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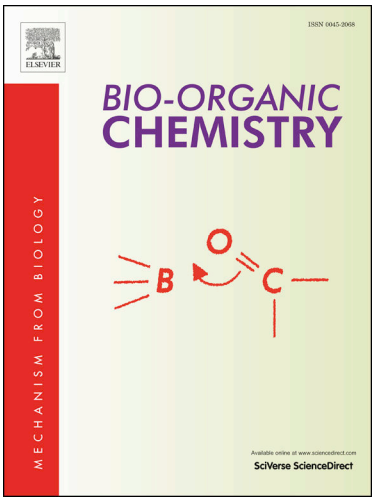

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Design and Synthesis of Novel Isatin-Based derivatives Targeting Cell Cycle Checkpoint Pathways as Potential Anticancer Agents

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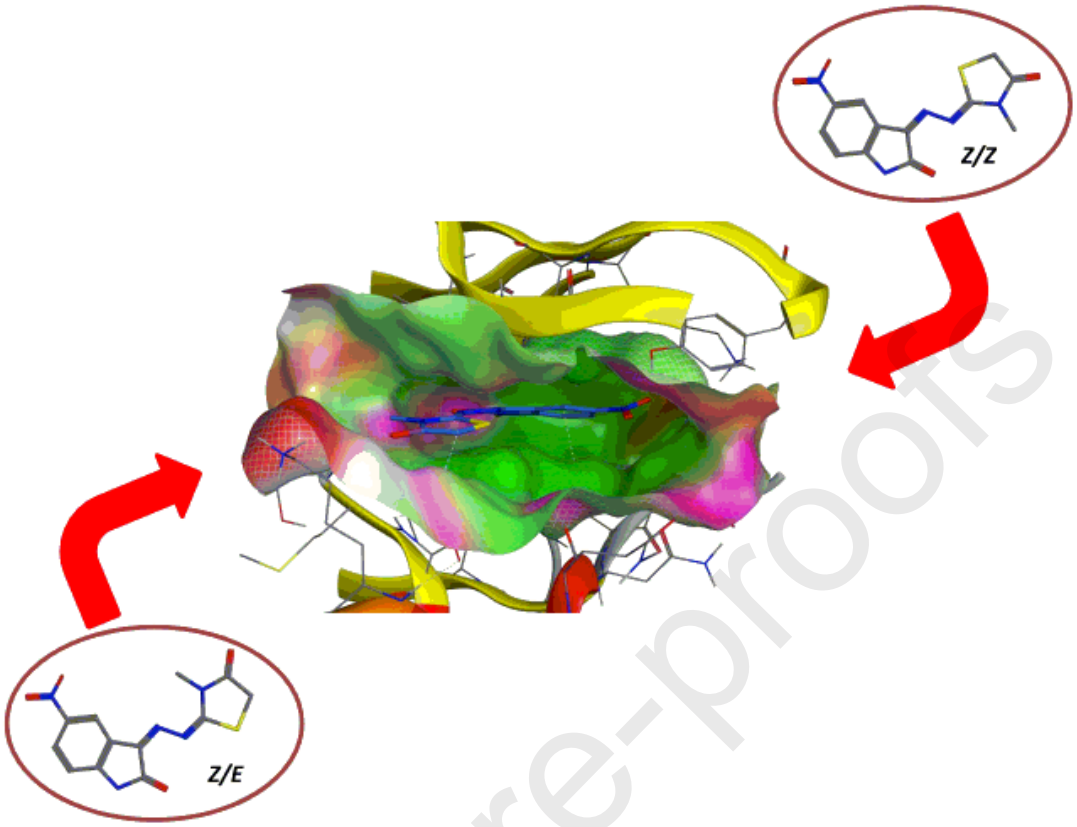
Graphical Abstract



Abstract

In recent years, cell cycle and checkpoint pathways regulation are offering new therapeutic approaches against cancer. Isatin, is a well exploited scaffold in the anticancer domain. Accordingly, the current work describes the design and synthesis of two series of (Z)-3-substituted-2-(((E/Z)-5-substituted-2-oxo-1-substituted-indolin-3-ylidene)hydrazinylidene)-thiazolidin-4-ones, 4(a-s) and (E/Z)-1-substituted-3-(((Z)-3-substituted-4-methylthiazol-2(3H)-ylidene)hydrazineylidene)-5-substituted-indolin-2-ones, 5(a-s). The structures of the synthesized molecules were confirmed by spectral and elemental methods of analyses. Pure diastereomers were further identified with ^{1}H-^{1}H-NOESY and confirmed with X-ray crystallography. The target compounds were tested *in vitro* for their cytotoxicity against three human epithelial cell lines, liver (HepG2), breast (MCF-7), and colon (HT-29) in addition to the diploid human normal cells (WI-38) compared to doxorubicin as a reference drug. Variable cytotoxic effects (IC_{50} 3.29 – 100 micromole) were reported on the three cancer cell lines with pronounced selectivity compared to the normal one WI-38. The potency of the most active compounds, 4o, 4s, 5e, 5f, 5l, 5m and 5o (IC_{50} 3.29 – 9.92 micromole), in both series associated with the (Z) configurations of N=thiazolidin/ene or one, however, the configuration of the N=isatin moiety seemed to be of no importance to the activity. The tested compounds were grouped for their possible mechanism of action into 4 categories. Compound 4o with no apparent effect on all genes examined. Compounds 4s and 5o affected all genes investigated and seem to have multiple cellular targets; induced the expression of p53 and caspases, and downregulated that of CDK1. Compounds 5l and 5m directly elevated the expression of initiator and effector caspases without going through p53 pathway. Finally, compounds 5e and 5f elevated the expression of p53 and inhibited CDK1. Docking studies on CDK1 revealed that the active molecules bind to the tested enzyme by the same manner of the co-crystallized ligands and the isatin-thiazoldinone/ene scaffold is essential for binding of these molecules.

Keywords: Isatin, Isatin-thiazolidine, Isatin-thiazolidinone, diastereomers, X-ray, cell cycle, check points, CDK1, p53, caspase-3, caspase-9, anticancer, docking.

1. Introduction

Cancer has become a fast growing threat across the globe. According to the WHO global health observatory report in 2018, about 9.6 million people worldwide have died from cancer. It is projected that by 2030, there will be ~26 million new cancer cases worldwide and 17 million cancer deaths per year [1,2]. Although cancer chemotherapy has progressed in major strides in recent years, there is still an unmet need for new anti-cancer agents with good potency, selectivity, and lower toxicity [3]. It has long been anticipated that the understanding of the basic principles of cell cycle control would result in effective cancer therapies [4].

Control of eukaryotic cell growth and division occurred at the three critical points: late G1, G2/M, and metaphase-to-anaphase transition. These critical steps are also known as "checkpoints" which ensure correct timing of cellular events. Therefore, Cell cycle checkpoints are surveillance mechanisms that monitor the order, integrity, and fidelity of the major events of the cell cycle [5].

Medicinal chemists face major challenges in designing new synthetic compounds with therapeutic importance. In particular, the therapy of complex and multifactorial diseases such as cancer may benefit from molecular design based on the multitarget ligand paradigm, i.e., by incorporation of various biologically active heterocyclic pharmacophores into a single molecule. The hybrid compounds thus generated, carrying more than one pharmacophoric entity and wherein each individual active unit may exert diverse modes of action, offering a new hope in the treatment of cancer. [6].

Isatin is a privileged scaffold in the modern medicinal chemistry which has a broad spectrum of biological activities and wide possibility to chemical modifications. Therefore, in the last several decades, increasing numbers of researchers from both industry and academia have embarked on the development of new isatin-based anticancer agents [1]. Interestingly, isatin is an important pharmacophore unit in several clinically approved anti-cancer drugs: sunitinib , toceranib phosphate and nintedanib, (Figure 1), are examples for isatin-based approved drugs [7]. On the other hand The thiazolidine moiety is also a well-known scaffold in medicinal chemistry and has been used to develop new potential anticancer agents [8,9].

Figure 1

In view of the above facts, and in continuation of our interest in the synthesis and investigating the anticancer activities of isatin derivatives [10,11], the current work describes

the synthesis of a novel series of isatin-thiazolidine derivatives. *In vitro* evaluation of cytotoxic activity of synthetic molecules against three human cancer cell lines was undertaken. In order to highlight the molecular mechanism(s) of the designed molecules, the most active compounds were tested on the targeted enzymes of the cell cycle checkpoints. For justification of the molecular mechanism and for future optimization of the biological activities, molecular docking studies seemed to be of interest.

2. Results and Discussion

2.1. Chemistry

The target molecules (4a-s) and (5a-s) were synthesized utilizing a three steps reaction (Scheme 1). The first step is the synthesis of *N*-propyl- and *N*-benzyl-isatins, 2(a-f), which was accomplished starting from commercially available isatin or its 5-methyl/nitro derivatives according to the reported procedure [1, 12,13]. In the following step, isatin-3-(*Z*)-thiosemicarbazone derivatives 3(a-r), the syntesones for the target compounds, by refluxing the appropriate isatin, **(1a-2f),** with 4-ethyl- or 4-methyl-3-thiosemicarbazides in ethanol in presence of catalytic amount of glacial acetic acid [14,15]. The final step was the preparation of the targeted compounds, (*Z*)-2-(((*E/Z*)-2-oxoindolin-3-ylidene)hydrazineylidene-thiazolidin-4-one 4(a-s) and (*E/Z*)-3-(((*Z*)-4-methylthiazol-2(3*H*)-ylidene)hydrazineylidene)-indolin-2-one 5(a-s). Cyclization of **3(a-r)** with chloroacetic acid in refluxing ethanol in presence of catalytic amount of anhydrous sod. acetate for 4(a-s). However, chloroacetone replaced the cholroacetic acid to obtain 5(a-s) [16-18].

The structures and purity of the prepared compounds were confirmed by spectral and elemental methods of analyses.

2.1.1. Structure elucidation of the target compounds.

It was reported that the reaction of isatin with thiosemicarbazide is stereoselective, as the Z-diastereomer only produced [19-24]. The chemical shifts of the prepared compounds 3(a-r) is comparable with the reported ^{1}H-NMR of Z-thiosemicarbazone [19,20]. They revealed a highly deshielded hydrazinic protons resonated at far downfield shift (around δ 12.55 ppm). This proton is a part of a characteristic six-membered ring resulted from intramolecular H-bond with the carbonyl group of isatin, figure 2. Other protons are resonated at the expected chemical shifts of 2-oxoindole derivatives.

Figure 2

As a general pattern, the ^{13}C-NMR resonance of isatinthiosemicarbazone compounds has three characteristics downfield peaks C=S, C=O and C=N around δ 177.24, 161.31 and 140.79 ppm, respectively, which are in consistent with the suggested structures and with the reported ^{13}C-NMR of isatinthiosemicarbazone derivatives [25].

It is proposed that the prepared compounds 4(a-s) have restricted rotation about the C=N of thiazol ring as well as of the imine C=N of indole that result in formation of E- and Z-diastereomers [26]. The prepared compounds showed duplicated peaks in ^{1}H-NMR and ^{13}C-NMR which confirm the existence of E/Z-diastereomers. The prepared compounds harbor two distinct double bonds; namely the C=N *exo* to thiazol ring and imine C=N *exo* to indole group which yield the possibility of four diastereoisomers; E_{ind} and Z_{ind} for isomers around double bond *exo* to indole moiety and E_{thiazo} and Z_{thiazo} for isomers around double bond *exo* to thiazole counterpart. Our ^{1}H-NMR and ^{13}C-NMR revealed the preferred formation of two isomers.

The set of proton peaks of the E-diastereomer is in general downfield shifted, approx. 0.1 ppm, compared to the Z-diastereomer. As a representative example, ^{1}H-NMR for compound 4b, confirms the formation of thiazolidinone ring with two characteristic singlet peaks for $SCH_2(E_{thiazo})$ and $SCH_2(Z_{thiazo})$ at δ 4.06 and 4.02 ppm, respectively.

The chemical shift of the C4 indole proton is very characteristic for the two diastereomers (E_{indo}/Z_{indo}). The indole C4 proton of the E-diastereomer appeared more downfield (approx. 0.6 ppm) as doublet of doublet around δ 8.18-8.16 ppm while the Z-diastereomer appeared relatively upfield as doublet of doublet around δ 7.54-7.52 ppm. The same pattern was observed for ^{13}C-NMR spectra, C4(E) and C4(Z) around δ 117.45 and 121.10 ppm, respectively.

The E_{thiazo} diastereomer of **4l**, showed as singlet peak for SCH_2 around δ 4.06 ppm and around δ 32.96 ppm in ^{1}H-NMR and ^{13}C-NMR spectra, respectively. The indole C4H showed doublet peak in ^{1}H-NMR around δ 9.04 ppm, and a singlet peak around δ 135.79 ppm in ^{13}C-NMR. These observations are consistent with the suggested structures and with the reported pattern of isatin-thiazolidinone derivatives [27].

The E-diastereomer and Z-diastereomer could be differentiated by 2D-NMR technique. $^{1}H^{1}H$-NOESY technique confirmed the presence of two diastereomers (E/Z) of isatin-thiazolidinone. E and Z protons could be assigned. Representative example is the E-diastereomer **4l**, showed NOE cross peak between NCH_3 and C4H, indicating that these two group are on the same plane. The Z-diastereomer 4m, however, showed no NOE cross peak between $C_{16}H_3$ and C_4H, as the NCH_3 and C4H are in different planes, fig. 3.

Figure 3

The **(*E*/*Z*)** diastereomers of isatin-thiazolidinene derivatives, **5(a-s),** showed the same patterns observed with isatin-thiazolidinone derivatives, **4(a-s)** in NMR spectra. This pattern is consistent with the suggested and reported structures of isatin-thiazolidinene derivatives [28-30].

X-Ray structures of two representative compounds, **5f** and **5g**, were determined in order to prove the allocated structures and to substantiate conformations of the synthesized compounds. For the X-ray structure determinations, X-ray quality single crystals of **5f and 5g** were obtained from *n*-hexane/ethyl acetate solution, **figures 4** and **5**. The X-ray analysis demonstrates that compound **5f** crystallizes in monoclinic crystal lattice with the "$Pna2_1/n_1$" space group and compound **5g** crystallizes orthorhombic crystal lattice with the "$Pna2_1$" space group. In these compounds, isatin and the central thiazolidene rings that are connected with each other through (–**C=N N=C**–) linkage lie nearly in the same plane. However, the propyl substituted on isatin moiety is tilted as compared to the plane of isatin ring with dihedral angle 87.67° for compound **5f** and 93.44° for compound **5g** (C5 N4 C14 C15) between the plane of propyl and isatin ring. In the isatin ring, the C(5)-O(1) bond shows a typical double bond character. For the two C-S bond lengths, C(1)-S1) and C(3)-S(1), the experimental values are in the standard range of C(Sp3)–S single bond [1.81 Å], [31].

Figure 4

Figure 5

While the theoretical values showed different bond lengths, the S(1) C(1) and S(1) C(3) bond lengths are 1.7379 Å and 1.734 Å for compound **5f** and 1.7365 Å and 1.7296 Å for compound **5g**, respectively. The C(1) S(1) C(3) bond angle being 89.70° for compound **5f** and

89.624° for compound **5g**. The most significant and interesting feature of compounds **5f** and **5g** is their molecular packing in the solid state, stabilized by hydrogen bond interactions, **figure 6**. In compound **5f,** each 1D-chain of this assembly is formed by the lateral arrangement of molecules by means of O…H [O(1)…H(13)C 2.3538 Å] interactions. The molecules in every chain of a layer with respect to another are inverted and perpendicular to each other. In compound **5g,** each 1D-chain of this assembly is formed by the lateral arrangement of molecules by means of O…H [O(1)…H(13B)C 2.4504 Å] and [O(1)…H(14A)C 2.5460 Å] interactions. The molecules in every chain of a layer with respect to another are inverted and on the same plane to each other. Other selected bond lengths and bond angles are presented in **table 1**.

Figure 6

Table 1

2.2. Biology.

2.2.1. Evaluation of anti-proliferative activity

The *in vitro* cytotoxicity of the targeted compounds **4(a-s)** and **5(a-s)** was evaluated using MTT colorimetric assay [32] on three cell lines; human liver cancer cell **(HepG2)**, breast cancer cell **(MCF-7)** and human colon cancer cell **(HT-29**) in addition to the diploid human normal cells **(WI-38)** according the protocol mentioned in the experimental section. **WI-38** are non-cancerous cells and were used to evaluate safety and selectivity of the tested compounds. Doxorubicin was used as a reference drug. The activities of the tested molecules are expressed as IC_{50} values (μmol/L) and are given in **table 2**.

Variable cytotoxic activities were shown by the tested compounds against the investigated cell lines. As a general pattern **isatin-thiazolidinenes**, **5(a-s)**, revealed relatively potent activities compared to **isatin-thiazolidinone**, **4(a-s)**.

Human colon cancer cell **(HT-29**) and human liver cancer cell **(HepG2**) were more sensitive to the tested compounds compared to the breast cancer cell **(MCF-7).** The worthy note is the pronounced selectivity of the tested compounds against the cancer cell lines compared to the normal ones (**WI-38**).

The most potent derivatives are among those with either 1-unsubstitued isatin or 1-propylisatin derivatives, while, the 1-benzylisatin derivatives revealed no considerable activity against the tested cell lines. The potency of isatin-thiazolidinone derivatives **4(a-s)** observed with the compounds bearing 5-nitroisatin moiety (**4o** and **4s**) however, the 5-substitued group seemed

to have no role in case of isatin-thiazolidinenes **5(a-s)** as revealed with the most potent compounds in this series of molecules (**5e, 5f, 5l, 5m** and **5o**).

Potency of the most active compounds (**4o, 4s, 5e, 5f, 5l, 5m and 5o)** in both series associated with the (*Z*) configurations of *N*=thiazolidin/ene or one, but the configuration of the *N*=isatin moiety seemed to be of no importance to the activity. Biological data of the tested compounds is not a sufficient data set for detailed SAR, however, the important result is the identification of 7 lead compounds for further development and optimization. At this point investigation of these 7 molecules on cell cycle checkpoint proteins is of interest to verify the hypotheses of the design.

Table 2

Most of compounds show different cytotoxic effect on the three cancer cell lines. Investigations of the cytotoxic activity against **HT-29** indicated that it was the most sensitive cell line to the influence of all synthesized compounds. Compound **4s** was found to be the most potent derivative against **HepG2**, as it was more potent than doxorubicin. Compound **5o** was found to be equipotent to doxorubicin against **HepG2**. Compounds **4o, 4s, 5e, 5f, 5l, 5m and 5o** were the most active compounds. Most compounds showed selectivity to cancer cell lines than normal cells (IC_{50} on WI-38 > 100 uM). Five of the most active compounds (**4s, 5e, 5f, 5l and 5m**) have R_2= propyl at isatin moiety, which indicates the significance of this group for the activity.

2.2.2. Evaluation of the tested compounds against some checkpoint genes

The most active compounds, **4o, 4s, 5e, 5f, 5l, 5m** and **5o**, were tested on the expression of ***CDK1, p53, caspase-3*** and ***caspase-9*** in order to highlight the possible molecular mechanism(s) of their antiproliferative efficacy**.** The selection of these genes is based on the fact that cell cycle progression is controlled by CDK/cyclin complexes. CDK1/cyclin A and CDK1/cyclin B complexes are the key molecules of G2/M checkpoint. The activation of CDK/cyclin complex promotes cell cycle progression, while most of DNA damage signals of cells induce cell cycle arrest by activating p53 *via* CHK2 to repair the damaged DNA. Activated p53 induces the transcription of p21, a CDK inhibitor, which can suppress G2/M transition by the inactivation of CDK/cyclin complex [5]. Apoptosis or programmed cell death, occurs in multicellular organisms and plays an important role in the regulation and maintenance of physiological conditions. It leads to various biochemical events including cell shrinkage, blebbing, nuclear fragmentation, and chromatin condensation. The mechanisms of apoptosis are divided into two pathways. One is the extrinsic pathway *via* death receptor and the other is

the mitochondrial intrinsic pathway. The ligation of death receptors and their ligands induces the formation of a death-inducing signalling complex, followed by the caspase-8 activation. Activated caspase-8 can transmit the apoptotic signals both directly *via* caspase-3 activation and indirectly *via* activation of pro- apoptotic B-cell lymphoma 2 (Bcl-2) family proteins. Activation of pro-apoptotic Bcl-2 family proteins can induce the mitochondrial permeabilization, resulting in the release of cytochrome c into the cytosol. In the intrinsic pathway, released cytochrome c can activate caspase-9, and then the activated caspase-9 induces the cleavage of procaspase-3. The cleaved caspase-3 through the extrinsic and intrinsic pathways can interact with its substrates, including PARP involved in DNA repair, finally resulting in cell death [33].

Accordingly, the human liver cancer cells **(HepG2)**, were treated with **4o, 4s, 5e, 5f, 5l, 5m and 5o** and the expression of **CDK1**, **p53**, **caspase-3** and **caspase-9** were monitored using real-time polymerase chain reaction technique (qRT-PCR), [34,35]. Doxorubicin was used as a reference drug, and the results are given in **table 3. HepG2** cells were selected since they were most responsive to the synthesized derivatives, they express many metabolizing enzymes, and in contrast to the breast cells, they express the investigated caspases. **MCF7** lack the expression of **caspase 3** [33].

Inspection of the data given in **table 3**, the tested compounds showed variable activities on the tested genes better than those observed with the reference drug in most cases. The tested compounds can be grouped for their possible mechanism of action into 4 categories. Compound **4o** with no apparent effect on all genes examined. Compounds **4s** and **5o** affected all genes investigated and seem to have multiple cellular targets; induced the expression of **p53** and caspases and inhibited that of **CDK1**. Compounds **5l** and **5m** directly elevated the expression of initiator and effector caspases without going through **p53** pathway. Finally, compounds **5e** and **5f** elevated the expression of **p53** and inhibited **CDK1**. These two compounds were unique in elevating the expression of execution **caspase-3** without inducing the expression of the upstream initiator **caspase 9**.

Many tumours express mutant or non-functional **p53** to avoid apoptosis [36,37]. Therefore, new molecules that directly induce **caspase-3** without going through **p53** and **caspase-9** are appraised and deserve more investigations as valuable leads. The limitations of the current study in cell lines versus real tumours in animal models and the limitations of q-PCR versus measuring the cleaved active **caspase-3** protein should be considered. However, the aim and scope of the current study are to investigate the antiproliferative activity of the novel isatin derivatives and explore their possible cellular mechanisms.

Table 3

2. 3. Molecular docking studies on CDK1

Modern molecular modelling techniques are remarkable tools in the search for potentially novel active agents by helping to understand and predict the behaviour of molecular systems, having assumed an important role in the development and optimisation of leading compounds. The aim of modelling methods is often to try to relate biological activity to structure. In drug designing process, molecular docking technique is usually used to investigate the most appropriate orientations and interactions of hit compound(s) at the active site of protein [38].

To reveal the binding mode of the most active compounds (**4o, 4s, 5e, 5f, 5i, 5m** and **5o**) on one of the targeted proteins (CDK1), docking studies were performed on commercial software Molecular Operating Environment (MOE). Structures of different protein crystal structures were retrieved from the Protein Data Bank (PDB) (www.rcsb.org/pdb).

Docking studies were done based on CDK1-ligand [*N*-(4-flurophenyl)-4-(2,6-flurobenzoylamino)-1*H*-pyrazole-3-carboxamide] co-crystallised complex (PDB 4Y72). The ligand, as shown in **Fig. 7,** interacts through three hydrogen bonds between pyrazole nitrogens and the carbonyl groups of Glu81 and Leu83. The aromatic ring showed a π-π stacking interaction with CDK1 backbone between Met85 and Asp86 amino acid residues [39].

Figure 7.

The binding interactions of the most active compounds (**4o**, **4s**, **5e**, **5f**, **5i**, **5m** and **5o**) with the active site residues were investigated *in silico*. It was found that the binding of compounds **4o,** with the highest binding affinity compared to the other tested compounds, is matched with the co-crystallized ligand, Fig. 8. The isatin carbonyl group, nitrogen and hydrogen atoms of the thiazolidinone ring of compound **4o,** bind to the enzyme active site residues Leu83, Glu81 and Asp86, respectively, through three hydrogen bonds. In addition to π-π interaction with the 4-nitrophenyl part of the isatin moiety conferring potential CDK1 inhibitory activity.

Figure 8.

Compound **5o** had less binding affinity due to missing of one hydrogen bond interaction on thiazolidinone moiety, Fig. 9.

Figure 9.

Based on the experimental results on cell lines, the least active compound **4j**, was docked on the CDK1 active sites for comparative study with **4o**. No binding interactions revealed between 4j with the active amino acid residues of the CDK1, Fig. 10.

Figure 10.

In conclusion the docking study supports the hypothesis of the mechanism of action of these molecules on checkpoints enzymes as represented with CDK1. Unsubstituted isatin nitrogen and potion 4- of the thiazolidinone ring are essential for hydrogen binding interaction with the CDK1 enzyme active sites and hence for activity. Finally it is worthy to mention that docking studies of *E*- and *Z*-diasteromers of the tested compounds revealed no differences in binding interactions or docking energy between the two diasteromers.

3. Materials and methods

3.1. General Notes

All chemicals were of commercially available reagent grades and were used without further purification. Precoated silica gel plates, 60G F254, obtained from Merck, Darmstadt (Germany), were used for thin layer chromatography (TLC). Spots were visualized using UV-lamp at λ_{max} 254 nm. Silica gel (60-120 mesh), was used for column chromatography and the developing systems used were: system (A): *n*-hexane/ethyl acetate (3:1), system (B): chloroform/ethyl acetate (2:1) and system (C): methylene chloride/ethyl acetate (2:1). Melting points (^{o}C) were determined on Stuart melting point apparatus (Stuart Scientific, England) and were uncorrected. NMR spectra were performed on Bruker Spectrophotometer (400 MHz for ^{1}H and 100 MHz for ^{13}C), Faculty of Science, Sohag University, Sohag, Egypt. Chemical shifts are expressed in δ values (ppm) relative to tetramethylsilane (TMS) as an internal standard. Coupling constants (*J*) for ^{1}H were given in Hz and expressed as (S) for singlet, (d) for doublet, (t) for triplet, (q) for quartet, (sxt) for sextet and (m) for multiplet. DMSO-d_6 was used as solvent. Deuterium oxide was used for the conformation of exchangeable protons and the exchangeable protons were assigned with (*). Elemental analyses were performed in the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. X-Ray crystallography was conducted at Department Chemie, Ludwig-Maximilians-Universität, München

Butenandtstr. 5-13, Haus D81377, München,German. The biological activity of the synthesized molecules were done at Department of Zoology, Faculty of Science, Ain Shams University, Cairo, Egypt. All molecular modelling studies were carried out using Molecular

Operating Environment (MOE 2019.0101, Chemical Computing Group, Canada) as the computational software, Department of Medicinal Chemistry, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

N-Benzyl- and propyl-isatins (1b, 1c, 2b, 2c, 3b, 3c) were prepared as described in literatures and the products were consistent with the reported physicochemical characters [38-41].

3.2. General procedures for synthesis of Isatin-Thiosemicarbazone derivatives 3(a-r)

Equimolar quantities of appropriate Isatin derivatives **(1a-3c)** and **4-substitued-3-Thiosemicarbazide** were dissolved in warm ethanol. The pH was adjusted to 4-5 with glacial acetic acid and heated under reflux for 1-2 hr. The reaction mixture was allowed to stand at room temperature and then poured into crushed ice, the yellow products were separated by filtration, dried and purified by flash column chromatography on silica gel using appropriate solvent system.

Isatin-Thiosemicarbazones (3a-d, 3g,3h, 3m and 3n) were reported and their physicochemical charters were consistent with reported ones [19,21,24,42].

(*Z*)-2-(1-propyl-2-oxoindolin-3-ylidene)-*N*-Ethylhydrazinecarbothioamide (3e)

Developing system of chromatography: A

Yield (%): 95

M.P. : 130-133

^{1}H-NMR: 12.52 (br s, 1H, NN<u>H</u>*), 9.23 (br s, 1H, <u>NH</u>*CH_2CH_3), 7.73 (dd, *J* = 7.3 Hz, 1H, C_4H), 7.44 (td, *J* = 7.3 Hz, 1H, C_5H), 7.19-7.21 (m, 1H, C_6H), 7.16 (dd, *J* = 7.3 Hz, 1H, C_7H), 3.73 (t, *J* = 7.3 Hz, 2H, N<u>CH_2</u>CH_2CH_3), 3.63-3.68 (m, 2H, NH<u>CH_2</u>CH_3), 1.68 (sxt, *J* =7.3 Hz, 2H, (NCH_2<u>CH_2</u>CH_3), 1.22 (t, *J* = 7.1 Hz, 3H, $NHCH_2$<u>CH_3</u>), 0.91 (t, *J* = 7.3 Hz, 3H, NCH_2CH_2<u>CH_3</u>).

^{13}C-NMR: 177.49 (C=S), 161.30 (C=O), 141.79 (C=N), 132.36, 131.90, 131.38, 121.53, 119.82, 110.32 (Ar C), 42.13 (C_8), 39.56 (C_{15}), 20.42 (C_9), 14.52 (C_{16}), 11.42 (C_{10})

Elemental analyses calculated for $C_{14}H_{18}N_4OS$: C, 57.91; H, 6.25; N, 19.29; S, 11.04. Found: C, 57.82; H, 6.10; N, 19.32; S, 10.99.

(*Z*)-2-(1-propyl-2-oxoindolin-3-ylidene)-*N*-ethylhydrazinecarbothioamide (3f)

Developing system of chromatography: A

Yield (%): 93

M.P. : 152-154

^{1}H-NMR: 12.55 (s, 1H, NNH*), 9.21 (s, 1H, NH*CH$_3$), 7.7 (dd, *J* = 7.1 Hz, 1H, C$_4$H), 7.41-7.45 (m, 1H, C$_5$H), 7.15-7.2 (m, 2H, C$_6$H, C$_7$H), 3.73 (t, *J* = 7.20, 2H, NCH$_2$CH$_2$CH$_3$), 3.11 (d, *J* = 3.4 Hz, 3H, NHCH$_3$), 1.73-1.63 (m, 2H, NCH$_2$CH$_2$CH$_3$), 0.91 (t, *J* = 7.20 Hz, 3H, NCH$_2$CH$_2$CH$_3$).

^{13}C-NMR: 176.49 (C=S), 160.30 (C=O), 142.79 (C=N), 133.34, 131.90, 131.38, 129.52, 117.82, 111.02 (Ar C), 43.23 (C$_8$) 32.56 (C$_{15}$), 20.50 (C$_9$), 11.50 (C$_{10}$)

Elemental analyses calculated for C$_{13}$H$_{16}$N$_4$OS: C, 56.50; H, 5.84; N, 20.27; S, 11.60. Found: C, 56.4; H, 5.72; N, 20.31; S, 11.73.

(*Z*)-2-(1-benzyl-5-methyl-2-oxoindolin-3-ylidene)-*N*-ethylhydrazinecarbo-thioamide (3i)

Developing system of chromatography: C

Yield (%): 92

M.P. : 200-203

^{1}H-NMR: 12.49 (s, 1H, NNH*), 9.30 (t, *J* = 4.9 Hz, 1H, NH*CH$_2$CH$_3$), 7.57 (s, 1H, C$_4$H), 7.38-7.26 (m, 5H, ph protons), 7.17 (d, *J* = 7.9 Hz, 1H, C$_6$H), 6.92 (d, *J* = 7.9 Hz, 1H, C$_7$H), 4.96 (s, 2H, NCH$_2$-ph), 3.67 (m, 2H, NHCH$_2$CH$_3$), 2.31 (s, 3H, C$_5$CH$_3$), 1.22 (t, *J* = 7.1 Hz, 3H, NHCH$_2$CH$_3$).

^{13}C-NMR: 177.24 (C=S), 161.31 (C=O), 140.79 (C=N), 136.26, 132.63, 131.75, 131.13, 129.6, 129.4, 128.03, 127.90, 127.86, 121.55, 119.19, 110.62 (Ar C), 43.05 (C$_8$), 39.58 (C$_{18}$), 21.01 (C$_{5a}$), 14.46 (C$_{19}$).

Elemental analyses calculated for C$_{19}$H$_{20}$N$_4$OS: C, 64.75; H, 5.72; N, 15.90; S, 9.10. Found: C, 64.84; H, 5.81; N, 15.85; S, 9.05.

(*Z*)-2-(1-benzyl-5-methyl-2-oxoindolin-3-ylidene)-*N*-methylhydrazinecarbo-thioamide (3j)

Developing system of chromatography: C

Yield (%): 95

M.P. : 260-262

^{1}H-NMR: 12.51 (s, 1H, NNH*), 9.28 (d, *J* = 3.9 Hz, 1H, NH*CH$_3$), 7.55 (s, 1H, C$_4$H), 7.38-7.26 (m, 5H, ph protons), 7.17 (d, *J* = 7.9 Hz, 1H, C$_6$H), 6.93 (d, *J* = 7.9 Hz, 1H, C$_7$H), 4.97 (s, 2H, NCH$_2$-ph), 3.11 (d, *J* = 3.90 Hz, 3H, NHCH$_3$), 2.31 (s, 3H, C$_5$CH$_3$).

^{13}C-NMR: 178.22 (C=S), 161.31 (C=O), 140.8 (C=N), 136.27, 132.63, 131.75, 131.07, 129.25, 129.15, 128.03, 127.90, 127.87, 121.44, 120.01, 110.65 (Ar C), 43.06 (C$_8$), 31.84 (C$_{18}$).

Elemental analyses calculated for C$_{18}$H$_{18}$N$_4$OS: C, 63.88; H, 5.36; N, 16.56; S, 9.47. Found: C, 63.52; H, 5.01; N, 16.91; S, 9.17.

(*Z*)-2-(1-propyl-5-methyl-2-oxoindolin-3-ylidene)-*N*-ethylhydrazinecarbo-thioamide (3k)

Developing system of chromatography: A

Yield (%): 95

M.P. : 203-206

^{1}H-NMR: 12.49 (s, 1H, NNH*), 9.26 (br. s, 1H, NH*CH$_2$CH$_3$), 7.54 (s, 1H, C$_4$H), 7.24 (d, *J* = 8.1 Hz, 1H, C$_6$H), 7.07 (d, *J* =8.1 Hz, 1H, C$_7$H), 3.71-3.63 (m, 4H, NCH$_2$CH$_2$CH$_3$, NHCH$_2$CH$_3$), 2.34 (s, 3H, C$_5$CH$_3$), 1.65 (sxt, *J* =7.2 Hz, 2H, (NCH$_2$CH$_2$CH$_3$), 1.21 (t, *J* =7.1 Hz, 3H, NHCH$_2$CH$_3$), 0.89 (t, *J* =7.2 Hz, 3H, NCH$_2$CH$_2$CH$_3$).

^{13}C-NMR: 177.24 (C=S), 161.28 (C=O), 141.17 (C=N), 132.34, 131.87, 131.31, 121.52, 119.82, 110.29 (Ar C), 39.55 (C$_{15}$), 21.01 (C$_{5a}$), 20.88 (C$_9$), 14.45 (C$_{16}$), 11.59 (C$_{10}$).

Elemental analyses calculated for C$_{15}$H$_{20}$N$_4$OS: C, 59.18; H, 6.62; N, 18.41; S, 10.53. Found: C, 59.33; H, 6.53; N, 18.49; S, 10.85.

(*Z*)-2-(1-propyl-5-methyl-2-oxoindolin-3-ylidene)-*N*-methylhydrazinecarbo-thioamide (3l)

Developing system of chromatography: A

Yield (%): 96

M.P. : 250-253

^{1}H-NMR: 12.53 (s, 1H, NNH*), 9.20 (br. s, 1H, NH*CH$_3$), 7.53 (s, 1H, C$_4$H), 7.24 (d, *J* = 7.9 Hz, 1H, C$_6$H), 7.07 (d, *J* = 7.9 Hz 1H, C$_7$H), 3.7 (t, *J* = 7 Hz, 2H, NCH$_2$CH$_2$CH$_3$), 3.11 (d, *J* = 4.2, Hz 3H, NHCH$_3$), 2.34 (s, 3H, C$_5$CH$_3$), 1.66 (sxt, *J* =7 Hz, 2H, (NCH$_2$CH$_2$CH$_3$), 0.9 (t, *J* =7 Hz, 3H, NCH$_2$CH$_2$CH$_3$).

^{13}C-NMR: 178.22 (C=S), 161.28 (C=O), 141.18 (C=N), 132.32, 131.84, 121.42, 119.77, 110.22 (Ar C), 41.23 (C$_8$), 39.55 (C$_{15}$), 31.83 (C$_{15}$), 21.02 (C$_{5a}$), 20.88 (C$_9$), 11.59 (C$_{10}$).

Elemental analyses calculated for C$_{14}$H$_{18}$N$_4$OS: C, 57.91; H, 6.25; N, 19.29; S, 11.04. Found: C, 57.88; H, 6.30; N, 19.10; S, 11.32.

(*Z*)-2-(1-benzyl-5-nitro-2-oxoindolin-3-ylidene)-*N*-ethylhydrazinecarbo-thioamide (3o)

Developing system of chromatography: C

Yield (%): 92

M.P. : 155-158

^{1}H-NMR: 12.30 (s, 1H, NNH*), 9.66 (br. t, *J* = 6.80 Hz, 1H, NH*CH$_2$CH$_3$), 8.63 (s, 1H, C$_4$H), 8.29 (d, *J* = 8.9 Hz, 1H, C$_6$H), 7.41-7.30 (m, 5H, ph protons), 7.26 (d, *J* = 8.90 Hz, 1H, C$_7$H), 5.09 (s, 2H, NCH$_2$-ph), 3.7 (p, *J* = 6.8 Hz, 2H, NHCH$_2$CH$_3$), 1.24 (t, *J* = 6.80 Hz, 3H, NHCH$_2$CH$_3$).

^{13}C-NMR: 177.21 (C=S), 161.69 (C=O), 147.52 (C=N), 143.81, 135.52, 129.30, 129.25, 129.06, 128.27, 127.92, 127.84, 127.04, 121.03, 116.39, 110.94 (Ar C), 43.51 (C_8), 18.86 (C_{18}), 14.28 (C_{19}).

Elemental analyses calculated for $C_{18}H_{17}N_5O_3S$: C, 56.39; H, 4.47; N, 18.27; S, 8.36. Found: C, 56.44; H, 4.50; N, 18.16; S, 8.43.

(*Z*)-2-(1-benzyl-5-nitro-2-oxoindolin-3-ylidene)-*N*-methylhydrazinecarbo-thioamide (3p)

Developing system of chromatography: C

Yield (%): 95

M.P. : 272-275

^{1}H-NMR: 12.32 (s, 1H, N<u>NH</u>*), 9.61 (br. q, *J* = 3.9 Hz, 1H, <u>NH</u>*CH_3), 8.61 (s, 1H, C_4H), 8.28 (dd, *J* = 8.8 Hz, 0.9 Hz, 1H, C_6H), 7.41-7.30 (m, 5H, ph protons), 7.26 (d, *J* = 8.8 Hz, 1H, C_7H), 5.08 (s, 2H, N<u>CH_2</u>-ph), 3.14 (d, *J* = 3.90 Hz, 3H, NH<u>CH_3</u>).

^{13}C-NMR: 178.1 (C=S). 161.68 (C=O), 147.55 (C=N), 143.76, 135.61, 129.28, 129.22, 128.96, 128.22, 127.92, 127.89, 127.02, 121.1, 116.24, 110.99 (Ar C), 43.45 (C_8), 31.91 (C_{18}).

Elemental analyses calculated for $C_{17}H_{15}N_5O_3S$: C, 55.28; H, 4.09; N, 18.96; S, 8.68. Found: C, 55.30; H, 4.12; N, 19.01; S, 8.82.

(*Z*)-2-(1-propyl-5-nitro-2-oxoindolin-3-ylidene)-*N*-ethylhydrazinecarbo-thioamide (3q)

Developing system of chromatography: A

Yield (%): 93

M.P. : 152-153

^{1}H-NMR: 12.30 (s, 1H, N<u>NH</u>*), 9.62 (br. t, *J* = 6.70 Hz, 1H, <u>NH</u>*CH_2CH_3), 8.61 (br. s, 1H, C_4H), 8.34 (dd, *J* = 8.6 Hz, 1.4 Hz, 1H, C_6H), 7.45 (d, *J* = 8.6 Hz, 1H, C_7H), 3.81 (t, *J* = 7.2 Hz, 2H, N<u>CH_2</u>CH_2CH_3), 3.68 (m, 2H, NH<u>CH_2</u>CH_3), 1.68 (sxt, *J* = 7.2 Hz, 2H, (NCH_2<u>CH_2</u>CH_3), 1.23 (t, *J* =6.70 Hz, 3H, $NHCH_2$<u>CH_3</u>), 0.92 (t, *J* = 7.20 Hz, 3H, NCH_2CH_2<u>CH_3</u>).

^{13}C-NMR: 177.11 (C=S), 161.74 (C=O), 148.06(C=N), 143.49, 129.22, 127.16, 120.79, 118.31, 110.71 (Ar C), 41.76 (C_8), 39.48 (C_{15}), 20.92 (C_9), 14.34 (C_{16}), 11.53 (**C**$_{10}$).

Elemental analyses calculated for $C_{14}H_{17}N_5O_3S$: C, 50.14; H, 5.11; N, 20.88; S, 9.56. Found: C, 50.19; H, 5.47; N, 21.01; S, 9.40.

(*Z*)-2-(1-propyl-5-nitro-2-oxoindolin-3-ylidene)-*N*-methylhydrazinecarbo-thioamide (3r)

Developing system of chromatography: A

Yield (%): 96

M.P. : 241-243

^{1}H-NMR: 12.32 (s, 1H, N<u>NH</u>*), 9.53 (br s, 1H, <u>NH</u>*CH_3), 8.58 (s, 1H, C_4H), 8.33 (d, *J* = 8.80 Hz, 1H, C_6H), 7.44 (d, *J* = 8.8 Hz, 1H, C_7H), 3.8 (t, *J* = 7.30 Hz, 2H, N<u>CH_2</u>CH_2CH_3), 3.13 (d, *J* = 4.3 Hz, 3H, NH<u>CH_3</u>), 1.69 (sxt, *J* = 7.30 Hz, 2H, (NCH_2<u>CH_2</u>CH_3), 0.92 (t, *J* = 7.3 Hz, 3H, NCH_2CH_2<u>CH_3</u>).

^{13}C-NMR: 177.12 (C=S), 161.77 (C=O), 148.03(C=N), 142.49, 129.20, 127.14, 120.76, 118.21, 110.69 (Ar C), 41.77 (C_8), 31.19 (C_{15}), 20.96 (C_9), 11.58 **(**C_{10}).

Elemental analyses calculated for $C_{13}H_{15}N_5O_3S$: C, 48.59; H, 4.71; N, 21.79; S, 9.98. Found: C, 48.49; H, 4.83; N, 21.91; S, 10.05.

3.3. General Procedures for Synthesis of (*Z*)-3-substituted-2-(((*E/Z*)-5-substituted-2-oxo-1-substituted-indolin-3-ylidene)hydrazineylid-ene)thiazolidin-4-one 4(a-s)

A mixture of appropriate isatin-3-(*Z*)-thiosemicarbazone, 3(a-r), (1 mmol), monochloroacetic acid (1 mmol) and anhydrous sodium acetate (2 mmol) in ethanol (50 ml) was refluxed for 24 hrs with occasional shaking. The reaction mixtures were cooled, poured into crushed ice, and the precipitates were filtered and dried to give yellow to orange products. The produced diastereomers (*E/Z*) either separated as mixture or resoluted on silica gel column using the appropriate developing system to the corresponding single diastereomer.

(Z)-3-Ethyl-2-(((*E*)-2-oxoindolin-3-ylidene)hydrazineylidene)thiazolidin-4-one (4a)

Developing system of chromatography: B

Yield (%): 22

M.P. : 270-273

^{1}H-NMR: 10.66 (s, 1H, <u>NH</u>*), 8.14-8.12 (m, 1H, C_4H), 7.39-7.35 (m, 1H, C_5H), 7.06-7.02 (m, 1H, C_6H), 6.90-6.89 (m, 1H, C_7H), 4.06 (s, 2H, S<u>CH_2</u>), 3.92 (q, *J* = 6.80 Hz, 2H, N<u>CH_2</u>CH_3), 1.28 (t, *J* = 6.80 Hz, 3H, NCH_2<u>CH_3</u>).

^{13}C-NMR: 172.68 (thiazol C=O), 172.42 (indole C=O), 165.24 (thiazol C=N), 149.14 (indole C=N), 144.81 (C_5), 133.39 (C_4), 128.51 (C_6), 122.61 (C_{7a}), 117.46 (C_{3a}), 111.01 (C_7), 38.88 (C_{13}), 33.04 (C_{12}), 12.56 (C_{14}).

Elemental analyses for calculated $C_{13}H_{12}N_4O_2S$: C, 54.16; H, 4.20; N, 19.43; S, 11.12. Found: C, 54.2; H, 4.35; N, 19.55; S, 11.05.

(*Z*)-3-methyl-2-(((*E/Z*)-2-oxoindolin-3-ylidene)hydrazineylidene)thiazolidin-4-one (4b)

Developing system of chromatography: B

Yield (%): 65

M.P. : 245-248

^{1}H-N-MR (*E/Z* %, 63:37): 10.66 (s, 1H, $\underline{NH}^*_{(E)}$) and 10.64 (s, 1H, $\underline{NH}^*_{(Z)}$), 8.18-8.16 (m, 1H, $C_4H_{(E)}$), 7.54-7.52 (m, 1H, $C_4H_{(Z)}$), 7.4-7.34 (m, 2H, $C_5H_{(E+Z)}$), 7.06-7.01 (m, 2H, $C_6H_{(E+Z)}$), 6.9-6.88 (m, 1H, $C_7H_{(E)}$), 6.86-6.84 (m, 1H, $C_7H_{(Z)}$), 4.06(s, 2H, $S\underline{CH_2}_{(E)}$), 4.02 (s, 2H, $S\underline{CH_2}_{(Z)}$), 3.33 (s, 3H, $N\underline{CH_3}_{(E)}$), 3.25 (s, 3H, $N\underline{CH_3}_{(Z)}$).

^{13}C-NMR: 172.86 (indole 2×C=$O_{(E+Z)}$), 172.86 (thiazol C=$O_{(E)}$), 169.97 (thiazol C=$O_{(Z)}$), 165.27 (thiazol C=$N_{(E)}$), 159.13 (thiazol C=$N_{(Z)}$), 149.27 (indole C=$N_{(E)}$), 147.07 (indole C=$N_{(Z)}$), 144.78 ($C_{4(E)}$), 143.92 ($C_{4(Z)}$), 133.35 ($C_{5(E)}$), 132.91 ($C_{5(Z)}$), 129.01 ($C_{6(E)}$), 122.60 ($C_{7a(Z)}$), 122.31 ($C_{7a(E)}$), 121.86 ($C_{6(Z)}$), 121.10 ($C_{3a(Z)}$), 117.45 ($C_{3a(E)}$), 110.94 ($C_{7(E)}$), 110.80 ($C_{7(Z)}$), 33.02 ($C_{13(E)}$), 32.79 ($C_{13(Z)}$), 30.26 ($C_{12(E)}$), 30.17 ($C_{12(Z)}$).

Elemental analyses for calculated $C_{12}H_{10}N_4O_2S$: C, 52.55; H, 3.67; N, 20.43; S, 11.69. Found: C, 52.39; H, 3.77; N, 20.51; S, 11.73.

(*Z*)-2-(((*E*)-1-benzyl-2-oxoindolin-3-ylidene)hydrazineylidene)-3-ethylthiaz-olidin-4-one (4c)

Developing system of chromatography: C

Yield (%): 22

M.P. : 248-250

^{1}H-NMR: 8.22-8.20 (m, 1H, C_4H), 7.41-7.33 (m, 5H, ph protons), 7.30-7.26 (m, 1H, C_5H), 7.13-7.09 (m, 1H, C_6H), 7.01-6.99 (m, 1H, C_7H), 4.98 (s, 2H, $N\underline{CH_2}$-ph), 4.09 (s, 2H, $S\underline{CH_2}$), 3.94 (q, J = 7 Hz, 2H, $N\underline{CH_2}CH_3$), 1.29 (t, J = 7 Hz, 3H, $NCH_2\underline{CH_3}$).

^{13}C-NMR: 173.18 (thiazol C=O), 172.70 (indole C=O), 164.05 (thiazol C=N), 148.08 (indole C=N), 144.83, 133.39, 136.65, 129.20, 129.16, 128.40, 127.93, 127.72, 127.68, 123.33, 116.97, 110.25(Ar C), 43.20 (C_8), 38.96 (C_{20}), 33.10 (C_{19}), 12.55 (C_{21}).

Elemental analyses calculated for $C_{20}H_{18}N_4O_2S$: C, 63.47; H, 4.79; N, 14.80; S, 8.47. Found: C, 63.39; H, 4.70; N, 14.75; S, 8.38.

(*Z*)-2-(((*E*)-1-benzyl-2-oxoindolin-3-ylidene)hydrazineylidene)-3-methyl-thiazolidin-4-one (4d)

Developing system of chromatography: C

Yield (%): 23

M.P. : 250-253

^{1}H-NMR: 8.26-8.24 (m, 1H, C_4H), 7.41-7.33 (m, 5H, ph protons), 7.30-7.26 (m, 1H, C_5H), 7.13-7.09 (m, 1H, C_6H), 7.01-6.99 (m, 1H, C_7H), 4.98 (s, 2H, N$\underline{CH_2}$-ph), 4.09 (s, 2H, S$\underline{CH_2}$), 3.35 (s, 3H, N$\underline{CH_3}$).

^{13}C-NMR: 173.20 (thiazol C=O), 172.73 (indole C=O), 163.98 (thiazol C=N), 147.99 (indole C=N), 144.72, 133.30, 136.60, 129.23, 129.20, 128.33, 127.50, 127.90, 127.88, 123.30, 116.80, 110.31(Ar C), 43.29 (C_8), 32.79 (C_{20}), 30.10 (C_{19}).

Elemental analyses for $C_{19}H_{16}N_4O_2S$: C, 62.62; H, 4.43; N, 15.37; S, 8.80. Found: C, 62.57; H, 4.51; N, 15.29; S, 8.68.

(*Z*)-2-(((*E*)-1-propyl-2-oxoindolin-3-ylidene)hydrazineylidene)-3-ethylthia-zolidin-4-one (4e)

Developing system of chromatography: A

Yield (%): 22

M.P. : 196-198

^{1}H-NMR: 8.20-8.18 (m, 1H, C_4H), 7.48-7.44 (m, 1H, C_5H), 7.15-7.11 (m, 1H, C_6H), 7.11-7.09 (m, 1H, C_7H), 4.07 (s, 2H, S$\underline{CH_2}$), 3.93 (q, J = 6.8 Hz, 2H, N$\underline{CH_2}CH_3$), 3.71 (t, J = 7.30 Hz, 2H, N$\underline{CH_2}CH_2CH_3$), 1.71-1.62 (m, 2H, $NCH_2\underline{CH_2}CH_3$), 1.3 (t, J = 6.80 Hz, 3H, $NCH_2\underline{CH_3}$), 0.91 (t, J = 7.3 Hz, 3H, $NCH_2CH_2\underline{CH_3}$).

^{13}C-NMR: 172.63 (thiazol C=O), 172.38 (indole C=O), 163.85 (thiazol C=N), 148.65 (indole C=N), 143.07 (C_5), 133.55 (C_4), 131.86 (C_6), 129.06 (C_{7a}), 120.27 (C_{3a}), 109.66 (C_7), 41.29 (C_8), 38.93 (C_{16}), 33.10 (C_{15}), 20.82 (C_9), 12.42(C_{17}), 11.57 (C_{10}).

Elemental analyses calculated for $C_{16}H_{18}N_4O_2S$: C, 58.16; H, 5.49; N, 16.96; S, 9.70. Found: C, 58.02; H, 5.55; N, 16.86; S, 9.85.

(*Z*)-2-(((*E*)-1-propyl-2-oxoindolin-3-ylidene)hydrazineylidene)-3-methylthia-zolidin-4-one (4f)

Developing system of chromatography: A

Yield (%): 20

M.P. : 202-205

^{1}H-NMR: 8.24-8.22 (m, 1H, C_4H), 7.47-7.44 (m, 1H, C_5H), 7.15-7.11 (m, 1H, C_6H), 7.11-7.09 (m, 1H, C_7H), 4.07 (s, 2H, S$\underline{CH_2}$), 3.7 (t, J = 6.9 Hz, 2H, N$\underline{CH_2}CH_2CH_3$), 3.33 (s, 3H, N$\underline{CH_3}$), 1.69-1.60 (m, 2H, $NCH_2\underline{CH_2}CH_3$), 0.9 (t, J = 6.9 Hz, 3H, $NCH_2CH_2\underline{CH_3}$).

^{13}C-NMR: 172.76 (thiazol C=O), 172.61 (indole C=O), 163.92 (thiazol C=N), 148.71 (indole C=N), 143.20 (C_5), 133.50 (C_4), 131.88 (C_6), 129.40 (C_{7a}), 116.92 (C_{3a}), 109.51 (C_7), 41.40 (C_8)**,** 32.96 (C_{15}), 30.18 (C_{16})**,** 20.82 (C_9)**,** 11.50 (C_{10})**.**

Elemental analyses calculated for $C_{15}H_{16}N_4O_2S$: C, 56.95; H, 5.10; N, 17.71; S, 10.13. Found: C, 56.86; H, 5.19; N, 17.66; S, 9.95.

(*Z*)-3-ethyl-2-(((*E*)-5-methyl-2-oxoindolin-3-ylidene)hydrazineylidene)thia-zolidin-4-one (4g)

Developing system of chromatography: B

Yield (%): 22

M.P. : 280-283

^{1}H-NMR: 10.60 (s, 1H, $\underline{NH}^{*}$), 7.97 (s, 1H, C_4H), 7.21-7.19 (m, 1H, C_6H), 6.79 (d, J = 7.8 Hz, 1H, C_7H), 4.07 (s, 2H, $S\underline{CH_2}$), 3.92 (q, J = 7.1 Hz, 2H, $N\underline{CH_2}CH_3$), 2.28 (s, 3H, $C_5\underline{CH3}$), 1.3 (t, J = 7.1 Hz, 3H, $NCH_2\underline{CH_3}$).

^{13}C-NMR: 172.64 (thiazol C=O), 171.94 (indole C=O), 165.35 (thiazol C=N), 149.47 (indole C=N), 142.49 (C_5), 133.72 (C_4), 131.37 (C_6), 129.08 (C_{7a}), 117.52 (C_{3a}), 110.80 (C_7), 38.90 (C_{13}), 33.04 (C_{12}), 21.14 (C_{5a})**,** 12.41 (C_{14}).

Elemental analyses calculated for $C_{14}H_{14}N_4O_2S$: C, 55.62; H, 4.67; N, 18.53; S, 10.60. Found: C, 55.59; H, 4.77; N, 18.60; S, 10.71.

(*Z*)-3-methyl-2-(((*E/Z*)-5-methyl-2-oxoindolin-3-ylidene)hydrazineylidene)-thiazolidin-4-one (4h)

Developing system of chromatography: B

Yield (%): 62

M.P. : 235-238

^{1}H-NMR (*E/Z* %, 64:36): 10.60 (s, 1H, $\underline{NH}^{*}_{(E)}$), 10.57 (s, 1H, $\underline{NH}^{*}_{(Z)}$), 7.98 (s, 1H, $C_4H_{(E)}$), 7.34 (s, 1H, $C_4H_{(Z)}$), 7.20-7.16 (m, 2H, $C_6H_{(E+Z)}$), 6.78 (d, J = 7.8 Hz, 1H, $C_7H_{(E)}$), 6.74 (d, J = 7.8 Hz, 1H, $C_7H_{(Z)}$), 4.06 (s, 2H, $S\underline{CH_{2(E)}}$), 4.02 (s, 2H, $S\underline{CH_{2(Z)}}$), 3.33 (s, 3H, $N\underline{CH_{3(E)}}$), 3.28 (s, 3H, $N\underline{CH_{3(Z)}}$), 2.28 (s, 6H, $C_5\underline{CH3}_{(E+Z)}$).

^{13}C-NMR: 172.89 (thiazol $C=O_{(E)}$), 172.62 (thiazol $C=O_{(Z)}$), 172.89 (indole $2\times C=O_{(E+Z)}$), 165.35 (thiazol $C=N_{(E)}$), 159.30 (thiazol $C=N_{(Z)}$), 149.43 (indole $C=N_{(E)}$), 147.28 (indole $C=N_{(Z)}$), 142.53 ($C_{5(E)}$), 141.70 ($C_{5(Z)}$), 133.69 ($C_{4(E)}$), 133.38 ($C_{4(Z)}$), 131.35 ($C_{6(E)}$), 131.29 ($C_{6(Z)}$), 129.43 ($C_{7a(E)}$), 121.10 ($C_{3a(Z)}$), 117.50 ($C_{3a(E)}$), 110.62 ($C_{7(Z)}$), 110.70 ($C_{7(E)}$), 33.00 ($C_{13(E)}$), 32.77 ($C_{13(Z)}$), 30.2 ($2\times C_{12(E+Z)}$), 21.19 ($C_{5a(E)}$), 20.93 ($C_{5a(Z)}$).

Elemental analyses calculated for $C_{13}H_{12}N_4O_2S$: C, 54.16; H, 4.20; N, 19.43; S, 11.12. Found: C, 54.27; H, 4.32; N, 19.55; S, 11.23.

(*Z*)-2-(((*E*)-1-benzyl-5-methyl-2-oxoindolin-3-ylidene)hydrazineylidene)-3-ethylthiazolidin-4-one (4i)

Developing system of chromatography: C

Yield (%): 22

M.P. : 255-258

^{1}H-NMR: 8.05 (s, 1H, C_4H), 7.35-7.27 (m, 5H, ph protons), 7.22-7.20 (m, 1H, C_6H), 6.89 (d, J = 8.1 Hz, 1H, C_7H), 4.95 (s, 2H, N$\underline{CH_2}$-ph.), 4.10 (s, 2H, S$\underline{CH_2}$), 3.94 (q, J = 6.8 Hz, 2H, N$\underline{CH_2}CH_3$), 2.28 (s, 3H, $\underline{C_5}\underline{CH3}$), 1.31 (t, J = 7.8 Hz, 3H, $NCH_2\underline{CH_3}$).

^{13}C-NMR: 172.75 (thiazol C=O), 172.67 (indole C=O), 148.44 (indole C=N), 164.05 (thiazol C=N), 142.62, 136.70, 133.46, 132.23, 129.14, 129.09, 127.90, 127.69, 127.65, 117.02, 110.08 (Ar C), 43.16 (C_8), 38.96 (C_{20}), 33.14 (C_{19}), 21.11 (C_{5a})**,** 12.42 (C_{21}).

Elemental analyses calculated for $C_{21}H_{20}N_4O_2S$: C, 64.27; H, 5.14; N, 14.28; S, 8.17. Found: C, 64.0; H, 5.10; N, 14.22; S, 8.12.

(*Z*)-2-(((*E/Z*)-1-benzyl-5-methyl-2-oxoindolin-3-ylidene)hydrazineylidene)-3-methylthiazolidin-4-one (4j)

Developing system of chromatography: C

Yield (%): 63

M.P. : 257-260

^{1}H-NMR (*E/Z* %, 85:15): 8.06 (s, 1H, $C_4H_{(E)}$), 7.41 (s, 1H, $C_4H_{(Z)}$), 7.35-7.27 (m, 10H, ph protons$_{(E+Z)}$), 7.21-7.18 (m, 2H, $C_6H_{(E+Z)}$), 6.90-6.85 (m, 2H, $C_7H_{(E+Z)}$), 4.95 (s, 2H, N$\underline{CH_2}$-ph$_{(E)}$), 4.90 (s, 2H, N$\underline{CH_2}$-ph$_{(Z)}$), 4.09 (s, 2H, S$\underline{CH_{2(E)}}$), 4.05 (s, 2H, S$\underline{CH_{2(Z)}}$), 3.35 (s, 6H, N$\underline{CH_{3(E+Z)}}$), 2.29 (s, 6H, $\underline{C_5}\underline{CH3}_{(E+Z)}$)

^{13}C-NMR: 173.47 (thiazol 2×C=O$_{(E+Z)}$), 172.95 (indole 2×C=O$_{(E+Z)}$), 164.08 (thiazol 2×C=N$_{(E+Z)}$), 148.38 (indole 2×C=N$_{(E+Z)}$), 142.26 (2×$C_{5(E+Z)}$), 136.72 (2×$C_{9(E+Z)}$), 133.45 (2×$C_{4(E+Z)}$), 132.27 (2×$C_{6(E+Z)}$), 129.38 (2×$C_{12(E+Z)}$), 129.14 (2×$C_{14(E+Z)}$), 129.08 (2×$C_{10(E+Z)}$), 127.90 ($C_{7a(E)}$), 127.73 ($C_{7a(Z)}$), 127.69 (2×$C_{11(E+Z)}$), 127.66 (2×$C_{13(E+Z)}$), 116.97 (2×$C_{3a(E+Z)}$), 110.01 (2×$C_{7(E+Z)}$), 43.18 (2×$C_{8(E+Z)}$), 33.08 ($C_{20(E)}$), 32.86($C_{20(Z)}$), 30.27 ($C_{19(E)}$), 30.19 ($C_{19(Z)}$), 21.14 ($C_{5a(E)}$), 20.88 ($C_{5a(Z)}$).

Elemental analyses calculated for $C_{20}H_{18}N_4O_2S$: C, 63.47; H, 4.79; N, 14.80; S, 8.47. Found: C, 63.39; H, 4.77; N, 14.62; S, 8.55.

(*Z*)-2-(((*E/Z*)-1-propyl-5-methyl-2-oxoindolin-3-ylidene)hydrazineylidene)-3-ethylthiazolidin-4-one (4k)

Developing system of chromatography: A

Yield (%): 67

M.P. : 185-187

^{1}H-NMR (*E/Z* %, 62:38): 8.03 (s, 1H, $C_4H_{(E)}$), 7.39 (s, 1H, $C_4H_{(Z)}$), 7.28-7.24 (m, 2H, $C_6H_{(E+Z)}$), 7.03 (d, *J* = 7.9 Hz, 1H, $C_7H_{(E)}$), 6.99 (d, *J* = 7.9 Hz, 1H, $C_7H_{(Z)}$), 4.08 (s, 2H, $S\underline{CH_2}_{(E)}$), 4.05 (s, 2H, $S\underline{CH_2}_{(Z)}$), 3.93 (q, *J* = 6.8 Hz, 2H, $N\underline{CH_2}CH_{3(Z)}$), 3.63 (q, *J* = 7 Hz, 2H, $N\underline{CH_2}CH_{3(Z)}$), 3.69-3.61 (m, 4H, $N\underline{CH_2}CH_2CH_{3(E+Z)}$), 2.32 (s, 3H, $C_5\underline{CH3}_{(E)}$), 2.30 (s, 3H, $C_5\underline{CH3}_{(Z)}$), 1.67-1.57 (m, 4H, $NCH_2\underline{CH_2}CH_{3(E+Z)}$), 1.32-1.25 (m, 6H, $NCH_2\underline{CH_3}_{(E+Z)}$), 0.91-0.87 (m, 6H, $NCH_2CH_2\underline{CH_3}_{(E+Z)}$).

^{13}C-NMR: 172.63 (thiazol $C=O_{(E)}$), 168.36 (thiazol $C=O_{(Z)}$), 172.38 (indole $2\times C=O_{(E+Z)}$), 163.85 (thiazol $C=N_{(E)}$), 157.59 (thiazol $C=N_{(Z)}$), 148.65 (indole $C=N_{(E)}$), 146.55 (indole $C=N_{(Z)}$), 143.07 ($C_{5(E)}$), 142.36 ($C_{5(Z)}$), 133.55 ($C_{4(E)}$), 133.30 ($C_{4(Z)}$), 131.86 ($2\times C_{6(E+Z)}$), 129.06 ($C_{7a(E)}$), 122.03 ($C_{7a(Z)}$), 120.27 ($C_{3a(E)}$), 116.86 ($C_{3a(Z)}$), 109.66 ($2\times C_{7(E+Z)}$), 41.29 ($C_{8(E)}$), 41.15 ($C_{8(Z)}$), 38.93 ($C_{16(E)}$), 38.74 ($C_{16(Z)}$), 33.10 ($C_{15(E)}$), 32.95 ($C_{15(Z)}$), 21.11 ($_{5a(E)}$), 20.88 ($_{5a(Z)}$), 20.82 ($2\times C_{9(E+Z)}$), 12.62 ($C_{17(Z)}$), 12.42($C_{17(E)}$), 11.63 ($C_{10(Z)}$), 11.57 ($C_{10(E)}$).

Elemental analyses calculated for $C_{17}H_{20}N_4O_2S$: C, 59.28; H, 5.85; N, 16.27; S, 9.31. Found: C, 59.20; H, 5.77; N, 16.22; S, 9.22.

(*Z*)-2-(((*E*)-1-propyl-5-methyl-2-oxoindolin-3-ylidene)hydrazineylidene)-3-methylthiazolidin-4-one (4l)

Developing system of chromatography: A

Yield (%): 15

M.P. : 228-230

^{1}H-NMR: 8.04 (s, 1H, C_4H), 7.27-7.25 (m, 1H, C_6H), 7.01 (d, *J* = 8.01 Hz, 1H, C_7H), 4.06 (s, 2H, $S\underline{CH_2}$), 3.67 (t, *J* = 6.9 Hz, 2H, $N\underline{CH_2}CH_2CH_3$), 3.34(s, 3H, $N\underline{CH_3}$), 2.31 (s, 3H, $C_5\underline{CH3}$), 1.64 (sxt, *J* = 6.9 Hz, 2H, $NCH_2\underline{CH_2}CH_3$), 0.9 (t, *J* = 6.9 Hz, 3H, $NCH_2CH_2\underline{CH_3}$).

^{13}C-NMR: 172.76 (thiazol C=O), 172.61 (indole C=O), 163.92 (thiazol C=N), 148.71 (indole C=N), 143.20 (C_5), 133.50 (C_4), 131.88 (C_6), 129.40 (C_{7a}), 116.92 (C_{3a}), 109.51 (C_7), 41.40 (C_8)**,** 32.96 (C_{15}), 30.18 (C_{16})**,** 21.08 (C_{5a})**,** 20.82 (C_9)**,** 11.50 (C_{10})**.**

Elemental analyses calculated for $C_{16}H_{18}N_4O_2S$: C, 58.16; H, 5.49; N, 16.96; S, 9.70. Found: C, 58.09; H, 5.55; N, 17.01; S, 9.77.

(*Z*)-2-(((*Z*)-1-propyl-5-methyl-2-oxoindolin-3-ylidene)hydrazineylidene)-3-methylthiazolidin-4-one (5m)

Developing system of chromatography: A

Yield (%): 6

M.P. : 196-198

^{1}H-NMR: 7.39 (s, 1H, C_4H), 7.25-7.23 (m, 1H, C_6H), 6.97 (d, J = 7.9 Hz, 1H, C_7H), 4.03 (s, 2H, S$\underline{CH_2}$), 3.63 (t, J = 7.20 Hz, 2H, N$\underline{CH_2}CH_2CH_3$), 3.26 (s, 3H, N$\underline{CH_3}$), 2.32 (s, 3H, $C_5\underline{CH3}$), 1.68-1.58 (m, 2H, $NCH_2\underline{CH_2}CH_3$), 0.89 (t, J = 7.2 Hz, 3H, $NCH_2CH_2\underline{CH_3}$).

^{13}C-NMR: 172.61 (indole C=O), 168.36 (thiazol C=O), 157.59 (thiazol C=N), 146.55 (indole C=N), 142.36 (C_5), 133.30 (C_4), 131.86 (C_6), 122.03 (C_{7a}), 116.86 (C_{3a}), 109.66 (C_7), 41.26 (C_8)**,** 32.94 (C_{15}), 20.85 (C_{5a})**,** 20.82 (C_9)**,** 11.56 (C_{10})**.**

Elemental analyses calculated for $C_{16}H_{18}N_4O_2S$: C, 58.16; H, 5.49; N, 16.96; S, 9.70. Found: C, 58.10; H, 5.53; N, 17.03; S, 9.67.

(*Z*)-3-ethyl-2-(((*E/Z*)-5-nitro-2-oxoindolin-3-ylidene)hydrazineylidene)thia-zolidin-4-one (4n)

Developing system of chromatography: B

Yield (%): 62

M.P. : 255-258

^{1}H-NMR (*E/Z* %, 68:32): 11.40 (s, 2H, $\underline{NH}^{*}_{(E+Z)}$), 8.96 (s, 1H, $C_4H_{(E)}$), 8.30-8.27 (m, 2H, $C_6H_{(E+Z)}$), 8.20 (s, 1H, $C_4H_{(E)}$), 7.08-7.03 (m, 2H, $C_7H_{(E+Z)}$), 4.12 (s, 2H, S$\underline{CH_2}_{(E)}$), 4.09 (s, 2H, S$\underline{CH_2}_{(Z)}$), 3.95 (q, J = 7 Hz, 2H, N$\underline{CH_2}CH_{3(E)}$), 3.84 (q, J = 7 Hz, 2H, N$\underline{CH_2}CH_{3(Z)}$), 1.34 (t, J = 7 Hz, 3H, $NCH_2\underline{CH_3}_{(E)}$), 1.26 (t, J = 7 Hz, 3H, $NCH_2\underline{CH_3}_{(Z)}$).

^{13}C-NMR: 174.59 (thiazol 2×C=$O_{(E+Z)}$), 172.68 (indole 2×C=$O_{(E+Z)}$), 165.43 (thiazol 2×C=$N_{(E+Z)}$), 149.93 (indole 2×C=$N_{(E+Z)}$), 149.03 ($C_{5(Z)}$), 147.28 ($C_{5(E)}$), 142.71 ($C_{4(Z)}$), 142.60($C_{4(E)}$), 129.27 ($C_{6(E)}$), 128.69 ($C_{6(Z)}$), 123.56 (2×$C_{7a(E+Z)}$), 117.29 ($C_{3a(E)}$), 116.95 ($C_{3a(Z)}$), 111.20 (2×$C_{7(E+Z)}$), 39.05 ($C_{13(E)}$), 38.91 ($C_{13(Z)}$), 33.31 ($C_{12(E)}$), 33.07 ($C_{12(Z)}$), 12.59 ($C_{14(Z)}$), 12.40 ($C_{14(E)}$).

Elemental analyses calculated for $C1_3H_{11}N_5O_4S$: C, 46.84; H, 3.33; N, 21.01; S, 9.62. Found: C, 46.79; H, 3.39; N, 21.09; S, 9.67.

(*Z*)-3-methyl-2-(((*E/Z*)-5-nitro-2-oxoindolin-3-ylidene)hydrazineylidene)-thiazolidin-4-one (4o)

Developing system of chromatography: B

Yield (%): 62

M.P. : 295-298

^{1}H-NMR (*E/Z* %, 44:56): 11.44 (s, 2H, $\underline{NH}^*_{(E+Z)}$), 8.92 (s, 1H, $C_4H_{(E)}$), 8.30-8.26 (m, 2H, $C_6H_{(E+Z)}$), 8.19 (s, 1H, $C_4H_{(Z)}$), 7.07-7.02 (m, 2H, $C_7H_{(E+Z)}$), 4.10 (s, 2H, $S\underline{CH_2}_{(E)}$), 4.07 (s, 2H, $S\underline{CH_2}_{(Z)}$), 3.36 (s, 1H, $N\underline{CH_3}_{(E)}$), 3.26 (s, 1H, $N\underline{CH_3}_{(Z)}$).

^{13}C-NMR: 174.59 (thiazol 2×C=$O_{(E+Z)}$), 172.68 (indole 2×C=$O_{(E+Z)}$), 165.43 (thiazol 2×C=$N_{(E+Z)}$), 149.93 (indole 2×C=$N_{(E+Z)}$), 149.03 ($C_{5(Z)}$), 147.28 ($C_{5(E)}$), 142.71 ($C_{4(Z)}$), 142.60 ($C_{4(E)}$), 129.27 ($C_{6(E)}$), 128.69 ($C_{6(E)}$), 123.56 (2×$C_{7a(E+Z)}$), 117.29 ($C_{3a(E)}$), 116.95 ($C_{3a(Z)}$), 111.20 (2×$C_{7(E+Z)}$), 33.31 ($C_{12(E)}$), 33.07 ($C_{12(Z)}$), 30.23 ($C_{13(E)}$), 30.14 ($C_{13(Z)}$).

Elemental analyses calculated for $C_{12}H_9N_5O_4S$: C, 45.14; H, 2.84; N, 21.93; S, 10.04. Found: C, 45.19; H, 2.95; N, 22.04; S, 9.93.

(*Z*)-2-(((*E/Z*)-1-benzyl-5-nitro-2-oxoindolin-3-ylidene)hydrazineylidene)-3-ethylthiazolidin-4-one (4p)

Developing system of chromatography: C

Yield (%): 60

M.P. : 190-193

^{1}H-NMR (*E/Z* %, 67:33): 9.04 (d, *J* = 2.1 Hz, 1H, $C_4H_{(E)}$), 8.33-8.29 (m, 2H, $C_6H_{(E+Z)}$), 8.27 (d, *J* = 2 Hz, 1H, $C_4H_{(Z)}$), 7.39-7.33 (m, 10H, ph $protons_{(E+Z)}$), 7.31-7.28 (m, 2H, $C_7H_{(E+Z)}$), 5.07 (s, 2H, $N\underline{CH_2}$-$ph_{(E)}$), 5.03 (s, 2H, $N\underline{CH_2}$-$ph_{(Z)}$), 4.15 (s, 2H, $S\underline{CH_2}_{(E)}$), 4.11 (s, 2H, $S\underline{CH_2}_{(Z)}$), 3.97 (q, *J* = 7 Hz, 2H, $N\underline{CH_2}CH_{3(E)}$), 3.87 (q, *J* = 7 Hz, 2H, $N\underline{CH_2}CH_{3(Z)}$), 1.36 (t, *J* = 7 Hz, 3H, $NCH_2\underline{CH_3}_{(E)}$), 1.28 (t, *J* = 7 Hz, 3H, $NCH_2\underline{CH_3}_{(Z)}$).

^{13}C-NMR: 175.38 (thiazol 2×C=$O_{(E+Z)}$), 172.77 (indole 2×C=$O_{(E+Z)}$), 164.43 (thiazol 2×C=$N_{(E+Z)}$), 149.52 (indole 2×C=$N_{(E+Z)}$), 143.16 (2×$C_{5(E+Z)}$), 135.67 (2×$C_{4(E+Z)}$), 129.29 (2×$C_{14(E+Z)}$), 129.27 (2×$C_{10(E+Z)}$), 129.23 (2×$C_{9(E+Z)}$), 129.09 (2×$C_{6(E+Z)}$), 128.17 (2×$C_{12(E+Z)}$), 127.66 (2×$C_{13(E+Z)}$), 127.62 (2×$C_{11(E+Z)}$), 123.30 (2×$C_{7a(E+Z)}$), 116.92 (2×$C_{3a(E+Z)}$), 110.42 (2×$C_{7(E+Z)}$), 43.64 ($C_{8(E)}$), 43.48 ($C_{8(Z)}$), 39.02(*E*) ($C_{20(E)}$), 38.88 ($C_{20(Z)}$), 33.36 ($C_{19(E)}$), 33.14 ($C_{19(Z)}$), 12.60 ($C_{21(Z)}$), 12.36 ($C_{21(E)}$).

Elemental analyses calculated for $C_{20}H_{17}N_5O_4S$: C, 56.73; H, 4.05; N, 16.54; S, 7.57. Found: C, 56.70; H, 3.97; N, 16.48; S, 7.51.

(*Z*)-2-(((*E*)-1-benzyl-5-nitro-2-oxoindolin-3-ylidene)hydrazineylidene)-3-methylthiazolidin-4-one (4q)

Developing system of chromatography: C

Yield (%): 21

M.P. : 230-233

^{1}H-NMR: 9.04 (d, *J* = 1.2 Hz, 1H, C_4H), 8.33 (dd, *J* = 8.8 Hz, 1.2 Hz, 1H, C_6H), 7.39-7.30 (m, 5H, ph protons), 7.23 (d, , *J* = 8.8 Hz, 1H, C_7H), 5.08 (s, 2H, N$\underline{CH_2}$-ph), 4.14 (s, 2H, S$\underline{CH_2}$), 3.39 (s, 3H, N$\underline{CH_3}$).

^{13}C-NMR: 175.49 (thiazol C=O), 172.93 (indole C=O), 164.45 (thiazol C=N), 149.64 (indole C=N), 143.19 (C_5), 135.79 (C_4), 129.29 (C_{14}), 129.26 (C_{10}), 129.20 (C_9), 129.11 (C_6), 128.14 (C_{12}), 127.70 (C_{13}), 127.67 (C_{11}), 123.55 (C_{7a}), 116.93 (C_{3a}), 110.44 (C_7), 65 (C_8), 33.31 (C_{19}), 30.23 (C_{20}).

Elemental analyses calculated for $C_{19}H_{15}N_5O_4S$: C, 55.74; H, 3.69; N, 17.11; S, 7.83. Found: C, 55.67; H, 3.72; N, 17.01; S, 7.88.

(*Z*)-2-(((*E*)-1-propyl-5-nitro-2-oxoindolin-3-ylidene)hydrazineylidene)-3-ethylthiazolidin-4-one (4r)

Developing system of chromatography: A

Yield (%): 18

M.P. : 196-199

^{1}H-NMR: 9.01 (d, *J* = 2.4 Hz, 1H, C_4H), 8.36 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H, C_6H), 7.39 (d, *J* = 8.8 Hz, 1H, C_7H), 4.13 (s, 2H, S$\underline{CH_2}$), 3.96 (q, *J* = 7.1 Hz, 2H, N$\underline{CH_2}CH_3$), 3.78 (t, *J* = 7.2 Hz, 2H, N$\underline{CH_2}CH_2CH_3$), 1.66 (sxt, *J* = 7.2 Hz, 2H, $NCH_2\underline{CH_2}CH_3$), 1.35 (t, *J* = 7.1 Hz, 3H, $NCH_2\underline{CH_3}$), 0.92 (t, *J* = 7.2 Hz, 3H, $NCH_2CH_2\underline{CH_3}$).

^{13}C-NMR: 172.61 (indole C=O), 168.36 (thiazol C=O), 157.59 (thiazol C=N), 146.55 (indole C=N), 142.36 (C_5), 133.30 (C_4), 131.86 (C_6), 122.03 (C_{7a}), 116.86 (C_{3a}), 109.66 (C_7), 41.26 (C_8)**,** 32.94 (C_{15}), 20.85 (C_{5a})**,** 20.82 (C_9)**,** 11.56 (C_{10})**.**

Elemental analyses calculated for $C_{16}H_{17}N_5O_4S$: C, 51.19; H, 4.56; N, 18.66; S, 8.54. Found: C, 51.22; H, 4.59; N, 18.75; S, 8.42.

(*Z*)-2-(((*E/Z*)-1-propyl-5-nitro-2-oxoindolin-3-ylidene)hydrazineylidene)-3-methylthiazolidin-4-one (4s)

Developing system of chromatography: A

Yield (%): 69

M.P. : 193-196

^{1}H-NMR (*E/Z* %, 67:33): 8.99 (s, 1H, $C_4H_{(E)}$), 8.37-8.33 (t, 2H, $C_6H_{(E+Z)}$), 8.24 (s, 1H, $C_4H_{(Z)}$), 7.39-7.34 (m, 2H, $C_7H_{(E+Z)}$), 4.12 (s, 2H, S$\underline{CH_2}_{(E)}$), 4.09 (s, 2H, S$\underline{CH_2}_{(Z)}$), 3.80-3.73 (m, 4H,

N$\underline{CH_2}CH_2CH_{3(E+Z)}$), 3.38 (s, 3H, N$\underline{CH_3}_{(E)}$), 3.28 (s, 3H, N$\underline{CH_3}_{(Z)}$), 1.73-1.63 (m, 4H, NCH$_2\underline{CH_2}CH_{3(E+Z)}$), 0.94-0.88 (m, 6H, NCH$_2CH_2\underline{CH_3}_{(E+Z)}$).

^{13}C-NMR: 175.63 (thiazol C=O$_{(E)}$), 173.36 (thiazol C=O$_{(Z)}$), 172.74 (indole 2×C=O$_{(E+Z)}$), 164.29 (thiazol C=N$_{(E)}$), 160.59 (thiazol C=N$_{(Z)}$), 150.08 (indole C=N$_{(E)}$), 148.20 (indole C=N$_{(Z)}$), 143.07 (C$_{5(E)}$), 142.36 (C$_{5(Z)}$), 133.55 (C$_{4(E)}$), 133.30 (C$_{4(Z)}$), 131.86 (2×C$_{6(E+Z)}$), 129.06 (C$_{7a(E)}$), 122.03 (C$_{7a(Z)}$), 120.27 (C$_{3a(E)}$), 116.77 (C$_{3a(Z)}$), 110.66 (2×C$_{7(E+Z)}$), 41.84 (C$_{8(E)}$), 41.70 (C$_{8(Z)}$), 33.32 (C$_{15(E)}$), 33.20 (C$_{15(Z)}$), 30.23 (C$_{16(E)}$), 30.19 (C$_{16(Z)}$), 20.66 (2×C$_{9(E+Z)}$), 11.57 (C$_{10(Z)}$), 11.51 (C$_{10(E)}$).

Elemental analyses calculated for $C_{15}H_{15}N_5O_4S$: C, 49.86; H, 4.18; N, 19.38; S, 8.87. Found: C, 49.75; H, 4.12; N, 19.30; S, 8.81.

3.4. General Procedures for Synthesis of (*E/Z*)-1-substituted-3-(((*Z*)-3-substituted-4-methylthiazol-2(3*H*)-ylidene)hydrazineylidene)-5-substituted-indolin-2-one 5(a-s)

A mixture of appropriate isatin-3-(*Z*)-thiosemicarbazone, **3(a-r),** (1 mmol), chloroacetone (1 mmol) and anhydrous sodium acetate (2 mmol) in ethanol (50 ml) was refluxed for 24 hrs with occasional shaking. The reaction mixtures were cooled, poured into crushed ice, the precipitates were filtered and dried to give yellow to orange products. The produced diastereomers (***E/Z***) either separtated as mixture or resoluted on silica gel column using the appropriate developing system to the corresponding single diastereomer.

(*E/Z*)-3-(((*Z*)-3-ethyl-4-methylthiazol-2(3*H*)-ylidene)hydrazineylidene)-indolin-2-one (5a)

Developing system of chromatography: B

Yield (%): 67

M.P. : 270-273

^{1}H-NMR (*E/Z* %, 38:62): 10.38 (br. s, 2H, $\underline{NH}^{*}_{(E+Z)}$), 8.20-8.18 (m, 1H, C$_4H_{(E)}$), 7.43-7.42 (m, 1H, C$_4H_{(Z)}$), 7.26-7.19 (m, 2H, C$_5H_{(E+Z)}$), 7.01-6.93 (m, 2H, C$_6H_{(E+Z)}$), 6.85-6.84 (m, 1H, C$_7H_{(E)}$), 6.80-6.78 (m, 1H, C$_7H_{(Z)}$), 6.48 (s, 1H, S$\underline{CH}_{(E)}$), 6.42 (s, 1H, S$\underline{CH}_{(Z)}$), 4.15-4.07 (m, 4H, N$\underline{CH_2}CH_{3(E+Z)}$), 2.29-2.27 (m, 6H, thiazol =C$_{11}\underline{CH_3}_{(E+Z)}$), 1.38-1.29 (m, 6H, NCH$_2\underline{CH_3}_{(E+Z)}$).

^{13}C-NMR: 175.62 (C=O$_{(Z)}$), 175.36 (C=O$_{(E)}$), 166.25 (indole 2×C=N$_{(E+Z)}$), 159.72 (thiazol 2×C=N$_{(E+Z)}$), 140.88 (C$_{5(Z)}$), 140.40 (C$_{5(E)}$), 139.36 (C$_{4(E)}$), 138.48 (C$_{4(Z)}$), 137.29 (2×C$_{6(E+Z)}$), 130.77 (C$_{11(E)}$), 130.45 (C$_{11(Z)}$), 127.33 (C$_{7a(E)}$), 122.99 (C$_{7a(Z)}$), 120.07 (C$_{3a(Z)}$), 118.75 (C$_{3a(E)}$),

109.30 (2×$C_{7(E+Z)}$), 101.17 ($C_{12(E)}$), 100.63 ($C_{12(Z)}$), 44.24 (2×$C_{13(E+Z)}$), 13.89 ($C_{11a(Z)}$), 13.82($C_{11a(E)}$), 13.28 (2×$C_{14(E+Z)}$).

Elemental analyses calculated for $C_{14}H_{14}N_4OS$: C, 58.72; H, 4.93; N, 19.57; S, 11.20. Found: C, 58.68; H, 4.84; N, 19.51; S, 11.07.

(*E/Z*)-3-(((*Z*)-3-methyl-4-methylthiazol-2(3*H*)-ylidene)hydrazineylidene)-indolin-2-one (5b)

Developing system of chromatography: B

Yield (%): 70

M.P. : 245-248

^{1}H-NMR (*E/Z* %, 68:32): 10.46 (s, 2H, $\underline{NH}^*_{(E+Z)}$), 8.22-8.21 (m, 1H, $C_4H_{(E)}$), 7.43-7.4 1 (m, 1H, $C_4H_{(Z)}$), 7.26-7.18 (m, 2H, $C_5H_{(E+Z)}$), 7.02-6.93 (m, 2H, $C_6H_{(E+Z)}$), 6.85-6.83 (m, 1H, $C_7H_{(E)}$), 6.79-6.77 (m, 1H, $C_7H_{(Z)}$), 6.50 (s, 1H, $S\underline{CH}_{(E)}$), 6.46 (s, 1H, $S\underline{CH}_{(Z)}$), 3.62 (s 3H, $N\underline{CH_3}_{(E)}$), 3.58 (s 3H, $N\underline{CH_3}_{(Z)}$), 2.27 (s, 3H, thiazol $=C_{11}\underline{CH_3}_{(E)}$), 2.25 (s, 3H, thiazol $=C_{11}\underline{CH_3}_{(Z)}$).

^{13}C-NMR: 176.51 ($C{=}O_{(Z)}$), 176.24 ($C{=}O_{(Z)}$), 166.28 (indole 2×$C{=}N_{(E+Z)}$), 159.77 (thiazol 2×$C{=}N_{(E+Z)}$), 140.38 ($C_{5(Z)}$), 139.28 ($C_{5(E)}$), 138.22 ($C_{4(E)}$), 137.97 ($C_{4(Z)}$), 130.75 ($C_{6(Z)}$), 130.48 ($C_{6(E)}$), 130.27 ($C_{11(E)}$), 130.06 ($C_{11(E)}$), 127.55 ($C_{7a(E)}$), 123.03 ($C_{7a(E)}$), 120.07 ($C_{3a(E)}$), 120.06 ($C_{3a(Z)}$), 118.69($C_{13(Z)}$), 109.81 (2×$C_{7(E+Z)}$), 100.84 ($C_{12(E)}$), 100.26 ($C_{12(Z)}$), 32.68 ($C_{13(E)}$), 32.45 ($C_{13(Z)}$), 14.09 ($C_{11a(Z)}$), 13.99 ($C_{11a(E)}$).

Elemental analyses calculated for $C_{13}H_{12}N_4OS$: C, 57.34; H, 4.44; N, 20.57; S, 11.77. Found: C, 57.42; H, 4.37; N, 20.46; S, 11.70.

(*E*)-1-benzyl-3-(((*Z*)-3-ethyl-4-methylthiazol-2(3*H*)-ylidene)hydrazineyl-idene)indolin-2-one (5c)

Developing system of chromatography: C

Yield (%): 25

M.P. : 248-250

^{1}H-NMR: 8.26-8.25 (m, 1H, C_4H), 7.43-7.26 (m, 5H, ph protons), 7.25-7.22 (m, 1H, C_5H), 7.07-7.03 (m, 1H, C_6H), 6.94-6.92 (m, 1H, C_7H), 6.45 (s, 1H, $S\underline{CH}$), 4.97 (s, 2H, $N\underline{CH_2}$-ph), 4.17 (q, *J* = 6.9 Hz, 2H, $N\underline{CH_2}CH_3$), 2.31 (s, 3H, thiazol $=C_{18}\underline{CH_3}$), 1.38 (t, *J* = 6.9 Hz, 3H, $NCH_2\underline{CH_3}$).

^{13}C-NMR: 176.16 (indole C=O), 164.85 (indole C=N), 157.78 (thiazol C=N), 142.54 (C_5), 139.03 (C_4), 137.54 (C_6), 137.28 (C_{18}), 130.22 (C_9), 129.02 (C_{10}), 129.07 (C_{14}), 127.64 (C_{13}), 127.59 (C_{11}), 126.34 (C_{12}), 122.65 (C_{7a}), 116.07 (C_{3a}), 109.30 (C_7), 101.90 (C19), 42.89 (C_8), 41.34 (C_{20}), 13.79 (C_{18a}), 13.49 (C_{21}).

Elemental analyses calculated for $C_{21}H_{20}N_4OS$: C, 67.00; H, 5.35; N, 14.88; S, 8.52. Found: C, 66.89; H, 5.39; N, 14.74; S, 8.43.

(*E/Z*)-1-benzyl-3-(((*Z*)-3-methyl-4-methylthiazol-2(3*H*)-ylidene)hydrazine-ylidene)indolin-2-one (5d)

Developing system of chromatography: C

Yield (%): 62

M.P. : 250-253

^{1}H-NMR (*E/Z* %, 30:70): 8.30-8.28 (m, 1H, $C_4H_{(E)}$), 7.51-7.49 (m, 1H, $C_4H_{(Z)}$), 7.33-7.26 (m, 10H, ph protons$_{(E+Z)}$), 7.26-7.23 (m, 1H, $C_5H_{(E)}$), 7.23-7.19 (m, 1H, $C_5H_{(Z)}$), 7.08-7.04 (m, 1H, $C_6H_{(E)}$), 7.02-6.98 (m, 1H, $C_6H_{(Z)}$), 6.94-6.92 (m, 1H, $C_7H_{(E)}$), 6.90-6.88 (m, 1H, $C_7H_{(Z)}$), 6.52 (s, 1H, S$\underline{CH}_{(E)}$), 6.47 (s, 1H, S$\underline{CH}_{(Z)}$), 4.97 (s, 2H, N$\underline{CH_2}$-ph$_{(E)}$), 4.94 (s, 2H, N$\underline{CH_2}$-ph$_{(Z)}$), 3.65 (s, 3H, N$\underline{CH_3}_{(E)}$), 3.62 (s, 3H, N$\underline{CH_3}_{(Z)}$), 2.29 (s, 3H, thiazol =$C_{18}\underline{CH_3}_{(E)}$), 2.2 7(s, 3H, thiazol =$C_{18}\underline{CH_3}_{(Z)}$).

^{13}C-NMR: 176.83 (C=O$_{(E)}$), 176.65 (C=O$_{(Z)}$), 164.91 (indole 2×C=N$_{(E+Z)}$), 162.43 (thiazol C=N$_{(Z)}$), 157.97 (thiazol C=N$_{(Z)}$), 139.42 ($C_{5(Z)}$), 139.25 ($C_{5(Z)}$), 137.48 ($C_{4(Z)}$), 137.38 ($C_{4(Z)}$), 131.48 (2×$C_{18(E+Z)}$), 130.44 (2×$C_{6(E+Z)}$), 129.79 (2×$C_{9(E+Z)}$), 129.09 (2×$C_{14(E+Z)}$), 129.04 (2×$C_{10(E+Z)}$), 127.88 (2×$C_{13(E+Z)}$), 127.70 (2×$C_{11(E+Z)}$), 127.40 (2×$C_{12(E+Z)}$), 122.30 (2×$C_{7a(E+Z)}$), 119.89 ($C_{3a(Z)}$), 118.07 ($C_{3a(E)}$), 109.14 ($C_{7(Z)}$), 109.04 ($C_{7(E)}$), 101.33 ($C_{19(E)}$), 100.74 ($C_{19(Z)}$), 42.92 ($C_{8(E)}$), 42.71 ($C_{8(Z)}$), 32.80 ($C_{20(Z)}$), 32.62 ($C_{20(E)}$), 14.08 ($C_{18a(E)}$), 13.89 ($C_{18a(Z)}$).

Elemental analyses calculated for $C_{20}H_{18}N_4OS$: C, 66.28; H, 5.01; N, 15.46; S, 8.85. Found: C, 66.22; H, 4.89; N, 15.65; S, 8.91.

(*E/Z*)-1-propyl-3-(((*Z*)-3-ethyl-4-methylthiazol-2(3*H*)-ylidene)hydrazineyl-idene)indolin-2-one (5e)

Developing system of chromatography: A

Yield (%): 69

M.P. : 202-205

^{1}H-NMR (*E/Z* %, 32:68): 8.25-8.24 (m, 1H, $C_4H_{(E)}$), 7.49-7.47 (m, 1H, $C_4H_{(Z)}$), 7.34-7.28 (m, 2H, $C_5H_{(E+Z)}$), 7.07-7.01 (m, 4H, C_6H, $C_7H_{(E+Z)}$), 6.51 (s, 1H, S$\underline{CH}_{(E)}$), 6.45 (s, 1H, S$\underline{CH}_{(Z)}$),

4.18-4.07 (m, 4H, N$\underline{CH_2}CH_{3(E+Z)}$), 3.72-3.65 (m, 4H, N$\underline{CH_2}CH_2CH_{3(E+Z)}$), 2.31 (s, 3H, thiazol =$C_{14}\underline{CH_{3(E)}}$), 2.28 (s, 3H, thiazol =$C_{14}\underline{CH_{3(Z)}}$), 1.68-1.59 (m, 4H, $NCH_2\underline{CH_2}CH_{3(E+Z)}$), 1.39-1.30 (m, 6H, $NCH_2\underline{CH_{3(E+Z)}}$), 0.91-0.88 (t, 6H, $NCH_2CH_2\underline{CH_{3(E+Z)}}$).

^{13}C-NMR: 175.78 ($C{=}O_{(Z)}$), 175.61 ($C{=}O_{(E)}$), 164.72 (thiazol $C{=}N_{(E)}$), 157.91 (thiazol $C{=}N_{(Z)}$), 140.99 (indole 2×$C{=}N_{(E+Z)}$), 139.99 ($C_{5(Z)}$), 139.87 ($C_{5(E)}$), 137.45 ($C_{4(Z)}$), 137.36 ($C_{4(E)}$), 130.98 ($C_{14(Z)}$), 130.78 ($C_{14(E)}$), 130.61 ($C_{6(E)}$), 129.99 ($C_{6(Z)}$), 127.26 ($C_{7a(E)}$), 122.13 ($C_{7a(Z)}$), 119.92 ($C_{3a(Z)}$), 117.99 ($C_{3a(E)}$), 108.71 ($C_{7(Z)}$), 108.63 ($C_{7(E)}$), 101.40 ($C_{15(E)}$), 100.83 ($C_{15(Z)}$), 41.31 ($C_{16(Z)}$), 41.00 (2×$C_{8(E+Z)}$), 40.83 ($C_{16(E)}$), 21.33 (2×$C_{9(E+Z)}$), 13.88 ($C_{14a(Z)}$), 13.80 ($C_{14a(E)}$), 13.26 (2×$C_{17(E+Z)}$), 11.70 ($C_{10(Z)}$), 11.60 ($C_{10(E)}$).

Elemental analyses calculated for $C_{17}H_{20}N_4OS$: C, 62.17; H, 6.14; N, 17.06; S, 9.76. Found: C, 62.10; H, 6.19; N, 17.12; S, 9.81.

(*E*)-1-propyl-3-(((*Z*)-3-methyl-4-methylthiazol-2(3*H*)-ylidene)hydrazineyl-idene)indolin-2-one (5f)

Developing system of chromatography: A

Yield (%): 14

M.P. : 220-222

^{1}H-NMR: 8.28-8.27 (m, 1H, C_4H), 7.33-7.29 (m, 1H, C_5H), 7.08-7.03 (m, 2H, C_6H, C_7H), 6.47 (s, 1H, S$\underline{CH}$), 3.7 (t, *J* = 7.30 Hz, 2H, N$\underline{CH_2}CH_2CH_3$), 3.64 (s, 3H, N$\underline{CH_3}$), 2.28 (s, 3H, thiazol =$C_{14}\underline{CH_3}$), 1.66 (sxt, *J* = 7.30 Hz, 2H, $NCH_2\underline{CH_2}CH_3$), 0.91 (t, *J* = 7.3 Hz, 3H, $NCH_2CH_2\underline{CH_3}$).

^{13}C-NMR: 177.15(C=O), 164.88 (thiazol C=N), 140.95 (indole C=N), 139.63 (C_5), 138.31 (C_4), 130.79 (C_{14}), 127.69 (C_6), 122.26 (C_{7a}), 119.37 (C_{3a}), 109.35 (C_7), 101.40 (C_{15}), 41.64 (C_8), 32.90 (C_{16}), 21.36 (C_9), 14.02 (C_{14a}), 11.62 (C_{10}).

Elemental analyses calculated for $C_{16}H_{18}N_4OS$: C, 61.12; H, 5.77; N, 17.82; S, 10.20. Found: C, 61.23; H, 5.87; N, 17.91; S, 10.17.

(*Z*)-1-propyl-3-(((*Z*)-3-methyl-4-methylthiazol-2(3*H*)-ylidene)hydrazineyl-idene)indolin-2-one (5g)

Developing system of chromatography: A

Yield (%): 6

M.P. : 280-283

^{1}H-NMR: 7.49-7.47 (m, 1H, C_4H), 7.30-7.26 (m, 1H, C_5H), 7.02-6.99 (m, 2H, C_6H, C_7H), 6.45 (s, 1H, S$\underline{CH}$), 3.67 (t, *J* = 7.3 Hz, 2H, N$\underline{CH_2}CH_2CH_3$), 3.60 (s, 3H, N$\underline{CH_3}$), 2.26 (s, 3H, thiazol = $C_{14}\underline{CH_3}$), 1.64 (sxt, *J* = 7.3 Hz, 2H, $NCH_2\underline{CH_2}CH_3$), 0.89 (t, *J* = 7.3 Hz, 3H, $NCH_2CH_2\underline{CH_3}$).
^{13}C-NMR: 176.37 (C=O), 166.22 (thiazol C=N), 142.47 (indole C=N), 140.23 (C_5), 138.08 (C_4), 130.44 (C_{14}), 126.69 (C_6), 121.97 (C_{7a}), 119.37 (C_{3a}), 110.04 (C_7), 101.40 (C_{15}), 41.70 (C_8), 32.76 (C_{16}), 21.36 (C_9), 14.01 (C_{14a}), 11.62 (C_{10}).
Elemental analyses calculated for $C_{16}H_{18}N_4OS$: C, 61.12; H, 5.77; N, 17.82; S, 10.20. Found: C, 61.26; H, 5.82; N, 17.95; S, 10.29.

(*E/Z*)-3-(((*Z*)-3-ethyl-4-methylthiazol-2(3*H*)-ylidene)hydrazineylidene)-5-methylindolin-2-one (5h)

Developing system of chromatography: B
Yield (%): 66
M.P. : 235-238
^{1}H-NMR (*E/Z* %, 66:34): 10.35 (s, 1H, $\underline{NH}^*_{(E)}$), 10.31 (s, 1H, $\underline{NH}^*_{(Z)}$), 8.04 (s, 1H, $C_4H_{(E)}$), 7.24 (s, 1H, $C_4H_{(Z)}$), 7.07-7.05 (m, 1H, $C_6H_{(E)}$), 7.02-7.00 (m, 1H, $C_6H_{(Z)}$), 6.74 (d, *J* = 7.8 Hz, 1H, $C_7H_{(E)}$), 6.69 (d, *J* = 7.8 Hz, 1H, $C_7H_{(Z)}$), 6.47 (s, 1H, S$\underline{CH}_{(E)}$), 6.41 (s, 1H, S$\underline{CH}_{(Z)}$), 4.14 (q, *J* = 7 Hz, 2H, N$\underline{CH_2}CH_{3(E)}$), 4.06 (q, *J* = 7 Hz, 2H, N$\underline{CH_2}CH_{3(Z)}$), 2.30-2.27 (m, 12H, thiazol =$C_{11}\underline{CH_3}_{(E+Z)}$, =$C_5\underline{CH_3}_{(E+Z)}$), 1.39 (t, *J* = 7 Hz, 3H, $NCH_2\underline{CH_3}_{(E)}$), 1.3 (t, *J* = 7 Hz, 3H, $NCH_2\underline{CH_3}_{(Z)}$).
^{13}C-NMR: 175.62 ($C{=}O_{(Z)}$), 175.36 ($C{=}O_{(E)}$), 166.25 (indole 2×$C{=}N_{(E+Z)}$), 159.72 (thiazol 2×$C{=}N_{(E+Z)}$), 140.88 ($C_{5(Z)}$), 140.40 ($C_{5(E)}$), 139.36 ($C_{4(E)}$), 138.48 ($C_{4(Z)}$), 137.29 (2×$C_{6(E+Z)}$), 130.77 ($C_{11(E)}$), 130.45 ($C_{11(Z)}$), 127.33 ($C_{7a(E)}$), 122.99 ($C_{7a(Z)}$), 120.07 ($C_{3a(Z)}$), 118.75 ($C_{3a(E)}$), 109.30 (2×$C_{7(E+Z)}$), 101.17 ($C_{12(E)}$), 100.63 ($C_{12(Z)}$), 44.24 (2×$C_{13(E+Z)}$), 21.39 ($C_{5a(E)}$), 21.14 ($C_{5a(Z)}$), 13.89 ($C_{11a(Z)}$), 13.82($C_{11a(E)}$), 13.28 (2×$C_{14(E+Z)}$).
Elemental analyses calculated for $C_{15}H_{16}N_4OS$: C, 59.98; H, 5.37; N, 18.65; S, 10.67. Found: C, 59.85; H, 5.41; N, 18.60; S, 10.74.

***(E/Z)*-3-(((*Z*)-3-methyl-4-methylthiazol-2(3*H*)-ylidene)hydrazineylidene)-5-methylindolin-2-one (5i)**

Developing system of chromatography: B
Yield (%): 65
M.P. : 255-258

^{1}H-NMR (*E/Z* %, 20:80): 10.33 (s, 1H, $\underline{NH}^*_{(E)}$), 10.30* (s, 1H, $\underline{NH}^*_{(Z)}$), 8.04 (s, 1H, $C_4H_{(E)}$), 7.24 (s, 1H, $C_4H_{(Z)}$), 7.07-7.05 (m, 1H, $C_6H_{(E)}$), 7.02-7.00 (m, 1H, $C_6H_{(Z)}$), 6.74 (d, *J* = 7.8Hz, 1H, $C_7H_{(E)}$), 6.68 (d, *J* = 7.8 Hz, 1H, $C_7H_{(Z)}$), 6.48 (s, 1H, $S\underline{CH}_{(E)}$), 6.43 (s, 1H, $S\underline{CH}_{(Z)}$), 3.62 (s, 3H, $N\underline{CH_3}_{(E)}$), 3.57 (s, 3H, $N\underline{CH_3}_{(Z)}$), 2.30-2.25 (m, 12H, thiazol $=C_{11}\underline{CH_3}_{(E+Z)}$, $=C_5\underline{CH_3}_{(E+Z)}$).

^{13}C-NMR: 176.51 ($C{=}O_{(Z)}$), 176.24 ($C{=}O_{(Z)}$), 166.28 (indole $2\times C{=}N_{(E+Z)}$), 159.77 (thiazol $2\times C{=}N_{(E+Z)}$), 140.38 ($C_{5(Z)}$), 139.28 ($C_{5(E)}$), 138.22 ($C_{4(E)}$), 137.97 ($C_{4(Z)}$), 130.75 ($C_{6(Z)}$), 130.48 ($C_{6(E)}$), 130.27 ($C_{11(E)}$), 130.06 ($C_{11(E)}$), 127.55 ($C_{7a(E)}$), 123.03 ($C_{7a(E)}$), 120.07 ($C_{3a(E)}$), 120.06 ($C_{3a(Z)}$), 109.81 ($2\times C_{7(E+Z)}$), 100.84 ($C_{12(E)}$), 100.26 ($C_{12(Z)}$), 32.68 ($C_{13(E)}$), 32.45 ($C_{13(Z)}$), 21.39 ($C_{5a(E)}$), 21.13 ($C_{5a(Z)}$),14.09 ($C_{11a(Z)}$), 13.99 ($C_{11a(E)}$).

Elemental analyses calculated for $C_{14}H_{14}N_4OS$: C, 58.72; H, 4.93; N, 19.57; S, 11.20. Found: C, 58.79; H, 5.01; N, 19.42; S, 11.28.

(*E/Z*)-1-benzyl-3-(((*Z*)-3-ethyl-4-methylthiazol-2(3*H*)-ylidene)hydrazineyl-idene)-5-methylindolin-2-one (5j)

Developing system of chromatography: C

Yield (%): 67

M.P. : 257-260

^{1}H-NMR (*E/Z* %, 25:75): 8.11 (s, 1H, $C_4H_{(E)}$), 7.35 (s, 1H, $C_4H_{(Z)}$), 7.33-7.24 (m, 10H, ph $protons_{(E+Z)}$), 7.07-7.05 (m, 1H, $C_6H_{(E)}$), 7.03-7.01 (m, 1H, $C_6H_{(Z)}$), 6.81 (d, *J* = 7.9 Hz, 1H, $C_7H_{(E)}$), 6.78 (d, *J* = 7.9 Hz, 1H, $C_7H_{(Z)}$), 6.53 (s, 1H, $S\underline{CH}_{(E)}$), 6.47 (s, 1H, $S\underline{CH}_{(Z)}$), 4.94 (s, 2H, $N\underline{CH_2}$-$ph_{(E)}$), 4.91 (s, 2H, $N\underline{CH_2}$-$ph_{(Z)}$), 4.19-4.08 (m, 2H, $N\underline{CH_2}CH_{3(E+Z)}$), 2.32-2.29 (m, 12H, thiazol $=C_{18}\underline{CH_3}_{(E+Z)}$, $=C_5\underline{CH_3}_{(E+Z)}$), 1.4 (t, *J* = 7.2 Hz, 3H, $NCH_2\underline{CH_3}_{(E)}$), 1.33 (t, *J* = 7.2 Hz, 3H, $NCH_2\underline{CH_3}_{(Z)}$).

^{13}C-NMR: 176.83 ($C{=}O_{(Z)}$), 176.01 ($C{=}O_{(E)}$), 164.89 (ind $2\times C{=}N_{(E+Z)}$), 161.99 (thiazol $C{=}N_{(Z)}$), 157.92 (thiazol $C{=}N_{(Z)}$), 139.53 ($2\times C_{5(E+Z)}$), 137.49 ($2\times C_{4(E+Z)}$), 131.37 ($2\times C_{18(E+Z)}$), 130.48 ($2\times C_{6(E+Z)}$), 129.87 ($2\times C_{9(E+Z)}$), 129.09 ($2\times C_{14(E+Z)}$), 129.04 ($2\times C_{10(E+Z)}$), 127.68 ($2\times C_{13(E+Z)}$), 127.60 ($2\times C_{11(E+Z)}$), 127.20 ($2\times C_{12(E+Z)}$), 122.27 ($2\times C_{7a(E+Z)}$), 119.91 ($C_{3a(Z)}$), 118.15 ($C_{3a(E)}$), 109.18 ($2\times C_{7(E+Z)}$), 101.67 ($C_{19(E)}$), 101.12 ($C_{19(Z)}$), 42.91 ($C_{8(E)}$), 42.72 ($C_{8(Z)}$), 41.39 ($C_{20(E)}$), 41.05 ($C_{20(Z)}$), 21.33 ($C_{5a(E)}$), 21.09 ($C_{5a(Z)}$), 13.91 ($C_{18a(E)}$), 13.82 ($C_{18a(Z)}$), 13.28 ($2\times C_{21(E+Z)}$).

Elemental analyses calculated for $C_{22}H_{22}N_4OS$: C, 67.67; H, 5.68; N, 14.35; S, 8.21. Found: C, 67.62; H, 5.71; N, 14.29; S, 8.33.

(*E/Z*)-1-benzyl-3-(((*Z*)-3-methyl-4-methylthiazol-2(3*H*)-ylidene)hydrazine-ylidene)-5-methylindolin-2-one (5k)

Developing system of chromatography: C

Yield (%): 66

M.P. : 185-188

^{1}H-NMR (*E/Z* %, 45:55): 8.10 (s, 1H, $C_4H_{(E)}$), 7.34 (s, 1H, $C_4H_{(Z)}$), 7.33-7.24 (m, 10H, phenyl protons$_{(E+Z)}$), 7.07-7.05 (m, 1H, $C_6H_{(E)}$), 7.03-7.01 (m, 1H, $C_6H_{(Z)}$), 6.82 (d, *J* = 7.9 Hz, 1H, $C_7H_{(E)}$), 6.78 (d, *J* = 7.9 Hz, 1H, $C_7H_{(Z)}$), 6.53 (s, 1H, $SCH_{(E)}$), 6.48 (s, 1H, $SCH_{(Z)}$), 4.94 (s, 2H, NCH_2-ph$_{(E)}$), 4.91 (s, 2H, NCH_2-ph$_{(Z)}$), 3.65 (s, 6H, $NCH_{3(E+Z)}$), 2.30-2.27 (m, 12H, thiazol $=C_{18}CH_{3(E+Z)}$, $=C_5CH_{3(E+Z)}$).

^{13}C-NMR: 176.83 ($C{=}O_{(E)}$), 176.65 ($C{=}O_{(Z)}$), 164.91 (indole 2×$C{=}N_{(E+Z)}$), 162.43 (thiazol $C{=}N_{(Z)}$), 157.97 (thiazol $C{=}N_{(Z)}$), 139.42 ($C_{5(Z)}$), 139.25 ($C_{5(Z)}$), 137.48 ($C_{4(Z)}$), 137.38 ($C_{4(Z)}$), 131.48 (2×$C_{18(E+Z)}$), 130.44 (2×$C_{6(E+Z)}$), 129.79 (2×$C_{9(E+Z)}$), 129.12 (2×$C_{14(E+Z)}$), 129.04 (2×$C_{10(E+Z)}$), 127.70 (2×$C_{13(E+Z)}$), 127.66 (2×$C_{11(E+Z)}$), 127.40 (2×$C_{12(E+Z)}$), 122.30 (2×$C_{7a(E+Z)}$), 119.89 ($C_{3a(Z)}$), 118.07 ($C_{3a(E)}$), 109.14 ($C_{7(Z)}$), 109.04 ($C_{7(E)}$), 101.33 ($C_{19(E)}$), 100.74 ($C_{19(Z)}$), 42.92 ($C_{8(E)}$), 42.71 ($C_{8(Z)}$), 32.62 ($C_{20(E)}$), 32.80 ($C_{20(Z)}$), 21.35 ($C_{5a(E)}$), 21.09 ($C_{5a(Z)}$), 14.08 ($C_{18a(E)}$), 13.89 ($C_{18a(Z)}$).

Elemental analyses calculated for $C_{21}H_{20}N_4OS$: C, 67.00; H, 5.35; N, 14.88; S, 8.52. Found: C, 67.12; H, 5.27; N, 14.81; S, 8.43.

(*E/Z*)-1-propyl-3-(((*Z*)-3-ethyl-4-methylthiazol-2(3*H*)-ylidene)hydrazine-ylidene)-5-methylindolin-2-one (5l)

Developing system of chromatography: A

Yield (%): 65

M.P. : 165-1688

^{1}H-NMR (*E/Z* %, 61:39): 8.10 (s, 1H, $C_4H_{(E)}$), 7.29 (s, 1H, $C_4H_{(Z)}$), 7.14-7.12 (m, 1H, $C_6H_{(E)}$), 7.10-7.08 (m, 1H, $C_6H_{(Z)}$), 6.94 (d, *J* = 7.8 Hz, 1H, $C_7H_{(E)}$), 6.89 (d, *J* = 7.8 Hz, 1H, $C_7H_{(Z)}$), 6.49 (s, 1H, $SCH_{(E)}$), 6.43 (s, 1H, $SCH_{(Z)}$), 4.15 (t, *J* = 7.1 Hz, 3H, $NCH_2CH_{3(E)}$), 4.1-4.06 (m, 3H, $NCH_2CH_{3(Z)}$), 3.68-3.62 (m, 4H, $NCH_2CH_2CH_{3(E+Z)}$), 2.31-2.28 (m, 12H, thiazol $=C_{14}CH_{3(E+Z)}$, $=C_5CH_{3(E+Z)}$), 1.66-1.57 (m, 4H, $NCH_2CH_2CH_{3(E+Z)}$), 1.39 (t, *J* = 7.1 Hz, 3H, $NCH_2CH_{3(E)}$), 1.31 (t, *J* = 7.1 Hz, 3H, $NCH_2CH_{3(Z)}$), 0.90-0.86 (m, 6H, $NCH_2CH_2CH_{3(E+Z)}$).

^{13}C-NMR: 175.78 ($C{=}O_{(Z)}$), 175.61 ($C{=}O_{(E)}$), 164.72 (thiazol $C{=}N_{(E)}$), 157.91 (thiazol $C{=}N_{(Z)}$), 140.99 (indole 2×$C{=}N_{(E+Z)}$), 139.99 ($C_{5(Z)}$), 139.87 ($C_{5(E)}$), 137.45 ($C_{4(Z)}$), 137.36 ($C_{4(E)}$), 130.98 ($C_{14(Z)}$), 130.78 ($C_{14(E)}$), 130.61 ($C_{6(E)}$), 129.99 ($C_{6(Z)}$), 127.26 ($C_{7a(E)}$), 122.13 ($C_{7a(Z)}$), 119.92

($C_{3a(Z)}$), 117.99 ($C_{3a(E)}$), 108.71 ($C_{7(Z)}$), 108.63 ($C_{7(E)}$), 101.40 ($C_{15(E)}$), 100.83 ($C_{15(Z)}$), 41.31 ($C_{16(Z)}$), 41.00 ($2\times C_{8(E+Z)}$), 40.83 ($C_{16(E)}$), 21.33 ($2\times C_{9(E+Z)}$), 21.08 ($2\times C_{5a(E+Z)}$), 13.88 ($C_{14a(Z)}$), 13.80 ($C_{14a(E)}$), 13.26 ($2\times C_{17(E+Z)}$), 11.70 ($C_{10(Z)}$), 11.60 ($C_{10(E)}$).

Elemental analyses calculated for $C_{18}H_{22}N_4OS$: C, 63.13; H, 6.48; N, 16.36; S, 9.36. Found: C, 63.19; H, 6.40; N, 16.42; S, 9.28.

(*E/Z*)-1-propyl-3-(((*Z*)-3-methyl-4-methylthiazol-2(3*H*)-ylidene)hydrazine-ylidene)-5-methylindolin-2-one (5m)

Developing system of chromatography: A

Yield (%): 62

M.P. : 153-155

^{1}H-NMR (*E/Z* %, 62:38): 8.09 (s, 1H, $C_4H_{(E)}$), 7.29 (s, 1H, $C_4H_{(Z)}$), 7.14-7.12 (m, 1H, $C_6H_{(E)}$), 7.10-7.08 (m, 1H, $C_6H_{(Z)}$), 6.95 (d, J = 7.8 Hz, 1H, $C_7H_{(E)}$), 6.9 (d, J = 7.8 Hz, 1H, $C_7H_{(Z)}$), 6.50 (s, 1H, S$\underline{CH}_{(E)}$), 6.45 (s, 1H, S$\underline{CH}_{(Z)}$), 3.71-3.64 (m, 4H, N$\underline{CH_2}CH_2CH_{3(E+Z)}$), 3.64 (s, 3H, N$\underline{CH_3}_{(E)}$), 3.58 (s, 3H, N$\underline{CH_3}_{(Z)}$), 2.32 (s, 3H, $C_5\underline{CH_3}_{(E)}$), 2.31 (s, 3H, $C_5\underline{CH_3}_{(Z)}$), 2.28 (s, 3H, thiazol =$C_{14}\underline{CH_3}_{(E)}$), 2.26 (s, 3H, thiazol =$C_{14}\underline{CH_3}_{(Z)}$), 1.66-1.57 (m, 4H, $NCH_2\underline{CH_2}CH_{3(E+Z)}$), 0.90-0.86 (m, 6H, $NCH_2CH_2\underline{CH_3}_{(E+Z)}$).

^{13}C-NMR: 176.66 (C=$O_{(Z)}$), 176.47 (C=$O_{(E)}$), 164.76 (thiazol C=$N_{(E)}$), 157.97 (thiazol C=$N_{(Z)}$), 140.95 (indole $2\times$C=$N_{(E+Z)}$), 139.91 ($C_{5(Z)}$), 139.63 ($C_{5(E)}$), 137.17 ($C_{4(E)}$), 137.07 ($C_{4(Z)}$), 131.02 ($C_{14(Z)}$), 130.79 ($C_{14(E)}$), 130.58 ($C_{6(E)}$), 129.93 ($C_{6(Z)}$), 127.47 ($C_{7a(E)}$), 122.18 ($C_{7a(Z)}$), 119.91 ($C_{3a(Z)}$), 117.92 ($C_{3a(E)}$), 108.72 ($C_{7(Z)}$), 108.61 ($C_{7(E)}$), 101.08 ($C_{15(E)}$), 100.48 ($C_{15(Z)}$), 41.01 ($2\times C_{8(E+Z)}$), 32.74 ($C_{16(E)}$), 32.55 ($C_{16(Z)}$), 21.36 ($2\times C_{9(E+Z)}$), 21.06 ($2\times C_{5a(E+Z)}$), 14.07 ($C_{14a(E)}$), 13.98 ($C_{14a(Z)}$), 11.67 ($C_{10(Z)}$), 11.62 ($C_{10(E)}$).

Elemental analyses calculated for $C_{17}H_{20}N_4OS$: C, 62.17; H, 6.14; N, 17.06; S, 9.76. Found: C, 62.28; H, 6.19; N, 17.11; S, 9.83.

(*Z*)-3-(((*Z*)-3-ethyl-4-methylthiazol-2(3*H*)-ylidene)hydrazineylidene)-5-nitrolindolin-2-one (5n)

Developing system of chromatography: B

Yield (%): 60

M.P. : 255-258

^{1}H-NMR: 11.20 (s, 1H, $\underline{NH}^*$), 8.14 (s, 1H, C_4H), 8.13-8.12 (m, 1H, C_6H), 7 (m, 1H, C_7H), 6.61 (s, 1H, S$\underline{CH}$), 4.14 (q, J = 7 Hz, 2H, N$\underline{CH_2}CH_3$), 2.32 (s, 3H, thiazol =$C_{11}\underline{CH_3}$), 1.33 (t, J = 7 Hz, 3H, $NCH_2\underline{CH_3}$).

^{13}C-NMR: 176.54 (C=O), 166.40 (thiazol C=N), 147.54 (indole C=N), 142.34 (C_5), 138.02 (C_4), 126.42 (C_6), 121.12 (C_{7a}), 118.42 (C_{11}), 113.93 (C_{3a}), 110.13 (C_7), 102.85 (C_{12}), 41.71 (C_{13}), 13.74 (C_{11a}), 13.26 (C_{14}).

Elemental analyses calculated for $C_{14}H_{13}N_5O_3S$: C, 50.75; H, 3.95; N, 21.14; S, 9.68. Found: C, 50.70; H, 3.89; N, 21.27; S, 9.74.

***(E/Z)*-3-(((*Z*)-3-methyl-4-methylthiazol-2(3*H*)-ylidene)hydrazineylidene)-5-nitroindolin-2-one (5o)**

Developing system of chromatography: B

Yield (%): 55

M.P. : 295-298

^{1}H-NMR (*E/Z* %, 33:67): 11.11 (s, 2H, $\underline{NH}^{*}_{(E+Z)}$), 9.00 (m, 1H, $C_4H_{(E)}$), 8.20-8.17 (m, 1H, $C_6H_{(E)}$), 8.15-8.11 (m, 2H, $C_4H_{(Z)}$, $C_6H_{(Z)}$), 7.02 (d, J = 8.3 Hz, 1H, $C_7H_{(E)}$), 6.97 (d, J = 8.3 Hz, 1H, $C_7H_{(Z)}$), 6.64 (s, 1H, S$\underline{CH}_{(E)}$), 6.62 (s, 1H, S$\underline{CH}_{(Z)}$), 3.71 (s, 3H, N$\underline{CH_3}_{(E)}$), 3.65 (s, 3H, N$\underline{CH_3}_{(Z)}$), 2.32 (s, 3H, thiazol =$C_{11}\underline{CH_3}_{(E)}$), 2.29 (s, 3H, thiazol =$C_{11}\underline{CH_3}_{(Z)}$).

^{13}C-NMR: 176.90 (2×C=$O_{(E+Z)}$), 166.47 (thiazol 2×C=$N_{(E+Z)}$), 147.47 (indole 2×C=$N_{(E+Z)}$), 142.38 (2×$C_{5(E+Z)}$), 138.76 (2×$C_{4(E+Z)}$), 126.43 (2×$C_{6(E+Z)}$), 121.25 (2×$C_{7a(E+Z)}$), 118.38 (2×$C_{11(E+Z)}$), 113.93 (2×$C_{3a(E+Z)}$), 110.06 (2×$C_{7(E+Z)}$), 102.55 ($C_{12(E)}$), 102.24 ($C_{12(Z)}$), 33.07 ($C_{13(E)}$), 32.49 ($C_{13(Z)}$), 13.98 (2×$C_{11a(E+Z)}$).

Elemental analyses calculated for $C_{13}H_{11}N_5O3S$: C, 49.21; H, 3.49; N, 22.07; S, 10.10. Found: C, 49.28; H, 3.55; N, 22.13; S, 10.18.

(*E/Z*)-1-benzyl-3-(((*Z*)-3-ethyl-4-methylthiazol-2(3*H*)-ylidene)hydrazine-ylidene)-5-nitroindolin-2-one (5p)

Developing system of chromatography: C

Yield (%): 57

M.P. : 190-193

^{1}H-NMR (*E/Z* %, 39:61): 9.09 (s, 1H, $C_4H_{(E)}$), 8.19 (s, 1H, $C_4H_{(Z)}$), 8.19-8.17 (m, 1H, $C_6H_{(E)}$), 8.15-8.13 (m, 1H, $C_6H_{(Z)}$), 7.37-7.28 (m, 10H, ph protons$_{(E+Z)}$), 7.17-7.13 (m, 2H, $C_7H_{(E+Z)}$), 6.70 (s, 1H, S$\underline{CH}_{(E)}$), 6.67 (s, 1H, S$\underline{CH}_{(Z)}$), 5.07 (s, 2H, N$\underline{CH_2}$-ph$_{(E)}$), 5.05 (s, 2H, N$\underline{CH_2}$-ph$_{(Z)}$), 4.25 (q, J = 7 Hz, 2H, N$\underline{CH_2}CH_{3(E)}$), 4.18 (q, J = 7 Hz, 2H, N$\underline{CH_2}CH_{3(Z)}$), 2.36 (s, 3H, thiazol =$C_{18}\underline{CH_3}_{(E)}$), 2.34 (s, 3H, thiazol =$C_{18}\underline{CH_3}_{(Z)}$), 1.45 (t, J = 7 Hz, 2H, 3H, $NCH_2\underline{CH_3}_{(Z)}$), 1.35 (t, J = 7 Hz, 2H, 3H, $NCH_2\underline{CH_3}_{(Z)}$).

^{13}C-NMR: 177.65 (C=$O_{(E)}$), 176.83 (C=$O_{(Z)}$), 165.18 (thiazol C=$N_{(Z)}$), 157.75 (thiazol C=$N_{(E)}$), 147.01 (indole C=$N_{(E)}$), 145.83 (indole C=$N_{(Z)}$), 142.82 (2×$C_{5(E+Z)}$), 138.26 (2×$C_{4(E+Z)}$), 129.22 (2×$C_{14(E+Z)}$), 129.19 (2×$C_{10(E+Z)}$), 127.98 (2×$C_{9(E+Z)}$), 127.72 (2×$C_{13(E+Z)}$), 127.68 (2×$C_{11(E+Z)}$), 126.12 (2×$C_{6(E+Z)}$), 124.93 (2×$C_{12(E+Z)}$), 120.80 (2×$C_{7a(E+Z)}$), 117.94 (2×$C_{18(E+Z)}$), 113.63 (2×$C_{3a(E+Z)}$), 109.27 (2×$C_{7(E+Z)}$), 103.45 ($C_{19(E)}$), 103.22 ($C_{19(Z)}$), 43.29 ($C_{8(E)}$), 43.10 ($C_{8(Z)}$), 41.88 ($C_{20(E)}$), 41.58 ($C_{20(Z)}$), 13.98 ($C_{21(E)}$), 13.77 ($C_{21(Z)}$), 13.31 (2×$C_{18a(E+Z)}$).

Elemental analyses calculated for $C_{21}H_{19}N_5O_3S$: C, 59.84; H, 4.54; N, 16.62; S, 7.61. Found: C, 59.79; H, 4.48; N, 16.70; S, 7.71.

(*E/Z*)-1-benzyl-3-(((*Z*)-3-methyl-4-methylthiazol-2(3*H*)-ylidene)hydrazine-ylidene)-5-nitroindolin-2-one (5q)

Developing system of chromatography: C

Yield (%): 65

M.P. : 230-233

^{1}H-NMR (*E/Z* %, 24:76): 9.07 (s, 1H, $C_4H_{(E)}$), 8.20 (s, 1H, $C_4H_{(Z)}$), 8.20-8.18 (m, 1H, $C_6H_{(E)}$), 8.16-8.13 (m, 1H, $C_6H_{(Z)}$), 7.40-7.25 (m, 10H, ph $protons_{(E+Z)}$), 7.19-7.14 (m, 2H, $C_7H_{(E+Z)}$), 6.71 (s, 1H, S$\underline{CH}_{(E)}$), 6.69 (s, 1H, S$\underline{CH}_{(Z)}$), 5.07 (s, 2H, N$\underline{CH_2}$-$ph_{(E)}$), 5.05 (s, 2H, N$\underline{CH_2}$-$ph_{(Z)}$), 3.75 (s, 3H, N$\underline{CH_3}_{(E)}$), 3.69 (s, 3H, N$\underline{CH_3}_{(Z)}$), 2.34 (s, 3H, thiazol =$C_{18}\underline{CH_3}_{(E)}$), 2.31 (s, 3H, thiazol =$C_{18}\underline{CH_3}_{(Z)}$).

^{13}C-NMR: 178.34 (C=$O_{(E)}$), 177.66 (C=$O_{(Z)}$), 165.21 (thiazol C=$N_{(Z)}$), 163.92 (thiazol C=$N_{(E)}$), 147.04 (indole C=$N_{(Z)}$), 145.69 (indole C=$N_{(E)}$), 142.80 (2×$C_{5(E+Z)}$), 139.06 ($C_{4(Z)}$), 138.99 ($C_{4(E)}$), 129.23 (2×$C_{14(E+Z)}$), 129.19 (2×$C_{10(E+Z)}$), 127.97 (2×$C_{9(E+Z)}$), 127.66 (2×$C_{13(E+Z)}$), 127.57 (2×$C_{11(E+Z)}$), 126.10 (2×$C_{6(E+Z)}$), 124.86 (2×$C_{12(E+Z)}$), 120.90 (2×$C_{7a(E+Z)}$), 117.86 (2×$C_{18(E+Z)}$), 113.59 (2×$C_{3a(E+Z)}$), 109.31 (2×$C_{7(E+Z)}$), 103.16 ($C_{19(E)}$), 102.83 ($C_{19(Z)}$), 43.30 ($C_{8(E)}$), 43.08 ($C_{8(Z)}$), 33.09 (2×$C_{20(E+Z)}$), 14.05 ($C_{18a(E)}$), 13.96 ($C_{18a(Z)}$).

Elemental analyses calculated for $C_{20}H_{17}N_5O_3S$: C, 58.96; H, 4.21; N, 17.19; S, 7.87. Found: C, 58.87; H, 4.33; N, 17.23; S, 7.95.

(*E/Z*)-1-propyl-3-(((*Z*)-3-ethyl-4-methylthiazol-2(3*H*)-ylidene)hydrazine-ylidene)-5-nitroindolin-2-one (5r)

Developing system of chromatography: A

Yield (%): 58

M.P. : 196-198

^{1}H-NMR (*E/Z* %, 61:39): 9.04 (s, 1H, $C_4H_{(E)}$), 8.23-8.18 (m, 2H, $C_6H_{(E+Z)}$), 8.15 (s, 1H, $C_4H_{(Z)}$), 7.29-7.23 (m, 2H, $C_7H_{(E+Z)}$), 6.67 (s, 1H, S$\underline{CH}_{(E)}$), 6.64 (s, 1H, S$\underline{CH}_{(Z)}$), 4.25-4.13 (m, 4H, N$\underline{CH_2}CH_{3(E+Z)}$), 3.79-3.73 (m, 4H, $NCH_2\underline{CH_2}CH_{3(E+Z)}$), 3.68-3.62 (m, 4H, N$\underline{CH_2}CH_2CH_{3(E+Z)}$), 2.35 (s, 3H, thiazol $=C_{11}\underline{CH_3}_{(E)}$), 2.33 (s, 3H, thiazol $=C_{11}\underline{CH_3}_{(Z)}$), 1.69-1.60 (m, 4H, $NCH_2\underline{CH_2}CH_{3(E+Z)}$), 1.44 (t, *J* = 7 Hz, 3H, $NCH_2\underline{CH_3}_{(E)}$), 1.34 (t, *J* = 7 Hz, 3H, $NCH_2\underline{CH_3}_{(Z)}$), 0.91-0.88 (m, 6H, $NCH_2CH_2\underline{CH_3}_{(E+Z)}$).

^{13}C-NMR: 176.74 ($2\times C{=}O_{(E+Z)}$), 165.21 (thiazol $2\times C{=}N_{(E+Z)}$), 147.58 (indole $C{=}N_{(Z)}$), 146.31 (indole $C{=}N_{(E)}$), 142.57 ($2\times C_{5(E+Z)}$), 138.18 ($C_{4(Z)}$), 136.48 ($C_{4(E)}$), 133.78 ($2\times C_{6(E+Z)}$), 126.24 ($C_{14(Z)}$), 125.05 ($C_{14(E)}$), 120.78 ($2\times C_{7a(E+Z)}$), 117.70 ($C_{3a(Z)}$), 113.81 ($C_{3a(E)}$), 108.97 ($C_{7(Z)}$), 108.91 ($C_{7(E)}$), 103.14 ($C_{15(E)}$), 102.97 ($C_{15(Z)}$), 41.79 ($2\times C_{8(E+Z)}$), 41.54 ($2\times C_{16(E+Z)}$), 21.09 ($2\times C_{9(E+Z)}$), 13.91 ($C_{14a(E)}$), 13.70 ($C_{14a(Z)}$), 13.25 ($2\times C_{17(E+Z)}$), 11.55 ($C_{10(Z)}$), 11.48 ($C_{10(E)}$).

Elemental analyses calculated for $C_{17}H_{19}N_5O_3S$: C, 54.68; H, 5.13; N, 18.75; S, 8.59. Found: C, 54.75; H, 5.22; N, 18.86; S, 8.61.

(*E/Z*)-1-propyl-3-(((*Z*)-3-methyl-4-methylthiazol-2(3*H*)-ylidene)hydrazine-ylidene)-5-nitrolindolin-2-one (5s)

Developing system of chromatography: A

Yield (%): 52

M.P. : 225-228

^{1}H-NMR (*E/Z* %, 44:56): 9.04 (s, 1H, $C_4H_{(E)}$), 8.25-8.23 (m, 1H, $C_6H_{(E)}$), 8.19-8.17 (m, 2H, $C_6H_{(Z)}$, $C_4H_{(Z)}$), 7.13 (d, *J* = 8.3 Hz, 1H, $C_7H_{(E)}$), 7.27 (d, *J* = 8.3 Hz, 1H, $C_7H_{(Z)}$), 6.68 (s, 1H, S$\underline{CH}_{(E)}$), 6.66 (s, 1H, S$\underline{CH}_{(Z)}$), 3.80-3.75 (m, 4H, N$\underline{CH_2}CH_2CH_{3(E+Z)}$), 3.73(*E*) and 3.67(*Z*) (s, 3H, N$\underline{CH_3}$), 2.33 (s, 3H, thiazol $=C_{14}\underline{CH_3}_{(E)}$), 2.30 (s, 3H, thiazol $=C_{14}\underline{CH_3}_{(Z)}$), 1.70-1.61 (m, 4H, $NCH_2\underline{CH_2}CH_{3(E+Z)}$), 0.92-0.87 (m, 6H, $NCH_2CH_2\underline{CH_3}_{(E+Z)}$).

^{13}C-NMR: 177.52 ($C{=}O_{(E)}$), 178.23 ($C{=}O_{(Z)}$), 165.12 (thiazol $C{=}N_{(E)}$), 157.84 (thiazol $C{=}N_{(Z)}$), 147.64 (indole $C{=}N_{(Z)}$), 146.28 (indole $C{=}N_{(E)}$), 142.49 ($C_{4(Z)}$), 138.86 ($2\times C_{5(E+Z)}$), 136.19 ($C_{4(E)}$), 133.54 ($2\times C_{6(E+Z)}$), 126.21 ($C_{14(Z)}$), 124.99 ($C_{14(E)}$), 120.93 ($2\times C_{7a(E+Z)}$), 117.64 ($C_{3a(Z)}$), 113.55 ($C_{3a(E)}$), 108.97 ($C_{7(Z)}$), 108.90 ($C_{7(E)}$), 102.91 ($C_{15(E)}$), 102.58 ($C_{15(Z)}$), 41.51 ($C_{8(Z)}$), 41.28 ($C_{8(E)}$), 33.02 ($2\times C_{16(E+Z)}$), 21.14 ($2\times C_{9(E+Z)}$), 14.04 ($C_{14a(E)}$), 13.95 ($C_{14a(Z)}$), 11.60 ($C_{10(Z)}$), 11.55 ($C_{10(E)}$).

Elemental analyses calculated for $C_{16}H_{17}N_5O_3S$: C, 53.47; H, 4.77; N, 19.49; S, 8.92. Found: C, 53.40; H, 4.68; N, 19.41; S, 9.03.

3.5. Anti-proliferative screening

The anti-proliferative activity of the compounds **4(a-s)** and **5(a-s)** was tested on the cell growth of liver **(HepG2)**, breast **(MCF-7)**, colon **(HT-29**) and lung **(WI-38)** cell lines using the MTT assay according to the reported protocol of Denizot and Lang [32]. The cells were treated with different concentrations of the targeted compounds **4(a-s)** and **5(a-s)**, and incubated for 24 h. After 24 h of treatment, 20 µl of MTT solution (5 mg/ml) was added and incubated for 4 h. DMSO (100 µl) was added to each well to dissolve the formazan formed and the optical density was recorded at 570 nm. The percentage of relative cell viability was calculated as follows;

(A_{570} of treated samples/$_{A570}$ of untreated sample) × 100. The IC_{50} of the tested compounds are given in **table 2**.

3.6. Screening of activities of selected compounds on gene expression of CDK1, p53, caspase-3 and caspase-9

Liver HepG2 cells were treated with the novel isatin-thiazolidine derivatives **4o, 4s, 5e, 5f, 5l, 5m and 5o** (at the IC_{50} in **table 2**) in DMSO for 6 h. Total RNA was isolated from liver cells using BIOZOL reagent (Bioer Technology Co., Ltd., China). Specific primers for p53, CDK1, caspase-3, caspase-9, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a house-keeping gene were used in reverse transcription (RT) and q-PCR, as shown in **table 9**. RT and q-PCR were executed as described by Rao and et al.[34]., using Maxima SYBR Green qPCR Master Mix (Bioline, London, UK). Each sample (n=4) was analysed in triplicate. The differences in gene expression between groups were determined using the $^{\Delta\Delta}$Ct (cycle threshold) method [32]. The changes were normalized against GAPDH of the same sample and expressed as fold change compared with the control untreated cells.

3.7. Statistical analysis

Results are expressed as mean ± SEM. The distribution of data was tested by the Kolmogorov-Smirnov test. Statistical analyses were performed with Instat-GraphPad software using One Way ANOVA for comparing different groups. Pairwise comparisons were executed using Tukey-HSD test. Differences were considered significant at $p < 0.05$.

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4. References

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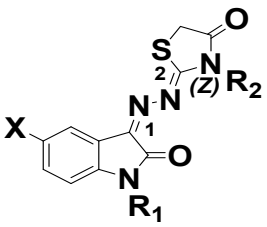
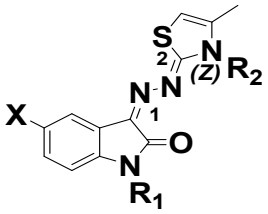
Table 1: Selected bond lengths and bond angles for **5f** and **5g**.

Atoms	Bond length (Å)		Atoms	Bond angle (°)	
	5f	5g		5f	5g
S(1) C(1)	1.7379	1.7365	C(1) S(1) C(3)	89.700	89.624
S(1) C(3)	1.7340	1.7296	C(2) N(1) C(3)	115.130	114.113
N(1) C(2)	1.3889	1.4011	C(2) N(1) C(13)	124.355	124.335
N(1) C(3)	1.3449	1.3515	C(3) N(1) C(13)	120.480	121.542
N(1) C(13)	1.4567	1.4558	C(3) N(2) N(3)	110.280	108.877
N(2) C(3)	1.3131	1.3250	N(2) N(3) C(4)	114.562	117.062
N(3) C(4)	1.2937	1.2955	C(5) N(4) C(6)	111.418	111.214
N(2) N(3)	1.3654	1.3598	C(5) N(4) C(14)	121.970	122.702
C(5) O(1)	1.2237	1.2167	C(6) N(4) C(14)	126.147	126.073
N(4) C(5)	1.3682	1.3801	N(1) C(3) N(2)	122.570	121.927
N(4) C(6)	1.4067	1.3998	S(1) C(3) N(2)	126.926	127.105
N(4) C(14)	1.4737	1.4565	N(3) C(4) C(5)	119.852	130.800

Table 2: *In vitro* cytotoxicity of the synthesized compounds on different cell lines.



4(a-s)



5(a-s)

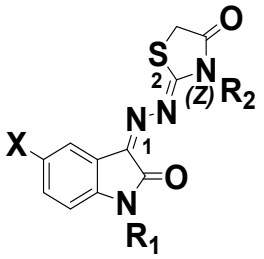
Compd.	X	R_1	R_2	IC50 (µM) ± S.E.			
				HepG2	MCF7	HCT-29	WI-38
Doxorubicin	-----	----------	------	4.50±0.2	4.17±0.2	4.01±0.4	5.21±0.3
4a	H	H	C_2H_5	40.12±2.7	28.04±2.3	21.01±2.3	64.20±6.2
4b	H	H	CH_3	93.08±5.7	76.07±4.9	81.81±6.4	>100
4c	H	$-CH_2C_6H_5$	C_2H_5	>100	91.86±5.4	89.50±7.1	>100
4d	H	$-CH_2C_6H_5$	CH_3	>100	79.95±4.9	78.01±8.4	>100
4e	H	$-CH_2CH_2CH_3$	C_2H_5	35.29±2.2	25.81±1.9	14.15±1.5	94.81± 6.2
4f	H	$-CH_2CH_2CH_3$	CH_3	68.84±4.3	47.29±3.5	40.11±2.7	>100
4g	CH_3	H	C_2H_5	86.27±5.4	53.56±3.8	51.21±5.8	>100
4h	CH_3	H	CH_3	90.68±5.5	69.45±4.5	58.57±6.7	>100
4i	CH_3	$-CH_2C_6H_5$	C_2H_5	41.65±3.2	30.90±2.4	28.33±1.8	90.00±5.8
4j	CH_3	$-CH_2C_6H_5$	CH_3	>100	>100	92.81±3.4	>100
4k	CH_3	$-CH_2CH_2CH_3$	C_2H_5	55.81±3.7	36.48±2.8	28.61±2.8	87.66±8.6
4l	CH_3	$-CH_2CH_2CH_3$	CH_3	74.76±4.6	65.38±4.3	60.14± 5.0	>100
4m	CH_3	$-CH_2CH_2CH_3$	CH_3	61.53±3.9	42.86±3.2	33.20±2.8	>100
4n	NO_2	H	C_2H_5	67.26±4.2	38.23±2.9	27.17±3.2	>100
4o	NO_2	H	CH_3	27.59±1.9	8.97±0.7	5.42±0.6	98.22±1.3
4p	NO_2	$-CH_2C_6H_5$	C_2H_5	81.35±5.0	87.42±5.1	74.51±6.5	>100
4q	NO_2	$-CH_2C_6H_5$	CH_3	29.16±2.0	23.87±1.8	17.11±0.3	88.50±6.3
4r	NO_2	$-CH_2CH_2CH_3$	C_2H_5	88.29±5.5	73.65±4.7	68.51±5.4	>100
4s	NO_2	$-CH_2CH_2CH_3$	CH_3	4.97±0.3	5.33±0.4	3.29±0.2	>100
5a	H	H	C_2H_5	16.38±1.3	12.68±1.2	10.05±1.2	>100

Table 2 (continued)

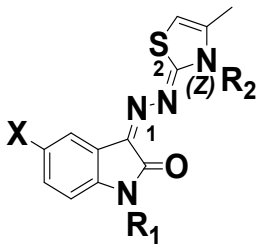
Compd.	X	R_1	R_2	IC50 (μM) ± S.E.			
				HepG2	MCF7	HCT-29	WI-38
5b	H	H	CH_3	51.33±3.5	34.93±2.7	29.90 ±2.4	>100
5c	H	$-CH_2C_6H_5$	C_2H_5	59.06±3.8	>100	90.11±10.0	>100
5d	H	$-CH_2C_6H_5$	CH_3	32.72±2.1	26.84±2.0	19.87±1.5	91.03±6.8
5e	H	$-CH_2CH_2CH_3$	C_2H_5	9.91±1.0	14.27±1.3	7.71±0.9	>100
5f	H	$-CH_2CH_2CH_3$	CH_3	9.02±1.0	10.48±1.1	6.24±0.4	>100
5g	H	$-CH_2CH_2CH_3$	CH_3	18.04±1.4	17.05±1.5	10.52±1.6	92.15±8.2
5h	CH_3	H	C_2H_5	13.49±1.2	15.44±1.3	11.16±2.0	>100
5i	CH_3	H	CH_3	22.87±1.6	19.35±1.6	25.10±3.1	>100
5j	CH_3	$-CH_2C_6H_5$	C_2H_5	37.67±2.3	33.80±2.6	41.20±3.8	>100
5k	CH_3	$-CH_2C_6H_5$	CH_3	48.10±3.4	44.16±3.3	38.25±2.7	>100
5l	CH_3	$-CH_2CH_2CH_3$	C_2H_5	7.38±0.8	9.51±0.9	5.71±0.7	94.50±6.1
5m	CH_3	$-CH_2CH_2CH_3$	CH_3	8.14±0.9	7.81±0.6	4.16±0.2	>100
5n	NO_2	H	C_2H_5	85.63±5.2	50.74±3.6	43.50±3.9	>100
5o	NO_2	H	CH_3	6.85±0.5	6.17±0.5	4.01±0.6	>100
5p	NO_2	$-CH_2C_6H_5$	C_2H_5	72.91±4.6	58.50±3.9	41.27±5.1	>100
5q	NO_2	$-CH_2C_6H_5$	CH_3	46.70±3.3	31.51±2.5	28.71±3.4	>100
5r	NO_2	$-CH_2CH_2CH_3$	C_2H_5	78.12±4.8	62.31±4.2	68.16±7.6	>100
5s	NO_2	$-CH_2CH_2CH_3$	CH_3	24.15±1.7	21.56±1.6	14.14±1.6	>100

IC_{50} (μmol/L) expressed as mean ± S.E.for two independent experiments.

Table 3: Effect of compound **4o**, **4s**, **5e**, **5f**, **5l**, **5m** and **5o** on the expression of **CDK1**, **p53**, **caspase-3** and **caspase-9** in **HepG2** after 6 hr. incubation.



4(a-s)



5(a-s)

Compd	X	R_1	R_2	IC_{50} (µM ± S.E.)			
				CDK1	p53	Caspase-3	Caspase-9
Doxorubicin	-----	----------	------	0.42 ± 0.07[a]	2.36± 0.25[a]	3.41 ± 0.25[a]	4.17 ± 0.60[a]
4o	NO_2	H	CH_3	1.13 ± 0.08	1.07 ± 0.33	1.19 ± 0.32	0.93 ± 0.27
4s	NO_2	$-CH_2CH_2CH_3$	CH_3	0.38 ± 0.04[a]	3.04± 0.22[a]	5.60 ± 0.49[a]	3.65 ± 0.47[a]
5e	H	$-CH_2CH_2CH_3$	C_2H_5	0.42 ± 0.06[a]	2.81± 0.31[a]	3.77 ± 0.26[a]	1.24 ± 0.16
5f	H	$-CH_2CH_2CH_3$	CH_3	0.51 ± 0.08[a]	2.39± 0.17[a]	3.22 ± 0.20[a]	1.17 ± 0.23
5l	CH_3	$-CH_2CH_2CH_3$	C_2H_5	1.07 ± 0.10	1.01 ± 0.26	3.44 ± 0.52[a]	3.13 ± 0.47[a]
5m	CH_3	$-CH_2CH_2CH_3$	CH_3	0.92 ± 0.11	1.12 ± 0.22	4.05 ± 0.63[a]	3.61 ± 0.43[a]
5o	NO_2	H	CH_3	0.43 ± 0.05[a]	3.31± 0.40[a]	3.78 ± 0.16[a]	3.72 ± 0.72[a]

[a] Significant difference ($p < 0.05$) as compared to untreated cells. The data are expressed as mean ± SEM for two independent experiments.

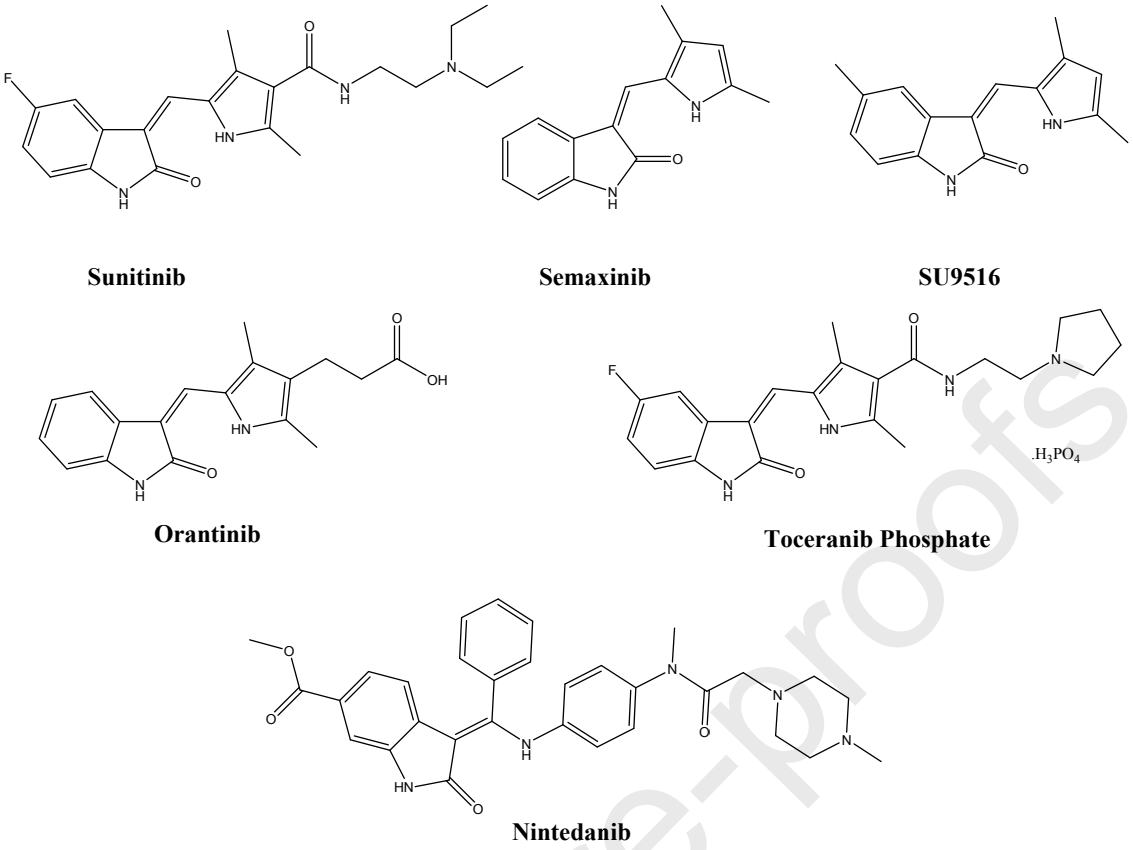


Figure 1. Chemical structure of some 2-indolone derivatives that at the stage of clinical trials or approved as anticancer agents.

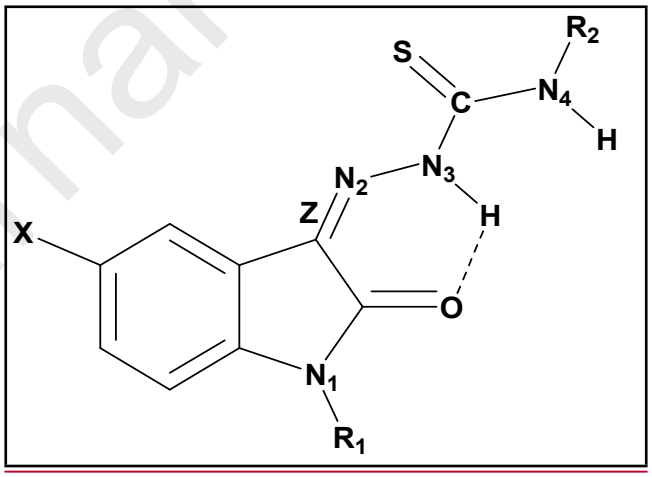


Figure 2. Possible isatin-3-(*Z*)-thiosemicarbazone structure.

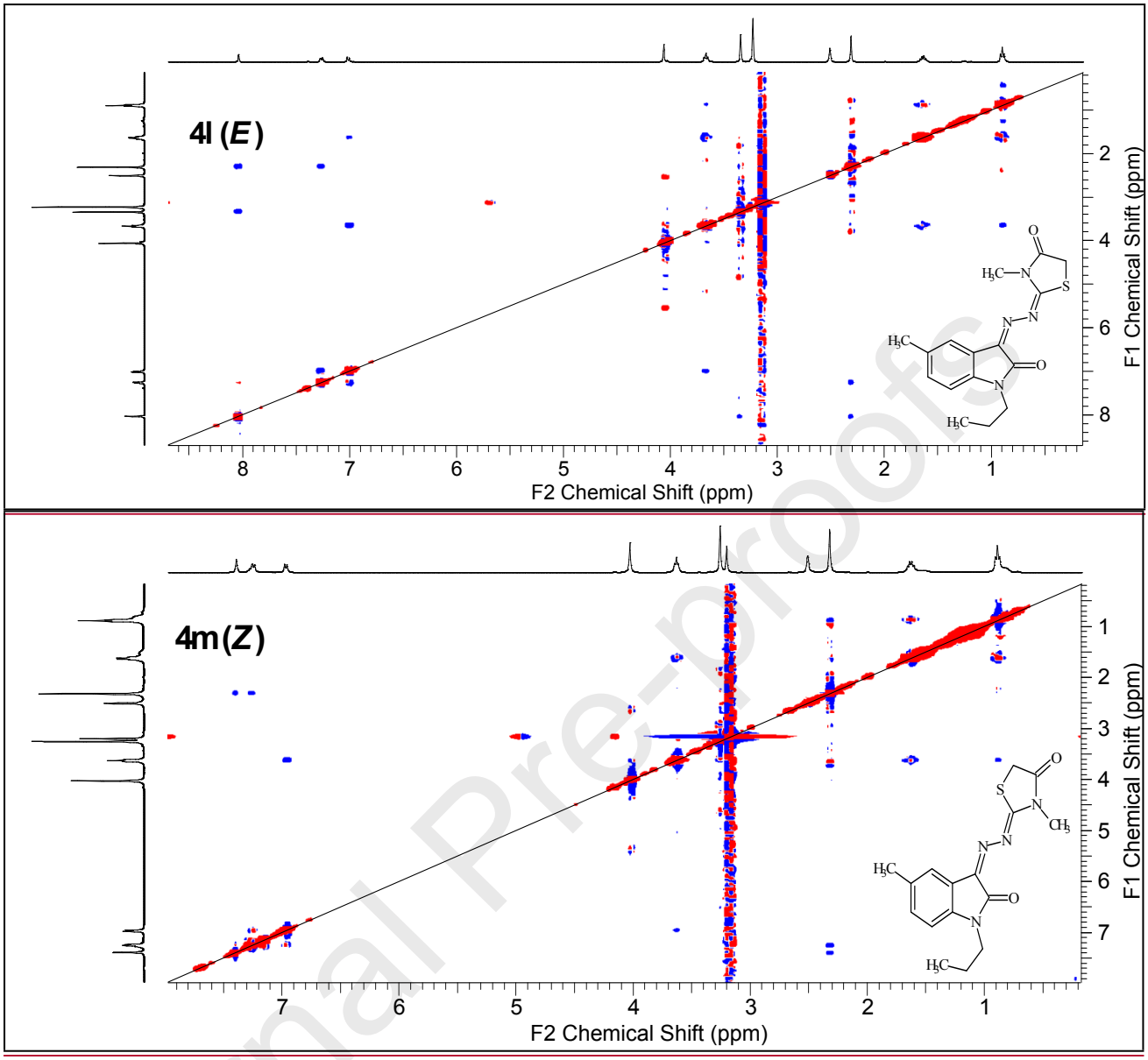


Figure 3. ^{1}H-^{1}H-NOESY spectra of compound **4l** and **4m**.

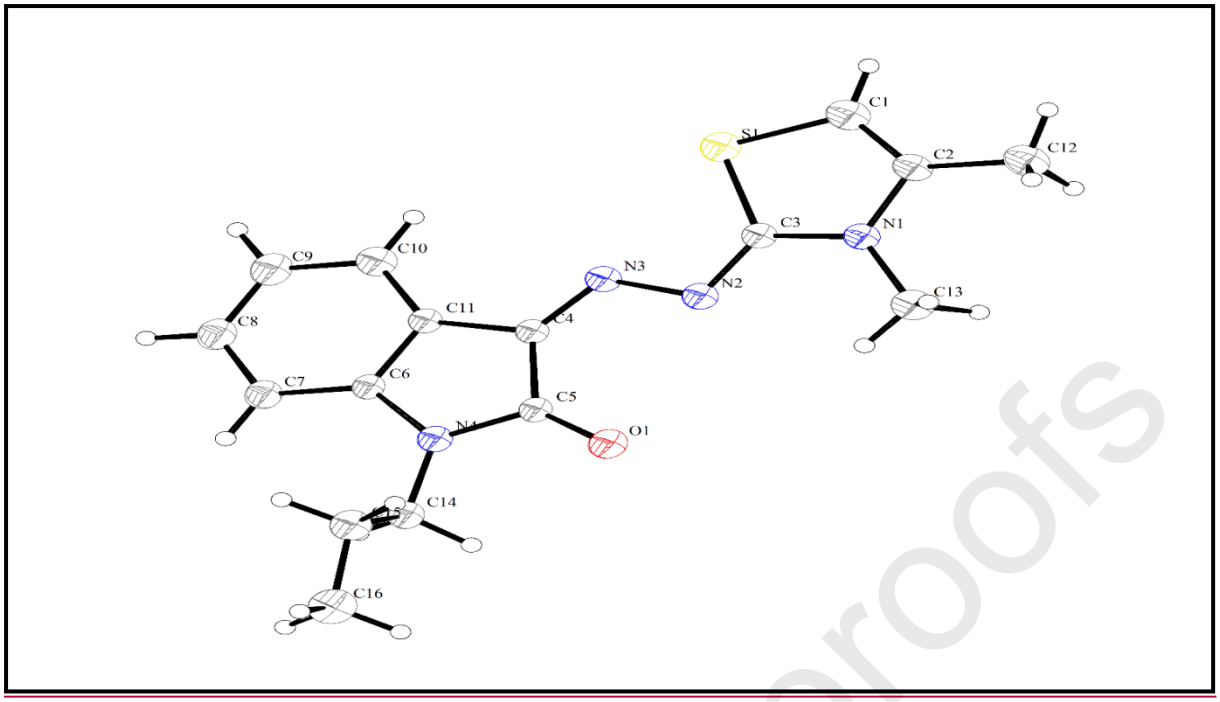


Figure 5. ORTEP diagram of **5f** showing the thermal ellipsoids at 30% probability

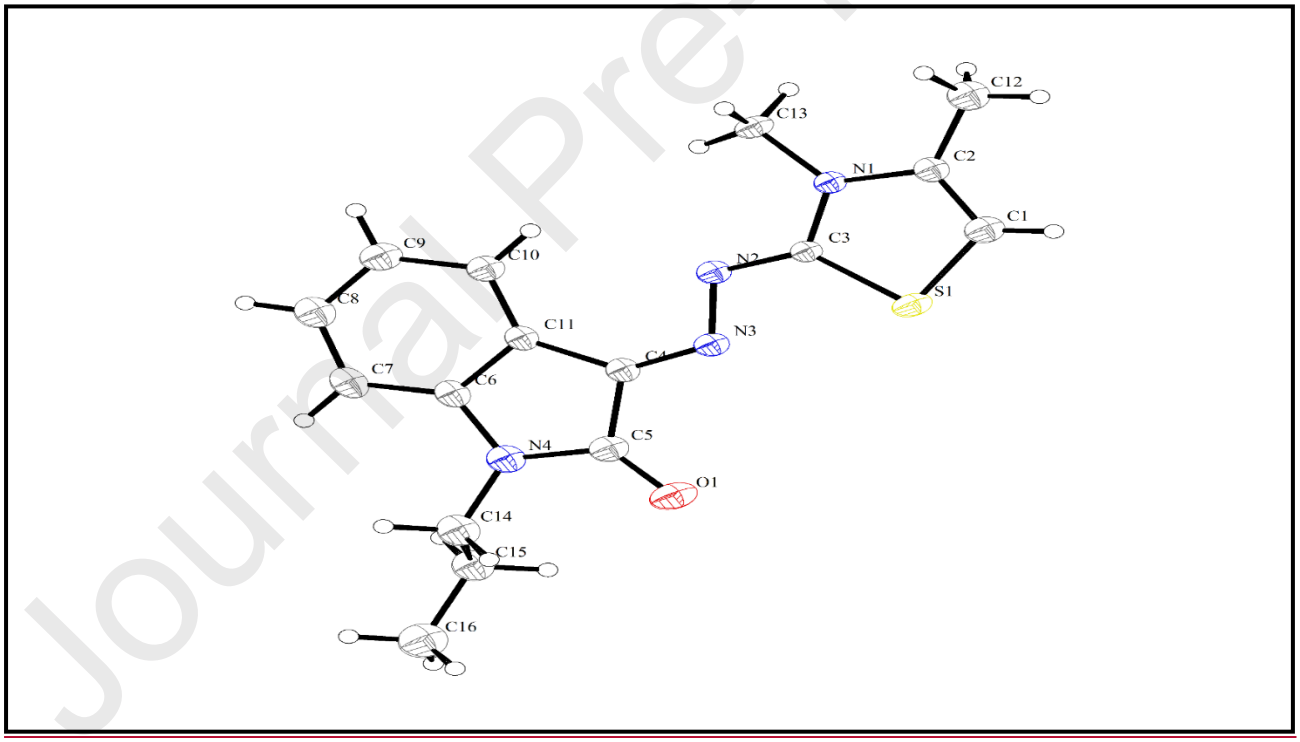
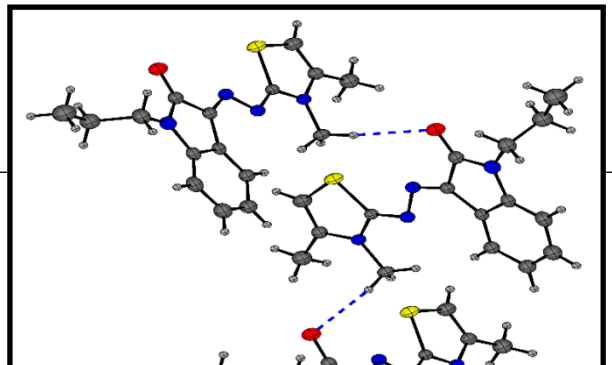


Figure 5. ORTEP diagram of **5g** showing the thermal ellipsoids at 30% probability



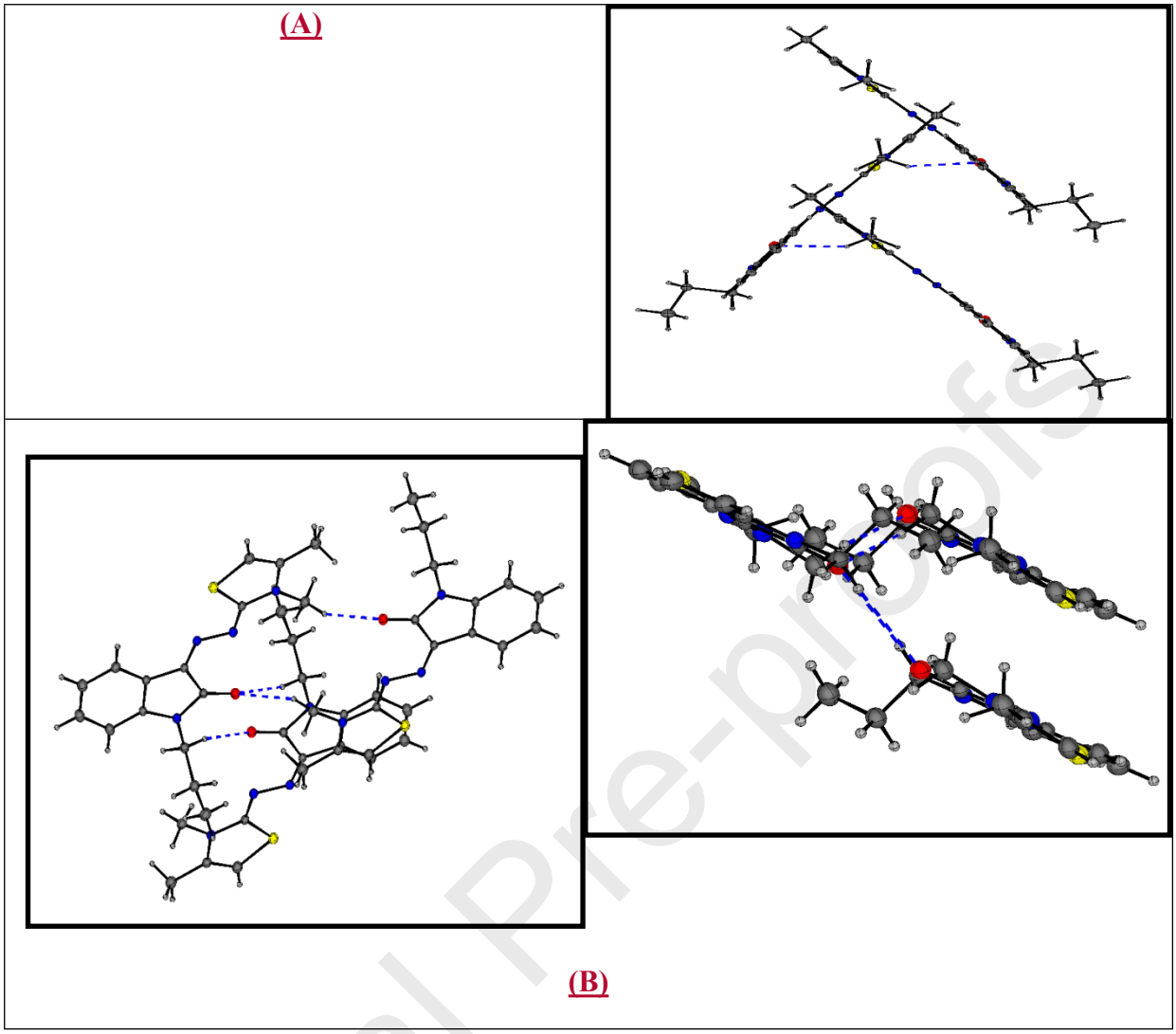


Figure 6. Crystal packing of **5f** (A) and **5g** (B).

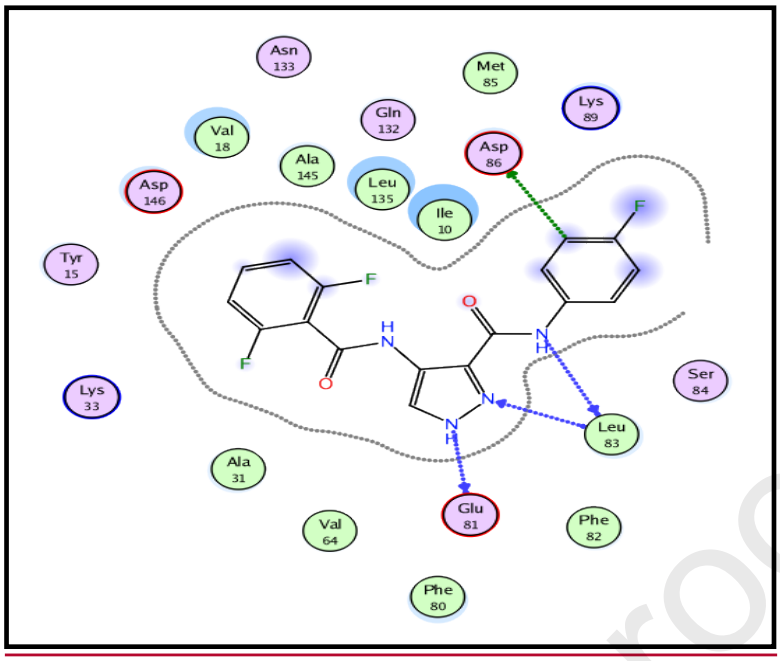


Figure 7. CDK1-ligand [*N*-(4-flurophenyl)-4-(2,6-flurobenzoylamino)-1*H*-pyrazole-3-carboxamide] binding interactions.

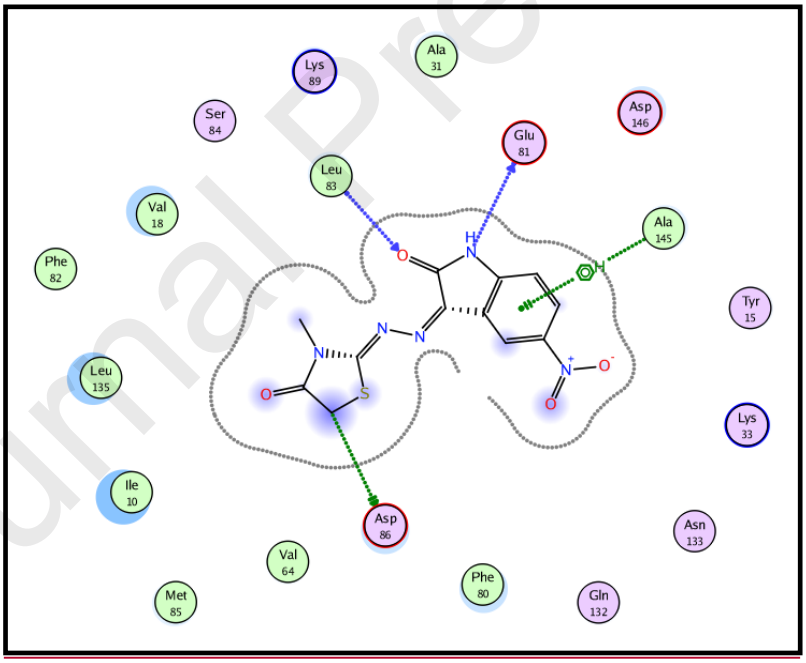


Figure 8. Docking of compound **4o** with CDK1 enzyme catalytic site.

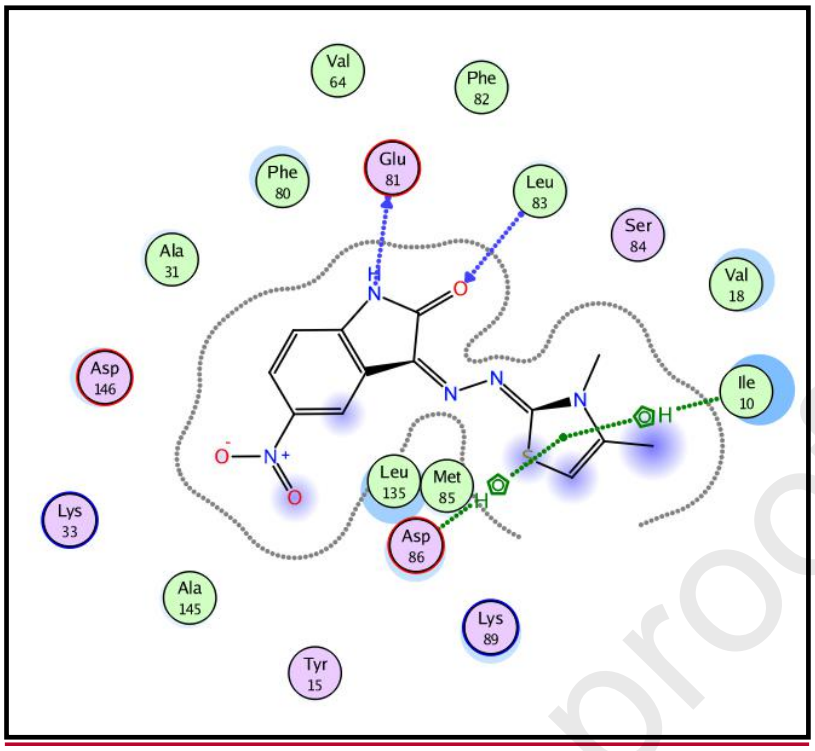


Figure 9. Docking of compound **5o** with CDK1 enzyme catalytic site.

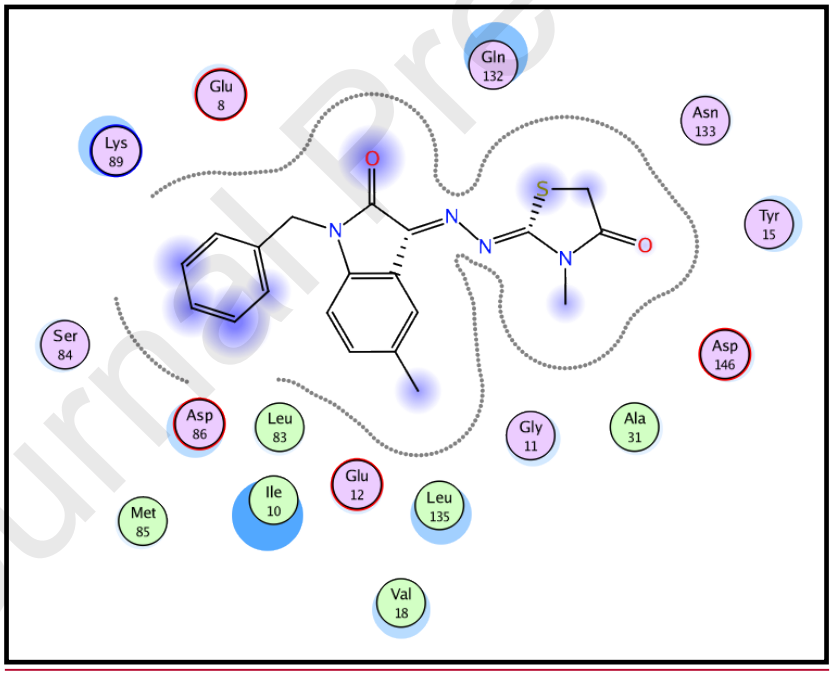
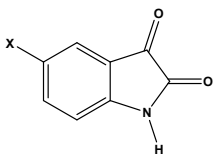


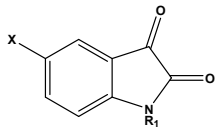
Figure 10. Docking of compound **4j** with CDK1 enzyme catalytic site.



1(a-c)

X = a, H; b, CH_3; c, NO_2

(A)

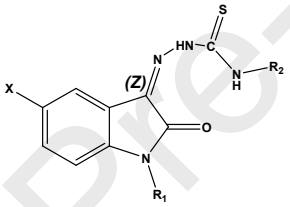


2(a-f)

a: X= H, $\mathbf{R_1}$= benzyl; **b**: X= H, $\mathbf{R_1}$= propyl; **c**: X= CH_3, $\mathbf{R_1}$= benzyl;
d: X= CH_3, $\mathbf{R_1}$= propyl; **e**: X= NO_2, $\mathbf{R_1}$= benzyl; **f**: X= NO_2, $\mathbf{R_1}$= propyl.

(B)

(B)

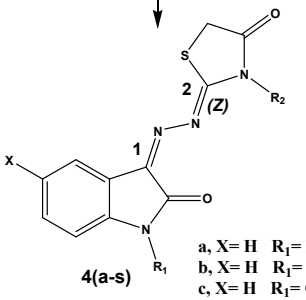


3(a-r)

(C)

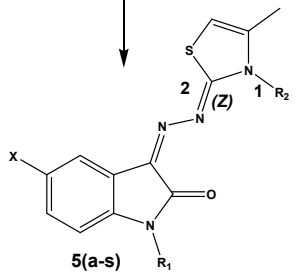
(D)

a, X= H R_1= H R_2= C_2H_5; **b, X= H R_1= H R_2= CH_3;** **c, X= H R_1= $CH_2C_6H_5$ R_2= C_2H_5;** **d, X= H R_1= $CH_2C_6H_5$ R_2= CH_3;**
e, X= H R_1= n-C_3H_7 R_2= C_2H_5; **f, X= H R_1= n-C_3H_7 R_2= CH_3;** **g, X= CH_3 R_1= H R_2= C_2H_5;** **h, X= CH_3 R_1= H R_2= CH_3;**
i, X= CH_3 R_1= $CH_2C_6H_5$ R_2= C_2H_5; **j, X= CH_3 R_1= $CH_2C_6H_5$ R_2= CH_3;** **k, X= CH_3 R_1= n-C_3H_7 R_2= C_2H_5;** **l, X=CH_3 R_1= C_3H_7 R_2= CH_3;**
m, X= NO_2 R_1= H R_2= C_2H_5; **n, X= NO_2 R_1= H R_2= CH_3;** **o, X= NO_2 R_1= $CH_2C_6H_5$ R_2= C_2H_5;** **p, X= NO_2 R_1= $CH_2C_6H_5$ R_2= CH_3;**
q, X= NO_2 R_1= n-C_3H_7 R_2= C_2H_5; **r, X= NO_2 R_1= n-C_3H_7 R_2= CH_3**



4(a-s)

a, X= H R_1= H R_2= C_2H_5;
b, X= H R_1= H R_2= CH_3;
c, X= H R_1= $CH_2C_6H_5$ R_2= C_2H_5;
d, X= H R_1= $CH_2C_6H_5$ R_2= CH_3;
e, X= H R_1= n-C_3H_7 R_2= C_2H_5;
f, X= H R_1= n-C_3H_7 R_2= CH_3;
g, X= H R_1= n-C_3H_7 R_2= CH_3;
h, X= CH_3 R_1= H R_2= C_2H_3;
i, X= CH_3 R_1= H R_2= CH_3;;
j, X= CH_3 R_1= $CH_2C_6H_5$ R_2= C_2H_5;
k, X= CH_3 R_1= $CH_2C_6H_5$ R_2= CH_3;
l, X=CH_3 R_1= n-C_3H_7 R_2= C_2H_5;
m, X=CH_3 R_1= n-C_3H_7 R_2= CH_3;
n, X= NO_2 R_1= H R_2= C_2H_3;
o, X= NO_2 R_1= H R_2= CH_3;
p, X= NO_2 R_1= $CH_2C_6H_5$ R_2= C_2H_5;
q, X= NO_2 R_1= $CH_2C_6H_5$ R_2= CH_3;
r, X= NO_2 R_1= n-C_3H_7 R_2= C_2H_3;
S, X= NO_2 R_1= n-C_3H_7 R_2= CH_3



5(a-s)

a, X= H R_1= H R_2= C_2H_5;
b, X= H R_1= H R_2= CH_3;
c, X= H R_1= $CH_2C_6H_5$ R_2= C_2H_5;
d, X= H R_1= $CH_2C_6H_5$ R_2= CH_3;
e, X= H R_1= n-C_3H_7 R_2= C_2H_5;
f, X= H R_1= n-C_3H_7 R_2= CH_3;
g, X= H R_1= n-C_3H_7 R_2= CH_3;
h, X= CH_3 R_1= H R_2= C_2H_5;
i, X= CH_3 R_1= H R_2= CH_3;
j, X= CH_3 R_1= $CH_2C_6H_5$ R_2= C_2H_5;
k, X= CH_3 R_1= $CH_2C_6H_5$ R_2= CH_3;
l, X=CH_3 R_1= n-C_3H_7 R_2= C_2H_5;
m, X=CH_3 R_1= n-C_3H_7 R_2= CH_3;
n, X= NO_2 R_1= H R_2= C_2H_5;
o, X= NO_2 R_1= H R_2= CH_3;
p, X= NO_2 R_1= $CH_2C_6H_5$ R_2= C_2H_5;
q, X= NO_2 R_1= $CH_2C_6H_5$ R_2= CH_3;
r, X= NO_2 R_1= n-C_3H_7 R_2= C_2H_5;
S, X= NO_2 R_1= n-C_3H_7 R_2= CH_3

Scheme 1: Synthetic routs of the target compounds 4(a-s) and 5(a-s); reagent and conditions: (A), R_1-NH_2, K_2CO_3 / DMF/KI / 80 ^oC, 45 mins.; (B), $R_2NHCSNHNH_2$/ethanol/reflux 2hrs.; (C), $ClCH_2CO_2H$ / anhydrous sod. acetate/ethanol / reflux 24 hrs.; (D), $ClCH_2COCH_3$ / anhydrous sod. acetate/ethanol / reflux 24 hrs.

- Two series of Isatin-thiazolidine, Isatin-thiazolidinone derivatives were designed and synthesized to target cell cycle checkpoint pathways.
- E-and Z-diasteromeres of the synthesized molecules were identified and there structures were confirmed.
- *In vitro* cytotoxicity of the prepared compounds was tested against three human epithelial cell lines, liver (HepG2), breast (MCF-7), and colon (HT-29) in addition to the diploid human normal cells (WI-38).
- The most active compounds, 4o, 4s, 5e, 5f, 5l, 5m and 5o (IC_{50} 3.29 – 9.92 mmole) were tested on the expression of CDK1, p53, caspase-3 and caspase-9
- Docking studies on CDK1 for the most active compounds were undertaken.