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# AdoMet Derivatives Induce the Production of Actinorhodin in *Streptomyces coelicolor*

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**Abstract** Exogenous *S*-adenosyl-L-methionine (AdoMet) enhances the production of actinorhodin in *Streptomyces coelicolor*. Thirty compounds related structurally with AdoMet were tested for their actinorhodin production. The relationships between the structures of the compounds tested and their actinorhodin production were analyzed using computational methods, and the molecules containing both bulky substituents at the C6 position of adenine and the long 5'-alkyl chain of adenosine were predicted to show high actinorhodin production.

**Key words:** Actinorhodin, *Streptomyces coelicolor*, structure-activity relationships

In *Streptomyces*, both morphological differentiation and secondary metabolism are influenced by their environmental conditions and controlled by the same signal transduction pathways [10]. Based on the results of the genome project of *Streptomyces coelicolor* A3(2), there are over thirty proteins with kinase catalytic domains. Proteins such as AfsK and AfsR participate in growth, morphogenesis, and secondary metabolism [7]. Although the regulation of secondary metabolism is not clear, the production of antibiotics is known to be related with morphological development [2]. *S. coelicolor* A3(2) produces two pigmented antibiotics, actinorhodin and tripyrrole undecylprodigiosin. Another species close to *S. coelicolor*, *S. lividans*, does not produce actinorhodin under its usual growth conditions [1]. Kim *et al.* [4] reported that *S*-adenosyl-L-methionine (AdoMet) synthetase affects the production of actinorhodin, and the exogenous addition of AdoMet affects actinorhodin production as well as morphological development in *S. lividans*. AdoMet is a known methyl donor, but Park *et al.* [10] found it to be a regulatory factor in the cell. Exogenous addition of AdoMet on *S. lividans* as well as *S. coelicolor* activates actinorhodin production [4, 9]. As mentioned above, morphological differentiation and antibiotic production are related with the same signal transduction pathways; that is, AdoMet enhances actinorhodin production and inhibits morphological differentiation [10].

If AdoMet can increase the production of actinorhodin, its derivatives may have a similar effect. Therefore, we tried to discover other compounds to enhance actinorhodin production. Actinorhodin belongs to the aromatic polyketides and benzoisochromanequinones and functions as an acid-base indicator depending on the pH of the medium; its color is blue in basic condition and red in acidic pH [1]. Because the addition of AdoMet on *S. coelicolor* can increase actinorhodin production, which results in killing microorganisms surrounding *S. coelicolor*, this phenomenon may be applied for biopesticides.

AdoMet contains an adenosine skeleton (Fig. 1). Thirty adenosine derivatives including adenosine and AdoMet, listed in Table 1, were selected to test actinorhodin production. *S. coelicolor* M145 was used for this study. R2YE medium was used for actinorhodin production [5] and actinorhdin was determined photometrically using the previous method published by Liao *et al.* [6]. The 30 AdoMet derivatives were prepared as 100 mM concentrations. The control without the addition of any compound showed an absorbance of 0.266 and the sample treated with AdoMet showed 0.702. Figure 2 shows the control plate and the plate treated with AdoMet, where the latter has more blue color. As published by Okamoto *et al.* [9] and Park *et al.* [10], the treatment of AdoMet on *S. coelicolor* causes

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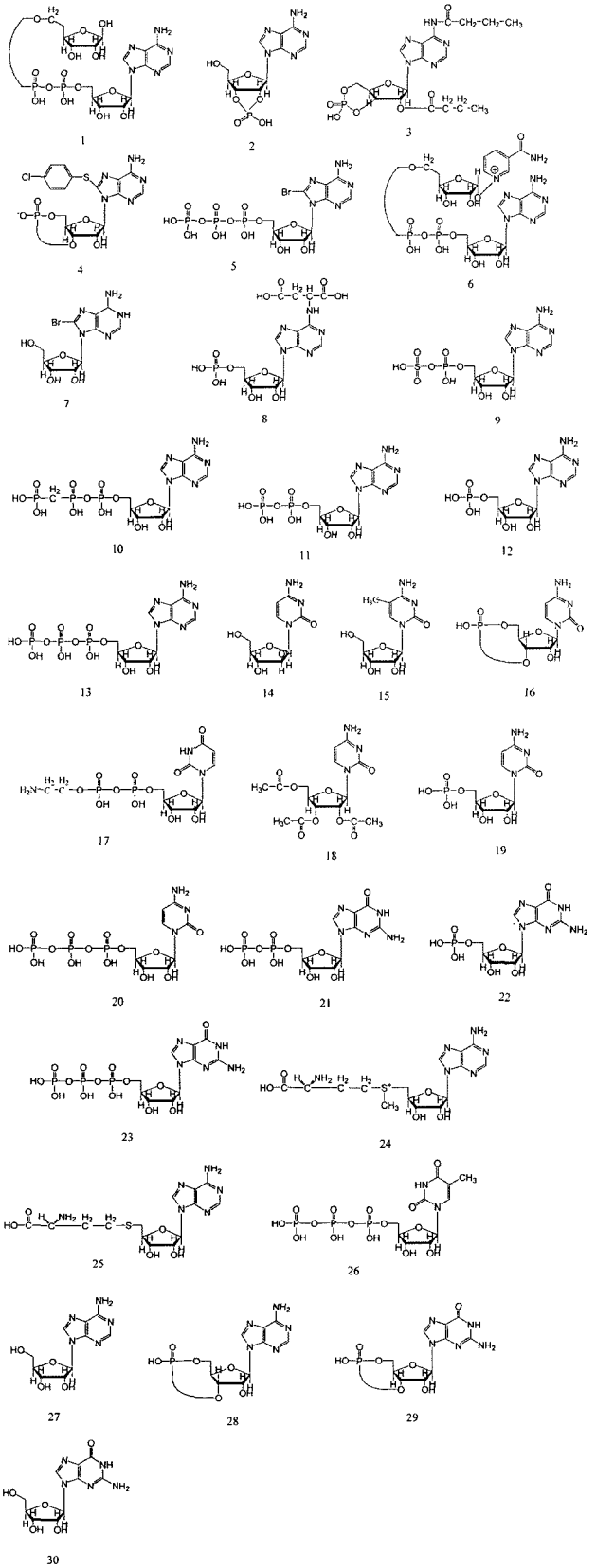

**Fig. 1.** Thirty compounds used for the training set of quantitative structure-activity relationships calculations.

overproduction of actinorhodin, but it does not affect the growth. The production data of actinorhodin by the treatment of adenosine derivatives are listed in Table 1.

Based on the actinorhodin production data, the compounds containing cytosine and guanosine as base did not enhance the production. Even though the compounds have the adenosine moiety, the compounds with more phosphates or sulfur at the 5'-position of the sugar moiety showed high production. In order to find the relationships between the structures of the compounds and their actinorhodin productions, we have performed a three-dimensional (3D) quantitative structure-activity relationship (QSAR) study by Comparative Molecular Field Analysis (CoMFA) [3]. In the absence of any information regarding the biological target, the indirect ligand-based approaches like 3D-QSAR can assist in understanding the SARs and also serve as a guide in the design of more potent inhibitors. A QSAR with CoMFA study was carried out using SYBYL 7.0 (Tripos, St. Louis, MO, U.S.A.) installed on a Pentium 3.2 GHz PC with the Linux OS (Red Hat Enterprise WS). The structures were built with the Sketcher module, and the energy was minimized by the conjugate gradient method using the Tripos force field. A set of 30 commercially available adenosine derivatives (Table 1 and Fig. 1) was used in this study. Of these, three molecules with bulky substituents on the adenine ring (**4**, Fig. 1) or 5'-alkyl chain (**1** and **6**, Fig. 1) were not included in the generation of the model. The remaining 27 molecules were divided into a training set (22 molecules) and a test set (5 molecules) by means of chemical as well as biological diversity. The most crucial input for the CoMFA is the alignment of the molecules. The molecule *S*-adenosyl-L-homocysteine (AdoHcy) with the highest activity was chosen as the template and all other molecules were aligned to it using the DATABASE ALIGNMENT method in SYBYL (Fig. 3). The steric and electrostatic fields in CoMFA were calculated at each lattice intersection of a regularly spaced grid of 1.0 Å in all three dimensions within the defined region, and the van der Waal's potential and Coulombic energy between the probe and the molecule were calculated using the standard Tripos force field. The final CoMFA model, which was obtained by using a standard protocol of the 3D-QSAR/CoMFA protocol [8], exhibits a cross-validated correlation coefficient ($q^2$) of 0.51, conventional correlation coefficient ($r^2$) of 0.99, and predictive correlation coefficient ($r^2_{pred}$) of 0.88, which indicates that the probability of chance correlation is less than 5% [8].

The colored CoMFA contours in the map represent areas in 3D space where change in the steric and electrostatic field values of a compound correlate strongly with a concomitant change in its biological activity. The contours for steric fields are shown in green (more bulk favored) and yellow (less bulk favored), whereas the electrostatic field contours are shown in red (electronegative substituents favored) and blue (electropositive substituent favored). The CoMFA

**Table 1.** Thirty compounds used to test actinorhodin production and their UV absorption values. The absorption of the control without the addition of any compounds is 0.266.

| Comp. | Name | Absorbance |
|---|---|---|
| 1 | Adenosine 5'-diphosphoribose sodium salt | 0.399 |
| 2 | Adenosine 2',3'-cyclic monophosphate sodium salt | 0.399 |
| 3 | N6,2-O-dibutyryladenosine 3',5'-cyclic monophosphate sodium salt | 0.599 |
| 4 | 8-(4-Chlorophenylthio)adenosine 3',5'-cyclic monophosphate sodium salt | 0.599 |
| 5 | 8-Bromoadenosine 5'-triphosphate sodium salt | 0.399 |
| 6 | α-nicotinamide adenine dinucleotide from *Saccharomyces cerevisiae* | 0.399 |
| 7 | 8-Bromoadenosine | 0.532 |
| 8 | Adenylosuccinic acid sodium | 0.532 |
| 9 | Adenosine 5'-phosphosulfate sodium salt | 0.266 |
| 10 | α,β-Methyleneadenosine 5'-triphosphate disodium salt | 0.532 |
| 11 | Adenosine diphosphate | 0.479 |
| 12 | Adenosine monophosphate | 0.665 |
| 13 | Adenosine triphosphate | 0.612 |
| 14 | Cytosine β-D-arabinofuranoside hydrochloride | 0.399 |
| 15 | 5-Methylcytidine | 0.266 |
| 16 | Cytidine 3',5'-cyclic monophosphate | 0.266 |
| 17 | Cytidine 5'-diphosphoethanolamine sodium salt | 0.532 |
| 18 | 2',3',5'-Tri-*O*-acetylcytidine hydrochloride | 0.532 |
| 19 | Cytosine-5'- monophosphate | 0.274 |
| 20 | Cytosine-5'-triphosphate | 0.274 |
| 21 | Guanosine-5'-diphosphate | 0.274 |
| 22 | Guanosine-5'-monophosphate | 0.266 |
| 23 | Guanosine-5'-triphosphate | 0.293 |
| 24 | *S*-(5'-Adenosyl)-L-methionine | 0.684 |
| 25 | *S*-(5'-Adenosyl)-homocysteine | 0.702 |
| 26 | Thymidine-5'-triphosphate | 0.271 |
| 27 | Adenosine | 0.399 |
| 28 | Adenosine 3',5'-cyclic monophosphate | 0.505 |
| 29 | Guanosine 3',5'-cyclic monophosphate | 0.266 |
| 30 | Guanosine | 0.266 |

steric and electrostatic contour plots of our model are shown in Figs. 4 and 5, respectively. Analysis of the steric contours (Fig. 4) revealed one green colored contour and two yellow colored contours. The favorable green contour was seen near the C6 substituent on the adenine ring. Molecules with bulky substituents at the C6 position exhibit good activity, which include **3** and **8**. The sterically unfavorable yellow contour away from the C2 and/or C8 positions of the adenine ring indicates that bulky substituents will not be favored at this position. Guanine analogs (**21–23**, **29–30**) and cytidine analogs (**14–20**) have substituents at the positions that correspond to the C2 and

**Fig. 2.** The control plate (left) and the plate treated with *S*-adenosy-L-methionine (right).

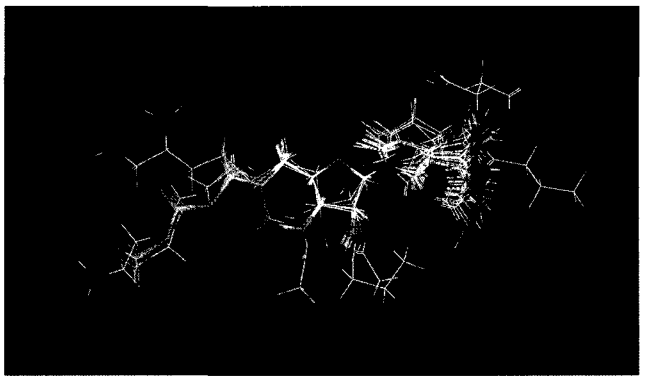

**Fig. 3.** The training set molecules aligned over each other using DATABASE ALIGNMENT.

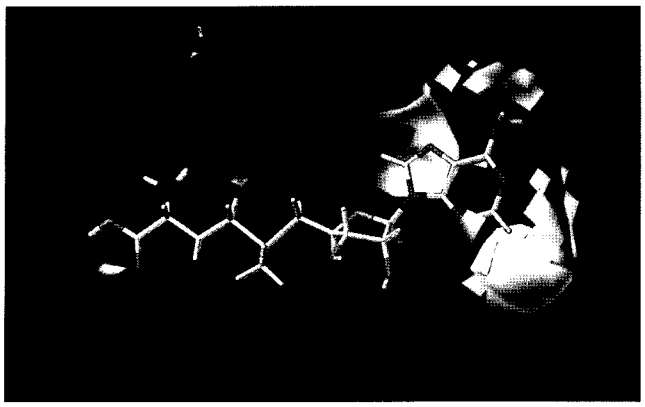

**Fig. 4.** CoMFA contour maps for steric fields drawn around AdoHcy.
The green colored contour favors steric bulk, and sites where steric bulk is disfavored are shown in yellow.

C7 positions of the adenine ring, respectively, and result in poor biological activity. Analysis of the electrostatic contours (Fig. 5) shows one red colored contour (electronegative substituents favored) and one blue colored contour (electropositive substituents favored) near the end of the long 5'-alkyl chain of adenosine, which implies that both electronegative and electropositive substituents would be making favorable interactions with its biological target. The CoMFA analysis thus provides deep structural insights into possible modifications of AdoMet that can improve the production of the antibiotic actinorhodin. The predictive ability of the 3D-QSAR with CoMFA model was determined from a set of 5 test molecules not included in the model generation.

Therefore, the molecule containing both bulky substituents at the C6 position of adenine and the long 5'-alkyl chain of adenosine are proposed to be a good signal molecule to produce actinorhodin. Attempts are currently underway in our laboratory to synthesize and evaluate the biological activities of the newly proposed structures.

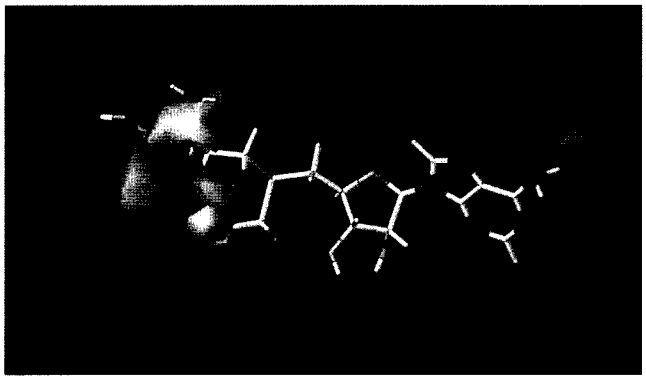

**Fig. 5.** CoMFA contour maps for electrostatic fields drawn around AdoHcy.
The red contour shows regions where electronegative substituents are favored, and the blue contour is associated with positions where electropositive substituents improve activity.

## Acknowledgments

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