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Synthesis, biological activities and molecular docking simulation of hydrazone scaffolds of carvacrol, thymol and eugenol

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Abstract In present work, we report the synthesis of 12 new hydrazones and sulfonyl hydrazones linkage containing carvacrol, thymol and eugenol derivatives by simple condensation reactions. Synthesized derivatives have been characterized by ^{1}H NMR, ^{13}C NMR, LC–MS, and X-ray single crystallography techniques, and these derivatives were screened for anticancer testing by using sulforhodamine B assay and anti-oxidant testing by using DPPH assay. Docking studies of all the derivatives against the active site of human heme oxygenase-1 indicated that interaction with the maximum site of the amino acid residue of human heme oxygenase-1 was crucial for anti-oxidant activity. The results show that all derivatives possess interesting biological activities.

Keywords Hydrazones · Sulfonyl hydrazones · SRB · DPPH · Carvacrol · Thymol · Eugenol

Introduction

Hydrazones linkage constitutes an imperative class of biologically active drug molecules which have attracted the attention of pharmaceutical chemists due to their huge range of medicinal properties [1, 2]. These compounds are being synthesized as drugs by many researchers in order to combat diseases with minimal toxicity and

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maximal effects [3]. These predictions have provided a therapeutic pathway to develop new effective biologically active hydrazones [4]. Many hydrazone derivatives has been reported to exert notably biological activities [5]. Throughout the world, medicinal chemists have carried out vast research work on hydrazones and established new scaffolds with better activity and low toxicity profile;, Fig. 1 shows hydrazone linkage-based bioactive molecules [6, 7]. Numerous synthetic methodologies have been developed for the synthesis of hydrazone derivatives and they have been found to be active against diverse pharmacological targets [8]. They are known to possess antimicrobial, anticancer, anti-inflammatory and antimalarial activities [9–11]. These observations have been key for the development of new hydrazones that possess diverse biological activities [12].

It is well known that the essential oils obtained from plants, a traditional Mediterranean spice, possess strong biological activity due to their very high contents of monoterpenes and oxygenated compounds [13]. Carvacrol, thymol and eugenol are present in essential oils of many plants. These three naturally occurring phenolic monoterpenoids are interesting resourceful molecules incorporated as useful constituents in several products [14, 15]. The wide range of pharmacological activities, including analgesic, antimicrobial, anti-inflammatory, anti-oxidant and anticancer, etc., have been well researched [16–18].

This has created an interest among researchers who have synthesized a variety of hydrazones and screened them for their various biological activities. In the present study, we report on 12 new hydrazone and sulfonyl hydrazone derivatives of carvacrol, thymol, and eugenol. All the synthesized compounds show excellent anti-oxidant and good anticancer activity.

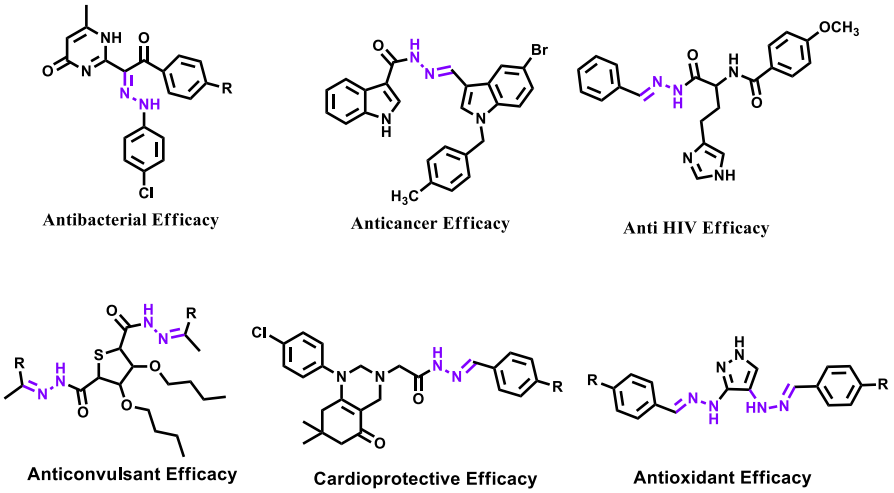


Fig. 1 Some biological active hydrazone linkage-based biological active molecules

Experimental

Chemicals, characterizations and procedures

All the chemicals and reagents necessary for the reactions were procured from Sigma-Aldrich and Fisher Scientific with purity 98% and used without further purification. The products were characterized using ^{1}H NMR, ^{13}C NMR, IR spectra and LC–MS. IR spectra were recorded with a Shimadzu FTIR spectrometer model using KBr disks. The NMR spectra ($CDCl_3$ and DMSO d6) were recorded on a Bruker AC-400 MHz spectrometer (for ^{1}H NMR use AC-400 MHz and ^{13}NMR use AC-100 MHz) with TMS as an internal standard. The crystal structure was examined using a Bruker APEX-II CCD diffractometer.

1. General procedure for the synthesis of ortho formyl phenolic monoterpenoids [29].
   Synthesis of ortho formyl phenolic monoterpenoids was carried out using a previously reported method.
2. General procedure for the synthesis of substituted hydrazine [30].
   Hydrazine hydrate (0.25 mol, 2.5 equiv) was added dropwise to a solution of 4,6 dichloro pyrimidine 3,6 dichloro pyridazine (0.1 mol, 1 equiv) in ethanol (100 mL) for 30 °C at room temperature. After stirring at 30 °C for 1 h, the reaction mixture after 1 h produced a creamy precipitate. After filtering the product (78% yield), it was used for the next step in the synthesis.
3. General procedure for the synthesis of substituted sulfonyl hydrazides [30].
   Hydrazine hydrate (0.25 mol, 2.5 equiv) was added dropwise to a solution of p-toluene sulfonyl chloride or 2, 4, 6 trimethyl benzene sulfonyl chloride (0.1 mol, 1 equiv) in THF (100 mL) at 0 °C under an inert atmosphere. After stirring for 0 to 5 °C for 30 min, ice-cold ethyl acetate (200 mL) was added to the cooled reaction mixture and the mixture was washed repeatedly with ice-cold 10% aqueous sodium chloride (5 × 150 mL). The organic layer was dried over sodium sulfate at 0 °C, and was then added slowly to a stirring solution of hexane (1.2 L) during 5 min. Substituted benzene sulfonyl hydrazide precipitated as a white solid and was collected by vacuum filtration. The filter cake was washed with hexanes (2 × 50 mL) and then dried in vacuo yielding the title compound as a white solid (17.61 g, 81%).
4. General procedure for the synthesis of phenolic monoterpenoid-based hydrazones [31].
   The hydrazones were prepared by reaction of equimolar quantities of substituted hydrazine (A or B) and ortho formyl thymol (or ortho formyl carvacrol or ortho formyl eugenol). Each reactant was dissolved in a minimum amount of ethanol, and then they were mixed together, adding 2–3 drops of acetic acid. The reaction mixture was refluxed for 2 h, then cooled to room temperature and poured into ice-cold water. The solid product obtained was collected by filtration and then dried using a drying oven at 70 °C. The product was crystallized from ethanol and dried to obtain the pure product (yield 80%).

5. General procedure for the synthesis of phenolic monoterpenoid-based sulfonyl hydrazones [31].
   The hydrazone were prepared by the reaction of equimolar quantities of substituted benzene sulfonyl hydrazide and ortho formyl thymol (or ortho formyl carvacrol or ortho formyl eugenol). Each reactant was dissolved in a minimum amount of ethanol, and then they were mixed together, adding 2–3 drops of acetic acid. The reaction mixture was refluxed for 2 h, then cooled to room temperature and poured into ice-cold water. The solid product obtained was collected by filtration and then dried using a drying oven at 70 °C. The product was crystallized from ethanol and dried to obtain the pure product (yield 80%).

Spectroscopic characterizations and physical properties of synthesized derivatives

(*E*)-2-((2-(6-chloropyridazine-3-yl)hydrazone)methyl)-3-isopropyl-6-methylphenol, (**IIA**), Color—white, mp- 110 °C. ^{1}**H NMR** (DMSO) δ 1.24–1.25 (d, $J = 4.0$ Hz, 6H), 2.19 (s, 3H), 3.21–3.34 (m, $J = 4.0$ Hz, 1H), 6.75 (d, $J = 8.0$ Hz, 1H), 6.78 (s, 1H), 7.10–7.12 (d, 1H), 8.44 (s, 1H) 8.80 (s, 1H for imine), 11.30 (s, 1H for OH D_2O exchangeable), 11.79 (s, 1H for NH D_2O exchangeable) ^{13}**C NMR** (DMSO) δ 15.39, 23.60, 27.96, 113.64, 115.30, 122.15, 132.22, 145.70, 156.09, LC–MS (methanol), *m*/*z*: $[M + 1]^+$ 305.50, $[M + 3]^+$ 307.51

(*E*)-2-((2-(6-chloropyridazine-3-yl)hydrazone)methyl)-6-isopropyl-3-methylphenol, (**IA**), Color—white, mp- 110 °C. ^{1}**H NMR** (DMSO) δ 1.33–1.35 (d, $J = 4.0$ Hz, 6H), 2.8 (s, 3H), 3.63–3.70 (m, $J = 4.0$ Hz, 1H), 6.80 (d, $J = 8.0$ Hz, 1H), 6.91 (s, 1H), 7.12–7.18 (d, 1H), 8.48 (s, 1H) 8.92 (s, 1H for imine), 11.34 (s, 1H for OH D_2O exchangeable), 11.69 (s, 1H for NH D_2O exchangeable) ^{13}**C NMR** (DMSO) δ 15.40, 23.63, 27.90, 114.22, 116.31, 126.20, 138.48, 146.10, 157.16, LC–MS (methanol), *m*/*z*: $[M + 1]^+$ 305.50, $[M + 3]^+$ 307.52

(*E*)-4-allyl-2-((2-(6-chloropyridazine-3-yl)hydrazone)methyl)-6-methoxyphenol, (**IIIA**), Color—yellow, mp- 108 °C. ^{1}**H NMR** ($CDCl_3$) δ 3.40 (d, 2H), 3.92 (s, 1H), 5.0–5.13 (t, 2H), 5.91–6.01 (m, 1H), 6.80–6.83 (s, 2H), 7.21 (s, 2H), 8.63 (s, 1H for imine), 9.72 (s, 1H for OH D_2O exchangeable), 11.43 (s, 1H for NH D_2O exchangeable) ^{13}**C NMR** ($CDCl_3$) δ 39.55, 56.22, 115.81, 116.21, 117.04, 123.40, 131.14, 137.13, 148.01, 148.24, 164.77, LC–MS (methanol), *m*/*z*: $[M + 1] +$ 319.47, $[M + 3] +$ 321.33

(*E*)-2-((2-(6-chloropyrimidin-4-yl)hydrazone)methyl)-3-isopropyl-6-methylphenol, (**IIB**), Color—white, mp- 112 °C. ^{1}**H NMR** (DMSO) δ 1.24–1.25 (d, $J = 4.0$ Hz, 6H), 2.19 (s, 3H), 3.21–3.34 (m, $J = 4.0$ Hz, 1H), 6.75 (d, $J = 8.0$ Hz, 1H), 6.78 (s, 1H), 7.10–7.12 (d, 1H), 8.44 (s, 1H) 8.80 (s, 1H for imine), 11.30 (s, 1H for OH D_2O

exchangeable), 11.79 (s, 1H for NH D_2O exchangeable) **^{13}C NMR** (DMSO) δ 15.39, 23.60, 27.96, 113.64, 115.30, 122.15, 132.22, 145.70, 156.09, LC–MS (methanol), *m/z*: $[M + 1]^+$ 305.50, $[M + 3]^+$ 307.51.

(*E*)-2-((2-(6-chloropyrimidin-4-yl)hydrazone)methyl)-6-isopropyl-3-methylphenol (**IIB**), Color—white; mp- 110 °C. **^{1}H NMR** (DMSO) δ 1.17–1.19 (d, $J = 4.0$ Hz, 6H), 2.35 (s, 3H), 3.26–3.35 (m, $J = 4.0$ Hz, 1H), 6.66 (d, $J = 8.0$ Hz, 1H), 6.77 (s, 1H), 7.07–7.09 (d, 1H), 8.43 (s, 1H), 8.65 (s, 1H for imine), 11.30 (s, 1H for OH D_2O exchangeable), 11.79 (s, 1H for NH D_2O exchangeable) **^{13}C NMR** (DMSO) δ 18.76, 22.28, 25.78, 115.16, 211.07, 127.58, 133.16, 135.06, 155.18, 158.38, LC–MS (methanol), *m/z*: $[M + 1]^+$ 350.50, $[M + 3]^+$ 307.52

(*E*)-4-allyl-2-((2-(6-chloropyrimidin-3-yl)hydrazone)methyl)-6-methoxyphenol (**IIIB**), Color—white; mp- 100 °C. **^{1}H NMR** (DMSO) δ 3.28–3.30 (d, 2H), 3.80 (s, 1H), 5.0–5.08 (t, 2H), 5.87–5.98(m, 1H), 6.72–6.73 (s, 1H), 7.00 (s, 1H), 7.08 (s, 1H), 8.33 (s, 1H), 8.41 (s, 1H for imine), 9.25 (s, 1H for OH D_2O exchangeable), 11.67 (s, 1H for NH D_2O exchangeable) **^{13}C NMR** (DMSO) δ 55.64, 101.30, 113.05, 155.34, 117.69, 119.74, 130.34, 137.54, 142.74, 144.74, 147.65, 157.79, 159.31, 161.75, LC–MS (methanol), *m/z*: $[M + 1]^+$ 319.47, $[M +3]^+$ 321.34.34

(*E*)-N-(2-hydroxy-6-isopropyl-3-methylbenzylidene)-4-methylbenezenesulfonohydrazide (**IID**), Color—white; mp- 98 °C. **^{1}H NMR** ($CDCl_3$) δ 1.18–1.20 (d, $J = 4.0$ Hz, 6H), 2.19 (s, 3H), 2.40–2.41 (s, 3H) 3.09–3.16 (m, $J = 4.0$ Hz, 1H), 6.69–6.71 (d, $J = 8.0$ Hz, 1H), 7.11–7.13 (d, $J = 8.0$ Hz, 1H), 7.32–7.34 (d, $J = 8.0$ Hz, 2H), 7.83–7.85 (d, $J = 8.0$ Hz, 2H), 8.00 (s, 1H for imine), 8.49 (s, 1H for OH D_2O exchangeable), 11.05 (s, 1H for NH D_2O exchangeable) **^{13}C NMR** ($CDCl_3$) δ 15.68, 21.66, 23.94 28.40, 112.93, 115.50, 123.67, 127.90, 128.39, 129.66, 130.07, 133.72, 134.52, 144.80, 146.82, 151.93, 157.40, LC–MS (methanol), *m/z*: $[M + 1]^+$ 347.49, $[M + Na^+]^+$ 369.47

(*E*)-N-(2-hydroxy-3-isopropyl-6-methylbenzylidene)-4-methylbenezenesulfonohydrazide (**ID**), Color—white; mp- 104 °C. **^{1}H NMR** (DMSO) δ 1.11–1.13 (d, $J = 4.0$ Hz, 6H), 2.26 (s, 3H), 2.34 (s, 3H) 3.19–3.22 (m, $J = 4.0$ Hz, 1H), 6.63–6.65 (d, $J = 8.0$ Hz, 1H), 7.06–7.08 (d, $J = 8.0$ Hz, 1H), 7.41–7.43 (d, $J = 8.0$ Hz, 2H), 7.76–7.80 (d, $J = 8.0$ Hz, 2H), 8.39 (s, 1H for imine), 11.31 (s, 1H for OH D_2O exchangeable), 11.74 (s, 1H for NH D_2O exchangeable) **^{13}C NMR** (DMSO) δ 18.53, 20.92, 22.21, 25.70, 114.70, 121.09, 127.07, 128.03, 129.89, 133.16, 135.35, 135.95, 143.90, 148.99, 155.22, LC–MS (methanol), *m/z*: $[M + 1]^+$ 3.47.50, $[M + Na^+]^+$ 369.47

(*E*)-N-(5-allyl-2-hydroxy-3-methoxybenzylidene)-4-methylbenzenesulfonohydrazide (**IIID**), Color—yellow; mp- 114 °C. **^{1}H NMR** ($CDCl_3$) δ 2.39 (S, 3H), 3.27–3.29 (d, 2H), 3.86 (s, 1H), 5.02–5.07 y7.82 (d, 2H), 7.93 (s, 1H for imine), 8.04 (s, s, 1H for OH D_2O exchangeable),10.01 (s, 1H for NH D_2O exchangeable) **^{13}C NMR** ($CDCl_3$) δ 21.64, 39.50, 56.12, 114.61, 116.76, 121.95, 127.93, 130.10,

131.12, 134.36, 137.11, 144.88, 146.01, 148.06, 152.34, LC–MS (methanol), *m/z*: $[M + 1]^+$ 361.46, $[M + Na^+]^+$ 383.42

(*E*)-N-(2-hydroxy-6-isopropyl-3-methylbenzylidene)-2,4,6-trimethylbenezenesulfonohydrazide (**IIC**), Color—white; mp- 114 °C. **^{1}H NMR** ($CDCl_3$) δ 1.16–1.18 (d, $J = 4.0$ Hz, 6H), 2.15 (s, 3H), 2.27–2.29 (s 3H), 2.70 (s, 6H), 3.08–3.19 (m, $J = 4.0$ Hz, 1H), 6.67–6.69 (d, $J = 8.0$ Hz, 1H), 6.99 (s, 2H), 7.07–7.09 (d, $J = 8.0$ Hz, 1H), 8.30 (s, 1H for imine), 8.44 (s, 1H for OH D_2O exchangeable), 10.82 (s, 1H for NH D_2O exchangeable) **^{13}C NMR** ($CDCl_3$) δ 15.66, 21.06, 23.13, 23.91, 28.38, 112.93, 113.02, 115.40, 123.54, 131.39, 132.38, 133.40, 140.22, 143.62, 146.56, 149.82, 157.13, LC–MS (methanol), *m/z*: $[M + 1]^+$ 375.51, $[M + Na^+]^+$ 397.47

(*E*)-N-(2-hydroxy-3-isopropyl-6-methylbenzylidene)-2,4,6-trimethylbenezenesulfonohydrazide (**IC**), Color—white; mp- 108 °C. **^{1}H NMR** (DMSO) δ 1.09–1.11 (d, $J = 4.0$ Hz, 6H), 2.23–2.26 (s, 6H), 2.62 (s, 6H), 3.13–3.20 (m, d, $J = 4.0$ Hz 1H), 6.63–6.65 (d, $J = 8.0$ Hz, 1H), 7.04 (d, $J = 8.0$ Hz, 1H), 7.06 (s, 2H), 8.43 (s, 1H for imine), 11.10 (s, 1H for OH D_2O exchangeable), 12.47 (s, 1H for NH D_2O exchangeable) **^{13}C NMR** (DMSO) δ 18.56, 20.35, 22.17, 22.21, 22.48, 25.65, 114.88, 121.12, 127.18, 131.81, 131.39, 132.51, 133.04, 165.27, 139.13, 142.65, 147.36, 154.90, LC–MS (methanol), *m/z*: $[M + 1]^+$ 375.51, $[M + Na^+]^+$ 397.48

(*E*)-N-(5-allyl-2-hydroxy-3-methoxybenzylidene)-2,4,6-trimethylbenzenesulfonohydrazide (**IIID**), Color—yellow; mp- 114 °C. **^{1}H NMR** ($CDCl_3$) δ 2.26 (S, 3H), 2.68 (s, 6H) 3.25–3.27 (d, 2H), 3.84 (s, 3H), 5.01–5.02 (s, 1H), 5.03–5.06 (t, 1H), 5.85–5.92 (m, 1H), 6.56 (s, 1H), 6.69–6.70 (s, 1H), 6.95 (s, 2H), 7.90 (s, 1H for imine), 8.49 (s, 1H for OH D_2O exchangeable), 9.74 (s, 1H for NH D_2O exchangeable) **^{13}C NMR** ($CDCl_3$) δ 21.03, 23.15, 39.49, 56.08, 114.31, 116.07, 116.97, 121.92, 131.06, 131.17, 132.27, 137.16, 140.25, 143.61, 145.64, 147.92, 150.44, LC–MS (methanol), *m/z*: $[M + 1]^+$ 389.48, $[M + Na^+]^+$ 411.44

Bioassay

Anticancer activity

In vitro anticancer activity of synthesized derivatives was performed using sulforhodamine B (SRB) assay on a panel of human cancer cell lines HCT-12 (colon cancer) and MIPaCa-2 (pancreatic cancer) [32]. The results are expressed as GI_{50} (growth inhibitory concentration at 50%), i.e. the concentration of the compounds which inhibits the tumor cell growth by 50%.

Anti-oxidant activity

DPPH radical-scavenging activity was performed by the reported method [33]. The concentrations of the sample necessary to decrease the DPPH concentration by 50% was obtained by interpolation from linear regression analysis and denoted as the

EC_{50} value (μg/mL). All determinations were carried out in triplicate. Butylated hydroxy toluene (BHT) was used as a reference compound.

Molecular docking studies

The docking study was performed by FRED (Open Eye) and used to determine the orientation of molecules bound in the active site of human heme oxygenase-1 (heme oxy-1, PDB code: 3CZY). The PDB files were downloaded from the Protein Data Bank (www.rsc.org). The ligands were drawn in Chem Draw Ultra 12.0 and the (Freeware) FRED docking programme was used to perform molecular docking. It is a shape-based docking approach. The steps performed by FRED along with the used parameters are as follows. Mostly, default parameters are used unless specified. Site box volume was 4818 Å. All possible poses of the ligand around the active site were enumerated by rigidly rotating and translating each conformer within the site. The resulting pose ensemble was filtered by rejecting poses that do not fit within the larger of the two volumes specified by the receptor file's shape potential grid and a contour level (referred to as the outer contour, volume = 1020 Å). The resulting pose ensemble was filtered by rejecting poses that do not have a least one heavy atom within the smaller of the two volumes specified by the receptor file's shape potential grid and a contour level (referred to as the inner contour, volume = 209 Å). The pose ensemble was filtered by rejecting poses that do not match any user-defined docking constraints [34].

Results and discussion

Chemistry of hydrazones

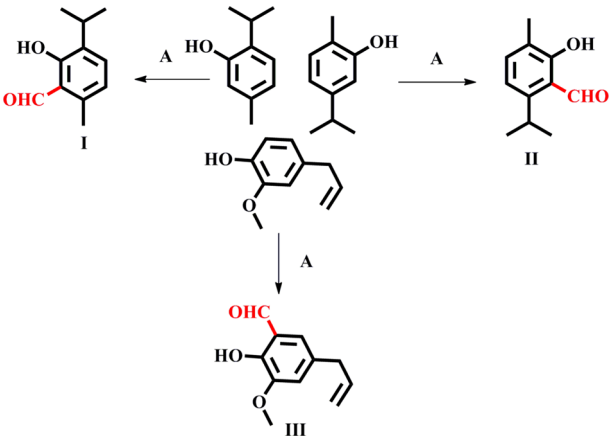
Hydrazones, possessing an azomethine –NHN=CH– group, constitute an important class of compounds as target structures for their biological activities [19]. These molecules are easily synthesized by the reaction of hydrazine or hydrazide or sometimes sulfonyl hydrazide with aldehydes and ketones. They are a class of organic compounds and widely used in organic synthesis [20]. They are not only intermediates but also very effective organic compounds when they are used as intermediates and coupling products which can be synthesized by using the active hydrogen component of the –CONHN=CH– azomethine group [21]. Many effective compounds, such as iproniazid and isocarboxazid, are synthesized by the reduction of hydrazide–hydrazones [22].

The targeted hydrazones and sulfonyl hydrazones were synthesized in three steps. The first step involved the synthesis of ortho formyl derivatives of natural phenolic monoterpenes (Part-I) via Reimer Tiemann reaction. The second step involved hydrazino and sulfonyl hydrazino analogs (A, B, C and D) of 4,6-dichloropyrimidine, 3,6-dichloropyridazine, p-toluene sulfonyl chloride and 2,4,6, trimethyl

benzene sulfonyl chloride (Part-II), which was further reacted with ortho formyl derivative of natural phenolic monoterpenes (Part-I) gave substituted hydrazones and sulfonyl hydrazones. (Part-III and Part-IV).

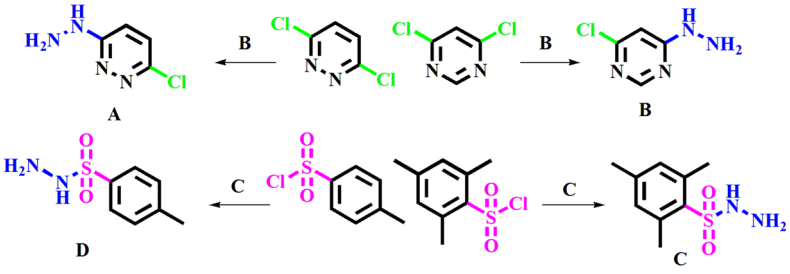
Reaction scheme

(Part-I) Synthesis of ortho formyl phenolic monoterpenoids



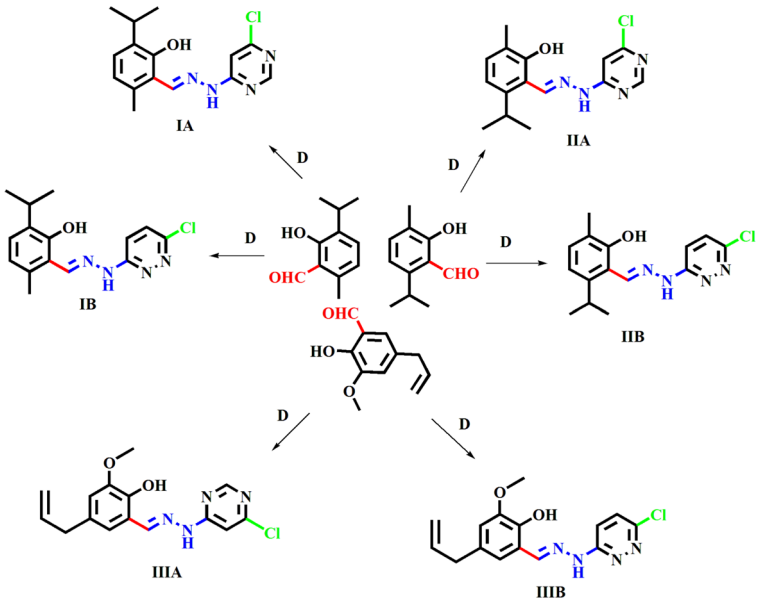
A = $CHCl_3$, NaOH in water reflux

(Part-II) Synthesis of substituted hydrazine and sulfonyl hydrazides



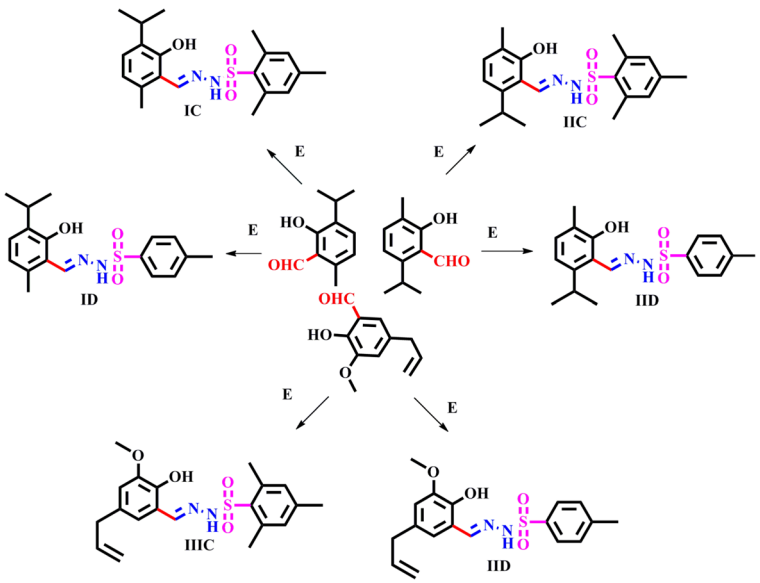
B = NH_2NH_2:H_2O in ethanol at RT for 2h. **C** = NH_2NH_2:H_2O in THF at 0 to 5 ^{O}C for 40 min.

(Part-III) Synthesis of phenolic monoterpenoid-based hydrazones



D= substituted hydrazine (A or B) in ethanol reflux at 80 °C for 2h.

(Part-IV) Synthesis of phenolic monoterpenoid-based sulfonyl hydrazones



E= substituted sulfonyl hydrazine (C or D) in ethanol reflux at 80 °C for 2h.

Biological evaluation

Cell growth inhibition was investigated by SRB assay and the results show that the some of compounds show an inhibitory effect on the proliferation of HCT15 (human colon cancer cell line) and MIAPaCa-2 (human pancreatic cancer cell line) in a dose-dependent manner (Table 1). The cytotoxic effect of **IB**, **IIIB**, **IIC** and **IID** derivatives against pancreatic cancer was excellent as compared to colon cancer: these six compounds were found to exhibit excellent cytotoxic potency (10 μg/mL) which is comparable with of Adriamycin (10 μg/mL) against pancreatic cancer on the MIAPaCa-2 cell line. Similarly, the compounds **IB**, **IIIB** and **IID** were found to possess moderate anticancer activity compared with that of Adriamycin (10 μg/mL) against colon cancer on the HCT-15 cell line. Of note is that the phenolic monoterpenoid-based derivatives show better activity against pancreatic cancer on the MIAPaCa-2 cell line as compared to colon cancer with the HCT-15 cell line. The total results suggested that, of all the compounds, there are six which possess excellent anticancer activity against pancreatic cancer. The theoretical reason behind the elevation in activity may be due to multiple influences involved in chemo-therapeutic mechanisms (Figs. 2, 3).

The DPPH radical scavenging activity of the compounds was found to be outstanding as compared to the standard BHT. In particular, the eugenol derivatives, i.e., **IIIA**, **IIIB**, **IIIC** and **IIID**, showed outstanding EC_{50} values which are comparable with STD, which may be due tothe free phenolic group and the allyl

Table 1 EC_{50} and GI_{50} values in anti-oxidant and anticancer activities of hydrazones and sulfonyl hydrazones

Sample no.	Compound code	Anti-oxidant test EC_{50} in μg/mL	Anticancer test	
			Colon cancer GI_{50} in μg/mL	Pancreatic cancer GI_{50} in μg/mL
1	**IA**	25.12 ± 0.123	>80	>80
2	**IIA**	22.54 ± 0.224	>80	>80
3	**IIIA**	**0.3608 ± 0.154**	>80	>80
4	**IB**	26.08 ± 0.145	69.2	**10**
5	**IIB**	18.02 ± 0.245	>80	>80
6	**IIIB**	**0.3883 ± 0.247**	19.2	**10**
7	**IC**	18.25 ± 0.115	>80	>80
8	**IIC**	20.8 ± 0.117	>80	**10**
9	**IIIC**	**0.3212 ± 0.138**	>80	54.74
10	**ID**	23.25 ± 0.123	>80	**10**
11	**IID**	19.06 ± 0.349	66.2	**10**
12	**IIID**	**0.3502 ± 0.350**	>80	**10**
13	**STD**	**0.1203 ± 0.213**	**10**	**10**
		Butylated hydroxy toluene (BHT)	Adriamycin (ADR)	Adriamycin (ADR)

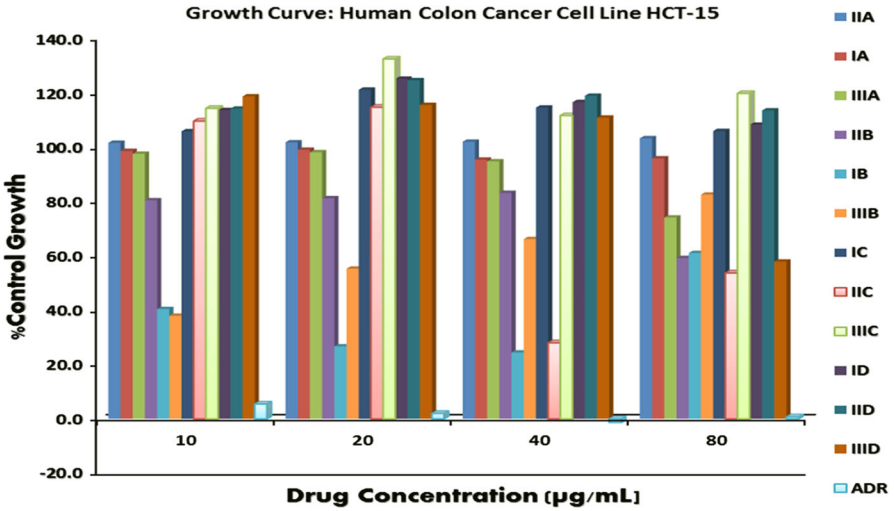


Fig. 2 Cytotoxicity effects of hydrazones and sulfonyl hydrazones against measured against colon on the HCT-15 cell line

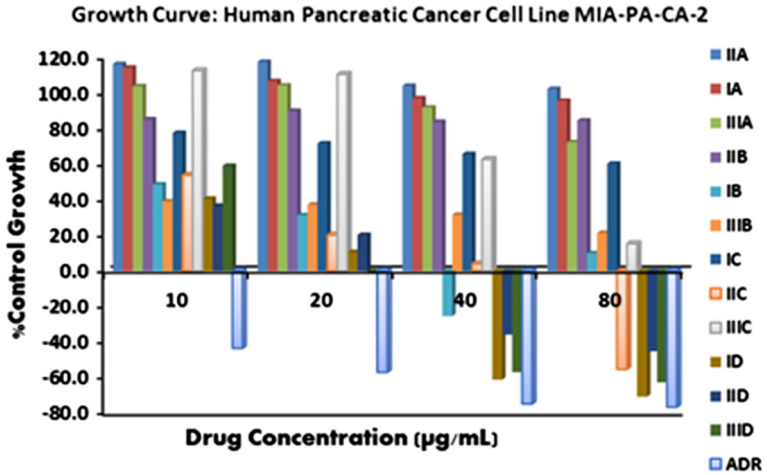


Fig. 3 Cytotoxicity effects of hydrazones and sulfonyl hydrazones against measured against pancreatic on the MIAPaCa-2 cell line

group present at the 5-position [23], while thymol derivatives, i.e., **IA**, **IIA**, **IC** and **ID**, demonstrated a decrease in % anti-oxidant activity with higher EC_{50} values. The lowest activity was noted for thymol derivatives at every concentration, perhaps due to the phenolic OH present in the steric crow [24]. The anti-oxidant activity of the compounds relates to their electron or hydrogen radical releasing abilities to DPPH so that they become stable diamagnetic molecules. This might be the reason for the higher or lower anti-oxidant activity [25], while carvacrol derivatives also showed inaudible anti-oxidant activity with high EC_{50} values, which is comparatively lower

than eugenol derivatives. All EC_{50} values for synthesizing derivatives are mentioned in Table 1.

Molecular docking outcomes

The docking of ligand molecules with human heme oxygenase-1 (3CZY) indicated that all the potent compounds exhibited the bonding with more than one amino acids in the active pocket (Fig. 4), and they may be considered as good inhibitors of heme oxygenase-1 (3CZY). The study also showed that the phenolic monoterpenoid-based hydrazone scaffolds are attached to the key residues, i.e., GLY143, ARG136, SER142, HEM140, ASP140 and GLN38 of the active pocket of oxygenase-1. Moreover, some of the compounds have minimum binding energy and docking scores comparable with 1(adamantan1yl) 2(1Himidazol1yl) ethanone from Tables 2 and 3, and hence could be considered to have a good affinity with heme oxygenase-1 (3CZY). The theoretical outcome highlighted that the minimum binding energy of the molecules with the targeted enzyme, which is lower than 1(adamantan1yl) 2(1Himidazol1yl) ethanone, suggests that the synthesized compounds **IA, IIA, IB** and **IC** are good inhibitors of heme oxygenase-1 (3CZY). Therefore, it is striking to state that the docking studies have extended the scope of developing phenolic monoterpenoid-based scaffolds as promising anti-oxidant and anticancer agents.

X-ray single crystallographic analysis

Appropriate crystals were selected and mounted on a Bruker APEX-II CCD diffractometer. During data collection, the crystal was kept at 296.15 K. The structure was solved using Olex2 [26] with the ShelXS [27] structure solution program by Direct Methods, and refined with the ShelXL [28] refinement package using least squares minimisation. The crystal structures of **IIC** and **IIA** with atomic

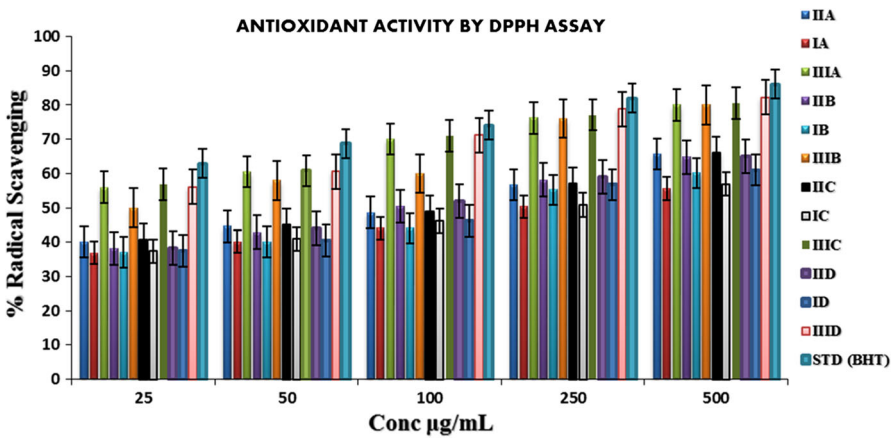


Fig. 4 Anti-oxidant activity of hydrazones and sulfonyl hydrazones determined by the DPPH free radical method at various concentrations

Table 2 Binding energies and entropies of protein, ligand and complex

Sample no.	Name	Binding energy	Complex × energy	Protein energy	Ligand energy	Entropic energy	Complex entropy	Protein entropy	Ligand entropy
1	**IA**	−32.22	−3416.13	−3394.88	10.97	19.58	−30.16	−30.15	−19.60
2	**IIA**	−43.16	−3426.96	−3394.88	11.08	19.51	−30.16	−30.15	−19.52
3	**IIIA**	−28.86	−3403.25	−3394.88	20.49	19.78	−30.16	−30.15	−19.79
4	**IB**	−33.95	−3402.46	−3394.88	26.37	19.63	−30.16	−30.15	−19.65
5	**IIB**	−26.55	−3400.85	−3394.88	20.58	19.50	−30.16	−30.15	−19.51
6	**IIIB**	−28.03	−3389.08	−3394.88	33.83	19.80	−30.17	−30.15	−19.82
7	**IC**	−35.60	−3390.89	−3394.88	39.59	20.02	−30.17	−30.15	−20.04
8	**IIC**	−25.30	−3355.81	−3394.88	44.37	20.00	−30.17	−30.15	−20.02
9	**IIIC**	−20.68	−3318.90	−3394.88	55.29	20.04	−30.17	−30.15	−20.06
10	**ID**	−23.23	−3393.40	−3394.88	24.71	19.90	−30.17	−30.15	−19.92
11	**IID**	−29.20	−3374.30	−3394.88	29.78	19.73	−30.16	−30.15	−19.75
12	**IIID**	−23.23	−3393.40	−3394.88	24.71	19.90	−30.17	−30.15	−19.92
13	**1(adamantan1yl) 2(1Himidazol1yl) ethanone (Std)**	−30.12	−3308.20	−3394.88	12.06	11.12	−30.20	−30.15	19.18

Table 3 Docking score, steric score and structural parameter of synthesized derivatives

Sample no.	Name	Docking score	Steric score	Desolvation	Hydrogen bond acceptor	Hydrogen bond donor
1	**IA**	−86.19	−91.13	8.01	0.00	−3.07
2	**IIA**	−78.81	−86.44	13.39	−3.36	−2.41
3	**IIIA**	−84.30	−87.58	13.54	−6.76	−3.50
4	**IB**	−82.44	−91.14	8.87	−0.17	0.00
5	**IIB**	−82.80	−77.56	10.53	−5.13	−10.64
6	**IIIB**	−81.93	−92.65	11.29	−0.47	−0.10
7	**IC**	−92.77	−103.05	10.28	0.00	0.00
8	**IIC**	−72.55	−79.07	11.41	−3.57	−1.31
9	**IIIC**	−83.62	−90.65	10.87	−3.81	−0.02
10	**ID**	−82.44	−91.14	8.87	−0.17	0.00
11	**IID**	−82.80	−77.56	10.53	−5.13	−10.64
12	**IIID**	−81.93	−92.65	11.29	−0.47	−0.10
13	**1(adamantan1yl) 2(1Himidazol1yl) ethanone (Std)**	−82.20	−88.20	9.47	−0.22	−8.4

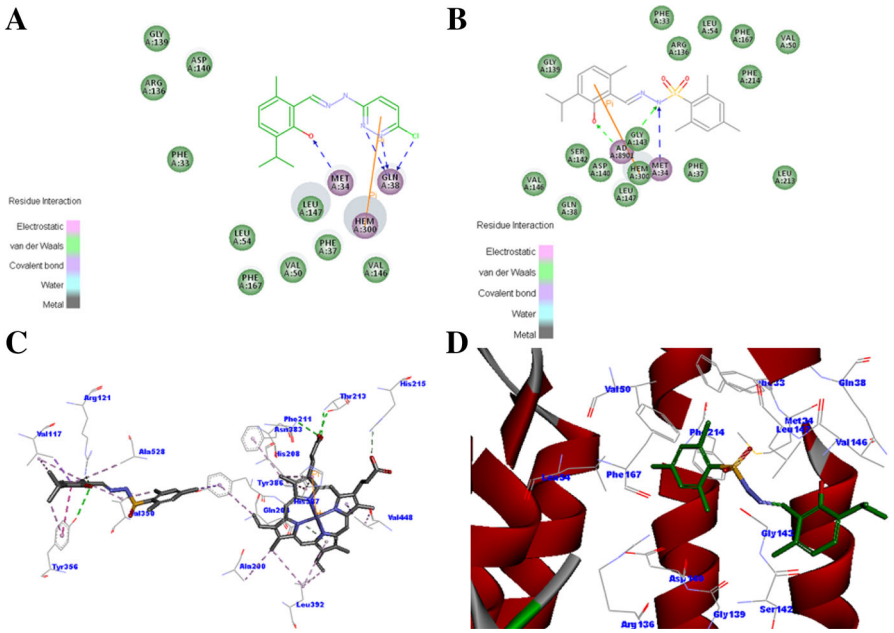


Fig. 5 2D binding interactions of **a** IB with structural amino acid residue, **b** IC with structural amino acid residue, **c** 3D binding interactions IC with structural amino acid residue and **d** 3D image of IC in the active site of heme oxygenase-1 (3CZY)

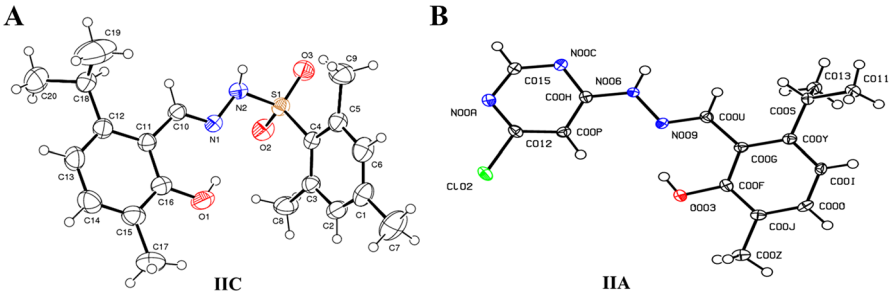


Fig. 6 ORTEPs representing 50% probability ellipsoids of **a** IIC and **b** IIA

numbering are presented in Fig. 5. The **IIC** crystal structures show a monoclinic crystal system and **IIA** crystal structures show a triclinic crystal system. The 3D representation diagrams of anhydrous **IIC** and **IIA** are shown in Fig. 6. The crystal structures indicate that structures of hydrazone and sulfonyl hydrazone scaffolds of phenolic monoterpenoids are connected through the substituted hydrazine and formyl functionality of phenolic monoterpenoids. The CCDC No. for **IIC** is 1515851 and for and **IIA** is 1501299.

Conclusion

In conclusion, novel derivatives of carvacrol- and thymol-containing hydrazone and sulfonyl hydrazone linkage have been synthesized by a multistep pathway with good practical yields. Anticancer and anti-oxidant activities were tested in vitro. The obtained results indicated that the free hydroxy as well as hydrazone and sulfonyl hydrazone groups in given scaffolds have significantly remarkable anticancer and anti-oxidant potency. All synthesized compounds displayed extensively in vitro anti-oxidant activities by DPPH assay. In an anticancer test by using a SRB assay against pancreatic cancer with the MIAPaCa-2 cell line and colon cancer with the HCT-15 cell line, the GI_{50} values of six compounds indicated their good anticancer efficacy which is comparable with that of STD (Adriamycin). All these results will be useful in the future to guide the design and modification of new natural phenolic monoterpenoid-based analogues as biologically active agents.

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Compliance with ethical standards

Conflict of interest The authors declare no competing financial interests.

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