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Design, synthesis, biological screenings and docking simulations of novel carvacrol and thymol derivatives containing acetohydrazone linkage

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Abstract Carvacrol and thymol are well-known phenolic monoterpenoids present as functional ingredients in numerous products. Keeping the diverse therapeutic activities of phenolic monoterpenes in mind, we attempted to synthesize a new series of acetohydrazone linkage containing carvacrol and thymol derivatives. All synthesized derivatives were characterized by spectroscopic techniques. Finally, all the derivatives were screened for their anti-oxidant activities by using DPPH assay, and anticancer activities by using SRB assay against pancreatic cancer with the MIAPaCa-2 cell line and colon cancer with the HCT-15 cell line. Themolecular docking studies of all the synthesized derivatives were carried out on the cyclooxygenase-2 (COX-2) protein enzyme. In the anti-oxidant test, EC_{50} values of all the compounds showed excellent anti-oxidant potency, and similarly the values of GI_{50} in the anticancer test displayed that most of the compounds possess good anticancer potency. Total docking results suggested that all the synthesized compounds exhibit good binding affinity towards receptors.

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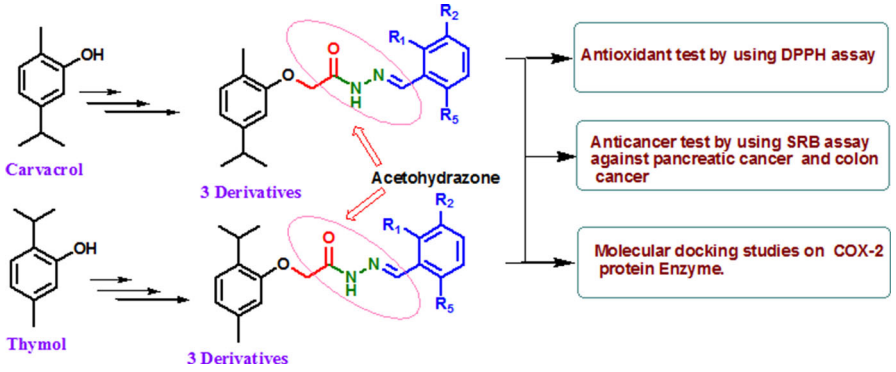
Graphical Abstract



Keywords Cyclooxygenase-2 · Molecular docking · HCT-15 · MIAPaCa-2 · SRB protocol · DPPH assay · Anti-oxidant and anticancer

Introduction

Organic syntheses are key tools for supplying rare compounds and are helping in the transformation of bioactive natural compounds into more drug-like derivatives [1, 2]. Some novel synthetic tactics have been developed in an effort to yield potential compounds with medicinal value [3–5]. Mainly, structural modifications of the natural core structures are achieved to increase selectivity and potency to provide other properties [6, 7] and to help their synthesis [8]. In addition, many novel synthetic approaches have been developed to increase structural diversity; in other words, to enlarge the chemical space of investigated molecules [9–11].

Thymol (2-iso-propyl- 5-methylphenol) and its isomer carvacrol (5-iso-propyl-2-methyl-phenol) are the most frequently occurring constituents of thyme plants, which possess a wide range of biological and pharmacological properties [12–14]. The rich essential oils present in thyme species are used for the treatment of several diseases as well as for the preservation of food [15]. These two naturally occurring phenolic monoterpenoids are outstanding resourceful molecules incorporated as functional ingredients in numerous products [16, 17]. Carvacrol and thymol have been used as precursors for the synthesis of various molecules, and their analogs are part of many biologically active molecules known to exhibit interesting biological activities [18, 19].

Hence, in the present investigation, we have synthesized six new carvacrol- and thymol-based hydrazone derivatives by the condensation of ortho formyl carvacrol, thymol and eugenol with substituted 2-acetohydrazide of carvacrol

and thymol as previously reported [20, 21]. Finally, all the synthesized derivatives were screened for their anti-oxidant activity by using DPPH assay, and anticancer activity by using SRB assay against pancreatic cancer and colon cancer with MIaPaCa-2 and HCT-15 cell lines, respectively. The molecular docking studies of all the synthesized derivatives were performed on the COX-2 protein enzyme.

Materials and methods

Experimental

All the chemicals and reagents necessary for the reactions were procured from Sigma-Aldrich and Fisher 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 on a Shimadzu FTIR spectrometer using KBr disks. The NMR spectra ($CDCl_3$) were recorded on a Bruker AC-400 MHz spectrometer with TMS as an internal standard.

Synthesis of acetate of carvacrol and thymol [22]

A mixture of carvacrol or thymol (0.02 mol), dry acetone (50 ml), and anhydrous K_2CO_3 (0.03 mol) in a 100-ml round-bottom flask was heated to reflux for 6 h. Upon cooling to room temperature, and the drop-wise addition of ethyl chloroacetate (0.02 mol) during 1 h, refluxing was continued for 4 h. The reaction mixture was kept overnight, the excess of solvent was recovered, and the residue was quenched onto crushed ice. The contents were then stirred for 30 min and extracted with diethyl ether. The organic layer was washed with water and dried with Na_2SO_4. The solvent was recovered under vacuum to obtain yellowish oils (yield: 79.55%), and the product was used in the next step without purification.

Synthesis of acetohydrazide of carvacrol and thymol [23]

Acetate of carvacrol or thymol (0.015 mol) and 99% hydrazine hydrate (0.023 mol) in ethanol (15 ml) were charged to a 50-ml round-bottom flask and refluxed for 2 h. Progress of the reaction was monitored by TLC. The resulting clear solution was concentrated under vacuum and the suspension formed was poured onto crushed ice. The product separated was filtered, washed with cold water, dried and recrystallized from ethanol–water (yield: 84%; m.p. 78–81 °C).

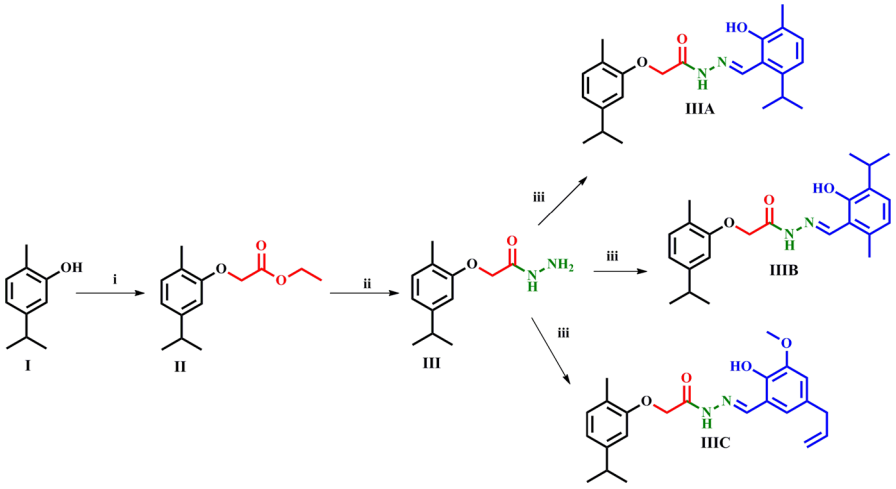
Synthesis of Acetohydrazone of carvacrol acetohydrazide and thymol acetohydrazide [24]

Acetohydrazone of carvacrol and thymol were conveniently synthesized via the condensation of acetohydrazide of carvacrol and thymol with ortho formyl carvacrol, thymol and eugenol [20]. The acetohydrazide of carvacrol or thymol (160 mg, 0.0103 mol) was treated with ortho formyl carvacrol or thymol or eugenol (120 mg, 0.01 mol) in anhydrous ethanol (20 mL). The reaction mixture was refluxed for 2 h. After cooling, the solid was collected and washed with anhydrous ethanol followed by drying to get a solid, which was dried and recrystallized from ethanol (yield: 78%).

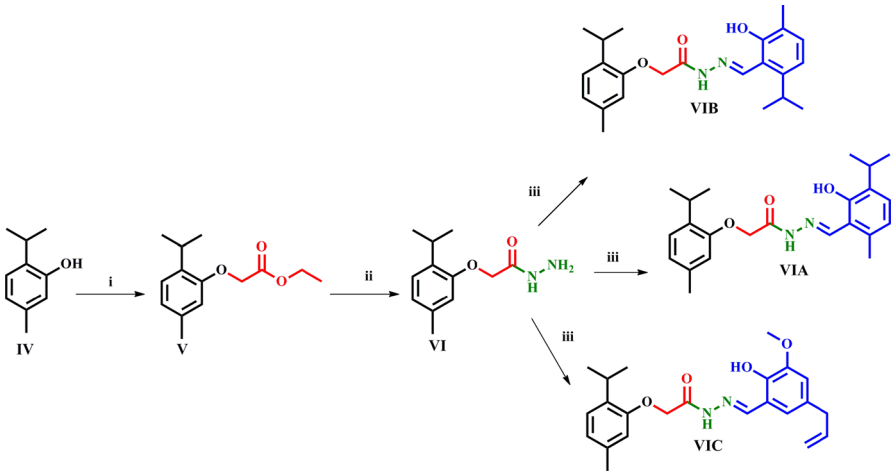
Results and discussion

Chemistry

The synthesis schemes involved a multi-step pathway leading to the formation of ix novel substituted acetohydrazones of carvacrol and thymol in excellent yields. The structures of the compounds obtained are shown in Schemes 1 and 2. Their analytical and spectroscopic data are in agreement with the predicted structures. The synthesis of acetohydrazone analogs of carvacrol and thymol by using 2-acetohydrazide prepared from carvacrol and thymol, which react with aldehyde of carvacrol, thymol and eugenol, has been previously reported by our group [24, 25].



Reaction Scheme 1 Synthesis of carvacrol hydrazones derivatives. Reaction condition: (*i*) K_2CO_3 in acetone and bromoethylaceate reflux at 60 °C for 4 h, (*ii*) $NH_2NH_2{:}H_2O$, ethanol, reflux at 80 °C for 2 h, (*iii*) respective aldehyde in ethanol, reflux at 80 °C for 2 h



Reaction Scheme 2 Synthesis of thymol hydrazones derivatives. Reaction condition: (*i*) K_2CO_3 in acetone and bromoethylaceate reflux at 60 °C for 4 h, (*ii*) $NH_2NH_2{:}H_2O$, ethanol, reflux at 80 °C for 2 h, (*iii*) respective aldehyde in ethanol, reflux at 80 °C for 2 h

The synthesis route of substituted acetohydrazones of carvacrol and thymol is shown in Schemes 1 and 2.

Spectroscopic Characterizations and Physical Properties of Synthesized Derivatives

(*E*)-*N*-(2-hydroxy-6-isoproyl-3-methylbenzylindine)-2-(5-isopropyl-2-methylphenoxy) *acetohydrazide, carvacrylacetohydrazone of carvacrol* **(IIIA)** Color creamy, mp-110 °C. ^{1}H NMR ($CDCl_3$) δ 1.21–1.24 (d, $J = 4.0$ Hz, 6H), δ 1.25–1.27 (*d*, $J = 4.0$ Hz, 6H), 2.16 (s, 3H), 2.31 (s, 3H), 2.84–2.89 (m, J = 4.0 Hz, 1H), 3.19–3.25 (m, J = 4.0 Hz, 1H), 4.69 (s, 2H), 6.71 (d, J = 8.0 Hz, 1H), 6.7 (s, 1H), 6.85 (d, 1H), 7.12–7.25 (d, J = 8.0 Hz, 2H), 8.90 (s, 1H for imine), 9.38 (s, 1H for OH + D_2O Exchangeable), 11.89 (s, 1H for NH + D_2O Exchangeable) ^{13}C NMR ($CDCl_3$) δ 15.75, 16.08, 24.01, 24.06, 28.46, 34.04, 67.86, 110.70, 112.98, 115.32, 120.32, 123.94, 124.01, 131.13, 133.61, 146.72, 148.81, 150.20, 155.27, 157.91, 164.27, LC–MS (methanol), m/z: [M + 1] + 383.43, [M + Na^+] + 405.40.

(E)-N-(2-hydroxy-3-isoproyl-6-methylbenzylindine)-2-(5-isopropyl-2-methylphenoxy) acetohydrazide, carvacryl acetohydrazone of thymol **(IIIB)** Color white, mp-100 °C. ^{1}H NMR ($CDCl_3$) δ 1.22 (d, J = 4.0 Hz, 6H), δ 1.24 (d, J = 4.0 Hz, 6H), 2.31 (s, 3H), 2.40 (s, 3H), 2.84–2.91 (m, J = 4.0 Hz, 1H), 3.35–3.42 (m, J = 4.0 Hz, 1H), 4.70 (s, 2H), 6.68 (d, J = 8.0 Hz, 1H), 6.71 (s, 1H), 6.86 (d, 2H), 7.12–7.16 (d, J = 8.0 Hz, 2H), 8.71 (s, 1H for imine), 9.35 (s, 1H for OH D_2O Exchangeable), 11.72 (s, 1H for NH + D_2O Exchangeable), ^{13}C NMR ($CDCl_3$) δ 16.07, 19.18, 22.40, 24.05, 26.50, 34.04, 67.79, 110.60, 114.52, 120.30, 121.00,

123.95, 129.08, 131.13, 134.80, 135.61, 148.82, 150.22, 155.25, 157.07, 164.13, LC–MS (methanol), m/z: [M + 1] + 383.43, [M + Na^+] + 405.40.

(E)-N-(5-allyl-2-hydroxy-3-methoxybenzylindine)-2-(5-isopropyl-2-methylphenoxy) acetohydrazide, carvacrylacetohydrazone of eugenol **(IIIC)** Color yellow, mp-108 °C. ^{1}H NMR ($CDCl_3$) δ 1.19 (d, J = 4.0 Hz, 6H), 2.11 (s, 3H), 2.21–2.85 (m, J = 4.0 Hz, 1H), 3.31–3.32 (d, 2H), 3.90 (s, 3H), 4.67 (s, 2H), 5.05–5.10 (m, 2H), 5.89–5.90 (m, 1H), 6.69 (s, 1H), 6.74 (d, J = 8.0 Hz, 1H), 6.77 (d, J = 8.0 Hz, 1H), 6.84 (d, 1H), 7.11–7.13 (d, 1H), 8.45 (s, 1H for imine), 9.41 (s, 1H for OH + D_2O Exchangeable), 10.50 (s, 1H for NH + D_2O Exchangeable), ^{13}C NMR ($CDCl_3$) δ 16.02, 24.06, 34.04, 39.57, 56.26, 67.57, 110.40, 114.18, 116.07, 117.04, 120.20, 121.99, 123.85, 13090, 131.10, 137.25, 146.63, 148.21, 148.76, 151.95, 155.13, 164.25. LC–MS (methanol), m/z: [M + 1] + 397.40, [M + Na^+] + 419.36.

(E)-N-(2-hydroxy-3-isoproyl-6-methylbenzylindine)-2-(2-isopropyl-5-methylphenoxy) acetohydrazide, thymylacetohydrazone of thymol **(VIA)** Color creamy, mp-112 °C. ^{1}H NMR ($CDCl_3$) δ 1.20–1.22 (d, J = 4.0 Hz, 6H), δ 1.24–1.297 (d, J = 4.0 Hz, 6H), 2.33 (s, 3H), 2.39 (s, 3H), 3.30–3.33 (m, J = 4.0 Hz, 1H), 3.37–3.40 (m, J = 4.0 Hz, 1H), 4.68 (s, 2H), 6.66–6.68 (d, J = 8.0 Hz, 2H), 6.68–6.88 (s, 1H), 7.14–7.25 (d, 2H), 8.70 (s, 1H for imine), 9.34 (s, 1H for OH D_2O + Exchangeable), 11.70 (s, 1H for NH + D_2O Exchangeable). ^{13}C NMR ($CDCl_3$) δ 19.12, 21.26, 22.41, 23.96, 26.51, 26.89, 68.10, 113.51, 114.50, 121.03, 123.48, 126.51, 129.11, 134.10, 134.79, 135.60, 137.26, 150.60, 154.26, 157.06, 164.22, LC–MS (methanol), m/z: [M + 1] + 383.43, [M + Na^+] + 405.40.

(E)-N-(2-hydroxy-6-isoproyl-3-methylbenzylindine)-2-(2-isopropyl-5-methylphenoxy) acetohydrazide, thymyl acetohydrazone of carvacrol **(VIB)** Color white, mp-102 °C. ^{1}H NMR ($CDCl_3$) δ 1.21–1.24 (d, J = 4.0 Hz, 6H), δ 1.26–1.29 (d, J = 4.0 Hz, 6H), 2.25 (s, 3H), 2.29 (s, 3H), 3.17–3.21 (m, J = 4.0 Hz, 1H), 3.22–3.28 (m, J = 4.0 Hz, 1H), 4.68 (s,2H), 6.68 (s, 1H), 6.73 (d, J = 8.0 Hz, 1H), 7.15–7.25 (d, 2H), 8.90 (s, 1H for imine), 9.35 (s, 1H for OH + D_2O Exchangeable), 11.84 (s, 1H for + NH D_2O Exchangeable). ^{13}C NMR ($CDCl_3$) δ 15.14, 21.26, 22.97, 23.96, 26.91, 28.53, 68.14, 112.96, 113.58, 115.35, 123.50, 123.95, 126.51, 133.63, 134.12, 137.27, 146.67, 150.37, 154.27, 157.90, 164.31, LC–MS (methanol), m/z: [M + 1] + 383.43, [M + Na^+] + 405.40.

(E)-N-(5-allyl-2-hydroxy-3-methoxybenzylindine)-2-(2-isopropyl-5-methylphenoxy) acetohydrazide, thymyl acetohydrazone of eugenol **(VIC)** Color yellow, mp-108 °C. ^{1}H NMR ($CDCl_3$) δ 1.18 (d, J = 4.0 Hz, 6H), 2.13 (s, 3H), 2.20–2.80 (m, J = 4.0 Hz, 1H), 3.31–3.32 (d, 2H), 3.90 (s, 3H), 4.67 (s,2H), 5.05–5.10 (m, 2H), 5.89–5.90 (m, 1H), 6.69 (s, 1H), 6.74 (d, J = 8.0 Hz, 1H), 6.77 (d, J = 8.0 Hz, 1H), 6.84 (d, 1H),7.11–7.13 (d, 1H), 8.40 (s, 1H for imine), 9.41 35 (s, 1H for OH + D_2O Exchangeable), 10.50 (s, 1H for NH + D_2O Exchangeable), ^{13}C NMR ($CDCl_3$) δ 16.08, 24.16, 34.14, 39.50, 56.20, 67.60, 110.40, 114.18, 116.07, 117.04, 120.20, 121.99, 123.85, 130.90, 131.10, 137.25, 146.63, 148.21, 148.76, 151.95, 155.13, 164.25. LC–MS (methanol), m/z: [M + 1] + 397.40, [M + Na^+] + 419.36.

Bioassay

Anticancer activity

In vitro anticancer activities of synthesized derivatives were performed using sulforhodamine B (SRB) assay on a panel of human cancer cell lines HCT-12 (colon cancer) and MIPaCa-2 (pancreatic cancer) [25]. The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For the present screening experiment, cells were inoculated into 96-well microtiter plates in 90 μL at 5000 cells per well. After cell inoculation, the micro-titer plates were incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of the experimental drugs, which were solubilized in an appropriate solvent to prepare stock of 10^{-2} concentration. At the time of experiment, four 10-fold serial dilutions were made using complete medium. Aliquots of 10 μl of these different drug dilutions were added to the appropriate micro-titer wells already containing 90 μl of medium, resulting in the required final drug concentrations. After addition of the compound, the plates were incubated in standard conditions for 48 h and the assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 μl of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant was discarded and the plates were washed five times with tap water and air-dried. Sulforhodamine B (SRB) solution (50 μl) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and the plates were incubated for 20 min at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid, and the plates were air-dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on an Elisa plate reader at a wavelength of 540 nm with 690-nm reference wavelength. The GI_{50} values, defined as the drug concentration required for inhibiting the growth of cell proliferation by 50%, were calculated from the percent growth and were expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells × 100. Using the six absorbance measurements [time zero (T), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. The results are expressed as GI_{50} (growth inhibitory concentration at 50%), i.e. the concentration of the compound which inhibits the tumor cell growth by 50%, and the data are presented in Table 1 and Figs. 1 and 2.

Cell growth inhibition was investigated by SRB assay and the results indicate that the some of compounds show an inhibitory effect on the proliferation of HCT-15 and MIAPaCa-2 cells in a dose-dependent manner (Table 1). Compound IIC is found to exhibit excellent cytotoxic potency (10 μg/ml) which is comparable with of adriamycin (10 μg/ml) against the HCT-15 cell. Similarly, the compounds **IIIB** and IIC were found to possess excellent cytotoxic potency (10 μg/ml) compared with adriamycin (10 μg/ml) against MIAPaCa-2. Of note is that the derivatives **IIIA**, **IIIC** and **IIIC** derived from 2-acetohydrazide of carvacrol show better activity than those derived from 2-acetohydrazide of thymol.

Table 1 Anti-oxidant and anticancer activity of synthetized compounds

Sr. no.	Name of derivatives	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.	Carvacryl acetohydrazone of carvacrol (**IIIA**)	0.1602	80	80
2.	Carvacryl acetohydrazone of Thymol (**IIIB**)	0.2011	80	10
3.	Carvacryl acetohydrazone of Eugenol (**IIIC**)	0.1224	10	10
4.	Thymyl acetohydrazone of Thymol (**VIA**)	18.22	80	80
5.	Thymyl acetohydrazone of carvacrol (**VIB**)	4.652	80	80
6.	Thymyl acetohydrazone of Eugenol (**VIC**)	0.1348	80	80
7.	STD	0.1203	10	10
		Butylated hydroxy toluene ([BHT)	Adriamycin (ADR)	Adriamycin (ADR)

* All calculations related to EC_{50} and GI_{50} are performed from graphpad prism (http://www.graphpad.com/scientific-software/prism/)

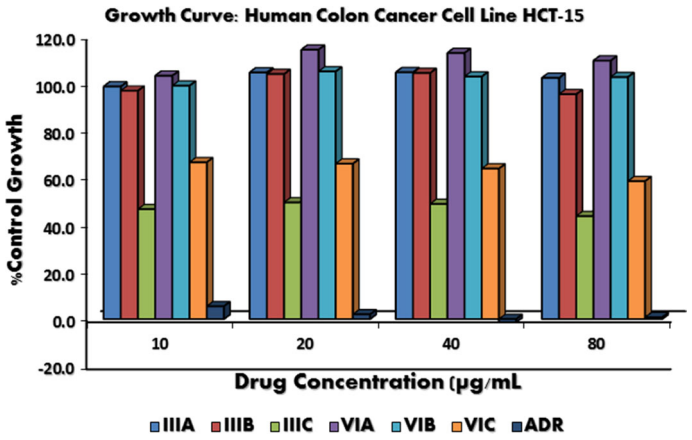


Fig. 1 Cytotoxicity effects of acetohydrazones measured against colon cancer on the HCT-15 cell line

Anti-oxidant activity

DPPH radical-scavenging activity was performed by the reported method [26]. For each determination, the stock solution (1 mg/ml) was diluted by serial dilution (25 μg–500 μg/ml) with 60% (v/v) ethanol. An aliquot of each dilution (0.5 mL) was mixed with a methanolic solution of DPPH (5 mL, 0.06 mM). The mixtures

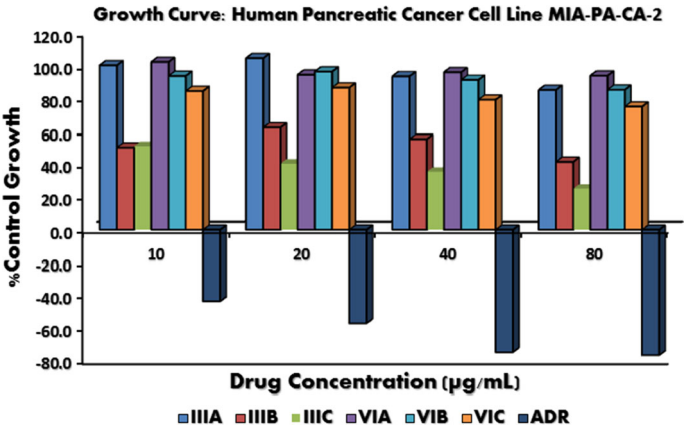


Fig. 2 Cytotoxicity effects of acetohydrazones measured against pancreatic cancer on the MIAPaCa cell line

were shaken vigorously and incubated at 37 °C in the dark for 30 min. At the same time, a control containing 60% (v/v) ethanol (0.5 mL) and methanolic solution of DPPH (5 mL, 0.06 mM) was run. The absorbance was measured at 517 nm. The percentage of DPPH scavenging versus concentration of samples was plotted. The concentration 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 the reference compound. Figure 3 demonstrates the % radical scavenging against the concentration of the entity.

DPPH radical scavenging activity of the compounds was found to be good to outstanding as compared to the standard BHT. In particular, compounds **IIIA**, **IIIB**, IIIE and **VIC** showed outstanding EC_{50} values which are comparable with STD, while **VIA** and **VIB** demonstrated a decrease in % anti-oxidant activity with higher EC_{50} values. The lowest activity was noted for VIA at every concentration; perhaps

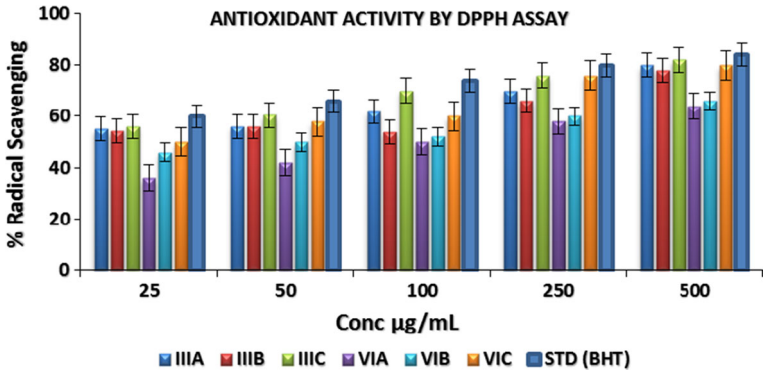


Fig. 3 Anti-oxidant activity of acetohydrazones determined by the DPPH free radical method at various concentrations

due to the phenolic OH present in the steric crow. The anti-oxidant activity of the compounds is related 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. **IIIC** showed the highest anti-oxidant activity with a remarkable EC_{50} value which is comparatively lower than STD. All EC_{50} values for synthesized derivatives are shown in Table 1.

Molecular docking studies

The docking study was performed by FRED (Open Eye) and used to determine the orientation of inhibitors bound in the active site of cyclooxygenases-2 (COX-2, PDB code: 4PH9). The PDB files were downloaded from the Protein Data Bank (www.rsc.org). The ligands were drawn in Chem Draw Ultra 12.0 (Freeware) and the FRED docking programme was used to perform molecular docking [27]. The docking of ligand molecules with COX-2 indicated that all the inhibitor compounds exhibit bonding with more than one amino acid in the active pocket (Fig. 4) and that they may be considered as good inhibitors of cyclooxygenases-2 (COX-2). The study also showed that the phenolic monoterpenoids-based scaffolds are attached to the key residues , i.e. TYR356, ARG121, IBP601 and SER354 of the active pocket of cyclooxygenases (COX-2). Moreover, all the compounds have a minimum binding energy and docking scores comparable with Ibuprofen from Tables 2 and 3, and hence could be considered as having a good affinity with cyclooxygenases (COX-2). The theoretical outcome highlighted that the minimum binding energy of the molecules with the targeted enzyme suggests that the synthesized carvacrol- and thymol-based scaffolds are good inhibitors ofto cyclooxygenases-2 (COX-2). Therefore, it is striking to state that the docking studies have extended the scope of developing carvacrol and thymol derivatives as promising anti-oxidant and anti-cancer agents.

On the basis of the EC_{50} values, all the compounds have been found to possess excellent anti-oxidant potency, and similarly the values of GI_{50} in anticancer test indicated good anticancer potency for some of the compounds. The molecular docking study was done by FRED (Open Eye) on cyclooxygenases-2. Overall docking results suggested that the synthesized compounds exhibit good binding affinity towards receptors.

Conclusion

In conclusion, six novel derivatives of carvacrol and thymol moieties containing an acetohydrazone linkage have been synthesized by a multistep pathway with good practical yields of the synthesized compounds. Anticancer and anti-oxidant activities were tested in vitro. The obtained results indicated that the free hydroxy group in a given scaffold has significantly improved anti-oxidant potency. All the synthesized compounds displayed extensive in vitro anti-oxidant activities by DPPH assay. In the anticancer test using 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

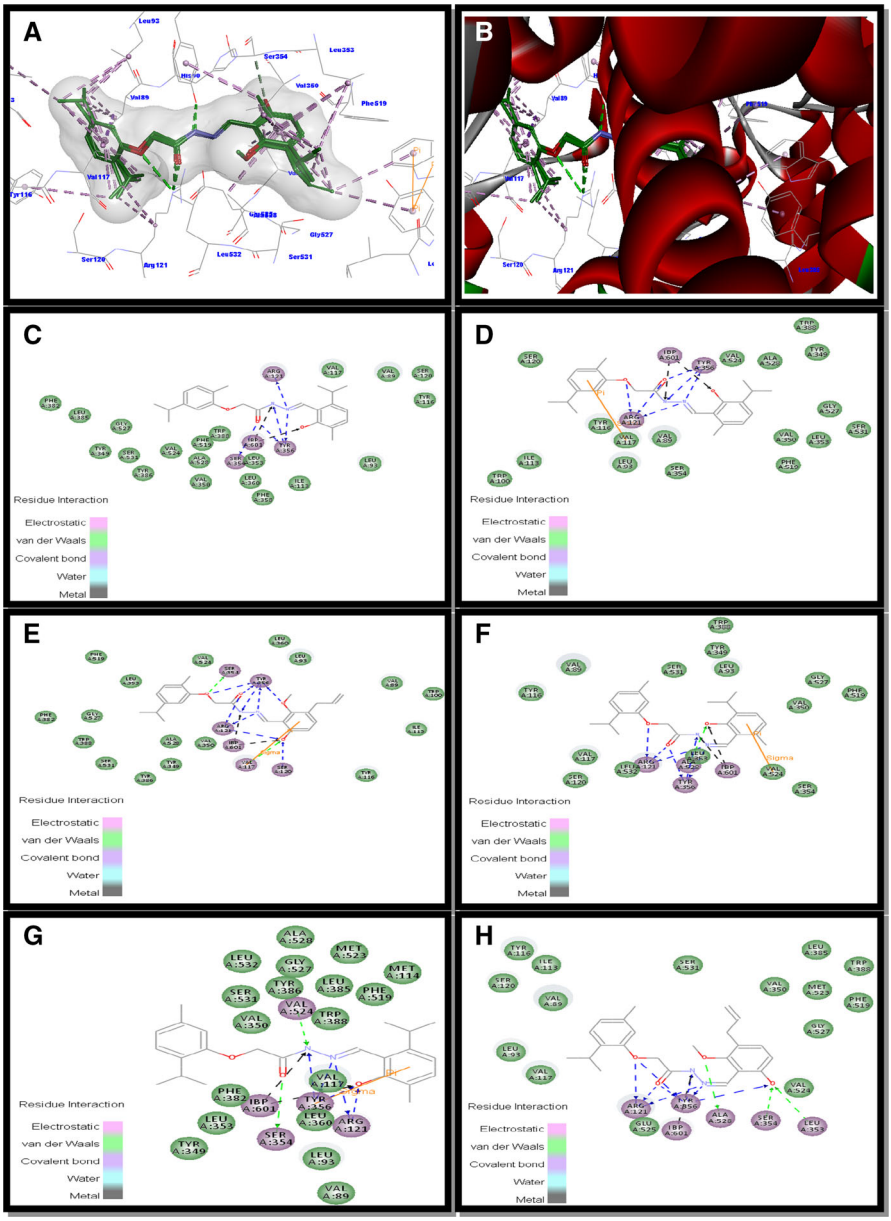


Fig. 4 **a** General molecule in the active pocket site of cyclooxygenases-2 (COX-2). **b** Binding mode of all inhibitors in active site of cyclooxygenases (COX-2). **c** Interaction of **IIIA** with cyclooxygenases (COX-2). **d** Binding of **IIIA** with amino acids of cyclooxygenases (COX-2). **e** Binding of **IIIC** with amino acids of cyclooxygenases (COX-2). **f** Binding of VIA with amino acids of cyclooxygenases (COX-2). **g** Binding of **VIB** with amino acids of cyclooxygenases (COX-2). **h** Binding of **VIC** with amino acids of cyclooxygenases (COX-2)

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

Sr. no.	Name	Binding energy	Complex energy	Protein energy	Ligand energy	Entropic energy	Complex entropy	Protein entropy	Ligand entropy
1	**IIIA**	−18.37	−9214.63	−9222.35	26.09	20.35	−32.41	−32.40	−20.36
2	**IIIB**	−44.40	−9252.98	−9222.35	13.77	20.37	−32.41	−32.40	−20.38
3	**IIIC**	−5.20	−9186.64	−9222.35	40.91	20.43	−32.41	−32.40	−20.44
4	**VIA**	−46.15	−9253.31	−9222.35	15.19	20.34	−32.41	−32.40	−20.35
5	**VIB**	54.22	−9120.36	−9222.35	47.77	20.25	−32.40	−32.40	−20.25
6	**VIC**	−47.69	−9241.88	−9222.35	28.17	20.42	−32.41	−32.40	−20.43
7	Ibuprofen	−51.34	−9250.99	−9222.35	22.70	20.19	−32.41	−32.40	−20.18

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

Sr. No.	Name	Docking score	Steric score	Desolvation	Hydrogen bond acceptor	Hydrogen bond donor
1	**IIIA**	−64.18	−76.85	13.21	−0.54	0.00
2	**IIIB**	−79.40	−82.73	12.95	−9.29	−0.32
3	**IIIC**	−67.96	−75.08	14.82	−6.22	−1.48
4	**VIA**	−78.71	−87.63	13.06	−2.30	−1.85
5	**VIB**	−49.92	−57.09	12.01	−4.59	−0.25
6	**VIC**	−82.45	−85.69	14.90	−9.47	−2.19
7	Ibuprofen	−81.28	−82.91	10.35	−8.72	0.00

two compounds, i.e. **IIIB** and **IIIC**, indicated their good anticancer efficacy. The entire results of the molecular docking studies of the synthesized derivatives carried on the COX-2 enzyme suggested that all the synthesized derivatives exhibit excellent binding affinity towards it.

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