A series of chalcone analogous compounds were designed and synthesized. Replacing/substituting the enone or ethylenic bridge of the parent chalcone with rigid heterocyclic moieties or substituted aromatic amines gave nineteen target compounds. Their cytotoxic activities were screened against both breast and liver cancer cells as well as breast and liver normal cells. Target compounds were also evaluated for their inhibition activity of tubulin beta polymerization. Target compound $2e, 3a, 3b, 3c, 4a-4d, 5a, 5b$ and $6$ showed broad spectrum excellent anticancer activity against both MCF-7 and HepG2. Compound $4a$ showed the most TUBb inhibition activity.

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structural classes of CSI, based on consistent structural features and recurring tubulin–ligand interactions [1,5,6]. Three hydrogen bond acceptors, one hydrogen bond donor, two hydrophobic centers and one planar group were the 7-point pharmacophores. Hydrophobic centers and planar groups’ points represented the rigid portion of the molecular scaffold, while the other features (the different heterocyclic centers and planar groups’ points represented the rigid portion of the one planar group were the 7-point pharmacophore, suggesting that the discovery of more potent tubulin destabilizing agents could be a feasible task [1,5,6].

The target of this work was the development of novel compounds bearing chalcone moieties hybridized with epoxides, pyrimidines or pyrazoles via modification of the chalcones’ structure involved either the enone (type 1)- or the alkene (type 2) functionalized parts of parent chalcones. (Fig. 2).

All the synthesized compounds (Scheme 1) were characterized by two aromatic rings (A&C) linked by various fragments e.g. heterocyclic three, five or six membered rings and chalcone derivatives.

2. Results and discussion

2.1. Chemistry

In the present study, compounds (E)-3-(4-substituted phenyl)-1–n-(methoxyphenyl) prop-2- en-1-ones (1a-i) were obtained by the Claisen-Schmidt condensation of substituted acetophenones and benzaldehydes, affording exclusively the E-isomer in high yields [14]. Replacing the enone moiety with pyrimidine and hydroxypryzaole heterocycles took place by reacting the parent chalcones 1a-i with thiourea to afford 2a-g or reacting the epoxide derivatives of the parent chalcone 3a-d with either phenyl hydrazine, hydrazine hydrate or thiourea to yield compounds 4a-f and compound A respectively (Scheme 1). Upon using the conventional method for preparing compounds 2a-g, it took very long time to complete the reaction with moderate yield. A novel highly efficient rapid method with very mild reaction conditions was used herein to synthesize compounds 2a-g in high yield, high atom economy (less chemical wastes) and following environmental friendly protocol. Under microwave irradiation, the reaction time reduced to 3–4 h and the product was afforded in almost high yield. The progress of the reaction was monitored by TLC after every 1 h (Scheme 1).

The structure of the compounds 2a-g was confirmed by element analysis, IR, 1H-NMR, 13CNMR and mass spectroscopy. The spectral data revealed the formation of the 3,4-dihydropyrimidinethione rather than the fully unsaturated pyrimidine. 1H-NMR of compounds 2a-g showed the appearance of two doublets corresponded to C4 proton due to geometrical isomerism and a doublet at δ 6.81–6.92 ppm due to C5 proton of the pyrimidine ring. 13CNMR showed the characteristic C==S signal at δ 173.7–175.7 ppm and the characteristic signal of C4 at δ 60.4–60.6 ppm.

Replacement of the ethylenic bridge with oxirane ring via epoxidation of α,β-unsaturated ketones gave 3-aryl-2-substituted-acetophenoyl-oxiranes 3a-d. Reacting compounds 1a-d with hydrogen peroxide 28% in a mixture of polar solvents (methanol and acetone) at 0°C afforded compounds 3a-d in excellent yield. The structure of the products 3a-c was confirmed by element analysis, IR, 1H-NMR, 13CNMR and mass spectroscopy. IR spectra of 3a-c showed an absorption band at 3446-3462 cm⁻¹ indicating the presence of C–H of oxirane ring functionality. 1H-NMR spectra of 3a-c showed two doublet signals at 4.13 ppm and 6.92 ppm due to C5 proton of the pyrimidine ring. Epoxides are versatile intermediate in organic synthesis as their ring can be easily opened by a variety of nucleophiles e.g. amines and hydrazines. To replace the enone bridge with hydroxypryzaole moiety; hydrazinolysis of the oxirane derivatives 3a-d using either phenyl hydrazine or hydrazine hydrates was carried out to give compounds 5-aryl-N1-substituted-3-(substituted-methoxy-phenyl)-4-hydroxypryzoalines 4a-f in good yield. The reaction of the hydrazine derivatives and epoxy chalcone analogues was believed to proceed via the formation of the intermediate hydrazone. IR, 1H-NMR, 13CNMR and mass spectroscopy and element
analysis were used to identify compounds 4a-f. The 1H NMR spectra of 4a-f showed signals corresponding to C4 proton, C5 proton of pyrazole ring and the hydroxyl group. Moreover and as a result of the geometrical isomerism, two adjacent singlet signals of the methoxy methyl group were appeared.

Replacing the Enone Bridge with fully saturated pyrimidinone thione compound A was believed to be via reacting the oxirane derivatives of chalcone with thiourea, but upon carrying out this reaction a different product was formed. The spectral data of the obtained products proved the formation of thiirane derivatives of the chalcone 5a-b rather than compound A. 1H NMR spectra of compound 5a-b showed four doublet signals at δ 4.78, 5.04, 5.84 and 6.36 ppm corresponds to CH=N, CH=O, OH and NH respectively.

2.2. Discussion of the cytotoxic activity of the target compounds

All the test compounds were evaluated for their cytotoxic activity against MCF-7 and HepG2 cell lines. They showed excellent cytotoxic activity, much more potent than or even as good as the reference drug colchicine.

2.2.1. Activity against the breast cancer cell line (MCF-7)

In the present study, we synthesized nineteen novel compounds. They were tested for their cytotoxic activity against MCF-7 cell line using colchicine as a reference drug.
Replacement of Enone Bridge of compounds 1a-i with 3,4-dihydropyrimidine-2-thione moiety (Type 1) represented compounds 2a-g which showed cytotoxic activity as potent as or more potent than the reference drug. Compounds 2d-g (with trimethoxynaphenyl (TMP) moiety (ring A)) showed higher cytotoxic activity against MCF-7 cell line than 2a-c. Compound 2e (IC50 0.0770 μM) with TMP (ring A) and bromophenyl (ring C) was the most active amongst compounds 2a-g nearly double the potency of the reference drug (IC50 0.1504 μM).

Replacing the ethylenic bridge with oxirane resulted in compounds 3a-d (type 2). Compounds 3a-c showed excellent cytotoxic activity against MCF-7 much more potent than colchicine. The most active amongst this group were 3b and 3c with IC50 0.0130 μM and IC50 0.0140 μM respectively, about ten folds more potent than colchicine (IC50 0.1504 μM). It was noticed that changing of the halogen group (ring C) from Br/F (compounds 3a-d) showed excellent activity as potent as compounds 2a-g.

Replacing the Enone Bridge by hydroxypropyrazole afforded compounds 4a-f (type 1) which were far more potent than the reference drug. Compounds 4a-d with N1-phenyl moiety revealed excellent antiproliferative activity against MCF-7 cell line (ten folds more potent), while 4e and 4f showed moderate activity in comparison to compound 4a, the parent of this group. In fact, compounds 4a and 4b were amongst the most active ones against MCF-7 of all the test compounds.

Upon replacing the ethylenic bridge with thirane heterocycle, compounds 5a and 5b resulted (type 2). The activity of compound 5a against MCF-7 was one and half the cytotoxic activity of compound 5b.

Substituting the ethylenic bridge with diphenyl ethanolamine moiety (type 3) resulted in one of the most potent test compounds 6 (Table 1).

2.2.2. Activity against human liver cancer cell line (HepG2)

Generally, all the novel synthesized compounds showed more potent cytotoxic activity against human liver carcinoma cell line (HepG2) than MCF-7 cell line. Replacing the enone bridge (Type 1) or the ethylenic bridge (Type 2 & 3) with either 3,4-dihydropyrimidine-2-thione, oxirane, pyrazoles, thirane or diphenyl ethanolamine afforded compounds 2a-g, 3a-d, 4a-f, 5a-b and 6 respectively.

In spite of being moderately active against MCF-7; compounds 2c, 2d, and 2e were the most active of this group (2a-g) with IC50 (0.0300, 0.0110 and 0.0095 μM) respectively against human hepatic carcinoma cell line (HepG2). Moreover, Compounds 3a and 3c showed remarkable antiproliferative activity with IC50 (0.0150 and 0.0170 μM), while compound 3b with IC50 (0.0300 μM) showed only one third of their activity. Concerning compounds 4a-f; Compounds 4a-d revealed a higher potency than compounds 4e and 4f. Compounds 4a, 4b and 4d have nearly the same cytotoxic activity against HepG2 cell line with IC50 (0.0110, 0.0099, 0.0130, and 0.0102 μM). Also compounds 5a (0.0130 μM) and 5b (0.0110 μM) showed excellent activity as potent as compounds 4a-d. Compound 6 disclosed very good antiproliferative activity (Table 1).

To summarize the cytotoxic activity of all the test compounds against both cell lines, compounds 2e, 3a, 3b, 3c, 4a-4d, 5a, 5b and 6 showed broad spectrum excellent anticancer activity against both MCF-7 and HepG2 (Table 1).

2.3. Enzyme-linked immunosorbent assay for tubulin beta (TUBb)

This assay was employed to predict the possible mode of action of representative target compounds. The percentage inhibition of tubulin polymerization of representative of the most active target compounds against cancer cell lines was measured. Compounds 3b, 3c, 4b, 4a, 5a, 5b and 6 (Fig. 3) with the highest cytotoxic activity against human breast cancer cell line (MCF-7) were tested for inhibiting tubulin beta (TUBb) polymerization using colchicine as a reference drug. All the selected tested compounds showed excellent TUBb polymerization inhibition activity. They were more potent than colchicine as TUBb polymerization inhibitors and this was in accordance with the in vitro cytotoxic activity of these compounds against MCF-7 cell line. Compounds 3b, 4a and 6 (Fig. 3) were excellent TUBb polymerization inhibitors with percentage inhibition 92.62997%, 92.29742% and 92.16634% respectively, while the percentage of TUBb inhibition of colchicine was 86.2803% (Table 2).

All the selected test compounds with outstanding TUBb polymerization inhibition activity have almost the same common features: 3′4′-methoxyphenyl (ring A), halo phenyl (ring C) and a bridge with a carbonyl group and/or a hydroxyl group (Fig. 3). Compounds 5a, 3c and 4b showed remarkable inhibition activity as well as great potencies against MCF-7.

In case of compounds that revealed excellent cytotoxic activity against human liver carcinoma (HepG2), TUBB polymerization inhibition assay was carried out for representative compounds 2a, 2c, 2d, 2e, 4a, 4b, 5a, 5b and 6. In spite of not being the most active compound amongst the representative target compounds, compound 4a showed outstanding inhibition activity for TUBB polymerization with percentage inhibition 93.02269%. As they showed excellent cytotoxic activity against HepG2, compounds 2e, 2d, 2c and 2a (Fig. 4) showed very high percentage of TUBB polymerization inhibition (92.77821%, 92.36795%, 91.43021% and 90.58723%) much more potent than colchicine (82.52266%). Compounds 5a, 6 and 4b were more potent as TUBB polymerization inhibitors than colchicine (Table 2).

2.4. Effect of representative target compounds on normal human breast cells and hepatic cells

One of the main problems of cancer chemotherapy is the unwanted damage to normal cells caused by the high toxicities of anticancer drugs. Representative target compounds that were the most active against MCF-7 and HepG2 and cause the highest percentage inhibition of TUBb polymerization were selected to be tested against normal breast cell line (HS 371T) and normal liver (AML12) cell line and their IC50 were determined using colchicine

Table 1

<table>
<thead>
<tr>
<th>Cpd. No.</th>
<th>R</th>
<th>R1</th>
<th>R2</th>
<th>MCF-7 (μM)</th>
<th>HepG2 (μM)</th>
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<tr>
<td>Colchicine</td>
<td>3-OCH3</td>
<td>Cl</td>
<td>–</td>
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<td>2c</td>
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<tr>
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<td>0.0150</td>
<td>0.0130</td>
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<td>5b</td>
<td>OCH3(C)</td>
<td>Br</td>
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<td>6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.0099</td>
<td>0.0372</td>
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</table>
as reference drug. Since all the target compounds were more potent against HepG2, six representative compounds were evaluated for their toxicity towards AML12 using colchicine as reference drug. Furthermore, three representative target compounds were evaluated for their cytotoxic effect on Hs 371.T (Breast N cells) via MTT assay. It was worthy to mention that, the IC50 doses of all the representative target compounds against normal breast and liver cells were very high in comparison to their IC50s doses of the cancer cell lines, 2.5–9 folds more than the anticancer does (Table 3).

3. Conclusion

In this study, a group of novel compounds were synthesized according to structure based-drug design to be colchicine site Fig. 3. Active compounds against MCF-7 cell line.

Table 2
Percentage inhibition of tubulin-b polymerization of selected target compounds.

<table>
<thead>
<tr>
<th>Cpd. No.</th>
<th>MCF-7 (µM)</th>
<th>Tubulin % inhibition</th>
<th>Cpd. No.</th>
<th>HepG2 (µM)</th>
<th>Tubulin % inhibition</th>
</tr>
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<tbody>
<tr>
<td>Colchicine</td>
<td>0.1504</td>
<td>86.2803</td>
<td>Colchicine</td>
<td>0.1239</td>
<td>82.52266</td>
</tr>
<tr>
<td>3b</td>
<td>0.0130</td>
<td>92.62997</td>
<td>2a</td>
<td>0.0540</td>
<td>90.58723</td>
</tr>
<tr>
<td>3c</td>
<td>0.0140</td>
<td>88.38521</td>
<td>2c</td>
<td>0.0300</td>
<td>91.43021</td>
</tr>
<tr>
<td>4a</td>
<td>0.0097</td>
<td>92.29742</td>
<td>2d</td>
<td>0.0110</td>
<td>92.36795</td>
</tr>
<tr>
<td>4b</td>
<td>0.0110</td>
<td>87.84939</td>
<td>2e</td>
<td>0.0095</td>
<td>92.77821</td>
</tr>
<tr>
<td>5a</td>
<td>0.0150</td>
<td>89.32233</td>
<td>4a</td>
<td>0.0110</td>
<td>93.02209</td>
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<tr>
<td>6</td>
<td>0.0099</td>
<td>92.16634</td>
<td>4b</td>
<td>0.0099</td>
<td>84.57606</td>
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<td>0.0372</td>
<td>88.4942</td>
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</tbody>
</table>

Fig. 4. Active compounds against Hep G2 cell line.

R R¹ R² R³
2a H Cl H OCH₃
2c H Br OCH₃ H
2d OCH₃ Cl OCH₃ OCH₃
2e OCH₃ Br OCH₃ OCH₃

2a IC₅₀ = 0.0540 µM (HepG2)
% TUBb inhibition= 90.58723%
2c IC₅₀ = 0.0300 µM (HepG2)
% TUBb inhibition= 91.43021%
2d IC₅₀ = 0.0110 µM (HepG2)
% TUBb inhibition= 92.36795%
2e IC₅₀ = 0.0095 µM (HepG2)
% TUBb inhibition= 92.77821%

3b IC₅₀ = 0.0130 µM (MCF-7)
% TUBb inhibition= 92.62997%
3c IC₅₀ = 0.0140 µM (MCF-7)
% TUBb inhibition= 88.38521%
3a IC₅₀ = 0.0150 µM (MCF-7)
% TUBb inhibition= 89.32233%
3a X = O 3- methoxy R¹ = Cl
3c X = O 4- methoxy R¹ = F
5a X = S 3-methoxy R¹ = Cl
inhibitors (CSI). Compounds were tested for their cytotoxic activity against MCF-7 and HepG2 cell lines. Target compounds exhibited potent inhibitory activity at nanomolar level. Generally, target compounds showed more potent activity against HepG2 cell line than MCF-7 cell line. Target compounds 2e, 3a, 3b, 3c, 3d, 4a-4d, 5a, 5b and 6 showed broad spectrum excellent anticancer activity against both MCF-7 and HepG2. Inhibition of TUBb polymerization test was used to confirm the mechanism of action of the representative broad spectrum antiproliferative active compounds. Compound 4a showed the most TUBb inhibition activity. It is worthy to mention that, the replacement of either the enone or ethylenic bridge of the chalcone with rigid heterocyclic moieties resulted in superior antitumor potential ligands that may eventually allow the design and synthesis of chalcone derivatives with superior anticancer activity. The effect of the most active target compounds against normal breast cells and normal hepatic cells was tested and it was clear that the IC50 doses of the target compounds against cancer cell lines were safe doses for normal cells.

4. Experimental section

4.1. Chemistry

Melting points were determined on Stuart apparatus and the values given are uncorrected. Column Chromatography was used for purification by using solid solvent benzene-acetone 90% (9:1). IR spectra were determined on Shimadzu IR 4000s spectrophotometer (KBr, cm−1) at Faculty of Pharmacy, Misr University for Science and Technology, Egypt. 1H NMR spectra were carried out using a Mercury, a Gemini 300-BB 300 MHz and Jeol (eca), 300 or 400 MHz spectrophotometers using TMS as internal standard. Chemical shift values were recorded in ppm on δ scale, Microanalytical center, Cairo University, Egypt, and Faculty of Pharmacy, Cairo University. Mass spectra were recorded on Hewlett Packard 5988 spectrometer, Microanalytical Center, Cairo University, Egypt. Elemental analyses were carried out at the Microanalytical Center, Faculty of Pharmacy, Al Azhar University, Egypt.

4.1.1. 4-(4-Chlorophenyl)-6-(3-methoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (2b). Yield, 85%; mp 212–214°C; IR (KBr) ν = 3399 (NH), 3189 (aromatic C–H) cm−1. 1H NMR (DMSO-d6) ppm δ: 3.85 (s, 3H, OCH3), 5.13, 5.42 (2d, 1H, J = 4.68, 4.44 Hz), 6.92 (d, 1H, J = 7.96 Hz) 7.00–7.99 (m, 8H, Ar–H), 9.13, 9.93 (2H, 2NH, D2O exchangeable). 13C NMR (DMSO-d6) ppm: 173.7, 159.6, 140.0, 135.1, 134.9, 129.9, 129.1, 128.8, 118.7, 115.5, 114.5, 114.4, 60.5, 55.6. GCMS m/z (% rel. abundance): 374.90 (M+, 100%). Anal. Calcd for C17BrH15N2OS (375.27): C, 54.41; H, 4.03; N, 7.46. Found: 54.60; H, 4.06; N, 7.41.

4.1.1.5. 4-(4-Bromophenyl)-6-(3,4,5-trimethoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (2d). Yield, 85%; mp 208–210°C; IR (KBr) ν = 3339 (NH), 3176 (aromatic C–H) cm−1. 1H NMR (DMSO-d6) ppm δ: 3.73, 3.82 (2s, 9H, 3OCH3), 5.21, 5.44 (2d, 1H, J = 4.68 Hz), 6.81 (d, 1H, J = 4.68 Hz), 7.23–7.52 (m, 6H, Ar–H), 9.11, 9.94 (2H, 2NH, D2O exchangeable). GCMS m/z (% rel. abundance): 375.95 (M+, 43.07%), 219.00 (100%). Anal. Calcd for C17BrH15N2OS (375.28): C, 54.41; H, 4.01; N, 7.52.

4.1.1.6. 4-(4-Chlorophenyl)-6-(4-methoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (2e). Yield, 50%; mp 210–212°C; IR (KBr) ν = 3389 (NH), 3176 (aromatic CH) cm−1. 1H NMR (DMSO-d6) ppm δ: 3.82 (s, 3H, OCH3), 5.09, 5.27 (2d, 1H, J = 4.68, 4.92 Hz), 6.98 (d, 1H, J = 4.92 Hz), 7.01–7.67 (m, 8H, Ar–H), 9.05, 9.88 (2H, 2NH, D2O exchangeable). GCMS m/z (% rel. abundance): 374.90 (M+, 100%). Anal. Calcd for C17ClH15N2OS (374.96): C, 54.37; H, 4.03; N, 7.46. Found: 54.60; H, 4.09; N, 7.41.

4.1.1.7. 4-(4-Bromophenyl)-6-(4-methoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (2f). Yield, 55%; mp 208–210°C; IR (KBr) ν = 3399 (NH), 3189 (aromatic C–H) cm−1. 1H NMR (DMSO-d6) ppm δ: 3.82 (s, 3H, OCH3), 5.09, 5.27 (2d, 1H, J = 4.68, 4.92 Hz), 6.92 (d, 1H, J = 7.96 Hz) 7.00–7.99 (m, 8H, Ar–H), 9.13, 9.93 (2H, 2NH, D2O exchangeable). 13C NMR (DMSO-d6) ppm: 175.7, 157.8, 159.6, 140.0, 135.1, 134.9, 129.9, 129.1, 128.8, 118.7, 115.5, 114.5, 114.4, 60.5, 55.6. GCMS m/z (% rel. abundance): 374.90 (M+, 100%). Anal. Calcd for C17BrH15N2OS (375.28): C, 54.41; H, 4.03; N, 7.46. Found: 54.60; H, 4.06; N, 7.41.

Table 3

<table>
<thead>
<tr>
<th>Cpd. No.</th>
<th>MCF-7 (µM)</th>
<th>Hs 371.T (Breast N cells) (µM)</th>
<th>Cpd. No.</th>
<th>HepG2 (µM)</th>
<th>AML 12 (Liver N cells) (µM)</th>
</tr>
</thead>
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<td>Colchicine</td>
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4.1.1.6. 4-(4-Fluorophenyl)-6-(3,4,5-trimethoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (2f). Yield, 60%; mp 179–181 °C; IR (KBr) ν = 3399 (NH), 1371 (aromatic C–H) cm⁻¹. ¹H NMR (DMSO-d₆) ppm: 3.77, 3.82, 3.91 (2s, 9H, 3OCH₃), 5.14, 5.45 (2d, 1H, J = 2.48, 4.44 Hz), 6.81 (d, 1H, J = 3.76 Hz, pyrimidine), 7.08–7.44 (m, 6H, Ar–H), 8.98, 9.85 (2s, 2H, 2NH, D₂O exchangeable). ¹³C NMR (DMSO-d₆) ppm: 175.4, 163.3, 160.9, 153.1, 140.8, 138.4, 134.7, 128.9, 115.8, 103.7, 101.0, 60.5, 56.4, 54.4. GCMS m/z (% rel. abundance): 435.95 (M⁺, 51.78%), 279.05 (100%). Anal. Calcld for C₁₉H₁₉NO₂S (435.33): C, 52.42; H, 4.40; N, 6.43. Found: 52.65; H, 4.10; N, 6.63.

4.1.2. General procedure for preparation of compounds 3a–c

To a well stirred solution of chalcone 1a, 1b, 1c or 1d (0.04 mol) in a mixture of acetone (25 mL) and methanol (10 mL) at 0 °C, a solution of hydrogen peroxide (28%, 10 mL) in sodium hydroxide solution (4 N, 5 mL) was added over a period of time. After completion of addition, the reaction mixture was stirred for an additional one hour, and then poured onto ice-cold water (100 mL). The separated product was collected by filtration, washed with water and crystallized from ethanol to give compounds 3a–d.

4.1.2.1. [3-(4-Chlorophenyl)oxiran-2-yl][3-(methoxyphenyl)methanone (3a). Yield, 95%; reaction time 1 h; mp 90–92 °C; IR (KBr) ν = 3462–3446 (C=H of oxirane ring), 1689 (C=O) cm⁻¹. ¹H NMR (DMSO-d₆) ppm: 3.80 (s, 3H, OCH₃), 4.16 (d, 1H, J = 2.1 Hz), 4.62 (d, 1H, J = 2.1 Hz), 7.24–7.63 (m, 8H, Ar–H). ¹³C NMR (DMSO-d₆) ppm: 129.6, 150.9, 136.5, 134.6, 133.5, 130.1, 128.5, 128.2, 120.8, 120.0, 112.6, 59.7, 57.9, 55.3. GCMS m/z (% rel. abundance): 386.05 (M⁺, 100%). Anal. Calcld for C₁₉BrH₂₂NO₂ (386.46): C, 62.16; H, 5.74; N, 7.25. Found: 62.55; H, 5.69; N, 7.27.

4.1.2.2. [3-(4-Chlorophenyl)oxiran-2-yl][3-(methoxyphenyl)methanone (3b). Yield, 95%; reaction time 1 h; mp 93–95 °C; IR (KBr) ν = 3446–3421 (C=H of oxirane ring), 1689 (C=O) cm⁻¹. ¹H NMR (DMSO-d₆) ppm: 3.81 (s, 3H, OCH₃), 4.15 (d, 1H, J = 2.1 Hz), 4.62 (d, 1H, J = 1.8 Hz), 7.29–7.63 (m, 8H, Ar–H). GCMS m/z (% rel. abundance): 333.90 (M⁺, 8.61%), 135.05 (100%). Anal. Calcld for C₁₉BrH₂₂NO (333.18): C, 57.68; H, 3.93. Found: 57.80; H, 3.91.

4.1.2.3. [3-(4-Fluorophenyl)oxiran-2-yl][4-(methoxyphenyl)methanone (3c). Yield, 71%; reaction time 4 h; mp 86–88 °C; IR (KBr) ν = 3426–3466 (C=H of oxirane ring), 1680 (C=O) cm⁻¹. ¹H NMR (DMSO-d₆) ppm: 3.85 (s, 3H, OCH₃), 4.13 (d, 1H, J = 2.4 Hz), 4.76 (d, 1H, J = 2.4 Hz), 7.06–7.27 (m, 7H, 4Ar–H), 7.47–7.52 (m, 2H, Ar–H), 8.01–8.04 (m, 2H, Ar–H). GCMS m/z (% rel. abundance): 272.00 (M⁺, 5.65%), 135.05 (100%). Anal. Calcld for C₁₉F₂₁O₃ (272.27): C, 70.58; H, 4.81. Found: 70.69; H, 4.89.

4.1.3. General procedure for preparation of compounds 4a-f

A solution of Oxirane 3a, 3b, 3c or 3d (0.02 mol) in absolute ethanol (30 mL) was refluxed with either phenyl hydrazine (0.02 mol, 1.97 mL) or hydrazine hydrate (99%, 0.02 mol, 0.64 mL) for 1–2 h. The solvent was evaporated under reduced pressure and the residue was purified either by crystallization from ethanol/ether mixture (2:1) for compounds 4a, 4b and 4e or by column chromatography using solvent system benzene: acetone (90%) for compounds 4c, 4d and 4f.
8H, Ar \(\text{CH})\), 1664 (C\(\text{OCH_3}\)), 4.15 (d, 1H, \(J = 8.40\) Hz, Ar\(\text{H})\), 1685 (C\(\text{OCH_3}\)), 4.16 (d, 1H, \(J = 2.8\) Hz). 13C NMR (DMSO-\(\text{d_6}\)) ppm: 3.80 (s, 3H, OCH\(_3\)), 4.16 (d, 1H, J = 2.8 Hz), 4.82 (d, 1H, J = 2.8 Hz), 7.26–7.64 (m, 8H, Ar–H). 13C NMR (DMSO-\(\text{d_6}\)) ppm: 192.5, 159.4, 136.5, 134.6, 133.4, 130.5, 129.8, 128.4, 120.7, 119.9, 112.6, 59.7, 57.8, 55.3. Anal. Calc'd for C\(_{16}\)BrH\(_{15}\)N\(_2\)O\(_2\) [349.24]: C, 55.03; H, 3.75.

1.5. 3-(4-Chloro-2-fluorophenyl)amino[2-hydroxy-1-(3-methyl-3-oxopropyl)]-1H-indole (5b) was heated with 3-chloro-4-hydroxy-1-(3-methoxyphenyl) propan-1-one (6) and crystallized from an ethanol to give compound 5a-b.

4.1.4.1. (3-(4-Chlorophenyl)thiiran-2-yl)(3-methoxyphenyl)methanone (5a).

4.2.1. Cell cultures

The newly synthesized compounds were evaluated on the in-vitro growth inhibition of two solid human tumor cell lines representing different tumor types, namely, breast adenocarcinoma (MCF-7) and liver hepatocellular carcinoma (HepG2) using colchicine as reference drug using SRB assay. Moreover, the toxic effect of representative target compounds was evaluated on normal breast (Hs. 371.T) and liver (AML 12) cell lines using MTT assay.

4.2. Cytotoxic activity

The newly synthesized compounds were evaluated on the in-vitro growth inhibition of two solid human tumor cell lines representing different tumor types, namely, breast adenocarcinoma (MCF-7) and liver hepatocellular carcinoma (HepG2) using colchicine as reference drug using SRB assay. Moreover, the toxic effect of representative target compounds was evaluated on normal breast (Hs. 371.T) and liver (AML 12) cell lines using MTT assay.


