Discovery of dual VEGFR-2 and tubulin inhibitors with *in-vivo* efficacy

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Table 5. Selectivity profile of VEGFR-2-inhibiting oxadiazoles.

Kinase	**14** IC_{50} (μM) [a]	**17** IC_{50} (μM) [a]	**23** IC_{50} (μM) [a]
VEGFR-2	0.17	0.21	0.30
VEGFR-1	7	3	11
VEGFR-3	>100	39	42
IGFR-1	58	22	28
Ins R	>100	20	13
c-Met	>100	>100	>100
ETK	>100	20	10
FGFR1	15	18	29
EGFR	>100	>100	>100
CDK1	N.D.[c]	10	24
Aurora A	N.D.[c]	34	39
PLK1	N.D.[c]	8	12

[a] Values are averages of two independent dose-response curves for each enzyme indicated.
[b] VEGFR-1, vascular endothelial growth factor receptor-1; VEGFR-3, vascular endothelial growth factor receptor-2; IGFR-1, insulin growth factor receptor-1; Ins R, insulin receptor; c-Met, c-Met gene product; ETK, epithelial and endothelial tyrosine kinase; FGFR1, fibroblast growth factor receptor 1; EGFR, epidermal growth factor receptor; CDK1, cyclin-dependent kinase 1; PLK1, polo-like kinase 1.
[c] N.D.- not determined.

Table 6. Rapid Assessment of Compound Exposure (RACE) for compounds **17** and **23**.[a]

	IP at 60mg/kg (nM)			PO at 60mg/kg (nM)			PO at 300mg/kg (nM)			
Time after dosing (hrs)	0.5	1	4	0.5	1	4	0.5	1	4	24
Cmpd **17**	7055	3588	2630	728	842	298	6669	2860	468	797
Cmpd **23**	750	647	187	N.D.[b]	N.D.[b]	N.D.[b]	N.D.[b]	N.D.[b]	N.D.[b]	N.D.[b]

[a] Average of three mice dosed at the indicated concentrations either injected IP or administered orally (PO). Compound prepared as a suspension in 5% Cremophore and 5% DMSO/Carboxymethyl cellulose for IP injection or 5% Ethanol, 5% Tween 80 and 5% PEG 400 in PBS for PO intubations.
[b] N.D.- not determined.

In vitro assay for VEGFR-2 (KDR) Kinase Inhibition

VEGFR tyrosine kinase inhibition is determined by measuring the phosphorylation level of poly-Glu-Ala-Tyr-biotin (pGAT-biotin) peptide in a Homogenous Time-Resolved Fluorescence (HTRF) assay. Into a black 96-well Costar plate is added 2μl/well of 25x compound in 100% DMSO (final concentration in the 50 μl kinase reaction is typically 1 nM to 10 μM). Next, 38 μl of reaction buffer (25 mM Hepes pH 7.5, 5mM $MgCl_2$, 5mM $MnCl_2$, 2mM DTT, 1mg/ml BSA) containing 0.5 mmol pGAT-biotin and 3-4ng KDR enzyme is added to each well. After 5-10 mins preincubation, the kinase reaction is initiated by the addition of 10μl of 10μM ATP in reaction buffer, after which the plate is incubated at room temperature for 45 minutes. The reaction is stopped by addition of 50μl of KF buffer, (50mM Hepes, pH 7.5, 0.5M KF, 1mg/ml BSA) containing 100mM EDTA and 0.36 μg/ml PY20K (Eu-cryptate labeled anti-phosphotyrosine antibody, CIS bio inenational) is added and after an additional 2 hour incubation at room temperature, the palte is read in a RUBYstar HTRF Reader.

Cell-based assay for VEGFR-2 inhibition

Transfection of 293 cells with DNA expressing FGFR1/VEGFR-2 chimera**:**

A chimeric construct containing the extracellular portion of FGFR1 and the intracellular portion of VEGFR-2 is transciently transfected into 293 adenovirus-transfected kidney cells. DNA for transfection is diluted to a final concentration of 5μg/ml DNA in serum-free medium and incubated at room temperature for 30 min. with 40μl/ml of Lipofectamine 2000, also in serum-free media. 250 μl of the Lipofectamine and DNA mixture is added to 293 cells suspended at $5x10^5$ cells/ml. 200 μl/well is dispensed into a 96-well plate, with subsequent overnight incubation. Within 24 hours, media is removed and 100 μl of media with 10% fetal bovine serum is added to the now adherent cells followed by an additional 24 hour incubation.

Target compounds are added to the individual wells with a final DMSO of 0.1%. Cells are lysed by resuspension in 100 µl Lysis buffer (150 mM NaCl, 50mM Hepes pH 7.5, 0.5% Triton X-100, 10mM NaPPi, 50mM NaF, 1mM Na_3VO_4) and rocked for 1h at 4 °C.

ELISA for detection of tyrosine-phosphorylated chimeric receptor :

96 well ELISA plates are coated using 100 µl/well of 10 µg/ml of αFGFR1 antibody, and incubated overnight at 4 °C. αFGFR1 is prepared in a buffer made with 16ml 0.2M Na_2CO_3 and 34ml 0.2M $NaHCO_3$ with pH adjusted to 9.6. Concurrent with lysis of the transfected cells, αFGFR1 coated ELISA plates are washed three times with PBS+0.1% Tween-20 and blocked by addition of 200□l/well of 3% BSA in PBS for 1h and washed again. 80 µl of lysate is then transferred to the coated and blocked wells and incubated for 1h at 4 °C. The plates are washed three times with PBS+0.1% Tween-20.

To detect bound phosphorylated chimeric receptor, 100 µl of anti-phosphotyrosine antibodies (RC20:HRP), Transduction Laboratories) was added per well (final concentration 0.5 µg/ml in PBS) and incubated for 1h. The plates are washed six times with PBS+0.1% Tween-20. Enzymatic activity of HRP is detected by adding 50µl/well of equal amounts of the Kirkegaard & Perry Laboratories (KPL) Substrate A and Substrate B. The reaction is stopped by addition of 50 µl/well of 0.1N H_2SO_4 and absorbance is detected at 450nm.

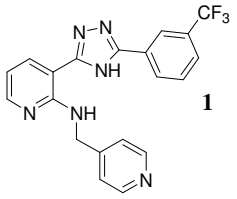
***In vitro* tubulin polymerization assay**

Tubulin polymerization *in vitro* was determined in a turbidity assay (measured at 340 nm). Lyophilized bovine tubulin (HTS02, Cytoskeleton Inc.) was re-suspended in G-PEM buffer (80 mM PIPES pH 7, 1 mM EGTA, 1 mM $MgCl_2$, 1 mM GTP, 5% glycerol) to a final concentration of 3 mg/mL and kept at 4˚C. Compounds in 100x stock solutions in DMSO were dotted onto pre-warmed 96-well plates (Corning Costar 3696), with the plates immediately transferred to a 37˚C plate reader (SPECTRAmax Plus, Molecular Devices). Cold tubulin was added to the wells, plates were mixed by shaking, and absorbance at 340 nm was read every minute for 30 minutes. Kinetic curves with 30 points each were collected for each compound, with a dynamic range between 0 and 0.4 OD units. Percentage inhibition values were calculated using the 30 minute data point, based on control samples (1% DMSO). This assay is a modified version of the screening assay developed by Cytoskeleton Inc. (Cytodynamix 12), formaximized throughput, and maintained sensitivity. EC50 values were determined by sigmoidal curve fitting using Excel-based software.

Cell cycle analysis for analysis of G2/M arrest

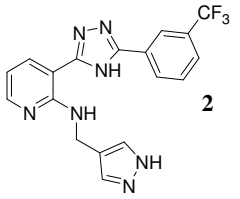
A431 cells (human epidermoid cancer cell line) were maintained in culture in D-MEM media with 10% FBS and 1 mg/ml glutamate. NCI/ADR cells were maintained in culture in RPMI media supplemented with 10% FBS. Cells were plated onto 6-well plates at a final density of 500,000 cells/well at the time of treatment, treated with compounds at 0.01-1 µM final concentrations (final 0.1% DMSO) for 24 hours, then trypsinized, collected, rinsed in PBS (phosphate buffered saline), and fixed in 70% cold ethanol overnight at 4°C. Cells were rinsed with PBS, re-suspended in PBS with 0.2% Tween, incubated with RNAse (final concentration of 1 µg/mL) at 37°C for 15 minutes, followed by addition of Propidium Iodide (final 50 µg/mL), and a 30 minute incubation at room temperature. DNA ploidy was analyzed using cell sorters (Epics Excel, Beckman-Coulter, or Guava PCA-96, Guava Technologies) and mitotic arrest characterized by accumulation of cells in the G2/M phase of the cell cycle. EC_{50} values were determined by sigmoidal curve fitting using Excel-based software.

Triazoles/oxadiazoles **1-5** were prepared according to reference 7.



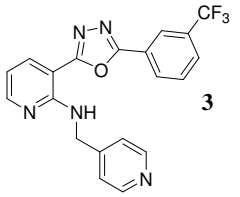
1

^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 14.63 (br. s, 1H), 8.41 (m, 6H), 7.80 (m, 2H), 7.27 (d, 2H), 6.75 (m, 2H), 4.68 (d, 2H). ^{13}C NMR (300 MHz, DMSO-d_6) δ (ppm) 160.5, 157.8, 157.2, 153.6, 148.6, 148.5, 134.2, 129.2, 129.0, 128.9, 128.5, 128.1, 125.2, 124.8, 121.2, 121.0, 110.9, 42.3; MS m/z: 397 (M+1); Formula $C_{20}H_{15}F_3N_6$: Calculated C 60.61, H 3.81, N 21.19; Found C 60.57, H 3.87, N 21.12.



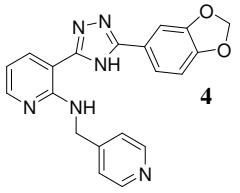
2

^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 14.60 (br. s, 1H), 13.57 (s, 1H), 8.51 (s, 1H), 7.89 (m, 1H), 7.21-7.68 (m, 6H), 6.82 (m, 2H), 4.41 (d, 2H). ^{13}C NMR (300 MHz, DMSO-d_6) δ (ppm) 160.4, 157.0, 156.6, 153.7, 133.1, 132.5, 130.1, 128.7, 128.4, 127.9, 124.8, 124.4, 121.0, 120.7, 110.8, 40.1; MS m/z: 386 (M+1); Formula $C_{18}H_{14}F_3N_7$: Calculated C 56.10, H 3.66, N 25.44; Found C 56.19, H 3.57, N 25.40.



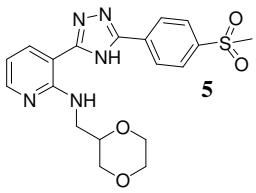
3

^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 8.45 (m, 6H), 7.79 (m, 2H), 7.22 (d, 2H), 6.81 (m, 2H), 4.60 (d, 2H). ^{13}C NMR (300 MHz, DMSO-d_6) δ (ppm) 161.6, 158.9, 157.8, 155.7, 150.5, 148.8, 134.1, 128.9, 129.1, 128.6, 128.3, 128.0, 125.2, 124.0, 121.7, 121.1, 110.8, 42.4; MS m/z: 398 (M+1); Formula $C_{20}H_{14}F_3N_5O$: Calculated C 60.45, H 3.55, N 17.62; Found C 60.50, H 3.51, N 17.74.



4

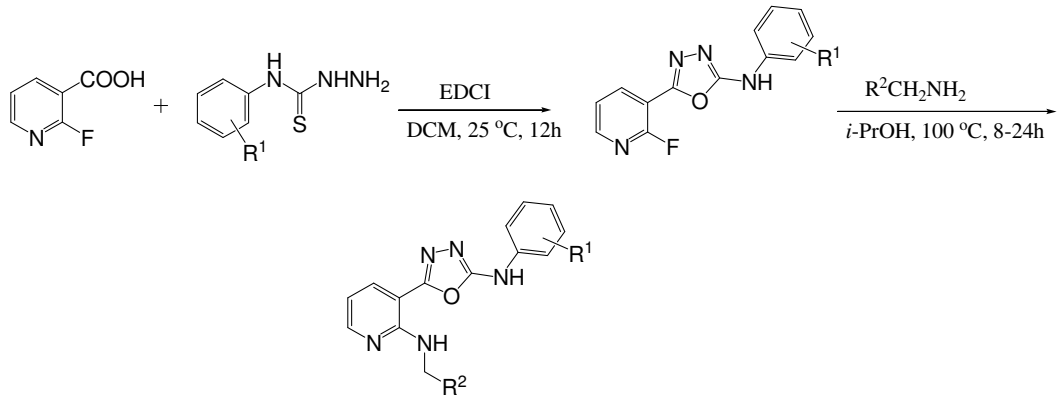
^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 14.63 (br. s, 1H), 8.39 (d, 2H), 7.71 (m, 2H), 7.27 (d, 2H), 7.05-7.14 (m, 4H), 6.77 (m, 2H), 6.09 (s, 2H), 4.68 (d, 2H). ^{13}C NMR (300 MHz, DMSO-d_6) δ (ppm) 160.1, 157.8, 157.0, 153.3, 148.1, 147.8, 130.7, 128.5, 127.6, 127.0, 126.5, 125.1, 124.6, 121.9, 121.1, 111.0, 101.8, 42.6; MS m/z: 373 (M+1); Formula $C_{20}H_{16}N_6O_2$: Calculated C 64.51, H 4.33, N 22.57; Found C 63.48, H 4.27, N 22.61.



5

^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 14.51 (br. s, 1H), 8.15 (m, 1H), 7.95 (d, 2H), 7.71 (d, 2H), 7.53 (m, 2H), 7.35 (br. s, 1H), 3.90 (m, 1H), 3.61 (m, 6H), 3.34 (m, 5H). ^{13}C NMR (300 MHz, DMSO-d_6) δ (ppm) 160.2, 157.8, 157.5, 150.3, 142.5, 140.8, 130.7, 128.9, 126.6, 126.1, 125.6, 124.9, 81.9, 74.9, 65.8, 65.0, 47.8, 43.3; MS m/z: 416 (M+1); Formula $C_{19}H_{21}N_5O_4S$: Calculated C 54.93, H 5.09, N 16.86; Found C 55.01, H 5.13, N 16.82.

Typical procedure for the preparation of oxadiazoles **6-23**:

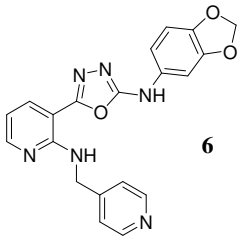


Step 1. Preparation of fluoro intermediate.

EDCI (1 eq.), thiosemicarbizide (4 eq.) and 2-fluoronicotinic acid (1 eq.) were stirred in approximately 100 mL of DCM at room temperature. The formed precipitate was filtered off, washed with DCM (10 mL) and dried to give a pure fluoro intermediate that was used for the next step without purification.

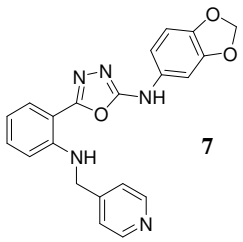
Step 2. Preparation of final oxadiazole.

Fluoro intermediate from the previous step (1 eq.) was suspended in anhydrous isopropanol (10 mL) and an amine of choice (1 eq.) was added to the reaction mixture. The suspension was stirred at 80-100 °C for 8 -24 h while monitoring by TLC. Upon completion, the reaction mixture was filtered off and the resulting precipitate was dried *in vacuo* to give a pure product.



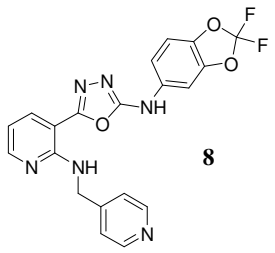
6

^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 10.66 (s, 1H), 8.48- 8.50 (m, 2H), 8.29- 8.33 (m, 1H), 8.16-8.21 (m, 1H), 7.88- 7.93 (m, 1H), 7.30-7.34 (m,3H), 7.01- 7.06 (m, 1H), 6.94 (d, J= 8.4 Hz, 1H), 6.75- 6.82 (m, 1H), 6.01 (s, 2H), 4.52 (d, J= 6.0 Hz, 2H); MS m/z: 389 (M+1); Formula $C_{20}H_{16}N_6O_3$: Calculated C 61.85, H 4.15, N 21.64; Found C 61.91, H 4.10, N 21.59.



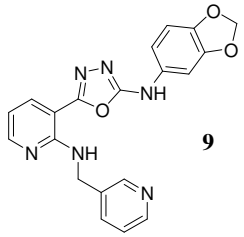
7

^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 10.58 (s, 1H), 8.51- 8.53 (m, 2H), 7.93-7.97 (m, 1H), 7.62-7.66 (m, 1H), 7.23-7.38 (m, 4H), 7.03- 7.10 (m, 1H), 6.90-6.94 (m, 1H), 6.65- 6.75 (m, 2H), 6.03 (s, 2H), 4.74-4.76 (m, 2H). MS m/z: 388 (M+1); Formula $C_{21}H_{17}N_5O_3$: Calculated C 65.11, H 4.42, N 18.08; Found C 65.17, H 4.37, N 18.01.



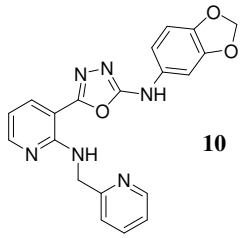
8

^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ (ppm) 10.60 (s, 1H), 8.51- 8.53 (m, 2H), 7.95-7.97 (m, 1H), 7.62-7.66 (m, 1H), 7.29-7.51 (m, 4H), 7.04-7.19 (m, 1H), 6.87-6.99 (m, 1H), 6.65-6.75 (m, 2H), 4.74-4.76 (m, 2H). MS m/z: 425 (M+1); Formula $C_{20}H_{14}F_2N_6O_3$: Calculated C 56.61, H 3.33, N 19.80; Found C 56.79, H 3.21, N 19.74.



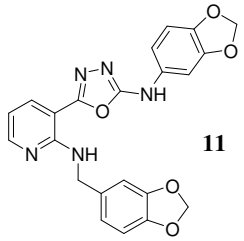
9

^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ (ppm) 10.69 (br. s, 1H), 8.62 (d, 1H), 8.45-8.47 (m, 1H), 8.21-8.28 (m, 2H), 7.86-7.89 (m, 1H), 7.78 (d, 1H), 7.31-7.38 (m, 2H), 7.02-7.06 (m, 1H), 6.89-6.92 (m, 1H), 6.77-6.81 (m, 1H), 6.00 (s, 2H), 4.82 (d, 2H). MS m/z: 389 (M+1); Formula $C_{21}H_{17}N_5O_3$: Calculated C 65.11, H 4.42, N 18.08; Found C 65.20, H 4.47, N 18.00.



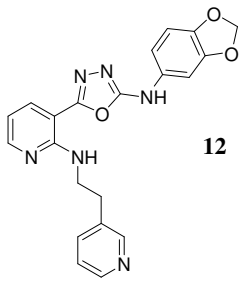
10

^{1}H NMR (300 MHz, DMSO-D$_6$) δ (ppm) 10.64 (s, 1H), 8.48-8.60 (m, 2H), 8.19-8.24 (m, 1H), 7.85-7.91 (m, 1H), 7.70- 7.80 (m, 1H), 7.26-7.40 (m,3H), 7.03-7.08 (m, 1H), 6.94 (d, J= 8.4 Hz, 1H), 6.75-6.82 (m, 1H), 6.01 (s, 2H), 4.88 (d, J=5.4 Hz, 2H); MS m/z: 389 (M+1); Formula $C_{21}H_{17}N_5O_3$: Calculated C 65.11, H 4.42, N 18.08; Found C 65.19, H 4.37, N 18.01.



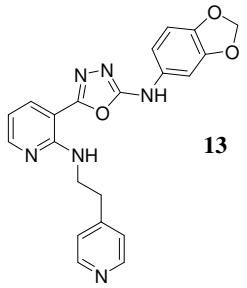
11

^{1}H NMR (300 MHz, DMSO-D$_6$) δ (ppm) 10.62 (s, 1H), 8.23-8.25 (m, 1H), 8.09 (t, 1H), 7.84-7.88 (m, 1H), 7.27-7.28 (d, 1H), 6.75-7.03 (m, 6H), 6.00 (s, 1H), 5.99 (s, 1H), 4.67 (d, 2H); MS m/z: 432 (M+1); Formula $C_{22}H_{17}N_5O_5$: Calculated C 61.25, H 3.97, N 16.23; Found C 61.19, H 4.06, N 16.26.



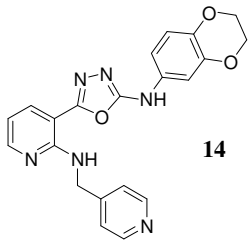
12

^{1}H NMR (300 MHz, DMSO-D_6) δ (ppm) 10.74 (s, 1H), 8.56 (d, 2H), 8.30 (m, 1H), 7.88-7.94 (m, 2H), 7.77-7.80 (m, 1H), 7.27-7.32 (m, 2H), 7.06-7.09 (m, 1H), 6.90-6.94 (m, 1H), 6.81-6.84 (m, 1H), 6.21 (s, 2H), 3.86-3.92 (m, 2H), 3.03-3.09 (m, 2H); MS m/z: 403 (M+1); Formula $C_{21}H_{18}N_6O_3$: Calculated C 62.68, H 4.51, N 20.88; Found C 62.78, H 4.57, N 21.01.



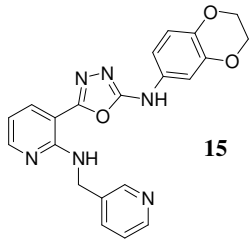
13

^{1}H NMR (300 MHz, DMSO-D_6) δ (ppm) 10.86 (s, 1H), 8.44-8.58 (m, 2H), 8.26-8.30 (m, 1H), 7.85-7.92 (m, 2H), 7.74-7.79 (m, 1H), 7.28-7.36 (m, 2H), 7.04-7.08 (m, 1H), 6.92-6.96 (m, 1H), 6.80-6.86 (m, 1H), 6.09 (s, 2H), 3.80-3.90 (m, 2H), 3.00-3.06 (m, 2H); MS m/z: 403 (M+1); Formula $C_{21}H_{18}N_6O_3$: Calculated C 62.68, H 4.51, N 20.88; Found C 62.75, H 4.42, N 20.76.



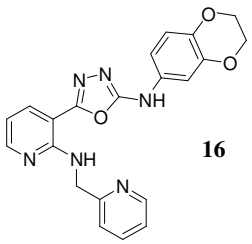
14

^{1}H NMR (300 MHz, DMSO-*d_6*) δ (ppm) 10.61 (s, 1H), 8.52 (d, J=5.7, 2H), 8.24- 8.40 (t, J= 6.0, 1H), 8.10- 8.24 (m, 1H), 7.81- 7.99 (m, 1H), 7.18- 7.45 (m, 3H), 6.94- 7.10 (m, 1H), 6.732- 6.92 (m, 2H), 4.76- 4.93 (d, J= 6.0, 2H), 4.14- 4.31 (m, 4H); MS m/z: 403 (M+1); Formula $C_{21}H_{18}N_6O_3$: Calculated C 62.68, H 4.51, N 20.88; Found C 62.61, H 4.58, N 20.97.



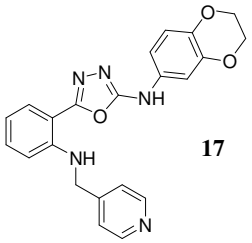
15

^{1}H NMR (300 MHz, DMSO-*d_6*) δ (ppm) 10.52 (s, 1H), 8.48 (d, 2H), 8.26- 8.36 (m, 1H), 8.13- 8.21 (m, 1H), 7.83- 7.96 (m, 1H), 7.29- 7.36 (m, 2H), 7.21- 7.29 (m, 1H), 6.95- 7.07 (m, 1H), 6.72- 6.92 (m, 2H), 4.85 (d, 2H), 4.14- 4.31 (m, 4H); MS m/z: 403 (M+1); Formula $C_{21}H_{18}N_6O_3$: Calculated C 62.68, H 4.51, N 20.88; Found C 62.62, H 4.63, N 20.81.



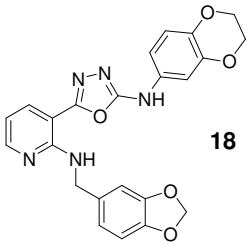
16

^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 10.55 (s, 1H), 8.44-8.66 (d, J= 4.8 Hz, 2H), 8.16- 8.27 (t, J= 5.4 Hz, 1H), 7.84- 7.95 (m, 1H), 7.69- 7.81 (m, 1H), 7.19- 7.43 (m, 3H), 6.95- 7.07 (m, 1H), 6.73- 6.91 (m, 2H), 4.83- 4.93 (d, J= 5.1 Hz, 2H), 4.16- 4.29 (m, 4H); MS m/z: 403 (M+1); Formula $C_{21}H_{18}N_6O_3$: Calculated C 62.68, H 4.51, N 20.88; Found C 62.59, H 4.44, N 20.97.



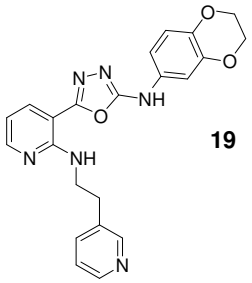
17

^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 10.58 (s, 1H), 8.57-8.63 (m, 2H), 7.86 (t, 1H), 7.58-7.64 (m, 1H), 7.20-7.33 (m, 4H), 6.96- 7.02 (m, 1H), 6.67- 6.88 (m, 3H), 4.65- 4.67 (m, 2H), 4.16- 4.25 (m, 4H); MS m/z: 402 (M+1); Formula $C_{22}H_{19}N_5O_3$: Calculated C 65.83, H 4.77, N 17.45; Found C 65.90, H 4.89, N 17.38.



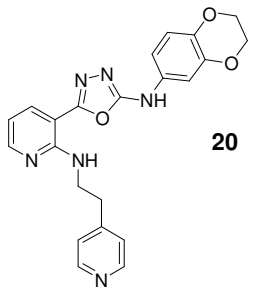
18

^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 10.48 (s, 1H), 8.19- 8.30 (m, 1H), 8.03- 8.16 (m, 1H), 7.79- 7.91 (m, 1H), 7.14- 7.28 (d, 1H), 6.92- 7.06 (m, 2H), 6.72- 6.91 (m, 4H), 5.92 (s, 2H), 4.68 (d, 2H), 4.14- 4.32 (m, 4H); MS m/z: 446 (M+1); Formula $C_{23}H_{19}N_5O_5$: Calculated C 62.02, H 4.30, N 15.72; Found C 61.97, H 4.37, N 15.63.



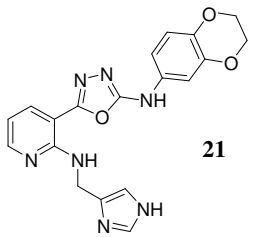
19

^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 10.50 (s, 1H), 8.30-8.45 (m, 2H), 8.15- 8.20 (m, 1H), 7.92-7.98 (m, 1H), 7.80- 7.90 (m, 2H), 7.61-7.66 (m, 1H), 7.12-7.28 (m, 2H), 6.65-6.80 (m, 2H), 4.05-4.20 (m, 4H), 3.71-3.76 (m, 2H), 2.85-2.92 (m, 2H); MS m/z: 417 (M+1); Formula $C_{22}H_{20}N_6O_3$: Calculated C 63.45, H 4.84, N 20.18; Found C 63.35, H 4.88, N 20.02.



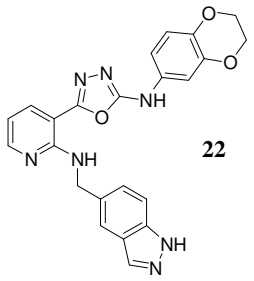
20

^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 10.57 (s, 1H), 8.59 (d, 2H), 8.19-8.22 (m, 1H), 7.97-8.00 (m, 1H), 7.86-7.91 (m, 2H), 7.60-7.63 (m, 1H), 7.19-7.29 (m, 2H), 6.69-6.81 (m, 2H), 4.08-4.23 (m, 4H), 3.70-3.76 (m, 2H), 2.89-2.97 (m, 2H); MS m/z: 417 (M+1); Formula $C_{22}H_{20}N_6O_3$: Calculated C 63.45, H 4.84, N 20.18; Found C 63.52, H 4.76, N 20.25.



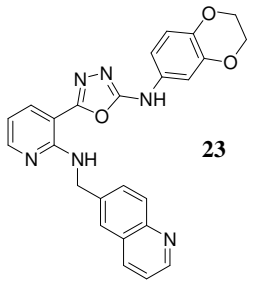
21

^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 13.42 (s, 1H), 10.95 (s, 1H), 8.29-8.37 (m, 1H), 8.19-8.28 (m, 1H), 7.83- 7.96 (m, 1H), 7.48 (s, 1H), 7.29-7.36 (m, 2H), 7.21-7.29 (m, 1H), 6.95 (s, 1H), 6.75 (m, 1H), 4.91 (d, 2H), 4.14- 4.31 (m, 4H); MS m/z: 392 (M+1); Formula $C_{19}H_{17}N_7O_3$: Calculated C 58.31, H 4.38, N 25.05; Found C 58.32, H 4.49, N 24.89.



22

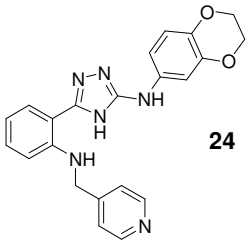
^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 12.86 (s, 1H), 10.65 (s, 1H), 8.32 (s, 1H), 8.12 (m, 1H), 8.06 (m, 1H), 7.80-7.91 (m, 2H), 7.12-7.24 (m, 3H), 6.97-7.03 (m, 1H), 6.70 (m, 2H), 4.57 (d, 2H), 4.14-4.31 (m, 4H); MS m/z: 442 (M+1); Formula $C_{23}H_{19}N_7O_3$: Calculated C 62.58, H 4.34, N 22.21; Found C 62.51, H 4.50, N 22.08.



23

^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 10.77 (s, 1H), 8.23 (d, 1H), 8.01-8.06 (m, 2H), 7.80-7.91 (m, 2H), 7.11-7.20 (m, 3H), 6.99 (m, 1H), 6.65 (m, 2H), 6.24 (m, 2H), 4.60 (d, 2H), 4.15-4.29 (m, 4H); MS m/z: 453 (M+1); Formula $C_{25}H_{20}N_6O_3$: Calculated C 66.36, H 4.46, N 18.57; Found C 66.22, H 4.34, N 18.65.

Triazoles **24-28** were obtained using procedure described in reference 6b.



24

^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 13.50 (s, 1H), 12.62 (s, 1H), 9.02 (s, 1H), 8.48- 8.52 (m, 2H), 7.80-7.83 (m, 1H), 7.30- 7.32 (m, 2H), 7.18-7.21 (m, 2H), 6.90-6.93 (m, 2H), 6.65-6.79 (m, 2H), 4.61 (d, 2H), 4.20- 4.32 (m, 4H); MS m/z: 401 (M+1); Formula $C_{22}H_{20}N_6O_2$: Calculated C 65.99, H 5.03, N 20.99; Found C 65.84, H 4.89, N 20.92.