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Antibacterial activity of noscapine analogs

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ABSTRACT

The antibacterial properties of close noscapine analogs have not been previously reported. We used our pDualrep2 double-reporter High Throughput Screening (HTS) platform to identify a series of noscapine derivatives with promising antibacterial activity. The platform is based on RPF (SOS-response/DNA damage) and Katushka2S (inhibition of translation) proteins and simultaneously provides information on antibacterial activity and the mechanism of action of small-molecule compounds against *E. coli*. The most potent compound exhibited an MIC of 13.5 μM (6.25 μg/ml) and a relatively low cytotoxicity against HEK293 cells (CC_{50} = 71 μM, selectivity index: ~5.5). Some compounds from this series induced average Katushka2S reporter signals, indicating inhibition of translation machinery in the bacteria; however, these compounds did not attenuate translation *in vitro* in a luciferase-based translation assay. The most effective compounds did not significantly arrest the mitotic cycle in HEK293 cells, in contrast to the parent compound in a flow cytometry assay. Several molecules showed activity against clinically relevant gram-negative and gram-positive bacterial strains. Compounds from the discovered series can be reasonably regarded as good templates for further development and evaluation.

Introduction

Noscapine (narcotine, Fig. 1) is a phthalideisoquinoline alkaloid that was first described in 1803 by Jean-François Derosne, who isolated crystalline substances containing noscapine and morphine from the opium poppy. Slightly later, in 1817, Pierre-Jean Robiquet successfully purified and characterized noscapine as an individual component of Derosne's salt.[1,2] Noscapine subsequently attracted little attention until 1930 and was mostly used as a tool compound. However, starting from the antitussive action of noscapine via the inhibition of the ACEI/bradykinin pathway,[3,4] a broad spectrum of potential clinical applications of noscapine were uncovered over the next few decades. Padmashree C. G. Rida and colleagues[5] recently provided a comprehensive overview of many proven and promising clinical applications of noscapine. For instance, noscapine is used to prevent ischemia–reperfusion injury[6,7] via the blockage of bradykinin receptors, thereby mitigating stroke sequelae. Numerous clinical studies have revealed the significant antineoplastic effect of noscapine against a wide range of cancers.[8] Thus, a relatively high concentration of noscapine induces apoptosis and metaphase arrest.[9] Noscapine has been found to mainly alter microtubule assembly dynamics without affecting the polymerization and polymer/monomer ratio.[10,11] Treating various cell types, including HeLa cells, with noscapine has been observed to reduce the tension across kinetochores and abnormalities in mitotic spindles, leading to the

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blockage of mitotic progression and chromosome congression during metaphase. Noscapine has been found to be effective against thymoma,[12] ovarian carcinoma,[13,14] glioblastoma,[15,16] nonsmall cell lung cancer,[17] gastric cancer,[18] human colon cancer,[19,20] prostate cancer,[21,22] and breast cancer.[23–27] The potential clinical benefits of noscapine are not restricted to antitussive and anticancer action. Noscapine has also been found to attenuate dopamine biosynthesis[28] and the NF-κB signaling pathway.[29] Noscapine inhibits HIF-1α and related VEGF expression[30] and possesses antifibrotic effects.[31] In particular, noscapine has no significant side effects and negligible toxicity to normal organs and tissues. The noscapine structure has at least five diversity points, and the large number of reported noscapine analogs have demonstrated similar pharmacological profiles, mostly as anticancer compounds with some improvements in pharmacokinetics and the target activity/selectivity profile. Integrity searching[32] has revealed 64 analogs of noscapine containing a 5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquinolin-4-ol core. Mitsubishi Chemical developed tritoqualine (hypostamine, livalfa) **I** (Fig. 1), an inhibitor of histidine decarboxylase and launched the drug in France against asthma,[33] and Hefei Qixing Pharmaceutical Technology developed the macrocyclic derivative cepharanthine **II**, which was evaluated in combination with azelastine and fexofenadine in clinics against hypereosinophilic syndrome.[34] The mechanism of action and related therapeutic applications for direct noscapine analogs has been investigated (Table 1). As shown in Tables 1 and 2, this class of compounds has generally been reported as microtubule destabilizers with anticancer activity. Despite the numerous potential therapeutic applications that have been published and claimed in the patent literature, we found no information on antibacterial activity for noscapine and its direct analogs. Razavi and colleagues demonstrated the inactivity of noscapine against many isolates of *Staphylococcus aureus*, with a minimum inhibitory concentration (MIC) in the range of 207–413 μM/ml.[35] Roger Frechette[36] reported a class of tetrahydroisoquinoline derivatives in 2009 as PPAT inhibitors and antibacterials. All the described molecules, including the structures presented as representative examples, are *N*-benzyl- or *N*-benzoyl-substituted analogs. The claimed synthetic route produces compounds with a phenyl substituent at position 5 of the tetrahydro-[1,3]dioxolo[4,5-g]isoquinoline core. Note that the most active compound containing this core exhibited an IC_{50} value of 30–50 μM against PPAT in a kinetic *in vitro* assay, and no corresponding MIC value was reported.

Table 1
Mechanism of action and therapeutic group for the reported noscapine analogues*. Therapeutic groups for noscapine analogues.

Mechanisms of action	cmpds	Therapeutic group	cmpds
Microtubule destabilizers	22	Oncolytic drugs	50
μ-Opioid receptor agonists	3	Antiasthmatic drugs	5
Histidine decarboxylase inhibitors	2	Antiviral drugs	3
Caspase 3 activators	2	Opioid analgesics	3
STPK13 inhibitors	1	Treatment of protozoal diseases	3
NFKB activation inhibitors	1	Allergic rhinitis therapy	2
LDHA inhibitors	1	Anti-inflammatory agents	2
		Dermatologic agents	2
		Ophthalmic drugs	2
		Treatment of atopic dermatitis	2
		Antimalarials, fibrosis, hematologic agents, hepatoprotectants, MLS, ND	6

* These data were collected based on a thorough search of the Integrity,[32] PubMed,[37] and SciFinder[38] databases; MLS - myotrophic lateral sclerosis, ND - neurodegenerative diseases

Table 2
HTS summary (ΔTolC *E. coli*) and the reporter response (na – not active, T - translation).

Activity	Inhibition area (mm)	Cmpds	%	Response	Signals	%
+++++	>25	1	0.2	High_SOS and High_SOS + T	1	1.0
++++	20–25	3	0.6	Average_SOS	6	5.9
+++	16–20	5	1.0	Average_T	1	1.0
++	11–16	13	2.7	Average_T + SOS	0	0
+	7–11	38	7.9	Low_SOS	41	40.6
+/-	4–7	44	9.1	Low_T	20	19.8
na	0–4	378	78.5	Low_T + SOS	32	31.7
Total		482	100	**Total**	101	100

Results and discussion

HTS platform

We used our pDualrep2 double-reporter HTS platform to screen>190,000 molecules with low structural similarity to the reported antibiotics for antibacterial activity.[39] Briefly, this platform is based on a specific double-reporter system (red fluorescent protein - RPF and far-red fluorescent protein - Katushka2S) that allows us to preliminarily investigate the mechanism of action of the tested compounds simultaneously. Thus, the expression of Katushka2S is observed mainly upon exposure to ribosome-stalling compounds (translation inhibitors), whereas the RPF signal is induced via the SOS response pathway (DNA damage). This system and the discovered antibacterial molecules have been previously described in more detail.[40–48]

Antibacterial activity

HTS and cytotoxicity

The antibacterial activity of the 482 noscapine analogs available in the InterBioScreen collection[49] was evaluated using the HTS platform at a concentration of 17–20 mg/ml (1–1.5 μl injection volume). As shown in Table 2, nine compounds (**1**–**9**, Fig. 2) were found to have strong

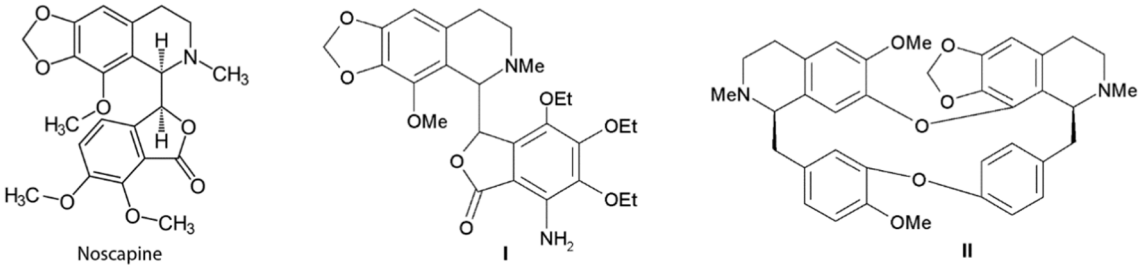

Fig. 1. The most advanced noscapine analogs reported to date. Compound **I** (livalfa) was launched against asthma in France, and compound **II** was evaluated in combination with azelastine and fexofenadine in clinics against hypereosinophilic syndrome.

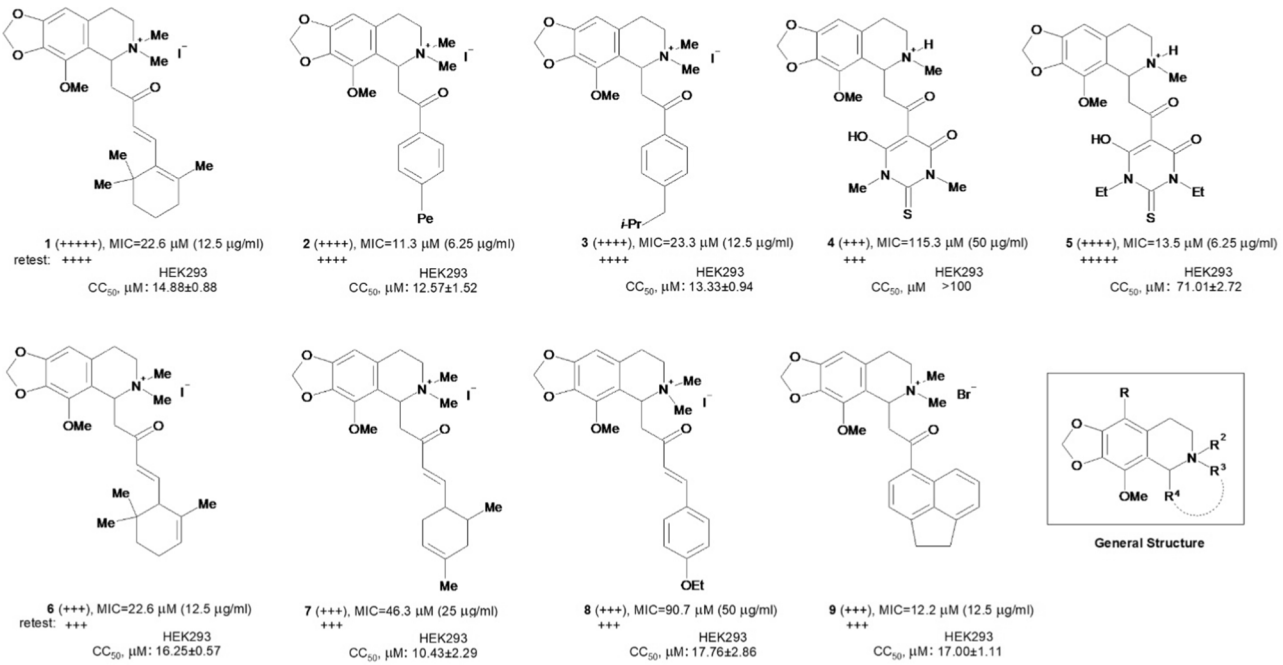

Fig. 2. General structure and the most active noscapine analogs revealed during HTS (structures and activities of other compounds from this class are presented in SI; retest means retesting under the same conditions).

inhibition potency (inhibition area > 16 mm, +++ and higher) against ΔTolC *E. coli*. Compound **2** showed a relatively high activity: the MIC of 11.3 μM (6.25 μg/ml was similar to that obtained for well-known antibiotics, e.g., erythromycin has an MIC of 5.32 μM (3.91 μg/ml). However, only three compounds (**2**, **4** and **7**) showed weak inhibition potency against wt *E. coli*, presumably because of poor permeation through the bacterial membrane. This issue can be addressed by modifying the hit structure via standard approaches, including adding a basic center, conferring rigidity, reducing globularity and improving the amphiphilicity index.[50] In particular, compounds that demonstrated moderate activity in HTS (++ or +++) exhibited relatively low MIC values in several cases, and the HTS output did not correlate exactly with the observed MIC values.

The antibacterial activity of compounds **1** and **6** can be partially mediated by attaching retinol and α-ionone substructures, respectively, at position 5 of the core fragment, where the antibacterial activity of the retinoid- and α-ionone-family compounds has been previously described [see review: [51–58]]. Hybrid structures **4** and **5** contain a 2-thiobarbituric acid moiety that can be found in several DNA topoisomerase II inhibitors and DNA-intercalating drugs, e.g., merbarone[59] and exhibits anticancer[60] and antibacterial activity.[61] However, only a weak SOS response was detected for these molecules, indicating other mechanisms of action distinct from gyrase inhibition or intercalation. Compound **2** showed relatively high inhibition activity against ΔTolC *E. coli* and

Table 3
The activity of compounds **1**–**9** against different microorganisms.

ID *Strain*	1	2	3	4	5	6	7	8	9
E. coli mx7[a,b,c,d]	+	++	++	±	±	–	+	±	–
E. cloacae O3[a,b,c,d]	+	+	+	±	–	–	±	±	–
E. coli ATCC 25,922	+	+++	++	±	–	–	+	±	–
Aeromonas sp. O9	+	++	++	–	–	–	–	±	–
M. morganii R36	+	+++	++	–	–	±	+	±	–
P. aeruginosa R42	+	++	++	±	–	–	±	±	–
K. pneumoniae 181210171-2[a,e]	+	++	+	–	–	–	±	±	–
Enterobacter sp. O54	±	++	+	+	–	–	±	±	–
Proteus sp. O14[a,b,c,d,e]	–	+	++	±	–	–	–	–	–
C. koseri R38	–	–	+	–	–	–	–	–	–
P. aeruginosa ATCC 27853[a,b]	±	±	–	–	–	–	–	–	–
H. alvei O31[a]	+	+++	++	±	–	–	±	±	–
P. vulgaris O41	–	+	+	–	–	–	–	+	–
C. albicans[a,b,c,d,e] 181210169-1	++	+++	++	++	±	–	++	±	–
E. aerogenes R41	+	+	+	±	–	±	±	±	–
K. oxytoca R48	+	±	+	±	–	–	±	±	–
*S. aureus**ATCC USA 206[e]	++	++	++	++	++	–	+	+	–
*B. subtilis** 3HM	++	++	++	++	+++++	+	+	±	++

E. coli – Escherichia coli, E. cloacae – Enterobacter cloacae, M. morganii – Morganella morganii, P. aeruginosa – Pseudomonas aeruginosa, K. pneumoniae – Klebsiella pneumoniae, C. koseri – Citrobacter koseri, H. alvei – Hafnia alvei, P. vulgaris – Proteus vulgaris, C. albicans – Candida albicans, E. aerogenes – Enterobacter aerogenes, K. oxytoca – Klebsiella oxytoca, S. aureus – Staphylococcus aureus, B. subtilis – Bacillus subtili.
[a]Ampicillin-resistant, [b]Cefotaxime-resistant, [c]Tetracycline-resistant, [d]Ceftriaxone-resistant, [e]Fosfomycin-resistant.
* – Gram-positive.

many clinically relevant microorganisms (Table 3). We did not observe the induction of reporters for more than 90% of the molecules, whereas several compounds were preliminarily classified as low-to-moderate inhibitors of translation machinery. We carried out an additional *in vitro* luciferase-based assay to elucidate the correct mechanism of action of these molecules; however, no response was observed (see SI, Table S1). The cytotoxicity of compounds **1**–**9** was investigated against HEK293 (epithelial embryonic kidney) cells. Although compound **4** with methyl substituents exhibited no cytotoxicity towards the tested cell line ($CC_{50} > 100$ μM) and weakly inhibited bacterial growth with an MIC of 115.3 μM (50 μg/ml), its close analog **5** bearing ethyl fragments was much more active and demonstrated an MIC of 13.5 μM (6.25 μg/ml) with low cytotoxicity against HEK293 ($CC_{50} \sim 70$ μM). The cytotoxicity of the tested compounds towards A549 (lung epithelial carcinoma) and MCF7 (breast epithelial adenocarcinoma) cells was also evaluated, and these results are summarized in Supporting Information (*SI*).

As shown in Table 3, compounds **1** and **2** were found to significantly inhibit the growth of many pathogenic microorganisms, including *M. morganii*, *H. alvei,* and *C. albicans* (fungi, pathogenic yeast), whereas compound **5** only inhibited the growth of two gram-positive strains (*B. subtilis* and *S. aureus*).

Considering that several noscapine analogs from the series demonstrated moderate cytotoxicity against the cell lines mentioned above, we investigated the effect of these compounds on the cell cycle. Noscapine has been well described as a tubulin binder and thereby influences microtubule dynamics and assembly, but has been shown to have low toxicity.[62,63] Noscapine and its close derivatives cause cell cycle arrest in a variety of mammalian cancer cells, resulting in antiproliferative activity via apoptosis and do not produce obvious side effects *in vivo*.[64] The FtsZ protein identified in *E. coli* is a close homolog of eukaryotic tubulin and contains the common tubulin 7-amino-acid motif. The FtsZ protein promotes the formation of a ring in the division zone between the newly forming offspring cells.[65] This protein has recently attracted considerable attention as a promising antibacterial target for developing novel antibiotics.[66] Moreover, berberine and its direct analogs containing the same noscapine scaffold have been found to inhibit GTPase activity and decrease FtsZ polymerization.[67] Consequently, we hypothesized that some of the compounds could exhibit antibacterial effects via the FtsZ pathway. Thus, we used flow cytometry to investigate the influence of compounds **1**, **2** and **5** on the cell cycle in HEK293 cells, as well as in the cancerogenous cell lines A549 and MCF-7 (see SI, Fig. S1). Treating HEK293 cells with compound **2** (12.6 μM) slightly increased the proportion of cells in the S phase after 24 h compared to the negative control (0.1% DMSO treatment), with subsequent accumulation of apoptotic cells in sub-G1 at 72 h (Fig. S1A). Compound **5** (71.0 μM) caused the HEK293 cells to undergo arrest in the S phase after 24 h, leading to an increase in apoptotic cells in sub-G1 at 48 h (Fig. S1B). However, compound **5** did not significantly affect the cell cycle compared to the negative control. In the same cell line, compound **1** (14.9 μM) slightly increased the G2/M phase cell population, accompanied by a moderate reduction in the number of cells in the G1 phase and a minor elevation of apoptotic cells (detected by the sub-G1 peak) within 24 h (Fig. S1C). Overall, these data indicate that some of the tested compounds could exhibit both cytostatic and cytotoxic activity, depending on the cell type.

Conclusion

We demonstrated antibacterial activity for direct noscapine analogs for the first time, where the most active molecule **2** exhibited an MIC of 11.3 μM (6.25 μg/ml). Some of these compounds exhibited an average translation response during HTS but did not inhibit translation *in vitro* in a luciferase-based assay. These compounds presumably induced Katushka2S signaling via an indirect translation blockade. Several compounds were found to be active against a panel of clinically relevant bacterial strains. Although some molecules were found to perturb the cell cycle in the model cell lines, compound **5** had a relatively low cytotoxicity and high antibacterial activity and exerted only a negligible effect on the cell cycle compared to the negative control. Thus, compound **5** is attractive for further research and development, with the primary focus on improving the compound permeability because compound **5** demonstrated weaker inhibition potency against wt *E.coli* in contrast to the ΔTolC strain, while showing considerable activity towards two gram-positive strains. The exact mechanism of action of this type of antibacterial compound should be investigated further.

Materials and methods

High through screening

Molecules were purchased from the IBS collection and dissolved in DMSO at a concentration of 17–20 mg/ml (for the first round of HTS). A 96-channel pipetting head of a Janus liquid handling station (PerkinElmer) was used to spot 1–1.5 μl of the solutions of each compound on agar plates with the reporter strain (ΔTolC and wt *E. coli*). Erythromycin (ERY, 1 μl) and levofloxacin (LVX, 1 μl) were added to each plate as control samples. The Petri plates were incubated for 16 h at 37 °C and then scanned by a ChemiDoc system (Bio-Rad). Antibacterial activity was preliminarily estimated by a thorough visual analysis. The most active compounds were then rescreened at a reduced concentration.

In vitro luciferase assay

In vitro transcribed firefly luciferase mRNA was translated in a cell-free system using a S30 cellular extract from *E. coli*. The samples were tested at a final concentration that was 100 times lower than that used in the cell-based assay. To investigate the effect of the selected molecules on the prokaryotic ribosome, isolated ribosomes were mixed with each compound and maintained at 37 °C for 5 min without mRNA. Then, mRNA (200 ng) was added to the reaction mixture, and translation was initiated in a 10-ml reaction volume at 37 °C for 30 min [68]. The translation of mRNA-encoding luciferase was evaluated by measuring the enzyme activity using 0.1 mM D-luciferin and a spectrophotometer (PerkinElmer). Two control samples were used: negative (1% DMSO was used to indicate no translation inhibition) and positive (ERY at a final concentration of 0.01 mg/ml was used as a translation inhibitor). All the measured values were normalized using the positive control baseline and expressed as a percentage.

Cytotoxicity

Cytotoxicity was assessed using the PrestoBlue® Cell Viability Reagent (Invitrogen, USA) following standard protocol. The cell lines were obtained from the Russian collection of cell cultures (the Institute of Cytology RAS, St. Petersburg). HEK293 ($25*10^3$ cells per well), A549, and MCF-7 ($12*10^3$ cells per well) cells were plated in DMEM (100 μl), FBS (10%), L-Glu (2 mM) and gentamicin (50 μg/ml) in a 96-well plate and incubated at 37 °C and 5% CO_2 for 24 h before treatment. Then, the tested compound (10 μM in a media/DMSO solution, with a final DMSO concentration of 0.1% in the media) was added, and the cell samples were incubated at 37 °C with 5% CO_2 for 28 h. After incubation, the PrestoBlue® reagent was added, and the fluorescence was measured at a wavelength of 590 nM using a multiplate analyzer, 2300 EnSpire® Multimode Plate Readers (PerkinElmer, USA). The percentage metabolic activity of the cells was calculated in relation to the control sample (0.1% DMSO, 100% cell viability). The CC_{50} value was calculated using GraphPad Prism 4 software (GraphPad Software, Inc., San Diego, CA). A thorough search of the SciFinder [38] database showed no claims of antibacterial activity for compounds **1**–**9** in the patent literature.

Minimum inhibitory concentration

The MICs in LB and M9 medium were determined using a broth microdilution assay [69]. The cell concentration was adjusted to approximately 5×10^5 cells/ml. The tested compound was serially diluted twofold in a 96-well microplate (100 μl per well). The microplates were covered and incubated at 37 °C with shaking. The OD600 of each well was measured, and the MIC was assigned as the lowest concentration of the tested compound that resulted in no growth after 16–20 h.

Cell cycle analysis

The HEK293, A549, and MCF-7 cell cycles were measured using a flow cytometry assay. Briefly, the cells were incubated with a vehicle (0.1% DMSO) or test compounds at their CC_{50} values and then harvested and centrifuged (400 × g, 5 min). The pellets were gently resuspended in 1 ml of ice-cold 70% ethanol and incubated for 24 h at −20 °C. After permeabilization, the cells were washed twice with PBS, resuspended in PBS containing RNase A (0.5 mg/ml; Sigma, USA), and incubated for 5 min at room temperature. Then, PI (propidium iodide, 50 μg/ml; Sigma, USA) was added, and the suspensions were incubated for another 30 min. The PI fluorescence of individual cells/nuclei was measured using a Novocyte 2060 flow cytometer (Acea Biosciences, Inc. USA) on a linear scale. Data analysis was performed using the cell cycle module of NovoExpress 1.3.0 software (Acea Biosciences, Inc. USA). The data were expressed as the mean ± S.E.M. The experiments were performed at least in duplicate. The cell cycle phases were compared using the Wilcoxon *t*-test (Statistica 6.1, StatSoft. Inc., USA).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128055.

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