesium Stearate	1

[0025] In another embodiment, the invention provides a pharmaceutical composition comprising the following components:

Twin Screw Wet Granule Blend	(%w/w)
Compound 1 Form I	80.0
Microcrystalline cellulose	13.6
Croscarmellose Sodium	2.5
Polyvinylpyrrolidone	3.1
Sodium Lauryl Sulfate	0.7

Tablet Composition	(%w/w)
Twin Screw Granule Blend	83
Microcrystalline cellulose	12
Croscarmellose Sodium	4
Magnesium Stearate	1

[0026] In another embodiment, the invention provides a pharmaceutical composition comprising the following components:

Twin Screw Granule Blend	(%w/w)
Compound 1 Form I	80.0

Microcrystalline cellulose	13.6
Croscarmellose Sodium	2.5
Polyvinylpyrrolidone	3.1
Sodium Lauryl Sulfate	0.7
Tablet Composition	(%w/w)
Twin Screw Granule Blend	83
Microcrystalline cellulose	12
Croscarmellose Sodium	4
Magnesium Stearate	1
Film Coated Tablet	(%w/w)
Core Tablet Composition	97
Film Coat	3
Wax	Trace

[0027] In another embodiment, the invention provides a pharmaceutical composition comprising the following components:

High Shear Granule Blend	mg
Compound 1 Form I	200
Microcrystalline cellulose	66
Mannitol	43
Croscarmellose Sodium	7
Polyvinylpyrrolidone	13
Sodium Lauryl Sulfate	3
Core Tablet Composition	mg

(200 mg dose)	
High Shear Granule Blend	332
Microcrystalline cellulose	56
Croscarmellose Sodium	8
Magnesium Stearate	4
Film Coated Tablet (200 mg dose)	mg
Core Tablet Composition	400
Film Coat	12
Wax	trace

[0028] In another embodiment, the invention provides a pharmaceutical composition comprising the following components:

Twin Screw Granule Blend	mg
Compound 1 Form I	200
Microcrystalline cellulose	66
Mannitol	43
Croscarmellose Sodium	7
Polyvinylpyrrolidone	13
Sodium Lauryl Sulfate	3
Core Tablet Composition (200 mg dose)	mg
Twin Screw Granule Blend	332
Microcrystalline cellulose	56

Croscarmellose Sodium	8
Magnesium Stearate	4

[0029] In another embodiment, the invention provides a pharmaceutical composition comprising the following components:

High Shear Granule Blend	mg
Compound 1 Form I	200
Microcrystalline cellulose	67
Mannitol	45
Croscarmellose Sodium	7
Polyvinylpyrrolidone	10.4
Sodium Lauryl Sulfate	2.6
Core Tablet Composition (200 mg dose)	**mg**
High Shear Granule Blend	332
Microcrystalline cellulose	56
Croscarmellose Sodium	8
Magnesium Stearate	4
Film Coated Tablet (200 mg dose)	**mg**
Core Tablet Composition	400
Film Coat	12
Wax	trace

[0030] In another embodiment, the invention provides a pharmaceutical composition comprising the following components:

High Shear Granule Blend	mg
Compound 1 Form I	300
Microcrystalline cellulose	99
Mannitol	64.5
Croscarmellose Sodium	10.5
Polyvinylpyrrolidone	19.5
Sodium Lauryl Sulfate	4.5
Core Tablet Composition (300 mg dose)	mg
High Shear Granule Blend	498
Microcrystalline cellulose	84
Croscarmellose Sodium	12
Magnesium Stearate	6
Film Coated Tablet (300 mg dose)	mg
Core Tablet Composition	600
Film Coat	18
Wax	trace

[0031] In another embodiment, the invention provides a pharmaceutical composition comprising the following components:

High Shear Granule Blend	mg

Compound 1 Form I	300
Microcrystalline cellulose	100.5
Mannitol	67.5
Croscarmellose Sodium	10.5
Polyvinylpyrrolidone	15.6
Sodium Lauryl Sulfate	3.9
Core Tablet Composition (300 mg dose)	mg
High Shear Granule Blend	498
Microcrystalline cellulose	84
Croscarmellose Sodium	12
Magnesium Stearate	6
Film Coated Tablet (300 mg dose)	mg
Core Tablet Composition	600
Film Coat	18
Wax	trace

[0032] In another embodiment, the invention provides a pharmaceutical composition comprising the following components:

High Shear Granule Blend	(%w/w)
Compound 1 Form I	70
Microcrystalline cellulose	12
Mannitol	11

Croscarmellose Sodium	2
Polyvinylpyrrolidone	4
Sodium Lauryl Sulfate	1
Tablet Composition	(%w/w)
High Shear Granule Blend	97.5
Croscarmellose Sodium	2.0
Magnesium Stearate	0.5

[0033] In another embodiment, the invention provides a pharmaceutical composition comprising the following components:

High Shear Granule Blend	(%w/w)
Compound 1 Form I or Form II	61
Microcrystalline cellulose	20.3
Mannitol	13.2
Croscarmellose Sodium	2
Polyvinylpyrrolidone	2.7
Sodium Lauryl Sulfate	0.7
Tablet Composition	(%w/w)
High Shear Granule Blend	83
Microcrystalline cellulose	14
Croscarmellose Sodium	2
Magnesium Stearate	1

[0034] In another embodiment, the invention provides a pharmaceutical composition comprising the following components:

High Shear Granule Blend	mg
Compound 1 Form I or Form II	100
Microcrystalline cellulose	33.3
Mannitol	21.7
Croscarmellose Sodium	3.3
Polyvinylpyrrolidone	4.4
Sodium Lauryl Sulfate	1.1
Core Tablet Composition (100 mg dose)	mg
High Shear Granule Blend	163.9
Microcrystalline cellulose	27.6
Croscarmellose Sodium	3.9
Magnesium Stearate	2.0

[0035] In another embodiment, the invention provides a pharmaceutical composition comprising the following components:

Twin Screw Granule Blend	mg
Compound 1 Form I	200
Microcrystalline cellulose	34.0
Croscarmellose Sodium	6.3
Polyvinylpyrrolidone	7.8
Sodium Lauryl Sulfate	1.8
Core Tablet Composition (200 mg dose)	mg

Twin Screw Granule Blend	249.9
Microcrystalline cellulose	36.1
Croscarmellose Sodium	12.0
Magnesium Stearate	3.0

[0036] In another embodiment, the invention provides a pharmaceutical composition comprising the following components:

Twin Screw Granule Blend	mg
Compound 1 Form I	400
Microcrystalline cellulose	68.0
Croscarmellose Sodium	12.6
Polyvinylpyrrolidone	15.6
Sodium Lauryl Sulfate	3.6
Core Tablet Composition (400 mg dose)	mg
Twin Screw Granule Blend	499.8
Microcrystalline cellulose	72.2
Croscarmellose Sodium	24.0
Magnesium Stearate	6.0

[0037] In another embodiment, the invention provides a pharmaceutical composition comprising the following components:

Twin Screw Granule Blend	mg
Compound 1 Form I	200

Microcrystalline cellulose	34.0
Croscarmellose Sodium	6.3
Polyvinylpyrrolidone	7.8
Sodium Lauryl Sulfate	1.8
Core Tablet Composition (200 mg dose)	mg
Twin Screw Granule Blend	249.9
Microcrystalline cellulose	36.1
Croscarmellose Sodium	12.0
Magnesium Stearate	3.0
Film Coated Tablet (200 mg dose, 310 mg total)	mg
Core Tablet Composition	301
Film Coat	9.0
Wax	trace

[0038] In another embodiment, the invention provides a pharmaceutical composition comprising the following components:

Twin Screw Granule Blend	mg
Compound 1 Form I	400
Microcrystalline cellulose	68.0
Croscarmellose Sodium	12.6
Polyvinylpyrrolidone	15.6
Sodium Lauryl Sulfate	3.6

Core Tablet Composition (400 mg dose)	mg
Twin Screw Granule Blend	499.8
Microcrystalline cellulose	72.2
Croscarmellose Sodium	24.0
Magnesium Stearate	6.0
Film Coated Tablet (400 mg dose, 620 mg total)	**mg**
Core Tablet Composition	602
Film Coat	18.0
Wax	trace

[0039] In another aspect, the invention provides a pharmaceutical composition in the form of a tablet that comprises Compound 1, and one or more pharmaceutically acceptable excipients, for example, a filler, a disintegrant, a surfactant, a diluent, a binder, a glidant, and a lubricant and any combination thereof, where the tablet has a dissolution of at least about 50% in about 30 minutes. In another embodiment, the dissolution rate is at least about 75% in about 30 minutes. In another embodiment, the dissolution rate is at least about 90% in about 30 minutes.

[0040] In another aspect, the invention provides a pharmaceutical composition consisting of a tablet that comprises a powder blend or granules comprising Compound 1; and, one or more pharmaceutically acceptable excipients, for example, a filler, a disintegrant, a surfactant, a diluent, a binder, a glidant, and a lubricant, wherein the tablet has a hardness of at least about 5 kP (kP = kilo Ponds; 1 kP = ~9.8 N). In another embodiment, the tablet has a target friability of less than 1.0% after 400 revolutions. In another aspect, the invention provides a pharmaceutical composition consisting of a tablet that comprises a powder blend or granules comprising Compound 1 Form II, Compound 1; and, one or more pharmaceutically acceptable excipients, for example, a filler, a disintegrant, a surfactant, a diluent, a binder, a glidant, and a lubricant,

wherein the tablet has a hardness of at least about 5 kP (kP = kilo Ponds; 1 kP = ~9.8 N). In another embodiment, the tablet has a target friability of less than 1.0% after 400 revolutions.

[0041] In another aspect, the invention provides a pharmaceutical composition as described herein further comprising an additional therapeutic agent. In some embodiments, the additional therapeutic agent is N-(5-hydroxy-2,4-ditert-butyl-phenyl)-4-oxo-1H-quinoline-3-carboxamide.

[0042] In another aspect, the invention provides a method of treating a CFTR mediated disease in a mammal comprising administering to the mammal an effective amount of a pharmaceutical composition as described herein. In some embodiments, the CFTR mediated disease is cystic fibrosis, emphysema, COPD, or osteoporosis. In other embodiments, the CFTR mediated disease is cystic fibrosis. This method may further comprise administering an additional therapeutic agent, wherein in some embodiments, the additional therapeutic agent is selected from a mucolytic agent, bronchodilator, an anti-biotic, an anti-infective agent, an anti-inflammatory agent, a CFTR potentiator, or a nutritional agent. In another embodiment, the additional therapeutic agent is N-(5-hydroxy-2,4-ditert-butyl-phenyl)-4-oxo-1H-quinoline-3-carboxamide. In another embodiment, the patient has a *F508del*-CFTR mutation. In another embodiment, the patient is homozygous for *F508del*. In another embodiment, the patient is heterozygous for *F508del*.

[0043] In another aspect, the invention features a kit comprising a tablet of the present invention, and a separate therapeutic agent or pharmaceutical composition thereof. In another embodiment, the Compound 1 in the tablet is in Form I. In another embodiment, the therapeutic agent is a cystic fibrosis corrector other than Compound 1. In another embodiment, the therapeutic agent is a cystic fibrosis potentiator. In another embodiment, the therapeutic agent is N-(5-hydroxy-2,4-ditert-butyl-phenyl)-4-oxo-1H-quinoline-3-carboxamide. In another embodiment, the tablet and the therapeutic agent are in separate containers. In another embodiment, the separate containers are bottles. In another embodiment, the separate containers are vials. In another embodiment, the separate containers are blister packs.

[0044] In another aspect, the invention provides a process for making the pharmaceutical compositions described herein by a roller compaction process comprising the steps of screening and weighing Compound 1 and excipients; blending Compound 1 and excipients for a suitable amount of time; roller compacting the blend into ribbons and milling the ribbons into granules;

blending the granules with extra-granular excipients for a suitable amount of time; compressing the blend into tablets; coating the tablets; and, optionally, printing a monogram on one or both tablet faces.

[0045] In another aspect, the invention provides a process for making the pharmaceutical compositions described herein by a high shear granulation process comprising the steps of screening and weighing Compound 1 and excipients; mixing Compound 1 and excipients while adding a granulation fluid comprising surfactant and a binder at a suitable mixing speed for a suitable amount of time and chopping the mixture into granules; drying the granules; blending the granules with extra-granular excipients for a suitable amount of time; compressing the blend into tablets; coating the tablets; and, optionally, printing a monogram on one or both tablet faces.

[0046] In another aspect, the invention provides a continuous or semi-continuous process for making the pharmaceutical compositions described herein by a twin screw wet granulation process comprising the steps of screening and weighing Compound 1 and excipients; mixing Compound 1 and excipients in a blender and feeding the blend into a continuous granulator while adding a granulation fluid comprising surfactant and a binder at a suitable rate for a suitable amount of time and chopping the mixture into granules; drying the granules; blending the granules with extra-granular excipients for a suitable amount of time; compressing the blend into tablets; coating the tablets; and, optionally, printing a monogram on one or both tablet faces.

BRIEF DESCRIPTION OF DRAWINGS

[0047] **Figure 1** is an X-ray diffraction pattern calculated from a single crystal structure of Compound 1 Form I.

[0048] **Figure 2** is an actual X-ray powder diffraction pattern of Compound 1 Form I.

[0049] **Figure 3** is an X-ray powder diffraction pattern of Compound 1 Form II.

[0050] **Figure 4** provides X-ray diffraction patterns of Compound 1 Form II's selected from:

1) Compound 1 Form II, Methanol Solvate;
2) Compound 1 Form II, Ethanol Solvate;
3) Compound 1 Form II, Acetone Solvate;
4) Compound 1 Form II, 2-Propanol Solvate;
5) Compound 1 Form II, Acetonitrile Solvate;

6) Compound 1 Form II, Tetrahydrofuran Solvate;

7) Compound 1 Form II, Methyl Acetate Solvate;

8) Compound 1 Form II, 2-Butanone Solvate;

9) Compound 1 Form II, Ethyl Formate Solvate; and

10) Compound 1 Form II, 2-Methyltetrahydrofuran Solvate.

[0051] **Figure 5** provides an X-ray diffraction pattern of Compound 1 Form II, Methanol Solvate.

[0052] **Figure 6** provides an X-ray diffraction pattern of Compound 1 Form II, Ethanol Solvate.

[0053] **Figure 7** provides an X-ray diffraction pattern of Compound 1 Form II, Acetone Solvate.

[0054] **Figure 8** provides an X-ray diffraction pattern of Compound 1 Form II, 2-Propanol Solvate.

[0055] **Figure 9** provides an X-ray diffraction pattern of Compound 1 Form II, Acetonitrile Solvate.

[0056] **Figure 10** provides an X-ray diffraction pattern of Compound 1 Form II, Tetrahydrofuran Solvate.

[0057] **Figure 11** provides an X-ray diffraction pattern of Compound 1 Form II, Methyl Acetate Solvate.

[0058] **Figure 12** provides an X-ray diffraction pattern of Compound 1 Form II, 2-Butanone Solvate.

[0059] **Figure 13** provides an X-ray diffraction pattern of Compound 1 Form II, Ethyl Formate Solvate.

[0060] **Figure 14** provides an X-ray diffraction pattern of Compound 1 Form II, 2-Methyltetrahydrofuran Solvate.

[0061] **Figure 15** is a differential scanning calorimetry (DSC) trace of Compound 1 Form II, Acetone Solvate.

[0062] **Figure 16** is a Thermogravimetric analysis (TGA) plot of Compound 1 Form II, Acetone Solvate.

[0063] **Figure 17** is a conformational image of Compound 1 Form II, Acetone Solvate based on single crystal X-ray analysis.

[0064] **Figure 18** is a conformational image of the dimer of Compound 1 HCl Salt Form A.

[0065] **Figure 19** is an X-ray diffraction pattern of Compound 1 HCl Salt Form A calculated from the crystal structure.

[0066] **Figure 20** is an ^{1}HNMR spectrum of Compound 1.

[0067] **Figure 21** is an ^{1}HNMR spectrum of Compound 1 HCl salt.

[0068] **Figure 22** is a differential scanning calorimetry (DSC) trace of Compound 1 Form I.

[0069] **Figure 23** is a conformational picture of Compound 1 Form I based on single crystal X-ray analysis.

[0070] **Figure 24** is a conformational image of Compound 1 Form II, Acetone Solvate, based on single crystal X-ray analysis.

[0071] **Figure 25** is a solid state ^{13}C NMR spectrum (15.0 kHz spinning) of Compound 1 Form II, Acetone Solvate.

[0072] **Figure 26** is a solid state ^{19}F NMR spectrum (12.5 kHz spinning) of Compound 1 Form II, Acetone Solvate.

[0073] **Figure 27** is an X-ray diffraction pattern of Compound 1 HCl Salt Form A calculated from the crystal structure.

[0074] **Figure 28** is a graph depicting Compound 1 pH gradient dissolution profiles for a tablet made by a high shear granulation (HSG) process and a twin screw wet granulation (TSWG) process (LOD stands for loss on drying, a measure to define the amount of water in a powder/granule).

DETAILED DESCRIPTION

DEFINITIONS

[0075] As used herein, "CFTR" stands for cystic fibrosis transmembrane conductance regulator.

[0076] As used herein, a "*ΔF508*" or "*F508del*" is a specific mutation within the CFTR protein. The mutation is a deletion of the three nucleotides that comprise the codon for amino acid phenylalanine at position 508, resulting in CFTR protein that lacks this particular phenylalanine.

[0077] As used herein, a patient who is "homozygous" for a particular mutation, e.g. *F508del*, has the same mutation on both alleles.

[0078] As used herein, a patient who is "heterozygous" for a particular mutation, e.g. *F508del*, has this mutation on one allele, and a different mutation on the other allele.

[0079] As used herein, the term "CFTR corrector" refers to a compound that augments or induces the amount of functional CFTR protein to the cell surface, resulting in increased functional activity.

[0080] As used herein, the term "CFTR potentiator" refers to a compound that augments or induces the channel activity of CFTR protein located at the cell surface, resulting in increased functional activity.

[0081] As used herein, the term "active pharmaceutical ingredient" or "API" refers to a biologically active compound. Exemplary APIs include 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid (Compound 1).

[0082] The terms "solid form", "solid forms" and related terms, when used herein to refer to 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl) benzoic acid (Compound 1), refer to a solid form e.g. crystals and the like, comprising Compound 1 which is not predominantly in a liquid or a gaseous state.

[0083] As used herein, the term "substantially amorphous" refers to a solid material having little or no long range order in the position of its molecules. For example, substantially

amorphous materials have less than about 15% crystallinity (e.g., less than about 10% crystallinity or less than about 5% crystallinity). It is also noted that the term 'substantially amorphous' includes the descriptor, 'amorphous', which refers to materials having no (0%) crystallinity.

[0084] As used herein, the term "substantially crystalline" (as in the phrase substantially crystalline Compound 1 Form I, Compound 1 Form II, or Compound 1 HCl Salt Form A) refers to a solid material having predominantly long range order in the position of its molecules. For example, substantially crystalline materials have more than about 85% crystallinity (e.g., more than about 90% crystallinity or more than about 95% crystallinity). It is also noted that the term 'substantially crystalline' includes the descriptor, 'crystalline', which refers to materials having 100% crystallinity.

[0085] The term "crystalline" and related terms used herein, when used to describe a substance, component, product, or form, means that the substance, component or product is substantially crystalline as determined by X-ray diffraction. (See, e.g., Remington: The Science and Practice of Pharmacy, 21st Ed., Lippincott Williams & Wilkins, Baltimore, Md. (2003); The United States Pharmacopeia, 23rd ed., 1843-1844 (1995)).

[0086] As used herein, the term "composition" generally refers to a composition of two or more components, usually one or more drugs (e.g., one drug (e.g., Compound 1 Form I, Compound 1 Form II, or Compound 1 HCl Salt Form A)) and one or more pharmaceutical excipients.

[0087] As used herein, the term "solid dosage form" generally refers to a pharmaceutical composition, which when used in an oral mode of 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.

[0088] As used herein, an "excipient" includes functional and non-functional ingredients in a pharmaceutical composition.

[0089] As used herein, a "disintegrant" is an excipient that hydrates a pharmaceutical composition and aids in tablet dispersion. As used herein, a "diluent" or "filler" is an excipient that adds bulkiness to a pharmaceutical composition.

[0090] As used herein, a "surfactant" is an excipient that imparts pharmaceutical compositions with enhanced solubility and/or wetability.

[0091] As used herein, a "binder" is an excipient that imparts a pharmaceutical composition with enhanced cohesion or tensile strength (e.g., hardness).

[0092] As used herein, a "glidant" is an excipient that imparts a pharmaceutical compositions with enhanced flow properties.

[0093] As used herein, a "colorant" is an excipient that imparts a pharmaceutical composition with a desired color. Examples of colorants include commercially available pigments such as FD&C Blue # 1 Aluminum Lake, FD&C Blue #2, other FD&C Blue colors, titanium dioxide, iron oxide, and/or combinations thereof. In one embodiment, the pharmaceutical composition provided by the invention is purple.

[0094] As used herein, a "lubricant" is an excipient that is added to pharmaceutical compositions that are pressed into tablets. The lubricant aids in compaction of granules into tablets and ejection of a tablet of a pharmaceutical composition from a die press.

[0095] As used herein, "cubic centimeter" and "cc" are used interchangeably to represent a unit of volume. Note that 1 cc = 1 mL.

[0096] As used herein, "kiloPond" and "kP" are used interchangeably and refer to the measure of force where a kP = approximately 9.8 Newtons.

[0097] As used herein, "friability" refers to the property of a tablet to remain intact and withhold its form despite an external force of pressure. Friability can be quantified using the mathematical expression presented in equation 1:

$$\%friabiliy = 100 \times \frac{\left(W_0 - W_f\right)}{W_0} \qquad (1)$$

wherein W_0 is the original weight of the tablet and W_f is the final weight of the tablet after it is put through the friabilator. Friability is measured using a standard USP testing apparatus that tumbles experimental tablets for 100 or 400 revolutions. Some tablets of the invention have a friability of less than 5.0%. In another embodiment, the friability is less than 2.0%. In another embodiment, the target friability is less than 1.0% after 400 revolutions.

[0098] As used herein, "mean particle diameter" is the average particle diameter as measured using techniques such as laser light scattering, image analysis, or sieve analysis. In one embodiment, the granules used to prepare the pharmaceutical compositions provided by the invention have a mean particle diameter of less than 1.0 mm.

[0099] As used herein, "bulk density" is the mass of particles of material divided by the total volume the particles occupy. The total volume includes particle volume, inter-particle void volume and internal pore volume. Bulk density is not an intrinsic property of a material; it can change depending on how the material is processed. In one embodiment, the granules used to prepare the pharmaceutical compositions provided by the invention have a bulk density of about 0.5-0.7 g/cc.

[00100] An effective amount or "therapeutically effective amount" of a drug compound of the invention may vary according to factors such as the disease state, age, and weight of the subject, and the ability of the compound of the invention to elicit a desired response in the subject. Dosage regimens may be adjusted to provide the optimum therapeutic response. An effective amount is also one in which any toxic or detrimental effects (e.g., side effects) of the compound of the invention are outweighed by the therapeutically beneficial effects.

[00101] As used herein, and unless otherwise specified, the terms "therapeutically effective amount" and "effective amount" of a compound mean an amount sufficient to provide a therapeutic benefit in the treatment or management of a disease or disorder, or to delay or minimize one or more symptoms associated with the disease or disorder. A "therapeutically effective amount" and "effective amount" of a compound mean an amount of therapeutic agent, alone or in combination with one or more other agent(s), which provides a therapeutic benefit in the treatment or management of the disease or disorder. The terms "therapeutically effective amount" and "effective amount" can encompass an amount that improves overall therapy, reduces or avoids symptoms or causes of disease or disorder, or enhances the therapeutic efficacy of another therapeutic agent.

[00102] "Substantially pure" as used in the phrase "substantially pure Compound 1 Form I, Compound 1 Form II, or Compound 1 HCl Salt Form A," means greater than about 90% purity. In another embodiment, substantially pure refers to greater than about 95% purity. In another

embodiment, substantially pure refers to greater than about 98% purity. In another embodiment, substantially pure refers to greater than about 99% purity.

[00103] With respect to Compound 1 (e.g., Compound 1 Form I, Compound 1 Form II, Compound 1 HCl Salt Form A), the terms "about" and "approximately", when used in connection with doses, amounts, or weight percent of ingredients of a composition or a dosage form, mean a dose, amount, or weight percent that is recognized by one of ordinary skill in the art to provide a pharmacological effect equivalent to that obtained from the specified dose, amount, or weight percent. Specifically the term "about" or "approximately" means an acceptable error for a particular value as determined by one of ordinary skill in the art, which depends in part on how the value is measured or determined. In certain embodiments, the term "about" or "approximately" means within 1, 2, 3, or 4 standard deviations. In certain embodiments, the term "about" or "approximately" means within 30%, 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, or 0.05% of a given value or range.

[00104] Unless otherwise specified, the term "Compound 1" includes, but is not limited to, the solid forms of Compound 1 as described herein, e.g. Compound 1 Form I, Compound 1 Form II, or Compound 1 HCl Salt Form A, as well as combinations thereof.

PHARMACEUTICAL COMPOSITIONS

[00105] The invention provides pharmaceutical compositions, pharmaceutical formulations and solid dosage forms comprising Compound 1 which may be in substantially crystalline form. In some embodiments, Compound 1 is in crystalline Form I (Compound 1 Form I). In some embodiments, Compound 1 is in crystalline Form II (Compound 1 Form II). In some embodiments, Compound 1 is in crystalline HCl salt form (Compound 1 HCl Salt Form A). In some embodiments of this aspect, the amount of Compound 1 that is present in the pharmaceutical composition is 25 mg, 50 mg, 75 mg, 100 mg, 125 mg, 150 mg, 200 mg, 250 mg, or 400 mg. In some embodiments of this aspect, weight/weight relative percent of Compound 1 that is present in the pharmaceutical composition is from 10 to 75 percent. In these and other embodiments, 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid is present as substantially pure Compound 1. "Substantially pure" means greater than ninety percent pure; preferably greater than 95 percent pure; more

preferably greater than 99.5 percent pure (i.e., not mixed with other crystalline forms of Compound 1).

[00106] Thus in one aspect, the invention provides a pharmaceutical composition comprising:

a. Compound 1;

b. a filler;

c. a disintegrant;

d. a surfactant;

e. a diluent;

f. a lubricant; and

g. a glidant or a binder.

[00107] In one embodiment of this aspect, the pharmaceutical composition comprises 25 mg of Compound 1. In another embodiment of this aspect, the pharmaceutical composition comprises 50 mg of Compound 1. In another embodiment of this aspect, the pharmaceutical composition comprises 100 mg of Compound 1. In another embodiment of this aspect, the pharmaceutical composition comprises 125 mg of Compound 1. In another embodiment of this aspect, the pharmaceutical composition comprises 150 mg of Compound 1. In another embodiment of this aspect, the pharmaceutical composition comprises 200 mg of Compound 1. In another embodiment of this aspect, the pharmaceutical composition comprises 250 mg of Compound 1. In another embodiment of this aspect, the pharmaceutical composition comprises 300 mg of Compound 1. In another embodiment of this aspect, the pharmaceutical composition comprises 400 mg of Compound 1.

[00108] In some embodiments, the pharmaceutical compositions comprises Compound 1, wherein Compound 1 is present in an amount of at least 15 wt% (e.g., at least 20 wt%, at least 30 wt%, at least 40 wt%, at least 50 wt%, at least 60 wt%, or at least 70 wt%) by weight of the composition.

[00109] In some embodiments, the pharmaceutical composition comprises Compound 1, a filler, a diluent, a disintegrant, a surfactant, a glidant, and a lubricant. In this embodiment, the composition comprises from about 20 wt% to about 50 wt% (e.g., about 25-35 wt%) of

Compound 1 by weight of the composition, and more typically, from 25 wt% to about 45 wt% (e.g., about 28-32 wt%) of Compound 1 by weight of the composition.

[00110] In some embodiments, the pharmaceutical composition comprises Compound 1, a filler, a diluent, a disintegrant, a surfactant, a binder, and a lubricant. In this embodiment, the composition comprises from about 30 wt% to about 60 wt% (e.g., about 40-55 wt%) of Compound 1 by weight of the composition, and more typically from 35 wt% to about 70 wt% (e.g., about 45-55 wt%) of Compound 1 by weight of the composition.

[00111] The concentration of Compound 1 in the composition depends on several factors such as the amount of pharmaceutical composition needed to provide a desired amount of Compound 1 and the desired dissolution profile of the pharmaceutical composition.

[00112] In another embodiment, the pharmaceutical composition comprises Compound 1, in which Compound 1 in its solid form has a mean particle diameter, measured by light scattering (e.g., using a Malvern Mastersizer available from Malvern Instruments in England) of 0.1 microns to 10 microns. In another embodiment, the particle size of Compound 1 is 1 micron to 5 microns. In another embodiment, Compound 1 has a particle size D50 of 2.0 microns.

[00113] As indicated, in addition to Compound 1, in some embodiments of the invention, the pharmaceutical compositions which are oral formulations also comprise one or more excipients such as fillers, disintegrants, surfactants, diluents, binders, glidants, lubricants, colorants, or fragrances and any combination thereof.

[00114] Fillers suitable for the invention are compatible with the ingredients of the pharmaceutical composition, i.e., they do not substantially reduce the solubility, the hardness, the chemical stability, the physical stability, or the biological activity of the pharmaceutical composition. Exemplary fillers include: celluloses, modified celluloses, (e.g. sodium carboxymethyl cellulose, ethyl cellulose hydroxymethyl cellulose, hydroxypropylcellulose), cellulose acetate, microcrystalline cellulose, calcium phosphates, dibasic calcium phosphate, starches (e.g. corn starch, potato starch), sugars (e.g., sorbitol) lactose, sucrose, or the like), or any combination thereof.

[00115] Thus, in one embodiment, the pharmaceutical composition comprises at least one filler in an amount of at least 5 wt% (e.g., at least about 20 wt%, at least about 30 wt%, or at least

about 40 wt%) by weight of the composition. For example, the pharmaceutical composition comprises from about 10 wt% to about 60 wt% (e.g., from about 20 wt% to about 55 wt%, from about 25 wt% to about 50 wt%, or from about 27 wt% to about 45 wt%) of filler, by weight of the composition. In another example, the pharmaceutical composition comprises at least about 20 wt% (e.g., at least 30 wt% or at least 40 wt%) of microcrystalline cellulose, for example MCC Avicel PH102, by weight of the composition. In yet another example, the pharmaceutical composition comprises from about 10 wt% to about 60 wt% (e.g., from about 20 wt% to about 55 wt% or from about 25 wt% to about 45 wt%) of microcellulose, by weight of the composition.

[00116] Disintegrants suitable for the invention enhance the dispersal of the pharmaceutical composition and are compatible with the ingredients of the pharmaceutical composition, i.e., they do not substantially reduce the chemical stability, the physical stability, the hardness, or the biological activity of the pharmaceutical composition. Exemplary disintegrants include croscarmellose sodium, sodium starch glycolate, or a combination thereof.

[00117] Thus, in one embodiment, the pharmaceutical composition comprises disintegrant in an amount of about 10 wt% or less (e.g., about 7 wt% or less, about 6 wt% or less, or about 5 wt% or less) by weight of the composition. For example, the pharmaceutical composition comprises from about 1 wt% to about 10 wt% (e.g., from about 1.5 wt% to about 7.5 wt% or from about 2.5 wt% to about 6 wt%) of disintegrant, by weight of the composition. In another example, the pharmaceutical composition comprises about 10 wt% or less (e.g., 7 wt% or less, 6 wt% or less, or 5 wt% or less) of croscarmellose sodium, by weight of the composition. In yet another example, the pharmaceutical composition comprises from about 1 wt% to about 10 wt% (e.g., from about 1.5 wt% to about 7.5 wt% or from about 2.5 wt% to about 6 wt%) of croscarmellose sodium, by weight of the composition. In some examples, the pharmaceutical composition comprises from about 0.1% to about 10 wt% (e.g., from about 0.5 wt% to about 7.5 wt% or from about 1.5 wt% to about 6 wt%) of disintegrant, by weight of the composition. In still other examples, the pharmaceutical composition comprises from about 0.5% to about 10 wt% (e.g., from about 1.5 wt% to about 7.5 wt% or from about 2.5 wt% to about 6 wt%) of disintegrant, by weight of the composition.

[00118] Surfactants suitable for the invention enhance the wettability of the pharmaceutical composition and are compatible with the ingredients of the pharmaceutical composition, i.e., they do not substantially reduce the chemical stability, the physical stability, the hardness, or the biological activity of the pharmaceutical composition. Exemplary surfactants include sodium lauryl sulfate (SLS), sodium stearyl fumarate (SSF), polyoxyethylene 20 sorbitan mono-oleate (e.g., Tween™), any combination thereof, or the like.

[00119] Thus, in one embodiment, the pharmaceutical composition comprises a surfactant in an amount of about 10 wt% or less (e.g., about 5 wt% or less, about 2 wt% or less, about 1 wt% or less, about 0.8 wt% or less, or about 0.6 wt% or less) by weight of the composition. For example, the pharmaceutical composition includes from about 10 wt% to about 0.1 wt% (e.g., from about 5 wt% to about 0.2 wt% or from about 2 wt% to about 0.3 wt%) of surfactant, by weight of the composition. In another example, the pharmaceutical composition comprises 10 wt% or less (e.g., about 5 wt% or less, about 2 wt% or less, about 1 wt% or less, about 0.8 wt% or less, or about 0.6 wt% or less) of sodium lauryl sulfate, by weight of the composition. In yet another example, the pharmaceutical composition comprises from about 10 wt% to about 0.1 wt% (e.g., from about 5 wt% to about 0.2 wt% or from about 2 wt% to about 0.3 wt%) of sodium lauryl sulfate, by weight of the composition.

[00120] Binders suitable for the invention enhance the tablet strength of the pharmaceutical composition and are compatible with the ingredients of the pharmaceutical composition, i.e., they do not substantially reduce the chemical stability, the physical stability, or the biological activity of the pharmaceutical composition. Exemplary binders include polyvinylpyrrolidone, dibasic calcium phosphate, sucrose, corn (maize) starch, modified cellulose (e.g., hydroxymethyl cellulose), or any combination thereof.

[00121] Thus, in one embodiment, the pharmaceutical composition comprises a binder in an amount of at least about 0.1 wt% (e.g., at least about 1 wt%, at least about 3 wt%, at least about 4 wt%, or at least about 5 wt%) by weight of the composition. For example, the pharmaceutical composition comprises from about 0.1 wt% to about 10 wt% (e.g., from about 1 wt% to about 10 wt% or from about 2 wt% to about 7 wt%) of binder, by weight of the composition. In another example, the pharmaceutical composition comprises at least about 0.1 wt% (e.g., at least about 1 wt%, at least about 2 wt%, at least about 3 wt%, or at least about 4 wt%) of

polyvinylpyrrolidone, by weight of the composition. In yet another example, the pharmaceutical composition comprises a glidant in an amount ranging from about 0.1 wt% to about 10 wt% (e.g., from about 1 wt% to about 8 wt% or from about 2 wt% to about 5 wt%) of polyvinylpyrrolidone, by weight of the composition.

[00122] Diluents suitable for the invention may add necessary bulk to a formulation to prepare tablets of the desired size and are generally compatible with the ingredients of the pharmaceutical composition, i.e., they do not substantially reduce the solubility, the hardness, the chemical stability, the physical stability, or the biological activity of the pharmaceutical composition. Exemplary diluents include: sugars, for example, confectioner's sugar, compressible sugar, dextrates, dextrin, dextrose, lactose, mannitol, sorbitol, cellulose, and modified celluloses, for example, powdered cellulose, talc, calcium phosphate, starch, or any combination thereof.

[00123] Thus, in one embodiment, the pharmaceutical composition comprises a diluent in an amount of 40 wt% or less (e.g., 35 wt% or less, 30 wt% or less, or 25 wt% or less, or 20 wt% or less, or 15 wt% or less, or 10 wt% or less) by weight of the composition. For example, the pharmaceutical composition comprises from about 40 wt% to about 1 wt% (e.g., from about 35 wt% to about 5 wt% or from about 30 wt% to about 7 wt%, from about 25 wt% to about 10 wt%, from about 20 wt% to about 15 wt%) of diluent, by weight of the composition. In another example, the pharmaceutical composition comprises 40 wt% or less (e.g., 35 wt% or less, 25 wt% or less, or 15 wt% or less) of mannitol, by weight of the composition. In yet another example, the pharmaceutical composition comprises from about 35 wt% to about 1 wt% (e.g., from about 30 wt% to about 5 wt% or from about 25 wt% to about 10 wt%) of mannitol, by weight of the composition.

[00124] Glidants suitable for the invention enhance the flow properties of the pharmaceutical composition and are compatible with the ingredients of the pharmaceutical composition, i.e., they do not substantially reduce the solubility, the hardness, the chemical stability, the physical stability, or the biological activity of the pharmaceutical composition. Exemplary glidants include colloidal silicon dioxide, talc, or a combination thereof.

[00125] Thus, in one embodiment, the pharmaceutical composition comprises a glidant in an amount of 2 wt% or less (e.g., 1.75 wt%, 1.25 wt% or less, or 1.00 wt% or less) by weight of the

composition. For example, the pharmaceutical composition comprises from about 2 wt% to about 0.05 wt% (e.g., from about 1.5 wt% to about 0.07 wt% or from about 1.0 wt% to about 0.09 wt%) of glidant, by weight of the composition. In another example, the pharmaceutical composition comprises 2 wt% or less (e.g., 1.75 wt%, 1.25 wt% or less, or 1.00 wt% or less) of colloidal silicon dioxide, by weight of the composition. In yet another example, the pharmaceutical composition comprises from about 2 wt% to about 0.05 wt% (e.g., from about 1.5 wt% to about 0.07 wt% or from about 1.0 wt% to about 0.09 wt%) of colloidal silicon dioxide, by weight of the composition.

[00126] In some embodiments, the pharmaceutical composition can include an oral solid pharmaceutical dosage form which can comprise a lubricant that can prevent adhesion of a granulate-bead admixture to a surface (e.g., a surface of a mixing bowl, a compression die and/or punch). A lubricant can also reduce interparticle friction within the granulate and improve the compression and ejection of compressed pharmaceutical compositions from a die press. The lubricant is also compatible with the ingredients of the pharmaceutical composition, i.e., they do not substantially reduce the solubility, the hardness, or the biological activity of the pharmaceutical composition. Exemplary lubricants include magnesium stearate, calcium stearate, zinc stearate, sodium stearate, stearic acid, aluminum stearate, leucine, glyceryl behenate, hydrogenated vegetable oil or any combination thereof. In one embodiment, the pharmaceutical composition comprises a lubricant in an amount of 5 wt% or less (e.g., 4.75 wt%, 4.0 wt% or less, or 3.00 wt% or less, or 2.0 wt% or less) by weight of the composition. For example, the pharmaceutical composition comprises from about 5 wt% to about 0.10 wt% (e.g., from about 4.5 wt% to about 0.5 wt% or from about 3 wt% to about 1 wt%) of lubricant, by weight of the composition. In another example, the pharmaceutical composition comprises 5 wt% or less (e.g., 4.0 wt% or less, 3.0 wt% or less, or 2.0 wt% or less, or 1.0 wt% or less) of magnesium stearate, by weight of the composition. In yet another example, the pharmaceutical composition comprises from about 5 wt% to about 0.10 wt% (e.g., from about 4.5 wt% to about 0.15 wt% or from about 3.0 wt% to about 0.50 wt%) of magnesium stearate, by weight of the composition.

[00127] Pharmaceutical compositions of the invention can optionally comprise one or more colorants, flavors, and/or fragrances to enhance the visual appeal, taste, and/or scent of the composition. Suitable colorants, flavors, or fragrances are compatible with the ingredients of the

pharmaceutical composition, i.e., they do not substantially reduce the solubility, the chemical stability, the physical stability, the hardness, or the biological activity of the pharmaceutical composition. In one embodiment, the pharmaceutical composition comprises a colorant, a flavor, and/or a fragrance. In one embodiment, the pharmaceutical compositions provided by the invention are purple.

[00128] In some embodiments, the pharmaceutical composition includes or can be made into tablets and the tablets can be coated with a colorant and optionally labeled with a logo, other image and/or text using a suitable ink. In still other embodiments, the pharmaceutical composition includes or can be made into tablets and the tablets can be coated with a colorant, waxed, and optionally labeled with a logo, other image and/or text using a suitable ink. Suitable colorants and inks are compatible with the ingredients of the pharmaceutical composition, i.e., they do not substantially reduce the solubility, the chemical stability, the physical stability, the hardness, or the biological activity of the pharmaceutical composition. The suitable colorants and inks can be any color and are water based or solvent based. In one embodiment, tablets made from the pharmaceutical composition are coated with a colorant and then labeled with a logo, other image, and/or text using a suitable ink. For example, tablets comprising pharmaceutical composition as described herein can be coated with about 3 wt% (e.g., less than about 6 wt% or less than about 4 wt%) of film coating comprising a colorant. The colored tablets can be labeled with a logo and text indicating the strength of the active ingredient in the tablet using a suitable ink. In another example, tablets comprising pharmaceutical composition as described herein can be coated with about 3 wt% (e.g., less than about 6 wt% or less than about 4 wt%) of a film coating comprising a colorant.

[00129] In another embodiment, tablets made from the pharmaceutical composition are coated with a colorant, waxed, and then labeled with a logo, other image, and/or text using a suitable ink. For example, tablets comprising pharmaceutical composition as described herein can be coated with about 3 wt% (e.g., less than about 6 wt% or less than about 4 wt%) of film coating comprising a colorant. The colored tablets can be waxed with Carnauba wax powder weighed out in the amount of about 0.01% w/w of the starting tablet core weight. The waxed tablets can be labeled with a logo and text indicating the strength of the active ingredient in the tablet using a suitable ink. In another example, tablets comprising pharmaceutical composition as described herein can be coated with about 3 wt% (e.g., less than about 6 wt% or less than about 4 wt%) of

a film coating comprising a colorant The colored tablets can be waxed with Carnauba wax powder weighed out in the amount of about 0.01% w/w of the starting tablet core weight. The waxed tablets can be labeled with a logo and text indicating the strength of the active ingredient in the tablet using a pharmaceutical grade ink such as a black ink (e.g., Opacode® S-1-17823, a solvent based ink, commercially available from Colorcon, Inc. of West Point, PA.).

[00130] One exemplary pharmaceutical composition comprises from about 15 wt% to about 70 wt% (e.g., from about 15 wt% to about 60 wt%, from about 15 wt% to about 50 wt%, or from about 15 wt% to about 40 wt%, or from about 20 wt% to about 70 wt%, or from about 30 wt% to about 70 wt%, or from about 40 wt% to about 70 wt%, or from about 50 wt% to about 70 wt%) of Compound 1, by weight of the composition. The aforementioned compositions can also include one or more pharmaceutically acceptable excipients, for example, from about 20 wt% to about 50 wt% of a filler; from about 1 wt% to about 5 wt% of a disintegrant; from about 2 wt% to about 0.3 wt% of a surfactant; from about 0.1 wt% to about 5 wt% of a binder; from about 1 wt% to about 30 wt% of a diluent; from about 2 wt% to about 0.05 wt% of a glidant; and from about 5 wt% to about 0.1 wt% of a lubricant. Or, the pharmaceutical composition comprises a composition containing from about 15 wt% to about 70 wt% (e.g., from about 20 wt% to about 40 wt%, from about 25 wt% to about 60 wt%, or from about 30 wt% to about 55 wt%) of Compound 1, by weight of the composition; and one or more excipients, for example, from about 20 wt% to about 50 wt% of a filler; from about 1 wt% to about 5 wt% of a disintegrant; from about 2 wt% to about 0.3 wt% of a surfactant; from about 0.1 wt% to about 5 wt% of a binder; from about 1 wt% to about 30 wt% of a diluent; from about 2 wt% to about 0.05 wt% of a glidant; and from about 5 wt% to about 0.1 wt% of a lubricant.

[00131] Another exemplary pharmaceutical composition comprises from about 15 wt% to about 70 wt% (e.g., from about 15 wt% to about 60 wt%, from about 15 wt% to about 50 wt%, or from about 15 wt% to about 40 wt% or from about 20 wt% to about 70 wt%, or from about 30 wt% to about 70 wt%, or from about 40 wt% to about 70 wt%, or from about 50 wt% to about 70 wt%) of Compound 1 by weight of the composition, and one or more excipients, for example, from about 20 wt% to about 50 wt% of a filler; from about 1 wt% to about 5 wt% of a disintegrant; from about 2 wt% to about 0.3 wt% of a surfactant; from about 0.1 wt% to about 5 wt% of a binder; from about 1 wt% to about 30 wt% of a diluent; from about 2 wt% to about 0.05 wt% of a glidant; and from about 2 wt% to about 0.1 wt% of a lubricant.

[00132] Another exemplary pharmaceutical composition comprises from about 15 wt% to about 70 wt% (e.g., from about 15 wt% to about 60 wt%, from about 15 wt% to about 50 wt%, or from about 15 wt% to about 40 wt% or from about 20 wt% to about 70 wt%, or from about 30 wt% to about 70 wt%, or from about 40 wt% to about 70 wt%, or from about 50 wt% to about 70 wt%) of Compound 1 by weight of the composition, and one or more excipients, for example, from about 20 wt% to about 50 wt% of a filler; from about 1 wt% to about 5 wt% of a disintegrant; from about 2 wt% to about 0.3 wt% of a surfactant; from about 0.1 wt% to about 5 wt% of a binder; from about 1 wt% to about 30 wt% of a diluent; from about 2 wt% to about 0.05 wt% of a glidant; and from about 2 wt% to about 0.1 wt% of a lubricant.

[00133] Another exemplary pharmaceutical composition comprises from about 15 wt% to about 70 wt% (e.g., from about 15 wt% to about 60 wt%, from about 15 wt% to about 50 wt%, or from about 15 wt% to about 40 wt% or from about 20 wt% to about 70 wt%, or from about 30 wt% to about 70 wt%, or from about 40 wt% to about 70 wt%, or from about 50 wt% to about 70 wt%) of Compound 1 and one or more excipients, for example, from about 20 wt% to about 50 wt% of a filler; from about 1 wt% to about 5 wt% of a disintegrant; from about 2 wt% to about 0.3 wt% of a surfactant; from about 0.1 wt% to about 5 wt% of a binder; from about 1 wt% to about 30 wt% of a diluent; from about 2 wt% to about 0.05 wt% of a glidant; and from about 2 wt% to about 0.1 wt% of a lubricant.

[00134] In one embodiment, the invention is a granular pharmaceutical composition comprising:

a. about 30 wt% of Compound 1 by weight of the composition;

b. about 42 wt% of microcrystalline cellulose by weight of the composition;

c. about 21 wt% of mannitol by weight of the composition;

d. about 3 wt% of sodium croscarmellose sodium by weight of the composition;

e. about 1 wt% of sodium lauryl sulfate by weight of the composition;

f. about 2 wt% of magnesium stearate by weight of the composition; and

g. about 0.5 wt% of colloidal silica by weight of the composition.

[00135] Another granular composition formulated into an oral formulation of the invention comprises:

a. about 50 wt% of Compound 1;

b. about 30 wt% of microcrystalline cellulose by weight of the composition;

c. about 13 wt% of mannitol by weight of the composition;

d. about 2 wt% of sodium croscarmellose sodium by weight of the composition;

e. about 4 wt% of polyvinylpyrrolidone by weight of the composition; and

f. about 1 wt% of sodium lauryl sulfate by weight of the composition.

[00136] In one embodiment, a pharmaceutical oral formulation of the invention comprises:

a. about 30 wt% of a Compound 1 by weight of the composition;

b. about 42 wt% of microcrystalline cellulose by weight of the composition;

c. about 21 wt% of mannitol by weight of the composition;

d. about 3 wt% of sodium croscarmellose sodium by weight of the composition;

e. about 1 wt% of sodium lauryl sulfate by weight of the composition;

f. about 2.5 wt% of magnesium stearate by weight of the composition; and

g. about 0.5 wt% of colloidal silica by weight of the composition.

[00137] Another pharmaceutical oral formulation of the invention comprises:

a. about 50 wt% of a Compound 1 by weight of the composition;

b. about 30 wt% of microcrystalline cellulose by weight of the composition;

c. about 13 wt% of mannitol by weight of the composition;

d. about 4 wt% of sodium croscarmellose sodium by weight of the composition;

e. about 4 wt% of polyvinylpyrrolidone by weight of the composition

f. about 1 wt% of sodium lauryl sulfate by weight of the composition; and

g. about 0.5 wt% of magnesium stearate by weight of the composition.

[00138] Another pharmaceutical oral formulation of the invention comprises:

a. about 60 wt% of a Compound 1 by weight of the composition;

b. about 20 wt% of microcrystalline cellulose by weight of the composition;

c. about 13 wt% of mannitol by weight of the composition;

d. about 4 wt% of sodium croscarmellose sodium by weight of the composition;

e. about 4 wt% of polyvinylpyrrolidone by weight of the composition

f. about 1 wt% of sodium lauryl sulfate by weight of the composition; and

g. about 0.5 wt% of magnesium stearate by weight of the composition.

[00139] Another pharmaceutical oral formulation of the invention comprises:

a. about 150 to 250 mg of Compound 1;

b. about 40 to 50 mg of mannitol;

c. about 120 to 130 mg of microcrystalline cellulose;

d. about 10 to 20 mg of croscarmellose sodium;

e. about 10 to 20 mg of polyvinylpyrrolidone;

f. about 1 to 5 mg of sodium lauryl sulfate; and

g. about 1 to 5 mg of magnesium stearate.

[00140] Another pharmaceutical oral formulation of the invention comprises:

a. about 200 mg of Compound 1;

b. about 43 mg of mannitol;

c. about 123 mg of microcrystalline cellulose;

d. about 15 mg of croscarmellose sodium;

e. about 13 mg of polyvinylpyrrolidone;

f. about 3 mg of sodium lauryl sulfate; and

g. about 4 mg of magnesium stearate.

[00141] Another pharmaceutical oral formulation of the invention comprises:

a. about 200 mg of Compound 1;

b. about 45 mg of mannitol;

c. about 123 mg of microcrystalline cellulose;

d. about 15 mg of croscarmellose sodium;

e. about 10.4 mg of polyvinylpyrrolidone;

f. about 2.6 mg of sodium lauryl sulfate; and

g. about 4 mg of magnesium stearate.

[00142] Another pharmaceutical oral formulation of the invention comprises:

a. about 70 wt% of a Compound 1 by weight of the composition;

b. about 12 wt% of microcrystalline cellulose by weight of the composition;

c. about 11 wt% of mannitol by weight of the composition;

d. about 4 wt% of sodium croscarmellose sodium by weight of the composition;

e. about 4 wt% of polyvinylpyrrolidone by weight of the composition

f. about 1 wt% of sodium lauryl sulfate by weight of the composition; and

g. about 0.5 wt% of magnesium stearate by weight of the composition.

[00143] The pharmaceutical compositions of the invention can be processed into a tablet form, capsule form, pouch form, lozenge form, or other solid form that is suited for oral administration. Thus in some embodiments, the pharmaceutical compositions are in tablet form.

[00144] In still another pharmaceutical oral formulation of the invention, a shaped pharmaceutical tablet composition having an initial hardness of 5-21 kP ± 20 percent comprises: about 30 wt% of Compound 1; about 42 wt% of microcrystalline cellulose by weight of the composition; about 21 wt% of mannitol by weight of the composition; about 3 wt% of sodium croscarmellose sodium by weight of the composition; about 1 wt% of sodium lauryl sulfate by weight of the composition; about 2.5 wt% of magnesium stearate by weight of the composition; and about 0.5 wt% of colloidal silica by weight of the composition. Wherein the amount of

Compound 1 in the shaped pharmaceutical tablet ranges from about 25 mg to about 250 mg, for example, 50 mg, or 75 mg, or 100 mg, or 150 mg, 200 mg, or 250 mg Compound 1 per tablet.

[00145] In still another pharmaceutical oral formulation of the invention, a shaped pharmaceutical tablet composition having an initial hardness of 5-21 kP ± 20 percent comprises: about 49 wt% of a Compound 1; about 29 wt% of microcrystalline cellulose by weight of the composition; about 12.6 wt% of mannitol by weight of the composition; about 4 wt% of sodium croscarmellose sodium by weight of the composition; about 4 wt% of polyvinylpyrrolidone by weight of the composition; about 1 wt% of sodium lauryl sulfate by weight of the composition; and about 0.5 wt% of magnesium stearate by weight of the composition. The amount of Compound 1 in the shaped pharmaceutical tablet ranges from about 25 mg to about 250 mg, for example, 50 mg, or 75 mg, or 100 mg, or 150 mg, 200 mg, or 250 mg Compound 1 per tablet.

[00146] In certain embodiments, the shaped pharmaceutical tablet contains about 100 mg of Compound 1. In certain embodiments, the shaped pharmaceutical tablet contains about 200 mg of Compound 1.

[00147] Another aspect of the invention provides a pharmaceutical formulation consisting of a tablet or capsule that includes a Compound 1 and other excipients (e.g., a filler, a disintegrant, a surfactant, a binder, a glidant, a colorant, a lubricant, or any combination thereof), each of which is described above and in the Examples below, wherein the tablet has a dissolution of at least about 50% (e.g., at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 99%) in about 30 minutes. In one example, the pharmaceutical composition consists of a tablet that includes Compound 1 in an amount ranging from 25 mg to 250 mg, for example, 25 mg, or 50 mg, or 75 mg, or 100 mg, or 150 mg, 200 mg, or 250 mg and one or more excipients (e.g., a filler, a disintegrant, a surfactant, a binder, a glidant, a colorant, a lubricant, or any combination thereof), each of which is described above and in the Examples below, wherein the tablet has a dissolution of from about 50% to about 100% (e.g., from about 55% to about 95% or from about 60% to about 90%) in about 30 minutes. In another example, the pharmaceutical composition consists of a tablet that comprises a composition comprising Compound 1; and one or more excipients from: a filler, a diluent, a disintegrant, a surfactant, a binder, a glidant, and a lubricant, wherein the tablet has a dissolution of at least about 50% (e.g.,

at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 99%) in about 30 minutes.

[00148] In one embodiment, the tablet comprises a composition comprising at least about 25 mg (e.g., at least about 30 mg, at least about 40 mg, or at least about 50 mg) of Compound 1; and one or more excipients from: a filler, a diluent, a disintegrant, a surfactant, a binder, a glidant, and a lubricant. In another embodiment, the tablet comprises a composition comprising at least about 25 mg (e.g., at least about 30 mg, at least about 40 mg, at least about 50 mg, at least about 100 mg, or at least 150 mg) of Compound 1 and one or more excipients from: a filler, a diluent, a disintegrant, a surfactant, a binder, a glidant, and a lubricant.

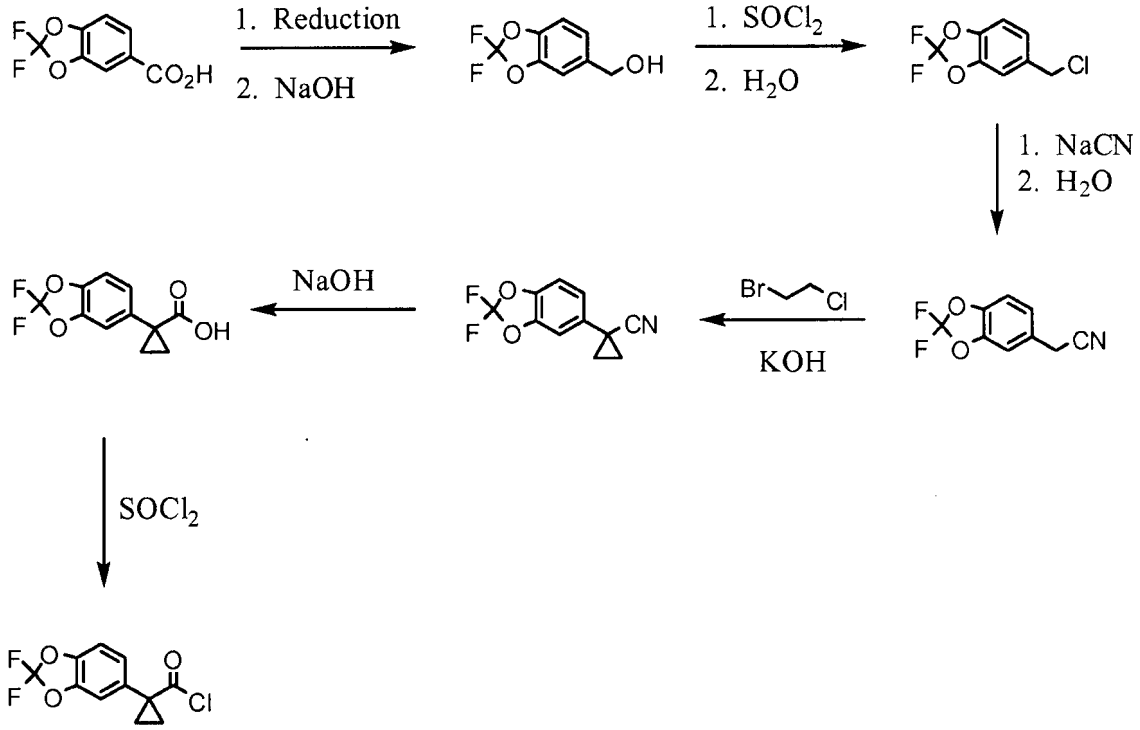
[00149] Dissolution can be measured with a standard USP Type II apparatus that employs a dissolution media of 0.1% CTAB dissolved in 900 mL of DI water, buffered at pH 6.8 with 50 mM potassium phosphate monoasic, stirring at about 50-75 rpm at a temperature of about 37 °C. A single experimental tablet is tested in each test vessel of the apparatus. Dissolution can also be measured with a standard USP Type II apparatus that employs a dissolution media of 0.7% sodium lauryl sulfate dissolved in 900 mL of 50 mM sodium phosphate buffer (pH 6.8), stirring at about 65 rpm at a temperature of about 37 °C. A single experimental tablet is tested in each test vessel of the apparatus. Dissolution can also be measured with a standard USP Type II apparatus that employs a dissolution media of 0.5% sodium lauryl sulfate dissolved in 900 mL of 50 mM sodium phosphate buffer (pH 6.8), stirring at about 65 rpm at a temperature of about 37 °C. A single experimental tablet is tested in each test vessel of the apparatus.

METHODS FOR MAKING COMPOUND 1, COMPOUND 1 FORM I, COMPOUND 1 FORM II, COMPOUND 1 HCl SALT FORM A

Compound 1

[00150] Compound 1 is used as the starting point for the other solid state forms and can be prepared by coupling an acid chloride moiety with an amine moiety according to Schemes 1-4.

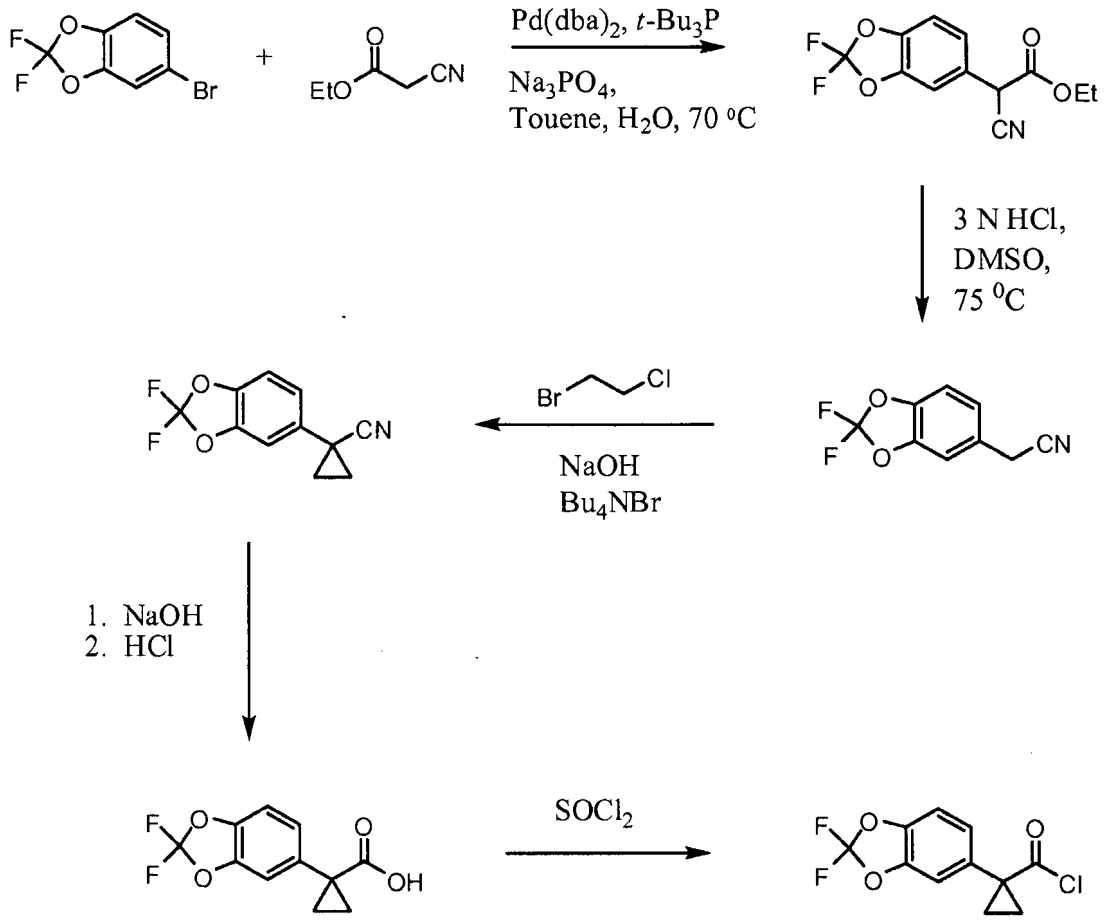
Scheme 1. Synthesis of the acid chloride moiety.



[00151] Scheme 1 depicts the preparation of 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarbonyl chloride, which is used in Scheme 3 to make the amide linkage of Compound 1.

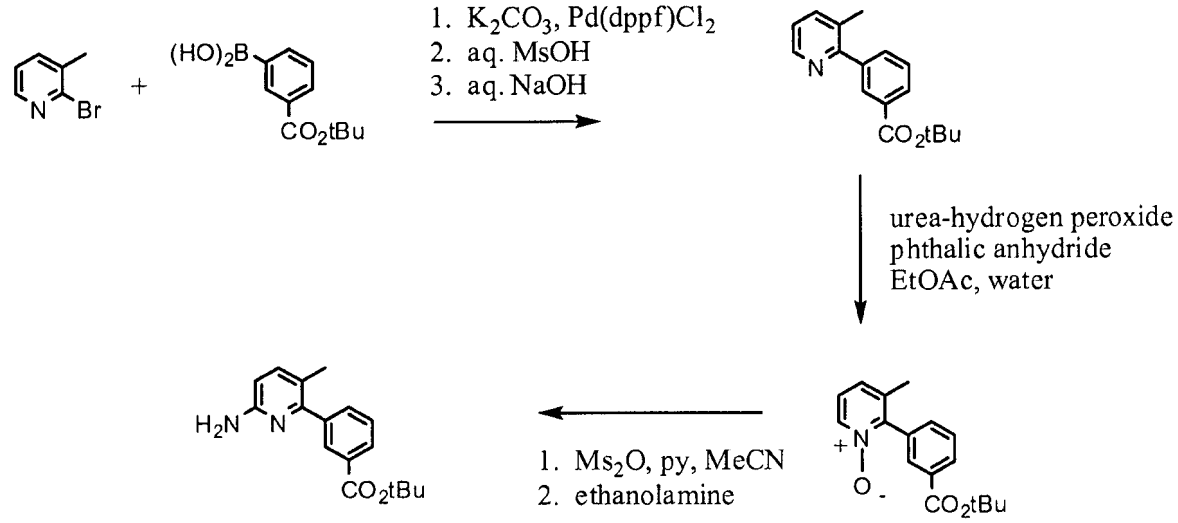
[00152] The starting material, 2,2-difluorobenzo[d][1,3]dioxole-5-carboxylic acid, is commercially available from Saltigo (an affiliate of the Lanxess Corporation). Reduction of the carboxylc acid moiety in 2,2-difluorobenzo[d][1,3]dioxole-5-carboxylic acid to the primary alcohol, followed by conversion to the corresponding chloride using thionyl chloride ($SOCl_2$), provides 5-(chloromethyl)-2,2-difluorobenzo[d][1,3]dioxole, which is subsequently converted to 2-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)acetonitrile using sodium cyanide. Treatment of 2-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)acetonitrile with base and 1-bromo-2-chloroethane provides 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarbonitrile. The nitrile moiety in 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarbonitrile is converted to a carboxylic acid using base to give 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid, which is converted to the desired acid chloride using thionyl chloride.

Scheme 2. Alternative synthesis of the acid chloride moiety.



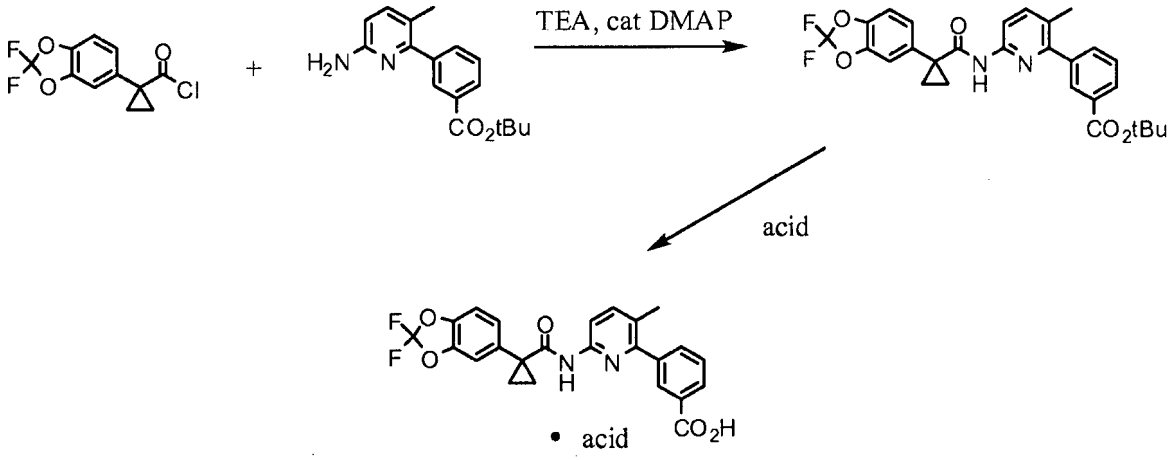
[00153] Scheme 2 depicts an alternative synthesis of the requisite acid chloride. 5-bromomethyl-2,2-difluoro-1,3-benzodioxole is coupled with ethyl cyanoacetate in the presence of a palladium catalyst to form the corresponding alpha cyano ethyl ester. Saponification of the ester moiety to the carboxylic acid gives the cyanoethyl compound. Alkylation of the cyanoethyl compound with 1-bromo-2-chloro ethane in the presence of base gives the cyanocyclopropyl compound. Treatment of the cyanocyclopropyl compound with base gives the carboxylate salt, which is converted to the carboxylic acid by treatment with acid. Conversion of the carboxylic acid to the acid chloride is then accomplished using a chlorinating agent such as thionyl chloride or the like.

Scheme 3. Synthesis of the amine moiety.



[00154] Scheme 3 depicts the preparation of the requisite tert-butyl 3-(6-amino-3-methylpyridin-2-yl)benzoate, which is coupled with 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarbonyl chloride in Scheme 3 to give Compound 1. Palladium-catalyzed coupling of 2-bromo-3-methylpyridine with 3-(tert-butoxycarbonyl)phenylboronic acid gives tert-butyl 3-(3-methylpyridin-2-yl)benzoate, which is subsequently converted to the desired compound.

Scheme 4. Formation of an acid salt of 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid.



[00155] Scheme 4 depicts the coupling of 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarbonyl chloride with tert-butyl 3-(6-amino-3-methylpyridin-2-yl)benzoate using triethyl amine and 4-dimethylaminopyridine to initially provide the tert-butyl ester of Compound 1.

Compound 1 Form I

[00156] Compound 1 Form I is prepared by dispersing or dissolving a salt form, such as the HCl salt, of Compound 1 in an appropriate solvent for an effective amount of time. Treatment of the tert-butyl ester with an acid such as HCl, gives the HCL salt of Compound 1, which is typically a crystalline solid. Compound 1 Form I may also be prepared directly from the t-butyl ester precursor by treatment with an appropriate acid, such as formic acid.

[00157] The HCl salt of 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid can be used to make Form I by dispersing or dissolving the HCl salt of 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid in an appropriate solvent for an effective amount of time. Other salts of 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid may be used, such as, for example, salts derived from other mineral or organic acids. The other salts result from acid-mediated hydrolysis of the t-butyl ester moiety. Salts derived from other acids may include, for example, nitric, sulfuric, phosphoric, boric, acetic, benzoic and malonic. These salt forms of 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid may or may not be soluble, depending upon the solvent used, but lack of solubility does not hinder formation of Form I. For example, in one embodiment, the appropriate solvent may be water or an alcohol/water mixture such as 50% methanol/water mixture, even though the HCl salt form of 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid is only sparingly soluble in water. In one embodiment, the appropriate solvent is water.

[00158] The effective amount of time for formation of Form I from the salt of 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid can be any time between 2 to 24 hours or greater. It is recognized that the amount of time needed is inversely proportional to the temperature. That is, the higher the temperature the less

time needed to affect dissociation of acid to form Form I. When the solvent is water, stirring the dispersion for approximately 24 hours at room temperature provides Form I in an approximately 98% yield. If a solution of the salt of 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid is desired for process purposes, an elevated temperature may be used. After stirring the solution for an effective amount of time at the elevated temperature, recrystallization upon cooling provides substantially pure Form I. In one embodiment, substantially pure refers to greater than about 90% purity. In another embodiment, substantially pure refers to greater than about 95% purity. In another embodiment, substantially pure refers to greater than about 98% purity. In another embodiment, substantially pure refers to greater than about 99% purity. The temperature selected depends in part on the solvent used and is well within the determination capabilities of one of ordinary skill in the art. In one embodiment, the temperature is between room temperature and about 80 °C. In another embodiment, the temperature is between room temperature and about 40 °C. In another embodiment, the temperature is between about 40 °C and about 60 °C. In another embodiment, the temperature is between about 60 °C and about 80 °C.

[00159] Compound 1 Form I may also be formed directly from 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)-t-butylbenzoate (cf. Scheme 3), which is a precursor to the salt of Compound 1. Thus, 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)-t-butylbenzoate is allowed to undergo reaction with an appropriate acid, such as, for example, formic acid under appropriate reaction conditions to give Compound 1 Form I.

[00160] Compound 1 Form I may be further purified by recrystallization from an organic solvent. Examples of organic solvents include, but are not limited to, toluene, cumene, anisol, 1-butanol, isopropyl acetate, butyl acetate, isobutyl acetate, methyl *t*-butyl ether, methyl isobutyl ketone and 1-propanol-water mixtures. The temperature may be as described above. For example, Form I is dissolved in 1-butanol at 75 °C until it is completely dissolved. Cooling down the solution to 10 °C at a rate of 0.2 °C/min yields crystals of Form I which may be isolated by filtration.

[00161] In one embodiment, Compound 1 Form I is characterized by one or more peaks at 15.2 to 15.6 degrees, 16.1 to 16.5 degrees, and 14.3 to 14.7 degrees in an X-ray powder diffraction

obtained using Cu K alpha radiation. In another embodiment, Compound 1 Form I is characterized by one or more peaks at 15.4, 16.3, and 14.5 degrees. In another embodiment, Compound 1 Form I is further characterized by a peak at 14.6 to 15.0 degrees. In another embodiment, Compound 1 Form I is further characterized by a peak at 14.8 degrees. In another embodiment, Compound 1 Form I is further characterized by a peak at 17.6 to 18.0 degrees. In another embodiment, Compound 1 Form I is further characterized by a peak at 17.8 degrees. In another embodiment, Compound 1 Form I is further characterized by a peak at 16.4 to 16.8 degrees. In another embodiment, Compound 1 Form I is further characterized by a peak at 16.4 to 16.8 degrees. In another embodiment, Compound 1 Form I is further characterized by a peak at 16.6 degrees. In another embodiment, Compound 1 Form I is further characterized by a peak at 7.6 to 8.0 degrees. In another embodiment, Compound 1 Form I is further characterized by a peak at 7.8 degrees. In another embodiment, Compound 1 Form I is further characterized by a peak at 25.8 to 26.2 degrees. In another embodiment, Compound 1 Form I is further characterized by a peak at 26.0 degrees. In another embodiment, Compound 1 Form I is further characterized by a peak at 21.4 to 21.8 degrees. In another embodiment, Compound 1 Form I is further characterized by a peak at 21.6 degrees. In another embodiment, Compound 1 Form I is further characterized by a peak at 23.1 to 23.5 degrees. In another embodiment, Compound 1 Form I is further characterized by a peak at 23.3 degrees. In some embodiments, Compound 1 Form I is characterized by a diffraction pattern substantially similar to that of Figure 1. In some embodiments, Compound 1 Form I is characterized by a diffraction pattern substantially similar to that of Figure 2.

[00162] In some embodiments, the particle size distribution of D90 is about 82 μm or less for Compound 1 Form I. In some embodiments, the particle size distribution of D50 is about 30 μm or less for Compound 1 Form I.

Compound 1 Form II

[00163] Compound 1 Form II is prepared by slurrying Compound 1 Form I in an appropriate solvent at a sufficient concentration for a sufficient time. The slurry is then filtered centrifugally or under vacuum and dried at ambient conditions for sufficient time to yield Compound 1 Form II.

[00164] In some embodiments, about 20 to 40 mg of Compound 1 Form I is slurried in about

400 to 600 μL of an appropriate solvent. In another embodiment, about 25 to 35 mg of Compound 1 Form I is slurried in about 450 to 550 μL of an appropriate solvent. In another embodiment, about 30 mg of Compound 1 Form I is slurried in about 500 μL of an appropriate solvent.

[00165] In some embodiments, the time that Compound 1 Form I is allowed to slurry with the solvent is from1 hour to four days. More particularly, the time that Compound 1 Form I is allowed to slurry with the solvent is from1 to 3 days. More particularly, the time is 2 days.

[00166] In some embodiments, the appropriate solvent is selected from an organic solvent of sufficient size to fit the voids in the crystalline lattice of Compound 1 Form II. In other embodiments, the solvate is of sufficient size to fit in voids measuring about 100 $Å^3$.

[00167] In other embodiments, the solvent is selected from the group consisting of methanol, ethanol, acetone, 2-propanol, acetonitrile, tetrahydrofuran, methyl acetate, 2-butanone, ethyl formate, and 2-methyl tetrahydrofuran.

[00168] In other embodiments, a mixture of two or more of these solvents may be used to obtain Compound 1 Form II. Alternatively, Compound 1 Form II may be obtained from a mixture comprising one or more of these solvents and water.

[00169] In some embodiments, the effective amount of time for drying Compound 1 Form II is 1 to 24 hours. More particularly, the time is 6 to 18 hours. More particularly, the time is about 12 hours.

[00170] In another embodiment, Compound 1 Form II is prepared by dispersing or dissolving a salt form of Compound 1, such as an HCl salt of Compound 1 in an appropriate solvent for an effective amount of time.

[00171] Compound 1 Form II as disclosed herein comprises a crystalline lattice of Compound 1 in which voids in the crystalline lattice are empty, or occupied, or partially occupied by one or more molecules of a suitable solvent. Suitable solvents include, but are not limited to, methanol, ethanol, acetone, 2-propanol, acetonitrile, tetrahydrofuran, methyl acetate, 2-butanone, ethyl formate, and 2-methyl tetrahydrofuran. Certain physical characterisics of Compound 1 isostructural solvate forms, such as X-ray powder diffraction, melting point and DSC, are not substantially affected by the particular solvent molecule in question.

[00172] In one embodiment, Compound 1 Form II is characterized by one or more peaks at 21.50 to 21.90 degrees, 8.80 to 9.20 degrees, and 10.80 to 11.20 degrees in an X-ray powder diffraction obtained using Cu K alpha radiation. In another embodiment, Compound 1 Form II is characterized by one or more peaks at 21.50 to 21.90 degrees, 8.80 to 9.20 degrees, 10.80 to 11.20 degrees, 18.00 to 18.40 degrees, and 22.90 to 23.30 degrees in an X-ray powder diffraction obtained using Cu K alpha radiation. In another embodiment, Compound 1 Form II is characterized by one or more peaks at 21.70, 8.98, and 11.04 degrees. In another embodiment, Compound 1 Form II is characterized by one or more peaks at 21.70, 8.98, 11.04, 18.16, and 23.06 degrees. In another embodiment, Compound 1 Form II is characterized by a peak at 21.50 to 21.90 degrees. In another embodiment, Compound 1 Form II is further characterized by a peak at 21.70 degrees. In another embodiment, Compound 1 Form II is further characterized by a peak at 8.80 to 9.20 degrees. In another embodiment, Compound 1 Form II is further characterized by a peak at 8.98 degrees. In another embodiment, Compound 1 Form II is further characterized by a peak at 10.80 to 11.20 degrees. In another embodiment, Compound 1 Form II is further characterized by a peak at 11.04. In another embodiment, Compound 1 Form II is further characterized by a peak at 18.00 to 18.40 degrees. In another embodiment, Compound 1 Form II is further characterized by a peak at 18.16 degrees. In another embodiment, Compound 1 Form II is further characterized by a peak at 22.90 to 23.30 degrees. In another embodiment, Compound 1 Form II is further characterized by a peak at 23.06 degrees. In another embodiment, Compound 1 Form II is further characterized by a peak at 20.40 to 20.80 degrees. In another embodiment, Compound 1 Form II is further characterized by a peak at 20.63 degrees. In another embodiment, Compound 1 Form II is further characterized by a peak at 22.00 to 22.40 degrees. In another embodiment, Compound 1 Form II is further characterized by a peak at 22.22 degrees. In another embodiment, Compound 1 Form II is further characterized by a peak at 18.40 to 18.80 degrees. In another embodiment, Compound 1 Form II is further characterized by a peak at 18.57 degrees. In another embodiment, Compound 1 Form II is further characterized by a peak at 16.50 to 16.90 degrees. In another embodiment, Compound 1 Form II is further characterized by a peak at 16.66 degrees. In another embodiment, Compound 1 Form II is further characterized by a peak at 19.70 to 20.10 degrees. In another embodiment, Compound 1 Form II is further characterized by a peak at 19.86 degrees.

[00173] In some embodiments, Compound 1 Form II is characterized by a diffraction pattern

substantially similar to that of Figure 3. In some embodiments, Compound 1 Form II is characterized by diffraction patterns substantially similar to those provided in Figure 4.

[00174] In another embodiment, the solvate that forms Compound 1 Form II is selected from the group consisting of methanol, ethanol, acetone, 2-propanol, acetonitrile, tetrahydrofuran, methyl acetate, 2-butanone, ethyl formate, and 2-methyl tetrahydrofuran. Diffraction patterns are provided for the following Compound 1 Form II: methanol (Figure 5), ethanol (Figure 6), acetone (Figure 7), 2-propanol (Figure 8), acetonitrile (Figure 9), tetrahydrofuran (Figure 10), methyl acetate (Figure 11), 2-butanone (Figure 12), ethyl formate (Figure 13), and 2-methytetrahydrofuran (Figure 14).

[00175] In another embodiment, the invention provides Compound 1 Form II which exhibits two or more phase transitions as determined by DSC or a similar analytic method known to the skilled artisan. In some embodiments, the DSC of Compound 1 Form II is substantially similar to the DSC trace depicted in Figure 15. In another embodiment of this aspect, the DSC gives two phase transitions. In another embodiment, the DSC gives three phase transitions. In another embodiment, one of the phase transitions occurs between 200 and 207 °C. In another embodiment, one of the phase transitions occurs between 204 and 206 °C. In another embodiment, one of the phase transitions occurs between 183 and 190 °C. In another embodiment, one of the phase transitions occurs between 185 and 187 °C. In another embodiment, the melting point of Compound 1, Solvate Form A is between 183 °C to 190 °C. In another embodiment, the melting point of Compound 1, Solvate Form A is between 185 °C to 187 °C.

[00176] In another embodiment, Compound 1 Form II comprises 1 to 10 weight percent (wt. %) solvate as determined by TGA. In some embodiments, the TGA of Compound 1 Form II is substantially similar to the TGA trace depicted in Figure 16. In another embodiment, Compound 1 Form II comprises 2 to 5 wt. % solvate as determined by TGA or a similar analytic method known to the skilled artisan.

[00177] In another embodiment, the conformation of Compound 1 Form II acetone solvate is substantially similar to that depicted in Figure 17, which is based on single X-ray analysis.

[00178] In another embodiment, Compound 1 Form II acetone solvate has a $P2_1/n$ space group, and the following unit cell dimensions:

a = 16.5235 (10) Å $\alpha = 90°$

b = 12.7425 (8) Å $\beta = 103.736\ (4)°$

c = 20.5512 (13) Å $\gamma = 90°$.

Compound 1 HCl Salt Form A

[00179] Compound 1 HCl Salt Form A can be prepared from the HCl salt of Compound 1, by dissolving the HCl salt of Compound 1 in a minimum of solvent and removing the solvent by slow evaporation. In another embodiment, the solvent is an alcohol. In another embodiment, the solvent is ethanol. Slow evaporation is generally carried out by impeding the evaporation of the solvent. For example, in one embodiment, slow evaporation involves dissolving the HCl salt of Compound 1 in a vial and covering the vial with parafilm that contains a hole poked in it.

[00180] In one embodiment, Compound 1 HCl Salt Form A is characterized by one or more peaks at 8.80 to 9.20 degrees, 17.30 to 17.70 degrees, and 18.20 to 18.60 degrees in an X-ray powder diffraction obtained using Cu K alpha radiation. In another embodiment, Compound 1 HCl Salt Form A is characterized by one or more peaks at 8.80 to 9.20 degrees, 17.30 to 17.70 degrees, 18.20 to 18.60 degrees, 10.10 to 10.50, and 15.80 to 16.20 degrees in an X-ray powder diffraction obtained using Cu K alpha radiation. In another embodiment, Compound 1 HCl Salt Form A is characterized by one or more peaks at 8.96, 17.51, and 18.45 degrees. In another embodiment, Compound 1 HCl Salt Form A is characterized by one or more peaks at 8.96, 17.51, 18.45. 10.33, and 16.01 degrees. In another embodiment, Compound 1 HCl Salt Form A is characterized by a peak at 8.80 to 9.20 degrees. In another embodiment, Compound 1 HCl Salt Form A is characterized by a peak at 8.96 degrees. In another embodiment, Compound 1 HCl Salt Form A is further characterized by a peak at 17.30 to 17.70 degrees. In another embodiment, Compound 1 HCl Salt Form A is characterized by a peak at 17.51 degrees. In another embodiment, Compound 1 HCl Salt Form A is further characterized by a peak at 18.20 to 18.60 degrees. In another embodiment, Compound 1 HCl Salt Form A is further characterized by a peak at 18.45degrees. In another embodiment, Compound 1 HCl Salt Form A is further characterized by a peak at 10.10 to 10.50 degrees. In another embodiment, Compound 1 HCl Salt Form A is further characterized by a peak at 10.33 degrees. In another embodiment, Compound 1 HCl Salt Form A is further characterized by a peak at 15.80 to 16.20 degrees. In another embodiment, Compound 1 HCl Salt Form A is further characterized by a peak at 16.01

degrees. In another embodiment, Compound 1 HCl Salt Form A is further characterized by a peak at 11.70 to 12.10 degrees. In another embodiment, Compound 1 HCl Salt Form A is further characterized by a peak at 11.94 degrees. In another embodiment, Compound 1 HCl Salt Form A is further characterized by a peak at 7.90 to 8.30 degrees. In another embodiment, Compound 1 HCl Salt Form A is further characterized by a peak at 8.14 degrees. In another embodiment, Compound 1 HCl Salt Form A is further characterized by a peak at 9.90 to 10.30 degrees. In another embodiment, Compound 1 HCl Salt Form A is further characterized by a peak at 10.10 degrees. In another embodiment, Compound 1 HCl Salt Form A is further characterized by a peak at 16.40 to 16.80 degrees. In another embodiment, Compound 1 HCl Salt Form A is further characterized by a peak at 16.55 degrees. In another embodiment, Compound 1 HCl Salt Form A is further characterized by a peak at 9.30 to 9.70 degrees. In another embodiment, Compound 1 HCl Salt Form A is further characterized by a peak at 9.54 degrees. In another embodiment, Compound 1 HCl Salt Form A is further characterized by a peak at 16.40 to 16.80 degrees. In another embodiment, Compound 1 HCl Salt Form A is further characterized by a peak at 16.55 degrees. In some embodiments, Compound 1 HCl Salt Form A is characterized as a dimer as depicted in Figure 18.

[00181] In some embodiments, Compound 1 HCl Salt Form A is characterized by a diffraction pattern substantially similar to that of Figure 19.

[00182] In another embodiment, the invention features crystalline Compound 1 HCl Salt Form A having a $P^{-}1$ space group, and the following unit cell dimensions:

$a = 10.2702\ (2)$ Å $\quad \alpha = 67.0270\ (10)°$

$b = 10.8782\ (2)$ Å $\quad \beta = 66.1810\ (10)°$

$c = 12.4821\ (3)$ Å $\quad \gamma = 72.4760\ (10)°$.

METHODS FOR MAKING THE PHARMACEUTICAL COMPOSITIONS

[00183] The dosage unit forms of the invention can be produced by compacting or compressing an admixture or composition, for example, a powder or granules, under pressure to form a stable three-dimensional shape (e.g., a tablet). As used herein, "tablet" includes compressed pharmaceutical dosage unit forms of all shapes and sizes, whether coated or uncoated.

[00184] The expression "dosage unit form" as used herein refers to a physically discrete unit of agent appropriate for the patient to be treated. In general, a compacted mixture has a density greater than that of the mixture prior to compaction. A dosage unit form of the invention can have almost any shape including concave and/or convex faces, rounded or angled corners, and a rounded to rectilinear shape. In some embodiments, the compressed dosage forms of the invention comprise a rounded tablet having flat faces. The solid pharmaceutical dosage forms of the invention can be prepared by any compaction and compression method known by persons of ordinary skill in the art of forming compressed solid pharmaceutical dosage forms. In particular embodiments, the formulations provided herein may be prepared using conventional methods known to those skilled in the field of pharmaceutical formulation, as described, e.g., in pertinent textbooks. See, e.g., Remington: The Science and Practice of Pharmacy, 21st Ed., Lippincott Williams & Wilkins, Baltimore, Md. (2003); Ansel et al., Pharmaceutical Dosage Forms And Drug Delivery Systems, 7th Edition, Lippincott Williams & Wilkins, (1999); The Handbook of Pharmaceutical Excipients, 4th edition, Rowe et al., Eds., American Pharmaceuticals Association (2003); Gibson, Pharmaceutical Preformulation And Formulation, CRC Press (2001), these references hereby incorporated herein by reference in their entirety.

Granulation and Compression

[00185] In some embodiments, solid forms, including powders comprising the active agent Compound 1 and the included pharmaceutically acceptable excipients (e.g. filler, diluent, disintegrant, surfactant, glidant, binder, lubricant, or any combination thereof) can be subjected to a dry granulation process. The dry granulation process causes the powder to agglomerate into larger particles having a size suitable for further processing. Dry granulation can improve the flowability of a mixture in order to be able to produce tablets that comply with the demand of mass variation or content uniformity.

[00186] Formulations as described herein may be produced using one or more mixing and dry granulations steps. The order and the number of the mixing and granulation steps do not seem to be critical. However, at least one of the excipients and Compound 1 can be been subject to dry granulation or wet high shear granulation before compression into tablets. Dry granulation of Compound 1 and the excipients made together prior to tablet compression seem, surprisingly, to be a simple, inexpensive and efficient way of providing close physical contact between the

ingredients of the present compositions and formulations and thus results in a tablet formulation with good stability properties. Dry granulation can be carried out by a mechanical process, which transfers energy to the mixture without any use of any liquid substances (neither in the form of aqueous solutions, solutions based on organic solutes, or mixtures thereof) in contrast to wet granulation processes, also contemplated herein. Generally, the mechanical process requires compaction such as the one provided by roller compaction. An example of an alternative method for dry granulation is slugging.

[00187] In some embodiments, roller compaction is a granulation process comprising highly intensive mechanical compacting of one or more substances. In some embodiments, a pharmaceutical composition comprising an admixture of powders is pressed, that is roller compacted, between 2 counter rotating rollers to make a solid sheet which is subsequently crushed in a sieve to form a particulate matter. In this particulate matter, a close mechanical contact between the ingredients can be obtained. An example of roller compaction equipment is Minipactor® a Gerteis 3W-Polygran from Gerteis Maschinen+Processengineering AG.

[00188] In some embodiments, tablet compression according to the invention can occur without any use of any liquid substances (neither in the form of aqueous solutions, solutions based on organic solutes, or mixtures thereof), i.e. a dry granulation process. In a typical embodiment the resulting core or tablet has a compressive strength in the range of 1 to 15 kP; such as 1.5 to 12.5 kP, preferably in the range of 2 to 10 kP.

Brief Manufacturing Procedure

[00189] In some embodiments, the ingredients are weighed according to the formula set herein. Next, all of the intragranular ingredients are sifted and mixed well. The ingredients can be lubricated with a suitable lubricant, for example, magnesium stearate. The next step can comprise compaction/slugging of the powder admixture and sized ingredients. Next, the compacted or slugged blends are milled into granules and sifted to obtain the desired size. Next, the granules can be further lubricated with, for example, magnesium stearate. Next the granular composition of the invention can be compressed on suitable punches into various pharmaceutical formulations in accordance with the invention. Optionally the tablets can be coated with a film, colorant or other coating.

[00190] Another aspect of the invention provides a method for producing a pharmaceutical composition comprising providing an admixture of a composition comprising Compound 1 and one or more excipients selected from: a filler, a diluent, a binder, a glidant, a surfactant, a lubricant, a disintegrant, and compressing the composition into a tablet having a dissolution of at least about 50% in about 30 minutes.

[00191] In another embodiment, a wet granulation process is performed to yield the pharmaceutical formulation of the invention from an admixture of powdered and liquid ingredients. For example, a pharmaceutical composition comprising an admixture of a composition comprising Compound 1 and one or more excipients selected from: a filler, a diluent, a binder, a glidant, a surfactant, a lubricant, a disintegrant, are weighed as per the formula set herein. Next, all of the intragranular ingredients are sifted and mixed in a high shear or low shear granulator or a twin screw granulator using water or water with a surfactant or water with a binder or water with a surfactant and a binder to granulate the powder blend. A fluid other than water can also be used with or without surfactant and/or binder to granulate the powder blend. Next, the wet granules can optionally be milled using a suitable mill. Next, water may optionally be removed from the admixture by drying the ingredients in any suitable manner. Next, the dried granules can optionally be milled to the required size. Next, extra granular excipients can be added by blending (for example a filler, a diluent, and a disintegrant). Next, the sized granules can be further lubricated with magnesium stearate and a disintegrant, for example, croscarmellose sodium. Next the granular composition of the invention can be compressed on suitable punches into various pharmaceutical formulations in accordance with the invention. Optionally, the tablets can be coated with a film, colorant or other coating.

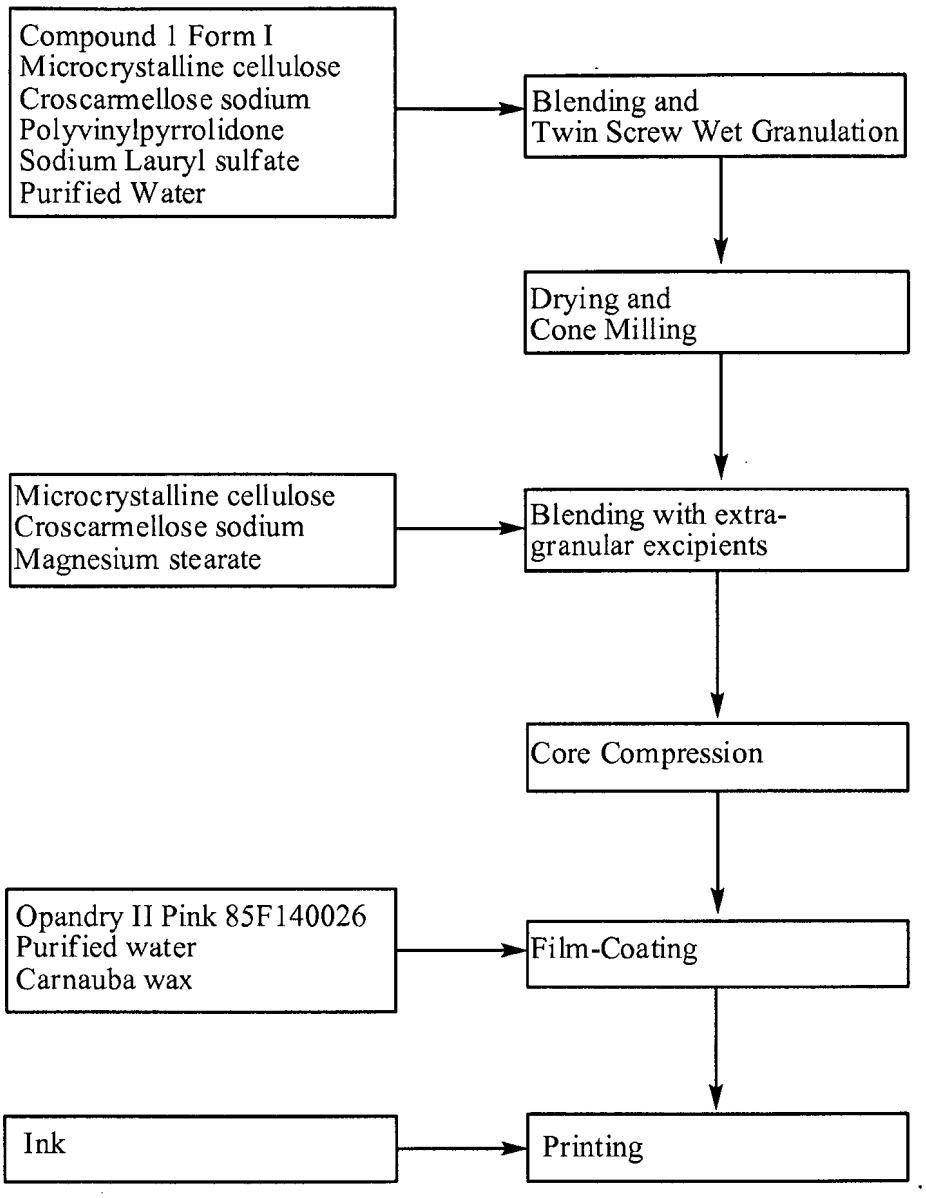
[00192] In a particularly favored embodiment, the pharmaceutical compositions of the present invention are prepared by a continuous twin screw wet granulation (TSWG) process. Continuous manufacturing delivers high quality and highly consistent product with on-line monitoring and control. Continuous manufacturing also facilitates quality by design development with a "data rich" design space and an easier to understand impact of upstream variables on the downstream process and final product quality. In addition, the pharmaceutical compositions of the present invention can be finalized early on commercial scale equipment which avoids scale-up risks and formulation changes late in development. Finally, continuous manufacturing has commercial manufacturing advantages such as improved process control,

reduced product handling, and real time release efficiencies. The overall result is a more robust, controllable, and scalable process that has fewer process checks resulting in increased product quality and therefore greater patient safety.

[00193] For example, high shear granulation (HSG), a common granulation technique is well known for the risk of over-granulation and poor process control. Scale-up of this process is very challenging and involves significant risk. Changing from a HSG process to a continuous TSWG process, allows scale-up using the same equipment to produce different batch sizes, by running for a longer time. This eliminates the scale-up risk commonly encountered with wet granulation processes. Additionally, it was found that the TSWG process is more robust, being less sensitive to over-granulation. As can be seen in Figure 28 for a Compound 1 tablet, the HSG process showed significant dissolution slow-down with increasing water content, while the TSWG process did not show a change for a similar range of water addition. Surprisingly, no performance changes were found with the tablet formulations comprising Compound 1 between 45-55 percent by weight and the tablet formulations comprising Compound 1 between 60-70 percent by weight using the twin screw wet granulation process. This was not the case with the HSG process. Additionally, this continuous and increased product quality process addresses a common complaint by the FDA regarding the lack of drug availability for patients in need thereof.

[00194] In one embodiment the continuous process starts with feeding individual excipients and Compound 1 into a continuous in-line blender through loss-in-weight feeding. From this blender, the material is continuously conveyed and processed through twin screw wet granulation, drying, milling, extra-granular excipient addition, blending, compression and film coating.

[00195] For example, in one embodiment, a tablet comprising Compound 1 may be prepared continuously according to the below flow chart.



[00196] Each of the ingredients of this exemplary admixture is described above and in the Examples below. Furthermore, the admixture can comprise optional additives, such as, one or more colorants, one or more flavors, and/or one or more fragrances as described above and in the Examples below. In some embodiments, the relative concentrations (e.g., wt%) of each of these ingredients (and any optional additives) in the admixture are also presented above and in the Examples below. The ingredients constituting the admixture can be provided sequentially or in any combination of additions; and, the ingredients or combination of ingredients can be provided in any order. In one embodiment, the lubricant is the last component added to the admixture.

[00197] In another embodiment, the admixture comprises a composition of Compound 1, and any one or more of the excipients; a binder, a glidant, a surfactant, a diluent, a lubricant, a disintegrant, and a filler, wherein each of these ingredients is provided in a powder form (e.g., provided as particles having a mean or average diameter, measured by light scattering, of 250 μm or less (e.g., 150 μm or less, 100 μm or less, 50 μm or less, 45 μm or less, 40 μm or less, or 35 μm or less)). For instance, the admixture comprises a composition of Compound 1, a diluent, a glidant, a surfactant, a lubricant, a disintegrant, and a filler, wherein each of these ingredients is provided in a powder form (e.g., provided as particles having a mean diameter, measured by light scattering, of 250 μm or less (e.g., 150 μm or less, 100 μm or less, 50 μm or less, 45 μm or less, 40 μm or less, or 35 μm or less)). In another example, the admixture comprises a composition of Compound 1, a diluent, a binder, a surfactant, a lubricant, a disintegrant, and a filler, wherein each of these ingredients is provided in a powder form (e.g., provided as particles having a mean diameter, measured by light scattering, of 250 μm or less (e.g., 150 μm or less, 100 μm or less, 50 μm or less, 45 μm or less, 40 μm or less, or 35 μm or less))

[00198] In another embodiment, the admixture comprises a composition of Compound 1, and any combination of: a binder, a glidant, a diluent, a surfactant, a lubricant, a disintegrant, and a filler, wherein each of these ingredients is substantially free of water. Each of the ingredients comprises less than 5 wt% (e.g., less than 2 wt%, less than 1 wt%, less than 0.75 wt%, less than 0.5 wt%, or less than 0.25 wt%) of water by weight of the ingredient. For instance, the admixture comprises a composition of Compound 1, a diluent, a glidant, a surfactant, a lubricant, a disintegrant, and a filler, wherein each of these ingredients is substantially free of water. In some embodiments, each of the ingredients comprises less than 5 wt% (e.g., less than 2 wt%, less than 1 wt%, less than 0.75 wt%, less than 0.5 wt%, or less than 0.25 wt%) of water by weight of the ingredient.

[00199] In another embodiment, compressing the admixture into a tablet is accomplished by filling a form (e.g., a mold) with the admixture and applying pressure to admixture. This can be accomplished using a die press or other similar apparatus. In some embodiments, the admixture of Compound 1 and excipients can be first processed into granular form. The granules can then be sized and compressed into tablets or formulated for encapsulation according to known methods in the pharmaceutical art. It is also noted that the application of pressure to the admixture in the form can be repeated using the same pressure during each compression or using

different pressures during the compressions. In another example, the admixture of powdered ingredients or granules can be compressed using a die press that applies sufficient pressure to form a tablet having a dissolution of about 50% or more at about 30 minutes (e.g., about 55% or more at about 30 minutes or about 60% or more at about 30 minutes). For instance, the admixture is compressed using a die press to produce a tablet hardness of at least about 5 kP (at least about 5.5 kP, at least about 6 kP, at least about 7 kP, at least about 10 kP, or at least 15 kP). In some instances, the admixture is compressed to produce a tablet hardness of between about 5 and 20 kP.

[00200] In some embodiments, tablets comprising a pharmaceutical composition as described herein can be coated with about 3.0 wt% of a film coating comprising a colorant by weight of the tablet. In certain instances, the colorant suspension or solution used to coat the tablets comprises about 20%w/w of solids by weight of the colorant suspension or solution. In still further instances, the coated tablets can be labeled with a logo, other image or text.

[00201] In another embodiment, the method for producing a pharmaceutical composition comprises providing an admixture of a solid forms, e.g. an admixture of powdered and/or liquid ingredients, the admixture comprising Compound 1 and one or more excipients selected from: a binder, a glidant, a diluent, a surfactant, a lubricant, a disintegrant, and a filler; mixing the admixture until the admixture is substantially homogenous, and compressing or compacting the admixture into a granular form. Then the granular composition comprising Compound 1 can be compressed into tablets or formulated into capsules as described above or in the Examples below. Alternatively, methods for producing a pharmaceutical composition comprises providing an admixture of Compound 1, and one or more excipients, e.g. a binder, a glidant, a diluent, a surfactant, a lubricant, a disintegrant, and a filler; mixing the admixture until the admixture is substantially homogenous, and compressing/compacting the admixture into a granular form using a roller compactor using a dry granulation composition as set forth in the Examples below or alternatively, compressed/compacted into granules using a high shear wet granule compaction process as set forth in the Examples below. Pharmaceutical formulations, for example a tablet as described herein, can be made using the granules prepared incorporating Compound 1 in addition to the selected excipients described herein.

[00202] In some embodiments, the admixture is mixed by stirring, blending, shaking, or the like using hand mixing, a mixer, a blender, any combination thereof, or the like. When ingredients or combinations of ingredients are added sequentially, mixing can occur between successive additions, continuously throughout the ingredient addition, after the addition of all of the ingredients or combinations of ingredients, or any combination thereof. The admixture is mixed until it has a substantially homogenous composition.

[00203] In another embodiment, the present invention comprises jet milling Compound 1, Compound 1 Form I, Compound 1 Form II, Compound 1 HCl Salt Form A in a suitable, conventional milling apparatus using air pressure suitable to produce particles having a significant particle size fraction between 0.1 microns and 50 microns. In another embodiment, the particle size is between 0.1 microns and 20 microns. In another embodiment, the particles size is between 0.1 microns and 10 microns. In another embodiment, the particle size is between 1.0 microns and 5 microns. In still another embodiment, Compound 1, Compound 1 Form I, Compound 1 Form II, Compound 1 HCl Salt Form A has a particle size D50 of 2.0 microns.

[00204] In various embodiments, a second therapeutic agent can be formulated together with Compound 1 to form a unitary or single dose form, for example, a tablet or capsule.

[00205] Dosage forms prepared as above can be subjected to in vitro dissolution evaluations according to Test 711 "Dissolution" in United States Pharmacopoeia 29, United States Pharmacopeial Convention, Inc., Rockville, Md., 2005 ("USP"), to determine the rate at which the active substance is released from the dosage forms. The content of active substance and the impurity levels are conveniently measured by techniques such as high performance liquid chromatography (HPLC).

[00206] In some embodiments, the invention includes use of packaging materials such as containers and closures of high-density polyethylene (HDPE), low-density polyethylene (LDPE) and or polypropylene and/or glass, glassine foil, aluminum pouches, and blisters or strips composed of aluminum or high-density polyvinyl chloride (PVC), optionally including a desiccant, polyethylene (PE), polyvinylidene dichloride (PVDC), PVC/PE/PVDC, and the like. These package materials can be used to store the various pharmaceutical compositions and formulations in a sterile fashion after appropriate sterilization of the package and its contents

using chemical or physical sterilization techniques commonly employed in the pharmaceutical arts.

METHODS FOR ADMINISTERING THE PHARMACEUTICAL COMPOSITIONS

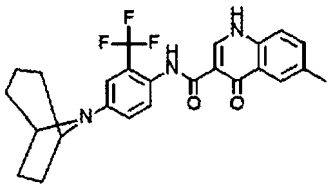
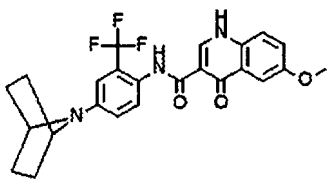
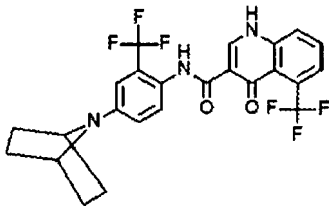
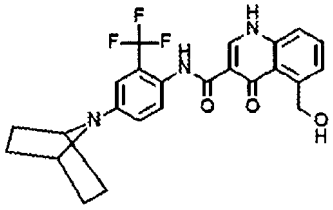
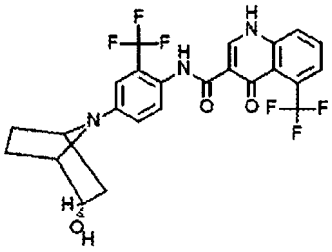
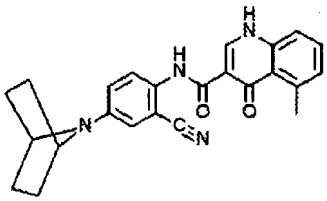
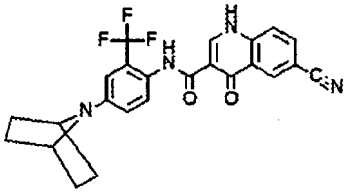
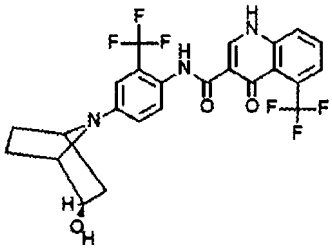
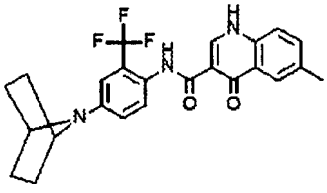
[00207] In one aspect, the pharmaceutical compositions of the invention can be administered to a patient once daily or about every twenty four hours. Alternatively, the pharmaceutical compositions of the invention can be administered to a patient twice daily or about every twelve hours. These pharmaceutical compositions are administered as oral formulations containing about 25 mg, 50 mg, 100 mg, 125 mg, 150 mg, 200 mg, 250 mg, or 400 mg of Compound 1. In this aspect, in addition to Compound 1, the pharmaceutical compositions comprise a filler; a diluent; a disintegrant; a surfactant; at least one of a binder and a glidant; and a lubricant. For instance, a dose of 400 mg of Compound 1, may comprise two tablets of the invention each containing 200 mg of Compound 1, or four tablets of the invention each containing 100 mg of Compound 1.

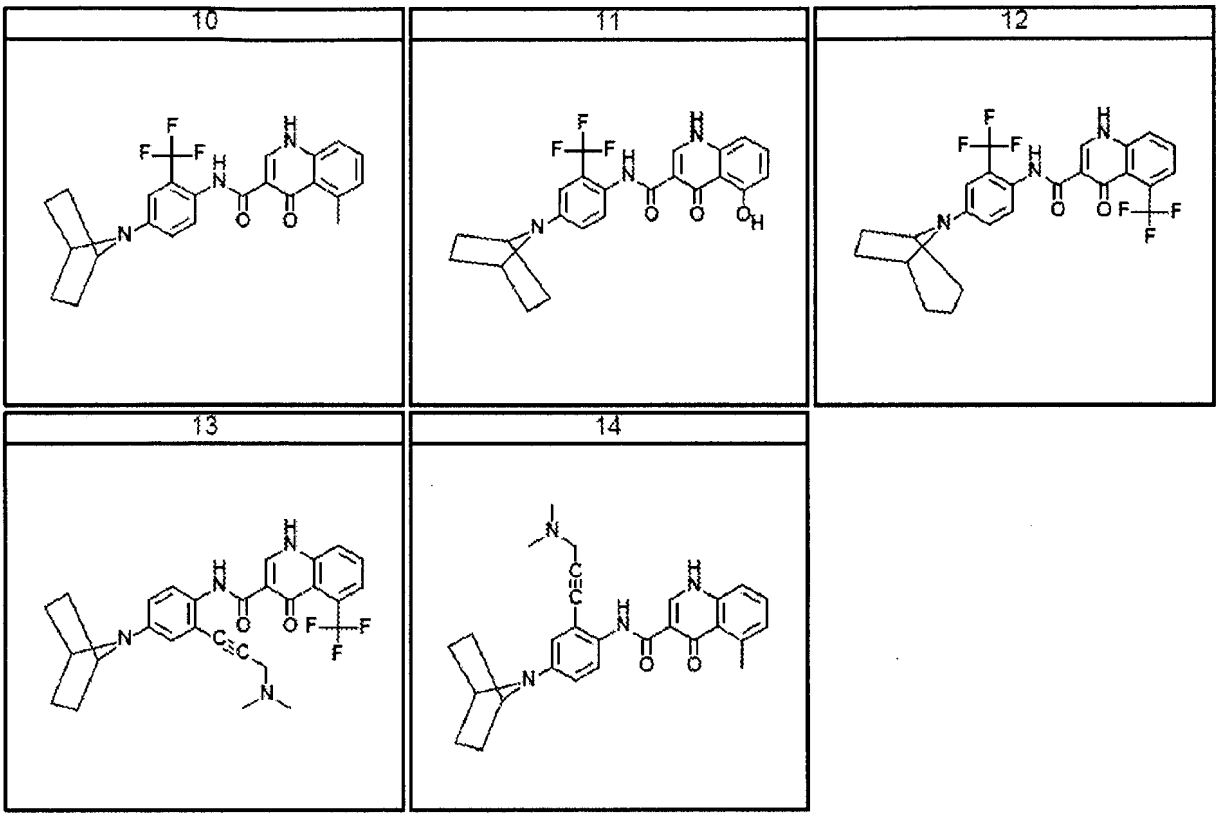
[00208] It will also be appreciated that the compound and pharmaceutically acceptable compositions and formulations of the invention can be employed in combination therapies; that is, Compound 1 and pharmaceutically acceptable compositions thereof can be administered concurrently with, prior to, or subsequent 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, for example, a CFTR mediated disease, or condition, are known as "appropriate for the disease or condition being treated."

[00209] In one embodiment, the additional therapeutic agent is selected from a mucolytic agent, bronchodialator, an antibiotic, an anti-infective agent, an anti-inflammatory agent, a CFTR modulator other than Compound 1 of the invention, or a nutritional agent.

[00210] In one embodiment, the additional agent is (*R*)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(1-(2,3-dihydroxypropyl)-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide. In another embodiment, the additional agent is N-(5-hydroxy-2,4-ditert-butyl-phenyl)-4-oxo-1H-quinoline-3-carboxamide. In another embodiment, the additional agent is selected from Table 1:

Table 1.

1	2	3
		
4	5	6
		
7	8	9
		



[00211] In another embodiment, the additional agent is any combination of the above agents. For example, the composition may comprise Compound 1, (*R*)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(1-(2,3-dihydroxypropyl)-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide, and N-(5-hydroxy-2,4-ditert-butyl-phenyl)-4-oxo-1H-quinoline-3-carboxamide. In another example, the composition may comprise Compound 1, N-(5-hydroxy-2,4-ditert-butyl-phenyl)-4-oxo-1H-quinoline-3-carboxamide, and any one of the compounds from Table 1, i.e. compounds 1 through 14 of Table 1, or any combination thereof.

[00212] In one embodiment, the additional therapeutic 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, levoflaxacin, including aerosolized formulations thereof, and combinations of two antibiotics, e.g., fosfomycin and tobramycin.

[00213] In another embodiment, the additional agent is a mucolyte. Exemplary mucolytes useful herein includes Pulmozyme®.

[00214] In another embodiment, the additional agent is a bronchodialator. Exemplary bronchodialtors include albuterol, metaprotenerol sulfate, pirbuterol acetate, salmeterol, or tetrabuline sulfate.

[00215] 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-dioxopyrimidin-1-yl)-3, 4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]oxy-hydroxyphosphoryl] hydrogen phosphate), or bronchitol (inhaled formulation of mannitol).

[00216] 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 simavastatin.

[00217] 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), and cobiprostone (7-{(2R, 4aR, 5R, 7aR)-2-[(3S)-1,1-difluoro-3-methylpentyl]-2-hydroxy-6-oxooctahydrocyclopenta[b]pyran-5-yl}heptanoic acid).

[00218] In another embodiment, the additional agent is a nutritional agent. Exemplary nutritional agents include pancrelipase (pancreating enzyme replacement), including Pancrease®, Pancreacarb®, Ultrase®, or Creon®, Liprotomase® (formerly Trizytek®), Aquadeks®, or glutathione inhalation. In one embodiment, the additional nutritional agent is pancrelipase.

[00219] In another embodiment, the additional agent is a compound selected from gentamicin, curcumin, cyclophosphamide, 4-phenylbutyrate, miglustat, felodipine, nimodipine, Philoxin B,

geniestein, Apigenin, cAMP/cGMP modulators such as rolipram, sildenafil, milrinone, tadalafil, amrinone, isoproterenol, albuterol, and almeterol, deoxyspergualin, HSP 90 inhibitors, HSP 70 inhibitors, proteosome inhibitors such as epoxomicin, lactacystin, etc.

[00220] In another embodiment, the additional agent is a compound selected from 3-amino-6-(4-fluoro-phenyl)-5-trifluoromethyl-pyridine-2-carboxylic acid (3,3,3-trifluoro-2-hydroxy -2-methyl-propyl)-amide; 5-amino-6′-methyl-3-trifluoromethyl-[2,3]bipyridinyl-6-carboxylic acid (3,3,3-trifluoro-2-hydroxy -2-methyl-propyl)-amide; 3-amino-6-cyclopropyl-N-(3,3,3-trifluoro-2-hydroxy-2-methylpropyl)-5-(trifluoromethyl)picolinamide; 3-amino-6-methoxy-N-(3,3,3-trifluoro-2-hydroxy-2-(trifluoromethyl)propyl)-5-(trifluoro methyl)picolinamide; 3-amino-6-(4-fluoro-phenyl)-5-trifluoromethyl-pyridine-2-carboxylic acid ((S)-3,3,3-trifluoro-2-hydroxy -2-methyl-propyl)-amide; 3-amino-6-methoxy-5-trifluoromethyl-pyridine-2-carboxylic acid((S-3,3,3-trifluoro-2-hydroxy-2-methyl-propyl)-amide; 3-amino-6-methoxy-5-trifluoromethyl-pyridine-2-carboxylic acid ((R)-3,3,3-trifluoro-2-hydroxy-2-methyl-propyl)-amide; 3-amino-6-(2,4-dichloro-phenyl)-5-trifluoromethyl-pyridine-2-carboxylic acid ((S)-3,3,3-trifluoro-2-hydroxy-2-methyl-propyl)-amide; 3-amino-6-(2,4-dichloro-phenyl)-5-trifluoromethyl-pyridine-2-carboxylic acid ((R)-3,3,3-trifluoro -2-hydroxy-2-methyl-propyl)-amide; 3-amino-6-(4-fluoro-phenyl)-5-trifluoromethyl-pyridine-2-carboxylic acid (2-hydroxy-2-methyl-propyl)-amide; 3-amino-5,6-bis-trifluoromethyl-pyridine-2-carboxylic acid ((S)-3,3,3-trifluoro-2-hydroxy-2-methyl-propyl)-amide; 3-amino-5,6-bis-trifluoromethyl-pyridine-2-carboxylic acid ((R)-3,3,3-trifluoro-2-hydroxy-2-methyl-propyl)-amide; (S)-3-amino-6-ethoxy-N-(3,3,3-trifluoro-2-hydroxy-2-methylpropyl)-5-(trifluoro methyl)picolinamide; 3-amino-6-methoxy-5-trifluoromethyl-pyridine-2-carboxylic acid ((S)-3,3,3-trifluoro -2-hydroxy-2-methyl-propyl)-amide; 3-amino-6-methoxy-5-trifluoromethyl-pyridine-2-carboxylic acid ((R)-3,3,3-trifluoro -2-hydroxy-2-methyl-propyl)-amide; 3-amino-6-(4-fluoro-phenyl)-5-trifluoromethyl-pyridine-2-carboxylic acid (3,3,3-trifluoro-2-hydroxy-2-methyl-propyl)-amide; 3-amino-5,6-bis-trifluoromethyl-pyridine-2-carboxylic acid ((S)-3,3,3-trifluoro -2-hydroxy-2-methyl-propyl)-amide; 3-amino-5,6-bis-trifluoromethyl-pyridine-2-carboxylic acid ((R)-3,3,3-trifluoro -2-hydroxy-2-methyl-propyl)-amide, or pharmaceutically acceptable salts thereof. In another embodiment, the additional agent is a compound disclosed in United States Patent No. 8,247,436 and International PCT Publication WO 2011113894, each incorporated herein in their entirety by reference.

[00221] In one embodiment, the additional agent is trimethylangelicin. In another embodiment, the additional agent is a compound disclosed in WO 2012171954, incorporated herein in its entirety by reference.

[00222] In other embodiments, the additional agent is a compound disclosed in WO 2004028480, WO 2004110352, WO 2005094374, WO 2005120497, or WO 2006101740. In another embodiment, the additional agent is a benzo[c]quinolizinium derivative that exhibits CFTR modulation activity or a benzopyran derivative that exhibits CFTR modulation activity. In another embodiment, the additional agent is a compound disclosed in U.S. Pat. No. 7,202,262, U.S. Pat. No. 6,992,096, US20060148864, US20060148863, US20060035943, US20050164973, WO2006110483, WO2006044456, WO2006044682, WO2006044505, WO2006044503, WO2006044502, or WO2004091502. In another embodiment, the additional agent is a compound disclosed in WO2004080972, WO2004111014, WO2005035514, WO2005049018, WO2006099256, WO2006127588, or WO2007044560. In another embodiment, the additional agent is N-(5-hydroxy-2,4-ditert-butyl-phenyl)-4-oxo-1H-quinoline-3-carboxamide.

[00223] In one embodiment, 600 mg of Compound 1 may be administered to a subject in need thereof followed by co-administration of 250 mg of N-(5-hydroxy-2,4-ditert-butyl-phenyl)-4-oxo-1H-quinoline-3-carboxamide (Compound 2). In these embodiments, the dosage amounts may be achieved by administration of one or more tablets of the invention. For example, administration of 600 mg of Compound 1 may be achieved by administering three tablets each containing 200 mg of Compound 1, four tablets each containing 150 mg of Compound 1, or one table of 400 mg Compound 1 and one tablet of 200 mg Compound 1. Compound 2 may be administered as a pharmaceutical composition comprising Compound 2 and a pharmaceutically acceptable carrier. The duration of administration may continue until amelioration of the disease is achieved or until a subject's physician advises, e.g. duration of administration may be less than a week, 1 week, 2 weeks, 3 weeks, or a month or longer. The co-administration period may be preceded by an administration period of just Compound 1 alone. For example, there could be administration of 600 mg of Compound 1 for 2 weeks followed by co-administration of 250 mg of Compound 2 for 1 additional week. In another embodiment, 600 mg of Compound 1 may be administered bid (twice daily) for 28 days followed by 250 mg of Compound 2 administered bid (twice daily) for 28 days. In another embodiment, 600 mg of Compound 1 may be administered qd (once a day) for 28 days followed by 250 mg of Compound 2 administered qd (once a day)

for 28 days. In another embodiment, 600 mg of Compound 1 may be administered qd (once a day) for 28 days followed by co-administration of 600 mg of Compound 1 qd (once a day) and 250 mg of Compound 2 q12h (once every 12 hours) for 28 days. In another embodiment, 600 mg of Compound 1 may be administered qd (once a day) and 250 mg of Compound 2 administered qd (once a day).

[00224] In one embodiment, 600 mg of Compound 1 may be administered to a subject in need thereof followed by co-administration of 450 mg of N-(5-hydroxy-2,4-ditert-butyl-phenyl)-4-oxo-1H-quinoline-3-carboxamide (Compound 2). In these embodiments, the dosage amounts may be achieved by administration of one or more tablets of the invention. For example, administration of 600 mg of Compound 1 may be achieved by administering three tablets each containing 200 mg of Compound 1, or four tablets each containing 150 mg of Compound 1. Compound 2 may be administered as a pharmaceutical composition comprising Compound 2 and a pharmaceutically acceptable carrier. The duration of administration may continue until amelioration of the disease is achieved or until a subject's physician advises, e.g. duration of administration may be less than a week, 1 week, 2 weeks, 3 weeks, or a month or longer. The co-administration period may be preceded by an administration period of just Compound 1 alone. For example, there could be administration of 600 mg of Compound 1 for 2 weeks followed by co-administration of 450 mg of Compound 2 for 1 additional week. In another embodiment, 600 mg of Compound 1 may be administered bid (twice daily) for 28 days followed by 450 mg of Compound 2 administered bid (twice daily) for 28 days.

[00225] In one embodiment, 400 mg of Compound 1 may be administered to a subject in need thereof followed by co-administration of 350 mg of N-(5-hydroxy-2,4-ditert-butyl-phenyl)-4-oxo-1H-quinoline-3-carboxamide (Compound 2). In these embodiments, the dosage amounts may be achieved by administration of one or more tablets of the invention. For example, administration of 400 mg of Compound 1 may be achieved by administering two tablets each containing 200 mg of Compound 1, or four tablets each containing 100 mg of Compound 1. Compound 2 may be administered as a pharmaceutical composition comprising Compound 2 and a pharmaceutically acceptable carrier. The duration of administration may continue until amelioration of the disease is achieved or until a subject's physician advises, e.g. duration of administration may be less than a week, 1 week, 2 weeks, 3 weeks, or a month or longer. The co-administration period may be preceded by an administration period of just Compound 1

alone. For example, there could be administration of 400 mg of Compound 1 for 2 weeks followed by co-administration of 350 mg of Compound 2 for 1 additional week. In another embodiment, 400 mg of Compound 1 may be administered q8h (every 8 hours) for 28 days followed by 350 mg of Compound 2 administered q8h (every 8 hours) for 28 days.

[00226] In one embodiment, 400 mg of Compound 1 may be administered to a subject in need thereof followed by co-administration of 250 mg of N-(5-hydroxy-2,4-ditert-butyl-phenyl)-4-oxo-1H-quinoline-3-carboxamide (Compound 2). In these embodiments, the dosage amounts may be achieved by administration of one or more tablets of the invention. For example, administration of 400 mg of Compound 1 may be achieved by administering two tablets each containing 200 mg of Compound 1, or four tablets each containing 100 mg of Compound 1. Compound 2 may be administered as a pharmaceutical composition comprising Compound 2 and a pharmaceutically acceptable carrier. The duration of administration may continue until amelioration of the disease is achieved or until a subject's physician advises, e.g. duration of administration may be less than a week, 1 week, 2 weeks, 3 weeks, or a month or longer. The co-administration period may be preceded by an administration period of just Compound 1 alone. For example, there could be administration of 400 mg of Compound 1 for 2 weeks followed by co-administration of 150 mg or 250 mg of Compound 2 for 1 additional week. In another embodiment, 400 mg of Compound 1 may be administered bid (twice daily) for 28 days followed by 250 mg of Compound 2 administered bid (twice daily) for 28 days. In another embodiment, 400 mg of Compound 1 may be administered bid (twice daily) for 28 days followed by 250 mg of Compound 2 administered qd (once daily) for 28 days. In another embodiment, 400 mg of Compound 1 may be administered qd (once a day) for 28 days followed by co-administration of 400 mg of Compound 1 qd (once a day) and 250 mg of Compound 2 q12h (once every 12 hours) for 28 days. In another embodiment, 400 mg of Compound 1 may be administered bid (twice daily) and 250 mg of Compound 2 administered qd (once daily).

[00227] In one embodiment, 400 mg of Compound 1 may be administered once a day to a subject in need thereof followed by co-administration of 150 mg of Compound 2 once a day. In these embodiments, the dosage amounts may be achieved by administration of one or more tablets of the invention. For example, administration of 400 mg of Compound 1 may be achieved by administering two tablets each containing 200 mg of Compound 1, or four tablets each containing 100 mg of Compound 1. Compound 2 may be administered as a pharmaceutical

composition comprising Compound 2 and a pharmaceutically acceptable carrier. The duration of administration may continue until amelioration of the disease is achieved or until a subject's physician advises, e.g. duration of administration may be less than a week, 1 week, 2 weeks, 3 weeks, or a month or longer. The co-administration period may be preceded by an administration period of just Compound 1 alone. For example, there could be administration of 400 mg of Compound 1 for 2 weeks followed by co-administration of 150 mg or 250 mg of Compound 2 for 1 additional week.

[00228] In one embodiment, 400 mg of Compound 1 may be administered once a day to a subject in need thereof followed by co-administration of 150 mg of Compound 2 every 12 hours. In another embodiment, 400 mg of Compound 1 may be administered once a day to a subject in need thereof followed by co-administration of 250 mg of Compound 2 every 12 hours. In these embodiments, the dosage amounts may be achieved by administration of one or more tablets of the invention. For example, administration of 400 mg of Compound 1 may be achieved by administering two tablets each containing 200 mg of Compound 1, or four tablets each containing 100 mg of Compound 1. Compound 2 may be administered as a pharmaceutical composition comprising Compound 2 and a pharmaceutically acceptable carrier. The duration of administration may continue until amelioration of the disease is achieved or until a subject's physician advises, e.g. duration of administration may be less than a week, 1 week, 2 weeks, 3 weeks, or a month or longer. The co-administration period may be preceded by an administration period of just Compound 1 alone. For example, there could be administration of 400 mg of Compound 1 for 2 weeks followed by co-administration of 150 mg or 250 mg of Compound 2 for 1 additional week.

[00229] In another embodiment, 200 mg of Compound 1 may be administered qd (once a day) for 28 days followed by co-administration of 200 mg of Compound 1 qd (once a day) and 250 mg of Compound 2 q12h (once every 12 hours) for 28 days.

[00230] In one embodiment, the 100 mg, 200 mg, and 300 mg of Compound 1 tablets may be combined to form a number of different dosage amounts. For example, dosage amounts of 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 700 mg, 800 mg, 900 mg, 1000 mg, 1100 mg, or 1200 mg of Compound 1 may be administered by using the 100 mg, 200 mg, and 300 mg tablet formulations and multiples thereof. For example, a dosage amount of 900 mg of Compound 1

may be administered using 3 300 mg tablets of Compound 1. A dosage amount of 600 mg of Compound 1 may be administered using 3 200 mg tablets of Compound 1 or 2 300 mg tablets of Compound 1. Any of the preceding dosage amounts of this paragraph my be administered with the amounts of Compound 2 and/or dosage schedules of the preceding 3 paragraphs.

[00231] These combinations are useful for treating the diseases described herein including cystic fibrosis. These combinations are also useful in the kits described herein.

[00232] 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.

[00233] In another aspect, the invention features a kit comprising a tablet of the present invention, and a separate therapeutic agent or pharmaceutical composition thereof. In another embodiment, the Compound 1 in the tablet is in Form I. In another embodiment, the therapeutic agent is a cystic fibrosis corrector other than Compound 1. In another embodiment, the therapeutic agent is a cystic fibrosis potentiator. In another embodiment, the therapeutic agent is N-(5-hydroxy-2,4-ditert-butyl-phenyl)-4-oxo-1H-quinoline-3-carboxamide. In another embodiment, the tablet and the therapeutic agent are in separate containers. In another embodiment, the separate containers are bottles. In another embodiment, the separate containers are vials. In another embodiment, the separate containers are blister packs.

THERAPEUTIC USES OF THE COMPOSITION

[00234] In one aspect, the invention also provides a method of treating, lessening the severity of, or symptomatically treating a disease in a patient, the method comprising administering an effective amount of the pharmaceutical composition of the invention to the patient, wherein the 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, myleoperoxidase 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 plasy, Pick's disease, several polyglutamine neurological disorders such as Huntington's, spinocerebullar ataxia type I, spinal and bulbar muscular atrophy, dentatorubal pallidoluysian, 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, 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.

[00235] Compound 1, as part of a combination with ivacaftor (N-(5-hydroxy-2,4-ditert-butyl-phenyl)-4-oxo-1H-quinoline-3-carboxamide), has been granted a Breakthrough Therapy Designation from the Food and Drug Administration (FDA) for the treatment of cystic fibrosis, one of only two such grants at the time of the filing of this application (the other being for ivacaftor). This demonstrates a significant unmet need for the effective treatment of the cause of cystic fibrosis over symptomatic treatments. Additionally, a common challenge for drugs approved by the FDA is the occasional lack of drug availability for patients in need thereof. Accordingly, a significant unmet need exists for the presently disclosed Compound 1 formulations and processes for preparing them in a continuous and controlled manner.

[00236] In one aspect, the invention also provides a method of treating, lessening the severity of, or symptomatically treating a disease in a patient comprising administering an effective amount of the pharmaceutical composition of the invention to the patient, wherein the disease is selected from generalized epilepsy with ferbrile seizures plus (GEFS+), general epilepsy with ferbile and aferbrile seizures, myotonia, paramyotonia congenital, potassium-aggravated myotonia, hyperkalemic periodic paralysis, LQTS, LQTS/Brugada syndrome, autosomal-dominant LQTS with deafness, autosomal-recessive LQTS, LQTS with dysmorphic features, congenital and acquired LQTS, Timothy syndrome, persistent hyperinsulinemic hypolglycemia of infancy, dilated cardiomyopathy, autosomal-dominant LQTS, Dent disease, Osteopetrosis, Bartter syndrome type III, central core disease, malignant hyperthermia, and catecholaminergic polymorphic tachycardia.

[00237] In one aspect, the present invention is directed to a method of treating, lessening the severity of, or symptomatically treating cystic fibrosis in a patient comprising administering an effective amount of the pharmaceutical composition of the invention to the patient, wherein the patient possesses the CFTR genetic mutation *N1303K*, *ΔI507*, or *R560T*.

[00238] In one aspect, the present invention is directed to a method of treating, lessening the severity of, or symptomatically treating cystic fibrosis in a patient comprising administering an effective amount of the pharmaceutical composition of the invention to the patient, wherein the patient possesses the CFTR genetic mutation *G551D*. In another embodiment, the patient is homozygous for *G551D*. In another embodiment, the patient is heterozygous for *G551D* wherein the other CFTR genetic mutation is any one of *F508del*, *G542X*, *N1303K*, *W1282X*, *R117H*, *R553X*, *1717-1G->A*, *621+1G->T*, *2789+5G->A*, *3849+10kbC->T*, *R1162X*, *G85E*, *3120+1G->A*, *ΔI507*, *1898+1G->A*, *3659delC*, *R347P*, *R560T*, *R334W*, *A455E*, *2184delA*, or *711+1G->T*.

[00239] In one aspect, the present invention is directed to a method of treating, lessening the severity of, or symptomatically treating cystic fibrosis in a patient comprising administering an effective amount of the pharmaceutical composition of the invention to the patient, wherein the patient possesses the CFTR genetic mutation *F508del*. In another embodiment, the patient is homozygous for *F508del*. In another embodiment, the patient is heterozygous for *F508del* wherein the other CFTR genetic mutation is any one of *G551D*, *G542X*, *N1303K*, *W1282X*,

R117H, R553X, 1717-1G->A, 621+1G->T, 2789+5G->A, 3849+10kbC->T, R1162X, G85E, 3120+1G->A, ΔI507, 1898+1G->A, 3659delC, R347P, R560T, R334W, A455E, 2184delA, or *711+1G->T*.

[00240] In certain embodiments, the pharmaceutically acceptable compositions of the present invention comprising Compound 1 are useful for treating, lessening the severity of, or symptomatically treating 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, *F508del*, as well as other mutations such as the *G551D* mutation, or the *R117H* mutation. In certain embodiments, the pharmaceutical compositions comprising Compound 1 are useful for treating, lessening the severity of, or symptomatically treating cystic fibrosis in patients who exhibit little to no residual CFTR activity. In certain embodiments, the pharmaceutical compositions comprising Compound 1 are useful for treating, lessening the severity of, or symptomatically treating cystic fibrosis in patients who exhibit little to no residual CFTR activity in the apical membrane of respiratory epithelia.

[00241] 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. Such a residual CFTR inducer or augmenter can be done using pharmacological methods. 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 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.

[00242] In one embodiment, pharmaceutical compositions of the present invention comprising Compound 1, as described herein, are useful for treating or lessening the severity of cystic fibrosis in patients within certain genotypes exhibiting residual CFTR activity, e.g., Class I mutations (not synthesized), class II mutation (misfolding), class III mutations (impaired regulation or gating), class IV mutations (altered conductance), or class V mutations (reduced synthesis).

[00243] In one embodiment, pharmaceutical compositions of the present invention comprising Compound 1, as described herein, are useful for treating, lessening the severity of, or symptomatically treating 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 sufficiency.

[00244] In one embodiment, pharmaceutical compositions of the present invention comprising Compound 1, as described herein, are useful for treating, lessening the severity of, or symptomatically treating patients diagnosed with pancreatic sufficiency, idiopathic pancreatitis and congenital bilateral absence of the vas deferens, or mild lung disease wherein the patient exhibits residual CFTR activity.

[00245] In one embodiment, pharmaceutical compositions of the present invention comprising Compound 1, as described herein, are useful for treating, lessening the severity of, or symptomatically treating patients diagnosed with pancreatic sufficiency, idiopathic pancreatitis and congenital bilateral absence of the vas deferens, or mild lung disease wherein the patient has wild type CFTR.

[00246] In addition to cystic fibrosis, modulation of CFTR activity may be beneficial for other diseases not directly caused by mutations in CFTR, such as secretory diseases and other protein folding diseases mediated by CFTR. These include, but are not limited to, chronic obstructive pulmonary disease (COPD), dry eye disease, and Sjögren's Syndrome. COPD is characterized by airflow limitation that is progressive and not fully reversible. The airflow limitation is due to mucus hypersecretion, emphysema, and bronchiolitis. Activators of mutant or wild-type CFTR offer a potential treatment of mucus hypersecretion and impaired mucociliary clearance that is common in COPD. Specifically, increasing anion secretion across CFTR may facilitate fluid

transport into the airway surface liquid to hydrate the mucus and optimized periciliary fluid viscosity. This would lead to enhanced mucociliary clearance and a reduction in the symptoms associated with COPD. Dry eye disease is characterized by a decrease in tear aqueous production and abnormal tear film lipid, protein and mucin profiles. There are many causes of dry eye, some of which include age, Lasik eye surgery, arthritis, medications, chemical/thermal burns, allergies, and diseases, such as cystic fibrosis and Sjögrens's syndrome. Increasing anion secretion via CFTR would enhance fluid transport from the corneal endothelial cells and secretory glands surrounding the eye to increase corneal hydration. This would help to alleviate the symptoms associated with dry eye disease. Sjögrens's syndrome is an autoimmune disease in which the immune system attacks moisture-producing glands throughout the body, including the eye, mouth, skin, respiratory tissue, liver, vagina, and gut. Symptoms, include, dry eye, mouth, and vagina, as well as lung disease. The disease is also associated with rheumatoid arthritis, systemic lupus, systemic sclerosis, and polymypositis/dermatomyositis. Defective protein trafficking is believed to cause the disease, for which treatment options are limited. Augmenters or inducers of CFTR activity may hydrate the various organs afflicted by the disease and help to elevate the associated symptoms.

[00247] In one embodiment, the invention relates to a method of augmenting or inducing anion channel activity *in vitro* or *in vivo*, comprising contacting the channel with a pharmaceutical composition of the present invention. In another embodiment, the anion channel is a chloride channel or a bicarbonate channel. In another embodiment, the anion channel is a chloride channel.

[00248] 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 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.

[00249] Anywhere in the present application where a name of a compound may not correctly describe the structure of the compound, the structure supersedes the name and governs.

EXAMPLES

[00250] XRPD (X-ray Powder Diffraction)

[00251] The X-Ray diffraction (XRD) data of Compound 1, Compound 1 Form I, Compound 1 Form II, or Compound 1 HCl Salt Form A were collected on a Bruker D8 DISCOVER powder diffractometer with HI-STAR 2-dimensional detector and a flat graphite monochromator. Cu sealed tube with Kα radiation was used at 40 kV, 35mA. The samples were placed on zero-background silicon wafers at 25°C. For each sample, two data frames were collected at 120 seconds each at 2 different θ_2 angles: 8° and 26°. The data were integrated with GADDS software and merged with DIFFRACT^{plus}EVA software. Uncertainties for the reported peak positions are ± 0.2 degrees.

[00252] Jet Milling Description

[00253] Unmicronized Compound 1, Compound 1 Form I, Compound 1 Form II, or Compound 1 HCl Salt Form A is sieved to de-lump it prior to placing it into the jet mill hopper. All sieves are disposable and received a wipe prior to use. Unmicronized Compound 1, Compound 1 Form I, Compound 1 Form II, or Compound 1 HCl Salt Form A is added to the jet mill hopper at a controlled feeding rate using compressed nitrogen gas. The gas pressure range is 40-45/45-70 (Venturi/Mill) PSI and the feeding rate range is 0.5-1.6 Kg/Hour. The Compound 1, Compound 1 Form I, Compound 1 Form II, or Compound 1 HCl Salt Form A is micronized in the mill through particle-particle and particle-wall collisions and the processed Compound 1, Compound 1 Form I, Compound 1 Form II, or Compound 1 HCl Salt Form A is emptied into the micronized product containers. It is believed that one of ordinary skill in the art may also achieve Compound 1, Compound 1 Form I, Compound 1 Form II, or Compound 1 HCl Salt

Form A with a favorable particle size through pin milling based in part on the conditions described above.

[00254] Differential Scanning Calorimetry (DSC)

[00255] The Differential scanning calorimetry (DSC) data of Compound 1, Compound 1 Form I, Compound 1 Form II, or Compound 1 HCl Salt Form A were collected using a DSC Q100 V9.6 Build 290 (TA Instruments, New Castle, DE). Temperature was calibrated with indium and heat capacity was calibrated with sapphire. Samples of 3-6 mg were weighed into aluminum pans that were crimped using lids with 1 pin hole. The samples were scanned from 25°C to 350°C at a heating rate of 1.0°C/min and with a nitrogen gas purge of 50 ml/min. Data were collected by Thermal Advantage Q SeriesTM version 2.2.0.248 software and analyzed by Universal Analysis software version 4.1D (TA Instruments, New Castle, DE). The reported numbers represent single analyses.

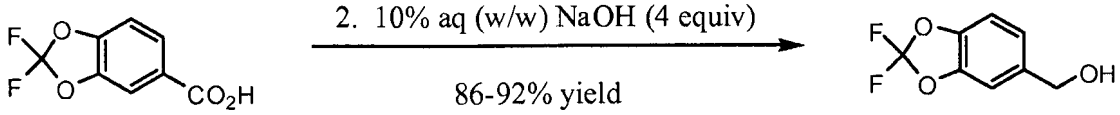
[00256] Compound 1 Form I, Compound 1 Form II, and Compound 1 HCl Salt Form A Single Crystal Structure Determination

[00257] Diffraction data were acquired on Bruker Apex II diffractometer equipped with sealed tube Cu K-alpha source and an Apex II CCD detector. The structure was solved and refined using SHELX program (Sheldrick, G.M., Acta Cryst., (2008) A64, 112-122). Based on systematic absences and intensities statistics the structure was solved and refined in $P2_1/n$ space group.

[00258] Vitride® (sodium bis(2-methoxyethoxy)aluminum hydride [or $NaAlH_2(OCH_2CH_2OCH_3)_2$], 65 wgt% solution in toluene) was purchased from Aldrich Chemicals.

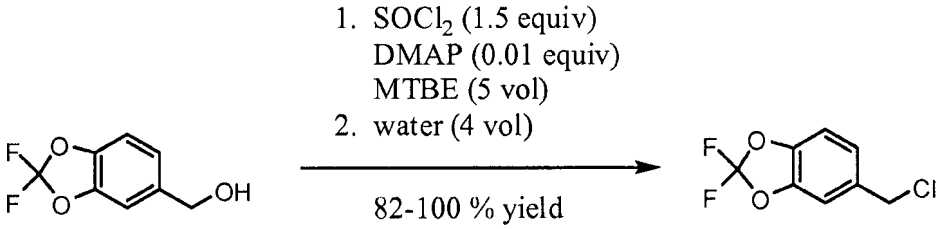
[00259] 2,2-Difluoro-1,3-benzodioxole-5-carboxylic acid was purchased from Saltigo (an affiliate of the Lanxess Corporation).

[00260] **Preparation of (2,2-difluoro-1,3-benzodioxol-5-yl)-methanol.**



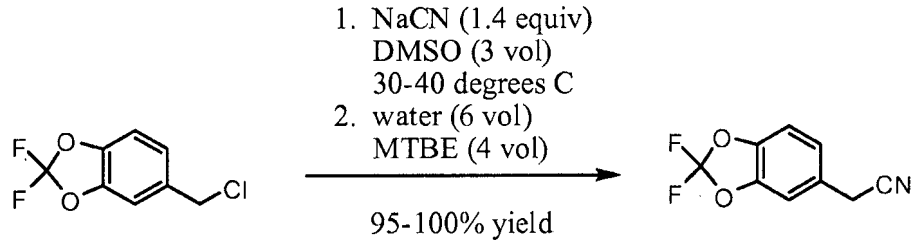
[00261] Commercially available 2,2-difluoro-1,3-benzodioxole-5-carboxylic acid (1.0 eq) was slurried in toluene (10 vol). Vitride® (2 eq) was added via addition funnel at a rate to maintain the temperature at 15-25 °C. At the end of the addition, the temperature was increased to 40 °C for 2 hours (h), then 10% (w/w) aqueous (aq) NaOH (4.0 eq) was carefully added via addition funnel, maintaining the temperature at 40-50 °C. After stirring for an additional 30 minutes (min), the layers were allowed to separate at 40 °C. The organic phase was cooled to 20 °C, then washed with water (2 x 1.5 vol), dried (Na_2SO_4), filtered, and concentrated to afford crude (2,2-difluoro-1,3-benzodioxol-5-yl)-methanol that was used directly in the next step.

[00262] **Preparation of 5-chloromethyl-2,2-difluoro-1,3-benzodioxole.**



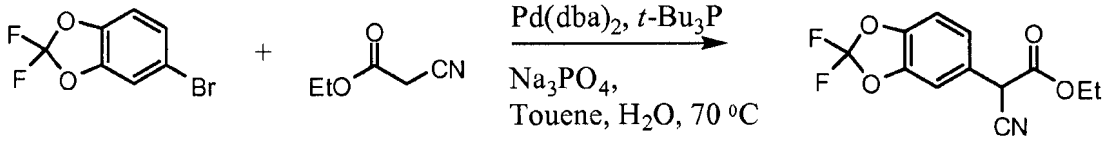
[00263] (2,2-difluoro-1,3-benzodioxol-5-yl)-methanol (1.0 eq) was dissolved in MTBE (5 vol). A catalytic amount of 4-(N,N-dimethyl)aminopyridine (DMAP) (1 mol %) was added and $SOCl_2$ (1.2 eq) was added via addition funnel. The $SOCl_2$ was added at a rate to maintain the temperature in the reactor at 15-25 °C. The temperature was increased to 30 °C for 1 h, and then was cooled to 20 °C. Water (4 vol) was added via addition funnel while maintaining the temperature at less than 30 °C. After stirring for an additional 30 min, the layers were allowed to separate. The organic layer was stirred and 10% (w/v) aq NaOH (4.4 vol) was added. After stirring for 15 to 20 min, the layers were allowed to separate. The organic phase was then dried (Na_2SO_4), filtered, and concentrated to afford crude 5-chloromethyl-2,2-difluoro-1,3-benzodioxole that was used directly in the next step.

[00264] **Preparation of (2,2-difluoro-1,3-benzodioxol-5-yl)-acetonitrile.**



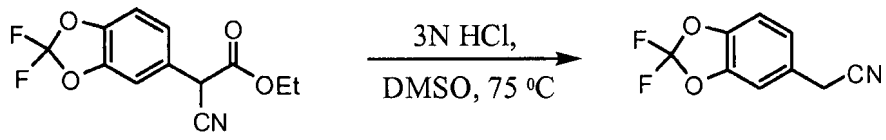
[00265] A solution of 5-chloromethyl-2,2-difluoro-1,3-benzodioxole (1 eq) in DMSO (1.25 vol) was added to a slurry of NaCN (1.4 eq) in DMSO (3 vol), while maintaining the temperature between 30-40 °C. The mixture was stirred for 1 h, and then water (6 vol) was added, followed by methyl *tert*-butyl ether (MTBE) (4 vol). After stirring for 30 min, the layers were separated. The aqueous layer was extracted with MTBE (1.8 vol). The combined organic layers were washed with water (1.8 vol), dried (Na_2SO_4), filtered, and concentrated to afford crude (2,2-difluoro-1,3-benzodioxol-5-yl)-acetonitrile (95%) that was used directly in the next step.

[00266] Synthesis of (2,2-difluoro-1,3-benzodioxol-5-yl)-1-ethylacetate-acetonitrile



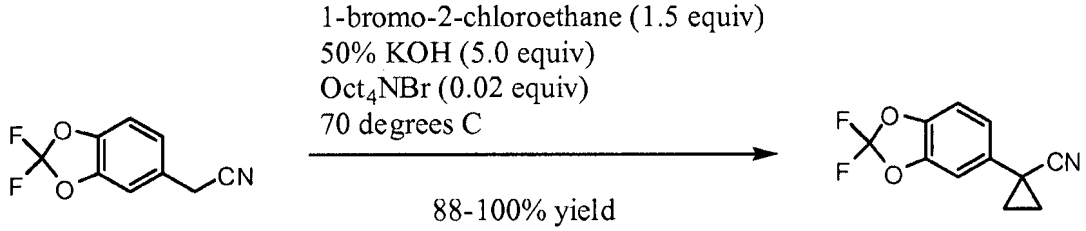
[00267] A reactor was purged with nitrogen and charged with 900 mL of toluene. The solvent was degassed via nitrogen sparge for no less than 16 h. To the reactor was then charged Na_3PO_4 (155.7 g, 949.5 mmol), followed by bis(dibenzylideneacetone) palladium (0) (7.28 g, 12.66 mmol). A 10% w/w solution of tert-butylphosphine in hexanes (51.23 g, 25.32 mmol) was charged over 10 min at 23 °C from a nitrogen purged addition funnel. The mixture was allowed to stir for 50 min, at which time 5-bromo-2,2-difluoro-1,3-benzodioxole (75 g, 316.5 mmol) was added over 1 min. After stirring for an additional 50 min, the mixture was charged with ethyl cyanoacetate (71.6 g, 633.0 mmol) over 5 min followed by water (4.5 mL) in one portion. The mixture was heated to 70 °C over 40 min and analyzed by HPLC every 1 – 2 h for the percent conversion of the reactant to the product. After complete conversion was observed (typically 100% conversion after 5 – 8 h), the mixture was cooled to 20 – 25 °C and filtered through a celite pad. The celite pad was rinsed with toluene (2 X 450 mL) and the combined organics were concentrated to 300 mL under vacuum at 60 – 65 °C. The concentrate was charged with 225mL DMSO and concentrated under vacuum at 70 – 80 °C until active distillation of the solvent ceased. The solution was cooled to 20 – 25 °C and diluted to 900 mL with DMSO in preparation for Step 2. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 – 7.10 (m, 2H), 7.03 (d, J = 8.2 Hz, 1H), 4.63 (s, 1H), 4.19 (m, 2H), 1.23 (t, J = 7.1 Hz, 3H).

[00268] Synthesis of (2,2-difluoro-1,3-benzodioxol-5-yl)-acetonitrile.



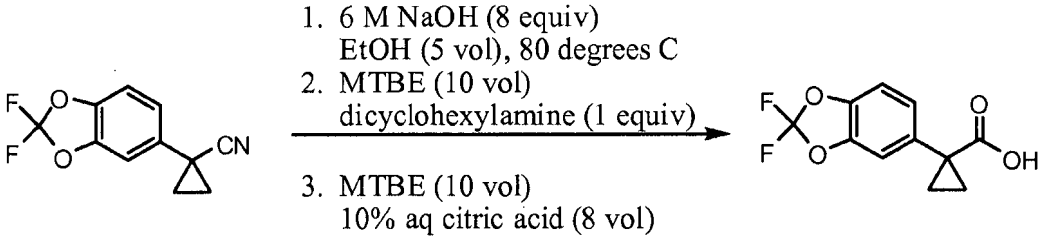
[00269] The DMSO solution of (2,2-difluoro-1,3-benzodioxol-5-yl)-1-ethylacetate-acetonitrile from above was charged with 3 N HCl (617.3 mL, 1.85 mol) over 20 min while maintaining an internal temperature < 40 °C. The mixture was then heated to 75°C over 1 h and analyzed by HPLC every 1 – 2 h for % conversion. When a conversion of > 99% was observed (typically after 5 – 6 h), the reaction was cooled to 20 – 25 °C and extracted with MTBE (2 X 525 mL), with sufficient time to allow for complete phase separation during the extractions. The combined organic extracts were washed with 5% NaCl (2 X 375 mL). The solution was then transferred to equipment appropriate for a 1.5 – 2.5 Torr vacuum distillation that was equipped with a cooled receiver flask. The solution was concentrated under vacuum at < 60°C to remove the solvents. (2,2-Difluoro-1,3-benzodioxol-5-yl)-acetonitrile was then distilled from the resulting oil at 125 – 130 °C (oven temperature) and 1.5 – 2.0 Torr. (2,2-Difluoro-1,3-benzodioxol-5-yl)-acetonitrile was isolated as a clear oil in 66% yield from 5-bromo-2,2-difluoro-1,3-benzodioxole (2 steps) and with an HPLC purity of 91.5% AUC (corresponds to a w/w assay of 95%). ^{1}H NMR (500 MHz, DMSO) δ 7.44 (br s, 1H), 7.43 (d, J = 8.4 Hz, 1H), 7.22 (dd, J = 8.2, 1.8 Hz, 1H), 4.07 (s, 2H).

[00270] Preparation of (2,2-difluoro-1,3-benzodioxol-5-yl)-cyclopropanecarbonitrile.



[00271] A mixture of (2,2-difluoro-1,3-benzodioxol-5-yl)-acetonitrile (1.0 eq), 50 wt % aqueous KOH (5.0 eq) 1-bromo-2-chloroethane (1.5 eq), and Oct_4NBr (0.02 eq) was heated at 70 °C for 1 h. The reaction mixture was cooled, then worked up with MTBE and water. The organic phase was washed with water and brine. The solvent was removed to afford (2,2-difluoro-1,3-benzodioxol-5-yl)-cyclopropanecarbonitrile.

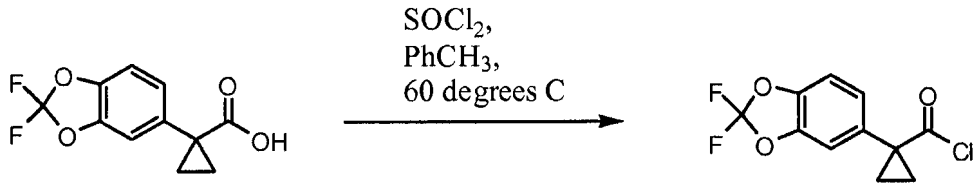
[00272] Preparation of 1-(2,2-difluoro-1,3-benzodioxol-5-yl)-cyclopropanecarboxylic acid.



69% yield

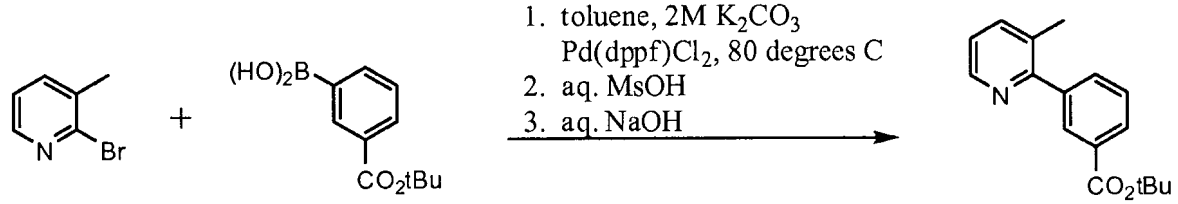
[00273] (2,2-difluoro-1,3-benzodioxol-5-yl)-cyclopropanecarbonitrile was hydrolyzed using 6 M NaOH (8 equiv) in ethanol (5 vol) at 80 °C overnight. The mixture was cooled to room temperature and the ethanol was evaporated under vacuum. The residue was taken up in water and MTBE, 1 M HCl was added, and the layers were separated. The MTBE layer was then treated with dicyclohexylamine (DCHA) (0.97 equiv). The slurry was cooled to 0 °C, filtered and washed with heptane to give the corresponding DCHA salt. The salt was taken into MTBE and 10% citric acid and stirred until all the solids had dissolved. The layers were separated and the MTBE layer was washed with water and brine. A solvent swap to heptane followed by filtration gave 1-(2,2-difluoro-1,3-benzodioxol-5-yl)-cyclopropanecarboxylic acid after drying in a vacuum oven at 50 °C overnight.

[00274] Preparation of 1-(2,2-difluoro-1,3-benzodioxol-5-yl)-cyclopropanecarbonyl chloride.



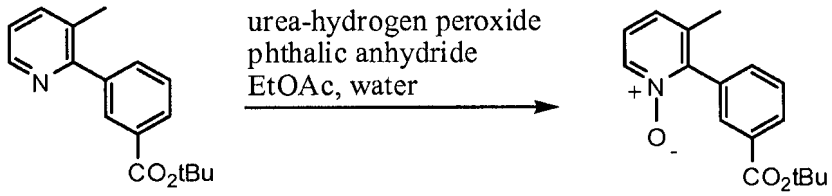
[00275] 1-(2,2-difluoro-1,3-benzodioxol-5-yl)-cyclopropanecarboxylic acid (1.2 eq) is slurried in toluene (2.5 vol) and the mixture was heated to 60 °C. $SOCl_2$ (1.4 eq) was added via addition funnel. The toluene and $SOCl_2$ were distilled from the reaction mixture after 30 minutes. Additional toluene (2.5 vol) was added and the resulting mixture was distilled again, leaving the product acid chloride as an oil, which was used without further purification.

[00276] Preparation of *tert*-butyl-3-(3-methylpyridin-2-yl)benzoate.



[00277] 2-Bromo-3-methylpyridine (1.0 eq) was dissolved in toluene (12 vol). K_2CO_3 (4.8 eq) was added, followed by water (3.5 vol). The resulting mixture was heated to 65 °C under a stream of N_2 for 1 hour. 3-(*t*-Butoxycarbonyl)phenylboronic acid (1.05 eq) and Pd(dppf)Cl_2·CH_2Cl_2 (0.015 eq) were then added and the mixture was heated to 80 °C. After 2 hours, the heat was turned off, water was added (3.5 vol), and the layers were allowed to separate. The organic phase was then washed with water (3.5 vol) and extracted with 10% aqueous methanesulfonic acid (2 eq MsOH, 7.7 vol). The aqueous phase was made basic with 50% aqueous NaOH (2 eq) and extracted with EtOAc (8 vol). The organic layer was concentrated to afford crude *tert*-butyl-3-(3-methylpyridin-2-yl)benzoate (82%) that was used directly in the next step.

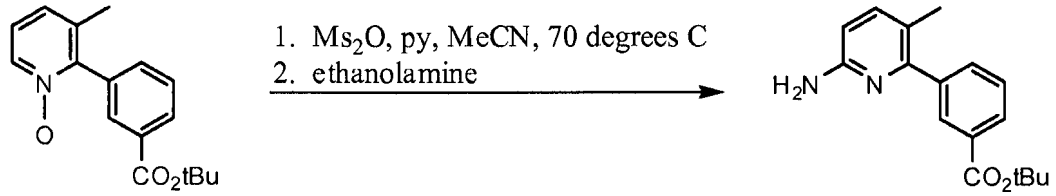
[00278] Preparation of 2-(3-(*tert*-butoxycarbonyl)phenyl)-3-methylpyridine-1-oxide.



[00279] *tert*-Butyl-3-(3-methylpyridin-2-yl)benzoate (1.0 eq) was dissolved in EtOAc (6 vol). Water (0. 3 vol) was added, followed by urea-hydrogen peroxide (3 eq). Phthalic anhydride (3 eq) was then added portionwise to the mixture as a solid at a rate to maintain the temperature in the reactor below 45 °C. After completion of the phthalic anhydride addition, the mixture was heated to 45 °C. After stirring for an additional 4 hours, the heat was turned off. 10% w/w aqueous Na_2SO_3 (1.5 eq) was added via addition funnel. After completion of Na_2SO_3 addition, the mixture was stirred for an additional 30 min and the layers separated. The organic layer was stirred and 10% wt/wt aqueous. Na_2CO_3 (2 eq) was added. After stirring for 30 minutes, the layers were allowed to separate. The organic phase was washed 13% w/v aq NaCl. The organic

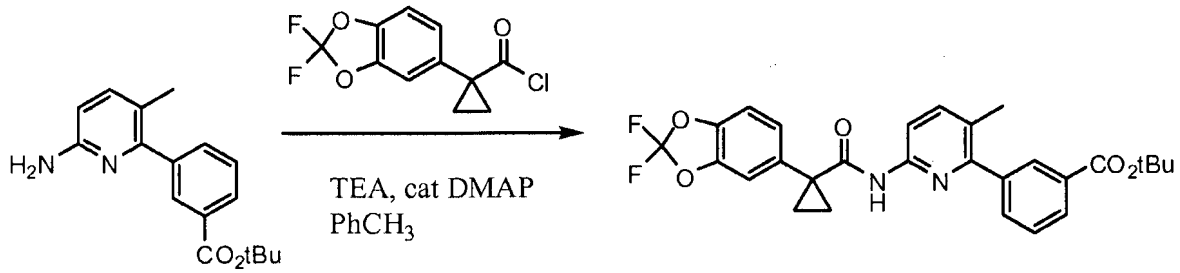
phase was then filtered and concentrated to afford crude 2-(3-(*tert*-butoxycarbonyl)phenyl)-3-methylpyridine-1-oxide (95%) that was used directly in the next step.

[00280] Preparation of *tert*-butyl-3-(6-amino-3-methylpyridin-2-yl)benzoate.



[00281] A solution of 2-(3-(*tert*-butoxycarbonyl)phenyl)-3-methylpyridine-1-oxide (1 eq) and pyridine (4 eq) in acetonitrile (8 vol) was heated to 70 °C. A solution of methanesulfonic anhydride (1.5 eq) in MeCN (2 vol) was added over 50 min via addition funnel while maintaining the temperature at less than 75 °C. The mixture was stirred for an additional 0.5 hours after complete addition. The mixture was then allowed to cool to ambient. Ethanolamine (10 eq) was added via addition funnel. After stirring for 2 hours, water (6 vol) was added and the mixture was cooled to 10 °C. After stirring for 3 hours, the solid was collected by filtration and washed with water (3 vol), 2:1 acetonitrile/water (3 vol), and acetonitrile (2 x 1.5 vol). The solid was dried to constant weight (<1% difference) in a vacuum oven at 50 °C with a slight N_2 bleed to afford *tert*-butyl-3-(6-amino-3-methylpyridin-2-yl)benzoate as a red-yellow solid (53% yield).

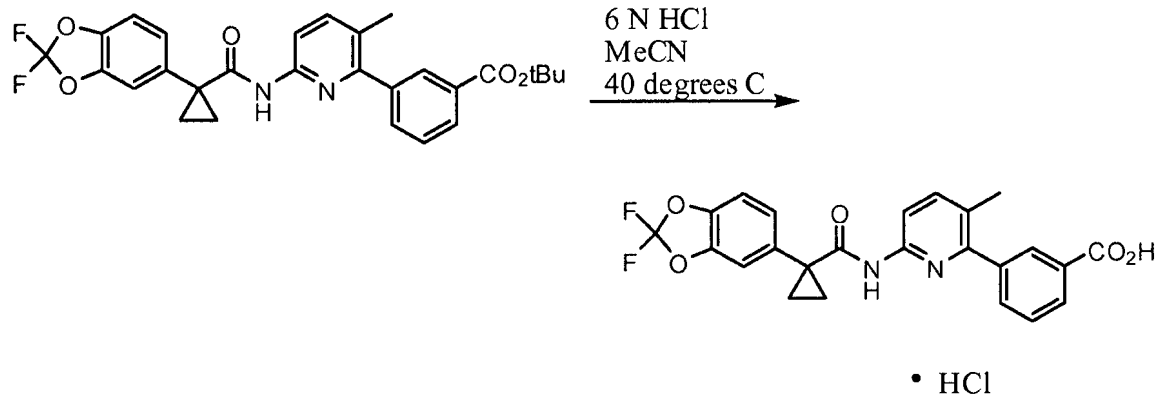
[00282] Preparation of 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-cyclopropanecarboxamido)-3-methylpyridin-2-yl)-t-butylbenzoate.



[00283] The crude acid chloride described above was dissolved in toluene (2.5 vol based on acid chloride) and added via addition funnel to a mixture of *tert*-butyl-3-(6-amino-3-methylpyridin-2-yl)benzoate (1 eq), DMAP, (0.02 eq), and triethylamine (3.0 eq) in toluene (4 vol based on *tert*-butyl-3-(6-amino-3-methylpyridin-2-yl)benzoate). After 2 hours, water (4 vol

based on *tert*-butyl-3-(6-amino-3-methylpyridin-2-yl)benzoate) was added to the reaction mixture. After stirring for 30 minutes, the layers were separated. The organic phase was then filtered and concentrated to afford a thick oil of 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)-t-butylbenzoate (quantitative crude yield). Acetonitrile (3 vol based on crude product) was added and distilled until crystallization occurs. Water (2 vol based on crude product) was added and the mixture stirred for 2 h. The solid was collected by filtration, washed with 1:1 (by volume) acetonitrile/water (2 x 1 volumes based on crude product), and partially dried on the filter under vacuum. The solid was dried to a constant weight (<1% difference) in a vacuum oven at 60 °C with a slight N_2 bleed to afford 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)-t-butylbenzoate as a brown solid.

[00284] Preparation of 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid • HCL salt.



[00285] To a slurry of 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)-t-butylbenzoate (1.0 eq) in MeCN (3.0 vol) was added water (0.83 vol) followed by concentrated aqueous HCl (0.83 vol). The mixture was heated to 45 ± 5 °C. After stirring for 24 to 48 h, the reaction was complete, and the mixture was allowed to cool to ambient. Water (1.33 vol) was added and the mixture stirred. The solid was collected by filtration, washed with water (2 x 0.3 vol), and partially dried on the filter under vacuum. The solid was dried to a constant weight (<1% difference) in a vacuum oven at 60 °C with a slight N_2 bleed to afford 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid • HCl as an off-white solid.

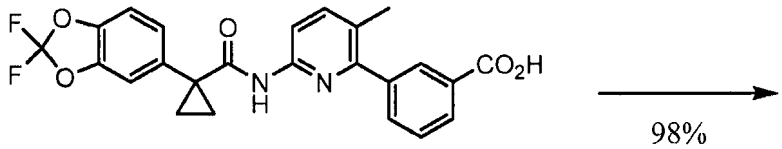
[00286] An ^{1}HNMR spectrum of Compound 1 is shown in Figure 20 and Figure 21 depicts an ^{1}HNMR spectrum of Compound 1 as an HCl salt.

[00287] Table 2 below recites the ^{1}HNMR data for Compound I.

Table 2.

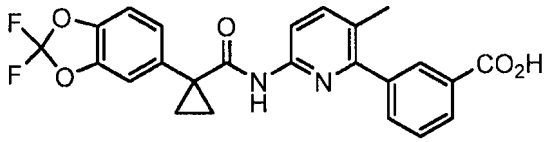
Compound No	LC/MS M + 1	LC/RT minutes	NMR
1	453.3	1.93	^{1}HNMR (400 MHz, DMSO-d6) 9.14 (s, 1H), 7.99-7.93 (m, 3H), 7.80-7.78 (m,1H), 7.74-7.72 (m,1H), 7.60-7.55 (m,2H), 7.41-7.33 (m,2H), 2.24 (s, 3H), 1.53-1.51 (m, 2H), 1.19-1.17 (m, 2H).

[00288] **Preparation of Compound 1 Form I, Method A.**



98%

• HCl

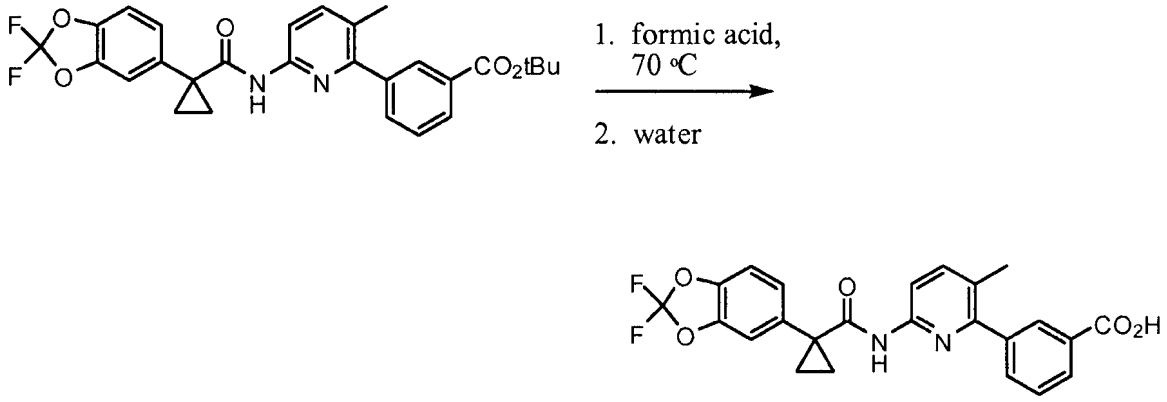


Form I

[00289] A slurry of 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid • HCl (1 eq) in water (10 vol) was stirred at ambient temperature. A sample was taken after stirring for 24 h. The sample was filtered and the solid was washed with water (2 times). The solid sample was submitted for DSC analysis. When DSC analysis indicated complete conversion to Form I, the solid was collected by filtration, washed with water (2 x 1.0 vol), and partially dried on a filter under vacuum. The solid was then dried to a constant weight (<1% difference) in a vacuum oven at 60 °C with a slight N_2 bleed to

afford Compound 1 Form I as an off-white solid (98% yield). ^{1}H NMR (400 MHz, DMSO-d6) 9.14 (s, 1H), 7.99-7.93 (m, 3H), 7.80-7.78 (m, 1H), 7.74-7.72 (m, 1H), 7.60-7.55 (m, 2H), 7.41-7.33 (m, 2H), 2.24 (s, 3H), 1.53-1.51 (m, 2H), 1.19-1.17 (m, 2H).

[00290] Preparation of Compound 1 Form I, Method B.



Form I

[00291] A solution of 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)-t-butylbenzoate (1.0 eq) in formic acid (3.0 vol) was heated with stirring to 70 ± 10 °C , for 8 h. The reaction was deemed complete when no more than 1.0% AUC by chromatographic methods of 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)-t-butylbenzoate) remained. The mixture was allowed to cool to ambient. The solution was added to water (6 vol), heated at 50 °C, and the mixture was stirred. The mixture was then heated to 70 ± 10 °C until the level of 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)-t-butylbenzoate was no more than 0.8% (AUC). The solid was collected by filtration, washed with water (2 x 3 vol), and partially dried on the filter under vacuum. The solid was dried to a constant weight (<1% difference) in a vacuum oven at 60 °C with a slight N_2 bleed to afford Compound 1 Form I as an off-white solid.

[00292] The DSC trace of Compound 1 Form I is shown in Figure 22. Melting for Compound 1 Form I occurs at about 204 °C.

[00293] An X-ray diffraction pattern was calculated from a single crystal structure of Compound 1 Form I and is shown in Figure 1. Table 3 lists the calculated peaks for Figure 1.

Table 3.

Peak Rank	2θ Angle [degrees]	Relative Intensity [%]
11	14.41	48.2
8	14.64	58.8
1	15.23	100.0
2	16.11	94.7
3	17.67	81.9
7	19.32	61.3
4	21.67	76.5
5	23.40	68.7
9	23.99	50.8
6	26.10	67.4
10	28.54	50.1

[00294] An actual X-ray powder diffraction pattern of Compound 1 Form I is shown in Figure 2. Table 4 lists the actual peaks for Figure 2.

Table 4.

Peak Rank	2θ Angle [degrees]	Relative Intensity [%]
7	7.83	37.7
3	14.51	74.9

4	14.78	73.5
1	15.39	100.0
2	16.26	75.6
6	16.62	42.6
5	17.81	70.9
9	21.59	36.6
10	23.32	34.8
11	24.93	26.4
8	25.99	36.9

[00295] Colorless crystals of Compound 1 Form I were obtained by cooling a concentrated 1-butanol solution from 75°C to 10 °C at a rate of 0.2 °C/min. A crystal with dimensions of 0.50 *x* 0.08 *x* 0.03 mm was selected, cleaned with mineral oil, mounted on a MicroMount and centered on a Bruker *APEX* II system. Three batches of 40 frames separated in reciprocal space were obtained to provide an orientation matrix and initial cell parameters. Final cell parameters were obtained and refined based on the full data set.

[00296] A diffraction data set of reciprocal space was obtained to a resolution of 0.82 Å using 0.5° steps using 30 s exposure for each frame. Data were collected at 100 (2) K. Integration of intensities and refinement of cell parameters were accomplished using APEXII software. Observation of the crystal after data collection showed no signs of decomposition.

[00297] A conformational picture of Compound 1 Form I based on single crystal X-ray analysis is shown in Figure 23. Compound 1 Form I is monoclinic, P_21/n, with the following unit cell dimensions: a=4.9626(7) Å, b=12.299(2) Å, c=33.075 (4) Å, β=93.938(9)°, V=2014.0 $Å^3$, Z=4. Density of Compound 1 Form I calculated from structural data is 1.492 g/cm^3 at 100 K.

[00298] Preparation of Compound 1 Form II from Compound 1 Form I.

[00299] Compound 1 Form I (approximately 30 mg) was slurried in 500 μL of an appropriate solvent (for example, methanol, ethanol, acetone, 2-propanol, acetonitrile, tetrahydrofuran, methyl acetate, 2-butanone, ethyl formate, and -methyl tetrahydrofuran for two days. The slurry was then filitered centrifugally or under vacuum and was left to dry at ambient temperature overnight to yield Compound 1 Form II.

[00300] The DSC trace of Compound 1 Form II Acetone Solvate is shown in Figure 15, showing two phase transitions. The melting point for Compound 1 Form II Acetone Solvate occurs at about 188 °C and 205 °C.

[00301] An actual X-ray powder diffraction pattern of Compound 1 Form II is shown in Figure 3. Table 5 lists the actual peaks for Figure 3 in descending order of relative intensity.

Table 5.

2θ Angle [degrees]	**Relative Intensity [%]**
21.70	100.0
8.98	65.5
11.04	57.4
18.16	55.9
23.06	55.4
20.63	53.1
22.22	50.2
18.57	49.1
16.66	47.2
19.86	35.0

[00302] Conformational depictions of Compound 1 Form II Acetone Solvate based on single crystal X-ray analysis are shown in Figure 24. The stoichiometry between Compound 1 Form II

and acetone is approximately 4.4:1 (4.48:1 calculated from ^{1}H NMR; 4.38:1 from X-ray). The crystal structure reveals a packing of the molecules where there are two voids or pockets per unit cell, or 1 void per host molecule. In the acetone solvate, approximately 92 percent of voids are occupied by acetone molecules. Compound 1 Form II is a monoclinic $P2_1/n$ space group with the following unit cell dimensions: a = 16.5235(10) Å, b = 12.7425(8) Å, c = 20.5512 (13) Å, α = 90°, β = 103.736(4)°, γ = 90°, V = 4203.3(5) Å^3, = 4. The density of Compound 1 in Compound 1 Form II calculated from structural data is 1.430/cm^3 at 100 K.

[00303] A solid state ^{13}C NMR spectrum of Compound 1 Form II Acetone Solvate is shown in Figure 25. Table 6 provides chemical shifts of the relevant peaks.

Table 6.

	Compound 1 Form II, Acetone Solvate ^{13}C Chem. Shifts	
Peak #	F1 [ppm]	Intensity
1	202.8	6.05
2	173.3	62.66
3	171.9	20.53
4	153.5	28.41
5	150.9	21.68
6	150.1	19.49
7	143.2	45.74
8	142.3	42.68
9	140.1	37.16
10	136.6	26.82
11	135.9	30.1
12	134.6	39.39
13	133.2	23.18
14	131.0	60.92
15	128.5	84.58
16	116.0	34.64
17	114.2	23.85
18	112.4	25.3
19	110.9	24.12
20	107.8	18.21
21	32.0	54.41
22	22.2	20.78
23	18.8	100

[00304] A solid state ^{19}F NMR spectrum of Compound 1 Form II Acetone Solvate is shown in Figure 26. Peaks with an asterisk denote spinning side bands. Table 7 provides chemical shifts of the relevant peaks.

Table 7.

	Compound 1 Form II, Acetone Solvate ^{19}F Chem. Shifts	
Peak #	F1 [ppm]	Intensity
1	-41.6	12.5
2	-46.4	6.77
3	-51.4	9.05

[00305] Preparation of Compound 1 HCl Salt Form A.

[00306] Colorless crystals of Compound 1 HCl Salt Form A were obtained by slow evaporation from a concentrated solution of the HCl salt of Compound 1 in ethanol. A crystal with dimensions of 0.30 *x* 1/5*x* 0.15 mm was selected, cleaned using mineral oil, mounted on a MicroMount and centered on a Bruker *APEX*II diffractometer. Three batches of 40 frames separated in reciprocal space were obtained to provide an orientation matrix and initial cell parameters. Final cell parameters were obtained and refined based on the full data set.

[00307] Figure 18 provides a conformational image of Compound 1 HCl Salt Form A as a dimer, based on single crystal analysis. An X-ray diffraction pattern of Compound 1 HCl Salt Form A calculated from the crystal structure is shown in Figure 27. Table 8 contains the calculated peaks for Figure 27 in descending order of relative intensity.

Table 8.

2θ [degrees]	**Relative Intensity [%]**
8.96	100.00
17.51	48.20
18.45	34.60
10.33	32.10

16.01	18.90
11.94	18.40
8.14	16.20
10.10	13.90
16.55	13.30
9.54	10.10
16.55	13.30

Exemplary Oral Pharmaceutical Formulations Comprising Compound 1

[00308] A tablet was prepared with the components and amounts listed in Table 9 for Exemplary Tablet 1A comprising 100mg of API, i.e. Compound 1 Form I. Exemplary Tablet 1A (formulated to have 100 mg of Compound 1) is prepared using a dry roller compaction device formulation process. In Table 9, grades/brands were microcrystalline cellulose: Avicel PH102; mannitol: Pearlitol SD 100; croscarmellose sodium: Acdisol; and colloidal silica: Cabosil.

Table 9.

Roller Compaction Granule Blend	(%w/w)
Compound 1 Form I	30
Microcrystalline cellulose	42.3
Mannitol	21.2
Croscarmellose Sodium	3
Sodium Lauryl Sulfate	1
Colloidal Silica	0.5
Magnesium Stearate	2
Tablet Composition (100 mg dose, 335 mg image)	(%w/w)

Roller Compaction Granule Blend	99.5
Magnesium Stearate	0.5

[00309] A tablet was prepared with the components and amounts listed in Table 10 for Exemplary Tablet 1B comprising 100mg of API, i.e. Compound 1 Form I. Exemplary Tablet 1B (formulated to have 100 mg of Compound 1 Form I) is prepared using a wet high shear granule formulation process. In Table 10, grades/brands were as follows. High Shear Granule Blend - microcrystalline cellulose: Avicel PH101; mannitol: Pearlitol C50; croscarmellose sodium: Acdisol; polyvinylpyrrolidone: Kollidon PVP K30; and in the Tablet Composition – croscarmellose sodium: Acdisol.

Table 10.

High Shear Granule Blend	(%w/w)
Compound 1 Form I	50
Microcrystalline cellulose	30
Mannitol	13
Croscarmellose Sodium	2
Polyvinylpyrrolidone	4
Sodium Lauryl Sulfate	1
Tablet Composition (100mg dose, 205 mg image)	(%w/w)
High Shear Granule Blend	97.5
Croscarmellose Sodium	2.0
Magnesium Stearate	0.5

[00310] A tablet was prepared with the components and amounts listed in Table 11 for Exemplary Tablet 1C comprising 100mg of API, i.e. crystalline Compound 1 Form I. Exemplary Tablet 1C (formulated to have 100 mg of crystalline Compound 1 Form I) is prepared

using a wet high shear granule formulation process. In Table 11, grades/brands were as follows. High Shear Granule Blend - microcrystalline cellulose: Avicel PH101; mannitol: Pearlitol C50; croscarmellose sodium: Acdisol; polyvinylpyrrolidone: Kollidon PVP K30; and in the Tablet Composition – croscarmellose sodium: Acdisol.

Table 11.

High Shear Granule Blend	(%w/w)
Compound 1 Form I	60
Microcrystalline cellulose	20
Mannitol	13
Croscarmellose Sodium	2
Polyvinylpyrrolidone	4
Sodium Lauryl Sulfate	1
Tablet Composition (100mg dose, 171 mg image)	(%w/w)
High Shear Granule Blend	97.5
Croscarmellose Sodium	2.0
Magnesium Stearate	0.5

[00311] A tablet was prepared with the components and amounts listed in Table 12 for Exemplary Tablet 1D comprising 200mg of API, i.e. crystalline Compound 1 Form I. Exemplary Tablet 1D (formulated to have 200 mg of crystalline Compound 1 Form I) is prepared using a wet high shear granule formulation process. In Table 12, grades/brands were as follows. High Shear Granule Blend - microcrystalline cellulose: Avicel PH101; mannitol: Pearlitol C50; croscarmellose sodium: Acdisol; polyvinylpyrrolidone: Kollidon PVP K30; and in the Tablet Composition – microcrystalline cellulose: Avicel PH200; croscarmellose sodium: Acdisol; and magnesium stearate: 5712.

Table 12.

High Shear Granule Blend	(%w/w)
Compound 1 Form I	60
Microcrystalline cellulose	20
Mannitol	13
Croscarmellose Sodium	2
Polyvinylpyrrolidone	4
Sodium Lauryl Sulfate	1
Tablet Composition (200mg dose, 402 mg image)	(%w/w)
High Shear Granule Blend	83
Microcrystalline cellulose	14
Croscarmellose Sodium	2
Magnesium Stearate	1

[00312] A tablet was prepared with the components and amounts listed in Table 13 for Exemplary Tablet 1E comprising 200 mg of API, i.e. crystalline Compound 1 Form I. Exemplary Tablet 1E (formulated to have 200 mg of crystalline Compound 1 Form I) is prepared using a wet high shear granule formulation process. In Table 13, grades/brands were as follows. High Shear Granule Blend - microcrystalline cellulose: Avicel PH101; mannitol: Pearlitol C50; croscarmellose sodium: Acdisol; polyvinylpyrrolidone: Kollidon PVP K30; and in the Core Tablet Composition – microcrystalline cellulose: Avicel PH200; croscarmellose sodium: Acdisol; and magnesium stearate: 5712; and in the film coat – film coat: Opadry II; wax: Carnauba.

Table 13.

High Shear Granule Blend	mg
Compound 1 Form I	200

Microcrystalline cellulose	66
Mannitol	43
Croscarmellose Sodium	7
Polyvinylpyrrolidone	13
Sodium Lauryl Sulfate	3
Core Tablet Composition (200 mg dose, 400 mg image)	mg
High Shear Granule Blend	332
Microcrystalline cellulose	56
Croscarmellose Sodium	8
Magnesium Stearate	4
Film Coated Tablet (200 mg dose, 412 mg image)	mg
Core Tablet Composition	400
Film Coat	12
Wax	trace

[00313] A tablet was prepared with the components and amounts listed in Table 14 for Exemplary Tablet 1F comprising 200 mg of API, i.e. crystalline Compound 1 Form I. Exemplary Tablet 1F (formulated to have 200 mg of crystalline Compound 1 Form I) is prepared using a wet high shear granule formulation process. In Table 14, grades/brands were as follows. High Shear Granule Blend - microcrystalline cellulose: Avicel PH101; mannitol: Pearlitol C50; croscarmellose sodium: Acdisol; polyvinylpyrrolidone: Kollidon PVP K30; and in the Core Tablet Composition – microcrystalline cellulose: Avicel PH200; croscarmellose sodium: Acdisol; and magnesium stearate: 5712; and in the film coat – film coat: Opadry II; wax: Carnauba.

Table 14.

High Shear Granule Blend	mg
Compound 1 Form I	200
Microcrystalline cellulose	67
Mannitol	45
Croscarmellose Sodium	7
Polyvinylpyrrolidone	10.4
Sodium Lauryl Sulfate	2.6
Core Tablet Composition (200 mg dose, 400 mg image)	mg
High Shear Granule Blend	332
Microcrystalline cellulose	56
Croscarmellose Sodium	8
Magnesium Stearate	4
Film Coated Tablet (200 mg dose, 412 mg image)	mg
Core Tablet Composition	400
Film Coat	12
Wax	0.04

[00314] A tablet was prepared with the components and amounts listed in Table 15 for Exemplary Tablet 1G comprising 100 mg of API, i.e. crystalline Compound 1 Form I. Exemplary Tablet 1G (formulated to have 100 mg of crystalline Compound 1 Form I) is prepared using a wet high shear granule formulation process. In Table 15, grades/brands were as follows. High Shear Granule Blend - microcrystalline cellulose: Avicel PH101; mannitol:

Pearlitol C50; croscarmellose sodium: Acdisol; polyvinylpyrrolidone: Kollidon PVP K30; and in the Tablet Composition – croscarmellose sodium: Acdisol.

Table 15.

High Shear Granule Blend	(%w/w)
Compound 1 Form I	70
Microcrystalline cellulose	12
Mannitol	11
Croscarmellose Sodium	2
Polyvinylpyrrolidone	4
Sodium Lauryl Sulfate	1
Tablet Composition (100mg dose, 147 mg image)	(%w/w)
High Shear Granule Blend	97.5
Croscarmellose Sodium	2.0
Magnesium Stearate	0.5

[00315] A tablet was prepared with the components and amounts listed in Table 16 for Exemplary Tablet 1H comprising 100 mg of API, i.e. crystalline Compound 1 Form I or Form II. Exemplary Tablet 1H (formulated to have 100 mg of crystalline Compound 1 Form I or Form II) is prepared using a wet high shear granule formulation process. In Table 16, grades/brands were as follows. High Shear Granule Blend - microcrystalline cellulose: Avicel PH101; mannitol: Pearlitol C50; croscarmellose sodium: Acdisol; polyvinylpyrrolidone: Kollidon PVP K30; and in the Core Tablet Composition – microcrystalline cellulose: Avicel PH200; croscarmellose sodium: Acdisol; and magnesium stearate: 5712.

Table 16.

High Shear Granule Blend	(%w/w)

Compound 1 Form I or Form II	61
Microcrystalline cellulose	20.3
Mannitol	13.2
Croscarmellose Sodium	2
Polyvinylpyrrolidone	2.7
Sodium Lauryl Sulfate	0.7
Tablet Composition (100 mg dose, 197 mg image)	(%w/w)
High Shear Granule Blend	83
Microcrystalline cellulose	14
Croscarmellose Sodium	2
Magnesium Stearate	1

[00316] A tablet was prepared with the components and amounts listed in Table 17 for Exemplary Tablet 1I comprising 100 mg of API, i.e. crystalline Compound 1 Form I or Form II. Exemplary Tablet 1I (formulated to have 100 mg of crystalline Compound 1 Form I or Form II) is prepared using a wet high shear granule formulation process. In Table 17, grades/brands were as follows. High Shear Granule Blend - microcrystalline cellulose: Avicel PH101; mannitol: Pearlitol C50; croscarmellose sodium: Acdisol; polyvinylpyrrolidone: Kollidon PVP K30; and in the Core Tablet Composition – microcrystalline cellulose: Avicel PH200; croscarmellose sodium: Acdisol; and magnesium stearate: 5712.

Table 17.

High Shear Granule Blend	mg
Compound 1 Form I or Form II	100
Microcrystalline cellulose	33.3

Mannitol	21.7
Croscarmellose Sodium	3.3
Polyvinylpyrrolidone	4.4
Sodium Lauryl Sulfate	1.1
Core Tablet Composition (100 mg dose, 197 mg image)	mg
High Shear Granule Blend	163.9
Microcrystalline cellulose	27.6
Croscarmellose Sodium	3.9
Magnesium Stearate	2.0

[00317] A tablet was prepared with the components and amounts listed in Table 18 for Exemplary Tablet 1J comprising 300 mg of API, i.e. crystalline Compound 1 Form I. Exemplary Tablet 1J (formulated to have 300 mg of crystalline Compound 1 Form I) is prepared using a wet high shear granule formulation process. In Table 18, grades/brands were as follows. High Shear Granule Blend - microcrystalline cellulose: Avicel PH101; mannitol: Pearlitol C50; croscarmellose sodium: Acdisol; polyvinylpyrrolidone: Kollidon PVP K30; and in the Core Tablet Composition – microcrystalline cellulose: Avicel PH200; croscarmellose sodium: Acdisol; and magnesium stearate: 5712; and in the film coat – film coat: Opadry II; wax: Carnauba.

Table 18.

High Shear Granule Blend	mg
Compound 1 Form I	300
Microcrystalline cellulose	99
Mannitol	64.5
Croscarmellose Sodium	10.5

Polyvinylpyrrolidone	19.5
Sodium Lauryl Sulfate	4.5
Core Tablet Composition (300 mg dose, 600 mg image)	mg
High Shear Granule Blend	498
Microcrystalline cellulose	84
Croscarmellose Sodium	12
Magnesium Stearate	6
Film Coated Tablet (300 mg dose, 618 mg image)	mg
Core Tablet Composition	600
Film Coat	18
Wax	0.06

[00318] A tablet was prepared with the components and amounts listed in Table 19 for Exemplary Tablet 1K comprising 300 mg of API, i.e. crystalline Compound 1 Form I. Exemplary Tablet 1K (formulated to have 300 mg of crystalline Compound 1 Form I) is prepared using a wet high shear granule formulation process. In Table 19, grades/brands were as follows. High Shear Granule Blend - microcrystalline cellulose: Avicel PH101; mannitol: Pearlitol C50; croscarmellose sodium: Acdisol; polyvinylpyrrolidone: Kollidon PVP K30; and in the Core Tablet Composition – microcrystalline cellulose: Avicel PH200; croscarmellose sodium: Acdisol; and magnesium stearate: 5712; and in the film coat – film coat: Opadry II; wax: Carnauba.

Table 19.

High Shear Granule Blend	mg
Compound 1 Form I	300

Microcrystalline cellulose	100.5
Mannitol	67.5
Croscarmellose Sodium	10.5
Polyvinylpyrrolidone	15.6
Sodium Lauryl Sulfate	3.9
Core Tablet Composition (300 mg dose, 600 mg image)	mg
High Shear Granule Blend	498
Microcrystalline cellulose	84
Croscarmellose Sodium	12
Magnesium Stearate	6
Film Coated Tablet (300 mg dose, 618 mg image)	mg
Core Tablet Composition	600
Film Coat	18
Wax	0.06

[00319] A tablet was prepared with the components and amounts listed in Table 20 for Exemplary Tablet 1L comprising 200 mg of API, i.e. crystalline Compound 1 Form I. Exemplary Tablet 1L (formulated to have 200 mg of crystalline Compound 1 Form I) is prepared using a twin screw wet granulation formulation process. In Table 20, grades/brands were as follows. Twin Screw Granule Blend - microcrystalline cellulose: Avicel PH101; croscarmellose sodium: Acdisol; polyvinylpyrrolidone: Kollidon PVP K30; and in the Core Tablet Composition – microcrystalline cellulose: Avicel PH200; croscarmellose sodium: Acdisol; and magnesium stearate: 5712; and in the film coat – film coat: Opadry II; wax: Carnauba.

Table 20.

Twin Screw Granule Blend	mg
Compound 1 Form I	200
Microcrystalline cellulose	34.0
Croscarmellose Sodium	6.3
Polyvinylpyrrolidone	7.8
Sodium Lauryl Sulfate	1.8
Core Tablet Composition (200 mg dose)	mg
Twin Screw Granule Blend	249.9
Microcrystalline cellulose	36.1
Croscarmellose Sodium	12.0
Magnesium Stearate	3.0
Film Coated Tablet (200 mg dose, 310 mg total)	mg
Core Tablet Composition	301

Film Coat	9.0
Wax	trace

[00320] A tablet was prepared with the components and amounts listed in Table 21 for Exemplary Tablet 1M comprising 400 mg of API, i.e. crystalline Compound 1 Form I. Exemplary Tablet 1M (formulated to have 400 mg of crystalline Compound 1 Form I) is prepared using a twin screw wet granule formulation process. In Table 21, grades/brands were as follows. Twin Screw Granule Blend - microcrystalline cellulose: Avicel PH101; croscarmellose sodium: Acdisol; polyvinylpyrrolidone: Kollidon PVP K30; and in the Core Tablet Composition – microcrystalline cellulose: Avicel PH200; croscarmellose sodium: Acdisol; and magnesium stearate: 5712; and in the film coat – film coat: Opadry II; wax: Carnauba.

Table 21.

Twin Screw Granule Blend	mg
Compound 1 Form I	400
Microcrystalline cellulose	68.0
Croscarmellose Sodium	12.6
Polyvinylpyrrolidone	15.6
Sodium Lauryl Sulfate	3.6
Core Tablet Composition (400 mg dose)	mg
Twin Screw Granule Blend	499.8
Microcrystalline cellulose	72.2
Croscarmellose Sodium	24.0
Magnesium Stearate	6.0
Film Coated Tablet	mg

(400 mg dose, 620 mg total)	
Core Tablet Composition	602
Film Coat	18.0
Wax	trace

[00321] Tablet Formation from Roller Compaction Granule Composition

[00322] Equipment/Process

[00323] *Equipment*

Roller Compactors: Alexanderwerk WP 120, Vector TF-Mini, or Vector TF-Labo.

[00324] *Screening/Weighing*

[00325] Compound 1 and excipients may be screened prior to or after weigh-out. Appropriate screen sizes are mesh 20, mesh 40, or mesh 60. Compound 1 may be pre-blended with one or more of the excipients to simplify screening.

[00326] *Blending*

[00327] Compound 1 and excipients may be added to the blender in different order. The blending may be performed in a Turbula blender or a v-shell blender. The components may be blended for 10 minutes without lubricant followed by additional blending with lubricant for 3 minutes.

[00328] *Roller Compaction*

[00329] The blend may be roller compacted in ribbons and milled into granules using an Alexanderwerk WP 120. The rolls used may be the 25 mm rolls using a compaction pressure of 18 to 50 bar, a roller speed of 3 to 12 RPM, and a screw feeder speed of 20 to 80 RPM. The screen sizes of the integrated mill may be 2 mm for the top screen and 0.8 mm for the bottom screen.

[00330] *Blending*

[00331] The roller compacted granules may be blended with extra-granular excipients such as fillers and lubricant using a V-shell blender. The blending time may be 5, 3 or 1 minute(s).

[00332] *Compression*

[00333] The compression blend has been compressed into tablets using a single station Riva MiniPress with 10 mm tooling. The weight of the tablets for a 100 mg dose may be about 200, 250, or 300 mg.

[00334] *Film Coating*

[00335] Tablets may be film coated using a pan coater, such as, for example an O'Hara Labcoat.

[00336] *Printing*

[00337] Film coated tablets may be printed with a monogram on one or both tablet faces with, for example, a Hartnett Delta printer.

[00338] **Tablet Formation from High Shear Granule Composition**

[00339] Equipment/Process

[00340] *Equipment*

Granulator: Procept MiPro with a 250 ml or 1 L granulation bowl.

[00341] *Screening/Weighing*

[00342] Compound 1 and excipients may be screened prior to or after weigh-out. Possible screen sizes are mesh 20, mesh 40, or mesh 60. Compound 1 may be pre-blended with one or more of the excipients to simplify screening.

[00343] *Granulation Operation*

Granulation Fluid – SLS and binder are added to purified water and mixed until dissolved. A suitable ratio is 2.5% w/w SLS and 10.0% w/w PVP K30 in water.

Granulation – The excipients and compound 1 are added to the granulation bowl. The order of addition may be Compound 1, disintegrant, diluent, and filler. The components may be mixed in the 250 ml bowl for 1 minute at impeller speed 1000 RPM and chopper speed 1000 RPM. Granulation may be performed at an impeller speed of 2000 RPM with a chopper speed of

4000 RPM while adding the granulation fluid with a syringe pump at 1.5 to 4.5 g/min. The fluid addition time may be 4 to 12 minutes. After the required binder fluid is added, the granules may be wet-massed for about 10 seconds to about 1 minute. One notable advantage of the present high shear granulation process is using a granulation fluid that comprises both a surfactant and the binder for better granulation through increased wettability. In one embodiment, the surfactant is SLS.

[00344] *Milling*

[00345] The granules may be reduced in size using a screen mill or a cone mill.

[00346] *Drying*

[00347] The granules may be dried using a vacuum oven, tray dryer, bi-conical dryer, or fluid bed drier. The granules have been dried using a vacuum oven with a nitrogen purge.

[00348] *Blending*

[00349] The granules may be blended with extra-granular excipients. The granules have been blended with extra-granular disintegrant, diluent, filler, and lubricant. The granules have been blended using the Turbula blender for 3 minutes pre-lubricant and 1 minute with lubricant. A larger scale blender such as a 4-quart V-shell blender may be used.

[00350] *Compression*

[00351] The compression blend has been compressed into tablets using a single station Riva MiniPress with 8 mm, or 10 mm tooling. The weight of the tablets for a 100 mg dose may be about 160, 200, or 250 mg.

[00352] *Film Coating*

[00353] Tablets may be film coated using a pan coater, such as, for example an O'Hara Labcoat.

[00354] *Printing*

[00355] Film coated tablets may be printed with a monogram on one or both tablet faces with, for example, a Hartnett Delta printer.

[00356] Tablet Formation from Continuous Twin Screw Wet Granulation Process

[00357] Equipment/Process

[00358] *Equipment*

Granulator: ConsiGma or Leistritz or Thermo Fisher twin screw granulator.

[00359] *Screening/Weighing*

[00360] Compound 1 and excipients may be screened prior to or after weigh-out. Possible screen sizes are mesh 20, mesh 40, or mesh 60. Compound 1 may be pre-blended with one or more of the excipients to simplify screening.

[00361] *Blending*

[00362] Compound 1 and excipients may be added to the blender in different order. The blending may be performed in a Turbula blender, a v-shell blender, a bin blender, or a continuous blender. The components may be blended for 10 minutes for batch blenders or continuously for a continuous blender.

[00363] *Granulation Operation*

Granulation Fluid – SLS and binder are added to purified water and mixed until dissolved. A suitable ratio is 2.5% w/w SLS and 10.0% w/w PVP K30 in water.

Granulation – The blend containing Compound 1 and excipients may be dosed into the twin screw granulator using a Loss in Weight feeder at a rate of 10 kg/hr. The granulation fluid may be added using a peristaltic pump at a rate of 3.5 kg/hr. The granulator may be run at a speed of 400 RPM. A notable advantage of the present twin screw wet granulation process is using a granulation fluid that comprises both a surfactant and the binder for better granulation through increased wettability. In one embodiment, the surfactant is SLS. Another notable advantage is that because the process is continuous and at any moment in time only a limited amount of material is processed, the process can be well controlled and results in a high quality product.

[00364] *Milling*

[00365] The granules may be reduced in size using a screen mill or a cone mill

[00366] *Drying*

[00367] The granules may be dried using a vacuum oven, tray dryer, bi-conical dryer, or fluid bed drier.

[00368] *Blending*

[00369] The granules may be blended with extra-granular excipients. The granules have been blended using a 300 liter bin blender for 60 revolutions.

[00370] *Compression*

[00371] The compression blend has been compressed into tablets using a Courtoy Modul P rotary press

[00372] *Film Coating*

[00373] Tablets may be film coated using a pan coater, such as, for example an O'Hara Labcoat.

[00374] *Printing*

[00375] Film coated tablets may be printed with a monogram on one or both tablet faces with, for example, a Hartnett Delta printer.

[00376] **Dosing Administration Schedule**

[00377] In another aspect, the invention relates to a method of treating a CFTR mediated disease in a subject comprising administering to a subject in need thereof an effective amount of the pharmaceutical composition provided by the invention. In another embodiment, the pharmaceutical composition is administered to the subject once every two weeks. In another embodiment, the pharmaceutical composition is administered to the subject once a week. In another embodiment, the pharmaceutical composition is administered to the subject once every three days. In another embodiment, the pharmaceutical composition is administered to the subject once a day. In one embodiment, when the pharmaceutical composition is a tablet according to Table 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 dosing is once a day.

ASSAYS

[00378] Assays for Detecting and Measuring *F508del*-CFTR Correction Properties of Compounds

[00379] Membrane potential optical methods for assaying *F508del*-CFTR modulation properties of compounds.

[00380] The optical membrane potential assay utilized 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).

[00381] These voltage sensitive assays are based on the change in fluorescence resonant energy transfer (FRET) between the membrane-soluble, voltage-sensitive dye, $DiSBAC_2(3)$, 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 $DiSBAC_2(3)$ to redistribute across the plasma membrane and the amount of energy transfer from CC2-DMPE changes accordingly. The changes in fluorescence emission were 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.

1. Identification of Correction Compounds

[00382] To identify small molecules that correct the trafficking defect associated with *F508del*-CFTR; a single-addition HTS assay format was developed. The cells were incubated in serum-free medium for 16 hrs at 37 °C in the presence or absence (negative control) of test compound. As a positive control, cells plated in 384-well plates were incubated for 16 hrs at 27 °C to "temperature-correct" *F508del*-CFTR. The cells were subsequently rinsed 3X with Krebs Ringers solution and loaded with the voltage-sensitive dyes. To activate *F508del*-CFTR, 10 μM forskolin and the CFTR potentiator, genistein (20 μM), were added along with Cl^--free medium

to each well. The addition of Cl^--free medium promoted Cl^- efflux in response to *F508del*-CFTR activation and the resulting membrane depolarization was optically monitored using the FRET-based voltage-sensor dyes.

2. Identification of Potentiator Compounds

[00383] To identify potentiators of *F508del*-CFTR, a double-addition HTS assay format was developed. During the first addition, a Cl^--free medium with or without test compound was added to each well. After 22 sec, a second addition of Cl^--free medium containing 2 - 10 μM forskolin was added to activate *F508del*-CFTR. The extracellular Cl^- concentration following both additions was 28 mM, which promoted Cl^- efflux in response to *F508del*-CFTR activation and the resulting membrane depolarization was optically monitored using the FRET-based voltage-sensor dyes.

3. Solutions

[00384] Bath Solution #1: (in mM) NaCl 160, KCl 4.5, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, pH 7.4 with NaOH.

[00385] Chloride-free bath solution: Chloride salts in Bath Solution #1 are substituted with gluconate salts.

[00386] CC2-DMPE: Prepared as a 10 mM stock solution in DMSO and stored at -20°C.

$DiSBAC_2(3)$: Prepared as a 10 mM stock in DMSO and stored at -20°C.

4. Cell Culture

[00387] NIH3T3 mouse fibroblasts stably expressing *F508del*-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 30,000/well in 384-well matrigel-coated plates and cultured for 2 hrs at 37 °C before culturing at 27 °C for 24 hrs 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.

[00388] *Electrophysiological Assays for assaying F508del-CFTR modulation properties of compounds*

1. Ussing Chamber Assay

[00389] Using chamber experiments were performed on polarized epithelial cells expressing *F508del*-CFTR to further characterize the *F508del*-CFTR modulators identified in the optical assays. $FRT^{\Delta F508\text{-}CFTR}$ epithelial cells grown on Costar Snapwell cell culture inserts were mounted in an Ussing chamber (Physiologic Instruments, Inc., San Diego, CA), and the monolayers were continuously short-circuited using a Voltage-clamp System (Department of Bioengineering, University of Iowa, IA, and, Physiologic Instruments, Inc., San Diego, CA). Transepithelial resistance was measured by applying a 2-mV pulse. Under these conditions, the FRT epithelia demonstrated resistances of 4 KΩ/ cm^2 or more. The solutions were maintained at 27 °C and bubbled with air. The electrode offset potential and fluid resistance were corrected using a cell-free insert. Under these conditions, the current reflects the flow of Cl^- through *F508del*-CFTR expressed in the apical membrane. The I_{SC} was digitally acquired using an MP100A-CE interface and AcqKnowledge software (*v*3.2.6; BIOPAC Systems, Santa Barbara, CA).

2. Identification of Correction Compounds

[00390] Typical protocol utilized a basolateral to apical membrane Cl^- concentration gradient. To set up this gradient, normal ringer 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. All experiments were performed with intact monolayers. To fully activate *F508del*-CFTR, forskolin (10 μM) and the PDE inhibitor, IBMX (100 μM), were applied followed by the addition of the CFTR potentiator, genistein (50 μM).

[00391] As observed in other cell types, incubation at low temperatures of FRT cells stably expressing *F508del*-CFTR increases the functional density of CFTR in the plasma membrane. To determine the activity of correction compounds, the cells were incubated with 10 μM of the test compound for 24 hours at 37°C and were subsequently washed 3X prior to recording. The cAMP- and genistein-mediated I_{SC} in compound-treated cells was normalized to the 27°C and

37°C controls and expressed as percentage activity. Preincubation of the cells with the correction compound significantly increased the cAMP- and genistein-mediated I_{SC} compared to the 37°C controls.

3. Identification of Potentiator Compounds

[00392] Typical protocol utilized a basolateral to apical membrane Cl^- concentration gradient. To set up this gradient, normal ringers was used on the basolateral membrane and was permeabilized with nystatin (360 μg/ml), 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. All experiments were performed 30 min after nystatin permeabilization. Forskolin (10 μM) and all test compounds were added to both sides of the cell culture inserts. The efficacy of the putative *F508del*-CFTR potentiators was compared to that of the known potentiator, genistein.

4. Solutions

[00393] Basolateral solution (in mM): NaCl (135), $CaCl_2$ (1.2), $MgCl_2$ (1.2), K_2HPO_4 (2.4), $KHPO_4$ (0.6), N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (10), and dextrose (10). The solution was titrated to pH 7.4 with NaOH.

[00394] Apical solution (in mM): Same as basolateral solution with NaCl replaced with Na Gluconate (135).

5. Cell Culture

[00395] Fisher rat epithelial (FRT) cells expressing *F508del*-CFTR ($FRT^{\Delta F508\text{-}CFTR}$) were used for Ussing chamber experiments for the putative *F508del*-CFTR modulators identified from our optical assays. The cells were cultured on Costar Snapwell cell culture inserts and cultured for five days at 37 °C and 5% CO_2 in Coon's modified Ham's F-12 medium supplemented with 5% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Prior to use for characterizing the potentiator activity of compounds, the cells were incubated at 27 °C for 16 - 48 hrs to correct for the *F508del*-CFTR. To determine the activity of corrections compounds, the cells were incubated at 27 °C or 37 °C with and without the compounds for 24 hours.

6. Whole-cell recordings

[00396] The macroscopic *F508del*-CFTR current ($I_{\Delta F508}$) in temperature- and test compound-corrected NIH3T3 cells stably expressing *F508del*-CFTR were monitored using the perforated-patch, whole-cell recording. Briefly, voltage-clamp recordings of $I_{\Delta F508}$ were performed at room temperature using an Axopatch 200B patch-clamp amplifier (Axon Instruments Inc., Foster City, CA). All recordings were acquired at a sampling frequency of 10 kHz and low-pass filtered at 1 kHz. Pipettes had a resistance of 5 – 6 MΩ when filled with the intracellular solution. Under these recording conditions, the calculated reversal potential for Cl^- (E_{Cl}) at room temperature was -28 mV. All recordings had a seal resistance > 20 GΩ and a series resistance < 15 MΩ. 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.). The bath contained < 250 μl of saline and was continuously perifused at a rate of 2 ml/min using a gravity-driven perfusion system,

7. Identification of Correction Compounds

[00397] To determine the activity of correction compounds for increasing the density of functional *F508del*-CFTR in the plasma membrane, we used the above-described perforated-patch-recording techniques to measure the current density following 24-hr treatment with the correction compounds. To fully activate *F508del*-CFTR, 10 μM forskolin and 20 μM genistein were added to the cells. Under our recording conditions, the current density following 24-hr incubation at 27°C was higher than that observed following 24-hr incubation at 37 °C. These results are consistent with the known effects of low-temperature incubation on the density of *F508del*-CFTR in the plasma membrane. To determine the effects of correction compounds on CFTR current density, the cells were incubated with 10 μM of the test compound for 24 hours at 37°C and the current density was compared to the 27°C and 37°C controls (% activity). Prior to recording, the cells were washed 3X with extracellular recording medium to remove any remaining test compound. Preincubation with 10 μM of correction compounds significantly increased the cAMP- and genistein-dependent current compared to the 37°C controls.

8. Identification of Potentiator Compounds

[00398] The ability of *F508del*-CFTR potentiators to increase the macroscopic *F508del*-CFTR Cl^- current ($I_{\Delta F508}$) in NIH3T3 cells stably expressing *F508del*-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).

9. Solutions

[00399] Intracellular solution (in mM): Cs-aspartate (90), CsCl (50), $MgCl_2$ (1), HEPES (10), and 240 μg/ml amphotericin-B (pH adjusted to 7.35 with CsOH).

[00400] Extracellular solution (in mM): N-methyl-D-glucamine (NMDG)-Cl (150), $MgCl_2$ (2), $CaCl_2$ (2), HEPES (10) (pH adjusted to 7.35 with HCl).

10. Cell Culture

[00401] NIH3T3 mouse fibroblasts stably expressing *F508del*-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.

11. Single-channel recordings

[00402] The single-channel activities of temperature-corrected *F508del*-CFTR stably expressed in NIH3T3 cells and activities of potentiator compounds were observed using excised inside-out membrane patch. Briefly, voltage-clamp recordings of single-channel activity were performed at room temperature with an Axopatch 200B patch-clamp amplifier (Axon Instruments Inc.). All recordings were acquired at a sampling frequency of 10 kHz and low-pass filtered at 400 Hz. Patch pipettes were fabricated from Corning Kovar Sealing #7052 glass (World Precision Instruments, Inc., Sarasota, FL) and had a resistance of 5 - 8 MΩ when filled

with the extracellular solution. The *F508del*-CFTR was activated after excision, by adding 1 mM Mg-ATP, and 75 nM of the cAMP-dependent protein kinase, catalytic subunit (PKA; Promega Corp. Madison, WI). After channel activity stabilized, the patch was perifused using a gravity-driven microperfusion system. The inflow was placed adjacent to the patch, resulting in complete solution exchange within 1 - 2 sec. To maintain *F508del*-CFTR activity during the rapid perifusion, the nonspecific phosphatase inhibitor F^- (10 mM NaF) was added to the bath solution. Under these recording conditions, channel activity remained constant throughout the duration of the patch recording (up to 60 min). Currents produced by positive charge moving from the intra- to extracellular solutions (anions moving in the opposite direction) are shown as positive currents. The pipette potential (V_p) was maintained at 80 mV.

[00403] 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 *F508del*-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.

12. Solutions

[00404] Extracellular solution (in mM): NMDG (150), aspartic acid (150), $CaCl_2$ (5), $MgCl_2$ (2), and HEPES (10) (pH adjusted to 7.35 with Tris base).

[00405] Intracellular solution (in mM): NMDG-Cl (150), $MgCl_2$ (2), EGTA (5), TES (10), and Tris base (14) (pH adjusted to 7.35 with HCl).

13. Cell Culture

[00406] NIH3T3 mouse fibroblasts stably expressing *F508del*-CFTR are used for excised-membrane patch-clamp 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 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.

[00407] Using the procedures described above, the activity, i.e., EC50s, of Compound 1 has been measured and is shown in Table 20.

Table 20.

IC50/EC50 Bins: +++ <= 2.0 < ++ <= 5.0 < +		
PercentActivity Bins: + <= 25.0 < ++ <= 100.0 < +++		
Cmpd. No.	BinnedEC50	BinnedMaxEfficacy
1	+++	+++

OTHER EMBODIMENTS

[00408] All publications and patents referred to in this disclosure are incorporated herein by reference to the same extent as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Should the meaning of the terms in any of the patents or publications incorporated by reference 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 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 tablet for oral administration comprising:

a. Compound 1, Compound 1 Form I, Compound 1 Form II, and/or Compound 1 HCl Salt Form A;

b. a filler;

c. a disintegrant;

d. a surfactant;

e. a lubricant; and

f. at least one of a binder or a glidant.

2. The tablet of claim 1, wherein Compound 1, Compound 1 Form I, Compound 1 Form II, and/or Compound 1 HCl Salt Form A is present in the tablet in an amount ranging from about 25 mg to about 500 mg.

3. The tablet of claim 1, wherein the amount of Compound 1, Compound 1 Form I, Compound 1 Form II, and/or Compound 1 HCl Salt Form A in the tablet ranges from about 15 wt% to about 75 wt% by weight of the tablet.

4. The tablet of claim 1, wherein the amount of Compound 1, Compound 1 Form I, Compound 1 Form II, and/or Compound 1 HCl Salt Form A in the tablet ranges from about 40 wt% to about 70 wt% by weight of the tablet.

5. The tablet of claim 1 having the following formulation:

Roller Compaction Granule Blend	(%w/w)
Compound 1	20-40
Microcrystalline cellulose	30-50
Mannitol	10-30
Croscarmellose Sodium	1-5
Sodium Lauryl Sulfate	0.1-2

Colloidal Silica	0.1-1
Magnesium Stearate	1-3
Tablet Composition	(%w/w)
Roller Compaction Granule Blend	99-99.9
Magnesium Stearate	0.1-1

6. The tablet of claim 1 having the following formulation:

High Shear Granule Blend	(%w/w)
Compound 1	60-70
Microcrystalline cellulose	5-15
Croscarmellose Sodium	1-5
Sodium Lauryl Sulfate	0.1-2
Polyvinylpyrrolidone	1-5
Tablet Composition	(%w/w)
High Shear Granule Blend	75-89
Microcrystalline cellulose	10-15
Croscarmellose Sodium	1-5
Magnesium Stearate	0.1-5

7. The tablet of claim 1 having the following formulation:

High Shear Granule Blend	(%w/w)
Compound 1 Form I	60-70
Microcrystalline cellulose	5-15
Croscarmellose Sodium	1-5

Polyvinylpyrrolidone	1-5
Sodium Lauryl Sulfate	0.1-2
Tablet Composition	(%w/w)
High Shear Granule Blend	78-89
Microcrystalline cellulose	10-15
Croscarmellose Sodium	1-5
Magnesium Stearate	0.1-2
Film Coated Tablet	(%w/w)
Core Tablet Composition	95-99
Film Coat	1-5
Wax	Trace

8. The tablet of claim 1 having the following formulation:

Roller Compaction Granule Blend	(%w/w)
Compound 1 Form I	30
Microcrystalline cellulose	42.3
Mannitol	21.2
Croscarmellose Sodium	3
Sodium Lauryl Sulfate	1
Colloidal Silica	0.5
Magnesium Stearate	2
Tablet Composition	(%w/w)
Roller Compaction Granule Blend	99.5
Magnesium Stearate	0.5

9. The tablet of claim 1 having the following formulation:

High Shear Granule Blend	(%w/w)
Compound 1 Form I	40-80
Microcrystalline cellulose	20-40
Mannitol	10-15
Croscarmellose Sodium	1-5
Polyvinylpyrrolidone	1-10
Sodium Lauryl Sulfate	0.1-2
Tablet Composition	**(%w/w)**
High Shear Granule Blend	95-99
Croscarmellose Sodium	1-4
Magnesium Stearate	0.1-1

10. The tablet of claim 1 having the following formulation:

High Shear Granule Blend	(%w/w)
Compound 1 Form I	50
Microcrystalline cellulose	30
Mannitol	13
Croscarmellose Sodium	2
Polyvinylpyrrolidone	4
Sodium Lauryl Sulfate	1
Tablet Composition	**(%w/w)**
High Shear Granule Blend	97.5

Croscarmellose Sodium	2.0
Magnesium Stearate	0.5

11. The tablet of claim 1 having the following formulation:

High Shear Granule Blend	(%w/w)
Compound 1 Form I	60
Microcrystalline cellulose	20
Mannitol	13
Croscarmellose Sodium	2
Polyvinylpyrrolidone	4
Sodium Lauryl Sulfate	1
Tablet Composition	**(%w/w)**
High Shear Granule Blend	97.5
Croscarmellose Sodium	2.0
Magnesium Stearate	0.5

12. The tablet of claim 1 having the following formulation:

High Shear Granule Blend	(%w/w)
Compound 1 Form I	60
Microcrystalline cellulose	20
Mannitol	13
Croscarmellose Sodium	2
Polyvinylpyrrolidone	4
Sodium Lauryl Sulfate	1

Tablet Composition	(%w/w)
High Shear Granule Blend	83
Microcrystalline cellulose	14
Croscarmellose Sodium	2
Magnesium Stearate	1

13. The tablet of claim 1 having the following formulation:

Twin Screw Granule Blend	(%w/w)
Compound 1 Form I	60
Microcrystalline cellulose	20
Mannitol	13
Croscarmellose Sodium	2
Polyvinylpyrrolidone	4
Sodium Lauryl Sulfate	1

Tablet Composition	(%w/w)
Twin Screw Granule Blend	83
Microcrystalline cellulose	14
Croscarmellose Sodium	2
Magnesium Stearate	1

14. The tablet of claim 1 having the following formulation:

Twin Screw Wet Granule Blend	(%w/w)
Compound 1 Form I	80.0
Microcrystalline cellulose	13.6

Croscarmellose Sodium	2.5
Polyvinylpyrrolidone	3.1
Sodium Lauryl Sulfate	0.7
Tablet Composition	(%w/w)
Twin Screw Granule Blend	83
Microcrystalline cellulose	12
Croscarmellose Sodium	4
Magnesium Stearate	1

15. The tablet of claim 1 having the following formulation:

Twin Screw Granule Blend	(%w/w)
Compound 1 Form I	80.0
Microcrystalline cellulose	13.6
Croscarmellose Sodium	2.5
Polyvinylpyrrolidone	3.1
Sodium Lauryl Sulfate	0.7
Tablet Composition	(%w/w)
Twin Screw Granule Blend	83
Microcrystalline cellulose	12
Croscarmellose Sodium	4
Magnesium Stearate	1
Film Coated Tablet	(%w/w)
Core Tablet Composition	97

Film Coat	3
Wax	Trace

16. The tablet of claim 1 having the following formulation:

High Shear Granule Blend	mg
Compound 1 Form I	200
Microcrystalline cellulose	66
Mannitol	43
Croscarmellose Sodium	7
Polyvinylpyrrolidone	13
Sodium Lauryl Sulfate	3
Core Tablet Composition (200 mg dose)	mg
High Shear Granule Blend	332
Microcrystalline cellulose	56
Croscarmellose Sodium	8
Magnesium Stearate	4
Film Coated Tablet (200 mg dose)	mg
Core Tablet Composition	400
Film Coat	12
Wax	trace

17. The tablet of claim 1 having the following formulation:

Twin Screw Granule Blend	mg
Compound 1 Form I	200
Microcrystalline cellulose	66
Mannitol	43
Croscarmellose Sodium	7
Polyvinylpyrrolidone	13
Sodium Lauryl Sulfate	3
Core Tablet Composition (200 mg dose)	mg
Twin Screw Granule Blend	332
Microcrystalline cellulose	56
Croscarmellose Sodium	8
Magnesium Stearate	4

18. The tablet of claim 1 having the following formulation:

High Shear Granule Blend	mg
Compound 1 Form I	200
Microcrystalline cellulose	67
Mannitol	45
Croscarmellose Sodium	7
Polyvinylpyrrolidone	10.4
Sodium Lauryl Sulfate	2.6
Core Tablet Composition (200 mg dose)	mg

High Shear Granule Blend	332
Microcrystalline cellulose	56
Croscarmellose Sodium	8
Magnesium Stearate	4
Film Coated Tablet (200 mg dose)	mg
Core Tablet Composition	400
Film Coat	12
Wax	trace

19. The tablet of claim 1 having the following formulation:

High Shear Granule Blend	mg
Compound 1 Form I	300
Microcrystalline cellulose	99
Mannitol	64.5
Croscarmellose Sodium	10.5
Polyvinylpyrrolidone	19.5
Sodium Lauryl Sulfate	4.5
Core Tablet Composition (300 mg dose)	mg
High Shear Granule Blend	498
Microcrystalline cellulose	84
Croscarmellose Sodium	12
Magnesium Stearate	6

Film Coated Tablet (300 mg dose)	mg
Core Tablet Composition	600
Film Coat	18
Wax	trace

20. The tablet of claim 1 having the following formulation:

High Shear Granule Blend	mg
Compound 1 Form I	300
Microcrystalline cellulose	100.5
Mannitol	67.5
Croscarmellose Sodium	10.5
Polyvinylpyrrolidone	15.6
Sodium Lauryl Sulfate	3.9
Core Tablet Composition (300 mg dose)	**mg**
High Shear Granule Blend	498
Microcrystalline cellulose	84
Croscarmellose Sodium	12
Magnesium Stearate	6
Film Coated Tablet (300 mg dose)	**mg**
Core Tablet Composition	600
Film Coat	18

Wax	trace

21. The tablet of claim 1 having the following formulation:

High Shear Granule Blend	(%w/w)
Compound 1 Form I	70
Microcrystalline cellulose	12
Mannitol	11
Croscarmellose Sodium	2
Polyvinylpyrrolidone	4
Sodium Lauryl Sulfate	1
Tablet Composition	**(%w/w)**
High Shear Granule Blend	97.5
Croscarmellose Sodium	2.0
Magnesium Stearate	0.5

22. The tablet of claim 1 having the following formulation:

High Shear Granule Blend	(%w/w)
Compound 1 Form I or Form II	61
Microcrystalline cellulose	20.3
Mannitol	13.2
Croscarmellose Sodium	2
Polyvinylpyrrolidone	2.7
Sodium Lauryl Sulfate	0.7
Tablet Composition	**(%w/w)**

High Shear Granule Blend	83
Microcrystalline cellulose	14
Croscarmellose Sodium	2
Magnesium Stearate	1

23. The tablet of claim 1 having the following formulation:

High Shear Granule Blend	mg
Compound 1 Form I or Form II	100
Microcrystalline cellulose	33.3
Mannitol	21.7
Croscarmellose Sodium	3.3
Polyvinylpyrrolidone	4.4
Sodium Lauryl Sulfate	1.1
Core Tablet Composition (100 mg dose)	mg
High Shear Granule Blend	163.9
Microcrystalline cellulose	27.6
Croscarmellose Sodium	3.9
Magnesium Stearate	2.0

24. The tablet of claim 1 having the following formulation:

Twin Screw Granule Blend	mg
Compound 1 Form I	200
Microcrystalline cellulose	34.0

Croscarmellose Sodium	6.3
Polyvinylpyrrolidone	7.8
Sodium Lauryl Sulfate	1.8
Core Tablet Composition (200 mg dose)	mg
Twin Screw Granule Blend	249.9
Microcrystalline cellulose	36.1
Croscarmellose Sodium	12.0
Magnesium Stearate	3.0

25. The tablet of claim 1 having the following formulation:

Twin Screw Granule Blend	mg
Compound 1 Form I	400
Microcrystalline cellulose	68.0
Croscarmellose Sodium	12.6
Polyvinylpyrrolidone	15.6
Sodium Lauryl Sulfate	3.6
Core Tablet Composition (400 mg dose)	mg
Twin Screw Granule Blend	499.8
Microcrystalline cellulose	72.2
Croscarmellose Sodium	24.0
Magnesium Stearate	6.0

26. The tablet of claim 1 having the following formulation:

Twin Screw Granule Blend	mg
Compound 1 Form I	200
Microcrystalline cellulose	34.0
Croscarmellose Sodium	6.3
Polyvinylpyrrolidone	7.8
Sodium Lauryl Sulfate	1.8
Core Tablet Composition (200 mg dose)	mg
Twin Screw Granule Blend	249.9
Microcrystalline cellulose	36.1
Croscarmellose Sodium	12.0
Magnesium Stearate	3.0
Film Coated Tablet (200 mg dose, 310 mg total)	mg
Core Tablet Composition	301
Film Coat	9.0
Wax	trace

27. The tablet of claim 1 having the following formulation:

Twin Screw Granule Blend	mg
Compound 1 Form I	400
Microcrystalline cellulose	68.0
Croscarmellose Sodium	12.6

Polyvinylpyrrolidone	15.6
Sodium Lauryl Sulfate	3.6
Core Tablet Composition (400 mg dose)	mg
Twin Screw Granule Blend	499.8
Microcrystalline cellulose	72.2
Croscarmellose Sodium	24.0
Magnesium Stearate	6.0
Film Coated Tablet (400 mg dose, 620 mg total)	mg
Core Tablet Composition	602
Film Coat	18.0
Wax	trace

28. The tablet of claim 1, wherein the tablet further comprises at least one additional therapeutic agent.

29. The tablet of claim 28, wherein the additional therapeutic agent is a CFTR modulator.

30. The tablet of claim 29, wherein the CFTR modulator is a CFTR potentiator.

31. The tablet of claim 29, wherein the CFTR modulator is N-(5-hydroxy-2,4-ditert-butyl-phenyl)-4-oxo-1H-quinoline-3-carboxamide.

32. The tablet of claim 1, wherein Compound 1 is in Form I characterized by one or more peaks at 15.2 to 15.6 degrees, 16.1 to 16.5 degrees, and 14.3 to 14.7 degrees in an X-ray powder diffraction obtained using Cu K alpha radiation.

33. The tablet of claim 32, wherein Compound 1 Form I is characterized by one or more peaks at 15.4, 16.3, and 14.5 degrees.

34. The tablet of claim 1, wherein Compound 1 is in Form I characterized by a diffraction pattern substantially similar to that of Figure 1.

35. The tablet of claim 1, wherein Compound 1 is in Form I characterized by a diffraction pattern substantially similar to that of Figure 2.

36. A method of treating or lessening the severity of a disease in a patient comprising administering to the patient the tablet of claim 1, wherein the disease is selected from cystic fibrosis, asthma, smoke induced COPD, chronic bronchitis, rhinosinusitis, constipation, pancreatitis, pancreatic insufficiency, male infertility, mild pulmonary disease, idiopathic pancreatitis, allergic bronchopulmonary aspergillosis (ABPA), liver disease, hereditary emphysema, hereditary hemochromatosis, coagulation-fibrinolysis deficiencies, protein C deficiency, Type 1 hereditary angioedema, lipid processing deficiencies, familial hypercholesterolemia, Type 1 chylomicronemia, abetalipoproteinemia, lysosomal storage diseases, I-cell disease/pseudo-Hurler, mucopolysaccharidoses, Sandhof/Tay-Sachs, Crigler-Najjar type II, polyendocrinopathy/hyperinsulemia, Diabetes mellitus, Laron dwarfism, myleoperoxidase 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, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, progressive supranuclear plasy, Pick's disease, several polyglutamine neurological disorders, Huntington's, spinocerebullar ataxia type I, spinal and bulbar muscular atrophy, dentatorubal pallidoluysian, myotonic dystrophy, spongiform encephalopathies, hereditary Creutzfeldt-Jakob disease, Fabry disease, Straussler-Scheinker syndrome, COPD, dry-eye disease, Sjogren's disease, Osteoporosis, Osteopenia, Gorham's Syndrome, chloride channelopathies, myotonia congenita, Bartter's syndrome type III, Dent's disease, hyperekplexia, epilepsy, hyperekplexia, lysosomal storage disease, Angelman syndrome, Primary Ciliary Dyskinesia (PCD), inherited disorders of the structure and/or function of cilia, PCD with situs inversus, PCD without situs inversus, or ciliary aplasia.

37. The method of claim 36, wherein the disease is cystic fibrosis, emphysema, COPD, or dry-eye disease.

38. The method of claim 36, wherein the disease is cystic fibrosis wherein the patient has a *F508del* CFTR mutation.

39. The method of claim 38, wherein the patient is homozygous for *F508del.*

40. The method of claim 38, wherein the patient is heterozygous for *F508del.*

41. The method of claim 36, wherein the method comprises administering an additional therapeutic agent.

42. The method of claim 41, wherein the therapeutic agent is selected from a mucolytic agent, bronchodilator, an anti-biotic, an anti-infective agent, an anti-inflammatory agent, a CFTR potentiator, or a nutritional agent.

43. The method of claim 41, wherein the additional therapeutic agent is N-(5-hydroxy-2,4-ditert-butyl-phenyl)-4-oxo-1H-quinoline-3-carboxamide.

44. A kit comprising the tablet of claim 1, and a separate therapeutic agent or pharmaceutical composition thereof.

45. The kit of claim 44, wherein the Compound 1 is in Form I.

46. The kit of claim 44, wherein the therapeutic agent is a cystic fibrosis corrector other than Compound 1.

47. The kit of claim 44, wherein the therapeutic agent is a cystic fibrosis potentiator.

48. The kit of claim 44, wherein the therapeutic agent is N-(5-hydroxy-2,4-ditert-butyl-phenyl)-4-oxo-1H-quinoline-3-carboxamide.

49. The kit of claim 44, wherein the tablet of claim 1 and the therapeutic agent are in separate containers.

50. The kit of claim 49, wherein the separate containers are bottles.

51. The kit of claim 49, wherein the separate containers are vials.

52. The kit of claim 49, wherein the separate containers are blister packs.

53. A continuous process for preparing a tablet comprising Compound 1 comprising the steps of:

a) mixing Compound 1, a filler, and a disintegrant in a blender to form a blend;

b) preparing a granulation solution with water, a binder, and a surfactant;

c) feeding the blend from step a) into a continuous twin screw granulator while adding the granulation solution from step b) to produce granules;

d) drying the granules from step c) and milling them;

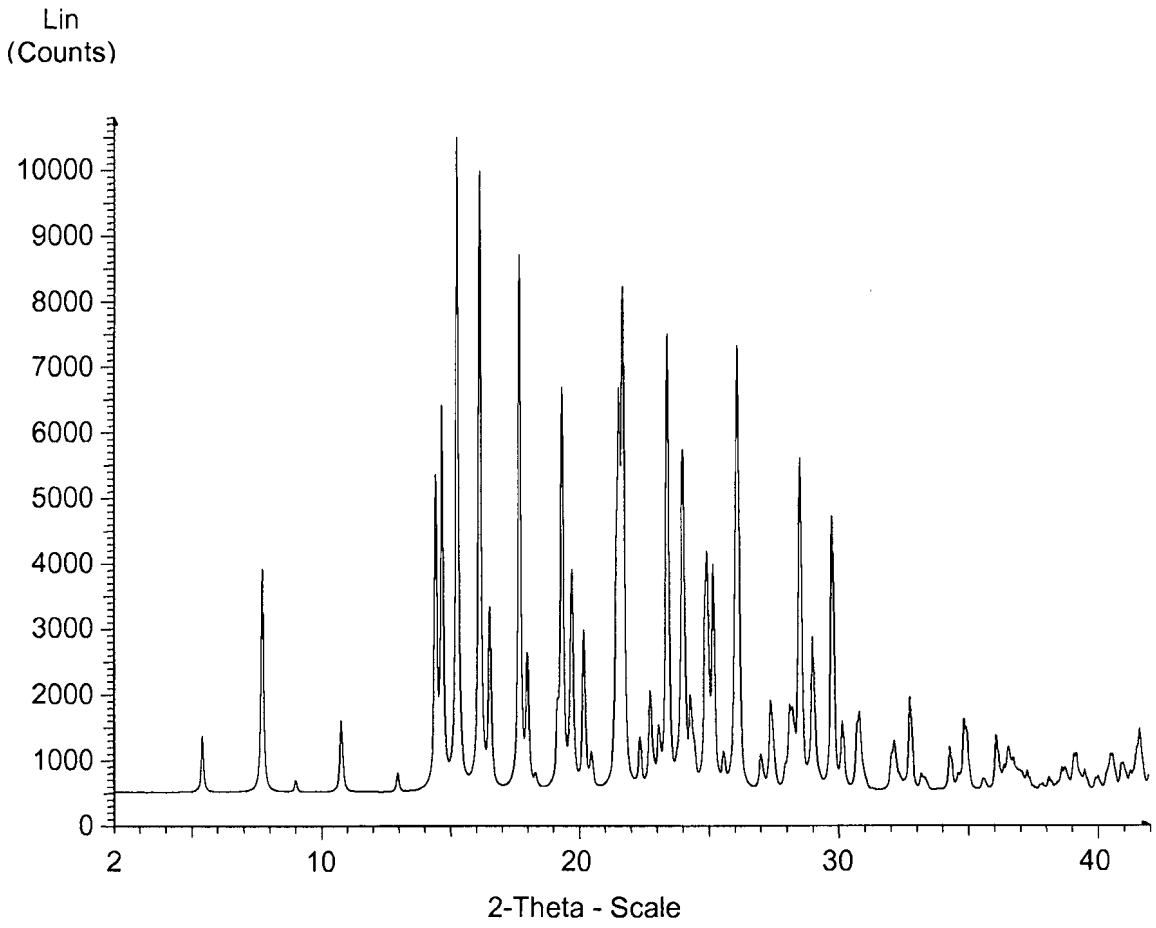
e) blending the milled granules from step d) with a filler, disintegrant, and lubricant to form a blend;

f) compressing the blend from step d) into a tablet; and

g) coating the tablet from step e).

54. The process of claim 53, wherein Compound 1 is in Form I.

Figure 1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(Counts)
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4000
3000
2000
1000
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2
10
20
30
40
2-Theta - Scale

Figure 2

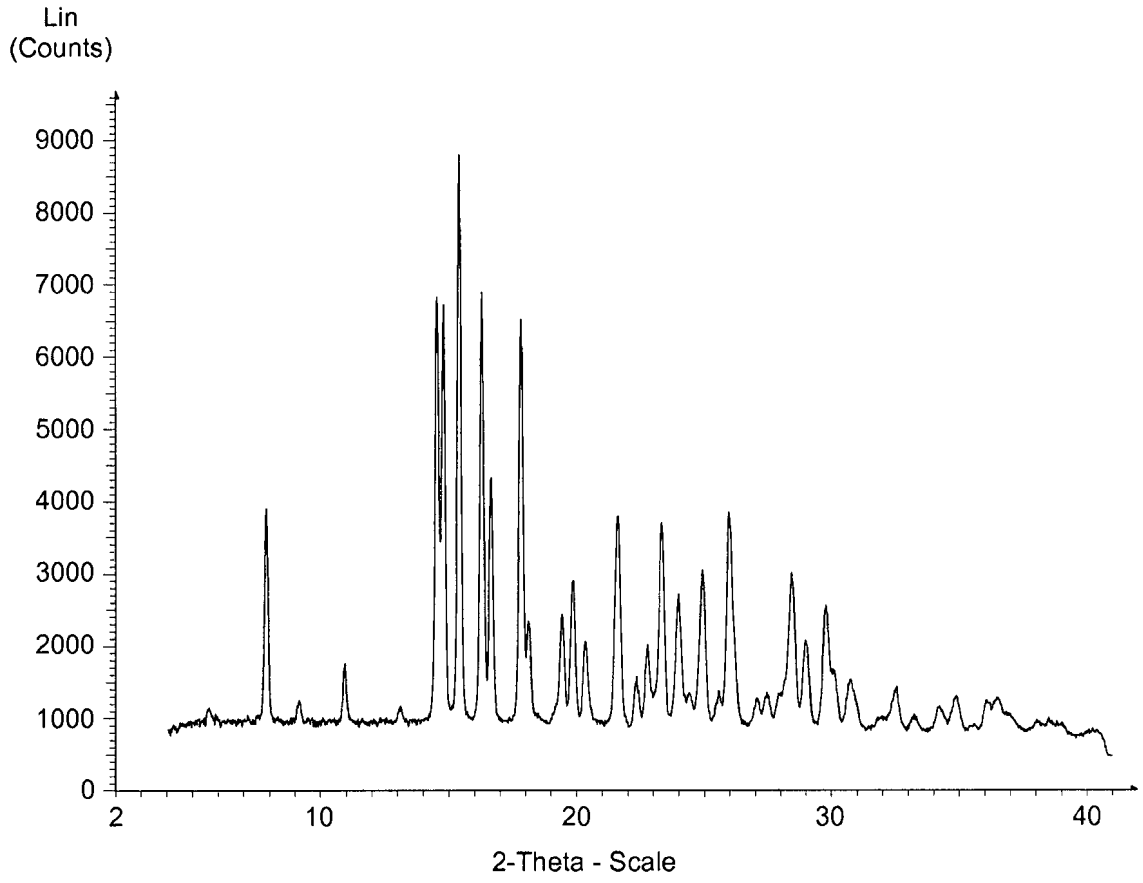
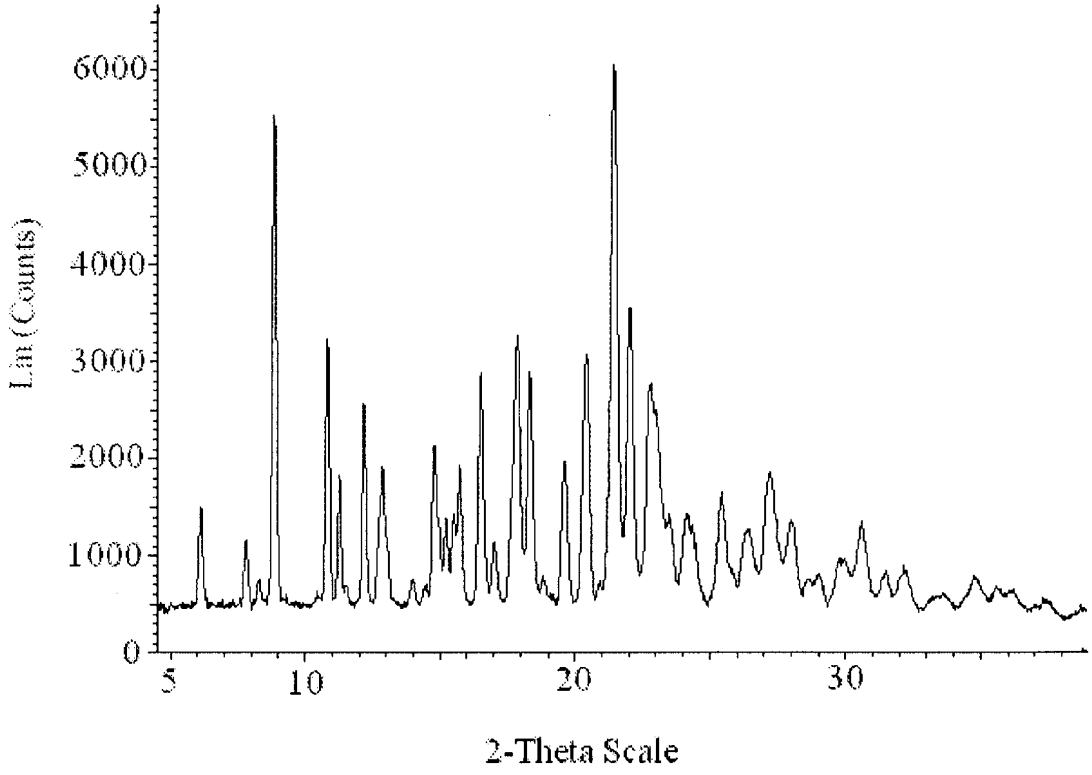

Lin
(Counts)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
2
10
20
30
40
2-Theta - Scale

Figure 3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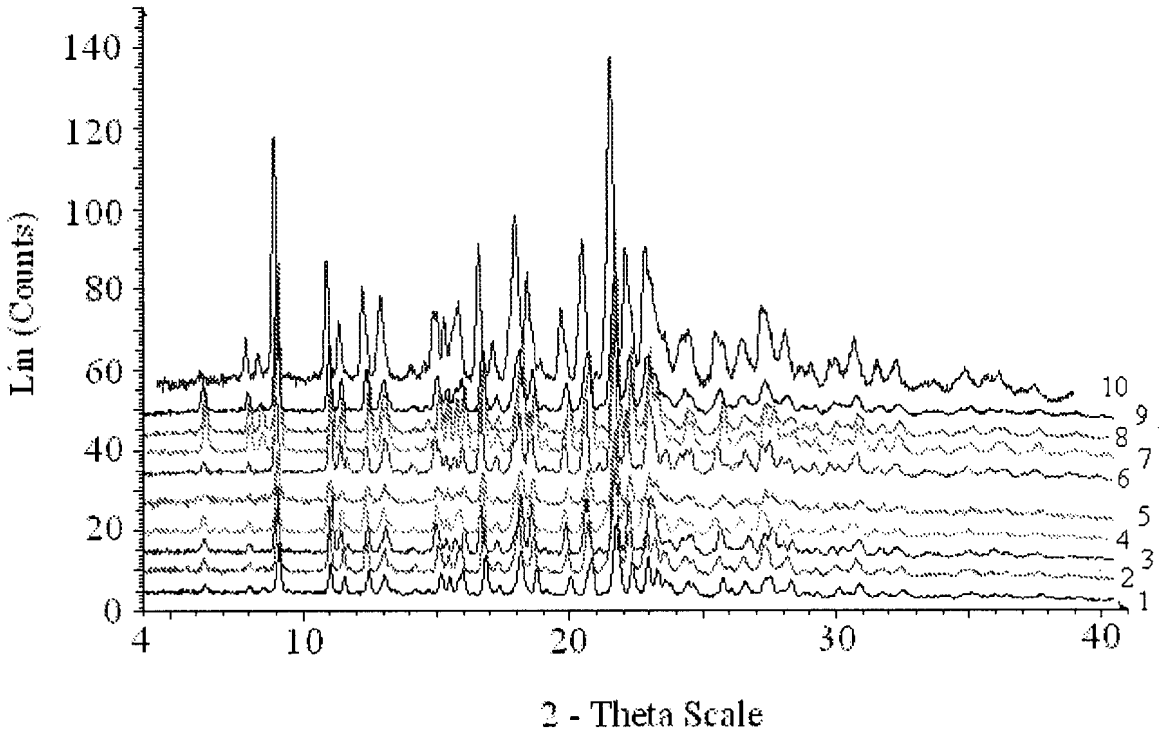
5000
4000
3000
2000
1000
0
Lin (Counts)
5
10
20
30
2-Theta Scale

Figure 4


Lin (Counts)
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100
80
60
40
20
0
4
10
20
30
40
2 - Theta Scale
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9
8
7
6
5
4
3
2
1

Figure 5

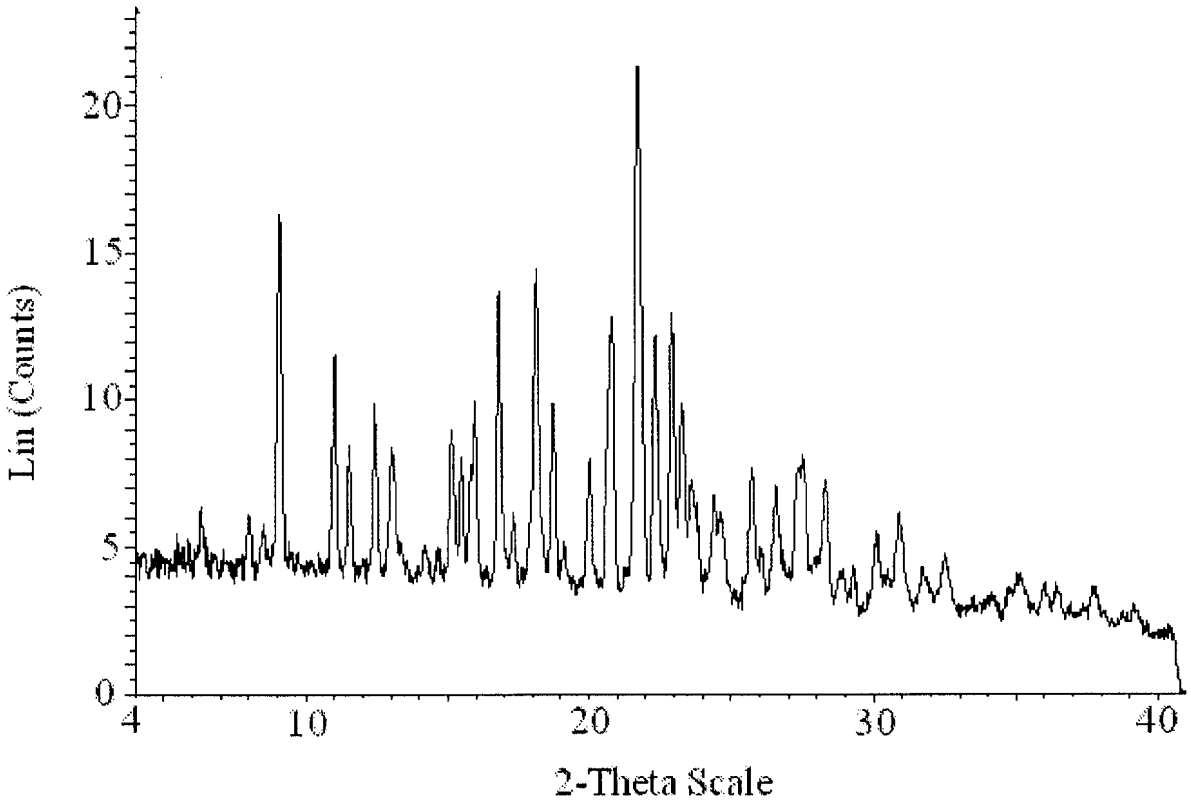


Figure 6

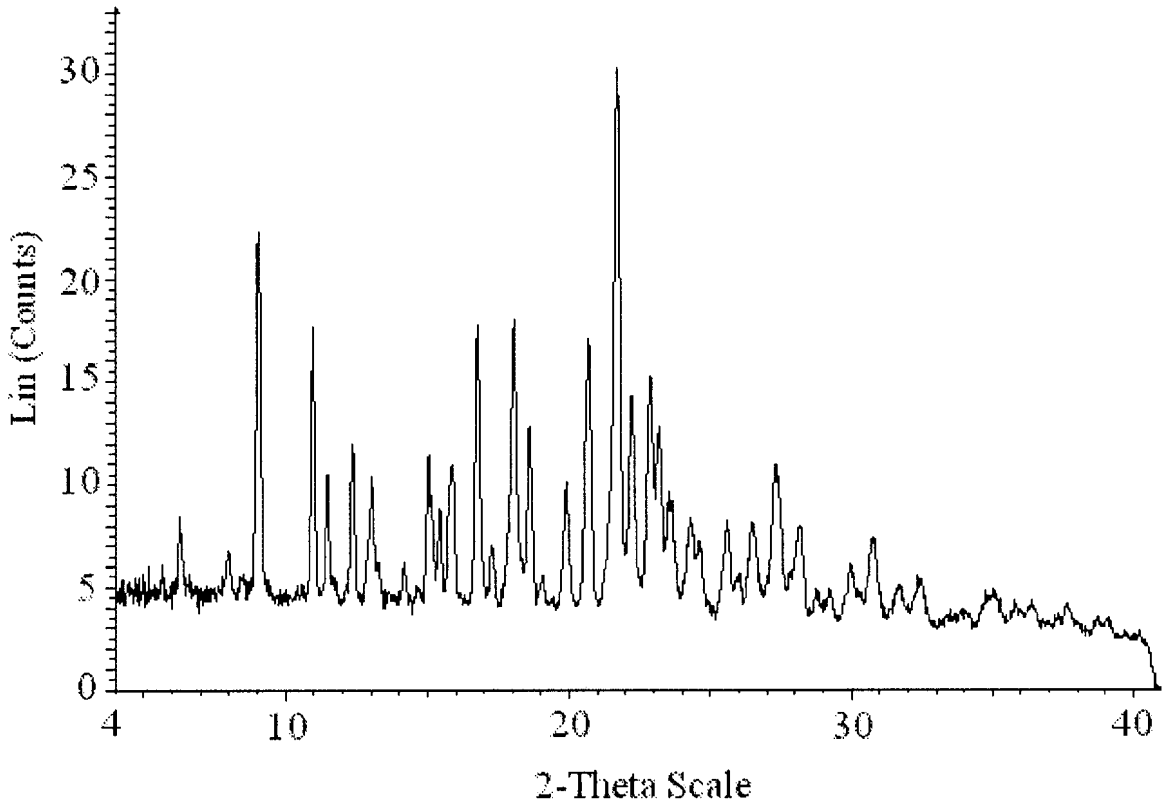


Figure 7

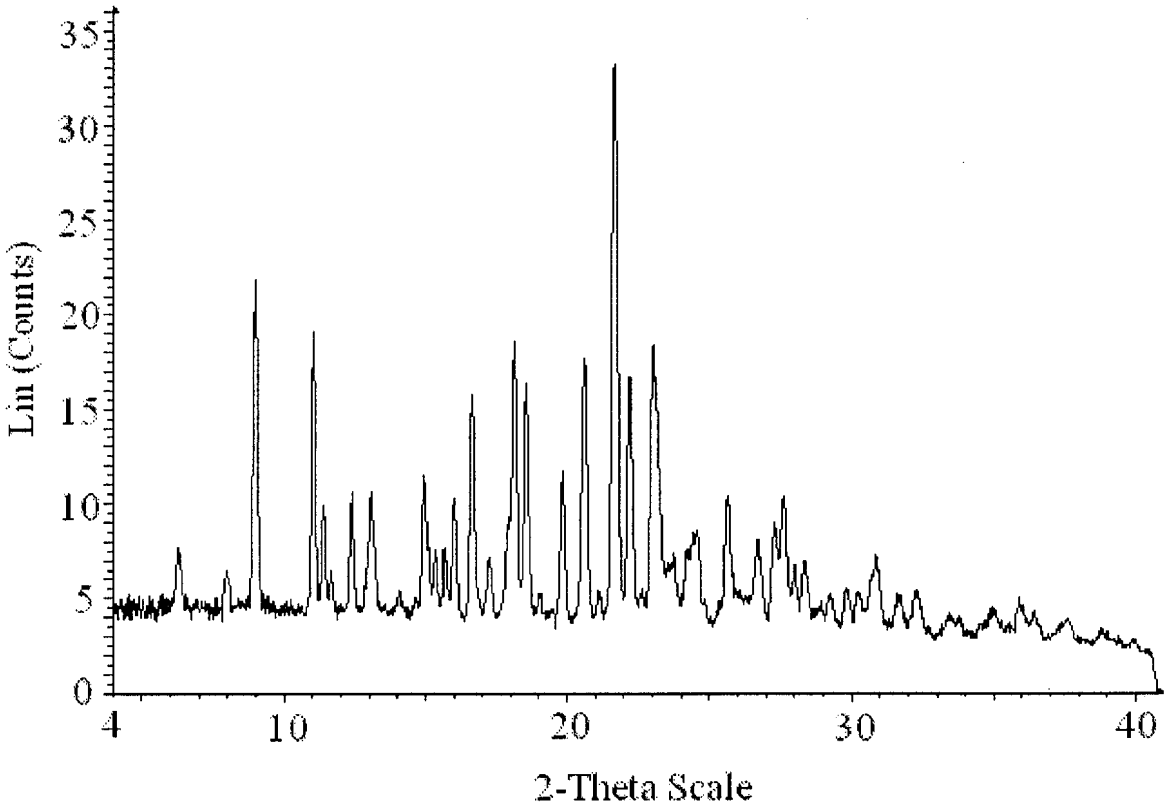


Figure 8

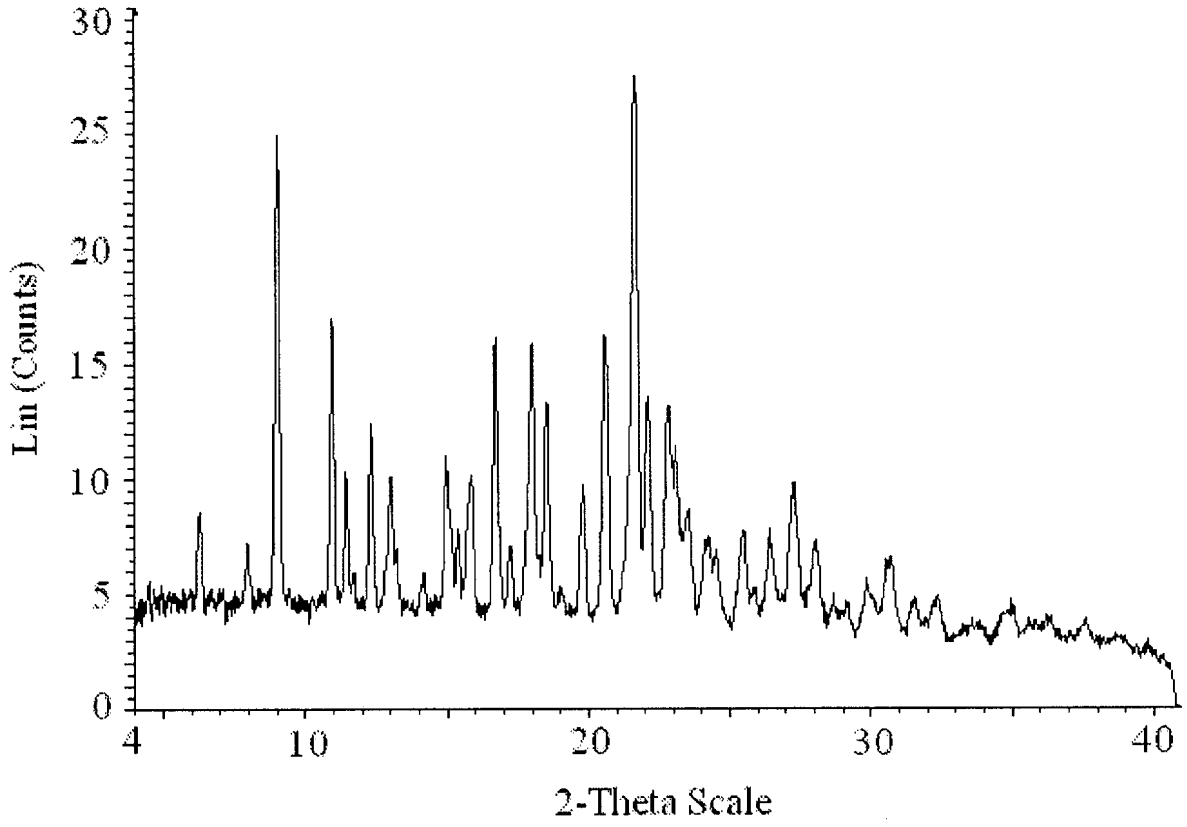


Figure 9

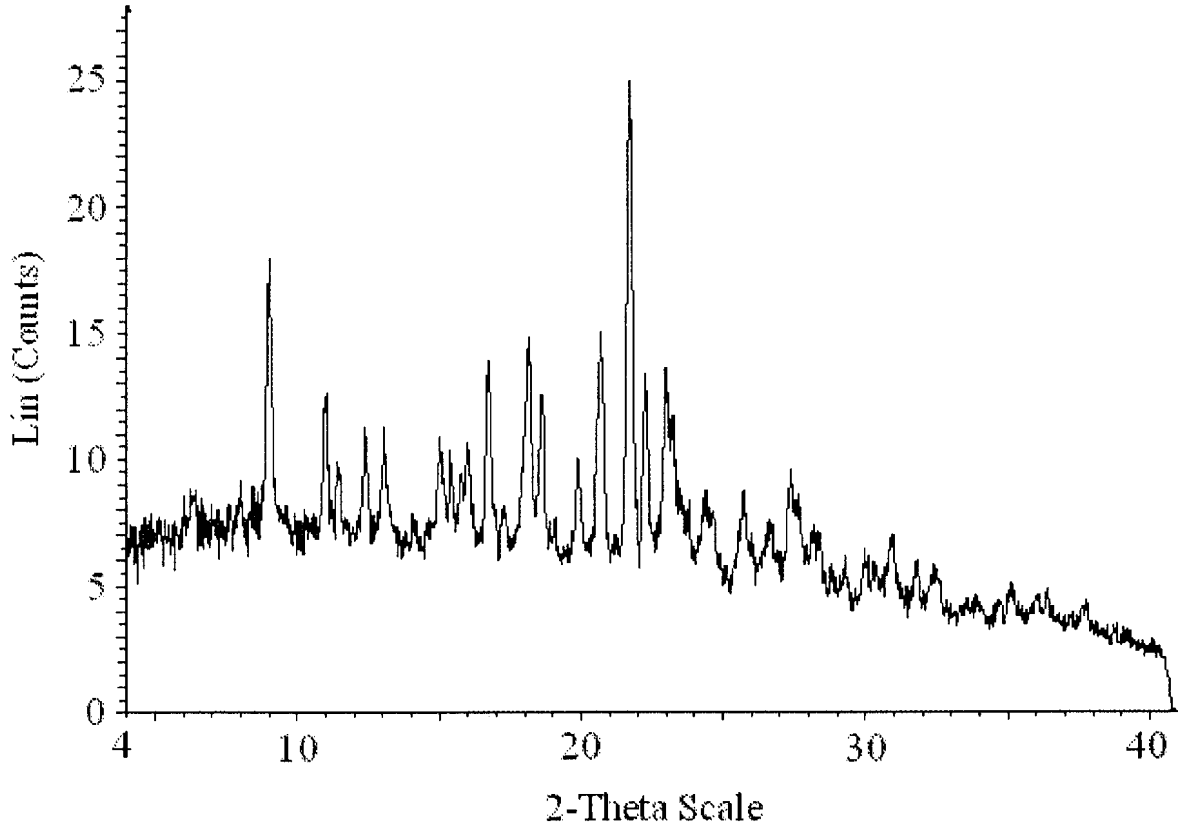


Figure 10

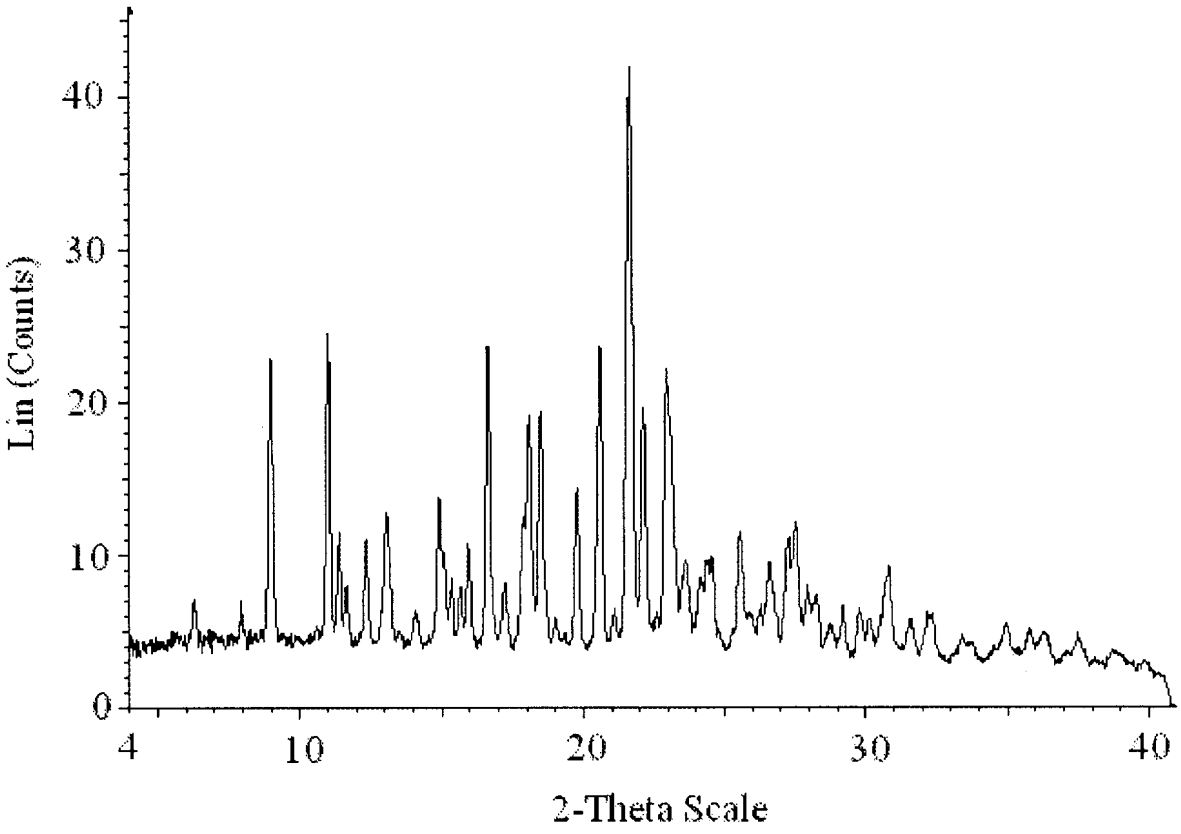


Figure 11

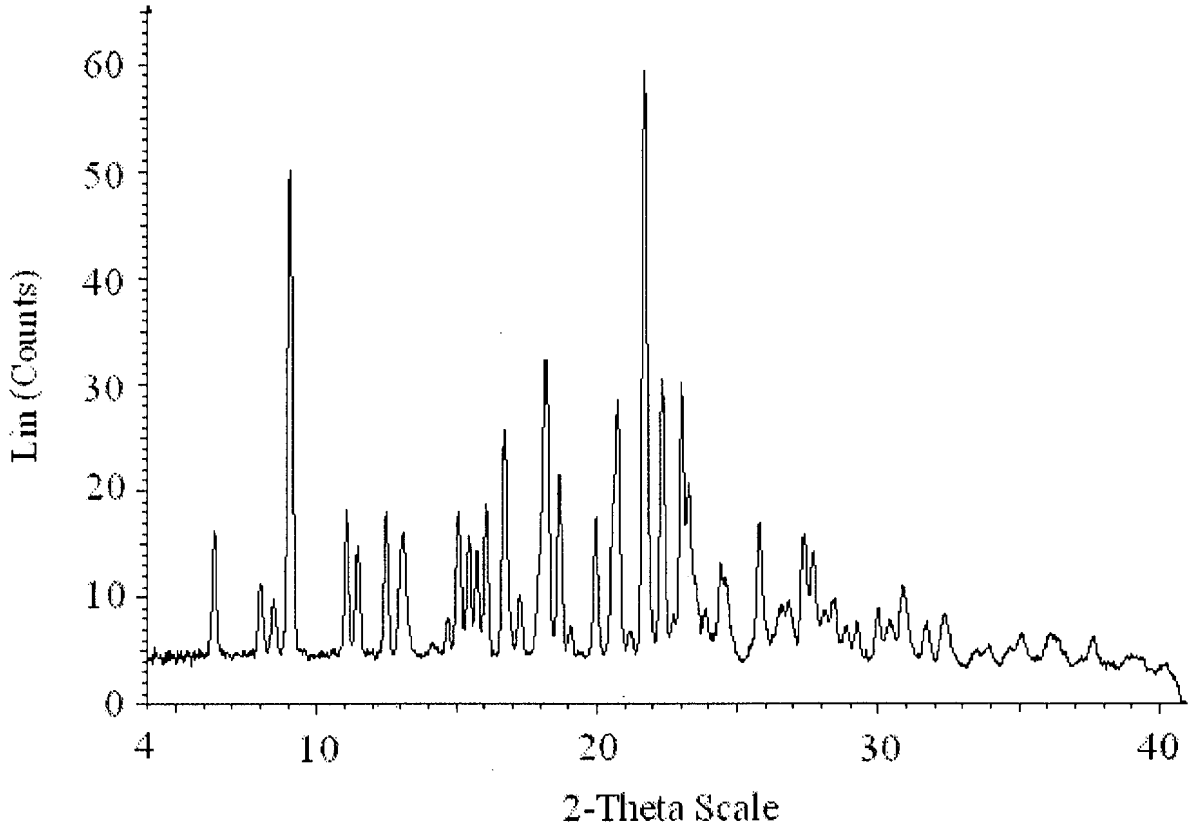


Figure 12

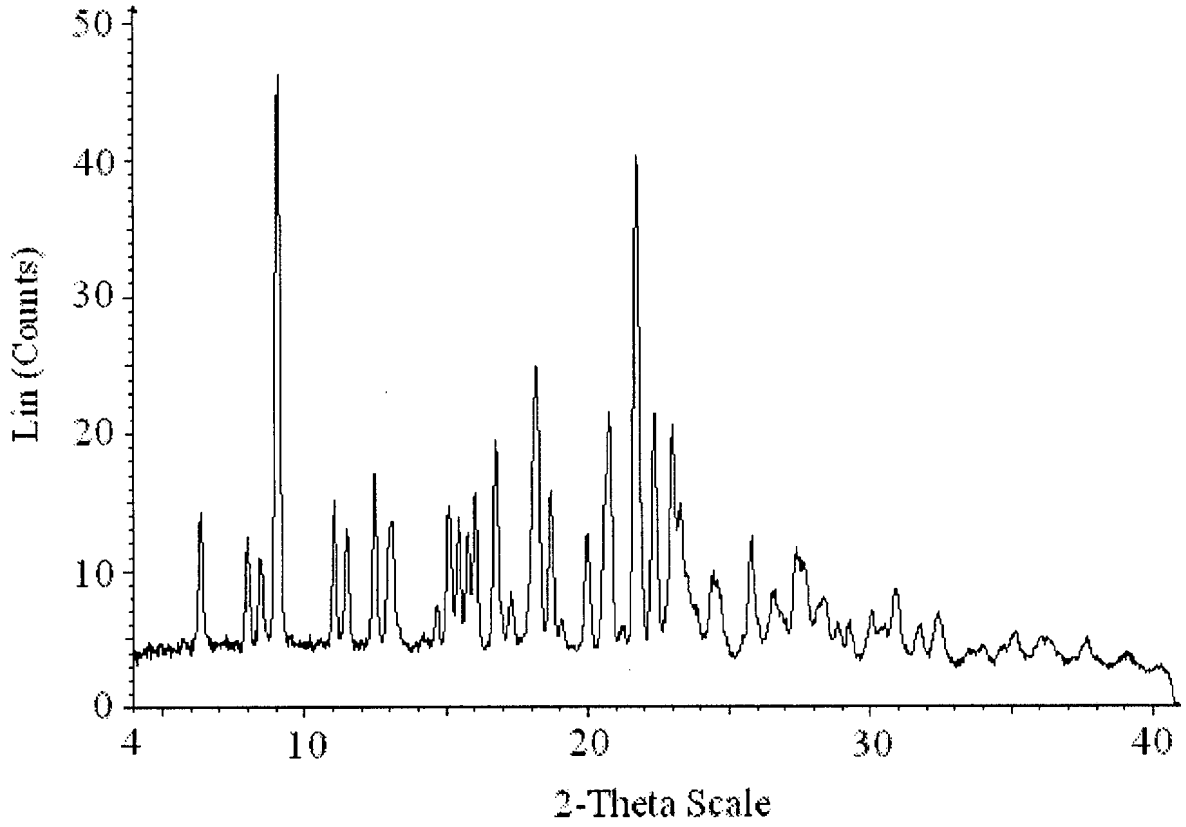


Figure 13

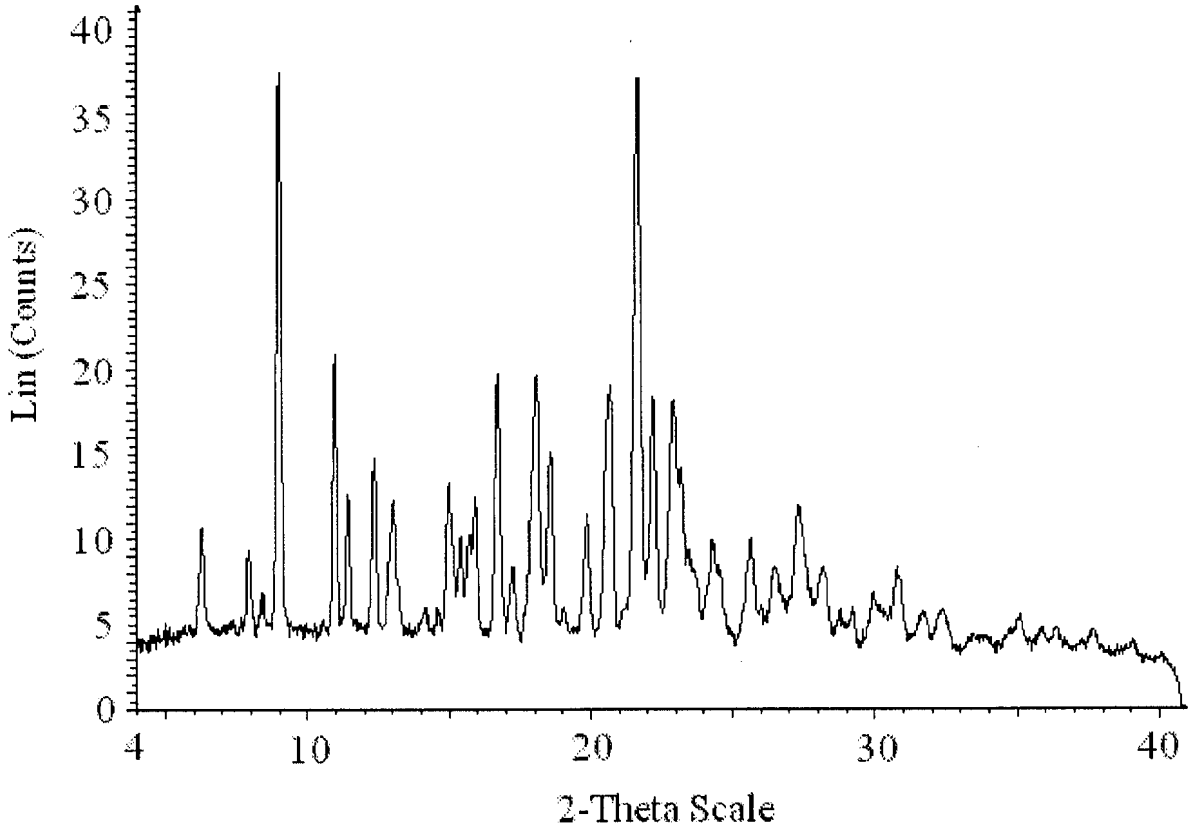
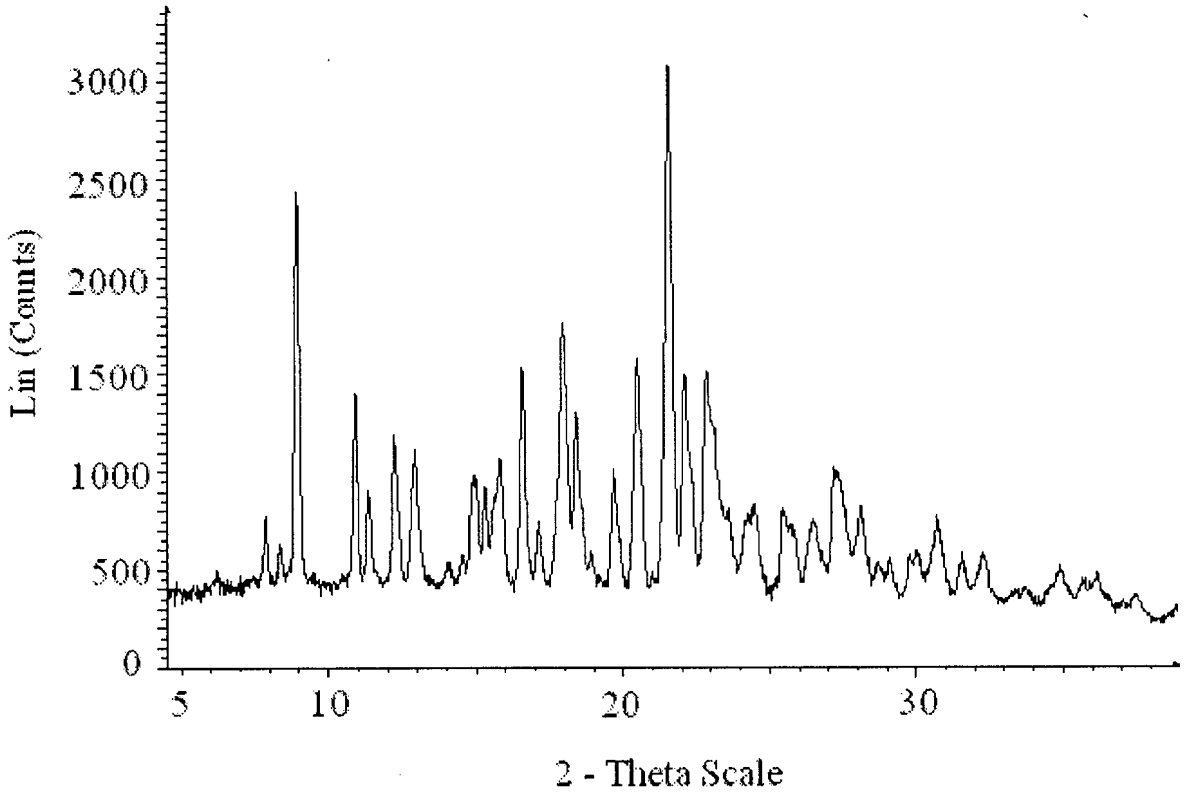


Figure 14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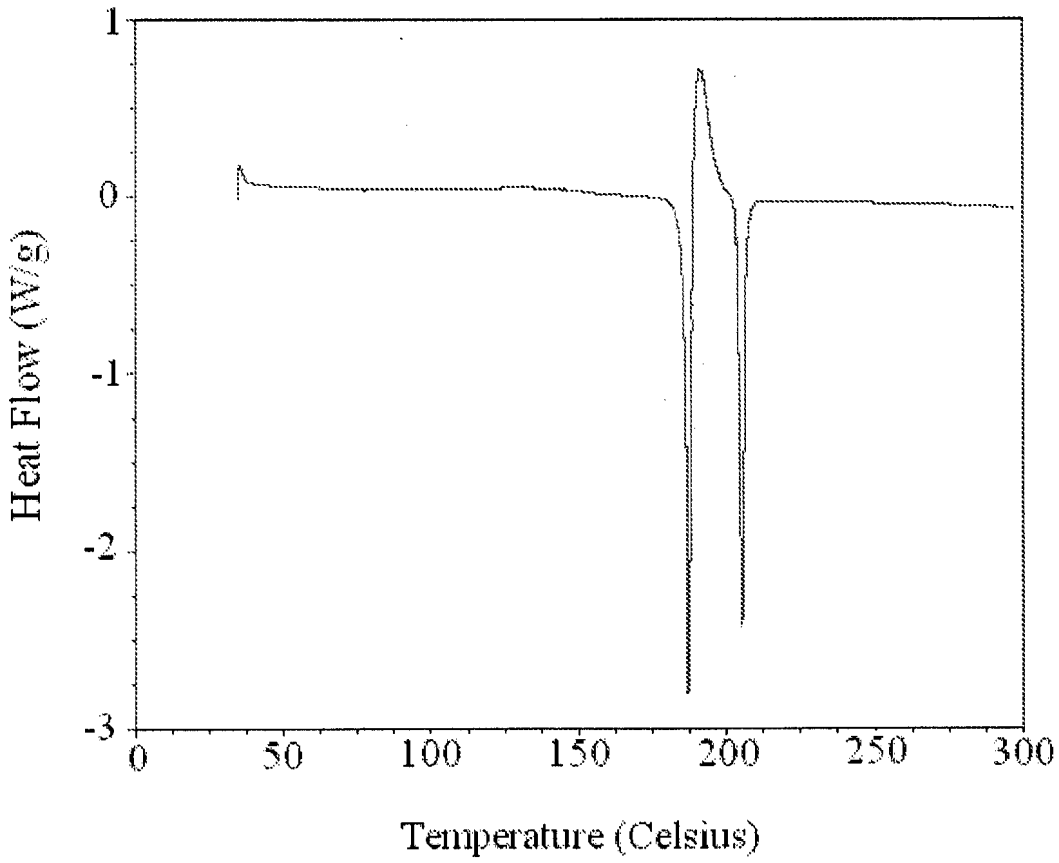
2500
2000
1500
1000
500
0
Lin (Counts)
5
10
20
30
2 - Theta Scale

Figure 15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0
-1
-2
-3
Heat Flow (W/g)
0
50
100
150
200
250
300
Temperature (Celsius)

Figure 16

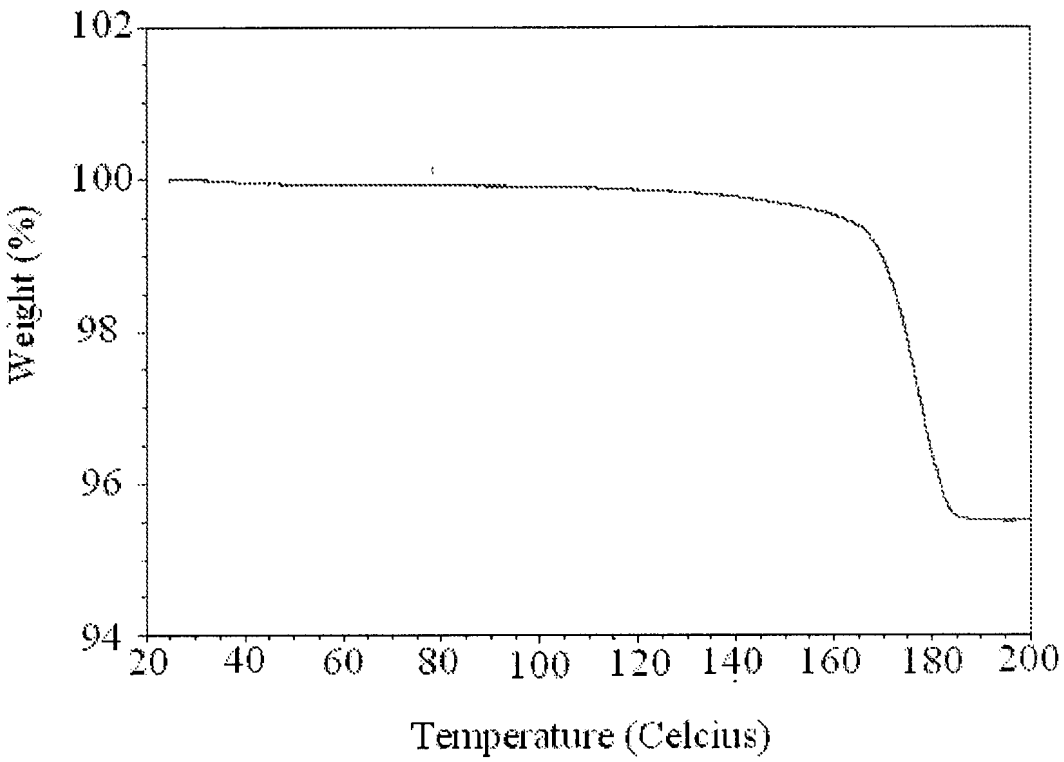

102
100
98
96
94
Weight (%)
20
40
60
80
100
120
140
160
180
200
Temperature (Celcius)

Figure 17

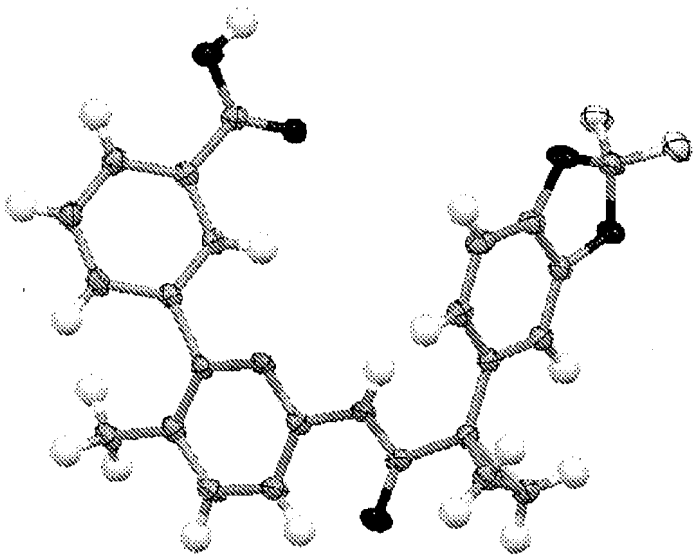


Figure 18

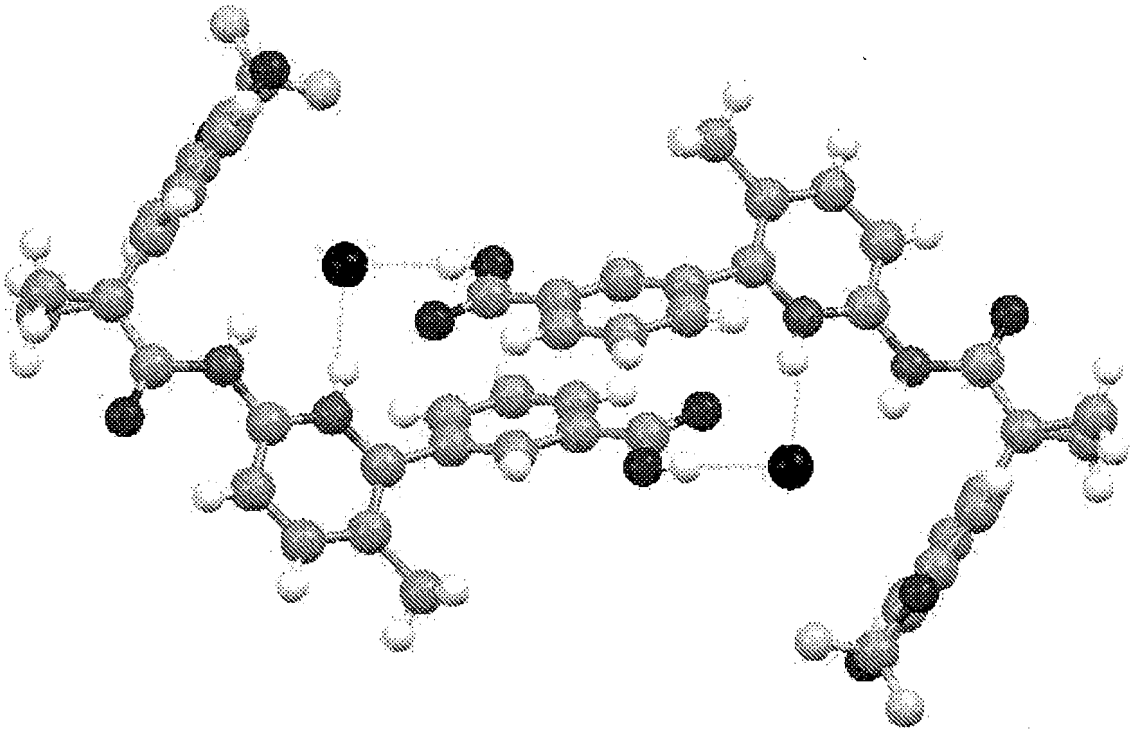
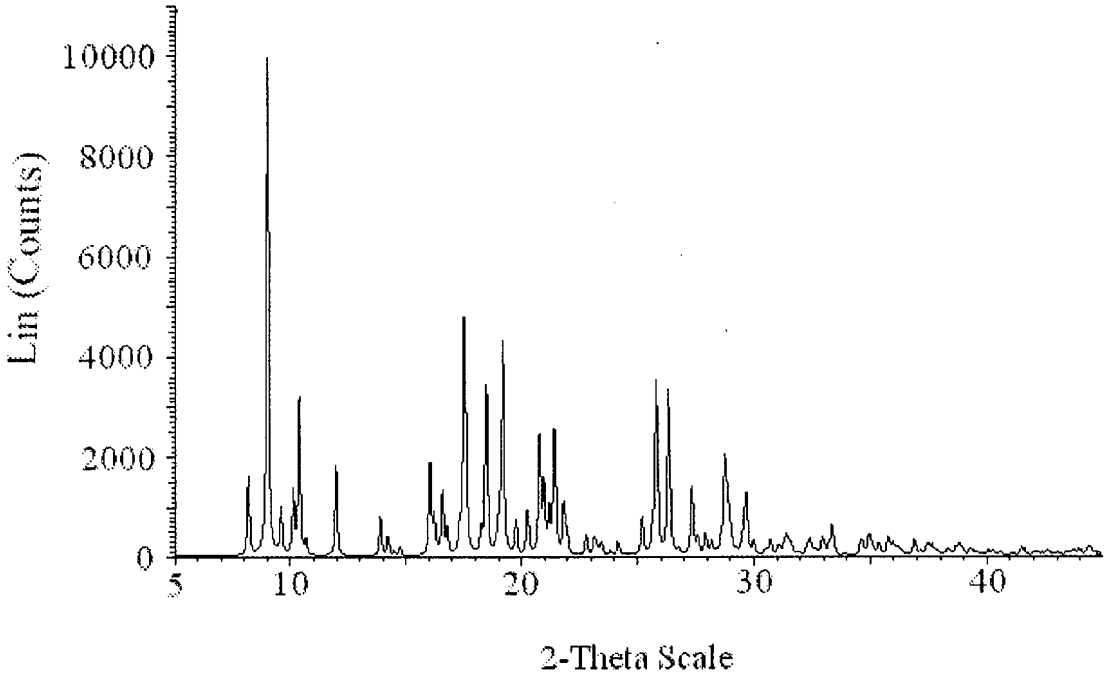


Figure 19


10000
8000
6000
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2000
0
Lin (Counts)
5
10
20
30
40
2-Theta Scale

Figure 20

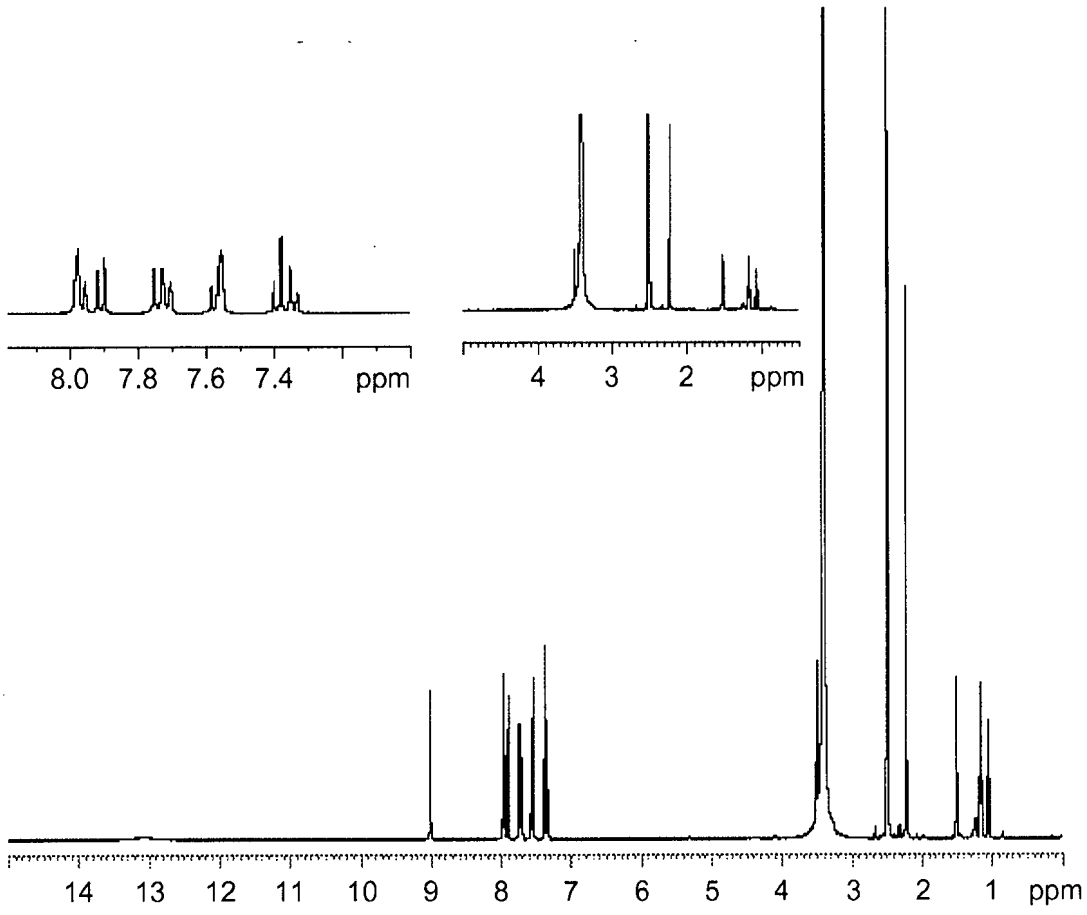


Figure 21

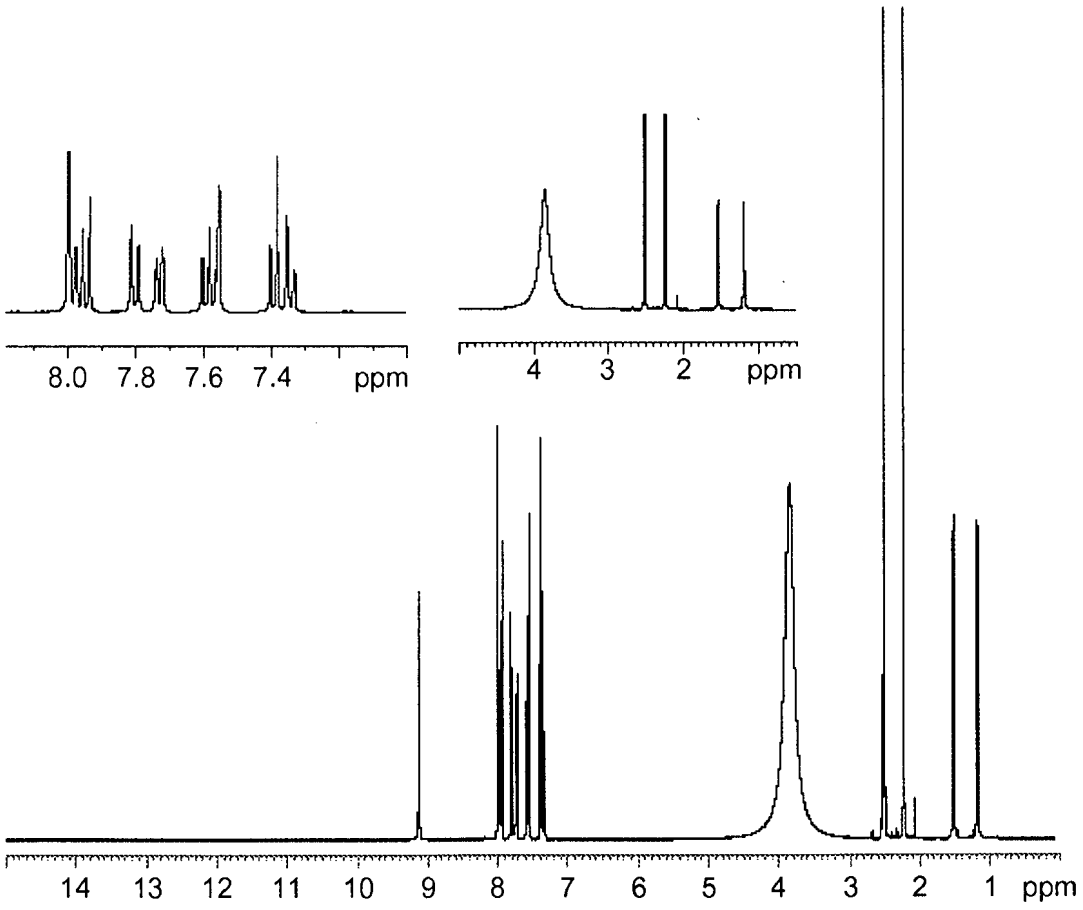


Figure 22

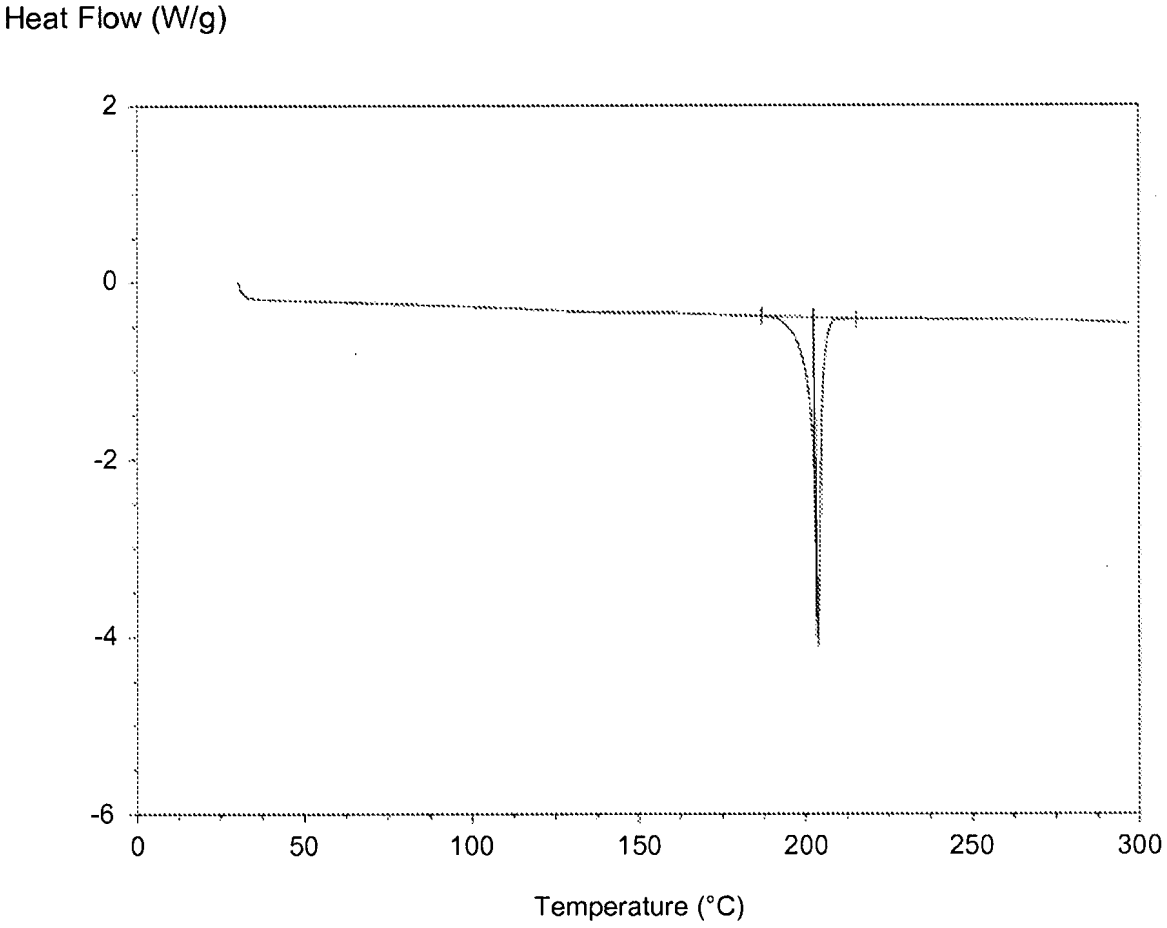


Figure 23

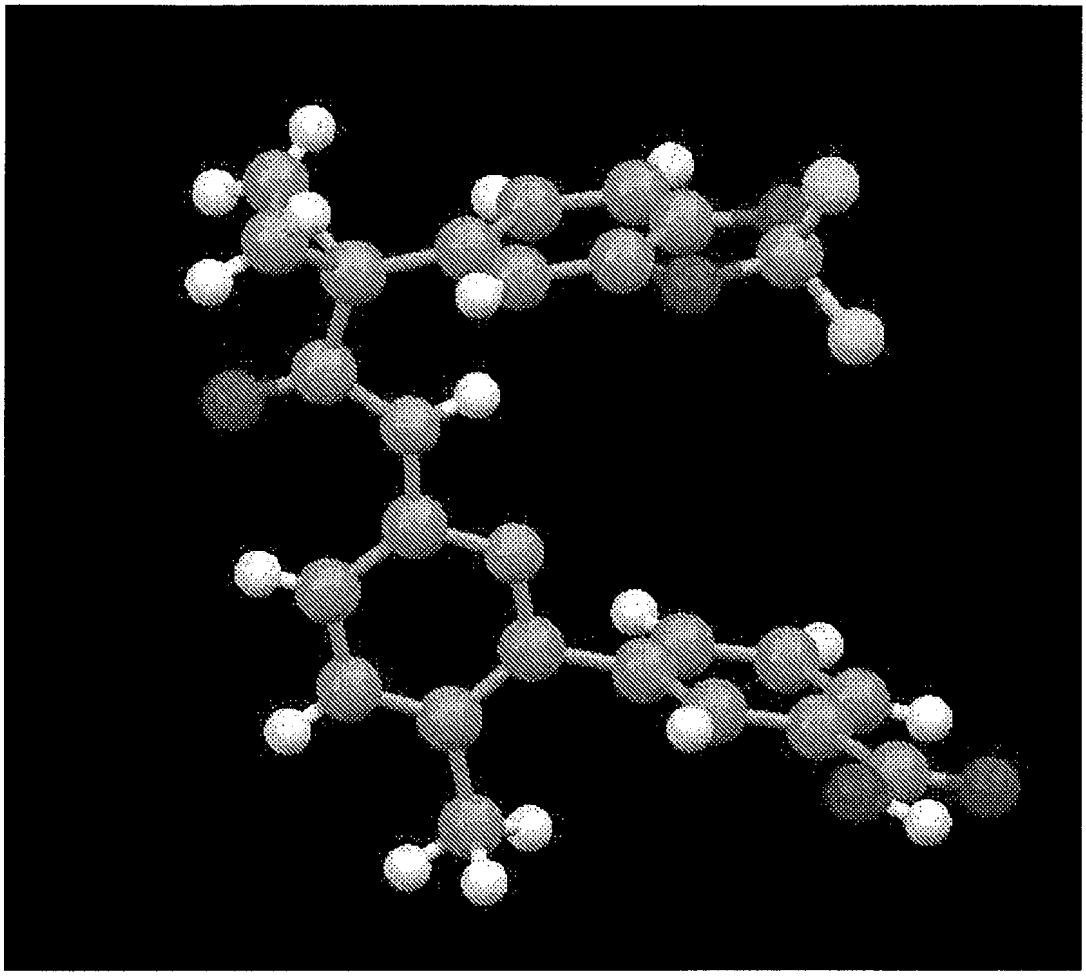


Figure 24

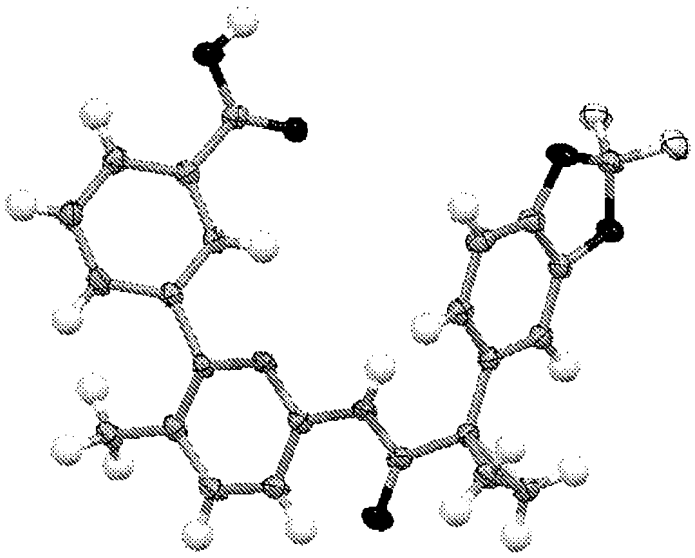


Figure 25

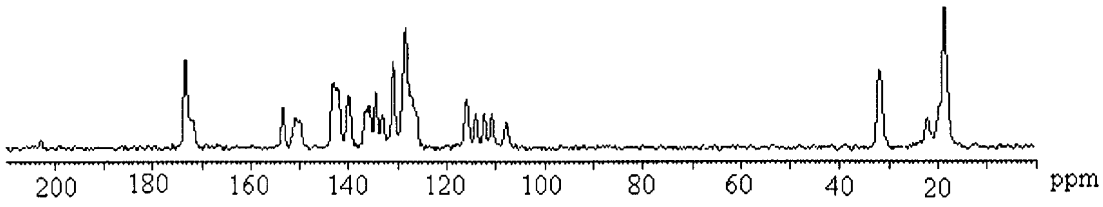


Figure 26

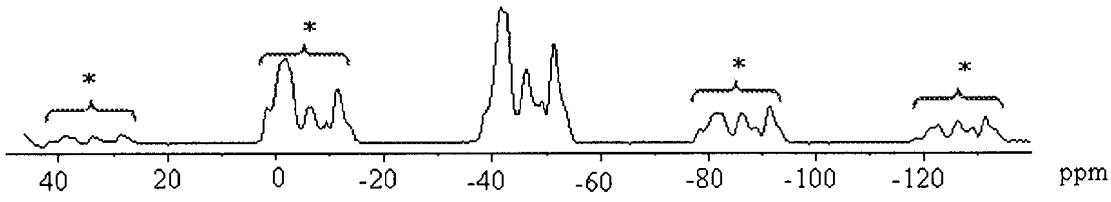


Figure 27

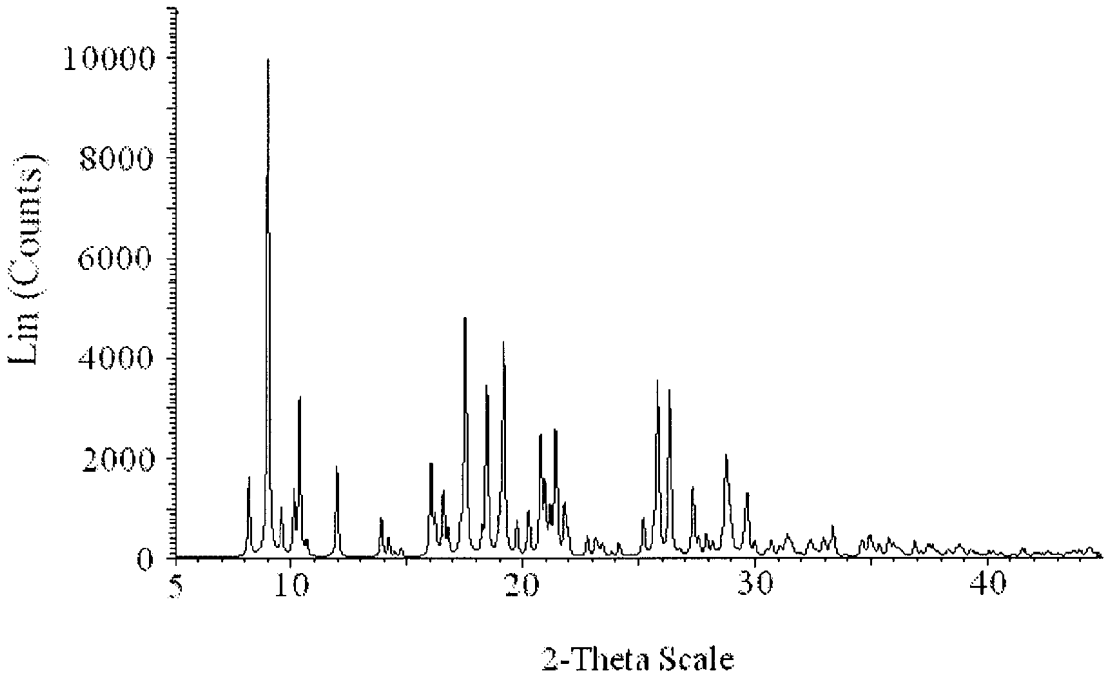
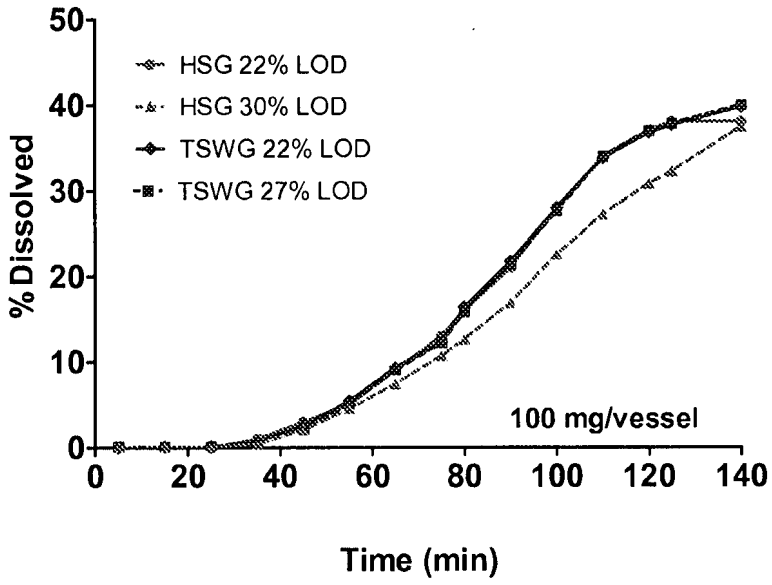

10000
8000
6000
4000
2000
0
Lin (Counts)
5
10
20
30
40
2-Theta Scale

Figure 28


50
40
30
20
10
0
% Dissolved
HSG 22% LOD
HSG 30% LOD
TSWG 22% LOD
TSWG 27% LOD
100 mg/vessel
0
20
40
60
80
100
120
140
Time (min)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/023100

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K9/16 A61K9/20 A61K9/28 C07D213/75 C07D405/12
C07D405/14 A61K31/443
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/037066 A2 (VERTEX PHARMA [US]; YOUNG CHRISTOPHER [US]) 1 April 2010 (2010-04-01) paragraphs [0020] - [0027], [0091], [0096], [0108]; claim 25; examples	1-54
X	WO 2009/073757 A1 (VERTEX PHARMA [US]; KESHAVARZ-SHOKRI ALI [US]; ZHANG BEILI [US]; KRAWI) 11 June 2009 (2009-06-11) paragraphs [0086], [0095] - [0097], [0105]	1-54
X	US 2011/256220 A1 (VERWIJS MARINUS JACOBUS [US] ET AL) 20 October 2011 (2011-10-20) paragraphs [0280], [0293], [0295]; claims; examples	1-54

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 March 2013	07/05/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Giménez Miralles, J

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2011/263654 A1 (KESHAVARZ-SHOKRI ALI [US] ET AL) 27 October 2011 (2011-10-27) paragraphs [0170], [0185], [0186]; claims; examples -----	1-54

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2013/023100

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010037066 A2	01-04-2010	AR 073709 A1	24-11-2010
		AU 2009296271 A1	01-04-2010
		CA 2736545 A1	01-04-2010
		CN 102164587 A	24-08-2011
		EP 2349223 A2	03-08-2011
		JP 2012504143 A	16-02-2012
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		RU 2011117177 A	10-11-2012
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		EP 2225230 A1	08-09-2010
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		AU 2011237494 A1	01-11-2012
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		TW 201139422 A	16-11-2011
		US 2011263654 A1	27-10-2011
		WO 2011127290 A2	13-10-2011

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C07D 405/12(2006.01)
C07D 405/14(2006.01)
A61K 31/443(2006.01)

权利要求书14页 说明书75页 附图22页

(54)发明名称

3-(6-(1-(2,2-二氟苯并[D][1,3]二氧杂环戊烯-5-基)环丙烷甲酰胺基)-3-甲基吡啶-2-基)苯甲酸的制剂

(57)摘要

本发明公开了一种药物组合物，所述药物组合物包含：化合物1，即(3-(6-(1-(2,2-二氟苯并[d][1,3]二氧杂环戊烯-5-基)环丙烷甲酰胺基)-3-甲基吡啶-2-基)苯甲酸)，以及至少一种选自填充剂、崩解剂、表面活性剂、粘合剂和润滑剂的赋形剂，所述组合物适于供对其有需要的患者口服以治疗诸如囊性纤维化的CFTR介导的疾病。本发明还公开了制备包含化合物1的药物组合物的工艺。

CN 104168890 A

1. 一种口服片剂，包含：

a. 化合物 1、化合物 1 形式 I、化合物 1 形式 II 和 / 或化合物 1HCl 盐形式 A；

b. 填充剂；

c. 崩解剂；

d. 表面活性剂；

e. 润滑剂；和

f. 粘合剂或助流剂中的至少一种。

2. 根据权利要求 1 所述的片剂，其中化合物 1、化合物 1 形式 I、化合物 1 形式 II 和 / 或化合物 1HCl 盐形式 A 以约 25mg 至约 500mg 范围内的量存在于所述片剂中。

3. 根据权利要求 1 所述的片剂，其中化合物 1、化合物 1 形式 I、化合物 1 形式 II 和 / 或化合物 1HCl 盐形式 A 在所述片剂中的量按所述片剂的重量计在约 15 重量％至 75 重量％的范围内。

4. 根据权利要求 1 所述的片剂，其中化合物 1、化合物 1 形式 I、化合物 1 形式 II 和 / 或化合物 1HCl 盐形式 A 在所述片剂中的量按所述片剂的重量计在约 40 重量％至约 70 重量％的范围内。

5. 根据权利要求 1 所述的片剂，具有以下配方：

辊压颗粒共混物	(%w/w)
化合物 1	20-40
微晶纤维素	30-50
甘露醇	10-30
交联羧甲基纤维素钠	1-5
月桂基硫酸钠	0.1-2
胶态二氧化硅	0.1-1
硬脂酸镁	1-3
片剂组合物	(%w/w)
辊压颗粒共混物	99-99.9
硬脂酸镁	0.1-1

6. 根据权利要求 1 所述的片剂，具有以下配方：

高剪切颗粒共混物	(%w/w)
化合物 1	60-70
微晶纤维素	5-15
交联羧甲基纤维素钠	1-5
月桂基硫酸钠	0.1-2
聚乙烯吡咯烷酮	1-5
片剂组合物	(%w/w)
高剪切颗粒共混物	75-89
微晶纤维素	10-15
交联羧甲基纤维素钠	1-5
硬脂酸镁	0.1-5

7. 根据权利要求 1 所述的片剂，具有以下配方：

高剪切颗粒共混物	(%w/w)
化合物 1 形式 I	60-70
微晶纤维素	5-15
交联羧甲基纤维素钠	1-5
聚乙烯吡咯烷酮	1-5
月桂基硫酸钠	0.1-2
片剂组合物	(%w/w)
高剪切颗粒共混物	78-89
微晶纤维素	10-15
交联羧甲基纤维素钠	1-5
硬脂酸镁	0.1-2
薄膜衣片剂	(%w/w)
片芯片剂组合物	95-99
薄膜衣	1-5
蜡	微量

8. 根据权利要求 1 所述的片剂，具有以下配方：

辊压颗粒共混物	(%w/w)
化合物 1 形式 I	30
微晶纤维素	42.3
甘露醇	21.2
交联羧甲基纤维素钠	3
月桂基硫酸钠	1
胶态二氧化硅	0.5
硬脂酸镁	2
片剂组合物	(%w/w)
辊压颗粒共混物	99.5
硬脂酸镁	0.5

9. 根据权利要求 1 所述的片剂，具有以下配方：

高剪切颗粒共混物	(%w/w)
化合物 1 形式 I	40-80
微晶纤维素	20-40
甘露醇	10-15
交联羧甲基纤维素钠	1-5
聚乙烯吡咯烷酮	1-10
月桂基硫酸钠	0.1-2
片剂组合物	(%w/w)
高剪切颗粒共混物	95-99
交联羧甲基纤维素钠	1-4
硬脂酸镁	0.1-1

10. 根据权利要求 1 所述的片剂，具有以下配方：

高剪切颗粒共混物	(%w/w)
化合物 1 形式 I	50
微晶纤维素	30
甘露醇	13
交联羧甲基纤维素钠	2
聚乙烯吡咯烷酮	4
月桂基硫酸钠	1
片剂组合物	(%w/w)
高剪切颗粒共混物	97.5
交联羧甲基纤维素钠	2.0
硬脂酸镁	0.5

11. 根据权利要求 1 所述的片剂，具有以下配方：

高剪切颗粒共混物	(%w/w)
化合物 1 形式 I	60
微晶纤维素	20
甘露醇	13
交联羧甲基纤维素钠	2
聚乙烯吡咯烷酮	4
月桂基硫酸钠	1
片剂组合物	(%w/w)
高剪切颗粒共混物	97.5
交联羧甲基纤维素钠	2.0
硬脂酸镁	0.5

12. 根据权利要求 1 所述的片剂，具有以下配方：

高剪切颗粒共混物	(%w/w)
化合物 1 形式 I	60
微晶纤维素	20
甘露醇	13
交联羧甲基纤维素钠	2
聚乙烯吡咯烷酮	4
月桂基硫酸钠	1
片剂组合物	(%w/w)
高剪切颗粒共混物	83
微晶纤维素	14
交联羧甲基纤维素钠	2
硬脂酸镁	1

13. 根据权利要求 1 所述的片剂，具有以下配方：

双螺杆颗粒共混物	(%w/w)
化合物 1 形式 I	60
微晶纤维素	20
甘露醇	13
交联羧甲基纤维素钠	2
聚乙烯吡咯烷酮	4
月桂基硫酸钠	1
片剂组合物	(%w/w)
双螺杆颗粒共混物	83
微晶纤维素	14
交联羧甲基纤维素钠	2
硬脂酸镁	1

14. 根据权利要求 1 所述的片剂，具有以下配方：

双螺杆湿颗粒共混物	(%w/w)
化合物 1 形式 I	80.0
微晶纤维素	13.6
交联羧甲基纤维素钠	2.5
聚乙烯吡咯烷酮	3.1
月桂基硫酸钠	0.7
片剂组合物	(%w/w)
双螺杆颗粒共混物	83
微晶纤维素	12
交联羧甲基纤维素钠	4
硬脂酸镁	1

15. 根据权利要求 1 所述的片剂，具有以下配方：

双螺杆颗粒共混物	(%w/w)
化合物 1 形式 I	80.0
微晶纤维素	13.6
交联羧甲基纤维素钠	2.5
聚乙烯吡咯烷酮	3.1
月桂基硫酸钠	0.7
片剂组合物	(%w/w)
双螺杆颗粒共混物	83
微晶纤维素	12
交联羧甲基纤维素钠	4
硬脂酸镁	1
薄膜衣片剂	(%w/w)
片芯片剂组合物	97
薄膜衣	3
蜡	微量

16. 根据权利要求 1 所述的片剂，具有以下配方：

高剪切颗粒共混物	mg
化合物 1 形式 I	200
微晶纤维素	66
甘露醇	43
交联羧甲基纤维素钠	7
聚乙烯吡咯烷酮	13
月桂基硫酸钠	3
片芯片剂组合物（200mg 剂量）	mg
高剪切颗粒共混物	332
微晶纤维素	56
交联羧甲基纤维素钠	8
硬脂酸镁	4
薄膜衣片剂（200mg 剂量）	mg
片芯片剂组合物	400
薄膜衣	12
蜡	微量

17. 根据权利要求 1 所述的片剂，具有以下配方：

双螺杆颗粒共混物	mg
化合物 1 形式 I	200
微晶纤维素	66
甘露醇	43
交联羧甲基纤维素钠	7
聚乙烯吡咯烷酮	13
月桂基硫酸钠	3
片芯片剂组合物（200mg 剂量）	mg
双螺杆颗粒共混物 微晶纤维素 交联羧甲基纤维素钠 硬脂酸镁	332 56 8 4

18. 根据权利要求 1 所述的片剂，具有以下配方：

高剪切颗粒共混物	mg
化合物 1 形式 I	200
微晶纤维素	67
甘露醇	45
交联羧甲基纤维素钠	7
聚乙烯吡咯烷酮	10.4
月桂基硫酸钠	2.6
片芯片剂组合物（200mg 剂量）	mg
高剪切颗粒共混物 微晶纤维素 交联羧甲基纤维素钠 硬脂酸镁	332 56 8 4
薄膜衣片剂（200mg 剂量）	mg
片芯片剂组合物 薄膜衣 蜡	400 12 微量

19. 根据权利要求 1 所述的片剂，具有以下配方：

高剪切颗粒共混物	mg
化合物 1 形式 I	300
微晶纤维素	99
甘露醇	64.5
交联羧甲基纤维素钠	10.5
聚乙烯吡咯烷酮	19.5
月桂基硫酸钠	4.5
片芯片剂组合物（300mg 剂量）	mg
高剪切颗粒共混物 微晶纤维素 交联羧甲基纤维素钠 硬脂酸镁	498 84 12 6
薄膜衣片剂（300mg 剂量）	mg
片芯片剂组合物 薄膜衣 蜡	600 18 微量

20. 根据权利要求 1 所述的片剂，具有以下配方：

高剪切颗粒共混物	mg
化合物 1 形式 I	300
微晶纤维素	100.5
甘露醇	67.5
交联羧甲基纤维素钠	10.5
聚乙烯吡咯烷酮	15.6
月桂基硫酸钠	3.9
片芯片剂组合物（300mg 剂量）	mg
高剪切颗粒共混物 微晶纤维素 交联羧甲基纤维素钠 硬脂酸镁	498 84 12 6
薄膜衣片剂（300mg 剂量）	mg
片芯片剂组合物 薄膜衣 蜡	600 18 微量

21. 根据权利要求 1 所述的片剂，具有以下配方：

高剪切颗粒共混物	(%w/w)
化合物 1 形式 I	70
微晶纤维素	12
甘露醇	11
交联羧甲基纤维素钠	2
聚乙烯吡咯烷酮	4
月桂基硫酸钠	1
片剂组合物	(%w/w)
高剪切颗粒共混物	97.5
交联羧甲基纤维素钠	2.0
硬脂酸镁	0.5

22. 根据权利要求 1 所述的片剂，具有以下配方：

高剪切颗粒共混物	(%w/w)
化合物 1 形式 I 或形式 II	61
微晶纤维素	20.3
甘露醇	13.2
交联羧甲基纤维素钠	2
聚乙烯吡咯烷酮	2.7
月桂基硫酸钠	0.7
片剂组合物	(%w/w)
高剪切颗粒共混物	83
微晶纤维素	14
交联羧甲基纤维素钠	2
硬脂酸镁	1

23. 根据权利要求 1 所述的片剂，具有以下配方：

高剪切颗粒共混物	mg
化合物 1 形式 I 或形式 II	100
微晶纤维素	33.3
甘露醇	21.7
交联羧甲基纤维素钠	3.3
聚乙烯吡咯烷酮	4.4
月桂基硫酸钠	1.1
片芯片剂组合物（100mg 剂量）	mg
高剪切颗粒共混物	163.9
微晶纤维素	27.6
交联羧甲基纤维素钠	3.9
硬脂酸镁	2.0

24. 根据权利要求 1 所述的片剂，具有以下配方：

双螺杆颗粒共混物	mg
化合物 1 形式 I	200
微晶纤维素	34.0
交联羧甲基纤维素钠	6.3
聚乙烯吡咯烷酮	7.8
月桂基硫酸钠	1.8
片芯片剂组合物（200mg 剂量）	mg
双螺杆颗粒共混物	249.9
微晶纤维素	36.1
交联羧甲基纤维素钠	12.0
硬脂酸镁	3.0

25. 根据权利要求 1 所述的片剂，具有以下配方：

双螺杆颗粒共混物	mg
化合物 1 形式 I	400
微晶纤维素	68.0
交联羧甲基纤维素钠	12.6
聚乙烯吡咯烷酮	15.6
月桂基硫酸钠	3.6
片芯片剂组合物 （400mg 剂量）	mg
双螺杆颗粒共混物	499.8
微晶纤维素	72.2
交联羧甲基纤维素钠	24.0
硬脂酸镁	6.0

26. 根据权利要求 1 所述的片剂，具有以下配方：

双螺杆颗粒共混物	mg
化合物 1 形式 I	200
微晶纤维素	34.0
交联羧甲基纤维素钠	6.3
聚乙烯吡咯烷酮	7.8
月桂基硫酸钠	1.8
片芯片剂组合物 （200mg 剂量）	mg
双螺杆颗粒共混物	249.9
微晶纤维素	36.1
交联羧甲基纤维素钠	12.0
硬脂酸镁	3.0
薄膜衣片剂 （200mg 剂量，310mg 总重）	mg
片芯片剂组合物	301
薄膜衣	9.0
蜡	微量

27. 根据权利要求 1 所述的片剂，具有以下配方：

双螺杆颗粒共混物	mg
化合物 1 形式 I	400
微晶纤维素	68.0
交联羧甲基纤维素钠	12.6
聚乙烯吡咯烷酮	15.6
月桂基硫酸钠	3.6
片芯片剂组合物 （400mg 剂量）	mg
双螺杆颗粒共混物 微晶纤维素 交联羧甲基纤维素钠 硬脂酸镁	499.8 72.2 24.0 6.0
薄膜衣片剂 （400mg 剂量，620mg 总重）	mg
片芯片剂组合物 薄膜衣 蜡	602 18.0 微量

28. 根据权利要求 1 所述的片剂，其中所述片剂还包含至少一种另外的治疗剂。

29. 根据权利要求 28 所述的片剂，其中所述另外的治疗剂为 CFTR 调节剂。

30. 根据权利要求 29 所述的片剂，其中所述 CFTR 调节剂为 CFTR 增效剂。

31. 根据权利要求 29 所述的片剂，其中所述 CFTR 调节剂为 N-(5- 羟基 -2,4- 二叔丁基苯基)-4- 氧代 -1H- 喹啉 -3- 甲酰胺。

32. 根据权利要求 1 所述的片剂，其中化合物 1 呈形式 I，其特征在于在使用 Cu Kα 辐射得到的 X 射线粉末衍射图中在 15.2 至 15.6 度、16.1 至 16.5 度和 14.3 至 14.7 度的一个或多个峰。

33. 根据权利要求 32 所述的片剂，其中化合物 1 形式 I 的特征在于在 15.4、16.3 和 14.5 度的一个或多个峰。

34. 根据权利要求 1 所述的片剂，其中化合物 1 呈形式 I，其特征在于基本上与图 1 类似的衍射图案。

35. 根据权利要求 1 所述的片剂，其中化合物 1 呈形式 I，其特征在于基本上与图 2 类似的衍射图案。

36. 一种治疗患者疾病或减轻所述疾病严重性的方法，包括向所述患者施用权利要求 1 所述的片剂，其中所述疾病选自囊性纤维化、哮喘、吸烟诱发的 COPD、慢性支气管炎、鼻窦炎、便秘、胰腺炎、胰腺功能不全、男性不育症、轻度肺病、特发性胰腺炎、变应性支气管肺曲菌病 (ABPA)、肝病、遗传性肺气肿、遗传性血色病、凝血 - 纤溶缺陷、蛋白 C 缺乏症、1 型遗传性血管性水肿、脂质加工缺陷、家族性高胆固醇血症、1 型乳糜微粒血症、无 β 脂蛋白血症、溶酶体贮积症、I- 细胞病 / 假性赫尔勒综合征、粘多糖症、桑德霍夫 / 泰 - 萨克斯病、克 - 纳综合征 II 型、多内分泌腺病 / 高胰岛素血症、糖尿病、拉伦侏儒症、髓过氧化物酶缺乏症、原发性甲状旁腺功能减退、黑素瘤、聚糖病 CDG 1 型、先天性甲状腺功能亢进症、成骨不全、遗传性低纤维蛋白原血症、ACT 缺乏症、尿崩症 (DI)、神经生长性 DI、肾性 DI、夏 - 马 - 图综合

征、佩－梅病、神经变性疾病、阿尔茨海默病、帕金森病、肌萎缩性侧索硬化、进行性核上性麻痹、皮克病、若干聚谷氨酰胺神经性障碍、亨廷顿病、I 型脊髓小脑性共济失调、脊髓与延髓肌肉萎缩症、齿状核红核苍白球丘脑下部核萎缩、肌强直性营养不良、海绵状脑病、遗传性克雅病、法布里病、施特劳斯综合征、COPD、干眼病、斯耶格伦氏综合征、骨质疏松症、骨质减少、戈勒姆综合征、氯离子通道病变、先天性肌强直、巴特综合征 III 型、登特病、过度惊跳症、癫痫症、过度惊跳症、溶酶体贮存病、安格曼综合征、原发性纤毛运动障碍 (PCD)、纤毛结构和 / 或功能遗传障碍、具有左右转位的 PCD、没有左右转位或纤毛发育不良的 PCD。

37. 根据权利要求 36 所述的方法，其中所述疾病为囊性纤维化、肺气肿、COPD 或干眼病。

38. 根据权利要求 36 所述的方法，其中所述疾病为囊性纤维化，其中所述患者具有 F508del CFTR 突变。

39. 根据权利要求 38 所述的方法，其中所述患者为 F508del 纯合的。

40. 根据权利要求 38 所述的方法，其中所述患者为 F508del 杂合的。

41. 根据权利要求 36 所述的方法，其中所述方法包括施用另外的治疗剂。

42. 根据权利要求 41 所述的方法，其中所述治疗剂选自溶粘蛋白剂、支气管扩张剂、抗生素、抗感染剂、抗炎剂、CFTR 增效剂或营养剂。

43. 根据权利要求 41 所述的方法，其中所述另外的治疗剂为 N-(5- 羟基 -2,4- 二叔丁基苯基)-4- 氧代 -1H- 喹啉 -3- 甲酰胺。

44. 一种试剂盒，包含权利要求 1 所述的片剂，和单独的治疗剂或其药物组合物。

45. 根据权利要求 44 所述的试剂盒，其中所述化合物 1 呈形式 I。

46. 根据权利要求 44 所述的试剂盒，其中所述治疗剂为化合物 1 之外的囊性纤维化纠正剂。

47. 根据权利要求 44 所述的试剂盒，其中所述治疗剂为囊性纤维化增效剂。

48. 根据权利要求 44 所述的试剂盒，其中所述治疗剂为 N-(5- 羟基 -2,4- 二叔丁基苯基)-4- 氧代 -1H- 喹啉 -3- 甲酰胺。

49. 根据权利要求 44 所述的试剂盒，其中权利要求 1 所述的片剂和所述治疗剂在单独的容器中。

50. 根据权利要求 49 所述的试剂盒，其中所述单独的容器为瓶子。

51. 根据权利要求 49 所述的试剂盒，其中所述单独的容器为小瓶。

52. 根据权利要求 49 所述的试剂盒，其中所述单独的容器为泡罩包装。

53. 一种用于制备包含化合物 1 的片剂的连续工艺，包括以下步骤：

a) 在共混机中混合化合物 1、填充剂和崩解剂以形成共混物；

b) 用水、粘合剂和表面活性剂制备制粒液；

c) 在添加得自步骤 b) 的所述制粒液的同时将得自步骤 a) 的所述共混物喂入连续双螺杆制粒机以产生颗粒；

d) 对得自步骤 c) 的所述颗粒进行干燥和研磨；

e) 将得自步骤 d) 的研磨后的颗粒与填充剂、崩解剂和润滑剂共混以形成共混物；

f) 将得自步骤 d) 的所述共混物压成片剂；以及

g) 对得自步骤 e) 的所述片剂进行包衣。

54. 根据权利要求 53 所述的工艺，其中化合物 1 呈形式 I。

3-(6-(1-(2,2-二氟苯并[D][1,3]二氧杂环戊烯-5-基)环丙烷甲酰胺基)-3-甲基吡啶-2-基)苯甲酸的制剂

技术领域

[0001] 本发明涉及包含3-(6-(1-(2,2-二氟苯并[d][1,3]二氧杂环戊烯-5-基)环丙烷甲酰胺基)-3-甲基吡啶-2-基)苯甲酸(化合物1)的药物组合物,用于制造此类组合物的方法以及用于施用包含其的药物组合物的方法。

背景技术

[0002] CFTR是在多种细胞类型,包括吸收和分泌上皮细胞中表达的cAMP/ATP-介导的阴离子通道,在这些细胞中其调节跨膜的阴离子通量以及其他离子通道和蛋白质的活性。在上皮细胞中,CFTR的正常运作对于保持电解质在整个身体(包括呼吸和消化组织)中的运输至关重要。CFTR由大约1480个氨基酸组成,这些氨基酸编码由串联重复的跨膜结构域组成的蛋白质,每个跨膜结构域含有六个跨膜螺旋和一个核苷酸结合域。两个跨膜结构域由大的、极性、调节性(R)-结构域连接,所述(R)-结构域具有多个调节通道活性和细胞运输的磷酸化位点。

[0003] 编码CFTR的基因已被鉴定和测序(参见Gregory,R.J.et al.(1990)Nature 347:382-386(Gregory,R.J.等人,1990年,《自然》,第347卷,第382-386页);Rich,D.P.et al.(1990)Nature 347:358-362(Rich,D.P.等人,1990年,《自然》,第347卷,第358-362页)),(Riordan,J.R.et al.(1989)Science 245:1066-1073(Riordan,J.R.等人,1989年,《科学》,第245卷,第1066-1073页))。该基因的缺陷引起CFTR突变,从而导致囊性纤维化("CF"),囊性纤维化是人类中最常见的致命的遗传性疾病。在美国,囊性纤维化影响着大约每2,500个婴儿中的一个。在一般的美国人口中,高达1000万人带有单拷贝的缺陷基因而没有明显的不良影响。相比之下,带有双拷贝的CF相关基因的个体遭受CF的衰弱和致命影响,包括慢性肺病。

[0004] 在患有囊性纤维化的患者中,在呼吸道上皮细胞中内源性表达的CFTR中的突变导致顶端阴离子分泌减少,从而造成离子和流体运输不平衡。所生成的阴离子运输减少有助于增强肺部的粘液积聚和伴随的微生物感染,这最终导致CF患者死亡。除了呼吸道疾病以外,CF患者通常患有胃肠道问题和胰腺功能不全,如果不进行治疗将导致死亡。另外,大多数患有囊性纤维化的男性不能生育并且在患有囊性纤维化的女性当中生育率下降。与双拷贝CF相关基因的严重影响形成对照,带有单拷贝CF相关基因的个体呈现对霍乱和对由于腹泻造成的脱水的耐受性增加,这也许可以解释在人口中存在相对高频率的CF基因。

[0005] 对CF染色体的CFTR基因的序列分析揭示了许多引起疾病的突变(Cutting,G.R.et al.(1990)Nature 346:366-369(Cutting,G.R.等人,1990年,《自然》,第346卷,第366-369页);Dean,M.et al.(1990)Cell61:863:870(Dean,M.等人,1990年,《细胞》,第61卷,第863-870页);以及Kerem,B-S.et al.(1989)Science 245:1073-1080(Kerem,B-S.等人,1989年,《科学》,第245卷,第1073-1080页);Kerem,B-S et al.(1990)Proc.Natl.Acad.Sci.USA 87:8447-8451(Kerem,B-S等人,1990年,《美国国家科学院院刊》,第87卷,

第 8447-8451 页))。迄今为止,据科学和医学文献报导,已在 CF 基因中鉴定出超过 1000 种引起疾病的突变。最普遍的突变是 CFTR 氨基酸序列的第 508 位的苯丙氨酸的缺失,并且一般称为 F508del-CFTR。这种突变出现在约 70%的囊性纤维化病例中并且与严重的疾病相关。其他突变包括 R117H 和 G551D。

[0006] F508del-CFTR 的第 508 位残基缺失阻止初生蛋白质的正确折叠。这导致突变蛋白质不能离开 ER 和运输到质膜。因此,存在于膜中的通道数目远少于在表达野生型 CFTR 的细胞中观察到的数目。除了运输受损,突变还导致通道门控缺陷。膜中的通道数目减少和门控缺陷一起导致阴离子跨上皮细胞运输减少,从而造成离子和流体运输缺陷。(Quinton, P. M. (1990), FASEB J. 4:2709-2727(Quinton, P. M., 1990 年,《美国实验生物学联合会杂志》,第 4 卷,第 2709-2727 页))。然而,研究显示,膜中的 F508del-CFTR 数目减少为功能性的,尽管少于野生型 CFTR。(Dalemans et al. (1991), Nature Lond. 354:526-528(Dalemans 等人,1991 年,《自然(伦敦)》,第 354 卷,第 526-528 页);Denning 等人,见上;Pasyk and Foskett(1995), J. Cell. Biochem. 270:12347-50(Pasyk 和 Foskett,1995 年,《细胞生物学杂志》,第 270 卷,第 12347-12350 页))。除了 F508del-CFTR 外,造成运输、合成和/或通道门控缺陷的 CFTR 中的其他致病突变可被上调或下调,以改变阴离子分泌并改变疾病进展和/或严重性。

[0007] 虽然 CFTR 除了阴离子之外也运输多种分子,但很明显,该作用(阴离子的运输)代表了运输离子和水通过上皮细胞的重要机制中的一个要素。其他要素包括上皮细胞的 Na^+ 通道、ENaC、$Na^+/2Cl^-/K^+$ 共转运蛋白、Na^+-K^+-ATP 酶泵,以及负责摄取氯离子进入细胞的基底外侧膜 K^+ 通道。

[0008] 这些要素共同起效以实现经其在细胞内的选择性表达和定位跨上皮细胞定向运输。氯吸收通过存在于顶端膜上的 ENaC 和 CFTR 以及在细胞的基底外侧表面上表达的 Na^+-K^+-ATP 酶泵和 Cl- 通道的协调活性而发生。氯从腔侧的继发性主动转运导致氯在细胞内积聚,这些氯然后可经 Cl^- 通道被动离开细胞,从而造成矢量运输。$Na^+/2Cl^-/K^+$ 共转运蛋白、Na^+-K^+-ATP 酶泵和基底外侧膜 K^+ 通道在基底外侧表面上的排列,并且在腔侧上的 CFTR 协调氯经腔侧上的 CFTR 的分泌。因为水自身可能从未主动转运,所以其跨上皮细胞的流动取决于钠和氯的总体流动产生的微小经上皮的渗透梯度。

[0009] 如上所述,据信 F508del-CFTR 的第 508 位残基缺失阻止初生蛋白质的正确折叠,从而导致这种突变蛋白质不能离开 ER 和运输至质膜。因此,在质膜上存在数量不足的成熟蛋白质,并且在上皮组织内的氯转运显著减少。事实上,已经表明,ATP- 结合盒 (ABC) 转运蛋白通过 ER 机构进行的有缺陷的内质网 (ER) 处理的这种细胞现象不仅是 CF 疾病的潜在基础,而且是多种其他孤立性和遗传性疾病的潜在基础。ER 机构可能发生故障的两种方式是通过失去与蛋白质的 ER 输出的偶合从而导致降解,或者通过这些有缺陷的/错误折叠的蛋白质的 ER 积聚 [Aridor M, et al., Nature Med., 5(7), pp745-751(1999)(Aridor M 等人,《自然医学》,第 5 卷第 7 期,第 745-751 页,1999 年);Shastry, B. S., et al., Neurochem. International, 43, pp 1-7(2003)(Shastry, B. S. 等人,《国际神经化学》,第 43 卷,第 1-7 页,2003 年);Rutishauser, J., et al., Swiss Med Wkly, 132, pp 211-222(2002)(Rutishauser, J. 等人,《瑞士医学周刊》,第 132 卷,第 211-222 页,2002 年);Morello, JP et al., TIPS, 21, pp. 466-469(2000)(Morello, JP 等人,《药理科学趋势》,第 21 卷,第 466-469

页，2000 年）；Bross P.，et al.，Human Mut.，14，pp. 186-198(1999)(Bross P. 等人，《人类突变》，第 14 卷，第 186-198 页，1999 年)]。

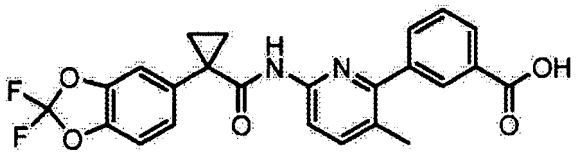
[0010] 盐形式的化合物 1 在国际 PCT 公布 WO 2007056341 中公开，该化合物作为 CFTR 活性的调节剂并因此作为 CFTR- 介导的疾病诸如囊性纤维化的有用治疗。基本上结晶并且不含盐的化合物 1 形式 I 在 2008 年 12 月 4 日提交的美国已公布的专利申请 US20090170905 中有所公开。化合物 1 形式 II 和化合物 1 的 HCl 盐形式 A 在 2011 年 4 月 7 日提交的美国已公布的专利申请 US20110263654 中有所公开。所有专利申请以全文引用方式并入本文。

[0011] 作为与 ivacaftor(N-(5- 羟基 -2,4- 二叔丁基苯基）-4- 氧代 -1H- 喹啉 -3- 甲酰胺）组合的一部分的化合物 1 已被美国食品和药物监督管理局 (FDA) 授予用于治疗囊性纤维化的突破性疗法认定，它是在提交本申请时仅有的两个此类授予中的一个（另一个为 ivacaftor)。这表明对于通过对症治疗而对囊性纤维化的原因进行有效治疗存在显著的尚未满足的需求。另外，经 FDA 批准的药物的共同挑战是对于有需要的患者而言有时无药可用。因此，对于目前公开的化合物 1 制剂以及以连续和受控的方式制备其的工艺存在明显尚未满足的需求。

发明内容

[0012] 本发明涉及包含 3-(6-(1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基）环丙烷甲酰胺基）-3- 甲基吡啶 -2- 基）苯甲酸（化合物 1）的药物组合物、药物制剂和固体剂型，化合物 1 具有以下结构：

[0013]



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[0014] 在一个方面，本发明提供了包含以下组分的药物组合物：

[0015] a. 化合物 1；

[0016] b. 填充剂；

[0017] c. 崩解剂；

[0018] d. 表面活性剂；

[0019] e. 润滑剂；和

[0020] f. 助流剂或粘合剂。

[0021] 在其他实施例中，化合物 1 为其基本上结晶的固体形式中的一种。在一个实施例中，化合物 1 为基本上结晶的形式 I（化合物 1 形式 I)。在一个实施例中，化合物 1 为基本上结晶的形式 II（化合物 1 形式 II)。在一个实施例中，化合物 1 为基本上结晶的 HCl 盐形式（化合物 1 的 HCl 盐形式 A)。应当理解，如全文所用的术语“化合物 1”除了其他形式（包括非结晶形式）之外包括以下固态形式：化合物 1 形式 I、化合物 1 形式 II 和 / 或化合物 1 的 HCl 盐形式 A。

[0022] 在一些实施例中，药物组合物包含 25mg 至 400mg。在一些实施例中，药物组合物包含 25mg 化合物 1。在一些实施例中，药物组合物包含 50mg 化合物 1。在一些实施例中，药

物组合物包含 100mg 化合物 1。在一些实施例中，药物组合物包含 125mg 化合物 1。在一些实施例中，药物组合物包含 150mg 化合物 1。在一些实施例中，药物组合物包含 200mg 化合物 1。在一些实施例中，药物组合物包含 250mg 化合物 1。在一些实施例中，药物组合物包含 300mg 化合物 1。在一些实施例中，药物组合物包含 400mg 化合物 1。

[0023] 在一个方面，本发明提供了包含以下组分的药物组合物：

[0024]

辊压颗粒共混物	(%w/w)
化合物 1	20-40
微晶纤维素	30-50
甘露醇	10-30
交联羧甲基纤维素钠	1-5
月桂基硫酸钠	0.1-2
胶态二氧化硅	0.1-1
硬脂酸镁	1-3
片剂组合物	(%w/w)
辊压颗粒共混物	99-99.9
硬脂酸镁	0.1-1

[0025] 在一个方面，本发明提供了包含以下组分的药物组合物：

[0026]

高剪切颗粒共混物	(%w/w)
化合物 1	60-70
微晶纤维素	5-15
交联羧甲基纤维素钠	1-5

[0027]

月桂基硫酸钠	0.1-2
聚乙烯吡咯烷酮	1-5
片剂组合物	(%w/w)
高剪切颗粒共混物	75-89
微晶纤维素	10-15
交联羧甲基纤维素钠	1-5
硬脂酸镁	0.1-5

[0028] 在另一个实施例中，本发明提供了包含以下组分的药物组合物：

[0029]

高剪切颗粒共混物	(%w/w)
化合物 1 形式 I	60-70
微晶纤维素	5-15
交联羧甲基纤维素钠	1-5
聚乙烯吡咯烷酮	1-5
月桂基硫酸钠	0.1-2
片剂组合物	(%w/w)
高剪切颗粒共混物	78-89
微晶纤维素	10-15
交联羧甲基纤维素钠	1-5
硬脂酸镁	0.1-2
薄膜衣片	(%w/w)
片芯片剂组合物	95-99
薄膜衣	1-5
蜡	微量

[0030] 在另一个实施例中，本发明提供了包含以下组分的药物组合物：

[0031]

辊压颗粒共混物	(%w/w)
化合物 1 形式 I	30
微晶纤维素	42.3
甘露醇	21.2
交联羧甲基纤维素钠	3
月桂基硫酸钠	1
胶态二氧化硅	0.5
硬脂酸镁	2
片剂组合物	(%w/w)
辊压颗粒共混物	99.5
硬脂酸镁	0.5

[0032] 在另一个方面，本发明提供了包含以下组分的药物组合物：

[0033]

高剪切颗粒共混物	(%w/w)
化合物 1 形式 I	40-80
微晶纤维素	20-40
甘露醇	10-15
交联羧甲基纤维素钠	1-5
聚乙烯吡咯烷酮	1-10
月桂基硫酸钠	0.1-2
片剂组合物	(%w/w)
高剪切颗粒共混物	95-99
交联羧甲基纤维素钠	1-4
硬脂酸镁	0.1-1

[0034] 在另一个实施例中，本发明提供了包含以下组分的药物组合物：

[0035]

高剪切颗粒共混物	(%w/w)
化合物 1 形式 I	50
微晶纤维素	30
甘露醇	13
交联羧甲基纤维素钠	2
聚乙烯吡咯烷酮	4
月桂基硫酸钠	1
片剂组合物	(%w/w)
高剪切颗粒共混物	97.5
交联羧甲基纤维素钠	2.0
硬脂酸镁	0.5

[0036] 在另一个实施例中，本发明提供了包含以下组分的药物组合物：

[0037]

高剪切颗粒共混物	(%w/w)
化合物 1 形式 I	60
微晶纤维素	20
甘露醇	13
交联羧甲基纤维素钠	2
聚乙烯吡咯烷酮	4
月桂基硫酸钠	1
片剂组合物	(%w/w)
高剪切颗粒共混物	97.5
交联羧甲基纤维素钠	2.0
硬脂酸镁	0.5

[0038] 在另一个实施例中，本发明提供了包含以下组分的药物组合物：

[0039]

高剪切颗粒共混物	(% w/w)
化合物 1 形式 I	60
微晶纤维素	20
甘露醇	13
交联羧甲基纤维素钠	2
聚乙烯吡咯烷酮	4
月桂基硫酸钠	1

片剂组合物	(% w/w)
高剪切颗粒共混物	83
微晶纤维素	14
交联羧甲基纤维素钠	2
硬脂酸镁	1

[0040] 在另一个实施例中，本发明提供了包含以下组分的药物组合物：

[0041]

双螺杆颗粒共混物	(%w/w)
化合物 1 形式 I	60
微晶纤维素	20
甘露醇	13
交联羧甲基纤维素钠	2
聚乙烯吡咯烷酮	4
月桂基硫酸钠	1
片剂组合物	(%w/w)
双螺杆颗粒共混物	83
微晶纤维素	14
交联羧甲基纤维素钠	2
硬脂酸镁	1

[0042] 在另一个实施例中，本发明提供了包含以下组分的药物组合物：

[0043]

双螺杆湿颗粒共混物	(%w/w)
化合物 1 形式 I	80.0
微晶纤维素	13.6
交联羧甲基纤维素钠	2.5
聚乙烯吡咯烷酮	3.1
月桂基硫酸钠	0.7
片剂组合物	(%w/w)

[0044]

双螺杆颗粒共混物	83
微晶纤维素	12
交联羧甲基纤维素钠	4
硬脂酸镁	1

[0045] 在另一个实施例中，本发明提供了包含以下组分的药物组合物：

[0046]

双螺杆颗粒共混物	(%w/w)
化合物 1 形式 I	80.0
微晶纤维素	13.6
交联羧甲基纤维素钠	2.5
聚乙烯吡咯烷酮	3.1
月桂基硫酸钠	0.7
片剂组合物	(%w/w)
双螺杆颗粒共混物	83
微晶纤维素	12
交联羧甲基纤维素钠	4
硬脂酸镁	1
薄膜衣片剂	(%w/w)
片芯片剂组合物	97
薄膜衣	3
蜡	微量

[0047] 在另一个实施例中，本发明提供了包含以下组分的药物组合物：

[0048]

高剪切颗粒共混物	mg
化合物 1 形式 I	200
微晶纤维素	66
甘露醇	43
交联羧甲基纤维素钠	7
聚乙烯吡咯烷酮	13
月桂基硫酸钠	3
片芯片剂组合物 (200mg 剂量)	mg
高剪切颗粒共混物	332
微晶纤维素	56
交联羧甲基纤维素钠	8
硬脂酸镁	4
薄膜衣片剂 (400mg 剂量)	mg
片芯片剂组合物	400
薄膜衣	12
蜡	微量

[0049] 在另一个实施例中，本发明提供了包含以下组分的药物组合物：

[0050]

双螺杆颗粒共混物	mg
化合物 1 形式 I	200
微晶纤维素	66
甘露醇	43
交联羧甲基纤维素钠	7
聚乙烯吡咯烷酮	13
月桂基硫酸钠	3
片芯片剂组合物（200mg 剂量）	mg
双螺杆颗粒共混物	332
微晶纤维素	56
交联羧甲基纤维素钠	8
硬脂酸镁	4

[0051] 在另一个实施例中，本发明提供了包含以下组分的药物组合物：

[0052]

高剪切颗粒共混物	mg
化合物 1 形式 I	200
微晶纤维素	67
甘露醇	45
交联羧甲基纤维素钠	7
聚乙烯吡咯烷酮	10.4
月桂基硫酸钠	2.6
片芯片剂组合物（200mg 剂量）	mg
高剪切颗粒共混物	332
微晶纤维素	56
交联羧甲基纤维素钠	8
硬脂酸镁	4
薄膜衣片剂（200mg 剂量）	mg
片芯片剂组合物	400
薄膜衣	12
蜡	微量

[0053] 在另一个实施例中，本发明提供了包含以下组分的药物组合物：

[0054]

高剪切颗粒共混物	mg
化合物 1 形式 I	300
微晶纤维素	99
甘露醇	64.5
交联羧甲基纤维素钠	10.5
聚乙烯吡咯烷酮	19.5
月桂基硫酸钠	4.5
片芯片剂组合物（300mg 剂量）	mg
高剪切颗粒共混物	498
微晶纤维素	84
交联羧甲基纤维素钠	12
硬脂酸镁	6
薄膜衣片剂（300mg 剂量）	mg
片芯片剂组合物	600
薄膜衣	18
蜡	微量

[0055] 在另一个实施例中，本发明提供了包含以下组分的药物组合物：

[0056]

高剪切颗粒共混物	mg
化合物 1 形式 I	300
微晶纤维素	100.5
甘露醇	67.5
交联羧甲基纤维素钠	10.5
聚乙烯吡咯烷酮	15.6
月桂基硫酸钠	3.9
片芯片剂组合物（300mg 剂量）	mg
高剪切颗粒共混物	498
微晶纤维素	84
交联羧甲基纤维素钠	12
硬脂酸镁	6
薄膜衣片剂（300mg 剂量）	mg
片芯片剂组合物	600
薄膜衣	18
蜡	微量

[0057] 在另一个实施例中，本发明提供了包含以下组分的药物组合物：

[0058]

高剪切颗粒共混物	(%w/w)
化合物 1 形式 I	70
微晶纤维素	12
甘露醇	11
交联羧甲基纤维素钠	2
聚乙烯吡咯烷酮	4
月桂基硫酸钠	1
片剂组合物	(%w/w)
高剪切颗粒共混物	97.5
交联羧甲基纤维素钠	2.0
硬脂酸镁	0.5

[0059] 在另一个实施例中，本发明提供了包含以下组分的药物组合物：

[0060]

高剪切颗粒共混物	(% w/w)
化合物 1 形式 I 或形式 II	61
微晶纤维素	20.3
甘露醇	13.2
交联羧甲基纤维素钠	2
聚乙烯吡咯烷酮	2.7
月桂基硫酸钠	0.7
片剂组合物	(% w/w)
高剪切颗粒共混物	83
微晶纤维素	14
交联羧甲基纤维素钠	2
硬脂酸镁	1

[0061] 在另一个实施例中，本发明提供了包含以下组分的药物组合物：

[0062]

高剪切颗粒共混物	mg
化合物 1 形式 I 或形式 II	100
微晶纤维素	33.3
甘露醇	21.7
交联羧甲基纤维素钠	3.3
聚乙烯吡咯烷酮	4.4
月桂基硫酸钠	1.1
片芯片剂组合物（100mg 剂量）	mg

[0063]

高剪切颗粒共混物	163.9
微晶纤维素	27.6
交联羧甲基纤维素钠	3.9
硬脂酸镁	2.0

[0064] 在另一个实施例中，本发明提供了包含以下组分的药物组合物：

[0065]

双螺杆颗粒共混物	mg
化合物 1 形式 I	200
微晶纤维素	34.0
交联羧甲基纤维素钠	6.3
聚乙烯吡咯烷酮	7.8
月桂基硫酸钠	1.8
片芯片剂组合物（200mg 剂量）	mg
双螺杆颗粒共混物	249.9
微晶纤维素	36.1
交联羧甲基纤维素钠	12.0
硬脂酸镁	3.0

[0066] 在另一个实施例中，本发明提供了包含以下组分的药物组合物：

[0067]

双螺杆颗粒共混物	mg
化合物 1 形式 I	400
微晶纤维素	68.0
交联羧甲基纤维素钠	12.6
聚乙烯吡咯烷酮	15.6
月桂基硫酸钠	3.6
片芯片剂组合物（400mg 剂量）	mg
双螺杆颗粒共混物	499.8
微晶纤维素	72.2
交联羧甲基纤维素钠	24.0
硬脂酸镁	6.0

[0068] 在另一个实施例中，本发明提供了包含以下组分的药物组合物：

[0069]

双螺杆颗粒共混物	mg
化合物 1 形式 I	200
微晶纤维素	34.0

[0070]

交联羧甲基纤维素钠	6.3
聚乙烯吡咯烷酮	7.8
月桂基硫酸钠	1.8
片芯片剂组合物（200mg 剂量）	mg
双螺杆颗粒共混物	249.9
微晶纤维素	36.1
交联羧甲基纤维素钠	12.0
硬脂酸镁	3.0
薄膜衣片剂（200mg 剂量，310mg 总重）	mg
片芯片剂组合物	301
薄膜衣	9.0
蜡	微量

[0071] 在另一个实施例中，本发明提供了包含以下组分的药物组合物：

[0072]

双螺杆颗粒共混物	mg
化合物 1 形式 I	400
微晶纤维素	68.0
交联羧甲基纤维素钠	12.6
聚乙烯吡咯烷酮	15.6
月桂基硫酸钠	3.6
片芯片剂组合物（400mg 剂量）	mg
双螺杆颗粒共混物	499.8
微晶纤维素	72.2
交联羧甲基纤维素钠	24.0
硬脂酸镁	6.0
薄膜衣片剂（400mg 剂量，620mg 总重）	mg
片芯片剂组合物	602
薄膜衣	18.0
蜡	微量

[0073] 在另一方面，本发明提供了片剂形式的药物组合物，所述片剂包含化合物 1，以及一种或多种药学上可接受的赋形剂，例如填充剂、崩解剂、表面活性剂、稀释剂、粘合剂、助流剂和润滑剂以及它们的任何组合，其中片剂在约 30 分钟内具有至少约 50％的溶出度。在另一个实施例中，溶出速率在约 30 分钟内为至少约 75％。在另一个实施例中，溶出速率在约 30 分钟内为至少约 90％。

[0074] 在另一个方面，本发明提供了由片剂组成的药物组合物，所述片剂包含含有化合物 1 的粉末共混物或颗粒；以及一种或多种药学上可接受的赋形剂，例如填充剂、崩解剂、表面活性剂、稀释剂、粘合剂、助流剂和润滑剂，其中片剂的硬度为至少约 5kP（kP ＝千磅；1kP ＝～ 9.8N）。在另一个实施例中，片剂在 400 转后的目标脆碎度小于 1.0％。在另一个方面，本发明提供了由片剂组成的药物组合物，所述片剂包含含有化合物 1 形式 II、化合物 1 的粉末共混物或颗粒；以及一种或多种药学上可接受的赋形剂，例如填充剂、崩解剂、表面活性剂、稀释剂、粘合剂、助流剂和润滑剂，其中片剂的硬度为至少约 5kP（kP ＝千磅；1kP ＝～ 9.8N）。在另一个实施例中，片剂在 400 转后的目标脆碎度小于 1.0％。

[0075] 在另一个方面，本发明提供了还包含另外的治疗剂的如本文所述的药物组合物。在一些实施例中，另外的治疗剂为 N-（5- 羟基 -2,4- 二叔丁基苯基）-4- 氧代 -1H- 喹啉 -3- 甲酰胺。

[0076] 在另一个方面，本发明提供了治疗哺乳动物中 CFTR 介导的疾病的方法，包括向哺乳动物施用有效量的如本文所述的药物组合物。在一些实施例中，CFTR 介导的疾病为囊性纤维化、肺气肿、COPD 或骨质疏松。在其他实施例中，CFTR 介导的疾病为囊性纤维化。该方法还可包括施用另外的治疗剂，其中在一些实施例中，另外的治疗剂选自溶粘蛋白剂、支气管扩张剂、抗生素、抗感染剂、抗炎剂、CFTR 增效剂或营养剂。在另一个实施例中，另外的治疗剂为 N-（5- 羟基 -2,4- 二叔丁基苯基）-4- 氧代 -1H- 喹啉 -3- 甲酰胺。在另一个实施例中，患者具有 F508del-CFTR 突变。在另一个实施例中，患者对于 F508del 是纯合的。在另

一个实施例中，患者对于 F508del 是杂合的。

[0077] 在另一个方面，本发明的特征在于包含本发明的片剂和单独的治疗剂或其药物组合物的试剂盒。在另一个实施例中，片剂中的化合物 1 呈形式 I。在另一个实施例中，治疗剂为化合物 1 之外的囊性纤维化纠正剂。在另一个实施例中，治疗剂为囊性纤维化增效剂。在另一个实施例中，治疗剂为 N-(5- 羟基 -2,4- 二叔丁基苯基)-4- 氧代 -1H- 喹啉 -3- 甲酰胺。在另一个实施例中，片剂和治疗剂处于单独的容器中。在另一个实施例中，单独的容器为瓶子。在另一个实施例中，单独的容器为小瓶。在另一个实施例中，单独的容器为泡罩包装。

[0078] 在另一个方面，本发明提供了通过辊压工艺制备本文所述的药物组合物的工艺，包括以下步骤：筛选并称取化合物 1 和赋形剂；将化合物 1 和赋形剂共混适当量的时间；将共混物辊压成带状物并将该带状物磨成颗粒；将颗粒与颗粒外的赋形剂共混适当量的时间；将共混物压成片剂；对片剂进行包衣；以及任选地在一个或两个片剂表面上印刷字母图案。

[0079] 在另一个方面，本发明提供了通过高剪切制粒工艺制备本文所述的药物组合物的工艺，包括以下步骤：筛选并称取化合物 1 和赋形剂；在添加含有表面活性剂和粘合剂的制粒流体的同时以合适的混合速度使化合物 1 和赋形剂混合适当量的时间并将混合物切碎成颗粒；干燥颗粒；将颗粒与颗粒外的赋形剂共混适当量的时间；将共混物压成片剂；对片剂进行包衣；以及任选地在一个或两个片剂表面上印刷字母图案。

[0080] 在另一个方面，本发明提供通过双螺杆湿法制粒工艺制备本文所述的药物组合物的连续或半连续工艺，包括以下步骤：筛选并称取化合物 1 和赋形剂；将化合物 1 和赋形剂在共混机中混合，并且在添加含有表面活性剂和粘合剂的制粒流体的同时以合适的速率使共混物进料至连续制粒机中持续适当量的时间，并将混合物切碎成颗粒；干燥颗粒；将颗粒与颗粒外的赋形剂共混适当量的时间；将共混物压成片剂；对片剂进行包衣；以及任选地在一个或两个片剂表面上印刷字母图案。

附图说明

[0081] 图 1 是由化合物 1 形式 I 的单个晶体结构计算的 X 射线衍射图。

[0082] 图 2 是化合物 1 形式 I 的实际 X 射线粉末衍射图。

[0083] 图 3 是化合物 1 形式 II 的 X 射线粉末衍射图。

[0084] 图 4 提供选自下列的化合物 1 形式 II 的 X 射线衍射图：

[0085] 1) 化合物 1 形式 II，甲醇溶剂化物；

[0086] 2) 化合物 1 形式 II，乙醇溶剂化物；

[0087] 3) 化合物 1 形式 II，丙酮溶剂化物；

[0088] 4) 化合物 1 形式 II，2- 丙醇溶剂化物；

[0089] 5) 化合物 1 形式 II，乙腈溶剂化物；

[0090] 6) 化合物 1 形式 II，四氢呋喃溶剂化物；

[0091] 7) 化合物 1 形式 II，乙酸甲酯溶剂化物；

[0092] 8) 化合物 1 形式 II，2- 丁酮溶剂化物；

[0093] 9) 化合物 1 形式 II，甲酸乙酯溶剂化物；以及

[0094] 10) 化合物 1 形式 II,2- 甲基四氢呋喃溶剂化物。

[0095] 图 5 提供了化合物 1 形式 II 甲醇溶剂化物的 X 射线衍射图。

[0096] 图 6 提供了化合物 1 形式 II 乙醇溶剂化物的 X 射线衍射图。

[0097] 图 7 提供了化合物 1 形式 II 丙酮溶剂化物的 X 射线衍射图。

[0098] 图 8 提供了化合物 1 形式 II2- 丙醇溶剂化物的 X 射线衍射图。

[0099] 图 9 提供了化合物 1 形式 II 乙腈溶剂化物的 X 射线衍射图。

[0100] 图 10 提供了化合物 1 形式 II 四氢呋喃溶剂化物的 X 射线衍射图。

[0101] 图 11 提供了化合物 1 形式 II 乙酸甲酯溶剂化物的 X 射线衍射图。

[0102] 图 12 提供了化合物 1 形式 II2- 丁酮溶剂化物的 X 射线衍射图。

[0103] 图 13 提供了化合物 1 形式 II 甲酸乙酯溶剂化物的 X 射线衍射图。

[0104] 图 14 提供了化合物 1 形式 II2- 甲基四氢呋喃溶剂化物的 X 射线衍射图。

[0105] 图 15 是化合物 1 形式 II 丙酮溶剂化物的差示扫描量热 (DSC) 曲线。

[0106] 图 16 是化合物 1 形式 II 丙酮溶剂化物的热重分析 (TGA) 图线。

[0107] 图 17 是基于单晶 X 射线分析的化合物 1 形式 II 丙酮溶剂化物的构象图。

[0108] 图 18 是化合物 1 的 HCl 盐形式 A 的二聚体的构象图。

[0109] 图 19 是由晶体结构计算的化合物 1 的 HCl 盐形式 A 的 X 射线衍射图。

[0110] 图 20 是化合物 1 的 ^{1}HNMR 光谱。

[0111] 图 21 是化合物 1 的 HCl 盐的 ^{1}HNMR 光谱。

[0112] 图 22 是化合物 1 形式 I 的差示扫描量热 (DSC) 曲线。

[0113] 图 23 是基于单晶 X 射线分析的化合物 1 形式 I 的构象图。

[0114] 图 24 是基于单晶 X 射线分析的化合物 1 形式 II 丙酮溶剂化物的构象图。

[0115] 图 25 是化合物 1 形式 II 丙酮溶剂化物的固态 ^{13}C NMR 光谱 (15.0kHz 自旋)。

[0116] 图 26 是化合物 1 形式 II 丙酮溶剂化物的固态 ^{19}F NMR 光谱 (12.5kHz 自旋)。

[0117] 图 27 是由晶体结构计算的化合物 1 的 HCl 盐形式 A 的 X 射线衍射图。

[0118] 图 28 是曲线图,示出了通过高剪切制粒 (HSG) 工艺和双螺杆湿法制粒 (TSWG) 工艺制备的片剂的化合物 1pH 梯度溶出曲线 (LOD 表示干燥失重,这是限定粉末 / 颗粒中的含水量的测量)。

具体实施方式

[0119] 定义

[0120] 如本文所用,“CFTR”表示囊性纤维化跨膜传导调节因子。

[0121] 如本文所用,“ΔF508”或“F508del”是 CFTR 蛋白质内的具体突变。该突变是包含氨基酸苯丙氨酸的密码子的三个核苷酸在位置 508 处的缺失,从而产生不含该特定苯丙氨酸的 CFTR 蛋白质。

[0122] 如本文所用,对于特定突变 (例如 F508del) 是“纯合的”患者在两个等位基因上具有相同的突变。

[0123] 如本文所用,对于特定突变 (例如 F508del) 是“杂合的”患者在一个等位基因上具有该突变,而在另一个等位基因上具有不同的突变。

[0124] 如本文所用,术语“CFTR 纠正剂”是指增加或诱导细胞表面功能性 CFTR 蛋白质的

量从而导致功能活性增加的化合物。

[0125] 如本文所用，术语“CFTR 增效剂”是指增加或诱导位于细胞表面的 CFTR 蛋白质的通道活性从而导致功能活性增加的化合物。

[0126] 如本文所用，术语“活性药物成分”或“API”是指生物活性化合物。示例性的 API 包括 3-(6-(1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基）环丙烷甲酰胺基）-3- 甲基吡啶 -2- 基）苯甲酸（化合物 1）。

[0127] 术语“固体形式”和相关术语在本文中用于指代 3-(6-(1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基）环丙烷甲酰胺基）-3- 甲基吡啶 -2- 基）苯甲酸（化合物 1）时是指包含了非主要处于液态或气态的化合物 1 的固体形式，例如晶体等等。

[0128] 如本文所用，术语“基本上无定形的”是指在其分子位置中具有很少或不具有长距秩序排列的固体材料。例如，基本上无定形的材料具有低于约 15％的结晶度（例如低于约 10％的结晶度或低于约 5％的结晶度）。还应注意，术语“基本上无定形的”包括描述语“无定形的”，其是指不具有 (0％）结晶度的材料。

[0129] 如本文所用，术语“基本上结晶的”（如在短语基本上结晶的化合物 1 形式 I、化合物 1 形式 II 或化合物 1 的 HCl 盐形式 A 中）是指在其分子位置中主要具有长距秩序排列的固体材料。例如，基本上结晶的材料具有大于约 85％的结晶度（例如大于约 90％的结晶度或大于约 95％的结晶度）。还应注意，术语“基本上结晶的”包括描述语“结晶的”，其是指具有 100％结晶度的材料。

[0130] 本文所用的术语“结晶的”和相关术语当用于描述物质、组分、产物或形式时是指该物质、组分或产物通过 X 射线衍射测定为基本上结晶的。（参见例如 Remington:The Science and Practice of Pharmacy,21st Ed.,Lippincott Williams&Wilkins,Baltimore,Md.(2003)（《雷明顿：药剂学科学与实践》，第 21 版，利平科特·威廉斯·威尔金斯出版公司，马里兰州巴尔的摩，2003 年）；The United States Pharmacopeia,23rd ed.,1843-1844(1995)（《美国药典》，第 23 版，第 1843-1844 页，1995 年））。

[0131] 如本文所用，术语“组合物”通常是指两种或更多种组分的组合物，所述组分通常为一种或多种药物（例如，一种药物（例如化合物 1 形式 I、化合物 1 形式 II 或化合物 1 的 HCl 盐形式 A））和一种或多种药物赋形剂。

[0132] 如本文所用，术语“固体剂型”通常是指药物组合物，当用于经口施用模式时其包括胶囊、片剂、丸剂、散剂和颗粒剂。在此类固体剂型中，将活性化合物与至少一种惰性的、药学上可接受的赋形剂或载体混合。

[0133] 如本文所用，“赋形剂”包括药物组合物中的功能性和非功能性成分。

[0134] 如本文所用，“崩解剂”是水合药物组合物并有助于片剂分散的赋形剂。如本文所用，“稀释剂”或“填充剂”是为药物组合物增添膨松度的赋形剂。

[0135] 如本文所用，“表面活性剂”是赋予药物组合物增强的溶解性和 / 或润湿性的赋形剂。

[0136] 如本文所用，“粘合剂”是赋予药物组合物增强的内聚力或拉伸强度（例如硬度）的赋形剂。

[0137] 如本文所用，“助流剂”是赋予药物组合物增强的流动性的赋形剂。

[0138] 如本文所用，“着色剂”是赋予药物组合物所需的颜色的赋形剂。着色剂的例子包括可商购获得的颜料，诸如 FD&C Blue#1 铝色淀、FD&C Blue#2、其他 FD&C Blue 颜色、二氧化钛、氧化铁和 / 或其组合。在一个实施例中，本发明提供的药物组合物为紫色的。

[0139] 如本文所用，“润滑剂”是加入到压成片剂的药物组合物中的赋形剂。润滑剂有助于将颗粒压成片剂并使药物组合物的片剂从模压机中脱模。

[0140] 如本文所用，“立方厘米”和“cc”可互换地用于表示体积的单位。注意，1cc = 1mL。

[0141] 如本文所用，“千磅”和“kP”可互换使用并表示力的度量，其中 1kP = 约 9.8 牛顿。

[0142] 如本文所用，“脆碎度”是指片剂不管外部压力而保持完整并维持其形式的性质。脆碎度可使用公式 1 所示的数学表达式来定量：

[0143]

$$\text{脆碎度\%} = 100 \times \frac{(W_0 - W_f)}{W_0} \qquad (1)$$

[0144] 其中 W_0 是片剂的初始重量，而 W_f 是片剂在放入通过脆碎度仪后的最终重量。脆碎度使用使实验片剂翻滚 100 或 400 转的标准 USP 测试设备进行测量。本发明的一些片剂的脆碎度小于 5.0%。在另一个实施例中，脆碎度小于 2.0%。在另一个实施例中，400 转之后的目标脆碎度小于 1.0%。

[0145] 如本文所用，“平均粒径”是使用诸如激光散射、图像分析或筛分分析的技术测得的平均粒径。在一个实施例中，用于制备本发明提供的药物组合物的颗粒具有小于 1.0mm 的平均粒径。

[0146] 如本文所用，“堆积密度”是材料的粒子的质量除以粒子占据的总体积。总体积包括粒子体积、粒间空隙体积和内部孔体积。堆积密度不是材料的固有性质；它可以根据材料的加工方式而有所变化。在一个实施例中，用于制备本发明提供的药物组合物的颗粒的堆积密度为约 0.5 至 0.7g/cc。

[0147] 本发明的药物化合物的有效量或“治疗有效量”可根据诸如以下因素而变化：疾病状态、受试者的年龄和体重以及本发明的化合物在受试者中引起所需响应的能力。可调整剂量方案以提供最佳的治疗响应。有效量还是治疗有益效果超过本发明的化合物的任何毒性或有害作用（例如副作用）的量。

[0148] 如本文所用，并且除非另外指明，否则术语化合物的“治疗有效量”和“有效量”是指在疾病或病症的治疗或管理中足以提供治疗有益效果或足以延缓或使与疾病或病症相关的一种或多种症状最小化的量。化合物的“治疗有效量”和“有效量”是指单独的或与一种或多种其他药剂结合的治疗剂的量，其在疾病或病症的治疗或管理中提供治疗有益效果。术语“治疗有效量”和“有效量”可涵盖改善总体治疗、减少或避免疾病或病症的症状或成因或增强另一种治疗剂的治疗效果的量。

[0149] 如在短语“基本上纯的化合物 1 形式 I、化合物 1 形式 II 或化合物 1 的 HCl 盐形式 A”中所用的“基本上纯的”是指大于约 90%的纯度。在另一个实施例中，基本上纯的是指大于约 95%的纯度。在另一个实施例中，基本上纯的是指大于约 98%的纯度。在另一个实施例中，基本上纯的是指大于约 99%的纯度。

[0150] 对于化合物 1（例如化合物 1 形式 I、化合物 1 形式 II、化合物 1 的 HCl 盐形式 A），

术语“约”和“大约”在结合组合物或剂型的成分的剂量、量或重量百分比使用时是指由本领域中的普通技术人员认为提供相当于指定的剂量、量或重量百分比获得的药理作用的剂量、量或重量百分比。具体地讲，术语“约”或“大约”是指由本领域中的普通技术人员确定的可接受的特定值的误差，其部分取决于该值的测量或测定方式。在某些实施例中，术语“约”或“大约”是指在 1、2、3 或 4 个标准偏差内。在某些实施例中，术语“约”或“大约”是指在给定值或范围的 30%、25%、20%、15%、10%、9%、8%、7%、6%、5%、4%、3%、2%、1%、0.5%、0.1%或 0.05%内。

[0151] 除非另外指明，否则术语“化合物 1”包括但不限于本文所述的化合物 1 的固体形式，例如化合物 1 形式 I、化合物 1 形式 II 或化合物 1 的 HCl 盐形式 A 及其组合。

[0152] 药物组合物

[0153] 本发明提供了包含化合物 1 的药物组合物、药物制剂和固体剂型，所述化合物 1 可以为基本上结晶的形式。在一些实施例中，化合物 1 为结晶形式 I（化合物 1 形式 I）。在一些实施例中，化合物 1 为结晶形式 II（化合物 1 形式 II）。在一些实施例中，化合物 1 为结晶的 HCl 盐形式（化合物 1 的 HCl 盐形式 A）。在该方面的一些实施例中，存在于药物组合物中的化合物 1 的量为 25mg、50mg、75mg、100mg、125mg、150mg、200mg、250mg 或 400mg。在该方面的一些实施例中，存在于药物组合物中的化合物 1 的重量 / 重量相对百分比为 10%至 75%。在这些和其他实施例中，3-(6-(1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基）环丙烷甲酰胺基）-3- 甲基吡啶 -2- 基）苯甲酸作为基本上纯的化合物 1 存在。“基本上纯的”是指大于 90%的纯度；优选地大于 95%的纯度；更优选地大于 99.5%的纯度（即，不与化合物 1 的其他结晶形式混合）。

[0154] 因此，在一个方面，本发明提供了包含以下组分的药物组合物：

[0155] a. 化合物 1；

[0156] b. 填充剂；

[0157] c. 崩解剂；

[0158] d. 表面活性剂；

[0159] e. 稀释剂；

[0160] f. 润滑剂；和

[0161] g. 助流剂或粘合剂。

[0162] 在该方面的一个实施例中，药物组合物包含 25mg 化合物 1。在该方面的另一个实施例中，药物组合物包含 50mg 化合物 1。在该方面的另一个实施例中，药物组合物包含 100mg 化合物 1。在该方面的另一个实施例中，药物组合物包含 125mg 化合物 1。在该方面的另一个实施例中，药物组合物包含 150mg 化合物 1。在该方面的另一个实施例中，药物组合物包含 200mg 化合物 1。在该方面的另一个实施例中，药物组合物包含 250mg 化合物 1。在该方面的另一个实施例中，药物组合物包含 300mg 化合物 1。在该方面的另一个实施例中，药物组合物包含 400mg 化合物 1。

[0163] 在一些实施例中，药物组合物包含化合物 1，其中按组合物的重量计，化合物 1 以至少 15 重量%（例如至少 20 重量%、至少 30 重量%、至少 40 重量%、至少 50 重量%、至少 60 重量%或至少 70 重量%）的量存在。

[0164] 在一些实施例中，药物组合物包含化合物 1、填充剂、稀释剂、崩解剂、表面活性剂、

助流剂和润滑剂。在该实施例中，按组合物的重量计，组合物包含约 20 重量％至约 50 重量％（例如约 25 至 35 重量％）的化合物 1，并且更典型地，按组合物的重量计，包含 25 重量％至约 45 重量％（例如约 28 至 32 重量％）的化合物 1。

[0165] 在一些实施例中，药物组合物包含化合物 1、填充剂、稀释剂、崩解剂、表面活性剂、粘合剂和润滑剂。在该实施例中，按组合物的重量计，组合物包含约 30 重量％至约 60 重量％（例如约 40 至 55 重量％）的化合物 1，并且更典型地，按组合物的重量计，包含 35 重量％至约 70 重量％（例如约 45 至 55 重量％）的化合物 1。

[0166] 化合物 1 在组合物中的浓度取决于若干因素，诸如提供所需量的化合物 1 所需要的药物组合物的量以及所需的药物组合物的溶出曲线。

[0167] 在另一个实施例中，药物组合物包含化合物 1，其中为其固体形式的化合物 1 具有通过光散射法（例如，使用可得自英国马尔文仪器有限公司 (Malvern Instruments, England) 的 Malvern Mastersizer) 测得的 0.1 微米至 10 微米的平均粒径。在另一个实施例中，化合物 1 的粒度为 1 微米至 5 微米。在另一个实施例中，化合物 1 的粒度 D50 为 2.0 微米。

[0168] 如所指出的那样，除了化合物 1 以外，在本发明的一些实施例中，为口服制剂的药物组合物还包含一种或多种赋形剂，诸如填充剂、崩解剂、表面活性剂、稀释剂、粘合剂、助流剂、润滑剂、着色剂或芳香剂以及它们的任何组合。

[0169] 适用于本发明的填充剂与药物组合物的成分相容，即，它们基本上不会降低药物组合物的溶解性、硬度、化学稳定性、物理稳定性或生物活性。示例性的填充剂包括：纤维素、改性纤维素（例如羧甲基纤维素钠、乙基纤维素、羟甲基纤维素、羟丙基纤维素）、醋酸纤维素、微晶纤维素、磷酸钙、磷酸氢钙、淀粉（例如玉米淀粉、马铃薯淀粉）、糖（例如山梨醇、乳糖、蔗糖等等）或它们的任何组合。

[0170] 因此，在一个实施例中，按组合物的重量计，药物组合物包含量为至少 5 重量％（例如至少约 20 重量％、至少约 30 重量％或至少约 40 重量％）的至少一种填充剂。例如，按组合物的重量计，药物组合物包含约 10 重量％至约 60 重量％（例如约 20 重量％至约 55 重量％、约 25 重量％至约 50 重量％或约 27 重量％至约 45 重量％）的填充剂。又如，按组合物的重量计，药物组合物包含至少约 20 重量％（例如至少 30 重量％或至少 40 重量％）的微晶纤维素，例如 MCC Avicel PH102。再如，按组合物的重量计，药物组合物包含约 10 重量％至约 60 重量％（例如约 20 重量％至约 55 重量％或约 25 重量％至约 45 重量％）的微晶纤维素。

[0171] 适用于本发明的崩解剂提高了药物组合物的分散性并与药物组合物的成分相容，即，它们基本上不会降低药物组合物的化学稳定性、物理稳定性、硬度或生物活性。示例性的崩解剂包括交联羧甲基纤维素钠、羟基乙酸淀粉钠或其组合。

[0172] 因此，在一个实施例中，按组合物的重量计，药物组合物包含量为约 10 重量％或更少（例如约 7 重量％或更少、约 6 重量％或更少或约 5 重量％或更少）的崩解剂。例如，按组合物的重量计，药物组合物包含约 1 重量％至约 10 重量％（例如约 1.5 重量％至约 7.5 重量％或约 2.5 重量％至约 6 重量％）的崩解剂。又如，按组合物的重量计，药物组合物包含约 10 重量％或更少（例如 7 重量％或更少、6 重量％或更少或 5 重量％或更少）的交联羧甲基纤维素钠。再如，按组合物的重量计，药物组合物包含约 1 重量％至约 10 重量％

（例如约 1.5 重量%至约 7.5 重量%或约 2.5 重量%至约 6 重量%）的交联羧甲基纤维素钠。在一些例子中，按组合物的重量计，药物组合物包含约 0.1%至约 10 重量%（例如约 0.5 重量%至约 7.5 重量%或约 1.5 重量%至约 6 重量%）的崩解剂。在另外其他例子中，按组合物的重量计，药物组合物包含约 0.5%至约 10 重量%（例如约 1.5 重量%至约 7.5 重量%或约 2.5 重量%至约 6 重量%）的崩解剂。

[0173] 适用于本发明的表面活性剂提高了药物组合物的润湿性并与药物组合物的成分相容，即，它们基本上不会降低药物组合物的化学稳定性、物理稳定性、硬度或生物活性。示例性的表面活性剂包括月桂基硫酸钠 (SLS)、硬脂富马酸钠 (SSF)、聚氧乙烯 20 脱水山梨糖醇单油酸酯（例如 Tween™）、它们的任何组合等等。

[0174] 因此，在一个实施例中，按组合物的重量计，药物组合物包含量为约 10 重量%或更少（例如约 5 重量%或更少、约 2 重量%或更少、约 1 重量%或更少、约 0.8 重量%或更少或约 0.6 重量%或更少）的表面活性剂。例如，按组合物的重量计，药物组合物包含约 10 重量%至约 0.1 重量%（例如约 5 重量%至约 0.2 重量%或约 2 重量%至约 0.3 重量%）的表面活性剂。又如，按组合物的重量计，药物组合物包含 10 重量%或更少（例如约 5 重量%或更少、约 2 重量%或更少、约 1 重量%或更少、约 0.8 重量%或更少或约 0.6 重量%或更少）的月桂基硫酸钠。再如，按组合物的重量计，药物组合物包含约 10 重量%至约 0.1 重量%（例如约 5 重量%至约 0.2 重量%或约 2 重量%至约 0.3 重量%）的月桂基硫酸钠。

[0175] 适用于本发明的粘合剂提高了药物组合物的片剂强度并与药物组合物的成分相容，即，它们基本上不会降低药物组合物的化学稳定性、物理稳定性或生物活性。示例性的粘合剂包括聚乙烯吡咯烷酮、磷酸氢钙、蔗糖、玉米（玉蜀黍）淀粉、改性纤维素（例如羟甲基纤维素）或它们的任何组合。

[0176] 因此，在一个实施例中，按组合物的重量计，药物组合物包含量为至少约 0.1 重量%（例如至少约 1 重量%、至少约 3 重量%、至少约 4 重量%或至少约 5 重量%）的粘合剂。例如，按组合物的重量计，药物组合物包含约 0.1 重量%至约 10 重量%（例如约 1 重量%至约 10 重量%或约 2 重量%至约 7 重量%）的粘合剂。又如，按组合物的重量计，药物组合物包含至少约 0.1 重量%（例如至少约 1 重量%、至少约 2 重量%、至少约 3 重量%、或至少约 4 重量%）的聚乙烯吡咯烷酮。再如，按组合物的重量计，药物组合物以约 0.1 重量%至约 10 重量%（例如约 1 重量%至约 8 重量%或约 2 重量%至约 5 重量%）范围内的聚乙烯吡咯烷酮的量包含助流剂。

[0177] 适用于本发明的稀释剂可为制剂增添必要的体积以制备所需大小的片剂并通常与药物组合物的成分相容，即，它们基本上不会降低药物组合物的溶解性、硬度、化学稳定性、物理稳定性或生物活性。示例性的稀释剂包括：糖，例如糖粉 (confectioner's sugar)、可压缩糖 (compressible sugar)、葡聚糖、糊精、右旋糖、乳糖、甘露醇、山梨醇、纤维素和改性纤维素，例如粉末状纤维素，滑石粉、磷酸钙、淀粉或它们的任何组合。

[0178] 因此，在一个实施例中，按组合物的重量计，药物组合物包含量为 40 重量%或更少（例如 35 重量%或更少、30 重量%或更少、或 25 重量%或更少、或 20 重量%或更少或 15 重量%或更少、或 10 重量%或更少）的稀释剂。例如，按组合物的重量计，药物组合物包含约 40 重量%至约 1 重量%（例如约 35 重量%至约 5 重量%或约 30 重量%至约 7 重量%、或 25 重量%至约 10 重量%、或 20 重量%至约 15 重量%）的稀释剂。又如，按组合物的重

量计，药物组合物包含 40 重量％或更少（例如 35 重量％或更少、25 重量％或更少或 15 重量％或更少）的甘露醇。再如，按组合物的重量计，药物组合物包含约 35 重量％至约 1 重量％（例如约 30 重量％至约 5 重量％或约 25 重量％至约 10 重量％）的甘露醇。

[0179] 适用于本发明的助流剂提高了药物组合物的流动性并与药物组合物的成分相容，即，它们基本上不会降低药物组合物的溶解性、硬度、化学稳定性、物理稳定性或生物活性。示例性的助流剂包括胶态二氧化硅、滑石粉或它们的组合。

[0180] 因此，在一个实施例中，按组合物的重量计，药物组合物包含量为 2 重量％或更少（例如 1.75 重量％、1.25 重量％或更少或 1.00 重量％或更少）的助流剂。例如，按组合物的重量计，药物组合物包含约 2 重量％至约 0.05 重量％（例如约 1.5 重量％至约 0.07 重量％或约 1.0 重量％至约 0.09 重量％）的助流剂。又如，按组合物的重量计，药物组合物包含 2 重量％或更少（例如 1.75 重量％、1.25 重量％或更少或 1.00 重量％或更少）的胶态二氧化硅。再如，按组合物的重量计，药物组合物包含约 2 重量％至约 0.05 重量％（例如约 1.5 重量％至约 0.07 重量％或约 1.0 重量％至约 0.09 重量％）的胶态二氧化硅。

[0181] 在一些实施例中，药物组合物可包括口服固体药物剂型，其可以包含可防止制粒微珠掺合剂粘附到表面（例如，混料罐、压模和／或冲床的表面）的润滑剂。润滑剂还可降低颗粒内的粒间摩擦，并改善药物组合物的压缩以及使压缩后的药物组合物从模压机中脱模。润滑剂也与药物组合物的成分相容，即，它们基本上不会降低药物组合物的溶解性、硬度或生物活性。示例性的润滑剂包括硬脂酸镁、硬脂酸钙、硬脂酸锌、硬脂酸钠、硬脂酸、硬脂酸铝、亮氨酸、甘油基二十二烷酸酯、氢化植物油或它们的任何组合。在一个实施例中，按组合物的重量计，药物组合物包含量为 5 重量％或更少（例如 4.75 重量％、4.0 重量％或更少、或 3.00 重量％或更少、或 2.0 重量％或更少）的润滑剂。例如，按组合物的重量计，药物组合物包含约 5 重量％至约 0.10 重量％（例如约 4.5 重量％至约 0.5 重量％或约 3 重量％至约 1 重量％）的润滑剂。又如，按组合物的重量计，药物组合物包含 5 重量％或更少（例如 4.0 重量％或更少、3.0 重量％或更少或 2.0 重量％或更少或 1.0 重量％或更少）的硬脂酸镁。再如，按组合物的重量计，药物组合物包含约 5 重量％至约 0.10 重量％（例如约 4.5 重量％至约 0.15 重量％或约 3.0 重量％至约 0.50 重量％）的硬脂酸镁。

[0182] 本发明的药物组合物可任选地包含一种或多种着色剂、矫味剂和／或芳香剂以增强组合物的视觉吸引力、口感和／或香味。合适的着色剂、矫味剂或芳香剂与药物组合物的成分相容，即，它们基本上不降低药物组合物的溶解性、化学稳定性、物理稳定性、硬度或生物活性。在一个实施例中，药物组合物包含着色剂、矫味剂和／或芳香剂。在一个实施例中，本发明提供的药物组合物为紫色。

[0183] 在一些实施例中，药物组合物包括片剂或可制成片剂，而片剂可用着色剂包衣并任选地使用合适的油墨标上徽标、其他图像和／或文字。在另外其他实施例中，药物组合物包括片剂或可制成片剂，而片剂可用着色剂包衣、打蜡并任选地使用合适的油墨标上徽标、其他图像和／或文字。合适的着色剂和油墨与药物组合物的成分相容，即，它们基本上不降低药物组合物的溶解性、化学稳定性、物理稳定性、硬度或生物活性。合适的着色剂和油墨可以为任何颜色并为基于水的或基于溶剂的。在一个实施例中，将由药物组合物制备的片剂用着色剂包衣然后使用合适的油墨标上徽标、其他图像和／或文字。例如，包含如本文所述的药物组合物的片剂可用约 3 重量％（例如低于约 6 重量％或低于约 4 重量％）的包含

着色剂的薄膜衣包衣。着色后的片剂可用合适的油墨标上徽标和文字，从而指明活性成分在片剂中的强度。又如，包含如本文所述的药物组合物的片剂可用约 3 重量%（例如低于约 6 重量%或低于约 4 重量%）的包含着色剂的薄膜衣包衣。

[0184] 在另一个实施例中，将由药物组合物制备的片剂用着色剂包衣、打蜡然后使用合适的油墨标上徽标、其他图像和 / 或文字。例如，包含如本文所述的药物组合物的片剂可用约 3 重量%（例如低于约 6 重量%或低于约 4 重量%）的包含着色剂的薄膜衣包衣。着色的片剂可用按起始片芯重量的约 0.01% w/w 的量称出的巴西棕榈蜡粉末打蜡。打蜡的片剂可用合适的油墨标上徽标和文字，从而指明活性成分在片剂中的强度。又如，可将包含如本文所述的药物组合物的片剂用约 3 重量%（例如低于约 6 重量%或低于约 4 重量%）的包含着色剂的薄膜衣包衣。着色的片剂可用按起始片芯重量的约 0.01% w/w 的量称出的巴西棕榈蜡粉末打蜡。打蜡的片剂可用诸如黑色油墨的药学级油墨（例如Opacode® S-1-17823，一种基于溶剂的油墨，可从宾夕法尼亚州西点卡乐康公司 (Colorcon, Inc., West Point, PA.) 商购获得）标上徽标和文字，从而指明活性成分在片剂中的强度。

[0185] 一种示例性药物组合物按组合物的重量计包含约 15 重量%至约 70 重量%（例如约 15 重量%至约 60 重量%、约 15 重量%至约 50 重量%、或约 15 重量%至约 40 重量%、或约 20 重量%至约 70 重量%、或约 30 重量%至约 70 重量%、或约 40 重量%至约 70 重量%、或约 50 重量%至约 70 重量%）的化合物 1。前述组合物还可以包含一种或多种药学上可接受的赋形剂，例如约 20 重量%至约 50 重量%的填充剂；约 1 重量%至约 5 重量%的崩解剂；约 2 重量%至约 0.3 重量%的表面活性剂；约 0.1 重量%至约 5 重量%的粘合剂；约 1 重量%至约 30 重量%的稀释剂；约 2 重量%至约 0.05 重量%的助流剂；以及约 5 重量%至约 0.1 重量%的润滑剂。或者，药物组合物包含：按组合物的重量计包含约 15 重量%至约 70 重量%（例如约 20 重量%至约 40 重量%、约 25 重量%至约 60 重量%、或约 30 重量%至约 55 重量%）的化合物 1 的组合物；以及一种或多种赋形剂，例如约 20 重量%至约 50 重量%的填充剂；约 1 重量%至约 5 重量%的崩解剂；约 2 重量%至约 0.3 重量%的表面活性剂；约 0.1 重量%至约 5 重量%的粘合剂；约 1 重量%至约 30 重量%的稀释剂；约 2 重量%至约 0.05 重量%的助流剂；以及约 5 重量%至约 0.1 重量%的润滑剂。

[0186] 另一种示例性药物组合物包含按组合物的重量计约 15 重量%至约 70 重量%（例如约 15 重量%至约 60 重量%、或 15 重量%至约 50 重量%、或约 15 重量%至约 40 重量%或约 20 重量%至约 70 重量%、或约 30 重量%至约 70 重量%、或约 40 重量%至约 70 重量%、或约 50 重量%至约 70 重量%）的化合物 1，以及一种或多种赋形剂，例如约 20 重量%至约 50 重量%的填充剂；约 1 重量%至约 5 重量%的崩解剂；约 2 重量%至约 0.3 重量%的表面活性剂；约 0.1 重量%至约 5 重量%的粘合剂；约 1 重量%至约 30 重量%的稀释剂；约 2 重量%至约 0.05 重量%的助流剂；以及约 2 重量%至约 0.1 重量%的润滑剂。

[0187] 另一种示例性药物组合物包含按组合物的重量计约 15 重量%至约 70 重量%（例如约 15 重量%至约 60 重量%、或 15 重量%至约 50 重量%、或约 15 重量%至约 40 重量%或约 20 重量%至约 70 重量%、或约 30 重量%至约 70 重量%、或约 40 重量%至约 70 重量%、或约 50 重量%至约 70 重量%）的化合物 1，以及一种或多种赋形剂，例如，约 20 重量%至约 50 重量%的填充剂；约 1 重量%至约 5 重量%的崩解剂；约 2 重量%至约 0.3 重

量%的表面活性剂；约 0.1 重量%至约 5 重量%的粘合剂；约 1 重量%至约 30 重量%的稀释剂；约 2 重量%至约 0.05 重量%的助流剂；以及约 2 重量%至约 0.1 重量%的润滑剂。

[0188] 另一种示例性药物组合物包含约 15 重量%至约 70 重量%（例如约 15 重量%至约 60 重量%、或 15 重量%至约 50 重量%、或约 15 重量%至约 40 重量%或约 20 重量%至约 70 重量%、或约 30 重量%至约 70 重量%、或约 40 重量%至约 70 重量%、或约 50 重量%至约 70 重量%）的化合物 1，以及一种或多种赋形剂，例如，约 20 重量%至约 50 重量%的填充剂；约 1 重量%至约 5 重量%的崩解剂；约 2 重量%至约 0.3 重量%的表面活性剂；约 0.1 重量%至约 5 重量%的粘合剂；约 1 重量%至约 30 重量%的稀释剂；约 2 重量%至约 0.05 重量%的助流剂；以及约 2 重量%至约 0.1 重量%的润滑剂。

[0189] 在一个实施例中，本发明为颗粒状药物组合物，其包含：

[0190] a. 按组合物的重量计约 30 重量%的化合物 1；

[0191] b. 按组合物的重量计约 42 重量%的微晶纤维素；

[0192] c. 按组合物的重量计约 21 重量%的甘露醇；

[0193] d. 按组合物的重量计约 3 重量%的交联羧甲基纤维素钠；

[0194] e. 按组合物的重量计约 1 重量%的月桂基硫酸钠；

[0195] f. 按组合物的重量计约 2 重量%的硬脂酸镁；以及

[0196] g. 按组合物的重量计约 0.5 重量%的胶态二氧化硅。

[0197] 本发明的配制成口服制剂的另一种颗粒状组合物包含：

[0198] a. 约 50 重量%的化合物 1；

[0199] b. 按组合物的重量计约 30 重量%的微晶纤维素；

[0200] c. 按组合物的重量计约 13 重量%的甘露醇；

[0201] d. 按组合物的重量计约 2 重量%的交联羧甲基纤维素钠；

[0202] e. 按组合物的重量计约 4 重量%的聚乙烯吡咯烷酮；以及

[0203] f. 按组合物的重量计约 1 重量%的月桂基硫酸钠。

[0204] 在一个实施例中，本发明的口服药物制剂包含：

[0205] a. 按组合物的重量计约 30 重量%的化合物 1；

[0206] b. 按组合物的重量计约 42 重量%的微晶纤维素；

[0207] c. 按组合物的重量计约 21 重量%的甘露醇；

[0208] d. 按组合物的重量计约 3 重量%的交联羧甲基纤维素钠；

[0209] e. 按组合物的重量计约 1 重量%的月桂基硫酸钠；

[0210] f. 按组合物的重量计约 2.5 重量%的硬脂酸镁；以及

[0211] g. 按组合物的重量计约 0.5 重量%的胶态二氧化硅。

[0212] 本发明的另一种口服药物制剂包含：

[0213] a. 按组合物的重量计约 50 重量%的化合物 1；

[0214] b. 按组合物的重量计约 30 重量%的微晶纤维素；

[0215] c. 按组合物的重量计约 13 重量%的甘露醇；

[0216] d. 按组合物的重量计约 4 重量%的交联羧甲基纤维素钠；

[0217] e. 按组合物的重量计约 4 重量%的聚乙烯吡咯烷酮；

[0218] f. 按组合物的重量计约 1 重量%的月桂基硫酸钠；以及

[0219] g. 按组合物的重量计约 0.5 重量%的硬脂酸镁。

[0220] 本发明的另一种口服药物制剂包含：

[0221] a. 按组合物的重量计约 60 重量%的化合物 1；

[0222] b. 按组合物的重量计约 20 重量%的微晶纤维素；

[0223] c. 按组合物的重量计约 13 重量%的甘露醇；

[0224] d. 按组合物的重量计约 4 重量%的交联羧甲基纤维素钠；

[0225] e. 按组合物的重量计约 4 重量%的聚乙烯吡咯烷酮；

[0226] f. 按组合物的重量计约 1 重量%的月桂基硫酸钠；并且

[0227] g. 按组合物的重量计约 0.5 重量%的硬脂酸镁。

[0228] 本发明的另一种口服药物制剂包含：

[0229] a. 约 150 至 250mg 化合物 1；

[0230] b. 约 40 至 50mg 甘露醇；

[0231] c. 约 120 至 130mg 微晶纤维素；

[0232] d. 约 10 至 20mg 交联羧甲基纤维素钠；

[0233] e. 约 10 至 20mg 聚乙烯吡咯烷酮；

[0234] f. 约 1 至 5mg 月桂基硫酸钠；以及

[0235] g. 约 1 至 5mg 硬脂酸镁。

[0236] 本发明的另一种口服药物制剂包含：

[0237] a. 约 200mg 化合物 1；

[0238] b. 约 43mg 甘露醇；

[0239] c. 约 123mg 微晶纤维素；

[0240] d. 约 15mg 交联羧甲基纤维素钠；

[0241] e. 约 13mg 聚乙烯吡咯烷酮；

[0242] f. 约 3mg 月桂基硫酸钠；以及

[0243] g. 约 4mg 硬脂酸镁。

[0244] 本发明的另一种口服药物制剂包含：

[0245] a. 约 200mg 化合物 1；

[0246] b. 约 45mg 甘露醇；

[0247] c. 约 123mg 微晶纤维素；

[0248] d. 约 15mg 交联羧甲基纤维素钠；

[0249] e. 约 10.4mg 聚乙烯吡咯烷酮；

[0250] f. 约 2.6mg 月桂基硫酸钠；以及

[0251] g. 约 4mg 硬脂酸镁。

[0252] 本发明的另一种口服药物制剂包含：

[0253] a. 按组合物的重量计约 70 重量%的化合物 1；

[0254] b. 按组合物的重量计约 12 重量%的微晶纤维素；

[0255] c. 按组合物的重量计约 11 重量%的甘露醇；

[0256] d. 按组合物的重量计约 4 重量%的交联羧甲基纤维素钠；

[0257] e. 按组合物的重量计约 4 重量%的聚乙烯吡咯烷酮；

[0258] f. 按组合物的重量计约 1 重量%的月桂基硫酸钠；以及

[0259] g. 按组合物的重量计约 0.5 重量%的硬脂酸镁。

[0260] 本发明的药物组合物可加工成片剂形式、胶囊形式、小袋形式、锭剂形式或适于口服的其他固体形式。因此，在一些实施例中，药物组合物为片剂形式。

[0261] 在本发明的又一种口服药物制剂中，初始硬度为 5-21kP±20%的成形药物片剂组合物包含：约 30 重量%的化合物 1；按组合物的重量计约 42 重量%的微晶纤维素；按组合物的重量计约 21 重量%的甘露醇；按组合物的重量计约 3 重量%的交联羧甲基纤维素钠；按组合物的重量计约 1 重量%的月桂基硫酸钠；按组合物的重量计约 2.5 重量%的硬脂酸镁；以及按组合物的重量计约 0.5 重量%的胶态二氧化硅。其中化合物 1 在成形药物片剂中的量在每片约 25mg 至约 250mg 的范围内，例如 50mg、或 75mg、或 100mg、或 150mg、200mg、或 250mg 的化合物 1。

[0262] 在本发明的又一种口服药物制剂中，初始硬度为 5-21kP±20%的成形药物片剂组合物包含：约 49 重量%的化合物 1；按组合物的重量计约 29 重量%的微晶纤维素；按组合物的重量计约 12.6 重量%的甘露醇；按组合物的重量计约 4 重量%的交联羧甲基纤维素钠；按组合物的重量计约 4 重量%的聚乙烯吡咯烷酮；按组合物的重量计约 1 重量%的月桂基硫酸钠；以及按组合物的重量计约 0.5 重量%的硬脂酸镁。化合物 1 在成形药物片剂中的量在每片约 25mg 至约 250mg 的范围内，例如 50mg、或 75mg、或 100mg、或 150mg、200mg、或 250mg 的化合物 1。

[0263] 在某些实施例中，成形药物片剂包含约 100mg 化合物 1。在某些实施例中，成形药物片剂包含约 200mg 化合物 1。

[0264] 本发明的另一个方面提供由片剂或胶囊剂组成的药物制剂，其包含化合物 1 和其他赋形剂（例如填充剂、崩解剂、表面活性剂、粘合剂、助流剂、着色剂、润滑剂或其任意组合），其每一者如上文和下文的实例中所述，其中该片剂在约 30 分钟内的溶出度为至少约 50%（例如至少约 60%、至少约 70%、至少约 80%、至少约 90%、或至少约 99%）。在一个例子中，药物组合物由片剂组成，该片剂包含 25mg 至 250mg 范围内（例如 25mg、或 50mg、或 75mg、或 100mg、或 150mg、200mg、或 250mg）的量的化合物 1 以及一种或多种赋形剂（例如填充剂、崩解剂、表面活性剂、粘合剂、助流剂、着色剂、润滑剂或其任意组合），其每一者如上文和下文的实例中所述，其中该片剂在约 30 分钟内的溶出度为约 50%至约 100%（例如约 55%至约 95%或约 60%至约 90%）。又如，药物组合物由片剂组成，该片剂包含含有化合物 1 以及一种或多种选自以下的赋形剂的组合物：填充剂、稀释剂、崩解剂、表面活性剂、粘合剂、助流剂和润滑剂，其中该片剂在约 30 分钟内的溶出度为至少约 50%（例如至少约 60%、至少约 70%、至少约 80%、至少约 90%、或至少约 99%）。

[0265] 在一个实施例中，该片剂包含含有至少约 25mg（例如至少约 30mg、至少约 40mg、或至少约 50mg）化合物 1 以及一种或多种选自以下的赋形剂的组合物：填充剂、稀释剂、崩解剂、表面活性剂、粘合剂、助流剂和润滑剂。在另一个实施例中，该片剂包含含有至少约 25mg（例如至少约 30mg、至少约 40mg、至少约 50mg、至少约 100mg、或至少 150mg）化合物 1 以及一种或多种选自以下的赋形剂的组合物：填充剂、稀释剂、崩解剂、表面活性剂、粘合剂、助流剂和润滑剂。

[0266] 可通过采用以下条件的标准美国药典 II 型仪器来测量溶出度：溶解在 900mL 去离

子水（用 50mM 磷酸二氢钾缓冲到 pH 6.8）中的 0.1% CTAB 作为溶出介质，在约 37℃的温度下以约 50-75rpm 搅拌。在仪器的每个测试容器中测试单个实验片剂。还可以通过采用以下条件的标准美国药典 II 型仪器来测量溶出度：溶解在 900mL 50mM 磷酸钠缓冲液（pH 6.8）中的 0.7%月桂基硫酸钠作为溶出介质，在约 37℃的温度下以约 65rpm 搅拌。在仪器的每个测试容器中测试单个实验片剂。还可以通过采用以下条件的标准美国药典 II 型仪器来测量溶出度：溶解在 900mL 50mM 磷酸钠缓冲液（pH 6.8）中的 0.5%月桂基硫酸钠作为溶出介质，在约 37℃的温度下以约 65rpm 搅拌。在仪器的每个测试容器中测试单个实验片剂。

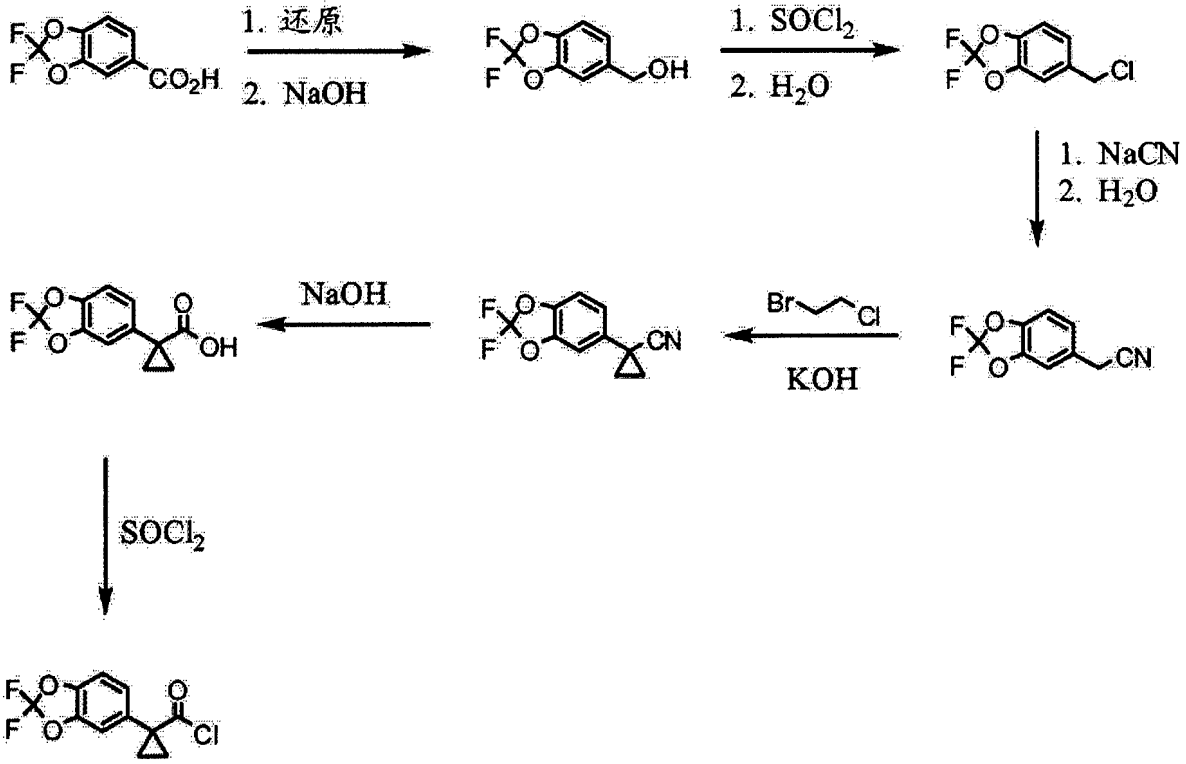
[0267] 制备化合物 1、化合物 1 形式 I、化合物 1 形式 II、化合物 1HCl 盐形式 A 的方法

[0268] 化合物 1

[0269] 化合物 1 用作其他固态形式的起点并可通过将酰氯部分与胺部分根据方案 1-4 偶联而制备。

[0270] 方案 1. 酰氯部分的合成。

[0271]



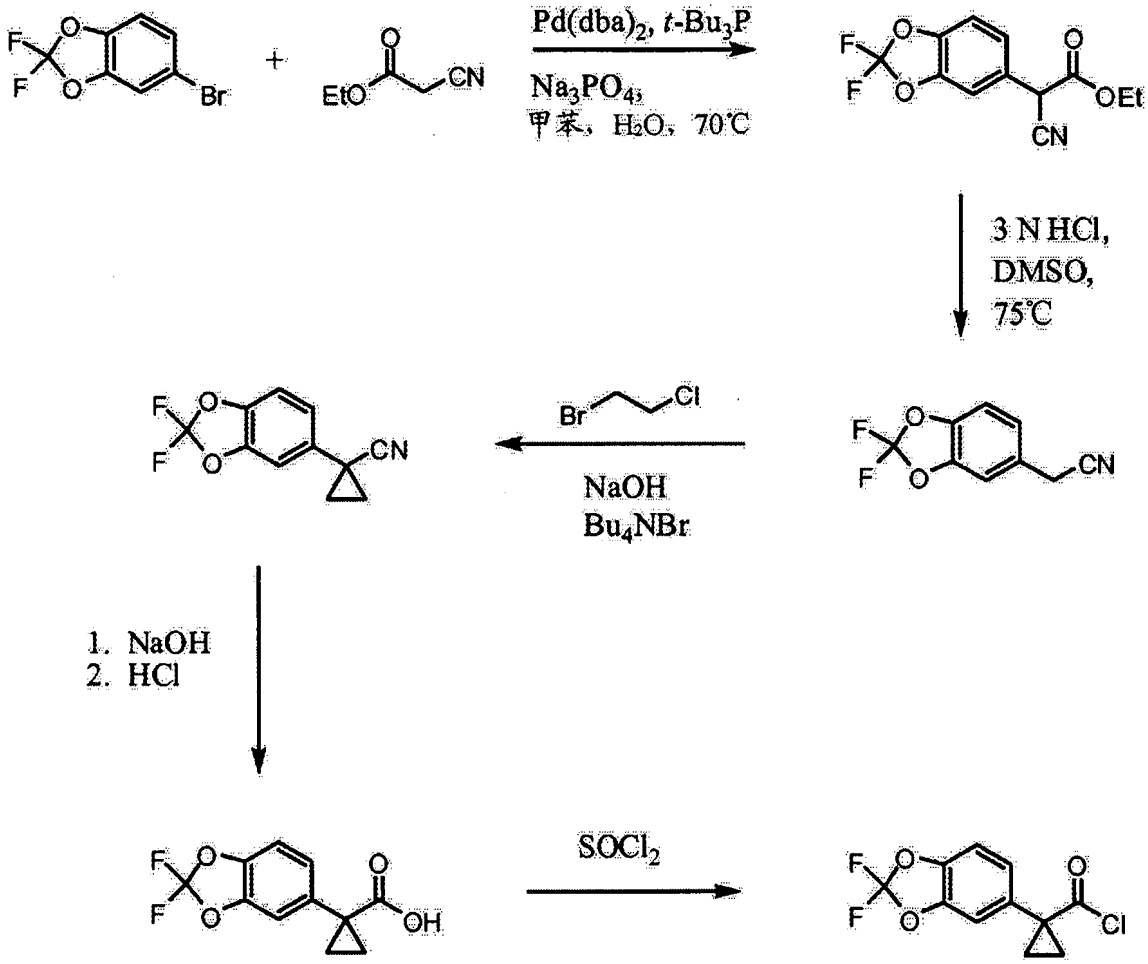
[0272] 方案 1 示出了 1-(2,2-二氟苯并[d][1,3]二氧杂环戊烯-5-基）环丙烷甲酰氯的制备，该物质用于方案 3 以形成化合物 1 的酰胺键合。

[0273] 原料 2,2-二氟苯并[d][1,3]二氧杂环戊烯-5-甲酸从赛拓（Saltigo）（朗盛（Lanxess Corporation）子公司）商购获得。将 2,2-二氟苯并[d][1,3]二氧杂环戊烯-5-甲酸中的羧酸部分还原成伯醇然后使用亚硫酰氯（$SOCl_2$）转化成相应的氯化物提供 5-（氯甲基）-2,2-二氟苯并[d][1,3]二氧杂环戊烯，随后使用氰化钠将其转化成 2-(2,2-二氟苯并[d][1,3]二氧杂环戊烯-5-基）乙腈。将 2-(2,2-二氟苯并[d][1,3]二氧杂环戊烯-5-基）乙腈用碱和 1-溴-2-氯乙烷处理提供 1-(2,2-二氟苯并[d][1,3]二氧杂环戊烯-5-基）环丙烷甲腈。将 1-(2,2-二氟苯并[d][1,3]二氧杂环戊烯-5-基）环丙

烷甲腈中的腈部分用碱转化成羧酸得到1-(2,2-二氟苯并[d][1,3]二氧杂环戊烯-5-基)环丙烷甲酸,将其用亚硫酰氯转化成所需的酰氯。

[0274] 方案2.酰氯部分的替代合成。

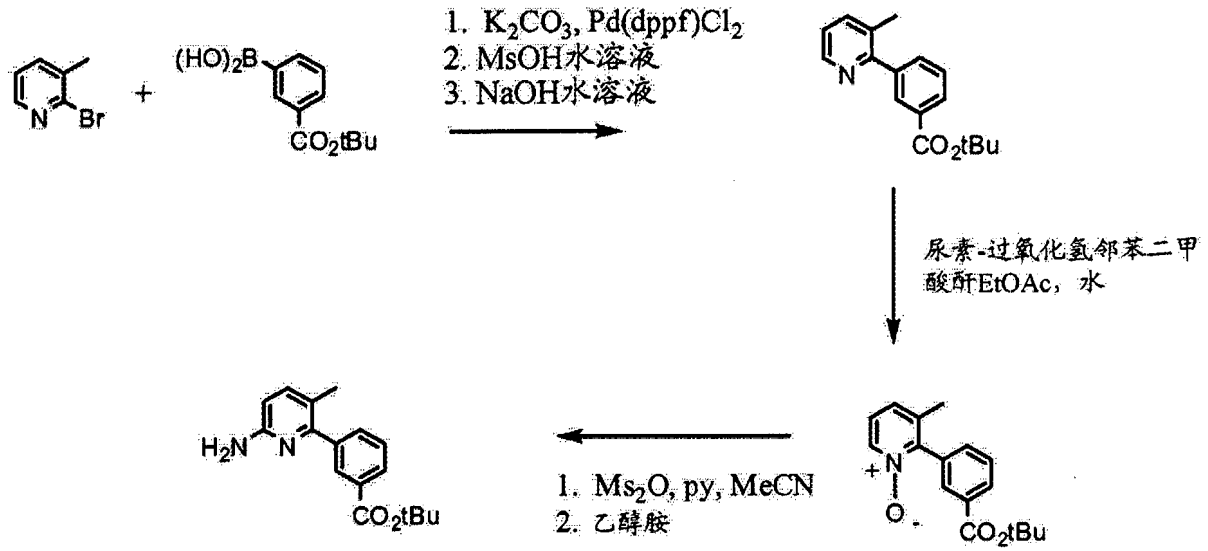
[0275]



[0276] 方案2示出了必需的酰氯的替代合成。将5-溴甲基-2,2-二氟-1,3-苯并二氧杂环戊烯在存在钯催化剂的情况下与氰基乙酸酯偶联以形成相应的α氰基乙酯。将酯部分皂化成羧酸得到氰基乙基化合物。将氰基乙基化合物用1-溴-2-氯乙烷在存在碱的情况下烷基化得到氰基环丙基化合物。将氰基环丙基化合物用碱处理得到羧酸盐,将其通过用酸处理而转化成羧酸。然后使用诸如亚硫酰氯等氯化剂实现羧酸向酰氯的转化。

[0277] 方案3.胺部分的合成。

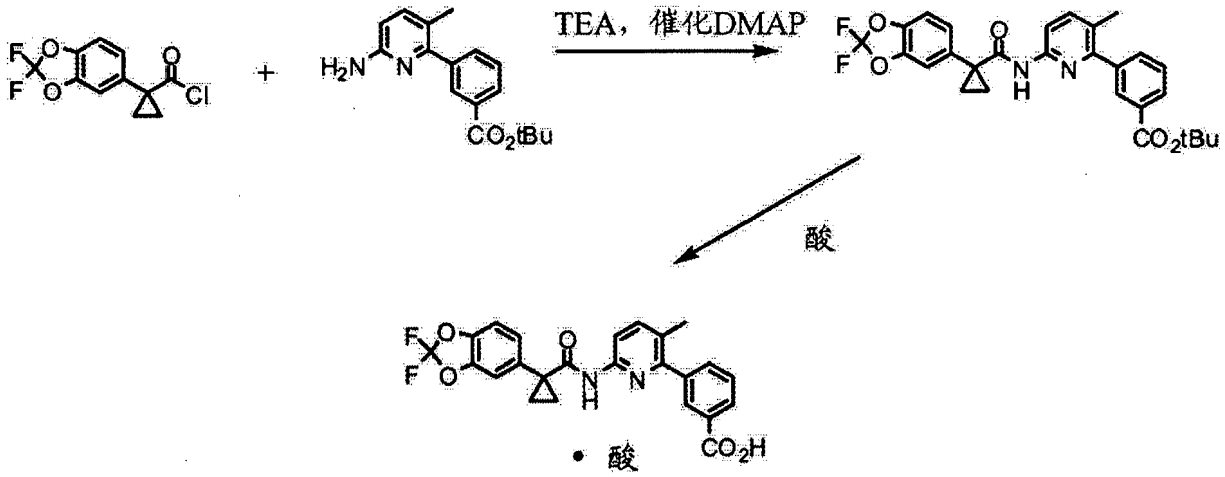
[0278]



[0279] 方案 3 示出了必需的 3-(6- 氨基 -3- 甲基吡啶 -2- 基）苯甲酸叔丁酯的制备，其在方案 3 中与 1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基）环丙烷甲酰氯偶联得到化合物 1。2- 溴 -3- 甲基吡啶与 3-（叔丁氧羰基）苯基硼酸的钯催化偶联得到 3-(3- 甲基吡啶 -2- 基）苯甲酸叔丁酯，其随后转化成所需的化合物。

[0280] 方案 4. 3-(6-(1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基）环丙烷甲酰胺基）-3- 甲基吡啶 -2- 基）苯甲酸的酸盐的形成。

[0281]



[0282] 方案 4 示出了使用三乙胺和 4- 二甲基氨基吡啶进行 1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基）环丙烷甲酰氯与 3-(6- 氨基 -3- 甲基吡啶 -2- 基）苯甲酸叔丁酯的偶联最初提供化合物 1 的叔丁酯。

[0283] 化合物 1 形式 I

[0284] 化合物 1 形式 I 通过将化合物 1 的盐形式（诸如 HCl 盐）在合适的溶剂中分散或溶解有效的时长而制备。将叔丁酯用诸如 HCl 的酸处理得到化合物 1 的 HCl 盐，其通常为结晶固体。化合物 1 形式 I 还可以通过用合适的酸（诸如甲酸）处理而直接由叔丁酯前体制备。

[0285] 3-(6-(1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基）环丙烷甲酰胺基）-3- 甲基吡啶 -2- 基）苯甲酸的 HCl 盐可用于通过将 3-(6-(1-(2,2- 二氟苯并 [d][1,3] 二氧杂

环戊烯 -5- 基）环丙烷甲酰胺基）-3- 甲基吡啶 -2- 基）苯甲酸的 HCl 盐在合适的溶剂中分散或溶解有效的时长而制备形式 I。可以使用 3-(6-(1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基）环丙烷甲酰胺基）-3- 甲基吡啶 -2- 基）苯甲酸的其他盐，诸如衍生自其他矿物酸或有机酸的盐。其他盐由叔丁酯部分的酸介导的水解而得到。衍生自其他酸的盐可包括例如硝酸、硫酸、磷酸、硼酸、乙酸、苯甲酸和丙二酸。3-(6-(1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基）环丙烷甲酰胺基）-3- 甲基吡啶 -2- 基）苯甲酸的这些盐形式可根据所用的溶剂为可溶的或不溶的，但溶解性不足不会阻碍形式 I 的形成。例如，在一个实施例中，合适的溶剂可以为水或醇 / 水混合物，诸如 50％甲醇 / 水混合物，即使 3-(6-(1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基）环丙烷甲酰胺基）-3- 甲基吡啶 -2- 基）苯甲酸的 HCl 盐形式仅微溶于水。在一个实施例中，合适的溶剂为水。

[0286] 由 3-(6-(1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基）环丙烷甲酰胺基）-3- 甲基吡啶 -2- 基）苯甲酸的盐形成形式 I 的有效时长可以为 2 至 24 小时的任何时间或更长。已经认识到，所需的时长与温度成反比。也就是说，温度越高，实现酸解离形成形式 I 所需的时间越短。当溶剂为水时，在室温下将分散体搅拌约 24 小时以大约 98％的产率提供形式 I。如果出于工艺目的需要 3-(6-(1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基）环丙烷甲酰胺基）-3- 甲基吡啶 -2- 基）苯甲酸的盐的溶液，则可以使用升高的温度。在升高的温度下将溶液搅拌有效的时长后，在冷却时的重结晶提供基本上纯的形式 I。在一个实施例中，基本上纯的是指大于约 90％的纯度。在另一个实施例中，基本上纯的是指大于约 95％的纯度。在另一个实施例中，基本上纯的是指大于约 98％的纯度。在另一个实施例中，基本上纯的是指大于约 99％的纯度。所选的温度部分地取决于所用的溶剂，并完全在本领域普通技术人员的确定能力范围内。在一个实施例中，该温度在室温与约 80℃之间。在另一个实施例中，该温度在室温与约 40℃之间。在另一个实施例中，该温度在约 40℃与约 60℃之间。在另一个实施例中，该温度在约 60℃与约 80℃之间。

[0287] 化合物 1 形式 I 也可直接由作为化合物 1 的盐的前体的 3-(6-(1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基）环丙烷甲酰胺基）-3- 甲基吡啶 -2- 基）苯甲酸叔丁酯（参阅方案 3）形成。因此，使 3-(6-(1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基）环丙烷甲酰胺基）-3- 甲基吡啶 -2- 基）苯甲酸叔丁酯在合适的反应条件下与合适的酸（诸如甲酸）发生反应以得到化合物 1 形式 I。

[0288] 化合物 1 形式 I 可通过从有机溶剂中重结晶而进一步纯化。有机溶剂的例子包括但不限于甲苯、异丙基苯、茴香醚、1- 丁醇、醋酸异丙酯、醋酸丁酯、醋酸异丁酯、甲基叔丁基醚、甲基异丁基酮和 1- 丙醇 - 水混合物。温度可以为如上所述的温度。例如，将形式 I 在 75℃下溶于 1- 丁醇直至其完全溶解。将溶液以 0.2℃ /min 的速率冷却到 10℃得到形式 I 的晶体，其可以通过过滤而分离。

[0289] 在一个实施例中，化合物 1 形式 I 的特征在于在使用 Cu Kα 辐射得到的 X 射线粉末衍射图中的 15.2 至 15.6 度、16.1 至 16.5 度以及 14.3 至 14.7 度的一个或多个峰。在另一个实施例中，化合物 1 形式 I 的特征在于在 15.4、16.3 和 14.5 度的一个或多个峰。在另一个实施例中，化合物 1 形式 I 的特征还在于在 14.6 至 15.0 度的峰。在另一个实施例中，化合物 1 形式 I 的特征还在于在 14.8 度的峰。在另一个实施例中，化合物 1 形式 I 的特征还在于在 17.6 至 18.0 度的峰。在另一个实施例中，化合物 1 形式 I 的特征还在于在 17.8

度的峰。在另一个实施例中，化合物 1 形式 I 的特征还在于在 16.4 至 16.8 度的峰。在另一个实施例中，化合物 1 形式 I 的特征还在于在 16.4 至 16.8 度的峰。在另一个实施例中，化合物 1 形式 I 的特征还在于在 16.6 度的峰。在另一个实施例中，化合物 1 形式 I 的特征还在于在 7.6 至 8.0 度的峰。在另一个实施例中，化合物 1 形式 I 的特征还在于在 7.8 度的峰。在另一个实施例中，化合物 1 形式 I 的特征还在于在 25.8 至 26.2 度的峰。在另一个实施例中，化合物 1 形式 I 的特征还在于在 26.0 度的峰。在另一个实施例中，化合物 1 形式 I 的特征还在于在 21.4 至 21.8 度的峰。在另一个实施例中，化合物 1 形式 I 的特征还在于在 21.6 度的峰。在另一个实施例中，化合物 1 形式 I 的特征还在于在 23.1 至 23.5 度的峰。在另一个实施例中，化合物 1 形式 I 的特征还在于在 23.3 度的峰。在一些实施例中，化合物 1 形式 I 的特征在于基本上与图 1 类似的衍射图案。在一些实施例中，化合物 1 形式 I 的特征在于基本上与图 2 类似的衍射图案。

[0290] 在一些实施例中，对于化合物 1 形式 I，D90 粒度分布为约 82μm 或更少。在一些实施例中，对于化合物 1 形式 I，D50 粒度分布为约 30μm 或更少。

[0291] 化合物 1 形式 II

[0292] 化合物 1 形式 II 通过使化合物 1 形式 I 在合适的溶剂中以足够的浓度浆化足够的时间而制备。然后将浆液进行离心过滤或在真空下过滤，并在环境条件下干燥足够的时间以得到化合物 1 形式 II。

[0293] 在一些实施例中，将约 20 至 40mg 化合物 1 形式 I 在约 400 至 600μL 合适的溶剂中浆化。在另一个实施例中，将约 25 至 35mg 化合物 1 形式 I 在约 450 至 550μL 合适的溶剂中浆化。在另一个实施例中，将约 30mg 化合物 1 形式 I 在约 500μL 合适的溶剂中浆化。

[0294] 在一些实施例中，将化合物 1 形式 I 用溶剂进行浆化的时间为 1 小时至四天。更具体地讲，将化合物 1 形式 I 用溶剂进行浆化的时间为 1 至 3 天。更具体地讲，该时间为 2 天。

[0295] 在一些实施例中，合适的溶剂选自大小足以配合化合物 1 形式 II 的晶格中的空隙的有机溶剂。在其他实施例中，溶剂化物具有足以配合在尺寸为约 $100Å^3$ 的空隙中的大小。

[0296] 在其他实施例中，溶剂选自甲醇、乙醇、丙酮、2- 丙醇、乙腈、四氢呋喃、醋酸甲酯、2- 丁酮、甲酸乙酯和 2- 甲基四氢呋喃。

[0297] 在其他实施例中，可将这些溶剂中的两种或更多种的混合物用于获得化合物 1 形式 II。作为另外一种选择，化合物 1 形式 II 可得自包含这些溶剂中的一种或多种和水的混合物。

[0298] 在一些实施例中，干燥化合物 1 形式 II 的有效时长为 1 至 24 小时。更具体地讲，该时间为 6 至 18 小时。更具体地讲，该时间为约 12 小时。

[0299] 在另一个实施例中，化合物 1 形式 II 通过将化合物 1 的盐形式（诸如化合物 1 的 HCl 盐）分散或溶解在合适的溶剂中有效的时长而制备。

[0300] 如本文所公开的化合物 1 形式 II 包含化合物 1 的晶格，其中晶格中的空隙为空的，或被合适溶剂的一个或多个分子占据或部分占据。合适的溶剂包括但不限于甲醇、乙醇、丙酮、2- 丙醇、乙腈、四氢呋喃、醋酸甲酯、2- 丁酮、甲酸乙酯和 2- 甲基四氢呋喃。化合物 1 同构溶剂化物形式的某些物理特性（诸如 X 射线粉末衍射、熔点和 DSC）基本上不受所考虑的特定溶剂分子影响。

[0301] 在一个实施例中，化合物 1 形式 II 的特征在于在使用 Cu Kα 辐射得到的 X 射线粉末衍射图中的 21.50 至 21.90 度、8.80 至 9.20 度以及 10.80 至 11.20 度的一个或多个峰。在另一个实施例中，化合物 1 形式 II 的特征在于在使用 Cu Kα 辐射得到的 X 射线粉末衍射图中的 21.50 至 21.90 度、8.80 至 9.20 度、10.80 至 11.20 度、18.00 至 18.40 度和 22.90 至 23.30 度的一个或多个峰。在另一个实施例中，化合物 1 形式 II 的特征在于在 21.70、8.98 和 11.04 度的一个或多个峰。在另一个实施例中，化合物 1 形式 II 的特征在于在 21.70、8.98、11.04、18.16 和 23.06 度的一个或多个峰。在另一个实施例中，化合物 1 形式 II 的特征在于在 21.50 至 21.90 度的峰。在另一个实施例中，化合物 1 形式 II 的特征还在于在 21.70 度的峰。在另一个实施例中，化合物 1 形式 II 的特征还在于在 8.80 至 9.20 度的峰。在另一个实施例中，化合物 1 形式 II 的特征还在于在 8.98 度的峰。在另一个实施例中，化合物 1 形式 II 的特征还在于在 10.80 至 11.20 度的峰。在另一个实施例中，化合物 1 形式 II 的特征还在于在 11.04 度的峰。在另一个实施例中，化合物 1 形式 II 的特征还在于在 18.00 至 18.40 度的峰。在另一个实施例中，化合物 1 形式 II 的特征还在于在 18.16 度的峰。在另一个实施例中，化合物 1 形式 II 的特征还在于在 22.90 至 23.30 度的峰。在另一个实施例中，化合物 1 形式 II 的特征还在于在 23.06 度的峰。在另一个实施例中，化合物 1 形式 II 的特征还在于在 20.40 至 20.80 度的峰。在另一个实施例中，化合物 1 形式 II 的特征还在于在 20.63 度的峰。在另一个实施例中，化合物 1 形式 II 的特征还在于在 22.00 至 22.40 度的峰。在另一个实施例中，化合物 1 形式 II 的特征还在于在 22.22 度的峰。在另一个实施例中，化合物 1 形式 II 的特征还在于在 18.40 至 18.80 度的峰。在另一个实施例中，化合物 1 形式 II 的特征还在于在 18.57 度的峰。在另一个实施例中，化合物 1 形式 II 的特征还在于在 16.50 至 16.90 度的峰。在另一个实施例中，化合物 1 形式 II 的特征还在于在 16.66 度的峰。在另一个实施例中，化合物 1 形式 II 的特征还在于在 19.70 至 20.10 度的峰。在另一个实施例中，化合物 1 形式 II 的特征还在于在 19.86 度的峰。

[0302] 在一些实施例中，化合物 1 形式 II 的特征在于基本上与图 3 类似的衍射图案。在一些实施例中，化合物 1 形式 II 的特征在于基本上与图 4 中所提供的那些衍射图案类似的衍射图案。

[0303] 在另一个实施例中，形成化合物 1 形式 II 的溶剂化物选自甲醇、乙醇、丙酮、2- 丙醇、乙腈、四氢呋喃、醋酸甲酯、2- 丁酮、甲酸乙酯和 2- 甲基四氢呋喃。提供了以下化合物 1 形式 II 的衍射图案：甲醇（图 5）、乙醇（图 6）、丙酮（图 7）、2- 丙醇（图 8）、乙腈（图 9）、四氢呋喃（图 10）、醋酸甲酯（图 11）、2- 丁酮（图 12）、甲酸乙酯（图 13）和 2- 甲基四氢呋喃（图 14）。

[0304] 在另一个实施例中，本发明提供化合物 1 形式 II，其表现出通过 DSC 或技术人员已知的类似分析方法测得的两个或更多个相变。在一些实施例中，化合物 1 形式 II 的 DSC 基本上类似于图 15 中所示的 DSC 曲线。在该方面的另一个实施例中，DSC 给出两个相变。在另一个实施例中，DSC 给出三个相变。在另一个实施例中，相变之一发生在 200 与 207℃之间。在另一个实施例中，相变之一发生在 204 与 206℃之间。在另一个实施例中，相变之一发生在 183 与 190℃之间。在另一个实施例中，相变之一发生在 185 与 187℃之间。在另一个实施例中，化合物 1 溶剂化物形式 A 的熔点在 183℃至 190℃之间。在另一个实施例中，

化合物 1 溶剂化物形式 A 的熔点在 185℃至 187℃之间。

[0305] 在另一个实施例中，化合物 1 形式 II 包含通过 TGA 测得的 1 至 10 重量％（wt.％）的溶剂化物。在一些实施例中，化合物 1 形式 II 的 TGA 基本上类似于图 16 中所示的 TGA 曲线。在另一个实施例中，化合物 1 形式 II 包含通过 TGA 或技术人员已知的类似分析方法测得的 2 至 5wt.％的溶剂化物。

[0306] 在另一个实施例中，化合物 1 形式 II 丙酮溶剂化物的构象基本上类似于基于单 X 射线分析的图 17 中所示的构象。

[0307] 在另一个实施例中，化合物 1 形式 II 丙酮溶剂化物具有 $P2_1/n$ 空间群和以下单位晶胞尺寸：

[0308] $a = 16.5235(10)$ Å $\alpha = 90°$

[0309] $b = 12.7425(8)$ Å $\beta = 103.736(4)°$

[0310] $c = 20.5512(13)$ Å $\gamma = 90°$ 。

[0311] 化合物 1HCl 盐形式 A

[0312] 化合物 1HCl 盐形式 A 可由化合物 1 的 HCl 盐来制备，方法是通过将化合物 1 的 HCl 盐溶于最少的溶剂并通过缓慢蒸发除去溶剂。在另一个实施例中，溶剂为醇。在另一个实施例中，溶剂为乙醇。缓慢蒸发通常通过阻碍溶剂的蒸发而进行。例如，在一个实施例中，缓慢蒸发涉及将化合物 1 的 HCl 盐溶于小瓶然后将小瓶盖上其中戳有孔的石蜡膜。

[0313] 在一个实施例中，化合物 1HCl 盐形式 A 的特征在于在使用 Cu Kα 辐射得到的 X 射线粉末衍射图中的 8.80 至 9.20 度、17.30 至 17.70 度和 18.20 至 18.60 度的一个或多个峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征在于在使用 Cu Kα 辐射得到的 X 射线粉末衍射图中的 8.80 至 9.20 度、17.30 至 17.70 度、18.20 至 18.60 度、10.10 至 10.50 度和 15.80 至 16.20 度的一个或多个峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征在于在 8.96、17.51 和 18.45 度的一个或多个峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征在于在 8.96、17.51、18.45、10.33 和 16.01 度的一个或多个峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征在于在 8.80 至 9.20 度的峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征在于在 8.96 度的峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征还在于在 17.30 至 17.70 度的峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征在于在 17.51 度的峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征还在于在 18.20 至 18.60 度的峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征还在于在 18.45 度的峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征还在于在 10.10 至 10.50 度的峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征还在于在 10.33 度的峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征还在于在 15.80 至 16.20 度的峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征还在于在 16.01 度的峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征还在于在 11.70 至 12.10 度的峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征还在于在 11.94 度的峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征还在于在 7.90 至 8.30 度的峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征还在于在 8.14 度的峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征还在于在 9.90 至 10.30 度的峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征还在于在 10.10 度的峰。在另一个实施例中，化合物 1HCl 盐

形式 A 的特征还在于在 16.40 至 16.80 度的峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征还在于在 16.55 度的峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征还在于在 9.30 至 9.70 度的峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征还在于在 9.54 度的峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征还在于在 16.40 至 16.80 度的峰。在另一个实施例中，化合物 1HCl 盐形式 A 的特征还在于在 16.55 度的峰。在一些实施例中，将化合物 1HCl 盐形式 A 表征为如图 18 中所示的二聚体。

[0314] 在一些实施例中，化合物 1HCl 盐形式 A 的特征在于基本上与图 19 类似的衍射图案。

[0315] 在另一个实施例中，本发明的特征在于具有 $P^{-}1$ 空间群和以下单位晶胞尺寸的结晶化合物 1HCl 盐形式 A：

[0316] $a = 10.2702\ (2)\ \text{Å}$ $\alpha = 67.0270(10)^{\circ}$

[0317] $b = 10.8782\ (2)\ \text{Å}$ $\beta = 66.1810(10)^{\circ}$

[0318] $c = 12.4821\ (3)\ \text{Å}$ $\gamma = 72.4760(10)^{\circ}$ 。

[0319] 制备药物组合物的方法

[0320] 本发明的剂量单位形式可以通过在压力下压缩或压制配混物或组合物（例如粉末或颗粒）以形成稳定的三维形状（例如片剂）来制备。如本文所用，“片剂”包括所有形状和大小的压制的药物剂量单位形式，不论是包衣的还是未包衣的。

[0321] 如本文所用的表述“剂量单位形式”是指适合于待治疗的患者的药剂的物理离散单元。通常，压缩的混合物的密度大于压缩之前的该混合物的密度。本发明的剂量单位形式可以具有几乎任何形状，包括凹状和 / 或凸状表面、圆形或楔形角和圆形至直线形状。在一些实施例中，本发明的压制剂型包括具有平表面的圆形片剂。本发明的固体药物剂型可以通过本领域普通技术人员已知的形成压制固体药物剂型的任何压缩和压制方法来制备。在特定的实施例中，本文提供的制剂可以使用药物制剂领域技术人员已知的常规方法来制备，如例如在相关教科书中所述。参见例如 Remington:The Science and Practice of Pharmacy, 21st Ed., Lippincott Williams&Wilkins, Baltimore, Md. (2003)（《雷明顿：药剂学科学与实践》，第 21 版，利平科特 • 威廉斯 • 威尔金斯出版公司，马里兰州巴尔的摩，2003 年）；Ansel et al., Pharmaceutical Dosage Forms And Drug Delivery Systems, 7th Edition, Lippincott Williams&Wilkins, (1999) (Ansel 等人，《药物剂型和药物递送系统》，第 7 版，利平科特 • 威廉斯 • 威尔金斯出版公司，1999 年）；The Handbook of Pharmaceutical Excipients, 4th edition, Rowe et al., Eds., American Pharmaceuticals Association (2003)（《药物赋形剂手册》，第 4 版，Rowe 等人编辑，美国医药协会，2003 年）；Gibson, Pharmaceutical Preformulation And Formulation, CRC Press (2001) (Gibson，《药物预制剂和制剂》，CRC 出版社，2001 年），这些参考文献据此整体以引用方式并入本文。

[0322] 制粒和压制

[0323] 在一些实施例中，可使包含活性剂化合物 1 并包含药学上可接受的赋形剂（例如填充剂、稀释剂、崩解剂、表面活性剂、助流剂、粘合剂、润滑剂或其任意组合）的固体形式（包括粉末）接受干法制粒工艺。干法制粒工艺导致粉末聚集成具有适于进一步加工的大小的较大粒子。干法制粒可以提高混合物的流动性，从而能够制备符合质量变化或含量均

匀性要求的片剂。

[0324] 本文所述的制剂可以使用一个或多个混合和干法制粒步骤来制备。混合和制粒步骤的顺序和数量似乎不是关键性的。然而，可以使赋形剂和化合物 1 中的至少一种接受干法制粒或湿法高剪切制粒，而后压制成片剂。在压片之前，使化合物 1 和赋形剂结合在一起进行的干法制粒似乎令人惊讶的是在本发明组合物和制剂的成分之间提供紧密物理接触的简单、低成本且有效的方法，并由此产生具有良好稳定性的片剂制剂。干法制粒可以通过机械过程进行，与本文还考虑的湿法制粒工艺相反，该过程将能量转移至混合物，而不使用任何液体物质（不是水性溶液的形式、基于有机溶质的溶液形式也不是其混合物形式）。通常，该机械过程需要压缩，诸如辊压所提供的压缩。干法制粒的替代方法的例子是击压法。

[0325] 在一些实施例中，辊压是包括对一种或多种物质进行高强度机械压缩的制粒工艺。在一些实施例中，在 2 个反向旋转的辊之间，将包含粉末配混物的药物组合物压缩，即辊压，以产生固体薄片，随后将其在筛网中粉碎，从而形成颗粒物。在这种颗粒物中，可以获得成分之间的紧密机械接触。辊压设备的例子是得自 Gerteis Maschinen+Processengineering AG 的 Minipactor® a Gerteis 3W-Polygran。

[0326] 在一些实施例中，可以在不使用任何液体物质（不是水性溶液的形式、基于有机溶质的溶液形式也不是其混合物形式）的条件下，进行根据本发明的压片，即，干法制粒工艺。在一个典型的实施例中，得到的片芯或片剂具有 1 至 15kP 范围内的压缩强度；例如，1.5 至 12.5kP，优选 2 至 10kP 范围内的压缩强度。

[0327] 制造程序简述

[0328] 在一些实施例中，按照本文所示的配方称取各成分。接下来，将所有颗粒内成分过筛，并充分混合。可以用合适的润滑剂（例如硬脂酸镁）润滑这些成分。下一步可以包括压缩 / 击压粉末配混物和过筛后的成分。接下来，将压缩或击压后的共混物碾磨成颗粒，并过筛，以得到所需的大小。接下来，进一步用例如硬脂酸镁润滑颗粒。接下来，可以在合适的冲头上压制本发明的颗粒组合物以形成根据本发明的各种药物制剂。任选地，可以用薄膜衣、着色剂或其他包衣对片剂包衣。

[0329] 本发明的另一个方面提供了制备药物组合物的方法，该方法包括：提供组合物的配混物，其中该组合物包含化合物 1 和一种或多种选自以下的赋形剂：填充剂、稀释剂、粘合剂、助流剂、表面活性剂、润滑剂、崩解剂；以及将该组合物压制成片剂，这种片剂在约 30 分钟内的溶出度为至少约 50％。

[0330] 在另一个实施例中，进行湿法制粒工艺，以由粉末成分和液体成分的配混物得到本发明的药物制剂。例如，按照本文所示的配方称取包含组合物（该组合物包含化合物 1 和一种或多种选自以下的赋形剂：填充剂、稀释剂、粘合剂、助流剂、表面活性剂、润滑剂、崩解剂）的配混物的药物组合物。接下来，将所有颗粒内成分过筛，并在高剪切或低剪切制粒机或双螺杆制粒机中使用水或含表面活性剂的水或含粘合剂的水或含表面活性剂和粘合剂的水混合，以使粉末共混物形成颗粒。也可以使用非水流体，其含或不含表面活性剂和 / 或粘合剂，以使粉末共混物形成颗粒。接下来，可以任选地使用合适的碾磨机来碾磨湿润的颗粒。接下来，可以任选地通过以任何合适的方式干燥成分从而从配混物中除去水。接下来，可以任选地将干燥的颗粒碾磨至所需的大小。接下来，可以通过共混的方式加入颗粒外赋形剂（例如填充剂、稀释剂和崩解剂）。接下来，可以用硬脂酸镁和崩解剂（例如交联羧

甲纤维素钠）进一步润滑大小已确定的颗粒。接下来，可以在合适的冲头上压制本发明的颗粒组合物以形成根据本发明的各种药物制剂。任选地，可以用薄膜衣、着色剂或其他包衣对片剂包衣。

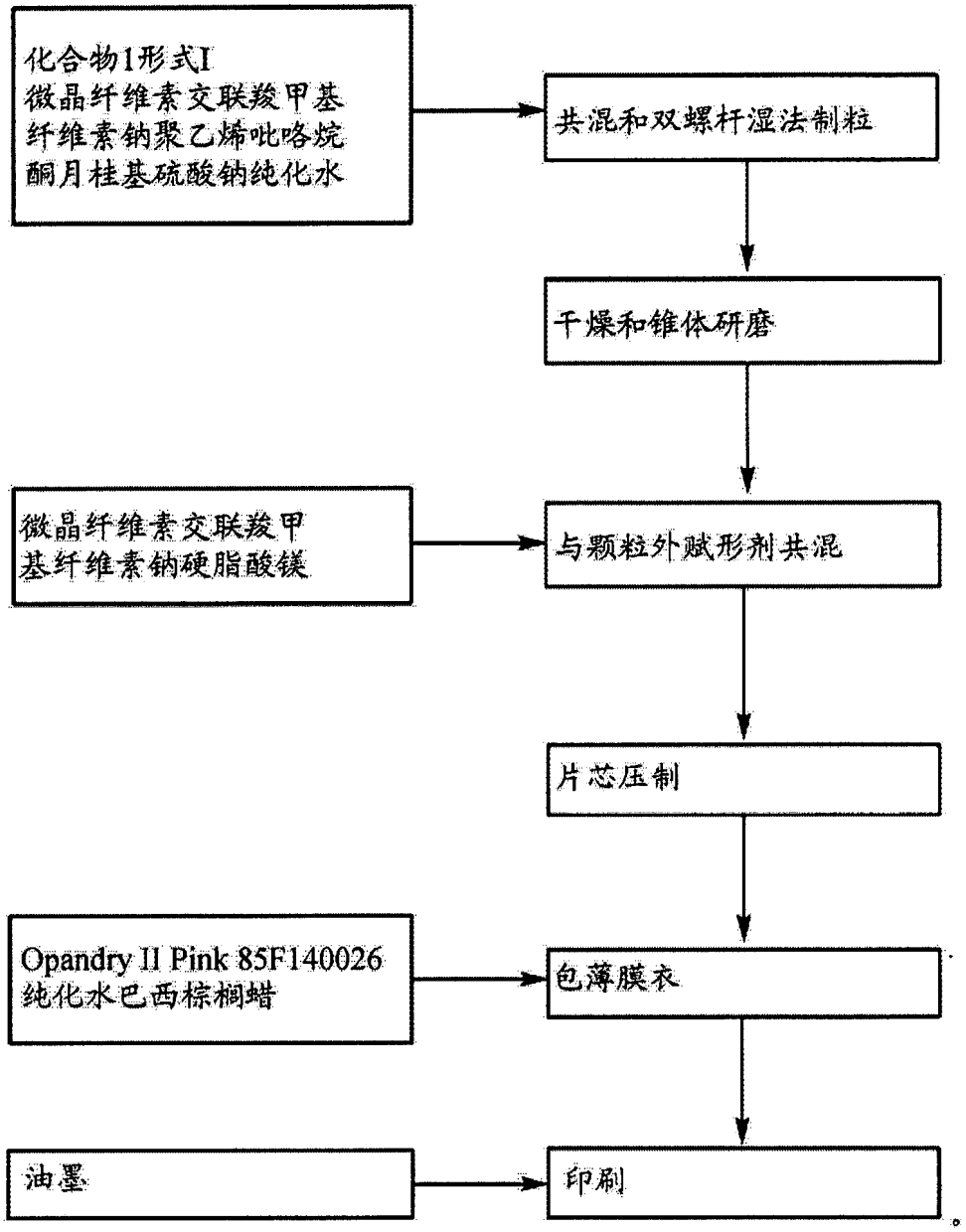
[0331] 在一个尤其有利的实施例中，本发明的药物组合物通过连续双螺杆湿法制粒(TSWG)工艺制备。连续制造通过在线监测和控制提供高质量和高度一致的产品。连续制造还通过具有“数据丰富的”设计空间的设计开发而有利于质量，并易于理解上游变量对下游工艺和最终产品质量的影响。此外，本发明的药物组合物可及早在商业规模的设备上定型，这避免了工艺放大风险和开发晚期的配方变化。最后，连续制造具有商业制造优点，诸如改进的工艺控制、减少的产品处理以及实时放行效率。总体结果是更稳健、可控和可放大的工艺，这种工艺具有更少的过程检查，从而实现产品质量的升高并因此实现更大的患者安全性。

[0332] 例如，高剪切制粒(HSG)(一种常见的制粒技术）熟知的是存在过度制粒和工艺控制不良的风险。该工艺的放大极具挑战性，并涉及重大风险。从HSG工艺变成连续TSWG工艺允许使用相同的设备进行放大以通过运行更长的时间产生不同的批量。这消除了湿法制粒工艺通常遇到的放大风险。另外，据发现，TSWG工艺更稳健、不易制粒过度。如在化合物1片剂的图28中可以看出，HSG工艺显示出随着水含量的增加溶出的明显减缓，而TSWG工艺对于类似的水添加范围未显示出变化。令人惊讶的是，对于使用双螺杆湿法制粒工艺的包含45-55重量%的化合物1的片剂制剂和包含60-70重量%的化合物1的片剂制剂未发现性能的变化。而HSG工艺则并非如此。另外，这种连续的且高产品质量的工艺解决了FDA关于对其有需要的患者无药可用的普遍抱怨。

[0333] 在一个实施例中，该连续工艺以单独的赋形剂和化合物1通过失重式进料进入连续在线共混机而开始。从该共混机，材料按以下过程连续传输并加工：双螺杆湿法制粒、干燥、研磨、颗粒外添加赋形剂、共混、压片和包薄膜衣。

[0334] 例如，在一个实施例中，可根据以下流程图连续制备包含化合物1的片剂。

[0335]



[0336] 这种示例性配混物的每种成分如上文和下文的实例所述。此外，该配混物可以包含任选的添加剂，诸如上文和下文的实例描述的一种或多种着色剂、一种或多种矫味剂和/或一种或多种芳香剂。在一些实施例中，上文和下文的实例还提供了该配混物中的这些成分（和任何任选的添加剂）的每一者的相对浓度（例如wt%）。可以按顺序或以任何添加组合来提供构成该配混物的成分；并且可以按任何顺序提供这些成分或成分的组合。在一个实施例中，润滑剂是最后加入配混物的组分。

[0337] 在另一个实施例中，配混物包含化合物1以及任何一种或多种以下赋形剂的组合物：粘合剂、助流剂、表面活性剂、稀释剂、润滑剂、崩解剂和填充剂，其中这些成分的每一者以粉末形式提供（例如，作为具有通过光散射法测得的250μm或更低（例如150μm或更低、100μm或更低、50μm或更低、45μm或更低、40μm或更低或35μm或更低）的平均直径的粒子而提供）。例如，配混物包含化合物1、稀释剂、助流剂、表面活性剂、润滑剂、崩解剂和填充剂的组合物，其中这些成分的每一者以粉末形式提供（例如，作为具有通过光散射法测得的250μm或更低（例如150μm或更低、100μm或更低、50μm或更低、45μm或更

低、40 μm 或更低或 35 μm 或更低）的平均直径的粒子而提供）。又如，配混物包含化合物 1、稀释剂、粘合剂、表面活性剂、润滑剂、崩解剂和填充剂的组合物，其中这些成分的每一者以粉末形式提供（例如，作为具有通过光散射法测得的 250 μm 或更低（例如 150 μm 或更低、100 μm 或更低、50 μm 或更低、45 μm 或更低、40 μm 或更低或 35 μm 或更低）的平均直径的粒子而提供）。

[0338] 在另一个实施例中，配混物包含化合物 1 与以下物质的任意组合的组合物：粘合剂、助流剂、稀释剂、表面活性剂、润滑剂、崩解剂和填充剂，其中这些成分的每一者基本上不含水。这些成分的每一者按所述成分的重量计包含低于 5 重量%（例如低于 2 重量%、低于 1 重量%、低于 0.75 重量%、低于 0.5 重量%、或低于 0.25 重量%）的水。例如，配混物包含化合物 1、稀释剂、助流剂、表面活性剂、润滑剂、崩解剂和填充剂的组合物，其中这些成分的每一者基本上不含水。在一些实施例中，这些成分的每一者按所述成分的重量计包含低于 5 重量%（例如低于 2 重量%、低于 1 重量%、低于 0.75 重量%、低于 0.5 重量%、或低于 0.25 重量%）的水。

[0339] 在另一个实施例中，将配混物压制成片剂通过如下方式实现：用配混物填充模板（例如模具），并对配混物施加压力。这可以使用模压机或其他类似的设备来实现。在一些实施例中，可以首先将化合物 1 和赋形剂的配混物加工成颗粒形式。然后，可以根据制药领域已知的方法筛分颗粒并压制成片剂或进行配制以用于包封。还应注意，对模板中的配混物施加压力可以在每次压制期间使用相同的压力进行重复，或在压制期间使用不同的压力进行。又如，可以使用施加足够压力的模压机来压制粉状成分或颗粒的配混物，以形成在约 30 分钟时溶出约 50%或更多（例如，在约 30 分钟时溶出约 55%或更多，或在约 30 分钟时溶出约 60%或更多）的片剂。例如，使用模压机来压制配混物，以产生至少约 5kP（至少约 5.5kP、至少约 6kP、至少约 7kP、至少约 10kP 或至少 15kP）的片剂硬度。在一些情况下，压缩配混物以产生约 5 与 20kP 之间的片剂硬度。

[0340] 在一些实施例中，包含如本文所述的药物组合物的片剂可以用按片剂的重量计约 3.0 重量%的薄膜衣进行包衣，其中薄膜衣包含着色剂。在某些情况下，用于对片剂包衣的着色剂悬浮液或溶液包含按着色剂悬浮液或溶液重量计约 20% w/w 的固体。在其他情况下，可以将包衣片标上徽标、其他图像或文字。

[0341] 在另一个实施例中，制备药物组合物的方法包括：提供固体形式的配混物，例如，粉末和 / 或液体成分的配混物，该配混物包含化合物 1 和一种或多种选自以下的赋形剂：粘合剂、助流剂、稀释剂、表面活性剂、润滑剂、崩解剂和填充剂；将配混物混合，直到配混物基本上均匀为止，并将配混物压制或压缩成颗粒形式。然后，如上文或下文的实例中所述，可以将包含化合物 1 颗粒组合物压制成片剂，或配制成胶囊剂。作为另外一种选择，制备药物组合物的方法包括：提供化合物 1 和一种或多种下列赋形剂的配混物：例如粘合剂、助流剂、稀释剂、表面活性剂、润滑剂、崩解剂和填充剂；将配混物混合，直到配混物基本上均匀为止，并使用辊压机使用如下文实例所示的干法制粒组合物将配混物压制 / 压缩成颗粒形式，或者，使用如下文实例所示的高剪切湿法颗粒压缩工艺将配混物压制 / 压缩成颗粒。药物制剂（例如，如本文所述的片剂）可以用颗粒来制备，所述颗粒除了结合选定的本文所述的赋形剂之外还结合了化合物 1 而制备。

[0342] 在一些实施例中，使用手工混合、混合机、共混机、其任何组合等通过搅拌、共混、

摇动等方式对配混物进行混合。当按顺序添加成分或成分的组合时，混合可以在顺序添加之间进行、在添加成分的整个过程中连续进行、在添加所有成分或成分的组合之后进行，或以其任何组合形式进行。对配混物进行混合，直到其具有基本上均匀的组成为止。

[0343] 在另一个实施例中，本发明包括以适于产生具有高比率的 0.1 微米与 50 微米之间粒度的粒子的空气压力在合适的、常规碾磨设备中喷射研磨化合物 1、化合物 1 形式 I、化合物 1 形式 II、化合物 1HCl 盐形式 A。在另一个实施例中，该粒度在 0.1 微米与 20 微米之间。在另一个实施例中，该粒度在 0.1 微米与 10 微米之间。在另一个实施例中，该粒度在 1.0 微米与 5 微米之间。在又一个实施例中，化合物 1 化合物 1 形式 I、化合物 1 形式 II、化合物 1HCl 盐形式 A 的粒度 D50 为 2.0 微米。

[0344] 在不同的实施例中，可以与化合物 1 一起配制第二治疗剂，以形成单一或单剂量形式，例如片剂或胶囊剂。

[0345] 如上所述制备的剂型可接受根据 Test 711 “Dissolution”in United States Pharmacopoeia 29,United States Pharmacopeial Convention,Inc.,Rockville,Md.,2005(“USP”)(《美国药典》第 29 版 711 溶出度试验，马里兰州罗克维尔美国药典委员会，2005 年）的体外溶出度评价，以测定活性物质从该剂型中释放的速率。通过诸如高效液相色谱 (HPLC) 的技术方便地测定活性物质的含量和杂质水平。

[0346] 在一些实施例中，本发明包括使用包装材料，诸如高密度聚乙烯 (HDPE)、低密度聚乙烯 (LDPE) 和 / 或聚丙烯和 / 或玻璃的容器和密封盒，玻璃纸薄片，铝箔袋和由铝或高密度聚氯乙烯 (PVC)（任选地包括干燥剂）、聚乙烯 (PE)、聚偏二氯乙烯 (PVDC)、PVC/PE/PVDC 等组成的泡罩或条板。在使用制药领域常用的化学或物理灭菌技术将包装及其内含物进行适当的灭菌之后，这些包装材料可用于以无菌方式储存各种药物组合物和制剂。

[0347] 施用药物组合物的方法

[0348] 在一个方面，可每天或大约每 24 小时向患者施用本发明的药物组合物一次。作为另外一种选择，可每天两次或大约每 12 小时一次向患者施用本发明的药物组合物。以口服制剂施用这些药物组合物，所述口服制剂包含约 25mg、50mg、100mg、125mg、150mg、200mg、250mg 或 400mg 化合物 1。在该方面，除了化合物 1 之外，药物组合物还包含填充剂、稀释剂、崩解剂、表面活性剂、粘合剂和助流剂中的至少一种以及润滑剂。例如，400mg 化合物 1 的剂量可包括两粒本发明的片剂，每粒包含 200mg 化合物 1，或者包括四粒本发明的片剂，每粒包含 100mg 化合物 1。

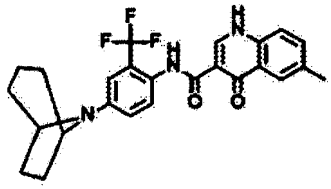
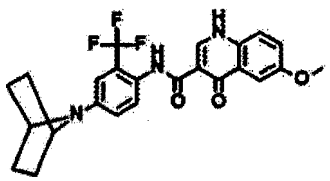
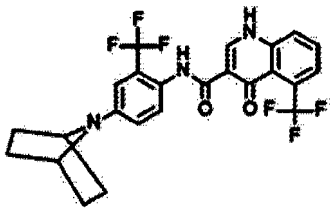
[0349] 还将认识到，本发明的化合物以及药学上可接受的组合物和制剂可以在联合疗法中使用；也就是说，化合物 1 及其药学上可接受的组合物可与一种或多种其他所需的治疗剂或医疗程序同时、在其之前或在其之后施用。用于联合方案的疗法（治疗剂或程序）的具体组合将考虑所需治疗剂和 / 或程序的相容性以及待实现的所需治疗效果。还将认识到，所用的疗法对于相同的障碍可以实现所需的效果（例如，本发明的化合物可以与另一种治疗相同障碍所用的药剂同时施用），或者它们可以实现不同的效果（例如，控制任何副作用）。如本文所用，通常施用来治疗或预防特定疾病（例如 CFTR 介导的疾病）或病症的另外的治疗剂被称为“适于所治疗的疾病或病症”。

[0350] 在一个实施例中，该另外的治疗剂选自溶粘蛋白剂、支气管扩张剂、抗生素、抗感染剂、抗炎剂、本发明化合物 1 之外的 CFTR 调节剂或营养剂。

[0351] 在一个实施例中，该另外的药剂为 (R)-1-(2,2-二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基)-N-(1-(2,3- 二羟基丙基)-6- 氟 -2-(1- 羟基 -2- 甲基丙烷 -2- 基)-1H- 吲哚 -5- 基) 环丙烷甲酰胺。在另一个实施例中，该另外的药剂为 N-(5- 羟基 -2,4- 二叔丁基苯基)-4- 氧代 -1H- 喹啉 -3- 甲酰胺。在另一个实施例中，该另外的药剂选自表 1：

[0352] 表 1。

[0353]

1	2	3
		
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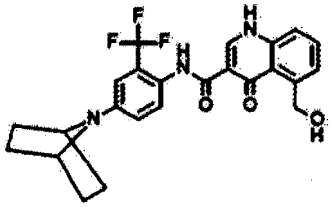
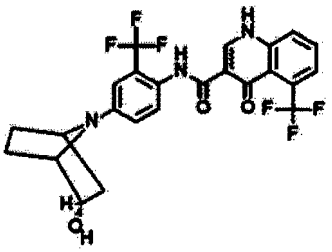
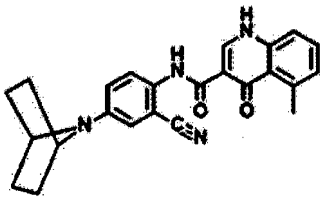
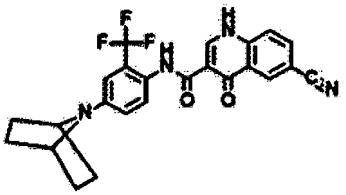
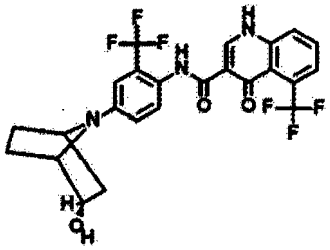
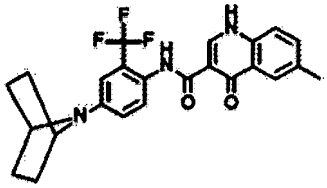
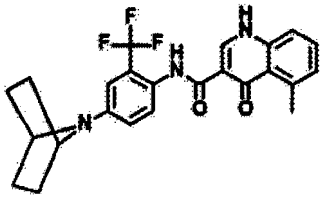
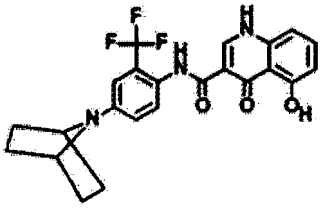
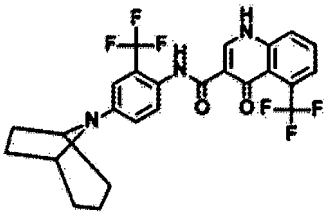
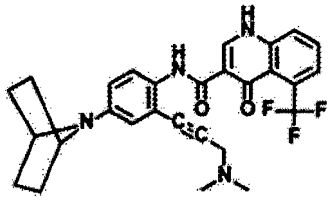
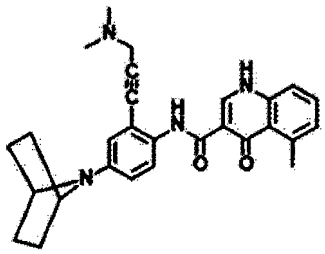
		

[0354]

7	8	9
		
10	11	12
		
13	14	
		

[0355] 在另一个实施例中，该另外的药剂为上述药剂的任意组合。例如，组合物可包含化合物 1、(R)-1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基)-N-(1-(2,3- 二羟基丙基)-6- 氟 -2-(1- 羟基 -2- 甲基丙烷 -2- 基)-1H- 吲哚 -5- 基) 环丙烷甲酰胺和 N-(5- 羟基 -2,4- 二叔丁基苯基)-4- 氧代 -1H- 喹啉 -3- 甲酰胺。又如，组合物可包含化合物 1、N-(5- 羟基 -2,4- 二叔丁基苯基)-4- 氧代 -1H- 喹啉 -3- 甲酰胺以及表 1 中的化合物 (即表 1 的化合物 1 至 14) 的任一者或其任意组合。

[0356] 在一个实施例中，该另外的治疗剂为抗生素。可用于本文的示例性抗生素包括妥布霉素 (包括妥布霉素吸入粉末 (TIP))、阿奇霉素、氨曲南 (包括雾化形式的氨曲南)、阿米卡星 (包括其脂质体制剂)、环丙沙星 (包括其适于吸入给药的制剂)、左氧氟沙星 (包括其雾化制剂) 以及两种抗生素的组合 (例如磷霉素和妥布霉素)。

[0357] 在另一个实施例中，该另外的药剂为粘液溶解剂 (mucolyte)。可用于本文的示例性粘液溶解剂包括 **Pulmozyme®**。

[0358] 在另一个实施例中，该另外的药剂为支气管扩张剂。示例性支气管扩张剂包括沙

丁胺醇、硫酸奥西那林 (metaprotenerol sulfate)、醋酸吡布特罗、沙美特罗或硫酸特布他林 (tetrabuline sulfate)。

[0359] 在另一个实施例中，该另外的药剂对于恢复肺气管表面液体有效。此类药剂改善盐进出细胞，从而使得在肺气管中的粘液更加水化，并因此更容易地清除。示例性的此类药剂包括高渗盐水、地纽福索钠 ([[(3S,5R)-5-(4- 氨基 -2- 氧嘧啶 -1- 基)-3- 羟基氧杂戊环 -2- 基] 甲氧基 - 羟基磷酰基][[[(2R,3S,4R,5R)-5-(2,4- 二氧嘧啶 -1- 基)-3,4- 二羟基氧杂戊环 -2- 基] 甲氧基 - 羟基磷酰基] 氧杂 - 羟基磷酰基] 磷酸氢盐) 或 bronchitol(甘露醇的吸入制剂)。

[0360] 在另一个实施例中，该另外的药剂为抗炎剂，即，可减少肺部炎症的药剂。可用于本文的示例性的此类药剂包括 :布洛芬、二十二碳六烯酸 (DHA)、西地那非、吸入的谷胱甘肽、吡格列酮、羟基氯喹或辛伐他汀。

[0361] 在另一个实施例中，该另外的药剂为化合物 1 之外的 CFTR 调节剂，即，具有调节 CFTR 活性的作用的药剂。示例性的此类药剂包括 ataluren(“PTC124®” ;3-[5-(2- 氟苯基)-1,2,4- 噁二唑 -3- 基] 苯甲酸)、西那普肽、兰考韦泰、地来司他 (人重组嗜中性粒细胞弹性蛋白酶抑制剂) 和考前列酮 (7-{(2R,4aR,5R,7aR)-2-[(3S)-1,1- 二氟 -3- 甲基戊基]-2- 羟基 -6- 氧代八氢环戊 [b] 吡喃 -5- 基 } 庚酸)。

[0362] 在另一个实施例中，该另外的药剂为营养剂。示例性营养剂包括胰脂肪酶 (胰腺酶替代)，包括 Pancrease®、Pancreacarb®、Ultrase® 或 Creon®、Liprotomase® (以前称为 Trizytek®)、Aquadeks® 或谷胱甘肽吸入剂。在一个实施例中，该另外的营养剂为胰脂肪酶。

[0363] 在另一个实施例中，该另外的药剂为选自以下的化合物 :庆大霉素、姜黄素、环磷酰胺、4- 苯基丁酸酯、麦格司他、非洛地平、尼莫地平、Philoxin B、染料木黄酮、芹菜素、cAMP/cGMP 调节剂诸如咯利普兰、西地那非、米力农、他达拉非、氨力农、异丙肾上腺素、沙丁胺醇和沙美特罗、脱氧精胍菌素、HSP 90 抑制剂、HSP 70 抑制剂、蛋白体抑制剂诸如环氧酶素 (epoxomicin)、乳胞素等。

[0364] 在另一个实施例中，该另外的药剂为选自以下的化合物 :3- 氨基 -6-(4- 氟 - 苯基)-5- 三氟甲基 - 吡啶 -2- 甲酸 (3,3,3- 三氟 -2- 羟基 -2- 甲基 - 丙基)- 酰胺 ;5- 氨基 -6′ - 甲基 -3- 三氟甲基 -[2,3] 联吡啶 -6- 甲酸 (3,3,3- 三氟 -2- 羟基 -2- 甲基 - 丙基)- 酰胺 ;3- 氨基 -6- 环丙基 -N-(3,3,3- 三氟 -2- 羟基 -2- 甲基丙基)-5-(三氟甲基) 吡啶酰胺 ;3- 氨基 -6- 甲氧基 -N-(3,3,3- 三氟 -2- 羟基 -2-(三氟甲基) 丙基)-5-(三氟甲基) 吡啶酰胺 ;3- 氨基 -6-(4- 氟 - 苯基)-5- 三氟甲基 - 吡啶 -2- 甲酸 ((S)-3,3,3- 三氟 -2- 羟基 -2- 甲基 - 丙基)- 酰胺 ;3- 氨基 -6- 甲氧基 -5- 三氟甲基 - 吡啶 -2- 甲酸 ((S-3,3,3- 三氟 -2- 羟基 -2- 甲基 - 丙基)- 酰胺 ;3- 氨基 -6- 甲氧基 -5- 三氟甲基 - 吡啶 -2- 甲酸 ((R)-3,3,3- 三氟 -2- 羟基 -2- 甲基 - 丙基)- 酰胺 ;3- 氨基 -6-(2,4- 二氯 - 苯基)-5- 三氟甲基 - 吡啶 -2- 甲酸 ((S)-3,3,3- 三氟 -2- 羟基 -2- 甲基 - 丙基)- 酰胺 ;3- 氨基 -6-(2,4- 二氯 - 苯基)-5- 三氟甲基 - 吡啶 -2- 甲酸 ((R)-3,3,3- 三氟 -2- 羟基 -2- 甲基 - 丙基)- 酰胺 ;3- 氨基 -6-(4- 氟 - 苯基)-5- 三氟甲基 - 吡啶 -2- 甲酸 (2- 羟基 -2- 甲基 - 丙基)- 酰胺 ;3- 氨基 -5,6- 双 - 三氟甲基 - 吡啶 -2- 甲酸 ((S)-3,3,3- 三氟 -2- 羟

基-2-甲基-丙基)-酰胺;3-氨基-5,6-双-三氟甲基-吡啶-2-甲酸((R)-3,3,3-三氟-2-羟基-2-甲基-丙基)-酰胺;(S)-3-氨基-6-乙氧基-N-(3,3,3-三氟-2-羟基-2-甲基丙基)-5-(三氟甲基)吡啶酰胺;3-氨基-6-甲氧基-5-三氟甲基-吡啶-2-甲酸((S)-3,3,3-三氟-2-羟基-2-甲基-丙基)-酰胺;3-氨基-6-甲氧基-5-三氟甲基-吡啶-2-甲酸((R)-3,3,3-三氟-2-羟基-2-甲基-丙基)-酰胺;3-氨基-6-(4-氟-苯基)-5-三氟甲基-吡啶-2-甲酸(3,3,3-三氟-2-羟基-2-甲基-丙基)-酰胺;3-氨基-5,6-双-三氟甲基-吡啶-2-甲酸((S)-3,3,3-三氟-2-羟基-2-甲基-丙基)-酰胺;3-氨基-5,6-双-三氟甲基-吡啶-2-甲酸((R)-3,3,3-三氟-2-羟基-2-甲基-丙基)-酰胺或其药学上可接受的盐。在另一个实施例中,该另外的药剂为美国专利No.8,247,436和国际PCT公布WO 2011113894中所公开的化合物,各专利均整体以引用方式并入本文。

[0365] 在一个实施例中,该另外的药剂为三甲基异补骨脂素。在另一个实施例中,该另外的药剂为整体以引用方式并入本文的WO 2012171954中所公开的化合物。

[0366] 在其他实施例中,该另外的药剂是在WO 2004028480、WO2004110352、WO 2005094374、WO 2005120497或WO 2006101740中公开的化合物。在另一个实施例中,该另外的药剂是表现出CFTR调节活性的苯并[c]喹嗪鎓衍生物或表现出CFTR调节活性的苯并吡喃衍生物。在另一个实施例中,该另外的药剂是在美国专利No.7,202,262、美国专 利No.6,992,096、US20060148864、US20060148863、US20060035943、US20050164973、WO2006110483、WO2006044456、WO2006044682、WO2006044505、WO2006044503、WO2006044502或WO2004091502中公开的化合物。在另一个实施例中,该另外的药剂是在WO2004080972、WO2004111014、WO2005035514、WO2005049018、WO2006099256、WO2006127588或WO2007044560中公开的化合物。在另一个实施例中,该另外的药剂是N-(5-羟基-2,4-二叔丁基苯基)-4-氧代-1H-喹啉-3-甲酰胺。

[0367] 在一个实施例中,可将600mg化合物1施用给对其有需要的受试者,然后联合施用250mg N-(5-羟基-2,4-二叔丁基苯基)-4-氧代-1H-喹啉-3-甲酰胺(化合物2)。在这些实施例中,剂量可通过施用一粒或多粒本发明的片剂而实现。例如,施用600mg化合物1可通过施用三粒片剂(每粒包含200mg化合物1)、四粒片剂(每粒包含150mg化合物1)或一粒400mg化合物1的片剂与一粒200mg化合物1的片剂而实现。化合物2可作为包含化合物2和药学上可接受的载体的药物组合物施用。施用的持续时间可持续直到实现疾病的改善或直到受试者的医生建议为止,例如施用的持续时间可短于一周、为1周、2周、3周或一个月或更长。联合施用期可在单独的化合物1的施用期之后。例如,可以施用600mg化合物1持续2周,然后联合施用250mg化合物2再持续1周。在另一个实施例中,可将600mg化合物1以bid(每天两次)的频率施用28天,然后将250mg化合物2以bid(每天两次)的频率施用28天。在另一个实施例中,可将600mg化合物1以qd(每天一次)的频率施用28天,然后将250mg化合物2以qd(每天一次)的频率施用28天。在另一个实施例中,可将600mg化合物1以qd(每天一次)的频率施用28天,然后将600mg化合物1以qd(每天一次)的频率以及将250mg化合物2以q12h(每12小时一次)的频率联合施用28天。在另一个实施例中,可将600mg化合物1以qd(每天一次)的频率施用并将250mg化合物2以qd(每天一次)的频率施用。

[0368] 在一个实施例中,可将600mg化合物1施用给对其有需要的受试者,然后联合施用

450mg N-(5- 羟基 -2,4- 二叔丁基苯基)-4- 氧代 -1H- 喹啉 -3- 甲酰胺 (化合物 2)。在这些实施例中,剂量可通过施用一粒或多粒本发明的片剂而实现。例如,施用 600mg 化合物 1 可通过施用三粒片剂 (每粒包含 200mg 化合物 1) 或四粒片剂 (每粒包含 150mg 化合物 1) 而实现。化合物 2 可作为包含化合物 2 和药学上可接受的载体的药物组合物施用。施用的持续时间可持续直到实现疾病的改善或直到受试者的医生建议为止,例如施用的持续时间可短于一周、为 1 周、2 周、3 周或一个月或更长。联合施用期可在单独的化合物 1 的施用期之后。例如,可以施用 600mg 化合物 1 持续 2 周,然后联合施用 450mg 化合物 2 再持续 1 周。在另一个实施例中,可将 600mg 化合物 1 以 bid(每天两次) 的频率施用 28 天,然后将 450mg 化合物 2 以 bid(每天两次) 的频率施用 28 天。

[0369] 在一个实施例中,可将 400mg 化合物 1 施用给对其有需要的受试者,然后联合施用 350mg N-(5- 羟基 -2,4- 二叔丁基苯基)-4- 氧代 -1H- 喹啉 -3- 甲酰胺 (化合物 2)。在这些实施例中,剂量可通过施用一粒或多粒本发明的片剂而实现。例如,施用 400mg 化合物 1 可通过施用两粒片剂 (每粒包含 200mg 化合物 1) 或四粒片剂 (每粒包含 100mg 化合物 1) 而实现。化合物 2 可作为包含化合物 2 和药学上可接受的载体的药物组合物施用。施用的持续时间可持续直到实现疾病的改善或直到受试者的医生建议为止,例如施用的持续时间可短于一周、为 1 周、2 周、3 周或一个月或更长。联合施用期可在单独的化合物 1 的施用期之后。例如,可以施用 400mg 化合物 1 持续 2 周,然后联合施用 350mg 化合物 2 再持续 1 周。在另一个实施例中,可将 400mg 化合物 1 以 q8h(每 8 小时一次) 的频率施用 28 天,然后将 350mg 化合物 2 以 q8h(每 8 小时一次) 的频率施用 28 天。

[0370] 在一个实施例中,可将 400mg 化合物 1 施用给对其有需要的受试者,然后联合施用 250mg N-(5- 羟基 -2,4- 二叔丁基苯基)-4- 氧代 -1H- 喹啉 -3- 甲酰胺 (化合物 2)。在这些实施例中,剂量可通过施用一粒或多粒本发明的片剂而实现。例如,施用 400mg 化合物 1 可通过施用两粒片剂 (每粒包含 200mg 化合物 1) 或四粒片剂 (每粒包含 100mg 化合物 1) 而实现。化合物 2 可作为包含化合物 2 和药学上可接受的载体的药物组合物施用。施用的持续时间可持续直到实现疾病的改善或直到受试者的医生建议为止,例如施用的持续时间可短于一周、为 1 周、2 周、3 周或一个月或更长。联合施用期可在单独的化合物 1 的施用期之后。例如,可以施用 400mg 化合物 1 持续 2 周,然后联合施用 150mg 或 250mg 化合物 2 再持续 1 周。在另一个实施例中,可将 400mg 化合物 1 以 bid(每天两次) 的频率施用 28 天,然后将 250mg 化合物 2 以 bid(每天两次) 的频率施用 28 天。在另一个实施例中,可将 400mg 化合物 1 以 bid(每天两次) 的频率施用 28 天,然后将 250mg 化合物 2 以 qd(每天一次) 的频率施用 28 天。在另一个实施例中,可将 400mg 化合物 1 以 qd(每天一次) 的频率施用 28 天,然后将 400mg 化合物 1 以 qd(每天一次) 的频率以及将 250mg 化合物 2 以 q12h(每 12 小时一次) 的频率联合施用 28 天。在另一个实施例中,可将 400mg 化合物 1 以 bid(每天两次) 的频率施用并将 250mg 化合物 2 以 qd(每天一次) 的频率施用。

[0371] 在一个实施例中,可将 400mg 化合物 1 以每天一次的频率施用给对其有需要的受试者,然后以每天一次的频率联合施用 150mg 化合物 2。在这些实施例中,剂量可通过施用一粒或多粒本发明的片剂而实现。例如,施用 400mg 化合物 1 可通过施用两粒片剂 (每粒包含 200mg 化合物 1) 或四粒片剂 (每粒包含 100mg 化合物 1) 而实现。化合物 2 可作为包含化合物 2 和药学上可接受的载体的药物组合物施用。施用的持续时间可持续直到实现疾

病的改善或直到受试者的医生建议为止，例如施用的持续时间可短于一周、为 1 周、2 周、3 周或一个月或更长。联合施用期可在单独的化合物 1 的施用期之后。例如，可以施用 400mg 化合物 1 持续 2 周，然后联合施用 150mg 或 250mg 化合物 2 再持续 1 周。

[0372] 在一个实施例中，可将 400mg 化合物 1 以每天一次的频率施用给对其有需要的受试者，然后以每 12 小时一次的频率联合施用 150mg 化合物 2。在另一个实施例中，可将 400mg 化合物 1 以每天一次的频率施用给对其有需要的受试者，然后以每 12 小时一次的频率联合施用 250mg 化合物 2。在这些实施例中，剂量可通过施用一粒或多粒本发明的片剂而实现。例如，施用 400mg 化合物 1 可通过施用两粒片剂（每粒包含 200mg 化合物 1）或四粒片剂（每粒包含 100mg 化合物 1）而实现。化合物 2 可作为包含化合物 2 和药学上可接受的载体的药物组合物施用。施用的持续时间可持续直到实现疾病的改善或直到受试者的医生建议为止，例如施用的持续时间可短于一周、为 1 周、2 周、3 周或一个月或更长。联合施用期可在单独的化合物 1 的施用期之后。例如，可以施用 400mg 化合物 1 持续 2 周，然后联合施用 150mg 或 250mg 化合物 2 再持续 1 周。

[0373] 在另一个实施例中，可将 200mg 化合物 1 以 qd（每天一次）的频率施用 28 天，然后将 200mg 化合物 1 以 qd（每天一次）的频率以及将 250mg 化合物 2 以 q12h（每 12 小时一次）的频率联合施用 28 天。

[0374] 在一个实施例中，可将 100mg、200mg 和 300mg 化合物 1 片剂合并以形成许多不同的剂量。例如，100mg、200mg、300mg、400mg、500mg、600mg、700mg、800mg、900mg、1000mg、1100mg 或 1200mg 化合物 1 剂量可通过使用 100mg、200mg 和 300mg 片剂制剂及其多粒片剂而施用。例如，900mg 化合物 1 剂量可使用 3 粒 300mg 化合物 1 片剂而施用。600mg 化合物 1 剂量可使用 3 粒 200mg 化合物 1 片剂或 2 粒 300mg 化合物 1 片剂而施用。该段落的任何前述剂量可与化合物 2 的多种量和 / 或前面 3 段的剂量方案一起施用。

[0375] 这些组合可用于治疗本文所述的疾病，包括囊性纤维化。这些组合还可用于本文所述的试剂盒。

[0376] 存在于本发明组合物中的另外的治疗剂的量将不超过在包含该治疗剂作为唯一活性剂的组合物中通常施用的量。优选地，在本发明所公开的组合物中的另外的治疗剂的量将在包含该药剂作为唯一治疗活性剂的组合物中通常存在的量的约 50％至 100％的范围内。

[0377] 在另一方面，本发明的特征在于包含本发明的片剂和单独的治疗剂或其药物组合物的试剂盒。在另一个实施例中，该片剂中的化合物 1 呈形式 I。在另一个实施例中，该治疗剂为化合物 1 之外的囊性纤维化纠正剂。在另一个实施例中，该治疗剂为囊性纤维化增效剂。在另一个实施例中，该治疗剂为 N-(5- 羟基 -2,4- 二叔丁基苯基)-4- 氧代 -1H- 喹啉 -3- 甲酰胺。在另一个实施例中，该片剂和治疗剂在单独的容器中。在另一个实施例中，单独的容器为瓶子。在另一个实施例中，单独的容器为小瓶。在另一个实施例中，单独的容器为泡罩包装。

[0378] 组合物的治疗性用途

[0379] 在一个方面，本发明还提供治疗患者的疾病、减轻所述疾病的严重性或对症治疗所述疾病的方法，该方法包括向患者施用有效量的本发明的药物组合物，其中该疾病选自：囊性纤维化、哮喘、吸烟诱发的 COPD、慢性支气管炎、鼻窦炎、便秘、胰腺炎、胰腺功能不全、

先天性双侧输精管缺失 (CBAVD) 导致的男性不育症、轻度肺病、特发性胰腺炎、变应性支气管肺曲菌病 (ABPA)、肝病、遗传性肺气肿、遗传性血色病、凝血 - 纤溶缺陷（诸如蛋白 C 缺乏症）、1 型遗传性血管性水肿、脂质加工缺陷（诸如家族性高胆固醇血症）、1 型乳糜微粒血症、无 β 脂蛋白血症、溶酶体贮积症（诸如 I- 细胞病 / 假性赫尔勒综合征）、粘多糖症、桑德霍夫 / 泰 - 萨克斯病、克 - 纳综合征 II 型、多内分泌腺病 / 高胰岛素血症、糖尿病、拉伦侏儒症、髓过氧化物酶缺乏症、原发性甲状旁腺功能减退、黑素瘤、聚糖病 CDG 1 型、先天性甲状腺功能亢进症、成骨不全、遗传性低纤维蛋白原血症、ACT 缺乏症、尿崩症 (DI)、神经生长性 DI、肾性 DI、夏 - 马 - 图综合征、佩 - 梅病、神经变性疾病（诸如阿尔茨海默病）、帕金森病、肌萎缩性侧索硬化、进行性核上性麻痹、皮克病、若干聚谷氨酰胺神经性障碍（例如亨廷顿病）、I 型脊髓小脑性共济失调、脊髓与延髓肌肉萎缩症、齿状核红核苍白球丘脑下部核萎缩和肌强直性营养不良，以及海绵状脑病，诸如遗传性克雅病（由朊病毒蛋白加工缺陷导致）、法布里病、格 - 斯 - 施综合征、COPD、干眼病或斯耶格伦氏综合征、骨质疏松症、骨质减少、骨愈合与骨生长（包括骨修复、骨再生、骨吸收减少和骨吸收增加）、戈勒姆综合征、氯离子通道病变例如先天性肌强直（汤姆森和贝克尔型）、巴特综合征 III 型、登特病、过度惊跳症、癫痫症、溶酶体贮存病、安格曼综合征和原发性纤毛运动障碍 (PCD)（用于纤毛结构和 / 或功能遗传障碍的术语），包括具有左右转位的 PCD（也称作卡特金纳综合征）、没有左右转位和纤毛发育不良的 PCD。

[0380] 作为与 ivacaftor(N-(5- 羟基 -2,4- 二叔丁基苯基)-4- 氧代 -1H- 喹啉 -3- 甲酰胺）的组合的一部分的化合物 1 已被美国食品和药品监督管理局 (FDA) 授予用于治疗囊性纤维化的突破性疗法认定，它是在提交本申请之时唯一两个这种授予中的一个（另一个为 ivacaftor)。这表明了相对于对症治疗而有效治疗治疗囊性纤维化成因的明显尚未满足的需求。另外，得到 FDA 审批的药物的一项共同挑战是对于有需要的患者而言有时无药可用。因此，对于本发明所公开的化合物 1 制剂以及以连续和受控的方式制备所述制剂的工艺存在明显尚未满足的需求。

[0381] 在一个方面，本发明还提供治疗患者的疾病、减轻所述疾病的严重性或对症治疗所述疾病的方法，该方法包括向患者施用有效量的本发明的药物组合物，其中该疾病选自：全面性癫痫伴热性惊厥附加症 (GEFS+)、全面性癫痫伴热性和非热性惊厥、肌强直、先天性副肌强直、钾加重肌强值、高钾型周期性麻痹、LQTS、LQTS/ 布鲁加综合征、常染色体显性 LQTS 伴耳聋、常染色体隐性 LQTS、LQTS 伴生理缺陷、先天性和获得性 LQTS、提摩西综合征、持续性幼儿型胰岛素过度分泌低血糖症、扩张型心肌病、常染色体显性 LQTS、Dent 病、骨硬化病、巴特综合征 III 型、中央轴空病、恶性高热和儿茶酚胺敏感性多形性心动过速。

[0382] 在一个方面，本发明涉及治疗患者中的囊性纤维化、减轻囊性纤维化的严重性或对症治疗囊性纤维化的方法，包括向患者施用有效量的本发明的药物组合物，其中该患者具有 CFTR 遗传突变 N1303K、ΔI507 或 R560T。

[0383] 在一个方面，本发明涉及治疗患者中的囊性纤维化、减轻囊性纤维化的严重性或对症治疗囊性纤维化的方法，包括向患者施用有效量的本发明的药物组合物，其中该患者具有 CFTR 遗传突变 G551D。在另一个实施例中，患者为 G551D 纯合的。在另一个实施例中，患者为 G551D 杂合的，其中另一个 CFTR 基因突变为 F508del、G542X、N1303K、W1282X、R117H、R553X、1717-1G->A、621+1G->T、2789+5G->A、3849+10kbC->T、R1162X、G85E、3120+1G->A、

ΔI507、1898+1G->A、3659delC、R347P、R560T、R334W、A455E、2184delA 或 711+1G->T 中的任一者。

[0384] 在一个方面，本发明涉及治疗患者中的囊性纤维化、减轻囊性纤维化的严重性或对症治疗囊性纤维化的方法，包括向患者施用有效量的本发明的药物组合物，其中该患者具有 CFTR 遗传突变 F508del。在另一个实施例中，患者为 F508del 纯合的。在另一个实施例中，患者为 F508del 杂合的，其中另一个 CFTR 基因突变为 G551D、G542X、N1303K、W1282X、R117H、R553X、1717-1G->A、621+1G->T、2789+5G->A、3849+10kbC->T、R1162X、G85E、3120+1G->A、ΔI507、1898+1G->A、3659delC、R347P、R560T、R334W、A455E、2184delA 或 711+1G->T 中的任一者。

[0385] 在某些实施例中，包含化合物 1 的本发明的药学上可接受的组合物可用于治疗患者中的囊性纤维化、减轻囊性纤维化的严重性或对症治疗囊性纤维化，所述患者在呼吸和非呼吸性上皮细胞的顶端膜中表现出残余的 CFTR 活性。在上皮细胞表面存在残余的 CFTR 活性可以容易地使用本领域已知的方法检测，例如标准电生理学、生物化学或组织化学技术。此类方法使用体内或离体电生理学技术，汗液或唾液中 Cl^- 浓度的测量，或者离体生物化学或组织化学技术来监测细胞表面密度从而确定 CFTR 活性。使用此类方法，可以容易地在为多种不同突变杂合或纯合的患者中检测残余的 CFTR 活性，包括为最常见的突变 F508del 以及诸如 G551D 突变或 R117H 突变的其他突变纯合或杂合的患者。在某些实施例中，包含化合物 1 的药物组合物可用于治疗患者中的囊性纤维化、减轻囊性纤维化的严重性或对症治疗囊性纤维化，所述患者表现出很低的或不表现出残余 CFTR 活性。在某些实施例中，包含化合物 1 的药物组合物可用于治疗患者中的囊性纤维化、减轻囊性纤维化的严重性或对症治疗囊性纤维化，所述患者在呼吸性上皮细胞的顶端膜中表现出很低的或不表现出残余 CFTR 活性。

[0386] 在另一个实施例中，本发明的化合物和组合物可用于治疗具有诱导的或增强的残余 CFTR 活性的患者中的囊性纤维化或减轻所述囊性纤维化的严重性。这种残余 CFTR 诱导剂或增强剂可使用药理学方法实现。在另一个实施例中，本发明的化合物和组合物可用于治疗具有使用基因疗法诱导的或增强的残余 CFTR 活性的患者中的囊性纤维化或减轻所述囊性纤维化的严重性。此类方法增加存在于细胞表面的 CFTR 的量，从而在患者中诱导迄今不存在的 CFTR 活性或在患者中增强残余 CFTR 活性的现有水平。

[0387] 在一个实施例中，如本文所述的包含化合物 1 的本发明药物组合物可用于治疗患者中的囊性纤维化或减轻其严重性，该患者具有某些表现出残余 CFTR 活性的表型，例如 I 类突变（不合成）、II 类突变（错折叠）、III 类突变（调节或门控受损）、IV 类突变（传导改变）或 V 类突变（合成减少）。

[0388] 在一个实施例中，如本文所述的包含化合物 1 的本发明的药物组合物可用于在具有某些临床表型（例如一般与上皮细胞顶端膜中残余 CFTR 活性的量有关的中度到轻度临床表型）的患者中治疗囊性纤维化、减轻囊性纤维化的严重性或对症治疗囊性纤维化。此类表型包括表现出胰腺充足的患者。

[0389] 在一个实施例中，如本文所述的包含化合物 1 的本发明药物组合物可用于治疗经诊断患有以下疾病的患者、减轻所述疾病的严重性或对症治疗所述疾病：胰腺功能不全、特发性胰腺炎和先天性双侧输精管缺失或轻度肺病，其中该患者表现出残余 CFTR 活性。

[0390] 在一个实施例中，如本文所述的包含化合物 1 的本发明药物组合物可用于治疗经诊断患有以下疾病的患者、减轻所述疾病的严重性或对症治疗所述疾病：胰腺功能不全、特发性胰腺炎和先天性双侧输精管缺失或轻度肺病，其中该患者具有野生型 CFTR。

[0391] 除了囊性纤维化以外，CFTR 活性的调节还可有益于其他不直接由 CFTR 突变所导致的疾病，诸如分泌性疾病和其他由 CFTR 介导的蛋白折叠疾病。这些疾病包括但不限于慢性阻塞性肺病 (COPD)、干眼病和斯耶格伦氏综合征。COPD 的特征在于进行性的并且不完全可逆的气流受限。气流受限是由于粘液分泌过多、肺气肿和细支气管炎。突变或野生型 CFTR 的活化剂提供 COPD 中常见的粘液分泌过多和粘膜纤毛清除力受损的潜在治疗。具体地讲，增加跨 CFTR 的阴离子分泌可有利于体液转运进气道表面液体，以水化粘液并优化纤毛周围的体液粘度。这将引起粘膜纤毛清除力增强和与 COPD 有关的症状的减轻。干眼病的特征在于泪水的产生减少和异常泪膜脂质、蛋白和粘蛋白行为。干眼有很多原因，其中一些包括年龄、Lasik 眼手术、关节炎、药物治疗、化学 / 热灼伤、变态反应和疾病，诸如囊性纤维化和斯耶格伦氏综合征。增加经由 CFTR 的阴离子分泌将增强体液从角膜内皮细胞和眼周围分泌腺体的转运，从而增加角膜的水化作用。这将有助于缓解与干眼病有关的症状。斯耶格伦氏综合征是一种自身免疫疾病，其中免疫系统攻击体内各处产生水分的腺体，包括眼、口、皮肤、呼吸组织、肝、阴道和肠。症状包括眼、口和阴道干燥以及肺部疾病。该疾病也与类风湿性关节炎、系统性狼疮、系统性硬化和多肌炎 / 皮肌炎有关。有缺陷的蛋白运输据信会导致该疾病，其治疗选择是有限的。CFTR 活性的增强剂或诱导剂可以水化各种受疾病影响的器官，并帮助改善有关症状。

[0392] 在一个实施例中，本发明涉及在体外或体内增强或诱导阴离子通道活性的方法，包括使该通道与本发明的药物组合物接触。在另一个实施例中，阴离子通道为氯离子通道或碳酸氢根通道。在另一个实施例中，阴离子通道为氯离子通道。

[0393] 所需的确切量将在受试者之间有所不同，这取决于受试者的物种、年龄和一般状况、感染的严重性、具体的药剂、其施用模式等。优选将本发明化合物配制成单位剂型以易于施用和保持剂量均匀。本文所用的表达“单位剂型”是指适合于待治疗的患者的药剂的物理离散单元。然而，应当理解，本发明的化合物和组合物的总每日用量将由主治医师在合理的医学判断范围内决定。用于任何特定患者或生物体的具体有效剂量水平将取决于包括如下各项在内的多种因素：所治疗的病症和该病症的严重性；所使用的具体化合物的活性；所使用的具体组合物；患者的年龄、体重、总体健康情况、性别和饮食；所使用的具体化合物的施用时间、施用途径以及排泄速率；治疗持续时间；与所使用的具体化合物联合或同时使用的药物，以及医学领域熟知的类似因素。本文所用的术语“患者”意指动物，优选哺乳动物，并且最优选人。

[0394] 在本申请中如果化合物的名称未能正确描述化合物的结构，则结构代替名称，以结构为准。

[0395] 实例

[0396] XRPD(X 射线粉末衍射)

[0397] 化合物 1、化合物 1 形式 I、化合物 1 形式 II 或化合物 1HCl 盐形式 A 的 X 射线衍射 (XRD) 数据在具有 HI-STAR 2 维检测器和扁平石墨单色器的 Bruker D8DISCOVER 粉末衍射仪上采集。在 40kV、35mA 下使用具有 Kα 辐射的 Cu 密封管。在 25℃下将样品置于零背

景的硅晶片上。对每一样品而言，各自以 2 个不同的 θ_2 角在 120 秒采集两个数据帧 :8° 和 26° 。用 GADDS 软件对数据积分并用 DIFFRACT^{plus}EVA 软件合并。所报告的峰位置的不确定值为 ±0.2 度。

[0398] 喷射研磨描述

[0399] 在将未微粉化的化合物 1、化合物 1 形式 I、化合物 1 形式 II 或化合物 1HCl 盐形式 A 置于喷磨机料斗之前将其过筛以除去团块。所有筛子都是一次性的，用前擦净。采用压缩氮气控制加料速率，将未微粉化的化合物 1、化合物 1 形式 I、化合物 1 形式 II 或化合物 1HCl 盐形式 A 加入喷磨机料斗中。气体压力范围为 40-45/45-70(Venturi/Mill)PSI，加料速率范围为 0.5-1.6Kg/ 小时。将化合物 1、化合物 1 形式 I、化合物 1 形式 II 或化合物 1HCl 盐形式 A 在磨机中通过颗粒 - 颗粒和颗粒 - 壁之间的碰撞微粉化，将加工后的化合物 1、化合物 1 形式 I、化合物 1 形式 II 或化合物 1HCl 盐形式 A 倒入微粉化产品容器中。据信，通过部分基于上述条件的针孔研磨法，本领域的普通技术人员也可获得具有有利粒度的化合物 1、化合物 1 形式 I、化合物 1 形式 II 或化合物 1HCl 盐形式 A。

[0400] 差示扫描量热法 (DSC)

[0401] 化合物 1、化合物 1 形式 I、化合物 1 形式 II 或化合物 1HCl 盐形式 A 的差示扫描量热法 (DSC) 数据用 DSC Q100V9.6Build 290(特拉华州纽卡斯尔 TA 仪器公司 (TA Instruments, New Castle, DE) 采集。温度用铟校准，而热容用蓝宝石校准。称取 3-6mg 样品到铝盘中，将铝盘轧上带有 1 个针孔的褶皱盖。在 25℃至 350℃范围内以 1.0℃ /min 的加热速率并以 50ml/min 的氮气吹扫进行样品扫描。数据通过 Thermal Advantage Q Series™ 软件 2.2.0.248 版采集并通过 Universal Analysis 软件 4.1D 版（特拉华州纽卡斯尔 TA 仪器公司）分析。报告的数值代表单次分析。

[0402] 化合物 1 形式 I、化合物 1 形式 II 和化合物 1HCl 盐形式 A 单晶结构测定

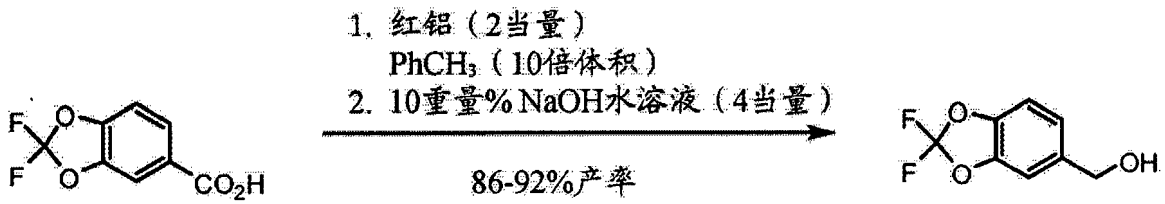
[0403] 衍射数据在配备有密封管 Cu Kα 源和 Apex II CCD 检测器的 Bruker Apex II 衍射仪上获得。使用 SHELX 程序 (Sheldrick, G.M., Acta Cryst., (2008) A64, 112-122(Sheldrick, G.M.,《晶体学报》,2008 年，第 A64 期，112-122 页)) 对结构进行解析和精修。基于系统消光和强度统计，解析和精修 $P2_1/n$ 空间群中的结构。

[0404] Vitride® （二氢双 (2- 甲氧乙氧基) 铝酸钠 [或 $NaAlH_2(OCH_2CH_2OCH_3)_2$]，65wt% 的甲苯溶液）购自德里奇化学公司 (Aldrich Chemicals)。

[0405] 2,2- 二氟 -1,3- 苯并二氧杂环戊烯 -5- 甲酸购自赛拓（朗盛子公司）。

[0406] (2,2- 二氟 -1,3- 苯并二氧杂环戊烯 -5- 基)- 甲醇的制备。

[0407]

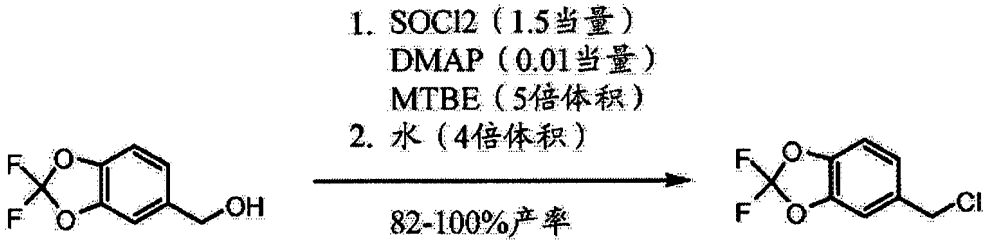


[0408] 使市售 2,2- 二氟 -1,3- 苯并二氧杂环戊烯 -5- 甲酸 (1.0 当量）在甲苯 (10 倍体积）中浆化。将 Vitride® (2 当量）经由加料漏斗以将温度维持在 15-25℃的速率加入。在添加结束时，将温度增至 40℃维持 2 小时 (h)，然后将 10% (w/w) 的 NaOH 水溶液 (aq, 4.0

当量）经由加料漏斗小心加入，将温度维持在 40-50℃。再搅拌 30 分钟 (min) 后，让层在 40℃下分离。将有机相冷却到 20℃，然后用水 (2×1.5 倍体积）洗涤、干燥 (Na_2SO_4)、过滤并浓缩得到直接用于下一步的粗 (2,2- 二氟 -1,3- 苯并二氧杂环戊烯 -5- 基）- 甲醇。

[0409] 5- 氯甲基 -2,2- 二氟 -1,3- 苯并二氧杂环戊烯的制备。

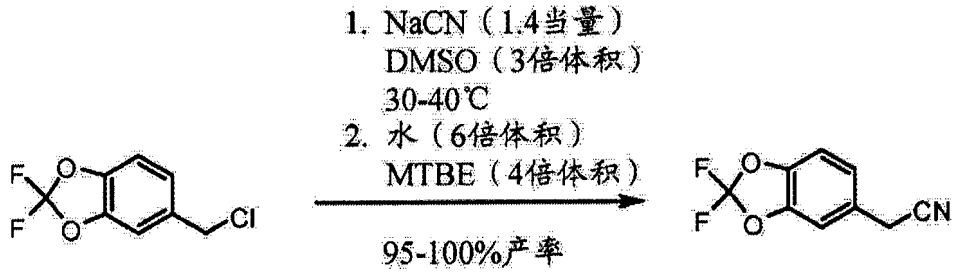
[0410]



[0411] 将 (2,2- 二氟 -1,3- 苯并二氧杂环戊烯 -5- 基）- 甲醇 (1.0 当量）溶于 MTBE(5 倍体积）。将催化量的 4-(N,N- 二甲基）氨基吡啶 (DMAP)(1mol％）加入并经由加料漏斗将 $SOCl_2$(1.2 当量）加入。将 $SOCl_2$ 以将反应器中的温度维持在 15-25℃的速率加入。将温度增至 30℃保持 1h，然后冷却到 20℃。将水 (4 倍体积）经加料漏斗加入，同时将温度维持在 30℃以下。再搅拌 30min 后，让层分离。对有机层进行搅拌，并将 10％ (w/v) 的 NaOH 水溶液 (4.4 倍体积）加入。搅拌 15 至 20min 后，让层分离。然后对有机相进行干燥 (Na_2SO_4)、过滤并浓缩，得到直接用于下一步的粗 5- 氯甲基 -2,2- 二氟 -1,3- 苯并二氧杂环戊烯。

[0412] (2,2- 二氟 -1,3- 苯并二氧杂环戊烯 -5- 基）- 乙腈的制备。

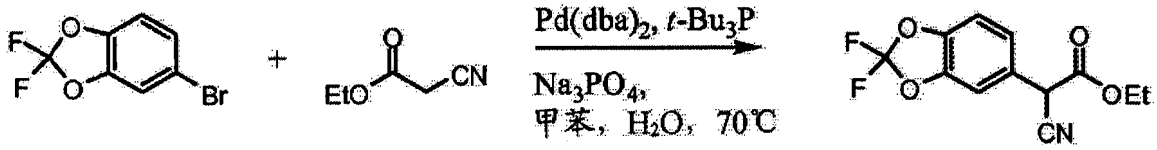
[0413]



[0414] 将 5- 氯甲基 -2,2- 二氟 -1,3- 苯并二氧杂环戊烯 (1 当量）在 DMSO(1.25 倍体积）中的溶液加入 NaCN(1.4 当量）在 DMSO(3 倍体积）中的浆液，同时将温度维持在 30-40℃之间。将混合物搅拌 1h，然后将水 (6 倍体积）加入，再将甲基叔丁基醚 (MTBE)(4 倍体积）加入。搅拌 30min 后，将层分离。将含水层用 MTBE(1.8 倍体积）萃取。将合并的有机层用水 (1.8 倍体积）洗涤、干燥 (Na_2SO_4)、过滤并浓缩得到直接用于下一步的粗 (2,2- 二氟 -1,3- 苯并二氧杂环戊烯 -5- 基）- 乙腈 (95％）。

[0415] (2,2- 二氟 -1,3- 苯并二氧杂环戊烯 -5- 基）-1- 乙酸乙酯 - 乙腈的合成

[0416]

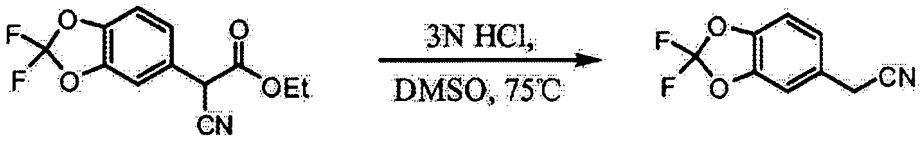


[0417] 将反应器用氮气吹扫，然后加入 900mL 甲苯。将溶剂经由不短于 16h 的氮气曝气进行脱气。然后向反应器中加入 Na_3PO_4(155.7g,949.5mmol)，再加入双（二亚苄基丙酮）钯

(0)(7.28g,12.66mmol)。将10% w/w的叔丁基膦的己烷溶液(51.23g,25.32mmol)在23℃下经10min通过用氮气吹扫过的加料漏斗加入。将混合物搅拌50min,此时将5-溴-2,2-二氟-1,3-苯并二氧杂环戊烯(75g,316.5mmol)经1min加入。再搅拌50min后,向混合物中经5min加入氰基乙酸乙酯(71.6g,633.0mmol),然后将水(4.5mL)一次性加入。将混合物经40min加热到70℃,每1-2h通过HPLC分析反应物向产物的转化百分比。在观察到转化完成后(通常在5-8h后转化率达到100%),将混合物冷却到20-25℃,然后通过硅藻土垫过滤。将硅藻土垫用甲苯(2×450mL)冲洗,将合并的有机物在真空和60-65℃下浓缩到300mL。向浓缩物中加入225mL DMSO,在真空和70-80℃下浓缩,直到活跃的溶剂蒸馏停止。将溶液冷却到20-25℃,用DMSO稀释到900mL,以为步骤2做准备。^{1}H NMR(500MHz,$CDCl_3$) δ 7.16-7.10(m,2H),7.03(d,J = 8.2Hz,1H),4.63(s,1H),4.19(m,2H),1.23(t,J = 7.1Hz,3H)。

[0418] (2,2-二氟-1,3-苯并二氧杂环戊烯-5-基)-乙腈的合成。

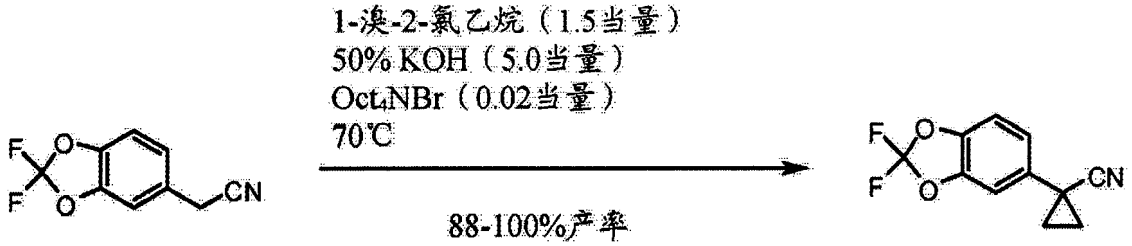
[0419]



[0420] 经20min向上面得到的(2,2-二氟-1,3-苯并二氧杂环戊烯-5-基)-1-乙酸乙酯-乙腈的DMSO溶液加入3N HCl(617.3mL,1.85mol),同时将内部温度维持在<40℃。然后经1h将混合物加热到75℃,并每1-2h通过HPLC分析转化百分比。当观察到转化率>99%时(通常在5-6h后),将反应物冷却到20-25℃,用MTBE(2×525mL)萃取足够的时间,以允许在萃取过程中实现完全的相分离。将合并的有机萃取物用5% NaCl(2×375mL)洗涤。然后将溶液转移到配备经冷却的接收烧瓶的适于1.5-2.5托真空蒸馏的设备。将溶液在真空和<60℃下浓缩以除去溶剂。然后在125-130℃(炉温)和1.5-2.0托下将(2,2-二氟-1,3-苯并二氧杂环戊烯-5-基)-乙腈从所得的油中蒸馏出来。(2,2-二氟-1,3-苯并二氧杂环戊烯-5-基)-乙腈作为澄清的油以66%的产率从5-溴-2,2-二氟-1,3-苯并二氧杂环戊烯(2步)中分离,HPLC纯度为91.5% AUC(对应于95%的重量比测定)。^{1}H NMR(500MHz,DMSO) δ 7.44(br s,1H),7.43(d,J = 8.4Hz,1H),7.22(dd,J = 8.2,1.8Hz,1H),4.07(s,2H)。

[0421] (2,2-二氟-1,3-苯并二氧杂环戊烯-5-基)-环丙烷甲腈的制备。

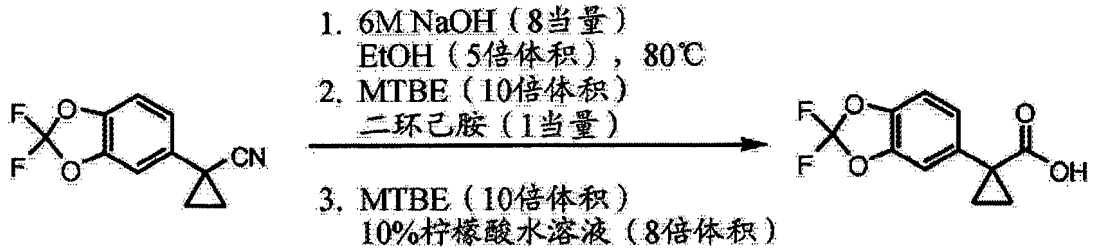
[0422]



[0423] 将(2,2-二氟-1,3-苯并二氧杂环戊烯-5-基)-乙腈(1.0当量)、50wt%的KOH水溶液(5.0当量)、1-溴-2-氯乙烷(1.5当量)和Oct_4NBr(0.02当量)的混合物在70℃下加热1h。将反应混合物冷却,然后用MTBE和水后处理。将有机相用水和盐水洗涤。除去溶剂得到(2,2-二氟-1,3-苯并二氧杂环戊烯-5-基)-环丙烷甲腈。

[0424] 1-(2,2-二氟-1,3-苯并二氧杂环戊烯-5-基)-环丙烷甲酸的制备。

[0425]

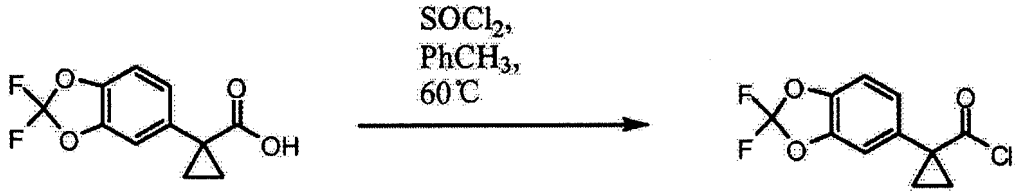


69%产率

[0426] 将(2,2-二氟-1,3-苯并二氧杂环戊烯-5-基)-环丙烷甲腈用6M的NaOH(8当量)的乙醇(5倍体积)溶液在80℃下水解过夜。将混合物冷却到室温,将乙醇在真空下蒸发。使残余物吸收到水和MTBE中,将1M HCl加入,然后对层进行分离。然后将MTBE层用二环己基胺(DCHA)(0.97当量)进行处理。将浆液冷却到0℃、过滤并用庚烷洗涤,得到相应的DCHA盐。使盐吸收到MTBE和10%柠檬酸中并搅拌直至所有固体溶解。对层进行分离,将MTBE层用水和盐水洗涤。溶剂交换成庚烷后过滤,在真空炉中在50℃干燥过夜后得到1-(2,2-二氟-1,3-苯并二氧杂环戊烯-5-基)-环丙烷甲酸。

[0427] 1-(2,2-二氟-1,3-苯并二氧杂环戊烯-5-基)-环丙烷甲酰氯的制备。

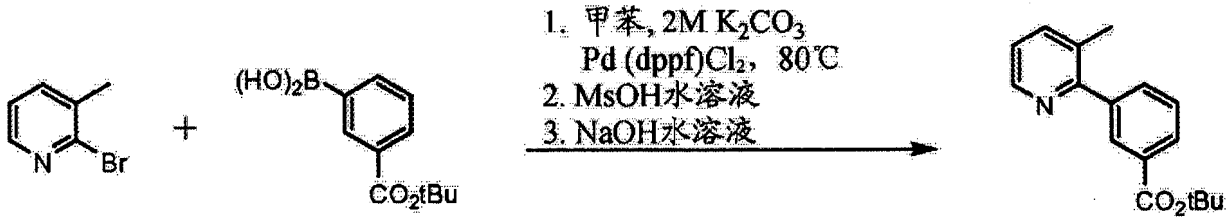
[0428]



[0429] 将1-(2,2-二氟-1,3-苯并二氧杂环戊烯-5-基)-环丙烷甲酸(1.2当量)在甲苯(2.5倍体积)中浆化,将混合物加热到60℃。将$SOCl_2$(1.4当量)经由加料漏斗加入。30分钟后将甲苯和$SOCl_2$从反应混合物中蒸馏出来。将另外的甲苯(2.5倍体积)加入,对所得的混合物再次蒸馏,留下作为油状物的酰氯,其无需进一步纯化而使用。

[0430] 3-(3-甲基吡啶-2-基)苯甲酸叔丁酯的制备。

[0431]

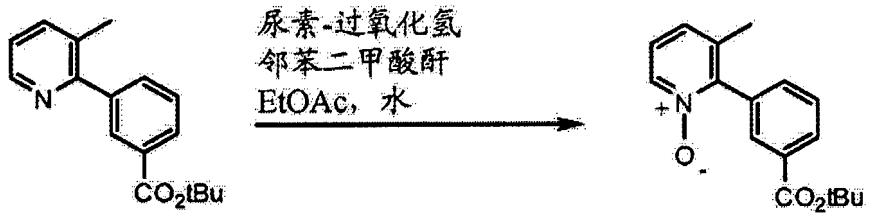


[0432] 将2-溴-3-甲基吡啶(1.0当量)溶于甲苯(12倍体积)。将K_2CO_3(4.8当量)加入,然后将水(3.5倍体积)加入。将所得的混合物在N_2流下加热到65℃维持1小时。然后将3-(叔丁氧羰基)苯基硼酸(1.05当量)和$Pd(dppf)Cl_2 \cdot CH_2Cl_2$(0.015当量)加入并将混合物加热到80℃。2小时后,停止加热,将水加入(3.5倍体积),并让层分离。然后将有机相用水(3.5倍体积)洗涤,并用10%的甲磺酸水溶液(2当量MsOH,7.7倍体积)萃取。通过50%的NaOH水溶液(2当量)使水相呈碱性,并用EtOAc(8倍体积)萃取。对有

机层进行浓缩得到直接用于下一步的粗 3-(3- 甲基吡啶 -2- 基) 苯甲酸叔丁酯 (82%)。

[0433] 2-(3-(叔丁氧羰基) 苯基)-3- 甲基吡啶 -1- 氧化物的制备。

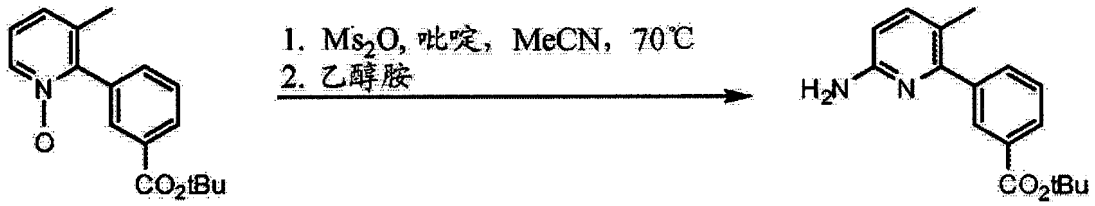
[0434]



[0435] 将 3-(3- 甲基吡啶 -2- 基) 苯甲酸叔丁酯 (1.0 当量) 溶于 EtOAc(6 倍体积)。将水 (0.3 倍体积) 加入，然后将过氧化氢合尿素 (3 当量) 加入。然后将邻苯二甲酸酐 (3 当量) 作为固体以将反应器中的温度维持在 45℃以下的速率分部分加入混合物。在完成邻苯二甲酸酐的添加后，将混合物加热到 45℃。再搅拌 4 小时后，停止加热。将 10% w/w 的 Na_2SO_3 水溶液 (1.5 当量) 经由加料漏斗加入。在完成 Na_2SO_3 的添加后，将混合物再搅拌 30min，然后对层进行分离。对有机层进行搅拌，并将 10% w/w 的 Na_2CO_3 水溶液 (2 当量) 加入。在搅拌 30 分钟后，让层分离。将有机相用 13% w/v 的 NaCl 水溶液洗涤。然后对有机相进行过滤并浓缩，得到直接用于下一步的粗 2-(3-(叔丁氧羰基) 苯基)-3- 甲基吡啶 -1- 氧化物 (95%)。

[0436] 3-(6- 氨基 -3- 甲基吡啶 -2- 基) 苯甲酸叔丁酯的制备。

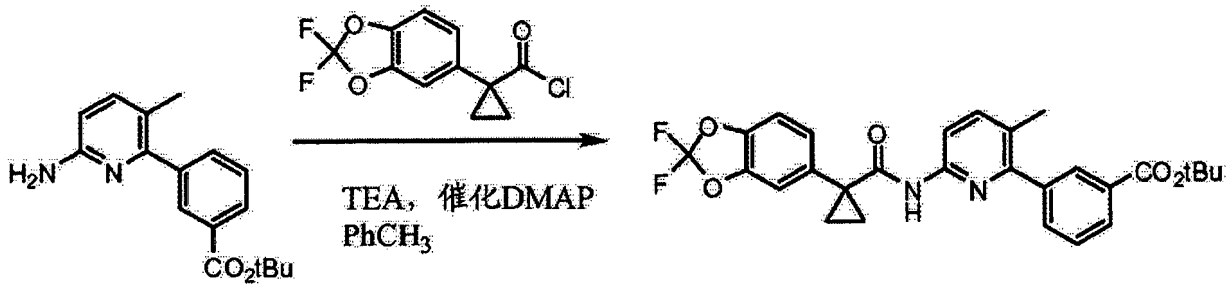
[0437]



[0438] 将 2-(3-(叔丁氧羰基) 苯基)-3- 甲基吡啶 -1- 氧化物 (1 当量) 和吡啶 (4 当量) 的乙腈 (8 倍体积) 溶液加热到 70℃。将甲磺酸酐 (1.5 当量) 的 MeCN(2 倍体积) 溶液经 50min 经由加料漏斗加入，同时将温度维持在 75℃以下。完成添加后，将混合物再搅拌 0.5 小时。然后使混合物冷却到环境温度。将乙醇胺 (10 当量) 经由加料漏斗加入。搅拌 2 小时后，将水 (6 倍体积) 加入并将混合物冷却到 10℃。搅拌 3 小时后，通过过滤收集固体，用水 (3 倍体积)、2:1 乙腈 / 水 (3 倍体积) 和乙腈 (2×1.5 倍体积) 洗涤。将固体在真空炉中在 50℃下通过轻微的 N_2 流干燥到恒重 (<1% 的差值)，得到作为红 - 黄色固体的 3-(6- 氨基 -3- 甲基吡啶 -2- 基) 苯甲酸叔丁酯 (53% 的产率)。

[0439] 3-(6-(1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基)- 环丙烷甲酰胺基)-3- 甲基吡啶 -2- 基)- 苯甲酸叔丁酯的制备。

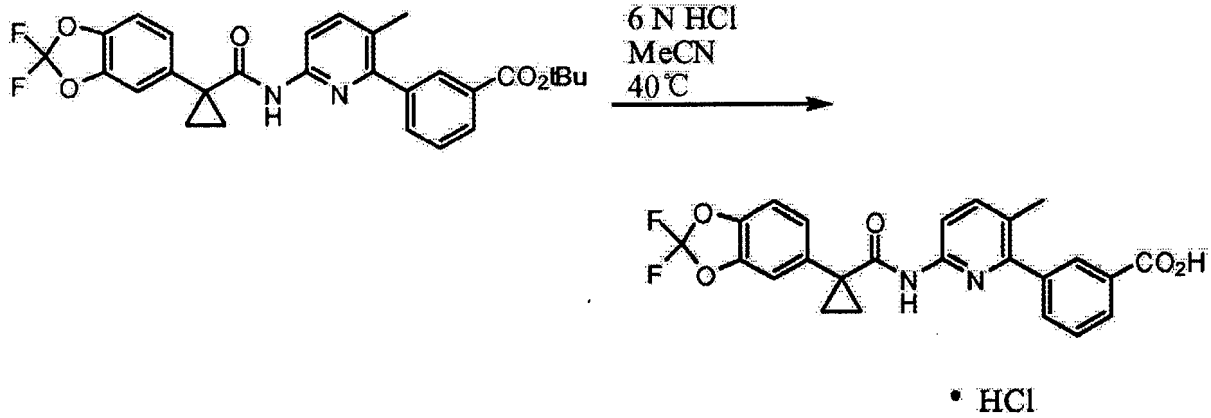
[0440]



[0441] 将上文所述的粗酰氯化物溶于甲苯（按酰氯化物计为2.5倍体积），并经由加料漏斗加入3-(6-氨基-3-甲基吡啶-2-基）苯甲酸叔丁酯（1当量）、DMAP(0.02当量）和三乙胺（3.0当量）在甲苯（按3-(6-氨基-3-甲基吡啶-2-基）苯甲酸叔丁酯计为4倍体积）的混合物中。2小时后，将水（按3-(6-氨基-3-甲基吡啶-2-基）苯甲酸叔丁酯计为4倍体积）加入反应混合物。搅拌30分钟后，对层进行分离。然后对有机相进行过滤，并浓缩得到3-(6-(1-(2,2-二氟苯并[d][1,3]二氧杂环戊烯-5-基）环丙烷甲酰胺基)-3-甲基吡啶-2-基)-苯甲酸叔丁酯（定量粗产率）的浓稠油。将乙腈（按粗产物计为3倍体积）加入，并蒸馏直至发生结晶。将水（按粗产物计为2倍体积）加入，并将混合物搅拌2h。通过过滤收集固体，用1:1（体积比）的乙腈/水（按粗产物计为2×1倍体积）洗涤，然后在过滤器上在真空下部分干燥。将固体在真空炉中在60℃下通过轻微的N_2流干燥到恒重（<1%的差值），得到作为棕色固体的3-(6-(1-(2,2-二氟苯并[d][1,3]二氧杂环戊烯-5-基）环丙烷甲酰胺基)-3-甲基吡啶-2-基)-苯甲酸叔丁酯。

[0442] 3-(6-(1-(2,2-二氟苯并[d][1,3]二氧杂环戊烯-5-基）环丙烷甲酰胺基)-3-甲基吡啶-2-基）苯甲酸·HCL盐的制备。

[0443]



·HCl

[0444] 向3-(6-(1-(2,2-二氟苯并[d][1,3]二氧杂环戊烯-5-基）环丙烷甲酰胺基)-3-甲基吡啶-2-基)-苯甲酸叔丁酯（1.0当量）在MeCN(3.0倍体积）中的浆液加入水（0.83倍体积），然后加入浓盐酸水溶液（0.83倍体积）。将混合物加热到45±5℃。搅拌24至48h后，反应完成，并让混合物冷却到环境温度。将水（1.33倍体积）加入，对混合物进行搅拌。通过过滤收集固体，用水（2×0.3倍体积）洗涤，然后在过滤器上在真空下部分干燥。将固体在真空炉中在60℃下通过轻微的N_2流干燥到恒重（<1%的差值），得到作为灰白色固体的3-(6-(1-(2,2-二氟苯并[d][1,3]二氧杂环戊烯-5-基）环丙烷甲酰胺基)-3-甲基吡啶-2-基）苯甲酸·HCl。

[0445] 化合物 1 的 ^{1}HNMR 光谱在图 20 中示出，而图 21 显示了作为 HCl 盐的化合物 1 的 ^{1}HNMR 光谱。

[0446] 下表 2 列出了化合物 I 的 ^{1}HNMR 数据。

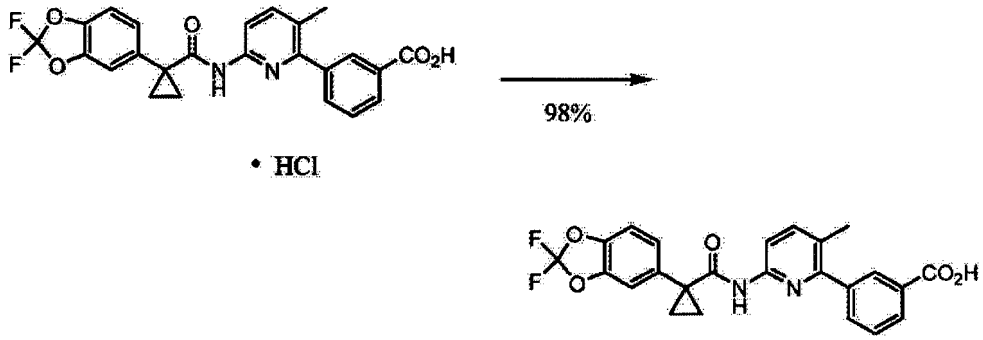
[0447] 表 2。

[0448]

化合物编号	LC/MS M+1	LC/RT 分钟	NMR
1	453.3	1.93	^{1}HNMR (400MHz, DMSO-d6) 9.14 (s, 1H), 7.99-7.93 (m, 3H), 7.80-7.78 (m,1H), 7.74-7.72 (m,1H), 7.60-7.55 (m,2H), 7.41-7.33 (m,2H), 2.24 (s, 3H), 1.53-1.51 (m, 2H), 1.19-1.17 (m, 2H)。

[0449] 化合物 1 形式 I 的制备，方法 A。

[0450]

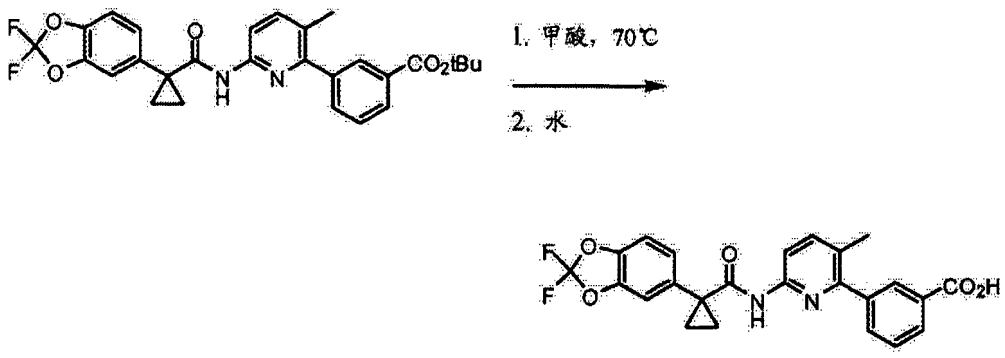


形式I

[0451] 将 3-(6-(1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基）环丙烷甲酰胺基）-3- 甲基吡啶 -2- 基）苯甲酸 •HCl(1 当量）在水（10 倍体积）中的浆液在环境温度下搅拌。在搅拌 24h 后采集样品。对样品过滤，将固体用水洗涤 (2 次）。使固体样品接受 DSC 分析。当 DSC 分析表明完全转化成形式 I 后，通过过滤收集固体，用水 (2×1.0 倍体积）洗涤，并在过滤器上在真空下部分干燥。然后将固体在真空炉中在 60℃下通过轻微的 N_2 流干燥到恒重 (<1%的差值），得到作为灰白色固体的化合物 1 形式 I(98%产率）。^{1}H NMR(400 MHz, DMSO-d6) 9.14(s, 1H), 7.99-7.93(m, 3H), 7.80-7.78(m, 1H), 7.74-7.72(m, 1H), 7.60-7.55(m, 2H), 7.41-7.33(m, 2H), 2.24(s, 3H), 1.53-1.51(m, 2H), 1.19-1.17(m, 2H)。

[0452] 化合物 1 形式 I 的制备，方法 B。

[0453]



形式I

[0454] 将 3-(6-(1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基）环丙烷甲酰胺基）-3- 甲基吡啶 -2- 基）- 苯甲酸叔丁酯 (1.0 当量）的甲酸 (3.0 倍体积）溶液在搅拌下加热到 70±10℃维持 8h。当色谱法表明不超过 1.0% AUC 的 3-(6-(1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基）环丙烷甲酰胺基）-3- 甲基吡啶 -2- 基）- 苯甲酸叔丁酯）剩余时，将反应视为完成。使混合物冷却到环境温度。将溶液加入水 (6 倍体积）中，在 50℃下加热，并对混合物进行搅拌。然后将混合物加热到 70±10℃，直到 3-(6-(1-(2,2- 二氟苯并 [d][1,3] 二氧杂环戊烯 -5- 基）环丙烷甲酰胺基）-3- 甲基吡啶 -2- 基）- 苯甲酸叔丁酯的含量不超过 0.8% (AUC)。通过过滤收集固体，用水 (2×3 倍体积）洗涤，然后在过滤器上在真空下部分干燥。将固体在真空炉中在 60℃下通过轻微的 N_2 流干燥到恒重 (<1% 的差值），得到作为灰白色固体的化合物 1 形式 I。

[0455] 化合物 1 形式 I 的 DSC 曲线在图 22 中示出。化合物 1 形式 I 的熔化发生在约 204℃。

[0456] X 射线衍射图从化合物 1 形式 I 的单晶结构计算而得，并在图 1 中示出。表 3 列出了图 1 的计算峰。

[0457] 表 3。

[0458]

峰排序	2θ 角 [度]	相对强度 [%]
11	14.41	48.2
8	14.64	58.8
1	15.23	100.0
2	16.11	94.7
3	17.67	81.9
7	19.32	61.3
4	21.67	76.5

5	23.40	68.7
9	23.99	50.8
6	26.10	67.4
10	28.54	50.1

[0459] 化合物 1 形式 I 的实际 X 射线粉末衍射图在图 2 中示出。表 4 列出了图 2 的实际峰。

[0460] 表 4。

[0461]

峰排序	2θ 角［度］	相对强度［%］
7	7.83	37.7
3	14.51	74.9
4	14.78	73.5
1	15.39	100.0
2	16.26	75.6
6	16.62	42.6
5	17.81	70.9
9	21.59	36.6
10	23.32	34.8
11	24.93	26.4
8	25.99	36.9

[0462]

[0463] 通过将浓缩的 1- 丁醇溶液以 0.2℃ /min 的速率从 75℃冷却到 10℃得到了化合物 1 形式 I 的无色晶体。选择尺寸为 0.50×0.08×0.03mm 的晶体，用矿物油清洁，安装在 MicroMount 上并在 Bruker APEX II 系统上居中。得到了在倒易空间中分离的三批 40 帧，以提供取向矩阵和初始晶胞参数。获得了最终晶胞参数，并基于完整数据集进行了精化。

[0464] 以每帧 30s 暴露使用 0.5° 步进获得了倒易空间的衍射数据集，达到 0.82Å 的分辨率。在 100(2)K 采集数据。强度的积分和晶胞参数的精化使用 APEXII 软件完成。在采集数据后观测晶体表明无分解迹象。

[0465] 基于单晶 X 射线分析的化合物 1 形式 I 的构象图在图 23 中示

出。化合物 1 形式 I 为单斜、P_21/n，具有以下单位晶胞尺寸：a=4.9626(7) Å、b=12.299(2) Å、c=33.075 (4) Å、β = 93.938(9)°、V=2014.0Å^3、Z = 4。从结构数据计算的化合物 1 形式 I 的密度在 100K 时为 1.492g/cm^3。

[0466] 由化合物 1 形式 I 制备化合物 1 形式 II。

[0467] 将化合物 1 形式 I（约 30mg）在 500μL 适当的溶剂（例如甲醇、乙醇、丙酮、2- 丙醇、乙腈、四氢呋喃、乙酸甲酯、2- 丁酮、甲酸乙酯和 - 甲基四氢呋喃）中浆化两天。然后对浆液进行离心或真空过滤，在环境温度下静置干燥过夜，得到化合物 1 形式 II。

[0468] 化合物 1 形式 II 丙酮溶剂化物的 DSC 曲线在图 15 中示出，显示了两个相变。化合物 1 形式 II 丙酮溶剂化物的熔点出现在约 188℃和 205℃。

[0469] 化合物 1 形式 II 的实际 X 射线粉末衍射图在图 3 中示出。表 5 以相对强度的降序列出了图 3 的实际峰。

[0470] 表 5。

[0471]

2θ 角 [度]	相对强度 [%]
21.70	100.0
8.98	65.5
11.04	57.4
18.16	55.9
23.06	55.4
20.63	53.1
22.22	50.2
18.57	49.1
16.66	47.2
19.86	35.0

[0472]

[0473] 基于单晶 X 射线分析的化合物 1 形式 II 丙酮溶剂化物的构象图在图 24 中示出。化合物 1 形式 II 与丙酮之间的化学计量为约 4.4:1（由 ^{1}H NMR 计算为 4.48:1；由 X 射线计算为 4.38:1）。晶体结构揭露出分子堆积，其中每个单位晶胞存在两个空隙或袋，或每个主分子存在 1 个空隙。在丙酮溶剂化物中，约 92% 的空隙被丙酮分子占据。化合物 1 形式 II 为单斜 $P2_1/n$ 空间群，具有以下单位晶胞尺寸：a = 16.5235(10) Å、b = 12.7425(8) Å、c = 20.5512 (13) Å、α = 90°、β =

103.736(4)°、γ = 90°、V = 4203.3(5) Å^3, = 4。从结构数据计算出的为化合物 1 形式 II 的化合物 1 的密度在 100K 时为 1.430/cm^3。

[0474] 化合物 1 形式 II 丙酮溶剂化物的固态 ^{13}C NMR 光谱在图 25 中示出。

[0475] 表 6 提供相关峰的化学位移。

[0476] 表 6。

[0477]

	化合物 1 形式 II 丙酮溶剂化物 ^{13}C 化学位移	
峰号	F1 [ppm]	强度
1	202.8	6.05
2	173.3	62.66
3	171.9	20.53
4	153.5	28.41
5	150.9	21.68
6	150.1	19.49
7	143.2	45.74
8	142.3	42.68
9	140.1	37.16
10	136.6	26.82
11	135.9	30.1
12	134.6	39.39
13	133.2	23.18
14	131.0	60.92
15	128.5	84.58
16	116.0	34.64
17	114.2	23.85
18	112.4	25.3
19	110.9	24.12

[0478]

20	107.8	18.21
21	32.0	54.41
22	22.2	20.78
23	18.8	100

[0479] 化合物 1 形式 II 丙酮溶剂化物的固态 ^{19}F NMR 光谱在图 26 中示出。带星号的峰表示旋转边带。表 7 提供相关峰的化学位移。

[0480] 表 7。

[0481]

	化合物 1 形式 II 丙酮溶剂化物 ^{19}F 化学位移	
峰号	F1 [ppm]	强度
1	-41.6	12.5
2	-46.4	6.77
3	-51.4	9.05

[0482] 化合物 1HCl 盐形式 A 的制备。

[0483] 通过由化合物 1 的 HCl 盐的浓缩乙醇溶液进行缓慢蒸发得到了化合物 1HCl 盐形式 A 的无色晶体。选择尺寸为 0.30×1/5×0.15mm 的晶体，用矿物油清洁，安装在 MicroMount 上并在 Bruker APEXII 衍射仪上居中。得到了在倒易空间中分离的三批 40 帧，以提供取向矩阵和初始晶胞参数。获得了最终晶胞参数，并基于完整数据集进行了精化。

[0484] 图 18 提供了基于单晶分析的作为二聚体的化合物 1HCl 盐形式 A 的构象图。从晶体结构计算的化合物 1HCl 盐形式 A 的 X 射线衍射图在图 27 中示出。表 8 包含以相对强度的降序排列的图 27 的计算峰。

[0485] 表 8。

[0486]

2θ [度]	相对强度 [%]
8.96	100.00
17.51	48.20
18.45	34.60
10.33	32.10
16.01	18.90
11.94	18.40
8.14	16.20
10.10	13.90
16.55	13.30
9.54	10.10
16.55	13.30

[0487] 包含化合物 1 的示例性口服药物制剂

[0488] 对于包含 100mg API（即化合物 1 形式 I）的示例性片剂 1A，以表 9 所列的组分和量制备了片剂。示例性片剂 1A（配制成具有 100mg 化合物 1）用干法辊压设备制剂工艺制备。在表 9 中，等级 / 品牌为 :微晶纤维素 :Avicel PH102 ;甘露醇 :Pearlitol SD 100 ;交联羧甲基纤维素钠 :Acdisol ;和胶态二氧化硅 :Cabosil。

[0489] 表 9。

[0490]

辊压颗粒共混物	(%w/w)
化合物 1 形式 I	30
微晶纤维素	42.3
甘露醇	21.2
交联羧甲基纤维素钠	3
月桂基硫酸钠	1
胶态二氧化硅	0.5
硬脂酸镁	2
片剂组合物（100mg 剂量，335mg 图像）	(%w/w)
辊压颗粒共混物	99.5
硬脂酸镁	0.5

[0491] 对于包含 100mg API（即化合物 1 形式 I）的示例性片剂 1B，以表 10 所列的组分和量制备了片剂。示例性片剂 1B（配制成具有 100mg 化合物 1 形式 I）用湿法高剪切颗粒制剂工艺制备。在表 10 中，等级 / 品牌如下。高剪切颗粒共混物 - 微晶纤维素 :Avicel PH101 ；甘露醇 :Pearlitol C50 ；交联羧甲基纤维素钠 :Acdisol ；聚乙烯吡咯烷酮 :Kollidon PVP K30 ；以及在片剂组合物中 - 交联羧甲基纤维素钠 :Acdisol。

[0492] 表 10。

[0493]

高剪切颗粒共混物	(%w/w)
化合物 1 形式 I	50
微晶纤维素	30
甘露醇	13
交联羧甲基纤维素钠	2
聚乙烯吡咯烷酮	4
月桂基硫酸钠	1

[0494]

片剂组合物（100mg 剂量，205mg 图像）	(%w/w)
高剪切颗粒共混物	97.5
交联羧甲基纤维素钠	2.0
硬脂酸镁	0.5

[0495] 对于包含 100mg API（即结晶化合物 1 形式 I）的示例性片剂 1C，以表 11 所列的组分和量制备了片剂。示例性片剂 1C（配制成具有 100mg 结晶化合物 1 形式 I）用湿法高剪切颗粒制剂工艺制备。在表 11 中，等级 / 品牌如下。高剪切颗粒共混物 - 微晶纤维素 : Avicel PH101 ；甘露醇 :Pearlitol C50 ；交联羧甲基纤维素钠 :Acdisol ；聚乙烯吡咯烷酮 : Kollidon PVP K30 ；以及在片剂组合物中 - 交联羧甲基纤维素钠 :Acdisol。

[0496] 表 11。

[0497]

高剪切颗粒共混物	(%w/w)
化合物 1 形式 I	60
微晶纤维素	20
甘露醇	13
交联羧甲基纤维素钠	2
聚乙烯吡咯烷酮	4
月桂基硫酸钠	1
片剂组合物（100mg 剂量，171mg 图像）	(%w/w)
高剪切颗粒共混物	97.5
交联羧甲基纤维素钠	2.0
硬脂酸镁	0.5

[0498] 对于包含 200mg API（即结晶化合物 1 形式 I）的示例性片剂 1D，以表 12 所列的组分和量制备了片剂。示例性片剂 1D（配制成具有 200mg 结晶化合物 1 形式 I）用湿法高剪切颗粒制剂工艺制备。在表 12 中，等级 / 品牌如下。高剪切颗粒共混物 - 微晶纤维素：Avicel PH101；甘露醇：Pearlitol C50；交联羧甲基纤维素钠：Acdisol；聚乙烯吡咯烷酮：Kollidon PVP K30；以及在片剂组合物中 - 微晶纤维素：Avicel PH200；交联羧甲基纤维素钠：Acdisol；和硬脂酸镁：5712。

[0499] 表 12。

[0500]

高剪切颗粒共混物	(%w/w)
化合物 1 形式 I	60
微晶纤维素	20
甘露醇	13
交联羧甲基纤维素钠	2
聚乙烯吡咯烷酮	4
月桂基硫酸钠	1
片剂组合物（200mg 剂量，402mg 图像）	(%w/w)
高剪切颗粒共混物	83
微晶纤维素	14
交联羧甲基纤维素钠	2
硬脂酸镁	1

[0501] 对于包含 200mg API（即结晶化合物 1 形式 I）的示例性片剂 1E，以表 13 所列的组分和量制备了片剂。示例性片剂 1E（配制成具有 200mg 结晶化合物 1 形式 I）用湿法高剪切颗粒制剂工艺制备。在表 13 中，等级 / 品牌如下。高剪切颗粒共混物 - 微晶纤维素：Avicel PH101；甘露醇：Pearlitol C50；交联羧甲基纤维素钠：Acdisol；聚乙烯吡咯烷酮：Kollidon PVP K30；以及在片芯片剂组合物中 - 微晶纤维素：Avicel PH200；交联羧甲基纤维素钠：Acdisol；和硬脂酸镁：5712；以及在薄膜衣中 - 薄膜衣：Opadry II；蜡：巴西棕榈蜡。

[0502] 表 13。

[0503]

高剪切颗粒共混物	mg
化合物 1 形式 I	200
微晶纤维素	66
甘露醇	43
交联羧甲基纤维素钠	7
聚乙烯吡咯烷酮	13
月桂基硫酸钠	3
片芯片剂组合物（200mg 剂量，400mg 图像）	mg
高剪切颗粒共混物	332
微晶纤维素	56
交联羧甲基纤维素钠	8
硬脂酸镁	4

[0504]

薄膜衣片剂（200mg 剂量，412mg 图像）	mg
片芯片剂组合物	400
薄膜衣	12
蜡	微量

[0505] 对于包含 200mg API（即结晶化合物 1 形式 I）的示例性片剂 1F，以表 14 所列的组分和量制备了片剂。示例性片剂 1F（配制成具有 200mg 结晶化合物 1 形式 I）用湿法高剪切颗粒制剂工艺制备。在表 14 中，等级 / 品牌如下。高剪切颗粒共混物 - 微晶纤维素：Avicel PH101；甘露醇：Pearlitol C50；交联羧甲基纤维素钠：Acdisol；聚乙烯吡咯烷酮：Kollidon PVP K30；以及在片芯片剂组合物中 - 微晶纤维素：Avicel PH200；交联羧甲基纤维素钠：Acdisol；和硬脂酸镁：5712；以及在薄膜衣中 - 薄膜衣：Opadry II；蜡：巴西棕榈蜡。

[0506] 表 14。

[0507]

高剪切颗粒共混物	mg
化合物 1 形式 I	200
微晶纤维素	67
甘露醇	45
交联羧甲基纤维素钠	7
聚乙烯吡咯烷酮	10.4
月桂基硫酸钠	2.6
片芯片剂组合物（200mg 剂量，400mg 图像）	mg
高剪切颗粒共混物	332
微晶纤维素	56
交联羧甲基纤维素钠	8
硬脂酸镁	4
薄膜衣片剂（200mg 剂量，412mg 图像）	mg
片芯片剂组合物	400
薄膜衣	12
蜡	0.04

[0508] 对于包含 100mg API（即结晶化合物 1 形式 I）的示例性片剂 1G，以表 15 所列的组分和量制备了片剂。示例性片剂 1G（配制成具有 100mg 结晶化合物 1 形式 I）用湿法高剪切颗粒制剂工艺制备。在表 15 中，等级 / 品牌如下。高剪切颗粒共混物 - 微晶纤维素：Avicel PH101；甘露醇：Pearlitol C50；交联羧甲基纤维素钠：Acdisol；聚乙烯吡咯烷酮：Kollidon PVP K30；以及在片剂组合物中 - 交联羧甲基纤维素钠：Acdisol。

[0509] 表 15。

[0510]

高剪切颗粒共混物	(%w/w)
化合物 1 形式 I	70
微晶纤维素	12
甘露醇	11
交联羧甲基纤维素钠	2
聚乙烯吡咯烷酮	4
月桂基硫酸钠	1
片剂组合物（100mg 剂量，147mg 图像）	(%w/w)
高剪切颗粒共混物	97.5
交联羧甲基纤维素钠	2.0
硬脂酸镁	0.5

[0511] 对于包含 100mg API（即结晶化合物 1 形式 I 或形式 II）的示例性片剂 1H，以表 16 所列的组分和量制备了片剂。示例性片剂 1H（配制成具有 100mg 结晶化合物 1 形式 I 或形式 II）用湿法高剪切颗粒制剂工艺制备。在表 16 中，等级 / 品牌如下。高剪切颗粒共混物 - 微晶纤维素：Avicel PH101；甘露醇：Pearlitol C50；交联羧甲基纤维素钠：Acdisol；聚乙烯

吡咯烷酮 :Kollidon PVP K30 ;以及在片芯片剂组合物中 - 微晶纤维素 :Avicel PH200 ;交联羧甲基纤维素钠 :Acdisol ;和硬脂酸镁 :5712。

[0512] 表 16。

[0513]

高剪切颗粒共混物	(%w/w)
化合物 1 形式 I 或形式 II	61
微晶纤维素	20.3
甘露醇	13.2
交联羧甲基纤维素钠	2
聚乙烯吡咯烷酮	2.7
月桂基硫酸钠	0.7
片剂组合物 (100mg 剂量；197mg 图像)	(%w/w)
高剪切颗粒共混物	83
微晶纤维素	14
交联羧甲基纤维素钠	2
硬脂酸镁	1

[0514] 对于包含 100mg API(即结晶化合物 1 形式 I 或形式 II) 的示例性片剂 1I,以表 17 所列的组分和量制备了片剂。示例性片剂 1I(配制成具有 100mg 结晶化合物 1 形式 I 或形式 II) 用湿法高剪切颗粒制剂工艺制备。在表 17 中,等级 / 品牌如下。高剪切颗粒共混物 - 微晶纤维素 :Avicel PH101 ;甘露醇 :Pearlitol C50 ;交联羧甲基纤维素钠 :Acdisol ;聚乙烯吡咯烷酮 :Kollidon PVP K30 ;以及在片芯片剂组合物中 - 微晶纤维素 :Avicel PH200 ;交联羧甲基纤维素钠 :Acdisol ;和硬脂酸镁 :5712。

[0515] 表 17。

[0516]

高剪切颗粒共混物	mg
化合物 1 形式 I 或形式 II	100
微晶纤维素	33.3
甘露醇	21.7
交联羧甲基纤维素钠	3.3
聚乙烯吡咯烷酮	4.4
月桂基硫酸钠	1.1
片芯片剂组合物 (100mg 剂量；197mg 图像)	mg
高剪切颗粒共混物	163.9
微晶纤维素	27.6
交联羧甲基纤维素钠	3.9
硬脂酸镁	2.0

[0517] 对于包含 300mg API(即结晶化合物 1 形式 I) 的示例性片剂 1J,以表 18 所列的组分和量制备了片剂。示例性片剂 1J(配制成具有 300mg 结晶化合物 1 形式 I) 用湿法高剪切颗粒制剂工艺制备。在表 18 中,等级 / 品牌如下。高剪切颗粒共混物 - 微晶纤维素 :

Avicel PH101 ;甘露醇 :Pearlitol C50 ;交联羧甲基纤维素钠 :Acdisol ;聚乙烯吡咯烷酮 : Kollidon PVP K30 ;以及在片芯片剂组合物中 - 微晶纤维素 :Avicel PH200 ;交联羧甲基纤维素钠 :Acdisol ;和硬脂酸镁 :5712 ;以及在薄膜衣中 - 薄膜衣 :Opadry II ;蜡 :巴西棕榈蜡。

[0518] 表 18。

[0519]

高剪切颗粒共混物	mg
化合物 1 形式 I	300
微晶纤维素	99
甘露醇	64.5
交联羧甲基纤维素钠	10.5
聚乙烯吡咯烷酮	19.5
月桂基硫酸钠	4.5
片芯片剂组合物 (300mg 剂量；600mg 图像)	mg
高剪切颗粒共混物	498
微晶纤维素	84
交联羧甲基纤维素钠	12
硬脂酸镁	6
薄膜衣片剂 (300mg 剂量；618mg 图像)	mg
片芯片剂组合物	600
薄膜衣	18
蜡	0.06

[0520] 对于包含 300mg API(即结晶化合物 1 形式 I) 的示例性片剂 1K, 以表 19 所列的组分和量制备了片剂。示例性片剂 1K(配制成具有 300mg 结晶化合物 1 形式 I) 用湿法高剪切颗粒制剂工艺制备。在表 19 中, 等级 / 品牌如下。高剪切颗粒共混物 - 微晶纤维素 : Avicel PH101 ;甘露醇 :Pearlitol C50 ;交联羧甲基纤维素钠 :Acdisol ;聚乙烯吡咯烷酮 : Kollidon PVP K30 ;以及在片芯片剂组合物中 - 微晶纤维素 :Avicel PH200 ;交联羧甲基纤维素钠 :Acdisol ;和硬脂酸镁 :5712 ;以及在薄膜衣中 - 薄膜衣 :Opadry II ;蜡 :巴西棕榈蜡。

[0521] 表 19。

[0522]

高剪切颗粒共混物	mg
化合物 1 形式 I	300
微晶纤维素	100.5
甘露醇	67.5
交联羧甲基纤维素钠	10.5
聚乙烯吡咯烷酮	15.6
月桂基硫酸钠	3.9

[0523]

片芯片剂组合物（300mg 剂量，600mg 图像）	mg
高剪切颗粒共混物	498
微晶纤维素	84
交联羧甲基纤维素钠	12
硬脂酸镁	6
薄膜衣片剂（300mg 剂量，618mg 图像）	mg
片芯片剂组合物	600
薄膜衣	18
蜡	0.06

[0524] 对于包含 200mg API（即结晶化合物 1 形式 I）的示例性片剂 1L，以表 20 所列的组分和量制备了片剂。示例性片剂 1L（配制成具有 200mg 结晶化合物 1 形式 I）用双螺杆湿法颗粒制剂工艺制备。在表 20 中，等级 / 品牌如下。双螺杆颗粒共混物 - 微晶纤维素：Avicel PH101；交联羧甲基纤维素钠：Acdisol；聚乙烯吡咯烷酮：Kollidon PVP K30；以及在片芯片剂组合物中 - 微晶纤维素：Avicel PH200；交联羧甲基纤维素钠：Acdisol；和硬脂酸镁：5712；以及在薄膜衣中 - 薄膜衣：Opadry II；蜡：巴西棕榈蜡。

[0525] 表 20。

[0526]

双螺杆颗粒共混物	mg
化合物 1 形式 I	200
微晶纤维素	34.0
交联羧甲基纤维素钠	6.3
聚乙烯吡咯烷酮	7.8
月桂基硫酸钠	1.8
片芯片剂组合物（200mg 剂量）	mg
双螺杆颗粒共混物	249.9
微晶纤维素	36.1
交联羧甲基纤维素钠	12.0
硬脂酸镁	3.0
薄膜衣片剂（200mg 剂量，310mg 总重）	mg
片芯片剂组合物	301
薄膜衣	9.0
蜡	微量

[0527] 对于包含 400mg API（即结晶化合物 1 形式 I）的示例性片剂 1M，以表 21 所列的组分和量制备了片剂。示例性片剂 1M（配制成具有 400mg 结晶化合物 1 形式 I）用双螺杆湿法颗粒制剂工艺制备。在表 21 中，等级 / 品牌如下。双螺杆颗粒共混物 - 微晶纤维素：Avicel PH101；交联羧甲基纤维素钠：Acdisol；聚乙烯吡咯烷酮：Kollidon PVP K30；以及在片芯片剂组合物中 - 微晶纤维素：Avicel PH200；交联羧甲基纤维素钠：Acdisol；和硬

脂酸镁 :5712 ;以及在薄膜衣中 - 薄膜衣 :Opadry II ;蜡 :巴西棕榈蜡。

[0528] 表 21。

[0529]

双螺杆颗粒共混物	mg
化合物 1 形式 I	400
微晶纤维素	68.0
交联羧甲基纤维素钠	12.6
聚乙烯吡咯烷酮	15.6
月桂基硫酸钠	3.6
片芯片剂组合物（400mg 剂量）	mg
双螺杆颗粒共混物	499.8
微晶纤维素	72.2
交联羧甲基纤维素钠	24.0
硬脂酸镁	6.0
薄膜衣片剂（400mg 剂量，620mg 总重）	mg
片芯片剂组合物	602
薄膜衣	18.0
蜡	微量

[0530] 由辊压颗粒组合物形成片剂

[0531] 设备 / 工艺

[0532] 设备

[0533] 辊压机 :Alexanderwerk WP 120、Vector TF-Mini 或 Vector TF-Labo。

[0534] 过筛 / 称重

[0535] 可在称取之前或之后对化合物 1 和赋形剂过筛。适当的筛号为 20 目、40 目或 60 目。化合物 1 可与赋形剂中的一种或多种预混以简化过筛。

[0536] 共混

[0537] 化合物 1 和赋形剂可按不同的顺序加入共混机中。共混在 Turbula 共混机或 V 形壳共混机中进行。可将组分在无润滑剂的情况下共混 10 分钟，然后与润滑剂一起再共混 3 分钟。

[0538] 辊压

[0539] 使用 Alexanderwerk WP 120 将共混物辊压成带并将带磨成颗粒。所用的辊为 25mm 辊，其采用 18 至 50 巴的压实压力、3 至 12RPM 的辊速和 20 至 80RPM 的螺旋进料机速度。集成磨机的筛网尺寸为 :顶部筛网 2mm，底部筛网 0.8mm。

[0540] 共混

[0541] 将辊压颗粒与颗粒外赋形剂（诸如填料和润滑剂）用 V 形壳共混机共混。共混时间可以为 5、3 或 1 分钟。

[0542] 压制

[0543] 将压缩共混物用具有 10mm 模具的单冲 Riva MiniPress 压成片。100mg 剂量的片

重可以为约 200、250 或 300mg。

[0544] 包薄膜衣

[0545] 将片剂用包衣锅（诸如 O' Hara Labcoat）包薄膜衣。

[0546] 印刷

[0547] 通过例如 Hartnett Delta 印刷机将薄膜衣片在片剂的一个或两个表面上印上字母图案。

[0548] 由高剪切颗粒组合物形成片剂

[0549] 设备 / 工艺

[0550] 设备

[0551] 制粒机：具有 250mL 或 1L 制粒槽的 Procept MiPro。

[0552] 过筛 / 称重

[0553] 可在称取之前或之后对化合物 1 和赋形剂过筛。可能的筛号为 20 目、40 目或 60 目。化合物 1 可与赋形剂中的一种或多种预混以简化过筛。

[0554] 制粒操作

[0555] 制粒液 - 将 SLS 和粘合剂加入纯化水，并混合直至溶解。合适的比率为 2.5% w/w 的 SLS 水溶液和 10.0% w/w 的 PVP K30 水溶液。

[0556] 制粒 - 将赋形剂和化合物 1 加入制粒槽。添加顺序可以为化合物 1、崩解剂、稀释剂和填充剂。可将组分在 250mL 槽中以 1000RPM 的叶轮速度和 1000RPM 的切碎机速度混合 1 分钟。制粒可以 2000RPM 的叶轮速度与 4000RPM 的切碎机速度进行，同时以 1.5 至 4.5g/min 的注射泵速度添加制粒液。制粒液添加时间可以为 4 至 12 分钟。添加所需的粘合剂液后，可将颗粒湿法和团约 10 秒至约 1 分钟。本发明的高剪切制粒工艺的一个值得注意的优点是使用包含表面活性剂和粘合剂两者的制粒液，以通过增强的可润湿性更好地制粒。在一个实施例中，表面活性剂为 SLS。

[0557] 研磨

[0558] 用筛网磨机或锥形磨使颗粒尺寸减小。

[0559] 干燥

[0560] 可将颗粒用真空炉、托盘烘干机、双锥形烘干机或流化床烘干机干燥。将颗粒用真空炉在氮气流下进行了干燥。

[0561] 共混

[0562] 可将颗粒与颗粒外赋形剂共混。将颗粒与颗粒外崩解剂、稀释剂、填充剂和润滑剂进行了共混。将颗粒用 Turbula 共混机在无润滑剂的情况下共混了 3 分钟，然后与润滑剂共混了 1 分钟。可以使用更大规模的共混机，诸如 4-quart V 形壳共混机。

[0563] 压制

[0564] 将压缩共混物用具有 8mm 或 10mm 模具的单冲 Riva MiniPress 压成片。100mg 剂量的片重可以为约 160、200 或 250mg。

[0565] 包薄膜衣

[0566] 将片剂用包衣锅（诸如 O' Hara Labcoat）包薄膜衣。

[0567] 印刷

[0568] 通过例如 Hartnett Delta 印刷机将薄膜衣片在片剂的一个或两个表面上印上字

母图案。

[0569] 由连续双螺杆湿法制粒工艺形成片剂

[0570] 设备 / 工艺

[0571] 设备

[0572] 制粒机 :ConsiGma 或 Leistritz 或 Thermo Fisher 双螺杆制粒机。

[0573] 过筛 / 称重

[0574] 可在称取之前或之后对化合物 1 和赋形剂过筛。可能的筛号为 20 目、40 目或 60 目。化合物 1 可与赋形剂中的一种或多种预混以简化过筛。

[0575] 共混

[0576] 化合物 1 和赋形剂可按不同的顺序加入共混机中。共混可在 Turbula 共混机、V 形壳共混机、料箱共混机 (bin blender) 或连续共混机中进行。对于分批共混机而言,可将组分共混 10 分钟,对于连续共混机而言,可连续共混。

[0577] 制粒操作

[0578] 制粒液 - 将 SLS 和粘合剂加入纯化水,并混合直至溶解。合适的比率为 2.5% w/w 的 SLS 水溶液和 10.0% w/w 的 PVP K30 水溶液。

[0579] 制粒 - 可将含有化合物 1 和赋形剂的共混物使用失重式进料机以 10kg/h 的速率定量加入双螺杆制粒机。可使用蠕动泵以 3.5kg/h 的速率添加制粒液。制粒机可以 400RPM 的速度运行。本发明的双螺杆湿法制粒工艺的一个值得注意的优点是使用包含表面活性剂和粘合剂两者的制粒液,以通过增强的可润湿性更好地制粒。在一个实施例中,表面活性剂为 SLS。另一个值得注意的优点在于,由于该工艺是连续的并且在任何时间只加工有限量的材料,因此该工艺可得到很好的控制并产生高质量的产品。

[0580] 研磨

[0581] 用筛网磨机或锥形磨使颗粒尺寸减小。

[0582] 干燥

[0583] 可将颗粒用真空炉、托盘烘干机、双锥形烘干机或流化床烘干机干燥。

[0584] 共混

[0585] 可将颗粒与颗粒外赋形剂共混。将颗粒用 300 升料箱共混机共混了 60 转。

[0586] 压制

[0587] 将压缩共混物用 Courtoy Modul P 旋转压片机压成片。

[0588] 包薄膜衣

[0589] 将片剂用包衣锅 (诸如 O' Hara Labcoat) 包薄膜衣。

[0590] 印刷

[0591] 通过例如 Hartnett Delta 印刷机将薄膜衣片在片剂的一个或两个表面上印上字母图案。

[0592] 给药方案

[0593] 在另一方面,本发明涉及治疗受试者中的 CFTR 介导的疾病的方法,包括向对其有需要的受试者施用有效量的本发明所提供的药物组合物。在另一个实施例中,将药物组合物以每两周一次的频率施用给受试者。在另一个实施例中,将药物组合物以每周一次的频率施用给受试者。在另一个实施例中,将药物组合物以每三天一次的频率施用给受试者。在

另一个实施例中，将药物组合物以每天一次的频率施用给受试者。在一个实施例中，当药物组合物为根据表 9、10、11、12、13、14、15、16、17、18 或 19 的片剂时，给药为一天一次。

[0594] 测定法

[0595] 用于检测和测量化合物的 F508del-CFTR 纠正特性的测定法

[0596] 用于测定化合物的 F508del-CFTR 调节特性的膜电位光学法。

[0597] 光学膜电位测定法采用 Gonzalez 和 Tsien 所述的电压敏感性 FRET 传感器（参见 Gonzalez, J. E. and R. Y. Tsien (1995) "Voltage sensing by fluorescence resonance energy transfer in single cells" Biophys J 69(4):1272-80 (Gonzalez, J. E. 和 R. Y. Tsien, 1995 年，"在单细胞中通过荧光共振能量转移感测电压"，《生物物理杂志》，第 69 期第 4 卷，第 1272-1280 页），以及 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 (Gonzalez, J. E. 和 R. Y. Tsien, 1997 年，"使用荧光共振能量转移的细胞膜电位的改进指标"，《化学与生物学》，第 4 期第 4 卷，第 269-277 页））连同测量荧光变化的仪器，诸如电压 / 离子探针读数器 (VIPR)（参见 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 (Gonzalez, J. E.、K. Oades 等人，1999 年，"基于细胞的测定法和筛选离子通道靶标的仪器"，《当今药物发现》，第 4 期第 9 卷，第 431-439 页））。

[0598] 这些电压敏感性测定法基于膜溶性、电压敏感性染料 $DiSBAC_2(3)$ 与荧光磷脂 CC2-DMPE 之间的荧光共振能量转移 (FRET) 的变化，所述荧光磷脂连接到质膜的外部小叶并充当 FRET 供体。膜电位 (V_m) 的变化导致带负电的 $DiSBAC_2(3)$ 跨越质膜重新分布，并且从 CC2-DMPE 转移的能量的量相应地改变。荧光发射的变化用 VIPR™II 加以监测，它是一种集成的液体处理器和荧光检测器，被设计用来在 96 或 384 孔微量滴定板中进行基于细胞的筛选。

[0599] 1. 纠正化合物的鉴定

[0600] 为了鉴定纠正与 F508del-CFTR 相关的运输缺陷的小分子，开发了单一添加 HTS 测定方式。在 37℃下将细胞在存在或不存在（阴性对照）供试化合物的情况下在不含血清的培养基中孵育 16 小时。作为阳性对照，将在 384 孔板中铺板的细胞在 27℃下孵育 16 小时至"温度 - 校正"F508del-CFTR。然后用 Krebs Ringers 溶液将细胞冲洗 3 次并加载电压敏感性染料。为了活化 F508del-CFTR，将 10 μM 福司可林和 CFTR 增效剂染料木黄酮 (20 μM) 与不含 Cl^- 的培养基加到各孔中。添加不含 Cl^- 的培养基促进了响应于 F508del-CFTR 活化的 Cl^- 外流，并使用基于 FRET 的电压传感器染料以光学方式监测所产生的膜去极化。

[0601] 2. 增效剂化合物的鉴定

[0602] 为了鉴定 F508del-CFTR 的增效剂，开发了双重添加 HTS 测定方式。在第一次添加过程中，在存在或不存在供试化合物的情况下向各孔中添加不含 Cl^- 的培养基。22 秒后，第二次添加不含 Cl^- 的含 2-10 μM 福司可林的培养基以活化 F508del-CFTR。两次添加后的胞外 Cl^- 浓度为 28mM，这促进了响应于 F508del-CFTR 活化的 Cl^- 外流，并使用基于 FRET 的电压传感器染料以光学方式监测所产生的膜去极化。

[0603] 3. 溶液

[0604] 浴液 #1 :(单位 mM)NaCl 160、KCl 4.5、$CaCl_2$2、$MgCl_2$1、HEPES10,用 NaOH 调至 pH 7.4。

[0605] 不含氯化物的浴液 :浴液 #1 中的氯化物盐被葡糖酸盐代替。

[0606] CC2-DMPE :制备成 10mM 的 DMSO 储备溶液并储存在 -20℃下。

[0607] $DiSBAC_2(3)$:制备成 10mM 的 DMSO 储备溶液并储存在 -20℃下。

[0608] 4. 细胞培养

[0609] 将稳定表达 F508del-CFTR 的 NIH3T3 小鼠成纤维细胞用于膜电位的光学测量。将细胞维持在 37℃、5% CO_2 和 90%湿度下的 175cm^2 培养烧瓶中的达尔伯克氏改良伊格尔培养基中,该培养基补充了 2mM 谷氨酰胺、10%胎牛血清、1X NEAA、β -ME、1X 青霉素 / 链霉素和 25mM HEPES。对于所有光学测定,均以 30,000 个 / 孔将细胞接种在 384 孔基质胶包被的板上并在 37℃下培养 2h,然后在 27℃下培养 24h,以用于增效剂测定。对于纠正测定,将细胞在 27℃或 37℃下与和不与化合物一起培养 16-24 小时。

[0610] 用于测定化合物的 F508del-CFTR 调节性质的电生理测定法

[0611] 1.Using 室测定

[0612] 对表达 F508del-CFTR 的极化上皮细胞进行 Using 室实验,以进一步表征在光学测定法中鉴定的 F508del-CFTR 调节剂。将生长在 Costar Snapwell 细胞培养插管上的 $FRT^{\Delta F508-CFTR}$ 上皮细胞固定在 Ussing 室内 (加利福利亚州圣迭戈生理仪器公司 (Physiologic Instruments, Inc., San Diego, CA)),并利用电压钳系统 (爱荷华州爱荷华大学生物工程系 (Department of Bioengineering, University of Iowa, IA) 和加利福利亚州圣迭戈生理仪器公司) 连续使单层短路。通过施加 2mV 脉冲测量跨上皮电阻。在这些条件下,FRT 上皮表现出 4KΩ/cm^2 或更高的电阻。将溶液维持在 27℃下,并鼓入空气。利用无细胞插管校正电极偏移电位和流体电阻。在这些条件下,电流反映 Cl^- 通过在顶端膜中所表达的 F508del-CFTR 的流动。利用 MP100A-CE 界面和 AcqKnowledge 软件 (v3.2.6 ;加利福尼亚州圣巴巴拉 BIOPAC 系统公司 (BIOPAC Systems, Santa Barbara, CA)) 以数字方式获取 I_{SC}。

[0613] 2. 纠正化合物的鉴定

[0614] 典型的方案采用基底外侧至顶端膜的 Cl^- 浓度梯度。为了建立这种梯度,在基底外侧膜上使用正常的套环,而顶端 NaCl 被等摩尔葡糖酸钠代替 (用 NaOH 滴定至 pH7.4),以得到跨上皮的大 Cl^- 浓度梯度。所有实验均用完整单层进行。为了完全活化 F508del-CFTR,施加福司可林 (10μM) 和 PDE 抑制剂 IBMX(100μM),然后添加 CFTR 增效剂染料木黄酮 (50μM)。

[0615] 正如在其他细胞类型中所观察到的,在低温下孵育稳定表达 F508del-CFTR 的 FRT 细胞会增加 CFTR 在质膜中的功能密度。为了测定纠正化合物的活性,将细胞与 10μM 供试化合物在 37℃下孵育 24 小时,随后洗涤 3 次,然后记录。将 cAMP- 和染料木黄酮 - 介导的化合物处理细胞中的 I_{SC} 归一化到 27℃和 37℃对照,并以百分比活性表示。与 37℃对照相比,细胞用纠正化合物预孵育显著增加了 cAMP- 和染料木黄酮 - 介导的 I_{SC}。

[0616] 3. 增效剂化合物的鉴定

[0617] 典型的方案采用基底外侧至顶端膜的 Cl^- 浓度梯度。为了建立这种梯度,在基底外侧膜上使用正常的套环并用制霉菌素 (360μg/mL) 透化,而顶端 NaCl 被等摩尔葡糖酸钠

代替（用 NaOH 滴定至 pH7.4），以得到跨越上皮的大 Cl^- 浓度梯度。所有实验均在制霉菌素透化后 30min 进行。向细胞培养插管两侧施加福司可林（10μM）和所有供试化合物。将推定的 F508del-CFTR 增效剂的功效与已知增效剂染料木黄酮的功效进行比较。

[0618] 4. 溶液

[0619] 基底外侧溶液（单位 mM）：NaCl(135)、$CaCl_2$(1.2)、$MgCl_2$(1.2)、K_2HPO_4(2.4)、$KHPO_4$(0.6)、N-2- 羟基乙基哌嗪 -N'-2- 乙磺酸（HEPES）(10) 和右旋糖（10）。用 NaOH 将该溶液滴定至 pH7.4。

[0620] 顶端溶液（单位 mM）：与基底外侧溶液相同，但 NaCl 被葡糖酸钠（135）替代。

[0621] 5. 细胞培养

[0622] 将表达 F508del-CFTR($FRT^{\Delta F508\text{-}CFTR}$) 的 Fisher 大鼠上皮（FRT）细胞用于从我们的光学测定法鉴定的推定 F508del-CFTR 调节剂的 Ussing 室实验。在 Costar Snapwell 细胞培养插管上培养细胞并在 37℃和 5% CO_2 下在 Coon's 改良 Ham's F-12 培养基中培养五天，该培养基补充了 5% 胎牛血清、100U/mL 青霉素和 100μg/mL 链霉素。在用于表征化合物的增效剂活性前，将细胞在 27℃孵育 16-48h 以纠正 F508del-CFTR。为了测定纠正化合物的活性，将细胞在 27℃或 37℃与和不与化合物一起孵育 24 小时。

[0623] 6. 全细胞记录

[0624] 使用穿孔的膜片全细胞记录监测温度和供试化合物纠正的稳定表达 F508del-CFTR 的 NIH3T3 细胞中的宏观 F508del-CFTR 电流（$I_{\Delta F508}$）。简而言之，在室温下用 Axopatch 200B 膜片钳放大器（加利福利亚州福斯特城 Axon 仪器公司 (Axon Instruments Inc.,Foster City,CA)）进行 $I_{\Delta F508}$ 的电压钳记录。全部记录均在 10kHz 采样频率下获取并在 1kHz 下低通过滤。微电极在充满胞内溶液时具有 5-6MΩ 的电阻。在这些记录条件下，在室温下计算的 Cl^- 逆转电位（E_{Cl}）为 -28mV。全部记录均具有 >20GΩ 的密封电阻和 <15MΩ 的串联电阻。使用配备了 Digidata 1320A/D 界面与 Clampex 8(Axon 仪器公司）的 PC 进行脉冲发生、数据采集和分析。浴包含 <250μL 的盐水并使用重力驱动的灌注系统以 2mL/min 的速率连续灌注。

[0625] 7. 纠正化合物的鉴定

[0626] 为了测定纠正化合物增加质膜中功能性 F508del-CFTR 密度的活性，我们利用上述开孔膜片记录技术测量用纠正化合物处理 24 小时后的电流密度。为了完全活化 F508del-CFTR，向细胞加入 10μM 福司可林和 20μM 染料木黄酮。在我们的记录条件下，在 27℃下孵育 24 小时后的电流密度高于在 37℃下孵育 24 小时后的观测值。这些结果与低温孵育对质膜中 F508del-CFTR 密度的已知影响是一致的。为了测定纠正化合物对 CFTR 电流密度的影响，将细胞与 10μM 供试化合物在 37℃下孵育 24 小时，与 27℃和 37℃对照比较电流密度（活性%）。在记录之前，将细胞用细胞外记录介质洗涤 3 次，以除去任何剩余的供试化合物。与 37℃对照相比，用 10μM 纠正化合物预孵育显著增加了 cAMP- 和染料木黄酮 - 依赖性电流。

[0627] 8. 增效剂化合物的鉴定

[0628] 还用穿孔膜片记录技术研究了 F508del-CFTR 增效剂增加稳定表达 F508del-CFTR 的 NIH3T3 细胞中宏观 F508del-CFTR Cl^- 电流（$I_{\Delta F508}$）的能力。从光学测定法中鉴定的增效剂引起了与在光学测定法中观察到的类似效力和功效的 $I_{\Delta F508}$ 剂量依赖性增加。在所有

研究的细胞中，施加增效剂之前和过程中的逆转电位为约 -30mV，它是计算的 E_{Cl}(-28mV)。

[0629] 9. 溶液

[0630] 胞内溶液（单位 mM）：天冬氨酸铯 (90)、CsCl(50)、$MgCl_2$(1)、HEPES(10) 和 240 μg/mL 两性霉素 -B（用 CsOH 将 pH 调至 7.35）。

[0631] 胞外溶液（单位 mM）：N- 甲基 -D- 葡糖胺 (NMDG)-Cl(150)、$MgCl_2$(2)、$CaCl_2$(2)、HEPES(10)（用 HCl 将 pH 调至 7.35）。

[0632] 10. 细胞培养

[0633] 将稳定表达 F508del-CFTR 的 NIH3T3 小鼠成纤维细胞用于全细胞记录。将细胞维持在 37℃、5% CO_2 和 90% 湿度下的 175cm^2 培养烧瓶中的达尔伯克氏改良伊格尔培养基中，该培养基补充了 2mM 谷氨酰胺、10% 胎牛血清、1X NEAA、β -ME、1X 青霉素 / 链霉素和 25mM HEPES。对于全细胞记录，将 2,500-5,000 个细胞接种在聚 -L- 赖氨酸包被的盖玻片上并在 27℃下培养 24-48h，然后用于测试增效剂的活性；并且与或不与纠正化合物一起在 37℃孵育，以便测量纠正剂的活性。

[0634] 11. 单通道记录

[0635] 使用切下的膜内侧翻外膜片观察在 NIH3T3 细胞中稳定表达的温度校正的 F508del-CFTR 的单通道活性以及增效剂化合物的活性。简而言之，在室温下用 Axopatch 200B 膜片钳放大器 (Axon 仪器公司）进行单通道活性的电压钳记录。全部记录均在 10kHz 采样频率下获取并在 400Hz 下低通过滤。膜片微电极由 Corning Kovar Sealing#7052 玻璃（佛罗里达州萨拉索塔世界精密仪器公司 (World Precision Instruments, Inc., Sarasota, FL)）制成并在充满胞外溶液时具有 5-8MΩ 的电阻。在切下后通过添加 1mM Mg-ATP 和 75nM cAMP 依赖性蛋白激酶催化亚单位 (PKA；威斯康星州麦迪逊普洛麦格公司 (Promega Corp. Madison, WI)) 活化了 F508del-CFTR。在通道活性稳定后，使用重力驱动的微量灌注系统灌注膜片。使内流接近膜片，从而导致在 1-2s 内完全交换溶液。为了维持快速灌注过程中的 F508del-CFTR 活性，将非特异性磷酸酶抑制剂 F^-(10mM NaF) 加入浴液中。在这些记录条件下，通道活性在整个膜片记录期间保持恒定（至多 60min）。从胞内溶液运动至胞外溶液（阴离子以相反方向运动）的正电荷所产生的电流显示为正电流。将微电极电位 (V_p) 维持在 80mV。

[0636] 从包括≤ 2 个活性通道的膜片分析通道活性。同时开放的最大值决定了实验过程中活性通道的数量。为了测定单通道电流振幅，在 100Hz 下“离线”过滤从 120s 的 F508del-CFTR 活性记录的数据，然后用于构建所有点的振幅直方图，该直方图用 Bio-Patch 分析软件（法国比奥罗杰公司 (Bio-Logic Comp. France)）通过多高斯函数拟合。通过 120s 的通道活性确定总微观电流和开放概率 (P_o)。使用 Bio-Patch 软件或由关系式 $P_o = I/i(N)$ 测定 P_o，其中 I = 平均电流，i = 单通道电流振幅，并且 N = 膜片中活性通道的数量。

[0637] 12. 溶液

[0638] 胞外溶液（单位 mM）：NMDG(150)、天冬氨酸 (150)、$CaCl_2$(5)、$MgCl_2$(2) 和 HEPES(10)（用 Tris 碱将 pH 调至 7.35）。

[0639] 胞内溶液（单位 mM）：NMDG-Cl(150)、$MgCl_2$(2)、EGTA(5)、TES(10) 和 Tris 碱 (14)（用 HCl 将 pH 调至 7.35）。

[0640] 13. 细胞培养

[0641] 将稳定表达 F508del-CFTR 的 NIH3T3 小鼠成纤维细胞用于切下膜的膜片钳记录。将细胞维持在 37℃、5% CO_2 和 90%湿度下的 175cm^2 培养烧瓶中的达尔伯克氏改良伊格尔培养基中，该培养基补充了 2mM 谷氨酰胺、10%胎牛血清、1X NEAA、β -ME、1X 青霉素 / 链霉素和 25mM HEPES。对于单通道记录，将 2,500-5,000 个细胞接种在聚 -L- 赖氨酸包被的盖玻片上并在 27℃下培养 24-48h 后使用。

[0642] 用上述程序测量了化合物 1 的活性（即 EC50）并且如表 20 所示。

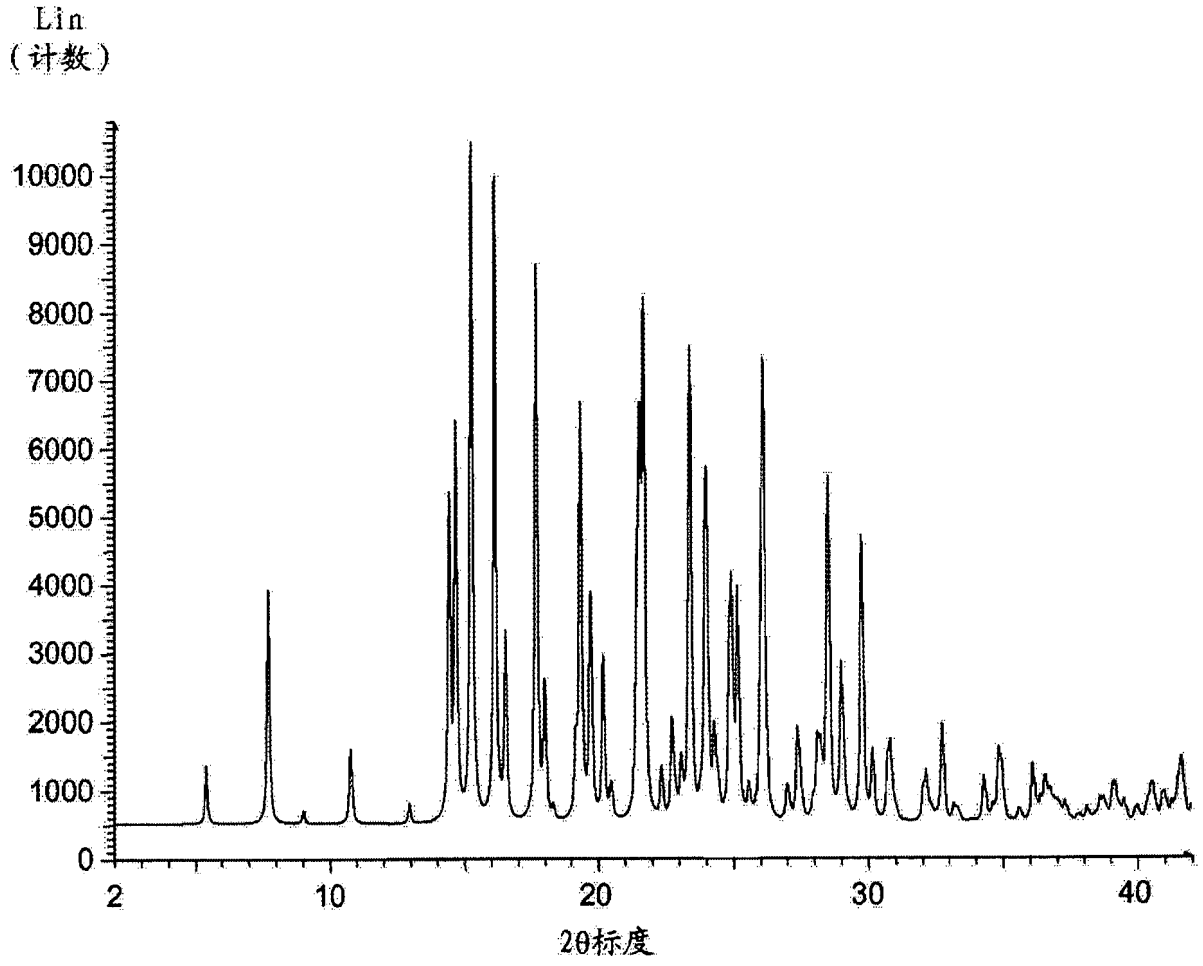
[0643] 表 20。

[0644]

IC50/EC50 Bin: +++ <= 2.0 < ++ <= 5.0 < +		
百分比活性 Bin: + <= 25.0 < ++ <= 100.0 < +++		
化合物编号	BinnedEC50	Binned 最大功效
1	+++	+++

[0645] 其他实施例

[0646] 本公开中提及的所有出版物和专利均以引用方式并入本文，达到如同各个出版物或专利申请具体且单独地表明以引用方式并入的相同程度。以引用方式并入的任何专利或出版物中的术语的含义与本公开中所用的术语的含义冲突时，旨在以本公开中的术语的含义为准。此外，前述讨论公开和描述的仅仅是本发明的示例性实施例。本领域的技术人员将容易从这些讨论中以及从附图和权利要求中认识到：在不背离以下权利要求书中所定义的本发明的精神和范围的情况下可以做出各种变化、修改和变更。


Lin
(计数)
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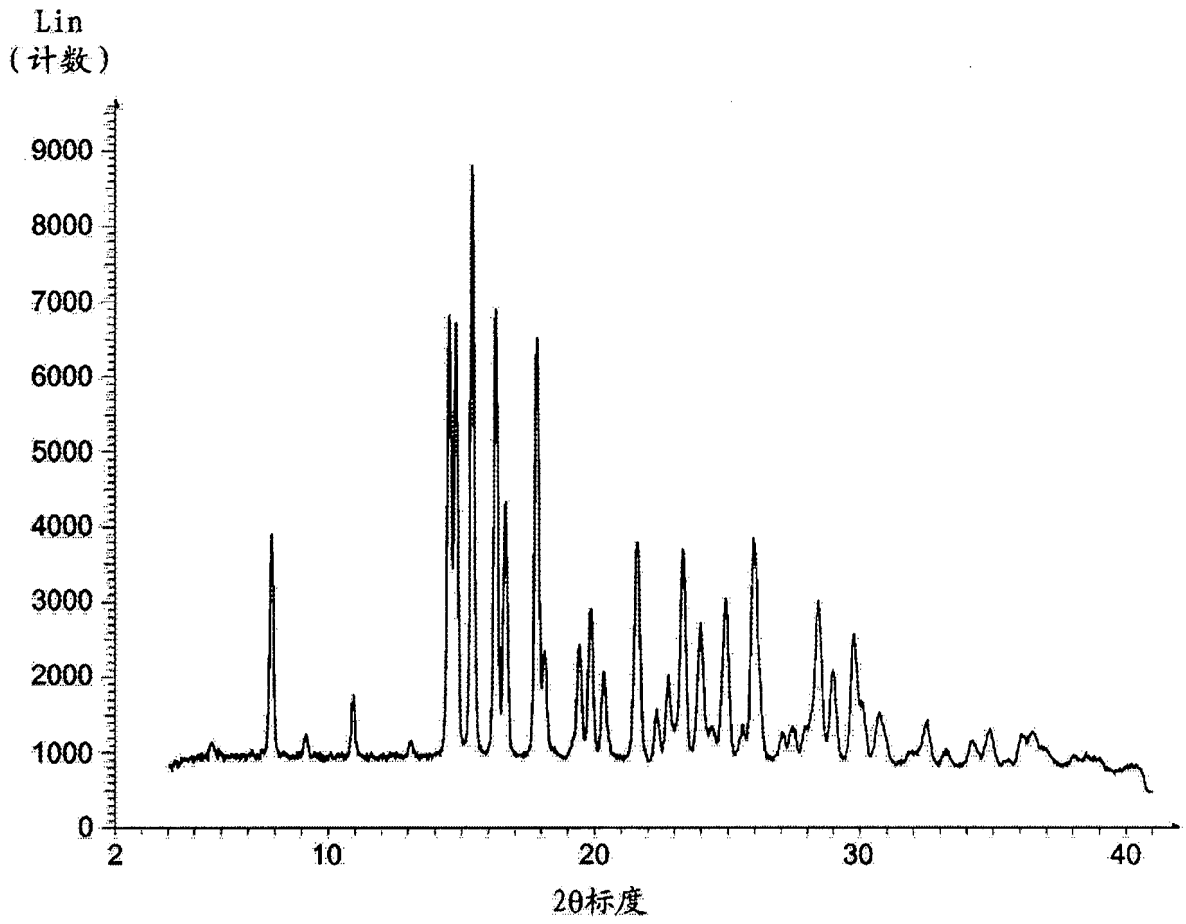
8000
7000
6000
5000
4000
3000
2000
1000
0
2
10
20
30
40
2θ标度

图 1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(计数)
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7000
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5000
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3000
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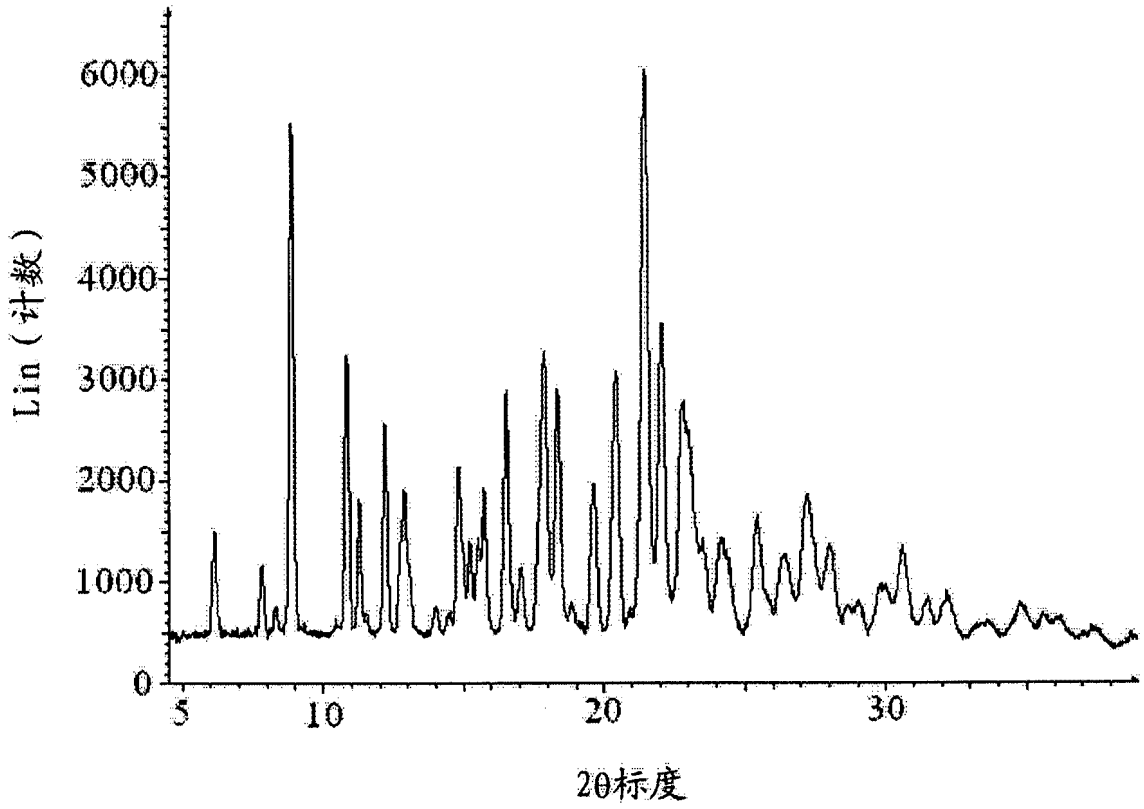
1000
0
2
10
20
30
40
2θ标度

图 2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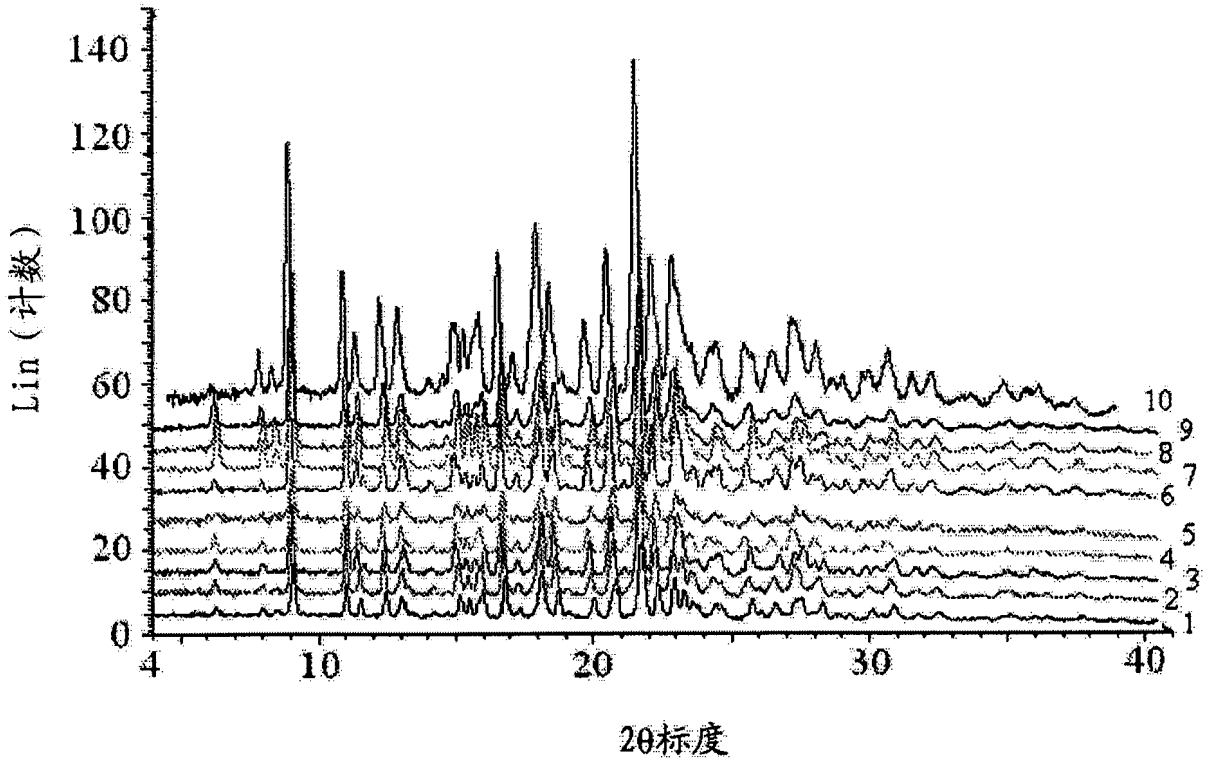
5000
4000
3000
2000
1000
0
Lin（计数）
5
10
20
30
2θ标度

图 3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120
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80
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40
20
0
Lin（计数）
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20
30
40
2θ标度
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8
7
6
5
4
3
2
1

图 4

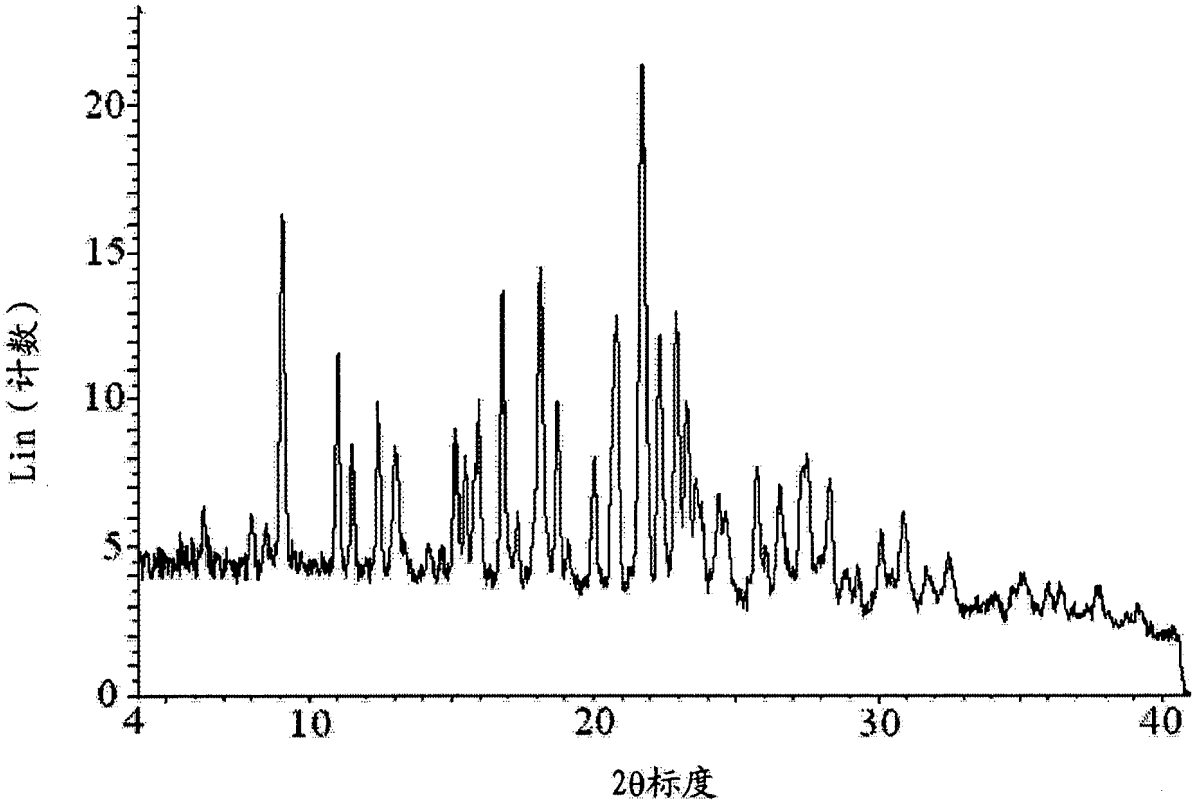

图 5

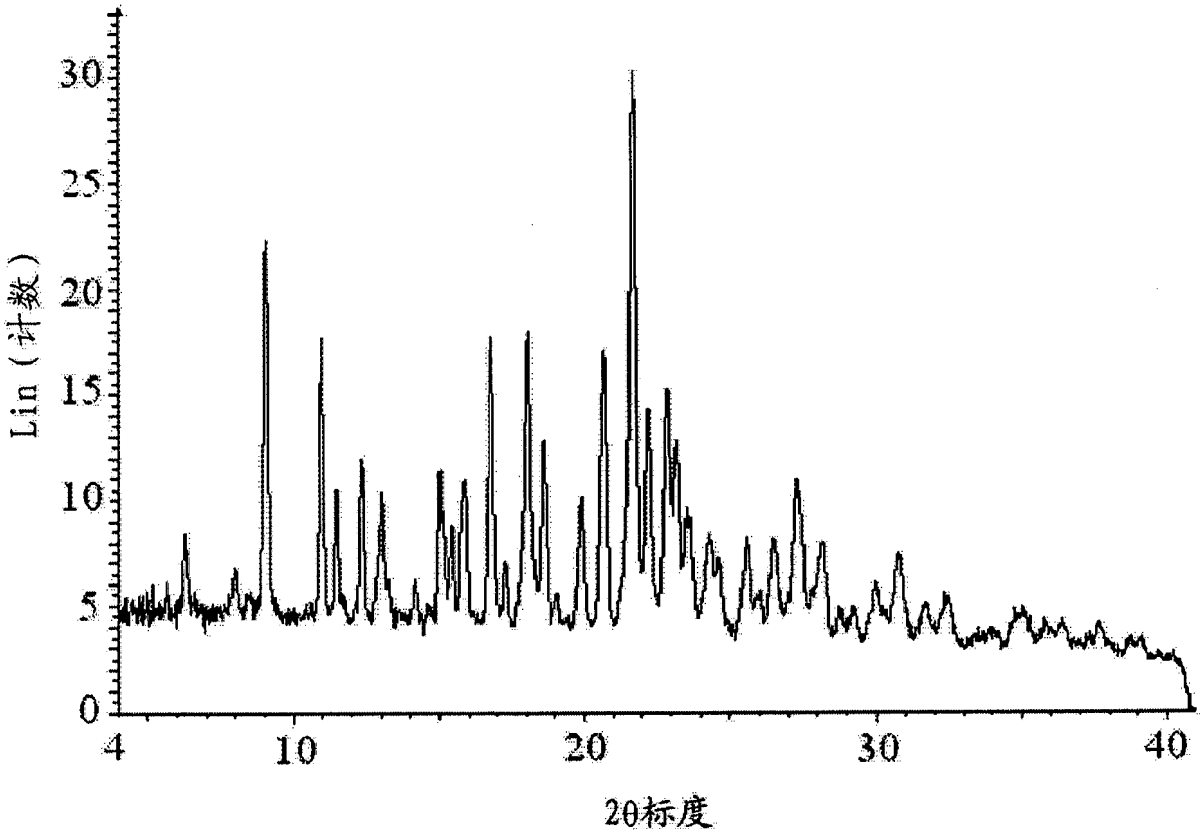

图 6

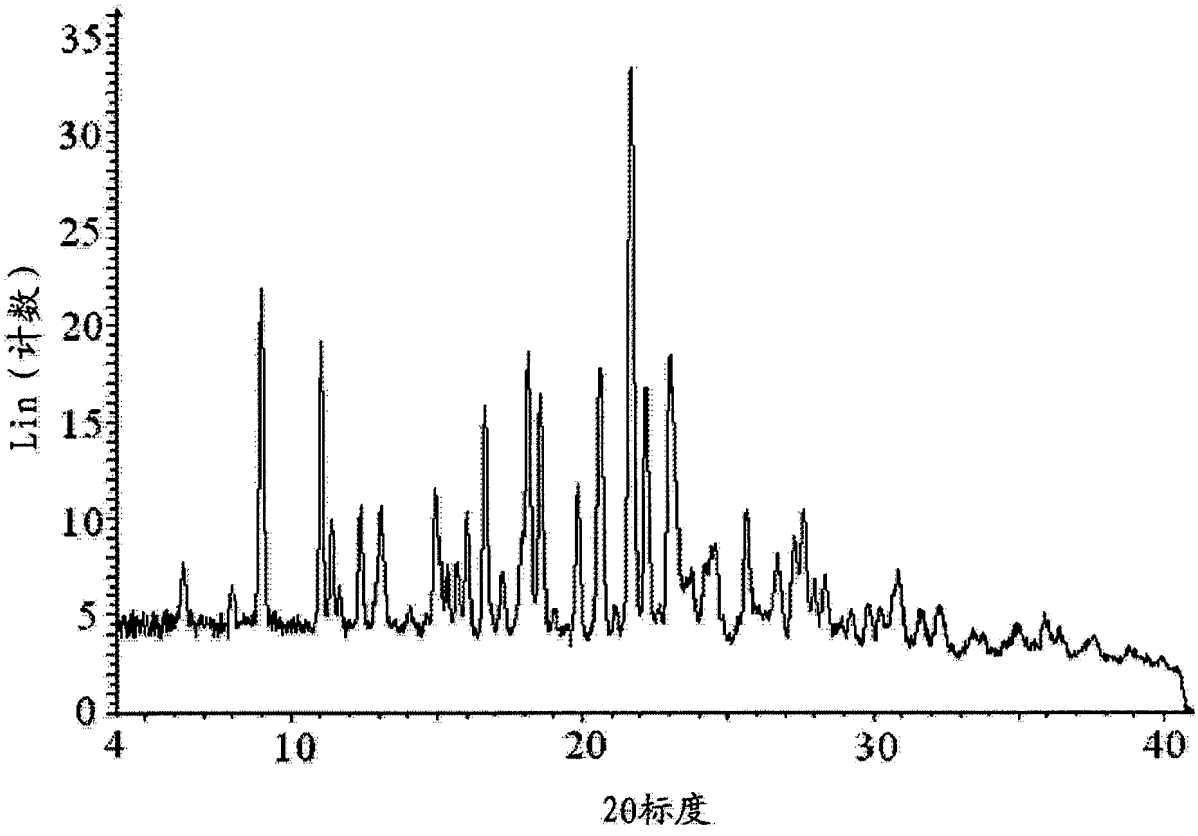

图 7

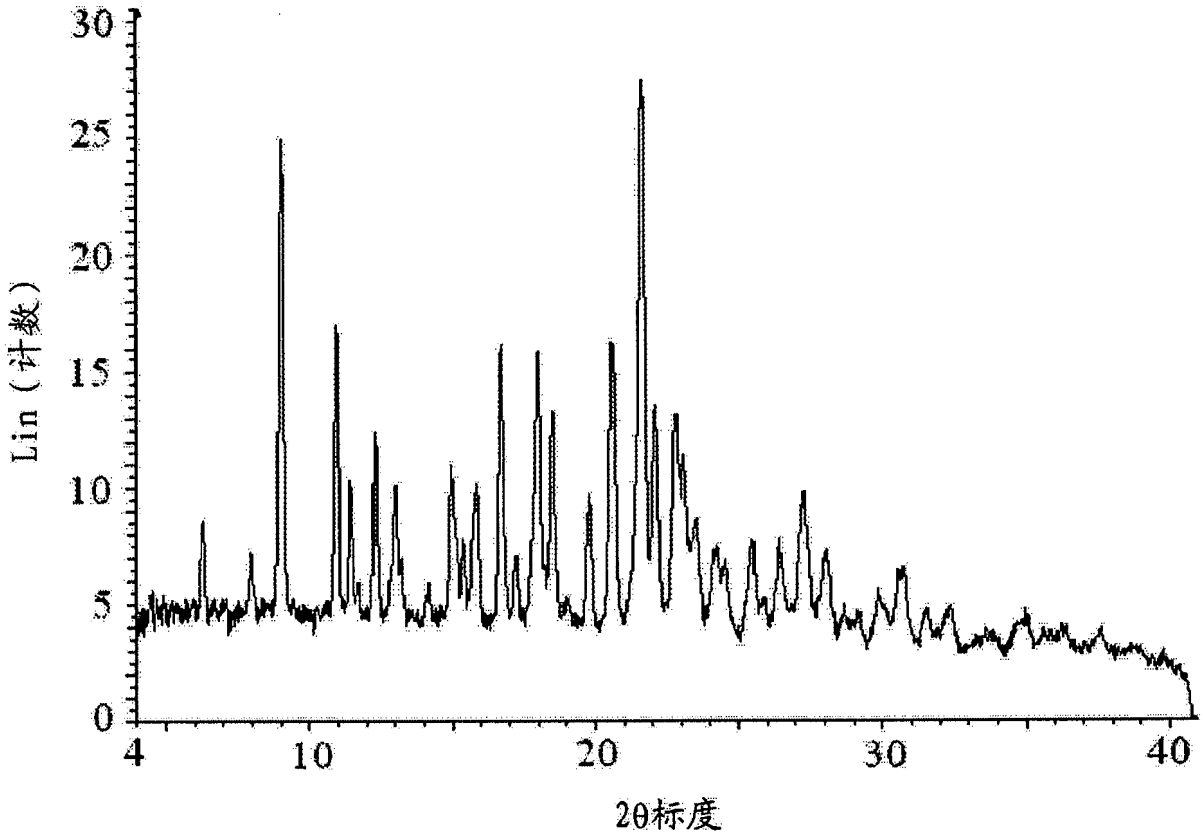

图 8

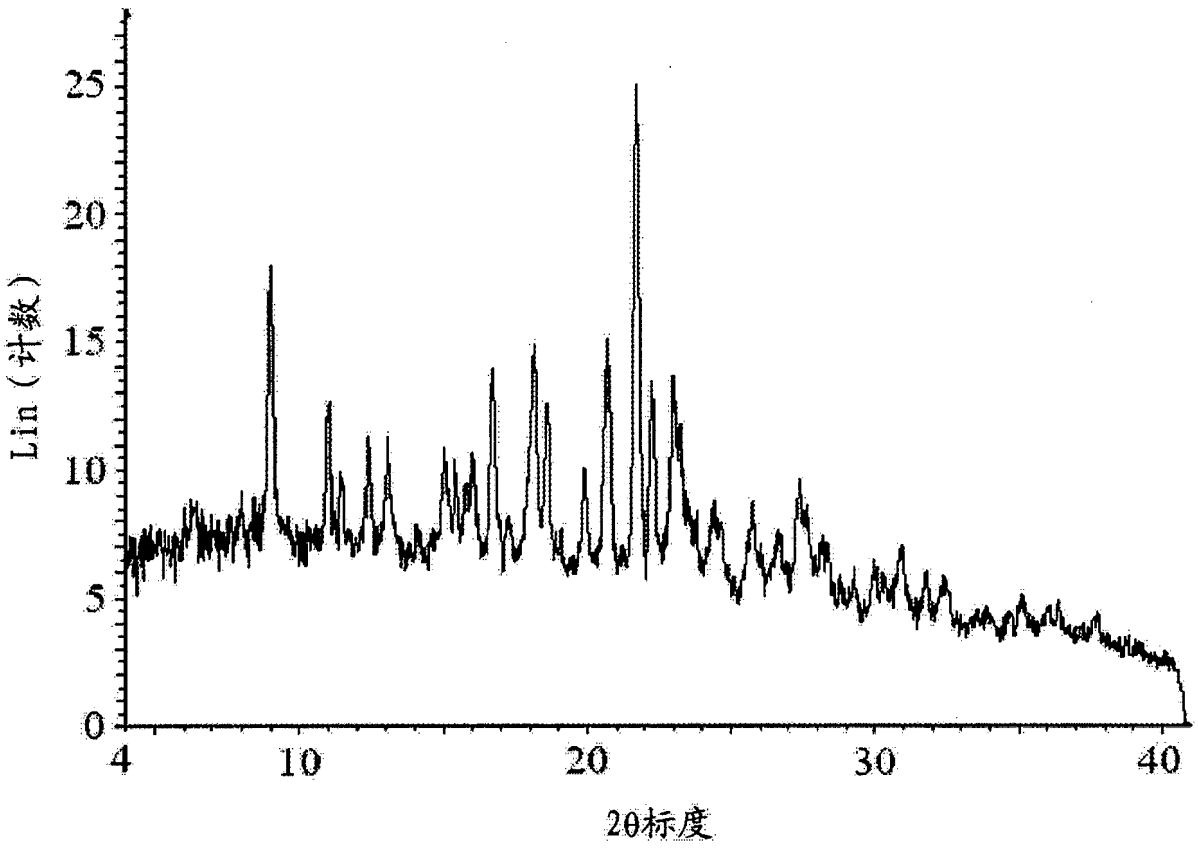

图 9

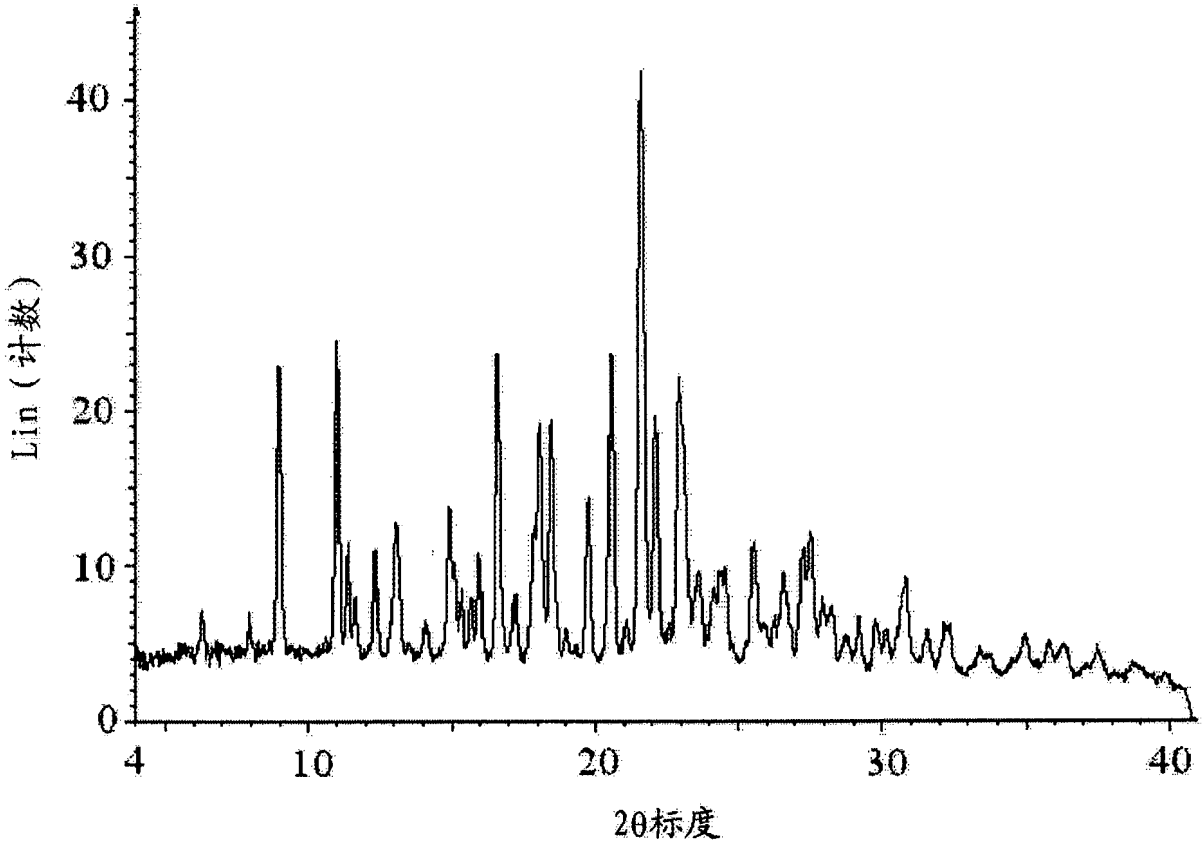

图 10

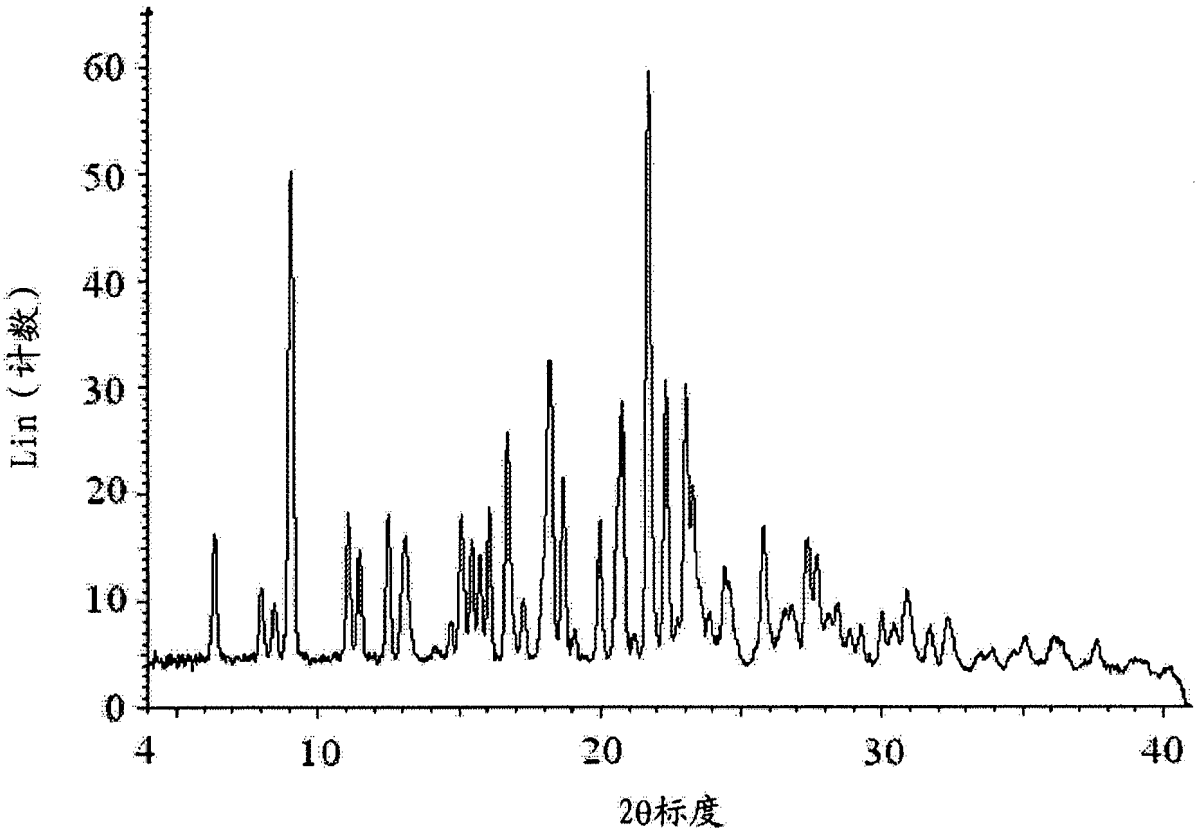

图 11

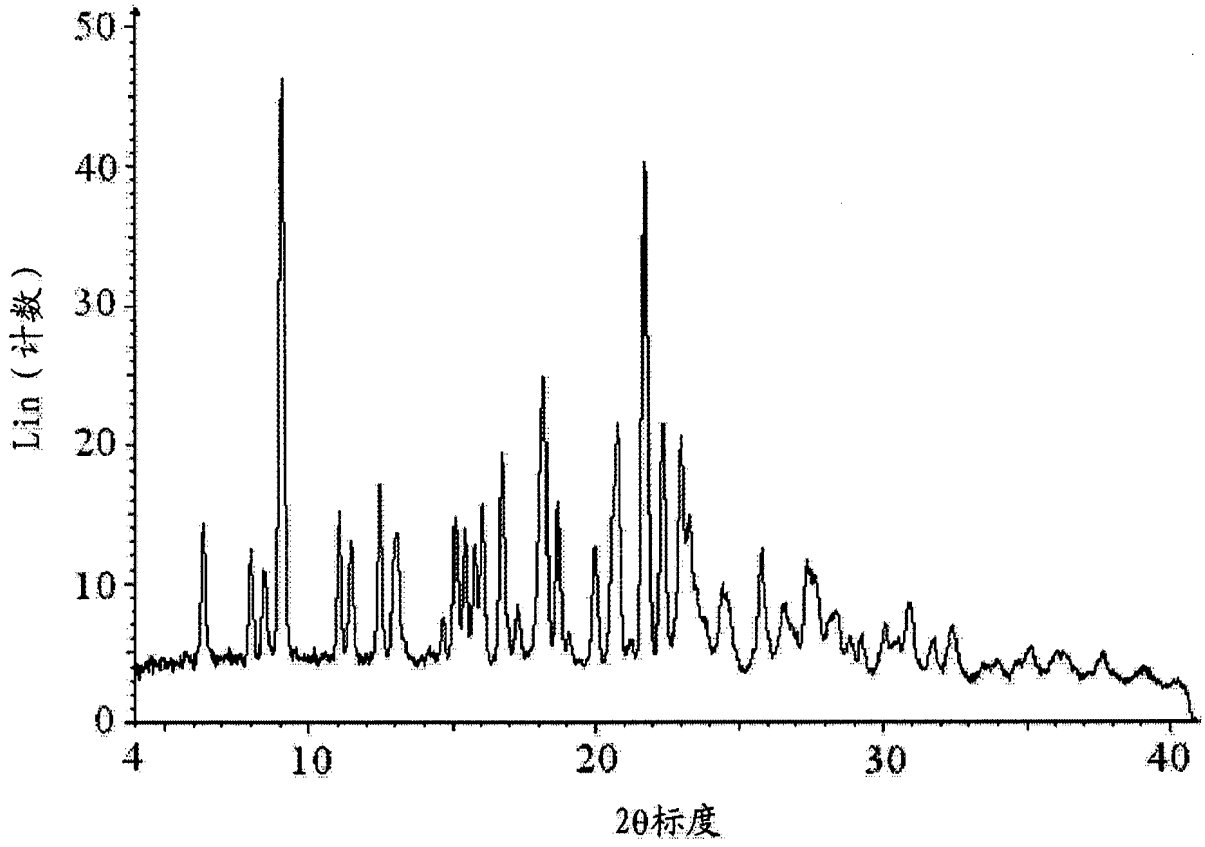

图 12

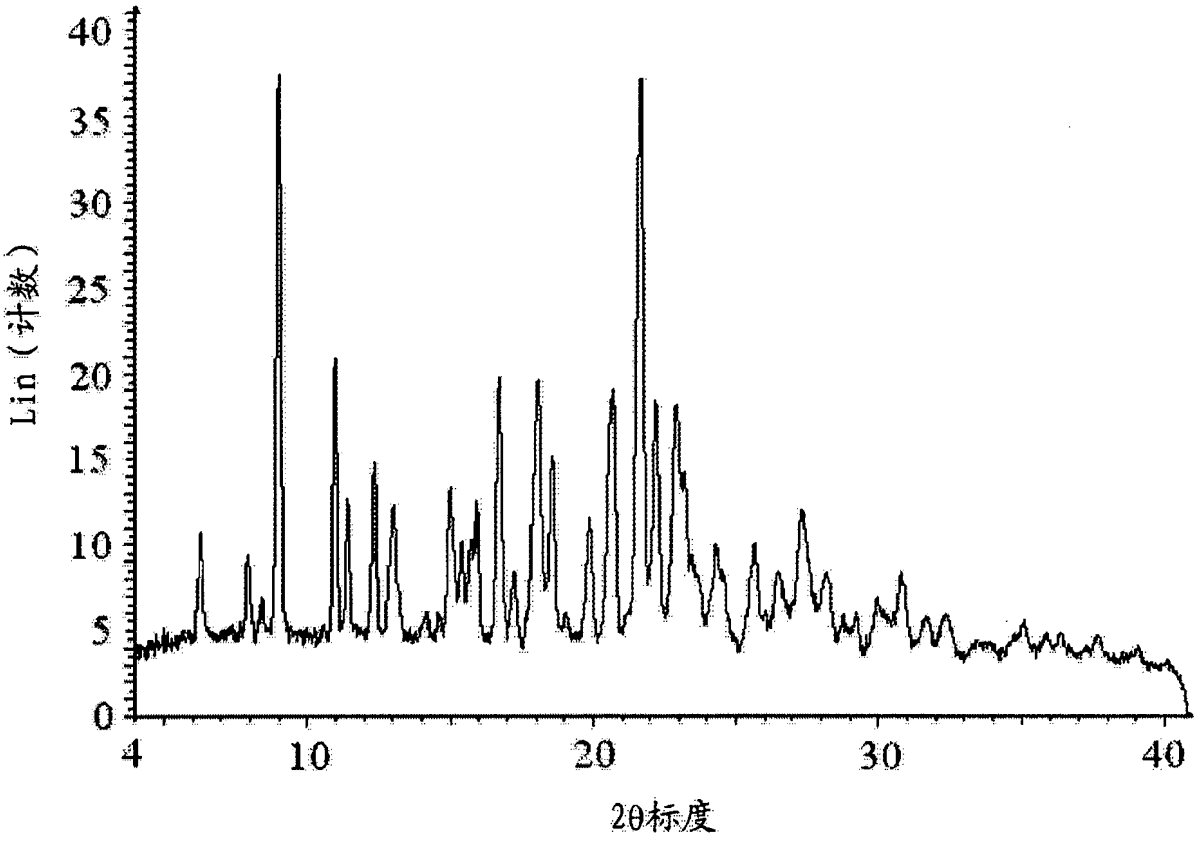

图 13

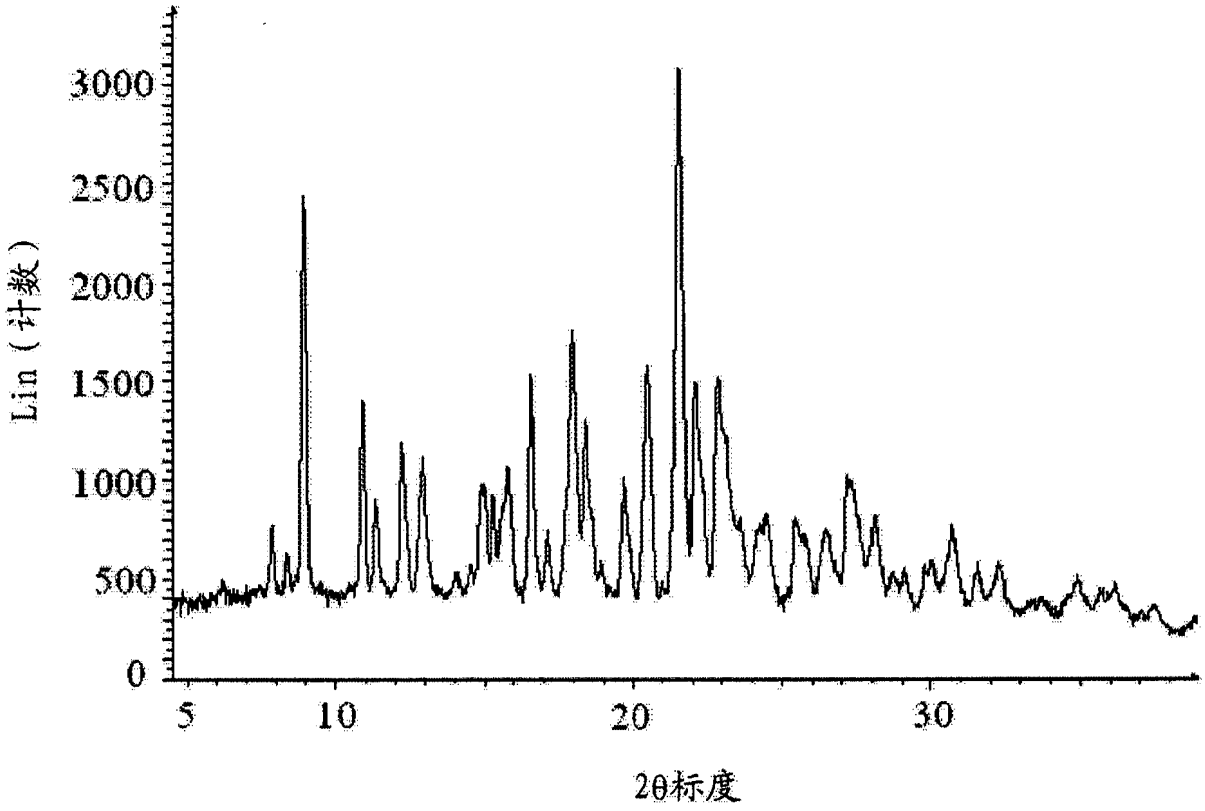

图 14

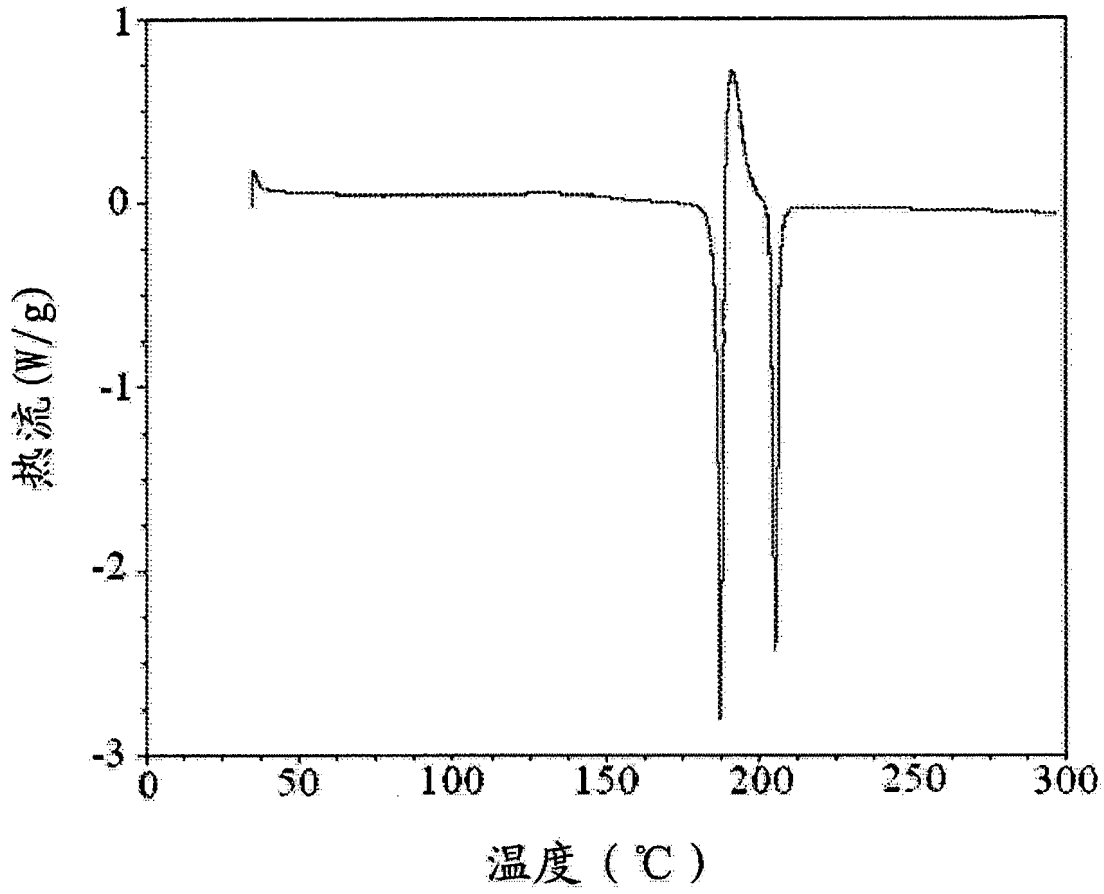

图 15

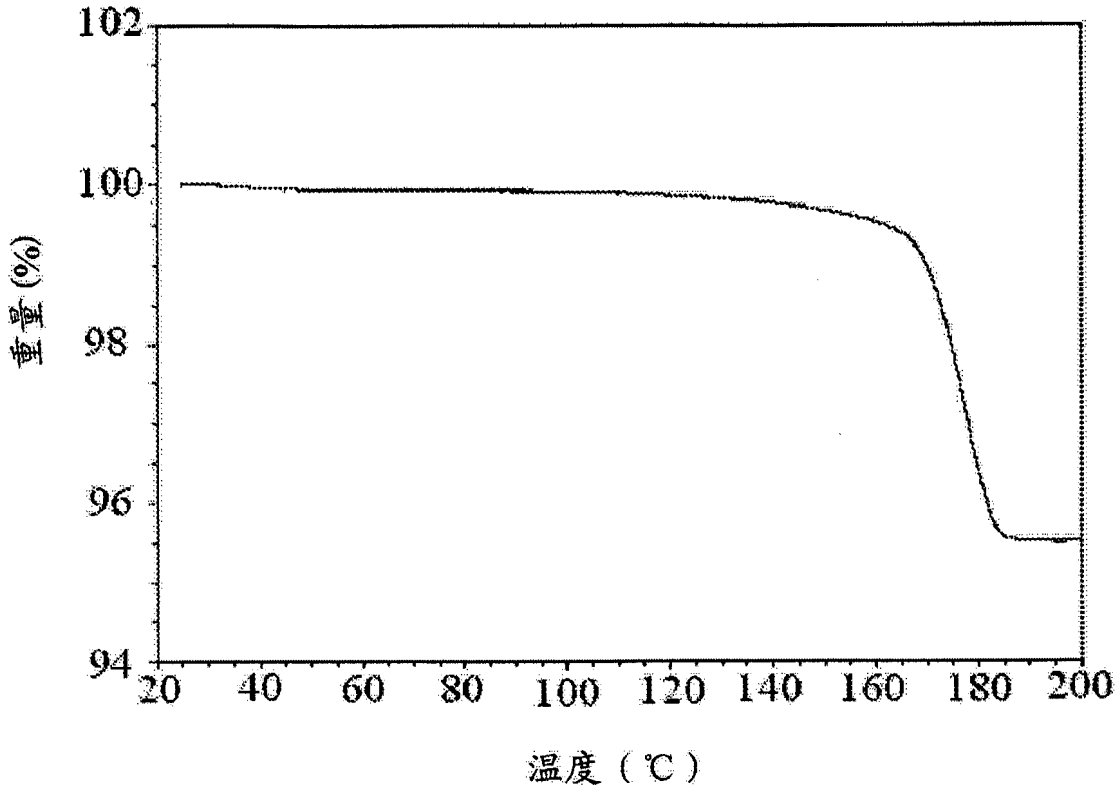

图 16

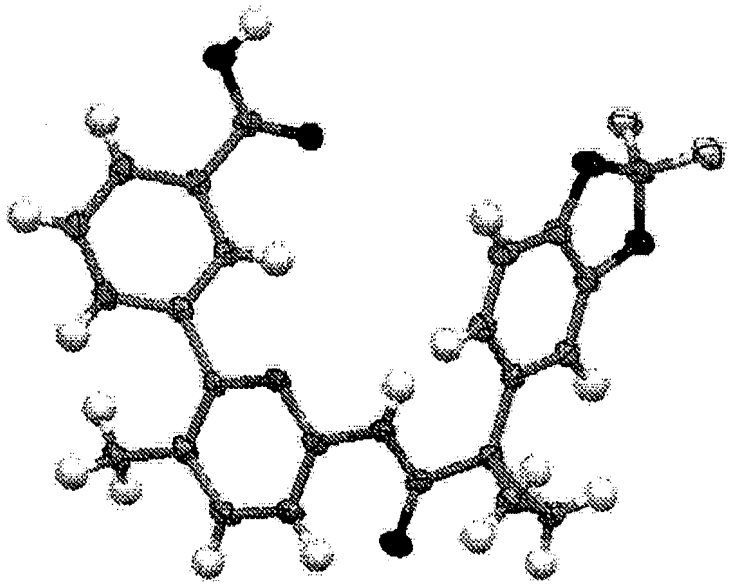

图 17

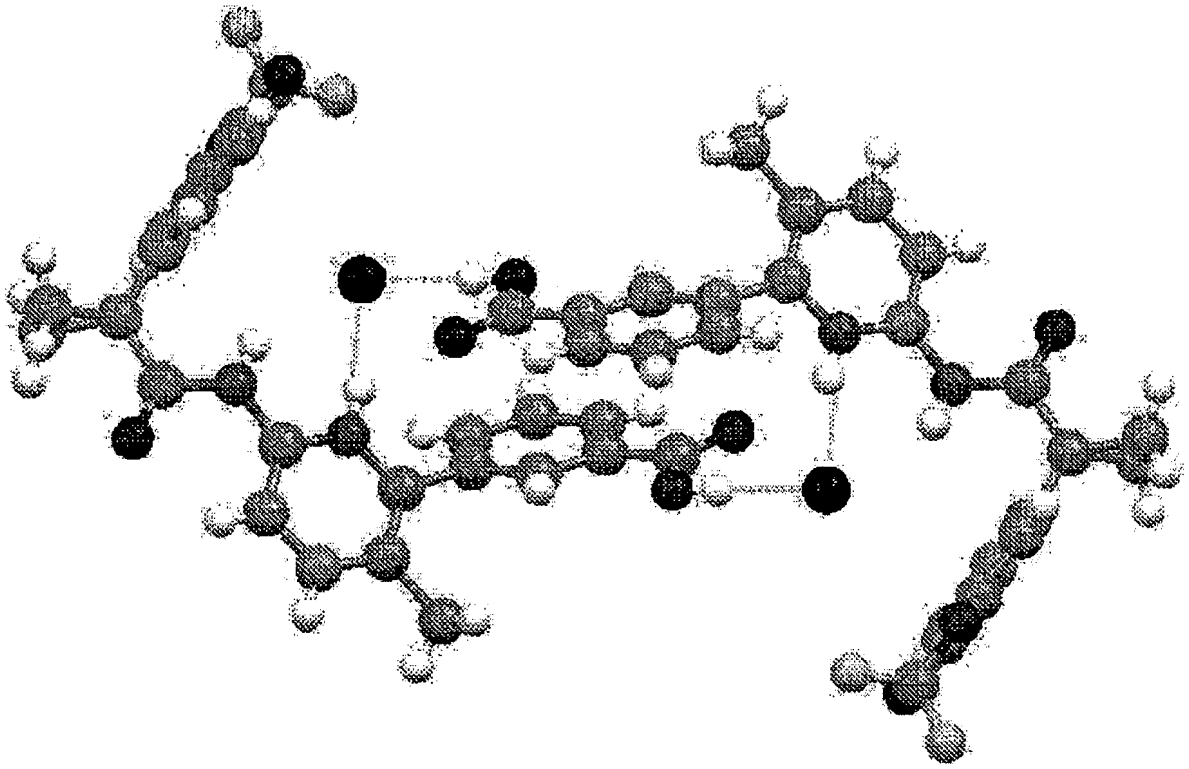

图 18

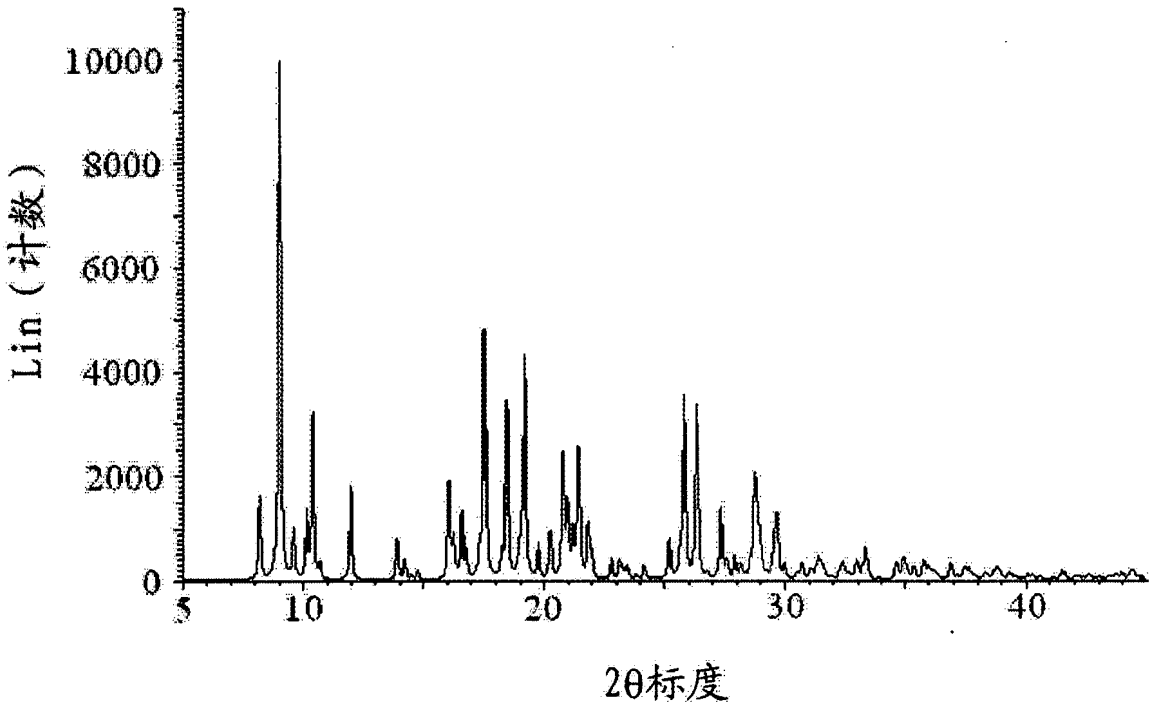

图 19

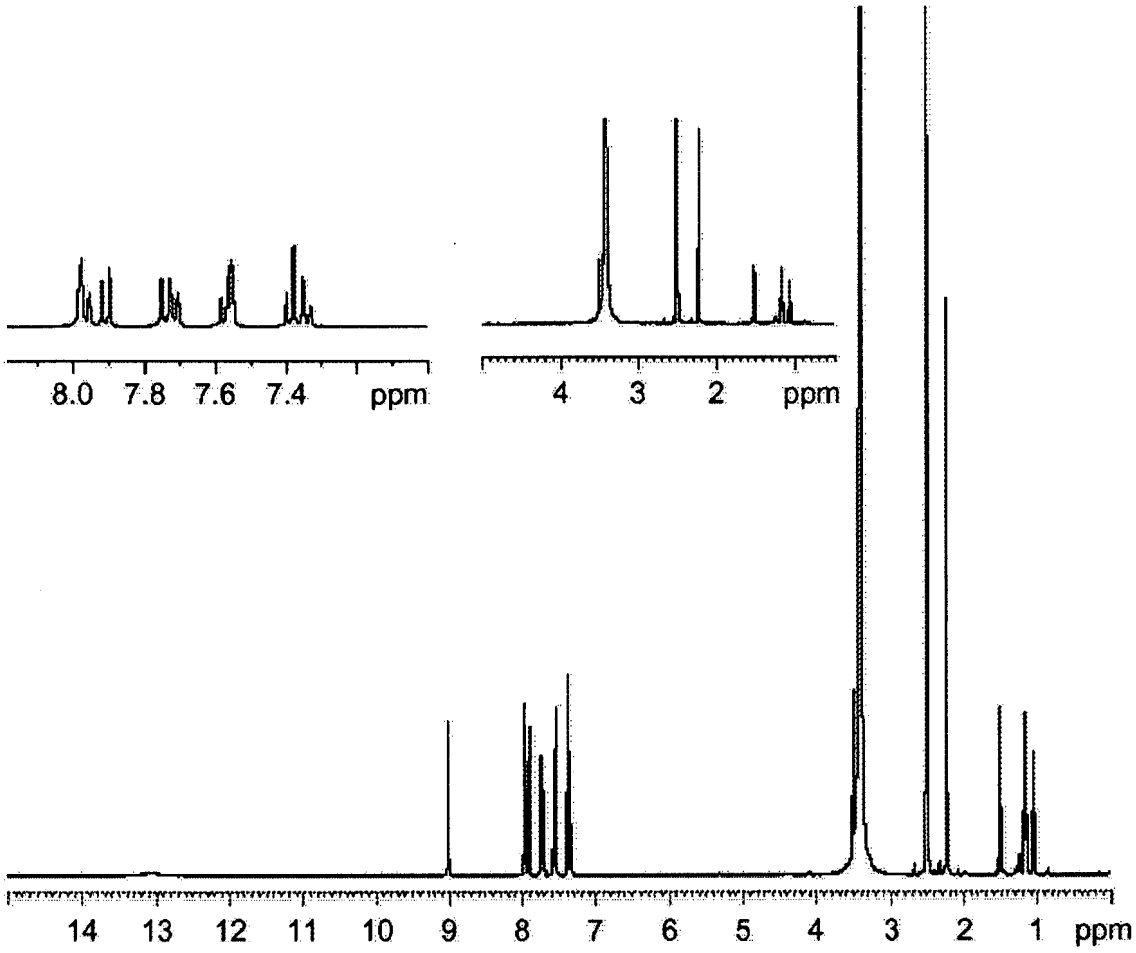

图 20

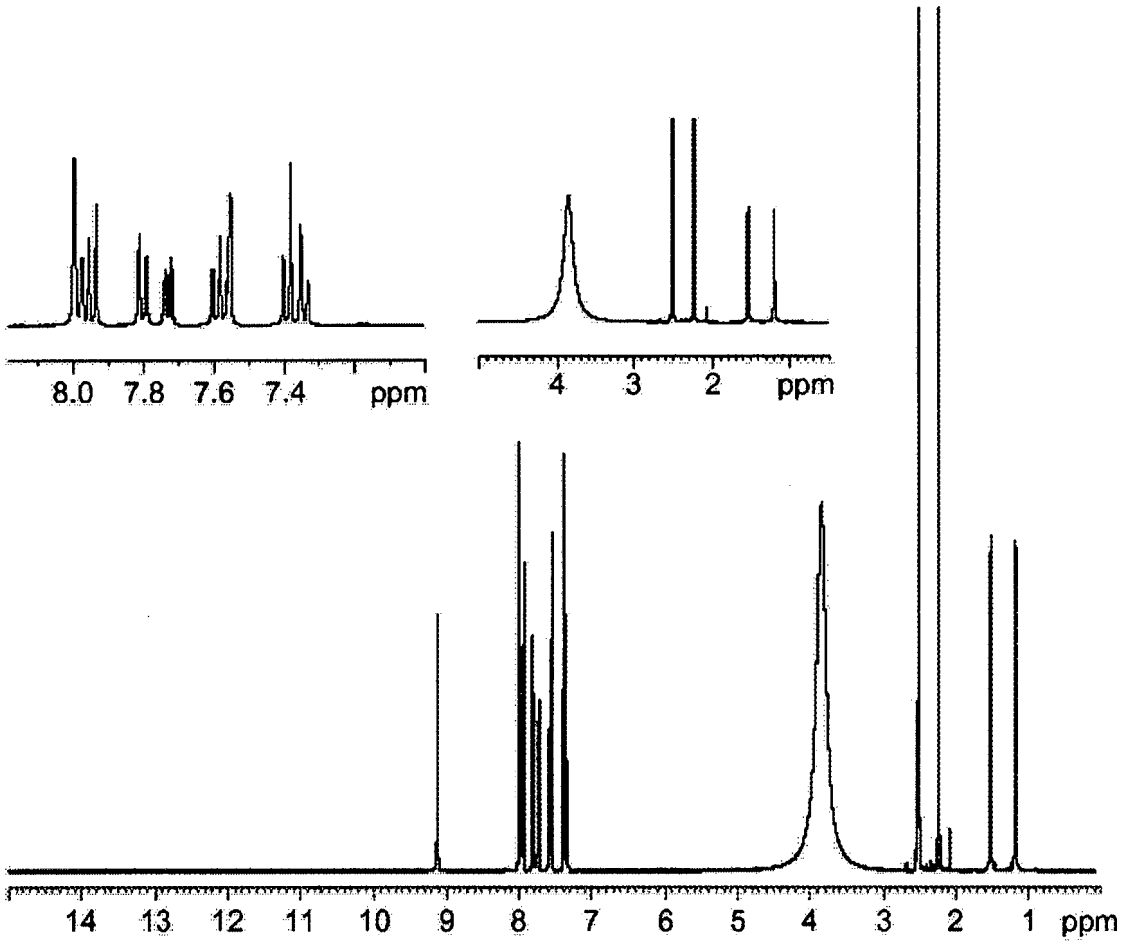

图 21

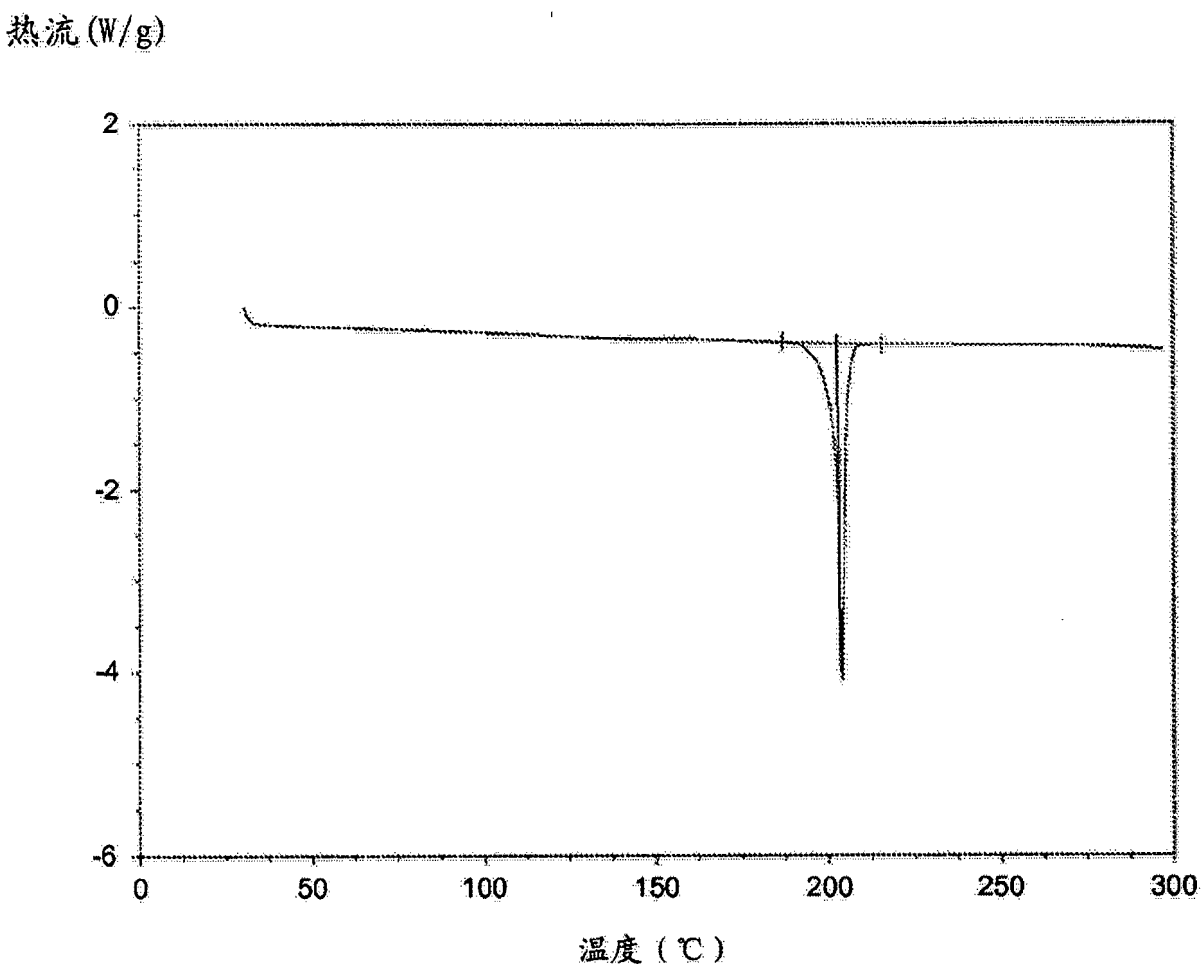

图 22

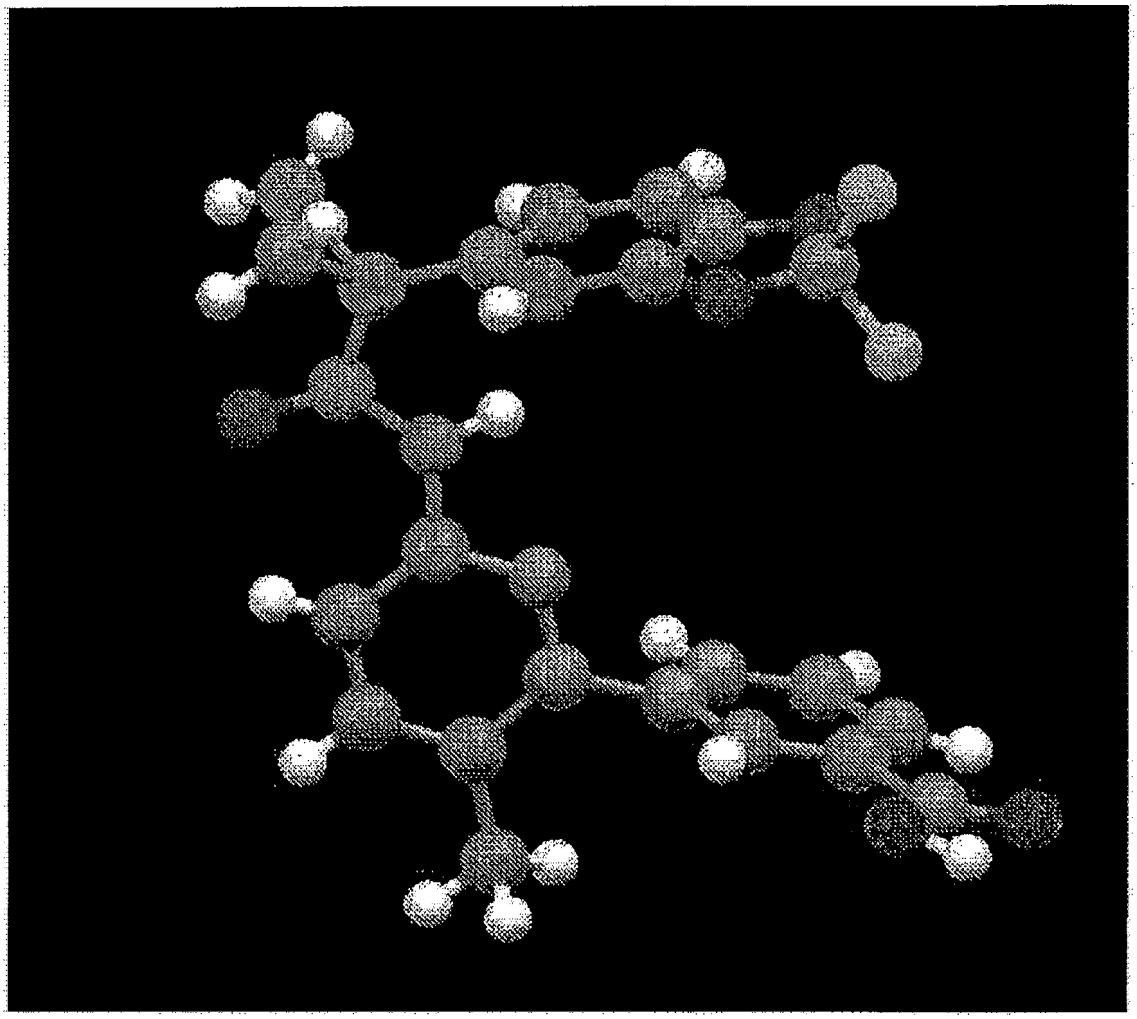

图 23

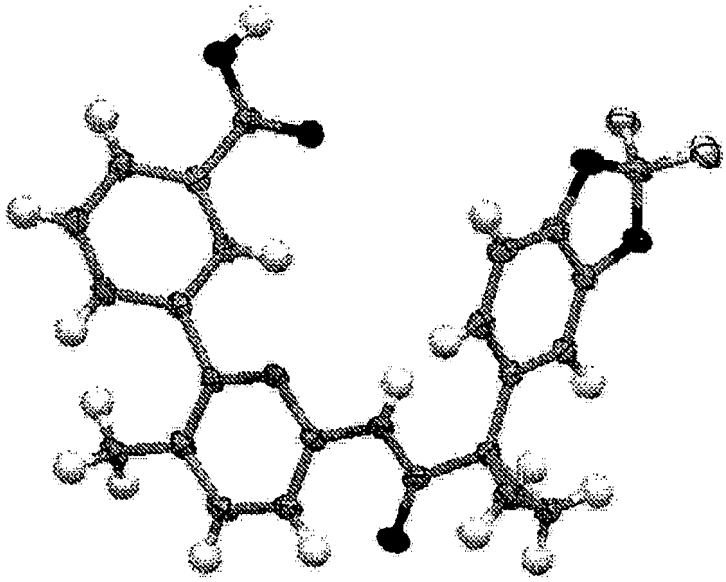

图 24

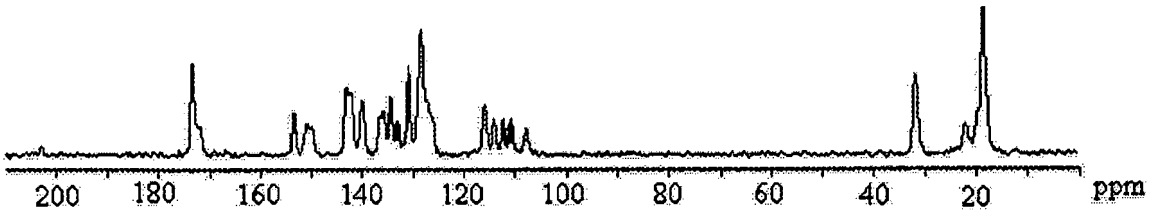
200
180
160
140
120
100
80
60
40
20
ppm

图 25

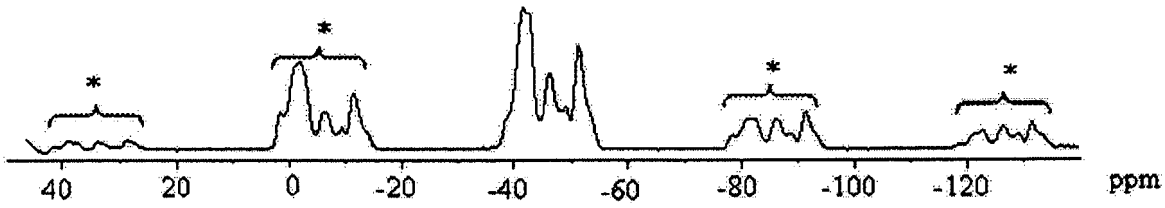
*
*
*
*
40
20
0
-20
-40
-60
-80
-100
-120
ppm

图 26

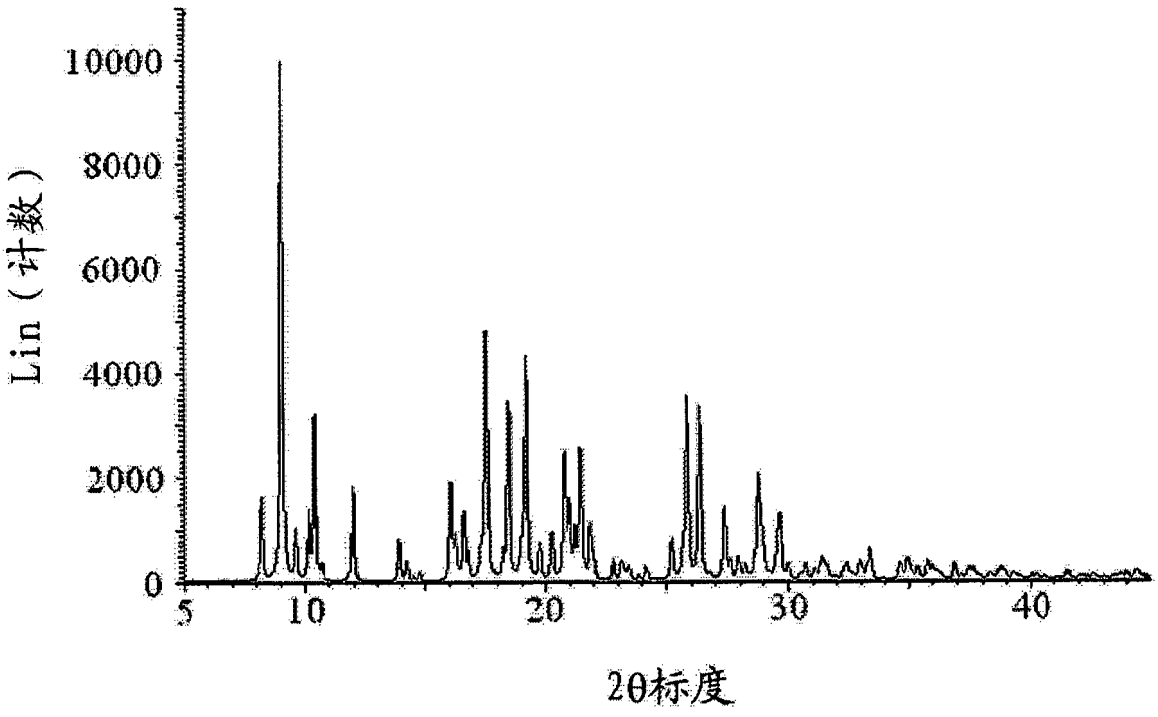
10000
8000
6000
4000
2000
0
Lin（计数）
5
10
20
30
40
2θ标度

图 27

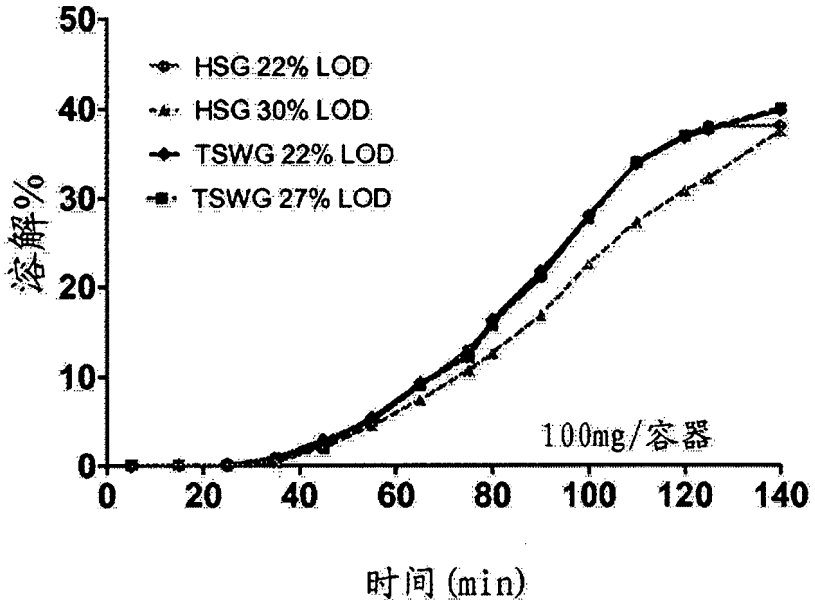
50
40
30
20
10
0
溶解%
HSG 22% LOD
HSG 30% LOD
TSWG 22% LOD
TSWG 27% LOD
100mg/容器
0
20
40
60
80
100
120
140
时间(min)

图 28