Biochemistry & Analytical Biochemistry
ISSN: 2161-1009

Shantharam et al., Biochem Anal Biochem 2017, 6:2
DOI: 10.4172/2161-1009.1000314

Research Article Open Access

Synthesis and SAR Studies of Potent Antioxidant and Anti-Inflammatory Activities of Imidazole Derived Schiff Base Analogues

Shantharam CS[1], Swaroopa M[2], Darshini N[3], Mallesha N[3]* and Rakesh KP[3]*

[1]Department of Chemistry, Pooja Bhagavath Memorial Mahajana Education Centre, Mysore-570016, Karnataka, India
[2]Department of Pharmaceutical Chemistry, Bharathi College of Pharmacy, Mandya-571422, Karnataka, India
[3]Sri Ram Chem, R & D Centre, Plot No 31, JCK Industrial Park, Belagola Industrial Estate, Mysore-570016, Karnataka, India

Abstract

A novel sequence of imidazole derived Schiff base analogues 4-23 were synthesized and characterized by spectroscopic and analytical techniques. The *in vitro* antioxidant activities of these compounds were evaluated by using DPPH, ABTS and DMPD assay. The results exposed that the IC_{50} of compounds 9, 10, 11, 15, 16, 22 and 23 were lower than that of standards in all the three tested antioxidant assays indicating good activities of these compounds. *In vitro* anti-inflammatory activities of the synthesized compounds were tested and the outcomes of results were confirmed that the compounds 5, 6, 7, 8, 12, 13, 14 and 21 exhibited excellent anti-inflammatory activity. Preliminary structure-activity relationship revealed that the compounds 9, 10, 11, 15, 16, 22 and 23 with electron donating moiety (OH, OCH_3) were found to be excellent antioxidants and compounds 5, 6, 7, 8, 12, 13, 14 and 21 with electron withdrawing moiety (Cl, F, NO_2 and Br) were found to be excellent anti-inflammatory agents.

Keywords: Imidazole; Anti-oxidants; Anti-inflammatory activity; Electronic properties

Introduction

Medicinal chemistry is an interdependenty established science that encompasses the innovation, progress, recognition and revelation of the mode of action of biologically dynamic compounds at the molecular level [1]. Heterocycles form by far the most of classical divisions of organic chemistry and are of vast use in biologically and industrially. Heterocyclic nucleus imparts an essential function in medicinal chemistry and serves as a key template for the improvement of various therapeutic agents [2]. Imidazoles have in use a sole arrangement in heterocyclic chemistry, and its derivatives have attracted significant interests in recent years for their multipurpose properties in chemistry and pharmacology. It improves pharmacokinetic characteristics of pilot analogues and thus is used as a remedy to optimize solubility and bioavailability parameters of projected unsuccessfully soluble lead molecules. The imidazole derivatives possess extensive spectrum of biological activities such as anti-inflammatory [3], anti-oxidant [4], anti-bacterial [5], anti-cancer [6], anti-tubercular [7] and anti-HIV [8] activities etc.

Literature Review

Antioxidant participate a fundamental role in the defense mechanism against oxidative damage induced by free radicals and reactive oxygen species (ROS). Reasonable ROS invention and detoxification in a common cellular metabolism is significant to keep the mammalian cells in healthy condition. When a cell fails to detoxify the excessive ROS generated as a result of destructive species or low level of antioxidants, they enter into a state of oxidative stress and are smashed [9]. High levels of ROS can cause injure to cell arrangement, nucleic acids, membrane lipids and proteins [10]. They in addition damage purine and pyrimidine bases of DNA molecule, thus most important to mutation [11]. Oxidative stress on a cell due to high level of ROS can lead to several disorders including cancer, neurodegenerative disorder, atherosclerosis and aging [12]. A lot of studies have recommended that antioxidants that can deactivate free radicals may be of essential interest in the preclusion of vascular diseases and some forms of cancer [13].

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly approved medications in the earth. They are mainly used for the treatment of pain, fever and inflammation, particularly arthritis [14]. Rheumatic diseases are the most common causes of disability in European countries and NSAIDs are still commonly used remedies. Chronic use might cause several serious adverse effects, the most important one being gastric injury and renal complications. Gastro-intestinal (GI) injure from NSAIDs is usually recognized to two factors: local irritation by the straight contact of the free carboxylic acid (COOH) moiety of NSAIDs with GI mucosal cells and decreased tissue prostaglandin invention in tissues [15].

In view of above facts and in extension of our drug development series [16-18], the present work involves the synthesis of a new sequence of imidazole derived Schiff's bases as potential anti-inflammatory and antioxidants. A number of reported effective antioxidant and anti-inflammatory activities of Schiff's bases are shown in Figure 1 [19-21].

Results and Discussion

Chemistry

Syntheses of title compounds were achieved according to the steps illustrated in Scheme 1. 4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carboxylic acid (1) were ethylated using TMS-Cl and ethanol at room temperature, which upon reaction with excess of hydrazine hydrate afforded the corresponding imidazole hydrazide

***Corresponding authors:** Mallesha N, Sri Ram Chem, R & D Centre, Plot No 31, JCK Industrial Park, Belagola Industrial Estate, Mysore-570016, Karnataka, India, Tel: +91-821-4255588; E-mail: research@sriramchem.com

Rakesh KP, Sri Ram Chem, R & D Centre, Plot No 31, JCK Industrial Park, Belagola Industrial Estate, Mysore-570016, Karnataka, India, Tel: +91-821-4255588; E-mail: rakeshasg@gmail.com

Received: December 26, 2016; **Accepted:** April 17, 2017; **Published** April 20, 2017

Citation: Shantharam CS, Swaroopa M, Darshini N, Mallesha N, Rakesh KP (2017) Synthesis and SAR Studies of Potent Antioxidant and Anti-Inflammatory Activities of Imidazole Derived Schiff Base Analogues. Biochem Anal Biochem 6: 314. doi: 10.4172/2161-1009.1000314

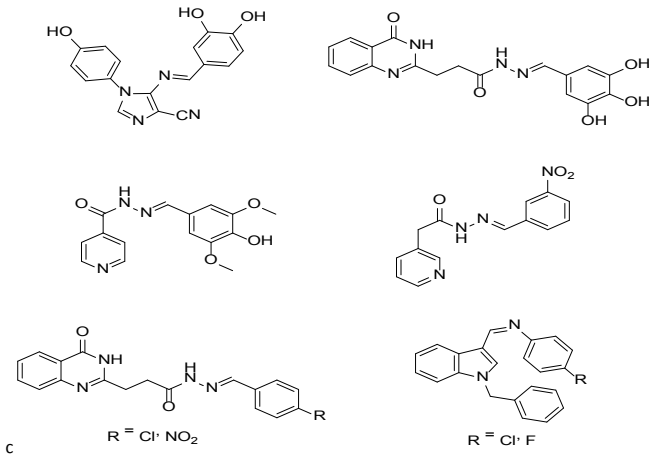
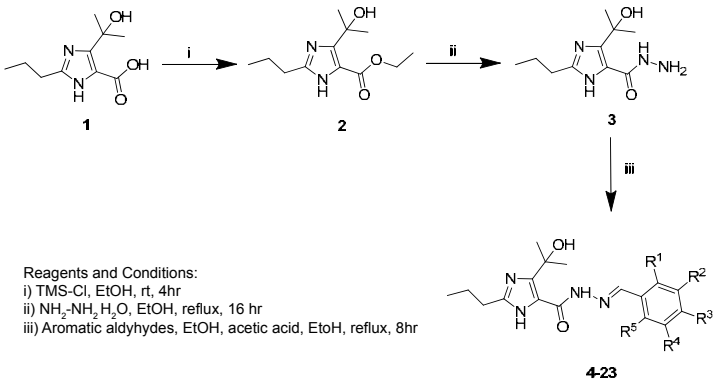


Figure 1: Some of the potent anti-oxidant and anti-inflammatory Schiff's base analogues.



Entry	R^1	R^2	R^3	R^4	R^5	Entry	R^1	R^2	R^3	R^4	R^5
4	H	H	H	H	H	14	NO_2	H	NO_2	H	H
5	H	H	Cl	H	H	15	H	OH	OH	H	H
6	H	H	NO_2	H	H	16	H	OCH_3	OCH_3	H	H
7	H	H	F	H	H	17	H	Br	OH	Br	H
8	H	H	Br	H	H	18	H	Br	OH	H	H
9	H	H	OCH_3	H	H	19	H	Br	OCH_3	H	H
10	H	H	OH	H	H	20	H	OCH_3	OH	Br	H
11	H	OCH_3	OH	H	H	21	Cl	H	H	H	F
12	Cl	H	Cl	H	H	22	H	OCH_3	OCH_3	OCH_3	H
13	F	H	F	H	H	23	H	OH	OH	OH	H

Scheme 1: Synthesis of target compounds 4-23.

(3). The Schiff's bases (4-23) were obtained by reacting 3 with several aromatic aldehydes in presence of catalytic amount of glacial acetic acid. All the analogues were obtained in good yield. The structures of all the synthesized compounds as well as intermediates were confirmed by IR, ^{1}HNMR, ^{13}CNMR and mass spectral analysis. The formations of ethyl ester (2) were confirmed by the appearance of a triplet at 1.30-1.33δ for ethyl CH_3 and multiplet at 4.30-4.35δ for ethyl CH_2 and absence of COOH proton peak at 11.80δ in ^{1}HNMR spectrum. In IR spectra, bands at 3334 and 3220cm^{-1} for NH_2-NH groups indicates the conversion of ethyl esters into hydrazides. The formation of Schiff's bases were confirmed by the presence of absorption at 1604-1635 for imines i.e., -N=CH- in IR spectra. The occurrence of all necessary peaks and lack of irrelevant peaks in ^{1}HNMR and ^{13}CNMR confirms the structures.

Biology

Antioxidant activities: *In vitro* antioxidant activities of all the synthesized compounds were evaluated by DPPH [22], ABTS[23] and DMPD[24] cation radical scavenging assay. A lower IC_{50} value indicated good antioxidant activity. The results were shown in Table 1. Some of the synthesized compounds showed strong antioxidant activities.

Entry	Antioxidant activity [a]			Anti-inflammatory activity [a] IC_{50} (μM)
	DPPH IC_{50} (μM)	**ABTS** IC_{50} (μM)	**DMPD** IC_{50} (μM)	
1	443 ± 2.01	424 ± 1.05	396 ± 0.46	-
2	375 ± 1.06	366 ± 1.05	358 ± 0.16	-
3	380 ± 1.65	389 ± 1.56	407 ± 2.31	407 ± 2.01
4	280 ± 1.26	267 ± 1.20	286 ± 1.65	267 ± 0.56
5	194 ± 0.23	177 ± 1.50	194 ± 1.89	100 ± 1.26
6	172 ± 0.55	189 ± 0.26	167 ± 0.47	77 ± 0.45
7	186 ± 1.02	204 ± 1.26	192 ± 1.26	78 ± 0.16
8	162 ± 2.94	172 ± 2.18	192 ± 1.26	86 ± 1.05
9	110 ± 1.02	122 ± 1.46	116 ± 0.45	174 ± 1.08
10	90 ± 1 .04	109 ± 0.49	103 ± 0.45	206 ± 1.04
11	50 ± 0.46	72 ± 1.48	61 ± 0.46	161 ± 1.07
12	182 ± 1.07	193 ± 0.14	203 ± 0.16	46 ± 1.26
13	222 ± 0.23	205 ± 1.56	205 ± 1.89	57 ± 1.45
14	183 ± 0.56	193 ± 0.56	198 ± 0.56	44 ± 0.17
15	57 ± 0.16	69 ± 0.18	57 ± 0.13	173 ± 0.14
16	58 ± 0.18	68 ± 0.43	64 ± 0.16	165 ± 0.19
17	155 ± 0.14	143 ± 1.04	147 ± 0.15	128 ± 0.11
18	151 ± 0.15	166 ± 1.06	156 ± 0.11	122 ± 0.19
19	141 ± 1.26	137 ± 0.17	142 ± 0.46	132 ± 0.14
20	118 ± 0.48	132 ± 1.09	136 ± 1.09	129 ± 0.18
21	196 ± 1.21	191 ± 1.04	213 ± 2.06	54 ± 0.10
22	49 ± 1.08	69 ± 0.43	54 ± 0.17	168 ± 0.12
23	44 ± 0.11	55 ± 0.64	49 ± 0.47	182 ± 0.19
BHT	116 ± 0.88	130 ± 1.04	127 ± 0.95	-
BHA	125 ± 0.88	121 ± 0.66	131 ± 0.28	-
Aspirin	-	-	-	200 ± 0.65
Indomethacin	-	-	-	112 ± 0.47

[a] Values are mean of three determinations, the ranges of which are <5% of the mean in all cases.

Table 1: Anti-oxidant and anti-inflammatory activities of the synthesized Imidazole-Schiff's base derivatives.

Compounds 9, 10, 11, 15, 16, 22 and 23 showed excellent radical scavenging activities with IC_{50} values 110 μM, 90 μM, 50 μM, 57 μM, 58 μM, 49 μM and 44 μM respectively in DPPH assay much better than the standards BHT (IC_{50}=116 μM) and BHA (IC_{50}=125 μM). In $ABTS^{+}$ radical scavenging assay, the compounds 9, 10, 11, 15, 16, 22 and 23 showed potent antioxidant activity with IC_{50} values 122 μg/mL, 109 μg/mL, 72 μg/mL, 69 μg/mL, 68 μg/mL, 69 μg/mL and 55 μg/mL respectively which is much better than the commercial standards BHT (IC_{50}=130 μM) and BHA (IC_{50}=121 μM). The compounds 9, 10, 11, 15, 16, 22 and 23 also exhibited potent antioxidant activity with IC_{50} values 116 μM, 103 μM, 61 μM, 57 μM, 64 μM, 54 μM and 49 μM respectively which is better than the standards BHT (IC_{50}=127 μM) and BHA (IC_{50}=131 μM) in DMPD assay.

In outlook of the above points, compounds having –OH (phenolic) and $-OCH_3$ (anisole) groups in the phenyl ring (9, 10, 11, 15, 16, 22 and 23) were found to be the most potent antioxidants. In phenyl ring the number of hydroxy and methoxy group increases the activities also increases [16]. The compounds with electron withdrawing Cl, F, NO_2 and Br substituents (5-8, 12-14 and 21) showed least antioxidants activity and IC_{50} values are higher than the standards. The compounds 17-20 were containing both electron donating and withdrawing groups shown that average activity.

Anti-inflammatory activity: *In vitro* anti-inflammatory activities of the synthesized compounds were also evaluated by using well-known literature procedure in human erythrocytes [23-25]. A huge number of compounds have been recognized exhibiting excellent to moderate activity compared to standard drugs aspirin and indomethacin. IC_{50} was determined for the compounds showing more than 50% inhibition concentration (Table 1). The compounds 5, 6, 7, 8, 12, 13, 14 and 21 showed excellent activity with IC_{50} values 100 μM, 77 μM, 78 μM, 86 μM, 46 μM, 57 μM, 44 μM and 54 μM respectively much better than the standard aspirin (IC_{50}=200 μM) and Indomethacin (IC_{50}=112 μM). Other compounds 4, 9-11, 15-20, 22 and 23 showed least and moderate activity. It is clear from the results that the compounds bearing electron withdrawing groups Cl, F, NO_2 and Br (5, 6, 7, 8, 12, 13, 14 and 21) are excellent anti-inflammatory agents and electron donating (OH and OCH_3) groups are least anti-inflammatory activity [16]. In phenyl ring the number of electron withdrawing group increases the anti-inflammatory activity also increases.

Experimental Data

General process

All necessary chemicals and reagents obtained from Merck (India) and Avra Synthesis (India) were used without further purification. Melting points were determined on a Thermonik melting point apparatus (Mumbai) and are uncorrected. FT-IR was performed using a Jasco spectrometer (Japan) using nujol media. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Agilent Technologies (USA) using DMSO (d_6) as solvent. High resolution mass spectroscopic analysis was performed on a Bruker MicroTOF QII mass spectrometer in positive mode. Development of the reaction was monitored by aluminum coated TLC plates with the solvent system comprising chloroform/methanol in the ratio 98:02 (R_f^a) and 95:05 (R_f^b) and the compounds on the TLC plates were detected by under UV light.

Chemistry

5-(1- Hydroxy-1- methyl-ethyl)- 2-propyl- 3H-imidazole-4-carboxylic acid ethyl ester (2)

To a solution of imidazole (10.6g, 0.05 mol) in ethanol (100 mL), trimethylsilylchloride (5.4g, 0.05 mol) was added slowly. The reaction mixture was stirred for 4 hrs to complete the reaction (monitored by TLC). The solvent was removed under reduced pressure and the resultant precipitate was washed with ice cold water and filtered to yield the desired products **2.** Yield 90.9%, R_f^a = 0.71, R_f^b = 0.8, m.p. 184-185 °C, IR KBr (cm^{-1}): 1750, 3214, 3315, 3510; ^{1}H NMR (DMSO-d_6) δ ppm: 0.91-0.95 (t, 3H, CH_3); 1.30 (t, 3H, CH_3); 1.59 (s, 6H, $2CH_3$); 1.68-1.73 (m, 2H, CH_2); 2.62-2.66 (t, 2H, CH_2); 4.30-4.35 (t, 2H, CH_2); 5.84 (s, 1H, OH); 9.9 (s, 1H, ring NH), HRMS m/z: 241.1750 [M+1]

5-(1- Hydroxy-1- methyl-ethyl)- 2-propyl- 3H-imidazole- 4-carboxylic acid hydrazide (3)

To a solution of **2** (10.5g, 0.043 mol) in ethanol (100 mL), hydrazine hydrate (2.6g, 0.052 mol,) was added. The reaction mixture was refluxed for 16 hrs for completion of the reaction (monitored by TLC). The solvent was removed under reduced pressure and cooled by adding ice cold water. The resulting precipitate was filtered, washed with cold water and recrystallized from ethanol to get the desired compounds **3.** Yield 85%, R_f^a = 0.39, R_f^b = 0.42, m.p. 201-202 °C, IR KBr (cm^{-1}): 1614, 1750, 3214, 3315, 3510; ^{1}H NMR (DMSO-d_6) δ ppm: 0.97 (t, 3H, CH_3); 1.58 (s, 6H, $2CH_3$); 1.67-1.69 (m, 2H, CH_2); 2.58-2.62 (t, 2H, CH_2); 4.09-4.11 (d, 2H, NH_2); 7.09 (s, 1H, OH); 8.37 (t, 1H, NH); 8.84 (s, 1H, ring NH); HRMS m/z, (M+1): 227.1758

General procedure for the synthesis of Schiff's bases (4-23)

Compounds 3 (1 mmol) was dissolved in ethanol (10 mL/g of compound) and treated with appropriate aldehydes (1 mmol) in the presence of catalytic amount of glacial acetic acid. The reaction mass were refluxed for 7–8 hr and the end of reaction was monitored by TLC. After end of the reaction, the solvent was removed under reduced pressure and cooled by adding ice cold water. The resulting precipitate was filtered, washed with water and recrystallized from ethanol to obtain the desired Schiff's bases (4-23).

***N′*-Benzylidene-4-(2-hydroxyprpan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (4)**

Yield 88.40%, R_f^a = 0.64, R_f^b = 0.71, m.p. 189-190 °C, IR KBr (cm^{-1}): 1614, 1750, 3214, 3315, 3510; ^{1}H NMR (DMSO-d_6) δ ppm: 0.98 (s, 3H, CH_3), 1.27 (s, 6H, $(CH_3)_2$), 1.71 (m, 2H, CH_2), 2.92 (t, *J*=7.2 Hz, 2H, CH_2), 7.41-7.85 (m, 5H, Ar-H), 7.71 (s, 1H, -N=CH), 8.14 (s, 1H, OH), 9.70 (s, 1H, NH), 10.41 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.9, 24.1, 29.8, 31.3, 76.2, 128.6, 129.4, 131.1, 133.7, 136.4, 142.8, 143.6, 157.1, 160.1; HRMS m/z: 315.1548 [M+1]

***N′*-(4-Chlorobenzylidene-4-(2-hydroxyprpan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (5)**

Yield 91.10%, R_f^a = 0.52, R_f^b = 0.56, m.p. 159-160 °C, IR KBr (cm^{-1}): 1619, 1768, 3215, 3320, 3560; ^{1}H NMR (DMSO-d_6) δ ppm: 0.90 (s, 3H, CH_3), 1.29 (s, 6H, $(CH_3)_2$), 1.75 (m, 2H, CH_2), 2.87 (t, *J*=6.8 Hz, 2H, CH_2), 7.32-7.80 (m, 4H, Ar-H), 7.88 (s, 1H, -N=CH), 9.01 (s, 1H, OH), 10.21 (s, 1H, NH), 11.52 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.5, 24.4, 29.5, 31.8, 75.9, 128.9, 129.8, 132.1, 136.0, 136.9, 143.1, 144.2, 157.2, 160.1; HRMS m/z: 349.1236 [M+1], 351.6245 [M+3]

***N′*-(4-Nitrobenzylidene-4-(2-hydroxyprpan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (6)**

Yield 86.23%, R_f^a = 0.42, R_f^b = 0.50, m.p. 172-174 °C, IR KBr (cm^{-1}): 1606, 1730, 3212, 3355, 3588; ^{1}H NMR (DMSO-d_6) δ ppm: 0.85 (s, 3H, CH_3), 1.30 (s, 6H, $(CH_3)_2$), 1.74 (m, 2H, CH_2), 2.72 (t, *J*=7.6 Hz, 2H, CH_2), 7.60-8.12 (m, 4H, Ar-H), 7.88(s, 1H, -N=CH), 8.90 (s, 1H, OH), 10.09 (s, 1H, NH), 11.12 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.6, 23.9, 28.6, 31.4, 76.2, 124.6, 125.7, 130.1, 136.1, 143.0, 143.9, 151.3, 157.8, 159.9; HRMS m/z: 360.6215 [M+1]

***N′*-(4-Fluorobenzylidene-4-(2-hydroxyprpan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (7)**

Yield 88.10%, R_f^a = 0.49, R_f^b = 0.51, m.p. 168-169 °C, IR KBr (cm^{-1}): 1610, 1770, 3250, 3370, 3568; ^{1}H NMR (DMSO-d_6) δ ppm: 0.88 (s, 3H, CH_3), 1.27 (s, 6H, $(CH_3)_2$), 1.77 (m, 2H, CH_2), 2.80 (t, *J*=8.2 Hz, 2H, CH_2), 7.12-7.42 (m, 4H, Ar-H), 7.95 (s, 1H, -N=CH), 8.51 (s, 1H, OH), 10.17 (s, 1H, NH), 11.30 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 12.9, 23.8, 29.1, 31.6, 75.8, 115.6, 128.7, 129.1, 136.7, 142.1, 144.5, 157.0, 157.9, 164.1; HRMS m/z: 333.4512 [M+1]

***N′*-(4-Bromobenzylidene-4-(2-hydroxyprpan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (8)**

Yield 86.20%, R_f^a = 0.39, R_f^b = 0.42, m.p. 185-187 °C, IR KBr (cm^{-1}): 1628, 1745, 3269, 3377, 3590; ^{1}H NMR (DMSO-d_6) δ ppm: 0.88 (s, 3H, CH_3), 1.32 (s, 6H, $(CH_3)_2$), 1.83 (m, 2H, CH_2), 2.69 (t, *J*=7.6 Hz, 2H, CH_2), 7.55-7.80 (m, 4H, Ar-H), 7.99(s, 1H, -N=CH), 8.79 (s, 1H, OH), 10.56 (s, 1H, NH), 11.31 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.0, 24.1, 28.5, 30.5, 76.0, 124.9, 125.6, 131.1, 132.6, 136.5, 142.0, 143.5, 156.9, 160.1; HRMS m/z: 394.1254 [M+1], 396.5642 [M+3]

4-(2-Hydroxypropan-2-yl)-*N′*-(4-methoxybenzylidene--2-propyl-1*H*-imidazole-5-carbohydrazide (9)

Yield 85.24%, R_f^a = 0.45, R_f^b = 0.51, m.p. 180-182 °C, IR KBr (cm^{-1}): 1606, 1733, 3310, 3395, 3555; ^{1}H NMR (DMSO-d_6) δ ppm: 0.92 (s, 3H, CH_3), 1.35 (s, 6H, $(CH_3)_2$), 1.90 (m, 2H, CH_2), 2.88 (t, *J*=6.8 Hz, 2H, CH_2), 3.78 (s, 3H, OMe), 7.10-7.82 (m, 4H, Ar-H), 7.91 (s, 1H, -N=CH), 8.56 (s, 1H, OH), 10.68 (s, 1H, NH), 11.12 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.5, 24.3, 29.4, 31.6, 55.6, 75.9, 114.9, 125.5, 130.6, 136.5, 142.8, 144.1, 157.0, 160.3, 163.5; HRMS m/z: 345.1254 [M+1]

***N′*-(4-Hydroxybenzylidene-4-(2-hydroxyprpan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (10)**

Yield 90.56%, R_f^a = 0.35, R_f^b = 0.39, m.p. 166-168 °C, IR KBr (cm^{-1}): 1610, 1740, 3260, 3380, 3565, 3590; ^{1}H NMR (DMSO-d_6) δ ppm: 0.90 (s, 3H, CH_3), 1.35 (s, 6H, $(CH_3)_2$), 1.90 (m, 2H, CH_2), 2.87 (t, *J*=7.0 Hz, 2H, CH_2), 6.80-7.45 (m, 4H, Ar-H), 7.88 (s, 1H, -N=CH), 8.90 (s, 1H, OH), 9.20 (s, 1H, OH), 10.61 (s, 1H, NH), 11.03 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.7, 24.6, 29.5, 31.3, 76.8, 116.9, 126.5, 130.2, 136.1, 142.5, 144.6, 157.5, 160.0, 160.9; HRMS m/z: 331.2364 [M+1]

***N′*-(4-Hydroxy-3-methoxybenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (11)**

Yield 82.15%, R_f^a = 0.41, R_f^b = 0.47, m.p. 158-160 °C, IR KBr (cm^{-1}): 1612, 1780, 3336, 3341, 3562, 3585; ^{1}H NMR (DMSO-d_6) δ ppm: 0.88 (s, 3H, CH_3), 1.31 (s, 6H, $(CH_3)_2$), 1.86 (m, 2H, CH_2), 2.81 (t, *J*=7.8 Hz, 2H, CH_2), 3.78 (s, 3H, OMe), 6.91-7.62 (m, 3H, Ar-H), 8.02 (s, 1H, -N=CH), 9.12 (s, 1H, OH), 9.45 (s, 1H, OH), 10.37 (s, 1H, NH), 11.16 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 14.1, 25.2, 30.3, 31.0, 56.2, 76.1, 112.1, 117.0, 122.6, 130.6, 136.1, 143.5, 144.3, 149.8, 151.6, 157.6, 160.1; HRMS m/z: 361.4562 [M+1]

***N′*-(2,4-dichlorobenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (12)**

Yield 87.28%, R_f^a = 0.43, R_f^b = 0.50, m.p. 175-176 °C, IR KBr (cm^{-1}):

1622, 1758, 3312, 3379, 3545; ^{1}H NMR (DMSO-d_6) δ ppm: 0.89 (s, 3H, CH_3), 1.35 (s, 6H, $(CH_3)_2$), 1.80 (m, 2H, CH_2), 2.84 (t, *J*=6.8 Hz, 2H, CH_2), 7.20-7.82 (m, 3H, Ar-H), 8.10 (s, 1H, -N=CH), 9.52 (s, 1H, OH), 10.52 (s, 1H, NH), 11.13 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.3, 24.5, 30.6, 31.4, 76.3, 126.9, 128.2, 129.4, 129.9, 131.4, 132.0, 136.1, 140.5, 142.3, 157.6, 160.3; HRMS m/z: 384.1546 [M+1], 386.4569 [M+3]

N′-(2,4-difluorobenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (13)

Yield 84.20%, R_f^a = 0.46, R_f^b = 0.52, m.p. 181-182 °C, IR KBr (cm^{-1}): 1630, 1755, 3324, 3318, 3569; ^{1}H NMR (DMSO-d_6) δ ppm: 0.85 (s, 3H, CH_3), 1.32 (s, 6H, $(CH_3)_2$), 1.83 (m, 2H, CH_2), 2.79 (t, *J*=7.0 Hz, 2H, CH_2), 6.92 (s, 1H, Ar-H), 7.13-7.70 (m, 2H, Ar-H), 7.84 (s, 1H, -N=CH), 8.99 (s, 1H, OH), 10.60 (s, 1H, NH), 11.17 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 14.3, 24.9, 29.5, 31.1, 76.1, 111.3, 112.9, 113.4, 132.1, 136.4, 142.8, 143.6, 157.2, 160.3, 161.4, 163.2; HRMS m/z: 351.4521 [M+1]

N′-(2,4-Dinitrobenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (14)

Yield 81.98%, R_f^a = 0.51, R_f^b = 0.57, m.p. 191-192 °C, IR KBr (cm^{-1}): 1622, 1768, 3375, 3398, 3514; ^{1}H NMR (DMSO-d_6) δ ppm: 0.89 (s, 3H, CH_3), 1.29 (s, 6H, $(CH_3)_2$), 1.77 (m, 2H, CH_2), 2.86 (t, *J*=7.8 Hz, 2H, CH_2), 7.92 (s, 1H, N=CH), 8.20-840 (m, 2H, Ar-H), 8.84 (s, 1H, Ar-H), 9.99 (s, 1H, OH), 10.29 (s, 1H, NH), 11.03 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.9, 23.9, 29.3, 31.5, 76.4, 120.5, 130.4, 131.2, 133.1, 136.1, 142.4, 143.9, 148.2, 151.6, 157.2, 160.3; HRMS m/z: 405.3542 [M+1]

N′-(3,4-Dihydroxybenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (15)

Yield 86.77%, R_f^a = 0.32, R_f^b = 0.37, m.p. 166-167 °C, IR KBr (cm^{-1}): 1610, 1710, 3318, 3374, 3520, 3599; ^{1}H NMR (DMSO-d_6) δ ppm: 0.84 (s, 3H, CH_3), 1.22 (s, 6H, $(CH_3)_2$), 1.82 (m, 2H, CH_2), 2.78 (t, *J*=7.4 Hz, 2H, CH_2), 6.80-7.45 (m, 3H, Ar-H), 7.88 (s, 1H, -N=CH), 8.84 (s, 1H, 0H), 9.57 (s, 2H, OH), 10.56 (s, 1H, NH), 11.09 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.8, 23.7, 29.3, 31.0, 76.1, 116.2, 118.3, 123.5, 131.1, 136.3, 142.7, 143.1, 148.1, 151.0, 157.3, 160.7; HRMS m/z: 347.6524 [M+1]

N′-(3,4-Dimethoxybenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (16)

Yield 83.15%, R_f^a = 0.44, R_f^b = 0.51, m.p. 157-158 °C, IR KBr (cm^{-1}): 1612, 1770, 3317, 3384, 3566; ^{1}H NMR (DMSO-d_6) δ ppm: 0.89 (s, 3H, CH_3), 1.20 (s, 6H, $(CH_3)_2$), 1.90 (m, 2H, CH_2), 2.90 (t, *J*=6.6 Hz, 2H, CH_2), 3.82 (s, 6H, 2OMe), 6.92-7.51 (m, 3H, Ar-H), 7.93 (s, 1H, -N=CH), 8.99 (s, 1H, OH), 10.12 (s, 1H, NH), 11.16 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.9, 23.8 29.4, 31.4, 56.4, 76.7, 109.3, 111.3, 122.6, 130.8, 136.8, 142.8, 144.2, 150.1, 152.4, 157.8, 160.4; HRMS m/z: 375.2654 [M+1]

N′-(3,5-Dibromo-4-hydroxybenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (17)

Yield 85.10%, R_f^a = 0.40, R_f^b = 0.44, m.p. 168-169 °C, IR KBr (cm^{-1}): 1608, 1778, 3330, 3398, 3547; ^{1}H NMR (DMSO-d_6) δ ppm: 0.93 (s, 3H, CH_3), 1.28 (s, 6H, $(CH_3)_2$), 1.94 (m, 2H, CH_2), 2.84 (t, *J*=6.8 Hz, 2H, CH_2), 7.60-7.72 (m, 2H, Ar-H), 7.88 (s, 1H, -N=CH), 8.78 (s, 1H, OH), 9.45 (s, 1H, OH), 10.19 (s, 1H, NH), 11.12 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.4, 23.6 29.5, 31.3, 76.3, 110.3, 129.3, 130.6, 136.8, 142.8, 145.8, 157.2, 158.1, 160.6; HRMS m/z: 489.2314 [M+1], 491.2654 [M+3]

N′-(3-Bromo-4-hydroxybenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (18)

Yield 87.10%, R_f^a = 0.46, R_f^b = 0.51, m.p. 174-175 °C, IR KBr (cm^{-1}): 1616, 1788, 3320, 3399, 3565; ^{1}H NMR (DMSO-d_6) δ ppm: 0.89 (s, 3H, CH_3), 1.33 (s, 6H, $(CH_3)_2$), 1.87 (m, 2H, CH_2), 2.74 (t, *J*=8.0 Hz, 2H, CH_2), 6.90-7.77 (m, 3H, Ar-H), 7.97 (s, 1H, -N=CH), 8.88 (s, 1H, OH), 9.45 (s, 1H, OH), 10.82 (s, 1H, NH), 11.13 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 14.4, 24.1 29.8, 31.6, 76.4, 113.3, 118.6, 128.4, 129.4, 130.4, 136.7, 142.7, 145.4, 157.4, 158.4, 160.9; HRMS m/z: 410.2654 [M+1], 412.2654 [M+3]

N′-(3-Bromo-4-methoxybenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (19)

Yield 88.17%, R_f^a = 0.51, R_f^b = 0.57, m.p. 179-181 °C, IR KBr (cm^{-1}): 1607, 1729, 3314, 3347, 3558; ^{1}H NMR (DMSO-d_6) δ ppm: 0.91 (s, 3H, CH_3), 1.37 (s, 6H, $(CH_3)_2$), 1.88 (m, 2H, CH_2), 2.82 (t, *J*=7.2 Hz, 2H, CH_2), 3.81 (s, 3H, OMe), 6.92-7.71 (m, 3H, Ar-H), 7.89 (s, 1H, -N=CH), 9.10 (s, 1H, OH), 10.22 (s, 1H, NH), 11.14 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.5, 24.3 29.7, 31.3, 56.4, 76.5, 111.3, 112.6, 128.1, 129.0, 129.4, 136.0, 142.3, 144.1, 157.1, 158.3, 160.6; HRMS m/z: 424.1264 [M+1], 426.4597 [M+3]

N′-(3-Bromo-4-hydroxy-5-methoxybenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (20)

Yield 89.27%, R_f^a = 0.42, R_f^b = 0.47, m.p. 165-168 °C, IR KBr (cm^{-1}): 1611, 1735, 3322, 3354, 3560; ^{1}H NMR (DMSO-d_6) δ ppm: 0.87 (s, 3H, CH_3), 1.34 (s, 6H, $(CH_3)_2$), 1.84 (m, 2H, CH_2), 2.77 (t, *J*=7.0 Hz, 2H, CH_2), 3.77 (s, 3H, OMe), 7.32-7.42 (m, 2H, Ar-H), 7.87 (s, 1H, -N=CH), 9.10 (s, 1H, OH), 9.88 (s, 1H, OH), 10.21 (s, 1H, NH), 11.22 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.2, 24.1 29.6, 31.8, 56.2, 76.1, 111.0, 114.4, 122.1, 129.9, 136.7, 142.1, 143.4, 145.6, 153.6, 157.4, 159.9; HRMS m/z: 440.1654 [M+1], 442.1564 [M+3]

N′-(2-Chloro-6-fluorobenzylidene)-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carbohydrazide (21)

Yield 86.38%, R_f^a = 0.52, R_f^b = 0.59, m.p. 170-171 °C, IR KBr (cm^{-1}): 1602, 1758, 3310, 3359, 3566; ^{1}H NMR (DMSO-d_6) δ ppm: 0.89 (s, 3H, CH_3), 1.39 (s, 6H, $(CH_3)_2$), 1.89 (m, 2H, CH_2), 2.90 (t, *J*=5.8 Hz, 2H, CH_2), 7.22-7.49 (m, 3H, Ar-H), 7.90 (s, 1H, -N=CH), 9.28 (s, 1H, OH), 10.12 (s, 1H, NH), 11.01 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.8, 24.3 29.1, 31.0, 76.0, 113.8 118.4, 125.7, 134.5, 135.4, 136.9, 142.0, 143.7, 156.5, 160.5, 161.5; HRMS m/z: 367.4521 [M+1], 369.2451 [M+3]

4-(2-hydroxypropan-2-yl)-2-propyl-N′-(3,4,5-trimethoxybenzylidene)-1*H*-imidazole-5-carbohydrazide (22)

Yield 85.41%, R_f^a = 0.42, R_f^b = 0.48, m.p. 168-169 °C, IR KBr (cm^{-1}): 1610, 1766, 3352, 3369, 3588; ^{1}H NMR (DMSO-d_6) δ ppm: 0.88 (s, 3H, CH_3), 1.36 (s, 6H, $(CH_3)_2$), 1.84 (m, 2H, CH_2), 2.77 (t, *J*=8.2 Hz, 2H, CH_2), 3.82 (s, 9H, 3OMe), 7.12-7.18 (m, 2H, Ar-H), 7.99 (s, 1H, -N=CH), 9.38 (s, 1H, OH), 10.11 (s, 1H, NH), 11.56 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.2, 24.8 29.7, 31.6, 56.2, 60.6, 76.7, 104.2, 128.4, 136.4, 141.5, 142.4, 144.9, 153.5, 156.0, 160.4; HRMS m/z: 405.1265 [M+1]

4-(2-hydroxypropan-2-yl)-2-propyl-N′-(3,4,5-trihydroxybenzylidene)-1*H*-imidazole-5-carbohydrazide (23)

Yield 84.09%, R_f^a = 0.30, R_f^b = 0.34, m.p. 174-175 °C, IR KBr (cm^{-1}): 1615, 1719, 3349, 3359, 3562; ^{1}H NMR (DMSO-d_6) δ ppm: 0.84

(s, 3H, CH_3), 1.30 (s, 6H, $(CH_3)_2$), 1.80 (m, 2H, CH_2), 2.81 (t, *J*=7.6 Hz, 2H, CH_2), 5.01 (s, 1H, OH), 6.88-7.10 (m, 2H, Ar-H), 7.78 (s, 1H, -N=CH), 8.12 (s, 2H, 2OH), 9.38 (s, 1H, OH), 10.11 (s, 1H, NH), 11.56 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 13.1, 24.5 29.6, 31.0, 76.4, 108.2, 129.4, 136.1, 138.5, 141.7, 143.3, 146.1, 157.5, 160.1; HRMS m/z: 363.1265 [M+1]

Biological Screening

Antioxidant activities

DPPH (1,1-diphenyl-2-picryl-hydrazyl) test [22]: The radical scavenging activity of DPPH free radicals by synthesized compounds was determined according to the reported method. Briefly, 50 µL of test compounds was mixed at different concentrations (20-100 µM/mL) with 1 mL of 0.1 mM DPPH in methanol solution and 450 µL of 50 mM Tris HCl buffer (pH 7.4) and as an experimental control methanol (50 µL) only was used. After 30 min of incubation at room temperature and reading was recorded spectrophotometrically at 517 nm.

Percent inhibition was calculated from the following equation:

$$\% \text{ Inhibition} = \left[\frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}}\right] \times 100$$

ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sufonic acid) test: The ability of the test sample to scavenge $ABTS^{\cdot+}$ radical cation was determined according to the literature method [23]. The $ABTS^{\cdot+}$ radical cation was pregenerated by mixing 7 mM $ABTS^{\cdot+}$ stock solution with 2.45 mM potassium persulfate and incubating for 12–16 hrs in the dark room at normal temperature till the colors changes from green to blue, then the absorbance was stable. Using distilled water, the absorbance of the $ABTS^{\cdot+}$ solution was equilibrated to 0.70 (± 0.02), then 2 mL was mixed with different concentration of the test sample (20 to 100 µg/mL) and after 6-8 min the absorbance was measured at 734 nm.

The scavenging effect of $ABTS^{\cdot+}$ radical was calculated using the following formula:

$$ABTS^{\cdot+} \text{ scavenging effect (\%)} = [(A_c - A_s)/A_c] \times 100$$

Where, A_c is the initial concentration of the $ABTS^{\cdot+}$ and A_s is the absorbance of the remaining concentration of $ABTS^{\cdot+}$ in the presence of compounds.

DMPD (*N, N*-dimethyl-*p*-phenylenediamine) test: The DMPD radical scavenging ability of synthesized compounds was determined by the Fogliano et al., method [24] with slight modification. The 5 mL solution of DMPD (105mg) in distilled water was prepared. Then, 1 mL of this solution was added to 100 mL of 0.1 M acetate buffer (pH 5.3). 0.3 mL ferric chloride (0.05 M) was to the solution to produce $DMPD^{\cdot+}$. Different concentrations of standard antioxidants or synthesized compounds (20-100 µg/mL) were added, and the total volume was adjusted to 1 mL with distilled water. 1 mL of the $DMPD^{\cdot+}$ solution was added to the reaction mixture. The reaction mixtures were incubated in the dark for 15 min. The absorbance was measured at 505 nm.

$$\% \text{ Inhibition} = \left[\frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}}\right] \times 100$$

Anti-inflammatory activity

Human erythrocyte suspension: The human blood was collected from a healthy volunteer who had not taken any NSAIDs for 2 weeks prior to the experiment and collected in heparinzed vacutainer. The collected healthy human blood was washed 0.9% saline and centrifuged for 10 minutes at 3000 rpm. The packed cells were washed with 0.9% saline and 40% v/v suspension made by isotonic phosphate buffer of 154 mM NaCl in 10 mM Sodium Phosphate Buffer at pH 7.4 used as Stock erythrocyte or RBC suspension.

Hypotonic solution-induced haemolysis: The activity of the synthesized compounds was performed according to the reported method.[25] The test sample consisted of stock erythrocyte (RBC) suspension 0.5 mL mixed with 5 mL of hypotonic solution (50 mM NaCl in 10 mM Sodium Phosphate Buffered saline at pH 7.4) containing different concentrations of sample (20, 40, 60, 80 and 100 µM/mL). The control consists of 0.5 mL RBC suspension mixed with 5 mL of hypotonic buffered solution alone. The mixtures were incubated for 10 minutes at room temperature, centrifuged for 10 minutes at 3000 rpm and supernatant was measured by spectrophotometrically at 540 nm. The % inhibition of haemolysis was calculated from the following formula.

$$\% \text{ Inhibition of haemolysis} = \left[\frac{A_1 - A_2}{A_1}\right] \times 100$$

Where:

A_1 = Absorbance of hypotonic buffered solution alone.

A_2 = Absorbance of test /standard sample in hypotonic solution.

Conclusion

In conclusion, we have synthesized a sequence of small and simple imidazole derived Schiff's base derivatives with various groups in benzene ring. All the synthesized analogues, compounds 9, 10, 11, 15, 16, 22 and 23 with OH and OCH_3 groups in benzene ring exhibited stronger radical scavenging activities than BHT and BHA in all the three assays performed. Compounds 5, 6, 7, 8, 12, 13, 14 and 21 with Cl, F, NO_2 and Br in benzene ring demonstrated excellent anti-inflammatory activity than aspirin and indomethacin.

Acknowledgement

All authors are thankful to Sri Ram Chem management for their continuous encouragement towards research.

References

1. Timmerman H (2007) Reflection of medicinal chemistry since the 1950s. Compr Med Chem 8: 7-15.
2. Kashyap SJ, Sharma PK, Garg VK, Dudhe R, Kumar N (2011) Review on synthesis and various biological potential of thiazolopyrimidine derivatives. J Adv Sci Res 2: 18-24.
3. Husain A, Drabu S, Kumar N, Alam MM, Bawa S (2013) Synthesis and biological evaluation of di- and tri-substituted imidazoles as safer anti-inflammatory-antifungal agents. J Pharm Bioallied Sci 5: 154-161.
4. Sorrenti V, Salerno L, Giacomo C, Acquaviva R, Siracusa MA, et al. (2006) Imidazole derivatives as antioxidants and selective inhibitors of Nnos. Nitric Oxide 14: 45-50.
5. Vijesh AM, Isloor AM, Telkar S, Peethambar SK, Rai S, et al. (2011) Synthesis, characterization and antimicrobial studies of some new pyrazole incorporated imidazole derivatives. Eur J Med Chem 46: 3531-3536.
6. Yang X, Wan W, Deng X, Li Y, Yang L, et al. (2012) Design, synthesis and cytotoxic activities of novel hybrid compounds between 2-phenylbenzofuran and imidazole. Bioorg Med Chem Lett 22: 2726-2729.
7. Lu X, Liu X, Wan B, Franzblau SG, Chen L, et al. (2012) Synthesis and evaluation of anti-tubercular and antibacterial activities of new 4-(2,6-dichlorobenzyloxy) phenyl thiazole, oxazole and imidazole derivatives. Part 2. Eur J Med Chem 49: 164-171.
8. Zhan P, Liu X, Zhu J, Fang Z, Li Z, et al. (2009) Synthesis and biological evaluation of imidazole thioacetanilides as novel non-nucleoside HIV-1 reverse transcriptase inhibitors. Bioorg Med Chem 17: 5775-5781.

9. Sorg O (2004) Oxidative stress: A theoretical model or a biological reality. Biol 327: 649-662.

10. Valko M, Rhodes CJ, Monocol J, Izakovic M, Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 160: 1-40.

11. Halliwell B, Gutteridge JMC (1999) Free Radicals in Biology and Medicine, (3rd edn) Oxford University Press.

12. Bandgar BP, Adsul LK, Chavan HV, Jalde SS, Shringare SN, et al. (2012) Synthesis, biological evaluation, and docking studies of 3-(substituted)-aryl-5-(9-methyl-3-carbazole)-1H-2-pyrazolines as potent anti-inflammatory and antioxidant agents. Bioorg Med Chem Lett 22: 5839-5844.

13. Nakayama T, Yamada M, Osawa T, Kawakishi S (1993) Suppression of active oxygen-induced cytotoxicity by flavonoids. Biochem Pharmacol 45: 265-267.

14. Palomer A, Cabre F, Pascual J, Campos J, Trugillo MA, et al. (2002) Identification of novel cyclooxygenase-2 selective inhibitors using pharmacophore models. J Med Chem 45: 1402-1411.

15. Gupta SPBN, Moorthy NSHN (2007) Synthesis and physicochemical characterization of mutual prodrug of indomethacin. Trends Appl Sci Res 2: 165-169.

16. Rakesh KP, Manukumar HM, Gowda DC (2015) Schiff's bases of quinazolinone derivatives: Synthesis and SAR studies of a novel series of potential anti-inflammatory and antioxidants Bioorg Med Chem Lett 25: 1072-1077.

17. Rakesh KP, Shantharam CS, Manukumar HM (2016) Synthesis and SAR studies of potent H+/K+-ATPase inhibitors of quinazolinone-Schiff's base analogues. Bioorg Chem 68: 1-8.

18. Rakesh KP, Suhas R, Chandan S, Manukumar HM, Gowda DC (2015) Quinazolinones linked amino acids derivatives as a new class of promising antimicrobial, antioxidant and anti-inflammatory agents. Eur J Chem 6: 254-260.

19. Kaur J, Bhardwaj A, Huany Z, Knaus EE (2012) N-1 and C-3 substituted indole Schiff bases as selective COX-2 inhibitors: synthesis and biological evaluation. Bioorg Med Chem Lett 22: 2154-2159.

20. Correia C, Leite C, Proenca MF, Carvalha MA (2014) Synthesis and radical scavenging activity of phenol-imidazole conjugates. Bioorg Med Chem Lett 24: 2768-2772.

21. Narang R, Sharma S, Narasimhan B (2012) Evaluation of Anti-inflammatory activity of acid Hydrazide derivatives. Hygeia J Drug Med 4: 15-20.

22. Blois MS (1958) Antioxidant determinations by the use of a stable free radical. Nature 181: 1199-1200.

23. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, et al. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Radical Bio Med 26: 1231-1237.

24. Fogliano V, Verde V, Randazzo G, Ritieni A (1999) Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. J Agric Food Chem 47: 1035-1040.

25. Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, et al. (1999) Membrane stabilizing activity: A possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. Fitoterapia 70: 251-257.