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Design, synthesis, and evaluation of 4,5,6,7-tetrahydrobenzo[d]thiazole-based novel dual kinase inhibitors of CK2 and GSK3β†

Triveni R. Pardhi,^a Manishkumar S. Patel,^b
V. Sudarsanam^c and Kamala K. Vasu^{*ac}

Casein kinase 2 (CK2) and glycogen synthase kinase-3β (GSK3β) are responsible for the phosphorylation of a tumor suppressor protein (PTEN) in a cooperative manner which causes its deactivation. Thus, it is essential to inhibit both kinases simultaneously to prevent PTEN deactivation more efficiently. In this study, we have designed a novel lead from Hit15 which was identified *in silico* as a dual kinase inhibitor against CK2 and GSK3β through our previous study. The dataset of structural analogs of the lead was designed and confirmed by pharmacophore mapping and molecular docking. The screened analogs were considered further and a series of “tetrahydrobenzo[d]thiazoles” were synthesized. Compound **1g** has shown highest dual kinase inhibitory activity at a concentration of 1.9 μM against CK2 and 0.67 μM against GSK3β. Our results suggest that the presence of a carboxyl group at the *meta* position of the phenyl ring plays a vital role in dual kinase inhibition.

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Introduction

The tumor suppressor protein PTEN (Phosphatase and TENSin homolog) was first identified in 1997 through deletion mapping of brain, breast and prostate cancers.¹ PTEN serves as a critical regulator of growth factor signaling by inhibiting the PI3K pathway and thus is able to regulate important cellular processes such as cell proliferation, apoptosis, protein synthesis and glucose metabolism.² Hence, PTEN performs its tumor suppressor job by acting as a negative regulator of the PI3K signaling pathway.³ PTEN is regulated by phosphorylation which requires the addition of phosphate groups to the amino acids in the C-tail region to turn off its activity. Bolduc *et al.* have found that addition of phosphate groups to the C-tail reduced PTEN's affinity for the plasma membrane. Interactions between the C-tail and C2 domains of PTEN induced a conformational change in PTEN. This converts PTEN into enzymatically inactive closed-closed form

which renders it incapable of interacting with the membrane. Eventually, PTEN will no longer be available for the inhibition of the PI3K pathway.⁴

CK2, a pro-oncogenic protein, is overexpressed in most human malignancies and has been linked to the activation of the PI3K signaling pathway through its ability to phosphorylate and thereby inactivate PTEN.⁵ It is reported that CK2 activity has been increased in many solid human tumors like colorectal,⁶ breast,⁷ and prostate carcinoma,^{8,9} as well as in various types of squamous cell carcinomas¹⁰ and also in hematological malignancies.¹¹ The first clinical stage inhibitor of protein kinase CK2 for the treatment of cancer is 5-(3-chlorophenylamino)benzo[c][2,6]naphthyridine-8-carboxylic acid (silmiteceritinib or CX-4945) which is an ATP-competitive inhibitor of CK2 with known antitumor activity in breast, pancreatic and prostate cancer xenograft mouse models.¹² GSK-3β plays essential roles in the development and cancer cell maintenance in different cancers, and its inhibition has been studied as a possible therapeutic strategy for cancer treatment. Inhibitors of GSK-3β showed antiproliferative activity against various cancers such as pancreatic,¹³ prostate,¹⁴ colon,¹⁵ colorectal,¹⁵ ovarian,¹⁶ glioblastoma,¹⁷ neuroblastoma,¹⁸ and leukemia.¹⁹ Moreover, the NF-κB pathway²⁰ and the mitochondrial apoptosis pathway²¹ were found to be involved in the antiproliferative effects of GSK-3β inhibitors against cancer cells. Many reports have suggested that CK2 or GSK-3β inhibitors can be promising therapeutics for cell death induction in combination with other therapies.^{22,23}

^a Department of Natural Products, National Institute of Pharmaceutical Education and Research (NIPER)-Ahmedabad, Opp. Air force station, Palaj, Gandhinagar-382355, Gujarat, India. E-mail: triveni.pardhi@gmail.com; Fax: +917927450449; Tel: +917927439375

^b Department of Biotechnology, National Institute of Pharmaceutical Education and Research (NIPER)-Ahmedabad, Gandhinagar-382355, Gujarat, India

^c Department of Medicinal Chemistry, B. V. Patel Pharmaceutical Education & Research Development (PERD) Centre, Ahmedabad-380054, Gujarat, India. E-mail: kamalav@perdcentre.com

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Phosphorylation of a bunch of Ser/Thr residues on PTEN's C-terminal tail has been implicated in the modulation of its tumor suppressor functions and has gained considerable attention in studies on PTEN regulation.²⁴ Casein kinase 2 (CK2) is a protein kinase that phosphorylates PTEN on Ser370, Ser380, Thr382, Thr383, and Ser385.²⁵ CK2-mediated phosphorylation stabilizes PTEN but creates an inactive conformation that decreases its interactions with binding partners and reduces its plasma membrane localization.²⁶ Meanwhile, glycogen synthase kinase 3 β (GSK3 β) phosphorylates PTEN at Ser362 and Thr366 which decreases its phosphatase activity.²⁷ It is also reported that prior phosphorylation of PTEN at Ser-370 by CK2 enhanced the GSK3 β mediated phosphorylation at Thr-366 which indicates that CK2 and GSK3 β may have synergistic effects during PTEN phosphorylation.²⁷ This results in conformational changes in PTEN^{24,28} and altered protein–protein interactions^{29,30} which decrease its activity.

Since the reduction in the level and activity of PTEN is sufficient to drive oncogenesis,³¹ activation of PTEN would be a rational therapeutic modality for a large number of cancers that show compromised PTEN activity or hyperactivation of the oncogenic PI3K/AKT/mTOR pathway. It becomes necessary to inhibit both kinases (CK2 and GSK3 β) simultaneously to prevent PTEN phosphorylation more efficiently.⁴ We have thought of targeting CK2 and GSK3 β to increase PTEN activity through a multi-target inhibitor strategy. The aspect of multi-targeting in drug discovery is of growing importance, as evidenced by the development of resistance to existing anticancer³² and antimicrobial agents.³³

Based on the existing studies, we have planned a strategy for the simultaneous inhibition of CK2 and GSK3 β (Fig. 1). It

involved the development of a single chemical entity using a computational drug designing approach. In our previous study, we have reported the design of dual kinase inhibitors *via* a combination of pharmacophore modeling and molecular docking approaches.³⁴ As a result of sequential screening, we have found Hit15 as a potential dual kinase inhibitor with good binding affinity and reactivity *in silico*.³⁴ In the present study, we have designed a novel lead molecule from *in silico* screened Hit15 (ZINC20464516, a molecule from the ZINC database) and further generated the library of structural analogs of the lead. The structurally diversified molecules were mapped on the best pharmacophore models (Pharm18 and Hypo1)³⁴ and docked on both CK2 and GSK3 β proteins. Finally, *in silico* screened structural analogs were synthesized and biological evaluation was carried out. Herein, we aim to design and synthesize a new series of dual kinase inhibitors and carry out a biological evaluation of their activity against CK2 and GSK3 β .

Results and discussion

In our previous report,³⁴ we have identified the common features and 3DQSAR pharmacophore models from known inhibitors of CK2 and GSK3 β . Using these pharmacophore models, we have identified Hit15 as a potent dual kinase inhibitor. The workflow of our *in silico* virtual screening process was summarized and explained in our previous report.³⁴ The virtually identified Hit15 is a molecule from the ZINC database, from which a novel lead has been designed in this study. The idea behind the hit-to-lead generation is to design in-house lead molecules. So, the current research was focused on designing a novel lead molecule and its structural analogs from *in silico* screened Hit15.

Fig. 1 Postulated strategy for the activation of PTEN. PTEN contains PBM: PIP2 binding module, PD: phosphatase domain, C2D: C2 domain, C-terminal region or tail (in brown attached to the C2 domain), and a PDZ binding domain (in blue attached to the other end of the C-tail).

Hit-to-lead discovery: pharmacophore modeling-based design

In the hit-to-lead designing process, the important structural features of Hit15 were considered for developing a lead molecule with similar features (Fig. 2). The designed lead mimics the *in silico* screened Hit15 as follows: (1) the thiazole fragment in the lead is a bioisostere of imidazole, but both heterocycles have different physicochemical properties in terms of their pK_a values. The pK_a of imidazole is 7 while that of thiazole is 2.5. Hence, thiazole has been substituted at the 2-position with a basic amino functional group to increase its pK_a . (2) Aminocyclohexane is a bioisostere of piperidine. Both are 6-membered rings and their basic centers are also the same. The amino group is located outside the ring in the lead structure while it is part of the ring system in Hit15. Various reports described the antitumor activity of kinase inhibitors containing a diaryl urea functional group.^{35,36} Sorafenib, a diaryl urea multi-targeted inhibitor of several kinases, is a well-known example of a drug used for the treatment of advanced renal cell carcinoma (RCC) and unresectable hepatocellular carcinoma (HCC).³⁷ The diaryl urea portion of kinase inhibitors is highly conserved and shared by most type II kinase inhibitors. The introduction of a urea linker also provides flexibility between the ring systems. Hence, we have also checked the effect of the urea linker by replacing it with a methylene linker. The designed lead was subjected to structural diversification to build a database of 301 novel molecules with similar functionalities to those of Hit15. These diversified molecules were checked for their mapping on the best pharmacophore models, Pharm18 and Hypo1, which were designed in our previous study,³⁴ and molecular docking on both target proteins. Finally, 36 molecules with good fit values (ESI^+) and docking scores (ESI^+) were selected for synthesis and then evaluated for their biological activity.

Chemistry

As adumbrated in Scheme 1, the desired 1-(6-(1,3-dioxoisindolin-2-yl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)-3-

(substituted phenyl)urea derivatives (1a–1f) were prepared from 2-(2-amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)-isindoline-1,3-dione and aromatic isocyanates. Reactions of isocyanates with amines go very fast without catalysis. The basicity and hence reactivity of aromatic amines are comparatively less than those of aliphatic ones due to the larger electronegativity of aromatic ring substituents. We have noticed that *ortho*-substituted aromatic isocyanates were comparatively less reactive due to the steric hindrance effect. The compounds were separated and purified by column chromatography. The purified esters 1d and 1e were subjected to hydrolysis using bases like LiOH or NaOH. We have found LiOH/THF–H₂O as an effective base for hydrolysis of ethyl esters with better yields. After hydrolysis of compounds 1d and 1e, we obtained compounds 1g and 1h, respectively, which were different from the expected ones. Here, the expected product of the hydrolysis reaction was an acid (mol. wt. 462.5) from the respective ethyl ester (mol. wt. 490.5). But we got a different compound with a $[M + H]^+$ peak at 509.3. This unexpected product of the reaction was a hemiketal type of compound (1g–1h) which was confirmed further by ¹H and ¹³C NMR spectroscopy. The compound was obtained by nucleophilic addition of an *in situ* generated ethanol to the carbonyl group of urea. The stability of this compound could be explained by the existence of a hydrogen bond between OH and thiazole nitrogen which is quite strong due to the electron withdrawing nature of C=N of the thiazole moiety.

The 3-phenyl-1-(2-(3-phenylureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)-1-propylurea derivatives (2a–2f) were synthesized by a nucleophilic addition reaction of 2 with substituted aromatic isocyanates (2 moles) as illustrated in Scheme 2. When we tried to synthesize the compounds having a mono-urea group with 1 mole of isocyanate, it was difficult to get a single compound. It always resulted in a mixture of compounds as the starting material *N*⁶-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine which possesses two reactive basic centers. The isocyanate probably reacts with either alicyclic secondary amine, aromatic primary amine or both, yielding a mixture of compounds. So instead of using

Fig. 2 Design of a novel lead from Hit15.

Scheme 1 Synthesis of compounds **1a–1h**. Reagents & conditions: (a) substituted isocyanates, DMF and DCM, rt, 1.5–2 h; for **1a**: 2-fluorophenyl isocyanate; for **1b**: 3-fluorophenyl isocyanate; for **1c**: 4-fluorophenyl isocyanate; for **1d**: ethyl 3-isocyanatobenzoate; for **1e**: ethyl 4-isocyanatobenzoate; for **1f**: 4-methoxyphenyl isocyanate; (b) for **1g** and **1h**: LiOH, THF–H₂O, rt, 3 h.

Scheme 2 Synthesis of compounds **2a–2h**. Reagents & conditions: (a) substituted isocyanates (2 moles), DMF and DCM, rt, 2.5–3 h; for **2a**: 2-fluorophenyl isocyanate; for **2b**: 3-fluorophenyl isocyanate; for **2c**: 4-fluorophenyl isocyanate; for **2d**: ethyl 3-isocyanatobenzoate; for **2e**: ethyl 4-isocyanatobenzoate; for **2f**: 4-methoxyphenyl isocyanate; (b) for **2g** and **2h**: LiOH, THF–H₂O, rt, 5–6 h.

one mole, we have used two moles of isocyanates. One mole forms a ureido linkage with a primary amine of thiazole while the other forms a second ureido linkage with an alicy-

clie secondary amine to obtain diaryl diureido derivatives of compound **2**. The pure compounds were obtained by column chromatography. The purified compounds **2d** and **2e** were

subjected to LiOH/THF-H₂O hydrolysis followed by neutralization with dil. HCl to get their diacid analogs (**2g-2h**).

The 1,1'-(4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diyl)bis(3-phenylurea) derivatives (**3a-3f**) were synthesized through a nucleophilic addition reaction of **3** with aromatic isocyanates as shown in Scheme 3. The diamino compound **3** was not soluble in *N,N*-dimethylformamide at room temperature, hence it was heated at 80 °C to form a clear solution, and then 2 moles of isocyanates were added. The pure diaryl diureido derivatives were obtained by column chromatography. The purified compounds **3d** and **3e** were subjected to hydrolysis with a LiOH/THF-H₂O solution. The reaction was quite sluggish, and required heating for around 10–12 h to achieve complete hydrolysis. After neutralization with dil. HCl, the respective diacid analogs were obtained (**3g-3h**).

Scheme 4 represents the detailed synthetic route for the preparation of ethyl 4-(((6-(3-phenyl-1-propylureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)amino)methyl)benzoate (**4a-4f**) and their hydrolyzed products, benzoic acid derivatives (**4g-4l**). The aim was to replace the benzyl group of alicyclic amine in the lead (Fig. 2) with aryl urea and retain the benzyl group on the other side to synthesize our desired compounds as predicted by pharmacophore mapping and molecular docking studies. But it is difficult to obtain the mono-urea derivatives because of the presence of two reactive basic centers. For this reason, we tried an alternative approach. First, we opted for imine synthesis, a reaction which is selective towards primary amines (thiazole NH₂ of compound **2**). The imine intermediate (**4**) was synthesized *via* condensation of *N*⁶-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (**2**) with methyl 4-formylbenzoate. Subsequently, the imine was reduced with a mild reducing agent, sodium cyanoborohydride, in an acidic environment to give the benzyl

group (**5**) and then alicyclic NH was reacted with aromatic isocyanate to yield the desired mono-urea derivatives (**4a-4f**). The column purified compounds were subjected to a hydrolysis reaction in LiOH/THF-H₂O medium to generate the corresponding acid derivatives (**4g-4l**).

CK2 and GSK3β kinase inhibition assays

All newly synthesized compounds were tested *in vitro* for their inhibitory activity against CK2 and GSK3β. The activity screening was performed using Z'-LYTE™ kinase fluorescence-based assay kits – Ser/Thr 11 peptide (for CK2) and Ser/Thr 9 peptide (for GSK3β). Initially, primary screening of all 36 compounds was carried out for the evaluation of dual kinase inhibitory activity. Since we aimed to identify dual kinase inhibitors of CK2 and GSK3β, IC₅₀ values were determined only for those compounds that showed greater than thirty percent inhibition of both kinases in the primary screening. TBB (CK2 inhibitor) and AR-A014418 (GSK3β inhibitor) were used as positive controls. The IC₅₀ values of the positive controls were determined to be 1480 ± 0.21 nM and 207 ± 0.33 nM under these assay conditions.

An analysis of the IC₅₀ values is summarized in Table 1. Among the 4,5,6,7-tetrahydrobenzo[d]thiazole derivatives, compounds **1g** and **2g** showed IC₅₀ values less than 3 μM (Fig. 3A–D, Table 1), whereas compounds **1d** and **1h** showed IC₅₀ values less than 8 μM (Table 1). Compound **1g** was the most effective in inhibiting both kinases, CK2 (IC₅₀, 1.9 μM) and GSK3β (IC₅₀, 0.67 μM). Notably, the acid group-containing compounds were significantly more potent than other compounds from all four series. Meanwhile, fluoro- or methoxy-group substituted compounds showed less than thirty percent inhibition of CK2 and GSK3β in the primary screening. This fact indicates that the acid

Scheme 3 Synthesis of compounds **3a-3h**. Reagents & conditions: (a) substituted isocyanates (2 moles), DMF, 80 °C for 1 h then rt for 1–2 h; for **3a**: 2-fluorophenyl isocyanate; for **3b**: 3-fluorophenyl isocyanate; for **3c**: 4-fluorophenyl isocyanate; for **3d**: ethyl 3-isocyanatobenzoate; for **3e**: ethyl 4-isocyanatobenzoate; for **3f**: 4-methoxyphenyl isocyanate; (b) for **3g** and **3h**: LiOH, THF-H₂O, 60 °C, 10–12 h.

Scheme 4 Synthesis of compounds **4a–4l**. Reagents & conditions: (a) methyl 4-formylbenzoate, MeOH, reflux, 14–16 h; (b) NaBH₃CN, CH₃COOH, MeOH & DCM, rt, 3–4 h; (c) substituted isocyanates, DMF, rt, 1.5–2 h; for **4a**: 2-fluorophenyl isocyanate, for **4b**: 3-fluorophenyl isocyanate, for **4c**: 4-fluorophenyl isocyanate, for **4d**: ethyl 3-isocyanatobenzoate, for **4e**: ethyl 4-isocyanatobenzoate, for **4f**: 4-methoxyphenyl isocyanate; (d) for **4g–4l**: LiOH, THF–H₂O, rt, 3–4 h.

Table 1 Kinase inhibition against CK2 and GSK3β (IC₅₀ values in μM)

<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> GS of series 1 (for 1d, 1g and 1h) </div> <div style="text-align: center;"> GS of series 2 and 3 (for 2g, 3d and 3g) </div> <div style="text-align: center;"> GS of series 4 (for 4d and 4j) </div> </div>					
Cpd	X	R ₁	R ₂	IC ₅₀ ^a CK ₂ (μM)	IC ₅₀ ^a GSK3β (μM)
1d		<i>m</i> -COOEt	—	7.9 ± 0.12	5.3 ± 0.31
1g		<i>m</i> -COOH	—	1.9 ± 0.05	0.67 ± 0.27
1h		<i>p</i> -COOH	—	4.9 ± 0.09	4.6 ± 0.22
2g		<i>m</i> -COOH	<i>m</i> -COOH	2.8 ± 0.05	1.5 ± 0.04
3d	H	<i>m</i> -COOEt	<i>m</i> -COOEt	20.4 ± 0.35	2.5 ± 0.23
3g	H	<i>m</i> -COOH	<i>m</i> -COOH	12.4 ± 0.19	6.0 ± 0.15
4d	—	<i>p</i> -COOMe	<i>m</i> -COOEt	4.6 ± 0.05	13.6 ± 0.59
4j	—	<i>p</i> -COOH	<i>m</i> -COOH	4.0 ± 0.15	11.4 ± 0.17

GS: general structure, Cpd: compound. ^a *In vitro* protein kinase assay. Data are mean ± SEM of triplicate measurements.

Fig. 3 Kinase inhibitory activity of (A and B) compound **1g** and (C and D) compound **2g** against CK2 and GSK3 β . Inhibition curves were obtained by using nonlinear regression analysis using GraphPad Prism software. The calculated IC_{50} values are shown in Table 1.

group may be favorable for targeting the CK2 and GSK3 β binding sites. Further examining all the compounds shown in Table 1, it was observed that a carboxyl group at the *m*-position of the benzene ring is more suitable than that at the *p*-position for enhancing the kinase inhibitory activity. Specifically, for GSK3 β inhibition, a carboxylic acid group at the *m*-position of the benzene ring plays an important role. However, the compounds with a fluoro group at the *o*-, *m*-, or *p*-position or with a methoxy group at the *p*-position of the benzene ring have shown no significant kinase inhibitory activity in the primary screening. The designed compounds from the first three series were composed of urea-based linkers while in the fourth series these were replaced with methyl linkers, which may be the reason behind a decrease in GSK3 β inhibitory activity.

Considering the potency of these dual kinase inhibitors against CK2 and GSK3 β , some structure–activity relationships (SARs) can be inferred as follows. (1) A carboxylic acid group on the *meta* position of the phenyl ring elicits better activity than that on the *para* position; (2) localization of sp^3 hybridized carbon at the linker site (such as in **1g**) was preferred over sp^2 hybridized carbon; (3) the availability of a hydrogen bond donor at the linker site displayed a noticeable effect on dual kinase inhibition (Fig. 4). The same effect was observed in **1g** because of

the presence of a hydroxyl group instead of a ketone group which may contribute to the increase in its activity.

Most of the compounds listed in Table 1 showed good activity against GSK3 β compared to CK2 while compounds with a methyl linker (compounds of series 4 *viz.* **4d** and **4j**) have the reverse effect. Considering the important features responsible for dual kinase inhibition which are present in **1g** (active compound), the reason behind the shift in the activity of **4j** could be explained. Compound **4j** possesses the following properties: (i) NH attached to the thiazole, as a hydrogen bond donor, (ii) sp^3 hybridized carbon in the methyl linker, (iii) a carboxylic acid group at the *p*-position and (iv) a urea linker instead of a methyl linker. The (i), (ii) and (iv) points describe similar features to those of compound **1g**. The only variation is in the position of the carboxylic acid group. The activity of **4j** against CK2 has been compromised to some extent compared to **1g**, and it may be due to the change in the position of the carboxyl group from *meta* to *para* of the phenyl ring. Hence, it may be concluded that carboxylic acid at the *m*-position of the phenyl ring may be crucial for potent CK2 inhibitory activity.

The comparative decrease in the GSK3 β inhibitory activity of compound **4j** could be due to the replacement of the urea linker with a methyl linker or due to the change in the

Urea linker necessary for GSK3 β activity

Fig. 4 Structure–activity relationships of synthesized dual kinase inhibitors.

position of carboxylic acid from *meta* to *para* of the phenyl ring. It has been suggested that decreasing the length of the linker or the *para* position of the carboxylic acid group may have a negative effect on the GSK3 β inhibitory activity. The variation of the linker length connecting two aromatic rings significantly influenced the efficiency of intermolecular interactions between the receptor and the ligand, and thus we have pointed out the decisive role of urea-linker interactions.

Compound **1g** has shown fit values of 3.71 and 11.06 when mapped on Pharm18 and Hypo1, respectively (ESI[†]). Mapping of compound **1g** is shown in Fig. 5A and B. Compound **1g** has better mapping on common pharmacophore models compared to standard inhibitors of CK2³⁸ and GSK3 β .³⁹ Molecular docking studies of dual kinase inhibitors with both proteins were performed to understand and interpret the bioassay results.

The docking analysis of the most active compound **1g** (Fig. 5C and D) in the binding pocket of the CK2 and GSK3 β proteins indicated higher dock scores in comparison with the reported inhibitors of CK2 (CX-5279)³⁸ and GSK3 β (3-amino-6-(4-([2-(dimethylamino)ethyl] sulfamoyl)phenyl)-*n*-pyridin-3-ylpyrazine-2-carboxamide).⁴⁰ We found common interacting amino acids between our active dual kinase inhibitors and known inhibitors of CK2 and GSK3 β (Table 2). These amino acids were LEU45, VAL53, VAL66, LYS68, ILE95, PHE113, VAL116, HIS160, MET163, ILE174, and ASP175 with known CK2 inhibitors, and ALA83, GLU97, TYR134, VAL135, PRO136, TYR140, ARG141, GLN185, LEU188, and CYS199 with known GSK3 β inhibitors.

Fig. 5C represents the interactions between compound **1g** and CK2 protein. The amino acids involved in the interactions are discussed as follows. (1) The interaction of the H-bond from the hydroxyl group (H-acceptor) of **1g** with the

carboxyl group (H-donor) of SER51 with 2.077 Å H-bond distance was observed. The other important H-bond interaction was found between the carboxyl group (H-acceptor) of **1g** and the protein chain amine (H-donor) of LYS77 with 2.072 Å H-bond distance; (2) the intramolecular H-bond interaction between thiazole nitrogen (H-acceptor) and the hydroxyl group (H-donor) of **1g** having a distance of 2.139 Å was observed, which may be an important characteristic feature responsible for the stability of this compound; (3) electrostatic pi-cation and pi-donor type hydrogen bond interactions between the carboxyl group and the thiazole ring of **1g** with LYS68 having 3.175 Å distance were found. (4) Hydrophobic alkyl and pi-alkyl type of interactions from the ethyl or pi-orbitals of **1g** with that of the alkyl group of VAL53, VAL66, LYS68, ILE174, PHE113, LEU45, and LYS49 were found. Fig. 5D represents the interactions between compound **1g** and GSK3 β protein. The amino acids involved in the interactions are discussed below. (1) The interaction of the H-bond from the carboxylic acid (H-acceptor) of **1g** with the protein chain amine (H-donor) of GLN72 with 2.307 Å H-bond distance was observed. The other H-bond interaction was found between the hydroxyl group (H-acceptor) at the linker site of **1g** and the hydroxyl group (H-donor) of TYR134 with 2.408 Å H-bond distance. The important H-bond interaction between the ethoxy oxygen group (H-acceptor) at the linker site of **1g** and the amino group (H-donor) of ARG141 oriented with 2.365 Å H-bond distance was also observed. (2) Electrostatic pi-cation and pi-donor type hydrogen bond interactions were found between the carboxyl group of **1g** and LYS60 having a distance of 2.429 Å. (3) Hydrophobic pi-sigma, alkyl and pi-alkyl type of interactions from the ethyl or pi-orbitals of **1g** with that of the alkyl group of ILE62,

Fig. 5 Pharmacophore mapping. (A) Mapping of compound **1g** on Pharm18; and (B) mapping of compound **1g** on Hypo1. In the pharmacophore model, green represents HBA, orange represents RA and cyan represents HY features. Molecular docking. Active compound **1g** (C) on the CK2 protein and (D) on the GSK3 β protein. The active compound **1g** is shown in cyan. Hydrogen bond interactions are shown in green dotted lines.

VAL70, ALA83, LEU188, CYS199, VAL110, and LEU132 were also observed.

Conclusions

We have successfully designed a novel lead molecule possessing similar properties to Hit15 (ZINC20464516, a molecule from the ZINC database) which was identified in our previous study.³⁴ The *in silico* screened structural analogs of the lead were synthesized, and among them, compound **1g** has shown the highest dual kinase inhibitory activity against CK2 and GSK3 β during the *in vitro* kinase inhibition assay. The important features that may be responsible for the dual kinase inhibitory activity of **1g** are the carboxylic acid at the 3-position of the phenyl ring, sp^3 hybridized carbon and the hydroxyl group at the linker site. Furthermore, it is worthwhile to investigate the role of compound **1g** in preventing the phosphorylation of PTEN *via* inhibition of CK2 and GSK3 β at the cellular level.

Materials and methods

Computational studies

Molecular modeling studies were carried out on an Intel® Xeon® Processor X5660 equipped with 32 GB RAM running Windows 7 Professional. The HypoGen and HipHop algorithms implemented in the Accelrys Discovery Studio 4.0 program package were employed for the pharmacophore modeling. The LibDock docking module in DS4.0 was used for molecular docking studies as described in the previous report.³⁴ Open Babel 2.3.1 was used for interconversion of chemical data from one format to another.

Chemistry

All reagents and solvents were of analysis or synthesis grade purchased from commercial vendors. ^1H and ^{13}C NMR spectra were recorded on a Bruker AVANCE II 500 spectrometer at 500 and 125 MHz, respectively, in DMSO. Chemical shifts are

Table 2 Interacting amino acids of CK2 and GSK3 β proteins with their known inhibitors and active compounds

Cpd	Dock score		Interacting amino acids in CK2	Interacting amino acids in GSK3 β
	CK2	GSK3 β		
Known CK2 inhibitor ³⁸	160.1 (ref. 34)		LEU45, VAL53, VAL66, LYS68, ILE95, PHE113, VAL116, HIS160, MET163, ILE174, ASP175, GLU114, HIS115 (ref. 34)	
Known GSK3 β inhibitor ⁴⁰		116.5 (ref. 34)		ALA83, GLU97, TYR134, VAL135, ARG141, LEU188, CYS199, PRO136, TYR140, GLN185, ARG143 (ref. 34)
1d	160.5	142.4	LEU45, VAL53, VAL66, LYS68, ILE95, PHE113, HIS160, MET163, ILE174, SER51, LYS49	ALA83, TYR134, VAL135, ARG141, LEU188, CYS199, PRO136, GLN185, VAL70, LYS85
1g	174.7	148.9	LEU45, VAL53, VAL66, LYS68, PHE113, HIS160, MET163, ILE174, ASP175, SER51, LYS77, GLY177, TYR50, LYS49	ALA83, TYR134, VAL135, ARG141, LEU188, CYS199, GLN72, ILE62, VAL110, LEU132
1h	163.7	143.4	VAL53, VAL66, LYS68, VAL116, HIS160, MET163, ILE174, ASP175, LYS49, TYR50, SER51, LEU70, GLU81	ALA83, TYR134, VAL135, ARG141, LEU188, CYS199, ILE62, VAL110, LEU132
2g	168.6	148.6	LEU45, VAL53, VAL66, LYS68, PHE113, VAL116, MET163, ILE174, ASP175, TRP176, GLY46, ARG47, LYS49	GLU97, TYR134, VAL135, ARG141, LEU188, CYS199, GLN185, ASP133, ASP200, PHE201, ILE62, LYS85, VAL110, LEU132, TYR140
3d	157.9	146.5	LEU45, VAL53, VAL66, ILE95, PHE113, HIS160, MET163, ILE174, ASP175, ASP156, GLY46, ARG47, LYS49, TYR50, LEU85, TRP176, LEU178	ALA83, TYR134, PRO136, ARG141, LEU188, CYS199, VAL61, ASN64, ILE62, LYS85
3g	160.0	145.7	VAL53, VAL66, LYS68, PHE113, HIS160, ILE174, ASP175, SER51, LYS77, GLY177, TYR50, LEU178	ALA83, TYR134, VAL135, ARG141, GLN185, LEU188, CYS199, LYS85, LYS183, ILE62, VAL70, LEU132
4d	162.9	138.4	LEU45, VAL53, VAL66, LYS68, ILE95, PHE113, HIS115, VAL116, MET163, ILE174, LYS49, TYR50, SER51, ASN118	ALA83, GLU97, TYR134, VAL135, TYR140, ARG141, LEU188, CYS199, LYS85, ASP133, ARG144, ASP200, ILE62, MET101, VAL110, LEU132, PHE201
4j	161.7	142.5	VAL53, LYS68, PHE113, HIS160, MET163, ILE174, ASP120, PHE121, LYS49, TYR50	ALA83, TYR134, VAL135, TYR140, ARG141, LEU188, CYS199, LYS85, ASP133, ARG144, ASP200, ILE62, VAL110, LEU132

Note: amino acids marked in bold are the common interacting features between reported known inhibitors of CK2 and GSK3 β and our designed dual kinase inhibitors. Underlined amino acids are involved in hydrogen bond interactions with the receptor while the remaining are involved in hydrophobic and electrostatic interactions.

reported in parts per million (δ) downfield from tetramethylsilane as an internal standard, and J values are given in Hertz. ^{19}F NMR spectra were recorded on a Bruker AVANCE III HD 500 spectrometer at 500 MHz. Positive-ion electrospray ionization (ESI) mass spectra were measured on a Perkin Elmer Applied Biosystems Sciex API 2000 instrument. High-resolution mass spectrometry (HRMS) measurements were performed using a Waters Micromass ESI-Q-TOF micro instrument with the Waters Alliance 2795 Separation Module at Panjab University, Chandigarh, Punjab. The purities of the assayed compounds were established by reversed-phase analytical HPLC and were found to be >95%. HPLC analyses were performed on a Shimadzu HPLC LC-2010C HT liquid chromatograph fitted with a Phenomenex Gemini C-18 column (4.6 mm $\times$ 250 mm, 10 μm particle size) with phosphate buffer (pH 4) (A) and CH_3CN (B) solvent mixtures and equipped with a photodiode array detector. Infrared spectra were obtained using KBr pellets. FT-IR spectra were recorded using a Bruker ALPHA FT-IR spectrometer. Melting points (mp) were measured in a Buchi melting point M-560 apparatus and are uncorrected. The reactions were monitored by thin-layer chromatography (TLC) on silica gel (precoated F254 Merck plates), and compounds were visual-

ized by UV (254 nm). Column chromatographic separations were carried out on silica gel (230–400 mesh). Organic solutions were dried over anhydrous Na_2SO_4 . All yields refer to isolated compounds.

General procedure A for the synthesis of compounds 1a–1h

A1: substituted phenyl isocyanates (1.0 equiv.) diluted in DCM were added dropwise to the solution of 2-(2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)isoindoline-1,3-dione (1.0 equiv.) in DMF under a nitrogen atmosphere. The mixture was stirred at room temperature for 1.5–2 h, and the reaction progress was monitored by TLC. After completion of the reaction, the low boiling point solvent was evaporated under vacuum and the reaction mixture was poured into ice cold water to give the precipitate. The resultant solid was filtered through a high vacuum suction pump, dried and purified by column chromatography on silica gel to get the desired product (1a–1f) (Scheme 1).

A2: the purified esters (comp 1d and 1e) were then stirred at room temp. with lithium hydroxide solution in a THF–water mixture for 3 h. THF was removed under vacuum and cold water was added to this mixture which was acidified

with dilute hydrochloric acid resulting in the precipitation of the acid. The acid was filtered and dried. The compound was again washed with diethyl ether to get the pure white product (**1g-1h**) (Scheme 1).

1-(6-(1,3-Dioxoisindolin-2-yl)-4,5,6,7-tetrahydrobenzo[d]-thiazol-2-yl)-3-(2-fluoro-phenyl)urea (1a). Compound **1a** was synthesized according to general procedure A1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 3 : 7 (v:v) as the eluent, to furnish **1a** as a white solid. Yield: 66%, mp 261–263 °C. IR (film) cm^{-1} 3366, 3219, 2966, 2860, 1767, 1711, 1624, 1208, 715 cm^{-1} . ^1H NMR ($\text{DMSO-}d_6$) δ : 1.99–2.05 (m, 1H), 2.55–2.58 (m, 1H), 2.74–2.75 (m, 2H), 2.85–2.89 (dd, J = 4.5 and 15.5 Hz, 1H), 3.17–3.18 (dd, J = 5.5 Hz, 1H), 4.42–4.47 (m, 1H), 7.05–7.09 (m, 1H), 7.16–7.19 (m, 1H), 7.26–7.29 (m, 1H), 7.85–7.90 (m, 4H), 8.11–8.14 (m, 1H), 9.05 (bs, 1H), 10.61 (s, 1H). ^{13}C NMR ($\text{DMSO-}d_6$) δ : 25.5, 25.6, 26.3, 47.1, 114.9, 115.1, 118.2, 120.6, 122.9 (2C), 123.3, 124.6, 126.5, 126.6, 131.4 (2C), 134.4 (2C), 151.1, 153.0, 167.7 (2C). MS (ESI): $[\text{M} + \text{H}]^+$, 437.1. HPLC purity: 98.5% (C-18 reverse phase, MeCN–phosphate buffer (NaH_2PO_4) pH 4, 70 : 30).

1-(6-(1,3-Dioxoisindolin-2-yl)-4,5,6,7-tetrahydrobenzo[d]-thiazol-2-yl)-3-(3-fluoro-phenyl)urea (1b). Compound **1b** was synthesized according to general procedure A1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 3 : 7 (v:v) as the eluent, to furnish **1b** as a white solid. Yield: 69%, mp 234–236 °C. IR (film) 3461, 3370, 2993, 2855, 1772, 1711, 1606, 1292, 1217, 714 cm^{-1} . ^1H NMR ($\text{DMSO-}d_6$) δ : 2.02–2.05 (m, 1H), 2.56–2.60 (m, 1H), 2.69–2.74 (m, 2H), 2.84–2.87 (dd, 1H), 3.26–3.33 (dd, 1H), 4.43–4.45 (m, 1H), 6.82–6.85 (m, 1H), 7.18–7.20 (m, 1H), 7.31–7.35 (m, 1H), 7.49–7.51 (m, 1H), 7.86–7.88 (m, 4H), 9.24 (s, 1H), 10.47 (bs, 1H). ^{13}C NMR ($\text{DMSO-}d_6$) δ : 25.2, 25.5, 26.2, 47.1, 105.0, 105.2, 108.7, 108.9, 114.2, 122.9 (2C), 130.3, 131.4 (2C), 134.4 (2C), 140.6, 140.7, 161.3, 163.2, 167.7 (2C). ^{19}F NMR (500 MHz, $\text{DMSO-}d_6$) δ (^1H decoupled): –112.06 (s); δ (^1H coupled): –112.07 (s). HRMS calculated for $\text{C}_{22}\text{H}_{17}\text{FN}_4\text{O}_3\text{S}$ $[\text{M} + \text{H}]^+$, 437.10; found, 437.0973. MS (ESI): $[\text{M} + \text{H}]^+$, 437.3. HPLC purity: 99.5% (C-18 reverse phase, MeCN–phosphate buffer (NaH_2PO_4) pH 4, 70 : 30).

1-(6-(1,3-Dioxoisindolin-2-yl)-4,5,6,7-tetrahydrobenzo[d]-thiazol-2-yl)-3-(4-fluoro-phenyl)urea (1c). Compound **1c** was synthesized according to general procedure A1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 3 : 7 (v:v) as the eluent, to furnish **1c** as a white solid. Yield: 58%, mp 220–222 °C. IR (film) 3400, 3101, 2980, 2924, 1770, 1709, 1622, 1581, 1207, 718 cm^{-1} . ^1H NMR ($\text{DMSO-}d_6$) δ : 2.02–2.04 (m, 1H), 2.55–2.58 (m, 1H), 2.73–2.74 (m, 2H), 2.84–2.87 (dd, 1H), 3.26–3.28 (dd, 1H), 4.43–4.46 (m, 1H), 7.13–7.17 (m, 2H), 7.49–7.51 (m, 2H), 7.85–7.90 (m, 4H), 9.04 (s, 1H), 10.35 (bs, 1H). ^{13}C NMR ($\text{DMSO-}d_6$) δ : 25.4, 25.5, 26.2, 47.1, 114.9, 115.3, 115.4, 117.6, 117.8, 120.3 (2C), 122.9 (2C), 131.4 (2C), 134.4 (2C), 135.1, 156.7, 158.6, 167.7 (2C). MS (ESI): $[\text{M} + \text{H}]^+$, 437.1. HPLC purity: 98.9% (C-18 reverse phase, MeCN–phosphate buffer (NaH_2PO_4) pH 4, 70 : 30).

Ethyl 3-(6-(1,3-dioxoisindolin-2-yl)-4,5,6,7-tetrahydrobenzo[d]-thiazol-2-yl)ureido)benzoate (1d). Compound **1d** was synthesized according to general procedure A1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 3.5 : 6.5 (v:v) as the eluent, to furnish **1d** as a white solid. Yield: 68%, mp 197–198 °C. IR (film) 3462, 3369, 2980, 2933, 1770, 1716, 1683, 1555, 1288, 722 cm^{-1} . ^1H NMR ($\text{DMSO-}d_6$) δ : 1.31–1.34 (t, J = 7 Hz, 3H), 2.03–2.05 (m, 1H), 2.54–2.63 (m, 1H), 2.69–2.74 (m, 2H), 2.84–2.88 (dd, J = 4.5, 15.5 Hz, 1H), 3.27–3.29 (dd, J = 13.5 Hz, 1H), 4.31–4.35 (q, J = 7 Hz, 2H), 4.44–4.46 (m, 1H), 7.44–7.47 (m, 1H), 7.61–7.62 (m, 1H), 7.69–7.71 (m, 1H), 7.85–7.90 (m, 4H), 8.18 (s, 1H), 9.26 (s, 1H), 10.42 (bs, 1H). ^{13}C NMR ($\text{DMSO-}d_6$) δ : 14.1, 25.3, 25.5, 26.2, 47.1, 60.8, 117.6, 118.7, 122.9 (2C), 123.1, 129.2, 130.5, 131.4 (2C), 134.4 (2C), 139.2, 152.2, 152.8, 157.8, 157.9, 165.5, 167.7 (2C). HRMS calculated for $\text{C}_{25}\text{H}_{22}\text{N}_4\text{O}_5\text{S}$ $[\text{M} + \text{H}]^+$, 491.13; found, 491.1301. MS (ESI): $[\text{M} + \text{H}]^+$, 491.2. HPLC purity: 98.8% (C-18 reverse phase, MeCN–phosphate buffer (NaH_2PO_4) pH 4, 70 : 30).

Ethyl 4-(3-(6-(1,3-dioxoisindolin-2-yl)-4,5,6,7-tetrahydrobenzo[d]-thiazol-2-yl)ureido)benzoate (1e). Compound **1e** was synthesized according to general procedure A1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 3.5 : 6.5 (v:v) as the eluent, to furnish **1e** as a white solid. Yield: 64%, mp 226–228 °C. IR (film) 3360, 3247, 2981, 2930, 1770, 1715, 1674, 1592, 1533, 1247, 719 cm^{-1} . ^1H NMR ($\text{DMSO-}d_6$) δ : 1.30–1.33 (t, J = 7 Hz, 3H), 2.02–2.05 (m, 1H), 2.59–2.60 (m, 1H), 2.74–2.75 (m, 2H), 2.84–2.88 (dd, J = 15.5 Hz, 1H), 2.96–2.97 (dd, 1H), 4.28–4.29 (q, J = 7 Hz, 2H), 4.44–4.46 (m, 1H), 7.62–7.64 (m, 2H), 7.85–7.91 (m, 6H), 9.40 (s, 1H), 10.48 (bs, 1H). ^{13}C NMR ($\text{DMSO-}d_6$) δ : 14.2, 25.2, 25.5, 26.1, 47.0, 60.3, 117.6 (2C), 123.0 (2C), 123.3, 130.4 (2C), 131.4 (2C), 134.4 (2C), 143.4, 152.3, 152.6, 157.9, 158.6, 165.3, 167.7 (2C). MS (ESI): $[\text{M} + \text{H}]^+$, 491.2. HPLC purity: 96.1% (C-18 reverse phase, MeCN–phosphate buffer (NaH_2PO_4) pH 4, 70 : 30).

1-(6-(1,3-Dioxoisindolin-2-yl)-4,5,6,7-tetrahydrobenzo[d]-thiazol-2-yl)-3-(4-methoxyphenyl)urea (1f). Compound **1f** was synthesized according to general procedure A1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 3.5 : 6.5 (v:v) as the eluent, to furnish **1f** as a white solid. Yield: 62%, mp 188–190 °C. IR (film) 3400, 3217, 2995, 2955, 1770, 1707, 1678, 1512, 1244, 1110, 718 cm^{-1} . ^1H NMR ($\text{DMSO-}d_6$) δ : 2.01–2.04 (m, 1H), 2.55–2.59 (m, 1H), 2.72–2.74 (m, 2H), 2.83–2.86 (dd, J = 14 Hz, 1H), 3.26–3.28 (dd, J = 13.5 Hz, 1H), 3.72 (s, 3H), 4.43–4.45 (m, 1H), 6.88–6.90 (dd, J = 8 Hz, 2H), 7.36–7.38 (dd, J = 8.5 Hz, 2H), 7.86–7.88 (m, 4H), 8.83 (s, 1H), 10.25 (bs, 1H). ^{13}C NMR ($\text{DMSO-}d_6$) δ : 25.4, 25.5, 26.3, 47.2, 55.1, 114.0 (2C), 117.6, 120.3 (2C), 122.9 (2C), 131.4 (2C), 131.6, 134.4 (2C), 142.7, 151.8, 154.9, 157.6, 167.7 (2C). MS (ESI): $[\text{M} + \text{H}]^+$, 449.3. HPLC purity: 98.9% (C-18 reverse phase, MeCN–phosphate buffer (NaH_2PO_4) pH 4, 70 : 30).

Ethoxy((6-phthalimido-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)amino)(3-carboxyphenylamino)methanol (1g). Compound **1g** was synthesized according to general procedure A2. The

hydrolyzed product was repeatedly washed with diethyl ether to get the pure white product. Yield: 66%, mp 199–200 °C. IR (film) 3419, 3272, 3067, 2943, 1718, 1627, 1600, 1545, 1289, 1225, 753 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ : 1.31–1.34 (t, *J* = 7.2 Hz, 3H), 1.79–1.87 (m, 1H), 2.02–2.04 (m, 1H), 2.56–2.64 (m, 2H), 2.69–2.72 (dd, *J* = 16.5 Hz, 1H), 2.94–2.98 (dd, *J* = 4.25, 15.75 Hz, 1H), 4.18–4.20 (m, 1H), 4.30–4.35 (q, *J* = 7.2 Hz, 2H), 7.39–7.45 (m, 1H), 7.46–7.48 (m, 1H), 7.49–7.51 (m, 1H), 7.56–7.61 (m, 2H), 7.68–7.70 (dd, *J* = 8.5 Hz, 1H), 7.78–7.80 (dd, *J* = 7.5 Hz, 1H), 8.18 (s, 1H), 8.46 (bs, 1H), 9.32 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ : 14.2, 24.1, 27.9, 28.0, 45.3, 60.8, 117.8, 118.8, 123.0 (2C), 127.7, 128.9, 129.2 (2C), 130.5, 131.2 (2C), 138.8, 139.4, 152.5, 157.8, 165.6, 167.9, 168.4 (2C) (ESI⁺). HRMS calculated for C₂₅H₂₄N₄O₆S [M + H]⁺, 509.14; found, 509.1328. MS (ESI): [M + H]⁺, 509.3. HPLC purity: 99.3% (C-18 reverse phase, MeCN–phosphate buffer (NaH₂PO₄) pH 4, 45:55).

Ethoxy((6-phthalimido-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)amino)(4-carboxyphenylamino)methanol (1h). Compound **1h** was synthesized according to general procedure A2. The hydrolyzed product was repeatedly washed with diethyl ether to get the pure white product. Yield: 60%, mp 174–176 °C. IR (film) 3452, 3070, 2983, 2932, 1705, 1632, 1602, 1545, 1283, 1252, 770 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ : 1.29–1.32 (t, *J* = 7.0 Hz, 3H), 1.79–1.87 (m, 1H), 2.02–2.04 (m, 1H), 2.56–2.64 (m, 2H), 2.69–2.72 (dd, *J* = 16 Hz, 1H), 2.95–2.97 (dd, *J* = 11 Hz, 1H), 4.18–4.20 (m, 1H), 4.26–4.30 (q, *J* = 7.0 Hz, 2H), 7.39–7.41 (m, 1H), 7.49–7.52 (m, 1H), 7.56–7.59 (m, 1H), 7.63–7.64 (dd, *J* = 8.5 Hz, 2H), 7.78–7.80 (m, 1H), 7.89–7.91 (dd, *J* = 8.5 Hz, 2H), 8.43–8.45 (d, *J* = 7.5 Hz, 1H), 9.45 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ : 14.7, 24.3, 28.2, 28.4, 45.8, 61.2, 118.2 (2C), 124.0 (2C), 128.2, 129.8, 129.9, 130.5, 131.0 (2C), 132.1 (2C), 138.9, 143.8, 152.8, 158.4, 166.2, 168.4, 169.4. MS (ESI): [M + H]⁺, 509.3. HPLC purity: 99.2% (C-18 reverse phase, MeCN–phosphate buffer (NaH₂PO₄) pH 4, 45:55).

General procedure B for the synthesis of compounds 2a–2h

B1: substituted phenyl isocyanates (2.0 equiv.) diluted in DCM were added dropwise to the solution of *N*⁶-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (1.0 equiv.) in DMF under a nitrogen atmosphere. The mixture was stirred at room temperature for 2.5–3 h, and the reaction progress was monitored by TLC. After completion of the reaction, the low boiling point solvent was evaporated under vacuum and the reaction mixture was poured into ice cold water to give the precipitate. The resultant solid was filtered through a high vacuum suction pump, dried and purified by column chromatography on silica gel to get the desired product (2a–2f) (Scheme 2).

B2: the purified esters (comp 2d and 2e) were then stirred at room temp. with lithium hydroxide solution in a THF–water mixture for 5–6 h. THF was removed under vacuum and cold water was added to this mixture which was acidified with dilute hydrochloric acid resulting in the precipitation of the acid. The acid was filtered and dried. The compound was

again washed with diethyl ether to get the pure white product (2g–2h) (Scheme 2).

3-(2-Fluorophenyl)-1-(2-(3-(2-fluorophenyl)ureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)-1-propylurea (2a). Compound **2a** was synthesized according to general procedure B1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 4:6 (v:v) as the eluent, to furnish **2a** as a white solid. Yield: 60%, mp 165–166 °C. IR (film) 3400, 3329, 3304, 3192, 3044, 2966, 2937, 1719, 1672, 1614, 1599, 1531, 1281, 752 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ : 0.87–0.90 (t, *J* = 7.0 Hz, 3H), 1.59–1.62 (m, 2H), 1.90–1.92 (m, 1H), 2.05–2.09 (m, 1H), 2.71–2.78 (m, 2H), 2.83–2.85 (m, 1H), 2.87–2.88 (m, 1H), 3.24–3.25 (dd, *J* = 6 Hz, 2H), 4.34–4.36 (m, 1H), 7.07–7.18 (m, 5H), 7.26–7.28 (m, 1H), 7.42–7.44 (m, 1H), 8.03 (s, 1H), 8.11–8.13 (m, 1H), 9.05 (bs, 1H), 10.56 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ : 11.1, 23.1, 25.9, 26.3, 27.4, 44.2, 52.3, 114.9, 115.2, 115.3, 119.0, 120.7, 123.3, 123.8, 124.6, 124.9, 126.6, 127.6, 127.7, 151.1, 153.1, 154.6, 154.9, 156.6. MS (ESI): [M + H]⁺, 486.2. HPLC purity: 99.7% (C-18 reverse phase, MeCN–phosphate buffer (NaH₂PO₄) pH 4, 60:40).

3-(3-Fluorophenyl)-1-(2-(3-(3-fluorophenyl)ureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)-1-propylurea (2b). Compound **2b** was synthesized according to general procedure B1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 4:6 (v:v) as the eluent, to furnish **2b** as a white solid. Yield: 68%, mp 143–144 °C. IR (film) 3501, 3330, 3310, 3086, 2964, 2934, 1710, 1640, 1603, 1545, 1288, 774 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ : 0.85–0.89 (dd, *J* = 7.25, 14.75 Hz, 3H), 1.52–1.61 (m, 2H), 1.87–1.89 (m, 1H), 2.05–2.09 (m, 1H), 2.69–2.71 (m, 2H), 2.75–2.76 (m, 1H), 2.81–2.86 (m, 1H), 3.23–3.26 (t, *J* = 7.5, 2H), 4.36–4.37 (m, 1H), 6.72–6.76 (m, 1H), 6.82–6.85 (m, 1H), 7.18–7.19 (m, 1H), 7.22–7.28 (m, 2H), 7.30–7.35 (m, 1H), 7.44–7.51 (m, 2H), 8.45 (s, 1H), 9.22 (s, 1H), 10.39 (bs, 1H). ¹³C NMR (DMSO-*d*₆) δ : 11.2, 23.1, 25.5, 26.3, 27.4, 43.9, 52.2, 105.1, 106.3, 107.8, 108.7, 114.2, 115.4, 118.4, 129.5, 130.3, 140.7, 142.5, 152.4, 154.5, 161.1, 161.4, 163.0, 163.3. ¹⁹F NMR (500 MHz, DMSO-*d*₆) δ (¹H decoupled): –113.14 (s), –112.07 (s); δ (¹H coupled): –113.18–113.12 (m), –112.08 (bs). HRMS calculated for C₂₄H₂₅F₂N₅O₂S [M + H]⁺, 486.17; found, 486.1704. MS (ESI): [M + H]⁺, 486.2. HPLC purity: 99.0% (C-18 reverse phase, MeCN–phosphate buffer (NaH₂PO₄) pH 4, 60:40).

3-(4-Fluorophenyl)-1-(2-(3-(4-fluorophenyl)ureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)-1-propylurea (2c). Compound **2c** was synthesized according to general procedure B1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 4.5:5.5 (v:v) as the eluent, to furnish **2c** as a white solid. Yield: 67%, mp 171–173 °C. IR (film) 3400, 3330, 3300, 2964, 2936, 1720, 1670, 1610, 1607, 1555, 1215, 831 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ : 0.86–0.89 (t, *J* = 7.25 Hz, 3H), 1.53–1.59 (m, 2H), 1.87–1.89 (m, 1H), 2.06–2.08 (m, 1H), 2.70–2.72 (m, 2H), 2.73–2.74 (m, 1H), 2.80–2.82 (m, 1H), 3.21–3.24 (t, *J* = 7.75, 2H), 4.35–4.36 (m, 1H), 7.05–7.08 (m, 2H), 7.13–7.16 (m, 2H), 7.44–7.50 (m, 4H), 8.29 (s, 1H), 9.03 (s, 1H), 10.31 (bs, 1H). ¹³C NMR (DMSO-*d*₆) δ : 11.2, 23.2, 25.7, 26.4, 27.5, 43.9, 52.1, 114.5, 114.7, 115.3, 115.4, 118.5,

120.3, 120.4, 122.0, 122.1, 135.1, 136.8, 152.2, 154.9, 156.5, 156.7, 158.3, 158.6. MS (ESI): $[M + H]^+$, 486.2. HPLC purity: 98.4% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 60:40).

Ethyl 3-(3-(6-(3-(ethoxycarbonyl)phenyl)-1-propylureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)ureido)benzoate (2d). Compound 2d was synthesized according to general procedure B1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 4.5:5.5 (v:v) as the eluent, to furnish 2d as a white solid. Yield: 65%, mp 178–179 °C. IR (film) 3367, 3290, 3070, 2980, 1720, 1690, 1627, 1596, 1554, 1288, 756 cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$) δ : 0.87–0.90 (t, $J = 7.25$ Hz, 3H), 1.30–1.34 (m, 6H), 1.56–1.61 (m, 2H), 1.89–1.91 (m, 1H), 2.08–2.09 (m, 1H), 2.71–2.73 (m, 2H), 2.77–2.78 (m, 1H), 2.83–2.85 (dd, $J = 12.0$ Hz, 1H), 3.24–3.29 (dd, $J = 7.75$, 15.75 Hz, 2H), 4.29–4.35 (m, 4H), 4.39–4.41 (m, 1H), 7.36–7.39 (dd, $J = 8.0$ Hz, 1H), 7.44–7.47 (dd, $J = 8.0$ Hz, 1H), 7.53–7.55 (dd, $J = 7.5$ Hz, 1H), 7.60–7.62 (dd, $J = 7.5$ Hz, 1H), 7.69–7.70 (dd, $J = 7.0$ Hz, 1H), 7.79–7.81 (dd, $J = 8.0$ Hz, 1H), 8.10 (s, 1H), 8.17 (s, 1H), 8.53 (s, 1H), 9.24 (s, 1H), 10.36 (bs, 1H). ^{13}C NMR ($\text{DMSO}-d_6$) δ : 11.2, 14.1, 14.2, 23.1, 25.6, 26.4, 27.4, 43.9, 52.2, 60.6, 60.7, 118.5, 118.7, 120.4, 122.3, 122.9, 123.0, 124.5, 128.4, 129.2, 129.9, 130.4, 139.3, 140.9, 152.2, 154.6, 157.8, 157.9, 165.5, 165.8. MS (ESI): $[M + H]^+$, 594.3. HPLC purity: 99.0% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 60:40).

Ethyl 4-(3-(6-(3-(4-(ethoxycarbonyl)phenyl)-1-propylureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)ureido)benzoate (2e). Compound 2e was synthesized according to general procedure B1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 4.5:5.5 (v:v) as the eluent, to furnish 2e as a white solid. Yield: 58%, mp 179–180 °C. IR (film) 3400, 3341, 2970, 2934, 1720, 1689, 1620, 1599, 1519, 1282, 771 cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$) δ : 0.86–0.89 (t, $J = 7.5$ Hz, 3H), 1.29–1.33 (m, 6H), 1.53–1.60 (m, 2H), 1.89–1.91 (m, 1H), 2.08–2.11 (m, 1H), 2.71–2.73 (m, 2H), 2.77–2.78 (m, 1H), 2.84–2.88 (dd, $J = 12.5$ Hz, 1H), 3.25–3.28 (dd, $J = 7.75$ Hz, 2H), 4.25–4.31 (m, 4H), 4.37–4.39 (m, 1H), 7.63–7.65 (m, 4H), 7.83–7.85 (dd, $J = 9.0$, 9.0 Hz, 2H), 7.89–7.91 (dd, $J = 8.5$, 8.5 Hz, 2H), 8.64 (s, 1H), 9.38 (s, 1H), 10.43 (s, 1H). ^{13}C NMR ($\text{DMSO}-d_6$) δ : 11.2, 14.2, 14.5, 23.1, 25.5, 26.3, 27.3, 44.1, 52.3, 60.2, 60.3, 117.6 (2C), 118.5 (2C), 122.4 (2C), 123.3, 129.7 (2C), 130.3 (2C), 143.5, 145.3, 152.1, 154.3, 158.3, 158.4, 165.3, 165.5. MS (ESI): $[M + H]^+$, 594.3. HPLC purity: 99.5% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 60:40).

3-(4-Methoxyphenyl)-1-(2-(3-(4-methoxyphenyl)ureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)-1-propylurea (2f). Compound 2f was synthesized according to general procedure B1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 4:6 (v:v) as the eluent, to furnish 2f as a white solid. Yield: 59%, mp 139–141 °C. IR (film) 3319, 3287, 2961, 2934, 1693, 1607, 1034, 827 cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$) δ : 0.86–0.89 (t, $J = 7.25$ Hz, 3H), 1.53–1.59 (m, 2H), 1.86–1.88 (m, 1H), 2.03–2.07 (m, 1H), 2.69–2.71 (m, 2H), 2.73–2.74 (m, 1H), 2.78–2.83 (dd, $J = 12.75$ Hz, 1H),

3.21–3.22 (dd, $J = 7.0$ Hz, 2H), 3.70 (s, 3H), 3.72 (s, 3H), 4.35–4.37 (m, 1H), 6.80–6.82 (dd, $J = 9.0$ Hz, 2H), 6.87–6.89 (dd, $J = 9.0$ Hz, 2H), 7.32–7.34 (dd, $J = 9.0$ Hz, 2H), 7.36–7.38 (dd, $J = 9.0$ Hz, 2H), 8.09 (s, 1H), 8.82 (s, 1H), 10.20 (s, 1H). ^{13}C NMR ($\text{DMSO}-d_6$) δ : 11.2, 23.3, 25.8, 26.4, 27.6, 43.9, 51.9, 55.0, 55.1, 113.4 (2C), 114.0 (2C), 118.5, 120.3 (2C), 122.3 (2C), 131.7, 133.5, 151.8, 154.5, 154.9, 155.1 (2C), 157.5. MS (ESI): $[M + H]^+$, 510.2. HPLC purity: 99.0% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 45:55).

3-(3-(6-(3-(3-Carboxyphenyl)-1-propylureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)ureido)benzoic acid (2g). Compound 2g was synthesized according to general procedure B2. The hydrolyzed product was repeatedly washed with diethyl ether to get the pure white product. Yield: 67%, mp 228–230 °C. IR (film) 3445, 3080, 2965, 2930, 1703, 1638, 1594, 1550, 1300, 758 cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$) δ : 0.87–0.90 (t, $J = 6.75$ Hz, 3H), 1.55–1.61 (m, 2H), 1.90–1.92 (m, 1H), 2.07–2.11 (m, 1H), 2.72–2.78 (m, 3H), 2.83–2.85 (m, 1H), 3.25–3.27 (m, 2H), 4.38–4.40 (m, 1H), 7.35 (t, $J = 8.0$ Hz, 1H), 7.43 (t, $J = 7.75$ Hz, 1H), 7.51–7.53 (d, $J = 7.5$ Hz, 1H), 7.58–7.60 (d, $J = 7.5$ Hz, 1H), 7.66–7.68 (d, $J = 7.5$ Hz, 1H), 7.77–7.79 (d, $J = 8.0$ Hz, 1H), 8.09 (s, 1H), 8.15 (s, 1H), 8.49 (s, 1H), 9.33 (s, 1H). ^{13}C NMR ($\text{DMSO}-d_6$) δ : 11.2, 23.2, 25.5, 26.4, 27.4, 44.0, 52.2, 118.7, 119.1, 120.9, 122.6 (2C), 123.4, 124.2, 128.4, 129.1, 130.8, 131.4, 139.1, 140.9, 152.1, 154.7, 157.8, 167.2, 167.5 (2C). MS (ESI): $[M + H]^+$, 538.2. HPLC purity: 97.0% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 35:65).

4-(3-(6-(3-(4-Carboxyphenyl)-1-propylureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)ureido)benzoic acid (2h). Compound 2h was synthesized according to general procedure B2. The hydrolyzed product was repeatedly washed with diethyl ether to get the pure white product. Yield: 63%, mp 230–232 °C. IR (film) 3422, 3080, 2965, 2934, 1698, 1647, 1602, 1540, 1247, 773 cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$) δ : 0.87–0.90 (t, $J = 7.0$ Hz, 3H), 1.56–1.59 (m, 2H), 1.90–1.92 (m, 1H), 2.07–2.09 (m, 1H), 2.71–2.78 (m, 3H), 2.84–2.89 (m, 1H), 3.27–3.29 (m, 2H), 4.38–4.40 (m, 1H), 7.59–7.61 (d, $J = 8.0$ Hz, 4H), 7.81–7.83 (d, $J = 8.0$ Hz, 2H), 7.87–7.89 (d, $J = 8.0$ Hz, 2H), 8.61 (s, 1H), 9.38 (s, 1H). ^{13}C NMR ($\text{DMSO}-d_6$) δ : 11.2, 23.1, 25.5, 26.3, 27.3, 44.1, 52.3, 117.5 (2C), 118.5, 118.8 (2C), 123.3, 124.2, 129.9 (2C), 130.5 (2C), 143.1, 144.9, 152.2, 154.4, 157.9, 166.9, 167.1 (2C). MS (ESI): $[M + H]^+$, 538.2. HPLC purity: 96.9% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 35:65).

General procedure C for the synthesis of compounds 3a–3h

C1: the compound 4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (1.0 equiv.) was dissolved in DMF by heating at 80 °C. Substituted phenyl isocyanates (2.0 equiv.) diluted in DCM were added dropwise to the hot solution under a nitrogen atmosphere. The mixture was stirred at the same temperature for 1 h and then at room temperature for 1–2 h. The reaction progress was monitored by TLC, and after completion, the reaction mixture was poured into ice cold water to give

the precipitate. The resultant solid was filtered through a high vacuum suction pump, dried and purified by column chromatography on silica gel to get the desired product (3a–3f) (Scheme 3).

C2: the purified esters (comp 3d and 3e) were then stirred with lithium hydroxide solution in a THF–water mixture at 60 °C for 10–12 h. THF was removed under vacuum and cold water was added to this mixture which was acidified with dilute hydrochloric acid resulting in the precipitation of the acid. The acid was filtered and dried. The compound was again washed with diethyl ether to get the pure white product (3g–3h) (Scheme 3).

1,1'-(4,5,6,7-Tetrahydrobenzo[d]thiazole-2,6-diyl)bis(3-(2-fluorophenyl)urea) (3a). Compound 3a was synthesized according to general procedure C1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 5.5:4.5 (v:v) as the eluent, to furnish 3a as a white solid. Yield: 55%, mp 214–215 °C. IR (film) 3440, 3323, 3042, 2924, 2847, 1722, 1640, 1619, 1599, 1539, 1256, 747 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 1.84–1.87 (m, 1H), 1.92–1.94 (m, 1H), 2.55–2.59 (dd, *J* = 4.25, 15.75 Hz, 1H), 2.66–2.73 (m, 2H), 2.97–2.99 (dd, *J* = 13.5 Hz, 1H), 4.09–4.11 (m, 1H), 6.88–6.92 (m, 2H), 7.07–7.09 (m, 2H), 7.15–7.19 (m, 2H), 7.25–7.29 (m, 1H), 8.12–8.18 (m, 2H), 8.28 (s, 1H), 9.08 (bs, 1H), 10.62 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 23.2, 27.8, 29.1, 44.4, 114.8, 115.0, 118.3, 119.8, 120.7, 121.3, 123.4, 124.3, 124.5, 126.6, 128.3, 150.5, 151.1, 152.4, 153.1, 154.3, 156.7. MS (ESI): [M + H]⁺, 443.9. HPLC purity: 99.7% (C-18 reverse phase, MeCN–phosphate buffer (NaH₂PO₄) pH 4, 55:45).

1,1'-(4,5,6,7-Tetrahydrobenzo[d]thiazole-2,6-diyl)bis(3-(3-fluorophenyl)urea) (3b). Compound 3b was synthesized according to general procedure C1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 5.5:4.5 (v:v) as the eluent, to furnish 3b as a white solid. Yield: 64%, mp 231–233 °C. IR (film) 3406, 3314, 3084, 2995, 2847, 1719, 1678, 1603, 1547, 1298, 773 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 1.81–1.87 (m, 1H), 1.93–1.95 (m, 1H), 2.53–2.57 (dd, *J* = 6.5, 16 Hz, 1H), 2.62–2.68 (m, 2H), 2.94–2.97 (dd, *J* = 3.5, 12 Hz, 1H), 4.02–4.06 (m, 1H), 6.46–6.48 (m, 1H), 6.68–6.72 (m, 1H), 6.82–6.85 (m, 1H), 6.98–6.99 (m, 1H), 7.18–7.26 (m, 2H), 7.31–7.35 (m, 1H), 7.45–7.51 (m, 2H), 8.62 (s, 1H), 9.24 (s, 1H), 10.43 (bs, 1H). ¹³C NMR (DMSO-*d*₆) δ: 23.2, 27.9, 29.0, 44.6, 104.1, 104.3, 105.1, 105.3, 107.3, 107.5, 109.0, 113.2, 114.3, 130.3, 130.5, 140.7, 142.2, 154.5, 161.5, 163.5 (2C). ¹⁹F NMR (500 MHz, DMSO-*d*₆) δ (¹H decoupled): –112.38 (s), –112.07 (s); δ (¹H coupled): –112.42–112.36 (m), –112.07 (bs). MS (ESI): [M + H]⁺, 444.1. HPLC purity: 99.7% (C-18 reverse phase, MeCN–phosphate buffer (NaH₂PO₄) pH 4, 60:40).

1,1'-(4,5,6,7-Tetrahydrobenzo[d]thiazole-2,6-diyl)bis(3-(4-fluorophenyl)urea) (3c). Compound 3c was synthesized according to general procedure C1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 5.5:4.5 (v:v) as the eluent, to furnish 3c as a white solid. Yield: 58%, mp 263–266 °C. IR (film) 3460, 3314, 3080, 2940, 2850, 1724, 1634, 1554, 1509, 1213, 796 cm⁻¹. ¹H

NMR (DMSO-*d*₆) δ: 1.81–1.87 (m, 1H), 1.93–1.95 (m, 1H), 2.52–2.57 (dd, *J* = 10.25, 15.75 Hz, 1H), 2.65–2.74 (m, 2H), 2.94–2.97 (dd, *J* = 14.5 Hz, 1H), 4.06–4.08 (m, 1H), 6.34–6.35 (dd, *J* = 7.0 Hz, 1H), 7.05–7.08 (m, 2H), 7.14–7.17 (m, 2H), 7.38–7.40 (m, 2H), 7.50–7.52 (m, 2H), 8.43 (s, 1H), 9.05 (s, 1H), 10.41 (bs, 1H). ¹³C NMR (DMSO-*d*₆) δ: 23.3, 28.1, 29.1, 44.6, 115.0, 115.2, 115.3, 115.5, 117.8, 119.1 (2C), 120.3 (2C), 135.1, 136.7, 154.7, 155.9, 156.8, 157.8 (2C), 158.7. MS (ESI): [M + H]⁺, 444.1. HPLC purity: 99.5% (C-18 reverse phase, MeCN–phosphate buffer (NaH₂PO₄) pH 4, 55:45).

Ethyl 3-(3-(6-(3-(ethoxycarbonyl)phenyl)ureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)ureido)benzoate (3d). Compound 3d was synthesized according to general procedure C1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 6.5:3.5 (v:v) as the eluent, to furnish 3d as a white solid. Yield: 60%, mp 217–219 °C. IR (film) 3480, 3395, 3318, 2985, 2935, 1722, 1683, 1639, 1556, 1282, 752 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 1.30–1.34 (m, 6H), 1.84–1.87 (m, 1H), 1.95–1.97 (m, 1H), 2.55–2.59 (dd, *J* = 5.5, 16.0 Hz, 1H), 2.64–2.66 (m, 2H), 2.95–2.98 (dd, *J* = 13.5 Hz, 1H), 4.06–4.08 (m, 1H), 4.28–4.35 (m, 4H), 6.38–6.39 (dd, *J* = 7.5 Hz, 1H), 7.35–7.38 (m, 1H), 7.44–7.47 (m, 1H), 7.49–7.50 (m, 1H), 7.56–7.58 (m, 1H), 7.60–7.62 (m, 1H), 7.69–7.71 (m, 1H), 8.09 (s, 1H), 8.18 (s, 1H), 8.66 (s, 1H), 9.25 (s, 1H), 10.38 (bs, 1H). ¹³C NMR (DMSO-*d*₆) δ: 14.1 (2C), 23.2, 27.9, 28.9, 44.7, 60.6, 60.8, 117.8, 118.2, 118.5, 121.7, 121.9, 122.9, 123.1, 128.9, 129.2, 130.3, 130.5, 139.3, 140.7, 151.4, 152.1, 154.5, 157.4, 165.5, 165.7. MS (ESI): [M + H]⁺, 552.3. HPLC purity: 99.8% (C-18 reverse phase, MeCN–phosphate buffer (NaH₂PO₄) pH 4, 60:40).

Ethyl 4-(3-(6-(3-(4-(ethoxycarbonyl)phenyl)ureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)ureido)benzoate (3e). Compound 3e was synthesized according to general procedure C1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 6.5:3.5 (v:v) as the eluent, to furnish 3e as a white solid. Yield: 57%, mp 220–222 °C. IR (film) 3480, 3346, 3320, 2986, 2939, 1709, 1645, 1600, 1549, 1283, 769 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 1.28–1.33 (m, 6H), 1.84–1.87 (m, 1H), 1.94–1.96 (m, 1H), 2.55–2.58 (dd, *J* = 17.5 Hz, 1H), 2.64–2.66 (m, 2H), 2.95–2.98 (dd, *J* = 14.5 Hz, 1H), 4.06–4.08 (m, 1H), 4.24–4.30 (m, 4H), 6.51–6.53 (dd, *J* = 7.5 Hz, 1H), 7.49–7.51 (m, 2H), 7.62–7.64 (m, 2H), 7.82–7.84 (m, 2H), 7.89–7.91 (m, 2H), 8.82 (s, 1H), 9.40 (s, 1H), 10.48 (bs, 1H). ¹³C NMR (DMSO-*d*₆) δ: 14.2 (2C), 23.1, 27.8, 28.9, 44.6, 60.1, 60.3, 116.6 (2C), 117.6 (2C), 122.0 (2C), 123.3, 130.3 (4C), 143.5, 144.9, 152.2, 154.2 (2C), 157.6, 165.3, 165.5. MS (ESI): [M + H]⁺, 552.4. HPLC purity: 99.7% (C-18 reverse phase, MeCN–phosphate buffer (NaH₂PO₄) pH 4, 55:45).

1,1'-(4,5,6,7-Tetrahydrobenzo[d]thiazole-2,6-diyl)bis(3-(4-methoxyphenyl)urea) (3f). Compound 3f was synthesized according to general procedure C1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 7:3 (v:v) as the eluent, to furnish 3f as a white solid. Yield: 52%, mp 230–232 °C. IR (film) 3330, 3307, 2923, 2837, 1723, 1638, 1604, 1547, 1243, 826 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ: 1.79–1.83 (m, 1H), 1.90–1.94 (m, 1H), 2.54–2.55

(dd, $J = 5.5$ Hz, 1H), 2.62–2.64 (m, 2H), 2.92–2.95 (dd, $J = 14.0$ Hz, 1H), 3.69 (s, 3H), 3.72 (s, 3H), 4.02–4.04 (m, 1H), 6.22–6.23 (dd, $J = 7.0$ Hz, 1H), 6.80–6.82 (m, 2H), 6.87–6.89 (m, 2H), 7.26–7.28 (m, 2H), 7.36–7.38 (m, 2H), 8.18 (s, 1H), 8.82 (s, 1H), 10.23 (s, 1H). ^{13}C NMR (DMSO- d_6) δ : 23.5, 28.2, 29.1, 44.6, 55.1 (2C), 113.8 (2C), 114.0 (2), 117.8, 119.2 (2C), 120.2 (2C), 131.7, 133.5, 151.7, 153.9 (2C), 154.9 (2C), 157.3. MS (ESI): $[\text{M} + \text{H}]^+$, 468.2. HPLC purity: 99.5% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 55 : 45).

3-(3-(6-(3-(3-Carboxyphenyl)ureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)ureido)benzoic acid (3g). Compound 3g was synthesized according to general procedure C2. The hydrolyzed product was repeatedly washed with diethyl ether to get the pure pinkish product. Yield: 58%, mp 238–240 °C. IR (film) 3480, 3347, 2936, 2849, 1700, 1679, 1649, 1600, 1556, 1228, 756 cm^{-1} . ^1H NMR (DMSO- d_6) δ : 1.85–1.87 (m, 1H), 1.94–1.96 (m, 1H), 2.56–2.59 (dd, $J = 13.0$ Hz, 1H), 2.65–2.66 (m, 2H), 2.96–2.99 (dd, $J = 15.0$ Hz, 1H), 4.06–4.07 (m, 1H), 6.40–6.44 (m, 1H), 7.32–7.33 (m, 1H), 7.41–7.42 (m, 1H), 7.48–7.49 (m, 1H), 7.56–7.59 (m, 2H), 7.67–7.68 (m, 1H), 8.05 (s, 1H), 8.17 (s, 1H), 8.64 (s, 1H), 9.63 (s, 1H). ^{13}C NMR (DMSO- d_6) δ : 23.4, 28.1, 29.1, 44.8, 117.9, 118.4, 119.2, 121.5, 122.0, 122.4, 123.4, 128.8, 128.9, 131.9, 132.4, 139.3, 140.6, 151.3, 152.4, 154.7 (2C), 157.8, 167.9 (2C). MS (ESI): $[\text{M} + \text{H}]^+$, 496.1. HPLC purity: 97.6% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 30 : 70).

4-(3-(6-(3-(4-Carboxyphenyl)ureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)ureido)benzoic acid (3h). Compound 3h was synthesized according to general procedure C2. The hydrolyzed product was repeatedly washed with diethyl ether to get the pure pinkish product. Yield: 52%, mp 239–241 °C. IR (film) 3470, 3339, 2945, 2850, 1712, 1691, 1642, 1600, 1548, 1250, 771 cm^{-1} . ^1H NMR (DMSO- d_6) δ : 1.85–1.87 (m, 1H), 1.94–1.96 (m, 1H), 2.55–2.58 (dd, $J = 16.5$ Hz, 1H), 2.65 (m, 2H), 2.95–2.98 (dd, $J = 15.0$ Hz, 1H), 4.07–4.08 (m, 1H), 6.53–6.55 (dd, $J = 7.5$ Hz, 1H), 7.47–7.49 (m, 2H), 7.59–7.61 (m, 2H), 7.80–7.82 (m, 2H), 7.88–7.89 (m, 2H), 8.81 (s, 1H), 9.47 (s, 1H). ^{13}C NMR (DMSO- d_6) δ : 23.2, 27.7, 29.1, 44.7, 116.6 (2C), 117.6 (2C), 117.9, 122.9, 124.3, 130.4 (4C), 143.2, 144.7, 152.3, 154.4 (2C), 157.7, 167.1 (2C). MS (ESI): $[\text{M} + \text{H}]^+$, 496.0. HPLC purity: 97.3% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 30 : 70).

General procedure D for the synthesis of compounds 4a–4l

D1: to a solution of N^6 -propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (1 equiv.) in methanol was added methyl 4-formylbenzoate (1 equiv.). The mixture was refluxed for about 14–16 h. The progress of the reaction was monitored by TLC. After completion of the reaction, methanol was evaporated under vacuum. The residue was washed with hexane for the removal of traces of nonpolar impurities to get the imine, methyl 4-(((6-(propylamino)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)imino)methyl)benzoate (4). To the solution of compound 4 in DCM:MeOH (1:1), NaBH_3CN was added slowly with vigorous shaking. A catalytic amount of glacial

acetic acid was added to the reaction mixture to maintain the reaction pH of 6–7. The mixture was stirred at room temperature for 3–4 h. The progress of the reaction was monitored by TLC, and after completion of the reaction, the reaction solvents were evaporated. The residue obtained was washed with a sodium bicarbonate solution. The precipitate of the desired product was filtered, washed with diethyl ether and then dried to obtain methyl 4-(((6-(propylamino)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)amino)methyl) benzoate (5). A dilute solution of substituted phenyl isocyanates (in DCM) was added dropwise to a solution of compound 5 in DMF under a nitrogen atmosphere. The mixture was stirred at room temperature for 1.5–2 h. After completion of the reaction, the low boiling point solvent was evaporated under vacuum and the reaction mixture was poured into ice cold water to give the precipitate of the desired product (4a–4f). The resultant solid was filtered through a high vacuum suction pump, dried and purified by column chromatography on silica gel.

D2: the compound in the above step was stirred at room temperature with lithium hydroxide solution in a THF–water mixture for 3–4 h to hydrolyze the ester. THF was removed under vacuum, and cold water was added to this mixture which was acidified with dilute hydrochloric acid to precipitate the acid. The acid was filtered and dried. The compound was again washed with diethyl ether to get the pure white product (4g–4l) (Scheme 4).

Methyl 4-(((6-(3-(2-fluorophenyl)-1-propylureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)amino)methyl)benzoate (4a). Compound 4a was synthesized according to general procedure D1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 2 : 8 (v : v) as the eluent, to furnish 4a as a white solid. Yield: 61%, mp 92–94 °C. IR (film) 3467, 3207, 2959, 2875, 1715, 1667, 1614, 1530, 1453, 1280, 754 cm^{-1} . ^1H NMR (DMSO- d_6) δ : 0.85–0.88 (t, $J = 7.25$ Hz, 3H), 1.56–1.58 (m, 2H), 1.81–1.83 (m, 1H), 1.97–2.01 (m, 1H), 2.54–2.55 (m, 2H), 2.58–2.61 (dd, $J = 13.5$ Hz, 1H), 2.69–2.74 (dd, $J = 12.25$ Hz, 1H), 3.18–3.21 (dd, $J = 6.25, 9.25$ Hz, 2H), 3.84 (s, 3H), 4.30–4.31 (m, 1H), 4.48–4.49 (d, $J = 5.5$ Hz, 2H), 7.10–7.11 (m, 2H), 7.16–7.18 (m, 1H), 7.42–7.46 (m, 3H), 7.92–7.96 (m, 3H), 8.00 (s, 1H). ^{13}C NMR (DMSO- d_6) δ : 11.2, 23.2, 26.3, 26.6, 27.5, 44.2, 46.9, 52.0, 52.4, 113.0, 115.4, 123.9, 124.9, 126.5, 127.3 (2C), 128.2, 129.2 (2C), 144.2, 145.3, 154.6, 154.9, 156.5, 166.1, 166.5. MS (ESI): $[\text{M} + \text{H}]^+$, 497.2. HPLC purity: 99.4% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 65 : 35).

Methyl 4-(((6-(3-(3-fluorophenyl)-1-propylureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)amino)methyl)benzoate (4b). Compound 4b was synthesized according to general procedure D1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 2 : 8 (v : v) as the eluent, to furnish 4b as a white solid. Yield: 67%, mp 79–81 °C. IR (film) 3340, 2957, 2875, 1721, 1642, 1602, 1536, 1438, 1281, 755 cm^{-1} . ^1H NMR (DMSO- d_6) δ : 0.86 (s, 3H), 1.52–1.55 (m, 2H), 1.80–1.82 (m, 1H), 1.98–2.02 (m, 1H), 2.54–2.55 (m, 2H), 2.59–2.61 (dd, 1H), 2.71–2.73 (dd, $J = 11.0$ Hz, 1H), 3.20–3.22 (dd, 2H), 3.84 (s, 3H), 4.32 (m, 1H), 4.49 (s, 2H), 6.73 (m,

1H), 7.24–7.25 (m, 2H), 7.44–7.46 (m, 3H), 7.92–7.94 (m, 3H), 8.43 (s, 1H). ^{13}C NMR (DMSO- d_6) δ : 11.3, 23.2, 26.3, 26.7, 27.6, 44.0, 47.1, 52.2, 52.4, 106.7, 107.9, 113.1, 115.6, 127.4 (2C), 128.3, 129.3 (2C), 142.6, 144.3, 145.4, 154.7, 161.3, 163.2, 166.3, 166.7. MS (ESI): $[\text{M} + \text{H}]^+$, 497.2. HPLC purity: 98.0% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 65:35).

Methyl 4-(((6-(3-(4-fluorophenyl)-1-propylureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)amino)methyl)benzoate (4c). Compound 4c was synthesized according to general procedure D1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 2:8 (v:v) as the eluent, to furnish 4c as a white solid. Yield: 58%, mp 84–85 °C. IR (film) 3404, 2957, 2875, 1721, 1639, 1612, 1536, 1414, 1282, 755 cm^{-1} . ^1H NMR (DMSO- d_6) δ : 0.86 (s, 3H), 1.52–1.55 (m, 2H), 1.80–1.82 (m, 1H), 1.98–2.02 (m, 1H), 2.54–2.55 (m, 2H), 2.59–2.61 (dd, 1H), 2.69–2.71 (dd, 1H), 3.18–3.20 (dd, 2H), 3.84 (s, 3H), 4.31 (m, 1H), 4.48 (s, 2H), 7.04–7.06 (m, 2H), 7.43–7.45 (m, 4H), 7.92–7.93 (m, 3H), 8.26 (s, 1H). ^{13}C NMR (DMSO- d_6) δ : 11.3, 23.3, 26.3, 26.8, 27.7, 44.0, 47.1, 52.2, 52.3, 113.2, 114.6, 122.2 (2C), 127.4 (2C), 128.3, 129.3 (2C), 136.9, 144.3, 145.4, 155.1, 156.6, 158.5, 166.3, 166.7. MS (ESI): $[\text{M} + \text{H}]^+$, 497.4. HPLC purity: 98.4% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 65:35).

Ethyl 3-(3-(2-((4-(methoxycarbonyl)benzyl)amino)-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)-3-propylureido)benzoate (4d). Compound 4d was synthesized according to general procedure D1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 2.5:7.5 (v:v) as the eluent, to furnish 4d as a white solid. Yield: 68%, mp 138–140 °C. IR (film) 3419, 2960, 2934, 1717, 1665, 1540, 1281, 754 cm^{-1} . ^1H NMR (DMSO- d_6) δ : 0.85–0.88 (t, $J = 7.25$ Hz, 3H), 1.30–1.33 (t, $J = 7.0$ Hz, 3H), 1.52–1.58 (m, 2H), 1.81–1.83 (m, 1H), 1.99–2.03 (m, 1H), 2.54–2.56 (m, 2H), 2.59–2.63 (dd, $J = 5.0, 15.0$ Hz, 1H), 2.69–2.74 (dd, $J = 12.75$ Hz, 1H), 3.21–3.22 (dd, 2H), 3.84 (s, 3H), 4.29–4.33 (q, $J = 7.0$ Hz, 2H), 4.34–4.35 (m, 1H), 4.48–4.49 (d, $J = 5.5$ Hz, 2H), 7.34–7.38 (m, 1H), 7.44–7.46 (m, 2H), 7.52–7.54 (m, 1H), 7.79–7.80 (m, 1H), 7.92–7.96 (m, 3H), 8.09 (s, 1H), 8.50 (s, 1H). ^{13}C NMR (DMSO- d_6) δ : 11.1, 14.1, 23.1, 26.2, 26.6, 27.5, 43.9, 46.9, 51.9, 52.2, 60.5, 112.9, 120.4, 122.2, 124.4, 127.2 (2C), 128.1, 128.4, 129.1 (2C), 129.8, 141.0, 144.2, 145.3, 154.6, 165.8, 166.0, 166.4. MS (ESI): $[\text{M} + \text{H}]^+$, 551.2. HPLC purity: 99.6% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 70:30).

Ethyl 4-(3-(2-((4-(methoxycarbonyl)benzyl)amino)-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)-3-propylureido)benzoate (4e). Compound 4e was synthesized according to general procedure D1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 2.5:7.5 (v:v) as the eluent, to furnish 4e as a white solid. Yield: 59%, mp 143–145 °C. IR (film) 3429, 2961, 2933, 1713, 1642, 1526, 1280, 770 cm^{-1} . ^1H NMR (DMSO- d_6) δ : 0.85–0.88 (t, $J = 7.25$ Hz, 3H), 1.30–1.33 (t, $J = 7.0$ Hz, 3H), 1.52–1.58 (m, 2H), 1.80–1.82 (m, 1H), 1.99–2.03 (m, 1H), 2.54–2.56 (m, 2H), 2.73 (dd, 1H), 2.89 (dd, 1H), 3.21–3.22 (dd, 2H), 3.84 (s, 3H), 4.25–4.27 (q, 2H), 4.28–4.29 (m, 1H), 4.48–4.49 (d, 2H), 7.45–7.47 (m,

2H), 7.62–7.64 (m, 2H), 7.81–7.83 (m, 2H), 7.92–7.95 (m, 3H), 8.62 (s, 1H). ^{13}C NMR (DMSO- d_6) δ : 11.1, 14.2, 23.1, 26.2, 26.6, 27.5, 43.9, 46.9, 51.9, 52.4, 60.1, 111.6, 118.7 (2C), 122.4, 127.2 (2C), 128.1, 129.1 (2C), 129.7 (2C), 144.1, 145.3, 145.4, 154.2, 165.5, 166.0, 166.4. MS (ESI): $[\text{M} + \text{H}]^+$, 551.2. HPLC purity: 99.2% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 65:35).

Methyl 4-(((6-(3-(4-methoxyphenyl)-1-propylureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)amino)methyl)benzoate (4f). Compound 4f was synthesized according to general procedure D1. The crude product was purified by column chromatography using ethyl acetate/petroleum ether 2.5:7.5 (v:v) as the eluent, to furnish 4f as a white solid. Yield: 60%, mp 88–90 °C. IR (film) 3402, 2954, 2930, 1721, 1635, 1512, 1282, 755 cm^{-1} . ^1H NMR (DMSO- d_6) δ : 0.84–0.87 (t, $J = 6.5$ Hz, 3H), 1.51–1.55 (m, 2H), 1.78–1.81 (m, 1H), 1.96–1.99 (m, 1H), 2.53–2.55 (m, 2H), 2.59–2.64 (dd, 1H), 2.67–2.72 (dd, $J = 12.0$ Hz, 1H), 3.17 (dd, 2H), 3.69 (s, 3H), 3.84 (s, 3H), 4.31–4.33 (m, 1H), 4.48–4.49 (d, $J = 4.5$ Hz, 2H), 6.79–6.81 (m, 2H), 7.31–7.32 (m, 2H), 7.44–7.46 (m, 2H), 7.92–7.93 (m, 3H), 8.07 (s, 1H). ^{13}C NMR (DMSO- d_6) δ : 11.2, 23.3, 26.3, 26.7, 27.6, 43.8, 46.9, 51.9, 52.0, 55.0, 113.1, 113.3 (2C), 122.2 (2C), 127.2 (2C), 128.1, 128.9 (2C), 133.5, 144.2, 145.3, 154.4, 155.0, 166.0, 166.4. MS (ESI): $[\text{M} + \text{H}]^+$, 509.2. HPLC purity: 97.6% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 65:35).

4-(((6-(3-(2-Fluorophenyl)-1-propylureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)amino)methyl)benzoic acid (4g). Compound 4g was synthesized according to general procedure D2. The product obtained was washed with diethyl ether to furnish a pure white solid. Yield: 65%, mp 186–188 °C. IR (film) 3460, 2964, 2932, 1640, 1614, 1528, 1256, 755 cm^{-1} . ^1H NMR (DMSO- d_6) δ : 0.86–0.88 (t, $J = 7.25$ Hz, 3H), 1.57–1.59 (m, 2H), 1.83–1.85 (m, 1H), 1.98–2.06 (m, 1H), 2.58–2.59 (m, 2H), 2.63–2.64 (dd, $J = 4.5$ Hz, 1H), 2.70–2.75 (dd, $J = 12.75$ Hz, 1H), 3.19–3.22 (dd, $J = 7.5$ Hz, 2H), 4.29–4.30 (m, 1H), 4.54 (s, 2H), 7.10–7.12 (m, 2H), 7.15–7.19 (m, 1H), 7.40–7.45 (m, 3H), 7.91–7.93 (m, 2H), 8.01 (s, 1H), 8.65 (bs, 1H). ^{13}C NMR (DMSO- d_6) δ : 11.2, 23.2, 25.3, 26.5, 27.0, 44.3, 47.4, 52.2, 113.2, 115.3, 115.4, 123.9, 125.0, 126.7, 127.3 (2C), 129.5 (2C), 140.9, 143.6, 154.7, 154.9, 156.6, 166.9, 167.2. MS (ESI): $[\text{M} + \text{H}]^+$, 483.6. HPLC purity: 99.6% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 40:60).

4-(((6-(3-(3-Fluorophenyl)-1-propylureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)amino)methyl)benzoic acid (4h). Compound 4h was synthesized according to general procedure D2. The product obtained was washed with diethyl ether to furnish a pure white solid. Yield: 68%, mp 177–179 °C. IR (film) 3404, 2964, 2933, 1641, 1603, 1536, 1278, 773 cm^{-1} . ^1H NMR (DMSO- d_6) δ : 0.84–0.87 (t, $J = 7.0$ Hz, 3H), 1.52–1.53 (m, 2H), 1.80–1.82 (m, 1H), 2.00–2.02 (m, 1H), 2.54–2.55 (m, 2H), 2.60–2.61 (dd, 1H), 2.69–2.74 (dd, $J = 12.25$ Hz, 1H), 3.20–3.22 (dd, 2H), 4.32–4.33 (m, 1H), 4.47 (s, 2H), 6.72–6.73 (m, 1H), 7.23–7.25 (m, 2H), 7.40–7.45 (m, 3H), 7.89–7.94 (m, 3H), 8.44 (s, 1H). ^{13}C NMR (DMSO- d_6) δ : 11.2, 23.1, 26.2, 26.7, 27.5, 43.9, 47.0, 52.3, 106.3, 106.5, 107.7,

112.9, 115.4, 127.0 (2C), 129.3 (2C), 142.6, 144.2, 144.4, 154.5, 161.1, 163.0, 166.5, 167.7. MS (ESI): $[M + H]^+$, 483.3. HPLC purity: 98.7% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 40:60).

4-(((6-(3-(4-Fluorophenyl)-1-propylureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)amino)methyl)benzoic acid (4i). Compound 4i was synthesized according to general procedure D2. The product obtained was washed with diethyl ether to furnish a pure white solid. Yield: 52%, mp 220–222 °C. IR (film) 3411, 2965, 2934, 1632, 1613, 1535, 1212, 756 cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$) δ : 0.84–0.87 (t, $J = 7.25$ Hz, 3H), 1.52–1.55 (m, 2H), 1.80–1.82 (m, 1H), 1.96–2.04 (m, 1H), 2.56–2.57 (m, 2H), 2.60–2.61 (dd, $J = 4.0$ Hz, 1H), 2.69–2.74 (dd, $J = 12.25$ Hz, 1H), 3.19 (dd, 2H), 4.31–4.33 (m, 1H), 4.49 (s, 2H), 7.04–7.07 (m, 2H), 7.43–7.46 (m, 4H), 7.90–7.92 (m, 2H), 8.12 (bs, 1H), 8.28 (s, 1H). ^{13}C NMR ($\text{DMSO}-d_6$) δ : 11.2, 23.2, 26.0, 26.7, 27.5, 43.9, 47.1, 52.2, 113.1, 114.5, 114.7, 122.1 (2C), 127.2 (2C), 129.4 (2C), 136.8, 143.4, 144.5, 154.9, 156.5, 158.4, 166.6, 167.3. ^{19}F NMR (500 MHz, $\text{DMSO}-d_6$) δ (^1H decoupled): –121.77 (s); δ (^1H coupled): –121.80–121.74 (m). MS (ESI): $[M + H]^+$, 483.2. HPLC purity: 99.2% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 40:60).

3-(3-(2-(((4-Carboxybenzyl)amino)-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)-3-propylureido)benzoic acid (4j). Compound 4j was synthesized according to general procedure D2. The product obtained was washed with diethyl ether to furnish a pure white solid. Yield: 63%, mp 177–178 °C. IR (film) 3393, 2965, 2934, 1699, 1627, 1537, 1237, 760 cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$) δ : 0.85–0.88 (t, $J = 7.25$ Hz, 3H), 1.52–1.56 (m, 2H), 1.81–1.83 (m, 1H), 1.99–2.03 (m, 1H), 2.54–2.56 (m, 2H), 2.59–2.63 (dd, $J = 5.0, 10.0$ Hz, 1H), 2.69–2.72 (dd, $J = 11.5$ Hz, 1H), 3.21–3.23 (dd, $J = 7.0$ Hz, 2H), 4.34–4.35 (m, 1H), 4.47–4.48 (d, $J = 5.5$ Hz, 2H), 7.31–7.34 (m, 1H), 7.41–7.43 (dd, $J = 8.0$ Hz, 2H), 7.50–7.51 (m, 1H), 7.75–7.76 (m, 1H), 7.89–7.91 (dd, $J = 8.0$ Hz, 2H), 7.92–7.94 (dd, $J = 6.0$ Hz, 1H), 8.07 (s, 1H), 8.45 (s, 1H). ^{13}C NMR ($\text{DMSO}-d_6$) δ : 11.2, 23.2, 26.3, 26.7, 27.6, 43.9, 47.0, 52.3, 113.0, 120.9, 122.5, 124.1, 127.1 (2C), 128.3, 129.4 (2C), 129.7, 131.0, 140.9, 144.2, 144.6, 154.7, 166.5, 167.3, 167.5. MS (ESI): $[M + H]^+$, 509.2. HPLC purity: 97.6% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 45:55).

4-(3-(2-(((4-Carboxybenzyl)amino)-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)-3-propylureido)benzoic acid (4k). Compound 4k was synthesized according to general procedure D2. The product obtained was washed with diethyl ether to furnish a pure white solid. Yield: 55%, mp 181–183 °C. IR (film) 3375, 2966, 2935, 1696, 1615, 1521, 1240, 773 cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$) δ : 0.85–0.88 (t, $J = 7.25$ Hz, 3H), 1.54–1.61 (m, 2H), 1.82–1.84 (m, 1H), 2.01–2.03 (m, 1H), 2.56–2.58 (m, 2H), 2.63 (dd, 1H), 2.72–2.74 (dd, 1H), 3.21–3.22 (dd, 2H), 4.33–4.34 (m, 1H), 4.50 (s, 2H), 7.44 (dd, 2H), 7.59 (dd, 2H), 7.80 (dd, 2H), 7.91 (m, 3H), 8.59 (s, 1H). ^{13}C NMR ($\text{DMSO}-d_6$) δ : 11.2, 23.1, 25.8, 26.6, 27.3, 44.1, 47.2, 52.4, 113.1, 117.3, 118.8 (2C), 123.4, 127.2 (2C), 129.4 (2C), 129.9 (2C), 142.8, 144.3, 145.1, 154.4, 166.7, 167.1, 167.2. MS (ESI): $[M + H]^+$, 509.4. HPLC

purity: 96.0% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 30:70).

4-(((6-(3-(4-Methoxyphenyl)-1-propylureido)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)amino)methyl)benzoic acid (4l). Compound 4l was synthesized according to general procedure D2. The product obtained was washed with diethyl ether to furnish a pure white solid. Yield: 59%, mp 185–187 °C. IR (film) 3415, 2962, 2935, 1704, 1613, 1512, 1236, 757 cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$) δ : 0.86 (s, 3H), 1.52–1.55 (m, 2H), 1.81–1.83 (m, 1H), 1.99–2.00 (m, 1H), 2.58–2.69 (m, 4H), 3.17–3.19 (dd, 2H), 3.69 (s, 3H), 3.84 (s, 3H), 4.30–4.32 (m, 1H), 4.53 (s, 2H), 6.79–6.81 (m, 2H), 7.31–7.32 (m, 2H), 7.44–7.46 (m, 2H), 7.92–7.93 (m, 2H), 8.08 (s, 1H), 8.50 (bs, 1H). ^{13}C NMR ($\text{DMSO}-d_6$) δ : 11.2, 23.3, 25.5, 26.6, 27.2, 43.9, 47.3, 51.9, 55.1, 113.2, 113.3 (2C), 122.3 (2C), 127.3 (2C), 129.5 (2C), 129.6, 133.4, 141.6, 143.8, 154.5, 155.1, 166.8, 167.2. MS (ESI): $[M + H]^+$, 495.4. HPLC purity: 96.2% (C-18 reverse phase, MeCN-phosphate buffer (NaH_2PO_4) pH 4, 40:60).

CK2 and GSK3 β kinase inhibition assays

Kinase inhibition assays were performed using Invitrogen's Z'-LYTE™ Kinase Assay Kits – Ser/Thr 11 Peptide (Cat. No. PV3670) and Ser/Thr 9 Peptide (Cat. no. PV3324) for CK2 and GSK3 β , respectively. The substrates for the assay of the CK2 α recombinant human protein (Cat. no. PV3248) and GSK3 β recombinant human protein (Cat. no. PV3365) were procured from Invitrogen. 4,5,6,7-Tetrabromobenzotriazole (TBB) and *N*-(4-methoxybenzyl)-*N'*-(5-nitro-1,3-thiazol-2-yl)urea (AR-A014418) were used as the standard inhibitor (or positive control) of CK2 and GSK3 β , respectively. This assay employs a FRET-based, coupled-enzyme format and is based on the differential sensitivity of phosphorylated and non-phosphorylated peptides to proteolytic cleavage. The assay uses a synthetic peptide substrate (the Z'-LYTE™ peptide substrate) that is labeled with a donor fluorophore (coumarin) and an acceptor fluorophore (fluorescein) that make up the FRET pair. The assay uses a ratiometric method to quantify kinase reactions by calculating the ratio of donor emissions to acceptor emissions after excitation of the donor fluorophore at 400 nm. Three assay controls were used along with kinase reactions, the 0% phosphorylation control, 100% phosphorylation control, and 0% inhibition control. The first two controls establish the minimal and maximal emission ratio values, used to calculate the percent phosphorylation achieved in a specific reaction well and 0% inhibition control wells. The first and third controls define the dynamic range in a given assay. Control wells contain no kinase inhibitor. All dilutions were made in kinase buffer A provided with the kits. The final 1 $\times$ concentration of Kinase Buffer A contains 50 mM HEPES (pH 7.5), 1 mM EGTA, 0.01% Brij-35, and 10 mM MgCl_2 . In the kinase reaction, the final 10 μL reaction volume contains 2.5 μL 4 $\times$ test compound (in 4% DMSO), 5 μL Kinase/peptide mixture (1:1) and 2.5 μL 4 $\times$ ATP solution and the reaction was incubated for 1 hour at 20–25 °C. Then, 5 μL of the development reagent A (a site-specific protease)

was added at an optimized concentration, and the assay plate was incubated for another 1 hour at the same temperature. In the next step, 5 μ L of the stop reagent was added only for the GSK3 β assay. Further, the coumarin (ex. 400 nm, em. 445 nm) and fluorescein (ex. 400 nm, em. 520 nm) emission signals were measured on a fluorescence plate reader (Thermo Scientific Varioskan Flash) for both assays. Percent phosphorylation and percent inhibition were calculated using the following equation:

Percent phosphorylation

$$= 1 - \left(\frac{(\text{emission ratio} \times F_{100\%}) - C_{100\%}}{(C_{0\%} - C_{100\%}) + [\text{emission ratio} \times (F_{100\%} - F_{0\%})]} \right) \times 100\%$$

where: emission ratio = ratio of coumarin/fluorescein emission signal intensities from sample wells. $C_{100\%}$ = average coumarin emission signal intensity of the 100% phosphorylation control. $C_{0\%}$ = average coumarin emission signal intensity of the 0% phosphorylation control. $F_{100\%}$ = average fluorescein emission signal intensity of the 100% phosphorylation control. $F_{0\%}$ = average fluorescein emission signal intensity of the 0% phosphorylation control.

Percent inhibition

$$= 100 \times \left(1 - \left(\frac{\text{Percent phosphorylation of test compound well}}{\text{Percent phosphorylation of 0\% inhibition control}} \right) \right)$$

We have performed *in vitro* kinase inhibition assays in two steps. During the primary screening, we have screened all the compounds at a concentration of 11 μ M. Compounds that showed more than thirty percent inhibition of both kinases at this concentration were selected further for the secondary screening (IC₅₀ determination).

Conflicts of interest

The authors declare that they have no competing interests.

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