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4-Hydroxy-*N*-[3,5-bis(trifluoromethyl)phenyl]-1,2,5-thiadiazole-3-carboxamide: a novel inhibitor of the canonical NF-κB cascade†

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The NF-κB signaling pathway is a validated oncological target. Here, we applied scaffold hopping to IMD-0354, a presumed IKKβ inhibitor, and identified 4-hydroxy-*N*-[3,5-bis(trifluoromethyl)phenyl]-1,2,5-thiadiazole-3-carboxamide (**4**) as a nM-inhibitor of the NF-κB pathway. However, both **4** and IMD-0354, being potent inhibitors of the canonical NF-κB pathway, were found to be inactive in human IKKβ enzyme assays.

NF-κB is a ubiquitously expressed family of transcription factors, known to be key regulators of immune response, cell proliferation, cell death and inflammation.[1] NF-κB dimers are normally inhibited in the cytoplasm of resting cells by proteins called IκB. Following cell stimulation, the inhibitory IκB proteins are rapidly phosphorylated by the so-called IKK kinases, and subsequently degraded by the 26S proteasome. This is followed by the translocation of the transcription factors to the nuclei and subsequent activation of the corresponding gene expression (the so-called canonical pathway of NF-κB activation). NF-κB signaling has been found to be constitutively activated in a variety of malignancies, leading to uncontrolled apoptosis, cell cycle deregulation and metastatic growth.[1] These observations validated the NF-κB pathway as an oncologic target, in particular in breast[2] and thyroid cancer.[3] The trimeric IKK complex involved in the activation of the canonical NF-κB pathway contains two catalytic subunits, IKKα and IKKβ kinase, and a regulatory protein IKKγ (also called NEMO). In a parallel, so-called non-canonical pathway,[4] TNF-receptor superfamily members selectively activate a different set of kinases, NF-κB-inducing kinase (NIK) and IκB kinase 1 (IKK1).[4]

The efforts aimed at discovering new NF-κB inhibitors, and particularly inhibitors of IKKβ, have been intensified in recent years.[5] Recently, the X-ray structures of IKKβ in a ligand-free form and in complex with an inhibitor have been reported,[6,7] thus providing new opportunities for the design of new potent inhibitors.[1] Although no IKKβ inhibitors have reached the human pharmacopoeia yet, some interesting molecules have been studied. Among those is PS-1145 (Fig. 1), a β-carboline analogue known to potently and selectively inhibit the endogenous IKK complex with an IC_{50} of 150 nM.[8–10] Other examples include, BMS-345541[11] which acts by allosterically inhibiting IKKβ, and IMD-0354, which reached the stage of clinical trials[12] (Fig. 1). IMD-0354 was designed by the *Institute of Medicinal Molecular Design Inc* (Tokyo, Japan) and is claimed to be a selective inhibitor of IKKβ.[13] The compound has completed phase 1 clinical trials as an anti-inflammatory, anti-allergic and anti-microbial agent,[14] while its prodrug, called IMD-1041[15] was evaluated in clinical trials that are currently awaiting proof-of-concept results (NCT00883584).[14]

The biological activity of IMD-0354 has been described in a variety of assays related to metabolic diseases and cardiovascular diseases.[15–27] In particular, it has been reported that

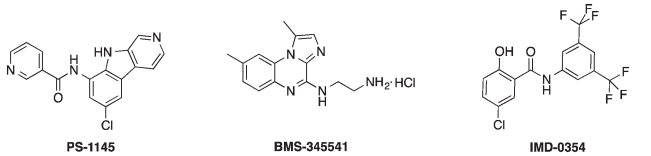

Fig. 1 PS-1145, BMS-345541 and IMD-0354 as examples of IKKβ inhibitors.

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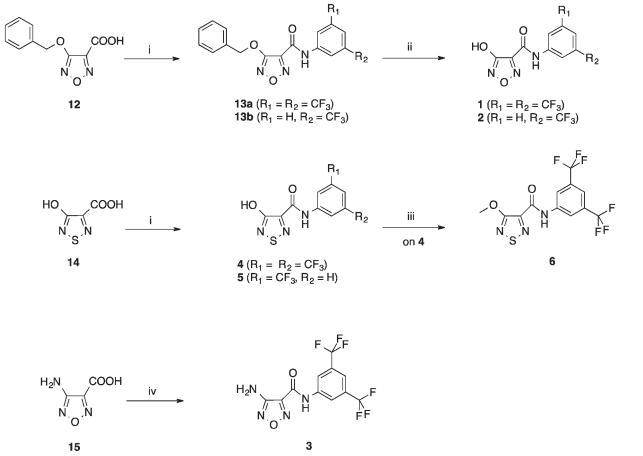
IMD-0354 is able to suppresses neoplastic proliferation of human mast cells with constitutively activated c-kit receptor,[28] and inhibits the growth of human breast cancer cells MDA-MB-231, HMC1-8 and MCF-7.[29]

Bio(iso)steric replacement is a widely used approach in medicinal chemistry, aimed at improving the characteristics of a lead compound, such as bioavailability, selectivity, and potency.[30] As an example of the use of the method, we recently designed a new generation of potent *h*DHODH inhibitors[31] using hydroxylated azoles. In these systems, the substitution of the azole ring allowed fine-tuning of the accessible chemical space,[32] thus increasing the probability of triggering the desired change in biological activity. Here, we applied a similar approach to the phenolic substructure in IMD-0354 to design new IKKβ inhibitors. The compounds **1** to **11** (Fig. 2) have been designed using four different acidic azoles: *hydroxyoxadiazole*, *hydroxythiadiazole*, *hydroxytriazole* and *hydroxypyrazole*. The four hydroxyazole systems have been selected to bioisosterically modulate the phenolic moiety of IMD-0354 by their different acidic and lipophilic properties.[33] Notably, *hydroxytriazole* and *hydroxypyrazole* scaffolds can be modified by the addition of protruding substituents, which may be designed to occupy the surrounding chemical space in several directions. Schemes 1 and 2 outline the synthetic methodologies used for the preparation of the target compounds **1–11**.

In some cases, a benzyloxy-protected azolecarboxylate (**12**, **16**, **20**) was transformed into the corresponding acyl chloride, which was allowed to react with the appropriate aniline, thus obtaining the corresponding amide (compounds **13**, **17** and **21**). The removal of the protecting benzyl group was accomplished by applying room pressure hydrogenation conditions (**1**, **2**, **7**, **8**, **10**, **11**). In the case of triazole analogue **9** (Scheme 2), the coupling reaction was conducted on the *p*-methoxybenzyl protected precursor **18**, previously obtained from its corresponding ethyl ester.[33] The resulting amide **19** was then deprotected under acidic conditions (TFA).



Scheme 1 Synthesis of 1,2,5-oxadiazole and thiadiazole analogues **1–6**: i) a) oxalyl chloride, DMF, dry THF, b) substituted aniline, dry pyridine, dry THF; ii) H_2, Pd/C, dry THF; iii) CH_3I, Cs_2CO_3, dry THF; iv) HBTU, DMAP, 3,5-bis(trifluoromethyl)aniline, dry DMF.

Other compounds (**4**, **5** and **3**, Scheme 1) were obtained by coupling unprotected 4-hydroxythiadiazole-3-carboxylic acyl chloride of the corresponding acid **14**[34] or commercial available 4-aminofurazan-3-carboxylic acid **15** with the corresponding aniline. Compound **4** was then methylated with methyl iodide to obtain compound **6**. Synthetic procedures and spectral characterization of the final compounds **1–11** are shown in the ESI.†

The designed compounds **1–11** (Fig. 2) were evaluated both by enzymatic and cellular assays and compared to IMD-0354[35] and PS-1145,[36] the latter used as IKKβ reference inhibitor[8] (Table 1).

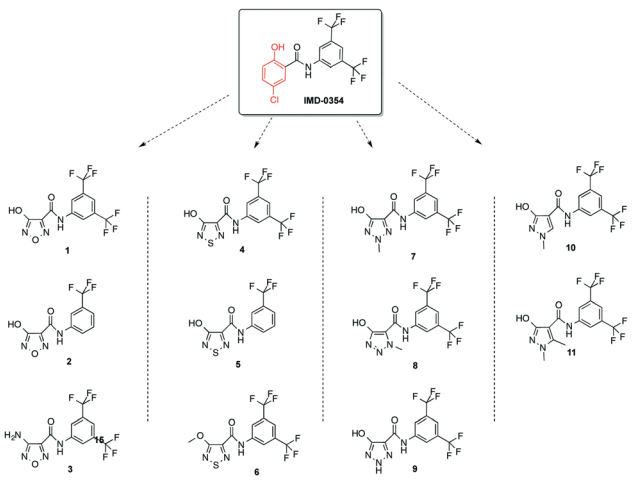
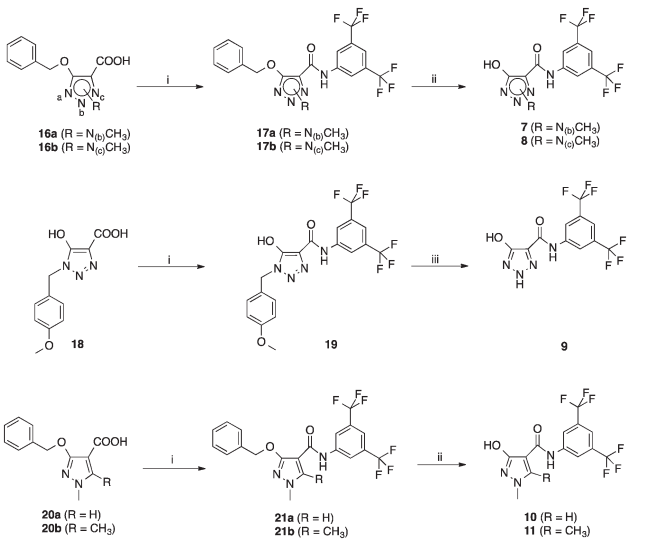


Fig. 2 The bioisosteric scaffold hopping strategy applied to IMD-0354 using *hydroxyoxadiazole*, *hydroxythiadiazole*, *hydroxytriazole* and *hydroxypyrazole* scaffolds.



Scheme 2 Synthesis of triazole and pyrazole analogues (**7–11**). i) a) Oxalyl chloride, DMF, dry THF; b) 3,5-bis(trifluoromethyl)aniline, dry pyridine, dry THF; ii) H_2, Pd/C, dry THF; iii) TFA, 45 °C.

Table 1 The effects of IMD-0354, PS-1145 and the designed compounds on: ATP-based kinase assays for IKKβ, IKKα, IKKε and NIK (expressed as IC_{50} value, μM); IκBα degradation assay in Jurkat cells (expressed as IC_{50} value, μM). All experiments were performed in triplicate, and data represent means ± standard deviation (SD)

Compound	IKKβ IC_{50} ± SD[a] (μM) (% inhib at 100 μM)	IKKα IC_{50} ± SD[a] (μM) (% inhib at 100 μM)	IKKε IC_{50} ± SD[a] (μM) (% inhib at 100 μM)	NIK IC_{50} ± SD[a] (μM) (% inhib at 100 μM)	IC_{50} (μM) on IκBα degradation assay[b]
IMD-0354	>100 (5.43)	>100 (3.45)	>100 (30.5)	>100 (13.8)	0.218 ± 0.007
PS-1145	0.087 ± 0.005	>100	>100	>100	0.186 ± 0.008
1	>100 (12.2)	>100 (18.6)	58.5 ± 0.4	>100 (19.3)	6.93 ± 0.12
2	>100 (2.06)	>100 (−2.5)	>100 (32.1)	>100 (11.8)	1.72 ± 0.11
3	>100 (31.6)	>100 (29.3)	44.8 ± 0.8	>100 (−9.20)	>100
4	>100 (−10.3)	>100 (25.2)	>100 (30.2)	>100 (4.34)	0.143 ± 0.005
5	>100 (−0.94)	35.5 ± 0.6	>100 (25.8)	>100 (−1.1)	>100
6	>100 (8.51)	>100 (24.1)	>100 (21.9)	>100 (−1.9)	>100
7	>100 (5.19)	>100 (46.9)	>100 (6.92)	>100 (9.1)	>100
8	>100 (11.2)	>100 (13.1)	58.5 ± 0.3	>100 (12.1)	>100
9	>100 (5.14)	>100 (24.5)	>100 (15.3)	>100 (−9.5)	>100
10	>100 (12.7)	>100 (12.1)	>100 (17.2)	>100 (7.23)	25.9 ± 0.4
11	>100 (13.9)	>100 (13.2)	>100 (14.2)	>100 (24.1)	1.32 ± 0.21

[a] ATP-based kinase assays for IKKβ, IKKα, IKKε and NIK (expressed as IC_{50} value, μM). [b] IκBα degradation assay in Jurkat cells (expressed as IC_{50} value, μM).

At the enzymatic level, the activity was assessed on recombinant human IKKβ. Surprisingly, the IMD-0354 *lead*, as well as the designed compounds **1–11**, were found to be inactive in the assays (Table 1). This result does not agree with the earlier proposed mechanism of action of IMD-0354, which assumes that the compound is a potent inhibitor of IKKβ.[28,29,37] In particular, its molecular structure was originally designed by analysing the binding mode of *aspirin* to IKKβ at the APB IKK-2 binding site,[28] and based on that it was suggested to compete with ATP for binding to IKKβ.[38] Based on an NF-κB-IKKβ reporter assay that uses a constitutively active IKKβ mutant,[17] the compound's mechanism of action was suggested to involve the inhibition of phosphorylation of IκB.

Indeed, IMD-0354 was found to inhibit the activated expression of NF-κB in a dose-dependent manner in HepG2 cells transfected with pFLAG-CMV-IKKβ (S177E/S181E) vector, and subsequent verification of IκBα degradation by Western blot analysis of cytosolic phospho-IκBα. The authors concluded[17] that the results were consistent with IKKβ inhibition although IMD-0354 was not assayed on isolated enzyme. In recent years, Azucena Gomez-Cabrero *et al.*[39] defined IMD-0354 as "*an indirect inhibitor of NF-κB*", probably due to the lack of strong evidence supporting an IKKβ related mechanism. In order to get a broader overview of the action of IMD-0354, we assayed both IMD-0354 and PS-1145, as well as compounds **1–11**, against the other three kinases involved in the *canonical* and *non-canonical* NF-κB activation pathways (IKKα, IKKε and NIK). In this assay the newly synthesized compounds and IMD-0354 were found to be essentially inactive (only modest activity of compounds **1**, **3** and **5** in the μM range was observed, Table 1).

Since IMD-0354 has been described as potent inhibitor of the NF-κB pathway in cellular assays, we also evaluated the ability of IMD-0354 and compounds **1–11** to block the NF-κB pathway. In these experiments the capacity of the compounds to inhibit the degradation of IκBα after inflammatory stimulus was evaluated in Jurkat cells. In agreement with earlier results,[17] these assays showed IMD-0354 to be a potent NF-κB inhibitor with IC_{50} = 0.218 μM (Table 1 and Fig. 3). Among the new compounds, the hydroxythiadiazole **4** was the most active, with IC_{50} of 0.143 μM (Table 1 and Fig. 3). Compounds **1**, **2**, **10** and **11** were active in the low μM range.

The effects of PS-1145, IMD-0354 and compound **4** on NF-κB gene reporter assay in Jurkat (TIB-152, ATCC) and MDA-MB-231 (ACC-732, DSMZ) cells were also evaluated (Fig. 4).

All three compounds were found active on TNFα-activated Jurkat cells 6 h post-treatment, with compound **4** having higher potency than IMD-0354 and PS-1145 (residual activity at 1 μM: IMD-0354, 23.48%; PS-1145, 38.14%; **4**, 15.91%). However, both compounds **4** and IMD-0354 were almost completely inactive on MDA-MB-231 cells 6 h post-treatment (residual activity at 10 μM: IMD-0354, 98.99%; compound **4**, 96.08%) and showed an appreciable activity 24 h post-treatment (residual activity at 10 μM: IMD-0354, 58.27%; compound **4**, 63.01%). Conversely, 10 μM PS-1145 showed potent activity not only at 24 h but also at 6 h (residual activity at 10 μM: 19.52% (6 h) and 8.67% (24 h)). The different inhibitory activity showed of IMD-0354 and compound **4** in gene reporter assay, carried-out in Jurkat and MDA-MB-231

Table 2 Antiproliferative and cytotoxic effects of compound **4**, IMD-0354 and PS-1145 on MDA-MB-231 cells. Cells were exposed to inhibitors for 72 h. Data obtained for IMD-0354 agree with previously reported results.[29] Data obtained for PS-1145 agree with previously reported antiproliferation assays in MDA-MB-231 cells.[40] Values are means ± SD of three independent experiments

Compound	Antiproliferative effect IC_{50} ± SD (μM)	Cytotoxicity IC_{50} ± SD (μM)
4	1.29 ± 0.08	53 ± 2
IMD-0354	0.64 ± 0.04	0.85 ± 0.01
PS-1145	0.90 ± 0.07	>100

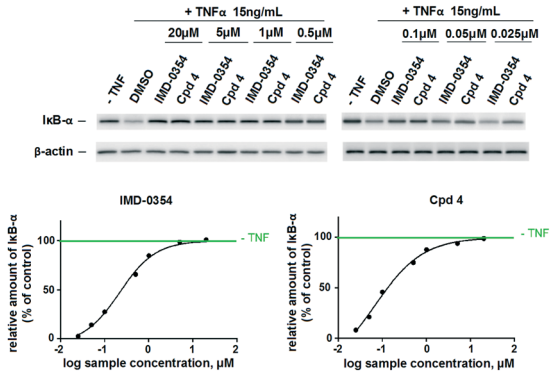


Fig. 3 Effects of compound 4 on IκBα degradation in Jurkat cells stimulated by TNF-α. IκBα protein expression was evaluated by immunoblotting. One representative immunoblot of three independent experiments is shown. Graphs represent IκBα relative band intensity quantified by densitometric analysis after normalization using β-actin as reference. Values are means ± SD of three independent experiments.

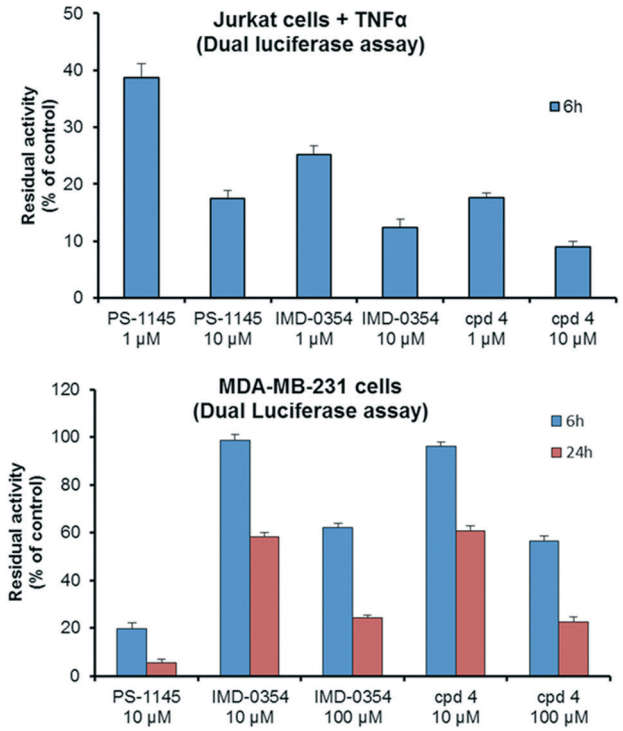


Fig. 4 Effects of PS-1145, IMD-0354 and compound 4 on NF-κB gene reporter assay in Jurkat and MDA-MB-231 cells.

cells, is probably a consequence of the activation status of the NF-κB pathway. In fact, in Jurkat cells the NF-κB pathway was activated through the treatment with TNFα whilst in MDA-MB-231 cells the NF-κB signaling pathway was reported to be constitutively activated and driven by both IKKβ and IKKα.[40]

The anti-inflammatory effects of compound 4 were evaluated in LPS-stimulated THP-1 (ACC-16, DSMZ) in which the canonical NF-κB signaling pathway controls the expression of several pro-inflammatory cytokines.[41] Compound 4 prevented in a dose-dependent manner the LPS-induced degradation of IκBα (Fig. 5).

These results also agree with earlier experiments, which showed that IMD-0354 is able to specifically block the NF-κB pathway when induced by proinflammatory cytokines, such as TNFα and IL-1β.[15,29] The presented experiments show that compound 4 potently blocks the NF-κB cascade, particularly in conditions of TNFα activation. However, similarly to IMD-0354, compound 4 does not inhibit IKKβ at enzymatic level (Table 1).

For a better understanding of the possible role of IKKβ in the IMD-0354 mechanism of action, we assayed the compound against the trimeric IKKβ-IKKα-Nemo complex, isolated from Jurkat cells treated with TNFα. In this assay, IMD-0354 showed only weak activity (40% inhibition at 100 μM, see ESI†), which does not explain the claimed potency of the compound on the NF-κB cascade. Moreover, in Jurkat cells exposed to IMD-0354 and treated with TNFα, the IKKs of the trimeric complex were found to be phosphorylated (ESI†), indicating that the TNFα stimulus did reach them. These results suggest that the mechanism of inhibition of the canonical NF-κB pathway by IMD-0354 is probably more complex than it was thought. However, the elucidation of this mechanism is outside the scope of this publication.

Finally, we also studied antiproliferative activity and cytotoxicity of compounds 4 on MDA-MB-231 cells, comparing them to IMD-0354 and PS-1145 (Table 2). Quantitation of DNA content and a fluorescent assay to assess cell membrane integrity were used to evaluate cell proliferation and cytotoxicity, respectively.

It can be seen from the data in Table 2 that compound 4 has antiproliferative effect in the low μM range (1.29 μM), slightly lower than that of the two lead compounds. On the other hand, this compound is not cytotoxic as IMD-0354, showing an effect more reminiscent of that of PS-1145, able to block the NF-κB cascade without significant cytotoxicity. In contrast to compound 4 and PS-1145, IMD-0354 has similar IC_{50} in both proliferation and cytotoxicity assays.

In conclusion, here we introduce the hydroxythiadiazole 4 as a nanomolar inhibitor of the canonical NF-κB cascade. The compound was designed through a bioisosteric scaffold hopping approach applied to the phenolic moiety of IMD-0354. When compared to IMD-0354, compound 4 showed

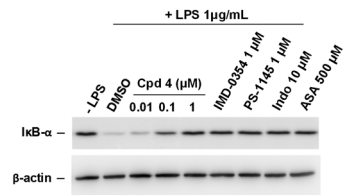


Fig. 5 Effects of compound 4, IMD-0354, PS-1145, aspirin (ASA) and indomethacin (Indo) on IκBα degradation in THP-1 cells stimulated by LPS. IκBα protein expression was evaluated by immunoblotting. One representative immunoblot of three independent experiments is shown.

similar mode of action, although with higher potency in blocking the NF-κB cascade on Jurkat cells and lower cytotoxicity on MDA-MB-231 cells. Both **4** and the *lead* IMD-0354 were found to be inactive in IKKβ enzymatic assays, although both being able to inhibit the canonical NF-κB pathway after TNFα or LPS stimulus.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

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Notes and references

1 H. Park, Y. Shin, H. Choe and S. Hong, *J. Am. Chem. Soc.*, 2015, **137**, 337–348.
2 W. Wang, S. A. Nag and R. Zhang, *Curr. Med. Chem.*, 2015, **22**, 264–289.
3 N. Pozdeyev, A. Berlinberg, K. Wuensch, W. M. Wood, Q. Zhou, H. Shibata and B. R. Haugen, *PLoS One*, 2015, **10**, e0134901.
4 S.-C. Sun, *Cell Res.*, 2011, **21**, 71–85.
5 S. K. Arepalli, M. Choi, J. K. Jung and H. Lee, *Expert Opin. Ther. Pat.*, 2015, **25**, 319–334.
6 G. Xu, Y. Lo, Q. Li, G. Napolitano, X. Wu, X. Jiang, M. Dreano, M. Karin and H. Wu, *Nature*, 2011, **472**, 325.
7 S. Liu, Y. R. Misquitta, A. Olland, M. A. Johnson, K. S. Kelleher, R. Kriz, L. L. Lin, M. Stahl and L. Mosyak, *J. Biol. Chem.*, 2013, **288**, 22758.
8 A. C. Castro, L. C. Dang, F. Soucy, L. Grenier, H. Mazdiyasni, M. Hottelet, L. Parent, C. Pien, V. Palombella and J. Adams, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 2419–2422.
9 A. Yemelyanov, A. Gasparian, P. Lindholm, L. Dang, J. W. Pierce, F. Kisseljov, A. Karseladze and I. Budunova, *Oncogene*, 2006, **25**, 387–398.
10 T. Hideshima, D. Chauhan, P. Richardson, C. Mitsiades, N. Mitsiades, T. Hayashi, N. Munshi, L. Dang, A. Castro, V. Palombella, J. Adams and K. C. Anderson, *J. Biol. Chem.*, 2002, **277**, 16639–16647.
11 C. Gamble, K. McIntosh, R. Scott, K. H. Ho, R. Plevin and A. Paul, *Br. J. Pharmacol.*, 2012, **165**, 802–819.
12 J.-J. Huang, H.-X. Chu, Z.-Y. Jiang, X.-J. Zhang, H.-P. Sun and Q.-D. You, *Curr. Med. Chem.*, 2014, **21**, 3893–3917.
13 P. D. Coish, P. L. Wickens and T. B. Lowinger, *Expert Opin. Ther. Pat.*, 2006, **16**, 1–12.
14 http://www.immd.co.jp/en/development.html - pipeline.
15 A. Lennikov, N. Kitaichi, K. Noda, R. Ando, Z. Dong, J. Fukuhara, S. Kinoshita, K. Namba, M. Mizutani, T. Fujikawa, A. Itai, S. Ohno and S. Ishida, *Mol. Vision*, 2012, **18**, 2586–2597.
16 A. Sugita, H. Ogawa, M. Azuma, S. Muto, A. Honjo, H. Yanagawa, Y. Nishioka, K. Tani, A. Itai and S. Sone, *Int. Arch. Allergy Immunol.*, 2009, **148**, 186–198.
17 Y. Onai, J.-i. Suzuki, T. Kakuta, Y. Maejima, G. Haraguchi, H. Fukasawa, S. Muto, A. Itai and M. Isobe, *Cardiovasc. Res.*, 2004, **63**, 51–59.
18 R. Watanabe, R. W. Azuma, J.-i. Suzuki, M. Ogawa, A. Itai, Y. Hirata, I. Komuro and M. Isobe, *Am. J. Physiol.*, 2013, **305**, H1761–H1771.
19 Y. Onai, J.-i. Suzuki, Y. Maejima, G. Haraguchi, S. Muto, A. Itai and M. Isobe, *Am. J. Physiol.*, 2007, **292**, H530–H538.
20 H. Ogawa, M. Azuma, S. Muto, Y. Nishioka, A. Honjo, T. Tezuka, H. Uehara, K. Izumi, A. Itai and S. Sone, *Clin. Exp. Allergy*, 2011, **41**, 104–115.
21 J. Wei, M. Shi, W.-Q. Wu, H. Xu, T. Wang, N. Wang, J.-L. Ma and Y.-G. Wang, *World J. Gastroenterol.*, 2011, **17**, 5203–5213.
22 Y. Nishioka, *Kokyu*, 2007, **26**, 92–107.
23 J. Kamon, T. Yamauchi, S. Muto, S. Takekawa, Y. Ito, Y. Hada, W. Ogawa, A. Itai, M. Kasuga, K. Tobe and T. Kadowaki, *Biochem. Biophys. Res. Commun.*, 2004, **323**, 242–248.
24 M. Inayama, Y. Nishioka, M. Azuma, S. Muto, Y. Aono, H. Makino, K. Tani, H. Uehara, K. Izumi, A. Itai and S. Sone, *Am. J. Respir. Crit. Care Med.*, 2006, **173**, 1016–1022.
25 A. Tanaka and H. Matsuda, *Ensho to Men'eki*, 2007, **15**, 676–680.
26 S. Hosokawa, G. Haraguchi, A. Sasaki, H. Arai, S. Muto, A. Itai, S. Doi, S. Mizutani and M. Isobe, *Cardiovasc. Res.*, 2013, **99**, 35–43.
27 A. Tanaka, S. Muto, K. Jung, A. Itai and H. Matsuda, *J. Invest. Dermatol.*, 2007, **127**, 855–863.
28 A. Tanaka, M. Konno, S. Muto, N. Kambe, E. Morii, T. Nakahata, A. Itai and H. Matsuda, *Blood*, 2005, **105**, 2324–2331.
29 A. Tanaka, S. Muto, M. Konno, A. Itai and H. Matsuda, *Cancer Res.*, 2006, **66**, 419–426.
30 N. A. Meanwell, *J. Med. Chem.*, 2011, **54**, 2529–2591.
31 S. Sainas, A. C. Pippione, M. Giorgis, E. Lupino, P. Goyal, C. Ramondetti, B. Buccinna, M. Piccinini, R. C. Braga, C. H. Andrade, M. Andersson, A. C. Moritzer, R. Friemann, S. Mensa, S. Al-Kadaraghi, D. Boschi and M. L. Lolli, *Eur. J. Med. Chem.*, 2017, **129**, 287–302.
32 M. Lolli, S. Narramore, C. W. Fishwick and K. Pors, *Drug Discovery Today*, 2015, **20**, 1018–1026.
33 A. C. Pippione, F. Dosio, A. Ducime, A. Federico, K. Martina, S. Sainas, B. Frolund, M. Gooyit, K. D. Janda, D. Boschi and M. L. Lolli, *MedChemComm*, 2015, **6**, 1285–1292.
34 D. Lehmann, R. Eckardt, G. Faust, W. Fiedler, W. Poepel, H. J. Jaensch, H. J. Heidrich and D. Lohmann, P, in, VEB Arzneimittelwerk, Ger. Dem. Rep., DD263767A1, 1989.
35 t. H. Obtained from Sigma (code: I3159).
36 t. H. Obtained from Sigma (code: P6624).
37 A. Lennikov, M. Hiraoka, A. Abe, S. Ohno, T. Fujikawa, A. Itai and H. Ohguro, *Invest. Ophthalmol. Visual Sci.*, 2014, **55**, 6365–6373.

38 A. Sugita, H. Ogawa, M. Azuma, S. Muto, A. Honjo, H. Yanagawa, Y. Nishioka, K. Tani, A. Itai and S. Sone, *Int. Arch. Allergy Immunol.*, 2009, **148**, 186–198.
39 A. Gomez-Cabrero, W. Wrasidlo and R. A. Reisfeld, *PLoS One*, 2013, **8**, e73607.
40 N. Yamaguchi, T. Ito, S. Azuma, E. Ito, R. Honma, Y. Yanagisawa, A. Nishikawa, M. Kawamura, J. Imai, S. Watanabe, K. Semba and J. Inoue, *Cancer Sci.*, 2009, **100**, 1668–1674.
41 O. Sharif, V. N. Bolshakov, S. Raines, P. Newham and N. D. Perkins, *BMC Immunol.*, 2007, **8**, 1.