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Synthesis and Structure-Activity Relationship (SAR) Studies of Novel Pyrazolopyridine Derivatives as Inhibitors of Enterovirus Replication

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ABSTRACT: A series of novel pyrazolopyridine compounds have been designed and prepared by a general synthetic route. Their activities against the replication of poliovirus-1, EV-A71, and CV-B3 enteroviruses were evaluated. The comprehensive understanding of the Structure-Activity Relationship was obtained by utilizing the variation of four positions, namely N1, C6, C4 and linker unit. From the screened analogues, the inhibitors with the highest selectivity indices at 50% inhibition of viral replication (SI_{50}) were those with isopropyl at the N1 position and thiophenyl-2-yl unit at C6 position. Furthermore, the C4 position offered the greatest potential for improvement because many different N-aryl groups had better antiviral activities and compatibilities than the lead compound **JX001**. For example, **JX040** with a 2-pyridyl group was the analogue with the most potent activity against non-polio enteroviruses and **JX025**, possessing a 3-sulfamoylphenyl moiety, had the best activity against polioviruses. In addition,

analogue **JX037**, possessing a novel pyrazolopyridine heterocycle, was also shown to have good anti-enteroviral activity, which further enlarges the compound space for anti-enteroviral drug design.

■ INTRODUCTION

The human enteroviruses (EVs) are a group of more than 110 distinct viruses, each composed of a single stranded RNA genome packaged within a protein capsid. Enteroviruses were originally classified into groups of polioviruses, coxsackieviruses, echoviruses, and later simply assigned consecutive numbers as they were discovered.[1] Poliovirus circulation and poliomyelitis have been nearly eliminated by immunization but other EVs, now organized into alphabetically organized species[1], remain clinically and economically significant pathogens with global impact. For example, enterovirus 71 (EV-A71) has been the cause of numerous epidemics of central nervous system infections in Europe and the Asia-Pacific Region over the last 15 years,[2] causing an estimated 7 million cases in China between 2008 and 2012.[3] Widespread reports of coxsackievirus B1 (CV-B1) myocarditis in the United States in 2007 highlighted the epidemic potential of enteroviruses and their danger to infants.[4,5] Similarly, a nationwide outbreak of enterovirus D68 (EV-D68) occurred in the summer of 2014. Beginning with reports in the midwest United States, EV-D68 was linked to severe respiratory illness, most often in young children. EV-D68 was also detected in respiratory specimens of some patients with polio-like paralysis, meningitis, and encephalitis.[6-8] In addition, enteroviruses are perennial causes of encephalitis, acute heart failure, sepsis in newborns, and other serious and life-threatening illnesses.[1]

No antiviral agents are currently approved to treat enterovirus infections and supportive care is the mainstay of treatment.[1,9] Extensive studies in pursuit of candidate antiviral agents have targeted the viral capsid, the virus-encoded RNA polymerase and proteases, and other viral proteins involved in replication.[9,10] Candidates that have reached preclinical or early clinical phases of development have included the viral capsid binding agent BTA-798 (vapendavir), the viral protease inhibitor AG7088 (rupintrivir), and the viral 3D polymerase inhibitor DTriP-22.[9-11] Two drugs, enviroxime and pleconaril, were unable to move beyond initial clinical studies due to limited efficacy or safety concerns, respectively.[10,12] Faced with this lack of progress, several recent studies have been performed in hopes of repurposing medications found to have *in vitro* antiviral activity against enteroviruses. For example, the selective serotonin reuptake inhibitor fluoxetine was found to have modest anti-enteroviral activity, likely reflecting its interference with the activity of the viral 2C protein.[13,14,15]

We previously applied a rapid, live virus assay to identify enterovirus inhibitors from nearly 86,000 compounds held by the Molecular Screening Shared Resource (MSSR) core facility of the CNSI (California NanoSystems Institute) at UCLA.[13,15] Using a commonly encountered enterovirus, CV-B3), we identified a novel group of anti-enteroviral compounds: 1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide derivatives.[15] These compounds are structurally unlike (distinct from) any previously described inhibitors of EV replication[9,10] and also exhibit antiviral activity against an array of clinically relevant enterovirus types at micromolar concentrations.[15] To identify the target of these compounds, we selected for resistance by intentionally exposing the molecularly cloned CVB3-H3 virus to them at sub-inhibitory concentrations. This resistance was genetically mapped to the coding domain for the viral 2C protein. Based on these data, we constructed a missense mutant, CVB3-H3-C179F. This virus replicated normally and was not

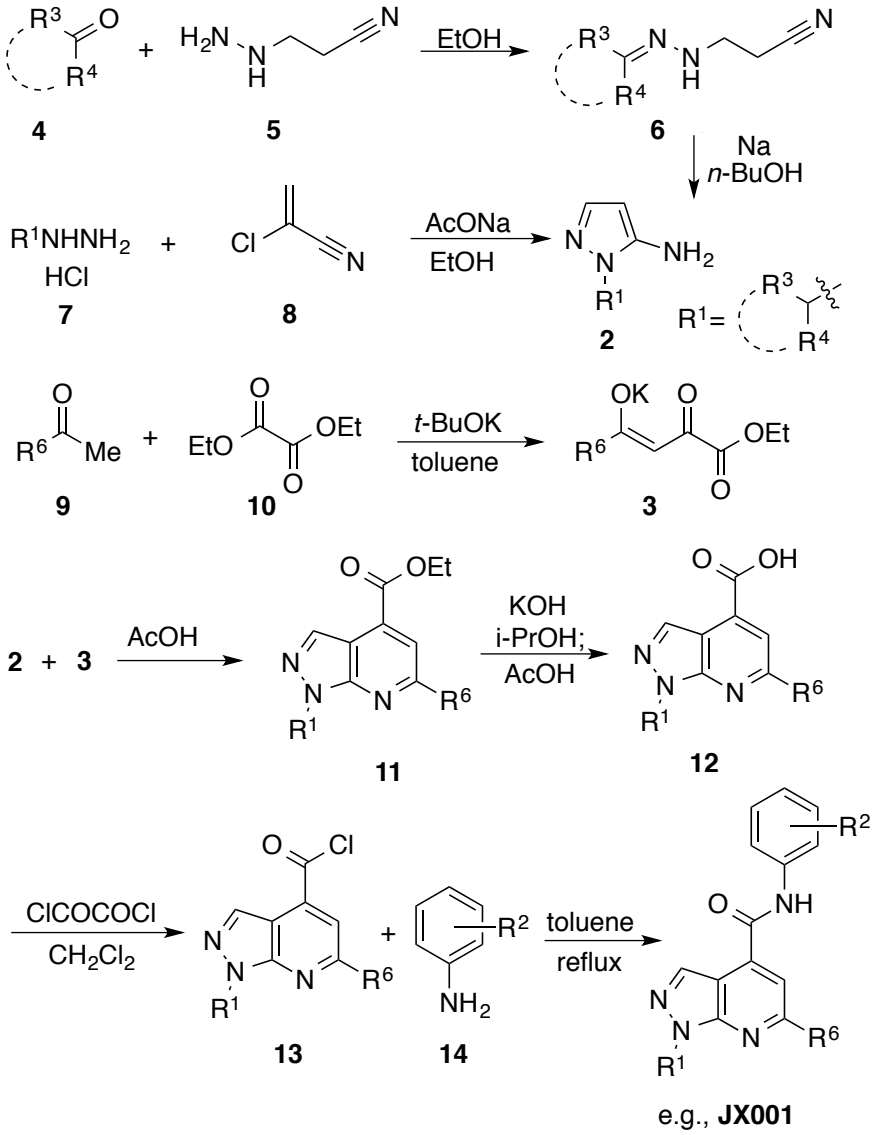
inhibited by these compounds, indicating that they interfere with the activities of the viral 2C protein, which plays a role in viral RNA replication and other processes.[13-15]

We herein describe an extensive structure-activity relationship (SAR) study of a series of pyrazolopyridine carboxamides and their structural analogues. We have developed a simple synthetic route to these compounds which allows one to prepare many analogues rapidly. All of these new compounds have been tested for their ability to inhibit the growth of 3 enteroviruses representing the major species of enteroviruses that infect humans. We have identified compounds with higher antiviral activity and lower cytotoxicity in vitro than the original lead compounds described previously.[15]

■ RESULTS AND DISCUSSION

Chemistry. The synthesis of these molecules, which were labeled **JX001** to **JX076**, was carried out as shown in the various schemes. The synthesis of the 1*H*-pyrazolo[3,4-*b*]pyridines are shown in Scheme 1. The key step was the condensation of the 1-alkyl-pyrazole-5-amine **2** with the 4-aryl-2,4-diketoester **3** to give the 1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxylic ester **11**. The components for this key coupling reaction were prepared as follows. Formation of the hydrazone **6** was easily accomplished by mixing the desired ketone **4** with 3-hydrazino-propanitrile **5**. Reaction of **6** with sodium butylate, prepared in situ, gave the desired 1-alkyl-pyrazole-5-amine **2**.[16] This compound could also be prepared by another route, namely reaction of the alkyl hydrazine hydrochloride salt **7** with commercially available 2-cholorpropenenitrile **8** to give **2**.[17] The second component **3** was synthesized by the condensation of an alkyl or aryl methyl ketone **9** with diethyl oxalate **10** to give the product of the Claisen condensation, the salt **3**.[18] Addition of **2** and **3** in acetic acid afforded good yields of the desired heterocycle **11**.[19] Basic hydrolysis of

the ester of **11** gave the acid **12**. Formation of the acid chloride **13** with oxalyl chloride was followed by addition of the desired aniline **14** to give the amides. These were labeled as **JX** compounds starting with **JX001**. Overall a total of 76 analogues in all series were prepared and tested.

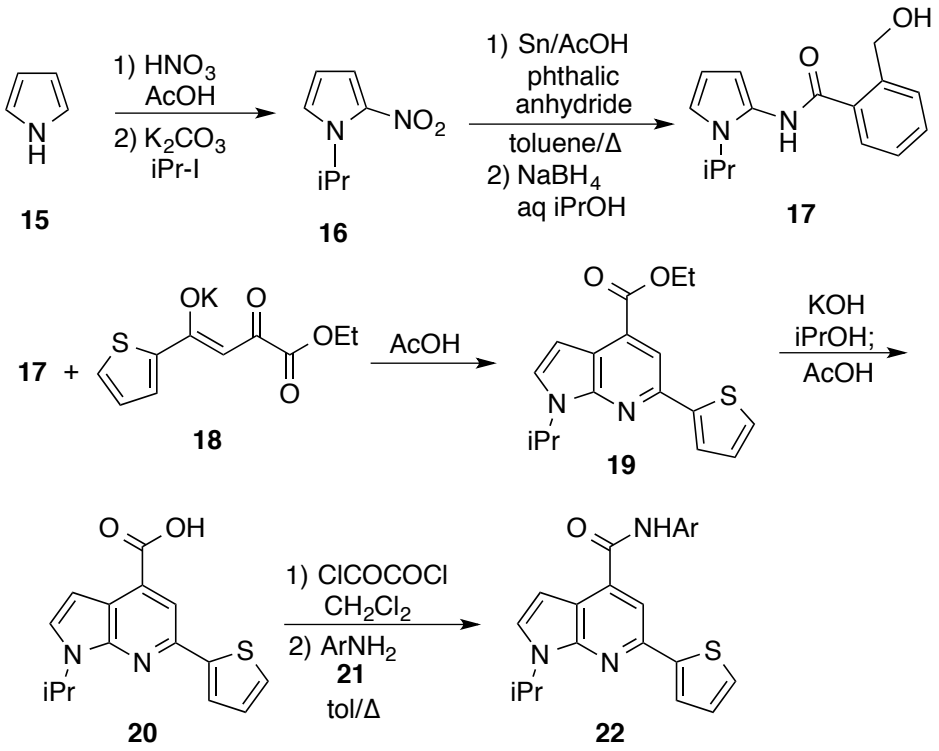


Scheme 1. Synthesis of 1*H*-Pyrazolo[3,4-*b*]pyridine-4-carboxamides, e.g., **JX001**.

In general, the yields of the synthesis were quite good and reasonable quantities of the materials were easily available. Although this general synthetic route was used for the synthesis of most of

the analogues, other methods could be used for specific substitution patterns and will be given in the experimental section. The compounds were purified by normal synthetic medicinal chemistry means, usually column chromatography and their structures were determined by high field NMR spectroscopy.

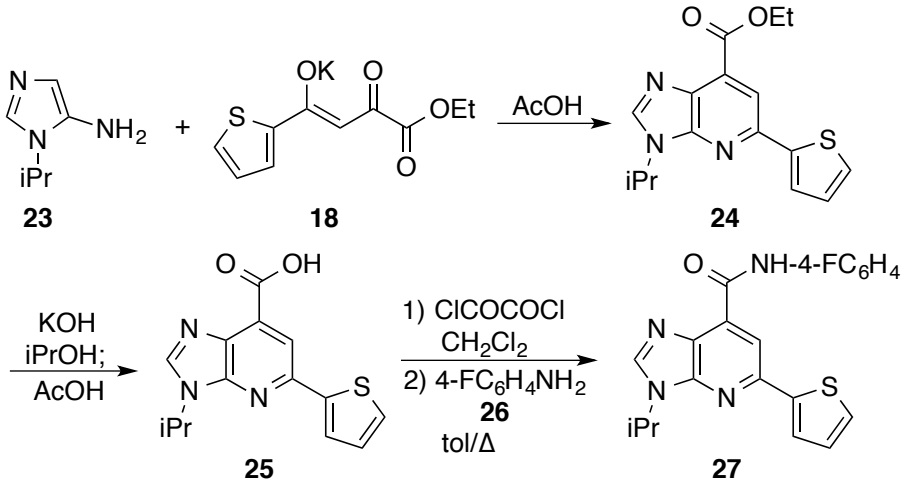
In addition, we also prepared several ring systems different from but similar to the 1*H*-pyrazolo[3,4-*b*]pyridine system by an analogous chemical synthesis, as shown in Scheme 2. Thus several 1*H*-pyrrolo[2,3-*b*]pyridine-4-carboxamides **22** were prepared by a route that involved a protected form of 2-aminopyrrole, namely the 2-(hydroxymethyl)benzamide **17**. This compound was prepared from pyrrole itself in four steps, namely nitration and isopropylation of **15** to give the 2-nitropyrrole **16** followed by tin reduction in the presence of phthalic anhydride and reduction of the imide to give **17**. Condensation of **17** with the 4-(2-thiophenyl)-2,4-



Scheme 2. Synthesis of 1*H*-Pyrrolo[2,3-*b*]pyridine-4-carboxamides, **22** (**JX037**, **JX062-63**, and **JX072**).

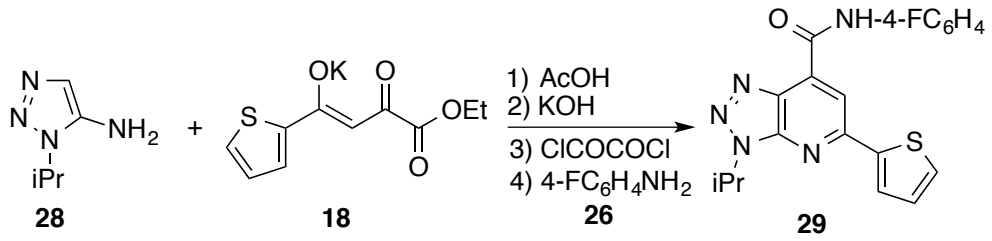
diketoester **18** (prepared as in Scheme 1) gave, via the free aminopyrrole formed in situ, the 1*H*-pyrrolo[2,3-*b*]pyridine-4-carboxylic ester **19**. The remainder of the synthesis follows that of Scheme 1, namely hydrolysis of the ethyl ester of **19** to give the acid **20** followed by activation to the acid chloride with oxalyl chloride and final coupling with one of several anilines **21** to affordthe analogues **22**. In particular, we varied the aniline to give the following analogues: Ar = 4-FC_6H_4: **JX037**; 2-pyridyl: **JX072**; 3-pyridyl: **JX062**; and 4-pyridyl: **JX063**.

In addition, one analogue in each of three additional bi-heterocyclic ring systems were prepared and tested. Again, a very similar route was used for the preparation of each of these three new analogues (Scheme 3). Thus beginning with the 5-amino-1-isopropyl-imidazole **23**, condensation with **18** gave the imidazopyridine **24**, which, after hydrolysis to give **25** and amide formation with 4-fluoroaniline **26**, afforded the desired 3*H*-imidazo[4,5-*b*]pyridine-7-carboxamide **27** (**JX034**).



Scheme 3. Synthesis of 3*H*-imidazo[4,5-*b*]pyridine-7-carboxamide, **27** (**JX034**).

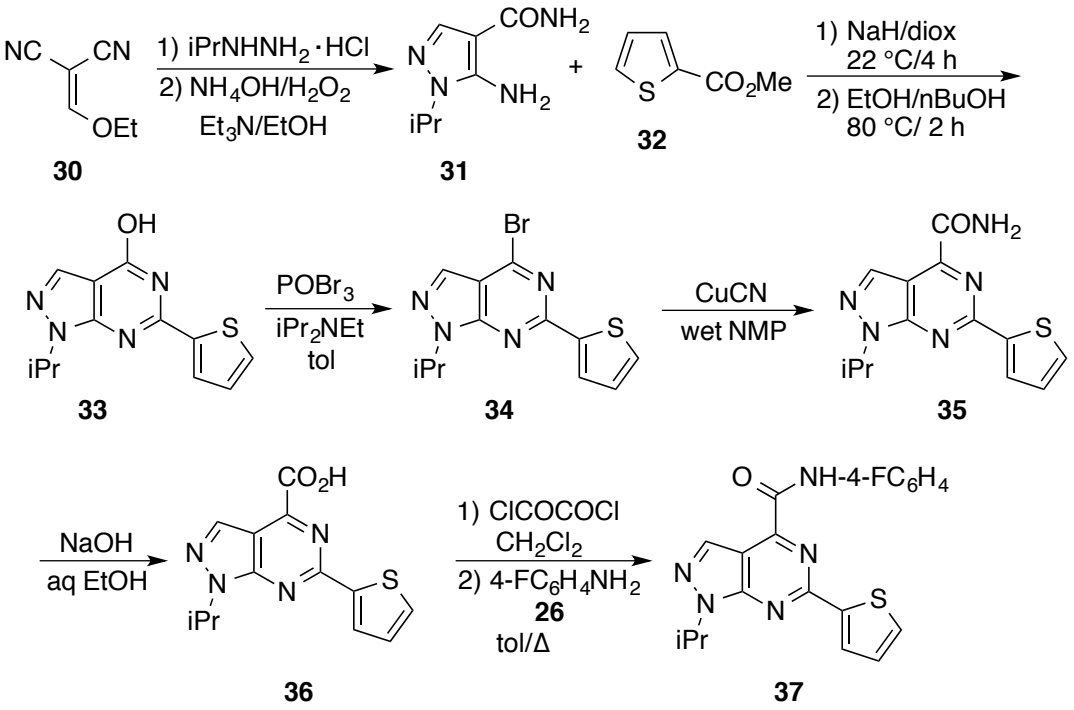
In a similar manner (Scheme 4), the known 1-isopropyl-5-aminotriazole **28** was condensed with



Scheme 4. Synthesis of 1*H*-Triazolo[2,3-*b*]pyridine-4-carboxamide, **29** (**JX035**).

18 to give, after hydrolysis and amide formation with **26**, the desired 3*H*-[1,2,3]triazolo[4,5-*b*]pyridine-7-carboxamide **29** (**JX035**).

Finally the 1*H*-pyrazolo[3,4-*d*]pyrimidine-4-carboxamide analogue, **37** (**JX036**), was prepared by a completely different route (Scheme 5). Thus reaction of 2-(ethoxyvinylidene)malononitrile **30** with isopropylhydrazine followed by treatment with basic hydrogen peroxide gave the known aminoamide **31**.[20] Condensation of this compound with methyl thiophene-2-carboxylate **32** afforded the desired 1*H*-pyrazolo[4,5-*d*]pyrimidin-ol **33**. Conversion of the hydroxyl to a bromide furnished **34** which was converted into the carboxamide **35** with copper cyanide and wet N-methylpyrrolidone (NMP). Hydrolysis of the amide gave the carboxylic acid **36** which was then converted, via the acid chloride, into the desired 4-fluorophenyl amide **37** (**JX036**).



Scheme 5. Synthesis of 1*H*-pyrazolo[3,4-*d*]pyrimidine-4-carboxamide, **37** (**JX036**)

BIOLOGICAL EVALUATION.

SAR analysis of original compounds. We recently described the identification of an array of chemical structures with antiviral activity against commonly encountered enteroviruses.[15] There were 144 1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide compounds tested in the primary screen, and twenty two of these were confirmed to be active against the enterovirus strain used (CVB3-H3) (Supplementary Table S1, structures footnoted with the letter b).[15] CV-B3 was chosen because it has been annually reported to the Center for Disease Control since 1975 as cause of severe disease,[1] and because the availability of the molecularly cloned pathogenic variant of CV-B3 (CVB3-H3) enhances the reproducibility of in vitro studies. The antiviral activity of this class of compounds is greatly affected by variations at the N1 (R^1), C6 (R^6) and C4 positions

(Figure 1, **1**). Among the 22 active library compounds, the number of variations decreased at the order of C4 > C6 > N1. In particular, the compound *N*-(2-fluorophenyl)-1-(propan-2-yl)-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide (Figure 1, **1a**) has shown to exhibit activity against 12 commonly encountered members of the enterovirus B species, as well as enterovirus A71 (EV species A) and two polioviruses (EV species C).[15]

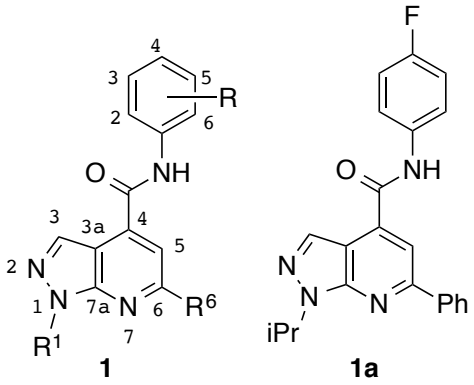


Figure 1. 1*H*-Pyrazolo[3,4-*b*]pyridine-4-carboxamides.

Here we used the 1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide **1a** as a reference compound and designed new compounds in the 1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide series **1** in order to vary the four most easily altered positions, namely: 1) the alkyl group at N1 (R^1); 2) the usually aryl or heteroaryl ring at C6 (R^6); 3) the aniline unit on the carboxamide at C4, varying the substituents at essentially every available carbon and introducing heterocyclic amines; and 4) a few changes in the amide linking unit between the two rings. Our goal was to identify candidate compounds for the development of anti-enteroviral drugs with significant improvements in potency and exhibiting reduced cytotoxicity. Specifically, we sought compounds that would inhibit the replication of enteroviruses in the EV-A, EV-B, and EV-C species at concentrations at less than 1 μM, yet with relatively low cytotoxicity.

Analogues at the N1 position (R^1). For the group attached to N1, we began with the isopropyl

group since it was the substituent in the lead compound **1a**. (henceforth referred to as **JX001**). We changed the substituent and examined aryl, secondary cycloalkyl, substituted methyl units, and even H. The compounds prepared with this variation were: R^1 = phenyl: **JX012**; *t*-butyl: **JX013**; cyclobutyl: **JX014**; 2,2,2-trifluoroethyl: **JX022**; cyclopentyl: **JX027**; cyclohexyl: **JX028**; cycloheptyl: **JX029**; 4-methoxybenzyl: **JX031**; and hydrogen: **JX032**. In general, those substituents were not as favorable for activity as the isopropyl group since none of the above analogs demonstrated antiviral activities against EV-A71, coxsackievirus B3(CV-B3), or poliovirus-1 (PV-1). This result is consistent with the SAR analysis of the initial 144 library compounds, in which the isopropyl group at the N1 position seemed crucial for the antiviral activity (Supplementary Table S1). The only two exceptions were those with an ethyl group replacing the isopropyl group, namely: *N*-(4-cyanophenyl)-1-ethyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide and 1-ethyl-*N*-(4-methyl-3-sulfamoylphenyl)-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide (highlighted groups in Supplementary Table S1). Therefore, we retained the isopropyl group at the N1 position in the other analogues in this study.

Analogues at the 6-position (R^6). The group attached at the 6-position in **1a (JX001)** is a thiophen-2-yl group; this was also present in 11 other active compounds of our primary screen (Supplementary Table S1). Besides, cyclopropyl, phenyl and isopropyl groups were also found at the 6-positions of active compounds. We tested the effect of variation at this position by changing R^6 to cycloalkyl, aryl, and heteroaryl units. New compounds prepared with this variation were: R^6 = cyclopropyl, **JX038**, **JX055**, **JX071**; phenyl, **JX002**, **JX070**; and heteroaryl, e.g., 2-pyridyl: **JX004**; 3-pyridyl: **JX005**; 4-pyridyl: **JX003**; 2-thiazolyl: **JX007**; 5-thiazolyl: **JX010**; 5-oxazolyl: **JX011**; 2-furyl: **JX026**; and thiophen-3-yl: **JX030**, **JX057-JX061**. Several

of these compounds also had different aniline units, namely: 4-fluorophenyl in **JX026**, **JX030** and **JX038**; 3-sulfamoylphenyl in **JX055**; 2-pyridyl in **JX070** and **JX071**. Those changes in the aniline units were generally associated with increased antiviral activities as discussed below. The highest activity was seen with thiophen-2-yl unit at the 6-position although the thiophen-3-yl unit was also associated with antiviral activity in low micromolar concentrations (Table 1). By contrast, the presence of azole groups - 2-thiazolyl (**JX007**), thiazolyl (**JX010**), and 5-oxazolyl (**JX011**) at R^6 clearly reduced antiviral activity. Other groups phenyl **(JX002** and **JX070**) and cyclopropyl (**JX038** and **JX055**) were also associated with decreases in antiviral activities. It seemed that cytotoxicity decreased significantly with 2-furyl (**JX026)** at the 6-position. Furthermore, we found that many derivatives with a thiophen-3-yl unit replacing the thiophen-2-yl unit: **JX030**, **JX057**, **JX058**, **JX059**, **and JX060** retained antiviral activity. The direct comparison of the effects of thiophen-2-yl and thiophen-3-yl units on antiviral activity was manifested in the following pairs: **JX017** vs. **JX030**, **JX040** vs. **JX057**, **JX041** vs. **JX058**, **JX042** vs. **JX061**, **JX043** vs. **JX059**, and **JX056** vs. **JX060**, where the thiophen-2-yl group was generally associated with higher antiviral activities (Table 1 and Table 2).

Table 1. Antiviral activities of analogues with various substituents at the 6-position.

Compound No. (JX)	**R^6**	**Anti-EV-A71 in LLC cells** (μM)[a]	**Anti- CV-B3 in HeLa cells** (μM)[a]	**Anti-PV-1 in HeLa cells** (μM)[a,b]
1a (or 001)	2-thiophenyl	EC_{50}: 2.3 ± 1.2 CC_{50}: 50.0 SI_{50}: 21.7	EC_{50}: 1.4 ± 0.6 CC_{50}: 12.5 SI_{50}: 8.9	EC_{50}: 7.0 ± 0.0 CC_{50}: 12.5 SI_{50}: 1.8
002	phenyl	EC_{50}: 5.7 ± 1.5 CC_{50}: > 200.0 SI_{50}: > 35.1	EC_{50}: 4.5 ± 1.5 CC_{50}: 37.5 SI_{50}: 8.3	EC_{50}: > 25.0 CC_{50}: 37.5 SI_{50}: N/A
003	4-pyridyl	EC_{50}: > 10.0 CC_{50}: 45.0 SI_{50}: N/A	EC_{50}: 5.1 ± 0.1 CC_{50}: 37.5 SI_{50}: 7.4	EC_{50}: > 25.0 CC_{50}: 37.5 SI_{50}: N/A

004	2-pyridyl	EC_{50}: > 10.0 CC_{50}: 100.0 SI_{50}: N/A	EC_{50}: 6.9 ± 0.7 CC_{50}: > 200.0 SI_{50}: > 29.0	EC_{50}: > 25.0 CC_{50}: > 200.0 SI_{50}: N/A
005	3-pyridyl	EC_{50}: > 10.0 CC_{50}: 50.0 SI_{50}: N/A	EC_{50}: 7.0 ± 2.4 CC_{50}: 37.5 SI_{50}: 5.4	EC_{50}: > 25.0 CC_{50}: 37.5 SI_{50}: N/A
007	2-thiazolyl	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A
010	5-thiazolyl	EC_{50}: 3.7 ± 0.2 CC_{50}: 100.0 SI_{50}: 27.0	EC_{50}: 3.3 ± 1.4 CC_{50}: > 200.0 SI_{50}: > 60.6	EC_{50}: > 25.0 CC_{50}: > 200.0 SI_{50}: N/A
011	5-oxazolyl	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A
026	2-furyl	EC_{50}: 2.6 ± 0.3 CC_{50}: > 200.0 SI_{50}: > 76.9	EC_{50}: 2.4 ± 0.0 CC_{50}: > 200.0 SI_{50}: > 83.0	EC_{50}: > 25.0 CC_{50}: > 200.0 SI_{50}: N/A
030	3-thiophenyl	EC_{50}: 1.4 ± 0.2 CC_{50}: > 200.0 SI_{50}: > 142.9	EC_{50}: 1.3 ± 0.1 CC_{50}: 25.0 SI_{50}: 19.2	EC_{50}: > 25.0 CC_{50}: 25.0 SI_{50}: N/A
038	cyclopropyl	EC_{50}: 3.2 ± 0.1 CC_{50}: 12.5 SI_{50}: 3.9	EC_{50}: 3.3 ± 0.1 CC_{50}: 10.0 SI_{50}: 3.0	EC_{50}: > 25.0 CC_{50}: 10.0 SI_{50}: N/A
055	cyclopropyl	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A
070	phenyl	EC_{50}: 0.7 CC_{50}: 12.5 SI_{50}: 17.9	EC_{50}: 1.6 ± 0.0 CC_{50}: 18.8 SI_{50}: 11.8	EC_{50}: > 25.0 CC_{50}: 18.8 SI_{50}: N/A
071	cyclopropyl	EC_{50}: 4.7 CC_{50}: 25.0 SI_{50}: 5.3	EC_{50}: 4.7 CC_{50}: 25.0 SI_{50}: 5.3	EC_{50}: > 25.0 CC_{50}: 25.0 SI_{50}: N/A

a. For compounds that were tested against certain viruses in 3 separate experiments, means ± s.d. are shown. Otherwise, a single value represents an average of triplicates in one experiment. b. Similar results were observed against anti-PV-1 and anti-PV-3 activities.

comparison of the effects of thiophen-2-yl and thiophen-3-yl units on antiviral activity was

manifested in the following pairs: **JX017** vs. **JX030**, **JX040** vs. **JX057**, **JX041** vs. **JX058**, **JX042** vs. **JX061**, **JX043** vs. **JX059**, and **JX056** vs. **JX060**, where the thiophen-2-yl group was generally associated with higher antiviral activities (Table 1 and Table 2).

Analogues at the aniline unit. In addition, we varied extensively the substitution pattern of the N-aryl group of the amide as shown in the Table 2. Many new compounds were prepared having halo substituted anilines (especially fluoro substituents), sulfamoyl and carbamoyl units, and especially heterocyclic amine units, pyridyl and pyrimidyl rings. We evaluated the effect of the position of a single fluorine atom on the phenyl ring in **JX017** and **JX021**. We also prepared analogues in which the position and number of fluorine atoms were varied: **JX008**, **JX009**, **JX015**, **JX016**, **JX018**, **JX019**, **JX020**, and **JX050**. Although nearly all of the analogues had antiviral activity against the virus test strains, and **JX017** with a 4-fluorophenyl unit had substantially lower cytotoxicity and lower EC_{50} concentrations against EV-A71 and CV-B3 compared to original lead **JX001** with a 2-fluorophenyl group. We also prepared and tested compounds with a sulfamoyl unit: **JX006**, **JX025**, **JX033** and **JX055**. Of these, the 3-sulfamoyl analogue **JX025** showed the best antiviral activity, with EC_{50} values for EV-A71 and CVB-B3 that were similar to those for lead **JX001,** in addition to a lower EC_{50} value against poliovirus, and generally lower cytotoxicity.

In light of this result, we synthesized other sulfamoyl analogues having also a fluorine atom, e.g., **JX068** and **JX069**, the 4-methylsulfonyl analogue **JX047**, the monomethyl- and dimethylsulfamoyl analogues **JX053** and **JX052**, and two carbamoyl analogues **JX064** and **JX073** to further explore the effect of such substituents on activity. All of these exhibited antiviral activity against EV-A71 and CV-B3 with the sulfone **JX047** and the 4-fluoro-3-sulfamoyl analogues, **JX069**, being the least cytotoxic of the compounds tested. They therefore

had the best selectivity indices against EV-A71 and CV-B3, but they were not active against polioviruses. The best antiviral activity (lowest EC_{50} values) against PV-1 was seen with the analogues **JX025**, **JX064** and **JX068**, which had either sulfamoyl or carbamoyl moieties at the 3-position of the phenyl ring.

In addition, we prepared many compounds with heterocyclic amines in the amides, some of which had excellent activity. The heterocyclic units prepared were: 2-pyridyl: **JX040**, **JX057**, **JX070**, **JX071**, **JX072** and **JX074**; 3-pyridyl: **JX056**, **JX060**, **JX062** and **JX065**; 4-pyridyl: **JX042**, **JX061** and **JX063**; 2-pyrimidyl: **JX041** and **JX058**; 4-pyrimidyl: **JX043** and **JX059**; and 5-pyrimidyl: **JX066**. As mentioned earlier, the five analogues **JX057**, **JX058**, **JX059**, **JX060** and **JX061** all had a 3-thiophenyl group at the 6-position. In general, analogues with pyridyl amides showed significantly better activity than those with substituted phenyl amides. Among the isomeric pyridyl analogues, generally the 2-pyridyl unit was associated with excellent antiviral activity and 4-pyridyl was associated with poor activity. In particular, the 2-pyridyl amide **JX040** had substantially lower EC_{50} values for EV-A71 and CV-B3 and higher CC_{50} concentration than the lead **1a (JX001)**, resulting in a markedly higher SI_{50}. Unfortunately, this substitution appeared to reduce activity against poliovirus. The effects of the pyrimidyl units were complicated as they were associated with excellent EC_{50} values and below average CC_{50} values. In this group, **JX059** was the best analogue as it had exceptional and excellent CC_{50} values.

Table 2. Antiviral activities of analogues with various substituents of the aniline units.

Compd No. (JX)	**N-aryl group**	**Anti-EV-A71 in LLC cells** (μM)[a]	**Anti- CV-B3 in HeLa cells** (μM)[a]	**Anti-PV-1 in HeLa cells** (μM)[a,b]

006	4-sulfamoylphenyl	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A
008	2,4-$F_2C_6H_3$	EC_{50}: 1.6 ± 0.4 CC_{50}: > 200.0 SI_{50}: > 125.0	EC_{50}: 1.4 ± 0.5 CC_{50}: > 200.0 SI_{50}: > 142.9	EC_{50}: > 25.0 CC_{50}: > 200.0 SI_{50}: N/A
009	2,6-$F_2C_6H_3$	EC_{50}: 3.4 ± 0.7 CC_{50}: > 200.0 SI_{50}: > 58.8	EC_{50}: 3.2 ± 0.5 CC_{50}: > 200.0 SI_{50}: > 62.5	EC_{50}: > 25.0 CC_{50}: > 200.0 SI_{50}: N/A
015	3,4-$F_2C_6H_3$	EC_{50}: > 25.0 CC_{50}: > 200.0 SI_{50}: N/A	EC_{50}: 1.3 ± 0.1 CC_{50}: > 200.0 SI_{50}: > 153.8	EC_{50}: > 10.0 CC_{50}: > 200.0 SI_{50}: N/A
016	3,5-$F_2C_6H_3$	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A
017	4-FC_6H_4	EC_{50}: 0.9 ± 0.3 CC50: > 200.0 SI_{50}: > 222.2	EC_{50}: 0.7 ± 0.2 CC_{50}: > 200.0 SI_{50}: > 285.7	EC_{50}: > 25.0 CC_{50}: > 200.0 SI_{50}: N/A
018	2,5-$F_2C_6H_3$	EC_{50}: 3.5 CC_{50}: 100.0 SI_{50}: 28.6	EC_{50}: 2.7 ± 0.4 CC_{50}: > 200.0 SI_{50}: > 74.1	EC_{50}: > 25.0 CC_{50}: > 200.0 SI_{50}: N/A
019	2,3-$F_2C_6H_3$	EC_{50}: 3.0 CC_{50}: 75.0 SI_{50}: 25.0	EC_{50}: 2.7 ± 0.4 CC_{50}: > 200.0 SI_{50}: > 74.1	EC_{50}: > 25.0 CC_{50}: > 200.0 SI_{50}: N/A
020	2,4,6-$F_3C_6H_2$	EC_{50}: 3.0 CC_{50}: > 200.0 SI_{50}: > 66.7	EC_{50}: 1.8 ± 0.7 CC_{50}: > 200.0 SI_{50}: > 111.1	EC_{50}: > 25.0 CC_{50}: > 200.0 SI_{50}: N/A
021	3-FC_6H_4	EC_{50}: 2.4 ± 0.8 CC_{50}: > 200.0 SI_{50}: > 83.3	EC_{50}: 0.8 ± 0.3 CC_{50}: > 200.0 SI_{50}: > 250.0	EC_{50}: > 25 CC_{50}: > 200.0 SI_{50}: N/A
023	2-$CF_3C_6H_4$	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A
024	4-$CF_3C_6H_4$	EC_{50}: > 25.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 25.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 25.0 CC_{50}: N/A SI_{50}: N/A
025	3-sulfamoylphenyl	EC_{50}: 1.3 ± 0.1	EC_{50}: 1.3 ± 0.2	EC_{50}: 5 ± 0.0

		CC_{50}: 25.0 SI_{50}: 19.2	CC_{50}: > 200.0 SI_{50}: 166.7	CC_{50}: 50.0 SI_{50}: 10.0
033	2-sulfamoylphenyl	EC_{50}: 2.5 ± 0.7 CC_{50}: 25.0 SI_{50}: 10.0	EC_{50}: 2.6 ± 0.4 CC_{50}: 10.0 SI_{50}: 3.8	EC_{50}: > 25.0 CC_{50}: 10.0 SI_{50}: N/A
040	2-pyridyl	EC_{50}: 0.5 ± 0.1 CC_{50}: > 200.0 SI_{50}: > 400.0	EC_{50}: 0.8 ± 0.3 CC_{50}: > 200.0 SI_{50}: > 250.0	EC_{50}: > 10.0 CC_{50}: > 200.0 SI_{50}: N/A
041	2-pyrimidyl	EC_{50}: 0.5 ± 0.1 CC_{50}: 75.0 SI_{50}: 150.0	EC_{50}: 0.6 ± 0.1 CC_{50}: 37.5 SI_{50}: 62.5	EC_{50}: > 10.0 CC_{50}: 37.5 SI_{50}: N/A
042	4-pyridyl	EC_{50}: 0.4 ± 0.0 CC_{50}: 6.3 SI_{50}: 15.8	EC_{50}: 0.6 ± 0.1 CC_{50}: 6.0 SI_{50}: 10.0	EC_{50}: > 10.0 CC_{50}: 6.0 SI_{50}: N/A
043	4-pyrimidyl	EC_{50}: 0.5 ± 0.1 CC_{50}: 50.0 SI_{50}: 100.0	EC_{50}: 0.6 ± 0.1 CC_{50}: 20.0 SI_{50}: 33.3	EC_{50}: > 10.0 CC_{50}: 20.0 SI_{50}: N/A
045	1-isopropyl-6-(2-thiophenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-4-yl	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A
047	4-methyl-sulfonylphenyl	EC_{50}: 0.5 ± 0.1 CC_{50}: 50.0 SI_{50}: 100.0	EC_{50}: 1.0 ± 0.4 CC_{50}: 200.0 SI_{50}: 200.0	EC_{50}: > 10.0 CC_{50}: 200.0 SI_{50}: N/A
050	2-Br-4-FC_6H_3	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A
052	3-N,N-dimethylsulf-amoylphenyl	EC_{50}: 0.8 ± 0.1 CC_{50}: > 200.0 SI_{50}: > 250.0	EC_{50}: 1.0 ± 0.4 CC_{50}: 12.0 SI_{50}: 12.0	EC_{50}: > 10.0 CC_{50}: 12.0 SI_{50}: N/A
053	3-N-methylsulf-oylphenyl	EC_{50}: 0.7 ± 0.1 CC_{50}: 25.0 SI_{50}: 35.7	EC_{50}: 0.5 ± 0.1 CC_{50}: 12.0 SI_{50}: 24.0	EC_{50}: > 10.0 CC_{50}: 12.0 SI_{50}: N/A
056	3-pyridyl	EC_{50}: 0.5 ± 0.1 CC_{50}: > 200.0 SI_{50}: > 400.0	EC_{50}: 0.4 ± 0.3 CC_{50}: 25.0 SI_{50}: 62.5	EC_{50}: 10.0 CC_{50}: 25.0 SI_{50}: 2.5
057	2-pyridyl	EC_{50}: 0.8 CC_{50}: 25.0	EC_{50}: 1.2 CC_{50}: 18.0	EC_{50}: > 10.0 CC_{50}: 18.0

		SI_{50}: 31.3	SI_{50}: 15.0	SI_{50}: N/A
058	2-pyrimidyl	EC_{50}: 1.2 CC_{50}: 50.0 SI_{50}: 41.7	EC_{50}: 1.2 CC_{50}: 40.0 SI_{50}: 33.3	EC_{50}: > 10.0 CC_{50}: 40.0 SI_{50}: N/A
059	4-pyrimidyl	EC_{50}: 0.8 CC_{50}: > 200.0 SI_{50}: > 250.0	EC_{50}: 1.2 ± 0.0 CC_{50}: > 200.0 SI_{50}: > 166.7	EC_{50}: > 10.0 CC_{50}: > 200.0 SI_{50}: N/A
060	3-pyridyl	EC_{50}: 2.4 CC_{50}: 37.0 SI_{50}: 15.4	EC_{50}: 0.6 CC_{50}: 18.0 SI_{50}: 30.0	EC_{50}: > 10.0 CC_{50}: 18.0 SI_{50}: N/A
061	4-pyridyl	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A
064	3-carbamoylphenyl	EC_{50}: 0.8 CC_{50}: 25.0 SI_{50}: 31.3	EC_{50}: 0.7 ± 0.1 CC_{50}: 18.0 SI_{50}: 25.7	EC_{50}: 6.3 CC_{50}: 18.0 SI_{50}: 2.9
066	5-pyrimidyl	EC_{50}: 0.4 CC_{50}: 20.0 SI_{50}: 50.0	EC_{50}: 0.7 ± 0.2 CC_{50}: 18.0 SI_{50}: 25.7	EC_{50}: 10.0 CC_{50}: 18.0 SI_{50}: 1.8
068	2-fluoro-5-sulfamoylphenyl	EC_{50}: 0.5 CC_{50}: 25.0 SI_{50}: 50.0	EC_{50}: 0.7 ± 0.1 CC_{50}: 18.0 SI_{50}: 25.7	EC_{50}: 6.3 CC_{50}: 18.0 SI_{50}: 2.9
069	4-fluoro-3-sulfamoylphenyl	EC_{50}: 0.8 CC_{50}: 50.0 SI_{50}: 62.5	EC_{50}: 1.1 ± 0.3 CC_{50}: > 200.0 SI_{50}: > 181.8	EC_{50}: > 10.0 CC_{50}: > 200.0 SI_{50}: N/A
073	2-fluoro-5-carbamoylphenyl	EC_{50}: 0.5 CC_{50}: 25.0 SI_{50}: 50.0	EC_{50}: 0.8 CC_{50}: 18.8 SI_{50}: 23.5	EC_{50}: 5.0 CC_{50}: 18.8 SI_{50}: 3.8
075	4-fluoro-2-pyridyl	EC_{50}: 1.2 CC_{50}: > 200.0 SI_{50}: > 166.7	EC_{50}: 0.7 ± 0.1 CC_{50}: 37.5 SI_{50}: 53.6	EC_{50}: > 10.0 CC_{50}: 37.5 SI_{50}: N/A

a. For compounds that were tested against certain viruses in 3 separate experiments, means ± s.d. are shown. Otherwise, a single value represents an average of triplicates in one experiment. b. Similar results were observed against anti-PV-1 and anti-PV-3 activities.

Analogues at the linker unit. We also synthesized analogues with linker units other than the original carboxamide, e.g., reverse amide: **JX054**; N-Me amide: **JX074**; imide: **JX039**;

thioamide: **JX046**; sulfonamide: **JX049**; reverse sulfonamide: **JX044**; and amidines: **JX065**, **JX067**. In addition two unusual linkers were made and tested, e.g., the benzoxazole: **JX051**; and the indoline amide: **JX048**. Testing of these analogues indicated that the normal amide linker was crucial for the antiviral activity since many new linker units failed in the tests of antiviral activity even though we put the most favorable groups at the N1, the R^6 and the N-aryl positions. **JX054** demonstrated modest antiviral activity, while **JX048** had comparable activity to the original compound **1a (JX001)** (Table 3). The indoline amide may be a promising alternative linker unit if the issue of compound stability might not allow a carboxamide in the antiviral drug design.

Table 3. Antiviral activities of analogues with different linker units.

Compound No. (JX)	**Structure**	**Anti-EV-A71in LLC cells** (μM)[a]	**Anti- CV-B3 in HeLa cells** (μM)[a]	**Anti-PV-1* in HeLa cells** (μM)[a,b]
044	reverse sulfonamide	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A
046	thioamide	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A
048	indolineamide	EC_{50}: 1.3 ± 0.2 CC_{50}: > 200.0 SI_{50}: > 153.4	EC_{50}: 1.9 ± 0.6 CC_{50}: 18.8 SI_{50}: 9.9	EC_{50}: 10.0 CC_{50}: 18.8 SI_{50}: 1.9
049	sulfonamide	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A
051	benzoxazole	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A
054	reverse amide	EC_{50}: 4.0 CC_{50}: 50.0 SI_{50}: 12.5	EC_{50}: 4.7 ± 0.0 CC_{50}: 12.0 SI_{50}: 2.6	EC_{50}: > 10.0 CC_{50}: 12.0 SI_{50}: N/A

065	amidine	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A
067	amidine	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A
074	N-methylamide	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 25.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A
076	7-azaindoline-amide	EC_{50}: 12.5 CC_{50}: 50.0 SI_{50}: 4.0	EC_{50}: 9.4 ± 0.0 CC_{50}: 37.5 SI_{50}: 4.0	EC_{50}: > 10.0 CC_{50}: 37.5 SI_{50}: N/A

a. For compounds that were tested against certain viruses in 3 separate experiments, means ± s.d. are shown. Otherwise, a single value represents an average of triplicates in one experiment. b. Similar results were observed against anti-PV-1 and anti-PV-3 activities.

New Heterocyclic Ring Systems. Finally, we also prepared several new heterocyclic ring systems other than the 1*H*-pyrazolo[3,4-*b*]pyridine system shown in compound **1**. In particular, we developed syntheses of the 1*H*-pyrrolo[2,3-*b*]pyridine-4-carboxamides, **22** (**JX037**, **JX062**, **JX063** and **JX072**), as shown in Scheme 2. We also prepared the 3*H*-imidazo[4,5-*b*]pyridine-7-carboxamide, **27** (**JX034**), depicted in Scheme 3 and the 1*H*-1,2,3-triazolo[4,5-*b*]pyridine-4-carboxamide, **29** (**JX035**), as shown in Scheme 4, changing the pyrazole unit for an imidazole and a 1,2,3-triazole, respectively. Finally a different synthetic route was used to make the 1*H*-pyrazolo[3,4-*d*]pyrimidine-4-carboxamide, **37** (**JX036**), depicted in Scheme 5, in which the pyrimidine unit was substituted for the pyridine. These changes also had profound impact on the activity. The first three heterocyclic ring systems - the imidazopyridine, the triazolopyridine, and the pyrazolopyrimidine abrogated antiviral activity in our test system. By contrast, pyrrolopyridine analogues were active with the best analogue, **JX062**, having EC50 concentrations for EV-A71 and CV-B3 that were similar to the original lead **1a** (Table 4).

Table 4. Antiviral activities of analogues with new heterocyclic ring systems.

Compound No. (JX)	Structure	Anti-EV-A71in LLC cells (μM)[a]	Anti- CV-B3 in HeLa cells (μM)[a]	Anti-PV-1 in HeLa cells (μM)[a,b]
034	3*H*-imidazo[4,5-*b*]pyridine-7-carboxamide	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A
035	1*H*-triazolo[4,5-*b*]pyridine-4-carboxamide	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50} > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A
036	1*H*-pyrazolo[3,4-*d*]pyrimidine-4-carboxamide	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A
037	1*H*-pyrrolo[2,3-*b*]pyridine-4-carboxamide	EC_{50}: > 25.0 CC_{50}: > 200.0 SI_{50}: N/A	EC_{50}: 3.1 ± 0.1 CC_{50}: > 200.0 SI_{50}: 64.5	EC_{50}: > 25.0 CC_{50}: > 200.0 SI_{50}: N/A
062	1*H*-pyrrolo[2,3-*b*]pyridine-4-carboxamide	EC_{50}: 0.8 CC_{50}: 25.0 SI_{50}: 31.3	EC_{50}: 1.3 ± 0.1 CC_{50}: 18.0 SI_{50}: 13.8	EC_{50}: > 10.0 CC_{50}: 18.0 SI_{50}: N/A
063	1*H*-pyrrolo[2,3-*b*]pyridine-4-carboxamide	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A	EC_{50}: > 10.0 CC_{50}: N/A SI_{50}: N/A
072	1*H*-pyrrolo[2,3-*b*]pyridine-4-carboxamide	EC_{50}: 1.2 CC_{50}: > 200.0 SI_{50}: > 166.7	EC_{50}: 2.2 ± 0.4 CC_{50}: 18.8 SI_{50}: 8.5	EC_{50}: > 10.0 CC_{50}: 18.8 SI_{50}: N/A

a. For compounds that were tested against certain viruses in 3 separate experiments, means ± s.d. are shown. Otherwise, a single value represents an average of triplicates in one experiment. b. Similar results were observed against anti-PV-1 and anti-PV-3 activities.

The direct of comparison of pyrazolopyridine and pyrrolopyridine was shown in the following pairs: **JX017** vs. **JX037**, **JX040** vs. **JX072**, **JX042** vs. **JX063** and **JX056** vs. **JX062** (Tables 2 and 4). The pyrrolopyridine analogues with either 4-fluorophenyl (**JX037**) or 3-pyridyl (**JX062**) had good antiviral activity against CV-B3, a commonly encountered representative of the EV-B species.[1] Thus, this new heterocyclic ring system represents a new class of antiviral compounds with a different heterocyclic core than the original lead **1a**.

■ CONCLUSIONS

In this report, we reported the synthesis and in vitro testing of novel pyrazolopyridine analogues for possible development into antiviral drugs for the treatment or prevention of enterovirus infections. We modified four sites around the core structure and carried out antiviral testing to establish a structure-activity relationship for this system. The best analogues with the highest SI_{50} values were those with isopropyl group at the 1-position and a thiophen-2-yl unit at the 6-position. The 4-position allowed the most variation since many different N-aryl groups had equal or better antiviral activity than 2-fluorophenyl unit in the lead compound **1a (JX001)**. The 4-fluorophenyl group (**JX017**) had the best antiviral activity in its class while the 3-sulfamoylphenyl moiety (**JX025**) also exhibited antiviral activity against polioviruses, albeit with an EC_{50} of 5 μM, which fell short of our target (1μM). Furthermore, several heterocyclic amines as the N-aryl group also had very favorable antiviral activities. Of all the pyridine and pyrimidine analogues, those with the 2-pyridyl group had perhaps the best overall activity, e.g., **JX040** had the greatest antiviral activity against non-polio enteroviruses, although it had weak antiviral activity against polioviruses. Given the fact that enteroviruses have more than 110 types and that many important pathogenic viruses may belong to different species (A-D), the diversity at the N-aryl position may provide options against different enteroviruses. The antiviral breadth of these variations at the N-aryl position will be studied in further research. We also screened different linker units and, while most of those had reduced activity compared to carboxamides, the indolineamide, reverse amide, and imide showed some activity. Finally, we changed the core structure from pyrazolopyridine to pyrrolopyridine with minimal loss in activity, thus further expanded the compound space for anti-enteroviral drugs. We infected cells with CVB3-H3 or CVB3-H3-C179F, which has a missense mutation in the 2C coding domain. As previously

described[15], CVB3-H3-C179F was resistant to JX001, which inhibited the wildtype virus. Similarly, CVB3-H3-C179F was not inhibited by JX017, JX034, and JX048 (data not shown), indicating that these compounds also target activities of the 2C protein.

We acknowledge that in vitro studies of the biological activity of antiviral compounds may not predict in vivo efficacy or toxicity; additional in vitro characterization and animal model studies are key to the pre-clinical development of antiviral agents.[10] In addition, we focused in this study on examining one representative each of the EV-A, EV-B, and EV-C species of enteroviruses, which are the most commonly encountered types in most of the world.[1] The recent outbreak of EV-D68 in the US re-emphasizes the need for anti-enteroviral drugs and several studies have looked at existing candidates such as fluoxetine.[10] Pyrazolopyridine analogues, which also target the viral 2C protein,[15] represent a novel class of antiviral candidates and their activity against EV-D68 warrants additional studies.

■ EXPERIMENTAL SECTION

General: Toluene was distilled from sodium under an argon atmosphere. Dichloromethane was distilled from calcium hydride under an argon atmosphere. All other solvents or reagents were purified according to literature procedures. ^{1}H NMR spectra were recorded on Bruker spectrometers at 400 MHz and are reported relative to deuterated solvent signals. Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz) and integration. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad. ^{13}C NMR spectra were recorded on Bruker Spectrometers at 100 MHz. Data for ^{13}C NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity and coupling constant (Hz). Splitting patterns are designated as the same in ^{1}H

NMR. High resolution mass spectrometry was taken on a Thermo Fisher Scientific Exactive Plus mass spectrometer equipped with an IonSense ID-CUBE DART ion source. The purity of all final compounds was determined to be >95% by analytical HPLC analysis. For most final compounds, purity was determined using a Shimadzu LC-20 HPLC with a Nova-Pak Silica 60Å 4μm HPLC Column (3.9 x 150 mm, Waters) and UV 254 nm detection. Elution at 0.5 mL/min with a mixture of CH_2Cl_2 (A) and EtOAc (B) isocratic at 90% A and 10% B, or CH_2Cl_2 (A) and MeOH (B) isocratic at 90% A and 10% B. The purity of compounds **JX042**, **JX056**, **JX060-063**, and **JX066-067** was determined using a Waters Acquity UPLC connected to a Waters LCT-Premier XE Time of Flight Instrument with an Acquity BEH C18 1.7μm UPLC Column (2.1 x 50 mm, Waters). Elution with a gradient of 0.4 mL/min H_2O/MeCN/0.3% Formic acid with a gradient of 3 to 90% MeCN between 0 and 5 min. Mass spectra were recorded from 70 to 2000 Daltons. All solvents were LC-MS/MS Grade and purchased from Fisher Scientific.

General procedure for the preparation of 2:

Method A: To a solution of compound **5** (10.0 mmol) in ethanol (10.0 mL) cooled to 0 °C , was added dropwise compound **4** (10.0 mmol) with stirring. The mixture was stirred overnight at 21 °C, then the solvent was evaporated in vacuo to give product **6** in almost quantitative yield. The product **6** was added to a solution of sodium (12.0 mmol) in *n*-butanol (20.0 ml), and the resulting mixture was refluxed for 12 h under an argon atmosphere, then cooled and the solvent evaporated in vacuo. The resulting residue was purified by flash column chromatography on silica gel (hexanes/ethyl acetate) to give the desired product **2**.

Method B: To 20.0 ml of ethanol were added successively 10.0 mmol of compound **7**, 20.0 mmol of sodium acetate, and 10.0 mmol of compound **8** at 21 °C, followed by stirring the

reaction mixture at 80 °C for 12 h under an argon atmosphere. After removing the solvent in vacuo, water was added to the residue. The mixture was neutralized with sodium bicarbonate, and extracted with ethyl acetate. The combined ethyl acetate solution was washed with brine, dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated in vacuo. The resulting residue was purified by flash column chromatography on silica gel (hexanes/ethyl acetate) to give the desired product **2**.

General procedure for the preparation of 3: Potassium *tert*-butoxide (24.0 mmol) was added to a solution of the substrate **9** (20.0 mmol) in anhydrous toluene (100 ml) at 0 °C under an argon atmosphere in one portion. The mixture was stirred at 0 °C for 15 min. Then diethyl oxalate **10** (4.0 ml) was added via syringe, and the resulting mixture was stirred at 21 °C for 12 h. The precipitated product was collected by filtration, washed with toluene and dried in vacuo to give the desired product **3**.

General procedure for the preparation of 11: To 25.0 ml of acetic acid were added successively 5.0 mmol of compound **2** and 5.0 mmol of compound **3** at 21 °C. The resulting mixture was stirred at 21 °C for 15 min, then refluxed for 4 h under an argon atmosphere. After removing the solvent in vacuo, the resulting residue was purified by flash column chromatography on silica gel (hexanes/ethyl acetate) to give the desired product **11**.

General procedure for the preparation of 12: To a solution of compound **11** (3.0 mmol) in 2-propanol (15.0 ml), was added potassium hydroxide (6.0 mmol) in one portion. The resulting mixture was stirred at 21 °C for 2 h. After removing the solvent in vacuo, the resulting residue was dissolved in water (100 ml) and neutralized with acetic acid. The precipitated product was

collected by filtration and dried in vacuo over phosphorus pentoxide to give the desired product **12**.

General procedure for the preparation of JX001 to JX076: To a solution of the substrate **12** (0.5 mmol) in anhydrous dichloromethane (5.0 ml) cooled to 0 °C was added dropwise oxalyl chloride (1.0 ml, 2.0 M in dichloromethane) with stirring under an argon atmosphere. Then a catalytic amount of DMF was added. The resulting mixture was stirred at 21 °C for 2 h. After removing the solvent in vacuo, product **13** was obtained in almost quantitative yield. The product **13** was dissolved in anhydrous toluene (15.0 ml) and compound **14** (2.5 mmol) was added at 21 °C. The resulting mixture was refluxed for 12 h under an argon atmosphere, then cooled to 21 °C, diluted with ethyl acetate, washed successively with 2 M hydrochloric acid and brine, dried over anhydrous sodium sulfate and the solvent was evaporated in vacuo. The resulting residue was purified by flash column chromatography on silica gel (hexanes/ethyl acetate) to give the desired JX compounds **(JX001** to **JX076)**. The yields are generally quite good: for example, the yield of **JX001** in this coupling of **13** and **14** was 82%.

Characterization data for JX001 to JX076:

***N*-(2-Fluorophenyl)-1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carbox-amide, JX001.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (t, *J* = 7.8 Hz, 1H), 8.34 (br s, 1H), 8.30 (s, 1H), 7.91 (s, 1H), 7.76 (d, *J* = 2.8 Hz, 1H), 7.46 (d, *J* = 4.8 Hz, 1H), 7.25-7.12 (m, 4H), 5.41-5.34 (m, 1H), 1.64 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.3, 152.8 (d, *J* = 242.4 Hz), 151.6, 150.2, 144.3, 135.9, 130.5, 128.9, 128.2, 126.6, 125.8 (d, *J* = 10.3 Hz), 125.3 (d, *J* = 7.6 Hz), 124.8 (d, *J* = 3.8 Hz), 122.0, 115.0 (d, *J* = 18.8 Hz), 111.7, 110.7, 49.2, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{16}FN_4OS$ ($[M-H]^-$): 379.1029, Found: 379.1031. mp 171-172 °C.

***N*-(2-Fluorophenyl)-1-isopropyl-6-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX002.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (td, *J* = 8.0, 1.2 Hz, 1H), 8.37 (m, 2H), 8.19-8.16 (m, 2H), 8.03 (s, 1H), 7.54-7.45 (m, 3H), 7.25-7.14 (m, 3H), 5.52-5.45 (m, 1H), 1.67 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.5, 156.6, 152.8 (d, *J* = 242.5 Hz), 150.7, 138.5, 136.0, 130.4, 129.8, 128.9, 127.5, 125.9 (d, *J* = 10.0 Hz), 125.3 (d, *J* = 7.7 Hz), 124.8 (d, *J* = 3.4 Hz), 122.0, 115.0 (d, *J* = 18.8 Hz), 112.9, 110.9, 49.0, 22.1 ; HRMS (ESI, *m*/*z*): calcd for $C_{22}H_{18}FN_4O$ ($[M-H]^-$): 373.1465, Found: 373.1465.

***N*-(2-Fluorophenyl)-1-isopropyl-6-(pyridin-4-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX003.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (d, *J* = 4.4 Hz, 2H), 8.62 (br s, 1H), 8.43-8.39 (m, 2H), 8.06 (s, 1H), 8.01 (d, *J* = 5.6 Hz, 2H), 7.22-7.13 (m, 3H), 5.48-5.41 (m, 1H), 1.65 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.2, 153.4, 153.0 (d, *J* = 242.9 Hz), 150.5, 150.4, 145.6, 136.5, 130.6, 125.65 (d, *J* = 10.3 Hz), 125.59 (d, *J* = 7.7 Hz), 124.8 (d, *J* = 3.5 Hz), 122.4, 121.4, 115.1 (d, *J* = 19.1 Hz), 112.7, 112.1, 49.3, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{21}H_{17}FN_5O$ ($[M-H]^-$): 374.1417, Found: 374.1414.

***N*-(2-Fluorophenyl)-1-isopropyl-6-(pyridin-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX004.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71-8.70 (m, 2H), 8.58 (d, *J* = 8.0 Hz, 1H), 8.48-8.44 (m, 3H), 7.86 (td, *J* = 7.8, 1.6 Hz, 1H), 7.38-7.34 (m, 1H), 7.24-7.13 (m, 3H), 5.48-5.42 (m, 1H), 1.67 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.6, 155.3, 155.1, 153.0 (d, *J* = 242.5 Hz), 150.4, 149.2, 137.0, 136.0, 131.8, 125.8 (d, *J* = 10.3 Hz), 125.3 (d, *J* = 7.7 Hz), 124.7 (d, *J* = 3.8 Hz), 124.4, 122.4, 121.5, 115.1 (d, *J* = 18.8 Hz), 112.9, 111.9, 49.0, 22.1; HRMS (ESI, *m*/*z*): calcd for $C_{21}H_{17}FN_5O$ ($[M-H]^-$): 374.1417, Found: 374.1419.

***N*-(2-Fluorophenyl)-1-isopropyl-6-(pyridin-3-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX005.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.37 (s, 1H), 8.67 (d, *J* = 4.0 Hz, 1H), 8.54 (d, *J* = 1.6 Hz, 1H), 8.48-8.42 (m, 2H), 8.38 (s, 1H), 8.03 (s, 1H), 7.44-7.41 (m, 1H), 7.24-7.14 (m, 3H), 5.48-5.41 (m, 1H), 1.65 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.3, 153.8, 152.9 (d, *J* = 242.4 Hz), 150.6, 150.5, 148.8, 136.5, 134.7, 134.1, 130.5, 125.8 (d, *J* = 9.9 Hz), 125.5 (d, *J* = 7.6 Hz), 124.8 (d, *J* = 3.5 Hz), 123.6, 122.2, 115.1 (d, *J* = 19.2 Hz), 112.5, 111.4, 49.3, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{21}H_{17}FN_5O$ ([M-H]$^-$): 374.1417, Found: 374.1417.

1-Isopropyl-*N*-(4-sulfamoylphenyl)-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX006. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.96 (s, 1H), 8.30 (s, 1H), 8.25 (s, 1H), 8.05 (d, *J* = 3.2 Hz, 1H), 7.98 (d, *J* = 8.8 Hz, 2H), 7.83 (d, *J* = 8.4 Hz, 2H), 7.75 (d, *J* = 4.8 Hz, 1H), 7.29 (s, 2H), 7.23 (t, *J* = 4.2 Hz, 1H), 5.24-5.18 (m, 1H), 1.52 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.4, 151.6, 150.0, 144.3, 141.9, 139.9, 137.1, 132.4, 130.3, 129.1, 128.3, 127.1, 120.7, 112.1, 111.8, 49.0, 22.4; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{18}N_5O_3S_2$ ([M-H]$^-$): 440.0851, Found: 440.0849.

***N*-(2-Fluorophenyl)-1-isopropyl-6-(thiazol-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX007.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48-8.44 (m, 3H), 8.37 (br s, 1H), 7.98 (d, *J* = 3.2 Hz, 1H), 7.53 (d, *J* = 3.2 Hz, 1H), 7.25-7.15 (m, 3H), 5.43-5.36 (m, 1H), 1.67 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.6, 163.0, 153.0 (d, *J* = 242.8 Hz), 150.1, 150.0, 144.2, 136.4, 132.0, 125.7 (d, *J* = 9.9 Hz), 125.5 (d, *J* = 7.6 Hz), 124.8 (d, *J* = 3.8 Hz), 122.4, 122.3, 115.1 (d, *J* = 19.1 Hz), 113.5, 110.8, 49.4, 22.1; HRMS (ESI, *m*/*z*): calcd for $C_{19}H_{15}FN_5OS$ ([M-H]$^-$): 380.0981, Found: 380.0984.

***N*-(2,4-Difluorophenyl)-1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX008.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46-8.40 (m, 1H), 8.28 (s, 1H), 8.21 (br s, 1H), 7.90 (s, 1H), 7.76 (dd, *J* = 3.6,1.2 Hz, 1H), 7.47 (dd, *J* = 5.2 Hz, 1.2 Hz, 1H), 7.15-7.13 (m, 1H), 6.99-6.93 (m, 2H), 5.41-5.34 (m, 1H), 1.65 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.4, 159.2 (dd, *J* = 246.3, 11.5 Hz), 153.0 (dd, *J* = 245.1, 11.9 Hz), 151.7, 150.2, 144.2, 135.7, 130.4, 129.0, 128.3, 126.6, 123.2 (dd, *J* = 9.2, 2.0 Hz), 122.1 (dd, *J* = 10.3, 3.8 Hz), 111.7, 111.5 (dd, *J* = 21.5, 3.5 Hz), 110.7, 104.0 (dd, *J* = 26.5, 23.0 Hz), 49.3, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{15}F_2N_4OS$ ([M-H]$^-$): 397.0935, Found: 397.0936.

***N*-(2,6-Difluorophenyl)-1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX009.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.92 (s, 1H), 7.76 (dd, *J* = 3.6, 0.8 Hz, 1H), 7.73 (br, 1H), 7.47 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.32-7.27 (m, 1H), 7.15-7.13 (m, 1H), 7.05-7.00 (m, 2H), 5.41-5.34 (m, 1H), 1.64 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.9, 157.8 (dd, *J* = 250.1, 4.6 Hz), 151.6, 150.2, 144.3, 134.9, 131.1, 128.9, 128.3 (t, *J* = 9.8 Hz), 126.5, 113.3 (t, *J* = 16.1 Hz), 112.0, 111.9 (d, *J* = 18.4 Hz), 111.8, 111.2, 49.2, 22.1; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{15}F_2N_4OS$ ([M-H]$^-$): 397.0935, Found: 397.0936.

***N*-(2-Fluorophenyl)-1-isopropyl-6-(thiazol-5-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX010.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 1H), 8.50 (s, 1H), 8.46 (t, *J* = 8.2 Hz, 1H), 8.42 (s, 1H), 8.33 (s, 1H), 7.93 (s, 1H), 7.25-7.14 (m, 3H), 5.39-5.32 (m, 1H), 1.64 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0, 155.4, 152.9 (d, *J* = 242.4 Hz), 150.1, 149.3, 142.0, 140.1, 136.4, 130.6, 125.7 (d, *J* = 9.9 Hz), 125.5 (d, *J* = 8.1 Hz), 124.8 (d, *J* = 3.9 Hz), 122.2, 115.1 (d, *J* = 19.2 Hz), 112.3, 111.3, 49.4, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{19}H_{15}FN_5OS$ ([M-H]$^-$): 380.0981, Found: 380.0986.

***N*-(2-Fluorophenyl)-1-isopropyl-6-(oxazol-5-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX011.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (t, *J* = 7.6 Hz, 1H), 8.39 (m, 2H), 8.03 (s, 1H), 7.91 (s, 1H), 7.85 (s, 1H), 7.24-7.14 (m, 3H), 5.43-5.36 (m, 1H), 1.63 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.9, 152.8 (d, *J* = 242.5 Hz), 151.7, 150.8, 150.2, 145.7, 136.4, 131.0, 126.4, 125.7 (d, *J* = 10.0 Hz), 125.5 (d, *J* = 7.7 Hz), 124.8 (d, *J* = 3.9 Hz), 122.1, 115.1 (d, *J* = 18.8 Hz), 111.7, 111.3, 49.1, 22.1; HRMS (ESI, *m*/*z*): calcd for $C_{19}H_{15}FN_5O_2$ ([M-H]$^-$): 364.1210, Found: 364.1214.

***N*-(2-Fluorophenyl)-1-phenyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX012.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51-8.47 (m, 2H), 8.38-8.36 (m, 2H), 8.33 (br s, 1H), 7.90 (s, 1H), 7.77 (dd, *J* = 4.0, 1.2 Hz, 1H), 7.57-7.52 (m, 2H), 7.49 (dd, *J* = 4.8, 1.2 Hz, 1H), 7.36-7.32 (m, 1H), 7.27-7.17 (m, 3H), 7.16-7.14 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0, 152.9 (d, *J* = 242.5 Hz), 152.5, 150.7, 144.1, 139.3, 136.5, 133.0, 129.5, 129.1, 128.4, 126.9, 126.3, 125.8 (d, *J* = 9.9 Hz), 125.6 (d, *J* = 7.7 Hz), 124.9 (d, *J* = 3.8 Hz), 122.2, 121.1, 115.1 (d, *J* = 19.2 Hz), 112.5, 111.8; HRMS (ESI, *m*/*z*): calcd for $C_{23}H_{14}FN_4OS$ ([M-H]$^-$): 413.0872, Found: 413.0874.

1-(*tert*-Butyl)-*N*-(2-fluorophenyl)-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX013. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (td, *J* = 8.0, 1.2 Hz, 1H), 8.31 (br s, 1H), 8.26 (s, 1H), 7.92 (s, 1H), 7.76 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.46 (dd, *J* = 4.8, 1.2 Hz, 1H), 7.25-7.14 (m, 4H), 1.91 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.4, 152.7 (d, *J* = 242.4 Hz), 150.8, 150.7, 144.8, 135.8, 129.1, 128.9, 128.3, 126.2, 125.9 (d, *J* = 10.0 Hz), 125.3 (d, *J* = 7.7 Hz), 124.8 (d, *J* = 3.8 Hz), 122.0, 115.0 (d, *J* = 18.7 Hz), 111.8, 111.0, 60.7, 29.2; HRMS (ESI, *m*/*z*): calcd for $C_{21}H_{18}FN_4OS$ ([M-H]$^-$): 393.1185, Found: 393.1190.

1-Cyclobutyl-*N*-(2-fluorophenyl)-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX014. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (t, *J* = 7.6 Hz, 1H), 8.33 (s, 1H), 8.31 (br s, 1H), 7.90 (s, 1H), 7.77 (d, *J* = 3.2 Hz, 1H), 7.48 (d, *J* = 4.8 Hz, 1H), 7.25-7.14 (m, 4H), 5.65-5.56 (m, 1H), 2.95-2.85 (m, 2H), 2.61-2.53 (m, 2H), 2.05-1.94 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.3, 152.8 (d, *J* = 242.5 Hz), 151.8, 150.5, 144.2, 136.0, 130.8, 129.0, 128.3, 126.6, 125.9 (d, *J* = 10.0 Hz), 125.4 (d, *J* = 7.7 Hz), 124.8 (d, *J* = 3.5 Hz), 122.0, 115.0 (d, *J* = 19.2 Hz), 111.8, 110.8, 51.0, 30.1, 15.1; HRMS (ESI, *m*/*z*): calcd for $C_{21}H_{16}FN_4OS$ ([M-H]$^-$): 391.1029, Found: 391.1031.

***N*-(3,4-Difluorophenyl)-1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX015.** ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H), 8.29 (s, 1H), 8.19 (s, 1H), 8.02 (dd, *J* = 4.0, 1.2 Hz, 1H), 7.98-7.92 (m, 1H), 7.74 (dd, *J* = 4.8, 1.2 Hz, 1H), 7.58-7.54 (m, 1H), 7.50-7.42 (m, 1H), 7.23 (dd, *J* = 4.8, 3.6 Hz, 1H), 5.24-5.17 (m, 1H), 1.52 (d, *J* = 6.8 Hz, 6H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.2, 151.5, 150.1, 149.5 (dd, *J* = 242.1, 13.0 Hz), 146.5 (dd, *J* = 241.3, 12.6 Hz), 144.4, 137.1, 136.0 (dd, *J* = 9.1, 3.0 Hz), 132.4, 130.3, 129.1, 128.1, 118.0 (d, *J* = 17.6 Hz), 117.4 (dd, *J* = 6.2, 3.5 Hz), 111.9, 111.8, 110.1 (d, *J* = 21.4 Hz), 49.0, 22.4 ppm; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{15}F_2N_4OS$ ([M-H]$^-$): 397.0935, Found: 397.0935.

***N*-(3,5-Difluorophenyl)-1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX016.** ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.93 (s, 1H), 8.29 (s, 1H), 8.18 (s, 1H), 8.01 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.72 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.55 (dd, *J* = 9.6, 2.4 Hz, 2H), 7.22 (dd, *J* = 5.2, 4.0 Hz, 1H), 6.97 (tt, *J* = 8.0, 2.4 Hz, 1H), 5.23-5.17 (m, 1H), 1.51 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.5, 163.0 (dd, *J* = 241.7, 15.0 Hz), 151.5, 150.1, 144.3, 141.5 (t, *J* = 13.8 Hz), 136.8, 132.3, 130.3, 129.1, 128.1, 112.0, 111.7, 103.8 (dd, *J* = 20.7, 8.5

Hz), 100.0 (t, *J* = 26.1 Hz), 49.0, 22.4; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{15}F_2N_4OS$ ([M-H]$^-$): 397.0935, Found: 397.0932.

***N*-(4-Fluorophenyl)-1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX017.** ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.67 (s, 1H), 8.28 (s, 1H), 8.19 (s, 1H), 8.00 (dd, *J* = 4.0, 1.2 Hz, 1H), 7.82-7.79 (m, 2H), 7.69 (dd, *J* = 5.2, 0.8 Hz, 1H), 7.21-7.16 (m, 3H), 5.25-5.18 (m, 1H), 1.52 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.8, 159.1 (d, *J* = 240.2 Hz), 151.4, 150.1, 144.4, 137.4, 135.2 (d, *J* = 2.7 Hz), 132.4, 130.0, 128.9, 127.8, 122.9 (d, *J* = 8.0 Hz), 115.7 (d, *J* = 22.2 Hz), 111.9, 111.7, 48.9, 22.3; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{16}FN_4OS$ ([M-H]$^-$): 379.1029, Found: 379.1023.

***N*-(2,5-Difluorophenyl)-1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX018.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (br, 1H), 8.36-8.31 (m, 1H), 8.25 (s, 1H), 7.86 (s, 1H), 7.72 (dd, *J* = 4.0, 1.2 Hz, 1H), 7.44 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.14-7.08 (m, 2H), 6.85-6.80 (m, 1H), 5.38-5.32 (m, 1H), 1.64 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.3, 158.6 (dd, *J* = 240.9, 2.3 Hz), 151.6, 150.1, 148.8 (dd, *J* = 237.9, 3.1 Hz), 144.1, 135.3, 130.3, 129.0, 128.2, 126.7 (t, *J* = 11.9 Hz), 126.6, 115.5 (dd, *J* = 21.8, 9.5 Hz), 111.7, 111.2 (dd, *J* = 24.1, 7.6 Hz), 110.5, 109.1 (d, *J* = 29.9 Hz), 49.2, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{15}F_2N_4OS$ ([M-H]$^-$): 397.0935, Found: 397.0934.

***N*-(2,3-Difluorophenyl)-1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX019.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (br, 1H), 8.27 (s, 1H), 8.26-8.22 (m, 1H), 7.89 (s, 1H), 7.75 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.46 (dd, *J* = 4.8, 1.2 Hz, 1H), 7.18-7.12 (m, 2H), 7.03-6.97 (m, 1H), 5.40-5.33 (m, 1H), 1.64 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.4, 151.6, 150.3 (dd, *J* = 246.3, 10.8 Hz), 150.2, 144.2, 141.7 (dd, *J* = 244.0, 15.0 Hz), 135.5,

130.4, 129.0, 128.3, 127.5 (dd, J = 7.3, 2.0 Hz), 126.6, 124.4 (dd, J = 7.3, 4.6 Hz), 117.1 (d, J = 3.5 Hz), 113.0 (d, J = 16.8 Hz), 111.7, 110.6, 49.3, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{15}F_2N_4OS$ ([M-H]$^-$): 397.0935, Found: 397.0936.

1-Isopropyl-6-(thiophen-2-yl)-*N*-(2,4,6-trifluorophenyl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX020. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.61 (s, 1H), 8.30 (s, 1H), 8.29 (s, 1H), 7.99 (dd, J = 3.6, 1.2 Hz, 1H), 7.73 (dd, J = 4.8, 1.2 Hz, 1H), 7.39-7.34 (m, 2H), 7.23 (dd, J = 5.2, 3.6 Hz, 1H), 5.25-5.18 (m, 1H), 1.52 (d, J = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.2, 161.0 (dt, J = 245.5, 15.0 Hz), 158.8 (ddd, J = 248.2, 15.7, 7.7 Hz), 151.7, 150.2, 144.3, 135.4, 132.5, 130.4, 129.1, 128.1, 112.2, 111.9, 111.5 (td, J = 17.2, 5.0 Hz), 101.7 (td, J = 26.4, 2.6 Hz), 49.0, 22.4; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{14}F_3N_4OS$ ([M-H]$^-$): 415.0840, Found: 415.0842.

***N*-(3-Fluorophenyl)-1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX021.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H), 8.20 (br, 1H), 7.80 (s, 1H), 7.71 (d, J = 3.2 Hz, 1H), 7.69-7.66 (m, 1H), 7.46 (d, J = 5.2 Hz, 1H), 7.38-7.34 (m, 2H), 7.12 (dd, J = 4.8, 3.6 Hz, 1H), 6.94-6.89 (m, 1H), 5.38-5.31 (m, 1H), 1.63 (d, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.8, 163.0 (d, J = 244.4 Hz), 151.5, 150.1, 144.3, 138.9 (d, J = 10.7 Hz), 136.2, 130.9, 130.3 (d, J = 9.2 Hz), 128.9, 128.2, 126.5, 115.6 (d, J = 3.1 Hz), 112.0 (d, J = 21.4 Hz), 111.2, 111.0, 107.9 (d, J = 26.4 Hz), 49.2, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{16}FN_4OS$ ([M-H]$^-$): 379.1029, Found: 379.1029.

***N*-(2-Fluorophenyl)-6-(thiophen-2-yl)-1-(2,2,2-trifluoroethyl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX022.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (t, J = 7.6 Hz, 1H), 8.44 (s, 1H), 8.25 (br s, 1H), 7.95 (s, 1H), 7.82 (d, J = 3.2 Hz, 1H), 7.52 (d, J = 4.4 Hz, 1H), 7.24-7.16 (m, 4H),

5.17 (q, *J* = 8.3 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.8, 152.8 (d, *J* = 242.5 Hz), 153.1, 152.0, 143.5, 136.7, 133.7, 129.7, 128.4, 127.3, 125.7 (d, *J* = 10.0 Hz), 125.6 (d, *J* = 7.6 Hz), 124.9 (d, *J* = 3.5 Hz), 123.2 (q, *J* = 278.4 Hz), 122.1, 115.1 (d, *J* = 19.2 Hz), 112.3, 111.0, 47.9 (q, *J* = 35.6 Hz); HRMS (ESI, *m*/*z*): calcd for $C_{19}H_{11}F_4N_4OS$ ([M-H]$^-$): 419.0590, Found: 419.0589.

1-Isopropyl-6-(thiophen-2-yl)-*N*-(2-(trifluoromethyl)phenyl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX023. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, *J* = 8.4 Hz, 1H), 8.42 (br s, 1H), 8.32 (s, 1H), 7.89 (s, 1H), 7.76 (dd, *J* = 3.2, 0.8 Hz, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.66 (t, *J* = 8.0 Hz, 1H), 7.47 (dd, *J* = 4.8, 0.8 Hz, 1H), 7.33 (t, *J* = 7.6 Hz, 1H), 7.15 (dd, *J* = 4.8, 3.6 Hz, 1H), 5.42-5.35 (m, 1H), 1.66 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.6, 151.6, 150.3, 144.2, 135.8, 134.7 (d, *J* = 1.5 Hz), 133.1, 130.6, 128.9, 128.3, 126.5, 126.3 (q, *J* = 5.2 Hz), 125.3, 124.5, 124.1 (q, *J* = 271.3 Hz), 120.5 (q, *J* = 29.5 Hz), 111.4, 110.7, 49.2, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{21}H_{16}F_3N_4OS$ ([M-H]$^-$): 429.0997, Found: 429.0996.

1-Isopropyl-6-(thiophen-2-yl)-*N*-(4-(trifluoromethyl)phenyl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX024. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.96 (s, 1H), 8.30 (s, 1H), 8.23 (s, 1H), 8.04-8.02 (m, 3H), 7.76-7.73 (m, 3H), 7.23 (dd, *J* = 4.8, 3.6 Hz, 1H), 5.24-5.17 (m, 1H), 1.52 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.5, 151.6, 150.1, 144.4, 142.7, 137.2, 132.4, 130.4, 129.1, 128.2, 126.6 (q, *J* = 3.8 Hz), 124.82 (q, *J* = 270.0 Hz), 124.77 (q, *J* = 32.2 Hz), 120.9, 112.1, 111.8, 49.0, 22.4; HRMS (ESI, *m*/*z*): calcd for $C_{21}H_{16}F_3N_4OS$ ([M-H]$^-$): 429.0997, Found: 429.0993.

1-Isopropyl-*N*-(3-sulfamoylphenyl)-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX025. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (s, 1H), 8.39 (s, 1H), 8.32 (s, 1H), 8.25

(s, 1H), 8.03 (dd, *J* = 3.6, 0.8 Hz, 1H), 7.98 (dt, *J* = 7.2, 2.2 Hz, 1H), 7.74 (dd, *J* = 5.2, 0.8 Hz, 1H), 7.62-7.56 (m, 2H), 7.41 (s, 2H), 7.23 (dd, *J* = 5.2, 3.6 Hz, 1H), 5.24-5.18 (m, 1H), 1.52 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.3, 151.6, 150.1, 145.2, 144.4, 139.4, 137.1, 132.4, 130.4, 130.0, 129.1, 128.2, 123.9, 121.9, 118.0, 112.0, 111.9, 49.0, 22.4; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{18}N_5O_3S_2$ ([M-H]$^-$): 440.0851, Found: 440.0844.

***N*-(4-Fluorophenyl)-6-(furan-2-yl)-1-isopropyl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX026.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (br s, 1H), 8.27 (s, 1H), 7.78 (s, 1H), 7.67-7.63 (m, 2H), 7.52 (d, *J* = 0.8 Hz, 1H), 7.17 (d, *J* = 3.2 Hz, 1H), 7.06 (t, *J* = 8.6 Hz, 2H), 6.56-6.55 (m, 1H), 5.37-5.30 (m, 1H), 1.59 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.7, 159.9 (d, *J* = 243.6 Hz), 153.2, 150.2, 147.9, 144.1, 136.3, 133.3 (d, *J* = 2.7 Hz), 131.3, 122.3 (d, *J* = 7.6 Hz), 115.9 (d, *J* = 22.6 Hz), 112.5, 111.2, 110.4, 110.3, 48.7, 22.1; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{16}FN_4O_2$ ([M-H]$^-$): 363.1257, Found: 363.1258.

1-Cyclopentyl-*N*-(4-fluorophenyl)-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX027. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.70 (s, 1H), 8.28 (s, 1H), 8.20 (s, 1H), 8.02 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.83-7.78 (m, 2H), 7.73 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.25-7.19 (m, 3H), 5.40-5.33 (m, 1H), 2.17-2.13 (m, 2H), 2.08-2.00 (m, 2H), 1.95-1.88 (m, 2H), 1.73-1.68 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.9, 159.2 (d, *J* = 239.4 Hz), 151.6, 150.5, 144.4, 137.5, 135.3 (d, *J* = 2.7 Hz), 132.5, 130.3, 129.1, 128.2, 123.0 (d, *J* = 7.6 Hz), 115.9 (d, *J* = 22.2 Hz), 112.0, 111.9, 57.9, 32.5, 24.9; HRMS (ESI, *m*/*z*): calcd for $C_{22}H_{18}FN_4OS$ ([M-H]$^-$): 405.1185, Found: 405.1184.

1-Cyclohexyl-*N*-(4-fluorophenyl)-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX028. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.72 (s, 1H), 8.27 (s, 1H), 8.20 (s, 1H), 8.03

(dd, *J* = 3.6, 0.8 Hz, 1H), 7.83-7.80 (m, 2H), 7.73 (dd, *J* = 5.2, 0.8 Hz, 1H), 7.25-7.20 (m, 3H), 4.82-4.75 (m, 1H), 2.04-1.95 (m, 4H), 1.88-1.84 (m, 2H), 1.72-1.69 (m, 1H), 1.52-1.43 (m, 2H), 1.31-1.24 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.9, 159.1 (d, *J* = 239.8 Hz), 151.5, 150.1, 144.4, 137.6, 135.4 (d, *J* = 2.7 Hz), 132.3, 130.3, 129.1, 128.2, 123.0 (d, *J* = 7.7 Hz), 115.9 (d, *J* = 22.2 Hz), 111.91, 111.86, 56.4, 32.5, 25.6, 25.5; HRMS (ESI, *m*/*z*): calcd for $C_{23}H_{20}FN_4OS$ ([M-H]$^-$): 419.1342, Found: 419.1339.

1-Cycloheptyl-*N*-(4-fluorophenyl)-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX029. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.69 (s, 1H), 8.26 (s, 1H), 8.18 (s, 1H), 8.02 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.82-7.78 (m, 2H), 7.73 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.25-7.19 (m, 3H), 5.07-4.99 (m, 1H), 2.18-2.13 (m, 2H), 2.04-1.99 (m, 2H), 1.86-1.81 (m, 2H), 1.67-1.57 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.9, 159.1 (d, *J* = 239.8 Hz), 151.5, 149.8, 144.4, 137.5, 135.4 (d, *J* = 2.7 Hz), 132.3, 130.3, 129.1, 128.1, 123.0 (d, *J* = 7.7 Hz), 115.9 (d, *J* = 22.2 Hz), 111.8 (2C), 58.3, 34.5, 28.5, 24.6; HRMS (ESI, *m*/*z*): calcd for $C_{24}H_{22}FN_4OS$ ([M-H]$^-$): 433.1498, Found: 433.1495.

***N*-(4-Fluorophenyl)-1-isopropyl-6-(thiophen-3-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX030.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (br s, 1H), 8.24 (s, 1H), 7.98 (d, *J* = 1.6 Hz, 1H), 7.77-7.76 (m, 2H), 7.65-7.61 (m, 2H), 7.39 (dd, *J* = 4.8, 3.2 Hz, 1H), 7.06 (t, *J* = 8.6 Hz, 2H), 5.40-5.33 (m, 1H), 1.61 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.0, 159.9 (d, *J* = 243.6 Hz), 152.3, 150.4, 141.6, 136.3, 133.2 (d, *J* = 2.7 Hz), 130.7, 126.59, 126.55, 125.1, 122.3 (d, *J* = 8.1 Hz), 115.9 (d, *J* = 22.6 Hz), 112.5, 110.8, 49.0, 22.1; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{16}FN_4OS$ ([M-H]$^-$): 379.1029, Found: 379.1027.

_N_-(4-Fluorophenyl)-1-(4-methoxybenzyl)-6-(thiophen-2-yl)-1_H_-pyrazolo[3,4-_b_]pyridine-4-carboxamide, JX031. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.70 (s, 1H), 8.29 (s, 1H), 8.22 (s, 1H), 8.05 (d, J = 2.8 Hz, 1H), 7.82-7.79 (m, 2H), 7.75 (d, J = 4.8 Hz, 1H), 7.30 (d, J = 8.4 Hz, 2H), 7.24-7.20 (m, 3H), 6.84 (d, J = 8.4 Hz, 2H), 5.59 (s, 2H), 3.65 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.8, 159.3, 159.2 (d, J = 239.7 Hz), 152.0, 150.7, 144.4, 137.7, 135.3 (d, J = 2.3 Hz), 133.0, 130.5, 129.9, 129.6, 129.2, 128.2, 123.0 (d, J = 8.1 Hz), 115.9 (d, J = 22.2 Hz), 114.4, 112.0, 111.8, 55.5, 50.4; HRMS (ESI, m/z): calcd for $C_{25}H_{18}FN_4O_2S$ ([M-H]$^-$): 457.1135, Found: 457.1122.

_N_-(4-Fluorophenyl)-6-(thiophen-2-yl)-1_H_-pyrazolo[3,4-_b_]pyridine-4-carboxamide, JX032. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.87 (s, 1H), 10.70 (s, 1H), 8.31 (s, 1H), 8.22 (s, 1H), 8.02 (dd, J = 3.6, 1.2 Hz, 1H), 7.84-7.80 (m, 2H), 7.72 (dd, J = 5.2, 1.2 Hz, 1H), 7.24-7.20 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.1, 159.2 (d, J = 239.8 Hz), 153.0, 151.9, 144.6, 137.3, 135.4 (d, J = 2.7 Hz), 134.0, 130.1, 129.1, 128.0, 123.0 (d, J = 8.0 Hz), 115.9 (d, J = 22.2 Hz), 111.7, 111.3; HRMS (ESI, m/z): calcd for $C_{17}H_{10}FN_4OS$ ([M-H]$^-$): 337.0559, Found: 337.0563.

1-Isopropyl-_N_-(2-sulfamoylphenyl)-6-(thiophen-2-yl)-1_H_-pyrazolo[3,4-_b_]pyridine-4-carboxamide, JX033. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.38-8.36 (m, 2H), 8.14 (s, 1H), 7.95-7.92 (m, 2H), 7.76-7.74 (m, 3H), 7.68 (td, J = 7.8, 1.6 Hz, 1H), 7.40 (td, J = 7.8, 1.2 Hz, 1H), 7.24 (dd, J = 5.2, 4.0 Hz, 1H), 5.26-5.19 (m, 1H), 1.53 (d, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.6, 151.6, 150.2, 144.2, 137.1, 134.9, 133.7, 133.6, 132.2, 130.5, 129.3, 128.7, 127.9, 125.6, 124.1, 111.64, 111.59, 49.1, 22.4; HRMS (ESI, m/z): calcd for $C_{20}H_{18}N_5O_3S_2$ ([M-H]$^-$): 440.0851, Found: 440.0851.

***N*-(4-Fluorophenyl)-3-isopropyl-5-(thiophen-2-yl)-3*H*-imidazo[4,5-*b*]pyridine-7-carboxamide, JX034.** ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.98 (s, 1H), 8.73 (s, 1H), 8.01 (s, 1H), 7.88 (d, *J* = 2.8 Hz, 1H), 7.78-7.74 (m, 2H), 7.59 (d, *J* = 4.8 Hz, 1H), 7.22 (t, *J* = 8.8 Hz, 2H), 7.13 (dd, *J* = 4.8, 3.6 Hz, 1H), 4.78-4.72 (m, 1H), 1.46 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.5, 159.2 (d, *J* = 239.8 Hz), 157.2, 147.5, 145.9, 145.2, 135.3 (d, *J* = 2.7 Hz), 130.7, 128.9, 128.5, 125.8, 122.5 (d, *J* = 8.0 Hz), 120.7, 116.1 (d, *J* = 22.2 Hz), 112.7, 49.1, 23.0; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{16}FN_4OS$ ([M-H]$^-$): 379.1029, Found:379.1036.

***N*-(4-Fluorophenyl)-3-isopropyl-5-(thiophen-2-yl)-3*H*-[1,2,3]triazolo[4,5-*b*]pyridine-7-carboxamide, JX035.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.97 (s, 1H), 8.48 (s, 1H), 7.87 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.86-7.83 (m, 2H), 7.52 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.18 (dd, *J* = 4.8, 3.6 Hz, 1H), 7.13-7.09 (m, 2H), 5.46-5.39 (m, 1H), 1.84 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.2, 159.7 (d, *J* = 243.2 Hz), 154.1, 146.0, 143.5, 134.0 (d, *J* = 2.6 Hz), 132.8, 131.9, 130.1, 128.6, 128.0, 122.0 (d, *J* = 8.0 Hz), 116.7, 115.8 (d, *J* = 22.2 Hz), 51.5, 22.1; HRMS (ESI, *m*/*z*): calcd for $C_{19}H_{15}FN_5OS$ ([M-H]$^-$): 380.0981, Found: 380.0972.

***N*-(4-Fluorophenyl)-1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-4-carboxamide, JX036.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.91 (s, 1H), 8.70 (s, 1H), 8.12 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.79-7.76 (m, 2H), 7.53 (dd, *J* = 4.8, 1.2 Hz, 1H), 7.18 (dd, *J* = 5.2, 3.6 Hz, 1H), 7.12-7.08 (m, 2H), 5.33-5.26 (m, 1H), 1.65 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.5, 159.7 (d, *J* = 243.2 Hz), 156.5, 154.3, 150.9, 142.6, 134.4, 133.0 (d, *J* = 3.1 Hz), 130.3, 129.5, 128.4, 121.5 (d, *J* = 8.1 Hz), 115.9 (d, *J* = 22.2 Hz), 109.9, 49.4, 21.9; HRMS (ESI, *m*/*z*): calcd for $C_{19}H_{15}FN_5OS$ ([M-H]$^-$): 380.0981, Found: 380.0991.

_N_-(4-Fluorophenyl)-1-isopropyl-6-(thiophen-2-yl)-1_H_-pyrrolo[2,3-_b_]pyridine-4-carboxamide, JX037. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.53 (s, 1H), 8.00 (s, 1H), 7.84 (dd, J = 3.6, 0.8 Hz, 1H), 7.82-7.78 (m, 2H), 7.74 (d, J = 3.6 Hz, 1H), 7.57 (dd, J = 5.2, 1.2 Hz, 1H), 7.22-7.18 (m, 2H), 7.15 (dd, J = 4.8, 3.6 Hz, 1H), 6.73 (d, J = 3.6 Hz, 1H), 5.12-5.05 (m, 1H), 1.50 (d, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.4, 158.9 (d, J = 239.4 Hz), 147.6, 145.8, 145.6, 135.7 (d, J = 2.3 Hz), 135.6, 128.8, 128.5, 127.9, 125.4, 122.7 (d, J = 7.7 Hz), 117.4, 115.8 (d, J = 21.9 Hz), 110.1, 100.5, 46.3, 22.8; HRMS (ESI, m/z): calcd for $C_{21}H_{17}FN_3OS$ ([M-H]$^-$): 378.1076, Found: 378.1078.

6-Cyclopropyl-_N_-(4-fluorophenyl)-1-isopropyl-1_H_-pyrazolo[3,4-_b_]pyridine-4-carboxamide, JX038. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (br s, 1H), 8.16 (s, 1H), 7.57-7.53 (m, 2H), 7.25 (s, 1H), 6.98-6.94 (m, 2H), 5.20-5.13 (m, 1H), 2.04-1.99 (m, 1H), 1.50 (d, J = 6.8 Hz, 6H), 1.12-1.08 (m, 2H), 1.01-0.96 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.3, 163.1, 159.7 (d, J = 243.7 Hz), 150.4, 135.2, 133.3 (d, J = 2.7 Hz), 130.7, 122.4 (d, J = 8.0 Hz), 115.7 (d, J = 22.2 Hz), 113.6, 110.4, 48.7, 21.8, 17.6, 11.1; HRMS (ESI, m/z): calcd for $C_{19}H_{19}FN_4O$ ([M-H]$^-$): 337.1465, Found: 337.1464.

4-Fluoro-_N_-(4-fluorobenzoyl)-_N_-(1-isopropyl-6-(thiophen-2-yl)-1_H_-pyrazolo[3,4-_b_]pyridin-4-yl)benzamide, JX039. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87-7.82 (m, 4H), 7.71 (s, 1H), 7.50 (dd, J = 4.0, 1.2 Hz, 1H), 7.41 (dd, J = 5.2, 1.2 Hz, 1H), 7.13 (s, 1H), 7.09-7.03 (m, 5H), 5.34-5.27 (m, 1H), 1.61 (d, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 165.6 (d, J = 254.7 Hz), 152.1, 150.8, 144.1, 141.4, 131.8 (d, J = 9.2 Hz), 129.9 (d, J = 3.1 Hz), 129.2, 128.8, 128.1, 126.2, 116.3 (d, J = 22.2 Hz), 111.3, 110.5, 49.3, 22.0; HRMS (ESI, m/z): calcd for $C_{27}H_{21}F_2N_4O_2S$ ([M+H]$^+$): 503.1353, Found: 503.1317.

1-Isopropyl-*N*-(pyridin-2-yl)-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX040. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1H), 8.45-8.43 (m, 1H), 8.37 (s, 1H), 8.23-8.21 (m, 1H), 7.88 (s, 1H), 7.80-7.76 (m, 1H), 7.70 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.45 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.12 (dd, *J* = 4.8, 3.6 Hz, 1H), 7.07 (ddd, *J* = 7.6, 5.2, 1.2 Hz, 1H), 5.39-5.33 (m, 1H), 1.64 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.8, 151.5, 151.1, 150.2, 148.0, 144.3, 138.6, 136.0, 131.3, 128.9, 128.2, 126.4, 120.5, 114.6, 111.2, 111.1, 49.1, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{19}H_{16}N_5OS$ ([M-H]$^-$): 362.1076, Found: 362.1086.

1-Isopropyl-*N*-(pyrimidin-2-yl)-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX041. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.39 (s, 1H), 8.63 (d, *J* = 4.8 Hz, 2H), 8.36 (s, 1H), 7.88 (s, 1H), 7.71 (dd, *J* = 3.6, 0.8 Hz, 1H), 7.42 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.09-7.05 (m, 2H), 5.35-5.32 (m, 1H), 1.61 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.3, 158.5, 157.4, 151.5, 150.2, 144.2, 135.7, 131.5, 128.8, 128.2, 126.5, 117.3, 111.2, 111.1, 49.1, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{18}H_{15}N_6OS$ ([M-H]$^-$): 363.1028, Found: 363.1034.

1-Isopropyl-*N*-(pyridin-4-yl)-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX042. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.46 (s, 1H), 8.45 (dd, *J* = 4.8, 1.6 Hz, 2H), 8.20 (s, 1H), 7.73 (s, 1H), 7.66 (dd, *J* = 4.8, 1.6 Hz, 2H), 7.56 (dd, *J* = 3.6, 0.8 Hz, 1H), 7.41 (dd, *J* = 4.8, 0.8 Hz, 1H), 7.05 (dd, *J* = 5.2, 3.6 Hz, 1H), 5.29-5.26 (m, 1H), 1.58 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.7, 151.3, 150.5, 150.0, 145.1, 144.0, 135.6, 131.0, 129.0, 128.2, 126.4, 114.3, 111.2, 110.9, 49.1, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{19}H_{16}N_5OS$ ([M-H]$^-$): 362.1076, Found: 362.1080.

1-Isopropyl-*N*-(pyrimidin-4-yl)-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX043. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.86 (d, *J* = 1.2 Hz, 1H), 8.69 (d, *J* =

5.6 Hz, 1H), 8.36 (dd, *J* = 6.0, 1.6 Hz, 1H), 8.31 (s, 1H), 7.85 (s, 1H), 7.71 (dd, *J* = 4.0, 1.2 Hz, 1H), 7.45 (dd, *J* = 4.8, 1.2 Hz, 1H), 7.11 (dd, *J* = 4.8, 3.6 Hz, 1H), 5.38-5.31 (m, 1H), 1.62 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.5, 158.8, 158.4, 156.9, 151.5, 150.2, 144.0, 134.7, 131.0, 129.1, 128.3, 126.6, 111.2, 110.8, 110.7, 49.3, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{18}H_{15}N_6OS$ ($[M-H]^-$): 363.1028, Found: 363.1034.

4-Fluoro-*N*-(1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridin-4-yl)benzenesulfonamide, JX044. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (m, 3H), 7.63 (dd, *J* = 4.0, 1.2 Hz, 1H), 7.46 (s, 1H), 7.42 (dd, *J* = 4.8, 1.2 Hz, 1H), 7.18-7.11 (m, 3H), 5.27-5.20 (m, 1H), 1.55 (d, *J* = 6.4 Hz, 6H), one low-field proton not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7 (d, *J* = 255.9 Hz), 152.4, 150.6, 144.8, 138.3, 134.4 (d, *J* = 3.5 Hz), 130.2 (d, *J* = 9.5 Hz),128.7, 128.4, 128.1, 126.0, 116.9 (d, *J* = 23.0 Hz), 106.0, 99.8, 48.9, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{19}H_{16}FN_4O_2S_2$ ($[M-H]^-$): 415.0699, Found: 415.0706.

1-Isopropyl-*N*-(1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridin-4-yl)-6-(thiophen-2-yl)-1*H*-pyra-zolo[3,4-*b*]pyridine-4-carboxamide, JX045. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 8.32 (s, 1H), 8.24 (s, 1H), 8.09 (s, 1H), 7.87 (s, 1H), 7.70-7.68 (m, 2H), 7.44-7.41 (m, 2H), 7.10-7.06 (m, 2H), 5.37-5.29 (m, 2H), 1.63 (d, *J* = 6.8 Hz, 6H), 1.62 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.2, 152.7, 151.6, 150.7, 150.1, 144.9, 144.0, 138.2, 135.3, 130.3, 129.1, 128.6, 128.34, 128.26, 128.1, 126.6, 126.2, 111.7, 110.6, 106.2, 102.7, 49.3, 49.0, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{27}H_{24}N_7OS_2$ ($[M-H]^-$): 526.1484, Found: 526.1488.

***N*-(4-Fluorophenyl)-1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carbothioamide, JX046.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.33 (s, 1H), 8.14 (s, 1H), 7.83-7.80 (m, 2H), 7.77 (s, 1H), 7.72 (d, *J* = 2.8 Hz, 1H), 7.45 (dd, *J* = 4.8, 0.8 Hz, 1H), 7.19-7.11 (m, 3H), 5.36-5.30 (m,

1H), 1.61 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.3, 161.0 (d, *J* = 246.7 Hz), 151.7, 150.1, 144.3, 143.8, 134.3 (d, *J* = 3.0 Hz), 130.6, 128.8, 128.2, 126.5, 125.6 (d, *J* = 8.0 Hz), 116.1 (d, *J* = 22.6 Hz), 111.0, 110.2, 49.2, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{16}FN_4S_2$ ([M-H]$^-$): 395.0800, Found: 395.0805.

1-Isopropyl-*N*-(4-(methylsulfonyl)phenyl)-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX047. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.04 (s, 1H), 8.30 (s, 1H), 8.24 (s, 1H), 8.07 (dt, *J* = 8.8, 2.0 Hz, 2H), 8.03 (dd, *J* = 4.0, 1.2 Hz, 1H), 7.95 (dt, *J* = 8.8, 2.0 Hz, 2H), 7.74 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.23 (dd, *J* = 4.8, 3.6 Hz, 1H), 5.24-5.18 (m, 1H), 3.18 (s, 3H), 1.52 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.6, 151.6, 150.0, 144.3, 143.6, 137.0, 136.2, 132.3, 130.4, 129.1, 128.6, 128.2, 120.8, 112.1, 117.8, 49.0, 44.3, 22.4; HRMS (ESI, *m*/*z*): calcd for $C_{21}H_{19}N_4O_3S_2$ ([M-H]$^-$): 439.0899, Found: 439.0882.

(5-Fluoroindolin-1-yl)(1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridin-4-yl)methanone, JX048. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (br s, 1H), 8.00 (br s, 1H), 7.69 (d, *J* = 2.8 Hz, 1H), 7.58 (s, 1H), 7.44 (dd, *J* = 5.2, 0.8 Hz, 1H), 7.11 (dd, *J* = 4.8, 4.0 Hz, 1H), 6.98-6.92 (m, 2H), 5.39-5.32 (m, 1H), 4.01 (br s, 12H), 3.11 (t, *J* = 7.0 Hz, 2H), 1.63 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.0, 159.9 (d, *J* = 243.6 Hz), 151.5, 149.7, 144.4, 138.3 (d, *J* = 10.7 Hz), 138.1, 134.2 (d, *J* = 3.5 Hz), 130.7, 128.7, 128.1, 126.3, 118.7 (d, *J* = 7.0 Hz), 113.9 (d, *J* = 23.8 Hz), 112.1 (d, *J* = 24.2 Hz), 110.6, 110.4, 50.5, 49.0, 28.3, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{22}H_{20}FN_4OS$ ([M+H]$^+$): 407.1342, Found: 407.1306.

***N*-(4-Fluorophenyl)-1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-sulfonamide, JX049.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 7.77 (s, 1H), 7.52 (s, 1H), 7.48 (d, *J* = 4.0 Hz, 1H), 7.26-7.22 (m, 2H), 7.03 (d, *J* = 4.0 Hz, 1H), 6.94-6.89 (m, 2H), 5.34-5.27 (m,

1H), 1.57 (d, *J* = 6.8 Hz, 6H), one low-field proton not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.7 (d, *J* = 244.4 Hz), 154.6, 148.9, 137.2, 136.9, 134.8, 132.2 (d, *J* = 3.1 Hz), 131.4, 128.0 (d, *J* = 25.2 Hz), 124.7 (d, *J* = 8.5 Hz), 116.2, 115.9, 113.7, 110.7, 49.5, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{19}H_{16}FN_4O_2S_2$ ($[M-H]^-$): 415.0699, Found: 415.0689.

***N*-(2-Bromo-4-fluorophenyl)-1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX050.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (dd, *J* = 9.2, 5.6 Hz, 1H), 8.53 (br s, 1H), 8.43 (s, 1H), 7.96 (s, 1H), 7.78 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.48 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.37 (dd, *J* = 7.6, 3.2 Hz, 1H), 7.17-7.12 (m, 2H), 5.42-5.36 (m, 1H), 1.65 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.3, 158.9 (d, *J* = 248.2 Hz), 151.7, 150.3, 144.3, 135.8, 131.9 (d, *J* = 3.0 Hz), 130.6, 129.0, 128.3, 126.6, 123.1 (d, *J* = 7.6 Hz), 119.6 (d, *J* = 25.7 Hz), 115.5 (d, *J* = 21.4 Hz), 114.0 (d, *J* = 9.2 Hz), 111.9, 110.5, 49.3, 22.1; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{17}BrFN_4OS$ ($[M+H]^+$): 459.0290, Found: 459.0251.

6-Fluoro-2-(1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridin-4-yl)benzo[*d*]oxazole, JX051. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H), 8.22 (s, 1H), 7.83-7.78 (m, 2H), 7.46 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.38 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.19-7.14 (m, 2H), 5.41-5.34 (m, 1H), 1.67 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.4 (d, *J* = 244.8 Hz), 161.1 (d, *J* = 3.5 Hz), 151.4, 150.7 (d, *J* = 15.0 Hz), 150.2, 144.6, 138.2 (d, *J* = 1.5 Hz), 132.4, 128.7, 128.2, 127.8, 126.4, 121.3 (d, *J* = 10.4 Hz), 113.4 (d, *J* = 24.9 Hz), 111.2, 110.5, 98.9 (d, *J* = 27.9 Hz), 49.1, 22.1; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{16}FN_4OS$ ($[M+H]^+$): 379.1029, Found: 379.0998.

***N*-(3-(*N*,*N*-Dimethylsulfamoyl)phenyl)-1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX052.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (br s, 1H), 8.29 (s, 1H), 8.15 (dt, *J* = 8.0, 0.6 Hz, 1H), 7.96 (t, *J* = 1.8 Hz, 1H), 7.88 (s, 1H), 7.77 (dd, *J* = 3.6, 1.2 Hz,

1H), 7.54-7.46 (m, 2H), 7.44 (dd, *J* = 4.8, 1.2 Hz, 1H), 7.10 (dd, *J* = 5.2, 4.0 Hz, 1H), 5.35-5.28 (m, 1H), 2.63 (s, 6H), 1.60 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.3, 151.5, 150.1, 144.3, 138.4, 136.2, 136.0, 131.4, 129.9, 128.9, 128.2, 126.7, 124.9, 123.7, 119.5, 111.2, 111.1, 49.1, 37.8, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{22}H_{22}N_5O_3S_2$ ([M-H]$^-$): 468.1164, Found: 468.1146.

1-Isopropyl-*N*-(3-(N-methylsulfamoyl)phenyl)-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX053. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.93 (s, 1H), 8.35 (t, *J* = 1.8 Hz, 1H), 8.32 (s, 1H), 8.25 (s, 1H), 8.05 (ddd, *J* = 8.0, 2.0, 0.8 Hz, 1H), 8.02 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.72 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.61 (t, *J* = 7.8 Hz, 1H), 7.54 (ddd, *J* = 8.0, 1.6, 1.2 Hz, 1H), 7.51-7.47 (m, 1H), 7.22 (dd, *J* = 4.8, 3.6 Hz, 1H), 5.25-5.18 (m, 1H), 2.44 (d, *J* = 4.8 Hz, 3H), 1.53 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.3, 151.5, 150.1, 144.4, 140.4, 139.7, 137.0, 132.4, 130.3, 130.2, 129.0, 128.1, 124.4, 122.7, 119.0, 112.0, 111.9, 49.0, 29.2, 22.4; HRMS (ESI, *m*/*z*): calcd for $C_{21}H_{20}N_5O_3S_2$ ([M-H]$^-$): 454.1008, Found: 454.0992.

4-Fluoro-*N*-(1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridin-4-yl)benzamide, JX054. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (br s, 1H), 8.24 (s, 1H), 8.05 (s, 1H), 7.95-7.91 (m, 2H), 7.67 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.40 (dd, *J* = 5.2, 0.8 Hz, 1H), 7.19-7.14 (m, 2H), 7.08 (dd, *J* = 5.2, 4.0 Hz, 1H), 5.34-5.28 (m, 1H), 1.60 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.3 (d, *J* = 252.8 Hz), 165.0, 152.7, 150.7, 145.1, 138.9, 130.1 (d, *J* = 3.0 Hz), 129.8 (d, *J* = 9.2 Hz), 128.7, 128.2, 128.0, 126.1, 116.1 (d, *J* = 21.9 Hz), 106.3, 102.5, 48.8, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{16}FN_4OS$ ([M-H]$^-$): 379.1029, Found: 379.1017.

6-Cyclopropyl-1-isopropyl-*N*-(3-sulfamoylphenyl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX055. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.82 (s, 1H), 8.39 (s, 1H), 8.22 (s, 1H), 7.95

(d, J = 7.6 Hz, 1H), 7.60-7.54 (m, 3H), 7.39 (s, 2H), 5.16-5.09 (m, 1H), 2.33-2.30 (m, 1H), 1.45 (d, J = 6.8 Hz, 6H), 1.16-1.09 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.7, 163.3, 150.4, 145.1, 139.5, 136.0, 131.9, 130.0, 123.8, 121.8, 117.9, 114.2, 111.2, 48.6, 22.3, 18.0, 11.5; HRMS (ESI, m/z): calcd for $C_{19}H_{20}N_5O_3S$ ([M-H]$^-$): 398.1287, Found: 398.1272.

1-Isopropyl-*N*-(pyridin-3-yl)-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX056. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.01 (s, 1H), 8.71 (d, J = 2.0 Hz, 1H), 8.34-8.32 (m, 2H), 8.23 (s, 1H), 7.79 (s, 1H), 7.61 (dd, J = 4.0, 1.2 Hz, 1H), 7.41 (dd, J = 5.2, 1.2 Hz, 1H), 7.32-7.29 (m, 1H), 7.06 (dd, J = 4.8, 4.0 Hz, 1H), 5.34-5.27 (m, 1H), 1.60 (d, J = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.4, 151.4, 150.1, 145.9, 144.1, 141.6, 135.8, 134.6, 131.1, 128.9, 128.2, 128.1, 126.4, 123.9, 111.2, 111.0, 49.2, 22.0; HRMS (ESI, m/z): calcd for $C_{19}H_{16}N_5OS$ ([M-H]$^-$): 362.1076, Found: 362.1066.

1-Isopropyl-*N*-(pyridin-2-yl)-6-(thiophen-3-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX057. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.18 (s, 1H), 8.45 (d, J = 6.8 Hz), 8.40 (s, 1H), 8.25 (s, 1H), 8.04 (dd, J = 2.8, 1.2 Hz, 1H), 7.88 (s, 1H), 7.83-7.77 (m, 2H), 7.43 (dd, J = 5.2, 2.8 Hz, 1H), 7.11-7.08 (m, 1H), 5.45-5.38 (m, 1H), 1.64 (d, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.0, 152.3, 151.1, 150.5, 148.0, 141.6, 138.7, 135.9, 131.2, 126.63, 126.59, 125.1, 120.5, 114.6, 112.4, 111.0, 48.9, 22.1; HRMS (ESI, m/z): calcd for $C_{19}H_{16}N_5OS$ ([M-H]$^-$): 362.1076, Found: 362.1066.

1-Isopropyl-*N*-(pyrimidin-2-yl)-6-(thiophen-3-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX058. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.31 (s, 1H), 8.65 (d, J = 4.8 Hz, 2H), 8.39 (s, 1H), 8.05 (dd, J = 3.2, 1.2, 1H), 7.89 (s, 1H), 7.82 (dd, J = 5.2, 1.2 Hz, 1H), 7.40 (dd, J = 4.8, 2.8 Hz, 1H), 7.08 (t, J = 4.8 Hz, 1H), 5.43-5.36 (m, 1H), 1.63 (d, J = 6.4 Hz, 6H); ^{13}C NMR (100

MHz, $CDCl_3$) δ 163.4, 158.5, 157.4, 152.3, 150.5, 141.6, 135.7, 131.4, 126.63, 126.56, 125.1, 117.3, 112.4, 111.1, 48.9, 22.1; HRMS (ESI, *m*/*z*): calcd for $C_{18}H_{15}N_6OS$ ([M-H]$^-$): 363.1028, Found: 363.1019.

1-Isopropyl-*N*-(pyrimidin-4-yl)-6-(thiophen-3-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX059. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.08 (br s, 1H), 8.91 (d, *J* = 0.8 Hz, 1H), 8.73 (d, *J* = 5.6 Hz, 1H), 8.39 (dd, *J* = 6.0, 1.2 Hz, 1H), 8.36 (s, 1H), 80.8 (dd, *J* = 2.8, 1.2 Hz, 1H), 7.87 (s, 1H), 7.84 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.45 (dd, *J* = 5.2, 3.2 Hz, 1H), 5.45-5.38 (m, 1H), 1.64 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.6, 158.8, 158.5, 156.9, 152.4, 150.5, 141.4, 134.7, 130.9, 126.8, 126.6, 125.3, 112.5, 110.70, 110.68, 49.1, 22.1; HRMS (ESI, *m*/*z*): calcd for $C_{18}H_{15}N_6OS$ ([M-H]$^-$): 363.1028, Found: 363.1018.

1-Isopropyl-*N*-(pyridin-3-yl)-6-(thiophen-3-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX060. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (br s, 1H), 8.71 (d, *J* = 2.4 Hz, 1H), 8.35-8.32 (m, 2H), 8.27 (s, 1H), 7.97 (dd, *J* = 3.2, 1.2 Hz, 1H), 7.81 (s, 1H), 7.76 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.38 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.33-7.30 (m, 1H), 5.40-5.33 (m, 1H), 1.61 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.6, 152.3, 150.4, 145.9, 141.6, 141.5, 135.8, 134.6, 130.9, 128.0, 126.61, 126.56, 125.1, 123.9, 112.5, 110.9, 48.9, 22.1; HRMS (ESI, *m*/*z*): calcd for $C_{19}H_{16}N_5OS$ ([M-H]$^-$): 362.1076, Found: 362.1064.

1-Isopropyl-*N*-(pyridin-4-yl)-6-(thiophen-3-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX061. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (br s, 1H), 8.51 (dd, *J* = 5.2, 1.2 Hz, 2H), 8.26 (s, 1H), 7.96 (dd, *J* = 2.8, 1.2 Hz, 1H), 7.78 (s, 1H), 7.76 (dd, *J* = 4.8, 1.2 Hz, 1H), 7.66 (dd, *J* = 4.8, 1.6 Hz, 2H), 7.40 (dd, *J* = 4.8, 2.8 Hz, 1H), 5.40-5.33 (m, 1H), 1.61 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.7, 152.3, 150.7, 150.4, 144.8, 141.5, 135.6, 130.7, 126.7, 126.6,

125.1, 114.2, 112.5, 110.7, 49.0, 22.1; HRMS (ESI, *m*/*z*): calcd for $C_{19}H_{16}N_5OS$ ($[M-H]^-$): 362.1076, Found: 362.1065.

1-Isopropyl-*N*-(pyridin-3-yl)-6-(thiophen-2-yl)-1*H*-pyrrolo[2,3-*b*]pyridine-4-carboxamide, JX062. ^1H NMR (400 MHz, $CDCl_3$) δ 8.71 (br s, 1H), 8.52 (s, 1H), 8.37-8.34 (m, 2H), 7.83 (s, 1H), 7.59 (dd, *J* = 4.0, 1.2 Hz, 1H), 7.42 (s, 1H), 7.33-7.30 (m, 2H), 7.06 (dd, *J* = 5.2, 4.0 Hz, 1H), 6.71 (d, *J* = 3.6 Hz, 1H), 5.26-5.16 (m, 1H), 1.56 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 147.8, 146.1, 145.6, 145.5, 141.4, 134.7, 133.8, 128.0, 127.6, 127.2, 126.8, 124.3, 123.8, 116.2, 110.3, 98.8, 46.2, 22.7; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{17}N_4OS$ ($[M-H]^-$): 361.1123, Found: 361.1112.

1-Isopropyl-*N*-(pyridin-4-yl)-6-(thiophen-2-yl)-1*H*-pyrrolo[2,3-*b*]pyridine-4-carboxamide, JX063. ^1H NMR (400 MHz, $CDCl_3$) δ 8.80 (br s, 1H), 8.49 (d, *J* = 6.0 Hz, 2H), 7.77 (s, 1H), 7.64 (dd, *J* = 4.8, 1.6 Hz, 2H), 7.55 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.42 (d, *J* = 3.6 Hz, 1H), 7.33 (dd, *J* = 4.8, 1.2 Hz, 1H), 7.05 (dd, *J* = 4.8, 3.6 Hz, 1H), 6.69 (d, *J* = 3.2 Hz, 1H), 5.24-5.17 (m, 1H), 1.55 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6, 150.7, 147.8, 146.0, 145.4, 145.0, 133.6, 128.0, 127.3, 126.8, 124.3, 116.1, 113.9, 110.2, 98.8, 46.2, 22.7; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{17}N_4OS$ ($[M-H]^-$): 361.1123, Found: 361.1112.

***N*-(3-Carbamoylphenyl)-1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX064.** ^1H NMR (400 MHz, DMSO-d_6) δ 10.76 (s, 1H), 8.32 (s, 1H), 8.27 (s, 1H), 8.25 (s, 1H), 8.03 (d, *J* = 2.8 Hz, 1H), 7.99-7.97 (m, 2H), 7.74 (dd, *J* = 4.8, 0.4 Hz, 1H), 7.64 (d, *J* = 8.0 Hz, 1H), 7.46 (t, *J* = 8.0 Hz, 1H), 7.37 (s, 1H), 7.23 (dd, *J* = 4.8, 3.6 Hz, 1H), 5.24-5.17 (m, 1H), 1.52 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.2, 164.0, 151.6, 150.1,

144.4, 139.0, 137.3, 135.6, 132.5, 130.3, 129.1, 128.2, 123.8, 123.5, 120.8, 112.0, 111.9, 49.0, 22.4; HRMS (ESI, *m*/*z*): calcd for $C_{21}H_{18}N_5O_2S$ ([M-H]$^-$): 404.1181, Found: 404.1166.

(*Z*)-1-Isopropyl-*N*'-(pyridin-3-yl)-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboximidamide, JX065. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38-8.34 (m, 2H), 8.31 (s, 1H), 7.89 (s, 1H), 7.77 (d, *J* = 3.2 Hz, 1H), 7.45 (dd, *J* = 4.8, 0.8 Hz, 1H), 7.40-7.38 (m, 1H), 7.34-7.31 (m, 1H), 7.14 (dd, *J* = 4.8, 4.0 Hz, 1H), 5.40-5.33 (m, 1H), 5.26 (s, 2H), 1.63 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.7, 151.4, 150.2, 145.1, 144.9, 144.6, 143.1, 137.5, 131.6, 128.9, 128.6, 128.2, 126.3, 124.2, 111.6, 111.1, 49.0, 22.1; HRMS (ESI, *m*/*z*): calcd for $C_{19}H_{17}N_6S$ ([M-H]$^-$): 361.1235, Found: 361.1224.

1-Isopropyl-*N*-(pyrimidin-5-yl)-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX066. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.16 (s, 2H), 9.03 (s, 1H), 8.51 (br s, 1H), 8.23 (s, 1H), 7.79 (s, 1H), 7.69 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.46 (dd, *J* = 4.8, 1.2 Hz, 1H), 7.11 (dd, *J* = 5.2, 3.6 Hz, 1H), 5.36-5.29 (m, 1H), 1.61 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.3, 154.9, 151.5, 150.1, 148.4, 144.0, 134.9, 133.1, 130.8, 129.2, 128.3, 126.6, 111.3, 110.7, 49.3, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{18}H_{15}N_6OS$ ([M-H]$^-$): 363.1028, Found: 363.1019.

(*Z*)-*N*'-(4-Fluorophenyl)-1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboximidamide, JX067. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.88 (s, 1H), 7.77 (d, *J* = 2.4 Hz, 1H), 7.45 (dd, *J* = 5.2, 0.8 Hz, 1H), 7.15-7.08 (m, 3H), 7.03-6.99 (m, 2H), 5.39-5.33 (m, 1H), 5.10 (br s, 2H), 1.63 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.3 (d, *J* = 240.2 Hz), 153.3, 153.1, 151.3, 150.1, 144.6, 137.8, 131.6, 128.5, 128.1, 126.2, 122.5 (d, *J* = 8.1 Hz), 116.3 (d, *J* = 22.2 Hz), 111.6, 111.0, 48.9, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{17}FN_5S$ ([M-H]$^-$): 378.1189, Found: 378.1176.

***N*-(2-Fluoro-5-sulfamoylphenyl)-1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX068.** ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.79 (s, 1H), 8.32-8.26 (m, 3H), 8.02 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.77-7.73 (m, 2H), 7.58-7.53 (m, 1H), 7.49 (br s, 2H), 7.23 (dd, *J* = 4.8, 3.6 Hz, 1H), 5.25-5.18 (m, 1H), 1.52 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.3, 157.3 (d, *J* = 252.0 Hz), 151.6, 150.1, 144.3, 141.0 (d, *J* = 3.5 Hz), 136.2, 132.3, 130.4, 129.2, 128.2, 126.1 (d, *J* = 13.0 Hz), 125.4 (d, *J* = 8.8 Hz), 124.6 (d, *J* = 2.3 Hz), 117.2 (d, *J* = 21.5 Hz), 112.3, 111.9, 49.0, 22.4; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{17}FN_5O_3S_2$ ([M-H]$^-$): 458.0757, Found: 458.0761.

***N*-(4-Fluoro-3-sulfamoylphenyl)-1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX069.** ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (s, 1H), 8.35 (dd, *J* = 6.4, 2.8 Hz, 1H), 8.31 (s, 1H), 8.23 (s, 1H), 8.07-8.02 (m, 2H), 7.74 (dd, *J* = 4.8, 0.8 Hz, 1H), 7.71 (br s, 2H), 7.46 (t, *J* = 9.2 Hz, 1H), 7.23 (dd, *J* = 4.8, 3.6 Hz, 1H), 5.24-5.17 (m, 1H), 1.52 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.2, 154.8 (d, *J* = 249.0 Hz), 151.5, 150.1, 144.4, 137.0, 135.2 (d, *J* = 3.0 Hz), 132.4, 132.0 (d, *J* = 15.3 Hz), 130.4, 129.1, 128.2, 126.3 (d, *J* = 7.7 Hz), 120.7, 117.9 (d, *J* = 22.2 Hz), 112.0, 111.9 (d, *J* = 12.3 Hz), 49.0, 22.4; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{17}FN_5O_3S_2$ ([M-H]$^-$): 458.0757, Found: 458.0741.

1-Isopropyl-6-phenyl-*N*-(pyridin-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX070. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.21 (br s, 1H), 8.46-8.44 (m, 2H), 8.25-8.23 (m, 1H), 8.15-8.11 (m, 2H), 7.99 (s, 1H), 7.81-7.76 (m, 1H), 7.53-7.44 (m, 3H), 7.09-7.06 (m, 1H), 5.50-5.43 (m, 1H), 1.66 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.0, 156.5, 151.1, 150.7, 148.0, 138.6, 138.5, 136.0, 131.2, 129.8, 128.9, 127.5, 120.5, 114.5, 112.3, 111.3, 49.0, 22.1; HRMS (ESI, *m*/*z*): calcd for $C_{21}H_{18}N_5O$ ([M-H]$^-$): 356.1511, Found: 356.1503.

6-Cyclopropyl-1-isopropyl-*N*-(pyridin-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX071. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.37 (br s, 1H), 8.41 (d, *J* = 8.4 Hz, 1H), 8.30 (s, 1H), 8.11 (d, *J* = 3.6 Hz, 1H), 7.77-7.72 (m, 1H), 7.35 (s, 1H), 7.04-7.01 (m, 1H), 5.26-5.19 (m, 1H), 2.14-2.09 (m, 1H), 1.55 (d, *J* = 6.8 Hz, 6H), 1.16-1.12 (m, 2H), 1.07-1.02 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.3, 163.1, 151.2, 150.6, 147.8, 138.5, 134.9, 131.0, 120.3, 114.5, 113.6, 110.6, 48.7, 21.9, 17.7, 11.1; HRMS (ESI, *m*/*z*): calcd for $C_{18}H_{18}N_5O$ ([M-H]$^-$): 320.1511, Found: 320.1503.

1-Isopropyl-*N*-(pyridin-2-yl)-6-(thiophen-2-yl)-1*H*-pyrrolo[2,3-*b*]pyridine-4-carboxamide, JX072. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.09 (br s, 1H), 8.46 (dt, *J* = 8.4, 1.0 Hz, 1H), 8.23 (ddd, *J* = 5.2, 1.6, 0.8 Hz, 1H), 7.93 (s, 1H), 7.79-7.75 (m, 1H), 7.65 (dd, *J* = 4.0, 0.8 Hz, 1H), 7.44 (d, *J* = 3.6 Hz, 1H), 7.36 (dd, *J* = 4.8, 0.8 Hz, 1H), 7.11 (dd, *J* = 4.8, 3.6 Hz, 1H), 7.05 (ddd, *J* = 7.2, 4.8, 0.8 Hz, 1H), 6.86 (d, *J* = 3.6 Hz, 1H), 5.29-5.23 (m, 1H), 1.58 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.3, 151.4, 148.1, 147.9, 146.1, 145.6, 138.5, 133.9, 128.0, 127.1, 126.7, 124.3, 120.1, 116.3, 114.3, 110.3, 99.1, 46.2, 22.7; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{17}N_4OS$ ([M-H]$^-$): 361.1123, Found: 361.1110.

***N*-(5-Carbamoyl-2-fluorophenyl)-1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX073.** ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.67 (s, 1H), 8.31 (s, 1H), 8.29 (s, 1H), 8.22 (dd, *J* = 7.6, 2.0 Hz, 1H), 8.03-8.01 (m, 2H), 7.85-7.81 (m, 1H), 7.74 (dd, *J* = 4.8, 0.8 Hz, 1H), 7.44-7.40 (m, 2H), 7.23 (dd, *J* = 4.8, 3.6 Hz, 1H), 5.24-5.18 (m, 1H), 1.52 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.8, 167.0, 164.1, 157.9 (d, *J* = 250.9 Hz), 151.6, 150.1, 144.3, 136.3, 132.4, 131.3 (d, *J* = 3.4 Hz), 130.3, 129.2, 128.1, 127.32, 127.28 (d, *J* = 12.3 Hz), 125.3 (d, *J* = 13.1 Hz), 116.3 (d, *J* = 20.3 Hz), 112.0 (d, *J* = 13.8 Hz), 49.0, 22.4; HRMS (ESI, *m*/*z*): calcd for $C_{21}H_{17}FN_5O_2S$ ([M-H]$^-$): 422.1087, Found: 422.1094.

1-Isopropyl-*N*-methyl-*N*-(pyridin-2-yl)-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX074. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (ddd, *J* = 4.8, 2.0, 0.8 Hz, 1H), 7.91 (s, 1H), 7.52 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.42 (td, *J* = 7.6, 2.0 Hz, 1H), 7.40 (dd, *J* = 4.8, 1.2 Hz, 1H), 7.36 (s, 1H), 7.08 (dd, *J* = 5.2, 4.0 Hz, 1H), 7.02 (ddd, *J* = 7.6, 5.2, 1.2 Hz, 1H), 6.97 (d, *J* = 8.0 Hz, 1H), 5.28-5.22 (m, 1H), 3.66 (s, 3H), 1.56 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6, 155.6, 151.0, 149.6, 149.0, 144.5, 137.8, 131.2, 128.5, 128.1, 126.1, 121.7, 120.5, 112.4, 111.4, 48.9, 35.8, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{20}H_{20}N_5OS$ ($[M+H]^+$): 378.1389, Found: 378.1387.

***N*-(5-Fluoropyridin-2-yl)-1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxamide, JX075.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99 (br s, 1H), 8.45 (dd, *J* = 9.2, 4.0 Hz, 1H), 8.33 (s, 1H), 8.14 (d, *J* = 2.8 Hz, 1H), 7.86 (s, 1H), 7.74 (dd, *J* = 3.6, 0.8 Hz, 1H), 7.52 (ddd, *J* = 9.2, 7.6, 3.2 Hz, 1H), 7.46 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.13 (dd, *J* = 4.8, 3.6 Hz, 1H), 5.39-5.33 (m, 1H), 1.64 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.5, 156.7 (d, *J* = 251.2 Hz), 151.5, 150.2, 147.1 (d, *J* = 2.3 Hz), 144.2, 135.6, 135.4 (d, *J* = 30.2 Hz), 131.1, 128.9, 128.3, 126.5, 125.7 (d, *J* = 19.1 Hz), 115.4 (d, *J* = 4.2 Hz), 111.1, 111.0, 49.2, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{19}H_{17}FN_5OS$ ($[M+H]^+$): 382.1138, Found: 382.1128.

(2,3-Dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)(1-isopropyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridin-4-yl)methanone, JX076. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.71 (d, *J* = 4.8 Hz, 1H), 7.65-7.64 (m, 2H), 7.49 (dd, *J* = 7.2, 1.6 Hz, 1H), 7.41 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.09 (dd, *J* = 4.8, 3.6 Hz, 1H), 6.81 (dd, *J* = 7.6, 5.2 Hz, 1H), 5.38-5.31 (m, 1H), 4.34 (t, *J* = 8.4 Hz, 2H), 3.21 (t, *J* = 8.2 Hz, 2H), 1.62 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6, 154.9, 151.0, 149.8, 146.1, 145.0, 137.9, 133.7, 131.0, 128.2, 128.1, 126.0, 125.8, 119.1, 112.1,

111.6, 48.8, 47.3, 25.0, 22.0; HRMS (ESI, *m*/*z*): calcd for $C_{21}H_{20}N_5OS$ ($[M+H]^+$): 390.1389, Found: 390.1386.

Cells and viruses. HeLa-RW cells or LLC-MK2 cells were used as the host cells for the enteroviruses as described previously.[15] The cells were grown in DMEM supplemented with penicillin, streptomycin, glutamine, and 10% fetal bovine serum. . The strains and sources of CVB3-H3, EV-A71, and poliovirus-1 types used and the conditions for their propagation and quantification were described previously.[15]

***In vitro* evaluation of antiviral activities of the new compounds.** Each compound was dissolved in DMSO and tested at concentration of 10 μM in two separate experiments. The compounds that protected the cells from cytopathic effects (CPE) were further evaluated for their *in vitro* efficacies against the representative viruses EV-A71, CV-B3, and poliovirus-1. Serial 2-fold dilutions of each compound were prepared. Cells growing in 96 well plates were infected with enteroviruses at low multiplicities of infection pre-determined to result in 100% cytopathic effects (CPE) in the cultures after 3-4 days incubation. Each dilution of compound was tested in triplicate. Cultures were monitored daily for microscopic signs of typical CPE: rounding of cells and detachment. When CPE appeared maximal in the control wells without the antiviral compounds, the cells were fixed with 4% formaldehyde before staining with 0.25% crystal violet solution. Dead cells and debris were washed out and the remaining blue stain intensity in each well was quantified by spectrophotometry at a wavelength of 590nm (OD_{590}) as a measurement of viability. A 7-point dose-response curve was constructed and the EC_{50} value was estimated using four parameter model or sigmoidal model. For CC_{50} value determination, cells were incubated with serial 2-fold dilutions of a compound for the same period as the virus CPE assay, and then cells were fixed, stained and the plate was read; CC_{50} was not always quantified for a

compound if no antiviral activity was demonstrated. We report the EC_{50}, CC_{50}, and the calculated selectivity index ($SI_{50} = CC_{50}/EC_{50}$).

■ ASSOCIATED CONTENT

Supporting Information.

High field proton and carbon NMR spectra for compounds **JX001** – **JX076**. Supplementary Table S1. Molecular Formula Strings.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. Y. X. and J. Z. contributed equally.

Notes

The authors declare no competing financial interest. Y. X., J. Z., P. A. K., and M. E. J. are coauthors on a patent application that includes the molecules described in this manuscript.

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■ ABBREVIATIONS USED

EV, enterovirus; CV, coxsackievirus; SAR, structure-activity relationship; LLC, Lewis lung carcinoma; SI, selectivity index; DMF, dimethyl formamide.

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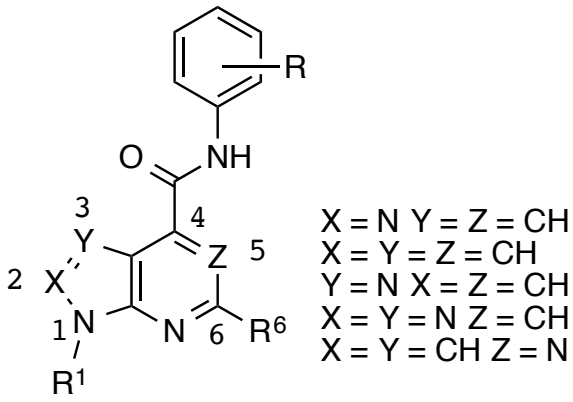
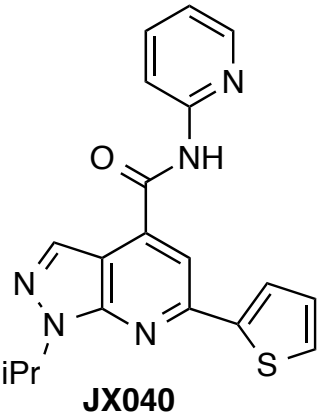
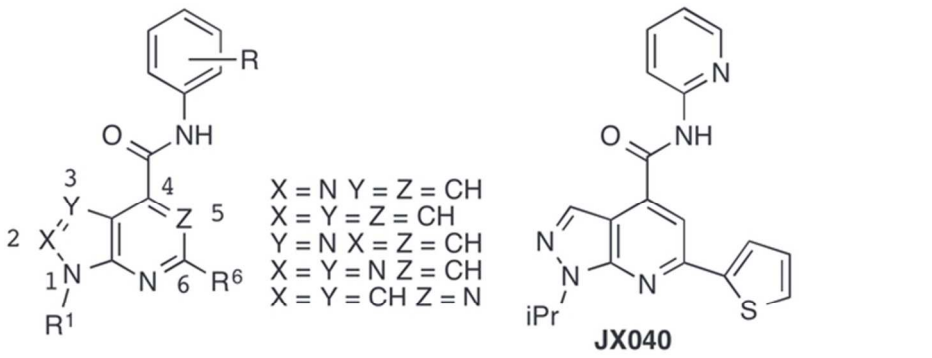
Tables of Contents Graphic





JX040

Strong activity vs enteroviruses, e.g., **JX040**, EC_{50} = 0.5 μM vs EV-A71 in LLC cells



JX040

Strong activity vs enteroviruses, e.g., **JX040**, EC_{50} = 0.5 μM vs EV-A71 in LLC cells

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