Original Article

Synthesis and biological evaluation of pyrazole clubbed pyrimidine bearing imidazole as linker for potent antioxidant agents

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ABSTRACT

The promoted synthesis of novel compound 2-(4,5-bis(1-phenyl-3-(4-substitutedphenyl)-1H-pyrazol-4-yl)-2H-imidazol-2-yl)pyrimidine incorporating three pharmacophoric heterocyclic was synthesized through one-pot reaction between pyrimidine-2-carboximidamide and 3-(4-substitutedphenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde. Full assignment of all ^{1}H and ^{13}C nuclear magnetic resonance chemical shifts has been unambiguously achieved. The proposed reaction mechanism is also discussed. Further, the synthesized compounds were evaluated for *in vitro* diphenyl-2-picryl-hydrazyl (DPPH) assay for antioxidant activity using ascorbic acid as standard. Compound 3b and 3e showed most promising antioxidant activity.

Keywords: 1,1-biphenyl-2-picrylhydrazyl, pyrazole, pyrimidine, antioxidant

INTRODUCTION

Free radicals are produced as results of many biochemical reactions in our body.[1] They are linked with majority of diseases such as cardiovascular diseases, liver cirrhosis, cancer, diabetes, and aging. Most common among them is partially reduced metabolites of oxygen and nitrogen. The common reactive oxygen species are superoxide anion (O_2•-), hydrogen peroxide (H_2O_2), peroxy radical (ROO•), and highly reactive hydroxyl radical (OH•). The reactive nitrogen free radicals species are of nitric oxide and peroxynitrite anion (ONOO•). Oxidation is the most common route for the generation of free radicals.[2-4] The process involved internal factors such as inflammation and external factors such as exposure to ultraviolet (UV) light and environmental pollution.[1] Thus, the preliminary prevention and treatment of the diseases associated with the free radicals is of immense importance today. Antioxidants are the substances that protect our body from the damaging oxidation reaction by reacting with free radicals and reactive oxygen species.

Imidazole derivatives received significant attention these days due to their wide applications. They exist in extract of naturally occurring herbs and also in substituted forms as antioxidant,[5-7] anticancer,[8] anti-inflammatory,[9] and antimicrobial.[6,10] Pyrimidine rings are of great interest for the researchers as antioxidants and other pharmacological activities.[11-13] One such fused pyrimidine heterocyclic is tirilazad mesylate (U-74006F) that has antioxidant potential and is associated with several research studies including ischemic stroke, acute spinal cord injury, and subarachnoid hemorrhage.[14-16] Pyrazoline rings and its derivatives also possess wide range of biological activities including antioxidant,[17-21] antimicrobial,[19] and many others.

The present work is focused toward design and synthesis of pyrazole clubbed pyrimidine bearing imidazole as linker for potent antioxidant. In this, the synthesized compounds include all these

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three pharmacophores in one result in compound with extended conjugation. The electron-donating capacity of the nitrogen in the rings and the potential of forming stabilized structures even after scavenging the free radicals due to extended conjugation were synthesized to provide compounds with improved antioxidants.

EXPERIMENTAL PROTOCOL

General

All the chemicals used were of laboratory grade and procured from E. Merck (Darmstadt, Germany) and S.D. Fine Chemicals (Mumbai, India). Melting points were determined by open capillary tubes in a Hicon melting point apparatus (Hicon, New Delhi, India) and are uncorrected. Purity of the compounds was checked by thin-layer chromatography (TLC) plates (silica gel G), which were visualized by exposing to iodine vapors and UV light. The Fourier transform infrared (FTIR) spectra were recorded on (IR affnity SHIMADZU) FTIR spectrophotometer using KBr pellets; v_{max} values are given in cm^{-1} and the ^{1}H-nuclear magnetic resonance (^{1}H-NMR). Spectra were taken on NMR spectra that were recorded on Bruker model DRX-400 MHz NMR spectrometer (^{1}H at 400 MHz, ^{13}C at 100 MHz) in DMSO-*d*6. Chemical shifts (δ) are expressed in ppm relative to tetramethylsilane as an internal standard and coupling constants (*J* values) are expressed in Hz. The splitting patterns abbreviated used are as follows: s, singlet; bs, broad singlet; d, doublet; dd, double doublet; t, triplet; q, quadruplet; and m, multiplet. Mass spectra were obtained on Water's Synapt LC/MS instrument using electron impact ionization presented as m/z.[22]

Synthesis

General procedure for the synthesis of 2-(4,5-bis(1-phenyl-3-(4-substitutedphenyl)-1H-pyrazol-4-yl)-2H-imidazol-2-yl)pyrimidine (3a)

A mixture of pyrimidine-2-carboximidamide (0.01 mol) and the appropriate intermediate 1,3-diphenyl-1H-pyrazole-4-carbaldehyde (0.02 mol) in dimethylformamide (DMF) (5–10 mL) was heated under reflux at 110°C for 6 h, monitored by single spot in TLC (Pet. Ether-EtOAc, 6:4). The solution was cooled and the poured into crushed ice. The obtained solid was filtered and then wash with sodium sulfate, and second wash with methanol and then recrystallized from ethanol.

2-(4,5-bis(1,3-diphenyl-1H-pyrazol-4-yl)-1H-imidazol-2-yl)pyrimidine (3a, $C_{37}H_{26}N_8$)

Yield 68%, orange solid, mp 250–252°C; R_f = 0.5 (pet ether:methanol (6:4)); ^{1}H-NMR (300 MHz, DMSO) δ (ppm): 6.71–6.74 (m, 1H, pyrimidine), 6.91–6.93, 7.10–7.13 (m, 2H, pyrimidine), 7.20–7.24, 8.10–8.13 (m, 10H, phenyl), 7.39–7.55 (m, 8H, phenyl), 8.29–8.35 (m, 2H, phenyl), 9.15 (s, 1H, pyrazole), 9.22 (s, 1H, pyrazole), 9.38 (s, 1H, -NH imidazole). IR (KBr) (cm^{-1}): 3429 (NH str), 3089 (CH str), 1690, 1685 (C=N str), ESI MS (m/z): 582 (M^+).

2-(4,5-bis(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1H-imidazol-2-yl)pyrimidine (3b, $C_{37}H_{24}Cl2N_8$)

Yield 65%, orange solid, mp 260–262°C; R_f = 0.7 (pet ether:methanol (6:4)); ^{1}H-NMR (300 MHz, DMSO) δ (ppm): 6.73–6.76 (m, 1H, pyrimidine), 6.83–6.90, 7.13-7.17 (m, 2H, pyrimidine), 7.20–7.24, 8.11–8.14 (m, 10H, phenyl), 7.37–7.56 (m, 8H, phenyl), 8.20–8.35 (m, 2H, phenyl), 9.14 (s, 1H, pyrazole), 9.25 (s, 1H, pyrazole), 9.38 (s, 1H, -NH imidazole). IR (KBr) (cm^{-1}): 3315 (NH str), 3082 (CH str), 1692, 1688 (C=N str), ESI MS (m/z): 650 (M^+).

2-(4,5-bis(3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl)-1H-imidazol-2-yl)pyrimidine (3c, $C_{37}H_{24}Br_2N_8$)

Yield 70%, orange solid, mp 265–267°C; R_f = 0.8 (pet ether:methanol (6:4)); ^{1}H-NMR (300 MHz, DMSO) δ (ppm): 6.64–6.69 (m, 1H, pyrimidine), 6.91–6.94, 7.12–7.15 (m, 2H, pyrimidine), 7.21–7.25, 8.12-8.15 (m, 10H, phenyl), 7.38–7.55 (m, 8H, phenyl), 8.29–8.34 (m, 2H, phenyl), 9.17 (s, 1H, pyrazole), 9.33 (s, 1H, pyrazole), 9.46 (s, 1H, -NH imidazole). IR (KBr) (cm^{-1}): 3300 (NH str), 3077 (CH str), 1682, 1677 (C=N str), ESI MS (m/z): 738 (M^+).

2-(4,5-bis(3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1H-imidazol-2-yl)pyrimidine (3d, $C_{37}H_{24}F_2N_8$)

Yield 67%, orange solid, mp 275–277°C; R_f = 0.65 (pet ether:methanol (6:4)); ^{1}H-NMR (300 MHz, DMSO) δ (ppm): 6.73–6.75 (m, 1H, pyrimidine), 6.83–6.92, 7.08–7.11 (m, 2H, pyrimidine), 7.19–7.23, 8.05–8.12 (m, 10H, phenyl), 7.38–7.61 (m, 8H, phenyl), 8.26–8.31 (m, 2H, phenyl), 9.15 (s, 1H, pyrazole), 9.24 (s, 1H, pyrazole), 9.40 (s, 1H, -NH imidazole). IR (KBr) (cm^{-1}): 3415 (NH str), 3067 (CHstr), 1695, 1682 (C=N str), ESI MS (m/z): 618 (M^+).

2-(4,5-bis(3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-1H-imidazol-2-yl)pyrimidine (3e, $C_{39}H_{30}N_8O_2$)

Yield 72%, orange solid, mp 270–272°C; R_f = 0.7 (pet ether:methanol (6:4)); ^{1}H-NMR (300 MHz, DMSO) δ (ppm): 3.61 (s, 3H, -OCH_3), 3.78 (s, 3H, -OCH_3), 6.69–6.72 (m, 1H, pyrimidine), 6.89–6.91, 7.05–7.07 (m, 2H, pyrimidine), 7.16–7.26, 8.01–8.09 (m, 8H, phenyl substituted with methoxy), 7.35–7.60 (m, 8H, phenyl), 8.24–8.30 (m, 2H, phenyl), 9.11 (s, 1H, pyrazole), 9.20 (s, 1H, pyrazole), 9.36 (s, 1H, -NH imidazole). IR (KBr) (cm^{-1}): 3433 (NH str), 3098 (CHstr), 2989 (CHstr), 1686, 1680 (C=N str), ESI MS (m/z): 642 (M^+).

Free radical scavenging effect

The scavenging effect of the title synthetic compounds 3(a-e) was screened by DPPH method.[23] Test compound in various concentrations (25, 50, 75, 100, and 250 mg/mL) with methanol (1 mL) was added into DPPH radical solution (4 mL, 0.125 mM). The mixture was shaken vigorously and incubated at room temperature for 30 min. The absorbance determined using Shimadzu UV-1601 spectrophotometer at 517 nm and percentage of inhibition (I%) of free radical production from DPPH was calculated by the following equation.

$$\%I = [(A_{control} - A_{sample})/A_{control})] \times 100$$

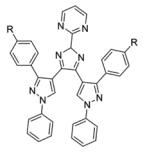
Where, $A_{control}$ is absorbance of DPPH methanol solution and A_{sample} is absorbance of test compound. Tests were carried out in triplicate and average.

RESULTS AND DISCUSSION

Chemistry

The synthetic schemes for the synthesis of 3(a-e) compounds are represented in Scheme 1. The intermediates 3-(4-substitutedphenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde 2(a-e) were synthesized according to the given procedure.[24] The compound 3(a-e) were prepared in one-pot synthesis from already prepared intermediates 2(a-e) with pyrimidine-2-carboximidamide under reflux in solvent DMF for more than 6 h.

Table 1:The compounds 3(a-e) with their scavenging effect on diphenyl-2-picryl-hydrazyl radical and IC_{50} values



Compd.	R	Concentration (µg/mL)			IC_{50} (µg/mL)
		50	75	100	
3a	-H	37.61	40.69	44.23	--
3b	-Cl	57.23	60.66	64.29	43.80
3c	-Br	45.82	51.62	55.71	69.02
3d	-F	44.52	48.96	53.51	81.58
3e	$-OCH_3$	60.56	63.91	65.34	41.24
Ascorbic acid		47.22	54.37	57.98	58.83

The FTIR bands at 3433, 3098, 2989, 1686, and 1680 confirm the presence of functioned group present in the potent compound 3e. The NMR singlet peaks at 9.36 confirmed for imidazole proton while singlet peaks at 9.20 and 9.11 confirm for the two protons of the pyrazole rings. Further, the peaks at 6.69–8.30 correspond for the aromatic protons. A distinct three protons each of the two methoxy groups are found to be at 3.61 and 3.78. The structures purity was confirmed through the melting points and single spot in TLC. The proposed mechanism of the final compounds is represented in Figure 1.

Free radical scavenging effect

Scavenging effect of the synthesized compounds was carried out on DPPH radical using ascorbic acid as standard. The results are represented in Table 1. Compounds 3b and 3e showed most promising antioxidant activities. The synthesized compounds have many electrons donating nitrogen and especially the nitrogen of the pyrazole ring after donating electron may exist in stabilized form due to conjugation effect in the structure. The compound 3e substituted with methoxy group to the *para* position of the phenyl attached to the pyrazole ring (IC_{50} = 41.24) showed the most potent activity followed by compound 3b (IC_{50} = 43.80) having *para* chloro group. The reasoned for the most potent activity of compound 3e is due to extended conjugation by the methoxy group, whereas the compound 3b showed activity because of weak donating effect of the chloro group toward the ring.

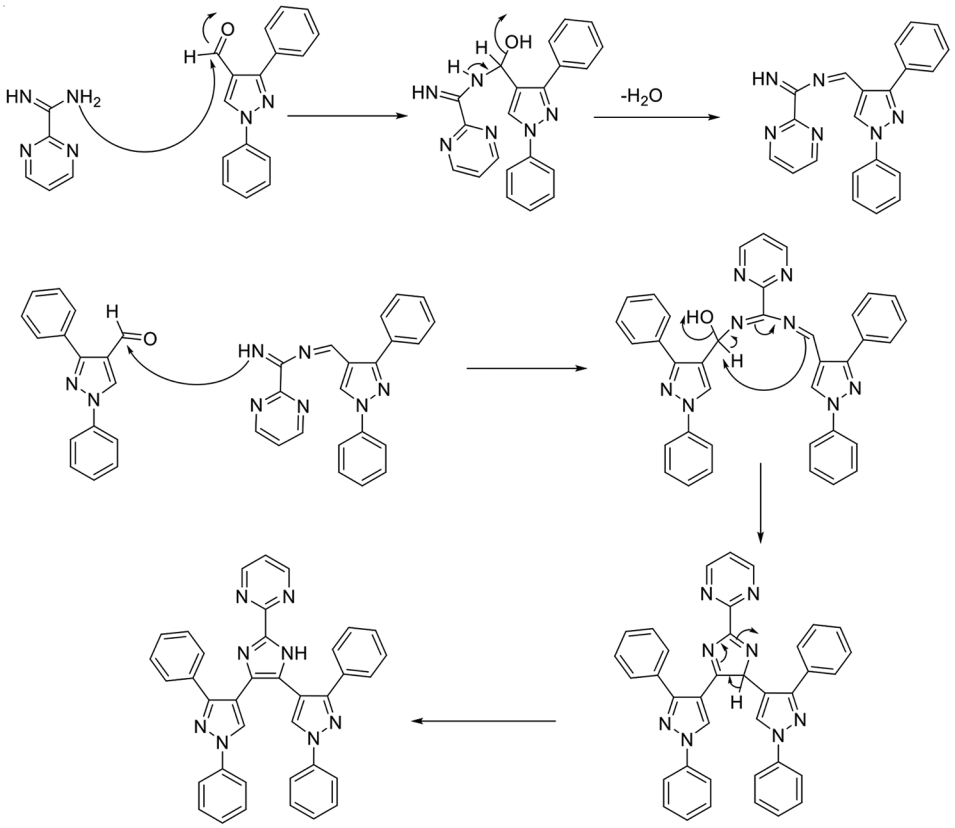
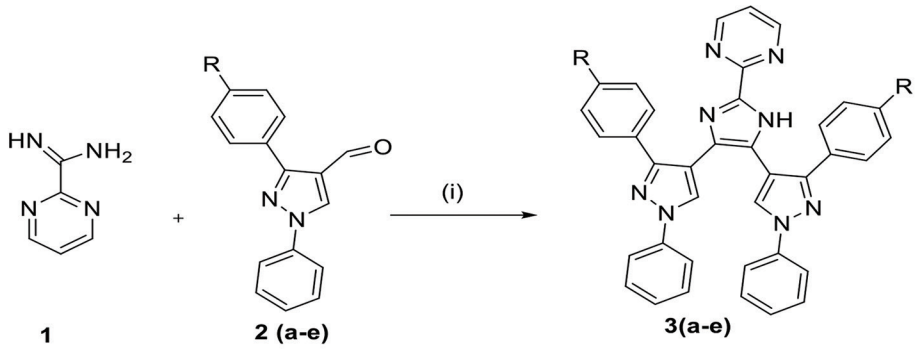


Figure 1: Proposed mechanism of the final compounds



Reaction and Condition:
(i) DMF, reflux at 110°C for 6 hr

Scheme 1. Protocol For Synthesis of Derivatives

Scheme 1: Protocol for synthesis of derivatives

CONCLUSION

A series of pyrazole clubbed pyrimidine with imidazole as linker were synthesized, having substituted aryl at the 3rd position of pyrazole. The compounds were tested for their antioxidant activity. The results showed potent antioxidant activity particularly with compound 3e and 3b with an IC_{50} value of 41.24 and 43.80. Other compounds 3c and 3d also showed moderate activity as compared to standard ascorbic acid. These compounds possess electron-donating nitrogen in the imidazole, pyrazole, and pyrimidine rings. Thus, the compounds have the potential to exist in radical form due to conjugation effect present in the structure. Compounds with methoxy and chloro group showed most potent effects due to their capacity to participate in extended conjugation.

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