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Synthesis, Biological Activity, and ADME Properties of Novel *S*-DABOs/*N*-DABOs as HIV Reverse Transcriptase Inhibitors

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Previous studies aimed at exploring the SAR of C2-functionalized *S*-DABOs demonstrated that the substituent at this position plays a key role in the inhibition of both wild-type RT and drug-resistant enzymes, particularly the K103N mutant form. The introduction of a cyclopropyl group led us to the discovery of a potent inhibitor with picomolar activity against wild-type RT and nanomolar activity against many key mutant forms such as K103N. Despite its excellent antiviral profile, this compound suffers from a suboptimal ADME profile typical of many *S*-DABO analogues, but it could, however, represent a promising candidate as an anti-HIV microbicide. In the present work, a new series of *S*-DABO/*N*-DABO derivatives were synthesized to obtain additional SAR information on the C2-position and in particular to improve ADME properties while maintaining a good activity profile against HIV-1 RT. In vitro ADME properties (PAMPA permeation, water solubility, and metabolic stability) were also experimentally evaluated for the most interesting compounds to obtain a reliable indication of their plasma levels after oral administration.

Introduction

Among the various anti-HIV agents, non-nucleoside reverse transcriptase inhibitors (NNRTIs) are now an established part of the therapy for treating HIV infection.[1,2] In addition, increased attention has been focused on the possibility of using NNRTIs as HIV microbicides to prevent or decrease HIV infections in women.[3–5] Microbicides in fact represent one of the most promising strategies for combating the HIV/AIDS epidemic in the absence of an effective vaccine. It is generally accepted that a microbicide should act prior to the integration of proviral DNA into the host cell DNA; therefore, the two major compound classes that can be used for this are entry inhibitors and RT inhibitors. Some NNRTIs exhibit high-affinity binding and slow dissociation from RT,[6–9] a characteristic that is well-suited to a potential microbicide. Examples of NNRTIs in preclinical and clinical evaluation as microbicides include dapivirine (TMC 120), MIV 150, UC 781, and dihydroalkylthiobenzyloxopyrimidines (DABOs).[10–13] In this context, DABOs have been the object of many studies aimed at the identification of novel analogues with potent inhibitory activity toward wild-type HIV-1 and especially drug-resistant mutants.[14] In fact, given the rapid selection for mutant strains, new drugs with activity against drug-resistant viruses are currently needed. Over the years, many derivatives characterized by various C2 side chains (*S*-DABOs and *N*-DABOs, Figure 1) with high activity profiles have been developed.[15–19] Structure–activity relationship (SAR) studies of *S*-DABOs along with molecular modeling investigations into their putative binding mode have identified a key hydrogen bond between the NH group at position 3 of the pyrimidinone and Lys 101 (Figure 1, zone C) and profitable hydrophobic interactions between the right portion of the molecule and a large pocket in RT defined by the residues in zones A and B, as illustrated in Figure 1.[20,21]

The substituent at position C2, which is characteristic for each DABO family, establishes a series of interactions within the HIV-1 RT non-nucleoside inhibitor binding pocket (NNBP) which are essential for the antiviral activity of these derivatives. Although computational studies have allowed rationalization of the activity profiles of many compounds, these simulations

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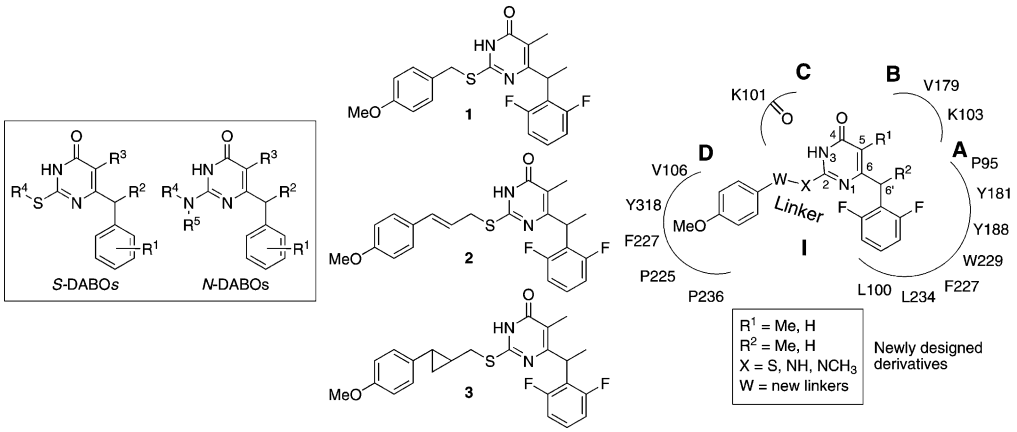

Figure 1. Structures of *S*-DABO, *N*-DABO, and hit compounds **1**–**3**. Interaction of a generic C2-*para*-methoxyarylalkyl DABO within the NNBP of HIV-1 RT and general structure (I) of the newly designed derivatives.

cannot explain the explicit role played by the substituent at position C2, owing to the considerable conformational flexibility of RT, which has complicated traditional structure-based drug design strategies for the discovery of novel NNRTIs.[20]

In a previous study dealing with the development of novel *S*-DABOs, we reported that the introduction of a *para*-methoxy arylalkyl moiety at C2 significantly increases the antiviral activity due to a profitable hydrophobic interaction with a large zone of the allosteric pocket (Figure 1, zone D).[21] In particular, the methoxy substituent that forms a hydrogen bond with N103 is able to disrupt the interaction of this residue with Y188 which in turn stabilizes the closed-pocket form of the K103N mutant, imparting resistance to NNRTIs (an analogous hydrogen bond contact is not present in the wild-type RT structure). In this manner, the C2 chain of the *S*-DABOs is able to penetrate into the NNBP, pushing residues V179 and Y181 away from Y188, which is known to be one of the major hurdles hampering the formation of the NNBP. Compound **1**, bearing a *para*-methoxybenzylthio chain at C2, was the first *S*-DABO derivative reported to be endowed with strong activity toward the K103N mutant due to its ability to open the NNBP. Subsequently, several analogues were synthesized, such as compound **2**,[22] with a cinnamylthio moiety, and its cyclopropyl bioisostere **3**,[23] which emerged as the most potent derivative (Figure 1). The latter compound, in fact, showed picomolar activity against wild-type RT and nanomolar activity against many key mutant viral strains. Kinetic studies also demonstrated that compound **3** dissociates very slowly, if at all, from the RT surface with a dissociation rate constant significantly lower than values obtained with the same technique for delavirdine, efavirenz, and nevirapine. Despite their excellent activity profiles, these compounds, as is the case for most of the DABO derivatives, suffer from poor absorption, distribution, metabolism, and excretion (ADME) properties. In their review, Sweeney and Klumpp[24] reported the importance of pharmacokinetic factors in the success of first-line anti-HIV therapies based on NNRTIs. A head-to-head comparison between nevirapine and efavirenz highlighted that even if the latter possesses higher in vitro antiviral activity, its poor pharmacokinetic profile makes it clinically similar to the less active nevirapine.

As a continuation of our research on the identification of DABO drug candidates for the treatment or prevention of HIV,[25] we report herein the synthesis of two series of novel DABO derivatives (*S*-DABOs and *N*-DABOs) bearing different linkers at position C2. We first focused on the synthesis of novel *S*-DABO derivatives by introducing various hydrophobic spacers at C2 and linkers containing hydrogen bond donor/acceptor moieties in order to evaluate the possibility of establishing additional profitable interactions with the NNBP. We next planned the synthesis of *N*-DABO derivatives with an acceptable ADME profile in terms of improved water solubility, permeation, and low first-pass metabolism.

Results and Discussion

S-DABOs

The initial series of novel *S*-DABO derivatives to be synthesized was characterized by the presence of a methyl group at C5 and C6′ ($R^1 = R^2 = CH_3$) and various linkers (W) at C2. First, lead compound **2** was modified by introducing an additional methyl group on the double bond of the linker to increase the lipophilicity and to further investigate the role played by this substituent on the left portion of the molecule (compound **7**). We then focused on the terminal alkyne derivative **10** to be used as a key intermediate for the synthesis of new analogues. The triple bond can be easily manipulated and converted into the triazole derivatives **14** and **15** by 1,3-dipolar cycloaddition, or in the case of diene **13**, by enyne cross-metathesis reaction. This latter compound was designed with the intent of exploring possible hydrophobic interactions between the diene feature and zone D of HIV RT, similar to those established by the cyclopropyl moiety of lead compound **3**. Moreover, the triple bond was reduced to *cis* alkene **20** to compare its activity with the corresponding *trans* analogue **2**. Finally, we decided to introduce an oxygenated moiety within the linker to evaluate the possibility of establishing hydrogen bond interactions within zone D of the NNBP. The other pharmacophoric groups of the *S*-DABO leads **1**–**3** were left unchanged, with the exception of the methyl group at C6′, which was removed from

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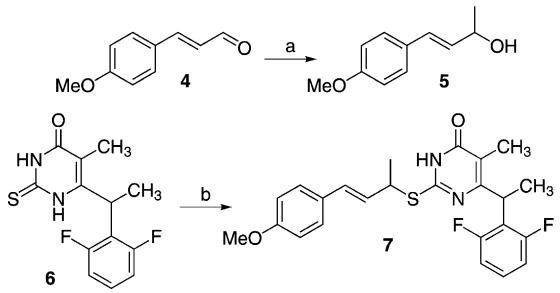
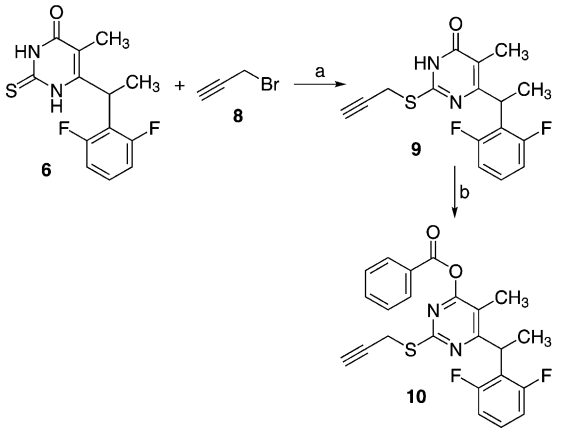
some derivatives to compare the activity of the diastereomeric mixtures with the corresponding racemates.

The first target compound **7** was prepared as shown in Scheme 1. The *trans*-4-methoxycinnamaldehyde was allowed to react with methylmagnesium bromide to afford the 1,2-addition product alcohol **5**, which in turn reacted with 6-(1-(2,6-difluorophenyl)ethyl)-5-methyl-3,4-dihydro-2-thioxopyrimidin-4(3*H*)-one **6** via microwave-assisted Mitsunobu reaction, leading to the desired compound **7** (Scheme 1). The key intermediate alkyne **10** was then synthesized from compound **6**, which was allowed to react with propargyl bromide in the presence of potassium carbonate in anhydrous DMF at 0 °C to obtain the desired compound **9**. Microwave-assisted conditions were not used in the latter alkylation to avoid formation of an undesired bicyclic side product.[26] The C4-carbonyl group was then protected as a benzoyl derivative (compound **10**) to avoid any side cyclization reaction during subsequent synthetic steps (Scheme 2). Sonogashira coupling of thiouracil **10** with the 4-iodoanisole followed by work-up deprotection with an aqueous mixture of ammonium chloride and ammonium hydroxide led to compound **12** (Scheme 3). Alternatively, intermediate **11** was isolated by a neutral work-up and allowed to react with ethylene in the presence of Grubbs' second-generation catalyst to give, after deprotection, diene **13**. Finally, copper-catalyzed azide–alkyne cycloaddition reactions using a microwave-assisted protocol developed in our laboratories afforded derivatives **14** and **15**. Compound **10** reacted with the *para*-methoxyphenyl azide and, after in situ deprotection, gave compound **14**. Reaction of **10** with sodium azide and *para*-methoxybenzyl chloride gave compound **15**, which differs from the previous **14** by the presence of an extra methylene bridge between the triazole ring and the *para*-methoxyphenyl moiety. The synthesis of *cis*-alkene derivative **20** was carried out as shown in Scheme 4. Propargyl alcohol **16** was allowed to react via Sonogashira coupling with the 4-iodoanisole to give compound **17**, which was further reduced with Lindlar catalyst to give *cis*-alkene **18**. This latter compound was finally converted into the corresponding bromide and reacted with thiouracil **6** to give desired pyrimidinone **20**. The synthesis of oxygenated derivatives **25**–**33** is illustrated in Scheme 5 and was performed by starting from derivatives with a propan-3-one spacer as versatile intermediates for further functionalization. The synthesis of key intermediates **25** and **26** was achieved by hetero-Michael condensation[27] between 1-(4-methoxyphenyl)prop-2-en-1-one **23** and pyrimidinones **6** and **24**. The synthesis of intermediate **25** was planned in order to obtain a series of non-diastereomeric derivatives **27**, **29**, **31** and non-chiral **33**.

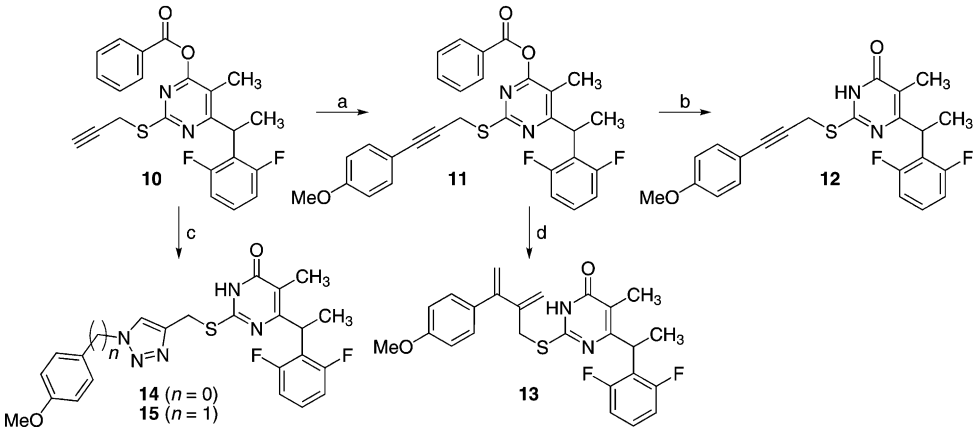
Starting from 4-methoxybenzaldehyde **21**, a Grignard reaction was performed with vinylmagnesium bromide to obtain compound **22**, which was then oxidized with manganese dioxide to give the desired 1-(4-methoxyphenyl)prop-2-en-1-one **23** (Scheme 5). The hetero-Michael condensation was finally



Scheme 1. *Reagents and conditions*: a) MeMgBr, THF, 0 °C, 1.5 h; b) **5**, $P(CH_3)_3$, DIAD, DMF, MW, 40 °C, 30 min.



Scheme 2. *Reagents and conditions:* a) K_2CO_3, DMF, 0 °C, 1 h; b) BzCl, py, r.t., 2 h.



Scheme 3. *Reagents and conditions*: a) 4-iodoanisole, CuI, $PdCl_2(PPh_3)_2$, Et_3N, DMF, r.t., 10 min; b) NH_4Cl/NH_4OH aq. solution, 30 min; c) 1. (for $n=0$): 4-$OMePhN_3$, sodium ascorbate/$Cu(SO)_4$, H_2O/*t*BuOH, MW, 120 °C, 10 min; (for $n=1$): NaN_3, BnCl, sodium ascorbate/$Cu(SO)_4$, H_2O/*t*BuOH, MW, 125 °C, 10 min; 2. NH_4Cl/NH_4OH aq. solution, 30 min; d) 1. $CH_2{=}CH_2$ 1 atm, Grubbs 2, toluene, 80 °C, 48 h; 2. NH_4Cl/NH_4OH aq. solution, 30 min.

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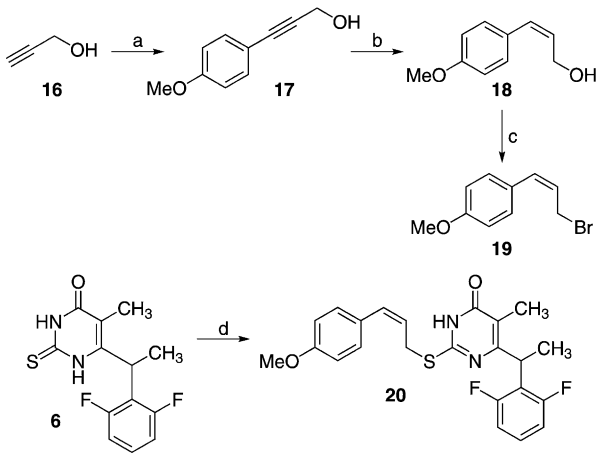


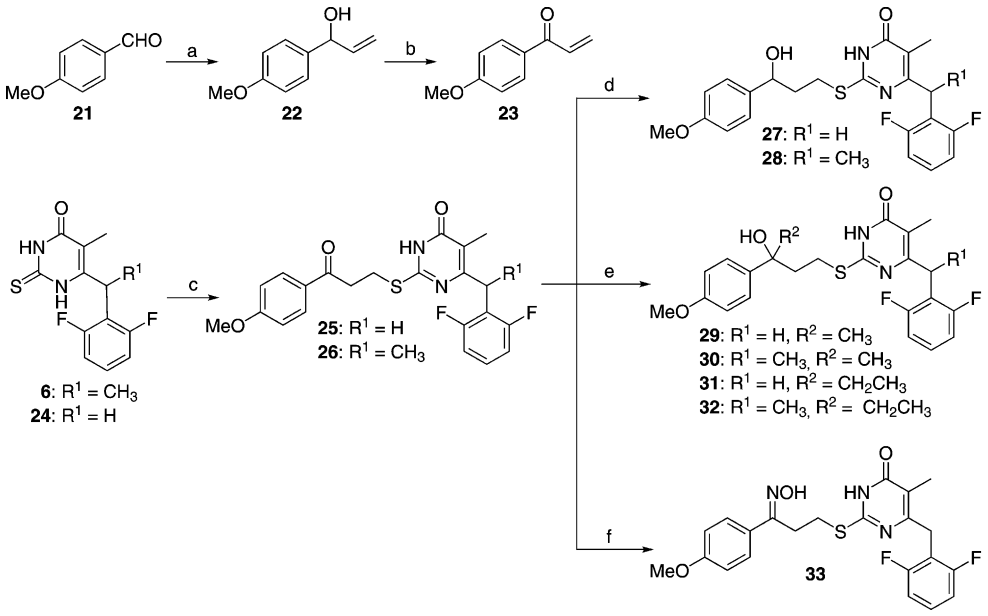
Scheme 4. *Reagents and conditions*: a) 4-iodoanisole, CuI, $PdCl_2(PPh_3)_2$, Et_3N, DMF, r.t., 5 min; b) H_2, Lindlar catalyst, quinoline, EtOAc, r.t., 48 h; c) PBr_3, Et_2O, 0 °C, 2 h; d) **19**, K_2CO_3, DMF, r.t., 3 h.

performed in anhydrous DMF using trifluoromethanesulfonic acid as catalyst to obtain the desired compounds in good yields. The ketone moiety of the side chain was manipulated to give secondary and tertiary alcohol derivatives **27**–**32**. Reduction with sodium borohydride led to compounds **27** and **28**. Grignard reaction with methyl- and ethylmagnesium bromide gave compounds **29**, **30** and **31**, **32**, respectively. Finally, we decided to treat compound **25** with hydroxyamine hydrochloride in ethanol to furnish the corresponding oxime **33** with the aim of evaluating the interactions of the oxime group with the RT binding site.

***N*-DABOs**

With the aim of obtaining improved RT inhibitors endowed with an acceptable ADME profile, we next focused our attention on the synthesis of *N*-DABO analogues of lead compounds **1**–**3**. Docking studies previously performed by us on this compound class revealed a further anchor point represented by the hydrogen bond between the NH function at C2 and the carbonyl backbone of Lys 101, which could compensate for the lack of hydrophobic interactions within the active site. Previously reported *N*-DABO derivatives were characterized primarily by alkyl or cycloalkyl substituents on the nitrogen atom at C2 and, despite the large amount of data on wild-type RT, very little information was reported for drug-resistant mutants such as K103N.[28] Considering the important role played by the 4-methoxyphenyl substituent in opening the NNBP of the K103N mutant in the case of the *S*-DABO series, we planned to synthesize new *N*-DABOs characterized by a 4-methoxybenzyl and 4-methoxyphenylcyclopropyl C2 linker bound to a primary or secondary amino group. In fact, while the NH group at C2 gives an additional hydrogen bond interaction with Lys 101, further functionalization of this nitrogen atom could give additional insight into the structure–activity relationships for this class of derivatives. The classical approaches for obtaining *N*-DABOs are a direct Pinner pyrimidine synthesis or a C2-nucleophilic substitution on DABOs or *S*-DABOs using the appropriate amines. The drawbacks of these procedures are long reaction times, high temperatures, and the use of nucleophiles in great excess; these limit their applicability with amines that are not readily accessible. We recently reported a versatile microwave-assisted domino Michael addition/cyclocondensation protocol for the preparation of pyrimidin-4(3*H*)-one derivatives for use in the synthesis of novel *N*-DABOs.[29] Following this new approach, we synthesized alkynone **38** as a key intermediate for our Michael addition/cyclocondensation protocol. Wittig olefination of the commercially available 1-(2,6-difluorophenyl)ethanone **34** with methoxymethyltriphenylphosphonium chloride and *n*-butyllithium at −78 °C in THF gave the corresponding enol ether **35**, which, after cleavage with boron tribromide followed by hydrolysis, gave aldehyde **36** in good yield (Scheme 6). Alkynone **38** was then synthesized through a one-pot/two-step sequence with an initial condensation of aldehyde **36** with the Ohira–Bestmann reagent[30] to obtain alkyne **37**, which was submitted directly to carbonylation to afford the key intermediate **38**.

The substituted guanidines **39a**–**d** required for the synthesis



Scheme 5. *Reagents and conditions*: a) vinylmagnesium bromide, THF, 0 °C, 1 h; b) MnO_2, Et_2O, r.t., 16 h; c) **23**, trifluoromethanesulfonic acid, DMF, r.t., 1 h; d) $NaBH_4$, MeOH, 0 °C→r.t., 15 min; e) R^2-MgBr, THF, 0 °C→r.t., 1 h; f) $NH_2OH{\cdot}HCl$, NaOAc, EtOH, r.t., 16 h.

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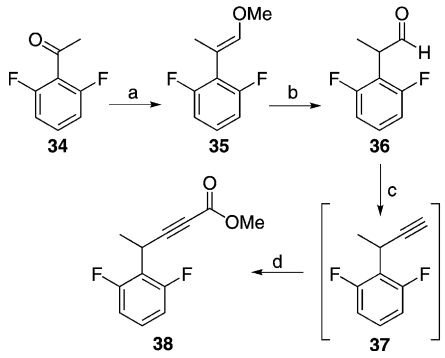
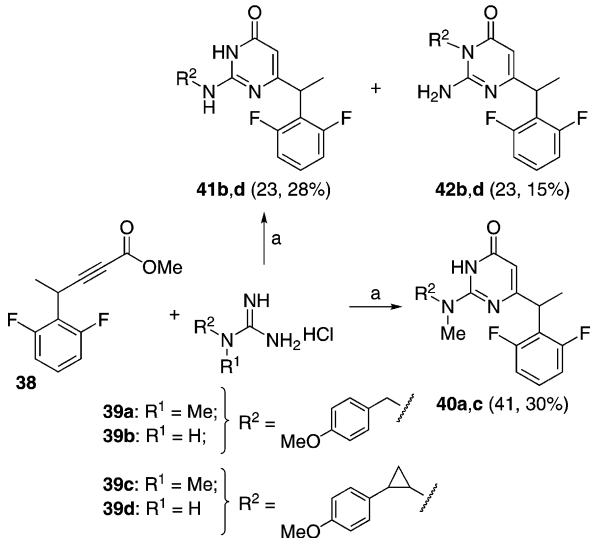


Scheme 6. *Reagents and conditions*: a) (methoxymethyl)triphenylphosphonium chloride, *n*BuLi, THF, −78 °C→r.t; b) BBr_3, CH_2Cl_2, −78 °C, 2 h; c) dimethyl 1-diazo-2-oxopropylphosphonate, K_2CO_3, MeOH, r.t., 12 h; d) $PdCl_2$, $CuCl_2$, CO, NaOAc, MeOH, r.t., 2 h.

of the desired *N*-DABO derivatives **40a**,**c** and **41b**,**d** (Scheme 7) were synthesized in a few steps and in high yields by starting from commercially available compounds. Guanidines reacted under Michael addition/cyclization conditions with **38** to give the desired *N*-DABO derivatives **40a**,**c** and **41b**,**d**. Compounds **40a**,**c** were obtained as single products in good yields, whereas derivatives **41b**,**d** were isolated together with their regioisomers **42b**,**d**.



Scheme 7. *Reagents and conditions*: a) Na_2CO_3, *t*BuOH, 120 °C, MW, 20 min (for R^2 = 4-methoxybenzyl) or 40 min (for R^2 = 4-methoxyphenylcyclopropyl).

Biological assays and in vitro ADME

The synthesized compounds were evaluated in enzyme assays for their ability to inhibit either wild-type (wt) or mutated RTs, as well as in assays with MT-4 cells to evaluate their cytotoxicity and anti-HIV activity as described previously,[31] in comparison with nevirapine (NVP) and efavirenz (EFV), used as reference drugs. In particular, the following mutants were used: K103N and Y181I for enzymatic tests, K103N, Y181C, and Y188L for tests on cell lines. The results of these assays are reported in Table 1. Among the novel *S*-DABOs, compound **7** retained an activity similar to or better than that of the leads **1**–**3**. Interestingly, derivatives **29**–**33**, while modestly active against wt RT and the K103N and Y118L mutants, showed very potent inhibitory activities against the replication of the Y18C mutant virus. The tertiary hydroxy moiety or oxime group on the linker of these compounds may play a role in the interactions with key residues of the latter mutant. On the other hand, compounds characterized by a secondary hydroxy group (**27** and **28**) did not show any selectivity for a specific RT mutant, and derivative **28** proved to be the most interesting derivative, endowed with good fold resistance. With the only exception of compound **40a**, the novel *N*-DABO derivatives (Entries 16–19) showed similar or even better activity profiles than leads **1**–**3**. More importantly, subsequent in vitro ADME studies showed improved properties for the synthesized *N*-DABOs over the corresponding *S*-DABOs (Table 2). In fact, the importance of optimizing ADME properties for potential drug candidates is widely recognized; the success of a potential drug is determined not only by good efficacy and specificity, but also by acceptable ADME and toxicity properties. Early assessment and optimization allow earlier corrections of property limitations, thereby decreasing the risk of drug failure in advanced phases.[32] In this regard, a few in vitro experiments can be easily conducted to quickly establish the absorption and stability of drug candidates in the early phase: aqueous solubility, parallel artificial membrane permeability assay (PAMPA),[33] and human liver microsome (HLM) stability determination. These experiments have proven to be reliable indicators of plasma exposure levels after oral administration. Accordingly, some of the most promising compounds previously identified were submitted to a thorough ADME study to identify promising drug candidates endowed with better aqueous solubility, passive permeation, and low phase 1 metabolism (Table 2).

Passive membrane permeability was initially evaluated in a PAMPA by using a validated protocol we recently applied to a family of pyrazolo[3,4-*d*]pyrimidines.[34] The aqueous solubility was evaluated by following the method developed by Avdeef,[35] and the results are expressed in $\mu g\,mL^{-1}$. Metabolic stability was finally evaluated by incubating the aforementioned compounds with 5 μL pooled HLM fractions for 1 h at 37 °C in order to simulate phase 1 metabolism. The parent drugs and metabolites were subsequently determined by LC–MS analysis (see Experimental Section). *N*-DABO derivatives **40a** and **41b** showed values of membrane permeation and metabolic stability similar to that of *S*-DABO lead **1**, yet also showed much improved aqueous solubility. In contrast, *S*-DABO derivative **27** did not show an improved ADME profile with respect to that of lead compound **1**.

Conclusions

In summary, we explored the effects of various linkers at position C2 of *S*- and *N*-DABO NNRTIs, derived from leads **1**–**3**, on their antiviral activity and in vitro ADME properties. Several compounds showed nanomolar inhibitory potency against

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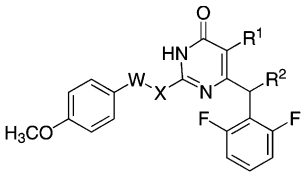
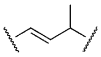
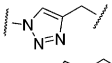
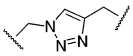
Table 1. Anti HIV-1 activity and cytotoxicity of the new *S*-/*N*-DABO derivatives.



Entry	Compd	X	W	R^1	R^2	IC_{50} [μM][a,b] wt	K103N	Y181I	EC_{50} [μM][a,c] NL4-3 wt	K103N	Y181C	Y188L	CC_{50} [μM][d]
1	**7**	X		CH_3	CH_3	0.02 ±0.003	0.04 ±0.01	0.8 ±0.1	0.0006 ±0.0001	0.52 ±0.05	0.16 ±0.06	0.045 ±0.007	37.3 ±0.9
2	**12**	S		CH_3	CH_3	0.06 ±0.006	0.36 ±0.06	0.47 ±0.07	0.01 ±0.001	0.1 ±0.01	0.02 ±0.003	0.4 ±0.05	>25
3	**13**	S		CH_3	CH_3	0.14 ±0.02	ND[e]	ND	0.08 ±0.01	0.9 ±0.1	0.085 ±0.005	>15.4	15.4 ±0.5
4	**14**	S		CH_3	CH_3	0.32 ±0.02	ND	ND	0.12 ±0.02	4.23 ±0.03	0.098 ±0.008	3.72 ±0.02	>25
5	**15**	S		CH_3	CH_3	1.11 ±0.01	ND	ND	0.16 ±0.03	>25	>25	>25	>25
6	**20**	S		CH_3	CH_3	>4	ND	ND	13.2 ±0.5	>25	0.13 ±0.01	>25	>25
7	**25**	S		CH_3	H	0.13 ±0.01	ND	ND	0.022 ±0.002	0.76 ±0.06	0.65 ±0.05	0.67 ±0.07	13.3 ±0.5
8	**26**	S		CH_3	CH_3	>4	ND	ND	>13.42	>13.42	>13.42	>13.42	13.4 ±0.5
9	**27**	S		CH_3	H	2.18 ±0.08	ND	ND	1.09 ±0.09	>11.28	0.6 ±0.05	>11.28	11.3 ±0.5
10	**28**	S		CH_3	CH_3	0.08 ±0.01	0.18 ±0.01	1 ±0.05	0.44 ±0.04	0.36 ±0.06	0.12 ±0.02	0.03 ±0.003	17.2 ±0.5
11	**29**	S		CH_3	H	0.48 ±0.08	ND	ND	>3.2	>3.2	0.28 ±0.08	>3.2	3.2 ±0.2
12	**30**	S		CH_3	CH_3	0.17 ±0.05	ND	ND	0.3 ±0.03	>3.1 ±0.1	0.015 ±0.005	>3.1	3.1 ±0.1
13	**31**	S		CH_3	H	0.22 ±0.02	ND	ND	0.4 ±0.04	>25	0.007 ±0.001	>25	>25
14	**32**	S		CH_3	CH_3	>4	ND	ND	>4.45	>4.45	0.079 ±0.009	>4.45	4.45 ±0.05
15	**33**	S		CH_3	H	0.76 ±0.06	ND	ND	0.45 ±0.05	>10.27	0.0015 ±0.0001	>25	10.27 ±0.03
16	**40a**	NCH_3	CH_2	H	CH_3	0.006 ±0.001	1.055	0.366 ±0.06	0.002 ±0.0002	0.003 ±0.0003	1.61 ±0.02	0.01 ±0.001	>5
17	**40c**	NCH_3		H	CH_3	0.010 ±0.004	0.885 ±0.007	0.666 ±0.006	0.003 ±0.0004	0.07 ±0.009	>5	2.95 ±0.05	>5
18	**41b**	NH	CH_2	H	CH_3	0.057 ±0.007	5	ND	0.003 ±0.0005	2.23 ±0.03	0.06 ±0.01	>5	>5
19	**41d**	NH		H	CH_3	0.01 ±0.001	0.88 ±0.08	0.313 ±0.005	0.001 ±0.0001	0.13 ±0.01	0.10 ±0.001	>5	>5
22	**1**	S	CH_2	CH_3	CH_3	0.003	45	>20	0.00003 ±0.00001	0.55 ±0.05	0.30	0.95	>12.43
23	**2**	S		CH_3	CH_3	0.292	3.7	>20	0.0002 ±0.00004	0.26 ±0.03	0.13	0.07	>11.7
24	**3**	S		CH_3	CH_3	0.04	3.00	6.00	0.0006	0.52	0.16	0.045	37.3
25	NVP	NH_2	–	–	–	0.40 ±0.05	8.00 ±0.1	20.0 ±0.9	0.47 ±0.07	>5	>5	>5	>5
26	EFV	–	–	–	–	0.04 ±0.06	0.40±0.01	0.10±0.1	0.0004 ±0.0002	0.01 ±0.003	0.56 ±0.0005	0.45 ±0.01	>1

[a] Data represent the mean of at least two experiments. [b] IC_{50}: inhibitory concentration 50; concentration required to inhibit 50% of the enzyme. [c] EC_{50}: effective concentration 50; concentration required to inhibit 50% of HIV-induced cell death, evaluated by the MTT method in MT-4 cells. [d] CC_{50}: cytotoxic concentration 50; concentration required to induce 50% death of uninfected cells evaluated by the MTT method in MT-4 cells. [e] ND: not determined.

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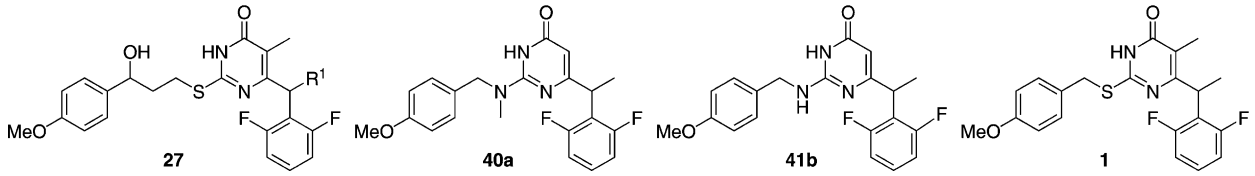
Table 2. ADME properties of *S*-/*N*-DABO derivatives.



Compd	QP-log *P*[a]	QP-log *S*[b]	QP-Caco [nm s^{-1}][c]	$P_{app}\times10^{-6}$ [cm s^{-1}][d]	Aq. Sol. [μg mL^{-1}]	log *S*	Met. Stab. [%][e]	Memb. Ret. [%][f]
27	4.44	−6.9	859	0.97	0.011	−7.6	99	0
40a	4.98	−6.1	2485	11.6	0.11	−6.5	99	32
41b	4.33	−6.1	1119	6.72	2.42	−5.18	98	6.1
1	4.8	−6.3	1845	10.5	<0.01	−7.5	99	1.7

[a] Predicted octanol/water partition coefficient; range of recommended values: (−2.0)–(+6.5). [b] Predicted aqueous solubility; range of recommended values: (−6.5)–(+0.5). [c] Predicted apparent Caco-2 cell permeability; range of recommended values: <25: poor, >500: great. [d] PAMPA: see Experimental Section for details. [e] Metabolic stability expressed as a percentage of unmodified parent drug after 60 min incubation with HLM protein. [f] Membrane retention expressed as percentage of compound retained within the phospholipid layer (50% DMSO).

wild-type HIV-1 and relevant drug-resistant mutants. Even if more detailed investigations are required to clearly understand the role of the C2 linker on the anti-HIV profile of *S*-DABO derivatives, the data reported herein give new insight into the chemical space that could be further explored around the C2 position of the *S*-DABO family. In addition, these preliminary results suggest that the conversion of the *S*-DABO derivatives into the corresponding *N*-DABOs may lead to new derivatives characterized by improved ADME profiles (especially aqueous solubility) for this potent family of antiviral agents, and will aid in the further rational design of more effective NNRTIs.

Experimental Section

General chemistry

Materials and methods: All commercially available reagents were purchased from Sigma–Aldrich and were used as received. Solvents were reagent grade and, when necessary, purified and dried by standard methods. Anhydrous reactions were run under positive pressure of dry N_2 or Ar gas. IR spectra were recorded on a PerkinElmer BX FTIR system, using KBr pellets. Thin-layer chromatography (TLC) was carried out using Merck TLC silica gel 60 F_{254} plates. Flash chromatographic purifications were performed on columns packed with Merck silica gel 60, 23–400 mesh. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz on a Bruker Avance DPX400, at 300 MHz on a Varian VXR-300, and at 200 MHz on a Bruker AC200F spectrometer. Chemical shifts are reported relative to tetramethylsilane at 0.00 ppm. Elemental analyses (C, H, N) were performed in house using a PerkinElmer Elemental Analyzer 240C and data are within 0.4% of the theoretical values. Melting points were taken with a Gallenkamp melting point apparatus and are uncorrected. MS data were obtained with an Agilent 1100 LC/MSD VL system (G1946C) at a flow rate of 0.4 mL min^{-1} using a binary solvent system of 95:5 MeOH/H_2O. UV detection was monitored at 254 nm. MS data were acquired in positive and negative mode, scanning over the mass range 50–1500. The following ion source parameters were used: drying gas flow, 9 mL min^{-1}; nebulizer pressure, 40 psig; drying gas temperature, 350 °C. All target compounds possessed a purity of ≥95% as verified by elemental analyses by comparison with the theoretical values.

Microwave irradiation experiments: Microwave irradiation experiments were conducted with a CEM Discover Synthesis Unit (CEM Corp., Matthews, NC, USA). The instrument consists of a continuous focused microwave power delivery system with operator-selectable power output from 0 to 300 W. The temperature of the vessel contents was monitored with a calibrated infrared temperature control unit mounted under the reaction vessel. All experiments were performed with a stirring option, whereby the contents of the vessel are stirred by means of a rotating magnetic plate located below the floor of the microwave cavity and a Teflon-coated magnetic stir bar in the vessel.

ADME assays

Chemicals: All solvents, reagents, and L-α-phosphatidylcholine were from Sigma–Aldrich Srl (Milan, Italy). Dodecane was purchased from Fluka (Milan, Italy). Pooled HLM fractions (20 mg mL^{-1}) from human male donors were obtained from BD Gentest-Biosciences (San Jose, CA, USA). Milli-Q purified water (Millipore, Milford, MA, USA) was used. Hydrophobic filter plates (MultiScreen-IP, Clear Plates, $\varnothing_{pore}$ = 0.45 μm), 96-well microplates, and 96-well UV-transparent microplates were obtained from Millipore (Bedford, MA, USA).

Parallel artificial membrane permeability assay (PAMPA): Donor solution (0.5 mM) was prepared by diluting 1 mM compound stock solution in DMSO with phosphate buffer (0.025 M, pH 7.4). Filters were coated with 5 μL 1% (*w*/*v*) solution of phosphatidylcholine in dodecane. Donor solution (150 μL) was added to each well of the filter plate. To each well of the acceptor plate were added 300 μL solution (50% DMSO in phosphate buffer). All compounds were tested in three different plates in different days. The sandwich was incubated for 5 h at room temperature under gentle shaking. After the incubation time, the sandwich plates were separated and samples were taken from both receiver and donor sides and analyzed by LC with UV detection. LC analyses were conducted by HPLC (PerkinElmer series 200) using a Polaris C_{18} column (150×4.6 mm, 5 μm particle size) at a flow rate of 0.8 mL min^{-1} with a mobile phase composed of CH_3CN/0.1% $HCOOH_{(aq)}$ (60:40 *v*/*v*) for each compound. Permeability (P_{app}) for PAMPA was calculated according to Equation (1), obtained from the equation of Wohnsland and Faller[36] and Sugano et al.[37] with some modification, in order to obtain permeability values in cm s^{-1}:

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$$P_{app} = \frac{V_D V_A}{(V_D + V_A)At} - \ln(1 - r) \quad (1)$$

for which V_A is the volume in the acceptor well, V_D is the volume in the donor well (cm^3), A is the "effective area" of the membrane (cm^2), t is the incubation time (s), and r is the ratio between drug concentration in the acceptor and equilibrium concentration of the drug in the total volume ($V_D + V_A$). Drug concentration is estimated by using peak area integration.

Microsomal stability assays: Each compound in DMSO solution was incubated at 37 °C for 60 min in 125 mM phosphate buffer (pH 7.4) and 5 μL HLM protein (0.2 mg mL^{-1}) in the presence of a NADPH-generating system at a final volume of 0.5 mL (final compound concentration: 50 μM); DMSO did not exceed 2% (final solution). The reaction was stopped by cooling in ice and adding 1.0 mL CH_3CN. The reaction mixtures were then centrifuged (10 min, 4 °C, 10000 rpm), and the parent drug and metabolites were subsequently determined by LC–MS. Chromatographic analyses were performed with an Agilent 1100 LC/MSD VL system (G1946C; Agilent Technologies, Palo Alto, CA, USA) consisting of a vacuum solvent degassing unit, a binary high-pressure gradient pump, an 1100 series UV detector, and an 1100 MSD model VL benchtop mass spectrometer. Chromatographic separation was carried out with a Varian Polaris 5 C_{18} A column (150–4.6 mm, 5 μm particle size) and gradient elution [eluent A: CH_3CN; eluent B: $HCOOH_{(aq)}$ (0.1%)]. The analysis started with 2% eluent A, which was rapidly increased to 70% over 10 min, then slowly increased to 98% over 15 min. The flow rate was 1.0 mL min^{-1}, and injection volume was 20 μL. The mass spectra detection (MSD) single-quadrupole instrument was equipped with orthogonal spray API-ES (Agilent Technologies). N_2 was used as both nebulizing and drying gas (pressure: 40 psi, flow rate: 9 mL min^{-1}, capillary voltage: 3000 V, fragmentor voltage: 70 V, vaporization temperature: 350 °C). UV detection was set at λ 254 nm. LC–ESIMS determination was performed by operating the MSD in the positive ion mode. Spectra were acquired over the scan range m/z 100–1500 using a step size of 0.1 u. The percentage of non-metabolized compound was calculated by comparison with reference solutions.

Water solubility assays: Each solid compound (1 mg) was added to 1 mL H_2O. Samples were shaken in a shaker bath at 20 °C for 24–36 h. The suspensions were filtered through a 0.45 μm nylon filter (Acrodisc), and the dissolved compound analyzed by LC–MS–MS. Determinations were performed in triplicate for each compound. An LC–MS system was used for quantification and consisted of a Varian apparatus including a vacuum solvent degassing unit, two pumps (212-LC), a triple quadrupole MSD (Model 320-LC) mass spectrometer with ES interface and Varian MS Workstation System Control Ver. 6.9 software. Chromatographic separation was carried out with a Pursuit C_{18} column (50×2.0 mm) (Varian) with 3 μm particle size and gradient elution [eluent A: CH_3CN; eluent B: $HCOOH_{(aq)}$ (0.1%)]; Analysis started with 0% eluent A, which was increased linearly to 70% over 10 min, then slowly increased to 98% over 15 min. The flow rate was 0.2 mL min^{-1}, and injection volume was 5 μL. The instrument was operated in positive mode and parameters were as follows: detector 1850 V, drying gas pressure 25.0 psi, desolvation temperature 300.0 °C, nebulizing gas 45.0 psi, needle 5000 V, and shield 600 V. N_2 was used as nebulizer and drying gas. Collision-induced dissociation was performed with Ar as the collision gas at a pressure of 1.8 mTorr in the collision cell. The transitions, capillary voltage, and collision energy used for each compound are summarized in Table 3. Quantification of a given compound was made by comparison with the appropriate calibration curves generated with standard solutions in MeOH.

Table 3. Chromatographic and MS parameters of selected compounds.

Compd	t_R [min][a]	Monitored Transition [m/z]	Collision Energy [eV]	Capillary Voltage [V]
27	5.9	433→147	−15	34
40a	5.9	386→121	−19.0	50
41b	5.3	372→121	−17.0	69
1	6.4	403→121	−17.0	40

[a] Retention time.

Biological methods

Chemicals: [^{3}H]dTTP (40 Ci mmol^{-1}) was obtained from Amersham, and unlabeled dNTPs were purchased from Boehringer. GF/C filters were supplied by Whatman. All other reagents were of analytical grade and were purchased from Merck or Fluka.

Nucleic acid substrates: The homopolymer poly(rA) (Pharmacia) was mixed at weight ratios in nucleotides of 10:1 to the oligomer oligo(dT)$_{12-18}$ (Pharmacia) in 20 mM Tris·HCl (pH 8.0) containing 20 mM KCl and 1 mM EDTA, heated at 65 °C for 5 min, and then slowly cooled to room temperature.

Expression and purification of recombinant HIV-1 RT forms: Recombinant heterodimeric wild-type RT, Leu100Ile, Val179Asp, Lys103Asn, and Tyr181Ile were expressed and purified to >95% purity (as determined by SDS-PAGE) as described.[25]

HIV-1 RT RNA-dependent DNA polymerase activity assays: RNA-dependent DNA polymerase activity was assayed as follows: a final volume of 25 μL contained buffer A (50 mM Tris·HCl pH 7.5, 1 mM DTT, 0.2 mg mL^{-1} BSA, 4% glycerol), 10 mM $MgCl_2$, 0.5 μg poly(rA)/oligo(dT) 10:1 (0.3 μM 3′-OH ends), 10 μM [^{3}H]dTTP (1 Ci mmol^{-1}), and 5–10 nM RT. Reactions were incubated for 10 min at 37 °C. Aliquots (20 μL) were then spotted onto GF/C glass fiber filters, which were immediately immersed in ice-cold 5% trichloroacetic acid (TCA). Filters were washed twice in ice-cold 5% TCA and once in EtOH for 5 min, dried, and the acid-precipitable radioactivity was measured by scintillation counting.

Inhibition assays: Reactions were performed under the conditions described for the HIV-1 RT RNA-dependent DNA polymerase activity assay. Incorporation of radioactive dTTP into poly(rA)/oligo(dT) was monitored in the presence of increasing amounts of inhibitor as indicated in the Figure legends. Data were analyzed according to Equation (2):

$$v = V_{max}/(1 + I/IC_{50}) \quad (2)$$

in which v is the apparent velocity of the reaction, V_{max} is the apparent maximum reaction rate (in absence of inhibitor), I is the inhibitor concentration, and IC_{50} is the concentration required to inhibit the enzymatic activity by 50%. Experiments were performed in triplicate, and mean values were fitted to the equation with GraphPad Prism 3.0 software.

Chemical synthesis

(*E*)-4-(4-Methoxyphenyl)but-3-en-2-ol (5): To a solution of *trans*-4-methoxycinnamaldehyde (1 g, 6.173 mmol) in 10 mL THF, MeMgBr

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(3.0 M in THF; 3.09 mL, 9.259 mmol) was added dropwise at 0 °C, and the mixture was stirred at this temperature for 1.5 h. The mixture was then diluted with Et_2O and quenched with a small amount of saturated $NH_4Cl_{(aq)}$. The resulting suspension was filtered through Celite, and to the filtered solution H_2O was added followed by two extractions with Et_2O. The organic layers were collected, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography (Et_2O/petroleum ether (PE), 40:60) to obtain pure product (990 mg, yield 90%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.29$ (d, $J = 7.2$ Hz, 1H), 6.82 (d, $J = 7.3$ Hz, 1H), 6.49 (d, $J_{trans} = 15.9$ Hz, 1H), 5.59–6.18 (dd, 1H, $J = 7.3$ Hz, $J_{trans} = 15.9$ Hz, 1H), 4.32–4.52 (m, 1H), 3.78 (s, 3H), 1.33 ppm (d, 3H, $J = 7.2$ Hz); Anal. calcd for $C_{11}H_{14}O_2$: C 74.13, H 7.92, found: C 74.25, H 7.98.

2-((*E*)-4-(4-Methoxyphenyl)but-3-en-2-ylthio)-6-(1-(2,6-difluorophenyl)ethyl)-5-methylpyrimidin-4(3*H*)-one (7): 6-(1-(2,6-Difluorophenyl)ethyl)-5-methyl-3,4-dihydro-2-thioxopyrimidin-4(3*H*)-one **6** (40 mg, 0.141 mmol) and compound **5** (38 mg, 0.212 mmol) were suspended in the minimal amount of dry DMF (1 mL) in the presence of trimethylphosphine (1 M solution in toluene, 212 μL, 212 mmol). The reaction mixture was cooled in an ice bath, and DIAD was added (42 μL, 0.212 mmol). The mixture was microwave irradiated at 40 °C for 30 min and was then diluted with H_2O (2 mL) and extracted with Et_2O (3×10 mL). Organic phases were collected, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was purified on silica gel by flash chromatography to obtain compound **7** (18 mg, yield 28%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.24$–7.03 (m, 3H), 6.79–6.72 (m, 4H), 6.42 (dd, $J_{trans} = 15.9$ Hz, 1H), 6.14 (dd, $J = 7.32$ Hz, $J_{trans} = 15.9$ Hz, 1H), 4.66–4.61 (q, $J = 7.8$ Hz, 1H), 4.54–4.52 (q, $J = 7.8$ Hz, 1H), 3.74 (s, 3H), 1.93 (s, 3H), 1.63–1.61 (d, $J = 7.1$ Hz, 3H), 1.48 ppm (dd, $J = 6.7$ Hz, 3H); MS (ESI) *m/z*: 443 $[M+H]^+$; Anal. calcd for $C_{24}H_{24}F_2N_2O_2S$: C 65.14, H 5.47, N 6.33, found: C 65.37, H 5.53, N 6.76.

6-(1-(2,6-Difluorophenyl)ethyl)-5-methyl-2-(prop-2-ynylthio)pyrimidin-4(3*H*)-one (9): Compound **6** (400 mg, 1.41 mmol) was suspended at 0 °C under Ar in a minimal amount of dry DMF, then K_2CO_3 (147 mg, 1.41 mmol) and propargyl bromide **15** (139 μL, 1.56 mmol) were added, and the mixture was stirred at 0 °C for 3 h. H_2O was added to the reaction mixture, which was then extracted with EtOAc, dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash chromatography (eluent: PE/EtOAc 2:1) to give the desired product **9** (382 mg, yield 84%); mp: 223–225 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.09$–7.05 (m, 1H), 6.83–6.79 (m, 2H), 4.57–4.51 (m, 1H), 3.91–3.87 (m, 2H), 2.10 (s, 1H), 1.97 (s, 3H), 1.60 ppm (d, 3H); MS (ESI) *m/z*: 321 $[M+H]^+$, 343 $[M+Na]^+$; Anal. calcd for $C_{16}H_{14}F_2N_2OS$: C 59.99, H 4.40, N 8.74, found: C 60.02, H 4.55, N 8.89.

6-(1-(2,6-Difluorophenyl)ethyl)-5-methyl-2-(prop-2-ynylthio)pyrimidin-4-yl benzoate (10): To a solution of compound **9** (378 mg, 1.18 mmol) in pyridine (19 mL), benzoyl chloride **19** (685 μL, 6 mmol) was added, and the mixture was stirred at room temperature for 2 h. The reaction mixture was quenched with saturated $NaHCO_{3(aq)}$ (50 mL), extracted with EtOAc (3×50 mL), and acidified with 1 N HCl. The combined extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the desired product **10** (451 mg, yield 90%); mp: 234–236 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.09$ (d, $J = 8$ Hz, 2H), 7.60–7.56 (m, 1H), 7.45–7.41 (m, 2H), 7.11–7.09 (m, 1H), 6.81–6.77 (m, 2H), 4.68–4.63 (m, 1H), 3.85–3.80 (m, 2H), 2.07 (s, 1H), 1.93 (s, 3H), 1.70 ppm (d, 3H); MS (ESI) *m/z*: 425 $[M+H]^+$, 447 $[M+Na]^+$; Anal. calcd for $C_{23}H_{18}F_2N_2O_2S$: C 65.08, H 4.27, N 6.60, found: C 65.21, H 4.34, N 6.69.

6-(1-(2,6-Difluorophenyl)ethyl)-2-(3-(4-methoxyphenyl)prop-2-ynylthio)-5-methylpyrimidin-4-yl benzoate (11) and 6-(1-(2,6-difluorophenyl)ethyl)-2-(3-(4-methoxyphenyl)prop-2-ynylthio)-5-methylpyrimidin-4(3*H*)-one (12): To a solution of compound **10** (54.3 mg,0.128 mmol) in dry DMF, 4-iodoanisole **9** (30 mg, 0.128 mmol), Et_3N (36 μL, 0.256 mmol), $PdCl_2(PPh_3)$ (9 mg, 0.0128 mmol), and CuI (7.3 mg, 0.038 mmol) were added. The reaction mixture was stirred for 10 min at room temperature and then extracted with EtOAc. The combined extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **11** (68 mg, yield >99%); mp: 231–233 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.75$ (d, $J = 8$ Hz, 2H), 7.49–7.44 (m, 3H), 7.25–7.20 (m, 2H), 7.09–7.06 (m, 1H), 6.76–6.73 (m, 4H), 4.58–4.56 (m, 1H), 4.15–4.00 (m, 2H), 3.73 (s, 3H), 1.95 (s, 3H), 1.63 ppm (d, 3H); MS (ESI) *m/z*: 531 $[M+H]^+$; Anal. calcd for $C_{30}H_{24}F_2N_2O_3S$: 67.91, H 4.56, N 5.28, found: C 67.87, H 4.50, N 5.19. Compound **11** was then dissolved in EtOAc, a solution of NH_4Cl/NH_4OH (10:1) was added, and the resulting mixture was stirred for 30 min. The organic phase was then separated, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification by flash chromatography (eluent: PE/EtOAc 4:1) give the final product **12** (54.5 mg, yield >99%); mp: 220–222 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 12.38$ (bs, 1H), 7.25–7.20 (m, 2H), 7.09–7.06 (m, 1H), 6.76–6.73 (m, 4H), 4.58–4.56 (m, 1H), 4.15–4.00 (m, 2H), 3.73 (s, 3H), 1.95 (s, 3H), 1.63 ppm (d, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 163.42$, 162.51 (dd, $J_1 = 246$ Hz, $J_2 = 9$ Hz, 2C), 154.75, 133.24, 128.18 (d, $J = 9$ Hz, 1C), 114.91, 113.83, 111.62, 111.36 (d, $J = 25$ Hz, 2C), 83.07, 82.36, 55.27, 34.05, 20.47, 17.66, 9.84 ppm; MS (ESI) *m/z*: 427 $[M+H]^+$, 449 $[M+Na]^+$; Anal. calcd for $C_{23}H_{20}F_2N_2O_2S$: C 64.77, H 4.73, N 6.57, found: C 64.92, H 4.69, N 6.48.

6-(1-(2,6-Difluorophenyl)ethyl)-2-(3-(4-methoxyphenyl)-2-methylenebut-3-enylthio)-5-methylpyrimidin-4(3*H*)-one (13): A solution of compound **11** (80 mg, 0.1509 mmol) in toluene and ruthenium carbene complex (Grubbs 2; 10 mol%) was stirred at 80 °C under ethylene gas (1 atm) for 48 h. The solvent was removed under reduced pressure, and then the crude product was dissolved in EtOAc and a solution of NH_4Cl/NH_4OH (10:1) was added. The mixture was stirred for an additional 30 min and then the organic layer was separated, dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash chromatography (eluent: Hex/Et_2O 1:1) to give the desired product **13** (10 mg, yield 15%); mp: 227–229 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.14$ (d, $J = 8$ Hz, 2H), 7.10–7.06 (m, 1H), 6.79–6.73 (m, 4H), 5.26 (s, 1H), 5.19 (s, 1H) 5.12 (s, 1H), 5.02 (s, 1H), 4.53–4.50 (m, 1H), 4.11–4.06 (m, 2H), 3.74 (s, 3H), 1.92 (s, 3H), 1.61 ppm (d, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 164.14$ (dd, $J_1 = 245$ Hz, $J_2 = 9$ Hz, 2C), 159.81, 155.29, 147.92, 143.32, 132.96, 129.39, 128.16 (d, $J = 9$ Hz, 1C), 119.41, 116.13, 114.29, 113.52, 111.63, 111.38 (d, $J = 26$ Hz, 2C), 55.27, 34.06, 29.70, 17.68, 9.89 ppm; MS (ESI) *m/z*: 455 $[M+H]^+$, 477 $[M+Na]^+$; Anal. calcd for $C_{25}H_{24}F_2N_2O_2S$: C 66.06, H 5.32, N 6.16, found: C 66.11, H 5.27, N 6.19.

6-(1-(2,6-Difluorophenyl)ethyl)-2-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methylthio)-5-methylpyrimidin-4(3*H*)-one (14): A mixture of 4-iodoanisole (468 mg, 2 mmol) NaN_3 (156 mg, 2.4 mmol), CuI (38 mg, 0.2 mmol) L-proline (48 mg, 0.4 mmol), and a solution of NaOH 20% in DMSO (4 mL) was heated at 60 °C under Ar for 18 h. The cooled mixture was partitioned between EtOAc and H_2O. The organic layer was separated, and the aqueous layer extracted with EtOAc twice. The combined organic layers were separated, washed with brine, dried (Na_2SO_4), filtered, and

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evaporated to give the crude 4-methoxyphenylazide. Then compound **10** (51.6 mg, 0.12 mmol) and freshly synthesized *para*-methoxyphenylazide (18 mg, 0.12 mmol) were suspended in a 1:1 mixture of H_2O and *t*BuOH (1.5 mL each) in a 10 mL glass vial equipped with a small magnetic stir bar. To this was added sodium ascorbate (0.1 equiv) and $CuSO_4{\cdot}5H_2O$ (0.01 equiv). The mixture was then irradiated for 10 min at 125 °C using an irradiation power of 100 W. After completion of the reaction a solution of NH_4Cl/NH_4OH (10:1) was added, stirring was continued for 30 min, followed by extraction with EtOAc, drying over Na_2SO_4, concentration under reduced pressure, and purification by flash chromatography (eluent: EtOAc) to give the desired product **14** (47 mg, yield 82%); mp: 241–243 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.49–7.46 (m, 1H), 7.10–7.04 (m, 1H), 6.77–6.73 (m, 3H), 6.62–6.60 (m, 2H), 6.49 (s, 1H), 4.44–4.42 (m, 1H), 3.96 (bs, 2H), 3.69 (s, 3H), 1.86 (s, 3H), 1.54 ppm (d, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.63, 161.13 (dd, J_1 = 245 Hz, J_2 = 9 Hz, 2C), 160.16, 140.25, 138.20, 128.27 (d, J = 8 Hz, 1C), 128.07, 116.37, 111.67, 111.41 (d, J = 26 Hz, 2C), 104.65, 55.31, 33.69, 31.28, 17.61, 10.17 ppm; MS (ESI) *m/z*: 470 $[M+H]^+$; Anal. calcd for $C_{23}H_{21}F_2N_5O_2S$: C 58.84, H 4.51, N 14.92, found: C 58.92, H 4.59, N 15.01.

6-(1-(2,6-Difluorophenyl)ethyl)-2-((1-(4-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methylthio)-5-methylpyrimidin-4(3*H*)-one (15): *para*-Methoxybenzyl chloride (17.4 μL, 0.13 mmol), compound **10** (54 mg, 0.13 mmol) and NaN_3 (9.22 mg, 0.14 mmol) were suspended in a 1:1 mixture of H_2O and *t*BuOH (1.5 mL each) in a 10 mL glass vial equipped with a small magnetic stir bar. To this were added sodium ascorbate (0.1 equiv) and $CuSO_4{\cdot}5H_2O$ (0.01 equiv), and the vial was microwave irradiated (100 W) for 10 min at 125 °C. After completion of the reaction, a solution of NH_4Cl/NH_4OH (10:1) was added, and stirring was continued for 30 min. The mixture was then extracted with EtOAc, dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash chromatography (eluent: PE/EtOAc 3:1) to give the desired product **15** (34.5 mg, yield 56%); mp: 217–219 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.20–7.18 (m, 1H), 7.09–7.04 (m, 2H), 6.77–6.73 (m, 3H), 6.49 (s, 1H), 5.05 (bs, 2H), 4.45–4.42 (m, 2H), 3.97 (s, 3H), 1.86 (s, 3H), 1.54 ppm (d, 3H,); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.54, 161.13 (dd, J_1 = 245 Hz, J_2 = 9 Hz, 2C), 160.08, 140.24, 128.27 (d, J = 8 Hz, 1C), 128.15, 116.31, 111.65, 111.40 (d, J = 26 Hz, 2C), 104.64, 65.84, 34.12, 33.68, 31.27, 17.58, 10.14 ppm; MS (ESI) *m/z*: 484 $[M+H]^+$; Anal. calcd for $C_{24}H_{23}F_2N_5O_2S$: C 59.61, H 4.79, N 14.48, found: C 59.73, H 4.82, N 14.55.

3-(4-Methoxyphenyl)prop-2-yn-1-ol (17): To a solution of 4-iodoanisole (1 g, 4.27 mmol) in dry DMF, propargyl alcohol (746 μL, 12.81 mmol), Et_3N (1.18 mL, 8.54 mmol), $PdCl_2(PPh_3)$ (300 mg, 0.42 mmol) and CuI (246.5 mg, 1.29 mmol) were added. The reaction mixture was stirred at room temperature for 5 min before quenching with H_2O (50 mL) and extraction with EtOAc (3×50 mL). The combined extract was dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and purified by flash chromatography (eluent: PE/EtOAc 3:1) to give the desired product **17** (700 mg, yield 92%); mp: 165–167 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.30 (d, J = 8 Hz, 2H), 6.76 (d, J = 8 Hz, 2H), 4.41 (s, 2H), 3.70 (s, 3H), 3.33 ppm (bs, 1H); MS (ESI) *m/z* 161 $[M-H]^-$; Anal. calcd for $C_{10}H_{10}O_2$: C 74.06, H 6.21, found: C 74.11, H 6.37.

(*Z*)-3-(4-Methoxyphenyl)prop-2-en-1-ol (18): To a solution of compound **17** (227 mg, 1.401 mmol) in EtOAc, quinoline (109 μL, 0.924 mmol) and Lindlar catalyst (227 mg) were added, and the mixture was stirred under H_2 for 48 h. The mixture was then filtered on a Celite pad and washed with a solution of 1 N HCl, dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash chromatography (eluent: Hex/Et_2O 1:1) to give the desired product **18** (57.4 mg, yield 25%); mp: 112–114 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.09 (d, J = 8 Hz, 2H), 6.81 (d, J = 8 Hz, 2H), 6.43 (d, J_{cis} = 12 Hz, 1H), 5.74–5.68 (m, J_{cis} = 12 Hz, 1H), 4.36 (d, 2H), 3.71 ppm (s, 3H); MS (ESI) *m/z*: 163 $[M-H]^-$; Anal. calcd for $C_{10}H_{12}O_2$: C 73.15, H 7.37, found: C 73.21, H 7.42.

(*Z*)-1-(3-Bromoprop-1-enyl)-4-methoxybenzene (19): To a solution of compound **18** (28.4 mg, 0.173 mmol) in Et_2O, PBr_3 (16 μL, 0.173 mmol) was added at 0 °C under Ar. The reaction mixture was stirred at 0 °C for 1 h, then quenched with H_2O (5 mL) and a saturated solution of $NaHCO_3$, and extracted with EtOAc (3×50 mL). The combined extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the desired product **19** (39 mg, yield >99%); mp: 116–118 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.09 (d, J = 8 Hz, 2H), 6.81 (d, J = 8 Hz, 2H), 6.38 (d, J_{cis} = 12 Hz, 1H), 5.70–5.63 (m, J_{cis} = 12 Hz, 1H), 3.98 (d, 2H), 3.70 ppm (s, 3H); MS (ESI) *m/z*: 226 $[M-H]^-$; Anal. calcd for $C_{10}H_{11}OBr$: C 52.89, H 4.88, found: C 52.80, H 4.79.

(*Z*)-6-(1-(2,6-Difluorophenyl)ethyl)-2-(3-(4-methoxyphenyl)allylthio)-5-methylpyrimidin-4(3*H*)-one (20): Compound **6** (33.4 mg, 0.12 mmol) was suspended under Ar in a minimal amount of dry DMF. Then K_2CO_3 (16.3 mg, 0.130 mmol) and compound **19** (29.6 mg, 1.56 mmol) were added, and the resulting mixture was stirred for 3 h at room temperature. H_2O was added to the reaction mixture, the organic layer was separated, and the aqueous phase was extracted with EtOAc (3×50 mL), dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash chromatography (eluent: PE/Hex 4:1) to give the desired product **20** (23 mg, yield 45%); mp: 220–222 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.79 (bs, 1H), 7.15 (d, J = 8 Hz, 2H), 7.07–7.04 (m, 1H), 6.77–6.73 (m, 4H), 6.41 (d, J_{cis} = 12 Hz, 1H), 6.03–5.97 (m, J_{cis} = 12 Hz, 1H), 4.57–4.52 (m, 1H), 3.97–3.91 (m, 2H), 3.75 (s, 3H), 1.95 (s, 3H), 1.62 ppm (d, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.71, 162.80 (dd, J_1 = 246 Hz, J_2 = 9 Hz, 2C), 155.58, 132.58, 130.12, 128.17 (d, J = 9 Hz, 1C), 127.61, 122.28, 113.92, 111.63 (d, J = 25 Hz, 2C), 55.29, 33.24, 29.70, 17.74, 9.84 ppm; MS (ESI) *m/z*: 427 $[M-H]^-$; Anal. calcd for $C_{23}H_{22}F_2N_2O_2S$: C 64.47, H 5.18, N 6.54, found: C 64.39, H 5.09, N 6.43.

1-(4-Methoxyphenyl)prop-2-en-1-ol (22): A solution of *para*-anisaldehyde (15 mmol) in dry THF (50 mL) at −15 °C was treated with vinylmagnesium bromide (45 mmol). The resulting mixture was allowed to warm to room temperature and stirred for 1 h. Saturated $NH_4Cl_{(aq)}$ (50 mL) was added, and the aqueous layer was extracted with EtOAc (3×50 mL). The organic layers were recollected, washed with brine (1×10 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2) to furnish compound **22** (1.603 g, yield 65%) as a yellow oil. TLC R_f (CH_2Cl_2) 0.53; ^{1}H NMR ($CDCl_3$): δ = 7.26 (d, J = 8.5 Hz, 2H), 6.86 (d, J = 8.5 Hz, 2H), 6.01 (sept, J_1 = 16.6 Hz, J_2 = 10.6 Hz, J_3 = 8.6 Hz, 1H), 5.29 (d, J = 16.6 Hz, 1H), 5.15 (d, J = 10.6 Hz, 1H), 5.10 (d, J = 8.6 Hz, 1H), 3.77 ppm (s, 3H); Anal. calcd for $C_{10}H_{12}O_2$: C 73.15, H 7.37, found: C 73.21, H 7.42.

1-(4-Methoxyphenyl)prop-2-en-1-one (23): A solution of **22** (16 mmol) in dry Et_2O (130 mL) was treated at room temperature with MnO_2 (160 mmol). The resulting mixture was stirred overnight at the same temperature. The black suspension was filtered on a Celite pad and evaporated under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 9:1) to furnish compound **23** (2.072 g, yield 80%) as a yellow oil. TLC R_f (CH_2Cl_2/PE 1:1) 0.66; ^{1}H NMR ($CDCl_3$): δ = 7.80 (d, J = 7.3 Hz, 2H), 7.00 (q, J_1 = 16.5 Hz, J_2 = 10.3 Hz, 1H), 6.76 (d, J = 7.3 Hz, 2H), 6.26 (d, J =

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16.5 Hz, 1H), 5.67 (d, $J=10.3$ Hz, 1H), 3.66 ppm (s, 3H); Anal. calcd for $C_{10}H_{10}O_2$: C 74.06, H 6.21, found: C 74.11, H 6.27.

6-(1-(2,6-Difluorophenyl)ethyl)-2-(3-(4-methoxyphenyl)-3-oxopropylthio)-5-methylpyrimidin-4(3*H*)-one (25): A solution of **6** (0.584 mmol) in dry DMF (8 mL) under Ar was treated with trifluoromethanesulfonic acid (0.117 mmol). After 15 min **23** (1.753 mmol) was added, and the solution stirred at room temperature for 1 h. EtOAc (12 mL) and saturated $NaHCO_{3(aq)}$ (5 mL) were then added, and the organic phase was washed with distilled H_2O (2×10 mL) and brine (2×10 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by flash chromatography (EtOAc/PE 1:3) to furnish compound **25** (126 mg, yield 50%) as a white solid; mp: 168 °C; TLC R_f (EtOAc/PE 2:3) 0.27; IR ($CHCl_3$): $\tilde{\nu}$1600, 1665 cm^{-1}; ^1H NMR ([D$_6$]DMSO): $\delta=7.76$ (d, $J=8.62$ Hz, 2H), 7.14 (dd, $J_1=7.70$ Hz, $J_2=6.65$ Hz, 1H), 7.02 (d, $J=8.62$ Hz, 2H), 6.90 (t, $J=7.70$ Hz, 2H), 3.87 (s, 2H), 3.84 (s, 3H), 3.01 (q, $J_1=10.85$ Hz, $J_2=4.90$ Hz, 4H), 2.01 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): $\delta=196.32$, 163.40, 163.24, 161.29 (dd, $J_1=245.21$ Hz, $J_2=8.98$ Hz, 2C), 158.26, 152.27, 130.15 (2C), 129.42, 128.91 (d, $J=8.98$ Hz), 128.76, 114.12, 114.03 (2C), 111.14 (d, $J=24.54$ Hz, 2C), 55.72, 37.60, 26.83, 24.42, 10.20 ppm; MS: *m/z* 431 $[M+H]^+$, 453 $[M+Na]^+$; Anal. calcd for $C_{22}H_{20}F_2N_2O_3S$: C 61.38, H 4.68, N 6.51, found: C 61.29, H 4.53, N 6.47.

6-(2,6-Difluorobenzyl)-2-(3-(4-methoxyphenyl)-3-oxopropylthio)-5-methylpyrimidin-4(3*H*)-one (26): A solution of **24** (0.553 mmol) in dry DMF (8 mL) under Ar was treated with trifluoromethanesulfonic acid (0.111 mmol). After 15 min **23** (1.753 mmol) was added, and the solution was stirred at room temperature for 1 h. EtOAc (12 mL) and saturated $NaHCO_{3(aq)}$ (5 mL) were then added, and the organic phase was washed with distilled H_2O (2×10 mL) and brine (2×10 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by flash chromatography (EtOAc/PE 1:3) to furnish compound **26** (167 mg, yield 68%) as a white solid; mp: 180 °C; TLC R_f (EtOAc/PE 2:3) 0.23; IR ($CHCl_3$): $\tilde{\nu}$1600, 1665 cm^{-1}; ^1H NMR ($CDCl_3$): $\delta=7.85$ (d, $J=8.78$ Hz, 2H), 7.04 (m, 1H), 6.91 (d, $J=8.78$ Hz, 2H), 6.75 (t, $J=8.37$ Hz, 2H), 4.59 (q, $J=7.23$ Hz, 1H), 3.87 (s, 3H), 3.55 (q, $J=6.60$ Hz, 2H), 3.29 (q, $J=6.60$ Hz, 2H), 2.02 (s, 3H), 1.64 ppm (d, $J=7.23$ Hz, 2H); ^{13}C NMR ($CDCl_3$): $\delta=196.59$, 165.02, 163.78, 161.75 (dd, $J_1=247.66$ Hz, $J_2=9.27$ Hz, 2C), 156.07, 130.24 (2C), 129.73, 128.22, 128.12 (d, $J=9.27$ Hz), 113.70 (3C), 111.45 (d, $J=26.12$ Hz, 2C), 55.48, 38.29, 34.13, 24.89, 17.65, 9.82 ppm; MS: *m/z* 467 $[M+Na]^+$; Anal. calcd for $C_{23}H_{23}F_2N_2O_3S$: C 62.15, H 4.99, N 6.30, found: C 62.21, H 5.03, N 6.37.

6-(2,6-Difluorobenzyl)-2-(3-(hydroxyimino)-3-(4-methoxyphenyl)-propylthio)-5-methylpyrimidin-4(3*H*)-one (27): A solution of **25** (0.046 mmol) in dry MeOH (1 mL) at 0 °C under Ar was treated with $NaBH_4$ (0.027 mmol) portionwise until TLC indicated complete consumption of starting material. The solvent was removed under reduced pressure. The residue was diluted with EtOAc (5 mL), and the organic layer was washed with distilled H_2O (2×5 mL) and brine (2×5 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by TLC (EtOAc/PE 1:1) to furnish compound **27** (20 mg, yield 100%) as a white solid. TLC R_f (EtOAc/PE 2:3) 0.30; ^1H NMR ($CDCl_3$): $\delta=7.22$ (d, $J=8.45$ Hz, 2H), 7.13 (m, 1H), 6.87 (d, $J=8.45$ Hz, 2H), 6.81 (t, $J=8.45$ Hz, 2H), 4.63 (q, $J=4.25$ Hz, 1H), 3.93 (s, 2H), 3.80 (s, 3H), 3.14–2.98 (m, 2H *diast.*), 2.14 (s, 3H), 1.99–1.77 ppm (m, 2H, *diast.*); ^{13}C NMR ([D$_6$]DMSO): $\delta=163.03$, 162.87, 161.16 (dd, $J_1=245.68$ Hz, $J_2=8.52$ Hz, 2C), 159.81, 158.16, 137.43, 128.65 (d, $J=8.52$ Hz), 126.85 (2C), 114.04 (d, $J=24.10$ Hz), 113.86 (2C), 110.09 (d, $J=24.10$ Hz, 2C), 70.31, 54.97, 38.22, 26.76, 26.53, 9.98 ppm; MS: *m/z* 455 $[M+Na]^+$; Anal. calcd for $C_{22}H_{22}F_2N_2O_3S$: C 61.10, H 5.13, N 6.48, found: C 61.17, H 5.24, N 6.52.

6-(1-(2,6-Difluorophenyl)ethyl)-2-(3-hydroxy-3-(4-methoxyphenyl)propylthio)-5-methylpyrimidin-4(3*H*)-one (28): A solution of **26** (0.046 mmol) in dry MeOH (1 mL) at 0 °C under Ar was treated with $NaBH_4$ (0.027 mmol) portionwise until TLC indicated complete consumption of starting material. The solvent was removed under reduced pressure. The residue was diluted with EtOAc (5 mL), and the organic layer was washed with distilled H_2O (2×5 mL) and brine (2×5 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by TLC (EtOAc/PE 1:1) to furnish compound **28** (20 mg, yield >99%) as a white solid. TLC R_f (EtOAc/PE 2:3) 0.30; ^1H NMR ($CDCl_3$): $\delta=7.25$ (d, $J=7.49$ Hz, 2H), 7.11 (m, 1H), 6.86 (d, $J=7.49$ Hz, 2H), 6.76 (t, $J=8.26$ Hz, 2H), 4.77 (m, 1H), 4.56 (m, 1H), 3.79 (s, 3H), 3.33–3.15 (m, 2H *diast.*), 2.20–1.99 (m, 2H *diast.*), 1.91 (d, 3H, *diast.*), 1.63 ppm (d, 3H, *diast.*); ^{13}C NMR ($CDCl_3$): $\delta=163.37$, 163.00, 161.28 (dd, $J_1=246.11$ Hz, $J_2=9.19$ Hz, 2C), 158.16, 155.68, 136.13, 128.48 (d, $J=9.19$ Hz), 127.29 (2C), 114.70 (d, $J=24.28$ Hz), 114.09 (2C), 111.34 (d, $J=24.28$ Hz, 2C), 71.90, 55.43, 38.67, 31.14, 27.44, 26.98, 10.57 ppm; MS: *m/z* 447 $[M+1]^+$, 469 $[M+Na]^+$; Anal. calcd for $C_{23}H_{24}F_2N_2O_3S$: C 61.87, H 5.42, N 6.27, found: C 61.93, H 5.50, N 6.31.

6-(2,6-Difluorobenzyl)-2-(3-hydroxy-3-(4-methoxyphenyl)butylthio)-5-methylpyrimidin-4(3*H*)-one (29) and 6-(1-(2,6-difluorophenyl)ethyl)-2-(3-hydroxy-3-(4-methoxyphenyl)butylthio)-5-methylpyrimidin-4(3*H*)-one (30): A solution of **25** or **26** (1 equiv) in dry THF (1 mL) at 0 °C under Ar was treated with MeMgBr (3 equiv). After 1 h, EtOAc (5 mL) and a saturated solution of NH_4Cl (5 mL) were added. The organic layer was washed with distilled H_2O (2×5 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by TLC (EtOAc/PE 1:1) to furnish compound **29** (yield 83%) as a white solid. ^1H NMR ($CDCl_3$): $\delta=7.30$ (d, $J=8.83$ Hz, 2H), 7.17 (m, 1H), 6.85 (d, $J=8.83$ Hz, 2H), 6.83 (m, 2H), 3.93 (s, 3H), 3.79 (s, 3H), 3.02–2.72 (dq, $J_1=72.01$ Hz, $J_2=7.23$ Hz, 2H *diast.*), 2.12 (s, 3H), 1.94 (t, $J=7.23$ Hz, 2H), 1.12 ppm (s, 3H); ^{13}C NMR ($CDCl_3$): $\delta=163.95$, 162.17, 161.60 (dd, $J_1=247.55$ Hz, $J_2=8.99$ Hz, 2C), 157.36, 155.12, 142.63, 127.05 (d, $J=8.99$ Hz), 126.77 (2C), 113.63 (2C), 113.44 (d, $J=24.48$ Hz), 110.88 (d, $J=23.96$ Hz, 2C), 75.01, 55.19, 34.13, 29.53, 26.13, 10.04 ppm; MS: *m/z* 429 $[M-H_2O]^+$, 469 $[M+Na]^+$; Anal. calcd for $C_{23}H_{24}F_2N_2O_3S$: C 61.87, H 5.42, N 6.27, found: C 61.92, H 5.49, N 6.34. Compound **30** (yield 95%), white solid; ^1H NMR ($CDCl_3$): $\delta=7.28$ (d, $J=8.54$ Hz, 2H), 7.08 (q, $J=6.83$ Hz, 1H), 6.82 (d, $J=8.29$ Hz, 2H), 6.76 (t, $J=8.29$ Hz, 2H), 4.45 (q, $J=7.07$ Hz,1H), 3.70 (s, 3H), 3.17–2.87 (m, 2H *diast.*), 2.14 (m, 2H), 1.88 (d, $J=3.90$ Hz, 2H *diast.*), 1.55 (t, $J=6.58$ Hz, 3H *diast.*), 1.50 ppm (d, $J=2.92$ Hz, 3H *diast.*); ^{13}C NMR ($CDCl_3$): $\delta=164.79$, 163.00, 161.28 (dd, $J_1=248.03$ Hz, $J_2=9.73$ Hz, 2C), 158.38, 155.76, 141.32, 128.15 (d, $J=9.73$ Hz), 125.94 (2C), 114.12 (2C), 113.64 (d, $J=23.96$ Hz), 111.58 (d, $J=23.96$ Hz, 2C), 74.19, 55.26, 43.70, 34.00, 30.30, 25.53, 17.72, 9.76 ppm; MS: *m/z* 461 $[M+1]^+$, 483 $[M+Na]^+$; Anal. calcd for $C_{24}H_{26}F_2N_2O_3S$: C 62.59, H 5.69, N 6.08, found: C 62.64, H 5.73, N 6.11.

6-(2,6-Difluorobenzyl)-2-(3-hydroxy-3-(4-methoxyphenyl)pentylthio)-5-methylpyrimidin-4(3*H*)-one (31): A solution of **25** (0.046 mmol) in dry THF (1 mL) at 0 °C under Ar was treated with EtMgBr (0.139 mmol). After 1 h, EtOAc (5 mL) and saturated $NH_4Cl_{(aq)}$ (5 mL) were added. The organic layer was washed with distilled H_2O (2×5 mL) and brine (2×5 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by TLC (EtOAc/PE 1:1) to furnish compound **31** (19 mg, yield 92%) as a white solid. TLC R_f (EtOAc/PE 1:1) 0.23; ^1H NMR ($CDCl_3$): $\delta=7.20$

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(d, $J=8.69$ Hz, 2H), 7.10 (q, $J=7.47$ Hz, 1H), 6.79 (m, 4H), 3.87 (s, 2H), 3.74 (s, 3H), 2.90–2.62 (m, 2H *diast.*), 2.05 (s, 3H), 1.99 (m, 2H *diast.*), 1.65 (m, 2H *diast.*), 0.66 ppm (t, $J=7.47$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta=164.14$, 162.82, 160.44 (dd, $J_1=244.92$ Hz, $J_2=9.07$ Hz, 2C), 159.63, 156.17, 136.57, 128.12 (d, $J=9.07$ Hz), 126.44 (2C), 113.38 (d, $J=24.22$ Hz), 111.47 (2C), 110.80 (d, $J=24.22$ Hz, 2C), 76.22, 55.13, 41.92, 35.62, 27.59, 25.10, 10.24, 7.56 ppm; MS: *m/z* 461 $[M+1]^+$, 483 $[M+Na]^+$; Anal. calcd for $C_{24}H_{26}F_2N_2O_3S$: C 62.59, H 5.69, N 6.08, found: C 62.52, H 5.62, N 5.99.

6-(1-(2,6-Difluorophenyl)ethyl)-2-(3-hydroxy-3-(4-methoxyphenyl)pentylthio)-5-methylpyrimidin-4(3*H*)-one (32): A solution of **26** (0.046 mmol) in dry THF (1 mL) at 0 °C under Ar was treated with EtMgBr (0.139 mmol). After 1 h, EtOAc (5 mL) and saturated $NH_4Cl_{(aq)}$ (5 mL) were added. The organic layer was washed with distilled H_2O (2×5 mL) and brine (2×5 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by TLC (EtOAc/PE 1:1) to furnish compound **32** (20 mg, yield 94%) as a white solid. TLC R_f (EtOAc/PE 1:1) 0.24; ^{1}H NMR ($CDCl_3$): $\delta=7.28$ (d, $J=8.52$ Hz, 2H), 7.13 (m, 1H), 6.86 (dd, $J_1=8.97$ Hz, $J_2=2.91$ Hz, 2H) 6.84 (d, $J=8.52$ Hz, 2H), 4.54 (q, $J=7.17$ Hz, 1H), 3.79 (ds, $J=3.78$ Hz, 3H), 3.24–2.82 (m, 2H *diast.*), 2.19 (m, 2H), 1.93 (ds, $J=9.19$ Hz, 3H), 1.84 (m, 2H *diast.*), 1.60 (q, $J=5.35$ Hz, 3H), 0.74 ppm (t, $J=5.35$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta=164.32$, 162.94, 161.22 (dd, $J_1=244.92$ Hz, $J_2=9.07$ Hz, 2C), 158.29, 155.70, 136.73, 128.11 (d, $J=9.07$ Hz), 126.42 (2C), 113.48 (d, $J=24.22$ Hz), 111.58 (2C), 111.32 (d, $J=24.22$ Hz, 2C), 55.14, 42.43, 42.23, 35.60, 33.90, 30.82, 25.24, 17.62, 9.70, 7.56 ppm; MS: *m/z* 475 $[M+1]^+$, 497 $[M+Na]^+$; Anal. calcd for $C_{25}H_{28}F_2N_2O_3S$: C 63.27, H 5.95, N 5.90, found: C 63.31, H 5.99, N 5.97.

6-(2,6-Difluorobenzyl)-2-(3-(hydroxyimino)-3-(4-methoxyphenyl)-propylthio)-5-methylpyrimidin-4(3*H*)-one (33): A solution of **25** (0.032 mmol) in dry EtOH (2 mL) was treated with $NH_2OH{\cdot}HCl$ (0.098 mmol) and NaOAc (0.098 mmol). The mixture was held at reflux overnight. Et_2O (5 mL) and saturated $NaHCO_{3(aq)}$ (5 mL) were added, and the organic layer was washed with distilled H_2O (2× 5 mL) and brine (2×5 mL), dried over Na_2SO_4, and evaporated under reduced pressure to give compound **33** as a white solid residue (15 mg, 100%). TLC R_f (EtOAc/PE 1:1) 0.21; ^{1}H NMR ([D_6]acetone): $\delta=7.41$ (d, $J=8.35$ Hz, 2H), 7.21 (m, 1H), 6.88 (t, $J=7.50$ Hz, 2H), 6.79 (d, $J=8.35$ Hz, 2H), 3.94 (s, 2H), 3.73 (s, 3H), 3.12 (t, $J=7.03$ Hz, 2H), 2.86 (t, $J=7.03$ Hz, 2H), 2.02 ppm (s, 3H); MS: *m/z* 468 $[M+Na]^+$; Anal. calcd for $C_{23}H_{23}F_2N_3O_3S$: C 60.12, H 5.05, N 9.14, found: C 60.21, H 5.17, N 9.19.

1,3-Difluoro-2-(1-methoxyprop-1-en-2-yl)benzene (35): To a suspension of methoxymethyl(triphenyl)phosphonium chloride (988 mg, 2.88 mmol) in THF (20 mL), a solution of *n*BuLi (2 M in hexane; 1.44 mL, 2.88 mmol) was added dropwise at −78 °C under Ar. The resulting orange solution was stirred for 30 min at −78 °C and then at room temperature for 30 min until a red color appeared. The solution was then cooled again to −78 °C, and a solution of 1-(2,6-difluorophenyl)ethanone (250 μL, 1.921) previously dissolved in THF (5 mL) was added dropwise. The mixture was stirred at room temperature for 18 h and was then quenched with H_2O, the organic phase separated, and the aqueous layer extracted twice with Et_2O. The organic phases were collected, washed with brine, dried over Na_2SO_4, and concentrated by rotary evaporation. The crude product was purified by flash chromatography (pentane/Et_2O 95:5) to obtain compound **35** (276 mg, yield 78%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.09$–7.04 (m, 1H), 6.82–6.75 (t, 2H), 6.08 (s, 1H), 3.64 (s, 3H), 1.88 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=161.08$ (dd, $J_1=245$ Hz, $J_2=9$ Hz, 2C), 137.34, 127.48 111.97 (d, $J=27$ Hz, 2C), 111.95 (d, $J=10$ Hz, 1C), 102.28, 59.84, 13.86 ppm; Anal. calcd for $C_{10}H_{10}F_2O$: C 65.21, H 5.47, found: C 65.33, H 5.52

2-(2,6-Difluorophenyl)propanal (36): Compound **35** (45 mg, 0.244 mmol) was dissolved in CH_2Cl_2, and the solution was cooled to −78 °C. Then BBr_3 (1 M in CH_2Cl_2; 244 μL, 0.244 mmol) was added dropwise, and the mixture was stirred at −78 °C for 2 h. H_2O was then added, the organic phase separated, and the aqueous layer extracted twice with CH_2Cl_2. The organic phases were collected, washed with $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated to obtain **36** in sufficient purity for use in the next step without purification (yield: quant.); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=$ 9.68 (s, 1H), 7.26–7.17 (m, 1H), 6.87–6.83 (m, 2H), 3.84–3.79 (q, $J=$ 7.4 Hz, 1H), 1.42–1.40 ppm (d, $J=7.3$ Hz, 3H); MS (ESI) *m/z*: 171 $[M+H]^+$; Anal. calcd for $C_9H_8F_2O$: C 65.21, H 5.47, found: C 65.29, H 5.42.

Methyl 4-(2,6-difluorophenyl)pent-2-ynoate (38): To a solution of **36** (41 mg, 0.241 mmol) in MeOH (5 mL), K_2CO_3 (67 mg, 0.482 mmol) and dimethyl-1-diazo-2-oxopropylphosphonate (43 μL, 0.289 mmol) were added, and the mixture was stirred at room temperature overnight under Ar. The mixture was then diluted with 15 mL MeOH, and NaOAc (19 mg, 0.234 mmol), $PdCl_2$ (0.5 mg, 0.03 mmol), and $CuCl_2$ (32 mg, 0.234 mmol) were added. The mixture was stirred under CO for 2 h (the color of the mixture turned from green to grey). Then mixture was filtered on Celite and dried by rotary evaporation. The crude product was purified by flash chromatography (EtOAc/Hex 90:10) to obtain compound **38** (13 mg, yield 34%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=$ 7.18–7.10 (m, 1H), 6.82–6.78 (m, 2H), 4.24–4.19 (q, $J=7.2$ Hz, 1H), 3.67 (s, 3H), 1.53–1.51 ppm (d, $J=7.2$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=161.91$ (dd, $J_1=245$ Hz, $J_2=9$ Hz, 2C), 154.08, 129.15, 117.18, 112.44 (d, $J=27$ Hz, 2C), 89.08, 72.31, 52.58, 20.94, 20.02 ppm; MS (ESI) *m/z*: 225 $[M+H]^+$; Anal. calcd for $C_{12}H_{10}F_2O_2$: C 64.28, H 4.50, found: C 64.31, H 4.62.

General procedure for the synthesis of 40 a,c 41 b,d and 42 b,d: In a microwave sealed tube, the appropriate substituted guanidine hydrochloride (1.2 equiv), compound **38** (1 equiv) and $NaHCO_3$ (2.4 equiv) in *t*BuOH (3 mL) were irradiated for 20 min (for $R^2=$4-methoxybenzyl) or 40 min (for $R^2=$4-methoxyphenylcyclopropyl) in a self-tunable CEM microwave synthesizer at 120 °C and finally allowed to cool to room temperature. The reaction mixture was directly evaporated to dryness to give a solid crude material. The residue was purified by flash chromatography (CH_2Cl_2/MeOH) to obtain final products.

2-(*N*-(4-Methoxybenzyl)-*N*-methylamino)-6-(1-(2,6-difluorophenyl)ethyl)pyrimidin-4(3*H*)-one (40 a): White solid (yield 41%); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=11.14$ (bs, NH), 7.09–7.05 (m, 1H), 7.00–6.98 (d, 2H, $J=8.4$ Hz), 6.77–6.69 (m, 4H), 5.59 (s, 1H), 4.67–4.44 (dd, 2H, $J=93.9$ Hz, 14.8 Hz), 4.24–4.19 (q, 1H, $J=7.2$ Hz), 3.71 (s, 3H), 2.95 (s, 3H), 1.56–1.55 ppm (d, 3H, $J=7.2$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=171.41$, 166.48, 162.90, 160.60, 159.01, 153.82, 129.30, 127.87, 119.94, 113.87, 111.37, 97.71, 55.25, 52.23, 36.24, 34.63, 16.73 ppm; MS (ESI) *m/z*: 386 $[M+H]^+$; Anal. calcd for $C_{21}H_{21}F_2N_3O_2$: C 65.44, H 5.49, N 10.90, found: C 65.52, H 5.55, N 11.05.

2-(4-Methoxybenzylamino)-6-(1-(2,6-difluorophenyl)ethyl)pyrimidin-4(3*H*)-one (41 b): White solid (yield 23%); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=11.98$ (bs, NH), 7.13–7.03 (m, 3H), 6.79–6.75 (t, 2H, $J=$ 8.3 Hz), 6.71–6.69 (d, 2H, $J=8.5$ Hz), 6.38 (bs, NH), 5.49 (s, 1H), 4.38–4.13 (m, 3H), 3.71 (s, 3H), 1.53–1.52 ppm (d, 3H, $J=7.1$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=172.6$, 166.2, 162.8, 160.4, 158.8, 153.6, 130.7, 129.3, 128.0, 119.7, 113.8, 111.5, 98.5, 55.2, 44.1, 29.7,

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16.7 ppm; MS (ESI) *m/z*: 372 $[M+H]^+$; Anal. calcd for $C_{20}H_{19}F_2N_3O_2$: C 64.68, H 5.16, N 11.31, found: C 64.71, H 5.19, N 11.42.

3-(4-Methoxybenzyl)-2-amino-6-(1-(2,6-difluorophenyl)ethyl)pyrimidin-4(3*H*)-one (42 b): White solid (yield 23%); ^{1}H NMR (400 MHz, CD_3OD): $\delta=7.15$–7.07 (m, 1H), 6.76–6.69 (m, 6H), 6.09 (s, 1H), 5.06–5.83 (dd, 2H, $J=92.2$ Hz, 18.1 Hz), 4.42–4.37 (q, 1H, $J=6.3$ Hz), 3.68 (s, 3H) 1.51–1.49 ppm (d, 3H, $J=6.5$ Hz); ^{13}C NMR (100 MHz, CD_3OD): $\delta=173.02$, 161.9, 159.5, 159.2, 158.0, 155.9, 129.8, 125.7, 125.4, 116.9, 113.9, 111.8, 105.8, 54.4, 47.7, 30.8, 17.9 ppm; MS (ESI) *m/z*: 372 $[M+H]^+$; Anal. calcd for $C_{20}H_{19}F_2N_3O_2$: C 64.68, H 5.16, N 11.31, found: C 64.73, H 5.19, N 11.45.

2-(*N*-((2-(4-Methoxyphenyl)cyclopropyl)methyl)-*N*-methylamino)-6-(1-(2,6-difluorophenyl)ethyl)pyrimidin-4(3*H*)-one (40 c): Colorless oil (yield 10%); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.07$–7.00 (m, 1H), 6.85–6.81 (m, 2H), 6.76–6.71 (m, 4H), 5.60 (s, 1H), 4.21–4.16 (q, 1H, $J=7.3$ Hz), 3.71 (s, 3H), 3.58–3.33 (m, 2H), 3.05 (s, 3H), 1.69–1.59 (m, 1H), 1.56–1.54 (d, 3H, $J=7.3$ Hz), 1.27–1.06 (m, 1H), 0.84–0.63 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=171.42$, 166.16, 163.11, 160.58, 157.92, 153.55, 134.35, 127.87, 126.92, 119.93, 113.80, 111.33, 97.48, 55.32, 53.16, 35.25, 29.70, 22.33, 21.04, 16.78, 14.05 ppm; MS (ESI) *m/z*: 426 $[M+H]^+$; Anal. calcd for $C_{24}H_{25}F_2N_3O_2$: C 67.75, H 5.92, N 9.88, found: C 67.81, H 5.97, N 9.93.

2-((2-(4-Methoxyphenyl)cyclopropyl)methylamino)-6-(1-(2,6-difluorophenyl)ethyl)pyrimidin-4(3*H*)-one (41 d): Colorless oil (yield 28%); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=11.80$ (bs, NH), 7.19–7.00 (m, 1H), 6.88–6.85 (m, 2H), 6.75–6.71 (m, 4H), 5.96 (bs, NH), 5.56 (s, 1H), 4.24–4.15 (q, 1H, $J=7.1$ Hz), 3.71 (s, 3H), 3.42–3.29 (m, 1H), 3.20–3.06 (m, 1H), 1.69–1.61 (m, 1H), 1.55–1.53 (d, 3H, $J=7.1$ Hz), 1.24–1.09 (m, 1H), 0.76–0.64 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=172.65$, 166.17, 162.91, 160.36, 157.73, 153.91, 134.46, 128.02, 127.12, 113.75, 111.40, 98.42, 55.31, 44.84, 36.39, 22.04, 21.23, 16.86, 13.80 ppm; MS (ESI) *m/z*: 412 $[M+H]^+$; Anal. calcd for $C_{22}H_{21}F_2N_3O_2$: C 66.49, H 5.33, N 10.57, found: C 66.54, H 5.41, N 10.59.

2-Amino-6-(1-(2,6-difluorophenyl)ethyl)-3-((2-(4-methoxyphenyl)cyclopropyl)methyl)pyrimidin-4(3*H*)-one (42 d): White solid (yield 15%); ^{1}H NMR (400 MHz, CD_3OD): $\delta=7.38$–7–11 (m, 1H), 7.00–6.79 (m, 4H), 6.72–6.70 (d, 2H, $J=8.3$ Hz), 5.97 (s, 1H), 4.49–4.45 (q, 1H, $J=7$ Hz), 3.97–3.88 (m, 1H), 3.71–3.61 (m, 4H), 1.94–1.69 (m, 2H), 1.59–1.54 (m, 3H), 1.29–0.75 ppm (m, 3H); ^{13}C NMR (100 MHz, CD_3OD): $\delta=172.99$, 168.41, 161.90, 158.20, 155.53, 133.06, 130.03, 126.71, 114.21, 113.50, 112.02, 105.73, 54.30, 47.01, 31.13, 21.71, 20.51, 17.90, 12.25 ppm; MS (ESI) *m/z*: 412 $[M+H]^+$; Anal. calcd for $C_{22}H_{21}F_2N_3O_2$: C 66.49, H 5.33, N 10.57, found: C 66.53, H 5.41, N 10.62.

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