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Development of Novel Thiazolopyrimidines as CDC25B Phosphatase Inhibitors

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The development of CDC25 phosphatase inhibitors is an interesting approach toward new antitumor agents, as CDC25 play key roles in cell-cycle regulation and are overexpressed in numerous cancers. We previously reported a novel compound belonging to the thiazolopyrimidine family that inhibits CDC25 activity with an IC_{50} value of 13 μM and displays cytotoxic properties against HeLa cells. Structural modifications were subsequently conducted on this new pharmacophore which led to a library of 45 thiazolopyrimidines. Regarding the in vitro effects, 14 compounds inhibit CDC25B with $IC_{50} < 20$ μM, with the most efficient inhibitor **44** improving the potency to 4.5 μM. Steady-state kinetics were performed and showed a mixed inhibition pattern for all tested compounds. Furthermore, **44** was able to revert the bypass of genotoxicity-induced G_2 arrest upon CDC25B overexpression, indicating that this compound targets the dual-specificity phosphatase in cultured cells. Finally, the cytotoxic activities of the compounds were determined against two human cancer cell lines. The results indicate that the prostatic LNCaP cell line is more sensitive to these derivatives than the pancreatic adenocarcinoma MiaPaCa-2 line. With its interesting enzymatic and cellular properties, compound **44** appears to be a promising CDC25B inhibitor for further development.

Introduction

Cell division cycle 25 (CDC25) phosphatases are key regulators of the cell cycle and its checkpoints.[1] They are required to dephosphorylate and thus activate the Cdk–cyclin complexes that trigger progression through the various cell-cycle phases.[2–3] Furthermore, they are essential key proteins in the checkpoint pathways, as they are inactivated when DNA damage occurs, thus leading to cell-cycle arrest.[4] CDC25 phosphatases are highly conserved proteins in eukaryotes. They belong to the tyrosine phosphatase family; their active site possesses the common HCX_5R motif, which contains the catalytic cysteine residue responsible for phosphate ester hydrolysis. In addition, CDC25 are major members of the dual-specificity phosphatase subclass, as they are able to dephosphorylate two contiguous phosphothreonine and phosphotyrosine residues of the Cdk subunit.

Three isoforms have been identified in the human genome, namely CDC25A, B, and C, and the presence of multiple splice variants has been reported for all of them.[5–9] CDC25A regulates the G_1/S transition by activating Cdk2–cyclin E and Cdk2–cyclin A complexes.[10–12] CDC25B and CDC25C are both required for entry into mitosis. CDC25B activates the mitosis-promoting factor Cdk1–cyclin B at the centrosome during the G_2/M transition[13] and is probably also involved in centrosome duplication,[14] while CDC25C enables the complete activation of this latter complex at the onset of mitosis.[15,16] Finally, all three isoforms are believed to cooperate during cell-cycle progression.

Overexpression of CDC25A and CDC25B has been demonstrated in a wide range of human tumors such as colorectal, breast, prostate, and ovarian cancers, and is often associated with aggressive tumor growth and poor clinical prognosis.[17,18] Moreover, CDC25A and B have been reported to present oncogenic properties because they are transcriptional targets of the *c-myc* oncogene.[19,20] CDC25 phosphatases may therefore represent attractive targets for anticancer therapy.[21] However, the inhibition of CDC25 activity still remains a challenging task.[22] Indeed, the catalytic cysteine is at the bottom of a shallow and small active site, located next to the so-called inhibitor binding pocket or 'swimming pool'.[23] This residue is highly reactive and may undergo oxidation or covalent modifications through sulfhydryl arylation. In addition, structural data are still lacking,

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although a peptide bound to the catalytic site has been co-crystallized by researchers at BASF.[24] Nevertheless, structurally diverse CDC25 inhibitors have been discovered over the past few years, the most potent of which are quinonoid-based derivatives with IC_{50} values in the sub-micromolar range. These compounds (Figure 1) act mainly through irreversible linkage or oxidation of the catalytic cysteine residue.[25]

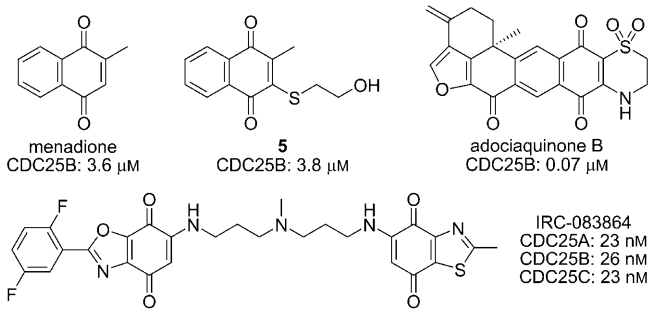


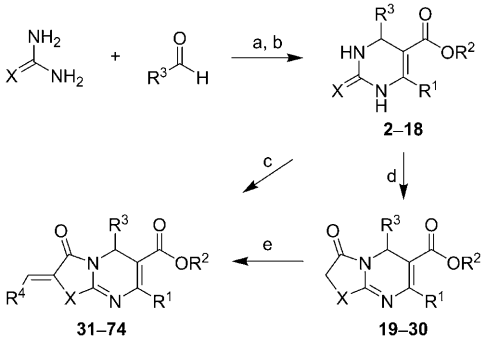
Figure 1. CDC25 inhibitors.

In an attempt to identify new and original scaffolds with CDC25 inhibitory activity, we conducted in silico/in vitro screening experiments on the CDC25B isoform,[26] targeting both the active site cavity and the inhibitor binding pocket.[23] Compound **1**, which belongs to the thiazolopyrimidine family, displays the most potent CDC25 inhibitory activity, with an IC_{50} value of 13 μM in the enzymatic assay, and exhibits cytotoxic and antiproliferative activity against HeLa cells.[26] Based on these results, and to improve the inhibitory potency of this compound class, a series of thiazolopyrimidine derivatives was synthesized and evaluated for CDC25B inhibitory activity. Their mechanism of inhibition was also investigated, and we examined whether our best inhibitor could target CDC25 in the cell. Finally, the cytotoxic effects of these compounds were also evaluated on two human cancer cell lines, LNCaP and MiaPaCa-2.

Results and Discussion

Chemistry

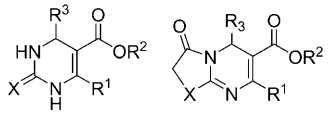
Thiazolopyrimidines **31**–**74** were easily prepared following a two- or three-step sequence involving multi-component reactions (Scheme 1). The first stage led to the formation of 3,4-dihydropyrimidine-2-(1*H*)-thione (DHPM) derivatives **2**–**18** by using the widely studied Biginelli condensation.[27] Here, compounds **2**–**18** were synthesized by reacting a substituted benzaldehyde, a thio- or selenourea (X = S or Se), and a β-keto ester in dioxane at reflux; *para*-toluenesulfonic acid (TsOH) was used as the catalyst (Table 1).[28] Preparation of homologue **6**, which bears a free carboxylic group at position 5, was achieved through the mild saponification of the corresponding 2-cyanoethyl ester Biginelli product **5**.[29,30]



Scheme 1. Reagents and conditions: a) TsOH, dioxane, reflux, 2 h; b) $R^1COCH_2CO_2R^2$, dioxane, reflux, 12 h; c) $ClCOCH_2Cl$, R^4CHO, NaOAc, Ac_2O/AcOH, reflux, 6 h; d) $ClCOCH_2Cl$, Na_2CO_3 (anhyd), dioxane, reflux, 4 h; e) R^4CHO, piperidine, MeOH, reflux, 2 h.

Condensation of DHPMs with chloroacetyl chloride and the appropriate benzaldehydes in the presence of sodium acetate in acetic acid/acetic anhydride medium at reflux provided the final thiazolopyrimidines **35**–**59** and **67**–**74** as *Z* isomers, as previously described (Scheme 1).[31–34] To avoid esterification of the phenolic functions introduced as R^4 and/or R^3 moieties, the 5-aryl-7-substituted-3-oxo-5*H*-thia(selena)zolo[3,2-*a*]pyrimidine intermediates **19**–**30** were isolated after cyclocondensation of chloroacetyl chloride with the corresponding DHPMs in dioxane at reflux (Table 1). Finally, thia- and selenazolopyrimidines

Table 1. Structures of DHPMs **2**–**18** and their corresponding thia- and selenazolo[3,2-*a*]pyrimidines **19**–**30**.



			2–18			19–30	
X	R^1	R^2	R^3	Compd	Inhibition [%][a]	Compd	Inhibition [%][a]
S	Me	Et	1,3-benzodioxol-5-yl	**2**	0	**19**	0
S	Ph	Et	1,3-benzodioxol-5-yl	**3**	12	**20**	0
S	Me	Bn	1,3-benzodioxol-5-yl	**4**	35	**21**	26
S	Me	$(CH_2)_2CN$	1,3-benzodioxol-5-yl	**5**	ND[b]	–	–
S	Me	H	1,3-benzodioxol-5-yl	**6**	ND[b]	**22**	ND[b]
Se	Me	Et	1,3-benzodioxol-5-yl	**7**	17	**23**	11
S	Me	Et	H	**8**	22	**24**	9
S	Me	Et	phenyl	**9**	0	**25**	ND[b]
S	Me	Et	3,4-dimethoxyphenyl	**10**	0	**26**	19
S	Me	Et	3,4-dichlorophenyl	**11**	ND[b]	**27**	13
S	Me	Et	3-hydroxyphenyl	**12**	20	**28**	9
S	Me	Et	4-hydroxyphenyl	**13**	13	**29**	15
S	Me	Et	3,4-dihydroxyphenyl	**14**	10	**30**	24
S	Me	Et	1,4-benzodioxol-6-yl	**15**	10	–	–
S	Me	Et	naphth-2-yl	**16**	45	–	–
S	Me	Et	4-methylnaphth-1-yl	**17**	48	–	–
S	Me	Et	3,4,5-trimethoxyphenyl	**18**	5	–	–

[a] Percent of CDC25B inhibition at 100 μM was calculated from two independent experiments with three determinations per tested concentration. [b] Not determined.

19–**30** were treated with the appropriate hydroxybenzaldehydes under classical Knoevenagel reaction conditions to afford the expected thiazolopyrimidines **31**–**34** and **60**–**66** (Scheme 1).[35]

In vitro enzymatic assay

Thiazolopyrimidine **1**, identified from our structure-based virtual ligand screen,[26] appeared as an attractive scaffold to explore the effects of pharmacophore modulations on CDC25 inhibitory activity. In this study, we were interested in modifying five points of chemical diversity around the thiazolopyrimidine core: R^1 and R^2, linked to the structure of the β-keto ester, R^3 and R^4, associated with the diversity of commercially available aldehydes, and X, which is a sulfur or selenium atom. Thus, we finally generated a library of 45 thiazolopyrimidines **31**–**74**.

All compounds were assayed for their inhibitory activity against the recombinant fusion protein MBP–CDC25B3 using fluorescein-3,6-diphosphate (FDP) as the artificial substrate. The fluorescent emission resulting from dephosphorylation was monitored for 30 min at 30 °C in the presence of increasing concentrations of inhibitors. Menadione and Cpd **5**[36] were used as reference and, under our assay conditions, displayed IC_{50} values of 2.2 and 3.7 μM, respectively. In addition, because some Biginelli derivatives have been reported to be biologically active,[27] we consequently evaluated some of the synthesized DHPMs for their anti-phosphatase activity at a concentration of 100 μM.

Results are reported in Tables 1–5. We initially observed that none of the DHPM derivatives was effective at inhibiting CDC25 activity, except compounds **16** and **17**, which bear a naphthyl ring, and showed ~50% inhibition at 100 μM (Table 1).

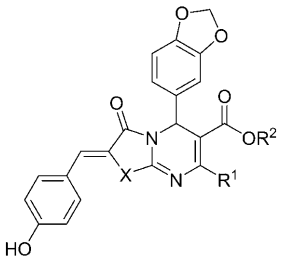
We first decided to investigate minor modifications on the lead compound **1** (Table 2). To evaluate the effects of R^1 at position 7, the methyl group was replaced by a phenyl moiety, which led to a slight enhancement of activity, as compound **31** displayed an IC_{50} value of 7.5 μM. Considering the ester function at position 6, the benzyl derivative **32** is as potent as compound **1**. In contrast, the introduction of a free carboxylic group (compound **33**) did not show enzymatic inhibition. Finally, replacing the sulfur atom by a selenium atom at position 1 did not affect activity; selenazolopyrimidine **34** showed an IC_{50} value of 15 μM.

Considering these preliminary results, we decided to keep a methyl group at the R^1 position, an ethyl group at R^2, and a sulfur atom at X, as on the original thiazolopyrimidine scaffold. To assess the importance of the R^4 substituent, the enzymatic activity of some of the thia- and selenazolopyrimidine intermediates **19**–**30** was examined (Table 1). None of the evaluated compounds displayed significant inhibitory activity against CDC25B at a concentration of 100 μM, indicating that a substituent is required for activity at position 2 of the thiazolopyrimidine core.

We next investigated the effects of pharmacophore modulations on R^4 and retained the benzodioxolane moiety of our starting compound **1** (Table 3). The phenyl derivative **35** showed no activity, indicating the need for substituted benzylidene moieties. Introduction of a nitro group at the *para* position of the phenyl ring (compound **36**) led to a total loss of activity. The position of the hydroxy group on the phenyl moiety was subsequently examined. Compound **1** was the most efficient, as *ortho* and *meta* substitutions led to **37** and **38** with IC_{50} values of 37.4 and 28.4 μM, respectively. Introducing a second hydroxy group (compounds **39**–**41**) retained activity, provided *para* substitution was present. Replacement of both hydroxy groups by methoxy groups (in **42**) or chlorine atoms (in **43**) led to a dramatic loss of activity, thereby underscoring the importance of the phenolic functions. Introducing two bromine atoms around the hydroxy group led to an increase in potency, as **44** is the most potent inhibitor, with an IC_{50} value of 4.5 μM. On the opposite end, compound **45**, bearing two electron-donating groups, displayed an IC_{50} value of 25.2 μM. Acetylation afforded derivatives **46**–**52**, which exhibited weak inhibitory activity relative to their corresponding free phenolic derivatives. Only compound **50**, with three acetoxy groups, was as active as **1**, with an IC_{50} value of 14.3 μM.

Replacing the hydroxy group by carboxylic functions (compounds **53** and **54**) led to a decrease in potency. Furthermore, the corresponding methyl ester analogues **55** and **56** were totally inactive. The effects of a free carboxylic function were further explored by introducing linkers to modify the orientation and distance from the phenyl ring. Compounds **57** and **58**, containing an *O*-methylene or an ethylene linker, were as potent as **1**, with IC_{50} values of 18 and 12.6 μM, respectively, suggesting novel interactions with the enzyme. Finally, introducing a heterocycle such as pyridin-3-yl (in **59**) was detrimental to activity.

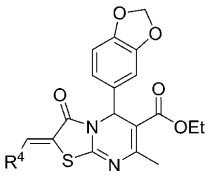
Table 2. Inhibitory activities of thia- and selenazolopyrimidines **31**–**34** toward CDC25.



Compd	X	R^1	R^2	$IC_{50} \pm$ SEM [μM][a] MBP–CDC25B3
menadione	–	–	–	2.2 ± 0.6
Cpd **5**	–	–	–	3.7 ± 0.2
1	S	Me	Et	13.0 ± 0.5
31	S	Ph	Et	7.5 ± 1.8
32	S	Me	Bn	15.2 ± 2.6
33	S	Me	H	NA[b]
34	Se	Me	Et	15.0 ± 0.8

[a] IC_{50} values ± SEM were calculated from three independent experiments with three determinations per tested concentration. [b] No activity; MBP–CDC25B3 inhibition is < 50% at 80 μM.

Table 3. Inhibitory activities of thiazolopyrimidines **35**–**59** toward CDC25.



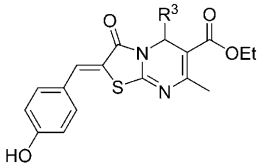
Compd	R^4	$IC_{50} \pm$ SEM [μM][a] MBP–CDC25B3
35	phenyl	NA[b]
36	4-nitrophenyl	NA[b]
37	2-hydroxyphenyl	37.4 ± 0.4
38	3-hydroxyphenyl	28.4 ± 0.4
39	2,4-dihydroxyphenyl	15.5 ± 0.1
40	3,4-dihydroxyphenyl	10.1 ± 2.1
41	2,5-dihydroxyphenyl	27.4 ± 9.2
42	3,4-dimethoxyphenyl	NA[b]
43	3,4-dichlorophenyl	NA[b]
44	3,5-dibromo-4-hydroxyphenyl	4.5 ± 0.2
45	4-hydroxy-3,5-dimethoxyphenyl	25.2 ± 1.7
46	2-acetoxyphenyl	NA[b]
47	3-acetoxyphenyl	NA[b]
48	4-acetoxyphenyl	46.5 ± 9.2
49	3,4-diacetoxyphenyl	31.1 ± 16.4
50	2,3,4-triacetoxyphenyl	14.3 ± 2.8
51	2,4,5-triacetoxyphenyl	NA[b]
52	3,4,5-triacetoxyphenyl	30.8 ± 4.2
53	3-carboxyphenyl	41.3 ± 7.6
54	4-carboxyphenyl	30.9 ± 2.7
55	3-methoxycarbonylphenyl	NA[b]
56	4-methoxycarbonylphenyl	NA[b]
57	4-(carboxymethoxy)phenyl	18.0 ± 0.3
58	4-(2-carboxyvinyl)phenyl	12.6 ± 1.9
59	pyridin-3-yl	NA[b]

[a] IC_{50} values ± SEM were calculated from three independent experiments with three determinations per tested concentration. [b] No activity; MBP–CDC25B3 inhibition is < 50% at 80 μM.

We then turned our attention toward R^3 and chose to develop two series of thiazolopyrimidine derivatives, with R^4 being either the 4-hydroxyphenyl moiety (Table 4) or the 3,4-diacetoxyphenyl moiety to improve cell penetration (Table 5). For both families, lack of an aromatic residue at position 5 on the thiazolopyrimidine core (compounds **60** and **67**) led to loss of activity. Considering both series, analogues with the same R^3 moieties showed similar potency (**61**–**63** versus **68**–**70**), with the 3,4-dichlorophenyl motif displaying IC_{50} values in the same range as **1**.

Regarding the 4-hydroxyphenyl subfamily, the introduction of hydrophilic groups on the phenyl ring led to the less efficient compounds **64**–**66**. In the 3,4-diacetoxyphenyl series, increasing the size of the substituent did not modify the inhibitory activity relative to the parent compound **49**, as the benzodioxol-6-yl derivative **71** exhibited an IC_{50} value of 31.3 μM. The naphth-2-yl derivative **72** was twofold more potent than **49**, whereas the 4-methylnaphth-1-yl core (compound **73**) was detrimental for activity. These results indicate that the orientation of the bicyclic moiety is essential for activity. Finally, introduction of a third methoxy group provided **74** with similar inhibitory activity as the dimethoxy derivative **69**.

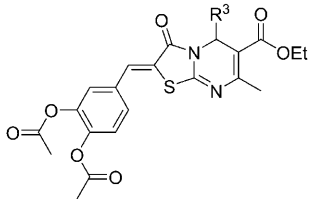
Table 4. Inhibitory activities of thiazolopyrimidines **60**–**66** toward CDC25.



Compd	R^3	$IC_{50} \pm$ SEM [μM][a] MBP–CDC25B3
60	H	NA[b]
61	phenyl	36.5 ± 0.2
62	3,4-dimethoxyphenyl	29.2 ± 3.0
63	3,4-dichlorophenyl	10.3 ± 0.1
64	3-hydroxyphenyl	18.8 ± 3.9
65	4-hydroxyphenyl	26.5 ± 1.9
66	3,4-dihydroxyphenyl	23.9 ± 4.0

[a] IC_{50} values ± SEM were calculated from three independent experiments with three determinations per tested concentration. [b] No activity; MBP–CDC25B3 inhibition is < 50% at 80 μM.

Table 5. Inhibitory activities of thiazolopyrimidines **67**–**74** toward CDC25.



Compd	R^3	$IC_{50} \pm$ SEM [μM][a] MBP–CDC25B3
67	H	NA[b]
68	phenyl	32.4 ± 11.9
69	3,4-dimethoxyphenyl	27.4 ± 2.0
70	3,4-dichlorophenyl	12.6 ± 3.3
71	1,4-benzodioxol-6-yl	31.3 ± 2.4
72	naphth-2-yl	14.9 ± 0.9
73	4-methylnaphth-1-yl	NA[b]
74	3,4,5-trimethoxyphenyl	31.2 ± 8.6

[a] IC_{50} values ± SEM were calculated from three independent experiments with three determinations per tested concentration. [b] No activity; MBP–CDC25B3 inhibition is < 50% at 80 μM.

Mechanism of inhibition

The mechanism of inhibition of four of our most potent inhibitors, namely compounds **1**, **44**, **58**, and **70** was investigated next. Because compounds **63** and **70** have similar potency, we chose to assess the latter, as it belongs to the 3,4-diacetoxy series. Enzyme kinetics assays were performed using inhibitor concentrations ranging from 0 to 10 μM, and the K_M value for FDP was 2.9 ± 0.3 μM toward CDC25B (data not shown). Interestingly, the inhibition kinetics of CDC25 activity by all compounds appeared to be most consistent with a mixed-competitive model (Figure 2). This result correlates with the predicted binding mode of compound **1**, which was proposed to contact both the active site of the enzyme and the inhibitor binding pocket.[26]

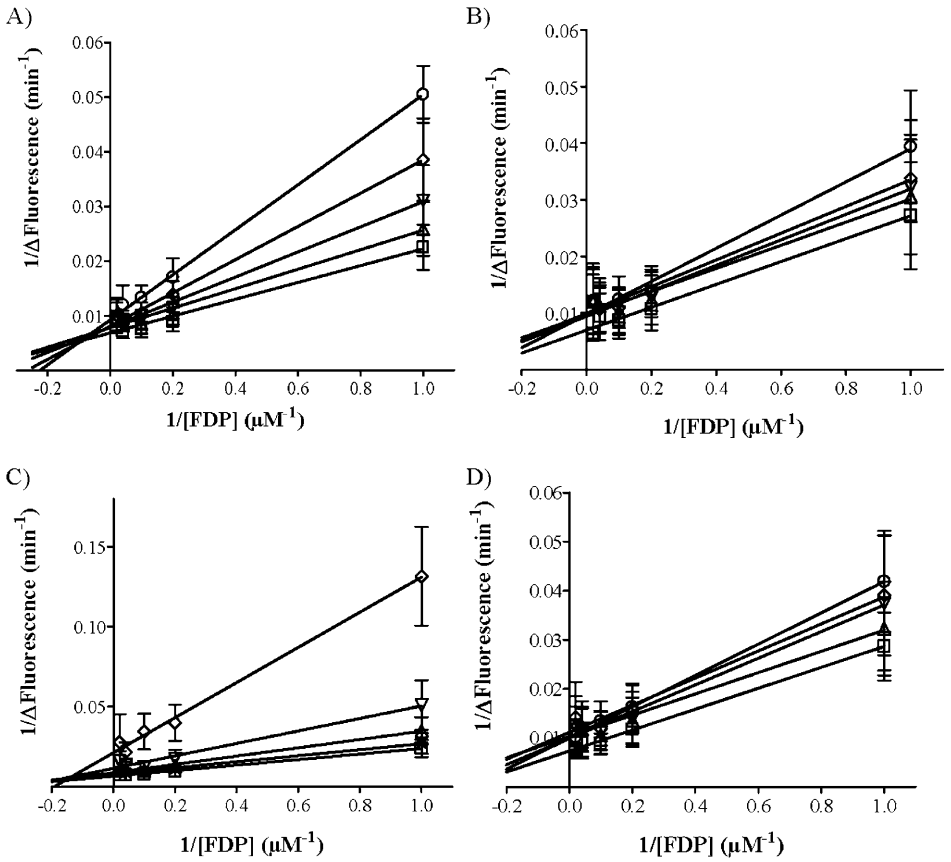


Figure 2. Lineweaver–Burk plots for the inhibition of CDC25B by compounds A) **70**, B) **1**, C) **44**, and D) **58**. The concentrations (in μM) of the inhibitors are 0(□), 1(△), 3(▽), 6(◇), and 10(○) for compounds **70**, **1**, and **58**, and 0(□), 0.3(*), 1(△), 3(▽), and 6(◇) for compound **44**.

pound **44** was neither sensitive to an increase in reducing agent concentration nor to the addition of catalase, suggesting a different mechanism of action.

Binding mode

We first investigated the docking of menadione, naphthofurandione 5169131,[40] and 1-(3,4-dihydroxyphenyl)-2-[2-(4-methoxyphenyl)-2*H*-tetrazol-5-ylsulfanyl]-ethanone.[41] Poses generated by Surflex were compatible with previously reported molecular modeling data, indicating that our docking protocol was appropriate to propose relevant binding modes for our inhibitors.

The binding modes of inhibitors **1** and **44** were then investigated in the context of the mutagenesis data and measured IC_{50} values. Prior to analysis of the docking modes, it is important to note that the binding site of CDC25 is complex and difficult to investigate structurally and energetically, with potential flexibility of some residues with long side chains such as arginine.[26] Considering the available data, however, we suggest that compound **1** could bind with its phenyldioxolane moiety located in the catalytic cavity and its remaining parts essentially anchored into the swimming pool (Figure 3 A). In this orientation, five hydrogen bonds (computed to be energetically relatively weak) could be found between the inhibitor and the protein, and several hydrophobic/aromatic contacts could also be replaced. The best inhibitor, **44**, could bind with its 3,5-di-

To further elucidate the binding mode of **44**, site-directed mutagenesis experiments were performed on R482 and R544, located at the junction between the active site and the inhibitor binding pocket. Both arginine residues are involved in the recognition of the Cdk phosphate group and were previously described to be engaged in strong interactions with quinonoid-based CDC25 inhibitors.[37,38] The R482A and R544Q mutants were constructed by gene synthesis (Genecust Europe) and subcloned in replacement of the wild-type gene. Results indicate that these residues do not play a critical role in the binding mode of this inhibitor, as these mutations do not modify the inhibitory activity, with IC_{50} values of 3.2 and 2.8 μM against R482A and R544Q, respectively.

The effects of increased dithiothreitol (DTT) concentrations on the inhibitory activity of compounds **1**, **44**, **58**, and **70** were then explored to investigate the mechanism of action of this series of inhibitors. Enzyme assays were performed as described in the Experimental Section by replacing the DTT concentration of 1 mM, normally used under our conditions, by 10 and 20 mM. The results are reported in Table 6 and indicate that an increase in DTT concentration correlates with a decrease in IC_{50} values for compounds **1**, **58**, and **70**, consistent with reactive oxygen species (ROS) generation in vitro. Such a mechanism has already been reported for quinonoid-based CDC25 inhibitors.[39] Interestingly, the inhibitory activity of com-

Table 6. Effect of catalase or various concentrations of DTT on the in vitro inhibition of CDC25B.

Compd	$IC_{50} \pm$ SEM [μM] or Percent MBP–CDC25B3 Inhibition[a] 1 mM DTT	10 mM DTT	20 mM DTT	Catalase[b]
menadione	2.2 ± 0.6	47%	26%	ND[c]
1	13.0 ± 0.5	36.1 ± 5.3	48%	ND[c]
44	4.5 ± 0.2	4.2 ± 0.1	5.8 ± 0.2	5.4 ± 2.1
58	12.6 ± 1.9	51.1 ± 3.5	40%	ND[c]
70	12.6 ± 3.3	70.9 ± 8.8	34%	ND[c]

[a] IC_{50} values ± SEM as well as the percent inhibition of CDC25B activity at 100 μM were calculated from two independent experiments with three determinations per tested concentration. [b] Catalase at 80 U mL^{-1}. [c] Not determined.

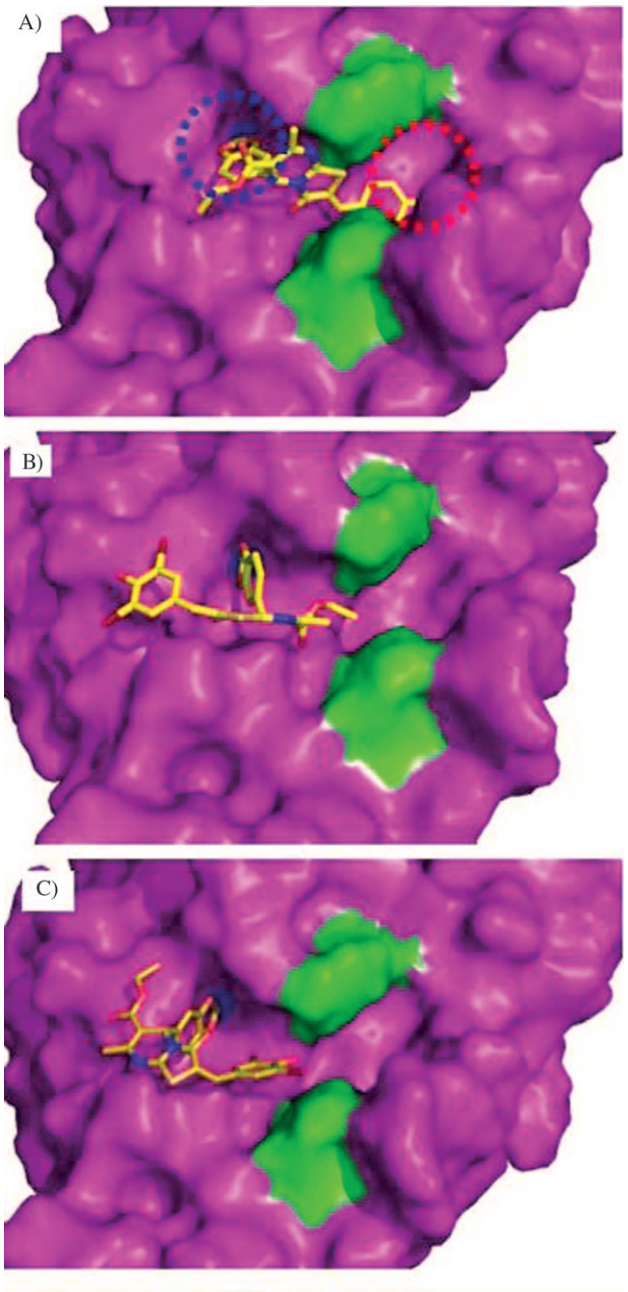


Figure 3. Proposed binding modes for compounds **1** and **44**. A) For compound **1** the CDC25 catalytic site is shown as a solid surface (magenta), the blue dashed circle highlights the catalytic site, and the red dashed circle shows the swimming pool region. R482 and R544 residues are highlighted in green. The catalytic C473 residue is shown in blue. Two orientations are proposed for compound **44**: B) In one, the dibromohydroxyphenyl moiety stacks against F475 with the phenyldioxolane moiety located in the catalytic site. C) Alternatively, the dibromohydroxyphenyl moiety is directed toward the swimming pool region, leaving the phenyldioxolane moiety in the catalytic cavity.

bromo-4-hydroxyphenyl moiety stacked against F475, next to the catalytic site, while the phenyldioxolane moiety would be oriented toward the catalytic cysteine as for compound **1** (Figure 3B). In this situation, compound **44** could form four strong hydrogen bonds with the protein (with atoms of the catalytic pocket and the side chain of N532, none with R482 and R544) and could make significant hydrophobic contacts and aromatic stacking interactions as well. Alternatively, the 3,5-dibromo-4-hydroxyphenyl moiety could point toward the swimming pool area without being able to fully insert into the cavity (Figure 3C). In this case, **44** could interact through five hydrogen bonds with the protein (four of favorable energy with atoms of the catalytic pocket and the side chain of N532, and one weak bond with the side chain of R544), and several hydrophobic/aromatic contacts were noted. Interestingly, in all cases, interactions with R482 and R544 are very limited, in agreement with site-directed mutagenesis data.

Compound 44 targets CDC25B in U2OS cells

To confirm that thiazolopyrimidine derivatives specifically inhibit CDC25 activity in cultured cells, we used an assay based on the reversion of the bypass of a G_2 checkpoint arrest induced by CDC25B overexpression in U2OS cells treated with the DNA-damaging agent etoposide.[42] In this assay, cells that overcome the G_2 checkpoint are identified by accumulation in mitosis following the addition of nocodazole (mitosis trap). U2OS cells overexpressing CDC25B were treated accordingly in the presence of increasing concentrations of **44** (Figure 4). As

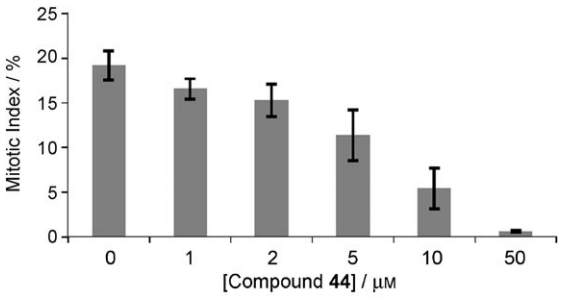


Figure 4. Compound **44** inhibits CDC25B activity in U2OS cells; these data are representative of four independent experiments.

shown, in the absence of CDC25 inhibitor, 20% of cells were trapped in mitosis, indicating that, as already reported, they are able to bypass the G_2/M checkpoint owing to CDC25B overexpression. We observed that treatment with compound **44** inhibited this CDC25B-dependent effect in a dose-dependent manner. At a concentration of 50 μM, compound **44** could totally revert CDC25B-dependent checkpoint bypass, with only 2% of cells showing a mitotic profile.

Cytotoxic assay

The cytotoxicity of compounds exhibiting inhibitory activity against CDC25B was evaluated on the human cancer cell lines MiaPaCa-2 (pancreatic adenocarcinoma) and LNCaP (hormone-dependent prostate carcinoma) by applying the WST-1 colorimetric cleavage assay. Viability of MiaPaCa-2 and LNCaP cells was determined after incubation periods of 48 and 72 h, respectively (Table 7). We previously reported that compound **1** is efficient at inhibiting clonal proliferation and exhibits cyto-

Table 7. Cytotoxic properties of thiazolopyrimidines with CDC25 inhibitory activity toward LNCaP and MiaPaCa-2 cell lines.

Compd	$IC_{50} \pm SEM$ [μM][a]	
	LNCaP	MiaPaCa-2
menadione	12.2 ± 2.1	14.5 ± 1.0
1	12.2 ± 3.8	36.0 ± 12.0
31	14.9 ± 2.6	NA[b]
32	9.6 ± 1.7	NA[b]
34	35.2 ± 19.8	NA[b]
37	10.5 ± 0.3	19.4 ± 3.8
38	9.5 ± 1.5	29.3 ± 9.9
39	7.1 ± 0.9	6.3 ± 0.9
40	7.1 ± 2.6	7.9 ± 1.6
41	14.9 ± 1.8	21.1 ± 1.5
44	3.4 ± 1.8	NA[b]
45	12.2 ± 2.3	NA[b]
48	16.6 ± 4.0	NA[b]
49	12.2 ± 0.6	53.7 ± 8.4
50	39.1 ± 2.3	NA[b]
52	41.8 ± 3.2	21.4 ± 0.1
53	17.1 ± 3.2	NA[b]
54	19.0 ± 8.7	NA[b]
57	49.0 ± 13.5	NA[b]
58	16.7 ± 6.0	NA[b]
61	41.6 ± 8.9	NA[b]
62	16.9 ± 8.9	48.8 ± 14.2
63	6.4 ± 1.3	NA[b]
64	10.9 ± 2.4	NA[b]
65	22.5 ± 4.0	NA[b]
66	32.3 ± 4.3	NA[b]
68	9.2 ± 0.7	31.3 ± 2.2
69	23.2 ± 6.7	33.2 ± 5.6
70	6.7 ± 2.5	15.5 ± 2.5
71	15.6 ± 2.1	NA[b]
72	15.1 ± 4.8	32.8 ± 2.2
74	21.1 ± 1.6	NA[b]

[a] IC_{50} values ± SEM were calculated from three independent experiments with three determinations per tested concentration. [b] No activity; cell viability is > 50% at 80 μM.

toxic activity against HeLa cells. Here, the thiazolopyrimidines were evaluated against LNCaP and MiaPaCa-2 cell lines, as CDC25B has been reported to be overexpressed in prostate and pancreatic cancers, respectively.[43,44] Menadione was tested under our assay conditions as the reference compound and displayed cytotoxic activity toward LNCaP and MiaPaCa-2 cell lines with IC_{50} values of 12.2 and 14.5 μM, respectively, in line with published results (Table 7).[45]

Our results show that LNCaP cells tend to be more sensitive to thiazolopyrimidines, as all 31 compounds tested displayed cytotoxic activity, with IC_{50} values ranging from 3.4 to 49.0 μM. The 3,5-dibromo-4-hydroxyphenyl derivative **44**, which is the most potent CDC25 inhibitor, also displayed the best cytotoxic potency against LNCaP, with an IC_{50} value of 3.4 μM, but was observed to be inactive against MiaPaCa-2. Several other potent in vitro inhibitors including **31**, **32**, **45**, **48**, **53**, **54**, **58**, **63**, **64**, and **71** shared the same profile. In contrast, no compound appeared to be selective toward MiaPaCa-2. On the other hand, compounds with hydroxy groups on the benzylidene moiety such as **37**, **39**–**41**, and **70** displayed cytotoxic activity toward both cell lines, except **44** and **45**. Therefore, the presence of a hydroxyphenyl moiety as the R_4 substituent seems to play an important role for cytotoxicity against MiaPaCa-2 cells.

Finally, several inhibitors displayed higher cytotoxic activities than their enzymatic potencies, suggesting that they might have targets other than CDC25 in the cell. Nonetheless, having more than one mechanism of action could be advantageous for a potential anticancer agent.

Conclusions

CDC25 phosphatases play critical roles in cell-cycle regulation and are attractive targets for anticancer therapies. Currently, the most advanced class of inhibitors are the quinonoid-based structures. Starting from in silico/in vitro screening experiments previously carried out on CDC25B, we developed a novel class of CDC25 inhibitors based on a thiazolopyrimidine scaffold. Our study presents 31 compounds with activity against CDC25 in the low-micromolar range for the most potent, as well as cytotoxic activity toward tumor cell lines. Furthermore, kinetics studies revealed that these compounds exhibit mixed-inhibition profiles, suggesting possible interactions with both the catalytic site and the inhibitor binding pocket. Among all synthesized thiazolopyrimidines, derivative **44**, which is the most potent in vitro inhibitor, appears as a new promising compound, as it may have a different mechanism of action, displays cytotoxic activity toward the LNCaP cell line, and targets CDC25B in cellular assays.

Experimental Section

General

Commercially available compounds were purchased from Acros and Aldrich, and were used without further purification. Solvents were obtained from Carlo Erba–SDS. Kieselgel 60 F_{254} plates (Merck) were used for analytical thin layer chromatography and were visualized with UV light ($\lambda = 254$ nm). Flash chromatography was performed on silica gel 60 (0.04–0.063 mm) purchased from Carlo Erba–SDS. All melting points were determined on a Kofler apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker WMFT 250 MHz spectrometer at the Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, Université Paris Descartes, Paris (France). Chemical shifts are expressed in parts per million (ppm) using tetramethylsilane as an internal standard, and coupling constants (*J*) are given in Hz. Elemental analyses (C, H, N) were performed at the Service de Microanalyse, Université Pierre et Marie Curie, Paris (France).

General procedure 1: Biginelli condensation for the preparation of compounds 2–18: Urea or selenourea (1.5 equiv) and *para*-toluenesulfonic acid (0.2 equiv) were added to a solution of the appropriate benzaldehyde dissolved in dioxane. The reaction mixture was heated at reflux for 2 h, and the β-keto ester (1.5 equiv) was added dropwise. After heating at reflux for 12 h, the solvent was removed under reduced pressure. The crude product was dissolved in EtOAc and washed with $NaHCO_3$. The organic layer was separated, and the aqueous phase was extracted (3×) with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and the solvent was removed in vacuo. The obtained

solids were either purified by column chromatography on silica gel or simply washed with Et_2O.

General procedure 2: Synthesis of 5-aryl-7-substituted-3-oxo-2,3-dihydro-5*H*-thia(selena)zolo[3,2-*a*]pyrimidine-6-carboxylates 19–30: Chloroacetyl chloride (1.5 equiv) and anhydrous Na_2CO_3 (2 equiv) were added to a solution of dihydropyrimidine in dioxane. The resulting reaction mixture was heated at reflux for 2 h. The solvent was removed under reduced pressure, and the crude product was dissolved in EtOAc. The organic layer was washed with H_2O (3×) and brine, dried over Na_2SO_4, and finally concentrated in vacuo. The crude products were either purified by column chromatography on silica gel or simply washed with Et_2O.

General procedure 3: Knoevenagel condensation for the preparation of compounds 1, 31–34, and 60–66: An equivalent mixture of thiazolopyrimidines **19--30** and hydroxybenzaldehydes was heated at reflux in MeOH with piperidine for 2 h. After complete reaction, the solid was filtered and washed with MeOH. The final 5-aryl-2-arylidene-7-substituted-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidin-2-ones were recrystallized from MeOH.

General procedure 4: One-pot synthesis of 5-aryl-2-arylidene-7-substituted-3-oxo-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyrimidine-6-carboxylates 35–59 and 67–74: Biginelli compounds (1 equiv), aldehyde (1 equiv), 2-chloroacetyl chloride (1.2 equiv), and NaOAc (1 equiv) were heated at reflux for 6 h in $AcOH/Ac_2O$. The resulting mixture was cooled to room temperature and poured into H_2O. The aqueous layer was extracted (3×) with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude products were purified by column chromatography on silica gel.

General procedure 5: Synthesis of thiazolopyrimidines 55 and 56: $SOCl_2$ (1 equiv) was added to a suspension of the corresponding thiazolopyrimidines **53** or **54** in MeOH at 0 °C. After complete reaction, the resulting precipitate was filtered and washed with MeOH.

Ethyl-4-(1,3-benzodioxol-5-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2):[46] According to procedure 1, compound **2** was obtained as a white powder (67%); mp: 175 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.12 (t, ^3J = 7.0, 3H, $CO_2CH_2CH_3$), 2.29 (s, 3H, 6-CH_3), 4.03 (q, ^3J = 7.0, 2H, $CO_2CH_2CH_3$), 5.10 (s, 1H, 4-H), 6.01 (s, 2H, O-CH_2-O), 6.68 (d, ^3J = 8.0, 1H, H_{ar}), 6.73 (s, 1H, H_{ar}), 6.88 (d, ^3J = 8.0, 1H, H_{ar}), 9.54 (s, 1H, NH), 10.06 ppm (s, 1H, NH); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 14.2, 17.4, 53.9, 59.8, 100.9, 101.3, 106.9, 108.3, 119.8, 137.7, 145.2, 146.9, 147.6, 165.3, 174.3 ppm.

Ethyl-4-(1,3-benzodioxol-5-yl)-6-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3): According to procedure 1, compound **3** was obtained as a white powder (75%); mp: 191–192 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 0.74 (t, ^3J = 7.0, 3H, $CO_2CH_2CH_3$), 3.75 (q, ^3J = 7.0, 2H, $CO_2CH_2CH_3$), 5.19 (s, 1H, 4-H), 6.03 (s, 2H, O-CH_2-O), 6.83–6.88 (m, 2H, H_{ar}), 6.93 (d, ^3J = 7.9, 1H, H_{ar}), 7.30–7.43 (m, 5H, H_{ar}), 9.72 (s, 1H, NH), 10.49 ppm (s, 1H, NH); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 13.9, 54.3, 60.0, 101.7, 102.4, 107.3, 108.9, 120.2, 128.3 (×2), 129.2 (×2), 129.7, 134.5, 137.5, 146.3, 147.4, 148.0, 165.4, 174.9 ppm; Anal. calcd for $C_{20}H_{18}N_2O_4S{\cdot}0.5\,H_2O$: C 61.38, H 4.86, N 7.16, found: C 61.63, H 4.98, N 7.27.

Benzyl-4-(1,3-benzodioxol-5-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4): According to procedure 1, compound **4** was obtained as a white powder (74%); mp: 204 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 2.33 (s, 3H, 6-CH_3), 5.05–5.14 (m, 2H, $CO_2CH_2C_6H_5$), 5.14 (s, 1H, 4-H), 6.01 (s, 2H, O-CH_2-O), 6.65–6.70 (m, 2H, H_{ar}), 6,85 (d, ^3J = 7.9, 1H, H_{ar}), 7.19 (m, 2H, H_{ar}), 7.30 (m, 3H, H_{ar}), 9.60 (s, 1H, NH), 10.36 ppm (s, 1H, NH); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 17.6, 54.1, 65.5, 100.6, 101.5, 107.3, 108.5, 120.2, 128.0 (×2), 128.2, 128.7 (×2), 136.7, 137.7, 146.2, 147.1, 147.8, 165.2, 174.3 ppm; Anal. calcd for $C_{20}H_{18}N_2O_4S{\cdot}0.5\,H_2O$: C 61.38, H 4.86, N 7.16, found: C 61.78, H 4.91, N 7.40.

2-Cyanoethyl-4-(1,3-benzodioxol-5-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (5): According to procedure 1, compound **5** was obtained as a white powder (75%); mp: 210 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 2.33 (s, 3H, 6-CH_3), 2.82–2.88 (m, 2H, $CO_2CH_2CH_2CN$), 4.13–4.26 (m, 2H, $CO_2CH_2CH_2CN$), 5.10 (s, 1H, 4-H), 6.00 (s, 2H, O-CH_2-O), 6.71–6.75 (m, 2H, H_{ar}), 6.86 (d, ^3J = 7.9, 1H, H_{ar}), 9.68 (s, 1H, NH), 10.41 ppm (s, 1H, NH); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 17.8 (×2), 54.0, 59.2, 100.4, 101.5, 107.1, 108.6, 119.0, 120.1, 137.7, 146.5, 147.2, 147.8, 165.0, 174.5 ppm; Anal. calcd for $C_{16}H_{15}N_3O_4S{\cdot}0.5\,H_2O$: C 54.23, H 4.52, N 11.86, found: C 54.13, H 4.55, N 11.15.

4-(1,3-Benzodioxol-5-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid (6): 2-Cyanoethyl-4-(1,3-benzodioxol-5-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **5** (150 mg, 0.43 mmol) was dissolved in MeOH (4 mL). The mixture was cooled to 0 °C before an aqueous solution of NaOH (26 mg in 0.2 mL) was added dropwise. After 4 h stirring, the reaction mixture was concentrated. The resulting aqueous phase was extracted with EtOAc after acidification with HCl (1 N). The combined organic layers were washed with brine, dried over Na_2SO_4, and the solvent was removed. The residue was washed with Et_2O. Compound **6** was obtained as a white solid (57%); mp: >260 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 2.28 (s, 3H, 6-CH_3), 5.08 (s, 1H, 4-H), 5.99 (s, 2H, O-CH_2-O), 6.66–6.73 (m, 2H, H_{ar}), 6.85 (d, ^3J = 7.8, 1H, H_{ar}), 9.52 (s, 1H, NH), 10.22 (s, 1H, NH), 12.20 ppm (s, 1H, COOH); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 17.1, 53.7, 101.0, 101.3, 106.7, 108.1, 119.5, 137.4, 144.4, 146.6, 147.3, 166.9, 174.1 ppm.

Ethyl-4-(1,3-benzodioxol-5-yl)-6-methyl-2-selenoxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (7): According to procedure 1, compound **7** was obtained as a pale-yellow powder (73%); mp: 106–108 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.06 (t, ^3J = 7.0, 3H, $CO_2CH_2CH_3$), 2.24 (s, 3H, 6-CH_3), 3.95 (q, ^3J = 7.0, 2H, $CO_2CH_2CH_3$), 5.05 (s, 1H, 4-H), 5.95 (s, 2H, O-CH_2-O), 6.60–6.67 (m, 2H, H_{ar}), 6.82–6.84 (d, ^3J = 8.2, 1H, H_{ar}), 10.10 (s, 1H, NH), 10.59 ppm (s, 1H, NH); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 14.5, 17.5, 54.4, 60.3, 101.7, 101.8, 107.3, 108.7, 120.3, 137.5, 144.6, 147.3, 148.0, 165.7, 170.7 ppm.

Ethyl-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (8):[47] According to procedure 1, compound **8** was obtained as a beige powder (66%); mp: 248 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.19 (t, ^3J = 7.0, 3H, $CO_2CH_2CH_3$), 2.18 (s, 3H, 6-CH_3), 3.89 (s, 2H, CH_2), 4.09 (q, ^3J = 7.0, 2H, $CO_2CH_2CH_3$), 8.95 (s, 1H, NH), 9.94 ppm (s, 1H, NH); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 14.2, 16.8, 40.8, 59.5, 96.0, 145.4, 165.1, 175.7 ppm.

Ethyl-6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (9):[47] According to procedure 1, compound **9** was obtained as a white powder (78%); mp: 210 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.08 (t, ^3J = 7.0, 3H, $CO_2CH_2CH_3$), 2.30 (s, 3H, 6-CH_3), 4.04 (q, ^3J = 7.0, 2H, $CO_2CH_2CH_3$), 5.18 (s, 1H, 4-H), 7.21–7.39 (m, 5H, H_{ar}), 9.65 (s, 1H, NH), 10.03 ppm (s, 1H, NH); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 13.7, 16.9, 53.8, 59.3, 100.5, 126.1 (×2), 127.4, 128.3 (×2), 143.2, 144.6, 164.9, 174.0 ppm.

Ethyl-4-(3,4-dimethoxphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (10):[48] According to procedure 1,

compound **10** was obtained as a white powder (66%); mp: 178 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.13 (t, ^3J = 7.1, 3H, $CO_2CH_2CH_3$), 2.30 (s, 3H, 6-CH_3), 3.73 (s, 6H, 2×O-CH_3), 4.03 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 5.13 (s, 1H, 4-H), 6.70 (d, ^3J = 8.2, 1H, H$_{ar}$), 6.84 (s, 1H, H$_{ar}$), 6.91 (d, ^3J = 8.2, 1H, H$_{ar}$), 9.59 (s, 1H, NH), 10.29 ppm (s, 1H, NH); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 13.8, 16.9, 53.3, 55.2 (×2), 59.3, 100.6, 110.2, 111.6, 117.9, 135.7, 144.5, 148.1, 148.3, 164.9, 173.9 ppm.

Ethyl-4-(3,4-dichlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (11):[49] According to procedure 1, compound **11** was obtained as a white powder (77%); mp: 126 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.12 (t, ^3J = 7.1, 3H, $CO_2CH_2CH_3$), 2.31 (s, 3H, 6-CH_3), 4.03 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 5.20 (s, 1H, 4-H), 7.19 (d, ^3J = 8.3, 1H, H$_{ar}$), 7.43 (s, 1H, H$_{ar}$), 7.65 (d, ^3J = 8.3, 1H, H$_{ar}$), 9.69 (s, 1H, NH), 10.45 ppm (s, 1H, NH).

Ethyl-4-(3-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (12):[50] According to procedure 1, compound **12** was obtained as a white powder (68%); mp: 152–154 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.13 (t, ^3J = 7.1, 3H, $CO_2CH_2CH_3$), 2.30 (s, 3H, 6-CH_3), 4.03 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 5.12 (s, 1H, 4-H), 6.66–6.69 (m, 3H, H$_{ar}$), 7.11–7.17 (m, 1H, H$_{ar}$), 9.45 (s, 1H, OH), 9.60 (s, 1H, NH), 10.30 ppm (s, 1H, NH); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 13.8, 16.9, 53.7, 59.3, 100.5, 113.0, 114.3, 116.7, 129.2, 144.5 (×2), 157.2, 164.9, 173.9 ppm.

Ethyl-4-(4-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (13):[51] According to procedure 1, compound **13** was obtained as a white powder (65%); mp: 202 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.15 (t, ^3J = 7.1, 3H, $CO_2CH_2CH_3$), 2.30 (s, 3H, 6-CH_3), 4.03 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 5.07 (s, 1H, 4-H), 6.72 (d, ^3J = 8.3, 2H, H$_{ar}$), 7.02 (d, ^3J = 8.3, 2H, H$_{ar}$), 9.43 (s, 1H, OH), 9.55 (s, 1H, NH), 10.24 ppm (s, 1H, NH); ^{13}C NMR (250 MHz, [D$_6$]acetone): δ = 14.6, 17.9, 55.7, 60.5, 103.3, 116.1 (×2), 129.0 (×2), 135.9, 144.7, 158.0, 166.2, 176.2 ppm.

Ethyl-4-(3,4-dihydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (14): According to procedure 1, compound **14** was obtained as a white powder (66%); mp: 212 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.13 (t, ^3J = 7.1, 3H, $CO_2CH_2CH_3$), 2.36 (s, 3H, 6-CH_3), 4.04 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 5.01 (s, 1H, 4-H), 6.47 (d, ^3J = 7.9, 1H, H$_{ar}$), 6.64–6.69 (m, 2H, H$_{ar}$), 8.80 (bs, 2H, 2×OH), 9.51 (s, 1H, NH), 10.20 ppm (s, 1H, NH); ^{13}C NMR ([D$_6$]DMSO): δ = 14.4, 22.9, 54.9, 60.6, 107.9, 114.5, 115.9, 118.3, 130.1, 142.5, 151.9, 158.0, 165.6, 171.5 ppm.

Ethyl-4-(2,3-dihydro-1,4-benzodioxin-6-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (15): According to procedure 1, compound **15** was obtained as a white powder (72%); mp: 158 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.15 (t, ^3J = 7.0, 3H, $CO_2CH_2CH_3$), 2.28 (s, 3H, 6-CH_3), 4.02 (q, ^3J = 7.0, 2H, $CO_2CH_2CH_3$), 4.21 (s, 4H, O-CH_2-CH_2-O), 5.06 (s, 1H, 4-H), 6.65–6.68 (m, 2H, H$_{ar}$), 6.82 (d, ^3J = 8.8, 1H, H$_{ar}$), 9.57 (s, 1H, NH), 10.30 ppm (s, 1H, NH); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 13.8, 22.5, 53.0, 59.3, 63.8 (×2), 100.5, 114.6, 116.7, 118.7, 136.2, 142.6, 142.8, 144.6, 164.8, 173.8 ppm; Anal. calcd for $C_{16}H_{18}N_2O_4S$: C 57.48, H 5.39, N 8.38, found: C 57.04, H 5.58, N 8.23.

Ethyl-6-methyl-4-(2-naphthyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (16):[48] According to procedure 1, compound **16** was obtained as a white powder (52%); mp: 177 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.07 (t, ^3J = 6.9, 3H, $CO_2CH_2CH_3$), 2.35 (s, 3H, 6-CH_3), 3.97 (q, ^3J = 6.9, 2H, $CO_2CH_2CH_3$), 5.36 (s, 1H, 4-H), 7.41–7.44 (m, 3H, H$_{ar}$), 7.54 (s, 1H, H$_{ar}$), 7.91–7.94 (m, 3H, H$_{ar}$), 9.74 (s, 1H, NH), 10.38 ppm (s, 1H, NH); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 14.5, 17.7, 54.8, 60.0, 101.0, 125.3, 125.4, 126.6, 126.9, 128.0, 128.4, 129.0, 132.9, 133.1, 141.3, 145.7, 165.6, 174.7 ppm.

Ethyl-6-methyl-4-(4-methyl-1-naphthyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (17): According to procedure 1, compound **17** was obtained as a white powder (72%); mp: 148–149 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 0.87 (t, ^3J = 7.0, 3H, $CO_2CH_2CH_3$), 2.41(s, 3H, 6-CH_3), 2.65 (s, 3H, CH_3), 3.88 (q, ^3J = 7.0, 2H, $CO_2CH_2CH_3$), 6.06 (s, 1H, 4-H), 7.25 (d, ^3J = 7.3, 1H, H$_{ar}$), 7.36 (d, ^3J = 7.3, 1H, H$_{ar}$), 7.60–7.63 (m, 2H, H$_{ar}$), 8.07 (d, ^3J = 5.4, 1H, H$_{ar}$), 8.39 (d, ^3J = 5.4, 1H, H$_{ar}$), 9.60 (s, 1H, NH), 10.35 ppm (s, 1H, NH); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 14.2, 17.5, 19.6, 50.1, 59.8, 101.1, 124.6, 124.8, 124.9, 126.1, 126.2, 126.8, 130.4, 132.7, 134.7, 137.6, 145.7, 165.5, 174.1 ppm; Anal. calcd for $C_{19}H_{20}N_2O_2S$: C 67.06, H 5.88, N 8.23, found: C 66.65, H 6.18, N 8.04.

Ethyl-6-methyl-4-(3,4,5-trimethoxyphenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (18):[52] According to procedure 1, compound **18** was obtained as a white powder (70%); mp: 202 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.13 (t, ^3J = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 2.28 (s, 3H, 6-CH_3), 3.62 (s, 3H, O-CH_3), 3.71 (s, 6H, 2×O-CH_3), 4.04 (q, ^3J = 7.2 Hz, 2H, $CO_2CH_2CH_3$), 5.15 (s, 1H, 4-H), 6.50 (s, 2H, H$_{ar}$), 9.58 (s, 1H, NH), 10.31 ppm (s, 1H, NH); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 14.6, 17.7, 54.4, 56.3, 56.4, 60.2, 60.5, 101.2, 104.0 (×2), 137.6, 139.6, 145.6, 153.4 (×2), 165.7, 175.0 ppm.

Ethyl-5-(1,3-benzodioxol-5-yl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (19): According to procedure 2, compound **19** was obtained as a pale-yellow powder (77%); mp: 213 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.14 (t, ^3J = 7.1, 3H, $CO_2CH_2CH_3$), 2.36 (s, 3H, 7-CH_3), 4.04 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 4.14 (s, 2H, CO-CH_2-S), 5.83 (s, 1H, 5-H), 6.02 (s, 2H, O-CH_2-O), 6.68–6.76 (m, 2H, H$_{ar}$), 6.87 ppm (d, ^3J = 8.4, 1H, H$_{ar}$); ^{13}C NMR (250 MHz, $CDCl_3$): δ = 14.5, 22.9, 33.0, 55.6, 61.0, 101.7, 108.6 (×2), 108.8, 122.4, 134.2, 148.2 (×2), 152.3, 160.4, 165.8, 170.7 ppm; Anal. calcd for $C_{17}H_{16}N_2O_5S{\cdot}0.5\,H_2O$: C 55.28, H 4.60, N 7.58, found: C 55.41, H 4.63, N 7.59.

Ethyl-5-(1,3-benzodioxol-5-yl)-3-oxo-7-phenyl-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (20): According to procedure 2, compound **20** was obtained as a pale-yellow powder (80%); mp: 201 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 0.82 (t, ^3J = 7.1, 3H, $CO_2CH_2CH_3$), 3.85 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 4.19 (s, 2H, CO-CH_2-S), 5.94 (s, 1H, 5-H), 6.05 (s, 2H, O-CH_2-O), 6.84–6.88 (m, 1H, H$_{ar}$), 6.92–6.96 (m, 2H, H$_{ar}$), 7.40–7.52 ppm (m, 5H, H$_{ar}$); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 13.4, 32.8, 55.1, 60.2, 101.5, 107.8, 108.5, 108.9, 121.3, 127.7 (×2), 128.3 (×2), 128.7, 134.0, 138.8, 147.6 (×2), 150.0, 160.6, 165.6, 171.2 ppm; Anal. calcd for $C_{22}H_{18}N_2O_5S$: C 62.56, H 4.26, N 6.63, found: C 63.39, H 4.56, N 6.79.

Benzyl-5-(1,3-benzodioxol-5-yl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (21): According to procedure 2, compound **21** was obtained as a beige powder (63%); mp: 195–196 °C; ^{1}H NMR (250 MHz, $CDCl_3$): δ = 2.46 (s, 3H, 7-CH_3), 5.00 (d, ^3J = 12.5, 1H, $CO_2CH_2C_6H_5$), 5.11 (d, ^3J = 12.5, 1H, CO-CH_2-S), 5.91 (s, 2H, O-CH_2-O), 5.96 (s, 1H, 5-H), 6.64–6.67 (m, 1H, H$_{ar}$), 6.76–6.79 (m, 2H, H$_{ar}$), 7.15–7.16 (m, 2H, H$_{ar}$), 7.27–7.28 ppm (m, 3H, H$_{ar}$); ^{13}C NMR (250 MHz, $CDCl_3$): δ = 23.0, 32.6, 55.4, 65.6, 101.4, 108.0, 108.4, 108.8, 122.3, 128.4 (×3), 128.7 (×2), 133.9, 135.8, 148.0 (×2), 153.1, 160.1, 165.4, 170.5 ppm; Anal. calcd for $C_{22}H_{18}N_2O_5S{\cdot}0.5\,H_2O$: C 61.25, H 4.40, N 6.50, found: C 61.83, H 4.36, N 6.87.

5-(1,3-Benzodioxol-5-yl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylic acid (22): According to procedure 2, compound **22** was obtained as a beige powder

(27%); mp: 194 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 2.33 (s, 3H, 7-CH$_3$), 4.11 (s, 2H, CO-CH$_2$-S), 5.80 (s, 1H, 5-H), 6.00 (s, 2H, O-CH$_2$-O), 6.71–6.73 (m, 1H, H$_{ar}$), 6.85–6.88 (m, 2H, H$_{ar}$), 12.51 ppm (bs, 1H,COOH); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 22.3, 32.6, 54.1, 101.2, 107.7, 107.9, 108.2, 121.0, 134.4, 147.2 (×2), 150.8, 160.2, 166.7, 171.1 ppm.

Ethyl-5-(1,3-benzodioxol-5-yl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]selenazolo[3,2-*a*]pyrimidine-6-carboxylate (23): According to procedure 2, compound **23** was obtained as a pale-yellow powder (70%); mp: 246 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.21 (t, ^3J = 7.0, 3H, CO$_2$CH$_2$CH$_3$), 2.45 (s, 3H, 7-CH$_3$), 3.74 (d, ^3J = 16.8, 1H, CO-CH$_2$-Se), 3.91 (d, 1H, ^3J = 16.8 Hz, CO-CH$_2$-Se), 4.08 (q, ^3J = 7.0, 2H, CO$_2$CH$_2$CH$_3$), 5.91 (s, 2H, O-CH$_2$-O), 5.96 (s, 1H, 5-H), 6.68 (d, ^3J = 7.8, 1H, H$_{ar}$), 6.81–6.85 ppm (m, 2H, H$_{ar}$); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 14.3, 22.7, 26.2, 56.0, 60.7, 101.4, 108.4, 108.6, 109.3, 122.1, 134.2, 148.0 (×2), 151.7, 156.0, 165.8, 171.9 ppm; Anal. calcd for C$_{17}$H$_{16}$N$_2$O$_5$Se: C 50.12, H 3.93, N 6.88, found: C 50.62, H 4.36, N 6.30.

Ethyl-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (24): According to procedure 2, compound **24** was obtained as a pale-yellow powder (74%); mp: 127 °C; ^{1}H NMR (250 MHz, CDCl$_3$): δ = 1.26 (t, ^3J = 7.0, 3H, CO$_2$CH$_2$CH$_3$), 2.35 (s, 3H, 7-CH$_3$), 3.88 (s, 2H, CH$_2$), 4.16 (q, ^3J = 7.0, 2H, CO$_2$CH$_2$CH$_3$), 4.39 ppm (s, 2H, CO-CH$_2$-S); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 13.9, 22.0, 32.2, 41.2, 59.6, 102.0, 151.7, 161.6, 164.9, 171.1 ppm; Anal. calcd for C$_{10}$H$_{12}$N$_2$O$_3$S: C 50.00, H 5.00, N 11.66, found: C 50.33, H 5.33, N 11.48.

Ethyl-7-methyl-3-oxo-5-phenyl-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (25):[53] According to procedure 2, compound **25** was obtained as a beige powder (80%); mp: 121 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.14 (t, ^3J = 7.1, 3H, CO$_2$CH$_2$CH$_3$), 2.36 (s, 3H, 7-CH$_3$), 4.04 (q, ^3J = 7.1, 2H, CO$_2$CH$_2$CH$_3$), 4.15 (s, 2H, CO-CH$_2$-S), 5.91 (s, 1H, 5-H), 7.28–7.39 ppm (m, 5H, H$_{ar}$); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 13.7, 22.2, 32.3, 54.3, 59.8, 107.0, 127.0 (×2), 128.2, 128.4 (×2), 140.5, 151.4, 160.8, 164.7, 170.8 ppm.

Ethyl-5-(3,4-dimethoxyphenyl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (26): According to procedure 2, compound **26** was obtained as a beige powder (77%); mp: 112–114 °C; ^{1}H NMR (250 MHz, CDCl$_3$): δ = 1.14 (t, ^3J = 7.1, 3H, CO$_2$CH$_2$CH$_3$), 2.39 (s, 3H, 7-CH$_3$), 3.75 (s, 3H, O-CH$_3$), 3.77 (s, 3H, O-CH$_3$), 3.98–4.07 (m, 4H, CO-CH$_2$-S and CO$_2$CH$_2$CH$_3$), 5.93 (s, 1H, 5-H), 6.70 (d, ^3J = 8.2, 1H, H$_{ar}$), 6.79–6.83 ppm (m, 2H, H$_{ar}$); ^{13}C NMR (250 MHz, CDCl$_3$): δ = 14.3, 22.8, 32.7, 55.4, 56.1 (×2), 60.7, 108.4, 111.2, 111.6, 120.6, 132.8, 149.0, 149.5, 152.1, 160.0, 165.7, 170.6 ppm.

Ethyl-5-(3,4-dichlorophenyl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (27): According to procedure 2, compound **27** was obtained as a beige powder (78%); mp: 197 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.12 (t, ^3J = 7.1, 3H, CO$_2$CH$_2$CH$_3$), 2.36 (s, 3H, 7-CH$_3$), 4.03 (q, ^3J = 7.1, 2H, CO$_2$CH$_2$CH$_3$), 4.14 (s, 2H, CO-CH$_2$-S), 5.87 (s, 1H, 5-H), 7.22 (d, ^3J = 8.3, 1H, H$_{ar}$), 7.46 (s, 1H, H$_{ar}$), 7.63 ppm (d, ^3J = 8.3, 1H, H$_{ar}$); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 13.8, 21.7, 33.1, 54.0, 60.2, 106.2, 128.0, 129.9, 131.0 (×2), 131.2, 140.9, 150.8, 162.8, 164.4, 171.0 ppm.

Ethyl-5-(3-hydroxyphenyl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (28): According to procedure 2, compound **28** was obtained as a white powder (77%); mp: 219 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.12 (t, ^3J = 7.1, 3H, CO$_2$CH$_2$CH$_3$), 2.31 (s, 3H, 7-CH$_3$), 4.08 (q, ^3J = 7.1, 2H, CO$_2$CH$_2$CH$_3$), 4.12 (s, 2H, CO-CH$_2$-S), 5.79 (s, 1H, 5-H), 6.63–6.65 (m, 3H, H$_{ar}$), 7.08–7.10 (m, 1H, H$_{ar}$), 9.50 ppm (s, 1H, OH); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 14.4, 22.9, 33.1, 54.9, 60.6, 107.9, 114.5, 115.9, 118.3, 130.1, 142.5, 151.9, 158.0, 161.6, 165.6, 171.5 ppm.

Ethyl-5-(4-hydroxyphenyl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (29): According to procedure 2, compound **29** was obtained as a white powder (81%); mp: 219 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.16 (t, ^3J = 7.1, 3H, CO$_2$CH$_2$CH$_3$), 2.36 (s, 3H, 7-CH$_3$), 4.03 (q, ^3J = 7.1, 2H, CO$_2$CH$_2$CH$_3$), 4.14 (s, 2H, CO-CH$_2$-S), 5.79 (s, 1H, 5-H), 6.70 (d, ^3J = 8.3, 2H, H$_{ar}$), 7.05 ppm (d, ^3J = 8.3, 2H, H$_{ar}$); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 14.4, 21.4, 33.8, 54.9, 60.7, 108.3, 115.8 (×2), 129.5 (×2), 131.0, 148.8, 158.2, 163.9, 165.1, 171.4 ppm; Anal. calcd for C$_{16}$H$_{16}$N$_2$O$_4$S·H$_2$O: C 54.85, H 5.14, N 8.00, found: C 54.27, H 4.83, N 7.45.

Ethyl-5-(3,4-dihydroxyphenyl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (30): According to procedure 2, compound **30** was obtained as a pale-yellow powder (75%); mp: 214 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.16 (t, ^3J = 7.1, 3H, CO$_2$CH$_2$CH$_3$), 2.35 (s, 3H, 7-CH$_3$), 4.01 (q, ^3J = 7.1, 2H, CO$_2$CH$_2$CH$_3$), 4.13 (s, 2H, CO-CH$_2$-S), 5.76 (s, 1H, 5-H), 6.52–6.55 (m, 1H, H$_{ar}$), 6.65–6.67 (m, 2H, H$_{ar}$), 8.97 (bs, 1H, OH), 9.03 ppm (s, 1H, OH); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 13.9, 22.3, 32.6, 54.1, 59.9, 107.7, 114.6, 115.3, 118.5, 131.6, 145.1, 145.6, 150.9, 160.6, 165.1, 171.0 ppm; Anal. calcd for C$_{16}$H$_{16}$N$_2$O$_5$S.: C 55.17, H 4.60, N 8.04, found: C 55.69, H 4.89, N 7.66.

Ethyl-(2*Z*)-5-(1,3-benzodioxol-5-yl)-2-(4-hydroxybenzylidene)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (1): According to procedure 3, compound **1** was obtained as a yellow powder (65%); mp: > 260 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.15 (t, ^3J = 6.7, 3H, CO$_2$CH$_2$CH$_3$), 2.40 (s, 3H, 7-CH$_3$), 4.06 (q, ^3J = 6.7, 2H, CO$_2$CH$_2$CH$_3$), 5.90 (s, 1H, 5-H), 6.01 (s, 2H, O-CH$_2$-O), 6.77–6.94 (m, 5H, H$_{ar}$), 7.47 (d, ^3J = 8.4, 2H, H$_{ar}$), 7.72 ppm (s, 1H, H$_{eth}$); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 14.5, 23.0, 55.0, 60.7, 101.8, 108.4, 108.8 (×2), 115.6, 117.0 (×2), 121.6, 124.3, 133.1 (×2), 134.1, 134.8, 147.9 (×2), 152.0, 156.3, 160.8, 165.1, 165.4 ppm.

Ethyl-(2*Z*)-5-(1,3-benzodioxol-5-yl)-2-(4-hydroxybenzylidene)-7-phenyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (31): According to procedure 3, compound **31** was obtained as an orange powder (67%); mp: 160 °C; ^{1}H NMR (250 MHz, CDCl$_3$): δ = 0.87 (t, ^3J = 7.5, 3H, CO$_2$CH$_2$CH$_3$), 3.91 (q, ^3J = 7.5, 2H, CO$_2$CH$_2$CH$_3$), 5.89 (s, 2H, O-CH$_2$-O), 6.20 (s, 1H, 5-H), 6.75–6.71 (m, 3H, H$_{ar}$), 6.98–7.02 (m, 2H, H$_{ar}$), 7.26–7.46 (m, 7H, H$_{ar}$), 7.69 ppm (s, 1H, H$_{eth}$); ^{13}C NMR (250 MHz, CDCl$_3$): δ = 13.7, 56.0, 61.0, 101.5, 108.6 (×2), 110.3, 116.7 (×3), 122.2, 125.5, 128.2 (×2), 128.4 (×2), 129.1, 132.6 (×2), 133.7, 134.5, 139.0, 148.2, 148.3, 151.5, 157.2, 158.9, 165.7, 166.3 ppm; Anal. calcd for C$_{29}$H$_{22}$N$_2$O$_6$S·H$_2$O: C 63.97, H 4.41, N 5.15, found: C 62.02, H 4.37, N 5.34.

Benzyl-(2*Z*)-5-(1,3-benzodioxol-5-yl)-2-(4-hydroxybenzylidene)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (32): According to procedure 3, compound **32** was obtained as a yellow powder (37%); mp: 238 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 2.41 (s, 3H, 7-CH$_3$), 5.04–5.18 (m, 2H, CO$_2$CH$_2$C$_6$H$_5$), 6.00 (s, 3H, 5-H and O-CH$_2$-O), 6.72–6.75 (m, 2H, H$_{ar}$), 6.82–6.93 (m, 3H, H$_{ar}$), 7.18–7.21 (m, 3H, H$_{ar}$), 7.31–7.34 (m, 3H, H$_{ar}$), 7.47 (d, ^3J = 8.5, 2H, H$_{ar}$), 7.72 ppm (s, 1H, H$_{eth}$); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 22.6, 54.4, 65.7, 101.3, 107.8, 108.1, 108.3, 115.0, 116.4 (×2), 121.2, 123.7, 127.9 (×2), 128.0, 128.3 (×2), 132.6 (×2), 133.8, 134.1, 135.9, 147.3, 147.4, 152.3, 156.0, 160.3, 164.6, 164.7 ppm; Anal. calcd for C$_{29}$H$_{22}$N$_2$O$_6$S·0.5 H$_2$O: C 65.04, H 4.30, N 5.23, found: C 65.45, H 4.29, N 5.35.

(2*Z*)-5-(1,3-Benzodioxol-5-yl)-2-(4-hydroxybenzylidene)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylic acid (33): According to procedure 3, compound **32** was obtained as a yellow powder (63%); mp: 182 °C; ^{1}H NMR (250 MHz, CD_3OD): δ = 2.58 (s, 3H, 7-CH_3), 5.98 (s, 2H, O-CH_2-O), 6.26 (s, 1H, 5-H), 6.81 (d, ^3J = 8.2, 1H, H_{ar}), 6.97–7.00 (m, 4H, H_{ar}), 7.56 (d, 2H, ^3J = 8.2, H_{ar}), 8.07 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, [D_6]DMSO): δ = 22.5, 54.9, 101.7, 108.3, 108.7, 109.4, 115.5, 116.9 (×2), 121.5, 124.1 (×2), 132.9, 134.1, 134.5, 147.7 (×2), 150.6, 156.0, 160.7, 165.0, 167.0 ppm; Anal. calcd for $C_{22}H_{16}N_2O_6S\cdot0.5\,H_2O$: C 59.32, H 3.82, N 6.29, found: C 59.09, H 4.29, N 6.59.

Ethyl-(2*Z*)-5-(1,3-benzodioxol-5-yl)-2-(4-hydroxybenzylidene)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]selenazolo[3,2-*a*]pyrimidine-6-carboxylate (34): According to procedure 3, compound **34** was obtained as a yellow powder (17%); mp: > 260 °C; ^{1}H NMR (250 MHz, [D_6]DMSO): δ = 1.12 (t, ^3J = 7.5, 3H, $CO_2CH_2CH_3$), 2.35 (s, 3H, 7-CH_3), 4.03 (q, ^3J = 7.5, 2H, $CO_2CH_2CH_3$), 5.94 (s, 1H, 5-H), 5.96 (s, 2H, O-CH_2-O), 6.72–6.82 (m, 3H, H_{ar}), 6.88 (d, ^3J = 7.5, 2H, H_{ar}), 7.35 (d, ^3J = 7.5, 2H, H_{ar}), 8.04 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, [D_6]DMSO): δ = 13.9, 22.2, 54.8, 60.1, 101.2, 107.7, 108.2, 109.0, 114.7, 116.4 (×2), 121.0, 124.2, 132.0 (×2), 134.3, 137.4, 147.3 (×2), 150.9, 154.0, 160.2, 165.0, 166.6 ppm; Anal. calcd for $C_{24}H_{20}N_2O_6Se\cdot0.5\,H_2O$: C 55.38, H 4.04, N 5.38, found: C 55.59, H 4.18, N 5.51.

Ethyl-(2*Z*)-5-(1,3-benzodioxol-5-yl)-2-benzylidene-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (35): According to procedure 4, compound **35** was obtained as a yellow powder (82%); mp: 185 °C; ^{1}H NMR (250 MHz, [D_6]DMSO): δ = 1.18 (t, 3H, ^3J = 7.3, $CO_2CH_2CH_3$), 2.41 (s, 3H, 7-CH_3), 4.08 (q, ^3J = 7.3, 2H, $CO_2CH_2CH_3$), 6.00 (s, 3H, 5-H and O-CH_2-O), 6.79–6.91 (m, 3H, H_{ar}), 7.45–7.64 (m, 5H, H_{ar}), 7.83 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, $CDCl_3$): δ = 14.3, 23.0, 55.4, 60.8, 101.4, 108.4, 108.7, 109.4, 120.5, 122.2, 129.5 (×2), 130.3 (×2), 130.7, 133.4, 133.6, 134.0, 148.1 (×2), 152.4, 156.2, 165.5, 165.7 ppm; Anal. calcd for $C_{25}H_{20}N_2O_5S$: C 65.21, H 4.34, N 6.08, found: C 65.83, H 4.41, N 6.51.

Ethyl-(2*Z*)-5-(1,3-benzodioxol-5-yl)-7-methyl-2-(4-nitrobenzylidene)-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (36): According to procedure 4, compound **36** was obtained as a yellow powder (53%); mp: 150 °C; ^{1}H NMR (250 MHz, [D_6]DMSO): δ = 1.15 (t, ^3J = 7.1, 3H, $CO_2CH_2CH_3$), 2.42 (s, 3H, 7-CH_3), 4.07 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 6.01 (s, 1H, 5-H), 6.03 (s, 2H, O-CH_2-O), 6.80–6.91 (m, 3H, H_{ar}), 7.87–7.94 (m, 3H, H_{ar}), 8.35–8.38 ppm (d, ^3J = 8.9 Hz, 2H, H_{ar}); ^{13}C NMR (250 MHz, $CDCl_3$): δ = 14.5, 23.0, 56.0, 61.1, 101.7, 108.7, 108.8, 110.2, 122.5, 124.8, 125.5 (×2), 130.3, 130.8 (×2), 139.7, 148.4 (×2), 152.1, 154.8, 165.0, 165.7 ppm; Anal. calcd for $C_{24}H_{19}N_3O_7S$: C 57.37, H 3.98, N 8.36, found: C 58.13, H 4.33, N 7.89.

Ethyl-(2*Z*)-5-(1,3-benzodioxol-5-yl)-2-(2-hydroxybenzylidene)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (37): According to procedure 3, compound **37** was obtained as an orange powder (64%); mp: 123 °C; ^{1}H NMR (250 MHz, [D_6]DMSO): δ = 1.15 (t, ^3J = 7.1, 3H, $CO_2CH_2CH_3$), 2.39 (s, 3H, 7-CH_3), 4.06 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 5.98 (s, 1H, 5-H), 6.01 (s, 2H, O-CH_2-O), 6.77–6.98 (m, 5H, H_{ar}), 7.30–7.39 (m, 2H, H_{ar}), 7.98 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, [D_6]DMSO): δ = 14.3, 22.7, 55.6, 61.2, 102.3, 108.2, 108.7, 108.8, 116.6, 118.7, 120.1(×2), 121.5, 129.1, 132.3, 133.1, 134.6, 147.7 (×2), 151.9, 156.1, 157.6, 165.1, 165.3 ppm; Anal. calcd for $C_{24}H_{20}N_2O_6S\cdot H_2O$: C 59.75, H 4.56, N 5.80, found: C 58.28, H 4.65, N 5.60.

Ethyl-(2*Z*)-5-(1,3-benzodioxol-5-yl)-2-(3-hydroxybenzylidene)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (38): According to procedure 3, compound **38** was obtained as a yellow powder (20%); mp: 210 °C; ^{1}H NMR (250 MHz, [D_6]DMSO): δ = 1.15 (t, ^3J = 7.0, 3H, $CO_2CH_2CH_3$), 2.41 (s, 3H, 7-CH_3), 4.07 (q, ^3J = 7.0, 2H, $CO_2CH_2CH_3$), 5.98 (s, 1H, 5-H), 6.01 (s, 2H, O-CH_2-O), 6.78–7.07 (m, 5H, H_{ar}), 7.35 (t, ^3J = 7.9, 1H, H_{ar}), 7.72 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, [D_6]DMSO): δ = 13.9, 22.3, 54.6, 60.1, 101.3, 107.9, 108.3, 108.7, 115.9, 118.0, 119.5, 121.2 (×2), 130.4, 133.1, 134.0 (×2), 147.3 (×2), 151.1, 155.3, 157.9, 164.3, 164.8 ppm; Anal. calcd for $C_{24}H_{20}N_2O_6S\cdot H_2O$: C 59.75, H 4.56, N 5.80, found: C 58.65, H 4.22, N 5.60.

Ethyl-(2*Z*)-5-(1,3-benzodioxol-5-yl)-2-(2,4-dihydroxybenzylidene)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (39): According to procedure 3, compound **39** was obtained as a yellow powder (28%); mp: > 240 °C; ^{1}H NMR (250 MHz, [D_6]DMSO): δ = 1.15 (t, 3H, ^3J = 7.0, $CO_2CH_2CH_3$), 2.38 (s, 3H, 7-CH_3), 4.06 (q, ^3J = 7.0, 2H, $CO_2CH_2CH_3$), 5.96 (s, 1H, 5-H), 6.01 (s, 2H, O-CH_2-O), 6.41–6.43 (m, 2H, H_{ar}), 6.76–6.79 (m, 3H, H_{ar}), 6.87 (d, 1H, ^3J = 7.9 Hz, H_{ar}), 7.94 (s, 1H, H_{eth}), 10.25 (s, 1H, OH), 10.56 ppm (s, 1H, OH); ^{13}C NMR (250 MHz, [D_6]DMSO): δ = 13.7, 22.0, 54.5, 59.5, 101.9, 103.2, 108.6, 108.8, 109.0, 109.4, 112.3, 114.0, 121.9, 130.2, 131.5, 135.6, 148.5 (×2), 153.1, 157.7, 160.2, 163.5, 166.4 ppm (×2); Anal. calcd for $C_{24}H_{20}N_2O_7S\cdot0.5\,H_2O$: C 58.90, H 4.29, N 5.72, found: C 59.64, H 4.30, N 5.53.

Ethyl-(2*Z*)-5-(1,3-benzodioxol-5-yl)-2-(3,4-dihydroxybenzylidene)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (40): According to procedure 3, compound **40** was obtained as an orange powder (63%); mp: > 260 °C; ^{1}H NMR (250 MHz, CD_3OD): δ = 1.23 (t, ^3J = 6.9, 3H, $CO_2CH_2CH_3$), 2.48 (s, 3H, 7-CH_3), 4.13 (q, ^3J = 6.9, 2H, $CO_2CH_2CH_3$), 5.95 (s, 2H, O-CH_2-O), 6.10 (s, 1H, 5-H), 6.76–7.04 (m, 6H, H_{ar}), 7.68 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, [D_6]DMSO): δ = 14.3, 22.8, 54.8, 60.5, 101.6, 108.2, 108.5, 108.6, 114.5, 116.5, 116.7, 121.5, 123.9, 124.8, 134.5, 134.7, 146.6, 147.7 (×2), 150.5, 151.2, 156.4, 164.9, 165.3 ppm; Anal. calcd for $C_{24}H_{20}N_2O_7S\cdot0.5\,H_2O$: C 58.90, H 4.30, N 5.72, found: C 59.56, H 4.74, N 5.59.

Ethyl-(2*Z*)-5-(1,3-benzodioxol-5-yl)-2-(2,5-dihydroxybenzylidene)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (41): According to procedure 3, compound **41** was obtained as a yellow powder (33%); mp: 128 °C; ^{1}H NMR (250 MHz, [D_6]DMSO): δ = 1.15 (t, 3H, ^3J = 7.0, $CO_2CH_2CH_3$), 2.36 (s, 3H, 7-CH_3), 4.06 (q, ^3J = 7.0, 2H, $CO_2CH_2CH_3$), 5.99 (s, 1H, 5-H), 6.03 (s, 2H, O-CH_2-O), 6.81–6.92 (m, 6H, H_{ar}), 7.96 (s, 1H, H_{eth}), 9.23 (s, 1H, OH), 9.93 ppm (s, 1H, OH); ^{13}C NMR (250 MHz, [D_6]DMSO): δ = 13.7, 22.2, 54.2, 59.9, 101.0, 107.6, 108.0, 108.2, 112.9, 116.9, 117.4, 119.5, 120.1, 120.8, 128.2, 133.9, 147.1 (×2), 149.9, 150.2, 151.1, 155.5, 164.3, 164.6 ppm.

Ethyl-(2*Z*)-5-(1,3-benzodioxol-5-yl)-2-(3,4-dimethoxybenzylidene)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (42): According to procedure 4, compound **42** was obtained as a yellow powder (12%); mp: 160 °C; ^{1}H NMR (250 MHz, [D_6]DMSO): δ = 1.16 (t, 3H, ^3J = 7.1, $CO_2CH_2CH_3$), 2.40 (s, 3H, 7-CH_3), 3.82 (s, 3H, O-CH_3), 3.85 (s, 3H, O-CH_3), 4.06 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 5.99 (s, 1H, 5-H), 6.01 (s, 2H, O-CH_2-O), 6.78–6.81 (m, 1H, H_{ar}), 6.87–6.91 (m, 2H, H_{ar}), 7.13–7.24 (m, 3H, H_{ar}), 7.78 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, $CDCl_3$): δ = 14.1, 22.6, 55.2, 56.0 (×2), 60.5, 101.2, 108.1, 108.4, 108.9, 111.4, 112.0, 117.4, 121.9, 124.7, 126.1 (×2), 133.9, 147.8 (×2), 149.4, 151.3, 151.9, 156.5, 165.2, 165.4 ppm.

Ethyl-(2*Z*)-5-(1,3-benzodioxol-5-yl)-2-(3,4-dichlorobenzylidene)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (43): According to procedure 4, compound **43** was obtained as a yellow powder (28%); mp: 166 °C; ^{1}H NMR (250 MHz,

[D_6]DMSO): $\delta = 1.15$ (t, $^3J = 7.1$, 3H, $CO_2CH_2CH_3$), 2.40 (s, 3H, 7-CH_3), 4.08 (q, $^3J = 7.1$, 2H, $CO_2CH_2CH_3$), 5.98 (s, 1H, 5-H), 6.01 (s, 2H, O-CH_2-O), 6.78–6.90 (m, 3H, H_{ar}), 7.56 (d, $^3J = 8.4$, 1H, H_{ar}), 7.81–7.83 (m, 2H, H_{ar}), 7.92 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, $CDCl_3$): $\delta =$ 14.3, 23.0, 55.4, 60.8, 101.3, 108.3, 108.4, 109.5, 122.0, 122.5, 128.7, 130.4, 131.3, 131.6, 133.3, 133.5, 133.8, 134.6, 147.9 (×2), 152.0, 155.0, 164.9, 165.5 ppm; Anal. calcd for $C_{24}H_{18}Cl_2N_2O_5S$: C 55.70, H 3.48, N 5.41, found: C 56.65, H 3.75, N 6.05.

Ethyl-(2*Z*)-5-(1,3-benzodioxol-5-yl)-2-(3,5-dibromo-4-hydroxybenzylidene)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (44): According to procedure 3, compound **44** was obtained as a yellow powder (57%); mp: 219 °C; ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.19$ (t, $^3J = 7.1$, 3H, $CO_2CH_2CH_3$), 2.48 (s, 3H, 7-CH_3), 4.08 (q, $^3J = 7.1$, 2H, $CO_2CH_2CH_3$), 5.89 (s, 2H, O-CH_2-O), 6.09 (s, 1H, 5-H), 6.67–6.71 (d, $^3J = 8.3$, 1H, H_{ar}), 6.86–6.90 (m, 2H, H_{ar}), 7.51 (s, 1H, H_{eth}), 7.55 ppm (s, 2H, H_{ar}); ^{13}C NMR (250 MHz, $CDCl_3$): $\delta =$ 14.4, 23.3, 45.3, 60.6, 101.4, 108.3, 108.7 (×2), 114.1, 115.8 (×2), 120.7 (×2), 122.1, 132.8, 134.5, 134.6, 147.9, 148.0, 152.9, 156.9, 162.3, 165.8, 165.9 ppm; Anal. calcd for $C_{24}H_{18}Br_2N_2O_6S$: C 46.30, H 2.89, N 4.50, found: C 46.29, H 3.75, N 5.08.

Ethyl-(2*Z*)-5-(1,3-benzodioxol-5-yl)-2-[4-hydroxy-3,5-dimethoxybenzylidene]-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (45): According to procedure 3, compound **45** was obtained as an orange powder (23%); mp: 112 °C; ^{1}H NMR (250 MHz, [D_6]DMSO): $\delta = 1.15$ (t, $^3J = 7.1$, 3H, $CO_2CH_2CH_3$), 2.39 (s, 3H, 7-CH_3), 3.82 (s, 6H, 2×O-CH_3), 4.08 (q, $^3J = 7.1$, 2H, $CO_2CH_2CH_3$), 6.01 (s, 3H, 5-H and O-CH_2-O), 6.73–6.90 (m, 5H, H_{ar}), 7.73 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, $CDCl_3$): $\delta =$ 14.1, 22.6, 55.2, 56.3 (×2), 60.6, 101.2, 107.2 (×2), 108.2, 108.4, 109.0, 117.4, 121.9, 124.7, 133.8, 134.0, 137.6, 147.4 (×2), 147.8 (×2), 151.7, 156.0, 165.1, 165.4 ppm; Anal. calcd for $C_{26}H_{24}N_2O_8S{\cdot}0.5\,H_2O$: C 58.53, H 4.69, N 5.25, found: C 58.63, H 5.04, N 5.25.

Ethyl-(2*Z*)-2-[2-acetoxybenzylidene]-5-(1,3-benzodioxol-5-yl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (46): According to procedure 4, compound **46** was obtained as a yellow powder (24%); mp: 178 °C; ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.23$ (t, $^3J = 7.3$, 3H, $CO_2CH_2CH_3$), 2.40 (s, 3H, OC(O)CH_3), 2.58 (s, 3H, 7-CH_3), 4.13 (q, $^3J = 7.3$, 2H, $CO_2CH_2CH_3$), 5.98 (s, 2H, O-CH_2-O), 6.16 (s, 1H, 5-H), 6.76–6.79 (m, 1H, H_{ar}), 6.91–6.94 (m, 2H, H_{ar}), 7.21 (d, $^3J = 7.8$, 1H, H_{ar}), 7.37–7.54 (m, 2H, H_{ar}), 7.59 (d, $^3J =$ 7.3, 1H, H_{ar}),7.88 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, $CDCl_3$): $\delta =$ 14.3, 21.2, 22.9, 55.6, 60.8, 101.5, 108.4, 108.7, 109.6, 122.2, 122.8, 123.6, 126.4, 126.6, 126.7, 128.6, 131.7, 133.9, 148.1 (×2), 150.1, 152.1, 155.8, 165.1, 165.7, 169.2 ppm; Anal. calcd for $C_{26}H_{22}N_2O_7S$: C 61.66, H 4.34, N 5.53, found: C 61.24, H 4.84, N 6.47.

Ethyl-(2*Z*)-2-[3-acetoxybenzylidene]-5-(1,3-benzodioxol-5-yl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (47): According to procedure 4, compound **47** was obtained as an orange powder (23%); mp: 78 °C; ^{1}H NMR (250 MHz, [D_6]DMSO): $\delta = 1.16$ (t, $^3J = 7.1$, 3H, $CO_2CH_2CH_3$), 2.31 (s, 3H, OC(O)CH_3), 2.42 (s, 3H, 7-CH_3), 4.07 (q, $^3J = 7.1$, 2H, $CO_2CH_2CH_3$), 5.99 (s, 1H, 5-H), 6.02 (s, 2H, O-CH_2-O), 6.78–6.90 (m, 3H, H_{ar}), 7.27 (d, $^3J = 7.4$, 1H, H_{ar}), 7.41 (s, 1H, H_{ar}), 7.49–7.63 (m, 2H, H_{ar}), 7.82 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, [D_6]DMSO): $\delta =$ 14.3, 21.2, 22.8, 55.1, 60.6, 101.7, 108.3, 108.6, 109.2, 121.4, 121.6, 123.5, 124.5, 127.4, 130.9, 132.2, 134.3, 134.7, 147.8 (×2), 151.3 (×2), 155.4, 164.5, 165.2, 169.4 ppm; Anal. calcd for $C_{26}H_{22}N_2O_7S$: C 61.66, H 4.34, N 5.53, found: C 62.14, H 4.77, N 5.39.

Ethyl-(2*Z*)-2-[4-acetoxybenzylidene]-5-(1,3-benzodioxol-5-yl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (48): According to procedure 4, compound **48** was obtained as an orange powder (36%); mp: >260 °C; ^{1}H NMR (250 MHz, [D_6]DMSO): $\delta = 1.13$ (t, $^3J = 7.5$, 3H, $CO_2CH_2CH_3$), 2.34 (s, 3H, OC(O)CH_3), 2.46 (s, 3H, 7-CH_3), 4.08 (q, $^3J = 7.5$, 2H, $CO_2CH_2CH_3$), 5.99 (s, 1H, 5-H), 6.01 (s, 2H, O-CH_2-O), 6.77–6.90 (m, 3H, H_{ar}), 7.35 (d, $^3J = 8.6$, 2H, H_{ar}), 7.69 (d, $^3J = 8.6$, 2H, H_{ar}), 7.83 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, $CDCl_3$): $\delta =$ 14.3, 21.3, 22.9, 55.5, 60.8, 101.4, 108.4, 108.7, 109.4, 120.6, 122.2, 122.7 (×2), 131.1, 131.4 (×2), 132.5, 134.0, 148.1 (×2), 152.2, 152.3, 156.0, 165.4, 165.7, 169.1 ppm; Anal. calcd for $C_{26}H_{22}N_2O_7S{\cdot}0.5\,H_2O$: C 60.52, H 4.46, N 5.43, found: C 60.82, H 4.50, N 5.42.

Ethyl-(2*Z*)-2-[3,4-bis(acetoxy)benzylidene]-5-(1,3-benzodioxol-5-yl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (49): According to procedure 4, compound **49** was obtained as a yellow powder (32%); mp: 98 °C; ^{1}H NMR (250 MHz, [D_6]DMSO): $\delta = 1.16$ (t, $^3J = 7.0$, 3H, $CO_2CH_2CH_3$), 2.33 (s, 6H, 2×OC(O)CH_3), 2.41 (s, 3H, 7-CH_3), 4.07 (q, $^3J = 7.0$, 2H, $CO_2CH_2CH_3$), 6.00 (s, 1H, 5-H), 6.03 (s, 2H, O-CH_2-O), 6.79–6.91 (m, 3H, H_{ar}), 7.46–7.59 (m, 3H, H_{ar}), 7.82 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, $CDCl_3$): $\delta =$ 14.6, 21.1 (×2), 23.2, 55.7, 61.0, 101.7, 108.6, 108.8, 109.8, 121.9, 122.4, 124.8, 125.3, 128.5, 131.8, 132.3, 134.0, 143.0, 143.9, 148.2, 148.3, 152.4, 155.9, 165.4, 165.8, 168.3 ppm (× 2); Anal. calcd for $C_{28}H_{24}N_2O_9S$: C 59.57, H 4.25, N 4.96, found: C 60.07, H 4.33, N 4.56.

Ethyl-(2*Z*)-2-[2,3,4-tris(acetoxy)benzylidene]-5-(1,3-benzodioxol-5-yl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (50): According to procedure 4, compound **50** was obtained as a yellow powder (11%); mp: 100 °C; ^{1}H NMR (250 MHz, [D_6]DMSO): $\delta = 1.17$ (t, $^3J = 7.1$, 3H, $CO_2CH_2CH_3$), 2.32 (s, 3H, OC(O)CH_3), 2.35 (s, 3H, OC(O)CH_3), 2.39 (s, 3H, OC(O)CH_3), 2.41 (s, 3H, 7-CH_3), 4.07 (q, $^3J = 7.1$, 2H, $CO_2CH_2CH_3$), 5.97 (s, 1H, 5-H), 6.03 (s, 2H, O-CH_2-O), 6.79–6.82 (m, 2H, H_{ar}), 6.89 (d, $^3J = 8.0$, 1H, H_{ar}), 7.46 (d, $^3J = 7.1$, 1H, H_{ar}), 7.57–7.61 ppm (m, 2H, H_{ar} and H_{eth}); ^{13}C NMR (250 MHz, $CDCl_3$): $\delta =$ 14.2, 20.3 (×2), 20.8, 22.8, 55.5, 60.7, 101.4, 108.3, 108.5, 109.6, 121.5, 122.1, 123.5, 125.1, 125.2, 127.1, 133.6, 135.9, 143.4, 144.9, 148.0 (×2), 151.9, 155.2, 164.8, 165.5, 166.6, 167.6 ppm (×2); Anal. calcd for $C_{30}H_{26}N_2O_{11}S{\cdot}0.5\,H_2O$: C 57.05, H 4.27, N 4.43, found: C 56.53, H 4.55, N 4.55.

Ethyl-(2*Z*)-2-[2,4,5-tris(acetoxy)benzylidene]-5-(1,3-benzodioxol-5-yl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (51): According to procedure 4, compound **51** was obtained as a yellow powder (32%); mp: 214 °C; ^{1}H NMR (250 MHz, [D_6]DMSO): $\delta = 1.17$ (t, $^3J = 7.1$, 3H, $CO_2CH_2CH_3$), 2.33–2.35 (s, 9H, 3×OC(O)CH_3), 2.40 (s, 3H, 7-CH_3), 4.07 (q, $^3J = 7.1$, 2H, $CO_2CH_2CH_3$), 5.97 (s, 1H, 5-H), 6.02 (s, 2H, O-CH_2-O), 6.77–6.81 (m, 2H, H_{ar}), 6.89 (d, $^3J = 7.8$, 1H, H_{ar}), 7.42 (s, 1H, H_{ar}), 7.53 (s, 1H, H_{ar}), 7.60 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, $CDCl_3$): $\delta =$ 14.2, 20.3 (× 2), 20.8, 22.8, 55.5, 60.7, 101.6, 108.5, 108.8, 109.7, 118.9, 122.2, 122.8, 123.6, 124.6, 125.2, 133.9, 140.1, 143.7, 147.5, 148.3 (×2), 152.0, 155.3, 164.9, 165.6, 167.6, 168.0, 168.7 ppm; Anal. calcd for $C_{30}H_{26}N_2O_{11}S$: C 57.88, H 4.18, N 4.50, found: C 58.14, H 4.65, N 5.07.

Ethyl-(2*Z*)-2-[3,4,5-tris(acetoxy)benzylidene]-5-(1,3-benzodioxol-5-yl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (52): According to procedure 4, compound **52** was obtained as a yellow powder (16%); mp: 111 °C; ^{1}H NMR (250 MHz, [D_6]DMSO): $\delta = 1.16$ (t, $^3J = 7.1$, 3H, $CO_2CH_2CH_3$), 2.33 (m, 9H, 3×OC(O)CH_3), 2.41 (s, 3H, 7-CH_3), 4.08 (q, $^3J = 7.1$, 2H, $CO_2CH_2CH_3$), 6.00 (s, 1H, 5-H), 6.03 (s, 2H, O-CH_2-O), 6.79–6.91 (m, 3H, H_{ar}), 7.57 (s, 2H, H_{ar}), 7.79 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, $CDCl_3$): $\delta =$ 14.3, 21.0 (×3), 22.3, 56.0, 61.1, 101.7, 108.7, 108.8, 109.9, 122.3 (×2), 122.4, 122.9, 131.8 (×2), 133.9, 136.4, 144.4 (×2),

148.3, 148.4, 152.0, 155.8, 165.1, 165.7, 167.0, 167.9 ppm (×2); Anal. calcd for $C_{30}H_{26}N_2O_{11}S$: C 57.88, H 4.18, N 4.50, found: C 58.14, H 5.02, N 3.72.

3-{(*Z*)-[5-(1,3-Benzodioxol-5-yl)-6-(ethoxycarbonyl)-7-methyl-3-oxo-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidin-2(3*H*)-ylidene]methyl}benzoic acid (53): According to procedure 4, compound **53** was obtained as a yellow powder (33%); mp: 255 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.15 (t, ^3J = 7.1, 3H, $CO_2CH_2CH_3$), 2.41 (s, 3H, 7-CH_3), 4.07 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 6.01 (s, 3H, 5-H and O-CH_2-O), 6.79–6.90 (m, 3H, H_{ar}), 7.63–7.70 (m, 1H, H_{ar}), 7.83 (d, ^3J = 8.0, 1H, H_{ar}), 7.90 (s, 1H, H_{eth}), 8.01 (d, ^3J = 8.2, 1H, H_{ar}), 8.17 ppm (s, 1H, H_{ar}); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 14.8, 23.3, 55.6, 61.1, 102.3, 108.8, 109.2, 109.8, 121.9, 122.2, 130.7, 131.2, 131.9, 132.0, 132.7, 134.2, 134.8 (×2), 148.3 (×2), 151.8, 155.8, 165.0, 165.7, 167.4 ppm; Anal. calcd for $C_{25}H_{20}N_2O_7S{\cdot}0.5\,H_2O$: C 59.88, H 4.19, N 5.59, found: C 60.23, H 4.42, N 5.49.

4-{(*Z*)-[5-(1,3-Benzodioxol-5-yl)-6-(ethoxycarbonyl)-7-methyl-3-oxo-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidin-2(3*H*)-ylidene]methyl}benzoic acid (54): According to procedure 4, compound **54** was obtained as a yellow powder (18%); mp: 235 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.15 (t, ^3J = 7.1, 3H, $CO_2CH_2CH_3$), 2.41 (s, 3H, 7-CH_3), 4.05 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 5.99 (s, 1H, 5-H), 6.01 (s, 2H, O-CH_2-O), 6.78–6.82 (m, 3H, H_{ar}), 7.71 (d, ^3J = 7.5, 2H, H_{ar}), 7.86 (s, 1H, H_{eth}), 8.05 (d, ^3J = 7.5, 2H, H_{ar}), 13.2 ppm (s, 1H, COOH); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 14.4, 22.8, 55.2, 60.7, 101.2, 108.3, 108.7, 109.4, 121.7, 122.6, 130.4 (×2), 130.5 (×2), 132.0, 132.4, 134.3, 137.2, 147.8, 147.9, 151.3, 155.3, 164.6, 165.2, 167.0 ppm; Anal. calcd for $C_{25}H_{20}N_2O_7S{\cdot}0.5\,H_2O$: C 59.88, H 4.19, N 5.59, found: C 59.22, H 4.09, N 5.49.

Ethyl-(2*Z*)-5-(1,3-benzodioxol-5-yl)-2-[3-methoxycarbonylbenzylidene]-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*] pyrimidine-6-carboxylate (55): According to procedure 5, compound **55** was obtained as a yellow powder (60%); mp: 170 °C; ^{1}H NMR (250 MHz, CDCl$_3$): δ = 1.24 (t, ^3J = 7.4, 3H, $CO_2CH_2CH_3$), 2.57 (s, 3H, 7-CH_3), 3.99 (s, 3H, CO_2CH_3), 4.16 (q, ^3J = 7.4, 2H, $CO_2CH_2CH_3$), 5.97 (s, 2H, O-CH_2-O), 6.18 (s, 1H, 5-H), 6.75–6.78 (d, ^3J = 8.1, 1H, H_{ar}), 6.92–6.96 (m, 2H, H_{ar}), 7.56 (m, 1H, H_{ar}), 7.67 (d, ^3J = 7.9, 1H, H_{ar}), 7.83 (s, 1H, H_{eth}), 8.11 (d, ^3J = 7.6, 1H, H_{ar}), 8.20 ppm (s, 1H, H_{ar}); ^{13}C NMR (250 MHz, CDCl$_3$): δ = 14.3, 22.9, 55.4, 60.7, 65.8, 101.4, 108.3, 108.5, 109.5, 122.0, 122.1, 129.5, 130.9, 131.4, 132.1 (×2), 133.7, 133.8, 133.9, 148.0 (×2), 152.2, 155.6, 165.1, 165.5, 166.2 ppm; Anal. calcd for $C_{26}H_{22}N_2O_7S$: C 61.66, H 4.34, N 5.53, found: C 61.63, H 4.54, N 5.35.

Ethyl-(2*Z*)-5-(1,3-benzodioxol-5-yl)-2-[4-methoxycarbonylbenzylidene]-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (56): According to procedure 5, compound **56** was obtained as a yellow powder (62%); mp: 149 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.16 (t, ^3J = 7.1, 3H, $CO_2CH_2CH_3$), 2.42 (s, 3H, 7-CH_3), 3.18 (s, 3H, CO_2CH_3), 4.07 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 6.03 (s, 3H, 5-H and O-CH_2-O), 6.80–6.91 (m, 3H, H_{ar}), 7.73–7.76 (d, ^3J = 8.2, 2H, H_{ar}), 7.88 (s, 1H, H_{eth}), 8.06–8.09 (d, ^3J = 8.2, 2H, H_{ar}); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 14.0, 22.3, 54.8, 59.7, 60.2, 101.3, 107.9, 108.3 (×2), 109.0, 121.3, 122.2, 130.0 (×3), 130.1, 131.9, 133.8, 136.8, 147.4 (×2), 150.8, 154.9, 164.1, 164.8, 166.5; Anal. calcd for $C_{26}H_{22}N_2O_7S$: C 61.59, H 4.34, N 5.53, found: C 63.37, H 4.36, N 4.95.

(4-{(*Z*)-[5-(1,3-Benzodioxol-5-yl)-6-(ethoxycarbonyl)-7-methyl-3-oxo-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidin-2(3*H*)-ylidene]methyl}phenoxy)acetic acid (57): According to procedure 4, compound **57** was obtained as an orange powder (65%); mp: 124 °C; ^{1}H NMR (250 MHz, CDCl$_3$): δ = 1.24 (t, ^3J = 7.2, 3H, $CO_2CH_2CH_3$), 2.55 (s, 3H, 7-CH_3), 4.14 (q, ^3J = 7.2, 2H, $CO_2CH_2CH_3$), 4.69 (d, ^3J = 10, 1H, O-CH_2), 4.74 (d, ^3J = 10, 1H, O-CH_2), 5.95 (s, 2H, O-CH_2-O), 6.15 (s, 2H, 5-H), 6.74 (d, ^3J = 7.8, 1H, H_{ar}), 6.90–7.04 (m, 4H, H_{ar}), 7.43 (d, ^3J = 7.5, 2H, H_{ar}), 7.69 (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, CDCl$_3$): δ = 14.5, 22.8, 55.6, 61.0, 78.0, 101.6, 108.6, 108.9, 109.5, 115.8 (×2), 118.2, 122.4, 127.2 (×2), 132.5 (×2), 134.2, 148.2 (×2), 152.1, 157.0, 159.7, 165.8 (×2), 169.3 ppm.

(2*Z*)-3-(4-{(*E*)-[5-(1,3-Benzodioxol-5-yl)-6-(ethoxycarbonyl)-7-methyl-3-oxo-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidin-2(3*H*)-ylidene]methyl}phenyl)acrylic acid (58): According to procedure 4, compound **58** was obtained as an orange powder (32%); mp: >260 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.15 (t, ^3J = 7.1, 3H), 2.41 (s, 3H, 7-CH_3), 4.07 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 5.99 (s, 1H, 5-H), 6.01 (s, 2H, O-CH_2-O), 6.62–6.90 (m, 4H, H_{ar}), 7.59–7.66 (m, 3H, H_{ar}), 7.83–7.88 ppm (m, 3H, H_{ar}); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 13.6, 23.3, 54.7, 60.2, 101.3, 108.1, 108.2, 108.9, 120.8, 121.6 (×2), 128.8 (×2), 130.3 (×2), 132.0, 134.1, 134.9, 136.6, 143.1, 147.8 (×2), 150.8, 155.1, 164.4, 165.3, 167.4 ppm; Anal. calcd for $C_{27}H_{22}N_2O_7S{\cdot}H_2O$: C 60.44, H 4.47, N 5.22, found: C 56.57, H 4.08, N 5.08.

Ethyl-(2*Z*)-2-[pyridin-3-ylmethylene]-7-methyl-5-(1,3-benzodioxol-5-yl)-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (59):[54] According to procedure 4, compound **59** was obtained as a yellow powder (27%); mp: 198 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.16 (t, 3H, ^3J = 7.0, $CO_2CH_2CH_3$), 2.41 (s, 3H, 7-CH_3), 4,08 (q, ^3J = 7.0, 2H, $CO_2CH_2CH_3$), 6.00 (s, 3H, 5-H and O-CH_2-O), 6.79–6.91 (m, 3H, H_{ar}), 7.59 (m, 1H, H_{ar}), 7.86 (s, 1H, H_{eth}), 8.01 (d, ^3J = 7.7, 1H, H_{ar}), 8.64 (d, ^3J = 7.2, 1H, H_{ar}), 8.85 ppm (s, 1H, H_{ar}); ^{13}C NMR (250 MHz, CDCl$_3$): δ = 14.5, 23.1, 55.8, 61.1, 101.7, 108.6, 108.8, 110.0, 122.4, 123.3, 124.3, 129.7, 129.8, 133.9, 136.2, 148.3 (×2), 151.1, 151.7, 152.3, 155.3, 165.1, 165.8 ppm; Anal. calcd for $C_{23}H_{19}N_3O_5S$: C 61.45, H 4.23, N 9.35, found: C 61.06, H 4.20, N 9.35.

Ethyl-(2*Z*)-2-(4-hydroxybenzylidene)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (60): According to procedure 3, compound **60** was obtained as a yellow powder (66%); mp: >260 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.26 (t, ^3J = 7.5, 3H, $CO_2CH_2CH_3$), 2.28 (s, 3H, 7-CH_3), 4.18 (q, ^3J = 7.5, 2H, $CO_2CH_2CH_3$), 4.39 (s, 2H, CH_2), 6.93 (d, ^3J = 8.3, 2H, H_{ar}), 7.51 (d, ^3J = 8.3, 2H, H_{ar}), 7.74 (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 13.8, 18.2, 41.3, 59.7, 103.3, 114.6, 116.3 (×2), 122.8, 131.9, 132.0 (×2), 151.7, 156.8, 160.9, 164.7, 164.8 ppm; Anal. calcd for $C_{17}H_{16}N_2O_4S$: C 55.92, H 4.64, N 8.13, found: C 55.97, H 4.63, N 7.70.

Ethyl-(2*Z*)-2-(4-hydroxybenzylidene)-7-methyl-5-phenyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (61): According to procedure 3, compound **61** was obtained as a yellow powder (60%); mp: 257 °C;^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.14 (t, ^3J = 7.0, 3H, $CO_2CH_2CH_3$), 2.40 (s, 3H, 7-CH_3), 4.07 (q, ^3J = 7.0, 2H, $CO_2CH_2CH_3$), 6.06 (s, 1H, 5-H), 6.90 (d, ^3J = 8.5, 2H, H_{ar}), 7.32–7.36 (m, 5H, H_{ar}), 7.47 (d, ^3J = 8.5, 2H, H_{ar}), 7.70 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 14.3, 22.9, 55.2, 60.6, 108.8, 115.4, 116.9 (×2), 124.1, 127.8 (×2), 128.9, 129.1 (×2), 133.0 (×2), 134.1, 141.0, 151.9, 156.5, 160.8, 165.0, 165.4 ppm; Anal. calcd for $C_{23}H_{20}N_2O_4S{\cdot}0.5\,H_2O$: C 66.11, H 5.03, N 6.70, found: C 65.44, H 4.94, N 6.62.

Ethyl-(2*Z*)-2-(4-hydroxybenzylidene)-5-(3,4-dimethoxyphenyl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (62): According to procedure 3, compound **62** was obtained as a yellow powder (25%); mp: 237 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.17 (t, ^3J = 7.5, 3H, $CO_2CH_2CH_3$), 2.41 (s, 3H, 7-CH_3), 3.74 (s, 6H, 2×O-CH_3), 4.10 (q, ^3J = 7.5, 2H, $CO_2CH_2CH_3$), 6.02 (s, 1H, 5-H), 6.78–6.82 (m, 2H, H_{ar}), 6.89–6.95 (m, 4H, H_{ar}), 7.48 (d, ^3J = 7.5,

2H, H_{ar}), 7.74 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 13.7, 22.2, 54.1, 55.2 (×2), 59.9, 108.2, 111.2, 111.7, 114.9, 116.2 (×2), 119.1, 123.5, 132.3 (×2), 132.8, 133.2, 148.1, 148.7, 151.0, 155.6, 160.0, 164.4, 164.7 ppm; Anal. calcd for $C_{25}H_{24}N_2O_6S$: C 62.42, H 4.99, N 5.82, found: C 62.98, H 4.85, N 6.32.

Ethyl-(2*Z*)-5-(3,4-dichlorophenyl)-2-(4-hydroxybenzylidene)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (63): According to procedure 3, compound **63** was obtained as a yellow powder (44%); mp: >250 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.14 (t, ^3J = 6.9, 3H, $CO_2CH_2CH_3$), 2.43 (s, 3H, 7-CH_3), 4.07 (q, ^3J = 6.9, 2H, $CO_2CH_2CH_3$), 6.06 (s, 1H, 5-H), 6.92 (d, ^3J = 8.4, 2H, H_{ar}), 7.30 (d, ^3J = 8.5, 1H, H_{ar}), 7.48 (d, ^3J = 8.4, 2H, H_{ar}), 7.56 (s, 1H, H_{ar}), 7.63 (d, ^3J = 8.5, 1H, H_{ar}), 7.74 (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 13.9, 22.6, 54.1, 60.3, 107.2, 114.8, 116.5 (×2), 123.7 (×2), 127.8, 130.0, 131.0, 131.1, 131.2, 132.6, 134.0, 141.2, 152.4, 156.2, 160.4, 164.5, 164.6 ppm; Anal. calcd for $C_{23}H_{18}Cl_2N_2O4S{\cdot}0.5H_2O$: C 55.37, H 3.81, N 5.61, found: C 55.68, H 3.73, N 5.66.

Ethyl-(2*Z*)-2-(4-hydroxybenzylidene)-5-(3-hydroxyphenyl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (64): According to procedure 3, compound **64** was obtained as a yellow powder, (26%); mp: 175 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.15 (t, ^3J = 7.1, 3H, $CO_2CH_2CH_3$), 2.39 (s, 3H, 7-CH_3), 4.08 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 5.99 (s, 1H, 5-H), 6.67–6.72 (m, 3H, H_{ar}), 6.93 (d, ^3J = 7.8, 2H, H_{ar}), 7.14 (t, ^3J = 7.5, 1H, H_{ar}), 7.49 (d, ^3J = 7.5, 2H, H_{ar}), 7.73 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 14.3, 22.7, 55.6, 61.2, 108.6, 114.0, 115.0, 115.5, 116.6 (×2), 117.9, 123.9, 129.8, 132.6 (×2), 133.5, 141.9, 151.3, 156.0, 157.5, 161.3, 164.5, 165.1 ppm; Anal. calcd for $C_{23}H_{20}N_2O_5S{\cdot}0.5H_2O$: C 62.02, H 4.71, N 6.29, found: C 62.76, H 5.20, N 6.23.

Ethyl-(2*Z*)-2-(4-hydroxybenzylidene)-5-(4-hydroxyphenyl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (65): According to procedure 3, compound **65** was obtained as a yellow powder (31%); mp: 187 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.14 (t, ^3J = 6.5, 3H, $CO_2CH_2CH_3$), 2.39 (s, 3H, 7-CH_3), 4.08 (q, ^3J = 6.5, 2H, $CO_2CH_2CH_3$), 5.97 (s, 1H, 5-H), 6.69–6.72 (m, 4H, H_{ar}), 7.09 (d, ^3J = 8.5, 2H, H_{ar}), 7.37 (d, ^3J = 8.5, 2H, H_{ar}), 7.63 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 13.9, 21.4, 54.3, 60.7, 108.7, 115.2 (×2), 116.4 (×2), 123.7, 128.7 (×2), 130.9, 131.2, 132.5 (×2), 151.0, 155.7, 157.5, 160.2, 164.5, 165.0, 167.4 ppm; Anal. calcd for $C_{23}H_{20}N_2O_5S$: C 63.23, H 4.58, N 6.41, found: C 63.29, H 4.92, N 6.23.

Ethyl-(2*Z*)-2-(4-hydroxybenzylidene)-5-(3,4-dihydroxyphenyl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (66): According to procedure 3, compound **66** was obtained as an orange powder (29%); mp: 123 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.15 (t, ^3J = 7.1, 3H, $CO_2CH_2CH_3$), 2.39 (s, 3H, 7-CH_3), 4.08 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 5.92 (s, 1H, 5-H), 6.56–6.71 (m, 3H, H_{ar}), 6.93 (d, ^3J = 7.9, 2H, H_{ar}), 7.48 (d, ^3J = 7.9, 2H, H_{ar}), 7.72 ppm (s, 1H, H_{eth});^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 13.0, 21.4, 53.4, 59.0, 107.8, 113.6, 114.3 (×2), 115.4 (×2), 117.5, 122.8 (×2), 130.4, 131.5, 132.4, 144.1, 144.6, 149.7, 154.7, 159.2, 163.6, 164.1 ppm; Anal. calcd for $C_{23}H_{20}N_2O_6S$: C 61.06, H 4.42, N 6.19, found: C 62.38, H 4.17, N 6.39.

Ethyl-(2*Z*)-2-[3,4-bis(acetoxy)benzylidene]-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (67): According to procedure 4, compound **67** was obtained as a yellow powder (26%); mp: 179 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.26 (t, ^3J = 7.1, 3H, $CO_2CH_2CH_3$), 2.30 (s, 3H, 7-CH_3), 2.32 (s, 6H, 2× OC(O)CH_3), 4.18 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 4.42 (s, 2H, CH_2), 7.45 (d, ^3J = 7.5, 1H, H_{ar}), 7.58–7.61 (m, 2H, H_{ar}), 7.84 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, $CDCl_3$): δ = 14.3, 20.6 (×2), 22.0, 41.7, 60.3, 104.3, 120.9, 124.0, 124.6, 127.7, 130.3, 131.7, 141.5, 143.1, 152.3, 155.7, 164.3, 164.4, 167.4 ppm (×2); Anal. calcd for $C_{21}H_{20}N_2O_7S{\cdot}0.5H_2O$: C 55.56, H 4.63, N 6.17, found: C 55.85, H 4.54, N 6.58.

Ethyl-(2*Z*)-2-[3,4-bis(acetoxy)benzylidene]-7-methyl-5-phenyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (68): According to procedure 4, compound **68** was obtained as a yellow powder (29%); mp: 160 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.14 (t, ^3J = 7.1, 3H, $CO_2CH_2CH_3$), 2.32 (s, 6H, 2×OC(O)CH_3), 2.41 (s, 3H, 7-CH_3), 4.08 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 6.07 (s, 1H, 5-H), 7.34–7.37 (m, 5H, H_{ar}), 7.48 (d, ^3J = 8.3, 1H, H_{ar}), 7.55–7.58 (m, 2H, H_{ar}), 7.80 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, $CDCl_3$): δ = 14.0, 20.6 (×2), 22.6, 55.6, 60.5, 109.3, 121.5, 124.3, 124.8, 128.0 (×3), 128.6 (× 2), 128.7, 131.4, 131.9, 139.8, 142.6, 143.5, 152.0, 155.7, 164.8, 165.3, 167.7, 167.8 ppm; Anal. calcd for $C_{27}H_{24}N_2O_7S$: C 62.23, H 4.61, N 5.38, found: C 62.15, H 4.86, N 5.50.

Ethyl-(2*Z*)-2-[3,4-bis(acetoxy)benzylidene]-5-(3,4-dimethoxyphenyl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (69): According to procedure 4, compound **69** was obtained as a yellow powder (19%); mp: 118 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.09 (t, ^3J = 7.2, 3H, $CO_2CH_2CH_3$), 2.25 (s, 6H, 2×OC(O)CH_3), 2.34 (s, 3H, 7-CH_3), 3.66 (s, 6H, 2×OCH_3), 3.99 (q, ^3J = 7.2, 2H, $CO_2CH_2CH_3$), 5.95 (s, 1H, 5-H), 6.74 (d, ^3J = 7.8, 1H, H_{ar}), 6.81–6.88 (m, 2H, H_{ar}), 7.39 (d, ^3J = 8.3, 1H, H_{ar}), 7.48 (s, 2H, H_{ar}), 7.74 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, [D$_6$]DMSO): δ = 14.3, 20.7 (×2), 22.7, 54.5, 55.8 (×2), 60.5, 109.3, 111.7, 112.1, 119.8, 121.3, 125.0, 125.4, 128.4, 131.4, 131.9, 133.0, 142.7, 143.7, 148.6, 149.2, 151.0, 155.4, 164.5, 165.2, 168.3, 168.4 ppm; Anal. calcd for $C_{29}H_{28}N_2O_9S{\cdot}0.5H_2O$: C 59.01, H 4.92, N 4.75, found: C 59.10, H 5.00, N 4.72.

Ethyl-(2*Z*)-2-[3,4-bis(acetoxy)benzylidene]-5-(3,4-dichlorophenyl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (70): According to procedure 4, compound **70** was obtained as a yellow powder (34%); mp: 95 °C; ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.23 (t, ^3J = 7.1, 3H, $CO_2CH_2CH_3$), 2.33 (s, 6H, 2×OC(O)CH_3), 2.54 (s, 3H, 7-CH_3), 4.15 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 6.15 (s, 1H, 5-H), 7.25–7.42 (m, 5H, H_{ar}), 7.51 (s, 1H, H_{ar}), 7.69 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, $CDCl_3$): δ = 14.3, 20.9 (×2), 23.1, 54.9, 61.0, 108.5, 121.3, 124.6, 125.1, 127.6, 128.3, 130.3, 130.9, 131.9, 132.0, 133.1, 133.2, 140.0, 142.9, 143.9, 153.3, 155.8, 165.0, 165.3, 168.0 ppm (×2); Anal. calcd for $C_{27}H_{22}Cl_2N_2O_7S$: C 55.02, H 3.73, N 4.75, found: C 55.88, H 4.43, N 4.09.

Ethyl-(2*Z*)-2-[3,4-bis(acetoxy)benzylidene]-5-(2,3-dihydro-1,4-benzodioxin-6-yl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo-[3,2-*a*]pyrimidine-6-carboxylate (71): According to procedure 4, compound **71** was obtained as a yellow powder (30%); mp: 106 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.16 (t, ^3J = 7.1, 3H, $CO_2CH_2CH_3$), 2.24 (s, 6H, 2×OC(O)CH_3), 2.39 (s, 3H, 7-CH_3), 4.07 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 4.21 (s, 4H, O-CH_2-CH_2-O), 5.96 (s, 1H, 5-H), 6.74–6.84 (m, 3H, H_{ar}), 7.45–7.57 ppm (m, 3H, H_{ar}), 7.81 (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, $CDCl_3$): δ = 14.3, 20.7 (×2), 22.7, 54.7, 60.6, 64.4 (×2), 109.3, 116.5, 117.6, 120.6, 121.3, 125.1, 125.5, 128.5, 131.9, 133.5, 142.8, 143.5, 143.8, 144.0, 151.3, 155.5, 164.5, 165.2 (× 2), 168.5 ppm (×2).

Ethyl-(2*Z*)-2-[3,4-bis(acetoxy)benzylidene]-7-methyl-5-(2-naphthyl)-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (72): According to procedure 4, compound **72** was obtained as an orange powder (24%); mp: 96–98 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.13 (t, ^3J = 7.1, 3H, $CO_2CH_2CH_3$), 2.31 (s, 6H, 2×OC(O)CH_3), 2.44 (s, 3H, 7-CH_3), 4.08 (q, ^3J = 7.1, 2H,

$CO_2CH_2CH_3$), 6.23 (s, 1H, 5-H), 7.44–7.55 (m, 7H, H_{ar}), 7.76 (s, 1H, H_{eth}), 7.87–7.96 ppm (m, 3H, H_{ar}); ^{13}C NMR (250 MHz, $CDCl_3$): δ = 14.4, 21.0 (×3), 55.0, 61.2, 109.0, 121.7, 123.8, 124.1, 124.8, 125.3, 126.6, 128.5, 132.2, 132.4, 134.6, 138.5, 142.5, 142.8, 143.0, 144.0, 148.1, 153.2, 165.1, 165.4, 168.2, 168.4 ppm; Anal. calcd for $C_{31}H_{26}N_2O_7S{\cdot}0.5\,H_2O$: C 64.24, H 4.66, N 4.83, found: C 64.61, H 4.77, N 4.93.

Ethyl-(2*Z*)-2-[3,4-bis(acetoxy)benzylidene]-7-methyl-5-(4-methylnaphthyl)-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (73): According to procedure 4, compound **73** was obtained as a yellow powder (24%); mp: 132 °C; ^{1}H NMR (250 MHz, [D_6]DMSO): δ = 0.84 (t, ^3J = 6.9, 3H, $CO_2CH_2CH_3$), 2.30 (s, 6H, 2× $OC(O)CH_3$), 2.43 (s, 3H, 7-CH_3), 2.62 (s, 3H, 4′-CH_3), 3.81 (q, ^3J = 6.9, 2H, $CO_2CH_2CH_3$), 6.91 (s, 1H, 5-H), 7.37 (s, 2H, H_{ar}), 7.47–7.50 (m, 3H, H_{ar}), 7.60–7.63 (m, 2H, H_{ar}), 7.65 (s, 1H, H_{eth}), 8.01 (d, ^3J = 6.9, 1H), 8.57 ppm (m, 1H, H_{ar}); ^{13}C NMR (250 MHz, [D_6]DMSO): δ = 13.6, 19.3, 20.4 (×2), 22.4, 53.6, 60.1, 110.3, 120.5 (×2), 124.3, 124.7, 125.0, 125.8 (×2), 126.6, 128.0, 130.5, 131.0, 131.5 (×2), 131.7, 135.4 (×2), 142.4, 143.4, 150.2, 154.8, 164.2, 164.9, 168.0, 168.1 ppm; Anal. calcd for $C_{32}H_{28}N_2O_7S{\cdot}0.5\,H_2O$: C 64.68, H 4.88, N 4.71, found: C 64.76, H 5.03, N 4.53.

Ethyl-(2*Z*)-2-[3,4-bis(acetoxy)benzylidene]-5-(3,4,5-trimethoxyphenyl)-7-methyl-3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxylate (74): According to procedure 4, compound **74** was obtained as a yellow powder (27%); mp: 174 °C; ^{1}H NMR (250 MHz, [D_6]DMSO): δ = 1.18 (t, ^3J = 7.1, 3H, $CO_2CH_2CH_3$), 2.32 (s, 6H, $OC(O)CH_3$), 2.40 (s, 3H, 7-CH_3), 3.64 (s, 3H, O-CH_3), 3.74 (s, 6H, 2×O-CH_3), 4.09 (q, ^3J = 7.1, 2H, $CO_2CH_2CH_3$), 6.03 (s, 1H, 5-H), 6.55 (s, 2H, H_{ar}), 7.45–7.59 (m, 3H, H_{ar}), 7.83 ppm (s, 1H, H_{eth}); ^{13}C NMR (250 MHz, $CDCl_3$): δ = 14.0, 20.6 (×2), 22.6, 55.6, 56.6 (×3), 60.5, 104.6 (×2), 108.9, 121.2, 124.0, 124.7, 127.2, 131.0, 131.6, 135.0, 137.9, 142.2, 143.2, 151.7, 152.9 (×2), 155.5, 164.8, 165.1, 167.8 ppm (×2); Anal. calcd for $C_{30}H_{30}N_2O_{10}S$: C 59.01, H 4.92, N 4.59, found: C 59.12, H 5.15, N 4.98.

Enzymes, cell culture, and chemicals

The recombinant wild-type and mutant MBP–CDC25B3 proteins were produced in bacteria as previously described.[45] Human cancer cell lines LNCaP and MiaPaCa-2 were obtained from ATCC (Rockville, MD, USA). Cells were cultured at 37 °C in ATCC-recommended media: either in RPMI 1640 complemented with 10% fetal bovine serum (FBS) or in Dulbecco's modified Eagle's medium complemented with 10% FBS, 2.5% horse serum, and penicillin/streptomycin (100 U mL^{-1}) in a humidified atmosphere of 5% CO_2. The tetrazolium salt WST-1 was purchased from Roche Diagnostics (Mannheim, Germany). U2OS cells expressing HA–CDC25B3 under a tetracycline-regulated promoter were grown and induced as previously described.[55]

In vitro enzymatic assays and steady-state kinetics: The activity of the MBP–CDC25B3 recombinant enzyme was monitored using fluorescein diphosphate. The assay was performed in 96-well plates in a final volume of 200 μL. MBP–CDC25B3 was diluted in assay buffer [30 mM Tris-HCl (pH 8.2), 75 mM NaCl, 0.67 mM EDTA, 0.033% BSA, 1 mM DTT] so that the final concentration was 90 ng well^{-1}. The reaction was initiated by the addition of fluorescein diphosphate (25 μM) followed by immediate measurement of fluorescein phosphate emission with a Fluoroskan Ascent (Lab Systems; excitation filter: 485 nm, emission filter: 530 nm). For each compound, the drug concentration required for 50% inhibition (IC_{50}) was determined from a sigmoid dose–response curve using GraphPad Prism 4 (GraphPad Software, San Diego, CA, USA). Compounds **1**, **58**, and **70** were added to the wells at final concentrations of 1, 3, 6, and 10 μM. Compound **44** was added to the wells at final concentrations of 0.3, 1, 3, and 6 μM. The substrate concentration was varied between 1 and 50 μM (K_M = 2.9 μM). Results are expressed as the mean of at least two independent experiments with three determinations per tested concentration and per experiment.

Bypass of a genotoxicity-induced G_2/M checkpoint arrest by overexpression of CDC25B: U2OS cells expressing CDC25B under control of the tetracycline-repressible promoter were grown for 24 h in the absence of tetracycline to allow CDC25B expression,[56] and then treated for 1 h with etoposide (40 μM). The cells were washed and further incubated in the presence of nocodazole (345 nM) together with compound **44** at the indicated concentrations. After 16 h, cells were fixed, and the mitotic index was determined by flow cytometry using the 3-12-I-22 mitotic specific monoclonal antibody.[57]

Cell proliferation assay: The inhibition of cell proliferation was determined by a colorimetric assay based on the cleavage of the WST-1 tetrazolium salt by mitochondrial dehydrogenase activity in viable cells, leading to the formation of formazan. At day 0, MiaPaCa-2 and LNCaP cells were plated at 5000 cells per well in 96-well culture plates with 95 μL medium per well. At day 1, MiaPaCa-2 and LNCaP cells were treated for 48 and 72 h, respectively, with 5 μL of increasing concentrations of drug. At day 3 (MiaPaCa-2) or 4 (LNCaP), after the addition of WST-1 (10 μL per well), cells were incubated for 2 h at 37 °C in a humidified atmosphere of 5% CO_2. Absorbance was measured at 430 nm with a microplate reader (Bio-Rad). The results are expressed as the mean of three independent experiments with three determinations per tested concentration and per experiment. For each compound, the IC_{50} value was determined from a sigmoid dose–response curve using GraphPad Prism (GraphPad Software, San Diego, CA, USA).

Computational chemistry

The Cdc25 X-ray structure (PDB code: 1CWT),[58] as modified in our previous study,[26] was used for docking experiments. SurflexDock 2.1 was used for the docking computations.[59] A protomol (an idealized ligand or molecular probes used to dock the ligand fragments) was generated that allows exploration of the catalytic site and the swimming pool areas. Compounds were drawn using JChemPaint (provided by Prof. C. Steinbeck), and SMILES strings were created. The 3D structure for each molecule was generated using the smi2sdf Java utility kindly provided by Dr. R. Guha. Molecules were then energy minimized before docking, and afterward in the context of the protein environment with Surflex, and 20 poses were saved (that is, the -pgeom parameter regime was turned on) and analyzed interactively. Docked ligands were also investigated using the MolDock scoring and re-scoring energy functions.[60] Energy refinement of the molecules docked by Surflex and rescoring, as well as visual inspection of favorable and unfavorable interaction energy and energy strains in the docked ligand structures, were also computed with MolDock. Figures were prepared with PyMol (DeLano Scientific, Palo Alto, CA, USA).

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