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Microwave assisted synthesis and docking study of *N*-(2-oxo-2-(4-oxo-2-substituted thiazolidin-3ylamino)ethyl)benzamide derivatives as anticonvulsant agents

Anna Pratima G. Nikalje [a,*], Arfat N. Shaikh [a], Sameer I. Shaikh [a], Firoz A. Kalam Khan [a], Jaiprakash N. Sangshetti [a], Devanand B. Shinde [b]

[a] Department of Pharmaceutical Chemistry, Y.B. Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Rauza Bagh, P.B. No. 33, Aurangabad 431001, M.S., India
[b] Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad 431004, M.S., India

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ABSTRACT

Herewith, we report the design and synthesis of a series of *N*-(2-oxo-2((4-oxo-2-substituted thiazolidin-3yl)amino)ethyl) benzamide derivatives **7**(**a**–**j**) under microwave irradiation, based on four component pharmacophoric model to get structural prerequisite indispensable for anticonvulsant activity. The synthesized derivatives were investigated in maximal electroshock seizure (MES), subcutaneous pentylenetetrazole (sc-PTZ) induced seizure and neurotoxicity screening. All the test compounds were administered at a dose of 30, 100 and 300 mg/kg body weight at the time interval of 0.5 h and 4 h. The compounds were also evaluated for behavioral activity and toxicity study. The compound **7h** was found to be most active in MES model. The anticonvulsant screening data shows that 65% of the compounds were found active against MES model when compared to 35% sc-PTZ model. The computational parameter such as docking study, log*P* determination and ADME prediction were performed to exploit the results.

Epilepsy is a common neurological condition, affecting 0.5–1% of the population worldwide.[1,2] Current drug treatments for epilepsy suffers from a number of disadvantages including the convulsions of approximately 25% of epileptics that are not controlled by medication.[3] In recent years, the field of antiepileptic drug development has become quite dynamic, providing many promising research opportunities.[4] The search for new anticonvulsant drugs continues to be an active area of investigation in medicinal chemistry.

Benzamide derivatives and related compounds constitute an important class of compounds and are known to possess a wide range of biological activities such as muscle relaxant, antiarrhythmic, antiemetic and antipsychotic.[5,6] Several patents and articles reported the anticonvulsant activity of benzamide containing compounds.[7–12] On the other side, thiazolidinone nucleus is also a potential candidate for anticonvulsant activity and is contained in ralitoline Figure 1 (**8**). Ralitoline is found to be effective in preclinical anticonvulsant evaluation. Various research groups have reported the anticonvulsant activity of thiazolidinone and its mechanism due to inhibition of oxidation of the substrates of the tricarboxylic acid cycle like pyruvate, α-ketoglutarate and citrate, and β-hydroxybutyrate.[13–17] Structures of some anticonvulsant agents bearing benzamide and thiazolidinone nucleus were reported in literature and are represented in Figure 1. Based on these facts of available literature and as a part of our continuous investigation in the area of anticonvulsant synthesis and its biological evaluation[18–20] herein, we designed and synthesized a series of compounds having benzamide group coupled with thiazolidinone ring via amide linkage which serves as a hydrogen binding domain with the possibility to get optimal pharmacological profile. A four point pharmacophoric model was proposed for studying structurally different compounds with anticonvulsant activity with different mechanism of action.[21] Based on this proposition, our synthesized compounds also comprises of this four point pharmacophoric model (Fig. 2) as follows:

1. *Hydrogen Binding Domain (HBD):* amide group which is attached to phenyl ring, that is, benzamide ring system.
2. *Electron donor moiety (D):* keto group near thiazolidinone ring system acts as electron donor.
3. *Distal aryl domain (A):* phenyl ring of benzamide ring system can perform function of distal aryl domain.

* Corresponding author. Tel.: +91 9823619992; fax: +91 0240 2381129.
E-mail address: annapratimanikalje@gmail.com (A.P.G. Nikalje).

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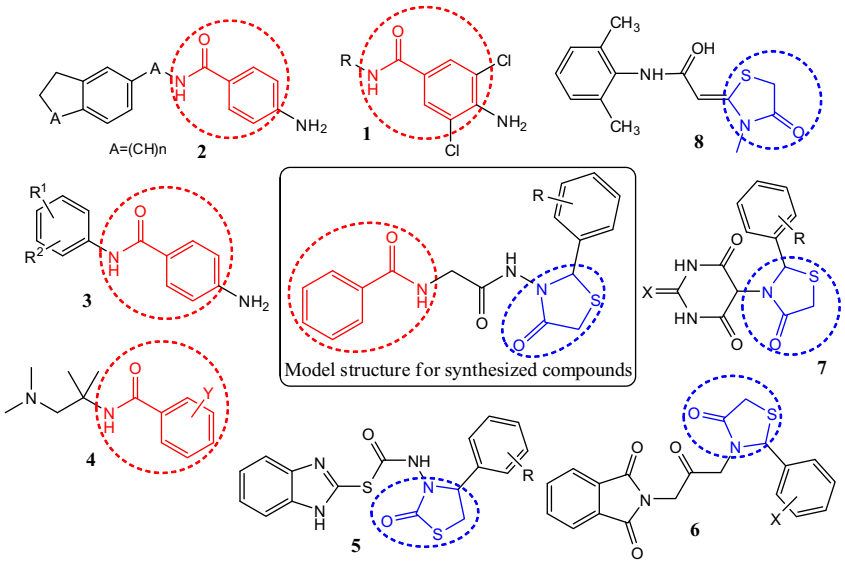


Figure 1. Structures of some anticonvulsants reported in literature **1–8**.

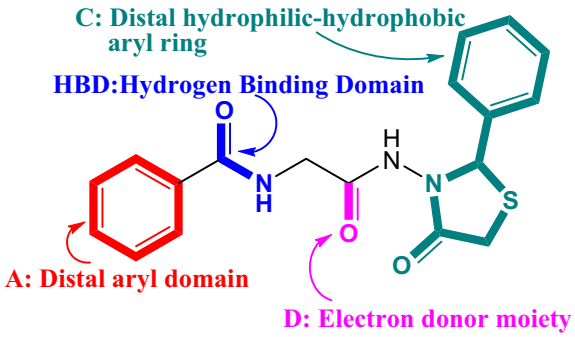


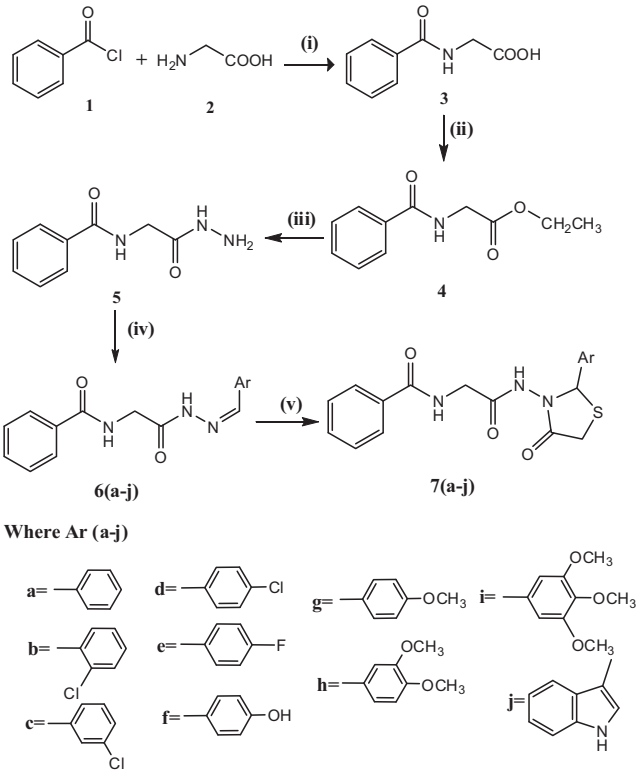
Figure 2. Structural requirement for anticonvulsant model bearing four component pharmacophoric model.

4. *Distal hydrophilic–hydrophobic aryl ring (C):* thiazolidinone nucleus with phenyl ring attached can serve as distal hydrophilic–hydrophobic aryl ring.

The computational parameter like molecular docking study, pharmacokinetic properties (ADME) and log*P* were determined. Along with the synthesis and anticonvulsant evaluation of titled compounds, toxicity study was also evaluated.

Target compounds **7**(**a**–**j**) were obtained according to Scheme 1. In first step, 2-benzamidoacetic acid **3** was synthesized by stirring benzoyl chloride **1** with glycine **2** in aq NaOH in ice-bath. Then compound **3** was esterified by refluxing in ethanol and concn H_2SO_4 for 6–8 h to get ethyl 2-benzamido acetate **4**. Compound **4** was further refluxed with hydrazine hydrate in ethanol for 3 h to obtain (2-hydrazinyl-2-oxoethyl)benzamide **5**.[22] Schiff's bases **6**(**a**–**j**) were prepared by refluxing compound **5** with various aromatic and heterocyclic aldehydes in the presence of glacial acetic acid using ethanol as solvent.[23] In final step, respective Schiff's bases **6**(**a**–**j**) were cyclized with thioglycolic acid in presence of anhydrous zinc chloride as a catalyst in DMF as solvent under microwave irradiation for 12–17 min to obtain titled compounds **7**(**a**–**j**). The structures of the synthesized compounds were confirmed by their physical characterization and spectral data, given in Supplementary material.

The anticonvulsant activity of titled compounds **7**(**a**–**j**) were assessed by anticonvulsant drug development (ADD) program



Scheme 1. Synthesis of titled compounds **7**(**a**–**j**). Reagents and conditions: (i) aq NaOH, HCl, Stirring 1–2 h, (ii) concn H_2SO_4, ethanol, reflux 6–8 h, (iii) $NH_2NH_2{\cdot}H_2O$, ethanol, reflux 3 h (iv) Ar-CHO, Gla. acetic acid, ethanol, reflux 6–8 h, (v) $SHCH_2COOH$, anhydrous $ZnCl_2$, DMF, microwave irradiation 12–17 min.

protocol.[24,25] The anticonvulsant potential of test compounds were performed by two models namely, maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole (sc-PTZ), at the dose of 30, 100 and 300 mg/kg. Neurotoxicity screening was performed by measuring minimal motor impairment using rotorod test[26] and behavioral study was evaluated by actophotometer.[27] The toxicity study on mice liver with ip administration of test compounds was also performed.[28]

The MES and sc-PTZ results of titled compounds were shown in Table 1. In MES, compounds **7f** and **7h** showed protection at 30 mg/kg while compounds **7b**, **7d** and **7i** showed protection against at the dose of 100 mg/kg. The compound **7h** showed protection at the dose of 30 mg/kg at 0.5 h and 4 h time interval indicating the compound to be highly potent and long acting. Similarly compound **7f** was showed protection against 30 mg/kg at 0.5 h indicating that it was highly potent and short acting as requires 4 h protection at the dose of 100 mg/kg. The compound **7d** showed protection at the dose of 100 mg/kg at both time intervals indicating that the compound is potent and long acting. In sc-PTZ screening, compounds **7a**, **7g**, and **7h** showed protection against 100 mg/kg at 0.5 h time interval, while compounds **7a**, **7h** and **7i** showed protection the dose of 300 mg/kg at 4 h time interval. None of the compounds was found potent in sc-PTZ test at the minimum dose 30 mg/kg. The data is shown in Table 1. The anticonvulsant screening data shows that 65% of the compounds were found active against MES model when compared to 35% sc-PTZ model.

As observed through data analysis, the compounds with smaller electron donating polar groups like dimethoxy and hydroxy attached to phenyl ring show good anticonvulsant activity. Substitution of 3,4-dimethoxy group on aromatic ring **7h** was found to be the most effective in MES model and sc-PTZ model. In MES model, **7h** was found to be highly potent and of long duration of action. Hydroxy substitution at *para* position **7f** of phenyl ring was found to be potent and short acting. When aromatic ring was replaced by heterocyclic ring (-indolyl), there is loss in anticonvulsant activity. During the study most of the synthesized derivatives were more effective in the MES test and the MES test is known to be sensitive to sodium channel inhibitors (e.g., Phenytoin), which suggested that tested compounds may inhibit voltage-gated ion channels (particularly sodium channels).[18,29]

In neurotoxicity screening (Table 1), compounds **7b** and **7i** showed neurotoxicity at 100 mg/kg in both intervals while the compound **7c** showed neurotoxicity at the dose 30 mg/kg in both time intervals. Some compounds were found to be less neurotoxic as compared to phenytoin. In behavioral activity (Table 2) using actophotometer (locomotor activity), all the titled compounds showed no behavioral despair effect when compared to diazepam at 0.5 h. The compound **7d** showed decreased locomotor activity in the 4 h interval but no significant effect on behavioral despair at 0.5 h interval when compared to standard diazepam. All other compounds **7a**, **7b**, **7c**, **7e**, **7f**, **7g**, **7h**, **7i** and **7j** were found to decrease locomotor activity of the animals as compared to diazepam at 4 h time interval.

Table 1
Anticonvulsant and neurotoxicity screening of compounds

Entry	MES screen		scPTZ screen		Neurotoxicity	
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
7a	300	300	100	300	100	300
7b	100	300	—	—	100	100
7c	300	—	—	—	30	30
7d	100	100	300	—	—	—
7e	—	—	—	—	—	300
7f	30	100	—	—	—	—
7g	300	—	100	—	300	300
7h	30	30	100	300	—	—
7i	100	300	300	300	100	100
7j	300	—	—	—	300	100
Phenytoin	30	30	×	×	—	100
Sodium valproate	×	×	300	—	—	—

Doses of 30, 100, 300 mg/kg of the compound were administered intraperitoneally the protection and neurotoxicity measured after 0.5 h and 4 h. The above figure clearly indicates that the minimal dose required causing protection or neurotoxicity in 50% or more of the animals. The dash (—) indicates the absence of anticonvulsant activity or neurotoxicity (×) denotes not tested.

Table 2
Behavioral study of compounds by using actophotometer

Entry	Activity score by using actophotometer		
	Control (24 h before)	Post treatment[a]	
		0.5 h	4 h
7a	119.62 ± 16.53	74.00 ± 3.715**	94.80 ± 4.893**
7b	133.25 ± 2.30	108.80 ± 7.324**	110.80 ± 7.324**
7c	170.41 ± 8.34	102.60 ± 9.770**	108.20 ± 1.463**
7d	115.39 ± 3.80	103.60 ± 2.713**	71.60 ± 8.875**
7e	182.36 ± 3.58	64.60 ± 7.501**	94.00 ± 9.659**
7f	177.68 ± 7.98	38.20 ± 3.184ns	99.60 ± 2.159**
7g	157.22 ± 2.76	77.80 ± 3.277**	80.20 ± 5.987**
7h	118.33 ± 15.33	42.00 ± 11.375**	88.60 ± 9.532**
7i	140.28 ± 2.85	92.60 ± 3.776**	101.00 ± 2.345**
7j	210.71 ± 9.62	102.60 ± 3.265**	120.00 ± 4.506**
Diazepam[b]	118.20 ± 9.22**	33.30 ± 2.557**	75.40 ± 13.309**

[a] Denotes each value represents the mean ± SEM significantly different from the control at p <0.05, ns denotes not significant at p <0.05 (Student's t test).
[b] Denotes the compound was tested at dose level of 4 mg/kg (ip).
** Indicates p value is between 0.001 and 0.01 and very significant.

In toxicity study we evaluated the histopathology of mice liver (T.S. of liver) given in Figure 3. The Figure 3(a) indicates the effect of control group (0.5% Tween 80) on albino mice liver which shows no inflammation and no toxicity and only fatty changes were observed. Figure 3(b) indicates the effect of standard drug (phenytoin), which shows hepatotoxicity on mice liver at dose 300 mg/kg. Section from liver shows lobules of the liver with extensive necrosis and fatty changes. The Figure 3(c) indicates that the effect of compound **7h** shows fatty changes and lesser inflammation seen at some places at dose 300 mg/kg. Finally in Figure 3(d) the effect of compound **7a** shows inflammation and extensive necrosis on albino mice at dose 300 mg/kg.

Titled compounds were docked against the crystal structure of sodium channel receptor (PDB ID: 1BYY)[30] using VLife MDS 4.3 package.[31] The docking study results revealed that the compound binds to the active site of sodium channel receptor by forming various hydrophobic and Van der Waal's interactions. The compound **7h** has shown good binding energy, that is, −36.45 kcal/mol. Benzamide coupled with thiazolidinone core is located in the hydrophobic binding cleft lined with PHE1489, MET1490, THR1491, and LYS1495. Also, the 3,4-dimethoxy phenyl group of compound **7h** has formed hydrophobic interactions with amino acid residues PHE1489, MET1490, THR1491 and LYS1495. The docking pose for compound **7h** was shown in Figure 4. The oxygen of $-OCH_3$ group at para position has formed hydrogen bond with amino acid MET1490 (2.578 Å). The compound has formed various Van der Waal's interactions with surrounding amino acid residues like PHE1489, MET1490, THR1491 and LYS1495. Thus compound **7h** has potential to inhibit sodium channel receptor and can be processed further to develop as a lead compound. Log$P$ values of titled compounds were determined by measuring the partition coefficient between octanol and phosphate buffer at room temperature using UV spectroscopy.[32] We found that the experimental values of titled compound are in good agreement with theoretical values and are given in Table 3. The prediction of ADME properties were determined by Molinspiration online property toolkit[33] and are given in Table 4. The properties such as total polar surface area (TPSA), number of rotatable bonds (n-ROTB), number of hydrogen bond donor (n-OHNH) and acceptors (n-ON) and log$P$ was determined. The compounds exhibited highest% absorption (ABS) ranging from 72.37% to 81.92%. None of the compounds synthesized violated Lipinski's parameters. From the above results, it can be concluded that titled compounds **7**(**a**–**j**) possess property of drug-likeness. The details of experimental procedures, pharmacology, toxicity study and computational parameters studied such as

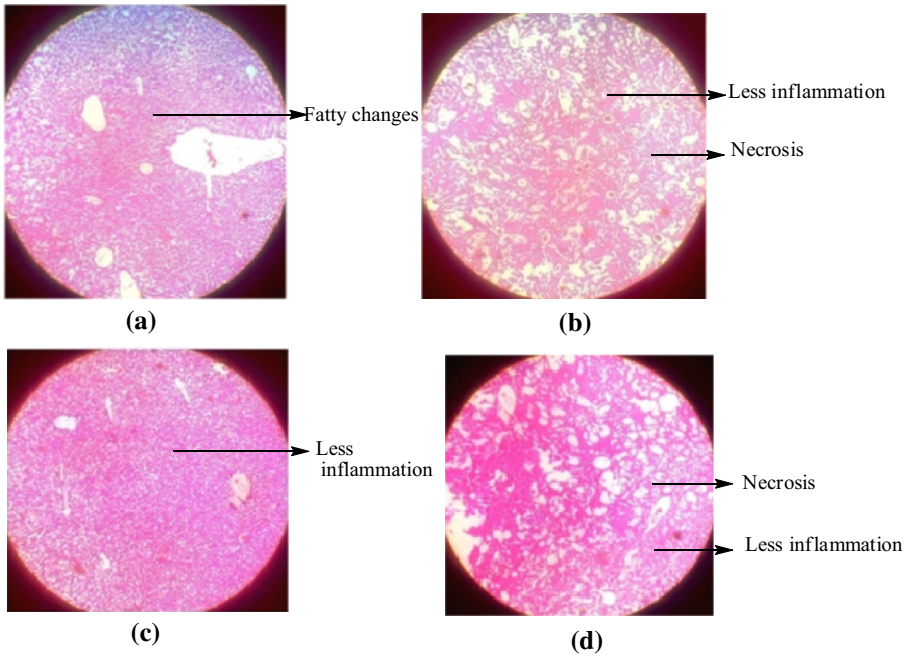


Figure 3. Histopathology of mice liver (T.S. of liver) showing effect of control (a), standard drug (b), compound **7h** (c) and compound **7a** (d).

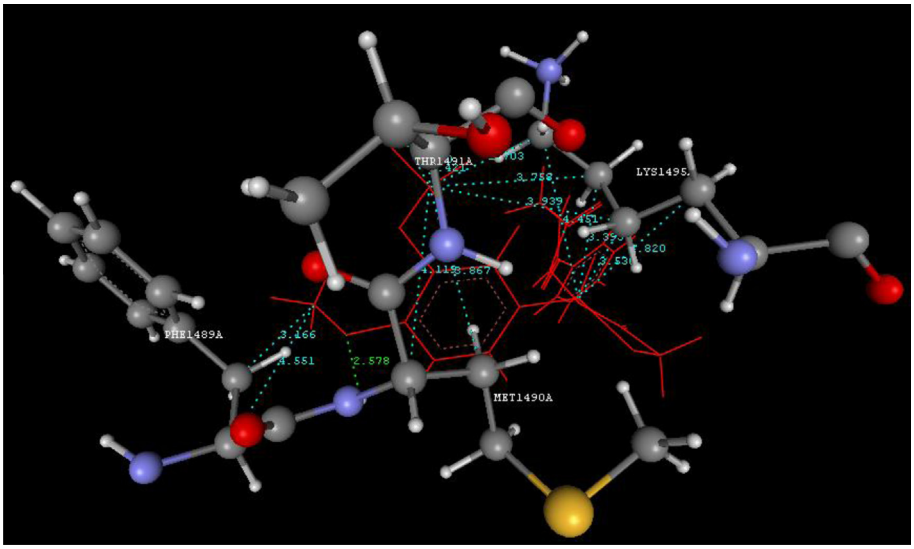


Figure 4. Docking image of compound **7h**.

Table 3
Log*P* for synthesized compounds

Entry	Experimental log*P*	Theoretical log*P*
7a	1.95	1.81
7b	2.30	2.36
7c	2.80	2.36
7d	2.87	2.36
7e	2.10	1.96
7f	1.75	1.45
7g	1.85	1.68
7h	1.85	1.55
7i	1.65	1.48
7j	1.70	1.45

Theoretical log*P* was calculated by Molinspiration online property toolkit.

docking, log*P* calculations and ADME properties are given in Supplementary material.

In conclusion, a series of titled compounds **7**(**a**–**j**) were designed and synthesized by efficient microwave irradiation in good to excellent yields. In this study, we validated the four component pharmacophoric model which is important for anticonvulsant activity. Preliminary anticonvulsant evaluation was analyzed by electrical test (MES model) and chemical test (sc-PTZ test) in animal mice model. The compound **7h** showed potent anticonvulsant activity in MES model while compounds **7a** and **7h** showed significant anticonvulsant activity in sc-PTZ model. The structure activity relationship revealed that the compounds having 3,4-dimethoxy phenyl and 4-hydroxy phenyl group attached to 2nd position of thiazolidinone ring were responsible for enhancing anticonvulsant activity in MES model and can be developed as lead candidates. The docking study exhibited good binding properties,

Table 4
Pharmacokinetic parameter important for good oral bioavailability of compounds

Entry	%ABS	TPSA (A^2)	*n*-ROTB	MW	MV	*n*-OHNH donors	*n*-ON acceptors	Lipinski's violations
Rule	—	—	—	<500	—	<5	<10	⩽1
7a	81.92	78.50	5	355.41	307.70	2	6	0
7b	81.92	78.50	5	389.86	321.24	2	6	0
7c	81.92	78.50	5	389.86	321.24	2	6	0
7d	81.92	78.50	5	389.86	321.24	2	6	0
7e	81.92	78.50	5	373.48	312.64	2	5	0
7f	74.93	98.73	5	371.41	315.72	3	7	0
7g	75.55	96.97	6	385.44	333.25	2	7	0
7h	75.55	96.97	7	415.47	358.80	2	8	0
7i	72.37	106.20	8	445.49	384.34	2	9	0
7j	76.47	94.29	5	394.45	336.68	3	7	0

%ABS, percentage of absorption; TPSA, topological polar surface area; *n*-ROTB, number of rotatable bonds; MW, molecular weight; MV, molecular volume; *n*-OHNH, number of hydrogen bond donors; *n*-ON, number of hydrogen bond acceptors.

thus indicating that the most active compound **7h** acts by inhibiting sodium channel receptor. Also, none of the synthesized compounds have violated Lipinski's rule of five, thus showing good drug like properties.

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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.2014.11.016.

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