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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 tumour 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 toward 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] (Fig. 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

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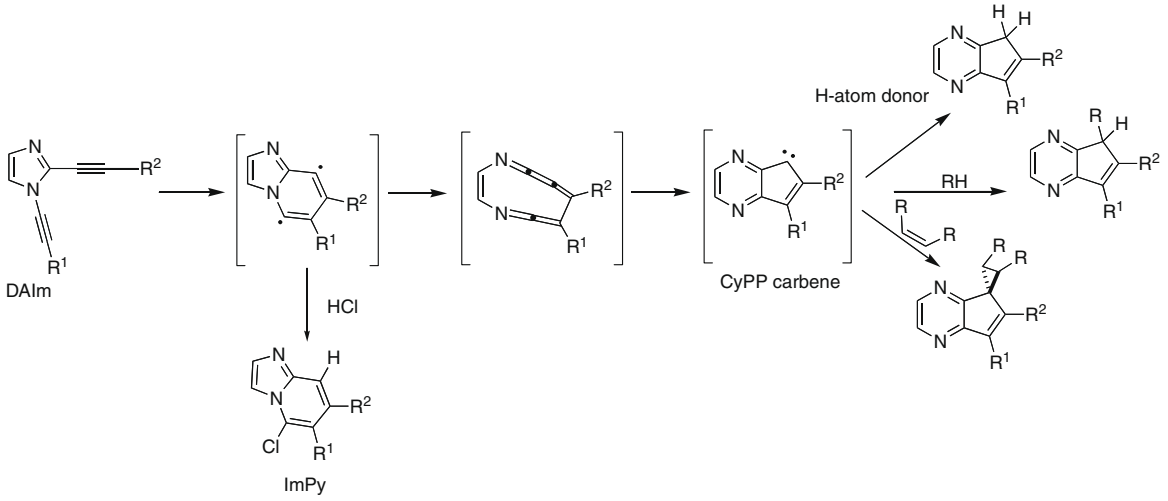


Scheme 1. Thermal cyclization and rearrangement of 1,2-dialkynylimidazoles.

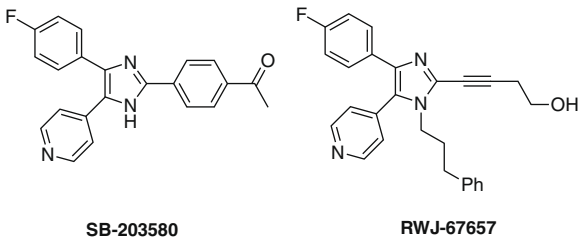


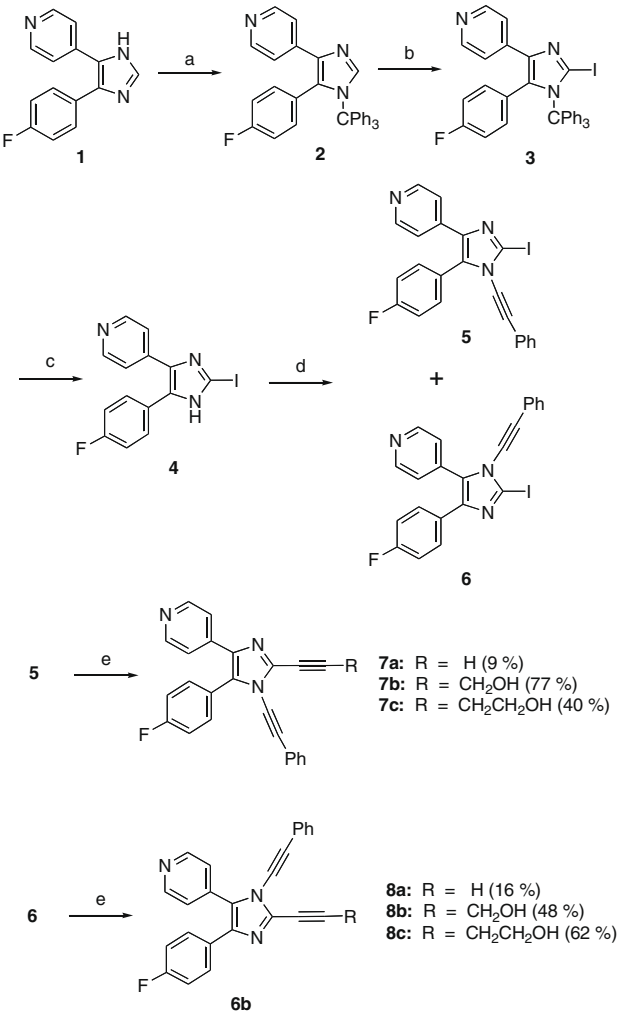
Figure 1. Examples of 4,5-diarylimidazole p38α inhibitors.

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 homo-propargyl 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 dialkynylimidazole **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



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.

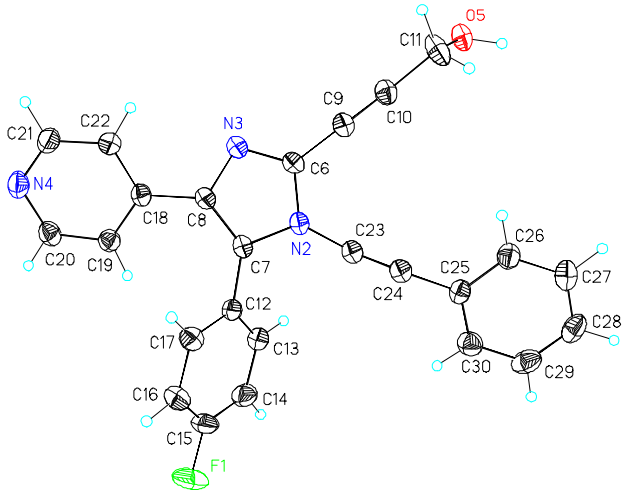


Figure 2. X-ray crystal structure of dialkynylimidazole **7b**.

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 p38β (5.4 μM) is >25-fold higher. Dialkynylimidazole **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% and 90% inhibition, see Supplementary data). The cyclized **15** also inhibited p38α (IC_{50} = 370 nM).

Table 1
In vitro activity of 1,2-dialkynylimidazoles against p38α

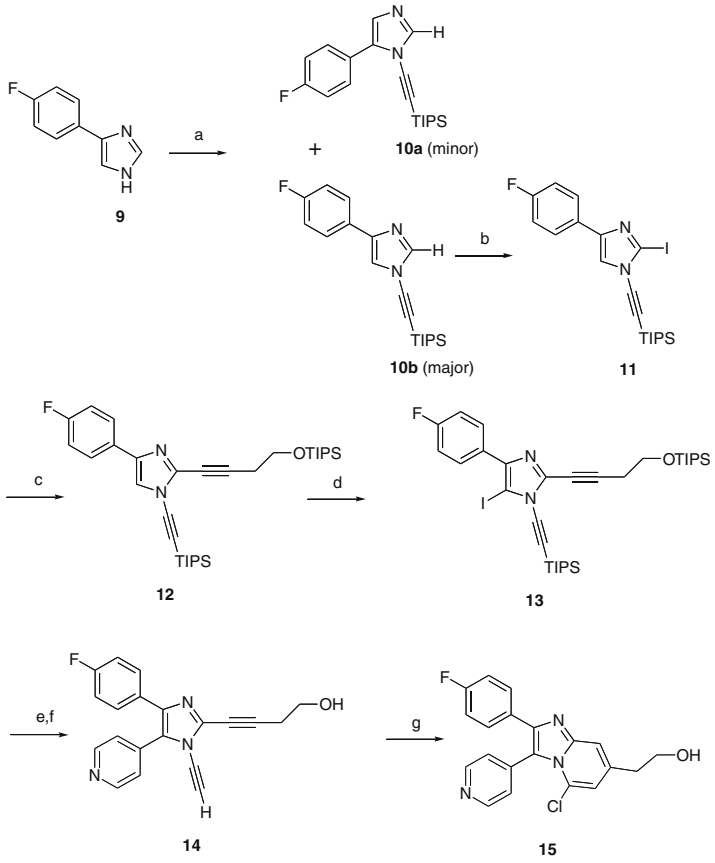
Compound	p38α % inhibition (@ 10 μM)[a]
7a	19
8a	28
7b	63
8b	83
7c	53
8c	75
14	100

[a] Tests were carried out in duplicate.

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 = 41,896, which corresponds to addition of a single molecule of **14** (MW = 331) to p38α, was observed (~25% adduct) (Fig. 3). Under identical conditions but with 1 mM DTT present, the adduct was the predominant species observed (Supplementary data).

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-substi-



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 **10a**/**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%).

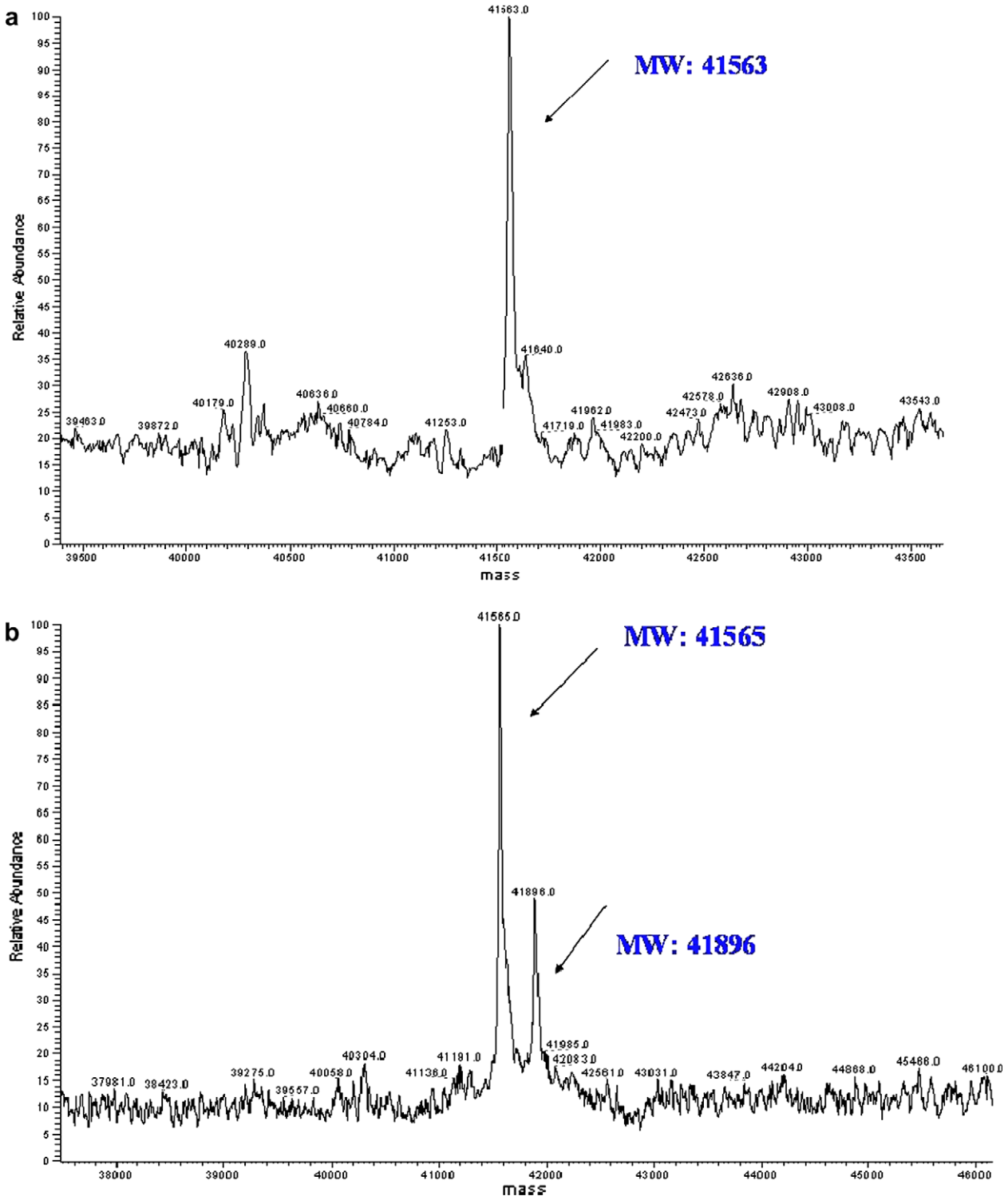


Figure 3. (a) ESI-MS spectrum of unphosphorylated p38α incubated for 12 h at 37 °C; (b) ESI-MS spectrum of unphosphorylated p38α incubated with dialkynylimidazole **14** for 12 h at 37 °C, followed by extensive dialysis.

tuted 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.[30] 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.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.09.094.

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