Received: 2 March 2018 | Revised: 25 April 2018 | Accepted: 2 May 2018
DOI: 10.1002/ardp.201800062

FULL PAPER

DPhG ARCH PHARM
Archiv der Pharmazie

Synthesis, cytotoxic characterization, and SAR study of imidazo[1,2-*b*]pyrazole-7-carboxamides

András Demjén[1,2] | **Róbert Alföldi**[1] | **Anikó Angyal**[1,2] | **Márió Gyuris**[1] | **László Hackler Jr.**[1] | **Gábor J. Szebeni**[3] | **János Wölfling**[2] | **László G. Puskás**[1,3] | **Iván Kanizsai**[1]

[1] AVIDIN Ltd., Szeged, Hungary

[2] Department of Organic Chemistry, University of Szeged, Szeged, Hungary

[3] Laboratory of Functional Genomics, Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

Correspondence
Dr. Iván Kanizsai, AVIDIN Ltd., Alsó kikötő sor 11/D, Szeged H-6726, Hungary.
Email: i.kanizsai@avidinbiotech.com

Funding information
National Research, Development and Innovation Office (NKFI), Grant numbers: GINOP-2.3.2-15-2016-00030, GINOP-2.3.2-15-2016-00001; Hungarian Academy of Sciences, Grant number: BO/00139/17/8

Abstract

The synthesis and *in vitro* cytotoxic characteristics of new imidazo[1,2-*b*]pyrazole-7-carboxamides were investigated. Following a hit-to-lead optimization exploiting 2D and 3D cultures of MCF-7 human breast, 4T1 mammary gland, and HL-60 human promyelocytic leukemia cancer cell lines, a 67-membered library was constructed and the structure–activity relationship (SAR) was determined. Seven synthesized analogues exhibited sub-micromolar activities, from which compound **63** exerted the most significant potency with a remarkable HL-60 sensitivity (IC_{50} = 0.183 μM).

KEYWORDS
anticancer agents, cytotoxic, Groebke–Blackburn–Bienaymé reaction, imidazo[1,2-*b*]pyrazole, multicomponent reaction

1 | INTRODUCTION

Pyrazoles are versatile pharmacophore scaffolds possessing a wide range of biological activities including anticancer property.[1–5] In particular, their amino-substituted derivatives have attracted considerable attention as extensively studied templates for antitumor agents.[6–12] The related aminopyrazole moiety is featured in several promising drug candidates, such as PHA-533533 (cyclin-dependent kinase inhibitor),[13,14] tozasertib (Aurora kinase A inhibitor),[15,16] and barasertib (Aurora kinase B inhibitor).[17]

Furthermore, the incorporation of the aminopyrazole scaffold into condensed heterocycles has emerged as a powerful strategy for novel anticancer drug development. Numerous pyrazolo[1,5-*a*]pyrimidines,[18–23] pyrazolo[3,4-*d*]pyrimidines,[24–31] pyrazolo[1,5-*a*][1,3,5]-triazines,[32,33] pyrazolo[5,1-*c*][1,2,4]triazoles,[34,35] and other aminopyrazole-fused bicycles[36–42] display remarkable cancer-related enzyme inhibitory activities. Despite several synthetic routes are available for the construction of imidazo[1,2-*b*]pyrazoles, their pharmacological properties are barely studied, and only a limited number of reports focused on their antitumor potential.[43–49] In terms of anticancer activity, 3-aminoimidazo[1,2-*b*]pyrazole-7-carbonitriles **1** were shown to inhibit spleen tyrosine kinase (Syk) with IC_{50} in sub-micromolar range,[50] while ethyl ester analogues **2** acted as potent topoisomerase IIα catalytic inhibitors (Figure 1).[51] Recently, some imidazo[1,2-*b*]pyrazole-7-carboxamides **3** were identified as Bruton's tyrosine kinase inhibitors (Btk; useful in the treatment of hematological malignancies),[52,53] while a series of C-7 aminomethylated derivatives **4** was synthesized and showed considerable antitumor activity against five human (A549, Hs683, MCF-7, SKMEL28, U373) and a murine (B16F10) cancer cell types.[54]

The most facile approach for the assembly of the imidazo[1,2-*b*]-pyrazole framework is the Groebke–Blackburn–Bienaymé three-component (GBB-3CR) reaction.[55–57] By employing this one-step operation, highly functionalized imidazo[1,2-*b*]pyrazoles can be accessed from 3-aminopyrazoles, aldehydes, and isocyanides as starting materials in the presence of Brønsted or Lewis acid catalysts.

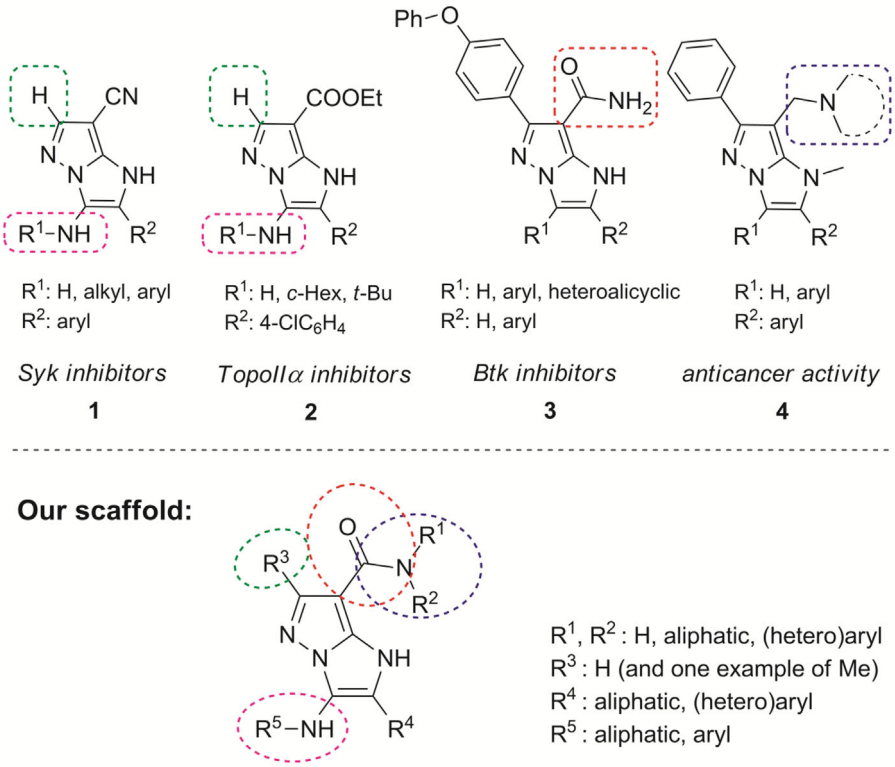


FIGURE 1 Previously reported imidazo[1,2-*b*]pyrazoles with anticancer activity and design strategy

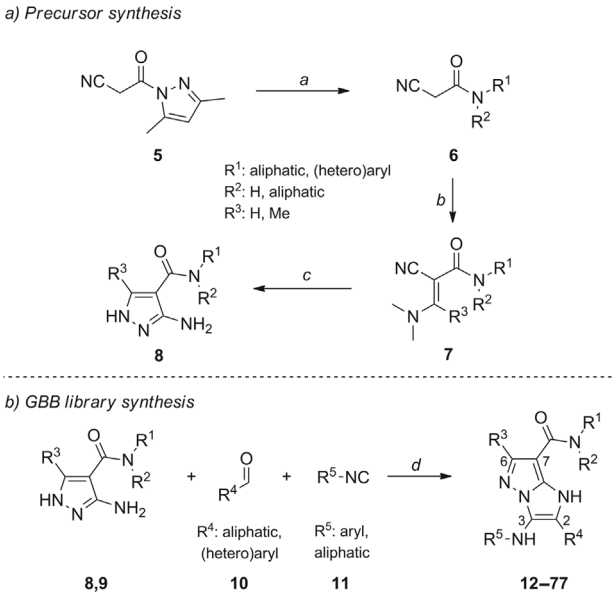
Cell-based phenotypic screening has emerged as a promising approach for the discovery of new drug candidates.[58] *In vitro* cellular screening protocols to identify cytoprotective or cytotoxic compounds are commonly based on two-dimensional (2D) assays.[59–61] However, cells grown on 2D plastic surfaces may result in higher drug sensitivity compared to three-dimensional (3D) culturing methods.[62,63] To apply a more accurate model system, the 2D plastic surfaces can be supplemented by different 3D culturing techniques for better read-outs and more reliable microenvironment modeling.

Herein we report the synthesis and *in vitro* cytotoxic activity of a novel 67-membered imidazo[1,2-*b*]pyrazole-7-carboxamide library. Cytotoxicity of imidazo[1,2-*b*]pyrazole-7-carboxamides was tested on non-adherent acute promyelocytic cell line HL-60 and on adherent breast cancer cell lines 4T1 and MCF-7. In this study, we supplemented the conventional 2D cytotoxicity test with a scaffold-free 3D culturing method using MCF-7 human breast cancer cells and 4T1 mouse mammary gland tumor cells.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The synthesis of the imidazo[1,2-*b*]pyrazole-7-carboxamide library was accomplished by the general synthetic route outlined in Scheme 1. First, the reaction of 3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3-oxopropanenitrile (**5**) with a series of alicyclic-, alkyl- or (hetero)aryl-substituted amines in toluene or DMF resulted in cyanoacetamide intermediates **6**,



SCHEME 1 Synthesis of the imidazo[1,2-*b*]pyrazole-7-carboxamide library and pyrazole precursors. Reagents and conditions: (a) R^1R^2NH, toluene or DMF, 80°C, 6–12 h, 25–97%; (b) *N*,*N*-dimethylformamide dimethyl acetal (R^3 = H) or *N*,*N*-dimethylacetamide dimethyl acetal (R^3 = Me), toluene, 80°C, 6 h, 49–98%; (c) $N_2H_4 \cdot H_2O$, EtOH, reflux, 12 h, 35–91%; (d) $HClO_4$ (20 mol%), MeCN, rt, 6 h, 23–85%

from which condensation reaction with the appropriate *N*,*N*-dimethylamide dimethyl acetal in toluene led to synthones **7**. The successive cyclization step performed with hydrazine monohydrate in refluxing EtOH furnished secondary and tertiary 3-aminopyrazole-4-carboxamides **8**. The 3-aminopyrazole-4-carboxamide (**9**) precursor ($R^1 = R^2 = R^3 = H$) was obtained by a known procedure.[64]

Target compounds **12**–**77** were prepared through GBB-type one-pot three-component assembly of pyrazoles **8** or **9**, aldehydes **10**, and isocyanides **11**. Reactions were performed under a common GBB condition (MeCN as solvent, $HClO_4$ as catalyst). Since an oxidative minor side reaction yielding dehydrogenated 3-imino derivatives of the target products was observed, argon atmosphere was employed. Derivative **78** was accessed by Eschweiler–Clarke reductive methylation of **63**. NMR analysis of **12**–**78** confirmed the exclusive presence of the tautomeric form shown in Scheme 1b.

2.2 | Biological evaluation and structure–activity relationship (SAR)

The *in vitro* antitumor activity of the synthesized imidazo[1,2-*b*]-pyrazole-7-carboxamide library was evaluated against two human (acute promyelocytic leukemia HL-60 and breast adenocarcinoma MCF-7) and a murine (mammary carcinoma 4T1) cancer cell lines using resazurin cell viability colometric assay with doxorubicin as positive control. For a more relevant comparison and hit identification, the traditional 2D MCF-7 and 4T1 screens were supplemented with *in vivo*-like 3D culture models. The reported IC_{50} values are averages of at least two independent experiments and represent the concentration that is required for 50% growth inhibition after an exposure time of 72 h. Of the 67 compound tested 7 possessed sub-micromolar anticancer activities against HL-60 cells, while 14 displayed an IC_{50} in the single-digit μM range in all cell lines. The development of the 67-membered chemical library was divided into two stages: First C-7 primary carboxamides (group 1; modification of R^4 and R^5) were evaluated, followed by C-7 secondary/tertiary carboxamides (group 2; modification of R^1 and R^2).

2.2.1 | SAR of C-7 primary carboxamides (group 1)

At the outset of the study, the substituent effect of R^5 was investigated with a fixed R^4 (phenyl) group. However, no outstanding *in vitro* cytotoxic activities were established (Table 1, compounds **12**–**16**). Only compound **14** ($R^5 = CH_2COOMe$) displayed a moderate efficacy against HL-60 cell line ($IC_{50} = 9.73$ μM). Next we modified R^4 with various (hetero)aryl groups bearing electron-donating and electron-withdrawing substituents (Table 1, compounds **17**–**26**). Most of the tested analogues, independently from the electronic nature of the aromatic ring, showed insignificant or no antitumor activities. The best, however, still unsatisfactory IC_{50} values were obtained for *tert*-octylamino analogues **17** ($R^4 = 4\text{-Me-}C_6H_4$) and **23** ($R^4 = 4\text{-}F_3C\text{-}C_6H_4$).

Replacing the R^4 phenyl group to aliphatic substituents, such as the cyclohexyl moiety, was beneficial in terms of cytotoxicity (Table 2, compound **27**). In contrast, the unbranched *n*-heptyl-containing analogue **28** was proved to be inactive in the tested range. Gratifyingly, by introducing *tert*-butyl group in R^4, appreciable IC_{50} values (<10 μM) could be achieved in all cell lines (compound **29**). Therefore, we turned our attention to the optimization of R^5 keeping *tert*-butyl for R^4. It was found that the replacement of the flexible cyclohexyl with the more compact *tert*-butyl group (homologue **30**) had negative influence on the activity against MCF-7 and 4T1 cells, while compounds with aromatic substituents (**31**: $R^5 = 4\text{-MeO-}C_6H_4$ and **32**: $R^5 = 4\text{-F-}C_6H_4$) completely lost the cytotoxic potency. Interestingly, compound **33** bearing the longer and sterically more demanding *tert*-octyl moiety in R^5 provided better growth inhibitory effects in terms of both the 2D and 3D culture cells than compound **29**. Afterwards, a series of other aliphatic substituents were used as R^4, while R^5 was maintained as *tert*-octyl. Note that the incorporation of smaller cyclopropyl (**34**) or ethyl (**35**) groups led to the dramatic loss of the the overall activity compared to that of **33**, while isopropyl-substituted analogue **36** exhibited moderate, but still lower efficacies than **33**. On the other hand, either the elongation of R^4 in **33** by a vinyl group (compound **37**) or the application of a more bulky substituent (compound **38**) resulted in no improvements in the antitumor potency. From these SAR results, product **33** (R^4 = *t*-Bu, R^5 = *t*-octyl) provided the best bioactivity and, therefore, it was selected for the basis of further hit-to-lead structure optimization.

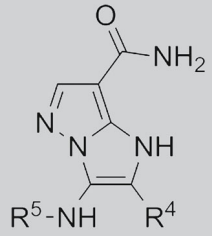
2.2.2 | SAR of C-7 secondary/tertiary carboxamides (group 2)

In this group, the C-7 carboxamide functionality as a new diversity point of the imidazo[1,2-*b*]pyrazole scaffold was exploited. By the variation of R^1 and R^2, a series of simple alkyl-, alicyclic-, benzylic-, and (hetero)aryl-substituted carboxamides were designed and tested for

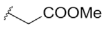
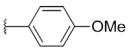
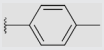
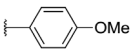
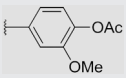
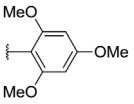
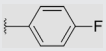
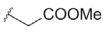
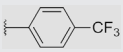
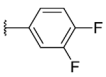
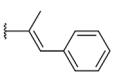
in vitro antitumor activity and compared with hit compound **33** (Table 3). Small aliphatic substituents such as methyl or cyclopropyl were well tolerated (compounds **39** and **42**), and only slight decreases in IC_{50} values were observed compared to those of **33**. In contrast, introduction of the larger *n*-butyl or bulky *t*-butyl moieties led to an apparent reduction in the overall potency (analogues **40** and **41**). Further increase in the size of R^1/R^2 resulted in compounds exhibiting limited solubility or no activity (saturated cyclic derivatives **43**–**45**). Introducing the benzyl group was also detrimental for cytotoxicity (compound **46**). However, when a phenyl group was attached to the carboxamide functionality, a nanomolar HL-60 growth inhibitory activity was established (**47**: $R^1 = H$, $R^2 = C_6H_5$, $IC_{50} = 0.604$ μM). Note that **47** displayed only moderate efficacy against 4T1 and MCF-7 cells ($IC_{50} = 28.5$ and 18.3 μM, respectively). Incorporation of 2-pyridyl moiety provided analogue **48** with two to fivefold lower activities, while thiazole-, isoxazole-, or other pyridine-substituted carboxamides (**49**–**52**) exhibited similar IC_{50} values than hit compound **33**.

Since all synthesized derivatives **39**–**52** displayed lower potencies against 4T1 and MCF-7 cancer cell lines than reference hit compound **33**, hereafter we performed only 2D screens and focused mainly on

TABLE 1 Structure and *in vitro* growth inhibitory concentrations of R^4 = aryl imidazo[1,2-*b*]pyrazole C-7 primary carboxamides **12**–**26** (group 1)



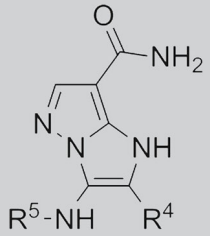
12–26

Compound	R^4	R^5	4T1[a]	3D 4T1[a]	MCF-7[a]	3D MCF-7[a]	HL-60[a]
12			>50	>50	>50	>50	>50
13			>50	>50	>50	>50	50.5
14			>50	>50	>50	>50	9.73
15			>50	>50	>50	47.6	22.7
16			>50	>50	>50	>50	>50
17			12.3	11.4	17.0	11.3	12.6
18			25.2	38.0	18.6	29.5	19.0
19			34.1	37.5	40.6	36.0	26.6
20			>50	>50	>50	>50	>50
21			>50	>50	>50	>50	17.5
22			>50	>50	>50	>50	15.9
23			20.9	17.1	12.7	10.9	13.2
24			>50	>50	>50	>50	>50
25			>50	>50	37.5	>50	>50
26			>50	19.5	>50	24.6	29.2
Doxorubicin			0.057	0.141	0.024	0.063	0.052

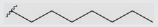
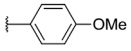
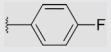
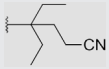
[a]IC_{50} values in μM.

improving the activity against myelocytic leukemia. Encouraged by the significant HL-60 selectivity and cytotoxicity observed for **47** (R^1 = H, R^2 = C_6H_5), we generated several phenyl-substituted carboxamide analogues and examined the effect of the electronic nature of the benzene ring on the anticancer potency (Table 4, compounds **53**–**72**). In comparison with unsubstituted **47**, the introduction of electron-donating alkyl or methoxy groups had a negative influence on activity. Notably, alkyl substituents were better tolerated at *ortho*, while the methoxy group at *para* position (compound **53** vs. **54**/**55** and compound **56** vs. **57**). Incorporation of the electron-withdrawing

TABLE 2 Structure and *in vitro* growth inhibitory concentrations of R^4 = aliphatic imidazo[1,2-*b*]pyrazole C-7 primary carboxamides **27**–**38** (group 1)



27–38

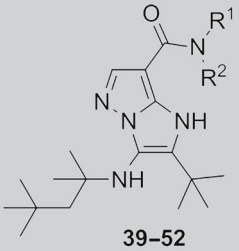
Compound	R^4	R^5	4T1[a]	3D 4T1[a]	MCF-7[a]	3D MCF-7[a]	HL-60[a]
27			12.3	11.5	11.1	13.1	9.35
28			>50	>50	>50	>50	>50
29			**4.24**	**9.09**	**4.07**	**8.91**	**2.95**
30			23.9	13.8	27.9	5.11	4.99
31			>50	>50	>50	>50	8.80
32			>50	>50	40.5	26.5	24.4
33			**1.88**	**2.97**	**1.49**	**3.79**	**1.24**
34			>50	>50	>50	>50	5.49
35			>50	>50	>50	>50	27.9
36			3.63	15.6	10.6	31.3	7.79
37			**9.95**	**4.94**	**8.29**	**5.12**	**1.37**
38			11.6	10.1	18.0	11.8	14.8
Doxorubicin			**0.057**	**0.141**	**0.024**	**0.063**	**0.052**

[a]IC_{50} values in μM. Bold values represent the compounds displaying an IC_{50} < 10 μM in all cell lines tested.

trifluoromethyl moiety also led to a dramatic loss of efficacy, regardless of its position (isomers **58**–**60**). To our surprise, systematic positioning of a fluorine atom on the phenyl ring provided HL-60 sensitive *para*-fluorophenyl derivative **63** with an excellent IC_{50} value of 0.183 μM (R^1 = H, R^2 = 4-F-C_6H_5). It should be highlighted that *ortho* **61** (IC_{50} = 2.04 μM) and mostly *meta* **62** (IC_{50} = 4.47 μM) regioisomers displayed markedly lower levels of cytotoxic activity from a HL-60 point of view. Replacing *para*-fluorine by other halogens, such as chlorine and bromine-induced activities lower by one and two orders of magnitude, respectively (**64**: IC_{50} = 3.05 μM, **65**: IC_{50} = 19.7 μM). Replacement by electron-withdrawing nitro (**66**), cyano (**67**), and ethyl ester (**68**) or electron-donating methylthio (**69**) functionalities was also detrimental for the efficacy against HL-60 cells (IC_{50} = 1.50–8.50 μM). Derivative **70** bearing the *para*-dimethylamino group exhibited a potency and selectivity toward myelocytic leukemia cells similar to that of **63**. Introducing an additional fluorine atom into *ortho* position yielded 2,4-difluorophenyl analogue **71** with an excellent selectivity for HL-60 cancer cells in conjunction with sub-micromolar activity (IC_{50} = 0.628 μM). As expected from the tendency of cytotoxicity observed for mono-fluorinated derivatives **61**–**63** (4-F > 2-F > 3-F), an additional fluorine atom at *meta* position was less tolerated (**72**: R^1 = H, R^2 = 3,4-F-C_6H_4, IC_{50} = 2.41 μM).

Afterwards, several modifications were performed on compound **63** to gain further insight into SARs (Table 5). For example, insertion of a methylene spacer between the carboxamide and the 4-fluorophenyl units resulted in an efficacy against HL-60 cells lower

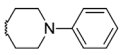
TABLE 3 Structure and *in vitro* growth inhibitory concentrations of imidazo[1,2-*b*]pyrazole C-7 secondary/tertiary carboxamides **39**–**52** (group 2)



39–52

Reference: ***33*** *(R^1=R^2=H)*

IC_{50}	2D 4T1	3D 4T1	2D MCF-7	3D MCF-7	HL60
(μM)	1.88	2.97	1.49	3.79	1.24

Comp.	R^1	R^2	4T1[a]	3D 4T1[a]	MCF-7[a]	3D MCF-7[a]	HL-60[a]
39	H	Me	4.66	3.25	4.46	3.52	1.52
40	H		>50	>50	34.1	31.3	2.72
41	H		>50	>50	>50	>50	>50
42	H		4.01	4.39	1.69	3.50	1.61
43	H		ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
44			ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
45			>50	>50	>50	>50	>50
46	H		9.31	8.72	8.57	7.38	6.26
47	H		28.5	34.7	18.3	15.2	**0.604**
48	H		6.91	6.34	8.35	5.09	4.38
49	H		2.59	2.22	1.90	1.81	**0.934**
50	H		4.34	4.32	2.55	4.20	2.71
51	H		2.41	3.62	1.95	2.81	2.86
52	H		2.85	2.21	1.70	2.07	**0.935**
Doxorubicin			0.057	0.141	0.024	0.063	**0.052**

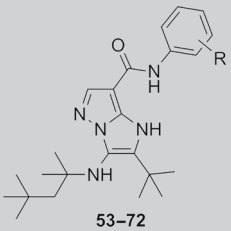
[a]IC_{50} values in μM. Bold values represent compounds displaying an IC_{50} < 1 μM in HL-60 cell line.
[b]ND: not determined because of solubility issues.

by one order of magnitude (**73**, IC_{50} = 8.70 μM). Incorporation of a nitrogen atom at *meta* position (compound **75**) was advantageous for the growth inhibitory activity against 4T1 cells. At the same time, however, it reduced the efficacy in myelocytic leukemia cells (IC_{50} = 0.501 μM). As expected from earlier observations (see Table 3), 2-pyridyl analogue **74** proved to be less effective. *N*-Methylation on the nitrogen of carboxamide or the *tert*-octylamino moiety revealed the importance of the hydrogen-bond donor NH functionalities (compounds **76** and **78**). Interestingly, while tertiary carboxamide **76** gave rise to a lower but moderate overall activity (IC_{50} = 2.50–9.06 μM), the presence of methyl group on the *tert*-octylamino side chain led to dramatic loss of potency, especially from the HL-60 point of view. Installing a methyl substituent at the C-6 position of the imidazo[1,2-*b*]pyrazole scaffold was also detrimental for the overall cytotoxicity (compound **77**).

2.2.3 | Real-time *in vitro* toxicity measurements

Using a cell microelectronic sensing technique, we have determined the kinetics of cell growth inhibition of two selected compounds on MCF-7 cells (Figure 2). A sudden decrease in signal was observed at the highest applied concentrations, while

TABLE 4 Structure and *in vitro* growth inhibitory concentrations of phenyl substituted C-7 carboxamides **53**–**72** (group 2)



53–72

Reference: ***47*** *(R = H)*

IC_{50} (μM)	4T1	MCF-7	HL60
	28.5	18.3	0.604

Compound	R	4T1[a]	MCF-7[a]	HL-60[a]
53	2-Me	>50	30.6	2.12
54	3,5-Me	>50	>50	>50
55	4-iPr	>50	>50	>50
56	4-OMe	24.7	17.8	1.11
57	2,4-OMe	>50	>50	>50
58	2-CF_3	>50	>50	>50
59	3-CF_3	35.4	>50	11.4
60	4-CF_3	>50	29.0	>50
61	2-F	>50	>50	2.04
62	3-F	23.5	17.3	4.47
63	4-F	7.43	2.35	**0.183**
64	4-Cl	30.7	29.3	3.05
65	4-Br	>50	>50	19.7
66	4-NO_2	27.1	9.94	7.64
67	4-CN	>50	9.71	1.50
68	4-COOEt	>50	>50	8.50
69	4-SMe	>50	21.9	3.93
70	4-NMe_2	11.8	10.0	**0.297**
71	2,4-F	>50	>50	**0.628**
72	3,4-F	19.0	10.3	2.41
Doxorubicin		0.057	0.024	**0.052**

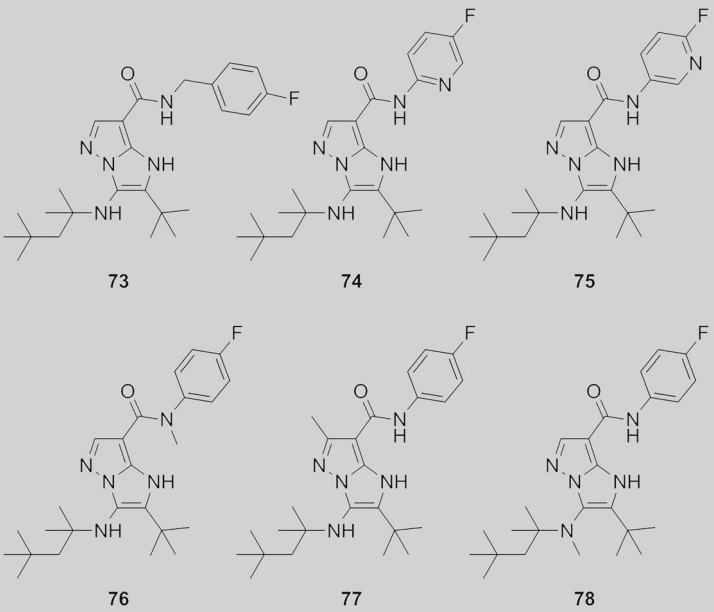
[a]IC_{50} values in μM. Bold values represent compounds displaying an IC_{50} < 1 μM in HL-60 cell line.

treatments at a lower concentration delayed the effect for several hours after treatment. IC_{50} values determined for the 72-h time points were comparable to values determined by the fluorimetric endpoint assay (Tables 2 and 3).

2.2.4 | Imidazo[1,2-*b*]pyrazole-7-carboxamides induce apoptotic cell death of HL-60 cells

In order to reveal the type of cytotoxic effect and distinguish apoptosis from necrosis we performed Annexin V and propidium iodide (PI) staining on HL-60 cells with hit compounds **33** and **63** (Figure 3). For comparison, two moderately active derivatives (**42**: IC_{50} = 1.61, **76**: IC_{50} = 2.50 μM) were also selected for Annexin V assay. Imidazo[1,2-*b*]-pyrazole-7-carboxamides caused dose-dependent increase in Annexin V staining proportional to phosphatidylserine exposure, an early marker of apoptotic cell death. Upon treatment by the selected compounds for 24 h both the early ($AnnV^+/PI^-$) and late apoptotic ($AnnV^+/PI^+$) populations increased without the appearance of the only PI positive necrotic population. Tertiary carboxamide **76** (*N*-methylated derivative of **63**) showed less apoptotic activity, hardly detected at 5 μM, while cyclopropyl-substituted secondary carboxamide analogue **42** induced apoptotosis in 20 and 40% of the treated cells at 2.5 and 5 μM, respectively. On the other hand, compound **33** was more effective inducing apoptosis at 2.5 and 5 μM (40 and 55%, respectively). Superior apoptotic activity was observed for hit compound **63**: 38% of the treated cells were apoptotic at 0.625 μM, which went to saturation at 1.25 μM.

TABLE 5 Structural analogues of compound **63**



Reference: **63**

IC_{50} (μM)	4T1	MCF-7	HL60
	7.43	2.35	0.183

Compound	4T1[a]	MCF-7[a]	HL-60[a]
73	15.6	10.1	8.70
74	14.6	11.8	2.38
75	3.21	2.34	**0.501**
76	9.06	8.44	2.50
77	>50	>50	16.6
78	43.8	11.8	>50
Doxorubicin	0.057	0.024	**0.052**

[a]IC_{50} values in μM. Bold values represent compounds displaying an IC_{50} < 1 μM in HL-60 cell line.

3 | CONCLUSION

A 67-membered library of novel imidazo[1,2-*b*]pyrazole-7-carboxamides has been synthesized and tested *in vitro* against MCF-7 human breast, 4T1 mammary gland, and HL-60 human promyelocytic leukemia cancer cell lines using 2D and 3D culture models. SARs were established and the crucial moieties on the imidazo[1,2-*b*]-pyrazole framework were defined. Among primary carboxamides (group 1, 27 compounds), compound **33** exerted the most significant overall cytotoxic activity with IC_{50} values of 1.24–3.79 μM. From secondary/tertiary carboxamides (group 2, 40 compounds), seven compounds exhibited sub-micromolar activity against HL-60 cells, of which derivative **63** demonstrated the highest potency (IC_{50} = 0.183 μM) with a remarkable HL-60 sensitivity. Annexin V PI assay revealed that the most potent derivatives **33** and **63** induced apoptosis in HL-60 cells. Kinetics of cell growth inhibition induced by **33** on MCF-7 cells were also determined. Further investigations to elucidate the molecular targets and understand the detailed mechanism of action are currently in progress.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

All NMR spectra were recorded in deuterated solvents at 298 K on a Bruker Avance 500 or Bruker Avance Neo 500 spectrometer. All chemical shifts (δ) are reported in ppm relative to the internal standard (TMS) or the residual solvent signal. The following abbreviations are used for NMR multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; p, pentet; sx, sextet; m, multiplet; br, broad, with coupling constants (*J*) given in Hertz (Hz). Melting points were determined on a Stuart SMP10 apparatus and are uncorrected. MS spectra were recorded on an

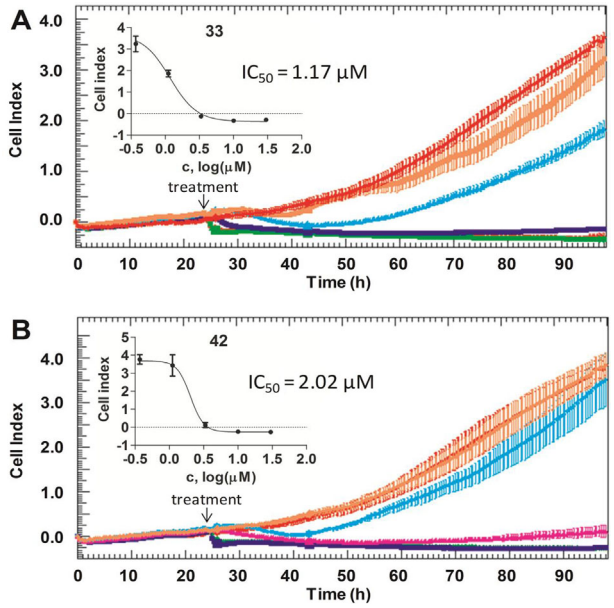


FIGURE 2 Kinetics of cell growth inhibition induced by imidazo[1,2-*b*]pyrazole-7-carboxamides in MCF-7 cells. Red, orange, cyan, magenta, blue, and green traces correspond to 0, 0.37, 1.1, 3.3, 10, and 30 µM concentrations, respectively. Inserts on panels show values determined at 72 h after treatment and the corresponding IC_{50} values for **33** (panel A) and **42** (panel B)

Agilent G1946D mass spectrometer equipped with Multimode Source in positive ESI or APCI mode. TLC was performed on aluminum sheets coated with silica gel 60 F254 (Merck, 1.05554). Visualization was done under UV light (254 nm) or by using potassium permanganate dip. Column chromatography was carried out using silica gel (Merck, 60 Å, 0.063–0.200 mm). Flash column chromatography was performed on a Teledyne Isco CombiFlash Rf system using RediSep Rf columns. Aromatic isocyanides (4-fluorophenyl isocyanide and 4-methoxyphenyl isocyanide) were obtained according to a known procedure.[65] All other reagents and solvents were commercially available and used without further purification.

The NMR spectra and the InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

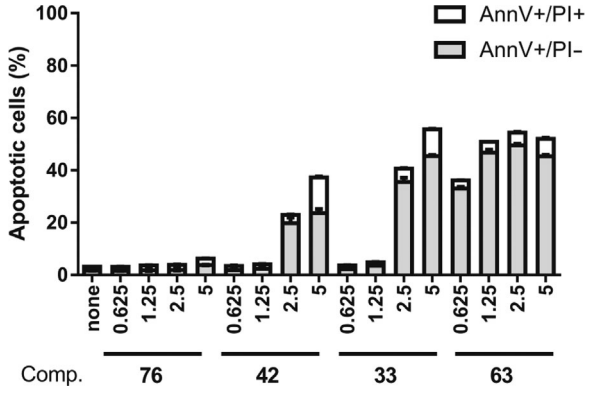


FIGURE 3 Imidazo[1,2-*b*]pyrazole-7-carboxamides induced phosphatidylserine exposure on HL-60 cells after 24 h. Cells were incubated with imidazo[1,2-*b*]pyrazole-7-carboxamides with the indicated concentrations (µM). The results are shown as arithmetic mean values of the summary of early ($AnnV^+/PI^-$, gray column) and late apoptosis ($AnnV^+/PI^+$, white column) of two samples ± SD

4.1.2 | General procedure for the synthesis of aminopyrazoles 8 through intermediates 6 and 7

Pyrazoles **8** were synthesized from cyanoacetic acid derivative **5** through intermediates **6** and **7** by adopting previously published protocols.[66–70]

Step 1: A mixture of 3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3-oxopropanenitrile (**5**) (4.00 mmol, 653 mg) and the corresponding amine (4.80 mmol) in toluene (2 mL) or DMF (if the amine was poorly soluble in toluene; 2 mL) was heated at 80°C for 6–12 h. After the reaction was completed (monitored by TLC), the reaction mixture was poured into aqueous HCl solution (30 mL, 0.5 M) and extracted with EtOAc (3 × 50 mL). The organic layers were combined, washed with saturated Na_2CO_3 followed by brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The obtained crude intermediate **6** was purified either by recrystallization or column chromatography on silica gel (eluent: hexane/EtOAc gradient), or used without further purification in the subsequent reaction. Pyridine derivatives were purified by column chromatography with the omission of the acidic extraction step.

Step 2: Afterwards, *N*,*N*-dimethylformamide dimethyl acetal (for R^3 = H; 3.9 mmol) or *N*,*N*-dimethylacetamide dimethyl acetal (for R^3 = Me; 3.90 mmol) was added to a suspension of cyanoacetamide **6** (3.00 mmol) in toluene (9 mL) and stirred at 80°C for 6 h. The precipitated solid was filtered, washed with toluene, and used directly in the subsequent reaction without characterization. In some cases, further purification by column chromatography on silica gel was necessary.

Step 3: To a solution of crude intermediate **7** (2.00 mmol) in EtOH (4 mL) hydrazine monohydrate (2.20 mmol) was added and stirred at reflux temperature for 12 h. The solvent was removed under vacuum and the residue was purified by column chromatography on silica gel (eluent: chloroform/methanol gradient) to afford pure pyrazoles **8**.

3-Amino-*N*-methyl-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 39)

White solid; yield: 69% (calcd. for *Step 3*); m.p. 178–179°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.74 (s, 1H), 7.68 (s, 1H), 7.63 (s, 1H), 5.71 (bs, 2H), 2.67 (d, *J* = 4.6 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.4, 25.6; ESI-MS (*m*/*z*): 141.0 ($M+H^+$).

3-Amino-*N*-butyl-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 40)

Light beige solid; yield: 63% (calcd. for *Step 3*); m.p. 138–139°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.73 (s, 1H), 7.73 (s, 1H), 7.62 (s, 1H), 5.72 (bs, 2H), 3.15 (q, *J* = 6.6 Hz, 2H), 1.44 (p, *J* = 7.3 Hz, 2H), 1.30 (sx,

J = 7.3 Hz, 2H), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.8, 38.2, 32.2, 20.1, 14.2. ESI-MS (*m/z*): 182.9 ($M+H^+$).

3-Amino-*N*-(*tert*-butyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 41)

Gray solid; yield: 51% (calcd. for *Step 3*); m.p. 199°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.76 (s, 0.4H), 11.63 (s, 0.6H), 8.01 (s, 0.4H), 7.71 (s, 0.6H), 6.90 (s, 1H), 5.85 (s, 1.2H), 5.25 (s, 0.8H), 1.34 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.1, 50.6, 29.5. ESI-MS (*m/z*): 183.1 ($M+H^+$).

3-Amino-*N*-cyclopropyl-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 42)

White solid; yield: 68% (calcd. for *Step 3*); m.p. 92°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.74 (s, 1H), 7.68 (bs, 2H), 5.83 (bs, 2H), 2.74–2.60 (m, 1H), 0.63 (s, 2H), 0.46 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.1, 22.5, 6.3. ESI-MS (*m/z*): 167.0 ($M+H^+$).

3-Amino-*N*-cyclopentyl-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 43)

Gray solid; yield: 48% (calcd. for *Step 3*); m.p. 194°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.76 (s, 0.4H), 11.68 (s, 0.6H), 7.95 (s, 0.4H), 7.70 (s, 0.6H), 7.45 (s, 1H), 5.87 (s, 1.2H), 5.29 (s, 0.8H), 4.14 (q, *J* = 7.2 Hz, 1H), 1.91–1.78 (m, 2H), 1.73–1.61 (m, 2H), 1.58–1.38 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.5, 50.3, 32.8, 24.0. ESI-MS (*m/z*): 195.1 ($M+H^+$).

(3-Amino-1*H*-pyrazol-4-yl)(piperidin-1-yl)methanone (pyrazole 8 leading to product 44)

Light beige solid; yield: 37% (calcd. for *Step 3*); m.p. 168°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.94 (s, 0.4H), 11.77 (s, 0.6H), 7.63 (s, 0.4H), 7.39 (s, 0.6H), 5.78 (s, 1.2H), 5.07 (s, 0.8H), 3.59–3.49 (m, 4H), 1.66–1.57 (m, 2H), 1.54–1.43 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.0, 26.3, 24.7. ESI-MS (*m/z*): 195.1 ($M+H^+$).

(3-Amino-1*H*-pyrazol-4-yl)(4-phenylpiperazin-1-yl)methanone (pyrazole 8 leading to product 45)

Gray solid; yield: 35% (calcd. for *Step 3*); m.p. 197–198°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.02 (s, 0.4H), 11.82 (s, 0.6H), 7.74 (s, 0.4H), 7.51 (s, 0.6H), 7.24 (t, *J* = 7.9 Hz, 2H), 6.96 (d, *J* = 8.1 Hz, 2H), 6.81 (t, *J* = 7.2 Hz, 1H), 5.86 (s, 1.2H), 5.16 (s, 0.8H), 3.74 (t, *J* = 5.1 Hz, 4H), 3.18 (t, *J* = 5.2 Hz, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.3, 151.3, 129.5, 119.6, 116.1, 49.0. ESI-MS (*m/z*): 272.2 ($M+H^+$).

3-Amino-*N*-benzyl-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 46)

Gray solid; yield: 57% (calcd. for *Step 3*); m.p. 146°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.76 (bs, 1H), 8.24 (s, 1H), 7.76 (bs, 1H), 7.36–7.27 (m, 4H), 7.23 (t, *J* = 7.0 Hz, 1H), 5.89 (s, 1.2H), 5.39 (s, 0.8H), 4.39 (d, *J* = 6.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.9, 140.8, 128.7, 127.6, 127.1, 41.9. ESI-MS (*m/z*): 217.1 ($M+H^+$).

3-Amino-*N*-phenyl-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 47)

White solid; yield: 63% (calcd. for *Step 3*); m.p. 206°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.04 (s, 0.3H), 11.83 (s, 0.7H), 9.49 (s, 0.3H), 9.38 (s, 0.7H), 8.23 (s, 0.3H), 7.91 (s, 0.7H), 7.68 (d, *J* = 7.9 Hz, 2H), 7.30 (t, *J* = 7.7 Hz, 2H), 7.02 (t, *J* = 7.3 Hz, 1H), 6.09 (s, 1.4H), 5.42 (s, 0.6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.5, 151.8, 140.0, 138.2, 129.0, 123.0, 120.2. ESI-MS (*m/z*): 203.0 ($M+H^+$).

3-Amino-*N*-(pyridin-2-yl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 48)

Yellow solid; yield: 74% (calcd. for *Step 3*); m.p. 216°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.09 (s, 0.35H), 11.82 (s, 0.65H), 10.04 (bs, 1H), 8.44 (s, 0.35H), 8.32 (dd, *J* = 4.9, 1.9 Hz, 1H), 8.16 (d, *J* = 8.4 Hz, 1H), 8.10 (s, 0.65H), 7.84–7.58 (m, 1H), 7.07 (dd, *J* = 7.3, 4.9 Hz, 1H), 6.17 (s, 1.3H), 5.48 (s, 0.7H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.8, 153.1, 148.1, 138.3, 119.2, 114.5. ESI-MS (*m/z*): 204.1 ($M+H^+$).

3-Amino-*N*-(pyridin-3-yl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 49)

Pale yellow solid; yield: 74% (calcd. for *Step 3*); m.p. 196–198°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.08 (s, 0.3H), 11.86 (s, 0.7H), 9.60 (s, 1H), 8.84 (d, *J* = 2.6 Hz, 1H), 8.23 (dd, *J* = 4.7, 1.5 Hz, 1H), 8.10 (dt, *J* = 8.3, 1.9 Hz, 1H), 7.91 (s, 0.7H), 7.34 (dd, *J* = 8.3, 4.7 Hz, 1H), 6.12 (s, 1.4H), 5.45 (s, 0.6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.7, 144.1, 141.8, 136.7, 127.0, 123.9. ESI-MS (*m/z*): 204.1 ($M+H^+$).

3-Amino-*N*-(pyridin-4-yl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 50)

Yellow solid; yield: 82% (calcd. for *Step 3*); m.p. 248–250°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.10 (s, 0.3H), 11.89 (s, 0.7H), 9.72 (s, 1H), 8.40 (d, *J* = 6.5 Hz, 2H), 7.94 (s, 1H), 7.69 (d, *J* = 6.6 Hz, 2H), 6.18 (s, 1.4H), 5.51 (s, 0.6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.0, 150.5, 146.8, 113.8. ESI-MS (*m/z*): 204.1 ($M+H^+$).

3-Amino-*N*-(thiazol-2-yl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 51)

Light brown; yield: 75% (calcd. for *Step 3*); m.p. 212°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.83 (bs, 2H), 8.17 (s, 1H), 7.47 (d, *J* = 3.6 Hz, 1H), 7.15 (d, *J* = 3.6 Hz, 1H), 6.03 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.3, 159.1, 137.8, 113.3. APCI-MS (*m/z*): 209.9 ($M+H^+$).

3-Amino-*N*-(isoxazol-3-yl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 52)

Light yellow solid; yield: 81% (calcd. for *Step 3*); m.p. 198°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.08 (s, 0.3H), 11.86 (s, 0.7H), 10.68 (s, 1H), 8.76 (d, *J* = 1.7 Hz, 1H), 8.04 (s, 1H), 7.00 (d, *J* = 1.8 Hz, 1H), 6.16 (s, 1.4H), 5.55 (s, 0.6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.9, 160.0, 158.6, 100.0. APCI-MS (*m/z*): 194.2 ($M+H^+$).

3-Amino-*N*-(o-tolyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 53)

Beige solid; yield: 64% (calcd. for *Step 3*); m.p. 190–191°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.83 (bs, 1H), 9.07 (s, 1H), 7.90 (bs, 1H), 7.33 (d, *J* = 7.8 Hz, 1H), 7.24 (d, *J* = 7.4 Hz, 1H), 7.18 (t, *J* = 7.3 Hz, 1H), 7.11 (t, *J* = 7.1 Hz, 1H), 5.96 (s, 1.4H), 5.41 (s, 0.6H), 2.23 (s, 3H). ^{13}C NMR

(126 MHz, DMSO-d_6) δ 163.5, 136.9, 133.6, 130.7, 126.8, 126.3, 125.7, 18.4. APCI-MS (*m/z*): 217.1 ($M+H^+$).

3-Amino-*N*-(3,5-dimethylphenyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 54)

Light beige solid; yield: 80% (calcd. for *Step 3*); m.p. 103°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.01 (s, 0.35H), 11.81 (s, 0.65H), 9.32 (s, 0.35H), 9.22 (s, 0.65H), 8.21 (s, 0.35H), 7.90 (s, 0.65H), 7.32 (s, 2H), 6.67 (s, 1H), 6.08 (s, 1.3H), 5.41 (s, 0.7H), 2.25 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.5, 151.7, 139.9, 138.2, 137.8, 124.6, 118.0, 21.6. APCI-MS (*m/z*): 231.2 ($M+H^+$).

3-Amino-*N*-(4-isopropylphenyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 55)

Gray solid; yield: 61% (calcd. for *Step 3*); m.p. 196°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.99 (s, 0.3H), 11.81 (s, 0.7H), 9.34 (s, 1H), 8.15 (s, 0.3H), 7.90 (s, 0.7H), 7.58 (d, J = 8.4 Hz, 2H), 7.17 (d, J = 8.3 Hz, 2H), 6.05 (s, 1.4H), 5.41 (s, 0.6H), 2.84 (p, J = 6.9 Hz, 1H), 1.19 (d, J = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.4, 143.1, 137.7, 126.7, 120.3, 33.3, 24.5. ESI-MS (*m/z*): 245.1 ($M+H^+$).

3-Amino-*N*-(4-methoxyphenyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 56)

White solid; yield: 58% (calcd. for *Step 3*); m.p. 207°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.96 (s, 0.3H), 11.82 (s, 0.7H), 9.31 (s, 1H), 8.11 (bs, 0.3H), 7.89 (bs, 0.7H), 7.76–7.38 (m, 2H), 7.21–6.65 (m, 2H), 6.03 (bs, 1.4H), 5.42 (bs, 0.6H), 3.73 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.3, 155.4, 133.0, 121.9, 114.1, 55.6. ESI-MS (*m/z*): 233.1 ($M+H^+$).

3-Amino-*N*-(2,4-dimethoxyphenyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 57)

White solid; yield: 63% (calcd. for *Step 3*); m.p. 103°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.00 (s, 0.3H), 11.79 (s, 0.7H), 8.74 (s, 0.3H), 8.61 (s, 0.7H), 8.16 (s, 0.3H), 7.82 (s, 0.7H), 7.47 (d, J = 8.6 Hz, 1H), 6.62 (d, J = 2.7 Hz, 1H), 6.50 (dd, J = 8.7, 2.7 Hz, 1H), 5.97 (s, 1.4H), 5.32 (s, 0.6H), 3.80 (s, 3H), 3.76 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.5, 157.7, 153.4, 126.5, 120.4, 104.5, 99.2, 56.1, 55.8. ESI-MS (*m/z*): 263.1 ($M+H^+$).

3-Amino-*N*-(2-(trifluoromethyl)phenyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 58)

White solid; yield: 53% (calcd. for *Step 3*); m.p. 171°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.08 (s, 0.3H), 11.85 (s, 0.7H), 9.35 (s, 0.3H), 9.21 (s, 0.7H), 8.15 (s, 0.3H), 7.81 (s, 0.7H), 7.76 (d, J = 7.9 Hz, 1H), 7.69 (t, J = 7.7 Hz, 1H), 7.52 (d, J = 8.0 Hz, 1H), 7.47 (t, J = 7.7 Hz, 1H), 6.01 (s, 1.4H), 5.33 (s, 0.6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.1, 151.62, 138.4, 136.3, 133.3, 131.4, 127.1, 126.8 (q, J = 5.1 Hz), 126.2 (q, J = 28.9 Hz), 124.2 (q, J = 273.3 Hz). ESI-MS (*m/z*): 271.1 ($M+H^+$).

3-Amino-*N*-(3-(trifluoromethyl)phenyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 59)

White solid; yield: 85% (calcd. for *Step 3*); m.p. 168–169°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.08 (s, 0.3H), 11.87 (s, 0.7H), 9.70 (s, 1H), 8.19 (s, 1H), 7.93 (dd, J = 8.2, 2.0 Hz, 1H), 7.54 (t, J = 8.0 Hz, 1H), 7.36 (d, J = 7.5 Hz, 1H), 6.14 (s, 1.4H), 5.47 (s, 0.6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.7, 140.9, 130.2, 129.7 (q, J = 31.2 Hz), 124.7 (q, J = 272.2 Hz), 123.4, 119.2, 116.0 (q, J = 4.1 Hz). ESI-MS (*m/z*): 271.0 ($M+H^+$).

3-Amino-*N*-(4-(trifluoromethyl)phenyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 60)

White solid; yield: 78% (calcd. for *Step 3*); m.p. 196°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.12 (s, 0.3H), 11.88 (s, 0.7H), 9.80 (s, 0.3H), 9.71 (s, 0.7H), 8.25 (s, 0.3H), 7.92 (d, J = 8.1 Hz, 2.7H), 7.66 (d, J = 8.4 Hz, 2H), 6.17 (s, 1.4H), 5.47 (s, 0.6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.67, 152.10, 138.24, 126.28, 125.0 (q, J = 271.2 Hz), 119.72. ESI-MS (*m/z*): 271.0 ($M+H^+$).

3-Amino-*N*-(2-fluorophenyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 61)

White solid; yield: 44% (calcd. for *Step 3*); m.p. 186°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.08 (s, 0.3H), 11.84 (s, 0.7H), 9.29 (bs, 1H), 8.21 (s, 0.3H), 7.89 (s, 0.7H), 7.62 (d, J = 8.8 Hz, 1H), 7.30–7.22 (m, 1H), 7.23–7.11 (m, 2H), 6.06 (s, 1.4H), 5.40 (s, 0.6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.4, 155.8 (d, J = 244.1 Hz), 127.2, 126.4, 126.3 (d, J = 7.6 Hz), 124.6 (d, J = 3.5 Hz), 116.1 (d, J = 19.6 Hz). ESI-MS (*m/z*): 221.0 ($M+H^+$).

3-Amino-*N*-(3-fluorophenyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 62)

White solid; yield: 82% (calcd. for *Step 3*); m.p. 189–190°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.04 (s, 0.3H), 11.86 (s, 0.7H), 9.58 (s, 1H), 7.92 (bs, 1H), 7.70 (d, J = 12.2 Hz, 1H), 7.43 (dd, J = 8.2, 1.8 Hz, 1H), 7.33 (q, J = 7.8 Hz, 1H), 6.84 (td, J = 8.5, 2.6 Hz, 1H), 6.11 (s, 1.4H), 5.47 (s, 0.6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.6, 162.6 (d, J = 240.6 Hz), 141.9 (d, J = 11.2 Hz), 130.5 (d, J = 9.4 Hz), 115.7, 109.4 (d, J = 21.1 Hz), 106.7 (d, J = 26.5 Hz). ESI-MS (*m/z*): 221.1 ($M+H^+$).

3-Amino-*N*-(4-fluorophenyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 63)

White solid; yield: 69% (calcd. for *Step 3*); m.p. 113°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.03 (s, 0.3H), 11.84 (s, 0.7H), 9.47 (s, 1H), 8.16 (s, 0.3H), 7.89 (s, 0.7H), 7.68 (dd, J = 8.9, 5.1 Hz, 2H), 7.14 (t, J = 8.8 Hz, 2H), 6.08 (s, 1.4H), 5.43 (s, 0.6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.4, 158.21 (d, J = 239.0 Hz), 136.3, 121.96 (d, J = 7.6 Hz), 115.49 (d, J = 22.0 Hz). ESI-MS (*m/z*): 221.1 ($M+H^+$).

3-Amino-*N*-(4-chlorophenyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 64)

Light beige solid; yield: 83% (calcd. for *Step 3*); m.p. 221°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.04 (s, 0.3H), 11.86 (s, 0.7H), 9.54 (s, 1H), 7.92 (bs, 1H), 7.72 (d, J = 8.9 Hz, 2H), 7.35 (d, J = 8.9 Hz, 2H), 6.09 (s, 1.4H), 5.47 (s, 0.6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.5, 139.0, 128.9, 126.6, 121.6. ESI-MS (*m/z*): 237.0 and 239.0 ($M+H^+$).

3-Amino-*N*-(4-bromophenyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 65)

Gray solid; yield: 63% (calcd. for *Step 3*); m.p. 235°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.04 (s, 0.3H), 11.84 (s, 0.7H), 9.52 (s, 1H),

8.16 (s, 0.3H), 7.90 (s, 0.7H), 7.67 (d, *J* = 8.9 Hz, 2H), 7.48 (d, *J* = 8.8 Hz, 2H), 6.10 (s, 1.4H), 5.43 (s, 0.6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.5, 139.4, 131.8, 122.0. ESI-MS (*m/z*): 281.0 and 283.0 ($M+H^+$).

3-Amino-*N*-(4-nitrophenyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 66)

Yellow solid; yield: 57% (calcd. for *Step 3*); m.p. 270°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.94 (bs, 1H), 9.97 (s, 1H), 8.22 (d, *J* = 8.9 Hz, 2H), 7.97 (d, *J* = 8.9 Hz, 2H), 6.21 (bs, 1.4H), 5.64 (bs, 0.6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.7, 146.7, 142.0, 125.3, 119.3. APCI-MS (*m/z*): 248.1 ($M+H^+$).

3-Amino-*N*-(4-cyanophenyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 67)

Gray solid; yield: 59% (calcd. for *Step 3*); m.p. 268°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.08 (bs, 0.3H), 11.91 (bs, 0.7H), 9.79 (s, 1H), 7.90 (d, *J* = 8.3 Hz, 2H), 7.76 (d, *J* = 8.4 Hz, 2H), 6.17 (s, 1.4H), 5.56 (s, 0.6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.7, 144.5, 133.5, 119.8, 119.8, 104.5. APCI-MS (*m/z*): 228.1 ($M+H^+$).

Ethyl 4-(3-amino-1*H*-pyrazole-4-carboxamido)benzoate (pyrazole 8 leading to product 68)

Gray solid; yield: 55% (calcd. for *Step 3*); m.p. 213°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.11 (s, 0.3H), 11.87 (s, 0.7H), 9.70 (s, 1H), 8.25 (s, 0.3H), 7.95 (s, 0.7H), 7.92 (d, *J* = 8.6 Hz, 2H), 7.85 (d, *J* = 8.5 Hz, 2H), 6.17 (s, 1,4H), 5.47 (s, 0.6H), 4.29 (q, *J* = 7.1 Hz, 2H), 1.32 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.9, 163.6, 144.7, 130.5, 123.8, 119.2, 60.8, 14.7. ESI-MS (*m/z*): 275.1 ($M+H^+$).

3-Amino-*N*-(4-(methylthio)phenyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 69)

Light beige solid; yield: 69% (calcd. for *Step 3*); m.p. 230°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.02 (s, 0.3H), 11.83 (s, 0.7H), 9.42 (s, 1H), 8.17 (s, 0.3H), 7.90 (s, 0.7H), 7.65 (d, *J* = 8.5 Hz, 2H), 7.23 (d, *J* = 8.5 Hz, 2H), 6.08 (s, 1.4H), 5.43 (s, 0.6H), 2.45 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.4, 137.6, 131.4, 127.6, 120.9, 16.1. APCI-MS (*m/z*): 249.1 ($M+H^+$).

3-Amino-*N*-(4-(dimethylamino)phenyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 70)

Gray solid; yield: 53% (calcd. for *Step 3*); m.p. 270°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.82 (s, 1H), 9.20 (s, 1H), 7.95 (s, 1H), 7.46 (d, *J* = 8.7 Hz, 2H), 6.70 (d, *J* = 8.7 Hz, 2H), 5.92 (bs, 2H), 2.85 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.1, 147.3, 129.7, 122.0, 113.1, 41.1. APCI-MS (*m/z*): 246.2 ($M+H^+$).

3-Amino-*N*-(2,4-difluorophenyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 71)

Beige solid; yield: 91% (calcd. for *Step 3*); m.p. 196°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.07 (s, 0.3H), 11.84 (s, 0.7H), 9.31 (bs, 1H), 8.17 (s, 0.3H), 7.85 (s, 0.7H), 7.57 (q, *J* = 8.1 Hz, 1H), 7.32 (ddd, *J* = 11.2, 9.2, 2.8 Hz, 1H), 7.12–7.03 (m, 1H), 6.04 (s, 1.4H), 5.38 (s, 0.6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.5, 159.6 (d, *J* = 243.7 Hz), 159.5 (d, *J* = 243.6 Hz), 128.6, 122.9 (d, *J* = 10.6 Hz), 111.4 (dd, *J* = 21.8, 3.7 Hz), 104.6 (t, *J* = 25.7 Hz). ESI-MS (*m/z*): 238.8 ($M+H^+$).

3-Amino-*N*-(3,4-difluorophenyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 72)

Beige solid; yield: 69% (calcd. for *Step 3*); m.p. 137°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.04 (s, 0.3H), 11.87 (s, 0.7H), 9.61 (s, 1H), 7.88 (ddd, *J* = 13.8, 7.6, 2.1 Hz, 1H), 7.45–7.30 (m, 2H), 6.11 (s, 1.4H), 5.47 (s, 0.6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.5, 149.3 (dd, *J* = 242.1, 13.1 Hz), 145.3 (dd, *J* = 240.3, 12.9 Hz), 137.1 (d, *J* = 8.5 Hz), 117.6 (d, *J* = 17.8 Hz), 116.1 (dd, *J* = 3.7, 3.2 Hz), 108.9 (d, *J* = 21.9 Hz). ESI-MS (*m/z*): 238.8 ($M+H^+$).

3-Amino-*N*-(4-fluorobenzyl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 73)

White solid; yield: 61% (calcd. for *Step 3*); m.p. 149°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.76 (bs, 1H), 8.26 (s, 1H), 7.72 (bs, 1H), 7.32 (dd, *J* = 8.4, 5.6 Hz, 2H), 7.15 (t, *J* = 8.7 Hz, 2H), 5.90 (s, 1.4H), 5.37 (s, 0.6H), 4.36 (d, *J* = 5.9 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.7 (d, *J* = 295.0 Hz), 160.6, 137.0, 129.6 (d, *J* = 8.2 Hz), 115.4 (d, *J* = 21.2 Hz). ESI-MS (*m/z*): 235.1 ($M+H^+$).

3-Amino-*N*-(5-fluoropyridin-2-yl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 74)

Light beige solid; yield: 88% (calcd. for *Step 3*); m.p. 233°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.10 (bs, 0.35H), 11.83 (bs, 0.65H), 10.27 (bs, 0.35H), 10.17 (bs, 0.65H), 8.41 (bs, 0.35H), 8.36–8.30 (m, 1H), 8.24–8.15 (m, 1H), 8.08 (bs, 0.65H), 7.80–7.67 (m, 1H), 6.17 (bs, 1.4H), 5.48 (bs, 0.7H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.6, 155.8 (d, *J* = 247.2 Hz), 152.2, 149.6, 138.7, 135.3 (d, *J* = 24.8 Hz), 125.5 (d, *J* = 19.3 Hz), 115.6 (d, *J* = 4.4 Hz). ESI-MS (*m/z*): 222.1 ($M+H^+$).

3-Amino-*N*-(6-fluoropyridin-3-yl)-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 75)

Beige solid; yield: 52% (calcd. for *Step 3*); m.p. 149°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.07 (bs, 0.3H), 11.88 (bs, 0.7H), 9.70 (bs, 1H), 8.49 (d, *J* = 2.7 Hz, 1H), 8.22 (ddd, *J* = 9.6, 7.6, 2.8 Hz, 1H), 7.89 (bs, 1H), 7.15 (dd, *J* = 8.9, 3.1 Hz, 1H), 6.12 (s, 1.4H), 5.47 (s, 0.6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.5, 158.8 (d, *J* = 231.6 Hz), 138.5 (d, *J* = 15.5 Hz), 135.0, 133.7 (d, *J* = 7.6 Hz), 109.5 (d, *J* = 39.2 Hz). ESI-MS (*m/z*): 222.0 ($M+H^+$).

3-Amino-*N*-(4-fluorophenyl)-*N*-methyl-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 76)

Gray solid; yield: 37% (calcd. for *Step 3*); m.p. 198°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.66 (s, 1H), 7.40–7.34 (m, 2H), 7.35–7.27 (m, 2H), 6.20 (s, 1.6H), 5.77 (s, 0.4H), 5.53 (s, 1H), 3.22 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.1, 161.5 (d, *J* = 244.8 Hz), 141.7, 130.7 (d, *J* = 8.8 Hz), 117.0 (d, *J* = 22.3 Hz), 37.9. ESI-MS (*m/z*): 235.1 ($M+H^+$).

3-Amino-*N*-(4-fluorophenyl)-5-methyl-1*H*-pyrazole-4-carboxamide (pyrazole 8 leading to product 77)

Light beige solid; yield: 69% (calcd. for *Step 3*); m.p. 201°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.84 (bs, 0.6H), 11.53 (bs, 0.4H), 9.05 (bs, 1H), 7.70–7.58 (m, 2H), 7.14 (t, *J* = 8.8 Hz, 2H), 5.76 (bs, 0.8H), 5.09 (bs, 1.2H), 2.37 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.9, 158.3 (d,

J = 239.3 Hz), 136.2, 122.2 (d, *J* = 7.9 Hz), 115.5 (d, *J* = 22.1 Hz). ESI-MS (*m/z*): 235.2 ($M+H^+$).

4.1.3 | General procedure for the synthesis of imidazo[1,2-*b*]pyrazoles 12–77

To a suspension of pyrazole **8** or **9** (0.50 mmol) in MeCN (0.5 mL) aldehyde **10** (0.55 mmol), $HClO_4$ (20 mol%), and isocyanide **11** (0.55 mmol) were added and stirred at room temperature for 6 h. Then the crude mixture was purified by filtration followed by washing with cold MeCN or by column chromatography on silica gel (eluent: hexane/EtOAc or chloroform/methanol gradient) to afford pure products **12**–**77**.

3-(*tert*-Butylamino)-2-phenyl-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (12)

White solid; yield: 69%; m.p. 246–248°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.61 (s, 1H), 7.99 (d, *J* = 7.8 Hz, 2H), 7.94 (s, 1H), 7.39 (t, *J* = 7.6 Hz, 2H), 7.26 (t, *J* = 7.4 Hz, 1H), 7.08 (bs, 1H), 6.87 (bs, 1H), 4.04 (bs, 1H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.9, 142.4, 137.1, 130.6, 128.2, 127.1, 126.7, 124.1, 121.8, 94.0, 54.7, 30.1; ESI-MS (*m/z*): 298.2 ($M+H^+$).

2-Phenyl-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (13)

Pale yellow solid; yield: 51%; m.p. 154–156°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.64 (s, 1H), 7.99 (s, 1H), 7.92 (d, *J* = 7.2 Hz, 2H), 7.43 (t, *J* = 7.7 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 1H), 1.57 (s, 2H), 1.03 (s, 6H), 0.99 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.3, 142.6, 137.5, 131.0, 128.6, 127.8, 127.6, 125.0, 122.1, 94.4, 59.3, 56.0, 32.1, 31.7, 29.5. ESI-MS (*m/z*): 354.2 ($M+H^+$).

Methyl 2-((7-carbamoyl-2-phenyl-1*H*-imidazo[1,2-*b*]pyrazol-3-yl)amino)acetate (14)

Gray solid; yield: 46%; m.p. 209–210°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.45 (s, 1H), 7.95 (s, 1H), 7.83 (d, *J* = 7.7 Hz, 2H), 7.41 (t, *J* = 7.8 Hz, 2H), 7.25 (t, *J* = 7.4 Hz, 1H), 7.12 (bs, 1H), 6.82 (bs, 1H), 5.59 (s, 1H), 4.22 (s, 2H), 3.55 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.9, 163.8, 143.0, 137.3, 130.3, 128.5, 126.4, 126.0, 124.2, 115.3, 93.9, 51.5, 46.0. ESI-MS (*m/z*): 314.1 ($M+H^+$).

3-(Cyclohexylamino)-2-phenyl-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (15)

White solid; yield: 51%; m.p. 240–241°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.49 (s, 1H), 7.95 (s, 1H), 7.91 (d, *J* = 8.0 Hz, 2H), 7.46–7.35 (m, 2H), 7.28–7.19 (m, 1H), 7.12 (bs, 1H), 6.82 (bs, 1H), 4.53 (bs, 1H), 1.81–1.69 (m, 2H), 1.64–1.55 (m, 2H), 1.50–1.40 (m, 1H), 1.24–1.03 (m, 5H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.8, 142.8, 137.1, 130.4, 128.4, 126.6, 125.8, 123.5, 119.9, 94.0, 54.2, 33.2, 25.5, 24.3. ESI-MS (*m/z*): 324.1 ($M+H^+$).

3-((4-Methoxyphenyl)amino)-2-phenyl-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (16)

Gray solid; yield: 48%; m.p. 229–231°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.97 (s, 1H), 7.93 (s, 1H), 7.79 (s, 3H), 7.37 (s, 2H), 7.26 (s, 1H), 6.85 (s, 2H), 6.71 (s, 2H), 6.51 (s, 2H), 3.61 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.9, 152.3, 142.5, 139.5, 137.7, 129.4, 128.5, 127.5, 126.0, 124.9, 117.6, 114.7, 114.3, 94.7, 55.3. ESI-MS (*m/z*): 348.2 ($M+H^+$).

2-(*p*-Tolyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (17)

White solid; yield: 66%; m.p. 218–219°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.54 (s, 1H), 7.92 (s, 1H), 7.81 (d, *J* = 7.8 Hz, 2H), 7.20 (d, *J* = 7.7 Hz, 2H), 6.82 (bs, 1H), 3.95 (bs, 1H), 3.49 (bs, 1H), 2.30 (s, 3H), 1.54 (s, 2H), 0.98 (s, 6H), 0.97 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.9, 142.2, 136.9, 136.5, 128.7, 127.8, 127.1, 124.4, 121.2, 93.9, 58.8, 55.5, 31.7, 31.3, 29.1, 20.9. ESI-MS (*m/z*): 368.3 ($M+H^+$).

2-(4-Methoxyphenyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (18)

Pale yellow solid; yield: 85%; m.p. 124–125°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.43 (s, 1H), 7.91 (s, 1H), 7.86 (d, *J* = 8.3 Hz, 2H), 7.00 (d, *J* = 8.3 Hz, 2H), 6.91–6.74 (bs, 2H), 3.87 (s, 1H), 3.81 (s, 3H), 1.58 (s, 2H), 1.03 (s, 6H), 1.00 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.4, 159.0, 142.5, 137.3, 129.1, 124.8, 123.6, 121.2, 114.1, 94.4, 59.1, 56.1, 55.7, 32.1, 31.8, 29.6. ESI-MS (*m/z*): 384.3 ($M+H^+$).

4-(7-Carbamoyl-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazol-2-yl)-2-methoxyphenyl acetate (19)

White solid; yield: 50%; m.p. 190–191°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.65 (s, 1H), 7.95 (s, 1H), 7.68 (s, 1H), 7.58 (d, *J* = 8.5 Hz, 1H), 7.34–6.97 (m, 1H), 7.09 (d, *J* = 8.3 Hz, 1H), 6.76 (s, 1H), 4.08 (s, 1H), 3.84 (s, 3H), 2.24 (s, 3H), 1.57 (s, 2H), 1.03 (s, 6H), 0.96 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.5, 164.0, 150.5, 142.1, 138.3, 137.3, 129.4, 123.8, 122.5, 121.6, 119.5, 111.7, 94.0, 58.7, 56.0, 55.6, 31.6, 31.3, 29.1, 20.4. ESI-MS (*m/z*): 442.3 ($M+H^+$).

Methyl 2-((7-carbamoyl-2-(2,4,6-trimethoxyphenyl)-1*H*-imidazo[1,2-*b*]pyrazol-3-yl)amino)acetate (20)

Yellow solid; yield: 74%; m.p. 226–227°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.90 (s, 1H), 7.87 (s, 1H), 6.91 (s, 1H), 6.71 (s, 1H), 6.28 (s, 2H), 4.68 (t, *J* = 7.1 Hz, 1H), 3.82 (s, 5H), 3.71 (s, 6H), 3.45 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.4, 163.9, 161.8, 159.6, 142.1, 136.5, 124.9, 105.8, 99.3, 93.4, 90.7, 55.7, 55.4, 51.3, 45.9. ESI-MS (*m/z*): 404.1 ($M+H^+$).

2-(4-Fluorophenyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (21)

Pale yellow solid; yield: 69%; m.p. 229–230°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.66 (s, 1H), 8.08–7.78 (m, 3H), 7.23 (t, *J* = 8.7 Hz, 2H), 6.84 (bs, 2H), 3.97 (s, 1H), 1.52 (s, 2H), 0.98 (s, 6H), 0.95 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.0, 161.3 (d, *J* = 244.7 Hz), 142.1, 137.2, 129.5 (d, *J* = 8.1 Hz), 127.2, 123.6, 121.4, 115.0 (d, *J* = 21.4 Hz), 94.0, 58.7, 55.5, 31.6, 31.3, 29.0. ESI-MS (*m/z*): 372.3 ($M+H^+$).

Methyl 2-((7-carbamoyl-2-(4-fluorophenyl)-1*H*-imidazo[1,2-*b*]pyrazol-3-yl)amino)acetate (22)

White solid; yield: 53%; m.p. 229–230°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.49 (s, 1H), 7.95 (s, 1H), 7.88 (dd, *J* = 8.6, 5.3 Hz, 2H), 7.25 (t, *J* = 8.7 Hz, 2H), 7.11 (bs, 1H), 6.79 (bs, 1H), 5.55 (t, *J* = 6.2 Hz, 1H), 4.19 (d, *J* = 6.2 Hz, 2H), 3.55 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.9, 163.8, 160.9 (d, *J* = 244.5 Hz), 142.8, 137.3, 128.3 (d, *J* = 5.0 Hz), 126.8, 123.8, 115.3 (d, *J* = 21.3 Hz), 115.1, 93.9, 51.5, 46.0. ESI-MS (*m/z*): 332.1 (M+H$^+$).

2-(4-(Trifluoromethyl)phenyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (23)

White solid; yield: 69%; m.p. 192–193°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.78 (s, 1H), 8.19 (d, *J* = 8.2 Hz, 2H), 8.01 (s, 1H), 7.77 (d, *J* = 8.2 Hz, 2H), 1.60 (s, 2H), 1.06 (s, 6H), 0.99 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.3, 143.0, 138.3, 135.2, 128.0, 127.7 (q, *J* = 32.0 Hz), 125.4 (q, *J* = 3.5 Hz), 124.8 (q, *J* = 271.7 Hz), 123.4, 94.5, 59.5, 56.0, 32.0, 31.7, 29.5. ESI-MS (*m/z*): 422.3 (M+H$^+$).

3-(*tert*-Butylamino)-2-(3,4-difluorophenyl)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (24)

White solid; yield: 80%; m.p. 256–258°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.73 (s, 1H), 8.12 (ddd, *J* = 13.0, 7.8, 2.2 Hz, 1H), 7.97 (s, 1H), 7.92–7.84 (m, 1H), 7.46 (dt, *J* = 10.7, 8.7 Hz, 1H), 7.13 (bs, 1H), 6.85 (bs, 1H), 4.25 (bs, 1H), 1.05 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.0, 149.2 (dd, *J* = 243.6, 12.7 Hz), 148.3 (dd, *J* = 246.6, 12.4 Hz), 142.3, 137.6, 128.2, 123.6, 122.3, 117.34 (d, *J* = 17.1 Hz), 115.51 (d, *J* = 19.5 Hz), 94.1, 54.8, 30.1. ESI-MS (*m/z*): 334.4 (M+H$^+$).

2-(Pyridin-3-yl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (25)

White solid; yield: 82%; m.p. 226–228°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.82 (s, 1H), 9.11 (s, 1H), 8.46 (s, 1H), 8.27 (d, *J* = 8.1 Hz, 1H), 7.97 (s, 1H), 7.43 (t, *J* = 6.6 Hz, 1H), 7.14 (bs, 1H), 6.80 (bs, 1H), 4.18 (s, 1H), 1.51 (s, 2H), 0.98 (s, 6H), 0.94 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.0, 147.9, 147.6, 142.2, 138.0, 134.6, 126.9, 123.2, 122.4, 121.7, 94.1, 58.7, 55.5, 31.6, 31.3, 29.1. ESI-MS (*m/z*): 355.3 (M+H$^+$).

(*E*)-3-(*tert*-Butylamino)-2-(1-phenylprop-1-en-2-yl)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (26)

White solid; yield: 65%; m.p. 190–191°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.33 (s, 1H), 7.92 (s, 1H), 7.40–7.34 (m, 4H), 7.28–7.19 (m, 1H), 7.02 (s, 1H), 6.77 (bs, 1H), 4.15 (bs, 1H), 3.40 (bs, 1H), 2.29 (s, 3H), 1.11 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.0, 142.2, 137.2, 136.7, 128.9, 128.5, 128.3, 127.2, 127.0, 126.6, 121.9, 93.8, 54.5, 30.0, 16.7. ESI-MS (*m/z*): 338.2 (M+H$^+$).

2-Cyclohexyl-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (27)

White solid; yield: 39%; m.p. 190–192°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.15 (s, 1H), 7.81 (s, 1H), 6.96 (bs, 1H), 6.70 (bs, 1H), 3.75 (s, 1H), 2.71 (t, *J* = 12.2 Hz, 1H), 1.79–1.68 (m, 4H), 1.67–1.57 (m, 3H), 1.54 (s, 2H), 1.32–1.19 (m, 3H), 1.12 (s, 6H), 1.00 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.3, 140.8, 136.9, 130.1, 119.3, 93.6, 57.2, 55.1, 33.9, 31.7, 31.5, 31.3, 29.0, 26.4, 25.3. ESI-MS (*m/z*): 360.3 (M+H$^+$).

3-(*tert*-Butylamino)-2-heptyl-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (28)

White solid; yield: 44%; m.p. 203–204°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.18 (s, 1H), 7.82 (s, 1H), 7.00 (bs, 1H), 6.69 (bs, 1H), 3.84 (bs, 1H), 1.60 (t, *J* = 7.7 Hz, 2H), 1.32–1.18 (m, 10H), 1.09 (s, 9H), 0.88–0.78 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.4, 140.5, 137.2, 125.5, 120.7, 93.7, 53.6, 31.2, 30.1, 28.8, 28.6, 28.4, 24.2, 22.1, 14.0. ESI-MS (*m/z*): 320.3 (M+H$^+$).

2-(*tert*-Butyl)-3-(cyclohexylamino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (29)

White solid; yield: 43%; m.p. 148–149°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.67 (s, 1H), 7.79 (s, 1H), 6.99 (bs, 1H), 6.63 (bs, 1H), 3.80 (s, 1H), 3.25 (s, 1H), 1.76–1.57 (m, 4H), 1.49 (s, 1H), 1.33 (s, 9H), 1.10 (s, 4H), 0.94 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.9, 141.8, 135.7, 129.6, 120.6, 93.6, 54.4, 33.3, 31.8, 30.0, 25.6, 24.5. ESI-MS (*m/z*): 304.3 (M+H$^+$).

2-(*tert*-Butyl)-3-(*tert*-butylamino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (30)

White solid; yield: 49%; m.p. 221°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.72 (s, 1H), 7.79 (s, 1H), 7.01 (bs, 1H), 6.71 (bs, 1H), 3.59 (s, 1H), 1.36 (s, 9H), 1.17 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.0, 141.5, 135.9, 132.4, 119.7, 93.5, 52.4, 32.0, 30.7, 30.2. ESI-MS (*m/z*): 278.2 (M+H$^+$).

2-(*tert*-Butyl)-3-((4-methoxyphenyl)amino)-1*H*-imidazo[1,2-*b*]-pyrazole-7-carboxamide (31)

Pale yellow solid; yield: 41%; m.p. 260–262°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.19 (s, 1H), 7.79 (s, 1H), 7.22 (s, 1H), 7.11 (bs, 1H), 6.83 (bs, 1H), 6.68 (d, *J* = 8.3 Hz, 2H), 6.38 (d, *J* = 8.4 Hz, 2H), 3.60 (s, 3H), 1.31 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.9, 151.8, 141.6, 141.0, 136.5, 133.9, 115.4, 114.6, 113.7, 94.3, 55.3, 32.0, 29.4. ESI-MS (*m/z*): 328.2 (M+H$^+$).

2-(*tert*-Butyl)-3-((4-fluorophenyl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (32)

Gray solid; yield: 54%; m.p. 249–250°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.28 (s, 1H), 7.82 (s, 1H), 7.55 (s, 1H), 7.14 (bs, 1H), 6.91 (t, *J* = 8.6 Hz, 2H), 6.77 (bs, 1H), 6.43 (dd, *J* = 8.5, 4.5 Hz, 2H), 1.32 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.9, 155.3 (d, *J* = 232.6 Hz), 143.6, 141.7, 136.5, 134.2, 115.4 (d, *J* = 22.3 Hz), 114.8, 113.8 (d, *J* = 7.2 Hz), 94.5, 32.0, 29.4. ESI-MS (*m/z*): 316.1 (M+H$^+$).

2-(*tert*-Butyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (33)

White solid; yield: 54%; m.p. 155–156°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.73 (s, 1H), 7.81 (s, 1H), 7.01 (s, 1H), 6.72 (s, 1H), 3.42 (s, 1H), 1.66 (s, 2H), 1.38 (s, 9H), 1.21 (s, 6H), 1.00 (s, 9H). ^{13}C NMR (126 MHz,

DMSO-d_6) δ 164.0, 141.5, 135.9, 132.4, 119.5, 93.5, 56.5, 56.1, 32.0, 31.8, 31.4, 30.2, 29.6. ESI-MS (*m/z*): 334.3 ($M+H^+$).

2-Cyclopropyl-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (34)

White solid; yield: 56%; m.p. 205–207°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.80 (s, 1H), 7.81 (s, 1H), 6.90 (bs, 1H), 6.73 (bs, 1H), 3.60 (bs, 1H, overlap with water), 2.00–1.92 (m, 1H), 1.59 (s, 2H), 1.14 (s, 6H), 1.02 (s, 9H), 0.91–0.87 (m, 2H), 0.85–0.80 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.1, 141.1, 136.2, 126.1, 121.5, 93.8, 58.1, 55.3, 31.8, 31.4, 29.2, 7.1, 6.6. ESI-MS (*m/z*): 318.2 ($M+H^+$).

2-Ethyl-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (35)

White solid; yield: 51%; m.p. 207–209°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.19 (s, 1H), 7.82 (s, 1H), 7.01 (bs, 1H), 6.65 (bs, 1H), 3.68 (s, 1H), 2.55–2.50 (m, 2H), 1.54 (s, 2H), 1.20–1.13 (m, 3H), 1.10 (s, 6H), 1.00 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.4, 140.5, 137.3, 126.6, 120.1, 93.6, 57.4, 55.2, 31.8, 31.4, 29.1, 17.7, 13.8. ESI-MS (*m/z*): 306.3 ($M+H^+$).

2-Isopropyl-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (36)

White solid; yield: 42%; m.p. 132–134°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.17 (s, 1H), 7.82 (s, 1H), 6.97 (bs, 1H), 6.70 (bs, 1H), 3.70 (bs, 1H), 3.12–3.02 (m, 1H), 1.55 (s, 2H), 1.22 (d, *J* = 7.1 Hz, 6H), 1.12 (s, 6H), 1.00 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.3, 140.8, 137.2, 130.7, 119.0, 93.7, 57.2, 55.2, 31.8, 31.4, 29.1, 24.0, 21.8. ESI-MS (*m/z*): 320.4 ($M+H^+$).

2-(2-Methylpent-4-en-2-yl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (37)

White solid; yield: 45%; m.p. 161–163°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.72 (s, 1H), 7.81 (s, 1H), 7.04 (s, 1H), 6.72 (s, 1H), 5.67–5.55 (m, 1H), 5.05–4.89 (m, 2H), 3.43 (s, 1H), 1.65 (s, 2H), 1.35 (s, 6H), 1.22 (s, 6H), 0.99 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.0, 141.5, 136.0, 135.5, 131.0, 120.5, 117.3, 93.5, 56.4, 56.1, 46.2, 35.2, 31.8, 31.4, 29.6, 27.7. ESI-MS (*m/z*): 360.3 ($M+H^+$).

2-(1-Cyano-3-ethylpentan-3-yl)-3-((2,4,4-trimethylpentan-2-yl)-amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (38)

White solid; yield: 35%; m.p. 184–186°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.61 (s, 1H), 7.83 (s, 1H), 7.05 (bs, 1H), 6.74 (bs, 1H), 3.56 (d, *J* = 2.1 Hz, 1H), 2.32–2.22 (m, 2H), 2.19–2.13 (m, 2H), 1.76 (q, *J* = 7.2 Hz, 4H), 1.66 (s, 2H), 1.27 (s, 6H), 1.00 (s, 9H), 0.68 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.9, 141.7, 136.2, 128.2, 122.8, 121.0, 93.7, 56.2, 56.1, 42.4, 31.8, 31.4, 29.6, 26.6, 11.5, 7.9. ESI-MS (*m/z*): 401.4 ($M+H^+$).

2-(*tert*-Butyl)-*N*-methyl-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (39)

White solid; yield: 31%; m.p. 173–174°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.75 (s, 1H), 7.81 (s, 1H), 7.46 (d, *J* = 6.2 Hz, 1H), 3.46 (s, 1H), 2.75 (d, *J* = 4.4 Hz, 3H), 1.68 (s, 2H), 1.41 (s, 9H), 1.23 (s, 6H), 1.03 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.2, 141.1, 136.2, 132.8, 119.9, 94.0, 56.9, 56.5, 32.5, 32.2, 31.9, 30.7, 30.1, 25.8. ESI-MS (*m/z*): 348.4 ($M+H^+$).

2-(*tert*-Butyl)-*N*-butyl-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (40)

White solid; yield: 43%; m.p. 147–148°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.73 (s, 1H), 7.84 (s, 1H), 7.52 (t, *J* = 5.9 Hz, 1H), 3.45 (s, 1H), 3.22 (q, *J* = 6.7 Hz, 2H), 1.68 (s, 2H), 1.48 (q, *J* = 7.2 Hz, 2H), 1.41 (s, 9H), 1.33 (q, *J* = 7.5 Hz, 2H), 1.23 (s, 6H), 1.03 (s, 9H), 0.91 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.7, 140.8, 136.5, 132.9, 119.9, 94.2, 56.9, 56.5, 38.4, 32.5, 32.2, 31.9, 30.7, 30.1, 20.1, 14.3. ESI-MS (*m/z*): 390.3 ($M+H^+$).

***N*,2-Di-*tert*-butyl-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (41)**

White solid; yield: 66%; m.p. 177–178°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.74 (s, 1H), 7.93 (s, 1H), 6.94 (s, 1H), 3.43 (s, 1H), 1.67 (s, 2H), 1.40 (s, 9H), 1.38 (s, 9H), 1.23 (s, 6H), 1.03 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ^{13}C NMR (126 MHz, DMSO) δ 163.1, 140.2, 137.6, 132.9, 119.7, 95.1, 57.0, 56.5, 50.6, 32.5, 32.2, 31.8, 30.7, 30.1, 29.7. ESI-MS (*m/z*): 390.4 ($M+H^+$).

2-(*tert*-Butyl)-*N*-cyclopropyl-3-((2,4,4-trimethylpentan-2-yl)-amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (42)

White solid; yield: 57%; m.p. 161°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.76 (s, 1H), 7.82 (s, 1H), 7.64–7.55 (m, 1H), 2.75–2.68 (m, 1H), 1.67 (s, 2H), 1.40 (s, 9H), 1.23 (s, 6H), 1.03 (s, 9H), 0.69–0.64 (m, 2H), 0.52–0.48 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.9, 140.9, 136.6, 132.9, 119.9, 94.0, 56.9, 56.5, 32.5, 32.2, 31.9, 30.7, 30.1, 22.7, 6.5. ESI-MS (*m/z*): 374.4 ($M+H^+$).

2-(*tert*-Butyl)-*N*-cyclopentyl-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (43)

White solid; yield: 38%; m.p. 182–183°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.75 (s, 1H), 7.90 (s, 1H), 7.40 (s, 1H), 4.19 (s, 1H), 3.44 (s, 1H), 1.89 (s, 2H), 1.69 (s, 4H), 1.51 (s, 4H), 1.41 (s, 9H), 1.24 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.3, 140.5, 136.6, 133.2, 120.0, 93.4, 57.2, 56.5, 50.9, 32.2, 31.9, 31.2, 31.0, 29.4, 29.1, 23.5. ESI-MS (*m/z*): 402.4 ($M+H^+$).

(2-(*tert*-Butyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazol-7-yl)(piperidin-1-yl)methanone (44)

White solid; yield: 60%; m.p. 164–165°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.69 (s, 1H), 7.63 (s, 1H), 3.80–3.56 (m, 4H), 1.70 (s, 2H), 1.67–1.59 (m, 2H), 1.56–1.47 (m, 4H), 1.40 (s, 9H), 1.24 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.4, 140.4, 138.5, 133.0, 119.7, 92.7, 56.9, 56.5, 32.5, 32.3, 31.9, 30.7, 30.1, 26.4, 24.8. ESI-MS (*m/z*): 402.4 ($M+H^+$).

(2-(*tert*-Butyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazol-7-yl)(4-phenylpiperazin-1-yl)methanone (45)

White solid; yield: 59%; m.p. 170°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.77 (s, 1H), 7.74 (s, 1H), 7.24 (t, *J* = 7.9 Hz, 2H), 6.97 (d, *J* = 8.1 Hz,

2H), 6.81 (t, *J* = 7.2 Hz, 1H), 3.83–3.79 (m, 4H), 3.49 (s, 1H), 3.21–3.17 (m, 4H), 1.72 (s, 2H), 1.41 (s, 9H), 1.25 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.6, 151.4, 140.7, 138.5, 133.1, 129.5, 119.8, 119.7, 116.2, 92.4, 56.9, 56.5, 49.1, 32.5, 32.3, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 479.4 ($M+H^+$).

N-Benzyl-2-(*tert*-butyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (46)

White solid; yield: 49%; m.p. 118–119°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.81 (s, 1H), 8.14 (t, *J* = 6.1 Hz, 1H), 7.91 (s, 1H), 7.35–7.30 (m, 4H), 7.28–7.20 (m, 1H), 4.46 (d, *J* = 6.0 Hz, 2H), 3.48 (s, 1H), 1.69 (s, 2H), 1.41 (s, 9H), 1.24 (s, 6H), 1.03 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.8, 141.1, 141.1, 136.6, 132.9, 128.7, 127.6, 127.0, 120.0, 93.9, 56.9, 56.5, 42.2, 32.5, 32.3, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 424.3 ($M+H^+$).

2-(*tert*-Butyl)-*N*-phenyl-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (47)

White solid; yield: 55%; m.p. 147°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.06 (s, 1H), 9.42 (s, 1H), 8.13 (s, 1H), 7.73 (d, *J* = 7.2 Hz, 2H), 7.31 (t, *J* = 7.9 Hz, 2H), 7.02 (t, *J* = 7.4 Hz, 1H), 3.52 (s, 1H), 1.70 (s, 2H), 1.43 (s, 9H), 1.25 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.5, 140.8, 140.4, 137.5, 133.3, 129.0, 122.9, 120.1, 120.1, 94.4, 57.0, 56.5, 32.6, 32.2, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 410.3 ($M+H^+$).

2-(*tert*-Butyl)-*N*-(pyridin-2-yl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (48)

White solid; yield: 29%; m.p. 144–145°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.17 (s, 1H), 10.05 (s, 1H), 8.33 (d, *J* = 4.8 Hz, 1H), 8.29 (s, 1H), 8.22 (d, *J* = 8.5 Hz, 1H), 7.76 (t, *J* = 8.0 Hz, 1H), 7.07 (t, *J* = 6.1 Hz, 1H), 3.53 (s, 1H), 1.69 (s, 2H), 1.43 (s, 9H), 1.25 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.6, 153.5, 148.1, 141.9, 138.2, 137.2, 133.2, 120.2, 119.0, 114.3, 94.0, 57.0, 56.5, 32.6, 32.3, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 411.3 ($M+H^+$).

2-(*tert*-Butyl)-*N*-(pyridin-3-yl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (49)

White solid; yield: 31%; m.p. 186–187°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.14 (s, 1H), 9.63 (s, 1H), 8.93 (d, *J* = 2.4 Hz, 1H), 8.23 (dd, *J* = 4.7, 1.5 Hz, 1H), 8.15 (s, 1H), 8.13–8.10 (m, 1H), 7.35 (dd, *J* = 8.3, 4.7 Hz, 1H), 3.54 (s, 1H), 1.70 (s, 2H), 1.43 (s, 9H), 1.25 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.6, 143.8, 141.7, 140.9, 137.5, 137.1, 133.4, 126.8, 123.9, 120.2, 94.0, 57.0, 56.5, 32.6, 32.2, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 411.3 ($M+H^+$).

2-(*tert*-Butyl)-*N*-(pyridin-4-yl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (50)

Pale yellow solid; yield: 23%; m.p. 185–186°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.24 (s, 1H), 9.80 (s, 1H), 8.40 (d, *J* = 5.5 Hz, 2H), 8.17 (s, 1H), 7.73 (d, *J* = 5.4 Hz, 2H), 3.56 (s, 1H), 1.69 (s, 2H), 1.42 (s, 9H), 1.24 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.9, 150.5, 147.2, 141.0, 137.6, 133.5, 120.2, 113.7, 94.1, 57.0, 56.4, 32.6, 32.2, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 411.3 ($M+H^+$).

2-(*tert*-Butyl)-*N*-(thiazol-2-yl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (51)

White solid; yield: 44%; m.p. 125–127°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.86 (s, 1H), 11.35 (s, 1H), 8.31 (s, 1H), 7.48 (d, *J* = 3.6 Hz, 1H), 7.14 (d, *J* = 3.5 Hz, 1H), 3.55 (s, 1H), 1.69 (s, 2H), 1.42 (s, 9H), 1.24 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.1, 159.5, 141.8, 137.8, 137.3, 133.4, 120.4, 113.1, 92.5, 57.0, 56.5, 32.6, 32.2, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 417.3 ($M+H^+$).

2-(*tert*-Butyl)-*N*-(isoxazol-3-yl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (52)

Gray solid; yield: 40%; m.p. 185–186°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.21 (s, 1H), 10.66 (s, 1H), 8.77 (d, *J* = 1.8 Hz, 1H), 8.23 (s, 1H), 7.05 (d, *J* = 1.7 Hz, 1H), 3.53 (s, 1H), 1.68 (s, 2H), 1.42 (s, 9H), 1.24 (s, 6H), 1.03 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.7, 159.9, 158.9, 141.8, 137.3, 133.3, 120.3, 99.9, 93.4, 57.0, 56.5, 32.5, 32.2, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 401.3 ($M+H^+$).

2-(*tert*-Butyl)-*N*-(*o*-tolyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (53)

White solid; yield: 30%; m.p. 172°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.96 (s, 1H), 8.93 (s, 1H), 8.04 (s, 1H), 7.37 (d, *J* = 7.8 Hz, 1H), 7.26 (d, *J* = 7.4 Hz, 1H), 7.20 (t, *J* = 7.6 Hz, 1H), 7.12 (t, *J* = 7.4 Hz, 1H), 3.53 (s, 1H), 2.26 (s, 3H), 1.71 (s, 2H), 1.42 (s, 9H), 1.26 (s, 6H), 1.05 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.2, 141.5, 137.3, 136.8, 133.8, 133.1, 130.7, 126.8, 126.3, 125.7, 120.1, 94.0, 57.0, 56.5, 32.5, 32.3, 31.9, 30.7, 30.1, 18.6. ESI-MS (*m/z*): 424.3 ($M+H^+$).

2-(*tert*-Butyl)-*N*-(3,5-dimethylphenyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (54)

Gray solid; yield: 51%; m.p. 180–181°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.02 (s, 1H), 9.28 (s, 1H), 8.11 (s, 1H), 7.38 (d, *J* = 1.6 Hz, 2H), 6.67 (s, 1H), 3.51 (s, 1H), 2.25 (s, 6H), 1.70 (s, 2H), 1.42 (s, 9H), 1.25 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.4, 140.7, 140.2, 137.8, 137.5, 133.2, 124.4, 120.1, 117.9, 94.5, 57.0, 56.5, 32.5, 32.2, 31.9, 30.7, 30.1, 21.6. ESI-MS (*m/z*): 438.4 ($M+H^+$).

2-(*tert*-Butyl)-*N*-(4-isopropylphenyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (55)

White solid; yield: 49%; m.p. 155–156°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.04 (s, 1H), 9.35 (s, 1H), 8.12 (s, 1H), 7.64 (d, *J* = 8.1 Hz, 2H), 7.18 (d, *J* = 8.2 Hz, 2H), 3.51 (s, 1H), 2.85 (hept, *J* = 7.0 Hz, 1H), 1.70 (s, 2H), 1.43 (s, 9H), 1.25 (s, 6H), 1.20 (d, *J* = 6.9 Hz, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.3, 142.8, 140.8, 138.1, 137.5, 133.2, 126.7, 120.1, 120.0, 94.4, 57.0, 56.5, 33.3, 32.6, 32.2, 31.9, 30.7, 30.1, 24.5. ESI-MS (*m/z*): 452.4 ($M+H^+$).

2-(*tert*-Butyl)-*N*-(4-methoxyphenyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (56)

White solid; yield: 59%; m.p. 187–188°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.00 (s, 1H), 9.29 (s, 1H), 8.08 (s, 1H), 7.61 (d, *J* = 8.7 Hz, 2H), 6.90 (d, *J* = 8.6 Hz, 2H), 3.51 (s, 1H), 1.70 (s, 2H), 1.43 (s, 9H), 1.25 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.2, 155.3,

140.8, 137.3, 133.4, 133.2, 121.9, 120.0, 114.1, 94.3, 57.0, 56.5, 55.6, 32.5, 32.2, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 440.4 ($M+H^+$).

2-(*tert*-Butyl)-*N*-(2,4-dimethoxyphenyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (57)

Light beige solid; yield: 39%; m.p. 127°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.95 (s, 1H), 8.51 (s, 1H), 8.00 (s, 1H), 7.50 (d, *J* = 8.7 Hz, 1H), 6.64 (s, 1H), 6.52 (d, *J* = 8.8 Hz, 1H), 3.81 (s, 3H), 3.77 (s, 3H), 3.52 (s, 1H), 1.70 (s, 2H), 1.42 (s, 9H), 1.25 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.3, 157.7, 153.5, 141.3, 136.7, 133.0, 126.5, 120.7, 120.1, 104.5, 99.2, 94.1, 56.9, 56.5, 56.1, 55.8, 32.5, 32.3, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 470.4 ($M+H^+$).

2-(tert-Butyl)-*N*-(2-(trifluoromethyl)phenyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (58)

White solid; yield: 34%; m.p. 106°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.01 (s, 1H), 9.11 (s, 1H), 8.02 (s, 1H), 7.77 (d, *J* = 7.9 Hz, 1H), 7.71 (t, *J* = 7.6 Hz, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.48 (t, *J* = 7.7 Hz, 1H), 3.54 (s, 1H), 1.71 (s, 2H), 1.42 (s, 9H), 1.26 (s, 6H), 1.05 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.9, 141.5, 136.9, 136.7, 133.3, 133.1, 131.4, 126.9, 126.8 (q, *J* = 4.6 Hz), 126.2 (q, *J* = 29.1 Hz), 124.3 (d, *J* = 273.6 Hz), 120.2, 93.6, 57.0, 56.5, 32.5, 32.3, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 478.3 ($M+H^+$).

2-(*tert*-Butyl)-*N*-(3-(trifluoromethyl)phenyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (59)

White solid; yield: 54%; m.p. 180°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.15 (s, 1H), 9.78 (s, 1H, 8.34 (s, 1H), 8.16 (s, 1H), 7.91 (d, *J* = 8.1 Hz, 1H), 7.55 (t, *J* = 8.0 Hz, 1H), 7.35 (d, *J* = 7.6 Hz), 3.54 (s, 1H), 1.70 (s, 2H), 1.43 (s, 9H), 1.25 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.7, 141.3, 140.7, 137.7, 133.4, 130.1, 129.7 (q, *J* = 31.3 Hz), 124.8 (q, *J* = 272.3 Hz), 123.2, 120.2, 119.0 (q, *J* = 3.7 Hz), 115.9 (q, *J* = 4.3 Hz), 94.1, 57.0, 56.4, 32.6, 32.2, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 478.4 ($M+H^+$).

2-(*tert*-Butyl)-*N*-(4-(trifluoromethyl)phenyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (60)

White solid; yield: 39%; m.p. 155–156°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.17 (s, 1H), 9.78 (s, 1H), 8.17 (s, 1H), 7.97 (d, *J* = 8.5 Hz, 2H), 7.67 (d, *J* = 8.5 Hz, 2H), 3.54 (s, 1H), 1.70 (s, 2H), 1.43 (s, 9H), 1.25 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.6, 144.2, 140.9, 137.6, 133.4, 126.3 (d, *J* = 3.5 Hz), 125.1 (q, *J* = 271.0 Hz), 122.7 (q, *J* = 31.7 Hz), 120.2, 119.6, 94.2, 57.0, 56.5, 32.6, 32.2, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 478.4 ($M+H^+$).

2-(*tert*-Butyl)-*N*-(2-fluorophenyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (61)

White solid; yield: 39%; m.p. 153–154°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.91 (s, 1H), 9.08 (s, 1H), 8.09 (s, 1H), 7.74–7.65 (m, 1H), 7.29–7.22 (m, 1H), 7.22–7.16 (m, 2H), 3.46 (s, 1H), 1.73 (s, 2H), 1.44 (s, 9H), 1.27 (s, 6H), 1.06 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.2, 155.8 (d, *J* = 245.3 Hz), 141.6, 137.1, 133.2, 127.0, 126.9, 126.0 (d, *J* = 7.5 Hz), 124.5 (d, *J* = 3.3 Hz), 120.3, 116.0(d, *J* = 20.2 Hz), 93.8, 57.1, 56.6, 32.5, 32.2, 31.8, 30.7, 30.1. ESI-MS (*m/z*): 428.3 ($M+H^+$).

2-(*tert*-Butyl)-*N*-(3-fluorophenyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (62)

White solid; yield: 47%; m.p. 159°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.13 (s, 1H), 9.62 (s, 1H), 8.14 (s, 1H), 7.78 (dt, *J* = 12.3, 2.4 Hz, 1H), 7.56–7.42 (m, 1H), 7.39–7.26 (m, 1H), 6.83 (td, *J* = 8.4, 2.6 Hz, 1H), 3.53 (s, 1H), 1.70 (s, 2H), 1.43 (s, 9H), 1.25 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.6, 162.6 (d, *J* = 240.2 Hz), 142.3 (d, *J* = 11.2 Hz), 140.8, 137.6, 133.3, 130.5 (d, *J* = 9.7 Hz), 120.1, 115.5, 109.1 (d, *J* = 21.2 Hz), 106.5 (d, *J* = 26.5 Hz), 94.2, 57.0, 56.5, 32.6, 32.2, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 428.4 ($M+H^+$).

2-(*tert*-Butyl)-*N*-(4-fluorophenyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (63)

White solid; yield: 49%; m.p. 188°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.06 (s, 1H), 9.48 (s, 1H), 8.10 (s, 1H), 7.80–7.67 (m, 2H), 7.15 (t, *J* = 8.9 Hz, 2H), 3.52 (s, 1H), 1.70 (s, 2H), 1.42 (s, 9H), 1.25 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.4, 158.1 (d, *J* = 238.7 Hz), 140.8, 137.4, 136.7, 133.3, 121.8 (d, *J* = 7.9 Hz), 120.1, 115.5 (d, *J* = 22.0 Hz), 94.2, 57.0, 56.5, 32.6, 32.2, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 428.4 ($M+H^+$).

2-(*tert*-Butyl)-*N*-(4-chlorophenyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (64)

White solid; yield: 50%; m.p. 188°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.09 (s, 1H), 9.55 (s, 1H), 8.12 (s, 1H), 7.77 (d, *J* = 8.8 Hz, 2H), 7.36 (d, *J* = 8.7 Hz, 2H), 3.52 (s, 1H), 1.70 (s, 2H), 1.42 (s, 9H), 1.25 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.4, 140.8, 139.4, 137.5, 133.3, 128.9, 126.4, 121.5, 120.1, 94.19, 57.0, 56.5, 32.6, 32.2, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 444.3 and 446.3 ($M+H^+$).

***N*-(4-Bromophenyl)-2-(*tert*-butyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (65)**

Pale yellow solid; yield: 42%; m.p. 153–154°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.10 (s, 1H), 9.55 (s, 1H), 8.12 (s, 1H), 7.72 (d, *J* = 8.5 Hz, 2H), 7.49 (d, *J* = 8.5 Hz, 2H), 3.53 (s, 1H), 1.70 (s, 2H), 1.42 (s, 9H), 1.25 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.4, 140.8, 139.8, 137.5, 133.3, 131.8, 121.9, 120.1, 114.3, 94.2, 57.0, 56.5, 32.6, 32.2, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 488.2 and 490.3 ($M+H^+$).

2-(*tert*-Butyl)-*N*-(4-nitrophenyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (66)

Yellow solid; yield: 38%; m.p. 199°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.27 (s, 1H), 10.06 (s, 1H), 8.24 (d, *J* = 9.2 Hz, 2H), 8.19 (s, 1H), 8.01 (d, *J* = 9.1 Hz, 2H), 3.57 (s, 1H), 1.69 (s, 2H), 1.43 (s, 9H), 1.25 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.6, 147.1, 141.9, 141.2, 137.7, 133.5, 125.2, 120.4, 119.2, 94.1, 57.1, 56.5, 32.6, 32.2, 31.8, 30.7, 30.1. ESI-MS (*m/z*): 455.3 ($M+H^+$).

2-(*tert*-Butyl)-*N*-(4-cyanophenyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (67)

White solid; yield: 48%; m.p. 199°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.20 (s, 1H), 9.85 (s, 1H), 8.16 (s, 1H), 7.94 (d, *J* = 8.8 Hz, 2H), 7.76 (d, *J* = 8.7 Hz, 2H), 3.55 (s, 1H), 1.70 (s, 2H), 1.43 (s, 9H), 1.25 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.6, 144.9, 141.0, 137.6, 133.5, 133.4, 120.2, 119.8, 119.7, 104.2, 94.1, 57.0, 56.5, 32.6, 32.2, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 435.3 (M+H$^+$).

Ethyl 4-(2-(*tert*-butyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamido)benzoate (68)

White solid; yield: 60%; m.p. 127–128°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.17 (s, 1H), 9.78 (s, 1H), 8.17 (s, 1H), 7.97–7.83 (m, 4H), 4.29 (q, *J* = 7.1 Hz, 2H), 3.54 (s, 1H), 1.70 (s, 2H), 1.43 (s, 9H), 1.32 (t, *J* = 7.1 Hz, 3H), 1.25 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.9, 161.6, 145.1, 140.9, 137.6, 133.4, 130.5, 123.6, 120.2, 119.1, 94.3, 60.8, 57.0, 56.5, 32.6, 32.2, 31.9, 30.7, 30.1, 14.7. ESI-MS (*m/z*): 482.4 (M+H$^+$).

2-(*tert*-Butyl)-*N*-(4-(methylthio)phenyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (69)

White solid; yield: 28%; m.p. 169–170°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.06 (s, 1H), 9.44 (s, 1H), 8.11 (s, 1H), 7.70 (d, *J* = 8.3 Hz, 2H), 7.25 (d, *J* = 8.3 Hz, 2H), 3.52 (s, 1H), 2.46 (s, 3H), 1.70 (s, 2H), 1.43 (s, 9H), 1.25 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.4, 140.8, 138.0, 137.5, 133.3, 131.1, 127.7, 120.8, 120.1, 94.3, 57.0, 56.5, 32.6, 32.2, 31.9, 30.7, 30.1, 16.2. ESI-MS (*m/z*): 456.3 (M+H$^+$).

2-(*tert*-Butyl)-*N*-(4-(dimethylamino)phenyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (70)

Gray solid; yield: 37%; m.p. 176–177°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.96 (s, 1H), 9.16 (s, 1H), 8.06 (s, 1H), 7.51 (d, *J* = 8.6 Hz, 2H), 6.72 (d, *J* = 8.5 Hz, 2H), 3.50 (s, 1H), 3.35 (s, 6H), 1.70 (s, 2H), 1.43 (s, 9H), 1.25 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.1, 147.2, 140.8, 137.2, 133.1, 130.1, 121.9, 120.0, 113.2, 94.5, 57.0, 56.5, 41.1, 32.5, 32.3, 31.9, 30.7, 30.1. APCI-MS (*m/z*): 453.3 (M+H$^+$).

2-(*tert*-Butyl)-*N*-(2,4-difluorophenyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (71)

White solid; yield: 45%; m.p. 174°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.03 (s, 1H), 9.22 (s, 1H), 8.08 (s, 1H), 7.62 (td, *J* = 8.9, 6.2 Hz, 1H), 7.33 (ddd, *J* = 10.6, 9.1, 2.9 Hz, 1H), 7.18–7.03 (m, 1H), 3.54 (s, 1H), 1.70 (s, 2H), 1.42 (s, 9H), 1.25 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.2, 159.4 (dd, *J* = 243.6, 11.5 Hz), 156.2 (dd, *J* = 248.5, 12.5 Hz), 141.5, 137.0, 133.2, 128.47 (dd, *J* = 9.6, 3.0 Hz), 123.28 (dd, *J* = 12.1, 3.5 Hz), 120.2, 111.42 (dd, *J* = 21.7, 3.3 Hz), 104.6 (dd, *J* = 26.1, 24.9 Hz), 93.5, 57.0, 56.5, 32.5, 32.2, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 446.3 (M+H$^+$).

2-(*tert*-Butyl)-*N*-(3,4-difluorophenyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (72)

White solid; yield: 34%; m.p. 162–164°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.12 (s, 1H), 9.64 (s, 1H), 8.11 (s, 1H), 7.95 (ddd, *J* = 13.8, 7.6, 2.4 Hz, 1H), 7.47–7.33 (m, 2H), 3.53 (s, 1H), 1.69 (s, 2H), 1.42 (s, 9H), 1.24 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.5, 149.31 (dd, *J* = 242.0, 13.0 Hz), 145.16 (dd, *J* = 240.2, 12.7 Hz), 140.8, 137.58 (dd, *J* = 9.3, 2.5 Hz), 137.5, 133.3, 120.1, 117.61 (d, *J* = 17.6 Hz), 115.93 (dd, *J* = 5.0, 3.1 Hz), 108.68 (d, *J* = 21.9 Hz), 94.0, 57.0, 56.5, 32.6, 32.2, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 446.3 (M+H$^+$).

2-(*tert*-Butyl)-*N*-(4-fluorobenzyl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (73)

White solid; yield: 33%; m.p. 163–164°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.82 (s, 1H), 8.16 (t, *J* = 6.1 Hz, 1H), 7.90 (s, 1H), 7.46–7.25 (m, 2H), 7.21–7.01 (m, 2H), 4.43 (d, *J* = 6.0 Hz, 2H), 3.48 (s, 1H), 1.68 (s, 2H), 1.40 (s, 9H), 1.24 (s, 6H), 1.03 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.8, 161.5 (d, *J* = 241.7 Hz), 141.0, 137.3, 136.6, 132.9, 129.5 (d, *J* = 8.1 Hz), 120.0, 115.4 (d, *J* = 21.2 Hz), 93.8, 56.9, 56.5, 41.5, 32.5, 32.2, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 442.4 (M+H$^+$).

2-(*tert*-Butyl)-*N*-(5-fluoropyridin-2-yl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (74)

White solid; yield: 34%; m.p. 140°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.16 (s, 1H), 10.17 (s, 1H), 8.34 (d, *J* = 3.1 Hz, 1H), 8.28 (s, 1H), 8.25 (dd, *J* = 9.2, 4.2 Hz, 1H), 7.74 (td, *J* = 8.8, 3.1 Hz, 1H), 3.53 (s, 1H), 1.69 (s, 2H), 1.43 (s, 9H), 1.24 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.4, 155.7 (d, *J* = 247.1 Hz), 150.0, 141.9, 137.2, 135.3 (d, *J* = 24.7 Hz), 133.2, 125.5 (d, *J* = 19.4 Hz), 120.2, 115.4 (d, *J* = 4.0 Hz), 93.8, 57.0, 56.5, 32.6, 32.2, 31.9, 30.7, 30.1. ESI-MS (*m/z*): 429.2 (M+H$^+$).

2-(*tert*-Butyl)-*N*-(6-fluoropyridin-3-yl)-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (75)

White solid; yield: 25%; m.p. 177–178°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.15 (s, 1H), 9.72 (s, 1H), 8.58 (s, 1H), 8.29–8.19 (m, 1H), 8.12 (s, 1H), 7.17 (dd, *J* = 9.0, 3.0 Hz, 1H), 3.54 (s, 1H), 1.70 (s, 2H), 1.43 (s, 9H), 1.25 (s, 6H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.48, 158.64 (d, *J* = 231.2 Hz), 140.91, 138.29 (d, *J* = 15.4 Hz), 137.43, 135.37, 133.37, 120.17, 109.53 (d, *J* = 39.4 Hz), 93.77, 56.99, 56.46, 32.57, 32.23, 31.86, 30.69, 30.10. ESI-MS (*m/z*): 429.2 (M+H$^+$).

2-(*tert*-Butyl)-*N*-(4-fluorophenyl)-*N*-methyl-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (76)

White solid; yield: 42%; m.p. 102°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.67 (s, 1H), 7.41 (dd, *J* = 8.7, 4.9 Hz, 2H), 7.31 (t, *J* = 8.5 Hz, 2H), 5.95 (s, 1H), 3.39 (s, 1H), 3.29 (s, 3H), 1.62 (s, 2H), 1.39 (s, 9H), 1.16 (s, 6H), 0.99 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.3, 161.4 (d, *J* = 244.7 Hz), 141.7, 140.3, 139.1, 133.0, 130.7 (d, *J* = 8.6 Hz), 119.7, 116.9 (d, *J* = 22.5 Hz), 93.8, 56.8, 56.4, 37.8, 32.5, 32.2, 31.8, 30.6, 30.0. ESI-MS (*m/z*): 442.4 (M+H$^+$).

2-(*tert*-Butyl)-*N*-(4-fluorophenyl)-6-methyl-3-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (77)

White solid; yield: 52%; m.p. 179°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.68 (s, 1H), 8.82 (s, 1H), 7.63 (dd, J = 8.9, 5.1 Hz, 2H), 7.16 (t, J = 8.7 Hz, 2H), 3.49 (s, 1H), 2.44 (s, 3H), 1.73 (s, 2H), 1.43 (s, 9H), 1.25 (s, 6H), 1.05 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.9, 158.4(d, J = 239.0 Hz), 151.9, 136.3, 136.3, 131.8, 123.3 (d, J = 7.5 Hz), 120.0, 115.3 (d, J = 22.0 Hz), 91.4, 56.9, 56.5, 32.4, 32.2, 31.9, 30.8, 30.1, 15.7. ESI-MS (m/z): 442.3 ($M+H^+$).

4.1.4 | Synthesis of imidazo[1,2-*b*]pyrazole 78

To a solution of **63** (0.25 mmol) in a MeCN/AcOH mixture (1:1, 1.5 mL) formaldehyde (37 wt.% in H_2O, 0.375 mmol) was added and stirred at room temperature for 15 min. Then $NaCNBH_3$ (0.375 mmol) was added in portions and stirred further for 1 h. The reaction mixture was poured slowly into saturated aqueous Na_2CO_3 solution (30 mL, 0.5 M) and extracted with EtOAc (3 × 50 mL). The organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluent: hexane/EtOAc gradient) to afford pure **78**.

2-(*tert*-Butyl)-*N*-(4-fluorophenyl)-3-(methyl(2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (78)

White solid; yield: 25%; m.p. 212°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.18 (s, 1H), 9.52 (s, 1H), 8.11 (s, 1H), 7.73 (dd, J = 8.8, 5.0 Hz, 2H), 7.15 (t, J = 8.7 Hz, 2H), 2.71 (s, 3H), 2.08 (d, J = 14.4 Hz, 1H), 1.54 (d, J = 14.3 Hz, 1H), 1.41 (s, 12H), 1.15 (s, 3H), 0.98 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.3, 158.1 (d, J = 238.9 Hz), 141.0, 137.8, 136.7, 133.7, 124.2, 121.8 (d, J = 7.6 Hz), 115.5 (d, J = 22.0 Hz), 94.4, 59.2, 52.7, 35.3, 32.6, 32.2, 31.4, 30.3, 27.3, 26.5. ESI-MS (m/z): 442.4 ($M+H^+$).

4.2 | Biological evaluation

4.2.1 | Cell culture

Cells were purchased from the American Type Culture Collection (ATCC, Manassas, Virginia, USA). The human breast adenocarcinoma cell line, MCF-7 cells were maintained in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12, Gibco, Paisley, UK) 10% fetal calf serum (FCS, Gibco, Paisley, UK). Mouse mammary carcinoma 4T1 and human promyelocytic leukemia HL-60 cells were maintained in Roswell Park Memorial Institute 1640 medium (RPMI-1640, Gibco, Paisley, UK) 10% FCS. Media were supplemented with 2 mM GlutaMAX, 100 U/mL penicillin, and 100 µg/mL streptomycin (Life Technologies, Carlsbad, CA, USA). Cell cultures were maintained at 37°C in a humidified incubator in an atmosphere of 5% CO_2 (Sanyo, Osaka, Japan).

4.2.2 | 3D spheroid production, pellet culture system

The spheroid production using pellet culture system was previously described.[71] Briefly, MCF-7 and 4T1 cells were gently digested from the flasks by trypsin, washed with PBS, counted and then resuspended for a final density of 6000 cells per well. Cellular suspensions (80 µL/well) were dispensed into U-bottom CellStar® 96 microplates (Cellstar® Cell-Repellent Microplate, Greiner Bio-One Hungary, Mosonmagyaróvár) and centrifuged at 1200×*g* for 10 min. Plates were incubated at 37°C, 5% CO_2 for 24 h before drug treatments. The obtained spherical aggregates (one spheroid/well) were directly used for the cytotoxicity assay.

4.2.3 | *In vitro* toxicity

In vitro toxicity of the sythesized molecules was determined on MCF-7, 4T1, and HL-60 cells using the fluorescent Resazurin assay as described previously.[72]

Briefly, cells (MCF-7 and 4T1: 6000, HL-60: 120.000 cells/well) were seeded into 96-well plates (Corning Life Sciences, Tewksbury, MA, USA) in media and incubated overnight. Test compounds were dissolved in dimethyl sulfoxide (DMSO). Cells were treated with an increasing concentration of test compounds (156 nM–100 µM). The highest applied DMSO content of the treated cells was 0.4%. Cell viability was determined after 72 h incubation. Resazurin reagent (Sigma–Aldrich, Budapest, Hungary) was added at a final concentration of 25 µg/mL. After a 2-h incubation for 2D or 12 h for 3D cultures at 37°C 5%, CO_2 fluorescence (530 nm excitation/580 nm emission) was recorded on a multimode microplate reader (Cytofluor4000, PerSeptive Biosytems, Framingham, MA, USA). Viability was calculated with relation to untreated control cells and blank wells containing media without cells. IC_{50} values (50% inhibiting concentration) were calculated by GraphPad Prism® 5 (La Jolla, CA, USA).

4.2.4 | Real-time cell electronic sensing (RT-CES) *in vitro* toxicity measurement

RT-CES protocol was performed as described previously[73] with slight modifications. RT-CES 96-well E-plate (Roche, Budapest, Hungary) was coated with gelatin solution (0.2% in PBS) for 20 min at 37°C, then gelatin was washed twice with PBS solution. A total of 50 µL growth media was then gently dispensed into each well of the 96-well E-plate for background readings by the RT-CES system prior to addition of 50 µL of MCF-7 cell suspension for a final density of 4000 cells/well. Devices containing the cell suspension were kept at room temperature in a tissue culture hood for 30 min prior to insertion into the RT-CES device in the incubator to allow cells to settle and, after 20 h, they were treated with test compounds. Treated and control wells were dynamically monitored before and after treatment over 92 h by measurements of electrical impedance every 15 min. Continuous recording of impedance in cells was reflected by cell index value. The raw plate reads for each titration point were normalized relative to the cell index status right before treatment. Each treatment was repeated

in 4 wells per during the experiment. Viability of cells was calculated relative to wells containing non-treated cells. IC_{50} values were calculated by GraphPad Prism® 5.

4.2.5 | Detection of phosphatidylserine exposure

Apoptosis was assayed as described previously.[72] Briefly, HL-60 cells (200000) were plated in 24-well tissue culture plates (Corning Life Sciences) and treated with the indicated compounds and concentrations in the figures in 500 µL media. After 24 h the supernatants were harvested. Cells were harvested with the corresponding supernatant and fuged down (2000 rpm, 5 min, Eppendorf, Wien, Austria). Pellet was resuspended in Annexin V binding buffer (0.01 M HEPES, 0.14 M NaCl, and 2.5 mM $CaCl_2$). Annexin V-Alexa Fluor® 488 (Life Technologies, 2.5:100) was added to the cells, which were then kept for 15 min in the dark at room temperature. Before the acquisition, propidium iodide (10 µg/mL, Sigma–Aldrich) was added in Annexin V binding buffer to dilute Annexin V-Alexa Fluor® 488 5x. Cells (10000 events) were analyzed on a FACSCalibur flow cytometer using CellQuest software (Becton Dickinson, Franklin Lakes, NJ, USA). The percentage of the FL1 (530/30 nm filter, Annexin V-Alexa Fluor® 488) positive and FL3 (670 nm filter, propidium iodide) negative early apoptotic cells and FL1 positive and FL3 positive late apoptotic cells were determined. The total apoptotic population includes both early and late apoptotic cells. Column charts were created by GraphPad Prism® 5.

ACKNOWLEDGMENTS

This work was partly supported by the following grants: GINOP-2.3.2-15-2016-00030 and GINOP-2.3.2-15-2016-00001 from the National Research, Development and Innovation Office (NKFI), Hungary. Gábor J. Szebeni was supported by János Bolyai Research Scholarship of the Hungarian Academy of Sciences (BO/00139/17/8).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ORCID

Iván Kanizsai http://orcid.org/0000-0003-0109-7097

REFERENCES

[1] M. F. Khan, M. M. Alam, G. Verma, W. Akhtar, M. Akhter, M. Shaquiquzzaman, *Eur. J. Med. Chem.* **2016**, *120*, 170.
[2] Ş. Küçükgüzel, S. Şenkardeş, *Eur. J. Med. Chem.* **2015**, *97*, 786.
[3] V. Kumar, K. Kaur, G. K. Gupta, A. K. Sharma, *Eur. J. Med. Chem.* **2013**, *69*, 735.
[4] H. Kumar, D. Saini, S. Jain, N. Jain, *Eur. J. Med. Chem.* **2013**, *70*, 248.
[5] S. Kumari, S. Paliwal, R. Chauhan, *Synth. Commun.* **2014**, *44*, 1521.
[6] S. Howard, V. Berdini, J. A. Boulstridge, M. G. Carr, D. M. Cross, J. Curry, L. A. Devine, T. R. Early, L. Fazal, A. L. Gill, M. Heathcote, S. Maman, J. E. Matthews, R. L. McMenamin, E. F. Navarro, M. A. O'Brien, M. O'Reilly, D. C. Rees, M. Reule, D. Tisi, G. Williams, M. Vinković, P. G. Wyatt, *J. Med. Chem.* **2009**, *52*, 379.
[7] S.-F. Wang, Y.-L. Zhu, P.-T. Zhu, J. A. Makawana, Y.-L. Zhang, M.-Y. Zhao, P.-C. Lv, H.-L. Zhu, *Bioorg. Med. Chem.* **2014**, *22*, 6201.
[8] M.-h. Kim, M. Kim, H. Yu, H. Kim, K. H. Yoo, T. Sim, J.-M. Hah, *Bioorg. Med. Chem.* **2011**, *19*, 1915.
[9] Y. Zheng, M. Zheng, X. Ling, Y. Liu, Y. Xue, L. An, N. Gu, M. Jin, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3523.
[10] G. M. Nitulescu, C. Draghici, O. T. Olaru, *Int. J. Mol. Sci.* **2013**, *14*, 21805.
[11] G. C. Fletcher, R. D. Brokx, T. A. Denny, T. A. Hembrough, S. M. Plum, W. E. Fogler, C. F. Sidor, M. R. Bray, *Mol. Cancer Ther.* **2011**, *10*, 126.
[12] P. Cankar, I. Frisova, V. Krystof, R. Lenobel, J. Slouka, M. Strnad, P. Fisher (Institute of Experimental Botany of the Academy of Sciences of the Czech Republic; Cyclacel Limited). WO2006/024858 A1. **2006**.
[13] P. Pevarello, M. G. Brasca, P. Orsini, G. Traquandi, A. Longo, M. Nesi, F. Orzi, C. Piutti, P. Sansonna, M. Varasi, A. Cameron, A. Vulpetti, F. Roletto, R. Alzani, M. Ciomei, C. Albanese, W. Pastori, A. Marsiglio, E. Pesenti, F. Fiorentini, J. R. Bischoff, C. Mercurio, *J. Med. Chem.* **2005**, *48*, 2944.
[14] P. Pevarello, P. Orsini, G. Traquandi, R. Amiei, M. Villa, C. Piutti, M. Varasi, A. Longo, US 2004/0019046 A1. **2004**.
[15] D. Bebbington, H. Binch, J.-D. Charrier, S. Everitt, D. Fraysse, J. Golec, D. Kay, R. Knegtel, C. Mak, F. Mazzei, A. Miller, M. Mortimore, M. O'Donnell, S. Patel, F. Pierard, J. Pinder, J. Pollard, S. Ramaya, D. Robinson, A. Rutherford, J. Studley, J. Westcott, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3586.
[16] E. A. Harrington, D. Bebbington, J. Moore, R. K. Rasmussen, A. O. Ajose-Adeogun, T. Nakayama, J. A. Graham, C. Demur, T. Hercend, A. Diu-Hercend, M. Su, J. M. C. Golec, K. M. Miller, *Nat. Med.* **2004**, *10*, 262.
[17] A. A. Mortlock, K. M. Foote, N. M. Heron, F. H. Jung, G. Pasquet, J.-J. M. Lohmann, N. Warin, F. Renaud, C. De Savi, N. J. Roberts, T. Johnson, C. B. Dousson, G. B. Hill, D. Perkins, G. Hatter, R. W. Wilkinson, S. R. Wedge, S. P. Heaton, R. Odedra, N. J. Keen, C. Crafter, E. Brown, K. Thompson, S. Brightwell, L. Khatri, M. C. Brady, S. Kearney, D. McKillop, S. Rhead, T. Parry, S. Green, *J. Med. Chem.* **2007**, *50*, 2213.
[18] E. J. Hanan, A. van Abbema, K. Barrett, W. S. Blair, J. Blaney, C. Chang, C. Eigenbrot, S. Flynn, P. Gibbons, C. A. Hurley, J. R. Kenny, J. Kulagowski, L. Lee, S. R. Magnuson, C. Morris, J. Murray, R. M. Pastor, T. Rawson, M. Siu, M. Ultsch, A. Zhou, D. Sampath, J. P. Lyssikatos, *J. Med. Chem.* **2012**, *55*, 10090.
[19] M. P. Dwyer, K. Paruch, M. Labroli, C. Alvarez, K. M. Keertikar, C. Poker, R. Rossman, T. O. Fischmann, J. S. Duca, V. Madison, D. Parry, N. Davis, W. Seghezzi, D. Wiswell, T. J. Guzi, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 467.
[20] M. Labroli, K. Paruch, M. P. Dwyer, C. Alvarez, K. Keertikar, C. Poker, R. Rossman, J. S. Duca, T. O. Fischmann, V. Madison, D. Parry, N. Davis, W. Seghezzi, D. Wiswell, T. J. Guzi, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 471.
[21] L. Ren, E. R. Laird, A. J. Buckmelter, V. Dinkel, S. L. Gloor, J. Grina, B. Newhouse, K. Rasor, G. Hastings, S. N. Gradl, J. Rudolph, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1165.
[22] T. Kosugi, D. R. Mitchell, A. Fujino, M. Imai, M. Kambe, S. Kobayashi, H. Makino, Y. Matsueda, Y. Oue, K. Komatsu, K. Imaizumi, Y. Sakai, S. Sugiura, O. Takenouchi, G. Unoki, Y. Yamakoshi, V. Cunliffe, J. Frearson, R. Gordon, C. J. Harris, H. Kalloo-Hosein, J. Le, G. Patel, D. J. Simpson, B. Sherborne, P. S. Thomas, N. Suzuki, M. Takimoto-Kamimura, K.-i. Kataoka, *J. Med. Chem.* **2012**, *55*, 6700.
[23] M. R. Shaaban, T. S. Saleh, A. S. Mayhoub, A. M. Farag, *Eur. J. Med. Chem.* **2011**, *46*, 3690.
[24] M. Radi, E. Dreassi, C. Brullo, E. Crespan, C. Tintori, V. Bernardo, M. Valoti, C. Zamperini, H. Daigl, F. Musumeci, F. Carraro, A. Naldini, I. Filippi, G. Maga, S. Schenone, M. Botta, *J. Med. Chem.* **2011**, *54*, 2610.
[25] P. Dinér, J. P. Alao, J. Söderlund, P. Sunnerhagen, M. Grøtli, *J. Med. Chem.* **2012**, *55*, 4872.
[26] J.-Y. Le Brazidec, A. Pasis, B. Tam, C. Boykin, C. Black, D. Wang, G. Claassen, J.-H. Chong, J. Chao, J. Fan, K. Nguyen, L. Silvian, L. Ling, L. Zhang, M. Choi, M. Teng, N. Pathan, S. Zhao, T. Li, A. Taveras, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2070.

[27] G. T. Wang, R. A. Mantei, R. D. Hubbard, J. L. Wilsbacher, Q. Zhang, L. Tucker, X. Hu, P. Kovar, E. F. Johnson, D. J. Osterling, J. Bouska, J. Wang, S. K. Davidsen, R. L. Bell, G. S. Sheppard, *Bioorg. Med. Chem. Lett*. **2010**, *20*, 6067.
[28] S. T. Staben, T. P. Heffron, D. P. Sutherlin, S. R. Bhat, G. M. Castanedo, I. S. Chuckowree, J. Dotson, A. J. Folkes, L. S. Friedman, L. Lee, J. Lesnick, C. Lewis, J. M. Murray, J. Nonomiya, A. G. Olivero, E. Plise, J. Pang, W. W. Prior, L. Salphati, L. Rouge, D. Sampath, V. Tsui, N. C. Wan, S. Wang, C. Weismann, P. Wu, B.-Y. Zhu, *Bioorg. Med. Chem. Lett*. **2010**, *20*, 6048.
[29] M. Soth, S. Abbot, A. Abubakari, N. Arora, H. Arzeno, R. Billedeau, N. Dewdney, K. Durkin, S. Frauchiger, M. Ghate, D. M. Goldstein, R. J. Hill, A. Kuglstatter, F. Li, B. Loe, K. McCaleb, J. McIntosh, E. Papp, J. Park, M. Stahl, M.-L. Sung, R. Suttman, D. C. Swinney, P. Weller, B. Wong, H. Zecic, T. Gabriel, *Bioorg. Med. Chem. Lett*. **2011**, *21*, 3452.
[30] M. Radi, C. Brullo, E. Crespan, C. Tintori, F. Musumeci, M. Biava, S. Schenone, E. Dreassi, C. Zamperini, G. Maga, D. Pagano, A. Angelucci, M. Bologna, M. Botta, *Bioorg. Med. Chem. Lett*. **2011**, *21*, 5928.
[31] L.-L. Yang, G.-B. Li, H.-X. Yan, Q.-Z. Sun, S. Ma, P. Ji, Z.-R. Wang, S. Feng, J. Zou, S.-Y. Yang, *Eur. J. Med. Chem*. **2012**, *56*, 30.
[32] F. Popowycz, G. Fournet, C. Schneider, K. Bettayeb, Y. Ferandin, C. Lamigeon, O. M. Tirado, S. Mateo-Lozano, V. Notario, P. Colas, P. Bernard, L. Meijer, B. Joseph, *J. Med. Chem*. **2009**, *52*, 655.
[33] Z. Nie, C. Perretta, P. Erickson, S. Margosiak, J. Lu, A. Averill, R. Almassy, S. Chu, *Bioorg. Med. Chem. Lett*. **2008**, *18*, 619.
[34] S. Bondock, S. Adel, H. A. Etman, F. A. Badria, *Eur. J. Med. Chem*. **2012**, *48*, 192.
[35] G. Q. Hu, L. L. Hou, Y. Yang, L. Yi, S. Q. Xie, G. Q. Wang, N. N. Duan, T. Y. Chao, X. Y. Wen, W. L. Huang, *Chin. Chem. Lett*. **2011**, *22*, 804.
[36] S. Bindi, D. Fancelli, C. Alli, D. Berta, J. A. Bertrand, A. D. Cameron, P. Cappella, P. Carpinelli, G. Cervi, V. Croci, M. D'Anello, B. Forte, M. Laura Giorgini, A. Marsiglio, J. Moll, E. Pesenti, V. Pittalà, M. Pulici, F. Riccardi-Sirtori, F. Roletto, C. Soncini, P. Storici, M. Varasi, D. Volpi, P. Zugnoni, P. Vianello, *Bioorg. Med. Chem*. **2010**, *18*, 7113.
[37] P. M. Lukasik, S. Elabar, F. Lam, H. Shao, X. Liu, A. Y. Abbas, S. Wang, *Eur. J. Med. Chem*. **2012**, *57*, 311.
[38] M. A. El-borai, H. F. Rizk, M. F. Abd-Aal, I. Y. El-Deeb, *Eur. J. Med. Chem*. **2012**, *48*, 92.
[39] H. Yu, Y. Jung, H. Kim, J. Lee, C.-H. Oh, K. H. Yoo, T. Sim, J.-M. Hah, *Bioorg. Med. Chem. Lett*. **2010**, *20*, 3805.
[40] H. Kim, M. Kim, J. Lee, H. Yu, J.-M. Hah, *Bioorg. Med. Chem*. **2011**, *19*, 6760.
[41] D. Raffa, B. Maggio, M. V. Raimondi, S. Cascioferro, F. Plescia, G. Cancemi, G. Daidone, *Eur. J. Med. Chem*. **2015**, *97*, 732.
[42] M. Li, B.-X. Zhao, *Eur. J. Med. Chem*. **2014**, *85*, 311.
[43] E. Vanotti, F. Fiorentini, M. Villa, *J. Het. Chem*. **1994**, *31*, 737.
[44] S. M. Sondhi, N. Singhal, M. Johar, B. S. N. Reddy, J. W. Lown, *Curr. Med. Chem*. **2002**, *9*, 1045.
[45] A. Terada, K. Wachi, H. Miyazawa, Y. Iizuka, K. Hasegawa, K. Tabata (Sankyo Company Limited). US 5,232,939 A. **1993**.
[46] M. V. Murlykina, M. N. Kornet, S. M. Desenko, S. V. Shishkina, O. V. Shishkin, A. A. Brazhko, V. I. Musatov, E. V. Van der Eycken, V. A. Chebanov, *Beilstein J. Org. Chem*. **2017**, *13*, 1050.
[47] A. O. Abdelhamid, E. K. A. Abdelall, Y. H. Zaki, *J. Het. Chem*. **2010**, *47*, 477.
[48] B. Frey, R. Hufton, M. Harding, A. G. Draffan (Biota Scientific Management Pty Ltd). WO 2013/036994 A1. **2013**.
[49] D. Elleder, J. Young, T. Baiga, J. Noel (The Salk Instituite for Biological Studies). WO 2009/061856 A1. **2009**.
[50] J. Zhang, R. Singh, D. Goff, T. Kinoshita, US 2010/0316649 A1. **2010**.
[51] A. T. Baviskar, C. Madaan, R. Preet, P. Mohapatra, V. Jain, A. Agarwal, S. K. Guchhait, C. N. Kundu, U. C. Banerjee, P. V. Bharatam, *J. Med. Chem*. **2011**, *54*, 5013.
[52] Y. Guo, Z. Wang (Beigene, Ltd.). WO 2014/173289 A1. **2014**.
[53] Z. Wang, Y. Guo (BeiGene, Ltd.). US 2017/0073349 A1. **2017**.
[54] S. Grosse, V. Mathieu, C. Pillard, S. Massip, M. Marchivie, C. Jarry, P. Bernard, R. Kiss, G. Guillaumet, *Eur. J. Med. Chem*. **2014**, *84*, 718.
[55] A. Demjén, M. Gyuris, J. Wölfling, L. G. Puskás, I. Kanizsai, *Beilstein J. Org. Chem*. **2014**, *10*, 2338.
[56] S. Shaaban, B. F. Abdel-Wahab, *Mol. Divers*. **2016**, *20*, 233.
[57] Z-Q. Liu, *Curr. Org. Synth*. **2015**, *12*, 20.
[58] F. Darvas, G. Dormán, P. Krajcsi, L. G. Puskás, Z. Kovári, Z. Lörincz, L. Urge, *Curr. Med. Chem*. **2004**, *11*, 3119.
[59] B. Ozsvári, L. G. Puskás, L. I. Nagy, I. Kanizsai, M. Gyuris, R. Madácsi, L. Z. Fehér, D. Gerö, C. Szabó, *Int. J. Mol. Med*. **2010**, *25*, 525.
[60] P. M. Forde, C. M. Rudin, *Expert Opin. Pharmacother*. **2012**, *13*, 1195.
[61] L. G. Puskás, L. Z. Fehér, C. Vizler, F. Ayaydin, E. Rásó, E. Molnár, I. Magyary, I. Kanizsai, M. Gyuris, R. Madácsi, G. Fábián, K. Farkas, P. Hegyi, F. Baska, B. Ozsvári, K. Kitajka, *Lipids Health Dis*. **2010**, *9*, 56.
[62] A. Abbott, *Nature* **2003**, *424*, 870.
[63] B. Weigelt, A. T. Lo, C. C. Park, J. W. Gray, M. J. Bissell, *Breast Cancer Res. Treat*. **2010**, *122*, 35.
[64] X. Ouyang, E. L. Piatnitski, V. Pattaropong, X. Chen, H.-Y. He, A. S. Kiselyov, A. Velankar, J. Kawakami, M. Labelle, L. Smith, J. Lohman, S. P. Lee, A. Malikzay, J. Fleming, J. Gerlak, Y. Wang, R. L. Rosler, K. Zhou, S. Mitelman, M. Camara, D. Surguladze, J. F. Doody, M. C. Tuma, *Bioorg. Med. Chem. Lett*. **2006**, *16*, 1191.
[65] J. C. A. Boeyens, L. M. Cook, Y. Ding, M. A. Fernandes, D. H. Reid, *Org. Biomol. Chem*. **2003**, *1*, 2168.
[66] N. Edraki, O. Firuzi, A. Foroumadi, R. Miri, A. Madadkar-Sobhani, M. Khoshneviszadeh, A. Shafiee, *Bioorg. Med. Chem*. **2013**, *21*, 2396.
[67] Y. Kobayashi, T. Nakatani, R. Tanaka, M. Okada, E. Torii, T. Harayama, T. Kimachi, *Tetrahedron* **2011**, *67*, 3457.
[68] H. Behbehani, H. M. Ibrahim, S. Makhseed, *ARKIVOC* **2010**, *2010*, 267.
[69] M. A. Gouda, M. A. Berghot, A. I. Shoeib, A. M. Khalil, *Eur. J. Med. Chem*. **2010**, *45*, 1843.
[70] T. Nasr, S. Bondock, S. Eid, *Eur. J. Med. Chem*. **2014**, *84*, 491.
[71] B. Johnstone, T. M. Hering, A. I. Caplan, V. M. Goldberg, J. U. Yoo, *Exp. Cell. Res*. **1998**, *238*, 265.
[72] G. J. Szebeni, Á. Balázs, I. Madarász, G. Pócz, F. Ayaydin, I. Kanizsai, R. Fajka-Boja, R. Alföldi, L. Hackler, L. G. Puskás, *Int. J. Mol. Sci*. **2017**, *18*, 2105.
[73] O. Antal, L. Hackler, J. Shen, I. Mán, K. Hideghéty, K. Kitajka, L. G. Puskás, *Lipids Health Dis*. **2014**, *13*, 142.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Demjén A, Alföldi R, Angyal A, et al. Synthesis, cytotoxic characterization, and SAR study of imidazo[1,2-*b*]pyrazole-7-carboxamides. *Arch Pharm Chem Life Sci*. 2018;1–21.
https://doi.org/10.1002/ardp.201800062