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Synthesis, Characterization of Some Novel Pyrazoline incorporated Imidazo[1,2-a]pyridines for anti-inflammatory and anti-bacterial activities

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ABSTRACT

Different phenacyl bromides were reacted with 2-amino pyridine to give 2-aryl imidazo[1,2-a] pyridines, which on subsequent reaction with Vilsmeier-Haack reagent gave 2-aryl imidazo[1,2-a] pyridine carbaldehydes **(4a-4b).** Claisen-Schmidt condensation of **4a** and **4b** with different substituted acetophenones afforded chalcones **(5a-5f).** The reaction of chalcones with phenyl hydrazine gave six new derivatives of pyrazolyl imidazo[1,2-a]pyridines (**6a-6f**) in good yields. The synthesized compounds were characterised on the basis of physical and spectral data. The compounds were evaluated for anti-inflammatory and anti-bacterial activities. The compounds **6a, 6b,** and **6c** exhibited potent anti-inflammatory activity and results are comparable with standard drug, ibuprofen. None of the compounds showed any significant antimicrobial activity even at a concentration of 1000 µg/ml against Gram +ve and Gram –ve organisms.

Keywords: Imidazo[1,2-a] pyridine, Pyrazoline, Claisen-Schmidt condensation, Chalcone, Anti-inflammatory activity and Anti-bacterial activity.

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INTRODUCTION

Imidazo[1,2-a]pyridines are bridge-head heterocycles and are considered to be an important scaffolds in medicinal chemistry because of their diverse biological activities like anxiolytic[1-3], analgesic and anti-inflammatory[4,5] , anti-convulsant[6,7],CDK1 & CDK2 inhibitor activity[8,9] , anti-ulcer[[10,11]],anti-bacterial[12,13], anti-microbial[14,15] and amoebicidal. Pyrazolines are an important class of five membered heterocyclic compounds, and are widely found as the core structures in a large variety of compounds that possess important agrochemical and pharmaceutical activities. Pyrazoline derivatives are reported to have anti-microbial[16],anti-inflammatory & analgesic[17-19], antipyretic,anti-depressant[20], anti-tubercular[21] and anti-cancer activities[22].The combination of structural features of both into a single scaffold is expected to provide new chemical entities with noteworthy pharmacological properties. In view of this, in the present study, we synthesized a new series of imidazopyridine incorporated pyrazolines through chalcone intermediates . The resulted compounds were screened for anti-inflammatory and antimicrobial activities since pyrazole is the basic nucleus in many of the marketed anti-inflammatory drugs.

MATERIAL AND METHOD

All the solvents and chemicals used were of synthetic grade from SD fine chemicals, E.Merck, NR chemicals and Aldrich chemicals. Completion of the reactions was monitored by analytical thin layer chromatography (TLC) using E- Merck 0.25 mm silica gel plates. Visualization was accomplished with UV light (256 nm) and iodine chamber. Purification of synthesized compounds was done by re-crystallization process. The purity of the compounds was checked by a single spot in TLC. Melting points were determined in open capillary tubes using ANALAB melting point apparatus and are uncorrected. All the ^{1}H NMR spectra were recorded on AVANCE 300 MHz spectrometer using DMSO as solvent and tetra methyl silane (TMS) as an internal standard. Chemical shift values are listed in δ scale. The IR spectra were recorded on Shimadzu FTIR spectrophotometer by using 1% potassium bromide discs. Mass spectra of the compounds were recorded on Agilent 6430 mass spectrophotometer.

General procedure for synthesis of 2-(4-aryl) imidazo[1, 2-a] pyridine-3-carbaldehydes (4a& 4b)

To Vilsmeier-Haack reagent (0.02 mol), 2-(4-aryl) imidazo [1,2-a] pyridine (0.01mol) was added slowly in 10 ml of chloroform with stirring and then refluxed for 10-12 h at 60-70^{0}c.The reaction was monitored by TLC and after completion, the reaction mixture was concentrated to remove chloroform and poured into ice cold water. Neutralized with aq. sodium bicarbonate and followed

by extraction with chloroform. The chloroform layer was dried over anhydrous sodium sulphate and concentrated in rotary evaporator in order to give the crude product. The product was recrystallised from aq. ethanol.

General procedure for synthesis of (E)-1-(4-aryl)-3-(2-(4-aryl)imidazo[1,2-a]pyridin-3-yl)prop-2-en-1-ones (5a-5f)

To equimolar quantities of imidazo [1, 2-a] pyridine-3-carbaldehyde (0.01mol) and substituted acetophenone (0.01mol) in 20-25 ml of methanol, was added 5 ml of 10% NaOH and stirred on a magnetic stirrer for 10-12 h at room temperature in order to obtain a precipitate. After completion of reaction, the mixture was poured into ice cold water. The crude product was filtered, washed thoroughly with water and without drying, the product was recrystallised from aq.ethanol.

General procedure for synthesis of 2-(4- aryl)-3-(3-(4- aryl)-1-phenyl-4, 5-dihydro-1H-pyrazol-5-yl) imidazo[1,2-a]pyridines (6a-6f)

Compound 5 (0.01 mol) was taken in a mixture of alcohol and THF , phenyl hydrazine (0.01 mol) was added with few drops of acetic acid and stirred on magnetic stirrer at 100°c for 2-3 h. The reaction mixture was cooled and added to ice cold water. The precipitate obtained was filtered and washed thoroughly with water. The product was recrystallised from aq. ethanol.

Physical and Spectral Data of compounds (6a-6f)

2-(4-chlorophenyl)-3-(3-(4-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)imidazo[1,2-a] pyridine[6a]

White powder; m.p. 115-117^{0}C; yield:76%;$C_{28}H_{21}N_4Cl_2$; IR(KBr,cm^{-1}):1633(C=N),780(C-Cl);^{1}H NMR (DMSO,400 MHz ppm) 7.1-8.2(17H,Ar-H),5.6(dd,1H,CH of pyrazoline),3.2(dd,1H,CH_2 of pyrazoline), 3.5(dd,1H,CH_2 of pyrazoline); Mass(m/z):484.10 $[M+1]^+$; Anal. Calcd. for $C_{28}H_{21}N_4Cl_2$ Calculated C: 69.41, H: 4.37, N: 11.57. Found C: 69.89, H: 4.8, N: 11.86.

2-(4-chlorophenyl)-3-(1-phenyl-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-5-yl)imidazo[1,2-a]pyridine [6b]

White powder; m.p.114-116^{0}C; yield:73%; $C_{29}H_{24}N_4Cl$;IR(KBr,cm^{-1}):1630(C=N),780(C-Cl);^{1}H NMR (DMSO,400 MHz,δ ppm) 6.9-7.8(17H,Ar-H),5.5(dd,1H,CH of pyrazoline),3.2(dd,1H,CH_2 of pyrazoline), 3.6(dd,1H,CH_2 of pyrazoline),2.5(s,3H,CH_3); Mass(m/z):463.8 $[M+1]^+$; Anal.Calcd. for $C_{29}H_{24}N_4Cl$ Calculated C: 75.06, H: 5.22, N: 12.08. Found C: 75.09, H: 5.32, N: 12.45.

3-(1-phenyl-3-(3-p-tolyl-4,5-dihydro-1H-pyrazol-5-yl)-2-(p-tolyl)imidazo[1,2-a]pyridine [6c]

White powder; m.p. 117-119^{0}C; yield:74%; $C_{30}H_{27}N_4$; IR(KBr,cm^{-1}):1633(C=N),2921(CH_3);^{1}H NMR (DMSO,400 MHz,δ ppm) 6.8-7.6(17H,Ar-H),5.5(dd,1H,CH of pyrazoline),3.3(dd,1H,

pyrazoline H),3.45(dd,1H,of pyrazoline),2.4(s,6H,CH_3); Mass(m/z):443 $[M+2]^+$; Anal.Calcd. for $C_{30}H_{27}N_4$ Calculated C: 81.22, H: 6.14, N: 12.64. Found C: 81.24, H: 6.20, N: 12.70.

3-(3-(4-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-2-p-tolyl)imidazo[1,2-a]pyridine (6d)

White powder; m.p. 120-123^{0}C; yield:75% ;$C_{28}H_{24}N_4Cl$; IR(KBr,cm^{-1}):1631(C=N),780(C-Cl);^{1}H NMR (DMSO,400 MHz,δ ppm) 6.8-7.6 (17H,Ar-H),5.5(dd,1H,CH of pyrazoline),3.3(dd,1H, pyrazoline H),3.45(dd,1H,of pyrazoline); Mass(m/z):463.5 $[M+1]^+$; Anal.Calcd. for $C_{29}H_{24}N_4Cl$ Calculated C: 75.22, H: 5.01, N: 12.11. Found C: 75.26, H: 5.08, N: 12.48.

2-(4-chlorophenyl)-3-(1,3-diphenyl-4,5-dihydro-1H-pyrazol-5-yl)imidazo[1,2-a]pyridine (6e)

White powder; m.p. 122-124^{0}C; yield: 83%; $C_{28}H_{21}N_4Cl$; IR(KBr,cm^{-1}):1633(C=N),780(C-Cl); ^{1}H NMR (DMSO,400 MHz,δ ppm) 6.8-7.6(17H,Ar-H),5.6(dd,1H,CH of pyrazoline),3.2(dd,1H,CH_2 of pyrazoline) ; Mass(m/z):452 $[M+1]^+$; Anal.Calcd. for $C_{28}H_{21}N_4Cl$ Calculated C: 74.89, H: 4.72, N: 12.49. Found C: 75.02, H: 4.8, N: 12.62.

3-(1,3-diphenyl-4,5-dihydro-1H-pyrazol-5-yl)-2-(p-tolyl)imidazo[1,2-a]pyridine (6f)

White powder; m.p. 132-134^{0}C; yield:68% ; $C_{29}H_{24}N_4$; IR(KBr,cm^{-1}):1633(C=N),780(C-Cl);^{1}H NMR (DMSO,400 MHz,δ ppm) 6.8-7.6(17H,Ar-H),5.5(dd,1H,CH of pyrazoline),3.3(dd,1H, pyrazoline H),3.45(dd,1H, of pyrazoline),2.4(s,6H,CH_3); Mass(m/z):428 $[M+1]^+$; Anal. Calcd. for $C_{29}H_{24}N_4$ Calculated C: 81.27, H: 5.65, N: 13.08. Found C: 81.32, H: 5.72, N: 13.52.

BIOLOGICAL EVALUATION

Anti-inflammatory activity

All the synthesized compounds were screened for in vivo anti-inflammatory activity by carrageenan induced paw edema method. Albino wistar rats of either sex (150- 200 g) were divided into eight groups of six animals each. Animals were deprived of food for 12 h prior to the experiment and only water was given . First group was used as a control group and received 1 ml of 1% w/v sodium carboxymethyl cellulose in saline, the second group received the standard drug, ibuprofen at a concentration of 10 mg/kg body weight orally, while the other groups received test compounds at a dose of 100 mg/kg body weight orally. One hour after the administration of the compounds, carrageenan suspension (0.1 ml of 1% w/v suspension in 0.9% saline solution) was injected into the sub planter region of left hind paw of animals. Immediately, the paw volume was measured using plethysmometer with one hour intervals. The difference between initial and final readings gave the change in oedema volume for the corresponding time. Oedema volume of control (V_c) and Oedema volume of treated (V_t) were used to calculate percentage (%) inhibition by using following formula.

% Inhibition = $(V_c-V_t)/V_c \times 100$

Anti-bacterial activity

The inhibition of growth of microorganisms against staphylococcus aureus & Bacillus subtilis (Gram +ve) and Escherichia coli & Pseudomonas aeruginosa (Gram –ve) was measured as the Zone of inhibition produced by test and as well as standard drugs using Cup-Plate method .The prepared liquefied agar medium (25 ml) was inoculated with 10^{-7} to 10^{-8} cfu/ml seeded broth. The suspension containing microorganisms was added at a temperature between 40-50 ^{0}C and vortexed to distribute the microorganisms evenly throughout the medium and poured immediately into petri dishes to give a depth of 3-4 mm. The petri dishes were placed on a flat surface to ensure that the layers of the medium were of uniform thickness. Using a sterile bore, cylindrical cavities of 8 mm diameter were made on the medium. 5 bores were made on each petri dish. 50 µl of test and standard solutions were transferred into cylindrical cavities using a micropipette, aseptically. The petri dishes were allowed to stand for 1 h at room temperature, as a pre incubation diffusion, to minimize the effects of variation in time between the applications of different solutions. Later, the plates were incubated for 24 h at 37 ^{0}C and the circular inhibition zone was measured. Ciproflaxin was used as standard drug for comparison.

RESULTS AND DISCUSSION

Chemistry

The route for the synthesis of compounds is shown under Scheme-1. Substituted acetophenones were brominated with cupric bromide to give different phenacyl bromides 2(a-b) .The phenacyl bromides were condensed with 2-aminopyridine to give 2-aryl imidazo[1,2-a] pyridines 3(a-b). Vilsmeier-Haack reaction of 2-aryl imidazo[1,2-a] pyridines afforded 2-aryl imidazo[1,2-a] pyridine 3-carbaldehydes 4(a-b) in good yields. In FTIR, the compounds showed peak around 1635 cm^{-1} due to the carbonyl group of CHO. Chalcones 5(a-f) were synthesized by Clasien-Schmidt condensation of 2-aryl imidazo [1,2-a] pyridine 3-carbaldehydes 4(a-b) with substituted acetophenones. In FTIR the compounds showed strong absorption peaks at 1660 cm^{-1} confirming the chalcones formation. The compounds were further confirmed with mass spectral data. The chalcones were cyclised with phenyl hydrazine in methanol and THF which affords 2-(4- aryl)-3-(3-(4- aryl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl) imidazo[1,2-a]pyridines 6(a-f). The structures of compounds were confirmed by FTIR, ^{1}H NMR and mass spectral data. The physical data of compounds is given in Table1.3

SCHEME-1

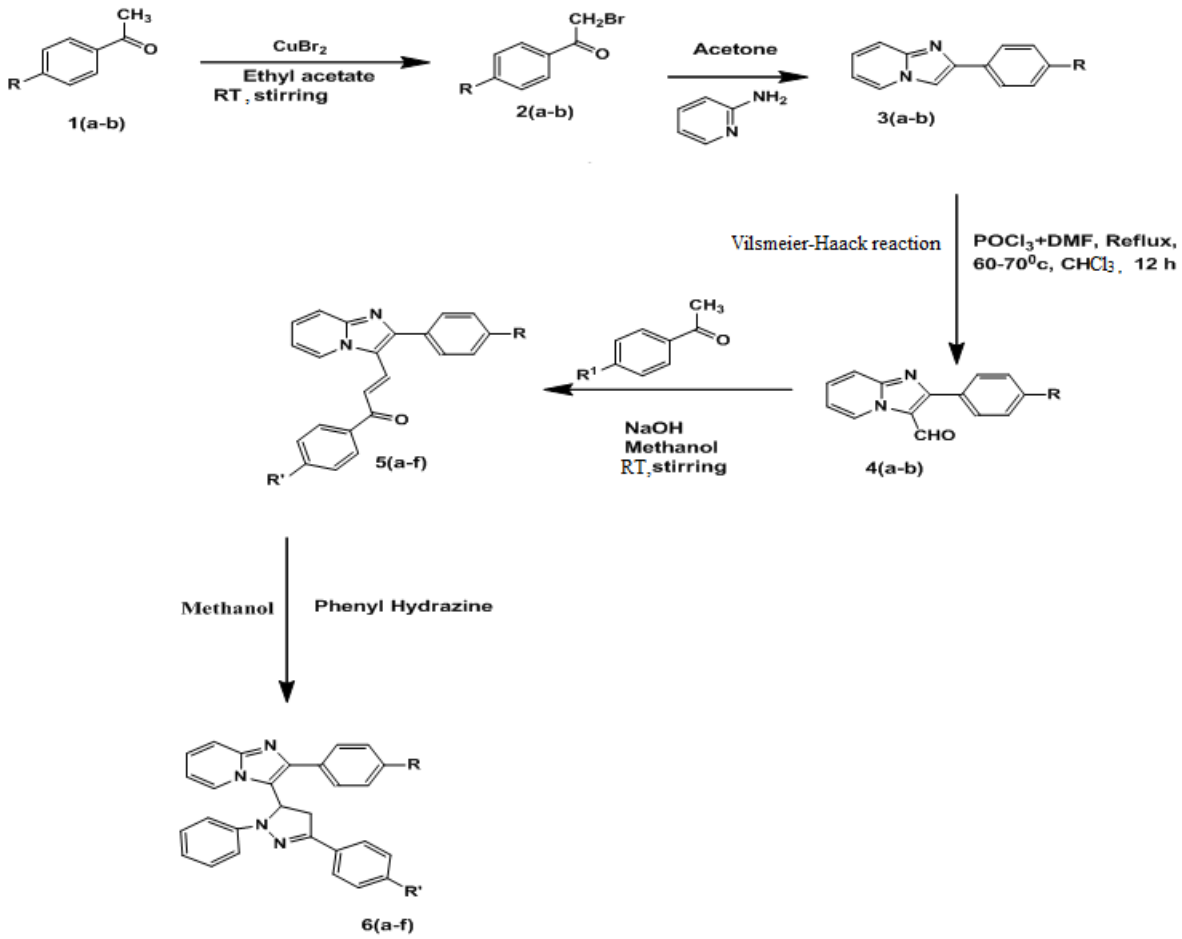

Table 1: Physical data of synthesized compounds

Compounds	Ar	Mol .formula	Mol.wt	M.P (^{0}C)	(%) Yield
6a	$R_1 = R_2 = Cl$	$C_{28}H_{21}N_4Cl_2$	484	115-118	76
6b	$R_1 = Cl$, $R_2 = CH_3$	$C_{29}H_{24}N_4Cl$	463.5	114-116	73
6c	$R_1 = R_2 = CH_3$	$C_{30}H_{27}N_4$	443	115-119	74
6d	$R_1 = CH_3$, $R_2 = Cl$	$C_{29}H_{24}N_4Cl$	463.5	120-123	75
6e	$R_1 = Cl$, $R_2 = H$	$C_{28}H_{21}N_4Cl$	448.5	122-125	83
6f	$R_1 = CH_3$, $R_2 = H$	$C_{29}H_{24}N_4$	428	132-135	68

Anti-inflammatory activity

All the synthesized compounds were screened for anti-inflammatory activity by carrageenan induced paw edema method. The anti-inflammatory data of synthesized compounds is shown in Table 2. Among the nine compounds **6a,6b&6c** exhibited 70 ,69 and 62% protection against edema formation at 4th hour and the values are comparable to standard drug, ibuprofen. The remaining compounds exhibited moderate activity. From the data obtained, the mean edema volume and percentage reduction in edema was calculated.

Table 2: Anti-inflammatory activity of synthesized compounds

Treatment	1 h	% red	2h	% red	3h	%red	4h	
	% red							
Control	2.25±0.02		2.75±0.04		2.290±0.08		3.10±0.08	
ibuprofen(std)	1.67±0.04	33	1.22±0.01	58	0.80±0.01	74	0.50±0.018	77
6a	1.60±0.02	32	1.30±0.15	54	1.10±0.023	59	0.90±0.03	**70**
6b	1.60±0.06	31	1.15±0.02	56	1.10±0.08	59	1.00±0.05	**69**
6c	1.60±0.05	34	1.55±0.02	47	1.48±0.08	53	1.22±0.036	**62**
6d	0.22±0.01	14	0.25±0.011	20	0.26±0.01	46	0.22±0.017	30
6e	0.21±0.17	19	0.22±0.019	29	0.17±0.018	65	0.20±0.019	39
6f	0.22±0.15	16	0.23±0.018	25	0.22±0.016	54	0.23±0.017	30

Values are in Mean ± Standard Deviation. Statistically significant ($p<0.05$) difference in comparison to control. Each group contains six animals.

Anti-bacterial activity

All the synthesized compounds were screened at a concentration of 150 µg/ ml,250 µg/ml , 500 µg/ml and 1000 µg/ml against two Gram positive bacteria (staphylococcus aureus & Bacillus subtilis) and two Gram negative bacteria (Escherichia coli & pseudomonas aeruginosa) using Cup-Plate method. Ciprofloxacin was used as standard and DMSO was used as control. None of the compounds exhibited any significant activity.

CONCLUSION

In present study six new derivatives of pyrazolyl imidazo[1,2-a]pyridines were synthesized and characterized by physical and spectral data. The synthesized compounds were screened for anti-inflammatory and anti-bacterial activities. In anti-inflammatory screening, among six compounds **6a, 6b** and **6c** exhibited good activity at 100 mg/kg body weight concentration and the results are comparable to standard drug, ibuprofen. The synthesized compounds have not exhibited any significant anti-bacterial activity against Gram positive bacteria and Gram negative bacteria even at 1000 µg/ml concentration. In conclusion the synthesized compounds might emerge as potent anti-inflammatory agents in further research.

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