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Synthesis and biological evaluation of p38α kinase-targeting dialkynylimidazoles

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Abstract

Based on the mild, thermal rearrangement of 1,2-dialkynylimidazoles to reactive carbene or diradical intermediates, a series of 1,2-dialkynylimidazoles were designed as potential irreversible p38 MAP kinase α-isoform (p38α) inhibitors. The synthesis of these dialkynylimidazoles and their kinase inhibition activity is reported. The 1-ethynyl-substituted dialkynylimidazole **14** is a potent (IC_{50} = 200 nM) and selective inhibitor of p38α. Moreover, compound **14** covalently modifies p38α as determined by ESI-MS after 12 h incubation at 37 °C. The unique kinase inhibition, covalent kinase adduct formation, and minimal CYP450 2D6 inhibition by compound **14** demonstrate that dialkynylimidazoles are a new, promising class of p38α inhibitors.

p38 MAP kinase (p38α) belongs to a family of serine/theronine kinases that serve as important mediators of inflammatory cytokines including tumor necrosis factor alpha (TNFα) and interleukin-1 beta (IL-1β).[1,2] Elevated levels of the pro-inflammatory cytokines are associated with a number of diseases, such as toxic shock syndrome, rheumatoid arthritis, osteoarthritis, diabetes and inflammatory bowel disease.[3] Therefore, inhibition of p38α is considered to be a potential therapeutic strategy.[4] A number of p38α inhibitors have been synthesized and characterized.[5] Although these compounds show good inhibition of p38α, many also inhibit other protein kinases with similar or greater potency.[6]

There has been a growing interest in irreversible inhibitors of protein kinases,[7] and a number of these drugs are in clinical trials.[8] Advantages of irreversible kinase inhibition include increased selectivity,[9] duration,[10] and therapeutic utility, especially against kinases that are resistant to competitive, ATP-binding pocket-targeting drugs.[11] Additionally, irreversible inhibitors and related selective, covalent kinase modifying small molecules are of interest as probes for chemical genetics studies.[12] While certain natural products and ATP analogs irreversibly inhibit kinases,[13] none are selective towards p38α. Thus, there is a need to develop selective and irreversible inhibitors that target p38α. We have discovered a novel thermal cyclization and rearrangement of 1,2-dialkynylimidazoles (DAIms) (Scheme 1). Mild thermolysis of DAIms in the presence of chlorinated solvents or HCl leads to the isolation of imidazo[1,2-*a*]pyridine (ImPy) products, which may result from trapping of an initially-formed diradical intermediate via aza-Bergman cyclization.[13]

Thermolysis under neutral conditions in non-halogenated solvents affords products derived from trapping cyclopentapyrazine (CyPP) carbene intermediates by H-atom abstraction, C–H

bond insertion, and alkene addition reactions.[14–16] The CyPP carbene is proposed to be derived from an intermediate cyclic cumulene that results from collapse of the diradical.[14] Non-covalent association between DAIms and a kinase may facilitate the rate-determining aza-Bergman cyclization.

The formation of reactive diradical and carbene intermediates under mild conditions from DAIms has led us to propose that DAIms can be designed to undergo kinase binding-induced cyclization and covalent inactivation of specific kinase targets. Specifically, the structural similarity between DAIms and the known p38α inhibitors such as SB-203580[17] and RWJ-67657[18] (Figure 1) has inspired the design and inhibition studies of p38α-targeting DAIms described here.

An initial route to kinase-targeting dialkynylimidazoles is shown in Scheme 2. The known 4(5)-(4-fluorophenyl)-5(4)-(4-pyridyl)imidazole **1**[19] was protected with trityl group. Interestingly, this reaction only afforded one regioisomer, which was assigned as the 5-(4-fluorophenyl)-4-(4-pyridyl)imidazole **2** based on COSY and NOESY NMR. Compound **2** was deprotonated with *n*-BuLi at 0 °C, and quenched with I_2 to give the 2-iodo-imidazole **3**, which was deprotected in aqueous TFA to afford **4**. Coupling of the lithium anion of imidazole **4,** formed by deprotonation with LHMDS, with phenyl(phenylethynyl)-iodonium tosylate[20] afforded a 15 % yield of a 1:1 mixture of the regioisomeric *N*-alkynyl-2-iodoimidazoles **5** and **6**. The separated regioisomers were subjected to Sonogashra coupling with various terminal acetylene partners to provide the regioisomeric dialkynylimidazoles **7** and **8**. The regiochemical assignments within this series were made based on the X-ray crystal structure of **7b** shown in Figure 2.[21]

Although providing access to select kinase-targeting dialkynylimidazoles, the synthetic route shown in Scheme 2 suffers from a number of limitations associated with the alkynyliodonium coupling reaction. Only the phenylethynyl and TMS-ethynyl iodonium reagents could be employed in this coupling,[22] and even in these cases, the yields are poor and mixtures of regioisomers are produced.

An improved synthetic route to these dialkynylimidazoles employing the recently reported copper-catalyzed *N*-alkynylation of imidazoles with bromoalkynes was devised (Scheme 3). Treating 4-fluorophenylimidazole **9**[23] with TIPS-protected bromo-acetylene in the presence of catalytic CuI and 2-acetyl-cyclohexanone as ligand affords a 9:1 mixture of regioisomeric alkynylimidazoles **10b** and **10a**, respectively, in 79 % yield.[22] Iodination of the 2-position of **10b** affords the 2-iodoimidazole **11**, which undergoes Sonogashira coupling with *O*-TIPS-protected homopropargyl alcohol to give the dialkynylimidazole **12** in 73 % yield.[24] Deprotonation of **12** with *n*-BuLi followed by iodine quench affords the 5-iodoimidazole **13** in 74 % yield. A final Suzuki-Miyaura coupling of the 5-iodo imidazole **13** with pyridine-4-boronic acid followed by TBAF deprotection gives **the dialkynyl-imidazole **14**.[25] Mild thermolysis of **14** at 80° C under acidic conditions in the presence of chloride afforded **15**, the product of HCl addition to the diradical, in 50 % yield.

All of these 1,2-dialkynylimidazoles were assayed against p38α MAPK at a fixed time-point of 60 min (Table 1).[26] Compounds **7a–c** and **8a–c** display modest inhibition at 10 μM concentration. In this series there is little difference in activity between the 1-alkynyl-5-fluorophenyl regioisomers **7a–c** and the 1-alkynyl-5-pyridylisomers **8a–c**, in contrast to reported 1-substituted pyridylimidazole p38α inhibitors.[28] Interestingly, the 1-ethynyl-substituted analog **14** is a potent inhibitor of p38α. Compound **14** completely inhibits p38α at 10 μM (Table 1), and has an IC_{50} for p38α of 200 nM.[27] In comparison, the IC_{50} of **14** against p38 (5.4 μM) is >25-fold higher. Dialkyny-limidazole **14** was also assayed at concentration of 20 μM against a panel of 53 additional human kinases. Only one kinase, (MAPK4/HGK) was

strongly inhibited (>90% inhibition at 20 μM, IC_{50} = 4.2 μM), while six additional kinases were moderately inhibited (between 50–90% inhibition, see Supporting Information). The cyclized **15** also inhibited p38α (IC_{50} = 370 nM).

Dialkynylimidazole **14** (100 μM) was incubated with non-phosphoryated p38α (5 μM) at 37 °C in 50 mM HEPES, 10 mM $MgCl_2$, 2 mM DTT, 1 mM EGTA, pH 7.5 for 12 h, followed by extensive dialysis, and the sample was analyzed by ESI- MS. A new peak in the mass spectrum at *m/z* = 41896, which corresponds to addition of a single molecule of **14** (MW = 331) to p38α, was observed (~25 % adduct) (Figure 3). Under identical conditions but with 1 mM DTT present, the adduct was the predominant species observed (Supporting Information).

A common concern for pyridinylimidazole MAPK inhibitors such as RWJ 67657 and SB-203580 is their inhibition of cytochrome P_{450} (CYP450) enzymes, which may be linked to hepatotoxicity.[29] Interestingly, the dialkynylimidazole **14** displays a much lower level of inhibition of CYP450 2D6 (4% inhibition at 10 μM) compared to SB-203580 (78% inhibition at 10 μM).

In summary, novel p38α-targeting dialkynylimidazoles were designed, synthesized and evaluated. Although 1-phenethynyl-substituted dialkynylimidazoles **7a–c** and **8a–c** are only modest inhibitors of p38α, the 1-ethynyl-substituted dialkynylimidazole **14** is a potent and selective inhibitor. Commensurate with the increased facility of rearrangement of 1-ethynyl-substituted dialkynylimidazols relative to 1-phenethynyl analogues,[16] compound **14** forms a covalent adduct with p38α. However, the conditions for p38α adduct formation (12 h at 37 °C) are much milder than those required for cyclization/trapping of **14** to afford **15** (5 days at 80 °C), indicating that the kinase may facilitate the cyclization of **14**. Further studies on the site and mechanism of this covalent modification of p38α by 1-ethynyl-substituted dialkynylimidazoles are on-going. The unique kinase inhibition, covalent kinase adduct formation, and minimal CYP450 2D6 inhibition by compound **14** demonstrate that dialkynylimidazoles are a new, promising class of p38α inhibitors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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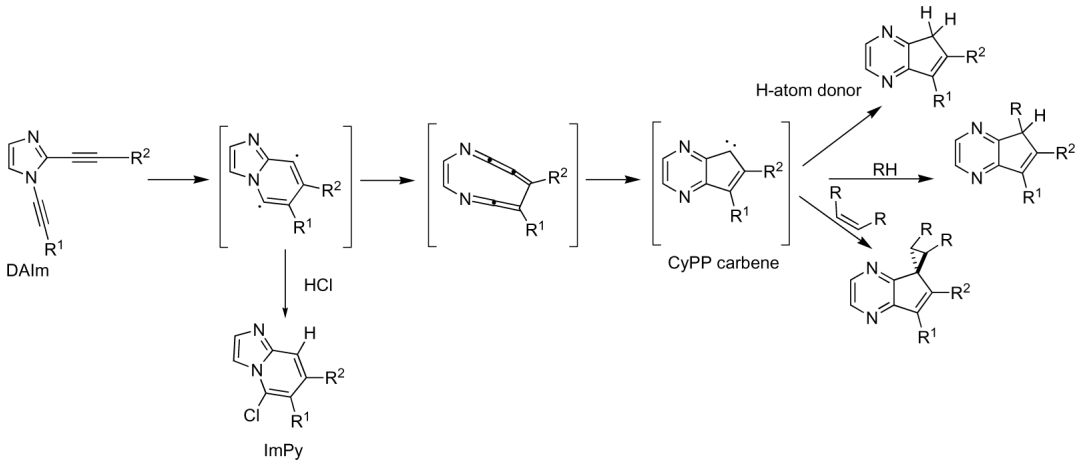
References and Notes

1. Lee JC, Laydon JT, McDonnell PC, Gallagher TF, Kumar S, Green D, McNulty D, Blumenthal MJ, Heys JR, Landvatter SW, et al. Nature 1994;372:739. [PubMed: 7997261]
2. Lee JC, Young PR. J. Leuk. Biol 1996;59:152–7.
3. Feldmann M, Brennan FM, Maini RN. Annu. Rev. Immunol 1996;14:397. [PubMed: 8717520]
4. Lee JC, Kassis S, Kumar S, Badger A, Adams JL. Pharmacol. Ther 1999;82:389. [PubMed: 10454214]
5. Pettus LH, Wurz RP. Curr. Top. Med. Chem 2008;8:1452. [PubMed: 18991731]
6. Bain J, Plater L, Elliott M, Shpiro N, Hastie CJ, McLauchlan H, Klevernic I, Arthur JS, Alessi DR, Cohen P. Biochem. J 2007;408:297. [PubMed: 17850214]
7. (a) Denny WA. Pharmacol. Therap 2002;93:253–261. [PubMed: 12191617] (b) Rastelli G, Rosenfeld R, Reid R, Santi DV. J. Struct. Biol 2008;164:18. [PubMed: 18571434]
8. Mukherji D, Spicer J. Expert Opin. Invest. Drugs 2009;18:293.
9. Cohen MS, Zhang C, Shokat KM, Taunton J. Science 2005;308:1318. [PubMed: 15919995]

10. (a) Tsou HR, Overbeek-Klumpers EG, Hallett WA, Reich MF, Floyd MB, Johnson BD, Michalak RS, Nilakantan R, Discafani C, Golas J, Rabindran SK, Shen R, Shi XQ, Wang YF, Upeslacis J, Wissner A. J. Med. Chem 2005;48:1107. [PubMed: 15715478] (b) Tummino PJ, Copeland RA. Biochemistry 2008;47:5481. [PubMed: 18412369] (c) Smith AJT, Zhang XY, Leach AG, Houk KN. J. Med. Chem 2009;52:225. [PubMed: 19053779]
11. Michalczyk, Anja; Klueter, Sabine; Rode, Haridas B.; Simard, Jeffrey R.; Gruetter, Christian; Rabiller, Matthias; Rauh, Daniel. Bioorg. Med. Chem 2008;16:3482. [PubMed: 18316192]
12. Blair JA, Rauh D, Kung C, Yun CH, Fan QW, Rode H, Zhang C, Eck MJ, Weiss WA, Shokat KM. Nat. Chem. Biol 2007;3:229. [PubMed: 17334377]
13. (a) Khandekar SS, Feng BB, Yi T, Chen S, Laping N, Bramson N. J Biomol. Screen 2005;10:447. [PubMed: 16093554] (b) Barluenga S, Dakas PY, Boulifa M, Moulin E, Winssinger N. C. R. Chim 2008;11:1306.
14. Nadipuram AK, Kerwin SM. Tetrahedron 2006;62:3798.
15. Nadipuram AK, David WM, Kumar D, Kerwin SM. Org. Lett 2002;4:4543. [PubMed: 12465933]
16. Kerwin SM, Nadipuram A. Synlett 2004:1404.
17. Gallagher TF, Fier-Thompson SM, Garigipati RS, Sorenson ME, Smietana JM, Lee D, Bender PE, Lee JC, Laydon JT, Griswold DE, Chabot-Fletcher MC, Breton JJ, Adams JL. Bioorg. Med. Chem. Lett 1995;5:1171–1176.
18. Wadsworth SA, Cavender DE, Beers SA, Lalan P, Schafer PH, Malloy EA, Wu W, Fahmy B, Olini GC, Davis JE, Pellegrino-Gensey JL, Wachter MP, Siekierka JJ. J. Phamacol. Exp. Therap 1999;291:680.
19. Boehm JC, Smietana JM, Sorenson ME, Garigipati RS, Gallagher TF, Sheldrake PL, Bradbeer J, Badger AM, Laydon JT, Lee JC, Hillegass LM, Griswold DE, Breton JJ, ChabotFletcher MC, Adams JL. J. Med. Chem 1996;39:3929. [PubMed: 8831759]
20. Koser GF, Rebrovic L, Wettach RH. J. Org. Chem 1981;46:4324.
21. Crystallographic data for compound **7b** have been deposited with the Cambridge Crystallographic Data Centre as CCDC740499. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223-336033 or deposit@ccdc.cam.ac.uk].
22. Laroche C, Li J, Freyer MW, Kerwin SM. J. Org. Chem 2008;73:6462. [PubMed: 18646827]
23. Ho K-K, Auld DS, Bohnstedt AC, Conti P, Dokter W, Erickson S, Feng D, Inglese J, Kingsbury C, Kultgen SG, Liu R-Q, Masterson CM, Ohlmeyer M, Rong Y, Rooseboom M, Roughton A, Samama P, Smit M-J, Son E, Van der Louw J, Vogel G, Webb M, Wijkmans J, You M. Bioorg. Med. Chem. Lett 2006;16:2724. [PubMed: 16540318]
24. Laroche C, Kerwin SM. Tetrahedron Lett 2009;50:5194.
25. See Supporting Information for characterization data for all compounds.
26. All kinase inhibition studies were performed by Invitrogen using the Z'-lyte™ assay at [ATP] = $K_{m[app]}$ and protein substrate concentration of 20 μM.
27. The inhibition due to **14** in these assays is primarily due to non-covalent inhibition: preincubation of the kinase with **14** for 60 min prior to the addition of ATP did not change the IC_{50} value.
28. Laufer S, Wagner G, Kotschenreuther D. Angew. Chem. Int. Ed 2002;41:2290.
29. Adams JL, Boehm JC, Kassis S, Gorycki PD, Webb EF, Hall R, Sorenson M, Lee JC, Ayrton A, Griswold DE, Gallagher TF. Bioorg. Med. Chem. Lett 1998;8:3111. [PubMed: 9873686]



Scheme 1.
Thermal cyclization and rearrangement of 1,2-dialkynylimidazoles

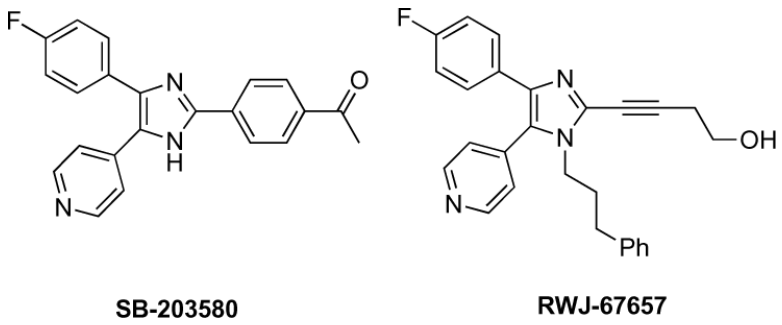
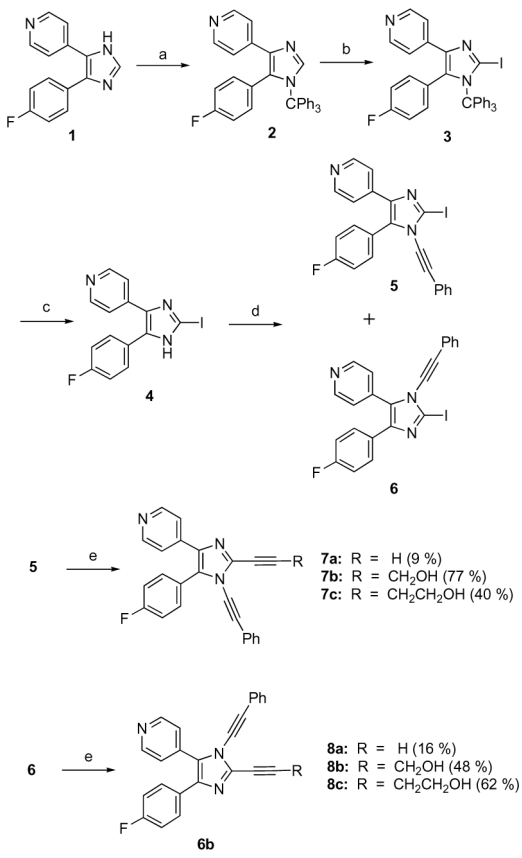


Figure 1.
Examples of 4,5-diarylimidazole p38a inhibitors.



Scheme 2.

Reagents and conditions: (a) Et_3N, Ph_3CCl, CH_2Cl_2 (58 %); (b) i) *n*-BuLi, ii) I_2, THF, 0 °C (60 %); (c) TFA, H_2O (83 %); (d) LHMDS, $PhI^+CCPhTsO^-$; (e) RCCH, $Pd(PPh_3)_4$, CuI, Et_3N.

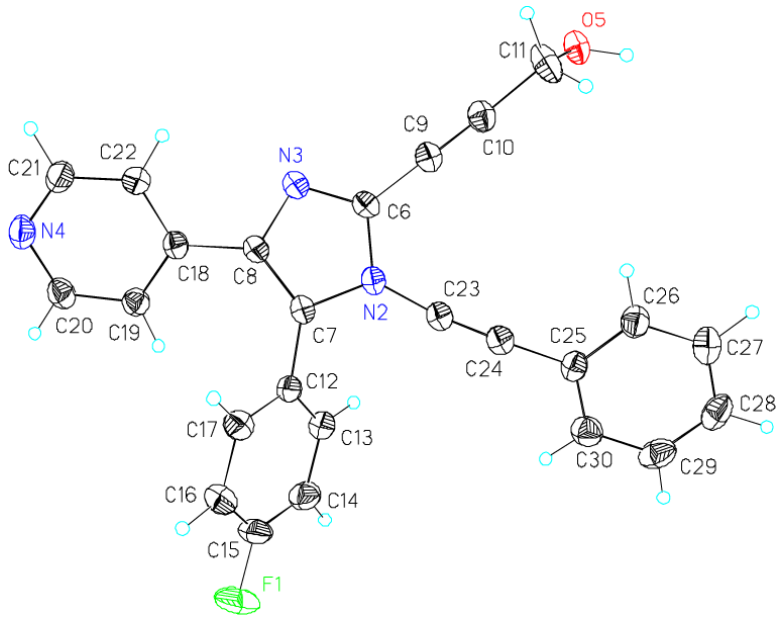
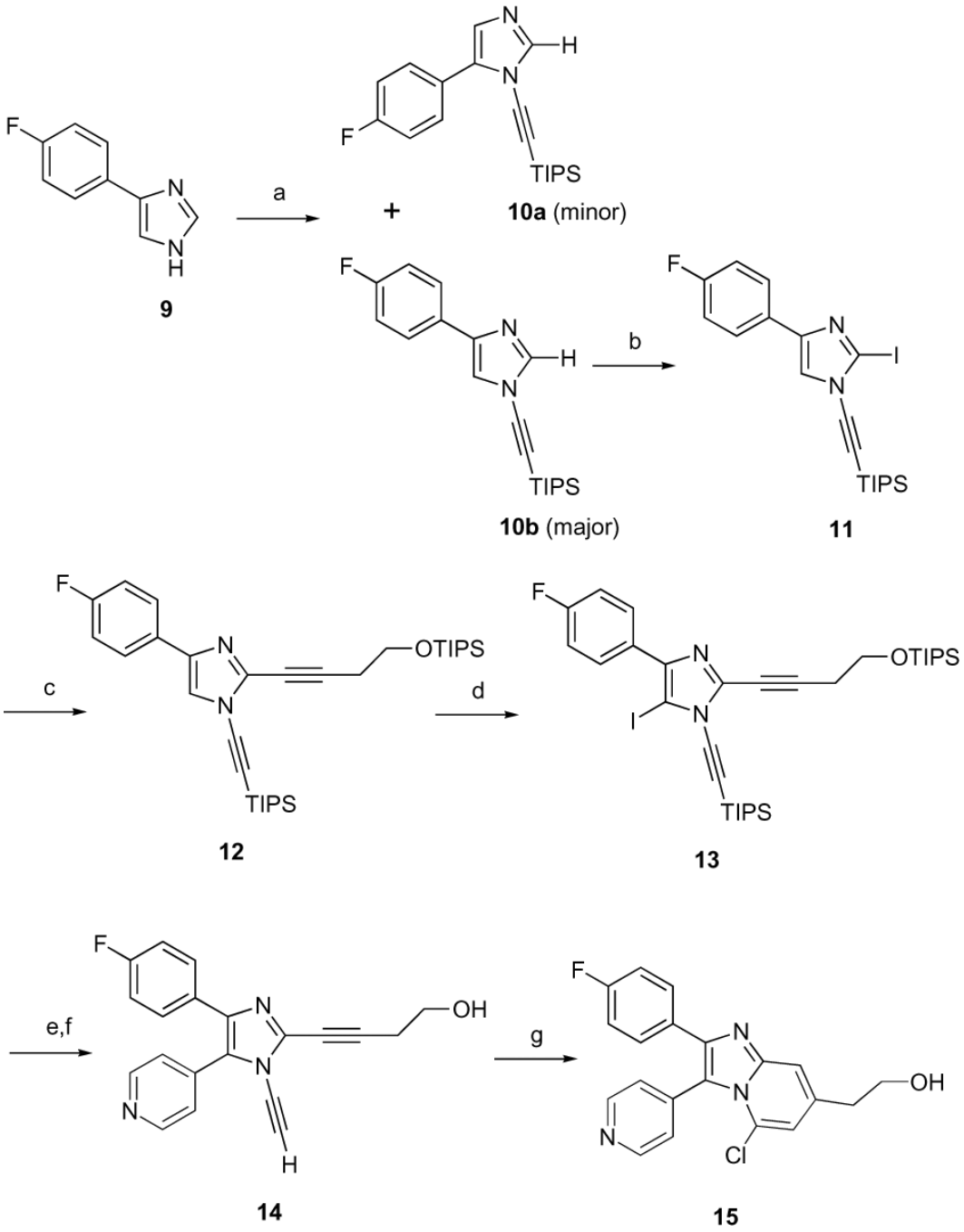


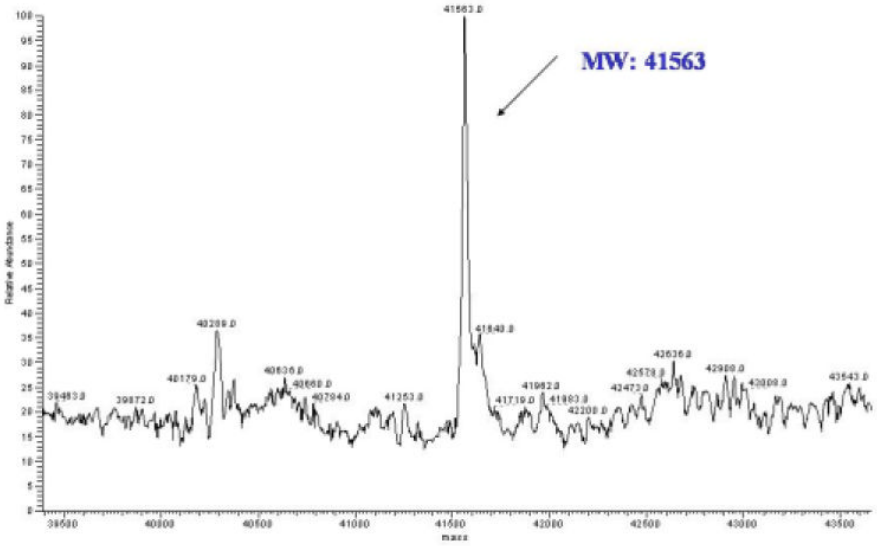
Figure 2.
X-ray crystal structure of dialkynylimidazole **7b**.



Scheme 3.

Reagents and conditions: (a) BrCCTIPS, CuI, AcC, Cs_2CO_3, dioxane, 50 °C overnight followed by reflux for 4 h (79 %, 1:9 1**0a**/**10b**); (b) i) *n*-BuLi, ii) I_2, THF, −78 °C (91 %); (c) $TIPSOCH_2CH_2CCH$, $Pd(PPh_3)_4$, CuI, Et_3N (73 %); (d) i) *n*-BuLi, ii) I_2, THF, −78 °C (74 %); (e) pyridine 4-boronic acid, $Pd(PPh_3)_4$, K_2CO_3 (41 %); (f) TBAF, THF, −78 °C (89 %); (g) Me_4NCl, TfOH, DMF, 80 °C, 5 days (50 %).

(a)



(b)

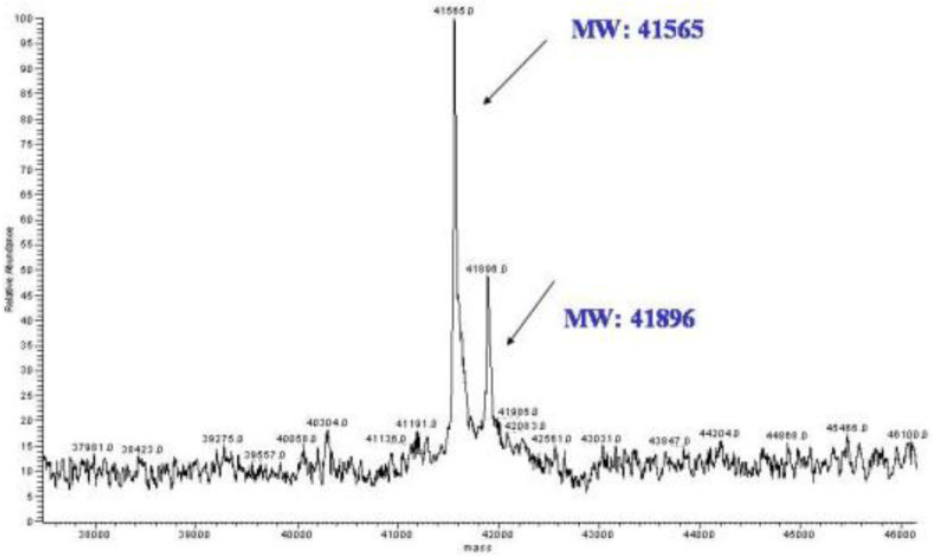


Figure 3.

(a) ESI-MS spectrum of unphosphorylated p38α incubated for 12 h at 37 °C; (b) ESI-MS spectrum of unphosphorylated p38α incubated with dialkynylimidazole **14** for 12 h at 37 °C, followed by extensive dialysis.

Table 1

In vitro activity of 1,2-dialkynylimidazoles against p38α

Compound	p38α % inhibition (@ 10 μM)[a]
7a	19
8a	28
7b	63
8b	83
7c	53
8c	75
14	100

[a]Tests were carried out in duplicate.