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

Pascal Marchand ¹,*

¹ Université de Nantes, Cibles et médicaments des infections et du cancer, IICiMed, EA 1155, F-44000, Nantes, France.

* Corresponding author: pascal.marchand@univ-nantes.fr
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$_3$

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
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.
Introduction: structure of cercosporamide

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

Enantiopure (S)-(—)-cercosporamide

\[ \alpha_D = -26^\circ \]

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 CaPkc1
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[\textit{b,d}]furan derivatives

\(-\)-Cercosporamide

\text{Pim-1 } IC_{50} = 732 \text{ nM} \\
\text{Pim-2 } IC_{50} = 1430 \text{ nM}

Dibenzo[\textit{b,d}]furans

\text{R}_1 = \text{H, NO}_2, \text{ NH}_2 \\
\text{R}_2 = \text{H, OH} \\
\text{R}_3 = \text{H, COCH}_3, \text{ F, CF}_3

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. \textit{Molecules} 2021, 26, 6572. https://doi.org/10.3390/molecules26216572

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-\textit{d}]pyrimidines inspired from cercosporamide \textit{Ca}Pkc1 inhibitor: Synthesis and evaluation of fluconazole susceptibility restoration. \textit{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.; Denevaut-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\(_3\)CN, 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\(_4\)Cl, CH\(_3\)OH, 80 °C, 2 h; (iv) 1) NaNO\(_2\), H\(_2\)SO\(_4\), 0 °C, 45 min, 2) Cu(0), H\(_2\)SO\(_4\), 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$_3$CN, 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$_4$Cl, CH$_3$OH, 80 °C, 2 h, 62% (for 16) and 49% (for 18); (v) NaNO$_2$, H$_2$SO$_4$, 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

Scheme 3. Reagents and conditions: (i) 3,5-Dimethoxyphenol 4, Cs$_2$CO$_3$, CuI, DMF, 110 °C, MW, 1 h; (ii) Pd(OAc)$_2$, AgOAc, PivOH, 130 °C, 4-24 h; (iii) CSI, CH$_3$CN, 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$_2$CH$_2$Cl, rt, 1 h, 74%. 

Scheme 3.
Results and discussion: kinase selectivity profile of dibenzo\[b,d\]furane derivatives

![Chemical structures](image)

| Entry | Compound | Series | R₁   | R₂   | R₃   | CDK5/ p25 | CDK9/ CyclinT | Pim-1 | CLK1 |
|-------|----------|--------|------|------|------|-----------|---------------|-------|------|
| 1     | Cerco ³  | A      | NO₂  | H    | H    | 5.60      | 0.22          | 0.73  | >10  |
| 2     | 14       | A      | NO₂  | H    | H    | >10       | >10           | 3.21  | >10  |
| 3     | 15       | B      | NO₂  | H    | H    | >10       | >10           | 0.18  | 1.22 |
| 4     | 18       | A      | NH₂  | H    | H    | >10       | >10           | >10   | >10  |
| 5     | 16       | B      | NH₂  | H    | H    | >10       | >10           | 0.13  | 0.45 |
| 6     | 19       | A      | H    | H    | H    | >10       | >10           | >10   | >10  |
| 7     | 17       | B      | H    | H    | H    | >10       | >10           | >10   | >10  |
| 8     | 38       | A      | OCH₃ | COCH₃| COCH₃| >10       | >10           | >10   | >10  |
| 9     | 43       | B      | H    | OH   | COCH₃| >10       | >10           | >10   | >10  |
| 10    | 39       | A      | OCH₃ | H    | COCH₃| >10       | >10           | >10   | >10  |
| 11    | 44       | B      | H    | OH   | H    | >10       | >10           | 0.06  | 0.026|
| 12    | 40       | A      | H    | H    | COCH₃| >10       | >10           | >10   | >10  |
| 13    | 45       | B      | H    | H    | COCH₃| >10       | >10           | 0.28  | 0.14 |
| 14    | 41       | A      | H    | H    | F    | >10       | >10           | >10   | >10  |
| 15    | 46       | B      | H    | H    | F    | 0.92      | 1.20          | 0.62  | 0.85 |
| 16    | 42       | A      | H    | H    | CF₃  | >10       | >10           | >10   | >10  |
| 17    | 47       | B      | H    | H    | CF₃  | >10       | >10           | 2.35  | >10  |

¹ IC₅₀ 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 phosphatase 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: Proviial 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

![Chemical Structure Diagram]

| Entry | Compound | R$_1$ | R$_2$ | R$_3$ | IC$_{50}$ (µM) |
|-------|----------|------|------|------|---------------|
| 1     | Cerco$^2$ |      |      |      | 0.73          |
| 2     | 15       | NO$_2$ | H    | H    | 0.18          |
| 3     | 16       | NH$_2$ | H    | H    | 0.13          |
| 4     | 17       | H      | H    | H    | 0.23          |
| 5     | 43       | H      | OH   | COCH$_3$ | 0.82 |
| 6     | 44       | H      | OH   | H    | 0.06          |
| 7     | 45       | H      | H    | COCH$_3$ | 0.28 |
| 8     | 46       | H      | H    | F    | 1.2           |
| 9     | 47       | H      | H    | CF$_3$ | 2.35 |

$^1$ 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

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

| Entry | Compound | R₁ | R₂ | R₃ | MV4-11  | KU812    | K562     | MCF-7    | HeLa     | L929     |
|-------|----------|----|----|----|---------|----------|----------|----------|----------|----------|
| 1     | 15       | NO₂| H  | H  | 30.7 ± 1.1| 78.4 ± 2.1| >100     | >100     | 24.7 ± 6.7| 49.2 ± 1.0|
| 2     | 16       | NH₂| 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 | COCH₃| 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  | COCH₃| 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  | CF₃| >100     | >100     | >100     | -        | -        | -        |
| 9     | Cerco³   | H  | H  |     | 31.5 ± 4.7| >100     | >100     | 44.3 ± 2.1| 7.5 ± 3.1| -        |
| 10    | Doxo³    |     |     |     | -        | -        | -        | 0.50 ± 0.02| 2.7 ± 0.2| 2.4 ± 0.4|
| 11    | SGI-1776 |     |     |     | 0.030 ± 0.003| 3.5 ± 0.6| 3.7 ± 1.5| -        | -        | -        |

¹ IC₅₀ HT-29 >100 μM for all the compounds and Cerco IC₅₀ = 10.4 ± 2.5 μM; Doxo IC₅₀ = 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 EC₅₀ 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:

- Cercosporamide is a valuable starting point for medicinal chemistry investigations.
- The replacement of the carboxamide group will be investigated.

(-)-Cercosporamide

Pim-1 IC$_{50}$ = 732 nM  
Pim-2 IC$_{50}$ = 1430 nM  

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

(+)-Usnic acid

Pim-1 IC$_{50}$ = 210 nM  
Pim-2 IC$_{50}$ = 580 nM
Acknowledgments

EA1155 – IICiMed, Nantes - France

Dr. Viet Hung Dao
Dr. Isabelle Ourliac-Garnier
Dr. Marc-Antoine Bazin
Dr. Cédric Logé
Jérome Thiéfaine

Screening platform: KISSf

Dr. Stéphane Bach
Blandine Baratte
Thomas Robert

Federal University of Pernambuco
Recife - Brazil

Prof. Teresinha G. da Silva

University College Cork
Ireland

Dr. Florence O. McCarthy

University of Tours - France
Dr. Caroline Denevault-Sabourin
Dr. Fabrice Gouilleux
Dr. Marie Brachet-Botineau