HETEROCYCLE, Vol. 56, 2002, pp. 519-524, Received, 30th May, 2001

SYNTHESIS OF NON-PEPTIDIC TRYPTAMINE DERIVATIVE (THS-12) WHICH STIMULATES TPO RESPONSIVE CELL GROWTH (SYNTHETIC STUDIES ON INDOLES AND RELATED COMPOUNDS 51[1])

Hideharu Suzuki,[a] Tomoko Furukawa,[a] Chiaki Yamada,[a] Ichiro Shibuya,[b] Masateru Kurumi,[b] Tetsuo Yokoyama,[c] and Yasuoki Murakami*[a]

a) School of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama, Funabashi, Chiba 274-8510, Japan
b) Research Laboratories, Torii Pharmaceutical Co., Ltd.,[2] 2183-1, Teranosaku, Oota, Sakura, Chiba, 285-0808, Japan
c) JCR Pharmaceutical Co., Ltd. Laboratories for Bioengineering, 2-2-10, Murotani, Nishi-ku, Kobe 651-2241, Japan

Abstract- A number of synthetic indole compounds were examined for *in vitro* TPO responsive cell proliferation assay using BaF/mpl cell. Among these compounds 3-(2-aminoacetyl)-1-phenylsulfonylindole hydrochloride (**7**, THS-12), which was synthesized *via* α-bromination of 3-acetyl-1-phenylsulfonylindole followed by amination with hexamethylenetetramine, stimulated strongly cell growth. During the synthetic study we found that the Friedel-Crafts reaction of 1-phenylsulfonylindole (**1**) with chloroacetyl chloride using $AlCl_3$ unexpectedly gave 6-chloroacetyl-1-phenylsulfonylindole as a main product.

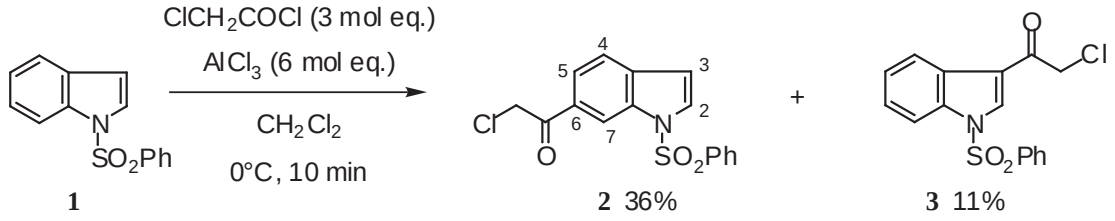
In chemotherapeutic and radiotherapeutic treatments for cancer, reduction of thrombocytes is a crucial problem for cancer patients. Therefore, thrombocyte growth factors have been required and expected as a curative for the treatments. Thrombopoietin (TPO), which acts as a ligand for a protein encoded by *c-mpl* (TPO receptor),[3] is cytokine-active at many stages in the development of megakaryocytic precursors leading to the production of platelets.[3,4] TPO treatment could be useful during the chemotherapeutic and radiotherapeutic treatments for cancer.[5] Currently, TPO is also expected as a new medicine for thrombocytepenia.[6] However, since TPO is a large protein consisting of 332 amino acid residues, TPO might easily be degraded by the digestive tract. So, it would be difficult to administer TPO orally.[7]

This paper is dedicated to Professor James P. Kutney in celebration of his 70th birthday.

In addition, a recent report describes that injected TPO raises an anti-TPO antibody, which causes another problem for the patient. Therefore, it is worthy to find a TPO mimetic non-peptidic compound for medical use.[6,7,8] On the other hand, we have been long studying and synthesizing various indole compounds for medical use.[1] These compounds were examined for *in vitro* TPO responsive cell proliferation assay to find the TPO mimetic activity. As a result, we have found a tryptamine derivative, 3-(2-aminoacetyl)-1-phenylsulfonylindole hydrochloride (THS-12, **7**) which strongly stimulates cell growth. In this report we describe the synthesis of **7** and the result of the proliferation assay of THS-12.[9]

Gribble *et al*. reported[10] the C3-selective Friedel-Crafts acylation of 1-phenylsulfonylindole (**1**). In order to synthesize the tryptamine derivative (**7**), we attempted C3-chloroacetylation using chloroacetyl chloride with aluminum chloride according to Gribble's method. However, the reaction gave a 6-chloroacetylindole (**2**, 36%) as a major product along with the target 3-chloroacetylindole (**3**, 11%).

Scheme 1



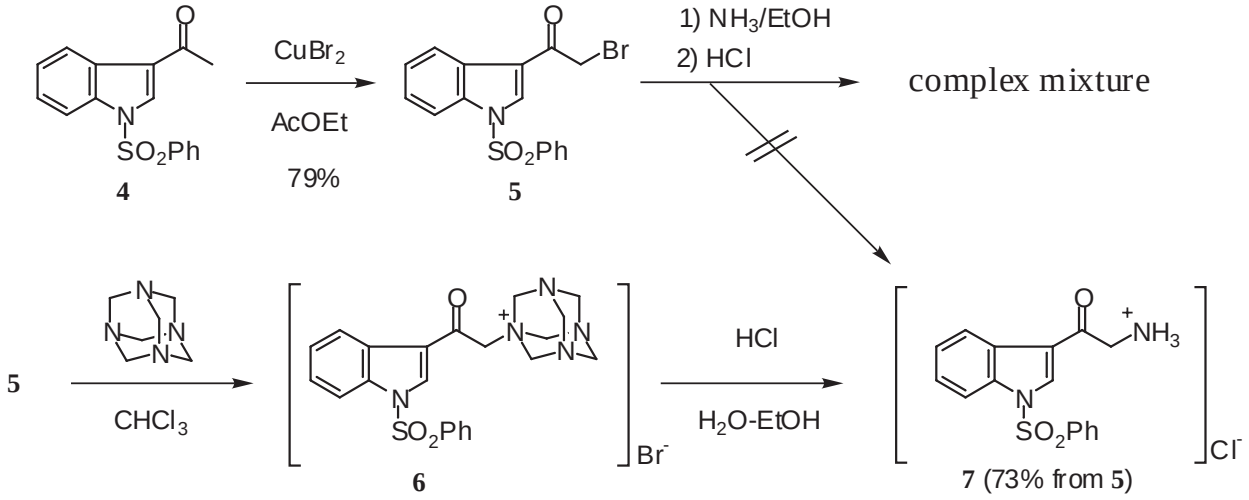
1 **2** 36% **3** 11%

The acylated position of the abnormal compound (**2**) was elucidated clearly by assigning the 8.62 ppm (dd, *J*=1.5 and 0.8 Hz) of C7-H signal and the 6.73 ppm (dd, *J*=3.7 and 0.8 Hz) of C3-H one. The long range coupling (0.8 Hz) of the two signals was confirmed by 2D DQF COSY spectrum. Furthermore, this C7-H signal and 7.77 ppm (d, *J*=3.7 Hz) of C2-H signal showed a NOE correlation toward the 7.93 ppm (2H, br d, *J*=8.0 Hz) of SO_2Ar-H signal on the 2D phase sensitive NOESY spectrum. Nakatsuka[11a] and Ottoni[11b] reported the abnormal Friedel-Crafts acylation of *N*-acylindole, which also gave 6-acylindole, while the C3-position was unoccupied. In this reaction, we found that the *N*-sulfonyl indole (**1**) has a same reactivity to the *N*-acylindoles, and that the α-haloacyl chloride mostly reacted at the benzene part of the indole ring rather than at the C3-position. However, it is very interesting that the abnormal Friedel-Crafts products from *N*-sulfonylindole (**1**) and *N*-acylindole[11] are 6-substituted indoles, whereas, our previously reported[12] abnormal Friedel-Crafts product from ethyl indole-2-carboxylate was 5-substituted indole. As the *N*-sulfonyl indole (**1**) and the $AlCl_3$ complex of the *N*-acylindole reported by Ottoni[11b] have a strong electronwithdrawing group at *N*1-position, these functional groups may act as "meta" directing groups on the benzene-part of the indole ring.

Thus we tried a stepwise process in the synthesis of 3-haloacetylindole. To synthesize the 3-bromoacetylindole (**5**), we chose the α-selective bromination[13,14] of the 3-acetyl-1-phenylsulfonylindole (**4**) prepared easily according to Gribble's method.[10] The reaction of **4** with 2.0 mol eq. of copper (II)

bromide[14] gave the monobromide (**5**) in a 79% yield. Next, we attempted direct aminolysis of 3-bromoacetylindole (**5**) using a large excess of ammonia gas in EtOH. However, the reaction gave a complex mixture. Therefore, the bromide (**5**) was reacted with hexamethylenetetramine[13] to give the quaternary salt (**6**). Then, the compound (**6**) was hydrolyzed under acidic condition to give 3-(2-aminoacetyl)-1-phenylsulfonylindole hydrochloride (THS-12, **7**).

Scheme 2



Proliferation assay was done as follows. Briefly, cultured IL-3 dependent mouse Ba/F3[15] cells and BaF/mpl (Ba/F3 cell) transfected with human *c-mpl* gene[3] cells were separately starved of IL-3 by incubation in a medium without exogenous cytokines for 24 h. Starved cells were then plated out in a 96-well micro plate at a cell density of 4 x 10^4 cells/mL in the presence of various concentrations (20-100 μM) of the synthesized compounds and incubated for several hours. After incubation, cell viability of each well was measured using MTT assay (Promega Co. LTD.). Among the synthesized compounds, THS-12 (**7**) showed the highest cell viability (76 %) at a concentration of 100 μM of the BaF/mpl cells. Thus, THS-12 acted as TPO through the TPO receptor in the BaF/mpl cell, and would be similarly mimic TPO in thrombocyte. We hope that THS-12 (**7**) may serve as a useful lead compound for the development of a chemotherapeutically and radiotherapeutically effective thrombopoietic agent.

EXPERIMENTAL

All melting points were determined on a micro melting point hot stage apparatus (Yanagimoto) and are uncorrected. IR spectra were recorded on a Shimadzu IR-400 spectrophotometer in Nujol mull. ^{1}H-NMR spectra were recorded on a Hitachi R-24B (60 MHz) and JEOL EX-400 (400 MHz) spectrometers in deuteriochloroform with tetramethylsilane as an internal reference unless otherwise stated. 2D ^{1}H-NMR

and ^{13}C-NMR spectra were recorded on a JEOL ECP-500 (500 MHz) spectrometer. MS spectra were measured on JEOL JMS D-300 spectrometer with a direct inlet system. For column chromatography, silica gel 60 (70 - 230 mesh ASTM, Merck), was used. For TLC, silica gel 60F_{254} (Merck) was used.

6-Chloroacetyl-1-phenysulfonylindole (2) and 3-Chloroacetyl-1-phenysulfonylindole (3)

To a suspension of aluminum chloride (805 mg, 6.0 mmol) in CH_2Cl_2 (6.5 mL), was added chloroacetyl chloride (343 mg, 3.0 mmol) under an argon atmosphere at 0°C and the mixture was stirred for 10 min. Then 1-phenylsulfonylindole (**1**),[16] (257 mg, 1.0 mmol) in CH_2Cl_2 (2.0 mL) was added to the above mixture under ice-cooling and the whole was stirred for 10 min. The reaction mixture was poured into ice-water, followed by extracted with AcOEt. The organic layer was washed with H_2O, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and evaporated to dryness *in vacuo*. The residual solid (293 mg) was separated into Fr-A and Fr-B by column chromatography (hexane : AcOEt ; 5 : 1). The Fr-A gave 3-chloroacetyl-1-phenysulfonylindole (**3**) as crystals (37 mg, 11%). Recrystallization from benzene-hexane gave colorless prisms (mp 137-138°C). *Anal.* Calcd for $C_{16}H_{12}NO_3ClS$; C:57.57, H:3.62, N:4.20, Found; C: 57.64; H, 3.67; N, 4.23. IR υ_{max}cm^{-1}: 1678 (C=O). ^{1}H-NMR (400 MHz) δ : 4.58 (2H, s, $COCH_2Cl$), 7.38 (1H, br t, *J*=7.5 Hz, C^5 or C^6-H), 7.41 (1H, br t, *J*=7.5 Hz, C^5 or C^6-H), 7.52 (2H, br t, *J*=8.0 Hz, SO_2Ar-H), 7.63 (1H, br t, *J*=8.0 Hz, SO_2Ar-H), 7.94 (1H, br d, *J*=7.5 Hz, C^4 or C^7-H), 7.97 (2H, br d, *J*=8.0 Hz, SO_2Ar-H), 8.31 (1H, br d, *J*=7.5 Hz, C^4 or C^7-H), 8.33 (1H, s, C^2-H). MS *m/z*; 335 (M^++2, 11%), 333 (M^+, 29%), 284(BP).

The Fr-B gave 6-chloroacetyl-1-phenysulfonylindole (**2**) as crystals (119 mg, 36%). Recrystallization from benzene-hexane gave colorless prisms (mp 142.5-143°C). *Anal*. Calcd for $C_{16}H_{12}NO_3ClS$; C:57.57, H:3.62, N:4.20, Found; C: 57.37; H, 3.57; N, 4.32. IR υ_{max}cm^{-1}: 1705 (C=O). ^{1}H-NMR (400 MHz) δ : 4.77 (2H, s, $COCH_2Cl$), 6.73 (1H, dd, *J*=3.7 and 0.8 Hz, C^3-H), 7.47 (2H, br t, *J*=8.0 Hz, SO_2Ar-H) 7.58 (1H, br t, *J*=8.0 Hz, SO_2Ar-H), 7.63 (1H, d, *J*=8.2 Hz, C^4-H), 7.77 (1H, d, *J*=3.7 Hz, C^2-H), 7.85 (1H, dd, *J*=8.2 and 2.0 Hz, C^5-H), 7.93 (2H, br d, *J*=8.0 Hz, SO_2Ar-H), 8.62 (1H, dd, *J*=1.5 and 0.8 Hz, C^7-H). ^{13}C-NMR (125MHz) δ : 45.8, 109.0, 114.5, 121.7, 123.5, 126.9, 129.5, 130.1, 130.6, 134.2, 134.3, 135.1, 137.8, 190.7. MS *m/z*; 335 (M^++2, 8%), 333 (M^+, 20%), 284(BP).

3-Bromoacetyl-1-phenylsulfonylindole (5)

To a suspension of $CuBr_2$ (18.90 g, 84.6 mmol) in AcOEt (300 mL), was added 3-acetyl-1-phenylsulfonylindole (**4**)[7] (12.6 mg, 42.1 mmol) in AcOEt (380 mL) under an argon atmosphere at rt. The mixture was stirred under reflux for 50 min. After cooling, the precipitates were removed by filtration and washed with ethyl acetate. Combined filtrates were washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and evaporated to dryness *in vacuo*. The residual solid was recrystallized from benzene-hexane to give **5** as colorless plates (12.61 g, 71%, mp 130.5-131.0°C).

Anal. Calcd for $C_{16}H_{12}NO_3BrS$; C:50.81, H:3.20, N:3.70, Found; C: 50.69; H, 3.16; N, 3.75. IR $\upsilon_{max}cm^{-1}$: 1678 (C=O). ^{1}H-NMR (60 MHz) δ : 4.32 (2H, s, $COCH_2Br$), 7.10-8.41 (9H, m, Ar-H), 8.28 (1H, s, C^2-H). MS *m/z*; 379 (M^++2, 14%), 377 (M^+, 13%), 77(BP).

1-[2-(1-Benzenesulfonyl-1*H*-indol-3-yl)-2-oxoethyl] hexamethylenetetraminium Bromide (6)

To a solution of hexamethylenetetramine (2.01 g, 14.3 mmol) in $CHCl_3$ (40 mL), was added 3-bromoacetyl-1-phenysulfonylindole (**5**, 4.94 g, 13.1 mmol) in $CHCl_3$ (20 mL) at rt. The mixture was stirred at rt for 1 h, and the colorless precipitates were collected by filtration to give crude compound (**6**) (5.81 g, 86%). This compound was hydrolyzed without further purification because of its instability.

3-(2-Aminoacetyl)-1-phenylsulfonylindole hydrochloride (7)

To a solution of the crude compound (**6**) (5.81 g, 11.2 mmol) in H_2O-MeOH (2:1, 100 mL), was added concentrated HCl (9.1 mL, 0.11 mol) at rt. The mixture was stirred at 65°C for 1 h, and evaporated to dryness under reduced pressure. A residual pale yellow solid was suspended in ether, shaked with 5% aqueous NaOH and brine, and dried over anhydrous $MgSO_4$. The ether layer was bubbled by dry HCl gas, and the resulted precipitates were collected by filtration. The crude solid was recrystallized from EtOH-Et_2O to give colorless prisms (3.35 g, 73% from **5**, mp 215-218 °C). *Anal*. Calcd for $C_{16}H_{15}N_2O_3ClS$; C:54.78, H:4.31, N:7.99, Found; C: 54.37; H, 4.69; N, 7.51. IR $\upsilon_{max}cm^{-1}$: 3365 (NH_2), 1680 (C=O). ^{1}H-NMR (DMSO-d_6, 60 MHz) δ : 3.30-3.58 (3H, m, $COCH_2N^+\underline{H}_3$), 4.58 (2H, br, $COC\underline{H}_2N^+H_3$), 7.10-8.35 (9H, m, Ar-H), 9.00 (1H, s, C^2-H). MS *m/z*; 314 (M^+, 14%), 284 (BP).

REFERENCES

1. Part 50: “IMPROVED SYNTHESIS OF 7, 9-DIDECARBOXY-METHOXATHINE” H. Suzuki, T. Watanabe, Y. Yokoyama, and Y. Murakami, *Heterocycles*, 2002, **56**, *in press.* : Part 49: “Unexpected Formation of Quinolone Derivatives in Reissert Indole Synthesis” H. Suzuki, H. Gyoutoku, H. Yokoo, M. Shinba, Y. Sato, H. Yamada, and Y. Murakami, *Synlett*, 2000, **8**, 1196.
2. Pharmacological study was carried out in Research and Laboratories of Torii Pharmaceutical Co., Ltd. from 1998 to 1999.
3. I. Vigon, J. P. Mornon, L. Cocault, M. T. Mitjavila, P. Tambourin, S. Gisselbrecht, and M. Souyri, *Proc. Natl. Acad. Sci.*, 1992, **89**, 5640.
4. a) K. Kaushansky, S. Lok, R. D. Holly, V. C. Broudy, N. Lin, M. C. Bailey, J. W. Forstrom, M. M. Buddle, P. J. Oort, F. S. Hagen, G. J. Roth, T. Papayannopoulou, and D. C. Foster, *Nature*, 1994, **369**, 568. b) T. D. Bartley, J. Bogenberger, P. Hunt, Y. S. Li, H. S. Lu, F. Martin, M. S. Chang, B. Samal, J. L. Nichol, S. Swift, M. J. Johnson, R. Y. Hsu, P. Parker, S. Suggs, J. D. Skrine, L.

A. Merewether, C. Clogston, E. Hsu, M. M. Hokom, A. Hornkohl, E. Choi, M. Pangelinan, Y. Sun, V. Mar, J. McNinch, L. Simonet, F. Jacobsen, C. Xie, J. Shutter, H. Chute, R. Basu, L. Selander, D. Trollinger, L. Sieu, D. Padilla, G. Trail, G. Elliott, R. Izumi, T. Covey, J. Crouse, A. Garcia, W. Xu, J. Del Castillo, J. Biron, S. Cole, M. C.-T. Hu, R. Pacifici, I. Ponting, C. Saris, D. Wen, Y. P. Yung, H. Lin, and R. A. Bosselman, *Cell*, 1994, **77**, 1117. c) S. Lok, K. Kaushansky, R. D. Holly, J. L. Kuijper, C. E. Lofton-Day, P. J. Oort, F. J. Grant, M. D. Heipei, S. K. Burkhead, J. M. Kramer, L. A. Bell, C. A. Sprecher, H. Blumberg, R. Johnson, D. Prunkard, A. F. T. Ching, S. L. Mathewes, M. C. Bailey, J. W. Forstrom, M. M. Buddle, S. G. Osborn, S. J. Evans, P. O. Sheppard, S. R. Presnell, P. J. O'Hara, F. S. Hagen, G. J. Roth, and D. C. Foster , *Nature*, 1994, **369**, 565. d) T. Kato, K. Ogami, Y. Shimada, A. Iwamatsu, Y. Sohma, H. Akahori, K. Horie, A. Kokubo, Y. Kudo, E. Maeda, K. Kobayashi, H. Ohashi, T. Ozawa, H. Inoue, K. Kawamura, and H. Miyazaki, *Biochem*., 1995, **118**, 229.

5 J. G. Drachman and K. Kaushansky, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 2350.

6. S. E. Cwirla, P. Balasubramanian, D. J. Duffin, C. R. Wagstrom, C. M. Gates, S. C. Singer, A. M. Davis, R. L. Tansik, L. C. Mattheakis, C. M. Boytos, P. J. Schatz, D. P. Baccanari, N. C. Wrighton, R. W. Barrett, and W. J. Dower, *Science*, 1997, **276**, 1696.

7. T. Kimura, H. Kaburaki, S. Miyamoto, J. Katayama, and Y. Watanabe, *J. Biochem.*, 1997, **122**, 1046.

8 T. Kimura, H. Kaburaki, T. Tsujino, Y. Ikeda, H. Kato, and Y. Watanabe, *FEBS Lett.* 1998, **428**, 250.

9. Y. Murakami, H. Suzuki, Y. Watanabe, and T. Yokoyama, Japan. Kokai Tokkyo Koho JP 2001-097948 (2001) [*Chem. Abstr*., 2001, **134**, 266201].

10. D. M. Ketcha and G. W. Gribble, *J. Og. Chem*., 1985, **50**, 5451.

11. a) S. Nakatsuka, T. Teranishi, and T. Goto, *Tetrahedron Lett*., 1994, **35**, 2699. b) R. P. A. Cruz, O. Ottoni, C. A. M. Abella, and L. B. Aquino, *Tetrahedron Lett.*, 2001, **42**, 1467.

12. M. Tani, T. Aoki, S. Ito, S. Matsumoto, M. Hideshima, K. Fukushima, R. Nozawa, T. Maeda, M. Tashiro, Y. Yokoyama, and Y. Murakami, *Chem., Pharm. Bull*., 1990, **38**, 3261.

13. H. Suzuki, K. Shinpo, T. Yamazaki, S. Niwa, Y. Yokoyama, and Y. Murakami, *Heterocycles*, 1996, **42**, 83.

14. M. Matsumoto, Y. Ishida, and N. Watanabe, *Heterocycles*, 1985, **23**, 165.

15. R. Palacios and M. Steinmetz, *Cell*, 1985, **41**, 727.

16. M. G. Saulnier and G. W. Gribble, *J. Org. Chem.*, 1982, **47**, 757.