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Discovery of 2-(1*H*-indol-5-ylamino)-6-(2,4-difluorophenylsulfonyl)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7ao) as a potent selective inhibitor of Polo like kinase 2 (PLK2)

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Abstract

Several families of protein kinases have been shown to play a critical role in the regulation of cell cycle progression, particularly progression through mitosis. These kinase families include the Aurora kinases, the Mps1 gene product and the Polo Like family of protein kinases (PLKs). The PLK family consists of five members and of these, the role of PLK1 in human cancer is well documented. PLK2 (SNK), which is highly homologous to PLK1, has been shown to play a critical role in centriole duplication and is also believed to play a regulatory role in the survival pathway by physically stabilizing the TSC1/2 complex in tumor cells under hypoxic conditions. As a part of our research program, we have developed a library of novel ATP mimetic chemotypes that are cytotoxic against a panel of cancer cell lines. We show that one of these chemotypes, the 6-arylsulfonyl pyridopyrimidinones, induces apoptosis of human tumor cell lines in nanomolar concentrations. The most potent of these compounds, **7ao**, was found to be a highly specific inhibitor of PLK2 when profiled against a panel of 288 wild type, 55 mutant and 12 lipid kinases. Here, we describe the synthesis, structure activity relationship, in vitro kinase specificity and biological activity of the lead compound, **7ao**.

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Supplementary data

Kinase inhibition profile of **7ao** (Reaction Biology Corp) and Kinome map for **7ao**. This material is available free of charge via the Internet at http://sciencedirect.com. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.11.045.

Keywords

PLK; Kinase inhibitor; Apoptosis; Cytotoxicity

1. Introduction

In 2006, Weinstein and Joe[1] proposed that cancer cells contain multiple genetic and epigenetic abnormalities and that despite this complexity, their growth and survival can often be impaired by the inactivation of a single oncogene. They proposed that this phenomenon, called 'oncogene addiction,' provides a rationale for molecular targeted therapy. The success of imatinib provides strong experimental support for the 'Oncogene Addiction' theory[2] and has also created a great impetus for the discovery of putative 'addictive kinases' in various tumor model systems.

Since growth of most human tumors is dictated by the activation of kinase cascades, we designed an approach in which we combined the identification of 'addictive kinases' with drug discovery. To enable us to identify possible oncogenic kinases to which a tumor cell might be addicted, we synthesized a large number (approximately 300) of novel ATP mimetics that can act as kinase inhibitors and screened this compound library for compounds that can preferentially induce the death of human tumor cells without affecting normal cell viability. Our approach was to first identify a compound that is effective in tumor cell killing and then determine the identities of the kinases that are inhibited by this compound. This approach allowed two important aspects of drug discovery to occur simultaneously: the development of novel pharmaceuticals and the identification of new therapeutic targets. Here we report the synthesis of **7ao**, which blocks tumor cell cycle progression in mitosis, causing apoptotic cell death and also inhibits PLK2 selectively.

There are five mammalian Polo kinases, termed Polo-like kinase (*Plk*) 1–5 which share two 30 amino acid conserved motifs, termed polo boxes, as well as additional residues in the kinase domain.[3] PLKs play a critical role in cell cycle progression and are known to mediate various stages of mitosis including kinetochore division, centriole duplication and cytokinesis.

Within the *PLK* family, *PLK1* is the best-characterized member and is the most closely related to yeast and *Drosophila* polo kinases.[4] *PLK1* is relevant to cancer biologists due to its over-expression in a variety of human tumor types and is linked to poor prognosis.[5,6] *PLK2*, or *Snk*, was identified as an immediate-early gene product and a p53 target gene.[7] PLK2 plays an important role in cell cycle control through the specific phosphorylation of centrosome-associated substrates. Specifically, PLK-2 regulates centriole duplication that occurs at the G_1/S border and is coordinately regulated by CDK2/cyclin E and CDK2/cyclin A complexes, as well as PLK4.[8,9] Studies have shown that inhibiting the expression of this gene by siRNA leads to mitotic catastrophe in paclitaxel-treated cells.[10]

While several pan-PLK inhibitors have been described in literature, no PLK2-specific small molecule inhibitor has been described. Here we report that **7ao (ON1231320)** is a selective inhibitor of PLK2 and blocks tumor cell cycle progression in mitosis, causing apoptotic cell

death. **7ao** synergizes with paclitaxel, and is efficacious in inhibiting tumor growth in vivo suggesting that PLK2 is drug-gable kinase and a new selective target for cancer therapy.

2. Chemistry

The compounds presented in this report were prepared following the general approach as illustrated in Scheme 1, that has been described previously.[11] Pyrimidines **2a**–**g** were prepared from commercially available 4-chloro-2-methylthio-5-pyrimidinecarboxylic acid ethyl ester (**1**) by replacement of chlorine with ammonia or methylamine or ethylamine or propylamine or cyclopropylamine or cyclopentyl amine or cyclohexyl amine. The ester functional group was converted to an aldehyde via a two-step reduction–oxidation sequence employing lithium aluminum hydride followed by manganese(IV) oxide to give aldehydes **4a**–**g**. Aldehydes **4a**–**g** were converted to pyrido[2,3-*d*]-pyrimidin-7-ones **5a**–**z** using Knoevenagel condensation. Thus, each aldehyde was treated with active methylene compounds (arylsulfonylacetic acid or arylmethanesulfonylacetic acid or *N*-arylmalonamic acid or *N*-arylsulfamoylacetic acid) in the presence of benzylamine to generate their corresponding intermediates **5a**–**z**. The methyl sulfides **5a**–**z** were oxidized to methyl sulfoxides **6a**–**z** using *m*-chloroperbenzoic acid (*m*-CPBA). The methyl sulfoxide was then replaced with different aryl/heteroaryl amines, to obtain the target pyridopyrimidine compounds **7** as described in Scheme 1.

The compounds **6aa**–**6ac** were prepared by oxidation of 5**aa**–**5ac** with *m*-CPBA, which in turn was prepared by alkylation of **5u** with corresponding alkyl iodides in the presence of Potassium carbonate in *N*,*N*-Dimethylformamide (DMF) at 50 °C as shown in Scheme 2.

As shown in Scheme 3, the biotin conjugated compound (**7u**) was prepared by treating the compound **7t** with biotin in the presence of EDC and DMAP at room temperature.

The active methylene compounds, arylsulfonylacetic acids (**10a**–**j**), arylmethanesulfonylacetic acids (**13a** and **13b**), *N*-arylmalonamic acids (**16a** and **16b**) and *N*-arylsulfamoylacetic acids (**18a** and **18b**) were prepared as shown in Schemes 4–7 using the procedures reported earlier.[12–15] As shown in Scheme 4, Arylthiols (**8a**–**j**) were treated with chloroacetic acid in the presence of NaOH and subsequent oxidation with 30% H_2O_2 to produce arylsulfanyl acetic acids (**9a**–**j**) and arylsulfonyl acetic acids (**10a**–**j**) respectively.

Arylmethyl bromides (**11a** and **11b**) were treated with thioglycolic acid in the presence of sodium hydroxide to produce corresponding arylmethylsulfanyl acetic acids (**12a** and **12b**). Oxidation of **12a** and **12b** with 30% H_2O_2 in acetic acid yielded the corresponding arylmethanesulfonyl acetic acids (**13a** and **13b**) as reported in Scheme 5.

The reaction of aromatic amines (**14a** and **14b**) with methyl-3-chloro-3-oxopropionate in the presence of triethylamine generated 3-anilino-3-oxopropionic acid methyl esters (**15a** and **15b**), which, when subjected to hydrolysis, resulted in the formation of 3-anilino-3-oxopropionic acids (**16a** and **16b**) as illustrated in Scheme 6.

As depicted in Scheme 7, chlorosulfonylacetic acid ethyl ester was treated with aromatic amines (**14a** and **14b**) in the presence of triethylamine in DCM to obtain the

arylsulfamoylacetic acid ethyl esters (**17a** and **17b**) in good yields. Hydrolysis of **17a** and **17b** with 10% NaOH in water generated the corresponding arylsulfamoylacetic acids (**18a** and **18b**) in high yields.

Methyl arylsulfonyl esters were prepared as shown in Scheme 8. Aromatic nucleophilic substitution of fluorine in 3,4-difluoronitrobenzene (**19**) and 2,5-difluoronitrobenzene (**20**) by methyl 2-mercaptoacetate in the presence of DIPEA produced methyl 2-(2-fluoro-4-nitrophenylthio)acetate (**21**) and methyl (2-(4-fluoro-2-nitrophenylthio)acetate (**22**) respectively.

The thio compounds **21** and **22** were subjected to oxidation with 30% H_2O_2 in acetic acid to generate corresponding sulfonyl compounds, methyl 2-(2-fluoro-4-nitrophenylsulfonyl)acetate (**23**) and methyl (2-(4-fluoro-2-nitrophenylsulfonyl)acetate (**24**). The resulting compounds **23** and **24** were dissolved in methanol and reduced to corresponding amines **25** and **26** using 10% Pd/C in the presence of hydrogen gas.

3. Results and discussion

3.1. Structure–activity relationships (SARs)

The cytotoxic data from the Tables 1–4 shows that the structure activity relationship (SAR) of these pyridopyrimidines (**7a**–**7aar**) is tightly regulated. Slight variation of X (*N*-8 position), Y (*C*-6 position) and Z (*C*-2 position) substitutions in **7a**–**7aar** drastically affected cytotoxic activity of the molecules against tumor cell lines. Initially, when we started the synthesis of these pyridopyrimidines, we kept the methyl group constant at *N*-8 position of **7** and varied the *C*-6 position (Y) with different arylsulfonyl groups and then introduced different aromatic and heteroaryl amines at the Z (*C*-2 position) position of the pyridopyrimidine ring. Cytotoxicity data from Table 1 shows that keeping the arylsulfonyl groups constant at the *C*-6 position and introducing aromatic amines (**7a**–**c**) and heteroaryl amines (**7d**–**7ap**) at the *C*-2 position yielded heteroaryl amino compounds which were more active than the aryl amino substituted derivatives. Among the heterocyclic derivatives of **7d**–**7ap**, bicyclic heterocyclic compounds (**7d**–**7ak** and **7an**–**ap**) were found to be more active than monocyclic heterocyclic compounds (**7al** and **7am**). Of the various bicyclic-heterocycles (**7d**–**7ak** and **7an**–**ap**) tested, amino indoles (**7d**, **7e**, **7h**, **7i**, **7l**–**o**, **7t**–**7x**, **7ac**–**ak**, and **7an**–**ap**) have shown better cytotoxicity compared to other benzo-fused five or six membered heterocyclic compounds (**7f**, **7g**, **7j**, **7k**, **7p**–**s** and **7y**–**7ab**). Among the amino indoles tested for their cytotoxicity, 5-aminoindoles (**7d**, **7i**, **7l**, **7n**, **7t**, **7u**, **7w**, **7ad**–**ak** and **7ao**) showed higher activity compared to 4-amino indoles (**7h**, **7m**, **7v**, **7ac** and **7an**) and 6-amino indoles (**7e**, **7o**, **7x** and **7ap**). These results suggest that 5-amino indole group is essential for the cytotoxic activity of the molecule and replacement of this moiety either by similar analogs (**7f**, **7j**, **7p**–**s**, **7y** and **7z**) or with corresponding 4th and 6th positional isomers (**7e**, **7h**, **7m**, **7o**, **7v**, **7x**, **7ac**, **7an** and **7ap**) results in substantial loss of activity of these compounds. Since 5-amino indole compounds showed best cytotoxic activity compared to the other analogs, this group was fixed at Z-position for subsequent SAR studies.

After optimizing the position of Z in **7** with 5-amino indole, we then focused our efforts on varying the Y position with different substitutions on the aromatic ring of the arylsulfonyl group. The cytotoxicity data (Table 1) indicated that arylsulfonyl ring with different substitutions exhibit moderate to excellent activity. Monosubstituted arylsulfonyl compounds (**7i**, **7l**, **7n**, **7t**, **7u**, **7w** and **7ad**) and di-substituted arylsulfonyl compounds (**7af–ak** and **7ao**) were found to be more active than un-substituted arylsulfonyl compound (**7d**). Among mono-substituted compounds, bromo (**7l**) and carboxy (**7ae**) substituted compounds showed substantially less cytotoxic activity compared to chloro (**7i**), fluoro (**7n**), hydroxyl (**7t**), biotiniloxy (**7u**), methyl (**7w**) and methoxy (**7ad**) substituted compounds. Of the various di-substituted arylsulfonyl compounds, 2,4-dichloro (**7ag**), 2-fluoro-4-nitro (**7aj**) and 2,4-difluoro (**7ao**) compounds showed excellent activity compared to di-substituted 4-fluoro-2-nitro (**7ah**), 2-amino-4-fluoro (**7ai**) and 4-amino-2-fluoro (**7ak**) compounds. Surprisingly reduction of the nitro compounds (**7ah** and **7aj**) to an amino group (**7ai** and **7ak**) resulted in 10 fold less cytotoxic activity towards cancer cells. A comparison of the activities of all of the arylsulfonyl compounds listed in Table 1 showed that the molecule (**7ao**) with 2,4-difluoro arylsulfonyl at Y and 5-amino indole at Z position exhibits the best cytotoxic activity. In our attempts to achieve even more potent molecule than **7ao**, we replaced arylsulfonyl group of the pyridopyrimidine ring with benzylsulfonyl (**7aq** and **7ar**), arylcarbamoyl (**7as** and **7at**) and arylsulfamyl (**7au** and **7av**) (Table 2) groups. All these changes resulted in a drastic loss of biological activity indicating that 2,4-difluoro arylsulfonyl group is indispensable at that position for the cytotoxic activity of these compounds. Similar attempts were made to produce molecules that were more active than **7ao** by introducing other bicyclic-heterocycles (**7aw–7aai**) (Table 3) at Z position of the pyridopyrimidine ring. However, these compounds were found to be substantially less active than **7ao**. Only a few compounds (**7ax**, **7az**, **7aaa** and **7aai**) showed a moderate cytotoxic activity indicating that 5-amino indole is critical for the activity at the Z position and is irreplaceable. Additional efforts were made to produce a more potent molecule than **7ao** by varying the substitutions on the nitrogen at *N*-8 position of the pyridopyrimidine ring (**7aaj–aar**) (Table 4). The cytotoxicity data from Table 4 shows that any modifications at *N*-8 position of the ring other than methyl group results in a severe loss of activity. Only *N*-ethyl substitution (**7aak**) at *N*-8 position showed a moderate cell killing activity, which however, was 10 fold less than **7ao**.

Results from the cytotoxicity data (Tables 1–4) suggested that **7ao** was the best molecule in this class of compounds and hence we performed all subsequent in vitro and in vivo biological studies using this compound.

3.2. Biological results

3.2.1. In vitro anti-tumor activity of 7n, 7t, 7u and 7ao—We next tested the cytotoxicity of the most active compounds in tissue culture growth inhibitory (GI_{50}) assays against a panel of 16 cancer cell lines (Table 5). These cell lines were chosen based upon the tissue of origin and aggressiveness of tumors propagated in xenograft models. This screen revealed four structurally related compounds (**7n**, **7t**, **7u** and **7ao**) that inhibited the growth of all the 16 tumor cell lines tested, at concentrations between 25 and 2500 nM (Table 5). As can be seen from the data, compound **7ao** was the most potent inhibitor of cancer cell

growth followed by compounds **7n**, **7t** and **7u.** We then tested **7ao**, the most active of the four compounds, in an extended panel of tumor cell lines (Table 6) and observed growth inhibition of multiple tumor cell types, suggesting that **7ao** inhibits cell proliferation by blocking one or more key component(s) of growth signaling pathways.

3.2.2. Kinase inhibition profile of 7n and 7ao—To understand the molecular basis for the high potency exhibited by compounds **7n** and **7ao**, we tested in vitro kinase inhibitory activities of these two compounds against a panel of 355 functional protein kinases (Reaction Biology Corp) (see Supporting information Table 1 and Fig. 1 for **7ao**). Interestingly, this kinase profiling study revealed that both compounds are highly specific inhibitors of Polo like kinase 2 (PLK2).[10] We next examined the ability of all of the compounds described in Tables 1–4 to inhibit PLK2 in in vitro kinase assays using recombinant PLK2 kinase. The results of these experiments are shown in Table 7. The kinase assays performed with the three structurally related compounds **7n**, **7t** and **7u** are shown in Figure 1. These studies suggest that inhibition of PLK2 is detrimental for tumor cell proliferation and/or survival. It is also interesting to note that **7ao**, which is the most potent inhibitor of this kinase amongst the compounds tested, also exhibits highest cytotoxicity against tumor cells, suggesting a functional relationship between PLK2 inhibitory activity and cytotoxicity. Further in vitro kinase and differential scanning fluorimetric (DSF)[16,17] testing revealed that **7ao** is a selective inhibitor of PLK2 with no inhibitory activity against PLK1, PLK3 and PLK4 (Fig. 2A and B).

3.2.3. Prediction of 7ao binding to the PLK2 ATP binding pocket—To gain a better understanding of the possible mode of **7ao** binding to the PLK2 kinase domain which permits specificity, molecular docking and energy minimization studies were conducted using the PLK2-1C8 (PDB 4I6H) X-ray co-crystal structure. Prediction of the binding of the inhibitor to the PLK2 kinase domain shows that multiple interactions stabilize the binding of **7ao** to PLK2 (Fig. 3A and B). Positioning of the difluoro phenyl moiety by van der Waals interactions with the side chains of residues Asn210, Phe212, Asp223 and the α-nitrogen atom within the main chain of Asp223 appear to be of high importance. In particular several of these interactions are observed with fluorine at position 2, noting the contribution of this atom for the observed binding. Phe212 is predicted to also contribute to the stabilization of pyridopyrimidine ring. In addition, Nitrogen atom at positions 1 and methyl group at positions 8 of the pyridopyrimidine ring are predicted to interact with Leu88. The carbonyl oxygen atom position 7 may be stabilized by interactions with the side chain of Cys96. The sulfone moiety of **7ao** is predicted to be within a distance that permits van der Waal interactions with the side chains of Lys111 and the PLK2 gatekeeper residue, Leu159. Another main interaction in the form of a hydrogen bond is predicted to form between the nitrogen atom at position 1 of the pyridopyrimidine ring and the nitrogen atom in the main chain of Cys162. A second hydrogen bond is also likely to form between the amino group located between the pyridopyrimidine and the indole ring with the oxygen atom in the main chain of Cys162. Positioning of the amino indole group is mainly governed by interactions with side chains of Leu88, Tyr161 and Arg165. Overall, these predicted interactions result in the optimal positioning of **7ao** to the ATP binding pocket of the PLK2 kinase domain.

Positioning of **7ao** in the ATP binding pocket of the PLK2 kinase domain is dictated by multiple and distinct interactions that permit high affinity and specificity for this member of the PLK family (Fig. 3A and B). In particular, PLK2 Tyr161, which corresponds to PLK1 Leu132, seems to be a major determinant for selectivity within the PLK family. The predicted proximity of Tyr161 to the 5-amino indole moiety of **7ao** will most likely facilitate an edge-to-face interaction, as has been described for another PLK2 ATP-mimetic inhibitor.[18] This type of interaction will not occur with PLK1 (Fig. 3C). Another determinant for selectivity could be due to the higher flexibility of the PLK2 P-loop as compared to that which is seen in PLK-1 co-crystal structures. The position of the amino group at position 5 of the indole moiety in **7ao** also appears to be critical for binding and specificity, since substitution at a different position (i.e., at position 4) may cause a steric clash with the hinge loop in PLK2. At the center of the molecule, the presence of carbonyl oxygen atom and a methyl group at positions 7 and 8 of the pyridopyrimidine ring are predicted to aid in the stabilization of the molecule in the PLK2 ATP binding site. The 2,4-difluoro benzene moiety at the other end of the small molecule fits deep within the binding site and is adequately accommodated between the side chains of three amino acid residues (Asn210, Phe212, Asp223). Other spatial orientations of this moiety do not appear to be favorable. Furthermore, given the predicted interactions of the difluoro benzene, substitution or removal of the highly electronegative fluorides will have a detrimental effect in the binding and activity of the molecule, which was seen with compounds **7d**, **7l**, **7ae**. Finally, in addition to directly interacting with PLK2, the sulfone moiety might participate in electron delocalization from the adjacent difluoro benzene ring. The importance of the functionality of these groups at specific positions of the molecule is denoted by a loss of SAR activity as observed in the kinase and cytotoxicity assays and is supported by the modeling studies.

3.3. 7n and 7ao activate programmed cell death in human tumor cells

The cytotoxicity exhibited by these compounds against cancer cells prompted us to investigate the mechanism by which these compounds induced cell death. We employed a fluorescent/luminescent assay combination (Cell Titer Blue/Caspase Glo-3/7, Promega Corp) that measures both cell viability and Caspase 3/7 activity (a measure of apoptosis) to determine the effects of compounds **7n** and **7ao** on U2OS human osteosarcoma cells following a 24 h exposure (Fig. 4). The results show that compounds **7n** and **7ao** increase the activity of Caspases 3/7 in a dose-dependent manner, suggesting that both compounds induce apoptosis.

3.4. 7ao does not inhibit tubulin polymerization

To test the possibility that these compounds exert their growth inhibitory effect by directly inhibiting tubulin polymerization, we examined their effect on tubulin polymerization in a cell free system. MAP-rich tubulin (Cytoskeleton Inc., Denver, CO) was reconstituted in polymerization buffer and its polymerization kinetics assayed by UV/VIS spectrophotometry at 305 nm. The results of this study (Fig. 5) show that while compounds **7n** and **7t** inhibit tubulin polymerization in a vincristine-like manner (albeit at a 10-fold higher concentration), compound **7u** only shows partial inhibitory activity. In contrast, compound **7ao** did not appreciably inhibit tubulin polymerization, suggesting that this compound is likely to be less

toxic to normal cells which tend to be sensitive to the toxic effects of tubulin depolymerizing agents.

3.5. In vivo antitumor efficacy of 7ao

To determine the efficacy of compound **7ao** in vivo using a tumor xenograft model, MDAMB-231 triple negative breast cancer cells were injected subcutaneously into nude mice. Once the tumors reached an average volume of 70 mm^3, **7ao** was administered on alternate days (Q2D) via intraperitoneal (IP) injection at a dose of 75 mg/kg body weight. This treatment with **7ao** resulted in significant inhibition of tumor growth (86.5%) as compared to the control group (Fig. 6A), and no overt signs of toxicity were observed in the **7ao** treated group (body weights shown in Fig. 6B). The potent in vivo anticancer efficacy of compound **7ao** and its low toxicity profile in mice portend well for its development for potential clinical use.

3.6. Conclusion

To identify possible oncogenic kinases to which tumor cells might be addicted, we screened our compound library of ATP-mimetic kinase inhibitors for candidate compounds that can preferentially induce the death of human tumor cells without affecting normal cell viability. This approach led to the identification of a novel kinase inhibitor, **7ao**, an arylsulfonyl pyrido-pyrimidinone, that specifically inhibits PLK2 isoform (SNK) and **7ao** also causes mitotic catastrophe and apoptosis of cultured tumor cell lines. Here, we describe the synthesis of 6-arylsulfonyl pyridopyrimidinones and their structure/function characteristics, which suggest that the cytotoxic activity of 6-arylsulfonyl pyridopyrimidinones is dependent on the nature and position of substitutions at C-2, C-6 and N-8—positions. Compound **7ao**, with a 5-aminoindolyl group at the C-2 position, 2,4-difluorophenyl group at the C-6 position and methyl group at the N-8 position, showed optimum biological activity. Kinase profiling revealed that **7ao** inhibited PLK2 with a high degree of specificity. **7ao** could not inhibit the kinase activities of PLK1, PLK3, or PLK4 in vitro, even at concentrations as high as 10 μM. Incubation of human tumor cell lines with **7ao** resulted in a dose-dependent increase in Caspase 3/7 activity suggesting that this compound activates programmed cell death in human tumor cells. In tubulin polymerization assays, **7ao** did not inhibit tubulin polymerization, indicating that this compound's mechanism of action is distinct from tubulin poisons such as paclitaxel and vincristine. **7ao** is well tolerated in mice, and reduces tumor burden in mice carrying subcutaneous and orthotopic xenografts of human tumor cells. Together, these studies demonstrate that PLK2 may be an important cancer target and suggest that its inhibitors can be used in cancer therapy.

4. Experimental section

4.1. Chemistry

All reagents and solvents were obtained from commercial suppliers and used without further purification unless otherwise stated. Solvents were dried using standard procedures and reactions requiring anhydrous conditions were performed under N_2 atmosphere. Reactions were monitored by Thin Layer Chromatography (TLC) on preloaded silica gel F254 plates (Sigma–Aldrich) with a UV indicator. Column chromatography was performed using Merck

70–230 mesh silica gel 60 Å. Yields were of purified product and were not optimized. Melting points were determined using an Electro thermal Mel-Temp 3.0 micro melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained using a Bruker AVANCE 300, 400 and 600 MHz spectrometer. Chemical shifts are reported in parts per million (δ) downfield using tetramethylsilane ($SiMe_4$) as internal standard. Spin multiplicities are given as s (singlet), d (doublet), t (triplet), dd (double doublet), br s (broad singlet), m (multiplet), and q (quartet). All LC/MS data were gathered on an Agilent 1200 LC with Agilent 6410 triple quadrupole mass spectrometer detectors. The compound solution was infused into the electrospray ionization source operating positive and negative modes in methanol/water/TFA (50:50:0.1% v/v) at 0.4 mL/min. The sample cone (declustering) voltage was set at 100 V. The instrument was externally calibrated for the mass range m/z 100 to m/z 1000. The purity of the final compounds was determined by HPLC and is 95% or higher unless specified otherwise. ZorbaxExlipse XDB C18 (150 × 4.6 mm, 5 μm particle size) using gradient elution of acetonitrile in water, 20–90% for 25 min at a flow rate of 1 mL/min with detection at 235 nm wavelength. For all samples 0.00154% $AcONH_{-4}$ was added to water. The aldehydes (**4a**–**g**),[11] and the active methylene compounds **10**,[12] **13**,[13] **16**,[14] and **18**[15] were prepared as per the reported procedures.

4.1.1. General procedure for the preparation of 2-(arylthio) acetic acid (9) (Scheme 4)—

The following 2-(arylthio)acetic acids were prepared according to the procedure reported in the literature.[12]

4.1.1.1. 2-(Phenylthio)acetic acid (9a): Alkylation of thiophenol with chloroacetic acid yielded 65% 2-(phenylthio)acetic acid (**9a**); mp 59–61 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.58 (s, 2H), 7.08–7.15 (m, 3H), 7.30 (d, J = 8.1 Hz, 2H), 11.89 (br s, 1H). MS found (M +H)+ (m/z), 169.03; calcd for $C_8H_8O_2S$ m/z, 168.02.

4.1.1.2. 2-((4-Fluorophenyl)thio)acetic acid (9b): Alkylation of 4-fluorothiophenol with chloroacetic acid yielded 63% 2-((4-fluorophenyl) thio)acetic acid (**9b**); mp 78–80 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.46 (s, 2H), 6.68–6.73 (m, 2H), 6.89–6.93 (m, 2H), 12.20 (br s, 1H). MS found (M+H)+ (m/z), 187.03; calcd for C_8H_7-FO_2S m/z, 186.02.

4.1.1.3. 2-((4-Chlorophenyl)thio)acetic acid (9c): Alkylation of 4-chlorothiophenol with chloroacetic acid yielded 63% 2-((4-chlorophenyl)thio)acetic acid (**9c**); mp 96–99 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.44 (s, 2H), 7.52 (d, J = 8.4 Hz, 2H), 7.77 (d, J = 8.2 Hz, 2H), 11.96 (br s, 1H). MS found (M+H)+ (m/z), 202.98; calcd for $C_8H_7ClO_2S$ m/z, 201.99.

4.1.1.4. 2-((4-Bromophenyl)thio)acetic acid (9d): Alkylation of 4-bromothiophenol with chloroacetic acid yielded 67% 2-((4-bromophenyl)thio)acetic acid (**9d**); mp 113–115 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.57 (s, 2H), 7.48 (d, J = 8.0 Hz, 2H), 7.65 (d, J = 8.1 Hz, 2H), 12.45 (br s, 1H). MS found (M+H)+ (m/z), 246.97; calcd for $C_8H_7BrO_2S$ m/z, 245.94.

4.1.1.5. 2-((4-Hydroxyphenyl)thio)acetic acid (9e): Alkylation of 4-mercaptophenol with chloroacetic acid yielded 55% 2-((4-hydroxyphenyl)thio)acetic acid (**9e**); mp 132–136 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.51 (s, 2H), 4.35 (br s, 1H), 6.72 (d, J = 8.1 Hz, 2H),

7.23 (d, J = 8.1 Hz, 2H), 12.07 (br s, 1H). MS found (M+H)+ (m/z), 185.03; calcd for $C_8H_8O_3S$ m/z, 184.02.

4.1.1.6. 2-(p-Tolylthio)acetic acid (9f): Alkylation of 4-methylthiophenol with chloroacetic acid yielded 65% 2-((*p*-tolylthio) acetic acid (**9f**); mp 77–78 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.53 (s, 3H), 3.47 (s, 2H), 7.38 (d, J = 8.4 Hz, 2H), 7.51 (d, J = 8.2 Hz, 2H), 12.44 (br s, 1H). MS found (M+H)+ (m/z), 183.04; calcd for $C_9H_{10}O_2S$ m/z, 182.04.

4.1.1.7. 2-((4-Methoxyphenyl)thio)acetic acid (9g): Alkylation of 4-methoxythiophenol with chloroacetic acid yielded 64% 2-((4-methoxyphenyl)thio)acetic acid (**9g**); mp 68–70 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.57 (s, 2H), 3.96 (s, 3H), 7.31 (d, J = 7.8 Hz, 2H), 7.47 (d, J = 7.8 Hz, 2H), 12.70 (br s, 1H). MS found (M+H)+ (m/z), 199.06; calcd for $C_9H_{10}O_3S$ m/z, 198.04.

4.1.1.8. 2-((4-Carboxyphenyl)thio)acetic acid (9h): Alkylation of 4-mercaptobenzoic acid with chloroacetic acid yielded 60% 2-((4-carboxyphenyl)thio)acetic acid (**9h**); mp 274–276 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.68 (s, 2H), 7.48 (d, J = 8.0 Hz, 2H), 7.92 (d, J = 8.0 Hz, 2H), 12.89 (br s, 2H). MS found (M+H)+ (m/z), 213.04; calcd for $C_9H_8O_4S$ m/z, 212.01.

4.1.1.9. 2-((2,4-Difluorophenyl)thio)acetic acid (9i): Alkylation of 2,4-difluorothiophenol with chloroacetic acid yielded 60% 2-((2,4-difluorophenyl)thio)acetic acid (**9i**); mp 78–80 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.57 (s, 2H), 6.86–6.87 (m, 2H), 7.49–7.50 (m, 1H). MS found (M+H)+ (m/z), 205.10; calcd for $C_8H_6F_2O_2S$ m/z, 204.01.

4.1.1.10. 2-((2,4-Dichlorophenyl)thio)acetic acid (9j): Alkylation of 2,4-dichlorothiophenol with chloroacetic acid yielded 60% 2-((2,4-dichlorophenyl)thio)acetic acid (**9j**); mp 110–112 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.68 (s, 2H), 7.19–7.21 (dd, J = 8.4 and 2.4 Hz, 1H), 7.34 (d, J = 8.4 Hz, 1H), 7.42 (d, J = 2.4 Hz, 1H). MS found (M+H)+ (m/z), 236.95; calcd for $C_8H_6Cl_2O_2S$ m/z, 235.95.

4.1.2. General procedure for the preparation of 2-(arylsulfonyl) acetic acid (10) (Scheme 4)—The following 2-(arylsulfonyl)acetic acids were prepared according to the procedure reported in the literature.[12]

4.1.2.1. 2-(Phenylsulfonyl)acetic acid (10a): Oxidation of 2-(phenylthio)acetic acid **9a** with 30% hydrogen peroxide yielded 83% of corresponding 2-(phenylsulfonyl)acetic acid (**10a**); mp 108–110 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.38 (s, 2H), 7.64–7.72 (m, 3H), 7.89 (d, J = 7.8 Hz, 2H), 12.24 (br s, 1H). MS found $(M+H)^+$ (m/z), 201.01; calcd for $C_8H_8O_4S$ m/z, 200.01.

4.1.2.2. 2-((4-Fluorophenyl)sulfonyl)acetic acid (10b): Oxidation of 2-((4-fluorophenyl)-thio)acetic acid **9b** with 30% hydrogen peroxide yielded 75% of corresponding 2-((4-fluorophenyl) sulfonyl)acetic acid (**10b**); mp 120–122 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.54 (s, 2H), 7.48–7.52 (m, 2H), 7.99–8.02 (m, 2H), 13.13 (br s, 1H). MS found $(M+H)^+$ (m/z), 219.02; calcd for $C_8H_7FO_4S$ m/z, 218.00.

4.1.2.3. 2-((4-Chlorophenyl)sulfonyl)acetic acid (10c): Oxidation of 2-((4-chlorophenyl)-thio)acetic acid **9c** with 30% hydrogen peroxide yielded 90% of corresponding 2-((4-chlorophenyl)sulfonyl)acetic acid (**10c**); mp 123–124 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.53 (s, 2H), 7.71 (d, J = 8.1 Hz, 2H), 7.91 (d, J = 8.0 Hz, 2H), 13.30 (br s, 1H). MS found $(M+H)^+$ (m/z), 234.97; calcd for $C_8H_7ClO_4S$ m/z, 233.98.

4.1.2.4. 2-((4-Bromophenyl)sulfonyl)acetic acid (10d): Oxidation of 2-((4-bromophenyl)-thio)acetic acid **9d** with 30% hydrogen peroxide yielded 86% of corresponding 2-((4-bromophenyl) sulfonyl)acetic acid (**10d**); mp 136–138 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.46 (s, 2H), 7.63 (d, J = 8.4 Hz, 2H), 7.88 (d, J = 8.3 Hz, 2H), 13.12 (br s, 1H). MS found $(M+H)^+$ (m/z), 278.95; calcd for $C_8H_7BrO_4S$ m/z, 277.92.

4.1.2.5. 2-((4-Hydroxyphenyl)sulfonyl)acetic acid (10e): Oxidation of 2-((4-hydroxyphenyl)-thio)acetic acid **9e** with 30% hydrogen peroxide yielded 70% of corresponding 2-((4-hydroxyphenyl) sulfonyl)acetic acid (**10e**); mp 182–184 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.04 (s, 2H), 6.70 (d, J = 9.0 Hz, 2H), 6.21 (br s, 1H), 7.47 (d, J = 8.7 Hz, 2H), 11.59 (br s, 1H). MS found $(M+H)^+$ (m/z), 217.01; calcd for $C_8H_8O_5S$ m/z, 216.01.

4.1.2.6. 2-Tosylacetic acid (10f): Oxidation of 2-((4-tolyl)thio)acetic acid **9f** with 30% hydrogen peroxide yielded 78% of corresponding 2-tosyl acetic acid (**10f**); mp 98–101 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.58 (s, 3H), 4.39 (s, 2H), 7.03 (d, J = 8.3 Hz, 2H), 7.55 (d, J = 8.1 Hz, 2H), 12.57 (br s, 1H). MS found $(M+H)^+$ (m/z), 215.03; calcd for $C_9H_{10}O_4S$ m/z, 214.03.

4.1.2.7. 2-((4-Methoxyphenyl)sulfonyl)acetic acid (10g): Oxidation of 2-((4-methoxyphenyl)-thio)acetic acid **9g** with 30% hydrogen peroxide yielded 83% of corresponding 2-((4-methoxyphenyl)sulfonyl)acetic acid (**10g**); mp 113–114 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.73 (s, 3H), 4.28 (s, 2H), 7.03 (d, J = 7.9 Hz, 2H), 7.72 (d, J = 8.1 Hz, 2H), 12.96 (br s, 1H). MS found $(M+H)^+$ (m/z), 231.00; calcd for $C_9H_{10}O_5S$ m/z, 230.02.

4.1.2.8. 2-((4-Carboxyphenyl)sulfonyl)acetic acid (10h): Oxidation of 2-((4-carboxyphenyl)-thio)acetic acid **9h** with 30% hydrogen peroxide yielded 74% of corresponding 2-((4-carboxyphenyl) sulfonyl)acetic acid (**10h**); mp 163–165 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.64 (s, 2H), 8.11 (d, J = 7.7 Hz, 2H), 8.20 (d, J = 7.8 Hz, 2H), 13.04 (br s, 2H). MS found $(M+H)^+$ (m/z), 245.02; calcd for $C_9H_8O_6S$ m/z, 244.00.

4.1.2.9. 2-((2,4-Difluorophenyl)sulfonyl)acetic acid (10i): Oxidation of 2-((2,4-difluoro-phenyl)thio)acetic acid **9i** with 30% hydrogen peroxide yielded 70% of corresponding 2-((2,4-difluorophenyl) sulfonyl)acetic acid (**10i**); mp 120–122 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.54 (s, 2H), 7.33–7.38 (m, 1H), 7.59–7.64 (m, 1H), 7.91–7.97 (m, 1H). MS found $(M+H)^+$ (m/z), 237.00; calcd for $C_8H_6F_2O_4S$ m/z, 236.00.

4.1.2.10. 2-((2,4-Dichlorophenyl)sulfonyl)acetic acid (10j): Oxidation of 2-((2,4-dichloro-phenyl)thio)acetic acid **9j** with 30% hydrogen peroxide yielded 73% of corresponding 2-

((2,4-dichlorophenyl)sulfonyl)acetic acid (**10j**); mp 140–142 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 4.62 (s, 2H), 7.71 (d, J = 8.4, 1H), 7.98–8.00 (m, 2H). MS found $(M+H)^+$ (m/z), 268.94; calcd for $C_8H_6Cl_2O_4S$ m/z, 267.94.

4.1.3. General procedure for the preparation of benzylthioacetic acids (Scheme 5)—The following benzylthioacetic acids were prepared according to the procedure reported in the literature.[13]

4.1.3.1. 4-Fluorobenzylthioacetic acid (12a): Condensation of 4-fluorobenzyl bromide **11a** with mercaptoacetic acid yielded 96% of the corresponding 4-fluorobenzylthioacetic acid (**12a**); mp 41–42 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.17 (s, 2H), 3.84 (s, 2H), 7.12–7.14 (m, 2H), 7.31–7.34 (m, 2H), 11.58 (br s, 1H). MS found $(M+H)^+$ (m/z), 201.03; calcd for $C_9H_9FO_2S$ m/z, 200.03.

4.1.3.2. 2,4-Difluorobenzylthioacetic acid (12b): Condensation of 2,4-difluorobenzyl bromide **11b** with mercaptoacetic acid yielded 90% of the corresponding 2,4-difluorobenzylthioacetic acid (**12b**); mp 58–60 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.19 (s, 2H), 3.88 (s, 2H), 6.83–6.89 (m, 2H), 7.32–7.36 (m, 1H), 11.44 (br s, 1H). MS found $(M+H)^+$ (m/z), 219.03; calcd for $C_9H_8F_2O_2S$ m/z, 218.02.

4.1.4. General procedure for the preparation of benzylsulfonylacetic acids (13) (Scheme 5)—The following benzylsulfonylacetic acids were prepared according to the procedure reported in the literature.[13]

4.1.4.1. 4-Fluorobenzylsulfonylacetic acid (13a): Oxidation of 4-fluorobenzylthioacetic acid **12a** with 30% hydrogen peroxide yielded 93% of the corresponding 4-fluorobenzylsulfonylacetic acid **13a**; mp 158–160 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.89 (s, 2H), 4.58 (s, 2H), 6.94–6.99 (m, 2H), 7.48–7.52 (m, 2H), 11.89 (br s, 1H). MS found $(M+H)^+$ (m/z), 233.02; calcd for $C_9H_9FO_4S$ m/z, 232.02.

4.1.4.2. 2,4-Difluorobenzylsulfonylacetic acid (13b): Oxidation of 2,4-difluorobenzylthioacetic acid **12b** with 30% hydrogen peroxide yielded 90% of the corresponding 2,4-difluorobenzylsulfonylacetic acid **13b**; mp 116–118 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.99 (s, 2H), 4.61 (s, 2H), 6.93–7.00 (m, 2H), 7.51–7.55 (m, 1H), 11.48 (br s, 1H). MS found $(M+H)^+$ (m/z), 251.00; calcd for $C_9H_8F_2O_4S$ m/z, 250.01.

4.1.5. General procedure for the preparation of methyl 3-((aryl) amino)-3-oxopropanoate (15) (Scheme 6)—The following methyl 3-((aryl)amino)-3-oxopropanoates (**15**) were prepared according to the procedure reported in the literature.[14]

4.1.5.1. Methyl 3-((4-fluorophenyl)amino)-3-oxopropanoate (15a): Reaction of methyl 3-chloro-3-oxopropionate with 4-fluoroaniline **14a** produced methyl 3-((4-fluorophenyl)amino)-3-oxopropionate **15a** in 76% yield. Mp 109–112 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.50 (s, 2H), 3.82 (s, 3H), 7.03–7.05 (m, 2H), 7.52–7.54 (m, 2H), 9.22 (s, 1H). MS found $(M+H)^+$ (m/z), 212.13; calcd for $C_{10}H_{10}FNO_3$ m/z, 211.06.

4.1.5.2. Methyl 3-((2,4-difluorophenyl)amino)-3-oxopropanoate (15b): Reaction of methyl 3-chloro-3-oxopropionate with 2,4-difluoroaniline **14b** produced methyl 3-((2,4-difluoro-phenyl)amino)-3-oxopropionate **15b** in 77% yield. Mp 128–130 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.39 (s, 2H), 3.78 (s, 3H), 7.05–7.08 (m, 1H), 7.32–7.36 (m, 1H), 7.89–7.91 (m, 1H), 9.98 (s, 1H). MS found $(M+H)^+$ (m/z), 230.18; calcd for $C_{10}H_9F_2NO_3$ m/z, 229.05.

4.1.6. General procedure for the preparation of 2-(arylcarbamoyl)acetic acid (16) (Scheme 6)—The following 2-(arylcarbamoyl)acetic acids (**16**) were prepared according to the procedure reported in the literature.[14]

4.1.6.1. 2-(4-Fluorophenylcarbamoyl)acetic acid (16a): Hydrolysis of methyl 3-((4-fluorophenyl)amino)-3-oxopropionate **15a** with 10% NaOH followed by neutralization with diluted HCl resulted 2-(4-fluorophenylcarbamoyl)acetic acid (**16a**) in 75% yield. Mp 175–176 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.31 (s, 2H), 7.07–7.10 (m, 2H), 7.51–7.53 (m, 2H), 10.24 (s, 1H), 12.86 (br s, 1H). MS found $(M+H)^+$ (m/z), 198.04; calcd for $C_9H_8FNO_3$ m/z, 197.05.

4.1.6.2. 2-(2,4-Difluorophenylcarbamoyl)acetic acid (16b): Hydrolysis of methyl 2-(2,4-difluoroarylcarbamoyl)acetate **15b** with 10% NaOH followed by neutralization with diluted HCl resulted 2-(2,4-difluorophenylcarbamoyl)acetic acid (**16b**) in 73% yield. Mp 183–184 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.36 (s, 2H), 7.01–7.04 (m, 1H), 7.30–7.33 (m, 1H), 7.85–7.87 (m, 1H), 9.95 (s, 1H), 12.86 (br s, 1H). MS found $(M+H)^+$ (m/z), 216.08; calcd for $C_9H_7F_2NO_3$ m/z, 215.04.

4.1.7. General procedure for the preparation of ethyl phenylsulfamoyl acetate (17) (Scheme 7)—The following ethyl phenylsulfamoyl acetates (**17**) were prepared according to the procedure reported in the literature.[15]

4.1.7.1. Ethyl 2-(*N*-(4-fluorophenyl)sulfamoyl)acetate (17a): Addition of ethyl 2-(chlorosulfonyl)acetate to 4-fluoroaniline **14a** yielded the corresponding ethyl 2-(*N*-(4-fluorophenyl)sulfamoyl) acetate (**17a**) in 72%. Mp 65–66 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.33 (t, 3H), 3.93 (s, 2H), 4.29 (q, 2H), 7.03–7.13 (m, 2H), 7.18 (br s, 1H, NH), 7.32–7.40 (m, 2H). MS found $(M+H)^+$ (m/z): 262.05; Calcd for $C_{10}H_{12}FNO_4S$ m/z, 261.05.

4.1.7.2. Ethyl 2-(*N*-(2,4-difluorophenyl)sulfamoyl)acetate (17b): Addition of ethyl 2-(chlorosulfonyl)acetate to 2,4-difluoroaniline **14b** yielded the corresponding ethyl 2-(***N***-(2,4-difluorophenyl)sulfamoyl)acetate (**17b**) in 70%. Mp 44–46 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.33 (t, 3H), 4.03 (s, 2H), 4.28–4.31 (q, 2H), 6.92–6.95 (m, 2H), 7.03 (br s, 1H), 7.58–7.62 (m, 1H). MS found $(M+H)^+$ (m/z), 280.04; calcd for $C_{10}H_{11}F_2NO_4S$ m/z, 279.04.

4.1.8. General procedure for the preparation of phenylsulfamoyl acetic acid (18) (Scheme 7)—The following ethyl phenylsulfamoylacetic acids (**18**) were prepared according to the procedure reported in the literature.[15]

4.1.8.1. 2-(*N*-(4-Fluorophenyl)sulfamoyl)acetic acid (18a): Hydrolysis followed by neutralization of ethyl 2-(*N*-(4-fluorophenyl)sulfamoyl)acetate **17a** resulted in the corresponding 2-(*N*-(4-fluorophenyl)sulfamoyl)acetic acid (**18**a) in 80%. Mp 114–116 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.05 (s, 2H), 7.17–7.28 (m, 4H), 10.04 (br s, 1H), 12.70 (br s, 1H). MS found $(M+H)^+$ (m/z): 234.02; Calcd for $C_8H_8FNO_4S$ m/z, 233.02.

4.1.8.2. 2-(*N*-(2,4-Difluorophenyl)sulfamoyl)acetic acid (18b): Hydrolysis followed by neutralization of ethyl 2-(*N*-(2,4-difluorophenyl) sulfamoyl)acetate **17b** resulted in the corresponding 2-(*N*-(2,4-difluorophenyl)-sulfamoyl)acetic acid (**18b**) in 76%. Mp 138–139 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ ^{1}H NMR (600 MHz, DMSO-d_6): δ 4.01 (s, 2H), 6.95–6.98 (m, 2H), 7.56–7.58 (m, 1H), 10.08 (br s, 1H), 12.68 (br s, 1H). MS found $(M+H)^+$ (m/z), 252.03; calcd for $C_8H_7F_2NO_4S$ m/z, 251.01.

4.1.9. General procedure for the preparation of compounds 21 and 22 (Scheme 8)—Methyl thioglycolate (62.8 mmol) was added to the compound **19**/**20** (62.8 mmol) dissolved in tetrahydrofuran (30 mL) and stirred the reaction mixture for 2 min at room temperature. *N*,*N*-Diisopropylethylamine (75.4 mmol) dissolved in tetrahydrofuran (10 mL) was added drop-wise to the above reaction mixture at room temperature and continued the reaction for 15 h. The solvent was removed under reduced pressure, ice-cold water was added to the crude product and stirred the reaction mixture for 5 min. Filtered the solid separated, washed with water and dried to get pure product **21**/**22**.

4.1.9.1. Methyl 2-((2-fluoro-4-nitrophenyl)thio)acetate (21): The reaction of 3,4-difluoronitrobenzene **19** with methyl thioglycolate yielded methyl 2-(2-fluoro-4-nitrophenylthio)acetate **21** in 96% yield. Mp 68–70 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.77 (s, 3H), 3.80 (s, 2H), 7.52 (t, 1H), 7.85–7.87 (m, 1H), 8.02–8.04 (m, 1H). MS found $(M+H)^+$ (m/z), 246.02; calcd for $C_9H_8FNO_4S$ m/z, 245.02.

4.1.9.2. Methyl 2-((4-fluoro-2-nitrophenyl)thio)acetate (22): The reaction of 2,5-difluoronitrobenzene **20** with methyl thioglycolate yielded methyl 2-(4-fluoro-2-nitrophenylthio)acetate **22** in 85% yield. Mp 62–64 °C; ^{1}H NMR (600MHz, DMSO-d_6): δ 3.62 (s, 3H), 4.45 (s, 2H), 7.82–7.84 (m, 1H), 8.01–8.02 (m, 1H), 8.17–8.19 (m, 1H). MS found $(M+H)^+$ (m/z), 246.02; calcd for $C_9H_8FNO_4S$ m/z, 245.02.

4.1.10. General procedure for the preparation of the compounds 23 and 24 (Scheme 8)—The compounds **23**–**24** were prepared according to the procedure reported in the literature.[13]

4.1.10.1. Methyl 2-((2-fluoro-4-nitrophenyl)sulfonyl)acetate (23): Oxidation of methyl 2-((2-fluoro-4-nitrophenyl)thio)acetate **21** gave methyl 2-((2-fluoro-4-nitrophenyl)sulfonyl)acetate **23** in 99% yield. Mp 94–96 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.73 (s, 3H), 4.39 (s, 2H), 8.14–8.16 (m, 1H), 8.18–8.21 (m, 1H), 8.24–8.25 (m, 1H). MS found $(M+H)^+$ (m/z), 278.01; calcd for $C_9H_8FNO_6S$ m/z, 277.01.

4.1.10.2. Methyl 2-((4-fluoro-2-nitrophenyl)sulfonyl)acetate (24): Oxidation of methyl 2-((4-fluoro-2-nitrophenyl)thio)acetate **22** gave methyl 2-((4-fluoro-2-

nitrophenyl)sulfonyl)acetate **24** in 90% yield. Mp 104–106 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.64 (s, 3H), 4.87 (s, 2H), 7.85–7.87 (m, 1H), 8.17–8.19 (m, 1H), 8.23–8.24 (m, 1H). MS found $(M+H)^+$ (*m*/*z*), 278.01; calcd for $C_9H_8FNO_6S$ *m*/*z*, 277.01.

4.1.11. General procedure for the preparation of the compounds 25 and 26 (Scheme 8)—10% Pd/C was added (500 mg) was added to the solution of the compound **23**/**24** (18 mmol) in methanol (100 mL) and then continued the reaction in the presence of hydrogen gas at room temperature for 3 h. After completion of the reaction (monitored by TLC), the reaction mixture was filtered on celite and washed the celite pad thoroughly with a mixture of methanol and chloroform. The solvent was removed under reduced pressure and the crude product was treated with 20% diethyl ether in hexanes. The separated solid was filtered, washed with 20% diethyl ether in hexanes and dried to get pure product **25**/**26**.

4.1.11.1. Methyl 2-(4-amino-2-fluorophenylsulfonyl)acetate (25): Methyl 2-((2-fluoro-4-nitrophenyl) sulfonyl)acetate **23** on reduction with iron produced methyl 2-(4-amino-2-fluoro-phenylsulfonyl) acetate **25** in 86% yield. Mp 99–101 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.72 (s, 3H), 4.22 (s, 2H), 4.48 (s, 2H), 6.39–6.42 (m, 1H), 6.46–6.48 (m, 1H), 7.62 (m, 1H). MS found $(M+H)^+$ (*m*/*z*), 248.06; calcd for $C_9H_{10}FNO_4S$ *m*/*z*, 247.03.

4.1.11.2. Methyl 2-(2-amino-4-fluorophenylsulfonyl)acetate (26): Methyl 2-((4-fluoro-2-nitrophenyl) sulfonyl)acetate **24** on reduction with iron produced methyl 2-(2-amino-4-fluoro-phenylsulfonyl) acetate **26** in 93% yield. Mp 85–86 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.74 (s, 3H), 4.16 (s, 2H), 5.21 (br s, 2H), 6.45–6.48 (m, 1H), 6.53–6.57 (m, 1H), 7.71–7.73 (m, 1H). MS found $(M+H)^+$ (*m*/*z*), 248.09; calcd for $C_9H_{10}FNO_4S$ *m*/*z*, 247.03.

4.1.12. General procedure for 8-alkyl/cycloalkyl-2-methylsulfanyl-6-substitued-7-oxo-7,8-dihydropyrido[2,3-*d*] pyrimidine (5)—Mixture of 4-alkylamino-2-methylsulfanyl-pyrimidine-5-carbaldehyde **4** (4.2 mmol),[11] 1.2 equiv of active methylene compound and catalytic amount of benzylamine was taken into acetic acid and refluxed for about 6 h. After completion of the reaction (checked with TLC), the reaction mixture was cooled to room temperature, the precipitated product was filtered. The solid was washed with saturated $NaHCO_3$, water and dried over vacuum. The crude product was recrystallized in 2-propanol to get pure product (**5**).

4.1.12.1. 8-Methyl-2-(methylthio)-6-(phenylsulfonyl)pyrido [2,3-*d*]pyrimidin-7(8*H*)-one (5a): Starting from **4b** and 2-(phenylsulfonyl)acetic acid (**10a**), 58% of **5a** was obtained according to the method described for the synthesis of **5**. Mp 232–236 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.60 (s, 3H), 3.50 (s, 3H), 7.61–7.63 (m, 2H), 7.71–7.74 (m, 1H), 7.80–7.83 (m, 2H), 8.70 (s, 1H), 8.81 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 348.06; calcd for $C_{15}H_{13}N_3O_3S_2$ *m*/*z*, 347.04.

4.1.12.2. 6-((4-Fluorophenyl)sulfonyl)-8-methyl-2-(methylthio) pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5b): Starting from **4b** and 2-(4-fluorophenylsulfonyl)acetic acid (**10b**), 62% of **5b** was obtained according to the method described for the synthesis of **5**. Mp 182–185 °C; ^{1}H NMR (400 MHz, $CDC1_3$): δ 2.54 (s, 3H), 3.61 (s, 3H), 7.32 (d, J = 8.2 Hz,

2H), 8.12 (d, *J* = 8.2 Hz, 2H), 8.63 (s, 1H), 8.77 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 366.01; calcd for $C_{15}H_{12}FN_3O_3S_2$ *m*/*z*, 365.03.

4.1.12.3. 6-((4-Chlorophenyl)sulfonyl)-8-methyl-2-(methylthio) pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5c): Starting from **4b** and 2-(4-chlorophenylsulfonyl)acetic acid (**10c**), 55% of **5c** was obtained according to the method described for the synthesis of **5**. Mp 296–298 °C; ^{1}H NMR (400 MHz, $CDC1_3$): δ 2.57 (s, 3H), 3.60 (s, 3H), 7.25 (d, *J* = 7.8 Hz, 2H), 8.00 (d, *J* = 7.8 Hz, 2H), 8.64 (s, 1H), 8.73 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 382.10; calcd for $C_{15}H_{12}ClN_3O_3S_2$ *m*/*z*, 381.00.

4.1.12.4. 6-((4-Bromophenyl)sulfonyl)-8-methyl-2-(methylthio) pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5d): Starting from **4b** and 2-(4-bromophenylsulfonyl)acetic acid (**10d**), 58% of **5d** was obtained according to the method described for the synthesis of **5**. Mp 254–256 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.62 (s, 3H), 3.51 (s, 3H), 7.86 (d, *J* = 8.0 Hz, 2H), 7.91 (d, *J* = 8.0 Hz, 2H), 9.03 (s, 1H), 9.20 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 425.92; calcd for $C_{15}H_{12}BrN_3O_3S_2$ *m*/*z*, 424.95.

4.1.12.5. 6-((4-Hydroxyphenyl)sulfonyl)-8-methyl-2-(methylthio) pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5e): Starting from **4b** and 2-(4-hydroxyphenylsulfonyl)acetic acid (**10e**), 42% of **5e** was obtained according to the method described for the synthesis of **5**. Mp 302–304 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.56 (s, 3H), 3.48 (s, 3H), 6.90 (d, *J* = 8.7 Hz, 2H), 7.81 (d, *J* = 9.0 Hz, 2H), 8.90 (s, 1H), 9.12 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 364.02; calcd for $C_{15}H_{13}N_3O_4S_2$ *m*/*z*, 363.03.

4.1.12.6. 8-Methyl-2-(methylthio)-6-tosylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5f): Starting from **4b** and 2-tosylacetic acid (**10f**), 59% of **5f** was obtained according to the method described for the synthesis of **5**. Mp 228–230 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.39 (s, 3H), 2.61 (s, 3H), 3.50 (s, 3H), 7.43 (d, *J* = 8.4 Hz, 2H), 7.89 (d, *J* = 8.4 Hz, 2H), 8.99 (s, 1H), 9.10 (s, lH). MS found $(M+H)^+$ (*m*/*z*), 362.10; calcd for $C_{16}H_{15}N_3O_3S_2$ *m*/*z*, 361.06.

4.1.12.7. 6-((4-Methoxyphenyl)sulfonyl)-8-methyl-2-(methylthio) pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5g): Starting from **4b** and 2-(4-methoxyphenylsulfonyl)acetic acid (**10g**), 61% of **5g** was obtained according to the method described for the synthesis of **5**. Mp 208–210 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.60 (s, 3H), 3.51 (s, 3H), 3.84 (s, 3H), 7.14 (d, *J* = 8.8 Hz, 2H), 7.94 (d, *J* = 8.0 Hz, 2H), 8.96 (s, 1H), 9.17 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 378.03; calcd for $C_{16}H_{15}N_3O_4S_2$ *m*/*z*, 377.05.

4.1.12.8. 4-((8-Methyl-2-(methylthio)-7-oxo-7,8-dihydropyrido [2,3-*d*]pyrimidin-6-yl)sulfo-nyl)benzoic acid (5h): Starting from **4b** and 2-(4-carboxyphenylsulfonyl)acetic acid (**10h**), 58% of **5h** was obtained according to the method described for the synthesis of **5**. Mp 236–240 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 2.60 (s, 3H), 3.48 (s, 3H), 8.03 (d, *J* = 8.7 Hz, 2H), 8.10 (d, *J* = 8.5 Hz, 2H), 9.04 (s, 1H), 9.19 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 366.12; calcd for $C_{16}H_{13}N_3O_5S_2$ *m*/*z*, 391.03.

4.1.12.9. 6-((2,4-Difluorophenyl)sulfonyl)-8-methyl-2-(methylthio) pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5i): Starting from **4b** and 2-(2,4-difluorophenylsulfonyl)acetic acid

(**10i**), 60% of **5i** was obtained according to the method described for the synthesis of **5**. Mp 280–283 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.61 (s, 3H), 3.50 (s, 3H), 7.40–7.56 (m, 2H), 8.12–8.19 (m, 1H), 9.07 (s, 1H), 9.24 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 384.02; calcd for $C_{15}H_{11}F_2N_3$-O_3S_2 *m*/*z*, 383.02.

4.1.12.10. 6-((2,4-Dichlorophenyl)sulfonyl)-8-methyl-2-(methylthio) pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5j): Starting from **4b** and 2-(2,4-dichlorophenylsulfonyl)acetic acid (**10j**), 51% of **5j** was obtained according to the method described for the synthesis of **5**. Mp 118–122 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 2.59 (s, 3H), 3.51 (s, 3H), 7.95 (s, 1H), 8.49 (d, J = 8.2 Hz, 1H), 8.57 (d, J = 8.0 Hz, 1H), 9.41 (s, 1H), 9.72 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 415.95; calcd for $C_{15}H_{11}Cl_2N_3O_3S_2$ *m*/*z*, 414.96.

4.1.12.11. 6-((4-Fluoro-2-nitrophenyl)sulfonyl)-8-methyl-2-(methylthio)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5k): Starting from **4b** and methyl 2-(4-fluoro-2-nitrophenylsulfonyl) acetate (**24**), 52% of **5k** was obtained according to the method described for the synthesis of **5**. Mp 260–262 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 2.62 (s, 3H), 3.54 (s, 3H), 7.87–7.90 (m, 1H), 8.13–8.14 (m, 1H), 8.47–8.49 (m, 1H), 8.93 (s, 1H), 9.28 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 411.02; calcd for $C_{15}H_{11}FN_4O_5S_2$ *m*/*z*, 410.02.

4.1.12.12. 6-((2-Amino-4-fluorophenyl)sulfonyl)-8-methyl-2-(methylthio)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5l): Starting from **4b** and methyl 2-(2-amino-4-fluorophenylsulfonyl) acetate (**26**), 67% of **5l** was obtained according to the method described for the synthesis of **5**. Mp 262–264 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 2.67 (s, 3H), 3.96 (s, 3H), 7.28–7.30 (m, 1H), 7.36–7.38 (m, 1H), 8.05–8.07 (m, 1H), 9.08 (s, 1H), 9.13 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 381.05; calcd for $C_{15}H_{13}FN_4O_3S_2$ *m*/*z*, 380.04.

4.1.12.13. 6-((2-Fluoro-4-nitrophenyl)sulfonyl)-8-methyl-2-(methylthio)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5m): Starting from **4b** and methyl 2-(2–fluoro–4-nitrophenylsulfonyl)acetate (**23**), 45% of **5m** was obtained according to the method described for the synthesis of **5**. Mp 314–316 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 2.62 (s, 3H), 3.51 (s, 3H), 8.28–8.39 (m, 3H), 9.15 (s, 1H), 9.26 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 411.02; calcd for $C_{15}H_{11}FN_4O_5S_2$ *m*/*z*, 410.02.

4.1.12.14. 6-((4-Amino-2-fluorophenyl)sulfonyl)-8-methyl-2-(methylthio)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5n): Starting from **4b** and methyl 2-(4-amino-2-fluorophenylsulfonyl) acetate (**25**), 32% of **5n** was obtained according to the method described for the synthesis of **5**. Mp 328–330 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 2.61 (s, 3H), 3.51 (s, 3H), 6.26–6.28 (m, 1H), 6.47–6.49 (m, 1H), 6.52 (s, 2H), 7.62–7.64 (m, 1H), 8.91 (s, 1H), 9.21 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 381.05; calcd for C_{15}-$H_{13}FN_4O_3S_2$ *m*/*z*, 380.04.

4.1.12.15. 6-((4-Fluorobenzyl)sulfonyl)-8-methyl-2-(methylthio) pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5o): Starting from **4b** and 4-fluorobenzylsulfonylacetic acid (**13a**), 55% of **5o** was obtained according to the method described for the synthesis of **5**. Mp 170–174 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.58 (s, 3H), 3.74 (s, 3H), 4.71 (s, 2H), 6.80–6.94

(m, 2H), 7.24–7.33 (m, 2H), 8.20 (s, 1H), 8.59 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 380.10; calcd for $C_{16}H_{14}FN_3O_3S_2$ *m*/*z*, 379.05.

4.1.12.16. 6-((2,4-Difluorobenzyl)sulfonyl)-8-methyl-2-(methylthio)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5p): Starting from **4b** and 2,4-fluorobenzylsulfonylacetic acid (**13b**), 46% of **5p** was obtained according to the method described for the synthesis of **5**. Mp: 264–266 °C; ^{1}H NMR (600 MHz; DMSO-d_6): δ 2.65 (s, 3H), 3.69 (s, 3H), 4.89 (s, 2H), 7.09–7.11 (m, 1H), 7.26–7.29 (m, 1H), 7.43–7.46 (m, 1H), 8.63 (s, 1H), 9.12 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 398.08; calcd for $C_{16}H_{13}F_2N_3O_3S_2$ *m*/*z*, 397.04.

4.1.12.17. *N*-(4-Fluorophenyl)-8-methyl-2-(methylthio)-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidine-6-carboxamide (5q): Starting from **4b** and 2-(4-fluorophenylcarbamoyl)acetic acid (**16a**), 56% of **5q** was obtained according to the method described for the synthesis of **5**. Mp 240–242 °C; ^{1}H NMR (600 MHz; $CDCl_3$): δ 2.69 (s, 3H), 3.88 (s, 3H), 7.08 (t, 2H), 7.71–7.73 (m, 2H), 8.86 (s, 1H), 8.94 (s, 1H), 11.60 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 345.08; calcd for $C_{16}H_{13}FN_4O_2S$ *m*/*z*, 344.07.

4.1.12.18. *N*-(2,4-Difluorophenyl)-8-methyl-2-(methylthio)-7-oxo-7,8-dihydropyrido[2,3-*d*]-pyrimidine-6-carboxamide (5r): Starting from **4b** and 2-(2,4-difluorophenylcarbamoyl)acetic acid (**17b**), 53% of **5r** was obtained according to the method described for the synthesis of **5**. Mp 276–278 °C; ^{1}H NMR (600 MHz; $CDCl_3$): δ 2.66 (s, 3H), 3.74 (s, 3H), 7.15–7.16 (m, 1H), 7.45–7.47 (m, 1H), 8.45–8.46 (m, 1H), 9.04 (s, 1H), 9.25 (s, 1H), 11.92 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 363.07; calcd for $C_{16}H_{12}F_2N_4O_2S$ *m*/*z*, 362.06.

4.1.12.19. *N*-(4-Fluorophenyl)-8-methyl-2-(methylthio)-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidine-6-sulfonamide (5s): Starting from **4b** and 2-(4-fluorophenylsulfamoyl)acetic acid (**18a**), 62% of **5s** was obtained according to the method described for the synthesis of **5**. Mp 202–204 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 2.59 (s, 3H), 3.61 (s, 3H), 7.02–7.06 (m, 2H), 7.08–7.11 (m, 2H), 8.71 (s, 1H), 9.06 (s, 1H), 10.31 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 381.09; calcd for $C_{15}H_{13}FN_4O_3S_2$ *m*/*z*, 380.04.

4.1.12.20. *N*-(2,4-Difluorophenyl)-8-methyl-2-(methylthio)-7-oxo-7,8-dihydropyrido[2,3-*d*]-pyrimidine-6-sulfonamide (5t): Starting from **4b** and 2-(2,4-difluorophenylsulfamoyl)acetic acid (**18b**), 51% of **5t** was obtained according to the method described for the synthesis of **5**. Mp 230–232 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 2.63 (s, 3H), 3.66 (s, 3H), 6.99–7.01 (m, 1H), 7.25–7.29 (m, 2H), 8.57 (s, 1H), 9.06 (s, 1H), 10.02 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 399.10; calcd for $C_{15}H_{12}F_2N_4O_3S_2$ *m*/*z*, 398.03.

4.1.12.21. 6-((2,4-Difluorophenyl)sulfonyl)-2-(methylthio)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5u): Starting from **4a** and 2-(2,4-difluorophenylsulfonyl)acetic acid (**10i**), 53% of **5u** was obtained according to the method described for the synthesis of **5**. Mp 338–340 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 2.58 (s, 3H), 7.39–7.41 (m, 1H), 7.11–7.15 (m, 1H), 8.10–8.15 (m, 1H), 9.02 (s, 1H), 9.20 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 370.02; calcd for $C_{14}H_9F_2N_3O_3S_2$ *m*/*z*, 369.01.

4.1.12.22. 6-((2,4-Difluorophenyl)sulfonyl)-8-ethyl-2-(methylthio)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5v): Starting from **4c** and 2-(2,4-difluorophenylsulfonyl)acetic acid (**10i**), 56% of **5v** was obtained according to the method described for the synthesis of **5**. Mp 215–217 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ1.32–1.27 (t, 3H), 2.66 (s, 3H), 4.44–4.37 (q, 2H), 6.92–6.85 (m, 1H), 7.17–7.11 (m, 1H), 8.33–8.28 (m, 1H), 8.85 (s, 1H), 8.74 (s, 1H). MS found $(M+H)^+$ (*m*/*z*): 398.10, Calcd for $C_{16}H_{13}F_2N_3O_3S_2$ *m*/*z*: 397.04.

4.1.12.23. 6-((2,4-Difluorophenyl)sulfonyl)-2-(methylthio)-8-propylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5w): Starting from **4d** and 2-(2,4-difluorophenylsulfonyl)acetic acid (**10i**), 64% of **5w** was obtained according to the method described for the synthesis of **5**. Mp 196–198 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.93–0.96 (m, 3H), 1.68–1.71 (m, 2H), 2.64 (s, 3H), 4.25–4.32 (m, 2H), 6.86–6.89 (m, 1H), 7.11–7.15 (m, 1H), 8.29–8.33 (m, 1H), 8.77 (s, 1H), 8.84 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 412.14; calcd for $C_{17}H_{15}F_2N_3O_3S_2$ *m*/*z*, 411.05.

4.1.12.24. 8-Cyclopropyl-6-((2,4-difluorophenyl)sulfonyl)-2-(methylthio)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5x): Starting from **4e** and 2-(2,4-difluorophenylsulfonyl)acetic acid (**10i**), 55% of **5x** was obtained according to the method described for the synthesis of **5**. Mp 236–238 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 0.75–0.77 (m, 2H), 1.12–1.14 (m, 2H), 2.62 (s, 3H), 2.80–2.81 (m, 1H), 7.40–7.54 (m, 2H), 8.14–8.16 (m, 1H), 9.01 (s, 1H), 9.18 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 410.20; calcd for $C_{17}H_{13}F_2N_3O_3S_2$ *m*/*z*, 409.04.

4.1.12.25. 8-Cyclopentyl-6-((2,4-difluorophenyl)sulfonyl)-2-(methylthio)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5y): Starting from **4f** and 2-(2,4-difluorophenylsulfonyl)acetic acid (**10i**), 60% of **5y** was obtained according to the method described for the synthesis of **5**. Mp 209–210 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.71–1.62 (m, 2H), 1.85–1.78 (m, 2H), 2.11–1.99 (m, 2H), 2.29–2.23 (m, 2H), 2.65 (s, 3H), 5.81–5.75 (m, 1H), 6.92–6.85 (m, 1H), 7.17–7.11 (m, 1H), 8.34–8.27 (m, 1H), 8.73 (s, 1H), 8.83 (s, 1H). MS found $(M+H)^+$ (*m*/*z*): 438.10, Calcd for $C_{19}H_{17}F_2N_3O_3S_2$ *m*/*z*: 437.07.

4.1.12.26. 8-Cyclohexyl-6-((2,4-difluorophenyl)sulfonyl)-2-(methylthio)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5z): Starting from **4g** and 2-(2,4-difluorophenylsulfonyl)acetic acid (**10i**), 55% of **5z** was obtained according to the method described for the synthesis of **5**. Mp 247–249 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.12–1.32 (m, 4H), 1.55–1.64 (m, 4H), 1.78–1.80 (m, 2H), 2.49–2.50 (m, 1H), 2.63 (s, 3H), 7.41–7.44 (m, 1H), 7.53–7.56 (m, 1H), 8.12–8.16 (m, 1H), 9.03 (s, 1H), 9.21 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 452.12; calcd for $C_{20}H_{19}F_2N_3O_3S_2$ *m*/*z*, 451.08.

4.1.13. General procedure for the synthesis of compounds (5aa–5ac) from 6-((2,4-difluorophenyl)sulfonyl)-2-(methylthio) pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5u) (Scheme 2)—The compound **5u** (5 mmol) was taken into DMF and stirred at 50 °C for 10 min until to get the clear solution, and then K_2CO_3 (7.5 mmol) was added and stirred about 5 min. The reaction mixture was brought to room temperature and was added alkyliodide (6.5 mmol) and the stirring was continued for 1 h. After completion of the reaction, the reaction mixture was quenched with water and filtered the crude product. The

crude product was purified with flash column chromatography using ethyl acetate and hexanes as eluents.

4.1.13.1. Methyl 2-(6-(2,4-difluorophenylsulfonyl)-2-(methylthio)-7-oxopyrido[2,3-*d*]pyrimidin-8(7*H*)yl)acetate (5aa): Starting from **5u** and methyl 2-bromoacetate, 55% of **5aa** was obtained according to the method described for the synthesis of **5aa**–**5ac**. Mp 225–227 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 2.58 (s, 3H),3.73 (s, 3H), 5.06 (s, 2H), 6.90–6.93 (m, 1H), 7.09–7.16 (m, 1H), 8.25–8.32 (m, 1H), 8.83 (s, 1H), 8.87 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 442.10; calcd for $C_{17}H_{13}F_2N_3O_5S_2$ *m*/*z*, 441.03.

4.1.13.2. 8-(Cyclopropylmethyl)-6-((2,4-difluorophenyl)sulfonyl)-2-(methylthio)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5ab): Starting from **5u** and (iodomethyl)cyclopropane, 60% of **5ab** was obtained according to the method described for the synthesis of **5aa**–**5ac**. Mp 204–205 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.28– 0.22 (m, 4H), 1.16–1.08 (m, 1H), 2.42 (s, 3H), 4.02 (d, 2H), 6.69–6.62 (m, 1H), 7.06–6.88 (m, 1H), 8.13–8.05 (m, 1H), 8.56 (s, 1H), 8.63 (s, 1H). MS found $(M+H)^+$ (*m*/*z*): 424.10, Calcd for $C_{18}H_{15}F_2N_3$-O_3S_2 *m*/*z*: 423.05.

4.1.13.3. 8-(Cyclopentylmethyl)-6-((2,4-difluorophenyl)sulfonyl)-2-(methylthio)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (5ac): Starting from **5u** and (iodomethyl)cyclopentane, 60% of **5ac** was obtained according to the method described for the synthesis of **5aa**–**5ac**. Mp 190–191 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.30–1.26 (m, 2H), 1.55–1.49 (m, 2H), 1.70–1.60 (m, 4H), 2.35 (m, 1H), 2.73 (s, 3H), 4.33 (d, 2H),, 6.91–6.84 (m, 1H), 7.17–7.11 (m, 1H), 8.36–8.28 (m, 1H), 8.77 (s, 1H), 8.84 (s, 1H). MS found $(M+H)^+$ (*m*/*z*): 452.10, Calcd for $C_{20}H_{19}F_2N_3O_3S_2$ *m*/*z*: 451.08.

4.1.14. General procedure for the synthesis of 8-substituted-2-methylsulfinyl-6-substituted-7-oxo-7,8-dihydro-pyrido[2,3-*d*] pyrimidines (6a–ac) (Schemes 1 and 2)—A solution of 8-substituted-2-methylsulfanyl-6-substitued-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidine **5a**–**ac** (3.5 mmol), and 3-chloro-perbenzoic acid (m-CPBA) (4.4 mmol) in CH_2Cl_2 was stirred at room temperature for about 4 h. After completion of the reaction, the reaction mixture was washed with saturated $NaHCO_3$, organic layer was dried over Na_2SO_4 and concentrated to produce the mixture of methylsulfoxide and methylsulfonyl product **6a**–**ac** and was used for next step without further purification.

4.1.15. General procedure for 8-alkyl/cycloalkyl-2-(aryl/heteroarylamino)-6-substiuted-7-oxo-7,8-dihydro-pyrido[2,3-*d*]pyrimidine (7) (Scheme 1)—The mixture of 8-alkyl-2-methylsulfinyl-6-substituted-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidine **6** (2 mmol) and aryl/heteroaryl amines (2.25 mmol) in toluene was stirred at 100 °C for overnight. The reaction mixture was cooled and solid was collected by filtration. The crude product was washed with toluene and purified by flash chromatography to get pure product.

4.1.15.1. 2-((4-Chlorophenyl)amino)-6-((4-chlorophenyl)sulfonyl)-8-methylpyrido[2,3-*d*]-pyrimidin-7(8*H*)-one (7a): Starting from **6c** and 4-chlorobenzenamine, 72% of **7a** was obtained as per the method described for the synthesis of **7**; mp >300 °C; ^{1}H NMR (400

MHz; DMSO-d_6): δ 3.48 (s, 3H), 7.41 (d, J = 8.8 Hz, 2H), 7.70 (d, J = 8.4 Hz, 2H), 7.82 (d, J = 8.4 Hz, 2H), 7.99 (d, J = 8.8 Hz, 2H), 8.88 (s, 1H), 9.10 (s, 1H), 10.40 (s, 1H). MS found $(M+H)^+$ (m/z), 461.02; calcd for $C_{20}H_{14}Cl_2N_4O_3S$ m/z, 460.02.

4.1.15.2. 6-((4-Chlorophenyl)sulfonyl)-2-((4-methoxyphenyl)amino)-8-methylpyrido[2,3-*d*]-pyrimidin-7(8*H*)-one (7b): Starting from **6c** and 4-methoxybenzenamine, 63% of **7b** was obtained as per the method described for the synthesis of **7**; mp 286–290 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.64 (s, 3H), 3.75 (s, 3H), 6.90 (d, J = 9.0 Hz, 2H), 7.50 (d, J = 8.0 Hz, 2H), 7.60 (d, J = 8.0 Hz, 2H), 7.72 (d, J = 8.8 Hz, 2H), 7.99 (s, 1H), 8.10 (s, 1H), 10.20 (s, 1H). MS found $(M+H)^+$ (m/z), 457.06; calcd for $C_{21}H_{17}ClN_4O_4S$ m/z, 456.07.

4.1.15.3. 2-((4-Methoxyphenyl)amino)-8-methyl-6-tosylpyrido [2,3-*d*]pyrimidin-7(8*H*)-one (7c): Starting from **6f** and 4-methoxybenzenamine, 68% of **7c** was obtained as per the method described for the synthesis of **7**; mp 336–340 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 2.39 (s, 3H), 3.47 (s, 3H), 3.75 (s, 3H), 6.41 (d, J = 8.0 Hz, 2H), 6.88 (d, J = 7.8 Hz, 2H), 7.41 (d, J = 8.0 Hz, 2H), 7.88 (d, J = 7.8 Hz, 2H), 8.80 (s, 1H), 9.05 (s, 1H), 10.20 (s, 1H). MS found $(M+H)^+$ (m/z), 437.10; calcd for $C_{22}H_{20}N_4O_4S$ m/z, 436.12.

4.1.15.4. 2-((1*H*-Indol-5-yl)amino)-8-methyl-6-(phenylsulfonyl)pyrido[2,3-*d*]pyrimidin-7-(8*H*)-one (7d): Starting from **6a** and 1*H*-indol-5-amine, 62% of **7d** was obtained as per the method described for the synthesis of **7**. Mp 340–344 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.47 (s, 3H), 6.41 (s, 1H), 7.13–7.24 (m, 3H), 7.33–7.38 (m, 2H), 7.46–7.48 (m, 2H), 7.67–7.71 (m, 2H), 8.82 (s, 1H), 9.03 (s, 1H), 10.49 (s, 1H), 11.08 (s, 1H). MS found $(M+H)^+$ (m/z), 432.08; calcd for $C_{22}H_{17}N_5O_3S$ m/z, 431.11.

4.1.15.5. 2-((1*H*-Indol-6-yl)amino)-8-methyl-6-(phenylsulfonyl)pyrido[2,3-*d*]pyrimidin-7-(8*H*)-one (7e): Starting from **6a** and 1*H*-indol-6-amine, 65% of **7e** was obtained as per the method described for the synthesis of **7**; mp >350 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.29 (s, 3H), 6.12 (s, 1H), 6.90–7.04 (m, 3H), 7.21–7.44 (m, 3H), 7.74–7.94 (m, 3H), 8.59 (s, 1H), 8.81 (s, 1H), 10.38 (s, 1H), 10.90 (s, 1H). MS found $(M+H)^+$ (m/z), 432.10; calcd for $C_{22}H_{17}N_5O_3S$ m/z, 431.11.

4.1.15.6. 2-((1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)amino)-8-methyl-6-(phenylsulfonyl)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7f): Starting from **6a** and 1*H*-pyrrolo[2,3-*b*]pyridin-5-amine, 68% of **7f** was obtained as per the method described for the synthesis of **7**; mp: 280–284 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.49 (s, 3H), 6.49 (s, 1H), 7.14–7.29 (m, 2H), 7.50–7.77 (m, 3H), 8.06–8.07 (m, 3H), 8.89 (s, 1H), 9.10 (s, 1H), 10.65 (s, 1H), 11.67 (s, 1H). MS found $(M+H)^+$ (m/z), 433.12; calcd for $C_{21}H_{16}N_6O_3S$ m/z, 432.10.

4.1.15.7. 8-Methyl-6-(phenylsulfonyl)-2-(quinolin-6-ylamino)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7g): Starting from **6a** and quinolin-6-amine, 65% of **7g** was obtained as per the method described for the synthesis of **7**; mp 348–350 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.50 (s, 3H), 6.80 (s, 1H), 7.42–7.49 (m, 2H), 7.62–7.71 (m, 3H), 7.82–

7.86 (m, 3H), 8.10–8.23 (m, 2H), 8.65 (s, 1H), 8.83 (s, 1H), 11.18 (s, 1H). MS found (M+H)$^+$ (*m*/*z*), 444.07; calcd for $C_{23}H_{17}N_5O_3S$ *m*/*z*, 443.11.

4.1.15.8. 2-((1*H*-Indol-4-yl)amino)-6-((4-chlorophenyl)sulfonyl)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7h): Starting from **6c** and 1*H*-indol-4-amine, 54% of **7h** was obtained as per the method described for the synthesis of **7**; mp 320–324 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.30 (s, 3H), 6.44 (s, 1H), 6.80–7.08 (m, 4H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.91 (d, *J* = 8.4 Hz, 2H), 8.75 (s, 1H), 8.98 (s, 1H), 10.30 (s, 1H), 11.07 (s, 1H). MS found (M+H)$^+$ (*m*/*z*), 466.10; calcd for $C_{22}H_{16}ClN_5O_3S$ *m*/*z*, 465.07.

4.1.15.9. 2-((1*H*-Indol-5-yl)amino)-6-((4-chlorophenyl)sulfonyl)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7i): Starting from **6c** and 1*H*-indol-5-amine, 58% of **7i** was obtained as per the method described for the synthesis of **7**; mp 316–320 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.40 (s, 3H), 6.40–6.43 (m, 1H), 7.29–7.30 (m, 3H), 7.36–7.37 (m, 2H), 8.01–8.18 (m, 3H), 8.80 (s, 1H), 9.03 (s, 1H), 10.30 (s, 1H), 11.07 (s, 1H). MS found (M+H)$^+$ (*m*/*z*), 466.08; calcd for $C_{22}H_{16}ClN_5O_3S$ *m*/*z*, 465.07.

4.1.15.10. 2-((1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)amino)-6-((4-chlorophenyl)sulfonyl)-8-methyl-pyrido[2,3-*d*]pyrimidin-7 (8*H*)-one (7j): Starting from **6c** and 1*H*-pyrrolo[2,3-*b*]pyridin-5-amine, 66% of **7j** was obtained as per the method described for the synthesis of **7**; mp 296–298 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.45 (s, 3H), 6.40 (s, 1H), 7.08–7.26 (m, 4H), 7.69–7.74 (m, 1H), 7.92–8.04 (m, 2H), 8.85 (s, 1H), 9.40 (s, 1H), 10.63 (s, 1H), 11.49 (s, 1H). MS found (M+H)$^+$ (*m*/*z*), 467.11; calcd for $C_{21}H_{15}ClN_6O_3S$ *m*/*z*, 466.06.

4.1.15.11. 6-((4-Chlorophenyl)sulfonyl)-8-methyl-2-(quinolin-3-ylamino)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7k): Starting from **6c** and quinolin-3-amine, 61% of **7k** was obtained as per the method described for the synthesis of **7**; mp: 304–306 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.40 (s, 3H), 7.12 (s, 1H), 7.42 (s, 1H),7.50–7.51 (m, 1H), 7.55–7.56 (m, 1H), 7.62 (d, *J* = 8.0 Hz, 2H), 7.83 (s, 1H), 7.86 (s, 1H), 8.25 (s, 2H), 8.88 (s, 1H), 9.12 (s, 1H), 10.38 (s, 1H). MS found (M+H)$^+$ (*m*/*z*), 478.05; calcd for $C_{23}H_{16}ClN_5O_3S$ *m*/*z*, 477.07.

4.1.15.12. 2-((1*H*-Indol-5-yl)amino)-6-((4-bromophenyl)sulfonyl)-8-methylpyrido[2,3-*d*]-pyrimidin-7(8*H*)-one (7l): Starting from **6d** and 1*H*-indol-5-amine, 63% of **7l** was obtained as per the method described for the synthesis of **7**; mp: 316–320 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.53 (s, 3H), 6.47 (s, 1H), 7.38–7.52 (m, 3H), 7.88 (d, *J* = 8.8 Hz, 2H), 7.98 (d, *J* = 8.8 Hz, 2H), 8.08 (s, 1H), 8.87 (s, 1H), 9.09 (s, 1H), 10.58 (s, 1H), 11.14 (s, 1H). MS found (M+H)$^+$ (*m*/*z*), 510.04; calcd for $C_{22}H_{16}BrN_5O_3S$ *m*/*z*, 509.02.

4.1.15.13. 2-((1*H*-Indol-4-yl)amino)-6-((4-fluorophenyl)sulfonyl)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7m): Starting from **6b** and 1*H*-indol-4-amine, 57% of **7m** was obtained as per the method described for the synthesis of **7**; mp >350 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.93 (s, 3H), 6.61–6.65 (m, 1H), 7.08–7.14 (m, 3H), 7.41–7.56 (m, 3H), 8.04–8.08 (m, 2H), 8.80 (s, 1H), 9.07 (s, 1H), 10.35 (s, 1H), 11.16 (s, 1H). MS found (M+H)$^+$ (*m*/*z*), 450.12; calcd for $C_{22}H_{16}FN_5O_3S$ *m*/*z*, 449.10.

4.1.15.14. 2-((1*H*-Indol-5-yl)amino)-6-((4-fluorophenyl)sulfonyl)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7n): Starting from **6b** and 1*H*-indol-5-amine, 63% of **7n** was obtained as per the method described for the synthesis of **7**; mp: 278–282 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.58 (s, 3H), 6.51 (s, 1H), 7.42–7.47 (m, 3H), 7.53–7.57 (m, 2H), 8.15–8.19 (m, 3H), 8.90 (s, 1H), 9.10 (s, 1H), 10.50 (s, 1H), 11.10 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 450.20; calcd for $C_{22}H_{16}FN_5O_3S$ *m*/*z*, 449.10.

4.1.15.15. 2-((1*H*-Indol-6-yl)amino)-6-((4-fluorophenyl)sulfonyl)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7o): Starting from **6b** and 1*H*-indol-6-amine, 56% of **7o** was obtained as per the method described for the synthesis of **7**; mp >350 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.30 (s, 3H), 6.12 (s, 1H), 7.05–7.23 (m, 5H), 7.83–7.91 (m, 3H), 8.58 (s, 1H), 8.80 (s, 1H), 10.18 (s, 1H), 10.89 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 450.10; calcd for $C_{22}H_{16}FN_5O_3S$ *m*/*z*, 449.10.

4.1.15.16. 2-((1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)amino)-6-((4-fluorophenyl)sulfonyl)-8-methyl-pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7p): Starting from **6b** and 1*H*-pyrrolo[2,3-*b*]pyridin-5-amine, 53% of **7p** was obtained as per the method described for the synthesis of **7**; mp 274–280 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.34 (s, 3H), 6.44–6.50 (m, 1H), 7.44–7.49 (m, 4H), 8.02–8.09 (m, 3H), 8.84 (s, 1H), 9.07 (s, 1H),10.70 (s, 1H), 11.50 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 451.07; calcd for $C_{21}H_{15}FN_6O_3S$ *m*/*z*, 450.09.

4.1.15.17. 2-((1*H*-Indazol-5-yl)amino)-6-((4-fluorophenyl)sulfonyl)-8-methylpyrido[2,3-*d*]-pyrimidin-7(8*H*)-one (7q): Starting from **6b** and 1*H*-indazol-5-amine, 65% of **7q** was obtained as per the method described for the synthesis of **7**; mp 160–165 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.35 (s, 3H), 7.31–7.35 (m, 4H), 7.91–7.94 (m, 4H), 8.69 (s, 1H), 8.92 (s, 1H),10.30 (s, 1H), 12.50 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 451.10; calcd for $C_{21}H_{15}FN_6O_3S$ *m*/*z*, 450.09.

4.1.15.18. 2-((1*H*-Benzo[d]imidazol-5-yl)amino)-6-((4-fluorophenyl)sulfonyl)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7r): Starting from **6b** and 1*H*-benzo[*d*]imidazol-5-amine, 70% of **7r** was obtained as per the method described for the synthesis of **7**; mp: 320–324 °C; ^{1}H NMR (600 MHz; DMSO-d_6): δ 3.61 (s, 3H), 5.38 (s, 1H), 6.70–6.72 (m, 1H), 6.84–6.85 (m, 1H), 7.28–7.30 (m, 3H), 6.84–6.85 (m, 1H), 8.09–8.11 (m, 3H), 9.08 (s, 1H), 9.40 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 451.08; calcd for $C_{21}H_{15}FN_6O_3S$ *m*/*z*, 450.09.

4.1.15.19. 2-(Benzofuran-5-ylamino)-6-((4-fluorophenyl)sulfonyl)-8-methylpyrido[2,3-*d*]-pyrimidin-7(8*H*)-one (7s): Starting from **6b** and benzofuran-5-amine, 68% of **7s** was obtained as per the method described for the synthesis of **7**; mp 296–298 °C; ^{1}H NMR (600 MHz; DMSO-d_6): δ 3.48 (s, 3H), 6.96 (s, 1H), 7.44 (t, 2H), 7.57–7.66 (m, 2H), 7.96 (s, 1H), 8.05–8.06 (m, 2H), 8.13 (s, 1H), 8.83 (s, 1H), 9.06 (s, 1H), 10.66 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 451.10; calcd for $C_{22}H_{15}FN_4O_4S$ *m*/*z*, 450.08.

4.1.15.20. 2-((1*H*-Indol-5-yl)amino)-6-((4-hydroxyphenyl)sulfonyl)-8-methylpyrido[2,3-*d*]-pyrimidin-7(8*H*)-one (7t): Starting from **6e** and 1*H*-indol-5-amine, 58% of **7t** was

obtained as per the method described for the synthesis of **7**; mp 288–290 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.55 (s, 3H), 6.39 (s, 1H), 6.89 (d, J = 8.0 Hz, 2H), 7.01–7.70 (m, 4H), 7.80 (d, J = 8.2 Hz, 2H), 8.72 (s, 1H), 9.08 (s, 1H), 10.55 (s, 1H), 11.29 (s, 1H). MS found $(M+H)^+$ (m/z), 448.08; calcd for $C_{22}H_{17}N_5O_4S$ m/z, 447.10.

4.1.15.21. Synthesis of 4-(2-(1*H*-indol-5-ylamino)-7,8-dihydro-8-methyl-7-oxopyrido[2,3-*d*]pyrimidin-6-ylsulfonyl)phenyl 5-(hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazol-6-yl)pentan-oate (7u): EDC (1-ethyl-3-[(3-dimethylamino)propyl]-carbodiimide) (4 mmol) was added to the solution of 2-((1*H*-indol-5-yl)amino)-6-((4-hydroxyphenyl)sulfonyl)-8-methylpyrido[2,3-*d*]pyrimidin-7 (8*H*)-one **7t** (2.15 mmol), biotin (2 mmol) and 4-dimethylaminopyridine (2 mmol) in DMF at °C and stirred the reaction for 24 h at room temperature. After completion of the reaction (monitored by LC–MS) the reaction mixture was filtered and the filtrate was concentrated. The crude product was purified using combiflash to get the pure product. Yield 45%, mp: 310–312 °C; ^{1}H NMR (600 MHz; DMSO-d_6): δ 1.35–1.62 (m, 6H), 2.20 (t, 2H), 2.57 (d, J = 12.4 Hz, 1H), 2.80–2.83 (m, 1H), 3.08–3.11 (m, 1H), 3.58 (s, 3H), 4.10–4.12 (m, 1H), 4.28–4.30 (m, 1H), 6.37 (br s, 1H), 6.37 (s, 1H), 6.45 (br s, 1H), 6.88 (d, J = 8.2 Hz, 2H), 7.08–7.75 (m, 4H), 7.84 (d, J = 8.2 Hz, 2H), 8.73 (s, 1H), 9.11 (s, 1H), 10.58 (s, 1H), 11.27 (s, 1H). MS found $(M+H)^+$ (m/z), 674.18; calcd for $C_{32}H_{31}N_7O_6S_2$ m/z, 673.18.

4.1.15.22. 2-((1*H*-Indol-4-yl)amino)-8-methyl-6-tosylpyrido [2,3-*d*]pyrimidin-7(8*H*)-one (7v): Starting from **6f** and 1*H*-indol-4-amine, 70% of **7v** was obtained as per the method described for the synthesis of **7**; mp 295–300 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 2.38 (s, 3H), 3.53 (s, 3H), 6.47 (s, 1H), 7.39–7.54 (m, 5H), 7.94 (d, J = 8.0 Hz, 2H), 8.09 (s, 1H), 8.85 (s, 1H), 9.08 (s, 1H), 10.54 (s, 1H), 11.13 (s, 1H). MS found $(M+H)^+$ (m/z), 446.10; calcd for $C_{23}H_{19}N_5O_3S$ m/z, 445.12.

4.1.15.23. 2-((1*H*-Indol-5-yl)amino)-8-methyl-6-tosylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7w): Starting from **6f** and 1*H*-indol-5-amine, 67% of **7w** was obtained as per the method described for the synthesis of **7**; mp 310–314 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 2.39 (s, 3H), 3.42 (s, 3H), 6.67–6.69 (m, 1H), 7.12–7.42 (m, 5H), 7.70 (s, 1H), 7.90 (d, J = 7.2 Hz, 1H), 8.84 (s, 1H), 9.07 (s, 1H), 10.36 (s, 1H), 11.18 (s, 1H). MS found $(M+H)^+$ (m/z), 446.11; calcd for $C_{23}H_{19}N_5O_3S$ m/z, 445.12.

4.1.15.24. 2-((1*H*-Indol-6-yl)amino)-8-methyl-6-tosylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7x): Starting from **6f** and 1*H*-indol-6-amine, 70% of **7x** was obtained as per the method described for the synthesis of **7**; mp 334–336 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 2.38 (s, 3H), 3.54 (s, 3H), 6.38 (s, 1H), 7.04–7.49 (m, 5H), 7.90–8.01 (m, 2H), 8.17 (s, 1H), 8.85 (s, 1H), 9.05 (s, 1H), 10.58 (s, 1H), 11.15 (s, 1H). MS found $(M+H)^+$ (m/z), 446.08; calcd for $C_{23}H_{19}N_5O_3S$ m/z, 445.12.

4.1.15.25. 2-((1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)amino)-8-methyl-6-tosylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7y): Starting from **6f** and 1*H*-pyrrolo[2,3-*b*]pyridin-5-amine, 62% of **7y** was obtained as per the method described for the synthesis of **7**; mp 252–255 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 2.43 (s, 3H), 3.46 (s, 3H), 6.60 (s, 1H), 7.25–7.37 (m, 2H),

7.54–7.59 (m, 2H), 7.98–8.03 (m, 3H), 8.89 (s, 1H), 9.08 (s, 1H), 10.55 (s, 1H), 11.23 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 447.13; calcd for $C_{22}H_{18}N_6O_3S$ *m*/*z*, 446.12.

4.1.15.26. 8-Methyl-2-((2-oxo-2,3-dihydro-1*H*-pyrrolo[2,3-*b*] pyridin-5-yl)amino)-6-tosyl-pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7z): Starting from **6f** and 5-amino-1*H*-pyrrolo[2,3-*b*]pyridin-2(3*H*)-one, 62% of **7z** was obtained as per the method described for the synthesis of **7**; mp 280 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 2.39 (s, 3H), 3.47 (s, 3H), 3.69 (s, 2H), 6.31–6.67 (m, 2H), 7.03–7.48 (m, 3H), 7.61–7.74 (m, 2H), 8.67 (s, 1H), 8.90 (s, 1H), 9.91 (s, 1H), 10.27 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 463.07; calcd for $C_{22}H_{18}N_6O_4S$ *m*/*z*, 462.11.

4.1.15.27. 8-Methyl-2-(quinolin-8-ylamino)-6-tosylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7aa): Starting from **6f** and quinolin-8-amine, 58% of **7aa** was obtained as per the method described for the synthesis of **7**; mp >350 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 2.22 (s, 3H), 3.50 (s, 3H), 7.40–7.43 (m, 4H), 7.55–7.56 (m, 2H), 7.74 (d, J = 8.0 Hz, 2H), 8.25–8.32 (m, 2H), 8.68 (s, 1H), 8.82 (s, 1H), 10.20 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 458.10; calcd for $C_{24}H_{19}N_5O_3S$ *m*/*z*, 457.12.

4.1.15.28. 8-Methyl-2-(quinolin-6-ylamino)-6-tosylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7ab): Starting from **6f** and quinolin-6-amine, 59% of **7ab** was obtained as per the method described for the synthesis of **7**; mp 325–330 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 2.34 (s, 3H), 3.40 (s, 3H), 6.80 (s, 1H), 7.42–7.43 (m, 3H), 7.48–7.49 (m, 1H), 7.75 (d, J = 8.2 Hz, 2H), 7.82–7.83 (m, 1H), 8.15–8.25 (m, 2H), 8.60 (s, 1H), 8.81 (s, 1H), 10.60 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 458.04; calcd for $C_{24}H_{19}N_5O_3S$ *m*/*z*, 457.12.

4.1.15.29. 2-((1*H*-Indol-4-yl)amino)-6-((4-methoxyphenyl)sulfonyl)-8-methylpyrido[2,3-*d*]-pyrimidin-7(8*H*)-one (7ac): Starting from **6g** and 1*H*-indol-4-amine, 62% of **7ac** was obtained as per the method described for the synthesis of **7**; mp 289–292 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.31 (s, 3H), 3.79 (s, 3H), 6.60 (s, 1H), 7.03–7.50 (m, 6H), 7.87–7.90 (m, 2H), 8.75 (s, 1H), 9.01 (s, 1H), 10.28 (s, 1H), 11.10 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 462.15; calcd for $C_{23}H_{19}N_5O_4S$ *m*/*z*, 461.12.

4.1.15.30. 2-((1*H*-Indol-5-yl)amino)-6-((4-methoxyphenyl)sulfonyl)-8-methylpyrido[2,3-*d*]-pyrimidin-7(8*H*)-one (7ad): Starting from **6g** and 1*H*-indol-5-amine, 63% of **7ad** was obtained as per the method described for the synthesis of **7**; mp 260–262 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.25 (s, 3H), 3.60 (s, 3H), 6.17 (s, 1H), 6.74–7.38 (m, 6H), 7.62–7.79 (m, 2H), 8.53 (s, 1H), 8.78 (s, 1H), 10.23 (s, 1H), 10.84 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 462.11; calcd for $C_{23}H_{19}N_5O_4S$ *m*/*z*, 461.12.

4.1.15.31. 4-((2-((1*H*-Indol-5-yl)amino)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidin-6-yl)sulfonyl)benzoic acid (7ae): Starting from **6h** and 1*H*-indol-5-amine, 69% of **7ae** was obtained as per the method described for the synthesis of **7**; mp 306–310 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.87 (s, 3H), 6.39 (s, 1H), 7.35–7.46 (m, 4H), 8.01–8.12 (m, 4H), 8.84 (s, 1H), 9.03 (s, 1H), 10.53 (s, 1H), 11.07 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 476.12; calcd for $C_{23}H_{17}N_5O_5S$ *m*/*z*, 475.10.

4.1.15.32. 2-((1*H*-Indol-5-yl)amino)-6-((2,4-dichlorophenyl)sulfonyl)-8-methylpyrido[2,3-*d*]-pyrimidin-7(8*H*)-one (7af): Starting from **6j** and 1*H*-indol-5-amine, 69% of **7af** was obtained as per the method described for the synthesis of **7**; mp 323–326 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.46 (s, 3H), 6.41 (s, 1H), 7.34–7.49 (m, 4H), 7.76–7.83 (m, 2H), 8.03–8.26 (m, 2H), 8.88 (s, 1H), 9.08 (s, 1H), 10.59 (s, 1H), 11.09 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 500.90; calcd for $C_{22}H_{15}Cl_2N_5O_3S$ *m*/*z*, 499.03.

4.1.15.33. 6-((2,4-Dichlorophenyl)sulfonyl)-8-methyl-2-((1-methyl-1*H*-indol-5-yl)amino)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7ag): Starting from **6j** and 1-methyl-1*H*-indol-5-amine, 6a% of **7ag** was obtained as per the method described for the synthesis of **7**;mp 290–292 °C; ^{1}H NMR (600 MHz; DMSO-d_6): δ 3.44 (s, 3H), 3.76 (s, 3H), 6.39 (s, 1H), 7.30 (s, 1H), 7.41 (d, J = 9.0 Hz, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.75 (d, J = 8.4 Hz, 1H), 7.82 (s, 1H), 7.99 (s, 1H), 8.24 (d, J = 9.0 Hz, 1H), 8.87 (s, 1H), 9.07 (s, 1H), 10.60 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 514.02; calcd for $C_{23}H_{17}Cl_2N_5O_3S$ *m*/*z*, 513.04.

4.1.15.34. 2-((1*H*-Indol-5-yl)amino)-6-((4-fluoro-2-nitrophenyl)-sulfonyl)-8-methylpyrido-[2,3-*d*]pyrimidin-7(8*H*)-one (7ah): Starting from **6k** and 1*H*-indol-5-amine, 62% of **7ah** was obtained as per the method described for the synthesis of **7**; mp 290–292 °C; ^{1}H NMR: (600 MHz, DMSO-d_6): δ 3.51 (s, 3H), 6.41 (s, 1H), 7.33–7.47 (m, 3H), 7.85 (s, 1H), 8.03–8.09 (m, 2H), 8.47 (s, 1H), 8.68 (s, 1H), 9.11 (s, 1H), 10.62 (s, 1H), 11.08 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 495.10; calcd for $C_{22}H_{15}FN_6O_5S$ *m*/*z*, 494.08.

4.1.15.35. 2-((1*H*-Indol-5-yl)amino)-6-((2-amino-4-fluorophenyl)sulfonyl)-8-methylpyrido-[2,3-*d*]pyrimidin-7(8*H*)-one (7ai): Starting from **6l** and 1*H*-indol-5-amine, 63% of **7ai** was obtained as per the method described for the synthesis of **7**; mp 330–332 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.94 (s, 3H), 6.45 (s, 1H), 7.21–7.51 (m, 5H), 8.01–8.07 (m, 2H), 8.89 (s, 1H), 9.04 (s, 1H), 10.66 (s, 1H), 11.11 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 465.11; calcd for $C_{22}H_{17}FN_6\ O_3S$ *m*/*z*, 464.11.

4.1.15.36. 2-((1*H*-Indol-5-yl)amino)-6-((2-fluoro-4-nitrophenyl)-sulfonyl)-8-methylpyrido-[2,3-*d*]pyrimidin-7(8*H*)-one (7aj): Starting from **6m** and 1*H*-indol-5-amine, 67% of **7aj** was obtained as per the method described for the synthesis of **7**; mp 338–340 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.46 (s, 3H),6.41 (s, 1H), 7.34–7.38 (m, 2H), 7.47–7.49 (m, 1H), 8.03 (s, 1H), 8.30–8.36 (m, 3H), 8.91 (s, 1H), 9.10 (s, 1H), 10.62 (s, 1H), 11.08 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 495.08; calcd for $C_{22}H_{15}FN_6O_5S$ *m*/*z*, 494.08.

4.1.15.37. 2-((1*H*-Indol-5-yl)amino)-6-((4-amino-2-fluorophenyl)sulfonyl)-8-methylpyrido-[2,3-*d*]pyrimidin-7(8*H*)-one (7ak): Starting from **6n** and 1*H*-indol-5-amine, 69% of **7ak** was obtained as per the method described for the synthesis of **7**; mp 292–294 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.49 (s, 3H),6.27 (m, 1H), 6.39–6.41 (m, 3H), 6.47 (d, J = 8.4 Hz, 1H), 7.33–7.37 (m, 2H), 7.47–7.49 (m, 1H), 7.61–7.64 (m, 1H), 8.03 (s, 1H), 8.71 (s, 1H), 9.04 (s, 1H), 10.41 (s, 1H), 11.04 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 465.11; calcd for $C_{22}H_{17}FN_6O_3S$ *m*/*z*, 464.11.

4.1.15.38. 6-((2,4-Difluorophenyl)sulfonyl)-8-methyl-2-(pyridin-2-ylamino)pyrido[2,3-*d*]-pyrimidin-7(8*H*)-one (7al): Starting from **6i** and 2-aminopyridine, 68% of **7al** was

obtained as per the method described for the synthesis of **7**; mp >350 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.50 (s, 3H), 7.17 (d, J = 8.4 Hz, 1H), 7.23–7.25 (m, 1H), 7.38–7.40 (m, 1H), 7.52–7.54 (m, 2H), 8.11–8.12 (m, 2H), 8.26 (d, J = 8.4 Hz, 1H), 8.97 (s, 1H), 9.21 (s, 1H), 10.87 (s, 1H). MS found $(M+H)^+$ (m/z), 430.07; calcd for $C_{19}H_{13}F_2N_5O_3S$ m/z, 429.07.

4.1.15.39. 6-(2,4-difluorophenylsulfonyl)-8-methyl-2-(pyrimidin-4-ylamino)pyrido[2,3-*d*]-pyrimidin-7(8*H*)-one (7am): Starting from **6i** and 2-aminopyrimidine, 60% of **7am**, was obtained as per the method described for the synthesis of **7**; mp >330 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.52 (s, 3H), 6.68–6.74 (m, 2H), 7.27–7.29 (m, 1H), 7.35–7.48 (m, 1H), 7.54–7.57 (m, 1H), 8.21 (d, J = 7.8 Hz, 1H), 8.95 (s, 1H), 9.25 (s, 1H), 10.89 (s, 1H). MS found $(M+H)^+$ (m/z), 431.07; calcd for $C_{18}H_{12}F_2N_6O_3S$ m/z, 430.07.

4.1.15.40. 2-((1*H*-Indol-4-yl)amino)-6-((2,4-difluorophenyl)sulfonyl)-8-methylpyrido[2,3-*d*]-pyrimidin-7(8*H*)-one (7an): Starting from **6i** and 1*H*-indol-4-amine, 71% of **7an** was obtained as per the method described for the synthesis of **7**; mp 334–336 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.34 (s, 3H), 6.62–6.75 (m, 1H), 7.09–7.58(m, 6H), 8.12–8.15 (m, 1H), 8.89 (s, 1H), 9.12 (s, 1H), 10.45 (s, 1H), 11.17 (s, 1H). MS found $(M+H)^+$ (m/z), 468.09; calcd for $C_{22}H_{15}F_2N_5O_3S$ m/z, 467.09.

4.1.15.41. 2-((1*H*-Indol-5-yl)amino)-6-((2,4-difluorophenyl)sulfonyl)-8-methylpyrido[2,3-*d*]-pyrimidin-7(8*H*)-one (7ao): Starting from **6i** and 1*H*-indol-5-amine, 86% of **7ao** was obtained as per the method described for the synthesis of **7**; mp >350 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.60 (s, 3H), 6.50 (s, 1H), 7.20–8.40 (m, 7H), 8.80 (s, 1H), 9.10 (s, 1H), 10.50 (s, 1H), 11.30 (s, 1H). MS found $(M+H)^+$ (m/z), 468.10; calcd for $C_{22}H_{15}F_2N_5O_3S$ m/z, 467.09.

4.1.15.42. 2-((1*H*-Indol-6-yl)amino)-6-((2,4-difluorophenyl)sulfonyl)-8-methylpyrido[2,3-*d*]-pyrimidin-7(8*H*)-one (7ap): Starting from **6i** and 1*H*-indol-6-amine, 75% of **7ap** was obtained as per the method described for the synthesis of **7**; mp >350 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.54 (s, 3H), 6.37 (s, 1H), 7.30–7.32 (m, 2H), 7.38–7.41 (m, 1H), 7.47–7.52 (m, 2H), 8.13–8.17 (m, 2H), 8.88 (s, 1H), 9.10 (s, 1H), 10.67 (s, 1H), 11.16 (s, 1H). MS found $(M+H)^+$ (m/z), 468.09; calcd for $C_{22}H_{15}F_2N_5O_3S$ m/z, 467.09.

4.1.15.43. 2-((1*H*-Indol-5-yl)amino)-6-((4-fluorobenzyl)sulfonyl)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7aq): Starting from **6o** and 1*H*-indol-5-amine, 68% of **7aq** was obtained as per the method described for the synthesis of **7**; mp 260–262 °C; ^{1}H NMR (400 MHz; DMSO-d_6): δ 3.66 (s, 3H), 4.81 (s, 2H), 6.44 (s, 1H), 7.16 (t, 2H), 7.30–7.33 (m, 5H), 7.35–7.40 (m, 1H), 8.37 (s, 1H), 8.92 (s, 1H), 10.25 (s, 1H), 11.10 (s, 1H). MS found $(M+H)^+$ (m/z), 464.10; calcd for $C_{23}H_{18}FN_5O_3S$ m/z, 463.11.

4.1.15.44. 2-((1*H*-Indol-5-yl)amino)-6-((2,4-difluorobenzyl)sulfonyl)-8-methylpyrido[2,3-*d*]-pyrimidin-7(8*H*)-one (7ar): Starting from **6p** and 1*H*-indol-5-amine, 77% of **7ar** was obtained as per the method described for the synthesis of **7**; mp 228–230 °C; ^{1}H NMR (600 MHz; DMSO-d_6): δ 3.65 (s, 3H), 4.84 (s, 2H), 6.44 (s, 1H), 7.10–

7.49 (m, 6H), 8.04–8.05 (m, 1H), 8.40 (s, 1H), 8.94 (s, 1H), 10.55 (s, 1H), 11.11 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 482.10; calcd for $C_{23}H_{17}F_2N_5O_3S$ *m*/*z*, 481.10.

4.1.15.45. 2-((1*H*-Indol-5-yl)amino)-N-(4-fluorophenyl)-8-methyl-7-oxo-7,8-dihydropyrido-[2,3-*d*]pyrimidine-6-carboxamide (7as): Starting from **6q** and 1*H*-indol-5-amine, 72% of **7as** was obtained as per the method described for the synthesis of **7**; mp 284–286 °C; ^{1}H NMR (600 MHz; DMSO-d_6): δ 3.69 (s, 3H), 6.43 (s, 1H), 7.20–7.49 (m, 5H), 7.75–8.06 (m, 3H), 8.81 (s, 1H), 9.04 (s, 1H), 10.45 (s, 1H), 11.08 (s, 1H), 11.73 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 429.14; calcd for $C_{23}H_{17}FN_6O_2$ *m*/*z*, 428.14.

4.1.15.46. 2-((1*H*-Indol-5-yl)amino)-*N*-(2,4-difluorophenyl)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidine-6-carboxamide (7at): Starting from **6r** and 1*H*-indol-5-amine, 64% of **7at** was obtained as per the method described for the synthesis of **7**; mp 322–324 °C; ^{1}H NMR (600 MHz; DMSO-d_6): δ 3.68 (s, 3H), 6.43 (s, 1H), 7.11–7.49 (m, 5H), 8.05–8.06 (m, 1H), 8.47–8.48 (m, 1H), 8.83 (s, 1H), 9.05 (s, 1H), 10.47 (s, 1H), 11.09 (s, 1H), 12.02 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 447.13; calcd for $C_{23}H_{16}F_2N_6O_2$ *m*/*z*, 446.13.

4.1.15.47. 2-((1*H*-Indol-5-yl)amino)-*N*-(4-fluorophenyl)-8-methyl-7-oxo-7,8-dihydropyrido-[2,3-*d*]pyrimidine-6-sulfonamide (7au): Starting from **6s** and 1*H*-indol-5-amine, 75% of **7au** was obtained as per the method described for the synthesis of **7**; mp 324–326 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.55 (s, 3H), 6.39 (s, 1H), 7.05–7.12 (m, 4H), 7.32–7.43 (m, 3H), 7.99 (s, 1H), 8.52 (s, 1H), 8.90 (s, 1H), 10.10 (s, 1H), 10.41 (s, 1H), 11.06 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 465.12; calcd for $C_{22}H_{17}FN_6O_3S$ *m*/*z*, 464.11.

4.1.15.48. 2-((1*H*-Indol-5-yl)amino)-*N*-(2,4-difluorophenyl)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidine-6-sulfonamide (7av): Starting from **6t** and 1*H*-indol-5-amine, 65% of **7av** was obtained as per the method described for the synthesis of **7**; mp 332–334 °C; ^{1}H NMR (600 MHz; DMSO-d_6): δ 3.62 (s, 3H), 6.42 (s, 1H), 7.00–7.48 (m, 6H), 8.04 (s, 1H), 8.38 (s, 1H), 8.89 (s, 1H), 9.74 (s, 1H), 10.43 (s, 1H), 11.08 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 483.10; calcd for $C_{22}H_{16}F_2N_6O_3S$ *m*/*z*, 482.10.

4.1.15.49. 2-((1*H*-Indazol-5-yl)amino)-6-((2,4-difluorophenyl)-sulfonyl)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7aw): Starting from **6i** and 1*H*-indazol-5-amine, 68% of **7aw** was obtained as per the method described for the synthesis of **7**; mp 284–286 °C; ^{1}H NMR (300 MHz; DMSO-d_6): δ 3.51 (s, 3H), 7.35–7.53 (m, 3H), 7.61–7.66 (m, 1H), 7.93–8.24 (m, 3H), 8.87 (s, 1H), 9.17 (s, 1H), 10.71 (s, 1H), 13.02 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 469.10; calcd for $C_{21}H_{14}F_2N_6O_3S$ *m*/*z*, 468.08.

4.1.15.50. 2-(Benzo[*b*]thiophen-5-ylamino)-6-((2,4-difluorophenyl)sulfonyl)-8-methylpyrido-[2,3-*d*]pyrimidin-7(8*H*)-one (7ax): Starting from **6i** and benzo[*b*]thiophenl-5-amine, 61% of **7ax** was obtained as per the method described for the synthesis of **7**; mp 320–322 °C; ^{1}H NMR (600 MHz; DMSO-d_6): δ 3.50 (s, 3H), 7.39–7.50 (m, 3H), 7.71–7.75 (m, 2H), 7.97 (d, J = 8.0 Hz, 1H), 8.13–8.14 (m, 1H), 8.41 (s, 1H), 8.90 (s, 1H), 9.14 (s, 1H), 10.79 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 485.10; calcd for $C_{22}H_{14}F_2N_4O_3S_2$ *m*/*z*, 484.05.

4.1.15.51. 2-(Benzofuran-5-ylamino)-6-((2,4-difluorophenyl)-sulfonyl)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7ay): Starting from **6i** and benzofuran-5-amine, 64% of **7ay** was obtained as per the method described for the synthesis of **7**; mp 318–320 °C; ^{1}H NMR (600 MHz; DMSO-d_6): δ 3.47 (s, 3H), 6.97 (s, 1H), 7.38 (t, 1H), 7.49 (t, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.66 (s, 1H), 7.97 (d, J = 1.8 Hz, 1H), 8.09–8.14 (m, 2H), 8.88 (s, 1H), 9.12 (s, 1H), 10.72 (s, 1H). MS found $(M+H)^+$ (m/z), 469.10; calcd for $C_{22}H_{14}F_2N_4O_4S$ m/z, 468.07.

4.1.15.52. 6-((2,4-Difluorophenyl)sulfonyl)-8-methyl-2-((1-methyl-1*H*-indol-5-yl)amino)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7az): Starting from **6i** and 1-methyl-1*H*-indol-5-amine, 72% of **7az** was obtained as per the method described for the synthesis of **7**; mp 330–332 °C; ^{1}H NMR (600 MHz; DMSO-d_6): δ 3.45 (s, 3H), 3.76 (s, 3H), 6.39 (s, 1H), 7.30–7.49 (m, 5H), 7.99 (s, 1H), 8.11–8.13 (m, 1H), 8.84 (s, 1H), 9.07 (s, 1H), 10.57 (s, 1H). MS found $(M+H)^+$ (m/z), 482.20; calcd for $C_{23}H_{17}F_2N_5O_3S$ m/z, 481.10.

4.1.15.53. 6-((2,4-Difluorophenyl)sulfonyl)-8-methyl-2-((2-methyl-1*H*-indol-5-yl)amino)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7aaa): Starting from **6i** and 2-methyl-1*H*-indol-5-amine, 70% of **7aaa** was obtained as per the method described for the synthesis of **7**; mp 288–290 °C; ^{1}H NMR (300 MHz; DMSO-d_6): δ 2.36 (s, 3H), 3.46 (s, 3H), 6.10 (s, 1H), 7.23–8.15 (m, 6H), 8.85 (s, 1H), 9.07 (s, 1H), 10.52 (s, 1H), 10.90 (s, 1H). MS found $(M+H)^+$ (m/z), 482.07; calcd for $C_{23}H_{17}F_2N_5O_3S$ m/z, 481.10.

4.1.15.54. 2-(((1*H*-Indol-5-yl)methyl)amino)-6-((2,4-difluorophenyl)sulfonyl)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7aab): Starting from **6i** and (1*H*-indol-5yl)methanamine, 75% of **7aab** was obtained as per the method described for the synthesis of **7**; mp 216–220 °C; ^{1}H NMR (600 MHz; DMSO-d_6): δ 3.42 (s, 3H), 4.64 (d, J = 6.0 Hz, 2H), 7.06–7.51 (m, 6H), 8.09 (t, 1H), 8.76 (s, 1H), 8.93 (s, 1H), 9.02 (s, 1H), 9.10 (t, 1H), 11.01 (s, 1H). MS found $(M+H)^+$ (m/z), 482.20; calcd for $C_{23}H_{17}F_2N_5O_3S$ m/z, 481.10.

4.1.15.55. Methyl 2-(5-((6-((2,4-difluorophenyl)sulfonyl)-8-methyl-7-oxo-7,8-dihydropyrido-[2,3-*d*]pyrimidin-2-yl)amino)-1*H*-indol-1-yl)acetate (7aac): Starting from **6i** and methyl 2-(5-amino-1*H*-indol-1yl)acetate, 65% of **7aac** was obtained as per the method described for the synthesis of **7**; mp 296–298 °C; ^{1}H NMR (600 MHz; DMSO-d_6): δ 3.48 (s, 3H), 3.80 (s, 3H), 5.05 (s, 2H), 6.46 (s, 1H), 7.35–7.59 (m, 5H), 8.03–8.16 (m, 2H), 8.85 (s, 1H), 9.10 (s, 1H), 10.62 (s, 1H). MS found $(M+H)^+$ (m/z), 540.11; calcd for $C_{25}H_{19}F_2N_5O_5S$ m/z, 539.11.

4.1.15.56. 5-((6-((2,4-Difluorophenyl)sulfonyl)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-*d*]-pyrimidin-2-yl)amino)indoline-2,3-dione (7aad): Starting from **6i** and methyl 5-aminoindolin-2,3-dione, 64% of **7aad** was obtained as per the method described for the synthesis of **7**; mp 312–314 °C; ^{1}H NMR (600 MHz; DMSO-d_6): δ 3.55 (s, 3H), 3.80 (s, 3H), 6.61–6.70 (m, 4H), 6.71–6.81 (m, 1H), 7.27–7.34 (m, 1H), 8.20 (s, 1H), 8.91 (s, 1H), 10.56 (s, 1H), 10.96 (s, 1H). MS found $(M+H)^+$ (m/z), 498.10; calcd for $C_{22}H_{13}F_2N_5O_5S$ m/z, 497.06.

4.1.15.57. 6-((2,4-Difluorophenyl)sulfonyl)-2-((8-hydroxyquinolin-2-yl)amino)-8-methyl-pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7aae): Starting from **6i** and 2-aminoquinolin-8-ol, 61% of **7aae** was obtained as per the method described for the synthesis of **7**; mp 320–322 °C. ^{1}H NMR (600 MHz; DMSO-d_6): δ 3.49 (s, 3H), 6.71–6.81 (m, 1H), 6.93–6.96 (m, 2H), 7.15–7.82 (m, 4H), 8.20 (d, J = 8.2 Hz, 1H), 9.06 (s, 1H), 9.18 (s, 1H), 10.60 (s, 1H), 10.64 (s, 1H). MS found(M+H)$^+$ (*m*/*z*), 496.09; calcd for $C_{23}H_{15}F_2N_5O_4S$*m*/*z*, 495.08.

4.1.15.58. 6-((2,4-Difluorophenyl)sulfonyl)-8-methyl-2-((2-oxoindolin-5-yl)amino)pyrido-[2,3-*d*]pyrimidin-7(8*H*)-one (7aaf): Starting from **6i** and 5-aminoindolin-2-one, 67% of **7aaf** was obtained as per the method described for the synthesis of **7**; mp 308–310 °C; ^{1}H NMR (600 MHz; DMSO-d_6): δ 3.42 (s, 2H), 3.48 (s, 3H), 6.53–6.79 (m, 2H), 6.53–6.79 (m, 4H), 9.25 (s, 1H), 9.42 (s, 1H), 10.28 (s, 1H), 11.12 (s, 1H). MS found (M+H)$^+$ (*m*/*z*), 484.05; calcd for $C_{22}H_{15}F_2N_5O_4S$ *m*/*z*, 483.08.

4.1.15.59. 5-((6-((2,4-Difluorophenyl)sulfonyl)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-*d*]-pyrimidin-2-yl)amino)-1*H*-indole-2-carboxylic acid (7aag): Starting from **6i** and 5-amino-1*H*-indole-2-carboxylic acid, 62% of **7aag** was obtained as per the method described for the synthesis of **7**; mp 240–244 °C; ^{1}H NMR (600 MHz; DMSO-d_6): δ 3.46 (s, 3H), 6.68–6.76 (m, 2H), 7.07–7.13 (m, 2H), 7.37–7.59 (m, 2H), 8.13 (s, 1H), 8.85 (s, 1H), 9.08 (s, 1H), 10.60 (s, 1H), 11.25 (s, 1H), 11.73 (s, 1H). MS found (M+H)$^+$ (*m*/*z*), 512.03; calcd for $C_{23}H_{15}F_2N_5O_5S$ *m*/*z*, 511.08.

4.1.15.60. 6-((2,4-Difluorophenyl)sulfonyl)-8-methyl-2-((4-(4-methylpiperazin-1-yl)phenyl)-amino)pyrido[2,3-*d*]pyrimidin-7 (8*H*)-one (7aah): Starting from **6i** and 4-(4-methylpiperazin-1-yl)benzenamine, 74% of **7aah** was obtained to method described for the synthesis of **7**; mp 246–250 °C; ^{1}H NMR (600 MHz; DMSO-d_6): δ 2.21 (s, 3H), 2.40–2.44 (m, 4H), 3.07–3.10 (m, 4H), 3.44 (s, 3H), 6.93 (d, J = 8.4 Hz, 2H), 7.38–7.64 (m, 4H), 8.11–8.14 (m, 1H), 8.84 (s, 1H), 9.05 (s, 1H), 10.50 (s, 1H). MS found (M+H)$^+$ (*m*/*z*), 527.73; calcd for $C_{25}H_{24}F_2N_6O_3S$ *m*/*z*, 526.16.

4.1.15.61. 2-((1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)amino)-6-((2,4-difluorophenyl)sulfonyl)-8-methylpyrido[2,3-*d*]pyrimidin-7 (8*H*)-one (7aai): Starting from **6i** and 1*H*-pyrrolo[2,3-*b*]-pyridine-5-amine, 74% of **7aai** was obtained as per the method described for the synthesis of **7**; mp 320–322 °C; ^{1}H NMR (600 MHz; DMSO-d_6): δ 3.42 (s, 3H), 6.43 (s, 1H), 7.37–7.47 (m, 3H), 8.12–8.51 (m, 3H), 8.87 (s, 1H), 9.10 (s, 1H), 10.64 (s, 1H), 11.60 (s, 1H). MS found (M+H)$^+$ (*m*/*z*), 469.09; calcd for $C_{21}H_{14}F_2N_6O_3S$ *m*/*z*, 468.08.

4.1.15.62. 2-((1*H*-Indol-5-yl)amino)-6-((2,4-difluorophenyl)sulfonyl)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7aaj): Starting from **6u** and 1*H*-indol-5-amine, 59% of **7aaj** was obtained as per the method described for the synthesis of **7**; mp >350 °C; ^{1}H NMR (600 MHz; DMSO-d_6): δ 6.38 (s, 1H), 7.31–7.58 (m, 5H), 8.09–8.25 (m, 2H), 8.81 (s, 1H), 9.03 (s, 1H), 10.41 (s, 1H), 11.04 (s, 1H), 12.55 (s, 1H). MS found (M+H)$^+$ (*m*/*z*), 454.07; calcd for $C_{21}H_{13}F_2N_5O_3S$ *m*/*z*, 453.07.

4.1.15.63. 2-(1*H*-Indol-5-ylamino)-6-(2,4-difluorophenylsulfonyl)-8-ethylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7aak): Starting from **6v** and 1*H*-indol-5-amine, 65% of **7aak** was obtained as per the method described for the synthesis of **7**; mp 278–280 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ1.19 (t, 3H), 4.19 (br s, 2H), 6.40 (br s, 1H),7.54–7.47 (m, 5H), 8.18–8.09 (m, 2H), 8.86 (s, 1H), 9.09 (s, 1H), 10.57 (br s, 1H), 11.09 (br s, 1H). MS found $(M-H)^+$ (*m*/*z*), 480.10; Calcd for $C_{23}H_{17}F_2N_5O_3S$ *m*/*z*, 481.10.

4.1.15.64. 2-((1*H*-Indol-5-yl)amino)-6-((2,4-difluorophenyl)sulfonyl)-8-propylpyrido[2,3-*d*]-pyrimidin-7(8*H*)-one (7aal): Starting from **6w** and 1*H*-indol-5-amine, 64% of **7aal** was obtained as per the method described for the synthesis of **7**; mp 218–220 °C; ^{1}H NMR: (600 MHz, DMSO-d_6): δ 0.85–0.92 (m, 3H), 1.55–1.62 (m, 2H), 3.95–4.09 (m, 2H), 6.37 (s, 1H), 7.23–7.52 (m, 5H), 7.95–8.12 (m, 2H), 8.85 (s, 1H), 9.08 (s, 1H), 10.58 (s, 1H), 11.09 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 496.12; calcd for $C_{24}H_{19}F_2N_5O_3S$ *m*/*z*, 495.12.

4.1.15.65. Methyl 2-(2-(1*H*-indol-5-ylamino)-6-(2,4-difluorophenylsulfonyl)-7-oxopyridio-[2,3-*d*]pyrimidin-8(7H)-yl)acetate (7aam): Starting from **6aa** and 1*H*-indol-5-amine, 64% of **7aam** was obtained as per the method described for the synthesis of **7**; mp 270–272 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.67 (s, 3H), 4.92 (br s, 2H), 6.38 (br s, 1H), 7.43–7.37 (m, 4H), 7.56–7.50 (m, 1H), 7.91 (br s, 1H), 8.16–8.08 (m, 1H), 8.96 (s, 1H), 9.13 (s, 1H), 10.68 (br s, 1H), 11.09 (br s, 1H). MS found $(M-H)^+$ (*m*/*z*), 524.10; Calcd for $C_{24}H_{17}F_2N_5O_5S$ *m*/*z*, 525.09.

4.1.15.66. 2-((1*H*-Indol-5-yl)amino)-8-cyclopropyl-6-((2,4-difluorophenyl)sulfonyl)pyrido-[2,3-*d*]pyrimidin-7(8*H*)-one (7aan): Starting from **6x** and 1*H*-indol-5-amine, 73% of **7aan** was obtained as per the method described for the synthesis of **7**; mp 262–264 °C; ^{1}H NMR: (600 MHz, DMSO-d_6): δ 0.77–0.79 (m, 2H), 1.17–1.18 (m, 2H), 2.79–2.81 (m, 1H), 6.40 (s, 1H), 7.32–7.50 (m, 5H), 8.12–8.14 (m, 1H), 8.30 (s, 1H), 8.79 (s, 1H), 9.03 (s, 1H), 10.51 (s, 1H), 11.06 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 494.11; calcd for $C_{24}H_{17}F_2N_5O_3S$ *m*/*z*, 493.10.

4.1.15.67. 2-(1*H*-Indol-5-ylamino)-6-(2,4-difluorophenylsulfonyl)-8-cyclopentylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7aao): Starting from **6y** and 1*H*-indol-5-amine, 70% of **7aao** was obtained as per the method described for the synthesis of **7**; mp 241–242 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.36 (br s, 2H), 1.62–1.54 (m, 4H), 2.09–2.06 (m, 2H), 5.62–5.56 (br s, 1H), 6.37 (br s, 1H), 7.55–7.24 (m, 5H), 7.85 (br s, 1H), 8.16–8.08 (m, 1H), 8.82 (s, 1H), 9.07 (s, 1H), 10.45 (br s, 1H), 11.09 (br s, 1H). MS found $(M+H)^+$ (*m*/*z*), 522.10; Calcd for $C_{26}H_{21}F_2N_5O_3S$ *m*/*z*, 521.13.

4.1.15.68. 2-((1*H*-Indol-5-yl)amino)-8-cyclohexyl-6-((2,4-difluorophenyl)sulfonyl)pyrido-[2,3-*d*]pyrimidin-7(8*H*)-one (7aap): Starting from **6z** and 1*H*-indol-5-amine, 60% of **7aap** was obtained as per the method described for the synthesis of **7**; mp 282–284 °C; ^{1}H NMR: (600 MHz, DMSO-d_6): δ 1.12–1.43 (m, 4H), 1.48–1.61 (m, 4H), 1.70–1.81 (m, 2H), 2.27–2.30 (m, 1H), 6.36 (s, 1H), 7.20–7.52 (m, 6H), 8.10–8.12 (m,

1H), 8.80 (s, 1H), 9.06 (s, 1H), 10.86 (s, 1H), 11.10 (s, 1H). MS found $(M+H)^+$ (*m*/*z*), 536.17; calcd for $C_{27}H_{23}F_2N_5O_3S$ *m*/*z*, 535.15.

4.1.15.69. 2-(1*H*-Indol-5-ylamino)-6-(2,4-difluorophenylsulfonyl)-8-(cyclopropylmethyl)-pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7aaq): Starting from **6ab** and 1*H*-indol-5-amine, 67% of **7aaq** was obtained as per the method described for the synthesis of **7**; mp 210–212 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.13–0.06 (m, 4H), 1.02–0.93 (m, 1H), 3.81 (br s, 2H), 6.14 (m, 1H), 7.26–7.12 (m, 5H), 7.91–7.84 (m, 2H), 8.62 (s, 1H), 8.85 (s, 1H), 10.32 (br s, 1H), 10.84 (br s, 1H). MS found $(M+H)^+$ (*m*/*z*), 508.10; Calcd for $C_{25}H_{19}F_2N_5O_3S$ *m*/*z*, 507.12.

4.1.15.70. 2-(1*H*-Indol-5-ylamino)-6-(2,4-difluorophenylsulfonyl)-8-(cyclopentylmethyl)-pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (7aar): Starting from **6ac** and 1*H*-indol-5-amine, 70% of **7aar** was obtained as per the method described for the synthesis of **7**; mp 230–232 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.23 (br s, 2H),1.32 (br s, 2H), 1.52 (br s, 4H), 2.44 (m, 1H), 4.13 (d, 2H),, 6.37 (br s, 1H), 7.53–7.34 (m, 5H), 8.18–8.09 (m, 2H), 8.85 (s, 1H), 9.08 (s, 1H), 10.56 (br s, 1H), 11.08 (br s, 1H). MS found $(M+H)^+$ (*m*/*z*), 536.10; Calcd for $C_{27}H_{23}F_2N_5O_3S$ *m*/*z*, 535.15.

5. Biology

5.1. Materials and methods

Unless otherwise stated, all chemicals used in these experiments were either from Sigma–Aldrich Co, St. Louis, MO or Fisher Scientific, Fair Lawn, NJ.

5.1.1. Cell culture—K562 and GRANTA-519 cells were maintained in RPMI medium 1640 (CellGro) supplemented with 10% FBS (Cellgeneration, CO) and 1 unit/mL penicillin–streptomycin (Invitrogen). All other cells were maintained in DMEM (CellGro) supplemented with 10% FBS and 1 unit/mL penicillin–streptomycin. Cells were incubated at 37 °C under humidified conditions and 5% CO_2.

5.1.2. Growth inhibition and GI_{50} determination—Cells were grown in the presence of increasing concentrations of **7n**, **7t**, **7u**, **or 7ao** for 72 h. Cell viability was measured by trypan blue exclusion. Growth inhibition and GI_{50} determination were performed as previously described.[19]

5.1.3. Kinase assays and IC_{50} determination—10 ng recombinant PLK2 (Life Technologies, Grand Island, NY) was diluted with kinase buffer (20 Mm Tris pH 7.5, 10 mM $MgCl_2$, 0.01% NP-40, 2 mM DTT) and incubated with the indicated concentrations of each inhibitor at room temperature for 30 min. The kinase reactions were initiated by the addition of 1 μg (1.5 μM) of dephosphorylated bovine alpha-casein, 5 μM ATP and 10 μCi γ-^{32}P-ATP. The reactions were incubated at 30 °C for 20 min, terminated by the addition of 3% phosphoric acid, spotted onto PE-30 filter mat, washed, dried and taken for scintillation counts. The data were plotted (after background subtraction) as a function of log-drug concentration using Prism 4 Graph pad software and IC_{50} values determined by plotting sigmoidal non-linear regression curves with variable slopes.

5.1.4. In vitro tubulin polymerization assay—Tubulin polymerization was performed as previously described[20] using MAP-rich bovine tubulin 1.5 mg/mL (Cytoskeleton Inc., Denver, CO) reconstituted in a buffer containing 80 mM PIPES pH 6.9, 1 mM GTP, 0.5 mM EGTA, and 2 mM $MgCl_2$. Reaction mixtures were briefly incubated in 1.5 mL tubes with DMSO, compounds **7n**, **7t** and **7u**, **7ao** or Vincristine, transferred to UV-cuvettes and placed in a preheated (37 °C) spectrophotometer to analyze polymerization. Polymer formation was measured by monitoring changes in absorbance at 340 nm and recorded every 2 min over a 30 min period ($n = 3$).

5.1.5. Apoptosis assays—U2OS cells were plated at a density of $1–3 \times 10^4$ cells/well in Optilux black-walled clear-bottom tissue culture microtiter plates (BD Biosciences, San Jose, CA). After an overnight incubation, the cells were treated with increasing concentrations of compound **7n** or **7ao** for 24 h. DMSO was used as the control. Cell viability was measured using the Cell Titer Blue reagent (Promega Corporation, Madison, WI). Induction of apoptotic effect or CASPASEs 3/7 activity was assayed using the Caspase-Glo reagent (Promega Corporation, Madison, WI) using the fluorescence and luminescence modes, respectively, of Promega's Glomax Multi detection System.

5.1.6. Nude mouse xenograft studies—1×10^7 MDAMB-231 cells were subcutaneously implanted into the right flank of 6–8 week old NCR nu/nu female mice. When the tumors reached an average volume of 75 mm^3, the mice were weighed and randomized into treatment groups ($n = 10$) and treated Q2D with either placebo, or the indicated amounts of **7n** or **7ao**. Tumors volumes were measured using electronic Vernier calipers and calculated by the formula 0.4 ($short^2 \times long$).[21]

5.1.7. Molecular modeling—7ao small molecule was generated using ChemDraw (PerkinElmer). Molecule was then imported and prepared in Maestro (Schrödinger suite). Binding positions of **7ao** were predicted using GLIDE[22–24] and the coordinates of the X-ray co-crystal structure of PLK2-1C8 (4I6H). The ligand of the co-crystal (1C8) was used for grid generation. Multiple poses were generated. Binding position with high docking score was visualized and graphed using PyMOL. The PLK1 kinase domain (2RKU) was superimposed on PLK2 and analyzed using PyMOL.

5.1.8. Differential scanning fluorimetry (DSF)—Binding of **7ao** to PLK2 or PLK1 kinase domain was assessed by incubating different concentrations of the drug with 1 μg of protein for 5 min and analyzing drug induced thermal shifts by DSF assays.[16,17]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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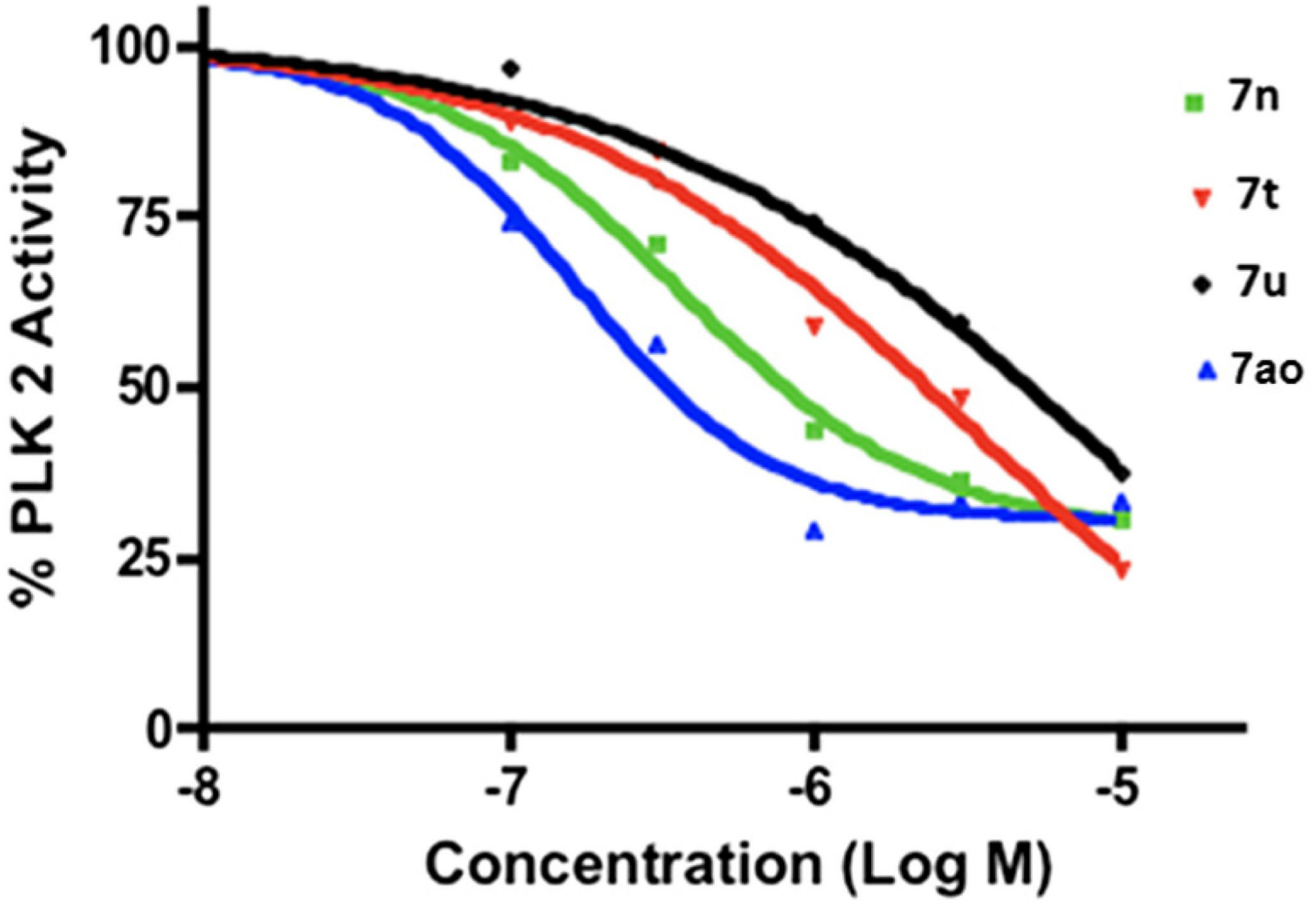
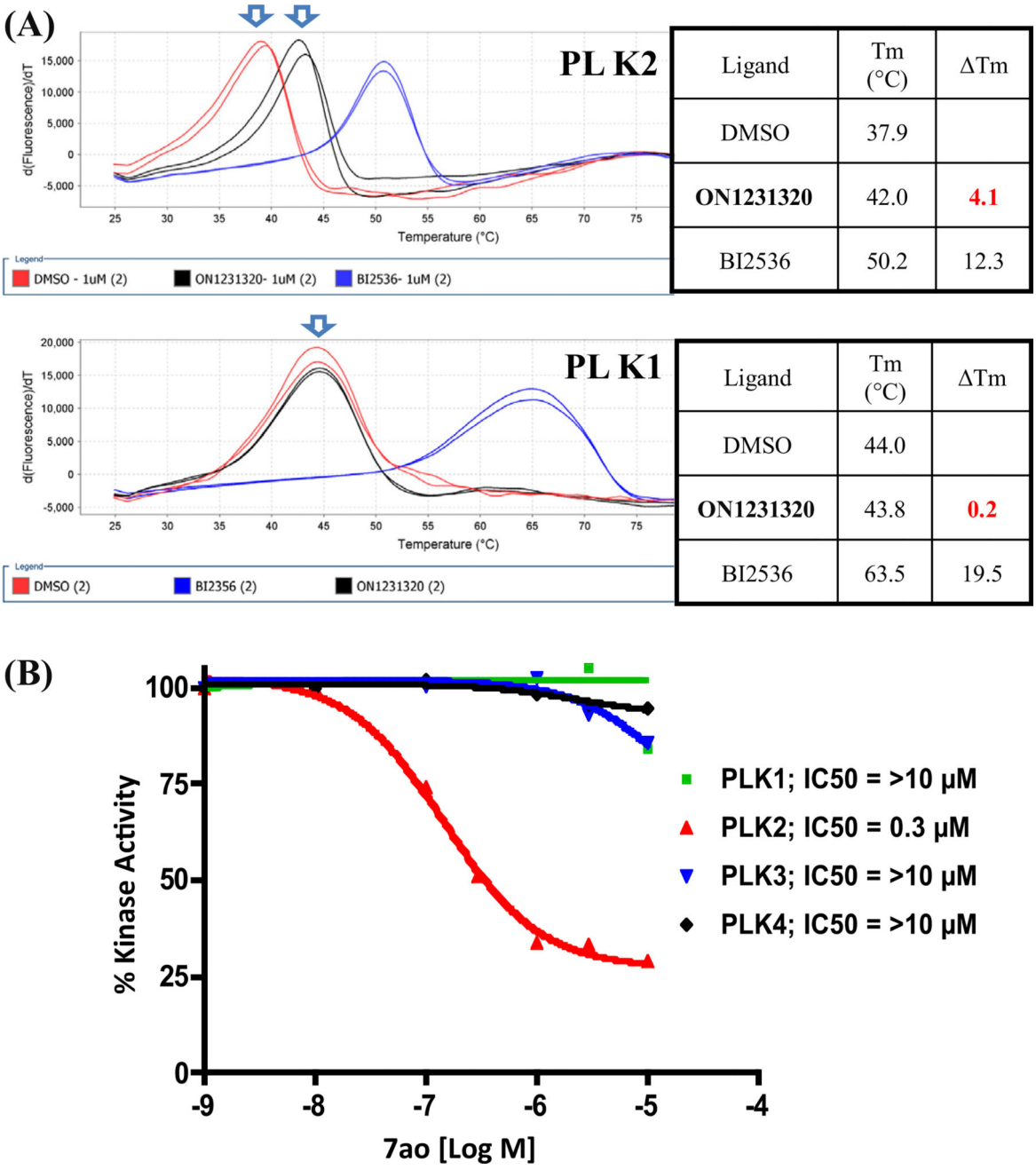


Figure 1.

In vitro PLK2 kinase inhibitory activity of **7n**, **7t**, **7u** and **7ao**. Ten nano grams of recombinant PLK2 was mixed with the indicated concentrations of compounds **7n**, **7t**, **7u** and **7ao**. Kinase assays were performed as described in Section 5.1 using dephosphorylated α-casein as substrate.



Ligand	Tm (°C)	ΔTm
DMSO	37.9	
ON1231320	42.0	**4.1**
BI2536	50.2	12.3

Ligand	Tm (°C)	ΔTm
DMSO	44.0	
ON1231320	43.8	**0.2**
BI2536	63.5	19.5

Figure 2.

(A) Binding of **7ao** (ON1231320) is specific towards PLK2 and not PLK1-recombinant proteins (PLK2 or PLK1 kinase domain) were incubated with different small molecules and assayed for binding by differential scanning fluorimetry (DSF). **7ao** shows specific binding to PLK2 and not to PLK1. Controls using BI2536, show both proteins binding to the PLK pan inhibitor. (B) Recombinant human PLKs were incubated with the indicated concentrations of the compounds. The kinase activity was assayed by using recombinant casein or synuclein as substrate in the presence of γ^{32}P-ATP. IC_{50} values were calculated from non-sigmoidal regression plots with variable slope using GraphPad Prism software.

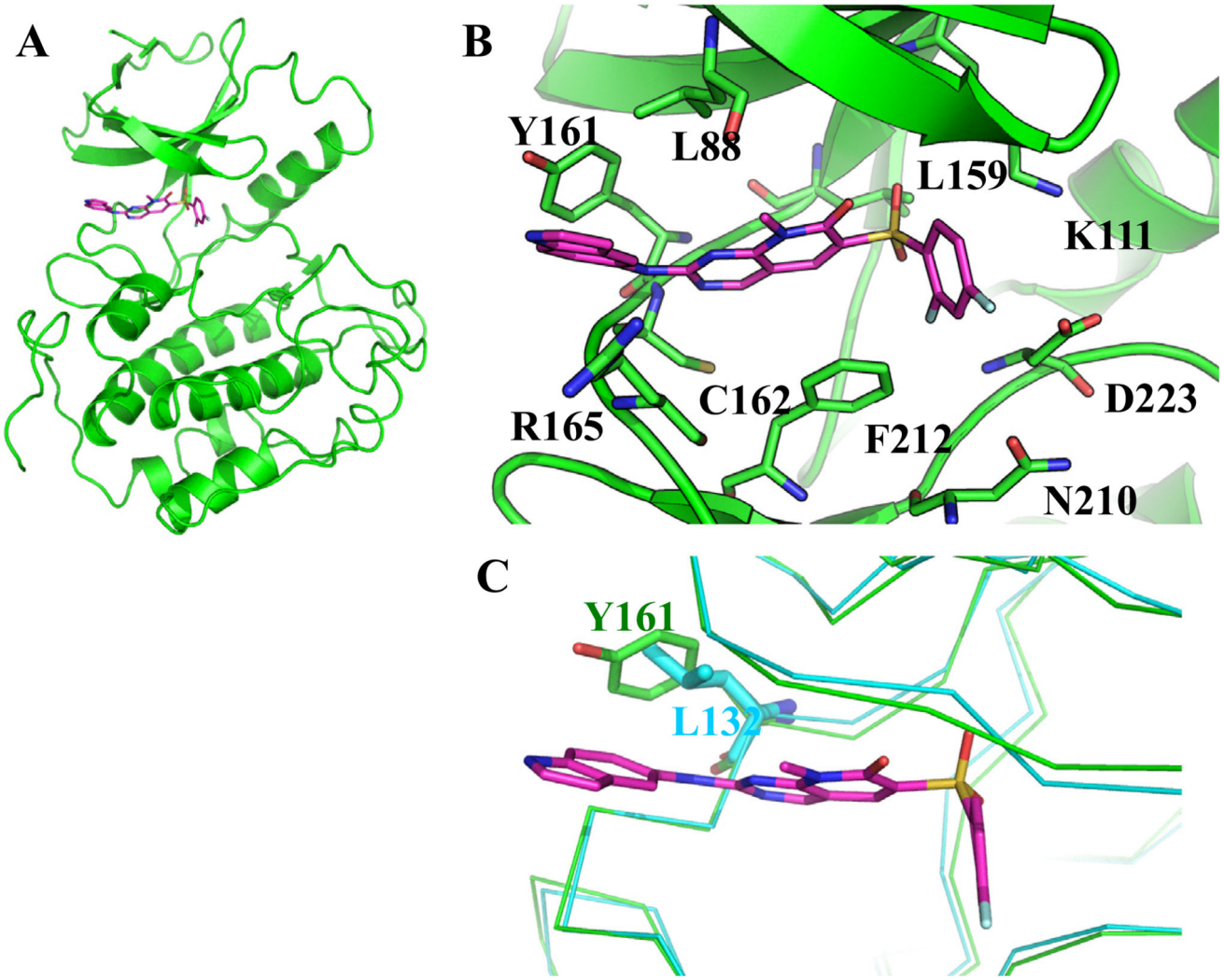


Figure 3.

Model of **7ao** binding to PLK2. **7ao** binding to the kinase domain of PLK2 was predicted by docking and energy minimization using the X-ray crystal structure of PLK2-1C8 (4I6H) as a reference. Representations of the predicted lowest energy binding (PLK2/**7ao**) were prepared using PyMOL. (A) Ribbon representation of PLK2 kinase domain (green) bound to **7ao** (magenta). **7ao** is shown as stick. (B) Close up view showing proximal residues of PLK2 to **7ao** predicted to be important for binding. (C) Superimposition of PLK1 crystal structure (2RKU) onto PLK2. PLK1 and PLK2 are shown as cyan and green ribbons, respectively.

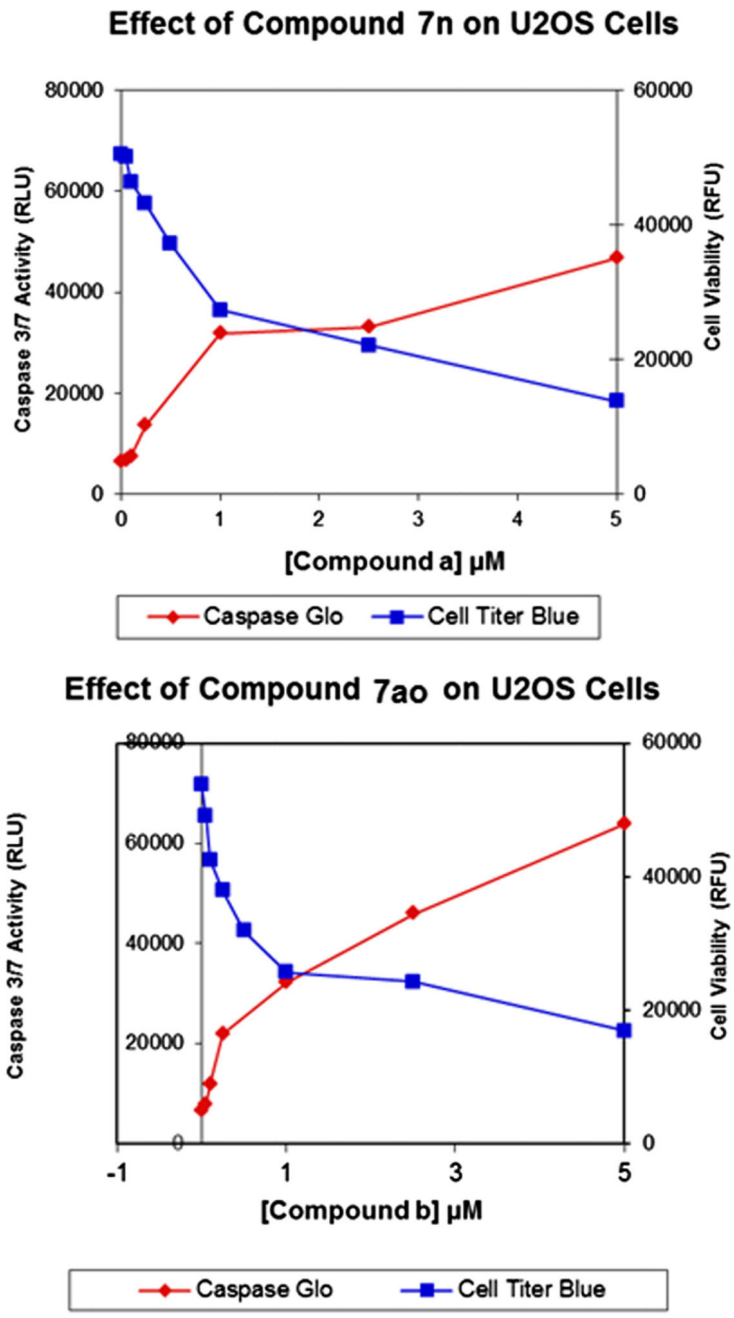


Figure 4.

Induction of caspase activity by **7n** and **7ao**. U2OS cells were treated with the indicated concentrations of each compound for 24 h. Cell viability (blue) and the induction of Caspase 3/7 activity (red) were measured as described in Section 5.1.

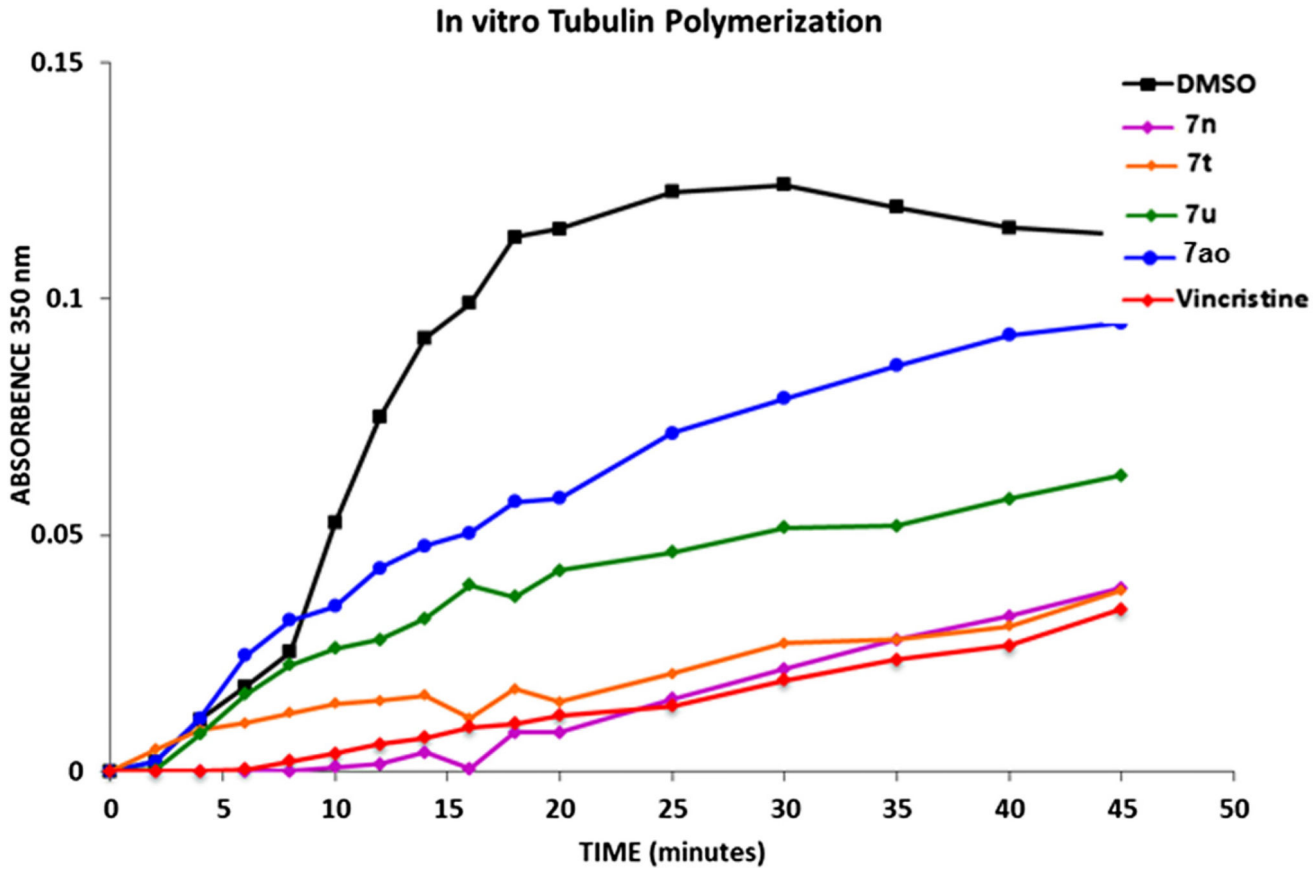


Figure 5.

7ao does not affect in vitro tubulin polymerization. MAP-rich Tubulin was polymerized in the presence of DMSO, 1 μM Vincristine (positive control for inhibition of polymerization) or 10 μM of compounds **7n**, **7t**, **7u** and **7ao** as described in Section 5.1.

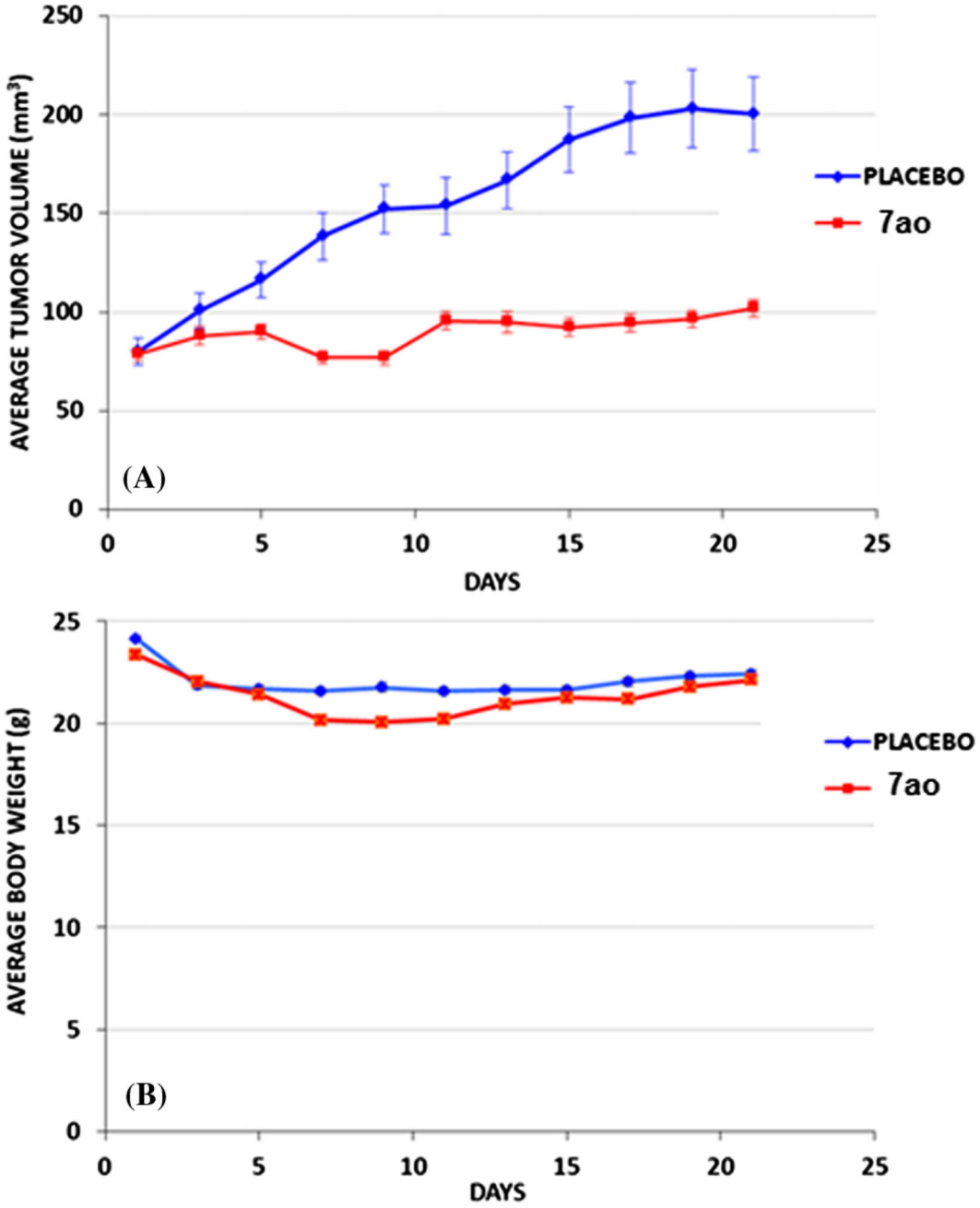
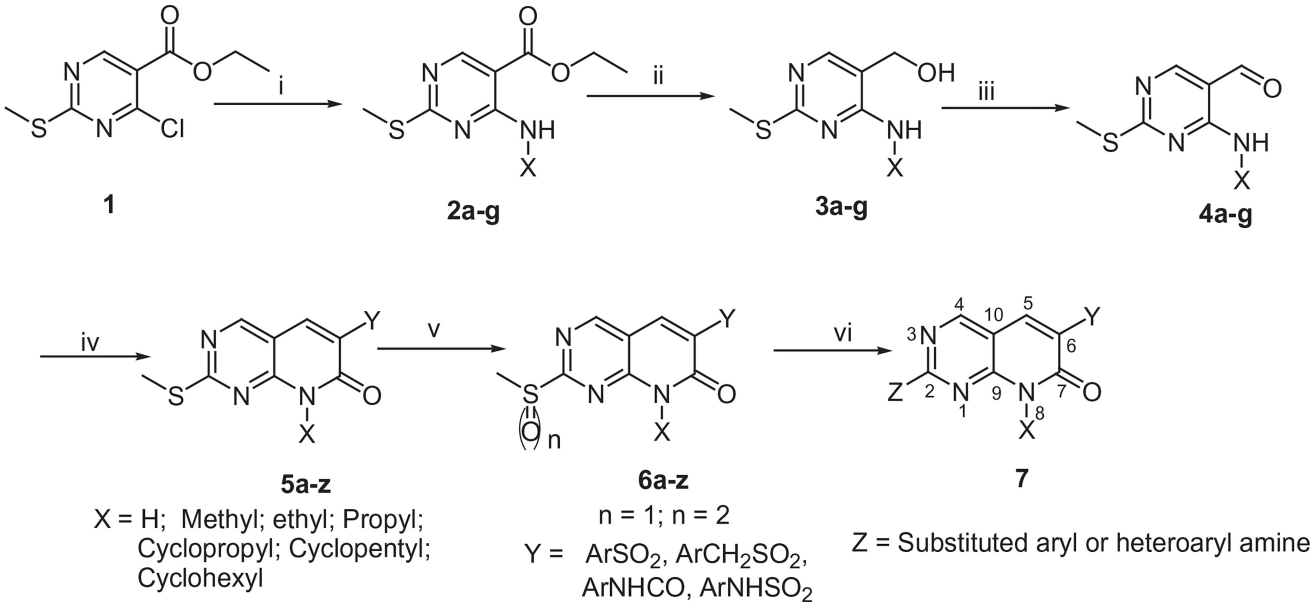


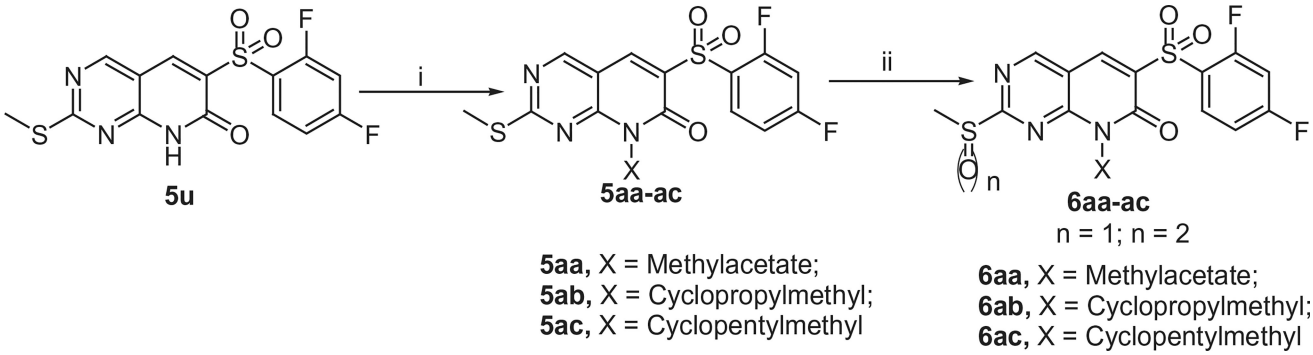
Figure 6.

In vivo efficacy of **7ao** in subcutaneous MDAMB-231 xenografts. Subcutaneous MDAMB-231 triple negative breast cancer xenografts were established in nude mice ($n = 10$ per group). Treatment was started when the average tumor volume reached 75 mm^3. Compound **7ao** at 75 mg/kg or vehicle was administered ip every other day (Q2D) and the tumor volumes (A) and body weights (B) measured over the course of the experiment.



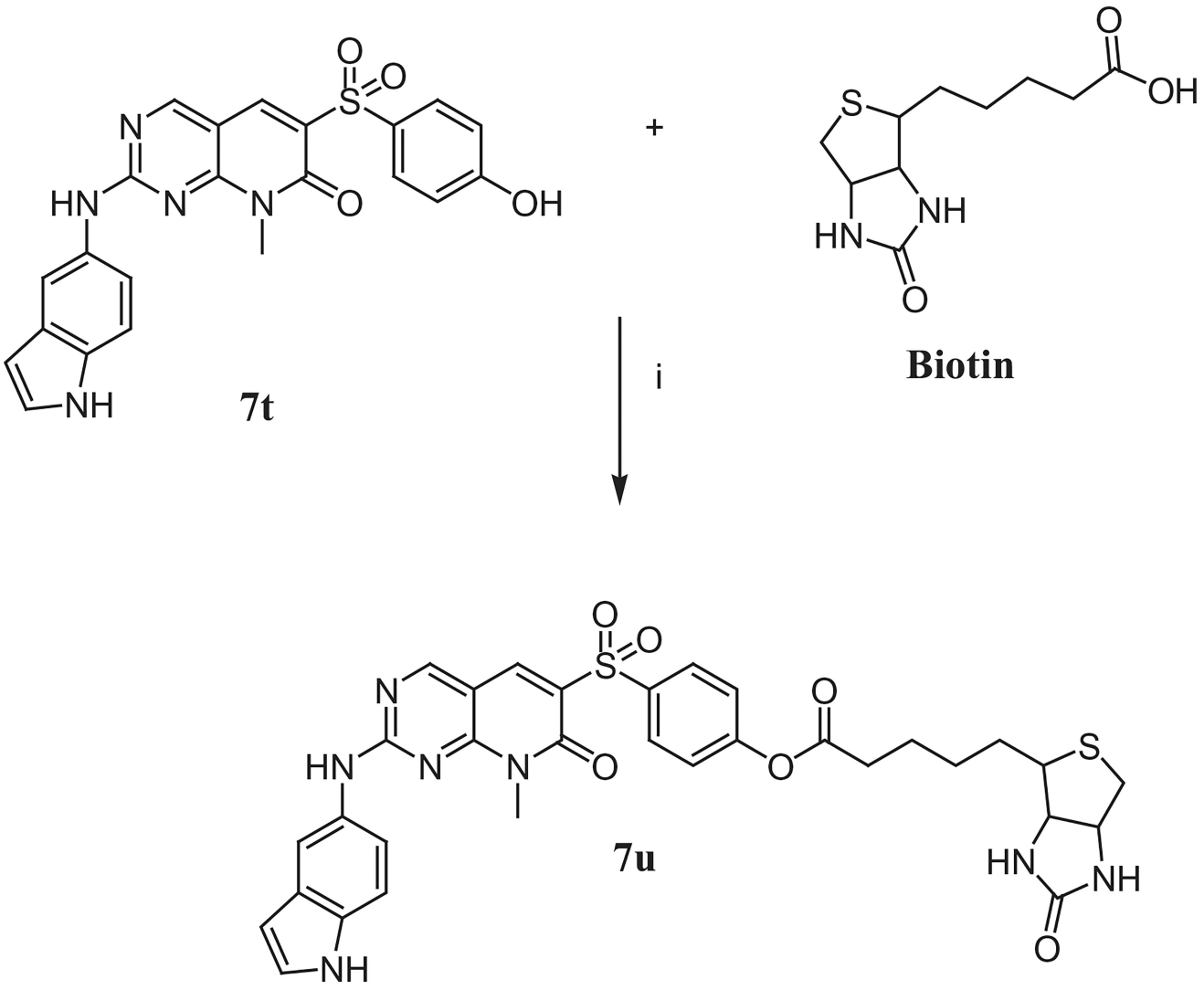
Scheme 1.

Synthesis of pyrido[2,3-*d*]pyrimidines. Reagents and conditions: (i) X-NH_2, Et_3N, THF; rt, 1–3 h, 80–95%; (ii) $LiAlH_4$, THF, −10 °C–rt, 1 h, 80–86%; (iii) MnO_2, $CHCl_3$, rt, 24 h, 70–90%; (iv) active methylene compounds, $BnNH_2$, AcOH, 100 °C, 6 h, 32–67%; (v) *m*-CPBA, CH_2Cl_2, rt, 3 h, 54–86%; (vi) *Z*, DMSO or toluene, 100 °C, 3–8 h, 45–65%.



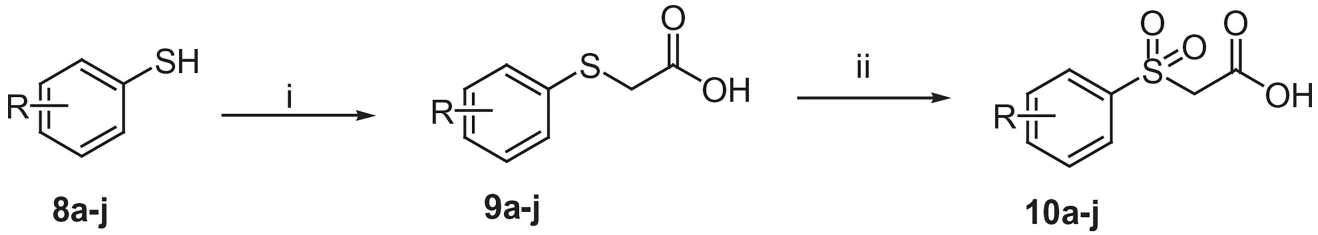
Scheme 2.

Alkylation of 6-((2,4-difluorophenyl)sulfonyl)-2-(methylthio)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (**5u**). Reagents and conditions: (i) X-I, NaH, DMF, 50 °C, 1 h, 55–60%; (ii) *m*-CPBA, CH_2Cl_2, rt, 3 h, 50–60%.



Scheme 3.

Synthesis of 4-(2-(1*H*-indol-5-ylamino)-7,8-dihydro-8-methyl-7-oxopyrido[2,3-*d*]pyrimidin-6-ylsulfonyl)phenyl-5-(hexahydro-2-oxo-1*H*-thieno[3,4-*d*] imidazol-6-yl)pentanoate (**7u**). Reagents and conditions: (i) EDC, DMAP, rt, 45%.

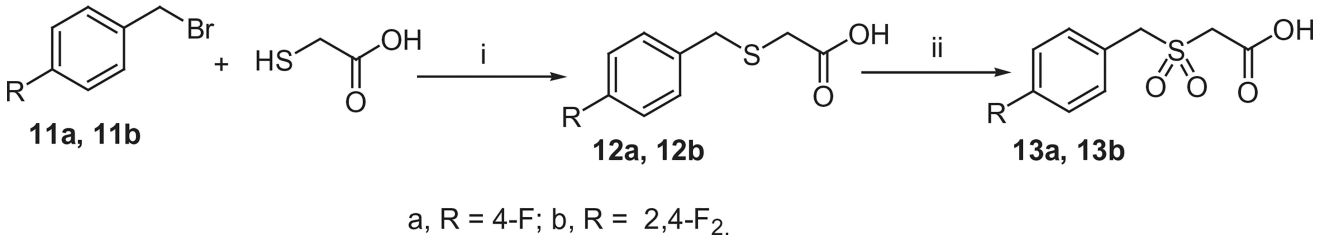


8a-j **9a-j** **10a-j**

a, R = H; b, R = 4-F; c, R = 4-Cl; d, R = 4-Br; e, R = 4-OH; f, R = 4-Me; g, R = 4-OMe; h, R = 4-CO_2H; i, R = 2,4-F_2; j, R = 2,4-Cl_2.

Scheme 4.

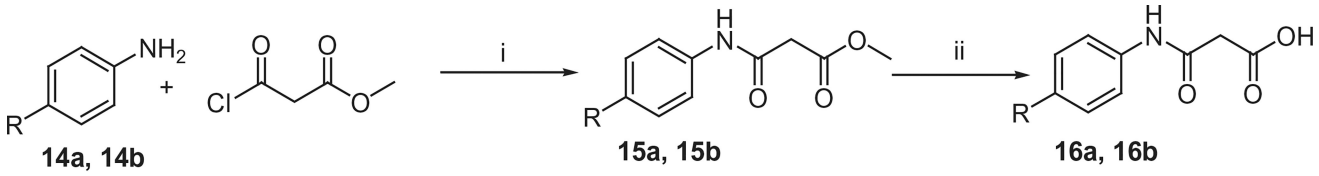
Synthesis of arylsulfonyl acetic acids. Reagents and conditions: (i) $ClCH_2CO_2H$, NaOH, MeOH, HCl, rt, 3 h, 55–66%; (ii) 30% H_2O_2, AcOH, rt, 24 h, 70–90%.



a, R = 4-F; b, R = 2,4-F_2.

Scheme 5.

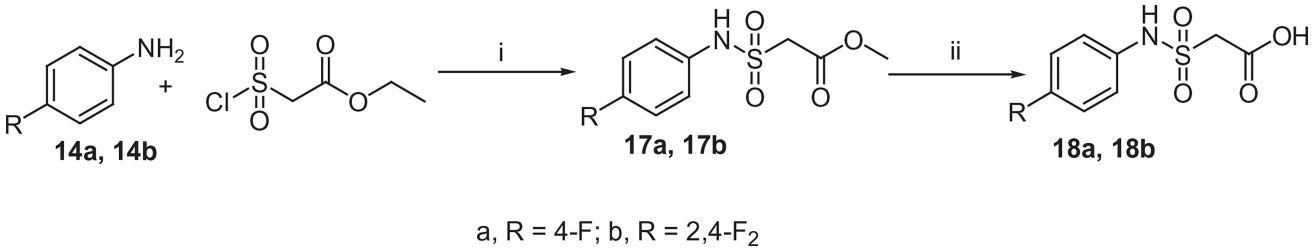
Synthesis of arylmethanesulfonyl acetic acids. Reagents and conditions: (i) NaOH, MeOH, rt, 3 h, 90–96%; (ii) 30% H_2O_2, AcOH, 24 h, 90–93%.



a, R = 4-F; b, R = 2,4-F_2.

Scheme 6.

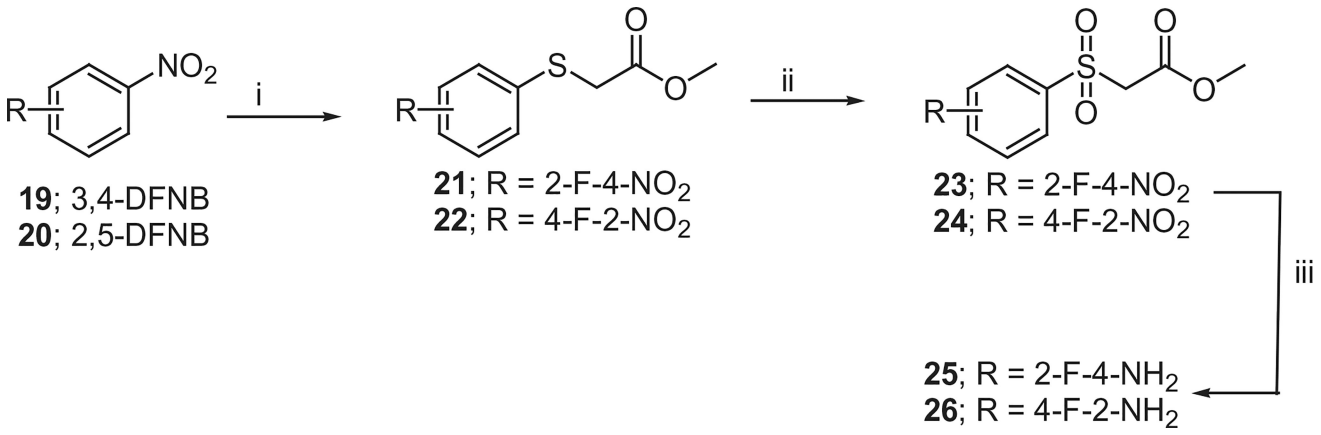
Synthesis of *N*-arylmalonamic acids. Reagents and conditions: (i) Et_3N, DCM, rt, 3 h, 76–77%; (ii) NaOH, rt, 1 h, 73–75%.



a, R = 4-F; b, R = 2,4-F_2

Scheme 7.

Synthesis of *N*-arylsulfamoylacetic acid. Reagents and conditions: (i) Et_3N, DCM, rt, 3 h, 70–72%; (ii) 10% NaOH, rt, 1 h, 76–80%.

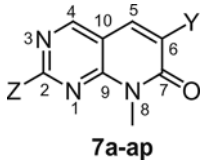


Scheme 8.

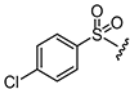
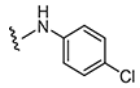
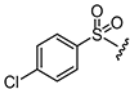
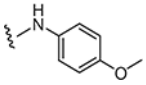
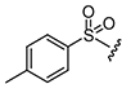
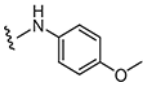
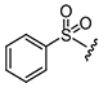
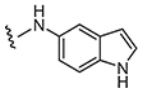
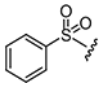
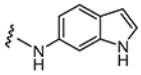
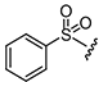
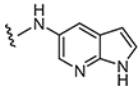
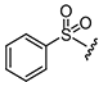
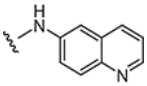
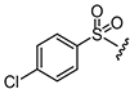
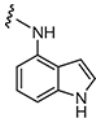
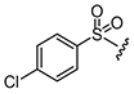
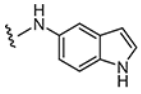
Synthesis of methyl arylsulfonyl esters. Reagents and conditions: (i) $HSCH_2CO_2Me$, DIPEA, THF, rt, 15 h, 85–96%; (ii) 30% H_2O_2, AcOH, 60 °C, 6 h, 90–99%; (iii) 10% Pd/C, H_2, MeOH, rt, 4 h, 86–93%.

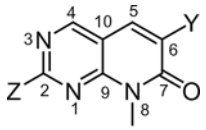
Table 1

In vitro cytotoxicity of pyrido[2,3-*d*]pyrimidine (**7a**–**ap**) with variables at C_2 and C_6 positions

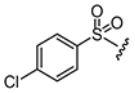
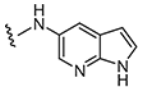
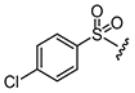
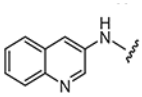
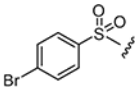
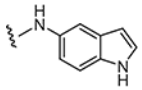
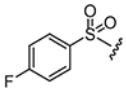
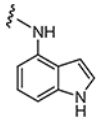
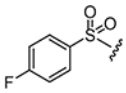
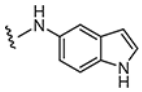
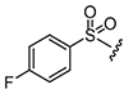
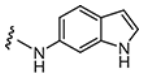
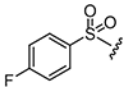
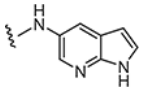
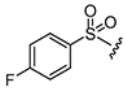
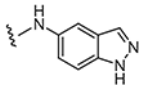
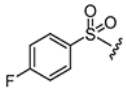
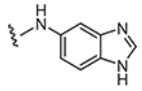
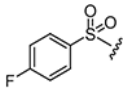
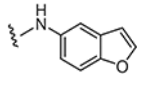


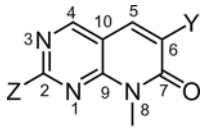
7a-ap

Compd	Y	Z	IC_{50} (μM)	
			K562	**DU145**
7a			15	15
7b			15	30
7c			75	100
7d			1	5
7e			10	10
7f			5	1
7g			100	50
7h			5	5
7i			0.1	0.25

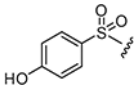
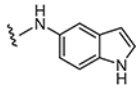
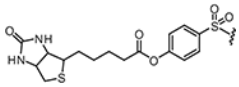
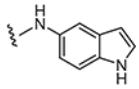
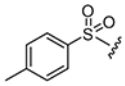
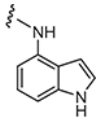
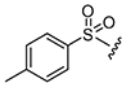
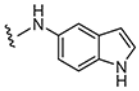
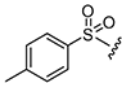
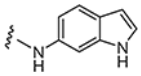
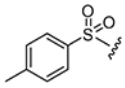
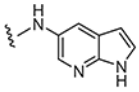
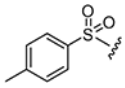
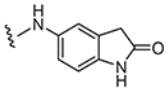
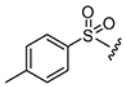
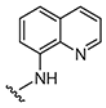
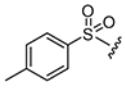
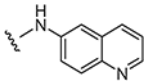
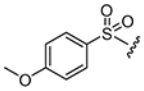
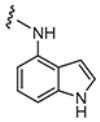


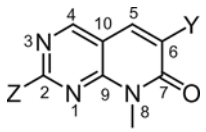
7a-ap

Compd	Y	Z	IC_{50} (μM)	
			K562	DU145
7j			5	5
7k			15	15
7l			5	5
7m			0.75	0.75
7n			0.2	0.3
7o			10	10
7p			0.75	0.5
7q			10	5
7r			10	10
7s			5	5

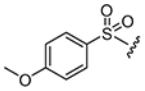
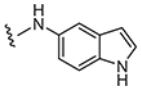
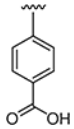
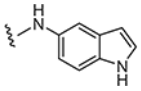
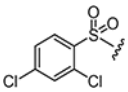
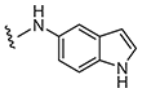
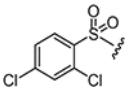
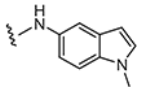
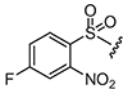
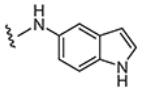
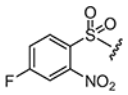
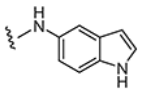
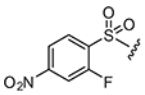
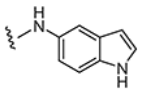
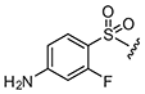
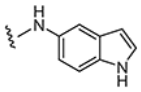
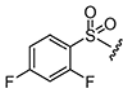
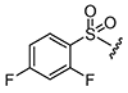


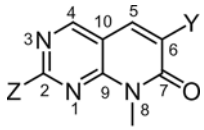
7a-ap

Compd	Y	Z	IC_{50} (μM)	
			K562	DU145
7t			0.1	0.25
7u			0.6	0.15
7v			5	10
7w			0.75	0.75
7x			15	15
7y			15	25
7z			15	15
7aa			10	10
7ab			10	10
7ac			5	5



7a-ap

Compd	Y	Z	IC_{50} (μM)	
			K562	DU145
7ad			0.3	0.5
7ae			5	10
7af			1	1
7ag			0.75	0.75
7ah			0.9	1.5
7ai			2	3
7aj			0.2	0.4
7ak			1.5	8
7al			35	75
7am			30	50



7a-ap

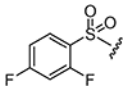
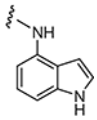
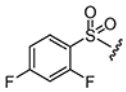
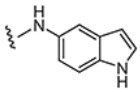
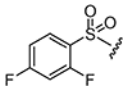
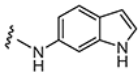
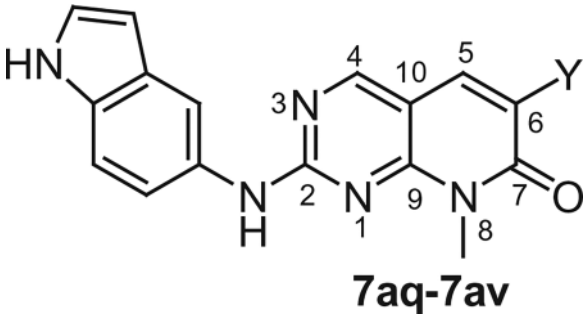
Compd	Y	Z	IC_{50} (μM)	
			K562	DU145
7an			1.5	0.75
7ao			0.075	0.075
7ap			5	5

Table 2

In vitro cytotoxicity and kinase profile of pyrido[2,3-*d*]pyrimidine (**7aq**–**av**) with variables at C_6 positions



7aq-7av

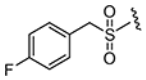
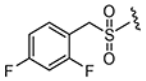
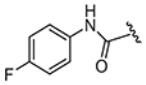
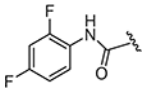
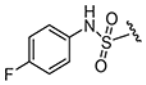
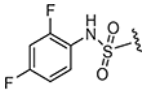
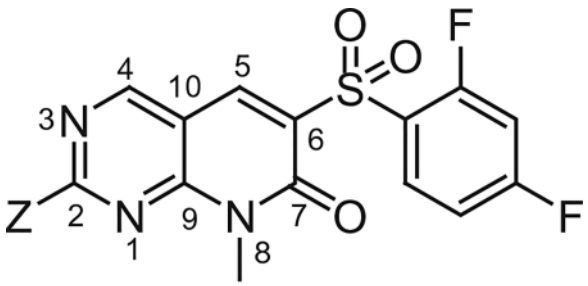
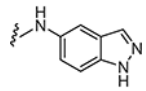
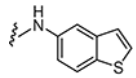
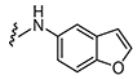
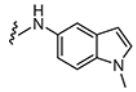
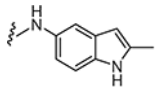
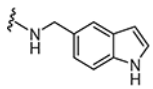
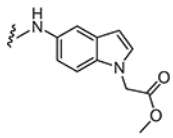
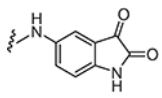
Compd	Y	IC_{50} (μM)	
		K562	DU145
7aq		30	30
7ar		1	1
7as		5	5
7at		5	5
7au		5	5
7av		5	5

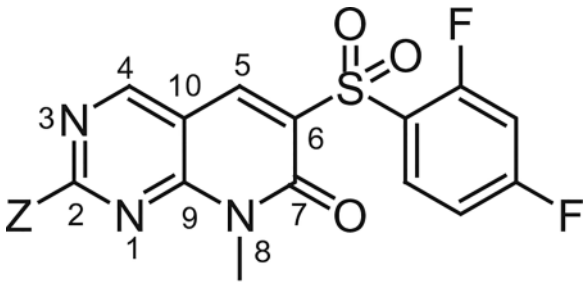
Table 3

In vitro Cytotoxicity and kinase profile of pyrido[2,3-*d*]pyrimidine (**7aw**–**aai**) with variable at C_2 position



7aw-aai

Compd	Z	IC_{50} (μM)	
		K562	DU145
7aw		50	30
7ax		0.5	0.5
7ay		15	15
7az		0.75	0.75
7aaa		0.75	0.75
7aab		75	100
7aac		5	5
7aad		30	75



7aw-aai

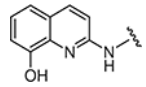
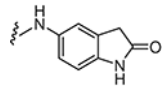
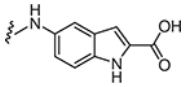
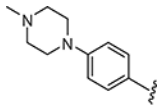
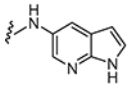
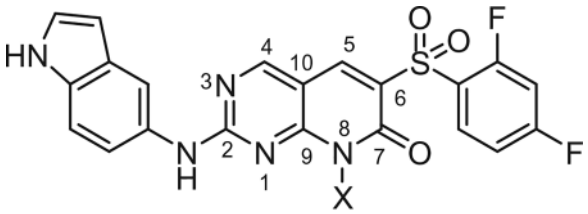
Compd	Z	IC_{50} (μM)	
		K562	DU145
7aae		5	5
7aaf		15	100
7aag		10	10
7aah		5	5
7aai		0.75	0.75

Table 4

In vitro cytotoxicity and kinase profile of pyrido[2,3-*d*]pyrimidine (**7aaj**–**aar**) with variables at N_8 position



7aaj-aar

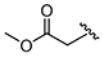
Compd	X	IC_{50} (μM)	
		K562	DU145
7aaj	H	15	15
7aak		0.75	0.75
7aal		1	1
7aam		5	10
7aan		0.5	0.5
7aao		5	5
7aap		10	10
7aaq		1	1
7aar		7	10

Table 5

Evaluation of **7n**, **7t**, **7u** and **7ao** against a panel of human tumor cell lines

Cell line	GI_{50} (μM)			
	7n	**7t**	**7u**	**7ao**
DU145	0.3	0.75	0.75	0.075
MCF-7	0.25	0.5	0.5	0.075
BT474	0.2	0.2	0.25	0.1
SK-OV-3	0.2	0.75	0.4	0.075
MIA-PaCa-2	0.075	0.1	0.1	0.075
SK-MEL-28	0.3	0.5	0.5	0.2
A549	0.2	0.75	2.5	0.075
U87	0.4	1	1	0.02
COLO-205	0.2	0.25	0.5	0.075
HELA	0.075	0.75	0.5	0.05
H1975	0.1	0.75	0.75	0.075
RAJI	0.1	0.5	0.4	0.05
U205	0.055	0.35	0.35	0.035
K562	0.1	0.5	0.5	0.075
GRANTA-519	0.06	0.25	0.5	0.04

Table 6

Evaluation of **7ao** against a panel of human tumor cell lines

Cell line	Tumor type	7ao GI_{50} (μM)
K562	Chronic myelogenous leukemia	0.075
DU145	Prostate	0.075
RAJI	Burkitt's lymphoma (B-cell)	0.05
SK-MEL-28	Skin melanoma	0.2
SK-OV-3	Ovarian	0.075
MCF-7	Breast	0.075
MIA-PaCa-2	Pancreatic	0.075
A549	Non small cell lung carcinoma	0.075
U87	Glioblastoma	0.2
COLO-205	Colo-rectal	0.075
BT-474	Breast	0.1
HELA	Cervical	0.075
GRANTA-519	Mantle cell lymphoma	0.04
JURKAT	acute t cell leukemia	0.025
U2OS	Osteosarcoma	0.05
A431	Epidermoid	0.03
WI-38	Normal fibroblast-lung	>5.0
HFL-1	Normal fibroblast-lung	>2.0
H460	Lung (large cell carcinoma)	0.1
BxPC-3	Pancreatic	0.075
SU.86.86	Pancreatic	0.4
BT-549	Lung	0.25
H292	Non-small cell lung carcinoma	0.15
H1650	Non-small cell lung carcinoma	0.2
UM-UC-3	Bladder	0.3
5637	Bladder	0.125
T24	Bladder	0.15
SW780	Bladder	0.15
U-937	Histiocytic lymphoma (monocytic)	0.125
DAUDI	Burkitt's lymphoma (B-cell)	0.075
HL-60	Acute myeloid leukemia	0.125
TCC-SUP	Bladder	0.25
RT-4	Bladder	0.5
PC-3	Prostate	0.15
HCT-116	Colo-rectal	0.15
CEM	T-ALL	0.05
Z-138	Mantle cell lymphoma	0.2
PANC 10.05	Pancreatic	0.4
PANC 03.25	Pancreatic	0.2

Cell line	Tumor type	7ao GI_{50} (μM)
ASPC-1	Pancreatic	0.3
HPAF-II	Pancreatic	0.25
Capan-1	Pancreatic	0.075
LNCaP	Prostate	0.04
BEL-7402	Hepatocellular carcinoma	0.05
SNU-449	Hepatocellular carcinoma	0.4
SNU-398	Hepatocellular carcinoma	0.2
PLC/PRF/5	Hepatocellular carcinoma	0.75
SNU-428	Hepatocellular carcinoma	0.75
SNU-398	Hepatocellular carcinoma	0.15
SNU-475	Hepatocellular carcinoma	7.5
Hep-G2	Hepatocellular carcinoma	0.5
SK-HEP-1	Liver adenocarcinoma (metastatic)	0.1
H359	Non-small cell lung carcinoma	0.15
H23	Non-small cell lung carcinoma	0.2
H1734	Non-small cell lung carcinoma	0.15
H827	Non-small cell lung carcinoma	0.5
H1975	Non-small cell lung carcinoma	0.2
MDA-MB-436	Breast	0.15
MDA-MB-453	Breast	0.075
HCC1806	Breast	0.1

Table 7

PLK2 inhibitory activity of **7d**, **7m**, **7n**, **7t**, **7ad** and **7ao**

Compound	PLK2 IC_{50} (μM)
7d	1.23
7m	6.08
7n	1.07
7t	0.47
7ad	0.35
7ao	0.31