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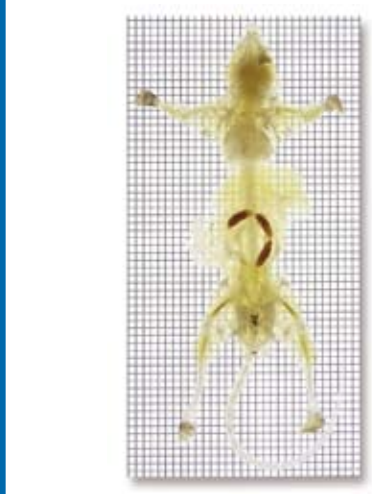


CONTENTS

TCI Product Feature

CUBIC Reagents

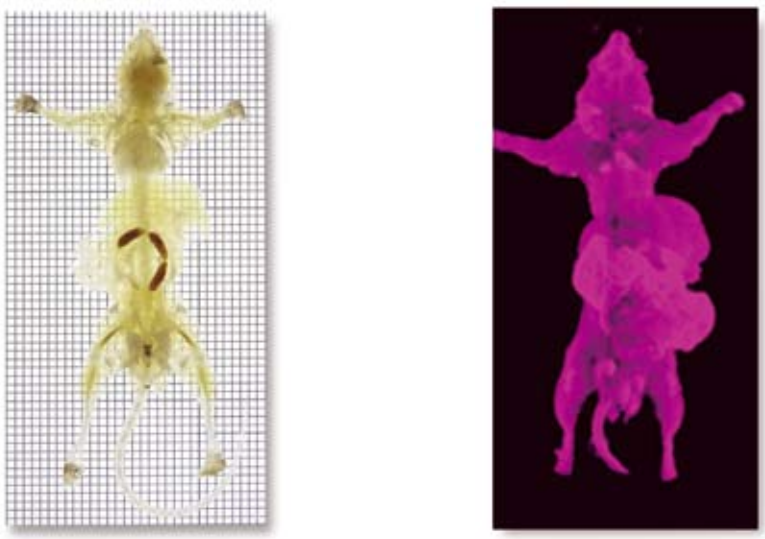
- for Animal Tissue, Whole-Organ and Whole-Body Clearing -

A tissue clearing method "CUBIC" has been developed by Prof. Hiroki R. Ueda and coworkers at The University of Tokyo / RIKEN. The CUBIC technique enables cyclopedic imaging at a single-cell resolution following whole-body and whole-organ clearing. CUBIC reagents (Product Number: T3740, T3741) that can be used in the tissue clearing method are provided by TCI.

● Advantages of clearing by CUBIC reagents

- Whole-body clearing is achieved using two reagents, T3740 CUBIC-L (for delipidation and decoloring) and T3741 CUBIC-R+ (for RI matching).
- The quenching of fluorescence signal is low.
- The period of sample treatment is shorter.
- The combination with light-sheet fluorescent microscopy (LSFM) or confocal laser-scanning microscopy (CLSM) enables the whole-organ / body imaging at a cellular resolution.

● Example : Mouse whole-body clearing



Fligure 1. Whole-body clearing (Left), Whole-body clearing with propidium iodide staining (Right)

Mouse whole-body clearing procedure

Pre-treatment	Delipidation	Wash x 3	Pre-treatment	RI match
50% CUBIC-L > 6 hr	CUBIC-L > 5 days	PBS > 2hr x 3	50% CUBIC-R+ 1 day	CUBIC-R+ > 1 day

Process	Reagent	Temp.	Time	Notes
Perfusion fixation	PBS 4% PFA in PBS			Finally, the mouse should be perfused with 50% CUBIC-L which is a 1:1 mixture of water and CUBIC-L.
Perfusion	PBS 50% CUBIC-L			
Pre-treatment	50% CUBIC-L	37℃	> 6 hr	Immerse the whole body of the mouse with gentle shaking (same in following steps). This step can be omitted.
Delipidation	CUBIC-L	37℃	> 5 days	Refresh CUBIC-L on day 1, day 2 and every 2 days after day 4
Wash x 3	PBS	RT	> 2hr x 3	Total 1 day
Pre-treatment	50% CUBIC-R+	RT	1 day	1:1 mixture of water and CUBIC-R+
RI matching	CUBIC-R+	RT	> 1 day	

Work samples in a tube in which whole-body can be contained.
PFA : paraformaldehyde, RT : room temperature

Mouse whole-body clearing procedure for staining

Example : nuclear staining by propidium iodide (PI)

Pre-treatment	Delipidation / Staining	Wash x 3	Pre-treatment	RI match
50% CUBIC-L > 6 hr	PI in CUBIC-L > 7 days	PBS > 2hr x 3	50% CUBIC-R+ 1 day	CUBIC-R+ > 1 day

Process	Reagent	Temp.	Time	Notes
Perfusion fixation	PBS 4% PFA in PBS			Finally, the mouse should be perfused with 50% CUBIC-L which is a 1:1 mixture of water and CUBIC-L.
Perfusion	PBS 50% CUBIC-L			
Pre-treatment	50% CUBIC-L	37℃	> 6 hr	Immerse the whole body of the mouse with gentle shaking (same in following steps). This step can be omitted.
Delipidation / Staining	5 μg/mL PI in CUBIC-L	37℃	> 7 days	Refresh PI CUBIC-L on day 1, day 2, and every 2 days after day 4
Wash x 3	PBS	RT	> 2hr x 3	Total 1 day
Pre-treatment	50% CUBIC-R+	RT	1 day	1:1 mixture of water and CUBIC-R+
RI matching	CUBIC-R+	RT	> 1 day	

Example : nuclear staining by RedDot2

Pre-treatment	Delipidation	Wash	Staining	Wash	Pre-treatment	RI match
50% CUBIC-L > 6 hr	in CUBIC-L > 5 days	PBS > 2hr x 3	RedDot2 in PBS > 3 days	PBS > 2hr x 3	50% CUBIC-R+ 1 day	CUBIC-R+ > 1 day

Process	Reagent	Temp.	Time	Notes
Perfusion fixation	PBS 4% PFA in PBS			Finally, the mouse should be perfused with 50% CUBIC-L which is 1:1 mixture of water and CUBIC-L.
Perfusion	PBS 50% CUBIC-L			
Pre-treatment	50% CUBIC-L	37℃	> 6 hr	Immerse the whole body of the mouse with gentle shaking (same in following steps). This step can be omitted.
Delipidation	CUBIC-L	37℃	> 5 days	Refresh CUBIC-L on day 1, day 2 and every 2 days after day 4
Wash	PBS	RT	> 2hr x 3	Total 1 day
Staining	1 : 100 diluted RedDot2 in PBS*	RT	> 3 days	*Comprised of 0.5% Triton X-100 and 0.25% casein
Wash x 3	PBS	RT	> 2hr x 3	Total 1 day
Pre-treatment	50% CUBIC-R+	RT	1 day	1:1 mixture of water and CUBIC-R+
RI matching	CUBIC-R+	RT	> 1 day	

● Example : Mouse whole-organ clearing

Figure 2. Whole-brain clearing (Left), Whole-brain clearing with RedDot 2 staining and immunostaining (Right)

Mouse whole-organ clearing procedure

Fix	Wash x 3	Pre-treatment	Delipidation	Wash x 3	Pre-treatment	RI match
4% PFA 1 day	PBS > 2hr x 3	50% CUBIC-L 6 – 24 hr	CUBIC-L > 2 days	PBS > 2hr x 3	50% CUBIC-R+ 6 – 24 hr	CUBIC-R+ > 2 days

Process	Reagent	Temp.	Time	Notes
Tissue excision				After perfusion fixation
Tissue Fix	4% PFA in PBS	4℃	1 day	
Wash x 3	PBS	RT	> 2hr x 3	Shake gently (same in following steps). Total 1 day
Pre-treatment	50% CUBIC-L	37℃	6 – 24 hr	1:1 mixture of water and CUBIC-L This step can be omitted.
Delipidation	CUBIC-L	37℃	> 2 days	Refresh CUBIC-L on day 1, day 2 and every 2 days after day 4
Wash x 3	PBS	RT	> 2hr x 3	Total 1 day
Pre-treatment	50% CUBIC-R+	RT	6 – 24 hr	1:1 mixture of water and CUBIC-R+
RI matching	CUBIC-R+	RT	> 2 days	

Work in a tube whose diameter is a little larger than that of organs. Appropriate liquid volume is essential as most of the organs are immersed in the liquid when the tube is in a horizontal position.

Mouse whole-organ clearing procedure for staining

Example : immunostaining

Fix	Wash x 3	Pre-treatment	Delipidation	Wash x 3	Staining	Wash x 3	Pre-treatment	RI match
4% PFA 1 day	PBS > 2hr x 3	50% CUBIC-L 6 – 24 hr	CUBIC-L > 2 days	PBS > 2hr x 3	antibodies > 3 days	PBS > 2hr x 3	50% CUBIC-R+ 6 – 24 hr	CUBIC-R+ > 2 days

Process	Reagent	Temp.	Time	Notes
Tissue excision				After perfusion fixation
Tissue Fix	4% PFA in PBS	4℃	1 day	
Wash x 3	PBS	RT	> 2hr x 3	Shake gently (same in following steps). Total 1 day
Pre-treatment	50% CUBIC-L	37℃	6 – 24 hr	1:1 mixture of water and CUBIC-L This step can be omitted.
Delipidation	CUBIC-L	37℃	> 2 days	Refresh CUBIC-L on day 1, day 2 and every 2 days after day 4
Wash x 3	PBS	RT	> 2hr x 3	Total 1 day
Staining	Antibody*1 in PBS*2	RT	> 3 days	*1 Use the fluorescent labeled antibody as a primary antibody. *2 Comprised of 0.5% Triton X-100, 0.25% casein and 0.01% sodium azide.
Wash x 3	PBS	RT	> 2hr x 3	Total 1 day
Pre-treatment	50% CUBIC-R+	RT	6 – 24 hr	1:1 mixture of water and CUBIC-R+
RI matching	CUBIC-R+	RT	> 2 days	

The staining protocol is not yet optimized completely. Please follow the latest publications.

References

1) Whole-body profiling of cancer metastasis with single-cell resolution
S. I. Kubota, K. Takahashi, J. Mishida, Y. Morishita, S. Ehata, K. Tainaka, K. Miyazono, H. R. Ueda, *Cell Reports* **2017**, *20*, 236.
2) Whole-brain imaging with single-cell resolution using chemical cocktails and computational analysis
E. A. Susaki, K. Tainaka, D. Perrin, F. Kishino, T. Tawara, T. M. Watanabe, C. Yokoyama, H. Onoe, M. Eguchi, S. Yamaguchi, T. Abe, H. Kiyonari, Y. Shimizu, A. Miyawaki, H. Yokota, H. R. Ueda, *Cell* **2014**, *157*, 726.
3) Whole-body imaging with single-cell resolution by tissue decolorization
K. Tainaka, S. I. Kubota, T. Q. Suyama, E. A. Susaki, D. Perrin, M. Ukai-Tadenuma, H. Ukai, H. R. Ueda, *Cell* **2014**, *159*, 911.
4) RIKEN Quantitative Biology Center, CUBIC protocol and etc.
http://cubic.riken.jp/

The pictures are provided by Prof. Hiroki R. Ueda.

CUBIC Reagents

T3740	Tissue-Clearing Reagent CUBIC-L [for Animals]	25mL 100mL
T3741	Tissue-Clearing Reagent CUBIC-R+ [for Animals]	25mL 100mL

These products are under invention licenses by RIKEN, Japan.
Both of CUBIC-L and CUBIC-R+ are required for tissue-clearing.

Perovskite Precursor: High Quality Tin (II) Iodide (SnI_2)

T3449 Tin(II) Iodide [for Perovskite precursor] (1) 1g 5g

In recent years, perovskite solar cells (PSCs) have received much attention as a novel solar cell type, and display power conversion efficiencies over 20% seen via PSC devices whose active layers were mainly consisted of lead.[1] On the other hand, lead-free or mixed metal PSCs have also been heavily researched (Figure 1)[2] due to the concerns of lead toxicity. Tin is one of the possible lead alternatives, and tin(II) iodide can be readily used as a precursor of perovskites. Using a high quality precursor without contamination from tin(IV) compounds (e.g. SnI_4) is required in order to fabricate a good perovskite layer.[3] Subsequently, TCI offers product **1** as high quality tin(II) iodide for perovskite precursor with extremely low tin(IV) content to meet purity requirement needs. In addition, **1** is suitable for solution-processed solar cells because its water content is low (<100 ppm) and it provides clear DMF solutions (Figure 3).

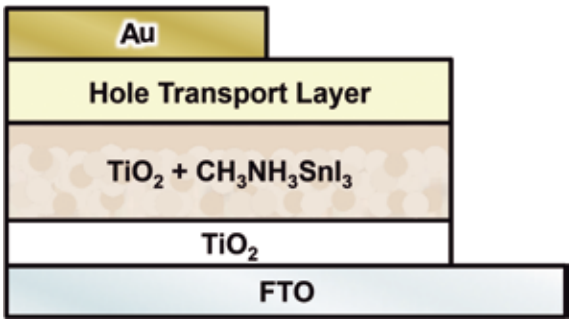


Figure 1. A typical device structure of lead-free perovskite solar cells[3]

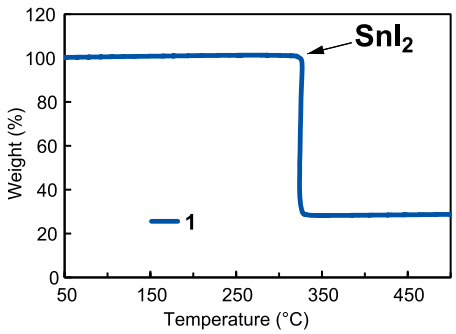


Figure 2. Thermogravimetric (TG) analysis of **1**

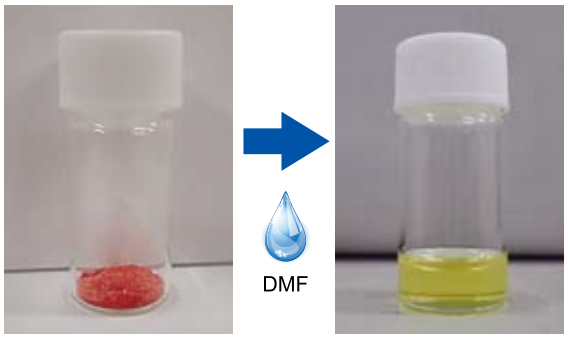


Figure 3. Crystalline solid (left) and DMF solution (right) of **1**

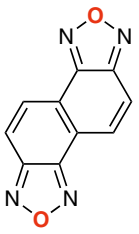
References

1) High-performance photovoltaic perovskite layers fabricated through intramolecular exchange
W. S. Yang, J. H. Noh, N. J. Jeon, Y. C. Kim, S. Ryu, J. Seo, S. I. Seok, *Science* **2015**, *348*, 1234.
2) Current advancements in material research and techniques focusing on lead-free perovskite solar cells
C. Zhang, L. Gao, S. Hayase, T. Ma, *Chem. Lett.* **2017**, *46*, 1276.
3) Lead-free solid-state organic–inorganic halide perovskite solar cells
F. Hao, C. C. Stoumpos, D. H. Cao, R. P. H. Chang, M. G. Kanatzidis, *Nat. Photonics* **2014**, *8*, 489.

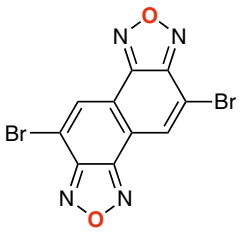
Organic Semiconductor Building Blocks with Benzoxadiazole Core

N1137	Naphtho[1,2-*c*:5,6-*c*']bis([1,2,5]oxadiazole) (1)	200mg
D5496	5,10-Dibromonaphtho[1,2-*c*:5,6-*c*']bis([1,2,5]oxadiazole) (2)	100mg
B5774	5,10-Bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-naphtho[1,2-*c*:5,6-*c*']bis([1,2,5]oxadiazole) (3)	100mg

Naphtho[1,2-*c*:5,6-*c*']bis([1,2,5]oxadiazole) (NOz) analogs (**1**-**3**) are used as electron-acceptor type building blocks for the constructing organic semiconductors. They are more electron-deficient compared to the naphtho[1,2-*c*:5,6-*c*']bis([1,2,5]thiadiazole) (NTz) analogs. Donor-acceptor conjugated polymers containing the NOz structures are expected to have deeper HOMO and LUMO levels than those of the NTz-based polymers. In fact, it has been reported that a donor-acceptor conjugated polymer (PNOz4T) and subsequently deep energy levels and high crystallinity, provides high hole mobility properties (μ_h = 0.55 cm^2/Vs, on triethoxy-1*H*,1*H*,2*H*,2*H*-heptadecafluorodecylsilane-treated substrates) and ambipolar behavior (μ_h = 0.27 cm^2/Vs and μ_e = 0.17 cm^2/Vs, on octadecyltriethoxysilane-treated substrates).[1)]



1



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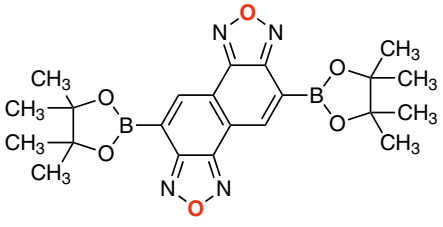
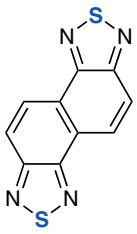
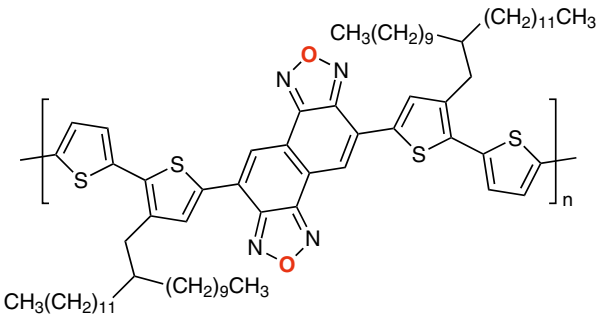


3



NTz



PNOz4T[1)]

Reference

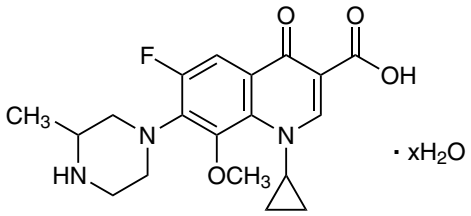
1) Effect of chalcogen atom on the properties of naphthobischalcogenadiazole-based π-conjugated polymers
K. Kawashima, I. Osaka, K. Takimiya, *Chem. Mater.* **2015**, *27*, 6558.

TCI Related Products

N1105	Naphtho[1,2-*c*:5,6-*c*']bis([1,2,5]thiadiazole)	200mg
D5288	5,10-Dibromonaphtho[1,2-*c*:5,6-*c*']bis([1,2,5]thiadiazole)	100mg
B5470	5,10-Bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)naphtho[1,2-*c*:5,6-*c*']bis([1,2,5]thiadiazole)	100mg

Topoisomerase Inhibitor

G0325 Gatifloxacin Hydrate (1) 1g



1

Gatifloxacin (**1**) is one of the fluoroquinolone antibiotics and shows a broad spectrum of antibacterial activity against gram-positive and gram-negative bacteria.[1] **1** inhibits bacterial DNA gyrase and topoisomerase IV (Table 1).[2] Furthermore, **1** promotes short-term self-renewal and differentiation of human embryonic stem cells.[3]

Table 1. Inhibition of type II topoisomerase by Gatifloxacin[2]

Enzyme	IC_{50} (μg/ml)
Topoisomerase IV from *S. Aureus* MS5935	13.8
DNA gyrase from *E.Coli* NIHJ JC-2	0.109
Topoisomerase II from HeLa cell	265

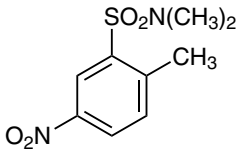
This product is for research purpose only.

References

1) In vitro and in vivo antibacterial activities of AM-1155, a new 6-fluoro-8-methoxy quinolone
M. Hosaka, T. Yasue, H. Fukuda, H. Tomizawa, H. Aoyama, K. Hirai, *Antimicrob. Agents Chemother.* **1992**, *36*, 2108.
2) Inhibitory activities of Gatifloxacin (AM-1155), a newly developed fluoroquinolone, against bacterial and mammalian type II topoisometases
M. Takei, H. Fukuda, T. Yasue, M. Hosaka, Y. Oomori, *Antimicrob. Agents Chemother.* **1998**, *42*, 2678.
3) High-throughput screening assay for the identification of compounds regulating self-renewal and differentiation in human embryonic stem cells
S. C. Desbordes, D. G. Placantonakis, A. Ciro, N. D. Socci, G. Lee, H. Djaballah, L. Studer, *Cell Stem Cell* **2008**, *2*, 602.

Phosphodiesterase 7 Inhibitor

T3112 BRL 50481 (1) **200mg 1g**



1

BRL 50481 (**1**) is a selective inhibitor of phosphodiesterase 7 (PDE7).[1] **1** inhibits human recombinant PDE7 in a competitive manner (Ki = 180 nM). **1** is much less potent against PDE3 and PDE4 (Table 1). In addition, treatment of human mesenchymal stem cell-derived osteoblasts with **1** increased mineralization.[2]

Table 1. Selectivity of BRL 50481 (**1**) to PDE isoenzymes[1]

	Inhibition of Cyclic Nucleotide Hydrolysis [IC_{50} (μM)]						
	hrPDE7A1	PDE1B	PDE1C	PDE2	PDE3	hrPDE4A4	PDE5
cAMP/cGMP concentration							
0.05 μM	0.26	>100	>100	>100	490	62	>100
1 μM	2.4	>100	>100	>100	>1000	92	>100

This product is for research purpose only.

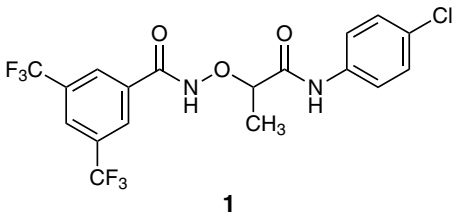
References

1) Discovery of BRL 50481 [3-(*N,N*-dimethylsulfonamido)-4-methyl-nitrobenzene], a selective inhibitor of phosphodiesterase 7: In vitro studies in human monocytes, lung macrophages, and $CD8^+$ T-lymphocytes
S. J. Smith, L. B. Cieslinski, R. Newton, L. E. Donnelly, P. S. Fenwick, A. G. Nicholson, P. J. Barnes, M. S. Barnette, M. A. Giembycz, *Mol. Pharmacol.* **2004**, *66*, 1679.

2) Effects of phosphodiesterase 7 inhibition by RNA interference on the gene expression and differentiation of human mesenchymal stem cell-derived osteoblasts
M. Pekkinen, M. E. B. Ahlström, U. Riehle, M. M. Huttunen, C. J. E. Lamberg-Allardt, *Bone* **2008**, *43*, 84.

RhoA Transcriptional Signaling Inhibitor

C3449 CCG-1423 (1) **25mg 100mg**



1

CCG-1423 (**1**) is an inhibitor of Rho signaling.[1] The target molecule is myocardin-related transcription factor A (MRTF-A).[2] **1** inhibits MRTF-A binding to importin α/β1.

Mouse embryonic stem cells (mESCs) are differentiated to BMP-7-positive cells by treatment in combination with **1** and a PI3K inhibitor, LY294002.[3] The combination of these small molecules is also used for differentiation of human induced pluripotent stem cells (iPSCs) to renal progenitors.[4]

BMP-7: Bone morphogenetic protein-7

This product is for research purpose only.

References

1) CCG-1423: A small-molecule inhibitor of RhoA transcriptional signaling
C. R. Evelyn, S. M. Wade, Q. Wang, M. Wu, J. A. Iñiguez-Lluhí, S. D. Merajver, R. R. Neubig, *Mol. Cancer Ther.* **2007**, *6*, 2249.

2) RPEL proteins are the molecular targets for CCG-1423, an inhibitor of Rho signaling
K. Hayashi, B. Watanabe, Y. Nakagawa, S. Minami, T. Morita, *PLoS ONE* **2014**, *9*, e89016.

3) Combination of small molecules enhances differentiation of mouse embryonic stem cells into intermediate mesoderm through BMP7-positive cells
S. Mae, S. Shirasawa, S. Yoshie, F. Sato, Y. Kanoh, H. Ichikawa, T. Yokoyama, F. Yue, D. Tomotsune, K. Sasaki, *Biochem. Biophys. Res. Commun.* **2010**, *393*, 877.

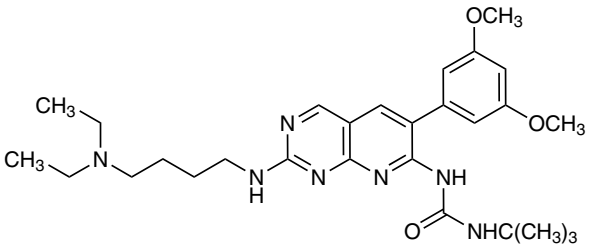
4) Renal progenitors derived from human iPSCs engraft and restore function in a mouse model of acute kidney injury
B. Imberti, S. Tomasoni, O. Ciampi, A. Pezzotta, M. Derosas, C. Xinaris, P. Rizzo, E. Papadimou, R. Novelli, A. Benigni, G. Remuzzi, M. Morigi, *Sci. Rep.* **2015**, *5*, 8826.

TCI Related Product

M2410 LY294002 25mg

FGFR/VEGFR Tyrosine Kinase Inhibitor

P2474 PD173074 (1) **10mg 50mg**



1

PD173074 (**1**) inhibits the tyrosine kinase activities of the fibroblast growth factor receptor (FGFR) and vascular endothelial growth factor receptor (VEGFR) (Table 1).[1] The inhibitory manner of **1** is ATP-competitive and the inhibitory constants (Ki) of **1** to FGFR1 and its cytoplasmic domain are 45.2 ± 4.8 nM and 36.4 ± 3.6 nM, respectively.

1 also inhibits the differentiation of mouse and human embryo stem cells[2,3] and human induced pluripotent stem cells.[4]

Table 1. Inhibitory activity of PD173074

Protein Kinase	IC_{50}
FGFR1	21.5 ± 0.8 nM
FGFR1 (cytoplasmic domain)	28.9 ± 1.9 nM
PDGFR (cytoplasmic domain)	17.6 ± 1.9 μM
C-Src	19.8 ± 2.3 μM
EGFR	>50 μM
InsR	>50 μM
MAPK	>50 μM
PKC	>50 μM

PDGFR, platelet-derived growth factor receptor; EGFR, epidermal growth factor receptor; InsR, insulin receptor; MAPK, mitogen-activated protein kinase; PKC, protein kinase C

This product is for research purpose only.

References

1) Crystal structure of an angiogenesis inhibitor bound to the FGF receptor tyrosine kinase domain
M. Mohammadi, S. Froum, J. H. Hamby, M. C. Schroeder, R. L. Panek, G. H. Lu, A. V. Eliseenkova, D. Green, J. Schlessinger, S. R. Hubbard, *EMBO J.* **1998**, *17*, 5896.
2) The ground state of embryonic stem cell self-renewal
Q.-L. Ying, J. Wray, J. Nichols, L. Batlle-Morera, B. Doble, J. Woodgett, P. Cohen, A. Smith, *Nature* **2008**, *453*, 519.
3) Human embryonic stem cells with biological and epigenetic characteristics similar to those of mouse ESCs
J. Hanna, A. W. Cheng, K. Saha, J. Kim, C. J. Lengner, F. Soldner, J. P. Cassady, J. Muffat, B. W. Carey, R. Jaenisch, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 9222.
4) Virus-free induction of pluripotency and subsequent excision of reprogramming factors
K. Kaji, K. Norrby, A. Paca, M. Mileikovsky, P. Mohseni, K. Woltjen, *Nature* **2009**, *458*, 771.

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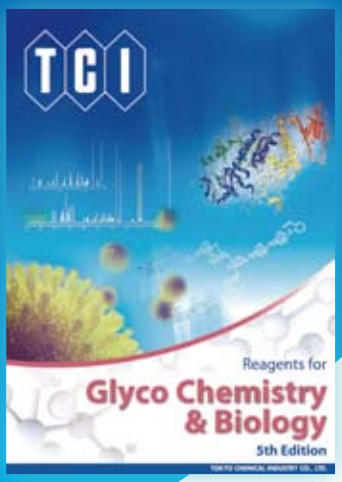

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