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SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF NOVEL INDOLEHYDRAZONES DERIVATIVES

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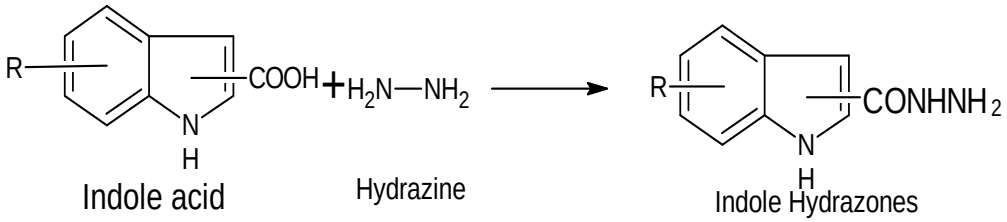
ABSTRACT
The indole hydrazine derivatives were syntehsised by using the indoleacetic acids in the presence of hydrazine to form indole hydrazones. The obtained compounds were identified by using spectral analysis. The antioxidant activity of compounds was done by using the DPPH method. In this method the compound 3e shows better acitivy than the other compounds. Teh results were compare with the standard compound α-tocopherol.

KEYWORDS: Indole acid, hydrazines. Anti oxidant activity, DPPH method.

INTRODUCTION
The indole scaffold represents one of the most important structural units prevalent in various naturally occurring and bioactive compounds. Recently, there has been an increasing interest in the indole derivatives to identify new drug candidates, particularly possessing five-membered heterocycles.[1,2] Many natural and synthetic bioactive indolyl heterocycles have demonstrated the critical role of indole scaffold in their biological activities.[3,4] Indolylazoles (1a) such as 5-(30-indolyl) oxazoles (Labradorins 1 & 2) and indolylthiazoles are known for their cytotoxic activities against NCIeH 460 (lung-NSC) human cancer cell line.[5] Marine indole alkaloids, Meridianins (1b) and their synthetic analogues have shown prominent anticancer activities against various cancer cell lines with best IC50 values against MCF-7 breast cancer cell line (0.25 mg/mL).[6] A diverse variety of indolylazoles with five/six-membered heterocyclic ring were also evaluated for their anticancer activities [5,7e10]. 1,3,4-Thiadiazoles are an important class of heterocyclic compounds with various biological activities such as antiviral[11], antimicrobial[12], fungicidal[13], antiinflammatory[14], antihypertensive[15], antituberculosis[16], antileishmanial[17], antidepressant[18] and anticancer.[19] Some of the marketed drugs with 1,3,4-thiadiazole scaffold such as Acetazolamide, Methazolamide and Globucid are well-known for their therapeutic applications.[20]

Experimental work
All the laboratory grade reagents were obtained commercially. The reaction was monitored by thin layer chromatography, which was performed on Merck pre-coated plates (silica gel. 60 F254, 0.25 mm) and was visualized by fluorescence quenching under UV light (254 nm). Recrystallization was done by commercially available absolute ethanol. Solvents were evaporated using Büchi rotary evaporator. Melting points were determined with electrothermal capillary melting point apparatus (E-Z melting). 1H NMR spectra were recorded on a Bruker Advance II (400 MHz) spectrometer. The coupling constant (J) values are in Hz. Mass spectra were measured on a 'Hewlette Packard' model HP GS/MS 5890/5972.

General procedure for the synthesis of indole-2(3)-carbohydrazides and indole-3-acetohydrazide derivatives
To a solution of indole carboxylic acid 2 (1 mmol) in ethanol (20 mL) was added catalytic amount of concentrated sulphuric acid (0.2 mL) and allowed to reflux for 20 h. After completion of the reaction, ethanol was removed and the residue was extracted with ethyl acetate (30 mL) and washed with saturated sodium bicarbonate solution (25 mL). Organic layer was dried over sodium sulphate and evaporated to give corresponding ester in good yields (85e95%). The solution of an appropriate ester (1 mmol) and hydrazine hydrate (2 mmol) in ethanol (15 mL) was refluxed for 4 h. Reaction mixture was cooled and the solid obtained was filtered and recrystallized from ethanol to obtain pure hydrazides 3aec, 3g and 3h.



R=H,5-OCH_3,6-OCH_3,1-CH_3,4-C6H4-OCH3, 1-C6H4-Cl

Scheme.

Antioxidant activity: 21

Antioxidant behaviour of these indole hydrazine derivatives (1-06) is measured *in vitro* by the inhibition of generated stable 2,2-diphenyl- 1-picrylhydrazyl (DPPH) free radical. Methods vary greatly as to the generated radical, the reproducibility of the generation process, and the end point that is used for the determination.

Procedure

The DPPH solution was prepared by dissolving accurately weighed 22 mg of DPPH in 100 ml of ethanol. From this stock solution, 18 ml was diluted to 100 ml with ethanol to obtain 100 µM DPPH solutions. The sample solution was prepared by accurately weighed 2.1 mg of each of the compounds and dissolved in 1 ml of freshly distilled DMSO separately to obtain solutions of 2.1 mg/ml concentration and the standard solution of was prepared by accurately weighed 10.5 mg of α-Tocopherol and dissolved in 1 ml of freshly distilled DMSO to get 10.5 mg/ml concentration.

A solution of test compound in ethanol (500 µl) was added to the ethanolic solution of DPPH radical. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm against the corresponding blank solution. The final concentration of the samples and standard α-Tocopherol solutions used is 100µg/ml. The percentage scavenging DPPH radical inhibitions were calculated by using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

Where, Abs control was the absorbance of DPPH radical and ethanol, Abs sample was the absorbance of DPPH radical and sample/standard.

The scavenging activity was expressed in terms of IC50, the concentration of the samples required to give a 50% reduction in the intensity of the signal of the DPPH radical. The results were done at least in triplicate.

RESULTS AND DISCUSSION

Indole-3-carbohydrazide (2a)

Yield 85%; White solid, mp. 232e234 _C. 1H NMR (400 MHz, DMSO-d6, d ppm): d 11.31 (s, 1H), 9.14 (s, 1H), 8.17 (d, J ¼ 7.10 Hz, 1H), 7.95 (d, J ¼ 2.80 Hz, 1H), 7.41 (d, J ¼ 8.10 Hz, 1H), 7.20e6.99 (m, 2H), 3.95 (s, 2H). IR (KBr, v cm_1): 3256, 3109, 1660, 1607, 1583, 1523, 1433, 1240, 736. MS (ESI) m/z calcd for C9H9N3O (M)þ 175.1, obsd 175.2.

5-Methoxyindole-3-carbohydrazide (2b)

Yield 90%; White solid, mp.178e179 _C. 1H NMR (400 MHz, DMSO-d6, d ppm): d 11.68 (s, 1H), 9.64 (s, 1H), 7.61 (s, 1H), 7.49 (s, 1H), 7.40 (d, J ¼ 8.00 Hz, 2H),4.18 (s, 2H), 3.86 (s, 3H). IR (KBr, n cm_1): 3340, 3290, 3050, 2920, 1646, 1605, 1545, 778, 724. MS (ESI) m/z calcd for C10H11N3O2 (M þ H)þ 206.1, obsd 206.2.

6-Methoxyindole-3-carbohydrazide (2c).

Yield 90%; White solid, mp 213e214 _C. 1H NMR (400 MHz, DMSO-d6, d ppm): d 11.72(s, 1H), 9.58 (s, 1H), 7.64 (s, 1H), 7.53 (s, 1H), 7.38 (d, J ¼ 8.00 Hz, 2H), 4.18 (s, 2H), 3.83 (s, 3H). IR (KBr, n cm_1): 3345, 3265, 2935, 1653, 1610, 1543, 736, 720. MS (ESI) m/z calcd for C10H11N3O2 (M)þ 205.1, obsd 205.1.

1-Methyl-indole-3-carbohydrazide (3d).

Yield 80%; White solid, mp 149e150 _C. 1H NMR (400 MHz, DMSO-d6, d ppm): d 9.18(s, 1H), 8.14 (d, J ¼ 7.80 Hz, 1H), 7.92 (d, J ¼ 7.80 Hz, 1H), 7.37 (d,J ¼ 8.10 Hz, 1H), 7.20e6.99 (m, 2H), 4.03 (s, 2H), 3.65 (s, 3H). 13C NMR (100 MHz, DMSO-d6, d ppm): d 160.88, 153.05, 141.03, 136.82, 130.96, 129.00, 125.32 124.60, 122.62, 121.58, 120.90, 117.21, 110.46, 105.69, 33.07. IR (KBr, v cm_1): 3212, 3098, 1658, 1604, 1582, 1520, 1465, 1235, 740. MS (ESI) m/z calcd for C10H11N3O (M)þ 189.1, obsd 189.1.

1-(4-Chlorobenzyl)-indole-3-carbohydrazide (3e).

Yield 75%; White solid, mp 173e174 _C. 1H NMR (400 MHz, DMSO-d6, d ppm): d 9.08 (s, 1H), 8.19 (d, J ¼ 8.0

Hz, 1H), 7.98 (s, 1H), 7.37 (d, J ¼ 6.2 Hz, 1H), 7.15 (d, J ¼ 7.0 Hz, 4H), 6.83 (d, J ¼ 7.45 Hz, 2H), 5.28 (s, 2H), 4.43 (s, 2H). IR (KBr, v cm_1): 3117, 3098, 1648, 1601, 1576, 1518, 1456, 1228, 764. MS (ESI) m/z calcd for C16H14ClN3O (Mþ H)þ 300.0, obsd 300.0.

1-(4-Methoxybenzyl)-indole-3-carbohydrazide (3f).
Yield 75%; White solid, mp 193e194 _C. 1H NMR (400 MHz, CDCl3, d ppm): d 8.20 (d, J ¼ 7.3 Hz, 1H), 7.83 (s, 1H), 7.33e7.23 (m, 6H), 7.06e7.09 (m, 2H), 5.30 (s, 2H), 4.45 (s, 2H), 3.91 (s, 3H). IR (KBr, v cm_1): 3112, 3098, 1650, 1602, 1580, 1521, 1448, 1223, 750. MS (ESI) m/z calcd for C17H17N3O2 (M þ H)þ 296.1, obsd 296.1.

Table 2: Anti oxidant activity by DPPH method.

S. No	CODE	Antioxidant activity (%inhibition)
1	3a	62
2	3b	84
3	3c	74
4	3d	61
5	3e	**51**
	3f	**58**
6	**α-Tocopherol**	**48**

DISCUSSION

The compound 3e shows better activity at IC_{50} value of 51 % inhibition than compare other molecules, less active than the standard molecule **α-Tocopherol** at IC 50 value of 48% inhibition.

CONCLUSION

The compound 3e shows better activity than compare other molecules, less active than the standard molecule **α-Tocophero.**

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