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Research Article

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Cytotoxicity effect and antioxidant potential of 5-Hydroxymethyl Furfural (5-HMF) analogues-An advance approach

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ABSTRACT

Rivea hypocrateriformis (Desr.) Choisy is a profound medicinal belongs to the family Convolvulaceae. Natural products are considered as an alternative source for a positive approach to the drug design and drug discovery. *R. hypocrateriformis* is becoming the most important natural source to produce diverse phytometabolites with varying biochemical activities. Therefore, in the proposed study, we utilizing *R. hypocrateriformis* for isolating the 5-hydroxymethyl-2-furfural (5-HMF) and characterized it by different scientifically approved spectroscopic techniques namely ^{1}HNMR, ^{13}C NMR, FTIR and mass spectroscopy respectively. As a part of this study, the synthesis of chemical analogues has been achieved by coupling 5-HMF with quinoline derivatives and it was also studied for their antioxidant and anticancer potentials. The results demonstrated that amongst the test compounds, 3d and 3b have shown significant free radical scavenging assay followed by 3e and 3a with a maximum inhibitory effect, 76.69 %, 75.90 %, 67.60 % and 56.07 % respectively at 50 µg/mL. The anticancer activity studied through SRB assay showed that, compound 3a was effective at low concentration (10 µg/mL) against the Colo-205 cell line. This study demonstrated the applicability of *R. hypocrateriformis* against the cytotoxicity and antioxidant potential of 5-HMF. It can further be utilized by the researcher and pharmaceutical industry to design a potential drug candidate to treat cellular toxicity.

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INTRODUCTION

Medicinal plants are the most useful sources for drug discovery and development against various diseases (Kabra *et al.*, 2019; Ayaz *et al.*, 2019). *Rivea hypocrateriformis* (Desr.) Choisy is less acknowledging herbal candidate belongs to the family Convolvulaceae (Mukim *et al.*, 2022). It is an important source of various phytometabolites, and cited as a medicinal herb by the researchers (Shivalingappa *et al.*, 2002). Previous reports showed that the presence of many important metabolites, such as glycosides (Choudhury *et al.*, 2013), flavonoids (Sneha *et al.*, 2013), phenolics (Loganayaki *et al.*, 2010) and coumarins (Patel *et al.*, 2019). The plant has various pharmacological activities, such as anti-inflammatory, hepatoprotective, anticancer, antimicrobial, antifungal, and wound healing (Saboo *et al.*, 2012; Godipurge *et al.*, 2015, 2016) and antioxidant activities (Borkar *et al.*, 2015). The medicinal value of *R. hypocrateriformis* fascinated us to utilize it for further study in chemistry as well as in the bioactive field. Earlier studies revealed that 5-HMF was isolated chemically from medicinal plants such as *Lycium Chinese* (Yamada, P, *et al.*, 2011) and *Centella asiatica* (Bhuyar *et al.*, 2021) respectively due to its medicinal values. In this study we isolated 5-HMF from the leaves of *R. hypocrateriformis*. Hence, it is the most interesting candidate for further screening and scientific reports offered significant proof related to 5-HMF for its potential importance and application (Fu *et al.*, 2008). Previous study revealed that when one heterocyclic arrangement paired with another, a compound with marked biopotential activity formed (Giri *et al.*, 2010). In this regard we attempted to enhance the potential capacity of 5-HMF by coupling it with quinoline derivatives. Quinoline moieties are regarded as potent drug regimens and are particularly used as

antimalarial, antimicrobial and anticancer agents (Solomon & Lee, 2011). The quinoline derivatives also possess broad spectrum pharmacological activities (Kumar *et al.*, 2009; Ambala & Lincoln, 2020). Based on this principle, quinoline derivatives are selected to support and enhance the pharmacological activities of 5-HMF using Schiff base condensation.

MATERIAL AND METHODS

Organic and Inorganic Chemicals

The dimethyl sulphoxide (DMSO), (1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyl anisole (BHA) a standard antioxidant compound and laboratory grade methanol (95% v/v) was utilized as an organic solvent. All other organic and inorganic chemicals were procured from (Sigma Aldrich-Germany). The accuracy of selected materials and the advancement of the reaction were confirmed on previously developed silica gel TLC plates. ^{1}H NMR (400MHz) and ^{13}C NMR (100MHz) spectrum were recorded on a JEOL FT400 NMR spectrometer, using DMSO-d_6 as solvent (2.50 ppm in ^{1}H and 40 ppm in ^{13}C NMR). IR spectrum was documented with the inert KBr disc method on Nicolet-Impact-410 FT-IR spectrometer. MS spectra were obtained on a JEOL SX102/DA- 6000 (10 kV) FAB mass spectrometer. The standard chemical shifts are acknowledged in δ units, while the coupling constant *J* was calculated in Hz. The melting points of the compounds were determined in an open glass capillary. Extraction of *Rivea hypocrateriformis* leaves was carried out using the glass extraction unit (Shital Scientific Ind., Mumbai, India). Separation of the compounds was done by optimum glass column chromatography via Merck Silica gel60 (70-230 mesh).

Extraction and Isolation of 5–HMF from *R. hypocrateriformis* Leaves

The sample of *R. hypocrateriformis* was physically collected from Kalaburgi District, India. The specimens were authenticated by the botanist and Voucher specimen (No. HGUG 90) was deposited in the Department of Botany, Kalaburgi University, India. The collected sample was washed thoroughly with tap water to remove the unwanted material. In brief, the material was dried and then ground to fine material using an electronic grinder (Philips-Netherlands). The powder (100 g) material was extracted with 80% of methanol (500 mL v/v), using a hot extraction method for 72 hr. The solvent was evaporated to dryness under reduced pressure and controlled temperature of 40-50 °C using a rotary evaporator to calculate the significant percentage yield (w/w %) of *R. hypocrateriformis* leaf extract. The collected methanol extract was suspended in water and extracted with *n*-hexane (50 mL x 4); dichloromethane (DCM) (100 mL x 2) and treated it with ethyl acetate (EtOAc) (50 mL x 4) sequentially. The obtained n-hexane layers were discarded while the DCM layers were combined, evaporated under reduced pressure, and dried. In the similar fashion the collected EtOAc layer was evaporated under reduced pressure and, dried separately. The residue of EtOAc extract was submitted to column chromatography (230-400 mesh, 3x20 cm) over silica gel (60 g), and eluted successively with n-hexane: ethyl acetate gradually. In the end, 12 fractions were identified by TLC which was stored for subsequent study. Further the fractionation of EtOAc extract residue was proceeding and fraction 5 was again subjected to silica gel column chromatography for isolation of compounds and purified by HPLC to obtain a significant amount of compound. The melting point was determined in glass open capillary at 34-35 °C.

Synthesis of 5-Hmfderivative 3(A-E)

General method for synthesis of 2-(furan-2-yl) quinoline-4-carbohydrazide derivatives

The synthesis of starting material, 2-(furan-2-yl) quinoline-4-carbohydrazides was carried out after slight modification to the earlier method (Xu *et al.*, 2017).

General method for synthesis 6-chloro-2-(furan-2-yl)-N'-{(E)-[5-(hydroxymethyl) furan-2-yl] methylidene} quinoline-4-carbohydrazide (3a-e).

The accurately weighed (0.1 g, 0.3 mmol) of 2-(furan-2-yl) quinoline-4-carbohydrazides and equivalent amount of 5-HMF (>99.90%, Sigma Aldrich, USA), (0.038 g) were dissolved in 15 mL of ethanol, using glacial acetic acid as a catalyst. The obtained reaction mixture was refluxed (4-6 hr), and was supplied on to the trampled ice for crystallization. The obtained solid mass was filtered, dried and recrystallized with ethyl alcohol.

Spectral details

Based on the spectral analysis, a compound was identified as furan derivative, 5-hydroxymethyl-2-furfural (5-HMF), as shown in Figure 1.

6-chloro-2-(furan-2-yl)-N'-{(E)-[5-(hydroxyl methyl) furan-2-yl] methylidene} quinoline-4carbohydrazide (3a) Yield:74%; m.p.:238-240 °C; ^{1}HNMR (400 MHz, DMSO-d_6, δ,ppm): 8.173-8.175(d,3H, *J*=0.8Hz), 8.06-8.07(d,1H,*J*=4Hz),7.94-7.96(t,1H,*J*=8Hz), 7.84-7.85(d,1H, *J*=4Hz), 7.49-7.50(d,1H,*J*=4Hz), 6.91-6.92(d,1H,*J*=4Hz), 6.75-6.76(d,1H,*J*=4Hz), 6.45-6.46(d,1H, *J*=4Hz), 5.43-5.44(t, 1H,*J*=4Hz), 4.44-4.46(d,2H,*J*=8Hz); ^{13}CNMR (100 MHz, DMSO-d6,δ, ppm):162.3, 158.8, 152.6, 148.8, 148.6, 146.2, 140.4, 139.0, 132.1, 131.7, 131.5, 124.4, 124.3, 117.3, 116.2, 113.3, 112.6, 109.7, 56.1; LC-MS(*m/z*):396 (M+1).

6-bromo-2-(furan-2-yl)-N'-{(E)-[5-(hydroxymethyl) furan-2-yl] methylidene} quinoline-4-carbohydrazide (3b) Yield: 78%; m.p.: 176-178 °C; ^{1}HNMR (400 MHz, DMSO-

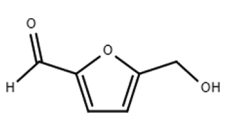


Figure 1: 5-HMF(5-hydroxymethyl-2-furfural) structure

d_6,δ,ppm):12.21(s,1H, NH proton), 8.26-8.30 (d,1H, *J*=4Hz), 8.16(s,2H), 7.91-7.92(m,4H), 7.49-7.50(d,1H,*J*=4Hz), 6.91-6.92 (d,1H,*J*=4Hz), 6.45-6-46(d,1H, *J*=4Hz), 5.43-5.44 (t,1H, *J*=4Hz,-OH proton), 4.44-4.46 (d,2H,*J*=8Hz,-CH_2 protons); ^{13}CNMR (100 MHz, DMSO-d6,δ,ppm): 162.3(C=O-NH), 158.8, 152.6, 148.8, 147.0, 146.2, 140.3, 139.1, 134.1, 131.8, 127.6, 124.8, 120.8, 117.2, 116.1, 113.3, 112.6, 109.7, 56.2; LC-MS (*m/z*): 441(M+1).

8-fluoro-2-(furan-2-yl)-N'-{(E)-[5-(hydroxyl methyl) furan-2-yl] methylidene} quinoline-4-carbohydrazide (3c)Yield:81%; m.p.:216-218 °C; ^{1}HNMR (400MHz, DMSOd_6,δ,ppm):12.19(s,1H,-NH proton), 8.19(s,1H) 7.96-7.99 (t,2H,*J*=12Hz),7.89-7.91(d,1H,*J*=8Hz),7.66-7.68(m,3H), 7.47-7.48 (d, 1H, *J*=4Hz), 6.910-6.918 (d,1H,*J*=3.2Hz), 6.760-6.764 (d,1H,*J*=1.6Hz), 6.45 6.46 (d,1H,*J*=4Hz), 5.44-5.45 (t,1H, *J*=4Hz) 4.44-4.45 (d,2H,*J*=4Hz,-CH_2 protons); ^{13}C NMR (100MHz, DMSO-d_6,δ,ppm):167.2, 163.5, 161.1, 157.4, 153.4, 153.3, 151.0, 146.3, 143.7, 132.4, 130.0, 126.3, 122.0, 120.8, 120.0, 119.9, 118.0, 117.6, 114.4, 60.6; LC-MS (*m/z*): 380.05(M+1).

6-chloro-N'-{(E)-[5-(hydroxymethyl) furan-2-yl] methylidene}-2-(5-methylfuran-2-yl) quinoline-4-carbohydrazide (3d) Yield:76%;m.p.:156-158 °C;^{1}HNMR (400MHz, DMSO-d_6,δ,ppm):12.19(s,1H,-NHproton),8.72-8.73(d,1H,*J*=4Hz), 8.27(s,1H), 8.02-8.04(m,3H), 7.77-7.76 (d,2H,*J*=4Hz), 7.34-7.35(d,1H,*J*=4Hz), 6.35-6.36(d,2H,*J*=4Hz), 4.45(s,2H), 2.41-2.46 (d,3H,*J*=16Hz); ^{13}CNMR(100MHz,DMSO d_6,δ,ppm):167.3, 162.3, 158.8, 155.5, 151.1, 148.9, 147.4, 146.8, 139.1, 132.1, 131.6, 131.1, 124.4, 119.5, 117.1, 116.1, 114.0, 113.6, 109.7, 56.2, 14.0; LCMS (*m/z*):410.04(M+1).

6-bromo-N'-{(E)-[5-(hydroxymethyl) furan-2-yl] methylidene}-2-(5-methylfuran-2yl) quinoline-4-carbohydrazide (3e) Yield: 80%; m.p.:138-140 °C;^{1}HNMR(400MHz, DMSO-d_6,δ,ppm):12.19(s,1H,-NHproton), 8.253-8.258 (d,1H,*J*=2.0Hz), 8.16(s,1H), 8.09(s,1H),7.89-7.90(m,4H), 7.40-7.41 (d,1H,*J*=4Hz), 6.91-6.92(d,1H,*J*=4Hz), 6.45-6.46(d,1H,*J*=4Hz), 6.37-6.38(d,1H,*J*=4Hz), 5.44(s,1H), 4.44-4.46(d,2H,*J*=8Hz), 247(s,3H); ^{13}CNMR (100MHz, DMSO-d_6,δ,ppm):158.8, 155.6, 151.1, 148.6, 147.0, 140.2, 139.1, 133.9, 131.6, 127.6, 124.5, 120.3, 117.0, 116.1, 114.1, 109.7, 56.2, 14.1; LC-MS(*m/z*): 455(M+1).

Antioxidant Potential

5-HMF derivatives scavenging outcome on DPPH free radical

The freshly synthesized derivatives have been appraised for their antioxidant potential using DPPH free radical scavenging assay (Blois, 1958). 5-HMF analogs were prepared at different concentrations (10, 25, 50 µg/mL) and adjusted to 500 µL with methanol. To this, 4.5 mL of 0.1 mM of methanolic solution of DPPH was added. The spectroscopic absorbance of the reactive components was recorded at 517 nm.

Statistical analysis

The values are expressed for control and treated groups in mean±standard deviation (SD). The obtained readings were fed in to the Microsoft Excel 2007 free software to determine the SD.

In-vitro cytotoxicity analysis

The modified Sulfurhodamine B dye cellular assay was used for *in-vitro* screening of anticancer agents (Skehan *et al.*, 1990). For primary screening, SK-MEL-2, B16-F10, Colo-205 and MCF-7 cell lines were used. The cell lines under investigation were full-grown in RPMI 1640 culture including 10% fetal bovine serum and 2 mM of L-glutamine. The cells were transferred and inoculated into 96 well micro titer plastic plates in 100 µL at plating densities. After 24 hr incubation, the test compounds were solubilized in suitable organic solvent, dimethyl sulfoxide (DMSO) at 100 mg/mL and diluted further by water to 1 mg/mL then hoarded to frozen. During sampling, 1 mg/mL of frozen component was diluted to 100, 200, 400 and 800 µg/mL. Aliquots of 10 µL of these different dilutions were supplied to the microtiter wells prefilled with 90 µL of medium, ensuing in the required last concentrations i.e., 9.5, 19.5, 35.5, and 79.5 µg/mL. After 48 hrs incubation of cell lines with test compounds, each well was fixed with 40 µL of 50% trichloroacetic acid (TCA) solution and further incubated for 1 hr at 4 °C. The plates were scientifically washed with distilled water and then air-dried for 24 hr. 50 µL of SRB solution in 1% acetic acid was supplied to each well and plates were nurtured for 30 min at laboratory temperature. After incubation, the unreacted dye was recovered and the residual dye was disinterested with washing with 1% acetic acid. The plates were air-dried and 100 µL of 10 mM tris-base solution (pH 9.5) was added to each of the wells to solubilize the dye and shaken for 30 min. The absorbance was observed at 540 nm with reference wavelength 690 nm. All the investigation was performed in triplicates.

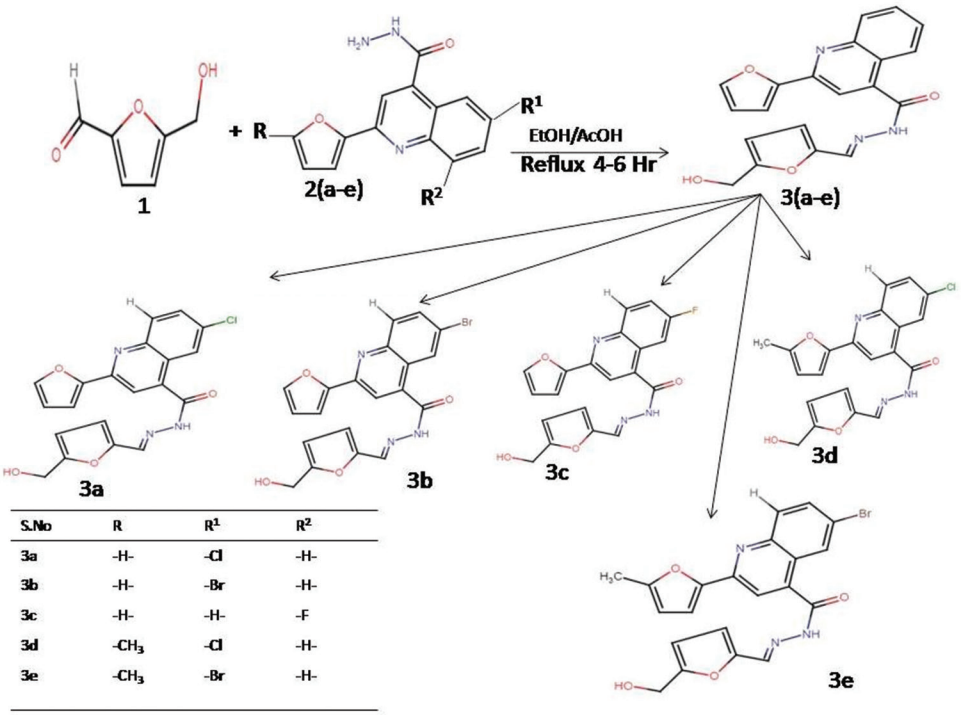
RESULTS AND DISCUSSION

Isolation and Elucidation Structure

The results revealed a significant percentage yield (0.15% w/w) of the leaf extract of *R. hypocrateriformis*. Based on the spectral analysis, the compound was elucidated as furan derivative, 5-hydroxymethyl-2-furfural (5-HMF), as shown in Figure 1. Qualitative analysis confirms the isolated 5-HMF was brownish in colour; 1H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.5 (s, 1H,-OH proton), 7.46-7.47 (d, 1H, *J*=4 Hz), 6.579-6.571 (d, 1H, *J*=3.2 Hz), 5.54-5.55 (t, 1H, *J*=4Hz), 4.46-4.48 (d, 2H, *J*=8Hz). 13C NMR (100 MHz, DMSO d6, δ, ppm):178.4, 162.6, 152.1, 124.8, 110.1, 56.3; LCMS (*m/z*): 125 g/mL (M^{+1}).

Chemistry to Obtain the 5-HMF

The synthetic pathway to obtain the titled compounds is shown in Scheme-1 (Figure 2). The compounds 2-(furan-2-yl)-N'-{(E)-[5-(hydroxylmethyl) furan-2-yl] methylidene} quinoline 4carbohydrazide derivatives 3(a-e) were synthesized in good yield in one step by Schiff base condensation of 5-hydroxy



S.No	R	R¹	R²
3a	-H-	-Cl	-H-
3b	-H-	-Br	-H-
3c	-H-	-H-	-F
3d	$-CH_3$	-Cl	-H-
3e	$-CH_3$	-Br	-H-

Figure 2: Scheme for the synthesis of 5-HMF (5-hydroxymethyl-2-furfural) derivative 3(a-e) R, R^1 and R^2 are the possible positions for the substitutions

Table 1: Percentage DPPH free radical scavenging activity of 5-HMF analogues

concentratio µg/mL	% Free radical scavenging activity					BHA
	3a	3b	3c	3d	3e	
10	16.23±1.01	21.48±1.03	13.05±0.95	36.36±1.06	41.64±1.04	54.27±1.02
25	26.07±1.01	64.59±2.04	25.66±2.06	43.26±1.01	56.30±1.95	70.10±1.01
50	56.07±1.01	75.90±1.02	35.19±2.04	76.69±2.09	67.60±2.05	91.82±1.02

Values are expressed in % of inhibition and mean±SD. SD-Standard deviation (n=3), BHA- Butylated Hydroxyl Anisole

methyl furfural (5-HMF) and different substituted 2-(furan-2-yl) quinoline-4 carbohydrazide in the presence of ethanol as solvent with the few drops of glacial acetic acid. This Schiff base (3a) was characterized using IR and NMR spectra. IR spectra of the compound showed an absorption band at 1728 cm^{-1} due to the identical C=O group. The ^1H NMR spectra of (3a) showed a singlet at δ 4.4 ppm corresponding to ethyl protons of aliphatic ethyl, the remaining peaks which appeared at δ 8.17 to 7.50 ppm corresponding to Ar-H and protons of the quinoline ring. The ^{13}C NMR spectra of (3a) showed a peak at δ 162.32 ppm corresponding to C=O carbon of the amide, the peaks between δ 162.32 to 109.79 ppm corresponding to aromatic carbon and a peak at δ 56.19 ppm corresponding to ethyl carbon. Moreover, the mass spectrum of (3a) showed a molecular ion peak at *m/z* =410.04 (M+1) corresponding to a molecular formula $C_{20}H_{14}ClN_3O_4$.

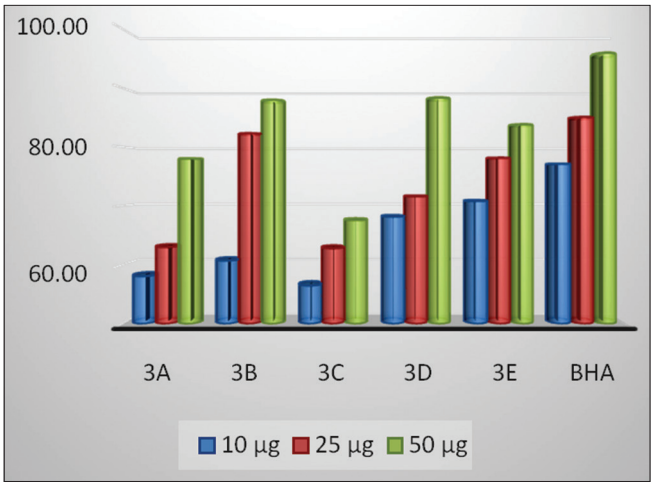


Figure 3: Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of 5-HMF (5-hydroxymethyl-2-furfural) analogues at 517 nm

In-Vitro Biological Activity

Anti-oxidant potential

The DPPH free radical radicals scavenging action of 3(a-e) derivatives are presented in Table 1 and Figure 3. Among the test compounds, (3d) and (3b) have shown better free radical scavenging assay followed by (3e) and (3a) with a maximum inhibition of 76.69%, 75.90%, 67.60% and 56.07% respectively at 50 µg/mL which is comparable to 91.82% for BHA at the same concentration.

Table 2: The *in-vitro* cytotoxic effects of synthesized molecules (3a, 3b, 3c, 3d, and 3e) on MCF-7 human breast cancer cell line and Colo-205 Human colon cancer cell line

Cell line			Human Breast Cancer Cell Line MCF-7				Human Colon Cancer Cell Line Colo-205			
S. No.	Drug	Conc (μg/mL)	Activity	Growth inhibition values for testmolecule(μg/mL)			Activity	Growth inhibition values for test molecule (μg/mL)		
			%viability	LC_{50}	TGI	GI_{50}	%viability	LC_{50}	TGI	GI_{50}
		10	10.3±0.75				14.1±8.12			
1	ADR	20	-6.9±2.11	NE	16.3	<10	12.0±10.85	NE	NE	<10
		40	17.2±3.93				-1.9±6.23			
		80	30.4±1.29				4.0±2.94			
		10	93.2±1.83				41.9±4.90			
2	3a	20	84.2±3.79	NE	NE	>80	32.3±2.63	NE	NE	<10
		40	78.4±4.32				26.5±1.3			
		80	58.1±3.24				34.1±5.66			
		10	94.2±2.92				65.6±6.90			
3	3b	20	87.3±5.12	NE	36.3	>80	63.5±21.21	NE	NE	76.6
		40	77.5±5.11				53.9±15.06			
		80	61.0±4.89				50.8±10.0			
		10	90.9±1.83				58.3±4.90			
4	3c	20	81.4±3.79	NE	NE	76.4	47.8±2.63	NE	NE	15.9
		40	68.2±4.32				29.0±1.3			
		80	49.3±3.24				22.7±5.66			
		10	95.6±2.92				68.1±6.90			
5	3d	20	91.2±5.12	NE	>80	54.0	74.1±21.21	NE	NE	61.1
		40	51.2±5.11				60.6±15.06			
		80	28.4±4.89				40.2±10.0			
		10	97.1±2.92				64.0±6.90			
6	3e	20	88.7±5.12	NE	NE	59.0	70.4±21.21	NE	NE	NE
		40	59.3±5.11				67.7±15.06			
		80	33.9±4.89				52.5±10.0			

The results shown are averages of three independent experiments, values are mean±SE. LC50-concentration of drug causing 50% cell kill, GI50-Concentration of drug causing 50% inhibition of cell growth, TGI-Concentration of drug causing total inhibition of cell growth, ADR-Adriamycin, Positive control compound, NE-Non-evaluable.

Table 3: The *in vitro* cytotoxic effects of synthesized molecules (3a, 3b, 3c, 3d, and 3e) on SK-MEL-2 Human melanoma cell line and B16-F10 Mouse skin melanoma cell line.

Cell line			Human Breast Cancer Cell Line MCF-7				Human Colon Cancer Cell Line Colo-205			
S. No.	Drug	Conc (μg/mL)	Activity	Growth inhibition values for test molecule (μg/mL)			Activity	Growth inhibition values for test molecule (μg/mL)		
			%viability	LC_{50}	TGI	GI_{50}	%viability	LC_{50}	TGI	GI_{50}
		10	-26.4±0.75				-77.1±8.12			
1	ADR	20	-3.4±2.11	NE	<10	<10	-78.2±10.85	NE	<10	<10
		40	-66.0±3.93				-79.5±6.23			
		80	-67.7±1.29				-71.6±2.94			
		10	95.7±1.83				97.1±4.90			
2	3a	20	114.6±3.79	NE	NE	>80	97.0±2.63	NE	NE	NE
		40	117.5±4.32				96.4±1.3			
		80	121.3±3.24				98.6±5.66			
		10	97.3±2.92				98.1±6.90			
		20	116.6±5.12	NE	NE	>80	97.8±21.21	NE	NE	NE
3	3b	40	114.8±5.11				98.3±15.06			
		80	131.0±4.89				103.5±10.0			
		10	98.6±1.83				96.5±4.90			
4	3c	20	120.2±3.79	NE	NE	>80	99.2±2.63	NE	NE	NE
		40	118.8±4.32				100.8±1.3			
		80	116.2±3.24				105.6±5.66			
		10	98.3±2.92				99.5±6.90			
5	3d	20	124.0±5.12	NE	NE	>80	97.8±21.21	NE	NE	NE
		40	124.4±5.11				99.4±15.06			
		80	112.8±4.89				74.2±10.0			
		10	95.4±2.92				100.7±6.90			
6	3e	20	123.7±5.12	NE	NE	>80	100.7±21.21	NE	NE	NE
		40	122.1±5.11				98.0±15.06			
		80	123.6±4.89				78.6±10.0			

The results shown are averages of three independent experiments, values are mean±SE. LC_{50}-concentration of drug causing 50% cell kill, GI_{50}-Concentration of drug causing 50% inhibition of cell growth, TGI-Concentration of drug causing total inhibition of cell growth, ADR-Adriamycin, Positive control compound, NE-Non-evaluable.

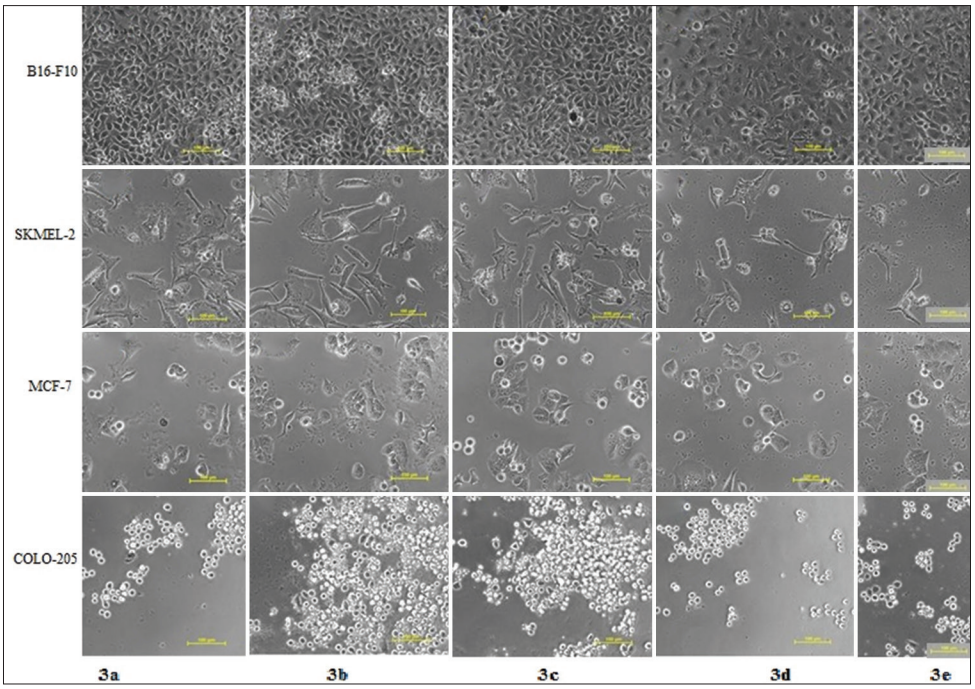


Figure 4: Photographic representation of anti-proliferative activity of (3a, 3b, 3c, 3d and 3e) compounds against B16-F10, SK-MEL-2, MCF-7and Colo-205 cell lines

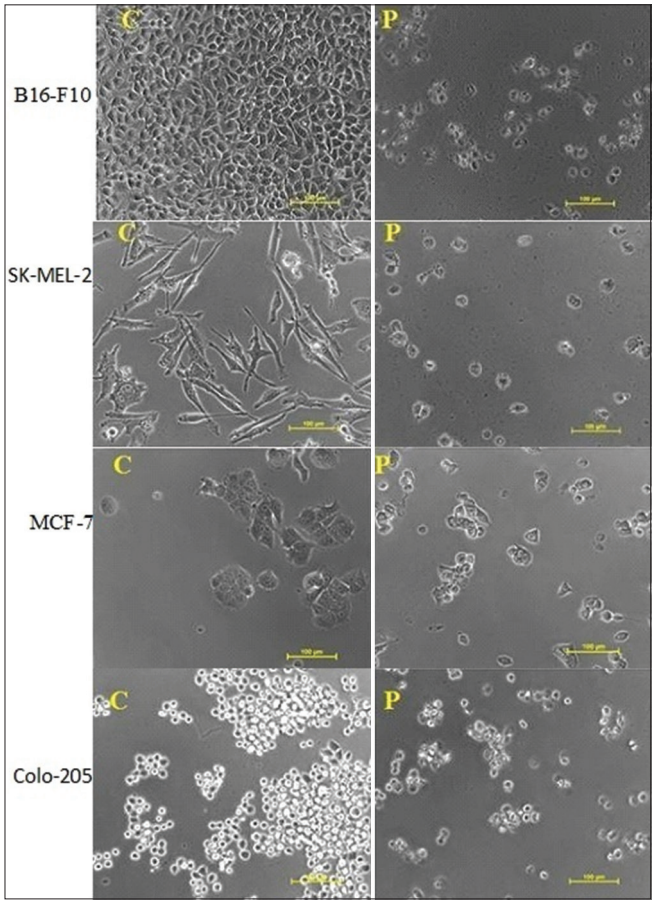


Figure 5: Photographic representation of anti-proliferative activity of C-Control, P-Positive control (Adriamycin) against B16-F10, SK-MEL-2, MCF-7 and Colo-205 cell lines

In-vitro cytotoxicity assay

All the synthesized compounds were investigated at four different concentrations i.e., 10, 20, 40 and 80 μg/mL against four cancer cell lines (Tables 2 & 3, Figures 3 & 4) with positive control (Adriamycin) (Figure 5). It was noticed that only (3a) showed effective less than 10 μg/ml concentration against Colo-205 cell line, whereas the (3b), (3c) and (3d) showed 76.6%, 15.9% and 61.1% growth inhibition against the Colo 205. It was observed that (3c), (3d) and (3e) showed 76.4%, 54.0% and 59.0% against MCF-7 cell line, and none against B16-F10 and SK-MEL-2 cell lines. Several paths are proposed to be involved in the death of cancer cells by anti-cancer drugs. The literature survey shows that, the drugs can kill cells either by activation of death receptors mediated pathways (Wilson, 1998), or by activation of caspases via mitochondrial-dependent pathways (Sun *et al*., 1999), more likely by a pro-oxidant one which may initially involve the generation of free radicals such as reactive oxygen/nitrogen species, etc., ultimately leading to the induction of DNA fragmentation, and hence cell death (Ghafourifar *et al*., 2001; Kellner & Zunino, 2004; Li & Wogan, 2005; Szewczyk & Wojtczak, 2002; Minotti *et al*., 2004). Besides, several agents may induce generation of free radicals and one such example is doxorubicin (Szewezk, Wojtczak, 2002; Minotti *et al*., 2004). In SRB dye assay, it was observed that the proliferation of cell lines was inhibited by 3(a-e) compounds. Growth of some cancer cell lines *viz*., Colo205 was inhibited significantly. Some anticancer agents stabilize an enzyme–DNA covalent intermediate by forming a ternary DNA-drug enzyme complex, resulting in an increase of DNA cleavage levels in living cells (Capranico & Binaschi, 1998).

CONCLUSION

5-HMF a well-known heterocyclic compound has various biochemical and biological activities. In the present study, an attempt is made to explore the efficiency of 5-HMF by synthesized different 5-HMF analogues and predict their biological activity. The present study indicated that some of 5-HMF derivatives were found to possess significant cytotoxicity effects, antioxidant and DNA cleavage activity. This study demonstrated the applicability of *R. hypocrateriformis* against cytotoxicity and antioxidant potential of 5-HMF. It can further be utilized by the researcher and pharmaceutical industry to design a potential drug candidate to treat cellular toxicity.

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