2014

Synthesis and Biological Evaluation of 5′-O-Dicarboxylic Fatty Acyl Monoester Derivatives of Anti-HIV Nucleoside Reverse Transcriptase Inhibitors

Bhanu Pemmaraju
University of Rhode Island

Hitesh K. Agarwal
University of Rhode Island

See next page for additional authors

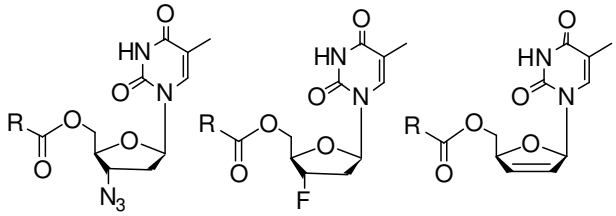
Citation/Publisher Attribution
Pemmaraju, B., Agarwal, H. K., Oh, D., Buckhei, K. W., Buckheit, R. W., Jr.,
Tiwari, R., & Parang, K. (2014, February 10). Synthesis and biological evaluation of 5′-O-dicarboxylic fatty acyl monoester derivatives of anti-HIV nucleoside reverse transcriptase inhibitors. *Tetrahedron Letters*. http://dx.doi.org/10.1016/j.tetlet.2014.02.001
Available at: http://dx.doi.org/10.1016/j.tetlet.2014.02.001

Graphical Abstract

Synthesis and Biological Evaluation of 5′-*O*-Dicarboxylic Fatty Acyl Monoester Derivatives of Anti-HIV Nucleoside Reverse Transcriptase Inhibitors

Leave this area blank for abstract info.

Bhanu Pemmaraju,[1] Hitesh K Agarwal,[1] Donghoon Oh,[1] Karen W. Buckheit,[2] Robert W. Buckheit Jr.,[2] Rakesh Tiwari,[2,3] Keykavous Parang[2,3,*]



R = -$(CH_2)_n$COOH n = 6, 8, 10

Synthesis and Biological Evaluation of 5′-*O*-Dicarboxylic Fatty Acyl Monoester Derivatives of Anti-HIV Nucleoside Reverse Transcriptase Inhibitors

Bhanu Pemmaraju,[1] Hitesh K Agarwal,[1] Donghoon Oh,[1] Karen W. Buckheit,[2] Robert W. Buckheit Jr.,[2] Rakesh Tiwari,[2,3] Keykavous Parang[2,3,*]

[1]*Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI 02881, United States*
[2]*ImQuest BioSciences Inc. 7340 Executive Way, Suite R, Frederick, MD, 21704, United States*
[3]*School of Pharmacy, Chapman University, Orange, CA, 92618, United States*

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:

Anti-HIV agents
Fatty acids
Lipophilicity
Nucleosides
Reverse Transcriptase

ABSTRACT

A number of 5′-*O*-dicarboxylic fatty acyl monoester derivatives of 3'-azido-3'-deoxythymidine (zidovudine, AZT), 2',3'-didehydro-2',3'-dideoxythymidine (stavudine, d4T), and 3'-fluoro-3'-deoxythymidine (alovudine, FLT) were synthesized to improve the lipophilicity and potentially the cellular delivery of parent polar 2′,3′-dideoxynucleoside (ddN) analogs. The compounds were evaluated for their anti-HIV activity. Three different fatty acids with varying chain length of suberic acid (octanedioic acid), sebacic acid (decanedioic acid), and dodecanedioic acid were used for the conjugation with the nucleosides. The compounds were evaluated for anti-HIV activity and cytotoxicity. All dicarboxylic ester conjugates of nucleosides exhibited significantly higher anti-HIV activity than that of the corresponding parent nucleoside analogs. Among all the tested conjugates, 5′-*O*-suberate derivative of AZT (EC_{50} = 0.10 nM) was found to be the most potent compound and showed 80-fold higher anti-HIV activity than AZT without any significant toxicity (TC_{50} > 500 nM).

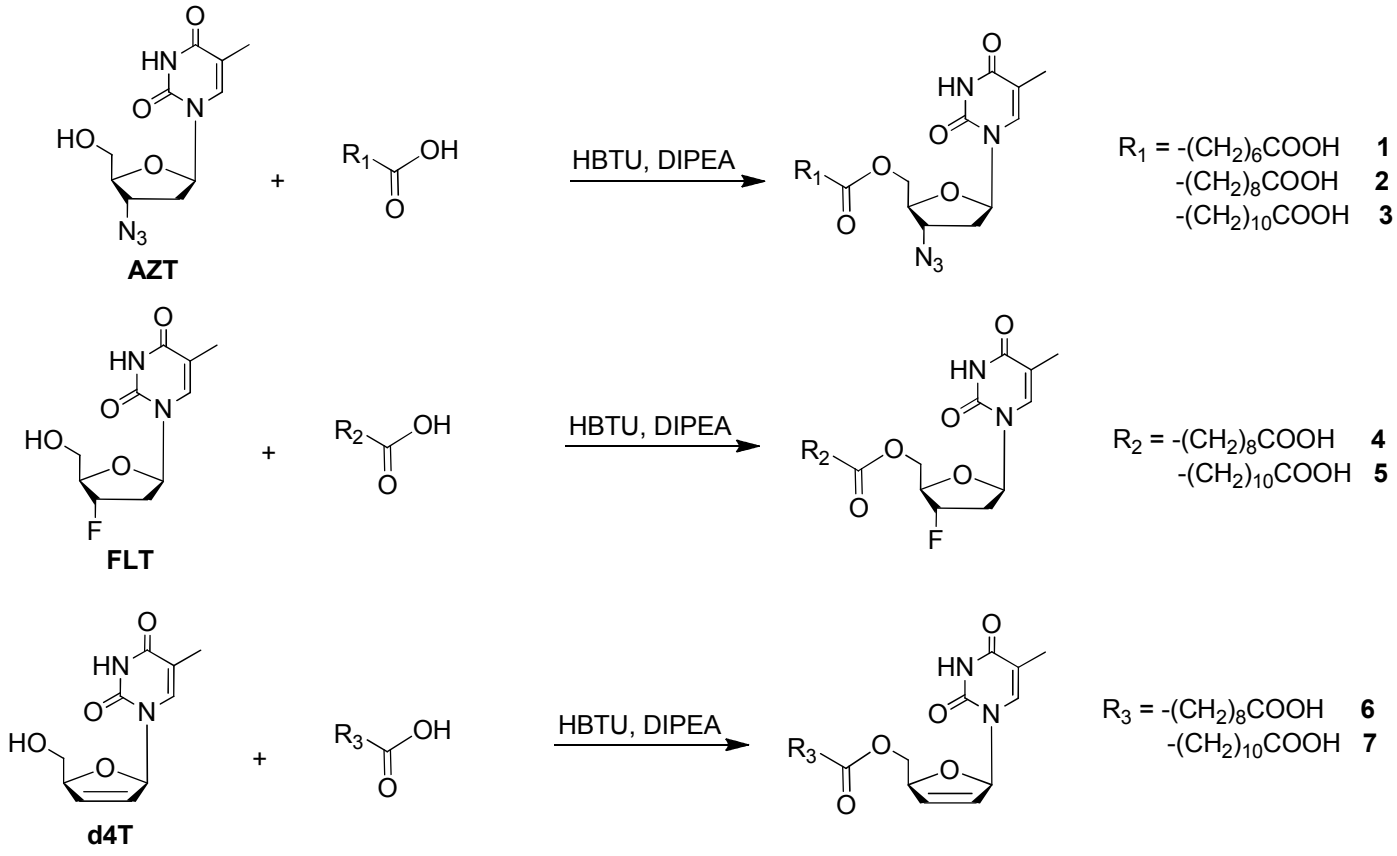
Highly Active Antiretroviral Therapy (HAART) for the treatment for human immunodeficiency virus (HIV) includes using different classes of anti-HIV agents, including nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) along with protease inhibitors (Agarwal, 2008). 2′,3′-Dideoxynucleoside (ddN) analogs are similar to naturally occurring nucleosides, but they cannot be used for the synthesis of new DNA as they lack the 3′-hydroxyl group. These agents act as nucleoside reverse transcriptase inhibitors (NRTIs) and thereby cause DNA chain termination. NRTIs have shown severe adverse effects, mitochondrial toxicity, and resistance to multi-drug resistant HIV.[1-4] Furthermore, nucleoside analogs are polar in nature and have limited cellular uptake. Novel anti-HIV agents are urgently needed with a better safety and resistance profile for the prevention and treatment of HIV infection.

Prevention of HIV transmission to women is essential to avoid transmission of the virus to the newborn. Currently there are no vaccines developed to provide protection against HIV. Thus, it is a necessity to develop additional safe, effective treatment and preventative strategies.

***Corresponding author.** 7 Greenhouse Road, Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, Rhode Island, 02881, USA; Tel.: +1-401-874-4471; Fax: +1-401-874-5787; E-mail address: kparang@uri.edu.

Microbicidal agents are agents which are topically applied to the vagina or rectum prevent the transmission of sexually transmitted organisms., Microbicides which are focused on the prevention of HIV transmissiontarget vaginal, cervical or rectal mucosa and may act via several mechanisms, such as the non-specific direct inactivation of the virus.[6] Currently known microbicides in development, such as UC-781 (thiocarboxanilide), TMC-120 (dapivirine), MIV-150, and tenofovir, act specifically via a single mechanism of the inhibition of reverse transcriptase enzyme.[7] Thus, they have the potential to induce viral resistance and are less effective against cell-associated virus. Developing new anti-HIV microbicides that are multifunctional anti-HIV agents is urgently needed to reduce the incidence of drug resistance. Furthermore, the lipophilic microbicides could bind tightly or irreversibly to the HIV envelope, leading to inactivation of cell-free, or cell-associated virus or both resulting in a significant decrease in virus transmission.

Furthermore, there have been several reports on lipids acting as antiviral and antibacterial agents.[8] The medium length chain saturated fatty acids are one of those agents that inhibit enveloped viruses like HIV and herpes simplex virus type 1 (HSV-1).[9] Microbicidal hydrogels of these fatty acids have been developed and evaluated, and they possibly act by disrupting the viral lipid membrane.[9] HIV-1 replication is inhibited by heteroatom substituted myristic acid analogs without showing any significant cellular toxicity. Myristoylated HIV proteins include $PR160^{gag\text{-}pol}$, $Pr55^{gag}$, $p17^{gag}$, and $p27^{nef}$. Myristic acid analogs inhibit the *N*-myristoyl transferase (NMT) that catalyzes the *N*-myristoylation of HIV proteins.[10] 2-Methoxydodecanoic acid, 4-oxatetradecanoic acid, and 12-thioethyldodecanoic acid have shown to reduce HIV-1 replication in acutely infected



Scheme 1. Synthesis of 5′–mono-substituted fatty acyl ester nucleoside conjugates of FLT, AZT, and d4T (**1-6**).

T-lymphocytes.[11] For example, 12-thioethyldodecanoic acid derivative was moderately active (EC_{50} = 9.4 μM) against HIV-infected T4 lymphocytes.

Several studies have demonstrated the use of lipids as active intravaginal microbicide agents to protect against various sexually transmitted infections (STIs).[8-10] When lipid-associated drugs are administered subcutaneously, they are usually distributed throughout the lymphoid system with improved stability, and the drug can be delivered at higher concentrations.[12] This is a major advantage since HIV is hidden in the lymphatic system not accessible by many commercially available polar anti-HIV nucleosides.

Thus, designing lipophilic anti-HIV nucleosides for developing anti-HIV agents or anti-HIV microbicides is a subject of major interest. The most predominant approach to this strategy is the esterification strategy between nucleosides and fatty acids. The *in vitro* chemical stability of ester conjugates helps to produce formulations with adequate shelf lives. The esters also act as the substrates for esterase enzyme and, therefore, are labile *in vivo*.[13] The 5′-*O*-hydroxyl esterification of the anti-HIV nucleosides also provides a viable approach to increase cellular delivery of polar nucleosides. We have previously shown that fatty acyl monocarboxylic ester derivatives of 3'-azido-3'-deoxythymidine (zidovudine, AZT),[14,15] 3'-fluoro-3'-deoxythymidine (FLT),[16] (-)-2',3'-dideoxy-3'-thiacytidine (3TC),[17] 5-fluoro-(-)-2',3'-dideoxy-3'-thiacytidine (FTC),[18] and 2',3'-didehydro-2',3'-dideoxythymidine (stavudine, d4T)[19] with enhanced anti-HIV activity against CXC4-tropic, CCR5-tropic cell-associated, and/or multi-drug resistant strains of virus. The fatty acid substitution with FTC and 3TC showed a significant increase in their cellular uptake in comparison to their parent nucleosides.[17,18]

Herein, we report the synthesis and evaluation of novel 5′-*O*-fatty acyl ester derivatives of AZT, d4T, and FLT using dicarboxylic fatty acids. As shown before for other conjugates, fatty acids were expected to improve the lipophilicty of polar nucleoside analogs and cellular uptake and to generate lipophilic agents with higher anti-HIV activity. Dicarboxylic acids instead of monocarboxylic fatty acids were selected to generate more amphipathic property in the structure of conjugates due to the presence of additional polar free carboxylic acid.

The synthesis of seven mono-substituted 5′-*O*-(fatty acyl)esters of nucleosides is shown in Scheme 1. Three nucleosides, FLT, AZT, and d4T, and three different dicarboxylic fatty acids were used for esterification. The conjugates were synthesized by reacting nucleosides and dicarboxylic fatty acids in *N*,*N*-dimethylformamide (DMF) in the presence of 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 1-hydroxybenzotriazole (HOBt) and diisopropylcarbodiimide (DIC) as coupling reagents and *N,N*-diisopropylethylamine (DIPEA) as a base. The reaction mixtures were stirred at room temperature overnight. The final products were purified by HPLC on C-18 column using water and acetonitrile as the solvent system in order to achieve more than 95% purity. The chemical structures of the final products were characterized by nuclear magnetic resonance spectrometry (^{1}H NMR and ^{13}C NMR), and were confirmed by a high–resolution time-of-flight electrospray mass spectrometer.

All the synthesized conjugates were evaluated for their inhibitory activity of HIV–1 (subtype B, US/92/727) replication in human peripheral blood mononuclear (PBMC) cells.[20] Table 1 illustrates the anti-HIV-1 activity (EC_{50}) and cytotoxicity (TC_{50}) of the nucleoside ester conjugates compared with their corresponding parent nucleosides. No cytotoxicity was observed up to the highest tested concentration for both the parent nucleosides and the synthesized conjugates (TC_{50} > 500 nM) (**1-7**).

Table 1. Anti-HIV activity of dicarboxylic acid ester conjugates of nucleoside conjugates (**1-7**).

Compd.	Chemical Name	PBMC/HIV-$1_{US/92/727}$			Log P[d]
		EC_{50} (nM)[a]	TC_{50} (nM)[b]	TI[c]	
AZT	3'-azido-2',3'-dideoxythymidine	8.00	>1000	>125	-0.24[e]
FLT	3'-fluoro-2',3'-deoxythymidine	2.00	>500	>250	-0.41
d4T	2',3'-didehydro-2',3'-dideoxythymidine	90.0	>500	>5.6	-0.34
1	8-[(3′-azido-2′,3′-dideoxythymidinyl)-5′-yl]octandioate	0.10	>500	>5000	1.97[e]
2	10-[(3′-azido-2′,3′-dideoxythymidinyl)-5′-yl]decandioate	0.31	>500	>1613	3.03[e]
3	12-[(3′-azido-2′,3′-dideoxythymidinyl)-5′-yl]dodecandioate	0.33	>500	>1516	4.09[e]
4	10-[5′-*O*-(3′-fluoro-2′,3′-dideoxythymidinyl)]decandioate	0.26	>500	>1923	1.99
5	12-[5′-*O*-(3′-fluoro-2′,3′-dideoxythymidinyl)]dodecandioate	0.25	>500	>2000	2.83
6	10-[(2',3'-didehydro-2',3'-dideoxythymidine)-5′-yl]decandioate	1.98	>500	>253	2.06
7	12-[(2',3'-didehydro-2',3'-dideoxythymidine)-5′-yl]dodecandioate	18.30	>500	>27	2.90

[a]EC_{50} (50% effective concentration), All the assays were carried out in triplicate (n = 3); [b]TC_{50} (50% toxic concentration), All the assays were carried out in triplicate (n = 3); [c]Therapeutic index(TC_{50}/EC_{50}); [d]Calculated Partition coefficient by ChemDraw Ultra 12.0; [e]CLogP calculated by ChemDraw Ultra 12.0.

The AZT conjugates (**1-3**, EC_{50} = 0.1-0.3 nM) exhibited consistently higher anti-HIV activity than that of AZT (EC_{50} = 8.0 nM). For example, octandioate (suberate) ester derivative of AZT (**1**, EC_{50} = 0.1 nM) showed 80 times higher anti-HIV activity than the parent nucleoside. AZT conjugates having longer chain fatty acids also showed enhancement in anti-HIV activity than AZT while the ratio of improvement was less than that of compound **1**. The decandioate ester of AZT (**2**, EC_{50} = 0.31 nM) was 26-fold more potent than that of AZT. The activity of dodecandioate ester of AZT (**3**) was 24 times higher when compared to AZT. Among the AZT conjugates, AZT-suberate conjugate (**1**) showed the highest anti-HIV activity. These data suggest that conjugation of AZT with dicarboxylic acids significantly enhances the anti-HIV activity with higher potency seen in conjugates with shorter chain length.

Similarly, dicarboxylic ester conjugates of d4T (**6** and **7**, EC_{50} = 1.98-18.3 nM) showed better anti-HIV activity from that of d4T (EC_{50} = 90 nM) in the PBMC assay against HIV−$1_{US/92/727}$. The decanedioate ester of d4T (**6**, EC_{50} = 1.98 nM) exhibited 45 times more anti-HIV activity than d4T. The dodecandioate ester of d4T (**7**, EC_{50} = 18.3 nM) showed 5 times higher anti-HIV activity when compared to that of its parent nucleoside. These results indicate that the anti-HIV activity of the carboxylic esters of nucleoside depends on the chain length of the carboxylic acid, and shorter length dicarboxylate monoester conjugates are more potent.

A similar trend was observed for FLT ester conjugates compared to the parent nucleoside. Amongst all the FLT conjugates (EC_{50} = 0.25-0.26 nM), the decanedioate ester of FLT (**4**, EC_{50} = 0.26 nM) and dodecanedioate ester of FLT (**5**, EC_{50} = 0.25 nM) showed an 8-fold increase in anti-HIV activity when compared to FLT (EC_{50} = 2 nM), but the difference in anti-HIV activity between the two conjugates was not significant.

There was a significant increase in the therapeutic index of AZT and FLT derivatives when compared to that of the respective parent nucleosides. The increased therapeutic index indicates synergistic effect of conjugation of dicarboxylic acids with ddNs. Among all the synthesized dicarboxylic acid ester derivatives of AZT, FLT, and d4T, the decandioate monoester conjugate of AZT (**1**) showed the highest anti-HIV activity.

This study indicates that NRTIs when in conjugation with long chain dicarboxylic acids exhibit higher anti-HIV activity possibly due to the improved lipophilicity thereby increasing the cellular uptake of NRTIs. As shown by calculated partition coefficient values (Table 1), the ester conjugates were more lipophilic that their parent analogs. We have shown previously for other fatty acyl derivatives of nucleosides that the highly lipophilic conjugates could have higher cellular uptake contributing to their improved anti-HIV activity.[17,18] However, for dicarboxylic monoester conjugates, an appropriate lipophilicty is required for an optimal anti-HIV activity since highly lipophilic conjugates with longer chain length were less potent than those with shorter chain length. We have previously shown higher cellular uptake and the intracellular hydrolysis of several fatty acyl ester derivatives of 3TC, d4T, and FTC.[17-19] Similarly, it is expected that when the conjugate esters enter the cells, they undergo intracellular hydrolysis by esterases and release the anti-HIV nucleosides and fatty acids targeting different stages of the HIV life cycle possibly contributing to enhanced anti-HIV activity. Nucleoside and fatty acid analogs are known to target the reverse transcriptase and the NMT enzymes. More mechanistic studies are required to determine the exact mechanism of activity of the ester conjugates of the nucleosides.

In conclusion, several lipophilic 5′-*O*-fatty acyl dicarboxylic monoester derivatives of the nucleoside reverse transcriptase inhibitors, AZT, FLT, and d4T were synthesized and evaluated

for their anti-HIV activity. The fatty acid substitution at the 5′-*O*-position enhanced the lipophilicity of the anti-HIV nucleoside analogs. All conjugates exhibited higher anti-HIV activity when compared to their parent nucleosides. The improved viral inhibition by the AZT, d4T, and FLT fatty acyl derivatives versus the parent nucleoside is presumably due to the enhanced cellular uptake of the lipophilic conjugates. These conjugates have the potential to be used as potent anti-HIV agents and/or lipophilic anti-HIV microbicides after further optimization.

Acknowledgments

The authors wish to acknowledge the technical contribution of Dr. Lu Yang and Ms. Ashlee Boczar for the antiviral assays described herein. We also acknowledge National Center for Research Resources, NIH, and Grant Number 8 P20 GM103430-12 for sponsoring the core facility.

References and notes

1. Otto, M. J. *Curr. Opinion Pharmacol.*, **2004**, *4*, 431-36.
2. Tan, X., Chu, C. K., Boudinot, F. D. *Advanced Drug Delivery Reviews* **1999**, *39*, 117-51.
3. Lewis, W., Kohlera, J. J., Hosseinia, S. H., Haasea, C. P., Copelandb, W. C., Bienstockb, R. J., Ludawaya, T., McNaughta, J., Russa, R., Stuarta, T., Santoiannia, R. *AIDS* **2006**, *20*, 675-84.
4. Lund, K. C.; Peterson, L. L.; Wallace, K. B. *Antimicrob. Agents Chemother*. **2007**, *51*, 2531-39.
5. Lederman, M. M., Offord, R. E., Hartley, O. *Nat. Rev. Immunol.,* **2006**, *6*, 371-82.
6. Balzarini, J., Damme, L. V. *Lancet*, **2007**, *369*, 787-97.
7. Mauck, C., Doncel, G. *Current Infectious Disease Reports,* **2001**, *3*, 561-68.
8. Thormar, H., Issacs, C. E., Brown, H. R., Barshatzky, M. R., Pessolano, T. *Antimicrob. Agents Chemother.,* **1987**, *31*, 27-31.
9. Kristmundsdottir, T., Arnadottir, S. G., Bergsson, G., Thormar, H. *J. Pharm. Sci.,* **1999**, *88*, 1011-15.
10. Langner, C. A., Travis, J. K., Caldwell, S. J., Tianbao, J. E., Li, Q., Bryant, M. L., Devadas, B., Gokel, G. W., Kobayashi, G. S., Gordon, J. I. *J. Biol. Chem.,* **1992**, *267*, 17159-69.
11. Parang, K., Wiebe, L. I., Knaus, E. E., Huang, J. S., Tyrrell, D. L., Csizmadia, F. *Antiviral Res.,* **1997**, *34*, 75-90.
12. Kinman, L., Brodie, S. J., Tsai, C. C., Bui, T., Larsen, K., Schmidt, A., Anderson, D., Morton, W. R., Hu, S. L., Ho, R. J. *J. Acquir. Immune Defic. Syndr.,* **2003**, *34*, 387-97.
13. Sriram, D., Yogeeswari, P., Srichakravarthy, N., Bal, T. R. *Bioorg. Med. Chem. Lett.,* **2004**, *14*, 1085-87.
14. Parang, K., Knaus, E. E., Wiebe, L. I. *Antiviral. Chem. Chemother.* **1998**, *9*, 311-23.
15. Parang, K., Wiebe, L. I., Knaus, E. E. *Curr. Med. Chem.,* **2000**, *7*, 995-1039.
16. Parang, K., Knaus, E. E., Wiebe, L. I. *Nucleosides & Nucleotides* **1998**, *17*, 987-1008.
17. Agarwal, H. K., Chhikara, B. S., Hanley, M. J., Ye, G., Doncel, G. F., Parang, K. *J. Med. Chem.* **2012**, *55*, 4861-71.
18. Agarwal, H. K., Chhikara, B. S., Bhavaraju, S., Mandal, D., Doncel, G. F., Parang, K. *Mol. Pharmaceutics* **2013**, *10*, 467-76.
19. Agarwal, H. K., Loethan, K., Mandal, D., Doncel, G. F., Parang, K. *Bioorg. Med. Chem. Lett.* **(2011)** *21*, 1917-21.
20. Watson, K. M., Buckheit, C. E., Buckheit, R. W., Jr. *Antimicrob. Agents Chemother.* **2008**, *52*, 2787-96.

Supplementary Material

Supplementary data associated with this article can be found in the online version.