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RAPID COMMUNICATION

Synthesis and Fluorescence Properties of Donor-Acceptor-Substituted Novel Dipyrazolo[3,4-*b*:3′,4′-*d*]Pyridines (DPP)

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Abstract A rapid and efficient method for the synthesis of novel dipyrazolo[3,4-*b*:3′,4′-*d*]pyridines (DPP) from pyrazolo[3,4-*b*]pyridine was successfully developed. The DPP derivative was further N-alkylated (**6**, **8**) as well as N-linked with amino acids (**13**) and their photophysical properties were studied along with N-aryl DPP **4** and observed that the chromophores at C_4 position in the aryl ring changed the absorption and emission λ_{max}.

Keywords Pyrazolo[*3,4-b*]pyridine · Dipyrazolo[*3,4-b:3′,4′-d*]pyridines (DPP) · Amino acids linked DPP · Absorption and Emission · Quantum yield · HOMO-LUMO

Introduction

Electroluminescent (EL) devices based on organic materials have received considerable attention in recent years since the successes reported by Tang and Vanslyke [1]. The advantage that have been reported in using organic materials to fabricate electroluminescent devices are their high brightness, high efficiency and potential color tuning as well as their low cost of fabrication [2–5]. These new technologies have shown great commercial potential. There is increasing interest in the development of efficient fluorescence materials particularly those emitting in the blue spectral region. The common characteristics of blue emitters and their large optical band gaps, as these are required in order to achieve an emission at relatively high energy. This may consequently restrict the injection characteristics and the conductivity as result of limited delocalization.

The 4-N,N-dimethylaminophenyl derivatives of bis-pyrazolo[3,4-*b*:4′,3′-*e*]pyridine (DMA-DMPP) are further representatives of bulky π-electron donor-acceptor compounds and were recently investigated in some detail both experimentally and semi-empirically [6–9]. Compounds like 3,5-dimethyl-1,7-diphenyl-bis-pyrazolo[3,4-*b*:4′3′-*e*] pyridine derivatives with electron donating substituents at C_4 on phenyl ring (Fig. 1a) showing intense fluorescence in comparison with electron withdrawing substituents in the blue-green region and are considered for application as fluorescence standards and luminophors in organic light emitting diodes [10–12]. In earlier communication, we have reported the fluorescence properties of dipyrazolo[3,4-*b*:3′,4′-*d*]pyridine-3(2*H*)-one [13] (Fig. 1b), Pyrazolo[3,4-*b*]pyrrolo[2,3-*d*]pyridines (PPP) [14] (Fig. 1c). All these compounds show effect of substituents on absorption and emission properties. In present communication, we are reporting the effect of donor and acceptor chromophores on 'N_1' of newly annulated pyrazolo ring to understand the electronic effect of electron donating and electron withdrawing substitutes on the light emitting properties of new dipyrazolo[3,4-*b*:3′,4′-*d*]pyridine derivatives (DPP) **4**. The 3D picture of one of the representative **4b** is shown in (Fig. 2).

Results and discussion

The synthesis of tricyclic heterocycles such as new dipyrazolo[3,4-*b*:3′,4′-*d*]pyridine (DPP) derivatives reported

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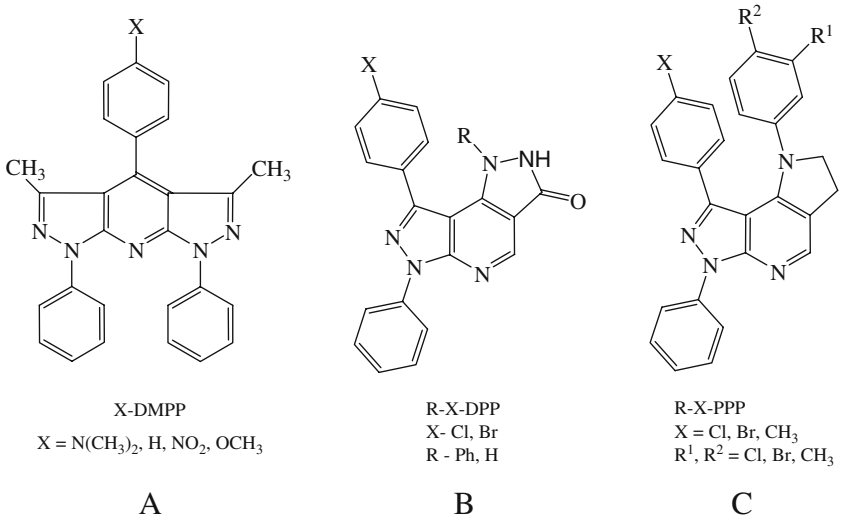
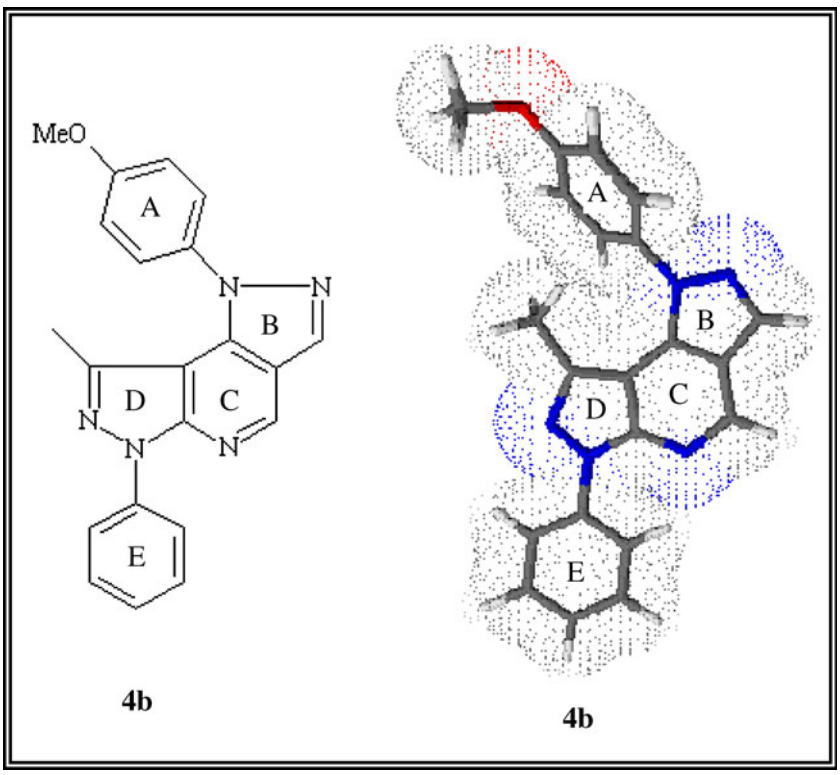
Fig. 1 Structure of DMPP, R-X-DPP, R-X-PPP



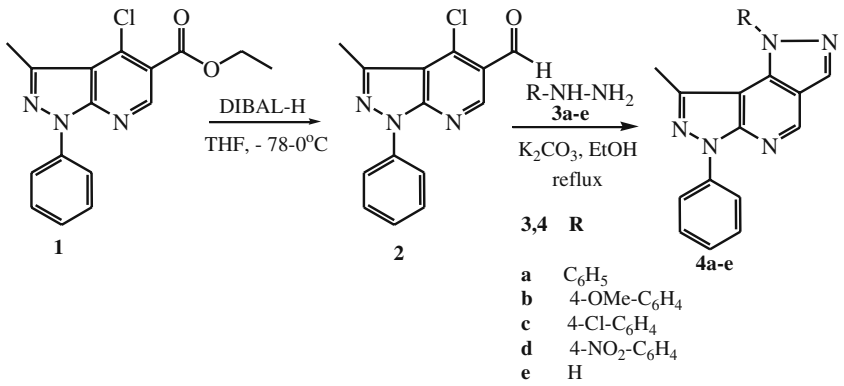
in this article was started with the chloroester **1** [15]. The compound **1** on reaction with diisobutylaluminium hydride (DIBAL-H) in dry tetrahydrofuran at −78 °C, furnished expected aldehyde **2** in 75% yield (Scheme 1).

The annulations of pyrazolo ring on pyridine moiety in **2** was achieved by the condensation with aryl hydrazines **3a–d** or hydrazine hydrate **3e** in ethanol at reflux temperature for 8–9 h to yield the dipyrazolo[3,4-*b*:3′,4′-*d*]pyridines (DPP) **4a–e** in 61–65% yield. The DPP derivatives (**4a–d**) formed in the above synthetic scheme were functionalized with substituted aryl groups and whereas **4e** having free NH group subjected for subsequent alkylation. The structures **4** were confirmed by IR, ^{1}H NMR, ^{13}C NMR, mass spectroscopy, and elemental analysis. For example, the ^{1}H NMR spectrum of **4e** showed singlet at δ 8.45 corresponding to C_4-H, singlet at δ 9.06 corresponding to C_3-H, singlet at δ 14.12 to -NH and remaining aromatic protons showed expected chemical shifts and splitting patterns. The mass spectrum of **4e** revealed a molecular ion peak *m*/*z* at 249. The ^{13}C NMR spectrum of this compound is in agreement with the structure proposed. The photophysical properties of **4** were studied and given in Table 1.

Fig. 2 Molecular modeling of dipyrazolo[3,4-*b*:3′,4′-*d*]pyridine **4b**



Scheme 1 Synthesis of dipyrazolopyridines (DPP) with N-aryl groups **4**



These finding revels that the compounds **4a**, **4b**, **4c** and **4d** (N-aryl DPP) exhibit remarkable fluorescence characters with high quantum yield in comparison with other derivatives of DPP with respect to quinine sulfate which is used as a reference standard for the present study. In the comparisons of **4a–d**, **4b** showed absorption and emission maximum equal to 375, 428 nm and quantum yields (Φ_F) 0.203 than **4a**, **4c** and **4d** showed absorption and emission maximum equal to (365, 415), (364, 411) and (368, 404 nm) and quantum yields (Φ_F) 0.182, 0.187 and 0.171 respectively. Generally, It could be observed that the attachment of an electron donating group (**4b**) to the phenyl function at N-1 position of dipyrazolo[3,4-*b*:3′,4′-*d*]pyridine enhance the fluorescence properties as well as the quantum yield than electron withdrawing group (**4c** and **4d**) at the same position. The qualitative and quantitative screening of **4a**, **4b**, **4c** and **4d** under fluorescent lamp is shown in Fig. 3. It is also observed that dipyrazolo[3,4-*b*:3′,4′-*d*]pyridine (DPP) derivatives with N-aryl substituents carrying electron donating group at para position may be very useful fluorophores that are well suited as fluorescence standard.

Table 1 The Photophysical data for electronic absorption (*abs*) and fluorescence (*flu*) of DPP **4**, **6**, **8** & **13** measured for 0.1 M Conc. in DMSO

Compd	λ_{abs} (DMSO)	λ_{flu} (DMSO)	Φ_F (DMSO)
4a	365	415	**0.182**
4b	375	428	**0.203**
4c	364	411	**0.187**
4d	368	404	**0.171**
4e	359	396	0.161
6a	328	381	0.144
6b	321	379	0.148
6c	337	386	0.137
8a	348	403	0.128
8b	357	414	0.131
8c	348	405	0.127
8d	350	406	0.126
8e	349	401	0.125
8f	352	399	0.128
13a	347	402	0.154
13b	345	404	0.159

To explore the fluorescence behavior of DPP derivatives **4a–d**, we introduced alkyl and analides on 'N-1' of newly annulated pyrazolo ring in **4e** and studied their fluorescence properties. The DPP derivative **4e** having secondary amine group was N-alkylated with different alkylating agents such as alkyl halide **5a–c** or 2-bromo-N-arylacetamide **7a–f** in dimethyl formamide at 55–60 °C for 6 h to afford N-alkyl linked compounds **6a–c** and **8a–f** respectively in 68–70% yield (Scheme 2). The fluorescence behavior of compounds **6** and **8** were studied and given in Table 1. Thus, in comparison with **4**, compounds **6** and **8** are showed lower absorption and emission maxima. For instance compound **6c** showed absorption maximum equal to 337 nm, emission maximum at 386 nm and quantum yield (Φ_F) = 0.137.

The fluorescent peptides [16–19] have large number of applications in biochemistry and biology, namely in studies of protein interaction and conformational analysis. Hence, we designed a strategy to link dipyrazolo [3,4-*b*:3′,4′-*d*]pyridine **4** with different peptides (amino acids) via active ester **11** and studied their photophysical properties. Thus, **4e** was first alkylated with ethyl 2-bromoacetate in DMF to obtain ethyl ester compound **9** in 74% yield, on hydrolysis using sodium hydroxide in aqueous ethanol as a solvent medium furnished the acid **10** in 87% yield, which was subsequently transformed into reactive succinimidoyl active ester **11** (OSu ester) by treating **10** with N-hydroxy succinimide in dry tetrahydrofuran and diisopropylcarbodiimide as a water scavenger. Having active OSu ester **11** in hand, we then displaced OSu with amino acids, to yield **13a–b**. Thus reaction of OSu ester **11** with glycine **12a** in aqueous dimethylsulfoxide as the solvent and aqueous pH 7 buffer as the base afforded amino-linked DPP **13a** in 52% yield. Analogously, the reaction of L-valine **12b** afforded **13b** in 54% yield (Scheme 3). These compounds were characterized using spectroscopic techniques, and further studied for their photophysical properties. The peptide linked DPP **13** may be

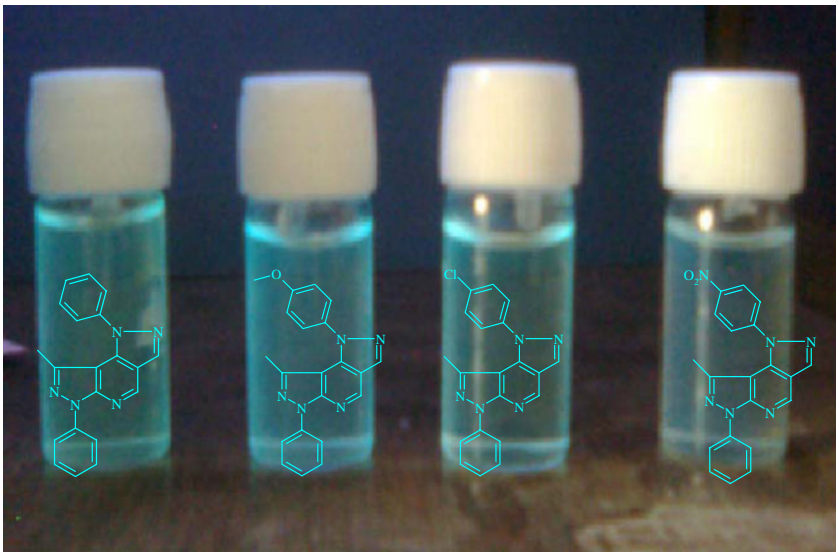


Fig. 3 "Hits" identified by the qualitative and subsequent quantitative screening of the Fluorescence under fluorescence lamp: of compounds **4a**, **4b**, **4c** and **4d**

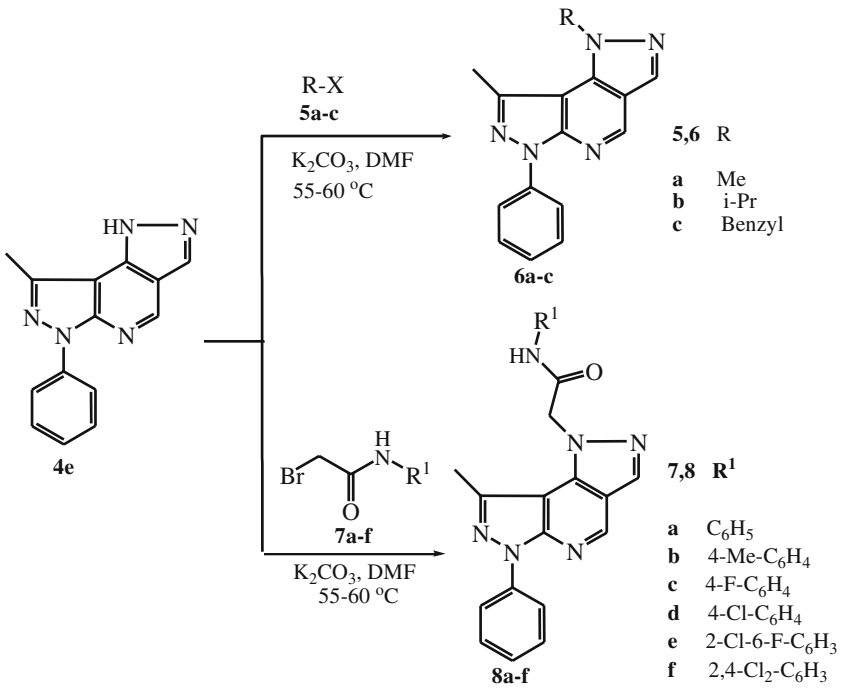
useful to study of protein interaction and conformational analysis.

The photophysical evaluations were determined and given in Table 1. It was clearly observed that peptide linked DPP **13** also showed lower absorption and emission as compared to **4**.

Semi-empirical study of 3, 6, 8 and 13

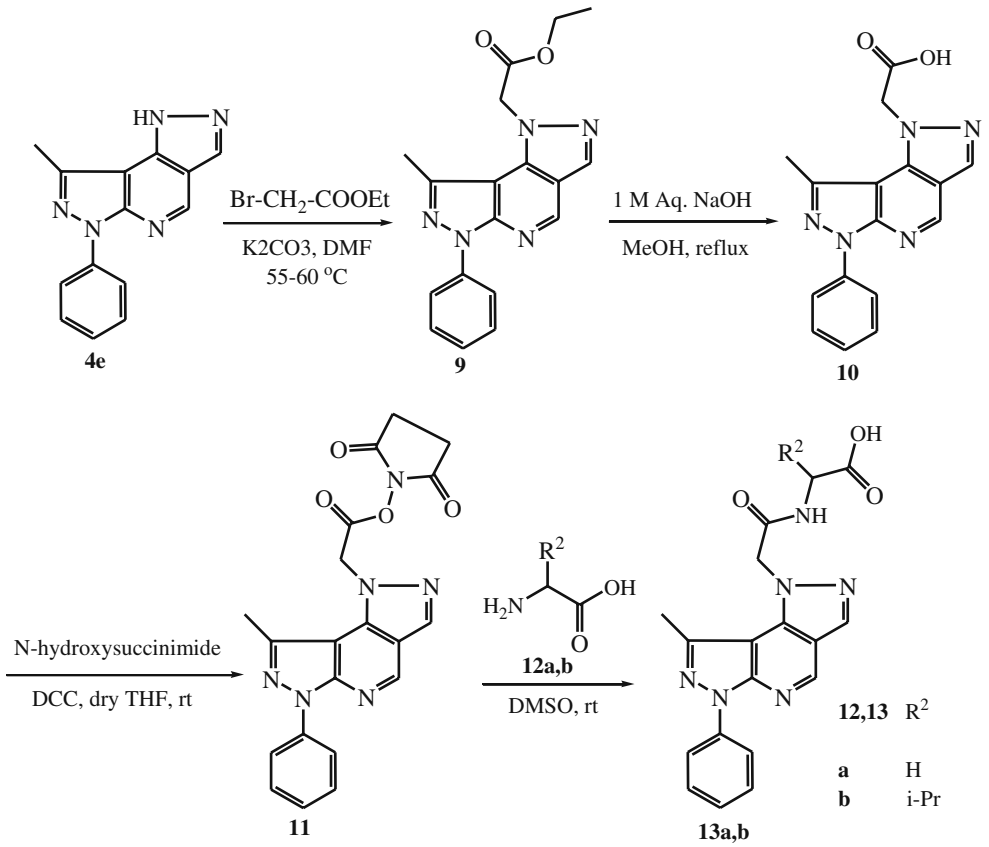
The semi-empirical study of dipyrazolo[3,4-*b*:3′,4′-*d*]pyridines (DPP) i.e. N-aryl DPP **4a–d** and N-alkyl DPP **6**, **8** and **13** has been discussed below:

The compound **4b** having donor group which shows low GAP values and high heat of formation and hence more stability. The compounds **4c** and **4d** are having acceptor group show high GAP values, comparatively low stability and low reactivity. There is more overlapping between the HOMO-LUMO for **4b**, which shows high quantum yields (Table 2). The charge is more concentrated on ring **B** compare to ring **D**. The donor chromophores on ring **A** is playing important roll in increasing electron density of ring **B.** The ring **B** is directly linked to the ring **C** and **D**, which make facile charge delocalization in the molecule, increases the stability and reactivity of the molecules and hence



Scheme 2 Synthesis of dipyrazolopyridines (DPP) with N-alkyl **6** and N-analides **8** at N_1

Scheme 3 Synthesis of dipyrazolopyridine (DPP) derivatives with amino acids **13** at N_1



shows high quantum yields in these compounds. The HOMO-LUMO calculation of **4c** and **4d** having inductively or mesomerically electron withdrawing chromophores increase the GAP values i.e. lower overlapping of atomic orbital. The N-alkyl linked DPP compounds **6**, **8** and **13** presented high GAP value. This shows low stability (low heat of formation), less charge distribution. Therefore N-alkyl / amino acids DPP **6**, **8** and **13** shows low quantum

Table 2 The molecular electronic properties (HOMO-LUMO energy, GAP) of the DPP **4**, **6**, **8** & **13**

Compd.	R	Heat of Formation (K CAL.)	Ionization Potential	HOMO	LUMO	GAP
3a	Ph	−157.29	8.682	−8.683	−0.918	9.501
3b	*p*–OCH_3–C_6H_4	−116.24	8.622	−8.623	−0.751	9.374
3c	*p*–Cl–C_6H_4	−148.09	8.979	−8.980	−0.919	9.899
3d	*p*–NO_2–C_6H_4	−127.29	9.122	−9.023	−1.197	10.22
3e	–H	−126.03	8.789	−8.789	−0.912	9.701
6a	CH_3	−125.83	8.704	−8.704	−0.806	9.51
6b	–CH $(CH_3)_2$	−104.22	8.698	−8.699	−0.851	9.55
6c	–CH_2–Ph	−141.68	8.773	−8.774	−0.935	9.709
8a	–CH_2–CO–NH–C_6H_5	−97.74	8.833	−8.834	−1.153	9.987
8b	–CH_2–CO–NH–*p*–CH_3–C_6H_4	−88.40	8.568	−8.568	−1.122	9.69
8c	–CH_2–CO–NH–*p*–F–C_6H_4	−50.39	8.934	−8.934	−1.215	10.149
8d	–CH_2–CO–NH–p–Cl–C_6H_4	−87.75	8.868	−8.869	−1.232	10.101
8e	–CH_2–CO–NH–2–F, 6–Cl–C_6H_3	−47.43	8.893	−8.893	−1.116	10.009
8f	–CH_2–CO–NH–4,6–*di*Cl–C_6H_3	−88.42	9.638	−9.639	−2.589	12.228
13a	–CH_2–CO–NH–CH_2–COOH	−16.92	8.845	−8.846	−1.099	9.945
13b	–CH_2–CO–NH–CH[$CH(CH_3)_2$]–(COOH)	−34.19	8.885	−8.885	−1.095	9.98

GAP = E_{LUMO}–E_{HOMO}

yield than N-aryl DPP **4a–d**. The practical results obtained are in agreement with the HOMO LUMO, Heat of formation obtained by semi empirical PM3/PM6 methods.

Conclusion

The reactions reported here represent the facile synthesis of new class of fluorescent dipyrazolo[3,4-*b*:3′,4′-*d*] pyridine (DPP) derivatives. Thermal analysis of **5**, **7**, **9** and **14** by differential scanning calorimetry (DSC) revealed that they are thermally stable compounds up to 300 °C. Most important of all, fluorescence quantum yields are almost independent of solvents and pH. The fluorescence properties of these compounds depends upon the nature of substituents present on nitrogen atom of pyrazolo nucleus of dipyrazolo[3,4-*b*:3′,4′-*d*]pyridine (DPP). The donor chromophore C_4-OCH_3 show absorption and emission maximum to red shift (bathochromic shift). While in case of acceptor chromophore C_4-NO_2 the absorption and emission maximum showed to blue shift (hypsochromic shift).

Experimental

General

Melting points were determined on Gallenkamp Melting Point Apparatus, Mod. MFB-595 in open capillary tubes and is uncorrected. The ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Varian XL-300 spectrometer. Chemical shifts are reported in parts per million using tetramethylsilane as internal standard and are given in δ units. The solvent for NMR spectra was $CDCl_3$ and DMSO-d_6 unless otherwise stated. Infrared spectra were taken on Shimadzu IR-408, a Shimadzu FTIR instrument in potassium bromide pellets unless otherwise stated. UV spectra were recorded on a Shimadzu UV-1601 UV-VIS Spectrophotometer. Fluorescence spectra were recorded using RF-5301 PC Spectrofluorophotometer. Compounds for UV and fluorescence measurements were dissolved in DMSO. Mass spectrum was recorded on Shimadzu GC-MS QP mass spectrometer with an ionization potential of 70 eV. UV and fluorescence scans were recorded from 200 to 600 nm. Elemental analysis was performed on a Hosli CH-Analyzer and is within ±0.3 of the theoretical percentage. All reactions were monitored by thin layer chromatography, carried out on 0.2 nm silica gel 60 F_{254} (Merck) plates using UV light (254 and 366 nm) for detection. Common reagents grade chemicals are either commercially available and were used without further purification or prepared by standard literature procedures. The GAMESS software is used for HOMO-LUMO, Heat of formation etc. by semi empirical PM3/PM6 methods.

(4-chloro-3-methyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-5-yl)methanol (2) A solution of diisobutylaluminium hydride (1.0 M toluene solution; 42.75 mL, 42.75 mmol) was added dropwise to a solution of compound **1** (4.5 g, 14.25 mmol) in methylene chloride (30 mL) at −78 °C. The reaction mixture was stirred for 2 h at the same temperature. The reaction mixture was quenched with brine solution at −78 °C and allowed to attain room temperature. The reaction mixture was filtered through celite, filtrate was dried over anhydrous sodium sulfate, evaporated under reduced pressure and recrystallized from ethanol to obtain colorless solid. Yield: 2.91 g (75.19%), mp 156–157 °C. IR (KBr): 3,057 m, 2,947 m, 2,717 m, 1,726 m, 1,606 m, 1,495 s, 1,232 m, 765w cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.70 (s, 3H, CH_3), 7.36 (t, *J*=7.8 Hz, 1H, Ar–H), 7.54 (t, *J*= 7.8 Hz, 2H, Ar–H), 8.06 (d, *J*=7.8 Hz, 2H, Ar–H), 8.84 (s, 1H, Ar–H), 10.34 (s, 1H, CH=O). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 15.5, 115.2, 120.4 (2 C's), 127.7, 129.7 (2 C's), 130.9, 138.8, 143.2, 145.3, 151.9, 152.6, 189.1. MS (70 eV) m/z (%): 273 (33) [M+2], 271 (100) [M$^+$], 242 (24), 235 (17), 194 (16), 180 (21), 91 (19), 77 (28). Anal. Calcd. for $C_{14}H_{10}ClN_3O$ (271.71): C, 61.89; H, 3.71; N, 15.47. Found: C, 62.06; H, 3.63; N, 15.56.

General procedure for the preparation of (4a–d) A solution of **2** (0.1 g, 0.368 mmol), aryl hydrazine **3a–d** (0.404 mmol) and anhydrous potassium carbonate (0.061 g, 0.44 mmol) in ethanol (3 mL) was heated at reflux for 8 h. The reaction mass was cooled at room temperature, ice cold water (1 ml) was added into the reaction mass, the solid obtained was filtered, dried and recrystallized to afford compound **4** in good yield.

8-Methyl-1,6-diphenyl-1,6-dihydrodipyrazolo[3,4-b:3',4'-d] pyridine (4a) Yield: 0.063 g (52.94%), recrystallized from ethanol to afford colorless solid, mp 162–163 °C. IR (KBr): 3,050 m, 2,923 m, 1,598 m, 1,509 s, 1,438 m, 1,221 m, 767w cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.79 (s, 3H, CH_3), 7.35 (t, *J*=7.4 Hz, 2H, Ar–H), 7.55 (t, *J*=7.4 Hz, 4H, Ar–H), 8.17 (d, *J*=7.4 Hz, 4H, Ar–H), 9.17 (s, 1H, Ar–H), 9.58 (s, 1H, Ar–H). MS (70 eV) m/z (%): 325 (100) [M$^+$], 234 (20), 248 (18), 171 (23), 91 (17), 77 (30). Anal. Calcd. for $C_{20}H_{15}N_5$ (325.38): C, 73.83; H, 4.65; N, 21.52. Found: C, 73.97; H, 4.74; N, 21.34.

1-(4-Methoxyphenyl)-8-methyl-6-phenyl-1,6-dihydrodipyrazolo[3,4-b:3',4'-d]pyridine (4b) Yield: 0.069 g (53.07%), recrystallized from ethanol to afford colorless solid; mp 189–190 °C. IR (KBr): 3,050, 2,924, 1,595, 1,501, 1,423, 1,230, 1,145, 754 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.86 (s, 3H, CH_3), 3.81 (s, 3H, CH_3), 6.73 (d, *J*=7.4 Hz,

2H, Ar–H), 7.32 (t, *J*=7.8 Hz, 1H, Ar–H), 7.41 (d, *J*= 7.4 Hz, 2H, Ar–H), 7.54 (t, *J*=7.8 Hz, 2H, Ar–H), 8.19 (d, *J*=7.8 Hz, 2H, Ar–H), 8.63 (s, 1H, Ar–H), 9.14 (s, 1H, Ar–H); MS (70 eV) m/z (%): 355 (100) [M^+], 340 (19), 324 (31), 264 (24), 121 (16), 107 (19), 91 (20), 77 (29). Anal. Calcd. for $C_{21}H_{17}N_5O$ (355.40): C, 70.97; H, 4.82; N, 19.71. Found: C, 70.79; H, 4.93; N, 19.79.

1-(4-Chlorophenyl)-8-methyl-6-phenyl-1,6-dihydrodipyrazolo[3,4-b:3',4'-d]pyridine (4c) Yield: 0.077 g (52.02%), recrystallized from ethanol to afford colorless solid; mp 173–174 °C. IR (KBr): 3,064 m, 2,934 m, 1,604 m, 1,501 s, 1,433 m, 1,230 m, 759w cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.73 (s, 3H, CH_3), 7.32 (t, *J*= 7.8 Hz, 1H, Ar–H), 7.47 (d, *J*=8.4 Hz, 2H, Ar–H), 7.55 (d, *J*=8.4 Hz, 2H, Ar–H), 7.62 (t, *J*=7.8 Hz, 2H, Ar–H), 8.11 (d, *J*=7.8 Hz, 2H, Ar–H), 9.19 (s, 1H, Ar–H), 9.64 (s, 1H, Ar–H). MS (70 eV) m/z (%): 361 (34) [M+2], 359 (100) [M^+], 324 (26), 248 (22), 268 (24), 112 (17), 91 (25), 77 (36). Anal. Calcd. for $C_{20}H_{14}ClN_5$ (359.82): C, 66.76; H, 3.92; N, 19.46. Found: C, 66.97; H, 3.84; N, 19.34.

1-(4-Nitroophenyl)-8-methyl-6-phenyl-1,6-dihydrodipyrazolo[3,4-b:3',4'-d]pyridine (4d) Yield: 0.067 g (50.75%), recrystallized from ethanol to afford colorless solid; mp 168–169 °C (ethanol). IR (KBr): 3,042 m, 2,919 m, 1,595 m, 1,501 s, 1,457 m, 1,325 s, 1,230 m, 751w cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 2.81 (s, 3H, CH_3), 7.37 (t, *J*=7.2 Hz, 1H, Ar–H), 7.42 (d, *J*=7.4 Hz, 2H, Ar–H), 7.51 (d, *J*=7.4 Hz, 2H, Ar–H), 7.57 (t, *J*=7.2 Hz, 2H, Ar–H), 8.19 (d, *J*=7.2 Hz, 2H, Ar–H), 9.18 (s, 1H, Ar–H), 9.59 (s, 1H, Ar-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 15.6, 112.9, 115.2, 121.1 (2 C's), 122.3 (2 C's), 125.2, 125.8, 128.1 (2 C's), 128.9 (2 C's), 132.3, 136.4, 138.6, 140.0, 143.2, 147.3, 150.7. MS (70 eV) m/z (%): 370 (100) [M^+], 324 (26), 248 (22), 268 (24), 112 (17), 91 (25), 77 (36). Anal. Calcd. for $C_{20}H_{14}ClN_5$ (359.82): C, 64.86; H, 3.81; N, 22.69. Found: C, 64.97; H, 3.74; N, 22.44.

8-Methyl-6-phenyl-1,6-dihydrodipyrazolo[3,4-b:3',4'-d]pyridine (4e) A solution of **2** (1 gm, 3.68 mmol), hydrazine hydrate **3e** (0.20 gm, 4.04 mmol) and anhydrous potassium carbonate (0.61 gm, 4.4 mmol) in ethanol (10 ml) were mixed and heated at reflux for 9 h. The reaction mass was cooled at room temperature and the solvent was removed under reduced pressure. The residue was purified by column chromatography (n-Hexane: ethyl acetate = 5:1) to afford colorless solid. Yield: 0.6 g (65.43%); mp 207–208 °C. IR (KBr): 3,345 m, 3,196 m, 3,087 m, 2,941 m, 1,607 m, 1,504 s, 1,447 m, 1,227 m, 754w cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 2.79 (s, 3H, CH_3), 7.32 (t, *J*=7.8 Hz, 1H, Ar–H), 7.54 (t, *J*=7.8 Hz, 2H, Ar–H), 8.26 (d, *J*=7.8 Hz, 2H, Ar–H), 8.45 (s, 1H, Ar–H), 9.06 (s, 1H, Ar–H), 14.12 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 15.6, 103.4, 116.3, 119.9 (2 C's), 125.7, 129.1 (2 C's), 131.8, 132.6, 140.4, 143.2, 147.5, 149.9. MS (70 eV) m/z (%): 249 (100) [M^+], 234 (25), 158 (21), 91 (21), 77 (37). Anal. Calcd. for $C_{14}H_{11}N_5$ (249.28): C, 67.46; H, 4.45; N, 28.09. Found: C, 67.29; H, 4.54; N, 19.24.

General procedure for the preparation of (6a–c) A solution of **4e** (0.1 g, 0.40 mmol), alkyl halide **5a–c** (0.44 mmol) and anhydrous potassium carbonate (0.066 gm, 0.48 mmol) in dimethyl formamide (3 mL) was heated at 55–60 °C for 6 h. The reaction mass was cooled at room temperature, ice cold water (1 ml) was added into the reaction mass, the solid obtained was filtered, dried and recrystallized to afford **6** in good yield.

1,8-Dimethyl-6-phenyl-1,6-dihydrodipyrazolo[3,4-b:3',4'-d]pyridine (6a) Yield: 0.074 g (70.47%), recrystallized from ethanol to afford colorless solid; mp 181–182 °C. IR (KBr): 3,041 m, 2,931 m, 1,605 m, 1,509 s, 1,439 m, 1,277 m, 781w cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 2.88 (s, 3H, CH_3), 4.29 (s, 3H, CH_3), 7.31 (t, *J*=7.5 Hz, 1H, Ar–H), 7.52 (t, *J*–7.5 Hz, 2H, Ar–H), 8.15 (d, *J*–7.5 Hz, 2H, Ar–H), 8.17 (s, 1H, Ar–H), 8.96 (s, 1H, Ar–H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 15.3, 39.7, 111.2, 115.9, 120.7 (2 C's), 123.5, 125.6, 129.8 (2 C's), 133.2, 138.9, 143.7, 148.8, 151.4. MS (70 eV) m/z (%): 263 (100) [M^+], 248 (29), 234 (19), 172 (24), 91 (19), 77 (31). Anal. Calcd. for $C_{15}H_{13}N_5$ (263.30): C, 68.43; H, 4.98; N, 26.60. Found: C, 68.57; H, 5.06; N, 26.48.

1-Isopropyl-8-methyl-6-phenyl-1,6-dihydrodipyrazolo[3,4-b:3',4'-d]pyridine (6b) Yield: 0.08 g (68.96%), recrystallized from ethanol to afford colorless solid; mp 165–166 °C. IR (KBr): 3,028 m, 2,921 m, 1,594 m, 1,504 s, 1,416 m, 1,249 m, 746w cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.70 (d, *J*=5.4 Hz, 6H, 2 x CH_3), 2.89 (s, 3H, CH_3), 4.89 (m, 1H, CH), 7.31 (t, *J*=7.5 Hz, 1H, Ar–H), 7.53 (t, *J*=7.5 Hz, 2H, Ar–H), 8.16 (d, *J*=7.5 Hz, 2H, Ar–H), 8.22 (s, 1H, Ar–H), 8.95 (s, 1H, Ar–H). MS (70 eV) m/z (%): 291 (100) [M^+], 276 (25), 248 (31), 91 (25), 77 (41), 57 (14). Anal. Calcd. for $C_{17}H_{17}N_5$ (291.36): C, 70.08; H, 5.88; N, 24.04. Found: C, 69.97; H, 5.81; N, 24.22.

1-Benzyl-8-methyl-6-phenyl-1,6-dihydrodipyrazolo[3,4-b:3',4'-d]pyridine (6c) Yield: 0.095 g (69.85%), recrystallized from ethanol to afford colorless solid; mp 197–198 °C. IR (KBr): 3,064 m, 2,936 m, 1,591 m, 1,499 s, 1,423 m, 1,221 m, 754w cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 2.89 (s, 3H, CH_3), 5.67 (s, 2H, CH_2), 7.26–7.45 (m, 6H, Ar–H), 7.52 (t, *J*=7.8 Hz, 2H, Ar–H), 8.08 (s, 1H, Ar–H), 8.14 (d, *J*=7.8 Hz, 2H, Ar–H), 8.92 (s, 1H, Ar–H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 15.5, 62.1, 111.8, 115.2, 120.4 (2 C's),

124.9, 125.2, 127.2, 127.9 (2 C's), 128.7 (2 C's), 129.6 (2 C's), 133.4, 133.5, 139.1, 144.4, 148.5, 149.9. MS (70 eV) m/z (%): 339 (100) [M^+], 262 (29), 248 (25), 119 (17), 105 (21), 91 (23), 77 (38). Anal. Calcd. for $C_{21}H_{17}N_5$ (339.40): C, 74.32; H, 5.05; N, 20.63. Found: C, 74.44; H, 4.94; N, 20.52.

General procedure for the synthesis of (8a–f) A solution of **4e** (0.1 g, 0.40 mmol), **7a–f** (0.44 mmol) and anhydrous potassium carbonate (0.066 g, 0.48 mmol) in dimethyl formamide (3 mL) was heated at 55–60 °C for 6 h. The reaction mass was cooled at room temperature, ice cold water (1 mL) was added into the reaction mass, the solid obtained was filtered, dried and recrystallized to afford **8** in good yield.

2-(8-methyl-6-phenyldipyrazolo[3,4-b:3',4'-d]pyridin-1(6H)-yl)-N-phenylacetamide (8a) Yield: 0.102 g (66.66%), recrystallized from ethanol to afford colorless solid; mp 239–240 °C. IR (KBr): 3,263 m, 3,036 m, 2,923 m, 1,665 m, 1,601 m, 1,508 s, 1,429 m, 1,234 m, 753w cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.74 (s, 3H, CH_3), 5.41 (s, 2H, CH_2), 7.24–7.31 (m, 6H, Ar–H), 7.43–7.50 (m, 4H, Ar–H), 8.65 (s, 1H, Ar–H), 9.06 (s, 1H, Ar–H), 10.67 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 14.8, 56.1, 109.9, 115.5, 120.6 (2 C's), 121.1 (2 C's), 123.9, 124.5, 125.9, 128.7 (2 C's), 129.6 (2 C's), 135.8, 137.9, 139.5, 143.5, 148.2, 149.1, 165.4. MS (70 eV) m/z (%): 382 (100) [M^+], 290 (41), 262 (26), 248 (23), 148 (16), 134 (22), 91 (17), 77 (33). Anal. Calcd. for $C_{22}H_{18}N_6O$ (382.43): C, 69.10; H, 4.74; N, 21.98. Found: C, 69.28; H, 4.82; N, 22.19.

2-(8-methyl-6-phenyldipyrazolo[3,4-b:3',4'-d]pyridin-1(6H)-yl)-N-p-tolylacetamide (8b) Yield: 0.107 g (67.29%), recrystallized from ethanol to afford colorless solid; mp 251–252 °C. IR (KBr): 3,289 m, 3,048 m, 2,915 m, 1,673 m, 1,594 m, 1,505 s, 1,432 m, 1,226 m, 758w cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.24 (s, 3H, CH_3), 2.71 (s, 3H, CH_3), 5.44 (s, 2H, CH_2), 7.12 (d, J=8.2 Hz, 2H, Ar–H), 7.32 (t, J=7.8 Hz, 1H, Ar–H), 7.48 (d, J=8.2 Hz, 2H, Ar–H), 7.54 (t, J=7.8 Hz, 2H, Ar–H), 8.22 (d, J= 7.8 Hz, 2H, Ar–H), 8.87 (s, 1H, Ar–H), 9.14 (s, 1H, Ar–H), 10.65 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 14.5, 23.2, 55.4, 108.8, 115.1, 120.8 (2 C's), 121.6 (2 C's), 124.2, 125.1, 128.9 (2 C's), 129.5 (2 C's), 133.6, 134.7, 135.1, 138.6, 143.9, 147.7, 150.6, 164.8. MS (70 eV) m/z (%): 396 (100) [M^+], 305 (16), 290 (34), 262 (19), 248 (23), 134 (25), 106 (19), 91 (26), 77 (37). Anal. Calcd. for $C_{23}H_{20}N_6O$ (396.46): C, 69.68; H, 5.08; N, 21.20. Found: C, 69.86; H, 4.99; N, 21.06.

N-(4-fluorophenyl)-2-(8-methyl-6-phenyldipyrazolo[3,4-b:3',4'-d]pyridin-1(6H)yl)acetamide (8c) Yield: 0.107 g (66.87%), recrystallized from ethanol to afford colorless solid; mp 247–248 °C. IR (KBr): 3,278 m, 3,061 m, 2,939 m, 1,659 m, 1,590 m, 1,507 s, 1,428 m, 1,247 m, 818w cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.91 (s, 3H, CH_3), 5.26 (s, 2H, CH_2), 7.02 (t, J=8.4 Hz, 2H, Ar–H), 7.34 (t, J=7.8 Hz, 1H, Ar–H), 7.44 (m, 2H, Ar–H), 7.53 (t, J=7.8 Hz, 2H, Ar–H), 8.14 (d, J=7.8 Hz, 2H, Ar–H), 8.35 (s, 1H, Ar–H), 9.06 (s, 1H, Ar–H), 9.14 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 14.0, 55.8, 103.5, 115.4, 115.7, 115.9, 121.1 (2 C's), 121.3, 125.8, 129.0 (2 C's), 130.6, 134.9, 139.4, 141.3, 144.2, 147.5, 148.2, 151.2, 153.5, 164.5. MS (70 eV) m/z (%): 400 (100) [M^+], 309 (18), 290 (41), 262 (26), 95 (20), 91 (23), 77 (34). Anal. Calcd. for $C_{22}H_{17}FN_6O$ (400.42): C, 65.99; H, 4.28; N, 20.99. Found: C, 66.14; H, 4.17; N, 21.09.

N-(4-chlorophenyl)-2-(8-methyl-6-phenyldipyrazolo[3,4-b:3',4'-d]pyridin-1(6H)yl)acetamide (8d) Yield: 0.111 g (66.46%), recrystallized from ethanol to afford colorless solid; mp 241–242 °C. IR (KBr): 3,314 m, 3,064 m, 2,935 m, 1,666 m, 1,595 m, 1,500 s, 1,454 m, 1,230 m, 760w cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.71 (s, 3H, CH_3), 5.48 (s, 2H, CH_2), 7.32–7.39 (m, 3H, Ar–H), 7.53–7.64 (m, 4H, Ar–H), 8.21 (d, J=7.4 Hz, 2H, Ar–H), 8.87 (s, 1H, Ar–H), 9.13 (s, 1H, Ar–H), 10.70 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 14.1, 55.6, 108.5, 115.9, 120.6 (2 C's), 121.3 (2 C's), 125.8, 127.4, 128.9 (2 C's), 129.0 (2 C's), 130.6, 134.6, 137.4, 139.4, 144.2, 148.2, 152.3, 169.7. MS (70 eV) m/z (%): 418 (33) [M+2], 416 (100) [M^+], 290 (27), 248 (21), 126 (20), 91 (25), 77 (32). Anal. Calcd. for $C_{22}H_{17}ClN_6O$ (416.87): C, 63.39; H, 4.11; N, 20.16. Found: C, 63.23; H, 4.03; N, 20.07.

N-(2-chloro-6-fluorophenyl)-2-(8-methyl-6-phenyldipyrazolo[3,4-b:3',4'-d]pyridin-1(6H)-yl) acetamide (8e) Yield: 0.114 g (65.51%), recrystallized from ethanol to afford colorless solid; mp 268–269 °C. IR (KBr): 3,279 m, 3,027 m, 2,941 m, 1,654 m, 1,598 m, 1,503 s, 1,431 m, 1,246 m, 783w cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.69 (s, 3H, CH_3), 5.56 (s, 2H, CH_2), 7.31–7.40 (m, 4H, Ar–H), 7.53 (t, J= 7.4 Hz, 2H, Ar–H), 8.22 (d, J=7.4 Hz, 2H, Ar–H), 8.89 (s, 1H, Ar–H), 9.10 (s, 1H, Ar–H), 10.31 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 14.1, 55.0, 103.5, 114.9, 115.9, 121.3 (2 C's), 125.4, 125.8, 126.9, 129.0 (2 C's), 130.5, 132.1, 139.4, 141.4, 143.9, 146.8, 148.2, 150.6, 154.1, 165.0. MS (70 eV) m/z (%): 436 (35) [M+2], 434 (100) [M^+], 343 (21), 248 (28), 144 (26), 105 (16), 77 (29). Anal. Calcd. for $C_{22}H_{16}ClFN_6O$ (434.86): C, 60.77; H, 3.71; N, 19.33. Found: C, 60.98; H, 3.81; N, 19.21.

N-(2,4-dichlorophenyl)-2-(8-methyl-6-phenyldipyrazolo[3,4-b:3',4'-d]pyridin1(6H)yl) acetamide (8f) Yield: 0.12 g (66.29%), recrystallized from ethanol to afford colorless

solid; mp 257–258 °C. IR (KBr): 3,249 m, 3,095 m, 2,927 m, 1,657 m, 1,596 m, 1,497 s, 1,460 m, 1,232 m, 747w cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 2.72 (s, 3H, CH_3), 5.58 (s, 2H, CH_2), 7.32–7.43 (m, 3H, Ar–H), 7.53 (t, J=8.2 Hz, 2H, Ar–H), 7.69 (d, J=1.8 Hz, 1H, Ar–H), 8.21 (d, J=8.2 Hz, 2H, Ar–H), 8.88 (s, 1H, Ar–H), 9.14 (s, 1H, Ar–H), 10.17 (s, 1H, NH). MS (70 eV) m/z (%): 454 (14) [M + 4], 452 (65) [M + 2], 450 (100) [M^+], 345 (18), 248 (24), 160 (29), 91 (23), 77 (39). Anal. Calcd. for $C_{22}H_{16}Cl_2N_6O$ (451.32): C, 58.55; H, 3.57; N, 18.62. Found: C, 58.74; H, 3.68; N, 18.49.

Ethyl 2-(8-methyl-6-phenyldipyrazolo[3,4-b:3',4'-d]pyridin-1(6H)-yl)acetate (9) A solution of **4e** (0.3 g, 1.2 mmol), anhydrous potassium carbonate (0.061 g, 1.44 mmol) and ethyl bromoacetate (0.22 g, 1.32 mmol) in dimethyl formamide (5 mL) was heated at 60–65 °C for 4 h. The reaction mass was cooled at room temperature, ice cold water (2 mL) was added into the reaction mass, the solid obtained was filtered, dried and recrystallized from ethanol to afford colorless solid. Yield: 0.3 g (74.44%), recrystallized from ethanol to afford colorless solid; mp 175–176 °C. IR (KBr): 3,261 m, 3,067 m, 2,963 m, 1,736 m, 1,601 m, 1,491 s, 1,192 m, 761w cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 1.23 (t, J=6.2 Hz, 3H, CH_3), 2.71 (s, 3H, CH_3), 4.19 (q, J=6.2 Hz, 2H, CH_3), 5.52 (s, 2H, CH_2), 7.34 (t, J=7.2 Hz, 1H, Ar–H), 7.54 (t, J=7.2 Hz, 2H, Ar–H), 8.20 (d, J=7.2 Hz, 2H, Ar–H), 8.85 (s, 1H, Ar–H), 9.14 (s, 1H, Ar–H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 13.6, 14.8, 54.7, 59.9, 111.5, 114.7, 120.7 (2 C's), 125.3, 125.9, 129.0 (2 C's), 136.4, 138.9, 143.8, 148.4, 151.3, 165.7. MS (70 eV) m/z (%): 335 (100) [M^+], 290 (29), 262 (22), 248 (26), 234 (19), 171 (15), 105 (21), 77 (40). Anal. Calcd. for $C_{18}H_{17}N_5O_2$ (335.37): C, 64.47; H, 5.11; N, 20.88. Found: C, 64.61; H, 5.20; N, 21.00.

2-(8-Methyl-6-phenyldipyrazolo[3,4-b:3',4'-d]pyridin-1(6H)-yl)acetic acid (10) A solution of the compound **9** (0.25 g, 0.74 mmol) in ethanol (5 mL) was treated with 1 M aq. sodium hydroxide solution (1 mL). The reaction mixture was heated at 85 °C for 3 h and ethanol was removed under reduced pressure. The aqueous residue was acidified with 2 N hydrochloric acid, the precipitate obtained is filtered off and dried under reduced pressure to give colorless solid. Yield: 0.20 g (87.33%), recrystallized from ethanol to afford colorless solid; mp 218–219 °C. IR (KBr): 3,424 m, 3,264 m, 3,067 m, 2,981 m, 2,925 m, 1,721 m, 1,594 m, 1,498 s, 1,178 m, 751w cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 2.77 (s, 3H, CH_3), 5.40 (s, 2H, CH_2), 7.32 (t, J=7.8 Hz, 1H, Ar–H), 7.54 (t, J=7.8 Hz, 2H, Ar–H), 8.19 (d, J=7.8 Hz, 2H, Ar–H), 8.83 (s, 1H, Ar–H), 9.13 (s, 1H, Ar–H), 12.1 (br, s, 1H, –COOH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 15.0, 56.2, 111.2, 115.1, 120.4 (2 C's), 124.9, 126.2, 129.1 (2 C's), 136.8, 139.1, 144.0, 148.3, 150.7, 179.4. MS (70 eV) m/z (%): 307 (100) [M^+], 290 (27), 248 (29), 199 (17), 143 (23), 91 (27), 77 (35). Anal. Calcd. for $C_{16}H_{13}N_5O_2$ (307.31): C, 62.53; H, 4.26; N, 22.79. Found: C, 62.36; H, 4.15; N, 22.99.

2,5-dioxopyrrolidin-1-yl-2-(8-methyl-6-phenyldipyrazolo[3,4-b:3',4'-d]pyridin-1(6H)yl) acetate (11) N-Hydroxysuccinimide (0.065 g, 0.57 mmol) was added slowly to a solution of **10** (0.175 g, 0.57 mmol) in dry tetrahydrofuran (7 mL) at 0 °C while stirring. Then N,N-diisopropylcarbodiimide (0.071 g, 0.57 mmol) was added at 0–5 °C dropwise under stirring. Yellowish-white precipitate obtained was stirred at 0–5 °C for about 15 h, and the solvent was removed under reduced pressure. The obtained solid was filtered by suction and washed with dry tetrahydrofuran. The solid was then stirred in dry ethanol (10 ml) at 20 °C for 0.5 h to remove N,N-diisopropyl urea formed during the reaction suction filtration to afford colorless solid. Yield: 0.147 g (63.91%), recrystallized from ethanol to afford colorless solid; mp 224–225 °C. IR (KBr): 3,276 m, 3,037 m, 2,932 m, 1,719 m, 1,654 m, 1,600 m, 1,503 s, 1,429 m, 1,183 m, 755w cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 2.60 (s, 4H, 2 x CH_2), 2.74 (s, 3H, CH_3), 5.42 (s, 2H, CH_2), 7.35 (t, J=7.2 Hz, 1H, Ar–H), 7.57 (t, J=7.2 Hz, 2H, Ar–H), 8.22 (d, J=7.2 Hz, 2H, Ar–H), 8.86 (s, 1H, Ar–H), 9.15 (s, 1H, Ar–H). MS (70 eV) m/z (%): 404 (100) [M^+], 306 (46), 248 (31), 201 (20), 84 (16), 91 (26), 77 (37). Anal. Calcd. for $C_{20}H_{16}N_6O_4$ (404.39): C, 59.40; H, 3.99; N, 20.78. Found: C, 59.55; H, 4.11; N, 20.59.

General procedure for the synthesis of compounds 13a, b To a solution of glycine **12a** or L-valine **12b** (0.1 mmol) in 90% aqueous dimethylsulfoxide (1.5 mL) was added a solution of succinimidoyl active ester **11** (0.04 g, 0.1 mmol) in 90% aqueous dimethylsulfoxide (1.5 mL) dropwise at 20 °C. Then, aqueous pH 7 buffer solution (0.75 mL) was added and the mixture was stirred for 14 h at 20 °C. Reaction mixture was poured into water (5 mL), acidified with concentrated HCl to pH = 1–2, a solid separated was stirred and filtered by suction and washed with an excess amount of water.

2-(2-(8-methyl-6-phenyldipyrazolo[3,4-b:3',4'-d]pyridin-1(6H)yl)acetamido)aceticacid (13a) Yield: 0.019 g (52.14%), recrystallized from ethanol to afford colorless solid; mp 255–256 °C. IR (KBr): 3,427 m, 3,306 m, 3,234 m, 3,136 m, 3,057 m, 2,974 m, 2,929 m, 1,718 m, 1,648 m, 1,604 m, 1,510 s, 1,187 m, 781w cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 2.69 (s, 3H, CH_3), 5.19 (s, 2H, CH_2), 5.37 (s, 2H, CH_2), 7.31 (t, J=7.8 Hz, 1H, Ar–H), 7.49 (t, J=7.8 Hz, 2H, Ar–H), 8.19 (d, J=7.8 Hz, 2H, Ar–H), 8.82 (s, 1H, Ar–H), 9.07 (s, 1H, Ar–H), 10.61 (s, 1H, –

NH), 12.56 (s, 1H, –COOH). MS (70 eV) m/z (%): 364 (100) [M^+], 319 (17), 290 (22), 262 (29), 248 (31), 143 (23), 105 (19), 91 (22), 77 (32). Anal. Calcd. for $C_{18}H_{16}N_6O_3$ (364.37): C, 59.34; H, 4.43; N, 23.06. Found: C, 59.14; H, 4.56; N, 23.22.

3-methyl-2-(2-(8-methyl-6-phenyldipyrazolo[3,4-b:3',4'-d] pyridin-1(6H)-yl) acetamido)butanoic acid (13b) Yield: 0.022 g (54.18%), recrystallized from ethanol to afford colorless solid; mp 248–249 °C. IR (KBr): 3,446 m, 3,372 m, 3,278 m, 3,067 m, 2,967 m, 2,914 m, 1,715 m, 1,654 m, 1,602 m, 1,502 s, 1,183 m, 743w cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 1.60 (d, J=5.4 Hz, 6H, 2 x CH_3), 2.73 (s, 3H, CH_3), 4.24 (m, 1H, CH), 5.37 (s, 2H, CH_2), 5.58 (d, J=5.8 Hz, 1H, CH), 7.36 (t, J=7.4 Hz, 1H, Ar–H), 7.56 (t, J=7.4 Hz, 2H, Ar–H), 8.25 (d, J=7.4 Hz, 2H, Ar–H), 8.84 (s, 1H, Ar–H), 9.15 (s, 1H, Ar–H), 10.03 (s, 1H, –NH), 12.82 (s, 1H, –COOH). MS (70 eV) m/z (%): 406 (100) [M^+], 391 (31), 376 (16), 361 (28), 248 (23), 91 (27), 77 (42), 58 (16). Anal. Calcd. for $C_{21}H_{22}N_6O_3$ (406.45): C, 62.06; H, 5.46; N, 20.68. Found: C, 62.29; H, 5.56; N, 20.87.

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