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Synthesis of 5,10,15,20-Tetra(N-methyl-6-quinolyl)-21,23-dithia-porphyrin Chloride as Cationic Core-modified Porphyrin

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Abstract: First cationic 6-quinolyl substituted dithiaporphyrin was synthesized using Skraup quinoline methodology from thiaporphyrin bearing 4-acetamidophenyl prepared by condensation reaction of aromatic aldehyde with pyrrole.

Keywords: Porphyrin, thiaporphyrin, telomerase inhibitor, dilithiophen.

Telomeres are specialized DNA-protein complexes at ends of chromosomes, which represent a potential target for novel anti-cancer agents[1]. Since Zahler and co-workers demonstrated in 1991 that K^+-stabilized G-quadruplex structures were able to inhibit telomerase, G-quadruplex DNA has emerged as an attractive target for telomerase inhibitors[2]. Several groups of leading compounds have been identified and their interaction with G-quadruplexes has been studied extensively[3,4]. It has been widely shown that cationic porphyrins based on 5,10,15,20-tetra(N-methyl-4-pyridyl)porphyrin (TMPyP4) can interact with quadruplex DNA structures by stacking externally to quinine tetrads[5]. As a result of this close association, cationic porphyrins have the ability to inhibit the action of telomerase in cell-free assays, by the stabilization of quadruplex DNA structures.

A wide range of TMPyP4 analoges have been synthesized and assayed against telomerase. However, they have the potential problem of photo-induced skin toxicity, which may affect their clinical use. In order to overcome this disadvantage, one of the most interesting and promising approaches involves performing specific atom replacements at the porphyrin core. Recent reports show that the core modified porphyrins containing thiophene have no photoinduced skin toxicity. An example of a core modified porphyrin is the recently synthesized 21-thiaporphyrin analog 10,15-bis(2-methoxy-4-sulphophenyl)-21-thiaporphyrin (STSP). This new PDT agent has been shown to be a highly effective photosensitization agent both in *vitro* and in *vivo*. In contrast, STSP was reported to show no photoinduced skin damage[6]. The reason for the lack of skin toxicity for STSP is not entirely clear. It may simply be that STSP does not

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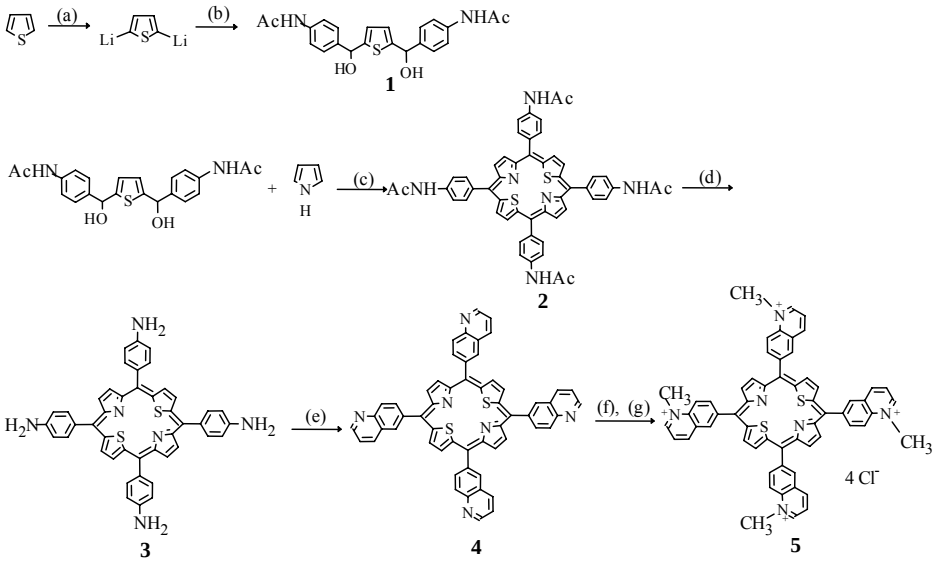
accumulate well in skin tissue. It can be expected that these types of mixed porphyrins will have less clinical side effects.

The typical synthesis of symmetrically and unsymmetically substituted tetraphenyl-21,23-dithiaporphyrins was reported by Ulman[7]. All the thiaporphyrins with neutral or electron donating groups were prepared in this way by refluxing the mixture of substituted 2,5-bis(arylhydroxymethyl)thiophens **1** and pyrrole in propionic acid. Up to date, dialcohols bearing some electron withdrawing groups, *e.g*. pyridyl or quinolyl, have not been reported yet. And also, no publications seem to exist on the synthesis of thiaporphyrins with cationic electron withdrawing groups.

The intermediate thiophen dialcohol **1** were also applied to the formation of thiaporphyrins with cationic pyridyl or quinolyl group. The typical methodologies for the synthesis of dithiaporphyrins were attempted under varing reaction conditions. However, no formation of thiaporphyrins bearing pyridyl or quinolyl ring could be substantiated.

Because *meso*-tetra(6-quinolyl)thiaporphyrin is of interest in the development of a library of core-modified porphyrins by replacement of nitrogen with sulfur as telomerase inhibitors. The other approach to make it has been investigated. The key intermediate 2,5-bis(α-hydroxy-α-phenylmethyl) thiophene was synthesized by reacting thiophene with *n*-butyllithium, then with acetamidobenzaldehyde in very poor yield. The resulting compound condensed with pyrrole in propionic acid to give *meso*-tetra (4-acetamidophenyl) thiaporphyrin. After hydrolysis in TFA/HCl, the *meso*-tetra (6-quinolyl) thiaporphyrin was made by Skraup quinoline synthesis[8] (**Scheme 1**). Quaternization of the free base was accomplished by reaction with alkyliodides in chloroform. The salt was converted to the chloride by ion exchange to give the final cationic thiaporphyrin.

Scheme 1



Reaction conditions: a) *n*-butyllithium, hexane; b) 4-acetamidobenzaldehyde, THF; c) propionic acid; d) TFA, HCl; e) glycerol, $PhNO_2$; f) iodomethane, $CHCl_3$; g) ion-exchange.

Preliminary biological studies showed that target compound is effective for inhibiting telomerase, especially stronger interaction with G-quadruplex DNA, but does not inhibit photoinduced skin damage.

Experimental

All chemicals unless otherwise stated were purchased from Sigma-Aldrich Company Ltd (USA). Silica gel chromatography was performed using 230-400 mesh silica purchased from EM science. NMR spectra were recorded on a Bruker AC250 NMR spectrometer. *J* values are given in Hz throughout. Mass spectra were performed by the Mass Spectrometry Center at The University of Texas at Austin. Low-resolution mass spectra were obtained with Bell and Howell 21-491 instrument.

Preparation of 2,5-bis(4-acetamidophenylhydroxymethyl)thiophene **1.** To a three-necked, round-bottomed flask flushed with argon was added anhydrous hexane (80 mL), TMEDA (7.6 mL, 0.05 mol) and *n*-butyllithium, (2.5 mol/L in hexane) (20 mL, 0.05 mol), thiophene (1.61 mL, 0.02 mol) was then added at room temperature, the mixture was refluxed for 1 h. After cooling to room temperature, the suspension formed was slowly transferred *via* needle to a degassed solution of 4-acetamidobenzaldehyde (6.52 g, 0.04 mol) in anhydrous THF (200 mL) in an ice-bath. After the addition was completed, the mixture was allowed to warm to room temperature and stirred for further 30 min. MeOH (20 mL) and ice-cold NH_4Cl (40 mL, 5mol/L) were added separately with stirring. The phases were separated and the water layer was extracted with chloroform. The organic layers were combined, washed with water and dried over Na_2SO_4. After removal of solvents, the residue was purified by chromatography on silica gel using $CHCl_3$/MeOH (8:1) as eluent giving **1** (5%). ^{1}HNMR (DMSO-d_6, δ_{ppm}) 9.89 (s, 2H), 7.48 (d, 4H, J=8.41Hz), 7.25 (d, 4H, J=8.24Hz), 6.59 (d, 2H, J=6.02Hz), 6.01 (d, 2H, J=4.17Hz), 5.73 (s, 2H), 2.00 (s, 6H); ^{13}CNMR (DMSO-d_6, δ_{ppm}) 168.1, 149.8, 139.8, 138.2, 126.3, 122.8, 118.7, 70.2, 24.0; MS (CI) 411(M+H).

Preparation of 5,10,15,20-tetra(4-acetamidophenyl)-21,23-dithiaporphyrin **2**. A mixture of 1.32 g (3.21 mmol) of compound **1** and 0.215 g (3.21 mmol) of pyrrole was dissolved in 500 mL of propionic acid. The mixture was heated to reflux for 1 h. After cooling to room temperature, the solvent was evaporated to dryness under high vacuum. The residue was purified by chromatography on silica gel using $CHCl_3$/MeOH (8:1 to 6:1) as eluent gave **2** (5%). ^{1}HNMR ($CDCl_3$/CD_3OD, δ_{ppm}) 9.44 (s, 4H), 8.40 (s, 4H), 7.90 (d, 8H, J=8.4Hz), 7.75 (d, 8H, J=8.3Hz), 2.03 (s, 12H); MS (CI) 877(M).

Preparation of 5,10,15,20-tetra(4-aminophenyl)-21,23-dithiaporphyrin **3.** 215 mg of crude compound **2** was dissolved in 25 mL of TFA and 30 mL of conc. HCl was added at room temperature. The resulting mixture was heated at 80-85°C for 24 h, then cooled to 0°C. It was diluted with 10 mL of water, neutralized with 1 mol/L NaOH to pH 8-9, extracted with chloroform, and then the organic layer was dried over Na_2SO_4. After removal of solvent, the residue was purified by chromatography on silica gel using chloroform-methanol (8:1) as eluent to give **3** (80%). HRMS (CI) (M+H) calcd. for $C_{44}H_{32}N_6S_2$, 709.2208; Found 709.2208.

Preparation of 5,10,15,20-tetra(6-quinolyl)-21,23-dithiaporphyrin **4.** To a stirred

mixture of 111 mg (0.9 mmol) of nitrobenzene, 138 mg (1.5 mmol) of glycerol and 53 mg (0.075 mmol) of compound **3** heated at 120°C, sulphuric acid was added in portion, the resulting mixture then was maintained at 140°C for 5 h, 2 mol/L NaOH was added until pH 9-10. The mixture was extracted with $CHCl_3$, and the organic layer was dried over Na_2SO_4. After removal of solvent, the residue was separated with PTLC ($CHCl_3$/MeOH 95:5) to give **4** (23.7%). ^{1}HNMR ($CDCl_3$, δ_{ppm}) 9.69 (s, 4H), 9.15 (d, 4H, J=3.03Hz), 8.68-8.64 (m, 12H), 8.52 (d, 4H, J=8.90Hz), 8.42 (d, 4H, J=8.44Hz), 7.62 (dd, 4H, J=4.23Hz, J=4.23Hz); HRMS (CI) (M+H) calcd. for $C_{56}H_{33}N_6S_2$, 853.2208; Found 853.2224.

Preparation of 5, 10, 15, 20- tetra (N-methyl-6-quinolyl)- 21, 23-dithiaporphyrin chloride **5.** 13.2 mg (0.155 mmol) of compound **4** was dissolved in 4.0 mL of chloroform and diluted with 3.0 mL of nitromethane. 3.0 mL of iodomethane was added and the mixture was heated at reflux under argon for 6 h and then stirred overnight. After removal of solvent to dryness, 5.0 mL of water was added to the residue and treated with 2.0 g of Dowex 1x2-200 anion exchange resin in the chloride form, shaking slowly for 2 h. The resin was filtrated off, washed with water, and the filtrate lyophilized to give the chloride salt (70%). The salt could be further purified by chromatography on lipophilic sephadex using methanol as eluent. ^{1}HNMR (DMSO-d_6, δ_{ppm}) 9.88-9.80 (m, 8H), 9.63 (br d, 4H, J=6.76Hz), 9.43 (d, 4H, J=9.42Hz), 9.20 (br t, 4H, J=9.81Hz), 9.04 (d, 4H, J=7.84Hz), 8.69 (br s, 4H), 8.42 (m, 4H), 4.95 (s, 12H); HRMS (FAB) (M) calcd. for $C_{60}H_{44}N_6S_2$, 912.3069; Found 912.3077.

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