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Thiohydantoin derivatives incorporating a pyrazole core: Design, synthesis and biological evaluation as dual inhibitors of topoisomerase-I and cycloxygenase-2 with anti-cancer and anti-inflammatory activities

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ABSTRACT

A new series of hybrid structures **14a–l** containing thiohydantoin as anti-cancer moiety and pyrazole core possessing SO_2Me pharmacophore as selective COX-2 moiety was designed and synthesized to be evaluated for both anti-inflammatory and anti-cancer activities. The synthesized compounds were evaluated for their COX inhibition, *in vivo* anti-inflammatory activity, ulcerogenic liability, *in vitro* cytotoxic activity and human topoisomerase-1 inhibition. All compounds were more selective for COX-2 isozyme and showed good *in vivo* anti-inflammatory activity. Also, all derivatives were significantly less ulcerogenic (ulcer indexes = 2.64–3.87) than ibuprofen (ulcer index = 20.25) and were of acceptable ulcerogenicity when compared with the non-ulcerogenic reference drug celecoxib (ulcer index = 2.99). Regarding anti-cancer activity, most of the target derivatives showed activities against A-549, MCF-7 and HCT-116 cell lines (IC_{50} = 5.32–17.90, 3.67–19.04 and 3.19–14.87 μM respectively) in comparison with doxorubicin (IC_{50} = 0.20, 0.50 and 2.44 μM respectively). Compound **14a** inhibited the human topoisomerase-1 with IC_{50} = 29.7 μg/ml while **14b** and **14c** showed more potent inhibitory activity with IC_{50} = 26.5 and 23.3 μg/ml. respectively in comparison with camptothecin (IC_{50} = 20.2 μg/ml). Additionally, COX-2 and human topoisomerase-1 docking studies were carried out to explain the interaction of the synthesized hybrid structures **14a–l** with the target enzymes.

1. Introduction

Cancer is one of the most leading causes of death all over the world. Thus, several trials were made by the researchers to find effective clinical approaches for cancer treatment [1]. Cancer cells differ from their normal counterparts in the type and the number of the biochemical processes that proceeded during cell growth and division. Chemotherapeutic agents generally act on the metabolically active rapid proliferating cells and they can't distinguish between cancer and normal cells [2] (see Figs. 1 and 2).

Within the last two decades, cyclooxygenase (COX), the essential enzyme in the up-regulation of the inflammation process, was introduced as a novel target for cancer treatment as COX-2 is overexpressed in malignant cells of colon and breast cancer [3–5]. COX-2 enzyme contributes to cancer through several mechanisms such as apoptosis inhibition, increasing angiogenesis, malignant cell proliferation, migration, inducing invasion and regulation of signal transduction pathways and cell to cell adhesion through upregulation of prostaglandin synthesis, which has both autocrine and paracrine properties on tumor cells proliferation and migration [6,7].

Pyrazole containing compounds has great attention due to its pharmacological effects especially as anti-inflammatory [8–12] (e.g. celecoxib, **1**) and antiproliferative (e.g. AT 7519, **2**) [13–15]. In our recent work, two series **(3, 4)** of Y-shaped pyrazole containing compounds were synthesized and showed good anti-inflammatory activity as potent celecoxib analogs [16,17].

On the other hand, DNA-topoisomerases are important enzymes responsible for solving all topological problems associated with DNA replication and transcription. There are two types of topoisomerases, type-1 (Topo-1) and type-2 (Topo-2), in various tumor cells Topo-1 is expressed much higher than in normal cells, it acts by causing a transient break in one DNA strand followed by resealing of the DNA strands after bathing one strand through each other hence, Topo-1 inhibitors could be used as anticancer agents [18–21]. Topo-1 inhibitors as

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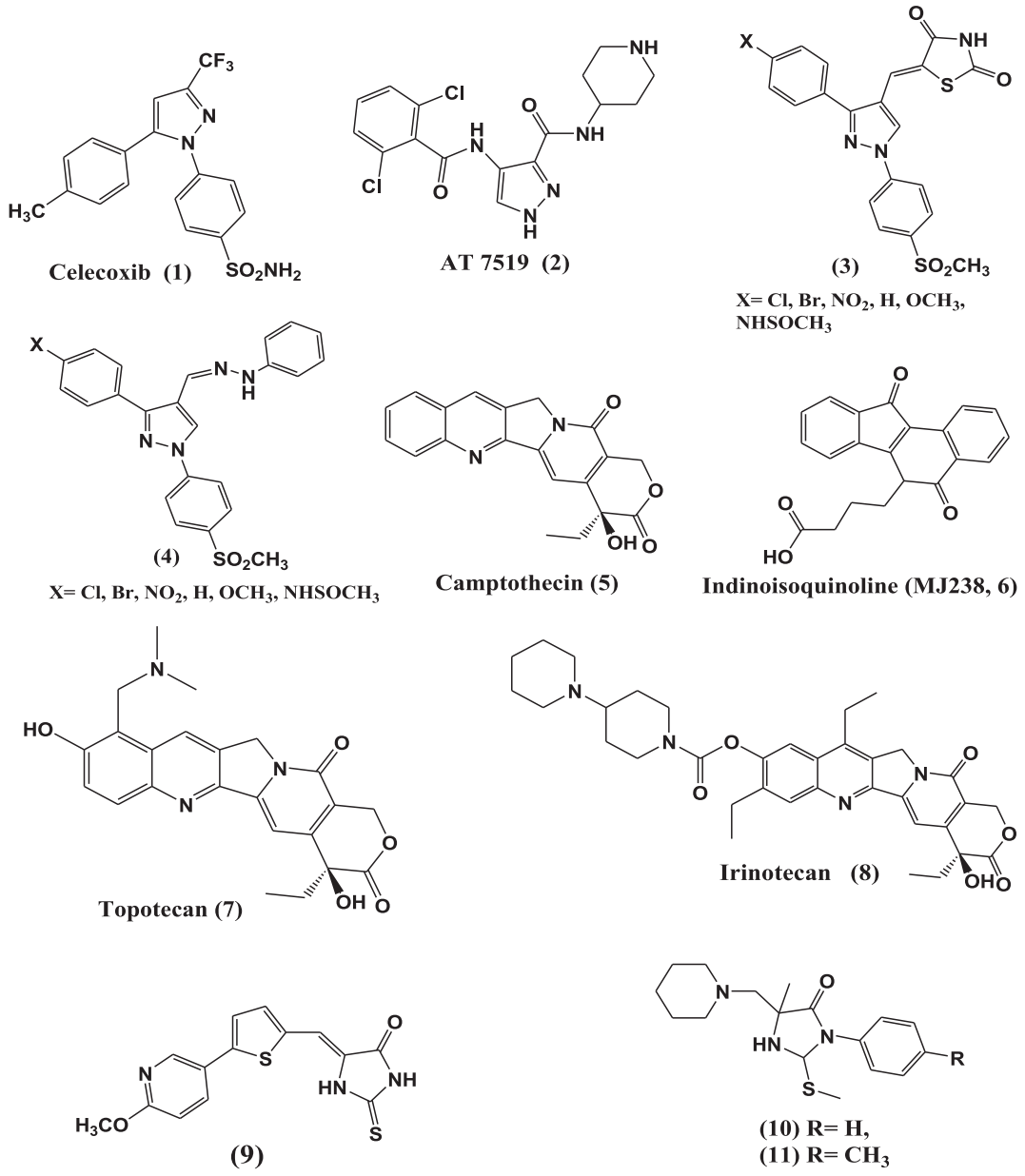


Fig. 1. Chemical structures of some reported drugs and lead compounds as COX-2 or/ and human Topo-1 inhibitors.

Camptothecin **(5)** and Indinoisoquinoline **(6)** perform their inhibitory action through stabilization of Topo-1 DNA cleavage complexes leading to cell death. Although traditional Topo-1 inhibitors as camptothecin **(5)** and its various analogs (topotecan, **7** and irinotecan, **8)** have many limitations due to their unstable chemical structures, rapid cellular efflux via membrane pumps and the occurrence of cellular resistant that lead to the need for the design and synthesis of non-camptothecin Topo-1 inhibitors [22–24]. Recently, 2-thioxoimidazolidinones (thiohydantoins) have been synthesized and evaluated as anticancer agents, focusing on their ability to inhibit Topo-1. From this study, it is revealed experimentally and through molecular docking that one derivative of 2-thioxoimidazolidinones **(9)** exhibited potent inhibition of human Topo-1 through stabilization of Topo-1-DNA cleavage complex with IC_{50} = 10.08 μM and showed cytotoxicity activity against MCF-7 (human breast cancer cell line) with IC_{50} = 11.4 μM [25]. Also, our group synthesized a group of 2-thioxoimidazolidinones which were effective as anti-proliferative agents against both prostate and breast cancer cell lines through their inhibitory activity against Topo-1 and it was found that two compounds **(10**, **11)** from this group were potent as Topo-1 inhibitors with IC_{50} 19.22 and 30.34 μg/ml respectively [26].

Combination of pharmacophoric moieties of different bioactive substances to produce a new hybrid compound with improved affinity and efficacy when compared to the parent drugs is considered as a new trend in drug design and discovery called molecular hybridization. This policy was used for the synthesis of novel bioactive hybrid drug with dual biological activities [27,28].

Based on the previous data in addition to the existing need for developing superior anticancer/anti-inflammatory agents, we herein describe our efforts to design new hybrid molecules (**14a–l**) aiming at achieving both activities by a combination of the thiohydantoin moiety as Topo-1 inhibitor with pyrazole core as a COX-2 inhibitor scaffold. According to the earlier displayed reports on the biological activities of both thiohydantoin and Y-shaped pyrazole core of celecoxib analogs, the newly designed target compounds (**14a–l**) would exhibit anti-inflammatory activity through selective inhibition of COX-2 enzyme and also showing anti-cancer activity directly through inhibition of Topo-1 enzyme and indirectly through inhibition of COX-2 enzyme.

Also, the synthesized target compounds **14a–l** were subjected to *in*

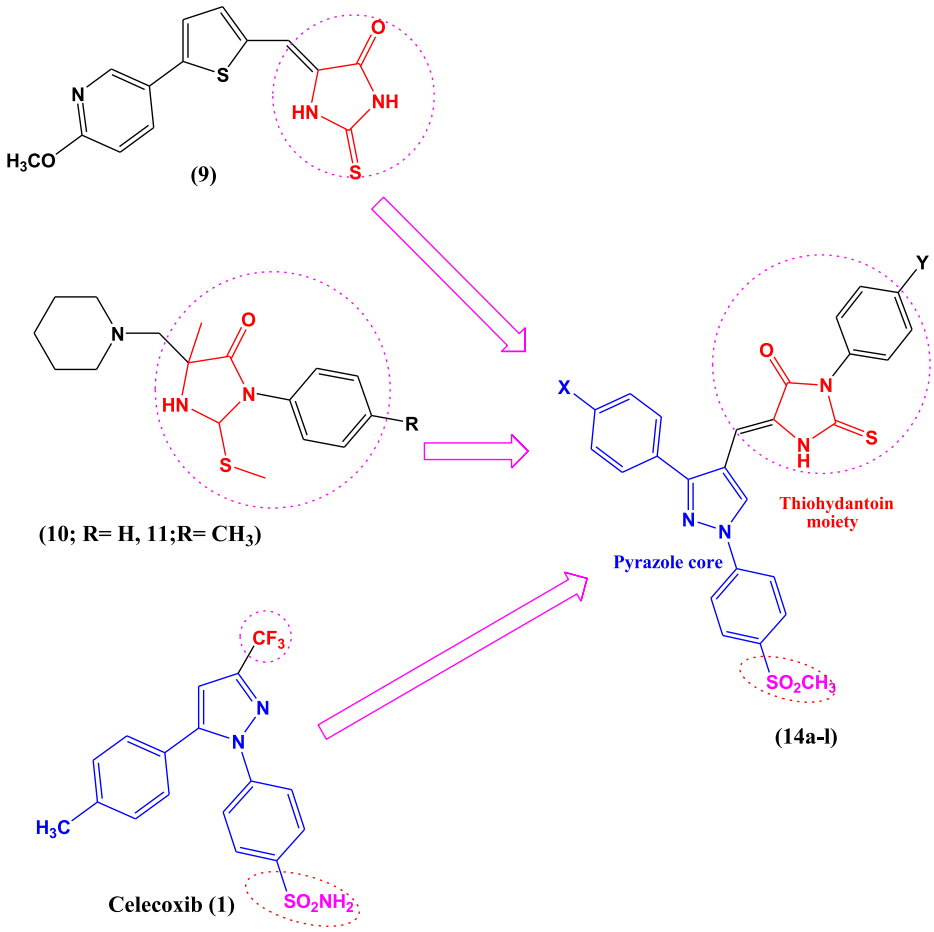


Fig. 2. Design of the target hybrid structures **14a–l** from celecoxib (**1**) and our reported thiohydantoin derivatives (**9**, **10** and **11**).

vitro evaluation as COX-1/COX-2 inhibitors, *in vivo* anti-inflammatory activity (AI), ulcerogenic liability, *in vitro* antiproliferative activity against MCF-7 (human breast carcinoma), HCT-116 (human colon cancer) and A549 (human lung carcinoma) cell lines. Additionally, we conducted molecular docking studies for the most potent derivatives against COX-2 and human Topo-1 enzymes in comparison with celecoxib (**1**) and the potent human Topo-1 inhibitor (topotecan, **7**). To obtain our goal in developing a new COX-2/human Topo-1 inhibitors as anti-cancer agents, we also evaluated the inhibitory effect of the most potent anti-cancer derivatives against human Topo-1.

2. Results and discussion

2.1. Chemistry

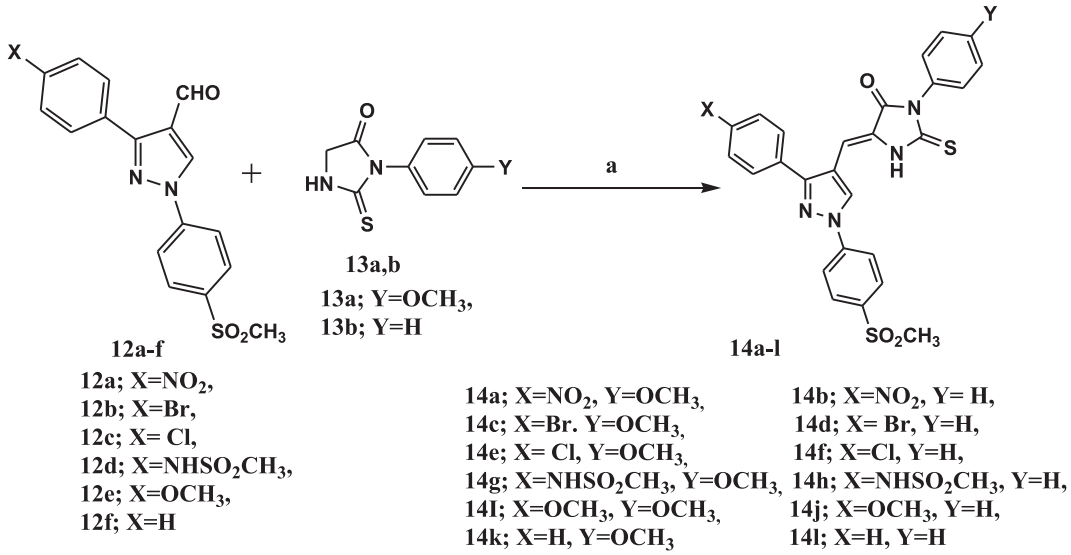
The pyrazole aldehydes **12a–f** were prepared starting from 4-methylsulfonylphenylhydrazine hydrochloride and acetophenone derivatives followed by Vilsmeier-Haack reaction according to our reported procedures [16]. Also, the *N*-aryl-thiohydantoin derivatives **13a,b** were synthesized from glycine and the respective arylisothiocyanate as reported [29]. Upon reaction **12a–f** with the respective *N*-aryl-thiohydantoin derivatives **13a,b** (Scheme 1) under basic conditions obtained by piperidine, the active methylene of the thiohydantoin ring was condensed with the carbonyl group of the pyrazole aldehyde derivative to give the target pyrazole derivatives **14a–l** in good yield (65–87%). The formation of the target compounds was confirmed by the presence of a singlet peak for CH=C– olefinic H at 6.31–6.72 ppm in ^{1}H NMR spectra in addition to appearance of a signal at 118.12–119.68 ppm for **CH**=C– olefinic carbon in ^{13}C NMR also the formation of the target pyrazole derivatives **14a–l** was confirmed by mass spectra and elemental analyses.

2.2. Biological evaluation

2.2.1. Anti-inflammatory

2.2.1.1. In vitro cyclooxygenase inhibition assay. The *in vitro* COX-1/COX-2 isozyme inhibition activity studies tested the ability of the target thiohydantoin-pyrazole hybrid derivatives **14a–l** to inhibit ovine COX-1 and human recombinant COX-2 using an enzyme immunoassay (EIA) [30]. The shown results in Table 1 revealed that the target derivatives had non-selective effects against COX-1 isozyme (IC_{50} = 4.22–9.87 μM range) but showed high COX-2 isozyme effects (IC_{50} = 0.56–1.72 μM range) with COX-2 selectivity indexes (5.34–10.72) in comparison to celecoxib, the COX-2 selective reference drug, (COX-1 IC_{50} = 7.23 μM, COX-2 IC_{50} = 0.84 μM and S.I. = 8.60). Within all compounds, the chloro analogue **14f** showed the highest COX-2 selectivity index (S.I. = 10.72) while the methoxy analogue **14k** showed the lowest COX-2 selectivity index (S.I. = 5.34).

2.2.1.2. In vivo anti-inflammatory activity. The *in vivo* AI activity of the synthesized derivatives **14a–l** and celecoxib (as a reference drug) was assayed using carrageenan-induced rat paw edema method according to the reported procedure [31] using a dose of 50 mg/kg body weight. The AI was then calculated based on changes of paw-volume at 1, 3 and 5 h after carrageenan injection as presented in Table 2. All derivatives significantly decreased inflammation when compared with carrageenan at the used time intervals. After 1 h, the target compounds showed moderate anti-inflammatory activity (AI = 41.33–72.00%) in



Scheme 1. Synthesis of the target compounds **14a–l**.

Table 1
In vitro COX-1 and COX-2 inhibitory activity of the target hybrid structures **14a-l** and reference drug celecoxib.

Compound	COX-1 IC_{50} (μM)[a]	COX-2 IC_{50} (μM)[a]	COX-2 S.I.[b]
14a	4.78	0.65	7.35
14b	9.87	1.72	5.73
14c	6.72	0.85	7.90
14d	7.32	0.84	8.71
14e	5.22	0.56	9.32
14f	8.15	0.76	10.72
14g	4.81	0.71	6.77
14h	8.12	0.98	8.28
14i	4.22	0.61	6.91
14j	6.17	0.82	7.52
14k	8.39	1.57	5.34
14l	5.49	0.60	9.15
Celecoxib	7.23	0.84	8.60

[a] The concentration of test compound produce 50% inhibition of COX-1, COX-2 enzyme, the result is the mean of two values obtained by assay of enzyme kits obtained from (Cayman Chemicals Inc., Ann Arbor, MI, USA).
[b] The *in vitro* COX-2 selectivity index (COX-1/COX-2).

comparison to celecoxib (AI = 71.73%). After 3 h of compounds administration, the anti-inflammatory activity was 23.25–69.33% in comparison to celecoxib (AI = 71.73%). While after 5 h, the AI was 42.66–69.33% and for celecoxib = 89.00%. It was noted that; for all compounds **14a–l** and at all time intervals 1, 3 and 5 h, The methoxy substituted analogues (**14a**, **14c**, **14e**, **14g**, **14i** and **14k**) revealed higher AI activities than the respective unsubstituted analogues (**14b**, **14d**, **14f**, **14h**, **14j** and **14l**).

Additionally, ED_{50} (the dose causing 50% edema inhibition) was calculated for the most potent AI analogues **14a**, **14b**, **14c, 14i** and **14k** in comparison to celecoxib. One of these potent derivatives is unsubstituted (**14b**) showed approximately similar ED_{50} (78.90 μmol/kg) to celecoxib (ED_{50} = 78.53 μmol/kg). The other potent analogues are methoxy substituted and three of them **14a**, **14c** and **14k** (ED_{50} = 62.61, 55.83 and 58.49 μmol/kg respectively) were more potent than celecoxib while the fourth one **14i** showed comparable potency (ED_{50} = 88.28 μmol/kg) to celecoxib (Table 3).

2.2.1.3. Ulcerogenic liability. Furthermore, we determined the ulcerogenicity for the most potent derivatives **14a**, **14b**, **14c, 14i** and **14k** using 50 mg/kg dose in comparison to the same dose of the reference drug celecoxib and a smaller dose of ibuprofen (120 μmol/kg) [32]. The data shown in Table 4 showed that all tested derivatives were significantly less ulcerogenic (ulcer indexes = 2.64–3.87) than the ulcerogenic drug ibuprofen (ulcer index = 20.25) and were of acceptable ulcerogenicity to the non-ulcerogenic reference drug celecoxib (ulcer index = 2.99). Among these five potent derivatives,

Table 2
In vivo anti-inflammatory activity of the target hybrid structures **14a–l** and reference drug celecoxib.

Compound No.	Mean value of paw edema thickness (cm) ± SEM (% of inhibition)		
	1 h	3 h	5 h
14a	0.23 ± 0.025 (69.33%)	0.25 ± 0.0200 (66.66%)	0.23 ± 0.0152 (69.33%)
14b	0.24 ± 0.036 (68.00%)	0.34 ± 0.0264 (54.66%)	0.33 ± 0.0152 (56%)
14c	0.21 ± 0.015* (72.00%)	0.23 ± 0.0458* (69.33%)	0.29 ± 0.0152* (61.33%)
14d	0.44 ± 0.0321 (41.33%)	0.47 ± 0.0057 (37.33%)	0.43 ± 0.0251 (42.66%)
14e	0.34 ± 0.032 (54.66%)	0.33 ± 0.0208 (56%)	0.30 ± 0.0305 (60.00%)
14f	0.34 ± 0.0082* (54.6%)	0.35 ± 0.0208 * (53.33%)	0.32 ± 0.0152 (57.33%)
14g	0.37 ± 0.0251* (50.66%)	0.32 ± 0.0208* (57.33%)	0.37 ± 0.0100* (50.66%)
14h	0.35 ± 0.036 (52.00%)	0.44 ± 0.0378 (23.25%)	0.42 ± 0.0152 (44.00%)
14i	0.14 ± 0.020 (72.33%)	0.22 ± 0.0208 (70.66%)	0.24 ± 0.0458 (68%)
14j	0.40 ± 0.040* (46.66%)	0.41 ± 0.0208* (45.33%)	0.41 ± 0.0230* (45.33%)
14k	0.27 ± 0.028 (64.00%)	0.24 ± 0.0300 (68.00%)	0.25 ± 0.0204 (66.66%)
14l	0.31 ± 0.0321 (58.66%)	0.46 ± 0.01528 (38.66%)	0.33 ± 0.0100 (56.00%)
Celecoxib	0.21 ± 0.0057 (71.73%)	0.22 ± 0.152 (70.38%)	0.23 ± 0.01 (69%)

Values represent means ± SEM of four animals for each group.
* Means significant difference with celecoxib at $p < 0.05$.

Table 3
ED_{50} for the most active compounds **14a**, **14b**, **14c**, **14i**, **14k**, and reference drug celecoxib.

Compound	% of inhibition			ED_{50} (μmol/kg)[a]
	10 mg/kg	25 mg/kg	50 mg/kg	
14a	13.22	38.31	66.66	**62.61**
14b	17.83	30.82	54.66	**78.90**
14c	12.41	35.92	69.33	**55.83**
14i	11.19	22.54	49.66	**88.28**
14k	16.41	42.69	68.00	**58.49**
Celecoxib	18.00	44.28	84.38	78.53

[a] Inhibitory activity in a carrageenan-induced rat paw edema assay. The results are expressed at 3 h after oral administration of the test compound.

Table 4
Ulcerogenic liability for the most active compounds **14a**, **14b**, **14c**, **14i**, **14k** and reference drugs (celecoxib and ibuprofen).

Compound	Average severity	Average no of ulcer[a]	% incidence/10	Ulcer index
14a	0.56 ± 0.033	0.3 ± 0.018***	3	3.21
14b	0.54 ± 0.023	0.4 ± 0.02 ***	3	3.77
14c	0.45 ± 0.017***	0.4 ± 0.016***	3	3.87
14i	0.40 ± 0.018***	0.3 ± 0.015***	2	2.64
14k	0.73 ± 0.042***	0.3 ± 0.016	2	3.02
Celecoxib	0.61 ± 0.039	0.3 ± 0.017	2	2.99
Ibuprofen	2.25 ± 0.13	8.0	10	20.25

*** Means significant difference with celecoxib at $p < 0.001$.
[a] Values represent means ± SEM of ten animals for each group.

Compound **14i** (the highest COX-2 potency with IC_{50} = 0.61 μM) was the least ulcerogenic derivative (ulcer index = 2.64) even less ulcerogenic than the non-ulcerogenic reference drug celecoxib.

2.2.2. Anti-cancer activity

2.2.2.1. In vitro cytotoxicity screening. The anti-cancer activity of the newly synthesized compounds **14a–l** was evaluated in a panel of different cancer cell lines including human lung cancer cell line (A549), human breast cancer cell line (MCF-7) and human colon cancer cell line (HCT-116). Doxorubicin was used as a positive control [33]. The MTT cell viability assay was performed to determine the half maximal inhibitory concentration (IC_{50}). Compounds **14a–l** showed an anti-cancer activity with IC_{50} of 5.32–17.90, 3.67–19.04 and 3.19–14.87 μM in A549, MCF-7 and HCT-116 cell lines, in comparison with doxorubicin (IC_{50} = 0.2, 0.5 and 2.44 μM) respectively (Table 5). Within all tested compounds, **14a**, **14b** and **14c** exhibited the highest potency with IC_{50} of 5.32, 7.23 and 7.30 μM; respectively in A549, IC_{50} of 3.94, 5.63 and 3.67 μM respectively in MCF-7 and IC_{50} of 3.73, 3.19 and 2.78 μM, respectively in HCT-116. On the other hand, exceptions were observed in A549, in which the IC_{50} of **14f**, **14g** and **14l** was not determined. Similarly, **14f** and **14g** didn't show anti-cancer activity in MCF-7 cells. In a similar manner to the anti-inflammatory activity, the methoxy substituted derivatives (**14a**, **14c**, **14e**, **14g**, **14i** and **14k**) have higher cytotoxic activities than the respective unsubstituted derivatives (**14b**, **14d**, **14f**, **14h**, **14j** and **14l**) with only one exception. The methoxy derivative **14g** was ineffective against A549, MCF-7 and had approximately similar potency against HCT-116 to its unsubstituted analogue **14h** with IC_{50} of 5.90 and 5.21 μM respectively.

2.2.2.2. Human topoisomerase-1 inhibitory assay. To investigate whether the cytotoxic activities of the target compounds **14a–l** were associated with inhibitory activity to human Topo-I, the inhibitory activity of the most cytotoxic derivatives **14a**, **14b** and **14c** compounds to human Topo-I was analyzed by human DNA topoisomerase-1 assay using the highly selective Topo-1 inhibitor (camptothecin, **5**) as a reference. The three derivatives significantly inhibited the DNA relaxation activity of human Topo-1 in a dose-dependent manner in concentrations comparable to camptothecin (Table 6). Compound **14a** inhibited the human Topo-1 enzymatic activity with IC_{50} = 29.7 μg/ml while **14b** and **14c** showed more potent inhibitory activity with IC_{50} = 26.5 and 23.3 μg/ml respectively in comparison with camptothecin (IC_{50} = 20.2 μg/ml). These results suggested that the evaluated compounds surely bound with human Topo-1 and possessed inhibitory activity to the human Topo-1. These results were also consistent with the molecular docking study, as illustrated later.

Table 5
In vitro cytotoxicity data for target hybrid structures **14a–l** and doxorubicin as a reference drug.

IC_{50} ± SD (μM)			
	A-549	MCF-7	HCT-116
14a	5.32 ± 0.94	3.94 ± 0.22	3.73 ± 0.39
14b	7.23 ± 0.87	5.63 ± 0.31	3.19 ± 0.36
14c	7.30 ± 1.10	3.67 ± 0.62	2.78 ± 0.08
14d	12.73 ± 1.21	8.61 ± 0.37	7.01 ± 0.65
14e	11.97 ± 1.07	9.19 ± 0.84	8.34 ± 0.74
14f	> 20	> 20	6.25 ± 0.67
14g	> 20	> 20	5.90 ± 0.55
14h	17.23 ± 1.43	18.20 ± 0.75	5.21 ± 0.17
14i	16.94 ± 0.97	18.74 ± 1.01	6.39 ± 0.13
14j	16.17 ± 0.21	19.04 ± 1.67	12.74 ± 0.44
14k	17.90 ± 0.89	17.21 ± 0.64	12.33 ± 0.92
14l	> 20	18.49 ± 0.36	14.87 ± 1.07
Doxorubicin	0.2 ± 0.05	0.5 ± 0.02	2.44 ± 0.36

Cells were treated with the test compounds or vehicle for 48 h. Data were reported as mean ± S.D. (n = 6). Three human cancer cell lines were used; MCF-7 (human breast carcinoma), HCT-116 (human colon cancer) and A549 (human lung carcinoma). Doxorubicin was used as a positive control in the anticancer screening.

2.3. Molecular modeling

2.3.1. COX-2 docking study

To know the plausible mode of interactions of the synthesized derivatives **14a–l** within the COX-2 isozymes, molecular docking was done using X-ray crystal structure data for COX-2 obtained from the protein data bank (PDB: ID 2AW1) using celecoxib as the reference ligand [34]. The docking data consisting of the energy associated with intermolecular interactions (affinity in kcal/mol) obtained upon computational docking for all derivatives and celecoxib within COX-2 active sites and the binding interactions between the amino acid residues and functional groups of the different derivatives are shown in Table 7. The binding energies were found to be in a range of 16.5 to −20.4 compared to −17.6 kcal/mol observed for celecoxib. Compounds 14e, 14f and 14l exhibited one hydrogen bonding interactions with Lys 68. Compound 14c is the only compound that interacted with COX-2 active site with two hydrogen bonding through Lys 68 and Tyr 108. Finally, four derivatives (14g, 14h, 14i and 14k) exhibited three hydrogen bonding interactions and the last four derivatives (14a, 14b, 14d and 14j) interacted with four hydrogen bonding in comparison to two hydrogen bonding interactions achieved by celecoxib with Ser 516 and Tyr 371 (Fig. 3).

2.3.2. Human topoisomerase-1 docking study

Also, molecular docking study was conducted on the target compounds **14a–l** in comparison with topotecan (**7**) into human Topo-1 enzyme using Flex X module in Lead IT 2.1.8 software-package [25].

From the docking results (Table 8), all compounds showed good binding interactions (affinity in Kcal/mol from −13.79 to −15.81 with one to two Hydrogen bonding interactions either with amino acids of

Table 6
In vitro human Topo-1 inhibitory activity for **14a–c** and reference drug camptothecin (**5**).

Compound	% of inhibition							IC_{50} μg/ml
	1.5 μg/ml	3.25 μg/ml	6.25 μg/ml	12.5 μg/ml	25 μg/ml	50 μg/ml	100 μg/ml	
14a	2.5	15.1	24.2	38.2	44.4	70.6	79.6	**29.7**
14b	3.6	14.3	26.3	37.7	50.9	66.2	74.8	**26.5**
14c	4.8	16.2	22.8	36.5	52.6	75.3	78.4	**23.3**
Camptothecin	6.9	18.6	27.3	39.5	60.2	80.3	91.2	**20.2**

Table 7
Molecular modeling data for the target hybrid structures **14a–l** and celecoxib in COX-2 (PDB: ID 2AW1) active sites.

Compound no	E-Score	Hydrogen bonding residues	No of H-bond	Function group
14a	−14.6	Tyr 341, Tyr 371, Ser 516	4	SO_2CH_3, C=O
14b	−16.5	Tyr 341, Tyr 371, Ser 516, Arg 106	4	SO_2CH_3, C=O
14c	−20.4	Tyr 108, Lys 68	2	OCH_3
14d	−16.8	Tyr 341, Tyr 371, Ser 516, Arg 106	4	SO_2CH_3, C=O
14e	−17.9	Lys 68	1	OCH_3
14f	−18.9	Lys 68	1	SO_2CH_3
14g	−18.9	Tyr 341, Arg 106, Lys 68	3	SO_2CH_3, C=O
14h	−18.7	Tyr 108, Arg 516, Lys 68	3	SO_2CH_3, C=O
14i	−17.6	Lys 68, Lys 68, Tyr 108	3	SO_2CH_3
14j	−18.7	Lys 68, Lys 68, Tyr108, Arg 499	4	SO_2CH_3, C=O
14k	−18.5	Tyr 101, Ser105, Lys 68	3	C=O, OCH_3
14l	−18.6	Lys 68	1	SO_2CH_3
Celecoxib	−17.6	Ser 516, Tyr 371	2	SO_2NH_2

protein or with DNA nucleobases and with two to six π-π stacking interactions with DNA base pairs either through arene-arene or arene-cation interactions when compared with topotecan (−17.13 with one Hydrogen bonding interaction with Asp-533 and two π-π stacking interactions with DNA base pairs through arene-arene interactions with deoxyadenosine DA113). It was noted that the hydrogen bonding interactions for these compounds **14a–l** were mainly through two functional groups (COX-2 pharmacophore SO_2CH_3 and OCH_3) suggesting the importance of these functional groups for binding with human topoisomerase-1 covalently joined to double-stranded DNA (see Fig. 4).

3. Conclusion

A new series of thiohydantoin analogues containing pyrazole core **14a–l** was prepared as hybrid structures to be tested for their COX inhibitory activity, AI activity, ulcerogenicity, *in vitro* cytotoxic activity and the human Topo-1 inhibitory evaluation. The obtained biological data in correlation with the structure revealed that; (i) all compounds were more potent against COX-2 than COX-1 and nine of them had equal or higher COX-2 inhibitory activity (IC_{50} = 0.56–0.84 μM range) in comparison with COX-2 selective drug celecoxib (IC_{50} = 0.84 μM), (ii) all derivatives **14a-l** showed good AI activities at all used time intervals (1, 3 and 5 h). The methoxy substituted derivatives (**14a**, **14c**, **14e**, **14g**, **14i** and **14k**) showed higher AI activities than the respective unsubstituted derivatives (**14b**, **14d**, **14f**, **14h**, **14j** and **14l**), (iii) all tested compounds were significantly less ulcerogenic (ulcer indexes = 2.64–3.87) than ibuprofen (ulcer index = 20.25) and were of acceptable ulcerogenicity when compared with the non-ulcerogenic reference drug celecoxib (ulcer index = 2.99), (iv) most of the target derivatives showed activities against A-549, MCF-7 and HCT-116 cell lines (IC_{50} = 5.32–17.90, 3.67–19.04 and 3.19–14.87 μM respectively) in comparison with doxorubicin (IC_{50} = 2.84, 2.11 and 2.44 μM respectively). Also, the methoxy substituted analogues showed higher cytotoxic activities than the respective unsubstituted derivatives, (v) compound **14a** inhibited the human Topo-1 enzymatic activity with IC_{50} = 29.7 μg/ml while **14b** and **14c** showed more potent inhibitory activity with IC_{50} = 26.5 and 23.3 μg/ml. respectively in comparison with camptothecin (IC_{50} = 20.2 μg/ml), (vi) from previous biological data in addition to molecular docking results, it can be concluded that for this type of analogues, presence of *para* substituted methoxy in addition to the COX-2 pharmacophore SO_2CH_3 is important for binding with both COX-2 and human Topo-1 and meanwhile, (vi) also, it can be concluded that the combination of different pharmacophoric groups of different biologically active compounds in one hybrid structure can be applied as an efficient strategy for the preparation of analogues with dual biological activities.

4. Experimental section

4.1. Chemistry

General: Melting points were done using a Thomas-Hoover capillary apparatus and are uncorrected. Infrared spectra were determined using films of KBr plates and a Nicolet 550 Series II Magna FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were determined using a Bruker machine 400 MHz NMR Spectrophotometer at Faculty of Pharmacy, Beni-Suef University, Egypt in $CDCl_3$ or DMSO-d_6 with TMS as the internal standard, where coupling constant (*J*) values were estimated in Hertz (Hz). Mass spectra (MS) were measured on a Water's Micromass ZQ 4000 mass spectrometer using the electro-spray (ES) ionization mode. Microanalyses for C, H and N were recorded on Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA) at the microanalytical unit, Cairo University, Egypt. All analyzed derivatives were within ± 0.4% of the theoretical values. Compounds **12a–f** [16] and **13a,b** [29] were synthesized as previously reported.

4.1.1. Experimental procedures and spectral data for compounds *14a–l*

To the appropriate aldehydes **12a–f** (0.04 mol) in 20 ml ethanol, the appropriate thiohydantoin (**13a, 13b** 0.04 mol) was added with catalytic amount of piperidine. The obtained mixture was heated under reflux for 12 h. The formed precipitate was filtered, washed with ethanol and recrystallized from ethanol to give **14a–l**. The physical and spectral data for all synthesized derivatives are listed below:

4.1.1.1. *(Z)-3-(4-Methoxyphenyl)-5-((1-(4-(methylsulfonyl)phenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl)methylene)-2-thioxoimidazolidin-4-one* (**14a**): Yield 87%; yellow solid; m.p. 222–224 °C; IR (KBr): 3110 (NH), 3022 (C—H aromatic), 2953 (C—H aliphatic),1724 (C=O), 1593 (C=N), 1342, 1143, (SO_2CH_3) cm^1; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 3.27 (s, 3H, $SO_2\underline{CH_3}$), 3.83 (s, 3H, $O\underline{CH_3}$), 6.69 (s, 1H, olefinic C—H), 7.06 (d, 2H, *J* = 8.4 Hz, 4-methoxyphenyl H-2, H-6), 7.28 (d, 2H, *J* = 8.0 Hz, methylsulfonylphenyl H-2, H-6), 8.00 (d, 2H, *J* = 8.0 Hz methylsulfonylphenyl H-3, H-5), 8.08 (d, 2H, *J* = 8.4 Hz, 4-methoxyphenyl H-3, H-5), 8.14 (d, 2H, *J* = 8.0 Hz, 4-nitro-phenyl H-2, H-6) 8.20 (d, 2H, *J* = 8.0 Hz, 4-nitro-phenyl H-3, H-5), 9.59 (s, 1H, pyrazole H-5), 12.23 (s, 1H, −NH−, D_2O exchangeable); ^{13}C NMR (DMSO- d_6 100 MHz, δ, ppm): 44.05, 55.90, 107.22, 114.51, 115.87, 119.68, 124.53, 126.16, 128.98, 129.61, 130.39, 130.46, 130.68, 138.16, 139.48, 142.42, 148.07, 152.82, 159.81, 162.49, 175.87; MS *m/z* (ES$^+$)

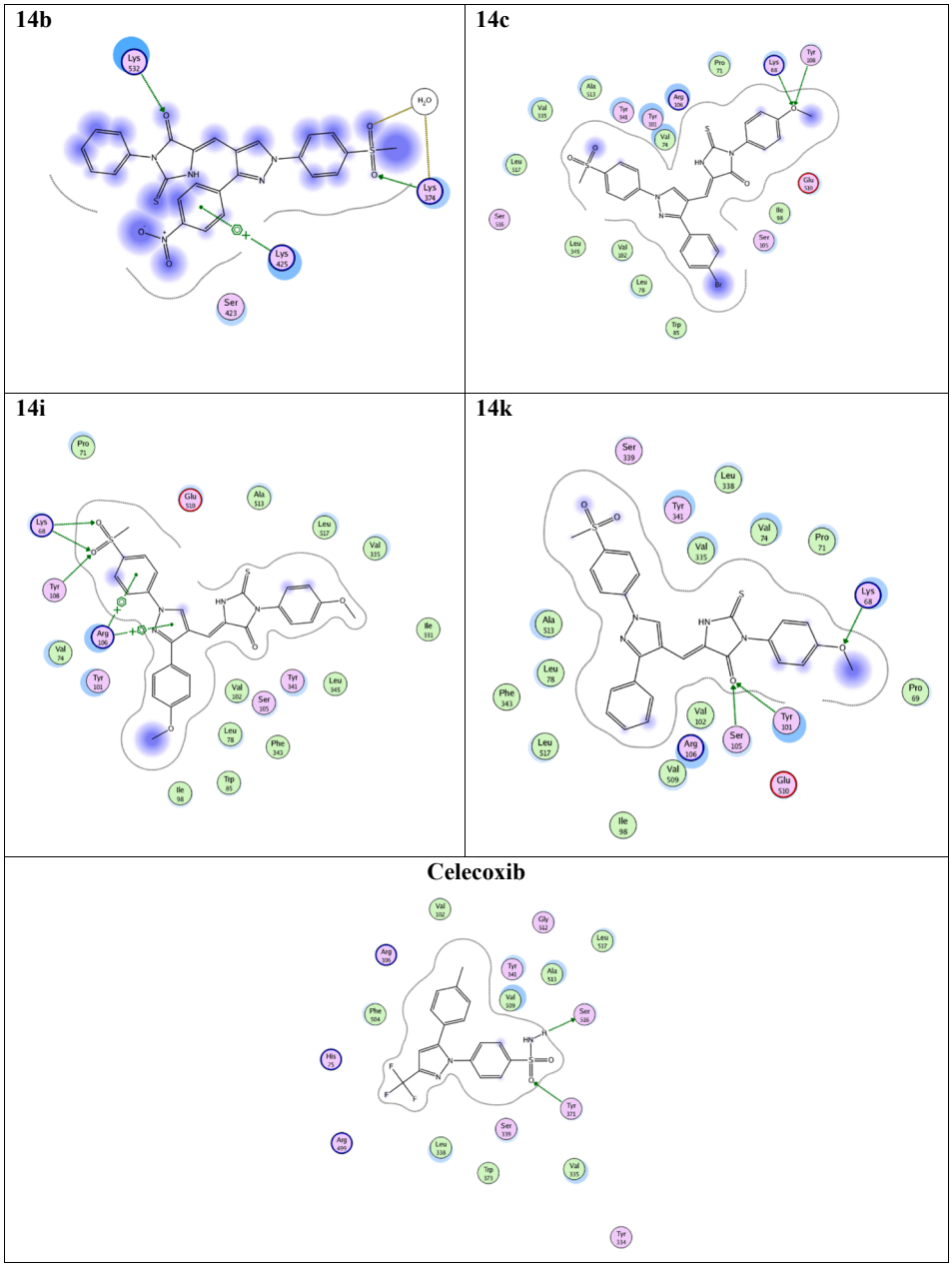


Fig. 3. Binding of compounds **14b, 14c, 14i, 14k** and celecoxib (**1**) into the active site of COX-2 as assessed by computer modeling studies.

575.62 (M+) (100%). Anal. Calcd. For $C_{27}H_{21}N_5O_6S_2$: C, 56.34; H, 3.68; N, 12.17; Found; C, 56.76; H, 3.39; N, 11.89.

4.1.1.2. *(Z)-5-((1-(4-(Methylsulfonyl)phenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl)methylene)-3-phenyl-2-thioxoimidazolidin-4-one* (**14b**): Yield 67%; yellow solid; m.p. 209–211 °C; IR (KBr): 3136 (NH), 3069 (C—H aromatic), 2926 (C—H aliphatic), 1735 (C=O), 1594 (C=N), 1342, 1147, (SO_2CH_3) cm^1; ^1H NMR (DMSO-d_6, 400 MHz, δ, ppm): 3.30 (s, 3H, $SO_2\underline{CH_3}$), 6.55 (s, 1H, olefinic C—H), 7.35 (d, 2H, J = 8.4 Hz, phenyl H-2, H-6), 7.48 (d, 2H, J = 8.0 Hz, methylsulfonylphenyl H-2, H-6), 8.02 (d, 2H, J = 8.0 Hz methylsulfonylphenyl H-3, H-5), 8.18 (m, 3H, phenyl H-3, H-4, H-5), 8.28 (d, 2H, J = 8.0 Hz, 4-nitro-phenyl H-2, H-6) 8.42 (d, 2H, J = 8.0 Hz, 4-nitro-phenyl H-3, H-5), 9.52 (s, 1H, pyrazole H-5), 12.27 (s, 1H, —NH—, D_2O exchangeable); ^{13}C NMR (DMSO- d_6 100 MHz, δ, ppm): 44.05, 107.12, 114.31, 115.66, 119.35, 124.65, 125.86, 128.98, 129.26, 130.06, 130.56, 130.68, 137.16, 139.55, 142.22, 147.07, 148.22, 159.31, 161.22, 175.67; MS m/z (ES^+) 545.59 (M+) (100%). Anal. Calcd. For $C_{26}H_{19}N_5O_5S_2$: C, 57.24; H, 3.51; N, 12.84; Found; C, 57.36; H, 3.44; N, 12.73.

4.1.1.3. *(Z)-5-((3-(4-Bromophenyl)-1-(4-(methylsulfonyl)phenyl)-1H-pyrazol-4-yl)methylene)-3-(4-methoxyphenyl)-2-thioxoimidazolidin-4-one* (**14c**): Yield 88%; yellow solid; m.p. 215–217 °C; IR (KBr): 3109 (NH), 3022 (C—H aromatic), 2925 (C—H aliphatic), 1702 (C=O), 1536 (C=N), 1375, 1142, (SO_2CH_3) cm^1; ^1H NMR (DMSO-d_6, 400 MHz, δ, ppm): 3.17 (s, 3H, $SO_2\underline{CH_3}$), 3.81 (s, 3H, $O\underline{CH_3}$), 6.67 (s, 1H, olefinic C—H), 7.04 (d, 2H, J = 8.0 Hz, 4-methoxy-phenyl H-2, H-6), 7.28 (d, 2H, J = 8.4 Hz, methylsulfonylphenyl H-2, H-6), 7.65 (d, 2H, J = 8.0 Hz 4-methoxy-phenyl H-3, H-5), 7.79 (d, 2H, J = 8.4 Hz, methylsulfonylphenyl H-3, H-5), 8.07 (d, 2H, J = 8.4 Hz, 4-bromo-phenyl H-2, H-6), 9.59 (s, 1H, pyrazole H-5), 12.25 (s, 1H, —NH—, D_2O exchangeable); ^{13}C NMR (DMSO- d_6 100 MHz, δ, ppm): 44.07, 55.83, 107.88, 114.51, 115.51, 119.41, 119.52, 123.17, 126.21, 129.49, 129.59, 130.32, 130.47, 131.35, 132.39, 132.48, 139.23, 142.54, 142.11, 159.80, 175.58; MS m/z (ES^+) 609.51 (M+) (100%). Anal. Calcd. For $C_{27}H_{21}BrN_4O_4S_2$: C, 53.20; H, 3.47; N, 9.19; Found; C, 53.43; H, 3.39; N, 8.89.

4.1.1.4. *(Z)-5-((3-(4-Bromophenyl)-1-(4-(methylsulfonyl)phenyl)-1H-pyrazol-4-yl)methylene)-3-phenyl-2-thioxoimidazolidin-4-one* (**14d**): Yield

Table 8
Molecular modeling data for the target hybrid structures **14a–l** and topotecan (**7**) in Topo-1 (PDB: ID 1K4T) active sites.

Compound no.	Score of energy (kcal/mol)	Interacting residues	Residues forming H-bond& type of hydrogen bond	Distance (Å)	Residues of other interactions
Topotecan	−17.13	DT10, TGP11, DC112, DA113, Arg364, Asp533, Thr18	ASP533 (H-donn)	2.14	2-arene-arene interaction with DA113
14a	−14.86	DT10, TGP11, DC112, DA113, DG12, Arg364, Asp533, Lys751, Ile535, Leu721, Asn722, Pro431	Lys751 (H-donn) TGP11 (H-ace)	2.50 1.94	2-arene-arene interaction with DA113& TGP11
14b	−14.28	DT10, TGP11, DC112, DA113, Arg364, Glu356, Trp416, Tyr426	Arg364 (H-ace)	2.31	3-arene-arene interaction 2 with DA113&1 with TGP11 1-arene-cation interaction with Arg364
14c	15.21	DT10, TGP11, DC112, DA113, Arg364, Asp533, Ile427, Asn352, Thr426, Thr718, Ala351, Lys532,	Met 428 (H-ace) Thr718 (H-ace)	2.84 2.80	3-arene-arene interaction 2with DA113& 1with TGP11
14d	−15.27	DT10, TGP11, DC112, DA113, DG12, Arg364, Asp533, Leu721, Asn722, Glu356	Arg364 (H-ace)	2.66	2-arene-arene interaction 2 with DA113 & 1-arene-cation interaction with Arg364
14e	−15.53	DT10, TGP11, DC112, DA113, DG12, Arg364, Asp533, Leu721, Asn722, Ile535, Lys751, Pro431	Arg364 (H-acc) Lys751 (H-ace)	2.63 2.61	2-arene-arene interaction with DA113 & 1-arene-cation interaction with Arg364
14f	−15.20	DT10, TGP11, DC112, DA113, Arg364, Asp533, Asn352, Lys532, Glu356, Tyr426, Thr718, Trp416	Lys532 (H-acc)	2.87	3-arene-arene interaction 1 with DA113 & 2 with TGP11
14g	−15.81	DT10, TGP11, DC112, DA113, Arg364, Asp533, Asn352, Lys532, Lys374, Lys751, Glu356, Leu721, Pro431	TGP11 (H-don) Lys374 (H-ace)	2.17 2.62	3-arene-arene interaction 2 with DA113 & 1 with TGP11
14h	−14.80	DT10, TGP11, DC112, DA113, DG12, Arg364, Asp533, Asn722, Lys751, Glu356, Leu721, Pro431, Ile535	TGP11 (H-don) Lys751 (H-ace)	1.98 2.53	2-arene-arene interaction with DA113 & TGP11
14i	−14.10	DT10, TGP11, DC112, DA113, DG12, Arg364, Asp533, Glu356, Ile535, His632, Gln633	–	–	4-arene-arene interaction 2 with DA113 & 2 with TGP11
14j	−14.00	DT10, TGP11, DC112, DA113, DG12, Arg364, Asp533, Asn722, Ile535, Thr718, Glu356, His632, Lys751, Leu721, Pro431	Lys575 (H-ace)	3.40	2-arene-arene interaction with DA113 & TGP11
14k	−13.79	DT10, TGP11, DC112, DA113, DG12, Arg364, Arg488, Asp533, Asn722, Asn531, Ile535, Thr718, Glu356, His632	–	–	2-arene-arene interaction with DA113 & TGP11
14l	−14.58	DT10, TGP11, DC112, DA113, DT9, Arg364, Asp533, Asn532, Lys532, PTR723, Ala351, Leu429, Met428, Thr718	DT10 (H-donn)	1.71	3-arene-arene interaction 2 with DA113 & 1 with TGP11

81%; yellow solid; mp 175–177 °C; IR (KBr): 3150 (NH), 3039 (C−H aromatic), 2926 (C−H aliphatic), 1720 (C═O), 1632 (C═N), 1318, 1145, (SO_2CH_3) cm^1; ^1H NMR (DMSO-d_6, 400 MHz, δ, ppm): 3.25 (s, 3H, $SO_2\underline{CH_3}$), 6.31 (s, 1H, olefinic C−H), 7.32 (d, 2H, J = 8.4 Hz, phenyl H-2, H-6), 7.45 (d, 2H, J = 8.0 Hz, methylsulfonylphenyl H-2, H-6), 7.69 (d, 2H, J = 8.0 Hz methylsulfonylphenyl H-3, H-5), 7.78 (m, 3H, phenyl H-3, H-4, H-5), 8.13 (d, 2H, J = 8.0 Hz, 4-bromo-phenyl H-2, H-6) 8.19 (d, 2H, J = 8.0 Hz, 4-bromo-phenyl H-3, H-5), 9.52 (s, 1H, pyrazole H-5), 12.27 (s, 1H, −NH−, D_2O exchangeable); ^{13}C NMR (DMSO- d_6 100 MHz, δ, ppm): 44.22, 104.88, 119.38, 124.55, 129.11, 129.61, 134.41, 138.26, 139.58, 139.55, 142.22, 142.88, 147.07 147.38, 148.22, 150.41, 151.22, 155.23, 159.55, 178.13; MS m/z (ES^+) 579.49 (M+) (100%). Anal. Calcd. For $C_{26}H_{19}BrN_4O_3S_2$: C, 53.89; H, 3.30; N, 9.67; Found; C, 53.76; H, 3.19; N, 9.79.

4.1.1.5. *(Z)-5-((3-(4-Chlorophenyl)-1-(4-(methylsulfonyl)phenyl)-1H-pyrazol-4-yl)methylene)-3-(4-methoxyphenyl)-2-thioxoimidazolidin-4-one* (**14e**): Yield 81%; yellow solid; mp 198–200 °C; IR (KBr): 3244 (NH), 3107 (C−H aromatic), 2929 (C−H aliphatic), 1718 (C═O), 1537 (C═N), 1375, 1143, (SO_2CH_3) cm^1; ^1H NMR (DMSO-d_6, 400 MHz, δ, ppm): 3.26 (s, 3H, $SO_2\underline{CH_3}$), 3.84 (s, 3H, $O\underline{CH_3}$), 6.69 (s, 1H, olefinic C−H), 7.06 (d, 2H, J = 8.0 Hz, 4-methoxy-phenyl H-2, H-6), 7.29 (d, 2H, J = 8.4 Hz, methylsulfonylphenyl H-2, H-6), 7.66 (d, 2H, J = 8.0 Hz 4-methoxy-phenyl H-3, H-5), 7.73 (d, 2H, J = 8.4 Hz, methylsulfonyl-phenyl H-3, H-5), 8.07 (d, 2H, J = 8.4 Hz, 4-chloro-phenyl H-2, H-6), 8.12 (d, 2H, J = 8.4 Hz, 4-chloro-phenyl H-3, H-5) 9.59 (s, 1H, pyrazole H-5), 12.10 (s, 1H, −NH−, D_2O exchangeable); ^{13}C NMR (DMSO- d_6 100 MHz, δ, ppm): 44.11, 55.92, 107.91, 114.54, 115.52, 119.55, 126.21, 128.30, 129.22, 129.45, 129.54, 130.44, 131.07, 134.50, 139.31, 142.56, 154.06, 159.84, 159.93, 162.44, 175.59; MS m/z (ES^+) 565.06 (M+) (100%). Anal. Calcd. For $C_{27}H_{21}ClN_4O_4S_2$: C, 57.39; H, 3.75; N, 9.92; Found; C, 57.43; H, 3.49; N, 9.89.

4.1.1.6. *(Z)-5-((3-(4-Chlorophenyl)-1-(4-(methylsulfonyl)phenyl)-1H-pyrazol-4-yl)methylene)-3-phenyl-2-thioxoimidazolidin-4-one* (**14f**): Yield 71%; white solid; mp 233–235 °C; IR (KBr): 3428 (NH), 3111(C−H aromatic), 2927 (C−H aliphatic), 1719 (C═O), 1536 (C═N), 1373, 1146, (SO_2CH_3) cm^1; ^1H NMR (DMSO-d_6, 400 MHz, δ, ppm): 3.28 (s, 3H, $SO_2\underline{CH_3}$), 6.71 (s, 1H, olefinic C−H), 7.40 (d, 2H, J = 8.0 Hz, phenyl H-2, H-6), 7.49 (m, 3H, phenyl H-3, H-4, H-5), 7.66 (d, 2H, J = 8.4 Hz, methylsulfonylphenyl H-2, H-6), 7.73 (d, 2H, J = 8.4 Hz, methylsulfonylphenyl H-3, H-5), 8.07 (d, 2H, J = 8.4 Hz, 4-chloro-phenyl H-2, H-6), 8.13 (d, 2H, J = 8.4 Hz, 4-chloro-phenyl H-3, H-5) 9.59 (s, 1H, pyrazole H-5), 12.42 (s, 1H, −NH−, D_2O exchangeable); ^{13}C NMR (DMSO- d_6 100 MHz, δ, ppm): 44.11, 55.91, 108.91, 114.83, 115.62, 119.59, 126.28, 128.30, 129.32, 129.47, 129.84, 130.39,

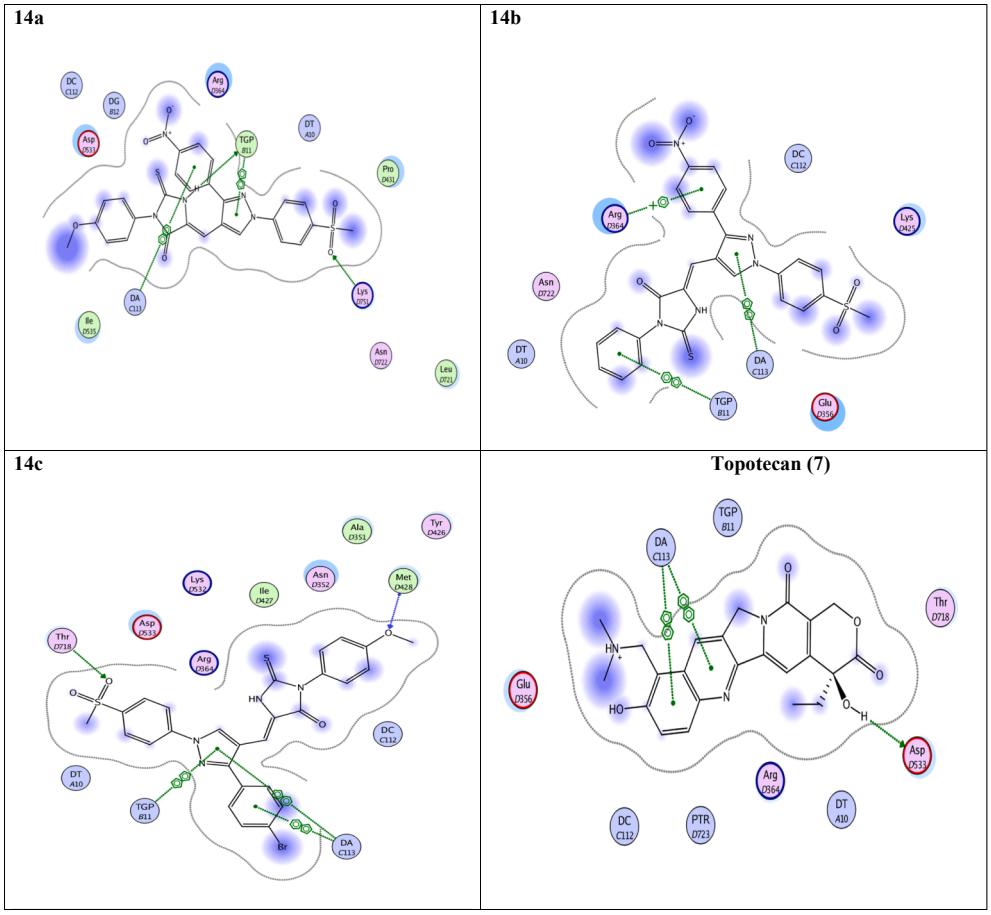


Fig. 4. Binding of compounds **14a**, **14b**, **14c** and topotecan (**7**) into the active site of Topo-1 as assessed by computer modeling studies showing the π-π stacking interactions of the compounds **14a–c** and the Topotecan 7 with the base pairs of DNA and hydrogen bod interaction with amino acids of protien.

131.10, 134.70, 138.31, 141.56, 154.06, 159.44, 159.93, 162.34, 175.51; MS *m/z* (ES^+) 535.04 (M+) (100%). Anal. Calcd. For $C_{26}H_{19}ClN_4O_3S_2$: C, 58.37; H, 3.58; N, 10.47; Found; C, 58.43; H, 3.49; N, 10.18.

4.1.1.7. *(Z)-3-(4-Methoxyphenyl)-5-((3-(4-methoxyphenyl)-1-(4-(methylsulfonyl)phenyl)-1H-pyrazol-4-yl)methylene)-2-thioxoimidazolidin-4-one* (**14g**): Yield 65%; yellow solid; mp 244–246 °C; IR (KBr): 3421 (NH), 3107 (C—H aromatic), 2924 (C—H aliphatic), 1717 (C═O), 1537 (C═N), 1374, 1143, (SO_2CH_3) cm^1; ^1H NMR (DMSO-d_6, 400 MHz, δ, ppm): 3.26 (s, 3H, $SO_2\underline{CH_3}$), 3.81 (s, 3H, $O\underline{CH_3}$), 3.85 (s, 3H, $O\underline{CH_3}$) 6.72 (s, 1H, olefinic C—H), 7.06 (d, 2H, *J* = 8.0 Hz, 4-methoxy-phenyl H-2, H-6), 7.15 (d, 2H, *J* = 8.0 Hz, 4-methoxy-phenyl H-2, H-6), 7.29 (d, 2H, *J* = 8.0 Hz 4-methoxy-phenyl H-3, H-5), 7.63 (d, 2H, *J* = 8.0 Hz 4-methoxy-phenyl H-3, H-5), 8.06 (d, 2H, *J* = 8.4 Hz, methylsulfonyl-phenyl H-2, H-6), 8.14 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl H-3, H-5), 9.60 (s, 1H, pyrazole H-5), 12.24 (s, 1H, —NH—, D_2O exchangeable); ^{13}C NMR (DMSO- d_6 100 MHz, δ, ppm): 44.11, 55.92, 55.95, 106.91, 112.54, 114.55, 118.21, 125.28, 127.32, 128.22, 129.45, 129.64, 130.44, 131.55, 134.30, 138.38, 142.56, 153.02, 158.81, 159.93, 161.44, 175.09; MS *m/z* (ES^+) 560.64 (M+) (100%). Anal. Calcd. For $C_{28}H_{24}N_4O_5S_2$: C, 59.98; H, 4.31; N, 9.99; Found; C, 59.55; H, 4.66; N, 10.33.

4.1.1.8. *(Z)-5-((3-(4-Methoxyphenyl)-1-(4-(methylsulfonyl)phenyl)-1H-pyrazol-4-yl)methylene)-3-phenyl-2-thioxoimidazolidin-4-one* (**14h**): Yield 71%; yellow solid; m.p. 247–249 °C; IR (KBr): 3325 (NH), 3114(C—H aromatic), 2911 (C—H aliphatic), 1742 (C═O), 1527 (C═N), 1403, 1147, (SO_2CH_3) cm^1; ^1H NMR (DMSO-d_6, 400 MHz, δ, ppm): 3.26 (s, 3H, $SO_2\underline{CH_3}$), 3.81 (s, 3H, $O\underline{CH_3}$), 3.82 (s, 3H, $O\underline{CH_3}$) 6.51 (s, 1H, olefinic C—H), 7.13 (d, 2H, *J* = 8.0 Hz, 4-methoxy-phenyl H-2, H-6), 7.38 (d, 2H, *J* = 8.0 Hz, 4-methoxy-phenyl H-2, H-6), 7.51 (m, 3H, phenyl H-3, H-4, H-5), 7.63 (d, 2H, *J* = 8.0 Hz 4- phenyl H-2, H-6), 8.15 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl H-2, H-6), 8.21 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl H-3, H-5), 9.46 (s, 1H, pyrazole H-5), 12.32 (s, 1H, —NH—, D_2O exchangeable); ^{13}C NMR (DMSO- d_6 100 MHz, δ, ppm): 44.16, 55.79, 102.94, 114.95, 118.64, 119.31, 123.96, 126.83, 129.21, 129.27, 129.40, 129.97, 130.38, 133.83, 139.03, 142.83, 154.44, 160.50, 163.99, 178.28; MS *m/z* (ES^+) 530.62 (M +) (100%). Anal. Calcd. For $C_{27}H_{22}N_4O_4S_2$: C, 61.12; H, 4.18; N, 10.56; Found; C, 61.45; H, 4.36; N, 10.84.

4.1.1.9. *(Z)-N-(4-(4-((1-(4-Methoxyphenyl)-5-oxo-2-thioxoimidazolidin-4-ylidene)methyl)-1-(4-(methylsulfonyl)phenyl)-1H-pyrazol-3-yl)phenyl)methanesulfonamide* (**14i**): Yield 83%; yellow solid; mp 266–268 °C; IR (KBr): 3445 (—$NHSO_2CH_3$), 3222 (NH), 3150 (C—H aromatic), 2924 (C—H aliphatic), 1743 (C═O), 1544 (C═N), 1398, 1148, (SO_2CH_3) cm^1; ^1H NMR (DMSO-d_6, 400 MHz, δ, ppm): 2.63 (s, 3H, $NHSO_2\underline{CH_3}$), 3.24 (s, 3H, $SO_2\underline{CH_3}$), 3.31 (s, 1H, $\underline{NH}SO_2CH_3$, D_2O exchangeable), 3.56 (s, 3H, $O\underline{CH_3}$), 6.72 (s, 1H, olefinic C—H), 7.60 (d, 2H, *J* = 8.0 Hz, 4-methoxy-phenyl H-2, H-6), 7.73 (d, 2H, *J* = 8.0 Hz 4-methoxy-phenyl H-3, H-5), 7.95 (d, 2H, *J* = 8.0 Hz, 4-aminomethanesulfonyl-phenyl H-2, H-6), 7.97 (d, 2H, *J* = 8.0 Hz 4-aminomethanesulfonyl-phenyl H-3, H-5), 8.01 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl H-2, H-6), 8.03 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl H-3, H-5), 9.65 (s, 1H, pyrazole H-5), 12.21 (s, 1H, —NH—, D_2O exchangeable); ^{13}C NMR (DMSO- d_6 100 MHz, δ, ppm): 15.32, 27.33, 43.60, 54.22, 54.90, 105.91, 111.54, 113.11, 118.26, 124.28, 127.32, 128.22, 129.41, 129.65, 129.97, 131.47, 131.73, 135.48, 139.74, 142.56, 156.91, 160.44, 175.19; MS *m/z* (ES^+) 623.72 (M+) (100%). Anal. Calcd. For $C_{28}H_{25}N_5O_6S_3$: C, 53.92; H, 4.04; N, 11.23; Found; C, 53.88; H, 3.96; N, 11.33.

4.1.1.10. *(Z)-N-(4-(1-(4-(Methylsulfonyl)phenyl)-4-((5-oxo-1-phenyl-2-thioxoimidazolidin-4-ylidene)methyl)-1H-pyrazol-3-yl)phenyl)methanesulfonamide* (**14j**): Yield 68%; yellow solid; mp 206–208 °C; IR (KBr): 3316 (-$NHSO_2CH_3$), 3229 (NH), 3111 (C—H aromatic), 2927 (C—H aliphatic), 1723 (C=O), 1540 (C=N), 1401, 1143, (SO_2CH_3) cm^1; ^1H NMR (DMSO-d_6, 400 MHz, δ, ppm): 3.24 (s, 3H, $NHSO_2\underline{CH_3}$), 3.29 (s, 3H, $SO_2\underline{CH_3}$), 3.31 (s, 1H, $\underline{NH}SO_2CH_3$, D_2O exchangeable), 6.54 (s, 1H, olefinic C—H), 7.36 (d, 2H, *J* = 8.0 Hz, phenyl H-2, H-6), 7.42 (m, 3H, phenyl H-3, H-4, H-5), 7.52 (d, 2H, *J* = 8.0 Hz, 4-aminomethanesulfonyl-phenyl H-2, H-6), 7.68 (d, 2H, *J* = 8.0 Hz 4-aminomethanesulfonyl-phenyl H-3, H-5), 8.16 (d, 2H, *J* = 8.4 Hz, methylsulfonyl-phenyl H-2, H-6), 8.25 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl H-3, H-5), 9.47 (s, 1H, pyrazole H-5), 12.26 (s, 1H, —NH—, D_2O exchangeable); ^{13}C NMR (DMSO- d_6 100 MHz, δ, ppm): 27.33, 43.60, 55.14, 55.87, 105.61, 111.34, 112.19, 118.12, 124.36, 126.12, 128.22, 129.66, 129.95, 129.97, 131.73, 139.74, 141.56, 156.85, 160.30, 174.88; MS *m/z* (ES^+) 593.70 (M+) (100%). Anal. Calcd. For $C_{27}H_{23}N_5O_5S_3$: C, 54.62; H, 3.90; N, 11.80; Found; C, 54.63; H, 3.56; N, 11.48.

4.1.1.11. *(Z)-3-(4-Methoxyphenyl)-5-((1-(4-(methylsulfonyl)phenyl)-3-phenyl-1H-pyrazol-4-yl)methylene)-2-thioxoimidazolidin-4-one* (**14k**): Yield 73%; yellow solid; mp 266–268 °C; IR (KBr): 3329 (NH), 3150 (C—H aromatic), 2939 (C—H aliphatic), 1720 (C=O), 1536 (C=N), 1372, 1145, (SO_2CH_3) cm^1; ^1H NMR (DMSO-d_6, 400 MHz, δ, ppm): 3.30 (s, 3H, $SO_2\underline{CH_3}$), 3.82 (s, 3H, $O\underline{CH_3}$), 6.42 (s, 1H, olefinic C—H), 7.02 (d, 2H, *J* = 8.0 Hz, phenyl H-2, H-6), 7.25 (d, 2H, *J* = 8.0 Hz, 4-methoxy-phenyl H-2, H-6), 7.55 (m, 3H, phenyl H-3, H-4, H-5), 7.71 (d, 2H, *J* = 8.0 Hz 4-methoxy-phenyl H-3, H-5), 8.15 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl H-2, H-6), 8.21 (d, 2H, *J* = 8.4 Hz, methylsulfonyl-phenyl H-3, H-5), 9.46 (s, 1H, pyrazole H-5), 12.20 (s, 1H, —NH—, D_2O exchangeable); ^{13}C NMR (DMSO- d_6 100 MHz, δ, ppm): 43.11, 55.43, 55.95, 106.73, 112.34, 114.37, 119.35, 125.58, 126.82, 127.22, 129.02, 129.43, 129.90, 130.29, 135.30, 137.58, 139.02, 142.88, 160.44, 176.04; MS *m/z* (ES^+) 530.62 (M^+) (100%). Anal. Calcd. For $C_{27}H_{22}N_4O_4S_2$: C, 61.12; H, 4.18; N, 10.56; Found; C, 61.45; H, 4.28; N, 10.71.

4.1.1.12. *(Z)-5-((1-(4-(Methylsulfonyl)phenyl)-3-phenyl-1H-pyrazol-4-yl)methylene)-3-phenyl-2-thioxoimidazolidin-4-one* (**14 l**)**:** Yield 69%; yellow solid; mp 251–253 °C; IR (KBr): 3426 (NH), 3151 (C—H aromatic), 2924 (C—H aliphatic), 1724 (C=O), 1536 (C=N), 1372, 1145, (SO_2CH_3) cm^1; ^1H NMR (DMSO-d_6, 400 MHz, δ, ppm): 3.28 (s, 3H, $SO_2\underline{CH_3}$), 6.45 (s, 1H, olefinic C—H), 7.36 (d, 2H, *J* = 8.0 Hz, phenyl H-2, H-6), 7.46 (m, 3H, phenyl H-3, H-4, H-5), 7.58 (m, 3H, phenyl H-3, H-4, H-5), 7.71 (d, 2H, *J* = 8.0 Hz phenyl H-2, H-6), 8.15 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl H-2, H-6), 8.22 (d, 2H, *J* = 8.4 Hz, methylsulfonylphenyl H-3, H-5), 9.47 (s, 1H, pyrazole H-5), 12.24 (s, 1H, —NH—, D_2O exchangeable); ^{13}C NMR (DMSO- d_6 100 MHz, δ, ppm): 44.16, 55.35, 106.43, 111.88, 115.37, 117.95, 119.38, 126.82, 127.22, 129.09, 129.44, 129.97, 134.33, 136.77, 139.02, 142.88, 161.44, 175.74; MS *m/z* (ES^+) 500.59 (M^+) (100%). Anal. Calcd. For $C_{26}H_{20}N_4O_3S_2$: C, 62.38; H, 4.03; N, 11.19; Found; C, 62.65; H, 4.39; N, 11.41.

4.2. Biological evaluation

4.2.1. Ant-inflammatory

4.2.1.1. In vitro cyclooxygenase inhibition assay. An enzyme immuno assay (EIA) kit (catalog no. 560131, Cayman Chemical, Ann Arbor, MI, USA) was used for determination the ability of the synthesized derivatives **14a–l** and the reference drug celecoxib to inhibit ovine COX-1 and human recombinant COX-2 (IC_{50} value, μM) according to the previously reported procedure (Table 1) [30].

4.2.1.2. In vivo anti-inflammatory assay. All synthesized derivatives **14a–l** and celecoxib as a reference drug were evaluated using the *in vivo* carrageenan-induced rat foot paw edema model (50 mg/kg) and the paw thickness was measured after 1, 3 and 5 h of carageenan injection as reported before. Additionally, the dose causing edema inhibition to half thickness (ED_{50}) was determined for the most potent AI analogues **14a**, **14b**, **14c, 14i** and **14k** in comparison to celecoxib [31]

4.2.1.3. Ulcerogenic liability. Ulcerogenicity of five compounds **14a**, **14b**, **14c, 14i** and **14k** (showed the most *in vivo* AI activity) in comparison with the non-ulcerogenic drug celecoxib (50 mg/kg) and ibuprofen (120 μmol/kg) was determined using the reported method [32].

4.2.2. Anti-cancer activity

4.2.2.1. Cell viability analysis. All the target derivatives **14a–l** were evaluated *in vitro* for their anti-proliferative activity against three different cancer cell lines, breast carcinoma (MCF-7), Non-Small Cell Lung Cancer (A-549) and human colon cancer (HCT-116**)** cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay as previously reported technique [33]. In brief, cells were seeded into 96-well tissue culture plates in DMEM containing 10% FBS to a final volume of 0.2 ml. The cells were subjected to different treatments after 24 h of seeding. The cells were then incubated for 48 h with doxorubicin as a positive control, test drugs or vehicle (DMSO). The media were then removed, replaced by 200 ml DMEM containing 0.5 mg/ml of MTT and cells were incubated for 2 h. Next, the supernatants were removed and the precipitated formazan was dissolved by adding 200 μl of DMSO. Absorbance at 570 nm was determined using a micro plate reader (Model 450 Micro plate Reader; Bio-Rad). Results were calculated by subtracting blank readings (Table 5).

4.2.2.2. Human Topoisomerase-1 inhibitory assay. The inhibitory activity of the synthesized compounds **14a–c** to human Topo1 was analyzed by human DNA Topoisomerase-1 assay kit (Profoldin, Hudson, MA, USA) with recombinant human DNA Topoisomerase-1 (Sigma-Aldrich, St, Louis, MO, USA) following the manufacturer's instructions.

The procedure was done by dissolving the reaction mixtures containing samples (serial dilutions of the tested compounds in 0.1% DMSO, 25 μg/ml super coiled DNA and 20 unit/ml human Topoisomerase- 1 (using 0.1% DMSO as a negative control and serial dilutions of Camptothecin (Abcam, Cambridge, UK) as a positive control). The reaction mixtures were incubated at 25 °C for 60 min. then, H19 dye was added followed by incubation for further 5 min. The super coiled DNA, Human Topoisomerase-1 and H19 dye were supplied with the assay kit (Profoldin), fluorescence intensity at 535 nm following excitation at 485 nm was measured by ARVOsx multiple counter (Perkin-Elmer, Downers grove II, USA). The inhibitory percentage was calculated as the fluorescence intensity without inhibitor being 0% and no enzyme 100% (Table 6) [26].

4.3. Molecular modeling

For both enzymes COX-2 and human Topo-1, The crystal structure of the reference drug celecoxib bound at the COX-2 active site (obtained from protein data bank at Research Collaboration for Structural Bioinformatics (RSCB) protein database [PDB] (entry 3LN1) [34]. And human Topo-1 (PDB ID: 1K4T) covalently joined to double-stranded DNA and bound to the clinically approved anticancer agent Topotecan. (Camptothecin derivative) were used in this work [25]. The preparation of the target derivatives for docking was done *via* their 3D structure built by MOE. Before docking, 3D protonation of the structures, running conformational analysis using systemic search, selecting the least energetic conformer and applying the same docking protocol used with ligand were done.

4.4. Statistical analysis

Significant difference among groups was assessed using two way ANOVA followed by Post hoc tukey. The results were expressed as mean ± standard error (SE).

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Declaration of Competing Interest

The authors have declared no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103132.

References

[1] M. De, K. Jessica, D.L. Glycinamide ribonucleotide transformylase (GAR TFase) as a target for cancer therapy, Boger, Drugs Future 33 (2008) 969–979.
[2] C.S.A. Kumar, S.N. Swamy, N.R. Thimmegowda, S.B.B. Prasad, G.W. Yip, K.S. Rangappa, Synthesis and evaluation of 1-benzhydryl-sulfonyl-piperazine derivatives as inhibitors of MDA-MB-231 human breast cancer cell proliferation, Med. Chem. Res. 16 (2007) 179–187.
[3] T.A. Chan, Nonsteroidal anti-inflammatory drugs, apoptosis, and colon-cancer chemoprevention, Lancet Oncol. 3 (2002) 166–174.
[4] M.J. Thun, S.J. Henley, C. Patrono, Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues, J. Natl. Cancer Inst. 94 (2002) 252–266.
[5] G. Gasparini, R. Longo, R. Sarmiento, A. Morabito, Inhibitors of cyclo-oxygenase 2: a new class of anticancer agents? Lancet Oncol. 4 (2003) 605–615; M.I. Patel, K. Subbaramaiah, B. Du, M. Chang, P. Yang, R.A. Newman, C. Cordon- Cardo, H.T. Thaler, A.J. Dannenberg, Celecoxib inhibits prostate cancer growth: evidence of a cyclooxygenase-2-independent mechanism, Clin. Cancer Res. 11 (2005) 1999–2007.
[6] M.I. Patel, K. Subbaramaiah, B. Du, M. Chang, P. Yang, R.A. Newman, C. Cordon-Cardo, H.T. Thaler, A.J. Dannenberg, Celecoxib inhibits prostate cancer growth: evidence of a cyclooxygenase-2-independent mechanism, Clin. Cancer Res. 11 (2005) 1999–2007.
[7] G.P. Nagaraju, B.F. El-Rayes, Cyclooxygenase-2 in gastrointestinal malignancies, Cancer (2019) 1–7.
[8] K. Ando, K. Kawamura, Sulfamoylheleroaryl pyrazole compounds as anti-inflammatory/analgesic agents, Google Patents (2003).
[9] M. Amir, S. Kumar, Synthesis and anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation activities of 3, 5-dimethyl pyrazoles, 3-methyl pyrazol-5-ones and 3,5-disubstituted pyrazolines, Indian J. Chem. 44B (2005) 2532–2537.
[10] A.A. Farghaly, A.A. Bekhit, J. Young Park, Design and synthesis of someoxadiazolyl, thiadiazolyl, thiazolidinyl, and thiazolyl derivatives of 1Hpyrazoleas anti-inflammatory antimicrobial agents, Arch. Pharm. 333 (2000) 53–57.
[11] R.B. Bakr, A.A. Azouz, K.R. Abdellatif, Synthesis, cyclooxygenase inhibition, anti-inflammatory evaluation and ulcerogenic liability of new 1-phenylpyrazolo [3, 4-d] pyrimidine derivatives, J. Enzyme Inhib. Med. Chem. 31 (2016) 6–12.
[12] K. Abdellatif, E. Abdelall, R. Bakr, Nitric oxide-NASIDS donor prodrugs as hybrid safe anti-inflammatory agents, Curr. Top. Med. Chem. 17 (2017) 941–955.
[13] I. Bouabdallah, L.A. M' Barek, A. Zyad, A. Ramdani, I. Zidane, A. Melhaoui, Anticancer effect of three pyrazole derivatives, Nat. Product Res. 20 (2006) 1024–1030.
[14] G.M. Nitulescu, C. Draghici, A.V. Missir, Synthesis of new pyrazole derivatives and their anticancer evaluation, Eur. J. Med. Chem. 45 (2010) 4914–4919.
[15] H. Kumar, D. Saini, S. Jain, N. Jain, Pyrazole scaffold: a remarkable tool in the development of anticancer agents, Eur. J. Med. Chem. 70 (2013) 248–258.
[16] K.R.A. Abdellatif, W.A.A. Fadaly, G.M. Kamel, Y.A.M.M. Elshaier, M.A. El-Magd, Design, synthesis, modeling studies and biological evaluation of thiazolidine derivatives containing pyrazole core as potential anti-diabetic PPAR-γ agonists and anti-inflammatory COX-2 selective inhibitors, Bioorg Chem. 82 (2019) 568–578.
[17] K.R.A. Abdellatif, W.A.A. Fadaly, Y.A.M.M. Elshaier, W.A.M. Ali, G.M. Kamel, Non-acidic 1,3,4-trisubstituted-pyrazole derivatives as lonazolac analogs with promising COX-2 selectivity, anti-inflammatory activity and gastric safety profile, Bioorg Chem. 77 (2018) 568–578.
[18] J.J. Champoux, DNA topoisomerases: structure, function, and mechanism, Annu. Rev. Biochem. 70 (2001) 369–413.
[19] J.C. Wang, DNA topoisomerases, Annu. Rev. Biochem. 65 (1996) 635–692.
[20] Z. Topcu, DNA topoisomerases as targets for anticancer drugs, J. Clin. Pharma. Ther. 26 (2001) 405–416.
[21] J.C. Wang, Cellular role of DNA topoisomerases: A molecular perspective, Nat. Rev. Mol. Cell Biol. 3 (2002) 430–440.
[22] Y. Pommier, Topoisomerase I inhibitors: camptothecins and beyond, Nat. Rev. Cancer 6 (2006) 789–802.
[23] Y.H. Hsiang, R. Hertzberg, S. Hecht, L.F. Liu, Camptothecin induces protein linked DNA breaks via mammalian DNA topoisomerase I, J. Biol. Chem. 260 (1985) 14873–14878.
[24] Y. Pommier, Drugging topoisomerases: lessons and challenges, ACS Chem. Biol. 8 (2013) 82–95.
[25] P. Majumdar, C. Bathula, S.M. Basu, S.K. Das, R. Agarwal, S. Hati, A. Singh, S. Sen, B.B. Das, Design, synthesis and evaluation of thiohydantoin derivatives as potent topoisomerase I (Top1) inhibitors with anticancer activity, Eur. J. Med. Chem. 102 (2015) 540–551.
[26] K.R.A. Abdellatif, M.M. Elbadawi, M.T. Elsaady, A.A. Abd El-hafeez, T. Fujimura, S. kawamoto, A.I. Khodair, Design, synthesis and cytotoxicity evaluation of new 3, 5-disubstituted-2- thioxoimidazolidinones. Anticancer Agents in Medicinal Chemistry 18 (2018) 573–582.
[27] L.K. Gediya, V.C. Njar, Promise and challenges in drug discovery and development of hybrid anticancer drugs, Expert Opin. Drug. Discovery 4 (2009) 1099–1111.
[28] A. Rialdi, L. Campisi, N. Zhao, A.C. Lagda, C. Pietzsch, J.S.Y. Ho, L. Martínez-Gil, R. Fenouil, X. Chen, M. Edwards, G. Metreveli, S. Jordan, Z. Peralta, C. Muñoz-Fontela, N. Bouvier, M. Merad, J. Jin, M. Weirauch, S. Heinz, C. Benner, H. van Bakel, C. Basler, A. García-Sastre, A. Bukreyev, I. Marazzi, Inhibition of topoisomerase 1 suppresses inflammatory genes and protected from death by inflammation, Science 352 (2016) 7993.
[29] F. Wu, H. Jiang, B. Zheng, M. Kogiso, Y. Yao, C. Zhou, X. Li, Y. Song, Inhibition of cancer-associated mutant isocitrate dehydrogenases by 2-thiohydantoin compounds, J. Med. Chem. 58 (2015) 6899–6908.
[30] B. Roschek, R.C. Fink, D. Li, M. McMichael, C.M. Tower, R.D. Smith, R.S. Alberte, J. Med. Food 12 (2009) 615–625.
[31] C.A. Winter, E.A. Risley, G.W. Nuss, Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs, Exp. Biol. Medi. 111 (3) (1962) 544–547.
[32] G.S. Hassan, S.M. Abou-Seri, G. Kamel, M.M. Ali, Celecoxib analogs bearing benzofuran moiety as cyclooxygenase-2 inhibitors: design, synthesis and evaluation as potential anti-inflammatory agents, Eur. J. Med. Chem. 76 (2014) 482–493.
[33] H.A. Omar, A. El-S, S.A. Arafa, H.H. Salama, C.-H. Arab, J.-R. Weng Wu, OSU-A9 inhibits angiogenesis in human umbilical vein endothelial cells via disrupting Akt–NF-κB and MAPK signaling pathways, Toxicol. Appl. Pharmacol 272 (2013) 616–624.
[34] J.L. Wang, D. Limburg, M.J. Graneto, J. Springer, J.R. Hamper, S. Liao, et al., The novel benzopyran class of selective cyclooxygenase-2 inhibitors. Part 2: the second clinical candidate having a shorter and favorable human half-life, Bioorg. Med. Chem. Lett. 20 (2010) 7159–7163.