A series of novel pyranoquinolinone-based Schiff’s bases were designed and synthesized. They were evaluated for topoisomerase IIβ (TOP2B) inhibitory activity, and cytotoxicity against breast cancer cell line (MCF-7) for the development of novel anticancer agents. A molecular docking study was employed to investigate their binding and functional properties as TOP2B inhibitors, using the DISCOVERY STUDIO 2.5 software, where they showed very interesting ability to intercalate the DNA–topoisomerase complex. Compounds 2a, 2c and 2f showed high docking score values (82.36% –29.98 kcal mol$^{-1}$ for compound 2a, 78.18% –26.98 kcal mol$^{-1}$ for compound 2c and 78.65, –28.11 kcal mol$^{-1}$ for compound 2f) and revealed the highest enzyme inhibition activity. The best hit compounds exhibited highly potent TOP2B inhibitors with submicromolar IC$50$ at 5 µM compared to the reference doxorubicin.

1. Introduction

Topoisomerases are crucial enzymes that control the higher-order structural state of DNA. By selective cleaving, the problems
of DNA are resolved by temporarily cleaving both strands of a DNA duplex to form a cleavage complex through which another DNA segment can be transported [1]. Topoisomerases can be classified in two general categories, termed type I or type II, depending on whether one or both DNA strands of a single duplex are cleaved during a catalytic cycle, respectively [2]. A variety of small-molecule agents capable of inducing such effects are widely prescribed as anti-cancer drugs which in turn increase the population of topoisomerase II (TOP2) breaking complexes, which leads to TOP2-mediated chromosome DNA cleavage and ultimately death of cancer cells [3]. Type II topoisomerases operate by a complicated mechanism that involves the controlled association and dissociation of subunit dimerization elements [4]. In humans TOP2 is further expressed into two type IIA topoisomerase isoforms, termed topoisomerase IIα (TOP2A) and topoisomerase IIβ (TOP2B) [5,6]. Type IIA topoisomerases share significant amino acid sequence similarity between species. TOP2A plays a crucial role in chromosome segregation and DNA replication [7,8], whereas TOP2B appears to be involved mainly in transcriptional regulation. The overall structure of the human TOP2B core dimer adopts a more open quaternary conformation than in other non-human DNA-bound structures reported [9]. The significant changes in the relative dimensional orientation between the main DNA-contacting domains of the two monomers decrease the buried surface area. Etoposide is one of the major inhibitors for TOP2B [9]. Because the active centre of TOP2 is assembled in trans with the catalytic tyrosine (located in the WHD domain) and the Mg$^{2+}$-chelating triad of acidic amino acid residues (E477, D557 and D559; situated in the TOPRIM domain) [10], the increase in distance between the active-site tyrosine and the Mg$^{2+}$-chelating residues suggests that etoposide stabilizes the cleavage complex. If one assumes that etoposide simply traps a pre-existing conformation of the TOP2 cleavage complex, this drug-bound structure may represent a functionally relevant quaternary conformation. The structure thus represents a putative intermediate between a closed post cleavage state [11,12] and the open conformation [13]. The quinoline scaffold exhibited DNA TOP2 inhibition [14], and polycyclic heteroaromatic systems which incorporates a quinoline moiety fused with another heterocycle demonstrated cytotoxicity against a large number of cancer cells [15–17]. Moreover, pyranoquinoline alkaloids showed a wide range of biological activities [18]; several of these alkaloids exhibited a selective cytotoxicity profile showing the greatest activity with oestrogen receptor-positive ZR-75-1 breast cancer cells [19]. On the other hand, a wide range of Schiff’s bases revealed interesting inhibitory activity against experimental tumour cells. It is also suggested that the Schiff’s bases could be hydrolysed selectively by the tumour cells to act as alkylating agents at the same time as the active amine becomes free to act as an antimetabolite [20]. The active centres of cell constituents are supposed to interact with azomethine’s nitrogen atom by forming a hydrogen bond which interferes with normal cell processes and results in the destruction of enzymatic activity of cancerous cells, thereby presenting Schiff’s bases as a potential target to discovering anti-cancer chemotherapeutics [21]. Based on the above findings, the aim in this research is to develop a novel series of Schiff’s bases containing pyranoquinolinone with potential anti-cancer activity.

2. Results and discussion
The 3-aminopyranoquinolinone 1 was prepared by the method reported by Hassanin et al. [22]. This amino derivative was condensed with some aromatic aldehydes in dry tetrahydrofuran (THF) to give the target Schiff’s bases 2a-g (scheme 1). The infrared (IR) spectra of compounds 2a-g show a newly stretching vibration absorption band around 1600–1630 cm$^{-1}$ attributed to (HC=N), in addition to the absence of the double stretching absorption bands of the amino group. Each 1H-nuclear magnetic resonance (NMR) spectrum of the synthesized Schiff bases exhibits a singlet signal in a region at 9.09–9.94 ppm attributed to a CH=N proton. The 13C-NMR spectra provide further support for the structural characterization of the Schiff bases. The number of signals found corresponds with the presence of magnetically non-equivalent carbon atoms. All the proposed structures were confirmed by liquid chromatography-mass spectrometry (LC-MS) or electrospray ionization-mass spectrometry (ESI-MS) techniques.

3. Molecular modelling study and biological evaluation
A molecular modelling study was initiated in order to support the assumed mode of action for tested compounds and optimize a reliable model for predicating novel effective antitumour hits. A docking study was carried out for the target compounds into TOP2B using the DISCOVERY STUDIO 2.5 software (Accelrys Inc., San Diego, CA, USA). X-ray crystal structure of TOP2B with the ligand molecule
etoposide (3QX3) was obtained from protein data bank PDB [23]. The prepared protein was used in determination of the important amino acids in the ATP-binding pocket. Interactive docking using the genetic optimization for ligand docking (GOLD) protocol was carried out for all the conformers of each compound of the test set (2a-g) to the selected active site, after energy minimization using the prepared ligand protocol. Re-docking lead compounds with the same binding site showed docking energy = \(-22.4 \text{ kcal mol}^{-1}\) with small root mean square deviation (RMSD) (0.904 Å) in comparison to its crystal structure. The small RMSD values proved the validity of the used docking processes (figure 1).

Each docked compound was assigned a score according to its mode of binding onto the binding site that predicted binding energies and the corresponding experimental values as outlined in table 2. The molecular docking simulation study revealed that the crucial residues of the main hinge region are Asp479 and Arg503, which are thought to be important in explaining the binding behaviour of etoposide toTOP2B as reported [14,15] (figure 2).
The etoposide lead binding mode indicated that the aromatic ring of the tetracyclic structure makes three $\pi$-interactions with different residues including Arg503, while the phenolic oxygen in the para position of the benzene ring is involved in H-bonding with Asp479 in the groove of the TOP2B binding site, while the extended chains are solvated and occupy a hydrophobic pocket. Further validation of our constructed active site of TOP2B was done by docking the etoposide molecule into the active site of and examining the binding mode. The binding mode of etoposide was matched with the reported data as shown in figure 3.
The binding behaviour of the molecule was analysed by docking of the pharmacologically active molecule into the active site of the protein. The residue involved in making the hydrogen bonds is Asp479 and that involved in pi interaction is Arg503 in the hinge region in the binding pocket. All interaction behaviours, as obtained from the docking studies, were found to be in accordance with the reported binding mode of the etoposide in the active site of TOP2B [23,24]. The active compounds adopted the same bioactive conformation of the lead compound and retained the same binding mode of the lead with higher interaction energy, and hence illustrated comparable biological activity which was verified by biological assay. The TOP2B inhibition per cent of the synthesized compounds and their docking scores are given in tables 1 and 2.

Compounds 2a, 2c and 2f which showed a similar binding mode to the lead compound with high docking score values (82.36% - 29.98 kcal mol\(^{-1}\)) for compound 2a, 78.18% - 26.98 kcal mol\(^{-1}\) for compound 2c and 78.65, -28.11 kcal mol\(^{-1}\) for compound 2f) revealed the highest enzyme inhibition activity (figures 4–6).

The general binding mode of the active hits that showed high inhibition per cent indicated that the carbonyl group in the active hits conserved the H-bond interaction with Asp479 in the hinge region and the butyl group extended chain occupied the hydrophobic pocket and solvated to form extra hydrogen bonding with more amino acids in the pocket region, which stabilizes the active conformer in addition to the chelating bond with Mg\(^{2+}\) in case of compound 2a, which showed higher inhibition than the reference compound doxorubicin (82.36 versus 74.18). The magnesium ions form salt bridges with both the protein and the DNA. Moreover, compound 2c which also showed higher inhibition per cent than the reference compound doxorubicin (78.22 versus 74.18) formed many hydrogen bonds with Arg503 which are much stronger than the pi interactions made by etoposide, which also is expected to highly stabilize the active conformer in the binding pocket. Interestingly, the results of compound 2f support the idea of the importance of the nitro group as an essential pharmacophoric feature. The two nitro moieties form multiple hydrogen bondings with the two crucial amino acids Asp479 and Arg503 required, which gain a compound potent activity of 78.65 versus 74.18. This information may aid to help explain the highly potent activity of compound 2a. It was observed that some of the compounds having

### Table 1. The per cent inhibition of the test set on TOP2B.

| compound | ser | cpd. code. | M.Wt g mol\(^{-1}\) | conc. (μM) | TOP2B % inhibition |
|----------|-----|------------|----------------------|-------------|-------------------|
| 1        | 1   | 2a         | 394                  | 10          | 82.36             |
| 2        | 2   | 2b         | 388                  | 10          | 60.67             |
| 3        | 3   | 2c         | 433                  | 10          | 78.22             |
| 4        | 4   | 2d         | 433                  | 10          | 67.098            |
| 5        | 5   | 2e         | 433                  | 10          | 66.83             |
| 6        | 6   | 2f         | 478                  | 10          | 78.65             |
| 7        | 7   | 2g         | 432                  | 10          | 70.15             |
| 8        | 8   | ref. (doxorubicin) | 579.98          | 10          | 74.18             |

### Table 2. Inhibition per cent of TOP2B and GOLD docking score for each compound in the test set.

| compound | etoposide | 2a | 2b | 2c | 2d | 2e | 2f | 2g |
|----------|-----------|----|----|----|----|----|----|----|
| % of inhibition | — | 82.36 | 60.67 | 78.22 | 67.098 | 66.83 | 78.65 | 70.15 |
| GOLD score (-Kcal mol\(^{-1}\)) | 22.4 | 29.98 | 18.97 | 26.98 | 20.94 | 20.02 | 28.11 | 21.28 |
Figure 4. Two- and three-dimensional interaction of compound 2a in the active site.

moderate inhibition activity like compound 2g (70.15%) although having high GOLD score fitness lacked the crucial interaction with Asp479, which may explain the reduction in activity as shown in figure 5.

These observed features support the attained SAR assumptions owing to enzymatic inhibitory activity data of the prepared compounds, which could be considered promising hits for developing good effective leads (figures 7 and 8).

To attain more information about the biological activity of the best hit compounds, the IC50 for each compound was measured and is displayed in table 4.

As seen from table 4, the hit compounds exhibited submicromolar IC50 at 5 µM compared to the reference doxorubicin that makes compounds 2a, 2c and 2f highly potent TOP2B cytotoxicity and can be further investigated in clinical trials as potential anti-cancer agents.

4. Conclusion

In this paper, a series of novel pyranoquinolinone based Schiff’s bases were synthesized and evaluated for TOP2B inhibitory activity and cytotoxicity against breast cancer cell line (MCF-7). Most of the compounds exhibited significant TOP2B inhibitory activity at 10 µM concentration compared to the reference doxorubicin. A molecular docking study was employed to investigate their binding and functional properties as TOP2B inhibitors. Compounds 2a, 2c and 2f showed similar binding mode to
Figure 5. Two- and three-dimensional interaction of compound 2c in the active site.

the lead compound with high docking score values (82.36% $-29.98$ kcal mol$^{-1}$ for compound 2a, 78.18% $-26.98$ kcal mol$^{-1}$ for compound 2c and 78.65, $-28.11$ kcal mol$^{-1}$ for compound 2f) and revealed higher inhibition per cent than the reference compound doxorubicin. Compounds 2a, 2c and 2f displayed significant TOP2B cytotoxicity in low micromolar range 5µM compared to the reference doxorubicin. The active compounds adopted the same bioactive conformation of the lead compound and retained the same binding mode of the lead with higher interaction energy and hence illustrated comparable biological activity which was verified by biological assay. Considering the previous results, we have concluded that we have three compounds 2a, 2c and 2f that can be further investigated in clinical trials as potential anticancer agents.

5. Experimental set-up

5.1. General

Thin layer chromatography (TLC) analysis of the reaction mixtures was performed using Fluka analytical silica gel 60 F254 nm TLC plates. Melting points were recorded on a Sanyo Gallenkamp MPD 350-BM
Table 3. Schiff bases.

| aldehyde derivative | amount of aldehyde | time (h) | m.poC | solvent | colour | yield     |
|---------------------|---------------------|----------|--------|---------|--------|-----------|
| 2a                  | 0.9 ml              | 10       | 180    | EtOH    | yellow | (2.7 g, 68.5%) |
| 2b                  | 1.06 ml             | 6        | 215    | EtOH    | yellow | (2.3 g, 59.3%) |
| 2c                  | 1.19 ml             | 4        | 235    | AcOH    | yellow | (2.4 g, 55.4%) |
| 2d                  | 1.19 ml             | 4        | 240    | AcOH    | yellow | (2.1 g, 48.5%) |
| 2e                  | 1.19 ml             | 1        | 296    | AcOH    | orange | (2.5 g, 57.7%) |
| 2f                  | 1.22 ml             | >300     |        | AcOH    | yellow | (3.1 g, 64.8%) |
| 2g                  | 1.48 g              | 6        | 231    | AcOH    | yellow | (2.5 g, 57.8%) |

3.5 Melting Point apparatus. A Thermo Nicolet Nexus 470 FT-IR spectrophotometer was used for IR analyses. $^1$H-NMR (400 MHz) and $^{13}$C-NMR (101 MHz) measurements were performed using a Varian-400 MHz spectrometer, and chemical shifts are expressed in $\delta$ (ppm) relative to tetramethylsilane (in CDCl$_3$ or DMSO-$d_6$ as solvent) as the internal standard. Elemental microanalyses were performed on a Perkin-Elmer CHN-2400II at the Chemical War Department, Ministry of Defence, Cairo, Egypt. Mass spectra were recorded on a Gas Chromatographic GCMSqp 1000 ex Shimadzu instrument at 70 ev., a triple–quadruple tandem mass spectrometer (Micromass W Quattro microTM, Waters Corp., Milford, MA, USA) or a Waters ZMD Quadrupole equipped with ESI.

5.2. General procedure for preparation of Schiff bases 2a–g

A mixture of compound 1 (3 g, 10 mmol) in dry THF (25 ml) and an equivalent amount of the appropriate aldehyde (10 mmol) in dry THF was heated under reflux. The progress of the reaction was monitored by TLC; our target product was precipitated hot. The reaction mixture was filtered and the filtrate was discarded. The solid material obtained was filtered, washed several times by water, dried and crystallized from a suitable solvent to give Schiff bases as illustrated in table 3.

5.2.1. 6-Butyl-4-hydroxy-3-(thiophen-2-ylmethylideneamino)-2H-pyrano[3,2-c]quinoline-2,5(6H)-dione (2a)

IR (KBr, cm$^{-1}$): 3415 broad band (OH), 3076 (CH$_{\text{aromatic}}$), 2948, 2918, 2860 (CH$_{\text{aliphatic}}$), 1714 (C=O$_{\text{-pyrone}}$), and 1668 (C=O$_{\text{quinoline}}$), 1609 (C=N), 1598 (C=C$_{\text{aromatic}}$). $^1$H NMR(400 MHz, CDCl$_3$) $\delta$ ppm 1.01 (t, $J = 8.00$ Hz, 3 H, C$_4$), 1.43–1.57 (m, 2 H, C$_3$, CH$_2$), 1.73–1.79 (m, 2 H, C$_2$, CH$_2$), 2.01 (t, $J = 8.00$ Hz, 2 H, C$_1$), 4.34 (t, $J = 8.00$ Hz, 1 H, C$_9$), 7.21 (t, $J = 8.00$ Hz, 1 H, C$_9$), 7.40 (t, $J = 8.00$ Hz, 1 H, C$_7$), 7.45 (d, $J = 8.00$ Hz, 1 H, C$_8$), 7.66 (t, $J = 8.00$ Hz, 1 H, C$_8$), 7.75–7.81 (m, 2 H, thiophene), 8.26 (dd, $J = 8.00$ Hz, 1 H, C$_{10}$), 9.94 (s, 1 H, C$_{10}$), 12.39 (s, 1 H, C$_{10}$), 12.39 (s, 1 H, C$_{10}$), 13.77 (s, 1 H, C$_{10}$), 20.19 (s, C$_3$), 29.62 (s, C$_2$), 42.28 (s, C$_1$), 101.52 (s, C$_3$), 113.95 (s, C$_4$), 114.91 (s, C$_1$), 115.23 (s, C$_7$), 123.60 (s, C$_{10}$), 124.30 (s, C$_9$), 129.15 (s, C$_{10}$), 130.47 (s, C$_{10}$), 131.82 (s, C$_8$), 136.40 (s, C$_6$), 139.99 (s, C$_{10}$), 143.02 (s, C$_{10}$), 146.28 (s, C$_{10}$), 150.01 (s, C$_{2}$), 162.82 (s, C$_{2}$), 163.25 (s, C$_{2}$), 163.58 (s, C$_{2}$). MS, m/z ($I_{%}$: 395(M$^+$+1; 25), 394 (M$^+$; 100).
Figure 6. Two- and three-dimensional interaction of compound 2f in the active site.

M.F: C_{21}H_{18}N_{2}O_{4}S, analysis calculated for C_{21}H_{18}N_{2}O_{4}S (394): C, 63.95%; H, 4.60%; N, 7.10%; S, 8.13%. Found: C, 63.90%; H, 4.58%; N, 7.04%; S, 8.11%.

5.2.2. 6-Butyl-4-hydroxy-3-((benzylidene)amino)-2H-pyran[3,2-c] quinoline-2,5(6H)-dione. (2b)

IR (KBr, cm\(^{-1}\)): 3408 broad band (OH), 3016 (CH\(_{\text{aromatic}}\)), 2945, 2864 (CH\(_{\text{aliphatic}}\)), 1715 (C=O\(_{\alpha}\)-pyrone), and 1663 (C=O\(_{\text{quinoline}}\)), 1615 (C=N), 1596 (C=C\(_{\text{aromatic}}\)). \(^1\)H NMR(400 MHz, CDCl\(_3\)) \(\delta\) ppm: 0.99 (t, \(J = 8.00\) Hz, 3 H, C\(_4\)'H\(_3\)), 1.41–1.49 (m, 2 H, C\(_3\)'CH\(_2\)), 1.70–1.81 (m, 2 H, C\(_2\)'CH\(_2\)), 4.44 (t, \(J = 8.00\) Hz, 2 H,C\(_1\)'NH-CH\(_2\)), 7.34 (t, \(J = 8.00\) Hz, 1 H, C\(_9\)-H), 7.40–7.53 (m, 2H\(_{\text{aromatic}}\)), 7.65–8.02 (m, 4H\(_{\text{aromatic}}\)), 8.28 (dd, \(J = 8.00, 0.8\) Hz, 1 H, C10-H), 9.31 (s, 1 H, CH=\(\text{N}\)), 13.98 (s, 1 H, C4-OHexchangeable in D\(_2\)O). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) ppm: 14.13 (s, C\(_4\)'), 19.96 (s, C\(_3\)'), 33.79 (s, C\(_2\)'), 42.28 (s, C 1'), 100.89 (s, C3), 112.44 (s, C4a), 115.03 (s, C7), 116.37 (s, C\(_{\text{phenyl}}\)), 119.43 (s, C\(_{\text{phenyl}}\)), 122.71 (s, C\(_{\text{phenyl}}\)), 123.63 (s, C9), 132.20 (s, C8), 133.35 (s, C\(_{\text{phenyl}}\)), 136.11 (s, C 6a), 136.29 (s, C\(_{\text{phenyl}}\)), 144.47 (s, C\(_{\text{phenyl}}\)), 151.27 (s, C 10b), 152.14 (s, C 2), 159.23 (s, C 4), 162.84 (s, C, C=\(\text{N}\)), 163.02 (s, C5). MS, m/z (I,%): 389 (M\(^{+}\)+1; 16), 388 (M\(^{+}\); 30), 368 (89), 364 (42), 358 (43), 339 (100). M.F: C\(_{23}\)H\(_{20}\)N\(_2\)O\(_6\), analysis calculated for C\(_{23}\)H\(_{20}\)N\(_2\)O\(_6\) (388.43): C, 71.12%; H, 5.19%; N, 7.21%. Found: C, 71.15%; H, 5.15%; N, 7.19%.
5.2.3. 6-Butyl-4-hydroxy-3-((2-nitrobenzylidene)amino)-2H-pyrano[3,2-c]quinoline-2,5(6H)-dione (2c)

IR (KBr, cm$^{-1}$): 3421 broad band (OH), 2985, 2925, 2872 (CH aliphatic), 1725 (C=O$\alpha$-pyrone), and 1669 (C=O quinoline). 1H NMR (400 MHz, CDCl$_3$) $\delta$ ppm: 1.01 (t, $J = 8.00$ Hz, 3 H, C$4'$), 1.43–1.59 (m, 2 H, C$3'$), 1.69–1.85 (m, 2 H, C$2'$), 4.36 (t, $J = 8.00$ Hz, 2 H, C$2'$), 7.45 (t, $J = 8.00$ Hz, 1 H C$9$-H), 7.50 (d, $J = 8.61$ Hz, 1 HC$7$-H), 7.56 (t, $J = 8.00$ Hz, 1 H phenyl), 7.68 (t, $J = 8.00$ Hz, 1 HC$8$-H), 7.78 (t, $J = 8.00$ Hz, 1 H phenyl), 8.01 (d, $J = 8.00$ Hz, 1 H phenyl), 8.31 (dd, $J = 8.00, 1.6$ Hz, 1 H phenyl), 8.33 (dd, $J = 8.00, 1.6$ Hz, 1 HC$10$-H), 9.87 (s, 1 H CH=N), 12.35 (s, 1 H, C$4$-OH). 13C NMR (101 MHz, CDCl$_3$) $\delta$ ppm: 13.75 (s, C$4'$), 20.61 (s, C$3'$), 29.57 (s, C$2'$), 42.53 (s, C$1'$), 100.28 (s, C$3$), 113.31 (s, C$4a$), 113.72 (s, C$10a$), 114.92 (s, C$7$), 115.19 (s, C phenyl), 123.52 (s, C$10$), 123.83 (s, C$9$), 124.91 (s, C phenyl), 129.62 (s, C phenyl), 130.74 (s, C phenyl), 131.84 (s, C$10$), 132.22 (s, C phenyl), 133.36 (s, C phenyl), 138.06 (s, C$6a$), 146.23 (s, C$10b$), 149.23 (s, C$2$), 157.41 (s, C=N), 162.52 (s, C$4$), 163.19 (s, C$5$). ESI-MS m/z: 434.7 [M+H]$^+$, 435.2 [M+2H]$^+$, 456.3 [M+Na]$^+$. M.F: C$_{23}$H$_{19}$N$_3$O$_6$, analysis calculated for C$_{23}$H$_{19}$N$_3$O$_6$ (433.42): C, 63.74%; H, 4.42%; N, 9.69%. Found: C, 63.70%; H, 4.45%; N, 9.64%.

5.2.4. 6-Butyl-4-hydroxy-3-((3-nitrobenzylidene)amino)-2H-pyrano[3,2-c]quinoline-2,5(6H)-dione (2d)

IR (KBr, cm$^{-1}$): 3403 broad band (OH), 3082 (CH aromatic), 2959, 2926, 2869 (CH aliphatic), 1715 (C=O$\alpha$-pyrone), and 1663 (C=O quinoline). 1H NMR (400 MHz, DMSO-$d_6$)
Figure 8. Alignment of compound 2a (with pink colour) and the lead compound (with green colour) in the active site.

Table 4. IC50 of the hit compounds at 5 µM.

| compound | M.Wt g mol⁻¹ | conc. (µM) | results |
|----------|-------------|------------|---------|
| MCF-7    | TOP2B-IC50  | µM         | µM      |
| 1        | 2a          | 394        | 5       | 0.42 ± 0.055 |
| 2        | 2c          | 433        | 5       | 0.83 ± 0.12  |
| 3        | 2f          | 478        | 5       | 0.6 ± 0.07   |
| 4        | doxorubicin | 579.98     | 5       | 1.17 ± 0.26  |

δ ppm: 0.92 (t, J = 8.00 Hz, 2 H,C 3′), 1.38–1.46 (m, 2 H,C 3′), 1.60–1.69 (m, 2 H, C 2′), 4.33 (t, J = 8.00 Hz, 2 H,C 2′), 7.50 (t, J = 8.00 Hz, 1 HC9-H), 7.74 (d, J = 8.00 Hz, 1 HC7-H), 7.82 (d, J = 8.00 Hz, 1 H phenyl), 8.15 (t, J = 8.00 Hz, 1 H phenyl), 8.27 (dd, J = 8.00, 1.76 Hz, 1 H phenyl), 8.65 (dd, J = 8.00, 1.76 Hz, 1 C10-H), 9.44 (s, 1 H CH=N), 11.94 (s, 1 H, C 4OH).

13C NMR (101 MHz, CDCl3) δ ppm: 13.72 (s, C4′), 20.16 (s, C3′), 29.57 (s, C 2′), 42.54 (s, C1′), 100.39 (s, C3), 112.69 (s, C4a), 113.76 (s, C10a), 115.18 (s, C7), 123.36 (s, C10), 124.21 (s, C9), 125.11 (s, C phenyl), 129.52 (s, C phenyl), 133.83 (s, C phenyl), 138.13 (s, C6a), 139.18 (s, C phenyl), 148.64 (s, C phenyl), 156.15 (s, C phenyl), 157.42 (s, C2), 157.79 (s, C=N), 162.97 (s, C4), 163.21 (s, C=N). ESI-MS m/z: 434.286 [M + H]+, 456.244 [M + Na]+. M:F: C23H19N3O6, analysis calculated for C23H19N3O6 (433.42): C, 63.74%; H, 4.42%; N, 9.69%. Found: C, 63.70%; H, 4.43%; N, 9.65%.

5.2.5. 6-Butyl-4-hydroxy-3-((4-nitrobenzylidene)amino)-2H-pyran[3,2-c]quinoline-2,5(6H)-dione. (2e)

IR (KBr, cm⁻¹): 3380 broad band (OH), 3082 (CH aromatic), 2959, 2921, 2848 (CH aliphatic), 1722 (C=O α-pyrole), 1666 (C=O quinoline), 1613 (C=N), 1572 (C=C aromatic). 1H NMR (400 MHz, DMSO-d6) δ ppm: 0.93 (t, J = 8.00 Hz, 3 H,C 4′), 1.36–1.43 (m, 2 H,C 3′), 1.60–1.67 (m, 2 H, C 2′), 4.35 (t, J = 8.00 Hz, 2 H,C 2′), 7.44 (t, J = 8.00 Hz, 1 HC9-H), 7.53 (d, J = 8.00 Hz, 1 HC7-H), 7.71 (d, J = 8.00 Hz, 1 H phenyl), 7.76 (d, J = 8.00 Hz, 1 H phenyl), 7.78 (t, J = 8.00 Hz, 1 HC8-H), 8.01 (dd, J = 8.00, 1.76 Hz, 1 H phenyl), 8.28 (dd, J = 8.00, 1.76 Hz, 1 H phenyl), 8.39 (dd, J = 8.00, 1.76 Hz, 1 C10-H), 9.48 (s, 1 H CH=N), 11.93 (s, 1 H, C 4OH). 13C NMR (101 MHz, CDCl3) δ ppm: 13.77 (s, C4′), 20.19 (s, C3′), 29.63 (s, C 2′), 42.28 (s, C 1′), 101.59 (s, C 3), 113.95 (s, C4a), 114.91 (s, C10a), 115.23 (s, C7), 123.60 (s, C10), 123.87 (s, C9),
124.30 (s, 2 C_{phenyl}), 129.15 (s, C_{phenyl}), 130.47 (s, 2 C_{phenyl}), 131.82 (s, C_{8}), 132.15 (s, C_{pipronal}), 136.34 (s, C_{6a}), 143.02 (s, C_{pipronal}), 144.39 (s, C_{pipronal}), 148.84 (s, C_{10b}), 153.19 (s, C_{pipronal}), 158.97 (s, C_{4}), 160.15 (s, C_{C}), 162.85 (s, C_{5}). M.F.: C_{23}H_{18}N_{4}O_{8}, the analysis calculated for C_{23}H_{18}N_{4}O_{8} (433.42): C, 63.74%; H, 4.42%; N, 11.71%. Found: C, 63.76%; H, 4.41%; N, 11.74%.

5.2.6. 6-Butyl-4-hydroxy-3-((3,5-dinitrobenzylidene) amino)-2H-pyrazone [3,2-c quinoline-2,5(6H)-dione. (2f)

IR (KBr, cm\(^{-1}\)): 3447, 3292 broad band (OH), 2958, 2932, 2876 (CH\(_{aliphatic}\).), 1728 (C=O\_pyrone). 1679 (C=O\_quinoline), 1613 (C=N), 1576 (C=\_aromatic). \(^1\)H NMR (400 MHz, DMSO-d\(_6\) \(\delta\) ppm: 0.92 (t, \(J = 8.00\) Hz, 3 H C{4}), 1.38–1.43 (m, 2 H, C{3}), 1.63–1.71 (m, 2 H, C{2}), 4.45 (t, \(J = 8.00\) Hz, 2 H C{2}), 7.15 (s, 1 H phenyl), 7.45 (d, \(J = 8.00\) Hz, 1 H C9-H), 7.53 (d, \(J = 8.00\) Hz, 1 H C7-H), 7.76 (s, 1 H phenyl), 7.78 (t, \(J = 8.00\) Hz, 1 H C8-H), 7.98 (s, 1 H phenyl), 8.25 (dd, \(J = 8.00, 1.76\) Hz, 1 H C10-H), 9.78 (s, 1 H CH=CH=), 13.53 (s, 1 H, C{4OH}). \(^{13}\)C NMR(101 MHz, CDCl\(_3\)) \(\delta\) ppm: 14.15 (s, C4{\prime}), 19.97 (s, C3{\prime}), 29.70 (s, 1 C{2}), 42.29 (s, 1 C{1}), 100.93 (s, 1 C{3}), 112.46 (s, C{4a}), 113.89 (s, C{10a}), 114.09 (s, C{7}), 116.43 (s, C_{phenyl}). 116.63 (s, C_{phenyl}), 119.42 (s, C_{phenyl}), 122.75 (s, C{10}), 123.30 (s, C{9}), 124.39 (s, C_{phenyl}), 126.43 (s, C_{phenyl}), 132.18 (s, C{8}), 133.35 (s, C{6a}), 136.15 (s, C_{phenyl}), 136.97 (s, C_{10b}), 152.17 (s, C{4}), 159.24 (s, C_{phenyl}), 162.82 (s, C{2}), 163.25 (s, C{phenyl}), 2H, 4.66%; N, 6.48%. Found: C, 66.62%; H, 3.75%; N, 11.74%.

5.2.7. 3-((Benzo[d][1,3]dioxol-5-ylmethylene) amino)-6-butyl-4-hydroxy-2H-pyrano[3,2-c quinoline-2,5(6H)-dione. (2g)

IR (KBr, cm\(^{-1}\)): 3082 (OH), 2955, 2919, 2872 (CH\(_{aliphatic}\).), 1724, 1613 (C=O\_pyrone). and 1666 (C=O\_quinoline), 1619 (C=N), 1593 (C=\_aromatic). \(^1\)H NMR (400 MHz, DMSO-d\(_6\) \(\delta\) ppm: 0.91 (t, \(J = 8.00\) Hz, 3 H C{4}), 1.35–1.45 (m, 2 H, C{3}), 1.58–1.68 (m, 2 H, C{2}), 4.30 (t, \(J = 8.00\) Hz, 2 H, C{1}), 6.09 (s, 2 H piperonal), 7.01 (d, \(J = 7.83\) Hz, 1 H piperonal), 7.33 (dd, \(J = 8.02, 1.37\) Hz, 1 H piperonal), 7.42 (t, \(J = 8.00\) Hz, 1 H C9-H), 7.73 (d, \(J = 7.60\) Hz, 1 H C7-H), 7.83 (t, \(J = 7.60\) Hz, 1 H C8-H), 8.02 (dd, \(J = 8.02, 1.37\) Hz, 1 H piperonal), 8.15 (dd, \(J = 8.02, 1.37\) Hz, 1 H piperonal), 8.25 (dd, \(J = 8.00, 1.20\) Hz, 1 H C10-H), 9.09 (s, 1 H CH=CH=), 12.27 (s, 1 H, C{4OH}). \(^{13}\)C NMR (101 MHz, DMSO-d\(_6\)) \(\delta\) ppm: 14.44 (s, C4), 19.94 (s, C3), 29.71 (s, C2), 42.08 (s, C{1}), 101.75 (s, C{3}), 102.77 (s, C{2piperonal}), 106.66 (s, C_{pipronal}), 109.01 (s, C_{pipronal}), 114.08 (s, C{4a}), 115.05 (s, C{10a}), 116.43 (s, C{7}), 122.73 (s, C{10}), 124.37 (s, C{8}), 129.05 (s, C_{pipronal}), 131.89 (s, C{9}), 132.15 (s, C_{pipronal}), 136.10 (s, C{6a}), 144.39 (s, C_{pipronal}), 148.84 (s, C{10b}), 153.19 (s, C_{pipronal}), 158.97 (s, C{4}), 160.15 (s, C_{C}), 160.80 (s, C{2}), 162.85 (s, C{5}). MS, \(m/z\) (I, %): 433 (M\(^{++}\)+1), 6, 432 (M\(^{+}\)+15), 417 (51), 393 (70), 378 (26), 372 (100). M.F.: C_{24}H_{20}N_{2}O_{6}, analysis calculated for C_{24}H_{20}N_{2}O_{6} (432.44): C, 66.66%; H, 4.66%; N, 6.48%. Found: C, 66.62%; H, 4.64%; N, 6.44%.

6. Molecular modelling

All molecular modelling studies were performed using the Accelrys DISCOVERY STUDIO 2.5 operating system, GOLD protocol at the Faculty of Pharmacy, Ain Shams University, Cairo, Egypt. Molecules were built within DS and conformational models for each compound were generated automatically. Docking study involved the following steps.

The docking analysis was carried out on TOP2B enzyme. The three-dimensional protein structure of TOP2B enzyme co-crystallized with lead etoposide (code: 3QX3) was downloaded from the Protein Data Bank of the Research Collaboration for Structural Bioinformatics (RCSB) website (www.rcsb.org). To evaluate the suitability of the crystal structure and the docking workflow, reference inhibitors were re-docked into the built active site and the RMSD value was calculated and binding interactions were compared to those found in the literature. For all compounds mainly poses inside the binding pocket were generated by the software.

The binding pocket was prepared for docking by clearing the protein, adding the missing hydrogens and side chains, with energy minimization according to the DS protocol. The binding pocket of the complexed lead compound with the connected amino acid residues was identified at a sphere of radius = 12 Å and then was used in docking of test compounds using the GOLD module. After that the GOLD fitness scores of the best fitted conformation of the docked molecule were recorded (table 2).
7. Biological techniques

MC7 cells were obtained from American Type Culture Collection; cells were cultured with RPMI (Invitrogen/Life Technologies) supplemented with 10% fetal bovine serum (Hyclone), 10 µg ml⁻¹ of insulin (Sigma) and 1% penicillin–streptomycin. All of the other chemicals and reagents were from Sigma or Invitrogen.

Plate cells (cells density 1.2–1.8 × 10,000 cells well⁻¹) were cultured in a volume of 100 µl of complete growth medium + 100 µl of the tested compound per well in a 96-well plate for 18–24 h before the enzyme assay.

Reader: ROBONIK P2000 ELISA READER wl 450 nm
Cell line: MCF-7
Kit used for enzyme-assay: Human DNA Topoisomerase 2B eia Kit MBS942146
Solvent: DMSO

Assay samples: cell culture supernatant

Data accessibility. The data of this work are available as the electronic supplementary material. Atomic coordinates and structure factors have been deposited in the Protein DataBank (PDB) under accession code 3QX3.

Authors' contributions. M.A.M. performed the experiments, shared in study design and samples analysis. All the authors gave their final approval for publication.

Competing interests. The authors declare no competing interests.

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