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Research Article

SYNTHESIS, DOCKING STUDIES AND PHARMACOLOGICAL EVALUATION OF IMIDAZOLE ANALOGUES OF ARECOLINE

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ABSTRACT

A series of novel analogues of arecoline were synthesized by incorporating substituted imidazole at the ester functionality of arecoline. Synthesis of the titled compounds initialized with a multicomponent, one-pot synthesis for achieving substituted imidazole with pyridine incorporated in the five-membered ring. Then successive N-methylation of pyridine using methyl iodide was executed to yield methyl iodide salt. Reduction of the salts completed the synthetic approach, achieving novel N-alkyl/aryl substituted imidazol-2-yl arecolines. The compounds were evaluated for *in vitro* anti-inflammatory by Human Red Blood Cell (HRBC) membrane stabilization method using Diclofenac as standard. Anthelmintic screening was also done on the novel analogues, with Albendazole as standard. Molecular Docking was performed for anti-inflammatory activity. It has been concluded that the computational values obtained after docking are in good agreement with the experimental values.

Keywords: Arecoline, Imidazole, Molecular Docking, p38 MAP kinase, Anti-inflammatory activity, Anthelmintic activity.

INTRODUCTION

The basic nucleus of arecoline (N-methyl-1,2,5,6-tetrahydropyridine) is blessed with a wide range of pharmacological activities. Arecoline and its' analogues have been established as Muscarinic M_1 receptor agonist in the treatment of Alzheimer's disease and other cognitive disorders[1-4]. Imidazole serves as a bio mimetic and reactive pharmacophores with broad spectrum of biological activities. Substituted imidazoles have been reported to possess antimycobacterial, antimicrobial, anthelmintic, anti-inflammatory, anticonvulsant and insecticidal properties[5-7]. In view of the important features, it was planned to design newer imidazole analogues of arecoline with better pharmacological properties. The lead was structurally modified by replacing substituted imidazole at the ester functionality of arecoline. The substitution pattern of the lead was rationalized so as to be correlated to the different heterocyclic templates of compounds. It involved the synthesis of novel N-alkyl/aryl substituted imidazol-2-yl arecoline derivatives and screening for its *in vitro* anti-inflammatory and anthelmintic activity. To predict the affinity and the activity of the analogues to the selected protein target of interest molecular docking was performed on p38 MAP kinase receptor for anti-inflammatory activity.

MATERIALS AND METHODS

All reagents and solvents were purchased from Sigma Aldrich, Loba Chemie and Nice Chemicals. Solvents were dried by standard procedures. The purity of the compounds was checked by TLC using precoated Silica Gel G plates. Melting points were determined in capillary tubes on Remi apparatus and were presented uncorrected. Infrared (IR) Spectra were recorded using KBr pellets on Jasco FTIR Model 4100 Type A. Nuclear magnetic resonance spectra were recorded on Bruker Ultrashield Model DPX 400 MHz spectrometer in Methanol (d_4) and TMS (Tetra methyl silane) as internal standard (chemical shifts in ppm). Mass spectra were recorded on a JOEL GCmate mass spectrometer. *In silico* modeling of the molecules was performed using various softwares. ACD Chemsketch 12.0 and Marvin Sketch were used for drawing, 3D optimizing and calculating various physicochemical properties of the proposed molecules. The docking score was calculated using Glide, a ligand binding program provided by Schrödinger under Maestro Molecular modeling environment.

Experimental

Synthesis of 3-(1,4,5-trisubstituted-1H-imidazol-2-yl) pyridine (5a-e)

A mixture of 10 mmol benzil, 10 mmol alkyl/aryl amine, 10 mmol pyridine-3-carboxaldehyde, 10 mmol ammonium acetate and 5 mol % $FeCl_3$ was refluxed in 10 ml ethanol for 90 minutes. After completion of the reaction (monitored by TLC), the mixture was filtered to separate the catalyst, then cooled to room temperature, and precipitated products were separated by filtration. The resulting residue was purified by recrystallization from ethanol.[8]

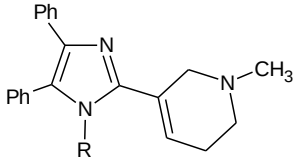
Synthesis of 1-methyl-3-(1,4,5-trisubstituted-1H-imidazol-2-yl) pyridinium iodide (6a-e)

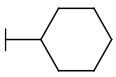
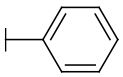
To a solution of the product obtained in first step (1 equivalent) in dry acetone, methyl iodide (2 equivalents) was added at 0°C and the mixture was stirred for 3 hr at 0°C. The product obtained was filtered and washed using cold acetone. The resultant was recrystallised from dry acetone.[5,9]

Synthesis of 1-methyl-3-(1,4,5-trisubstituted-1H-imidazol-2-yl)-1,2,5,6-tetrahydropyridine (7a-e)

To a solution of methyl iodide salt (1 equivalent) achieved in step 2 in methanol at 0°C, sodium borohydride (1.2 equivalent) was added portion wise and the mixture was stirred at -15°C using crushed ice and sodium chloride (1:1) as freezing mixture for 8-10 hrs. Methanol was removed under reduced pressure and the residue obtained was dried over anhydrous Sodium sulphate and purified by recrystallisation with methanol.[5,9]The physical characteristic of the compounds prepared are listed in **Table 1**.

Table 1: Physical Characterization data of all the synthesized derivatives



Compound	R	Mol. Formula	Melting point (°C)	Yield (%)	*R_f
7a	$-CH_3$	$C_{22}H_{23}N_3$	140-143	58	0.72
7b	$-CH_2CH_3$	$C_{23}H_{25}N_3$	156-158	55	0.76
7c	$-CH_2CH_2CH_3$	$C_{24}H_{27}N_3$	169-171	62	0.79
7d		$C_{27}H_{31}N_3$	163-165	59	0.74
7e		$C_{27}H_{25}N_3$	183-185	61	0.81

*Chloroform: Methanol (9.7:0.3)

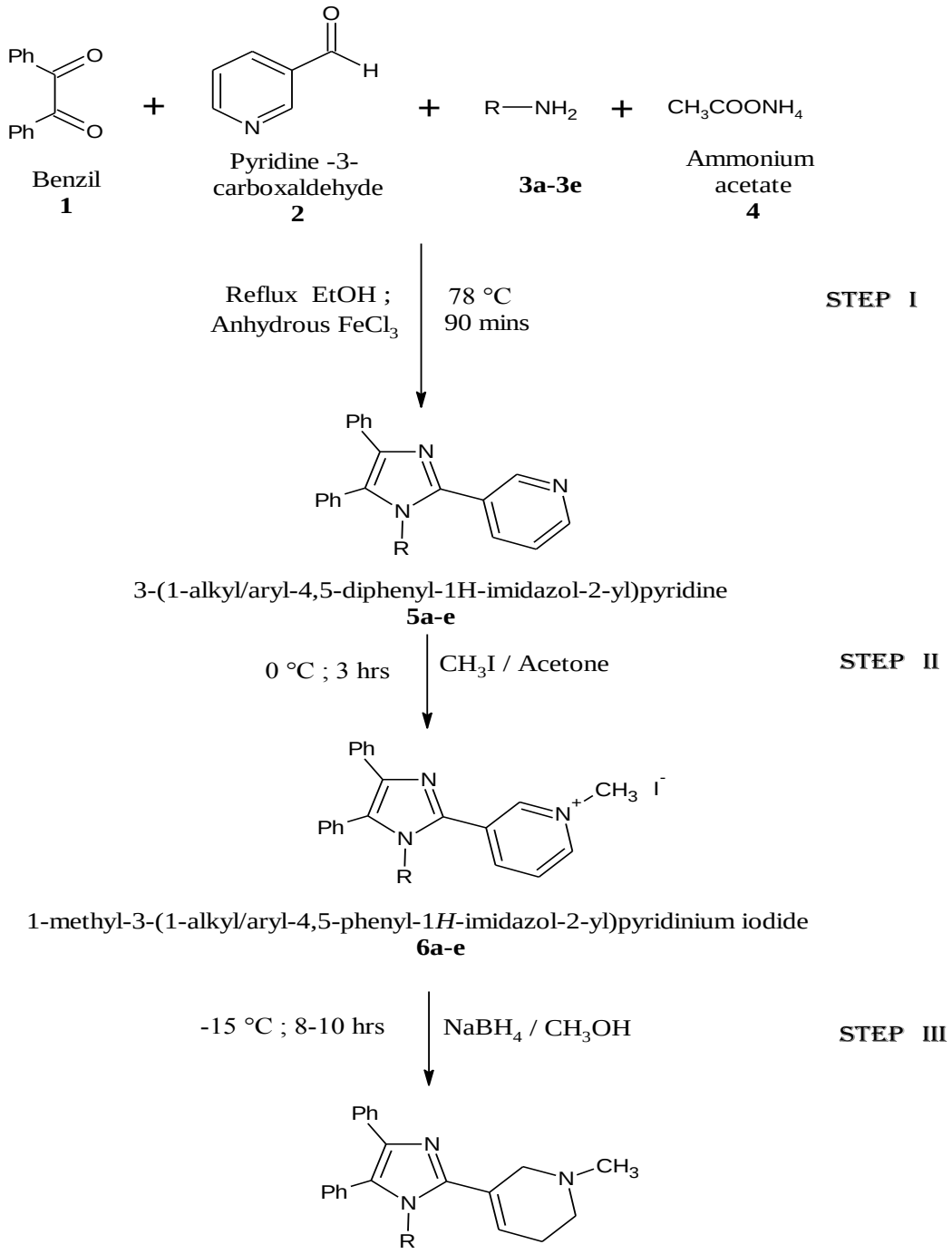


Fig. 1: Scheme for the synthetic route of 1-methyl-5-(1-substituted-4,5-diphenyl-1*H*-imidazol-2-yl)-1,2,5,6-tetrahydropyridine

3-(1-methyl-4,5-diphenyl-1*H*-imidazol-2-yl)- 1-methyl-1,2,5,6-tetrahydropyridine (7a)

FT IR (KBr, V_{max} cm^{-1}): 1671 (C=N str), 1498 (Ar C=C str), 1212 (C-N str). **^{1}H NMR** (Methanol-d_4, δ ppm): 1.89 (3H, tetrahydropyridine N-CH_3), 2.1 (3H, imidazole N-CH_3), 2.2-2.4 (6H, $3CH_2$), 6.9-7.9 (m 10H, Ar-H). **MS** m/z = 330.21 $(M+H)^+$.

3-(1-ethyl-4,5-diphenyl-1*H*-imidazol-2-yl)-1-methyl-1,2,5,6-tetrahydropyridine (7b)

FT IR (KBr, V_{max} cm^{-1}): 1601 (C=N str), 1452 (Ar C=C str), 1255 (C-N str) **^{1}H NMR** (Methanol-d_4, δ ppm): 2.2 (3H, tetrahydropyridine N-CH_3), 6.8-7.3 (m 10H, Ar-H). **MS** m/z = 344.28 $(M+H)^+$.

3-(4,5-diphenyl-1-propyl--1*H*-imidazol-2-yl)-1-methyl-1,2,5,6-tetrahydropyridine (7c)

FT IR (KBr, V_{max} cm^{-1}): 1625 (C=N str), 1499 (Ar C=C str), 1223 (C-N str) **^{1}H NMR** (Methanol-d_4, δ ppm): 0.63 (3H, CH_3), 1.24-1.32 (4H, $2CH_2$), 2.15 (3H, tetrahydropyridine N-CH_3), 7.5-7.9 (10H, Ar-H). **MS** m/z = 358.23 $(M+H)^+$.

3-(1-cyclohexyl-4,5-diphenyl-1*H*-imidazol-2-yl)-1-methyl-1,2,5,6-tetrahydropyridine (7d)

FT IR (KBr, V_{max} cm^{-1}): 2928 (Ar C-H str), 1597 (C=N str), 1447 (Ar C=C str) **^{1}H NMR** (Methanol-d_4, δ ppm): 1.9 (3H, tetrahydropyridine N-CH_3), 7.8-8.1 (10H, Ar-H). **MS** m/z = 398.26 $(M+H)^+$.

3-(1,4,5-triphenyl-1*H*-imidazol-2-yl)- 1-methyl-1,2,5,6-tetrahydropyridine (7e)

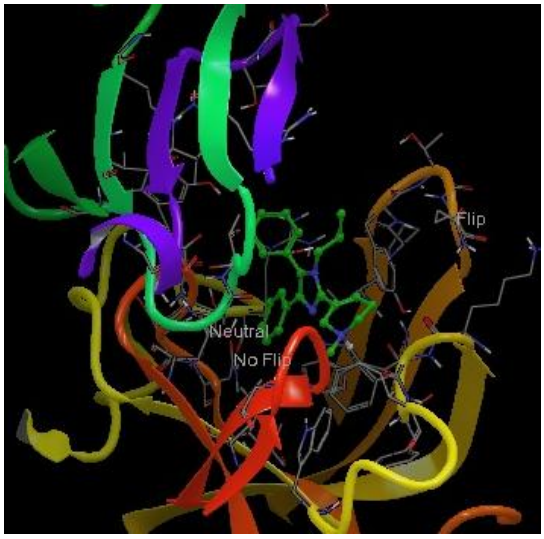
FT IR (KBr, V_{max} cm^{-1}): 1627 (C=N str), 1497 (Ar C=C str), 1169 (C-N str) **^{1}H NMR** (Methanol-d_4, δ ppm): 2.1 (3H, tetrahydropyridine N-CH_3), 6.8-7.6 (s 15H, Ar-H). **MS** m/z = 392.21 $(M+H)^+$.

Molecular Docking Methodology

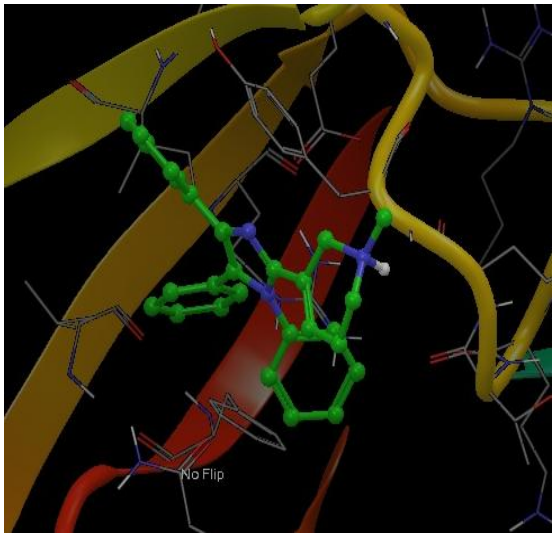
The crystal structure of p38 MAP kinase (PDB entry: 3FMJ) was retrieved from Protein Data Bank (http://www.rcbs.org). The Protein was edited using Schrödinger ligand preparation product LigPrep by adding of hydrogen for protonation of its ionizable residues, modification of tautomeric forms and repositioning of reorientable hydrogen to yield the optimized protein-ligand complex. All the synthesised derivatives were built using ACD Chemsketch version 12.0 and Marvin Sketch. After saving the molecules in the mol format, these were imported into the work space of Maestro version 9.2. The geometry was optimized by adding hydrogen atoms, generating ionization states, neutralizing the charged groups, generating tautomers and low-energy ring conformers. These energy minimized structures were further used for docking. The active sites of the protein MAP kinase (PDB entry: 3FMJ) were identified by Receptor Grid Generation Panel under Glide of Maestro. The options in each tab of this panel allows one to define the receptor structure by excluding any co-crystallized ligand that may be present, determining the position and size of the active site as it will be represented by receptor grids, set up Glide constraints, and set up flexible hydroxyl groups. Docking was executed between the prepared ligands and protein using the Ligand Docking option under Glide. In this study, flexible docking was conducted using XP Glide for extra precision. The resulting docking structures were visualized using Glide XP Visualiser. Suitable images were saved along with the dock score in terms of Gscore, shown in **Table 2**.

Table 2: Glide Scores For Anti-inflammatory Action [p38 MAP kinase (PDB entry: 3FMJ)]

COMPOUND	GLIDE SCORE
7c	-8.25
7b	-8.21
7e	-6.51
7a	-6.83
7d	-5.97



Compound 7d docked to 3FMJ



Compound 7a docked to 3FMJ

Fig. 2: Images of derivatives docked to the receptors

***In-Vitro* Anti-Inflammatory Activity[10]**

In-vitro Anti-Inflammatory Activity was performed on synthesised novel arecoline analogues by Human Red Blood Cell (HRBC) membrane stabilization method using Diclofenac sodium as standard.

5ml of human blood was collected from healthy volunteer and mixed with equal volume of sterilized Alsever solution (2% Dextrose, 0.8% sodium citrate, 0.05% Citric acid and 0.42% sodium chloride in distilled water). The blood was then centrifuged at 3000 rpm and packed cells were washed with isosaline 0.85% (pH7.21) and a 10% v/v suspension was made with isosaline. Drug concentrations of 50, 100, and 250 µg/ml were prepared. The desired concentration of the drug was mixed with 1ml phosphate buffer (0.15M, pH7.4), 2ml hypo saline (0.36%) and 0.5ml HRBC suspension. Diclofenac sodium was used as reference standard. Instead of hypo saline 2ml distilled water was used as the control.Haemoglobin content in the supernatant solution obtained after centrifugation was estimated at 560 nm. The percentage haemolysis was calculated by assuming the haemolysis produced in the presence of Distilled water as 100%. The percentage of HRBC membrane stabilization was calculated.

% Inhibition of haemolysis = 100 x [(OD_1- OD_2) / OD_1]

Where

OD_2 = optical density of sample

OD_1 = optical density of control

The results were analyzed for statistical significance by one way ANOVA followed by Dunnett's t test and reported in **Table 3**.

Anthelmintic Activity[11]

Anthelmintic screening was done on the synthesized novel 3-substituted arecoline analogues. Albendazole was used as standard.

Adult earthworms *Eudrilus eugenia*, washed with normal saline to remove all the faecal matter, were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol due to its anatomical and physiological resemblance with the intestinal roundworm parasites in human beings. Albendazole was diluted with normal saline to obtain 0.1, 0.25, 0.5 gm% as standards and poured into Petri dishes. All the test compounds were prepared in minimum quantity of DMSO and diluted to 15 ml with normal saline to obtain 0.1, 0.25, 0.5 gm% and taken into the Petri dishes. Normal saline serves as control for standard. Six earth worms of nearly equal size were placed in each Petri dish at room temperature. The time taken for complete paralysis and death were recorded. The mean paralysis time and mean lethal time for each sample were recorded. Observations were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colours. The results were analyzed for statistical significance by one way ANOVA followed by Dunnett's t test and reported in **Table 4**.

Table 3: *In-Vitro* Anti-Inflammatory Activity of Synthesized Analogues *

Sample	Conc. (µg/ml)	Mean absorbance	% inhibition
Control *(Distilled water)*	-	0.456 ± 0.03	-
Standard *(Diclofenac sodium)*	50	0.101 ± 0.02	77.85
	100	0.099 ± 0.018	78.29
	250	0.0869 ± 0.028	80.94
7a	50	0.188 ± 0.023	58.77
	100	0.186 ± 0.012	59.21
	250	0.182 ± 0.043	60.09
7b	50	0.113 ± 0.016	75.21
	100	0.111 ± 0. 014	75.66
	250	0.109 ± 0. 017	76.09
7c	50	0.157 ± 0. 013	65.57
	100	0.155 ± 0.06	66.01
	250	0.152 ± 0.0109	66.67
7d	50	0.178 ± 0.011	60.96
	100	0.175 ± 0.014	61.63
	250	0.174 ± 0.013	61.84
7e	50	0.168 ± 0.012	63.15
	100	0.167 ± 0.013	63.38
	250	0.164 ± 0.015	64.04

*Data are expressed as mean ± SEM. (n = 4) and results considered significant when P < 0.01.

Table 4: Anthelmintic activity of Synthesized analogues *

	Conc. (Gm %)	Mean Time taken for paralysis (Min.) *	Mean Time taken for complete death (Min.) *
Control**	**-**	No paralysis	No death
Standard (*Albendazole*)	0.1	27.13 ± 0.306	41.35 ± 0.206
	0.25	25.32 ± 0.174	38.63 ± 0.246
	0.5	23.03 ± 0.275	35.47 ± 0.294
7a	0.1	25.78 ± 0.375	38.75 ± 0.240
	0.25	23.8 ± 0.244	36.33 ± 0.154
	0.5	22.73 ± 0.191	35.25 ± 0.195
7b	0.1	26.28 ± 0.224	41.75 ± 0.523
	0.25	24.3 ± 0.132	37.23 ± 0.304
	0.5	23.18 ± 0.224	35.6 ± 0.292
7c	0.1	21.93 ± 0.236	33.38 ± 0.930
	0.25	17.92 ± 0.459	31.93 ± 0.499
	0.5	15.23 ± 0.247	28.1 ± 0.311
7d	0.1	22.58 ± 0.147	34.97 ± 0.154
	0.25	21.23 ± 0.267	33.95 ± 0.257
	0.5	17.38 ± 0.154	32. 3 ± 0.139
7e	0.1	24.07 ± 0.320	37.45 ± 0.175
	0.25	22.5 ± 0.336	36.13 ± 0.171
	0.5	20.27 ± 0.264	31.88 ± 0.171

*Data are expressed as mean ± SEM. (n = 6) and results considered significant when P < 0.01.

RESULTS AND DISCUSSION

The 1-methyl-5-(1-substituted-4,5-diphenyl-1*H*-imidazol-2-yl-1,2,5,6-tetrahydropyridine) derivatives were synthesized using appropriate synthetic route, further recrystallised using methanol and checked the purity by thin layer chromatography. Characterizations were done by R_f value, melting point, FTIR and ^{1}HNMR.

FTIR spectra of all synthesized compounds shows absorbance bands at range 1255-1169 cm^{-1} associated with C-N stretching vibration and bands at 1671-1597 $cm^{-1}$for C=N stretching.

The ^{1}HNMR spectrum of synthesized compounds exhibited peaks in the range of 1.89-2.2 ppm corresponding to N-methylated protons of tetrahydropyridine.

The results of *in vitro* anti-inflammatory and anthelmintic activity of test compounds given in table 3 and 4 shows that 7b and 7c showed significant anti-inflammatory and 7a and 7b exhibited significant anthelmintic activity on comparison with standards Diclofenac sodium and Albendazole respectively.

Molecular Docking for anti-inflammatory activity with protein p38 MAP kinase showed good binding affinity and the computed Gscore of the compounds was found to have good correlation with the experimented values obtained.

CONCLUSION

A series of 1-methyl-5-(1-substituted-4,5-diphenyl-1*H*-imidazol-2-yl-1,2,5,6-tetrahydropyridine) were successfully prepared as per the scheme and characterized. The titled compounds were further screened for *in vitro* anti-inflammatory and anthelmintic activity. Compound 7b and 7c showed significant *in vitro* anti-inflammatory activity on comparison to standard Diclofenac sodium. The derivatives also showed good binding affinity with p38 MAP kinase on docking. The computational values as Gscore obtained after docking are in good agreement with the experimental values. Compound 7a and 7b exhibited significant anthelmintic activity when compared with standard Albendazole. The analogues can be subjected to further detailed studies for consideration as drug candidates.

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