European Journal of Medicinal Chemistry 45 (2010) 3950–3959

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and docking studies of novel benzopyran-2-ones with anticancer activity

Magda M.F. Ismail [a,*], Heba S. Rateb [b], Mohammad M.M. Hussein [c]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy (Girls), 11754 Al-Azhar University, Cairo, Egypt
[b] Department of Pharmaceutical and Medicinal Chemistry, Pharmacy College, Misr University for Science and Technology, Cairo, Egypt
[c] Department of Pharmaceutical Chemistry; Faculty of Pharmacy, Cairo University, Cairo, Egypt

ARTICLE INFO

Article history:
Received 14 March 2010
Received in revised form
23 May 2010
Accepted 25 May 2010
Available online 1 June 2010

Keywords:
Benzopyran-2-one
Synthesis
Docking
Anticancer activity

ABSTRACT

Novel series of 7-substituted-benzopyran-2-ones was synthesized by incorporating heterocyclic rings as oxadiazole, triazole, pyrazole or pyrazolin-5-one to benzopyran-2-one nucleus at p-7 via methylene-oxy or acetoxy linker. In-vitro anticancer activity was evaluated for these hybrids; twelve compounds were selected by National Cancer Institute for anticancer screening. Among them, compound **9a** exhibited broad spectrum antitumor activity showing full panel median growth inhibition (GI_{50}) = 5.46 μM. According to docking results using Molsoft ICM 3.4-8c program, the target compounds may act through inhibition of topoismerase 1, where camptothecin is used as ligand.

1. Introduction

Improved understanding of cancer biology at the molecular level has led to a shift in treatment paradigm from the traditional cytotoxics toward more rationally designed targeted therapies.

Geiparvarin [1,2] (Fig. 1) a naturally occurring product bearing the benzopyran-2-one (coumarin) [3–6] residue possesses significant inhibitory activity against variety of cell lines including sarcoma 180, Lewis lung carcinoma P-388, lymphocytic leukemia and Walker 256 carcinosarcoma.

A series of geiparvarin analogs modified on the unsaturated alkenyloxy bridge, where a H-atom replaced the 3′-Me group, were synthesized and evaluated against a panel of human tumor cell lines [7,8]. These compounds demonstrated a stronger increase in growth inhibitory activity when compared to the parent compound, geiparvarin. In particular, the activity increased even further in the series of demethylated compounds when a Me substituent in the coumarin moiety is introduced [8]. Moreover; a variety of 7-[(1,5-dialkyl-1*H*-1,2,4-triazolyl) methoxy (and methyl)] benzopyran-2-ones were synthesized and showed anticancer (breast, lung, CNS cancers) properties *in vitro* [9] Besides, it has been well documented that pyrazole [10,11], pyrazolin-5-one [12] and oxadiazole [13,14], have cytotoxic activity.

In the present study, we have aimed to synthesize novel geiparvarin analogs by incorporating benzopyran-2-one and 5-membered heterocyclic residues (triazole/pyrazole/pyrazolin-5-one/oxzdiazole) via $-OCH_2-$/$-COCH_2O-$ bridge in a single molecule and investigate the anticancer activity of the resultant compounds.

Additionally, docking of the new compounds into human DNA topoisomerase I (Top1) [15] complexed with its bound inhibitor Camptothecin (Cpt) using Molsoft [16] ICM 3.4-8c program was performed in order to predict the affinity and orientation of the these compounds to the active site. Top1 is an essential enzyme participating to all those processes associated with separation of DNA strands. It manages superhelical tensions through the transient breakage of one strand of duplex DNA, followed by the unwinding of supercoiled DNA [17].

2. Results and discussion

2.1. Chemistry

The designed compounds are synthesized starting from 7-hydroxy-4-and/-8-substituted-benzopyran-2-ones by its reaction with ethylchloroacetate to give the corresponding esters, **1a–c**. Hydrazinolysis of the latter afforded the acid hydrazides **2a–c**; which were condensed with carbon disulphide, to furnish the target compounds, 5-mercapto-1,3,4-oxadiazoles, **3a–c**. Methylation of **3a**,**c** with CH_3I/anhyrous K_2CO_3 produced the corresponding 5-methyl-thio oxadiazoles **4a**,**b**.

* Corresponding author. Tel.: +20 2 22713531.
E-mail address: magdafathi_111@hotmail.com (M.M.F. Ismail).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.05.050

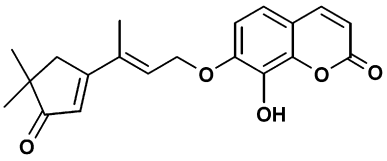


Fig. 1. Geiparvarin.

On the other hand, reaction of the key intermediates, **2a**–**c** with benzylisothiocyanate gave the thiosemi-carbazides, **5a**–**c** which were cyclized using sodium hydroxide to give 5-mercapto-1,3,4-triazoles, **6a**–**c**. Moreover, refluxing the intermediates, **2a**–**c** with benzoic acid/phosphorous oxychloride furnished the 5-aryl/phenyl-1,3,4-oxadiazoles, **7a**–**e** (Scheme 1). In addition, more target compounds were obtained by cyclocondensation of **2a**–**c** with active-methylene compounds such as acetylacetone, ethyl-acetoacetate or diethylmalonate to afford the corresponding pyrazoles **8a**–**c**, pyrazolin-5-ones, **9a**–**c** and pyrazolidinediones, **10a**, **b** respectively (Scheme 2).

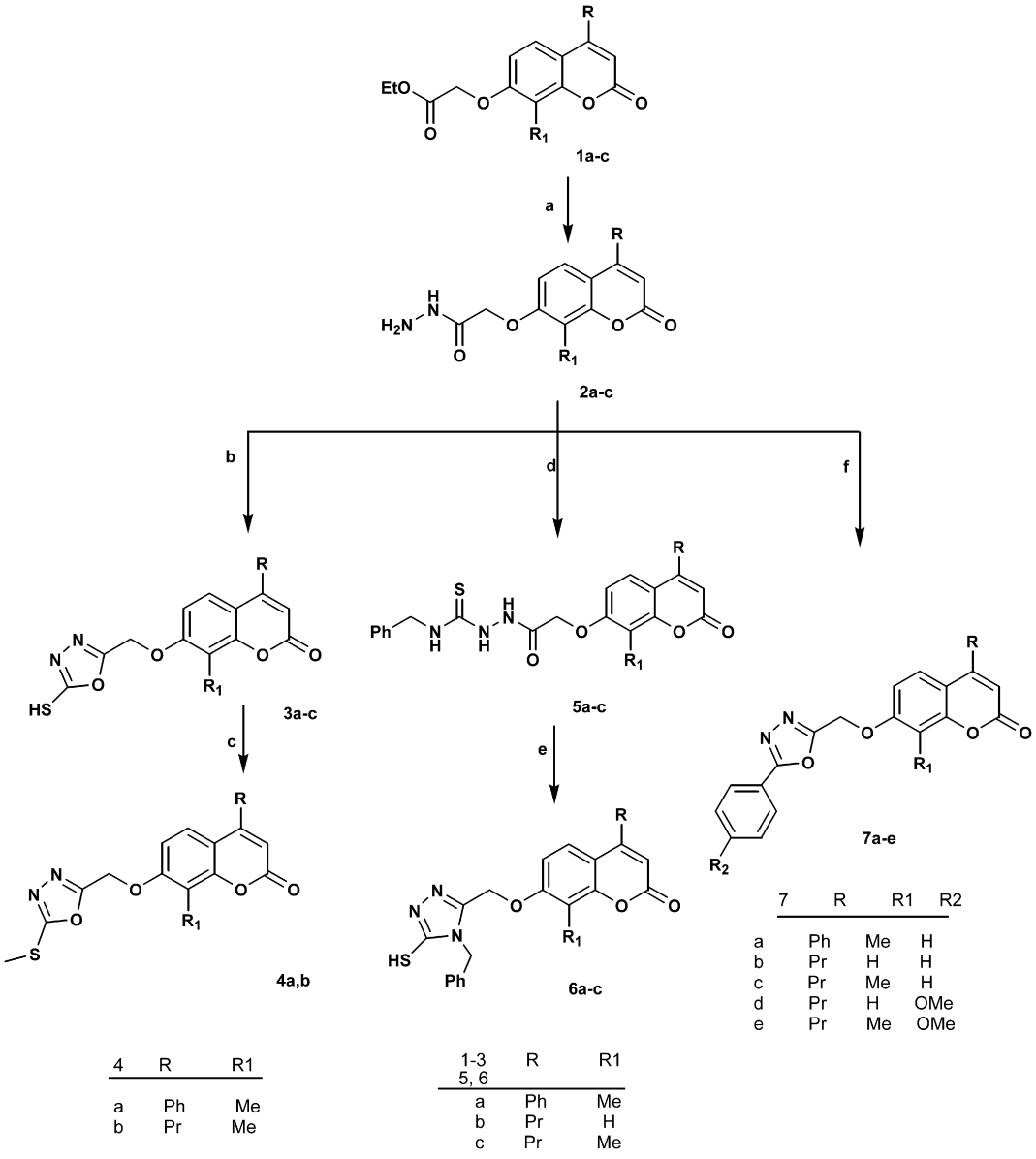
2.2. Pharmacological screening

2.2.1. Anticancer screening

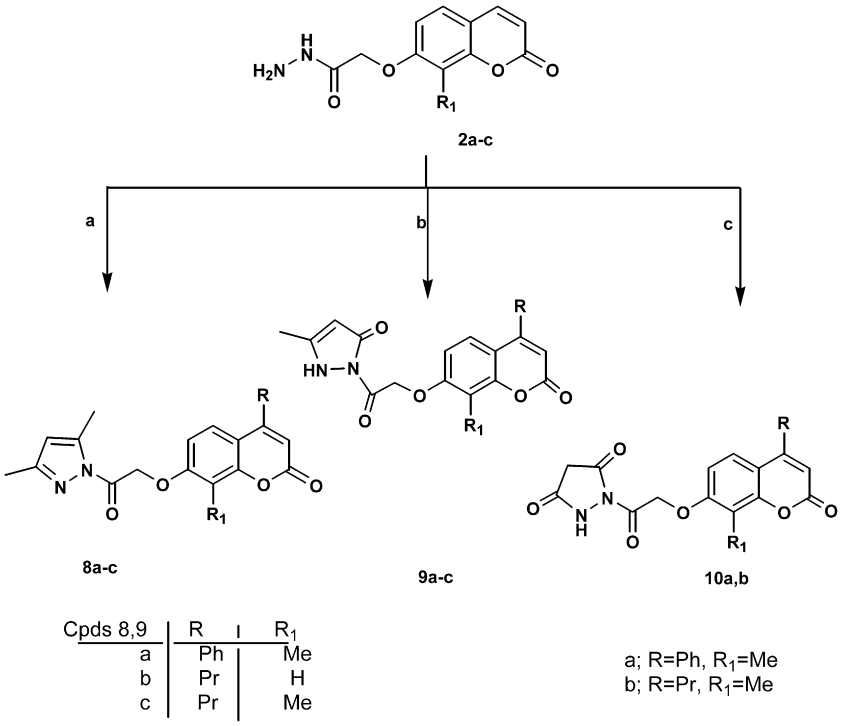
Development Therapeutic Program (DTP), Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI), Bethesda, Maryland, USA, has adopted an *in-vitro* anticancer screening.

Twelve compounds (**3a**–**c**, **4a**,**b**, **6a**,**b**, **7a**, **8a**–**c** and **9a**) were selected by NCI to be evaluated. The screening is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines representing cancers of lung, colon, brain, ovary, breast, prostate, kidney, leukemia, and melanoma [18] at a single dose of 10 μM (Table 1).

3-Methyl-1-(2-(8-methyl-4-phenyl-2*H*-1-benzopyran-2-one-7-yloxy)acetyl)-1,2-dihydropyrazol-5-one (**9a**) was passed on to further evaluation at five concentration levels (0.01–100 μM) [18–20]. It exhibited broad spectrum anticancer activity against all tested sub-panel tumor cell lines, with GI_{50} full panel mean-graph mid-point (MG-MID) = 5.46 μM (Table 2), TGI full panel



Scheme 1. Reagents and conditions: a; $NH_2NH_2 \cdot H_2O$/absolute EtOH/ref. 2 h. b; CS_2/ethanolic KOH/ref. 48 h. c; CH_3I/anhydrous K_2CO_3/dry acetone/ref. d; $PhCH_2NCS$/absolute EtOH/ref. 6 h. e; 2 N NaOH/ref.3 h. f; $R_2C_6H_5COOH/POCl_3$/ref. 2 h.



Scheme 2. Reagents and conditions: a; $CH_3COCH_2COCH_3$/AcOH/ref.6 h. b; CH_3COCH_2COOEt/AcOH/ref. 6 h. c; $EtOCOCH_2COOEt$/NaOEt/ref. 2 h.

MG-MID = 29.16 μM and LC_{50} full panel MG-MID = 60.87 μM (Table 2).

With regard to sensitivity against individual cell lines, it exhibited a super sensitivity profile toward Leukemia CCRF-CEM and HL-60(TB) with GI_{50} value lying in the nanomolar range ($GI_{50} < 0.04$ μM and TGI of 0.57 and 2.62 μM respectively). It also exhibited high activity against Colon Cancer HTC-116, Melanoma LOX IMVI, Ovarian Cancer OVCAR-4 ($GI_{50} = 1.94$, 1.99 and 1.95 μM) and (TGI = 3.76, 3.88 and 4.73 μM) respectively. It also showed high sensitivity against CNS cancer and Prostate Cancer with GI_{50} of 2.58, 2.38 μM and TGI values of 8.85 and 5.75 μM, respectively. The LC_{50} (cytotoxicity) values were >100 μM for most tested cell lines.

The ratio obtained by dividing the compounds' full panel MG-MID (μM) by its individual sub-panel MG-MID (μM) is considered as a measure of compound selectivity. Ratios between 3 and 6 refer to moderate selectivity, ratios >6 indicate high selectivity toward the corresponding cell line, while compounds meeting neither of these criteria are rated non-selective [15]. Compound **9a** proved to be non-selective with a broad spectrum of activity against the nine tumor subpanels tested with ratios ranging between 0.51 and 2.29 (Table 2).

5-Methyl thioether of 1,3,4-oxadiazole, **4b** showed good activity against CNS Cancer (SF-295) with growth % = 21.89. Moreover, 5-phenyl-1,3,4-oxadiazole, **7a** elicited anticancer activity against Leukemia (RPMI-8226) with growth % = 47.45.

5-Mercapto-4-benzyl-1,2,4-triazoles, **6a**,**b** elicited activity against 6 panels of cancer cell lines. They displayed high antitumor activities against Leukemia, Non-Small Cell Lung Cancer, CNS Cancer and Melanoma; **6a** showed further activity against Colon and Prostate Cancers; while further activities against Ovarian and Breast Cancers were elicited by **6b**.

In brief, compound **9a**, which is a hybrid of pyrazolin-5-one linked to benzopyran-2-one nucleus via acetoxy-linker proved to be the most potent cytotoxic agent, therefore, pyrazolin-5-one synergizes the activity of benzopyran-2-one rather than that for 1,3,4-oxadiazoles (**4b**, **7a**)/1,3,4-triazoles (**6a**, **6b**). On the other hand, the length of the acetoxy-linker (**9a**) favors the activity than methoxy-linker (**4b**, **6a**, **6b**, **7a**). Moreover, **9a** bearing the higher lipophilic substituents at p-4 (Ph) and p-8 (Me) displayed the highest cytotoxicity when compared to other analogs **9b**, **9c** which are not selected by NCI for preliminary cytotoxic screening.

2.3. Docking studies

To further rationalize the observed antitumor activity of the new compound**s**, a flexible ligand–receptor docking investigation was undertaken using "Internal coordinate Mechanics (Molsoft ICM 3.5-0a)".

Molecular modeling docking studies are performed and ICM score values [21–23] combined with hydrogen bonds formed with the surrounding amino acid residues help to predict the correct binding geometry for each binder at the active site. In order to compare the binding affinity of the of synthesized benzopyran-2-ones, we docked them into the empty binding site of the experimentally known crystal structure of the human DNA top-oisomerase I (top 1, 70 kDa) in complex with the poison Camptothecin (Cpt, ligand) and covalent complex with A 22 Base Pair DNA Duplex (1T8I) [15] as a target for anticancer compounds. ICM stochastic global optimization algorithm attempts to find the global minimum of the energy function that include five grid potentials describing interaction of the flexible ligand with the receptor and internal conformational energy of the ligand, during this process a stack of alternative low energy conformations is saved (Table 3).

Human top I is a multidomain enzyme that contains two highly conserved globular domains (the core and the COOH-terminal domain) that are crucial for catalytic activity, and two other regions (NH_2-terminal and linker) that are not strictly required for its catalytic and relaxation functions. The phosphate of the tyrosine–DNA phosphodiester bond makes close interactions with the guanidinium groups of Arg488 and Arg590. Both arginines are implicated in the reaction mechanism of the enzyme [24]. Arg488

Table 1
The mean growth percent, delta value and the growth percent of the synthesized compounds, **3**–**9**.

Cpd No	NSC-Number	Mean growth percent	Delta	Panel	Subpanel cell lines (growth %)
3a	749093/1	101.00	21.08	All panels[a]	_
3b	749094/1	101.83	24.55	All panels[a]	_
3c	749095/1	101.73	27.18	All panels[a]	_
4a	749105/1	95.34	28.88	All panels[a]	_
4b	749106/1	86.39	64.50	CNS cancer	SF-295 (21.89)
7a	749109/1	94.74	47.29	Leukemia	RPMI-8226 (47.45)
6a	749098/1	84.81	53.31	Leukemia	MOLT-4 (49.01), RPMI-8226 (31.50), K-562 (53.60).
				Non-small cell Lung cancer	NCI-H522 (56.62)
				Colon cancer	HT29 (57.15).
				CNS cancer	SF-295 (45.78)
				Prostate cancer	PC-3 (56.56)
				Melanoma	UACC-62 (59.87).
6b	749100/1	82.61	45.34	Leukemia	HL-60(TB) (59.74), MOLT-4 (58.74), RPMI-8226 (37.27), K-562 (46.91), SR (57.60).
				Non-small cell Lung cancer	EKVX (57.70)
				CNS cancer	SF-295 (53.03)
				Melanoma	SK-MEL-2 (53.36), UACC-62 (59.70).
				Ovarian cancer	OVCAR-4 (48.66),
				Breast cancer	T-47D (50.70)
8a	749090/1	102.17	19.04	All panels[a]	_
8b	749091/1	104.56	21.07	All panels[a]	_
8c	749092/1	102.35	18.81	All panels[a]	_
9a	749096/1	−20.25	72.70	Non-small cell Lung cancer	A549/ATCC (0.69), EKVX (−33.60), HOP-62 (−18.64), HOP-92(-64.41), NCI-H322M (−24.66), NCI-H460 (10.90), NCI-H522 (−39.18), NCI-H226 (0.36), NCI-H23 (1.72).
				Colon cancer	COLO 205 (6.21), HCC-2998 (17.35), HCT-116 (−65.87), HCT-15(38.32), HT29 (−44.22), KM12 (−11.98), SW-620 (−28.31).
				Breast cancer	MCF7 (−14.43), MDA-MB-468 (−8.13), MDA-MB-231/ATCC (−29.98), HS 578T (−4.17), BT-549 (−65.85), T-47D (−1.54)
				Ovarian cancer	IGROVI (−17.54), OVCAR-3 (−61.15), OVCAR-4 (−42.92), OVCAR-5 (10.22), OVCAR-8 (−30.48), SK-OV-3 (15.91).
				Leukemia	CCRF-CEM (13.46), HL-60(TB) (−66.59), MOLT-4 (22.70), RPMI-8226 (−18.20), K-562 (−24.06), SR (18.53).
				Renal cancer	786-0 (−58.84), A498 (−3.79), ACHN (41.59), SN12C (−69.30), RXF-393 (−36.95), TK-10 (−33.43), UO-31 (18.57).
				Melanoma	LOX IMVI (−61.79), M14 (17.58), MALME-3M (−8.61), MDA-MB-435 (−39.57), SK-MEL-2 (−12.23), SK-MEL-28 (−47.17), SK-MEL-5 (−31.48), UACC-257 (6.57), UACC-62 (−27.27).
				Prostate cancer	PC-3 (−92.95), DU-145 (−56.27).
				CNS cancer	SF-268 (2.94), SF-295 (−77.21), SF-539 (−78.24), SNB-19 (33.65), SNB-75 (−35.21), U251 (−72.36).

[a] Inactive.

makes non-bonded interaction with thymine nucleotide in minor groove (T16); Lys532 forms a hydrogen bond with thymine nucleotide (T10), another hydrogen bond formed between Thr718 and Guanine (G12). Asp533 forms non-bonded interaction with backbone sugar of Adenine (A114) whereas Tyr723 forms interaction with backbone sugar of Thymine (T10).

Table 2
GI_{50}, TGI, LC_{50} of sub-panel tumor cell lines, full panel MG-MID and selectivity ratio of compound **9a**.

Subpanel tumor cell lines[a]	GI_{50} (μM) MG-MID	TGI (μM) MG-MID	LC_{50} (μM) MG-MID	Selectivity ratio
I	3.44	50.75	93.97	1.58
II	3.69	37.40	65.07	1.48
III	8.98	32.64	64.52	0.61
IV	2.58	8.85	27.66	2.11
V	4.65	43.21	72.42	1.17
VI	8.53	28.73	52.03	0.64
VII	10.72	21.40	55.23	0.51
VIII	2.38	5.75	29.45	2.29
IX	4.23	33.71	87.50	1.29
Full panel MG-MID	5.46[b]	29.16[c]	60.87[d]	

[a] I, Leukemia; II, non-small cell lung cancer; III, colon cancer; IV, CNS cancer; V, melanoma; VI, ovarian cancer; VII, renal cancer; VIII, prostate cancer; IX, breast cancer.
[b] GI_{50} (μM) full panel mean-graph mid-point (MG-MID) = The average sensitivity of all cell lines toward the test agent.
[c] TGI (μM) full panel mean-graph mid-point (MG-MID) = The average sensitivity of all cell lines toward the test agent.
[d] LC_{50} (μM) full panel mean-graph mid-point (MG-MID).

As shown in Table 3, Cpt (ligand) reveals ICM score of −54.51 and forms eight H bonds with Arg488, Gly531, Arg590, Asn631 and Ala486 (Fig. 2); and Geiparvarin reveals ICM score of −79.36; and forms seven H bonds with Arg488, Arg488, Arg590, Arg590, Arg590, Arg590 and Cys630 (Table 3).

All the target compounds elicited higher binding affinities (ICM scores of ranges from −61.68 to −83.66) to the active site of top 1 than that for ligand.

2-Mercapto-oxadiazoles, **3a**–**c** are biologically inactive; they have high ICM scores of ranges from −61.68 to −66.78 but they bind incorrectly in the active site. 5-Methyl thioether of the latter, **4a**,**b** bind correctly to hinge region catalytic residues of top 1 (Arg488, Lys532 and Asn631) in the active site with 5H bonds; however compound is **4b** showed activity probably due to it is more stable (ICM = −73.89) than **4a** (ICM = −64.97). Analogously, 5-aryl/phenyl-1,3,4-oxadiazoles, **7a**–**e** have ICM scores of ranges from −68.89 to −77.97, but the only biologically active one is compound **7a** as it binds with the important amino acids: Arg488, Lys532, Asn631 and Thr718 by 7H bonds.

1,3,4-Triazoles, **6a**–**c** have ICM scores of ranges from −81.41 to −83.66; where both **6a**,**b** showed stability and affinity to the active site of Top 1 e.g. **6a** binds with 6H bonds to Lys532, Asp533, Ser534, Tyr723; the counterpart, **6b** binds by 7H bonds to Arg488 and

Table 3
ICM Scores of Camptothecin, Geiparvarin, the compounds, and hydrogen bonds formed with amino acid residues and their lengths.

Compounds	ICM scores	No. of H-bonds	Involved group of amino acid	Atom of ligand involved	Length of H-bond Å
Camptothecin	−54.51	8	Arg488···Hh11	O-4	2.77
			Arg488···Hh12	N-1	2.36
			Arg488···Hh21	N-1	1.78
			Gly531···Hn	O-2	2.79
			Asn631···Hd21	O-2	2.31
			Asn631···Hd22	O-2	2.56
			Arg590···Hh22	O-4	2.03
			Ala 486···O	H16	2.07
Geiparvarin	−79.36	7	Arg488···Hh12	O-2	1.80
			Arg488···Hh12	O-4	1.81
			Arg590···Hh21	O-2	1.57
			Arg590···Hh22	O-3	2.75
			Arg590···Hh22	O-1	1.60
			Arg590···Hh22	O-2	2.53
			Cys630···O	H5	1.39
3a	−61.68	4	Asn352···Hd21	O-4	2.31
			Glu356···Hn	N-2	2.62
			Lys374···Hz2	O-2	1.98
			Lys354···O	H14	1.91
3b	−61.90	2	Arg364···O	H9	1.49
			Ala499···Hn	N-2	2.04
3c	−66.78	4	Glu356···Hn	O-2	2.38
			Gln421···He21	O-4	1.79
			Lys425···Hz1	N-1	1.79
			Lys425···Hz3	N-1	2.66
4a	−64.97	5	Arg488···Hh11	N-2	2.78
			Arg488···Hh12	O-3	2.25
			Arg488···Hh12	O-4	2.79
			Lys532···Hn	N-2	1.91
			Asn631···Hd21	O-4	2.44
4b	−73.89	5	Arg488···Hh11	N-2	2.80
			Arg488···Hh12	O-3	2.66
			Lys532···Hn	O-3	2.73
			Lys532···Hn	N-2	1.89
			Asn631···Hd21	O-4	2.50
6a	−81.41	6	Lys532···Hz2	N-2	2.74
			Lys532···Hz2	N-3	1.99
			Asp533···Hn	O-2	2.28
			Ser534···Hn	O-2	1.98
			Ser534···Hg	O-2	2.46
			Tyr723···O-3	H14	2.63
6b	−82.01	**7**	Arg488···Hh12	O-1	2.53
			Arg488···Hh12	N-3	2.78
			Arg488···Hh21	N-2	1.53
			Arg488···Hh21	N-3	1.24
			Arg488···Hh22	N-2	1.73
			Arg488···Hh22	N-3	2.53
			Arg590···Hh22	O-2	2.35
6c	−83.66	4	Lys493···Hz1	N-2	2.49
			Lys493···Hz1	N-3	1.83
			Ala499···Hn	N-2	2.34
			His367···Nd1	H16	2.35
7a	−73.29	7	Arg488··· Hh12	O-3	2.16
			Arg488···Hh21	O-4	2.76
			Lys532···Hn	N-1	2.11
			Lys532···Hn	N-2	2.59
			Asn631···Hd21	O-4	2.65
			Thr718···Hg1	O-1	2.45
			Thr718···Hg1	O-2	2.62
7b	−70.08	2	Lys218···Hz1	N-2	2.05
			Lys218···Hz3	O-1	2.59
7c	−69.97	**1**	Lys443···Hz3	O-2	1.58
7d	−77.97	5	Asn491···Hn	N-1	2.60
			Asp533···Hn	O-1	2.78
			Ser534···Hn	O-1	2.62
			Ser534···Hg	O-2	1.42
			Ser534···Hg	O-1	2.67
7e	−68.89	2	Gly717···Hn	O-2	2.33
			Lys720···Hz3	O-1	2.72
8a	−62.49	3	Lys493···Hz2	O-2	2.58
			Thr50···Hn	O-2	2.28
			Thr50···Hg1	O-2	1.97
8b	−67.97	2	Val502···Hn	O-2	2.78
			Asp533···Hn	O-3	2.31
8c	−69.93	3	Lys374···Hz	N-2	2.39
			Arg375···Hh11	O-2	2.69
			Asn419···Hn	O-2	1.64
9a	−68.53	6	Arg488···Hh12	O-3	1.86
			Arg488···Hh12	N-2	2.69
			Arg488···Hh21	O-3	2.65
			Lys532···Hn	O-4	1.48
			Asn631···Hd21	N-2	2.50
			Thr718···Hg1	O-2	2.74
9b	−69.35	2	Asn491···Hn	O-4	2.54
			Asn491···Hn	O-5	1.53
9c	−75.37	3	Asn352···Hd21	O-4	1.95
			Asn352···Hd21	O-5	2.21
			Asn352···Hd22	O-5	2.76
10a	−66.15	2	Lys374···Hz2	O-2	2.05
			Tyr426···Hn	O-5	2.75
10b	−64.88	4	Gly214···Hn	O-3	2.47
			Arg434···Hh21	O-4	2.14
			Arg434···Hh22	O-4	1.36
			Ile215···O	H20	2.12

Arg590 which are implicated in the reaction mechanism of top 1.; however **6c** binds to a different amino acids (Table 3).

3,5- Dimethylpyrazoles, **8a**–**c** as well as pyrazolidine-3,5-diones, **10a**,**b** lack affinity to the active site of top 1 (Table 3).

Pyrazolin-5-one, **9a** binds (ICM = −68.53) to hinge region catalytic residues Arg488, Lys532, Asn631 with five H bonds and another H-bond with Thr718, one of residues of the carboxyl-terminal domain of the enzyme (Fig. 3). In addition, it binds to both of Thr718 that impedes the interaction with guanine (G12), Ala486 by hydrophobic interaction as well as Tyr723. **9a** develops disturbing interactions with these important residues. On the other hand; **9b**,**c** do not bind with the characteristic amino acids for enzyme inhibition (Table 3). These interactions and the corresponding induced fit in the active site conformation are compared with the one occurring with Cpt.

3. Experimental

3.1. Chemistry

All melting points are uncorrected and determined by the open capillary method using Gallencamp melting point apparatus (MFB-595-010 M). Microanalysis is carried out at the microanalytical center, Faculty of Science, Cairo University and the microanalytical center, Faculty of Science, Assiut University. Infrared spectra are determined using Shimadzu Infrared Spectrometer (IR-435). ^{1}H NMR spectra are carried out using A JEOL FX-200 MHz NMR spectrometer and A JEOL FX-300 MHz NMR spectrometer for ^{1}H NMR. Mass spectra are carried out using Finnigan MAT, SSQ 7000 mass spectrometer at 70 eV. TLC is carried out using Art.5735, Kieselgel 60 F_{245} sheets and spots are visualized by UV- 365, 254 nm. The starting materials, **1b**,**c** and the intermediates, **2a**,**b** were prepared according the reported procedures [25,26].

3.1.1. Ethyl (8-methyl-4-phenyl-2H-1-benzopyran-2-one-7-yloxy)-acetate (*1a*)

A mixture of 7-hydroxy-8-methyl-4-phenyl-2*H*-1-benzopyran-2-ones (0.1 mol), anhydrous potassium carbonate (2.76 g, 0.2 mol)

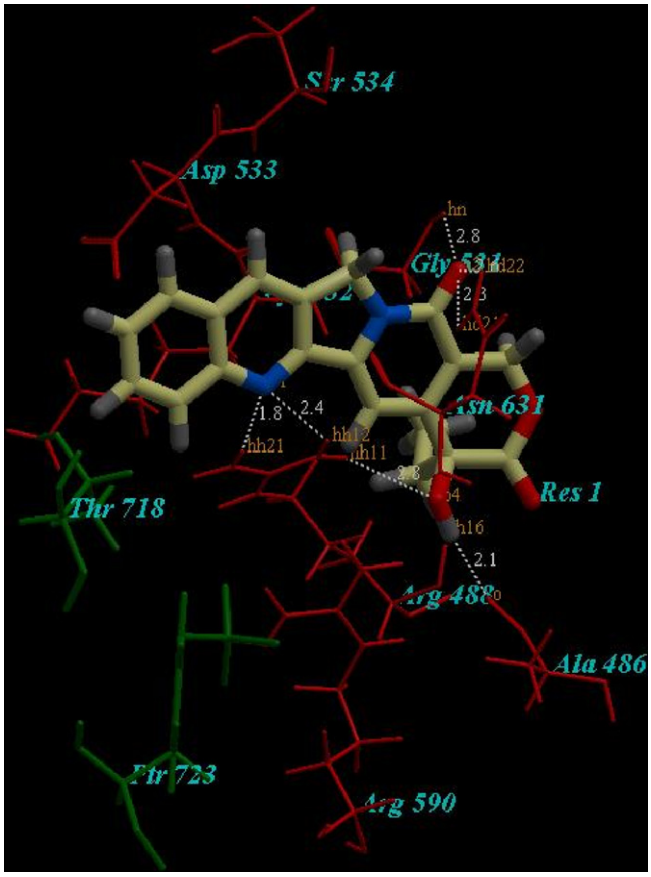


Fig. 2. Binding mode of camptothecin in the active site of human DNA topoisomerase 1.

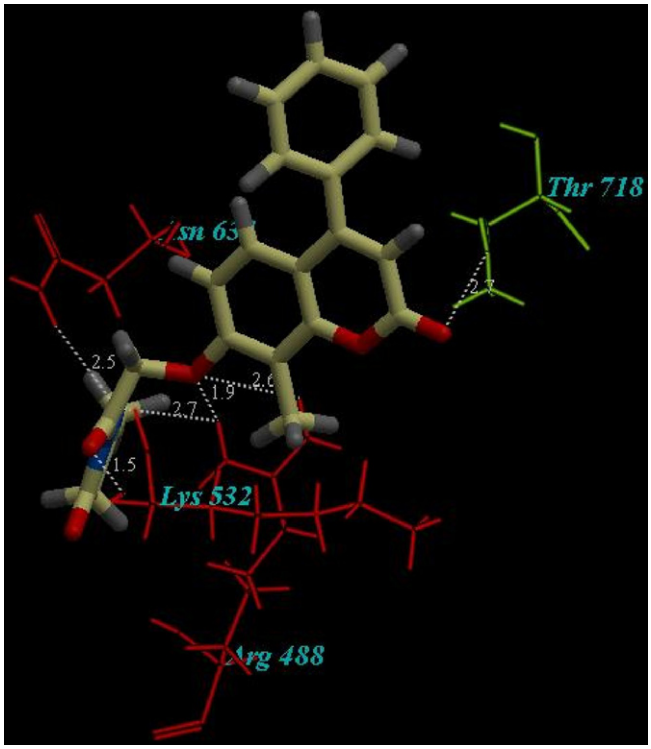


Fig. 3. Binding mode of **9a** in the active site of human DNA topoisomerase 1.

and ethylchloroacetate (14.64 g, 0.12 mol) in dry acetone (200 ml) was refluxed with continuous stirring for 24 h, cool then filter, wash with acetone. The combined filtrate and wash was concentrated. The remained residue was filtered then crystallized from isopropyl alcohol. Yield: 100%; m.p. 151–153 °C; IR (KBr cm^{-1}): 1745, 1709 (C=O of α-pyrone and OC=O); ^1H NMR (200 MHz, DMSO, δ ppm): δ = 1.2 (t, 3H, CH_2–CH_3, J = 7.2 Hz), 2.2 (s, 3H, CH_3, on C_8), 4.2 (q, 2H, CH_2–CH_3, J = 7.2 Hz), 4.9 (s, 2H, O–CH_2), 6.2 (s, 1H, C_3–H), 6.9 (d, 1H, C_6–H, J = 8.6 Hz), 7.2 (d,1H, C_5–H, J = 8.6 Hz), 7.5 (s, 5H, Ph–H). Anal. Calcd for $C_{20}H_{18}O_5$, C: 71.00; H: 5.36; found: C: 71.22, H: 5.12.

3.1.2. (8-Methyl-4-propyl-2H-1-benzopyran-2-one-7-yloxy)-acetic acid hydrazide (**2c**)

A mixture of ethyl (4,8-disubstituted-2*H*-1-benzopyran-2-one-7-yloxy)-acetate (**1a**–**c**) (0.01 mol) and hydrazine hydrate 99% (1 ml, 0.02 mol) in absolute ethanol (30 ml) was refluxed for 2 h. The precipitate was filtered off, washed with water and crystallized from dil. acetic acid. Yield: 97%; m.p. 207–209 °C, IR (KBr cm^{-1}): 3337, 3308, 3208 (NH_2, NH), 1714 (C=O, α-pyrone), 1675 (C=O, amide). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.05 (t, 3H, CH_3–CH_2–CH_2, J = 7.5 Hz), 1.7 (m, 2H, CH_3–CH_2–CH_2, J = 7.5 Hz), 2.7 (t, 2H, CH_3–CH_2–CH_2, J = 7.5 Hz), 2.4 (s, 3H, CH_3 at C_8), 4.0 (broad s, 2H, NH_2 disappeared upon deuteration), 4.7 (s, 2H, O–CH_2), 6.2 (s, 1H, C_3–H), 6.8 (d, 1H, C_6–H, J = 9 Hz), 7.5(d, 1H, C_5–H, J = 9 Hz), 7.6 (s, 1H, NH disappeared upon deuteration). MS (*m*/*z*, %): M^+ (290, 26.4). Anal. Calcd for $C_{15}H_{18}N_2O_4$, C: 62.06; H: 6.25; N: 9.65, found: C: 61.55, H: 6.78; N: 9.34.

3.1.3. 7-[(5-Mercapto-1,3,4-oxadiazol-2-yl)methoxy]-4,8-disubstituted-2H-1-benzopyran-2-ones (**3a**–**c**)

To a solution of KOH (0.56 g, 0.01 mol) in ethanol (50 ml) the acid hydrazide **2a**–**c** (0.01 mol) and carbon disulphide (1 g, 0.013 mol) were added. The mixture was refluxed for 48 h while stirring, the solvent was then removed in vacuo, the residue was dissolved in water and acidified with diluted HCl. The precipitate was washed with water and crystallized from ethanol.

3.1.4. 7-[(5-Mercapto-1,3,4-oxadiazol-2-yl)methoxy]-8-methyl-4-phenyl-2H-1-benzopyran-2-one (**3a**)

Yield: 98%; m.p. 232–235 °C, IR (KBr cm^{-1}): 3150 (NH), 1710 (C=O of α-pyrone); ^1H NMR (200 MHz, DMSO, δ ppm): δ 2.2 (s, 3H, CH_3 on C_8), 4.8 (s, 2H, O–CH_2), 6.2 (s, 1H, C_3–H), 6.3 (d, 1H, C_6–H, J = 9 Hz), 6.9 (d, 1H, C_5–H, J = 9 Hz), 7.1–7.5 (m, 5H, Ph–Hs), 10.2 (s, 1H, NH disappeared upon deuteration); MS (*m*/*z*, %): M^+ (366, 0.76). Anal. Calcd for $C_{19}H_{14}N_2O_4S$, C: 62.29; H: 3.85; N: 7.65, found: C: 61.75, H: 4.42; N: 7.23.

3.1.5. 7-[(5-Mercapto-1,3,4-oxadiazol-2-yl)methoxy]-4-propyl-2H-1-benzopyran-2-one (**3b**)

Yield: 96%; m.p. 197–199 °C, IR (KBr cm^{-1}): 3210 (NH), 1676 (C=O of α-pyrone); ^1H NMR (200 MHz, DMSO, δ ppm): δ 0.97 (t, 3H, CH_3–CH_2–CH_2, J = 7.4 Hz), 1.6 (m, 2H, CH_3–CH_2–CH_2, J = 7.4 Hz), 2.7 (t, 2H, CH_3–CH_2–CH_2, J = 7.4 Hz), 5.4 (s, 2H, O–CH_2), 6.2 (s, 1H, C_3–H), 7.1 (d, 1H, C_6–H, J = 8.8 Hz), 7.2 (s, 1H, C_8–H), 7.8 (d, 1H, C_5–H, J = 8.8 Hz), 10.3 (s, 1H, NH disappeared upon deuteration); MS (*m*/*z*, %): M^+ (318, 70.5). Anal. Calcd for $C_{15}H_{14}N_2O_4S$, C: 56.59; H: 4.43; N: 8.80, found: C: 56.20, H: 4.75; N: 8.33.

3.1.6. 7-[(5-Mercapto-1,3,4-oxadiazol-2-yl)methoxy]-4-propyl-2H-1-benzopyran-2-one (**3c**)

Yield: 97%; m.p. 252–255 °C, IR (KBr): 3306 (NH), 1709 (C=O of α-pyrone); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 1.03 (t, 3H, CH_3–CH_2–CH_2, J = 7.5 Hz), 1.7 (m, 2H, CH_3–CH_2–CH_2, J = 7.5 Hz), 2.3 (s, 3H, CH_3 at benzopyronyl C_8), 2.7 (t, 2H, CH_3–CH_2–CH_2, J = 7.5 Hz), 4.7 (s, 2H, OCH_2), 6.2 (s, 1H, benzopyronyl C_3–H), 6.8 (d, 1H, benzopyronyl C_6–H, J = 8.7 Hz) 7.5 (d, 1H, benzopyronyl C_5–H,

J = 8.7 Hz), 7.7 (s, 1H, NH disappeared upon deuteration); MS (*m*/*z*, %): M^+ (332, 2.6). Anal. Calcd for $C_{16}H_{16}N_2O_4S$, C: 57.82; H: 4.85; N: 8.43, found: C: 57.12, H: 4.70; N: 8.96.

3.2. General procedure for preparation of 7-[(5-methylthio-1,3,4-oxadiazol-2-yl)methoxy]-4,8-disubstituted-2H-1-benzopyran-2-ones (**4a**,**b**)

A mixture of **3** (0.01 mol), methyl iodide (2.8 g, 0.02 mol) and anhydrous potassium carbonate (2.8 g, 0.02 mol) in dry acetone (100 ml) was refluxed and stirred for 24 h. The product was filtered while hot, then the solvent was distilled off and the residue was crystallized from ethanol.

3.2.1. 7-[(5-Methylthio-1,3,4-oxadiazol-2-yl)methoxy]-8-methyl-4-phenyl-2H-1-benzopyran-2-one (**4a**)

Yield: 89%; m.p. 155–157 °C. IR (KBr cm^{-1}): 1719 (C=O of α-pyrone); ^1H NMR (200 MHz, DMSO, δ ppm): δ 2.3 (s, 3H, CH_3 at C_8), 2.6 (s, 3H, SCH_3), 4.8 (s, 2H, O–CH_2), 6.3 (s, 1H, C_3–H), 6.9 (d, 1H, C_6–H, J = 8.8 Hz), 7.2 (d, 1H, C_5–H, J = 8.8 Hz), 7.5 (m, 5H, Ph–H); MS (*m*/*z*, %): M^+ (380, 74.6). Anal. Calcd for $C_{20}H_{16}N_2O_4S$, C: 63.15; H: 4.24; N: 7.36, found: C: 62.66, H: 4.13, N: 7.17.

3.2.2. 7-[(5-Methylthio-1,3,4-oxadiazol-2-yl)methoxy]-8-methyl-4-propyl-2H-1-benzopyran-2-one (**4b**)

Yield: 91%; m.p. 160–163 °C, IR (KBr cm^{-1}): 1709 (C=O of α-pyrone); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 1.05 (t, 3H, CH_3–CH_2–CH_2, J = 7.5 Hz), 1.7 (m, 2H, CH_3–CH_2–CH_2, J = 7.5 Hz), 2.3 (s, 3H, CH_3 at C_8), 2.72 (t, 2H, CH_3–CH_2–CH_2, J = 7.5 Hz), 2.76 (s, 3H, SCH_3), 5.3 (s, 2H, O–CH_2), 6.2 (s, 1H, C_3–H), 7.0 (d, 1H, C_6–H, J = 9 Hz), 7.5 (d, 1H, C_5–H, J = 9 Hz). MS (*m*/*z*, %): M^+ (346, 100). Anal. Calcd for $C_{17}H_{18}N_2O_4S$, C: 58.94; H: 5.24; N: 8.09, found: C: 58.63, H: 5.12, N: 7.80.

3.3. General procedure for preparation of N-substituted-N1((8-methyl-4-phenyl-2H-benzopyran-2-one-7-yloxy)acetyl)-thiosemicarbazides (**5a**–**c**)

To a solution of the acid hydrazide **2a**,**b** (0.01 mol) in hot absolute ethanol (30 ml) the appropriate substituted isothiocyanate (0.01 mol) was added, the mixture was refluxed while stirring for 6 h. The separated solid was washed with ethanol and crystallized from ethanol/chloroform.

3.3.1. N-Benzyl-N1((8-methyl-4-phenyl-2H-benzopyran-2-one-7-yloxy)acetyl)-thiosemicarbazide (**5a**)

Yield: 78%; m.p. 230–232 °C, IR (KBr cm^{-1}): 3338, 3261, 3218 (3NH), 1698 (C=O of α-pyrone), 1602 (C=O, amide); ^1H NMR (200 MHz, DMSO, δ ppm): δ 2.3 (s, 3H, CH_3 at C_8), 4.8 (s, 2H, CH_2–C_6H_5), 4.9 (s, 2H, O–CH_2) 6.2 (s, 1H, C_3–H), 6.9 (d, 1H, C_6–H, J = 8.8 Hz), 7.1–7.5 (m, 11H, C_5–H, CH_2–C_6H_5, Ph–H), 8.6, 9.4, 10.1 (3s, 3H, 3NH disappeared upon deuteration). MS (*m*/*z*, %): M^+ (473, 0.37). Anal. Calcd for $C_{26}H_{23}N_3O_4S$, C: 65.95; H: 4.90; N: 8.87, found: C: 65.21, H: 4.68, N: 8.98.

3.3.2. N-Benzyl-N1((4-propyl-2H-benzopyran-2-one-7-yloxy) acetyl)-thiosemicarbazide (**5b**)

Yield: 90%; m.p. 180–183 °C, IR (KBr cm^{-1}): 3403, 3365, 3186 (3NH), 1730 (C=O of α-pyrone), 1612 (C=O, amide); ^1H NMR (200 MHz, DMSO, δ ppm): δ 0.97 (t, 3H, $-CH_2CH_2CH_3$, J = 7.2 Hz), 1.7 (m, 2H, $-CH_2CH_2CH_3$, J = 7.2 Hz), 2.8 (t, 2H, $-CH_2CH_2CH_3$, J = 7.2 Hz), 4.8 (s, 4H, O–CH_2 and $-CH_2-C_6H_5$), 6.2 (s, 1H, C_3–H), 7.0–7.2 (m, 6H, C_6–H, CH_2–C_6H_5), 7.7 (d, 1H, C_5–H, J = 9.4 Hz), 8.6, 9.4, 10.2 (3s, 3H, 3NH disappeared upon deuteration); MS (*m*/*z*, %): M^+ (425, 1.25). Anal. Calcd for $C_{22}H_{23}N_3O_4S$, C: 62.10; H: 5.45; N: 9.88, found: C: 62.73, H: 5.57, N: 9.40.

3.3.3. N-Benzyl-N1((8-methyl-4-propyl-2H-benzopyran-2-one-7-yloxy)acetyl)-thiosemicarbazide (**5c**)

Yield: 92%; m.p. 214–216 °C, IR (KBr cm^{-1}): 3336–3286 (3NH), 1697 (C=O of α-pyrone), 1605 (C=O, amide). ^1H NMR (200 MHz, DMSO): 0.97 (t, 3H, $-CH_2CH_2\underline{CH_3}$, J = 7.2 Hz), 1.7 (m, 2H, $-CH_2\underline{CH_2}CH_3$, J = 7.2 Hz) 2.3(s, 3H, CH_3 at C_8 of benzopyrone), 2.7 (t, 2H, $-\underline{CH_2}CH_2CH_3$, J = 7.2 Hz), 4.8 (s, 4H, O–CH_2 and $-\underline{CH_2}-C_6H_5$), 6.2 (s, 1H, benzopyronyl C_3–H), 6.9 (d, 1H, benzopyronyl C_6–H, J = 8.4 Hz), 7.3 (m, 5H, $CH_2-\underline{C_6H_5}$), 7.6(d, 1H, benzopyronyl C_5–H, J = 8.4 Hz), 8.6, 9.4, 10.1(3s, 3H, 3NH disappeared upon deuteration). MS *m*/*z* (%): M^+ (439, 0.18%), base peak (218,100%). Anal. Calcd for $C_{23}H_{25}N_3O_4S$, C: 62.85; H: 5.37; N: 9.56, found: C: 62.17, H: 5.80, N: 9.93.

3.3.4. 7-[(5-Mercapto-4-benzyl-1,2,4-triazol-3-yl)methoxy]-4,8-disubstituted-2H-1-benzopyran-2-ones (**6a**–**c**)

A mixture of **5a**–**c** (0.005 mol) and 2 N NaOH (10 ml) was refluxed and stirred for 3 h, cooled, acidified with HCl and the separated solid was crystallized from ethanol/water.

3.3.5. 7-[(5-Mercapto-4-benzyl-1,2,4-triazol-3-yl)methoxy]-8-methyl-4-phenyl-2H-1-benzopyran-2-one (**6a**)

Yield: 99%; m.p. 250–251 °C, IR (KBr cm^{-1}): 1722 (C=O, α-pyrone); ^1H NMR (200 MHz, DMSO, δ ppm): δ 2.5 (s, 3H, CH_3, C_8), 2.8 (s, 1H, SH), 5.2 (s, 2H, $-CH_2-C_6H_5$), 5.3 (s, 2H, O–CH_2), 6.2 (s, 1H, C_3–H), 7.0–7.6 (m, 12H, C_5, C_6–H, $CH_2-C_6H_5$ + Ph–H); MS: (*m*/*z*, %) = (455, 10.9). Anal. Calcd for $C_{26}H_{21}N_3O_3S$, C: 68.55; H: 4.65; N: 9.22, found: C: 68.14, H: 4.82, N: 9.32.

3.3.6. 7-[(5-Mercapto-4-benzyl-1,2,4-triazol-3-yl)-methoxy]-4-propyl-2H-1-benzopyran-2-one (**6b**)

Yield: 96%; m.p. 186–188 °C, IR (KBr cm^{-1}): 1730 (C=O, α-pyrone); ^1H NMR (200 MHz, DMSO, δ ppm): δ 0.96 (t, 3H, $-CH_2CH_2-CH_3$, J = 7.0 Hz), 1.6 (m, 2H, $-CH_2CH_2CH_3$, J = 7.0 Hz), 2.7 (t, 2H, $-CH_2CH_2-CH_3$, J = 7.0 Hz) 5.2 (s, 2H, $-CH_2-C_6H_5$), 5.3 (s, 2H, O–CH_2), 6.2 (s, 1H, C_3–H), 6.7 (d, 1H, C_6–H, J = 8.6 Hz), 7.2 (m, 5H, $CH_2-C_6H_5$), 7.7 (d, 1H C_5–H, J = 8.6 Hz); MS (*m*/*z*, %): M^+ (407, 0.01). Anal. Calcd for $C_{22}H_{21}N_3O_3S$, C: 64.85; H: 5.19; N: 10.31, found: C: 64.25, H: 5.97, N: 10.26.

3.3.7. 7-[(5-Mercapto-4-benzyl-1,2,4-triazol-3-yl)-methoxy]-8-methyl-4-propyl-2H-1-benzopyran-2-one (**6c**)

Yield: 97%, m.p. 187–189 °C; IR (cm^{-1}): 1712 (C=O, α-pyrone); ^1H NMR(DMSO); δ 1.0 (t, 3H, $-CH_2CH_2-\underline{CH_3}$, J = 7.2 Hz), 1.7 (m, 2H, $-CH_2\underline{CH_2}CH_3$, J = 7.2 Hz), 2.5 (s, 3H, $\underline{CH_3}$, on benzopyronyl C_8), 2.7 (t, 2H, $-\underline{CH_2}CH_2-CH_3$, J = 7.2 Hz) 5.2 (s, 2H, $-\underline{CH_2}-C_6H_5$), 5.3 (s, 2H, O–CH_2), 6.2 (s, 1H, benzopyronyl C_3–H), 7.0–7.3 (m, 6H, benzopyronyl, C_6–H and 5H, Ph–Hs), 7.6 (d, 1H, benzopyronyl, C_5–H, J = 9 Hz). MS *m*/*z* (%): M^+ (421, 2.9%), base peak (91, 100%). Anal. Calcd for $C_{23}H_{23}N_3O_3S$, C: 65.54; H: 5.50; N: 9.97, found: C: 65.33, H: 5.37, N: 9.51.

3.3.8. 7-{[5-(Aryl)-1,3,4-oxadiazol-2-yl] methoxy}-4,8-disubstituted-2H-1-benzopyran-2-ones (**7a**–**e**)

A mixture of the acid hydrazide **1a**–**c** (0.005 mol) and benzoic acid or *p*-methoxy benzoic acid (0.005 mol) in phosphorous oxychloride (5 ml) was heated under reflux temperature for 2 h, cooled down and slowly added to ice/water, the precipitated solid was filtered off, washed with water and crystallized from DMF/H_2O.

3.3.9. 7-{[5-(Phenyl)-1,3,4-oxadiazol-2-yl]methoxy}-8-methyl-4-phenyl-2H-1-benzopyran-2-one, **7a**

Yield: 95%, m.p. 127–130 °C; IR (cm^{-1}) 1710 (C=O of α-pyrone). ^1H NMR (DMSO); δ 2.2(s, 3H, CH_3 at benzopyronyl C_8), 4.9 (s, 2H, O–CH_2), 6.2 (s, 1H, benzopyronyl C_3–H), 6.9(d, 1H, benzopyronyl C_6–H, J = 9 Hz), 7.2(d, 1H, benzopyronyl C_5–H, J = 9 Hz), 7.4–7.6 (m, 10H, Ph–H). MS *m*/*z* (%): M^++1 (411, 0.02%), base peak (352, 100%).

Anal. Calcd for $C_{25}H_{18}N_2O_4$, C: 73.16; H: 4.42; N: 6.83, found: C: 72.87, H: 4.67; N: 6.76.

3.3.10. 7-{[5-(Phenyl)-1,3,4-oxadiazol-2-yl]methoxy}-4-propyl-2H-1-benzopyran-2-one, ***7b***

Yield: 100%, m.p. 205–207 °C; IR (cm^{-1}) 1709 (C=O of α-pyrone). ^1H NMR (DMSO); δ 0.97 (t, 3H, $\underline{CH_3}-CH_2-CH_2$), 1.2 (m, 2H, $CH_3-\underline{CH_2}-CH_2$), 1.6 (t, 2H, $CH_3-CH_2-\underline{CH_2}$), 4.8 (s, 2H, $O-CH_2$), 6.2 (s, 1H, benzopyronyl C_3–H), 6.9–8.0 (m, 8H, benzopyronyl C_8, C_5, C_6–H, +Ph–Hs). MS *m/z* (%): M^+ (362, 1.95%), base peak (135, 100%). Anal. Calcd for $C_{21}H_{18}N_2O_4$, C: 69.60; H: 5.01; N: 7.73, found: C: 69.15; H: 5.45; N: 7.29.

3.3.11. 7-{[5-(Phenyl)-1,3,4-oxadiazol-2-yl]methoxy}-8-methyl-4-propyl-2H-1-benzopyran-2-one, ***7c***

Yield: 84%, m.p. 157–160 °C; IR (cm^{-1}) 1722 (C=O of α-pyrone). ^1H NMR ($CDCl_3$); δ 1.05 (t, 3H, CH_3, $\underline{CH_3}-CH_2-CH_2$, $J = 7.5$ Hz), 1.3 (m, 2H, $CH_3-\underline{CH_2}-CH_2$, $J = 7.5$ Hz), 1.7 (t, 2H, $CH_3-CH_2-\underline{CH_2}$, $J = 7.5$ Hz), 2.4 (s, 3H, CH_3 at benzopyronyl C_8), 4.8 (s, 2H, $O-\underline{CH_2}$), 6.2 (s, 1H, benzopyronyl C_3–H), 6.7 (d, 1H, benzopyronyl C_6–H, $J = 9$ Hz), 7.3 (m, 5H, Ph–Hs), 7.4 (d, 1H, benzopyronyl C_5–H, $J = 9$ Hz). MS *m/z* (%): M^+ (376, 47.17%), base peak (221, 100%). Anal. Calcd for $C_{22}H_{20}N_2O_4$, C: 70.20; H: 5.36; N: 7.44, found: C: 69.85, H: 5.98; N: 7.89.

3.3.12. 7-{[5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl]methoxy}-4-propyl-2H-1-benzopyran-2-one, ***7d***

Yield: 99%, m.p. 228–230 °C; IR (cm^{-1}) 1718 (C=O of α-pyrone). ^1H NMR (DMSO); δ 0.97 (t, 3H, $\underline{CH_3}-CH_2-CH_2$), 1.2 (m, 2H, $CH_3-\underline{CH_2}-CH_2$), 1.6 (t, 2H, $CH_3-CH_2-\underline{CH_2}$), 2.3 (s, 3H, CH_3 at benzopyronyl C_8), 3.8 (s, 3H, $O-CH_3$), 4.8 (s, 2H, $O-CH_2$), 6.2 (s,1H, benzopyronyl C_3–H), 6.6–7.9 (m, 7H, benzopyronyl C_8, C_5, C_6–H, +Ph–Hs). MS *m/z* (%): M^+ (392, 10.62%), base peak (135, 100%). Anal. Calcd for $C_{22}H_{20}N_2O_5$, C: 67.34; H: 5.41; N: 7.14, found: C: 67.93, H: 5.55; N: 7.73.

3.3.13. 7-{[5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl]methoxy}8-methyl-4-propyl-2H-1-benzopyran -2-one, ***7e***

Yield: 88%, m.p. 137–140 °C; IR (cm^{-1}) 1718 (C=O of α-pyrone). ^1H NMR (DMSO); δ 0.97 (t, 3H, $\underline{CH_3}-CH_2-CH_2$, $J = 7.6$ Hz), 1.2(m, 2H, $CH_3-\underline{CH_2}-CH_2$, $J = 7.6$ Hz), 1.6 (t, 2H, $CH_3-CH_2-\underline{CH_2}$, $J = 7.6$ Hz), 2.2 (s, 3H, at benzopyronyl C_8), 3.8 (s, 3H, $O-CH_3$), 4.8 (s, 2H, $O-CH_2$), 6.2 (s, 1H, benzopyronyl C_3–H), 6.8–7.9 (m, 6H, benzopyronyl C_5, C_6–H, +Ph–H). Anal. Calcd for $C_{23}H_{22}N_2O_5$, C: 67.97; H: 5.46; N: 6.89, found: C: 67.53, H: 5.95; N: 7.14.

*3.3.14. 7-[2-(3,5-Dimethyl-1H-pyrazole-1-yl)-2-oxoethoxy]-4,8-disubstituted-2H-1-benzopyran-2-ones (****8a–c****)*

A mixture of the acid hydrazide **1a–c** (0.01 mol) and acetylacetone (2 ml, 0.02 mol) in acetic acid (10 ml) was refluxed for 6 h, the formed solid was filtered off and crystallized from ethyl alcohol.

3.3.15. 7-[2-(3,5-Dimethyl-1H-pyrazole-1-yl)-2-oxoethoxy]-8-methyl-4-phenyl-2H-1-benzopyran-2-one, ***8a***

Yield: 86%, m.p. 240–242 °C; IR (cm^{-1}) 1774 (C=O of α-pyrone), 1664(C=O of amide). MS *m/z* (%): M^+ (388, 0.04%), base peak (276, 100%). Anal. Calcd for $C_{23}H_{20}N_2O_4$, C: 71.12; H: 5.19; N: 7.21, found: C: 69.88, H: 5.56, N: 7.27.

3.3.16. 7-[2-(3,5-Dimethyl-1H-pyrazole-1-yl)-2-oxoethoxy]-4-propyl-2H-1-benzopyran-2-one, ***8b***

Yield: 78%, m.p. 180–182 °C; IR (cm^{-1}) 1764 (C=O of α-pyrone), 1664(C=O of amide). MS *m/z* (%): M^+ (341, 9.85%), base peak (45, 100%). Anal. Calcd for $C_{19}H_{20}N_2O_4$, C: 67.05; H: 5.92; N: 8.23, found: C: 67.54, H: 5.50, N: 7.94.

3.3.17. 7-[2-(3,5-Dimethyl-1H-pyrazole-1-yl)-2-oxoethoxy]-8-methyl-4-propyl-2H-1-benzopyran-2-one, ***8c***

Yield: 76%, m.p. 220–221 °C; IR (cm^{-1}) 1774 (CO of α-pyrone), 1663 (CO of amide). ^1H NMR (DMSO); δ 0.97 (t, 3H, $\underline{CH_3}-CH_2-CH_2$, $J = 7.2$ Hz), 1.6 (m, 2H, $CH_3-\underline{CH_2}-CH_2$, $J = 7.2$ Hz), 2.2 (s, 9H, $3CH_3$), 2.7 (t, 2H, $CH_3-CH_2-\underline{CH_2}$, $J = 7.2$ Hz), 4.8 (s, 3H, $O-CH_2$ + pyrazolyl–CH), 6.2 (s,1H, benzopyronyl C_3–H), 6.9(d, 1H, benzopyronyl C_6–H, $J = 8.4$ Hz), 7.6 (d, 1H, benzopyronyl C_5–H, $J = 8.4$ Hz). MS *m/z* (%) M^+ (354, 1.27%), base peak (189, 100%). Anal. Calcd for $C_{20}H_{22}N_2O_4$, C: 67.78; H: 6.26; N: 7.90, found: C: 67.80, H: 6.55, N: 7.54.

*3.3.18. 3-Methyl-1-(2-(4,8-disubstituted-2H-1-benzopyran-2-one-7-yloxy)acetyl)-1,2-dihydropyrazol-5-ones (****9a–c****)*

A mixture of the acid hydrazide **2a–c** (0.1 mol) and ethylacetoacetate (26 ml, 0.2 mol) in glacial acetic acid (30 ml) was refluxed for 6 h, the precipitated solid was filtered off and crystallized from DMF/H_2O.

*3.3.19. 3-Methyl-1-(2-(8-methyl-4-phenyl-2H-1-benzopyran-2-one-7-yloxy)acetyl)-1,2-dihydro- pyrazol-5-one (****9a****)*

Yield: 84%; m.p. 309–311 °C, IR (KBr cm^{-1}): 3318 (NH), 1718 (C=O of α-pyrone), 1616 (NC=O); ^1H NMR (200 MHz, DMSO, δ ppm): δ 2.3 (s, 6H, $2CH_3$), 4.8 (s, 2H, $O-CH_2$) 5.0 (s, IH, CH–pyrazolone), 6.2 (s, 1H, C_3–H), 6.9 (d, 1H, C_6–H, $J = 9.4$ Hz), 7.2 (d, 1H, C_5–H, $J = 9.4$ Hz), 7.5 (m, 5H, Ar–H),10.2 (s, 1H, NH disappeared upon deuteration). MS (*m/z*, %): M^+ (390, 0.39). Anal. Calcd for $C_{22}H_{18}N_2O_5$, C: 67.69; H: 4.28; N: 7.18, found: C: 67.34, H: 4.55; N: 7.12.

*3.3.20. 3-Methyl-1-(2-(4-propyl-2H-1-benzopyran-2-one-7-yloxy) acetyl)-1,2-dihydropyrazol-5-one (****9b****)*

Yield: 90%; m.p. 229–231 °C, IR (KBr cm^{-1}): 3319 (NH), 1712 (C=O of α-pyrone), 1648,1617 (2NC=O); ^1H NMR (200 MHz, DMSO, δ ppm): δ 0.97 (t, 3H, $CH_3-CH_2-CH_2$, $J = 7.2$ Hz), 1.6 (m, 2H, $CH_3-CH_2-CH_2$, $J = 7.2$ Hz), 2.1 (s, 3H, $-CH_3$ on pyrazolone), 2.7 (t, 2H, $CH_3-CH_2-CH_2$, $J = 7.2$ Hz), 4.8 (s, 2H, $O-CH_2$) 5.0 (s, IH, CH–pyrazolone), 6.2 (s, 1H, C_3–H), 6.8–7.0 (m, 2H, C_6, C_8–H), 7.2 (d, 1H, C_5–H, $J = 8.6$ Hz), 10.3 (s, 1H, NH disappeared upon deuteration). MS: (*m/z*, %) M^+ (342, 0.99). Anal. Calcd for $C_{18}H_{18}N_2O_5$, C: 63.15; H: 5.30; N: 8.18, found: C: 63.82, H: 5.62; N: 7.86.

*3.3.21. 3-Methyl-1-(2-(8-methyl-4-propyl-2H-1-benzopyran-2-one-7-yloxy)acetyl)-1,2-dihydro-pyrazol-5-one (****9c****)*

Yield: 92%; m.p. 303–306 °C IR (cm^{-1}): 3164 (NH), 1717 (C=O of α-pyrone), 1611 (2NC=O). ^1H NMR (DMSO); δ 0.97 (t, 3H, $\underline{CH_3}-CH_2-CH_2$, $J = 7.2$ Hz), 1.6 (m, 2H, $CH_3-\underline{CH_2}-CH_2$, $J = 7.2$ Hz), 2.3 (s, 3H, CH_3 at C_8 of benzopyrone), 2.5 (s, 3H, $-\underline{CH_3}$ on pyrazolone), 2.7 (t, 2H, $CH_3-CH_2-\underline{CH_2}$, $J = 7.2$ Hz), 4.8 (s, 3H, $O-CH_2$ + CH of pyrazolone), 6.2 (s, 1H, benzopyronyl C_3–H), 7.0 (d, 1H, benzopyronyl C_6–H, $J = 9$ Hz), 7.6 (d, 1H, benzopyronyl C_5–H, $J = 9$ Hz), 10.2 (s, 1H, NH disappeared upon deuteration). MS *m/z* (%): M^+ (356, 0.31%), base peak (218, 100%). Anal. Calcd for $C_{19}H_{20}N_2O_5$, C: 64.04; H: 5.66; N: 7.86, found: C: 64.01, H: 6.17; N: 7.49.

*3.3.22. 1-{[(4,8-Disubstituted-2H-1-benzopyran-2-one-7-yl)oxy] acetyl} pyrazolidine-3,5-diones (****10a,b****)*

To a solution of sodium ethoxide (made of 0.198 g of sodium in 20 ml of absolute ethanol) was added a freshly distilled diethylmalonate (0.68 g, 0.0424 mol) followed by addition of the acid hydrazide, **2** (0.028 mol). The reaction mixture was refluxed for 2 h, the residue was further heated at 150–155 °C for 5 h, it was dissolved in water, filtered off, acidified with 10% HCl. The precipitate was filtered off, washed with cold water then crystallized from ethanol.

*3.3.23. 1-{[(8-Methyl-4-phenyl-2H-1-benzopyran-2-one-7-yl)oxy] acetyl}pyrazolidine-3,5-dione (**10a**)*

Yield: 87%; m.p. 170–173 °C; IR (cm^{-1}): 3420 (OH), 1724 (C=O of α-pyrone), 1672 (3NC=O). ^1H NMR (DMSO); δ 2.3 (s, 3H, CH_3 on C_8 of benzopyranone), 2.4 (s, 2H, CH_2 of pyrazolone), 4.8 (s,4H, O–CH_2), 6.2 (s, 1H, benzopyronyl C_3–H), 6.9 (d, 1H, benzopyranonyl C_6–H, $J = 9$ Hz), 7.2 (d, 1H, benzopyronyl C_5–H, $J = 9$ Hz), 7.5 (m, 5H, Ph–Hs), 10.0 (s, 1H, OH, disappeared upon deuteration). MS *m*/*z* (%): M^+ (392, 0.2%), base peak (236, 100%). Anal. Calcd for $C_{21}H_{16}N_2O_6$, C: 64.28; H: 4.11; N: 7.14, found: C: 64.52, H: 4.43; N: 7.12.

*3.3.24. 1-{[(8-Methyl-4-propyl-2H-1-benzopyran-2-one-7-yl)oxy] acetyl}pyrazolidine-3,5-dione (**10b**)*

Yield: 84%; m.p. 208–210 °C; IR (cm^{-1}): 3400 (OH), 1772 (C=O of α-pyrone), 1662 (3NC=O). ^1H NMR ($CDCl_3$); δ 1.04 (t, 3H, $\underline{CH_3}$–CH_2–CH_2, $J = 7.5$ Hz), 1.7 (m, 2H, CH_3–$\underline{CH_2}$–CH_2, $J = 7.5$ Hz), 2.7 (t, 2H, CH_3–CH_2–$\underline{CH_2}$ $J = 7.5$ Hz), 2.3 (s, 3H, $\underline{CH_3}$ on C_8 of benzopyranone), 2.4 (s, 2H, $\underline{CH_2}$ of pyrazolone), 4.8 (s, 2H, O–CH_2), 6.2 (s, 1H, benzopyronyl C_3–H), 6.7 (d, 1H, benzopyranonyl C_6–H, $J = 8.7$ Hz), 7.5(d, 1H, benzopyronyl C_5–H, $J = 8.7$ Hz). Anal. Calcd for $C_{18}H_{18}N_2O_6$, C: 60.33; H: 5.06; N: 7.82, found: C: 60.96, H: 5.31; N: 7.83.

3.4. Antitumor screening

3.4.1. Methodology of the in vitro cancer screen

The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 μl at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two plates of each cell line are fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition. Experimental drugs are solubilized in dimethylsulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/ml gentamycin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 μl of these different drug dilutions are added to the appropriate microtiter wells already containing 100 μl of medium, resulting in the required final drug concentrations.

Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of 50 μl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μl) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 min at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 μl of 80% TCA (final concentration, 16% TCA).

Three response parameters (GI_{50}, TGI, and LC_{50}) were calculated for each cell line.

- The GI_{50} value (growth inhibitory activity) corresponds to the concentration of the compounds causing 50% decrease in net cell growth,
- The TGI value (cytostatic activity) is the concentration of the compounds resulting in total growth inhibition,
- The LC_{50} value (cytotoxic activity) is the concentration of the compounds causing net 50% loss of initial cells at the end of the incubation period (48 h).

Subpanel and full panel mean-graph mid-point values (MG-MID) for certain agents are the average of individual GI_{50}, TGI, or LC_{50} values of all cell lines in the sub-panel or the full panel, respectively.

The NCI antitumor drug discovery program was designed to distinguish between broad spectrum antitumor compounds and tumor or subpanel-selective agents.

3.5. Docking studies

All docking studies were performed using "Internal coordinate Mechanics (Molsoft ICM 3.5-0a)".

3.5.1. Generation of ligand and enzyme structures

The crystal structure of the human DNA topoisomerase I (1T8I) was retrieved from the Protein Data Bank (http://www.pdb.org/pdb/explore/explore.do?structureId=1T8I).

All bound waters ligands and cofactors were removed from the protein. The amino acids of the binding site where defined using data in pdbsum http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl.

3.5.2. Preparation of small molecule

A set of 7-substituted-benzopyran-2-ones, was designed to bind with human DNA topoisomerase I, ChemDraw 3D structures were constructed using ChemDraw 3D ultra 9.0 software [Cambridge Soft Corporation, USA (2004)], and then they were energetically minimized by using MOPAC (semi-empirical quantum mechanics) with MM2, Jop Type with 100 iterations and minimum RMS gradient of 0.01, and saved as MDL MolFile (*.mol).

3.5.3. Docking using Molsoft ICM 3.4-8C program [27,28]

3.5.3.1. Convert our PDB file 1T8I into an ICM object. This conversion involves addition of hydrogen bonds, assignment of atoms types, and charges from the residue templates. Click on MolMechanics/Convert/Protein, and then delete water molecules.

3.5.3.2. To perform ICM small molecule docking.

(a) Setup docking project:
  (i) Set Project Name: Click on Docking/Set project name, press OK.
  (ii) Setup the receptor: Click on Docking/Receptor Setup, enter the receptor molecule in the receptor molecule data entry box (a_*) will do, then click on identify the binding sites button to identify the potential ligand binding pockets, press OK. After the receptor setup is complete, the program normally displays the receptor with selected binding site residues highlighted in yellow xstick presentation.
  (iii) Review and adjust binding site: ICM makes a box around the ligand binding site based on the information entered in the receptor setup section. The position of the box encompasses the residues expected to be involved in ligand binding. Click on the menu Docking/Review/Adjust ligand/Box.
  (vi) Make receptor maps: The step now is to construct energy maps of the environment within the docking box. Click on

menu Docking/Make Receptor Maps, select the resolution of the map by entering a value into the grid cell size data entry box which is 0.5, this step takes few minutes.

(b) Start docking simulation: Use interactive docking to dock one ligand at a time. Click on menu Docking/Interactive docking/ Mol Table Ligand, use the drop down arrow to find the table of ligand and/or Compounds we wish to dock, and then enter the thoroughness which represent the length of simulation. Generally 1 is reasonable value, select Calc ICM Score, then select Display run which display the ligand sampling the energy in the ligand binding project.

3.5.3.3. Display the result: click docking/browse/stack conformations. ICM stochastic global optimization algorithm attempts to find the global minimum of the energy function that include five grid potentials describing interaction of the flexible ligand with the receptor and internal conformational energy of the ligand, during this process a stack of alternative low energy conformations is saved. The mode of interaction of Cpt within 1T8I was used as a standard docked model as well as for RMSD (Results differing by less than 1.0 Å in positional root-mean-square deviation) calculation. All inhibitors were compared according to the best binding free energy (minimum) obtained among all the run.

References

[1] K. Padmawinata, Acta Pharm. 4 (1973).
[2] J.P. Jerris, A.B. Smith, J. Org. Chem. 46 (1981) 577.
[3] E.B. Yang, Y.N. Zhang, P. Mack, Biochem. Biophys. Res. Commun. 260 (1999) 682.
[4] A. Lacy, R. O'Kennedy, Curr. Pharm. Res. 10 (2004) 3797.
[5] K.M. Khan, S.Z. Saify, M.Z. Khan, Z.- Ullah, M. Iqbal, et al., J. Enzym. Inhib. Med. Chem. 19 (2004) 373.
[6] S. Christmann-Franck, S. Fermandjian, G. Mirambeau, P.A. Garabedian, Biochimie 89 (2007) 468.
[7] S. Chimichi, M. Boccalini, A. Salvador, F. Dall'Acqua, G. Basso, G. Viola, ChemMedChem 4 (2009) 769.
[8] G. Viola, D. Vedaldi, F. dall'Acqua, G. Basso, S. Disarò, M. Spinelli, B. Cosimelli, M. Boccalini, S. Chimich, Chem. Biodiversity 1 (2004) 1265.
[9] Y.A. Al-Soud, I.A. Al-Masoudi, B. Safeed, U. Beifuss, N.A. Al-Masoudi, Chem. Heterocycl. Compounds 42 (5) (2006) 583.
[10] M. Popsavin, L. Torovic, S. Spaic, S. Stankov, V. Popsavin, Tetrahedron Lett. 41 (2000) 5737.
[11] R. Le-Blanc, J. Dickson, T. Brown, M. Stewart, N.H. Pati, et al., Bioorg. Med. Chem. 13 (2005) 6025.
[12] D. Seref, K. Ismail, Y. Leyla, A. Rana, J. Enz Inhib. Med. Chem. 25 (1) (2010) 74.
[13] M. Boiani, H. Cerecetto, M. González, M. Risso, C. Olea-Azar, et al., Eur. J. Med. Chem. 36 (2001) 771.
[14] M. Boiani, H. Cerecetto, M. González, Il Farmaco 59 (2004) 405.
[15] B.L. Staker, M.D. Feese, M. Cushman, Y. Pommier, D. Zembower, et al., J. Med. Chem. 48 (2005) 2336.
[16] M. Totrof, R. Abagyan, Proteins Struct. Funct. Genet. S1 (1997) 215.
[17] A. Lauria, M. Ippolito, A.M. Almerico, J. Mol. Model. 13 (2007) 393.
[18] A. Monk, D.A. Scudiero, P. Skehan, R.H. Shoemaker, K.D. Paull, et al., J. Natl. Cancer Inst. 83 (1991) 757.
[19] M.R. Grever, S.A. Schepartz, B.A. Chabner, Semin. Oncol. 19 (1992) 622.
[20] M.R. Boyed, K.D. Paull, Drug Dev. Res. 34 (1995) 91.
[21] C.N. Cavasotto, R.A. Abagyan, J. Mol. Biol. 337 (2004) 209.
[22] I. Halperin, B. Ma, H. Wolfson, R. Nussinov, Proteins: Struct. Funct. Genet. 47 (2002) 409.
[23] A. Anderson, Z. Weng, J. Mol. Graphics Model. 17 (1999) 180.
[24] M.R. Redinbo, L. Stewart, P. Kuhn, J.J. Champoux, W.G.J. Hol, Science 279 (1998) 1504.
[25] G. Sapna, A. Kavita, V.K. Tiwari, K. Diksha, R.P. Tripathi, A.K. Srivastava, R. D. Walter, Med. Chem. Res. 13 (8/9) (2004) 707.
[26] K. Diksha, T.V. Kumar, T.R. Pati, R.V. Mohan, B.S. Mishra, S.J. Kumar, Arzn.-Forsch./Drug Res. 53 (12) (2003) 857.
[27] R.A. Abagyan, M.M. Totrov, D.N. Kuznetsov, J. Mol. Biol. 235 (1994) 983.
[28] R.A. Abagyan, M.M. Totrov, D.N. Kuznetsov, J. Comp. Chem. 15 (1994) 488.