Osmium Complex Formation at 5-Methylcytosine: New Technology for Analysis of DNA Epigenetic Modification

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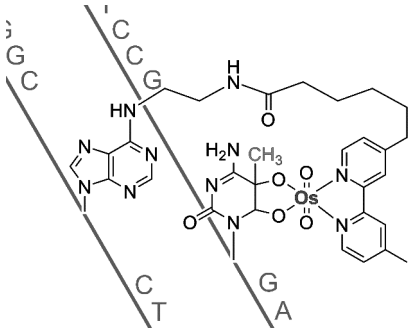
5-Methylcytosine (5mC) is a common C5-methylated cytosine base and plays an important role in a variety of epigenetic events. Analysis of the methylation status of cytosine (C) is required for understanding which genes are working. However, it is not easy to distinguish 5mC from C, i.e., to detect the existence of only one methyl group in a long DNA strand. Although bisulfide methods or immunoprecipitation methods are often used for 5mC analysis, they are not suitable for sequence-specific labeling of 5mC. In this paper, we report 5mC-selective DNA oxidation for DNA methylation analysis. Nucleic acids often acquire new functions by forming a variety of complexes with metal ions. Osmium, in an oxidized state, reacts with C5-methylated pyrimidines. We have developed the 5mC-selective osmium complex formation for effective detection of 5mC. One example of the reaction conditions is as follows: 5mM potassium osmate, 100 mM potassium hexacyanoferrate(III), and 100 mM 2,2'-bipyridine in 100 mM Tris-HCl buffer (pH = 7.7), 1 mM EDTA, and 10% acetonitrile. The reaction mixture is incubated with the target DNA at 0 °C for 5 min. 5mC was oxidized efficiently by exposure to a reaction mixture containing an osmium complex, making possible a clear distinction from very weak oxidation of C. Based on this reaction, we synthesized a bipyridine-attached adenine derivative for sequence-specific osmium complex formation. Sequence-specific osmium complex formation was achieved by the hybridization of a short DNA molecule containing this functional nucleotide to a target DNA sequence, and resulted in the formation of a crosslinked structure (Figure). The interstrand crosslink clearly distinguished 5mC from C, which was applied to quantification of the degree of methylation at a specific cytosine in a whole genome and to visualization of methylation regions in chromosomes (MeFISH).



Keywords Osmium; Oxidation; DNA; 5-Methylcytosine; Epigenetics

Biphenyl and Xanthone Derivatives from the Twigs of a *Garcinia* sp. (Clusiaceae)

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The genus of *Garcinia* is a well-known rich source of bioactive xanthones and benzophenones. However, some species of this genus also produce triterpenoids, flavonoids and biphenyls as major compounds. In this study, two new biphenyls, doitungbiphenyls A (**1**) and B (**2**), along with six known compounds including two known biphenyls, schomburgbiphenyl (**3**) and nigrolineabiphenyl B (**4**); and four known xanthones, 1,3,6-trihydroxy-8-isoprenyl-7-methoxyxanthone (**5**), morusignin K (**6**), 1,5-dihydroxyxanthone (**7**), and 1,7-dihydroxyxanthone (**8**), were isolated from the acetone extract of *Garcinia* sp. twigs. The structures of **1** and **2** were characterized extensively by 1D and 2D NMR spectroscopy and HR-EI-MS. The cytotoxicity of **1** and **2** against the oral cavity cancer (KB) and the breast cancer (MCF-7) cell lines was also evaluated.

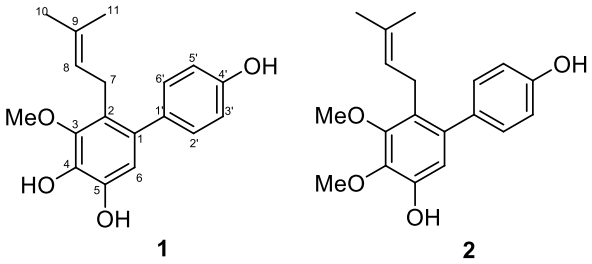

Figure 1. Structure of doitungbiphenyls A (**1**) and B (**2**)

Keywords *Garcinia*; Clusiaceae; Biphenyl derivative; Xanthone

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Combined Computational-biochemical Drug Discovery: A Novel Approach for Scoring and Predicting Efficacy of Compounds Targeting HIV-1 Integrase

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HIV-1 integrase (IN) is a crucial enzyme for HIV infectivity and is required for the insertion of the viral DNA into the host genome. The two key steps involved in the viral integration are '3'-processing' and 'strand transfer' reactions. The first step, 3'-processing, involves a cleavage of GT dinucleotides from the viral DNA to generate 3'-hydroxyl ends at the constant CA-3 terminal. The second step, known as 'strand transfer' or 'two-metal chelation', is a transesterification process between the 3'-ends of the invariant viral DNA and the phosphodiester backbone of the host DNA to attain a 'proviral DNA'. In this research, a modern drug discovery was re-designed by using molecular docking via AutoDock 4 and Vina. Molecular docking techniques were utilized in a receptor-based virtual screening and affirmative re-docking, which followed by data analyses employing advanced mathematical models, Partial Least Squares (PLS) and Self-Organizing Maps (SOMs), in order to score and predict the inhibitory activity of the docked ligands. Highly active compounds against HIV-1 integrase selected from the US NIH Clinical Collection were emphasized. After the computational methods, the predicted active agents were biochemically tested with an HIV-1 integrase assay to essentially validate the computational prediction. During this protocol is still being optimized and applied to identify lead compounds for several targets, a combination of this technique and a pharmacophore search approach, confirmed with biochemical assay, had led to the discovery of a compound 'NIH-Int01', a compound from the US NIH Clinical Collection. The agent at 10 μM concentration exhibited an initial inhibitory activity against HIV-1 integrase of 59.19%. Detailed biochemical assay confirmed an IC_{50} of 3.70 μM. These data, combined with molecular modeling, can also be used to further improve the activity of this lead compound. Especially, a pharmacophore search prior to virtual screening has proved essential for a more successful drug discovery.

Keywords HIV; HIV-1 integrase; Molecular docking; Virtual screening; AutoDock 4; AutoDock Vina; PLS; SOMs

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