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Synthesis of new pyrazoline derivatives and their cytotoxic and anti-inflammatory screening

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ABSTRACT

*Six new derivatives of pyrazoline incorporated imidazo(1,2-a)pyridines **6(a-f)** were synthesized and evaluated against anti inflammatory and invitro anti cancer activities. Claisen- Schmidt condensation of 2-aryl imidazo(1,2-a) carbaldehydes **(4a&4b)** with substituted acetophenones gave 2-aryl imidazo pyridinyl chalcones **5(a-f)**. Cyclization of **5(a-f)** with hydrazine hydrate afforded 2-(4- substituted phenyl)-3-(3-(4- substituted phenyl)4,5-dihydro-1H-pyrazol-5-yl)imidazo[1,2-a]pyridines **6(a-f)** in good yields. The structures of the synthesized compounds were confirmed on the basis of physical and spectral data. In MTT assay,some of the compounds have shown superior activity against MCF-7 and Hela cell lines and moderate activity aganist DU-147 cell lines. In anti-inflammmatory screening, 6e exhibited significant activity while the other compounds were moderately active.*

Key words: Chalcones, Pyrazolines, Imidazo(1,2-a) pyridines, Cytotoxic activity, Anti- inflammatory activity.

INTRODUCTION

In recent years, pyrazolines have generated a lot of interest because of their B-Raf kinase inhibitor activity and anti-tumour properties**(1-5)** besides anti-inflammatory**(6-8)**, anti-microbial**(9-12)**, anti- convulsant**(13-14)** etc., activities. Imidazo (1,2-a) pyridines are bridge- head nitrogen heterocycles and are the important building blocks in both natural and bioactive synthetic compounds with wide spectrum of therapeutic activities **(15-19)**. These observations have prompted us to synthesize new imidazo pyridine-pyrazoline hybrids with the hope of discovering new anti-tumour agents. In this context, intially we synthesized chalcones by Claisen-Schmidt condensation of imidazo(1,2-a) pyridine carbaldehydes with different acetophenones. Reaction of chalcones with hydrazine hydrate afforded 4,5-dihydro pyrazolyl imidazo(1,2-a) pyridines in good yields. The structures of the synthesized compounds were confirmed on the basis of physical and spectral data. Further, the compounds were evaluated for *invitro* cytotoxic activity against three cancer cell lines by MTT assay method. Anti-inflammatory activity was also performed for all the compounds using carrageenan induced rat paw edema method.

MATERIALS AND METHODS

All the solvents and chemicals used were of synthetic grade from SD fine chemicals Ltd., E.Merck, NR chemicals Ltd. 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-d_6 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 Schimadzu FTIR spectrophotometer by using 1% potassium bromide discs. Mass spectra of the compounds were recorded on Agilent 6430 triple quadruple LC-MS system and were given in mass units (m/z).

EXPERIMENTAL PROCEDURES

General procedure for synthesis of 2-aryl imidazo [1, 2-*a*] pyridines 3(a-b)

A mixture of 0.01 mol of phenacyl bromide and 0.01 mol of 2-amino pyridine was taken in 20-25 ml of acetone and kept for stirring at room temperature for nearly 1-2 hrs and the precipitate obtained was filtered. The filtrate on concentration in Rota evaporator gave the product which was washed thoroughly with acetone to remove traces of amino pyridine. The compound was purified by recrystallization from acetone. The percentage yield was 88%.

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

To an ice cold solution of DMF (0.3 mol), was added $POCl_3$ (0.036 mol) drop-wise and the temperature was maintained below 10°C since an exothermic reaction takes place. To the reaction mixture, an ice-cold solution of 2-aryl imidazo [1,2-*a*] pyridine (0.1 mol) dissolved in $CHCl_3$ was added slowly. After completion of addition, the reaction mixture was refluxed for about 12 hrs and the reaction was monitored by TLC. The reaction mixture was neutralised by using $NaHCO_3$ in cold condition and then washed with water and extracted with $CHCl_3$ and dried over anhyd.Na_2SO_4. The product obtained was purified by recrystallization from ethanol. The perc.entage yield was 70%.

General procedure for synthesis of chalcones 5(a-f)

To an equimolar quantities of carbaldehyde and substituted acetophenone, was added NaOH(5%) solution drop by drop and stirred on magnetic stirrer at room temperature for 4-6 hrs. The completion of the reaction was monitored by TLC. The precipitate obtained was filtered and recrystallized from 90% ethanol.

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

To 0.01 mole of Chalcone in methanol (20-25 ml), was added 0.05 mole of hydrazine hydrate and few drops of glacial acetic acid and heated under reflux for 12-16 hrs. After completion of the reaction, the mixture was poured into ice cold water. The precipitate obtained was filtered, dried and recrystallized from 90% ethanol.

BIOLOGICAL ACTIVITY

ANTI-INFLAMMATORY ACTIVITY

The anti-inflammatory activity of six derivatives was tested by Carrageenan induced rat paw edema method as per the procedure described in our earlier work (20) using ibuprofen as standard drug.

CYTOTOXIC ACTIVITY

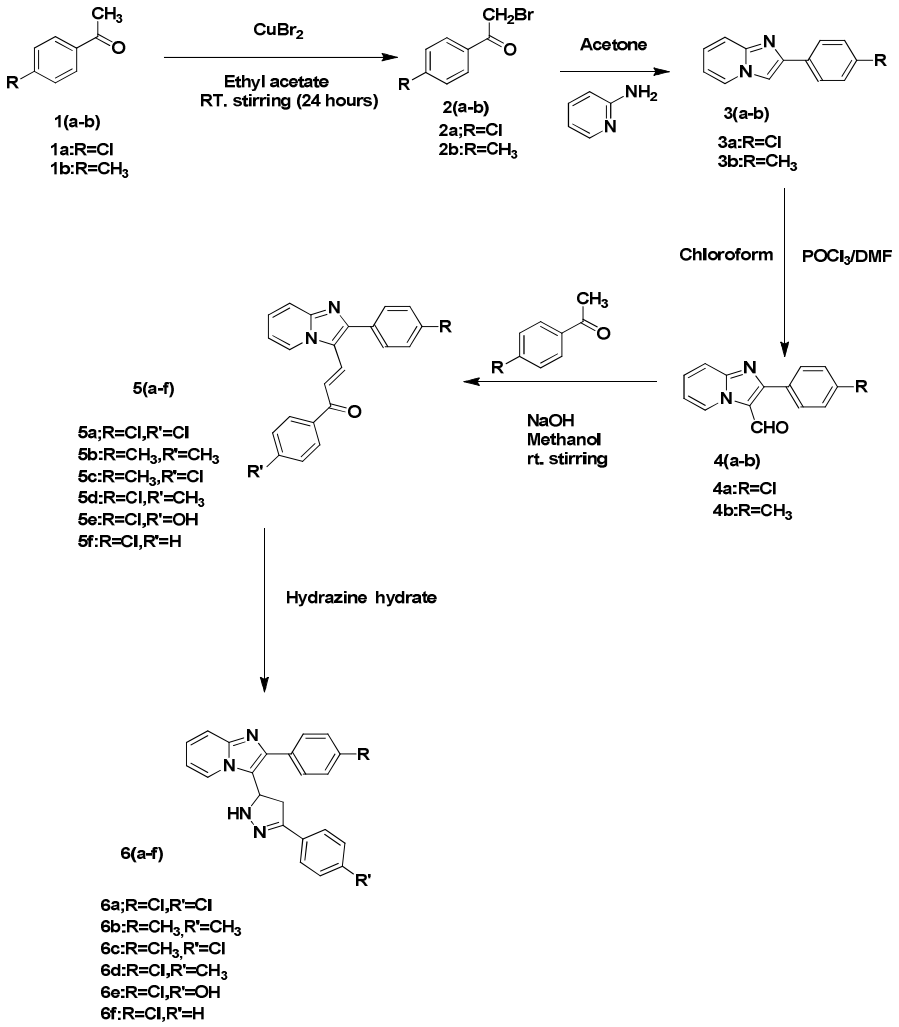
All the synthesized compounds were screened for MTT assay against DU-145, Hela, and MCF-7 cell lines and the test was performed at Natco Laboratories, Hyderabad. $1x10^4$ cells/well were seeded in 100 µl DMEM supplemented with 10% FBS in each well of 96 well microculture plates and incubated for 24 hr at 37^0C in a CO_2 incubator. After incubation, cells were treated with test compounds **6(a-f)** at 100,50,25,12.5,6.25 µg/ml concentrations for 48 hr. After 48 hr of incubation, media was removed and to each well 10 µl of MTT(5 mg/ml) was added and the plates were further incubated for 4 hrs. Supernatant liquid from each well was carefully removed and formazon crystals were dissolved in 100 µl of DMSO and absorbance was measured at 540 nm wavelength.

RESULTS AND DISCUSSION

The plan of the synthesis is given under scheme 1. Different acetophenoes were converted into phenacyl bromides using cupric bromide as brominating agent in ethyl acetate by stirring 12-15 hrs at room temperature. Reaction of phenacyl bromides with 2- amino pyridine in dry acetone gave 2–aryl imidazo (1,2-a) pyridines **3(a-b**). Vilsmeier-Haack reaction of **3(a-b)** gave 2–aryl imidazo (1,2 -a) pyridine carbaldehydes **4(a-b**) in good yields. The structures of imidazo pyridine carbaldehydes were confirmed by FTIR and mass spectral data. The compounds showed absorption peaks around 1640 cm^{-1} due to carbonyl absorption of aldehyde. Further, the mass ion peaks of 100% intensity in mass spectra corresponding to their molecular weights confirmed the structures. Claisen-Schmidt condensation of **4(a-b)** with substituted acetophenones gave chalcones **5(a-f)** in good yields. Structures were

confirmed with the help of FTIR, and mass spectral data. In IR, the **C=O** absorption of chalcones appeared around 1660 $cm^{-1}$and the mass ion peaks of 100% intensity in mass spectra corresponding to the molecular weights further confirmed the structures. Cyclization of chalcones with 99% hydrazine hydrate in presence of few drops of acetic acid as catalyst afforded 4,5-dihydro-pyrazolyl imidazo (1,2-a) pyridines **6(a-f)** in reasonable yields. Protons CH_2-CH of pyrazoline fragment in the ^{1}HNMR spectra exhibited characteristic patterns of AMX system. The appearance of three double doublets around δ 3.0, 3.6 and 5.5 due to three pyrazoline protons clearly indicated the formation of pyrazolines Further, the structures were confirmed on the basis of mass and IR spectral data. The synthesized compounds were evaluated for anti- inflammatory and cytotoxic studies against 3 cell lines.

Scheme for synthesis of compounds



Scheme-1

Physical and Spectral data of synthesized compounds

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

white coloured solid: Yield:76%: M.p: 115-119^{0}C; IR:(KBr)cm^{-1}: 3467,1633and780cm^{-1}:^{1}HNMR (300 MHz, $CDCl_3$): δ 8.23 (s,1H,NH), 7.2-7.6 (s,12H,Ar-CH) 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) :408$(M+H)^+$: Anal.Calcd. for $C_{22}H_{16}N_4Cl_2$: Calculated C,64.88; H, 3.96;N,13.76%. Found:C, 64.08; H, 3.90; N,13.86%.

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

Light yellow coloured solid : Yield:74%: M.p: 115-119 ^{0}C: IR:(KBr)cm^{-1}: 3477, 2987, 1654 cm^1; ^{1}HNMR (300 MHz, $CDCl_3$): δ8.23 (s,1H,NH), 6.9-7.8 (s,12H,Ar-H), 5.5 (dd,1H,CHof pyrazoline),3.2(dd,1H,CH_2

of pyrazoline), 3.6(dd, 1H,CH_2 of pyrazoline) 2.5(s,6H,2CH_3) ; Mass (m/z):366 $(M+H)^+$:Anal.calcd.for $C_{24}H_{22}N_4$: Calculated C,78.66; H, 6.05; N,15.29%.Found: C,78.05; H,5.96; N,15.25 %

(6c);3-(3-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)-2-(*p*-toyl)imidazo[1,2-a]pyridine
white coloured powder ;Yield:75%; M.p:120-123^{0}C; IR:(KBr)cm^{-1}3467, 1633,and 780cm^{-1}; ^{1}HNMR (300 MHzCDCl$_3$): δ 8.25 (s,1H,NH), 7.0-7.8 (s,12H,Ar-CH) 5.9(dd,1H,CH of pyrazoline),3.5(dd,1H,CH_2 of pyrazoline),3.7(dd,1H,CH_2 of pyrazoline), 2.4(s, 3H,CH_3); Mass (m/z):387 $(M+H)^+$: Anal.calcd.for $C_{23}H_{19}N_4Cl$: calculated: C,71.40; H, 4.95;Cl,9.16; N,14.48% Found:C, 70.35; H, 4.85; Cl,9.19; N,14.47%

(6d);2-(4-chlorophenyl)-3-(3-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-5-yl)imidazo[1,2-*a*]pyridine
white coloured solid: Yield: 73%: M.p: 115-119 ^{0}C ; IR:(KBr)cm^{-1}: 3467 , 1633 ,and 780 cm^{-1} ;^{1}HNMR(300MHz,CDCl$_3$):δ 8.23 (s,1H,NH), 6.8-7.6 (s,12H,Ar-H), 5.5 (dd,1H,CH of pyrazoline), 3.3 (dd,1H,CH_2 of pyrazoline), 3.45 (dd, 1H, CH_2 of pyrazoline), 2.4 (s,3H,CH_3); Mass (m/z) : 367 $(M+H)^+$ Anal.calcd.for $C_{23}H_{19}N_4Cl$: calculated C,71.40; H, 4.95; Cl,9.16; N,14.48% Found :C,70.37; H, 4.88; Cl,9.14; N,14.40 %

(6e)4-(5-(2-(4-chlorophenyl)imidazo[1,2-*a*]pyridin-3-yl)-4,5-dihydro-1*H*-pyrazol-3-yl)phenol
white coloured solid: Yield : 68%: M.p: 132-135^{0}C; IR:(KBr)cm^{-1}: 3467,1633and780cm^{-1} ; ^{1}HNMR (300 MHz, CDCl$_3$): δ8.26 (s,1H,NH), 7.2-7.8 (s,12H,Ar-CH) 5.8(dd,1H,CH of pyrazoline),3.3(dd,1H,CH_2 of pyrazoline),3.6(dd,1H,CH_2 of pyrazoline),4.8(s,1H,OH); Mass (m/z) :389 $(M+H)^+$: Anal.calcd.for $C_{22}H_{17}N_4ClO$: Calculated C,67.90; H, 4.41;Cl,9.12,N,14.41; O,4.11% Found: C, 67.80; H, 4.39; N,14.39; O,4.07%

(6f);2-(4-chlorophenyl)-3-(3-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)imidazo[1,2- a]pyridine
white coloured solid: Yield:76%: M.p: 115-119^{0}C: IR:(KBr)cm^{-1}: 3467,1633and780cm^{-1}:^{1}HNMR (300 MHz, CDCl$_3$): δ 8.23 (s,1H,NH), 7.2-7.6 (s,12H,Ar-CH) 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):372$(M+H)^+$: Anal.calcd.for $C_{22}H_{17}N_4Cl$: Calculated C,70.87; H, 4.60;N,15.03% Found: C, 70.85; H, 4.57; N,15.00%

Anti-inflammatory activity of synthesized compounds 6(a-f)
The mean edema volume and percentage inhibition were recorded and presented in Table 1. The results obtained in this investigation indicated that the percentage protection against edema formation with compound **6e** was significant, while the other compounds showed moderate protection. The test was done by using standard procedure against inflammation induced by carrageenan at 1, 2, 3 and 4 hr intervals.

Table-1

Compound	Paw edema(ml)(Mean±S.E)					% Inhibition After 3h
	Dose mg/kg	1 h	2h	3h	4h	
Control(sodium CMC)	-	2.25±0.02	2.75±0.04	2.290±0.08	3.10±0.08	-
Standard(Ibuprofen)	100	1.67±0.04	1.22±0.01	0.80±0.01	0.50±0.018	74
6a	100	1.60±0.02	1.30±0.15	1.10±0.023	0.90±0.03	59
6b	100	1.60±0.06	1.15±0.02	1.10±0.08	1.00±0.05	59
6c	100	1.60±0.05	1.55±0.02	1.48±0.08	1.22±0.036	53
6d	100	0.22±0.01	0.25±0.011	0.26±0.01	0.22±0.017	46
6e	100	0.21±0.17	0.22±0.019	0.17±0.018	0.20±0.019	**65**
6f	100	0.22±0.15	0.23±0.018	0.22±0.016	0.23±0.017	54
*Each value represents mean ±SE of six animals; *P < 0.05 was considered significant when compared to control*						

ANTICANCER ACTIVITY
In the present investigation, the synthesized compounds were screened against DU-145, Hela and MCF-7 cell lines using MTT assay and the IC_{50} values of the compounds are recorded in table 2. Among the six compounds, compounds **6c, 6d** and **6f** exhibited superior activity against MCF-7 cell lines with IC_{50} values in the range of 18.0 to 24.0 µM. Compound 6e has shown highest activity against Hela cell lines at IC_{50} value of 28.8 µM. All the compounds exhibited moderate cytotoxic activity aganist DU-145 cell lines. Doxorubicin was used as a reference drug.

IC_{50} values[a] (µM) of compounds from 6(a-f)

Table-2

Compound	[a]Cytotoxicity expressed as IC_{50}(µM) in cell lines		
	DU-145[b]	Hela [c]	MCF-7[d]
6a	76.83	58.33	33.08
6b	>100 (158.4)	60.38	44.56
6c	40.71	35.02	18.12
6d	52.60	33.86	22.18
6e	65.25	28.8	32.0
6f	40.83	34.16	24.08
Doxorubcin	3.1	1.544	1.964

[a]50%Inhibitory concentration after 48 hrs of drug treatment and the values are average of three individual experiments.
[b] Human prostate cancer
[c] Human cervical cancer
[d]Human breast cancer cell line

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