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Synthesis and Biological Activities of Tyroscherin Analogs

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The synthesis and biological evaluation of natural products and their analogs have been an important subject for many medicinal chemists in order to determine the pharmacophores of their activity and to search for a new chemical entity with more potent biological activity and less toxicity.[1] One such natural product is the alkaloid tyroscherin, which was isolated in 2004 from the mycelium of *Pseudallescheria* sp. as a potent and selective inhibitor of the insulin-like growth factor-1 (IGF-1)-induced growth of MCF-7 human breast cancer cells.[2] Up to now, the origin of the activity and the selectivity of the growth factor IGF-1 has not yet been disclosed.

The structure of tyroscherin features a long alkyl chain with *anti* amino alcohol at C2 and C3 with phenol at C1 originating from tryptophan. The long alkyl chain bears one *E*-olefin with two methyl groups at C8 and C10 (Figure 1). The configurations of all four stereocenters were identified by the total synthesis of the natural product tyroscherin by the Watanabe group.[3] Additional syntheses were reported by Maier and Crews.[4] Recently, the Crews group communicated the synthesis of several epimers with different stereochemistries of all four stereocenters located at the backbone chain and their biological activities.[5] What they found was a stereochemical tolerance for cytotoxic activity, which is quite unusual in most cases with spacially well-defined narrow phamacophores. They also tested one analog without a hydroxyl group in the phenyl ring of tyroscherin.[5] However, more analogs are needed to draw a solid conclusion as how important the hydroxy group at the phenyl ring is for its biological activity. In most cases changing the substituents at the phenyl ring alters its biological activity which is one routine to determine the pharmacophore and to design a better entity.[6]

All of the synthetic methods reported thus far have had more than one drawback, with difficulties obtaining analogs of the natural product tyroscherin with diverse substituents on the *para*-position of the phenyl ring. Substituted phenylalanine or its analogs have been required as a starting material with several reaction steps including the addition of alkyl chains and methylation of amine. Recently we reported a new synthetic method so called alkylative aziridine ring opening of *N*-methylaziridinium ion[7,8] and the formal synthesis of tyroscherin.[8a] This method allows us to afford an easy access for the preparation of tyroscherin analogs with diverse substituents on the *para*-position of the phenyl ring by using several different aryl nucleophiles on *N*-methylaziridinium ion[8] bearing a part of alkyl chain backbone. We presently describe the synthesis and biological activity of tyroscherin analogs with various substituents at the *para*-position of the phenyl ring such as H, Me, OMe and F. The biological evaluation of these analogs may provide implications of the cytotoxicity of tyroscherin.

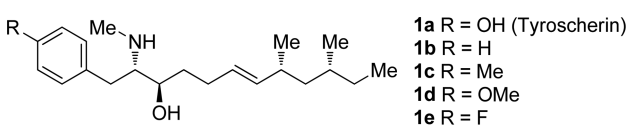
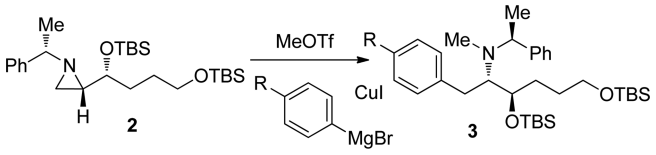


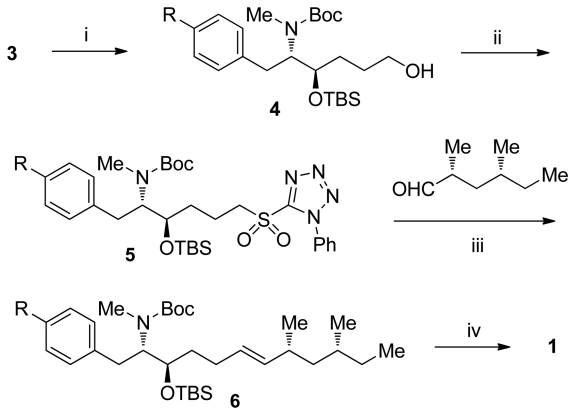
Figure 1. Structure of tyroscherin (**1a**) and its analogs (**1b-1e**).

All of the designed compounds were synthesized from bis-TBS protected 4-(aziridine-2-yl)butan-1,4-diol (**2**) elaborated from the commercially available (2*S*)-aziridine-2-carboxylate.[8b] This was subjected to the formation of *N*-methylaziridinium ions by methyl triflate[7] and the subsequent "alkylative aziridine-ring opening" of this aziridinium ion. The aziridine ring opening was executed not only by simple phenylmagnesium bromide but by arylmagnesium bromides with various substituents at the *para*-position of the aryl ring including H, Me, OMe and F for the compounds **3b**, **3c**, **3d**, and **3e**, which proceeded smoothly with 83, 65, 85 and 87% yields, respectively (Scheme 1).

The acyclic compound (**3**) deduced from ring opening was treated with HF to remove the terminal TBS group followed by hydrogenolysis to remove the benzyl group at the nitrogen in the presence of $(Boc)_2O$ to afford *N*-Boc-protected amino alcohol (**4**) (Scheme 2). The terminal alcohol was then converted to 1-phenyl-1*H*-tetrazole-5-ylsulfone (**5**) under Mitsunobu reaction conditions which was ready to be reacted with the (2*R*,4*R*)-2,4-dimethylhexanal for Julia coupling. The coupling reactions between the common dimethylhexanal



Scheme 1. Methylative and alkylative ring openings of **2** by MeOTf and various arylmagnesium bromides with CuI in dioxane (R = H (83%), R = Me (65%), R = OMe (85%), R = F (87%)).



Scheme 2. (i) (a) HF·Et_3N, Pyridine, rt; (b) $Pd(OH)_2$, H_2, Boc_2O, **4b** (92%), **4c** (83%), **4d** (89%), **4e** (90%); (ii) (a) DEAD, PPh_3, PTSH, THF, 0 °C; (b) 35% H_2O_2, $(NH_4)_6Mo_7O_{24}·4H_2O$, EtOH, **5b** (85%), **5c** (88%), **5d** (84%), **5e** (81%); (iii) KHMDS, THF, –78 °C to rt, EtOH, **6b** (82%), **6c** (83%), **6d** (75%), **6e** (80%); (iv) TFA, THF, MeOH, H_2O, 60 °C **1b** (82%), **1c** (97%), **1d** (95%), **1e** (98%).

unit[3] and the ring-opened products (**5**) yielded the (*E*)-olefin (**6**) with the TBS-protected alcohol. The final tyroscherin analog was generated by the simple removal of TBS at the alcohol and Boc group at the amine by trifluoroacetic acid. This synthetic method was rather efficient for obtaining tyroscherin analogs (**1b-1e**) with various substituents of the phenyl ring at the *para*-position in high yields.

All of the synthesized analogs (**1b-1e**) with various substituents at the *para*-position of the phenyl ring such as H, Me, OMe and F rather than OH for the natural product tyroscherin were evaluated as inhibitors of the growth of MCF-7 human breast cancer cells driven by IGF, FGF (Fibroblast growth factor) and EGF (Epidermal growth factor) using colorimetric MTS conversion assays.[9] All of the tested compounds (**1a-1e**) except for **1d** showed similar potency without noteworthy selectivity among the three growth factors tested (Figure 2).

The compound **1d** displayed weaker cell growth inhibitions induced by IGF and FGF than other analogs but inhibited EGF-induced cell growth in a similar level to other analogs. These results indicate that compound **1d** possesses selective inhibitory activity toward EGF-induced cell growth signaling pathway. Regarding the size effect of substituents, somehow less activity was observed from the compounds **1c** or **1d**, which bear substituents of either methyl or methoxy groups at the phenyl ring that are bigger than the hydroxy group of the natural product tyroscherin. The fluorine substituent as a hydroxyl bioisostere[10] with similar dipole moments to the hydroxyl group shows somewhat better activity in all cases tested. The IC_{50} values of the inhibition of the IGF induced cell proliferation of the compounds **1b**, **1c**, **1d** and **1e** were 9.5, 10.5, 16.0 and 7.5 μM, respectively.[11] The best anticancer activity among the analogs tested was obtained from the fluorinated analog **1e**, which implies that the fluorine served as a good bioisostere of hydroxide with possible strong hydrogen bonding capability.[12] However all of these biological data in Figure 2 showed us that the substituent at the *para*-position of the phenyl ring was not as significant as expected. This observation is quite unusual compared to most cases reported in the literature showing the significant activity difference by the substituent at the phenyl ring.[6] This raises a question of how important the phenyl ring is as an important pharmacophore for the anticancer activity of the natural product tyroscherin. This is parallel to the observation that the configurations of the alkyl side chain on the backbone of tyroscherin were not quite

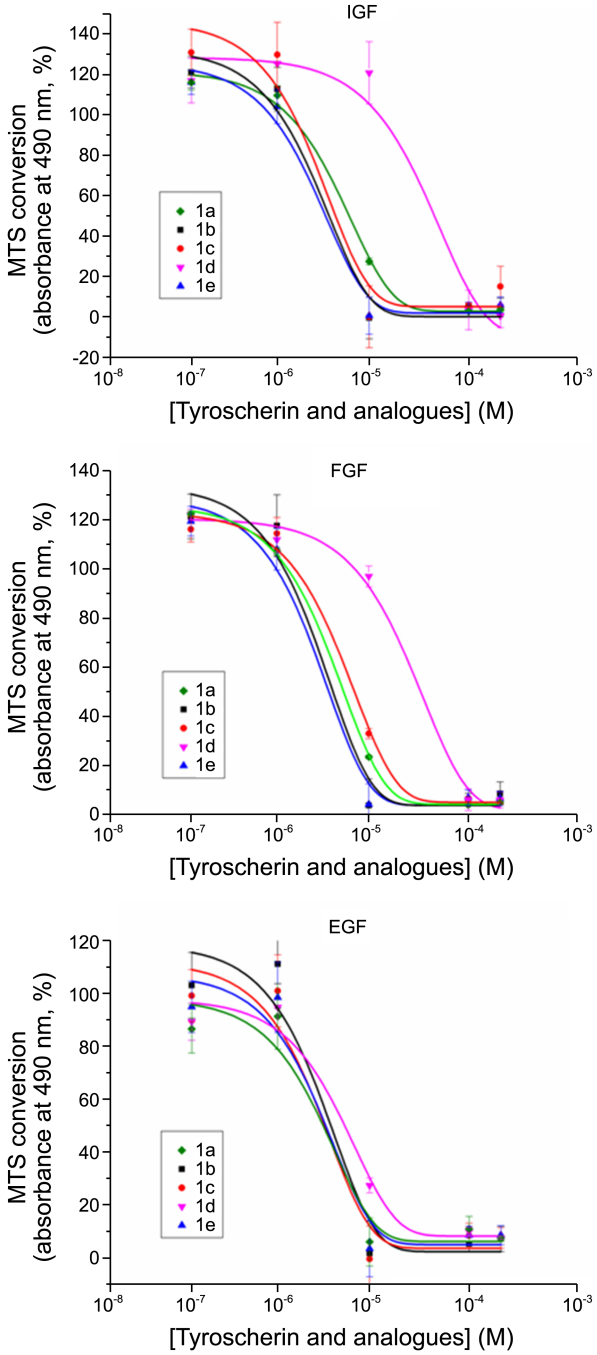


Figure 2. Potency to inhibit MCF-7 cell growth driven by IGF, basic FGF, and EGF by tyroscherin (**1a**) and its analogs (**1b-1e**) obtained using colorimetric MTS conversion assay.

sensitive retaining biological activity. The great tolerance of the stereochemistry of the side chain of tyroscherin in an earlier report[5] and the insignificant difference in activity due to the substituents of the phenyl ring tell us that the essential structural element for the biological activity of tyroscherin is still undisclosed.

In conclusion tyroscherin analogs were efficiently synthesized from the unique "alkylative ring opening reaction" of *N*-methylaziridinium ion to yield the compounds **1b**, **1c**, **1d**, and **1e** with various substituents at the *para*-position of the aryl ring including H, Me, OMe and F. No significant differences in their biological activities were observed other than the slightly better activity of the *para*-fluorinated analog.

Experimental Section

General Comments. Purification of reaction products was carried out by flash chromatography using Kieselgel 60 Art 9385 (230-400 mesh). ^{1}H-NMR and ^{13}C-NMR spectra were obtained using a Varian unity lNOVA 400WB (400 MHz). Optical rotations were obtained using a Rudolph Autopol IV digital polarimeter and optical rotation data was reported as follows: $[\alpha]_D$ (concentration c = g/100 mL, solvent). High resolution mass spectra were recorded on a 4.7 Tesla IonSpec ESI-FOFMS and JEOL (JMS-700). All synthesis were achieved by the reactions applying the corresponding arylmagnesium bromides with various substituents at the *para*-position of the aryl ring including H, Me, OMe and F. The detailed reaction procedures were well described in the previous report.[8a]

(1b): Colorless oil.; $[\alpha]_D = -12.5$ ($c = 0.94$, in $CHCl_3$). ^{1}H NMR (400MHz, CD_3OD) δ 7.45-7.14 (5H, m, Ph), 5.27 (2H, m), 3.83 (1H, m), 3.41 (1H, td, $J = 2.8, 7.1$ Hz), 2.99 (2H, dd, $J = 2.5, 7.2$ Hz), 2.63 (3H, S), 2.31-1.85 (2H, m) 1.84-1.03 (7H, m), 0.93-0.80 (9H, m). ^{13}C NMR (100 MHz, CD_3OD) δ 138.7, 138.6, 137.5, 130.2, 130.1, 128.3, 68.9, 66.4, 45.5, 35.7, 33.2, 33.1, 32.3, 31.1, 29.8, 22.3, 19.3, 11.6. HRMS *m/z* calcd for $C_{21}H_{35}NO$ $[M+H]^+$ 318.2793, found 318.2791.

(1c): Colorless oil.; $[\alpha]_D = -16.5$ ($c = 0.885$, in $CHCl_3$). ^{1}H NMR (400 MHz, CD_3OD) δ 7.16 (4H, m, Ph), 5.26 (2H, m), 3.79 (1H, m), 3.31 (1H, m), 2.90 (2H, m), 2.59 (3H, s), 2.31 (3H, s), 2.27-1.90 (2H, m), 1.67-0.95 (7H, m), 0.93-0.82 (9H, m). ^{13}C NMR (100 MHz, CD_3OD) δ 151.7, 138.6, 138.1, 134.2, 130.7, 130.1, 128.3, 68.7, 66.5, 45.5, 35.7, 33.1, 11.0, 32.8, 32.3, 31.1, 30.7, 29.7, 22.2, 21.1, 19.3, 11.6. HRMS *m/z* calcd for $C_{22}H_{37}NO$ $[M+H]^+$ 332.2949, found 332.2948.

(1d): Colorless oil.; $[\alpha]_D = -21.1$ ($c = 0.785$, in $CHCl_3$). ^{1}H NMR (400 MHz, CD_3OD) δ 7.21 (2H, d, $J = 8.1$ Hz, Ph), 6.91 (2H, d, $J = 8.1$ Hz, Ph), 5.27 (2H, m), 3.85 (1H, m), 3.82 (3H, s), 3.39 (1H, m), 2.99 (2H, m), 2.63 (3H, s), 2.31-1.85 (2H, m), 1.84-1.03 (7H, m), 0.93-0.80 (9H, m). ^{13}C NMR (100 MHz, CD_3OD) δ 160.0, 138.3, 131.1, 130.9, 128.8, 115.2, 70.1, 67.2, 55.6, 45.6, 35.7, 34.2, 33.8, 33.1, 32.9, 31.1, 30.0, 22.3, 19.3, 11.7. HRMS *m/z* calcd for $C_{22}H_{37}NO_2$ $[M+H]^+$ 348.2898, found 348.2893.

(1e): Colorless oil.; $[\alpha]_D = -17.8$ ($c = 2.025$, in $CHCl_3$). ^{1}H NMR (400 MHz, CD_3OD) δ 7.38-7.24 (2H, m, Ph), 7.13-7.06 (2H, m, Ph), 5.25 (2H, m), 3.79 (1H, m,), 3.29 (1H, m), 2.93 (2H, m), 2.56 (3H, s), 2.33-1.83 (2H, m) 1.55-1.95 (7H, m), 0.93-0.80 (9H, m). ^{13}C NMR (100 MHz, CD_3OD) δ 164.4, 162.2, 138.6, 133.7, 132.0, 131.9, 128.3, 116.8, 116.5, 68.9, 66.3, 45.5, 35.7, 33.2, 33.1, 32.6, 32.4, 31.1, 29.8, 22.2, 19.3, 11.6. HRMS *m/z* calcd for $C_{21}H_{34}FNO$ $[M+H]^+$ 336.2698, found 336.2696.

Cell Biological Assays. MCF-7 cells were purchased from Korean Cell Line Bank (Seoul, Korea) and were routinely grown in DMEM medium containing high glucose, L-glutamine, and sodium pyruvate and supplemented with 10% heat-inactivated fetal bovine serum. Cells were grown at 37 ºC in an atmosphere of 5% CO_2. Cell cytotoxicity assay was performed with a MTS assay kit (Promega, USA) as manufacturer's protocol. Briefly, MCF-7 cells were seeded into 96 well plates in serum-free medium at a density of 3×10^4 cells/well. Three different growth factors, human insulin-like growth factor 1 (IGF), epidermal growth factor (EGF), and basic fibroblast growth factor (bFGF), were added to the wells at the final concentration of 30 ng/mL with a series of increasing concentrations of tyroscherin analogs. After 72 h of incubation, 30 mL of MTS reagent was added to each well and the 96 well plates were further incubated for 2-3 h for MTS conversion by viable cells. Absorbance was measured at 490 nm. All the growth factors were purchased from Cell Signaling Technology (MA, USA).

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11. To determine the IC_{50} values of the tyroscherin analogs, more refined concentrations (0, 1, 5, 10, 20, 40, 70, and 100 μM) of tyroscherin analogs were used for MTS assay. All other assay conditions were the same as described in the experimental section.