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Discovery of an L-alanine ester prodrug of the Hsp90 inhibitor, MPC-3100

Se-Ho Kim [a,*,†], Rajendra Tangallapally [b,†], In Chul Kim [m,†], Richard Trovato [c,†], Daniel Parker [n,†], J. Scott Patton [d,†], Leslie Reeves [e,†], Chad Bradford [f,†], Daniel Wettstein [g,†], Vijay Baichwal [h,†], Damon Papac [i,†], Ashok Bajji [j,†], Robert Carlson [k,†], Kraig M. Yager [l,*,†]

[a] Orthobond Corporation, 675 U.S. 1, North Brunswick, NJ 08902, USA
[b] St Jude Children's Research Hospital, Department of Chemical Biology, 262 Danny Thomas Place, Memphis, TN 38105, USA
[c] Jacobs Engineering, 155 N, 400 W., Suite 550, Salt Lake City, UT 84103, USA
[d] Arup Laboratories, 500 Chipeta Way, Salt Lake City, UT 84108, USA
[e] Genysis Labs, 391 Orange St, Salt Lake City, UT 84104, USA
[f] Sera Prognostics, 2749 East Parleys Way, Suite 200, Salt Lake City, UT 84109, USA
[g] MediProPharma, 419 Wakara Way, #207B, Salt Lake City, UT 84108, USA
[h] Core Diagnostics, 2458 Embarcadero Way, Palo Alto, CA 94303, USA
[i] Navigen, 383 Colorow Dr, Salt Lake City, UT 84108, USA
[j] VioGen Biosciences, 1290 West, 2320 South, Suite E, Salt Lake City, UT 84119, USA
[k] Karyopharm Therapeutics, 85 Wells Ave., 2nd Floor, Newton, MA 02459, USA
[l] Myriad Genetic Laboratories, 320 Wakara Way, Salt Lake City, UT 84108, USA
[m] New Hope Baptist Church, 149 Highland Ave., East Lansing, MI 48823, USA
[n] AvidXchange, 6415 South, 3000 East, Suite 150, Salt Lake City, UT 84121, USA

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ABSTRACT

Various types of Hsp90 inhibitors have been and continue to undergo clinical investigation. One development candidate is the purine-based, synthetic Hsp90 inhibitor **1** (MPC-3100), which successfully completed a phase I clinical study. However, further clinical development of **1** was hindered by poor solubility and consequent formulation issues and promoted development of a more water soluble prodrug. Towards this end, numerous pro-moieties were explored in vitro and in vivo. These studies resulted in identification of L-alanine ester mesylate, **2i** (MPC-0767), which exhibited improved aqueous solubility, adequate chemical stability, and rapid bioconversion without the need for solubilizing excipients. Based on improved physical characteristics and favorable PK and PD profiles, **2i** mesylate was selected for further development. A convergent, scalable, chromatography-free synthesis for **2i** mesylate was developed to support further clinical evaluation.

Small-molecule drugs targeting a single altered cell signaling pathway have been successfully developed as cancer therapeutics. However, many of these drugs suffer reduced clinical efficacy over time due to emergence of drug resistance.[1] One approach to overcoming this limitation is to develop agents that inhibit multiple signaling pathways by inhibiting a critical, shared upstream effector. Heat shock protein 90 (Hsp90) is a molecular chaperone that represents one of the promising targets for such a strategy[2] due to its crucial function in cellular homeostasis (e.g., maturation, folding, anti-aggregation, cellular trafficking, and stabilization of signal transduction proteins). The value of Hsp90 as an anticancer target was largely overlooked until the pioneering work of Neckers[3] which revealed Hsp90 as the molecular target responsible for the antitumor activity of the natural product geldanamycin. In addition to geldanamycin, the natural product radicicol has provided further anticancer target validation, and together have paved the way for development of numerous synthetic Hsp90 inhibitor chemotypes. To date, approximately 17 Hsp90 inhibitors have entered clinical development.[4] These clinical-stage Hsp90 inhibitors target the N-terminal, nucleotide-binding site of Hsp90 thus blocking the ATP-binding and rate-limiting hydrolysis steps in the ATPase cycle.

* Corresponding authors. Tel.: +1 732 658 6235; fax: +1 732 745 7270 (S.-H.K.); tel.: +1 801 209 2225; fax: +1 801 584 3640 (K.M.Y.).
E-mail addresses: seho.kimkim@gmail.com (S.-H. Kim), kraig.yager@gmail.com (K.M. Yager).
† Former employees of Myrexis Inc., 305 Chipeta Way, Salt Lake City, UT 84108, USA.

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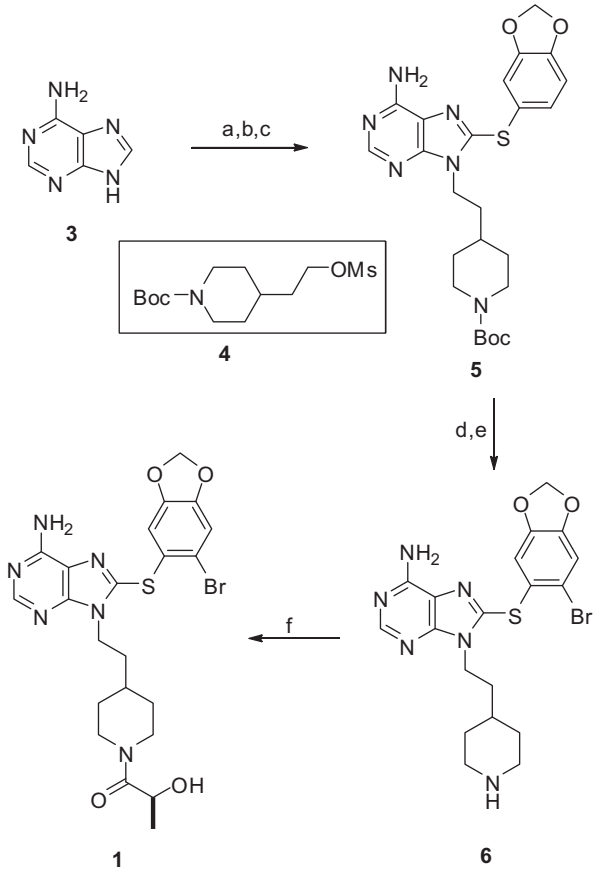
The tumor selectivity of Hsp90 inhibitors can be attributed to the high dependence of cancer cells on Hsp90 function.[5] Cancer cells are genetically unstable and rapidly adapt to stressful environments such as nutrient deprivation, hypoxia, and accumulation of misfolded proteins. Under these conditions, Hsp90 is upregulated and exists entirely in a multi-chaperone complex which preferentially stabilizes client proteins associated with oncogenic signaling pathways, and inhibition of Hsp90 can simultaneously deplete multiple oncogenic client proteins resulting in proteotoxic stress and cumulative antitumor effect. Thus, Hsp90 facilitates oncogene addiction along with non-oncogene addiction for stability and function of cancer cells. Taken together, targeting Hsp90 could be an effective strategy for treating cancer as a single agent or in combination with other chemotherapy drugs.[4,6]

Previously, we reported the discovery of **1** (MPC-3100), a purine-based synthetic Hsp90 inhibitor that was advanced into clinical development based on favorable pharmacokinetic (PK), pharmacodynamic (PD), and broad-spectrum antiproliferative activities in animal models.[7] In a phase I human clinical trial, **1** was well tolerated and exhibited PK/PD profiles similar to those observed in preclinical studies.[8] However, poor aqueous solubility (7.5 μg/mL in simulated intestinal fluid (SIF); pH 6.8) led to a dosage form with a high percentage of solubilizing excipient (40% Captisol) and consequently a development-limiting patient 'pill burden'. To facilitate development of oral dosage forms, a prodrug approach was explored with the goal of identifying derivatives of **1** demonstrating higher aqueous solubility and rapid bioconversion in the gastrointestinal tract. Herein, we describe the design, synthesis, and preclinical evaluation of candidate prodrugs that culminated in identification of L-alaninate mesylate salt **2i**, (MPC-0767).

To support clinical development of **1**, the scalable, six-step synthesis illustrated in Scheme 1 was developed and the details have been reported previously.[7] Briefly, N-alkylation of adenine (**3**) with readily available mesylate ester **4** followed by C8 bromination and displacement with potassium benzo[1,3]dioxole-5-thiolate provided thioether **5** in 48% overall yield. Selective C5′ bromination of benzodioxole **5** was achieved under mild conditions and preceded deprotection of the piperidine nitrogen with TFA and conversion to free base **6** with NH_4OH. EDCI mediated coupling of **6** with (L)-lactic acid afforded **1** in 74% yield.

The lactamide hydroxyl group of **1** presented a desirable point of attachment for solubilizing promoieties; straightforward synthetic chemistry and a physically robust bond potentially subject to hydrolysis by gastrointestinal esterases. To this end, the series of candidate ester prodrugs, **2a**–**k**, were synthesized by standard methods (Scheme 2). Three criteria were used to evaluate prodrug suitability: (1) solubility (>1 mg/mL in SIF, pH = 6.5–6.8), (2) acceptable oral bioavailability ($F\,\% > 40$), and (3) efficacy (>40% tumor regression in N-87 human tumor xenograft models). Two candidates that satisfied these criteria were alaninate and lysinate esters **2i** and **2k**, respectively. The lysine derivative **2k**, while providing exceptional solubility, could not be developed further due to poor physical and handling characteristics imparted by both hygroscopic and amorphous solid forms. Attempts to overcome these limitations via salt form variants (e.g., monohydrochloride, dihydrochloride, mesylate and maleate) proved ineffective. Additionally, during a maximum tolerated dose (MTD) study, one death was observed among the five mice ($nu^{+/-}$) treated with **2k**·3HCl (286 mg/kg po; equivalent to 200 mg/kg **1**). Conversely, **2i**·2HCl was well tolerated when administered at 252 mg/kg po (equivalent to 200 mg/kg dose of **1**). Consequently, variants of alaninate **2i** were the subject of further investigations.

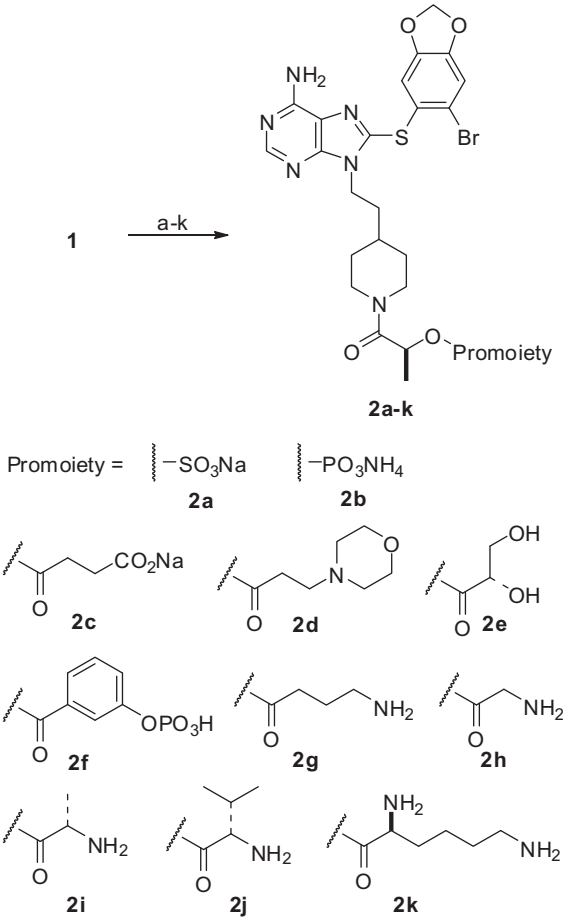
First, we sought to find an optimal salt form of **2i**. Selection of an optimal salt form is a critical element of early stage drug development; a poor choice can lead to challenging scale-up and drug product formulation campaigns and significant additional cost and protracted timelines. Systematic and rational strategies for optimal salt selection have been highlighted in the literature.[10]



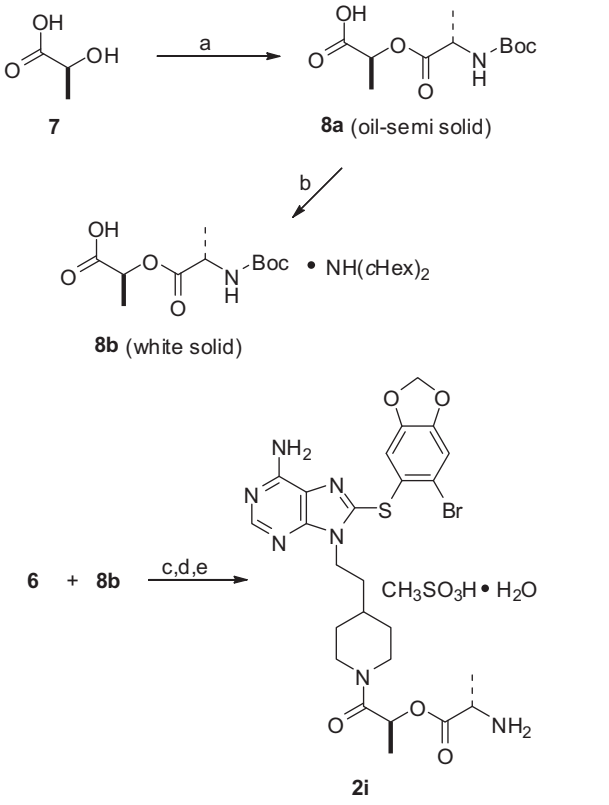
Scheme 1. Pilot plant synthesis of **1**. Reagents and conditions: (a) K_2CO_3, **4**, DMF, 35 °C, 65 h, 76%; (b) Br_2, NaOAc–AcOH, MeOH/THF, 20–25 °C, 1 h, 75%; (c) benzo[1,3]dioxole-5-thiol, K_2CO_3, DMF, 100 °C, 2.5 h, 85%; (d) NBS, AcOH, 20–25 °C, 3 h, 77%; (e) TFA, CH_2Cl_2, 20–25 °C, 3 h, then NH_4OH, 77%; (f) (L)-lactic acid, 1-hydroxybenzotriazole hydrate (HOBt), 1-(3-dimethylaminopropyl)-3-ethylcarbondiimide hydrochloride (EDCI), NEt_3, DMF/THF, 20–25 °C, 18 h, 74%.

Six salt forms of **2i** (HCl, HBr, sulfate, mesylate, fumarate, and maleate) were prepared using an approximately 1:1 stoichiometric ratio of drug and acid (Table 1). Hydrochloride, hydrobromide, and sulfate salts were found to be more hygroscopic than other salt forms as indicated by 8–10% weight gains upon 24 h exposure to conditions of 84% relative humidity (RH) at ambient temperature. In contrast, the phosphate, mesylate, maleate and fumarate salts exhibited relatively low moisture absorption with their water content staying relatively low and uniform at 3.5%, 1.9%, 3.7%, 3.1% and 0.4% for the phosphate, mesylate, fumarate, maleate, and succinate salts, respectively). The fumarate, maleate, and succinate salts, considered to have an acceptable hygroscopicity, displayed a lower melting point than parent **1** (203 °C, DSC), suggesting a less stable drug-counter ion complex.[10] These and the phosphate derivative also resulted in unacceptably low isolated yields or relatively poor purities. Therefore, the mesylate salt was selected for further evaluation.

To facilitate preclinical evaluation, a scalable, chromatography-free synthesis of **2i** mesylate was developed based on EDCI-mediated coupling of depsipeptide **8** and intermediate piperidine, **6** (Scheme 3). Depsipeptide **8a** was prepared from L-lactic acid (**7**) and Boc-L-alanine *N*-succinimidyl ester according to a literature procedure.[11] Ester **8a** was obtained as pale yellow oil and was used without purification in the EDCI-mediated coupling with **6**.



Scheme 2. Design and synthesis of prodrugs of **1**. Reagents and conditions: (a) **2a**, chlorosulfonic acid, pyridine, DMF, rt, then NaOEt, 26%; (b) **2b**, (1) bis(2,2,2-trichloroethyl)phosphoryl chloride, pyridine, rt, 31%, (2) $Cu(OAc)_2 \cdot H_2O$, Zn dust, AcOH, 100 °C, then acetylacetone, DMF, rt, 28%; (c) **2c**, succinic anhydride, DMAP, pyridine, rt, then NaOMe, 17%; (d) **2d**, 3-morpholin-4-yl-propionic acid HCl, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (EDCI), 4-(*N*,*N*-dimethylamino)pyridine (DMAP), DMF, rt, 45%; (e) (1) **2e**, (4*S*)-2,2-dimethyl-1,3-dioxolane-4-carboxylic acid, EDCI, DMAP, DMF, rt, 22%, (2) AcOH/H_2O (4:1, v/v), 50 °C, 34%; (f) **2f**, (1) 3-dimethoxyphosphoryloxybenzoic acid, EDCI, DMAP, DMF, rt, 74%, (2) TMSBr, CH_2Cl_2, rt, 39%; (g) **2g**, *N*-Boc-4-aminobutyric acid, EDCI, DMAP, DMF, then 4 M HCl in dioxane, 47%; (h) **2h**, Boc-glycine NHS ester, NEt_3, DMF, 60 °C, then 4 M HCl in dioxane, 22%; (i) **2i**, (2*S*)-2-([(*tert*-butoxy)carbonyl]amino)-propionic acid, EDCI, DMAP, DMF, rt, then 4 M HCl in dioxane, 37%; (j) **2j**, (2*S*)-2-([(*tert*-butoxy)carbonyl]amino)-3-methylbutanoic acid, EDCI, DMAP, DMF, rt, then 4 M HCl in dioxane, 42%; (k) **2k**, (*S*)-2,6-bis-*tert*-butoxycarbonylamino-hexanoic acid, EDCI, DMAP, DMF, rt, then 4 M HCl in dioxane, 72%.



Scheme 3. Reagents and conditions: (a) Boc-L-alanine *N*-succinimidyl ester, DMAP (0.1 equiv), pyridine (3.0 equiv), THF, rt, 1 d; (b) dicyclohexylamine, THF, rt then crystallization (aq MTBE), 65% (two steps); (c) EDCI, HOBt, DMF, rt, 8–12 h, 93%; (d) 4 M HCl, then NH_4OH; (e) methanesulfonic acid, THF, rt, 2 h, 75% (two steps).

Unfortunately, this streamlined approach led to impure (70–90%) **2i** mesylate and the introduction of preparative HPLC into the synthetic sequence. Ultimately, pure depsipeptide was required and this was achieved via crystallization of the dicyclohexylamine salt **8b** from aqueous methyl *t*-butyl ether (MTBE). EDCI-mediated coupling of **6** and **8b** proceeded in good yield and high purity (>95%). Deprotection and mesylate formation were uneventful and provided **2i** mesylate with excellent purity (>99% by HPLC) without chromatography.[9]

Karl Fisher analysis of prodrug candidate **2i** mesylate indicated a monohydrate form based on water content measurement (1.98%) and was supported by differential scanning calorimetry (DSC) which showed a small endothermic peak at around 95 °C.[9] In accordance with a general trend, **2i** mesylate had a higher melting point than the parent drug **1** (231 °C vs 203 °C, respectively). The monohydrate form was found to be very stable with essentially no water absorption after 7 days at 40 °C and 82% RH, and no measurable degradation as evidenced by HPLC analysis.

The kinetic solubility of **2i** mesylate was significantly greater (~52 fold) than the parent compound **1** (10.2 vs 536 μg/mL; pH 6.5, for **1** and **2i** mesylate, respectively), while the apparent permeability was dramatically reduced (420×10^{-6} vs 2.7×10^{-6} cm/sec at pH 6.2, for **1** and **2i** mesylate). The low permeability of **2i** mesylate suggests that bioconversion to parent should occur within the intestinal lumen (or apical side) and that systematic exposure to the prodrug should be minimal. Indeed, plasma concentration of **2i** was below the limit of detection at the earliest possible time points post oral dosing.

Table 1
Comparison of salt forms of **2i**

	Hydrochloride	Hydrobromide	Sulfate	Phosphate	Mesylate	Fumarate	Maleate	Succinate
Melting point (°C)	221.92	216.68	251.26	224.97	233.44	202.92	194.75	190.21
Weight change (%)[a]	8.0	8.4	9.7	3.5	1.9	3.7	3.1	0.4

[a] Saturated KCl solution was used to maintain 84% relative humidity (RH) conditions at room temperature. Weight change (%) of salt forms was determined after storage at 25 °C/84% relative humidity for 1 day.

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Table 2
Half-life of **2i** mesylate in EDTA plasma (min)

Mouse	Monkey[a]	Human	Phosphate buffer (pH 7.4)
<2	300	149	145

[a] Monkey plasma was incubated at room temperature and sampling time was extended to 240 min.

Table 3
Half-life of **2i** mesylate in liver microsomes (min)

Mouse[a]	Mouse[b]	Human[a]	Human[b]
<2	<2	2	2

[a] In the absence of NADPH.
[b] In the presence of NADPH.

Table 4
Half-life of **2i** mesylate in simulated gastric and intestinal fluid (min)

SGF		SIF	
pH 1.2	pH 1.2 + pepsin	pH 6.8	pH 6.8 + pancreatin
>240	>240	173	22

Table 5
PK profiles of **2i** mesylate and **1**

Compound	Route	Dose (mg/kg)	T_{max} (h)	C_{max} (ng/mL)	AUC (h * ng/mL)	F %
1[a]	iv	2.5	—	—	2106	—
1[b]	po	10	0.25	2802	3214	38
1[c]	po	200	1	24888	113035	67
1[d]	po	200	0.3	7366	35094	21
2i mesylate[d]	po	265	1	21562	94194	56

[a] Formulation: DMA/PEG300/EtOH/H_2O (3:40:12:45, v/v/v/v).
[b] Formulation: DMA/PEG300 (5:95, v/v).
[c] Formulation: 40% Captisol.
[d] Formulation: 2% CMC; analyses for parent compound **1**.

Candidate prodrug **2i** mesylate was evaluated in various matrices to examine its chemical stability and kinetics and mechanism of bioconversion. It was rapidly converted to parent **1** in mouse plasma and mouse and human liver microsomes, but was very stable in monkey and human plasma, similar to non-enzymatic hydrolysis in phosphate buffer (Tables 2 and 3). The facile hydrolysis of **2i** mesylate in mouse plasma versus primate plasma can be attributed to high plasma esterase activity in mice.[12] In SIF, **2i** mesylate was stable with and without added pepsin, indicating that **2i** mesylate would not likely be subject to non-enzymatic conversion in the stomach. In contrast, **2i** mesylate was rapidly converted to **1** in pancreatin-rich SIF (Table 4) supportive of a primarily enzyme-mediated cleavage process, and the assumption that **2i** is most likely bioconverted to parent **1** by enzymes present in the intestinal lumen prior to absorption.

The oral PK properties of **2i** were assessed in mice as described in Table 5. In these studies, the plasma concentration of parent **1** was quantified by LC–ESI–MS/MS and despite concerns over drug absorption, the PK profiles of prodrug **2i** formulated in 2% carboxymethylcellulose (CMC), were similar to that of parent **1** dosed in 40% Captisol (**1** F % = 67; 200 mg/kg, and **2i** mesylate F % = 56; 265 mg/kg, respectively). Prodrug **2i** mesylate (265 mg/kg; 200 mg/kg equivalent of **1**) also demonstrated comparable efficacy in an N-87 xenograft tumor model to that observed for parent **1** (46% tumor size reduction at 200 mg/kg) with no evidence of weight loss in the study animals. It is tempting to speculate that a super-saturated solution of parent **1**, produced as a result of **2i** bioconversion, may be responsible for superior absorption. Alternatively, it could be the result of precipitation of an amorphous parent drug which would be expected to a higher dissolution rate and thus net absorption.[13] Based on these data, L-alaninate **2i** mesylate was selected for further clinical evaluation.

In summary, numerous ester derivatives of the Hsp90 inhibitor **1** were designed and synthesized in an effort to address drug formulation issues imparted by its poor aqueous solubility. Evaluations of the potential prodrugs **2a**–**2k** both in vitro and in vivo led to the identification of L-alaninate **2i** mesylate that satisfied the criteria of aqueous solubility, chemical stability, bioconvertibility, and adequate PK and PD properties. Based on these characteristics, **2i** mesylate was selected for further clinical evaluation which was supported by an efficient, scalable, and chromatography-free synthesis.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.09.053.

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