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Article

2-((3,5-Dinitrobenzyl)thio)quinazolinones: Potent Antimycobacterial Agents Activated by Deazaflavin (F_{420})-Dependent Nitroreductase (Ddn)

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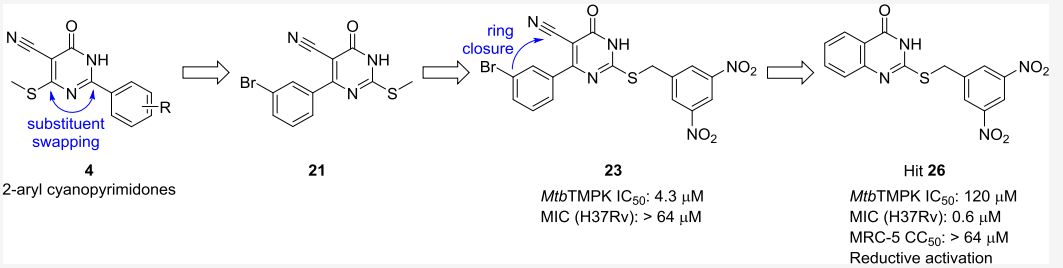

ABSTRACT: Swapping the substituents in positions 2 and 4 of the previously synthesized but yet undisclosed 5-cyano-4-(methylthio)-2-arylpyrimidin-6-ones **4**, ring closure, and further optimization led to the identification of the potent antitubercular 2-thio-substituted quinazolinone **26**. Structure−activity relationship (SAR) studies indicated a crucial role for both *meta*-nitro substituents for antitubercular activity, while the introduction of polar substituents on the quinazolinone core allowed reduction of bovine serum albumin (BSA) binding (**63c**, **63d**). While most of the tested quinazolinones exhibited no cytotoxicity against MRC-5, the most potent compound **26** was found to be mutagenic via the Ames test. This analogue exhibited moderate inhibitory potency against *Mycobacterium tuberculosis* thymidylate kinase, the target of the 3-cyanopyridones that lies at the basis of the current analogues, indicating that the whole-cell antimycobacterial activity of the present *S*-substituted thioquinazolinones is likely due to modulation of alternative or additional targets. Diminished antimycobacterial activity was observed against mutants affected in cofactor F_{420} biosynthesis (*fbiC*), cofactor reduction (*fgd*), or deazaflavin-dependent nitroreductase activity (*rv3547*), indicating that reductive activation of the 3,5-dinitrobenzyl analogues is key to antimycobacterial activity.

INTRODUCTION

Infection with *Mycobacterium tuberculosis* is currently one of the top 10 leading causes of death by a single pathogen worldwide and leads to an estimated 10 million new tuberculosis (TB) cases annually.[1] The airborne transmission of *M. tuberculosis* from an individual with active tuberculosis to a healthy individual typically occurs via droplet nuclei containing a few viable bacteria expelled through coughing. Although combination therapy with four first-line agents for the treatment of drug-sensitive TB has decreased TB mortality, control of the disease is hindered by the emergence of antibiotic-resistant strains as well as by the pathogen's ability to enter a persistent state, in which it tolerates conventional antibiotics, even in the absence of genetic drug resistance, necessitating long and complicated treatment regimens.[2,3] The newly approved agents bedaquiline,[4] delamanid,[5] and pretomanid[6] have provided new hope for afflicted patients. However, the first *M. tuberculosis* isolate resistant to bedaquiline and delamanid was reported shortly after their clinical introduction,[7] and pretomanid only has a narrow use as it is contraindicated in patients with hypersensitivity to bedaquiline or linezolid.[8] Thus, novel anti-TB agents are still needed to tackle the spread and drug resistance burden of *M. tuberculosis*.

M. tuberculosis thymidylate kinase (*Mtb*TMPK) is an enzyme involved in the synthesis of the DNA building block thymidine-5′-triphosphate and is thus indispensable for mycobacterial growth.[9−12] The reported co-crystal structures of *Mtb*TMPK indicate it as an attractive target for identifying new anti-TB agents.[13−15]

Through high-throughput screening, 3-cyanopyridones (Figure 1) were discovered as a new class of *Mtb*TMPK inhibitors by AstraZeneca in 2015.[14] They reported that replacement of the methylthio group of compound **1** with aromatic substituents improved the inhibitory potency (Figure 1, **1** and **2**).[14]

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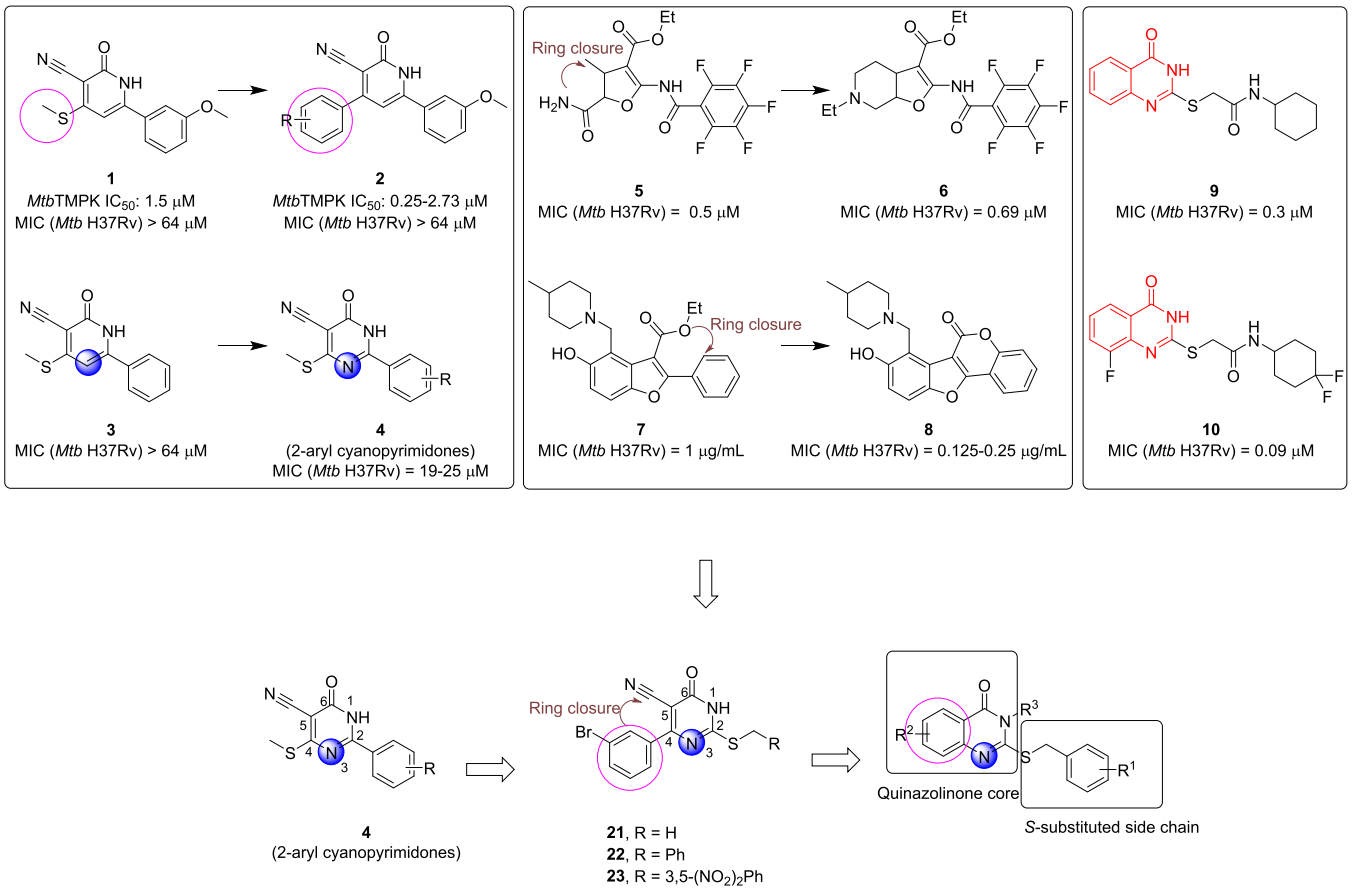
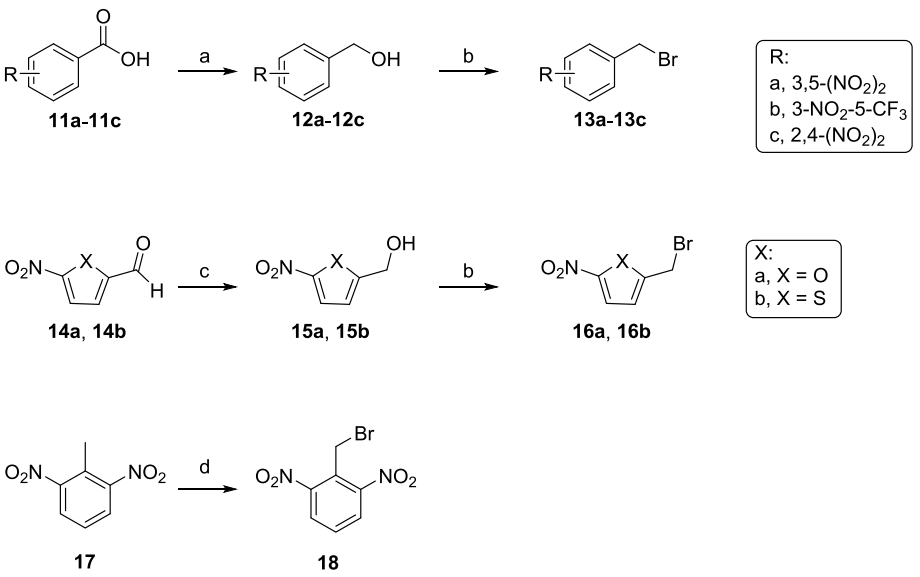


Figure 1. Structures of reported *Mtb*TMPK inhibitors (**1**–**4**), Pks13 inhibitors (**5**–**8**), and thioquinazolinone compounds (**9**, **10**), as well as compounds designed in this study.

Scheme 1. Synthesis of 13a–c, 16a, 16b, and 18[a]



[a]Reagents and conditions: (a) $NaBH_4$, $BF_3{\cdot}Et_2O$, tetrahydrofuran (THF); (b) CBr_4, PPh_3, ethyl acetate (EA); (c) $NaBH_4$, THF; (d) azobisisobutyronitrile (AIBN), DBDMH, CCl_4, reflux.

However, none of these derivatives possessed whole-cell activity.[14] Starting from the reported compound **3**, we introduced an additional N atom, which resulted in moderately active 2-aryl cyanopyrimidone analogues **4** (manuscript in preparation).

Inspired by these observations, we interchanged the 4-methylthio and 2-aromatic substituents of **4**, leading to cyanopyrimidone **21** (Figure 1). Moreover, the methylthio group was expanded with a phenyl and a 3,5-dinitrophenyl ring, a commonly used antimycobacterial pharmacophore,[16,17] since

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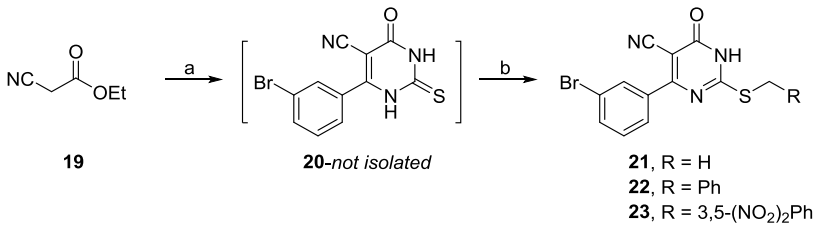
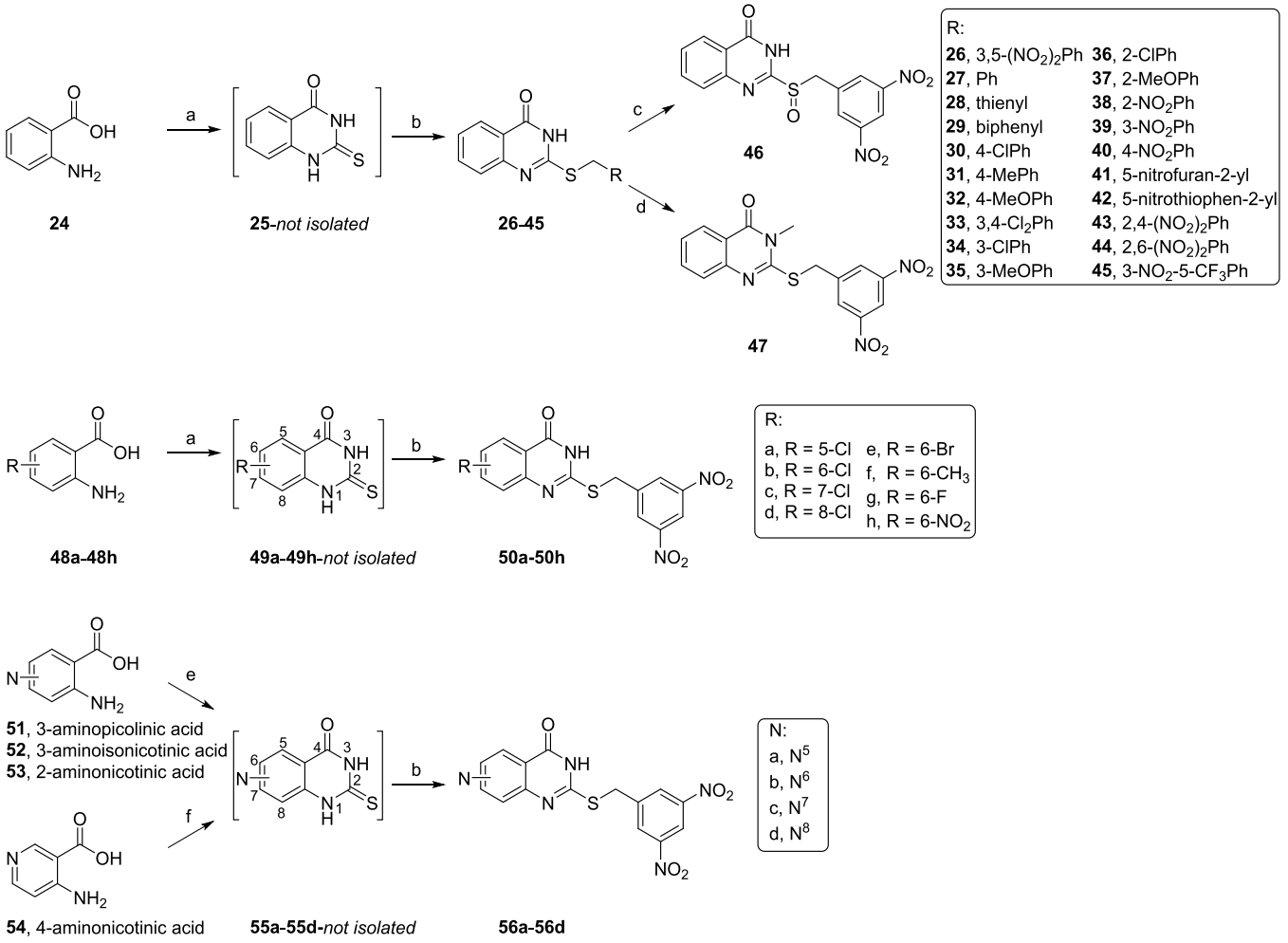
Scheme 2. Synthesis of 21−23[a]



[a]Reagents and conditions: (a) thiourea, K_2CO_3, 3-bromobenzaldehyde, EtOH; (b) R = H, CH_3I, NaOH, EtOH/H_2O (2/1, v/v); R = Ph/3,5-$(NO_2)_2$Ph, BnBr/**13a**, K_2CO_3, MeCN.

Scheme 3. Synthesis of 26−47, 50a−h, and 56a−d[a]



[a]Reagents and conditions: (a) (i) $SOCl_2$, 90 °C, (ii) NH_4SCN, acetone; (b) appropriate arylmethyl bromide, DMSO/MeCN (1/10, v/v), K_2CO_3; (c) *m*CPBA, THF; (d) CH_3I, K_2CO_3, MeCN; (e) thiourea, 200 °C; (f) (i)THF/dioxane (1/1, v/v), $SOCl_2$, catalytic dimethylformamide (DMF); (ii) NH_4SCN, acetone/dioxane (15/10, v/v).

sterically demanding groups in the same position of cyanopyridones were shown to be tolerated by AstraZeneca.[14] Encouraged by the finding that a cyclization strategy to form bicyclic (Figure 1, **5** → **6**)[18] or polycyclic motifs[19] (Figure 1, **7** → **8**) can successfully improve the antimycobacterial activity while maintaining inhibitory activity against the polyketide synthase 13 (Pks13) target, and that thioquinazolinone compounds such as **9** and **10** (Figure 1) were previously shown to possess potent antitubercular activity,[20,21] the phenyl ring was fused to the pyrimidone ring to generate novel (*S*)-quinazolinone derivatives.

RESULTS AND DISCUSSION

Chemistry. The synthesis of desired analogues **21−23**, **26−47**, **50a−h**, **56a−d**, and **63a−d** relied on the preparation of the desired heterocycle core, followed by S-alkylation to install the side chain (scheme 1−4). In cases where the required arylmethyl bromide for S-alkylation was not commercially available, it was prepared from either the corresponding alcohol (Scheme 1).[22] Arylmethyl alcohols **12a−c**, **15a**, and **15b** were prepared by $NaBH_4$ reduction of the corresponding commercially available acids or aldehydes.[23] Alternatively, **18** was prepared by radical

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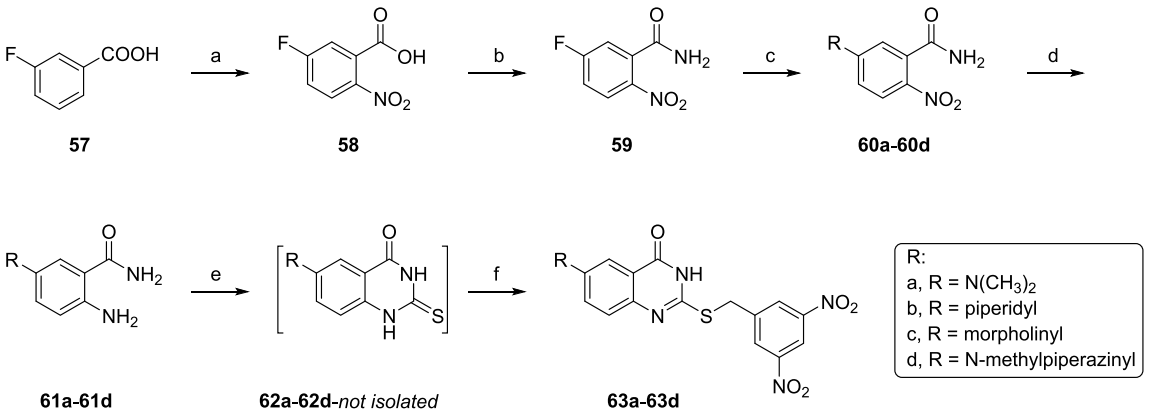
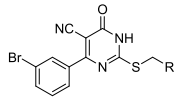
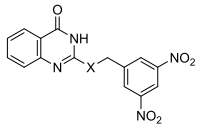
Scheme 4. Synthesis of 63a−d[a]



[a]Reagents and conditions: (a) sulfuric acid, fuming nitric acid; (b) (i) $SOCl_2$, reflux; (ii) NH_3, CH_2Cl_2; (c) K_2CO_3, dimethyl sulfoxide (DMSO), amine, 60 °C; (d) Pd/C, H_2, EtOH; (e) KOH, CS_2, EtOH/H_2O, reflux; (f) **13a**, DMSO/MeCN (1/10, v/v), K_2CO_3.

Table 1. *Mtb*TMPK Inhibitory and Antimycobacterial Activity of Cyanopyrimidone and Thioquinazolinone Compounds[c]

Structure	R/X	Comp.	*Mtb*TMPK IC_{50}	MIC[a] 7H9	MRC-5 CC_{50}
	R = H	**21**	82 ± 3	25	> 64
	R = Ph	**22**	49 ± 3	25	>36
	R = 3,5-$(NO_2)_2$Ph	**23**	4.3 ± 0.3	> 64	> 64
	X = S	**26**	120[b]	0.6	> 64
	X = S(O)	**46**	217 ± 20	50	> 64

[a]Minimum inhibitory concentration (MIC) is the minimum concentration required to inhibit >99% growth of *M. tuberculosis* H37Rv in 7H9/glucose liquid culture. Isoniazid was included as an internal control reference compound (MIC = 0.15 μM). [b]Approximate value reaches upper limit of solubility. [c]Values are given in micromolar (μM), *Mtb*TMPK IC_{50} values for which standard deviations (SD) are given are the calculated mean values of at least two measurement results.

bromination with 1,3-dibromo-5,5-dimethylhydantoin (DBDMH).[24]

The synthesis of **21−23** (Scheme 2) commenced with a Knoevenagel condensation of 3-bromobenzaldehyde with ethyl cyanoacetate, followed by a cyclization with thiourea under basic conditions[25] to afford the 4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine intermediate **20**, which was immediately alkylated[25] with iodomethane or (substituted) benzyl bromide to furnish **21−23**.

Envisioned relatively large series of thioquinazolinones with differently substituted arylmethyl moieties or various quinazolinone core modifications were synthesized through S-alkylation of thioquinazolinone with the appropriate arylmethyl bromides under basic conditions (Scheme 3).[25] Condensation of NH_4SCN with anthranilic acid chloride derivatives, obtained in situ from reaction of the (substituted) anthranilic acid with thionyl chloride, afforded the thioquinazolinone intermediates **25**, **49a−h**, which were selectively S-alkylated with arylmethyl bromides to furnish the desired compounds **26−45** and **50a−h**. Further oxidation[26] of **26** with *m*-chloroperoxybenzoic acid (*m*CPBA, 1 equiv) yielded sulfoxide **46**. Alternatively, **47** was obtained via methylation[27] of the same intermediate with CH_3I.

Due to the poor solubility of aminonicotinic/aminoisonicotinic/aminopiconilic acids in thionyl chloride, a different synthetic strategy was applied to gain access to the (*S*)-pyridopyrimidinones. The aminonicotinic/aminoisonicotinic/aminopiconilic acid was condensed directly with thiourea[20,28] at high temperatures to give **55a**, **55c**, and **55d**. Since this method only gave low conversion of 4-aminonicotinic acid to **55b** (monitored by liquid chromatography−mass spectrometry (LC−MS)), for this analogue, we returned to the $SOCl_2$-mediated acid chloride formation, in a THF/dioxane mixture. Alkylation of the resulting crude (*S*)-pyridopyrimidinone intermediates **55a−d** with **13a** afforded the desired compounds **56a−56d**.

The synthesis of 6-substituted analogues **63a−d** is shown in Scheme 4. The introduction of a nitro group in position 6 of 3-fluorobenzoic acid using fuming nitric acid led to intermediate **58**,[29] which was converted into an amide. After nucleophilic aromatic substitution of amide **59** with a secondary amine, Pd/

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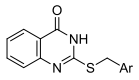
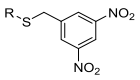
Table 2. Modifications of (*S*)-Dinitrobenzyl Side Chain[b]

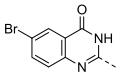
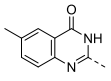
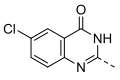
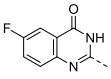
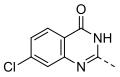
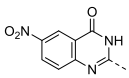


Ar	comp.	MIC[a] 7H9	MRC-5 CC_{50}	Ar	comp.	MIC 7H9	MRC-5 CC_{50}
3,5-$(NO_2)_2$Ph	**26**	0.6	>64	2-ClPh	**36**	2.3	>64
Ph	**27**	12.5	>64	2-MeOPh	**37**	>50	
2-thienyl	**28**	>50	>64	2-NO_2Ph	**38**	12.5	>64
biphenyl	**29**	>50	>64	3-NO_2Ph	**39**	>50	>64
4-ClPh	**30**	>50	>64	4-NO_2Ph	**40**	>50	>64
4-MePh	**31**	>50	>64	5-nitrofuran-2-yl	**41**	≥50	35
4-MeOPh	**32**	>50	>64	5-nitrothiophen-2-yl	**42**	≥50	>64
3,4-Cl_2Ph	**33**	>50	>64	2, 4-$(NO_2)_2$Ph	**43**	>50	>64
3-ClPh	**34**	3.13	>64	2, 6-$(NO_2)_2$Ph	**44**	>50	>64
3-MeOPh	**35**	0.78	>64	3-NO_2-5-CF_3Ph	**45**	>50	>64

[a]Minimum inhibitory concentration (MIC) is the minimum concentration required to inhibit >99% growth of *M. tuberculosis* H37Rv in 7H9/glucose liquid culture. [b]Values are given in μM.

Table 3. Modifications of Quinazolinone Core[b]



R	Comp.	MIC[a] 7H9	MRC-5 CC_{50}	R	Comp.	MIC[a] 7H9	MRC-5 CC_{50}
	**26**	0.6	> 64		**50d**	> 50	> 64
	**47**	1.56	> 64		**50e**	3.13	> 64
	**50a**	≥ 50	> 64		**50f**	1.56	> 64
	**50b**	0.78	> 64		**50g**	1.2	> 64
	**50c**	2.3	> 64		**50h**	6.25	34

[a]Minimum inhibitory concentration (MIC) is the minimum concentration required to inhibit >99% growth of *M. tuberculosis* H37Rv in 7H9/glucose liquid culture. [b]Values are given in μM.

C-mediated hydrogenation afforded the desired substituted anthranilic amide, which condensed[30] with CS_2 under basic conditions to yield 6-substituted thioquinazolinone intermediates (**62a−d**). Subsequently, the desired compounds (**63a−d**) were achieved through S-alkylation of **62a−d** with **13a**.

Biological Activity. *Enzymatic Activity and Antimycobacterial Activity against Wild-Type Strain.* The synthesized compounds were evaluated for their *Mtb*TMPK inhibitory potency (Table 1). Except for **23**, the cyanopyrimidones (**21**, **22**) showed moderate inhibitory activity. Quinazolinone **26** and the corresponding sulfoxide **46** only poorly inhibited *Mtb*TMPK.

Despite their poor enzyme inhibitory potency, these analogues mostly showed *in vitro* antimycobacterial activity (Table 1). Cyanopyrimidone analogues **21** and **22** showed moderate whole-cell activity, while introduction of two nitro groups in the meta position (**23**) led to activity loss. Surprisingly, the cyclized analogue **26** exhibited submicromolar antimycobacterial activity in 7H9 medium, without showing cytotoxicity against MRC-5 fibroblasts. Oxidation of **26** to the corresponding sulfoxide (**46**) resulted in a drastic decrease of antimycobacterial activity.

Encouraged by these results, we further focused on the structure−antimycobacterial activity relationship and optimization of **26** (Table 2; *Mtb*TMPK inhibitory activity in Table S2). Elimination of the nitro groups (**27**) led to a 20-fold decrease in activity, while the 2-thienyl isostere **28** and the *para*-biphenyl analogue **29** were inactive.

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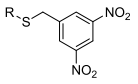
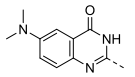
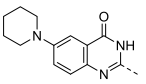
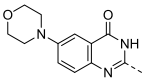
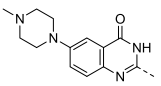
Table 4. Modification of Quinazolinone Core by Introduction of Polar Moieties[b]



R	Comp.	MIC[a] 7H9	MRC-5 CC_{50}	R	Comp.	MIC 7H9	MRC-5 CC_{50}
	**56a**	19	> 64		**63a**	> 50	14
	**56b**	> 50	> 64		**63b**	> 50	> 64
	**56c**	> 50	> 64		**63c**	4.7	> 64
	**56d**	≥ 50	> 64		**63d**	9.4	49

[a]Minimum inhibitory concentration (MIC) is the minimum concentration required to inhibit >99% growth of *M. tuberculosis* H37Rv in 7H9/glucose liquid culture. [b]Values are given in μM.

Substitution in para position of the phenyl ring failed to improve the antimycobacterial activity (**30**−**32**). In contrast, selected substituents in ortho or meta position improved activity (**34**−**36**). However, unlike analogue **26** which displayed antimycobacterial activity in all growth media tested, compounds **34**−**36** only showed activity when assayed in 7H9/glucose medium, potentially indicating that their antimycobacterial activity might be associated with carbon source metabolism[31] (Table 5, $SI_{7H9/glucose\ vs\ GAST/Fe}$ = 6, 64, and 16 for **34**, **35**, and **36**, respectively). Moreover, compounds **34**−**36** were inactive in media containing bovine serum albumin (BSA) (albumin, dextrose, and catalase (ADC) supplement and 7H9/glucose/BSA).[32]

Since the nitro groups of **26** proved important for antimycobacterial activity, we investigated the effects of mono-nitro-functionalized analogues. Remarkably, only ortho-substituted nitro analogue **38** displayed moderate activity. Also, analogues with moieties with higher reduction potentials that are more readily reduced such as nitrofuran (**41**) and nitrothiophene (**42**) failed to show antimycobacterial activity.

Finally, we investigated the optimal positional arrangement of the two nitro groups. We observed that the 3,5-dinitrophenyl pattern was superior to two other isomers (2,4-analogue **43** and 2,6-analogue **44**), which failed to inhibit antimycobacterial growth at the highest concentration (50 μM). Replacement of the 5-nitro with a trifluoromethyl group (**45**) also had a negative impact on the activity.

Next, we turned our attention to modifications in the quinazolinone core (Table 3). Methylation of the N^3-amino group of **26** (**47**) led to a small drop in activity (MIC = 1.6 μM). Additional Cl substitution of the phenyl ring of the quinazolinone core revealed that substitution on positions 7 and especially 6 is tolerated, while 5- or 8-substitution is detrimental to activity (compare **50b**/**50c** v. **50a**/**50d**). Changing the 6 Cl substituent of **50b** (compounds **50e**−**h**) did not result in improved activity, although still affording analogues with MIC values in the low micromolar range.

In an attempt to increase the polarity of **26**, we decided to investigate all different aza-isomers (i.e., pyridopyrimidinones **56a**−**d**, Table 4). Remarkably, only the 5-aza isomer (**56a**) showed some antimycobacterial activity, while all other isomers were inactive. Alternatively, several polar groups were introduced at the 6-position (**63a**−**d**). The morpholine and *N*-methylpiperazine derivatives (**63c** and **63d**, respectively) showed interesting whole-cell activity.

To further investigate whether the antimycobacterial activities of compounds were metabolic-dependent, active compounds were investigated under different growth conditions (Table 5). Most dinitro-containing analogues (**26**, **47**, **50b**, **50f**, **50g**, **63c**, **63d**) performed well under different test conditions, potentially reflecting less metabolic dependence, in contrast to non- or mono-nitro compounds (**27**−**38**), which only displayed activity in certain media. Comparison between BSA-free 7H9/glucose medium and BSA-containing medium suggests that the introduction of polar groups may effectively circumvent BSA binding (**63c**, **63d**). This observation was further supported by the correlation analysis between $R_{BSA/BSA\text{-}free}$ and clog *P* (Figure 2), where $R_{BSA/BSA\text{-}free}$ value is lower with improved clog *P*.

Mutagenicity of Compound ***26****.* Due to the mutagenic potential caused by nitro groups, a 384-well microplate "fluctuation" version of the classical reverse mutation *Salmonella typhimurium* Ames test was performed for **26** in the absence and presence of S9 metabolic activation (Table 6). Compound **26** exhibited genotoxic effect against TA98 strain in the absence of S9, while no genotoxic effect was observed in the presence of S9. Additionally, the genotoxic effect of **26** in the absence of S9 decreased with increased concentration, which may be due to the bactericidal activity of **26** against *S. typhimurium* TA 98 strain. With the TA100 strain, no genotoxicity was observed, regardless of metabolic activation.

Antimycobacterial Activity against Mutant Strains.[34−36] Based on the fact that **26** exhibited weak inhibitory potency against *Mtb*TMPK, we predicted that the whole-cell activity of this (and structurally related) thioquinazolinone was likely due

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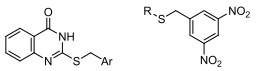
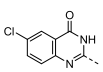
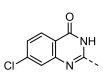
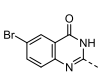
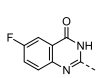
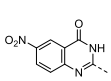
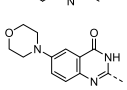
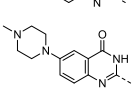
Table 5. Antimycobacterial Activity of Hits in Different Media[ab]



Ar/R	Comp.	MIC[a] 7H9		MIC	MIC	R[b]
		Glucose	ADC	GAST/Fe	7H9/ Glucose/ BSA	BSA/ BSA-free
	**26**	0.6	2.3	0.78	1.56	2.6
	**27**	12.5	> 50	19	> 50	> 4.0
	**34**	3.13	> 50	19	> 50	> 16
	**35**	0.78	> 50	50	> 50	> 64
	**36**	2.3	> 50	37	> 50	> 21.7
	**38**	12.5	> 50	25	> 50	> 4
	**47**	1.56	3.13	0.39	3.13	2.0
	**50b**	0.78	4.7	0.2	3.13	4.0
	**50c**	2.3	25	0.72	25	10.8
	**50e**	3.13	-	3.13	37	11.8
	**50f**	1.56	-	1.56	6.25	4.0
	**50g**	1.2	-	1.2	6.25	5.2
	**50h**	6.25	-	4.7	> 50	> 8.0
	**56a**	19	-	12.5	> 50	> 2.6
	**63c**	4.7	-	3.13	3.13	0.66
	**63d**	9.4	-	9.4	9.4	1.0

[a]Minimum inhibitory concentration (MIC) is the minimum concentration required to inhibit >99% growth of *M. tuberculosis* H37Rv in liquid culture and is given in μM. [b]$R_{BSA/BSA\text{-}free}$ = MIC (7H9/glucose/BSA)/MIC (7H9/glucose).

to modulation of alternative or additional targets. Since the most promising compounds possess a nitro group as in the anti-TB drug pretomanid, MIC determinations in mutants deficient in cofactor F_{420} biosynthesis (FbiC) or an enzyme involved in the regeneration of reduced F_{420} (Fgd), as well as a mutant in the

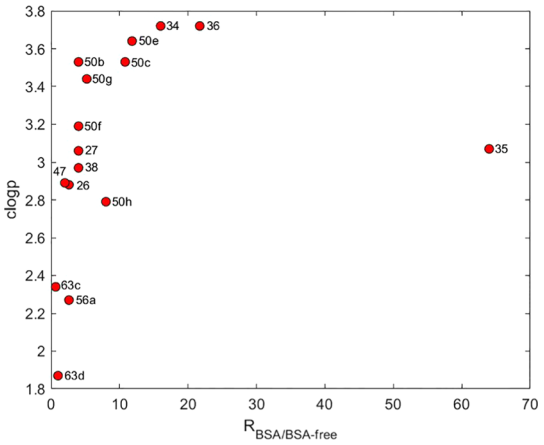


Figure 2. $R_{BSA/BSA\text{-}free}$ *vs* calculated clog *P* (created by MATLAB): *X*-axis, $R_{BSA/BSA\text{-}free}$. When $R_{BSA/BSA\text{-}free}$ is in a range, the minimum value was used; *Y*-axis, calculated clog *P* using SwissADME.[33] The numbers for each spot correspond to compound numbers in the structure–activity relationship (SAR) tables.

Table 6. Evaluation of Mutagenicity via the Ames Fluctuation Assay Performed with Compound 26 against TA100 and TA98 Strains (Highest Concentration, 349 μM)

comp.	Ames fluctuation assay[aa]		
	S9 activation	TA100	TA98
2-nitrofluorene	–	n.d.	+
4-nitroquinoline *N*-oxide	–	+	n.d.
aminoanthracene	+	+	+
26	–	–	+
26	+	–	–

[aa]–, negative mutagenicity; +, positive mutagenicity. n.d., not determined. For all compounds, the statistical significance related to mutagenicity was set at 0.05.

F_{420}-dependent nitroreductase Rv3547 and the Rv3547:A76E mutant, all of which are resistant to pretomanid, were performed to assess whether an activation mechanism is required for antimycobacterial activity (Table 7). All aforementioned mutant strains were resistant to all nitro-containing compounds studied, providing evidence that these compounds undergo a similar bioactivation mechanism as pretomanid[37] (reductive activation of the nitro group) (Table 7).

To support the notion that compound **26** and structurally related compounds are prodrugs activated by F_{420}-dependent nitroreductase, resistant mutants were generated by plating of *M. tuberculosis* on 7H11/OADC agar plates containing 3.75, 7.5, and 15 μM concentrations of compound **26**. As expected, the frequency of spontaneous resistance to compound **26** was very high, with a mutation frequency of 2.60×10^{-05} at 3.75 μM, 2.50×10^{-05} at 7.5 μM, and 1.50×10^{-06} at 15 μM (Supporting Information, Table S4). Thirty-four **26**-resistant mutants were tested for their level of resistance by MIC determination against compound **26** (Supporting information, Table S5), and all were found to be more than 40-fold resistant compared with the parental strain (H37Rv). Additionally, 33 out of 34 resistant mutants generated to compound **26** were cross-resistant to pretomanid and delamanid, while the susceptibility of these mutants remained unchanged relative to isoniazid and rifampicin, strongly further supporting that compound **26** is activated by a mechanism similar to that of pretomanid and delamanid.

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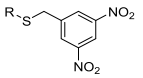
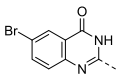
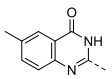
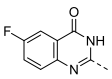
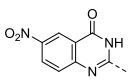
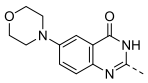
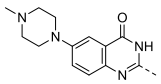
Table 7. Antimycobacterial Activities of 3,5-Dinitrobenzyl Compounds against Mutant Strains[a]



R	Comp.	MIC[a] 7H9				
		H37Rv	*fbiC* (5A1)	*fgd* (T3)	*rv3547* (14A1)	*rv3547*:A76E (T2)
	**26**	0.6	> 50	37	12.5	37
	**50e**	3.13	> 50	> 50	> 50	> 50
	**50f**	1.56	> 50	> 50	> 50	> 50
	**50g**	1.2	> 50	≥ 50	6.25	≥ 50
	**50h**	6.25	> 50	> 50	50	≥ 50
	**56a**	19	37	37	50	50
	**63c**	4.7	≥ 50	≥ 50	19	19
	**63d**	9.4	37	25	37	37

[a]*fbiC* mutant deficient in cofactor F_{420} biosynthesis; *fgd* mutant deficient in F_{420} reduction; *rv3547* and *rv3547*:A76E mutants deficient in F_{420}-dependent nitroreductase. Minimum inhibitory concentration (MIC) is the minimum concentration required to inhibit >99% growth of *M. tuberculosis* H37Rv in 7H9/glucose liquid culture, and is given in μM.

CONCLUSIONS

We synthesized a large series of **38** thioquinazolinone analogues structurally derived from an earlier cyanopyrimidone-type *Mtb*TMPK inhibitor. Several of these analogues displayed antimycobacterial activity, with the 3,5-dinitrobenzyl analogue **26** possessing submicromolar activity. SAR exploration of **26** indicated that ortho/meta-substitution of the (*S*)-benzyl side chain was tolerated, leading to the identification of the potent non-nitro analogue **35**, which, however, failed to display antimycobacterial activity when assayed in different growth media. Additionally, addition of BSA significantly lowered the antimycobacterial activity of **35**, suggesting high protein binding. Next, modifications of the quinazoline core of **26** were investigated. It was observed that substitution in position 3, 6, or 7 is tolerated, leading to the discovery of analogues **63c** and **63d** with lower clog *P*. Most of the active compounds were devoid of cytotoxicity against MRC-5, while the Ames fluctuation assay demonstrated that dinitrobenzyl analogue **26** induced statistically significant reverse mutations without metabolic activation in the TA98 strain.

Evaluations against selected mutants known to impede reduction of nitro groups were less sensitive to **26** and related compounds featuring identical *S*-(3,5-dinitrobenzyl) substituents, indicating that the antimycobacterial activities of these compounds relied on the reductive activation of 3,5-dinitrobenzyl moiety. The prodrug notion was further supported by the observation that resistant mutations to compound **26** were cross-resistant to known prodrugs pretomanid and delamanid. Taken together, thioquinazolinone analogues prove to be potent antimycobacterial agents. Further optimization efforts could be directed toward reducing the protein binding of **35** or reducing the mutagenicity of **26**.

EXPERIMENTAL SECTION

Enzymatic Assay. After expression and purification of *Mtb*TMPK as described by Munier-Lehmann et al.,[38] the enzymatic assay was conducted as previously reported.[15] Briefly, at fixed concentrations of ATP (0.5 mM) and dTMP (0.05 mM), compounds were evaluated at different concentrations using the spectrophotometric assay described by Blondin et al.[39] The reaction medium consists of 50 mM Tris−HCl, pH 7.4, 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM NADH, 1 mM phosphoenol pyruvate, and 2 units each of coupling enzymes (lactate dehydrogenase,

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pyruvate kinase, and nucleoside diphosphate kinase). The IC_{50} value was calculated using KaleidaGraph to plot and fit the experimental data points.

***In Vitro* Antitubercular Activity.** The MIC values of all compounds were determined as previously decribed.[40−42] In brief, *M. tuberculosis* H37Rv (ATCC 27294) and the mutant strains were grown to OD_{650nm} 0.2 in the respective medium prior to further 1000-fold dilution in fresh medium. Drugs were twofold serially diluted in duplicate in the medium of choice (50 μL/well) in a concentration range spanning 100−0.049 μM in sterile 96-well U-bottom clear polysteyrene microtiter plates. Isoniazid and DMSO were used as positive and negative controls, respectively. An equal volume (50 μL) of diluted cells was added to the plates with serial drug dilution. Plates were sealed in ziplock bags and incubated at 37 °C. After 7−14 days, the plates were read with enlarging inverted mirror plate reader. The MIC was recorded as the concentration that fully inhibited all visible growth.

Generation of Compound 26-Resistant Mutants. Two independent cultures of *M. tuberculosis* H37Rv (ATCC 27294) were grown to mid-logarithmic phase. At that stage, the cells were harvested and resuspended in fresh 7H9/ADC/Tween 80 medium to final cell densities approximating 1×10^6, 1×10^7, 1×10^8, and 1×10^9 colony-forming units (CFU)/mL. Dilutions of the suspensions were made, and the resulting dilutions (100 μL) were plated on drug-free plates to determine the actual cell density. To generate mutants against compound **26**, 100 μL of the four cell suspensions of the two independent cultures was plated on 7H11/OADC agar plates containing compound **26** at 3.75, 7.5, and 15 μM concentrations. The plates were incubated at 37 °C for 4 weeks, and the colonies were counted. Rifampicin was used as a control. A total of 34 colonies were picked and inoculated in 7H9/ADC/Tween, and MICs were determined for compound **26**, pretomanid, delamanid, isoniazid, and rifampicin to confirm their resistance to compound **26** and cross-resistant to pretomanid and delamanid.

***In Vitro* Genotoxicity Assay.**[43] Approximately 10 million bacteria were exposed in triplicate to the test agent compound **26** (six concentrations), a negative control (vehicle), and a positive control (2-nitrofluorene against TA 98 in the absence of S9, 4-nitroquinoline *N*-oxide against TA100 in the absence of S9, aminoanthracene against TA 98 and TA100 in the presence of S9) for 90 min in a medium containing a low concentration of histidine (sufficient for about two doublings). The cultures were then diluted into an indicator medium lacking histidine, and dispensed into 48 wells of 384-well plates (micro-plate format, MPF). The plates were incubated for 48 h at 37 °C, and cells that underwent a reversion grew, resulting in a color change in the wells with growth. The numbers of wells showing growth were counted and compared to the vehicle control. An increase in the number of colonies of at least twofold over baseline (mean + SD of the vehicle control) and a dose response indicate a positive response. An unpaired, one-sided Student's *t*-test was used to identify the conditions that were significantly different from the vehicle control. S9 fraction from the livers of Aroclor 1254-treated rats was included in the incubation at a final concentration of 4.5%. A reduced nicotinamide adenine dinucleotide phosphate (NADPH)-regenerating system was also included to ensure a steady supply of reducing equivalents. Strains used in this study: *S. typhimurium* TA98: hisD3052, rfa, uvrB/pKM101, detects frame-shift mutations; *S. typhimurium* TA100: hisG45, rfa, uvrB/pKM101, detects base-pair substitutions.

***In Vitro* Cytotoxicity Assay.** The cytotoxicity of compounds on MRC-5 fibroblasts was performed exactly as previously reported using neutral red uptake assay.[15]

Chemistry. All reagents and solvents were purchased from standard commercial sources and were of analytical grade. All synthetic compounds described in this study were checked with analytical thin-layer chromatography (TLC) (Machery−Nagel-precoated F_{254} aluminum plates), visualized under UV light at 254 nm, and purified by column chromatography (CC) on a Reveleris X2 (Grace) automated flash unit. NMR data were recorded on a Varian Mercury 300/75 MHz spectrometer or a Bruker Avance Neo 400/100 MHz spectrometer at 298.15 K using residual solvent signal as a reference. Confirmation of compound structure was conducted with ^{1}H, ^{13}C, heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) NMR spectrometry. Additionally, high-resolution mass spectrometry (HRMS) was performed on a Waters LCT Premier XE time-of-flight (TOF) mass spectrometer equipped with a standard electrospray ionization (ESI) and modular LockSpray interface. The purity (95%) of the tested compounds was determined by LC-MS analysis (Waters AutoPurification system: a Waters Cortecs C18 column (2.7 μm, 100 × 4.6 mm^2); a gradient system of formic acid in H_2O (0.2%, v/v)/MeCN; a flow rate of 1.44 mL/min; and a gradient of 95:5−0:100 in 10 min).

General Procedure A: Synthesis of (Substituted) Quinazolinone Intermediates. According to a literature report,[44] (substituted) aminobenzoic acid (6.0 mmol) was refluxed in thionyl chloride (10 mL) for 2 h. The excess thionyl chloride was removed in vacuo, and the crude acyl chloride was dissolved in acetone (10 mL) and added dropwise to a solution of NH_4SCN (6.9 mmol) in acetone (5.0 mL). The reaction mixture was stirred at room temperature for 1 h, and the obtained solid was collected through filtration. Then, the brown solid was suspended in 2 M aq NaOH (15 mL) and filtered. Water (15 mL) was added to the collected filtrate, and the mixture was acidified with 2 N aq HCl to pH 2. The obtained solid was collected by filtration, and additionally washed with H_2O/MeOH (1/1 v/v, 50 mL). The resulting solid was used in the next step without further purification.

General Procedure B: Synthesis of Final Compounds. The (substituted) thioquinazolinone/*S*-pyridopyrimidinone intermediate (0.50 mmol) and K_2CO_3 (1.5 mmol) were dissolved in DMSO/MeCN (1/10 v/v, 11 mL), and the appropriate arylmethyl bromide (0.45 mmol) was added in one portion. The resulting mixture was stirred at room temperature for 1 h. After the consumption of starting material was found by monitoring with TLC, water (100 mL) was added to the reaction mixture. The resulting mixture was acidified with 1 N aq HCl to pH 6. The generated solid was collected by filtration, dried in a high-vacuum oil pump, and purified to give the desired compound.

*1-(Bromomethyl)-3,5-dinitrobenzene (**13a**).* According to a literature report,[23] to a suspension of $NaBH_4$ (36 mg, 0.94 mmol) in dry THF (10 mL) at 0 °C was added 3,5-dinitrobenzoic acid (0.10 g, 0.47 mmol), followed by $BF_3{\cdot}Et_2O$ (0.16 mL, 1.2 mmol). After addition, the reaction mixture was warmed to room temperature and stirred for 1 h, after which the reaction was quenched with 1 N aq HCl (5.0 mL). The aqueous phase was extracted with CH_2Cl_2 (25 mL × 2), and the organic layer was dried, concentrated, and purified to give intermediate alcohol **12a** (eluent system: 25% EA in petroleum ether, 73 mg, 0.37 mmol, 78% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 4.98 (s, 2H, CH_2), 8.52−8.73 (m, 2H, Ph), 8.87−9.08 (m, 1H, Ph). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 63.0 (1C, CH_2), 117.7 (1C, Ph), 122.5 (1C, Ph), 126.4 (2C, Ph), 145.4 (2C, Ph). To a solution of the intermediate alcohol **12a**, CBr_4 (0.25 g, 0.74 mmol) in EA (10 mL) was added PPh_3 (0.19 g, 0.74 mmol), and the resulting mixture was stirred at room temperature for 1 h according to a literature report.[22] After completion of the reaction, the mixture was evaporated and the residue was purified to give **13a** (eluent system: 10% EA in petroleum ether, 70 mg, 0.27 mmol, 73% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 4.63 (s, 2H, CH_2), 8.60 (d, J = 2.1 Hz, 2H, Ph), 8.99 (t, J = 2.1 Hz, 1H, Ph). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 29.3 (1C, CH_2), 110.0 (1C, Ph), 118.6 (1C, Ph), 129.1 (2C, Ph), 142.0 (2C, Ph).

*1-(Bromomethyl)-3-nitro-5-(trifluoromethyl)benzene (**13b**).* Following the procedure described for **13a**, $NaBH_4$ (32 mg, 0.85 mmol), 3-nitro-5-(trifluoromethyl)benzoic acid (0.10 g, 0.43 mmol), and $BF_3{\cdot}Et_2O$ (0.14 mL, 1.1 mmol) in THF (10 mL) afforded **12b** (eluent system: 25% EA in petroleum ether, 85 mg, 0.38 mmol, 90% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 3.32 (s, 1H, OH), 4.88 (s, 2H, CH_2), 7.94 (s, 1H, Ph), 8.31 (s, 1H, Ph), 8.37 (s, 1H, Ph). ^{19}F NMR (282 MHz, $CDCl_3$) δ ppm −63.10 (s, 3 F). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 63.0 (1C, CH_2), 119.4 (q, J = 3.4 Hz, 1C, Ph), 122.7 (q, J = 271.0 Hz, 1C, CF_3), 124.2 (1C, Ph), 128.9 (q, J = 3.4 Hz, 1C, Ph), 132.1 (q, J = 34.8 Hz, 1C, Ph), 144.7 (1C, Ph), 148.2 (1C, Ph). Then, **12b**, CBr_4 (0.26 g, 0.77 mmol) and PPh_3 (0.20 g, 0.77 mmol) in EA (10 mL) gave **13b** (eluent system: 10% EA in petroleum ether, 93 mg, 0.33 mmol, 85% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 4.60 (s, 2H, CH_2), 8.00 (s, 1H, Ph), 8.42 (s, 1H, Ph), 8.46 (t, J = 1.8 Hz, 1H, Ph). ^{13}C NMR

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(75 MHz, $CDCl_3$) δ ppm 29.8 (1C, CH_2), 120.5 (q, J = 3.6 Hz, 1C, Ph), 122.5 (q, J = 272.9 Hz, 1C, CF_3), 127.0 (1C, Ph), 131.4 (q, J = 3.4 Hz, 1C, Ph), 132.8 (q, J = 33.4 Hz, 1C, Ph), 141.3 (1C, Ph), 148.5 (1C, Ph).

*1-(Bromomethyl)-2,4-dinitrobenzene (**13c**).* Following the procedure described for **13a**, $NaBH_4$ (36 mg, 0.94 mmol), 2,4-dinitrobenzoic acid (0.10 g, 0.43 mmol), and $BF_3{\cdot}Et_2O$ (0.16 mL, 1.2 mmol) in THF (10 mL) afforded **12c** (eluent system: 25% EA in petroleum ether, 56 mg, 0.28 mmol, 60% yield). ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 3.50 (s, 2H, CH_2), 6.63 (d, J = 8.6 Hz, 1H, Ph), 7.00 (dd, J = 8.6, 2.4 Hz, 1H, Ph), 7.31 (d, J = 2.4 Hz, 1H, Ph). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 61.8 (1C, CH_2), 120.4 (1C, Ph), 127.9 (1C, Ph), 130.3 (1C, Ph), 143.8 (1C, Ph), 146.9 (1C, Ph), 147.1 (1C, Ph). Then, **12c**, CBr_4 (0.19 g, 0.56 mmol) and PPh_3 (0.15 g, 0.56 mmol) in EA (10 mL) gave **13c** (eluent system: 10% EA in petroleum ether, 66 mg, 0.25 mmol, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.89 (s, 2H, CH_2), 7.85 (d, J = 8.5 Hz, 1H, Ph), 8.47 (dd, J = 8.5, 2.3 Hz, 1H, Ph), 8.90 (d, J = 2.3 Hz, 1H, Ph). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 26.9 (1C, CH_2), 121.0 (1C, Ph), 127.7 (1C, Ph), 133.9 (1C, Ph), 139.2 (1C, Ph), 147.7 (1C, Ph), 148.0 (1C, Ph).

*2-(Bromomethyl)-5-nitrofuran (**16a**).* To a solution of 5-nitrofuran-2-carbaldehyde (0.10 g, 0.71 mmol) in THF (10 mL) was added $NaBH_4$ (54 mg, 1.4 mmol), and the resulting mixture was stirred at room temperature for 2 h. After consumption of the aldehyde starting material, the reaction was quenched with saturated aq solution of Na^+/K^+ tartaric acid (25 mL), and the aqueous layer was extracted with CH_2Cl_2 (25 mL × 2). The organic layer was dried, concentrated, and purified to afford **15a** (eluent system: 25% EA in petroleum ether, 50 mg, 0.35 mmol, 50% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.27 (br. s., 1H, OH), 4.73 (s, 2H, CH_2), 6.56 (d, J = 3.8 Hz, 1H, furyl), 7.29 (d, J = 3.6 Hz, 1H, furyl). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 57.4 (1C, CH_2), 110.6 (1C, furyl), 112.4 (1C, furyl), 151.9 (1C, furyl), 157.4 (1C, furyl). Following the procedure described for **13a**, **15a**, CBr_4 (0.23 g, 0.70 mmol), and PPh_3 (0.18 g, 0.70 mmol) in EA (10 mL) gave **16a** (eluent system: 10% EA in petroleum ether, 59 mg, 0.28 mmol, 82% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.47 (s, 2H, CH_2), 6.63 (d, J = 3.6 Hz, 1H, furyl), 7.28 (d, J = 4.0 Hz, 1H, furyl). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 20.7 (1C, CH_2), 112.4 (1C, furyl), 112.5 (1C, furyl), 151.9 (1C, furyl), 153.4 (1C, furyl).

*2-(Bromomethyl)-5-nitrothiophene (**16b**).* Following the procedure described for **16a**, 5-nitrothiophene-2-carbaldehyde (0.10 g, 0.64 mmol) and $NaBH_4$ (48 mg, 1.3 mmol) in THF (10 mL) afforded **15b** (eluent system: 25% EA in petroleum ether, 76 mg, 0.48 mmol, 75% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.47 (s, 1H, OH), 4.88 (d, J = 0.9 Hz, 2H, CH_2), 6.93 (dt, J = 4.2, 1.0 Hz, 1H, thienyl), 7.81 (d, J = 4.1 Hz, 1H, thienyl). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 60.2 (1C, CH_2), 123.4 (1C, thienyl), 128.8 (1C, thienyl), 150.8 (1C, thienyl), 153.4 (1C, thienyl). Then, **15b**, CBr_4 (0.32 g, 0.96 mmol) and PPh_3 (0.25 g, 0.96 mmol) in EA (10 mL) gave **16b** (eluent system: 10% EA in petroleum ether, 70 mg, 0.31 mmol, 66% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.63 (s, 2H, CH_2), 7.07 (d, J = 4.3 Hz, 1H, thienyl), 7.79 (d, J = 4.3 Hz, 1H, thienyl). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 24.5 (1C, CH_2), 127.0 (1C, thienyl), 128.4 (1C, thienyl), 148.2 (1C, thienyl), 151.8 (1C, thienyl).

*2-(Bromomethyl)-1,3-dinitrobenzene (**18**).* According to a literature report,[24] 2,6-dinitrotoluene (0.97 g, 5.4 mmol), AIBN (30 mg, 0.18 mmol), and DBDMH (2.9 g, 10 mmol) were suspended in CCl_4 (50 mL), and the resulting mixture was refluxed overnight. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2 (0.15 L), concentrated in vacuo and the resulting residue was purified to give **18** (eluent system: 10% EA in petroleum ether, 0.38 g, 1.5 mmol, 27% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.95 (s, 2H, CH_2), 7.69 (t, J = 8.2 Hz, 1H, Ph), 8.11 (d, J = 8.1 Hz, 2H, Ph). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 19.9 (1C, CH_2), 127.5 (1C, Ph), 128.7 (2C, Ph), 130.1 (1C, Ph), 150.2 (2C, Ph).

*6-(3-Bromophenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**20**).* According to a literature report,[25] to a stirred solution of 3-bromobenzaldehyde (0.80 mL, 6.9 mmol), ethyl cyanoacetate (0.78 g, 6.9 mmol), and thiourea (0.52 g, 6.9 mmol) in EtOH was added K_2CO_3 (1.1 g, 8.3 mmol). Stirring was continued at reflux overnight. After cooling to room temperature, the pale yellow solid was collected, taken up with boiling water, and filtered. The aqueous phase was acidified to pH 5 with 1 N aq HCl, and the precipitate was collected by filtration and dried in vacuo to afford the desired compound, which was used in the next step without further purification.

*4-(3-Bromophenyl)-2-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**21**).* According to a literature report,[45] a solution of **20** (1.0 g, 3.2 mmol), CH_3I (0.20 mL, 3.2 mmol), and NaOH (0.13 g, 3.2 mmol) in EtOH/H_2O (10/5 mL) was stirred at 60 °C for 1 h. After cooling to room temperature, the reaction mixture was acidified to pH 5 with 1 N aq HCl. The obtained solid collected through filtration was washed with water, dried, and purified to give **21** (eluent system: 5% MeOH in CH_2Cl_2, 0.91 g, 2.8 mmol, 88% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.61 (s, 3H, CH_3), 7.54 (t, J = 7.9 Hz, 1H, Ph), 7.82 (ddd, J = 8.0, 2.0, 0.9 Hz, 1H, Ph), 7.95 (dt, J = 7.9, 1.3 Hz, 1H, Ph), 8.09 (t, J = 1.8 Hz, 1H, Ph), 13.86 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 13.3 (1C, CH_3), 93.5 (1C, C-5), 115.7 (1C, CN), 121.7 (1C, Ph), 127.7 (1C, Ph), 130.8 (1C, Ph), 131.1 (1C, Ph), 134.3 (1C, Ph), 137.6 (1C, Ph), 160.9 (1C, CO), 165.6 (1C, C-4), 167.1 (1C, C-2). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{12}H_8BrN_3OS + H]^+$ 323.9624, found 323.9643.

*2-(Benzylthio)-4-(3-bromophenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**22**).* According to a literature report,[25] to a stirred suspension of **20** (0.5 g, 1.6 mmol) and K_2CO_3 (0.67 g, 4.9 mmol) in MeCN (25 mL) was added BnBr (0.19 mL, 1.6 mmol). Stirring was continued overnight. The volatiles were removed in vacuum. The crude was taken up with water after it cooled to room temperature and acidified to pH 5 with 1 N aq HCl. The obtained solid was purified to give **22** (eluent system: 5% MeOH in CH_2Cl_2, 0.15 g, 0.37 mmol, 23% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 4.50 (s, 2H, CH_2), 7.20−7.36 (m, 3H, Ph), 7.36−7.43 (m, 2H, Ph), 7.52 (t, J = 15.8 Hz, 1H, Ph), 7.80 (ddd, J = 8.1, 2.1, 1.0 Hz, 1H, Ph), 7.90 (ddd, J = 7.8, 1.2, 1.2 Hz, 1H, Ph), 8.00 (t, J = 1.8 Hz, 1H, Ph). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 34.3 (1C, CH_2), 93.8 (1C, C-5), 115.5 (1C, CN), 121.7 (1C, Ph), 127.5 (1C, Ph), 127.7 (1C, Ph), 128.5 (2C, Ph), 129.0 (2C, Ph), 130.7 (1C, Ph), 131.2 (1C, Ph), 134.3 (1C, Ph), 136.4 (1C, Ph), 137.4 (1C, Ph), 160.9 (1C, CO), 165.7 (1C, C-4), 166.1 (1C, C-2). HRMS (ESI): m/z $[M - H]^-$ calcd for $[C_{18}H_{12}BrN_3OS - H]^-$ 395.98112, found 395.9755.

*4-(3-Bromophenyl)-2-((3,5-dinitrobenzyl)thio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**23**).* Following the same procedure described for **22**, **20** (0.50 g, 1.6 mmol), K_2CO_3 (0.67 g, 4.9 mmol), and **13a** (0.42 g, 1.6 mmol) in MeCN (25 mL) afforded **23** (eluent system: 5% MeOH in CH_2Cl_2, 0.18 g, 0.37 mmol, 23% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 4.69 (s, 2H, CH_2), 7.48 (t, J = 8.0 Hz, 1H, Ph), 7.75−7.85 (m, 2H, Ph), 7.90 (d, J = 1.8 Hz, 1H, Ph), 8.67 (s, 3H, Ph). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 32.8 (1C, CH_2), 115.9 (1C, CN), 117.3 (1C, Ph), 121.7 (1C, Ph), 127.5 (1C, Ph), 129.4 (2C, Ph), 130.7 (1C, Ph), 130.8 (1C, Ph), 134.0 (1C, Ph), 137.6 (1C, Ph), 142.6 (1C, Ph), 147.8 (2C, Ph), 166.0 (1C, C-4), 166.1 (1C, C-2). C (C-5) and C (CO) could not be observed. HRMS (ESI): m/z $[M - H]^-$ calcd for $[C_{18}H_{10}BrN_5O_5S - H]^-$ 485.9513, found 485.9518.

*2-Thioxo-2,3-dihydroquinazolin-4(1H)-one (**25**).* Following General Procedure A, anthranilic acid (0.82 g, 6.0 mmol) in thionyl chloride (10 mL) afforded acyl chloride, which was reacted with NH_4SCN (0.53 g, 6.9 mmol) in acetone (15 mL) to give **25**.

*2-((3,5-Dinitrobenzyl)thio)quinazolin-4(3H)-one (**26**).* Following General Procedure B, **25** (89 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol) and **13a** (0.12 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **26** (eluent system: 15% EA in toluene, 95 mg, 0.27 mmol, 59% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 4.67 (s, 2H, CH_2), 7.41 (t, J = 7.3 Hz, 1H, quinazolinone), 7.62 (d, J = 7.9 Hz, 1H, quinazolinone), 7.80 (t, J = 7.3 Hz, 1H, quinazolinone), 7.99 (dd, J = 7.9, 1.2 Hz, 1H, quinazolinone), 8.66 (t, J = 2.2 Hz, 1H, Ph), 8.88 (d, J = 2.1 Hz, 2H, Ph), 12.63 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 32.1 (1C, CH_2), 117.3 (1C, Ph), 120.0 (1C, quinazolinone), 125.8 (1C, quinazolinone), 125.9 (1C, quinazolinone), 126.0 (1C, quinazolinone), 130.1 (2C, Ph), 134.7 (1C, quinazolinone), 143.1 (1C, Ph), 147.6 (2C, Ph), 148.1 (1C, quinazolinone), 154.5 (1C,

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quinazolinone), 161.0 (1C, CO). HRMS (ESI): m/z $[M - H]^-$ calcd for $[C_{15}H_{10}N_4O_5S - H]^-$ 357.0299, found 357.0300.

*2-(Benzylthio)quinazolin-4(3H)-one (**27**).* Following General Procedure B, **25** (89 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and benzyl bromide (53 μL, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **27** (eluent system: 15% EA in toluene, 78 mg, 0.29 mmol, 65% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 4.49 (s, 2H, CH_2), 7.15−7.34 (m, 3H, Ph), 7.39 (t, J = 7.5 Hz, 1H, quinazolinone), 7.47 (d, J = 7.3 Hz, 2H, Ph), 7.58 (d, J = 7.9 Hz, 1H, quinazolinone), 7.73 (t, J = 8.0 Hz, 1H, quinazolinone), 8.04 (d, J = 7.9 Hz, 1H, quinazolinone), 12.59 (br. s., 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 33.6 (1C, CH_2), 120.0 (1C, quinazolinone), 125.6 (1C, quinazolinone), 126.0 (2C, quinazolinone), 127.3 (1C, Ph), 128.4 (2C, Ph), 129.2 (2C, Ph), 134.5 (1C, quinazolinone), 137.3 (1C, Ph), 148.3 (1C, quinazolinone), 155.2 (1C, quinazolinone), 161.2 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{15}H_{12}N_2OS + H]^+$ 269.0743, found 269.0753.

*2-((Thiophen-2-ylmethyl)thio)quinazolin-4(3H)-one (**28**).* Following General Procedure B, **25** (89 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and 2-(bromomethyl)thiophene (49 μL, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **28** (eluent system: 15% EA in toluene, 87 mg, 0.32 mmol, 70% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.74 (s, 2H, CH_2), 6.94 (dd, J = 5.1, 3.5 Hz, 1H, thienyl), 7.15 (d, J = 2.6 Hz, 1H, thienyl), 7.38 (dd, J = 5.1, 1.1 Hz, 1H, thienyl), 7.45 (t, J = 7.5 Hz, 1H, quinazolinone), 7.63 (d, J = 8.0 Hz, 1H, quinazolinone), 7.80 (t, J = 7.5 Hz, 1H, quinazolinone), 8.05 (dd, J = 7.9, 1.3 Hz, 1H, quinazolinone), 12.60 (br. s., 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 28.3 (1C, CH_2), 120.0 (1C, quinazolinone), 125.8 (1C, quinazolinone), 126.0 (1C, thienyl), 126.1 (2C, quinazolinone), 126.6 (1C, thienyl), 127.4 (1C, thienyl), 134.7 (1C, quinazolinone), 140.0 (1C, thienyl), 148.2 (1C, quinazolinone), 154.8 (1C, quinazolinone), 161.2 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{13}H_{10}N_2OS_2 + H]^+$ 275.0308, found 275.0303.

*2-(([1,1′-Biphenyl]-4-ylmethyl)thio)quinazolin-4(3H)-one (**29**).* Following General Procedure B, **25** (89 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and 4-(bromomethyl)biphenyl (0.11 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **29** (eluent system: 15% EA in toluene, 44 mg, 0.13 mmol, 28% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.55 (s, 2H, CH_2), 7.32−7.38 (m, 1H, Ph), 7.40−7.48 (m, 3H, quinazolinone, Ph), 7.56−7.67 (m, 7H, quinazolinone, Ph), 7.79 (ddd, J = 8.3, 7.1, 1.6 Hz, 1H, quinazolinone), 8.05 (dd, J = 7.9, 1.2 Hz, 1H, quinazolinone), 12.61 (br. s., 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 33.2 (1C, CH_2), 120.0 (1C, quinazolinone) 125.7 (1C, quinazolinone), 126.0 (2C, quinazolinone), 126.6 (2C, Ph), 126.7 (2C, Ph), 127.4 (1C, Ph), 128.9 (2C, Ph), 129.8 (2C, Ph), 134.7 (1C, quinazolinone), 136.7 (1C, Ph), 139.1 (1C, Ph), 139.7 (1C, Ph), 148.3 (1C, quinazolinone), 155.2 (1C, quinazolinone), 161.2 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{21}H_{16}N_2OS + H]^+$ 345.1056, found 345.1060.

*2-((4-Chlorobenzyl)thio)quinazolin-4(3H)-one (**30**).* Following General Procedure B, **25** (89 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and 4-chlorobenzyl bromide (92 mg, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **30** (eluent system: 15% EA in toluene, 0.10 g, 0.34 mmol, 75% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.49 (s, 2H, CH_2), 7.31−7.40 (m, 2H, Ph), 7.43 (ddd, J = 8.0, 7.1, 1.1 Hz, 1H, quinazolinone), 7.49−7.56 (m, 2H, Ph), 7.61 (d, J = 8.1 Hz, 1H, quinazolinone), 7.78 (ddd, J = 8.3, 7.0, 1.6 Hz, 1H, quinazolinone), 8.04 (dd, J = 7.9, 1.2 Hz, 1H, quinazolinone), 12.60 (br. s., 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 32.7 (1C, CH_2), 120.0 (1C, quinazolinone), 125.7 (1C, quinazolinone), 126.0 (2C, quinazolinone), 128.3 (2C, Ph), 131.0 (2C, Ph), 131.8 (1C, Ph), 134.7 (1C, quinazolinone), 136.8 (1C, Ph), 148.3 (1C, quinazolinone), 154.9 (1C, quinazolinone), 161.1 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{15}H_{11}ClN_2OS + H]^+$ 303.0354, found 303.0346.

*2-((4-Methylbenzyl)thio)quinazolin-4(3H)-one (**31**).* Following General Procedure B, **25** (89 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and *p*-methylbenzyl bromide (83 mg, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **31** (eluent system: 15% EA in toluene, 70 mg, 0.25 mmol, 55% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.26 (s, 3H, CH_3), 4.46 (s, 2H, CH_2), 7.12 (d, J = 7.9 Hz, 2H, Ph), 7.36 (d, J = 8.0 Hz, 2H, Ph), 7.43 (ddd, J = 8.0, 7.1, 1.1 Hz, 1H, quinazolinone), 7.60 (d, J = 8.1 Hz, 1H, quinazolinone), 7.78 (ddd, J = 8.3, 7.1, 1.6 Hz, 1H, quinazolinone), 8.04 (dd, J = 7.9, 1.13 Hz, 1H, quinazolinone), 12.56 (br. s., 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 20.7 (1C, CH_3), 33.4 (1C, CH_2), 120.0 (1C, quinazolinone), 125.7 (1C, quinazolinone), 126.0 (2C, quinazolinone), 129.0 (2C, Ph), 129.1 (2C, Ph), 134.1 (1C, Ph), 134.6 (1C, quinazolinone), 136.5 (1C, Ph), 148.3 (1C, quinazolinone), 155.3 (1C, quinazolinone), 161.1 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{16}H_{14}N_2OS + H]^+$ 283.0900, found 283.0898.

*2-((4-Methoxybenzyl)thio)quinazolin-4(3H)-one (**32**).* Following General Procedure B, **25** (89 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and 4-methoxybenzyl bromide (90 mg, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **32** (eluent system: 15% EA in toluene, 55 mg, 0.18 mmol, 41% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.71 (s, 3H, OCH_3), 4.44 (s, 2H, CH_2), 6.85−6.90 (m, 2H, Ph), 7.37−7.45 (m, 3H, quinazolinone, Ph), 7.60 (d, J = 8.1 Hz, 1H, quinazolinone), 7.78 (ddd, J = 8.3, 7.04, 1.6 Hz, 1H, quinazolinone), 8.03 (dd, J = 7.9, 1.3 Hz, 1H, quinazolinone), 12.54 (br. s., 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 33.1 (1C, CH_2), 55.0 (1C, OCH_3), 113.8 (2C, Ph), 120.0 (1C, quinazolinone), 125.7 (1C, quinazolinone), 126.0 (2C, quinazolinone), 128.9 (1C, Ph), 130.4 (2C, Ph), 134.6 (1C, quinazolinone), 148.3 (1C, quinazolinone), 155.4 (1C, quinazolinone), 158.5 (1C, Ph), 161.2 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{16}H_{14}N_2O_2S + H]^+$ 299.0849, found 299.0858.

*2-((3,4-Dichlorobenzyl)thio)quinazolin-4(3H)-one (**33**).* Following General Procedure B, **25** (89 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and 3,4-dichlorobenzyl bromide (65 μL, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **33** (eluent system: 15% EA in toluene, 0.11 g, 0.32 mmol, 72% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.49 (s, 2H, CH_2), 7.40−7.47 (m, 1H, quinazolinone), 7.49−7.54 (m, 1H, Ph), 7.55−7.63 (m, 2H, quinazolinone, Ph), 7.75−7.85 (m, 2H, quinazolinone, Ph), 8.04 (dd, J = 7.9, 1.3 Hz, 1H, quinazolinone), 12.63 (br. s., 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 32.2 (1C, CH_2), 120.0 (1C, quinazolinone), 125.7 (1C, quinazolinone), 125.8 (1C, quinazolinone), 126.1 (1C, quinazolinone), 129.6 (1C, Ph), 129.7 (1C, Ph), 130.5 (1C, Ph), 130.7 (1C, Ph), 131.3 (1C, Ph), 134.6 (1C, quinazolinone), 139.3 (1C, Ph), 148.1 (1C, quinazolinone), 155.0 (1C, quinazolinone), 161.3 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{15}H_{10}Cl_2N_2OS + H]^+$ 336.9964, found 336.9981.

*2-((3-Chlorobenzyl)thio)quinazolin-4(3H)-one (**34**).* Following General Procedure B, **25** (89 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and 3-chlorobenzyl bromide (59 μL, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **34** (eluent system: 15% EA in toluene, 96 mg, 0.32 mmol, 70% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.51 (s, 2H, CH_2), 7.29−7.39 (m, 2H, Ph), 7.41−7.51 (m, 2H, quinazolinone, Ph), 7.58−7.65 (m, 2H, quinazolinone, Ph), 7.74−7.84 (m, 1H, quinazolinone), 8.05 (dd, J = 7.9, 1.3 Hz, 1H, quinazolinone), 12.62 (br. s., 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 32.8 (1C, CH_2), 120.0 (1C, quinazolinone), 125.7 (1C, quinazolinone), 125.9 (1C, quinazolinone), 126.1 (1C, quinazolinone), 127.2 (1C, Ph), 127.9 (1C, Ph), 129.1 (1C, Ph), 130.2 (1C, Ph), 132.8 (1C, Ph), 134.7 (1C, quinazolinone), 140.3 (1C, Ph), 148.2 (1C, quinazolinone), 154.9 (1C, quinazolinone), 161.1 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{15}H_{11}ClN_2OS + H]^+$ 303.0354, found 303.0356.

*2-((3-Methoxybenzyl)thio)quinazolin-4(3H)-one (**35**).* Following General Procedure B, **25** (89 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and 3-methoxybenzyl bromide (63 μL, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **35** (eluent system: 15% EA in toluene, 80 mg, 0.27 mmol, 60% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.70 (s, 3H, OCH_3), 4.45 (s, 2H, CH_2), 6.80 (dd, J = 8.2, 2.1 Hz, 1H, Ph), 6.99−7.09 (m, 2H, Ph), 7.21 (t, J = 7.5 Hz, 1H, Ph), 7.41 (t, J = 7.5 Hz, 1H, quinazolinone), 7.59 (d, J = 8.2 Hz, 1H, quinazolinone), 7.76 (t, J = 8.2 Hz, 1H, quinazolinone), 8.01 (d, J = 7.6 Hz, 1H, quinazolinone), 12.55 (br. s., 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 33.5 (1C, CH_2), 55.0 (1C, OCH_3), 112.8 (1C, Ph), 114.8 (1C, Ph), 120.0 (1C, quinazolinone), 121.3 (1C, Ph), 125.7 (1C, quinazolinone), 126.0 (1C, quinazolinone), 126.0 (1C, quinazoli-

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none), 129.5 (1C, Ph), 134.7 (1C, quinazolinone), 138.8 (1C, Ph), 148.3 (1C, quinazolinone), 155.2 (1C, quinazolinone), 159.2 (1C, Ph), 161.1 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{16}H_{14}N_2O_2S + H]^+$ 299.0849, found 299.0848.

*2-((2-Chlorobenzyl)thio)quinazolin-4(3H)-one (**36**).* Following General Procedure B, **25** (89 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and 1-(bromomethyl)-2-chlorobenzene (58 μL, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **36** (eluent system: 15% EA in toluene, 99 mg, 0.33 mmol, 73% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.60 (s, 2H, CH_2), 7.28−7.36 (m, 2H, Ph), 7.41−7.47 (m, 1H, quinazolinone), 7.47−7.53 (m, 1H, Ph), 7.66 (d, J = 8.0 Hz, 1H, quinazolinone), 7.69−7.75 (m, 1H, Ph), 7.76−7.83 (m, 1H, quinazolinone), 8.04 (dd, J = 7.9, 1.3 Hz, 1H, quinazolinone), 12.61 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 31.6 (1C, CH_2), 120.0 (1C, quinazolinone), 125.8 (1C, quinazolinone), 126.0 (2C, quinazolinone), 127.3 (1C, Ph), 129.4 (2C, Ph), 131.7 (1C, Ph), 133.4 (1C, Ph), 134.7 (1C, Ph), 134.7 (1C, quinazolinone), 148.2 (1C, quinazolinone), 154.7 (1C, quinazolinone), 161.1 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{15}H_{11}ClN_2OS + H]^+$ 303.0354, found 303.0360.

*2-((2-Methoxybenzyl)thio)quinazolin-4(3H)-one (**37**).* Following General Procedure B, **25** (89 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and *o*-methoxybenzyl bromide (90 mg, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **37** (eluent system: 15% EA in toluene, 78 mg, 0.26 mmol, 58% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.84 (s, 3H, OCH_3), 4.45 (s, 2H, CH_2), 6.89 (td, J = 7.4, 0.9 Hz, 1H, Ph), 7.02 (d, J = 7.9 Hz, 1H, Ph), 7.28 (td, J = 7.9, 1.75 Hz, 1H, Ph), 7.43 (t, J = 7.5 Hz, 1H, quinazolinone), 7.48 (dd, J = 7.5, 1.6 Hz, 1H, Ph), 7.63 (d, J = 8.1 Hz, 1H, quinazolinone), 7.79 (ddd, J = 8.3, 7.1, 1.5 Hz, 1H, quinazolinone), 8.04 (dd, J = 8.0, 1.3 Hz, 1H, quinazolinone), 12.52 (br. s., 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 28.8 (1C, CH_2), 55.5 (1C, OCH_3), 110.9 (1C, Ph), 119.9 (1C, quinazolinone), 120.3 (1C, Ph), 124.6 (1C, Ph), 125.6 (1C, quinazolinone), 126.0 (2C, quinazolinone), 129.1 (1C, Ph), 130.6 (1C, Ph), 134.7 (1C, quinazolinone), 148.4 (1C, quinazolinone), 155.7 (1C, quinazolinone), 157.2 (1C, Ph), 161.2 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{16}H_{14}N_2O_2S + H]^+$ 299.0849, found 299.0848.

*2-((2-Nitrobenzyl)thio)quinazolin-4(3H)-one (**38**).* Following General Procedure B, **25** (89 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and 2-nitrobenzyl bromide (97 mg, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **38** (eluent system: 15% EA in toluene, 98 mg, 0.31 mmol, 70% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 4.75 (s, 2H, CH_2), 7.39 (t, J = 7.5 Hz, 1H, quinazolinone), 7.49 (t, J = 7.8, Hz, 1H, Ph), 7.57 (d, J = 7.9 Hz, 1H, quinazolinone), 7.67 (t, J = 7.5 Hz, 1H, Ph), 7.75 (t, J = 7.9 Hz, 1H, quinazolinone), 7.87 (d, J = 7.6 Hz, 1H, Ph), 7.94−8.03 (m, 2H, quinazolinone, Ph), 12.56 (br. s., 1 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 30.8 (1C, CH_2), 120.4 (1C, quinazolinone), 124.7 (1C, Ph), 125.8 (1C, quinazolinone), 125.9 (1C, quinazolinone), 126.0 (1C, quinazolinone), 128.9 (1C, Ph), 132.7 (1C, Ph), 133.2 (1C, Ph), 133.7 (1C, Ph), 134.6 (1C, quinazolinone), 148.1 (1C, quinazolinone), 148.4 (1C, Ph), 154.7 (1C, quinazolinone), 161.1 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{15}H_{11}N_3O_3S + H]^+$ 314.0594, found 314.0607.

*2-((3-Nitrobenzyl)thio)quinazolin-4(3H)-one (**39**).* Following General Procedure B, **25** (89 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and 1-(bromomethyl)-3-nitrobenzene (97 mg, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **39** (eluent system: 15% EA in toluene, 88 mg, 0.28 mmol, 62% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.62 (s, 2H, CH_2), 7.40−7.48 (m, 1H, quinazolinone), 7.57−7.68 (m, 2H, quinazolinone, Ph), 7.75−7.85 (m, 1H, quinazolinone), 7.99 (d, J = 7.8 Hz, 1H, Ph), 8.03 (dd, J = 7.9, 1.3 Hz, 1H, quinazolinone), 8.10 (dd, J = 8.1, 1.5 Hz, 1H, Ph), 8.43−8.49 (m, 1H, Ph), 12.64 (br. s., 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 32.5 (1C, CH_2), 120.0 (1C, quinazolinone), 122.1 (1C, Ph), 124.1 (1C, Ph), 125.8 (1C, quinazolinone), 125.9 (1C, quinazolinone), 126.0 (1C, quinazolinone), 129.8 (1C, Ph), 134.7 (1C, quinazolinone), 136.0 (1C, Ph), 140.5 (1C, Ph), 147.5 (1C, Ph), 148.2 (1C, quinazolinone), 154.7 (1C, quinazolinone), 161.1 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{15}H_{11}N_3O_3S + H]^+$ 314.0594, found 314.0595.

*2-((4-Nitrobenzyl)thio)quinazolin-4(3H)-one (**40**).* Following General Procedure B, **25** (89 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and *p*-nitrobenzyl bromide (97 mg, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **40** (eluent system: 15% EA in toluene, 0.10 g, 0.32 mmol, 72% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.61 (s, 2H, CH_2), 7.40−7.49 (m, 1H, quinazolinone), 7.62 (d, J = 8.1 Hz, 1H, quinazolinone), 7.76−7.83 (m, 3H, quinazolinone, Ph), 8.03 (dd, J = 7.9, 1.3 Hz, 1H, quinazolinone), 8.15−8.22 (m, 2H, Ph), 12.65 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 32.7 (1C, CH_2), 120.0 (1C, quinazolinone), 123.4 (2C, Ph), 125.8 (1C, quinazolinone), 126.0 (1C, quinazolinone), 126.1 (1C, quinazolinone), 130.5 (2C, Ph), 134.7 (1C, quinazolinone), 146.2 (1C, Ph), 146.5 (1C, Ph), 148.2 (1C, quinazolinone), 154.6 (1C, quinazolinone), 161.1 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{15}H_{11}N_3O_3S + H]^+$ 314.0594, found 314.0586.

*2-(((5-Nitrofuran-2-yl)methyl)thio)quinazolin-4(3H)-one (**41**).* Following General Procedure B, **25** (89 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **16a** (93 mg, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **41** (eluent system: 15% EA in toluene, 0.11 g, 0.35 mmol, 78% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.68 (s, 2H, CH_2), 6.87 (d, J = 3.8 Hz, 1H, furyl), 7.45 (td, J = 7.5, 1.1 Hz, 1H, quinazolinone), 7.59−7.67 (m, 2H, quinazolinone, furyl), 7.79 (ddd, J = 8.3, 7.0, 1.6 Hz, 1H, quinazolinone), 8.04 (dd, J = 7.9, 1.2 Hz, 1H, quinazolinone), 12.71 (br. s., 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 26.0 (1C, CH_2), 112.9 (1C, furyl), 114.2 (1C, furyl), 120.0 (1C, quinazolinone), 126.0 (1C, quinazolinone), 126.1 (2C, quinazolinone), 134.7 (1C, quinazolinone), 148.1 (1C, quinazolinone), 150.9 (1C, furyl), 155.8 (2C, quinazolinone, furyl), 161.1 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{13}H_9N_3O_4S + H]^+$ 304.0387, found 304.0377.

*2-(((5-Nitrothiophen-2-yl)methyl)thio)quinazolin-4(3H)-one (**42**).* Following General Procedure B, **25** (89 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **16b** (0.10 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **42** (eluent system: 15% EA in toluene, 31 mg, 0.097 mmol, 22% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.76 (s, 2H, CH_2), 7.28 (d, J = 4.1 Hz, 1H, thienyl), 7.48 (t, J = 7.4 Hz, 1H, quinazolinone), 7.67 (d, J = 8.0 Hz, 1H, quinazolinone), 7.84 (t, J = 7.4 Hz, 1H, quinazolinone), 7.97 (d, J = 4.1 Hz, 1H, thienyl), 8.07 (d, J = 7.6 Hz, 1H, quinazolinone), 12.73 (br. s., 1H, H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 28.5 (1C, CH_2), 120.1 (1C, quinazolinone), 125.9 (1C, quinazolinone), 126.1 (1C, quinazolinone), 126.2 (1C, quinazolinone), 127.9 (1C, thienyl), 129.4 (1C, thienyl), 134.9 (1C, quinazolinone), 147.9 (1C, quinazolinone), 150.0 (1C, thienyl), 150.7 (1C, thienyl), 154.5 (1C, quinazolinone), 161.1 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{13}H_9N_3O_3S_2 + H]^+$ 320.0158, found 320.0173.

*2-((2,4-Dinitrobenzyl)thio)quinazolin-4(3H)-one (**43**).* Following General Procedure B, **25** (89 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **13c** (0.12 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **43** (eluent system: 15% EA in toluene, 85 mg, 0.24 mmol, 53% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.86 (s, 2H, CH_2), 7.39−7.50 (m, 1H, quinazolinone), 7.59 (d, J = 7.9 Hz, 1H, quinazolinone), 7.80 (ddd, J = 8.3, 7.1, 1.6 Hz, 1H, quinazolinone), 8.00 (dd, J = 7.9, 1.1 Hz, 1H, quinazolinone), 8.21 (d, J = 8.6 Hz, 1H, Ph), 8.51 (dd, J = 8.6, 2.4 Hz, 1H, Ph), 8.73 (d, J = 2.5 Hz, 1H, Ph), 12.65 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 30.0 (1C, CH_2), 120.0 (1C, quinazolinone), 120.1 (1C, Ph), 125.9 (1C, quinazolinone), 126.0 (2C, quinazolinone), 127.6 (1C, Ph), 134.3 (1C, Ph), 134.7 (1C, quinazolinone), 140.3 (1C, Ph), 146.5 (1C, Ph), 148.0 (1C, Ph), 148.4 (1C, quinazolinone), 154.3 (1C, quinazolinone), 161.0 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{15}H_{10}N_4O_5S + H]^+$ 359.0445, found 359.0461.

*2-((2,6-Dinitrobenzyl)thio)quinazolin-4(3H)-one (**44**).* Following General Procedure B, **25** (89 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **18** (0.12 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **44** (eluent system: 15% EA in toluene, 0.13 g, 0.36 mmol, 81% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 5.00 (s, 2H, CH_2), 7.43 (t, J = 7.6 Hz, 1H, quinazolinone), 7.52 (d, J = 8.0 Hz, 1H, quinazolinone), 7.75−7.85 (m, 2H, quinazolinone, Ph), 8.00 (dd, J = 7.9, 1.2 Hz, 1H, quinazolinone), 8.33 (d, J = 8.3 Hz, 2H, Ph), 12.60 (s,

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1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 25.6 (1C, CH_2), 120.0 (1C, quinazolinone), 125.5 (1C, quinazolinone), 126.0 (1C, quinazolinone), 126.0 (1C, quinazolinone), 127.9 (1C, Ph), 129.4 (2C, Ph), 130.1 (1C, Ph), 134.6 (1C, quinazolinone), 147.9 (1C, quinazolinone), 150.4 (2C, Ph), 154.1 (1C, quinazolinone), 160.9 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{15}H_{10}N_4O_5S + H]^+$ 359.0445, found 359.0432.

*2-((3-Nitro-5-(trifluoromethyl)benzyl)thio)quinazolin-4(3H)-one (**45**).* Following General Procedure B, **25** (89 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **13b** (0.13 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **45** (eluent system: 15% EA in toluene, 0.11 g, 0.28 mmol, 60% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.66 (s, 2H, CH_2), 7.40−7.51 (m, 1H, quinazolinone), 7.61 (d, J = 8.1 Hz, 1H, quinazolinone), 7.75−7.88 (m, 1H, quinazolinone), 8.02 (dd, J = 7.9, 1.3 Hz, 1H, quinazolinone), 8.34 (s, 1H, Ph), 8.46 (s, 1H, Ph), 8.77 (s, 1H, Ph), 12.65 (br. s., 1H, NH). ^{19}F NMR (377 MHz, DMSO-d_6) δ ppm −61.35 (s, 3 F). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 32.3 (1C, CH_2), 119.1 (q, J = 4.5 Hz, 1C, Ph), 120.0 (1C, quinazolinone), 122.9 (q, J = 272.1 Hz, 1C, CF_3), 125.7 (1C, quinazolinone), 125.9 (1C, quinazolinone), 126.1 (1C, quinazolinone), 128.2 (1C, Ph), 129.9 (q, J = 33.8 Hz, 1C, Ph), 132.5 (q, J = 3.8 Hz, 1C, Ph), 134.7 (1C, quinazolinone), 142.8 (1C, Ph), 147.9 (1C, Ph), 148.1 (1C, quinazolinone), 154.6 (1C, quinazolinone), 161.1 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{16}H_{10}F_3N_3O_3S + H]^+$ 382.0468, found 382.0471.

*2-((3,5-Dinitrobenzyl)sulfinyl)quinazolin-4(3H)-one (**46**).* To a solution of **26** (0.10 g, 0.28 mmol) in THF (10 mL) was added *m*CPBA (75%, 63 mg, 0.28 mmol), and the resulting mixture was stirred at room temperature for 2 h. After concentration in vacuo, the residue was purified to give **46** (eluent system: 25% EA in toluene, 86 mg, 0.23 mmol, 83% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.76 (d, J = 12.9 Hz, 1H, CH_2), 4.95 (d, J = 12.9 Hz, 1H, CH_2), 7.53−7.74 (m, 2H, quinazolinone), 7.88 (t, J = 7.1 Hz, 1H, quinazolinone), 8.12 (d, J = 7.0 Hz, 1H, quinazolinone), 8.47 (d, J = 1.9 Hz, 2H, Ph), 8.78 (t, J = 2.0 Hz, 1H, Ph), 12.43 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 56.3 (1C, CH_2), 118.2 (1C, Ph), 121.8 (1C, quinazolinone), 126.3 (1C, quinazolinone), 126.9 (1C, quinazolinone), 128.1 (1C, quinazolinone), 131.0 (2C, Ph), 133.9 (1C, Ph), 135.0 (1C, quinazolinone), 146.8 (1C, quinazolinone), 147.5 (2C, Ph), 157.5 (1C, quinazolinone), 160.8 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{15}H_{10}N_4O_6S - H]^-$ 373.0248, found 373.0244.

*2-((3,5-Dinitrobenzyl)thio)-3-methylquinazolin-4(3H)-one (**47**).* To a solution of **26** (0.10 g, 0.28 mmol) and K_2CO_3 (0.12 g, 0.83 mmol) in MeCN (10 mL) was added iodomethane (34 μL, 0.55 mmol), and the resulting mixture was stirred at room temperature for 1 h. The reaction mixture was adjusted to pH 5−6 with 1 N aq HCl, and the concentrated mixture was purified to give **47** (eluent system: 15% EA in toluene, 38 mg, 0.10 mmol, 37% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.48 (s, 3H, CH_3), 4.77 (s, 2H, CH_2), 7.45 (t, J = 7.7 Hz, 1H, quinazolinone), 7.68 (d, J = 7.9 Hz, 1H, quinazolinone), 7.84 (t, J = 7.5 Hz, 1H, quinazolinone), 8.05 (dd, J = 7.9, 1.3 Hz, 1H, quinazolinone), 8.69 (t, J = 2.1 Hz, 1H, Ph), 8.95 (d, J = 2.1 Hz, 2H, Ph). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 30.1 (1C, CH_3), 33.7 (1C, CH_2), 117.4 (1C, Ph), 118.7 (1C, quinazolinone), 125.6 (1C, quinazolinone), 126.0 (1C, quinazolinone), 126.4 (1C, quinazolinone), 130.3 (2C, Ph), 134.7 (1C, quinazolinone), 142.7 (1C, Ph), 146.6 (1C, quinazolinone), 147.6 (2C, Ph), 156.1 (1C, quinazolinone), 160.6 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{16}H_{12}N_4O_5S + H]^+$ 373.0601, found 373.0603.

*5-Chloro-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (**49a**).* Following General Procedure A, 6-chloroanthranilic acid (1.0 g, 6.0 mmol) in thionyl chloride (10 mL) afforded acyl chloride, which was reacted with NH_4SCN (0.53 g, 6.9 mmol) in acetone (15 mL) to give **49a**.

*6-Chloro-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (**49b**).* Following General Procedure A, 5-chloroanthranilic acid (1.0 g, 6.0 mmol) in thionyl chloride (10 mL) afforded acyl chloride, which was reacted with NH_4SCN (0.53 g, 6.9 mmol) in acetone (15 mL) to give **49b**.

*7-Chloro-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (**49c**).* Following General Procedure A, 4-chloroanthranilic acid (1.0 g, 6.0 mmol) in thionyl chloride (10 mL) afforded acyl chloride, which was reacted with NH_4SCN (0.53 g, 6.9 mmol) in acetone (15 mL) to give **49c**.

*8-Chloro-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (**49d**).* Following General Procedure A, 3-chloroanthranilic acid (1.0 g, 6.0 mmol) in thionyl chloride (10 mL) afforded acyl chloride, which was reacted with NH_4SCN (0.53 g, 6.9 mmol) in acetone (15 mL) to give **49d**.

*6-Bromo-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (**49e**).* Following General Procedure A, 5-bromoanthranilic acid (1.3 g, 6.0 mmol) in thionyl chloride (10 mL) afforded acyl chloride, which was reacted with NH_4SCN (0.53 g, 6.9 mmol) in acetone (15 mL) to give **49e**.

*6-Methyl-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (**49f**).* Following General Procedure A, 5-methylanthranilic acid (0.91 g, 6.0 mmol) in thionyl chloride (10 mL) afforded acyl chloride, which was reacted with NH_4SCN (0.53 g, 6.9 mmol) in acetone (15 mL) to give **49f**.

*6-Fluoro-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (**49g**).* Following General Procedure A, 5-fluoroanthranilic acid (0.93 g, 6.0 mmol) in thionyl chloride (10 mL) afforded acyl chloride, which was reacted with NH_4SCN (0.53 g, 6.9 mmol) in acetone (15 mL) to give **49g**.

*6-Nitro-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (**49h**).* Following General Procedure A, 5-nitroanthranilic acid (1.1 g, 6.0 mmol) in thionyl chloride (10 mL) afforded acyl chloride, which was reacted with NH_4SCN (0.53 g, 6.9 mmol) in acetone (15 mL) to give **49h**.

*5-Chloro-2-((3,5-dinitrobenzyl)thio)quinazolin-4(3H)-one (**50a**).* Following General Procedure B, **49a** (0.11 g, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **13a** (0.12 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **50a** (eluent system: 15% EA in toluene, 0.11 g, 0.28 mmol, 59% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.67 (s, 2H, CH_2), 7.42 (dd, J = 7.8, 0.9 Hz, 1H, quinazolinone), 7.58 (dd, J = 8.2, 0.9 Hz, 1H, quinazolinone), 7.73 (t, J = 8.0 Hz, 1H, quinazolinone), 8.68 (t, J = 2.1 Hz, 1H, Ph), 8.88 (d, J = 2.0 Hz, 2H, Ph), 12.69 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 32.1 (1C, CH_2), 116.9 (1C, quinazolinone), 117.3 (1C, Ph), 125.4 (1C, quinazolinone), 128.3 (1C, quinazolinone), 130.1 (2C, Ph), 132.7 (1C, quinazolinone), 134.5 (1C, quinazolinone), 143.0 (1C, Ph), 147.7 (2C, Ph), 150.5 (1C, quinazolinone), 155.6 (1C, quinazolinone), 159.1 (1C, CO). HRMS (ESI): m/z $[M - H]^-$ calcd for $[C_{15}H_9ClN_4O_5S - H]^-$ 390.9909, found 390.9908.

*6-Chloro-2-((3,5-dinitrobenzyl)thio)quinazolin-4(3H)-one (**50b**).* Following General Procedure B, **49b** (0.11 g, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **13a** (0.12 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **50b** (eluent system: 15% EA in toluene, 91 mg, 0.23 mmol, 51% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.68 (s, 2H, CH_2), 7.45 (dd, J = 8.5, 2.1 Hz, 1H, quinazolinone), 7.70 (d, J = 2.0 Hz, 1H, quinazolinone), 7.99 (d, J = 8.5 Hz, 1H, quinazolinone), 8.68 (t, J = 2.1 Hz, 1H, Ph), 8.91 (d, J = 2.1 Hz, 2H, Ph), 12.79 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 32.3 (1C, CH_2), 117.3 (1C, Ph), 118.8 (1C, quinazolinone), 125.1 (1C, quinazolinone), 126.0 (1C, quinazolinone), 128.1 (1C, quinazolinone), 130.2 (2C, Ph), 139.2 (1C, quinazolinone), 142.9 (1C, Ph), 147.6 (2C, Ph), 149.1 (1C, quinazolinone), 156.5 (1C, quinazolinone), 160.4 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{15}H_9ClN_4O_5S + H]^+$ 393.0055, found 393.0045.

*7-Chloro-2-((3,5-dinitrobenzyl)thio)quinazolin-4(3H)-one (**50c**).* Following General Procedure B, **49c** (0.11 g, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **13a** (0.12 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **50c** (eluent system: 15% EA in toluene, 70 mg, 0.18 mmol, 40% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.69 (br. s., 2H, CH_2), 7.53−7.76 (m, 1H, quinazolinone), 7.77−7.90 (m, 1H, quinazolinone), 7.93 (br. s., 1H, quinazolinone), 8.68 (br. s., 1H, Ph), 8.89 (br. s., 2H, Ph), 12.84 (br. s., 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 32.2 (1C, CH_2), 117.3 (1C, Ph), 121.3 (1C, quinazolinone), 125.0 (1C, quinazolinone), 128.0 (1C, quinazolinone), 130.0 (1C, quinazolinone), 130.1 (2C, Ph), 134.8 (1C, quinazolinone), 142.9 (1C, Ph), 146.8 (1C, quinazolinone), 147.7 (2C, Ph), 155.4 (1C, quinazolinone), 160.1 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{15}H_9ClN_4O_5S + H]^+$ 393.0055, found 393.0063.

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*8-Chloro-2-((3,5-dinitrobenzyl)thio)quinazolin-4(3H)-one (**50d**).* Following General Procedure B, **49d** (0.11 g, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **13a** (0.12 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **50d** (eluent system: 15% EA in toluene, 0.12 g, 0.31 mmol, 68% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.72 (s, 2H, CH_2), 7.39 (t, J = 7.9 Hz, 1H, quinazolinone), 7.95 (ddd, J = 9.2, 7.9, 1.4 Hz, 2H, quinazolinone), 8.69 (t, J = 2.1 Hz, 1H, Ph), 8.94 (d, J = 2.1 Hz, 2H, Ph), 12.91 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 32.3 (1C, CH_2), 117.4 (1C, Ph), 121.7 (1C, quinazolinone), 125.1 (1C, quinazolinone), 126.1 (1C, quinazolinone), 129.3 (1C, quinazolinone), 129.7 (2C, Ph), 134.7 (1C, quinazolinone), 143.2 (1C, Ph), 144.5 (1C, quinazolinone), 147.8 (2C, Ph), 156.2 (1C, quinazolinone), 160.6 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{15}H_9ClN_4O_5S + H]^+$ 393.0054, found 393.0063.

*6-Bromo-2-((3,5-dinitrobenzyl)thio)quinazolin-4(3H)-one (**50e**).* Following General Procedure B, **49e** (0.13 g, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **13a** (0.12 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **50e** (eluent system: 15% EA in toluene, 0.14 g, 0.32 mmol, 63% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.69 (s, 2H, CH_2), 7.58 (d, J = 8.8 Hz, 1H, quinazolinone), 7.97 (dd, J = 8.7, 2.4 Hz, 1H, quinazolinone), 8.06 (d, J = 2.4 Hz, 1H, quinazolinone), 8.68 (t, J = 2.1 Hz, 1H, Ph), 8.89 (d, J = 2.1 Hz, 2H, Ph), 12.84 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 32.2 (1C, CH_2), 117.3 (1C, Ph), 118.1 (1C, quinazolinone), 121.7 (1C, quinazolinone), 128.1 (1C, quinazolinone), 128.2 (1C, quinazolinone), 130.1 (2C, Ph), 137.5 (1C, quinazolinone), 142.9 (1C, Ph), 147.1 (1C, quinazolinone), 147.7 (2C, Ph), 155.5 (1C, quinazolinone), 159.9 (1C, CO). HRMS (ESI): m/z $[M - H]^-$ calcd for $[C_{15}H_9BrN_4O_5S - H]^-$ 434.9404, found 434.9408.

*2-((3,5-Dinitrobenzyl)thio)-6-methylquinazolin-4(3H)-one (**50f**).* Following General Procedure B, **49f** (96 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **13a** (0.12 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **50f** (eluent system: 15% EA in toluene, 86 mg, 0.23 mmol, 51% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.40 (s, 3H, CH_3), 4.67 (s, 2H, CH_2), 7.55 (d, J = 8.3 Hz, 1H, quinazolinone), 7.64 (dd, J = 8.3, 1.8 Hz, 1H, quinazolinone), 7.80 (s, 1H, quinazolinone), 8.67 (t, J = 2.1 Hz, 1H, Ph), 8.89 (d, J = 2.0 Hz, 2H, Ph), 12.56 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 20.7 (1C, CH_3), 32.1 (1C, CH_2), 117.3 (1C, Ph), 119.7 (1C, quinazolinone), 125.4 (1C, quinazolinone), 125.7 (1C, quinazolinone), 130.1 (2C, Ph), 135.6 (1C, quinazolinone), 135.9 (1C, quinazolinone), 143.1 (1C, Ph), 146.2 (1C, quinazolinone), 147.6 (2C, Ph), 153.4 (1C, quinazolinone), 161.0 (1C, CO). HRMS (ESI): m/z $[M - H]^-$ calcd for $[C_{16}H_{12}N_4O_5S - H]^-$ 371.0455, found 371.0458.

*2-((3,5-Dinitrobenzyl)thio)-6-fluoroquinazolin-4(3H)-one (**50g**).* Following General Procedure B, **49g** (98 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **13a** (0.12 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **50g** (eluent system: 15% EA in toluene, 0.13 g, 0.35 mmol, 78% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.68 (s, 2H, CH_2), 7.65−7.76 (m, 3H, quinazolinone), 8.68 (t, J = 2.1 Hz, 1H, Ph), 8.90 (d, J = 2.0 Hz, 2H, Ph), 12.78 (s, 1H, NH). ^{19}F NMR (377 MHz, DMSO-d_6) δ ppm -114.34 (s, 1 F). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 32.2 (1C, CH_2), 110.8 (d, J = 23.3 Hz, 1C, quinazolinone), 117.3 (1C, Ph), 121.2 (d, J = 9.0 Hz, 1C, quinazolinone), 123.2 (d, J = 23.6 Hz, 1C, quinazolinone), 128.5 (d, J = 7.9 Hz, 1C, quinazolinone), 130.1 (2C, Ph), 143.0 (1C, Ph), 145.04 (1C, quinazolinone), 147.7 (2C, Ph), 154.1 (1C, quinazolinone), 158.2 (1C, CO), 160.6 (d, J = 16.9 Hz, 1C, quinazolinone). HRMS (ESI): m/z $[M - H]^-$ calcd for $[C_{15}H_9FN_4O_5S - H]^-$ 375.0205, found 375.0211.

*2-((3,5-Dinitrobenzyl)thio)-6-nitroquinazolin-4(3H)-one (**50h**).* Following General Procedure B, **49h** (0.11 g, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **13a** (0.12 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **50h** (eluent system: 15% EA in toluene, 0.11 g, 0.28 mmol, 63% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.74 (s, 2H, CH_2), 7.80 (d, J = 9.0 Hz, 1H, quinazolinone), 8.58 (dd, J = 8.9, 2.7 Hz, 1H, quinazolinone), 8.66−8.74 (m, 2H, quinazolinone, Ph), 8.91 (d, J = 2.1 Hz, 2H, Ph), 13.15 (br. s., 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 32.4 (1C, CH_2), 117.4 (1C, Ph), 120.1 (1C, quinazolinone), 122.3 (1C, quinazolinone), 128.2 (1C, quinazolinone), 128.9 (1C, quinazolinone), 130.1 (2C, Ph), 142.7 (1C, Ph), 144.1 (1C, quinazolinone), 147.7 (2C, Ph), 152.0 (1C, quinazolinone), 159.6 (1C, quinazolinone), 160.2 (1C, CO). HRMS (ESI): m/z $[M - H]^-$ calcd for $[C_{15}H_9N_5O_7S - H]^-$ 402.0150, found 402.0148.

*2-Thioxo-2,3-dihydropyrido[3,2-d]pyrimidin-4(1H)-one (**55a**).* According to a literature report,[20,28] a mixture of 3-aminopicolinic acid (0.83 g, 6.0 mmol) and thiourea (2.3 g, 30 mmol) was stirred at 200 °C for 2 h. After cooling to 40 °C, water (50 mL) was added to the reaction mixture and the resulting mixture was stirred at room temperature overnight. After filtration, the filtrate was collected and concentrated to give crude **55a**, which was used in the next step without purification.

*2-Thioxo-2,3-dihydropyrido[4,3-d]pyrimidin-4(1H)-one (**55b**).* To a solution of 4-aminonicotinic acid (0.83 g, 6.0 mmol) in THF/dioxane (1/1 v/v, 20 mL) were added $SOCl_2$ (1.0 mL, 21 mmol) and catalytic DMF (8.0 μL), and the resulting mixture was stirred at room temperature for 3 h.[46] After completion of acyl chloride formation, a solution of NH_4SCN (0.53 g, 6.9 mmol) in acetone (15 mL) and dioxane (10 mL) was added to the reaction mixture. The resulting mixture was stirred at 80 °C for 2 h and filtered when it was hot. The filter cake was washed with CH_2Cl_2 (20 mL) and dried to give **55b**, which was used in the next step without purification.

*2-Thioxo-2,3-dihydropyrido[3,4-d]pyrimidin-4(1H)-one (**55c**).* Following the procedure described for **55a**, 3-aminoisonicotinic acid (0.83 g, 6.0 mmol) and thiourea (2.3 g, 30 mmol) afforded **55c**, which was in the filter cake.

*2-Thioxo-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (**55d**).* Following the procedure described for **55a**, 2-aminonicotinic acid (0.83 g, 6.0 mmol) and thiourea (2.3 g, 30 mmol) afforded **55d**, which was in the filter cake.

*2-((3,5-Dinitrobenzyl)thio)pyrido[3,2-d]pyrimidin-4(3H)-one (**56a**).* Following General Procedure B, **55a** (90 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **13a** (0.12 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **56a** (eluent system: 5% MeOH in CH_2Cl_2, 79 mg, 0.22 mmol, 49% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.70 (s, 2H, CH_2), 7.81 (dd, J = 8.3, 4.3 Hz, 1H, pyridyl), 8.02 (d, J = 7.5 Hz, 1H, pyridyl), 8.62−8.75 (m, 2H, pyridyl, Ph), 8.90 (d, J = 1.8 Hz, 2H, Ph), 12.93 (br. s., 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 32.4 (1C, CH_2), 117.3 (1C, Ph), 129.0 (1C, pyridyl), 130.1 (2C, Ph), 133.9 (1C, pyridyl), 137.1 (1C, pyridyl), 142.9 (1C, Ph), 145.1 (1C, pyridyl), 147.7 (2C, Ph), 148.2 (1C, pyridyl), 155.6 (1C, NCS), 159.6 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{14}H_9N_5O_5S + H]^+$ 360.0397, found 360.0395.

*2-((3,5-Dinitrobenzyl)thio)pyrido[4,3-d]pyrimidin-4(3H)-one (**56b**).* Following General Procedure B, **55b** (90 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **13a** (0.12 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **56b** (eluent system: 5% MeOH in CH_2Cl_2, 44 mg, 0.12 mmol, 27% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.72 (s, 2H, CH_2), 7.50 (d, J = 5.6 Hz, 1H, pyridyl), 8.69 (t, J = 2.1 Hz, 1H, Ph), 8.81 (d, J = 5.6 Hz, 1H, pyridyl), 8.90 (d, J = 2.0 Hz, 2H, Ph), 9.14 (s, 1H, pyridyl), 13.02 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 32.4 (1C, CH_2), 115.9 (1C, pyridyl), 117.4 (1C, Ph), 119.2 (1C, pyridyl), 130.1 (2C, Ph), 142.6 (1C, Ph), 147.7 (2C, Ph), 149.4 (1C, pyridyl), 152.8 (1C, pyridyl), 153.6 (1C, pyridyl), 160.3 (1C, NCS), C (CO) could not be observed. HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{14}H_9N_5O_5S + H]^+$ 360.0397, found 360.0403.

*2-((3,5-Dinitrobenzyl)thio)pyrido[3,4-d]pyrimidin-4(3H)-one (**56c**).* Following General Procedure B, **55c** (90 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **13a** (0.12 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded crude **56c**. After filtration, the solid was suspended in DMSO (2.0 mL) and the suspension was stirred at room temperature for 0.5 h. **56c** was obtained through filtration (91 mg, 0.25 mmol, 56% yield). ^{1}H NMR (400 MHz, trifluoroacetic acid-*d*) δ ppm 4.74 (s, 2H, CH_2), 8.61−8.75 (m, 2H, pyridyl), 8.82 (s, 2H, Ph), 8.93 (br. s., 1H, Ph), 9.36 (s, 1H, pyridyl). ^{13}C NMR (101 MHz, trifluoroacetic acid-*d*) δ ppm 34.2 (1C, CH_2), 119.0 (1C, Ph), 126.1 (1C, pyridyl), 130.8 (2C, Ph), 132.9 (1C, pyridyl), 136.5 (1C, pyridyl), 141.8 (1C, Ph), 143.8 (1C, pyridyl), 146.9 (1C, pyridyl), 149.2 (2C, Ph), 160.9 (1C, NCS), 163.8 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{14}H_9N_5O_5S + H]^+$ 360.0397, found 360.0394.

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*2-((3,5-Dinitrobenzyl)thio)pyrido[2,3-d]pyrimidin-4(3H)-one (**56d**).* Following General Procedure B, **55d** (90 mg, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **13a** (0.12 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **56d** (eluent system: 5% MeOH in CH_2Cl_2, 0.12 g, 0.33 mmol, 72% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.77 (s, 2H, CH_2), 7.46 (dd, J = 7.9, 4.6 Hz, 1H, pyridyl), 8.40 (dd, J = 7.9, 2.0 Hz, 1H, pyridyl), 8.70 (t, J = 2.1 Hz, 1H, Ph), 8.86−8.93 (m, 3H, pyridyl, Ph), 12.98 (br. s., 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 32.1 (1C, CH_2), 115.3 (1C, pyridyl), 117.4 (1C, Ph), 121.7 (1C, pyridyl), 129.8 (2C, Ph), 135.7 (1C, pyridyl), 142.8 (1C, Ph), 147.8 (2C, Ph), 155.8 (1C, pyridyl), 157.7 (1C, pyridyl), 158.8 (1C, NCS), 161.8 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{14}H_9N_5O_5S + H]^+$ 360.0397, found 360.0402.

*5-Fluoro-2-nitrobenzoic Acid (**58**).* According to a literature report,[29] to a solution of 3-fluorobenzoic acid (0.79 g, 5.6 mmol) in sulfuric acid (5.0 mL) at 0 °C was added fuming nitric acid (0.31 mL, 6.7 mmol). The resulting mixture was stirred at room temperature for 1 h. Then, the solution was poured to water (75 mL) and extracted with EA (0.15 L × 2). The combined organic layers were dried and concentrated to give **58** without purification (0.79 g, 4.3 mmol, 76% yield). ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 7.45 (ddd, J = 9.0, 7.7, 2.8 Hz, 1H, Ph), 7.55 (dd, J = 8.3, 2.8 Hz, 1H, Ph), 8.03 (dd, J = 9.0, 4.6 Hz, 1H, Ph). ^{19}F NMR (377 MHz, methanol-d_4) δ ppm -106.2 (s, 1 F). ^{13}C NMR (101 MHz, methanol-d_4) δ ppm 118.1 (d, J = 23.5 Hz, 1C, Ph), 119.7 (d, J = 23.5 Hz, 1C, Ph), 128.2 (d, J = 9.3 Hz, 1C, Ph), 132.6 (d, J = 9.3 Hz, 1C, Ph), 146.1 (1C, Ph), 165.7 (d, J = 254.2 Hz, 1C, Ph), 167.2 (1C, CO).

*5-Fluoro-2-nitrobenzamide (**59**).* **58** (2.0 g, 11 mmol) was refluxed in thionyl chloride (10 mL) for 2 h. The excess thionyl chloride was removed in vacuo. The crude acyl chloride was dissolved in CH_2Cl_2 (25 mL), followed by addition of NH_3 (excess) through a balloon. The precipitate was filtered to afford **59** without purification (1.2 g, 6.5 mmol, 60% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.50−7.58 (m, 2H, Ph), 7.80 (br. s., 1H, NH_2), 8.13 (dd, J = 9.8, 4.8 Hz, 1H, Ph), 8.19 (br. s., 1H, NH_2). ^{19}F NMR (377 MHz, DMSO-d_6) δ ppm -104.3 (s, 1 F). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 116.2 (d, J = 24.8 Hz, 1C, Ph), 117.3 (d, J = 24.8 Hz, 1C, Ph), 127.3 (d, J = 11.0 Hz, 1C, Ph), 135.7 (d, J = 8.8 Hz, 1C, Ph), 143.3 (1C, Ph), 163.7 (d, J = 254.8 Hz, 1C, Ph), 165.4 (1C, CO).

*5-(Dimethylamino)-2-nitrobenzamide (**60a**).* According to a literature report,[47] to a solution of **59** (0.50 g, 2.7 mmol) and K_2CO_3 (0.79 g, 5.7 mmol) in DMSO (2.0 mL) at 60 °C was added dimethylamine hydrochloride (0.27 g, 3.3 mmol). After 1 h, the reaction was quenched with water (0.10 L). The resulting mixture was extracted with EA (0.10 L × 2), and the combined organic layers were dried, concentrated, and purified to afford **60a** (eluent system: 25% EA in petroleum ether, 0.30 g, 1.4 mmol, 52% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.08 (s, 6H, $N(CH_3)_2$), 6.58 (d, J = 2.9 Hz, 1H, Ph), 6.75 (dd, J = 9.4, 2.9 Hz, 1H, Ph), 7.49 (br. s., 1H, NH_2), 7.85 (br. s., 1H, NH_2), 7.96 (d, J = 9.4 Hz, 1H, Ph). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 39.8 (2C, $N(CH_3)_2$), 109.8 (1C, Ph), 110.5 (1C, Ph), 126.7 (1C, Ph), 133.0 (1C, Ph), 136.8 (1C, Ph), 153.3 (1C, Ph), 168.8 (1C, CO).

*2-Nitro-5-(piperidin-1-yl)benzamide (**60b**).* Following the procedure described for **60a**, **59** (0.50 g, 2.7 mmol), K_2CO_3 (0.56 g, 4.1 mmol), and piperidine (0.32 mL, 3.3 mmol) in DMSO (2.0 mL) afforded **60b** (eluent system: 25% EA in petroleum ether, 0.58 g, 2.3 mmol, 86% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.53−1.67 (m, 6H, piperidyl), 3.44−3.52 (m, 4H, piperidyl), 6.80 (d, J = 2.9 Hz, 1H, Ph), 6.97 (dd, J = 9.5, 2.9 Hz, 1H, Ph), 7.48 (s, 1H, NH_2), 7.84 (s, 1H, NH_2), 7.93 (d, J = 9.4 Hz, 1H, Ph). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 23.8 (1C, piperidyl), 24.9 (2C, piperidyl), 47.6 (2C, piperidyl), 111.5 (1C, Ph), 112.2 (1C, Ph), 126.9 (1C, Ph), 133.6 (1C, Ph), 136.9 (1C, Ph), 153.4 (1C, Ph), 168.6 (1C, CO).

*5-Morpholino-2-nitrobenzamide (**60c**).* Following the procedure described for **60a**, **59** (0.50 g, 2.7 mmol), K_2CO_3 (0.56 g, 4.1 mmol), and morpholine (0.29 mL, 3.3 mmol) in DMSO (2.0 mL) afforded **60c** (eluent system: 5% MeOH in CH_2Cl_2, 0.51 g, 2.0 mmol, 75% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.36−3.44 (m, 4H, morpholinyl), 3.66−3.76 (m, 4H, morpholinyl), 6.89 (d, J = 2.8 Hz, 1H, Ph), 7.02 (dd, J = 9.4, 2.9 Hz, 1H, Ph), 7.52 (br. s., 1H, NH_2), 7.87 (br. s., 1H, NH_2), 7.97 (d, J = 9.4 Hz, 1H, Ph). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 46.5 (2C, morpholinyl), 65.7 (2C, morpholinyl), 111.9 (1C, Ph), 112.5 (1C, Ph), 126.5 (1C, Ph), 135.1 (1C, Ph), 136.4 (1C, Ph), 153.8 (1C, Ph), 168.4 (1C, CO).

*5-(4-Methylpiperazin-1-yl)-2-nitrobenzamide (**60d**).* Following the procedure described for **60a**, **59** (0.50 g, 2.7 mmol), K_2CO_3 (0.56 g, 4.1 mmol), and N-methylpiperazine (0.36 mL, 3.3 mmol) in DMSO (2.0 mL) afforded **60d** (eluent system: 15% MeOH in CH_2Cl_2, 0.56 g, 2.1 mmol, 78% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.21 (s, 3H, CH_3), 2.34−2.46 (m, 4H, piperazinyl), 3.29−3.48 (m, 4H, piperazinyl), 4.85 (s, 2H, NH_2), 6.85 (d, J = 2.0 Hz, 1H, Ph), 6.93 (dd, J = 9.3, 2.3 Hz, 1H, Ph), 7.84 (d, J = 9.3 Hz, 1H, Ph). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 45.6 (1C, CH_3), 46.4 (2C, piperazinyl), 54.2 (2C, piperazinyl), 112.2 (1C, Ph), 112.3 (1C, Ph), 126.2 (1C, Ph), 135.4 (1C, Ph), 138.5 (1C, Ph), 153.5 (1C, Ph), 169.3 (1C, CO).

*6-(Dimethylamino)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (**62a**).* To a stirred solution of **60a** (0.29 g, 1.4 mmol) in EtOH (40 mL) was added Pd−C (10%) under N_2. H_2 gas was introduced to the reaction mixture using a double-folded balloon. Upon completion, the reaction mixture was filtered through a Celite pad and the filter cake was washed with MeOH (20 mL). The filtrate was concentrated to give **61a** without purification (0.23 g, 1.3 mmol, 93% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.73 (s, 6H, $N(CH_3)_2$), 5.88 (s, 2H, NH_2), 6.61 (d, J = 8.8 Hz, 1H, Ph), 6.80 (dd, J = 8.8, 2.8 Hz, 1H, Ph), 6.91 (d, J = 2.8 Hz, 1H, Ph), 7.01 (s, 1H, NH_2), 7.74 (s, 1H, NH_2). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 41.9 (2C, $N(CH_3)_2$), 113.7 (1C, Ph), 114.7 (1C, Ph), 117.6 (1C, Ph), 120.1 (1C, Ph), 141.6 (1C, Ph), 142.3 (1C, Ph), 171.5 (1C, CO). According to a literature report,[30] KOH (0.24 g, 4.3 mmol) was suspended in EtOH (10 mL) and the suspension was stirred at room temperature for 0.5 h to dissolve the KOH. Then, CS_2 (0.26 mL, 4.3 mmol) was added to the stirred solution and the resulting mixture was stirred for another 0.5 h to produce a yellow suspension. Then, **61a** (0.51 g, 2.9 mmol), EtOH (5.0 mL), and water (0.40 mL) were added to the mixture in succession. The reaction mixture was refluxed overnight. After cooling to room temperature, the volatile material was removed in vacuo. The mixture was adjusted to pH 6−7 with 1 N aq HCl. The precipitate was collected through filtration and dried over an oil pump to give **62a** without purification.

*6-(Piperidin-1-yl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (**62b**).* Following the procedure described for **62a**, **60b** (0.57 g, 2.3 mmol) and Pd−C (10%) in EtOH (40 mL) under H_2 afforded **61b** (0.48 g, 2.2 mmol, 96% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.39−1.54 (m, 2H, piperidyl), 1.61 (q, J = 5.6 Hz, 4H, piperidyl), 2.82−3.02 (m, 4H, piperidyl), 6.05 (br. s., 2H, NH_2), 6.59 (d, J = 8.8 Hz, 1H, Ph), 6.79−6.94 (m, 1H, Ph), 6.98 (br. s., 1H, NH_2), 7.06 (br. s., 1H, Ph), 7.72 (br. s., 1H, NH_2). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 23.8 (1C, piperidyl), 25.7 (2C, piperidyl), 51.9 (2C, piperidyl), 114.2 (1C, Ph), 117.2 (2C, Ph), 123.3 (1C, Ph), 142.2 (1C, Ph), 144.1 (1C, Ph), 171.4 (1C, CO). Then, KOH (0.18 g, 3.2 mmol), CS_2 (0.19 mL, 3.2 mmol) and **61b** (0.47 g, 2.1 mmol) in EtOH/water (15 mL/0.3 mL) gave **62b** without purification.

*6-Morpholino-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (**62c**).* Following the procedure described for **62a**, **60c** (0.51 g, 2.0 mmol) and Pd−C (10%) in EtOH (40 mL) under H_2 afforded **61c** (0.30 g, 1.4 mmol, 67% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.89−3.00 (m, 4H, morpholinyl), 3.68−3.74 (m, 4H, morpholinyl), 6.08 (s, 2H, NH_2), 6.62 (d, J = 8.9 Hz, 1H, Ph), 6.91 (dd, J = 8.8, 2.7 Hz, 1H, Ph), 7.01 (br. s., 1H, NH_2), 7.05 (d, J = 2.8 Hz, 1H, Ph), 7.74 (br. s., 1H, NH_2). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 50.6 (2C, morpholinyl), 66.3 (2C, morpholinyl), 114.1 (1C, Ph), 116.1 (1C, Ph), 117.4 (1C, Ph), 122.3 (1C, Ph), 141.0 (1C, Ph), 144.3 (1C, Ph), 171.3 (1C, CO). Then, KOH (0.11 g, 2.0 mmol), CS_2 (0.12 mL, 2.0 mmol) and **61c** (0.29 g, 1.3 mmol) in EtOH/water (15 mL/0.20 mL) gave **62c** without purification.

*6-(4-Methylpiperazin-1-yl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (**62d**).* Following the procedure described for **62a**, **60d** (0.55 g, 2.1 mmol) and Pd−C (10%) in EtOH (40 mL) under H_2 afforded **61d** (0.45 g, 1.9 mmol, 92% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.20 (br. s., 3H, CH_3), 2.43 (br. s., 4H, piperazinyl), 2.85−3.02 (m,

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4H, piperazinyl), 6.06 (br. s., 2H, NH_2), 6.61 (d, J = 8.6 Hz, 1H, Ph), 6.89 (d, J = 8.1 Hz, 1H, Ph), 7.00 (br. s., 1H, NH_2), 7.05 (br. s., 1H, Ph), 7.76 (br. s., 1H, NH_2). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 45.8 (1C, CH_3), 50.1 (2C, piperazinyl), 54.9 (2C, piperazinyl), 114.2 (1C, Ph), 116.3 (1C, Ph), 117.3 (1C, Ph), 122.5 (1C, Ph), 141.1 (1C, Ph), 144.1 (1C, Ph), 171.4 (1C, CO). Then, KOH (0.15 g, 2.8 mmol), CS_2 (0.17 mL, 2.8 mmol) and **61d** (0.43 g, 1.8 mmol) in EtOH/water (15 mL/0.30 mL) gave **62d** without purification.

*6-(Dimethylamino)-2-((3,5-dinitrobenzyl)thio)quinazolin-4(3H)-one (**63a**).* Following General Procedure B, **62a** (0.11 g, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **13a** (0.12 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **63a** (eluent system: 20% EA in toluene, 0.18 g, 0.44 mmol, 98% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.97 (s, 6H, $N(CH_3)_2$), 4.64 (s, 2H, CH_2), 7.08 (d, J = 3.0 Hz, 1H, quinazolinone), 7.31 (dd, J = 9.0, 3.0 Hz, 1H, quinazolinone), 7.51 (d, J = 9.0 Hz, 1H, quinazolinone), 8.66 (t, J = 2.1 Hz, 1H, Ph), 8.88 (d, J = 2.1 Hz, 2H, Ph), 12.37 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 32.0 (1C, CH_2), 40.1 (2C, $N(CH_3)_2$), 105.0 (1C, quinazolinone), 117.2 (1C, Ph), 120.6 (1C, quinazolinone), 120.6 (1C, quinazolinone), 126.7 (1C, quinazolinone), 130.0 (2C, Ph), 139.4 (1C, quinazolinone), 143.4 (1C, Ph), 147.6 (2C, Ph), 148.4 (1C, quinazolinone), 148.9 (1C, quinazolinone), 161.3 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{17}H_{15}N_5O_5S + H]^+$ 402.0867, found 402.0869.

*2-((3,5-Dinitrobenzyl)thio)-6-(piperidin-1-yl)quinazolin-4(3H)-one (**63b**).* Following General Procedure B, **62b** (0.13 g, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **13a** (0.12 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **63b** (eluent system: 15% EA in toluene, 0.18 g, 0.41 mmol, 91% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.55 (d, J = 4.4 Hz, 2H, piperidyl), 1.59−1.70 (m, 4H, piperidyl), 3.12−3.28 (m, 4H, piperidyl), 4.64 (s, 2H, CH_2), 7.28 (d, J = 1.8 Hz, 1H, quinazolinone), 7.45−7.57 (m, 2H, quinazolinone), 8.66 (t, J = 2.0 Hz, 1H, Ph), 8.87 (d, J = 2.0 Hz, 2H, Ph), 12.42 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 23.7 (1C, piperidyl), 25.0 (2C, piperidyl), 32.1 (1C, CH_2), 49.2 (2C, piperidyl), 108.3 (1C, quinazolinone), 117.2 (1C, Ph), 120.5 (1C, quinazolinone), 124.3 (1C, quinazolinone), 126.6 (1C, quinazolinone), 130.0 (2C, Ph), 140.9 (1C, quinazolinone), 143.3 (1C, Ph), 147.6 (2C, Ph), 149.4 (1C, quinazolinone), 150.2 (1C, quinazolinone), 161.2 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{20}H_{19}N_5O_5S + H]^+$ 442.1180, found 442.1184.

*2-((3,5-Dinitrobenzyl)thio)-6-morpholinoquinazolin-4(3H)-one (**63c**).* Following General Procedure B, **62c** (0.13 g, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **13a** (0.12 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **63c** (eluent system: 5% MeOH in CH_2Cl_2, 0.18 g, 0.42 mmol, 92% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.18 (br. s., 4H, morpholinyl), 3.75 (br. s., 4H, morpholinyl), 4.65 (br. s., 2H, CH_2), 7.31 (br. s., 1H, quinazolinone), 7.55 (br. s., 2H, quinazolinone), 8.67 (br. s., 1H, Ph), 8.89 (br. s., 2H, Ph), 12.47 (br. s., 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 32.1 (1C, CH_2), 48.2 (2C, morpholinyl), 66.0 (2C, morpholinyl), 108.1 (1C, quinazolinone), 117.3 (1C, Ph), 120.5 (1C, quinazolinone), 123.6 (1C, quinazolinone), 126.7 (1C, quinazolinone), 130.1 (2C, Ph), 141.5 (1C, quinazolinone), 143.3 (1C, Ph), 147.6 (2C, Ph), 149.0 (1C, quinazolinone), 150.7 (1C, quinazolinone), 161.1 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{19}H_{17}N_5O_6S + H]^+$ 444.0973, found 444.0966.

*2-((3,5-Dinitrobenzyl)thio)-6-(4-methylpiperazin-1-yl)-quinazolin-4(3H)-one (**63d**).* Following General Procedure B, **62d** (0.14 g, 0.50 mmol), K_2CO_3 (0.21 g, 1.5 mmol), and **13a** (0.12 g, 0.45 mmol) in DMSO/MeCN (1/10 v/v, 11 mL) afforded **63d** (eluent system: 5% MeOH in CH_2Cl_2, 93 mg, 0.20 mmol, 45% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.24 (s, 3H, CH_3), 2.41−2.59 (m, 4H, piperazinyl), 3.18−3.23 (m, 4H, piperazinyl), 4.64 (s, 2H, CH_2), 7.29 (d, J = 2.3 Hz, 1H, quinazolinone), 7.47−7.58 (m, 2H, quinazolinone), 8.66 (t, J = 2.1 Hz, 1H, Ph), 8.88 (d, J = 2.1 Hz, 2H, Ph), 12.01−12.81 (m, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 32.1 (1C, CH_2), 45.6 (1C, CH_3), 47.8 (2C, piperazinyl), 54.4 (2C, piperazinyl), 108.2 (1C, quinazolinone), 117.2 (1C, Ph), 120.4 (1C, quinazolinone), 123.9 (1C, quinazolinone), 126.6 (1C, quinazolinone), 130.1 (2C, Ph), 141.2 (1C, quinazolinone), 143.3 (1C, Ph), 147.6 (2C, Ph), 148.9 (1C, quinazolinone), 150.6 (1C, quinazolinone), 161.2 (1C, CO). HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{20}H_{20}N_6O_5S + H]^+$ 457.1289, found 457.1283.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c01374.

Determination of IC_{50} values of compounds **21**−**23**, **26**, **27**, **30**−**40**, **45**−**47**, **50b**, and **50c** (Table S1); antimycobacterial activity of compounds **21**−**23**, **28**−**33**, **37**, **39**−**46**, **50a**, **50d**, **56b**−**d**, **63a**, and **63b** under different media (Table S2); frequency of resistance for compound **26** and rifampicin (Table S3); MIC values of compound **26**, pretomanid, delamanid, isoniazid, and rifampicin against wild-type strain and mutants generated to compound **26** (Table S4); ^{1}H, ^{13}C, and ^{19}F NMR spectra of compounds **21**−**23**, **26**−**47**, **50a**−**50h**, **56a**−**56d**, and **63a**−**d** (Table S5); HSQC and HMBC spectra of compounds **46** and **47**; purity of final compounds (PDF)

Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

EA, ethyl acetate; ADC, albumin, dextrose, and catalase supplement; dTMP, thymidine monophosphate; Pks13, polyketide synthase 13

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