Bioorganic & Medicinal Chemistry Letters xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of NSC 106084 and NSC 14778 and evaluation of their DNMT inhibitory activity

Maxime Leroy[a], Léa Mélin[a], Steven R. LaPlante[b], José L. Medina-Franco[c], Alexandre Gagnon[a,*]

[a] Département de chimie, Université du Québec à Montréal, C.P. 8888, Succ. Centre-Ville, Montréal, Québec H3C 3P8, Canada
[b] Centre INRS-Armand Frappier, 531 boul. des Prairies, Laval, Québec H7V 1B7, Canada
[c] Facultad de Química, Departamento de Farmacia, Universidad Nacional Autónoma de México, Avenida Universidad 3000, Mexico City 04510, Mexico

ARTICLE INFO

Keywords:
DNA methylation
DNA methyltransferases (DNMTs)
DNA methyltransferase Inhibitors (DNMTi)
NSC 106084
NSC 14778

ABSTRACT

DNA methylation is an epigenetic modification that is performed by DNA methyltransferases (DNMTs) and that leads to the transfer of a methyl group from *S*-adenosylmethionine (SAM) to the C5 position of cytosine. This transformation results in hypermethylation and silencing of genes such as tumor suppressor genes. Aberrant DNA methylation has been associated with the development of many diseases, including cancer. Inhibition of DNMTs promotes the demethylation and reactivation of epigenetically silenced genes. NSC 106084 and 14778 have been reported to inhibit DNMTs in the micromolar range. We report herein the synthesis of NSC 106084 and 14778 and the evaluation of their DNMT inhibitory activity. Our results indicate that while commercial NSC 14778 is moderately active against DNMT1, 3A/3L and 3B/3L, resynthesized NSC 14778 is inactive under our assay conditions. Resynthesized 106084 was also found to be inactive.

DNA methylation is an epigenetic modification that leads to the installation of a methyl group at the C5 position of cytosine on DNA and that results in gene silencing.[1] This process plays a critical role in the regulation of gene expression, X-chromosome inactivation, genomic imprinting, cell differentiation, development and aging.[2] The methylation of DNA is performed by DNA methyltransferases (DNMTs), a class of epigenetic enzymes that catalyze the transfer of a methyl group from the methyl donor *S*-adenosylmethionine (SAM) to the DNA, mainly at CpG sites, producing 5-methylcytosine on DNA and *S*-adenosylhomocystein (SAH).[3] Five types of DNA methyltransferases have been identified in humans: DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L. DNMT1 performs the methylation of hemi-methylated DNA and is responsible for copying the methylation pattern of the parental strand to the daughter strand during DNA replication while DNMT3A and 3B are responsible for *de novo* methylation of unmethylated DNA. DNMT2, originally believed to be a DNA methyltransferase, actually performs the methylation of tRNA. Finally, although DNMT3L is catalytically inactive, it plays a crucial role by modulating the activity of DNMT3A and 3B.

Dysregulation of DNA methylation processes has been observed in many types of cancer, resulting in global DNA hypomethylation with concomitant hypermethylation and silencing of promoter regions of tumor suppressor genes.[4] Aberrant DNA methylation patterns have also been observed in many other diseases[5] including psychiatric disorders,[6] arthritis,[7] amyotrophic lateral sclerosis,[8] schizophrenia,[9] dementia and hearing loss,[10] and central nervous system disorders.[11] In addition, DNA methylation has been found to have an important role in memory formation,[12] emotional behavior,[13] and epigenetic programming induced by maternal behavior.[14] Contrary to genetic modifications, which are irreversible, epigenetic changes such as DNA methylation are dynamic and can be reversed by targeting DNA methyltransferases.[15] Indeed, reports have shown that inhibition of DNMTs leads to demethylation and reactivation of epigenetically-silenced genes,[16] thus demonstrating the value of DNMTs in drug discovery.

Vidaza (**1a**) and Dacogen (**1b**) are nucleoside DNMT inhibitors that have been approved by the FDA for the treatment of myelodysplastic syndrome (MDS), acute myeloid leukaemia (AML) and chronic myelomonocytic leukaemia (CML) (Fig. 1).[17] After being phosphorylated, these drugs are incorporated into the DNA where they react sequentially with the DNMT catalytic cysteine and then with SAM, resulting in the deactivation of the enzyme through formation of an irreversible complex that gets degraded by proteolysis. Although these drugs have

* Corresponding author.
E-mail address: gagnon.alexandre@uqam.ca (A. Gagnon).

https://doi.org/10.1016/j.bmcl.2019.01.022
Received 28 November 2018; Received in revised form 11 January 2019; Accepted 17 January 2019

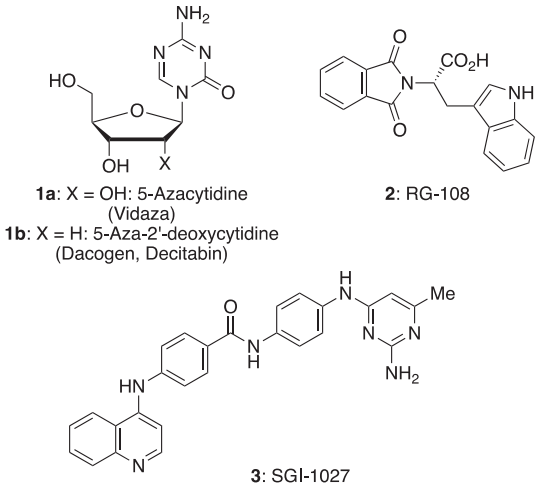


Fig. 1. Selected DNMT inhibitors.

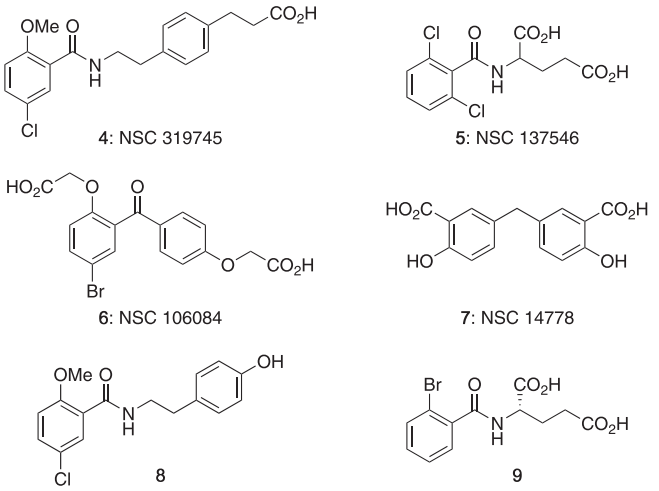


Fig. 2. NSC compounds reported as DNMT inhibitors.

demonstrated efficacy in the clinic, they suffer from several liabilities such as high toxicity, low chemical and plasmatic stability,[18] poor pharmacokinetic profiles, poor selectivity and lack of efficacy on solid tumors.[19] Derivatives of Vidaza and Dacogen have been prepared to improve their chemical stability, but these analogues also rely on DNA incorporation to unfold their activity.[20]

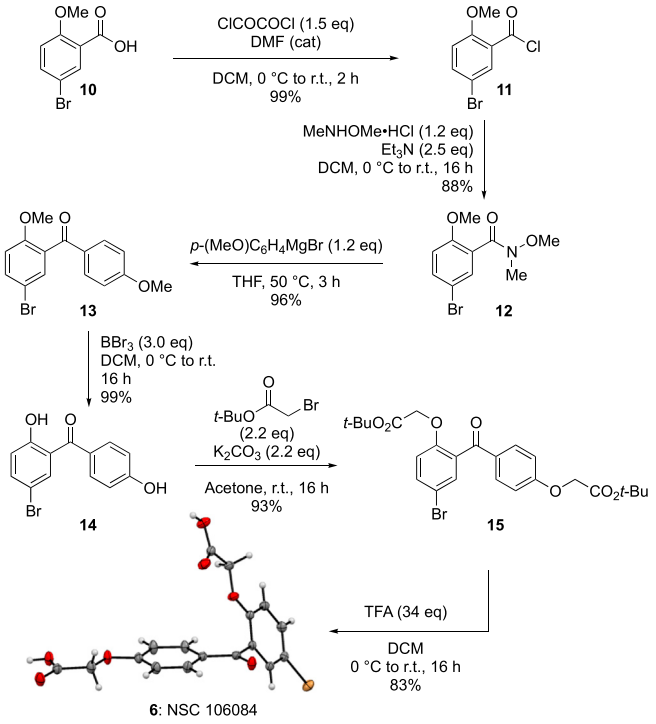
Several natural products have been reported to inhibit DNMTs.[21] However, in addition to having low potency and poor pharmacokinetic profiles, many of them operate via an unknown mode of action or show pan-activity against other targets. Procainamide, procaine, and hydralazine are drugs that have been approved for cardiac arrhythmias, for local anesthesia and for treatment of hypertension, respectively. Using a drug repurposing strategy, these compounds have been shown to moderately inhibit DNMTs[22] and lead optimization programs have resulted in improvement of their DNMT inhibitory activity.[23]

RG-108 (**2**)[24] and SGI-1027 (**3**),[25] two of the most well characterized non-nucleoside DNMT inhibitors, are commonly utilized as reference compounds in biochemical and cellular DNA methylation assays (Fig. 1). The activity of these compounds has been improved through SAR studies.[26] DNMT3A inhibitors with low micromolar potency based on a quinazoline-quinoline scaffold which mimicks the deoxycytidine substrate and the SAM cofactor have also been developed using a rational approach.[27] A limited number of other non-nucleoside DNMT inhibitors with moderate potency have been reported.[28] Despite decades of work on DNA methyltransferases, Vidaza and Dacogen remain the only two approved drugs that target these epigenetic enzymes. In light of the liabilities associated with current DNMT inhibitors, there is an urgent need for novel small molecules that can modulate the activity of DNMTs through reversible binding and that possess adequate biophysical and pharmacokinetic properties, low toxicity, and good chemical stability.

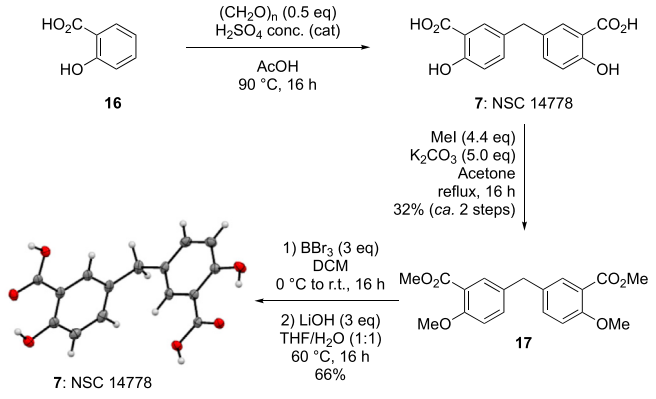
NSC 319745 (**4**), 137546 (**5**), 106084 (**6**) and 14778 (**7**) are DNMT inhibitors that were discovered using a docking-based virtual screening campaign of the National Cancer Institute (NCI) compound collection (Fig. 2). These virtual screening hits showed 34%, 27%, 22% and 29% inhibition against DNMT1 at 100 μM, respectively, in a ^{3}H-SAM-based biochemical assay.[29] Of these, only NSC 14778 inhibited DNMT3B (60% at 100 μM). These compounds were suggested to be potential starting points for hit-to-lead campaigns targeting DNMTs. Due to our interest in the development of DNMT inhibitors, we initiated in 2011 a program aimed at exploring the SAR of NSC 319745 (**4**). Although we were unable to reproduce the DNMT inhibitory activity of this compound under our assay conditions, our efforts led to the discovery of phenol derivative (**8**) that inhibited DNMT3A with an IC_{50} of 36 μM.[30] Recently, hydroxamic derivatives of NSC 319745 that showed dual inhibition against HDAC and DNMTs were reported.[31] NSC 137546 (**5**) also served as a starting point for lead discovery, resulting in compound **9** which inhibited DNMT1 and 3A in a concentration-dependent manner in the micromolar range.[32] To the best of our knowledge, the SAR of NSC 106084 (**6**) and 14778 (**7**) has never been explored. Even though these compounds possess chemical scaffolds shared by several PAINS[33] such as a benzophenone in NSC 106084 that could potentially act as a photoreactive moiety[34] and a diphenolic methylene structure in NSC 14778 that could lead to quinone methides, we felt that, once their activity confirmed, these compounds could be suitably modified by extensive SAR activities to remove or replace unwanted chemical features that are associated with PAINS. Therefore, we would like to report herein the synthesis of NSC 106084 and 14778 and the evaluation of their DNMT inhibitory activity.

The synthesis of NSC 106084 (**6**) commenced with the formation of acid chloride **11** by treatment of carboxylic acid **10** with oxalyl chloride in the presence of a catalytic amount of DMF (Scheme 1). Reacting **11** with *N*-methoxy-*N*-methylamine hydrochloride salt in the presence of triethylamine afforded the Weinreb amide **12** in 88% yield which was then reacted with 4-methoxy-phenylmagnesium bromide to afford the unsymmetrical diaryl ketone **13** in 96% yield. Demethylation of both methyl ethers using boron tribromide quantitatively afforded bisphenol **14** which was then reacted with *tert*-butyl bromo acetate in acetone in the presence of potassium carbonate to afford **15** in 93% yield. Lastly, removal of both *tert*-butyl groups by treatment with excess trifluoroacetic acid provided pure NSC 106084 (**6**) who's structure was confirmed by X-ray diffraction analysis.

Methylene disalicylic acid **7** has previously been prepared by condensation of salicylic acid **16** with formaldehyde in the presence of sulfuric acid.[35] Fluoro,[36] chloro,[37] methyl and trifluoromethyl[38] derivatives have also been obtained using this approach. Compound **7** has



Scheme 1. Synthesis of NSC 106084 (**6**).



Scheme 2. Synthesis of NSC 14778 (**7**).

also been prepared by dehalogenation of methylene dichlorodisalicylic acid via hydrogenation catalyzed by palladium on carbon.[37b] Another approach for the preparation of methylene disalicylic acids involves the condensation of salicylic acid methyl ester derivatives followed by saponification of the esters.[39] The synthesis of NSC 14778 (**7**) was attempted using a one-step procedure from the literature where salicylic acid **16** is heated with formaldehyde in acetic acid in the presence of a catalytic amount of fuming sulfuric acid (Scheme 2).[40] However, in our hand, this procedure failed to provide pure product **7** and purification of this highly polar compound by silica gel chromatography proved

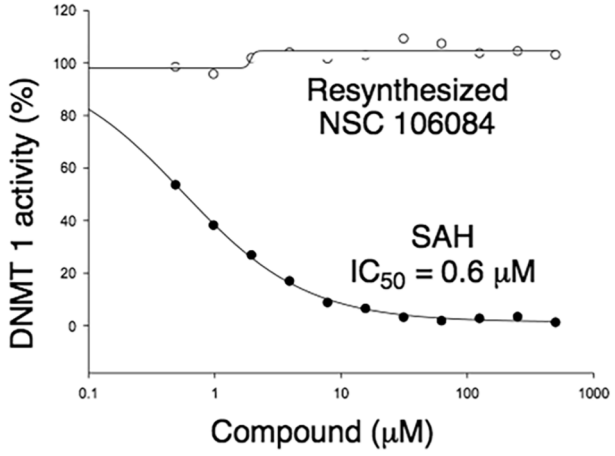


Fig. 3. Full inhibition curves of resynthesized NSC 106084 (**6**) and SAH against DNMT1 using a tritiated SAM-based assay.

difficult. To obtain sufficient quantities for characterization and biological studies, we favored the development of an alternative synthetic route over purification by preparative HPLC. In the event, all phenolic and carboxylic acid groups in crude **7** were methylated by treatment with excess iodomethane in refluxing acetone in the presence of potassium carbonate to afford **17**. Purification of **17** followed by sequential demethylation with boron tribromide and saponification with lithium hydroxide afforded pure **7** who's structure was confirmed by X-ray diffraction analysis on recrystallized material.

We then pursued with the evaluation of the DNMT inhibitory activity of resynthesized NSC 106084 (**6**) and 14778 (**7**). In a previous report, NSC 106084 was found to selectively inhibit DNMT1 over DNMT3B with an IC_{50} of 212 µM.[29] Under our assay conditions, resynthesized NSC 106084 (**6**) showed no DNMT inhibitory activity against DNMT1 in a ^{3}H-SAM-based assay at concentrations up to 1 mM (Fig. 3). The validity of the assay was confirmed using SAH which gave an IC_{50} of 0.6 µM against DNMT1.

We then evaluated the DNMT inhibitory activity of commercial and resynthesized NSC 14778 (**7**) against DNMT1, 3A/3L and 3B/3L. Interestingly, commercial NSC 14778 inhibited DNMT1, 3A/3L and 3B/3L with IC_{50} values of 47, 1.4, and 45 µM, respectively (Fig. 4). These values are in the same range as those previously reported (i.e. 92 and 17 µM against DNMT1 and 3B, respectively).[29] To investigate the mode of action of commercial NSC 14778 (**7**), we measured the IC_{50} against DNMT3A/3L under increasing concentrations of SAM and oligonucleotide and saw no changes in activity, suggesting that NSC 14778 is not a SAM nor a DNA-competitor (Supplementary Fig. S3 and S4). When commercial NSC 14778 was tested for possible DNA intercalation, no effect was observed at up to 1 mM (Supplementary Fig. S7). Evaluation of the binding affinity of commercial NSC 14778 to DNMT3A/3L by surface plasmon resonance (SPR) showed that the compound failed to reach a steady state (Supplementary Fig. S6). Because commercial NSC 14778 showed better potency against DNMT 3A/3L in our assay, we then tested our resynthesized NSC 14778 (**7**) against DNMT 3A/3L and were surprised to see no inhibition at concentrations up to 1 mM (Fig. 4).

To better understand the discrepancy between the activity of commercial and resynthesized NSC 14778 (**7**), we analyzed both samples by

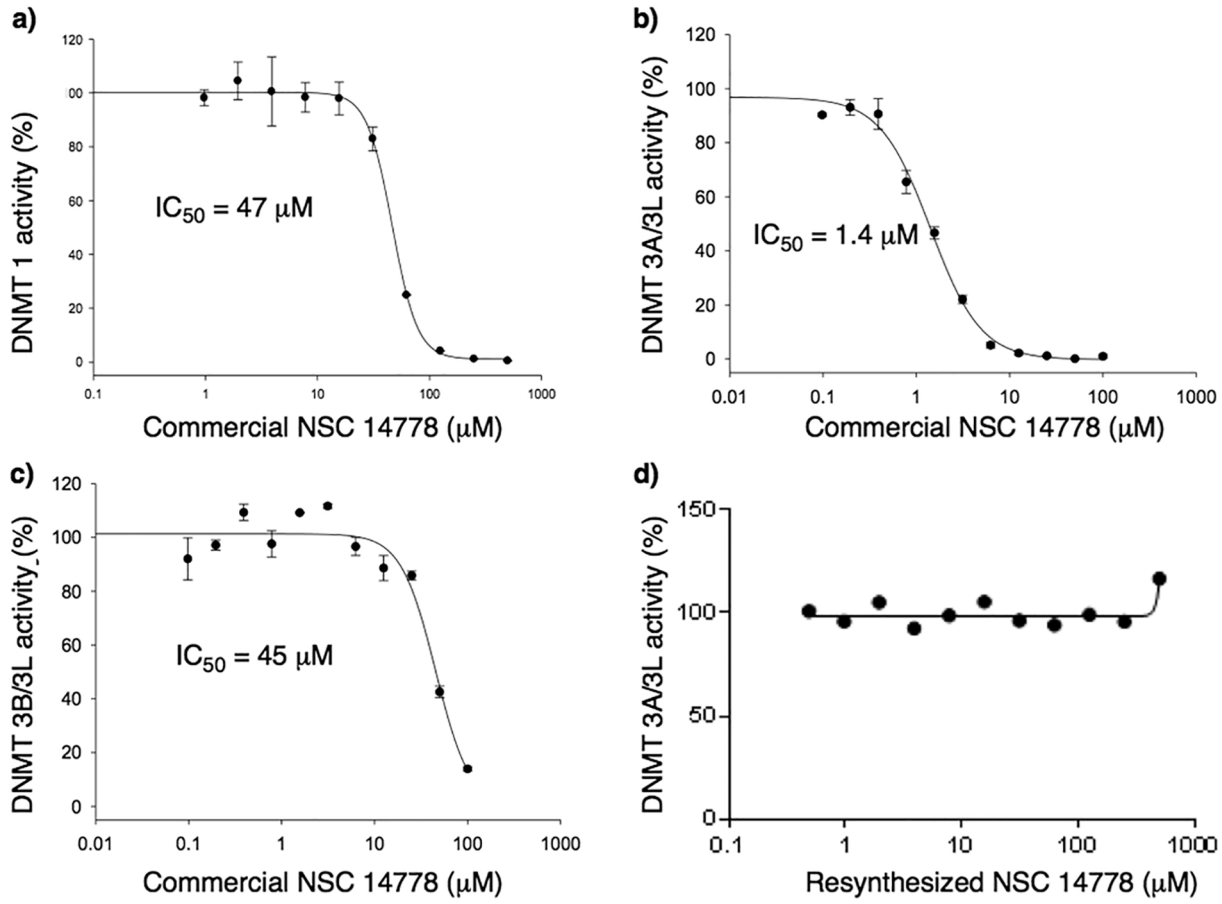


Fig. 4. DNMT inhibitory activity of commercial NSC 14778 against **a)** DNMT1; **b)** DNMT3A/3L; **c)** DNMT3B/3L and **d)** resynthesized NSC 14778 (**7**) against DNMT3A/3L.

reverse phase HPLC (Fig. 5) and ^{1}H NMR (Fig. 6) and observed that while our resynthesized compound is > 95% pure, the commercial material contains a very high number of impurities.

Interestingly, the HPLC chromatogram and ^{1}H NMR spectra of crude NSC 14778 (**7**) obtained in Scheme 2 via a literature protocol[40] was found to be very similar to the commercial material. Therefore, we evaluated the activity of crude resynthesized **7** against DNMT3A/3L but saw no inhibitory activity. Elemental analysis of commercial, crude and pure resynthesized NSC 14778 showed similar carbon and hydrogen content, suggesting that the impurities are possibly chemically related to NSC 14778 (Supplementary Table S11). Metal impurities are known to cause false positives in high-throughput screening campaigns.[41] To determine if the activity of the commercial NSC 14778 sample could derive from a metal impurity, we evaluated the metal content of commercial and synthetic crude and pure samples by ICP-MS. However, no significant difference was found between those three samples, ruling out the possibility of metal interference in the biological assays (Supplementary Table S12). Finally, purification of commercial NSC 14778 (**7**) by reverse phase preparative HPLC followed by lyophilization of each fraction showed that every single constituent is inactive towards DNMT 3A/3L. Altogether, our results suggest that the DNMT inhibitory activity observed with commercial NSC 14778 (**7**) either comes from a combination of impurities or from an impurity that cannot be isolated by preparative HPLC.

In conclusion, synthetic routes were developed for the synthesis of NSC 106084 (**6**) and NSC 14778 (**7**) and their structures were confirmed using X-ray diffraction analysis. Contrary to literature results, under our assay conditions, pure resynthesized NSC 106084 showed no activity against DNMT1. While commercial NSC 14778 showed micromolar activity against DNMT1, 3A/3L and 3B/3L, pure resynthesized NSC 14778 showed no DNMT inhibitory activity at up to 1 mM concentration. HPLC analysis of commercial NSC 14778 showed that this compound contains a high number of impurities. The activity of commercial NSC 14778 cannot be explained by differences in carbon and hydrogen content, metal contaminants, or by any of its single component. The activity of commercial NSC 14778 is thus believed to come from an intractable impurity or a combination of species. These results demonstrate the challenges inherent to the identification of non-nucleoside small-molecule DNMT inhibitors, the variations that exist between assays, the propensity of those assays to lead to false positives with apparent acceptable full inhibition curves and the high relevance of the purity of commercial samples in biological screenings. Further work is in progress in our laboratory to identify the origin of the activity of commercial NSC 14778 and results will be reported in due course.

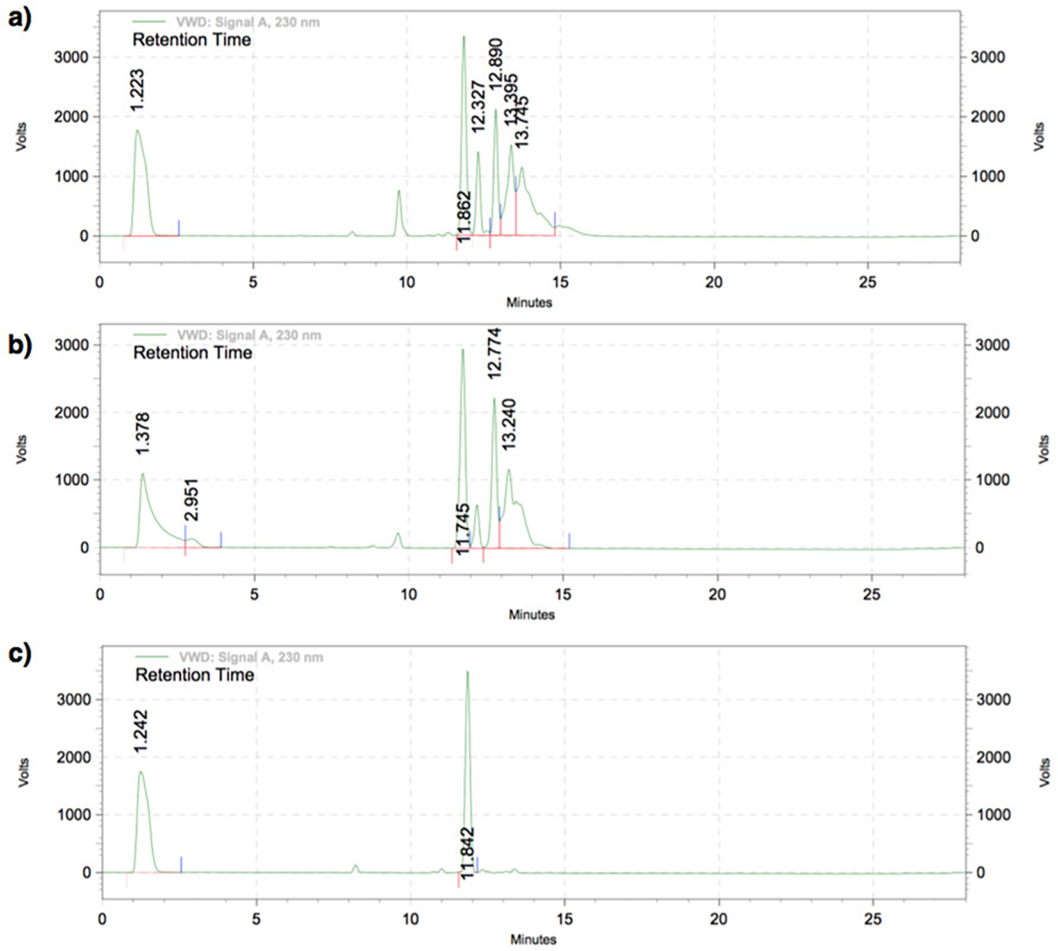


Fig. 5. HPLC chromatogram of **a)** commercial NSC 14778; **b)** crude resynthesized NSC 14778; and **c)** purified resynthesized NSC 14778. Conditions: Agilent Eclipse Plus C18 (3.5 mM, 4.6 × 100 mm), 0–100% acetonitrile in water (+0.06% TFA) over 20 min, absorbance measured at 230 nm.

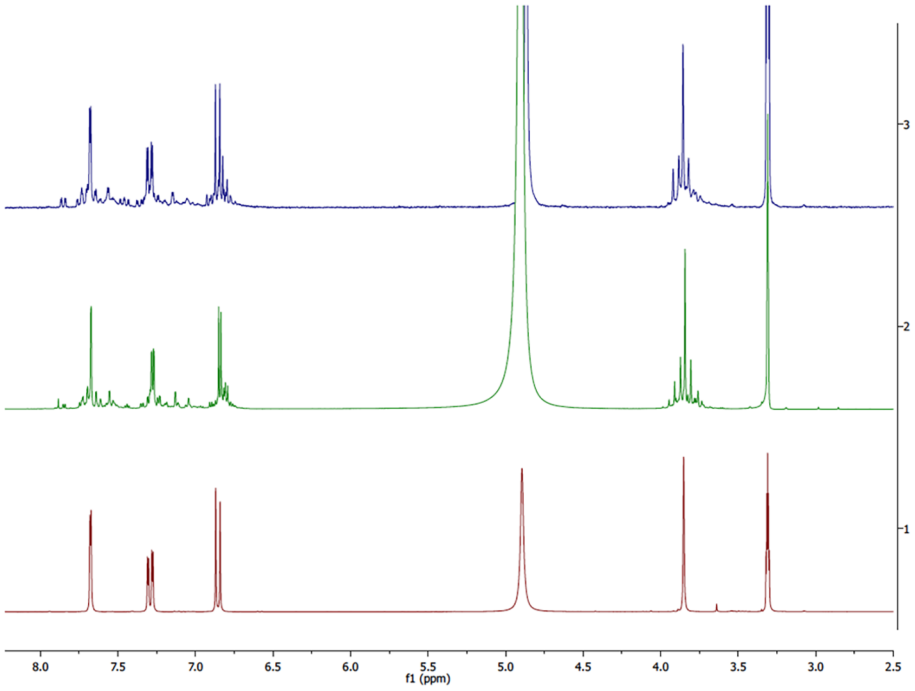


Fig. 6. ^{1}H NMR in methanol-d_4 of **top)** commercial NSC 14778; **middle)** resynthesized crude NSC 14778; **bottom)** resynthesized pure NSC14778.

Acknowledgments

This work was funded by Pharmaqam and the Réseau Québécois de Recherche sur le Médicament (RQRM). M.L. and L.M. would like to thank NSERC Create and Pharmaqam for post-graduate scholarships. J.L.M.-F. acknowledges funding from Consejo Nacional de Ciencia y Tecnología (CONACyT, Mexico) grant number 282785. We thank Dr. Abdellah Allali-Hassani, Dr. Masoud Vedadi, Dr. Peter Brown and Dr. Santha Santhakumar of the Structural Genomics Consortium (SGC) of Toronto for the biochemical and biophysical analyses. We thank Pr. Xavier Ottenwaelder from the department of chemistry and biochemistry of Concordia University for the X-ray analysis of compound 6 and Francine Gariépy-Bélanger, Michel Simard and Thierry Maris from the department of chemistry of the Université de Montréal for the X-ray analysis of compound 7. The crystallographic data for compound 6 and 7 were deposited at the Cambridge Crystallographic Data Centre (CCDC) and were assigned the numbers 1867725 and 1867726, respectively. We thank Pr. Kevin J. Wilkinson from the department of chemistry of the Université de Montréal for the ICP-MS analyses and Carole-Anne Létourneau from the department of chemistry of the Université de Sherbrooke for the elemental analyses.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.01.022.

References

1. Klose RJ, Bird AP. *Trends Biochem Sci.* 2006;31:89.
2. a) Ravichandran M, Jurkowska RZ, Jurkowski TP. *Org Biomol Chem.* 2018;16:1419;
   b) Bogdanović O, Lister R. *Curr Opin Genet Dev.* 2017;46:9;
   c) Smith ZD, Meissner A. *Nat Rev Genet.* 2013;14:204.
3. a) Lyko F. *Nat Rev Genet.* 2018;19:81;
   b) Jurkowska RZ, Jurkowski TP, Jeltsch A. *ChemBioChem.* 2011;12:206;
   c) Denis H, Ndlovu MN, Fuks F. *EMBO Rep.* 2011;12:647.
4. a) Jones PA, Issa J-PJ, Baylin S. *Nat Rev Genet.* 2016;17:630;
   b) Shen H, Laird PW. *Cell.* 2013;153:38;
   c) Azad N, Zahnow CA, Rudin CM, Baylin SB. *Nat Rev Clin Oncol.* 2013;10:256;
   d) Baylin SB, Jones PA. *Nat Rev Cancer.* 2011;11:726;
   e) Daniel FI, Cherubini K, Yurgel LS, Zancanaro de Figueiredo MAZ, Salum FG. *Cancer.* 2011;117:677;
   f) Gal-Yam EN, Saito Y, Egger G, Jones PA. *Annu Rev Med.* 2008;59:267.
5. Robertson KD. *Nat Rev Genet.* 2005;6:597.
6. Tsankova N, Renthal W, Kumar A, Nestler EJ. *Nat Rev Neurosci.* 2007;8:355.
7. Karouzakis E, Gay RE, Gay S, Neidhart M. *Nat Rev Rheumatol.* 2009;5:266.
8. Martin LJ, Wong M. *Neurotherapeutics.* 2013;10:722.
9. Gavin DP, Sharma RP. *Neurosci Biobehav Rev.* 2010;34:882.
10. Klein CJ, Botuyan M-V, Wu Y, et al. *Nat Genet.* 2011;43:595.
11. Feng J, Fan G. *Int Rev Neurobiol.* 2009;89:67.
12. Levenson JM, Sweatt JD. *Cell Mol Life Sci.* 2006;63:1009.
13. LaPlant Q, Vialou V, Covington III HE, et al. *Nat Neurosci.* 2010;13:1137.
14. Weaver ICG, Cervoni N, Champagne FA, et al. *Nat Neurosci.* 2004;7:847.
15. Ramchandani S, Bhattacharya SK, Cervoni N, Szyf M. *PNAS.* 1999;96:6107.
16. a) Karpf AR, Jones DA. *Oncogene.* 2002;21:5496;
   b) Ramchandani S, MacLeod AR, Pinard M, von Hofe E, Szyf M. *PNAS.* 1997;94:684.
17. a) Da Costa EM, McInnes G, Beaudry A, Raynald NJ-M. *The Cancer J.* 2017;23:270;
   b) Derissen EJB, Beijnen JH, Schellens JHM. *Oncologist.* 2013;18:619;
   c) Gnyszka A, Jastrzebski Z, Flis S. *Anticancer Res.* 2013;33:2989.
18. a) Rogstad DK, Herring JL, Theruvathu JA, et al. *Chem Res Toxicol.* 2009;22:1194;
   b) Stresemann C, Lyko F. *Int J Cancer.* 2008;123:8;
   c) Notari RE, DeYoung JL. *J Pharm Sci.* 1975;64:1148;
   d) Chan KK, Giannini DD, Staroscik JA, Sadee W. *J Pharm Sci.* 1979;68:807.
19. Linnekamp JF, Butter R, Spijker R, Medema JP, van Laarhoven HWM. *Cancer Treatment Rev.* 2017;54:10.
20. a) Aranda J, Attana F, Tuñón I. *ACS Catal.* 2017;7:1728;
   b) Chuang JC, Warner SL, Vollmer D, et al. *Mol Cancer Ther.* 2010;9:1443;
   c) Brueckner B, Rius M, Markelova MR, et al. *Mol Cancer Ther.* 2010;9:1256;
   d) Champion C, Guianvarc'h D, Sénamaud-Beaufort C, et al. *PLoS One.* 2010;5:e12388;
   e) Byun H-M, Choi SH, Laird PW, et al. *Cancer Lett.* 2008;266:238.
21. Zwergel C, Valente S, Mai A. *Curr Top Med Chem.* 2016;16:680.
22. a) Singh N, Dueñas-González A, Lyko F, Medina-Franco JL. *ChemMedChem.* 2009;4:792;
   b) Segura-Pacheco B, Perez-Cardenas E, Taja-Chayeb L, et al. *J. Trans. Med.* 2006;4:32;
   c) Zambrano P, Segura-Pacheco B, Perez-Cardenas E, et al. *BMC Cancer.* 2005;5:44;
   d) Villar-Garea A, Fraga MF, Espada J, Esteller M. *Cancer Res.* 2003;63:4984;
   e) Segura-Pacheco B, Trejo-Becerril C, Perez-Cardenas E, et al. *Clin Cancer Res.* 2003;9:1596.
23. a) Halby L, Champion C, Sénamaud-Beaufort C, et al. *ChemBioChem.* 2012;13:157;
   b) Castellano S, Kuck D, Viviano M, et al. *J Med Chem.* 2011;54:7663;
   c) Castellano S, Kuck D, Sala M, Novellino E, Lyko F, Sbardella G. *J Med Chem.* 2008;51:2321.
24. a) Siedlecki P, Garcia Boy R, Musch T, et al. *J Med Chem.* 2006;49:678;
   b) Brueckner B, Garcia Boy R, Siedlecki P, et al. *Cancer Res.* 2005;65:6305.
25. Datta J, Ghoshal K, Denny WA, et al. *Cancer Res.* 2009;69:4277.
26. a) Asgatay S, Champion C, Marloie G, et al. *J Med Chem.* 2014;57:421;
   b) Valente S, Liu Y, Schnekenburger M, et al. *J Med Chem.* 2014;57:701;
   c) Suzuki T, Tanaka R, Hamada S, Nakagawa H, Miyata N. *Bioorg Med Chem Lett.* 2010;20:1124.
27. Halby L, Menon Y, Rilova E, et al. *J Med Chem.* 2017;60:4665.
28. a) Pechalrieu D, Etievant C, Arimondo PB. *Biochem. Pharm.* 2017;129:1;
   b) Medina-Franco JL, Méndez-Lucio O, Dueñas-González A, Yoo J. *Drug Disc. Today.* 2015;20:569;
   c) Gros C, Fahy J, Halby L, et al. *Biochimie.* 2012;94:2280.
29. Kuck D, Singh N, Lyko F, Medina-Franco JL. *Bioorg Med Chem.* 2010;18:822.
30. Kabro A, Lachance H, Marcoux-Archambault I, et al. *MedChemComm.* 2013;4:1562.
31. Yuan Z, Sun Q, Li D, et al. *Eur J Med Chem.* 2017;134:281.
32. Garella D, Atlante S, Borretto E, et al. *Chem Biol Drug Des.* 2016;88:664.
33. a) Dahlin JL, Walters MA. *Assay Drug Dev Technol.* 2016;14:168;
   b) Dahlin JL, Walters MA. *Future Med Chem.* 2014;6:1265.
34. Pérez-Prieto J, Galian RE, Miranda MA. *Mini-Rev Org Chem.* 2006;3:117.
35. Reddy CS, Raghu M. *Chem Pharm Bull.* 2008;56:1732.
36. Casimiro-Garcia A, Micklatcher M, Turpin JA, et al. *J Med Chem.* 1999;42:4861.
37. a) Golebiewski WM, Bader JP, Cushman M. *Bioorg Med Chem Lett.* 1993;3:1739;
   b) Cushman M, Kanamathareddy S. *Tetrahedron.* 1990;46:1491.
38. Xu G, Micklatcher M, Silvestri MA, et al. *J Med Chem.* 2001;44:4092.
39. Shrestha S, Raj Bhattarai B, Chang KJ, Lee K-H, Cho H. *Bioorg Med Chem Lett.* 2007;17:2760.
40. Guieu S, Rocha J, Silva AMS. *Tetrahedron.* 2013;69:9329.
41. Hermann JC, Chen Y, Wartchow C, et al. *ACS Med Chem Lett.* 2013;4:197.