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Dibenzofuran derivatives inspired from cercosporamide as dual inhibitors of Pim and CLK1 kinases

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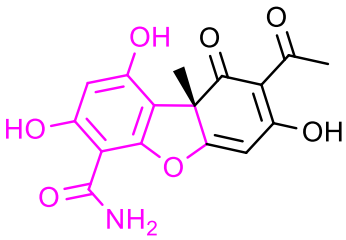
UNIVERSITÉ DE NANTES

Dibenzofuran derivatives inspired from cercosporamide as dual inhibitors of Pim and CLK1 kinases

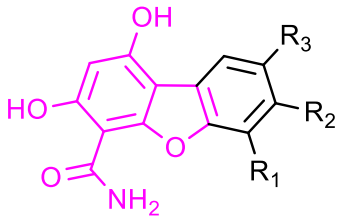
Graphical Abstract



(-)-Cercosporamide

Pim-1 IC_{50} = 732 nM

Pim-2 IC_{50} = 1430 nM

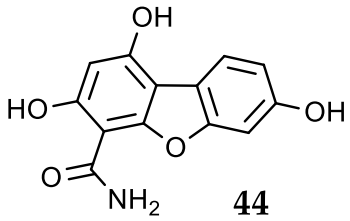


Dibenzo[*b*,*d*]furans

R_1 = H, NO_2, NH_2

R_2 = H, OH

R_3 = H, $COCH_3$, F, CF_3



44

Pim-1 IC_{50} = 60 nM

Pim-2 IC_{50} = 35 nM

CLK1 IC_{50} = 26 nM

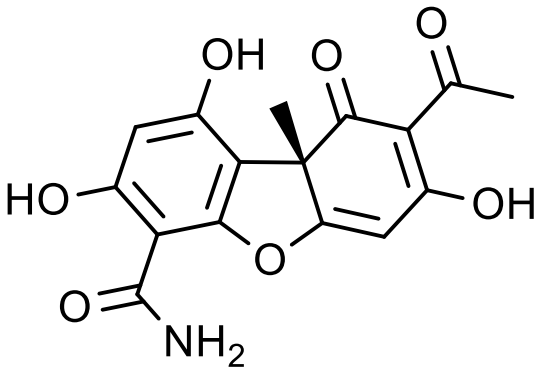
MV4-11 IC_{50} = 2.6 ± 0.4 μM

AML cell line

Abstract:
Pim kinases (proviral integration site for Moloney murine leukemia virus kinases) are overexpressed in various types of hematological malignancies and solid carcinomas, and promote cell proliferation and survival. Thus, Pim kinases are validated as targets for antitumor therapy. In this context, our combined efforts in natural product-inspired library generation and screening furnished very promising dibenzo[*b,d*]furan derivatives derived from cercosporamide. Among them, lead compound **44** was highlighted as a potent Pim-1/2 kinases inhibitor with an additional nanomolar IC_{50} value against CLK1 (cdc2-like kinases 1) and displayed a low micromolar anticancer potency towards the MV4-11 (AML) cell line, expressing high endogenous levels of Pim-1/2 kinases.
The design, synthesis, structure–activity relationship, and docking studies are reported herein and supported by enzyme, cellular assays, and *Galleria mellonella* larvae testing for acute toxicity.

Keywords: cercosporamide; dibenzo[*b*,*d*]furan; Pim & CLK1 kinases; kinase inhibitors; anticancer agents

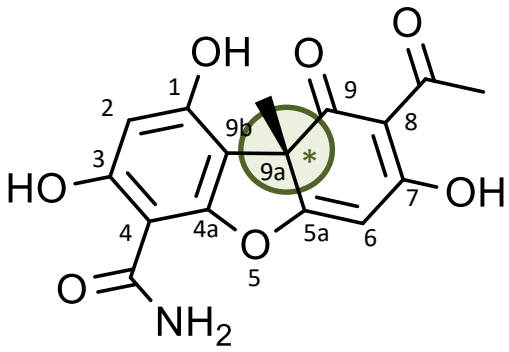
Introduction: discovery of cercosporamide



Cercosporamide, originally isolated in 1991 as a phytotoxin from the plant fungal pathogen of casava, *Cercosporidium henningsii*, was shown to have broad-spectrum antifungal activity.

Sugawara, F.; Strobel, S.; Strobel, G.; Larsen, R. D.; Berglund, D. L.; Gray, G.; Takahashi, N.; Coval, S. J.; Stout, T. J.; Clardy, J. *J. Org. Chem.* **1991**, *56*, 909-910

Introduction: structure of cercosporamide



(9a*S*)-8-acetyl-9,9a-dihydro-1,3,7-trihyroxy-9a-methyl-9-oxodibenzo[*b*,*d*]furan-4-carboxamide

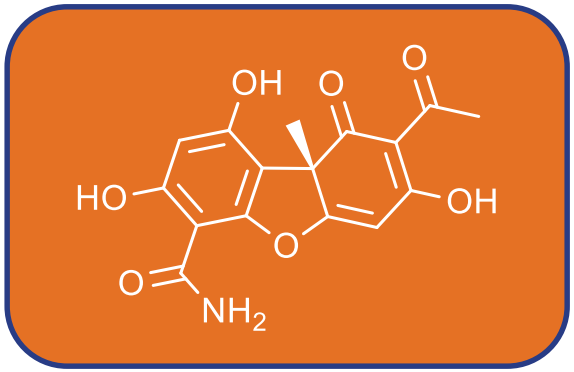
Enantiopure (*S*)-(—)-cercosporamide
$[\alpha]_D$ = -26°
Mp: 188-189 °C
Red crystals

(R)-isomer of cercosporamide not described in the literature.

Sugawara, F.; Strobel, S.; Strobel, G.; Larsen, R. D.; Berglund, D. L.; Gray, G.; Takahashi, N.; Coval, S. J.; Stout, T. J.; Clardy, J. *J. Org. Chem.* **1991**, *56*, 909-910

Introduction: biological properties of cercosporamide

potent selective inhibitor *Ca*Pkc1
Sussman *et al.*, 2004
LaFayette *et al.*, 2010



MAPK-interacting kinases (Mnk1/2) inhibitor in cancers
Konicek *et al.*, 2011
Hou *et al.*, 2012

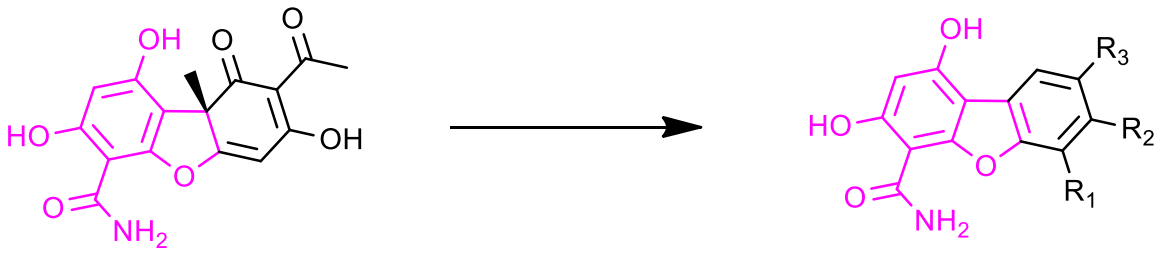
inhibition of bone morphogenetic protein receptor (BMPR) type I kinase

rare genetic disorder fibrodysplasia ossificans progressiva (FOP)
Hoeksma *et al.*, 2020

rare childhood brainstem tumor diffuse intrinsic pontine glioma (DIPG)
Hoeksma *et al.*, 2020

Dao, V.H.; Ourliac-Garnier, I.; Logé, C.; McCarthy, F.O.; Bach, S.; da Silva, T.G.; Denevault-Sabourin, C.; Thiéfaine, J.; Baratte, B.; Robert, T.; Gouilleux, F.; Brachet-Botineau, M.; Bazin M.-A.; Marchand, P. *Molecules* **2021**, *26*, 6572. https://doi.org/10.3390/molecules26216572

Introduction:
from cercosporamide to dibenzo[*b*,*d*]furan derivatives



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Dao, V.H.; Ourliac-Garnier, I.; Bazin, M.-A.; Jacquot, C.; Baratte, B.; Ruchaud, S.; Bach, S.; Grovel, O.; Le Pape, P.; Marchand, P. Benzofuro[3,2-*d*]pyrimidines inspired from cercosporamide *Ca*Pkc1 inhibitor: Synthesis and evaluation of fluconazole susceptibility restoration. *Bioorg. Med. Chem. Lett.* **2018**, *28*, 2250–2255.

Introduction: Pim kinases, active proto-oncogenic serine/threonine protein kinases

cell cycle progression

cell proliferation, survival, differentiation, and migration

apoptosis

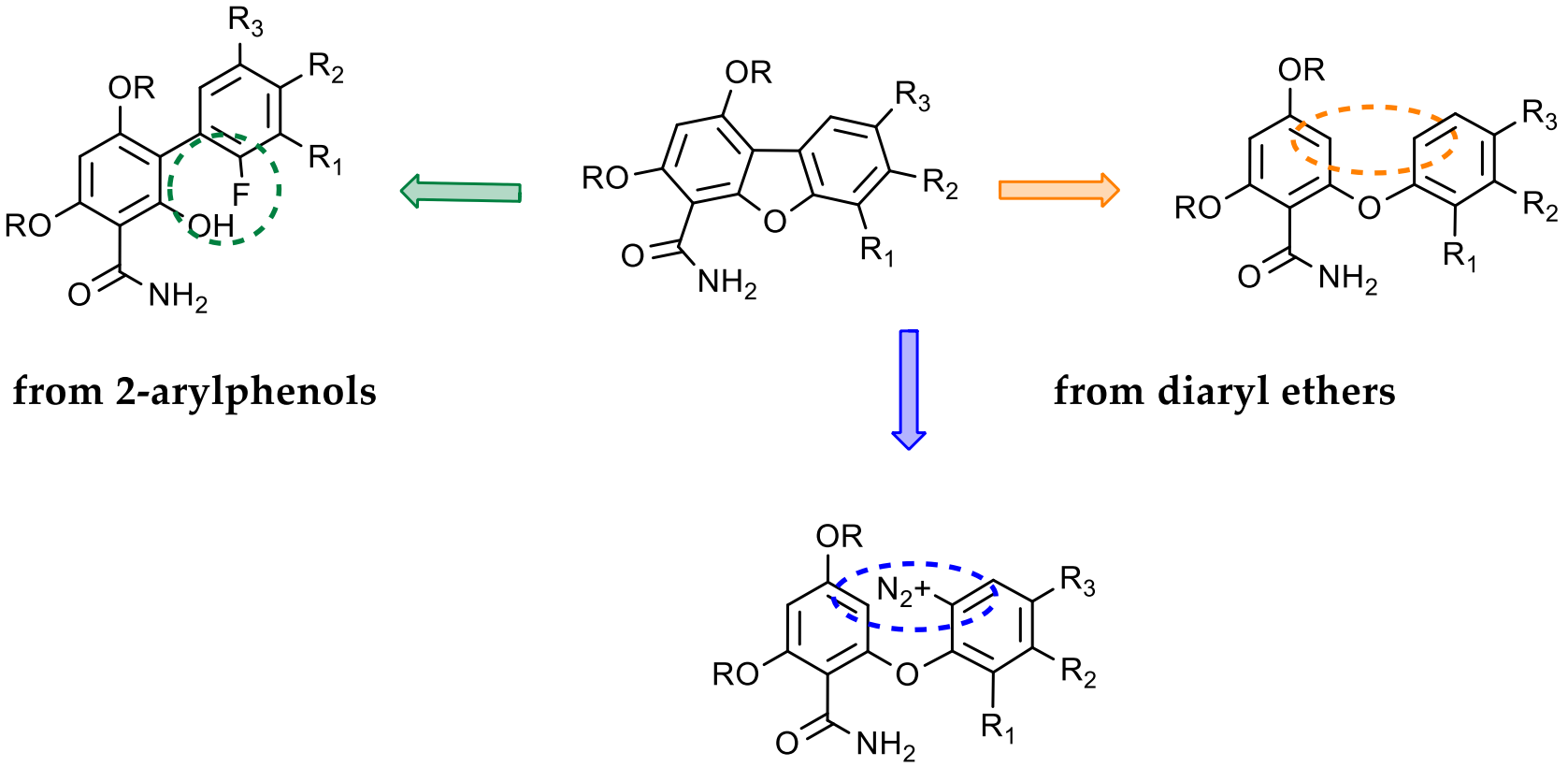
Proviral integration site for Moloney murine leukemia virus (Pim) kinases 1, 2 and 3

aberrantly up-regulated in a variety of hematologic and solid tumors

contribution to malignant transformation, cancer progression, metastasis, drug resistance

Dao, V.H.; Ourliac-Garnier, I.; Logé, C.; McCarthy, F.O.; Bach, S.; da Silva, T.G.; Denevault-Sabourin, C.; Thiéfaine, J.; Baratte, B.; Robert, T.; Gouilleux, F.; Brachet-Botineau, M.; Bazin M.-A.; Marchand, P. *Molecules* **2021**, *26*, 6572. https://doi.org/10.3390/molecules26216572

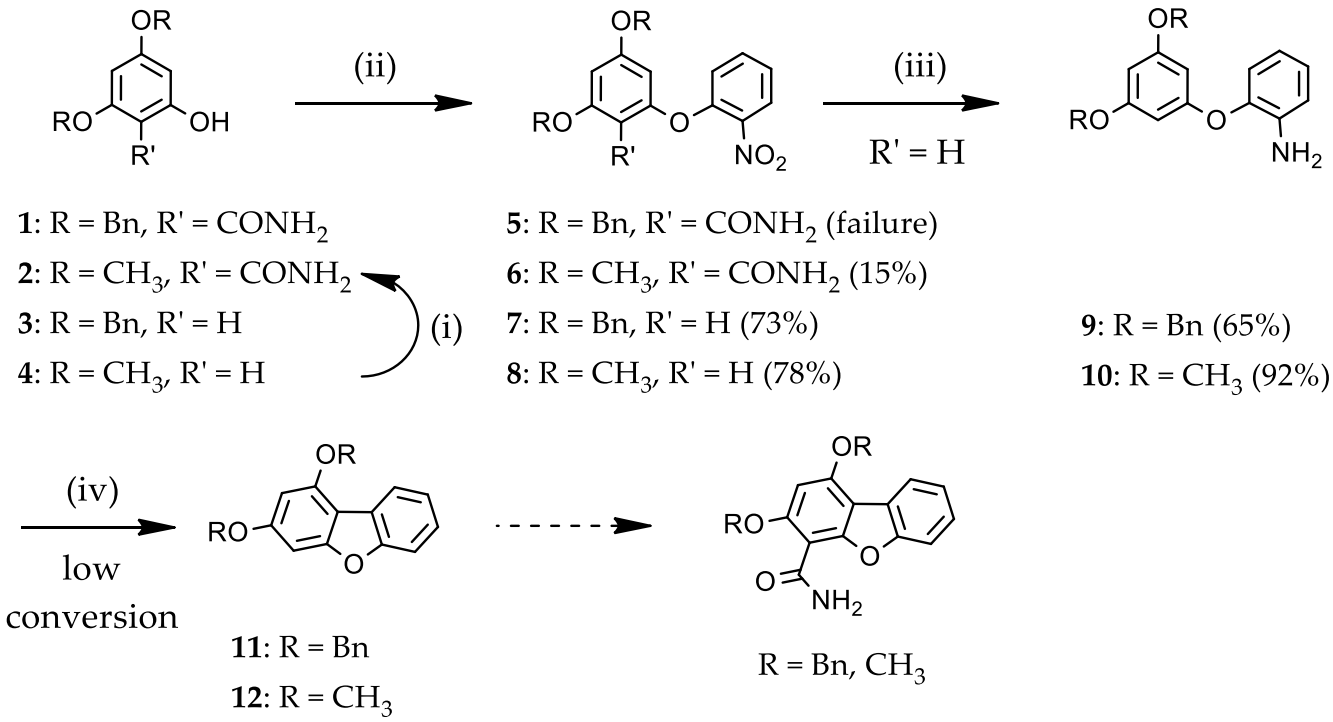
Introduction:
synthetic strategies to obtain dibenzofuran series



from 2-arylphenols

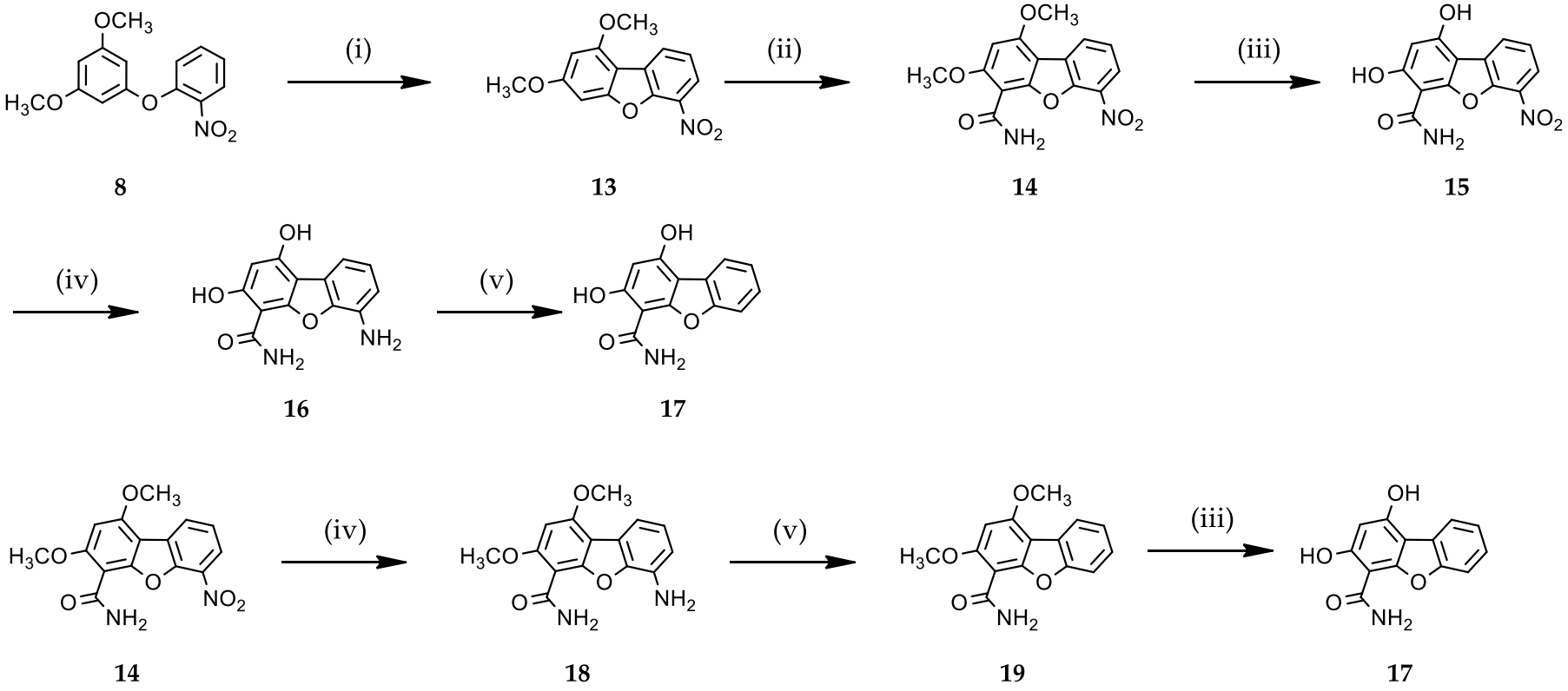
from diaryl ethers

Results and discussion:
synthesis via *ortho*-(aryloxy)aryldiazonium salts



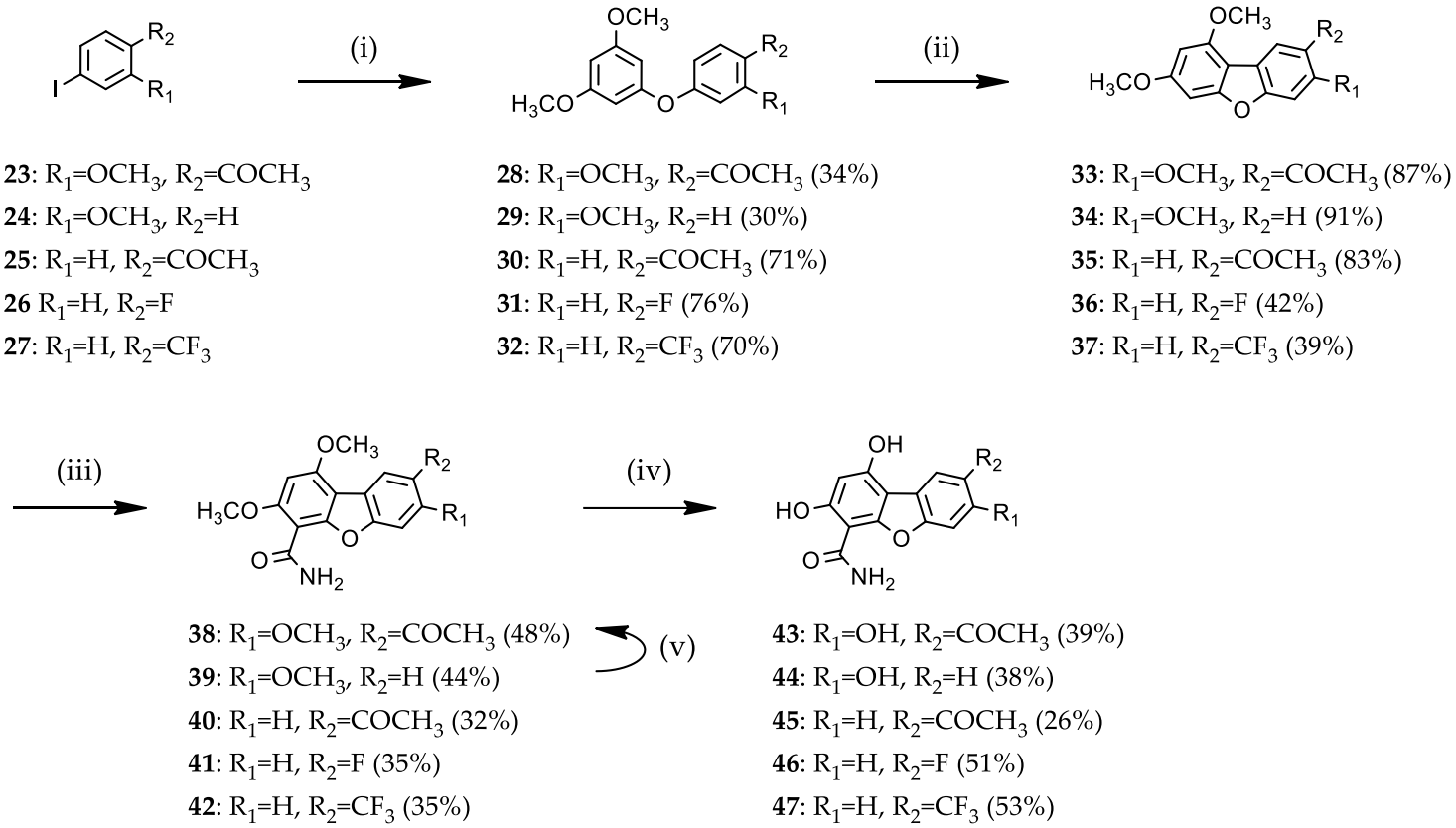
Scheme 1. Reagents and conditions: (i) CSI, CH_3CN, 0 °C, 10 min, then HCl 5M, rt, 10 h, 45% (ii) KOH, DMF, 120 °C, 30 min, then 1-iodo-2-nitrobenzene, Cu(0), 170 °C, 24 h (for **5** and **6**) and 2 h (for **7** and **8**); (iii) Zn dust, NH_4Cl, CH_3OH, 80 °C, 2 h; (iv) 1) $NaNO_2$, H_2SO_4, 0 °C, 45 min, 2) Cu(0), H_2SO_4, 60 °C, 24 h, 6% (for **11**) and 11% (for **12**) of conversion rates or $Pd(OAc)_2$, EtOH, 60 °C, 24 h, 8% (for **11**) and 28% (for **12**) of conversion rates.

Results and discussion: intramolecular palladium(II)-catalyzed oxidative carbon-carbon bond formation



Scheme 2. Reagents and conditions: (i) $Pd(OAc)_2$, AcOAg, PivOH, 130 °C, 4 h, 72%; (ii) CSI, CH_3CN, rt, 12 h, then HCl 5M, rt, 6 h, 53%; (iii) Pyridine.HCl, 200 °C, MW, 15 min, 51% (for **15**) and 48% (for **17**); (iv) Zn dust, NH_4Cl, CH_3OH, 80 °C, 2 h, 62% (for **16**) and 49% (for **18**); (v) $NaNO_2$, H_2SO_4, EtOH, 0 °C, 30 min, then 80 °C, 45 min, 52% (for **17**) and 46% (for **19**).

Results and discussion: intramolecular palladium(II)-catalyzed oxidative carbon-carbon bond formation



23: R_1=OCH_3, R_2=$COCH_3$
24: R_1=OCH_3, R_2=H
25: R_1=H, R_2=$COCH_3$
26 R_1=H, R_2=F
27: R_1=H, R_2=CF_3

28: R_1=OCH_3, R_2=$COCH_3$ (34%)
29: R_1=OCH_3, R_2=H (30%)
30: R_1=H, R_2=$COCH_3$ (71%)
31: R_1=H, R_2=F (76%)
32: R_1=H, R_2=CF_3 (70%)

33: R_1=OCH_3, R_2=$COCH_3$ (87%)
34: R_1=OCH_3, R_2=H (91%)
35: R_1=H, R_2=$COCH_3$ (83%)
36: R_1=H, R_2=F (42%)
37: R_1=H, R_2=CF_3 (39%)

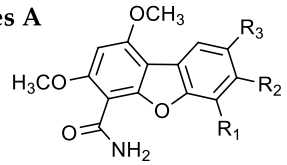
38: R_1=OCH_3, R_2=$COCH_3$ (48%)
39: R_1=OCH_3, R_2=H (44%)
40: R_1=H, R_2=$COCH_3$ (32%)
41: R_1=H, R_2=F (35%)
42: R_1=H, R_2=CF_3 (35%)

43: R_1=OH, R_2=$COCH_3$ (39%)
44: R_1=OH, R_2=H (38%)
45: R_1=H, R_2=$COCH_3$ (26%)
46: R_1=H, R_2=F (51%)
47: R_1=H, R_2=CF_3 (53%)

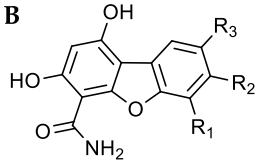
Scheme 3. Reagents and conditions: (i) 3,5-Dimethoxyphenol **4**, Cs_2CO_3, CuI, DMF, 110 °C, MW, 1 h; (ii) $Pd(OAc)_2$, AgOAc, PivOH, 130 °C, 4-24 h; (iii) CSI, CH_3CN, 0 °C-rt, 6-12 h, then HCl 5M, rt, 6-12 h; (iv) Pyridine.HCl, 200 °C, MW, 15 min; (v) AcCl, $AlCl_3$, Cl-CH_2CH_2Cl, rt, 1 h, 74%.

Results and discussion: kinase selectivity profile of dibenzo[*b*,*d*]furane derivatives

Series A



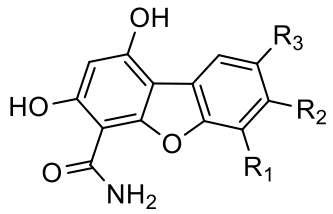
Series B



						Kinase Enzymatic IC_{50} (μM) [1,2]			
Entry	Compound	Series	R_1	R_2	R_3	CDK5/ p25	CDK9/ CyclinT	Pim-1	CLK1
1	Cerco [3]					5.60	**0.22**	**0.73**	>10
2	**14**	A	NO_2	H	H	>10	>10	3.21	>10
3	**15**	B	NO_2	H	H	>10	>10	**0.18**	**1.22**
4	**18**	A	NH_2	H	H	>10	>10	>10	>10
5	**16**	B	NH_2	H	H	>10	>10	**0.13**	**0.45**
6	**19**	A	H	H	H	>10	>10	>10	**0.75**
7	**17**	B	H	H	H	>10	>10	**0.23**	**0.26**
8	**38**	A	H	OCH_3	$COCH_3$	>10	>10	>10	>10
9	**43**	B	H	OH	$COCH_3$	>10	>10	**0.82**	**0.29**
10	**39**	A	H	OCH_3	H	>10	>10	>10	>10
11	**44**	B	H	OH	H	>10	>10	**0.06**	**0.026**
12	**40**	A	H	H	$COCH_3$	>10	>10	>10	>10
13	**45**	B	H	H	$COCH_3$	>10	>10	**0.28**	**0.14**
14	**41**	A	H	H	F	>10	>10	>10	**0.85**
15	**46**	B	H	H	F	>10	0.92	**1.20**	**0.62**
16	**42**	A	H	H	CF_3	>10	>10	>10	>10
17	**47**	B	H	H	CF_3	>10	>10	**2.35**	>10

[1] IC_{50} values were calculated from dose–response curves. Each inhibitor concentration was tested in duplicate. All protein kinases used here are human with the exception of DYRK1A (*Rattus norvegicus*) and CLK1 (*Mus musculus*). DYRK1A: dual specificity tyrosine phosphorylation regulated kinase 1A, CDK: cyclin-dependent kinase, Haspin: haploid germ cell-specific nuclear protein kinase, CLK1: cdc2-like kinase 1, CK1: casein kinase 1, GSK3β: glycogen synthase kinase 3, Pim: Proviral integration site for Moloney murine leukemia virus. [2] All the compounds remained inactive against Haspin, DYRK1A, GSK3 and CK1. Except compound **41**: Haspin (0.93 μM) and DYRK1A (1.33 μM) and compound **46**: Haspin (0.80 μM), DYRK1A (0.69 μM), and CK1 (0.68 μM). [3] Cercosporamide (**Cerco**) was used as the reference compound.

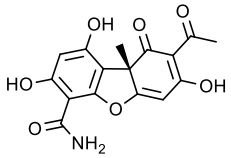
Results and discussion: enzymatic assays on human Pim-1 and Pim-2 kinases



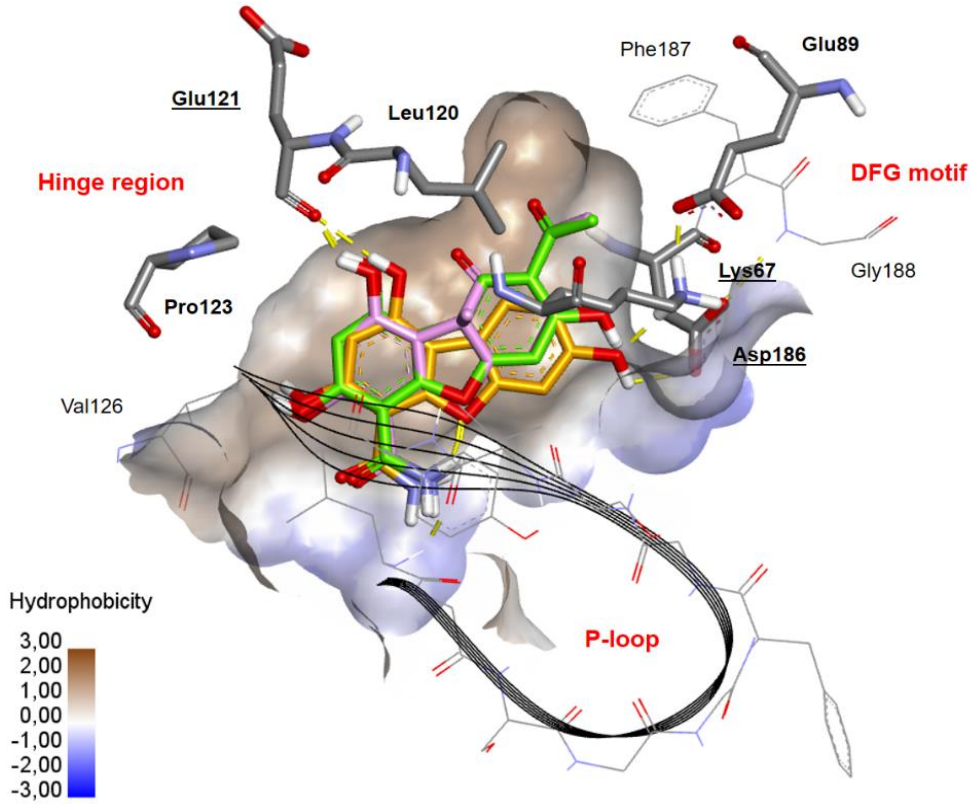
					IC$_{50}$ (μM) [1]	
Entry	Compound	R_1	R_2	R_3	Pim-1	Pim-2
1	Cerco [2]				0.73	1.43
2	**15**	NO_2	H	H	0.18	0.33
3	**16**	NH_2	H	H	0.13	0.085
4	**17**	H	H	H	0.23	0.20
5	**43**	H	OH	$COCH_3$	0.82	0.14
6	**44**	H	OH	H	0.06	0.035
7	**45**	H	H	$COCH_3$	0.28	0.12
8	**46**	H	H	F	1.2	0.25
9	**47**	H	H	CF_3	2.35	0.37

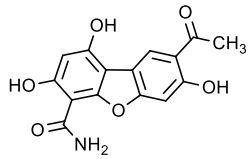
[1] IC$_{50}$ on Pim-1/2 kinase activity was calculated from dose–response curves. Each inhibitor concentration was tested in duplicate. Values are a mean of $n \geq 3$ independent experiments. [2] Cercosporamide (Cerco) was used reference compound.

Results and discussion: docking studies

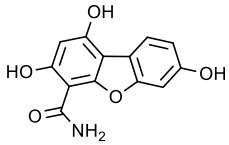


(-)-Cercosporamide





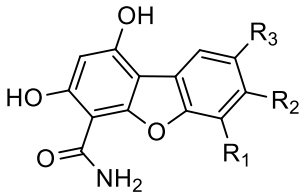
compound 43



compound 44

Binding pose found by the docking program GOLD for compound **44** (orange), compound **43** (green), and cercosporamide (pink) within the ATP pocket of Pim-1 (PDB ID 3A99). Hydrogen bonds are indicated as yellow lines.

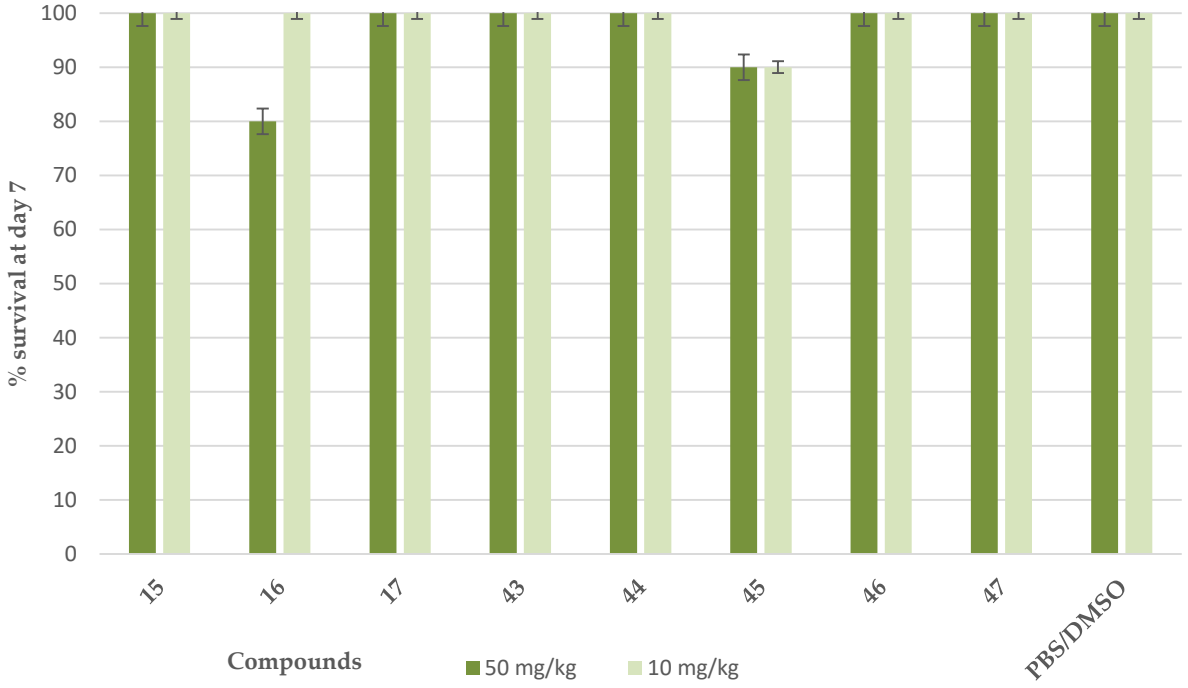
Results and discussion: cell-based assays of representative dibenzo[*b*,*d*]furane derivatives



					IC$_{50}$ (μM) [1,2]					
Entry	Compound	R_1	R_2	R_3	MV4-11	KU812	K562	MCF-7	HeLa	L929
1	**15**	NO_2	H	H	30.7 ± 1.1	78.4 ± 2.1	>100	>100	24.7 ± 6.7	49.2 ± 1.0
2	**16**	NH_2	H	H	10.1 ± 0.8	>100	>100	>100	14.6 ± 7.2	>100
3	**17**	H	H	H	14.3 ± 1.1	>100	>100	>100	9.5 ± 4.1	>100
4	**43**	H	OH	$COCH_3$	52.2 ± 1.7	>100	>100	>100	57.1 ± 7.6	>100
5	**44**	H	OH	H	2.6 ± 0.4	42.1 ± 1.3	75.7 ± 11.8	52.5 ± 1.2	10.2 ± 1.2	28.4 ± 1.1
6	**45**	H	H	$COCH_3$	8.8 ± 0.9	69.4 ± 12.9	>100	>100	59.6 ± 7.6	>100
7	**46**	H	H	F	16.1 ± 1.6	>100	>100	-	-	-
8	**47**	H	H	CF_3	>100	>100	>100	-	-	-
9	**Cerco** [3]				31.5 ± 4.7	>100	>100	44.3 ± 2.1	7.5 ± 3.1	-
10	**Doxo** [3]				-	-	-	0.50 ± 0.02	2.7 ± 0.2	2.4 ± 0.4
11	**SGI-1776** [3]				0.030 ± 0.003	3.5 ± 0.6	3.7 ± 1.5	-	-	-

[1] IC$_{50}$ HT-29 >100 μM for all the compounds and **Cerco** IC$_{50}$ = 10.4 ± 2.5 μM; **Doxo** IC$_{50}$ = 0.72 ± 0.5 μM. [2] Values are a mean of n ≥ 3 independent experiments. Cells were treated with concentrations ranging from 100 nM to 100 μM for 48 h or 72 h (MCF-7, Hela and L929). Cell viability was then determined by MTT assays, and EC$_{50}$ values were calculated using Graphpad PRISM 7 software (n = 3 in triplicate; data are in the mean ± SEM). -: Not determined. [3] Cercosporamide (**Cerco**) was used as the reference compound. Doxorubicin (**Doxo**) and SGI-1776 were used as positive controls.

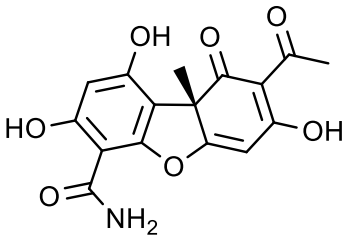
Results and discussion:
evaluation of *in vivo* cytotoxicity on *G. mellonella* model



Conclusions:

Mandatory for binding to Pim-1

Not essential for binding to Pim-1



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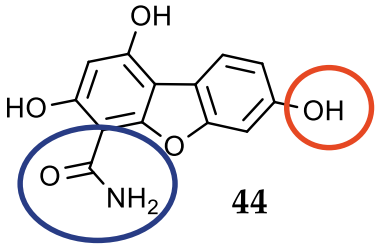


44

Pim-1 IC_{50} = 60 nM

Pim-2 IC_{50} = 35 nM

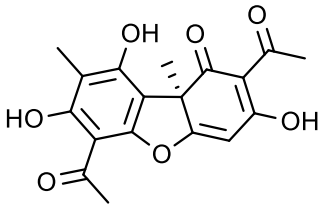
CLK1 IC_{50} = 26 nM

MV4-11 IC_{50} = 2.6 ± 0.4 μM

AML cell line

- Cercosporamide is a valuable starting point for medicinal chemistry investigations

- The replacement of the carboxamide group will be investigated



(+)-Usnic acid

Pim-1 IC_{50} = 210 nM

Pim-2 IC_{50} = 580 nM

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