Niaziminin, a Thiocarbamate from the Leaves of *Moringa oleifera*, Holds a Strict Structural Requirement for Inhibition of Tumor-Promoter-Induced Epstein-Barr Virus Activation

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Abstract: Three known thiocarbamate (TC)- and isothiocyanate (ITC)-related compounds have been isolated from the leaves of *Moringa oleifera*, a traditional herb in southeast Asia, as inhibitors of tumor promoter teleocidin B-4-induced Epstein-Barr virus (EBV) activation in Raji cells. Interestingly, only niaziminin among 10 TCs including 8 synthetic ones showed considerable inhibition against EBV activation. The structure-activity relationships indicated that the presence of an acetoxy group at the 4′-position of niaziminin is important and indispensable for inhibition. On the other hand, among the ITC-related compounds, naturally occurring 4-[(4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl]ITC and commercially available allyl- and benzyl-ITC significantly inhibited activation, suggesting that the isothiocyano group is a critical structural factor for activity.

Key words: Anti-tumor promoter, niaziminin, Epstein-Barr virus, thiocarbamate, isothiocyanate, *Moringa oleifera*, Moringaceae.

Abbreviations:
ACA: 1′-acetoxychavicol acetate
EBV: Epstein-Barr virus
FITC: fluorescence isothiocyanate
ITC: isothiocyanate
NPC: nasopharyngeal carcinoma
TC: thiocarbamate

Introduction

Inhibition of the tumor promotion stage in the multistages of chemical carcinogenesis is one of the promising strategies for cancer chemoprevention (1). To more efficiently search for hopeful anti-tumor promoters from edible plants, we have been conducting a convenient *in vitro* assay, the inhibitory test of tumor promoter-induced Epstein-Barr virus (EBV) activation in Raji cells (2). Our recent screening tests of edible Thai plants for this activity verified that they have high potential in terms of a natural source of new types of cancer chemopreventers as compared with edible plants in Japan, most of which are common with those in western countries (3–5).

So far, activity-guiding separation of the extracts from Thai plants afforded some notable EBV activation inhibitors: 1′-acetoxychavicol acetate (ACA) [*Languas galanga* (L.) Stuntz, Zingiberaceae] (6–9), cardamonin [*Boesenbergia pandurata* (Roxb.) Schult, Zingiberaceae] (4), geranial [*Cymbopogon citratus* (A.DC.) Stapf, Gramineae] (10), curcumin (*Zingiber cassumunary* Roxb., Zingiberaceae) (10), glyceroglycolipids (*Citrus hystrix* DC., Rutaceae) (11), and chlorophyll-related compounds (*Neptunia oleracea* Lour., Leguminosae) (12, 13). The 50% inhibition concentration values (IC_{50}: 0.43–16 μM) of these compounds in the EBV assay are much lower than those of representative, naturally occurring chemopreventive agents such as β-carotene (IC_{50} = 30 μM), quercetin (IC_{50} = 23 μM), or (−)-epigallocatechin gallate (IC_{50} = 68 μM) (10). In terms of *in vivo* cancer preventive potential as well, ACA, for instance, showed powerful chemopreventive activities in 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced skin tumor promotion in 7,12-dimethylbenz[*a*]anthracene-initiated mice (7), 4-nitroquinoline 1-oxide-induced rat tongue carcinogenesis (8), and azoxymethane-induced rat colonic aberrant crypt foci formation (9).

The methanol extract from the leaves of *Moringa oleifera* (Moringaceae), used as a food item and concurrently as a folk medicine to cure wounds or stop bleeding in Thailand (14), was suggested to contain EBV activation inhibitors (4). Here we describe the isolation and identification of new types of possible anti-tumor promoters from *M. oleifera* as well as inhibitory properties of their synthetic or commercially available analogs for EBV activation to discuss the structure-activity relationships.

Materials and Methods

General remarks

Analytical instruments used were as follows: HPLC, HITACHI 655A-11; $[\alpha]_D$, JASCO DIP-4; UV, Shimadzu UV 200; IR, Shimadzu IR-435; MS, JEOL JMS-DX300; and NMR, Bruker ARX-500 (500 MHz, int. ref. TMS). In the case of compounds **1**, **2**, and **4–10**, there is a geometric isomerism (*E* and *Z*) along with the ester bond, and thus the signals distinguishable as *E* and *Z* are labeled as described below. A voucher of the title plant is on deposit with the herbarium of CLGC of Kasetsart University, Thailand; specimen no. S-087-L.

Chemicals and cells

Teleocidin B-4 was isolated from *Streptoveritcillium blastmyceticum* N A 34-17 as previously reported (15). High-titer early antigen (EA)-positive sera from nasopharyngeal carcinoma patients (NPC) for EBV assay and Raji cells were a kind gift from Dr. T. Ohsato of Health Sciences University of Hokkaido. Fluorescence isothiocyanate (FITC)-labeled anti-human IgG was obtained from Dako Co. Ltd (Glostrup, Denmark). Allyl-ITC (**12**) and benzyl-ITC (**13**) were purchased from nacalai tesque (Kyoto, Japan). Other chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan).

Isolation and identification of active constituents

The fresh leaves of *M. oleifera* (710 g) were extracted with methanol, and the extract was partitioned with a solvent mixture of *n*-hexane : methanol : water = 5 : 9 : 1. The active polar fraction (lower layer) was separated by silica gel (1 kg, Wakogel C-200, Wako Pure Chemical Industries, Osaka, Japan) column chromatography (0 – 15 % methanol in chloroform, 2.5 % stepwise) to give active 5.0 and 7.5 % methanol fractions followed by preparative high-performance liquid chromatography (HPLC) on μBondasphere C_{18} (Waters, Milford, MA, 19 × 150 mm; mobile phase: 45 % acetonitrile in water; flow rate: 7.0 ml/min; detection: 254 nm) to give three compounds; niaziminin (**1**, 175 mg, t_R = 16 min), niazimicin (**2**, 258 mg, t_R = 13 min), and 4-(4′-*O*-acetyl-α-L-rhamonsyloxy)-benzylisothioyanate (**3**, 80 mg, t_R = 20 min). Their spectral data were in good agreement with those reported previously (16, 17); **1**, $[\alpha]_D^{25}$: − 28.3° (*c* 0.46, $CHCl_3$) (17); **2**, $[\alpha]_D^{25}$: − 96.8° (*c* 0.48, $CHCl_3$) (17); **3**, $[\alpha]_D^{25}$: − 107.0° (*c* 0.84, $CHCl_3$) (16).

Preparation of synthetic thiocarbamates

2′- (**4**) *and 3′-mono-O-acetylniazimicin* (**5**): After heating niaziminin (**1**, 17.5 mg) in 33 % acetonitrile in water at 70 – 75 °C for 7 h, 2′-*O*- (**4**, 4.5 mg) and 3′-*O*-acetylniazimicin (**5**, 3.2 mg) were purified by HPLC (μBondasphere C_{18}, 19 × 150 mm; mobile phase: 30 % acetonitrile in water; flow rate: 7.0 ml/min; detection: 254 nm); **4**, $[\alpha]_D^{25}$: − 51.5° (*c* 0.41, $CHCl_3$); IR (CH_2Cl_2) cm^{-1}: ν_{max} = 3560 (br.), 3380, 1750, 1510, 1230, 1060, 1032; UV (CH_2Cl_2) nm (ε): λ_{max} = 247 (17,700); EI-MS (probe, 70 eV): *m/z* (rel. int.) = 399 (M^+, $C_{18}H_{25}NO_7S$, 5), 211 (42), 189 ($C_8H_{13}O_5$, 100), 171 ($C_8H_{11}O_4$, 74), 107 (82). ^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.13 (1H, d, *J* = 6.3 Hz, 6′-CH_3), 1.20 (1H, t, *J* = 7.1, OCH_2CH_3, *Z*), 1.25 (1H, t, *J* = 7.1 Hz, OCH_2CH_3, *E*), 207 (3H, s, OAc), 3.24 (1H, dt, *J* = 9.4, 4.7 Hz, 4′-H), 3.81 (1H, br.m, 3′-H), 4.24 (2H, d, *J* = 6.3 Hz, 7-H, *Z*), 4.38 (1H, q, *J* = 7.1 Hz, OCH_2CH_3, *Z*), 4.39 (1H, q, *J* = 7.1 Hz, OCH_2CH_3, *E*), 4.56 (2H, d, *J* = 6.3 Hz, 7-H, *E*), 5.06 (1H, dd, *J* = 3.6, 1.7 Hz, 2′-H), 5.07 (1H, d, 4.7 Hz, 3′-OH), 5.11 (1H, d, *J* = 4.7 Hz, 4′-OH), 5.43 (1H, br.s, 1′-H), 7.00 (2H, d, *J* = 8.6 Hz, 2,6-H, *E*), 7.01 (2H, *J* = 8.6 Hz, 2,6-H, *Z*), 7.17 (2H, d, *J* = 8.6 Hz, 3,5-H, *E*), and 7.22 (2H, d, *J* = 8.6 Hz, 3,5-H, *Z*). **5**; $[\alpha]_D^{25}$: − 101.9° (*c* 0.63, $CHCl_3$). IR (CH_2Cl_2) cm^{-1}: ν_{max} = 3560 (br.), 3380, 1750, 1510, 1230, 1060, 1032; UV (CH_2Cl_2) nm (ε): λ_{max} = 247 (17,700); EI-MS (probe, 70 eV): *m/z* (rel. int.) = 399 (M^+, $C_{18}H_{25}NO_7S$, 5), 211 (42), 189 ($C_8H_{13}O_5$, 100), 171 ($C_8H_{11}O_4$, 74), 107 (82); ^{1}H-NMR (500 MHz, DMSO-d_6): δ = 0.99 (3H, d, *J* = 5.9 Hz, 6′-CH_3), 1.21 (3H, t, *J* = 7.1 Hz, OCH_2CH_3, *Z*), 1.26 (3H, t, *J* = 7.1 Hz, OCH_2CH_3, *E*), 2.06 (3H, s, OAc), 3.49 (1H, dt, *J* = 9.6, 6.5 Hz, 4′-H), 3.57 (1H, m, 5′-H), 4.24 (2H, d, *J* = 6.1 Hz, 7-H, *Z*), 4.39 4H, q, *J* = 7.1 Hz, OCH_2CH_3, *E,Z*), 4.56 (2H, d, *J* = 5.9 Hz, 7-H, *E*), 4.90 (1H, dd, *J* = 9.6, 3.1 Hz, 3′-H), 5.12 (1H, d, *J* = 5.0 Hz, 4′-OH), 5.35 (1H, br.s, 1′-H), 5.37 (1H, d, *J* = 4.5 Hz, 2′-OH), 7.00 (4H, d, *J* = 8.2 Hz, 2,6-H, *E,Z*), 7.17 (2H, d, *J* = 8.2 Hz, 3,5-H, *E*), 7.22 (2H, d, *J* = 8.2 Hz, 3,5-H, *Z*), and 9.49 (1H, br.m, NH).

2′,3′- (**6**), *2′,4′-* (**7**), *and 3′,4′-di-O-acetylniazimicin* (**8**): Acetic anhydride (32 μl) was added to a niazimicin solution (58.9 mg in 0.5 ml pyridine), and left standing for 24 h at room temperature. After working up in the same manner as described above, the diacetate mixture was purified by HPLC (μBondasphere C_{18}, 19 × 150 mm; mobile phase: 40 % acetonitrile in water; flow rate: 7.0 ml/min; detection: 254 nm) to give **6** (3.4 mg), **7** (2.9 mg), and **8** (4.5 mg). **6**, $[\alpha]_D^{25}$: − 63.0° (*c* 0.21, $CHCl_3$); IR ($CHCl_3$): ν_{max} = 3400, 3000, 1740, 1510, 1220 cm^{-1}; UV (CH_2Cl_2) nm (ε): λ_{max} = 248 (14,900); EI-MS (probe, 70 eV): *m/z* (rel. int.) = 441 (M^+ $C_{20}H_{27}NO_8S$, 3), 231 ($C_{10}H_{10}O_6$, 79), 171 ($C_8H_{11}O_4$, 88), 153 ($C_8H_9O_3$, 55), 111 ($C_6H_7O_2$, 100); ^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.16 (1H, d, *J* = 6.2 Hz, 6′-CH_3), 1.20 (3H, t, *J* = 7.0 Hz, OCH_2CH_3, *Z*), 1.25 (3H, t, *J* = 7.0 Hz, OCH_2CH_3, *E*), 1.99 (3H, s, 3′-OAc), 2.10 (3H, s, 2′-OAc), 3.43 (1H, m, 4′-H), 3.69 (1H, qd, *J* = 9.3, 6.2 Hz, 5′-H), 4.25 (2H, d, *J* = 6.1 Hz, 7-H, *Z*), 4.39 (2H, q, *J* = 7.0 Hz, OCH_2CH_3, *Z*), 4.40 (2H, q, *J* = 7.0 Hz, OCH_2CH_3, *E*), 4.57 (2H, d, *J* = 6.0 Hz, 7-H, *E*), 5.07 (1H, dd, *J* = 9.9, 3.5 Hz, 3′-H), 5.24 (1H, br.m, 2′-H), 5.39 (1H, d, *J* = 6.4 Hz, 4′-OH), 5.54 (1H, br.s, 1′-H), 7.04 (2H, d, *J* = 8.5 Hz, 2,6-H, *E*), 7.06 (2H, *J* = 8.5 Hz, 2,6-H, *Z*), 7.19 (2H, d, *J* = 8.5 Hz, 3,5-H, *E*), 7.24 (2H, d, *J* = 8.5 Hz, 3,5-H, *Z*), and 9.54 (br.t, *J* = 5.9 Hz, NH). **7**, $[\alpha]_D^{25}$: − 46.2° (*c* 0.11, $CHCl_3$); IR ($CHCl_3$): ν_{max} = 3400, 3000, 1740, 1510, 1220, 1030 cm^{-1}; UV (CH_2Cl_2) nm (ε): λ_{max} = 248 (16,200); EI-MS (probe, 70 eV): *m/z* (rel. int.) = 441 (M^+ $C_{20}H_{27}NO_8S$, 5), 231 ($C_{10}H_{10}O_6$, 100), 171 ($C_8H_{11}O_4$, 67), 111 ($C_6H_7O_2$, 34), 85 (80); ^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.03 (3H, d, *J* = 6.2 Hz, 6′-CH_3), 1.20 (3H, t, *J* = 7.0 Hz, OCH_2CH_3, *Z*), 1.25 (3H, t, *J* = 7.0 Hz, OCH_2CH_3, *E*), 2.06 (3H, s, 4′-OAc), 2.11 (3H, s, 2′-OAc), 3.75 (1H, qd, *J* = 9.9, 6.2 Hz, 5′-H), 4.05 (1H, m, 3′-H), 4.24 (2H, d, *J* = 6.3 Hz, 7-H, *Z*), 4.39 4H, q, *J* = 7.0 Hz, OCH_2CH_3, *E,Z*), 4.57 (2H, d, *J* = 6.1 Hz, 7-H, *E*), 4.80 (1H, t, *J* = 9.9 Hz, 4′-H), 5.13 (1H, br.m, 2′-H), 5.42 (1H, d, *J* = 6.0 Hz, 3′-OH), 5.52 (1H, br.s, 1′-H), 7.03 (2H, d, *J* = 8.5 Hz, 2,6-H, *E*), 7.05 (2H, *J* = 8.5 Hz, 2,6-H, *Z*), 7.18 (2H, d, *J* = 8.5 Hz, 3,5-H, *E*), 7.23 (2H, d, *J* = 8.5 Hz, 3,5-H, *Z*), and 9.54 (br.t, *J* = 5.9 Hz, NH). **8**, $[\alpha]_D^{25}$: − 106.5° (*c* 0.27, $CHCl_3$); IR ($CHCl_3$): ν_{max} = 3400, 3000, 1740, 1510, 1220, 1025 cm^{-1}; UV ($CHCl_3$) nm (ε): λ_{max} = 248 (18,100); EI-MS (probe, 70 eV): *m/z* (rel. int.) = 441 (M^+ $C_{20}H_{27}NO_8S$, 5), 231 ($C_{10}H_{10}O_6$, 100), 171 ($C_8H_{11}O_4$, 90), 111 ($C_6H_7O_2$, 66), 85 (54). ^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.03 (3H, d, *J* = 6.1 Hz, 6′-CH_3), 1.20 (3H, t, *J* = 7.0 Hz, OCH_2CH_3, *Z*), 1.25 (3H, t, *J* = 7.0 Hz, OCH_2CH_3, *E*), 2.02 (3H, s, 3′-OAc), 2.07 (3H, s, 4′-OAc), 3.45 (1H, qd, *J* = 10.0, 6.1 Hz, 5′-H), 4.08 (1H, br.m, 2′-H), 4.24 (2H, d, *J* = 6.2 Hz, 7-H, *Z*), 4.39 (2H, d, *J* = 7.0 Hz, OCH_2CH_3), *Z*), 4.40 (2H, d, *J* = 7.0 Hz, OCH_2CH_3, *E*), 4.57 (2H, d, *J* = 6.0 Hz, 7-H, *E*), 5.06 (1H, t, *J* = 10.0 Hz, 4′-H), 5.09 (1H, dd, *J* = 10.0, 2.9 Hz, 3′-H), 5.45 (1H, br.s, 1′-H), 5.70 (1H, d, *J* = 5.1 Hz, 2′-OH), 7.04 (2H, d, *J* = 8.5 Hz, 2,6-H, *E*), 7.06 (2H, *J* = 8.5 Hz, 2,6-H, *Z*), 7.18 (2H, d, *J* = 8.5 Hz, 3,5-H, *E*), 7.23 (2H, d, *J* = 8.5 Hz, 3,5-H, *Z*), and 9.54 (1H, br.t, *J* = 5.7 Hz, NH).

2′,3′,4′-Tri-O-acetylniazimicin (**9**): Acetic anhydride (1 ml) was added to a niazimicin solution (23.9 mg in 1 ml pyridine), and left standing for 1 h at room temperature. The reaction was stopped by the addition of ice, and the mixture was partitioned between ethyl acetate and water. The ethyl acetate layer was dried over Na_2SO_4, and worked up using the same procedure as mentioned above. The mixture thus obtained

was purified by HPLC (μBondasphere C_{18}, 19 × 150 mm; mobile phase: 65% acetonitrile in water; flow rate: 7.0 ml/min; detection: 254 nm) to give **9** (14.5 mg); $[\alpha]_D^{25}$: 61.1° (*c* 0.36, $CHCl_3$); IR (CH_2Cl_2): ν_{max} = 3380, 1740, 1510, 1370, 1220, 1190, 1030, 1040 cm^{-1}; UV (CH_2Cl_2) nm (ε): λ_{max} = 246 (17,400); EI-MS (probe, 70 eV): *m/z* (rel. int.) = 483 (M^+ $C_{22}H_{29}NO_9S$, 3), 231 ($C_{10}H_{15}O_6$, 100), 171 ($C_8H_{11}O_4$, 44), 111 ($C_6H_7O_2$, 89), 85 (70); ^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.08 (1H, d, *J* = 6.2 Hz, 6′-CH_3), 1.21 (3H, t, *J* = 7.0 Hz, OCH_2CH_3, *Z*), 1.26 (3H, t, *J* = 7.1 Hz, OCH_2CH_3, *E*), 1.97 (3H, s, 3′-OAc), 2.04 (3H, s, 4′-OAc), 2.14 (3H, s, 2′-OAc), 3.91 (1H, qd, *J* = 10.0, 6.2 Hz, 5′-H), 4.24 (2H, d, *J* = 6.2 Hz, 7-H, *Z*), 4.38 (2H, q, *J* = 7.0 Hz, OCH_2CH_3, *Z*), 4.39 (2H, q, *J* = 7.1 Hz, OCH_2CH_3, *E*), 4.57 (2H, d, *J* = 6.1 Hz, 7-H, *E*), 4.96 (1H, t, *J* = 10.0 Hz, 4′-H), 5.27 (1H, dd, *J* = 10.0, 3.5 Hz, 3′-H), 5.30 (1H, dd, *J* = 3.5, 1.7 Hz, 2′-H), 5.62 (1H, br. s, 1′-H), 7.06 (2H, d, *J* = 8.6 Hz, 2,6-H, *E*), 7.07 (2H, *J* = 8.6 Hz, 2,6-H, *Z*), 7.19 (2H, d, *J* = 8.6 Hz, 3,5-H, *E*), 7.24 (2H, d, *J* = 8.6 Hz, 2,6-H, *Z*), and 9.52 (1H, br. m, NH).

O-Ethyl allylthiocarbamate (**10**) *and O-ethyl benzylthiocarbamate* (**11**): Allyl-ITC (**12**, 23.1 mg) or benzyl-ITC (**13**, 27.5 mg) was dissolved in 0.25% sodium ethoxide in absolute ethanol, and left standing at room temperature for 30 min. After adjusting the pH to 5 with 50% acetic acid, the reaction mixture was partitioned between $CHCl_3$ and water. The $CHCl_3$ layer was dried over Na_2SO_4 and purified on preparative TLC (*n*-hexane : ethyl acetate = 10 : 1) to give *O*-ethyl allylTC (**10**, 20.0 mg) or *O*-ethyl benzylTC (**11**, 30.4 mg), respectively. **10**, IR (CH_2Cl_2): ν_{max} = 3390, 1510, 1390, 1320, 1190 cm^{-1}; UV (CH_2Cl_2) nm (ε): λ_{max} 246 (14,100); ^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.32 (3H, t, *J* = 7.1 Hz, OCH_2CH_3, *E*), 1.36 (3H, t, *J* = 7.1 Hz, OCH_2CH_3, *Z*), 3.89 (2H, m, CH-CH_2, *Z*), 4.20 (2H, m, CH-CH_2, *E*), 4.49 (2H, q, *J* = 7.1 Hz, OCH_2CH_3, *E*), 4.56 (2H, q, *J* = 7.1 Hz, OCH_2CH_3, *Z*), 5.17–5.29 (4H, m, CH_2 = CH, *E,Z*), 5.80 (1H, m, CH_2=CH, *Z*), 5.89 (1H, m, CH_2=CH, *E*), 6.26 (1H, br. s, NH), and 6.64 (1H, br. s, NH); **11**, IR (CH_2Cl_2): ν_{max} = 3400, 1510, 1390, 1190 cm^{-1}; UV (CH_2Cl_2) nm (ε): λ_{max} = 246 (20,400); ^{1}H-NMR (500 MHz, DMSO-d_6: δ = 1.26 (3H, t, *J* = 7.2 Hz, OCH_2CH_3, *E*), 1.33 (3H, t, *J* = 7.2 Hz, OCH_2CH_3, *Z*), 4.45 (2H, d, *J* = 5.8 Hz, CH_2, *Z*), 4.52 (2H, q, *J* = 7.2 Hz, OCH_2CH_3, *E*), 4.57 (2H, q, *J* = 7.2 Hz, OCH_2CH_3, *Z*), 4.75 (2H, d, *J* = 5.7 Hz, CH_2, *E*), and 7.27–7.37 (5H, m, phenyl).

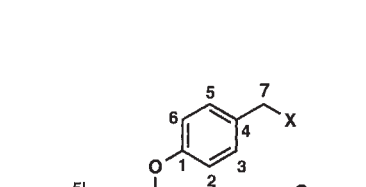
EBV activation test

The EBV activation test was done as previously reported (2). Human B-lymphoblastoid cells, Raji, were incubated in 1 ml of RPMI 1640 medium (supplemented with 10% fetal calf serum) containing *n*-butyric acid (3 mM), teleocidin B-4 (50 nM), and the test compound at 37 °C under 5% CO_2 atmosphere for 48 h. After the cytotoxicity of each test sample was measured by staining the cells with trypan blue, smears were made from the cell suspension. Then, EA-induced cells were stained by a conventional indirect immunofluorescence technique with high-titer EA-positive sera from NPC patients followed by FITC-labeled IgG. The rate of EA-induced cells was compared to that of a control experiment using only *n*-butyric acid and teleocidin B-4, in which the rate of EA-induced cells was ordinarily around 50%. The IC_{50} value of β-carotene, a representative chemopreventer, is 30 μM in this assay.

Results and Discussion

The leaves of *Moringa oleifera* (Moringaceae) were extracted with methanol at room temperature. The active principles were traced by using teleocidin B-4-induced Epstein-Barr virus (EBV) activation test in Raji cells; a short-term assay estimating anti-tumor promoting activity *in vitro*. By repeated column chromatography, two known TCs, niaziminin (**1**) and niazimicin (**2**), and a known ITC, 4-[4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl]isothiocyanate (**3**) (Fig. **1**), were isolated. Their structures were identified by comparison of their spectral data with those previously reported (16, 17). These TCs (**1**, **2**) and ITC (**3**) have been reported as hypotensive (16) and antibiotic (17) agents, respectively.

During the isolation procedure, **1** was found to be convertible to its isomers 2′-*O*-acetylniazimicin (**4**) or 3′-*O*-acetylniazimicin (**5**) (Fig. **1**) on heating in aqueous solutions through the acyl migration. To investigate the structure-activity relationships of niazimicin-related compounds, 2′,3′- (**6**), 2′,4′- (**7**), 3′,4′-di-*O*-acetylniazimicins (**8**), and 2′,3′,4′-tri-*O*-acetylniazimicin (**9**) (Fig. **1**) were prepared by usual acetylation. In addition, *O*-ethyl allylTC (**10**) and *O*-ethyl benzyl ITC (**11**) were obtained by the base catalyzed ethanolysis of allyl ITC (**12**) and benzyl ITC (**13**), respectively (Fig. **1**).



	R_1	R_2	R_3	X	IC_{50} (μM)
1	H	H	Ac	TC	1.3
2	H	H	H	TC	> 250
3	H	H	Ac	ITC	1.0
4	Ac	H	H	TC	120
5	H	Ac	H	TC	140
6	Ac	Ac	H	TC	> 250
7	Ac	H	Ac	TC	190
8	H	Ac	Ac	TC	190
9	Ac	Ac	Ac	TC	150

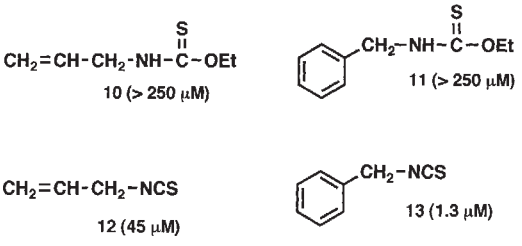


Fig. 1 Structures of thiocarbamates and isothiocyanates. Compounds **1**–**3** have been isolated from the leaves of *Moringa oleifera*, **4**–**11** were chemically synthesized, and **12** and **13** were commercially obtained. The values in parenthesis indicate the IC_{50} in the EBV activation test.

Niaziminin (**1**) showed a potent inhibition of EBV activation in a concentration-dependent manner (IC_{50} = 1.3 μM) (Figs. **1** and **2**). This inhibitory potential is comparable to that of ACA (IC_{50} = 1.3 μM), a prominent cancer chemopreventer in mouse skin, rat tongue and colon as reported previously (7–9). Therefore, **1** is anticipated to have cancer preventive activity in the rodent models.

Quite interestingly, niazimicin (**2**), an analogous compound to **1** lacking the acetyl group in the sugar moiety, exhibited no inhibitory activity (Figs. **1** and **2**). Also, two isomers of **1**, bearing the acetoxy group either at the 2′- (**4**) or 3′-position (**5**), showed little activity (Figs. **1** and **2**). These results clearly indicate that an acetoxy group in niazimicin-related compounds should be present at the 4′-position for inhibition of EBV activation. Furthermore, 2′,4′- (**7**), 3′,4′-di-*O*-acetylniazimicin (**8**), and 2′,3′,4′-tri-*O*-acetylniazimicin (**9**) were inactive (Figs. **1** and **2**). Thus, insertion of additional acetoxy group(s) to the 2′- and/or 3′-position results in loss of the activity of **1**. In addition, TCs (**10**, **11**) showed no inhibitory potential (Figs. **1** and **2**). Their inactivity is probably not due to a lack of the hydrophilic sugar moiety because all of the analogs (**2**, **4**–**9**) of **1** are inactive. Overall, only niaziminin (**1**) among all TCs tested remarkably inhibited EBV activation. We therefore conclude that one acetoxy group is required to occur at the 4′-position of the sugar moiety of niazimicin and concurrently no further acetoxy groups are allowed to occupy any other positions of the sugar moiety for activity exhibition. Such a strict structural requirement reminds us of the specific target(s) of niaziminin being involved in the action mechnaisms of EBV activation, and thus deserve further mechanistic studies.

It is noticeable that naturally-occurring ITC **3** (IC_{50} = 1.0 μM) also potently inhibited EBV activation (Figs. **1** and **2**). However, results suggest that the sugar moiety of **3** plays no substantial role in inhibition because benzyl-ITC (**13**) showed similar inhibition (IC_{50} = 1.3 μM). Accordingly, the ITC group in **3** or **13** might be a critical and minimal structural factor for activity exhibition. Allyl-ITC (**12**) showed relatively weak activity (IC_{50} = 45 μM) (Figs. **1** and **2**), possibly because the allyl group in **12** may contribute less than the benzyl group in **3** or **13** to cellular uptake on account of its lesser hydrophobicity.

ITCs, formed from glucosinolates by mirosinase in plants, are known to occur widely in the Cruciferae, e.g., cabbage, cauliflower or broccoli. ITCs have so far attracted the attention of researchers because of their inducibility of xenobiotic-metabolizing, phase II enzymes such as quinone reductase or glutathione *S*-transferase in experimental rodents. It is well-known that ITCs exhibit anti-carcinogenic actions in several animal models in which some carcinogens are given to rats or mice on the initiation phase (18). However, modifying effects of phase II enzyme inducers in the post-initiation or promotion phase remain to be addressed. The most distinct chemical characteristic of the ITC group is the electron-deficiency of the carbon atom (-N=C=S) (19), which, thus, is considered to attack a wide range of nucleophiles such as the OH and SH groups of macromolecules, especially those of proteins in biological systems (19). It is reasonable to assume that such reactions could be non-specific to the target molecules. This hypothesis could be supported by the data that all ITCs (**3**, **12**, **13**) examined in the present study considerably inhibited EBV activation probably because of cytotoxic or cytostatic interactions of the ITC group with cellular components. Thus, it is tempting to speculate that ITCs may not be appropriate

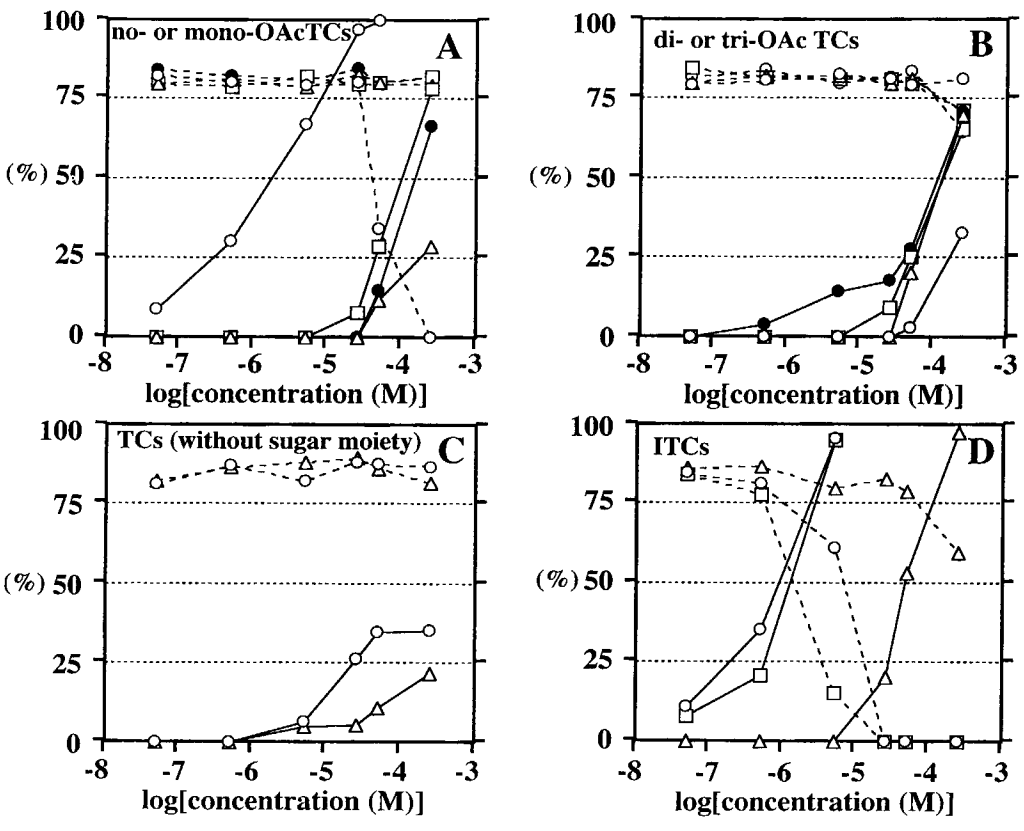


Fig. 2 Inhibitory activities of thiocarbamates and isothiocyanates toward EBV activation. Raji cells were incubated in 1 ml of RPMI 1640 medium containing *n*-butyric acid (3 mM), teleocidin B-4 (50 nM), and the test compound at 37 °C under 5 % CO_2 atmosphere for 48 hrs. Each experiment was done in duplicate, and the mean values are shown. Solid and break lines indicate EBV activation inhibition (%) and cell viability (%), respectively. A: **1** (○); **2** (△), **4** (□), **5** (●). B: **6** (○); **7** (△); **8** (□); **9** (●). C: **10** (○); **11** (△). D: **3** (○); **12** (△); **13** (□). TC, thiocarbamate; ITC, isothiocyanate; OAc, acetoxy group.

cancer preventive agents in humans though it is meaningful to note that the risk/benefit ratio should be regulated by the dose, target population (high risk or general), or timing of supplementation (initiation or post-initiation) in clinical trials.

In conclusion, niaziminin (**1**), the only active TC-related compound in the present study, is an interesting and useful tool for further cancer chemopreventive studies. As mentioned above, the structural requirement of **1** for activity is strikingly strict, i.e., the only one acetyl group should be present compulsorily at the oxygen of the 4′-position and yet no further acetyl groups should be attached to the remainnig hydroxy groups of the sugar moiety of **1** (Fig. **3**). Such chemical characteristics may provide clues to reveal the action mechanisms by which EBV is activated by tumor promoters.

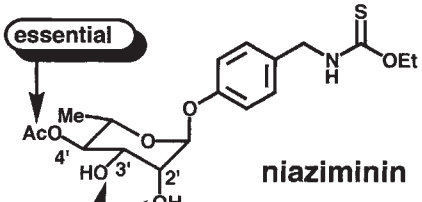


Fig. 3 Structure-activity relationships of niaziminin for the inhibition of EBV activation.

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