# Discovery of 8-Cyclopentyl-2-[4-(4-methyl-piperazin-1-yl)-phenylamino]-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7x) as a Potent Inhibitor of Cyclin-Dependent Kinase 4 (CDK4) and AMPK-Related Kinase 5 (ARK5)

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**S** *Supporting Information*

**ABSTRACT:** The success of imatinib, a BCR-ABL inhibitor for the treatment of chronic myelogenous leukemia, has created a great impetus for the development of additional kinase inhibitors as therapeutic agents. However, the complexity of cancer has led to recent interest in polypharmacological approaches for developing multikinase inhibitors with low toxicity profiles. With this goal in mind, we analyzed more than 150 novel cyano pyridopyrimidine compounds and identified



structure−activity relationship trends that can be exploited in the design of potent kinase inhibitors. One compound, 8-cyclopentyl-2-[4-(4-methyl-piperazin-1-yl)-phenylamino]-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7x), was found to be the most active, inducing apoptosis of tumor cells at a concentration of approximately 30−100 nM. In vitro kinase profiling revealed that 7x is a multikinase inhibitor with potent inhibitory activity against the CDK4/CYCLIN D1 and ARK5 kinases. Here, we report the synthesis, structure−activity relationship, kinase inhibitory profile, in vitro cytotoxicity, and in vivo tumor regression studies by this lead compound.
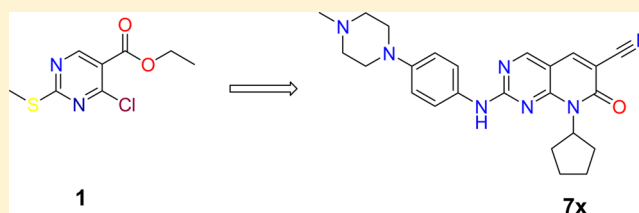
## ■ INTRODUCTION

Cancer is now believed to result from perturbations in cell cycle that result in unlimited proliferation and an inability of a cell to undergo differentiation and/or apoptosis.[1−5] The cell cycle is typically divided into four phases, $G_1$, S, $G_2$, and M, and it is apparent that the order and timing of each phase is critical for accurate transmission of genetic information. Consequently, a number of biochemical pathways have evolved to ensure that initiation of a particular cell cycle event is dependent on the accurate completion of another. These biochemical pathways have been termed "checkpoints."[1,2,5]
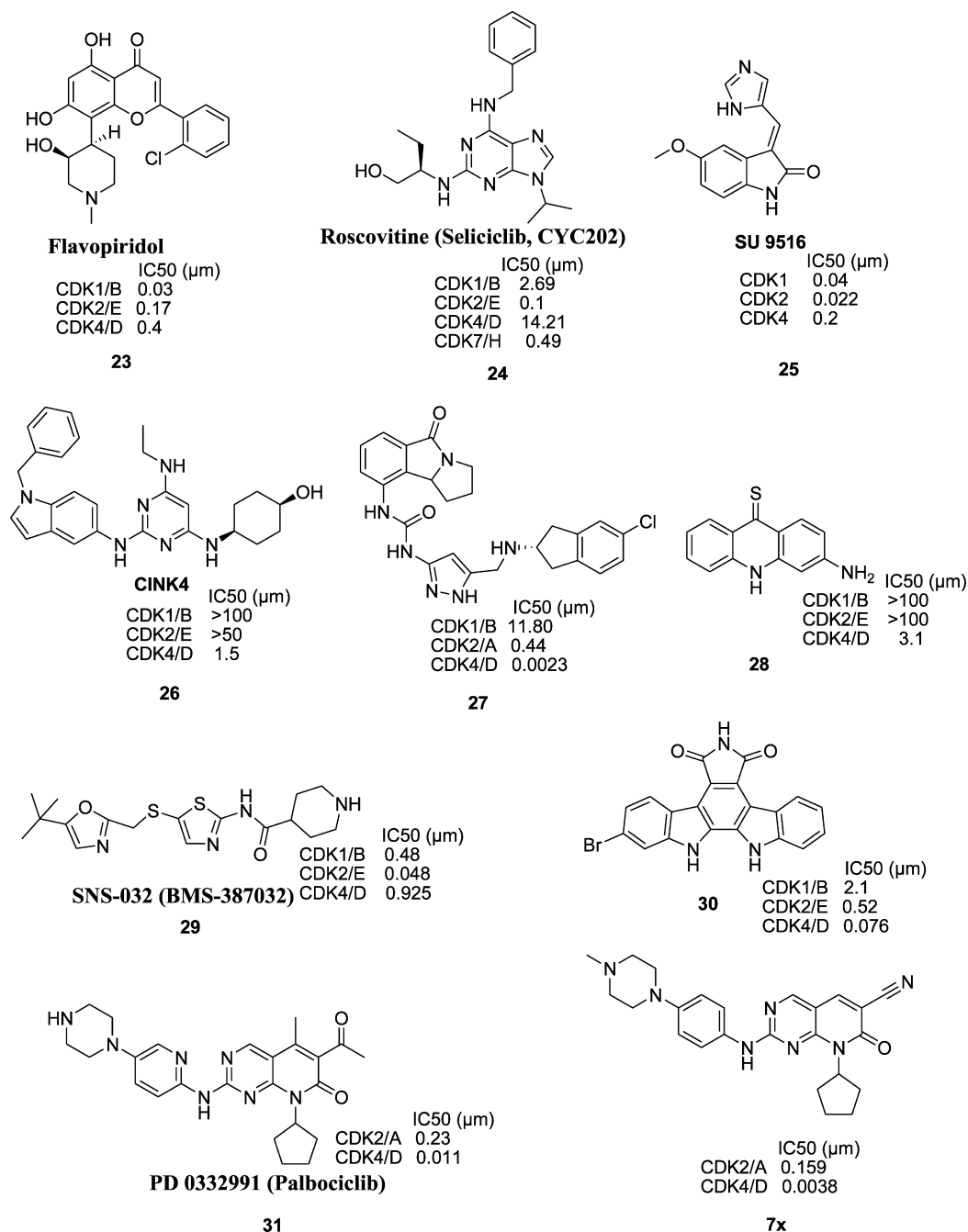
When cells proliferate, mitogenic growth factors bind to their cognate receptors and initiate a cascade of events that culminate in the expression and assembly of different kinase holoenzymes that are composed of a regulatory subunit, called a cyclin, and a catalytic subunit, termed a cyclin-dependent kinase (CDK).[1−3,5] CDKs are serine/threonine kinases that are inactive when they are under-phosphorylated and monomeric.[4] The primary mechanism of CDK activation is association with its partner cyclin. In the mammalian cell cycle, CDK4/6 associate with D-type cyclins and control progression through the $G_1$ phase when the cell prepares to initiate DNA synthesis.[5,6] Activation of CDK4/6/cyclin D complexes contribute to hyperphosphorylation of the retinoblastoma (RB) family of proteins, which results in the release of associated protein factors.[7,8] One key RB-binding partner is the E2F-1 transcription factor, which appears to activate the transcription of genes whose products are required for S-phase progression. E2F-1 and other members of the E2F family are known to bind to pRB and heterodimerize with DP-1 and -2, an interaction that is required for the DNA-binding capacity of E2F family proteins.[1−8] Once the cell has made the $G_1$/S transition, cyclin E/CDK2 phosphorylates the remaining residues on the RB family proteins that are critical for E2F activation. Activation of E2F-mediated transcription allows the cell to transit into S phase and to initiate DNA replication, which is controlled, in part, through cyclin A/CDK2. Cyclin A/CDK2 ultimately forces the cell through the $G_2$ phase prior to the assembly of the cyclin B/CDK1 complex and the initiation of mitosis.[5,8]

**Chart 1. CDK Inhibitors**



Flavopiridol

| | IC50 (µm) |
|---|---|
| CDK1/B | 0.03 |
| CDK2/E | 0.17 |
| CDK4/D | 0.4 |

**23**

Roscovitine (Seliciclib, CYC202)

| | IC50 (µm) |
|---|---|
| CDK1/B | 2.69 |
| CDK2/E | 0.1 |
| CDK4/D | 14.21 |
| CDK7/H | 0.49 |

**24**

SU 9516

| | IC50 (µm) |
|---|---|
| CDK1 | 0.04 |
| CDK2 | 0.022 |
| CDK4 | 0.2 |

**25**

CINK4

| | IC50 (µm) |
|---|---|
| CDK1/B | >100 |
| CDK2/E | >50 |
| CDK4/D | 1.5 |

**26**

| | IC50 (µm) |
|---|---|
| CDK1/B | 11.80 |
| CDK2/A | 0.44 |
| CDK4/D | 0.0023 |

**27**

| | IC50 (µm) |
|---|---|
| CDK1/B | >100 |
| CDK2/E | >100 |
| CDK4/D | 3.1 |

**28**

SNS-032 (BMS-387032)

| | IC50 (µm) |
|---|---|
| CDK1/B | 0.48 |
| CDK2/E | 0.048 |
| CDK4/D | 0.925 |

**29**

| | IC50 (µm) |
|---|---|
| CDK1/B | 2.1 |
| CDK2/E | 0.52 |
| CDK4/D | 0.076 |

**30**

PD 0332991 (Palbociclib)

| | IC50 (µm) |
|---|---|
| CDK2/A | 0.23 |
| CDK4/D | 0.011 |

**31**

| | IC50 (µm) |
|---|---|
| CDK2/A | 0.159 |
| CDK4/D | 0.0038 |

**7x**

There is considerable evidence showing that a vast majority of human tumors exhibit deregulation of the CDK4/6-cyclin D-RB pathway.[1,9,10] For example, CDK4/6 is hyperactivated in a number of human cancers as a result of overexpression of positive regulators such as cyclin D or deletion and/or epigenetic alterations of substrates such as RB.[1,9,10] In addition, mutations and chromosomal translocations in the CDK4 locus have also been described. One prominent example is the CDK4[R24C] mutation that results in insensitivity of CDK4 to INK4 family inhibitors and was first described in patients with familial melanoma.[11,12] Finally, CDK4/6 amplification or overexpression has been observed in a wide spectrum of tumors, including gliomas, sarcomas, lymphomas, melanomas, carcinomas of breast, squamous cell carcinomas, and leukemias.[13]

Because cyclin D, CDK4, and CDK6 activities are upregulated in a variety of tumor types, several groups have focused their efforts on the development of small molecule CDK4 inhibitors. Experimental evidence indicating that CDK4 is dispensable for development[14−17] suggests that inhibitors of this kinase might be both nontoxic and effective in the treatment of cancers that are dependent on CDK4 activity for proliferation. The first generation of CDK inhibitors, flavopiridol (23)[18] and roscovitine (CYC202) (24)[19] (see Chart 1), were potent CDK4 inhibitors but were nonselective and inhibited multiple kinases including CDK1 and CDK7 and caused severe toxic side effects in clinical trials.[20,21] Several other pan-CDK inhibitors have since entered clinical trials, but the therapeutic efficacy of these molecules has been modest due to dose-limiting toxicity and poor pharmacokinetics. Several early trials have since been discontinued.[22,23]

**Scheme 1. Synthesis of Pyrido[2,3-d]pyrimidines (7)**[a]
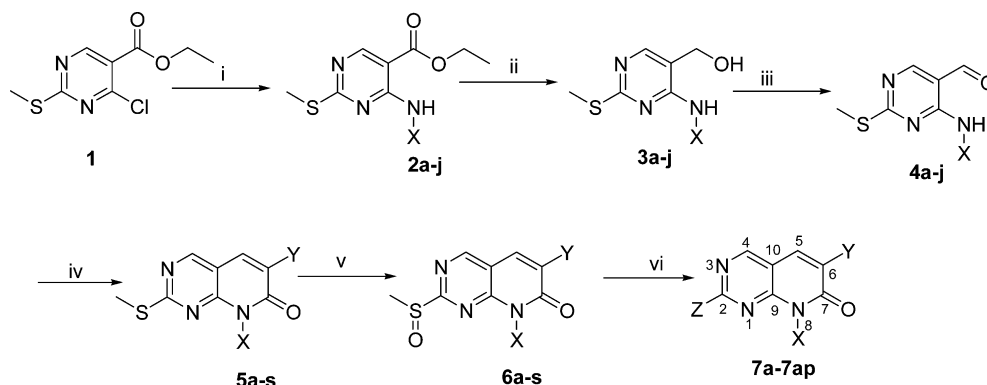


X = H, Methyl, Ethyl, Propyl, Isopropyl, Butyl, Pentyl, Cyclopropyl, Cyclopentyl, Cyclohexyl

Y = CN, $NO_2$, $CH_3SO_2$, $ArSO_2$, $ArCH_2SO_2$, ArNHCO

Z = Substituted aryl or heteroaryl amine

[a] Reagents and conditions (i) X-NH$_2$, Et$_3$N, THF, rt, 1−3 h, 80−95%; (ii) LiAlH$_4$, THF, −10 °C to rt, 1 h, 80−86%; (iii) MnO$_2$, CHCl$_3$, rt, 24 h, 70−90%; (iv) CNCH$_2$CO$_2$H or NO$_2$CH$_2$CO$_2$Et or **10** or **13** or **16**, BnNH$_2$, AcOH, 100 °C, 6 h, 62−73%; (v) *m*-CPBA, CH$_2$Cl$_2$, rt, 3 h, 87−94%; (vi) Z-H, toluene, 100 °C, 3−8 h, 40−65%.

In an attempt to overcome the toxicity profile of pan-CDK inhibitors, small molecules belonging to additional chemical classes such as oxindoles (**25**),[24] triaminopyrimidines (**26**),[25] diarylureas (**27**),[26] thioacridones (**28**),[27] aminothiazoles (**29**),[28] indolocarbazoles (**30**),[29] and pyrido[2,3-d]pyrimidines (**31**)[30−32] (see Chart 1) have been developed that are specific for individual CDKs. Some of these compounds exhibited a high degree of selectivity toward CDK4/6 by targeting the ATP binding site of CDK4/6-cyclin D complexes. Of these, one CDK4/6 selective compound, PD-0332991, which is a pyrido-[2,3-d]pyrimidine derivative, is highly specific for CDK4 and CDK6, inhibiting these two kinases with IC$_{50}$ values of 0.011 and 0.015 μM, respectively, with little or no inhibitory activity against a large panel of kinases including other CDKs and a wide variety of serine, threonine, and tyrosine kinases.[30,31] PD-0332991 has been extensively studied for its efficacy in tissue culture model systems as well as in mouse xenograft models of colorectal cancer, mantle cell lymphoma (MCL), and disseminated myeloma.[31−38] PD-0332991 causes G$_1$ arrest in cultured tumor cell lines and inhibits tumor growth in xenograft models of RB-positive human tumor cell lines derived from breast, ovarian, lung, colon, prostate, brain, and blood such as multiple myeloma and mantle cell lymphoma.[31−38] Therapeutic doses of PD-0332991 resulted in a reduction of both phosphorylated RB and the proliferative marker Ki-67 in the tumor tissue as well as downregulation of E2F-target genes.[37,38] On the basis of these promising results, this compound entered clinical trials in 2004 and results from phase I and phase II trials indicate that the side effects are tolerable.[39−42] Phase I studies with palbociclib (PD-0332991) indicated that the clinical response was mostly cytostatic where disease stabilization was observed in a significant number of patients (http://clinicaltrials.gov). However, few partial or complete remissions were observed in phase I and II clinical trials where PD-0332991 was used as a single agent. However, combination studies have yielded more promising results.[43−46] This drug is currently being evaluated in phase III trials in combination with letrozole for the treatment of ER+/HER2− advanced breast cancer.[45,46] These studies suggest that reduction of tumor burden in patients whose tumors express

high levels of cyclin D/CDK4 might require inhibition not only of CDK4 but of other aberrantly activated proteins. With this in mind, our goal was to identify potent inhibitors that possess sufficient cross-reactivity with a small number of kinases, such that the combined inhibition of these kinases will result in a more effective treatment of these tumors. Here, we describe the synthesis, structure−activity relationship (SAR), cytotoxic properties, kinase inhibition profile, and mechanism of action of **7x**. **7x**, which is a member of the pyridopyrimidine series of compounds, is a potent CDK4/6 inhibitor that exhibits cross-reactivity with a small number of kinases that play critical roles in mitogenic signaling. Mice treated with **7x** did not exhibit signs of toxicity and tumor formation in **7x**-treated xenograft nude mouse models was inhibited over an 18-day period. Together, these studies indicate that pyridopyrimidine compounds might represent a safe and effective chemotype to treat tumors whose cell cycle progression is altered as a result of CDK4/6-RB hyperactivity.
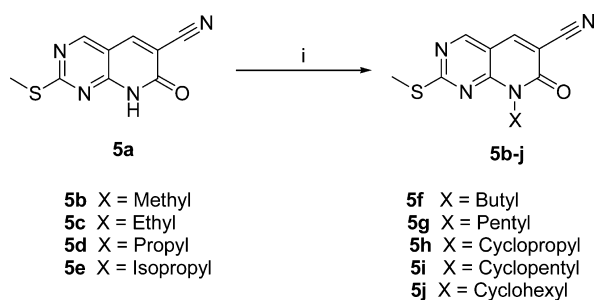
## ■ CHEMISTRY

To generate an ATP-competitive kinase inhibitor library, we used pyrido[2,3-d]pyrimidines (**7**) as the backbone because this class of compounds have been shown to possess kinase inhibitory activity.[47,48] To facilitate the synthesis of a large number of compounds, we developed a unique and simple method which is summarized in Scheme 1.

The commercially available compound 4-chloro-2-methyl-sulfanyl-pyrimidine-5-carboxylic acid ethyl ester **1** was treated with acyclic and cyclic amines in the presence of triethylamine (Et$_3$N) to yield **2a**−**2j**. The ester group in compounds **2a**−**2j** was then reduced with lithium aluminum hydride (LiAlH$_4$) to obtain the corresponding alcohols **3a**−**3j**, which when further oxidized with manganese dioxide (MnO$_2$), yielded the corresponding pyrimidine carbaldehyde **4a**−**4j**. The aldehyde **4a**−**4j** were converted to pyridopyrimidines **5a**−**5s** by Knoevenagel condensation, with each aldehyde treated with active methylene compounds (cyanoacetic acid or nitro-acetic acid ethyl ester or **10** or **13** or **16**) in the presence of benzylamine to generate their corresponding intermediates **5a**−**5s**. This approach permitted

the introduction of cyano, nitro, sulfonyl, and carboxamide groups at the *C*-6 position of the pyrido[2,3-*d*]pyrimidines. To replace methylsulfide at the *C*-2 position with substituted aryl/heteroaryl amines, it was oxidized to methyl sulfoxides **6a−6s** using *m*-chloroperbenzoic acid (*m*-CPBA). The methyl sulfoxide was then substituted with different aryl/heteroaryl amines to achieve the desired pyridopyrimidine compounds **7a−7ap** (Scheme 1).

Alternatively, the compounds **5b−5j** were also prepared by alkylation of **5a** with different alkyl iodides in the presence of sodium hydride (NaH) in *N,N*-dimethylformamide (DMF) at 50 °C as shown in Scheme 2.

**Scheme 2. Synthesis of 8-Alkyl/cycloalkyl-2-methylsulfanyl-7-oxo-7,8-dihydro[2,3-*d*]pyrimidine-6-carbonitrile (5b−5j) from 2-Methylsulfanyl-7-oxo-7,8-dihydro[2,3-*d*]pyrimidine-6-carbonitrile (5a)**[a]



| | |
|---|---|
| **5b**  X = Methyl | **5f**  X = Butyl |
| **5c**  X = Ethyl | **5g**  X = Pentyl |
| **5d**  X = Propyl | **5h**  X = Cyclopropyl |
| **5e**  X = Isopropyl | **5i**  X = Cyclopentyl |
| | **5j**  X = Cyclohexyl |

[a]Reagents and conditions: (i) X-I, NaH, DMF, 50 °C, 1 h, 64−75%.

Later, we evaluated the role of NH proton at the *C*-2 position of **7x** in cytotoxicity assays by acylating and benzoylating **7x** with acetic anhydride and 4-trifluoromethylbenzoyl chloride to obtain **7aq** and **7ar** respectively (Scheme 3).

The active methylene compounds, arylmethanesulfonylacetic acids (**10a−10c**), 4-chlorophenylsulfonylacetic acid (**13**), and *N*-arylmalonamic acids (**16a−16b**) were prepared as shown in Schemes 4, 5, and 6 as per the reported procedures.[49−51]

Arylmethyl bromides (**8a−8c**) were treated with thioglycolic acid in the presence of sodium hydroxide to produce corresponding arylmethylsulfanyl acetic acids (**9a−9c**). Deprotection of *tert*-butyldimethylsilyl ether (TBDMS) group in **9c** with tetrabutylammonium fluoride (TBAF) yielded **9d**.[49a] The oxidation of **9a−9b** and **9d** with 30% H$_2$O$_2$ in acetic acid yielded the corresponding arylmethylsulfonyl acetic acids (**10a−10c**) (Scheme 4).
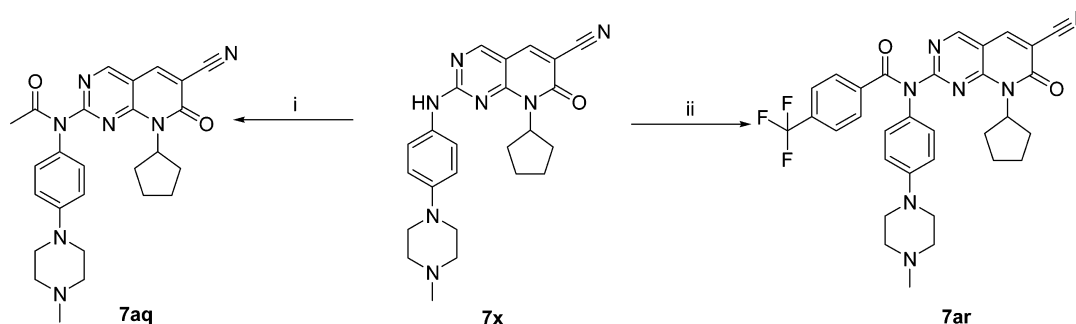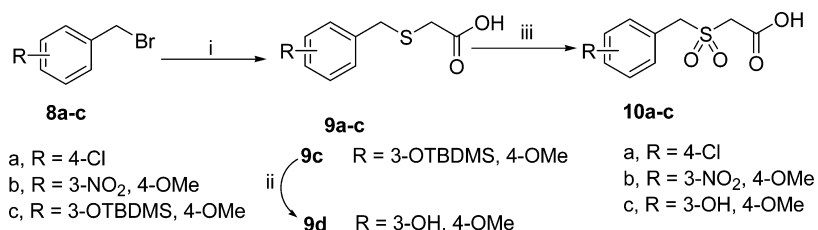
As shown in Scheme 5, 4-chlorothiophenol (**11**) was treated with chloroacetic acid in the presence of NaOH and subsequent oxidation with 30% H$_2$O$_2$ to produce 4-chlorophenylsulfanyl acetic acid (**12**) and 4-chlorophenylsulfonyl acetic acid (**13**), respectively.

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

Commercially unavailable bicyclic amines were prepared in two steps as shown in Scheme 7.[30] Aromatic nucleophilic substitution of halogen in 1-nitro-4-fluorobenzene (**17**) and 5-bromo-2-nitropyridine (**18**) by *N*-methylpiperazine was achieved under heating conditions. The resulting nitro compounds **19** and **20** were reduced to corresponding amines **21** and **22** with Pd/C in the presence of hydrazine hydrate in methanol.

## ■ STRUCTURE−ACTIVITY RELATIONSHIPS (SARS)

It is evident from Table1 that the nature of substituents X (*N*-8 position), Y (*C*-6 position), and Z (*C*-2 position) in the general structure (**7**) (Scheme 1) specify the cytotoxicity activity of the molecules on the cancer cells. Hence, by varying X, Y, and Z using various combinations of atoms or groups, we were able to generate compounds with excellent cytotoxicity. We initially kept X and Y constant, where X = C$_5$H$_9$ and Y = CN, and varied the substitutions at the Z position. In our initial attempts, we have included simple anilines at the Z position and placed C$_5$H$_9$ and CN at the X and Y positions, respectively. The cytotoxicity data from Table1 shows that 2-pyridine (**7m**) and 2-methoxy-6-quinoline (**7s**) at the Z-position showed better activity when compared to benzyl (**7a**), chlorophenyl (**7b**), cyanophenyl (**7c**), hydroxy phenyl (**7d**), methoxyphenyl (**7e−7l**), substituted pyridyl (**7n**), indolyl (**7o**, **7p**), and substituted quinolines (**7q**, **7r**). Encouraged by these results, we then attached bicyclic amines, such as substituted morpholino-aniline (**7t**), morpholino-pyridine (**7u**), piperazino-pyridine (**7v**), and pyridyl-piperazine (**7w**), which can bring both potency and water solubility to the molecule. These morpholino-anilines, morpholino-pyridines, and piperazino-pyridines easily form water-soluble salts of hydrochlorides, lactates, and citrates from hydrochloric acid, lactic acid, and citric acid. Although the salts of bicyclic amines (**7t**, **7u**, **7v**, and **7w**) showed enhanced water solubility when compared to the salts of monocyclic amines (**7m**

**Scheme 3. Acylation and Benzoylation of NH Group of 8-Cyclopentyl-2-[4-(4-methyl-piperazin-1-yl)-phenylamino]-7- oxo-7,8-dihydro-pyrido[2,3-*d*]pyrimidine-6-carbonitrile (7x)**[a]



[a]Reagents and conditions: (i) AC$_2$O, 120 °C, 3 h, 64%; (ii) 4-CF$_3$PhCOCl, NaH, DMF, rt, 3 h, 62%.

**Scheme 4. Synthesis of Arylmethanesulfonyl Acetic Acids**[a]



**8a-c**

a, R = 4-Cl
b, R = 3-NO$_2$, 4-OMe
c, R = 3-OTBDMS, 4-OMe

**9a-c**

9c    R = 3-OTBDMS, 4-OMe

ii

9d    R = 3-OH, 4-OMe

**10a-c**

a, R = 4-Cl
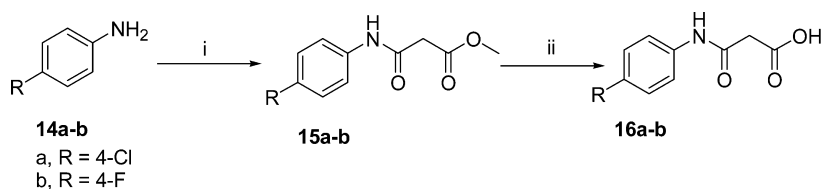b, R = 3-NO$_2$, 4-OMe
c, R = 3-OH, 4-OMe

[a]Reagents and conditions: (i) HSCH$_2$CO$_2$H, NaOH, MeOH, HCl, rt, 3 h, 90−95%; (ii) TBAF solution in THF, rt, 2 h, 50%; (iii) 30% H$_2$O$_2$, AcOH, rt, 24 h, 85−90%.

**Scheme 5. Synthesis of 4-Chlorophenylsulfonyl Acetic Acids**[a]



**11**      **12**      **13**
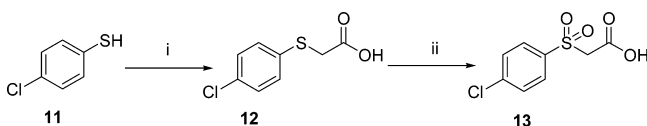
[a]Reagents and conditions: (i) ClCH$_2$CO$_2$H, NaOH, MeOH, HCl, rt, 3 h, 98%; (ii) 30% H$_2$O$_2$, AcOH, rt, 24 h, 80%.

and **7s**), the cytotoxicity properties of these compounds was decreased by several fold when compared to **7m** and **7s**. We further tested the effect of incorporating additional bicyclic amines at the Z position that might enhance the molecules' cytotoxicity properties. Substituted piperazino-anilines could readily be placed at the Z position, and all of the compounds tested, **7x** (Table 1), **7aq**, and **7ar** (Table 4), showed enhanced cytotoxic activity against these cancer cell lines. However, of these three compounds, **7x** showed superior cytotoxic activity against both leukemic (K562) and prostate (DU145) cancer cell lines when compared to any of the molecules listed in Table 1 and Table 4.

Following optimization of the Z position with *N*-methylpiperazino-aniline, we then focused our efforts on varying the X position using different substituents. The Y and Z positions were kept constant using cyano and *N*-methyl-piperazino-aniline groups on the pyridopyrimidine ring. To understand the significance and role of the alkyl group at the X position of the ring with respect to the cytotoxic properties of the molecule, we replaced the *N*-cyclopentyl group of **7x** with hydrogen (**7y**), methyl (**7z**), ethyl (**7aa**), *n*-propyl (**7ab**), isopropyl (**7ac**), butyl (**7ad**), pentyl (**7ae**), cyclopropyl (**7af**), and cyclohexyl (**7ag**) moieties (Table 2). The cytotoxic activities of the resulting molecules were then tested using our panel of cancer cell lines, and all were found to be substantially less active than the original compound (**7x**). These data clearly show that a cyclopentyl group at X position is the most optimal substituent, as compound **7x** showed the highest level of cytotoxicity when compared to other molecules (**7y**−**7ag**). Once we identified suitable substituents for the X and Z positions, we then focused our

**Scheme 7. Synthesis of *N*-Methylpiperazine Arylamines**[a]



**21**    **19**    **17** X = CH, R = F    **20**    **22**
              **18** X = N, R = Br
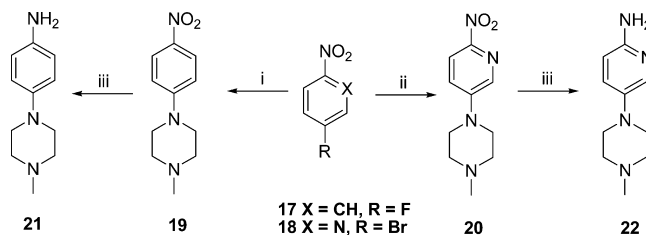
[a]Reagents and conditions: (i) *N*-methylpiperazine, acetonitrile, 100 °C, 5 h, 70%; (ii) *N*-methylpiperazine, tetra-*n*-butyl ammonium iodide, DMSO, 80 °C, overnight, 65%; (iii) 10% Pd/C, NH$_2$NH$_2$·H$_2$O, rt, 4 h, 75%.

efforts on optimizing the Y position of the pyridopyrimidine ring. Because **7x**, with a cyano group at Y position, is an active compound, we further explored the possibility of enhancing the antiproliferative activity by replacing the cyano group with other chemical moieties. As the cyano group is an electron withdrawing group, we considered replacing it with nitro (**7ah**), sulfonyl (**7ai**−**7an**), and carboxamide (**7ao** and **7ap**) groups (Table 3), all of which are electron withdrawers and are therefore similar to the cyano group with respect to that property. All of the resulting compounds were then tested in cytotoxicity assays using K562 and DU145 cells. The results of these studies showed that all of the molecules were several-fold less active than **7x**, suggesting that the cyano group at the Y position of the pyridopyrimidinone ring is critical for its activity. Furthermore, the polarized nitrogen atom of the moiety might also be interacting with the key amino acids of the enzymatic pocket of the target kinase. Because SAR analysis clearly shows that **7x** is best in this class, we performed all subsequent in vitro and in vivo biological studies using this compound.
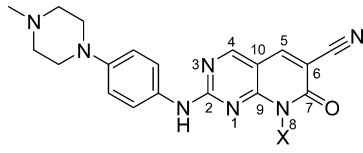
## ■ BIOLOGICAL RESULTS AND DISCUSSION

**In Vitro Antitumor Activity of Compound 7x.** We next tested the cyotoxic activity of the most active compound (**7x**) against a panel of human tumor cell lines. The results of this

**Scheme 6. Synthesis of *N*-Arylmalonamic Acids**[a]



**14a-b**      **15a-b**      **16a-b**

a, R = 4-Cl
b, R = 4-F

[a]Reagents and conditions: (i) ClCOCH$_2$CO$_2$Me, Et$_3$N, DCM, rt, 3 h, 90%; (ii) 10% NaOH, HCl, rt, 1 h, 78−80%.

## Table 1. In Vitro Cytotoxicity of Pyrido[2,3-d]pyrimidines (7a−7x) with Variables at C-2 Position
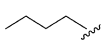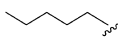


**7a-7x**

| Compd. | Z | IC$_{50}$ (μM) | | Compd. | Z | IC$_{50}$ (μM) | |
|---|---|---|---|---|---|---|---|
| | | K562 | DU145 | | | K562 | DU145 |
| 7a | | 100 | 75 | 7p | | 5 | 5 |
| 7b | | 30 | 30 | 7q | | 5 | 5 |
| 7c | | 75 | 5 | 7r | | 1 | 1 |
| 7d | | 15 | 15 | 7s | | 0.25 | 3 |
| 7e | | 15 | 30 | 7t | | 5 | 15 |
| 7f | | 5 | 0.5 | 7u | | 5 | 15 |
| 7g | | 75 | 25 | | | | |
| 7h | | 15 | 15 | 7v | | 5 | 15 |
| 7i | | 2 | 5 | | | | |
| 7j | | 15 | 15 | | | | |
| 7k | | 2.5 | 5 | 7w | | 30 | 100 |
| 7l | | 75 | 75 | | | | |
| 7m | | 0.5 | 3 | 7x | | 0.05 | 0.025 |
| 7n | | 5 | 30 | | | | |
| 7o | | 30 | 15 | PD0332991 (Palbociclib) | - | 2 | 7.5 |

**Table 2. In Vitro Cytotoxicity of Pyrido[2,3-d]pyrimidines (7y−7ag) with Variables at N-8 Position**



**7y -7ag**

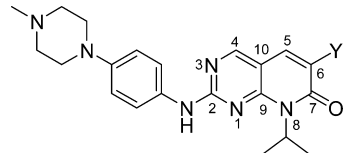| Compd. | X | IC$_{50}$ (μM) | |
|---|---|---|---|
| | | K562 | DU145 |
| **7y** | H | 30 | 30 |
| **7z** | | 75 | 75 |
| **7aa** | | 0.75 | 1.5 |
| **7ab** | | 5 | 15 |
| **7ac** | | 2.5 | 2.5 |
| **7ad** | | 0.75 | 0.75 |
| **7ae** | | 0.75 | 1.5 |
| **7af** | | 2.5 | 2.5 |
| **7ag** | | 1.5 | 50 |
| **7x** | | 0.05 | 0.025 |

**Table 3. In Vitro Cytotoxicity of Pyrido[2,3-d]pyrimidines (7ah−7ap) with Variables at C-6 Position**



**7ah -7ap**

| Compd. | Y | IC$_{50}$ (μM) | |
|---|---|---|---|
| | | K562 | DU145 |
| **7ah** | O$_2$N | 0.75 | 2.5 |
| **7ai** | | 5 | 5 |
| **7aj** | | 30 | 75 |
| **7ak** | Cl | 5 | 15 |
| **7al** | Cl | 10 | 75 |
| **7am** | OH | 2 | 2 |
| **7an** | NO$_2$ | 2 | 5 |
| **7ao** | Cl | 5 | 30 |
| **7ap** | F | 15 | 30 |
| **7x** | CN | 0.05 | 0.025 |
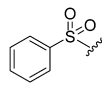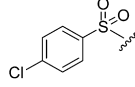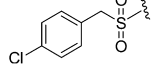
study, which are listed in Table 5, show that treatment with **7x** induces growth arrest of most tumor cell lines, with GI$_{50}$ values ranging from 0.025 to 2 μM (selected data is shown in Table 5). The evidence of growth inhibition across multiple tumor cell types suggests that this compound inhibits cellular proliferation by blocking key signaling pathways that are required for growth. A comparison of growth inhibitory activities of **7x** and PD-0332991 for a panel of breast cancer cell lines is given in Supporting Information Table 1.

It is interesting to note that this compound exhibited highest growth inhibitory activity against two mantle cell lymphoma cell lines, both of which are known to exhibit a chromosomal translocation that results in the overexpression of cyclin D1 and an associated increase in CDK4 activity. Because of its excellent potency, the 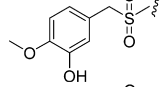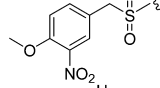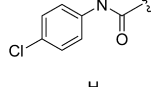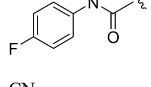kinase inhibition profile of **7x** was subsequently tested against a series of 285 functional kinases (Reaction Biology Corp.), the results of which are provided in Supporting Information Table 2. Interestingly, this study revealed that **7x** is a multikinase inhibitor, with the highest inhibitory activity against CDK4, CDK6, ARK5, FGFR1, PDGFRβ, and PI3K-δ, all of which are intimately associated with the growth, survival, and metastasis in human tumor cells.[52,53] The kinome inhibition map of 365 kinases encoded by the human genome, as well as the IC$_{50}$ values for selected kinases (as compared to the CDK4/6 inhibitor PD-0332991), are shown in Figure 1 and Table 6, respectively.

## ■ MOLECULAR MODELING OF 7X

**Docking Prediction of 7x to CDK6 Suggest Different Binding than PD-0332991.** To explain the difference in inhibitor potency between **7x** and PD-0332991, binding of **7x** to the kinase domain of CDK6 was predicted by molecular docking and energy minimization based upon the X-ray cocrystal structure of CDK6−Vcyclin−PD-0332991. CDK6 was used instead of CDK4 given the high amino acid similarity between these two CDKs and because CDK6 and not CDK4 has been crystallized in the presence of PD-0332991. In addition, a CDK4 small-molecule inhibitor X-ray cocrystal structure is not available to date. Prediction shows that **7x** may bind to the CDK6 active site in a different orientation than PD-0332991 (Figure 2A). This change in binding may be mainly achieved by new interactions formed by the cyano (CN) group of **7x**, which is substituted for an acetyl group (COCH$_3$) in PD-0332991. The nitrogen of the cyano group is in close contact with several residues of CDK6, in particular at a hydrogen bonding distance with the side chain ε-amino group of Lys43 and main chain α-nitrogen of Ala23

## Table 4. In Vitro Cytotoxicity of 7aq and 7ar Compounds



**7aq, 7ar**

| Compd. | R | IC$_{50}$ (μM) | |
|--------|---|------|------|
| | | K562 | DU145 |
| 7aq | (acetyl group) | 0.75 | 5 |
| 7ar | (4-trifluoromethyl benzoyl group) | 0.5 | 1 |
| 7x | H | 0.05 | 0.025 |

## Table 5. Evaluation of 7x against a Panel of Human Tumor Cell Lines

| cell line | tumor type | GI$_{50}$ (μM) |
|-----------|-----------|-------|
| DU145 | prostate (AR−) | 0.05 |
| K562 | CML | 0.1 |
| BT474 | breast (ER+) | 0.25 |
| SK-BR-3 | breast (ER−) | 0.15 |
| Granta-519 | mantle cell lymphoma | 0.025 |
| Z138C | mantle cell lymphoma | 0.025 |
| N87 | gastric carcinoma | 0.9 |
| SNU-5 | gastric carcinoma | 0.1 |
| MIA-Paca-2 | pancreatic | 0.25 |
| SK-OV3 | ovarian | 0.75 |
| U87 | glioblastoma | 0.1 |
| MCF-7 | breast (ER+) | 0.15 |
| Raji | Burkitt's lymphoma (B-cell) | 0.25 |
| Jurkat | acute T cell leukemia | 0.15 |
| U266 | multiple myeloma | 0.2 |
| N417 | SCLC | 0.25 |
| Hela | cervical | 0.75 |
| A549 | NSCLC | 0.2 |
| BT-20 | breast (ER−) | 0.1 |
| SNU-398 | hepatocellular carcinoma | 0.2 |
| SNU-449 | hepatocellular carcinoma | 0.75 |
| SNU-475 | hepatocellular carcinoma | 0.3 |
| A431 | epidermoid | 0.25 |
| DLD-1 | colorectal | 0.1 |
| SW-480 | colorectal | 0.09 |
| MDA-MB-468 | breast (triple negative) | 2 |
| Colo-205 | colorectal | 0.1 |
| HCC70 | breast | 1.5 |
| HCC1428 | breast | 0.3 |
| MDA-MB-231 | breast (triple negative) | 0.25 |
| MDA-MB-157 | breast (triple negative) | 1 |
| 2008 | ovarian | 1.5 |
| 2008/1714 | resistant ovarian | 1.5 |
| MES-SA | sarcoma | 0.25 |
| MES-SA/DX5 | resistant sarcoma | 0.25 |
| HCT15 | colorectal | 0.3 |
| CAPAN-1 | pancreatic | 0.5 |
| HFL | normal fibroblast | 5.0 |

(Figure 2B). Similar interactions in this binding mode might not be energetically favorable for PD-0332991 due to the perpendicular orientation seen in the cocrystal structure of the carbonyl group (CO) plus its lower hydrogen bond acceptor potential when compared with the cyano group in **7x** (Figure 2C). Moreover, the presence of the methyl group and the negative charges contributed by the side chain carbonyl groups of Glu61 and Asp163 might not favor the stabilization of the acetyl group in a position that is similar to the one predicted for the cyano group in **7x**. The presence of a rigid and more electron withdrawing cyano group instead of an acetyl group may be the main reason for the stabilization of the molecule in this new orientation and also may explain the higher potency observed for **7x** with another closely related member of the CDK family.

Positioning of PD-0332991 in the ATP binding pocket of CDK6 is mainly given by hydrogen bond interactions between N3 and N2−H to the backbone of Val101 and C6-acetyl group to the main chain amide of Asp163. Similar to what is observed in the X-ray structure of CDK6 and PD-0332991, **7x** docking prediction shows multiple residues with distances under 4.5 Å that may be involved in van der Waals interactions that aid in the stabilization of the small molecule. It is worth emphasizing that the difference in potency and mode of action in PD-0332991 and **7x** might be due to a change in binding orientation inside the ATP binding pocket of CDK6 and further explained by gain and loss of interactions. In this regard, **7x** does not present the same hydrogen bond interactions described above for PD-0332991 but, as predicted by the docking, present new ones implied by the presence of the cyano group in **7x** and residues Ala23 and Lys43 of CDK6. This slightly deeper binding of **7x** into the ATP binding site of CDK6 when compared with the binding of PD-0332991 may explain a difference in potency in the CDK family observed for the two compounds, where **7x** may interfere more efficiently with ATP binding and make it a better inhibitor of the CDK kinase activity.

Docking calculations of **7x** with ABL, FGFR, and FMS were performed to see the interactions of the cyano group with the amino acids in the ATP binding site of these kinases (given in

Supporting Information). Results of these docking studies show a great variability of binding between kinases, and because crystal structures of these kinases in complex with compound with similar structure to **7x** are not available for reference and comparison, docking simulations become hypothetical.

**Inhibition of CDK4 Kinase Activity and RB by 7x.** To further validate the results from RBC corporation (Figure 1) and molecular modeling studies, we independently tested the inhibitory activity of **7x** in an in vitro kinase assay using recombinant CDK4 (Figure 3A). Our results showed that **7x** is a potent inhibitor of CDK4 with an IC$_{50}$ of 3.87 nM, with little inhibitory activity against CDKs 1, 2, 5, 8, and 9 (data not shown). Flavopiridol, a pan-CDK inhibitor, and PD-0332991, a highly selective CDK4/6 inhibitor that is currently in clinical trials, were used as positive controls.[40,54] These assays showed that PD-0332991 showed a similar level of CDK4 inhibition, with an IC$_{50}$ of 5.36 nM. It is now well established that the retinoblastoma family of proteins (pRb, p107, and p130) are primary targets of CDK4. RB is hypophosphorylated in quiescent cells and becomes phosphorylated on Ser[780] and Ser[795] by
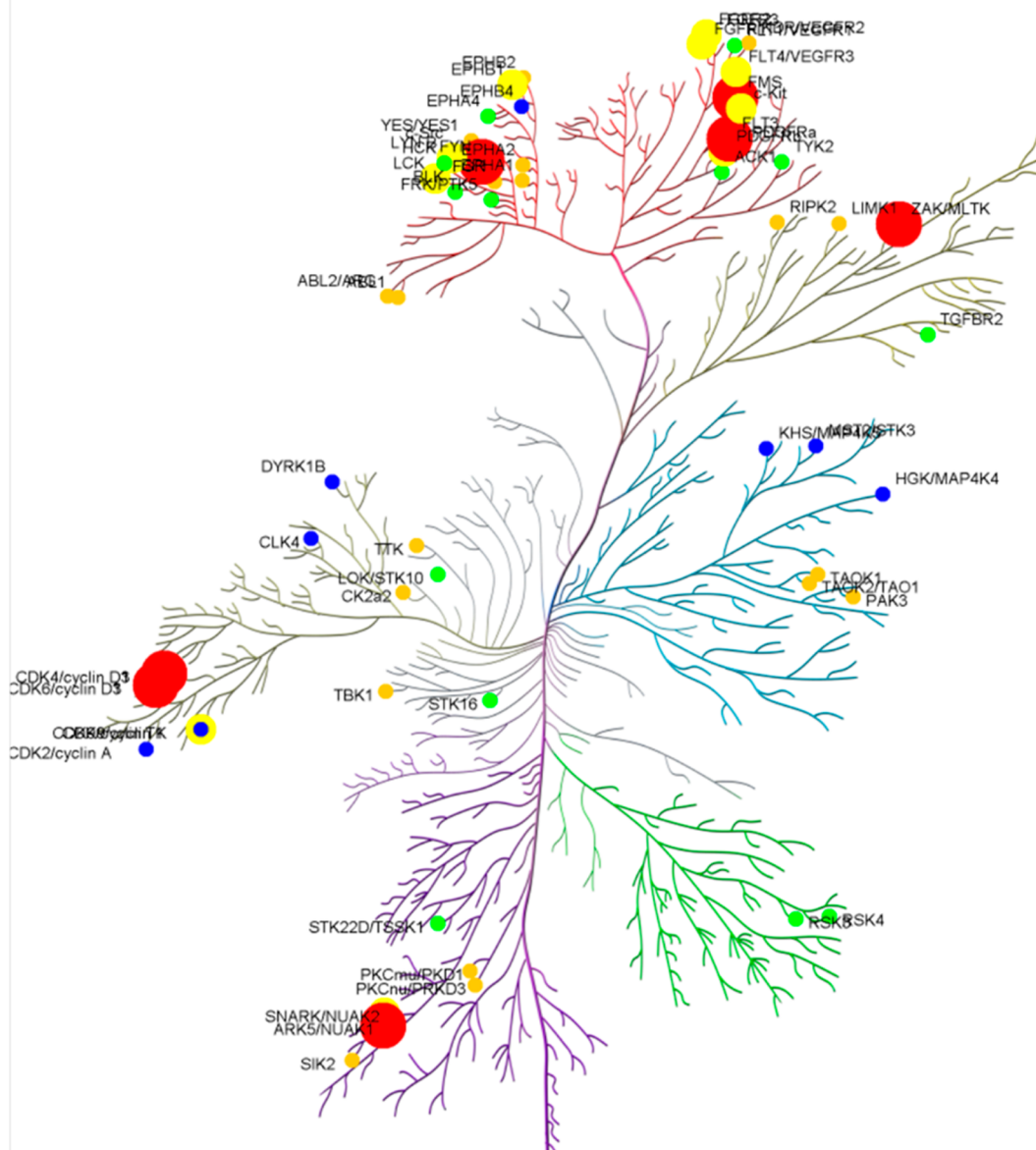
**Figure 1.** Kinome inhibition map of compound **7x**: The kinases targeted by **7x** are indicated. Red circles represent kinases targeted below 20 nM. Yellow, amber, green, and blue circles represent kinases inhibited between 20−50, 50−100, 100−150, and 150−250 nM, respectively. The human kinome map is adapted with permission from Reaction Biology Corp. (http://reactionbiology.com).

**Table 6. Kinase Inhibition Profile of 7x and PD-0332991**

| kinase | 7x IC$_{50}$ (nM) | PD-0332991 IC$_{50}$ (nM) |
|---|---|---|
| CDK4/cyclin D1 | 3.87 | 5.36 |
| CDK6/cyclin D1 | 9.82 | 3.76 |
| ARK5 | 4.95 | >5000 |
| FLT3 | 12.22 | >10000 |
| FYN | 11.09 | >10000 |
| FMS | 10.00 | >10000 |
| PDGFR$\beta$ | 26.00 | >10000 |
| FGFR1 | 26.00 | >10000 |
| ABL | 53.32 | >10000 |
| PI3K-$\delta$ | 144 | >10000 |

CDK4/CDK6 during mid to late G$_1$. The hypophosphorylated form of pRB associates with several cellular proteins and its phosphorylation results in the disassociation of RB from its binding partners.[55−57] To determine whether **7x** inhibits the activity of pRB in vivo, two human breast carcinoma cell lines, MCF-7 (Figure 3B) and MDA-MB-231 (Figure 3C), were incubated with increasing concentrations of **7x** for 24 h and the levels of phosphorylated RB (Ser[780]) determined by Western blot analysis. The results of this study (Figure 3) show that **7x** inhibits RB phosphorylation at Ser[780] as well as PD-0332991, confirming that CDK4 and CDK6 are targets of this compound (Figure 3).

**Effect of 7x on Cell Cycle Progression of Human Tumor Cells.** We next examined the effect of **7x** treatment on MCF-7 (Figure 4A) and MDA-MB-231 (Figure 4B) cell cycle kinetics. For these studies, cells were treated with **7x** or PD-0332991 for

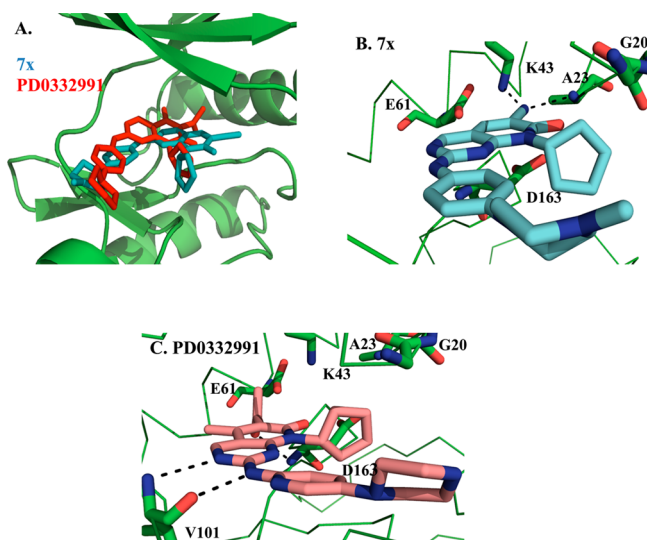**Figure 2.** Model of **7x** binding to CDK6. Small molecule **7x** binding was predicted by docking and energy minimization using the X-ray crystal structure of CDK6−Vcyclin−PD-0332991 (2EUF) as a reference. Representations of the superimposition of X-ray crystal structure (CDK6/PD-0332991) and predicted lowest energy binding (CDK6/**7x**) were prepared using PyMOL. (A) Ribbon representation of CDK6 (green) bound to PD-0332991 (red) and **7x** (cyan). Small molecules are shown as sticks. (B,C) Close up view showing proximal residues of CDK6 to **7x** (blue) and PD-0332991 (pink), respectively. Hydrogen bonds are shown as a dotted back lines.

**Figure 3.** (A) Inhibition of CDK4/cyclin D1 activity by **7x**: 10 ng of recombinant CDK4/cyclin D1 complex was incubated with the indicated concentrations of **7x**, flavopiridol (FP), or PD-0332991 for 30 min at room temperature. Kinase reactions were initiated by the addition of the substrate mixture (5 $\mu$M ATP, 10 $\mu$ci $\gamma^{32}$P-ATP, 10 mM MgCl$_2$, and 1 $\mu$g recombinant RB substrate) and incubated at 30 °C for 20 min. The reactions were terminated by the addition of 2× Laemmli sample buffer and heated at 95 °C for 3 min. Proteins were resolved by 12% SDS-PAGE and the resulting gel subjected to autoradiography. (B) **7x** inhibits RB phosphorylation at serine 780: An estrogen-dependent breast cancer cell line, MCF-7, and the triple negative (C) human breast carcinoma cell line, MDA-MB-231, were treated with increasing concentrations of **7x** or PD-0332991 (control) for 24 h. Western blot analysis was performed using antibodies directed against phosphorylated (Ser$^{780}$) and nonphosphorylated forms of the retinoblastoma protein. Both **7x** and PD-0332991 inhibit RB phosphorylation at Ser$^{780}$, a known substrate of CDK4.

24 h and subjected to flow cytometric analysis to determine the distribution of cells in various phases of the cell cycle. At time 0, the majority of cells were in the G$_1$ phase of the cell cycle, with smaller percentages of the population in the S and G$_2$ phases (data not shown). While there was no significant change in the profile of cells treated with DMSO throughout the course of the experiment, an accumulation in the G$_1$ phase was evident following treatment with **7x** and PD-0332991 (Figure 4). Prolonged treatment of cells with **7x** (greater than 48 h) resulted in the appearance of a sub-G$_1$ population, which is indicative of cells undergoing apoptosis. This population was absent in PD-0332991 treated cells.

**7x Activates Programmed Cell Death.** Because flow cytometric analysis indicated that cells treated with **7x** for longer periods of time might be undergoing apoptosis, we next determined the levels of poly-ADP ribose polymerase (PARP) cleavage, which is a marker of apoptosis. These studies show a dose-dependent accumulation of the ∼89 kDa cleaved PARP polypeptide in cells treated with 1 $\mu$M **7x** (Figure 5). PARP cleavage was not observed in cells treated with PD-0332991, suggesting that **7x** exhibits a strong pro-apoptotic activity that is not seen with PD-0332991.

**Inhibition of the PI3Kinase/AKT Pathway by 7x.** Kinase inhibition assays performed with **7x** show inhibition of two important growth factor receptors, PDGFR$\beta$ and FGFR1, with an IC$_{50}$ of 26 nM (Table 6). Because these kinases are involved in the activation of the PI3Kinase/AKT survival pathway, we next examined the ability of **7x** to inhibit PI3Kinase/AKT activity in cells treated with this compound. We accomplished this by examining the phosphorylation status of AKT (Figure 6), PI3Ks (see Supporting Information Table 3), and mTOR (data not shown) proteins, which are well established modulators of cell survival. The results presented in Figure 6 shows that **7x** (but not PD-0332991) inhibits the phosphorylation of AKT, which plays
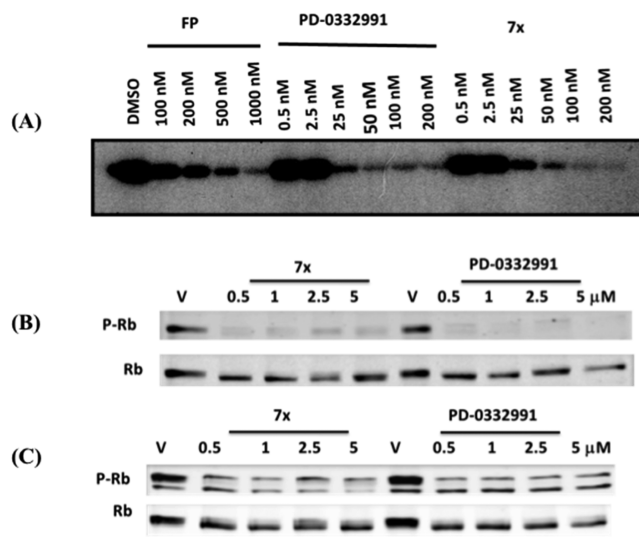
a critical role in the survival of tumor cells. This observation might explain the differential effects of **7x** and PD-0332991 on their ability to induce apoptotic death of breast tumor cells (Figure 5).

**Pharmacological Safety and in Vivo Efficacy of 7x.** We next carried out studies to determine the maximum tolerated dose of **7x** in mice. CD-1 female mice ($n$ = 3) received single doses of **7x** 100 or 200 mg/kg intraperitoneally and were monitored over a 7 day period. We observed no signs of toxicity or weight loss, with a survival rate of 100%. We next injected mice with 200 mg/kg of **7x** (ip) for 5 consecutive days and again monitored them for signs of toxicity. One hundred percent of the mice survived for more than 10 days after injection (data not shown). To determine the efficacy of **7x** in vivo using tumor xenograft models, MDA-MB-231 cells were orthotopically implanted into the mammary fat pads of 6−8 week old female nude mice. Once the tumors reached an average volume of 100 mm$^3$, either placebo or **7x** (50 mg/kg body weight) was administered on alternate days (Q2D) via IP injection. The results of this study (Figure 7A) showed that **7x** administered on this schedule led to a dose-dependent inhibition of tumor growth over a 21 day period. A decrease in tumor weight was also observed at the end-point of the study (data not shown). No overt signs of toxicity were observed in the **7x** treated groups (body weights shown in Figure 7B), indicating that the compound is well-tolerated. In vivo pharmacokinetic studies
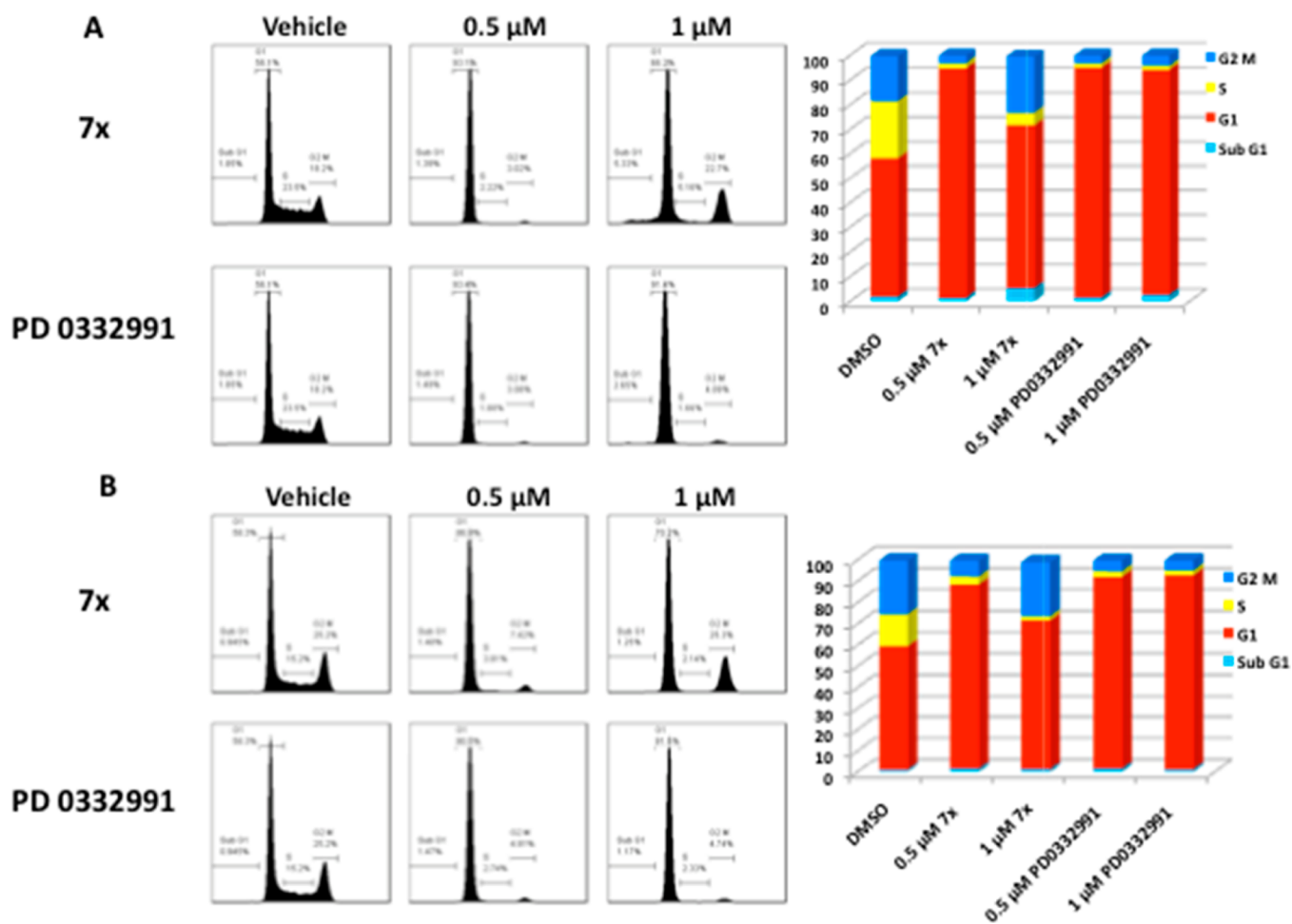
**Figure 4.** Effect of **7x** on cell cycle progression: MCF-7 (A) and MDA-MB-231 (B) human breast carcinoma cell lines were treated with increasing concentrations of **7x** or PD-0332991 (control) for 24 h. The cells were then harvested, fixed, and stained with propidium iodide prior to flow cytometric analysis. The percentage of cells at each phase of the cell cycle was calculated and represented as % cells in each phase in the bar graph.
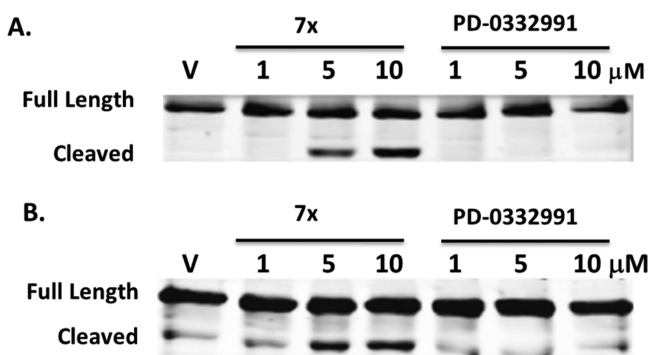


**Figure 5.** **7x** treatment induces apoptosis of breast carcinoma cell lines: MCF-7 (A) and MDA-MB-231 (B) human breast carcinoma cell lines were treated with increasing concentrations of **7x** or PD-0332991 (control) for 24 h. Total cell lysates were subjected to Western blot analysis using a PARP-specific antibody. The cleaved protein, which is expressed in **7x** treated cells, is indicative of apoptosis.

with **7x** exhibited favorable cytotoxicity, brain penetration, and better half-life.[58]

■ **CONCLUSION**

In this article, we describe the synthesis of pyrido[2,3-*d*]pyrimidine analogues that induce apoptotic death of a wide

variety of human tumor cell lines at nanomolar concentrations while exhibiting little or no in vivo toxicity. Structure−function studies described here suggest that the cytotoxic activity of pyrido[2,3-*d*]pyrimidines is dependent on the nature and position of substituents at *C*-2, *C*-6, and *N*-8 positions. Compound **7x**, with a 4-(4-methyl-piperazin-1-yl) phenylamine group at *C*-2 position, cyano group at *C*-6 position, and cyclopentyl at *N*-8 position showed optimum biological activity. The biochemical and biological studies presented here show that this compound is a potent inhibitor of CDK4 and CDK6 kinases and in this aspect is comparable to PD-0332991, a dual CDK4/6 inhibitor that is currently in clinical trials. However, unlike PD-0332991, **7x** inhibits other kinases such as ARK5, FGFR, and PDGFR$\beta$, which are known to play critical roles in proliferation and survival signaling in tumor cells. As has been stated in the Introduction, considerable evidence implicates the deregulation of the CDK4/Rb pathway in tumor cell growth, and up-regulation of this pathway is observed in greater than 90% of all human tumors. However, clinical trials with PD-0332991 suggest that reduction of tumor burden in human tumors that have increased levels of cyclin D/CDK4 activity might require inhibition of not only CDK4 but also other signaling pathways.[44−46] Because most kinase inhibitors are promiscuous in their target specificity and invariably bind to more than one kinase, we took advantage of this property to develop multitargeted kinase inhibitors that can inhibit multiple signaling
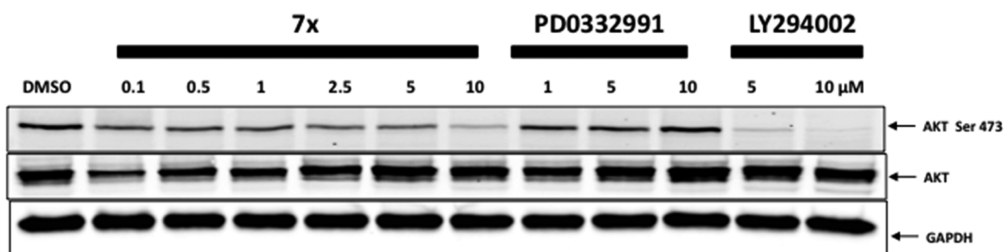
**Figure 6.** **7x** treatment inhibits AKT phosphorylation: MDA-MB-231 human breast carcinoma cells were treated with increasing concentrations of **7x** or PD-0332991 or LY294002 (2-morpholino-8-phenyl-4*H*-chromen-4-one) (PI3Kinase inhibitor) for 24 h. Total cell lysates were subjected to Western blot analysis using antibodies directed against phosphorylated (Ser[473]) and nonphosphorylated forms of AKT. GAPDH was used as a loading control.
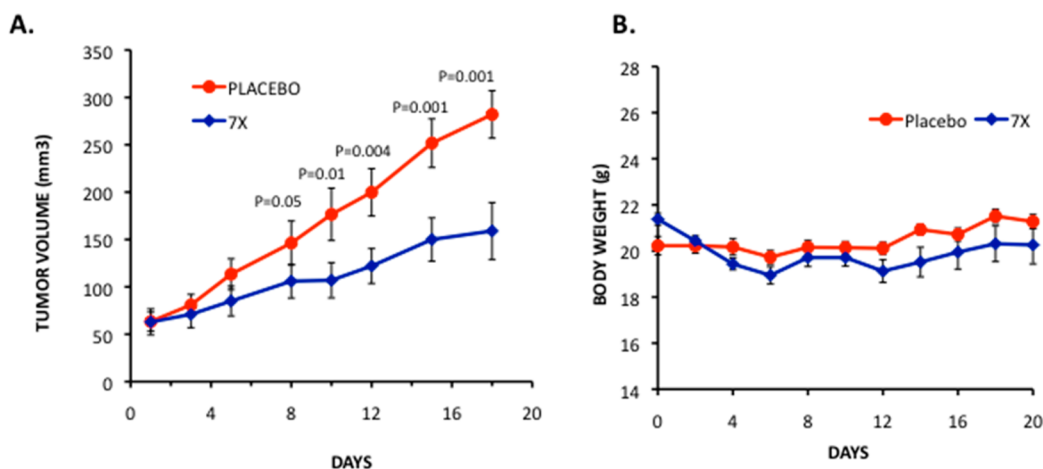


**Figure 7.** In vivo efficacy of **7x** against subcutaneous breast tumor xenografts: MDA-MB-231 cells were orthotopically implanted into the mammary fat pad of 6−8 week old female nude mice ($n = 11$ per group). Treatment was started when the average tumor volume reached 100 mm$^3$. **7x** (lactate salt dissolved in PBS) or vehicle was administered intraperitoneally every other day (Q2D). Tumor volumes (A) and body weights (B) were recorded every 2 days. All values represent mean ± SEM.

pathways that activated in a given tumor type. Our approach to kinase inhibitor design, which incorporates tumor cell growth inhibition as an integral parameter, led to the development of **7x**. This compound inhibits other pathways that are deregulated in tumor cells, such as the ARK5 and PI3K/AKT pathways, in addition to inhibition of CDK4/RB. As a result, tumor cells treated with **7x** underwent apoptosis, an effect that is not seen in PD-0332991-treated cells. The low toxicity profile and the potent tumor inhibitory activity observed in nude mouse xenograft assays highlight the potential value of this compound as a safe and targeted therapy for human cancers that overexpress cyclin D/CDK4 complexes.

## ■ EXPERIMENTAL SECTION

**Chemistry: General Methods.** All reagents and solvents were obtained from commercial suppliers and used without further purification unless otherwise stated. Solvents were dried using standard procedures, and reactions requiring anhydrous conditions were performed under N$_2$ atmosphere. Reactions were monitored by thin layer chromatography (TLC) on preloaded silica gel F254 plates (Sigma-Aldrich) with a UV indicator. Column chromatography was performed using Merck 70-230 mesh silica gel 60 Å. Yields were of purified product and were not optimized. Melting points were determined using an Electro-thermal Mel-Temp 3.0 micromelting point apparatus and are uncorrected. $^1$H NMR spectra were obtained using a Bruker AVANCE 300 and 400 MHz spectrometer. Chemical shifts are reported in parts per million ($\delta$) downfield using tetramethylsilane (SiMe$_4$) as internal standard. Spin multiplicities are given as s (singlet), d (doublet), t (triplet), dd (double doublet) bs (broad singlet), m (multiplet), and q (quartet). All LC/MS data were

gathered on an Agilent 1200 LC with Agilent 6410 triple quadrupole mass spectrometer detectors. The compound solution was infused into the electrospray ionization source operating positive and negative modes in methanol:water:TFA (50:50:0.1% v/v) at 0.4 mL/min. The sample cone (declustering) voltage was set at 100 V. The instrument was externally calibrated for the mass range $m/z$ 100−1000. The purity of the final compounds was determined by HPLC and is 95% or higher unless specified otherwise. Zorbax Exlipse XDB C18 (150 mm × 4.6 mm, 5 $\mu$m particle size) using gradient elution of acetonitrile in water, 20−90%, for 25 min at a flow rate of 1 mL/min with detection at 235 nm wavelength. For all samples 0.00154% AcONH$_4$ was added to water. The active methylene compounds **10**,[49] **13**,[50] and **16**[51] and amino compounds (**21** and **22**)[30] were prepared as per the reported procedures.

*General Procedure for the Synthesis of 4-Alkyl/cycloalkylamino-2-methylsulfanyl-pyrimidine-5-carboxylic Acid Ethyl Ester (2).* 4-Chloro-2-methylsulfanyl-pyrimidine-5-carboxylic acid ethyl ester **1** (107 mmol) was dissolved in THF to which triethylamine (322 mmol) and alkylamine (117 mmol) was added and stirred for overnight at room temperature. The precipitated salts were filtered and the solvent evaporated in vacuo. The resultant oil was dissolved in ethyl acetate and washed with sodium bicarbonate then dried over Na$_2$SO$_4$. The salts were filtered, and the solvent was evaporated in vacuum to obtain the product.

*4-Amino-2-methylsulfanyl-pyrimidine-5-carboxylic Acid Ethyl Ester (2a).* Starting from 4-chloro-2-methylsulfanyl-pyrimidine-5-carboxylic acid ethyl ester **1** and ammonium hydroxide, 90% of **2a** was obtained as solid according to the method described for the synthesis of **2**; mp 130−131 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.58 (s, 1H), 8.10 (bs, 2H), 4.30 (q, 2H), 2.45 (s, 3H), 1.25 (t, 3H). MS found (M + H)$^+$ ($m/z$), 214.10; calcd for C$_8$H$_{11}$N$_3$O$_2$S $m/z$, 213.06.

*4-Methylamino-2-methylsulfanyl-pyrimidine-5-carboxylic Acid Ethyl Ester (2b).* Starting from 4-chloro-2-methylsulfanyl-pyrimidine-

5-carboxylic acid ethyl ester **1** and methylamine (40 wt % solution in water), 80% of **2b** was obtained as solid according to the method described for the synthesis of **2**; mp 82−83 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.61 (s, 1H), 8.18 (bs, 1H), 4.33 (q, 2H), 3.09 (d, 3H), 2.55 (s, 3H), 1.37 (t, 3H). MS found (M + H)$^+$ ($m/z$), 228.10; calcd for C$_9$H$_{13}$N$_3$O$_2$S $m/z$, 227.07.

*4-Ethylamino-2-methylsulfanyl-pyrimidine-5-carboxylic Acid Ethyl Ester (2c).* Starting from 4-chloro-2-methylsulfanyl-pyrimidine-5-carboxylic acid ethyl ester **1** and ethylamine (70 wt % solution in water), 92% of **2c** was obtained as liquid according to the method described for the synthesis of **2**. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.59 (s, 1H), 8.18 (bs, 1H), 4.26 (q, 2H), 3.50 (q, 2H), 2.51 (s, 3H), 1.35 (t, 3H), 1.25 (t, 3H). MS found (M + H)$^+$ ($m/z$), 242.10; calcd for C$_{10}$H$_{15}$N$_3$O$_2$S $m/z$, 241.09.

*2-Methylsulfanyl-4-propylmino-pyrimidine-5-carboxylic Acid Ethyl Ester (2d).* Starting from 4-chloro-2-methylsulfanyl-pyrimidine-5-carboxylic acid ethyl ester **1** and propylamine, 89% of **2d** was obtained as liquid according to the method described for the synthesis of **2**. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.60 (s, 1H), 8.26 (bs, 1H), 4.25 (q, 2H), 3.47−3.54 (m, 2H), 2.52 (s, 3H), 1.65−1.61 (m, 2H), 1.35 (t, 3H), 1.00 (t, 3H). MS found (M + H)$^+$ ($m/z$), 256.10; calcd for C$_{11}$H$_{17}$N$_3$O$_2$S $m/z$, 255.10.

*4-Isopropylamino-2-methylsulfanyl-pyrimidine-5-carboxylic Acid Ethyl Ester (2e).* Starting from 4-chloro-2-methylsulfanyl-pyrimidine-5-carboxylic acid ethyl ester **1** and isopropylamine, 95% of **2e** was obtained as liquid according to the method described for the synthesis of **2**. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.58 (s, 1H), 8.15 (bs, 1H), 5.70−5.67 (m, 1H), 4.24 (q, 2H), 2.49 (s, 3H), 1.26 (t, 3H), 1.21 (d, 6H). MS found (M + H)$^+$ ($m/z$), 256.10; calcd for C$_{11}$H$_{17}$N$_3$O$_2$S $m/z$, 255.10.

*4-Butylamino-2-methylsulfanyl-pyrimidine-5-carboxylic Acid Ethyl Ester (2f).* Starting from 4-chloro-2-methylsulfanyl-pyrimidine-5-carboxylic acid ethyl ester **1** and butylamine, 95% of **2f** was obtained as liquid according to the method described for the synthesis of **2**. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.61 (s, 1H), 8.25 (bs, 1H), 4.43 (q, 2H), 3.59−3.52 (m, 2H), 2.53 (s, 3H), 1.65−1.60 (m, 2H), 1.46−1.35 (m, 5H), 0.96 (t, 3H). MS found (M + H)$^+$ ($m/z$), 270.20; calcd for C$_{12}$H$_{19}$N$_3$O$_2$S $m/z$, 269.12.

*2-Methylsulfanyl-4-pentylamino-pyrimidine-5-carboxylic Acid Ethyl Ester (2g).* Starting from 4-chloro-2-methylsulfanyl-pyrimidine-5-carboxylic acid ethyl ester **1** and amylamine, 95% of **2g** was obtained as liquid according to the method described for the synthesis of **2**. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.62 (s, 1H), 8.25 (bs, 1H), 4.35 (q, 2H), 3.58−3.51 (m, 2H), 2.53 (s, 3H), 1.67−1.62 (m, 2H), 1.40−1.34 (m, 7H), 0.93 (t, 3H). MS found (M + H)$^+$ ($m/z$), 284.20; calcd for C$_{13}$H$_{21}$N$_3$O$_2$S $m/z$, 283.14.

*4-Cyclopropylamino-2-methylsulfanyl-pyrimidine-5-carboxylic Acid Ethyl Ester (2h).* Starting from 4-chloro-2-methylsulfanyl-pyrimidine-5-carboxylic acid ethyl ester **1** and cylcopropylamine, 95% of **2h** was obtained as liquid according to the method described for the synthesis of **2**. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.59 (s, 1H), 8.48 (bs, 1H), 4.27 (q, 2H), 2.95−2.89 (m, 1H), 2.51 (s, 3H), 1.34 (t, 3H), 0.84−0.79 (m, 2H), 0.61−0.58 (m, 2H). MS found (M + H)$^+$ ($m/z$), 254.10; calcd for C$_{11}$H$_{15}$N$_3$O$_2$S $m/z$, 253.09.

*4-Cyclopentylamino-2-methylsulfanyl-pyrimidine-5-carboxylic Acid Ethyl Ester (2i).* Starting from 4-chloro-2-methylsulfanyl-pyrimidine-5-carboxylic acid ethyl ester **1** and cyclopentyl amine, 90% of **2i** was obtained as liquid according to the method described for the synthesis of **2**. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.60 (s, 1H), 8.25 (bs, 1H), 4.49−4.54 (m, 1H), 4.30 (q, 2H), 2.52 (s, 3H), 2.00−2.10 (m, 2H), 1.50−1.79 (m, 6H), 1.35 (t, 3H). MS found (M + H)$^+$ ($m/z$), 282.20; calcd for C$_{13}$H$_{19}$N$_3$O$_2$S $m/z$, 281.12.

*4-Cyclohexylamino-2-methylsulfanyl-pyrimidine-5-carboxylic Acid Ethyl Ester (2j).* Starting from 4-chloro-2-methylsulfanyl-pyrimidine-5-carboxylic acid ethyl ester **1** and cyclohexyl amine, 85% of **2j** was obtained as liquid according to the method described for the synthesis of **2**. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.60 (s, 1H), 8.22 (bs, 1H), 4.30 (q, 2H), 4.09−4.14 (m, 1H), 2.51 (s, 3H), 1.94−2.27 (m, 2H), 1.73−1.81 (m, 2H), 1.59−1.64 (m, 2H), 1.30−1.41 (m, 4H). MS found (M + H)$^+$ ($m/z$), 296.10; calcd for C$_{14}$H$_{21}$N$_3$O$_2$S $m/z$, 295.14.

*General Procedure for the Synthesis of (4-Alkyl/cycloalkylamino-2-methylsulfanyl-pyridine-5-yl)-methanol (3).* Lithium aluminum hydride (53.3 mmol) was suspended in THF under nitrogen atmosphere and cooled with dry ice. The compound **2** (35.5 mmol) was dissolved in THF and added dropwise to the cooled LiAlH$_4$ solution while keeping the reaction temperature bellow −10 °C. The reaction was brought to room temperature and stirred for 1 h. The reaction was quenched by the addition of water (5 mL), 15% NaOH (10 mL), and then water (15 mL) again. The white solid that precipitated was filtered and the filtrate evaporated in vacuo to afford the product.

*(4-Amino-2-methylsulfanyl-pyridine-5-yl)-methanol (3a).* Starting from **2a**, 85% of **3a** was obtained according to method described for the synthesis of **3**; mp 157−159 °C. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.85 (s, 1H), 6.70 (bs, 2H), 5.30 (bs, 1H), 4.25 (s, 2H), 2.56 (s, 3H). MS found (M + H)$^+$ ($m/z$), 172.00; calcd for C$_6$H$_9$N$_3$OS $m/z$, 171.05.

*(4-Methylamino-2-methylsulfanyl-pyridine-5-yl)-methanol (3b).* Starting from **2b**, 80% of **3b** was obtained according to method described for the synthesis of **3**; mp 145−146 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.64 (s, 1H), 5.93 (bs, 1H), 4.50 (s, 2H), 3.05 (d, 3H), 2.53 (s, 3H). MS found (M + H)$^+$ ($m/z$), 186.00; calcd for C$_7$H$_{11}$N$_3$OS $m/z$, 185.06.

*(4-Ethylamino-2-methylsulfanyl-pyridine-5-yl)-methanol (3c).* Starting from **2c**, 84% of **3c** was obtained according to method described for the synthesis of **3**; mp 155−157 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.63 (s, 1H), 6.55 (bs, 1H), 4.10 (s, 2H), 3.33 (q, 2H), 2.52 (s, 3H), 1.17 (t, 3H). MS found (M + H)$^+$ ($m/z$), 200.10; calcd for C$_8$H$_{13}$N$_3$OS $m/z$, 199.08.

*(2-Methylsulfanyl-4-propylamino-pyridine-5-yl)-methanol (3d).* Starting from **2d**, 83% of **3d** was obtained according to method described for the synthesis of **3**; mp 120−121 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.58 (s, 1H), 6.00 (bs, 1H), 4.48 (s, 2H), 3.42−3.37 (m, 2H), 2.49 (s, 3H), 1.55−1.68 (m, 2H), 0.97 (t, 3H). MS found (M + H)$^+$ ($m/z$), 214.10; calcd for C$_9$H$_{15}$N$_3$OS $m/z$, 213.09.

*(4-Isopropylamino-2-methylsulfanyl-pyridine-5-yl)-methanol (3e).* Starting from **2e**, 85% of **3e** was obtained according to method described for the synthesis of **3**; mp 127−129 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.60 (s, 1H), 5.58 (bs, 1H), 4.52−4.41 (m, 3H), 2.53 (s, 3H). 1.31 (d, 6H). MS found (M + H)$^+$ ($m/z$), 214.10; calcd for C$_9$H$_{15}$N$_3$OS $m/z$, 213.09.

*(4-Butylamino-2-methylsulfanyl-pyridine-5-yl)-methanol (3f).* Starting from **2f**, 83% of **3f** was obtained according to method described for the synthesis of **3**; mp 105−107 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.65 (s, 1H), 5.92 (bs, 1H), 4.50 (s, 2H), 3.54−3.48 (m, 2H), 2.52 (s, 3H), 1.64−1.57 (m, 2H), 1.45−1.37 (m, 2H), 0.96 (t, 3H). MS found (M + H)$^+$ ($m/z$), 228.20; calcd for C$_{10}$H$_{17}$N$_3$OS $m/z$, 227.11.

*(2-Methylsulfanyl-4-pentylamino-pyridine-5-yl)-methanol (3g).* Starting from **2g**, 86% of **3g** was obtained according to method described for the synthesis of **3**; mp 110−112 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.64 (s, 1H), 5.93 (bs, 1H), 4.50 (s, 2H), 3.53−3.46 (m, 2H), 2.51 (s, 3H), 1.66−1.59 (m, 2H), 1.40−1.33 (m, 4H), 0.95−0.92 (m, 3H). MS found (M + H)$^+$ ($m/z$), 242.20; calcd for C$_{11}$H$_{19}$N$_3$OS $m/z$, 241.12.

*(4-Cyclopropylmino-2-methylsulfanyl-pyridine-5-yl)-methanol (3h).* Starting from **2h**, 82% of **3h** was obtained according to method described for the synthesis of **3**; mp 135−137 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.69 (s, 1H), 6.08 (bs, 1H), 4.48 (s, 2H), 2.92−2.86 (m, 1H), 2.54 (s, 3H), 0.87−0.80 (m, 2H), 0.59−0.56 (m, 2H), MS found (M + H)$^+$ ($m/z$), 212.10; calcd for C$_9$H$_{13}$N$_3$OS $m/z$, 211.08.

*(4-Cyclopentylamino-2-methylsulfanyl-pyridine-5-yl)-methanol (3i).* Starting from **2i**, 84% of **3i** was obtained according to method described for the synthesis of **3**; mp 150−151 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.65 (s, 1H), 5.80 (bs, 1H), 4.52 (s, 2H), 4.45−4.50 (m, 1H), 2.50 (s, 3H), 2.00−2.15 (m, 2H), 1.62−1.78 (m, 4H), 1.40−1.49 (m, 2H). MS found (M + H)$^+$ ($m/z$), 240.10; calcd for C$_{11}$H$_{17}$N$_3$OS $m/z$, 239.11.

*(4-Cyclohexylamino-2-methylsulfanyl-pyridine-5-yl)-methanol (3j).* Starting from **2j**, 84% of **3j** was obtained according to method described for the synthesis of **3**; mp 175−177 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.59 (s, 1H), 5.80 (bs, 1H), 4.45 (s, 2H), 3.95−4.08 (m, 1H), 2.51 (s, 3H), 1.99−2.08 (m, 2H), 1.61−1.78 (m, 4H), 1.24−1.43 (m, 4H). MS found (M + H)$^+$ ($m/z$), 254.10; calcd for C$_{12}$H$_{19}$N$_3$OS $m/z$, 253.12.

*General Procedure for the Synthesis of 4-Alkyl/cycloalkylamino-2-methylsulfanyl-pyrimidine-5-carbaldehyde (4).* The compound **3** (20.8 mmol) was dissolved in chloroform to which manganese dioxide ($MnO_2$) (119 mmol) was added and stirred for overnight, and an additional portion of $MnO_2$ (31.3 mmol) was added and stirred for 12 h. The solids were removed by filtration through a Celite pad and washed with chloroform. The chloroform was evaporated in vacuum to get the product.

*4-Amino-2-methylsulfanyl-pyrimidine-5-carbaldehyde (4a).* Starting from **3a**, 72% of **4a** was obtained according to the procedure described for the synthesis of **4**; mp 186−188 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.80 (s, 1H), 8.45 (s, 1H), 8.20 (bs, 1H), 5.74 (bs, 1H), 2.55 (s, 3H). MS found (M + H)$^+$ (*m/z*), 170.00; calcd for C$_6$H$_7$N$_3$OS *m/z*, 169.03.

*4-Methylamino-2-methylsulfanyl-pyrimidine-5-carbaldehyde (4b).* Starting from **3b**, 75% of **4b** was obtained according to the procedure described for the synthesis of **4**; mp 98−99 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.71 (s, 1H), 8.59 (bs, 1H), 8.30 (s, 1H), 3.14 (d, 3H), 2.58 (s, 3H). MS found (M + H)$^+$ (*m/z*), 184.10; calcd for C$_7$H$_9$N$_3$OS *m/z*, 183.05.

*4-Ethylamino-2-methylsulfanyl-pyrimidine-5-carbaldehyde (4c).* Starting from **3c**, 72% of **4c** was obtained according to the procedure described for the synthesis of **4**; mp 60−61 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.72 (s, 1H), 8.64 (bs, 1H), 8.29 (s, 1H), 3.56 (q, 2H), 2.50 (s, 3H), 1.18 (t, 3H). MS found (M + H)$^+$ (*m/z*), 198.10; calcd for C$_8$H$_{11}$N$_3$OS *m/z*, 197.06.

*2-Methylsulfanyl-4-propylmino-pyrimidine-5-carbaldehyde (4d).* Starting from **3d**, 70% of **4d** was obtained according to the procedure described for the synthesis of **4**; mp 50−51 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.69 (s, 1H), 8.63 (bs, 1H), 8.28 (s, 1H), 3.53−3.57 (m, 2H), 2.52 (s, 3H), 1.61−1.73 (m, 2H), 0.86 (t, 3H). MS found (M + H)$^+$ (*m/z*), 212.10; calcd for C$_9$H$_{13}$N$_3$OS *m/z*, 211.08.

*4-Isopropylamino-2-methylsulfanyl-pyrimidine-5-carbaldehyde (4e).* Starting from **3e**, 90% of **4e** was obtained as low melting solid according to the procedure described for the synthesis of **4**; mp 54−55 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.69 (s, 1H), 8.48 (bs, 1H), 8.29 (s, 1H), 4.51−4.40 (m, 1H), 2.55 (s, 3H), 1.30 (d, 6H). MS found (M + H)$^+$ (*m/z*), 212.00; calcd for C$_9$H$_{13}$N$_3$OS *m/z*, 211.08.

*4-Butylamino-2-methylsulfanyl-pyrimidine-5-carbaldehyde (4f).* Starting from **3f**, 90% of **4f** was obtained as thick liquid according to the procedure described for the synthesis of **4**. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.70 (s, 1H), 8.63 (bs, 1H), 8.29 (s, 1H), 3.63−3.56 (m, 2H), 2.53 (s, 3H), 1.70−1.60 (m, 2H), 1.49−1.39 (m, 2H), 0.97 (t, 3H). MS found (M + H)$^+$ (*m/z*), 226.10; calcd for C$_{10}$H$_{15}$N$_3$OS *m/z*, 225.09.

*2-Methylsulfanyl-4-pentylmino-pyrimidine-5-carbaldehyde (4g).* Starting from **3g**, 87% of **4g** was obtained as thick liquid according to the procedure described for the synthesis of **4**. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.70 (s, 1H), 8.63 (bs, 1H), 8.29 (s, 1H), 3.61−3.55 (m, 2H), 2.55 (s, 3H), 1.70−1.74 (m, 2H), 1.40−1.34 (m, 4H), 0.95−0.91 (m, 3H). MS found (M + H)$^+$ (*m/z*), 240.10; calcd for C$_{11}$H$_{17}$N$_3$OS *m/z*, 239.11.

*4-Cyclopropylamino-2-methylsulfanyl-pyrimidine-5-carbaldehyde (4h).* Starting from **3h**, 80% of **4h** was obtained as low melting solid according to the procedure described for the synthesis of **4**; mp 69−70 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.61 (s, 1H), 8. 49 (bs, 1H), 8.23 (s, 1H), 2.98−2.92 (m, 1H), 2.50 (s, 3H), 0.85−0.78 (m, 2H), 0.60−0.57 (m, 2H). MS found (M + H)$^+$ (*m/z*), 210.10; calcd for C$_9$H$_{11}$N$_3$OS *m/z*, 209.06.

*4-Cyclopentylamino-2-methylsulfanyl-pyrimidine-5-carbalde-hyde (4i).* Starting from **3i**, 87% of **4i** was obtained as solid according to the procedure described for the synthesis of **4**; mp 41−42 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.65 (s, 1H), 8.60 (bs, 1H), 8.25 (s, 1H), 4.49−4.54 (m, 1H), 2.52 (s, 3H), 2.01−2.12 (m, 2H), 1.50−1.82 (m, 6H). MS found (M + H)$^+$ (*m/z*), 238.10; calcd for C$_{11}$H$_{15}$N$_3$OS *m/z*, 237.09.

*4-Cyclohexylamino-2-methylsulfanyl-pyrimidine-5-carbaldehyde (4j).* Starting from **3j**, 90% of **4j** was obtained as thick liquid according to the method described for the synthesis of **4**. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.62 (s, 1H), 8.55 (bs, 1H), 8.15 (s, 1H), 4.01−4.09 (m, 1H), 2.51 (s, 3H), 1.97−2.05 (m, 2H), 1.60−1.80 (m, 4H), 1.22−1.44 (m, 4H). MS found (M + H)$^+$ (*m/z*), 252.10; calcd for C$_{12}$H$_{17}$N$_3$OS *m/z*, 251.11.

*General Procedure for 8-Alkyl/cycloalkyl-2-methylsulfanyl-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidine (5).* A mixture of 4-alkylamino-2-methylsulfanyl-pyrimidine-5-carbaldehyde **4** (4.2 mmol), 1.2 equiv of active methylene compound, and a catalytic amount of benzylamine was taken in to acetic acid and refluxed for about 6 h. After completion of the reaction (checked with TLC), the reaction mixture was cooled to room temperature and the precipitated product was filtered. In some cases, the reaction mixture was diluted with hexane to get solid out. The solid was washed with saturated NaHCO$_3$ and water and dried over vacuo. The crude product was recrystallized in 2-propanol to get pure product (**5**).

*2-Methylsulfanyl-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidine-6-carbonitrile (5a).* Starting from **4a** and cyanoacetic acid, 65% of **5a** was obtained according to the method described for the synthesis of **5**; mp 328−330 °C. $^1$H NMR (300 MHz, DMSO-*d$_6$*) $\delta$ 13.12 (bs, 1H), 8.94 (s, 1H), 8.71 (s, 1H), 2.56 (s, 3H). MS found (M + H)$^+$ (*m/z*), 219.10; calcd for C$_9$H$_6$N$_4$OS *m/z*, 218.03.

*8-Methyl-2-methylsulfanyl-7-oxo-7,8-dihydropyrido[2,3-d]-pyrimidine-6-carbonitrile (5b).* Starting from **4b** and cyanoacetic acid, 67% of **5b** was obtained according to the method described for the synthesis of **5**; mp 292−294 °C. $^1$H NMR (300 MHz, DMSO-*d$_6$*) $\delta$ 8.95 (s, 1H), 8.79 (s, 1H), 3.61 (s, 3H), 2.51 (s, 3H). MS found (M + H)$^+$ (*m/z*), 233.10; calcd for C$_{10}$H$_8$N$_4$OS *m/z*, 232.04.

*8-Ethyl-2-methylsulfanyl-7-oxo-7,8-dihydropyrido[2,3-d]-pyrimidine-6-carbonitrile (5c).* Starting from **4c** and cyanoacetic acid, 70% of **5c** was obtained according to the method described for the synthesis of **5**; mp 244−245 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.71 (s, 1H), 8.15 (s, 1H), 4.51 (q, 2H), 2.61 (s, 3H), 1.37 (t, 3H). MS found (M + H)$^+$ (*m/z*), 247.10; calcd for C$_{11}$H$_{10}$N$_4$OS *m/z*, 246.06.

*2-Methylsulfanyl-7-oxo-8-propyl-7,8-dihydropyrido[2,3-d]-pyrimidine-6-carbonitrile (5d).* Starting from **4d** and cyanoacetic acid, 70% of **5d** was obtained according to the method described for the synthesis of **5**; mp 230−231 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.70 (s, 1H), 8.14 (s, 1H), 4.44−4.39 (m, 2H), 2.60 (s, 3H), 1.83−1.76 (m, 2H), 1.02 (t, 3H). MS found (M + H)$^+$ (*m/z*), 261.10; calcd for C$_{12}$H$_{12}$N$_4$OS *m/z*, 260.07.

*8-Isopropyl-2-methylsulfanyl-7-oxo-7,8-dihydropyrido[2,3-d]-pyrimidine-6-carbonitrile (5e).* Starting from **4e** and cyanoacetic acid, 70% of **5e** was obtained according to the method described for the synthesis of **5**; mp 200−202 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.68 (s, 1H), 8.10 (s, 1H), 5.89−5.80 (m, 1H), 2.67 (s, 3H), 1.65 (d, 6H). MS found (M + H)$^+$ (*m/z*), 261.10; calcd for C$_{12}$H$_{12}$N$_4$OS *m/z*, 260.07.

*8-Butyl-2-methylsulfanyl-7-oxo-7,8-dihydropyrido[2,3-d]-pyrimidine-6-carbonitrile (5f).* Starting from **4f** and cyanoacetic acid, 70% of **5f** was obtained according to the method described for the synthesis of **5**; mp 220−222 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.70 (s, 1H), 8.14 (s, 1H), 4.45 (t, 2H), 2.65 (s, 3H), 1.77−1.71 (m, 2H), 1.49−1.41 (m, 2H), 1.00 (t, 3H). MS found (M + H)$^+$ (*m/z*), 275.10; calcd for C$_{13}$H$_{14}$N$_4$OS *m/z*, 274.09.

*2-Methylsulfanyl-7-oxo-8-pentyl-7,8-dihydropyrido[2,3-d]-pyrimidine-6-carbonitrile (5g).* Starting from **4g** and cyanoacetic acid, 70% of **5g** was obtained according to the method described for the synthesis of **5**; mp 160−161 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.71 (s, 1H), 8.15 (s, 1H), 4.43 (t, 2H), 2.66 (s, 3H), 1.78−1.73 (m, 2H), 1.42−1.38 (m, 4H), 0.93 (t, 3H). MS found (M + H)$^+$ (*m/z*), 289.10; calcd for C$_{14}$H$_{16}$N$_4$OS *m/z*, 288.10.

*8-Cyclopropyl-2-methylsulfanyl-7-oxo-7,8-dihydropyrido[2,3-d]-pyrimidine-6-carbonitrile (5h).* Starting from **4h** and cyanoacetic acid, 71% of **5h** was obtained according to the method described for the synthesis of **5**; mp 158−159 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.70 (s, 1H), 8.14 (s, 1H), 2.99−2.97 (m, 1H), 2.65 (s, 3H), 1.27 (bs, 2H), 1.02 (bs, 2H). MS found (M + H)$^+$ (*m/z*), 259.10; calcd for C$_{12}$H$_{10}$N$_4$OS *m/z*, 258.06.

*8-Cyclopentyl-2-methylsulfanyl-7-oxo-7,8-dihydropyrido[2,3-d]-pyrimidine-6-carbonitrile (5i).* Starting from **4i** and cyanoacetic acid, 71% of **5i** was obtained according to the method described for the synthesis of **5**; mp 209−210 °C. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.68 (s, 1H), 8.10 (s, 1H), 5.89−5.98 (m, 1H), 2.64 (s, 3H), 2.23−2.35 (m, 2H), 2.09−2.16 (m, 2H), 1.83−1.96 (m, 2H), 1.63−1.76 (m, 2H). MS found (M + H)$^+$ (*m/z*), 287.10; calcd for C$_{14}$H$_{14}$N$_4$OS *m/z*, 286.09.

*8-Cyclohexyl-2-methylsulfanyl-7-oxo-7,8-dihydropyrido[2,3-d]-pyrimidine-6-carbonitrile (5j).* Starting from **4j** and cyanoacetic acid,

71% of **5j** was obtained according to the method described for the synthesis of **5**; mp 239−240 °C. $^1$H NMR (300 MHz, CDCl$_3$) δ 8.65 (s, 1H), 8.08 (s, 1H), 5.42 (bs, 1H), 2.62 (s, 3H), 1.90−1.95 (m, 2H), 1.67−1.76 (m, 6H), 1.34−1.45 (m, 2H). MS found (M + H)$^+$ (*m/z*), 301.10; calcd for C$_{15}$H$_{16}$N$_4$OS *m/z*, 300.10.

*8-Cyclopentyl-2-methylsulfanyl-6-nitro-8H-pyrido[2,3-d]-pyrimidin-7-one (5k).* Starting from **4i** and nitro-acetic acid ethyl ester, 70% of **5k** was obtained according to the method described for the synthesis of **5**; mp 182−84 °C. $^1$H NMR (300 MHz, CDCl$_3$) δ 8.79 (s, 1H), 8.42 (s, 1H), 6.05−6.00 (m, 1H), 2.67 (m, 3H), 2.34−2.30 (m, 2H), 2.15−2.10 (m, 2H), 1.99−1.91 (m, 2H), 1.78−172 (m, 2H). MS found (M + H)$^+$ (*m/z*), 307.10; calcd for C$_{13}$H$_{14}$N$_4$O$_3$S *m/z*, 306.08.

*8-Cyclopentyl-6-methanesulfonyl-2-methylsulfanyl-8H-pyrido-[2,3-d]pyrimidin-7-one (5l).* Starting from **4i** and methyl sulfonyl acetic acid, 73% of **5l** was obtained according to the method described for the synthesis of **5**; mp 190−191 °C. $^1$H NMR (300 MHz, CDCl$_3$) δ 8.76 (s, 1H), 8.49 (s, 1H), 5.88−6.00 (m, 1H), 3.34 (s, 3H), 2.65 (s, 3H), 2.28−2.41 (m, 2H), 2.09−2.16 (m, 2H), 1.84−1.99 (m, 2H), 1.67−1.73 (m, 2H). MS found (M + H)$^+$ (*m/z*), 340.20; calcd for C$_{14}$H$_{17}$N$_3$O$_3$S$_2$ *m/z*, 339.07.

*6-Benzenesulfonyl-8-cyclopentyl-2-methylsulfanyl-8H-pyrido-[2,3-d]pyrimidin-7-one (5m).* Starting from **4i** and benzene sulfonyl acetic acid, 70% of **5m** was obtained according to the method described for the synthesis of **5**; mp 184−185 °C. $^1$H NMR (300 MHz, CDCl$_3$) δ 8.87 (s, 1H), 8.70 (s, 1H), 7.54−7.80 (m, 5H), 5.69−5.72 (m, 1H), 2.66 (s, 3H), 2.20−2.33 (m, 2H), 1.99−2.11 (m, 2H), 1.78−1.88 (m, 2H), 1.61−1.69 (m, 2H). MS found (M + H)$^+$ (*m/z*), 402.10; calcd for C$_{19}$H$_{19}$N$_3$O$_3$S$_2$ *m/z*, 401.09.

*6-(4-Chloro-benzenesulfonyl)-8-cyclopentyl-2-methylsulfanyl-8H-pyrido[2,3-d]pyrimidin-7-one (5n).* Starting from **4i** and 4-chlorobenzene sulfonyl acetic acid **13**, 70% of **5n** was obtained according to the method described for the synthesis of **5**; mp 222−223 °C. $^1$H NMR (300 MHz, CDCl$_3$) δ 8.78 (s, 1H), 8.65 (s, 1H), 8.05−8.09 (m, 2H), 7.49−7.54 (m, 2H), 5.75−5.81 (m, 1H), 2.63 (s, 3H), 2.21−2.28 (m, 2H), 1.95−2.07 (m, 2H), 1.77−1.83 (m, 2H), 1.64−1.69 (m, 2H). MS found (M + H)$^+$ (*m/z*), 436.10; calcd for C$_{19}$H$_{18}$ClN$_3$O$_3$S$_2$ *m/z*, 435.05.

*6-(4-Chloro-phenylmethanesulfonyl)-8-cyclopentyl-2-methylsulfanyl-8H-pyrido[2,3-d] pyrimidin-7-one (5o).* Starting from **4i** and 4-chloro-phenylmethane sulfonyl acetic acid **10a**, 67% of **5o** was obtained according to the method described for the synthesis of **5**; mp 250−252 °C. $^1$H NMR (300 MHz, CDCl$_3$) δ 8.68 (s, 1H), 8.57 (s, 1H), 7.42 (d, 2H), 7.25 (d, 2H), 5.84−5.93 (m, 1H), 4.65 (s, 2H), 2.59 (s, 3H), 2.21−2.29 (m, 2H), 1.99−2.09 (m, 2H), 1.82−1.89 (m, 2H), 1.64−1.72 (m, 2H). MS found (M + H)$^+$ (*m/z*), 450.10; calcd for C$_{20}$H$_{20}$ClN$_3$O$_3$S$_2$ *m/z*, 449.06.

*8-Cyclopentyl-6-(3-hydroxy-4-methoxy-phenylmethanesulfonyl)-2-methylsulfanyl-8H-pyrido[2,3-d]pyrimidin-7-one (5p).* Starting from **4i** and 4-hydroxy-3-methoxy-phenylmethane sulfonyl acetic acid **10c**, 65% of **5p** was obtained according to the method described for the synthesis of **5**; mp 180−181 °C. $^1$H NMR (300 MHz, CDCl$_3$) δ 8.48 (s, 1H), 8.12 (s, 1H), 6.52−6.72 (m, 3H), 5.73−5.90 (m, 1H), 4.55 (s, 2H), 3.69 (s, 3H), 2.49 (s, 3H), 2.12−2.28 (m, 2H), 1.89−2.02 (m, 2H), 1.68−1.81 (m, 2H), 1.48−1.61 (m, 2H). MS found (M + H)$^+$ (*m/z*), 462.10; calcd for C$_{21}$H$_{23}$N$_3$O$_5$S$_2$ *m/z*, 461.11.

*8-Cyclopentyl-6-(4-methoxy-3-nitro-phenylmethanesulfonyl)-2-methylsulfanyl-8H-pyrido [2,3-d]pyrimidin-7-one (5q).* Starting from **4i** and 4-methoxy-3-nitro-phenylmethane sulfonyl acetic acid **10b**, 62% of **5q** was obtained according to the method described for the synthesis of **5**; mp 200−201 °C. $^1$H NMR (300 MHz, CDCl$_3$) δ 8.68 (s, 1H), 8.27 (s, 1H), 7.85 (s, 1H), 7.80−7.82 (m, 1H), 7.58−7.63 (m, 1H), 5.90−6.09 (m, 1H), 4.82 (s, 2H), 3.90 (s, 3H), 2.67 (s, 3H), 2.32−2.43 (m, 2H), 2.09−2.20 (m, 2H), 1.88−2.00 (m, 2H), 1.69−1.77 (m, 2H). MS found (M + H)$^+$ (*m/z*), 491.10; calcd for C$_{21}$H$_{22}$N$_4$O$_6$S$_2$ *m/z*, 490.10.

*8-Cyclopentyl-2-methylsulfanyl-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carboxylic Acid (4-Chloro-phenyl)-amide (5r).* Starting from **4i** and N-(4-chloro-phenyl)-malonamic acid **16a**, 65% of **5r** was obtained according to the method described for the synthesis of **5**; mp 260−262 °C. $^1$H NMR (300 MHz, CDCl$_3$) δ 11.71 (bs, 1H), 8.86 (s, 1H), 8.82 (s, 1H), 7.68−7.78 (m, 2H), 7.33−7.39 (m, 2H), 6.04−6.10 (m, 1H), 2.65 (s, 3H), 2.30−2.42 (m, 2H), 2.10−2.20 (m, 2H), 1.89−

2.00 (m, 2H), 1.79−1.81 (m, 2H). MS found (M + H)$^+$ (*m/z*), 415.10; calcd for C$_{20}$H$_{19}$ClN$_4$O$_2$S *m/z*, 414.09.

*8-Cyclopentyl-2-methylsulfanyl-7,8-dihydro-pyrido[2,3-d]-pyrimidine-6-carboxylic Acid (4-Fluoro-phenyl)-amide (5s).* Starting from **4i** and N-(4-fluoro-phenyl)-malonamic acid **16b**, 65% of **5s** was obtained according to the method described for the synthesis of **5**; mp 243−242 °C. $^1$H NMR (300 MHz, CDCl$_3$) δ 11.65 (bs, 1H), 8.87 (s, 1H), 8.83 (s, 1H), 7.69−7.76 (m, 2H), 6.98−7.10 (m, 2H), 6.01−6.13 (m, 1H), 2.66 (s, 3H), 2.31−2.42 (m, 2H), 2.09−2.20 (m, 2H), 1.89−2.00 (m, 2H), 1.72−1.81 (m, 2H). MS found (M + H)$^+$ (*m/z*), 399.10; calcd for C$_{20}$H$_{19}$FN$_4$O$_2$S *m/z*, 398.12.

*General Procedure for the Synthesis of Compounds (5b−5j) Form 2-Methylsulfanyl-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidine-6-carbonitrile (5a), (Scheme 2).* The compound **5a** (5 mmol) was taken into DMF and stirred at 50 °C for 10 min until obtaining the clear solution, and then NaH (5 mmol) was added and stirred about 5 min. The reaction mixture was brought to room temperature and alkyl iodides (6.5 mmol) were added. After 1 h stirring, the reaction was quenched with water and filtered the obtained product. The crude product was purified with flash column chromatography using 10−30% ethyl acetate in hexanes. All physical and spectral data matched with the compounds, which were prepared according to Scheme 1.

*General Procedure for the Synthesis of 8-Alkyl-2-methylsulfinyl-6-substituted-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidines (6).* A solution of 8-alkyl-2-methylsulfinyl-7-oxo-7,8-dihydropyrido[2,3-d]-pyrimidine **5** (3.5 mmol) and 3-chloro-benzenecarboperoxoic acid (*m*-CPBA) (4.4 mmol) in CH$_2$Cl$_2$ was stirred at room temperature for about 4 h. After completion of the reaction, the reaction mixture was washed with saturated NaHCO$_3$, and the organic layer was dried over Na$_2$SO$_4$ and concentrated to produce the corresponding methylsuloxide **6** and was purified with flash chromatography.

*2-Methylsulfinyl-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidine-6-carbonitrile (6a).* Starting from **5a** and 3-chloro-benzenecarboperoxoic acid, 90% of **6a** was obtained according to the method described for the synthesis of **6**. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 13.10 (bs, 1H), 8.93 (s, 1H), 8.72 (s, 1H), 2.92 (s, 3H). MS found (M + H)$^+$ (*m/z*), 235.10; calcd for C$_9$H$_6$N$_4$O$_2$S *m/z*, 234.02.

*2-Methylsulfinyl-8-methyl-7-oxo-7,8-dihydropyrido[2,3-d]-pyrimidine-6-carbonitrile (6b).* Starting from **5b** and 3-chloro-benzenecarboperoxoic acid, 92% of **6b** was obtained according to the method described for the synthesis of **6**. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 8.92 (s, 1H), 8.88 (s, 1H), 3.62 (s, 3H), 2.91 (s, 3H). MS found (M + H)$^+$ (*m/z*), 249.10; calcd for C$_{10}$H$_8$N$_4$O$_2$S *m/z*, 248.04.

*8-Ethyl-2-methylsulfinyl-7-oxo-7,8-dihydropyrido[2,3-d]-pyrimidine-6-carbonitrile (6c).* Starting from **5c** and 3-chloro-benzenecarboperoxoic acid, 90% of **6c** was obtained according to the method described for the synthesis of **6**. $^1$H NMR (300 MHz, CDCl$_3$) δ 8.73 (s, 1H), 8.17 (s, 1H), 4.50 (q, 2H), 2.92 (s, 3H), 1.37 (t, 3H). MS found (M + H)$^+$ (*m/z*), 263.10; calcd for C$_{11}$H$_{10}$N$_4$O$_2$S *m/z*, 262.05.

*2-Methylsulfinyl-7-oxo-8-propyl-7,8-dihydropyrido[2,3-d]-pyrimidine-6-carbonitrile (6d).* Starting from **5d** and 3-chloro-benzenecarboperoxoic acid, 92% of **6d** was obtained according to the method described for the synthesis of **6**. $^1$H NMR (300 MHz, CDCl$_3$) δ 8.72 (s, 1H), 8.13 (s, 1H), 4.42−4.38 (m, 2H), 2.94 (s, 3H), 1.84−1.76 (m, 2H), 1.02 (t, 3H). MS found (M + H)$^+$ (*m/z*), 277.10; calcd for C$_{12}$H$_{12}$N$_4$O$_2$S *m/z*, 276.07.

*8-Isopropyl-2-methylsulfinyl-7-oxo-7,8-dihydropyrido[2,3-d]-pyrimidine-6-carbonitrile (6e).* Starting from **5e** and 3-chloro-benzenecarboperoxoic acid, 89% of **6e** was obtained according to the method described for the synthesis of **6**. $^1$H NMR (300 MHz, CDCl$_3$) δ 8.67 (s, 1H), 8.12 (s, 1H), 5.87−5.79 (m, 1H), 2.95 (s, 3H), 1.63 (d, 6H). MS found (M + H)$^+$ (*m/z*), 277.10; calcd for C$_{12}$H$_{12}$N$_4$O$_2$S *m/z*, 276.07.

*8-Butyl-2-methylsulfinyl-7-oxo-7,8-dihydropyrido[2,3-d]-pyrimidine-6-carbonitrile (6f).* Starting from **5f** and 3-chloro-benzenecarboperoxoic acid, 90% of **6f** was obtained according to the method described for the synthesis of **6**. $^1$H NMR (300 MHz, CDCl$_3$) δ 8.69 (s, 1H), 8.15 (s, 1H), 4.43 (t, 2H), 2.96 (s, 3H), 1.75−1.70 (m, 2H), 1.50−1.43 (m, 2H), 1.02 (t, 3H). MS found (M + H)$^+$ (*m/z*), 291.10; calcd for C$_{13}$H$_{14}$N$_4$O$_2$S *m/z*, 290.08.

*2-Methylsulfinyl-7-oxo-8-pentyl-7,8-dihydropyrido[2,3-d]-pyrimidine-6-carbonitrile (6g).* Starting from **5g** and 3-chloro-benzenecarboperoxoic acid, 93% of **6g** was obtained according to the method described for the synthesis of **6**. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.72 (s, 1H), 8.14 (s, 1H), 4.44 (t, 2H), 2.93 (s, 3H), 1.77−1.72 (m, 2H), 1.43−1.37 (m, 4H), 0.92 (t, 3H). MS found (M + H)$^+$ (m/z), 305.10; calcd for C$_{14}$H$_{16}$N$_4$O$_2$S m/z, 304.10.

*8-Cyclopropyl-2-methylsulfinyl-7-oxo-7,8-dihydropyrido[2,3-d]-pyrimidine-6-carbonitrile (6h).* Starting from **5h** and 3-chloro-benzenecarboperoxoic acid, 91% of **6h** was obtained according to the method described for the synthesis of **6**. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.70 (s, 1H), 8.14 (s, 1H), 2.96−2.93 (m, 1H), 2.94 (s, 3H), 1.25 (bs, 2H), 1.03 (bs, 2H). MS found (M + H)$^+$ (m/z), 275.10; calcd for C$_{12}$H$_{10}$N$_4$O$_2$S m/z, 274.05.

*8-Cyclopentyl-2-methylsulfinyl-7-oxo-7,8-dihydropyrido[2,3-d]-pyrimidine-6-carbonitrile (6i).* Starting from **5i** and 3-chloro-benzenecarboperoxoic acid, 94% of **6i** was obtained according to the method described for the synthesis of **6**. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.69 (s, 1H), 8.13 (s, 1H), 5.98 (bs, 1H), 2.93 (s, 3H), 2.20−2.33 (m, 2H), 2.07−2.15 (m, 2H), 1.82−1.94 (m, 2H), 1.62−1.74 (m, 2H). MS found (M + H)$^+$ (m/z), 303.10; calcd for C$_{14}$H$_{14}$N$_4$O$_2$S m/z, 302.08.

*8-Cyclohexyl-2-methylsulfinyl-7-oxo-7,8-dihydropyrido[2,3-d]-pyrimidine-6-carbonitrile (6j).* Starting from **5j** and 3-chloro-benzenecarboperoxoic acid, 92% of **6j** was obtained according to the method described for the synthesis of **6**. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.69 (s, 1H), 8.11 (s, 1H), 5.43 (bs, 1H), 2.95 (s, 3H), 1.92−1.96 (m, 2H), 1.66−1.79 (m, 6H), 1.33−1.45 (m, 2H). MS found (M + H)$^+$ (m/z), 317.10; calcd for C$_{15}$H$_{16}$N$_4$O$_2$S m/z, 316.10.

*8-Cyclopentyl-2-methylsulfinyl-6-nitro-8H-pyrido[2,3-d]-pyrimidin-7-one (6k).* Starting from **5k** and 3-chloro-benzenecarboperoxoic acid, 91% of **6k** was obtained according to the method described for the synthesis of **6**. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.73 (s, 1H), 8.40 (s, 1H), 6.03 (bs, 1H), 2.94 (m, 3H), 2.35−2.32 (m, 2H), 2.16−2.09 (m, 2H), 1.99−1.91 (m, 2H), 1.76−171 (m, 2H). MS found (M + H)$^+$ (m/z), 323.10; calcd for C$_{13}$H$_{14}$N$_4$O$_4$S m/z, 322.07.

*8-Cyclopentyl-2-methylsulfinyl-6-methanesulfonyl-8H-pyrido-[2,3-d]pyrimidin-7-one (6l).* Starting from **5l** and 3-chloro-benzene-carboperoxoic acid, 92% of **6l** was obtained according to the method described for the synthesis of **6**. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.78 (s, 1H), 8.51 (s, 1H), 6.00 (bs, 1H), 3.34 (s, 3H), 2.95 (s, 3H), 2.28−2.40 (m, 2H), 2.10−2.15 (m, 2H), 1.86−1.98 (m, 2H), 1.66−1.71 (m, 2H). MS found (M + H)$^+$ (m/z), 356.10; calcd for C$_{14}$H$_{17}$N$_3$O$_4$S$_2$ m/z, 355.07.

*6-Benzenesulfonyl-8-cyclopentyl-2-methylsulfinyl-8H-pyrido[2,3-d]pyrimidin-7-one (6m).* Starting from **5m** and 3-chloro-benzenecar-boperoxoic acid, 90% of **6m** was obtained according to the method described for the synthesis of **6**. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.86 (s, 1H), 8.70 (s, 1H), 7.55−7.80 (m, 5H), 5.71 (bs, 1H), 2.95 (s, 3H), 2.19−2.31 (m, 2H), 1.99−2.10 (m, 2H), 1.80−1.87 (m, 2H), 1.60−1.70 (m, 2H). MS found (M + H)$^+$ (m/z), 418.10; calcd for C$_{19}$H$_{19}$N$_3$O$_4$S$_2$ m/z, 417.08.

*6-(4-Chloro-benzenesulfonyl)-8-cyclopentyl-2-methylsulfinyl-8H-pyrido[2,3-d]pyrimidin-7-one (6n).* Starting from **5n** and 3-chloro-benzenecarboperoxoic acid, 91% of **6n** was obtained according to the method described for the synthesis of **6**. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.81 (s, 1H), 8.68 (s, 1H), 8.02−8.06 (m, 2H), 7.50−7.57 (m, 2H), 5.77 (bs, 1H), 2.94 (s, 3H), 2.20−2.26 (m, 2H), 1.96−2.08 (m, 2H), 1.78−1.82 (m, 2H), 1.64−1.69 (m, 2H). MS found (M + H)$^+$ (m/z), 452.10; calcd for C$_{19}$H$_{18}$ClN$_3$O$_4$S$_2$ m/z, 451.04.

*6-(4-Chloro-phenylmethanesulfonyl)-8-cyclopentyl-2-methylsul-finyl-8H-pyrido[2,3-d]pyrimidin-7-one (6o).* Starting from **5o** and 3-chloro-benzenecarboperoxoic acid, 92% of **6o** was obtained according to the method described for the synthesis of **6**. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.70 (s, 1H), 8.56 (s, 1H), 7.43 (d, 2H), 7.24 (d, 2H), 5.83−5.91 (m, 1H), 4.67 (s, 2H), 2.96 (s, 3H), 2.20−2.28 (m, 2H), 1.97−2.10 (m, 2H), 1.81−1.88 (m, 2H), 1.65−1.73 (m, 2H). MS found (M + H)$^+$ (m/z), 466.10; calcd for C$_{20}$H$_{20}$ClN$_3$O$_4$S$_2$ m/z, 465.06.

*8-Cyclopentyl-6-(3-hydroxy-4-methoxy-phenylmethanesulfonyl)-2-methylsulfinyl-8H-pyrido[2,3-d]pyrimidin-7-one (6p).* Starting from **5p** and 3-chloro-benzenecarboperoxoic acid, 91% of **6p** was obtained according to the method described for the synthesis of **6**. ^{1}H NMR (300

MHz, CDCl$_3$) δ 8.50 (s, 1H), 8.14 (s, 1H), 6.51−6.72 (m, 3H), 5.74−5.91 (m, 1H), 4.58 (s, 2H), 3.67 (s, 3H), 2.97 (s, 3H), 2.13−2.27 (m, 2H), 1.88−2.02 (m, 2H), 1.66−1.80 (m, 2H), 1.49−1.60 (m, 2H). MS found (M + H)$^+$ (m/z), 478.10; calcd for C$_{21}$H$_{23}$N$_3$O$_6$S$_2$ m/z, 477.10.

*8-Cyclopentyl-2-methylsulfinyl-6-(4-methoxy-3-nitro-phenylme-thanesulfonyl)-2-methyl-sulfanyl-8H-pyrido [2,3-d]pyrimidin-7-one (6q).* Starting from **5q** and 3-chloro-benzene-carboperoxoic acid, 93% of **6q** was obtained according to the method described for the synthesis of **6**. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.67 (s, 1H), 8.30 (s, 1H), 7.83 (s, 1H), 7.81−7.83 (m, 1H), 7.59−7.63 (m, 1H), 5.93−6.09 (m, 1H), 4.85 (s, 2H), 3.90 (s, 3H), 2.96 (s, 3H), 2.30−2.42 (m, 2H), 2.08−2.18 (m, 2H), 1.89−2.01 (m, 2H), 1.67−1.78 (m, 2H). MS found (M + H)$^+$ (m/z), 507.10; calcd for C$_{21}$H$_{22}$N$_4$O$_7$S$_2$ m/z, 506.09.

*8-Cyclopentyl-2-methylsulfinyl-7,8-dihydro-pyrido[2,3-d]-pyrimidine-6-carboxylic Acid (4-Chloro-phenyl)-amide (6r).* Starting from **5r** and 3-chloro-benzenecarboperoxoic acid, 88% of **6r** was obtained according to the method described for the synthesis of **6**. ^{1}H NMR (300 MHz, CDCl$_3$) δ 11.68 (bs, 1H), 8.85 (s, 1H), 8.80 (s, 1H), 7.69−7.77 (m, 2H), 7.35−7.38 (m, 2H), 6.06 (bs, 1H), 2.95 (s, 3H), 2.33−2.43 (m, 2H), 2.13−2.21 (m, 2H), 1.92−2.00 (m, 2H), 1.77−1.80 (m, 2H). MS found (M + H)$^+$ (m/z), 431.10; calcd for C$_{20}$H$_{19}$ClN$_4$O$_3$S m/z, 430.09.

*8-Cyclopentyl-2-methylsulfinyl-7,8-dihydro-pyrido[2,3-d]-pyrimidine-6-carboxylic Acid (4-Fluoro-phenyl)-amide (6s).* Starting from **5s** and m-chloroperbenzoic acid, 87% of **6s** was obtained according to the method described for the synthesis of **6**. ^{1}H NMR (300 MHz, CDCl$_3$) δ 11.60 (bs, 1H), 8.86 (s, 1H), 8.81 (s, 1H), 7.71−7.77 (m, 2H), 6.96−7.09 (m, 2H), 6.03−6.13 (m, 1H), 2.96 (s, 3H), 2.33−2.42 (m, 2H), 2.13−2.21 (m, 2H), 1.92−2.00 (m, 2H), 1.71−1.80 (m, 2H). MS found (M + H)$^+$ (m/z), 415.10; calcd for C$_{20}$H$_{19}$FN$_4$O$_3$S m/z, 414.12.

*General Procedure for 8-Alkyl/cycloalkyl-6-(aryl/heteroarylami-no)-6-substituted-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine (7).* The mixture of 8-alkyl-2-methylsulfinyl-6-substituted-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidine **6** (1.65 mmol) and aryl/heteroaryl amines (2 mmol) in toluene was stirred at 100 °C for overnight. The reaction mixture was cooled, and solid was collected by filtration. The crude product was washed with toluene and purified by flash chromatography to get pure product.

*2-Benzylamino-8-cyclopentyl-7-oxo-7,8-dihydro-pyrido[2,3-d]-pyrimidine-6-carbonitrile (7a).* Starting from **6i** and benzylamine, 51% of **7a** was obtained according to the method described for the synthesis of **7**; mp 211−212 °C. ^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.45 (s, 1H), 8.35 (s, 1H), 7.36−7.42 (m, 5H), 5.80−5.86 (m, 1H), 2.48 (s, 2H), 2.10 (bs, 2H), 1.91 (m, 2H), 1.66 (m, 2H), 1.54 (m, 2H). MS found (M + H)$^+$ (m/z), 346.20; calcd for C$_{20}$H$_{19}$N$_5$O m/z, 345.16.

*2-(4-Chloro-phenylamino)-8-cyclopentyl-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7b).* Starting from **6i** and 4-chlorophenylamine, 50% of **7b** was obtained according to the method described for the synthesis of **7**; mp 272−273 °C. ^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.63 (s, 1H), 8.02 (s, 1H), 7.58−7.55 (m, 2H), 7.40−7.37 (m, 2H), 5.89−5.83 (m, 1H), 2.30−2.27 (m, 2H), 2.05 (bs, 2H), 1.90−1.87 (m, 2H), 1.71−1.67 (m, 2H). MS found (M + H)$^+$ (m/z), 366.20; calcd for C$_{19}$H$_{16}$ClN$_5$O m/z, 365.10.

*2-(4-Cyano-phenylamino)-8-cyclopentyl-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7c).* Starting from **6i** and 4-aminobenzonitrile, 55% of **7c** was obtained according to the method described for the synthesis of **7**; mp 285−287 °C. ^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.61 (s, 1H), 8.03 (s, 1H), 7.62−7.59 (m, 2H), 7.45−7.43 (m, 2H), 5.92−5.89 (m, 1H), 2.31−2.28 (m, 2H), 2.15 (bs, 2H), 1.89−1.86 (m, 2H), 1.75−1.72 (m, 2H). MS found (M + H)$^+$ (m/z), 357.10; calcd for C$_{20}$H$_{16}$N$_6$O m/z, 356.14.

*8-Cyclopentyl-2-(2-hydroxy-phenylamino)-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7d).* Starting from **6i** and 2-aminophenol, 42% of **7d** was obtained according to the method described for the synthesis of **7**; mp 303−305 °C. ^{1}H NMR (CDCl$_3$, 300 MHz) δ 9.69 (bs, 1H), 8.76 (s, 1H), 8.52 (s, 1H), 7.42−7.45 (m, 1H), 7.03−7.08 (m, 1H), 6.89−6.90 (m, 1H), 6.79−6.84 (m, 1H), 5.58−5.59 (m, 1H), 3.37 (bs, 1H), 2.14−2.21 (m, 2H), 1.59−1.65 (m, 4H), 1.39−1.43 (M, 2H). MS found (M + H)$^+$ (m/z), 348.20; calcd for C$_{19}$H$_{17}$N$_5$O$_2$ m/z, 347.14.

*8-Cyclopentyl-2-(2-methoxy-phenylamino)-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7e).* Starting from **6i** and 2-methoxyphenylamine, 54% of **7e** was obtained according to the method described for the synthesis of 7; mp 159−160 °C. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.60 (s, 1H), 8.31−8.33 (m, 1H), 7.99 (s, 1H), 7.10−7.16 (m, 1H), 6.96−7.06 (m, 2H), 5.89−5.98 (m, 1H), 3.90 (s, 3H), 2.28−2.36 (m, 2H), 2.04−2.10 (m, 2H), 1.85−1.96 (m, 2H), 1.64−1.75 (M, 2H). MS found (M + H)$^+$ (m/z), 362.20; calcd for C$_{20}$H$_{19}$N$_5$O$_2$ m/z, 361.15.

*8-Cyclopentyl-2-(3-methoxy-phenylamino)-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7f).* Starting from **6i** and 3-methoxyphenylamine, 50% of **7f** was obtained according to the method described for the synthesis of 7; mp 179−180 °C. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 8.61 (s, 1H), 8.00 (s, 1H), 7.56 (bs, 1H), 7.35−7.36 (m, 1H), 7.17−7.19 (m, 1H), 7.05−7.07 (m, 1H), 6.73−6.76 (m, 1H), 5.88−5.94 (m, 1H), 3.85 (s, 3H), 2.24−2.33 (m, 2H), 2.05 (bs, 2H), 1.83−1.90 (m, 2H), 1.62−1.67 (m, 2H). MS found (M + H)$^+$ (m/z), 362.20; calcd for C$_{20}$H$_{19}$N$_5$O$_2$ m/z, 361.15.

*8-Cyclopentyl-2-(4-methoxy-phenylamino)-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7g).* Starting from **6i** and 4-methoxyphenylamine, 54% of **7g** was obtained according to the method described for the synthesis of 7; mp 238−239 °C. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.57 (s, 1H), 7.97 (s, 1H), 7.42−7.47 (m, 2H), 6.92−6.97 (m, 2H), 5.83 (bs 1H), 3.84 (s, 3H), 2.21−2.30 (m, 2H), 1.85−2.05 (m, 4H), 1.61−1.74 (m, 2H). MS found (M + H)$^+$ (m/z), 362.30; calcd for C$_{20}$H$_{19}$N$_5$O$_2$ m/z, 361.15.

*8-Cyclopentyl-2-(2,4-dimethoxy-phenylamino)-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7h).* Starting from **6i** and 2,4-dimethoxyphenylamine, 52% of **7h** was obtained according to the method described for the synthesis of 7; mp 201−202 °C. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.57 (s, 1H), 7.96 (s, 1H), 7.19−7.23 (m, 1H), 6.51−6.56 (m, 2H), 5.85−5.91 (m, 1H), 3.91 (s, 3H), 3.85 (s, 3H), 2.30 (bs, 2H), 2.03 (bs, 2H), 1.86−1.90 (m, 2H), 1.66−1.69 (m, 2H). MS found (M + H)$^+$ (m/z), 392.20; calcd for C$_{21}$H$_{21}$N$_5$O$_3$ m/z, 391.16.

*8-Cyclopentyl-2-(3,4-dimethoxy-phenylamino)-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7i).* Starting from **6i** and 3,4-dimethoxyphenylamine, 50% of **7i** was obtained according to the method described for the synthesis of 7; mp 215−216 °C. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.58 (s, 1H), 7.98 (s, 1H), 7.16−7.26 (m, 1H), 6.90−6.98 (m, 1H), 6.84−6.87 (m, 1H), 5.84−5.95 (m, 1H), 3.91 (s, 6H), 2.20−2.29 (m, 2H), 1.84−2.13 (m, 4H), 1.61 (bs, 2H). MS found (M + H)$^+$ (m/z), 392.20; calcd for C$_{21}$H$_{21}$N$_5$O$_3$ m/z, 391.16.

*8-Cyclopentyl-2-(3,5-dimethoxy-phenylamino)-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7j).* Starting from **6i** and 3,5-dimethoxyphenylamine, 52% of **7j** was obtained according to the method described for the synthesis of 7; mp 150−151 °C. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.60 (s, 1H), 7.99 (s, 1H), 7.26 (s, 1H), 6.91−6.88 (m, 1H), 6.31−6.30 (m, 1H), 5.88−5.94 (m, 1H), 3.82 (s, 6H), 2.23−2.36 (m, 2H), 2.05−2.18 (m, 2H), 1.83−1.93 (m, 2H), 1.62−1.64 (m, 2H). MS found (M + H)$^+$ (m/z), 392.20; calcd for C$_{21}$H$_{21}$N$_5$O$_3$ m/z, 391.16.

*8-Cyclopentyl-2-(3,4,5-trimethoxy-phenylamino)-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7k).* Starting from **6i** and 3,4,5-trimethoxyphenylamine, 52% of **7k** was obtained according to the method described for the synthesis of 7; mp 169−170 °C. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.59 (s, 1H), 8.01 (s, 1H), 6.91 (s, 2H), 5.93−5.99 (m, 1H), 3.90 (s, 6H), 3.87 (s, 3H), 2.23−2.32 (m, 2H), 2.03−2.14 (m, 2H), 1.85−1.92 (m, 2H), 1.52−1.58 (m, 2H). MS found (M + H)$^+$ (m/z), 422.30; calcd for C$_{22}$H$_{23}$N$_5$O$_4$ m/z, 421.18.

*8-Cyclopentyl-2-(5-fluoro-2-methoxy-phenylamino)-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7l).* Starting from **6i** and 5-fluoro-2-methoxy-phenylamine, 50% of **7l** was obtained according to the method described for the synthesis of 7; mp 241−243 °C. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.65 (s, 1H), 8.02 (s, 1H), 7.16−7.19 (m, 1H), 6.76−6.89 (m, 2H), 5.85−8.97 (m, 1H), 3.94 (s, 3H), 2.33−2.36 (m, 2H), 2.05−2.13 (m, 2H), 1.90−1.98 (m, 2H), 1.71−1.75 (m, 2H). MS found (M + H)$^+$ (m/z), 380.20; calcd for C$_{20}$H$_{18}$FN$_5$O$_2$ m/z, 379.14.

*8-Cyclopentyl-7-oxo-2-(pyridin-2-ylamino)-7,8-dihydro-pyrido-[2,3-d]pyrimidine-6-carbonitrile (7m).* Starting from **6i** and 2-aminopyridine, 48% of **7m** was obtained according to the method described for the synthesis of 7; mp 284−286 °C. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.74

(s, 1H), 8.42−8.43 (m, 1H), 8.29−8.32 (m, 1H), 8.08 (s, 1H), 7.75−7.81 (m, 1H), 7.07−7.12 (m, 1H), 5.87−5.93 (m, 1H), 2.30−2.33 (m, 2H), 2.11−2.14 (m, 2H), 1.92−1.98 (m, 2H), 1.68−1.74 (m, 2H). MS found (M + H)$^+$ (m/z), 333.20; calcd for C$_{18}$H$_{16}$N$_6$O m/z, 332.14.

*2-(4-Cyano-pyridin-2-ylamino)-8-cyclopentyl-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7n).* Starting from **6i** and 2-amino-4-cyanopyridine, 40% of **7n** was obtained according to the method described for the synthesis of 7; mp 285−287 °C. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 11.29 (s, 1H), 8.96 (s, 1H), 8.65 (s, 1H), 8.59−8.61 (m, 1H), 8.49 (s, 1H), 7.54−7.56 (m, 1H), 5.72−5.80 (m, 1H), 2.18−2.25 (m, 2H), 1.83−1.93 (m, 4H), 1.56−1.68 (m, 2H). MS found (M + H)$^+$ (m/z), 358.20; calcd for C$_{19}$H$_{15}$N$_7$O m/z, 357.13.

*8-Cyclopentyl-2-(1H-indole-4-ylamino)-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7o).* Starting from **6i** and 4-aminoindole, 40% of **7o** was obtained according to the method described for the synthesis of 7; mp 320−322 °C. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 11.15 (bs, 1H), 10.36 (bs, 1H), 9.80 (s, 1H), 8.53 (s, 1H), 7.05−7.29 (m, 4H), 6.50 (bs, 1H), 5.62 (bs, 1H), 2.06 (bs, 2H), 1.35−1.60 (m, 6H). MS found (M + H)$^+$ (m/z), 371.30; calcd for C$_{21}$H$_{18}$N$_6$O m/z, 370.15.

*8-Cyclopentyl-2-(1H-indole-5-ylamino)-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7p).* Starting from **6i** and 5-aminoindole, 42% of **7p** was obtained according to the method described for the synthesis of 7; mp 192−193 °C. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 11.09 (bs, 1H), 10.57 (bs, 1H), 8.80 (s, 1H), 8.51 (s, 1H), 7.10−7.37 (m, 4H), 6.36 (s, 1H), 5.73−5.87 (m, 1H), 2.18−2.28 (m, 2H), 1.70−1.89 (m, 4H), 1.45−1.57 (m, 2H). MS found (M + H)$^+$ (m/z), 371.20; calcd for C$_{21}$H$_{18}$N$_6$O m/z, 370.15.

*8-Cyclopentyl-7-oxo-2-[quinolin-3-ylamino]-7,8-dihydro-pyrido-[2,3-d]pyrimidine-6-carbonitrile (7q).* Starting from **6i** and 3-amino-quinoline, 40% of **7q** was obtained according to the method described for the synthesis of 7; mp 212−213 °C. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 10.92 (bs, 1H), 8.07−9.08 (m, 1H), 8.88 (s, 1H), 8.69−8.70 (m, 1H), 8.62 (s, 1H), 7.99−8.09 (m, 1H), 7.81−7.85 (m, 1H), 7.58−7.68 (m, 2H), 5.85−5.87 (m, 1H), 2.10−2.28 (m, 2H), 1.85 (bs, 4H), 1.56 (bs, 2H). MS found (M + H)$^+$ (m/z), 383.30; calcd for C$_{22}$H$_{18}$N$_6$O m/z, 382.15.

*8-Cyclopentyl-7-oxo-2-[quinolin-8-ylamino]-7,8-dihydro-pyrido-[2,3-d]pyrimidine-6-carbonitrile (7r).* Starting from **6i** and 8-amino-quinoline, 40% of **7r** was obtained according to the method described for the synthesis of 7; mp 222−224 °C. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 10.84 (bs, 1H), 8.91 (s, 1H), 8.79−8.81 (m, 1H), 8.62 (s, 1H), 8.39−8.41 (m, 1H), 8.18−8.21 (m, 1H), 7.94−8.02 (m, 2H), 7.50−7.54 (m, 1H), 5.87−5.89 (m, 1H), 2.20−2.28 (m, 2H), 1.83−1.87 (m, 4H), 1.57−1.59 (m, 2H). MS found (M + H)$^+$ (m/z), 383.20; calcd for C$_{22}$H$_{18}$N$_6$O m/z, 382.15.

*8-Cyclopentyl-2-(2-methoxy-quinolin-6-ylamino)-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7s).* Starting from **6i** and 6-amino-2-methoxy-quinoline, 40% of **7s** was obtained according to the method described for the synthesis of 7; mp 223−224 °C. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 10.82 (bs, 1H), 8.64 (s, 1H), 8.10−8.11 (m, 1H), 8.01 (s, 1H), 7.90−7.96 (m, 1H), 7.86 (s, 1H), 7.68−6.72 (m, 1H), 6.95 (d, 1H), 5.86−5.98 (m, 1H), 4.09 (s, 3H), 2.29−2.36 (m, 2H), 2.01 (bs, 2H), 1.88−1.95 (m, 2H), 1.65 (bs, 2H). MS found (M + H)$^+$ (m/z), 413.20; calcd for C$_{23}$H$_{20}$N$_6$O$_2$ m/z, 412.16.

*8-Cyclopentyl-2-(4-morpholin-4-yl-phenylamino)-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7t).* Starting from **6i** and 4-morpholin-4-yl-phenylamine, 53% of **7t** was obtained according to the method described for the synthesis of 7; mp 294−296 °C. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.55 (s, 1H), 7.98 (s, 1H), 7.43−7.48 (m, 2H), 6.93−6.99 (m, 2H), 5.82−5.89 (m, 1H), 3.87−3.92 (m, 4H), 3.15−3.22 (m, 4H), 2.22−2.31 (m, 2H), 1.80−1.91 (m, 4H), 1.59−1.68 (m, 2H). MS found (M + H)$^+$ (m/z), 417.10; calcd for C$_{23}$H$_{24}$N$_6$O$_2$ m/z, 416.20.

*8-Cyclopentyl-2-(5-morpholin-4-yl-pyridin-2-ylamino)-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7u).* Starting from **6i** and 5-morpholin-4-yl-pyridin-2-ylamine, 40% of **7u** was obtained according to the method described for the synthesis of 7; mp 240−242 °C. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 10.30 (bs, 1H), 8.57 (s, 1H), 8.31 (s, 1H), 7.87−7.87 (m, 1H), 7.84−7.88 (m, 1H), 6.71 (d, 1H), 5.80 (bs, 1H), 3.88−3.84 (m, 4H), 3.55−3.52 (m, 4H), 2.22−2.06 (m, 2H),

1.63−1.29 (m, 6H). MS found (M + H)$^+$ (*m/z*), 418.20; calcd for C$_{22}$H$_{23}$N$_7$O$_2$ *m/z*, 417.19.

*8-Cyclopentyl-2-[5-(4-methyl-piperazin-1-yl)-pyridin-2-ylamino]-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7v).* Starting from **6i** and 5-(4-methyl-piperazin-1-yl)-pyridin-2-ylamine **22**, 50% of **7v** was obtained according to the method described for the synthesis of 7; mp 295−297 °C. $^1$H NMR (DMSO-*d$_6$*, 300 MHz) δ 10.53 (bs, 1H), 8.82 (s, 1H), 8.56 (s, 1H), 8.08 (d, 1H), 7.77 (d, 1H), 7.49−7.45 (m, 1H), 5.79−5.73 (m, 1H), 3.19−3.17 (m, 4H), 2.55−2.52 (m, 4H), 2.32 (s, 3H), 2.19−2.16 (m, 2H), 1.84−1.75 (m, 4H), 1.58−1.55 (m, 2H). MS found (M + H)$^+$ (*m/z*), 431.20; calcd for C$_{23}$H$_{26}$N$_8$O *m/z*, 430.22.

*8-Cyclopentyl-7-oxo-2-[4-(5-trifluoromethyl-pyridin-2-yl)-piperazin-1-yl]-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7w).* Starting from **6i** and 1-(5-trifluoromethyl-pyridin-2-yl)-piperazine, 40% of **7w** was obtained according to the method described for the synthesis of 7; mp 259−260 °C. $^1$H NMR (CDCl$_3$, 300 MHz) δ 8.53 (s, 1H), 8.44 (s, 1H), 7.94 (s, 1H), 7.68−7.71 (m, 1H), 6.68−6.71 (m, 1H), 5.82−5.88 (m, 1H), 4.09−4.14 (m, 4H), 3.82 (bs, 4H), 2.27−2.38 (m, 2H), 2.02−2.09 (m, 2H), 1.82−1.93 (m, 2H), 1.70−1.76 (m, 2H). MS found (M + H)$^+$ (*m/z*), 470.10; calcd for C$_{23}$H$_{22}$F$_3$N$_7$O *m/z*, 469.18.

*8-Cyclopentyl-2-[4-(4-methyl-piperazin-1-yl)-phenylamino]-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7x).* Starting from **6i** and 4-(4-methyl-piperazin-1-yl)-phenyl amine **21**, 55% of **7x** was obtained according to the method described for the synthesis of 7; mp 290−292 °C. $^1$H NMR (CDCl$_3$, 300 MHz) δ 8.55 (s, 1H), 7.79 (s, 1H), 7.40−7.45 (m, 2H), 6.91−6.99 (m, 2H), 5.83−5.84 (m, 1H), 3.23−3.27 (m, 4H), 2.63−2.66 (m, 4H), 2.43 (s, 3H), 2.21−2.30 (m, 2H), 1.85 (bs, 4H), 1.62 (bs, 2H). $^{13}$C NMR (CDCl$_3$, 300 MHz) δ 25.6, 28.0, 46.1, 49.2, 54.7, 55.0, 102.9, 105.6, 105.7, 115.3, 116.3, 123.0, 129.3, 143.4, 148.9, 157.0, 160.2. HPLC purity 99.63%, retention time 8.41 min. HRMS: *m/z* calcd [M + H], 430.2355; found, 430.2374. Anal. Calcd for C$_{24}$H$_{27}$N$_7$O: C 67.11, H 6.34, N 22.83. Found: C 67.00, H 6.27, N 22.77.

*2-[4-(4-Methyl-piperazin-1-yl)-phenylamino]-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7y).* Starting from **6a** and 4-(4-methyl-piperazin-1-yl)-phenylamine **21**, 45% of **7y** was obtained according to the method described for the synthesis of 7; mp >300 °C. $^1$H NMR (DMSO-*d$_6$*, 300 MHz) δ 13.10 (bs, 1H), 9.03 (s, 1H), 8.82 (s, 1H), 7.85−7.82 (m, 2H), 7.55−7.53 (m, 2H), 3.88−3.86 (m, 4H), 2.97−2.95 (m, 4H), 2.47 (s, 3H). MS found (M + H)$^+$ (*m/z*), 362.20; calcd for C$_{19}$H$_{19}$N$_7$O *m/z*, 361.17.

*8-Methyl-2-[4-(4-methyl-piperazin-1-yl)-phenylamino]-7-oxo-7,8-dihydro-pyrido[2,3-d] pyrimidine-6-carbonitrile (7z).* Starting from **6b** and 4-(4-methyl-piperazin-1-yl)-phenylamine **21**, 55% of **7z** was obtained according to the method described for the synthesis of 7; mp 211−212 °C. $^1$H NMR (DMSO-*d$_6$*, 300 MHz) δ 8.57 (s, 1H), 8.04 (s, 1H), 7.89 (bs, 1H), 7.58−7.54 (m, 2H), 7.12−7.09 (m, 2H), 3.30−3.24 (m, 4H), 2.65−2.61 (m, 4H), 2.65 (s, 3H), 2.44 (s, 3H). MS found (M + H)$^+$ (*m/z*), 376.20; calcd for C$_{20}$H$_{21}$N$_7$O *m/z*, 375.18.

*8-Ethyl-2-[4-(4-methyl-piperazin-1-yl)-phenylamino]-7-oxo-7,8-dihydro-pyrido[2,3-d] pyrimidine-6-carbonitrile (7aa).* Starting from **6c** and 4-(4-methyl-piperazin-1-yl)-phenylamine **21**, 60% of **7aa** was obtained according to the method described for the synthesis of 7; mp 262−264 °C. $^1$H NMR (DMSO-*d$_6$*, 300 MHz) δ 8.58 (s, 1H), 8.01 (s, 1H), 7.71 (bs, 1H), 7.53−7.50 (m, 2H), 6.99−6.96 (m, 2H), 4.44 (q, 2H), 3.27−3.24 (m, 4H), 2.67−2.63 (m, 4H), 2.40 (s, 3H), 1.36 (t, 3H). MS found (M + H)$^+$ (*m/z*), 390.20; calcd for C$_{21}$H$_{23}$N$_7$O *m/z*, 389.20.

*2-[4-(4-Methyl-piperazin-1-yl)-phenylamino]-7-oxo-8-propyl-7,8-dihydro-pyrido[2,3-d] pyrimidine-6-carbonitrile (7ab).* Starting from **6d** and 4-(4-methyl-piperzin-1-yl)-phenylamine **21**, 52% of **7ab** was obtained according to the method described for the synthesis of 7; mp 291−293 °C. $^1$H NMR (CDCl$_3$, 300 MHz) δ 8.56 (s, 1H), 8.07 (s, 1H), 7.51−7.57 (m, 2H), 6.93−6.99 (2H), 4.30 (t, 2H), 3.21−3.25 (m, 4H), 2.61−2.63 (m, 4H), 2.38 (s, 3H), 1.73−1.78 (m, 2H), 1.02 (t, 3H). MS found (M + H)$^+$ (*m/z*), 404.21; calcd for C$_{22}$H$_{25}$N$_7$O *m/z*, 403.21.

*8-Isopropyl-2-[4-(4-methyl-piperazin-1-yl)-phenylamino]-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7ac).* Starting from **6e** and 4-(4-methyl-piperazin-1-yl)-phenylamine **21**, 65% of **7ac** was obtained according to the method described for the synthesis of 7; mp 296−298 °C. $^1$H NMR (CDCl$_3$, 300 MHz) δ 8.56 (s, 1H), 7.96 (s,

1H), 7.47−7.44 (m, 2H,), 6.99−6.96 (m, 2H), 5.78−5.71 (m, 1H), 3.27−3.24 (bs, 4H), 2.66−2.62 (m, 4H), 2.40 (s, 3H), 1.60−1.57 (m, 6H). MS found (M + H)$^+$ (*m/z*), 404.30; calcd for C$_{22}$H$_{25}$N$_7$O *m/z*, 403.21.

*8-Butyl-2-[4-(4-methyl-piperazin-1-yl)-phenylamino]-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7ad).* Starting from **6f** and 4-(4-methyl-piperazin-1-yl)-phenylamine **21**, 60% of **7ad** was obtained according to the method described for the synthesis of 7; mp 282−283 °C. $^1$H NMR (DMSO-*d$_6$*, 300 MHz) δ 8.58 (s, 1H), 8.00 (s, 1H), 7.64 (bs, 1H), 7.54−7.52 (m, 2H), 6.98−6.95 (m, 2H), 4.37−4.32 (m, 2H), 3.30−3.26 (m, 4H), 2.73−2.70 (m, 4H), 2.45 (s, 3H), 1.75−1.68 (m, 2H), 1.49−126 (m, 2H), 1.01−0.94 (t, 3H). MS found (M + H)$^+$ (*m/z*), 418.30; calcd for C$_{23}$H$_{27}$N$_7$O *m/z*, 417.23.

*2-[4-(4-Methyl-piperazin-1-yl)-phenylamino]-7-oxo-8-pentyl-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7ae).* Starting from **6g** and 4-(4-methyl-piperazin-1-yl)-phenylamine **21**, 60% of **7ae** was obtained according to the method described for the synthesis of 7; mp 291−292 °C. $^1$H NMR (DMSO-*d$_6$*, 300 MHz) δ 10.48 (bs, 1H), 8.80 (s, 1H), 8.56 (s, 1H), 7.64−7.62 (m, 2H,), 6.95−6.92 (m, 2H), 4.18 (bs, 2H), 3.34 (bs, 4H), 3.14 (bs, 4H), 2.30 (s, 3H), 1.63 (bs, 2H), 1.33 (bs, 4H), 0.86 (bs, 3H). MS found (M + H)$^+$ (*m/z*), 432.40; calcd for C$_{24}$H$_{29}$N$_7$O *m/z*, 431.24.

*8-Cyclopropyl-2-[4-(4-methyl-piperazin-1-yl)-phenylamino]-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7af).* Starting from **6h** and 4-(4-methyl-piperazin-1-yl)-phenylamine **21**, 55% of **7af** was obtained according to the method described for the synthesis of 7; mp 285−287 °C. $^1$H NMR (DMSO-*d$_6$*, 300 MHz) δ 10.44 (bs, 1H), 8.74 (s, 1H), 8.50 (s, 1H), 7.84−7.82 (m, 2H,), 6.98−6.95 (m, 2H), 3.36−3.34 (bs, 4H), 3.15−3.12 (m, 4H), 2.91−2.85 (m, 1H), 2.29 (s, 3H), 1.25 (bs, 2H), 0.86 (bs, 2H). MS found (M + H)$^+$ (*m/z*), 402.30; calcd for C$_{22}$H$_{23}$N$_7$O *m/z*, 401.20.

*8-Cyclohexyl-2-[4-(4-methyl-piperazin-1-yl)-phenylamino]-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carbonitrile (7ag).* Starting from **6j** and 4-(4-methyl-piperazin-1-yl)-phenylamine **21**, 52% of **7ag** was obtained according to the method described for the synthesis of 7; mp 279−281 °C. $^1$H NMR (CDCl$_3$, 300 MHz) δ 8.53 (s, 1H), 7.94 (s, 1H), 7.45−7.66 (m, 2H), 6.95−6.98 (m, 2H), 5.43−5.47 (m, 1H), 3.22−3.26 (m, 4H), 2.62−2.65 (m, 4H), 2.39 (s, 3H), 1.88 (bs, 2H), 1.64 (bs, 4H), 1.33 (bs, 4H). MS found (M + H)$^+$ (*m/z*), 444.30; calcd for C$_{25}$H$_{29}$N$_7$O *m/z*, 443.24.

*8-Cyclopentyl-2-[4-(4-methyl-piperazin-1-yl)-phenylamino]-6-nitro-8H-pyrido[2,3-d]pyrimidin-7-one (7ah).* Starting from **6k** and 4-(4-methyl-piperazin-1-yl)-phenylamine **21**, 55% of **7ah** was obtained according to the method described for the synthesis of 7; mp 220−222 °C. $^1$H NMR (DMSO-*d$_6$*, 300 MHz) δ 8.67 (s, 1H), 8.46 (s, 1H), 7.72 (bs, 1H), 7.47−7.44 (m, 2H), 6.99−6.96 (m, 2H), 5.90 (bs, 1H), 3.28−3.25 (bs, 4H), 2.68−2.64 (m, 4H), 2.41 (s, 3H), 2.32−2.28 (m, 2H), 2.07 (bs, 2H), 1.88 (bs, 2H), 1.63 (bs, 2H). MS found (M + H)$^+$ (*m/z*), 450.20; calcd for C$_{23}$H$_{27}$N$_7$O$_3$ *m/z*, 449.22.

*8-Cyclopentyl-6-methylsulfonyl-2-[4-(4-methyl-piperazin-1-yl)-phenylamino]-8H-pyrido[2, 3-d]pyrimidin-7-one (7ai).* Starting from **6l** and 4-(4-methyl-piperazin-1-yl)-phenyl amine **21**, 52% of **7ai** was obtained according to the method described for the synthesis of 7; mp 255−257 °C. $^1$H NMR (CDCl$_3$, 300 MHz) δ 8.56 (s, 1H), 8.35 (s, 1H), 7.35 (d, 2H), 6.90 (d, 2H), 5.75 (bs, 1H), 3.25 (s, 3H), 3.14−3.23 (m, 4H), 2.52−2.55 (m, 4H), 2.30 (s, 3H), 2.12−2.27 (m, 2H), 1.75−1.97 (m, 2H), 1.57−1.76 (m, 4H). MS found (M + H)$^+$ (*m/z*), 483.20; calcd for C$_{24}$H$_{30}$N$_6$O$_3$S *m/z*, 482.21.

*6-Benzenesulfonyl-8-cyclopentyl-2-[4-(4-methyl-piperazin-1-yl)-phenylamino]-8H-pyrido [2,3-d]pyrimidine-7-one (7aj).* Starting from **6m** and 4-(4-methyl-piperazin-1-yl)-phenylamine **21**, 51% of **7aj** was obtained according to the method described for the synthesis of 7; mp 199−200 °C. $^1$H NMR (CDCl$_3$, 300 MHz) δ 8.65 (s, 1H), 8.58 (s, 1H), 8.09−8.13 (m, 2H), 7.55−7.63 (m, 3H), 7.37−7.46 (m, 1H), 7.13−7.24 (m, 1H), 6.80−6.96 (m, 2H), 5.82−5.84 (m, 1H), 3.22−3.32 (m, 4H), 2.63−2.71 (m, 4H), 2.46 (s, 3H), 2.18−2.33 (m, 2H), 1.45−1.76 (m, 6H). MS found (M + H)$^+$ (*m/z*), 545.30; calcd for C$_{29}$H$_{32}$N$_6$O$_3$S *m/z*, 544.23.

*6-(4-Chloro-benzenesulfonyl)-8-cyclopentyl-2-[4-(4-methyl-piperazin-1-yl)-phenylamino]-8H-pyrido[2,3-d]pyrimidine-7-one (7ak).* Starting from **6n** and 4-(4-methyl-piperazin-1-yl)-phenylamine

**21**, 50% of **7ak** was obtained according to the method described for the synthesis of **7**; mp 220−221 °C. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.64 (s, 1H), 8.62 (s, 1H), 8.04−8.06 (m, 2H), 7.49−7.50 (m, 2H), 7.37−7.40 (m, 2H), 6.92−6.95 (m, 2H), 5.65−5.69 (m, 1H), 3.23−3.27 (m, 4H), 2.67−2.70 (m, 4H), 2.42 (s, 3H), 2.09−2.2.20 (m, 2H), 1.71−1.82 (m, 4H), 1.51−1.63 (m, 2H). MS found (M + H)$^+$ (*m/z*), 579.30; calcd for C$_{29}$H$_{31}$ClN$_6$O$_3$S *m/z*, 578.19.

*6-(4-Chloro-phenylmethanesulfonyl)-8-cyclopentyl-2-[4-(4-methyl-piperazin-1-yl)-phenyl-amino]-8H-pyrido[2,3-d]pyrimidine-7-one (7al).* Starting from **6o** and 4-(4-methyl-piperazin-1-yl)-phenylamine **21**, 60% of **7al** was obtained according to the method described for the synthesis of **7**; mp 200−202 °C. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 10.59 (bs, 1H), 8.98 (s, 1H), 8.41 (s, 1H), 8.09−8.11 (m, 1H), 7.77−7.81 (d, 2H), 7.48−7.53 (m, 1H), 7.33−7.42 (m, 2H), 7.20−7.31 (m, 2H), 5.75−7.91 (m, 1H), 4.83 (s, 2H), 3.24−3.29 (m, 4H), 2.68−2.72 (m, 4H), 2.31−2.44 (s, 3H), 2.37 (m, 2H), 2.11−2.20 (m, 2H), 1.55−2.05 (m, 4H). MS found (M + H)$^+$ (*m/z*), 593.10; calcd for C$_{30}$H$_{33}$ClN$_6$O$_3$S *m/z*, 592.20.

*8-Cyclopentyl-6-(3-hydroxy-4-methoxy-phenylmethanesulfonyl)-2-[4-(4-methyl-piperzin-1-yl)-phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (7am).* Starting from **6p** and 4-(4-methyl-piperazin-1-yl)-phenylamine **21**, 53% of **7am** was obtained according to the method described for the synthesis of **7**; mp 165−166 °C. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 10.35 (bs, 1H), 9.08 (s, 1H), 8.88 (s, 1H), 7.46−7.51 (m, 2H), 6.95−6.92 (m, 2H), 6.83 (d, 1H), 6.67−6.68 (m, 1H), 6.56−6.60 (m, 1H), 5.89 (bs, 1H), 4.61 (s, 2H), 3.71 (s, 3H), 2.48−2.50 (m, 4H), 2.42−2.45 (m, 4H), 2.41 (s, 3H), 2.23−2.25 (m, 2H), 1.83−1.97 (m, 4H), 1.60 (bs, 2H). MS found (M + H)$^+$ (*m/z*), 605.30; calcd for C$_{31}$H$_{36}$N$_6$O$_5$S *m/z*, 604.25.

*8-Cyclopentyl-6-(4-methoxy-3-nitro-phenylmethanesulfonyl)-2-[4-(4-methyl-piperzin-1-yl)-phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (7an).* Starting from **6q** and 4-(4-methyl-piperazin-1-yl)-phenylamine **21**, 45% of **7an** was obtained according to the method described for the synthesis of **7**; mp 260−262 °C. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.52 (s, 1H), 8.12 (s, 1H), 7.80−7.81 (m, 1H), 7.59−7.62 (m, 1H), 7.38−7.42 (m, 2H), 7.02−7.05 (m, 1H), 6.91−1.94 (m, 2H), 5.89 (bs, 1H), 4.81 (s, 2H), 3.93 (s, 3H), 3.23−2.35 (m, 4H), 2.64−2.66 (m, 4H), 2.41 (s, 3H), 1.93−2.05 (m, 6H), 1.64−1.76 (m, 2H). MS found (M + H)$^+$ (*m/z*), 634.30; calcd for C$_{31}$H$_{35}$N$_7$O$_6$S *m/z*, 633.24.

*8-Cyclopentyl-2-[4-(4-methyl-piperazin-1-yl)-phenylamino]-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carboxylic Acid (4-Chloro-phenyl)-amide (7ao).* Starting from **6r** and 4-(4-methyl-piperazin-1-yl)-phenylamine **21**, 55% of **7ao** was obtained according to the method described for the synthesis of **7**; mp 301−302 °C. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 11.79 (s, 1H), 10.23 (bs, 1H), 8.77 (s, 1H), 8.67 (s, 1H), 7.71−7.74 (m, 2H), 7.44−7.61 (m, 2H), 7.31−7.33 (m, 2H), 6.91−6.99 (m, 2H), 5.89−6.03 (m, 1H), 3.47−3.499 (m, 4H), 3.22−3.25 (m, 4H), 2.63 (s, 3H), 2.28−2.38 (m, 2H), 1.89−2.05 (m, 4H), 1.67 (bs 2H). MS found (M + H)$^+$ (*m/z*), 558.30; calcd for C$_{30}$H$_{32}$ClN$_7$O$_2$ *m/z*, 557.23.

*8-Cyclopentyl-2-[4-(4-methyl-piperazin-1-yl)-phenylamino]-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidine-6-carboxylic Acid (4-Fluoro-phenyl)-amide (7ap).* Starting from **6s** and 4-(4-methyl-piperazin-1-yl)-phenylamine **21**, 55% of **7ap** was obtained according to the method described for the synthesis of **7**; mp 289−290 °C. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 11.63 (bs, 1H), 10.25 (bs, 1H), 9.01 (s, 1H), 8.77 (s, 1H), 7.76−7.77 (m, 2H), 7.43−7.52 (m, 2H), 7.19−7.29 (m, 2H), 6.92−6.95 (m, 2H), 5.88−5.97 (m, 1H), 3.08−3.11 (m, 4H), 2.50 (s, 3H), 2.45−2.48 (m, 4H), 2.20−2.30 (m, 2H), 1.81−1.96 (m, 4H), 1.46−1.60 (m, 2H). MS found (M + H)$^+$ (*m/z*), 542.30; calcd for C$_{30}$H$_{32}$FN$_7$O$_2$ *m/z*, 541.26.

*N-(6-Cyano-8-cyclopentyl-7-oxo-7,8-dihydro-pyrido[2,3-d]pyrimidin-2-yl)-N-[4-(4-methyl-piperazin-1-yl)-phenyl]-acetamide (7aq).* The compound **7x** (1 g, 2.3 mmol) was taken into acetic anhydride and stirred at 120 °C temperature for 3 h. The reaction mixture was cooled to room temperature, and the crude product was filtered. The pure product **7aq** was obtained by flash chromatography with 2% methanol in chloroform. Yield 64%; light orange solid, mp 223−224 °C. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.88 (s, 1H), 8.11 (s, 1H), 7.12−7.09 (m, 2H), 7.01−6.97 (m, 2H), 5.61−5.55 (m, 1H), 3.30−3.26 (m, 4H), 2.62−2.59 (m, 4H), 2.42 (s, 3H), 2.38 (s, 3H), 2.11−2.04 (m, 2H),

1.72−1.68 (m, 4H), 1.54−1.51 (m, 2H). MS found (M + H)$^+$ (*m/z*), 472.30; calcd for C$_{26}$H$_{29}$N$_7$O$_2$ *m/z*, 471.24.

*N-(6-Cyano-8-cyclopentyl-7-oxo-7,8-dihydro-pyrido[2,3-d]-pyrimidin-2-yl)-N-[4-(4-methyl-piperazin-1-yl)-phenyl]-4-trifluoro-methyl-benzamide (7ar).* The compound **7x** (1 g, 2.3 mmol) was taken into DMF and NaH was added at room temperature. After 10 min, 4-trifluoromethyl benzoyl chloride (0.58 g, 2.8 mmol) was added and stirring continued for about 1 h. The reaction mixture was quenched with water and filter off the crude product, and it was purified with column chromatography by using 1−2% methanol in dichloromethane as eluents. Yield 62%; brown solid, mp 155−156 °C. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.60 (s, 1H), 7.97 (s, 1H), 7.72 (d, 2H), 7.56 (d, 2H), 7.07−7.04 (m, 2H), 6.91−6.88 (m, 2H), 5.01−4.95 (m, 1H), 3.21−3.18 (m, 4H), 2.52−2.48 (m, 4H), 2.29 (s, 3H), 1.87−1.85 (m, 2H), 1.69−1.68 (m, 2H), 1.35−1.33 (m, 4H). MS found (M + H)$^+$ (*m/z*), 602.30; calcd for C$_{32}$H$_{30}$F$_3$N$_7$O$_2$ *m/z*, 601.24.

**Biology: Materials and Methods.** Cell lines were purchased from ATCC and were maintained in DMEM or RPM1 (CellGro) supplemented with 10% fetal bovine serum (Cellgeneration, CO) and 1 unit/mL penicillin−streptomycin (Invitrogen) at 37 °C under humidified conditions.

*Cytotoxicity Assays.* Cells were seeded at a cell density of $1.5 \times 10^3$ cells/0.1 mL/well in a 96-well plate. The compounds were added 24 h postplating at the indicated concentrations. Cell counts were determined from duplicate wells 96 h post-treatment. The total number of viable cells was determined using the Cell Titer Blue assay (Promega, WI) in conjunction with the GloMax plate reader (Promega, WI).

*Kinase Assays and IC$_{50}$ Determination.* First, 10 ng of recombinant CDK4/cyclin D1 (Life Technologies PV4204) was diluted in kinase buffer (20 mM Tris pH 7.5, 10 mM MgCl$_2$, 0.01% NP-40, 2 mM DTT) and incubated with the indicated concentration of inhibitor at room temperature for 30 min. Kinase reactions were initiated by the addition of 1 $\mu$g (1.5 $\mu$M) of recombinant Rb protein, 5 $\mu$M ATP, and 10 $\mu$Ci $\gamma$-$^{32}$P-ATP. The reactions were incubated at 30 °C for 20 min, terminated by the addition of 2× Laemmli sample buffer, heated at 95 °C for 3 min, resolved using 12% acrylamide SDS-PAGE, and subjected to autoradiography. The autoradiograms were scanned, and the band corresponding to the phosphorylated protein substrate was quantitated using a densitometer (Bio-Rad). The densitometric values obtained were plotted as a function of log drug concentration using Prism 4 Graphpad software and IC$_{50}$ values determined by plotting sigmoidal nonlinear regression curves with a variable slope.

*Flow Cytometry.* MCF-7 (human estrogen positive breast carcinoma) and MDA-MB-231 (human triple negative breast carcinoma) cells were plated onto 100 mm$^2$ dishes at a cell density of $1.0 \times 10^6$ cells/dish. All cells were treated with increasing concentrations of the indicated compounds 24 h postplating. Both nonadherent cells (floating) and adherent cells were harvested 24 h post-treatment, washed in phosphate buffered saline (PBS), and fixed in ice cold 70% ethanol for at least 24 h. The fixed cells were then washed with room temperature PBS and stained with propidium iodide (50 mg/mL) in the presence of RNase A (0.5 mg) for 30 min at 37 °C. The stained cells were then analyzed using a FACSCAN (BD Biosciences) and the resulting data analyzed with cell cycle analysis software (Modfit, BD).

*Western Blot Analysis.* Cells were treated with increasing concentrations of compound and harvested 24 h post-treatment. All cell pellets were frozen on dry ice before lysis. Cells were lysed in lysis buffer ((50 mM Tris-HCl, 0.1% Triton-X100, 250 mM NaCl, 5 mM EDTA, 50 mM NaF, 0.1 mM sodium orthovanadate (pH 7.4), and protease inhibitors), and 100 $\mu$g of clarified lysates were resolved by 10%-SDS-polyacrylamide gel electrophoresis. The resolved proteins were transferred onto nitrocellulose filter paper and hybridized with the following antibodies: phosphospecific Rb (Cell Signaling; catalogue no. 9307), Rb (Cell Signaling; catalogue no. 9309), AKT (Cell Signaling, catalogue no. 4691), phosphospecific AKT Ser473 (Cell Signaling; catalogue no. 9271), and PARP (BD Biosciences; catalogue no. 556362). Lysates used for Western blot analysis to detect cleaved PARP were obtained as above except that the cells were lysed in 1% NP40/PBS lysis buffer containing protease inhibitors. Following hybridization with primary antibodies, the blots were washed, treated

with secondary antibodies conjugated to infrared dyes (IRDye 800 or IRDye 680) and analyzed on an infrared scanning system (Odyssey, Li-Cor Biosciences, NE) according to the manufacturer's instructions.

*Orthotopic Nude Mouse Assay.* MDA-MB-231 triple negative breast cancer cells ($1 \times 10^6$) were injected bilaterally in the mammary fat pads of 7−8 week old female athymic nude mice (NCR nu/nu, Taconic, NY). Once the tumors grew to a volume of approximately 100 mm$^3$, they were placed into two treatment groups ($n = 6$, with a total tumor number of 11). The mice were treated daily for 15 days (QD × 15), a dose of 100 mg/kg (0.1 mL, intraperitoneally), or placebo (sterile PBS). Body weights and tumor size were determined every other day. Tumor measurements were used using a digital vernier caliper, and the volumes were determined using the following calculation: (short$^2$) × long × 0.5. Experiments were performed under an approved IACUC protocol according to federal and institutional guidelines and regulations.

*Statistical Analysis.* Statistical analysis was performed using a standard, unpaired, two-tailed Student's $t$ test. Data are graphed as mean ± SEM.

*Model of 7x Binding to CDK6.* Small molecule **7x** binding was predicted by docking and energy minimization using the X-ray crystal structure of CDK6−Vcyclin−PD-0332991 (2EUF) as a reference. Representations of the superimposition of X-ray crystal structure (CDK6/PD-0332991) and predicted lowest energy binding (CDK6/**7x**) were prepared using PyMOL (Figure 2). Figure 2A, ribbon representation of CDK6 (green) bound to PD-0332991 (red) and **7x** (cyan). Small molecules are shown as sticks. Figure 2B,C, closeup view showing proximal residues of CDK6 to **7x** (blue) and PD-0332991 (pink), respectively. Hydrogen bonds are shown as a dotted back lines.

## ASSOCIATED CONTENT

### Ⓢ Supporting Information

Effect of **7x** and PD-0332991 on a panel of human breast carcinoma cell lines, kinase inhibition profile of **7x** (Reaction Biology Corporation), inhibition of PI3K isoforms by **7x**, and docking models showing different binding positions of **7x** to Abl, FGFR, and FMS kinases. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Author Contributions

§B.A. and S.C.C. contributed equally.

### Notes

The authors declare the following competing financial interest(s): Dr. E. P. Reddy is a stockholder, Board member, grant recipient and paid consultant of Onconova Therapeutics Inc. Dr. M. V. R. Reddy is a stockholder and paid consultant of Onconova Inc. Dr. S. Cosenza is a paid consultant of Onconova Therapeutics Inc.

## ACKNOWLEDGMENTS

## ABBREVIATIONS USED

CDK, cyclin-dependent kinase; MPF, M-phase promoting factor; ARK5, AMPK-related protein kinase 5; FGFR, fibroblast growth factor receptor; PDGFR, platelet-derived growth factor receptor; PI3K, phosphatidylinositide 3-kinases; RB, retinoblastoma protein; SDS-PAGE, sodium dodecyl sulfate polyacryla-mide gel electrophoresis; PVDF, polyvinylidene difluoride; mTOR, mammalian target of rapamycin; FACS, fluorescence activated cell sorting; PARP, poly(ADP-ribose)polymerase; DMEM, Dulbecco's Modified Eagle Medium; RPMI, Roswell Park Memorial Institute; PBS, phosphate buffered saline

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