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An α_1-adrenergic receptor ligand repurposed as potent antiproliferative agent on head and neck squamous cell carcinoma

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Spectroscopic and analytical data for RN5-Me

General methods and instrumentation

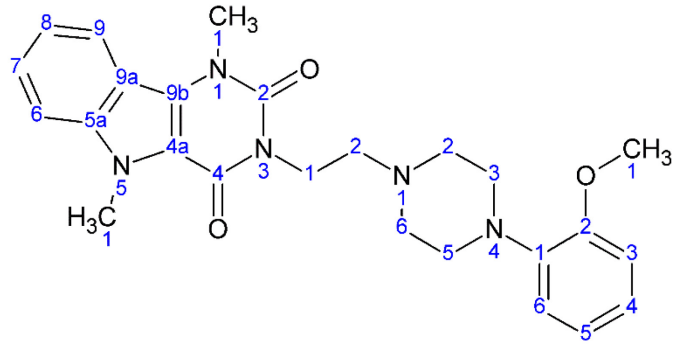
Melting point was determined in open capillary on a Büchi 530 apparatus and is uncorrected. Elemental analyses (C, H, N) was performed on a Carlo Erba CHNS-0 EA1106 analyser; the analytical results were within ±0.4% of the theoretical value assuring a purity ≥95%.

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 (^{1}H NMR: 2.50 ppm; ^{13}C NMR: 39.5 ppm). ^{1}H 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. ^{13}C 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 ^{1}H-^{1}H 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/cm^2). 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 ($C_{68}H_{78}N_4O_4$, 1014 Da), tetrakis(*p*-dodecanoxyphenyl)porphyrin

($C_{92}H_{126}N_4O_4$, 1350 Da) and a PEG sample of a known structure were used as external standards for m/z calibration.

RN5-Me data



3-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-1,5-dimethyl-1*H*-pyrimido[5,4-*b*]indole-2,4(3*H*,5*H*)-dione.

White powder, mp 175 °C (from EtOH/H_2O).

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)CH_2), 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. $C_{25}H_{29}N_5O_3$ requires C, 67.1; H, 6.5; N, 15.65%.

MALDI-TOF MS: *m/z* 448.83 MH^+; 470.70 MNa^+; 486.74 MK^+.

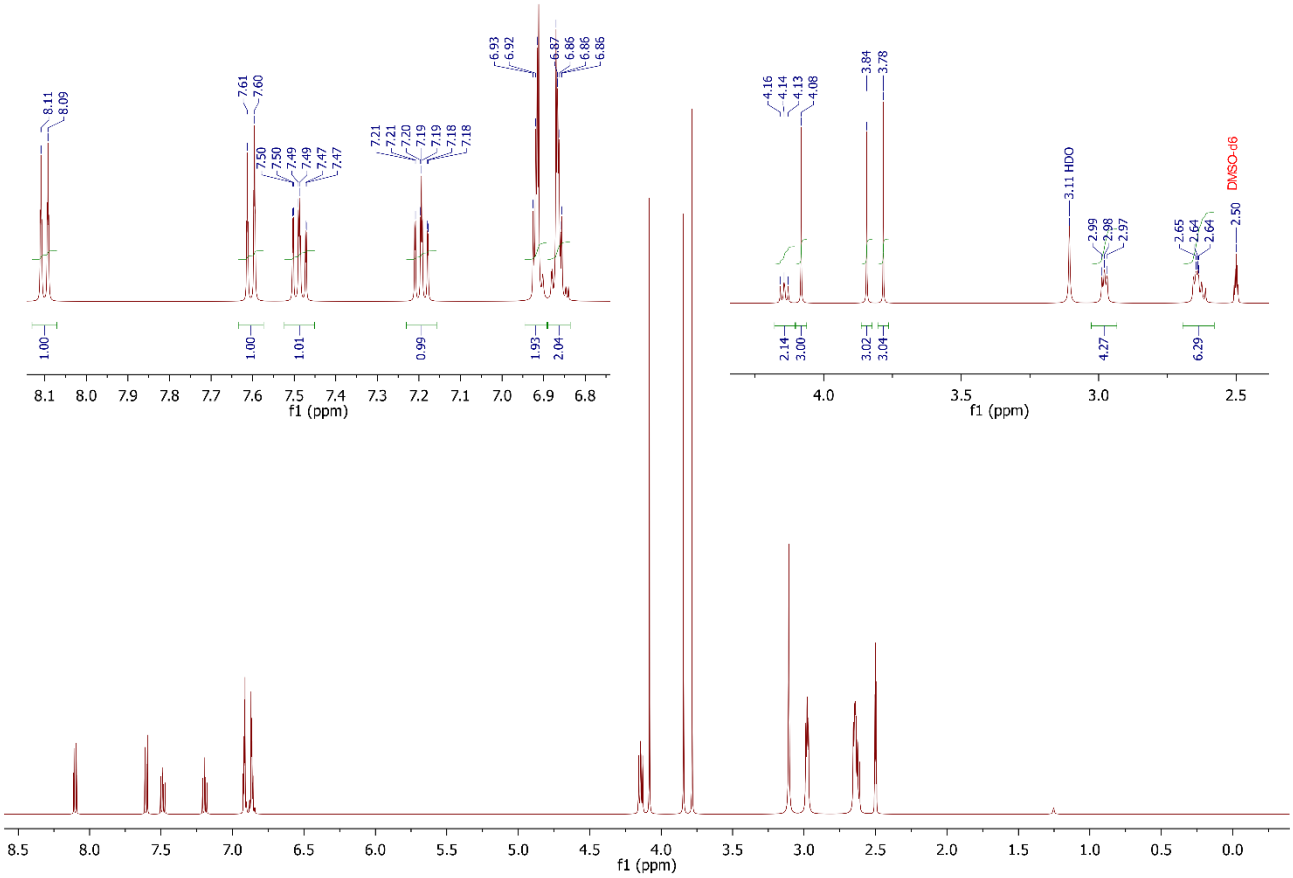


Fig. S1 ^{1}H NMR spectrum of RN5-Me.

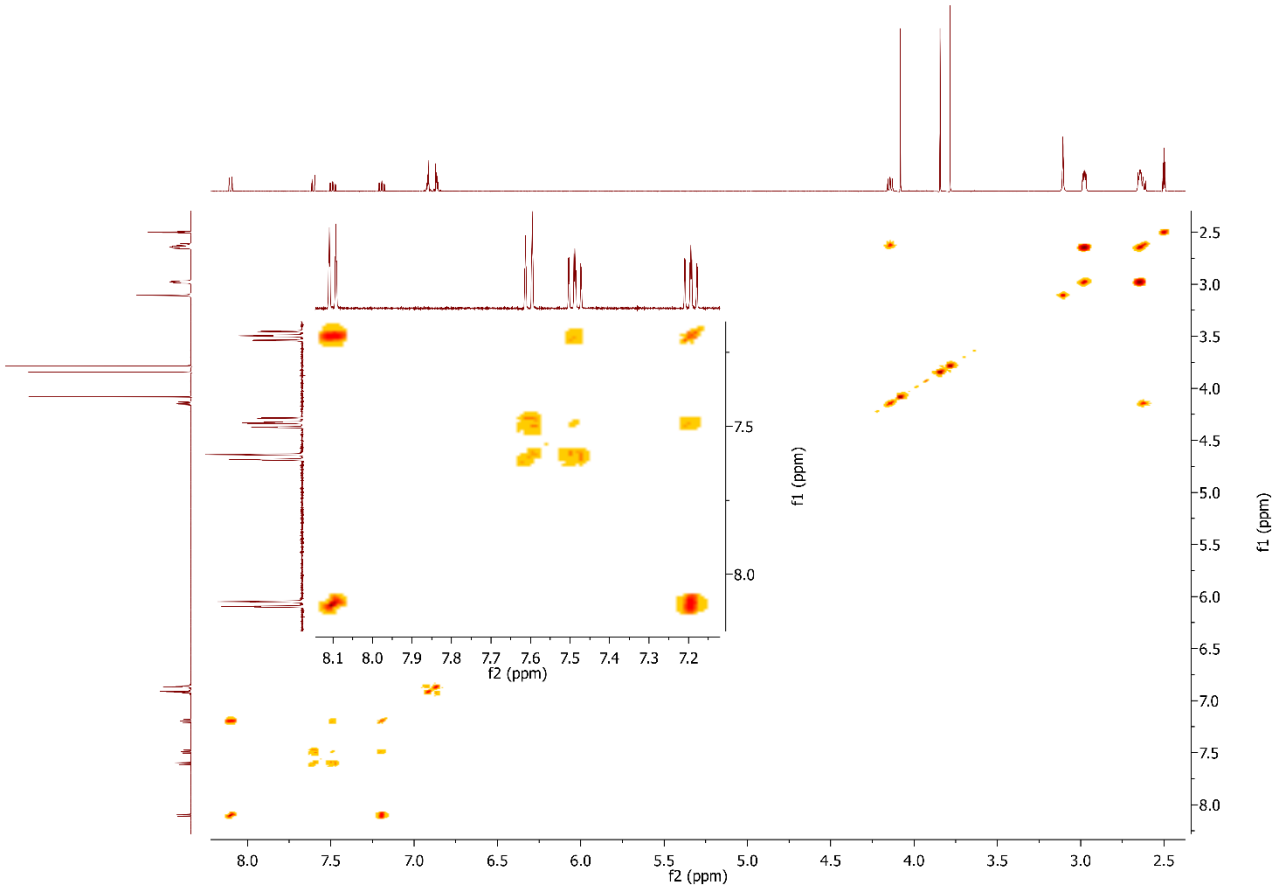


Fig. S2 gCOSY spectrum of RN5-Me.

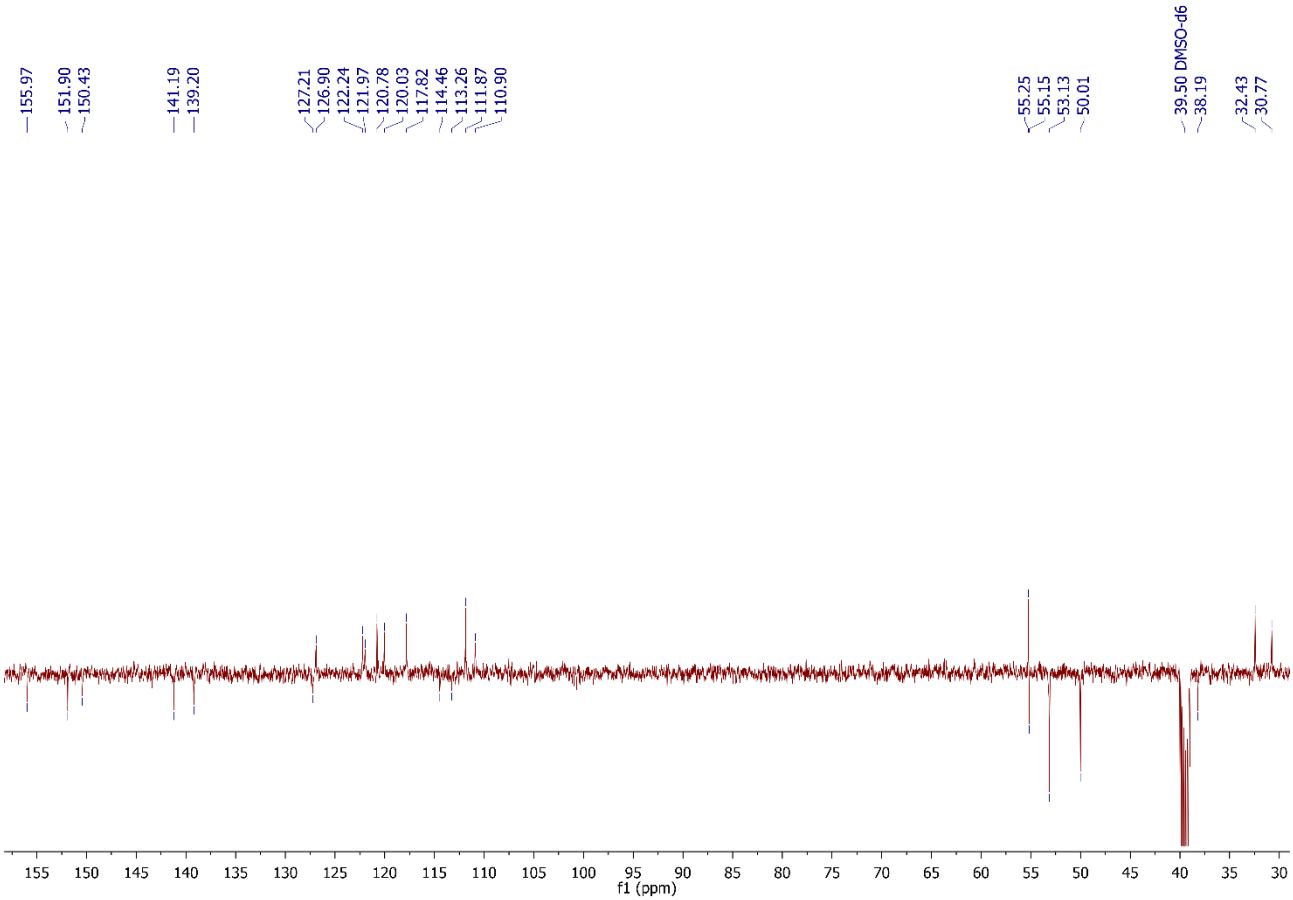


Fig. S3 APT spectrum of RN5-Me.

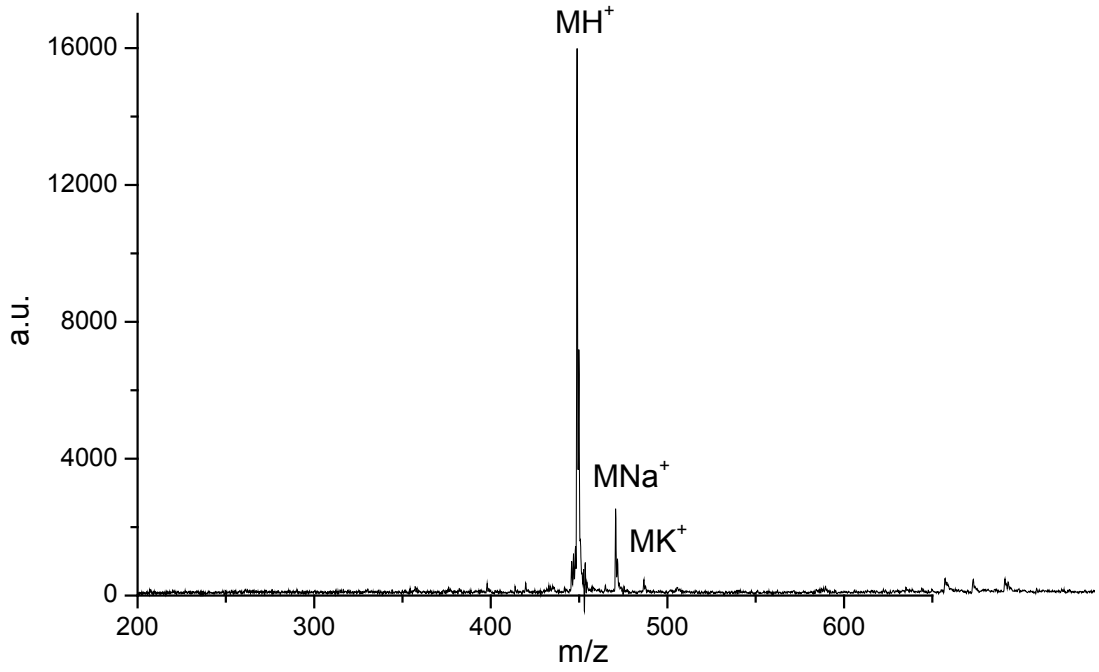


Fig. S4 MALDI-TOF mass spectrum of RN5-Me.