SYNTHESIS OF TETRASUBSTITUTD IMIDAZOLES CONTAINING INDOLE AND THEIR ANTIUREASE AND ANTIOXIDANT ACTIVITIES

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ABSTRACT

Indole-based tetraarylimidazoles were prepared by multicomponent reaction of substituted 2-arylindole-3-carbaldehydes, benzil, substituted anilines and ammonium acetate in acetic acid. The new compounds were evaluated for their antiurease and antioxidant activity. The synthesized compounds exhibited potent antiurease activity**.**

Keywords: Imidazole; Indole; antitumor; ammonium acetate.

INTRODUCTION

Indole skeleton is a building block of a number of natural and synthetic products found in plants and animals or synthesized in the laboratory. Indole derivatives fascinated chemists because of their significant range of pharmacological activities such as anticonvulsant[1], antitumor[2], anti-inflammatory[3], antimicrobial[4]and antifungal[5].

Further, it has been reported that substitution of different heterocyclic or aromatic moieties at 2 or 3- position of indole nucleus modulates various biological activities of such substituted indole derivatives[3]. The incorporation of the imidazole ring system is a synthetic approach in drug invention. The therapeutic properties of imidazole entity are a major source of attention for medicinal chemists. The imidazole ring system has received much attention because of its anti-inflammatory[6], antimicrobial[7], anticonvulsant[8], anticancer[9], analgesic[10], fungicidal[11] and antithrombotic activities[12]. Imidazole derivatives have also been reported in compounds which are used for electronics, photography and as fire retardants[13].

It is therefore thought worthwhile to synthesize some new indole derivatives by incorporating imidazole nucleus with the hope that it may exhibit still other interesting biological activities. We have recently reported the synthesis of a number of indole based triarylimidazoles[14] and tetraarylimidazoles[15] which possess excellent α-glucosidase inhibitory and antiurease activity respectively. In view of such importance of these imidazole and indole ring compounds, we would like to communicate our continuing efforts in preparing still more tetraarylimidazoles containing a 2-indol-3-yl substituents and their screening as antiurease and antioxidant agents.

Urease is an enzyme that catalyzes the hydrolysis of urea into ammonia and carbon dioxide. It is found in various types of bacteria, fungi and plants. It is the main source of pathology provoked by *Helicobacter pylori* (Gram negative bacterium) is found in the stomach and play an important part in the development of gastric and duodenal ulcers[16]. Urease has different functions and antiurease agents have received special interest over the past few years. Urease inhibitors are classified into two major classes of organic compounds such as Thiourea[17], Omeprazole[18] (imidazoles) (**Fig.1**), coumarins[19], quinines and triazoles[20] and as metal organic such as bishydrazone derivatives and its copper (II) complexes[21] (**Fig.2**) has been reported.

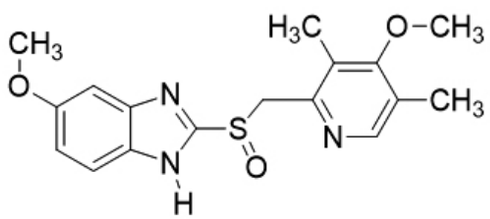

Fig.1 Omeprazole.

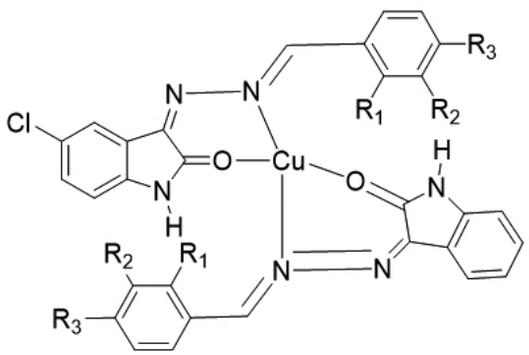

Fig.2 Structure of Cu(II) complexes with bishydrazones.

Antioxidant acts as a weapon to shield the body against oxidative stress. Antioxidants reduce degenerative diseases such as cancers and cardiovascular diseases by trapping free radicals. Certain imidazole derivatives[22] (**Fig.3**), indole derivatives[23] (**Fig.4**) and imidazolopyridinyl indoles[24] (**Fig.5**) have been found to possess antioxidant activity.

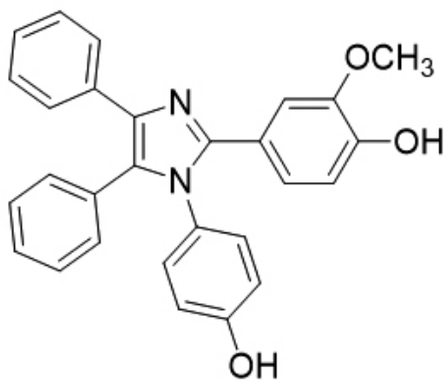

Fig.3 4-(1-(4-hydroxyphenyl) -4,5-diphenyl-1-1H- X=H,F R=Thiazolyl imidazole-2-yl)2-yl)-2-methoxyphenol.

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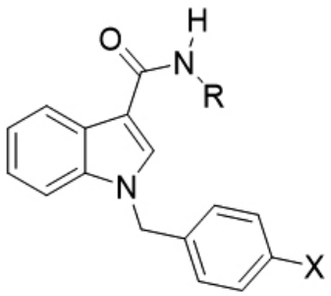


Fig.4 Indole-3-carboxamide R=Cl, Br, CH_3, OCH_3 R/=Ph

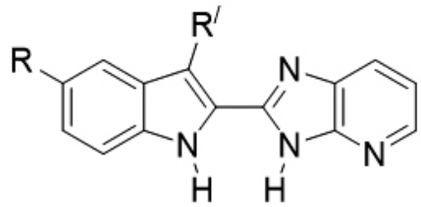


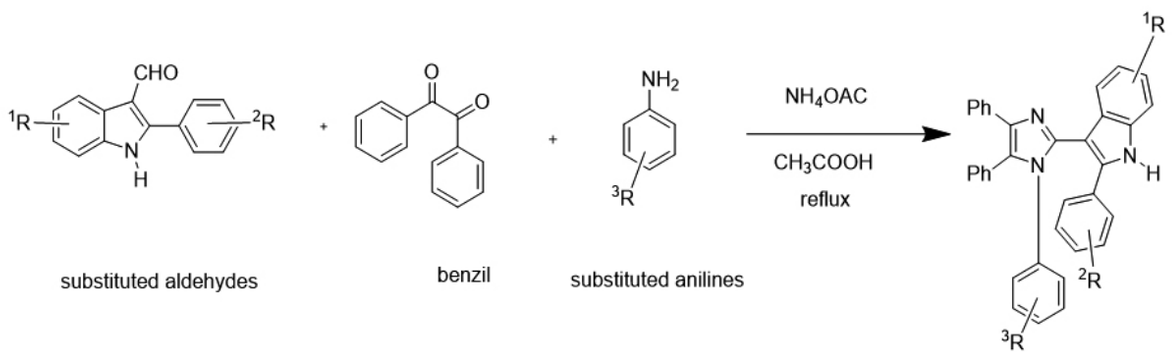
Fig.5 imidazolopyridinyl indole.

RESULTS AND DISCUSSIONS

Chemistry

Substituted 2-arylindoles were prepared by the method of Fischer indole synthesis[25] which were subsequently formylated using Vilsmeir-Haack[26] reaction. The 2-arylindole-3-carbaldehydes, ammonium acetate, benzil and substituted anilines were the reactants used in this MCR (Multi component reaction) to provide a new series of tetraarylimidazoles (Scheme 1,4a-4j).

The structures of these compounds were confirmed on the basis of spectral data. In their HRMS, the molecular ion peaks were found to correspond to their expected values. The ^{1}HNMR spectra showed a singlet for –NH of indole in the range of δ 11-12. The compounds substituted with –CH_3 and –OCH_3 (**4a, 4d, 4f, 4g,5h,5i,4j)** showed upfield singlet in the range of δ 2- 4. The splitting patterns of remaining protons of spectra were as expected, and were according to the substituents. The ^{13}CNMR spectra and FTIR were also consistent with the structures.



(1) **(2)** **(3)** **(4a-4j)**

^{1}R= H, Br

^{2} R= H, Cl, CH_3,Br ^{3} R= H, Br, F, Cl, CH_3, OCH_3

Scheme-1 Synthetic route for the synthesis of compounds (**4a-4j**).

Table 1: Antiurease and antioxidant activity by indole-imidazole derivatives.

Sr. No.	Sample code	^{1}R	^{2}R	^{3}R	Antiurease		Antioxidant	
					Inhibition (%) at 0.5 mM	IC_{50} (µM)	Inhibition (%) at 0.5 mM	IC_{50} (µM)
1	**4a**	H	Cl	CH_3	76.44±0.8	47.53**±0.12**	62.58±0.7	175.26±1.24
2	**4b**	H	Cl	Br	76.54±0.8	47.64**±0.15**	71.74±0.2	146.27±1.09
3	**4c**	Br	H	F	92.58±0.3	11.35±0.07	71.87±0.5	181.26±1.1
4	**4d**	H	Br	CH_3	87.44±0.8	9.95**±0.05**	90.39±0.5	148.26±1.2
5	**4e**	H	Br	Cl	89.44±0.3	20.25±0.03	20.97±0.5	-
6	**4f**	H	CH_3	H	85.77±0.6	4.85±0.07	67.61±0.3	162.27±1.2
7	**4g**	H	CH3	CH_3	86.73±0.7	0.16±0.04	44.21±0.7	-
8	**4h**	H	CH_3	Br	87.38±0.46	1.45±0.05	7.11±0.2	-
9	**4i**	H	CH_3	F	89.73±0.9	4.31±0.02	18.91±0.6	-
10	**4j**	H	CH_3	OCH_3	87.63±0.9	0.12±0.02	23.03±0.5	-
	Thiourea				88.7±0.8	21.25±0.15	-	-
	Quercetin				-		93.21±0.9	16.96±0.1

Antiurease activity.

The decreasing order of activity of these compounds is **4j>4g>4h>4i>4f>4d>4c>4e>4a>4b**

Most potent active compound **(4j)** has substituent R_1=H, R_2=CH_3 and R_3=OCH_3; Compounds substituted with R_1=H. R_2=Cl and R_3=CH_3 and R_1=H, R_2=Cl and R_3=Br**(4a, 4b)**displayed lesser inhibition towards this enzyme.

Outstanding activity was exhibited by compound **4j**. It was found 177.78 times more potent as compared to the standard and the compound **4g** (IC_{50} = 0.16) disubstituted with –CH_3 were 132.8 times more active than the reference. The compound **4h** (IC_{50} = 1.45) substituted with a CH_3 and a Br still 14.65 times more active than standard.

The compounds **4i** (IC_{50} = 4.31) substituted with a CH_3 and a F and compound **4f** (IC_{50} = 4.85) substituted with the CH_3 have been found 4.93 and 4.38 times, respectively more potent than the standard, while compounds **4d** (IC_{50} = 9.95) substituted with Br and CH_3, compound **4c** (IC_{50} = 11.35) substituted with Br and F and 4e(IC_{50} = 20.25) substituted with Cl and Br were 2.13, 1.87 and 1.04 times respectively more active than the standard.

The compound **4a** (IC_{50} = 47.53) substituted with CH_3 and Cl and compound **4b** (IC_{50} = 47.64) substituted with Cl and Br were found 2.23 and 2.24 times respectively less active as compared to the standard.

It seems that in general compounds containing electron donating groups (CH_3 and a halogen) in general are potent candidates as more active antiureases.

Antioxidant Activity

The compounds of this series were also tested for their antioxidant activities as compared to Quercetin against DPPH. The compounds **4a,4b,4c,4d,4f** were found active with decreasing order of this activity being **4b>4d>4f>4a>4c** .These compounds exhibited weak antioxidant activities and are listed in **table 1**. Among tested compounds the compound **4d** substituted with CH_3 and Br showed the highest antioxidant inhibition of 90.3 ± 0.57% at 0.5mM and IC_{50} 148.2 ± 1 mM and compound **4b** substituted with Cl and Br with inhibition of 71.7± 0.27% and IC50 146.27±1.09mM. This data suggest that such compounds are weak antioxidant agents.

Conclusions

A series of tetrasubstituted imidazoles containing a 2-arylindole substituent has been prepared by one-pot four component condensation reaction. The new compounds showed significant antiurease activity as compared to standard inhibitor, thiourea. However, low inhibition profiles were observed for the antioxidant activity.

MATERIALS AND METHODS

The chemicals and solvent used in this experimental work were of analytical grade and were purchased from Merck, Fluka and Aldrich and were used as such. Melting points were determined on a Gallen Kamp melting point apparatus in open capillary tubes and are uncorrected. High resolution mass spectra were recorded on Waters LCT Premier XE TOF-MS. Analytical TLC was performed on DC-Alufolien Silica Gel 60 F_{254} Merck. UV lamp of short and long wavelength (model UVGL-25 minor light multiband UV-254/366) was used to visualize TLC plates. IR spectra were recorded on Perkin Elmer Spectrum BX FT-IR. ^{1}H spectra were recorded at 500 MHZ and ^{13}C NMR spectra were recorded at 126 MHZ on a Brucker Avance AV11500B spectrometer.

General Procedure for the synthesis of Tetrasubstituted imidazoles containing a substituted indole (4a-4j)

A mixture of benzil (1.0 equiv), a substituted 2-phenylindole 3-carbaldehyde (1.0 equiv) an aromatic amine (1.0 equiv) and ammonium acetate (4.0 equiv) in acetic acid was heated at reflux for 5-6 hours[15]. After the completion of the reaction (monitored by TLC) and cooling to room temperature, the reaction mixture was poured into cold water. The precipitated product was filtered, washed with excess of water and recrystallized with EtOH to obtain pure (**4a-4j).**

The following compounds were prepared from this general method:

2-(*p*-Chlorophenyl)-3-(4,5-diphenyl-1-(p-tolyl)-1*H*-imidazol-2-yl)-1*H*-indole (4a)

Yield: 57% as a white solid.

mp: 265-270 ^{0}C;

IR (neat): 3063, 2356, 1514, 1389, 1366, 1095, 1020 cm^{-1}

HRMS (ES$^+$) calcd. for $C_{36}H_{27}ClN_3$ [M+H]$^+$ 536.1894 Found: 536.1882; m/z (%): 536.1882([M+H]$^+$, 100%), 538.1915 (30%)

^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.64 (s, 1H), 7.60 – 7.44 (m, 3H), 7.45 (d, *J* = 8.5 Hz, 2H), 7.39 (d, *J* = 7.9 Hz, 1H), 7.32 – 7.20 (m, 9H), 7.20 – 7.12 (m, 2H), 7.03-7.08 (m, 1H), 6.69 (d, *J* = 8.0 Hz, 2H), 6.46 (d, *J* = 6.8 Hz, 2H), 2.05 (s, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ: 142.69, 139.59, 139.33, 139.13, 137.59, 137.36, 136.58, 136.36, 135.35, 135.19, 133.90, 133.11, 131.68, 131.48, 131.29, 130.56, 130.32, 130.03, 129.65, 129.18, 129.12, 129.08, 128.85, 128.79, 128.74, 127.66, 127.58, 126.88, 123.07, 120.69, 120.08, 113.85, 112.06, 103.92, 21.14.

3-(1-(*p*-Bromophenyl)-4,5-diphenyl-1*H*-imidazol-2-yl)-2-(*p*-chlorophenyl)-1*H*-indole(4b)

Yield: 68% as a white solid.

mp: 265 ^{0}C;

IR (neat) 3065, 2359, 1490, 1386, 1224, 1070, 1015 cm^{-1};

HRMS (ES$^+$) calcd. for $C_{35}H_{24}BrClN_3$ [M+H]$^+$ 600.0842 Found: 600.0836; m/z (%): 600.0836([M+H]$^+$, 80%), 602.0800 (100%)

^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.71 (s, 1H), 7.62 (d, *J* = 7.9 Hz, 1H), 7.54 (d, *J* = 7.3 Hz, 2H), 7.45 – 7.39 (m, 3H), 7.35 – 7.17 (m, 12H), 7.11 – 7.06 (m, 2H), 6.47 (d, *J* = 6.2 Hz, 2H).

^{13}C NMR (126 MHz, DMSO-d_6) δ: 142.19, 136.78, 136.56, 135.55, 133.28, 131.68, 131.53, 130.36, 129.86, 129.25, 129.05, 128.70, 127.73, 126.93, 123.15, 120.72, 120.07, 112.12, 111.97, 109.55, 105.09.

5-Bromo-3-(1-(*p*-fluorophenyl)-4,5-diphenyl-1'*H*-imidazol-2-yl)-2-phenyl-1*H*-indole (4c)

Yield: 83% as a white solid.

mp: > 300 ^{0}C;

IR (neat): 3178, 1602, 1508, 1313, 1219, 1152, 1046 cm^{-1};

HRMS (ES$^+$) calcd. for $C_{35}H_{24}BrFN_3$ [M+H]$^+$ 584.1138 Found: 584.1136; m/z (%): 584.1136([M+H]$^+$, 100%), 586.1129 (100%)

^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.83 (s, 1H), 7.78 (s, 1H), 7.55 (d, *J* = 8.1 Hz, 2H), 7.39 – 7.18 (m, 15H), 6.72-6.68 (m, 2H), 6.48 (d, *J* = 6.7 Hz, 2H).

^{13}C NMR (126 MHz, DMSO-d_6) δ: 160.15, 142.31, 139.44, 137.71, 135.21, 135.18, 132.63, 131.93, 131.65, 131.28, 131.23, 130.46, 129.82, 129.74, 129.26, 129.17, 128.97, 128.93, 128.81, 127.86, 127.00, 126.93, 125.35, 122.15, 115.44, 115.26, 114.14, 113.20, 102.68.

2-(*p*-Bromophenyl)-3-(4,5-diphenyl-1-(*p*-tolyl)-1*H*-imidazol-2-yl)-1*H*-indole (4d)

Yield: 52% as a white solid.

mp: 278 ^{0}C;

HRMS (ES$^+$) calcd. for $C_{36}H_{27}BrN_3$ [M+H]$^+$ 580.1388 Found: 580.1380; m/z (%): 580.1 ([M+H]$^+$ 100%) 582.1(100 %)

IR (neat): 3056, 2360, 1514, 1074, 846 cm^{-1};

^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.66 (s, 1H), 7.63 – 7.50 (m, 5H), 7.39 (d, *J* = 7.9 Hz, 1H), 7.32 – 7.20 (m, 9H), 7.19 – 7.13 (m, 2H), 7.09-7.02 (m, 1H), 6.68 (d, *J* = 7.9 Hz, 2H), 6.48 (d, *J* = 6.8 Hz, 2H), 2.04 (s, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ: 142.45, 142.23, 137.72, 137.49, 136.58, 135.16, 132.13, 131.99, 131.69, 131.10, 130.59, 130.29, 129.45, 129.18, 129.12, 128.79, 128.74, 127.66, 127.59, 127.00, 126.89, 124.50, 122.73, 121.62, 120.66, 114.36, 112.06, 103.93, 21.00.

2-(*p*-Bromophenyl)-3-(1-(*p*-chlorophenyl)-4,5-diphenyl-1*H*-imidazol-2-yl)-1*H*-indole (4e)

Yield: 86% as a white solid.

mp: 276 ^{0}C;

IR (neat): 3068(C-H), 2364, 1602, 1494, 1388, 1240, 1094, 1017 cm^{-1};

HRMS (ES$^+$) calcd. for $C_{35}H_{24}BrClN_3$ [M+H]$^+$ 600.0842 Found: 600.0827; m/z (%): 600.0827([M+H]$^+$ 80%), 602.0825 (100%)

^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.71 (s, 1H), 7.65 – 7.52 (m, 5H), 7.41 (d, *J* = 8.2 Hz, 1H), 7.36 – 7.04 (m, 12H), 6.96 (d, *J* = 8.3 Hz, 2H), 6.55 (d, *J* = 6.7 Hz, 2H).

^{13}C NMR (75 MHz, DMSO-d_6) δ 141.93, 137.23, 136.01, 135.85, 134.63, 134.50, 131.93, 131.53, 131.32, 131.07, 130.90, 130.47, 129.93, 129.58, 128.92, 128.89, 128.79, 128.59, 128.52, 128.41, 128.13, 127.93, 127.31, 127.02, 126.39, 126.30, 122.52, 121.23, 121.21, 120.10, 119.99, 119.54, 111.50, 111.40,102.76.

2-(*p*-Tolyl)-3-(1,4,5-triphenyl-1*H*-imidazol-2-yl)-1*H*-indole (4f)

Yield: 54% as a yellow solid

mp: 260 ^{0}C;

IR (neat): 3068, 1598, 1496, 1392, 1089, 1050 cm^{-1};

HRMS (ES$^+$) calcd. for $C_{36}H_{28}N_3$ [M+H]$^+$ 502.2283 Found: 502.2269;

^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.48 (s, 1H), 7.57 – 7.49 (m, 3H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.30 – 7.09 (m, 13H), 7.04 – 6.85 (m, 4H), 6.56 (d, *J* = 7.0 Hz, 2H), 2.30 (s, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ: 143.15, 138.20, 137.90, 137.65, 136.58, 136.29, 135.45, 131.60, 131.42, 130.35, 129.82, 129.58, 129.08, 128.76, 128.50, 127.85, 127.50, 127.00, 126.86, 122.51, 120.40, 119.76, 111.91, 103.02, 21.39.

3-(4,5-Diphenyl-1-(*p*-tolyl)-1*H*-imidazol-2-yl)-2-(*p*-tolyl)-1*H*-indole (4g)

Yield: 68% as a yellow solid.

mp: 270 ^{0}C;

IR (neat): 3055, 1600, 1514, 1330, 1146, 1050 cm^{-1};

HRMS (ES$^+$) calcd. for $C_{37}H_{30}N_3$ [M+H]$^+$ 516.2440 Found: 516.2436;

^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.53 (s, 1H), 7.56 – 7.47 (m, 3H), 7.37 (d, *J* = 8.0 Hz, 1H), 7.32 – 6.99 (m, 14H), 6.68 (d, *J* = 7.9 Hz, 2H), 6.49 (d, *J* = 6.1 Hz, 2H), 2.31 (s, 3H), 2.03 (s, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ: 143.13, 138.07, 137.92, 137.47, 137.29, 136.14, 135.45, 134.06, 133.65, 133.62, 131.61, 129.85, 129.66, 129.12, 128.84, 128.73, 127.68, 127.40, 126.90, 126.78, 122.44, 120.40, 119.79, 111.90, 103.03, 21.42, 20.99.

3-(1-(*p*-Bromophenyl)-4, 5-diphenyl-1*H*-imidazol-2-yl)-2-(*p*-tolyl)-1*H*-indole (4h)

Yield: 48% as a yellow solid.

mp: 268 ^{0}C;

IR (neat): 3065, 1490, 1388, 1242, 1068 cm^{-1};

HRMS (ES$^+$) calcd. for $C_{36}H_{27}BrN_3$ [M+H]$^+$ 580.1388 Found: 580.1400; m/z (%): 580.1400([M+H]$^+$ 100%), 582.1371 (100%)

^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.57 (s, 1H), 7.60 – 7.51 (m, 3H), 7.39 (d, *J* = 7.9 Hz, 1H), 7.34 – 7.14 (m, 13H), 7.09 – 7.02 (m, 3H), 6.44 (d, *J* = 5.5 Hz, 2H), 2.31 (s, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ: 143.08, 138.19, 138.11, 137.85, 136.34, 135.83, 135.27, 131.64, 131.46, 131.22, 129.99, 129.80, 129.70, 129.61, 129.23, 129.13, 128.97, 128.75, 128.65, 128.50, 127.60, 127.00, 122.65, 121.02, 120.51, 119.86, 112.05, 102.56, 21.37.

3-(1-(*p*-Fluorophenyl)-4,5-diphenyl-1*H*-imidazol-2-yl)-2-(*p*-tolyl)-1*H*-indole (4i)

Yield: 54% as a yellow solid.

mp: 248-250 ^{0}C;

IR (neat): 3186, 1600, 1510, 1223, 1051 cm^{-1};

HRMS (ES$^+$) calcd. for $C_{36}H_{27}FN_3$ [M+H]$^+$ 520.2189 Found: 520.2169;

^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.54 (s, 1H), 7.60 – 7.51 (m, 3H), 7.38 (d, *J* = 7.9 Hz, 1H), 7.33 – 7.24 (m, 5H), 7.23 – 7.12 (m, 8H), 7.07 – 7.01 (m, 1H), 6.76 – 6.68 (m, 2H), 6.54 (bs, 2H), 2.31 (s, 3H).

^{13}C NMR (75 MHz, DMSO-d_6) δ: 162.12, 158.88, 142.56, 137.52, 137.44, 136.95, 135.64, 134.67, 132.20, 132.17, 130.99, 130.67, 129.62, 129.24, 129.10, 128.97, 128.55, 128.43, 128.27, 128.13, 128.00, 127.81, 127.04, 126.91, 126.30, 121.96, 119.82, 119.23, 114.88, 114.58, 111.31, 101.96, 20.78.

3-(1-(*p*-Methoxyphenyl)-4,5-diphenyl-1*H*-imidazol-2-yl)-2-(*p*-tolyl)-1*H*-indole (4j)

Yield: 46% as a white solid.

m.p: 260 ^{0}C;

HRMS (ES$^+$) calcd. for $C_{37}H_{30}N_3O$ [M+H]$^+$ 532.2389 Found: 532.2380;

^{1}H NMR (500 MHz, Acetone-d_6) δ: 10.53 (s, 1H), 7.70 – 7.63 (m, 3H), 7.39 (d, *J* = 8.1 Hz, 1H), 7.30 – 7.20 (m, 9H), 7.19 – 7.05 (m, 5H), 6.47 (d, *J* = 7.8 Hz, 2H), 6.37 (d, *J* = 7.9 Hz, 2H), 3.58 (s, 3H), 2.32 (s, 3H).

^{13}C NMR (75 MHz, DMSO-d_6) δ: 157.87, 142.67, 137.39, 137.28, 136.73, 135.52, 134.85, 130.95, 129.97, 129.18, 129.07, 129.06, 128.94, 128.69, 128.48, 128.44, 128.32, 128.09, 128.05, 127.98, 127.79, 127.02, 126.78, 126.23, 126.13, 121.83, 119.72, 119.13, 113.06, 11.22, 102.41, 54.97,20.75.

Urease assay

This assay was modified from Berthelot assay and was employed for the determination of urease activity[27].

Principle Hydrolysis of urea is catalyzed by urease enzyme as under:-

$(NH_2)_2CO + H_2O \rightarrow CO_2 + 2NH_3$

This reaction of urease is stopped when the phenol-hypochlorite is added to the reaction mixture. It gives a light blue colored complex between ammonia released and phenol-hypochlorite

METHOD

A total volume of 85 µl assay mixture contained 10 µl of phosphate buffer of pH 7.0 in each well in the 96-well plate followed by the addition of 10 µl of sample solution and 25 µl of enzyme solution (0.015 units). The contents were pre-incubated at 37ºC for 10 min. Then, 40 µl of urea stock solution (20 mM) was added to each well and incubation continued at 37ºC for further 10 min and take pre-read at 625nm. After taking pre-read at 625nm using the 96-well plate reader Synergy HT, 115 µl phenol hypochlorite reagent was added in each well (freshly prepared by mixing 45 µl phenol reagent with 70 µl of alkali reagent). For colour development, incubation was done at 37ºC for another 10 min. Absorbance (after read) was again measured at 625 nm using the 96-well plate reader Synergy HT. The percentage enzyme inhibition was calculated by the following formula:

Inhibition (%) = 100 – [(Abs. of test sample / Abs. of control) × 100]

IC_{50} values (concentration at which 50% enzyme catalyzed reaction occurs) of active compounds were determined by measuring activities at lower concentrations

(0.5, 0.25, 0.125, 0.06, 0.03 mM) and data was computed by using EZ-Fit Enzyme

Antioxidant assay

Antioxidant potential of all the derivatives was checked by the following method.

DPPH radical scavenging activity

The stable 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) was used for the determination of antioxidant activity according to a reported method[28]. Different concentrations of compounds in respective solvents were added at an equal volume (10µl) to 90 µl of 100 µM methanolic DPPH in a total volume of 100 µl in 96-well plates. The contents were mixed and incubated at 37 ^{o}C for 30 minutes. The absorbance was measured at 517nm using Synergy HT BioTek® USA microplate reader. Quercetin and L-ascorbic acid were used as standard antioxidants. The experiments were carried out in triplicates. IC_{50} values were calculated using EZ-Fit5 Perrella Scientific Inc. Amherst USA software. The decrease in absorbance indicates increased radical scavenging activity which was determined by the following formula.

Percent scavenging activity = [100 - (Abs of test compound / Abs of control) x 100

All the measurements were done in triplicate and statistical analysis was performed by Microsoft Excel 2003. Results are presented as mean ± sem.

ACKNOWLEDGEMENTS

The authors are thankful to Higher Education Commission of Pakistan (HEC) for the award of IRSIP fellowship. M. A. Khan puts on record his thanks for the generous support of HEC through their Analytical support provisions. Prof. Gary S. Molander of Department of Chemistry, University of Pennsylvania, USA, was gracious enough to provide necessary laboratory and other analytical facilities to Sadia Naureen and deserves our thanks.

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