An α₁-adrenergic receptor ligand repurposed as potent antiproliferative agent on head and neck squamous cell carcinoma

Chiara Zagni,ab Douglas Magno Guimarães,b Loredana Salerno,a Francesco Punzo,a Cristiane H. Squarize,b Placido G. Mineo,cd Giuseppe Romeo**a and Antonio Rescifina*a

a Dipartimento di Scienze del Farmaco, Università di Catania, V.le A. Doria 6, I-95126 Catania, Italy. E-mail: arescifina@unict.it; gromeo@unict.it; Fax: +3906233208980; Tel: +39390957385017
b Laboratory of Epithelial Biology, Department of Periodontics and Oral Medicine, University of Michigan, Ann Arbor, MI 48109-1078, USA
c CNR-IPCF Istituto per i Processi Chimico Fisici, Viale Ferdinando Stagno D’Alcontres, 37, 98158 Messina, Italy
d Dipartimento di Scienze Chimiche and I.N.S.T.M. UdR of Catania, Università di Catania, Viale A. Doria, 6, 95125 Catania, Italy

Spectroscopic and analytical data for RN5-Me

General methods and instrumentation

Melting point was determined in open capillary on a Büchi 530 apparatus and is uncorrected. Elemental analyses (C, H, N) was performed on a Carlo Erba CHNS-0 EA1106 analyser; the analytical results were within ±0.4% of the theoretical value assuring a purity ≥95%.

NMR spectra were measured with a Varian VNMR-S 500 MHz spectrometer (Varian, Palo Alto CA, USA) at 300 K using a 5 mm inverse detection broadband probe. A total of 10 mg of compound was dissolved in 0.7 mL of DMSO-d6. Chemical shifts were given in ppm relative to the remaining signals of DMSO as an internal reference (1H NMR: 2.50 ppm; 13C NMR: 39.5 ppm). 1H NMR spectrum was recorded at 499.883 MHz with the following parameters: 6.7 µs 90° pulse length, 4500 Hz spectral width, 64k data points, 24 scans, and relaxation delay 1 s. 13C NMR spectrum was recorded at 125.709 MHz with 1H WALTZ decoupling and APT macro; other parameters were chosen as follows: 26400 Hz spectral width, 64k data points, 4000 scans, relaxation delay 10 s, and decoupling field 2.5 kHz. 2D gradient enhanced 1H-1H correlated (gCOSY) spectrum was acquired with 16 scans per t1 value for 512 experiments of 2048 data points and processed with linear prediction.

The MALDI-TOF mass spectrum was acquired by a Voyager DE (PerSeptive Biosystem) using a delay extraction procedure (25 kV applied after 1000 ns with a potential gradient of 454 V/mm and a wire voltage of 25 V) with ion detection in linear mode. The instrument was equipped with a nitrogen laser (emission at 337 nm for 3 ns) and a flash AD converter (time base 2). To prevent fragmentation of the polymers, the laser irradiance was slightly above the threshold (ca. 106 W/cm²). Each spectrum was an average of 32 laser shots. The MALDI experiments were performed by loading a 0.1 mmol sample and 40 mmol matrix trans-3-indoleacrylic acid (IAA) onto the sample plate with DMF as the solvent. Both 5,10-di(p-dodecanoxyphenyl)-15,20-di(p-hydroxyphenyl) porphyrin (C₆₈H₇₈N₄O₄, 1014 Da), tetrakis(p-dodecanoxyphenyl)porphyrin
RN5-Me data

White powder, mp 175 °C (from EtOH/H₂O).

δ_H(500 MHz, DMSO-d₆, DMSO-d₆) 2.61–2.66 (6H, m), 2.97–2.99 (6H, m), 3.78 (3H, s, OMe), 3.84 (3H, s, NMe), 4.08 (3H, s, NMe), 4.14 (2H, dd, J = 6.8, 8.2 Hz, N(3)CH₂), 6.85–6.93 (4H, m, H(3–6) aromatics), 7.19 (1H, ddd, J = 1.1, 8.2, 8.4 Hz, H(8) aromatic), 7.49 (1H, ddd, J = 1.1, 8.2, 8.6 Hz, H(7) aromatic), 7.60 (1H, dt, J = 1.1, 8.6 Hz, H(6) aromatic), 8.10 (1H, dt, J = 1.1, 8.4 Hz, H(9) aromatic).

δ_C(125 MHz, DMSO-d₆, DMSO-d₆) 30.77, 32.43, 38.19, 50.01, 53.13, 55.15, 55.25, 110.90, 111.87, 113.26, 114.46, 117.82, 120.03, 120.78, 121.97, 122.24, 126.90, 127.21, 139.20, 141.19, 150.43, 151.90, 155.97.

Found: C, 67.3; H, 6.4; N, 15.9. C_{25}H_{29}N_{5}O_{3} requires C, 67.1; H, 6.5; N, 15.65%.

MALDI-TOF MS: m/z 448.83 MH⁺; 470.70 MNa⁺; 486.74 MK⁺.
Fig. S1 $^1$H NMR spectrum of RN5-Me.

Fig. S2 gCOSY spectrum of RN5-Me.
**Fig. S3** APT spectrum of RN5-Me.

**Fig. S4** MALDI-TOF mass spectrum of RN5-Me.