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**ORIGINAL ARTICLE**

# Synthesis and Antioxidant Properties of Two New Derivatives of Indeno-Benzofuran

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**KEYWORDS**

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Acetic Ionic Liquid;
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**ABSTRACT:** Ninhydrin reacts with poly-phenols in different ratios to produce tetracyclic adducts. Here, pyrogallol was used as a polyphenol compound. In the company of acidic ionic liquid (AIL), there was a selective reaction between the ortho-site of polyphenol and the ninhydrin's carbonyl group. Mono-adduct (1:1) 3 and bis-adduct (2:1) 4 were prepared as a solvent and a catalyst for the reaction of ninhydrin with pyrogallol in 1-(carboxymethyl)-3-methyl-1*H*-imidazolium chloride (mcmimCl). Purity of the products was approved by $^{1}$H NMR, $^{13}$C NMR, IR and Mass Spectroscopy. Moreover, the antioxidant activity of novel derivatives investigated in this paper by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical assay and butylated hydroxytoluene (BHT) was considered to be standard. The results indicated that mono-adduct showed the strongest antioxidant activity (IC50 = 5.289µg/ml).

## INTRODUCTION

Nowadays, 'green solvents' like room temperature ionic liquids (RTILs) have attracted a great deal of attention of many scientists all over the world. As a kind of useful ILs, imidazolium-based ionic liquids have exhibited unique aspects such as negligible vapor pressure, high thermal stability, non-flammability, high polarity and recyclability. Furthermore, ionic liquids have a high miscibility to both organic solvents and water [1].

Therefore, ionic liquids as special materials with excellent features that can result in improved rates of chemical reactions and provide higher selectivity compared to ordinary solvents. Thus, ILs has performed as green and useful solvents in chemical conversions [2-5].

Among the ionic liquids, acidic ionic liquids (AILs) are actually significant. The combination of acidic properties and ILs excellent features has provided researchers with new chances. It seems that, in presence of AIL, there have been communications of charged intermediates with cation/anion, by which facile proton or electron is transmitted by acidic ionic liquids [6].

To our best knowledge, there is no report of using ionic liquid 1-methyl-3-imidazolium acetic acid (Figure 1) as a catalyst for the preparation of phenol-ninhydrin adducts under mild condition.

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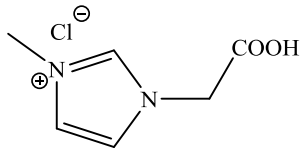


**Figure 1.** Chemical structure of 1-(carboxymethyl)-3-methyl-1H-imidazolium chloride [mcmim]Cl (AIL)

In this research, it is tried to present a novel, simple, and environment friendly method for the green synthesis of phenol-ninhydrin adducts (Figures. 2 and 3).

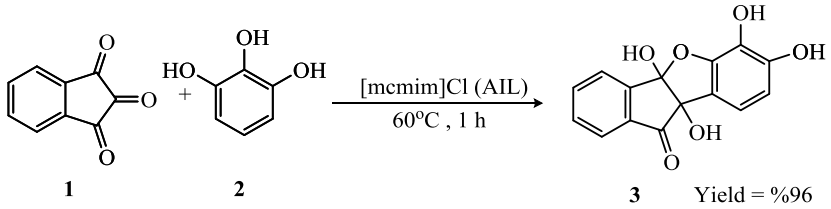


**Figure 2.** Synthesis of ninhydrin-pyrogallol monoadduct 3 in acetic ionic liquid

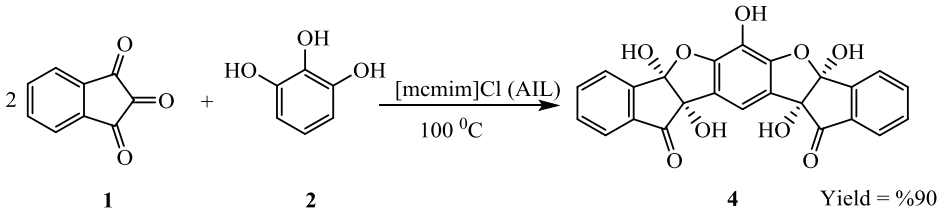


**Figure 3.** Synthesis of ninhydrin-pyrogallol bisadduct 4 in acetic ionic liquid.

Various natural and synthetic benzofuran derivatives have exhibited marvelous biological activities as pharmacological agents, such as antioxidant and antitumor properties. Therefore, due to all these points, these compounds have strongly attracted pharmacologists [7-11]. Antioxidants are supposed to show a very vital role against reactive oxygen species (ROS) in the body defense system [12-15]. They can donate their own electrons to ROS and thereby defuse the adverse effects; then antioxidants are hired to protect biomolecules from the destructive effects of ROS (for example, DNA mutation leading to cancer) [16]. In this research, antioxidative capacity and radical scavenging of anew-synthesized products are assessed by DPPH assay. There was violet ethanol solution made by an antioxidant assay based on electron-transfer. The free radical has been reduced in the presence of an antioxidant molecule; then, a colorless ethanol solution has been made. DPPH assay has provided a facile technique to evaluate antioxidants by spectrophotometry [17].

## MATERIALS AND METHODS

### *General*

Ninhydrin and pyrogallol obtained from Merck were used without further purification. The ILs used in this study, 1-metyl-3-carboxymethylimidazolium chloride [mcmim]Cl, was synthesized from the reaction of *N*-methylimidazole and monochloroacetic acid according to the procedure reported in the literature [18]. All melting points are corrected and determined by Electrothermal-9100 apparatus. IR spectra were recorded with a Shimadzu IR-460 spectrometer. $^{1}H$ and $^{13}C$ NMR spectra were recorded with a Bruker DRX-500 Avance instrument using $(CD_3)_2SO$ as the deuterated solvent containing tetramethylsilane as internal standard, at 500 and 75 MHz, respectively, in parts per million, and *J* in hertz. Electron impact ionization-mass spectroscopy (EI-MS) (70 eV): mass spectra were obtained with a Finnigan-MAT-8430 mass spectrometer (in *m/z)*. Elemental analyses (C, H and N) were obtained with a Heraeus CHN-O-Rapid analyzer.

***General procedure for preparation of AIL***

1-Methylimidazole (7.92 ml, 0.1 mol) and monochloroacetic acid (9.45 g, 0.1mol) were refluxed in acetonitrile (50 mL) for 5–6 h under stirring. After completion of the reaction (5-6 h; by TLC AcOEt/hexane 1:1)) and evaporation of the solvent, the AIL appeared to solid form and was used in the next step without further purification; yield: 16.7 g (95%).

***1-metyl-3-carboxymethylimidazolium chloride (AIL)***

White viscous oil, 16.7 g, yield 95%.$C_6H_9N_2O_2$, IR (KBr) ($\nu_{max}/cm^{-1}$): 2500-3340, 1725, 1607, 1520, 1230. 1H NMR (300 MHz, $CDCl_3$): $\delta$ = 3.40 (3 H, s, *Me*), 4.70 (2 H, s, $CH_2$), 7.03 (1 H, d, $^3J$ = 1.6 Hz, CH), 7.08 (1 H, d, $^3J$ = 1.6 Hz, CH), 8.42 (1 H, s, NCHN), 11.83 (1 H, br-s, COOH). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 34.8 (Me), 54.8 ($CH_2$), 118.9 (CH), 122.3 (CH), 134.6 (CH), 174.2 (C=O) ppm.

***General procedure for preparation of compound 3***

A stirred mixture of ninhydrin monohydrate (178 mg, 1 mmol) and pyrogallol (126 mg, 1 mmol) in [mcmim]Cl (1 mL) was heated to 60-70°C. After stirring for 2 h, 5 mL of water was added and white solid was deposited in the reaction mixture. Then, the reaction mixture was cooled down to room temperature and the solid materials were filtered and successively washed with cold water to afford the desired compound as a white solid, 274 mg (96%). Analytically pure sample was obtained by recrystallization from the mixture ethanol/EtOAc/n-hexane (Figure 2). The ionic liquid can be reused after extraction from the aqueous phase.

***4b,6,7,9b -Tetrahydroxy-4bH-indeno[1,2-b]benzofuran-10(9bH)-one (3)***

Colorless crystals, m.p. 265-266 °C; yield: 0.27g (96%). IR (KBr): 3387, 3324, 3200, 1713, 1635, 1603, 1225, 1138 cm-1. 1H NMR (DMSO): δ = 6.31 (1 H, d, 3J 8.1, CH), 6.35 (1 H, br-s, OH-alcoholic), 6.62 (1 H, d, 3J 8.1, CH), 7.57 (1 H, t, 3J 7.5, CH), 7.65 (1 H, d, 3J 7.5, CH), 7.79 (1H, br-s, OH-alcoholic), 7.82 (1 H, t, 3J 7.5, CH), 7.89 (1 H, d, 3J 7.5, CH), 8.53 (1H, br-s, OH-phenolic), 9.12 (1H, br-s, OH-phenolic) ppm. 13C NMR (DMSO): δ 83.4 (C-OH), 105.5 (C-OH), 110.2 (CH), 112.6 (CH), 119.3 (C), 123.8 (CH), 126.2 (CH), 128.2 (CH), 128.9 (CH), 132.5 (C), 139.6 (C), 160.3 (Carom-O), 164.2 (Carom-OH), 169.8 (Carom-OH), 199.8 (C=O) ppm. EI-MS: m/z (%) = 286 (M+, 76), 268 (21), 184 (22), 153 (95), 126 (43), 104 (100), 76 (78). Anal.Calcd for C15H10O6 (286.0): C, 62.94; H, 3.52. Found: C, 62.22; H, 3.85

***General procedure for preparation of compound 4***

A stirred mixture of ninhydrin monohydrate (356 mg, 2 mmol) and pyrogallol (126 mg, 1 mmol) in [mcmim]Cl (1 mL) was heated to 100-110°C. After stirring for 3 h, 10 mL of water was added and the white solid was deposited in the reaction mixture. After cooling the reaction mixture to room temperature, solid materials were filtered and successively washed with cold water to afford the desired compound as a white solid, 402 mg (90%). Analytically pured sample was obtained by recrystallization of the mixture ethanol/EtOAc (Figure 2). The ionic liquid can be reused after extraction from the aqueous phase.

***6,12a,13b,4b,7a-Pentahydroxy-12a,13b,4b,7a-tetrahydroindano[1,2-b]indano[2'',1''-5',4']furano[2',3'-5,4]benzo[d]furan-12,14-dione (4)***

Colorless crystals, m.p. 231-233°C; yield: 0.40g (90%). IR (KBr): 3431, 3330, 3071, 1726, 1605, 1499, 1233, 1108 cm-1. 1H NMR (DMSO): δ = 6.90 (1 H, s, CH), 6.32-6.35 (2 H, br-s, 2 OH-alcoholic), 7.55-7.57 (2 H, m, 2 CH), 7.87-7.89 (2 H, m, 2 CH), 7.77-7.79 (2 H, br-s, 2 OH-alcoholic), 7.82 (2 H, m, 2 CH), 7.89-8.02 (2 H, m, 2 CH), 8.73 (1H, br-s, OH-phenolic) ppm. 13C NMR (DMSO): δ 80.2 (2 C-OH), 102.6 (2 C-OH), 108.2 (CHp-arom), 119.2 (2 Cm-arom), 123.8 (2 CH), 127.1 (2 CH), 128.8 (2 CH), 129.3 (2 CH), 133.5 (2 C), 139.6 (2 C), 163.2 (2 Carom-O), 168.3 (Carom-OH), 197.9 (2 C=O) ppm. Anal.Calcd for C24H14O9 (446.3): C, 64.58; H, 3.16. Found: C, 65.02; H, 3.25.

***Antioxidant activity by DPPH assay***

BHT and DPPH were purchased from Sigma Chemical Co. The diluted DMSO solution of the samples (0.066 mg/ml) was prepared in diverse volumes (5, 10, 20, 30 µl). Then, 150 µl of freshly (80 µg/mL) DPPH methanol solution was combined with the above solution. The solution's absorbance was measured at 517 nm [19-20]. Then, the final mixture was incubated for 30 min at 37°C. The results suggest that absorbance decrease in samples led to the decreased activity of free radicals. Tests were occurred in triplicate. Diminution in absorption induced by the tested sample was compared with that of the control BHA, the ability to scavenge the DPPH. Radical has been calculated by the following equation:

Inhibition ratio (DPPH. scavenging effect) (%) = [(A control - A sample) / A control] × 100

## RESULTS AND DISCUSSION

The structure of products was confirmed by the spectroscopic data. For example, the $^1$H NMR spectrum of **3** showed two broad singlets at $\delta$ = 6.35, 7.79 ppm for the alcohol hydroxyl groups. The protons on the aromatic ring of pyrogallol moiety appeared as two doublets at $\delta$ = 6.31 and $\delta$ = 6.62 ppm. Two broad singlets were also observed at $\delta$ = 8.53 and $\delta$ = 9.12 ppm for the phenol hydroxyl groups. The protons on the aromatic ring of ninhydrin moiety appeared as two triplets ($\delta$ = 7.57, 7.82 ppm) and two doublets ($\delta$ = 7.65, 7.89 ppm). The $^1$H-decoupled $^{13}$C NMR spectrum of **3** showed 15 signals that is compatible with the proposed structure including one distinct resonance at $\delta$ = 199.8 ppm for the C=O group. The mass spectrum of **3** displayed the molecular ion peak at *m/z* =286. The $^1$H NMR spectrum of **4** showed the broad signals at $\delta$ = 6.32-6.35 and $\delta$ = 7.77-7.79 ppm for the alcohol hydroxyl groups, along with characteristic signals for phenol hydroxyl groups. A single proton on the aromatic ring of pyrogallol moiety appeared at $\delta$ = 6.90 ppm as singlet. Partial assignments of CH-aromatic resonances will be provided in the experimental section. In the $^1$H-decoupled $^{13}$C NMR spectrum of **4**, only 13 carbon signals were presented indicating a highly symmetric molecule. For example, two same carbonyl groups were appeared at 197.9 as one distinct resonance. The other aliphatic; and aromatic carbons of **4** also exhibited characteristic signals in the appropriate regions of the $^{13}$CNMR spectra.

Although the mechanistic details of the above reaction are unknown, a tentative mechanism for this transformation has been proposed as observed in Figure 4. Presumably, the electron-rich ortho-position of pyrogallol selectively reacts with the carbonyl group at the 2-position of ninhydrin and the intermediate **4** is formed in acidic ionic liquid media. This intermediate undergoes ring closure to produce product **3** with good yield (Figure 4).

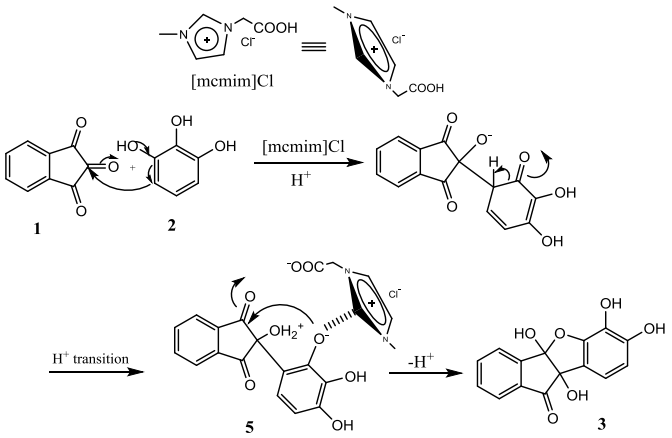


**Figure 4.** Proposed mechanism for the formation of ninhydrin-pyrogallol monoadduct **3**

The in vitro antioxidant activity of the tested compounds was determined with DPPH radical-scavenging activity. The compounds were tested for antioxidant activity at 1.65, 3.3, 6.6 and 9.9 microgram/ml (Table 1).

**Table 1.** In vitro antioxidant activity of Indeno-Benzofuran derivatives.

| Compd | %inhibition ± S.D.* 0.00165 mg/ml | %inhibition ± S.D. 0.0033 mg/ml | %inhibition ± S.D. 0.0066 mg/ml | %inhibition ± S.D. 0.0099 mg/ml |
|---|---|---|---|---|
| **BHA(std)** | 14.42771±2.486196 | 20.55383±2.170323 | 32.57606±1.231094 | 44.59829±1.903121 |
| **Mono-adduct (3)** | 26.53303±2.217015 | 41.69906±3.326833 | 65.68949±2.074393 | 86.65869±1.211764 |
| **Bis-adduct (4)** | 18.16937±1.084094 | 26.41339±0.380749 | 48.3398±1.505196 | 67.48429±0.784042 |

*Average of three determinations

The IC50 was calculated using the linear relation between the compound concentration and the probability of the percentage of DPPH inhibition (Table 2). Amongst the compounds screened for antioxidant activity, mono-adduct showed the most potent activity with IC50 value 5.289 µg/ml (Table 2). The results prove this fact that benzofuran may be a rich source for exploitation.

**Table 2.** IC50 of Mono and Bis-adduct benzofurans

| Compd | IC50 Value (microgram/ml) |
|---|---|
| **Mono-adduct** | 5.289 |
| **Bis-adduct** | 7.071 |
| **BHA (std)** | 11.383 |

## CONCLUSIONS

Results show the excellent antioxidant activity of the synthesized compounds. The free hydroxyl group on phenolic compound is responsible for free radicals scavenger [21]. This is because hydrogen from phenolic compound is donated to free radicals [22].

In the present research, this has been proven by testing

compounds 3and 4, indicating good antioxidant activity. Increasing the antioxidant activity of compound 3 (IC50= 5.289 µg/ml) relative to compound 4 (IC50= 7.071µg/ml) is probably due to the presence of two hydroxyl group in compound 3, easily donating hydrogen to free radicals, versus the only one hydroxyl group in compound 4.

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## REFERENCES

1. Wasserscheid P., Welton T., 2003. Ionic liquids in synthesis, Wiley, Weinheim.

2. Yavari I., Kowsari E., 2007. Ionic liquids as novel and recyclable reaction media for N-alkylation of amino-9,10-anthraquinones by trialkyl phosphites, Tetrahedron Lett. 48, 3753-3756.

3. Yavari I., Shahvelayati A.S., Ghanbari M., Ghazvini M., Piltan M., 2011. One-pot synthesis of functionalized α-acyloxythioamides from N-Protected a-amino acids as an acid component in the passerini reaction in an ionic liquid. J Iran Chem Soc. 8, 636-642

4. Shahvelayati A.S., Esmaeeli Z., 2012. Efficient synthesis of S-dipeptidothiouracil derivatives via a one-pot, five-component reactionunder ionic liquid condition. J Sulfur Chem. 33, 319-325.

5. Amarasekara A.S., 2016. Acidic Ionic Liquids. Chem Rev. 116(10), 6133-6183.

6. Vygodskii Y.S., Lozinskaya E.I., Shaplov A.S., 2002. Macromol Rapid Commun. 23, 676–679.

7. Rindhe S.S., Rode M., Karale B.K., 2010. New benzofuran derivatives as an antioxidant agent, Indian J. Pharm Sci. 27, 231-235.

8. Alwin, T., Reji T., 2017. Synthesis Antioxidant and Antibacterial Studies on 2-(2-arylamino-4-phenylthiazol-5-yl)benzofuran Derivatives. Int Res J Pure Appl Chem. 15(1), 1-8.

9. Chand K., Rajeshwari., Hiremathad A., Singh M., Santos MA., Keri RS., 2017. A review on antioxidant potential of bioactive heterocycle benzofuran: Natural and synthetic derivatives. Pharmacol Rep. 69, 281-295.

10. Naik R., Harmalkar DS., Xu X., Jang K., Lee K., 2015. Bioactive benzofuran derivatives: Moracins A-Z in medicinal chemistry. Eur J Med Chem. 90, 379-393.

11. Rangaswamy J., Kumar HV., Harini ST., Naik N., 2012. Synthesis of benzofuran based 1,3,5-substituted pyrazole derivatives: As a new class of potent antioxidants and antimicrobials-A novel accost to amend biocompatibility, Bioorganic and Medicinal Chemistry Letters. Bioorganic Med Chem Lett. 22, 4773-4777.

12. Yadav A., Yadav A., Kumari R., Mishra J.P., 2016. Antioxidants and its Functions in Human Body- A Review, Res. Environ Life Sci. 9, 1328-1331.

13. Sarvajeet G., Narendra S.T., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem. 48, 909-930.

14. Matés., José M. Pérez-Gómez., Cristina De Castro I.N., 1999. Antioxidant enzymes and human diseases, Clin Biochem. 32, 595-603.

15. Blokhina O., Virolainen E., Fagerstedt K., 2002. Antioxidants, oxidative damage and oxygen deprivation stress- A review, Ann Bot. 91, 179-194.

16. Kunwar A., 2011. Free radicals, oxidative stress and importance of antioxidants in human health Amit. J Med Allied Sci. 1(2), 53-60.

17. Huang D.J., Prior R.l., 2005. The chemistry behind antioxidant capacity assays. J Agric Food chem. 53, 1841-1856.

18. Vygodskii Y.S., Lozinskaya E.I., Shaplov A.S., 2002. Ionic Liquids as Novel Reaction Media for the Synthesis of Condensation Polymers. Macromol Rapid Commun. 23, 676–679.

19. Esmaeili M., Sonboli A., Ayyari Noushabadi M., 2010. Antioxidant and protective properties of six Tanacetum species against hydrogen peroxide-induced oxidative stress in K562 cell line: A comparative study. Food Chem. 121, 148-155.

20. Dastan D., Aliahmadi A., 2015. Antioxidant and antibacterial studies on different extracts of *Ornithogalum cuspidatum* Bertol from Iran. BFAIJ. 7(2), 1027-1072.