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Synthesis of new pyrazoles and pyrozolo [3,4-*b*] pyridines as anti-inflammatory agents by inhibition of COX-2 enzyme

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ABSTRACT

New pyrazoles and pyrazolo[3,4-*b*] pyridines were synthesized and their structure was confirmed by elemental analyses as well as IR, ^{1}H NMR, ^{13}C NMR, and mass spectral data. All the newly synthesized derivatives were evaluated *in vitro* for inhibitory activity against COX-1 and COX-2 enzymes and their IC_{50} values were calculated, most of the derivatives showed good inhibitory activity with derivatives **IVb, IVh and IVJ** showing inhibitory activity better than celecoxib. Moreover, the eight most potent derivatives **IVa, IVb, IVc, IVd, IVe, IVh, IVJ, and IVL** were selected for *in vivo* assay to measure their effect on paw edema in rates and their ulcerogenic effect. Compounds **IVa, IVb and IVc** were found to be the most active and selective as COX-2 inhibitors and most effective in protection from edema, they were also found to have lowest ulcerogenic effect among all derivatives.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) comprise a heterogeneous group of medications, with analgesic, antipyretic and anti-inflammatory activity [1]. They act as inhibitors of prostaglandin synthesis through non-selective inhibition of COX enzymes but as a side effect they cause mucosal damage, ulceration and ulcer complication [2]. There are two cyclooxygenase enzymes, one predominating at sites of inflammation (COX-2) and one constitutively expressed in the gastrointestinal tract (COX-1), this led to the important therapeutic development of COX-2 inhibitors [3]. COX-2 inhibitors were found to exert anti-inflammatory and analgesic effects without the complications associated with existing non-selective inhibitors [4]. Several selective COX-2 inhibitors have been reported and many of them have reached market as parecoxib sodium, valdecoxib, rofecoxib, and celecoxib as shown in Fig. 1 [5]. Pyrazole ring plays an important role in medicinal chemistry, the use of pyrazole cores in biologically active molecules have stimulated the need for efficient ways to make these heterocyclic lead [6]. Several pyrazole compounds have been reported to be potential therapeutic agents for the treatment of inflammation and not associated with adverse effects including the marketed selective COX-2 inhibitor drug like celecoxib [7]. Moreover, several pyrazole derivatives were reported as anti-inflammatory [8,9] in addition to pyrazolo [3,4-*b*] pyridine derivatives were synthesized and investigated for their anti-inflammatory agents and were recognized as promising multi-potent anti-inflammatory agents [10] Fig. 2.

Encouraged with the above survey, the present study aimed to develop and synthesize novel compounds bearing pyrazole and pyrazolo [3,4-*b*] pyridine rings and test their biological activity as anti-inflammatory agents by inhibition of COX-2 enzyme. In addition, to test their safety on the gastrointestinal tract.

2. Discussion

2.1. Chemistry

The synthesis of new derivatives was performed through Schemes 1, 2 and 3. The structure of the newly synthesized compounds was confirmed by elemental analyses and (IR, ^{1}H NMR, ^{13}C NMR, and MS) spectral data. First, 3-amino-1H-pyrazol-5(4H)-one **(I)** was synthesized

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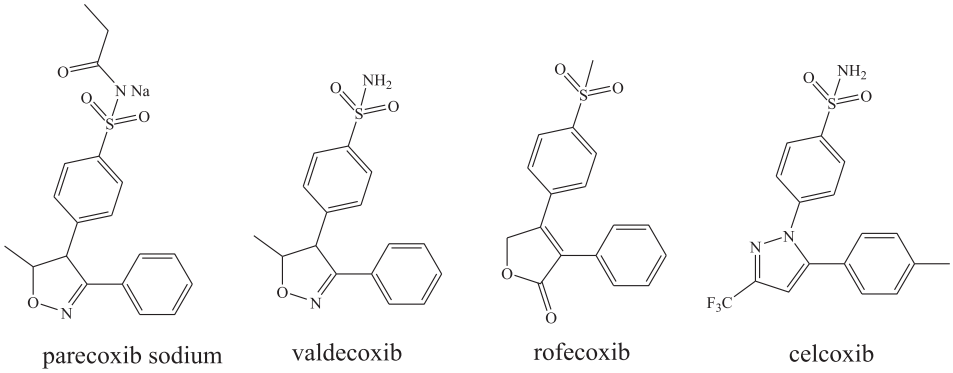


Fig. 1. Selective COX-2 inhibitors that have reached market.

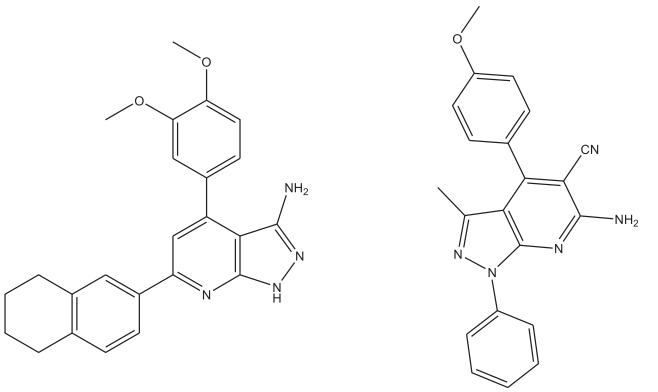


Fig. 2. Pyrazolo **[3,4-*b*]** pyridine derivatives reported as anti-inflammatory agents.

following reported procedure [11]followed by reaction of **(I)** with *o*-flouro aniline in water acidified with hydrochloric acid to produce **(II)** as reported [12,14]. Moreover**, (II)** was subjected to coupling reaction with diazonium salt of different aromatic amines in presence of sodium acetate to produce **(IIIa-f)** following a previously reported procedure for analogous compounds [15–17]. The structure of which was proven by the disappearance of CH aliphatic absorption band from IR spectra at 2966 cm^{-1}, and disappearance of single peak at 3.43 representing CH_2 of pyrazole ring and appearance of characteristic D_2O exchangeable signal at 13.42 of NH of the diazonium in ^1H NMR, also the appearance of triplet and quartet signals equivalent to ethyl group of **(IIIc)** and singlet signal at 3.78 of methoxy group of **(IIId).** In addition to singlet signal at 2.30 representing methyl group of **(IIIe),** finally **(IIIf)** showed the two methyl groups as singlet signal at 2.36 ppm. ^{13}C NMR showed the distinct signals of ethyl and methyl substitution at14.21, 23.55 and 16.67 also the 2(C═O) groups of **(IIIc and IIIe)** appeared at 159.27 and 159.28 ppm**.** Finally mass spectroscopy showed molecular ion peak of all derivatives.

The new derivatives **(IVa-L)** were synthesized by the mannich reaction procedure. Where **(IIIa-f)** reacted with a number of amines in formaline/ethanol mixture [18,19]. Structure elucidation of the new derivatives was performed, IR spectra showed the appearance of aliphatic peak equivalent to CH_2 of the mannich at 2997–2927 cm^{-1}. ^1H NMR showed the appearance of signal equivalent to the CH_2 of mannich at 4.92–5.47 ppm. In addition to the appearance of singlet signals of the two methyl groups of 2,6-dimethyl aniline at 3.17–3.56 ppm of **(IVg-L)**. ^{13}C NMR was used to confirm the structure of the new derivatives which showed signals at 49.07–46.54 equivalent to CH_2 of mannich base of **(IVa, IVc and IVg)** also signal of (C═O) group at 156.05–157.12 ppm. Moreover, signals at 21.33 and 21.39 representing the ethyl group of **(IVc).** Finally mass spectroscopy showed molecular ion peak of all derivatives.

Pyridine ring synthesis was reported to be performed by the reaction of substituted benzaldehyde, malononitrile and ammonium acetate with active methylene [20]. In our study, this procedure was used to synthesize prazolo [3,4-*b*] pyridine derivatives **(Va-f)** using pyazole ring **(II)** as the active methylene provider. The structure of **(Va-f)** was confirmed by the appearance of sharp peak at 2210–2202 cm^{-1} equivalent to the cyano group. Also by the appearance of forked peak of (NH_2) group at 3452–3336 cm^{-1}. A broad peak at 3518 of **(OH)** group confirmed **(Ve).** ^1H NMR proved the structure by the appearance of D_2O exchangeable signals at 7.16–7.69 and 7.54–7.98 ppm of the **(NH_2)** group. It also confirmed the structure of **(Ve and Vf)** by the D_2O exchangeable signal at 9.97 of (OH) group and singlet signal at 3.86 ppm of methoxy group. ^{13}C NMR showed signal equivalent to (CN) group at 75.69 and 75.46 ppm. Also the signal at 55.76 ppm equivalent to (OCH_3) group of **(Vf).** Mass spectroscopy showed molecular ion peak of all derivatives.

2.2. Biological activity

2.2.1. In vitro COX-1 and COX-2 assay

All newly synthesized compounds were subjected to *in vitro* COX-1 and COX-2 assay, the eight most potent derivatives were subjected to *in vivo* anti-inflammatory screening and ulcerogenic effect. First, the structure of the new derivatives was designed to follow structure activity relationship of celecoxib a selective COX-2 enzyme inhibitor [21] COX-2 enzyme shows larger space than COX-1 and so the bulkier inhibitors fit better to COX-2 showing selectivity [22]. Derivatives **(IVa-L)** were the bulkier among other derivatives and so showed highest activity with **IVb, IVh and IVJ** showing activity better than celecoxib with IC_{50} values of 0.048, 0.048 and 0.046 μM respectively compared to IC_{50} value of celecoxib 0.049 μM. while, **IVa, IVc, IVd, IVe, IVf, IVg, IVL and Vf** showed moderate activity relative to celecoxib with IC_{50} values 0.071, 0.055, 0.051, 0.067, 0.082. 0.093, 0.054 and 0.082 μM respectively but still more potent than NSAID drugs indomethacin and diclofenac sodium. From these results, the bulkier derivatives showed better activity than the less bulky ones.

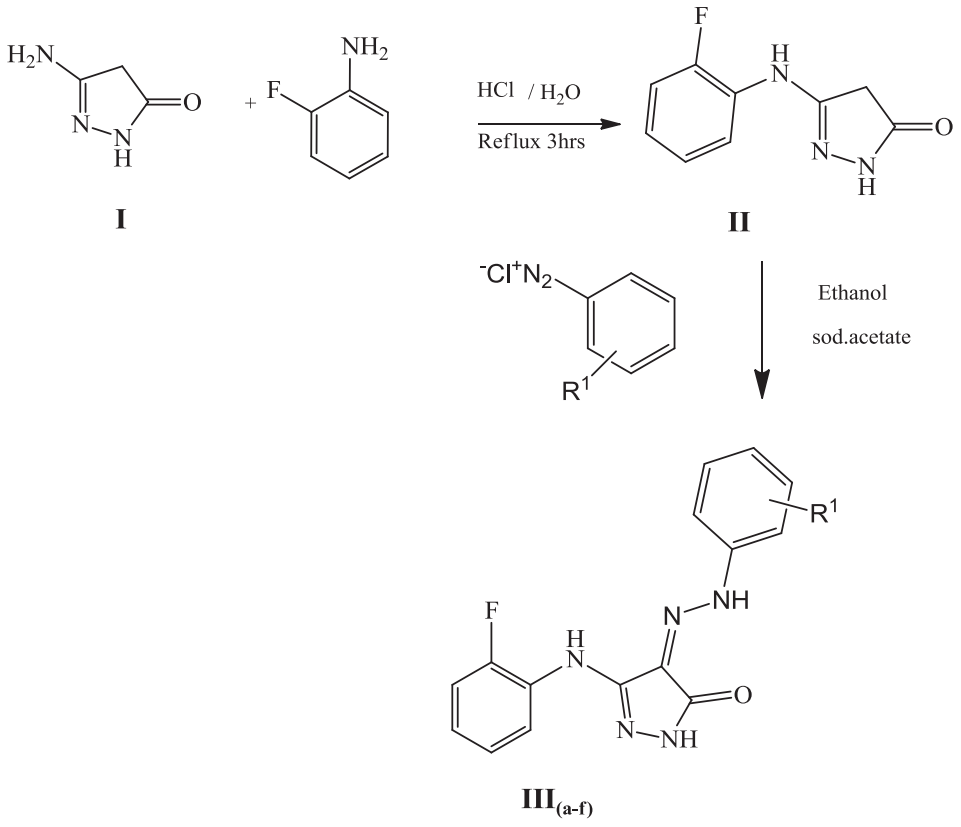
2.2.2. In vivo anti-inflammatory screening

The eight most potent derivatives **IVa, IVb, IVc, IVd, IVe, IVh, IVJ, IVL** were further subjected to *in vivo* anti-inflammatory screening based upon the ability of the tested compounds to inhibit the edema produced in the hind paw of rats by injection of carrageenan to produce experimental arthritis [13]. Derivatives **IVa, IVb and IVc** were found to be the most potent in protection from paw edema with percentage protection value of 61, 64, 62 compared to 69% of celecoxib and 70% of indomethacin.

2.2.3. Ulcerogenic effect

Finally ulcerogenic effect was tested to examine the safety of the eight most potent derivatives and **IVa, IVb, IVc and IVJ** showed the safest profile against ulcer compared to celecoxib.

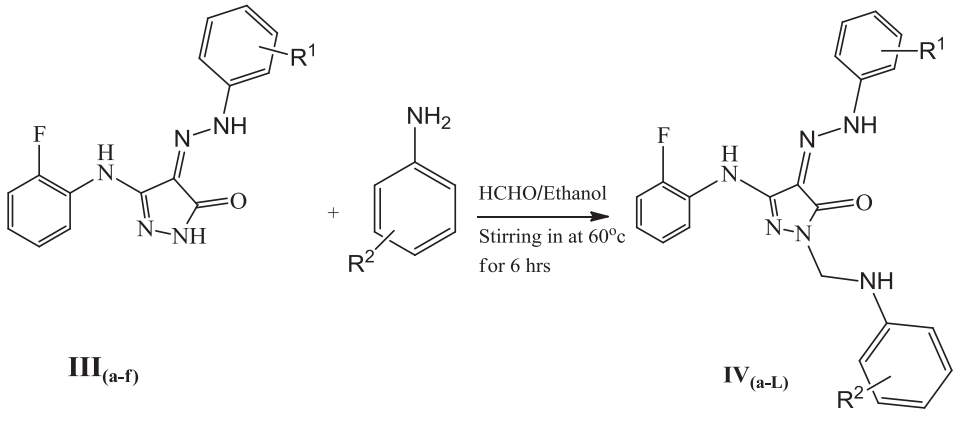
Scheme(1): Synthesis of derivatives IIIa-f



R^1 : H, 2-bromo, 2-ethyl, 4-methoxy, 2-methyl , 2,6-dimethyl

Scheme 1. Synthesis of derivatives **IIIa-f.**

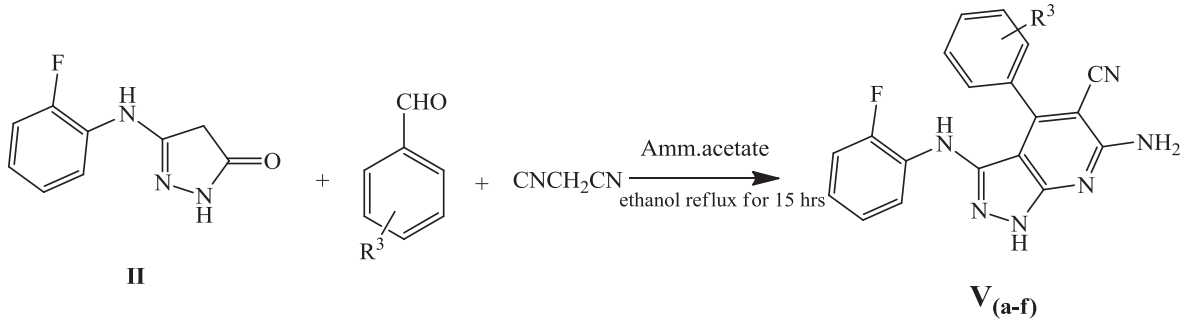
Scheme(II) synthesis of derivatives IVa-L



R^1:H, 2-bromo, 2-ethy , 4-methoxy , 2-methyl , ,2,6-dimethyl
R^2: H , 2,6-dimethyl

Scheme 2. Synthesis of derivatives **IVa-L.**

Scheme (III) Synthesis of derivatives Va-f



R^3 =H , *p*-Br , *p*-Cl , *p*-F, *p*-OH, *p*-OCH_3

Scheme 3. Synthesis of derivatives **Va-f.**

3. Conclusion

The synthesis of the new pyrazoles and pyrazolo[3,4-*b*] pyridines was achieved with the aim of finding new derivatives with anti-inflammatory activity by inhibition of COX-2 enzyme overcoming the side effects of non-selective anti-inflammatory drugs which was revealed by *in-vivo* determination of ulcerogenic effect. The most potent derivatives were found to be of the pyrazole ring derivatives, while the pyrazolo[3,4-*b*] pyridines were found to be of moderate activity in comparison to celecoxib and indomethacin as reference drugs.

4. Methods and materials

4.1. Chemistry

Melting points were determined on Stuart apparatus and the values given were uncorrected. IR spectra were determined on Shimadzu IR435 spectrophotometer at Micro analytical Center, Faculty of Pharmacy, Cairo University and values were represented in cm^{-1}. ^1H NMR spectra and ^{13}C NMR spectra were carried out using Bruker 400 MHz using tetramethylsilane (TMS) as internal standard and chemical shift values were recorded in ppm on δ scale, Micro analytical Center, Faculty of Pharmacy, Cairo University, Egypt. Mass spectra were carried out at the Regional center for Mycology and Biotechnology, Faculty of Pharmacy, Al Azhar University, Egypt. Element analyses were carried out at the Regional center for Mycology and Biotechnology, Faculty of Pharmacy, Al Azhar University, Egypt. The Progress of the reactions was monitored using TLC aluminum sheets precoated with UV fluorescent silica gel (Merck 60F 254) and were visualized using UV lamp.

3-amino-1H-pyrazol-5(4H)-one **(I)** and 3-((2-fluorophenyl)amino)-1H-pyrazol-5(4H)-one (**II**) were synthesized according to reported procedure [20,21].

4.1.1. General procedure of synthesis of compounds (IIIa-f)

A solution of sodium nitrite (0.012 mol, 0.9 g) in water (1 ml) was gradually added to a cold solution of appropriate aromatic amine (0.013 mol) in conc. HCl (4 ml). The diazonium salt obtained was added with continuous stirring to a cold solution of **II** (0.008 mol, 1.64 g) in absolute ethanol (42 ml) in presence of sodium acetate (3.4 g). The reaction mixture was stirred at 0 °C for 2 h and the coloured solid formed was filtered, washed with water and crystallized from ethanol.

4.1.1.1. 3-((2-Fluorophenyl)amino)-4-(2-phenylhydrazono)-1H-pyrazol-5(4H)-one (IIIa). Yield: 80%, m.p: 240 °C, IR(KBr) cm^{-1}: 3433, 3174 (NH), 3055 (CH aromatic), 1666 (C═O), 1624 (C═N). ^1H NMR (DMSO-d_6-400 MHz, ppm): 7.14–7.17 (m, 5H, Ar-H), 7.24–7.27 (m, 2H, Ar-H), 7.63 (d, *J* = *7.68* Hz, 1H, Ar-H), 8.01 (s, 1H, NH, D_2O exchangeable), 8.05 (t, 1H, Ar-H), 11.10 (s, 1H,NH, D_2O exchangeable), 13.42 (s, 1H, NH, D_2O exchangeable). MS *m/z* (% rel. abundance): 297(M^+. 59.58%), 93.10 (100%). Anal. Calcd. For $C_{15}H_{12}FN_5O$ (297):calcd. C, 60.60, H, 4.07, N, 23.56. Found: C, 60.75, H, 4.13, N, 23.89.

4.1.1.2. 4-(2-(2-Bromophenyl)hydrazono)-3-((2-fluorophenyl)amino)-1H-pyrazol-5(4H)-one (IIIb). Yield: 90.3%, m.p: 230 °C, IR(KBr)cm^{-1}: 3421,3174 (NH), 3066 (CH aromatic), 1678 (C═O), 1624 (C═N). ^1H NMR (DMSO-d_6-400 MHz, ppm): 7.13–7.09 (m, 3H, Ar-H),7.23–7.28 (m, 2H, Ar-H), 7.50 (t, 1H, Ar-H), 7.69 (d, *J* = *7.92* Hz, 1H, Ar-H), 8.03 (t, 1H, Ar-H), 8.34 (s, 1H, NH, D_2O exchangeable), 11.29 (s, 1H, NH, D_2O exchangeable), 13.38 (s, 1H, NH, D_2O exchangeable). MS *m/z* (% rel. abundance): 377 (M^{+2}. 96%), 375 (M^+0.100%). Anal. Calcd. For $C_{15}H_{11}BrFN_5O$ (375):calcd C, 47.89, H, 2.95, N, 18.62. Found: C, 48.16, H, 3.17, N, 18.89.

4.1.1.3. 4-(2-(2-Ethylphenyl)hydrazono)-3-((2-fluorophenyl)amino)-1H-pyrazol-5(4H)-one (IIIc). Yield: 85%, m.p: 200 °C, IR(KBr) cm^{-1}: 3421,3221 (NH), 3059 (CH aromatic), 2966 (CH aliphatic), 1670 (C═O), 1624(C═N). ^1H NMR (DMSO-d_6-400 MHz, ppm): 1.24 (t, 3H, CH_3), 2.68 (q, 2H, CH_2), 6.99–7.04 ((m, 4H, Ar-H), 7.29 (d, *J* = 8.70 Hz, 1H, Ar-H), 7.34 (t, 1H, Ar-H), 7.88 (d, *J* = 8.08 Hz, 1H, Ar-H), 8.06 (t, 1H, Ar-H), 8.22 (s, 1H, NH, D_2O exchangeable), 11.22 (s, 1H, NH, D_2O exchangeable), 13.55 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): 14.21, 23.55, 114.89, 115.52, 115.71, 121.70, 122.89, 123.64, 124.91, 124.94, 125.40, 127.79, 128.77, 130.53, 139.10, 145.96, 159.27. MS *m/z* (% rel. abundance): 325 (M^+0.9.16%), 120.04 (100%). Anal. Calcd. For $C_{17}H_{16}FN_5O$ (325): calcd C, 62.76, H, 4.96, N, 21.53. Found: C, 62.89, H, 4.93, N, 21.71.

4.1.1.4. 3-((2-fluorophenyl)amino)-4-(2-(4-methoxyphenyl)hydrazono)-1H-pyrazol-5(4H)-one (IIId). Yield: 92.5%, m.p: 230 °C, IR(KBr)cm^{-1}: 3429,3151 (NH), 3055 (CH aromatic), 2962(CH aliphatic), 1662 (C═O), 1624(C═N). ^1H NMR (DMSO-d_6-400 MHz, ppm): 3.78 (s, 3H, OCH_3), 7.02 (d, *J* = 9.00 Hz, 1H, Ar-H), 7.14 (t, 1H, Ar-H), 7.23–7.27 (m, 4H, Ar-H), 7.61 (d, *J* = *9.00* Hz, 1H, Ar-H), 8.07 (t, 1H, Ar-H), 8.09 (s, 1H, NH, D_2O exchangeable), 11.06 (s, 1H, NH, D_2O exchangeable), 13.07(s, 1H, NH, D2O exchangeable). MS *m/z* (% rel. abundance): 327 (M^+0.95.15%), 42.83 (100%). Anal. Calcd. For $C_{16}H_{14}FN_5O_2$(327): calcd C, 58.71, H, 4.31, N, 21.40. Found: C, 58.97, H, 4.24, N, 21.53.

4.1.1.5. 3-((2-Fluorophenyl)amino)-4-(2-(o-tolyl)hydrazono)-1H-pyrazol-5(4H)-one (IIIe). Yield: 84%, m.p: 220 °C, IR(KBr) cm^{-1}: 3429, 3163

(NH), 3062 (CH aromatic), 2970 (CH aliphatic), 1666 (C═O), 1624(C═N). ^{1}H NMR (DMSO-d_6-400 MHz, ppm): 2.3 (s, 3H, CH3), 7.12 (t, 1H, Ar-H), 7.18 (t, 1H, Ar-H), 7.25–7.33 (m, 4H, Ar-H), 7.87 (d, *J* = 8.00 Hz, 1H, Ar-H), 8.1 (t,1H, Ar-H), 8.22 (s, 1H, NH, D_2O exchangeable), 11.23 (s, 1H, NH, D_2O exchangeable), 13.40 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): 16.67, 114.53, 115.54, 115.73, 121.74, 122.80, 123.10, 123.66, 124.64, 124.94, 125.09, 127.78, 131.41, 139.80, 145.94, 159.28. MS *m/z* (% rel. abundance): 311 (M^+0.80.75%), 107.15 (100%). Anal. Calcd. For $C_{16}H_{14}FN_5O$ (311):calcdC, 61.73, H, 4.53, N:22.50. Found: C, 61.49, H, 4.67, N, 22.38.

4.1.1.6. 4-(2-(2,6-Dimethylphenyl)hydrazono)-3-((2-fluorophenyl) amino)-1H-pyrazol-5(4H)-one (IIIf). Yield: 74%, m.p: 210 °C, IR(KBr) cm^{-1}: 3421,3232 (NH), 3055 (CH aromatic), 2966 (CH aliphatic), 1670 (C═O), 1624(C═N). ^{1}H NMR (DMSO-d_6-400 MHz, ppm): 2.36 (s, 6H, $2CH_3$), 6.98 (d, *J* = 5.32 Hz, 1H, Ar-H), 7.07 (t, 1H, Ar-H), 7.14 (d, *J* = 6.80 Hz, 1H, Ar-H), 7.17 (d, *J* = 8.20 Hz, 1H, Ar-H), 7.21 (t, 2H, Ar-H), 7.73 (s, 1H, Ar-H), 8.00 (s, 1H, NH, D_2O exchangeable), 11.15 (s, 1H, NH, D_2O exchangeable), 13.10 (s, 1H, NH, D_2O exchangeable). MS *m/z* (% rel. abundance):325(M^+. 100%). Anal. Calcd. For $C_{17}H_{16}FN_5O$ (325):calcd. C, 62.76, H, 4.96, N, 21.53. Found: C, 62.95, H, 4.89, N, 21.76.

4.1.2. General procedure of synthesis of compounds (IVa-L)

A solution of (IIIa-f) (0.05 mol) in ethanol (10 ml) was added to solution of 40% formaldehyde (12 ml) and aniline (0.1 mol, 9.3 g) in ethanol (20 ml). The reaction mixture was heated in water bath at (60 °C) for 6 h. It was then cooled, poured on ice-cold water and the separated solid was filtered, dried and crystallized from ethanol

4.1.2.1. 3-((2-Fluorophenyl)amino)-1-((phenylamino)methyl)-4-(2-phenyl hydrazono) -1H-pyrazol-5(4H)-one (IVa). Yield: 81%, m.p: 220 °C, IR(KBr) cm^{-1}: 3433 (NH), 3055 (CH aromatic), 2947 (CH aliphatic), 1670 (C═O), 1624(C═N). ^{1}H NMR (DMSO-d_6-400 MHz, ppm): 4.97 (d, *J* = *5.20* Hz, 2H, CH2), 6.60 (t, 2H, Ar-H), 6.75 (s, 1H, NH, D_2O exchangeable), 6.86 (d, *J* = *7.72 Hz*, 2H, Ar-H), 7.08 (d, *J* = *7.12* Hz, 2H, Ar-H), 7.18 (d, *J* = *7.44* Hz, 2H, Ar-H), 7.25 (d, *J* = *6.76* Hz, 2H, Ar-H), 7.43 (s, 1H, Ar-H), 7.67 (s, 1H, Ar-H), 8.09 (d, *J* = *90*. Hz, 2H, Ar-H), 8.28 (s, 1H, NH, D2O exchangeable), 13.00 (s, 1H, NH, D2O exchangeable). ^{13}C NMR (DMSO-d_6): 52.11, 113.14, 115.83, 116.39, 116.46, 117.40, 121.84, 122.33, 122.47, 124.91, 125.46, 128.30, 129.35, 129.86, 142.11, 145.76, 146.94, 156.05. MS *m/z* (% rel. abundance): 402 (M^+0.17.92%), 296.95 (100%). Anal. Calcd. For $C_{22}H_{19}FN_6O$ (402): calcd. C, 65.66, H, 4.76, N, 20.88. Found: C, 65.38, H, 4.89, N, 21.17.

4.1.2.2. 4-(2-(2-Bromophenyl)hydrazono)-3-((2-fluorophenyl)amino)-1-((phenylamino)methyl)-1H-pyrazol-5(4H)-one (IVb). Yield: 78%, m.p: 190 °C, IR(KBr) cm^{-1}: 3425 (NH), 3062(CH aromatic), 2970 (CH aliphatic), 1670 (C═O), 1624(C═N).^{1}H NMR (DMSO-d_6-400 MHz, ppm): 4.98 (d, *J* = *12.72* Hz, 2H, CH2), 7.08–7.18 (m, 3H, Ar-H), 7.30 (s, 1H, NH, D_2O exchangeable), 7.50 (t, 3H, Ar-H), 7.72 (t, 3H, Ar-H), 8.00 (t, 2H, Ar-H), 8.03 (t, 2H, Ar-H), 8.58 (s, 1H, NH, D_2O exchangeable), 13.28 (s, 1H, NH, D2O exchangeable). MS *m/z* (% rel. abundance): 481(M^+. 2.31%), 483(M^{+2}. 3.72%), 347.00(100%). Anal. Calcd. For $C_{22}H_{18}BrFN_6O$ (481): calcd. C, 54.90, H, 3.77, N, 17.46. Found: C, 54.67, H, 3.81, N, 17.82.

4.1.2.3. 4-(2-(2-Ethylphenyl)hydrazono)-3-((2-fluorophenyl)amino)-1-((phenyl amino) methyl)-1H-pyrazol-5(4H)-one (IVc). Yield: 68%, m.p: 200 °C, IR(KBr) cm^{-1}: 3441 (NH), 3066(CH aromatic), 2962(CH aliphatic), 1674 (C═O), 1624(C═N). ^{1}H NMR (DMSO-d_6-400 MHz, ppm): 1.27 (t, 3H, CH_3), 2.60 (q, 2H, CH_2), 5.00 (d, *J* = *6.44* Hz, 2H, CH2), 6.86 (d, *J* = *7.88* Hz, 2H, Ar-H), 7.00 (d, *J* = *7.32* Hz, 1H, Ar-H), 7.11 (t, 3H, Ar-H), 7.16 (t, 3H, Ar-H) 7.21 (s, 1H, NH, D_2O exchangeable), 7.26–7.33 (m, 2H, Ar-H), 7.89 (d, *J* = *8.04* Hz, 1H, Ar-H), 8.07 (t, 1H, Ar-H), 8.39 (s, 1H, NH, D_2O exchangeable), 13.46 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): 17.03, 19.04, 19.13, 21.33, 21.39, 49.07, 113.21, 117.42, 119.55, 124.58, 124.90, 126.40, 128.55, 128.90, 129.32, 129.73, 130.08, 130.45, 137.56, 137.64, 145.58, 146.99, 153.58, 156.50. MS *m/z* (% rel. abundance): 430 (M^+0.11.54%), 339.14 (100%). Anal. Calcd. For $C_{24}H_{23}FN_6O$ (430):calcd. C, 66.96, H, 5.39, N, 19.52. Found: C, 67.14, H, 5.45, N, 19.68.

4.1.2.4. 3-((2-Fluorophenyl)amino)-4-(2-(4-methoxyphenyl)hydrazono)-1-((phenylamino)methyl)-1H-pyrazol-5(4H)-one (IVd). Yield: 92%, m.p: 205 °C, IR(KBr) cm^{-1}: 3429 (NH), 3043(CH aromatic), 2962 (CH aliphatic), 1662 (C═O), 1624 (C═N). ^{1}H NMR (DMSO-d_6-400 MHz, ppm): 3.78 (s, 3H, OCH_3), 5.47 (d, *J* = *8.56* Hz, 2H, CH_2), 6.70–6.87 (m, 2H, Ar-H), 6.88 (t, 2H, Ar-H), 7.01 (d, *J* = *8.92* Hz, 2H, Ar-H), 7.08 (s, 1H, NH, D_2O exchangeable), 7.15 (t, 1H, Ar-H), 7.64 (t, 3H, Ar-H), 8.09 (t, 2H, Ar-H), 8.19 (s, 1H, Ar-H), 8.21 (s, 1H, NH, D_2O exchangeable), 13.04 (s, 1H, NH, D_2O exchangeable). MS *m/z* (% rel. abundance): 432 (M^+0.3.74%), 354.99 (100%). Anal. Calcd. For $C_{23}H_{21}FN_6O_2$ (432): calcd. C, 63.88, H, 4.89, N, 19.43. Found: C, 63.96, H, 5.02, N, 19.69.

4.1.2.5. 3-((2-Fluorophenyl)amino)-1-((phenylamino)methyl)-4-(2-(o-tolyl) hydrazono)-1H-pyrazol-5(4H)-one (IVe). Yield: 76.9%, m.p: 220 °C, IR(KBr) cm^{-1}: 3429 (NH), 3055 (CH aromatic), 2978 (CH aliphatic), 1666 (C═O), 1624 (C═N). ^{1}H NMR (DMSO-d_6-400 MHz, ppm): 2.30 (s, 3H, CH_3), 5.00 (d, *J* = *6.24* Hz, 2H, CH_2), 6.60 (t, 1H, Ar-H), 6.76 (s, 1H, NH, D_2O exchangeable), 6.85 (d, *J* = *7.92* Hz, 2H, Ar-H), 7.08–7.13 (m, 2H, Ar-H), 7.17 (s, 2H, Ar-H), 7.24 (d, *J* = *7.32* Hz, 2H, Ar-H), 7.30 (d, *J* = *8.12* Hz, 2H, Ar-H) 7.87 (d, *J* = *8.2* Hz, 1H, Ar-H), 8.08 (t, 1H, Ar-H), 8.38 (s, 1H, NH, D2O exchangeable), 13.30 (s, 1H, NH, D_2Oexchangeable). MS *m/z* (% rel. abundance): 416 ($M^{+\cdot}$5.08%), 92.99 (100%). Anal. Calcd. For $C_{23}H_{21}FN_6O$ (416): calcd. C, 66.33, H, 5.08, N, 20.18. Found C, 66.62, H, 5.23, N, 20.42.

4.1.2.6. 4-(2-(2,6-Dimethylphenyl)hydrazono)-3-((2-fluorophenyl) amino)-1-((phenylamino)methyl)-1H-pyrazol-5(4H)-one (IVf). Yield: 92%, m.p: 160 °C, IR(KBr) cm^{-1}: 3429 (NH), 3066(CH aromatic), 2966(CH aliphatic), 1662 (C═O), 1604(C═N). ^{1}H NMR (DMSO-d_6-400 MHz, ppm): 2.37 (s, 6H, $2CH_3$), 4.98(d, *J* = 15.24 Hz, 2H, CH_2), 7.04 (d, *J* = 6.24 Hz, 2H, Ar-H), 7.12 (d, *J* = 6.00 Hz, 2H, Ar-H), 7.17 (d, *J* = 8.28 Hz, 2H, Ar-H), 7.20 (s, 1H, NH, D_2O exchangeable), 7.21–7.26 (m, 4H, Ar-H), 7.97 (d, *J* = 13.28 Hz, 2H, Ar-H), 8.01 (s, 1H, NH, D_2O exchangeable), 12.98 (s, 1H, NH, D_2O exchangeable).MS *m/z* (% rel. abundance): 430 (M^+. 0.4%), 325.15 (100%). Anal. Calcd. For $C_{24}H_{23}FN_6O$ (430): calcd. C, 66.96, H, 5.39, N, 19.52. Found: C, 66.85, H, 5.52, N, 19.73.

4.1.2.7. 1-(((2,6-Dimethylphenyl)amino)methyl)-3-((2-fluorophenyl) amino)-4-(2-phenylhydrazono)-1H-pyrazol-5(4H)-one (IVg). Yield: 62.5%, m.p: 162 °C, IR(KBr) cm^{-1}: 3429 (NH), 3043 (CH aromatic), 2997 (CH aliphatic), 1662 (C═O), 1624(C═N). ^{1}H NMR (DMSO-d_6-400 MHz, ppm): 3.17 (s, 6H, $2CH_3$), 4.92 (d, *J* = 7.48 Hz, 2H, CH_2), 7.07 (t, 1H, Ar-H), 7.20 (t, 2H, Ar-H), 7.25 (s, 1H, NH, D_2O exchangeable). 7.27 (t, 2H, Ar-H), 7.30 (t, 1H, Ar-H), 7.44 (t, 2H, Ar-H), 7.66 (s, 1H, Ar-H), 7.68 (d, *J* = 7.64 Hz, 1H, Ar-H), 8.03 (d, *J* = 7.32 Hz, 1H, Ar-H), 8.07 (s, 1H, Ar-H), 8.32 (s, 1H, NH, D_2O exchangeable), 12.95(s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): 56.54, 66.20, 74.35, 115.68, 115.86, 116.48, 121.89, 122.42, 123.53, 124.95, 124.99, 125.62, 128.35, 129.87, 142.06, 145.96, 152.23, 154.65, 157.12. MS *m/z* (% rel. abundance): 430 (M^+. 14.45%), 355.02 (100%). Anal. Calcd. For $C_{24}H_{23}FN_6O$ (430):calcd. C, 66.96, H, 5.39, N, 19.52. Found: C, 67.32, H, 5.60, N, 19.31.

4.1.2.8. 4-(2-(2-Bromophenyl)hydrazono)-1-(((2,6-dimethylphenyl) amino)methyl)-3-((2-fluorophenyl)amino)-1H-pyrazol-5(4H)-one (IVh). Yield: 69.2%, m.p: 180 °C, IR(KBr) cm^{-1}: 3421 (NH), 3074 (CH aromatic), 2974 (CH aliphatic), 1674 (C═O), 1624(C═N). ^{1}H NMR (DMSO-d_6-400 MHz, ppm): 3.33 (s, 6H, $2CH_3$), 4.96 (d, J = 9.08 Hz, 2H, CH_2), 7.06–7.11 (m, 2H, Ar-H), 7.16 (t, 1H, Ar-H), 7.21 (t, 1H, Ar-H), 7.28 (t, 1H, Ar-H), 7.51(t, 2H, Ar-H), 7.54 (s, 1H, NH, D_2O exchangeable), 7.71 (d, J = 7.12 Hz, 1H, Ar-H), 8.06–8.07 (m, 3H, Ar-H), 8.58 (s, 1H, NH, D_2O exchangeable), 13.26 (s, 1H, NH, D_2O exchangeable). MS *m/z* (% rel. abundance): 508(M^+0.14.38%), 510($M+2^+$. 9.63%), 375.03 (100%). Anal. Calcd. For $C_{24}H_{22}BrFN_6O$ (508): calcd. C, 56.59, H, 4.35, N, 16.50. Found: C, 56.82, H, 4.48, N, 16.31.

4.1.2.9. 1-(((2,6-Dimethylphenyl)amino)methyl)-4-(2-(2-ethylphenyl) hydrazono)-3-((2-fluorophenyl)amino)-1H-pyrazol-5(4H)-one (IVi). Yield: 78.5%, m.p: 150 °C, IR(KBr) cm^{-1}: 3421 (NH), 3070 (CH aromatic), 2974 (CH aliphatic), 1662 (C═O), 1624 (C═N). ^{1}H NMR (DMSO-d_6-400 MHz, ppm): 1.22 (t, 3H, CH_3), 2.67 (q, 2H, CH_2), 3.33 (s, 6H, $2CH_3$), 5.00 (s, 2H, CH_2), 7.07 (t, 1H, Ar-H), 7.15–7.20 (m, 1H, Ar-H), 7.23 (t, 2H, Ar-H), 7.25 (s, 1H, NH, D_2O exchangeable), 7.27–7.28 (m, 2H, Ar-H), 7.30 (d, J = 6.84 Hz, 1H, Ar-H), 7.35 (t, 2H, Ar-H), 7.91 (d, J = 8.12 HZ, 1H, Ar-H), 8.05 (t, 1H, Ar-H), 8.42 (s, 1H, NH, D_2O exchangeable), 13.38 (s, 1H, NH, D_2O exchangeable).MS *m/z* (% rel. abundance): 458 (M^+0.0.14%), 369 (100%). Anal. Calcd. For $C_{26}H_{27}FN_6O$ (458):calcd. C, 68.10, H, 5.94, N, 18.33. Found: C, 68.21, H, 6.02, N, 18.49.

4.1.2.10. 1-(((2,6-Dimethylphenyl)amino)methyl)-3-((2-fluorophenyl) amino)-4-(2-(4-methoxyphenyl)hydrazono)-1H-pyrazol-5(4H)-one (IVj). Yield: 92.9%, m.p: 150 °C, IR(KBr) cm^{-1}: 3421 (NH), 3078 (CH aromatic), 2931 (CH aliphatic), 1662 (C═O), 1624 (C═N). ^{1}H NMR (DMSO-d_6-400 MHz, ppm): 3.29 (s, 6H, $2CH_3$), 3.78 (s, 3H, OCH_3), 4.97 (d, J = 15.56 Hz, 2H,CH_2), 7.02 (d, J = 8.96 Hz, 4H, Ar-H), 7.19 (t, 2H, Ar-H), 7.24 (s, 1H, NH, D_2O exchangeable), 7.26 (t, 1H, Ar-H), 7.29 (s, 2H, Ar-H), 7.62–7.66 (m, 2H, Ar-H), 8.23 (s, 1H, NH, D_2O exchangeable), 13.02 (s, 1H, NH, D_2O exchangeable).MS *m/z* (% rel. abundance): 460 (M^+0.24.14%), 371.06 (100%). Anal. Calcd. For $C_{25}H_{25}FN_6O_2$ (460): calcd. C, 65.20, H, 5.47, N, 18.25. Found: C, 64.97, H, 5.66, N, 18.37.

4.1.2.11. 1-(((2,6-Dimethylphenyl)amino)methyl)-3-((2-fluorophenyl) amino)-4-(2-(o-tolyl)hydrazono)-1H-pyrazol-5(4H)-one (IVk). Yield: 71%, m.p: 140 °C, IR(KBr) cm^{-1}: 3421 (NH), 3078 (CH aromatic), 2970 (CH aliphatic), 1670 (C═O), 1624 (C═N). ^{1}H NMR (DMSO-d_6-400 MHz, ppm): 2.33 (s,3H,CH_3), 3.56 (s, 6H, $2CH_3$), 5.00 (s, 2H, CH_2), 7.05–7.10 (m, 4H, Ar-H), 7.12 (d, J = 7.40 Hz, 1H, Ar-H), 7.19 (t, 2H, Ar-H), 7.24 (s, 1H, NH, D_2O exchangeable), 7.28 (d, J = 7.88 Hz, 1H, Ar-H), 7.33 (t, 1H, Ar-H), 7.88 (d, J = 8.04 Hz, 1H, Ar-H), 8.06 (t, 1H, Ar-H), 8.38 (s, 1H, NH, D_2Oexchangeable), 13.22 (s, 1H, NH, D_2Oexchangeable). MS *m/z* (% rel. abundance): 444 (M^+0.3.01%), 373.13 (100%). Anal. Calcd. For $C_{25}H_{25}FN_6O$ (444): calcd. C, 67.55, H, 5.67, N, 18.91. Found: C, 67.89, H, 5.82, N, 19.24.

4.1.2.12. 1-(((2,6-Dimethylphenyl)amino)methyl)-4-(2-(2,6-dimethylphenyl) hydrazono)-3-((2-fluorophenyl)amino)-1H-pyrazol-5(4H)-one (IVL). Yield: 71.4%, m.p: 157 °C, IR(KBr) cm^{-1}: 3429 (NH), 3066 (CH aromatic), 2927 (CH aliphatic), 1662 (C═O), 1604(C═N). ^{1}H NMR (DMSO-d_6-400 MHz, ppm): 2.51 (s, 6H, $2CH_3$), 3.17 (s, 6H, $2CH_3$) 4.94 (d, J = 11.08 Hz, 2H, CH_2), 6.89 (d, J = 7.36 Hz, 2H, Ar-H), 6.96 (s, 1H, NH, D_2O exchangeable), 7.12 (d, J = 7.52 Hz, 2H, Ar-H), 7.17 (d, J = 7.64 Hz, 2H, Ar-H), 7.91 (s, 1H, Ar-H), 7.95–7.99 (m, 3H, Ar-H), 8.13 (s,1H, NH, D_2O exchangeable), 12.98 (s, 1H, NH, D_2O exchangeable). MS *m/z* (% rel. abundance): 458(M^+0.70.46%), 368.98(100%). Anal. Calcd. For $C_{26}H_{27}FN_6O$ (458): calcd. C, 68.10, H, 5.94, N, 18.33. Found: C, 68.38, H, 6.07, N, 18.17.

4.1.3. General procedure of synthesis of compounds (Va-f)

A mixture of II (0.01 mol, 1.93 g), appropriate aromatic aldehyde (0.01 mol), malononitrile (0.01 mol, 0.66 g) and ammonium acetate (0.03 mol, 2.31 g) in absolute ethanol (15 ml) was heated under reflux for 15 h. The reaction mixture was then cooled and the separated solid was washed with water, dried and crystallized from ethanol.

4.1.3.1. 6-Amino-3-((2-fluorophenyl)amino)-4-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (Va). Yield: 50%, m.p: 295 °C, IR(KBr) cm^{-1}: 3452, 3421 (forked peak of NH2), 3363 (NH), 3101 (CH aromatic), 2206 (CN), 1662 (C═N). ^{1}H NMR (DMSO-d_6-400 MHz, ppm): 7.26 (s, 1H, NH, D_2O exchangeable), 7.46 (d, J = 8.08 Hz, 2H, Ar-H), 7.52 (t, 3H, Ar-H), 7.63–7.67 (m, 4H, Ar-H), 7.68 (s, 1H, NH, D_2O exchangeable), 8.05 (s, 1H, NH, D_2O exchangeable), 10.66 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): 75.69, 80.25, 100.62, 116.93, 117.81, 126.69, 126.72, 128.19, 128.72, 129.65, 130.33, 130.45, 131.32, 132.54, 133.26, 133.34, 145.79, 156.14, 161.91. MS *m/z* (% rel. abundance): 344 (M^+0.50.34%), 234.96 (100%). Anal. Calcd. For $C_{19}H_{13}FN_6$ (344): calcd. C, 66.27, H, 3.81, N, 24.41. Found: C, 66.49, H, 4.03, N, 24.68.

4.1.3.2. 6-Amino-4-(4-bromophenyl)-3-((2-fluorophenyl)amino)-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (Vb). Yield: 48%, m.p: > 300 °C, IR(KBr) cm^{-1}: 3340 (NH), 3167 (CH aromatic), 2210 (CN), 1662 (C═N). ^{1}H NMR (DMSO-d_6-400 MHz, ppm): 7.45 (t, 1H, Ar-H), 7.53 (d, J = 8.76 Hz, 1H, Ar H), 7.58 (d, J = 8.40 Hz, 1H, Ar H), 7.65 (s, 1H, NH, D_2O exchangeable), 7.66 (s, 1H, NH, D_2O exchangeable), 7.68 (t, 4H, Ar-H), 7.73 (d, J = 8.36 Hz, 1H, Ar H), 8.11 (s, 1H, NH, D_2O exchangeable), 10.69 (s, 1H, NH, D_2O exchangeable). MS *m/z* (% rel. abundance): 423(M + 0.8.75%), 425 (M + 2 $^+$. 12.50%), 345.04 (100%). Anal. Calcd. For $C_{19}H_{12}BrFN_6$ (423): calcd. C, 53.92, H, 2.86, N, 19.86. Found: C, 54.21, H, 3.01, N, 20.12.

4.1.3.3. 6-Amino-4-(4-chlorophenyl)-3-((2-fluorophenyl)amino)-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (Vc). Yield: 58%, m.p: > 300 °C, IR(KBr) cm^{-1}: 3363 (NH), 3124(CH aromatic), 2210(CN), 1662 (C═N). ^{1}H NMR (DMSO-d_6-400 MHz, ppm): 7.45 (t, 4H, Ar-H),7.54 (s, 1H, NH, D_2O exchangeable), 7.59 (d, J = 8.32 Hz, 2H, Ar -H), 7.66 (d, J = 8. 20 Hz, 2H, Ar -H), 7.67 (s, 1H, NH, D_2O exchangeable), 8.11 (s, 1H, NH, D_2O exchangeable), 10.70 (s, 1H, NH, D_2O exchangeable). MS *m/z* (% rel. abundance): 378(M^+0.5.66%), 380 (M + 2$^+$. 3.92%), 361.04 (100%). Anal. Calcd. For $C_{19}H_{12}ClFN_6$(378): calcd. C, 60.25, H, 3.19, N, 22.19. Found: C, 60.43, H, 3.36, N, 22.42.

4.1.3.4. 6-Amino-4-(4-fluorophenyl)-3-((2-fluorophenyl)amino)-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (Vd). Yield: 68.4%, m.p: > 300 °C, IR(KBr) cm^{-1}: 3425 (NH), 3363, 3336 (forked peak of NH2), 3159(CH aromatic), 2202(CN), 1662(C═N). ^{1}H NMR (DMSO-d_6-400 MHz, ppm): 7.27 (s, 1H, NH, D_2O exchangeable), 7.33–7.34 (m, 2H,Ar H), 7.38 (d, J = 6.04 Hz, 2H, Ar H), 7.45 (t, 1H, Ar-H), 7.54 (s, 1H, NH, D_2O exchangeable), 7.56 (d, J = 10.20 Hz, 2H,Ar H), 7.67–7.72 (m, 1H, Ar-H), 8.08 (s, 1H, NH, D_2O exchangeable), 10.67 (s, 1H, NH, D_2O exchangeable). MS *m/z* (% rel. abundance): 362(M^+0.56.72%), 363.15 (100%). Anal. Calcd. For $C_{19}H_{12}F_2N_6$ (362): calcd. C, 62.98, H, 3.34, N, 23.19. Found: C, 63.15, H, 3.47, N, 23.51.

4.1.3.5. 6-Amino-3-((2-fluorophenyl)amino)-4-(4-hydroxyphenyl)-1H-pyrazolo [3,4-b] pyridine-5-carbonitrile (Ve). Yield: 39%, m.p: > 300 °C, IR(KBr)cm^{-1}: 3518 (OH), 3414, 3367 (forked peak of NH2), 3317(NH), 3097 (CH aromatic), 2210 (CN), 1643 (C═N). ^{1}H NMR (DMSO-d_6-400 MHz, ppm): 6.86 (d, J = 8.6 Hz, 2H, Ar-H), 7.16 (s, 1H, NH, D_2O exchangeable), 7.31 (d, J = 8.52 Hz, 1H, Ar-H), 7.44 (t, 2H, Ar-H), 7.53 (d, J = 8.52 Hz, 2H, Ar-H), 7.63–7.67 (m, 1H, Ar-H), 7.68 (s, 1H, NH, D_2O exchangeable), 7.94 (s, 1H, NH, D_2O exchangeable), 9.97 (s, 1H, OH, D_2O exchangeable), 10.59 (s, 1H, NH, D_2O exchangeable). MS *m/z* (% rel. abundance): 360(M^+0.69.88%), 361 (100%). Anal. Calcd. For

$C_{19}H_{13}FN_6O$ (360): calcd. C, 63.33, H, 3.64, N, 23.32. Found: C, 63.59, H, 3.08, N, 23.17.

4.1.3.6. 6-Amino-3-((2-fluorophenyl)amino)-4-(4-methoxyphenyl)-1H-pyrazolo [3,4-b]pyridine-5-carbonitrile (Vf). Yield: 79%, m.p: 290 °C, IR (KBr) cm^{-1}:. 3487 (NH), 3421, 3375 (forked peak of NH2), 3151 (CH aromatic), 2978 (CH aliphatic), 2202(CN), 1627 (C═N). ^{1}H NMR (DMSO-d_6-400 MHz, ppm): 3.86 (s, 3H, OCH_3), 7.05–7.10 (m, 3H, Ar-H), 7.19 (s, 1H, NH, D_2O exchangeable), 7.43 (d, J = 7.04 Hz, 2H,Ar H), 7.53 (t, 1H, Ar-H), 7.63–7.67 (m, 2H, Ar-H), 7.69 (s, 1H, NH, D_2O exchangeable), 7.98 (s, 1H, NH, D_2Oexchangeable), 10.62 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): 55.76, 75.46, 80.21, 100.18, 113.53, 114.39, 117.19, 124.38, 126.70, 127.50, 130.43, 131.33, 131.74, 145.86, 156.41, 161.33, 161.59, 162.05. MS *m/z* (% rel. abundance): 374 (M^+ 0.68.62%), 375.20 (100%). Anal. Calcd. For $C_{20}H_{15}FN_6O$ (374): calcd. C, 64.16, H, 4.04, N, 22.45. Found: C, 63.95, H, 4.17, N, 22.81.

4.2. Biological activity

4.2.1. In vitro COX-1 and COX-2 assay

All newly synthesized compounds were subject to *in vitro* COX-1 and COX-2 assay against celecoxib, indomethacin and diclofenac sodium as reference drugs using Cayman COX (ovine/human) inhibitor screening assay which directly measures PGF2α produced by $SnCl_2$ reduction of COX-derived PGH2. Results of COX-1 and COX-2 inhibitory activity of the tested compounds as well as reference drugs are shown in Table 1.

4.2.2. In vivo anti-inflammatory screening

Measure of the ability of eight tested compounds **IVa, IVb, IVc, IVd, IVe, IVh, IVJ, IVL** to inhibit the edema produced in the hind paw of rats by injection of carrageenan to produce experimental arthritis [22]. Male albino rats weighing 150–180 g divided into 11 groups of eight rats each, group 1 (control): given 5% DMSO. Group 2 and 3 (reference groups): were given indomethacin and celecoxib in dose of 10 mg/kg body weight, 1 h prior to carrageenan injection. Groups 4–11 were given the tested compounds in dose of 20 mg/kg body weight The volume of paw edema (in ml) was determined by means of a water plethysmometer immediately after injection of carrageenan and after 4 h later. The percentage protection against inflammation was calculated as follows: Vc − Vd/Vc × 100, where Vc is the increase in paw volume in the absence of the test compound (control) and Vd is the increase of paw volume after injection of the test compound. Data were expressed as means ± SEM. Significant differences between the control and the treated groups were obtained using one-way Anova followed by posthocTukey and kramer and p-values. The differences in results were considered significant when $p < 0.05$ the results shown in Table 2.

Table 1
Results of COX-1 and COX-2 inhibition activity assay.

Compounds	COX1 μm IC $_{50}$	COX2 μm IC $_{50}$	Si (selectivity index) = IC_{50} COX-1 IC_{50} COX-2
Celecoxib	15.1	0.049	308.163
Diclofenac sodium	3.8	0.84	4.524
Indomethacin	0.041	0.51	0.080
IIIa	6.8	0.342	19.883
IIIb	9.1	0.101	90.099
IIIc	8.1	0.108	75
IIId	4.9	0.232	21.121
IIIe	5.8	0.233	24.893
IIIf	7.3	0.192	38.021
IVa	9.5	0.071	133.803
IVb	12.4	0.048	258.333
IVc	13.2	0.055	240
IVd	13.1	0.051	256.863
IVe	11.2	0.067	167.164
IVf	10.4	0.082	126.829
IVg	10.4	0.093	111.828
IVh	14.3	0.048	297.917
IVi	8.7	0.162	53.704
IVJ	12.3	0.046	267.391
IVk	9.9	0.112	88.393
IVL	10.5	0.054	194.444
Va	5.1	0.312	16.346
Vb	6.4	0.220	29.091
Vc	7.3	0.160	45.625
Vd	6.3	0.340	18.529
Ve	4.9	0.291	16.554
Vf	8.6	0.082	104.878

Table 2
Results of *in vivo* anti-inflammatory activity.

Compounds	Increase in paw edema (ml) ± SEM	% protection	Activity relative to celecoxib
Control	1.01 ± 0.031	0.0	0.0
Indomethacin	0.30* ± 0.036	70	101
Celecoxib	0.31* ± 0.043	69	100
IVa	0.39* ± 0.045	61	88
IVb	0.36* ± 0.026	64	93
IVc	038* ± 0.047	62	90
IVd	0.41* ± 0.024	59	86
IVe	0.45* ± 0.042	55	80
IVh	0.44* ± 0.042	56	81
IVJ	0.49* ± 0.051	51	74
IVL	0.52* ± 0.042	49	71

4.2.3. Ulcerogenic effect

Eight of the newly synthesized compounds **IVa, IVb, IVc, IVd, IVe, IVh, IVJ, IVL** were subjected to *in vivo* testing to measure their ulcerogenic effect in comparison to celecoxib and indomethacin. Male albino rats (120–150 g) divided into 11 groups of eight rats each. Group 1 (control) received 0.2 ml DMSO orally. Group 2 and 3 (reference groups) received 5 mg/kg indomethacin and celecoxib. Groups 4–11 (test groups) received 10 mg/kg tested compounds orally for three successive days. Animals were sacrificed by diethyl ether 6 h after the last dose and the stomach was removed. An opening at the greater curvature was made and the stomach was cleaned by washing with cold saline and inspected with a three time magnifying lens for any evidence of hyperemia, hemorrhage, definite hemorrhagic erosion, or ulcer. An arbitrary scale was used to calculate the ulcer index which indicates the severity of the stomach lesions The % ulceration for each group was calculated as follows: % Ulceration = Number of animals bearing ulcer in a group/Total number of animals in the same group × 100. Results are shown in Table 3.

Table 3
Results of ulcerogenic effect.

Compounds	% ulceration
Control	0.0
Indomethacin	100
Celecoxib	10
IVa	20
IVb	20
IVc	20
IVd	30
IVe	30
IVh	30
IVJ	20
IVL	30

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Conflict of interest

The authors have declared no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.10.014. These data include MOL files and InChiKeys of the most important compounds described in this article.

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