Supporting Information

Phenylpyrazolo[1,5-*a*]quinolin-5(4*H*)-one: a suitable scaffold for the development of non-camptothecin Topoisomerase I (Top1) inhibitors.

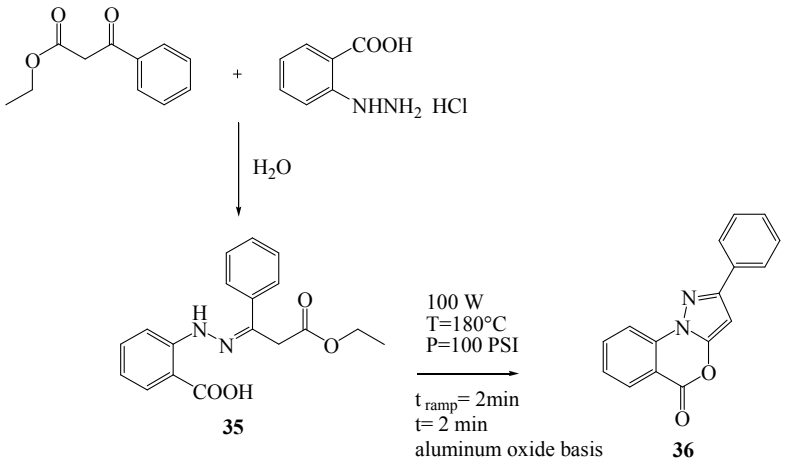
Sabrina Taliani, Isabella Pugliesi, Elisabetta Barresi, Silvia Salerno, Christophe Marchand, Keli Agama, Francesca Simorini, Concettina La Motta, Anna Maria Marini, Francesco Saverio Di Leva, Luciana Marinelli, Sandro Cosconati, Ettore Novellino, Yves Pommier, Roberto Di Santo, and Federico Da Settimo.

Table of Contents

Chemistry

The 2-phenyl-5*H*-pyrazolo[1,5-*a*][3,1]benzoxazin-5-one **36** (Scheme 1S) was obtained by a synthetic protocol that represents an improvement of reported procedures[1,2] and involved the use of microwaves (MW). MW-assisted synthesis is a technique widely used to perform and to accelerate organic reactions. This method offers a number of advantages, represented by shorter reaction time, lower temperature with potential energetic saving, solvent removal, allowing a green chemistry and cleaner reactions with minimal discarding and by-products. The commercially available 2-hydrazinobenzoic acid hydrochloride and ethylbenzoylacetate were reacted in water at 20 °C for two hours to yield the hydrazone **35**, that was then transformed into the lactone **36** by heating above its melting point, through the use of MW: at a temperature of 180 °C, with a pressure of 100 PSI, and power of 100 W for 2 minutes, using aluminum oxide basis as solid support (Scheme 1S). The aluminium oxide basis acts both as dispersing heat and as a carrier of the microwave, allowing a more homogeneous reaction environment.

Scheme 1S. Synthesis of 2-phenyl-5*H*-pyrazolo[1,5-*a*][3,1]benzoxazin-5-one **36.**

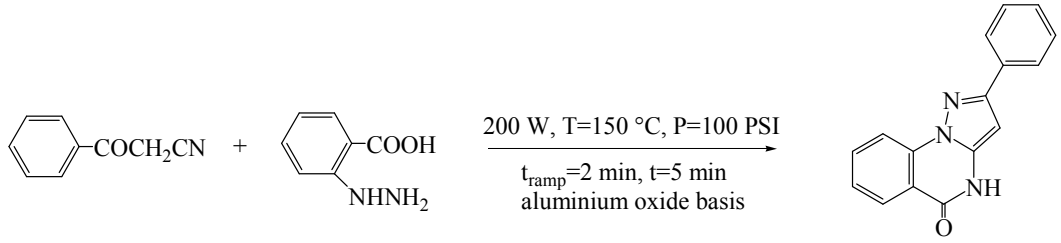


The phenylpyrazolo[1,5-*a*]quinazolin-5(4*H*)-ones **37-41** were obtained by improving synthetic procedures reported in the literature (Schemes 2S and 3S).[3,4] A one-pot MW-assisted solution phase protocol for the preparation of 2-phenylpyrazolo[1,5-*a*]quinazolin-5(4*H*)-one **37**, 3-(4-chlorophenyl)pyrazolo[1,5-*a*]quinazolin-5(4*H*)-one **40**, and 3-phenylpyrazolo[1,5-*a*]quinazolin-5(4*H*)-one **41** was described: condensation of 2-hydrazinobenzoic acid and the appropriate α-cyano-ketones in acetic acid through irradiation at 150 °C for 5-10 minutes,[3,4] and subsequent washing with ether of the precipitate formed yielded, for example, compound **37** with a total 85% yield.[3] We simplify the reaction work-up and optimized the yield to 95-98% by setting up a solvent-free procedure. For **37**, the two reagents (benzoylacetonitrile and 2-hydrazinobenzoic acid) were irradiated at a temperature of 150 °C, with a pressure of 100 PSI and power of 200 W for 5 minutes, using aluminium oxide basis as solid support (Scheme 2S).

For the 3-phenylpyrazolo[1,5-*a*]quinazolin-5(4*H*)-ones **38-41** the synthesis started from the easy preparation of the α-formyl-(4-substituted)-phenylacetonitriles **46-49** (Scheme 3S): a solution of the appropriate 4-substituted-phenylacetonitrile (**42-45**) and ethyl formate in anhydrous toluene was added dropwise to a solution of sodium methoxide in the same solvent, and the reaction mixture

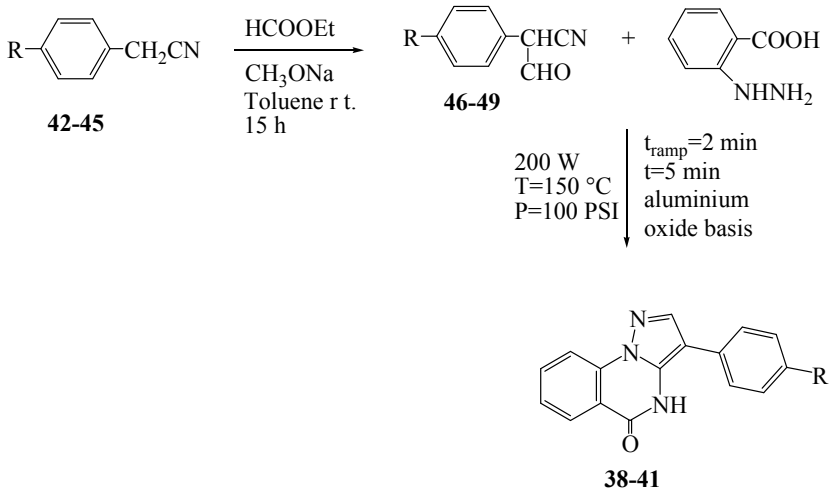
was stirred for 15 hours at room temperature.[5] The obtained α-cyano-aldehydes **46-49** were then reacted with 2-hydrazinobenzoic acid hydrochloride in the presence of aluminium oxide basis, under the same conditions utilized for the synthesis of the corresponding 2-phenyl derivative **37** (Scheme 3S), furnishing compounds **38-41** in 95-98% yields.

Scheme 2S. Synthesis of 2-phenylpyrazolo[1,5-*a*]quinazolin-5(4*H*)-one **37**.



37

Scheme 3S. Synthesis of 3-phenylpyrazolo[1,5-*a*]quinazolin-5(4*H*)-ones **38-41**



42-45

46-49

38-41

38, 42, 46: R = OCH_3
39, 43, 47: R = CF_3
40, 44, 48: R = Cl
41, 45, 49: R = H

General Chemistry Directions.

Melting points were determined using a Reichert Köfler hot-stage apparatus and are uncorrected. Infrared spectra were recorded with a Nicolet/Avatar 360 FT-IR spectrometer in Nujol mulls. Routine nuclear magnetic resonance spectra were recorded in DMSO-d_6 solution on a Varian Gemini 200 spectrometer operating at 200 MHz. Evaporation was performed in vacuo (rotary evaporator). Analytical TLC was carried out on Merck 0.2 mm precoated silica gel aluminum sheets (60 F-254). Microwaves-assisted synthesis were performed by CEM Discover labmate (DU5124). Combustion analyses on target compounds were performed by our Analytical Laboratory in Pisa. All compounds showed ≥ 95% purity.

Ethyl 2-(2-(3-ethoxy-3-oxo-1-phenylpropylidene)hydrazinyl)benzoate 35. Ethyl benzoylacetate (0.17 mL, 0.001 mol) was added to a solution of 2-hydrazinobenzoic acid (0.188 g, 0.001 mol) in 10 mL of water. The reaction mixture was allowed to stir for 2 h at room temperature. A precipitate formed, which was collected by vacuum filtration and purified by crystallization from Petroleum Ether 60-80 °C. Yield 81%; mp 162-164 °C, lit ref n.[1] 166-167 °C.

2-Phenyl-5*H*-pyrazolo[1,5-*a*][3,1]benzoxazin-5-one 36. Ethyl 2-(2-(3-ethoxy-3-oxo-1-phenylpropylidene)hydrazinyl)benzoate **35** (0.326 g, 0.001 moli) was irradiated at a temperature of 180 °C with a pressure of 100 PSI and power of 100 W for 2 minutes, using aluminium oxide basics as solid support. Then, the mixture was extracted with hot AcOEt and the organic solvent was evaporated to dryness. The product **36** was finally purified by crystallization from ethanol. Yield 76%; mp 195-197 °C, lit. ref n.[1]: mp 198-200 °C.

General procedure for the synthesis of 2-phenylpyrazolo[1,5-*a*]quinazolin-5(4*H*)-one 37 and 3-(4-substituted-phenyl)pyrazolo[1,5-*a*]quinazolin-5(4*H*)-ones 38-41. A mixture of the appropriate 2-formylacetonitrile **46-49** or benzoylacetonitrile (0.0012 moli) and 2-hydrazinobenzoic acid (0.452 g, 0.0024 moli) were irradiated at a temperature of 150 °C with a pressure of 100 PSI and power of 200 W for 5 minutes and using aluminium oxide basics as solid support. Then, the mixture was extracted with hot AcOEt and the organic solvent was evaporated to dryness. Products **37-41** were purified by crystallization from EtOH.

*2-Phenylpyrazolo[1,5-a]quinazolin-5(4H)-one **37***. Yield 98%; mp>300 °C. lit. ref n.[2]: mp 315 °C. IR (nujol, cm^{-1}): 3140, 3078, 1676, 1607, 1136, 750. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 6.38 (s, 1H, Ar-H); 7.39-7.54 (m, 4H, Ar-H); 7.86-7.99 (m, 3H, Ar-H); 8.13-8.18 (m, 2H, Ar-H); 12.34 (bs exch., 1H, NH). Anal. Calcd. for $C_{16}H_{11}N_3O$: C, 73.56; H, 4.21; N, 16.92. Found: C, 73.95; H, 4.17; N, 16.88.

*3-(4-Methoxyphenyl)pyrazolo[1,5-a]quinazolin-5(4H)-one **38***. Yield 95%; mp 258-260 °C. IR (nujol, cm^{-1}): 3160, 3058, 1662, 1611, 1119, 750. ^1H NMR (200 MHz, DMSO-d_6, δ ppm):3.80 (s, 3H, CH_3); 6.99 (dd, 2H, *J* = 7.2, 1.2 Hz, Ar-H); 7.47-7.55 (m, 3H, Ar-H); 7.91 (dt, 1H, *J* =8.6, 1.4 Ar-H); 8.04-8.19 (m, 3H, Ar-H); 12.05 (bs exch., 1H, NH). Anal. Calcd. for $C_{17}H_{13}N_3O_2$: C, 70.09; H, 4.50; N, 14.42. Found: C, 70.39; H, 4.65; N, 14.65.

*3-(4-Trifluoromethylphenyl)pyrazolo[1,5-a]quinazolin-5(4H)-one **39***. Yield 96%; mp>300 °C. IR (nujol, cm^{-1}): 3155, 3073, 1658, 1607, 1102, 753. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 7.51-7.58 (t, 1H, *J*=7.5 Hz, Ar-H); 7.77-7.85 (m, 4H, Ar-H); 7.93 (dt, 1H, *J*=8.4 ,1.2 Hz, Ar-H)8.13-8.24 (m, 3H, Ar-H); 12.27 (bs exch., 1H, NH). Anal. Calcd. for $C_{17}H_{10}N_3O$: C, 62.01; H, 3.06; N, 12.76. Found: C, 62.41; H, 3.86; N, 13.00.

*3-(4-Chlorophenyl)pyrazolo[1,5-a]quinazolin-5(4H)-one **40***.[4] Yield 98%; mp>300 °C. IR (nujol, cm^{-1}): 3140, 3072, 1676, 1614, 1095, 753. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 7.44-7.65 (m, 5H, Ar-H); 7.92 (dt, 1H, *J*=8.6, 1.4 Hz, Ar-H); 8.12-8.20 (m, 3H, Ar-H); 12.19 (bs exch., 1H, NH). Anal. Calcd. for $C_{16}H_{10}N_3OCl$: C, 64.98; H, 3.41; N, 14.21. Found: C, 65.22; H, 3.71; N, 14.71.

*3-Phenylpyrazolo[1,5-a]quinazolin-5(4H)-one **41***.[4] Yield 95%; mp 260-262 °C. IR (nujol, cm^{-1}): 3174, 3078, 1672, 1611, 1115, 750. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 7.28-7.62 (m, 6H, Ar-H); 7.92 (dt, 1H, *J*=7.2, 1.4 Hz, Ar-H,); 8.13-8-20 (m, 3H, Ar-H); 12.12 (bs exch., 1H, NH). Anal. Calcd. for $C_{16}H_{11}N_3O$: C, 73.56; H, 4.21; N, 16.92. Found: C, 72.22; H, 4.21; N, 15.89.

General procedure for the synthesis of 2-(4-substitutedphenyl)-2-formylacetonitrile derivatives 46-49. Sodium methoxyde was prepared by adding portionwise sodium (0.236 g, 0.01 mol) to 10 mL of MeOH at room temperature. A solution of the appropriate 4-substituted-phenylacetonitrile (0.01 mol) and ethyl formate (0.8 ml, 0.01 mol) in 20 ml of dry toluene was added dropwise. The mixture was stirring for 15 h at room temperature (TLC analysis) and then filtered. The collected precipitate was dissolved in water and the mixture was acidified with 20% HCl to pH=3.5. The precipitate collected gave a first portion of product. The toluene solution was extracted with water, and the aqueous solution was acidified with 20% HCl to pH=3.5. The precipitate collected yielded an additional amount of product. Compounds **46-49** resulted sufficiently pure to be used in the next reaction without further purification.

*2-(4-Methoxyphenyl)-2-formylacetonitrile **46.*** Yield 66%; mp 118-120 °C, lit. ref n.[6]: mp 100-102 °C.

*2-(4-Trifluoromethylphenyl)-2-formylacetonitrile **47.*** Yield 62%; mp 156-158 °C. IR (nujol, cm^{-1}): 3103, 1644, 1260, 1168, 1117, 840. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 7.62-7.90 (m, 4H, Ar-H); 8.26 (s, 1H, Ar-H); 12.54 (bs exch., 1H, CHO). Anal. Calcd. for $C_{10}H_6NO$: C, 56.35; H, 2.84; N, 6.57. Found: C, 56.75; H, 3.01; N, 6.69.

*2-(4-Chlorophenyl)-2-formylacetonitrile **48.*** Yield 70%; mp 150-152 °C, lit. ref n.[6]: mp 160-165 °C.

*2-Phenyl-2-formylacetonitrile **49.*** Yield 74%; mp 155-157 °C, lit. ref n.[7]: mp 158-160 °C.

General procedure for the synthesis of 5-chloro-2-phenylpyrazolo[1,5-*a*]quinazolin-5(4*H*)-one 50 and 5-chloro-3-(4-substituted-phenyl)pyrazolo[1,5-*a*]quinazolin-5(4*H*)-one derivatives 51-54. A mixture of the appropriate phenylpyrazolo[1,5-*a*]quinazolin-5(4*H*)-one **37-41** (0.001 mol) and 3 ml of $PhPOCl_2$ was heated at 180 °C for 4 h, under stirring and with exclusion of moisture. After cooling, the excess reagent was decomposed by addition of ice and water; the obtained aqueous suspension was neutralized with conc. ammonia, after which the desiderate products **50-54** were isolated in a pure state by filtration in the desired purity degree (≥ 95%). Samples of **50-54** were characterized after crystallization from ethanol.

*5-Chloro-2-phenylpyrazolo[1,5-a]quinazolin-5(4H)-one **50.*** Yield 99 %; mp 137-139 °C. IR (nujol, cm^{-1}): 3126, 1614, 1556, 1269, 969, 801, 760. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 7.42 (s, 1H, Ar-H); 7.45-7.58 (m, 3H, Ar-H); 7.74 (t, 1H, *J*=6.9 Hz, Ar-H); 8.09 (t, 1H, *J*=7.0 Hz, Ar-H); 8.12-8.17 (m, 2H, Ar-H); 8.28 (d, 1H, *J*=8.2 Hz, Ar-H); 8.51 (d, 1H, *J*=8.2 Hz, Ar-H). Anal. Calcd. for $C_{10}H_{10}ClN_3$: C, 68.70; H, 3.60; N, 15.02. Found: C, 69.01; H, 3.92; N, 15.35.

*5-Chloro-3-(4-methoxyphenyl)pyrazolo[1,5-a]quinazolin-5(4H)-one **51.*** Yield 97%; mp 228-230 °C. IR (nujol, cm^{-1}). ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 3.81 (s, 3H, OCH_3); 7.06-7.30 (m, 3H, Ar-H); 8.29 (d, 1H, *J*=8.0 Hz, Ar-H); 8.45 (d, 1H, *J*=8.2 Hz, Ar-H); 8.69 (s, 1H, Ar-H). Anal. Calcd. for $C_{17}H_9N_3$: C, 58.72; H, 2.61; N, 12.08. Found: C, 58.92; H, 2.81; N, 12.28.

*5-Chloro-3-(4-trifluoromethylphenyl)pyrazolo[1,5-a]quinazolin-5(4H)-one **52**.* Yield 100 %; mp 170-172 °C. IR (nujol, cm^{-1}): 3100, 1610, 1566, 1241, 1166, 770. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 7.75-7.88 (m, 3H, Ar-H); 8.12-8.35 (m, 4H, Ar-H); 8.49 (d, 1H, *J*=8.4 Hz, Ar-H); 8.89 (s, 1H, Ar-H). Anal. Calcd. for $C_{17}H_9ClF_3N_3$: C, 58.72; H, 2.61; N, 12.08. Found: C, 58.95; H, 2.84; N, 12.36.

*5-Chloro-3-(4-chlorophenyl)pyrazolo[1,5-a]quinazolin-5(4H)-one **53**.* Yield 88 %; mp 208-210 °C. IR (nujol, cm^{-1}): 3106, 1614, 1597, 1273, 1190, 760. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 7.53-7.91 (m, 3H, Ar-H); 8.09-8.13 (m, 3H, Ar-H); 8.30 (d, 1H, *J*=6.8 Hz, Ar-H); 8.44 (d, 1H, *J*=6.0 Hz, Ar-H); 8.77 (s, 1H, Ar-H). Anal. Calcd. for $C_{16}H_9ClN_3$: C, 61.17; H, 2.89; N, 13.38. Found: C, 61.28; H, 2.98; N, 13.43.

*5-Chloro-3-phenylpyrazolo[1,5-a]quinazolin-5(4H)-one **54**.* Yield 99 %; mp 180-182 °C. IR (nujol, cm^{-1}): 3147, 1603, 1563, 1269, 1142, 756. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 7.31-7.76 (m, 5H, Ar-H); 8.10-8.14 (m, 2H, Ar-H); 8.30 (d, 1H, *J*=8.2 Hz, Ar-H); 8.47 (d, 1H, *J*=8.2 Hz, Ar-H); 8.77 (s, 1H, Ar-H). Anal. Calcd. for $C_{10}H_{10}ClN_3$: C, 68.70; H, 3.60; N, 15.02. Found: C, 69.00; H, 3.90; N, 15.32.

Yields, Physical, and Spectral Data of Pyrazoloquinazoline Derivatives 1-34.

*4-(2-Dimethylaminoethyl)-2-phenylpyrazolo[1,5-a]quinazolin-5(4H)-one **1***. Yield 66 %, mp 113-115 °C. IR (nujol, cm^{-1}): 1672, 1617, 1562, 1337, 1030, 750. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 2.23 (s, 6H, $2CH_3$); 2.65 (t, 2H, *J*=6.5 Hz, CH_2); 4.20 (t, 2H, *J*=6.8 Hz, CH_2); 6.88 (s, 1H, Ar-H); 7.42-7.56 (m, 4H, Ar-H); 7.93-8.03 (m, 3H, Ar-H); 8.17-8.21 (m, 2H, Ar-H). Anal. Calcd. for $C_{20}H_{20}N_4O$: C, 72.27; H, 6.06; N, 16.86. Found: C, 72.21; H, 6.10; N, 17.01.

*4-(2-Dimethylamino-1-propyl)-2-phenylpyrazolo[1,5-a]quinazolin-5(4H)-one **2***. Yield 62 %, oil. IR (nujol, cm^{-1}): 1672, 1614, 1562, 1334, 1033, 743. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 1.82-1.93 (m, 2H, CH_2); 2.36 (t, 2H, *J*=6.9 Hz, CH_2); 4.13 (t, 2H, *J*=7.2 Hz, CH_2); 6.89 (s, 1H, Ar-H); 7.42-7.56 (m, 4H, Ar-H); 7.93-8.02 (m, 3H, Ar-H); 8.17-8.21 (m, 2H, Ar-H). Anal. Calcd. for $C_{21}H_{22}N_4O$: C, 72.81; H, 6.40; N, 16.17. Found: C, 73.01; H, 6.60; N, 16.37.

*4-(Imidazolyl-1-propyl)-2-phenylpyrazolo[1,5-a]quinazolin-5(4H)-one **3***. Yield 63%, mp 83-85 °C. IR (nujol, cm^{-1}):1648, 1597, 1562, 1334, 1023, 753. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 2.21 (t, 2H, *J*=7.5 Hz, CH_2); 4.12 (q, 4H, *J*=6.6 Hz, $2CH_2$); 6.71 (s, 1H, Ar-H); 6.91 (s, 1H, Ar-H); 7.26 (s, 1H, Ar-H); 7.42-7.55 (m, 4H, Ar-H); 7.68 (s, 1H, Ar-H); 7.88-7.99 (m, 3H, Ar-H); 8.16-8.22 (m, 2H, Ar-H). Anal. Calcd. for $C_{22}H_{19}N_5O$: C, 71.53; H, 5.18; N, 18.96. Found: C, 71.72; H, 5.38; N, 19.01.

*5-(2-Dimethylaminoethoxy)-2-phenylpyrazolo[1,5-a]quinazoline **4** hydrochloride.* Yield 63 %, mp 251-253 °C. IR (nujol, cm^{-1}): 3105, 2464, 1672, 1600, 1385, 756. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 2.91 (s, 6H, $2CH_3$); 3.51 (t, 2H, *J*=7 Hz, CH_2); 4.51 (t, 2H, *J*=6.8 Hz, CH_2); 7.14 (s, 1H, Ar-H); 7.43-7-58 (m, 4H, Ar-H); 7.91-8.01 (m, 3H, Ar-H); 8.20 (d, 2H, *J*=9.2 Hz, Ar-H,); 10.20 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{20}H_{21}ClN_4O$: C, 72.05; H, 6.35; N, 16.80. Found: C, 72.25; H, 6.52 ; N, 17.01.

*5-(2-Diethylaminoethoxy)-2-phenylpyrazolo[1,5-a]quinazoline **5** hydrochloride.* Yield 51 %, mp 241-243 °C. IR (nujol, cm^{-1}): 3092, 2409, 1672, 1597, 1334, 760. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 1.27 (t, 6H, *J*=7.1 Hz, $2CH_3$); 3.30 (m, 4H, $2CH_2$); 3.49 (t, 2H, *J*=7.2 Hz, CH_2); 4.54 (t, 2H, *J*=7.4 Hz, CH_2); 7.20 (s, 1H, Ar-H); 7.45-7.58 (m, 4H, Ar-H); 7.92-8.02 (m, 3H, Ar-H); 8.21 (d, 2H, *J*=8.2 Hz, Ar-H); 10.40 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{22}H_{25}ClN_4O$: C, 73.10; H, 6.97; N, 15.50. Found: C, 73.21; H, 7.03; N, 15.72.

*5-(2-Dimethylamino-1-propoxy)-2-phenylpyrazolo[1,5-a]quinazoline **6** hydrochloride.* Yield 63 %, mp 221-224 °C. IR (nujol, cm^{-1}): 3057, 2675, 1665, 1600, 1258, 746. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 2.15-2.19 (m, 2H, CH_2); 2.72 (s, 6H, $2CH_3$); 3.20 (t, 2H, *J*=7.7 Hz, CH_2); 4.20 (t, 2H, *J*=6.7 Hz, CH_2); 7.00 (s, 1H, Ar-H); 7.39-7.56 (m, 4H, Ar-H); 7.89-8.01 (m, 3H, Ar-H); 8.19 (d, 2H, *J*=8.4 Hz, Ar-H); 10.31 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{21}H_{23}ClN_4O$: C, 72.60; H, 6.67; N, 16.13. Found: C, 72.71; H, 6.85; N, 16.37.

*5-(2-Diethylaminoethylamino)-2-phenylpyrazolo[1,5-a]quinazoline **7** hydrochloride.* Yield 55 %, mp 126-128 °C. IR (nujol, cm^{-1}): 3296, 1665, 1620, 1456, 1313, 767. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 1.26 (t, 6H, *J*=4.2 Hz, $2CH_3$); 3.14-3.28 (m, 4H, $2CH_2$); 3.39 (t, 2H, *J*=5.6 Hz, CH_2); 3.93 (t, 2H, *J* = 4.8 Hz, CH_2); 6.68 (s, 1H, Ar-H); 7.35-7.55 (m, 4H, Ar-H); 7.89-8.03 (m, 3H, Ar-H); 8.27-8.33 (m, 2H, Ar-H); 8.68 (bs exch., 1H, NH^+); 10.45 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{22}H_{26}ClN_5$: C, 73.30; H, 7.27; N, 19.43. Found: C, 73.51; H, 7.33; N, 19.65.

5-(Imidazolyl-1-propyl)-2-phenylpyrazolo[1,5-a]quinazoline ***8*** *hydrochloride.* Yield 51%, mp 210-212 °C. IR (nujol, cm^{-1}): 3092, 2723, 1658, 1620, 1456, 1313, 763. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 2.25 (t, 2H, *J*=6.6 Hz, CH_2,); 3.53-3.58 (m, 2H, CH_2); 4.34 (t, 2H, *J*=6.6 Hz, CH_2,); 6.62 (s, 1H, Ar-H); 7.36-7.56 (m, 4H, Ar-H); 7.71 (s, 1H, Ar-H); 7.85-8.00 (m, 4H, Ar-H); 8.28 (d, 1H, *J*=8.2 Hz, Ar-H); 8.44-8.48 (m, 2H, Ar-H, NH); 9.28 (s, 1H, Ar-H); 14.6 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{22}H_{21}ClN_6$: C, 71.52; H, 5.73; N, 22.75. Found: C, 71.66; H, 5.89; N, 22.86.

3-(4-Methoxyphenyl)-5-(2-dimethylamino-1-ethoxy)pyrazolo[1,5-a]quinazoline ***9*** *hydrochloride.* Yield 55 %, mp 250-252 °C. IR (nujol, cm^{-1}): 3392, 2552, 1624, 1549, 1306, 760. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 2.92 (s, 6H, $2CH_3$); 3.71-3.74 (m, 2H, CH_2); 3.80 (s, 3H, CH_3); 4.94-4.99 (m, 2H, CH_2); 7.02 (d, 2H, *J*=8.8 Hz, Ar-H); 7.57 (t, 1H, *J*=7.4 Hz, Ar-H); 7.96-8.06 (m, 3H, Ar-H); 8.31 (d, 1H, *J*=8.4 Hz, Ar-H,); 8.44 (d, 1H, *J*=7.2 Hz, Ar-H); 8.52 (s, 1H, Ar-H); 10.91 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{21}H_{23}ClN_4O_2$: C, 69.40; H, 6.38; N, 15.42. Found: C, 69.60; H, 6.57; N, 15.70.

3-(4-Methoxyphenyl)-5-(2-diethylamino-1-ethoxy)pyrazolo[1,5-a]quinazoline ***10*** *hydrochloride.* Yield 53 %, mp 210-212 °C. IR (nujol, cm^{-1}): 3426, 2600, 1607, 1552, 1340, 763. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 1.31 (t, 6H, *J*=7.2 Hz, $2CH_3$); 3.25-3.34 (m, 4H, CH_2); 3.72-3.74 (m, 2H, CH_2); 3.81 (s, 3H, CH_3); 5.98-5.02 (m, 2H, CH_2); 7.03 (d, 2H, *J*=8.8 Hz, Ar-H); 7.64 (t, 1H, *J*=7.2 Hz, Ar-H); 7.99-8.06 (m, 3H, Ar-H); 8.32 (t, 2H, *J*=8.7 Hz, Ar-H); 8.52 (s, 1H, Ar-H); 10.18 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{23}H_{27}ClN_4O_2$: C, 70.56; H, 6.95; N, 14.31. Found: C, 70.78; H, 7.08; N, 14.52.

3-(4-Methoxyphenyl)-5-(2-dimethylamino-1-propoxy)pyrazolo[1,5-a]quinazoline ***11*** *hydrochloride.* Yield 58 %, mp 229-230 °C. IR (nujol, cm^{-1}): 3390, 2580, 1600, 1542, 1296, 763. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 2.28-2.36 (m, 2H, CH_2); 2.81 (d, 6H, *J*=4.8 Hz, $2CH_3$); 3.30-3.40 (m, 2H, CH_2); 3.79 (s, 3H, CH_3); 4.65-4.71 (m, 2H, CH_2); 7.01-7.04 (m, 3H, Ar-H); 7.60 (t, 1H, *J*=7.4 Hz, Ar-H); 7.95-8.05 (m, 2H, Ar-H); 8.21-8.32 (m, 2H, Ar-H); 8.43 (s, 1H, Ar-H); 10.91 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{22}H_{25}ClN_4O_2$: C, 70.00; H, 6.68; N, 14.84. Found: C, 70.20; H, 6.88; N, 14.98.

3-(4-Methoxyphenyl)-5-(2-dimethylaminoethylamino)pyrazolo[1,5-a]quinazoline ***12*** *hydrochloride.* Yield 60 %, mp 137-139 °C. IR (nujol, cm^{-1}): 3344, 2686, 1600, 1579, 1245, 753. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 2.85 (d, 6H, *J*=4.6 Hz, $2CH_3$); 3.45-3.47 (m, 2H, CH_2); 3.76 (s, 3H, CH_3); 3.97-4.00 (m, 2H, CH_2); 6.97 (d, 2H, *J*=8.6 Hz, Ar-H,); 7.52 (t, 1H, *J*=7.4 Hz, Ar-H,); 7.84 (t, 1H, *J*=7.8 Hz, Ar-H,); 8.01 (d, 2H, *J*=8.6 Hz, Ar-H); 8.21-8.30 (m, 2H, Ar-H); 8.47 (d, 1H, *J*=7.8 Hz, Ar-H); 8.62 (bs exch., 1H, NH); 10.56 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{21}H_{24}ClN_5O$: C, 69.59; H, 6.67; N, 19.32. Found: C, 69.79; H, 6.87; N, 19.43.

3-(4-Methoxyphenyl)-5-(2-diethylaminoethylamino)pyrazolo[1,5-a]quinazoline ***13*** *hydrochloride.* Yield 55%, mp 141-143 °C. IR (nujol, cm^{-1}): 3303, 2416, 1620, 1607, 1375, 753. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 1.22 (t, 6H, *J*=7.0 Hz, $2CH_3$); 3.20-3.26 (m, 4H, $2CH_2$); 3.41-3.44 (m, 2H, CH_2); 3.76 (s, 3H, CH_3); 3.94-4.04 (m, 2H, CH_2); 6.97 (d, 2H, *J*=8.6 Hz, Ar-H,); 7.53 (t, 1H, *J*=7.7 Hz, Ar-H,); 7.89 (t, 1H, *J*=7.8 Hz, Ar-H); 8.00 (d, 2H, *J*=8.6 Hz, Ar-H,); 8.22-8.31 (m, 2H, Ar-H); 8.55 (d, 1H, *J*=8.4 Hz, Ar-H); 8.67 (bs exch., 1H, NH); 10.49 (bs exch, 1H, NH^+). Anal. Calcd. for $C_{23}H_{28}ClN_5O$: C, 70.74; H, 7.23; N, 17.93. Found: C, 70.89; H, 7.51; N, 18.06.

3-(4-Methoxyphenyl)-5-(2-dimethylaminopropyl-1-amino)pyrazolo[1,5-a]quinazoline ***14*** *hydrochloride.* Yield 55 %, mp 256-258 °C. IR (nujol, cm^{-1}): 3269, 2587, 1620, 1600,1241, 753. ^1H

NMR (200 MHz, DMSO-d_6, δ ppm): 2.15-2.22 (m, 2H, CH_2); 2.74 (d, 6H, *J*=4.8 Hz, $2CH_3$); 3.15-3.25 (m, 2H, CH_2); 3.68-3.74 (m, 2H, CH_2); 3.78 (s, 1H, CH_3); 7.00 (d, 2H, *J*=8.8 Hz, Ar-H,); 7.53 (t, 1H, *J*=7.3 Hz, Ar-H); 7.88 (t, 1H, *J*=7.4 Hz, Ar-H); 8.04 (d, 2H, *J*=8.8 Hz, Ar-H);8.21-8.30 (m, 2H, Ar-H); 8.41-8.45 (m, 2H, Ar-H, NH); 10.58 (bs exch, 1H, NH^+). Anal. Calcd. for $C_{22}H_{26}ClN_5O$: C, 70.19; H, 6.96; N, 18.60. Found: C, 70.36; H, 6.25; N, 18.77.

3-(4-Methoxyphenyl)-5-(imidazolyl-1-propyl)pyrazolo[1,5-a]quinazoline ***15*** *hydrochloride.* Yield 61 %, mp 188-190 °C. IR (nujol, cm^{-1}): 3228, 2668, 1620, 1600, 828, 722. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 2.35 (t, 2H, *J*=6.4 Hz, CH_2); 3.59-3.65 (m, 2H, CH_2); 3.78 (s, 3H, Ar-H); 4.39 (t, 2H, *J*=6.7 Hz, CH_2); 6.96 (d, 2H, *J*=8.6 Hz, Ar-H,); 7.53 (t, 1H, *J*=7.4 Hz, Ar-H); 7.69 (s, 1H, Ar-H); 7.84-8.02 (m, 4H, Ar-H); 8.21-8.30 (m, 3H, Ar-H); 8.48 (bs exch., 1H, NH); 9.31 (s, 1H, Ar-H); 14.57 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{23}H_{23}ClN_6O$: C, 69.15; H, 5.80; N, 21.04. Found: C, 69.35; H, 5.96; N, 21.42.

5-(2-Dimethylamino-1-ethoxy)-3-(4-trifluoromethylphenyl)pyrazolo[1,5-a]quinazoline ***16*** *hydrochloride.* Yield 67 %, mp 240-242 °C. IR (nujol, cm^{-1}): 3421, 2607, 1608, 1552, 1330, 750. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 2.93 (s, 6H, $2CH_3$); 3.68-3.81 (m, 2H, CH_2); 4.99-5.04 (m, 2H, CH_2); 7.67 (t, 1H, *J*=7.4 Hz, Ar-H); 7.78 (d, 2H, *J*=8.4 Hz, Ar-H); 8.04 (t, 1H, *J*=7.9 Hz, Ar-H); 8.35 (d, 2H, *J*=8.2 Hz, Ar-H); 8.46 (d, 2H, *J*=7.2 Hz, Ar-H); 8.73 (s, 1H, Ar-H); 10.73 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{21}H_{20}ClF_3N_4O$: C, 62.84; H, 5.02; N, 13.96. Found: C, 62.98; H, 5.32; N, 14.21.

5-(2-Diethylamino-1-ethoxy)-3-(4-trifluoromethylphenyl)pyrazolo[1,5-a]quinazoline ***17*** *hydrochloride.* Yield 65 %, mp 238-240 °C. IR (nujol, cm^{-1}): 3400, 2552, 1610, 1549, 1303, 746. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 1.31 (t, 6H, *J*=7.2 Hz, $2CH_3$); 3.28-3.34 (m, 4H, CH_2); 3.70-3.77 (m, 2H, CH_2); 5.01-5.08 (m, 2H, CH_2); 7.67 (t, 1H, *J*=7.8 Hz, Ar-H); 7.78 (d, 1H, *J*=8.6 Hz, Ar-H); 8.05 (t, 2H, *J*=8.0 Hz, Ar-H); 8.31-8.37 (m, 4H, Ar-H); 8.73 (s, 1H, Ar-H); 10.63 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{23}H_{24}F_3N_4O$: C, 64.32; H, 5.63; N, 13.05. Found: C, 64.53; H, 5.84; N, 13.26.

5-(2-Dimethylamino-1-propoxy)-3-(4-trifluoromethylphenyl)pyrazolo[1,5-a]quinazoline ***18*** *hydrochloride.* Yield 70 %, mp 273-275 °C. IR (nujol, cm^{-1}): 3419, 2600, 1607, 1549, 1371, 750. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 2.32-2.39 (m, 2H, CH_2); 2.81 (s, 6H, $2CH_3$); 3.31-3.85 (m, 2H, CH_2); 7.64 (t, 1H, *J*=7.0 Hz, Ar-H); 7.79 (d, 2H, *J*=8.2 Hz, Ar-H); 8.03 (t, 1H, *J*=7.8 Hz, Ar-H); 8.25 (d, 2H, *J*=8.2 Hz, Ar-H); 8.34 (d, 2H, *J*=8.2 Hz, Ar-H); 8.69 (s, 1H, Ar-H); 10.66 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{22}H_{22}ClF_3N_4O$: C, 63.61; H, 5.34; N, 13.49. Found: C, 63.91; H, 5.58; N, 13.59.

3-(4-Trifluoromethylphenyl)-5-(2-dimethylaminoethylamino)pyrazolo[1,5-a]quinazoline ***19*** *hydrochloride.* Yield 77 %, mp 278-280 °C. IR (nujol, cm^{-1}): 3433, 2682, 1620, 1600, 1569, 753. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 2.88 (d, 6H, *J*=4.2 Hz, $2CH_3$); 3.49-3.52 (m, 2H, CH_2); 4.04-4.07 (m, 2H, CH_2); 7.60 (t, 1H, *J*=7.7 Hz, Ar-H); 7.75 (d, 2H, *J*=8.2 Hz, Ar-H); 7.94 (t, 1H, *J*=7.7 Hz, Ar-H,); 8.27-8.38 (m, 3H, Ar-H); 8.48-8.55 (m, 2H, Ar-H); 8.81 (bs exch., 1H, NH); 10.52 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{21}H_{21}F_3ClN_5$: C, 62.99; H, 5.29; N, 17.49. Found: C, 63.02; H, 5.52; N, 17.65.

3-(4-Trifluoromethylphenyl)-5-(2-diethylaminoethylamino)pyrazolo[1,5-a]quinazoline ***20*** *hydrochloride.* Yield 67 %, mp 258-260 °C. IR (nujol, cm^{-1}): 3358, 2416, 1600, 1545, 1378, 760. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 1.23 (t, 6H, *J*=7.0 Hz, $2CH_3$,); 3.23-3.29 (m, 4H, $2CH_2$);

3.45-3.47 (m, 2H, CH_2); 4.08-4.10 (m, 2H, CH_2); 7.60 (t, 1H, *J*=7.5 Hz, Ar-H); 7.76 (d, 2H, *J*=8.0 Hz, Ar-H); 7.95 (t, 1H, *J* = 7.7 Hz, Ar-H); 8.27-8.37 (m, 3H, Ar-H); 8.46-8.54 (m, 2H, Ar-H); 8.84 (bs exch., 1H, NH); 10.48 (bs exch, 1H, NH^+). Anal. Calcd. for $C_{23}H_{25}F_3ClN_5$: C, 64.47; H, 5.88; N, 16.34. Found: C, 67.65; H, 5.96; N, 16.41.

3-(4-Trifluoromethylphenyl)-5-(2-dimethylaminopropyl-1-amino)pyrazolo[1,5-a]quinazoline ***21*** *hydrochloride.* Yield 60 %, mp 233-235 °C. IR (nujol, cm^{-1}): 3433, 2675, 1600, 1545, 1316, 756. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 2.16-2.23 (m, 2H, CH_2); 2.75 (d, 6H, *J*=4.8 Hz, $2CH_3$,); 3.17-3.27 (m, 2H, CH_2); 3.72-3.75 (m, 2H, CH_2); 7.57 (t, 1H, *J*=7.4 Hz, Ar-H,); 7.75 (d, 2H, *J*=8.4 Hz, Ar-H); 7.91 (t, 1H, *J*=7.5 Hz, Ar-H); 8.24-8.37 (m, 3H, Ar-H); 8.49 (d, 2H, *J*=9.2 Hz, Ar-H,); 8.69 (bs exch., 1H, NH); 10.59 (bs exch, 1H, NH^+). Anal. Calcd. for $C_{22}H_{23}F_3ClN_5$: C, 63.76; H, 5.59; N, 16.90. Found: C, 63.95; H, 5.70; N, 13.95.

3-(4-Trifluoromethylphenyl)-5-(imidazolyl-1-propyl)pyrazolo[1,5-a]quinazoline ***22*** *hydrochloride.* Yield 55 %, mp 138-140 °C. IR (nujol, cm^{-1}): 3208, 2658, 1600, 1545, 849, 760. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 2.37 (t, 2H, *J*=6.6 Hz, CH_2); 3.67-3.70 (m, 2H, CH_2); 4.40 (t, 2H, *J*=6.4 Hz, CH_2,); 7.55-7.74 (m, 4H, Ar-H); 7.88-7.96 (m, 2H, Ar-H); 8.26-8.35 (m, 3H, Ar-H); 8.47-8.52 (m, 2H, Ar-H); 8.62 (bs exch., 1H, NH); 9.27 (s, 1H, Ar-H); 14.36 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{23}H_{20}F_3ClN_6$: C, 63.15; H, 4.61; N, 19.21. Found: C, 63.26; H, 4.85; N, 19.35.

3-(4-Chlorophenyl)-5-(2-dimethylaminoethoxy)pyrazolo[1,5-a]quinazoline hydrochloride ***23*** *hydrochloride.* Yield 57 %, mp 225-227 °C. IR (nujol, cm^{-1}): 3085, 2600, 1627, 1607, 1303, 750. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 2.95 (s, 6H, $2CH_3$); 3.70-3.73 (m, 2H, CH_2); 4.96-4.98 (m, 2H, CH_2); 7.50 (d, 2H, *J*=8.6 Hz, Ar-H); 7.67 (t, 1H, *J*=7.2 Hz, Ar-H); 8.05 (m, 1H, *J*=7.2 Hz, Ar-H); 8.15 (d, 2H, *J*=8.6 Hz, Ar-H); 8.34-8.43 (m, 2H, Ar-H); 8.64 (s, 1H, Ar-H); 10.12 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{20}H_{20}Cl_2N_4O$: C, 65.30; H, 5.48; N, 15.23. Found: C, 65.49; H, 5.65; N, 15.29.

3-(4-Chlorophenyl)-5-(2-diethylaminoethoxy)pyrazolo[1,5-a]quinazoline hydrochloride ***24*** *hydrochloride.* Yield 48 %, mp>300 °C. IR (nujol, cm^{-1}): 3071, 2573, 1624, 1607, 1303, 746. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 1.28 (t, 6H, *J*=7.2 Hz, $2CH_3$); 3.26-3.33 (m, 4H, CH_2); 3.70-3.72 (m, 2H, CH_2); 4.96-5.03 (m, 2H, CH_2); 7.50 (d, 2H, *J*=8.6 Hz, Ar-H); 7.67 (t, 1H, *J*=7.2 Hz, Ar-H); 8.05 (t, 1H, *J*=7.2 Hz, Ar-H); 8.15 (d, 2H, *J*=8.4 Hz, Ar-H); 8.29-8.38 (m, 2H, Ar-H); 8.59 (s, 1H, Ar-H);10.19 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{22}H_{24}Cl_2N_4O$: C, 66.74; H, 6.11; N, 14.15. Found: C, 66.86; H, 6.34; N, 14.35.

3-(4-Chlorophenyl)-5-(2-dimethylamino-1-propoxy)pyrazolo[1,5-a]quinazoline ***25*** *hydrochloride.* Yield 73 %, mp 241-243 °C. IR (nujol, cm^{-1}): 3066, 2590, 1647, 1610, 1311, 763. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 2.32 (m, 2H, CH_2); 2.84 (s, 6H, $2CH_3$); 3.36 (t, 2H, *J*=7.7 Hz, CH_2); 4.70 (t, 2H, *J*=5.6 Hz, CH_2); 7.50 (d, 2H, *J*=8.0 Hz, Ar-H); 7.65 (t, 1H, *J*=7.7 Hz, Ar-H); 8.04 (t, 1H, *J*=7.4 Hz, Ar-H); 8.15 (d, 2H, *J*=8.6 Hz, Ar-H); 8.24-8.37 (m, 2H, Ar-H); 8.59 (s, 1H, Ar-H); 10.32 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{21}H_{22}Cl_2N_4O$: C, 66.05; H, 5.81; N, 14.67. Found: C, 66.25; H, 5.96; N, 14.79.

3-(4-Chlorophenyl)-5-(2-dimethylaminoethylamino)pyrazolo[1,5-a]quinazoline ***26*** *hydrochloride.* Yield 70 %, mp 284-286 °C. IR (nujol, cm^{-1}): 3030, 2675, 1624, 1603, 1578, 750. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 2.89 (d, 6H, *J*=3.6 Hz, $2CH_3$); 3.47-3.49 (m, 2H, CH_2); 3.99-4.02 (m, 2H, Ar-H); 7.45 (d, 2H, *J*=8.4 Hz, Ar-H); 7.60 (t, 1H, *J*=7.6 Hz, Ar-H); 7.94 (t, 1H, *J*=7.7 Hz, Ar-H);

8.13-8.42 (m, 5H, Ar-H); 8.56 (bs exch., 1H, NH); 10.02 (bs, 1H, NH^+). Anal. Calcd. for $C_{20}H_{21}Cl_2N_5$: C, 65.48; H, 5.77; N, 19.09. Found: C, 65.68; H, 5.88; N, 19.32.

*3-(4-Chlorophenyl)-5-(2-diethylaminoethylamino)pyrazolo[1,5-a]quinazoline **27** hydrochloride.* Yield 62 %, mp 268-270 °C. IR (nujol, cm^{-1}): 3303, 2648, 1597, 1549, 838, 753. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 1.22 (t, 6H, *J*=7.1 Hz, $2CH_3$); 3.25 (m, 2H, CH_2); 3.47 (m, 2H, CH_2); 7.45 (d, 2H, *J*=8.4 Hz, Ar-H); 7.60 (t, 1H, *J*=7.6 Hz, Ar-H); 7.93 (t, 1H, *J*=7.8 Hz, Ar-H); 8.12-8.43 (m, 5H, Ar-H); 8.63 (bs exch., 1H, NH); 10.08 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{22}H_{25}Cl_2N_5$: C, 66.91; H, 6.38; N, 17.73. Found: C, 67.03; H, 6.62; N, 17.93.

*3-(4-Chlorophenyl)-5-(2-dimethylaminopropyl-1-amino)pyrazolo[1,5-a]quinazoline **28** hydrochloride.* Yield 60 %, mp 267-269 °C. IR (nujol, cm^{-1}): 3242, 2586, 1597, 1549, 828, 763. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 2.16 (m, 2H, CH_2); 2.76 (d, 6H, *J*=4.6 Hz, $2CH_3$,); 3.20 (m, 2H, CH_2); 3.70 (m, 2H, CH_2); 7.47 (d, 2H, *J*=8.4 Hz, Ar-H); 7.57 (t, 1H, *J*=7.8 Hz, Ar-H); 7.91 (t, 1H, *J*=7.8 Hz, Ar-H); 8.14-8.48 (m, 6H, Ar-H, NH); 10.03 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{21}H_{23}Cl_2N_5$: C, 66.22; H, 6.09; N, 18.39. Found: C, 66.42; H, 6.15; N, 18.57.

*3-(4-Chlorophenyl)-5-(imidazolyl-1-propyl)pyrazolo[1,5-a]quinazoline **29** hydrochloride.* Yield 65 %, mp 148-150 °C. IR (nujol, cm^{-1}): 3214, 2662, 1597, 1562, 828, 756. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 2.34 (t, 2H, *J*=6.6 Hz, CH_2); 3.63-3.66 (m, 2H, CH_2); 4.38 (t, 2H, *J*=6.8 Hz, CH_2); 7.43 (d, 2H, *J*=6.8 Hz, Ar-H); 7.57 (t, 1H, *J*=7.7 Hz, Ar-H,); 7.69 (s, 1H, Ar-H); 7.87-7.95 (m, 2H, Ar-H); 8.12 (d, 2H, *J*=8.8 Hz, Ar-H,); 8.26 (d, 2H, *J*=8.2 Hz, Ar-H,); 8.38-8.42 (m, 2H, Ar-H, NH); 9.22 (s, 1H, Ar-H); 14.36 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{22}H_{20}ClN_6$: C, 65.42; H, 4.99; N, 20.81. Found: C, 65.58; H, 5.12; N, 20.99.

*5-(2-Dimethylaminoethoxy)-3-phenylpyrazolo[1,5-a]quinazoline **30** hydrochloride.* Yield 70 %, mp 210-212 °C. IR (nujol, cm^{-1}): 3057, 2573, 1620, 1610, 1303, 753. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 2.91 (s, 6H, $2CH_3$); 3.69-3.75 (m, 2H, CH_2); 4.95-5.00 (m, 2H, CH_2); 7.24 (t, 1H, *J*=7.3 Hz, Ar-H); 7.44 (t, 2H, *J*=7.2 Hz, Ar-H); 7.63 (t, 1H, *J*=6.9 Hz, Ar-H); 7.98-8.13 (m, 3H, Ar-H); 8.31-8.45 (m, 2H, Ar-H); 8.59 (s, 1H, Ar-H); 10.52 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{20}H_{21}ClN_4O$: C, 72.05; H, 6.35; N, 16.80. Found: C, 72.25; H, 6.52 ; N, 17.01.

*5-(2-Diethylaminoethoxy)-3-phenylpyrazolo[1,5-a]quinazoline **31** hydrochloride.* Yield 66 %, mp 202-204 °C. IR (nujol, cm^{-1}): 3064, 2655, 1620, 1603, 1306, 750.^1H NMR (200 MHz, DMSO-d_6, δ ppm): 1.29 (t, 6H, *J*=7.2 Hz, $2CH_3$,); 3.25-3.31 (m, 4H, CH_2); 3.73-3.77 (m, 2H, CH_2); 4.95-5.05 (m, 2H, CH_2); 7.24 (t, 1H, *J*=7.3 Hz, Ar-H); 7.44 (t, 2H, *J*=7.7 Hz, Ar-H); 7.64 (t, 1H, *J*=7.6 Hz, Ar-H); 7.97-8.12 (m, 3H, Ar-H); 8.28-8.35 (m, 2H, Ar-H); 8.59 (s, 1H, Ar-H); 10.62 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{22}H_{25}ClN_4O$: C, 73.10; H, 6.97; N, 15.50. Found: C, 73.21; H, 7.03; N, 15.72.

*5-(2-Dimethylamino-1-propoxy)-3-phenylpyrazolo[1,5-a]quinazoline **32** hydrochloride.* Yield 61 %, mp 220-222 °C. IR (nujol, cm^{-1}): 3060, 2614, 1620, 1603, 1313, 763. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 2.30-2.40 (m, 2H, CH_2); 2.84 (s, 6H, $2CH_3$); 3.36 (t, 2H, *J*=6.0 Hz, CH_2); 4.70 (t, 2H, *J*=5.8 Hz, CH_2); 7.25 (t, 1H, *J*=7.5 Hz, Ar-H); 7.45 (t, 2H, *J*=7.7 Hz, Ar-H,); 7.63 (t, 1H, *J*=7.5 Hz, Ar-H); 7.98-8.14 (m, 3H, Ar-H); 8.24-8.36 (m, 2H, Ar-H); 8.59 (s, 1H, Ar-H); 10.31 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{21}H_{23}ClN_4O$: C, 72.60; H, 6.67; N, 16.13. Found: C, 72.71; H, 6.85; N, 16.37.

*5-(2-Diethylaminoethylamino)-3-phenylpyrazolo[1,5-a]quinazoline **33** hydrochloride.* Yield 63 %, mp 212-214 °C. IR (nujol, cm^{-1}): 3262, 2655, 1600, 1556, 1300, 767. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 1.26 (t, 6H, *J*=3.4 Hz, $2CH_3$); 3.13-3.24 (m, 4H, CH_2); 3.40-3.45 (m, 2H, CH_2); 4.00-4.10 (m, 2H, CH_2); 7.18 (t, 1H, *J*=7.3 Hz, Ar-H); 7.41 (t, 2H, *J*=7.7 Hz, Ar-H); 7.58 (t, 1H, *J*=7.1 Hz, Ar-H,); 7.92 (t, 1H, *J*=7.3 Hz, Ar-H); 8.10 (d, 1H, *J*=7.2 Hz, Ar-H); 8.27 (d, 1H, *J*=7.2 Hz, Ar-H); 8.43 (s, 1H, Ar-H); 8.68 (bs exch., 1H, NH); 10.27 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{22}H_{26}ClN_5$: C, 73.30; H, 7.27; N, 19.43. Found: C, 73.51; H, 7.33; N, 19.65.

*5-(Imidazolyl-1-propyl)-3-phenylpyrazolo[1,5-a]quinazoline **34** hydrochloride.* Yield 55 %, mp 156-158 °C. IR (nujol, cm^{-1}): 3064, 2723, 1600, 1559, 1538, 1303, 760. ^1H NMR (200 MHz, DMSO-d_6, δ ppm): 2.37 (t, 2H, *J*=6.6 Hz, CH_2); 3.64-3.68 (m, 2H, CH_2); 4.39 (t, 2H, *J*=7.0 Hz, CH_2); 7.17 (t, 1H, *J*=7.2 Hz, Ar-H); 7.39 (t, 2H, *J*=7.7 Hz, Ar-H); 7.57 (t, 1H, *J*=7.8 Hz, Ar-H); 7.70 (s, 1H, Ar-H); 7.87-7.95 (m, 2H, Ar-H); 8.08 (d, 2H, *J*=8.0 Hz, Ar-H); 8.27 (d, 2H, *J*=8.0 Hz, Ar-H); 8.38-8.42 (m, 2H, Ar-H, NH); 9.24 (s, 1H, Ar-H); 14.60 (bs exch., 1H, NH^+). Anal. Calcd. for $C_{22}H_{21}ClN_6$: C, 71.52; H, 5.73; N, 22.75. Found: C, 71.68; H, 5.87; N, 22.87.

Biological Tests.

Topoisomerase I-Mediated DNA Cleavage Reactions. Top1 reactions were performed as recently described.[8] Briefly, a 3′-[^{32}P]-end-labeled 117-bp DNA oligonucleotide (Integrated DNA Technologies) was incubated at 2 nM with recombinant Top1 in 20 μL of reaction buffer [10 mM Tris-HCl (pH 7.5), 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, and 15 μg/mL BSA] at 25 °C for 20 min in the presence of various concentrations of compounds. The reactions were terminated by adding SDS (0.5% final concentration) followed by the addition of two volumes of loading dye (80% formamide, 10 mM sodium hydroxide, 1 mM sodium EDTA, 0.1% xylene cyanol, and 0.1% bromphenol blue). Reactions were subjected to 20% denaturing PAGE. Gels were dried and visualized by using a Typhoon 8600 and ImageQuant software (Molecular Dynamics). Compounds are ranked for their ability to generate Top1-mediated cleavage complexes by a systematic visual analysis of the number of cleavage sites and their respective intensity in each lane as compared to the positive control lanes containing camptothecin or MJ-III-65 at 1 μM. A semi-quantitative ranking system is then used to rank the compounds from 0, not active; 0/+, trace of activity; +, weak activity; ++, moderate activity; +++, strong activity; to ++++, activity equivalent to 1 μM camptothecin or MJ-III-65.

Molecular Modeling

Hydrated docking. The new version of the docking program AutoDock (version 4.2, AD4),[9] as implemented through the graphical user interface called AutoDockTools (ADT), was used to perform docking studies. The ligand structures were built using the builder in the Maestro package of Schrödinger Suite 2012 and optimized using MacroModel (version 9.9).[10] The constructed compounds were converted into AD4 format files using ADT and hydrated using the wet.py python script. Protein structure (PDB code: 1K4T)[11] was prepared using the Protein Preparation Wizard through the graphical user interface of Maestro 9.3.[12] Water molecules were removed and hydrogen atoms were added and minimized using the all-atom OPLS-2005 force field. The receptor was also converted in the AD4 format file using ADT. Gasteiger-Marsili partial charges were then assigned to the ligands and the receptor. The docking area was centered around the ligand binding site. A set of grids of 80 × 80 × 80 with 0.375 Å spacing was calculated around the docking area for the ligand atom types using AutoGrid4.2. An additional grid map was calculated for the water molecules using the mapwater.py suite. For each ligand, 100 separate docking calculations were performed. Each docking calculation consisted of 10 million energy evaluations using the Lamarckian genetic algorithm local search (GALS) method. The GALS method evaluates a population of possible docking solutions and propagates the most successful individuals from each generation into the subsequent generation of possible solutions. A low-frequency local search according to the method of Solis and Wets is applied to docking trials to ensure that the final solution represents a local minimum. All dockings described in this paper were performed with a population size of 250, and 300 rounds of Solis and Wets local search were applied with a probability of 0.06. A mutation rate of 0.02 and a crossover rate of 0.8 were used to generate new docking trials for subsequent generations, and the best individual from each generation was propagated over the next generation. The docking results from each of the 100 calculations were clustered on the basis of root-mean square deviation (RMSD) (solutions differing by <2.0 Å) between the Cartesian coordinates of the atoms and were ranked on the basis of free energy of binding (ΔG_{AD4}). Because AD4 does not perform any structural optimization and energy minimization of the ligand-protein complexes, a molecular mechanics/energy minimization (MM/EM) approach using the OPLS-2005 force field was applied to refine the AD4 output. The MM/EM protocol consisted of 100,000 steps of the Polak–Ribière conjugate gradients (PRCG) or until the derivative convergence was 0.05 kJ·mol^{-1}. All pictures were rendered using PyMOL (http://www.pymol.org).

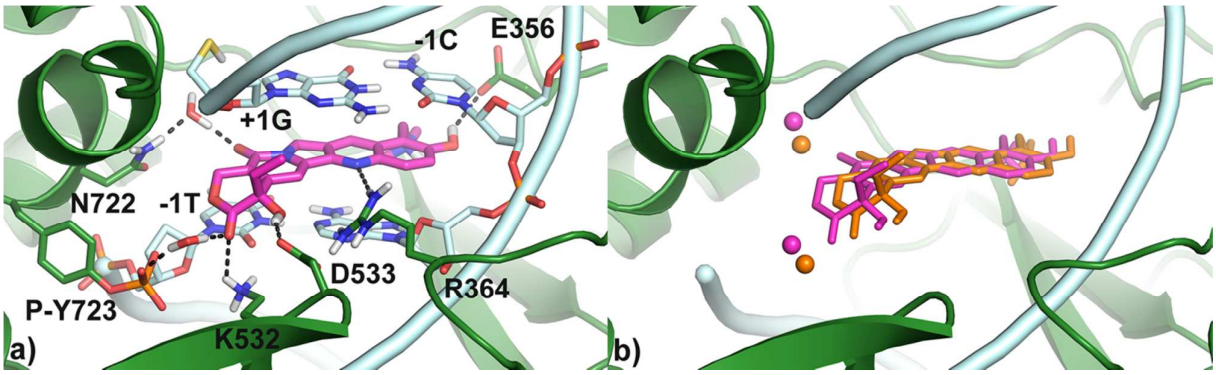


Figure S1. a) X-ray binding pose of Topotecan (**II**) within the Top1 DNA cleavage site (PDB code: 1K4T). The ligand is shown as magenta sticks. The enzyme and the substrate DNA are represented as green and cyan cartoons, respectively. Residues important for ligand binding are highlighted as sticks. H-bonds are depicted as dashed black lines. b) Superimposition of the X-ray binding pose (magenta sticks) of **II** and the docking conformation (orange sticks) as predicted by AD4. The enzyme and the substrate DNA are represented as green and cyan cartoons, respectively. Crystal and AD4 water molecules bridging between the ligand and the enzyme are shown as magenta and orange spheres, respectively.

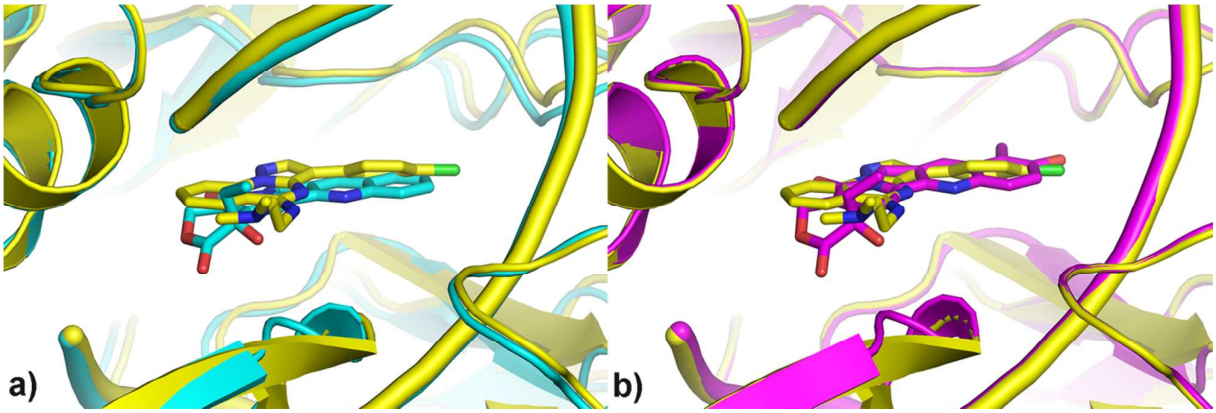


Figure S2. Superimposition of the docking pose of **26** as predicted by AD4 (yellow) and (a) the X-ray binding pose of Camptothecin (**I**) (cyan) and (b) Topotecan (**II**) (magenta) within the Top1 DNA cleavage site (PDB codes: 1T8I for **I**; 1K4T for **II**). The ligands are shown as sticks. The enzyme and the substrate DNA are represented as cartoons. Hydrogens are omitted for clarity.

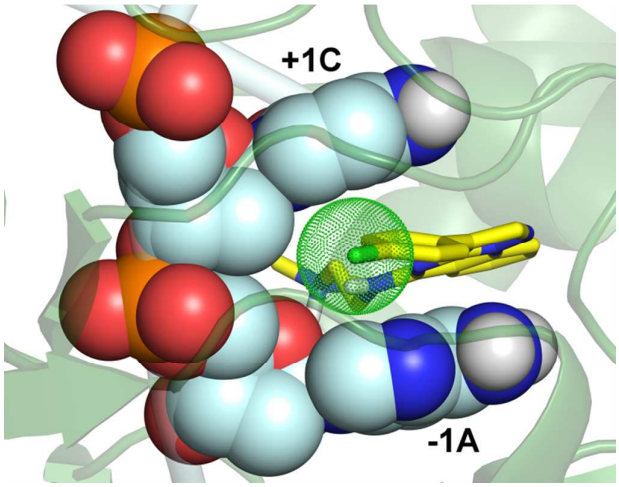


Figure S3. Side view of compound **26** at the Top1 (green cartoons) DNA (cyan cartoons) cleavage site. The +1C and -1A residues on the non-scissile strand are shown as spheres. The ligand is depicted as yellow sticks with its 4'-chlorine atom highlighted by a mesh of green dots representing its van der Waals radius.

REFERENCES

[1] Veibel, S.; Arnfred, N. H. Pyrazole Studies. II. Anhydrides of 1-(2'-carboxyphenyl)-3-phenyl-pyrazolone-5 and 1-(2'-carboxyphenyò)-3-methyl-4-ethyl-pyrazolone-5. *Acta Chem. Scand.* **1948**, 921-926.

[2] Michaelis, I. A. 1-Phenyl-5- and 3-pyrazolone-o-carboxylic Anhydrides. *Justus Liebigs Annalen der Chemie* **1910,** *373*, 129-212.

[3] Vasquez, T. E., Jr.; Nixey, T.; Chenera, B.; Gore, V.; Bartberger, M. D.; Sun, Y.; Hulme, C. One-pot microwave assisted preparation of pyrazoloquinazolinone libraries. *Mol. Divers.* **2003**, *7*, 161-164.

[4] Penning, T. D.; Thomas, S. A.; Hajduk, P. J.; Sauer, D. R.; Sarris, K.; Giranda, V. L. Preparation of 4*H*-pyrazolo[1,5-*a*]quinazolin-5-one derivatives as PARP inhibitors. *PCT Int. Appl. WO 2007149907,* **2007**.

[5] Beccalli, E. M.; La Rosa, C.; Marchesini, A. Oxidation of 4-aryl-substituted isoxazolin-5-ones. A new synthesis of 2,5-diaryl-1,3-oxazin-6-ones. *J. Org. Chem.* **1984**, *49*, 4287-4290.

[6] Russell, P. B.; Hitchings, G. H. 2,4-Diaminopyrimidines as Antimalarials. III. 5-Aryl Derivatives. *J. Am. Chem. Soc.* **1951**, *73*, 3763-3770.

[7] de Paulis, T.; Hemstapat, K.; Chen, Y.; Zhang, Y.; Saleh, S.; Alagille, D.; Baldwin, R. M.; Tamagnan, G. D.; Conn, P. J. Substituent effects of N-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamides on positive allosteric modulation of the metabotropic glutamate-5 receptor in rat cortical astrocytes. *J. Med. Chem.* **2006**, *49*, 3332-3344.

[8] Nguyen, T. X.; Morrell, A.; Conda-Sheridan, M.; Marchand, C.; Agama, K.; Bermingam, A.; Stephen, A. G.; Chergui, A.; Naumova, A.; Fisher, R.; O'Keefe, B. R.; Pommier, Y.; Cushman, M. Synthesis and Biological Evaluation of the First Dual Tyrosyl-DNA Phosphodiesterase I (Tdp1)-Topoisomerase I (Top1) Inhibitors. *J. Med. Chem.* **2012**, *55*, 4457–4478.

[9] Huey, R.; Morris, G. M.; Olson, A. J.; Goodsell, D. S. A semiempirical free energy force field with charge-based desolvation. *J. Comput. Chem.* **2007**, *28*, 1145-1152.

[10] MacroModel, v., Schrödinger, LLC, New York, NY, **2012**

[11] Staker, B. L.; Hjerrild, K.; Feese, M. D.; Behnke, C. A.; Burgin, A. B., Jr.; Stewart, L. The mechanism of topoisomerase I poisoning by a camptothecin analog. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 15387-15392.

[12] Maestro, v., Schrödinger, LLC, New York, NY, **2012**.