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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 5-[(2,5-DIHYDROXYBENZYL)AMINO]SALICYLIC ACID ANALOGS AS INHIBITORS OF EGF RECEPTOR-ASSOCIATED PROTEIN TYROSINE KINASE

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Abstract: The synthesis and biological activity of a series of 5-[(2,5-dihydroxybenzyl)amino]salicylic acid derivatives (**3**-**6**) as analogs of the active partial structure (**2**) of the potent EGF-R tyrosine kinase inhibitor lavendustin A (**1**) are described. Analogs with an electron-withdrawing group in place of the carboxyl group of **2** showed activity. The *N*-hexylsalicylamide analog **6b** (IC_{50}=0.9 μM) was about four times more potent than **2**.
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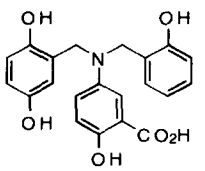
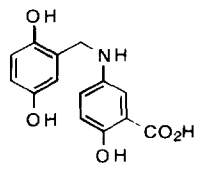
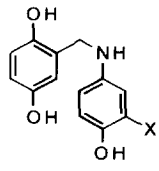
Activation of protein tyrosine kinase (PTK) is one of the main mechanisms by which external stimuli are transmitted into cells. PTK plays an important role in normal developmental and regenerative processes of cells, and overexpression of protein tyrosine kinase can result in the loss of growth control, leading to cancer and other disorders of cell proliferation.[1] For example, the epidermal growth factor receptor (EGF-R), which is endowed with tyrosine kinase activity, and its endogenous ligand, transforming growth factor α (TGFα), are overexpressed in several tumors.[2-5] Accordingly, specific inhibitors of PTK can be useful in investigating the mechanisms of carcinogenesis, cell proliferation and differentiation, and could be effective in prevention and chemotherapy of cancer.



Lavendustin A (**1**)



Active partial structure (**2**)



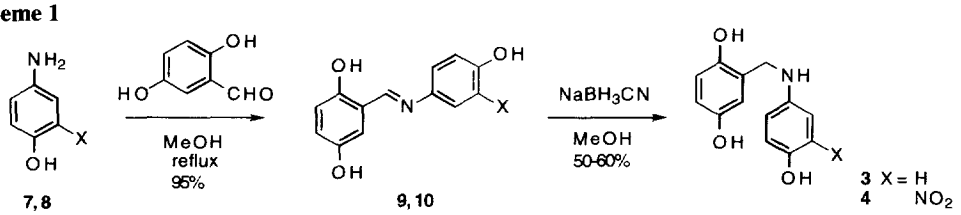
3 X = H
4 NO_2
5 SO_3H
6 CONHR

The novel microbial secondary metabolite levendustin A (**1**) and its active partial structure (**2**) were reported to inhibit potently the EGF-R tyrosine kinase in A431 cell-free extracts.[6] Recent studies of structure-activity relationships of **2**[7,8] indicated that the salicylate moiety might be important for activity, and chain elongation and the introduction of a branched alkyl substituent of ester alcohol could improve cellular bioavailability.[9] On the basis of these findings, we initiated an investigation of the role of the salicylate moiety by replacing the carboxyl group with electronically equivalent groups such as nitro, sulfonic acid and *N*-alkylsalicylamides.

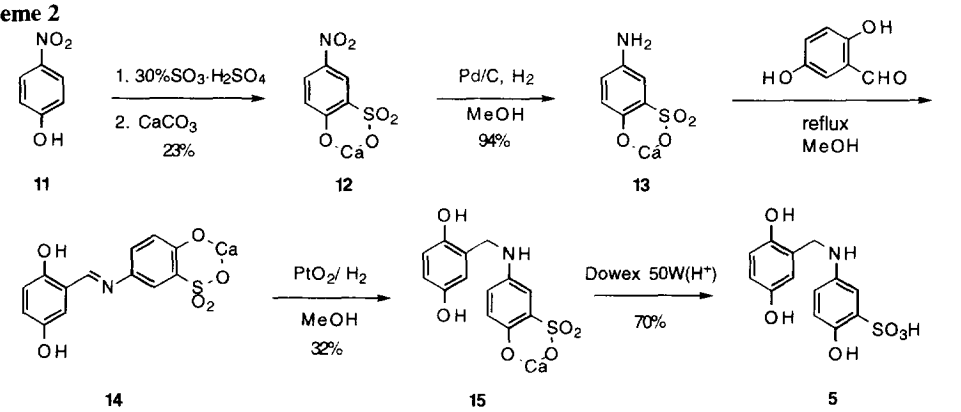
As shown in **Scheme 1**, analogs **3** and **4** were synthesized by the condensation of gentisinaldehyde with anilines **7** and **8** followed by the reduction of corresponding imines **9** and **10**, respectively.

Sulfonic acid analog **5** was prepared as shown in **Scheme 2**. Reduction of the calcium salt of 4-nitrophenol-2-sulfonic acid obtained by the procedures of King[10] provided the amine **13**, which was condensed with gentisinaldehyde to afford imine **14**. Reduction of **14** by catalytic hydrogenation gave **15**, which was treated with ion-exchange resin to give sulfonic acid **5**.

Scheme 1

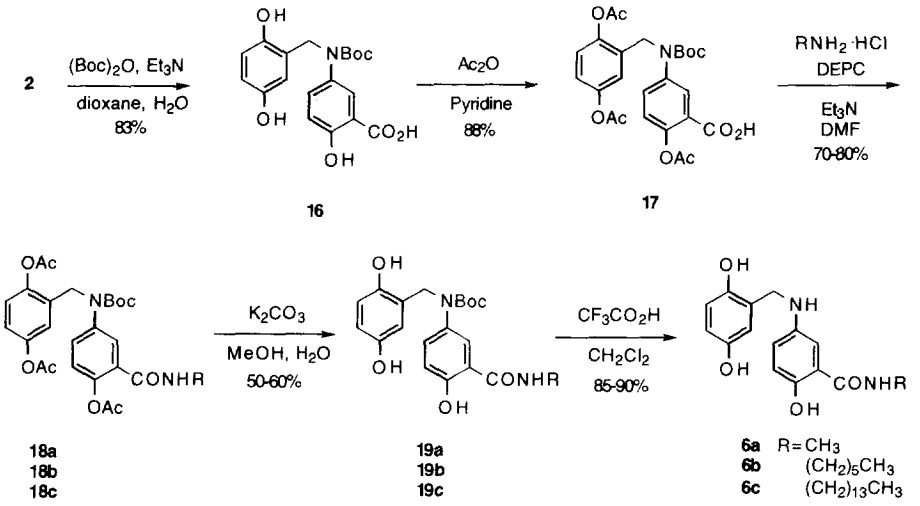


Scheme 2



Amide analogs **6a**, **6b** and **6c** were synthesized as shown in **Scheme 3**. Fully protected carboxylic acid **17** was condensed with the corresponding amines by DEPC, followed by the sequential deprotection to give **6a**, **6b** and **6c**, respectively.

Scheme 3



The inhibitory activity of these synthesized lavendustin A analogs *in vitro* was tested against EGF-R tyrosine kinase (IC_{50})[11,12] and the results are listed in **Table**.

Table **Inhibitory Activity of 2-6 towards EGF-R Tyrosine Kinase**

Compound[13]	X	IC_{50} (μM)
2	CO_2H	4.0
3	H	41.9
4	NO_2	5.4
5	SO_3H	10.3
6a	$CONHCH_3$	9.7
6b	CONH-*n*-C_6H_{13}	0.9
6c	CONH-*n*-$C_{14}H_{29}$	27.2

All of the compounds with an electron-withdrawing group proved to be moderate to effective inhibitors of EGF-R tyrosine kinase. The unsubstituted analog **3** showed decreased inhibitory activity. The nitro analog **4** and sulfonic acid analog **5** showed strong activity. The *N*-hexylsalicylamide analog **6b** exhibited striking activity, being about four times more potent than **2**, the active core structure of a natural inhibitor of EGF-R tyrosine kinase, lavendustin A (**1**). Interestingly, similar amide analogs with a shorter or longer side chain showed significant loss of activity. These results suggest that, in addition to the electron-withdrawing group, the hydrophobic side chain also plays an important role in strong inhibition of EGF-R tyrosine kinase. Further studies on other *N*-alkylsalicylamide analogs are in progress.

Acknowledgment:

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11. The EGF-R tyrosine kinase assay was performed as follows. See also ref. 6.
    Enzyme Assay: Tyrosine kinase reactions were carried out in a final volume of 50 µl containing HEPES buffer (20 mM, pH 7.2), $MnCl_2$ (1 mM), bovine serum albumin (BSA, 0.125 mg/ml), mouse EGF (100 ng/ml, Collaborative Research Inc.). the membrane fraction of A431 cells, histone (Sigma, 40 µg/ml), and [γ-^{32}P]ATP (12 Ci/mmol). Drugs were dissolved in DMSO at 10 mg/ml and diluted with H_2O. The EGF receptor was first incubated with EGF and the sample at 0°C for 10 min before assay of kinase activity. The kinase reactions were initiated by the addition of [γ-^{32}P]ATP (1 µM), and the reaction mixture was incubated for 30 min at 0°C. The reaction was terminated by adding 0.2 ml of 10% TCA and the mixture was filtered on Whatman 3MM paper in a cell harvester. The papers were washed extensively with 10%TCA and dried. The radioactivity of the filter was counted by the Cherenkov effect in a liquid scintillation counter.
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13. All new compounds gave satisfactory spectroscopic and analytical data. Representative data for **6b**. ^{1}H-NMR (500MHz, CD_3OD) δ: 0.85 (3H, t, J=7.0Hz), 1.29 (6H, m), 1.54 (2H, m), 3.30 (2H, t, J=7.0Hz), 4.16 (2H, s), 6.48 (1H, dd, J=2.5, 8.5Hz), 6.59 (1H, d, J=8.5 Hz), 6.67 (1H, d, J=2.5Hz), 6.68 (1H, d, J=8.5Hz), 6.78 (1H, dd, J=3.0, 8.5Hz), 7.10 (1H, d, J=3.0Hz); FABMS m/z: 358 $(M)^+$; HRFABMS. Calcd. for $C_{20}H_{26}N_2O_4$ $(M)^+$: 358.1893, found 358.1867; Anal. Calcd. for $C_{20}H_{26}N_2O_4$: C, 67.01; H, 7.33; N,7.82. Found: C, 66.66; H, 7.39; N, 7.76.

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