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Falcipain Inhibitors: Optimization Studies of the 2-Pyrimidinecarbonitrile Lead Series†

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Falcipain-2 and falcipain-3 are papain-family cysteine proteases of the malaria parasite *Plasmodium falciparum* that are responsible for host hemoglobin hydrolysis to provide amino acids for parasite protein synthesis. Different heteroarylnitrile derivatives were studied as potential falcipain inhibitors and therefore potential antiparasitic lead compounds, with the 5-substituted-2-cyanopyrimidine chemical class emerging as the most potent and promising lead series. Through a sequential lead optimization process considering the different positions present in the initial scaffold, nanomolar and subnanomolar inhibitors at falcipains 2 and 3 were identified, with activity against cultured parasites in the micromolar range. Introduction of protonable amines within lead molecules led to marked improvements of up to 1000 times in activity against cultured parasites without noteworthy alterations in other SAR tendencies. Optimized compounds presented enzymatic activities in the picomolar to low nanomolar range and antiparasitic activities in the low nanomolar range.

Introduction

Malaria is one of the major disease problems of the developing world. The most virulent malaria parasite is *Plasmodium falciparum*, which is the cause of hundreds of millions of cases of malaria and about 1 million deaths each year, mostly in African children.[1,2] One problem encountered in the treatment of malaria is the development of resistance to most available drugs. Thus, new antimalarials are urgently needed.

Plasmodium falciparum relies on hemoglobin hydrolysis to supply amino acids for protein synthesis and to maintain osmotic stability. Hemoglobin is transported to an acidic (pH 4−6) food vacuole, where it is hydrolyzed by multiple enzymes, including food vacuole cysteine, aspartic, and metalloproteases, before further action against hemoglobin fragments by a cytosolic aminopeptidase.[3,4] Hemoglobin degradation is blocked by cysteine protease inhibitors, causing a characteristic morphological abnormality in which the food vacuole fills with undegraded hemoglobin and parasite development is blocked.[5,6] Hence, plasmodial hemoglobinases constitute potential therapeutic targets. Efforts to identify the enzymes responsible for hemoglobin degradation led to the characterization of "falcipain" as a trophozoite food vacuole cysteine protease.[7,8] More recent advances identified and characterized a family of papain-like cysteine proteases comprising falcipain-1, falcipain-2, falcipain-2′, and falcipain-3.[9] Falcipain-2 and falcipain-3 (65% amino acid identity) are predominantly expressed in the acidic food vacuole of trophozoites and schizonts. Falcipain-3 is expressed later in the life cycle and appears to be a more efficient hemoglobinase than falcipain-2.[10−12] Importantly, cysteine protease inhibitors that inhibit falcipain-2 and falcipain-3 consistently block hemoglobin hydrolysis and parasite development, suggesting that these two proteases are key target enzymes. Knockout of falcipain-2 led to a block in hemoglobin hydrolysis in trophozoites, although parasites recovered from this defect, presumably because of expression of falcipain-3 later in the life cycle. Knockout of falcipain-3 was not successful, although replacement of the falcipain-3 gene with a functional copy was readily achieved, strongly suggesting that falcipain-3 is an essential enzyme in erythrocytic parasites.[13] Falcipain-2′, a nearly identical copy of falcipain-2, also has unknown function, as disruption of the falcipain 2′ gene did not lead to noticeable alterations in erythrocytic parasites.[14] Finally, the role of falcipain-1 is unknown; knockout of this protease led to no apparent changes in erythrocytic parasites.[15] In any event, cysteine protease inhibition, in particular the inhibition of falcipain-2 and falcipain-3, blocks parasite development. Falcipain-2 and related plasmodial cysteine proteases are thus logical targets for antiparasitic chemotherapy, and therefore, we were interested in the development of inhibitors of these enzymes as antiparasitic drugs.

Alkyl- and arylnitriles are well established inhibitors of cysteine proteases.[16−24] The active site of papain and related proteases is large and can be divided into pockets at each side

†Dedicated to Prof. Federico Gómez de las Heras, DDW-GlaxoSmithKline, on the occasion of his retirement.

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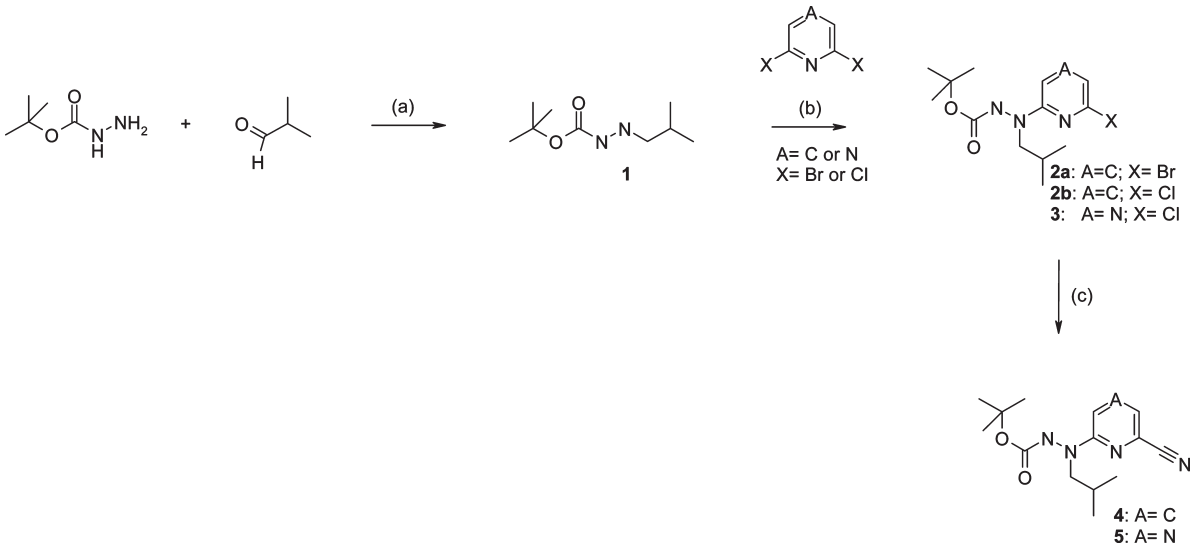
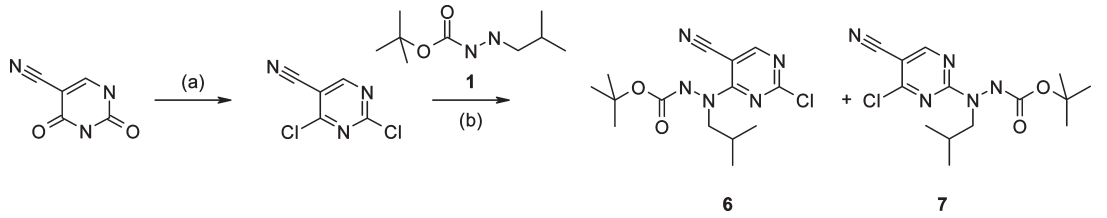
Scheme 1. Synthesis of Pyridine and Pyrimidine Derivatives[a]



[a] Reagents and conditions: (a) *i*-PrOH, 0 °C → rt, then H_2, PtO_2, rt; (b) $Pd_2(dba)_3$, BINAP, NaO^{t}Bu, toluene, rt; (c) $Zn(CN)_2$, $Pd_2(dba)_3$, dppf, Zn, DMA, 120 °C.

Scheme 2. Synthesis of 5-Cyanopyrimidine Derivatives[a]



[a] Reagents and conditions: (a) $POCl_3$, PCl_5, 115 °C; (b) DIPEA, toluene or *i*-PrOH, rt.

of the catalytic site, S1−S4 and S1′−S3′, with each pocket accommodating one amino acid residue of the peptide substrate.[25] The general structure of a cysteine protease inhibitor (Figure 1) should comprise an electrophilic moiety for interaction with the cysteine residue of the enzyme and one or two series of substituents, P1−P4 and/or P1′−P3′, responsible for interactions with the different pockets of the protease. Ideally, the X-ray structure of the complex formed between the enzyme and an inhibitor should be a valuable source of information about interactions in the complex and potential interactions to guide the design of new inhibitors. Despite the lack of X-ray structures at the time of initiation of this project, falcipains were perceived as novel and extremely promising targets, and we began lead optimization studies with the arylnitrile series following an empirical approach in which optimization of the initial hits took place considering interactions with both the active site cysteine and P1−P3 positions.

Chemistry

Synthesis of Heteroaryl Nitriles for Optimization of the Electrophilic Moiety and P1 Position. The first step of the optimization process was the identification of the most promising heteroarylnitrile designed to contain both the electrophilic moiety (nitrile group) and the P1 substituent (heteroaryl ring). In order to carry out a fair comparison among different compounds, substitutions at positions P2 (2-methylpropyl-) and P3 (*tert*-butyloxycarbonyl-) were fixed for all derivatives. The synthesis of *tert*-butyloxycarbonyl derivatives of heteroarylnitrile falcipain inhibitors is outlined in Schemes 1−13. Depending on the commercial availability of the appropriately substituted heteroaryl precursors, different approaches were followed.

Palladium catalyzed amination and cyanation reactions were necessary in the synthesis of pyridine and pyrazine derivatives starting out from the corresponding dihalogen derivatives (Scheme 1). Aromatic substitution of 2,6-dibromopyridine with the hydrazido derivative **1** failed when DIPEA[a] and isopropanol were used at 130 °C, while palladium-catalyzed aminations (both using palladium(II) acetate/BINAP/potassium carbonate and $Pd_2(dba)_3$/BINAP/sodium *tert*-butoxide) yielded the desired product when 2,6-dichloropyridine was used as starting material, diamination being

[a] Abbreviations: ACN, acetonitrile; AcO/OAc, acetate; AcOH, acetic acid; anh, anhydrous; aq, aqueous; BINAP, 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl; DABCO, 4-diazabicyclo[2.2.2]octane; dba, dibenzylideneacetone; DCM, dichloromethane; DIPEA, diisopropylethylamine; DMA, dimethylacetamide; DME, dimethoxyethane; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; dppf, 1,1′-bis(diphenylphosphino)ferrocene; DTT, dithiothreitol; EtOAc, ethyl acetate; Et_3N, triethylamine; EtOH, ethanol; FP-2, falcipain-2; FP-3, falcipain-3; g, gram; h, hour; H-D-VLR-AFC, HD-valyl-leucyl-arginyl-7-amido-4-trifluoromethylcoumarin; HetAr, heteroaryl; HMPA, hexamethylphosphoramide; HPLC, high performance liquid chromatography; IC_{50}, inhibitory concentration 50; *i*-PrOH, isopropanol; LCMS, liquid chromatography−mass spectrometry; M, molar; min, minute; MeOH, methanol; MHz, megahertz; mL, milliliter; mM, millimolar; mmol, millimole; N, normal; NBS, *N*-bromosuccinimide; ND, not determined; NaOEt/EtONa, sodium ethoxide; NaO^{t}Bu, sodium *tert*-butoxide; nM, nanomolar; NMR, nuclear magnetic resonance; °C, degree centigrade; PBS, phosphate buffer solution; ppm, parts per million; RPMI, Roswell Park Memorial Institute; rt, room temperature; sat., saturated; TFA, trifluoroacetic acid; THF, tetrahydrofuran; μM, micromolar; vs, versus.

observed when 2,6-dibromopyridine was subjected to these reaction conditions. Cyanation of the intermediate 1,1-dimethylethyl-2-(6-bromo-2-pyridinyl)-2-(2-methylpropyl)-hydrazinecarboxylate **2a** failed when nickel(II) bromide and sodium cyanide were used under the conditions described by Leadbeater and Arvela.[26] Negative results followed the exploration of different solvents as well as the use of other cyanating agents, such as copper(I) cyanide, sodium cyanide/copper(I) iodide/*N*,*N*′-dimethylethylenediamine/potassium iodide,[27] zinc(II) cyanide/tetrakis(triphenylphosphine)palladium(0) or sodium cyanide/tetrakis(triphenylphosphine)palladium(0)/copper(I) iodide. Finally, the use of zinc(II) cyanide/tris(dibenzylideneacetone)dipalladium(0)/dppf/Zn[28] yielded the desired product **4**. With these reaction conditions in hand, the corresponding pyrazine derivative **5** was synthesized starting out from 2,6-dichloropyrazine through intermediate **3**.

The 4-, 5- and 6-nitrile pyrimidine derivatives (Schemes 2 and 3) were prepared from the corresponding dichloropyrimidines by chlorine displacement with the hydrazide derivative following the literature procedure given by Bagley et al.[29] 5-Carbonitrilepyrimidines **6** and **7** were obtained by alkylation of 5-cyano-2,4-dichloropyrimidine with intermediate **1** (Scheme 2), while 4- and 6-nitrile substituted pyrimidines **8** and **9** were obtained following an alkylation−cyanation sequence starting from 4,6- and 2,4-dichloropyrimidine, respectively (Scheme 3).

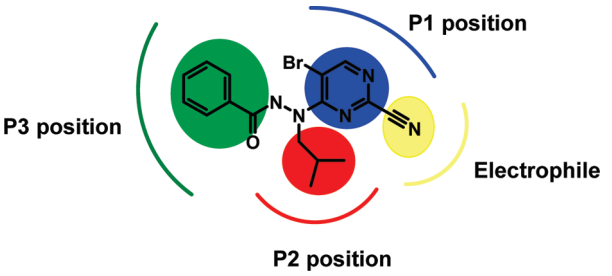


Figure 1. General structure of 2-pyrimidinecarbonitrile derivatives showing positions P1, P2, and P3.

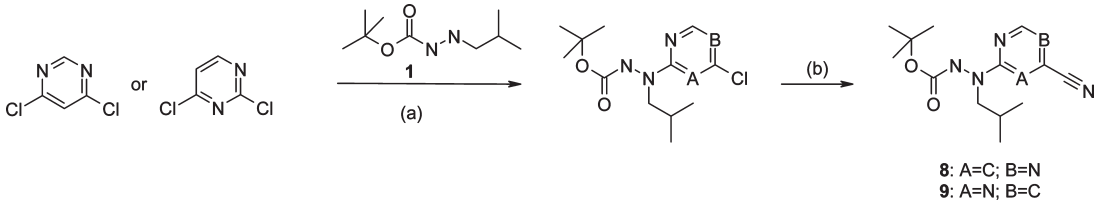
Triazine derivative **10** was synthesized using milder reaction conditions because of the reactivity of the starting 2,4-dichloro-6-methoxy-1,3,5-triazine (Scheme 4). Chlorine displacement with the hydrazido derivative **1** in the absence of base followed by cyanation using sodium cyanide afforded the desired product.

The synthesis of 2-pyrimidinenitrile **12** was accomplished using the synthetic route described in Scheme 5 through an alkylation and subsequent cyanation process starting from 2,6-dichloropyrimidine and compound **1**.

The *tert*-butyloxycarbonyl protected 2-pyrimidinenitriles presenting different substitutions in positions 5 and 6 of the ring are outlined in Schemes 6−13. Selection of the appropriate synthetic approach was made individually for each compound on the basis of commercial availability of the substituted pyrimidine precursors.

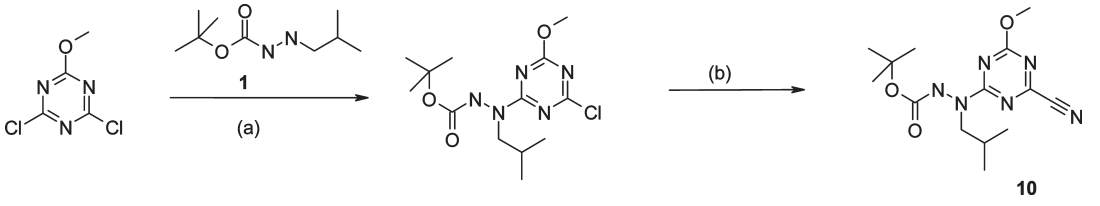
6-Substituted 2-cyanopyrimidines were prepared according to Schemes 6 and 7. 6-Substituted 2,4-dichloropyrimidines were used as starting materials, and chlorine displacement by the hydrazide derivative **1** was achieved following the literature procedure given by Bagley et al.[29] Intermediates **13a**,**b** were reacted with KCN in DMSO/H_2O to yield **14a**,**b**, respectively. 6-Methoxy substituted pyrimidine **17** was

Scheme 3. Synthetic Route for 4- and 6-Nitrile Substituted Pyrimidines[a]



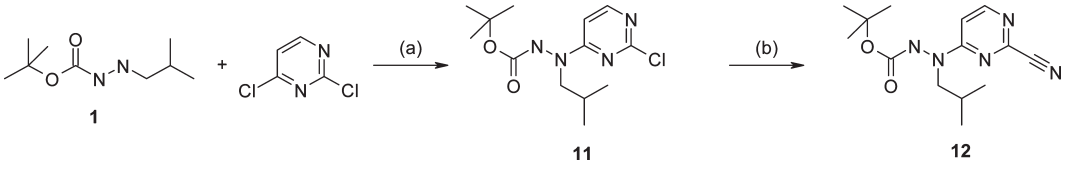
[a] Reagents and conditions: (a) DIPEA, toluene or isopropanol; (b) potassium cyanide, 1,4-diazabicyclo[2.2.2]octane, 85:15 DMSO−H_2O, 65−70 °C.

Scheme 4. Synthesis of Triazine Derivative **10**[a]



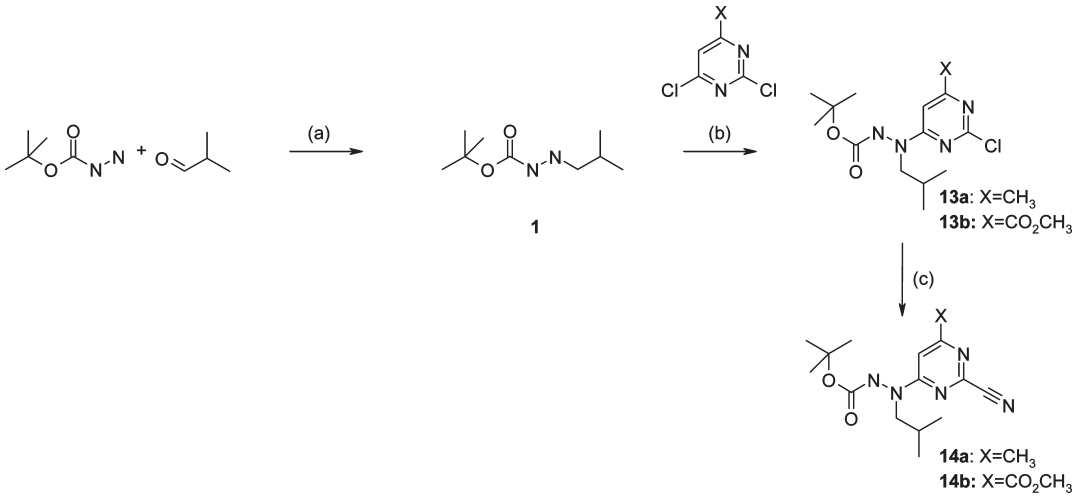
[a] Reagents and conditions: (a) THF, 0 °C to rt; (b) NaCN, DMSO.

Scheme 5. Synthetic Route for 2-Pyrimidinenitrile Derivative **12**[a]



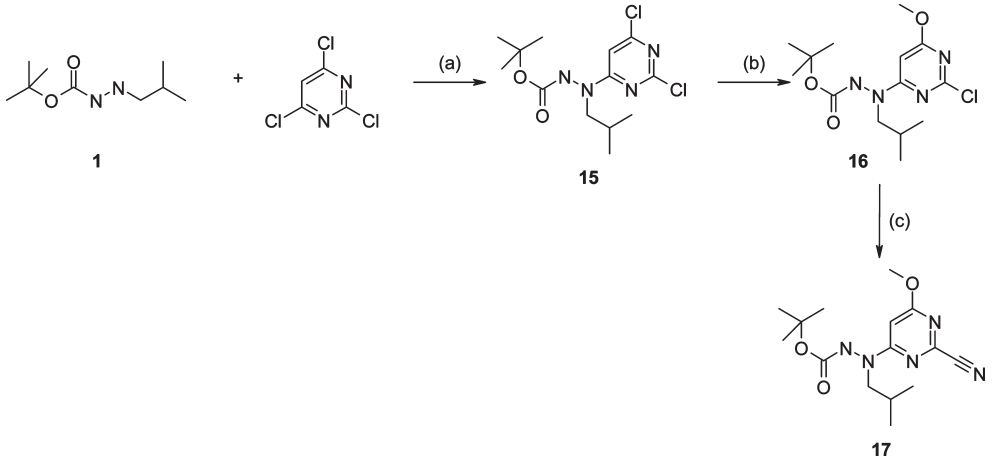
[a] Reagents and conditions: (a) triethylamine or DIPEA, EtOH or isopropanol; (b) potassium cyanide, 1,4-diazabicyclo[2.2.2]octane, 9:1 DMSO−H_2O.

Scheme 6. General Synthesis of 6-Substituted Pyrimidines[a]



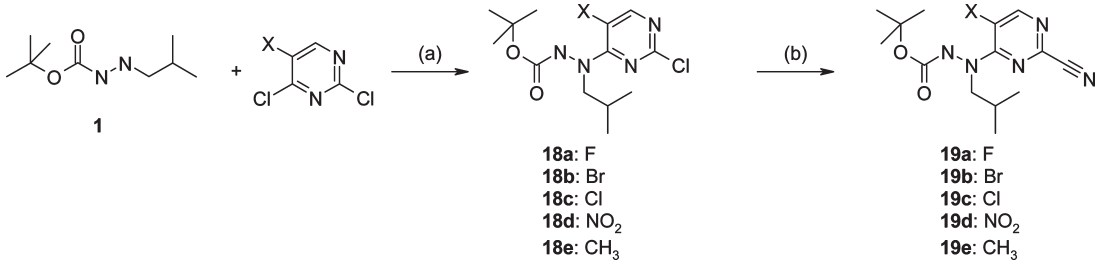
[a] Reagents and conditions: (a) *i*-PrOH, 0 °C → rt, then H_2, PtO_2, rt; (b) triethylamine, EtOH; (c) potassium cyanide, 1,4-diazabicyclo[2.2.2]octane, 85:15 DMSO−H_2O, 80−100 °C or tetraethylammonium cyanide, trimethylamine, THF, rt.

Scheme 7. Synthesis of 6-Methoxypyrimidine Derivative[a]



[a] Reagents and conditions: (a) EtOH, rt; (b) sodium methoxide, MeOH, rt; (c) tetraethylammonium cyanide, trimethylamine, ACN, rt.

Scheme 8. General Synthetic Route for 5-Substituted Pyrimidines[a]

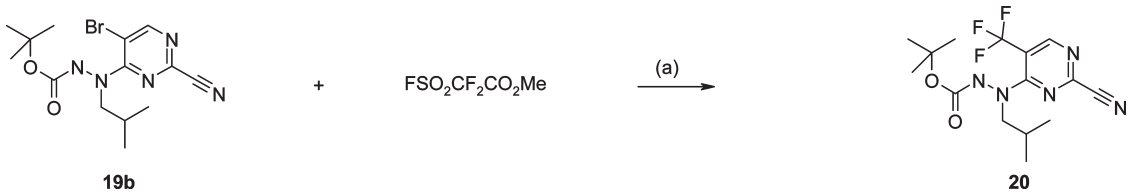


[a] Reagents and conditions: (a) triethylamine or DIPEA, EtOH or isopropanol; (b) potassium cyanide, 1,4-diazabicyclo[2.2.2]octane, 9:1 DMSO−H_2O.

synthesized (Scheme 7) by reaction of 2,4,6-trichloropyrimidine with the hydrazide intermediate **1** followed by chlorine displacement in intermediate **15** with sodium methoxide. Substitution at the 2-position was favored over the 6-position in a 9:2 ratio; however, no attempt was made to optimize these reaction conditions. Final cyanation of intermediate **16** took place using tetramethylammonium cyanide to yield compound **17**.

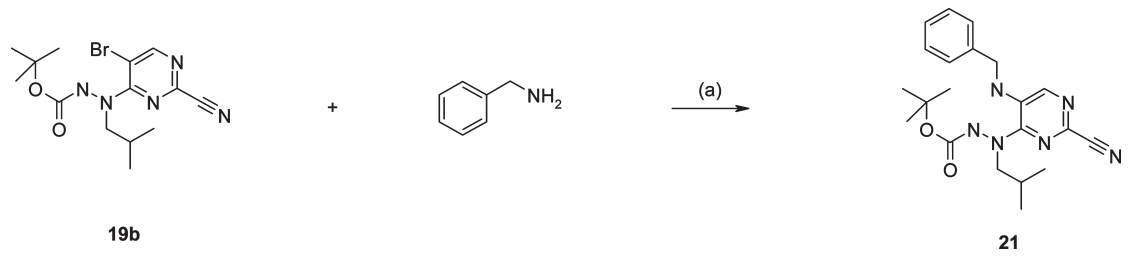
5-Substituted-2-cyanopyrimidine derivatives were prepared following the procedures described in Schemes 8−13. Whenever availability of starting materials made it possible, the chlorine atom at the 4-position was substituted by the hydrazide derivative **1** in a basic alcoholic medium. Remarkably, little or no substitution at the 2-position of the pyrimidine ring was observed with some of these derivatives, in particular halogens or nitro groups. Cyanation of intermediates

Scheme 9. Synthesis of 5-Trifluoromethyl Derivative[a]



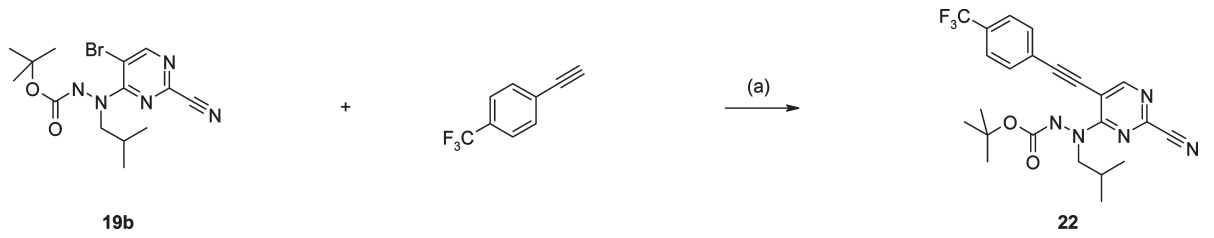
[a] Reagents and conditions: (a) copper(I) iodide, hexamethylphosphoramide, DMF, 80 °C.

Scheme 10. Synthesis of 5-Benzylamine Derivative[a]



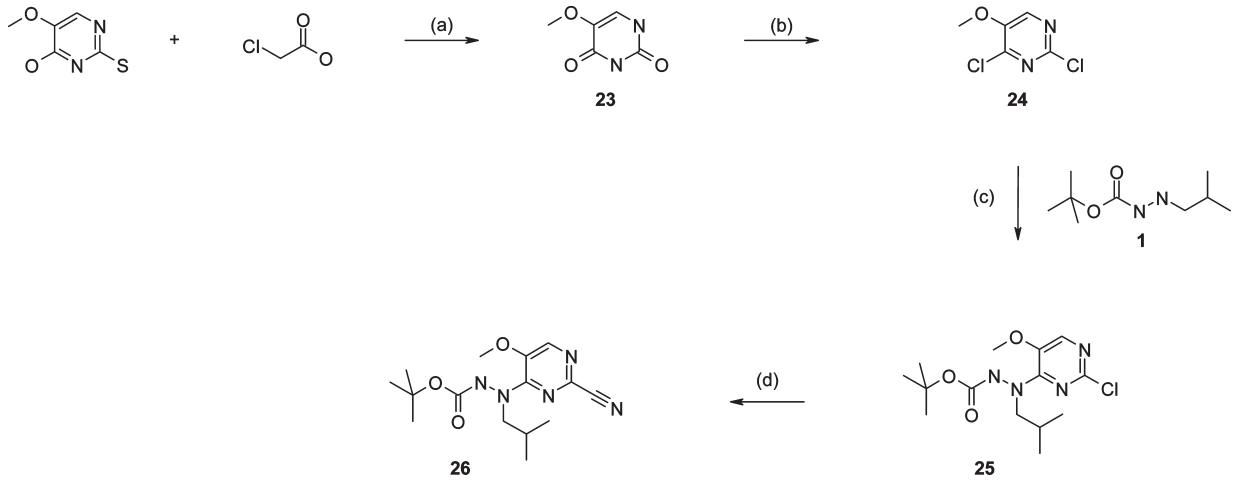
[a] Reagents and conditions: (a) $Pd(OAc)_2$, (±)-BINAP, K_2CO_3, DMF, microwave at 60 °C.

Scheme 11. Synthesis of 5-Alkyne Substituted Derivative[a]



[a] Reagents and conditions: (a) $Pd(PPh_3)_2Cl_2$, copper(I) iodide, Et_3N, DMF, 50 °C.

Scheme 12. Synthesis of 5-Methoxy Derivative[a]

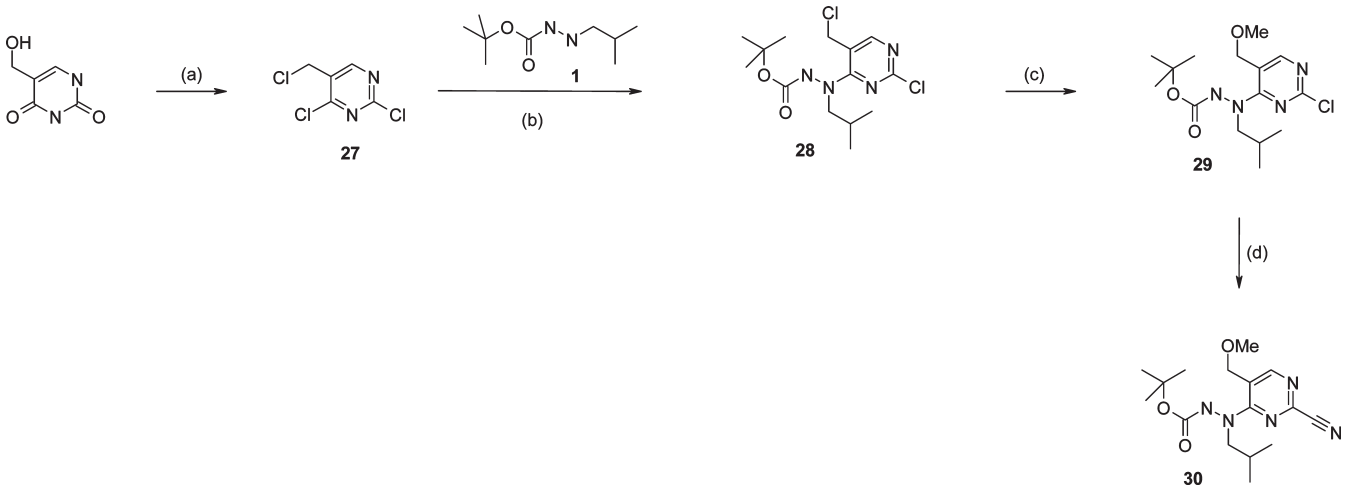


[a] Reagents and conditions: (a) conc HCl, H_2O, reflux; (b) $POCl_3$, dimethylaniline, reflux; (c) DIPEA, isopropanol, reflux; (d) potassium cyanide, 1,4-diazabicyclo[2.2.2]octane, 9:1 DMSO−H_2O, microwave at 60 °C.

18a−**e** using KCN afforded derivatives **19a**−**e**. A significant influence of the substituent present at the 5-position on the reaction temperature was observed.

By use of the corresponding 5-bromopyrimidine derivative **19b** as precursor, compounds **20**−**22** were prepared (Schemes 9−11). Reaction of **19b** with methyl 2,2-difluoro-2-(fluorosulfonyl)acetate in the presence of CuI and HMPA afforded the corresponding 5-trifluoromethyl derivative **20**, whereas coupling between **19b** and benzylamine using $Pd(OAc)_2$ and (±)-BINAP as catalyst and ligand, respectively, afforded compound **21** (Scheme 10). The preparation of **22** was performed by Sonogashira coupling of **19b** and commercially available 4-ethynyl-α,α,α-trifluorotoluene in the presence of $Pd(PPh_3)_2Cl_2$ and CuI catalysts. Cyclization

Scheme 13. Synthesis of 5-Methoxymethylene Derivative[a]



[a] Reagents and conditions: (a) $POCl_3$, PCl_5, reflux; (b) Cs_2CO_3, THF, rt; (c) MeONa, MeOH, rt; (d) potassium cyanide, 1,4-diazabicyclo[2.2.2]octane, 9:1 DMSO−H_2O, rt.

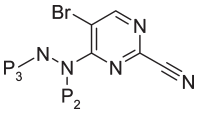


Figure 2. General structure of the P2 series of 2-cyanopyrimidine derivatives.

side products were observed when high palladium loadings were used and/or triphenylphosphine was used as ligand.

The corresponding 5-methoxy derivative **26** was synthesized starting out from 5-methoxy-2-sulfanyl-4-pyrimidinol (Scheme 12). Reaction with chloroacetic acid afforded the uracil derivative **23** which was then chlorinated using $POCl_3$ to yield the corresponding 2,4-dichloro-5-methoxypyrimidine **24**.[30] Subsequent chlorine displacement with hydrazide **1** and consecutive cyanation of intermediate **25** finally yielded compound **26**. The chlorine atom at the 2-position proved to be quite unreactive, requiring harsh but at the same time balanced reaction conditions to stop product decomposition. In an analogous manner, compound **30** was prepared from 5-(hydroxylmethyl)uracil hydrate (Scheme 13).[31] Prior to the final cyanation reaction, substitution at the chloromethylene end with MeONa to obtain intermediate **29** was accomplished.

Synthesis of *tert*-Butyloxy-2-pyrimidinenitrile Derivatives for Optimization of the P2 Position. The second step of the optimization process was the study of the P2 position (Figure 2). Following a similar approach to that used in the first step of the optimization process, substitutions at the P1 and P3 positions were fixed for comparative purposes as 2-nitrile-5-chloro(or bromo)-6-pyrimidyl and *tert*-butyloxycarbonyl, respectively. Synthesis of all derivatives included in this study, compounds **33**−**78**, was carried out following the procedure outlined in Scheme 14. Starting from the commercially available aldehydes or ketones, the corresponding imine was formed by reacting with *tert*-butyl carbazate, followed by reduction using H_2/PtO_2 or $NaBH_3CN$/AcOH. In those cases where the desired aldehyde was not available, it was prepared via Weinreb amide from the corresponding carboxylic acid or ester.

Derivative **37** was prepared from tetrahydro-2-furancarbaldehyde, reduction of the corresponding imine via hydrogenation resulting in an opening of the ring, thus yielding the corresponding hydrazine carboxylate which ultimately gave **37**. Likewise, compound **38** was synthesized from 2,2-dimethyloxirane, the corresponding hydrazinecarboxylate being formed via epoxide opening.

The corresponding hydrazine carboxylate derivative **31** was reacted with 5-bromo-2,4-dichloropyrimidine according to the literature procedure given in Bagley et al.[29] The corresponding intermediates **32** were reacted with KCN in DMSO−H_2O to yield compounds **33**−**78**, respectively.

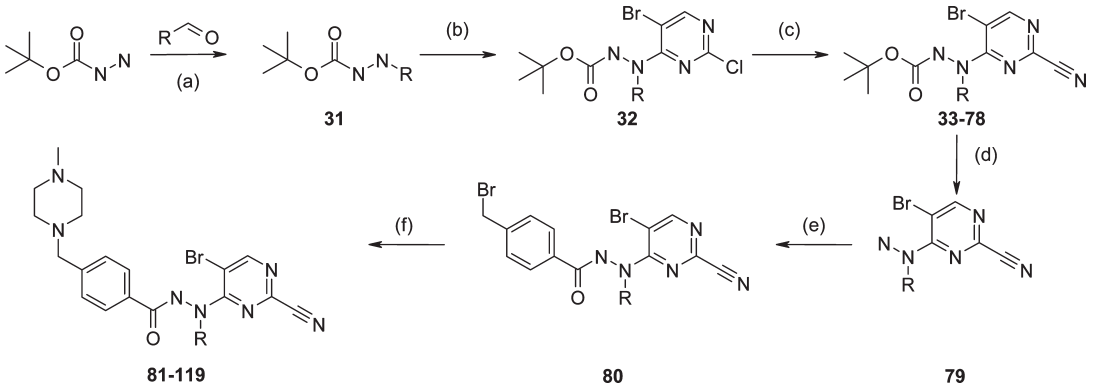
Derivatives **79** were obtained by hydrazine deprotection using *p*-toluenesulfonic acid, and subsequent coupling with 4-bromomethylbenzoyl bromide yielded the corresponding derivatives **80**. Nucleophilic substitution using *N*-methylpiperazine yielded the amine containing products **81**−**119**.

Synthesis of Isobutyl-2-pyrimidinenitrile Derivatives for Optimization of the P3 Position. For the optimization of the P3 position, the previously optimized substituents at positions P1 (2-nitrile-5-chloro(or bromo)-6-pyrimidyl) and P2 (2-methylpropyl) were selected (Figure 3). Derivatives **121**−**269** were synthesized following the procedure outlined in Scheme 15, analogous to that used for compounds **33**−**78**. Hence, starting from the commercially available isobutyraldehyde, the corresponding imine was formed by reaction with *tert*-butyl carbazate, followed by reduction using $NaBH_3CN$/AcOH. Then the corresponding hydrazinecarboxylate derivative **1** was reacted either with 5-bromo-2,4-dichloropyrimidine or 2,4,5-trichloropyrimidine[29] to yield the corresponding intermediates **18b**−**c** which were reacted with KCN in DMSO−H_2O to give compounds **19b** and **19c**, respectively. Intermediate **120** was obtained by hydrazine deprotection with *p*-toluenesulfonic acid, and subsequent coupling with the corresponding acid chloride or carboxylic acid yielded the final products **121**−**269**.

Results and Discussion

The known structures of cysteine proteases, in which a central nucleophilic moiety (SH group of the cysteine) is flanked by two sets of pockets where interactions with the side chains of peptidic substrates take place, make the rational design of potent enzymatic inhibitors a challenging task because a compound may interact with either or both sets of

Scheme 14. General Synthetic Procedure for 2-Cyanopyrimidines[a]



[a] Reagents and conditions: (a) aldehyde or ketone, $NaBH_3CN$, AcOH, rt; (b) 5-bromo-2,4-dichloropyrimidine, DIPEA, *i*-PrOH; (c) potassium cyanide, 1,4-diazabicyclo[2.2.2]octane, 9:1 DMSO−H_2O; (d) *p*-toluenesulfonic acid, ACN, rt; (e) 4-bromomethylbenzoyl bromide, DIPEA; (f) *N*-methylpiperazine, DIPEA, THF, rt.

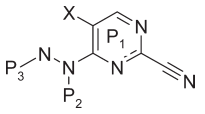


Figure 3. General structure of the P3 series of 2-cyanopyrimidine derivatives.

pockets. X-ray structures of complexes between proteases and small inhibitors are a useful tool for the design of improved inhibitors.

Cocrystallization of falcipains with small molecules to obtain diffraction quality crystals has proven to be difficult, hampering the rational design of potent inhibitors of these enzymes. However, a few X-ray structures of falcipain−inhibitor complexes have been published.[32−34] With limited available structures, we used an empirical optimization process starting from the electrophilic nitrile group and then considering the P1, P2 and P3 positions to obtain potent inhibitors of the falcipains.

Optimization Studies on the P1 Position. In order to study the influence of the heteroaromatic ring on the nitrile electrophilic nature, several derivatives were prepared and tested (Table 1). 2-Cyanopyridine derivative **4** presented submicromolar inhibition of falcipain-2 but proved to be inactive versus falcipain-3. When pyrazine was used instead, compound **5** demonstrated tens of nanomolar activity against falcipain-2 and micromolar activity against falcipain-3.

If the pyrazine was replaced by a pyrimidine ring, greater variability was found depending on the position of the nitrile group. No activity was observed when the hydrazine derivative and the cyano groups occupied positions 4 and 6, respectively (compound **8**), whereas falcipain-2 was moderately inhibited by derivative **6**, which bears the nitrile group at position 5, the hydrazine moiety at position 4, and a chlorine atom at the 2-position. On the other hand, better results were obtained when the cyano group was at the 5-position, the chlorine atom at position 4, and the hydrazine derivative at position 2 (compound **7**), with nanomolar inhibition against falcipain-2. The 2-cyano-4-hydrazinopyrimidine derivative **12** was the most potent inhibitor of this series, with submicromolar inhibition of both falcipain enzymes.

The importance of the relative positioning between the cyano and hydrazine groups can be illustrated by comparing compounds **6** and **7**. Both derivatives present the cyano group at the 5-position, although enhancement of the inhibitory capacity was observed when the hydrazine derivative was placed at position 2 and one of the pyrimidine nitrogen atoms was between the substituents. A similar effect, although milder, was observed for **9**, with the nitrile group at position 4 and hydrazine group at position 2, and for **12**, with the nitrile group at position 2 and hydrazine group at position 4. The latter derivative presented enzymatic inhibition values 3−5 times greater than the former.

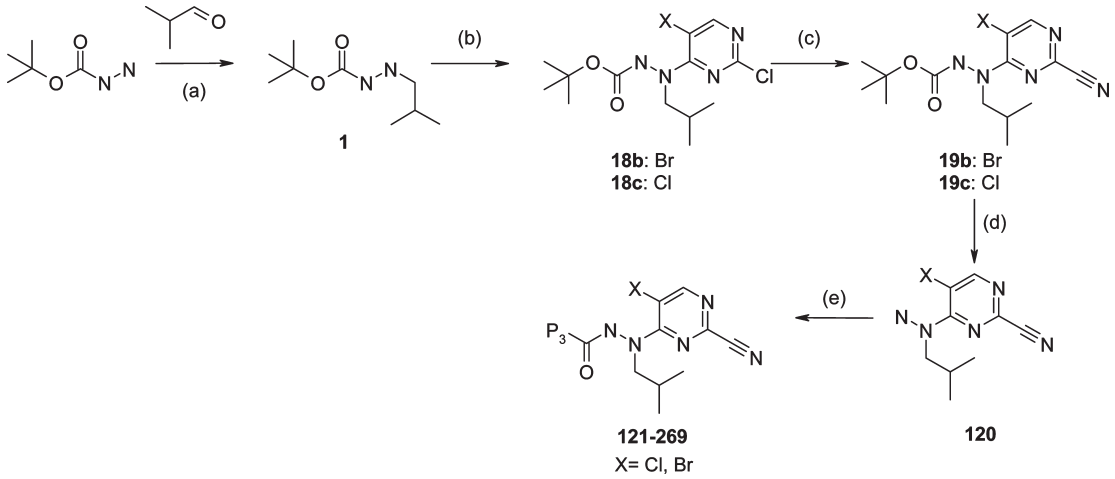
Replacement of the heteroaryl group by a triazine (compound **10**) yielded the most active compound of this set (Table 1), with activity against falcipain-2 in the tens of nanomolar range and against falcipain-3 in the hundreds of nanomolar range. These data correlate well with the different calculated electrophilicities of the cyano groups anticipated by Oballa et al.;[35] a nitrile substituent in a triazine moiety was an excellent electrophile, whereas the electrophilicity of a pyridine derivative was calculated as modest. Among pyrimidine derivatives, a nitrile group at the 2-position had the highest calculated electrophilicity.

Results in Table 1 indicate a clear advantage in terms of enzymatic activity for the triazine and 2-pyrimidine nitrile motifs, with values in the submicromolar range against both falcipain enzymes. The limited variability of results for substitution around the triazine moiety prompted us to focus our efforts on substitution around positions 5 and 6 of the pyrimidine ring in order to identify an improved scaffold for the inhibition of falcipains.

The effect of substitution at the 6-position of the pyrimidine ring on the enzymatic activity of these compounds is reflected in Table S1 (Supporting Information). Insertion of methyl or methoxy groups (compounds **14a** and **17**, respectively) had little effect on the inhibitory capacity of these compounds compared to the reference compound **12**; slightly decreased inhibition of falcipain-3 was observed in the case of the 6-methyl derivative. A moderate improvement in activity, 2 and 3 times against falcipain-2 and falcipain-3, respectively, was observed with the ester group in **14b**. Thus, substitution in position 6 of the pyrimidine ring did not exert enough influence on the enzymatic potency of 2-cyanopyrimidine derivatives to consider further studies on this position.

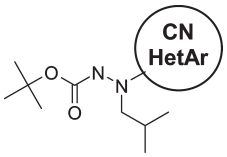
A greater influence on the enzymatic activity of these derivatives was observed when the substituent was placed at the 5-position (Table 2). An overall loss of falcipain inhibition was observed with a methoxymethylene group, **30**,

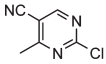
Scheme 15. General Synthetic Procedure for 2-Cyanopyrimidines[a]



[a] Reagents and conditions: (a) $NaBH_3CN$, AcOH, rt; (b) 5-bromo-2,4-dichloropyrimidine or 2,4,5-trichloropyrimidine, *N*,*N*-diisopropylethylamine, *i*-PrOH; (c) potassium cyanide, 1,4-diazabicyclo[2.2.2]octane, 9:1 DMSO−H_2O; (d) *p*-toluenesulfonic acid, ACN, rt; (e) P_3 derivative as acid or acid chloride.

Table 1. Effect on Falcipain Activity of Different Heterocyclic Rings

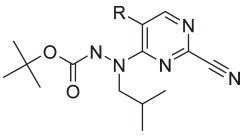


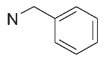
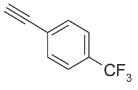
COMPOUND[a]	HetAr	FP2 IC50 (μM)	FP3 IC50 (μM)
4		0.609	>10
5		0.059	1.67
6		4.53	N.D.
7		0.064	0.99
8		>10	>10
9		0.196	3.57
10		0.012	0.204
12		0.060	0.870

[a] All compounds were characterized and purity was assessed using ^1H NMR and LCMS.

and to a lesser extent with an alkyne group, **22**, with falcipain-3 remarkably affected by these changes. Other electron-donating groups, such as benzylamine, **21**, or methoxy, **26**, also resulted in less active compounds, whereas a methyl substituent as in **19e** had a minimal impact on activity, correlating well with the electron-donating capacity of these functional groups.

Table 2. Cyanopyrimidines: Influence of the Substituent at Position-5 on in Vitro Enzymatic Activity

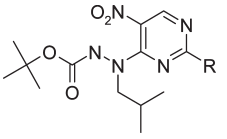


COMPOUND	R	FP2 IC50 (μM)	FP3 IC50 (μM)	σ_p
12	-H	0.060	0.870	0
19a	-F	0.024	0.583	0.15
19b	-Br	0.003	0.092	0.26
19c	-Cl	0.009	0.144	0.24
19d	$-NO_2$	0.002	0.026	0.81
19e	$-CH_3$	0.040	1.150	-0.14
20	$-CF_3$	0.003	0.066	0.53
21		0.616	2.07	-0.63[a]
22		0.102	5.33	
26	$-CH_3O$	0.079	2.39	-0.28
30	$-CH_2OCH_3$	8.40	>10	

[a] This value is in fact for a dimethylamino group, but it has been used for comparative purposes.

An enhancement of the inhibitory capacity of 2-cyanopyrimidine derivatives was observed for compounds bearing electron-withdrawing groups, **19a**−**d** and **20**. Within the 5-halogen derivatives, a 2- and 6-fold improvement against both enzymes was observed with fluorine and chlorine

Table 3. Comparison between 2-Chloro- and 2-Cyanopyrimidine Derivatives



compound	R	FP2 IC_{50} (μM)	FP3 IC_{50} (μM)
18d	Cl	> 25	> 25
19d	CN	0.002	0.026

substituents, whereas the bromine atom proved to have a greater effect, with an inhibitory capacity 10- and 20-fold better against falcipain-3 and falcipain-2, respectively. Replacement of Br by CF_3, **20**, yielded only a marginal increase in activity. A greater boost in activity was observed when a nitro group was introduced, **19d**, leading to a compound that was 30 times more potent.

Our inhibitor results correlated reasonably well with the Hammett parameter, σ_p, described for 4-substituted benzene rings,[36] suggesting that these substituents could be modulating the electrophilic nature of the nitrile group. Hence, augmentation of the electrophilic character of the nitrile group resulted in a more potent interaction with the nucleophilic thiol group of the falcipain enzymes, which in turn translated into better inhibitory activity. However, these observations raised concerns regarding inhibition due to the liberation of cyanide caused by the displacement of the cyano by the thiol group of the falcipains, an alternative to the expected mechanism of action in which the nucleophilic attack takes place on the carbon atom of the nitrile unit. In order to discard this hypothesis, the antiparasitic activity of intermediate **18d**, which would liberate chloride and thus be active if this hypothesis was confirmed, was tested against both falcipain enzymes (Table 3). The compound was inactive, thus proving that the observed activity was not triggered by cyanide release.

In summary, the most promising and developable nitrile-containing moieties in the P1 position were 2-cyano-5-chloro-6-pyrimidyl and 2-cyano-5-bromo-6-pyrimidyl. These two chemical units were used in subsequent optimization steps as the preferred P1 substituents.

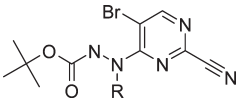
Optimization Studies on the P2 Position. For P2, in an attempt to optimize interaction with the S2 pocket, a wide range of different structural motifs were tested, while the *tert*-butyloxycarbonyl group was maintained as the substituent in position P3.

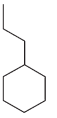
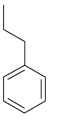
In order to study the depth of the S2 pocket in falcipain-2 and falcipain-3, compounds with different linear aliphatic substituents were synthesized and evaluated (Table S2, Supporting Information). Our results indicated that the S2 pocket of both enzymes accommodates well linear groups with up to five carbon atoms, with the optimal interaction observed when the chain was four carbon atoms long (**34**) and diminished inhibition with the six carbon atom substituent (**36**). Interestingly, substitution of the last carbon atom in compound **36** by a polar group (OH) in compound **37** led to the recovery of enzymatic activity up to the levels observed for compound **34**, suggesting the possibility of a polar interaction at the end of the S2 pocket.

In order to study the width of the S2 pocket in falcipain-2 and falcipain-3, compounds with different branched aliphatic chains were synthesized and evaluated (Table S3, Supporting Information). Not surprisingly, based on prior data, the leucine-like isobutyl moiety (compound **19b**) was the most potent derivative against both enzymes in the branched aliphatic chain class. Further changes in polarity (compounds **19b** and **38**), branching distance (compounds **19b**, **39** and **46**) or length of branches in either asymmetrical (compounds **19b**, **44**, and **45**) or symmetrical (compounds **19b**, **41**, **42**, and **43**) compounds, led to either similar inhibition profiles or loss of enzymatic activity but not to an improvement in activity. Hence, it seemed that additions to the chains should not be larger than a methylene, as larger moieties did not seem to fit in the P2 pocket. On the other hand, when the branched chain was directly attached to the nitrogen atom (**46**, **47**), a loss of activity was observed against both enzymes, even if the substituents were as small as a methyl group, as in **46**.

Replacement of the acyclic moiety in compound **19b** by alicyclic substituents (Table 4) showed that enzymatic

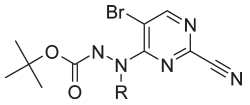
Table 4. Effect on in Vitro Falcipain Activity of Different P2 Alicyclic Substituents



COMPOUND[a]	R	FP2 IC_{50} (μM)	FP3 IC_{50} (μM)
48		0.007	0.413
49		0.003	0.062
50		**0.001**	**0.010**
51		0.024	0.146
52		0.025	0.121
53		0.257	0.860
54		0.064	0.504
55		0.017	0.347
56		0.093	0.557
57		0.002	0.035
58		0.002	0.041
59		0.012	0.311

[a] All compounds were characterized and purity was assessed using ^1H NMR and LCMS.

Table 5. Effect on in Vitro Falcipain Activity of Different P2 Cycloalkyl Substituents Directly Attached to the Nitrogen Atom



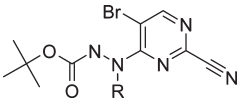
COMPOUND[a]	R	FP2 IC_{50} (μM)	FP3 IC_{50} (μM)
60		0.013	0.297
61		0.009	0.056
62		**0.001**	**0.008**
63		0.034	0.447
64		0.003	0.053
65 Cis-		0.058	0.407
66 Trans-		0.003	**0.006**
67		0.002	0.021

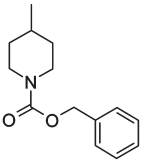
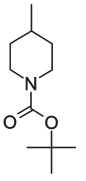
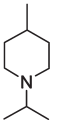
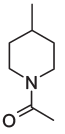
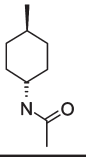
[a] All compounds were characterized and purity was assessed using ^{1}H NMR and LCMS.

activity against falcipain-2 and falcipain-3 increased with ring size up to the five-atom cycle (compounds **48**−**50**) and then decreased for the six-membered ring (compound **51**). Hence, the optimal interaction with the enzyme was found for cyclopentyl derivative **50**, with inhibition in the low nanomolar range. Further lengthening of the aliphatic moiety with an extra methylene (compounds **53** and **54**) led to a loss of activity against both enzymes. In addition, the use of aromatic moieties had a detrimental effect (**55** and **56**), and the introduction of unsaturation yielded mixed results with respect to the saturated compounds, with a similar activity profile for the five-membered rings (compare compounds **50** and **57**) and improvement in the case of the six-membered rings (compare compounds **51** and **58**).

Suppression of the methylene spacer yielding alicyclic moieties directly attached to the nitrogen atom maximized interaction with the lipophilic S2 pocket of the falcipains (compounds **60**−**67**, Table 5). Results obtained for these alicyclic compounds were somewhat surprising when compared with those for the corresponding acyclic derivatives (compounds **46** and **47**, Table S3 in Supporting Information), and an explanation for these differences remains elusive. The most favorable result was achieved with the cyclohexyl group (**62**), showing similar activity to that displayed by **50**. Introduction of methyl substituents in the cyclopentyl ring (**63**−**66**) only showed a clear positive effect when present at the 2-position trans to the nitrogen atom (compound **66**), and this effect was most pronounced against falcipain-3. The presence of unsaturation in the cyclopentyl ring (compound **67**) did not affect activity strongly.

Table 6. Effect on in Vitro Falcipain Activity of Different P2 Heterocyclic Substituents



COMPOUND[a]	R	FP2 IC_{50} (μM)	FP3 IC_{50} (μM)
68		0.014	0.112
69		0.007	0.098
70		0.008	0.085
71		0.008	0.140
72		0.006	0.183
73		**0.002**	**0.028**
74		0.002	0.038
75		0.026	0.135
76		0.902	5.235
77		**<0.0005**	**0.033**
78		0.007	0.067

[a] All compounds were characterized and purity was assessed using ^{1}H NMR and LCMS.

The study of heterocyclic moieties in the P2 position included oxygen-containing (compounds **68**−**73**) and nitrogen-containing (compounds **74**−**78**) derivatives (Table 6). The presence of oxygen yielded similar inhibitory capacities regardless of the nature of the cyclic moiety (aliphatic, aromatic, tetrahydrofuran or tetrahydropyran derivatives), with activities in the low nanomolar range against falcipain-2 and in the high tens to low hundreds of nanomolar range

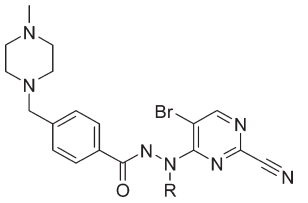
against falcipain-3. The nitrogen-containing group of inhibitors showed very different results depending on the nature of the nitrogen atom involved in the interaction. Good to excellent activity profiles were observed for compounds containing low polarity nitrogen atoms, as **74**, **77**, or **78**, and very modest activity profiles were seen for compounds where the nitrogen atom presented a more polar character, as in compound **76**. The latter result is not surprising given the known preference for leucine, reflecting the lipophilic character of the P2 pocket in related cysteine proteases.[37] Overall, the most potent inhibitors against both enzymes with heterocyclic moieties were the tetrahydropyran derivative directly attached to the nitrogen atom, **73**, and the corresponding *N*-acetylpiperidine derivative **77**, which was a picomolar falcipain-2 inhibitor.

In summary, some very active compounds against both falcipain enzymes (low nanomolar or picomolar inhibitors of falcipain-2 and units of nanomolar or low tens of nanomolar inhibitors of falcipain-3) were identified with modifications of position P2 of 2-cyano-5-bromo-6-pyrimidyl derivatives having a *tert*-butyloxycarbonyl group in the P3 position and using compound **19b** (P2: isobutyl) as reference. Preferred substitution at position P2 included the branched alkyl group cyclopentylmethyl (compound **50**; FP2 IC_{50} = 0.001 μM, FP3 IC_{50} = 0.01 μM), the cycloalkyl group directly attached to the nitrogen atom cyclohexyl (compound **62**; FP2 IC_{50} = 0.001 μM, FP3 IC_50 = 0.008 μM), the cycloalkyl group directly attached to the nitrogen atom *trans*-2-methylcyclopentyl (compound **66**; FP2 IC_{50} = 0.003 μM, FP3 IC_{50} = 0.006 μM), the heterocyclic group directly attached to the nitrogen atom tetrahydropyran (compound **73**; FP2 IC_{50} = 0.002 μM, FP3 IC_{50} = 0.028 μM), and the heterocyclic group directly attached to the nitrogen atom *N*-acetyl-4-piperidine (compound **77**; FP2 IC_{50} < 0.0005 μM, FP3 IC_{50} = 0.033 μM).

All of the compounds described above, despite their promising enzymatic inhibitory capacities, showed only modest antiparasitic activity in the micromolar range (data not shown). However, we had previously noticed that antiparasitic inhibitory capacity within the nitrile family improved when amino groups protonable at the food vacuole pH (4.0−6.0) were present. Lysosomatropism,[38] defined as the ability of compounds to accumulate in acidic compartments, is a property of weakly basic, lipophilic molecules. These neutral species are able to freely diffuse across the cell membrane but become membrane impermeable once protonated within the acidic compartment. The degree of compound accumulation is dependent on its pK_a and the pH difference between the cytosol and the lysosome (or food vacuole). In addition, compound lipophilicity can affect accumulation because of aggregation or interaction with lipid membranes. In order to test this "amine hypothesis" with the 2-cyanopyrimidine class, a benzylic *N*-methylpiperazinyl moiety was introduced in position P3 of the general structure and a similar SAR exploration of the P2 position was carried out for this chemical class. Activities against falcipain-2 and falcipain-3 and against cultured *P. falciparum* were tested (Tables 7−10, Table S4 Supporting Information).

These in vitro results confirmed the previously observed tendencies in terms of the best possible substituents for the P2 position. In addition, introduction of a protonable amine consistently led to a marked improvement in activity against cultured parasites, confirming the "amine hypothesis". When linear alkyl substituents were introduced (Table S4, Supporting Information), the best results in terms of enzymatic and antiparasitic activities were observed with the *n*-butyl chain (compound **82**). An improvement in enzymatic inhibition was observed when compared to *tert*-butyloxycarbonyl derivatives. The improvement was generally moderate but more noticeable for the *n*-hexyl derivative **84** (compare with compound **36**).

Table 7. Effect on in Vitro Antiparasitic Activity of Different P2 Branched Alkyl Substituents



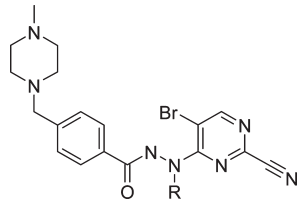
COMPOUND[a]	R	FP2 IC_{50} (μM)	FP3 IC_{50} (μM)	Pf W2 IC_{50} (μM)
85		**0.0004**	**0.055**	**0.015**
86		0.019	0.158	0.409
87		0.004	0.068	0.262
88		0.003	0.039	1.50
89		0.002	0.044	0.194
90		0.081	0.802	1.20
91		0.293	0.656	0.566
92		**0.0009**	**0.035**	**0.024**
93		0.002	0.052	0.272
94		0.029	1.37	0.401
95		0.187	2.04	1.61

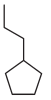
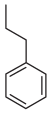
[a] All compounds were characterized and purity was assessed using ^{1}H NMR and LCMS.

In the case of branched alkyl substituents (Table 7), again the isobutyl group represented an advantageous interaction with the S2 pocket (compound **85**). Further augmentation of steric hindrance, as in the *tert*-butyl group (compound **86**), resulted in a loss of inhibitory capacity, especially against falcipain-3 and cultured parasites. Elongation of the methylene spacer as in **87** and **88** did not affect enzymatic activities as much as antiparasitic activities, the same effect being observed when the branch lengths were prolonged (**89**−**91**). Interaction with the lipophilic S2 pocket was not affected when only one of the branches was extended, with activities in the tens of nanomolar range (**92**). Further elongation or introduction of hindrance around the nitrogen atom (**93**−**95**) led to a diminution in potency. Overall, the introduction of the *N*-methylpiperazine moiety in these derivatives yielded more potent enzymatic inhibitors than *N*-*tert*-butyloxycarbonyl derivatives, which in turn rendered better inhibitors of parasite growth.

When cycloalkyl substituents were introduced (Table 8), improved enzymatic potencies over the *N*-*tert*-butyloxycarbonyl derivatives were again observed; the most noticeable effects

Table 8. Effect on in Vitro Antiparasitic Activity of Different P2 Cycloalkyl Substituents



COMPOUND[a]	R	FP2 IC_{50} (μM)	FP3 IC_{50} (μM)	*Pf*W2 IC_{50} (μM)
96		0.005	0.330	1.08
97		0.003	0.111	0.438
98		**0.0005**	**0.006**	**0.033**
99		0.002	0.016	0.263
100		0.005	0.017	1.49
101		0.004	0.045	0.177
102		0.005	0.335	2.37
103		0.023	0.105	2.18
104		0.0007	0.016	0.098

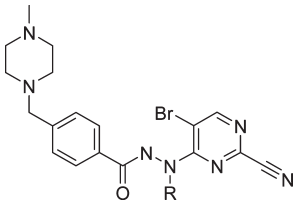
[a] All compounds were characterized and purity was assessed using ^{1}H NMR and LCMS.

were with compounds **99**−**101**. The cyclopentylmethyl derivative **98** and its unsaturated analogue **104** confirmed the tendency previously observed when no protonable amine was present (compounds **50** and **57**, respectively). These compounds were very potent enzyme inhibitors and showed good antiparasitic activities.

Our results also confirmed the advantage of having the cycloalkyl substituent directly attached to the nitrogen atom (Table 9) over the corresponding open chain substituent (compare, for example, compounds **105** and **94** or compounds **106** and **95**), as previously observed for the *N*-*tert*-butyloxy derivatives. Improvements in activity against both falcipains and cultured parasites were observed. Among the most active compounds identified in this group of compounds, equivalent potencies were observed for cyclohexyl and cyclopentyl derivatives (**106** and **107**), while the most potent derivative resulted from the introduction of a *trans*-methyl substituent at the 2-position of the cycloalkyl ring (**111**), yielding picomolar activity against falcipain-2 and single-digit nanomolar inhibition against falcipain-3 and against cultured parasites.

The introduction of a protonable amine on derivatives bearing heterocyclic substituents at P2 (Table 10) again had little effect on enzymatic activities when compared to the corresponding *N*-*tert*-butyloxy derivatives but led to markedly improved antiparasitic activity. The most potent derivatives of this group were the tetrahydropyran and *N*-acetylpiperidine (**117** and **118**), with activities in the picomolar range against falcipain-2 and in the tens of nanomolar range against both falcipain-3 and cultured parasites.

Table 9. Effect on in Vitro Antiparasitic Activity of Different P2 Cycloalkyl Substituents Directly Attached to the Nitrogen Atom



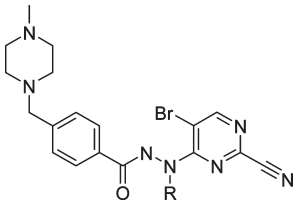
COMPOUND[a]	R	FP2 IC_{50} (μM)	FP3 IC_{50} (μM)	*Pf*W2 IC_{50} (μM)
105		0.002	0.173	0.090
106		**<0.0005**	**0.011**	**0.024**
107		**0.0005**	**0.006**	**0.027**
108		0.002	0.110	0.104
109		0.0009	0.045	0.068
110	 Cis-	0.002	0.041	0.082
111	 Trans-	**0.0002**	**0.003**	**0.005**

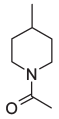
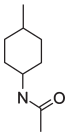
[a] All compounds were characterized and purity was assessed using ^{1}H NMR and LCMS.

In summary, the S2 subsite showed preference for a number of substituents in the P2 position, including 2-methylpropyl (compound **19b**), cyclopentylmethyl (compounds **50** and **57**), or cyclopentyl (compounds **61**, **64**, **66**, and **67**), all presenting enzymatic activities in the low nanomolar range against falcipain-2 and in the tens of nanomolar range against falcipain-3, with antiparasitic activities in the micromolar range. The introduction of a protonable amine markedly boosted the antiparasitic activity of these derivatives, with all being active against cultured parasites in the tens of nanomolar range (compounds **85**, **98**, **106**, **109**, and **111**). Furthermore, the protonable amine series confirmed the previously observed S2 subsite SAR for enzyme inhibition.

Optimization Studies on the P3 Position. The last step of the optimization process was the study of position P3. Substitution in the P1 position was kept as 2-cyano-5-chloro-(or bromo)-6-pyrimidyl on the basis of the results obtained in the optimization studies described above. Compounds bearing a phenyl substituent in position P3 demonstrated activity at units of nanomolar against falcipain-2 and tens of nanomolar against falcipain-3, regardless of the halogen substituent at the 5-position of the pyrimidine ring (compounds **121** and **122**, Table S5 Supporting Information). Selection of the most appropriate substitution at position P2

Table 10. Effect on in Vitro Antiparasitic Activity of Different P2 Heteroatom Containing Cycloalkyl Substituents



COMPOUND[a]	R	FP2 IC_{50} (μM)	FP3 IC_{50} (μM)	*Pf*W2 IC_{50} (μM)
112		0.014	0.099	1.88
113		0.002	0.075	0.193
114		0.011	0.135	0.310
115		0.004	0.180	0.721
116		0.002	0.133	0.333
117		**0.0009**	**0.017**	**0.059**
118		**<0.0005**	**0.026**	**0.028**
119		0.008	0.088	1.31

[a] All compounds were characterized and purity was assessed using ^{1}H NMR and LCMS.

was more problematic because of the number of available substituents presenting similarly good enzymatic and whole-cell activity profiles. We decided that the 2-methylpropyl moiety was the best balanced substituent among the potential candidates, considering lack of chirality, low lipophilicity, low molecular weight, and low predicted cost of goods. It is worth noting that whole-cell activity was as important as the enzymatic activity profile in our considerations because it could reflect the contribution of a substituent to membrane permeability or transport to the target enzyme, and this point was crucial for selection of 2-methylpropyl as the substituent in position P2. Thus, position P3 was studied using derivatives with the 2-cyano-5-chloro (or bromo)-6-pyrimidyl unit at position P1 and the 2-methylpropyl unit at position P2.

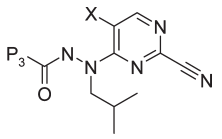
When six-membered aromatic rings were used (Table S5 Supporting Information, compounds **121**−**132**), no improvement of enzymatic activity with regard to reference compounds **121** and **122** was obtained, although some interesting trends could be observed. Substitution in the 4-position of the phenyl ring (compound **123**) did not affect the activity profile, while substitution in the 3-position of the ring (compounds **124** and **125**) led to a significant loss of activity versus both falcipain enzymes. The opposite effect was observed with replacement of one carbon atom of the phenyl ring by nitrogen; the activity profile of the 4-pyridyl derivative (compound **126**) was clearly worse than that for the 3-pyridyl derivative (compound **128**), and the 2-pyridyl derivative **129** was the best inhibitor of both enzymes in this series. Replacement of a second carbon atom by nitrogen did not affect activity against falcipain-2 but led to some differences in activity versus falcipain-3, as shown for compounds **130**, **131**, and **132**.

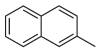
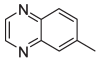
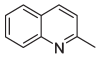
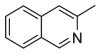
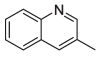
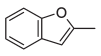
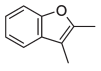
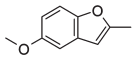
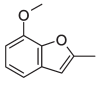
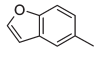
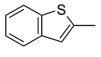
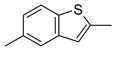
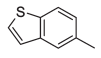
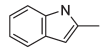
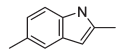
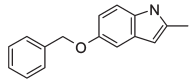
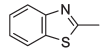
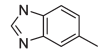
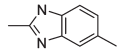
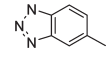
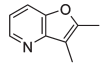
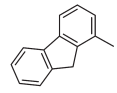
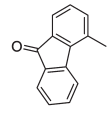
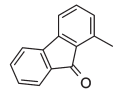
In the case of five-membered heteroaromatic rings (Table S6 Supporting Information, compounds **133**−**150**), most of the compounds studied showed activities against falcipain-2 in the tens of nanomolar range and against falcipain-3 in the tens to low hundreds of nanomolar range regardless of the heteroatom present (oxygen, sulfur, or nitrogen, e.g., compounds **133**, **136**, and **139**, respectively), the number of heteroatoms (one or two, e.g., compounds **139** and **146**), or their disposition within the ring (2- or 3-, e.g., compounds **133** and **134** or compounds **136** and **137**). Exceptions to this rule were compounds **147** and **150**. Derivatives bearing two heteroatoms in a relative 1,3-position (compounds **141**−**146**) exhibited similar activities. On the other hand, when the heteroatoms had a relative 1,2-disposition (compounds **147**−**150**), an enhancement of their enzymatic inhibitory capacity was observed when the oxygen atom was adjacent to the carbonyl group (compounds **148** and **149**).

The use of fused (hetero)-aromatic rings yielded variable results (Table 11, compounds **151**−**179**). When both rings were six-membered (compounds **151**−**157**), the introduction of nitrogen atoms distant relative to the carbonyl moiety ring (compound **152**) had a detrimental effect against falcipain-3. Similar activities were observed when the nitrogen atom was placed in the ring nearer the carbonyl attachment, regardless of its positioning (compounds **153**−**157**) and always representing a clear improvement in activity against falcipain-2 and a more modest enhancement against falcipain-3 over the reference compound **151**. No difference was observed when a 2-benzofuranyl moiety replaced the naphthyl group and substitution within the condensed rings was explored (compounds **159**−**161**). The introduction of a methyl group adjacent to the carbonyl attachment yielded a loss of inhibitory capacity (**159** vs **158**). A similar effect was observed when a 5-methoxy substituent was present, whereas moving this group to position 7 of the benzofuran substituent yielded improved inhibitory capacity (compounds **160** and **161**). An improvement in activity was observed when attachment to the carbonyl group was at position 5 (compound **162**), contrasting with the quinoxaline ring (compound **152**). If a benzothiophene group was used instead (compounds **163**−**165**), analogous results were observed, whereas an indole moiety yielded mixed results depending upon the substitution position in the bicyclic structure (compounds **166**−**170**).

Introduction of two heteroatoms, as in 1,2-benzoisoxazole (compound **171**), resulted in the loss of inhibitory capacity against falcipain-3, while a 2-benzothiazolyl group (compound **172**) yielded a quite potent inhibitor. The results previously observed, with benzofuranyl and benzothiophenyl groups affording more potent inhibitors when the heteroatom was placed in the distant ring, were confirmed with the 1*H*-benzimidazolyl and 1*H*-1,2,3-benzotriazolyl derivatives (compounds **173**−**175**), yielding the most potent derivatives of these series, with subnanomolar activities against falcipain-2. Comparing compounds **159** and **176**, the influence of the heteroatom on the second ring was once more seen, yielding a compound 2 orders of magnitude more potent against falcipain-2 and 30 times more potent against

Table 11. Effect on in Vitro Falcipain Inhibition of Different Fused (Hetero)Aromatic Ring Substituents as P_3



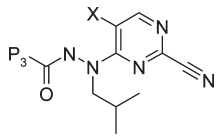
COMPOUND[a]	X	R	FP2 IC_{50} (μM)	FP3 IC_{50} (μM)
151	Br		0.065	0.645
152	Br		0.066	1.44
153	Cl		0.006	0.254
154	Cl		0.007	0.197
155	Cl		0.007	0.333
156	Cl		0.001	0.126
157	Cl		0.001	0.044
158	Br		0.065	0.563
159	Br		0.223	1.43
160	Br		0.183	1.24
161	Br		0.046	0.399
162	Br		0.0007	0.069
163	Br		0.241	0.365
164	Br		0.006	0.748
165	Br		0.001	0.154
166	Br		0.015	0.641
167	Br		0.033	1.53
168	Br		0.103	4.65
169	Br		0.004	0.311
170	Br		0.003	0.342
171	Br		0.604	>10
172	Cl		0.002	0.112
173	Cl		0.0005	0.093
174	Br		**0.0002**	**0.027**
175	Br		**0.0002**	**0.040**
176	Br		0.002	0.073
177	Br		0.004	0.329
178	Br		0.002	0.067
179	Br		0.025	0.831

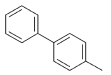
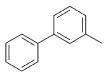
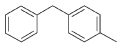
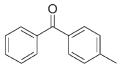
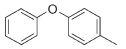
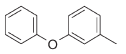
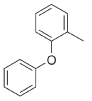
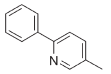
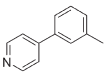
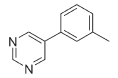
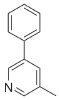
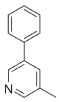
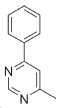
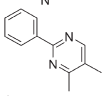
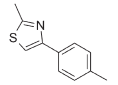
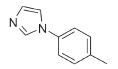
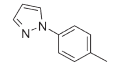
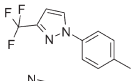
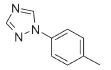
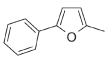
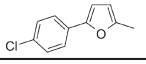
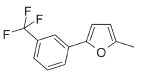
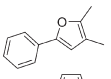
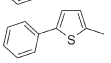
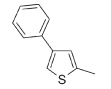
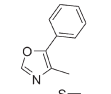
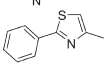
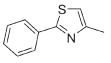
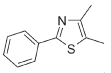
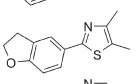
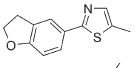
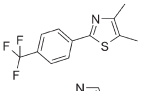
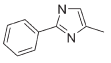
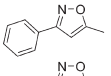
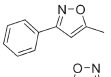
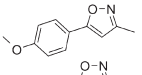
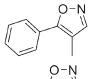
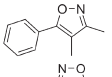
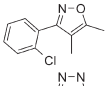
[a] All compounds were characterized and purity was assessed using ^{1}H NMR and LCMS.

falcipain-3. Using tricyclic derivatives such as fluorene or fluorenone (compounds **177**−**179**), the influence of the position of the carbonyl group in the tricyclic structure was apparent: the derivative with carbonyl groups further apart, **178**, was 10 times more potent against falcipain-2, but it was still modestly potent against falcipain-3.

When the two (hetero)aromatic cycles were not fused (Table 12, compounds **180**−**223**), greater variability in the results was observed. With two phenyl rings (compounds **180**−**186**), quite a significant improvement was detected when the two rings were angled toward each other (**180** vs **181**). This effect was also seen when an oxygen linker between the rings was present (**185** vs **186**), while the influence of the different substitution positions in the phenyl ring was only noticeable in the degree of inhibition of falcipain-3 (compounds **184**, **185**, and **186**). The introduction of one or two nitrogen atoms in any of the rings (compounds **187**−**193**) proved to be highly successful, with several derivatives presenting subnanomolar potencies against falcipain-2 and low tens of nanomolar activities against falcipain-3 (**188**−**191**). When phenyl

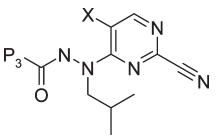
Table 12. Effect on in Vitro Falcipain Activity of Different Not Fused Bicyclic (Hetero)Aromatic Ring Substituents as P_3

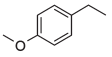
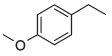
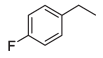
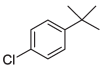
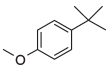
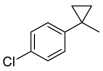
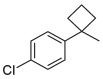
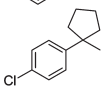
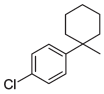


COMPOUND[a]	X	R	FP2 IC_{50} (μM)	FP3 IC_{50} (μM)
180	Br		0.028	1.46
181	Cl		0.001	0.037
182	Br		0.018	0.962
183	Br		0.003	0.115
184	Br		0.008	0.896
185	Br		0.004	0.116
186	Br		0.005	0.066
187	Br		0.003	0.104
188	Br		**<0.0005**	**0.015**
189	Br		**<0.0005**	**0.012**
190	Br		**<0.0005**	**0.004**
191	Cl		**0.0008**	**0.026**
192	Cl		0.001	0.030
193	Br		0.002	0.165
194	Br		0.0008	0.164
195	Cl		0.009	0.239
196	Cl		0.008	0.148
197	Cl		0.002	0.144
198	Br		0.003	0.052
199	Br		0.001	0.039
200	Br		0.020	0.351
201	Br		0.005	0.097
202	Br		0.025	0.837
203	Br		0.006	0.549
204	Br		0.003	0.374
205	Br		0.014	0.094
206	Cl		0.0015	0.054
207	Br		0.003	0.130
208	Cl		0.406	0.999
209	Cl		0.003	0.950
210	Cl		0.002	0.198
211	Br		0.012	1.54
212	Br		0.004	0.146
213	Cl		0.002	0.120
214	Br		0.010	0.359
215	Cl		0.004	0.692
216	Br		0.008	0.128
217	Cl		0.020	0.071
218	Br		0.100	0.571
219	Cl		0.003	0.352
220	Br		0.007	0.063
221	Br		0.676	1.10
222	Cl		0.004	0.218
223	Br		0.005	0.451

[a] All compounds were characterized and purity was assessed using ^{1}H NMR and LCMS.

Table 13. Effect on in Vitro Falcipain Inhibition of Different Benzylic Aromatic Ring Substituents as P_3



COMPOUND[a]	X	R	FP2 IC_{50} (μM)	FP3 IC_{50} (μM)
224	Br		**0.0015**	**0.006**
225	Cl		**0.001**	**0.005**
226	Br		**0.003**	**0.004**
227	Br		**0.0005**	**0.008**
228	Br		0.001	0.066
229	Br		0.001	0.021
230	Br		0.018	0.060
231	Br		0.004	0.018
232	Br		0.006	0.034
233	Br		0.006	0.102
234	Br		0.015	0.069
235	Br		0.012	0.216
236	Br		0.011	0.137
237	Br		0.012	0.184
238	Br		0.040	1.65
239	Br		0.007	0.034

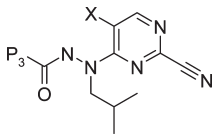
[a] All compounds were characterized and purity was assessed using ^1H NMR and LCMS.

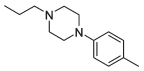
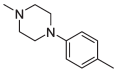
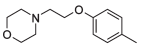
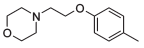
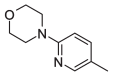
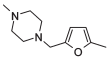
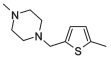
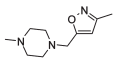
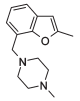
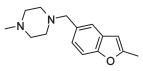
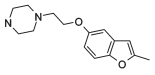
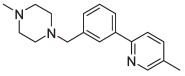
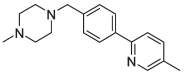
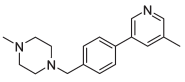
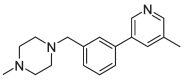
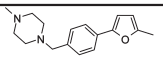
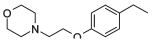
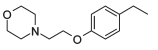
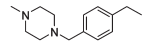
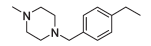
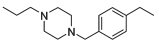
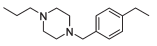
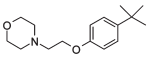
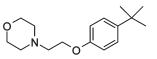
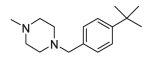
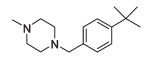
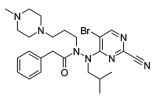
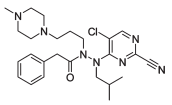
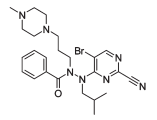
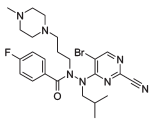
five-membered heterocyclic ring combinations were evaluated (compounds **194**−**198**), potent inhibitors of falcipain-2 and moderate inhibitors of falcipain-3 were obtained. When the order was reversed, i.e., five-membered heterocycle−phenyl combination (compounds **199**−**221**), a greater influence of the heteroatoms and/or substituents was detected. When the heterocycle was a furan or a thiophene ring, compounds with inhibition of falcipain-2 in the units to tens of nanomolar range and inhibition of falcipain-3 in the tens to hundreds of nanomolar range were obtained (**199**−**204**), although the furan seems to offer an advantage in terms of enzyme inhibition over the thiophene, particularly against falcipain-3 (**199** vs **203**). For oxazolyl, thiazolyl, and imidazolyl derivatives (**205**−**212**), no improvements in activity over the reference compound were observed, while a negative influence on the enzymatic activity of a methyl group in the position vicinal to the carbonyl substituent, especially against falcipain-3, was confirmed (compounds **202**, **208**, **209**, and **211**). The use of isoxazolyl derivatives with varying positioning and substitutions (compounds **213**−**218**) and of pyrazolyl (compounds **219**−**220**) and 1,2,3-triazolyl derivatives (compound **221**) showed no benefits. Also, substitution of the most distant phenyl ring by thiophene (compounds **222** and **223**) did not affect the inhibition of either falcipain-2 or falcipain-3.

Finally, benzylic derivatives were explored (Table 13, compounds **224**−**239**). Very potent inhibitors against both enzymes were obtained with very simple substitution of the phenyl ring (compounds **224**−**229**). Attempts to stabilize the benzylic position both with methyl groups or different rings (**230**, **233**, and **236**) resulted in a loss of activity against both enzymes. When para-chloro substituted phenyl derivatives were compared (**231**, **234**, **235**, **237**, and **238**), there seemed to be a small pocket around the benzylic position, with the optimal substituent being dimethyl, a cyclopropyl ring allowed, but with other aliphatic rings resulting in a loss of potency, in particular against falcipain-3.

All the P3-substituted compounds described above were micromolar inhibitors of cultured parasites, a situation similar to that observed in the optimization process for position P2. In order to confirm the "amine hypothesis" described above, different amines were introduced using the most potent scaffolds at P3 identified so far, and the activities of the resulting derivatives were tested against cultured malaria parasites (Table 14, compounds **240**−**269**). When the phenyl ring was attached to the carbonyl group (compounds **240**−**243**), very potent falcipain inhibitors were obtained, although their antiparasitic activity was variable. Directly attached *N*-alkylpiperazinyl derivatives such as compounds **240** and **241** showed superior antiparasitic activity compared to compounds with an oxygen−ethylene linker between the amine and the phenyl ring (compounds **242**−**243**). The amine hypothesis was once again confirmed with compound **244**; this compound had an enzymatic activity profile similar to that observed for compound **240** but was a much weaker inhibitor of cultured parasites (over 60 times less active), reflecting the lack of a basic amine in its structure. The use of five-membered heteroaromatic rings (compounds **245**−**247**) yielded very good antiparasitic activity, with minimal differences in their respective enzymatic activities observed. In the case of benzofuran derivatives (compounds **248**−**250**), no significant differences in enzymatic or whole-cell activities were observed for compounds with substitution at either position 5 or position 7. Pyridinyl−phenyl combinations (compounds **251**−**254**) were markedly active, yielding nanomolar antiparasitic inhibitors when the phenyl rings were angled toward each other, confirming the results previously observed for compounds lacking a basic amine (**180** vs **181**). This result suggests the importance of the relative disposition of the rings rather than the nature of the heteroatoms present in them, as the furanyl−phenyl derivative **255** was equipotent. Benzylic derivatives (compounds **256**−**265**) confirmed the tendency previously observed with directly attached phenyl rings; *N*-alkylpiperazinyl derivatives were the most potent. Attempts to stabilize the benzylic position with dimethyl groups once more resulted in a loss of activity in all cases; also, the overall lipophilicity of these

Table 14. Effect on in Vitro Antiparasitic Activity of Different Substituents as P_3 Bearing an Amine



COMPOUND[a]	X	R	FP2 IC_{50} (μM)	FP3 IC_{50} (μM)	*Pf*W2 IC_{50} (μM)
240	Br		<0.0005	0.032	0.025
241	Br		<0.0002	0.032	0.033
242	Br		0.003	0.145	0.622
243	Cl		0.002	0.090	0.870
244	Br		0.0002	0.053	1.53
245	Br		0.0007	0.012	0.021
246	Br		0.0002	0.012	0.010
247	Br		0.001	0.046	0.033
248	Br		0.0004	0.061	0.048
249	Br		<0.0005	0.029	0.030
250	Cl		0.001	0.107	0.076
251	Br		0.0002	0.038	0.022
252	Br		<0.0005	0.026	0.016
253	Br		**<0.0005**	**<0.003**	**0.001**
254	Br		**<0.0005**	**0.004**	**0.002**
255	Br		<0.0005	0.005	0.017
256	Br		0.0007	0.005	0.065
257	Cl		0.001	0.008	0.403
258	Br		0.0008	0.005	0.028
259	Cl		0.0007	0.005	0.043
260	Br		0.0002	0.003	0.018
261	Cl		0.0007	0.006	0.041
262	Br		0.003	0.013	0.802
263	Cl		0.004	0.018	0.806
264	Br		0.003	0.021	0.236
265	Cl		0.005	0.046	0.253
266	Br		0.006	0.027	0.214
267	Cl		0.011	0.078	0.108
268	Br		0.007	0.078	0.166
269	Br		0.009	0.105	0.346

[a] All compounds were characterized and purity was assessed using ^{1}H NMR and LCMS.

molecules was increased. Introduction of a protonable amine as a substituent of the second nitrogen atom of the hydrazide group (compounds **266**−**269**) yielded inhibitors in the low hundreds of nanomolar range against cultured parasites.

Conclusions

A potent new class of falcipain inhibitors based on a pyrimidine nitrile scaffold has been discovered and optimized. Optimization studies on the electrophile and P1 position led to identification of 2-cyano-5-chloropyrimidine and 2-cyano-5-bromopyrimidine scaffolds as the most promising, showing a clear advantage in terms of enzymatic activity over other nitriles. On the basis of these results and studies on the P2 position, compounds with substituents including 2-methylpropyl, cyclopentylmethyl, or cyclopentyl at the hydrazide nitrogen position, with improved enzymatic activity profiles, were obtained. Further studies on the P3 position using the

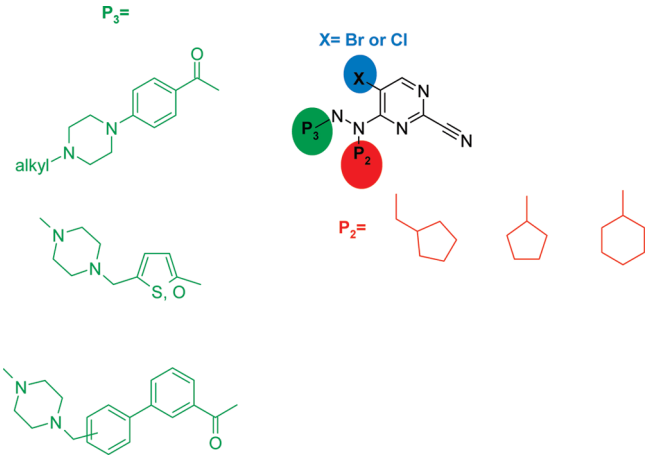


Figure 4. Most potent 2-cyanopyrimidine derivatives identified.

2-methylpropyl moiety as the reference P2 substitution led to identification of a group of promising falcipain inhibitors, with subnanomolar inhibition of falcipain-2 and nanomolar inhibition of falcipain-3, although inhibition of cultured parasites was in the micromolar range. Introduction of a protonable amine in the structure of these compounds confirmed the "amine hypothesis", with the presence of a protonable amine markedly improving antiparasitic activity. Compounds presenting excellent in vitro enzymatic and antiparasitic activities included **85**, **98**, **106**, **107**, **240**, **241**, **245**, **246**, **253**, and **254** (summarized in Figure 4). These results have encouraged further investigation of these compounds, including evaluation of in vitro and in vivo metabolic stability, selectivity against human cysteine proteases, toxicity, and in vivo efficacy evaluation. These results will be published in due course. On the whole, 2-cyanopyrimidines have afforded a broad range of potent in vitro antimalarials, with the variety of moieties allowed, especially at the P3 position, facilitating the modulation of the properties of these promising derivatives.

Experimental Section

Biological Assays. Enzyme Inhibition. Falcipain-2 and falcipain-3 were recombinantly expressed in *E. coli* and refolded to the active enzyme as previously described.[39] IC_{50} values against recombinant falcipain-2 and falcipain-3 were determined as follows. The substrate (H-D-Val-Leu-Arg-AFC), added to a final concentration of 30 μM for FP-2 and 150 μM for FP-3, was mixed at room temperature with different concentrations of tested inhibitors in the reaction buffer (100 mM sodium acetate, pH 5.5, 500 μM CHAPS, and 10 mM DTT). Inhibitor solutions were prepared from stocks in DMSO (maximum concentration of DMSO in the assay was 1%).

The reaction was started with the addition of the recombinant enzymes, 0.25 nM FP-2 and 2 nM FP-3. Fluorescence was monitored for 1 h at room temperature in a SpectraMax Plus 384 spectrofluorometer. IC_{50} values were determined from plots of percent activity over concentration using GraFit software.

Inhibition of Cultured Malaria Parasites. The effects of inhibitors were studied against W2-strain *P. falciparum*, obtained from the Malaria Research and Reference Reagent Resource Center and cultured with human erythrocytes at 2% hematocrit in RPMI medium with either 10% human serum or 0.5% AlbuMAX II serum substitute. Synchrony was maintained with sorbitol. Cultures were incubated with serial dilutions of test compounds from 1000× stocks in DMSO for 48 h beginning at the ring stage. The medium was changed after 24 h, with maintenance of the appropriate inhibitor concentration. After 48 h, when control cultures contained nearly all new ring-stage parasites, parasites were fixed with 1% formaldehyde in PBS, pH 7.4, for 48 h at room temperature and then labeled with YOYO-1 (1 nM; Molecular Probes) in 0.1% Triton X-100 in PBS. Parasitemias were determined from dot plots (forward scatter vs fluorescence) acquired on a FACSort flow cytometer using CELLQUEST software (Becton Dickinson). IC_{50} values for growth inhibition were determined with GraphPad Prism software from plots of percentage parasitemia compared to controls (untreated parasites) over inhibitor concentration. In each case, the goodness of curve fit was documented by R^2 values of ≥0.95.

Chemistry. Starting materials were obtained from commercial suppliers and used without further purification unless otherwise stated. Flash chromatography was carried out using prepacked Isolute Flash or Biotage silica gel columns as the stationary phase and analytical grade solvents as the eluent unless otherwise stated.

NMR spectra were determined on a Varian Unity spectrometer (300 MHz). Chemical shifts are reported as δ values (ppm) downfield from tetramethylsilane, used as an internal standard in the solvent indicated. All coupling constants are reported in hertz (Hz), and multiplicities are labeled br (broad), s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), td (triplet of doublets), ddd (double-doubledoublet), and m (multiplet).

Total ion current traces were obtained for electrospray positive and negative ionization (ES+/ES−) on a Waters ZMD 2000.

Analytical chromatographic conditions used for the LC/MS analysis were as follows. The column was ACE, 4.6 mm × 30 mm, and the stationary phase particle size was 3 μm. Solvent A was an aqueous solvent consisting of water + 0.1% formic acid. Solvent B was an organic solvent consisting of acetonitrile + 0.1% formic acid. The composition of the solvent over 5 min is shown in Table 15. Additional chromatographic parameters were as follows: flow rate, 1 mL/min; injection volume, 5 μL; column temperature, 30 °C; UV wavelength range, 220−330 nm.

The purity of all tested compounds was ≥95% using the analytical method described above unless stated otherwise.

1,1-Dimethylethyl 2-(2-Methylpropyl)hydrazinecarboxylate (1). A solution of 1,1-dimethylethyl hydrazinecarboxylate (9.2 g, 70 mmol) in isopropanol (50 mL) was treated at 0 °C with isobutyraldehyde (6.4 mL, 70 mmol) over 15 min, and the mixture was stirred at 0 °C for 2 h. Then the mixture was stirred for 5 h at room temperature. To this solution containing the intermediate hydrazone was added PtO_2, and the suspension was hydrogenated at room temperature and 2.6 bar for 48 h. The suspension was filtered and the solvent was removed under reduced pressure to give the title compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 6.02 (br s, 1H), 3.92 (br s, 1H), 2.66 (d, J = 6.7 Hz, 2H), 1.73 (m, 1H), 1.46 (s, 9H), 0.93 (d, J = 6.9 Hz, 6H). ESIMS m/z: 189 (MH^+).

1,1-Dimethylethyl 2-(6-Cyano-2-pyridinyl)-2-(2-methylpropyl)-hydrazinecarboxylate (4). Under a nitrogen atmosphere, $Pd_2(dba)_3$ (0.077 g, 0.08 mmol), (±)-2,2′-bis(diphenylphosphino)-1,1′-binaphthalene (BINAP, 0.01 g, 0.02 mmol), 1,1-dimethylethyl 2-(2-methylpropyl)hydrazinecarboxylate (**1**, 0.715 g, 3.8 mmol), 2,6-dibromopyridine (1 g, 4.22 mmol), and sodium *tert*-butoxide (0.568 g, 5.9 mmol) were dissolved in toluene (20 mL). The resulting reaction mixture was stirred at room temperature for 3 h. The mixture was then diluted with DCM (100 mL), filtered over a Celite pad, and concentrated in vacuo. The crude mixture was purified by flash chromatography on silica gel (eluting with Hex/EtOAc mixtures from 100:0 to 0:100%) and then by preparative HPLC (ACN/H_2O, 0.1%TFA, gradient 30−100%) to give 1,1-dimethylethyl 2-(6-bromo-2-pyridinyl)-2-(2-methylpropyl)hydrazinecarboxylate **2a** (0.34 g, 26%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 9.41−8.95 (br, 1H), 7.45 (t, J = 7.9 Hz, 1H), 6.83 (d, J = 7.5 Hz, 1H), 6.57 (d, J = 8.5 Hz, 1H), 3.77−3.15 (br, 2H), 1.98−1.7 (m, 1H), 1.42−1.28 (br, 9H), 0.87 (d, J = 6.7 Hz, 6H).

Table 15. Analytical Chromatography: Composition of Solvent over 5 min

time (min)	% solvent B
0	10
0.2	10
3.5	90
4	90
4.01	90
5	10

In a round-bottomed flask and under a nitrogen atmosphere, 1,1-dimethylethyl 2-(6-bromo-2-pyridinyl)-2-(2-methylpropyl)-hydrazinecarboxylate **2a** (0.1 g, 0.29 mmol), $Pd_2(dba)_3$ (0.053 g, 0.006 mmol), dppf (0.064 g, 0.01 mmol), zinc(II) cyanide (0.02 g, 0.17 mmol), and zinc dust (0.0023 g, 0.035 mmol) were dissolved in DMA (10 mL). The mixture was heated to 120 °C for 2 h and then diluted with DCM, filtered over a Celite pad, and concentrated to dryness. The residue was purified by flash chromatography on silica gel (eluting with Hex/EtOAc mixtures from 100:0 to 0:100%) to yield the title compound **4** (0.07 g, 83% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 9.51−9.06 (br, 1H), 7.77−7.71 (m, 1H), 7.27 (d, J = 7.2 Hz, 1H), 6.91 (d, J = 8.8 Hz, 1H), 3.79−3.33 (br, 2H), 2.01−1.85 (m, 1H), 1.43−1.27 (br, 9H), 0.89 (d, J = 6.6 Hz, 6H). ESIMS m/z: 235 ($MH^+ - 56$).

1,1-Dimethylethyl 2-(6-Cyano-2-pyrazinyl)-2-(2-methylpropyl)hydrazinecarboxylate (5). Following analogous procedures, compound **5** was synthesized. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 9.81−9.24 (br, 1H), 8.46−8.29 (br, 2H), 4.22−3.13 (br, 2H), 2.03−1.86 (m, 1H), 1.53−1.19 (br m, 9H), 0.9 (d, J = 6.6 Hz, 6H). ESIMS m/z: 292 (MH^+).

1,1-Dimethylethyl 2-(2-Chloro-5-cyano-4-pyrimidinyl)-2-(2-methylpropyl)hydrazinecarboxylate (6). 5-Cyanouracil (2 g, 14.6 mmol) and PCl_5 (11.68 g, 56.1 mmol) were added to $POCl_3$ (27 mL, 290.6 mmol), and the resulting mixture was heated to 115 °C for 4 h. Excess $POCl_3$ was distilled, and the mixture was added dropwise to a cooled solution of saturated aqueous $NaHCO_3$. Product was extracted with DCM several times, and the combined organic layers were washed with water and brine and dried over anhydrous Na_2SO_4. Solvent was evaporated under reduced pressure and the crude mixture was purified by flash chromatography on silica gel (eluting with Hex/EtOAc mixtures from 100:0 to 70:30%) to yield 2,4-dichloro-5-pyrimidinecarbonitrile as a white solid (1.63 g, 64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 8.63 (s, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ ppm: 192.7, 163.8, 163.3, 111.75, 109.2.

Intermediate 2,4-dichloro-5-pyrimidinecarbonitrile (0.4 g, 2.3 mmol) and 1,1-dimethylethyl 2-(2-methylpropyl)hydrazinecarboxylate (**1**, 0.315 g, 1.7 mmol) were dissolved in isopropanol (5 mL), and DIPEA (0.52 mL, 3.0 mmol) was added to the mixture. After stirring at room temperature for 22 h, solvent was evaporated in vacuo and the residue was taken up in DCM and washed with 1 N NH_4Cl, water, and brine. The organic layer was dried over anhydrous Na_2SO_4, and solvent was removed under reduced pressure. The crude mixture was purified by flash chromatography on silica gel (eluting with Hex/EtOAc mixtures from 100:0 to 70:30%) followed by preparative HPLC (ACN/H_2O, 0.1% TFA, gradient 20−100%) to yield the title compound **6** (0.121 g, 12%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 8.50 (s, 1H), 6.69−6.56 (br, 1H), 3.81−3.66 (m, 2H), 2.26−1.96 (m, 1H), 1.66−1.23 (m, 9H), 0.95 (d, J = 6.7 Hz, 6H). ^{13}C NMR (300 MHz, $CDCl_3$) δ ppm: 162.8, 162.6, 162.3, 155.1, 114.3, 98.3, 82.4, 58.1, 28.1, 26.6, 19.9. ESIMS m/z: 324 (MH^-).

1,1-Dimethylethyl 2-(4-Chloro-5-cyano-2-pyrimidinyl)-2-(2-methylpropyl)hydrazinecarboxylate (7). Replacement of isopropanol by toluene in the reaction between 2,4-dichloro-5-pyrimidinecarbonitrile and 1,1-dimethylethyl 2-(2-methylpropyl)-hydrazinecarboxylate yielded a mixture of **6** and **7**, the latter being the minor one. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 8.47 (s, 1H), 6.85 (s, 1H), 3.41−2.83 (br, 2H), 2.20−1.97 (m, 1H), 1.54 (br, 9H), 1.11−1.81 (br, 6H). ^{13}C NMR (300 MHz, $CDCl_3$) δ ppm: 164.6, 163.9, 162.3, 115.1, 98.6, 89.9, 83.6, 58.2, 28.1, 26.2, 20.0. ESIMS m/z: 326 (MH^+).

1,1-Dimethylethyl 2-(6-Cyano-4-pyrimidinyl)-2-(2-methylpropyl)hydrazinecarboxylate (8). 4,6-Dichloropyrimidine (0.15 g, 1 mmol) was dissolved in isopropanol (5 mL), and 1,1-dimethylethyl 2-(2-methylpropyl)hydrazinecarboxylate (**1**, 0.17 g, 0.9 mmol) and DIPEA (0.35 mL, 2.0 mmol) were added to the solution. The resulting mixture was heated under microwave irradiation at 130 °C for 90 min. Solvent was evaporated in vacuo and the residue was taken up in DCM and washed with 1 N NH_4Cl and brine. The organic layer was dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure to yield 1,1-dimethylethyl 2-(6-chloro-4-pyrimidinyl)-2-(2-methylpropyl)-hydrazinecarboxylate (0.23 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 8.44 (s, 1H), 6.77−6.31 (br, 2H), 3.97−3.25 (br, 2H), 2.18−1.94 (m, 1H), 1.68−1.23 (m, 9H), 0.95 (d, J = 6.7 Hz, 6H).

1,1-Dimethylethyl 2-(6-chloro-4-pyrimidinyl)-2-(2-methylpropyl)hydrazinecarboxylate (0.225 g, 0.75 mmol) was dissolved in a 85:15 DMSO−H_2O mixture (3 mL), and DABCO (0.084 g, 0.75 mmol) and KCN (0.098 g, 1.5 mmol) were added to the solution. The resulting mixture was heated to 65 °C for 30 h, and then it was poured into ice−water. The reddish solid that precipitated was filtered off and purified by flash chromatography on silica gel (eluting with a 50:1 DCM/MeOH mixture) first and then by preparative HPLC (ACN/H_2O, gradient 30−100%) to yield the title compound **8**. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 8.68 (s, 1H), 7.02 (br s, 1H), 6.71−6.40 (m, 1H), 4.31−2.99 (m, 2H), 2.16−1.96 (m, 1H), 1.81−1.32 (br, 9H), 0.97 (d, J = 6.3 Hz, 6H). ESIMS m/z: 290 (MH^-).

1,1-Dimethylethyl 2-(4-Cyano-2-pyrimidinyl)-2-(2-methylpropyl)hydrazinecarboxylate (9). A mixture of 1,1-dimethylethyl 2-(2-methylpropyl)hydrazinecarboxylate (**1**, 2.38 g, 12.6 mmol), 2,4-dichloropyrimidine (2.07 g, 13.9 mmol), and DIPEA (4.4 mL, 25.3 mmol) in toluene (10 mL) was refluxed for 14 h. Solvent was removed under reduced pressure, and the residue was taken up in DCM and washed with 1 N NH_4Cl and brine and dried over anhydrous $MgSO_4$. After evaporation of the solvent in vacuo, the crude mixture was purified by flash chromatography on silica gel (eluting with Hex/EtOAc mixtures from 10:1 to 1:1) to yield intermediate 1,1-dimethylethyl 2-(4-chloro-2-pyrimidinyl)-2-(2-methylpropyl)hydrazinecarboxylate (0.787 g, 21%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 9.31 (s, 1H), 8.37 (d, J = 4.97 Hz, 1H), 6.87 (d, J = 4.97 Hz, 1H), 3.83−3.37 (br, 2H), 2.04−1.85 (m, 1H), 1.43−1.24 (m, 9H), 0.92−0.85 (m, 6H). ESIMS m/z: 299 (MH^-).

DABCO (0.589 g, 5.25 mmol) and KCN (0.342 g, 5.25 mmol) were added to a solution of intermediate 1,1-dimethylethyl 2-(4-chloro-2-pyrimidinyl)-2-(2-methylpropyl)hydrazinecarboxylate (0.787 g, 2.6 mmol) in a 85:15 DMSO−H_2O mixture (12 mL) at room temperature. The resulting reaction mixture was then heated to 70 °C. After 5 h, the reaction mixture was cooled down to room temperature and then poured into ice−water and kept at low temperature in order to favor product precipitation. A gum was filtered off and redissolved in a DCM−MeOH mixture. Solvent was removed under reduced pressure to yield a brown oil which was purified by flash chromatography on silica gel (eluting with a 4:1 Hex/EtOAc mixture) to yield the title compound **9** (0.28 g, 37%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 9.37−8.98 (m, 1H), 8.77−8.68 (m, 1H), 7.36−7.27 (m, 1H), 3.83−3.16 (br, 2H), 2.03−1.87 (m, 1H), 1.43−1.26 (m, 9H), 0.98−0.78 (m, 6H). ESIMS m/z: 290 (MH^-).

1,1-Dimethylethyl 2-[4-Cyano-6-(methyloxy)-1,3,5-triazin-2-yl]-2-(2-methylpropyl)hydrazinecarboxylate (10). To a solution of 2,4-dichloro-6-(methyloxy)-1,3,5-triazine (1 g, 5.53 mmol) in anhydrous THF (40 mL) at 0 °C, 1,1-dimethylethyl 2-(2-methylpropyl)hydrazinecarboxylate (**1**, 1.05 g, 3.8 mmol) was added. The mixture was left to reach room temperature overnight. It was then evaporated to dryness and the crude was purified by

flash chromatography on silica gel (eluting with Hex/EtOAc mixtures from 0:100 to 50:50%) to yield 1,1-dimethylethyl 2-[4-chloro-6-(methyloxy)-1,3,5-triazin-2-yl]-2-(2-methylpropyl)hydrazinecarboxylate (0.975 g, 53% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 6.83−6.47 (br, 1H), 3.98−3.95 (m, 3H), 3.69−3.59 (br, 2H), 2.17−2.02 (m, 1H), 1.58−1.33 (br, 9H), 0.97 (d, J = 6.7 Hz, 6H).

1,1-Dimethylethyl 2-[4-chloro-6-(methyloxy)-1,3,5-triazin-2-yl]-2-(2-methylpropyl)hydrazinecarboxylate (0.109 g, 0.33 mmol) was dissolved in DMSO (3 mL). To the resulting solution, NaCN (0.033 g, 0.66 mmol) was added. The reaction mixture was stirred at room temperature for 18 h, and then it was diluted with EtOAc. The organic phase was washed with water several times and brine. Solvent was evaporated under reduced pressure and the crude mixture was purified by flash chromatography on silica gel (eluting with Hex/EtOAc mixtures from 95:5 to 40:60%) to yield the title compound **10** (0.06 g, 56% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 9.83−9.25 (br, 1H), 3.95−3.92 (m, 3H), 3.62−3.41 (br, 2H), 2.04−1.9 (m, 1H), 1.48−1.26 (m, 9H), 0.89 (d, J = 6.7 Hz, 6H). ESIMS m/z: 350 (MH^+).

1,1-Dimethylethyl 2-(2-Cyano-4-pyrimidinyl)-2-(2-methylpropyl)hydrazinecarboxylate (12). A mixture of compound **1** (12.07 g, 64 mmol), 2,4-dichloropyrimidine (19.07 g, 128 mmol), triethylamine (10.7 mL, 145 mmol), and dry EtOH (100 mL) was stirred at room temperature for 3 days. The mixture was concentrated under reduced pressure and the residue partitioned between DCM and 1 M NH_4Cl. The organic layer was treated with brine and dried over $MgSO_4$. The residue was purified by flash chromatography (eluent, Hex/AcOEt mixtures 87:13 to 50:50%) to give intermediate **11**. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 9.91−9.21 (br, 1H), 8.15 (d, J = 6.0 Hz, 1H), 6.72−6.46 (br, 1H), 4.02−3.56 (br, 1H), 3.42−3.00 (br, 1H), 2.05−1.8 (m, 1H), 1.53−1.17 (br, 9H), 1.02−0.73 (br, 6H). ESIMS m/z 299 (MH^-).

Potassium cyanide (3.5 g, 54.6 mmol) was added to a suspension of intermediate **11** (13.69 g, 45.51 mmol) and DABCO (5.10 g, 45.51 mmol) in a mixture of 85:15 DMSO−H_2O (195 mL) at room temperature. The reaction mixture was stirred at 100 °C for 1.7 h and poured into ice−water (250 mL). The white solid was filtered off and dried. The compound was purified by flash chromatography (eluent, Hex/AcOEt mixtures 4:1 to 3:1) to give compound **12**. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 10.06−9.23 (br s, 1H), 8.37 (br, 1H), 7.42−6.67 (br, 1H), 3.92−3.56 (br, 1H), 3.46−3.02 (br, 1H), 2.1−1.82 (m, 1H), 1.58−1.08 (br, 9H), 1.02−0.67 (br, 6H). ESIMS m/z 290 (MH^-).

1,1-Dimethylethyl 2-(2-Cyano-6-methyl-4-pyrimidinyl)-2-(2-methylpropyl)hydrazinecarboxylate (14a). To a solution of 2,4-dichloro-6-methylpyrimidine (1.43 g, 8.8 mmol) in EtOH (4 mL), a solution of **1** (0.82 g, 4.4 mmol) in EtOH (4 mL) and Et_3N (0.73 mL, 5.3 mmol) were added. The resulting reaction mixture was stirred at room temperature for 2 days and then refluxed overnight. The mixture was washed with saturated NH_4Cl and brine. The organic layer was dried over anhydrous $MgSO_4$. The residue was purified by chromatography on silica gel (eluting with Hex/EtOAc mixtures from 30:1 to1:1) followed by preparative HPLC purification (ACN/H_2O, 0.1% TFA, gradient 30−100%) to give 1,1-dimethylethyl 2-(2-chloro-6-methyl-4-pyrimidinyl)-2-(2-methylpropyl)hydrazinecarboxylate **13a** (0.5 g, 36%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 6.66−6.46 (br, 1H), 6.46−6.29 (br, 1H), 3.82−3.32 (br, 2H), 2.37 (s, 3H), 2.12−1.95 (m, 1H), 1.73−1.36 (br, 9H), 0.96 (d, J = 6.6 Hz, 6H).

To a solution of **13a** in a 9:1 DMSO−H_2O mixture (7 mL), DABCO (0.18 g, 1.6 mmol) and KCN (0. g, 3.1 mmol) were added, and the mixture was heated to 80 °C overnight. More KCN (0.1 g, 1.6 mmol) and DABCO (0.09 g, 0.8 mmol) were added, and stirring was kept overnight. The mixture was poured into ice−water, and the white precipitate was filtered off. The crude product was purified by flash chromatography on silica gel (eluting with Hex/EtOAc mixtures from 100:0 to 50:50%) to yield the above product **14a** (0.28 g, 58%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 9.93−9.70 (br, 1H), 6.78−6.58 (br, 1H), 3.93−3.57 (br, 1H), 3.45−3.11 (br, 1H), 2.35 (s, 3H), 2.04−1.86 (m, 1H), 1.56−1.22 (br, 9H), 1.02−0.72 (br, 6H). ^{1}H NMR (300 MHz, DMSO-d_6 + D_2O) δ ppm: 6.80−6.52 (br, 1H), 3.96−3.64 (br, 1H), 3.40−3.09 (br, 1H), 2.34 (s, 3H), 2.02−1.84 (m, 1H), 1.52−1.18 (br, 9H), 0.99−0.70 (br, 6H). ESIMS m/z: 306 (MH^+).

Methyl 2-Cyano-6-[2-{[(1,1-dimethylethyl)oxy]carbonyl}-1-(2-methylpropyl)hydrazino]-4-pyrimidinecarboxylate (14b). A solution of **1** (1 g, 5.3 mmol) in EtOH was added to methyl 2,6-dichloro-4-pyrimidinecarboxylate (1 g, 4.83 mmol) previously dissolved in EtOH (20 mL). The resulting reaction mixture was stirred at room temperature overnight. The mixture was then concentrated under vacuum and the crude mixture purified by flash chromatography on silica gel (eluting with 5:1 Hex/EtOAc) to yield intermediate **13b** (0.46 g, 26%).

A solution of **13b** (0.02 g, 0.06 mmol) and tetraethylammonium cyanide (0.04 g, 0.3 mmol) in THF (2 mL) was cooled at −78 °C. Then excess liquid trimethylamine was added and the mixture was allowed to warm to room temperature. After 24 h, the mixture was concentrated in vacuo and the residue taken up with DCM and washed with water. The organic layer was dried over anhydrous $MgSO_4$, filtered, and solvent was then removed under reduced pressure. The brown oil obtained was purified by flash chromatography on silica gel (eluting with 50:1 DCM/MeOH) to yield the title compound **14b** (0.02 g, quantitative yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 10.17−10.04 (br, 1H), 7.31−7.22 (br, 1H), 4.03−3.75 (br, 4H), 3.51−3.3 (br, 1H), 2.06−1.91 (m, 1H), 1.53−1.21 (br m, 9H), 1.01−0.79 (br, 6H). ESIMS m/z: 350 (MH^+).

1,1-Dimethylethyl 2-[2-Cyano-6-(methyloxy)-4-pyrimidinyl]-2-(2-methylpropyl)hydrazinecarboxylate (17). To a solution of 2,4,6-trichloropyrimidine (2.9 g, 16 mmol) in EtOH (30 mL), **1** (3 g, 16 mmol) was added. The mixture was stirred at room temperature. After 16 h, the mixture was concentrated under vacuum and the yellow solid obtained was purified by flash chromatography on silica gel (eluting with Hex/EtOAc mixtures from 6:1 to 2:1). The solid obtained was washed with EtOH to yield intermediate **15** (2.5 g, 47%) as a white solid.

To a solution of **15** (0.4 g, 1.2 mmol) in MeOH (10 mL), sodium methoxide (0.13 g, 2.4 mmol previously prepared from sodium and MeOH) was added. The resulting mixture was stirred at room temperature. After 16 h, the mixture was concentrated under vacuum and the residue partitioned between DCM and water. The organic layer was washed with brine and the crude product was purified by flash chromatography on silica gel (eluting with 10:1 Hex/EtOAc) to yield intermediate **16** (0.06 g, 15%).

A solution of **16** (0.05 g, 0.15 mmol) and tetraethylammonium cyanide (0.1 g, 0.6 mmol) in THF (3 mL) was cooled at −78 °C. Then excess liquid trimethylamine was added and the mixture was allowed to warm to room temperature. After 48 h, the mixture was concentrated in vacuo and the residue taken up with DCM and washed with water. The organic layer was washed with brine and solvent was removed under reduced pressure to yield the title compound **17** (0.04 g, 91%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 9.94−9.58 (br, 1H), 6.22−5.85 (br, 1H), 4.04−3.62 (br, 4H), 3.33−3.09 (br, 1H), 2.02−1.82 (m, 1H), 1.56−1.25 (br, 9H), 1.04−0.75 (br, 6H). ESIMS m/z: 322 (MH^+) .

1,1-Dimethylethyl 2-(2-Cyano-5-fluoro-4-pyrimidinyl)-2-(2-methylpropyl)hydrazinecarboxylate (19a). To a solution of 5-fluoro-2,4-dichloropyrimidine (1.5 g, 9.1 mmol) and **1** (1.9 g, 9.8 mmol) in *i*-PrOH (40 mL), DIPEA (2 mL, 11.3 mmol) was added. The resulting reaction mixture was refluxed overnight. The mixture was concentrated under reduced pressure and the residue partitioned between DCM and 1 M NH_4Cl. The organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. The residue was purified by flash chromatography on silica gel (elute, Hex/EtOAc 9:1) to give the intermediate **18a** as a colorless oil (2.88 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$)

δ ppm: 7.98 (d, J = 5.4 Hz, 1H), 6.77−6.65 (br, 1H), 3.68−3.45 (br, 2H), 2.18−1.96 (m, 1H), 1.65−1.31 (br, 9H), 0.95 (d, J = 6.6 Hz, 6H). ESIMS m/z: 319 (MH^+).

Potassium cyanide (0.5 g, 7.7 mmol) was added to a suspension of **18a** (2.2 g, 7 mmol) and DABCO (0.8 g, 7 mmol) in a 9:1 DMSO−H_2O mixture (65 mL) at room temperature. The reaction mixture was stirred at 65 °C for 5 h and then poured into ice−water. The cream color solid that precipitated was filtered off, washed abundantly with water, and dried under air. The compound was purified by flash chromatography (eluting with Hex/EtOAC mixtures from 19:1 to 4:1) to give the title compound **19a** as a white solid (0.26 g, 9%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 8.16 (d, J = 5.6 Hz, 1H), 6.86−6.56 (br, 1H), 3.83−3.45 (br, 2H), 2.20−1.98 (m, 1H), 1.72−1.31 (br, 9H), 0.97 (d, J = 6.7 Hz, 6H). ESIMS m/z: 310 (MH^+).

1,1-Dimethylethyl 2-(5-Bromo-2-cyano-4-pyrimidinyl)-2-(2-methylpropyl)hydrazinecarboxylate (19b). To a solution of 5-bromo-2,4-dichloropyrimidine (3.0 g, 13 mmol) and **1** (2.5 g, 13 mmol) in *i*-PrOH (80 mL), DIPEA (3 mL, 17 mmol) was added. The resulting reaction mixture was refluxed for 5 h. The mixture was concentrated under reduced pressure and the residue partitioned between DCM and 1 M NH_4Cl. The organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. The residue was purified by flash chromatography on silica gel (elute, Hex/EtOAc 9:1) to give intermediate 1,1-dimethylethyl 2-(5-bromo-2-chloro-4-pyrimidinyl)-2-(2-methylpropyl)hydrazinecarboxylate **18b** as a white solid (4.12 g, 82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 8.27 (br s, 1H), 6.85−6.40 (br, 1H), 4.15−3.00 (br, 2H), 2.16−1.96 (m, 1H), 1.55−1.30 (br, 9H), 0.97 (d, J = 6.6 Hz, 6H).

Potassium cyanide (0.9 g, 13 mmol) was added to a suspension of **18b** (4.1 g, 11 mmol) and DABCO (1.2 g, 11 mmol) in a 9:1 DMSO−H_2O mixture at room temperature. The reaction mixture was stirred at room temperature for 3 h and then poured into ice−water. The cream color solid that precipitated was filtered off, washed abundantly with water, and dried under air. The compound was purified by flash chromatography on silica gel (eluent, Hex/EtOAC 19:1) to give the title compound **19b** as a white foam (3.68 g, 92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 8.46 (br s, 1H), 6.85−6.45 (br, 1H), 4.15−3.0 (br, 2H), 2.17−1.95 (m, 1H), 1.52−1.31 (br, 9H), 0.97 (d, J = 6.6 Hz, 6H). ^{1}H NMR (300 MHz, DMSO-d_6, 80 °C) δ ppm: 9.94−9.43 (br, 1H), 8.59 (s, 1H), 3.94−3.23 (br, 2H), 2.11−1.97 (dt, J = 6.7, 13.5 Hz, 1H), 1.52−1.29 (br, 9H), 0.92 (d, J = 6.7 Hz, 6H). ^{13}C NMR (90 MHz, DMSO-d_6) δ ppm: 160.7, 159.6, 153.6, 140.6, 115.7, 104.7, 80.4, 58.6, 27.7, 25.5, 20.0. ESIMS m/z: 370 (MH^+).

1,1-Dimethylethyl 2-(5-Chloro-2-cyano-4-pyrimidinyl)-2-(2-methylpropyl)hydrazinecarboxylate (19c). To a solution of 2,4,5-trichloropyrimidine (0.5 g, 2.7 mmol) and **1** (0.6 g, 3.1 mmol) in *i*-PrOH (15 mL), DIPEA (0.7 mL, 3.7 mmol) was added. The resulting reaction mixture was refluxed for 3 h. The mixture was concentrated under reduced pressure and the residue partitioned between DCM and 1 M NH_4Cl. The organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. The residue was purified by flash chromatography (elute, Hex/EtOAc 19:1) to give intermediate **18c** as a white solid (0.76 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 8.11 (br s, 1H), 6.85−6.70 (br, 1H), 3.80−3.50 (br, 2H), 2.17−1.97 (m, 1H), 1.63−1.29 (br, 9H), 0.96 (d, J = 6.7 Hz, 6H). ESIMS m/z: 337 (MH^+).

Potassium cyanide (0.2 g, 2.7 mmol) was added to a suspension of **18c** (0.7 g, 2.2 mmol) and DABCO (1.2 g, 11 mmol) in a 9:1 DMSO−H_2O mixture (20 mL) at room temperature. The reaction mixture was stirred at room temperature for 9 h and then poured into ice−water. The light-yellow solid that precipitated was filtered off, washed abundantly with water, and dried under air. The compound was purified by flash chromatography on silica gel (eluting with Hex/EtOAC mixtures from 19:1 to 9:1) to give the title compound **19c** as a white solid (0.62 g, 85%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 10.09 (b s, 1H), 8.56−8.51 (br, 1H), 3.98−3.71 (br, 1H), 3.30−3.07 (br, 1H), 2.08−1.90 (m, 1H), 1.52−1.2 (br, 9H), 0.99−0.79 (br, 6H). ^{1}H NMR (300 MHz, DMSO-d_6, 80 °C) δ ppm: 9.95−9.5 (br, 1H), 8.47 (s, 1H), 3.80−3.34 (br, 2H), 2.11−1.95 (m, 1H), 1.41 (br s, 9H), 0.93 (d, J = 6.7 Hz, 6H). ESIMS m/z: 326 (MH^+).

1,1-Dimethylethyl 2-(2-Cyano-5-nitro-4-pyrimidinyl)-2-(2-methylpropyl)hydrazinecarboxylate (19d). To a solution of 2,4-dichloro-5-nitropyrimidine (0.2 g, 1 mmol) and **1** (0.2 g, 1 mmol) in *i*-PrOH (7 mL), DIPEA (0.2 mL, 1.1 mmol) was added. The resulting reaction mixture was stirred at room temperature for 1.5 h. The mixture was concentrated under reduced pressure and the residue partitioned between DCM and 1 M NH_4Cl. The organic layer was washed with water and brine and dried over anhydrous $MgSO_4$. The residue was purified by preparative HPLC (ACN/H_2O, 0.1%TFA, gradient 30−100%) to give intermediate 1,1-dimethylethyl 2-(2-chloro-5-nitro-4-pyrimidinyl)-2-(2-methylpropyl)hydrazinecarboxylate **18d** (0.22 g, 62%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 8.55 (s, 1H), 6.66−6.44 (br, 1H), 4.53−4.01 (br, 1H), 3.45−2.99 (br, 1H), 2.18−1.98 (m, 1H), 1.41 (br s, 9H), 1.00 (d, J = 6.4 Hz, 6H). ESIMS m/z: 346 (MH^+).

Potassium cyanide (0.05 g, 0.7 mmol) was added to a suspension of **18d** (0.2 g, 0.6 mmol) and DABCO (0.07 g, 0.6 mmol) in a 9:1 DMSO−H_2O mixture (7 mL) at room temperature. The reaction mixture was stirred at room temperature for 1.5 h and then poured into ice−water. The cream color solid that precipitated was filtered off, washed abundantly with water, and dried under air. Compound was further extracted from the aqueous filtrate with *n*-butanol. The resulting crude mixture of compounds was purified by preparative HPLC (ACN/H_2O, gradient 50−100%) to give the title compound **19d** as a yellow solid (0.07 g, 22%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 10.19−10.02 (br, 1H), 8.93 (br s, 1H), 4.05−3.85 (br, 1H), 3.22−3.06 (br, 1H), 2.10−1.92 (m, 1H), 1.49−1.20 (br, 9H), 1.03−0.75 (br, 6H). ESIMS m/z: 337 (MH^+).

1,1-Dimethylethyl 2-(2-Cyano-5-methyl-4-pyrimidinyl)-2-(2-methylpropyl)hydrazinecarboxylate (19e). To a solution of 2,4-dichloro-5-methylpyrimidine (1.5 g, 9 mmol) and **1** (0.8 g, 4 mmol) in EtOH (8 mL), triethylamine (0.7 mL, 5 mmol) was added. The resulting reaction mixture was stirred at room temperature for 72 h and then refluxed for 40 h to reach completion. The mixture was concentrated under reduced pressure and the residue partitioned between DCM and 1 M NH_4Cl. The organic layer was washed with water and brine and dried over anhydrous $MgSO_4$. The residue was purified by flash chromatography on silica gel (eluting with Hex/EtOAc mixtures from 100:0 to 1:1) to give intermediate **18e** (0.87 g, 63%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 9.83−9.41 (br, 1H), 8.01−7.91 (br, 1H), 4.02−3.71 (br, 1H), 3.12−2.92 (br, 1H), 2.14 (s, 3H), 2.01−1.85 (m, 1H), 1.49−1.23 (br, 9H), 1.00−0.75 (br, 6H).

Potassium cyanide (0.4 g, 6 mmol) was added to a suspension of **18e** (0.9 g, 3 mmol) and DABCO (0.3 g, 3 mmol) in a 85:15 DMSO−H_2O mixture (12 mL) at room temperature. The reaction mixture was stirred at 80 °C for 24 h, then poured into ice−water. The solid that precipitated was filtered off, washed abundantly with water, and dried under air. The compound was purified by flash chromatography on silica gel (eluting with Hex/EtOAC mixtures from 100:0 to 1:1) to give the title compound **19e** (0.16 g, 20%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 9.93−9.51 (br, 1H), 8.24−8.19 (br, 1H), 4.02−3.73 (br, 1H), 3.14−2.92 (br, 1H), 2.23 (s, 3H), 2.06−1.89 (m, 1H), 1.41−1.23 (br, 9H), 1.00−0.74 (br, 6H). ESIMS m/z: 306 (MH^+).

1,1-Dimethylethyl 2-[2-Cyano-5-(trifluoromethyl)-4-pyrimidinyl]-2-(2-methylpropyl)hydrazinecarboxylate (20). Under an inert atmosphere, **19b** (0.1 g, 0.27 mmol) and copper(I) iodide (0.07 g, 0.35 mmol) were dissolved in dry DMF (5 mL). The resulting solution was preheated to 80 °C, prior to the addition of methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (0.26 g, 1.34 mmol) and hexamethylphosphoramide (0.25 g, 1.38 mmol). The resulting

reaction mixture was heated at 80 °C for a further 3 h, before the reaction reached completion. Solvent was evaporated in vacuo and the residue partitioned between EtOAc and 1 M NH_4Cl. The organic layer was treated water, brine and dried over anhydrous Na_2SO_4. The residue was purified by flash chromatography on silica gel (eluting with Hex/EtOAc mixtures from 100:0 to 3:2) to give the title compound **20** as a colorless oil (0.02 g, 24%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 8.65 (s, 1H), 6.70 (s, 1H), 4.53−4.25 (br, 1H), 3.16−2.89 (br, 1H), 2.21−1.95 (m, 1H), 1.5−1.28 (br, 9H), 1.08−0.83 (br, 6H). ESIMS *m*/*z*: 360 (MH^+).

1,1-Dimethylethyl 2-{2-Cyano-5-[(phenylmethyl)amino]-4-pyrimidinyl}-2-(2-methylpropyl)hydrazinecarboxylate (21). In a microwave vial, **19b** (0.2 g, 0.5 mmol), benzylamine (0.24 mL, 2.2 mmol), palladium(II) acetate (0.06 g, 0.03 mmol), (±)-BINAP (0.02 g, 0.03 mmol), and potassium carbonate (0.15 g, 1.1 mmol) were dissolved in DMF (5 mL). The resulting mixture was heated under microwave irradiation at 60 °C for 30 min. Solvent was evaporated to dryness, and the residue was taken up with DCM and washed with 1 N NH_4Cl, saturated $NaHCO_3$, and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and solvent was removed in vacuo. The crude material was purified by preparative HPLC (ACN/H_2O, 0.1% TFA, gradient 30−100%) to give the title compound **21** (0.006 g, 3%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 9.53−9.21 (br, 1H), 7.72 (s, 1H), 7.47−7.17 (m, 5H), 6.44−6.24 (br, 1H), 4.46 (d, J = 6 Hz, 2H), 3.60−3.25 (br, 2H), 1.98−1.79 (m, 1H), 1.49−1.06 (br, 9H), 0.92 (d, J = 6.6 Hz, 6H). ESIMS *m*/*z*: 397 (MH^+).

1,1-Dimethylethyl 2-(2-Cyano-5-{[4-(trifluoromethyl)phenyl]-ethynyl}-4-pyrimidinyl)-2-(2-methylpropyl)hydrazinecarboxylate (22). Under inert atmosphere, **19b** (0.103 g, 0.3 mmol), copper(I) iodide (0.006 g, 0.03 mmol), and bis(triphenylphosphine)-palladium(II) chloride (0.01 g, 0.01 mmol) were dissolved in dry DMF (5 mL). The resulting reaction mixture was stirred at room temperature for 5 min before 4-ethynyl-α,α,α-trifluorotoluene (0.04 mL, 0.2 mmol) and triethylamine (0.06 mL, 0.4 mmol) were added. The resulting reaction mixture was then heated to 50 °C for 22 h. The mixture was concentrated under reduced pressure and the residue partitioned between EtOAc and 1 M NH_4Cl. The organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. The residue was purified by preparative HPLC (ACN/H_2O, 0.1%TFA, gradient 50−100%) to give the title compound **22** as a yellow solid (0.04 g, 24%). ^{1}H NMR (300 MHz, DMSO-d_6, 80 °C) δ ppm: 10.07−9.88 (br, 1H), 8.70−8.56 (br s, 1H), 7.94−7.72 (m, 4H), 4.01−3.46 (br, 2H), 2.17−1.98 (m, 1H), 1.27 (br s, 9H), 0.96 (d, J = 6.7 Hz, 6H). ESIMS *m*/*z*: 460 (MH^+).

1,1-Dimethylethyl 2-[2-Cyano-5-(methyloxy)-4-pyrimidinyl]-2-(2-methylpropyl)hydrazinecarboxylate (26). To a solution of 5-methoxy-2-sulfanyl-4-pyrimidinol (2 g, 12.7 mmol) in water (50 mL), chloroacetic acid (2 g, 21.3 mmol) was added. The resulting reaction mixture was refluxed for 2 h. After the mixture was cooled to room temperature, concentrated HCl (8 mL) was added and the mixture was heated overnight. A precipitate appeared once the mixture cooled to room temperature. It was filtered off, washed abundantly with water, and dried under air to yield intermediate 5-(methyloxy)-2,4(1*H*,3*H*)-pyrimidinedione **23** as a brown solid (1.65 g, 92%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.32−11.01 (br, 1H), 10.60−10.23 (br, 1H), 7.00 (s, 1H), 3.57 (s, 3H).

To a suspension of intermediate **23** (1.64 g, 11.5 mmol) in phosphorus oxychloride (8.3 mL), *N*,*N*-dimethylaniline (1.64 mL) was added. The resulting reaction mixture was heated to reflux for 20 h. A precipitate appeared upon cooling to room temperature. It was filtered off, and the mother liquors were extracted with Et_2O. The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. Intermediate 2,4-dichloro-5-(methyloxy)pyrimidine **24** (1.3 g, 64%) was obtained as a pale-yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 8.20 (s, 1H), 4.03 (s, 3H).

To a solution of intermediate **24** (0.97 g, 5.4 mmol) in *i*-PrOH (25 mL), intermediate **1** (1.2 g, 6.3 mmol) and DIPEA (1.2 mL, 7.1 mmol) were added. The mixture was refluxed overnight. Solvent was removed under reduced pressure, and the residue was partitioned between DCM and 1 N NH_4Cl. The organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. The crude material was purified by flash chromatography on silica gel (eluting with Hex/EtOAc mixtures from 100:0 to 60:40%) to yield intermediate 1,1-dimethylethyl 2-[2-chloro-5-(methyloxy)-4-pyrimidinyl]-2-(2-methylpropyl)-hydrazinecarboxylate **25** (1.7 g, 94%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 7.76 (s, 1H), 6.82−6.61 (br, 1H), 3.84 (s, 3H), 3.77−3.52 (br, 2H), 2.15−2.01 (m, 1H), 1.58−1.32 (br, 9H), 0.94 (d, J = 6.6 Hz, 6H).

In a microwave vial, intermediate **25** (0.05 g, 0.2 mmol), potassium cyanide (0.03 g, 0.5 mmol), and DABCO (0.02 g, 0.2 mmol) were dissolved in a 9:1 DMSO−H_2O mixture (0.5 mL). The resulting reaction mixture was heated under microwave irradiation to 60 °C for 90 min. Solvent was removed under vacuum and the crude material was purified by preparative HPLC (ACN/H_2O, 0.1%TFA, gradient 20−100%) to give the title compound **26** as a colorless oil (0.02 g). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 7.92 (s, 1H), 6.91−6.77 (br, 1H), 3.92 (s, 3H), 3.80−3.59 (br, 2H), 2.18−2.01 (m, 1H), 1.65−1.31 (br, 9H), 0.96 (d, J = 6.6 Hz, 6H). ESIMS *m*/*z*: 322 (MH^+).

1,1-Dimethylethyl 2-{2-Cyano-5-[(methyloxy)methyl]-4-pyrimidinyl}-2-(2-methylpropyl)hydrazinecarboxylate (30). 5-(Hydroxymethyl)uracil hydrate (2.5 g, 15.6 mmol) and phosphorus pentoxide (13.7 g, 65.7 mmol) were added to phosphorus oxychloride (31.5 mL, 339 mmol), and the resulting mixture was heated to 115 °C overnight. Excess $POCl_3$ was distilled, and the residue was carefully added dropwise into a saturated $NaHCO_3$ solution previously cooled at 0 °C. Product was extracted with DCM, and the combined organic layers were washed with water and brine and dried over anhydrous Na_2SO_4. The crude material was purified by flash chromatography on silica gel (elute, Hex/EtOAc 19:1) to yield intermediate 2,4-dichloro-5-(chloromethyl)-pyrimidine **27** (2.1 g, 69%) as a pearl colored solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 8.66 (s, 1H), 4.64 (s, 2H). ^{13}C NMR (90 MHz, $CDCl_3$) δ ppm: 161.73, 160.16, 160.11, 128.53, 38.80.

Cesium carbonate (1.66 g, 5.1 mmol) was added to a solution of **27** (0.99 g, 5 mmol) and **1** (0.98 g, 5.2 mmol) in THF (30 mL), and the resulting reaction mixture was stirred at room temperature for 48 h before more cesium carbonate (0.83 g, 2.55 mmol) was added. Stirring at room temperature continued for further 24 h, and more cesium carbonate (0.83 g, 2.55 mmol) was added to drive the reaction to completion. After further 6 h, solvent was removed under reduced pressure. The residue obtained was partitioned between DCM and 1 M NH_4Cl, and the aqueous phase was further extracted with DCM. The combined organic layers were washed with water and brine and dried over anhydrous Na_2SO_4. The residue was purified by flash chromatography on silica gel (elute, Hex/EtOAc 9:1) to give intermediate 1,1-dimethylethyl 2-[2-chloro-5-(chloromethyl)-4-pyrimidinyl]-2-(2-methylpropyl)hydrazinecarboxylate **28** (1 g, 57%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C) δ ppm: 8.80−8.63 (br, 1H), 8.07−7.37 (br, 1H), 4.06−3.65 (br, 2H), 2.66−2.49 (br, 2H), 1.72−1.56 (m, 1H), 1.49−1.08 (br, 9H), 0.97−0.81 (m, 6H). ^{1}H NMR (300 MHz, DMSO-d_6, 80 °C) δ ppm: 8.73 (s, 1H), 7.70−7.49 (br, 1H), 3.92 (br, 2H), 2.6 (d, J = 7.0 Hz, 2H), 1.76−1.63 (m, 1H), 1.27 (br s, 9H), 0.92 (d, J = 6.6 Hz, 6H). ^{13}C NMR (90 MHz, DMSO-d_6) δ ppm: 161.78, 157.44, 154.70, 129.57, 78.13, 65.14, 55.14, 28.10, 27.92, 25.94, 20.45.

To a solution of **28** (0.1 g, 0.3 mmol) in dry MeOH (5 mL), sodium methoxide (0.02 g, 0.3 mmol) was added. The mixture was stirred at room temperature for 16 h. More sodium methoxide (0.02 g, 0.4 mmol) was added, and stirring was continued for further 4 h. Solvent was removed under reduced pressure. The residue was taken up with DCM and washed with 1 N NH_4Cl, water, and brine, and it was dried over anhydrous Na_2SO_4. The

crude material was purified by flash chromatography on silica gel (eluting with Hex/EtOAc mixtures from 100:0 to 0:100%) to yield intermediate 1,1-dimethylethyl 2-{2-chloro-5-[(methyloxy)-methyl]-4-pyrimidinyl}-2-(2-methylpropyl)hydrazinecarboxylate **29** (0.04 g, 44%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 8.51−8.27 (br, 1H), 5.72−4.80 (br, 1H), 4.03 (s, 3H), 3.98−3.55 (br, 2H), 2.77−2.40 (br, 2H), 1.86−1.65 (m, 1H), 1.40 (s, 9H), 0.94 (d, J = 6.6 Hz, 6H).

To a solution of **29** (0.04 g, 0.1 mmol) in a 9:1 DMSO−H_2O mixture (2 mL), potassium cyanide (0.02 g, 0.2 mmol) and DABCO (0.02 g, 0.15 mmol) were added. The resulting reaction mixture was stirred at room temperature for 22 h. Product was extracted with *n*-butanol, and the combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. The crude material was purified by preparative HPLC (ACN/H_2O, 0.1% TFA, gradient 10−100%) to give the title compound **30** (0.02 g, 40%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 8.72−8.53 (br, 1H), 5.73−4.96 (br, 1H), 4.05 (s, 3H), 4.04−3.69 (br, 2H), 2.75−2.44 (br, 2H), 1.84−1.66 (m, 1H), 1.40 (s, 9H), 0.96 (d, J = 6.6 Hz, 6H). ESIMS m/z: 336 (MH^+), 334 (MH^-).

General Procedure for the Synthesis of *N*-*tert*-Butoxycarbonyl-2-cyanopyrimidine Derivatives 33−78. To a solution of *tert*-butyl hydrazinecarboxylate (1 mmol) in dry MeOH, the appropriate aldehyde or ketone (1 mmol), $NaBH_3CN$ (2 mmol), and glacial AcOH (0.2 mL/mmol) were added. The mixture was stirred at room temperature until disappearance of starting materials (16−18 h, TLC analysis). After the reaction mixture was cooled in an ice bath, it was neutralized with 2 N NaOH. Solvent was evaporated, and the residue was partitioned between DCM and water. The organic phase was washed with brine, dried and solvent evaporated. The residue was purified on silica gel column chromatography to yield the corresponding intermediate hydrazine carboxylate **31**.

To a solution of 5-bromo-2,4-dichloropyrimidine (1.2 mmol) and the corresponding intermediate hydrazine carboxylate **31** (1 mmol) in EtOH or *i*-PrOH, DIPEA (2 mmol) was added. The resulting reaction mixture was refluxed until disappearance of starting materials (3−18 h, HPLC analysis). The mixture was concentrated under reduced pressure and the residue partitioned between DCM and 1 M NH_4Cl. The organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. The residue was purified by flash chromatography on silica gel to give the corresponding intermediate *N*-*tert*-butoxycarbonyl-2-chloropyrimidine **32**.

1,4-Diazabicyclo[2.2.2]octane (1 mmol) was added to a suspension of the corresponding intermediate *N*-*tert*-butoxycarbonyl-2-chloropyrimidine **32** (1 mmol) in a 9:1 DMSO−H_2O mixture at room temperature. Potassium cyanide (1.2 mmol) was then added to the mixture which was then stirred at the appropriate temperature (room temperature to 100 °C) until reaction was complete. The reaction mixture was cooled to room temperature and then poured into ice−water and kept at low temperature in order to favor product precipitation. The solid was filtered off, washed abundantly with water, and dried to yield the title compound. Whenever further purification of the final product was necessary, HPLC or flash chromatography on silica gel was used and compounds **33−78** were obtained (Supporting Information Table S7).

General Procedure for the Synthesis 4-[(4-Methyl-1-piperazinyl)methyl]benzoic Acid 2-Cyanopyrimidine Derivatives 81−119. To a solution of the corresponding compounds **31−76** (1 mmol) in dry ACN, *p*-toluenesulfonic acid monohydrate (3 mmol) was added. The mixture was stirred at room temperature overnight. The reaction mixture was concentrated, and the residue was dissolved in DCM and washed with saturated $NaHCO_3$. The organic phase was dried over anhydrous $MgSO_4$, filtered and solvent evaporated. The residue was purified by flash chromatography on silica gel to yield intermediate hydrazine derivative **79**.

To a solution of the corresponding intermediate derivative **79** (1 mmol) in dry THF, 4-bromomethylbenzoyl bromide (1 mmol) and DIPEA (2 mmol) were added. The reaction mixture was stirred at room temperature overnight. Then solvent was removed in vacuo and the residue partitioned between DCM and water. The organic layer was washed with 1 N aqueous NaOH and brine, dried over anhydrous $MgSO_4$, and concentrated to dryness. The residue was dissolved in a minimum amount of DCM and the solution stirred with hexane, intermediate bromobenzoyl derivative **80** precipitating off and used without any further purification.

To a solution of bromobenzoyl derivative **80** (1 mmol) in THF, DIPEA (1.5 mmol) and *N*-methylpiperazine (1.21 mmol) were added. The resulting reaction mixture was stirred at room temperature until it reached completion. Solvent was removed under reduced pressure and the resulting crude product was purified by either HPLC or flash chromatography on silica gel to yield the final products **81−119** (Supporting Information Table S8).

General Procedure for the Synthesis of 2-Cyanopyrimidine Derivatives 121−269. To a solution of the corresponding intermediate **19b** or **19c** (1 mmol) in dry ACN, *p*-toluenesulfonic acid monohydrate (3 mmol) was added. The mixture was stirred at room temperature overnight. The reaction mixture was concentrated, and the residue was dissolved in DCM and washed with saturated $NaHCO_3$. The organic phase was dried over $MgSO_4$, filtered and solvent evaporated in vacuo. The residue was purified by flash chromatography on silica gel to yield intermediate hydrazine derivative **120**.

To a solution of the corresponding intermediate derivative **120** (1 mmol) in dry THF or DCM, the corresponding acid chloride (1 mmol) and DIPEA (2 mmol) were added. The reaction mixture was stirred at room temperature until reaction was complete. Then solvent was removed in vacuo and the residue partitioned between DCM and water. The organic layer was washed with 1 N aqueous NaOH and brine, dried over anhydrous $MgSO_4$, and concentrated to dryness. The resulting crude product was purified by either HPLC or flash chromatography on silica gel to yield the final products **121−269** (Table S9 in Supporting Information).

In cases where the corresponding carboxylic esters were commercially available, they were hydrolyzed to the corresponding acid using standard procedures unless stated otherwise. Whenever the corresponding esters, acids, or acyl chlorides were not commercially available, specific procedures were followed in order to synthesize compounds **239**, **245−248**, **250−255**, **266−269** (see Supporting Information).

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Supporting Information Available: Spectroscopic data and LCMS data for all listed compounds and specific synthetic procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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