Journal of Photochemistry and Photobiology A: Chemistry 289 (2014) 39–46

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

Unsymmetric indolylmaleimides: Synthesis, photophysical properties and amyloid detection

Manabu Nakazono[a,*], Konen Obayashi[b], Yuji Oshikawa[a], Kazushi Tani[a], Genki Suenaga[c], Yukio Ando[c], Shinkoh Nanbu[d], Ryoichi Kuwano[e]

[a] Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
[b] Diagnostic Unit for Amyloidosis, Department of Laboratory Medicine, Kumamoto University Hospital, 1-1-1 Honjo, Kumamoto 860-8556, Japan
[c] Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan
[d] Faculty of Science and Technology, Sophia University, 7-1 Kioi-Cho, Chiyoda-ku, Tokyo 102-8554, Japan
[e] Department of Chemistry, Graduate School of Sciences, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan

ARTICLE INFO

Article history:
Received 7 January 2014
Received in revised form 13 March 2014
Accepted 15 April 2014
Available online 2 June 2014

Keywords:
Fluorescence
Indolylmaleimide
π-Conjugation
Amyloid

ABSTRACT

Various unsymmetric indolylmaleimides were synthesized. Their photophysical properties and affinities for amyloid fibrils were evaluated. Some unsymmetric indolylmaleimides have large Stokes shifts of more than 120 nm, fluorescence emission maxima wavelengths of more than 550 nm and different emissions under UV irradiation at 365 nm. From the results of histopathologic methods using stains, 3-(1*H*-indol-3-yl)-1-methyl-4-phenyl-1*H*-pyrrole-2,5-dione has high selectivity for amyloid fibrils in senile systemic amyloidosis.

1. Introduction

Recently, indolylmaleimide (IM) derivatives have been a focus in the development of anticancer medicines based on inhibition of protein kinases [1–4]. Furthermore, IM compounds have been evaluated as luminescent materials for organic light-emitting diodes [5,6]. Some research groups [7–10], including us [11–14], have studied the fluorescence (FL) and chemiluminescence properties of symmetric bisindolylmaleimides. However, the potential use of IM derivatives in clinical diagnosis has been unexplored. There have been two studies on the FL properties of unsymmetric IMs [6,7]. A monosubstituted IM compound is obtained from the reaction of indolylmagnesium bromide and 2,3-dibromo-*N*-methylmaleimide [15]. The resulting monosubstituted IM is readily transformed to unsymmetric disubstituted IMs through Suzuki–Miyaura coupling. The advantage of this synthetic method is that various substituents, including fluorophores, can be introduced at the C=C bond of the maleimide moiety. When the indolylmaleimides are modified with a functional group possessing high affinity for a specific analyte, the utility of the unsymmetric IM derivatives as FL probes is drastically increased.

Amyloidosis is a disorder of protein metabolism in which normally soluble autologous proteins are deposited in tissues as abnormal insoluble fibrils, which cause structural and functional disruptions [16–21]. Among several histopathologic methods using stains, Congo red staining is one of the most popular detection methods of amyloid deposits in tissues [22,23]. However, Congo red-stained histochemical specimens are not always easily interpreted. In previous studies, styrylbenzene derivatives exhibited high selectivity for amyloid fibrils in systemic amyloidoses [24–27]. For example, (*trans*, *trans*)-1-bromo-2,5-bis-(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (BSB) was developed for in vivo detection of amyloid deposits in patients with various systemic and localized forms of amyloidosis [25]. Styrylbenzene derivatives such as BSB have π-conjugated structure. In this study, we designed and synthesized various π-conjugated unsymmetric indolylmaleimides, cleared their photophysical properties and carefully checked the reactivity of the compounds with amyloid fibrils.

2. Experimental

2.1. Chemicals

Ethylmagnesium bromide (tetrahydrofuran solution), *trans*-2-phenylvinyl boronic acid, 3-quinine boronic acid, 2,2′-bithiophene-5,5′-diboronic acid bis(pinacol)ester, 4-formylphenylboronic acid,

* Corresponding author. Tel.: +81 92 642 6597.
E-mail address: nakazono@phar.kyushu-u.ac.jp (M. Nakazono).

http://dx.doi.org/10.1016/j.jphotochem.2014.04.027
1010-6030/

pyrene-1-boronic acid, 9*H*-carbazole-2-boronic acid pinacol ester and 4,4′-biphenyl-diboronic acid were purchased from Aldrich (Milwaukee, USA). Indole, sodium hydride, phenylboronic acid, palladium(II) acetate, tetrakis(triphenylphosphine)palladium (0), naphthalene-2-boronic acid, sodium methoxide, di-*tert*-butyl dicarbonate, 4-bromomethyl-7-methoxycoumarin and 4-dimethylaminopyridine were purchased from Wako Chemicals (Osaka, Japan). Diethyl(4-iodobenzyl)phophonate and rhodamin B were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Cesium fluoride was purchased from Nakalai Tesque Inc. (Kyoto, Japan). 3-Bromo-4-(1*H*-indol-3-yl)-1-methyl-1*H*-pyrrole-2,5-dione and *tert*-butyl 3-(4-bromo-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl)-1*H*-indole-1-carboxylate were prepared by the reported method [15]. All other chemicals and solvents were of analytical reagent grade.

2.2. Apparatus

The ^{1}H NMR (500 MHz) and ^{13}C NMR (125.7 MHz) spectra were obtained using a Varian UNITY plus (USA) spectrometer. The HR ESI-TOF-MS spectra of compounds **1–13** were obtained using a Bruker/micrOTOF II (Karlsruhe, Germany). The FAB MS spectra were obtained using a JEOL JMS-HX110A (Tokyo, Japan). The absorbance and fluorescence spectra of compounds **1–13** were obtained using a Jasco V-530 absorptiometer and FP-6500 fluorometer (Tokyo, Japan). The slit widths at the excitation and emission of the fluorometer were 5 nm. All the FL spectra were corrected.

2.3. Synthesis and characterization

All reactions were done under an atmosphere of inert gas.

2.3.1. 3-(1H-indol-3-yl)-1-methyl-4-phenyl-1H-pyrrole-2,5-dione (*1*)

3-Bromo-4-(1*H*-indol-3-yl)-1-methyl-1*H*-pyrrole-2,5-dione (0.12 g, 0.39 mmol), phenylboronic acid (0.1 g, 0.82 mmol) and K_2CO_3 (0.08 g, 0.58 mmol) were added to dioxane-H_2O (28 mL, 6:1). Palladium(II) acetate (0.06 g, 0.27 mmol) was then added to the mixture, and the solution was refluxed for 17 h. Ethyl acetate (200 mL), 1 M aqHCl (100 mL) and 10% (w/v) aqNaCl (100 mL) were added to the mixture. The organic layer was dried with anhydrous Na_2SO_4. The filtrate was concentrated and purified by column chromatography ($CHCl_3$:CH_3OH = 20:1) to produce compound **1** as a red solid (0.04 g, 33% yield). M.p. 203–205 °C. ^{1}H NMR (500 MHz, $(CD_3)_2S{=}O$) 3.03 (s, 3H, CH_3), 6.3–6.32 (d, *J* = 8 Hz, 1H, ArH), 6.65–6.68 (t, *J* = 8 Hz, 1H, ArH), 7.03–7.06 (t, *J* = 8 Hz, 1H, ArH), 7.31–7.36 (m, 3H, ArH), 7.39–7.43 (m, 3H, ArH), 8 (s, 1H, ArH), 11.9 (s, 1H, indole NH). ^{13}C NMR (125.7 MHz, $(CD_3)_2S{=}O$) 24.0, 104.1, 112.1, 119.7, 121.1, 122.0, 123.8, 128.0, 128.6, 129.5, 130.6, 131.1, 132.0, 136.5, 171.0, 171.3. HRMS-ESI (*m*/*z*) calculated for $C_{19}H_{14}N_2O_2$ $[M+Na]^+$ 325.0953, found 325.0952.

2.3.2. 3-(1H-indol-3-yl)-1-methyl-4-styryl-1H-pyrrole-2,5-dione (*2*)

Tert-butyl 3-(4-bromo-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl)-1*H*-indole-1-carboxylate (0.4 g, 1 mmol), *trans*-2-phenylvinylboronic acid (0.22 g, 1.49 mmol) and K_2CO_3 (0.14 g, 1 mmol) were added to dioxane-H_2O (30 mL, 4:1). Tetrakis(triphenylphosphine)palladium (0) (0.12 g, 0.1 mmol) was then added to the mixture, and the solution was refluxed for 16.5 h. Ethyl acetate (200 mL), 0.5 M aqHCl (100 mL) and 10% (w/v) aqNaCl (100 mL) were added to the mixture. The organic layer was dried with anhydrous $MgSO_4$. The filtrate was concentrated and purified by column chromatography (ethyl acetate:hexane = 1:2 → $CHCl_3$:CH_3OH = 20:1) to produce compound **2** as a red solid (0.03 g, 9% yield). M.p. 166–168 °C. ^{1}H NMR (500 MHz, $(CD_3)_2S{=}O$) 3 (s, 3H, CH_3), 7.1–7.13 (t, *J* = 7 Hz, 1H, ArH), 7.2–7.24 (m, 2H, ArH), 7.29–7.37 (m, 3H, ArH), 7.48–7.53 (m, 3H, ArH), 7.62–7.64 (d, *J* = 8 Hz, 1H, ArH), 7.79–7.82 (d, *J* = 16.5 Hz, 1H, CH=CH), 7.88–7.89 (d, *J* = 2.5 Hz, 1H, ArH), 12 (s, 1H, indole NH). ^{13}C NMR (125.7 MHz, $(CD_3)_2S{=}O$) 23.7, 104.8, 112.5, 118.7, 120.5, 122.4, 125.3, 126.3, 126.6, 128.9, 129.0, 130.3, 131.7, 135.9, 136.6, 136.7, 170.6, 171.0. HRMS-ESI (*m*/*z*) calculated for $C_{21}H_{16}N_2O_2$ $[M+Na]^+$ 351.1109, found 351.1106.

2.3.3. 3-(1H-indol-3-yl)-1-methyl-4-(naphthalen-2-yl)-1H-pyrrole-2,5-dione (*3*)

3-Bromo-4-(1*H*-indol-3-yl)-1-methyl-1*H*-pyrrole-2,5-dione (0.12 g, 0.39 mmol), naphthalene-2-boronic acid (0.14 g, 0.8 mmol) and K_2CO_3 (0.08 g, 0.58 mmol) were added to dioxane-H_2O (28 mL, 6:1). Palladium(II) acetate (0.06 g, 0.27 mmol) was added to the mixture, and the solution was refluxed for 15 h. Ethyl acetate (200 mL), 1 M aqHCl (100 mL) and 10% (w/v) aqNaCl (100 mL) were added to the mixture. The organic layer was dried with anhydrous Na_2SO_4. The filtrate was concentrated and purified by column chromatography ($CHCl_3$:CH_3OH = 20:1) to produce compound **3** as a red solid (0.1 g, 71% yield). M.p. 230–232 °C. ^{1}H NMR (500 MHz, $(CD_3)_2S{=}O$) 3.06 (s, 3H, CH_3), 6.35–6.36 (d, *J* = 8.5 Hz, 1H, ArH), 6.51–6.54 (t, *J* = 8 Hz, 1H, ArH), 7–7.01 (m, 1H, ArH), 7.4–7.43 (m, 2H, ArH), 7.48–7.55 (m, 2H, ArH), 7.77–7.79 (d, *J* = 8.5 Hz, 1H, ArH), 7.83–7.85 (d, *J* = 8 Hz, 1H, ArH), 7.87–7.88 (d, *J* = 8 Hz, 1H, ArH), 8.02 (s, 1H, ArH), 8.09 (s, 1H, ArH), 11.94 (s, 1H, indole NH). ^{13}C NMR (125.7 MHz, $(CD_3)_2S{=}O$) 24.0 (CH_3), 104.2, 112.1, 119.7, 120.9, 122.0, 124.0, 126.4, 126.6, 126.8, 127.3, 127.5, 127.8, 128.1, 128.3, 129.2, 131.1, 132.2, 132.3, 132.5, 136.5, 171.0, 171.3. HRMS-ESI (*m*/*z*) calculated for $C_{23}H_{16}N_2O_2$ $[M+Na]^+$ 375.1109, found 375.1109.

2.3.4. 3-(1H-indol-3-yl)-1-methyl-4-(quinolin-3-yl)-1H-pyrrole-2,5-dione (*4*)

Tert-butyl 3-(4-bromo-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl)-1*H*-indole-1-carboxylate (0.6 g, 1.5 mmol), 3-quinoline boronic acid (0.35 g, 2.25 mmol) and Na_2CO_3 (1.2 g, 15.5 mmol) were added to dioxane-H_2O (120 mL, 4:1). Tetrakis(triphenylphosphine)palladium (0) (0.25 g, 0.22 mmol) was then added to the mixture, and the solution was refluxed for 20.5 h. Ethyl acetate (300 mL) and H_2O (200 mL) were added to the mixture. The organic layer was dried with anhydrous $MgSO_4$. The filtrate was concentrated and purified by column chromatography (ethyl acetate:hexane = 4:5) to produce compound **4** as a red solid (0.29 g, 56% yield). M.p. 249–251 °C. ^{1}H NMR (500 MHz, $(CD_3)_2S{=}O$) 3.08 (s, 3H, CH_3), 6.37–6.38 (d, *J* = 8 Hz, 1H, ArH), 6.56–6.59 (m, 1H, ArH), 7.01–7.04 (m, 1H, ArH), 7.45–7.47 (d, *J* = 8 Hz, 1H, ArH), 7.59–7.63 (m, 1H, ArH), 7.76–7.8 (m, 1H, ArH), 7.95–7.97 (m, 2H, ArH), 8.08 (s, 1H, ArH), 8.48 (s, 1H, ArH), 8.73 (s, 1H, ArH), 12.04 (s, 1H, indole NH). ^{13}C NMR (125.7 MHz, $(CD_3)_2S{=}O$) 24.1, 104.1, 112.4, 120.1, 120.6, 122.2, 123.7, 124.2, 124.6, 126.9, 127.1, 128.6, 128.7, 130.3, 131.7, 133.1, 136.2, 136.7, 146.6, 150.1, 170.7, 171.1. HRMS-ESI (*m*/*z*) calculated for $C_{22}H_{15}N_3O_2$ $[M+Na]^+$ 376.1062, found 376.1051.

2.3.5. 3-([2,2′-Bithiophen]-5-yl)-4-(1H-indol-3-yl)-1-methyl-1H-pyrrole-2,5-dione (*5*)

Tert-butyl 3-(4-bromo-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl)-1*H*-indole-1-carboxylate (0.21 g, 0.52 mmol) was added to dioxane (40 mL), and then tetrakis(triphenylphosphine)palladium (0) (0.06 g, 0.052 mmol) was added to the mixture. The solution was stirred for 10 min. 2,2′-Bithiophene-5,5′-diboronic acid bis(pinacol)ester (0.13 g, 0.3 mmol) and