

Research Article

Guanidine-Annellated Heterocycles: Design, Synthesis and Biological Evaluation of Novel 2-Aminopyridoimidazotriazines, Pyridoimidazopyrimidines, Pyridoimidazotriazoles and Pyridoimidazoles

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Abstract

Starting from 2,3-diaminopyridine, several novel 2-amino [2,3:4,5] imidazo [1,2-a] [1,3,5] triazines (2-6), 2- (1H-imidazo [4,5-b] pyridine-2-yl) imino)-2,3-dihydro-1H-imidazoles (7-11), pyrido [2,3,4,5] imidazo [1,2-a] pyrimidones (13-16), 9H [1,2,4] triazolo [4,3:1,2] imidazo [4,5-b] pyridines (20-21) and 1-(1H-imidazo [4,5-b] pyridine-2-yl) -3-methyl-1H-pyrazol-5-ol (22a) were synthesized in a very good yield. Compound 2-6, 7-11, 13-16, 20 and 21 were screened in vitro for their antibacterial and antifungal activity by the agar diffusion method and most of them showed a pronounced anti-inhibitory effect. Compound 22a showed pronounced analgesic activity by i.p. injection of 0.5-1.0 mg/kg in conscious rats in a hot plate testing procedure.

Keywords: Dihydroimidazoles; [1,3,5] -Triazines; Pyrimidine; Triazoles; Pyrazoles; Antimicrobial Activity; Analgesic Activity.

Introduction

In recent years, the mounting threat of bacterial resistance has heightened the urgency to discover and develop anti-infective agents with novel mechanism of action and enhanced activity profile [1]. Clinical use of potent drugs is limited in many cases due to their side effects. To overcome the clinical limitations, considerable research efforts have been directed to the discovery of high potency local antimicrobial agents with reduced or without systematic adverse effects.

Azole class of drugs, particularly fused imidazole occupy prominent place in medicinal chemistry because of their broad spectrum of pharmacological activities such as anti-inflammatory, analgesic, anticancer, antiulcer, antimicrobial, antiviral, pesticidal, cytotoxicity, and anti-arrhythmic activities [2-5]. Omeprazole, Mebendazole, and Albendazole are well known drugs in the market which contain fused imidazole as active core moiety.

1,3,5-Triazine derivatives which synthesized via heterocyclization of biguanides or their analogues using β -keto esters [6] such as Tretamines, Furazil, and Dioxadet have been known as anticancer drugs [7]. There has been increasing interest in the synthesis of heterocyclic compounds containing a 1,2,4-triazine ring because of their broad biological significance [7]. 1,2,4-Triazines are regarded as 6-aza analogues of pyrimidine bases, needless to say

that pyrimidines in general have a great biological importance [8].

Bioisosterism is a useful strategy for the lead optimization process and molecular modification for rational drug design [9]. The bioisostere concept is an over simplification of the role of scaffold's for activity, unless it plays a pivotal role for function or interaction such as for β -lactams in penicillins [9]. On the basis of these results and in continuation of our project directed towards the design and synthesis of biologically active isosteric heterocyclic lead compounds [10-13], we summarize that replacement of CH by N of the phenyl ring in the antibacterials [14] 3,4-dihydrobenzo[4,5] imidazo[1,2-a]triazine A and the dihydroimidazole derivatives B, could give their biological isosteres 2-6 and 7-11 respectively (Figure 1), with more potent antibacterial and antifungal activity. These were carried out by illustrating the old and well known isosterism pyridine/phenyl are having some similar geometry and electronic features [15]. Moreover, replacing the CH in the benzene ring of the analgesic derivative 5,6-dimethyl-2-(5-hydroxy-3-methyl-1-pyrazolyl)benzimidazole C. [16] could give compound 22a with more potent analgesic activity (Figure 1).

Imidazoles like metronidazole inhibit nucleic acid synthesis by disrupting DNA of microbial cells [17] and azoles like clotrimazole inhibit the synthesis of ergosterol (the main fungal sterol) [18]. In this work, our aim is also to confirm the mechanism by which our new pyridoimidazoles (2-6, 7-11) act as antibacterial and antifungal

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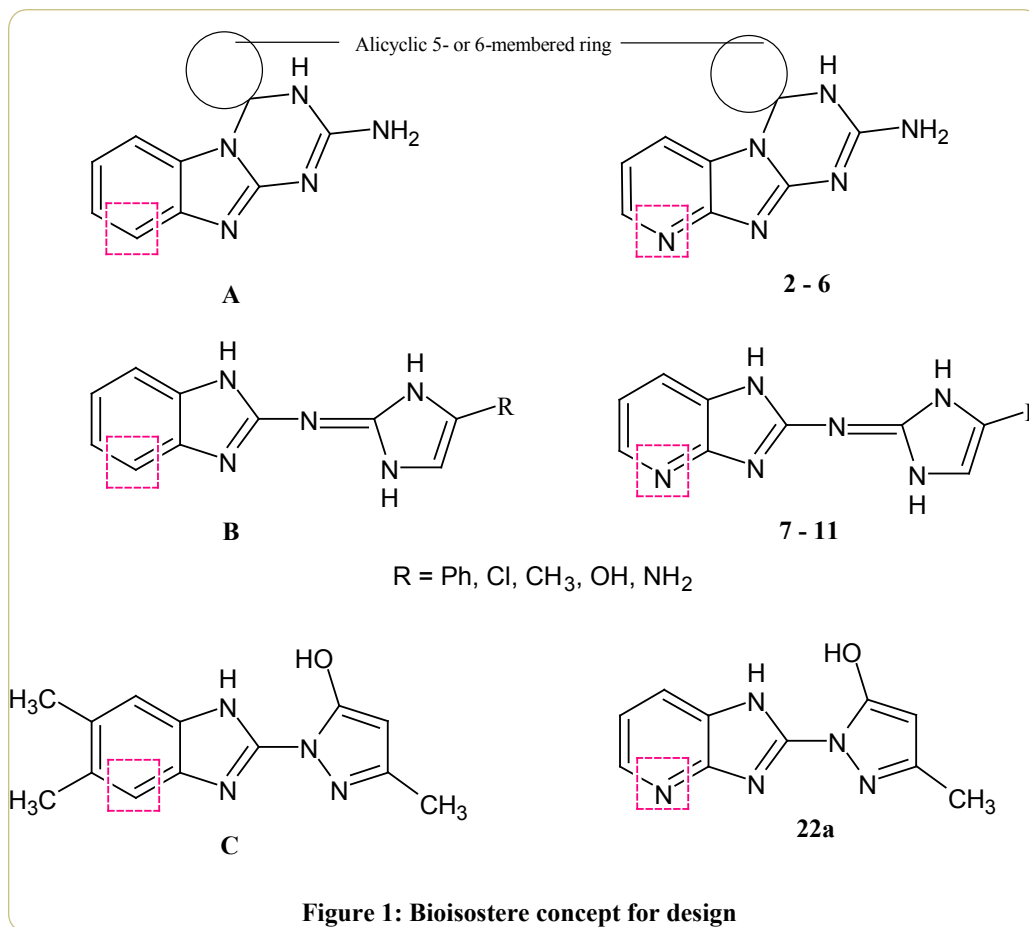
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agents. Moreover, fused benzimidazoles have attracted particular attention as new template for drug design and identification of novel anti-inflammatory and analgesic agents.

[1,3,5] triazines (2 - 6) respectively. The structures of these com



They have attracted particular attention owing to their diverse array of mechanism as anti-inflammatory and analgesics, by targeting of cyclooxygenase-2 (COX-2, prostaglandin inhibition), phosphodiesterase IV (PDE 4, cytokine inhibition) and tumor necrosis factor (TNF)-converting enzyme (TACE) as well as transient receptor potential vanilloid (TRPV1) antagonists [19]. In this work, our aim of interest is to synthesize pyridoimidazole containing pyrazole compound 22a in the hope to have a potent anti-inflammatory and analgesic activity and to expect its mechanism of action.

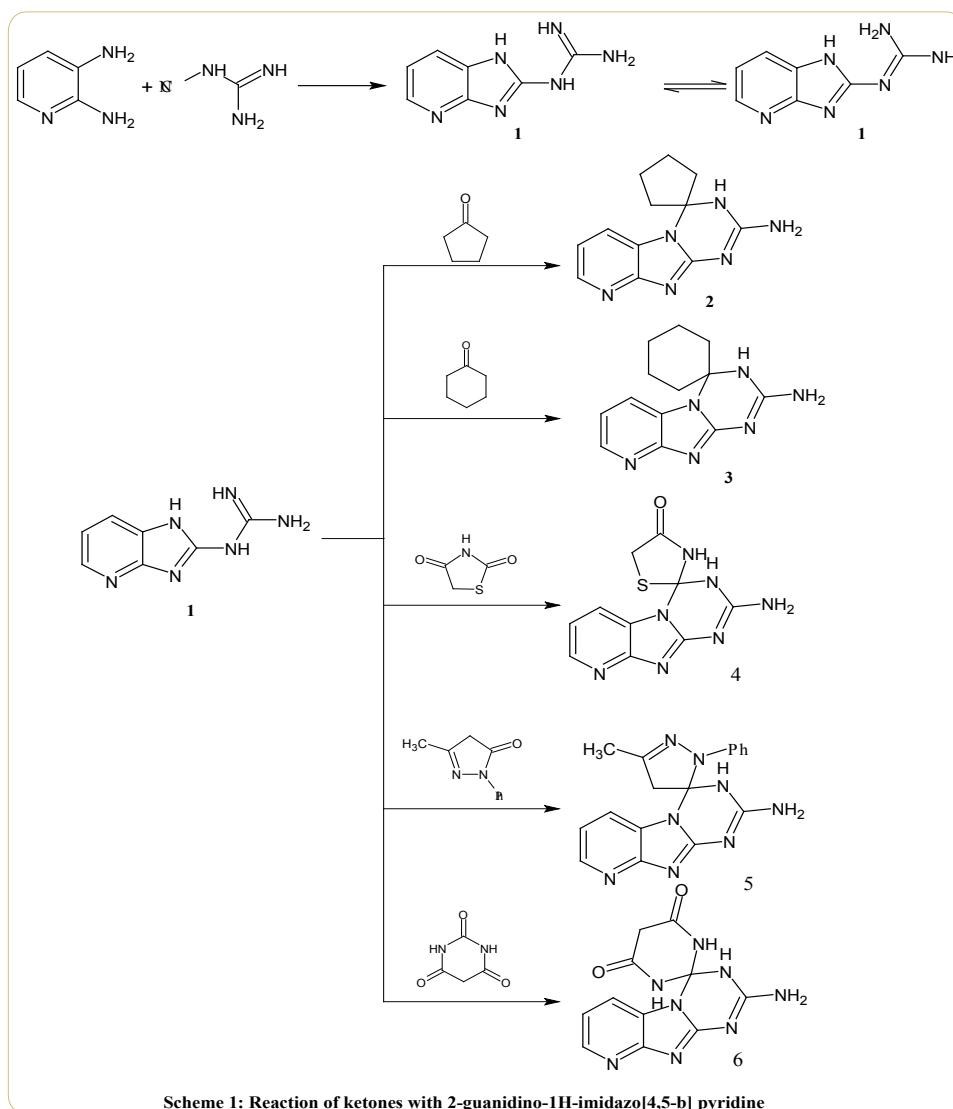
Results and discussion

Chemistry

Guanidino-1H-imidazo [4,5-b] pyridine 1 was prepared by the cyclocondensation of 2,3-diaminopyridine with cyanoguanidine adopting the method of Liu et al [20] (Scheme 1). Reaction of 1 with ketones namely cyclopentanone, cyclohexanone, thiazolidone, pyrazolone and barbituric acid in ethanol proceeded via (5+1) heterocyclization [14] and resulted in the formation of hitherto unknown 4-(het) aryl-2-aminopyrido [2,3:4,5] imidazo [1,2-a]

pounds are established on the basis of elemental analysis and spectral analysis. The IR spectra of the title compounds 2 - 6 are characterized by the presence of NH and NH₂ stretching bands at 3385, 3260, and 3180 cm⁻¹. In the ¹H-NMR spectra of 2 - 6, the sharp singlets corresponding to the protons of NH were found at δ 10.15 - 10.08 ppm and the broad NH₂ band at δ 4.80 - 4.61 ppm.

Reaction of compound 1 with halogenated active methylenes such as phenacyl bromide, chloroacetyl chloride, chloroacetone, ethyl bromoacetate and chloroacetonitrile in the presence of few drops of glacial acetic acid as a benign catalyst afforded the formation of pyrido-dihydroimidazole derivatives (7 - 11) respectively (Scheme 2). The reaction mechanism was proceeding via elimination of the corresponding halogen halide and elimination of either water molecules as in 7 - 9 or molecule of ethanol as in 10, while addition of cyano group resulted in ultimately the amino dihydroimidazole 11. IR spectra of 7 - 11 illustrated absorption peaks at a range between 3386 and 3185 cm⁻¹ corresponding to NH groups in dihydroimidazole ring and confirmed in ¹H-NMR spectra as appeared as singlet peak between 9.84 and 10.45 ppm while CH



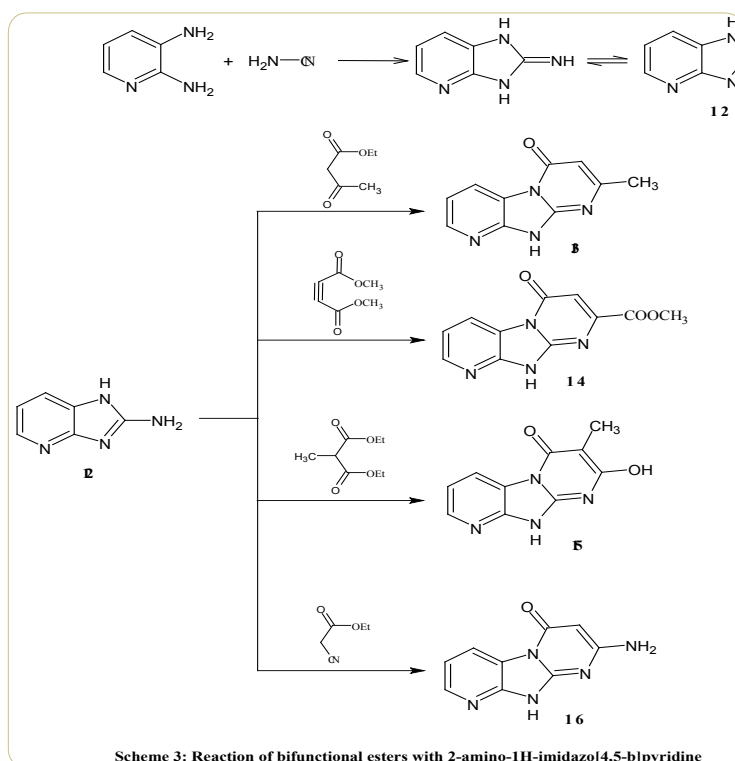
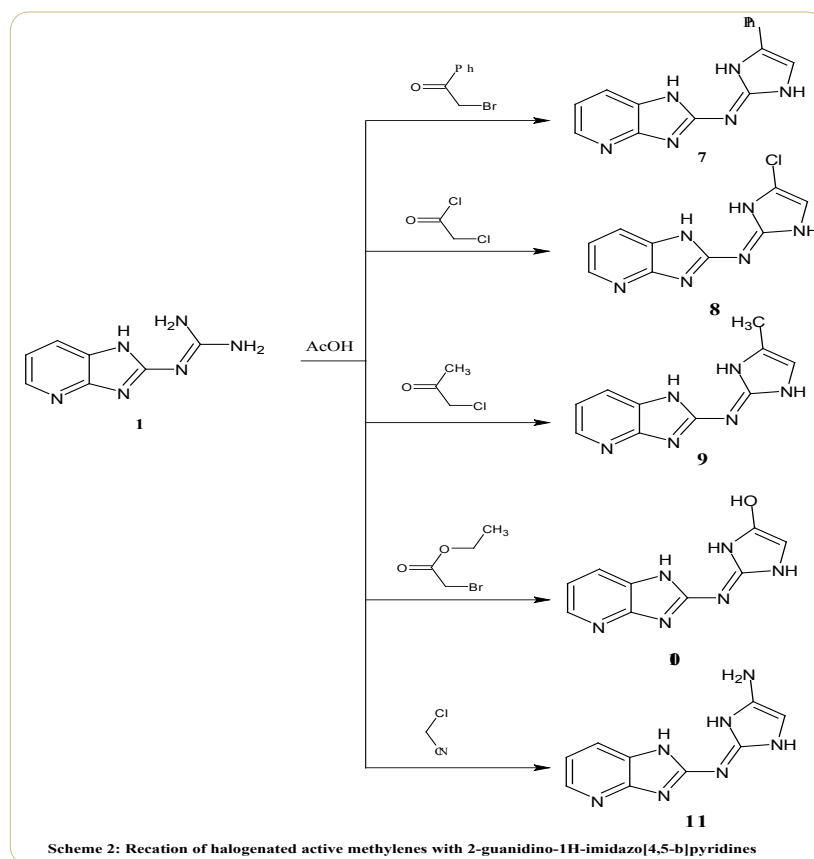
of dihydroimidazole ring was observed between δ 5.67 and 6.85 as singlet peak for compounds 9-11.

The starting material 2-amino-1H-imidazo[4,5-b]pyridine (12) was prepared in excellent yield by treating 2,3-diaminopyridine with cyanamide according to the method of Weiss et al [21]. As illustrated in the reaction Scheme 3, 12 might be produced in isomeric form. Since in both cases, the formation of constitutional isomers could arise from the subsequent condensation reaction. Compound 12 was used directly without further reaction analysis.

The reaction of compound 12 owing to the presence of two nucleophiles with the appropriate asymmetrical bifunctional esters might produce either 2-oxo or 4-oxo tricycles depending on the direction of annellation. We have carried out the reaction of 12 with a representative member of the appropriate esters namely acetoacetate, acetylenedicarboxylate, alkylmalonate and in

addition cyanoacetate either in glacial acetic acid or in ethanol in the presence of equivalent quantity of sodium ethoxide at refluxed temperature. In all cases, 4-oxo-tricyclic condensate was isolated as the unique product and on contrary to those instances mentioned [22-24], no alternative 2-oxo isomers were formed.

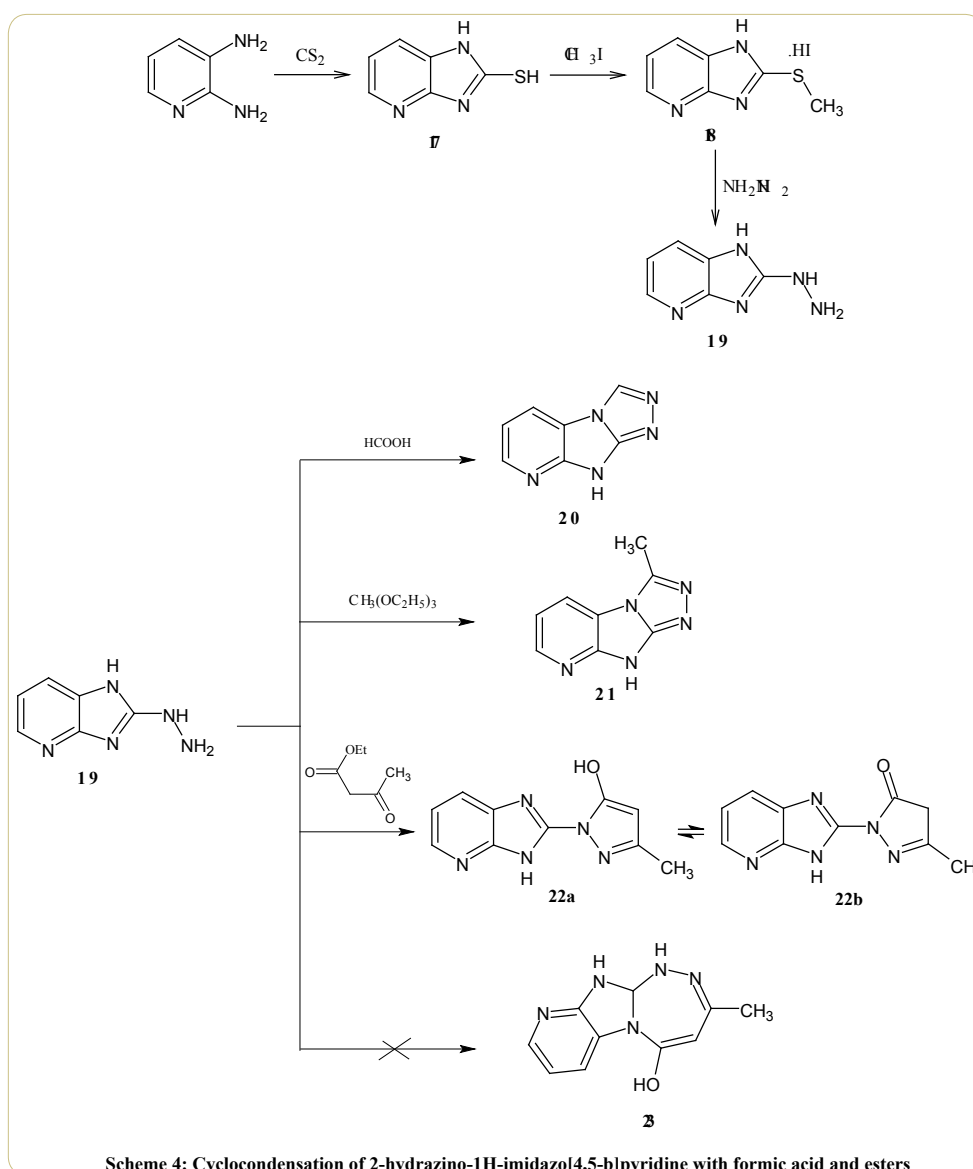
The structures of our products were determined from their IR and $^1\text{H-NMR}$ spectra. The IR spectra of these compounds are characterized by the presence of heterocyclic N-H band at $3480 - 3300 \text{ cm}^{-1}$ and a lactam C=O band at $1680 - 1660 \text{ cm}^{-1}$. In the $^1\text{H-NMR}$ spectra of compounds 13-16, a signal of one aromatic proton was shifted to lower field at about δ 8.12 – 8.29 ppm and indicated its position C-6 in proximity to the paramagnetic anisotropic 4-oxo function. Such an anisotropic effect of carbonyl group has generally been applied to differentiate between 2-oxo and 4-oxo structures of similarly annellated compounds [25].



As shown in Scheme 4, the synthetic experiments were carried out using 2,3-diaminopyridine as starting material which was first heated with carbon disulfide in alkaline solution to give 2-mercapto-1H-imidazo[4,5-b]pyridine (17) in a good yield. Adopting the reported procedure [26,27], methylation of this derivative with methyl iodide in ethanol provided 2-methylthiopyridoimidazole as hydroiodide (18). The hydroiodide salt was isolated in more satisfactory yield. Recation of 18 with hydrazine hydrate under reflux gave the corresponding 2-hydrazinyl-1H-imidazo[4,5-b]pyridine (19). Treatment of 19 with formic acid by direct heating and ethyl orthoacetate by refluxing in xylene afforded the corresponding cyclocondensation product 20 and 21, respectively, each in satisfactory yield. The structures of these derivatives are established on the basis of elemental analysis and spectral analysis. The IR spectra of the title compounds 20, 21 are characterized by the presence of N-H, =C-H stretching, C=N / C=C skeleton as well as

the =C-H deformation bands at 3240 cm^{-1} , 3120, 3100 – 3010, 1690 – 1460 and 870 – 730 cm^{-1} respectively. In the $^1\text{H-NMR}$ spectra of 21, the sharp singlets corresponding to the protons of methyl group were found at 2.32 ppm, broad singlet due to N-H were observed at 3.44 – 4.02 ppm. The mass spectra of compound 21 showed the intensive molecular ion peaks due to the presence of aromatic or heteroaromatic nuclei. The subsequent fragmentation followed also the general rule's .e.g. via β -cleavage of carbon heteroatom bond to give the rational prominent peaks. And above all good evidences were displayed by the consistent elemental analytical data of the isolated products.

The synthesis of the title compound 22 was accomplished by heating the hydrazine derivative 19 with excess amount of ethyl acetoacetate in ethanol at 65°C for 2 hours. It afforded the light yellow fine crystalline product in 82% yield after recrystallization



Scheme 4: Cyclocondensation of 2-hydrazino-1H-imidazo[4,5-b]pyridine with formic acid and esters

from methanol. As we have reported in similar report [28], the hydrazine derivatives on treating with the β -keto ester might be affected to form a hydrazine intermediate and then underwent an acylative cyclization to give either the pyrazole derivative 22 or the fused triazipinio derivative 23. The pure product gave agreeable elemental analytical data for both 22 and 23. To assure the integrity of the structure, spectral analysis was further applied. The IR spectrum exhibited an N-H stretching band at 3280 cm^{-1} , a broad shallow enolic O-H stretching band at 2620 cm^{-1} along with a carbonyl absorption at 1675 cm^{-1} . In $^1\text{H-NMR}$, a sharp singlet due to an olefinic proton was observed at $\delta\ 5.22\text{ ppm}$ following the expected signals of the methyl protons at $\delta\ 2.20\text{ ppm}$. A broad shallow singlet of the hydroxyl proton, which was exchangeable with deuterium oxide appeared at $\delta\ 8.86\text{ ppm}$, very closely to the multiplet of three pyridine residue protons at $\delta\ 8.05\text{ ppm}$. These data indicate that this product might exist in equilibrium between the keto and enol form and the later is more favorable over the former. This is reasonably attributed to the formation of an intramolecular hydrogen bonding [29] between the enolic hydroxyl and the ring nitrogen, and this phenomenon would not occur in structure 23. The mass fragmentation of the product provided further evidence

to support this point. The base molecular ion peak was observed at $m/e\ 215$ as expected and it then followed the general cleavage pattern by successive breakdown of the pyrazole ring to give the fragment ion peaks at $m/e\ 200\ (M^+ - \text{CH}_3)$, $174\ (M^+ - \text{CN})$, $132\ (M^+ - \text{C}_2\text{H}_2\text{O})$, respectively. Notably, no trace of methyltriazepinone ($m/e\ 123$) or its cleaved fragment, methylpyridazinone ($m/e\ 109$) could be visualized [30]. Based on these spectral findings, it is reasonable to assign the structure of the product a 1-(1H-imidazo[4,5-b]pyridine-2-yl)-3-methyl-1H-pyrazol-5-ol (22a), which furthermore showed the tendency to tautomerize to the 2-(1H-imidazo[4,5-b]pyridine-2-yl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one, 22b, as shown in the reaction Scheme 4.

Biological studies

Antimicrobial activity

The compounds were dissolved in DMSO. In order to ensure that the solvent had no effect on bacterial growth or enzymatic activity, negative control tests were performed using DMSO at the same concentration. The antimicrobial activity was carried out using agar diffusion method [31] against two bacterial strains,

Compound No.	Antibacterial activity		Antifungal activity	
	Gram + Bacillus subtilis	Gram - Escherichia coli	Aspergillusfumigatus	Candida albicans
2	+++	++	++	++
3	+++	+++	++	++
4	+++	++	++	++
5	+++	+++	++	++
6	+++	++	++	++
7	++	+	+	+
8	++	+	++	++
9	++	+	+	+
10	++	+	++	++
11	++	++	++	++
13	+++	++	+++	++
14	+++	++	++	++
15	+++	++	++	++
16	+++	+++	+++	++
20	++	++	++	++
21	++	++	++	++
Ampicillin	+++	+++	-	-
Clotrimazole	-	+++	+++	+++
DMSO	-	-	-	-

Table 1 Antimicrobial activity data of title compounds*

* The test done using the diffusion agar technique; well diameter = 0.06 cm; inhibition values = 0.1-0.5 cm beyond control = + (less active inhibition); inhibition values = 0.6-1.0 cm beyond control = ++ (moderate active); inhibition values 1.1 -1.5 cm beyond control = +++ (highly active); solvent control; DMSO for antibacterial; ampicillin for antibacterial; clotrimazole for antifungal.

namely *B. Subtilis* and *E. coli* and two fungal strains, namely, *A. fumigates* and *C. albicans*. The results of antimicrobial activity is summarized in Table 1. From the data it is clear that most of the synthesized compounds 2 – 11 & 13 – 16 & 20-21 were found to possess antimicrobial activities towards all microorganisms tested. In general, most of the synthesized compounds showed a greater inhibitory effect against both the bacterial and fungal strains. However, compounds 2-6 & 13-16 showed maximum antibacterial activity comparable to the standard drugs. We can conclude from the preliminary antibacterial screening that the compounds have enhanced antimicrobial properties due to the presence of bioactive moieties as 1,2,4-triazol, 1,3,5-triazine, pyrimidine and imidazole [32]. Moreover, the presence of pyridine moiety in all the synthesized compounds enhance their antimicrobial activity in comparison to their reported [14] bioisoteres A and B.

These better antibacterial and antifungal activities may be due to the presence of many functional groups in our new synthesized fused pyridoimidazoles which serve in bonding with organism cell membrane molecule and hence increases bacterial and fungal inhibition. These data are in accordance to the reported imidazoles with many functional groups [33].

* The test done using the diffusion agar technique; well diameter = 0.06 cm; inhibition values = 0.1-0.5 cm beyond control = + (less active inhibition); inhibition values = 0.6-1.0 cm beyond control = ++ (moderate active); inhibition values 1.1 -1.5 cm beyond control = +++ (highly active); solvent control; DMSO for antibacterial; ampicillin for antibacterial; clotrimazole for antifungal.

Analgesic activity

Compound 22a was evaluated for its analgesic activity on conscious Sprague – Dawley rats using the hot plate test procedure [26] at the dose levels of intraperitoneal injection of 0.5-1.0 mg/kg. It produced pronounced analgesic activity, which continued more than two hours as shown in Table 2. These results are in accordance to the reported data [19] in which hot plate induces pain sensation through prostaglandin (PG) biosynthesis and hence the writhing response is associated with increased level of PGE₂ and PEG_{2a}. We can summarize that the mechanism of action of compound 22a

is by targeting the cyclooxygenase-2 (prostaglandin inhibition). The further pharmacological and toxicological studies are still in progress and the results will be described subsequently.

Conclusion

It can be concluded that the replacement of CH by NH in the reported [14] antibacterial compound A and B has enhanced the biological activity and hence they are ideally suited for further modification to obtain more potent antimicrobial and antifungal compounds. Also compound 22a showed more potent analgesic activity than its bioisostere [16]. Hence compound 22a could be developed as lead compound in novel class of analgesics.

Experimental

Chemistry

All melting points are uncorrected and were recorded on Melt-Temp II melting point apparatus. IR spectra in cm⁻¹ were measured as KBr pellets on Shimadzu DR-8001 spectrophotometer. ¹H-NMR spectra were recorded on a Varian Gemini at 280 MHz using TMS as an internal reference and DMSO-d₆ as a solvent. Mass spectra were performed on a Shimadzu GCMS-QP1000 mass spectrometer at 70 eV. The elemental analysis were carried out on a Perkin Elmer 240 C microanalyser. All compounds were checked for their purity on TLC plates.

2-Guanidino-1H-imidazo[4,5-b]pyridine (1)

A solution of (0.55 moles) of cyanoguanidine in 50 ml of water was added drop wise during 20 minutes to a boiling solution of (0.50 moles) of 2,3-diaminopyridine in 50 ml of concentrated hydrochloric acid and the mixture was kept at 100°C for 1 hour. Then 45 ml of 50% sodium hydroxide was added and the whole was heated under reflux for 2 hours. The precipitate was isolated, washed free from chloride with water and dried in vacuum. It gave brown levlt of (1) in 80% yield. M.P. 253 – 255 °C; IR (KBr): 3335 (N-H); ¹H-NMR (DMSO-d₆), δ ppm: 8-8.11 (m, 3H, pyridine residue), 8.27 (4H, broad singlet, NNHC(=NH)NH₂), 11.53 (s, 1H, NH).

Compd. No.	Dose ^a (i.p.; mg/kg)	10 min.	25 min.	40 min.	55 min.	100 min.	130 min.
		22a	0.5	9.2±0.08	11.8±0.34*	14.2±0.16*	11.7±0.38*
	1.0	12.3±0.22*	20.4±0.17*	19.4±0.27*	17.6±0.28*	18.8±0.16*	17.4±0.04*
Control	b	6.6±0.05	7.7±0.38	7.8±0.24	7.6±0.47	5.2±0.06	4.7±0.021

Table 2 Analgesic Activity of 1-(1H-imidazo[4,5-b]pyridin-2-yl)-3-methyl-1H-pyrazol-5-ol (22a) on rats by hot plate test (55oC)

aCompound was administered in a mixture of N,N-dimethylacetamide and polyethylene glycol 200 (1:1) at a concentration of 5 mg/ml.

b = Animals in control group received the vehicle only

*p values less than 0.05 were found in comparison to control group by student's t-test.

General Procedure for compounds 2-6

A mixture of compound 1 (0.55 moles) and (0.55 moles) of cyclopentanone, cyclohexanone, thiazolidinone, pyrazolone and barbituric acid in ethanol and presence of a few drops of hydrochloric acid as a catalyst was refluxed, solid products were observed after 10 hours. After cooling, the solid crystalline products were filtered, washed and recrystallized from ethanol.

3H-Spiro[cyclopentane-1,4-pyrido[2,3:4,5]imidazo[1,2-a][1,3,5]triazin]-2-amine (2)

Yield 81%; M.P. 312-314 °C; IR (KBr): 3380, 3270, 3178 (NH₂, NH); ¹H-NMR (DMSO-d₆), δ (ppm): 1.93 (s, 4H, 2CH₂), 2.10 (s, 4H, 2CH₂), 4.77 (br, 2H, NH₂), 8.05-8.12 (m, 3H, pyridine residue), 9.91 (s, 1H, NH); Elemental analysis for C₁₂H₁₄N₆: Calcd. C, 59.49; H, 5.82; N, 34.69; Found: C, 59.51; H, 5.91; N, 34.52.

3H-Spiro[cyclohexane-1,4-pyrido[2,3:4,5]imidazo[1,2-a][1,3,5-triazin]-2-amine (3)

Yield 80%; M.P. 270-273 °C; IR (KBr): 3385, 3270, 3120 (NH₂, NH); ¹H-NMR (DMSO-d₆), δ (ppm): 1.80-1.51 (m, 10H, 5CH₂), 4.85 (br, 2H, NH₂), 8.01-8.17 (m, 3H, pyridine residue), 10.07 (s, 1H, NH); Elemental analysis for C₁₃H₁₆N₆ (256.31): Calcd. C, 60.92; H, 6.29; N, 32.79; Found: C, 60.55; H, 6.01; N, 32.81.

2-Amino-3H-spiro[pyrido[2,3:4,5]imidazo[1,2-a][1,3,5]triazine-4,2-thiazolidin]-4-one (4)

Yield 81%; M.P. 278-280 °C; IR (KBr): 3382, 3265, 3180 (NH₂, 2NH), 1690 (C=O); ¹H-NMR (DMSO-d₆), δ (ppm): 3.98 (s, 2H, CH₂), 4.85 (br, 2H, NH₂), 6.90 (s, 1H, NH), 8.05-8.15 (m, 3H, pyridine residue), 10.20 (s, 1H, NH); Elemental analysis for C₁₀H₉N₇OS (275.06): Calcd. C, 43.63; H, 3.30; N, 35.62; Found: C, 43.51; H, 3.45; N, 35.77.

4-Methyl-1-phenyl-1,3-dihydro-3H-spiro[pyrido[2,3:4,5]imidazo[1,2-a][1,3,5-triazine-4,2-pyrrol]-2-amine (5)

Yield 79%; M.P. 282-284 °C; IR (KBr): 3385, 3275, 3180 (NH₂, NH); ¹H-NMR (DMSO-d₆), δ (ppm): 2.28 (s, 6H, 2CH₃), 3.21 (s, 2H, CH₂), 4.65 (br, 2H, NH₂), 8.06-8.15 (m, 3H, pyridine residue), 10.1 (s, 1H, NH); Elemental analysis for C₁₈H₁₇N₇: Calcd. C, 65.24; H, 5.17; N, 29.59; Found: C, 65.11; H, 5.22; N, 29.61.

2-Amino-1H,3H-spiro[pyrido[2,3:4,5]imidazo[1,2-a][1,3,5]triazine-4,2-pyrimidine]-4,6-(3H,5H)-dione (6)

Yield 81%; M.P. 299-301 °C; IR (KBr): 3390, 3270, 3180 (NH₂, 3NH), 1690 (C=O), 1675 (C=O); ¹H-NMR (DMSO-d₆), δ (ppm): 3.26 (s, 2H, CH₂), 5.31 (br, 2H, NH₂), 8.05-8.10 (m, 3H, pyridine residue), 9.81 (s, 2H, 2NH), 10.33 (s, 1H, NH); Elemental analysis for C₁₁H₁₀N₈O₂ (286.26): Calcd. C, 46.16; H, 3.52; N, 39.15; Found: C, 46.01; H, 3.11; N, 39.44.

General Procedure (7-11)

A mixture of compound 1 (0.05 moles) and 10 ml of halogenated active methylenes such as phenacyl bromide, chloroacetyl chloride, chloro acetone, ethyl bromoacetate, and chloroacetonitrile in addition to few drops of catalytic glacial acetic acid was refluxed. The solid product was observed after reflux for further 4 to 10 hours. After cooling, the solid formed was collected by filtration, washed with cold ethanol and recrystallized from ethanol.

(Z)-N-(1H-Imidazo[4,5-b]pyridin-2-yl)-4-phenyl-1,3-dihydro-2H-imidazol-2-imine (7)

Yield 80%; M.P. 146-148 °C; IR (KBr): 3320, 3180 (3NH); ¹H-NMR (DMSO-d₆), δ (ppm): 6.15 (s, 1H, NH), 6.76 (s, 1H, CH), 7.71-7.21 (m, 5H, Ar-H), 7.88-7.79 (broad, 1H, NH), 9.76 (s, 1H, NH); Elemental analysis for C₁₅H₁₂N₆ (276.30): Calcd. C, 65.21; H, 4.38; N, 30.42; Found: C, 65.11; H, 4.21; N, 30.55.

(Z)-4-Chloro-N-(1H-imidazo[4,5-b]pyridin-2-yl)-1,3-dihydro-2H-imidazol-2-imine (8)

Yield 81%; M.P. 275-278 °C; IR (KBr): 3380, 3215 (3NH); ¹H-NMR (DMSO-d₆), δ (ppm): 6.18 (s, 1H, NH), 6.75 (s, 1H, CH), 7.49-7.55 (s, 1H, broad NH), 9.85 (s, 1H, NH); Elemental analysis for C₉H₇ClN₆ (234.65): Calcd. C, 46.07; H, 3.01; N, 35.82; Found: C, 46.11; H, 3.17; N, 35.55.

(Z)-N-(1H-Imidazo[4,5-b]pyridin-2-yl)-4-methyl-1,3-dihydro-2H-imidazol-2-imine (9)

Yield 79%; M.P. 301-303 °C; IR (KBr): 3340, 3195 (3NH); ¹H-NMR (DMSO-d₆), δ (ppm): 2.52 (s, 3H, CH₃), 6.21 (s, 1H, NH), 6.78 (s, 1H, CH), 7.55-7.41 (br, 1H, NH), 8.05-8.11 (m, 3H, pyridine residue), 10.23 (s, 1H, NH); Elemental analysis for C₁₀H₁₀N₆ (214.23): Calcd. C, 56.07; H, 4.71; N, 39.23; Found: C, 56.11; H, 4.88; N, 39.55.

(Z)-2-(1H-Imidazo[4,5-b]pyridin-2-yl)imino-2,3-dihydro-1H-imidazol-4-ol (10)

Yield 78%; M.P. 288-290 °C; IR (KBr): 3460 (O-H), 3290, 3180 (2N-H); ¹H-NMR (DMSO-d₆), δ (ppm): 6.20 (s, 1H, NH), 6.67 (s, 1H, CH), 7.51-7.48 (br, 1H, NH), 10.11 (s, 1H, NH), 11.4 (s, 1H, OH); Elemental analysis for C₉H₈N₆O (216.20): Calcd. C, 50.00; H, 3.73; N, 38.87; Found: C, 50.11; H, 3.78; N, 38.91.

(Z)-2-(1H-Imidazo[4,5-b]pyridin-2-yl)imino-2,3-dihydro-1H-imidazol-4-amine (11)

Yield 81%; M.P. 310-312 °C; IR (KBr): 3388, 3241, 3185 (3NH, NH₂); ¹H-NMR (DMSO-d₆), δ (ppm): 4.81 (s, 2H, NH₂), 5.75 (s, 1H, CH), 6.21 (s, 1H, NH), 7.77-7.51 (br, 1H, NH), 8.05-8.17 (m,

3H, pyridine residue), 9.85 (s, 1H, NH); Elemental analysis for $C_9H_9N_7$ (215.22): Calcd. C, 50.23; H, 4.22; N, 45.56; Found: C, 50.57; H, 4.33; N, 45.77.

2-Amino-1H-imidazo[4,5-b]pyridine (12)

A solution of (0.55 moles) of cyanamide in 50 ml of water was added dropwise during 20 minutes to a boiling solution of (0.5 moles) of 2,3-diairpyridine in 50 ml of concentrated hydrochloric acid and the mixture was kept at 100°C for 1 hour. Then 45 ml of 50% sodium hydroxide was added and the whole was heated under reflux for 2 hours. The precipitate was isolated, washed free from chloride with water and dried in vacuum. It gave pale brown leaflets of 12 in 90% yield; M.P. 241-243 °C. IR(KBr): 3300 (N-H), 3030 (=C-H), 1630 and 1550 (C=C, C=N ring).

8-Methylpyrido[2,3:4,5]imidazo[1,2-a]pyrimidin-6(10H)-one (13)

A mixture of 12 (0.04 moles) and wthylacetoacetate (0.04 moles) in 30 ml of glacial acetic acid was refluxed for 30 minutes and after cooling poured onto 200 ml of warm water. The brown precipitate was collected on a filter and washed with ethanol to give 13 in 45% yield. M.P. 290-292 °C IR (KBr): 3400 (N-H), 3020 (=C-H), 1670 (C=O), 1640, 1520 (C=C, C=N ring); ¹H-NMR (DMSO-d₆), δ (ppm): 2.72 (s, 3H, CH₃), 3.55 (s, NH, broad), 6.21 (s, 1H, CH=C), 8.05-8.15 (m, 3H, pyridine residue); Elemental analysis for $C_{10}H_8N_4O$ (200.20): Calcd. C, 59.99; H, 4.03; N, 27.99; Found: C, 59.55; H, 4.00; N, 27.71.

Methyl-6-oxo-6,10-dihydropyrido[2,3:4,5]imidazo[1,2-a]pyrimidine-8-carboxylate (14)

A mixture of (0.04 moles) of 12 and (0.04 moles) dimethylacetylenedicarboxylate in 40 ml of glacial acetic acid was refluxed for 4 hours. After cooling, the precipitate was collected on a filter and washed with ethanol to give 14 in 58% yield. M.P. 281-283 °C; IR (KBr): 3480 (N-H), 3070 (=C-H), 1740 (C=O ester), 1670 (C=O); ¹H-NMR (DMSO-d₆), δ (ppm): 3.1 (s, 3H, CH₃), 3.56 (s, NH, broad), 6.23 (s, 1H, CH=C), 8.05-8.12 (m, 3H, pyridine residue); Elemental analysis for $C_{11}H_8N_4O_3$ (244.21): Calcd. C, 54.10; H, 3.30; N, 22.94; Found: C, 54.11; H, 3.17; N, 23.14.

8-Hydroxy-7-methylpyrido[2,3:4,5]imidazo[1,2-a]pyrimidine-6(10H)-one (15)

A solution of (0.03 moles) of 12, (0.03 moles) of methyl diethylmalonate and (0.03 moles) of sodium ethoxide in 30 ml of absolute ethanol was refluxed for 4 hours. Then ethanol was evaporated under reduced pressure, and the residue was dissolved in 120 ml of warm water and acidified to pH 2 with 6N HCl. The precipitate was collected on a filter, washed with water and dried at 60°C in vacuum to give 15 in 60% yield. M.P. 298-300 °C; IR (KBr): 3400 (N-H), 3040 (=C-H), 1660 (C=O); ¹H-NMR (DMSO-d₆), δ

(ppm): 2.75 (s, 3H, CH₃), 3.55 (s, 1H, N-H, broad), 8.05-8.13 (m, 3H, pyridine residue); Elemental analysis for $C_{10}H_8N_4O_2$ (216.20): Calcd. C, 55.56; H, 3.73; N, 25.91; Found: C, 55.31; H, 3.55; N, 25.88.

8-Aminopyrido[2,3:4,5]imidazo[1,2-a]pyrimidine-6(10H)-one (16)

A solution of (0.04 moles) of 12, (0.04 moles) of ethyl cyanoacetate and (0.04 moles) of sodium ethoxide in 40 ml of absolute wthanol was refluxed for one hour. Then ethanol was evaporated under reduced pressure and the solid residue was dissolved in 200 ml of warm water and neutralized with dilute acetic acid. The precipitate was collected, washed with water and dried at 60°C in vacuum to give 16 in 52% yield. M.P. 191-193 °C; IR (KBr): 3300 (N-H), 3010 (=C-H), 1670 (C=O), 1620, 1580 (C=C, C=N ring); ¹H-NMR (DMSO-d₆), δ (ppm): 3.1 (s, 2H, NH₂), 3.55 (s, 1H, N-H, broad), 6.1 (s, 1H, CH=C), 8.05-8.13 (m, 3H, pyridine residue); Elemental analysis for $C_9H_7N_5O$ (201.19): Calcd. C, 53.73; H, 3.51; N, 34.81; Found: C, 53.55; H, 3.14; N, 35.15.

2-Mercapto-1H-imidazo[4,5-b]pyridine (17)

It was prepared according to the reported procedures [23, 24].

2-(Methylthio)-1H-imidazo[4,5-b]pyridine hydroiodide (18)

A solution of (0.1 mole) of 17 in 100 ml of methanol was added (0.1 mole) of methyl iodide under stirring. The reaction mixture was heated under reflux for 2 hours and then concentrated and allowed to stand at 4°C overnight, the solid product was separated, collected, washed with methanol and recrystallized from methanol. White needle crystals in 80% yield were obtained. M.P. 200 °C; R_f (silica gel G, benzene:acetone (9:1)) = 0.40; IR (KBr): 3220 (N-H), 1620 (C=N), MS (70 eV), m/e: 165 (M⁺-HI, 100), 128 (1HI, 79), 127 (I, 48), 118 (165-SCH₃), 35; Elemental analysis for $C_7H_8IN_3S$ (293.13): Calcd. C, 28.68; H, 2.75; N, 14.34; Found: C, 28.51; H, 3.11; N, 14.45.

2-Hydrazinyl-1H-imidazo[4,5-b]pyridine (19)

A solution of (0.05 moles) of 18 in (2.0 moles) of hydrazine was heated under reflux for 12 hours. After cooling to room temperature, the solid product separated was collected. A second crop was obtained from the mother liquid after concentration under reduced pressure. The combined crude product was recrystallized from a mixture of benzene and ethanol to give 18 as crystalline powder in 80% yield. M.P. 270-272 °C; R_f (silica gel G, benzene:acetone (9:1)) = 0.63; IR (KBr): 3320, 3280, 3220 (N-H), 1660 (C=N); MS (70 eV), m/e: 149 (M⁺, 59), 133 (M⁺-NH₂, 53), 118 (M⁺-N₂H₃, 44); Elemental analysis for $C_6H_7N_5$ (149.16): Calcd. C, 48.32; H, 4.73; N, 46.95; Found: C, 48.50; H, 4.81; N, 46.75.

9H-(1,2,4)Triazolo[4,3:1,2]imidazo[4,5-b]pyridine (20)

A solution of (0.01 mole) of 19 and 15 ml (0.4 moles) of formic

acid was heated under reflux for 5 hours. Then formic acid was distilled off under reduced pressure. The solid product obtained was collected and recrystallized from ethanol to give a crystalline white crystals of 20 in 73% yield. M.P. 238-240 °C; R_f (silica gel G, benzene:acetone (9:1)) = 0.64; IR (KBr): 3120 (N-H), 1690 (C=N/C=C); MS (70 eV), m/z: 159.15 (M^+), 118 (159- CHN_2), 100 (1H -NMR (DMSO- d_6), δ (ppm): 8.05-8.15 (m, 3H, pyridine residue), 8.30 (s, H-3), 8.59 (s, NH); Elemental analysis for $C_7H_5N_5$ (159.15): Calcd. C, 52.83; H, 3.17; N, 44.01; Found: C, 52.75; H, 3.51; N, 44.16.

3-Methyl-9H-(1,24)triazolo[4,3:1,2]imidazo[4,5-b]pyridine (21)

A solution of (0.01 mole) of 19 and 3.7 ml (0.02 moles) of ethyl orthoacetate in 20 ml of xylene was heated for 4 hours whilst ethanol was distilled off through a fractionating column. The reaction mixture was then allowed to stand at 4°C for night and the solid product was collected and recrystallized from n-butanol to give 21 as white crystals in 87% yield. M.P. 222-224 °C; R_f (silica gel G, benzene:acetone (9:1)) = 0.51; IR (KBr): 3230 (N-H), 1620 (C=N); MS (70 eV), m/z: 173 (M^+ , 41), 132 (M^+ - C_2H_3N , 22), 106 (132-CN, 56); 1H -NMR (DMSO- d_6), δ (ppm): 2.70 (s, CH_3), 3.44 (s, NH, broad), 8.05-8.16 (m, 3H, pyridine residue); Elemental analysis for $C_8H_7N_5$ (173.18): Calcd. C, 55.48; H, 4.07; N, 40.44; Found: C, 55.51; H, 4.17; N, 40.41.

1-(1H-Imidazo[4,5-b]pyridine-2-yl)-3-methyl-1H-pyrazol-5-ol (22a)

A solution of (0.02 moles) of 2-hydrazino-1H-imidazo[4,5-b]pyridine 19 and (0.04 moles) of ethyl acetoacetate in 100 ml of ethanol was heated at 65°C for 2 hours. After cooling, the precipitate was collected and recrystallized from ethanol to give compound 22a in 82% yield. M.P. 250-252 °C; R_f (silica gel G, methanol:ethanol:chloroform (1:1:3)) = 0.42; IR (KBr): 3280 (N-H), 2990 (=C-H), 2620 (=C-OH), 1675 (C=O), 1490 (C=N/C=C); 1H -NMR (DMSO- d_6), δ (ppm): 2.20 (s, 3H, N=C- CH_3), 5.22 (s, 1H, =C-H), 8.05 (m, 3H, pyridine residue), 8.86 (s, broad, 2H, NH, OH, exchangeable with D_2O); MS (70 eV), m/z: 215 (M^+ , 100), 200 (M^+ - CH_3 , 20), 174 (200-CN, 20), 132 (174- C_2H_2O , 50); Elemental analysis for $C_{10}H_9N_5O$ (215.22): Calcd. C, 55.81; H, 4.22; N, 32.54; Found: C, 55.50; H, 4.11; N, 32.51.

Biological studies

Antimicrobial screening

Representative compounds of the synthesized products, 2-11, 13-16, 20 and 21 were screened in vitro for their antibacterial activities against two strains of bacteria *Bacillus subtilis* and *Escherichia coli* and two strains of fungi, *Aspergillus fumigatus* and *Candida albicans* by the agar diffusion method [28]. The dishes were incubated at 37°C for 48 hours (for bacteria), and at 30°C for 72 hours (for fungi), where clear or inhibition zones were detected around each hole. Each 0.1 ml of DMSO alone was used as a control under the same conditions for each *microorganisms* and subtracting the

diameter of inhibition zone resulting with DMSO alone from that obtained in each case as a mean of three replicateMSO alone from that obtained in each case as a mean of three replicates (Table 1). Clotrimazole used as a standard antifungal agent and *Ampicillin* used as a standard antibacterial agent.

Analgesic activity

The analgesic evaluation of compound 22a was carried out in conscious Sprague – Dawley rats by intraperitoneal injection of 0.5 and 1.0 mg/kg in a solution of N,N-dimethylacetamide and polyethylene glycol 200 (1:1) at a concentration of 5 mg/ml, using the hot plate procedure [26].

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