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Supplementary Information

Discovery of novel JAK2 and EGFR inhibitors from a series of thiazole-based chalcone derivatives

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1. Figures S1

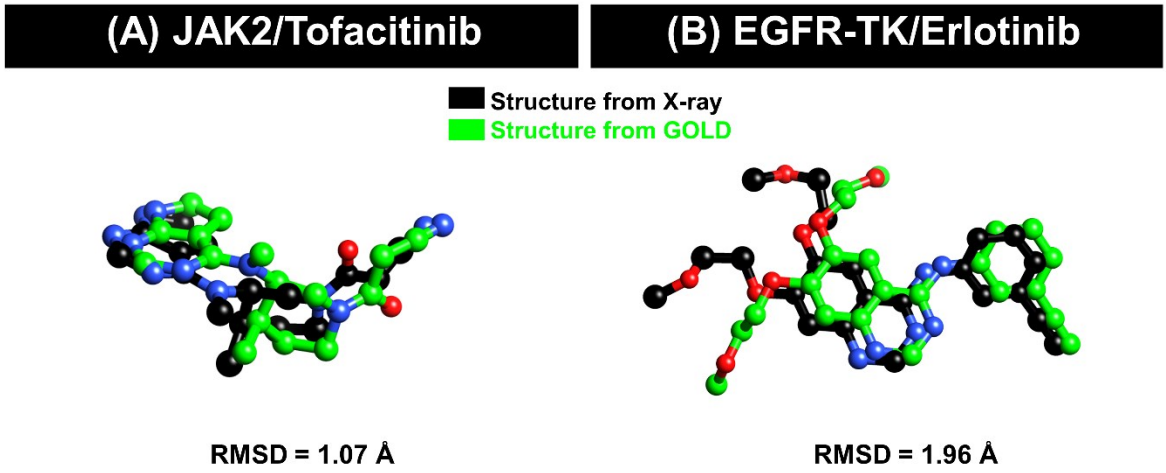

Figure S1. Superimposition of ligands between X-ray structure (black) and GOLD docking (green). JAK2 complexed with tofacitinib (A) and EGFR-TK complexed with erlotinib (B).

2. Figures S2

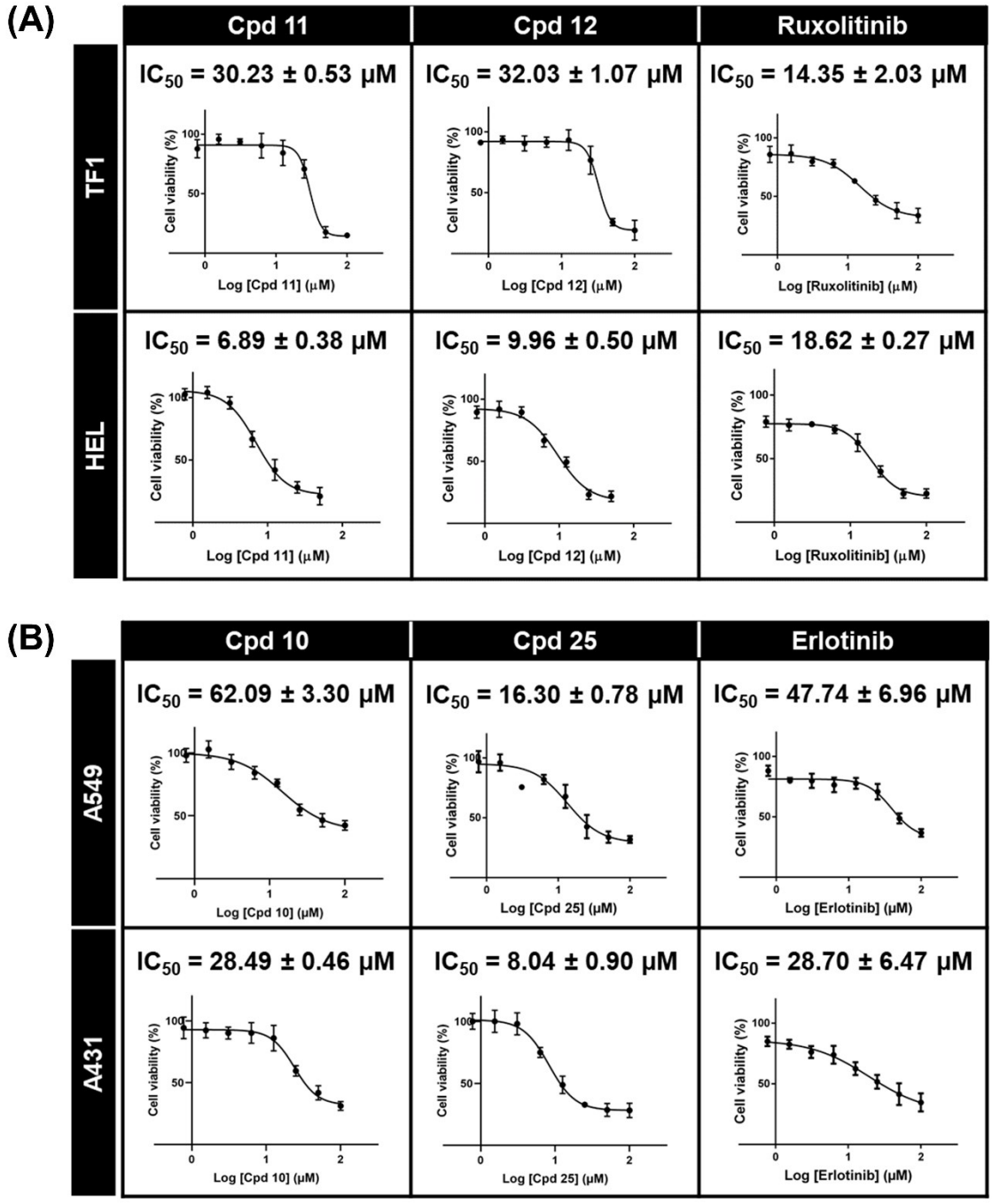

Figure S2. IC_{50} curves of compounds and know drugs towards focused cells (A) TF1, HEL and (B) A549, A431. Data are represented as means ± SEM of triplicate experiments.

3. Figure S3

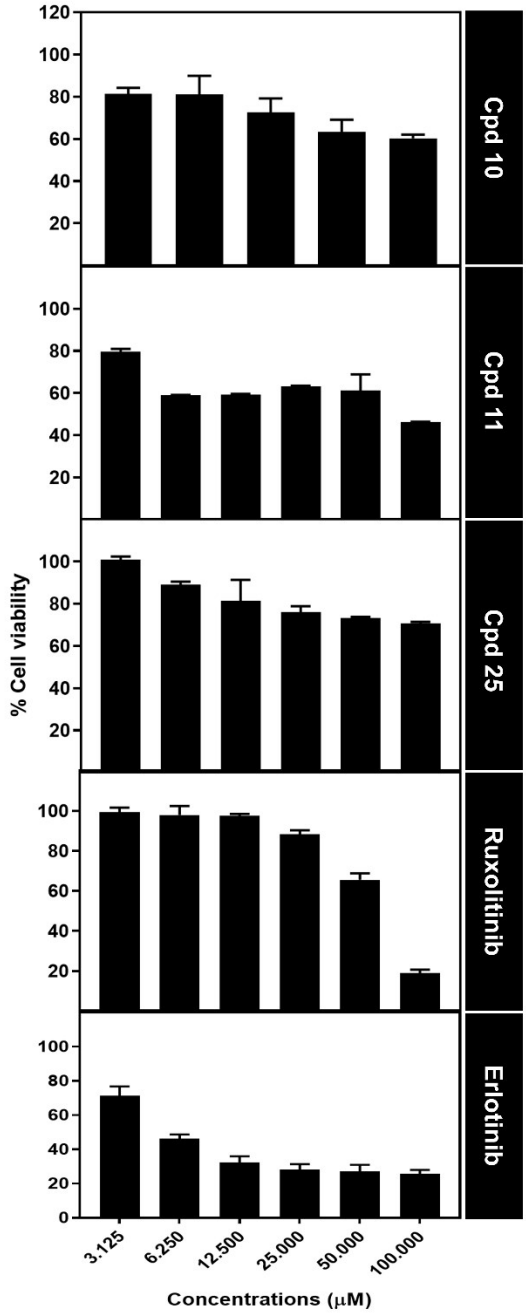

Figure S3. Cytotoxicity of compounds and know drugs towards Vero cells. Data are represented as means ± SEM of triplicate experiments.

4. Figures S4

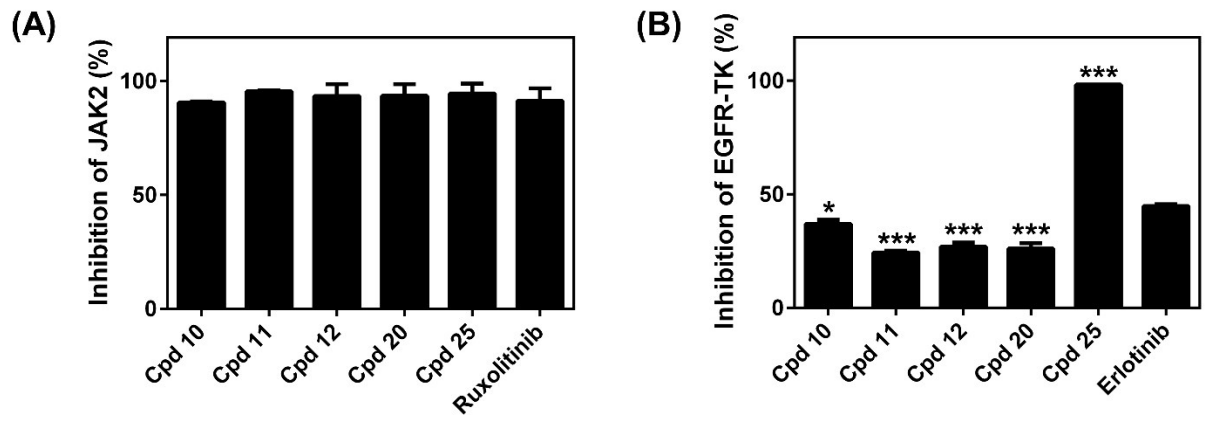

Figure S4. Kinase inhibitory activity screening of compounds towards JAK2 and EGFR-TK at 1 μM. Data are represented as means ± SEM from duplicate independent experiments (n=2). * $p \leq 0.05$ and *** $p \leq 0.001$ vs. erlotinib in EGFR-TK.

5. Figures S5

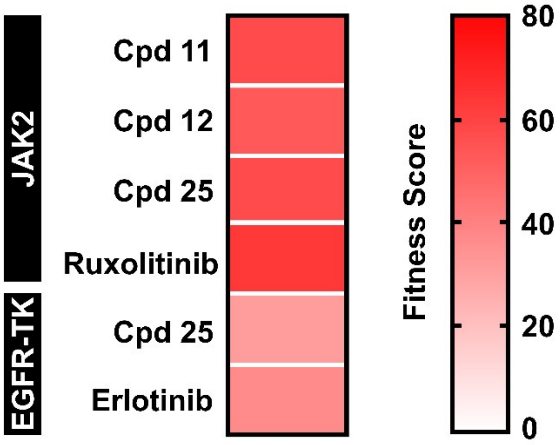

Figure S5. The fitness scores of focused thiazole derivatives towards JAK2 and EGFR-TK.

6. Table S1

Table S1. Interactions of thiazole-based chalcones derivatives as well as the known drugs towards JAK2 and EGFR-TK. Underlined text represents the overlapped residues with the known drugs.

Protein	Compounds	Interactions					
		H bond	Van der Waals	Pi-sulfur	Pi-sigma	Pi-Alkyl	Amide-Pi Stacked
JAK2	11	E930, L932	K857, S862, V911, M929, P933, G935, S936, G993	-	-	A880, V863, Y931, L983	G856, L855
	12	E930, L932	K857, V863, V911 M929, P933, G935 S936	-	G856	L855, A880, Y931, L983	-
	25	K857, L932	G858, E930, Y931, P933, G935, S936, D939, G993	-	G856	L855, V863, A880, V911, M929, L983	-
	Ruxolitinib	E930, L932	G856, K857, G858, G861, S862, K882, V911, Y931, P933, G935, S936, R980, N981, I982, G993, D994	M929	-	A880, L855, V863, L983	-
EGFR-TK	25	K721, M769, D831	V702, K704, A719, E738, M742, L764, T766, Q767, L768, P770, G772, C773, L820, T830	-	-	L694	-

Protein	Compounds	Interactions					
		H bond	Van der Waals	Pi-sulfur	Pi-sigma	Pi-Alkyl	Amide-Pi Stacked
	Erlotinib	M769	G695, G697, F699, V702, K704, A719, E738, M742, L753, L764, I765, T766, Q767, L768, P770, G772, L820, T830, D831	C751	-	L694, K721	-

7. Detail of synthesis and characterization of five compounds (10, 11, 12, 20 and 25)[34]

Synthesis and characterization of five compounds (10, 11, 12, 20 and 25) were published and obtained from Tratrat C. et al., 2019 [34]as follows:

7.1. General Method A (Basic Catalysis)

To a solution of aromatic aldehyde (1 mmol) in 10% NaOH (3-4 mL) at 0° a solution of appropriate 1-(4-methyl-2-alkylamino) thiazol-5-yl) ethanone (1 mmol) in methanol (4.0-4.1 mL), was added dropwise. The solution was maintained at 0 ^{o}C for 1.5 h and then was allowed to stir at room temperature. After some time (24-48 h), the solid started separating out. The solid was filtered under vacuum and recrystallized from dioxane or ethanol to give the title chalcones.

7.2 General Method B (Acid Catalysis)

To a solution of aromatic aldehyde (1mmol) in conc. H2SO4 (1 -1.5 mL at 0°, a solution of appropriate 1- (4-methyl-2- (alkylamino)thiazol-5-yl) ethanone (1 mmol) in methanol (4.0-4.1 mL), was added dropwise. The solution was maintained at 0 ^{o}C for 1.5 h and then was allowed to stir at room temperature. After some time (24-48 h), the solid started separating out. The solid was filtered under vacuum and recrystallized from dioxane or ethanol to give the title chalcones

7.3 (E)- 1-(4-methyl-2-(methylamino)thiazol-5-yl)-3-(thiophen-2-yl) prop-2-en-1-on (10)

Yield: 45.2%, m.p. 228- 229 oC, Rf: 0.46 (CHCl3:MeOH,9,5:0,5), IR: (cm-1, Nujol): 3203 (NH), 3068 (C-H vinyl.), 1628 (C=O), 1602 (C=C), 1559 (C-Harom.). 1 H-NMR: (δ ppm, DMSO-d6/CDCl3, 300 MHz): 2.52 (s, 3H, thiazole-4′- CH3), 2.88 (s, 3H, N-CH3), 6.91 (d, J=15 Hz, 1H, CO -CH), 7.09-7.11 (t, 1H, thiophene-4′), 7.42 (d, J=6 Hz, 1H, thiophene-3 ′), 7.55 (d, J=6 Hz, 1H, thiophene-5′), 7.67 (d, J=15 Hz, 1H, thiophene-2′-CH), 8.31 (s, 1H, NH). Anal. Calc. for C12H12N2OS2: C: 54.52; H: 4.58; N: 10.60%. Found: C: 54.55; H: 4.56; N: 10.82%.

7.4 (E)-1-(4-methyl-2 -(methylamino)thiazol-5-yl)-3-(thiazol-2-yl) prop-2-en-1-on (11)

Yield: 67.2%, m.p. 227- 228 oC, Rf: 0.32 (CHCl3: MeOH, 9.5:0.5). IR: (cm-1, Nujol): 3207 (NH), 3072 (C-H vinyl.), 1628 (C=O), 1605 (C=C), 1556 (C-H arom.). 1 H-NMR: (δ ppm, DMSO-d6/CDCl3, 300 MHz): 2.55 (s, 3H, thiazole-4′- CH3), 2.89 (s, 3H, CH3-N), 7.42 (d, J=15 Hz, 1H, CO-CH), 7.60 (d, J=15 Hz, 1H, thiazole-2′′- CH), 7.72 (d, J=3 Hz, 1H, thiazole 5′′), 7.92 (d, J=3 Hz, 1H, thiazole-4′′), 8.43 (s, 1H, NH). Anal. Calc. for C11H11N3OS2: C: 49.79; H: 4.18; N: 15.84%. Found: C: 49.50; H: 4.21; N: 15.61%.

7.5 (E)- 1-(4-methyl-2-(methylamino)thiazol-5-yl)-3-(thiophen-2-yl) prop-2-en-1-on (12)

Yield: 29.0%, m.p. 212-213 oC, Rf: 0.47 (CHCl3: MeOH, 9.5:0.5). IR: (cm-1, Nujol): 3198 (NH), 3082 (C-H vinyl.), 1634 (C=O), 1597 (C=C), 1560 (C-H arom.). H-NMR: (δ ppm, DMSO-d6, 300 MHz): 2.53 (s, 3H, thiazole-4′-CH3), 2.86 (s, 3H, CH3-N), 7.10 (d, J=15 Hz, 1H, CO-CH), 7.52-7.61 (m, 4H, thiphene-3′-CH,thiphene. 2′,4′,5′), 7.96 (s, 1H, NH). Anal. Calc. for C12H12N2OS2: C: 54.52, H: 4.58, N: 10.60%. Found: C: 54.10, H: 4.5, N: 10.44%.

7.6 (E)-1- (4--methyl-2-(ethylamino)thiazol-5-yl)-3-(4-hydroxyphenyl) prop-2-en-1-on (20)

Method B. Reaction time30 h. Yield: 25.5%, m.p. 246- 248 oC (dioxane), Rf: 0.44 (toluene:EtOH: 8/2). IR: (cm-1, KBr) 3265 (NH), 1645 (C=O).1H- NMR: (δ ppm, 250 MHz, DMSO-d 6) δ= 1.19 (t, J= 7,1 Hz, 3 H, CH3CH2N-), 2.56 (s, 3 H, thiazole 4'-CH3), 3.21- 3.36 (m, 2 H, N-CH2CH3), 6.82 (d, J=7.7 Hz, 2 H, Ar. 2',6'), 7.10 (d, J=15.1 Hz, 1 H, allylic CO-CH), 7.51 (d, J=16.1 Hz, 1 H, allylicAr-CH), 7.61 (d, J= 7.6 Hz, 2 H, Ar. 3',5'), 8.80 (br, 1 H, NH), 10.03 (s, 1 H, PhOH). Anal. Calc. for C15H16N2O2S (288.09): C:62.48; H: 5.59; N: 9.71%. Found: C:62.49; H: 5.57; N: 9.70%.

7.7 (E)-1-(4 --methyl-2-(methylamino)thiazol-5-yl)-3-(4-hydroxyphenyl) prop-2-en-1-on (25)

Method B. Reaction time 30 h. Yield: 17.1%, m.p. 253- 255 oC (dioxane), Rf: 0.41 (toluene:EtOH: 8/2). IR: (cm-1, KBr) 3209 (NH), 1650 (C=O). 1H-NMR: (δ ppm, 250 MHz, DMSO-d6) δ= 2,57 (s, 3 H, thiazole 4'-CH3), 2.93 (s, 3 H, N-CH3), 6.83 (d, J=8,2 Hz, 2H, Ar. 2',6'), 7.09 (d, J=15,3 Hz, 1H, allylic CO-CH), 7.52 (d, J=15.4 Hz, 1 H, allylic Ar -CH), 7.60 (d, J=8.2 Hz, 2 H, Ar. 3',5'), 8.91 (br, 1 H, NH). Anal. Calc. for for C14H14N2O2 S (274.8): C 61.29; H: 5.14; N: 10.21%. Found: C:61.32; H: 5.15; N: 10.22%.

8. Figure S6

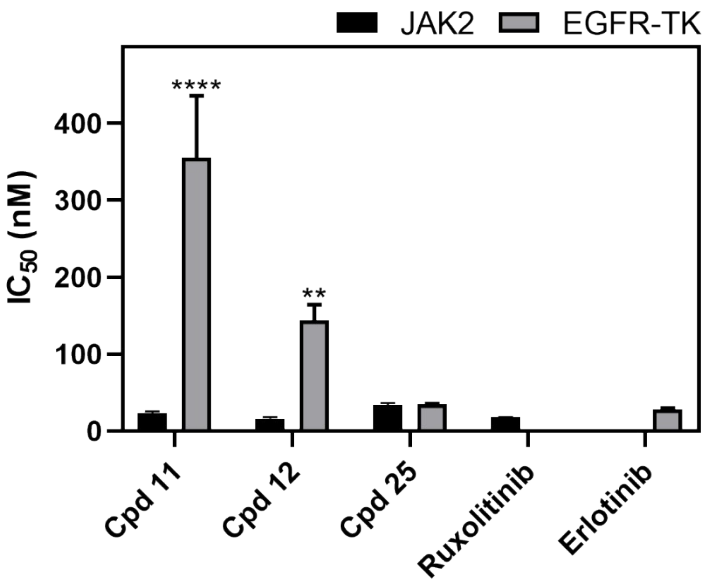

Figure S6. IC_{50} of kinase inhibitory activity of compounds towards JAK2 and EGFR-TK. Data are represented as means ± SEM from triplicate independent experiments (n=3). ** $p \leq 0.01$ and **** $p \leq 0.0001$ vs. erlotinib in EGFR-TK.