**Design, synthesis and biological of pyrazolo[3,4-d]pyrimidine derivatives as potential VEGFR-2 inhibitors**

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| **Compound No.** | **InChI** |
| I-1 | InChI=1S/C18H14N6O/c25-18(23-13-4-2-1-3-5-13)12-6-8-14(9-7-12)22-16-15-10-21-24-17(15)20-11-19-16/h1-11H,(H,23,25)(H2,19,20,21,22,24) |
| I-2 | InChI=1S/C19H16N6O/c1-12-4-2-3-5-16(12)24-19(26)13-6-8-14(9-7-13)23-17-15-10-22-25-18(15)21-11-20-17/h2-11H,1H3,(H,24,26)(H2,20,21,22,23,25) |
| I-3 | InChI=1S/C19H16N6O/c1-12-2-6-15(7-3-12)24-19(26)13-4-8-14(9-5-13)23-17-16-10-22-25-18(16)21-11-20-17/h2-11H,1H3,(H,24,26)(H2,20,21,22,23,25) |
| I-4 | InChI=1S/C18H13ClN6O/c19-12-3-7-14(8-4-12)24-18(26)11-1-5-13(6-2-11)23-16-15-9-22-25-17(15)21-10-20-16/h1-10H,(H,24,26)(H2,20,21,22,23,25) |
| I-5 | InChI=1S/C18H12F2N6O/c19-11-3-6-14(20)15(7-11)25-18(27)10-1-4-12(5-2-10)24-16-13-8-23-26-17(13)22-9-21-16/h1-9H,(H,25,27)(H2,21,22,23,24,26) |
| I-6 | O=C(NC1=CC(C(F)(F)F)=CC(C(F)(F)F)=C1)C2=CC=C(NC3=C4C(NN=C4)=NC=N3)C=C2 |
| II-1 | InChI=1S/C20H15ClN6O/c21-17-7-2-1-4-13(17)8-9-18(28)25-14-5-3-6-15(10-14)26-19-16-11-24-27-20(16)23-12-22-19/h1-12H,(H,25,28)(H2,22,23,24,26,27)/b9-8+ |
| II-2 | InChI=1S/C20H14Cl2N6O/c21-13-6-4-12(17(22)8-13)5-7-18(29)26-14-2-1-3-15(9-14)27-19-16-10-25-28-20(16)24-11-23-19/h1-11H,(H,26,29)(H2,23,24,25,27,28)/b7-5+ |
| II-3 | InChI=1S/C21H18N6O/c1-14-5-2-3-6-15(14)9-10-19(28)25-16-7-4-8-17(11-16)26-20-18-12-24-27-21(18)23-13-22-20/h2-13H,1H3,(H,25,28)(H2,22,23,24,26,27)/b10-9+ |
| II-4 | InChI=1S/C21H18N6O/c1-14-4-2-5-15(10-14)8-9-19(28)25-16-6-3-7-17(11-16)26-20-18-12-24-27-21(18)23-13-22-20/h2-13H,1H3,(H,25,28)(H2,22,23,24,26,27)/b9-8+ |
| II-5 | InChI=1S/C20H15FN6O/c21-14-7-4-13(5-8-14)6-9-18(28)25-15-2-1-3-16(10-15)26-19-17-11-24-27-20(17)23-12-22-19/h1-12H,(H,25,28)(H2,22,23,24,26,27)/b9-6+ |
| II-6 | InChI=1S/C22H20N6O3/c1-30-18-8-6-14(10-19(18)31-2)7-9-20(29)26-15-4-3-5-16(11-15)27-21-17-12-25-28-22(17)24-13-23-21/h3-13H,1-2H3,(H,26,29)(H2,23,24,25,27,28)/b9-7+ |

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| **Compound No.** | **Biological Activity a（μM）** | | | | | | |
| A375 | A549 | HepG2 | AGS | T47D | MDA-MB-231 | SW620 |
| I-1 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| I-2 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| I-3 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| I-4 | 6.33±0.86 | >20 | 8.34±1.38 | 6.21±0.14 | 5.57±1.55 | >20 | >20 |
| I-5 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| I-6 | 6.87±3.41 | 11.99±0.21 | 6.00±1.20 | 8.28±3.59 | 3.26±0.34 | 10.34±0.18 | 6.30±1.58 |
| II-1 | 8.82±3.92 | >20 | 5.90±0.06 | 13.37±3.53 | 5.57±1.55 | 12.55±0.17 | 5.85±1.10 |
| II-2 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| II-3 | 11.51±1.47 | >20 | 11.99±0.16 | 16.95±2.19 | >20 | >20 | 10.29±0.04 |
| II-4 | >20 | >20 | 18.17±0.08 | >20 | >20 | >20 | >20 |
| II-5 | >20 | >20 | 6.84±0.12 | >20 | >20 | >20 | >20 |
| II-6 | >20 | >20 | 8.19±2.14 | >20 | >20 | >20 | 10.25±2.87 |

a Cells were seeded in 96-well plates at density of 5000 to 8000 cells/well in regular DMEM, 1640 or L15 supplemented with 10% (v/v) FBS, 1% (v/v) penicillin-streptomycin. After 24 h incubation at 37 °C in a humidified atmosphere containing 5% CO2, complete growth medium was removed with fresh medium containing test compounds and incubated for further 48 h. After incubation, cell viability was determined by MTT assay. Briefly, following drug treatment, 10 µl MTT (5 mg/ml) was added to each well and incubated cell for 4 h at 37 °C. Next, the medium was discarded and 150 µL DMSO were added to each well to fully dissolve the formazan product. The absorbance was obtained by microplate reader (Biotek) at 490 nm. The IC50 values were calculated from the inhibition curves by nonlinear regression analysis using GraphPad Prism (GraphPad Software, Inc.).