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Wu et al.

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(45) **Date of Patent: Jan. 25, 2022**

(54) **INHIBITORS OF MITOCHONDRIAL FISSION**

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(73) Assignee: **Queen's University at Kingston**, Kingston (CA)

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Related U.S. Application Data

(60) Provisional application No. 62/826,247, filed on Mar.

OTHER PUBLICATIONS

Sieveking et al, Bioorganic & Medicinal Chemistry, 22, pp. 4609-4620 (Year: 2014).*

Nishiyama et al, European Journal of Medicinal Chemistry , vol. 136, pp. 1-13 (Year: 2017).*

Cassidy-Stone, A., et al., "Chemical Inhibition of the Mitochondrial Division Dynamin Reveals Its Role in Bax-Bak-Dependent

pp. 193-204, (2008).

Archer, S.L., "Mitochondrial Dynamics—Mtiochondrial Fission and Fusion in Human Diseases", New England Journal of Medicine, 369;23, pp. 2236-2251, (2013).

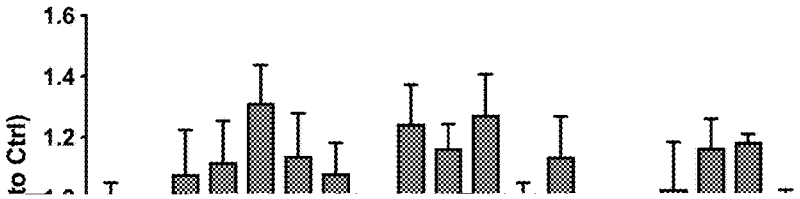
Bordt, E.A., et al., "The Putative Drp1 inhibitor mdivi-1 is a Reversible Mitochondrial Complex 1 Inhibitor that Modulates Reactive Oxygen Species", Dev. Cell, 40, pp. 583-594, (2017).

Kitamura, S., et al., "Drp1 expression levels correlate with clinical stage in extramammary Paget's disease", European Academy of Dermatology and Venereology, 34, pp. e433-e531, (2020).

Moran, M.D., et al., "KRAS Mutation Status is Associated with Enhanced Dependency on Folate Metabolism Pathways in Non-

1611-1624, (2014).

Yu, L. el al., "The expression and prognostic significance of Drp1 in lung cancer", Medicine, 96:48, pp. 1-11, (2019).


1.6
1.4
1.2
to Ctrl)

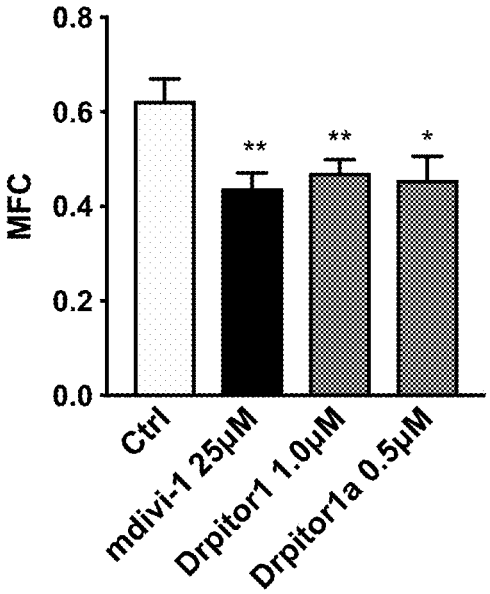
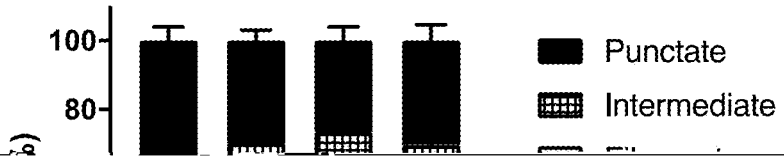
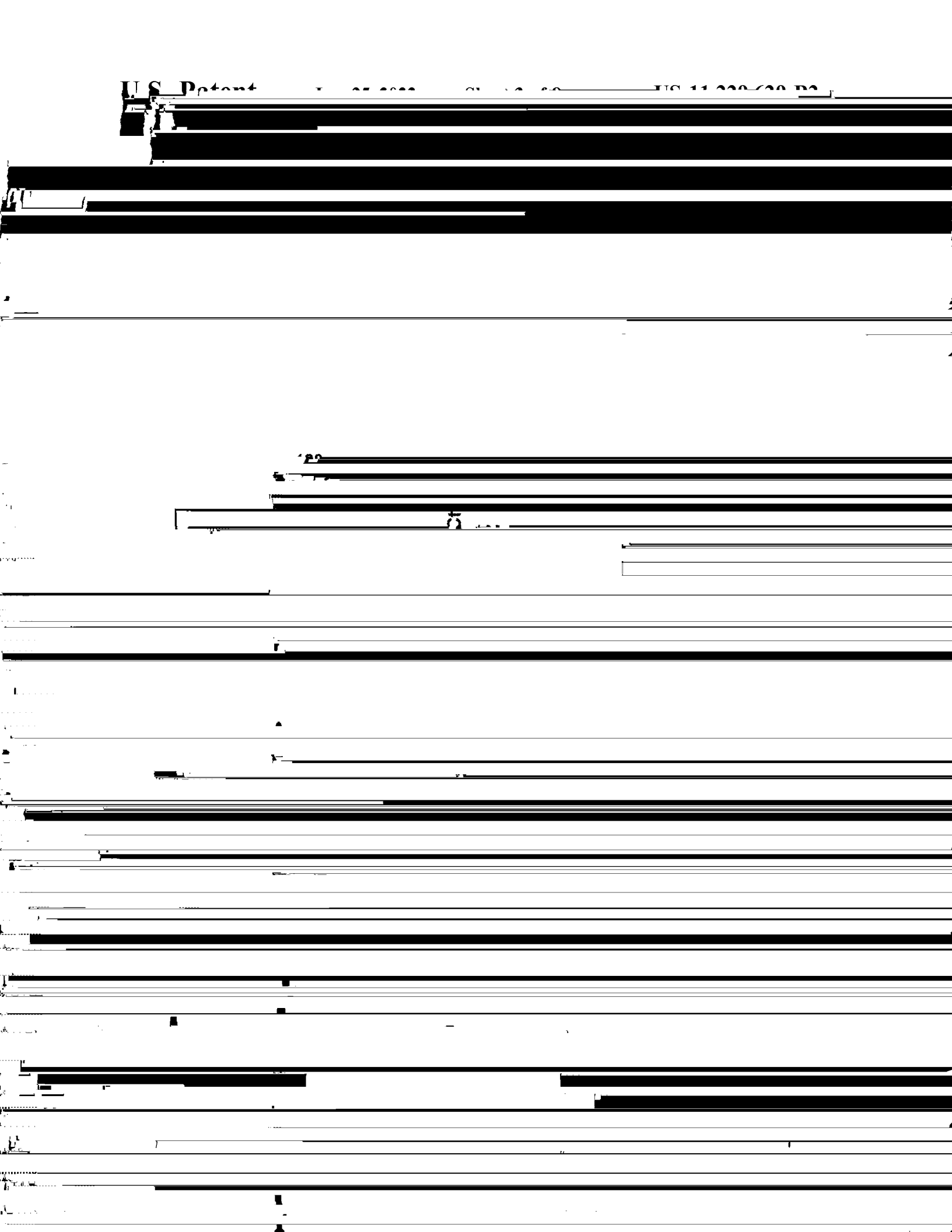
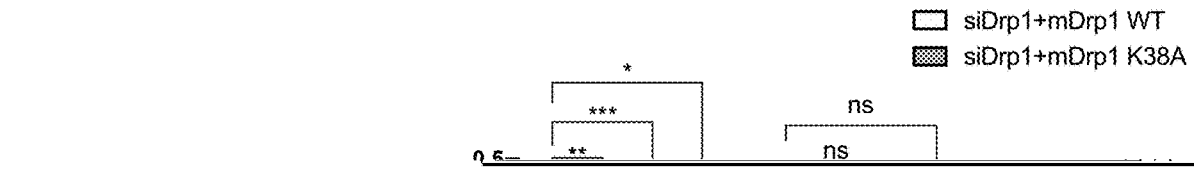
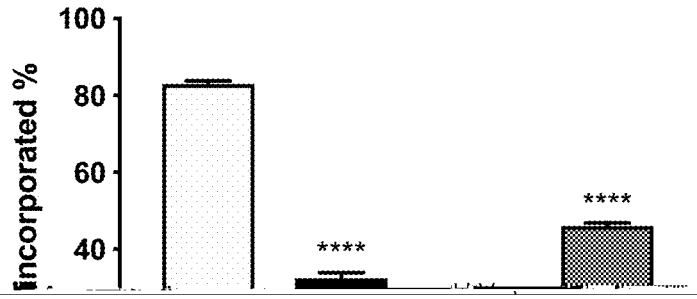

0.8
0.6
0.4
0.2
0.0
MFC
**
**
*
Ctrl
mdivi-1 25μM
Drpitor1 1.0μM
Drpitor1a 0.5μM

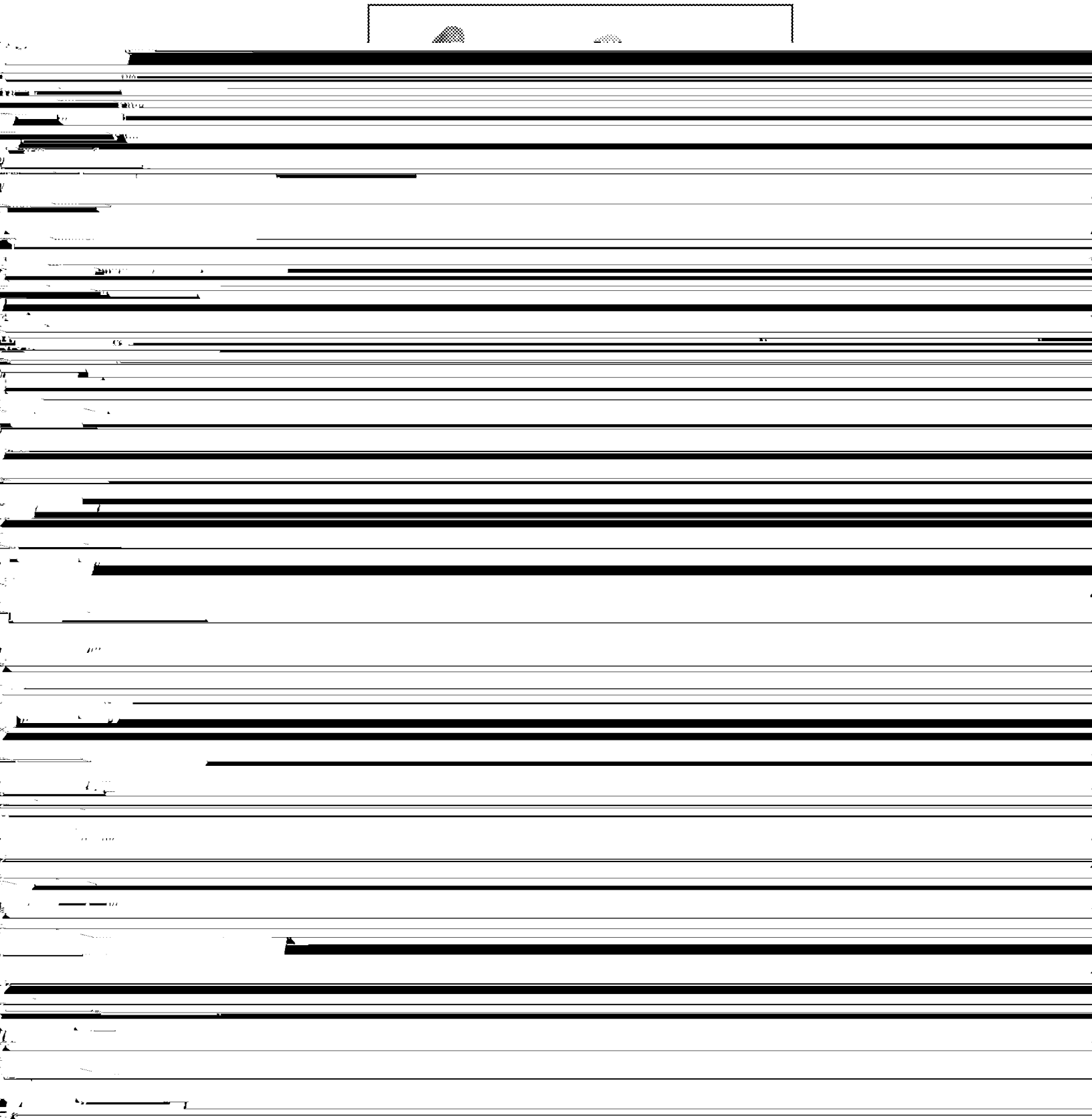
Fig. 3A


100
80
Punctate
Intermediate




siDrp1+mDrp1 WT
siDrp1+mDrp1 K38A
*
***
**
ns
ns
0.6


100
80
60
40
Incorporated %
****
****



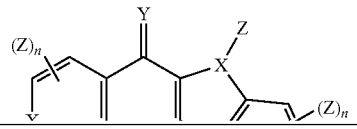

RVSP

INHIBITORS OF MITOCHONDRIAL FISSION

RELATED APPLICATION

This application claims the benefit of the filing date of U.S. Application No. 62/826,247, filed on Mar. 29, 2019, the

(1)



The invention relates to inhibitors of mitochondrial fission and uses thereof.

BACKGROUND

Mitochondria exist in a dynamic network, continuously joining together (a process termed mitochondrial fusion) and separating (termed mitochondrial fission). Fission and fusion, along with mitochondrial motility, are noncanonical

where X is N or C;

Y is O or S;

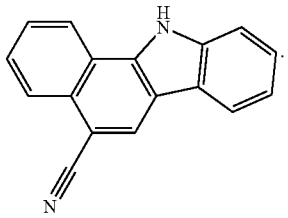
Z is a substituent that may be further substituted; and

n is 1-4,

wherein a substituent comprises alkyl, alkenyl, alkynyl, aryl, aryl-halide, heteroaryl, cyclyl, $Si(alkyl)_3$, $Si(alkoxy)_3$, halo, alkoxyl, amino, amide, amidine, hydroxyl, thioether, alkyl-

filamentous mitochondria was significantly increased for all

Compound 13



In one embodiment, the compound of Formula 1 is Drpitor1a. In one embodiment, the compound of claim **1** is present in an amount from 1 to 1000 mg.

In one aspect, the invention provides a method of reducing or inhibiting mitochondrial fission, comprising administering to a subject in need thereof a pharmaceutical composition comprising a compound of Formula (1). In one embodiment, the compound of Formula (1) is Compound 13

agents relative to control, n=15-20 per group; *P<0.05 vs Ctrl, **P<0.01 vs Ctrl.

FIG. **4**A is a bar graph depicting GTPase activity of Drp1 in A549 cells vs. treatment, wherein a significant decrease is shown for treatment with mdivi-1 (50 μM) (positive control), Dyngo4a (100 μM) (positive control), Drpitor1 (2.0 μM) or Drpitor1a (0.5 μM) for 6 hours, n=3 per group; ****P<0.0001 vs Ctrl.

FIG. **4**B is a bar graph depicting GTPase activity of dynamin 1 in A549 cells vs. treatment, wherein a significant decrease was seen for treatment with Dyngo4a (100 μM) (positive control), which was not seen for treatment with mdivi-1 (25 μM), Drpitor1 (1.0 μM) and Drpitor1a (0.5 μM), n=3 per group; ****P<0.0001 vs Ctrl, ns, not significant.

FIG. **5**A is a bar graph depicting MFC versus treatment, wherein a significantly reduction was seen for mdivi-1, Drpitor1 and Drpitor1a treatment in cells transfected with

FIG. **8**D is a bar graph that shows mitochondrial volume vs. treatment group, wherein mitochondrial volume was significantly increased by Drpitor1a treatment. n=9-19 per group; *P<0.05.

bonyl, alkylthiocarbonyl, phosphate, phosphate ester, phosphonato, phosphinato, cyano, acylamino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, dithiocarboxylate, sulfate, sulfato, sulfamoyl, sulfonamide, nitro, nitrile, azido,

DESCRIPTION

ester, or a combination thereof.

As used herein, the term "TMRM" refers to tetramethyl

sulfhydryl, alkylthio, arylthio, thiocarboxylate, dithiocarboxylate, sulfate, sulfato, sulfamoyl, sulfonamide, nitro,

The therapeutic compound may also be administered ocularly, via inhalation, topically, intravaginally, as well as

moieties, thioester, or a combination thereof. Table 1 shows embodiments of Formula 1 including a variety of substituents.

In particular, two representative examples of Formula 1 that are analogs of ellipticine were identified as specific Drp1 GTPase inhibitors that are more potent than standard Drp1 inhibitor mdivi-1. These two analogs, Drpitor1 and Drpitor1a (see Table 3), have utility through their inhibition of Drp1 and thereby reduction of mitochondrial fission. Accordingly, compounds of Formula 1 have therapeutic potential for treatment of cancer, pulmonary arterial hyper-

toneally, intraspinally, intrathecally, or intracerebrally). Dispersions can be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms. Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the composition must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be

polyethylene glycols, sodium lauryl sulfate, and mixtures thereof, or incorporated directly into the subject's diet. In the case of capsules, tablets and pills, the dosage form may also

to achieve the desired therapeutic effect, e.g. to prevent the spread of cancer and/or kill cancerous cells, to treatment and/or mitigate pulmonary arterial hypertension, cardiopro-

type may also be employed as fillers in soft and hard-filled

diseases, Parksinonism, Huntington's Chorea, Alzheimer's

bovine serum albumin (Center for Drug Evaluation and

Drpitor1 and Drpitor1a were tested in two distinct fisso-

(2002) *Estimating the safe starting dose in clinical trials for therapeutics in adult healthy volunteers*, U.S. Food and

increased mitotic fission (cancer) and a cell injury model, in which fission causes cell injury through ROS production.

wherein no significant change was seen for treatment with was extracted by Cell Lysis Buffer (9803, Cell Signaling

(25200114, Gibco, Burlington, ON, Canada) and reseeded in a 48-well plate at a density of 200 live cells/mL. After 7-10 days of culture, cells were fixed with 4% paraformaldehyde and then stained with 1% crystal violet (V5265, Millipore

median value between two groups as appropriate. One-way ANOVA was used to compare the means of three or more independent groups. Two-way ANOVA was used to compare

pound having an amine instead of a methoxymethyl group was synthesized (Drpitor1a, see FIG. **2**). FIG. **2** depicts

the Drpitors, endogenous Drp1 was first knocked down in A549 cells using siRNA and then restored by transfection

FIG. **2**) with s-BuLi under standard conditions followed by

mouse Drp1 plasmid (K38A), in which lysine 38 is substi-

7C). Drpitor1a did not reduce body weight, change tumor density or result in liver or kidney toxicity during 22 days of therapy (Table 8).

Example 8. Drpitor1a Preserves RV Diastolic Function in an RV-IR Model

ex vivo model. Test compound was added to the perfusate prior to IR. A dose of 0.5 μM was used for Drpitor1a. Drpitor1a-treated hearts, did not manifest the increase in right ventricular end-diastolic pressure (RVEDP) that was seen in control hearts after two periods of IR challenge (FIGS. **8**A and **8**B). After IR, RV myocardium was collected

ROS generation and calcium overload play important roles

IR compared to control RV, reflecting less production of

TABLE 1-continued

Identification of candidate compounds from in silico screening and predicted binding affinity and structural formula of compounds of FIG. 1

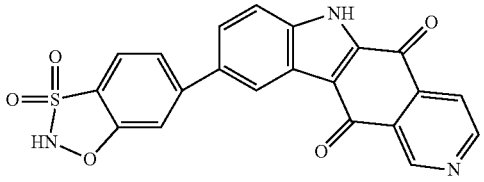
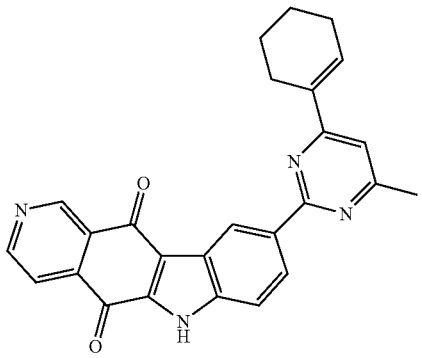
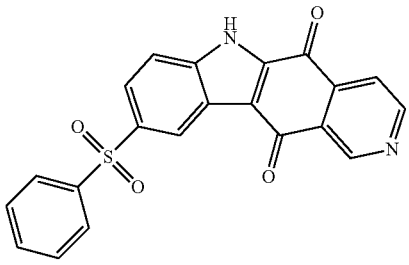
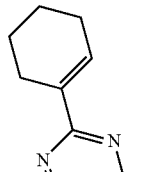
Compound Number if assigned	Structure	Predicted Binding Affinity
103		−11.3
104		−11.2
105		−11
106		−11

TABLE 1 continued

Identification of candidate compounds from in silico screening and predicted binding affinity and structural formula of compounds of FIG. 1

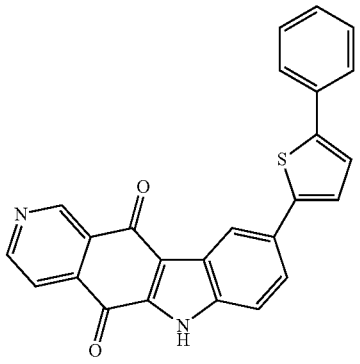
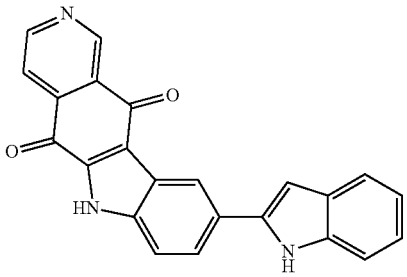
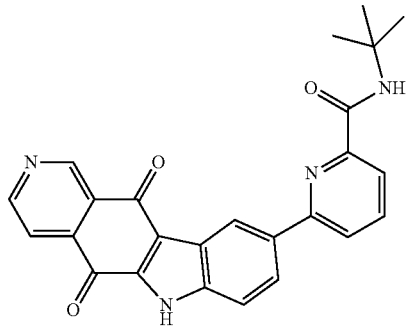
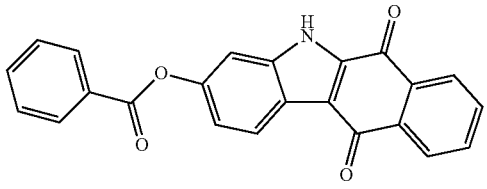
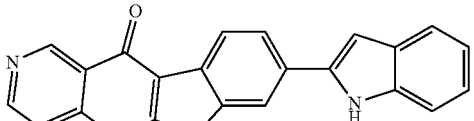
Compound Number if assigned	Structure	Predicted Binding Affinity
107		−10.9
108		−10.9
109		−10.8
110		−10.8
111		−10.8

TABLE 1-continued

Identification of candidate compounds from in silico screening and predicted binding affinity and structural formula of compounds of FIG. 1

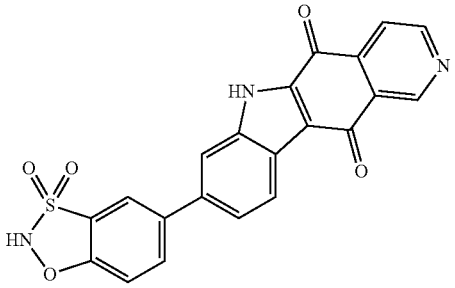
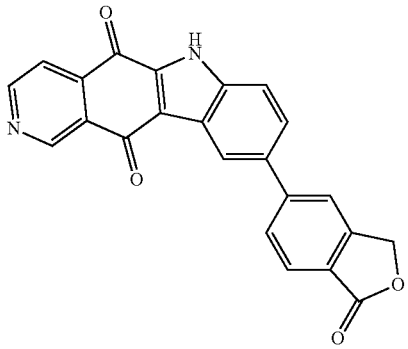
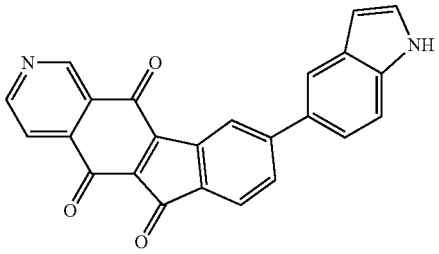
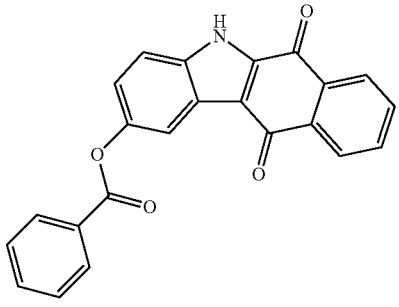
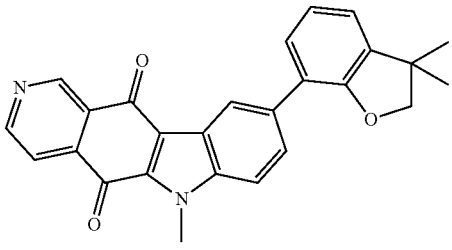
Compound Number if assigned	Structure	Predicted Binding Affinity
112		−10.8
113		−10.8
114		−10.8
115		−10.7
116		−10.6

TABLE 1 [illegible]

Identification of candidate compounds from in silico screening and predicted binding affinity and structural formula of compounds of FIG. 1

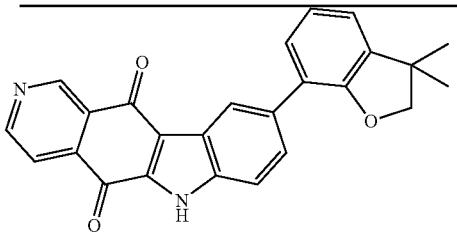
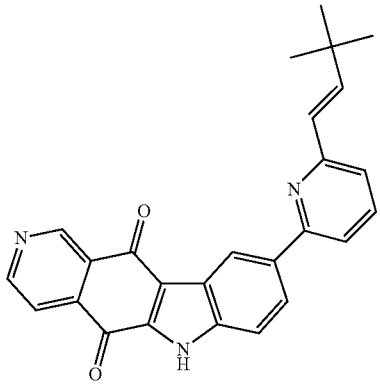
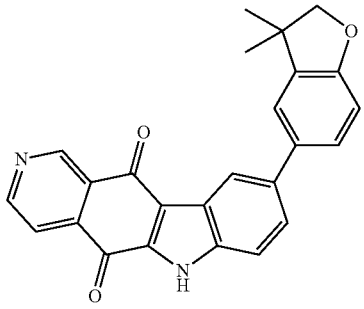
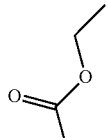
Compound Number if [illegible]	[illegible]	Predicted Binding [illegible]
117		−10.6
118		−10.4
119		−10.4
120		−10.1

TABLE 1 continued

Identification of candidate compounds from in silico screening and predicted binding affinity and structural formula of compounds of FIG. 1

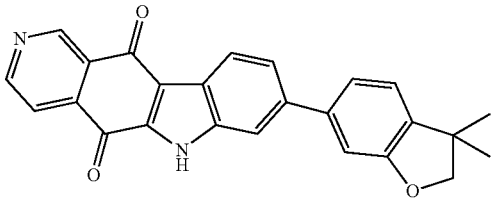
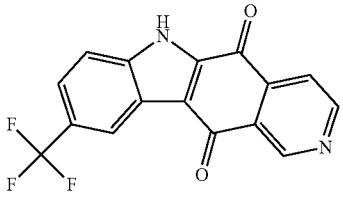
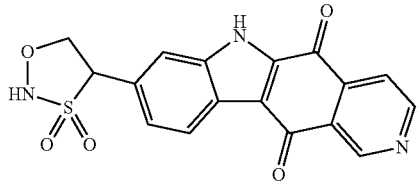
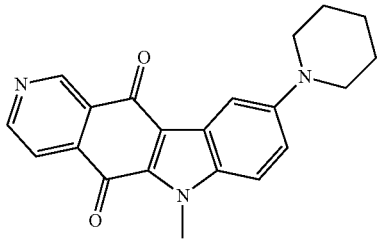
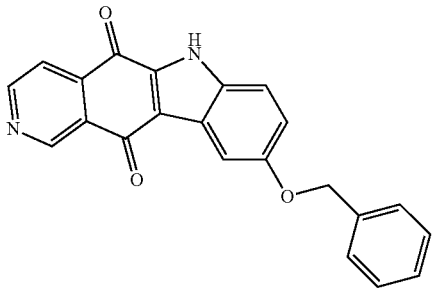
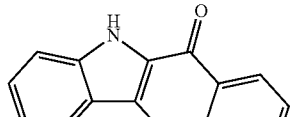
Compound Number if assigned	Structure	Predicted Binding Affinity
121		−10.1
122		−10
123		−9.9
124		−9.8
125		−9.8
126		−9.8

TABLE 1-continued

Identification of candidate compounds from in silico screening and predicted binding affinity and structural formula of compounds of FIG. 1

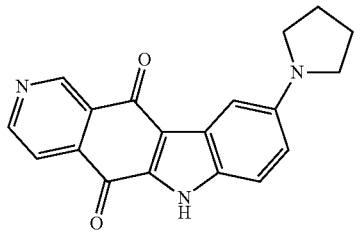
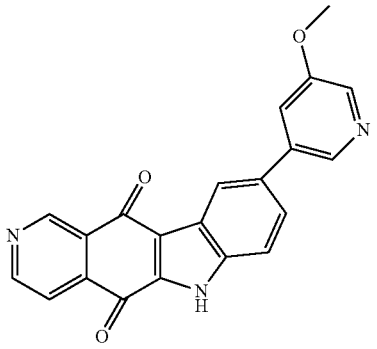
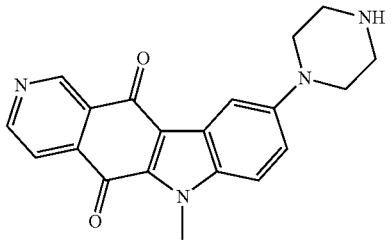
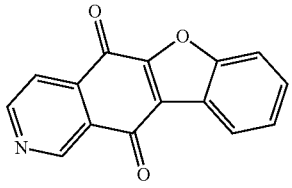
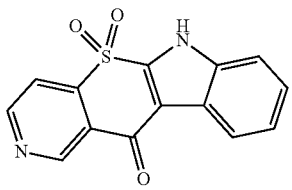
Compound Number if assigned	Structure	Predicted Binding Affinity
127		−9.7
128		−9.7
129		−9.7
130		−9.7
131		−9.7

TABLE 1-continued

Identification of candidate compounds from in silico screening and predicted binding affinity and structural formula of compounds of FIG. 1

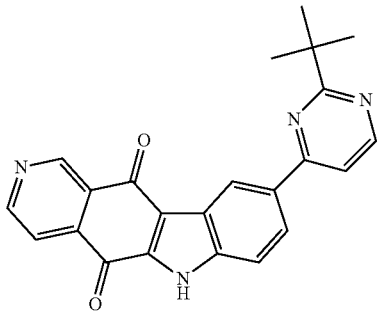
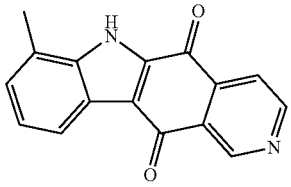
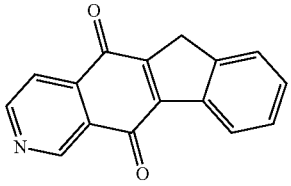
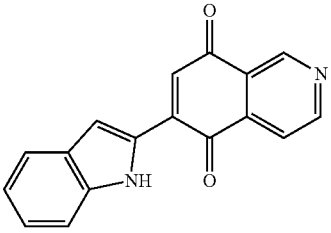
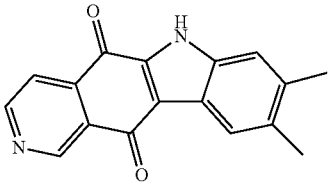
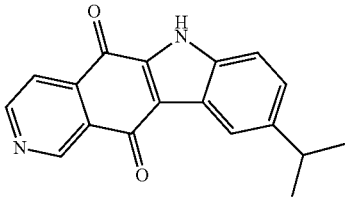
Compound Number if assigned	Structure	Predicted Binding Affinity
132		−9.6
133		−9.6
134		−9.6
135		−9.5
136		−9.5
137		−9.5

TABLE 1-continued

binding affinity and structural formula of compounds of FIG. 1

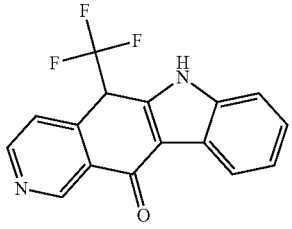
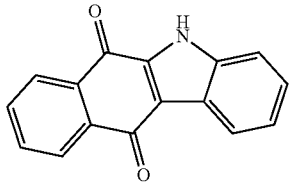
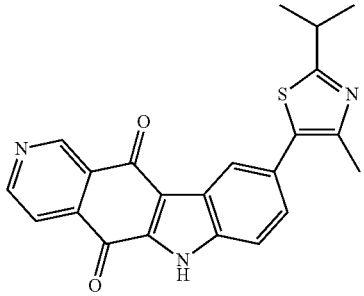
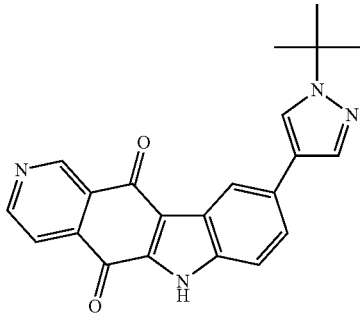
Compound Number if assigned	Structure	Predicted Binding Affinity
138		−9.5
139		−9.5
140		−9.5
141		−9.5
142		−9.4

TABLE 1-continued

Identification of candidate compounds from in silico screening and predicted binding affinity and structural formula of compounds of FIG. 1

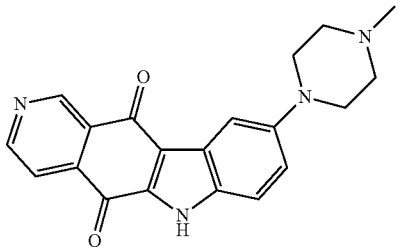
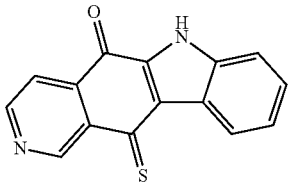
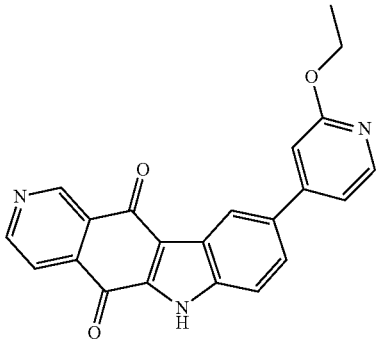
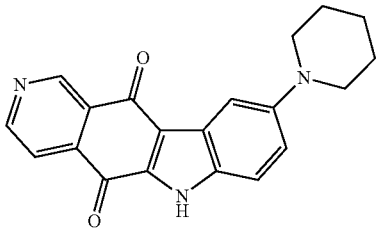
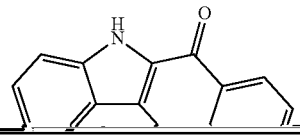
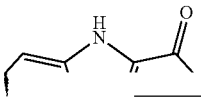
Compound Number if assigned	Structure	Predicted Binding Affinity
144		−9.3
145		−9.3
146		−9.2
147		−9.2
148		−9.2
149		−9.2

TABLE 1-continued

Identification of candidate compounds from in silico screening and predicted

151 –9

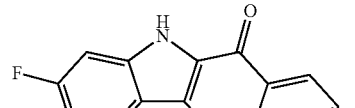


TABLE 1-continued

Identification of candidate compounds from in silico screening and predicted binding affinity and structural formula of compounds of FIG. 1

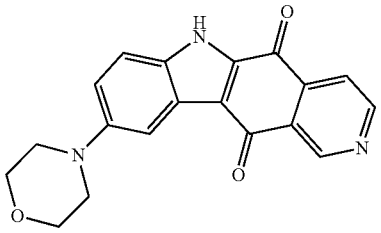
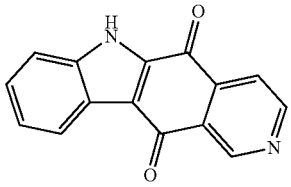
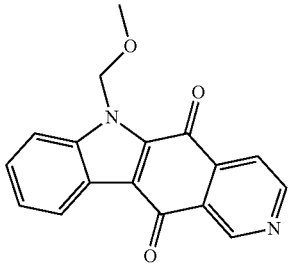
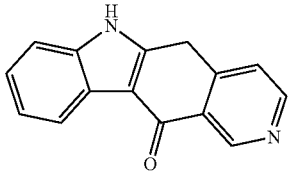
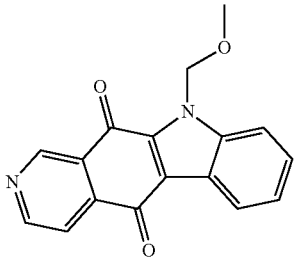
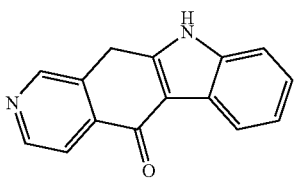
Compound Number if assigned	Structure	Predicted Binding Affinity
157		−8.8
158		−8.8
159		−8.8
160		−8.8
161		−8.8
162		−8.8

TABLE 1-continued

Identification of candidate compounds from in silico screening and predicted binding affinity and structural formula of compounds of FIG. 1

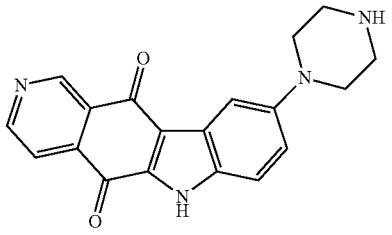
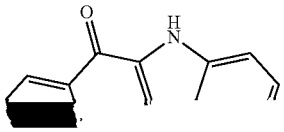
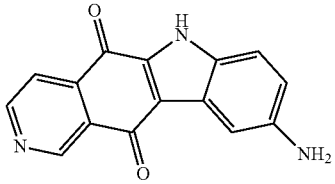
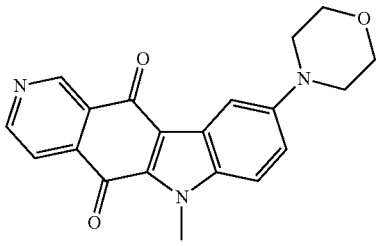
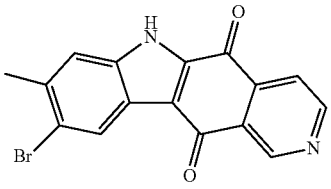
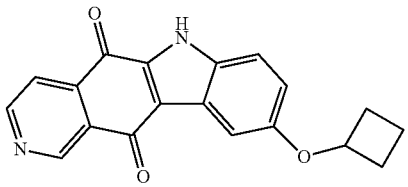
Compound Number if assigned	Structure	Predicted Binding Affinity
163		−8.7
164		−8.7
165		−8.7
166		−8.7
167		−8.7
168		−8.7
169		−8.6

TABLE 1-continued

binding affinity and structural formula of compounds of FIG. 1

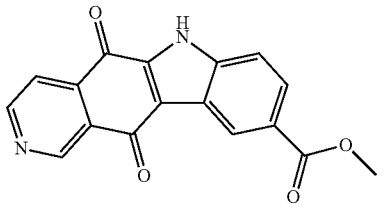
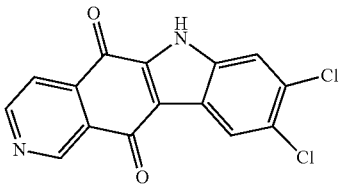
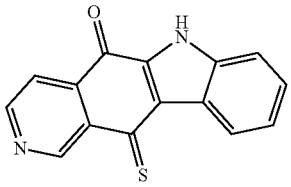
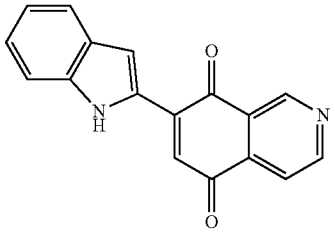
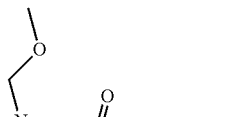
Compound Number if assigned	Structure	Predicted Binding Affinity
170		−8.6
171		−8.5
172		−8.5
173		−8.4
174		−8.3

TABLE 1-continued

Identification of candidate compounds from in silico screening and predicted binding affinity and structural formula of compounds of FIG. 1

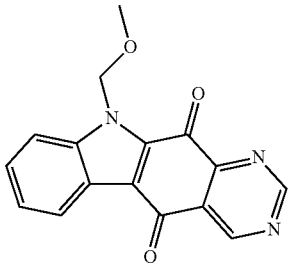
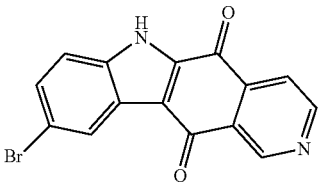
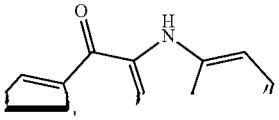
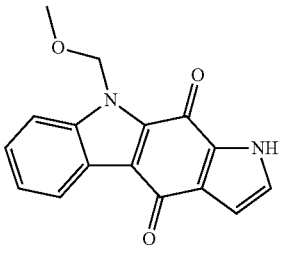
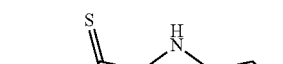
Compound Number if assigned	Structure	Predicted Binding Affinity
176		−8.2
177		−8
178		−8
180		−7.8
181		−7.5

TABLE 1-continued

Identification of candidate compounds from in silico screening and predicted

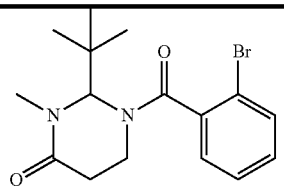
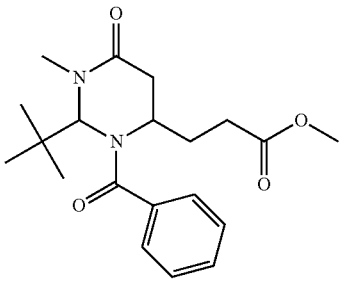
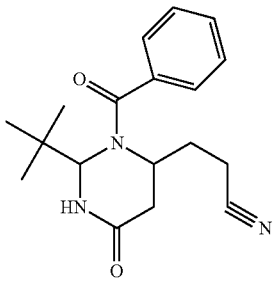
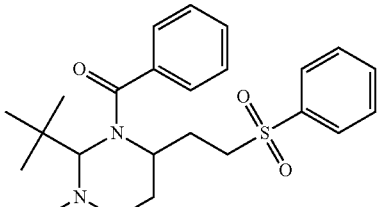
Compound Number if		Predicted Binding
1		
2		
3		
4		

TABLE 1-continued

binding affinity and structural formula of compounds of FIG. 1

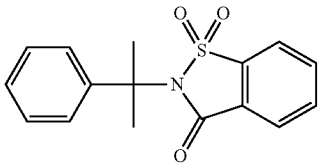
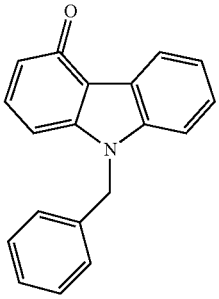
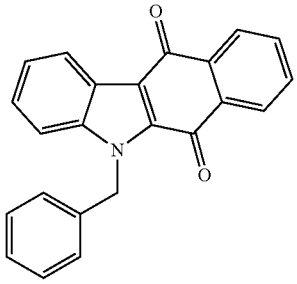
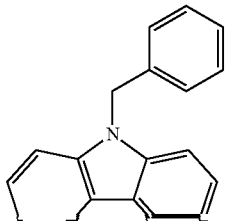
Compound Number if assigned	Structure	Predicted Binding Affinity
6		
7		
8		
9		

TABLE 1 continued

Identification of candidate compounds from in silico screening and predicted

binding affinity and structural formula of compounds of FIG. 1

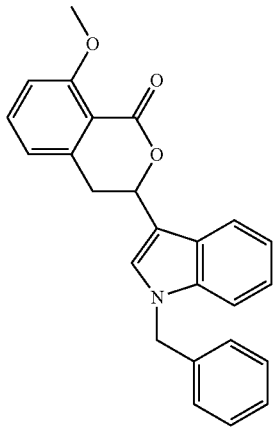
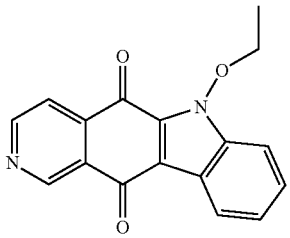
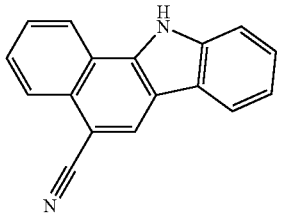
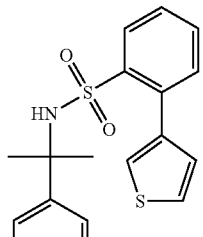
Compound Number if assigned	Structure	Predicted Binding Affinity
11		
12 "Drpitor1"		
13		
14		

TABLE 1-continued

Identification of candidate compounds from in silico screening and predicted binding affinity and structural formula of compounds of FIG. 1

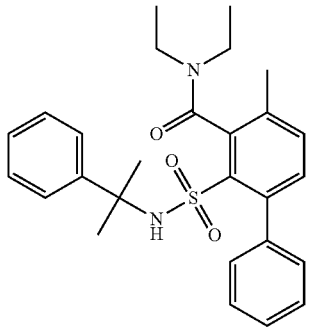
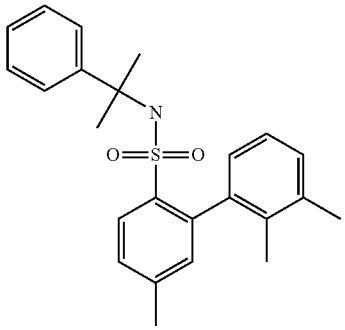
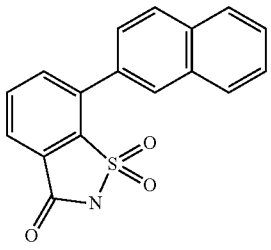
Compound Number if assigned	Structure	Predicted Binding Affinity
15		
16		
17		

TABLE 2

TABLE 2-continued

Molinspiration bioactivity score showing compound

Molinspiration bioactivity score showing compound

TABLE 3

Predicted binding energies of compound Drpitor1, Drpitor1a and mdivi-1 to Drp1 and Dynamin 1 and structural formulae of Dyngo4a

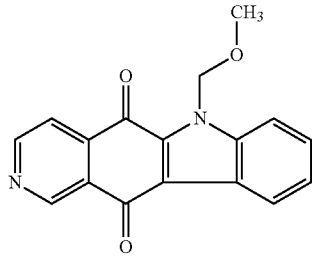
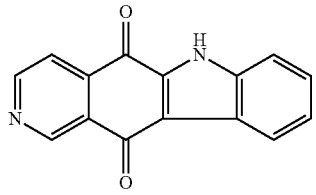
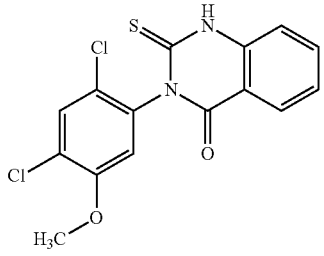
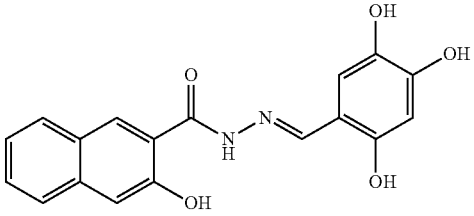
Compound	Structure	Drp1 (4H1V)	Dynamin 1 (5D3Q)
Drpitor1		−8.4	−7.8
Drpitor1a		−9.1	−8.3
mdivi-1		−7.2	−8.4
Dyngo4a			

TABLE 4

in cancer cell lines by Drpitor1a

Cell line	MFC (Ctrl)	MFC (Drpitor1a)	Significance (Ctrl vs Drpitor1a)
SK-MES-1	0.3186 ± 0.02578	0.2291 ± 0.02596	*P < 0.05
SK-LU-1	0.4654 ± 0.04816	0.3367 ± 0.03623	*P < 0.05
MCF7	0.8706 ± 0.1287	0.5479 ± 0.05737	*P < 0.05
SW 900	0.4836 ± 0.05773	0.3345 ± 0.03090	*P < 0.05

Ctrl, control; MFC, mitochondrial fragmentation count.
A dose of 0.1-0.5 μM of Drpitor1a was used.

TABLE 5

Inhibition of cell proliferation in cancer cell lines by Drpitors

Cell line	Cell proliferation (Ctrl)	Cell proliferation (mdivi-1)	Cell proliferation (Drpitor1)	Cell proliferation (Drpitor1a)	P value (Ctrl vs Drpitor1a)
A549	82.95 ± 0.8354	32.43 ± 1.481	21.83 ± 1.906	46 ± 0.8505	****P < 0.0001
SK-MES-1	45.23 ± 0.8373	24.97 ± 3.38	N/A	5.523 ± 0.3613	****P < 0.0001
SK-LU-1	71.2 ± 1.015	29.5 ± 1.652	N/A	5.04 ± 0.5525	****P < 0.0001
SW 900	82.6 ± 0.5568	50.1 ± 0.7211	N/A	28.37 ± 0.3712	****P < 0.0001
MCF7	68.23 ± 1.037	8.217 ± 1.256	N/A	3.437 ± 0.2325	****P < 0.0001

A dosage of 0.5 μM of Drpitor1a was used.

TABLE 6

Inhibition of cell survival in cancer cell lines by Drpitor1a

Cell line	Colony number (Ctrl)	Colony number (mdivi-1)	Colony number (Drpitor1a)	P value (Ctrl vs Drpitor1a)
A549	30.83 ± 4.799	0.5 ± 0.3416	1.5 ± 0.2236	****P < 0.0001
SK-MES-1	10.17 ± 0.8333	5.5 ± 0.5627	0 ± 0	****P < 0.0001
SK-LU-1	4.333 ± 0.8433	2.5 ± 0.4282	0.6667 ± 0.3333	***P < 0.001
MCF7	10.17 ± 0.7923	7.167 ± 0.7491	0 ± 0	****P < 0.0001

TABLE 8

Drpitor1a does not cause liver and kidney toxicity

Parameter	Ctrl	Drpitor1a	P value
Albumin (g/L)	40.33 ± 9.387	26.75 ± 0.9465	ns
Cholesterol (mmol/L)	8.04 ± 2.873	4.188 ± 0.3317	ns
BUN (mmol/L)	8.833 ± 0.2963	6.225 ± 1.186	ns

BUN, blood urea nitrogen; ns, not significant.
A dose of 10 mg/kg of Drpitor1a was used.

TABLE 9

Drpitor1a inhibits mitochondrial fragmentation of PAH PASMC cell lines

Cell line	MFC (Ctrl)	MFC (mdivi-1)	MFC (Drpitor1a)	P value (Ctrl vs Drpitor1a)
P1	0.2041 ± 0.01529	0.1394 ± 0.01072	0.1339 ± 0.01669	*P < 0.05
P3	0.3364 ± 0.02963	0.2667 ± 0.02021	0.2061 ± 0.01332	***P < 0.001
P7	0.3233 ± 0.04189	0.2354 ± 0.022	0.2095 ± 0.02134	*P < 0.05

PAH, pulmonary arterial hypertension; PASMC, pulmonary artery smooth muscle cells; MFC, mitochondrial fragmentation count

TABLE 10

Drpitor1a inhibits cell proliferation of PAH PASMC cell lines

Cell line	Cell proliferation (Ctrl)	Cell proliferation (mdivi-1)	Cell proliferation (Drpitor1a)	P value (Ctrl vs Drpitor1a)

-continued

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cccuagcugu aaucacuaaa cuuga 25

<210> SEQ ID NO 2
<211> LENGTH: 27
<212> TYPE: RNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

We claim:

2. The method of claim 1, wherein the mitochondrial