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Synthesis, antibacterial, and antioxidant studies of 7-amino-3-(4-fluorobenzoyl)indolizine-1-carboxylate derivatives

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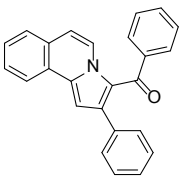
Indolizine, 4-aminopyridine, acetylene, anti-bacterial, antioxidant.

ABSTRACT

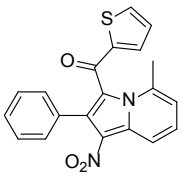
In the present work, the quaternary salts of 4-aminopyridine, i.e., 4-amino-1-[2-(4-bromophenyl)-2-oxoethyl]pyridin-1-ium bromides were obtained by stirring 4-aminopyridine with phenacyl bromides in acetone at room temperature separately. These quaternary salts of 4-aminopyridine were treated with acetylenes (electron deficient), in the presence of anhydrous possium carbonate in N,N-dimethylformamide solvent to get indolizine derivatives. The structures of newly synthesised compounds have been confirmed by spectroscopic techniques, such as liquid chromatography mass spectrometry, ^{1}H-NMR, and elemental analysis. Synthesized all compounds were screened for antibacterial and antioxidant activity. The compounds 2e, 2g, and 2j shows inhibition zone against bacteria and compounds 2a and 2f moderately active against bacteria. All compounds 2a to 2j show 1,1-diphenyl-2-picrylhydrazide radical free radical scavenging activity, Nitric Oxide free radical scavenging activity, Reducing power scavenging activity, and Lipid peroxidation inhibition activity.

INTRODUCTION

The heterocyclic chemistry is one of the important class of organic chemistry because of their diversity of activity in nature and significant of synthesized compounds (Mallikarjun *et al*., 2016, Nagesh *et al*., 2014; 2015; Rakshita *et al*., 2019; Siddesh *et al*., 2013; 2014a; 2014b; Thriveni *et al*., 2014). In this class, indolizine very interesting compound due to its wild range of biological activity, such as *in vitro* COX-2 inhibitory activity (Sandeep *et al*., 2018b), anti-tubercular activity (Swinborne *et al*., 2016), *in vitro* anticancer activity (Sandeep *et al*., 2016a), antimicrobial activity (Sandeep *et al*., 2013a), larvicidal activity against Anopheles arabiensis (Sandeep *et al*., 2018a, 2016b). Indolizine is an organic molecule contaning both a p-excessive pyrrole and a p-deficient pyridine ring with only one bridged nitrigen. The indolizine system being isomeric with indole (Flitsch *et al*., 1984; Venugopal *et al*., 2019) and its synthesis also take our attention (Sandeep *et al*., 2013b; 2014; Kemnitzer *et al*., 2008). Compound 1 shows antibacterial and antifungal activity and compound 2 shows antibacterial activity (Hazra *et al*., 2011). In this present work, we have undertaken the synthesis of indolizine derivatives 2a–j. The final targeted moiety has been achieved in two steps and depicted in Scheme 1.



Compound 1



compound 2

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MATERIALS AND METHODS

Chemistry

All the chemicals and anhydrous solvents used in this work of analytical reagent grade and purchased from Sigma-Aldrich. Synthesized compounds IR spectra recorded on Bruker alpha FT-IR spectrometer using Nujol. ^{1}H NMR spectra were recorded using $CDCl_3$ and DMSO as a solvent, in Bruker AV 300. All chemical shifts are expressed in δ ppm. Using Jole JMS-D 300 mass spectrometer mass spectra were recorded at 70 eV. Elemental analysis was done on an Elementar Vario EL analyzer Satisfactory C, H, N analyses were obtained for all the compounds. The progress of reactions was monitored and checked by TLC, and further purification was finished by column chromatography using 60–120 mesh silica gel. All the melting points were recorded in open capillary and were uncorrected. Physiochemical constants like molecular mass, cLogP, melting point and % of the yield of Ethyl 7-amino-3-(4-bromobenzoyl)indolizine-1-carboxylate derivatives (2a-2j) provided in table 1.

Preparation of 4-amino-1-(2-(4-bromophenyl)-2-oxethyl) pyridine-1-ium bromide (1d)

To a stirred of 4-Aminopyridine (1.0 g, 0.0160 mol) in anhydrous acetone (10 ml) was added 2,4-Dibromoacetophenone (2.93 g, 0.0106 mol) and stirred for 30 minutes at normal room temperature. Solid was obtained which filtered and under vasuum to get quartenary salt 4-Amino -1-(2-(4-bromophenyl)-2-oxethyl) pyridine-1-ium bromide (89% yield).

Preparation of ethyl-7-amino-3-(4-bromobenzyl)indolizine-1 -carboxylate (2g)

To a stirred solution of 4-Amino-1-(2-(4-bromophenyl)-2-oxoethyl)pyridine-1-ium bromide (0.5 g, 0.0013 mol) in dry DMF was added ethylpropiolate (0.1 g, 0.0013 mol) [for compound 2h, dimethyl but-2-ynedioate (0.1 g, 0.0013 mol)], K_2CO_3 (0.2 g, 0.0016 mol), and was stirred for 30 minutes in room temperature. The progrss of the reaction was monitored by TLC. After completion, the reaction mass was pour into container which contain crushed ice and filtered on the vacuum pump. The crude compound was purified by column chromatography using hexane:ehtlacetate as an eluent to afford ethyl-7-amino-3-(4-bromobenzoyl)indolizine-1-carboxylate (78% yield). The remaining indolizine derivatives were synthesized by using the same protocol. The series of reaction carried out have been represented in Scheme 1.

Ethyl 7-amino-3-(4-fluorobenzoyl)indolizine-1-carboxylate (2a)

^{1}H-NMR (600 MHz, DMSO-d_6); δ = 9.53–9.52 (1H, m), 7.77 (1H, s), 7.37 (2H, s), 7.35–7.27 (4H, m), 6.69–6.64 (3H, m), 4.22–4.18 (2H, *q*, *J* = 7.2 Hz), 1.28–1.26 (3H, *t*, *J* = 7.2 Hz); ^{13}C-NMR (150 MHz, DMSO-d_6); *δ* = 188.26, 186.60, 164.88, 163.76, 150.63, 143.52, 137.11, 131.33, 131.28, 130.58, 129.87, 120.65, 115.87, 115.73, 108.11, 101.77, 96.27, 59.54, and 14.94; MS (ESI, Positive): m/z = 327.2 (M+H)+; analytical calculated for $C_{18}H_{15}FN_2O_3$; C, 66.25, H, 4.63, 63, N, 8.58; found, C, 66.19, H, 4.59, N, 8.55.

Dimethyl 2-acetyl-7-amino-3-(4-fluorobenzoyl)indolizine-1,2 -dicarboxylate (2b)

^{1}H-NMR (600 MHz, DMSO-d_6); *δ* = 9.60–9.62 (1H, m), 7.78–7.72(2H,m),7.70–7.68(2H,m),7.41(1H,s),7.00–7.10(3H,m), 3.89 (3H, s), 3.84(3H, s); ^{13}C-NMR (150 MHz, DMSO-d_6); *δ* = 180.12, 169.23, 163.44, 162.98, 143.43, 137.98, 135.21, 133.45, 133.21, 126.2, 124.10, 116.80, 112.83, 108.17, 99.22, 52.59, and 51.44; MS (ESI, Positive): m/z = 371.2 $(M+H)^+$; analytical calculated for $C_{19}H_{15}FN_2O_5$; C, 61.62, H, 4.80, N, 7.56; found C, 61.09,H, 3.97,N, 7.18.

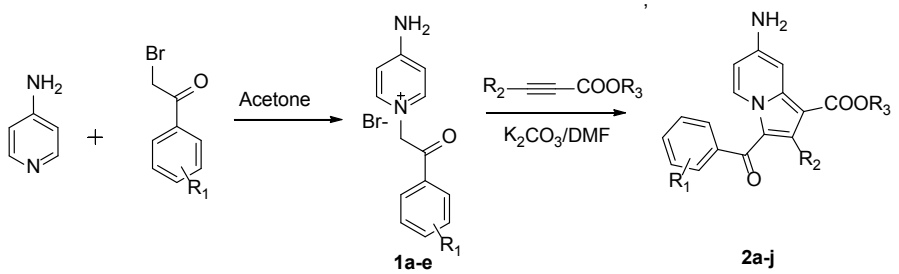
Ethyl 7-amino-3-(2-nitrobenzoyl)indolizine-1-carboxylate (2c)

1H-NMR (600, DMSO-d_6); *δ* = 9.48–9.47 (1H, m), 8.17 (1H, s), 7.87–73 (4H, m), 7.27–7.25 (1H, m), 6.737.70 (3H, m), 4.19–4.15 (2H, *q*, *J* = 7.2Hz), 1.23–1.20 (3H, *t*, *J* = 7.2 Hz); ^{13}C-NMR (150 MHz, DMSO-d_6,); *δ* = 178.83, 163.52, 151.05, 147.60, 143.74, 135.76, 134.42, 131.37, 130.54, 130.15, 129.49, 125.13, 120.42, 110.10, 108.44, 59.64, and 14.54; MS (ESI, Positive): m/z = 354 (M+H)+; analytical calculated for $C_{18}H_{15}N_3O_3$; C, 61.19, H 4.28, N, 11.89; found C, 61.15, H, 4.22, N, 11.82.

Dimethyl 7-amino-3-(2-nitrobenzoyl)indolizine-1,2 -dicarboxylate (2d)

1H-NMR (600 MHz, MNSO-d_6), *δ* = 9.60–9.59 (1H, m), 8.55 (1H, s), 8.43–8.25 (2H, m), 8.16-8.10 (1H, m), 7.86–7.77 (1H, m), 7.59–7.25 (1H, m), 6.80 (2H, bs), 3.87 (3H, s), 3.80 (3H, s); ^{13}C-NMR (150 MHz, DMSO-d_6); *δ* = 165.22, 163.05, 151.43, 146.14,

Scheme-1



R_1= 4-F, 4-Cl , 4-Br, 4-OCH_3, 2-NO_2
R_2=H, $COOCH_3$
R_3=CH_3, C_2H_5

141.89, 135.17, 134.79, 134.18, 133.73, 132.71, 131.47, 130.73, 130.64, 125.32, 124.66, 117.34, 109.27, 99.91, and 51.67; MS (ESI, Positive): m/z = 398 $(M+H)^+$; analytical calcuted for $C_{19}H_{15}N_3O_7$; C, 57.43, H, 3.81, N, 10.58, found; C, 57.40, H, 3.78, N, 10.52.

Ethyl 7-amino-3-(4-methoxybenzoyl)indolizine-1-carboxylate (2e)

1H-NMR (600 MHz, DMSO-d_6); δ = 9.52–9.51 (1H, m), 7.78 (1H, s), 7.35 (2H, s), 7.25–7.19 (4H, m), 7.05–6.95 (3H, m), 3.85 (3H, s), 4.22–4.18 (2H, *q*, *J*=7.2Hz), 1.28–1.26 (3H, *t*, *J* = 7.2Hz); ^{13}C-NMR(150 MHz, DMSO-d_6); δ = 188.26, 186.60, 164.88, 163.76, 150.63, 143.52, 137.11, 131.33, 131.28, 130.58, 129.87, 120.65, 115.87, 115.73, 108.11, 101.77, 96.27, 59.54, 55.86, and 14.94; MS (ESI Positive): m/z = 339.2 (M+H)+; analytical calculated for $C_{19}H_{18}N_2O_4$; C, 6.44, H, 5.36, N, 8.28; found; C, 66.19, H, 5.19, N, 8.05.

Dimethyl 2-acetyl-7-amino-3-(4-methoxybenzoyl)indolizine-1,2-dicarboxylate (2f)

^{1}H-NMR (600MHz, DMSO-d_6); δ = 9.67–9.65(1H, m), 7.88–7.82(2H, m), 7.80–7.78 (2H, m), 7.45 (1H, s), 7.10–7.12 (3H, m), 3.90 (3H, s), 3.85 (3H, s), 3.83 (3H, s), ^{13}C-NMR (150 MHz, DMSO-d_6); δ = 181.23, 166.22, 165.27, 164.98, 142.55, 138.23, 135.26, 131.25, 129.66, 126.24, 124.87, 114.66, 112.83, 108.67, 99.56, 56.35, 55.98, and 55.88; MS (ESI, Positive): m/z = 383.2 (M+H)+; analytical calculated for $C_{20}H_{18}N_2O_6$; C, 62.82, H, 4.74, N, 7.33, found; C, 62.09, H, 4.57, N, 7.05.

Ethyl 7-amino-3-(4-bromobenzoyl)indolizine-1-carboxylate (2g)

^{1}H-NMR (600 MHz, DMSO-d_6); δ = 9.53–9.52 (1H, m), 7.74–7.34 (2H, m), 7.65–7.63 (2H, m), 7.37 (1H, s), 7.27 (1H, s), 6.96–6.67 (3H, m), 4.22–4.18 (2H, *q*, *J* = 7.2Hz),.1.28–1.24 (3H, *t*, *J* = 7.2Hz); ^{13}C-NMR (150 MHz, DMSO-d_6); δ = 184.56, 163.71, 150.75, 143.59, 139.67, 131.85, 130.80, 130.63, 130.00, 124.91, 120.55, 108.15, 102.00, 96.33, 59.57, and 14.93; MS (ESI, Positive): m/z = 387 (M+H)+; analytical calculated for $C_{18}H_{15}N_2O_3$; C, 55.83, H, 3.90, N, 7.23, found, C, 55.80, H, 3.87, N, 7.18.

Dimethyl 2-acetyl-7-amino-3-(4-bromobenzoyl)indolizine-1,2-dicarboxylate (2h)

^{1}H-NMR (600 MHz, DMSO-d_6); δ = 9.59–9.58 (1H, m), 7.75–7.73 (2H, m), .7.70–7.68 (2H, m), 7.44 (1H, s), 7.11–7.07 (3H, m), 3.92 (3H, s), 3.86 (3H, s); ^{13}C-NMR-(150 MHz, DMSO-d_6) δ = 178.12, 168.43, 163.11, 142.56, 136.67, 134.33, 133.17, 132.89, 125.12, 124.00, 116.47, 112.49, 108.01, 99.78, 52.31, and 51.69; MS (ESI, Positive): m/z = 331.2 (M+H)+; analytical calculate for $C_{19}H_{15}BrN_2O_5$; C, 52.92, H, 3.51,N, 6.50; found; C, 52.09, H, 3.37, N, 6.08.

Ethyl 7-amino-3-(4-chlorobenzoyl)indolizine-1-carboxylate (2i)

1H-NMR (600 MHz, DMSO-d6); δ = 9.52–9.51 (1H, m), 7.71–7.69 (2H, m), 7.60–7.59 (2H, m), 7.46–7.44(1H, m), 7.27–7.37 (1H, m), 6.69–6.66 (3H, m), 4.22–4.18 (2H, *q*, *J* = 7.2 Hz), 1.22–1.19 (3H, *t*, *J* = 7.2Hz); ^{13}C-NMR (150 MHz, DMSO-d_6); δ = 181.29, 163.72, 150.74, 143.58, 139.32, 136.06, 130.61, 129.99, 128.93, 128.33, 120.59, 108.15, 101.97, 96.32, 59.57, and 14.93; MS (ESI, Positive): m/z = 343 (M+H)+, analytical calculated for $C_{18}H_{15}ClN_2O_3$; C, 63.07, H, 4.41, N, 8.17; found; C, 62.89, H, 4.38, N, 8.14.

Dimethyl 2-acetyl-7-amino-3-(4-chlorobenzoyl)indolizine-1,2-dicarboxylate (2j)

^{1}H-NMR (600MHz, DMSO-d_6); δ = 9.59–9.58 (1H, m), 7.76–7.74 (2H, m), 7.71–7.69 (2H, m), 7.43 (1H, s), 7.10–7.06 (3H, m), 3.90 (3H, s), 3.85 (3H, s), ^{13}C-NMR (150 MHz, DMSO-d_6) δ = 179.12, 169.43, 163.02, 142.43, 136.98, 134.21, 133.93, 133.41, 126.12, 124.10, 116.67, 112.79, 108.23, 99.01, 52.30, and 51.66; MS (ESI, Positive): m/z = 387.2 (M+H)+, analytical calculated for $C_{19}H_{15}ClN_2O_5$; C, 59.00, H, 3.91, N, 7.24; found; C, 58.09, H, 3.77, N, 7.08.

Pharmacology studies

Antibactrerial activity

The clean cultures obtained were revived and maintained in nutrient agar at 37ºC. The microorganisms were cultured in nutrient broth 37ºC overnight.

Determination of antibacterial activity by disk-diffusion method

The test organisms were subculture using nutrient agar medium. The tubes containg sterilized medium were inoculated the respective bacterial strain. After incubation at 37 ± 1ºC for 18 hours, they stored in a refrigerator. The nutrient agar medium was sterilized by autocleaving at 121º for 15 minutes. In to each sterilized petri plate, was poured with LB Agar

Table 1. Physiochemical constants of Ethyl 7-amino-3-(4-bromobenzyol)indolizine-1-carboxylate derivatives (2a -2j).

Compound no	Molicular formula (Mol mass)	R_1	R_2	R_3	Yield (%)	clogP	MP in ºC
2a	$C_{18}H_{15}FN_2O_3$(326.11)	4-F	H	C_2H_5	72	3.66	170–172
2b	$C_{19}H_{15}FN_2O_3$(370.10)	4-F	$COOCH_3$	CH_3	76	2.10	168–170
2c	$C_{18}H_{15}N_3O_5$(353.10)	2-NO_2	H	C_2H_5	74	3.18	183–185
2d	$C_{19}H_{15}N_3O_5$(397.09)	2-NO_2	$COOCH_3$	CH_3	84	1.62	174–176
2e	$C_{19}H_{18}N_2O_3$(338.13)	4-OMe	H	C_2H_5	80	3.49	191–193
2f	$C_{20}H_{18}N_2O_6$(382.12)	4-OMe	$COOCH_3$	CH_3	78	1.95	177–179
2g	$C_{18}H_{15}BrN_2O_3$(430.02)	4-Br	H	C_2H_5	75	3.42	169–171
2h	$C_{19}H_{15}BrN_2O_3$(386.03)	4-Br	$COOCH_3$	CH_3	78	2.82	173–175
2i	$C_{18}H_{15}ClN_2O_3$(386.07)	4-Cl	H	C_2H_5	81	4.23	163–165
2j	$C_{19}H_{15}ClN_2O_3$(342.08)	4-Cl	$COOCH_3$	CH_3	82	2.67	154–156

cLogP was calculated using ChemDraw Professional 16.0 v.
Melting point determined by open capillary method and uncorrected.

medium. The Plates were left at room temperature aseptically to allow the solidification. After solidification, the appropriate bacterial cultures were inoculated over the surface of the agar using a sterile cotton swabbing method. Discs measuring 6 mm in diameter were used. The discs of the each compounds was placed individually on the LB agar mediumwith fresh bacteria respectively.

The antibacterial result of the all synthesized 10 ethyl 7-amino-3-(4-bromobenzoyl)indolizine-1-carboxylate compounds is summarized in Table 2. Among 10 compounds, only four compounds exhibited inhibition to bacteria and the remaining six compounds not active against tested bacteria.

In vitro antioxidant studies

DPPH free radical inhibition assy

The stable 1,1-diphenyl-2-picrylhydrazide radical (DPPH) was used for determining free radical scavenging activity. Different concentations compounds were added at an equal volume to the silution of DPPH (500 µl). After the sample preparation, keep the sample for 20 minutes and the absorbance was recorded at 517 nm at room temperature, using Ultraviolet (UV) spectrophotometer. The experiment was repeated thrice. Ascorbic acid was used as standard control (Koleva *et al*., 2002).

The DPPH free radical scavenging activity was calculated using the fallowing formula:

$$\% \text{ Scavenging effect} = \frac{\left(\text{Control absorbtion} - \text{Test absorbance}\right)}{\text{Control absorbtion}} \times 100$$

All synthesized ethyl 7-amino-3-(4-bromobenzoyl) indolizine-1-carboxylate derivatives screened for their inhibition activity against DPPH free radical. The DPPH free radical scavenging result of the all synthesized ten ethyl 7-amino-3-(4-bromobenzoyl)indolizine-1-carboxylate compounds is summarized in Table 3 and graph of Table 3 is shown in Figure 1. All compounds moderately active against DPPH free radical with compare to standard ascorbic acid.

Nitric oxide radical scavenging activity

Nitric oxide was produced from sodium nitroprusside and measured by the Griess reagent. Nitric oxide spontaneously generated by sodium nitroprusside in aqueous solution at physiological pH, this relised nitric oxide react with oxygen to produce nitrite ions that can estimated by the Griess reagent. Scavengers of nitric play race with oxygen leading to reduce the production of nitric oxide. 5 mM of sodium nitropprusside in phosphate buffered saline was mixed with different aliquots of

Table 2. Antibacterial activity of Indolizines derivatives in agar-disc diffusion method.

Compound	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aerudinous*	*Staphylococcus.aureus*	*Bacillus cereus*	*Zymomonas mobilis*
2a	14 ± 0.81	00	10.5 ± 0.5	00	10.05 ± 0.5	13.0 ± 1.0
2b	00	00	00	00	00	00
2c	00	00	00	00	00	00
2d	00	00	00	00	00	00
2e	16.66 ± 1.15	14.66 ± 1.15	15.33 ± 1.15	14.33 ± 2.08	8.66 ± 1.15	15.66 ± 0.57
2f	10.0 ± 0.81	00	00	00	00	00
2g	12.66 ± 1.15	15.66 ± 1.15	9.33 ± 1.15	8.66 ± 1.15	10.33 ± 0.57	8.66 ± 1.15
2h	00	00	00	00	00	0
2i	00	00	00	00	00	00
2j	11.33 ± 1.15	15.33 ± 0.57	11.33 ± 1.15	11.66 ± 0.57	10.33 ± 0.57	11.33 ± 1.15

(Diameter of the inhibition zone in mm at 100 µg of Sample), $N = 3 \pm$ SD.

Table 3. DPPH free radical scavenging activity of synthesized dimethyl 7-amino-3-benzoylindolizine-1,2-dicarboxylate derivatives.

Compd. number	Inhibition in %				
Concentration (µg/ml)	20	40	60	80	100
2a	6.952194	20.0284	31.08308	33.67005	35.46838
2b	8.874152	11.58933	17.78623	19.02114	27.69117
2c	3.573405	8.906978	13.81798	14.62787	18.83489
2d	3.274101	13.22477	15.64046	17.19529	20.06601
2e	10.49616	30.28744	36.46627	38.52124	43.15716
2f	7.520194	12.2411	15.22927	18.33619	21.78378
2g	4.53826	16.44915	26.76646	30.91653	32.83957
2h	1.949414	3.367248	9.447937	11.71589	17.61575
2i	7.599865	22.64933	28.71759	34.22499	40.38501
2j	8.4359	15.60315	19.60633	27.39953	37.24206
Ascorbic acid	27.57077	45.46394	63.46551	78.89519	86.31934

2–10 µg the compound and incubated at temperature 29ºC for 2 hours. The compound reacted with Griess reagent. The absorbtion of the chromophore produced during the diazotiazation of nitrite with sulphanilamide and subsequent coupling with naphthyl ethylene diamine was read at 550 nm (Marcocci *et al*., 1994). The nitric oxide free radical scavenging activity was calculated using thr fallowing formula:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance control} - \text{Absorbance of sample})}{\text{Absorbance control}} \times 100$$

All synthesized ethyl 7-amino-3-(4-bromobenzoyl) indolizine-1-carboxylate derivatives screened for their inhibition activity against nitric oxide free radical. The nitric oxide free radical scavenging result of the all synthesized 10 ethyl 7-amino-3-(4-bromobenzoyl)indolizine-1-carboxylate compounds is summarised in Table 4 and graph of Table 4 is shown in Figure 2. All compounds moderately active against nitric oxide free radical with compare to standard ascorbic acid.

Reducing power assay

Different concentration of compound was mixed with an equal volume of (2.5 ml) of 0.2 mol phosphate buffer (6.6 pH) and 1% potassium ferricyanide (2.5 ml) and incubated at 50ºC for 20 minutes. 10% Trichloroacetic acid (2.5 ml) was added and centrifuged at 3,000 rpm for 10 minutes. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml of water and 0.1% ferric chloride of 0.5ml and absorbance was read at 700 nm. An increase in the absorbance of indicates increases in redusing power (Oyaizu *et al*., 1986).

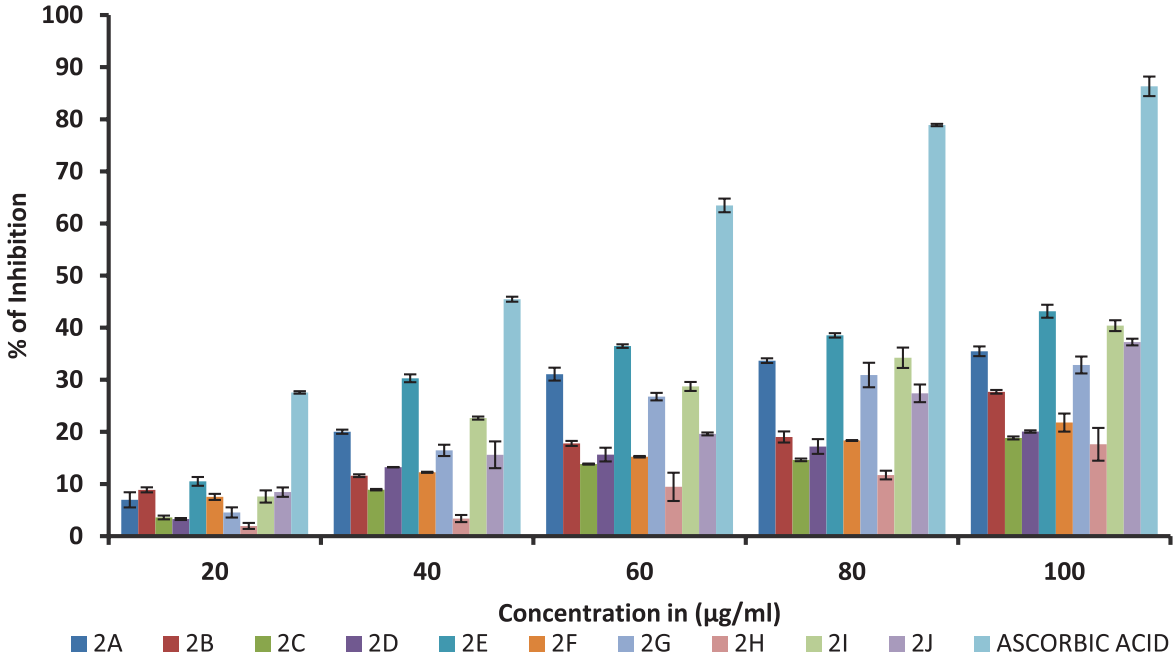


Figure 1. % of inhibition in free radical scavenging activity of dimethyl 7-amino-3-benzoylindolizine-1,2-dicarboxylate derivatives against DPPH free radical values were means ± SD of triplicate.

Table 4. Nitric oxide free radical scavenging acticity of synthesized dimethyl 7-amino-3-benzoylindolizine-1,2-dicarboxylate derivatives.

Compd. number	Inhibition in %				
Concentration (µg/ml)	20	40	60	80	100
2a	8.609501	11.28838	16.79519	21.30167	24.43246
2b	5.139437	8.355743	11.43443	16.06493	20.51322
2c	4.269065	13.22231	16.21296	18.22201	24.45212
2d	2.574787	3.45503	5.907833	7.573848	13.37522
2e	1.251929	5.826586	17.28959	17.15912	19.485
2f	1.549592	10.90729	11.75832	16.52459	18.80608
2g	4.72745	6.220635	9.695841	14.69706	17.38694
2h	4.683	7.423	8.369	12.204	16.153
2i	5.097399	11.43091	13.20099	14.02766	17.57459
2j	2.199841	4.47381	7.80029	9.311806	11.5696
Ascorbic acid	28.80784	42.53922	58.9574	72.23137	81.67745

All synthesised ethyl 7-amino-3-(4-bromobenzoyl) indolizine-1-carboxylate derivatives screened for their reducing power. The potassium ferricyanide reduction power of the all synthesized 10 ethyl 7-amino-3-(4-bromobenzoyl)indolizine-1-carboxylate compounds is summarized in Table 5 and graph of Table 5 is shown in Figure 3. All compounds moderately act as a reducing agents against ferric ion.

Lipid peroxidation inhibition assay

A reformed thiobarbituric acid-reactive species assy was used to measure the lipid peroxide formed. For this assay, egg yolk homogenates as lipid rich media. MDA (Malondialdihyde), a secondary end product of the oxidation of poly unsaturated fatty acids reacts with two molecules of TBA (thiobarbituric acid) yielding a pinkish red chromogen with an absorbance maximum at 532 nm. Egg homogenate 0.5 ml (10% in distilled water, v/v) and synthesized compound were mixed in a test tube and volume was made up to 1 ml by adding distilled water. Finally, 0.05 ml iron(II) sulfate (0.07 M) was added to the above mixture and incubated for 30 minutes to induce lipid peroxidation. Thereafter, 1.5 ml of 20% acetic acid (pH adujest to 3.5 with sodium hydroxide) and 1.5 ml of 0.8% thiobarbituric acid (w/v) (prepared in 1.1% sodim lauryl sulphate) and 0.05 ml 20% TCA was added, vortexes and then heted for 1 hour in a boling water bath. 5.0 ml of butan-1-ol was added to each tube in cool condition and centrifuged at 3,000 rpm for 10 minutes. The absorpbance of organic upper layer was measured at 532 nm using UV spectrophotometer (Ruberto *et al.*, 2000). The lipid peroxidation inhibition activity was calculated usingthe fallowing formula.

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

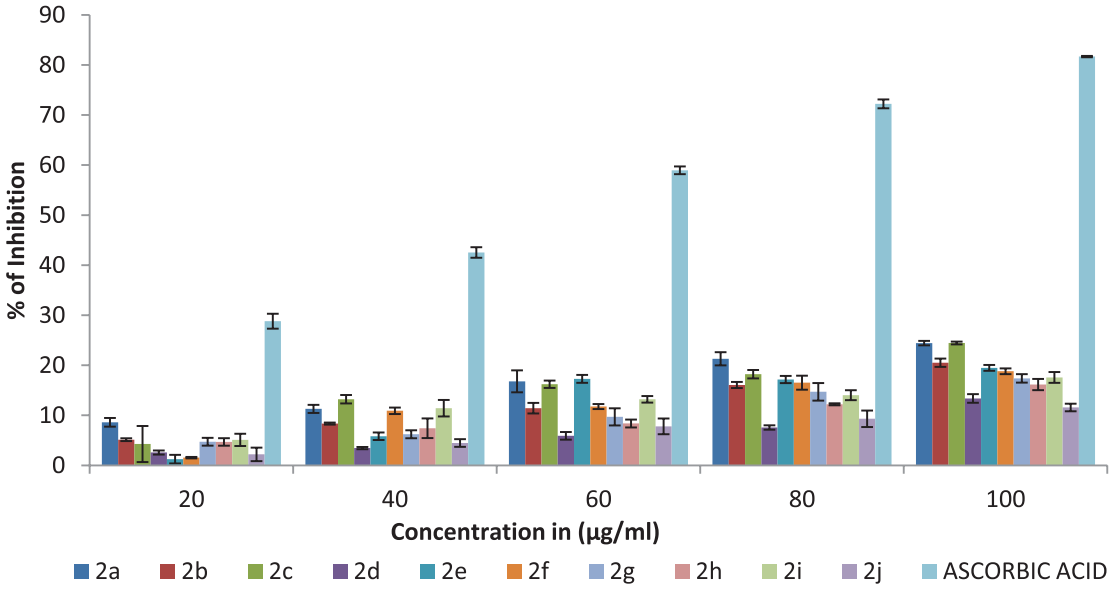


Figure 2. % of inhibition in free radical scavenging activity of dimethyl 7-amino-3-benzoylindolizine-1,2-dicarboxylate derivatives against Nitric oxide free radical. Values were means ± SD of triplicate.

Table 5. Reducing power scavenging activity of synthesized dimethyl 7-amino-3-benzoylindolizine-1,2-dicarboxylate derivatives.

Compd. number	Inhibition in %					
Concentration (µg/ml)	0	20	40	60	80	100
2a	0.109567	0.199967	0.2582	0.265533	0.3464	0.3797
2b	0.0964	0.124033	0.1107	0.141267	0.129033	0.139433
2c	0.0964	0.127167	0.171133	0.288267	0.288767	0.352767
2d	0.105733	0.124233	0.140167	0.1501	0.209767	0.247933
2e	0.1043	0.159633	0.316333	0.353633	0.396633	0.422033
2f	0.131667	0.212667	0.296733	0.323433	0.300767	0.419467
2g	0.106633	0.141333	0.144067	0.180667	0.1536	0.168367
2h	0.147933	0.1742	0.208567	0.259633	0.326967	0.313667
2i	0.088033	0.1182	0.1689	0.2211	0.253333	0.2984
2j	0.086233	0.089667	0.0984	0.0927	0.099767	0.1063
Ascorbic acid	0.145167	0.42767	0.688367	0.965	1.2361	1.329133

All synthesized ethyl 7-amino-3-(4-bromobenzoyl) indolizine-1-carboxylate derivatives screened for their lipid peroxidation inhibition activity. The lipid peroxidation inhibition of the all synthesized 10 ethyl 7-amino-3-(4-bromobenzoyl)indolizine-1-carboxylate compounds is summarized in Table 6 and graph of Table 6 is shown in Figure 4. All compounds moderately act as reducing agents against lipid peroxidation.

RESULTS AND DISCUSSION

Statistical analysis

The resultrs of the experiment was expressed as mean ± SD of triplicate. All stastical analysis and graphing of data were accomplished using MS-excel software

Chemistry

In this present work, the novel indolizine derivatives were synthesized with eco-friendly and reduced temperature method. The time required to complete the reaction was only 30 minutes. It was efficient to produce to 7-amino-3-benzoylindolizine-1, 2-dicarboxylate derivatives. Newly synthesized compounds have been purified by column chromatography and recrystallized from ethyl acetate. The structures have been confermed by elemental analysis and spectroscopic techniques like ^{1}H-NMR, ^{13}C-NMR, LC-MS.

Pharmacological studies

All synthesized compounds have been tested for antibacterial activity. Among 10 compounds only five compounds

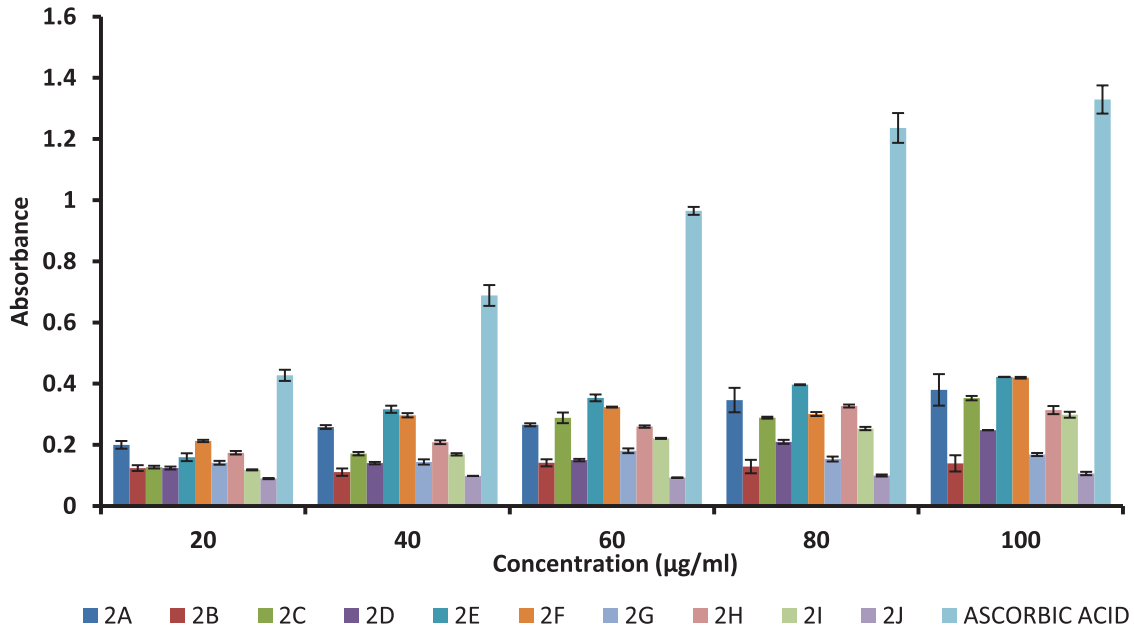


Figure 3. The antioxidant activity of dimethyl 7-amino-3-benzoylindolizine-1,2-dicarboxylate derivatives by reducing power. Values were means ± SD of triplicate.

Table 6. Lipid peroxidation inhibition activity of synthesized dimethyl 7-amino-3-benzoylindolizine-1,2-dicarboxylate derivatives.

Compd. number	Inhibition in %				
Concentration (µg/ml)	20	40	60	80	100
2a	10.71111	13.85815	15.34055	17.12565	18.85059
2b	11.11117	14.08192	17.25151	19.25151	22.25181
2c	13.14982	14.03798	18.95572	24.01957	24.73153
2d	10.39262	27.38557	32.74164	34.40364	35.06474
2e	18.85522	25.62413	27.83602	33.63016	37.10736
2f	10.45268	16.12327	18.25914	29.14443	39.39944
2g	5.362016	8.362016	13.85958	27.67104	42.07086
2h	6.797542	25.13023	26.84195	27.36313	28.33041
2i	16.55333	23.01244	26.77826	27.71957	29.86410
2j	13.26352	19.77354	25.78557	26.49128	29.93738
Ascorbic acid	31.65604	46.5659	62.92903	71.20411	77.75096

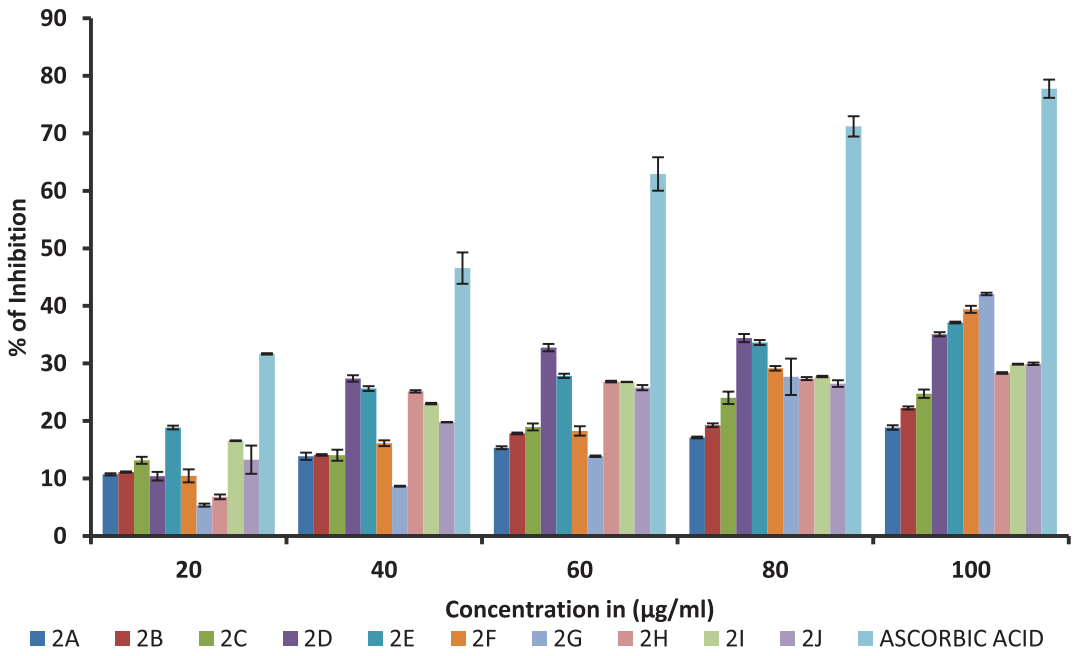


Figure 4. The lipid peroxidation inhibition activity of dimethyl 7-amino-3-benzoylindolizine-1,2-dicarboxylate derivatives. Values were means ± SD of triplicate.

shows inhibition activity and the other five compounds were non-toxic to bacteria. Compounds 2e, 2g, and 2i show the highest inhibition zone and compound 2a and 2f shows moderate inhibition zone. In Table 2, compounds inhibition zone against bacteria mentioned in mm to respective bacteria.

The synthesized compounds were screened for their antioxidant activity against DPPH free radical scavenging activity, Nitric oxide free radical scavenging activity, Reducing power scavenging activity and Lipid peroxidation inhibition activity. The 7-amino-3-benzoylindolizine-1,2-dicarboxylate derivatives antioxidant property have been studied by above mentioned four methods. The all the synthesized indolizine derivatives show moderate antioxidant activity in all the methods.

CONCLUSION

The present research work is concentrated on the efficient synthesis of indolizine derivatives. The eco-friendly reaction method was adapted to synthesized indolizine derivatives. All synthesized 7-amino-3-benzoylindolizine-1,2-dicarboxylate compounds have been tested for their anti-bacterial activity and five compounds possess remarkable activity against selected bacteria *Bacillus subtilis, Eschericha coli, Psseudomonas aeruginosa, Staphylococcous aureus, Baccillus cereus and Zymomonas mobilis*. The compound 2f only shows inhibition activity against *Bacillus subtilis*. The compound 2a does not shows inhibition activity against *Bacilluscereus* and it actively inhibits the all other reaming five bacteria. The compound 2e, 2g and 2j possess very remarkable activity against all selected bacteria with significant inhibition zone.

All synthesized 7-amino-3-benzoylindolizine-1, 2-dicarboxylate compounds have been tested for their antioxidant activity. All compounds significantly possess antioxidant property in DPPH free radical scavenging activity, Nitric oxide free radical scavenging activity, Reducing power scavenging activity, and Lipid peroxidation inhibition activity. In all the methods, synthesized compounds shows their activity less than standard. It means synthesized 10 compounds shows not negligible antioxidant property in each method.

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CONFLICT OF INTERESTS

The authors declared that they have no conflict of interest.

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