VOL. 54 NO. 10, OCT. 2001 THE JOURNAL OF ANTIBIOTICS pp. 827 — 830

YUA001, a Novel Aldose Reductase Inhibitor Isolated from Alkalophilic *Corynebacterium* sp. YUA25

II. Chemical Modification and Biological Activity

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(Received for publication May 22, 2001)

A series of novel *N*-substituted tyramine (2-*p*-hydroxyphenylethylamine) derivatives (**1**~**11**) were synthesized and evaluated for their inhibitory activity against pig kidney aldose reductase (EC 1, 1, 1, 21). Of these compounds, *N*-2-*p*-hydroxyphenylethyl maleamic acid (**10**) exhibits the strongest aldose reductase inhibitory activity, which is 22 times more potent than that of YUA001[1].

Aldose reductase (AR) (EC 1, 1, 1, 21) is an NADPH-specific aldehyde reductase to catalyze the conversion of glucose to sorbitol in the polyol pathway[2], and known to be involved in the onset of secondary diabetic complications such as cataract, neuropathy, retinopathy and nephropathy[3~5]. In a previous paper[1], BAHN *et al.* reported a novel aldose reductase inhibitior, YUA001, discovered from alkalophilic *Corynebacterium* sp. YUA25 to be identified as *N*-2-methylbutanoyl tyramine. Its inhibitory activity was not quite enough when compared to previously reported inhibitors.

So far many aldose reductase inhibitors (ARIs) have been developed to prevent those complications[6~8] mentioned above. Most of them possess lipophilic aromatic rings to interact with the side chains of aromatic amino acids, and ionizable groups such as carboxylate to be anchored to the positively charged binding region in the active site of AR[9,10]. YUA001 has aryl and amide group like other known ARIs, such as tolrestat, zopolrestat, alrestatin, *etc.*

Therefore we synthesized several derivatives (**1**~**11**) based on tyramine (2-*p*-hydroxyphenylethylamine) framework to enhance the AR inhibitory activity of YUA001. Through the chemical modifications of it, we found out that *N*-2-*p*-hydroxyphenylethyl maleamic acid (**10**) to have a potent AR inhibitory activity.

The present paper describes the chemical modifications, structural analyses and biological activities of the derivatives of YUA001 (**1**~**11**).

Materials and Methods

O.D. at 340 nm was recorded with Shimadzu UV1201 spectrophotometer. ^{1}H-NMR spectra were determined on a Varian Mercury 300 MHz, and ESI-MS (Electrospray Ionization Mass) on Fisons VG Platform.

Assay of Aldose Reductase Activity

The preparation of AR and *in vitro* AR inhibition assays were conducted according to the method in the previous publication[1]. Tolrestat and YUA001 were used as positive control.

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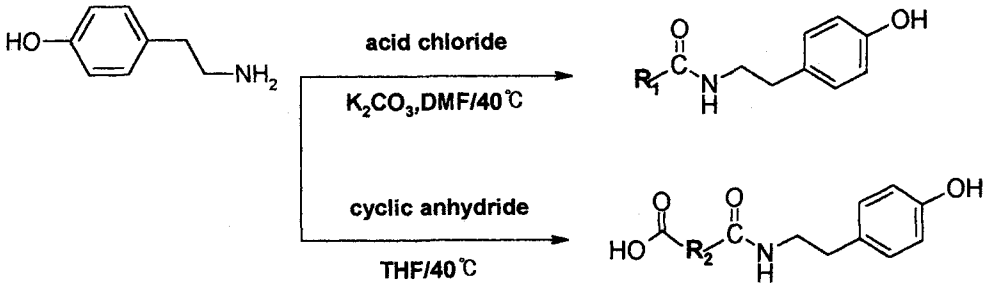
Fig. 1. Synthesis of *N*-substituted tyramine.



1 R_1 = -CH_3
2 R_1 = -$(CH_2)_3CH_3$
3 R_1 = -$(CH_2)_6CH_3$
4 R_1 = -$(CH_2)_8CH_3$
5 R_1 = -$(CH_2)_{10}CH_3$
6 R_1 = -$(CH_2)_{14}CH_3$

7 R_1 = 
8 R_1 = 

9 R_2 = -CH_2CH_2-
10 R_2 = -CH=CH-
11 R_2 = 

Preparation of *N*-substituted Tyramine Derivatives (**1**~**11**)

Tyramine framework was modified as follows (Fig. 1.).

N-Ethanoyl Tyramine (**1**)

Ethanoyl chloride (0.156 ml, 2.187 mmol) was added dropwise to a solution of tyramine (300 mg, 2.187 mmol) and K_2CO_3 (377 mg, 2.73 mmol) in DMF (30 ml) at 40°C. The mixture was stirred for 8 hours, poured into H_2O, and washed several times with EtOAc. The aqueous layer was concentrated and chromatographed on silica gel ($CHCl_3$: MeOH=4 : 1) to give **1** (213mg, 71%): ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 7.21 (2H, d, Ar-*H*), δ 7.03 (2H, d, Ar-*H*), δ 3.26 (2H, m, NHC$H_2$$CH_2$Ar), δ 2.72 (2H, t, NHC$H_2$$(CH_2)$Ar), δ 2.79 (3H, s, NHCH_3); ESI-MS *m/z* 179 (M^+).

N-Pentanoyl Tyramine (**2**)

The procedure was same as that of **1**. The mixture of pentanoyl chloride (0.259 ml, 2.187 mmol), tyramine (300 mg, 2.187 mmol) and K_2CO_3 (377 mg, 2.73 mmol) in DMF (30 ml) gave **2** (222 mg, 74%): ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 6.97 (2H, d, Ar-*H*), δ 6.68 (2H, d, Ar-*H*), δ 3.18 (2H, m, NHC$H_2$$CH_2$Ar), δ 2.56 (2H, t, NHC$H_2$$(CH_2)$Ar), δ 2.00 (2H, t, COC$H_2$$(CH_2)_2CH_3$), δ 1.45 (2H, t, COC$H_2$$(CH_2)_2CH_3$), δ 1.24 (2H, t, $CO(CH_2)_2CH_2CH_3$), δ 0.82 (3H, t, $CO(CH_2)_3CH_3$); ESI-MS *m/z* 221 (M^+).

N-Octanoyl Tyramine (**3**)

The procedure was same as that of **1**. The mixture of octanoyl chloride (0.374 ml, 2.187 mmol), tyramine (300 mg, 2.187 mmol) and K_2CO_3 (377 mg, 2.73 mmol) in DMF (30 ml) gave **3** (135 mg, 45%): ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.98 (2H, d, Ar-*H*), δ 6.51 (2H, d, Ar-*H*), δ 3.48 (2H, m, NHC$H_2$$CH_2$Ar), δ 2.79 (2H, t, NHCH_2CH_2Ar), δ 2.15 (2H, t, COC$H_2$$(CH_2)_5CH_3$), δ 1.26 (10H, br, $COCH_2(CH_2)_5CH_3$), δ 0.88 (3H, t, $CO(CH_2)_6CH_3$); ESI-MS *m/z* 263 (M^+).

N-Decanoyl Tyramine (**4**)

Decanoyl chloride (0.455 ml, 2.187 mmol) was added dropwise to a solution of tyramine (300 mg, 2.187 mmol) and K_2CO_3 (377 mg, 2.73 mmol) in DMF (30 ml) at room temperature. The mixture was stirred for 8 hours, poured into H_2O, and extracted several times with EtOAc. The combined extracts were dried over $MgSO_4$ and concentrated. The residual oil was chromatographed on silica gel ($CHCl_3$: MeOH=9 : 1) and recrystallized from ether to give **4** (171 mg, 57%): ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.01 (2H, d, Ar-*H*), δ 6.81 (2H, d, Ar-*H*), δ 3.48 (2H, m, NHC$H_2$$CH_2$Ar), δ 2.74 (2H, t, NHCH_2CH_2Ar), δ 2.16 (2H, t, COC$H_2$$(CH_2)_7CH_3$), δ 1.26 (14H, br, $COCH_2(CH_2)_7CH_3$), δ 0.88 (3H, t, $CO(CH_2)_8CH_3$); ESI-MS *m/z* 291 (M^+).

N-Dodecanoyl Tyramine (**5**)

The procedure was same as that of **4**. The mixture of dodecanoyl chloride (0.507 ml, 2.187 mmol), tyramine (300 mg, 2.187 mmol) and K_2CO_3 (377 mg, 2.73 mmol) in DMF (30 ml) gave **5** (222 mg, 74%): ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.01 (2H, d, Ar-*H*), δ 6.80 (2H, d, Ar-*H*), δ 3.45 (2H, m, $NHCH_2CH_2Ar$), δ 2.74 (2H, t, $NHCH_2CH_2Ar$), δ 2.17 (2H, t, $COCH_2(CH_2)_9CH_3$), δ 1.27 (18H, br, $COCH_2(CH_2)_9CH_3$), δ 0.88 (3H, t, $CO(CH_2)_{10}CH_3$); ESI-MS *m/z* 319 (M^+).

N-Hexadecanoyl Tyramine (**6**)

The procedure was same as that of **4**. The mixture of hexadecanoyl chloride (0.666 ml, 2.187 mmol), tyramine (300 mg, 2.187 mmol) and K_2CO_3 (377 mg, 2.73 mmol) in DMF (30 ml) gave **6** (267 mg, 89%): ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.03 (2H, d, Ar-*H*), δ 6.81 (2H, d, Ar-*H*), δ 3.45 (2H, m, $NHCH_2CH_2Ar$), δ 2.72 (2H, t, $NHCH_2CH_2Ar$), δ 2.18 (2H, t, $COCH_2(CH_2)_{13}CH_3$), δ 1.26 (26H, br, $COCH_2(CH_2)_{13}CH_3$), δ 0.86 (3H, t, $CO(CH_2)_{14}CH_3$); ESI-MS *m/z* 375 (M^+).

N-Benzoyl Tyramine (**7**)

The procedure was same as that of **4**. The mixture of benzoyl chloride (0.256 ml, 2.187 mmol), tyramine (300 mg, 2.187 mmol) and K_2CO_3 (377 mg, 2.73 mmol) in DMF (30 ml) gave **7** (168 mg, 56%): ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.79 (2H, d, CO*Ph*), δ 7.48 (3H, m, CO*Ph*), δ 7.03 (2H, d, Ar-*H*), δ 6.68 (2H, d, Ar-*H*), δ 3.42 (2H, m, $NHCH_2CH_2Ar$), δ 2.72 (2H, t, $NHCH_2CH_2Ar$); ESI-MS *m/z* 241 (M^+).

N-3,4,5-Trimethoxy Benzoyl Tyramine (**8**)

The procedure was same as that of **4**. The mixture of trimethoxy benzoyl chloride (0.156 ml, 2.187 mmol), tyramine (300 mg, 2.187 mmol) and K_2CO_3 (377 mg, 2.73 mmol) in DMF (30 ml) gave **7** (243 mg, 81%): ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 7.15 (2H, d, Ar-*H*), δ 7.02 (2H, d, Ar-*H*), δ 6.70 (2H, d, Ar-*H*), δ 3.81 (6H, s, *m*-OCH_3), δ 3.70 (3H, s, *p*-OCH_3), δ 3.40 (2H, m, $NHCH_2CH_2Ar$), δ 2.72 (2H, t, $NHCH_2CH_2Ar$); ESI-MS *m/z* 283 (M^+).

N-2-*p*-Hydroxyphenylethyl Succinamic Acid (**9**)

Succinic anhydride (262 mg, 2.622 mmol) was added to a suspension of tyramine (300 mg, 2.187 mmol) in acetonitrile (30 ml) at room temperature. The mixture was stirred for 10 hours in 40°C and concentrated. The residual oil was chromatographed on silica gel ($CHCl_3$: MeOH= 4 : 1) and recrystallized from acetonitrile to give **9** (123 mg, 41%): ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 6.96 (2H, d, Ar-*H*), δ 6.68 (2H, d, Ar-*H*), δ 3.19 (2H, m, $NHCH_2CH_2Ar$), δ 2.54 (2H, t, $NHCH_2CH_2Ar$), δ 2.43 (2H, t, $COCH_2CH_2COOH$), δ 2.39 (2H, t, $COCH_2CH_2COOH$); ESI-MS *m/z* 237 (M^+).

N-2-*p*-Hydroxyphenylethyl Maleamic Acid (**10**)

The procedure was same as that of **9**. The mixture of maleic anhydride (256 mg, 2.622 mmol) and tyramine (300 mg, 2.187 mmol) in acetonitrile (30 ml) gave **10** (144 mg, 48%): ^{1}H-NMR (DMSO-d_6, 300 MHz), δ 7.01 (2H, d, Ar-*H*), δ 6.68 (2H, d, Ar-*H*), δ 6.39 (1H, t, $COCH$=CHCOOH), δ 6.24 (1H, t, COCH=CHCOOH), δ 3.35 (2H, m, $NHCH_2CH_2Ar$), δ 2.66 (2H, t, $NHCH_2CH_2Ar$); ESI-MS *m/z* 235 (M^+).

N-2-*p*-Hydroxyphenylethyl Phthalamic Acid (**11**)

The procedure was same as that of **9**. The mixture of phthalic anhydride (390 mg, 2.622 mmol) and tyramine (300 mg, 2.187 mmol) in acetonitrile (30 ml) was chromatographed on silica gel ($CHCl_3$: MeOH=4 : 1) and recrystallized from acetone to give **11** (120 mg, 40%): ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 7.83 (4H, s, CO*Ph*COOH), δ 6.97 (2H, d, Ar-*H*), δ 6.63 (2H, d, Ar-*H*), δ 3.74 (2H, m, $NHCH_2CH_2Ar$), δ 2.79 (2H, t, $NHCH_2CH_2Ar$); ESI-MS *m/z* 285 (M^+).

Results

N-Substituted tyramine derivatives (**1**~**11**) were synthesized and evaluated for their inhibitory activities against pig kidney AR in a spectrometric assay with DL-glyceraldehyde as the substrate and NADPH as the cofactor. Among them, *N*-2-*p*-hydroxyphenylethyl maleamic acid (**10**) showed the strongest AR inhibitory activity with an IC_{50} value of 8.04×10^{-5} M, which is much lower than 1.80×10^{-3} M of YUA001 (Table 1).

Disussion

We were interested in the amide moiety of YUA001, which might be the essential portion to have AR inhibitory activity, and modified it with the elongation of aliphatic chain (**1**~**6**) as well as introducing the aromatic rings (**7**~**8**) or terminal carboxyl acids (**9**~**11**). All the derivatives (**1**~**11**) were found to have stronger activity than that of YUA001, but the chain length of acyl moiety did not affect their inhibitory activity significantly,

Table 1. Inhibitory activities against pig kidney aldose reductase of derivatives of YUA001 (**1**~**11**).

Inhibition	IC_{50}value(M)[a]
YUA001	1.8×10^{-3}
N-ethanoyl tyramine (**1**)	1.6×10^{-4}
N-pentanoyl tyramine (**2**)	1.3×10^{-4}
N-octanoyl tyramine (**3**)	1.2×10^{-4}
N-decanoyl tyramine (**4**)	1.1×10^{-4}
N-dodecanoyl tyramine (**5**)	1.1×10^{-4}
N-hexadecanoyl tyramine (**6**)	1.5×10^{-4}
N-benzoyl tyramine (**7**)	1.6×10^{-4}
N-3,4,5-trimethoxy benzoyl tyramine (**8**)	1.0×10^{-4}
N-2-*p*-hydroxyphenylethyl succinamic acid (**9**)	3.9×10^{-4}
N-2-*p*-hydroxyphenylethyl maleamic acid (**10**)	8.0×10^{-5}
N-2-*p*-hydroxyphenylethyl phthalamic acid (**11**)	3.4×10^{-4}
Tolrestat[b]	1.6×10^{-5}

[a] Evaluted *in vitro* against pig kidney aldose reductase

[b] *N*-[[6-Methoxy-5-(trifluoromethyl)-1-naphthalenyl]thioxomethyl]-N-methylglycine

as shown in Table 1. In these compounds, *N*-2-*p*-hydroxyphenylethyl maleamic acid (**10**) exhibited the most potent activity, which is comparable to that of tolrestat.

Further studies to find out the inhibitory mechanism of these derivatives (**1**~**11**) and enhance their activities are underway.

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