Supporting Information for:

Synthesis, biological evaluation and structure-activity relationships of diflapolin analogs as dual sEH/FLAP inhibitors

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Materials and methods

Bovine serum albumin (BSA) and Tris were obtained from AppliChem (Darmstadt, Germany); PGB1, 3-phenyl-cyano(6-methoxy-2-naphthalenyl)methylester-2-oxiraneacetic acid (PHOME) and MK886 from Cayman Chemical (Biomol, Hamburg, Germany); DMSO from Merck (Darmstadt, Germany); zileuton from Sequoia Research Products (Oxford, UK); Dulbecco's buffer substance (PBS) from SERVA Electrophoresis (Heidelberg, Germany); HPLC solvents were from VWR (Darmstadt, Germany); Ca^{2+}-ionophore A23187, dextrane, and all other chemicals were from Sigma-Aldrich (Taufkirchen, Germany), unless stated elsewhere.

Determination of 5-LO activity

Human neutrophils were collected from buffy coats of healthy adult fasted donors as described recently.[1] The use of human blood preparations was approved by the ethical commission of the Friedrich-Schiller-University Jena (approval number 4292-12/14) in accordance with relevant guidelines and regulations. Freshly isolated neutrophils were diluted with Dulbecco's PBS containing 0.1 % glucose and 1 mM $CaCl_2$ to a final density of 5 mio. cells/mL and pre-incubated for 10 min at 37 °C with test compounds or 0.1 % vehicle (DMSO), respectively. Cells were subsequently stimulated with 2.5 µM A23187 for 10 min at 37 °C and reactions were terminated using one volume of ice-cold methanol. 200 ng of internal PGB_1 standard was added to the acidified samples and solid-phase-extraction using C18 RP-columns (100 mg, UCT, Bristol, PA, USA) was performed. Samples were eluted with methanol and 5-LO product formation including 5-HpETE, the corresponding alcohol 5-HETE and all-trans-isomers of LTB_4, was analyzed by RP-HPLC with a C-18 Radial-PAK column (Waters, Eschborn, Germany) as described.[2]

Determination of sEH activity

Human recombinant sEH was expressed and purified as published.[3] Isolated sEH was diluted in 25 mM Tris buffer (pH 7) containing 0.1 mg/mL BSA to a final protein concentration of 0.5 µg/mL. Preincubation with test compounds or 0.1 % vehicle (DMSO) at room temperature for 10 min was followed by stimulation with 20 µM PHOME at RT for 60 min. Reactions were stopped with 200 mM $ZnSO_4$ and fluorescence was subsequently detected at λ_{em} 465 nm and λ_{ex} 330 nm. If required, a possible fluorescence of test compounds was subtracted from the read-out.

(n=3-4)

Docking

Docking simulations were performed on a windows 7 machine with an i5-4670S CPU using the CCDC's Gold software (version 5.2).[4] Gold was set to calculate ten poses per molecule. If the genetic algorithm leads to very similar poses, this a sign of a stable energetic minimum. The pdb entry 3koo[5] was selected as a crystal structure for docking. Inhibitor *N*-(2,4-dichlorobenzyl)-4-(pyrimidin-2-yloxy)piperidine-1-carboxamide was extracted from the structure and its binding site was used to define the docking site in an 9 Å radius. CHEMPLP was selected as a scoring function. The original ligand was prepared in the same workflow as the candidate molecules and redocked into the binding site, with an RMSD of 1.42.

The interactions between the docking poses and the binding site were calculated and visualized in Ligandscout 4.2. (www.inteligand.com)

Additional Figures:

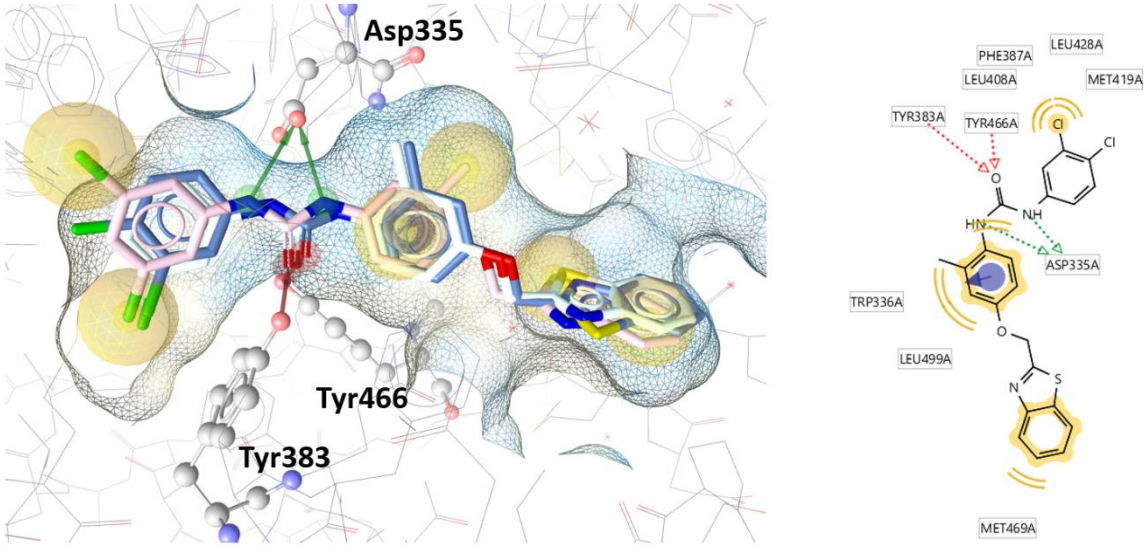


Figure S1. Binding pocket of diflapolin in sEH, showing the three key residues of the protein that form the binding site for the urea motif. Right: Binding pocket in 2D, showing all interacting residues.

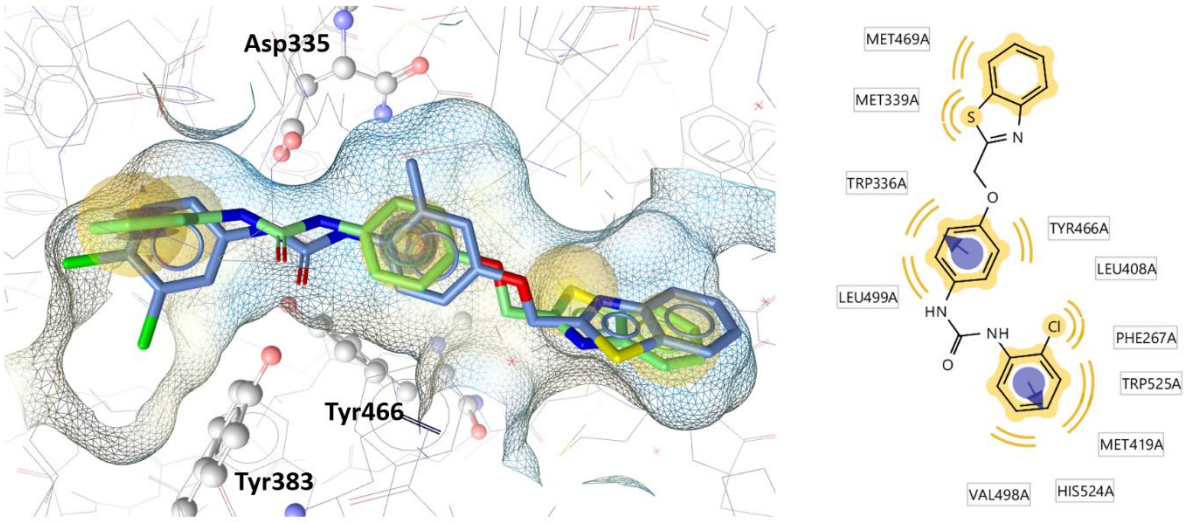


Figure S2. Comparison between diflapolin and compound 24 (in green), the methyl group in the ortho position tilts the molecule, so it can no longer form the interactions with the urea binding motif. Right: 2D depiction of the binding pocket.

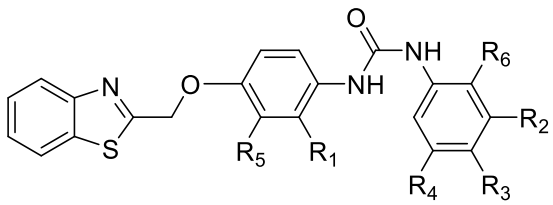
Free-Wilson Analysis

Free-Wilson analysis is a QSAR method to assign to each occurring rest group in a SAR dataset a contribution to the activity of the molecule. Following the equation

$$\text{Log } BA_i = \sum a_{jk} + X_{jk} + \mu$$

BA_i, the biological activity of a series (used in a logarithmic scale) is expressed as the sum of the biological activity contributions a_{jk} of the substituents R_k in each position j, μ is referring to the overall average activity value for the series. [6]

A Free-Wilson least squares model was calculated in Biovia's Pipeline Pilot, using the implemented script "Create Free-Wilson least squares model". The model was based on compounds **1** and **11-27**, which have the same core structure and only differ in the location of the methyl group on subunit III and the chlorine substitution on subunit V. The Free-Wilson predicted activity was based on the pIC_{50} of the measured compounds.



Core molecule for Free-Wilson analysis

Free-Wilson analysis for sEH

The Free-Wilson analysis with the pIC_{50} values for sEH activity led to a model with an RMSE 0.056805 and an R^2 of 0.9481. For the individual groups, the following contributions were calculated:

Constant μ	**-1.8327**

R1: CH_3	0.012368
R2: Cl	0.49865
R3: Cl	0.52163
R4: Cl	0.1231
R5: CH_3	1.0484
R6: Cl	-0.90979

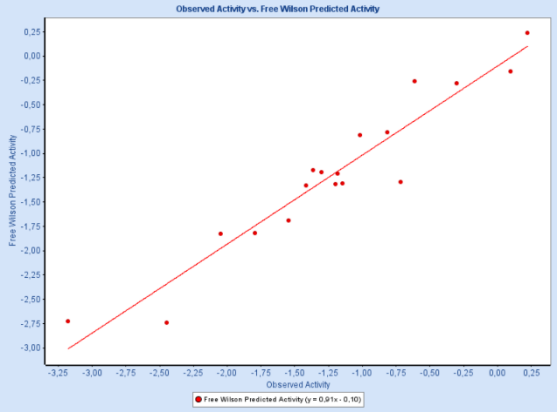


Figure S3. Predicted Free-Wilson Activity for sEH vs observed activity.

While most substituents are acceptable for sEH activity, the 2-Cl substitution clearly damages sEH activity, 3-methyl is preferred but not required for sEH activity.

Free-Wilson analysis for FLAP

The Free-Wilson analysis with the pIC_{50} values for FLAP activity resulted in a model with an RMSE 0.10795 and an R^2 of 0.76320. For the individual groups, the following contributions were calculated:

Constant μ	**0.29196**
R1: CH_3	0.01186
R2: Cl	0.41063
R3: Cl	-0.40362
R4: Cl	0.45669
R5: CH_3	-0.923
R6: Cl	0.24246

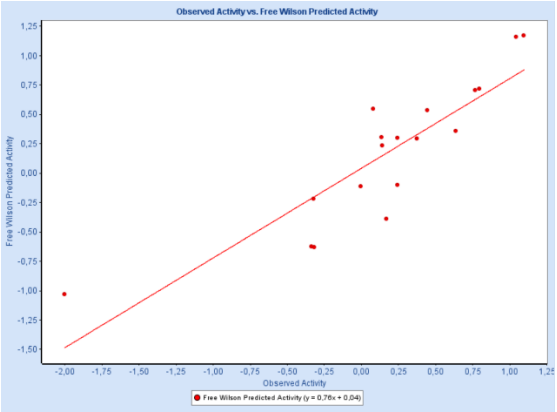


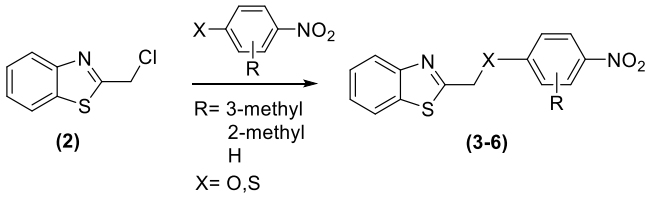
Figure S4. For FLAP activity the model showed that the 3-methyl modification is lowering FLAP activity. Also the 4-Cl modification is not beneficial for FLAP activity, while Cl-substitution in the meta position results in a positive activity contribution.

Organic synthesis

Reactions were monitored by TLC using Polygram® SIL G/UV254 (Macherey-Nagel) plastic-backed plates (0.25 mm layer thickness), column chromatography was performed using silica gel 60 (40-63 µm). The yields are not optimized. Melting points were determined with a Kofler hot-stage microscope (Reichert) and are uncorrected. IR spectra were recorded on a Bruker ALPHA FT-IR apparatus equipped with a Platinum ATR module. ^{1}H-NMR spectra were recorded on a Varian Gemini 200 spectrometer 199.98 MHz. ^{13}C-NMR spectra were recorded on a Bruker Avance II 600 spectrometer (Bruker) operating at 150.91 MHz. The center of the solvent multiplet (DMSO-d_6) was used as internal standard (chemical shifts in δ ppm), which was related to TMS with δ 2.49 ppm (^{1}H) and 39.5 ppm (^{13}C). Elemental analyses were performed by Mag. J. Theiner, 'Mikroanalytisches Laboratorium', Faculty of Chemistry, University of Vienna, Austria

Compounds **29** and **30** were prepared according to the literature by reaction of 2-methylbenzothiazole with 4-nitrobenzaldehyde in the presence of acetic acid and acetic anhydrate and subsequent reduction of the nitro function.[7]

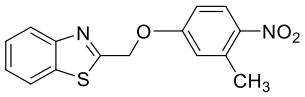
Preparation of compounds 3-6



The synthesis of the compounds was performed according to a procedure described in reference[8].

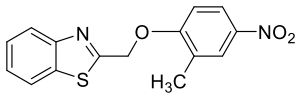
A suspension of 2-chloromethylbenzothiazole (**2**) (11 mmol, 1 equiv.) with the appropriate 4-nitro(thio)phenol (11 mmol, 1 equiv.) in the presence of caesium carbonate (10.7 mmol, 0.9 equiv.), sodium carbonate (11 mmol, 0.9 equiv.) and potassium iodide (3.3 mmol, 0.3 equiv.) in acetone (70 mL) was heated under reflux until the reaction *was* completed (monitored by TLC). The mixture was filtered and acetone was partially reduced under vacuo. After addition of water, the solid thus obtained was isolated and dried to give the pure product.

2-((3-Methyl-4-nitrophenoxy)methyl)benzothiazole (3) (CAS Registry Number 197364-74-2)



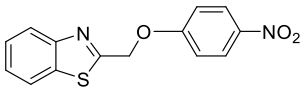
Yield: 1.99 g (61 %), light brown crystals. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 8.14-8.00 (m, 3H, Ar-H); 7.58-7.48 (m, 2H, Ar-H); 7.22 (d, J = 2.8 Hz, 1H, Ar-H); 7.16-7.10 (dd, J = 9.2 Hz, J = 2.8 Hz, 1H, Ar-H); 5.74 (s, 2H, CH_2); 2.54 (s, 3H, CH_3).

2-((2-Methyl-4-nitrophenoxy)methyl)benzothiazole (4)



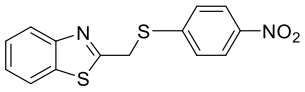
Yield: 3.07 g (94 %), light brown solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 8.13 (d, J = 7.6 Hz, 2H, Ar-H); 8.02 (d, J = 7.6 Hz, 1H, Ar-H); 7.59-7.42 (m, 2H, Ar-H); 7.32 (d, J = 8.8 Hz, 1H, Ar-H); 5.78 (s, 2H, CH_2); 2.36 (s, 3H, CH_3).

2-((4-Nitrophenoxy)methyl)benzothiazole (5) (CAS Registry Number 100537-61-9)



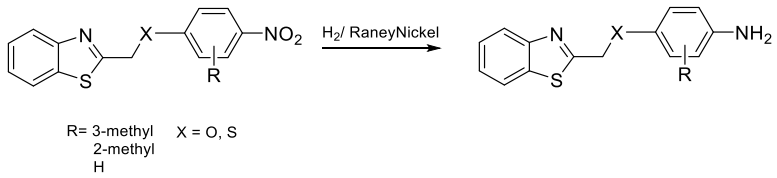
Yield: 1.82 g (85 %), beige solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 8.24 (d, J = 9.4 Hz, 2H, Ar-H); 8.15-8.11 (dd, J = 7.3 Hz, J = 1.9 Hz, 1H, Ar-H); 8.05-8.01 (dd, J = 7.6 Hz, J = 1.8 Hz, 1H, Ar-H); 7.59-7.42 (m, 2H, Ar-H); 7.32 (d, J = 9.4 Hz, 2H, Ar-H); 5.78 (s, 2H, CH_2).

2-((4-Nitrophenylthio)methyl)benzothiazole (6) (CAS Registry Number 100537-48-2)



Yield: 1.64 g (98 %), dark green solid ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 8.11 (d, J = 9.1 Hz, 2H); 8.04-8.00 (dd, J = 1.8 Hz, J = 7.6 Hz, 1H, Ar-H); 7.96-7.91 (dd, J = 1.8 Hz, 7.6 Hz, 1H, Ar-H); 7.62 (d, J = 9.1 Hz, 2H, Ar-H); 7.53-7.35 (m, 2H, Ar-H); 4.97 (s, 2H, CH_2).

Preparation of compounds 7-10

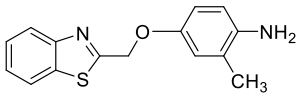


R= 3-methyl
2-methyl
H

X = O, S

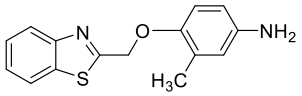
A mixture of the appropriate nitro compounds (10.0 mmol) in methanol (200-250 mL) was reduced on a Parr hydrogenator at 40 psi using RaneyNickel as catalyst until the reduction was completed (determined by TLC). To remove the catalyst, the resulting mixture was filtered. Then, the solution was evaporated under reduced pressure to yield the pure products.

2-(4-Amino-3-methylphenoxymethyl)benzothiazole (7) (CAS Registry Number 197364-75-3)



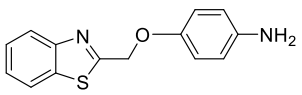
Yield: 2.71 g (93 %), white solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 8.10 (d, *J* = 7.6 Hz, 1H, Ar-H); 7.98 (d, *J* = 7.6 Hz, 1H, Ar-H); 7.56-7.39 (m, 2H, Ar-H); 6.75-6.65 (m, 2H, Ar-H); 6.54 (d, *J* = 8.6 Hz, 1H, Ar-H); 5.41 (s, 2H, CH_2); 4.48 (s, 2H, exchangeable with D_2O, NH_2); 2.02 (s, 3H, CH_3).

2-(4-Amino-2-methylphenoxymethyl)benzothiazole (8) (CAS Registry Number 1917245-90-9)



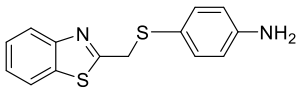
Yield: 2.64 g (94 %), white solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 8.11 (d, *J* = 7.3 Hz, 1H, Ar-H); 7.99 (d, *J* = 7.3 Hz, 1H, Ar-H); 7.56-7.40 (m, 2H, Ar-H); 6.78 (d, *J* = 8.6 Hz, 1H, Ar-H); 5.39 (s, 2H, CH_2); 4.63 (s, 2H, exchangeable with D_2O, NH_2); 2.16 (s, 3H, CH_3).

2-(4-Aminophenoxymethyl)benzothiazole (9) (CAS Registry Number 197364-64-0)



Yield: 2.08 g (89 %), white solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 8.12-8.07 (dd, *J* = 7.7 Hz, *J* = 2.1 Hz, 1H, Ar-H); 8.02-7.97 (dd, *J* = 7.6 Hz, *J* = 1.6 Hz, 1H, Ar-H); 7.56-7.39 (m, 2H, Ar-H); 6.81 (d, *J* = 8.9 Hz, 2H, Ar-H); 6.52 (d, *J* = 8.9 Hz, 2H, Ar-H); 5.41 (s, 2H, CH_2); 4.72 (s, 2H, exchangeable with D_2O, NH_2).

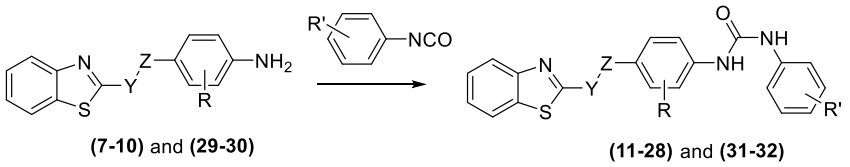
2-(4-Aminophenylthiomethyl)benzothiazole (10)



Yield: 1.33 g (98 %), dark brown solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 8.02 (d, *J* = 8.0 Hz, 1H, Ar-H); 7.86 (d, *J* = 8.0 Hz, 1H, Ar-H); 7.49-7.34 (q, *J* = 7.2 Hz, 2H, Ar-H); 7.10 (d, *J* = 8.5 Hz, 2H, Ar-H); 6.45 (d, *J* = 8.5 Hz, 2H, Ar-H); 5.30 (s, 2H, CH_2); 4.40 (s, 2H, exchangeable with D_2O, NH_2).

Preparation of compounds 11-28 and 31-32

To a solution of the amino compounds (1 equiv.) in tetrahydrofuran (5 mL), a solution of the appropriate phenylisocyanate (1.2 equiv.) in tetrahydrofuran was added dropwise. The resulting mixture was stirred at room temperature for 2-4 h. The obtained solid was filtered, washed with diethyl ether, and dried. The product thus obtained was purified by treatment with acetone.

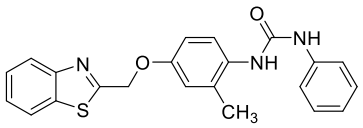


R= 3-methyl
2-methyl
H

Y-Z= CH_2-O; CH_2-S; CH_2-CH_2; CH=CH

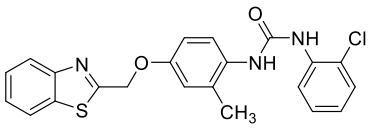
R'= H; 3,4-Cl_2; 2-Cl; 3-Cl; 4-Cl; 3,5-Cl_2

1-(4-(Benzo[*d*]thiazol-2-ylmethoxy)-2-methylphenyl)-3-phenylurea (11)



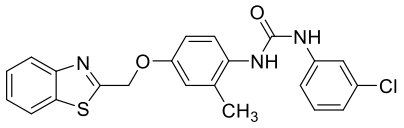
Yield: 0.212 g (75 %), white solid. ^{1}H-NMR (DMSO-d_6, 200 MHz) δ: 8.86 (s, 1H, exchangeable with D_2O, NH); 8.13-8.09 (m, 1H, Ar-H); 8.01 (dd, *J* = 7.2 Hz, *J* = 1.4 Hz, 1H, Ar-H); 7.81 (s, 1H, exchangeable with D_2O, NH); 7.62-7.41 (m, 5H, Ar-H); 7.25 (t, *J* = 7.8 Hz, 2H, Ar-H); 6.96-6.88 (m, 3H, Ar-H); 5.55 (s, 2H, CH_2); 2.21 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6, δ): 168.8, 153.3, 152.9, 152.5, 140.0, 134.4, 131.3, 130.8, 128.8, 126.3, 125.3, 123.7, 122.7, 122.4, 121.5, 117.9, 116.7, 112.4, 67.4, 18.0. Anal. Calcd for $C_{22}H_{19}N_3O_2S$: C, 67.85; H, 4.92; N, 10.79; S, 8.23. Found: C, 67.75; H, 4.95; N, 10.80; S, 8.21. IR (cm^{-1}): 3290, 1634. mp 226-229 °C.

1-(4-(Benzo[*d*]thiazol-2-ylmethoxy)-2-methylphenyl)-3-(2-chlorophenyl)urea (12)



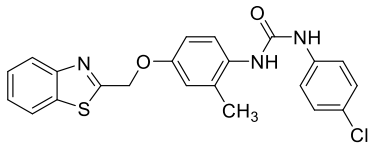
Yield: 0.237 g (75 %), white solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 8.54 (s, 1H, exchangeable with D_2O, NH); 8.49 (s, 1H, exchangeable with D_2O, NH); 8.14-8.09 (m, 2H, Ar-H), 8.03-7.99 (m, 1H, Ar-H), 7.56-7.40 (m, 4H, Ar-H); 7.30-7.22 (m, 1H, Ar-H); 7.03-6.87 (m, 3H, Ar-H); 5.55 (s, 2H, CH_2); 2.22 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6, δ): 168.8, 153.7, 152.8, 152.5, 136.3, 134.4, 131.4, 130.9, 129.2, 127.5, 126.3, 125.3, 124.4, 123.0, 122.7, 122.4, 121.8, 121.4, 116.7, 112.4, 67.3, 18.1. Anal. Calcd for $C_{22}H_{18}ClN_3O_2S \times 0.2\ H_2O$: C, 61.81; H, 4.34; N, 9.83; S, 7.50 Found: C, 61.89; H, 4.38; N, 9.80; S, 7.43. IR (cm^{-1}): 3282, 1639. mp 239-241 °C.

1-(4-(Benzo[*d*]thiazol-2-ylmethoxy)-2-methylphenyl)-3-(3-chlorophenyl)urea (13)



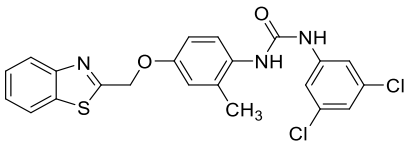
Yield: 0.229 g (73 %), white solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 9.07 (s, 1H, exchangeable with D_2O, NH); 8.11 (d, *J* = 7.6 Hz, 1H, Ar-H); 8.01 (d, *J* = 8.0 Hz, 1H, Ar-H); 7.89 (s, 1H, exchangeable with D_2O, NH); 7.71 ('s', 1H, Ar-H); 7.57-7.41 (m, 3H, Ar-H); 7.31-7.19 (m, 2H, Ar-H); 6.98-6.87 (m, 3H, Ar-H); 5.55 (s, 2H, CH_2); 2.20 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6, δ): 168.8, 153.6, 152.8, 152.5, 141.6, 134.4, 133.2, 131.3, 131.0, 130.4, 126.3, 125.3, 124.1, 122.7, 122.4, 121.1, 117.3, 116.7, 116.3, 112.4, 67.3, 18.0. Anal. Calcd for $C_{22}H_{18}ClN_3O_2S$: C, 62.33; H, 4.28; N, 9.91; S, 7.56. Found: C, 62.20; H, 4.22; N, 9.85; S, 7.50. IR (cm^{-1}): 3285, 1640. mp 231-232 °C.

1-(4-(Benzo[*d*]thiazol-2-ylmethoxy)-2-methylphenyl)-3-(4-chlorophenyl)urea (14)



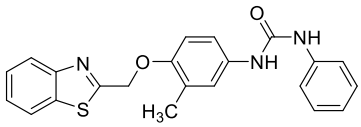
Yield: 0.203 g (65 %), white solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 9.00 (s, 1H, exchangeable with D_2O, NH); 8.13-8.08 (m, 1H, Ar-H); 8.01 (d, *J* = 7.6 Hz, 1H, Ar-H); 7.85 (s, 1H, exchangeable with D_2O, NH); 7.58-7.41 (m, 5H, Ar-H); 7.29 (d, *J* = 8.8 Hz, 2H, Ar-H); 6.97-6.86 (m, 2H, Ar-H); 5.55 (s, 2H, CH_2); 2.20 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6, δ): 168.8, 153.5, 152.9, 152.5, 139.0, 134.4, 131.1, 131.1, 128.6, 126.3, 125.3, 125.0, 123.9, 122.7, 122.4, 119.4, 116.8, 112.4, 67.4, 18.0. $C_{22}H_{18}ClN_3O_2S$ x 0.2 H_2O: C, 61.81; H, 4.34; N, 9.83; S, 7.50. Found: C, 62.19; H, 4.34; N, 9.41; S, 7.51. IR (cm^{-1}): 3311, 3273, 1634. mp 248-250 °C.

1-(4-(Benzo[*d*]thiazol-2-ylmethoxy)-2-methylphenyl)-3-(3,5-dichlorophenyl)urea (15)



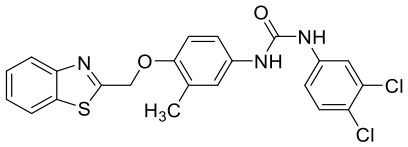
Yield: 0.280 g (83 %), white solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 9.24 (s, 1H, exchangeable with D_2O, NH); 8.13-8.08 (m, 1H, Ar-H); 8.03-7.99 (m, 2H, 1H exchangeable with D_2O, NH, Ar-H); 7.57-7.40 (m, 5H, Ar-H); 7.11 (t, *J* = 1.8 Hz, 1H, Ar-H); 6.98-6.87 (m, 2H, Ar-H); 5.56 (s, 2H, CH_2); 2.20 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6, δ): 168.7, 154.0, 152.7, 152.5, 142.6, 134.4, 134.1, 132.0, 130.6, 126.3, 125.3, 124.7, 122.7, 122.4, 120.6, 116.7, 116.0, 112.4, 67.3, 17.9. Anal. Calcd for $C_{22}H_{17}Cl_2N_3O_2S$: C, 57.65; H, 3.74; N, 9.17; S, 6.99. Found: C, 57.41; H, 3.67; N, 9.03; S, 6.71. IR (cm^{-1}): 3288, 1635. mp 235-236 °C.

1-(4-(Benzo[*d*]thiazol-2-ylmethoxy)-3-methylphenyl)-3-phenylurea (16)



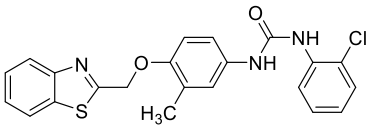
Yield: 0.271 g (94 %), white solid. ^{1}H-NMR (200 MHz, DMSO-d$_6$, δ) 8.57 (s, 1H, exchangeable with D$_2$O, NH); 8.44 (s, 1H, exchangeable with D$_2$O, NH); 8.14-8.09 (m, 1H, Ar-H); 8.00 (d, *J* = 7.8 Hz, 1H, Ar-H); 7.57-7.41 (m, 4H, Ar-H); 7.29-7.18 (m, 4H, Ar-H); 7.03-6.95 (m, 2H, Ar-H); 5,54 (s, 2H, CH$_2$); 2.27 (s, 3H, CH$_3$). ^{13}C-NMR (DMSO-d$_6$, δ): 169.2, 152.7, 152.6, 150.7, 139.9, 134.3, 133.4, 128.7, 126.4, 126.3, 125.3, 122.7, 122.4, 121.6, 121.4, 118.1, 117.0, 112.5, 67.6, 16.2. Anal. Calcd for $C_{22}H_{19}N_3O_2S$: C, 67.85; H, 4.92; N, 10.79; S, 8.23. Found: C, 67.62; H, 4.94; N, 10.59; S, 8.07. IR (cm^{-1}): 3267, 1639. mp 235-240 °C.

1-(4-(Benzo[*d*]thiazol-2-ylmethoxy)-3-methylphenyl)-3-(3,4-dichlorophenyl)urea (17)



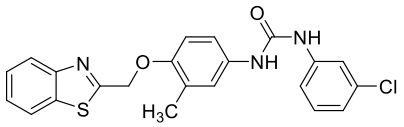
Yield: 0.310 g (91 %), white solid. ^{1}H-NMR (200 MHz, DMSO-d$_6$, δ) 8.91 (s, 1H, exchangeable with D$_2$O, NH); 8.58 (s, 1H, exchangeable with D$_2$O, NH); 8.12 (dd, *J* = 7.8 Hz, *J* = 1.0 Hz, 1H, Ar-H); 8.02-7.98 (m, 1H, Ar-H); 7.86 (d, *J* = 2.4 Hz, 1H, Ar-H); 7.57-7.40 (m, 3H, Ar-H); 7.31-7.17 (m, 3H, Ar-H); 7.07 (d, *J* = 9.0 Hz, 1H, Ar-H); 5.54 (s, 2H, CH$_2$); 2.26 (s, 3H, CH$_3$). ^{13}C-NMR (DMSO-d$_6$, δ): 169.1, 152.6, 152.4, 151.0, 140.1, 134.3, 132.9, 131.0, 130.5, 126.4, 126.3, 125.2, 122.8, 122.7, 122.4, 121.8, 119.1, 118.2, 117.4, 112.5, 67.6, 16.1. Anal. Calcd for $C_{22}H_{17}Cl_2N_3O_2S$: C, 57.65; H, 3.74; N, 9.17; S, 6.99. Found: C, 57.68; H, 3.72; N, 9.08; S, 6.90. IR (cm^{-1}): 3272, 1632. mp 255-258 °C.

1-(4-(Benzo[*d*]thiazol-2-ylmethoxy)-3-methylphenyl)-3-(2-chlorophenyl)urea (18)



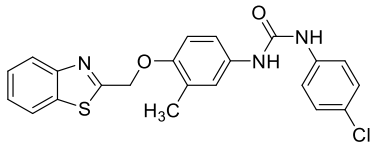
Yield: 0.294 g (94 %), white solid. ^{1}H-NMR (200 MHz, DMSO-d$_6$, δ) 9.23 (s, 1H, exchangeable with D$_2$O, NH); 8.22 (s, 1H, exchangeable with D$_2$O, NH); 8.18-8.10 (m, 2H, Ar-H); 8.01 (d, *J* = 7.6 Hz, 1H, Ar-H); 7.57-7.20 (m, 6H, Ar-H); 7.05-6.95 (m, 2H, Ar-H); 5.55 (s, 2H, CH$_2$); 2.28 (s, 3H, CH$_3$). ^{13}C-NMR (DMSO-d$_6$, δ): 169.1, 152.6, 152.2, 150.9, 136.1, 134.3, 133.1, 129.1, 127.5, 126.5, 126.3, 125.2, 123.0, 122.7, 122.4, 121.7, 121.3, 121.1, 116.9, 112.5, 67.6, 16.2. Anal. Calcd for $C_{22}H_{18}ClN_3O_2S$: C, 62.33; H, 4.28; N, 9.91; S, 7.56. Found: C, 62.03; H, 4.23; N, 9.76; S, 7.30. IR (cm^{-1}): 3272, 1638. mp 238-240 °C.

1-(4-(Benzo[*d*]thiazol-2-ylmethoxy)-3-methylphenyl)-3-(3-chlorophenyl)urea (19)



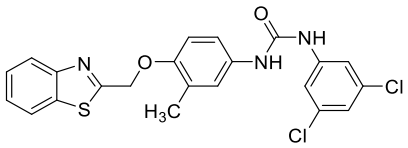
Yield: 0.278 g (89 %), white solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 8.80 (s, 1H, exchangeable with D_2O, NH); 8.53 (s, 1H, exchangeable with D_2O, NH); 8.14-8.10 (m, 1H, Ar-H); 8.03-7.99 (m, 1H, Ar-H); 7.71-7.70 (m, 1H, Ar-H); 7.57-7.41 (m, 2H, Ar-H); 7.31-7.18 (m, 4H, Ar-H); 7.04-6.95 (m, 2H, Ar-H); 5.55 (s, 2H, CH_2); 2.27 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6, δ): 169.1, 152.6, 152.5, 150.9, 141.4, 134.3, 133.1, 133.0, 130.3, 126.4, 126.3, 125.2, 122.6, 122.4, 121.6, 121.2, 117.4, 117.2, 116.5, 112.5, 67.6, 16.2. Anal. Calcd for $C_{22}H_{18}ClN_3O_2S$: C, 62.33; H, 4.28; N, 9.91; S, 7.56. Found: C, 62.29; H, 4.22; N, 9.82; S, 7.52. IR (cm^{-1}): 3264, 1640. mp 241-242 °C.

1-(4-(Benzo[*d*]thiazol-2-ylmethoxy)-3-methylphenyl)-3-(4-chlorophenyl)urea (20)



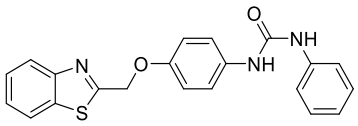
Yield: 0.299 g (95 %), white solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 8.73 (s, 1H, exchangeable with D_2O, NH); 8.48 (s, 1H, exchangeable with D_2O, NH); 8.14-8.09 (m, 1H, Ar-H); 8.03-7.98 (m, 1H, Ar-H); 7.57-7.41 (m, 4H, Ar-H); 7.31-7.18 (m, 4H, Ar-H); 7.01 (d, J = 9.0 Hz, 1H, Ar-H); 5.54 (s, 2H, CH_2); 2.26 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6, δ): 169.1, 152.6, 152.5, 150.9, 138.9, 134.3, 133.2, 128.6, 126.4, 126.3, 125.2, 125.1, 122.7, 122.4, 121.5, 119.6, 117.1, 112.5, 67.6, 16.2. Anal. Calcd for $C_{22}H_{18}ClN_3O_2S$: C, 62.33; H, 4.28; N, 9.91; S, 7.56. Found: C, 62.26; H, 4.31; N, 9.70; S, 7.38. IR (cm^{-1}): 3285, 1634. mp 260-262 °C.

1-(4-(Benzo[*d*]thiazol-2-ylmethoxy)-3-methylphenyl)-3-(3,5-dichlorophenyl)urea (21)



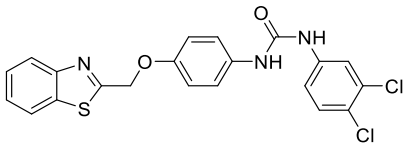
Yield: 0.305 g (90 %), white solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 8.96 (s, 1H, exchangeable with D_2O, NH); 8.64 (s, 1H, exchangeable with D_2O, NH) 8.13-8.09 (m, 1H, Ar-H); 8.00 (d, J = 7.6 Hz, 1H, Ar-H); 7.56-7.00 (m, 8H, Ar-H); 5.54 (s, 2H, CH_2); 2.27 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6, δ): 169.1, 152.6, 152.3, 151.1, 142.4, 134.3, 134.0, 132.8, 126.4, 126.3, 125.2, 122.7, 122.4, 121.9, 120.6, 117.5, 116.2, 112.4, 67.6, 16.1. Anal. Calcd for $C_{22}H_{17}Cl_2N_3O_2S$: C, 57.65; H, 3.74; N, 9.17; S, 6.99. Found: C, 57.44; H, 3.78; N, 9.06; S, 6.84. IR (cm^{-1}): 3281, 1639. mp 256-257 °C.

1-(4-(Benzo[*d*]thiazol-2-ylmethoxy)phenyl)-3-phenylurea (22)



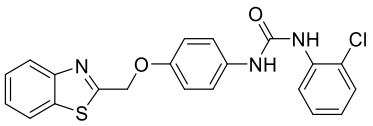
Yield: 0.205 g (70 %), white solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 8.59 (s, 1H, exchangeable with D_2O, NH); 8.52 (s, 1H, exchangeable with D_2O, NH); 8.11 (d, J = 8.0 Hz, 1H, Ar-H); 8.01 (d, J = 7.6 Hz, 1H, Ar-H); 7.56-7.21 (m, 8H, Ar-H); 7.05-6.90 (m, 3H, Ar-H); 5.55 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6, δ): 168.8, 152.7, 152.6, 152.5, 139.8, 134.4, 133.8, 128.8, 126.3, 125.3, 122.7, 122.4, 121.7, 119.9, 118.1, 115.3, 67.5. Anal. Calcd for $C_{21}H_{17}N_3O_2S$: C, 67.18; H, 4.56; N, 11.19; S, 8.54. Found: C, 67.07; H, 4.44; N, 11.04; S, 8.42. IR (cm^{-1}): 3293, 1631. mp 238-242 °C.

1-(4-(Benzo[*d*]thiazol-2-ylmethoxy)phenyl)-3-(3,4-dichlorophenyl)urea (23)



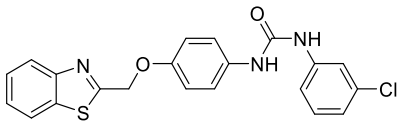
Yield: 0.419 g (63 %), white solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 8.94 (s, 1H, exchangeable with D_2O, NH); 8.67 (s, 1H, exchangeable with D_2O, NH); 8.13-8.09 (m, 1H, Ar-H); 8.03-7.99 (m, 1H, Ar-H); 7.86 (d, J = 2.4 Hz, 1H, Ar-H); 7.57-7.27 (m, 6H, Ar-H); 7.04 (d, J = 9.2 Hz, 2H, Ar-H); 5.55 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6, δ): 168.7, 152.8, 152.5, 152.4, 140.1, 134.4, 133.3, 131.0, 130.5, 126.3, 125.3, 122.9, 122.7, 122.4, 120.3, 119.2, 118.2, 115.3, 67.5. Anal. Calcd for $C_{21}H_{15}Cl_2N_3O_2S$: C, 56.77; H, 3.40; N, 9.46; S, 7.22. Found: C, 56.62; H, 3.33; N, 9.31; S, 7.03. IR (cm^{-1}): 3338, 3245, 1637. mp 246-248 °C.

1-(4-(Benzo[*d*]thiazol-2-ylmethoxy)phenyl)-3-(2-chlorophenyl)urea (24)



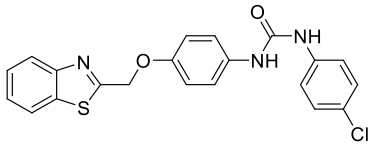
Yield: 0.231 g (72 %), white solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 9.29 (s, 1H, exchangeable with D_2O, NH); 8.24 (s, 1H, exchangeable with D_2O, NH); 8.17-8.09 (m, 2H, Ar-H); 8.03-7.99 (m, 1H, Ar-H); 7.57-7.23 (m, 6H, Ar-H); 7.07-6.95 (m, 3H, Ar-H); 5.56 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6, δ): 168.7, 152.7, 152.5, 152.2, 136.1, 134.4, 133.5, 129.2, 127.5, 126.3, 125.3, 123.1, 122.7, 122.4, 121.7, 121.1, 119.8, 115.4, 67.5. Anal. Calcd for $C_{21}H_{16}ClN_3O_2S$: C, 61.54; H, 3.93; N, 10.25; S, 7.82. Found: C, 61.37; H, 3.88; N, 10.08; S, 7.82. IR (cm^{-1}): 3278, 1636. mp 273-275 °C.

1-(4-(Benzo[*d*]thiazol-2-ylmethoxy)phenyl)-3-(3-chlorophenyl)urea (25)



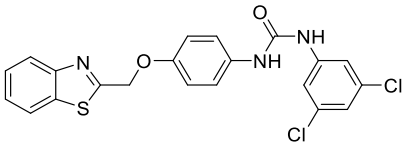
Yield: 0.213 (67 %), white solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 8.82 (s, 1H, exchangeable with D_2O, NH); 8.62 (s, 1H, exchangeable with D_2O, NH); 8.13-8.09 (m, 1H, Ar-H); 8.03-7.99 (m, 1H, Ar-H); 7.70-7.69 (m, 1H, Ar-H); 7.57-7.22 (m, 6H, Ar-H); 7.06-6.96 (m, 3H, Ar-H); 5.55 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6, δ): 168.7, 152.7, 152.5, 141.4, 134.4, 133.4, 133.1, 130.3, 126.3, 125.3, 122.7, 122.4, 121.2, 120.2, 117.4, 116.5, 115.3, 67.5. Anal. Calcd for $C_{21}H_{16}ClN_3O_2S$: C, 61.54; H, 3.93; N, 10.25; S, 7.82. Found: C, 61.37; H, 3.87; N, 10.16; S, 7.70. IR (cm^{-1}): 3273, 1635. mp 233-235 °C.

1-(4-(Benzo[*d*]thiazol-2-ylmethoxy)phenyl)-3-(4-chlorophenyl)urea (26)



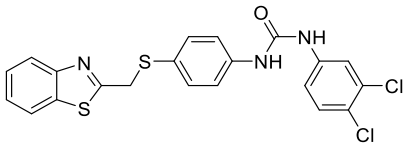
Yield: 0.260 g (81 %), white solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 8.76 (s, 1H, exchangeable with D_2O, NH); 8.58 (s, 1H, exchangeable with D_2O, NH); 8.13-8.09 (m, 1H, Ar-H); 8.03-7.99 (m, 1H, Ar-H); 7.57-7.26 (m, 8H, Ar-H); 7.03 (d, J = 8.8 Hz, 2H, Ar-H); 5.55 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6, δ): 168.7, 152.6, 152.5, 152.5, 138.8, 134.4, 133.6, 128.6, 126.3, 125.3, 125.1, 122.7, 122.4, 120.1, 119.6, 115.3, 67.5. Anal. Calcd for $C_{21}H_{16}ClN_3O_2S$: C, 61.54; H, 3.93; N, 10.25; S, 7.82. Found: C, 61.29; H, 3.79; N, 10.04; S, 7.57. IR (cm^{-1}): 3276, 1630. mp 256-258 °C.

1-(4-(Benzo[*d*]thiazol-2-ylmethoxy)phenyl)-3-(3,5-dichlorophenyl)urea (27)



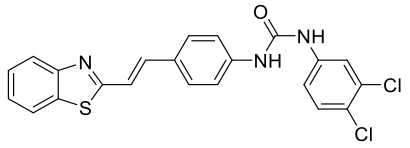
Yield: 0.332 g (95 %), white solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 8.99 (s, 1H, exchangeable with D_2O, NH); 8.73 (s, 1H, exchangeable with D_2O, NH); 8.12-8.08 (m, 1H, Ar-H); 8.03-7.98 (m, 1H, Ar-H); 7.57-7.34 (m, 6H, Ar-H); 7.13-7.02 (m, 3H, Ar-H); 5.55 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6, δ): 168.7, 152.9, 152.5, 152.3, 142.4, 134.4, 134.0, 133.2, 126.3, 125.3, 122.7, 122.4, 120.7, 120.4, 116.2, 115.3, 67.5. Anal. Calcd for $C_{21}H_{15}Cl_2N_3O_2S$: C, 56.77; H, 3.40; N, 9.46; S, 7.22. Found: C, 56.61; H, 3.37; N, 9.39; S, 7.20. IR (cm^{-1}): 3288, 1634. mp 241-243 °C.

1-(4-((Benzo[*d*]thiazol-2-ylmethyl)thio)phenyl)-3-(3,4-dichlorophenyl)urea (28)



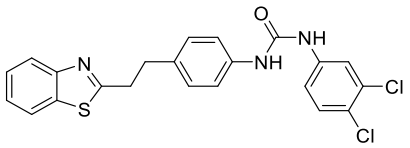
Yield: 0.274 g (81 %), yellowish solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 8.98 (s, 1H, exchangeable with D_2O, NH); 8.88 (s, 1H, exchangeable with D_2O, NH); 8.04-8.00 (m, 1H, Ar-H); 7.92-7.87 (m, 1H, Ar-H); 7.84 (d, *J* = 2.4 Hz, 1H, Ar-H); 7.51-7.27 (m, 8H, Ar-H); 4.64 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6, δ): 170.2, 152.6, 152.2, 139.8, 138.7, 135.1, 131.2, 131.0, 130.6, 126.2, 126.1, 125.1, 123.2, 122.4, 122.2, 119.3, 119.1, 118.4, 36.3. Anal. Calcd for $C_{21}H_{15}Cl_2N_3OS_2$: C, 54.79; H, 3.28; N, 9.13; S, 13.93. Found: C, 54.55; H, 3.23; N, 9.01; S, 14.03. IR (cm^{-1}): 3285, 1636. mp 202-206 °C.

(*E*)-1-(4-(2-(Benzo[*d*]thiazol-2-yl)vinyl)phenyl)-3-(3,4-dichlorophenyl)urea (31)



Yield: 0.313 g (86 %), yellow solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 9.07 (s, 1H, exchangeable with D_2O, NH); 9.05 (s, 1H, exchangeable with D_2O, NH); 8.08-8.04 (m, 1H, Ar-H); 7.94 (d, *J* = 7.6 Hz, 1H, Ar-H); 7.88 (d, *J* = 2.6 Hz, 1H, Ar-H); 7.73-7.31 (m, 10H, Ar-H, CH=CH). ^{13}C-NMR (DMSO-d_6, δ): 166.8, 153.5, 152.1, 140.7, 139.8, 137.3, 133.9, 131.1, 130.6, 129.1, 128.6, 126.5, 125.3, 123.3, 122.4, 122.1, 119.8, 119.4, 118.5, 118.4. Anal. Calcd for $C_{22}H_{15}Cl_2N_3OS$: C, 60.01; H, 3.43; N, 9.54; S, 7.28. Found: C, 59.94; H, 3.46; N, 9.50; S, 7.18. IR (cm^{-1}): 3281, 1633. mp 318-320 °C.

1-(4-(2-(Benzo[*d*]thiazol-2-yl)ethyl)phenyl)-3-(3,4-dichlorophenyl)urea (32)



Yield: 0.201 g (58 %), white solid. ^{1}H-NMR (200 MHz, DMSO-d_6, δ) 8.94 (s, 1H, exchangeable with D_2O, NH); 8.71 (s, 1H, exchangeable with D_2O, NH); 8.04-7.99 (m, 1H, Ar-H); 7.92 (d, *J* = 7.8 Hz, 1H, Ar-H); 7.86 (d, *J* = 2.4 Hz, 1H, Ar-H); 7.51-7.18 (m, 8H, Ar-H); 3.40 (t, *J* = 7.4 Hz, 2H, CH_2), 3.08 (t, *J* = 7.4 Hz, 2H, CH_2). ^{13}C-NMR (DMSO-d_6, δ): 170.9, 152.7, 152.3, 140.0, 137.4, 134.7, 134.0, 131.0, 130.6, 128.8, 126.0, 124.8, 123.0, 122.2, 122.1, 119.2, 118.6, 118.3, 35.1, 33.9. Anal. Calcd for $C_{22}H_{17}Cl_2N_3OS$: C, 59.73; H, 3.87; N, 9.50; S, 7.25. Found: C, 59.88; H, 3.88; N, 9.46; S, 7.22. IR (cm^{-1}): 3275, 1632. mp 208-211 °C.

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