



Synthesis of new thiazolo-pyrrolidine–(spirooxindole) tethered to 3-acylindole as anticancer agents

Mohammad Shahidul Islam^a, Hussien Mansur Ghawas^a, Fardous F. El-Senduny^b,
Abdullah Mohammed Al-Majid^a, Yaseen A.M.M. Elshaier^c, Farid A. Badria^d, Assem Barakat^{a,e,*}

^a Department of Chemistry, College of Science, King Saud University, P. O. Box 2455, Riyadh 11451, Saudi Arabia

^b Department of Chemistry, Faculty of Science, Mansura University, Mansura, Egypt

^c Department of organic and medicinal chemistry, Faculty of Pharmacy, University of Sadat City, Menofia, Egypt

^d Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt

^e Department of Chemistry, Faculty of Science, Alexandria University, P.O. Box 426, Ibrahimia, Alexandria 21321, Egypt

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ABSTRACT

Anticancer therapeutics with profiles of high potency, low toxicity, and low resistance is of considerable interest. A new series of functionalized spirooxindole linked with 3-acylindole scaffold is reported, starting from chalcones derived from 3-acetyl indole with isatin, and l-4-thiazolidinecarboxylic acid. The reactions proceeded regioselectivity, stereoselectivity, without side products in high yield (71–89%). The new spirooxindole hybrids have been evaluated *in vitro* for their antiproliferative effects against colon cancer (HCT-116), hepatocellular carcinoma (HepG2) and prostate cancer (PC-3). The selectivity of their activity was evaluated. Some of the synthesized compounds showed considerable anticancer activities. Compound **4k** proved to retain a high cytotoxic activity and selectivity against colon cancer cells HCT-116 ($IC_{50} = 7 \pm 0.27 \mu\text{M}$, SI: 3.7), and HepG2 ($IC_{50} = 5.5 \pm 0.2 \mu\text{M}$, SI: 4.7) in comparison to ($IC_{50} = 12.6 \pm 0.5$, SI: 0.4 and $5.5 \pm 0.3 \mu\text{M}$, SI: 0.9, respectively). Compound **4k** was less active ($IC_{50} = 6 \pm 0.3 \mu\text{M}$, SI: 4.3) than cisplatin ($IC_{50} = 5 \pm 0.56 \mu\text{M}$, SI: 1.0) but showed greater selectivity towards prostate cancer cells PC-3 in comparison to cisplatin. The details of the binding mode of the active compounds were clarified by molecular docking. Ligand Efficiency (LE) and Ligand Lipophilic Efficiency (LLE) were evaluated and revealed that compound **4k** had acceptable value.

1. Introduction

Cancer is the second cause of death worldwide after heart diseases according to the Surveillance, Epidemiology, and End Results (SEER) program of the National Cancer Institute [1,2]. The estimated new cases in colon cancer in both sexes are 95,520 (47,700 in men and 47,820 in women), followed by 40,710 (29,200 in men and 11,510 in women) new cases in liver cancer. The prostate cancer new estimated cases in 2017 were reported to be 161,360 and expected deaths were 26,730 [2]. Despite the available chemotherapeutic, hormonal or radiotherapy for treating cancer, there is relapse in the disease and acquire of resistance of recurrent disease. Therefore, there is still need for the discovery of alternative drugs with less side effects to the normal cells. Colon cancer is the second cause of deaths among cancer types in men and the 3rd in females [2]. Different factors are attributed to the development of colorectal cancer. Among them, dietary components (high alcohol consumption, low methionine and folate intake) [3], obesity [4]

and non-steroidal anti-inflammatory drugs [5]. Chemotherapy is the most common used method in treating colorectal cancer but in metastatic cancer, the chemo-resistance emerge leading to the failure of chemotherapy [6]. Therefore, there is an urgent need for discovering alternative options for treatment of colorectal cancer.

Liver cancer is considered as the 5th cause of deaths among men in 2017 and the 8th in female [2]. Food contamination with aflatoxins carcinogen [7], hepatitis B and C viruses [8–10], alcohol intake [11] even the non-alcoholic fatty liver disease are considered as the major contributors for the development of liver cancer. The first line in liver treatment is the surgical resection followed by conventional chemotherapy and immunotherapy [12]. At advanced stages, trans-arterial chemo-embolization (TACE) and trans-arterial radio-embolization or systemic chemotherapy are applied in order to increase the survival of the patient [13]. Despite the varieties of the offered options in the liver cancer treatment, the resistance of cancer cells still is an obstacle in the increase of curative rate. Therefore, there is a need for finding new

* Corresponding author at: Department of Chemistry, College of Science, King Saud University, P. O. Box 2455, Riyadh 11451, Saudi Arabia.
E-mail address: ambarakat@ksu.edu.sa (A. Barakat).

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anticancer agents for treatment of this disease.

Prostate cancer is the 3rd cause of deaths due to malignancies and its incidence rate is high in modern industrialized countries [2,14]. Family history with prostate cancer is linked to the high incidence rate due to genetic predisposition [15]. Moreover, dietary factors are also contribute to the development of prostate cancer (meat-rich diet, fats, less fibers, low fruit and vegetables intake) [16,17]. Change in hormonal level, infection and presence of corpora amylacea in prostate glands are also risk factors for prostate cancer development [18]. Chemotherapy and hormonal therapy are among the available treatment regimen for prostate cancer [19]. The metastatic disease usually respond to androgen-deprivation therapy. However the relapse of the disease in patients within one and half to two years [20] leading to the poor survival of patients due to the emergence of resistance. Finding new leads in treating prostate cancer is highly required. Mutated or deleted p53 is highly correlated to the poor prognosis of different types of aggressive cancers [21–32]. Therefore, reactivation of p53 pathway is a valuable target for treating different kinds of cancers. Reactivation of P53 by blocking the MDM2-P53 interaction using non-peptide small molecules is an exciting new strategy for treatment of cancer. Under normal conditions, MDM2, ubiquitin ligase enzyme, keeps p53 level low by inducing the degradation of p53 [21]. Under DNA damage, p53 is phosphorylated and is released from its MDM2 inhibitor and translocated to the nucleus where it promotes the expression of DNA repair genes. If the DNA damage is unfixable and severe, p53 induces the expression of apoptotic genes and the cell cycle will be arrested and undergoes the programmed cell death apoptosis [22,23]. Therefore, discovery of small non-peptide molecules for activation of p53 is highly desirable.

Spirooxindole alkaloids have been identified as a promising anticancer agent due to the excellent ability to bind with several cellular receptors [33,34]. Especially, the tetra-substituted carbon at position 3 of the oxindole or spirooxindole framework has been found as a core structure in a large family of unnatural/natural heterocyclic bioactive compounds with different pharmacological targets (e.g., spirotryprostatin A, rhynchophylline, coerule-scine, horsifiline, elacomine,

and mitraphylline, etc., Fig. 1). Spirotryprostatin A, a natural product extracted from *Aspergillus fumigatus*, is responsible for disruption of microtubule assembly and inhibition of serotonin receptor [35,36]. The simplest example in nature for spirooxindole alkaloid is coerule-scine and exhibits a local anesthetic effect [37,38]. Additionally, mitraphylline and rhynchophylline are now in advanced preclinical studies as antioxidant and antigenotoxic drugs [39,40].

Naturally or synthetically spirooxindoles exhibit different biological activities such as anticancer, antimycobacterial [41], antimigraine, contraceptive [42], sedative [43], acetyl cholinesterase [44] inhibition activities. MDM2, p53 tumor suppressor regulator, is a target for spirooxindole derivatives such as MI-888, MI-219, and NITD609 [45–49]. Rhynchophylline and isorhynchophylline showed neuroprotective activities *via* the noncompetitive inhibition for *N*-methyl-D-aspartate (NMDA) receptor [50].

Various synthetic derivatives of spirooxindole have been produced in an attempt to obtain more potent anticancer agents profile intact [51]. In this regard, we have synthesized a new series of the spirooxindole-based frameworks. The skeleton is constructed from scaffolds; (1) spirooxindole moiety, (2) thiazolo-pyrrolidine system, (3) 3-acylindole ring (Fig. 2). Their anticancer activities against colon cancer (HCT-116), liver cancer (HepG2) and prostate cancer (PC-3) were determined. Their cytotoxicity against normal kidney epithelial cells extracted from an African green monkey was also evaluated (Vero cells). The possible molecular binding with MDM2-p53 complex as well as lipophilicity were investigated.

2. Results and discussion

2.1. Synthesis of 4a–n

The use of low cost, one-pot reaction, and readily available core structure for the preparation of highly functionalized complex molecules is of high importance in organic chemistry. The target spirooxindole analogous were synthesized using a powerful method of multi-component reactions (Scheme 1).

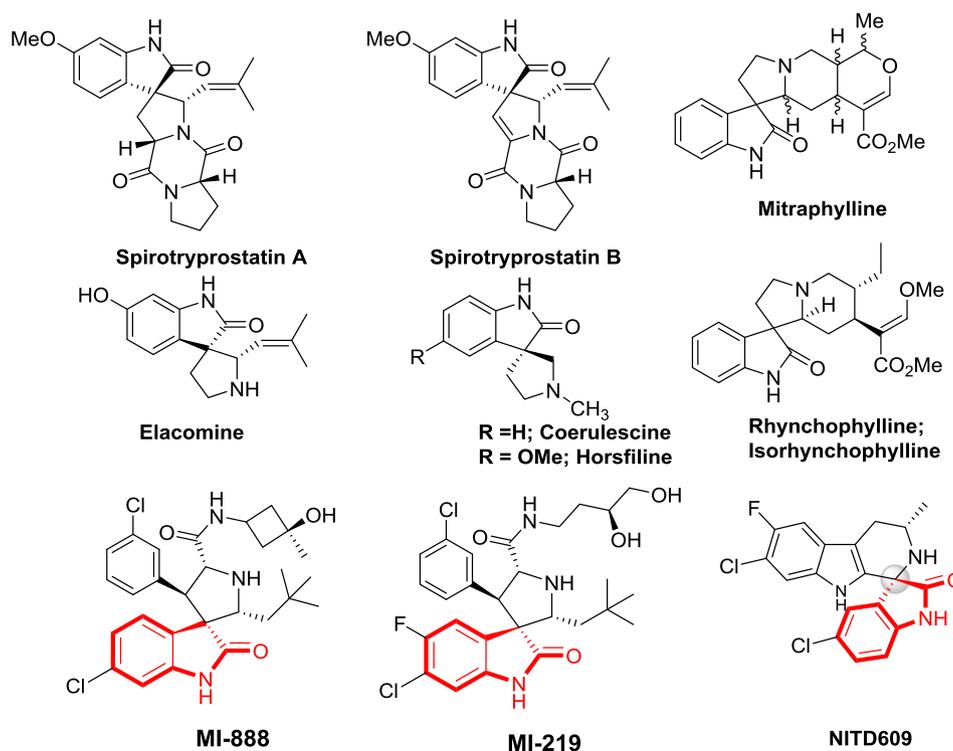


Fig. 1. Spirooxindole backbones with highly biologically importance.

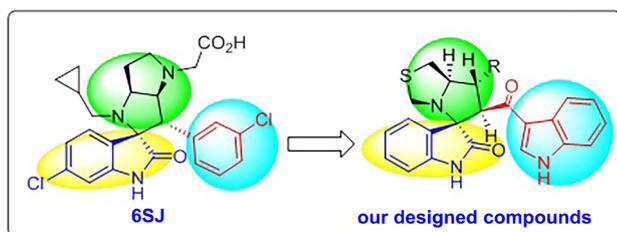
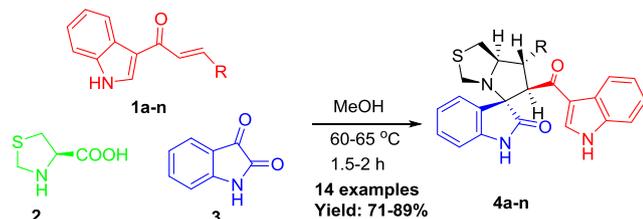
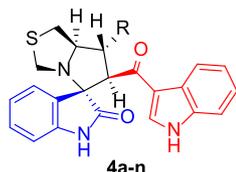


Fig. 2. Standard spirooxindole (6SJ) and general formula for the target compounds.



Scheme 1. The synthesis of the target thiazolo-pyrrolidine/spirooxindole derivative 4a-n.

Table 1
Synthesis of thiazolo-pyrrolidine/spirooxindole 4a-n.



Entry	4a-n	R	Yield (%)
1.	4a	C ₆ H ₅	88
2.	4b	<i>p</i> -MeC ₆ H ₄	87
3.	4c	<i>p</i> -ClC ₆ H ₄	81
4.	4d	2,4-Cl ₂ C ₆ H ₃	80
5.	4e	<i>p</i> -MeOC ₆ H ₄	85
6.	4f	<i>p</i> -BrC ₆ H ₄	79
7.	4g	<i>p</i> -FC ₆ H ₄	81
8.	4h	<i>m</i> -FC ₆ H ₄	77
9.	4i	<i>m</i> -MeC ₆ H ₄	83
10.	4j	<i>m</i> -BrC ₆ H ₄	71
11.	4k	<i>p</i> -CF ₃ C ₆ H ₄	83
12.	4l	2-Thiophene	88
13.	4m	2-Furan	89
14.	4n	3,4,5-tri-MeOC ₆ H ₂	83

The chalcone 1a-n was synthesized from 3-acetyl indole with aldehydes according to our previous reported method [51]. 14 spirooxindole derivatives 4a-n were selectively synthesized by the one-pot reaction of chalcone derivative 1a-n, active carbonyl compounds (isatin, 3), and secondary amino acid (*L*-4-thiazolidinecarboxylic acid, 2) in high yield (71–89%) in boiling MeOH for 1.5–2.0 h (Scheme 1). The result is summarized in Table 1. All spectrophotometric tools including ¹H NMR, ¹³C NMR, mass spectrometry (MS), infrared (IR) spectroscopy, elemental analysis is provided in the supplementary information to confirm the assignment of the chemical structures of the desired targets.

The key intermediate for the three component reaction is the formation of azomethine ylide (isatin and carboxylic acid group of *L*-thio-proline). The dipolar cycloaddition reaction of azomethine ylide therefore preferably proceed via the *E*-β-exo transition state (Scheme 2). This reaction, which sets four contiguous stereogenic centres, constructs the entire spirooxindole moiety in a single and a simple operation.

2.2. Biological activity

In order to evaluate the possible anticancer activity of the new series of spirooxindole derivatives, an initial screening was performed against colon (HCT-116), liver (HepG2) and prostate (PC-3) cancer. Additionally, the selectivity to cancer cells rather than normal cells was evaluated and compared to the standard chemotherapeutic drug cisplatin. The results showed that all tested new compounds have variable anticancer activities. Compound 4k (IC₅₀ = 7 ± 0.27 μM, Table 2) was the most active one against colon cancer cells in comparison to cisplatin (IC₅₀ = 12.6 ± 0.5 μM). Compound 4k was highly selective to colon cancer cells (SI > 3.7) while cisplatin showed less selectivity (SI = 0.4). Compound 4b, 4d, 4h, and 4j were also more active and more selective than cisplatin towards colon cancer cells (Table 2).

Among the tested new compounds against hepatocellular carcinoma, only compound 4k (IC₅₀ = 5.5 ± 0.2 μM, Table 3) showed activity equal to the standard cisplatin but again with superior selectivity to cancer cells (SI > 4.7 vs SI = 0.9, respectively). Despite that, compounds 4d, 4j, 4f and 4h were less active than cisplatin against hepatocellular carcinoma, their selectivity was greater (SI > 3.7, 3, 2.9 and 2.9, respectively) (Table 3) than cisplatin (SI = 0.9).

In between the tested compounds 4d, 4k, 4j, 4f and 4h against PC-3 revealed the following activities (IC₅₀ = 6 ± 0.3, 6 ± 0.23, 7 ± 0.15, 8 ± 0.5 and 8 ± 0.45 μM, respectively) which are less than the standard drug cisplatin (IC₅₀ = 5 ± 0.12 μM) but with possessing stronger selectivity (SI > 4.3, 4.3, 3, 2.9, and 2.9) towards PC-3 leaving normal cells unaffected (Table 4). The current results present new spirooxindole series as a promising anticancer agents and requires further mechanistic studies to determine the mechanism of anticancer activity and their selectivity to cancer cells.

2.3. Structure activity relationship (SAR)

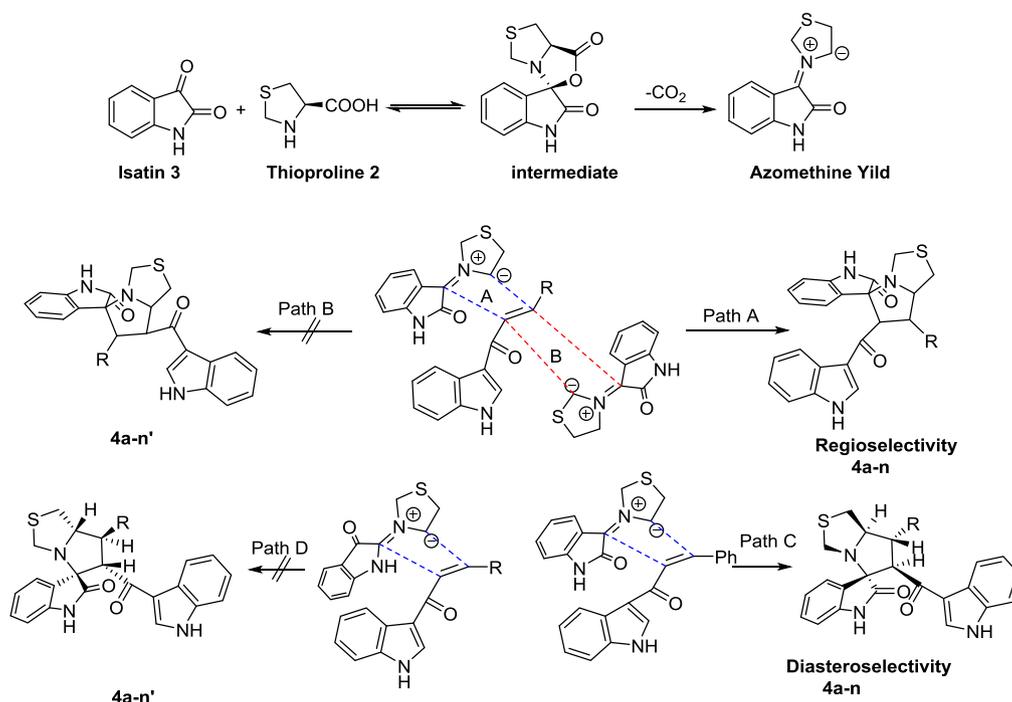
Seemingly, all structural features of the spirooxindole compounds were active against colon cancer cells (HCT-116). The activities are varied due to the variation in the substituent of the aromatic ring. We have noticed that *p*-CF₃ (4k, IC₅₀ = 7 ± 0.27 μM, Table 2) was found to be the most active compound in this series. With *p*-methyl (4b); *p*-chloro (4c); 2,4-dichloro (4d); *p*-bromo (4f); *m*-fluoro; (4h) and *m*-bromo (4j) displayed more potent anticancer activity (4b: 9 ± 1.78; 4c: 11.5 ± 2; 4d: 9 ± 1.5; 4f: 11.3 ± 1; 4h: 9 ± 0.98; 4j: 9 ± 1 μM, respectively) comparable to standard drug cisplatin (IC₅₀ = 12.6 ± 0.5 μM). Without any substitution on the benzene ring 4a, or having electron donating group such as methoxy group 4e or 4n and also if the phenyl ring replaced by heterocyclic aromatic rings such as thiophene 4l or furan 4m, the reactivity decreases dramatically.

In case of hepatocellular carcinoma (HepG2), only compound with *p*-CF₃ substituent (4k: IC₅₀ = 5.5 ± 0.2 μM, Table 3) showed activity equal to the standard drug cisplatin. On the other hand, 2,4-dichloro (4d); *p*-bromo (4f); and *m*-bromo (4j) displayed good anticancer activity (4d: 7 ± 1; 4f: 8 ± 1.56; 4j: 7 ± 1.5 μM, respectively) but less active than cisplatin with greater selectivity.

Finally in case of prostate cancer cells (PC-3); we noticed that the following variation 2,4-dichloro (4d); *p*-CF₃ (4k); *m*-bromo (4j); *p*-bromo (4f); and *m*-fluoro (4h) exhibited lower activity (IC₅₀ = 6 ± 0.3, 6 ± 0.23, 7 ± 0.15, 8 ± 0.5 and 8 ± 0.45 μM, respectively) than cisplatin (IC₅₀ = 5 ± 0.12 μM) but with stronger selectivity (SI > 4.3, 4.3, 3, 2.9, and 2.9 Table 4). Our attention was then directed to understand the structure activity relationship for these compounds with emphasize on the molecular weight and lipophilicity descriptors by molecular docking study.

2.4. Molecular docking study

Based on shape similarity of our compounds with reported spirooxindole standards and our previous finding [51], these compounds



Scheme 2. Plausible mechanism for the synthesized compounds 4a-n.

Table 2

The cytotoxic activity of the synthesized new spirooxindole derivatives against colorectal cancer cells (HCT-116). SI; selectivity index, IC₅₀; the concentration that is required to kill 50% of cells. SD ± represent the standard deviation of triplicates.

#	4a-n	HCT-116 (IC ₅₀ , μM)	HCT-116 (IC ₅₀ , μg/ml)	VERO-B (IC ₅₀ , μM)	SI
1	4a	30 ± 3	13.9	68	2.7
2	4b	9 ± 1.78	4.3	28	3.1
3	4c	11.5 ± 2	5.7	40	3.5
4	4d	9 ± 1.5	4.8	26	2.9
5	4e	26 ± 2	12.9	50	1.9
6	4f	11.3 ± 1	6.1	22	1.9
7	4g	13 ± 1.2	6.3	30	2.3
8	4h	9 ± 0.98	4.3	22	2.4
9	4i	15.3 ± 2	7.3	32	2.1
10	4j	9 ± 1	4.9	20	2.2
11	4k	7 ± 0.27	3.7	26	3.7
12	4l	90 ± 8	42.4	40	0.4
13	4m	40 ± 5	18.2	33	0.8
14	4n	> 40 ± 3	> 18	42	< 0.8
STD	cisplatin	12.6 ± 0.5	3.8	5	0.4

have the ability to inhibit MDM2-P53 interactions. The designed compounds (4a-n) and references spirooxindole drugs [46,48,52–55] were subjected to docking study with MDM2 that was retrieved from protein data bank (PDB code: 5law) [56]. Docking study was operated using Open Eye® scientific software [57].

To validate our study, the docking method started with standard spirooxindole (6SJ) [55]. 6SJ interacts in the receptor with consensus score 8 and showed hydrogen bond (HB) towards binding site of MDM2 coming from the NH of indole moiety (donor) with Leu 54: AA (acceptor) (supplementary data). This result was similar to its co-crystallized pose [55].

Most of compounds lay in the active site with similar pose to the standard drug 6SJ but it showed hydrophobic-hydrophobic interactions without formation of HB. In particular compounds 4j and 4d (Figs. 3 and 4, respectively), which are active compounds, showed the best docking mode in this series of compounds with consensus score 76 and

Table 3

The cytotoxic activity of the synthesized compounds against hepatocellular carcinoma (HepG2) cells. SI; selectivity index, IC₅₀; the concentration that is required to kill 50% of cells. SD ± represent the standard deviation of triplicates.

#	4a-n	HepG2 (IC ₅₀ , μM)	HepG2 (IC ₅₀ , μg/ml)	VERO-B (IC ₅₀ , μM)	SI
1	4a	30 ± 2.4	13.9	68	2.3
2	4b	10 ± 2	4.8	28	2.8
3	4c	15 ± 1.1	7.5	40	2.7
4	4d	7 ± 1	3.7	26	3.7
5	4e	30 ± 3	14.9	50	1.7
6	4f	8 ± 1.56	4.4	22	2.8
7	4g	12 ± 1	5.8	30	2.5
8	4h	8 ± 2.1	3.9	22	2.8
9	4i	12 ± 2	5.8	32	2.7
10	4j	7 ± 1.5	3.8	20	2.9
11	4k	5.5 ± 0.2	2.9	26	4.7
12	4l	35 ± 4	16.5	40	1.1
13	4m	30 ± 2	13.7	33	1.1
14	4n	35 ± 3	15.9	42	1.2
STD	cisplatin	5.5 ± 0.3	1.7	5	0.9

68, respectively. Other compounds interact in the receptor active site and showed hydrogen bond (HB) towards binding site of MDM2. Compound 4k interacts with the receptor with consensus score 73 and forms HB coming from the NH of indole moiety (donor) with Leu 54:AA (acceptor). (Fig. 5). This interaction suggested that presence of substituted phenyl fragment acts as an arm and so we can speculate that it is an important new pharmacophore.

Judging from these docking results and previous reported data [58–66], these class of compounds may be considered as non-peptide small molecules that inhibit the MDM2-p53 interaction and so highly effective in activation of p53 function.

2.5. Ligand efficiency (LE) and ligand lipophilic efficiency (LLE)

Determination of ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties, with special emphasis on the

Table 4

The cytotoxic activity of the synthesized compounds against prostate cancer (PC-3) cell line. SI; selectivity index, IC₅₀; the concentration that is required to kill 50% of cells. SD ± represent the standard deviation of triplicates.

#	4a-n	PC-3 (IC ₅₀ , μM)	PC-3 (IC ₅₀ , μg/ml)	VERO-B (IC ₅₀ , μM)	SI
1	4a	30 ± 3.4	13.9	68	2.3
2	4b	15.3 ± 2.3	7.3	28	1.8
3	4c	18.4 ± 1.5	9.2	40	2.2
4	4d	6 ± 0.3	3.2	26	4.3
5	4e	24.6 ± 3	12.2	50	2.0
6	4f	8 ± 0.5	4.4	22	2.8
7	4g	16.8 ± 2	8.1	30	1.9
8	4h	8 ± 0.45	3.9	22	2.8
9	4i	17.7 ± 3	8.5	32	1.8
10	4j	7 ± 0.15	3.8	20	2.9
11	4k	6 ± 0.23	3.2	26	4.3
12	4l	25 ± 2.9	11.8	40	1.6
13	4m	18.5 ± 1.86	8.4	33	1.8
14	4n	18.5 ± 2	8.4	42	2.4
STD	cisplatin	5 ± 0.56	1.5	5	1

lipophilicity, is an essential parameters in drug development and drug design [67]. Currently, various helpful parameters “optimization measures” are used to correlate between the activities of compounds (pIC₅₀) with their molecular size and lipophilicity (cLogP). These optimization assessments include ligand efficiency (LE) and lipophilic ligand efficiency (LLE) and the rule of three, [68,69].

2.5.1. Ligand efficiency (LE) scores

Medicinal chemists utilize LE, in drug discovery, to construct compounds with optimal combinations of physicochemical and pharmacological properties [67–69]. LE is used to assess the efficiency of compounds and to calculate its binding affinity (in terms of binding energy or pIC₅₀) in relation to the number of heavy atoms in a molecule. This approach guides to compare the affinity of molecules corrected for their size. LE measures the average binding energy contribution per atom (non-hydrogen atom) instead of considering the potency or binding

affinity of the whole compound. It can be calculated as showed in the following equations:

$$LE = \Delta G \div NHA$$

$$\text{or } LE = (pIC_{50} \times 1.37) \div NHA$$

where

ΔG = Gibb's free energy

IC₅₀ = half-maximal inhibitory concentration (in term of molar concentration)

NHA = non-hydrogen atom

The recommended LE value should be in the range of 0.3. The acceptable LE value should be higher than 0.3. Performing ligand efficiency analyses has practical utility in “lead optimization” towards “drug-like candidate” space. LE values for target compounds are presented in Table 5.

2.5.2. Ligand lipophilic efficiency (LLE)

LLE provides a way to determine the affinity of a compound with respect to its lipophilicity. LLE is defined as the difference between the potency and log P as illustrated in the following equation [69]:

$$LLE = pIC_{50} - cLogP$$

The challenge in drug discovery is to improve the activity with keeping lipophilicity in constant value. For this, LLE is consider as an effective and practical tools of keeping lipophilicity under control to avoid any “molecular obesity” during drug optimization process. An acceptable lead compound should have LLE value ≥ 3 while LLE value ≥ 5 recommend a compound as a drug-like candidate. Monitoring LLE metric during lead optimization can highlight the price paid in lipophilicity on the expense of potency or binding affinity.

LE and LLE calculations, as illustrated in Table 5, were calculated based on the compounds IC₅₀ against every cell line namely HCT-116, HepG2 and PC-3. Results indicated that most of these compounds

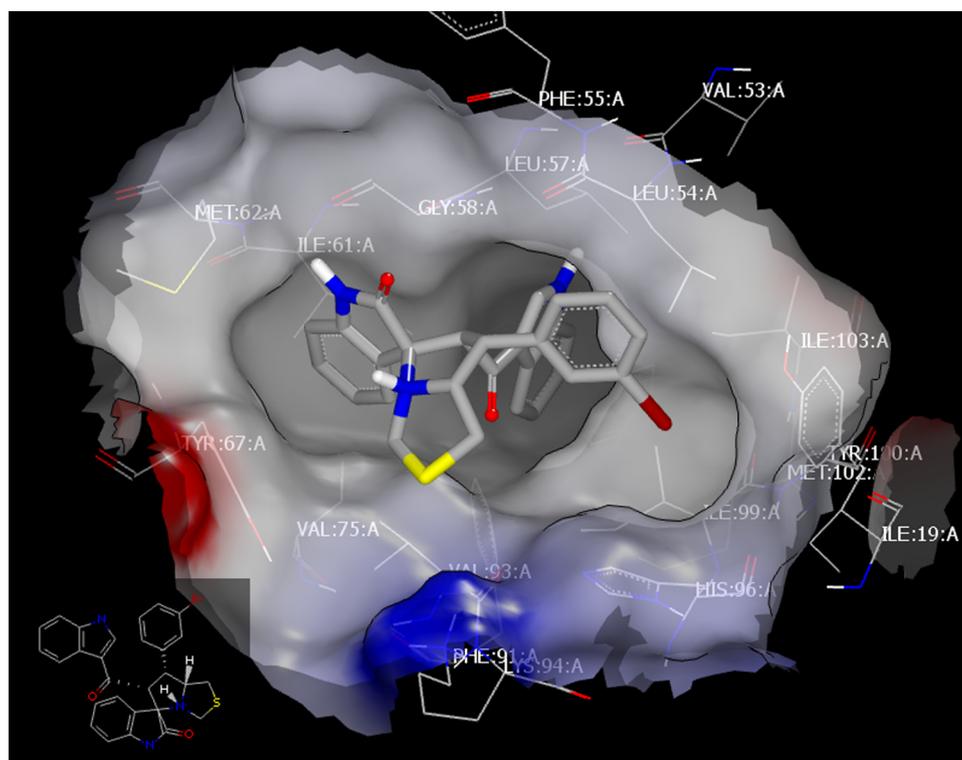


Fig. 3. Snap shot visualization of 4j docked with ID: 5law, showed hydrophobic-hydrophobic interaction without formation of HB interaction as illustrated by Vida.

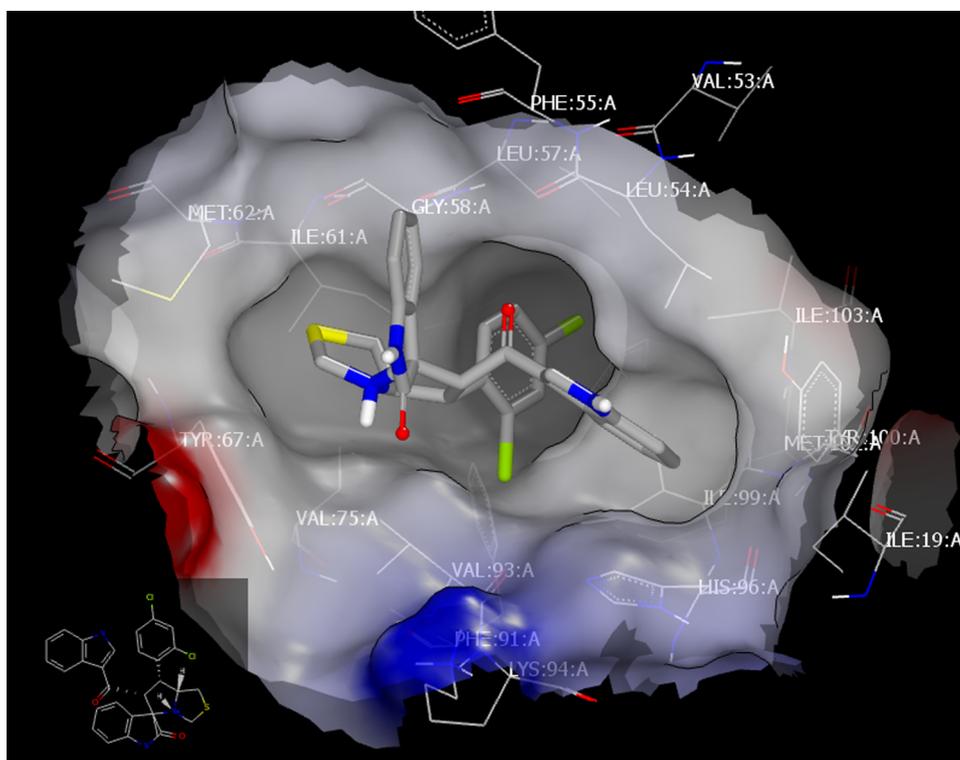


Fig. 4. Snap shot visualization of **4d** docked with ID: 5law, showed hydrophobic-hydrophobic interaction without formation of HB interaction as illustrated by Vida.

revealed LE values 0.16–0.2. Compounds **4d**, **4k**, and **4j** have reasonable LE values (0.2). Compound **4m** has an acceptable LEE value (above 3) and compounds **4g**, **4h**, and **4l** exhibited LEE values close to 3.

However, LLE values for other compounds were beyond the recommended limit (1.80–2.39) which means that there is a price paid in lipophilicity on the expense of the efficiency of the final compounds.

The pIC_{50} , LE and LLE values for **4d** and **4k** indicated that optimization using phenyl substituent with low number of NHA.

3. Conclusion

In summary, the application of 1,3 dipolar cycloaddition provides

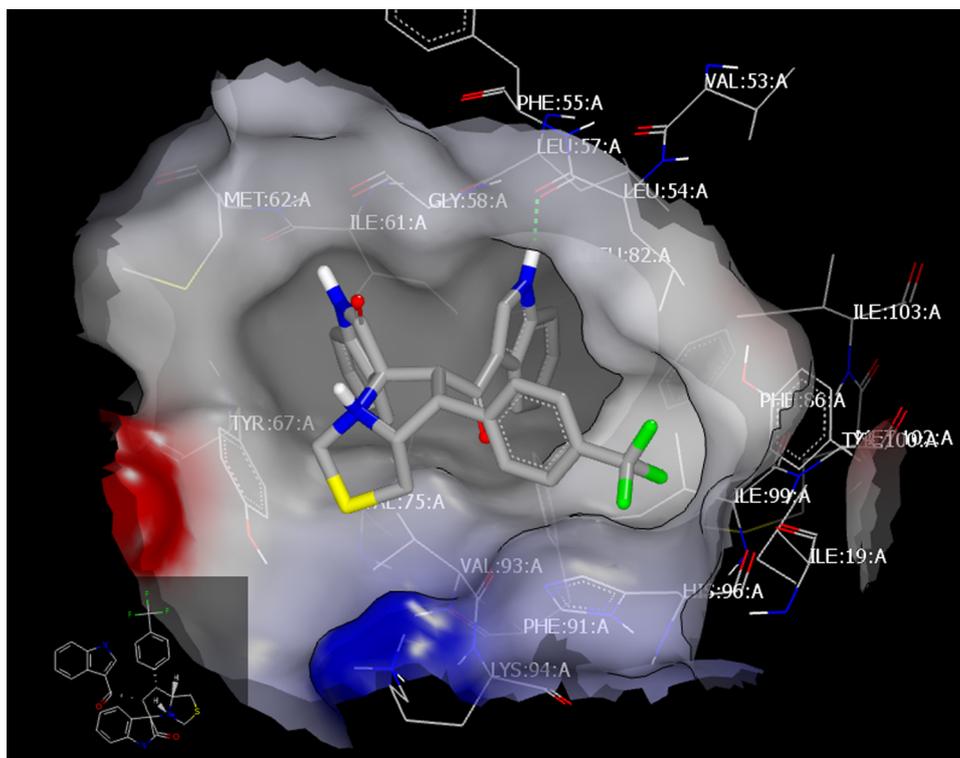


Fig. 5. Snap shot visualization of compound **4k** docked with ID: 5law, showed HB interaction with Leu 54:AA (green dotted line) as illustrated by Vida.

Table 5
Summary of ligand efficiency scores for the target compounds.

	NHA	cLog P	HCT-116			HepG2			PC-3		
			pIC ₅₀	LE	LLE	pIC ₅₀	LE	LEE	pIC ₅₀	LE	LEE
4a	34	2.03	4.52	0.18	2.49	4.52	0.18	2.49	4.52	0.18	2.49
4b	35	2.53	5.04	0.20	2.51	4.52	0.18	1.99	4.18	0.16	1.65
4c	35	2.74	4.94	0.19	2.2	4.82	0.19	2.08	4.73	0.19	1.99
4d	36	3.45	5.04	0.19	1.6	5.15	0.20	1.7	5.22	0.20	1.77
4e	36	1.95	4.58	0.17	2.63	4.52	0.17	2.57	4.6	0.18	2.65
4f	35	2.89	4.95	0.19	2.1	5.09	0.20	2.2	5.09	0.20	2.2
4g	35	2.17	4.88	0.19	2.71	4.29	0.17	2.12	4.77	0.19	2.6
4h	35	2.17	5.04	0.20	2.87	5.09	0.20	2.92	5.09	0.20	2.92
4i	35	2.53	4.81	0.19	2.28	4.29	0.17	1.76	4.75	0.19	2.22
4j	35	2.89	5.04	0.20	2.15	5.15	0.20	2.26	5.15	0.20	2.26
4k	38	2.91	5.15	0.19	2.24	5.52	0.20	2.61	5.22	0.19	2.31
4l	33	1.67	4.05	0.17	2.38	4.5	0.19	2.83	4.60	0.19	2.93
4m	33	1.2	4.39	0.18	3.19	4.52	0.19	3.32	4.73	0.20	3.5
6SJ	34	2.84									

access to new series of spirooxindole derivatives in an efficient one-pot multicomponent reaction. This approach appears well-suited in preparing several analogues that may prove to generate potential anticancer derivatives of this class of unique spirooxindole alkaloids. Compounds **4k**, **4d**, **4j**, **4f** and **4h** revealed broad activity against the tested three cancer cell lines with greater selectivity. The synthesized compounds interact with the receptor inside the active sites through hydrophobic-hydrophobic interaction as compound **4d** and **4b**. Most of compounds overlay with reference ligands. Compound **4k** showed ligand protein interactions as **6SJ** reference ligand through HB with Leu 54: AA. LE and LLE analysis indicate that compounds **4k** and **4d** can be considered as good candidates as drug target. Our designed compounds showed high SI indicating that these compounds have low toxicity and in advantage of other spirooxindoles with known high toxicity. In this study, new series of spirooxindole have been synthesized and showed binding affinity in MDM2 binding site for p53. Further studies are required to investigate the molecular mechanism of the binding and the anticancer activities.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bioorg.2018.10.036>.

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