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Regioselective *N*-alkylation of some imidazole-containing heterocycles and their *in vitro* anticancer evaluation

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Regioisomer
NOESY (nuclear overhauser effect spectroscopy)

ABSTRACT

Imidazole-containing heterocycles: Imidazopyridines, imidazopyrimidines and imidazopyra-zines can exist more tautomeric forms than benzimidazoles. Their regioselectivities were determined for *N*-alkylations with 4-fluorobenzyl bromide under basic conditions (K_2CO_3) in DMF. We observed that, regioisomers were mainly formed as a mixture in this reaction and *N*-benzylation occurs at a higher ratio on six membered heterocycles. Their structural assignments were made with the use of two-dimensional $^1H-^1H$ NOE (nuclear overhauser effect spectroscopy, NOESY). Complementary structural information was provided by 2D-HMBC spectra of the compounds. Synthesized compounds were tested for *in vitro* cytotoxic activities against Human colon cancer cell line (HCT-116) and leukemia cell lines (K562 and HL-60) by MTT test. Among them, imidazopyridine analogue **10**, bearing bromine atom at the C-6 position of the pyridine moiety, gave the lowest IC_{50} value with 6–7 μg/mL against all three cancer cell lines.

1. Introduction

Imidazopyridines, imidazopyrimidines (purine) and imidazopyrazines are formed by fusing imidazole ring with pyridine, pyrimidine and pyrazine moieties. Furthermore imidazopyridines have various isomeric forms like imidazo[4,5-*b*]pyridines, imidazo[4,5-*c*]pyridines, imidazo[1,5-*a*]pyridines and imidazo[1,2-*a*]pyridines. In 2017, a review was reported about pharmacological profiles of imidazopyridines such as anti-tumoral, anti-microbial, anti-inflammatory, anti-diabetic, anti-hypertensive and other pharmacological properties [1]. In US Patent number 2017/0210741, Southern Research Institute claimed a series of benzimidazole and imidazopyridine analogues that are capable of inhibiting Wnt signaling. Although Wnt/β-catenin signaling plays a critical role in embryonic development, it can lead to tumor formation and disease when it is aberrantly activated. Therefore Wnt signaling pathways are therapeutic targets in cancer therapies. The patent exemplified 38 compounds in the structure of benzimidazole and imidazopyridine with (4-(piperazine/pyrrolidine/piperidin-1-yl) phenyl substitution at the 2-position and several halogens substitution on the benzene moiety. Examples are represented in Fig. 1 (1) [2]. One of the compound was similarly reported as Rheb inhibitors with moderate activity in patent number WO2018/191146 [3].

In 2008, Astrazeneca AB also patented a series of 3*H*-imidazo [4,5-b]pyridine-7-carboxamides which were glycogen synthase kinase 3 (GSK3) inhibitors. As it is known GSK3 inhibitors, inhibit growth and survival of solid tumors in pancreatic, colon and prostate cancers. A representative example is given in Fig. 1 **(2)** showing best Ki value with 390 nM [4].

In patent number WO2009/111277, Array BioPharma Inc. claimed a series of imidazo[4,5-b] pyridines as B-Raf inhibitors. Certain hyperproliferative disorders are characterized by over activation of Raf kinase function caused by mutation or over expression of the protein. So discovery of this type of inhibitors is useful in treatment of cancer disease. In this patent, 42 compounds with several benzamides substitution at the 6-position were exemplified and some of their IC_{50}'s were found less than 1 μM. Example is represented in Fig. 1 (3) [5].

Purine ring system is undoubtedly among the most ubiquitous of all the heterocyclic compounds. The two most common well

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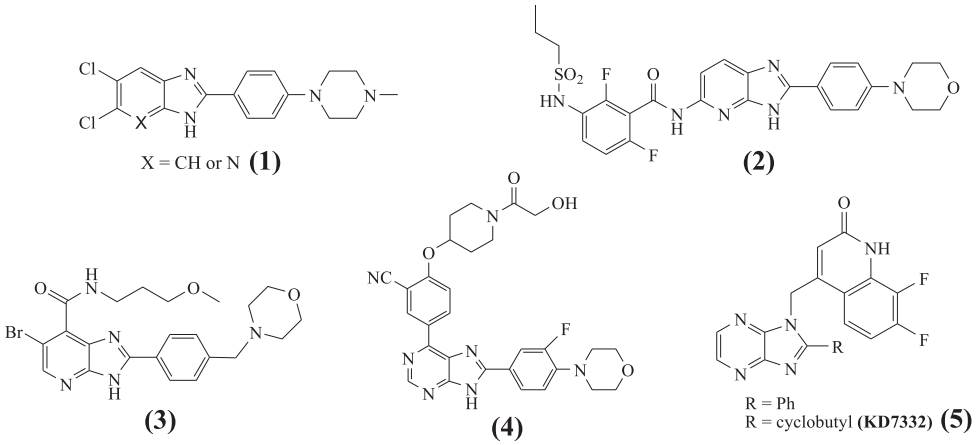


Fig. 1. The structures of previously reported 2-phenyl imidazole containing heterocycles.

known purines are adenine and guanine which constitute the structure of DNA and RNA. Other purines like xanthine, hypoxanthine and uric acid are also important naturally occurring metabolites, although they are far less abundant than adenine and guanine. Purine analogues are commonly used to treat cancer by interfering with DNA replication [6], such as Cladribine, Tioguanine, Clofarabine, Mercaptopurine, Fludarabine and Nelarabine. Gilead Sciences disclosed a library of imidazo-(pyrimidine/pyridine) derivatives as inhibitors of TBK1 and/or IKKε. All the compounds were characterized by the presence of extended substituents on C-4. A representative example is given in Fig. 1 (**4**), which shows IC50 values of 1.299 and 2.637 nM on TBK1 and IKKε, respectively [7].

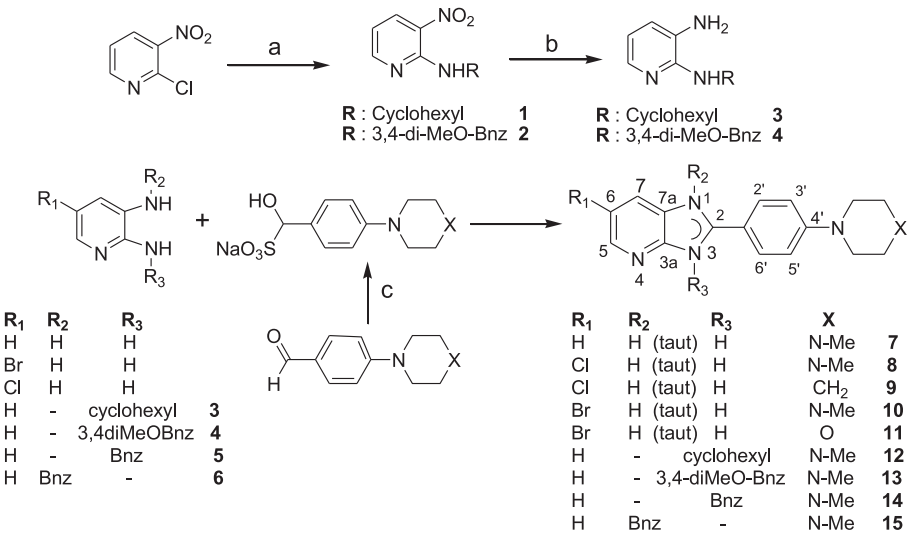
On the other hand, imidazopyrazines have also various isomeric forms like imidazopyridines some of which were found highly attractive as anticancer agents. In 2011, Mortensen et al [8] reported the discovery of the imidazo[4,5-b]pyrazin-2-one series as selective Mammalian Target of Rapamycin (mTOR) kinase inhibitors. Recently, new 1*H*-imidazo[4,5-b]pyrazine derivatives were published as potent and selective inhibitor of Mesenchymal-epithelial transition factor (c-Met) kinase by Zhao et al. [9] and Cui et al. [10]. Payne et al. [11,12] reported a congeneric series of 1*H*-imidazo [4,5-b]pyrazines as dual inhibitors of Inducible/Neuronal Nitric Oxide Synthase (iNOS/nNOS) simultaneously. These compounds were primarily found effective to chronic pain state associated with asthma, inflammatory bowel disease, arthritis and neuropathy. Compound KD7332 Fig. 1 **(5)** was confirmed as one of the several potent inhibitors.

Despite the importance of these structures, bicyclic heterocycles are difficult to prepare in a regioselective manner, especially with substitution at the N-positions. The electrons of these rings are extensively delocalized, hence the alkylation of these unsubstituted imidazole cores is remarkably unselective. The design of improved, high efficient and regioselective approaches for these condensed heterocycles's preparation is of prime importance. These cores with unsubstituted NH groups exhibit fast prototropic tautomerism leading to equilibrium mixtures of asymmetrically substituted compounds. The existence of this tautomerism has been proved by several approaches including NMR spectroscopy. This migration is not found when the *N*-attached hydrogen is replaced by an alkyl group. In our recently published papers, we described the synthesis of some regioisomers of benzimidazoles [13] and imidazopyridines [14] and elucidated their structure by selective synthesis and/or 2D-NMR data such as nuclear overhauser effect spectroscopy (NOESY), and heteronuclear multiple bond correlation (HMBC) experiments. These bicyclic heterocycles were shown to have inhibitory effect on various kinases as anti-cancer agents.

Prompted by the findings from the studies mentioned above, in this research we set out to develop and synthesize new bicyclic derivatives (imidazopyridines, imidazopyrimidines and imidazopyrazines which of their structures are given in Fig. 1) and investigate their inhibitory activities against some cancer cell lines. The other aim of this study is to investigate the regioisomer selectivity of the synthesized compounds during the *N*-alkylation reaction.

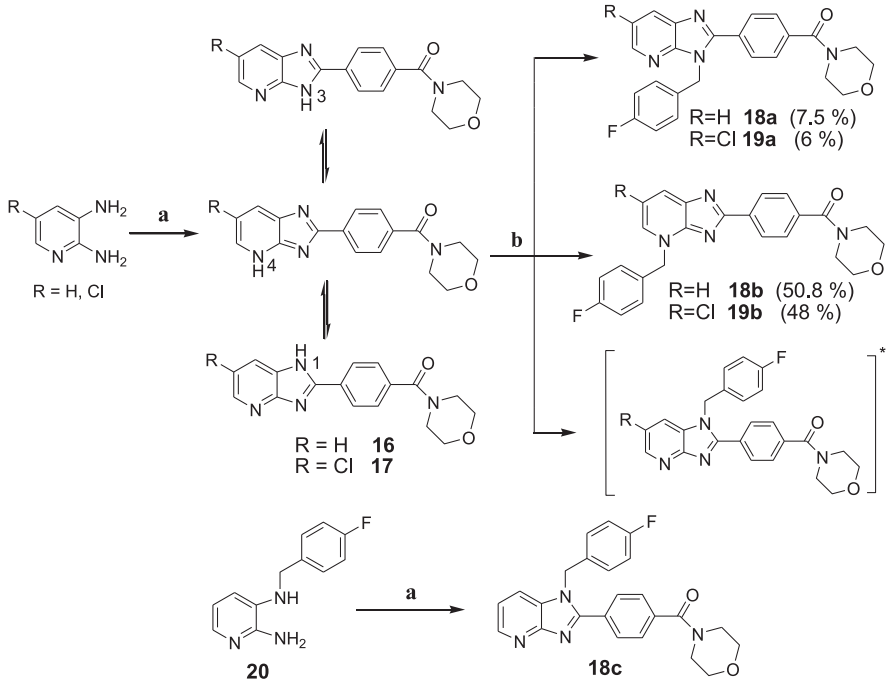
2. Results and discussion

Targeted compounds were prepared using the methods outlined in Scheme 1- 5. Cyclization of related (o-diamino)pyridine, pyrimidine and pyrazine derivatives with sodyum metabisulfite adduct of corresponding benzaldehydes, gave required imidazopyridines **7**—**16, 24, 25,** imidazopyrimidines **27, 28** and imidazopyrazine **33**. In these condensed systems, nitrogen bearing a hydrogen atom ($N^{1,3}$) resembles a pyrole *N*-atom; the other nitrogen atoms ($N^{4,5,6,7}$) resemble a pyridine *N*-atom. Hydrogen atom attached to nitrogen in the 1,3-position can easily tautomerise in several positions as in compounds **16, 21, 25, 28** and **33** shown in Schemes 2—5. Because of these tautomeric forms, both ^{1}H and ^{13}C NMR spectra of unsubstituted analogues cannot be clearly seen unless hexamethylphosphoramide-d_{18} (HMPA-d_{18}) [15] is used as a deuterated solvent. Both appearance of some proton and carbon signals as broad peaks and unobservable some hinge carbon signals are normal. Elimination of NH proton and substitution of this nitrogen atom would prevent tautomerism and can lead to a separable mixture of regioisomers [14]. The regioselective alkylation of one within other nitrogen atoms is difficult in many cases and giving rise to mixtures of the two regioisomers. We reported the *N*-alkylation reaction of some imidazo [4,5-b]pyridines in our previous study [14]. It was declared that formation ratio of the regioisomers could be changed with reaction conditions and solvent effects. We aimed to explain some conflict resolutions on the formation of regioisomers. In the literature there were two different results about the regiosomer formation ratio under same conditions. Zeinyeh et al. [16,17] stated that at RT using K_2CO_3 in DMF, benzylation of imidazo [4,5-*b*] pyridine-4-oxide and 2-methyl- imidazo[4,5-*b*]pyridine-4-oxide with benzyl bromide or benzyl iodide gave the *N*-1/*N*-3 regioisomers in slightly different ratios. But they never mentioned about the formation of *N*-4 regioisomer. In contrast, Ouzidan et al. [18—20] and Bourichi et al. [21] reported obtaining *N*-4 regiosomers with X-Ray Data by using the same reaction. Similarly, when we attempted [14] alkylation of



Reagents : a) Cyclohexylamine or 3,4-*di*-MeO-benzyl amine b) H_2. Pd/C c) $Na_2S_2O_5$

Scheme 1. Synthesis of 2-substituted imidazo[4,5-*b*]pyridine analogues.

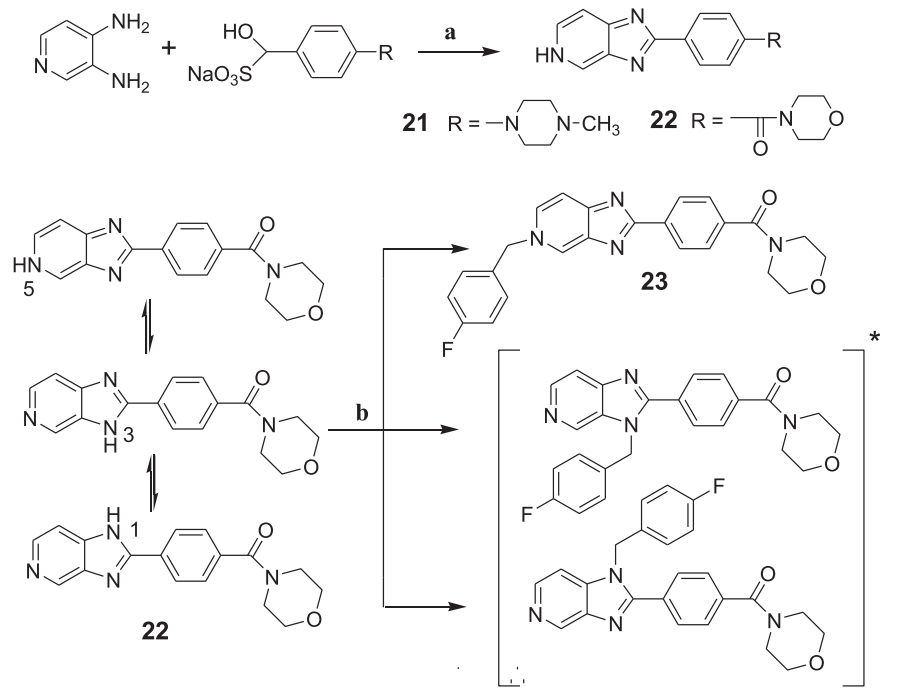


Reagents : * Could not be isolated a) Sodium bisulfide adduct of 4-(morpholin-4-ylcarbonyl)-benzaldehyde b) Potassium carbonate / 4-F-benzyl bromide

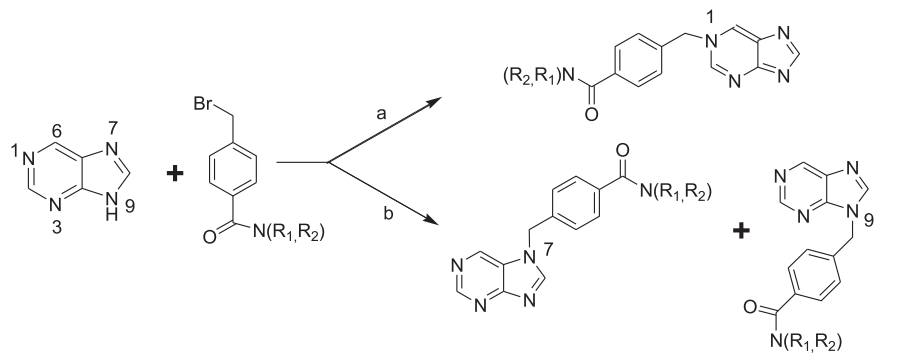
Scheme 2. *N*-Benzylation of 2-substitutedimidazo[4,5-*b*]pyridine (**16**,**17**) analogues.

some imidazo [4,5-b]pyridine with *n*-butyl bromide and 4-fluorobenzyl bromide under same conditions, alkylations were formed mainly in *N*-4 position. Hence, we are totally in agreement with literature [18–21]. Meantime same researchers also reported in other studies [22–24] that *N*-3 regioisomers were obtained. It is concluded that both properties of the previously existing substituents and nature of the alkylating agent can affect formation of regioisomers. This was proved in our previous study not only by advanced NMR techniques but also by regioselective synthesis [14].

In this study we have prepared a series of some new 2-(piperazine, piperidine and morpholin-substituted) phenylimidazo [4,5-b]pyridines **7**–**19** to investigate their anti-cancer activities (Schemes 1 and 2). The chlorine atom of 2-chloro-3-nitropyridine was converted to alkylamino group by aromatic nucleophilic substitution reaction. Reduction of the nitro groups in **1** and **2** with H_2/Pd–*C* gave pyridinediamines **3** and **4** (Scheme 1). By condensation of the respective substituted pyridinediamines **3**–**6** with the corresponding $Na_2S_2O_5$ adducts of various arylaldehydes in DMF, we



Scheme 3. *N*-Benzylation of 2-substitutedimidazo[4,5-*c*]pyridine (**22**).



Scheme 4. *N*-alkylation of purines reported by Khanna et al. [29].

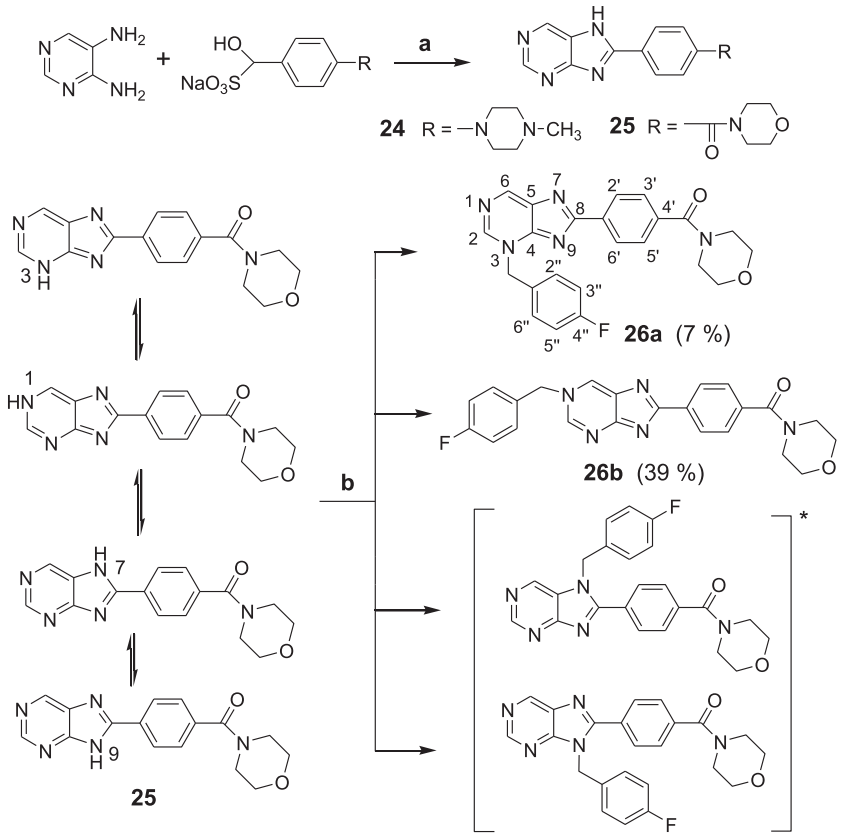
reached the targeted compounds **7**—**19,** (Scheme 1 and 2). When we attempted alkylation of **16** and **17** with 4-fluorobenzyl bromide under basic conditions (K_2CO_3, DMF), alkylations were formed mainly in *N*-4 position. **18b** (50.8%) and **19b** (48%) were obtained as *N-4* isomer similar to our previous study [14]. In same reaction medium. **18a** (7.5%) and **19a** (6%) were isolated as *N*-3 regioisomer in less amount (Scheme 2) by using column chromatography. **18c** (*N*-1) which is the third regioisomer of **18** and its' analogue in **19** were not detected in LC-MS chromatograms of **18** and **19.** This is why we prepared **18c** by selective regioisomer synthesis method, between the reaction product **20** and sodium metabisulfite adduct of 4-(morpholin-4-ylcarbonyl)benzaldehyde. It can be seen in LC-MS chromatogram of compound **18** (Fig. 2), while R_t values of **18c** (*N-1)* (5.22 min) and **18a** (*N-3)* (5.36 min) regioisomers are very close to each other, R_t value of **18b** *(N-4)* (4.55 min) regioisomer is far from them (*N-1* and *N-3)* and the peak area of *N-4* is much more than other isomer.

Hence, *N-4* regioisomer could be easily separated from *N-3* regioisomer as it is shown in our experiments. Characterisation of the individual isomeric products was determined by observation of 2D-NOESY enhancements between the *N*—CH_2 and H-5,7 protons.

In the NOESY spectra of compound;

- **18b** (Fig. 3a) and **19b,** strong correlations were observed between benzylic and H-5 protons.
- **18c,** NOE cross peaks between *N*—CH_2 and H-7 protons were observed.
- **18a** and **19a**, while there was no NOE contours between benzylic protons and pyridine protons, strong correlations were seen between benzylic and H-2′ and/or H-6′ as expected.

Complementary structural information was provided by 2D-



Reagents : a) DMF, 120°C b) Potassium carbonate / 4-F-benzyl bromide
* Could not be isolated

Scheme 5. *N*-Benzylation of 2-substitutedimidazo[4,5-*c*]pyrimidine (**25**).

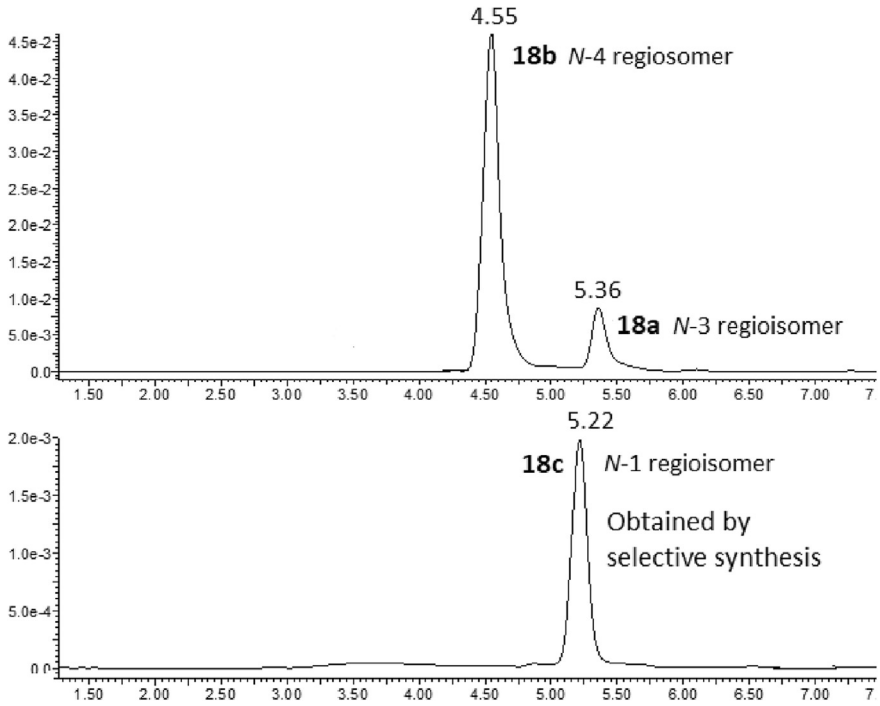


Fig. 2. LC-MS chromatogram (ESI+ and SIR techniques for [M+H]. 417 m/e) for **18a**, **18 b** at the endpoint of synthesis and **18c.**

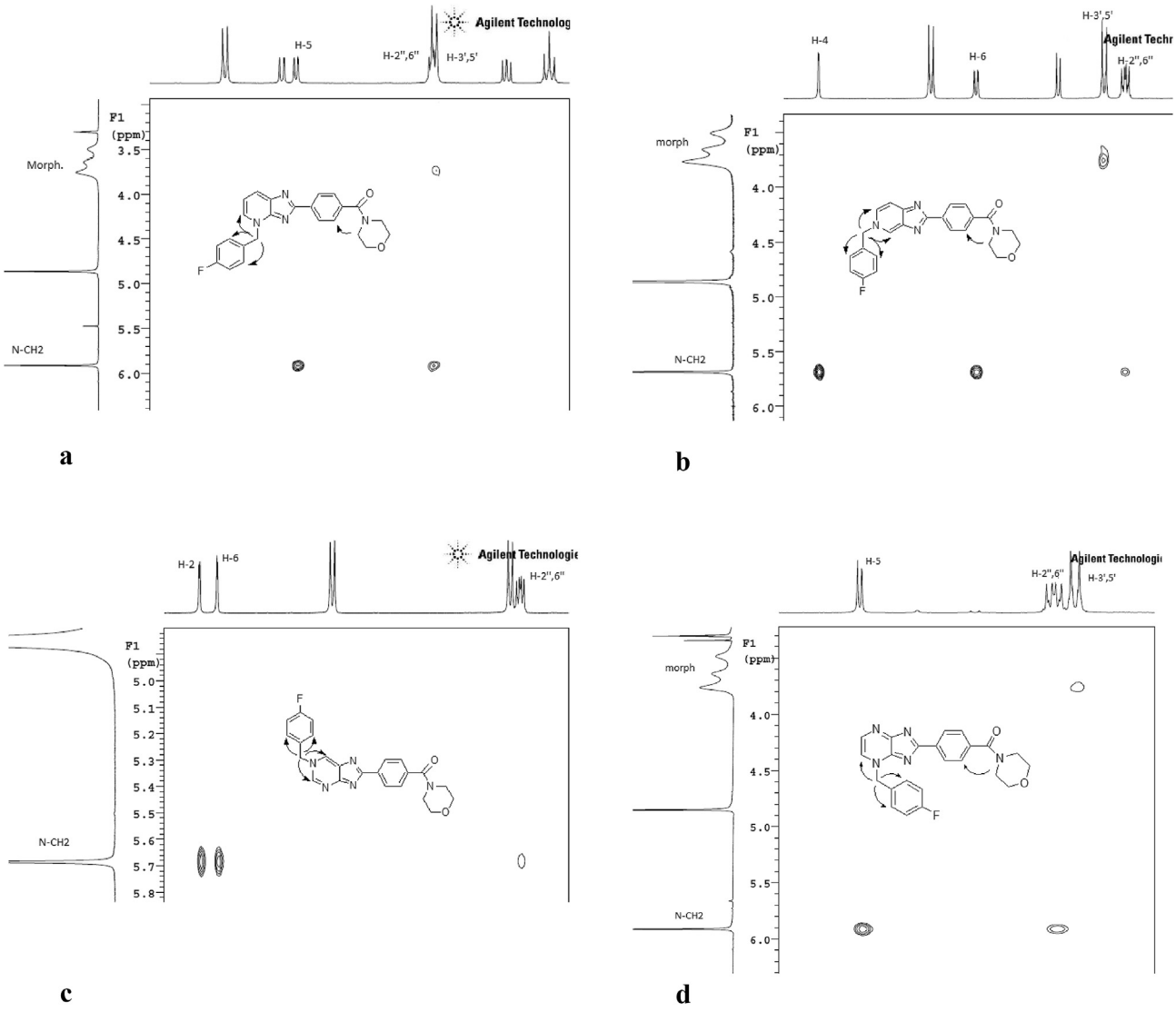


Fig. 3. aPartial NOESY spectrum of compound **18 b b** Partial NOESY spectrum of compound **23. c**. Partial NOESY spectrum of compound **26b. d.** Partial NOESY spectrum of compound **30b**.

HMBC spectra of **18(a-c**). It was possible to determine the chemical shift values (δ ppm) of C-3a and C-7a, by their correlations with pyridine hydrogens in HMBC spectra. Subsequently, it could be available to determine other possible correlations (whether N—CH_2: C-3a or N—CH_2: C-7a) in separation of regioisomers.

The results obtained from the HMBC spectra of **18 (a—c)** and **19a** (Fig. 4)**, 19b** provided all expected correlations.

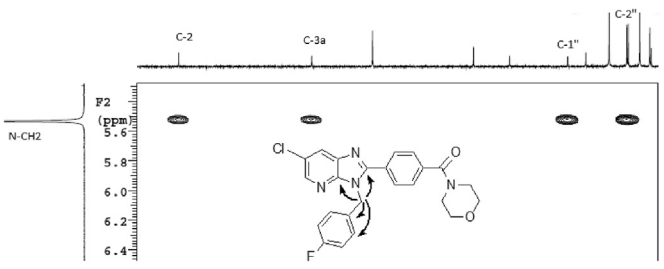


Fig. 4. Partial gHMBC spectrum of compound **19a** showing. cross peaks between N—CH_2/C-2, C-3a, C-1″, C-2″,6'.'.

In the same conditions, 4-fluorobenzylation of **22** gave only 5*H* isomer **23** (Scheme 3) as previously reported [14,25,26]. Therefore very strong NOE interactions were seen between N—CH_2 and H-4,6 in the NOESY spectra of **23** (Fig. 3b). These results were also confirmed by HMBC correlations.

Purine is generally represented when a hydrogen atom is attached to *N*-9 and it is called *N*(9)H tautomer. It is obvious that structures may be considered in which the proton is attached to the other nitrogens of the molecule, yielding the tautomers *N*(7)H; *N*(3)H and *N*(l)H [27]. Alkylation of purines usually results with the formation of *N*-9 and *N*-7 alkylpurines and frequently *N*-9 regiosomer is formed as major product. Bookser et al. [28] reported that alkylation of some purines with CH_3I and NaHMDS in THF or DMSO resulted with the formation of *N*-7 and *N*-9 regiosomers. Khanna et al. [29] reported that benzylation of purine with potassium hydride formed mixture of possible *N*-7 and *N*-9 alkylated products. In contrast, without potassium hydride, only *N*-1 alkylated product was obtained (Scheme 4). In US 2006/0052602, Gilead sciences, Inc. claimed a series of imidazo [4,5-d]pyrimidine analogues for their antiviral activities [30]. In this study, when 8-(substitutedphenyl) purines were alkylated with several benzyl

chlorides in aqueous NaOH medium, alkylation was formed at *N*-1 position of purines.

In similiar conditions (with dry K_2CO_3 in DMF, instead of aqueous NaOH), we achieved the same result and compound **26b** (39%) was obtained as *N*-1 regioisomer (Scheme 5). In addition, *N*-3 regioisomer **26a** (7%) was also isolated in our conditions. *N*-3 regioisomers of purines are scarcely reported, Mitsunobu reactions could be given as an example [31,32]. In NOESY spectra of **26a** and **26b** (Fig. 3c), very strong NOE interactions was detected with *N*–CH_2: only H-2 and *N*–CH_2: H-2,6, respectively. Another detail supporting this finding was the absence of cross peaks between *N*–CH_2: H-2′ or/and 6'. It clarified that these products could not be *N*-7 or *N*-9 alkylated products. HMBC correlations also confirmed the proposed structures of both **26a** and **26b**.

Finally, to our knowledge no study has been made about alkylation of 1*H*-imidazo [4,5-b] pyrazines yet. So their alkylations were also examined in this study. **27** was prepared by the nucleophilic substitution reaction in sealed tube [33] (Scheme 6).

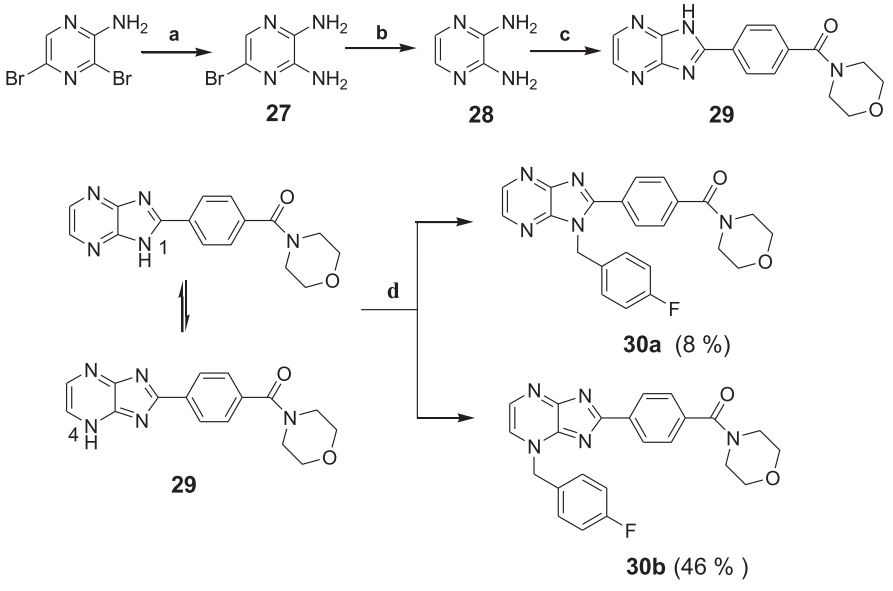
Bromine atom was removed by catalytic hydrogenation in Parr apparatus. Finally by the cyclization of **28** with sodyum metabisulfite adduct of 4-(morpholin-4-ylcarbonyl] benzaldehyde gave **29**. The same alkylation reaction was carried out to **29**, and it was observed that alkylation of **29** favorably formed *N*4-(4-F-benzyl) **30b** (71%). Because of the symmetrical position of **29**, second and last choice, regioisomer **30a** (11%) was formed. NOESY and HMBC correlations confirmed the proposed structures of both **30a** and **30b** (Fig. 3d).

All described imidazole-containing heterocycles **7**–**30b** were tested for *in vitro* cytotoxic activities against human leukemia cell lines (K562 and HL-60) and human colon cancer cell line (HCT-116) by MTT test in triplicate (Table 1). Camptotechin and imatinib mesylate were used as reference cytotoxic drugs in the experiments. The results were expressed as growth inhibitory concentration (IC_{50}) values. It represents the compound concentrations required to produce 50% growth inhibition of cells growth after of incubation compared with untreated controls. The synthesized compounds and reference drugs were dissolved in DMSO and further dilutions were made with culture medium solution.

Table 1
In vitro cytotoxic activities of compounds **7**–**30b** was assessed by MTT.

Compound	IC_{50} (μM)		
	K562	HL-60	HCT-116
7	>100	>100	>100
8	8.28 ± 0.99	8.45 ± 0.297	9.74 ± 3.82
9	>100	3.61 ± 0.627	3.34 ± 0.52
10	7.05 ± 0.28	7.28 ± 0.417	6.22 ± 0.83
11	>100	>100	>100
12	33.96 ± 1.44	41.53 ± 1.37	73.23 ± 0.53
13	>100	>100	>100
14	>100	>100	>100
15	>100	>100	>100
16	>100	>100	>100
17	>100	>100	15.49 ± 2.35
18b	78.9 ± 5.35	>100	>100
18c	>100	>100	>100
19b	>100	>100	>100
21	>100	64.50 ± 4.79	>100
22	>100	>100	>100
23	>100	>100	>100
24	>100	>100	>100
25	>100	>100	>100
26b	>100	>100	>100
30a	>100	>100	>100
30b	>100	>100	>100
Camptotechin	–	0.118 ± 0.007	0.441 ± 0 .016
Imatinib	0.502 ± 0.09	–	–

None of the compounds exhibited cell growth inhibition as much as reference compounds. However, some compounds showed remarkable inhibitory activities. Compounds **8**–**10** having halogen atom on the 6 position of the pyridine moiety were the most active derivatives with the IC_{50} values 3.3–9.7 μM. Compound **12,** bearing a cyclohexyl group on N^3 position, also showed moderate activity against selected cancer cell lines. The other benzyl N^3-substituted analogues did not show significant inhibitory activities. It can be concluded that tautomeric NH and halogens substitution on pyridine moiety can be important to determine inhibitory



Reagents : a) NH_4OH (25 %) b) Pd.C/ H_2 c) Sodium metabisulfite adduct of 4-(morpholin-4-ylcarbonyl)benzaldehyde d) Potassium carbonate / 4-F-benzyl bromide

Scheme 6. *N*-alkylation 2-substitutedimidazo[4,5-*b*]pyrazine (**29**).

activities.

3. Conclusion

It was found that *N*−benzylation of imidazo[4,5-*b*]pyridine, imidazo[4,5-*c*]pyridine, imidazo[4,5-*d*]pyrimidine (Purine) and imidazo [4,5-b]pyrazine favorably was realized on the six membered *N*-heterocycles in presence of anhydrous K_2CO_3 in DMF. Benzylation of imidazole nitrogen ($N^{1,3,7,9}$) atoms occur at a lower ratio secondarily. Since polarities of imidazole *N*-alkylated products are not much different from each other, they cannot be easily separated by column chromatography. However, it is easy to separate imidazole *N*-alkylated regioisomers from *N*-alkylated six membered heterocycles. Since *N*-alkylated six membered heterocycles adhere more strongly to silica gel, they move slower than imidazole *N*-alkylated regioisomers having a weaker adhesion on the column chromatography. For this reason, imidazole *N*-alkylated regioisomers are obtained first. By continuing to this elution, *N*-alkylated six membered heterocycles (polarity of mobil phase was increased gradually using MeOH) are obtained. NOESY experiment is the primary method for structural elucidation of these types of regioisomers. The cross peaks of *N*−CH_2 (at around 5−6 δ ppm) and aromatic protons confirm their spatial proximity as an evidence for which nitrogen atom is substituted. HMBC technique can be an effective alternative method for assignment of regioisomers. The complete structure elucidation of all synthesized compounds were performed using 1D and 2D NMR experiments including COSY, NOESY, gHSQC, and gHMBC techniques.

4. Material and method

4.1. Chemistry

Uncorrected melting points were measured using a Büchi B-540 capillary melting point apparatus. All NMR experiments were carried out using VARIAN (Agilent) MERCURY 400 MHz (Varian, Palo Alto, CA, USA) at a proton resonance frequency of 400 and 100 MHz for carbon. The NMR spectrum optimisation was conducted using Agilent VnmrJ version 3.2 revision A software and all parameters were set in it. The samples (5−15 mg) were prepared in 0.7 mL of DMSO-d_6, $CDCl_3$ and CD_3OD. TMS was used as an internal standart. The liquid chromatography mass spectrometry (LC-MS) spectra were taken on a Waters Micromass ZQ connected with Waters Alliance HPLC (Waters Corporation, Milford, MA, USA), using the ESI(+) method with a C-18 column (XTerra®, 4.6 × 250 mm, 5 μm). Analytical conditions of mass spectrometry were as follows: capillary voltage, 3.11 kV; cone voltage, 29 V; source temperature, 100 °C; and desolvation temperature, 300 °C. Because of the tautomeric effect of imidazole ring, some nmr data did not give satisfactory results, in order to eliminate the tautomeric effect, the related benzimidazoles were dissolved in CD_3OD or DMSO-d_6, followed by a tiny amount of dry NaH, and 2−3 drops of D_2O were added to the nmr tube and stirred well. As it is reported in the experimental part, this time very satisfactory nmr results have been observed since the tautomeric effect was totally disappeared. The HCl salt of the synthesized compounds were prepared using ethanolic HCl. Compounds **5** and **6** [14], **20** [34] were prepared according to the literature methods.

4.1.1. General synthesis of ***1*−*2***

The mixture of 2-chloro-3-nitropyridine (3 mmol), corresponding anilines (3 mmol) and K_2CO_3 (3 mmol) were heated in DMF at 120 °C, for 3−4 h. The reaction mixture was allowed to cool to room temperature then diethyl ether was added. The organic layer was washed with water dried over $MgSO_4$, filtered and evaporated. The residue was crystallised from ethylacetate/n-hexane.

*4.1.1.1. N^2-cyclohexyl-3-nitro-2-amino pyridine **(1)**.* Prepared from 2-chloro-3-nitropyridine (0.47 g), cyclohexylamine (0.34 g) and K_2CO_3 (0.41 g) as described in general method. Yield. 0.19 g, (30%), yellow oily liquid. **^{1}H-NMR** δ ppm ($CDCl_3$): 1.22−1.49 (m,5H), 1.59−1.74 (m,1H), 1.75−1.79 (m,2H), 2.01−2.05 (m,2H), 4.21 (m,1H), 6.56 (m,1H), 8.20 (br.s,1H), 8.36−8.38 (m,2H). **^{13}C-NMR** δ ppm ($CDCl_3$): 24.7, 25.6, 32.9, 49.4, 111.2, 127.6, 135.3, 152.0, 155.9. **MS** (ESI+) *m*/*z*: 222(M + H, 100%), $C_{11}H_{15}N_3O_2$.

*4.1.1.2. N^2-(3,4-dimethoxybenzyl)-3-nitro-2-amino pyridine **(2)**.* Prepared from 2-chloro-3-nitropyridine (0.47 g), 3,4-dimethoxybenzylamine (0.45 mL) and K_2CO_3 (0.41 g), as described in general method. Yield, 0.29 g, (34%), mp 85−88 °C. **^{1}H-NMR** δ ppm ($CDCl_3$): 3.87 (s,6H), 4.77 (d,2H,J = 5.2 Hz), 6.67 (dd,1H,J = 8 & 4.8 Hz), 6.84 (d, 1H, J = 8 Hz), 6.91−6.94 (m,2H), 8.42−8.45 (m,3H). **^{13}C-NMR** δ ppm ($CDCl_3$): 44.9, 55.8, 55.9, 111.1, 111.3, 112.0, 120.0, 128.1, 130.6, 135.3, 148.5, 149.1, 152.3, 155.7. **MS** (ESI+) *m*/*z*: 290(M + H, 100%), $C_{14}H_{15}N_3O_4$.

4.1.2. General synthesis of ***3*−*4***

Compounds **1**−**2** (1 mmol) in EtOH (25 mL) was reduced by hydrogenation using 50 psi of H_2 and 10% Pd−C (15 mg) until cessation of H_2 uptake. The catalyst was filtered on a bed of Celite, washed with EtOH and the filtrate was concentrated in vacuo. The crude amines were used for the further steps without purification.

*4.1.2.1. N^2-cyclohexylpyridine-2,3-diamine (**3**).* Prepared from N^2-cyclohexyl-3-nitro-2-amino pyridine (0.22 g) as described in general method. Yield, 0.17 g, (90%), dark purple colored powder. **^{1}H-NMR** δ ppm (CD_3OD): 1.14−1.29 (m,3H), 1.35−1.46 (m,2H), 1.62−1.65 (m,1H), 1.72−1.77 (m,2H), 1.99−2.02 (m,2H), 3.70−3.78 (m,1H), 6.43 (dd,1H,J = 7.6 & 5.6 Hz), 6.87 (dd,1H,J = 7.6 & 0.8 Hz), 7.38 (dd,1H,J = 5.6 & 0.8 Hz). **^{13}C-NMR** δ ppm (CD_3OD): 26.3, 26.9, 34.4, 51.0, 113.5, 121.5, 131.8, 134.7, 148.9. **MS** (ESI+) *m*/*z*: 192(M + H, 100%), $C_{11}H_{17}N_3$.

*4.1.2.2. N^2-(3,4-dimethoxybenzyl)pyridine-2,3-diamine **(4)**.* Prepared from N^2-(3,4-dimethoxybenzyl)-3-nitro-2-amino pyridine (0.28 g) as described in general method. Yield, 0.26 g, (92%), dark brown colored powder. **^{1}H-NMR** δ ppm (CD_3OD): 3.77 (s,6H), 4.57 (d,2H,J = 5.2 Hz), 6.60 (dd,1H,J = 7.6 & 6 Hz), 6.86 (d,1H,J = 8 Hz), 6.92 (dd, 1H,J = 8.4 & 1.6 Hz), 6.99−7.02 (m,2H), 7.35−7.37 (m,1H). **^{13}C-NMR** δ ppm (CD_3OD): 46.6, 56.50, 56.53, 112.9, 113.1, 114.4, 121.3, 121.4, 129.3, 131.5, 133.7, 147.3, 150.0, 150.6. **MS** (ESI+) *m*/*z*: 260(M + H, 100%), $C_{14}H_{17}N_3O_2$.

4.1.3. General synthesis of sodium metabisulphite adduct of benzaldehydes

Related benzaldehydes (7.5 mmol) was dissolved in EtOH (25 mL) and sodium metabisulfite (0.8 g) (in 5 mL of water) was added in portions. The reaction mixture was stirred vigorously and more EtOH was added. The mixture was kept in a refrigerator for a while. The white precipitate, the obtained salts were gained by filtration, dried and used for the further steps without purification and characterisation.

4.1.4. General synthesis of 7−16, 21, 24, 25, 27, 28, 33

The mixture of sodium metabisulphite adduct of benzaldehydes (1 mmol) and corresponding 2,3-diamines (1 mmol), in DMF (2 mL) were heated at 120 °C, for 3−4 h. The reaction mixture was cooled, poured into water. The resulting precipitate was collected by filtration and dried. If the product was not solid, it was purified with

column chromatography.

*4.1.4.1. 2-[4-(4-Methylpiperazin-1-yl)phenyl]-3H-imidazo [4,5-b] pyridine HCl (**7**).* Prepared from 2,3-diaminopyridine (0.1 g) and $Na_2S_2O_5$ adduct of 4-(4-methylpiperazin-1-yl) benzaldehyde (0.308 g) as described in general method. Crude product was converted to HCl salt, yield 0.14 g, (35%), mp > 300 °C. **^{1}H-NMR** δ ppm (DMSO-d_6+NaH + D_2O): 2.23 (s,3H), 2.49 (t,4H,J = 4.8 Hz), 3.20 (t,4H,J = 4.8 Hz), 6.77 (dd,1H,J = 7.6 & 4.8 Hz,H-6), 6.95 (d,2H,J = 6.8 Hz), 7.64 (dd,1H,J = 7.2 & 1.2 Hz), 7.89 (dd,1H,J = 4.8 & 2 Hz), 8.15 (d,2H,J = 6.8 Hz). **^{13}C-NMR** (DMSO-d_6+NaH + D_2O): 46.1, 48.2, 54.9, 113.5, 115.1, 121.8, 127.9, 128.2, 138.7, 139.5, 150.8, 160.8, 163.1. **MS** (ESI+) *m/z*: 294(M + H, 37%), 148 (100%). Anal Calcd for $C_{17}H_{19}N_5 \cdot 2HCl \cdot 2.25H_2O$: C, 50.18; H, 6.31; N, 17.21. Found: C, 50.13; H, 6.60; N, 17.36.

*4.1.4.2. 6-Chloro-2-[4-(4-methylpiperazin-1-yl)phenyl]-3H-imidazo [4,5-b]pyridine (**8**).* Prepared from 5-chloro-2,3-diaminopyridine (0.14 g) and $Na_2S_2O_5$ adduct of 4-(4-methylpiperazin-1-yl)benzaldehyde (0.308 g) as described in general method. Resulting precipitate was purified with column chromatography using CH_2Cl_2: MeOH (100 : 0.5) as eluant, yield 0.245 g, (75%), mp > 300 °C. **^{1}H-NMR** δ ppm (DMSO-d_6+NaH + D_2O): 2.19 (s,3H), 2.44 (t,4H,J = 4,8 Hz), 3.16 (t,4H,J = 4,8 Hz), 6.90 (d,2H,J = 8.8 Hz), 7.58 (d,1H,J = 2.4 Hz), 7.81 (d, 1H,J = 2.4 Hz), 8.09 (d,2H,J = 8.8 Hz). **^{13}C-NMR** (DMSO-d_6+NaH + D_2O): 46.0, 48.1, 54.8, 115.0, 119.9, 120.5, 127.3, 128.3, 136.5, 140.3, 151.1, 159.8, 165.3. **MS** (ESI+) *m/z*: 328(M + H, 42%), 330.7(M + H+2, 15%), 165(100%). Anal Calcd for $C_{17}H_{18}ClN_5 \cdot 0.5H_2O$: C, 60.62; H, 5.68; N, 20.79. Found: C, 60.73; H, 5.78; N, 20.61.

*4.1.4.3. 6-Chloro-2-[4-(piperidin-1-yl)phenyl]-3H-imidazo [4,5-b] pyridine (**9**).* Prepared from 5-chloro-2,3-diaminopyridine (0.14 g) and $Na_2S_2O_5$ adduct of 4-(piperidin-1-yl)benzaldehyde (0.29 g) as described in general method. Resulting precipitate was purified with column chromatography using CH_2Cl_2: MeOH (100 : 0.8) as eluant, yield 0.27 g, (88%), mp, 280–282 °C. **^{1}H-NMR** δ ppm (CD_3OD + NaH + D_2O): 1.60–1.62 (m,2H), 1.71–1.74 (m,4H), 3.20–3.23 (m,4H), 7.08 (dd,2H,J = 7.2 & 2 Hz), 7.76 (d,1H,J = 2 Hz), 7.97 (d,1H,J = 2 Hz), 8.13 (d,2H,J = 6.4 & 2 Hz). **^{13}C-NMR** (CD_3OD + NaH + D_2O): 25.2, 26.4, 51.9, 117.8, 122.8, 122.9, 127.7, 129.3, 138.6, 140.9, 153.8, 159.5, 167.0. **MS** (ESI+) *m/z*: 313(M + H, 98%), 315(M + H+2, 35%). Anal Calcd for $C_{17}H_{17}ClN_4 \cdot 0.25H_2O$: C, 64.35; H, 5.55; N, 17.66. Found: C, 64.25; H, 5.54; N, 17.85.

*4.1.4.4. 6-Bromo-2-[4-(4-methylpiperazin-1-yl)phenyl]-3H-imidazo [4,5-b]pyridine (**10**).* Prepared from 5-bromo-2,3-diaminopyridine (0.14 g) and $Na_2S_2O_5$ adduct of 4-(4-methylpiperazin-1-yl)benzaldehyde (0.308 g) as described in general method. Resulting precipitate was purified with column chromatography using CH_2Cl_2: MeOH (100 : 0.5) as eluant, yield 0.245 g, (75%), mp > 300 °C. **^{1}H-NMR** δ ppm (DMSO-d_6+NaH + D_2O): 2.23 (s,3H), 2.48 (t,4H,J = 4.8 Hz), 3.20 (t,4H,J = 4.8 Hz), 6.94 (d,2H,J = 8.4 Hz), 7.72 (d,1H,J = 2 Hz), 7.90 (d, 1H,J = 2.4 Hz), 8.13 (d,2H,J = 8.8 Hz). **^{13}C-NMR** (DMSO-d_6+NaH + D_2O): 45.9, 48.0, 54.7, 108.1, 114.8, 122.9, 127.4, 128.1, 138.2, 141.1, 150.9, 159.9, 165.0. **MS** (ESI+) *m/z*: 372(M + H, %100), 374(M + H+2, %95). Anal Calcd for $C_{17}H_{18}BrN_5 \cdot 0.5H_2O$: C, 53.55; H, 5.02; N, 18.36. Found: C, 54.08; H, 5.06; N, 18.59.

*4.1.4.5. 6-Bromo-2-[4-(morpholin-4-yl)phenyl]-3H-imidazo [4,5-b] pyridine (**11**).* Prepared from 5-bromo-2,3-diaminopyridine (0.18 g) and $Na_2S_2O_5$ adduct of 4-(morpholino) benzaldehyde (0.29 g) as described in general method. Resulting precipitate (0.28 g) was purified with column chromatography using CH_2Cl_2: MeOH (100 : 0.5) as eluant, yield 0.19 g, (55%), mp > 300 °C. **^{1}H-NMR** δ ppm (DMSO-d_6+NaH + D_2O): 3.18 (t,4H,J = 4.8 Hz), 3.77 (t,4H,J = 4.8 Hz), 6.98 (d,2H,J = 8.8 Hz), 7.80 (d,1H,J = 2 Hz), 7.94 (d,1H,J = 2.4 Hz), 8.14 (d, 2H,J = 8.8 Hz). **^{13}C-NMR** (DMSO-d_6+NaH + D_2O): 48.7, 66.6, 108.7, 115.1, 123.7, 127.4, 128.5, 138.8, 141.1, 151.5, 159.6, 165.1. **MS** (ESI+) *m/z*: 359(M + H, 100%), 361(M + H+2, 98%). Anal Calcd for $C_{16}H_{15}BrN_4O \cdot 0.25H_2O$: C, 52.83; H, 4.29; N, 15.40. Found: C, 53.04; H, 4.22; N, 15.79.

*4.1.4.6. 3-Cyclohexyl-2-(4-(4-methylpiperazin-1-yl)phenyl)-3H-imidazo [4,5-b]pyridine (**12**).* Prepared from **3** (0.19 g) and $Na_2S_2O_5$ adduct of *4-(4-methylpiperazin*-1-yl)benzaldehyde (0.308 g) as described in general method. Resulting precipitate was purified with column chromatography using CH_2Cl_2: EtOH (100 : 0.5) as eluant, yield 0.15 g, (40%), mp 151–154 °C. **^{1}H-NMR** δ ppm (CD_3OD): 1.26–1.40 (m,3H), 1.67–1.70 (m,1H), 1.82–1.90 (m,4H), 2.36 (s,3H), 2.62 (t,4H,J = 4.4 Hz), 2.74–2.82 (m,2H), 3.35 (t,4H,J = 4.4 Hz), 4.36–4.42 (m,1H), 7.11 (d,2H,J = 8.8 Hz), 7.26 (dd,1H,J = 7.6 & 5.2 Hz,H-6), 7.52 (d,2H,J = 8.8 Hz), 7.96 (dd,1H,J = 7.6 & 1.2 Hz), 8.32 (dd,1H,J = 5.2 &1.6 Hz). **^{13}C-NMR** (CD_3OD): 26.1, 27.1, 31.7, 46.1, 48.6, 55.8, 58.8, 116.2, 119.4, 120.9, 127.3, 131.5, 136.0, 144.2, 149.4, 153.9, 156.7. **MS** (ESI+) *m/z*: 376(M + H, 58%), 147(100%). Anal Calcd for $C_{23}H_{29}N_5 \cdot 1.5H_2O \cdot 0.25C_2H_5OH$: C, 68.16; H, 8.15; N, 16.91. Found: C, 68.28; H, 7.83; N, 16.82.

*4.1.4.7. 3-(3,4-Dimethoxybenzyl)-2-(4-(4-methylpiperazin-1-yl) phenyl)-3H-imidazo [4,5-b]pyridine (**13**).* Prepared from **4** (0.25 g) and $Na_2S_2O_5$ adduct of *4-(4-methylpiperazin*-1-yl)benzaldehyde (0.308 g) as described in general method. Resulting precipitate was purified with column chromatography using CH_2Cl_2: MeOH (100 : 0.5) as eluant, yield 0.23 g, (52%), mp 192–194 °C. **^{1}H-NMR** δ ppm ($CDCl_3$): 2.47 (s,3H), 2.73(br.t,4H), 3.42 (t,4H,J = 4.4 Hz), 3.74 (s,3H), 3.83 (s,3H), 5.54 (s,2H), 6.64 (dd,1H,J = 8 & 2 Hz), 6.75–6.77 (m,2H), 6.96 (d,2H,J = 7.2 Hz), 7.26 (dd, 1H,J = 7.2 & 5.6 Hz,H-6), 7.65 (d,2H,J = 6.8 Hz), 8.06 (dd,1H,J = 7.6 & 1.2 Hz), 8.38 (dd,1H,J = 4.8 & 1.2 Hz). **^{13}C-NMR** ($CDCl_3$): 45.9, 46.5, 47.7, 54.7, 55.7, 55.8, 110.0, 111.3, 114.9, 118.5, 118.8, 120.0, 126.6, 129.5, 130.3, 135.3, 143.5, 148.4, 149.0, 149.1, 152.1, 155.2. **MS** (ESI+) *m/z*: 444(M + H, 100%). Anal Calcd for $C_{26}H_{29}N_5O_2$: C, 70.41; H, 6.59; N, 15.79. Found: C, 70.54; H, 6.85; N, 15.78.

*4.1.4.8. 3-Benzyl-2-(4-(4-methylpiperazin-1-yl)phenyl)-3H-imidazo [4,5-b]pyridine (**14**).* Prepared from **5** (0.199 g) and $Na_2S_2O_5$ adduct of *4-(4-methylpiperazin*-1-yl)benzaldehyde (0.308 g) as described in general method. Resulting precipitate was purified with column chromatography using CH_2Cl_2: MeOH (100 : 0.5) as eluant, yield 0.21 g, (55%), mp 159–161 °C. **^{1}H-NMR** δ ppm ($CDCl_3$): 2.41 (s,3H), 2.66(br.t,4H), 3.37 (t,4H,J = 4.8 Hz) 5.60 (s,2H), 6.92 (d, 2H,J = 8.8 Hz), 7.12 (d,2H,J = 7.2 Hz), 7.23–7.31 (m,4H), 7.60 (d,2H,J = 8.4 Hz), 8.06 (dd,1H,J = 8 & 1.6 Hz), 8.36 (dd,1H,J = 5.2 & 1.6 Hz). **^{13}C-NMR** ($CDCl_3$): 45.8, 46.8, 47.6, 54.6, 115.1, 118.6, 120.1, 126.3, 126.7, 127.5, 128.8, 130.3, 135.3, 137.1, 143.6, 149.1, 151.9, 155.1. **MS** (ESI+) *m/z*: 384(M + H, 100%). Anal Calcd for $C_{24}H_{25}N_5 \cdot 0.25H_2O$: C, 74.29; H, 6.62; N, 18.05. Found: C, 74.40; H, 6.98; N, 17.74.

*4.1.4.9. 1-Benzyl-2-[4-(4-methylpiperazin-1-yl)phenyl]-1H-imidazo [4,5-b]pyridine (**15**).* Prepared from **6** (0.199 g) and $Na_2S_2O_5$ adduct of *4-(4-methylpiperazin*-1-yl)benzaldehyde (0.308 g) as described in general method. Resulting precipitate was purified with column chromatography using CH_2Cl_2: MeOH (100 : 0.5) as eluant, yield 0.23 g, (60%), mp 236–238 °C. **^{1}H-NMR** δ ppm ($CDCl_3$): 2.41 (s,3H), 2.66(br.t,4H), 3.36 (t,4H,J = 4.8 Hz), 5.48 (s,2H), 6.94 (d,2H,J = 8.8 Hz), 7.08–7.12 (m,3H), 7.26–7.37 (m,3H), 7.43 (dd,1H,J = 7.6 & 1.2 Hz), 7.67 (d,2H,J = 8.8 Hz), 8.54 (dd,1H,J = 4.8 &

1.6 Hz). **^{13}C-NMR** ($CDCl_3$): 45.8, 47.6, 48.6, 54.6, 115.1, 117.6, 117.9, 119.5, 125.9, 127.9, 128.6, 129.2, 130.6, 135.9, 145.1, 152.1, 156.2, 156.6. **MS** (ESI+) *m/z*: 384(M + H, 100%). Anal Calcd for $C_{24}H_{25}N_5$: C, 75.17; H, 6.57; N, 18.26. Found: C, 74.77; H, 6.78; N, 18.10.

*4.1.4.10. [4-(4H-imidazo [4,5-b]pyridin-2-yl)phenyl](morpholin-4-yl) methanone (**16**).* Prepared from 2,3-diaminopyridine (0.1 g) and $Na_2S_2O_5$ adduct of 4-(morpholin-4-ylcar-bonyl)benzaldehyde (0.323 g) as described in general method. Resulting precipitate was boiled in MeOH, cooled and filtered, yield 0.21 g, (68%), mp, 264–268 °C. **^{1}H-NMR** δ ppm (CD_3OD): 3.49–3.78 (m,8H), 7.33 (dd,1H,*J* = 8 & 4.8 Hz,H-6), 7.64 (d,2H,*J* = 8.4 Hz), 8.04 (d,1H,*J* = 7.6 Hz), 8.25 (m,2H,*J* = 8.4 Hz), 8.38 (dd,1H,*J* = 5.2 & 1.2 Hz). **^{13}C-NMR** (CD_3OD): 44.0, 50.9, 58.6, 61.0, 67.8, 70.8, 120.0, 128.5, 129.0, 132.1, 138.8, 145.6, 171.6. **MS** (ESI+) *m/z*: 309(M + H, 100%). Anal Calcd for $C_{17}H_{16}N_4O_2 \cdot 0.75H_2O$: C, 63.44; H, 5.48; N, 17.40. Found: C, 63.53; H, 5.19; N, 17.20.

*4.1.4.11. [4-(6-Chloro-4H-imidazo [4,5-b]pyridin-2-yl)phenyl](-morpholin-4-yl)methanone (**17**).* Prepared from 5-chloro-2,3-diaminopyridine (0.144 g) and $Na_2S_2O_5$ adduct of 4-(morpholin-4- ylcarbonyl) benzaldehyde (0.323 g) as described in general method. Resulting precipitate was boiled in MeOH, cooled and filtered, yield 0.215 g, (63%), mp > 300 °C. **^{1}H-NMR** δ ppm (CD_3OD): 3.29–3.77(8H), 7.77 (br.s,2H), 8.28 (br.s,2H), 8.41 (s,1H), 8.67 (s,1H). **^{13}C-NMR** (CD_3OD): 67.9, 124.9, 126.6, 128.8, 129.7, 129.9, 130.3, 141.7, 146.0, 146.7, 154.7, 170.6. **MS** (ESI+) *m/z*: 343(M + H, 100%), 345(M + H+2, 31%). Anal Calcd for $C_{17}H_{15}ClN_4O_2 \cdot 0.5H_2O$: C, 58.04; H, 4.58; N, 15.92. Found: C, 58.40; H, 4.41; N, 16.32.

*4.1.4.12. 2-[4-(4-Methylpiperazine-1-yl)phenyl]-3H-imidazo [4,5-c] pyridine (**21**).* Prepared from 3,4-diaminopyridine (0.1 g) and $Na_2S_2O_5$ adduct of 4-(4-methylpiperazin-1-yl)benzaldehyde (0.308 g) as described in general method. Resulting precipitate was used without purification, yield 0.135 g, (46%), mp > 280 °C. **^{1}H-NMR** δ ppm (DMSO-d_6+NaH + D_2O): 2.23 (s,3H), 2.49 (t,4H,*J* = 4.8 Hz), 3.21 (t,4H,*J* = 4.8 Hz), 6.95 (d,2H,*J* = 8.8 Hz), 7.32 (dd,1H,*J* = 5.6 & 1.2 Hz,H-6), 7.81 (d,1H,*J* = 5.2 Hz,H-7), 8.15 (d,2H,*J* = 8.8 Hz), 8.57 (d,1H, *J* = 1.2 Hz,H-4). **^{13}C-NMR** (DMSO-d_6+NaH + D_2O): 45.9, 48.1, 54.8, 111.2, 115.1, 127.5, 128.4, 136.4, 137.6, 145.3, 150.9, 151.6, 163.5. **MS** (ESI+) *m/z*: 294.5(M + H, 100%). Anal Calcd for $C_{17}H_{19}N_5 \cdot 1.5H_2O$: C, 63.72; H, 6.92; N, 21.85. Found: C, 64.13; H, 7.03; N, 21.66.

*4.1.4.13. [4-(5H-imidazo [4,5-c]pyridin-2-yl)phenyl](morpholin-4-yl) methanone (**22**).* Prepared from 3,4-diaminopyridine (0.1 g) and $Na_2S_2O_5$ adduct of 4-(morpholin-4-ylcarbonyl) benzaldehyde (0.323 g) as described in general method. Resulting precipitate was purified with column chromatography using CH_2Cl_2: MeOH (100 : 5) as eluant yield 0.21 g, (65%), mp, 257–260 °C. **^{1}H-NMR** δ ppm (DMSO-d_6+NaH + D_2O): 3.48–3.67(8H), 7.46 (dd,1H,*J* = 5.6 & 1.2 Hz,H-6), 7.48 (d,2H,*J* = 8.4 Hz), 7.92 (d,1H,*J* = 5.6 Hz,H-7), 8.34 (d,2H,*J* = 8 Hz), 8.69 (d,1H,*J* = 1.2 Hz,H-4). **^{13}C-NMR** (DMSO-d_6+NaH + D_2O): 66.9, 112.5, 127.8, 128.1, 135.0, 137.5, 137.9, 138.9, 145.3, 151.8, 162.7, 170.8. **MS** (ESI+) *m/z*: 309(M + H, 100%) Anal Calcd for $C_{17}H_{16}N_4O_2 \cdot 2H_2O$: C, 59.29; H, 5.85; N, 16.26. Found: C, 59.13; H, 5.70; N, 16.11.

*4.1.4.14. 8-[4-(4-Methylpiperazin-1-yl)phenyl]-9H-purine HCl (**24**).* Prepared from 4,5-diaminopyrimidine (0.11 g) and $Na_2S_2O_5$ adduct of 4-(4-methylpiperazin-1-yl)benzaldehyde (0.308 g) as described in general method. Crude product was converted to HCl salt in MeOH, yield 0.24 g, (55%), mp > 300 °C. **^{1}H-NMR** δ ppm (DMSO-d_6+ D_2O): 2.18 (s,3H), 2.45 (t,4H,*J* = 4.8 Hz), 3.18 (t,4H,*J* = 4.8 Hz), 6.95 (d,2H,*J* = 8.8 Hz), 8.12 (d,2H, *J* = 8.8 Hz), 8.39 (s,1H), 8.55 (s,1H). **^{13}C-NMR** (DMSO-d_6+D_2O): 46.1, 48.1, 54.8, 115.3, 126.3, 129.1, 138.4, 141.1, 148.5, 151.9, 164.1, 166.3. **MS** (ESI+) *m/z*: 295(M + H, 100%). Anal Calcd for $C_{16}H_{18}N_6 \cdot 4HCl$: C, 43.65; H, 5.03; N, 19.09. Found: C, 43.58; H, 4.68; N, 19.13.

*4.1.4.15. Morpholin-4-yl[4-(9h-purin-8-yl)phenyl]methanone (**25**).* Prepared from 4,5-diaminopyrimidine (0.11 g) and $Na_2S_2O_5$ adduct of 4-(morpholin-4-yl)car-bonyl)benzaldehyde (0.323 g) as described in general method. Resulting precipitate was boiled in MeOH, cooled and filtered, yield 0.24 g, (77%), mp 246–249 °C. **^{1}H-NMR** δ ppm (DMSO-d_6 +D_2O): 3.41–3.62(8H), 7.63 (d,2H,*J* = 8 Hz), 8.31 (d,2H,*J* = 8.4 Hz), 8.91 (s,1H), 9.12 (s,1H). **^{13}C-NMR** (DMSO-d_6+D_2O): 65.9, 127.1, 127.7, 129.7, 132.9, 138.0, 145.1, 152.1, 153.8, 168.2. **MS** (ESI+) *m/z*: 310(M + H, 100%). Anal Calcd for $C_{16}H_{15}N_5O_2 \cdot 2H_2O$: C, 55.64; H, 5.54; N, 20.27. Found: C, 55.35; H, 5.68; N, 20.20.

*4.1.4.16. (4-(1H-imidazo [4,5-b]pyrazin-2-yl)phenyl)(morpholino) methanone (**29**).* 5-Bromopyrazine-2,3-diamine (**27**) was prepared from 2-amino-3,5-dibromopyrazine and NH_4OH (25%) in sealed tube according to lit.[33] **^{1}H-NMR** δ ppm (CD_3OD): 4.90 (s,4H,NH_2), 7.24 (s,2H,H-5,6). **^{13}C-NMR** (CD_3OD): 123.3, 129.8, 144.3, 145.9. MS (ESI+) *m/z*: 189(M + H, 100%), 191(M + H+2, 97%). **27** (0.303 g, 1.6 mmol) in EtOH (10 mL) was reduced by hydrogenation using 40 psi of H_2 and 10% Pd–C (15 mg) for 8 h. The catalyst was filtered on a bed of Celite, the filtrate was concentrated in vacuo. Purple colored powder **28** were used for the further steps without purification. **^{1}H-NMR** δ ppm (CD_3OD): 4.90 (s,4H,NH_2), 7.24 (s,2H, H-5,6). **^{13}C-NMR** (CD_3OD): 123.7, 145.1. **MS** (ESI+) *m/z*: 110(M + H, 100%). **29** was prepared from **28** (0.11 g) and $Na_2S_2O_5$ adduct of 4-(morpholin-4-ylcarbonyl) benzaldehyde (0.323 g) as described in general method. Resulting precipitate (0.155 g) was purified with column chromatography using CH_2Cl_2: MeOH (95 : 5) as eluant, yield 0.056 g, (18%), mp > 300 °C. **^{1}H-NMR** δ ppm (DMSO-d_6+D_2O): 3.27–3.61 (m,8H), 7.62 (d,2H,*J* = 8.4 Hz), 8.33 (d,2H,*J* = 8.4 Hz), 8.40 (s,2H,H-5,6). **^{13}C-NMR** (DMSO-d_6+D_2O): 66.0, 127.3, 127.8, 129.9, 138.2, 139.0, 154.7, 168.2. **MS** (ESI+) *m/z*: 310(M + H, 100%). Anal Calcd for $C_{16}H_{15}N_5O_2 \cdot H_2O$: C, 58.70; H, 5.23; N, 21.39. Found: C, 58.97; H, 5.01; N, 20.91.

4.1.4.17. General synthesis of 18a-b, 19a-b, 23, 26a-b, 30a-b. K_2CO_3 (0.069 g, 0.5 mmol) was added to a suspension of the **16**, **17**, **22, 25** and **29** (0.36 mmol) in DMF (0.5 mL) and stirred. One hour later, 4-fluorobenzyl bromide (0.095 g, 0.5 mmol) was added. After overnight stirring at rt, water was added and precipitate was filtered, if precipitate was not occurred, it was extracted with the mixture CH_2Cl_2: MeOH (95 : 5).

*(4-(3-(4-Fluorobenzyl)-3H-imidazo [4,5-b]pyridin-2-yl)phenyl)(-morpholino)methanone (**18a**) & (4-(4-(4-Fluorobenzyl)-4H-imidazo [4,5-b]pyridin-2-yl)phenyl)(morpholino)methanone (**18b**)*

Prepared from **16** (0.11 g) as described in general method. Purification (Silicagel, CH_2Cl_2: MeOH 100 : 2) of crude product first afforded **18a** as oily semi-solid, yield 0.011 g (7.5%). **^{1}H-NMR** δ ppm (CD_3OD): 3.44, 3.65 and 3.77 (br.s,8H,morph.), 5.68 (s,2H,*N*–CH_2), 6.93–6.97 (m,2H,H-3″,5″), 7.00–7.04 (m,2H,H-2″,6″), 7.43 (dd,1H,*J* = 8 & 5.2 Hz,H-6), 7.58 (d, 2H,*J* = 8.4 Hz,H-3′,5′), 7.78 (d,2H,*J* = 8 Hz,H-2′,6′), 8.15 (dd,1H,*J* = 8 & 1.2 Hz,H-7), 8.45 (dd, 1H,*J* = 5.2 & 1.6 Hz,H-5). **COSY**: (H-5/H-6), (H-6/H-7), (H-2′,6′/H-3′,5′), (H-2″,6″/H-3″,5″). **NOESY**: (*N*–CH_2/H-2′,6′), (*N*–CH_2/H-2″,6″), (morph./H-3′,5′). **^{13}C-NMR** & **HSQC** & **HMBC** δ ppm (CD_3OD): 43.9 & 67.7(morph.), 47.0(*N*–CH_2), 116.6(d,*J* = 22 Hz,CH-3″,5″), 120.7(CH-6), 128.4(CH-7), 128.8(CH-3′,5′), 129.9(d,*J* = 8.4 Hz,CH-2″,6″), 130.8(CH-2′,6′), 132.2(C-1′), 134.0(d,*J* = 3.2 Hz,C-1″), 135.7(C-7a), 138.8(C-4′), 145.9(CH-5), 149.5(C-3a), 155.4(C-2),

163.6(d,J = 244 Hz,C-4″), 171.4(CO). **MS** (ESI+) m/z: 417(M + H, 100%), $C_{24}H_{21}FN_4O_2$.

Continued elution (CH_2Cl_2: MeOH 100 : 4) provided **18b** as a white solid, yield 0.074 g, (50.8%), mp 78–80 °C (bubling), HCl salt mp 260–265 °C. **^{1}H-NMR** δ ppm (CD_3OD): 3.5, 3.62 and 3.76 (br.s,8H,morph.), 5.47 (s,2H,N–CH_2), 7.05–7.096 (m,2H,H-3″,5″), 7.26 (dd,1H,J = 7.6 & 6.4 Hz,H-6), 7.56–7.59 (m,4H,H-2″,6″,3′,5′), 8.16 (d,1H,J = 6.4 Hz,H-5), 8.23 (dd,1H,J = 8 & 0.8 Hz,H-7), 8.46 (d,2H,J = 8 Hz,H-2′,6′). **COSY**: (H-5/H-6), (H-6/H-7), (H-2′,6'/H-3′,5′), (H-2″,6''/H-3″,5″). **NOESY**: (N–CH_2/H-5), (N–CH_2/H-2″,6″), (morph./H-3′,5′). **^{13}C-NMR** & **HSQC** & **HMBC** δ ppm (CD_3OD): 43.9 & 67.8(morph.), 57.0(N–CH_2), 115.5(CH-6), 116.8(d,J = 21.8 Hz,CH-3″,5″), 128.6(CH-3′,5′), 129.45(CH-2′,6′), 129.7(CH-7), 132.0(d,J = 8.4 Hz,CH-2″,6″), 132.6(d,J = 3.3 Hz,C-1″), 133.1(CH-5), 136.9(C-1′), 137.9(C-4′), 145.7(C-7a), 154.6(C-3a), 164.4(d,J = 245 Hz,C-4″), 168.7(C-2), 172.1(CO). **MS** (ESI+) m/z: 417(M + H, 100%). Anal. Calcd for $C_{24}H_{21}FN_4O_2 \cdot H_2O$: C, 66.34; H, 5.33; N, 12.89. Found: C, 66.72; H, 5.37; N, 13.21.

(4-(6-Chloro-3-(4-fluorobenzyl)-3H-imidazo [*4,5-b*]*pyridin-2* yl*) phenyl)(morpholino) methanone (**19a**)* and *(4-(6-Chloro-4-(4-fluorobenzyl)-4H-imidazo* [*4,5-b*]*pyridin-2*-yl*)phenyl) (morpholino) methanone (**19b**)*

Prepared from **17** (0.125 g) as described in general method. Purification (Silicagel, CH_2Cl_2: MeOH 100 : 2) first afforded compound **19a** as a white solid, yield 0.010 g, (6%), mp 132–135 °C. **^{1}H-NMR** δ ppm ($CDCl_3$): 3.42, 3.63 and 3.78 (br.s,8H,morph.), 5.53 (s,2H,N–CH_2), 6.95–6.99 (m,2H,H-3″,5″), 7.05–7.09 (m,2H,H-2″,6″), 7.52 (d,2H,J = 8.4 Hz,H-3′,5′), 7.7 (d,2H, J = 8.4 Hz,H-2′,6′), 8.09 (dd,1H,J = 2 Hz,H-7), 8.38 (d,1H,J = 2.4 Hz,H-5). **COSY**: (H-5/H-7, secondary), (H-2′,6'/H-3′,5′), (H-2″,6''/H-3″,5″). **NOESY**: (N–CH_2/H-2′,6′), (N–CH_2/H-2″,6″), (morph./H-3′,5′). **^{13}C-NMR** & **HSQC** & **HMBC** δ ppm ($CDCl_3$): 42.6, 48.1 and 66.8(morph.), 46.4(N–CH_2), 115.9(d,J = 21.8 Hz,CH-3″,5″), 127.0(C-6), 127.1(CH-7), 127.6 (CH-3′,5′), 128.35(d,J = 8.4 Hz,CH-2″,6″), 129.5(CH-2′,6′), 130.9(C-1′), 131.9(d,J = 3.2 Hz, C-1″), 135.4(C-7a), 137.5(C-4′), 143.5(CH-5), 147.1(C-3a), 155.0(C-2), 162.3(d,J = 246 Hz, C-4″), 169.3(CO). **MS** (ESI+) m/z: 451(M + H, 100%). Anal. Calcd for $C_{24}H_{20}ClFN_4O_2 \cdot H_2O$: C, 61.47; H, 4.72; N, 11.94. Found: C, 61.87; H, 4.69; N, 12.15.

Continued elution (CH_2Cl_2: MeOH 100 : 4) provided compound **19b** as a white solid (0.079 g, 48%), mp 118–122 °C. **^{1}H-NMR** δ ppm (CD_3OD): 3.5, 3.67 and 3.8 (br.s,8H,morph.), 5.77 (s,2H,N–CH_2), 7.03–7.08(2H,H-3″,5″), 7.45–7.49 (m,2H,H-2″,6″), 7.5 (d,2H,J = 8 Hz, H-3′,5′), 7.64 (d,1H,J = 1.6 Hz,H-5), 8.07 (d,1H,J = 1.6 Hz,H-7), 8.50 (d,2H,J = 8 Hz,H-2′,6′). **COSY**: (H-5/H-7 secondary), (H-2′,6'/H-3′,5′), (H-2″,6''/H-3″,5″). **NOESY**: (N–CH_2/H-5), (N–CH_2/H-2″,6″), (morph./H-3′,5′). **^{13}C-NMR** & **HSQC** & **HMBC** δ ppm (CD_3OD): 42.65, 48.15 & 66.8(morph.), 56.2(N–CH_2), 116.3(d,J = 22 Hz,CH-3″,5″), 120.5(C-6), 127.1 (CH-5), 127.4(CH-3′,5′), 127.8(CH-7), 128.6(CH-2′,6′), 129.6(d,J = 3.8 Hz,C-1″), 130.8(d, J = 8.4 Hz, CH-2″,6″), 135.3(C-1′), 136.8(C-4′), 146.0(C-7a), 153.6(C-3a), 163.2(d,J = 244 Hz, C-4″), 170.1(CO), 170.7(C-2). **MS** (ESI+) m/z: 451(M + H, 100%). Anal. Calcd for $C_{24}H_{20}ClFN_4O_2 \cdot H_2O$: C, 61.47; H, 4.72; N, 11.94. Found: C, 61.23; H, 4.86; N, 12.04.

*4.1.4.18. (4-(5-(4-Fluorobenzyl)-5H-imidazo [4,5-c]pyridin-2-yl)phenyl)(morpholino)methanone (**23**).* Prepared from **22** (0.11 g) as described in general method. The resulting precipitate was crystallised from EtOAc–MeOH as a white solid, yield 0.067 g (45%), mp 109–112 °C.

^{1}H-NMR δ ppm (CD_3OD): 3.51, 3.66 and 3.78 (m,8H,morp), 5.68 (s,2H,N–CH_2), 7.13–7.17 (m, 2H,H-3″,5″), 7.46–7.49 (m,2H,H-2″, 6″), 7.58 (d,2H,J = 8.4 Hz,H-3′,5′), 7.79 (d,1H,J = 7.2 Hz, H-7), 8.18 (dd,1H,J = 6.8 & 1.6 Hz,H-6), 8.39 (d,2H,J = 8.8 Hz,H-2′,6′), 8.93 (d,1H,J = 1.2 Hz,H-4). **COSY**: (H-6/H-7), (H-2′,6'/H-3′,5′), (H-2″,6''/H-3″,5″), (H-4/H-6 secondary). **NOESY**: (N–CH_2/H-4,6), (N–CH_2/H-2″,6″), (morph./H-3′,5′). **^{13}C-NMR** & **HSQC** & **HMBC** δ ppm (CD_3OD): 43.9 & 67.8(morph), 63.0(N–CH_2), 114.1(CH-7), 117.2(d,J = 21.8 Hz,CH-3″,5″), 128.7(CH-3′,5′), 129.5(CH-2′,6′), 131.6(d,J = 8.3 Hz,CH-2″,6″), 132.9(d,J = 3.2 Hz,C-1″), 133.2 (CH-4), 133.6(CH-6), 136.6(C-1′), 138.0(C-4′), 145.9(C-3a), 156.6(C-7a), 164.5 (d,J = 245 Hz, C-4″), 171.6 (C-2), 172.1(CO). **MS** (ESI+) m/z: 417(M + H, 100%). Anal. Calcd for $C_{24}H_{21}FN_4O_2 \cdot 3H_2O$: C, 61.27; H, 5.78; N, 11.90. Found: C, 61.35; H, 5.69; N, 12.07.

*4.1.4.19. (4-(3-(4-Fluorobenzyl)-3H-purin-8-yl)phenyl)(morpholino) methanone 26a & (4-(1-(4-fluorobenzyl)-1H-purin-8-yl)phenyl)(-morpholino)methanone **26b**.* Prepared from **25** (0.11 g) as described in general method. Purification (Silicagel, CH_2Cl_2: MeOH 100 : 3.5) first afforded compound **26a** as a oily semi-solid, yield 0.011 g, (7.3%), **^{1}H-NMR** δ ppm (CD_3OD): 3.5, 3.66 & 3.78 (br.s,8H,morph), 5.9 (s,2H,N–CH_2), 7.09–7.14 (m,2H, H-3″,5″), 7.59 (d,2H,J = 8.4 Hz,H-3′,5′), 7.66–7.69 (m,2H,H-2″,6″) 8.5 (d,2H, J = 8.4 Hz,H-2′,6′), 8.94 (s,1H,H-6), 8.99 (s,1H,H-2). **COSY**: (H-2′,6'/H-3′,5′), (H-2″,6''/H-3″,5″). **NOESY**: (N–CH_2/H-2), (N–CH_2/H-2″,6″), (morph./H-3′,5′). **^{13}C-NMR** & **HSQC** & **HMBC** δ ppm (CD_3OD): 43.9 and 67.8(morph.), 54.8(N–CH_2), 116.9(d, J = 21.8 Hz,CH-3″,5″), 128.7 (CH-3′,5′), 129.9(CH-2′,6′), 131.9(d,J = 3.2 Hz,C-1″), 132.2(d,J = 8.3 Hz,CH-2″,6″), 136.0 (C-1′), 138.75(C-4′), 143.15(C-2), 143.3(C-5), 144.8(C-6), 157.0(C-4), 164.3(d,J = 246 Hz, C-4″), 171.2(C-8), 172.0(CO). **MS** (ESI+) m/z: 418(M + H, 100%), $C_{23}H_{20}FN_5O_2$.

Continued elution (CH_2Cl_2: MeOH 100 : 5) provided compound **26b** as a white solid (0.059 g, 39%), mp 114–117 °C. **^{1}H-NMR** δ ppm (CD_3OD): 3.48, 3.46 and 3.76 (br.s,8H, morph), 5.68 (s,2H,N–CH_2), 7.14–7.19 (m,2H,H-3″,5″), 7.51–7.54 (m,2H,H-2″,6″), 7.57 (d,2H,J = 8.4 Hz,H-3′,5′), 8.41 (d,2H,J = 8.4 Hz,H-2′,6′), 8.95 (d,1H,J = 2 Hz,H-6), 9.04 (d,1H,J = 1.6 Hz,H-2) **COSY**: (H-2′,6'/H-3′,5′), (H-2″,6''/H-3″,5″) **NOESY**: (N–CH_2/H-2,6), (N–CH_2/H-2″,6″), (morph./H-3′,5′). **^{13}C-NMR** & **HSQC** & **HMBC** δ ppm (CD_3OD): 43.9 and 67.7(morph.) 60.1(N–CH_2), 117.3(d,J = 22.5 Hz,CH-3″,5″), 128.7(CH-3′,5′), 129.7(CH-2′,6′), 131.8(d,J = 8.3 Hz,CH-2″,6″), 132.2(d,J = 3.2 Hz,C-1″), 135.15(C-2), 135.8(C-1′), 137.7(C-5), 138.7(C-4′), 145.9(C-6), 164.6(d,J = 245 Hz,C-4″), 166.7(C-4), 171.8(CO), 173.8(C-8). **MS** (ESI+) m/z: 418(M + H, 100%). Anal. Calcd for $C_{23}H_{20}FN_5O_2 \cdot 3.25H_2O$: C, 58.04; H, 5.61; N, 14.71. Found: C, 58.07; H, 5.62; N, 14.60.

(4-(1-(4-Fluorobenzyl)-1H-imidazo [*4,5-b*]*pyrazin-2*-yl*)phenyl)(-morpholino)methanone* **(30a)** *&* *4-(4-(4-fluorobenzyl)-4H-imidazo* [*4,5-b*]*pyrazin-2*-yl*)phenyl)(morpholino)methanone* **(30b)**

Prepared from **29** (0.11 g) as described in general method. Purification (Silicagel, EtOAc: MeOH 100 : 4) first afforded compound **30a** as a white solid, yield 0.012 g, (8%), mp 138–142 °C. **^{1}H-NMR** δ ppm (CD_3OD): 3.44, 3.63 and 3.77 (m,8H,morph), 5.70 (s,2H,N–CH_2), 6.95–6.99 (m,2H,H-3″,5″), 7.06–7.09 (m,2H,H-2″,6″), 7.60 (d,2H,J = 8.4 Hz,H-3′,5′), 7.85 (d,2H,J = 8 Hz,H-2′,6′), 8.47 (d,1H,J = 2.8 Hz,H-5), 8.57 (d,1H,J = 2.8 Hz,H-6). **COSY**: (H-5/H-6), (H-2′,6'/H-3′,5′), (H-2″,6''/H-3″,5″). **NOESY**: (N–CH_2/H-2′,6′), (N–CH_2/H-2″,6″), (morph./H-3′,5′). **^{13}C-NMR** & **HSQC** & **HMBC** δ ppm (CD_3OD): 43.9 and 67.8(morph), 47.4(N–CH_2), 116.7(d,J = 21.8 Hz,CH-3″,5″), 128.8(CH-3′,5′), 130.1(d,J = 8.3 Hz,CH-2″,6″), 130.9 (CH-2′,6′), 131.7(C-1′), 133.4(d,J = 3.2 Hz,C-1″), 139.3(C-4′), 140.6(CH-5), 141.8(CH-6), 143.2(C-3a), 149.4(C-7a), 158.5(C-2), 163.8(d,J = 244 Hz,C-4″), 171.3(CO). **MS** (ESI+) m/z: 418(M + H, 100%). Anal. Calcd for $C_{23}H_{20}FN_5O_2 \cdot 2H_2O$: C, 60.91; H, 5.33; N, 15.44. Found: C, 61.00; H, 5.57; N, 15.30.

Continued elution (EtOAc: MeOH 100 : 5) provided compound **30b** as a white solid (0.07 g, 46.6%) mp 85–88 °C. **^{1}H-NMR** δ ppm (CD_3OD): 3.48, 3.64 and 3.76 (m,8H,morph), 5.91 (s,2H,N–CH_2),

7.08−7.13 (m,2H,H-3″,5″), 7.59 (d,2H,J = 8.4 Hz,H-3′,5′), 7.62−7.66 (m,2H, H-2″,6″), 8.09 (d,1H,J = 3.6 Hz,H-5), 8.35 (d,1H,J = 4 Hz,H-6), 8.54 (d,2H,J = 8.4 Hz,H-2′,6′). **COSY**: (H-5/H-6), (H-2′,6′/H-3′,5′), (H-2″,6″/H-3″,5″). **NOESY**: (*N*−CH_2/H-5), (*N*−CH_2/H-2″,6″), (morph./H-3′,5′). **^{13}C-NMR** & **HSQC** & **HMBC** δ ppm (CD_3OD): 43.9 & 67.8(morph), 57.3(*N*−CH_2), 117.0(d,J = 22 Hz,CH-3″,5″), 124.4(CH-5), 128.7(CH-3′,5′), 130.2(CH-2′,6′), 131.5(d,J = 3.2 Hz,C-1″), 132.5(d,J = 8.4 Hz,CH-2″,6″), 135.8(CH-6), 135.9 (C-1′), 139.3(C-4′), 145.6(C-3a), 161.7(C-7a), 164.6(d,J = 246 Hz,C-4″), 171.8(CO), 174.4 (C-2). **MS** (ESI+) *m/z*: 418(M + H, 100%). Anal. Calcd for $C_{23}H_{20}FN_5O_2 \cdot H_2O$: C, 63.43; H, 5.09; N, 16.08. Found: C, 63.10; H, 5.44; N, 15.86.

*4.1.4.20. (4-(1-(4-Fluorobenzyl)-1H-imidazo [4,5-b]pyridin-2-yl) phenyl)(morpholino)methanone (**18c**).* A mixture of **20** (0.044 g, 0.2 mmol) and $Na_2S_2O_5$ adduct of 4-(morpholin-4-ylcarbonyl) benzaldehyde (0.065 g, 0.2 mmol) in DMF (0.35 mL) was heated at 120 °C for 2.5 h. Water was added, the resulting precipitate was filtered, dried. Precipitate was purified with column chromatography using CH_2Cl_2: MeOH (100 : 3) as eluant, mp 212−215 °C, yield 0.051 g (60%). **^{1}H-NMR** δ ppm (CD_3OD): 3.45, 3.64 and 3.76 (br.s,8H,morph.), 5.62 (s,2H,*N*−CH_2), 6.99−7.07 (m,4H,H-3″,5″,2″,6″), 7.34 (dd,1H,J = 8 & 5.2 Hz,H-6), 7.60 (d,2H,J = 8 Hz,H-3′,5′), 7.83 (d,2H,J = 8 Hz,H-2′,6′), 7.91 (dd,1H,J = 8.4 & 1.6 Hz,H-7), 8.48 (dd,1H,J = 5.2 & 1.6 Hz,H-5). **COSY**: (H-5/H-6), (H-6/H-7), (H-2′,6′/H-3′,5′). **NOESY**: (*N*−CH_2/H-7), (*N*−CH_2/H-2′,6′), (*N*−CH_2/H-2″,6″), (morph./H-3′,5′). **^{13}C-NMR** & **HSQC** & **HMBC** δ ppm (CD_3OD): 43.9 & 67.7(morph), 49.5(*N*−CH_2), 116.9(d,J = 21.8 Hz,CH-3″,5″), 120.15(CH-6), 121.5(CH-7), 128.8(CH-3′,5′), 129.5(d,J = 8.3 Hz,CH-2″,6″), 130.0(C-7a), 131.0(CH-2′,6′), 131.9(C-1′), 133.2(d,J = 3.3 Hz,C-1″), 138.8(C-4′), 146.1(CH-5), 155.8(C-3a), 156.9(C-2), 163.8(d, J = 244 Hz,C-4″), 171.3(CO). **MS** (ESI+) *m/z*: 417(M + H, 100%). Anal. Calcd for $C_{24}H_{21}FN_4O_2 \cdot H_2O$: C, 66.34; H, 5.33; N, 12.9. Found: C, 66.72; H, 5.37; N, 13.21.

4.2. Biology

4.2.1. Cell culture

Human colon cancer cell line (HCT-116) and leukemia cell lines (K562 and HL-60) were cultured in RPMI 1640 medium with 2 mmol/L L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and inactive fetal bovine serum (10%) in 75 cm^2 flasks, incubated at 37 °C, in 5% CO_2 and passaged upon reaching 80% confluence.

4.2.2. Preparation of compounds

Compounds were dissolved in DMSO and further dilutions were made with culture medium. Final DMSO concentrations never exceeded 0.5%. Untreated cells which contain 0.5% DMSO were used as controls. Imatinib was used as a positive control in K562 cells whereas camptotechin was used in HL- 60 and HCT-116 cells.

4.2.3. Cell viability assay

To test the cytotoxic activities of compounds against HCT-116, HL-60 and K562 cells, MTT test was performed by T. Mosmann [35]. For this purpose, cells (2×10^4 cells/well) were seeded into 96 well plates. After 24 h incubation, cells were treated with compounds (0.01−100 μM) for 72 h. Maximum concentration of compounds applied to cells differed due to different solubility. After proper treatment of cells with compounds, cells were further incubated with MTT solution for 4 h before dissolving the insoluble formazan crystals with acidified SDS solution. Absorbance at 550 nm was measured using microplate reader (Thermo Scientific). Untreated cells were used as a control group and the IC_{50} was calculated using GraphPad Prism 7.0 software (GraphPad Software, USA). NE indicates that no IC_{50} value was determined in the concentration range in which the cytotoxic activities were screened.

4.3. Statistical analysis

One-way ANOVA variance test of StatistiXL program (Broadway−Nedlands, Western Australia) was used to determine statistical differences among groups. A p value of <0.05 was considered as significant and results were expressed as mean ± standard deviation.

CRediT authorship contribution statement

Cigdem Karaaslan: Investigation, Data curation, Writing - review & editing. **Fatima Doganc:** Investigation. **Mehmet Alp:** Investigation. **Asli Koc:** Investigation. **Arzu Zeynep Karabay:** Investigation. **Hakan Göker:** Conceptualization, Methodology, Supervision, Writing - review & editing.

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Appendix A. Supplementary data

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