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Etidronic Acid Catalyzed Efficient Synthesis of 1H-imidazo[1,2-b] pyrazole via Sequential One-pot Strategy

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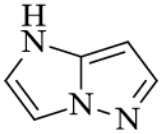
ABSTRACT

In present work we report a convenient and efficient synthesis of 1H-imidazo[1,2-b]pyrazole library by multicomponent reaction of 2-cyano-N-cyclohexyl-3,3-bis(methylthio)acrylamide, hydrazine hydrate, aldehyde and tert-butyl isocyanides. Here 2-cyano-N-cyclohexyl-3,3-bis(methylthio)acrylamide was synthesized by condensation reaction of ethylcyanoacetate and cyclohexylamine in toluene at reflux temperature and followed by reaction with CS2 in DMF in presence of potassium carbonate at room temperature. The newly synthesized compounds were characterized by IR, Mass, 1H NMR, 13C NMR spectroscopy and elemental analysis. All the synthesized compounds were evaluated for their antimicrobial activity. The investigation of antimicrobial and antifungal screening data revealed that all the tested compounds showed moderate to significant activity.

Keywords : 1H-imidazo[1,2-b]pyrazole,

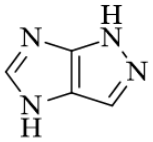
I. INTRODUCTION

Biheterocycles have attracted significant attention from the scientific community because of their relevance in medicinal chemistry. Many bicyclic pyrazole fused with various heterocycles such as imidazole, pyrimidine and pyridine are well recognized for their potent and diverse biological activities[1-7] and have been used as a key pharmacophore. Generally imidazopyrazoles are synthesized by annelation of imidazole to the pyrazole ring. Mainly two different possible isomers 1*H*-imidazo[1,2-*b*]pyrazole (1) and 1,4-dihydroimidazo[4,5-*c*]pyrazole (2) exist. Among these two isomers 1*H*-imidazo[1,2-*b*]pyrazole is most studied one and show anticancer, antimicrobial, anti-inflammatory activities, while imidazo[4,5-*c*]pyrazole remains largely unexplored and show promise as antineurodegenerative drugs.[8,9]



(1)

1*H*-imidazo[1,2-*b*]pyrazole



(2)

1,4-dihydroimidazo[4,5-*c*]pyrazole

The fused pyrazole nucleus is present in wide variety of biologically interesting compounds, which exhibited anti-inflammatory, antipyretic, antibacterial, anticancer activities. As we described, very tremendous biological activity mainly anticancer activity of imidazo[1,2-*b*]pyrazole scaffolds have attracted many chemist to synthesize this class of compounds. Thus the practical synthesis of structurally divers imidazopyrazoles based molecules is of great significance.

II. EXPERIMENTAL

All chemicals and solvents were purchased from Spectrochem Pvt Ltd., Mumbai of AR grade and were used without further purification. Thin-layer chromatography was accomplished on 0.2-mm precoated plates of silica gel G60 F^{254} (Merck). Visualization was made with UV light (254 and 365nm)

or with an iodine vapor. IR spectra were recorded on a FTIR-8400 spectrophotometer using DRS prob. ^{1}H (400 MHz), ^{13}C (100 MHz) NMR spectra were recorded on a Bruker AVANCE II spectrometer in $CDCl_3$ and DMSO. Chemical shifts are expressed in δ *ppm* downfield from TMS as an internal standard. Mass spectra were determined using direct inlet probe on a GCMS-QP 2010 mass spectrometer (Shimadzu). Solvents were evaporated with a BUCHI rotary evaporator. Melting points were measured in open capillaries and are uncorrected. Physical constants of the synthesized compounds **MB-3a to MB-3o** are shown in Table 1.

Procedure for the synthesis of 2-cyano-N-cyclohexylacetamide

A mixture of Cycloheamine (10 mmol) and ethyl cyanoacetate (10 mmol) was reflux at 100 ^{o}C for the approximately 12-15 h in toluene. The reaction was monitored by TLC. After completion of reaction, the solvent was removed under vaccuo and the solid or oil was crystallized from methanol which afforded pure products.

Procedure for the synthesis of 2-cyano-*N*-cyclohexyl-3,3-bis(methylthio)acrylamide

To a well-stirred suspension of potassium carbonate (30 mmol) and 2-cyano-N-cyclohexylacetamide (15 mmol) in DMF (15 mL) at 0-5 ºC was added CS2 (15 mmol) in dropwise over a period of 30 min. After completion of the addition, the reaction mixture was stirred at 0-5 ºC for 1.0 h. appearance of reddish solid in the reaction medium indicated the formation of disodium salt. To this reaction, a solution of methyl iodide (30 mmol) was added dropwise within 15 min at 0-5 ºC. The mixture was allowed to warm to room temperature and stirred for 5-6 h, and then poured onto crushed ice under stirring. The separated solid was collected by filtration, washed with water (2 × 100 mL), dried in vacuo and crystallized from chloroform to furnish the analytically pure products in excellent yield.

Procedure for the synthesis of 5-amino-*N*-cyclohexyl-3-(methylthio)-1*H*-pyrazole-4-carboxamide (MB-3a-o)

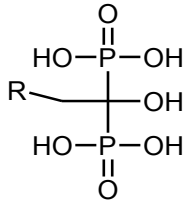
To a suspension of 2-cyano-N-cyclohexyl-3,3-bis(methylthio)acrylamide (10 mmol) in water (25 mL), hydrazine hydrate 80% (20 mmol) was added and the reaction mixture was refluxed for 3-4 hrs with constant stirring. After completion of the reaction, the reaction mixtures were cooled to room temperature and add cold water (50 mL). The separated solid was filtered, washed with water (2 × 50 mL), dried and crystallized from methanol to afford analytically pure products which were used for next step without further purification.

Procedure for the synthesis of 5-amino-*N*-cyclohexyl-3-(methylthio)-1*H*-pyrazole-4-carboxamide (MB-3a-o)

To the solution of 5-amino-*N*-cyclohexyl-3-(methylthio)-1*H*-pyrazole-4-carboxamide (170 mg, 1 mmol) in Methanol:Water (1:1) (5 mL) added aryl aldehyde (1 mmol) at 25-30 ^{0}C in presence of etidronic acid (20 mol%) and stirred for 15 min. Then after *t*-butyl isonitrile was added in one portion and stirring was continued until starting material was consumed completely. Reaction was poured on to ice chilled water and separated solid was filtered off through whatmann filter paper under vacuum. The crude was washed with 1 N $NaHCO_3$ solution (30 mL) and then 1 N HCl solution (30 mL). Finally the crude solid was recrystallized from methanol. Check TLC in (7:3) Hex:EA.Solid material was triturated and recrystallized in Methanol to afford **MB-3a-o** (Overall yield 60-80%).

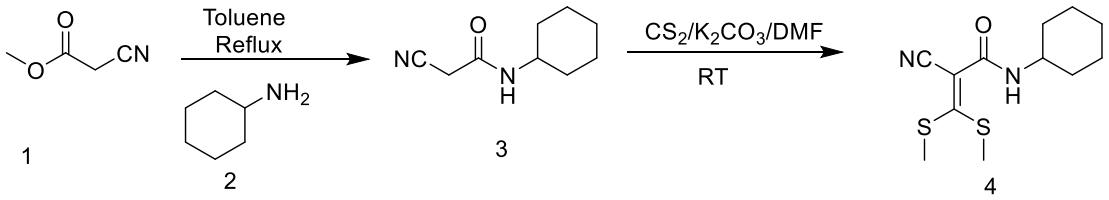
III. REACTION SCHEME

Etidronic acid [(1-hydroxyethylidene) bisphosphonic acid] is one of the bisphosphonic acid derivative and also known as bisphosphonate having molecular formula $C_2H_8O_7P_2$. The two PO_3 (phosphonate) groups covalently linked to carbon atom



It is differ from Polyphosphate ester and polyphosphoric acid. Various bisphosphonic acids are known.[51,52] Etidronic acid is mild enough as compare to another strong acid such as polyphosphoric acid etc. moreover, the catalyst did not affect acid sensitive aldehydes.

Scheme-1. Synthesis of 2-cyano-*N*-cyclohexyl-3,3-bis(methylthio)acrylamide (4)



Scheme-2. Synthesis of Imidazo[1,2-*b*]pyrazole pyrazole derivative

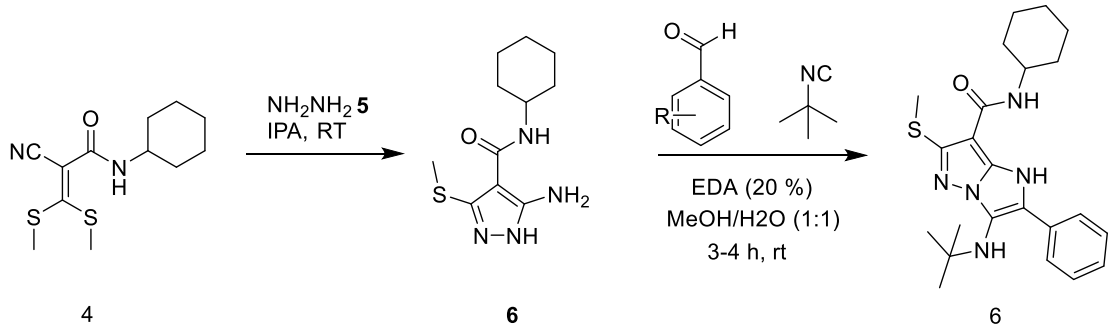


Table 1. Optimization of the reaction condition for the synthesis of MB -3a-o

Entry[a]	Solvent	Catalyst (mol%)	Yield[b] (%)
1	MeOH	HCl	62
2	MeOH	H_2SO_4	45
3	MeOH	TFA	55
4	MeOH	*p*TSA	75
5	MeOH	EDA	67
6	MeOH	PPA	44
7	CAN	EDA	75
8	1,4-Dioxane	EDA	74
9	DMF	EDA	55
10	Water	EDA	50
11	**Methanol:Water (1:1)**	**EDA**	**83**

[a]All the reactions were performed with 0.2 mmol of 4, 0.22 mmol of 5, 0.22 mmol of 6, catalyst 10 mol% and 5 mL of solvent at room temperature of the respective solvent used and for appropriate time. [b]Isolated yield after purification.

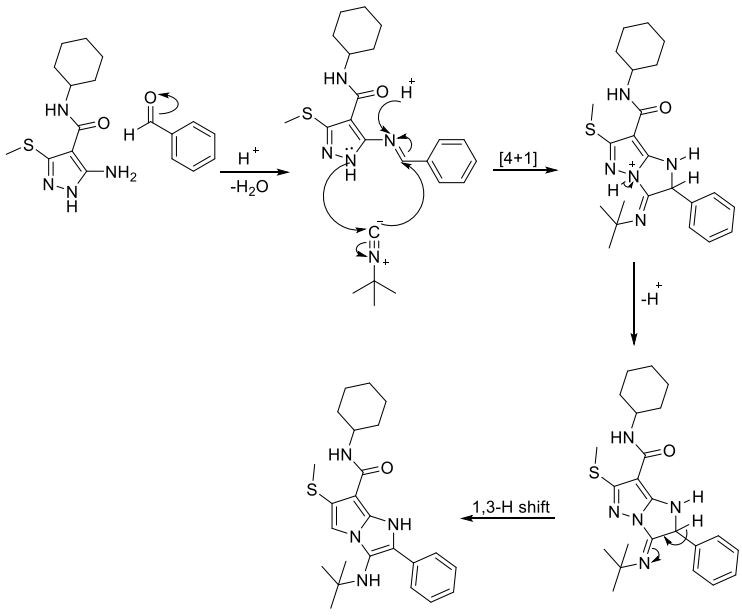
For optimization, the synthesis of **MB-3a-o** were investigated under different catalytic conditions (Table-1). No reaction occurred in the absence of the catalyst (Table-1, entry 1). The GBB-3CR catalyzed by

various acids, including HCL, PTSA, H_2SO_4, TFA, PPA and EDA showed different outputs. Among them EDA demonstrated better yield in comparison to other acid catalysts (Table-1, entries 1-6). On moving further to observe solvent effect, water-methanol mixture displayed improved yield of the desired compound. With this optimized condition in hand, we explored variations of substitution, the result demonstrated well toleration for electron withdrawing functional groups and halide groups as compared to electron donating on the aromatic part

Table 2. Physical Data of The synthesized Synthesis of 1*H*-imidazo[1,2-*b*]pyrazoles derivatives

Entry	R	Reaction time h	M.W.	Yield %	mp ^{0}C
MB-3a	H	3.0	425	72	188
MB-3b	4-OCH_3	3.0	455	71	202
MB-3c	4-CH_3	4.0	439	66	218
MB-3d	2,5-di-OCH_3	3.5	485	61	162
MB-3e	4-$N(CH_3)_2$	3.5	468	69	192
MB-3f	4-OH	4.0	441	58	248
MB-3g	4-Cl	3.0	459	79	210
MB-3h	4-F	4.5	443	70	220
MB-3i	4-Br	3.0	504	81	222
MB-3j	2-Cl	4.0	459	72	224
MB-3k	2-Br	4.0	504	67	236
MB-3l	4-CN	3.5	450	83	230
MB-3m	4-NO_2	3.0	470	81	248
MB-3n	2-NO_2	3.0	470	73	192
MB-3o	-C_3H_7	3.5	391	64	138

Plausible mechanism for the formation of 1*H*-imidazo[1,2-*b*]pyrazole



4. Biological activity

***Microorganisms* used in the study**

The following microorganisms were used in this study: *Staphylococcus aureus* (ATCC29737), *Streptococcus pyogenes (*MTCC443), *Escherichia coli* (NCIM2931), *Pseudomonas aeruginosa (*MTCC 441), *Candida albicans (*MTCC 227), *Aspergillus Niger (*MTCC 282), all the strains were acquired from National chemical Laboratory (NCL), Pune, India. The micro organisms were maintained at 4ºC.

Antibiotics used in the study

All antibiotics were purchased from Hi-Media Laboratory Pvt. Ltd., (Mumbai, india) viz. Chloromphenicol, Ciprofloxacin, Nystatin, Greseofulvin were used for MIC.

Antimicrobial assay

The minimum inhibitory concentration (MIC) values of compounds (1-24) were determined by using the broth microdilution method with 96 micro test plate (Andrews, 2001). The samples were dissolved in DMSO at 1250 µg/mL, and were then diluted to achieve concentrations in the range 30 to 500 μg/ mL for each compound. Two fold dilutions of Chloromphenicol and Ciprafloxacin, Nystatin and Greseofulvin (1-32 µg/mL) were used for a positive control.

A 150 µL volume of Muller-Hinton broth was introduced into all the 96 wells and 20 µL of the varying concentration of the test compounds added in decreasing order along with 30 µL of the test organism suspension. A final volume of 200 µL was achieved in each well (150 µL Muller-Hinton suspension and 20 µL compounds/ antibiotic). Three control wells were maintained for each test batch. The positive control, sterility control and organism control. Plates were incubated at 37 ºC for 24 hours.

After incubation, 40 µL of 2-(4-Iodo phenyl)-3-(4-nitro phenyl)5-phenyltetrazolium chloride (I. N. T) solution (0.2 mg/ml) dissolved in sterile distilled water then added to each well. The plates were incubated for 30 mins and estimated visually for any change in color in to pink indicating reduction of the dye due to bacterial growth. The highest dilution (Lowest concentration) that retained clear corresponded to the MIC.

Table-3. Antimicrobial evaluation data

Compounds and standard drugs	Antibacterial activity				Antifungal acitivity	
	Minimum inhibitory concentration µg/ml				Minimum inhibitory concentration µg/ml	
	Gram +Ve Bacteria		Gram -Ve Bacteria			
	S. aureus	***S.pyogenes***	***E.coli***	***P.aeruginosa***	***C.albicans***	***A.niger***
Ciprofloxacin	7.8	7.8	15.625	15.625	-	-
Chloramphenicol	7.8	7.8	7.8	7.8	-	-

Nystatin	-	-	-	-	31.25	31.25
Greseofulvin					15.625	15.625
MB-3a	33.25	32.25	**13.625**	**11.625**	**13.625**	**31.25**
MB-3b	17.625	16.625	**12.625**	**6.81**	**32.25**	**31.25**
MB-3c	38.25	63.5	33.25	31.25	**14.625**	63.5
MB-3d	68.5	63.5	32.25	63.5	**14.625**	63.5
MB-3e	18.625	32.25	16.625	63.5	**33.25**	63.5
MB-3f	8.81	33.25	**6.81**	63.5	**33.25**	63.5
MB-3g	19.625	16.625	**11.625**	63.5	**33.25**	63.5
MB-3h	18.625	63.5	31.25	32.25	**33.25**	63.5
MB-3i	**9.81**	32.25	**12.625**	**14.625**	**12.625**	**12.625**
MB-3j	39.25	33.25	31.25	**13.625**	**12.625**	**12.625**
MB-3k	35.25	16.625	32.25	32.25	**12.625**	63.5
MB-3l	36.25	**8.8**	32.25	32.25	**12.625**	**30.25**
MB-3m	37.25	34.25	32.25	**6.81**	**12.625**	**30.25**
MB-3n	38.25	63.5	**12.625**	**7.81**	**34.25**	**31.25**
MB-3o	19.625	19.625	**11.625**	**12.625**	**34.25**	**30.25**

IV. CONCLUSION

In summary, we have developed a highly efficient etidronic acid (EDA) promoted synthesis of 1*H*-imidazo[1,2-*b*]pyrazoles *via* multicomponent Groebke–Blackburn–Bienaymè reaction. This synthetic approach has various prominent features such as less reaction steps, good yield, simple reaction conditions, green solvent media and easy purification process. Biological screening of synthesized compounds is currently under progress and will be reported in due course.

6. Spectral data of the synthesized compounds

3-(tert-butylamino)-*N*-cyclohexyl-6-(methylthio)-2-phenyl-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (MB-3a): Off white solid; mp 188 °C; R_f 0.53 (3:7–EtOAc-hexane); IR (KBr): 3317, 3219, 3167, 2964, 2210, 1618, 1473, 1361, 1232, 1195, 1097, 1030, 974, 842, 758, 688, 640, 551 cm^{-1}; MS (*m/z*): 425 (M^+); Elemental analysis: Calcd. for $C_{23}H_{31}N_5OS$: C, 64.91; H, 7.34; N, 16.46; Found: C, 64.82; H, 6.91; N, 16.25.

3-(tert-butylamino)-*N*-cyclohexyl-2-(4-methoxyphenyl)-6-(methylthio)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (MB-3b): White solid; mp 202 °C; R_f 0.56 (3:7–EtOAc-hexane); IR (KBr): 3232, 3082, 2966, 2926, 2829, 2214, 1616, 1519, 1469, 1433, 1361, 1292, 1249, 1186, 1095, 1033, 970, 839, 773, 736, 682, 596, cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.28 (s, 1H, Imidazole-NH), 7.65 (s, 1H, amide -NH), 6.79 (s, 2H, Ar-H), 6.59 (s, 2H, Ar-H), 4.03 (s, 1H, Cyclohexyl-H), 3.56 (s, 3H, -OCH_3), 3.13 (s, 1H, tertbutyl-NH), 2.21 (s, 3H, -SCH_3), 1.49 (m, 6H, Cyclohexyl-H), 1.08 (s, 6H, Cyclohexyl-H), 0.83 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (400 MHz, DMSO-d_6): 160.77, 158.42, 149.98, 136.49, 128.05, 122.99, 122.77, 120.98, 114.46, 113.47, 91.67, 55.64, 55.09, 54.39, 47.30, 32.89, 32.67, 30.08, 27.98, 25.30, 24.88, 14.55; MS (*m/z*): 455 (M^+); Elemental analysis: Calcd. for $C_{24}H_{33}N_5O_2S$: C, 63.27; H, 7.30; N, 15.37; Found: C, 63.48; H, 7.47; N, 15.07.

3-(tert-butylamino)-*N*-cyclohexyl-6-(methylthio)-2-(*p*-tolyl)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (MB-3c): Off white solid; mp 218 °C; R_f 0.57 (3:7–EtOAc-hexane); IR (KBr): 3188, 2972, 2858, 2220, 1624, 1519, 1477, 1384, 1357, 1234, 1201, 823, 775, 678, 648, 588 cm^{-1}; MS (*m/z*): 439 (M^+); Elemental analysis: Calcd. for $C_{24}H_{33}N_5OS$: C, 65.57; H, 7.57; N, 15.93; Found: C, 65.27; H, 7.82; N, 16.12.

3-(*tert*-butylamino)-*N*-cyclohexyl-2-(2,5-dimethoxyphenyl)-6-(methylthio)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (MB-3d): Off white solid; mp 162 °C; R_f 0.51 (3:7–EtOAc-hexane); IR (KBr): 3099, 3049, 2852, 2274, 1737, 1697, 1543, 1410, 1332, 1286, 1159, 1112, 1074, 995, 881, 711, 657, 551 cm^{-1}; MS (*m/z*): 485 (M^+); Elemental analysis: Calcd. for $C_{25}H_{35}N_5O_3S$: C, 61.83; H, 7.26; N, 14.42; Found: C, 60.71; H, 7.42; N, 14.56

3-(*tert*-butylamino)-*N*-cyclohexyl-2-(4-(dimethylamino)phenyl)-6-(methylthio)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (MB-3e): White solid; mp 192 °C; R_f 0.53 (3:7–EtOAc-hexane); IR (KBr): 3244, 3192, 2951, 2939, 2214, 1610, 1527, 1475, 1361, 1323, 1228, 1095, 952, 873, 817, 682, 651, 574 cm^{-1}; MS (*m/z*): 468 (M^+); Elemental analysis:

Calcd. for $C_{25}H_{36}N_6OS$: C, 64.07; H, 7.74; N, 17.93; Found: C, 64.24; H, 7.49; N, 18.16.

3-(*tert*-butylamino)-*N*-cyclohexyl-2-(4-hydroxyphenyl)-6-(methylthio)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (MB-3f): Off white solid; mp 248 °C; R_f 0.46 (3:7–EtOAc-hexane); IR (KBr): 3335, 3176, 2966, 2224, 1616, 1518, 1475, 1388, 1323, 1278, 1234, 1199, 835, 786, 729, 680, 592 cm^{-1}; MS (*m/z*): 441 (M^+); Elemental analysis: Calcd. for $C_{23}H_{31}N_5O_2S$: C, 62.56; H, 7.08; N, 15.86; Found: C, 62.34; H, 7.32; N, 15.64.

3-(*tert*-butylamino)-2-(4-chlorophenyl)-*N*-cyclohexyl-6-(methylthio)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (MB-3g): White solid; mp 210 °C; R_f 0.49 (3:7–EtOAc-hexane); IR (KBr): 3263, 3200, 2970, 2926, 2866, 2210, 1618, 1556, 1498, 1467, 1388, 1232, 1199, 1091, 1014, 977, 879, 837, 748, 630, 586 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 11.65 (s, 1H, Imidazole-NH), 8.47 (d, *J*= 8.0 Hz, 2H, Ar-H), 8.00 (d, *J*= 8.0 Hz, 2H, Ar-H), 6.83 (s, 1H, amide-NH), 4.43 (s, 1H, Cyclohexyl-H), 3.37 (s, 1H, tertbutyl-NH), 2.51 (s, 3H, $-SCH_3$), 1.72 (m, 6H, Cyclohexyl-H), 1.34 (m, 6H, Cyclohexyl-H), 1.29 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (400 MHz, $CDCl_3$): 160.70, 150.49, 137.05, 131.92, 131.51, 129.29, 128.77, 128.31, 128.12, 122.27, 121.88, 91.80, 54.67, 47.32, 32.85, 30.07, 27.94, 25.29, 24.83, 24.35, 14.59; MS (*m/z*): 459 (M^+); Elemental analysis: Calcd. for $C_{23}H_{30}ClN_5OS$: C, 60.05; H, 6.57; N, 15.22; Found: C, 59.87; H, 6.34; N, 14.97.

3-(*tert*-butylamino)-*N*-cyclohexyl-2-(4-fluorophenyl)-6-(methylthio)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (MB-3h): Off white solid; mp 220 °C; R_f 0.58 (3:7–EtOAc-hexane); IR (KBr): 3250, 3211, 3186, 2210, 1620, 1514, 1469, 1367, 1315, 1234, 1195, 1095, 972, 881, 839, 673, 638, 594, 534 cm^{-1}MS (*m/z*): 443 (M^+); Elemental analysis: Calcd. for $C_{23}H_{30}FN_5OS$: C, 62.28,; H, 6.82; N, 15.79; Found: C, 63.98; H, 6.08; N, 15.86.

2-(4-bromophenyl)-3-(*tert*-butylamino)-*N*-cyclohexyl-6-(methylthio)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (MB-3i): Off white solid; mp 222 °C; R_f 0.53 (3:7–EtOAc-hexane); IR (KBr): 3255, 3203, 3180, 2970, 2210, 1620, 1494, 1467, 1388, 1361, 1230, 1076, 1008, 979, 875, 835, 783, 746, 651, 582 cm^{-1}; MS (*m/z*): 504 (M^+); Elemental analysis: Calcd. for $C_{23}H_{30}BrN_5OS$: C, 54.76; H, 5.99; N, 13.88; Found: C, 54.92; H, 5.74; N, 13.67.

3-(*tert*-butylamino)-2-(2-chlorophenyl)-*N*-cyclohexyl-6-(methylthio)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (MB-3j): Off white solid; mp 224 °C; R_f 0.52 (3:7–EtOAc-hexane); IR (KBr): 3101, 3043, 2868, 2220, 1618, 1579, 1504, 1444, 1371, 1298, 1192, 1139, 1103, 1070, 1001, 981, 875, 827, 773, 750, 677, 534 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 12.45 (s, 1H, Imidazole-NH), 7.63 (t, 2H, Ar-H), 7.51-7.44 (m, 2H, Ar-H), 4.16 (s, 1H, *tert*-butyl-NH), 2.59 (s, 3H, $-SCH_3$), 0.93 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (400 MHz, DMSO-d_6): 152.82, 138.68, 133.14, 130.73, 129.73, 128.51, 127.22, 123.93, 121.30, 114.46, 66.19, 54.21, 29.66, 14.93; MS (*m/z*): 459 (M^+); Elemental analysis: Calcd. for $C_{23}H_{30}ClN_5OS$: C, 60.05; H, 6.57; N, 15.22; Found: C, 59.83; H, 6.42; N, 15.46.

2-(2-bromophenyl)-3-(*tert*-butylamino)-*N*-cyclohexyl-6-(methylthio)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (MB-3k): Off white solid; mp 236 °C; R_f 0.61 (3:7–EtOAc-hexane); IR (KBr): 3263, 3213, 3176, 2959, 2221, 1648, 1487, 1421, 1376, 1308, 1242, 1094, 1021, 967, 864, 813, 768, 725, 654, 523 cm^{-1}; MS (*m/z*): 504 (M^+); Elemental analysis: Calcd. for $C_{23}H_{30}BrN_5OS$: C, 54.76; H, 5.99; N, 13.88; Found: C, 54.62; H, 6.14; N, 13.58

3-(*tert*-butylamino)-2-(4-cyanophenyl)-*N*-cyclohexyl-6-(methylthio)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (MB-3l): White solid; mp 230 °C; R_f 0.47 (3:7–EtOAc-hexane); IR (KBr): 3300, 3198, 3053, 2962, 2216, 1737, 1697, 1614, 1512, 1471, 1363, 1199, 1097, 981, 883, 848, 781, 732, 619, 576 cm^{-1}; MS (*m/z*): 450 (M^+); Elemental analysis: Calcd. for $C_{24}H_{30}N_6OS$: C, 63.97; H, 6.71; N, 18.65; Found: C, 63.75; H, 6.53; N, 18.45.

3-(*tert*-butylamino)-2-(4-cyanophenyl)-*N*-cyclohexyl-6-(methylthio)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (MB-3m): White solid; mp 248 °C; R_f 0.55 (3:7–EtOAc-hexane); IR (KBr): 3371, 3215, 3194, 2970, 2208, 1627, 1597, 1510, 1469, 1334, 1232, 1199, 1112, 974, 846, 750, 690, 623, 551 cm^{-1}; MS (*m/z*): 470 (M^+); Elemental analysis: Calcd. for $C_{17}H_{18}N_6O_2S$: C, 63.97; H, 6.71; N, 18.65; Found: C, 64.21; H, 6.48; N, 18.35.

3-(*tert*-butylamino)-*N*-cyclohexyl-6-(methylthio)-2-(2-nitrophenyl)-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (MB-3n): White solid; mp 192 °C; R_f 0.48 (3:7–EtOAc-hexane); IR (KBr): 3326, 3086, 2933, 2846, 2214, 1637, 1556, 1545, 1467, 1354, 1235, 1143, 1047, 956, 898, 812, 753, 682, 654 cm^{-1}; MS (*m/z*): 470 (M^+); Elemental analysis: Calcd. for $C_{23}H_{30}N_6O_3S$: C, 58.70; H, 6.43; N, 17.86; Found: C, 58.92; H, 6.73; N, 17.58.

3-(*tert*-butylamino)-*N*-cyclohexyl-6-(methylthio)-2-propyl-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide

(MB-3o): White solid; mp 138 °C; R_f 0.56 (3:7–EtOAc-hexane); IR (KBr): 3335, 3254, 3188, 2960, 2218, 1610, 1467, 1359, 1300, 1263, 1205, 1043, 954, 885, 744, 692, 599, 555 cm^{-1}; MS (*m/z*): 391 (M^+); Elemental analysis: Calcd. for $C_{20}H_{33}N_5OS$: C, 61.35; H, 8.49; N, 17.89; Found: C, 61.48; H, 8.32; N, 17.64.

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