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Communications to the Editor

(2*E*)-5-[3-[(Phenylsulfonyl)amino]phenyl]-pent-2-en-4-ynohydroxamic Acid and Its Derivatives as Novel and Potent Inhibitors of *ras* Transformation

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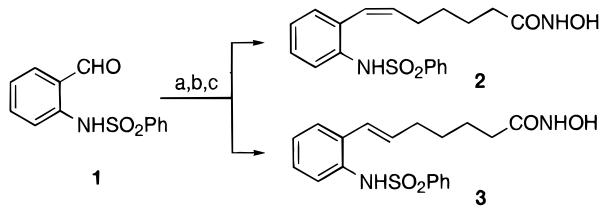
The *ras* oncogene has been reported to be expressed in many human tumors and tumor cell lines.[1] The product of *ras* oncogene is a kind of GTP-binding protein, and its GTPase activity can be correlated with the transforming activity.[2] There have been several reports on small molecule inhibitors against *ras* oncogene. Oxanosine was found to suppress the function of *ras* by decreasing the pool of guanine nucleotides inside the cells by inhibiting IMP dehydrogenase.[3] Azatyrosine reversed the phenotype of the *ras*-transformed cells, by selecting the flat revertant type after long-term culture.[4] Farnesyltransferase inhibitors, such as manumycin,[5] tetrapeptide analogue,[6] farnesyl diphosphate inhibitor,[7] farnesyl carboxylic derivative,[8] and benzodiazepine peptidomimetics,[9] inhibited the growth of *ras*-transformed cells by suppressing the modification of *ras* protein specifically in the process of signal transduction.

Described here are the synthesis and *in vitro* biological activity of novel synthetic compounds, substituted aromatic unsaturated hydroxamic acids which reversibly induce the flat phenotype of K_i-*ras*-transformed NIH3T3 cells, indicating reversion of tumor characteristic to normal one.

In the course of screening compounds exhibiting *ras* transformation inhibition, an aromatic sulfonamido **2** showed interesting activity with an MIC value of 17 μM.

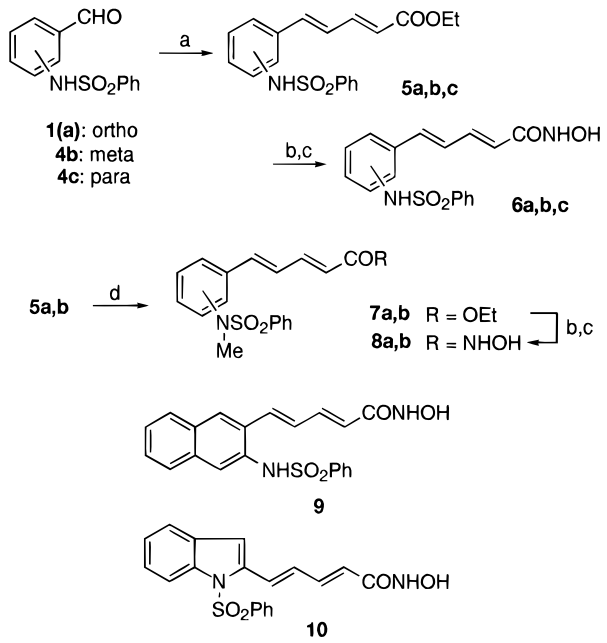
Chemistry. The target compounds were prepared using a straightforward process known in the literature. (6*Z*)- and (6*E*)-6-[2-[(phenylsulfonyl)amino]phenyl]hept-6-enohydroxamic acids were prepared using 2-[(phenylsulfonyl)amino]benzaldehyde (**1**) as a starting material (Scheme 1). Wittig reaction gave a mixture which was separated using silica gel, and the usual hydroxamic acid formation produced **2** and **3** in 13% and 17% (overall yield), respectively. Regioisomers, *i.e.*, (2*E*,4*E*)-[*o*-, *m*-, and *p*-[(phenylsulfonyl)amino]phenyl]penta-2,4-diene derivatives (**6a**−**c**) were obtained using [(2*E*)-3-(ethoxycarbonyl)prop-2-enyl]triphenylphosphonium bromide as the Wittig reagent in 38%, 15%, and 9% overall yield from **1**, **4b**, and **4c**, respectively (Scheme 2). Esterification of **5a**,**b** with diazomethane gave *N*-methyl

Scheme 1[a]



[a] (a) $[Ph_3P^+(CH_2)_5COOH]Br^-$, KO^{t}Bu; (b) SiO_2 separation; (c) HOSu, DCC, $NH_2OH\cdot HCl$.

Scheme 2[a]



[a] (a) $[Ph_3P^+CH_2CH{=}CHCOOH]Br^-$, KO^{t}Bu; (b) NaOH; (c) $(COCl)_2$, $NH_2OH\cdot HCl$; (d) CH_2N_2.

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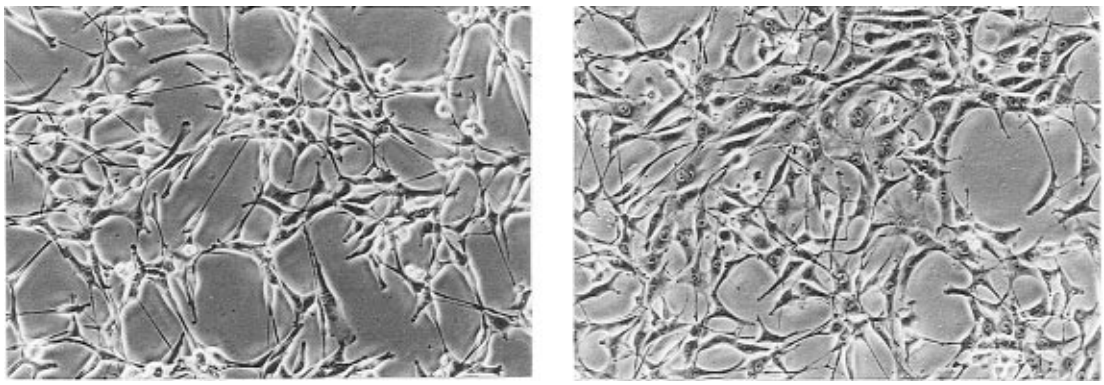
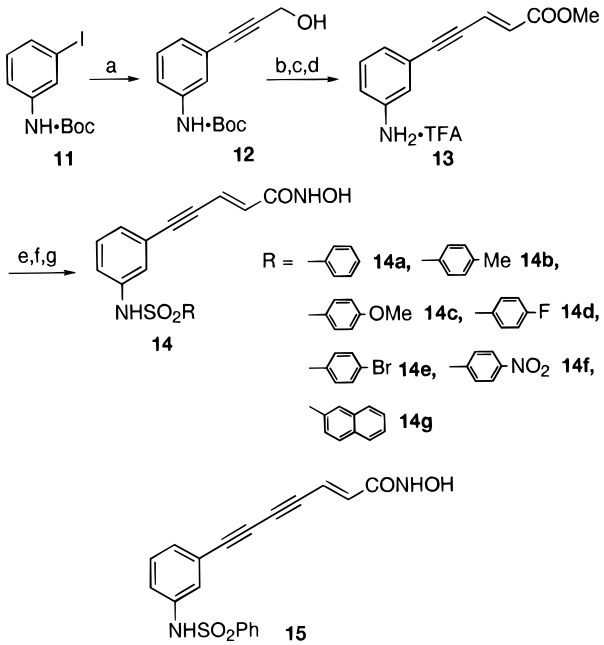


Figure 1. Induction of the flat morphology of DT cells by **14a**. DT cells (5×10^5) were seeded in Dulbecco's modified Eagle medium (DMEM) containing fetal calf serum (FCS) (10%) in 10 cm (diameter) dishes and cultured overnight and then incubated for 24 h without (left) or with (right) **14a** (1 μM). Magnification 25× (reproduced at 60% of original size).

Scheme 3[a]



[a] (a) $HC{\equiv}C{-}CH_2OH$, $Pd(PPh_3)_4$, CuI, Et_3N; (b) Swern oxidation; (c) $(EtO)_2P(O)CH_2COOMe$, NaH; (d) TFA; (e) RSO_2Cl; (f) NaOH; (g) $(COCl)_2$, $NH_2OH{\cdot}HCl$.

derivatives **7a**,**b**, and hydroxamic acid formation afforded **8a** and **8b** in 43% and 52% yield from **5a**,**b**, respectively. Naphthalene derivative **9** and indole derivative **10** were obtained in a manner similar to that of the phenyl derivative in 34% and 7% yield from starting aldehydes, respectively. The coupling reaction of aromatic iodide **11** with propargyl alcohol in the presence of Pd(0) catalyst gave **12** in 52% yield (Scheme 3). Swern oxidation followed by Horner−Emmons reaction and deprotection of the amino protective group (Boc) afforded trifluoroacetic acid salt **13** in 65% yield. Sulfonamide formation with typical sulfonyl chlorides followed by hydroxamic acid production gave the desired compounds **14a**−**g** in good yield. The diyne compound **15** was prepared in a manner similar to that of **14** using (tetrahydropyranyloxy)penta-2,4-diyne[10] in place of propargyl alcohol.

Biological Results and Discussion. As a result of research for biological mechanism, compound **2** was found to reverse the morphology of the cells transformed by *ras* oncogene *via* increased expression of transcription factor JunD.[11,12]

Table 1. *ras* Transformation Inhibition of Aromatic Conjugated Hydroxamic Acids

compound	MIC (μM)[a]	compound	MIC (μM)[a]
2	17	**14a**	0.040
3	4.3	**14b**	0.16
6a	1.6	**14c**	0.080
6b	0.30	**14d**	0.080
6c	1.6	**14e**	0.16
8a	0.80	**14f**	0.64
8b	0.60	**14g**	0.080
9	0.32	**15**	—[b]
10	0.40		

[a] The values show the minimum inhibitory concentration (MIC) for inhibition of transformation in *ras*-transfomed cells (ras/NIH3T3). [b] Not effective at the highest concentration tested (100 μM).

The double bond isomer (*E*-isomer, **3**) lowered its MIC value, and *E*-dienes **6a**−**c** and **8a,b** having conformationally straight and restricted structures showed improved activity (Table 1). Changing the phenyl nucleus to naphthalene **9** or indole **10** had no effect on the inhibitory activity. Among regio isomers, *meta*-substituted compound **6b** showed superior activity to *ortho* (**6a**) or *para* isomers (**6c**). *N*-Methylation (**8a,b**) afforded no significant improvement of activity over that of **6a,b**. Introduction of a triple bond to modify the structures to those having straighter and more rigid ones gave significantly enhanced activity as shown by compound **14a**. It reversed the phenotype of *ras*-transformed cells to the flat one at the concentration of 0.04 μM, and the effect was observed completely at 8 h after addition of **14a** to the medium, showing that **14a** acts directly on the cell to be flat and never selects resistant cells (Figure 1). It also suppresses anchorage-independent growth of *ras*-transformed cells. The synthesis of *ras*-mRNA does not decrease with treatment by **14a** at the concentration which can reverse the transformed morphology, showing that the induction of the flat morphology using **14a** is not caused by inhibition of *ras*-mRNA synthesis.[12] Its mode of action is to induce the gene for transcription factor JunD, which leads to interfere with *ras*-dependent transformation.[12]

The optimum length of the side chain is very strictly restricted, which is shown by the fact that the diyne derivative **17** completely loses its activity. On the basis of the above results, modification to improve the activity on the phenyl ring of the side chain of **14a** was tried and most of the products were found to be very potent inhibitors. Among them, **14c**, **14d**, and **14g** showed activity comparable to that of **14a**, and no notable effect

of substituents on the side chain phenyl ring was observed in these compounds.

In conclusion, we have reported the synthesis and inhibitory activity for *ras* transformation of aromatic sulfonamide hydroxamates, which induce a flat phenotype in K_i-*ras*-transformed NIH3T3 cells. Among these inhibitors, (2*E*)-5-[3-[(phenylsulfonyl)amino]phenyl]pent-2-en-4-ynohydroxamic acid (**14a**) proved to be the most potent inhibitor and was categorized as the first compound inducing genes with products that can interfere with *ras*-dependent transformation.

Acknowledgment. The authors gratefully acknowledge Dr. Hikaru Sonoda for helpful discussions.

Supporting Information Available: Experimental details with spectral data (14 pages). Ordering information is given on any current masthead page.

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(10) (Tetrahydropyranyloxy)pent-2,4-diyne was prepared from propargyl alcohol. THP−propargyl alcohol was subjected to Heck reaction using (*E*)-1,2-dichloroethene and treated with $^{n}Bu_4NF$ to remove hydrogen chloride, giving the above diyne in 24% overall yield.
(11) v-K_i-*ras*-transformed NIH3T3 (ras/NIH3T3) cells were grown in Dulbecco's modified minimum essential medium (D-MEM) supplemented with 10% fetal bovine serum. Inhibition of cell growth by the reported compounds was determined according to a method using MTT as a dye. The morphological change of the cells was observed under a microscope.
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