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LETTERS

Regioselective synthesis of indole-thiazolidine-2,4-dione coupled isoxazoles as *in vitro* tubulin polymerization inhibitors

Sunitha Boda, Ravinder Manchal*

Department of Chemistry, Chaitanya (Deemed to be University), Hanumakonda, Telangana, India

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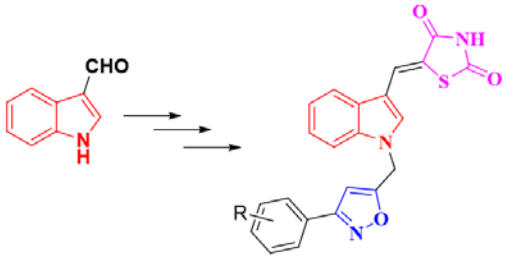
Article

ABSTRACT

Herein we synthesized new indole-thiazolidine-2,4-dione coupled isoxazoles (7a-n) via simple reactions like N-propargylation, Knoevenagel condensation and copper (I) catalysed one pot regioselective reactions. All the newly synthesized compounds were characterized by 1H-NMR, 13C-NMR and Mass spectra and they were screened for *in vitro* anticancer activity against three human cancer cell lines like A549 (lung), MCF-7 (breast), and SKOV3 (ovarian) using MTT assay and etoposide was used as the standard drug. As per the results the compounds **7e**, **7f** and **7g** where shown selectivity towards A549 cell line with IC_{50} values of 5.27 µM, 3.14 µM and 6.25 µM respectively and they are high active than etoposide. Further *in vitro* tubulin polymerization assay on three potent compounds (**7e**, **7f** and **7g**) revealed that compounds 7e and 7f have exhibited potency than standard combretastatin A-4 with IC_{50} values 0.82 and .044 µM respectively.



Keywords: Indole, thiazolidine 2,4-dione, isoxazole, in vitro anticancer activity, in vitro tubulin polymerization assay

INTRODUCTION

Cancer is a lethal illness regarded as uncontrolled growing and spread of abnormal cells. As per the data of year 2018, around 6 lakhs cancer demises arise in United States and based on statistics of 2006–2015 decease proportion decayed by the 1.5% both in men and women.[1] Hereafter, the development new cancer treatments or active anticancer drugs have always been essential and motivating task to the current medicinal chemistry researchers. In view of this, the selection of indole pharmacophore in the development of new anticancer agents was found to be suitable choice, as it provided many anticancer agents work with diverse mechanism of actions.[2] The indole framework is also available in few natural alkaloids, plant's hormones and living organisms and in many pharmacological active analogues.[3–6] Along with that, the thiazolidine-2,4-dione ring has been recognized as vital framework in the drug design and discovery.[7,8] Remarkably, several anticancer agents containing thiazolidine-2,4-dione as key pharmacophore are also reported in the literature.[9–13] Few thiazolidine-2,4-dione compounds named by GSK1059615, AZD1208 and SMI-4a are now in clinical trials for the cancer treatment.[13–15] On the other aspect, owing to the less cytotoxicity and relatively easy synthesis,[16] the isoxazole core has always been a prevalent scaffolds in the development of new compounds with different biological activities including anti-inflammatory, antimicrobial, anticonvulsant, antiviral and antidiabetic. Particularly, isoxazole core incorporated to other pharmacophores showed diverse anticancer activities.[17–26]

Bearing all the above findings in our mind, herein, we combined the indole, thiazolidine-2,4-dione and isoxazole pharmacophores as single framework using the concept of pharmacophore hybridization[27–29] and studied their antiproliferative activity against A549 (lung), MCF-7 (breast), and SKOV3 (ovarian) cells. We have also carried out the *in vitro* tubulin polymerization for the compounds **7e**, **7f** and **7g** which found to be potent in the antiproliferative activity.

RESULTS AND DISCUSSION

Chemistry

The entire synthesis of anticipated indole-thiazolidine-2,4-dione-isoxazoles (**7a-7n**) was achieved in two main steps as outline in **schemes 1** and **2**. At first, the 1*H*-indole-3-carbaldehyde (**1**) was treated with propargyl bromide (**2**) using Cs_2CO_3 in DMF at RT for 3 h to give 1-(prop-2-yn-1-yl)-1*H*-indole-3-carbaldehyde (**3**). Then, the Knoevenagel condensation reaction of intermediate **3** with thiazolidine-2,4-dione (**4**) under piperidine catalysis in MeOH under reflux for 21 h gave the (Z)-5-((1-(prop-2-yn-1-yl)-1*H*-indol-3-yl)methylene)thiazolidine-2,4-dione (**5**) (**Scheme 1**).[30]

*Corresponding Author: Prof. Ravinder Manchal
Department of chemistry, Chaitanya (Deemed to be University), Hanumakonda, Telangana, India.
Email: visitravi76@gmail.com

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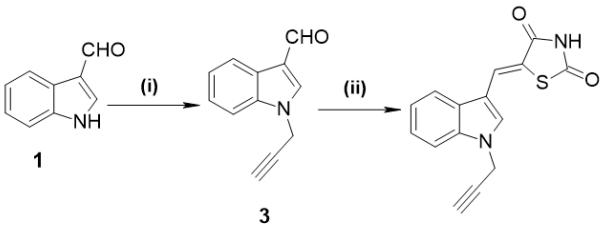


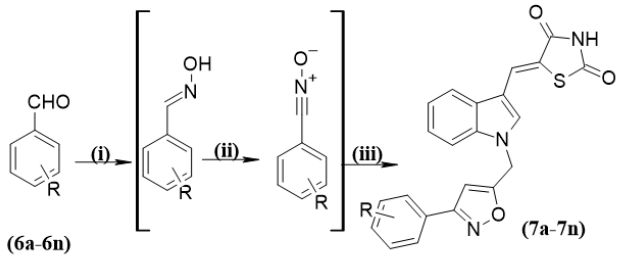
Reagents and conditions

(i) Propargyl bromide (2), Cs_2CO_3, DMF, RT, 3 h;

(ii) Thiazolidine-2,4-dione (4), Piperidene, MeOH, Reflux, 21 h;

Scheme 1. Synthesis of (Z)-5-((1-(prop-2-yn-1-yl)-1H-indol-3-yl)methylene)thiazolidine-2,4-dione (5)

According to **scheme 2**, at the outset, aromatic aldehydes (**6a-6n**) were treated with NH_2OH. HCl and NaOH in aq. ^{t}BuOH solvent media at RT for 2 h to give consistent *in situ* aldoximes that subsequently transformed into corresponding *in situ* nitrile oxides by adding chloramine T trihydrate slowly for 20 min to them. Finally, the *in situ* formed Cu(I) (generated by combining $CuSO_4$ and sodium ascorbate) promoted [3+2]-cycloaddition between nitrile oxides and intermediate (**5**) at RT for an additional 12 h provided the anticipated compounds (**7a-7n**).[31]



7a R= H ; **7b** R= 4-Me ; **7c** R= 3,5-di-Me ; **7d** R= 4-OMe ;

7e R= 3,5-di-OMe ; **7f** R= 3,4,5-tri-OMe ; **7g** R= 4-Cl-3,5-di-OMe ;

7h R= 4-Cl ; **7i** R=3,5-di-Cl ; **7j** R= 4-Br ; **7k** R=4-F ;

7l R=4-NO_2 ; **7m** R= 3-NO_2 ; **7n** R= 4-CN

Reagents & conditions:

(i) NH_2OH. HCl, NaOH, ^{t}BuOH:H_2O (1:1), RT, 2 h. (ii) Chloramine T trihydrate 20 min, (iii) Comp. (5), $CuSO_4.5H_2O$, Sodium ascorbate, RT, 12 h.

Scheme 2. Synthesis of indole-thiazolidine-2,4-dione-isoxazoles (7a-7n)

In vitro anticancer activity

The newly synthesized compounds (**7a-7n**) were screened for their *in vitro* anticancer activity against A549 (lung), MCF-7 (breast), and SKOV3 (ovarian) cell lines using MTT assay and etoposide was used as the standard drug (**Table 1**). From the results, it was observed that the compounds **7e, 7f** and **7g** showed more activity than etoposide against A549 cell line with IC_{50} values of 5.27 µM, 3.14 µM and 6.25 µM respectively. But the same compounds have shown slightly less activity than the reference drug against remaining two cell lines MCF-7 and SKOV3 cell lines. We have analyzed the structure–activity relationships (SAR) to identify the effect of substituent on the phenyl ring attached to isoxazole. In the case of compounds with electron donating substituents on phenyl ring, the compound **7f** (having 3,4,5-trimethoxy substituent) have shown highest activity compared to the remaining compounds. Similarly, the compound **7e** (having 3,5-dimethoxy substituent) has ranked second in the series. Introducing halogen at 4th position of **7f** resulted **7g** (having 4-chloro-3,5-dimethoxy substituent) with decreased activity. The compound **7d** (having 4-methoxy substituent) has shown less activity compared to **7e**, **7f** and **7g**. The compound **7c** with two methyl substituents has exhibited greater activity than **7b** (with 4-methyl substituent) and **7a** (without any substituent). The overall order of activity of the compounds with electron releasing groups was found to be 3,4,5-tri-OMe (**7f**)>3,5-di-OMe (**7e**)> 4-Cl-3,5-di-OMe (**7g**)> 4-OMe (**7d**)> 3,5-di-OMe (**7c**)> 4-Me (**7b**)> **7a**. In case of compounds with electron donating groups (**7h-7n**), **7n** having 4-cyano substituent has shown highest activity but the remaining compounds exhibited lower activities. In case of mono halogenated compounds, the order was found to be 4-Cl> 4-F> 4-Br. The compound **7i** with two chlorine atoms at 3rd and 5th exhibited less activity than **7h** with on chlorine at 4th position. The compounds **7l** and **7m** with nitro groups at 3rd and 4th positions displayed less activity than halogenated compounds.

Table 1: *In vitro* anticancer activity of newly developed indole-thiazolidine-2,4-dione-isoxazoles (**7a-n**) with IC_{50} in µM[a]

Entry	R	[b]A549	[c]MCF-7	[d]SKOV3
7a	H	56.15±1.14	65.42±1.65	54.75±1.85
7b	4-Me	44.85±1.51	58.27±1.46	48.74±1.42
7c	3,5-di-Me	34.59±1.34	35.82±1.40	44.57±1.65
7d	4-OMe	26.51±0.2	55.64±1.35	37.58±2.02
7e	3,5-di-OMe	5.27±0.2	24.27±0.85	28.08±1.02
7f	3,4,5-tri-OMe	3.14±0.1	14.15±0.42	15.22±0.98
7g	4-Cl-3,5-di-OMe	6.25±0.2	26.14±1.05	19.56±1.13
7h	4-Cl	29.81±2.12	37.84±1.10	48.08±1.22
7i	3,5-di-Cl	89.72±1.21	98.42±1.56	NI
7j	4-Br	68.85±2.07	NI	63.24±1.34
7k	4-F	48.62±1.72	58.21±1.15	54.82±1.52
7l	4-NO_2	75.15±1.07	NI	NI
7m	3-NO_2	82.56±1.54	NI	87.45±1.15
7n	4-CN	16.12±1.51	25.46±1.22	32.26±1.28
Etoposide	-	7.6±0.1	9.8±0.2	8.4±0.1

[a]Each data represents as mean ±S.D values; [b]A549: Human lung cancer cell line; [c]MCF-7: Human breast cancer cell line; [d]SKOV3: Human ovarian cancer cell line. NI = IC_{50} >100 µM

In vitro tubulin polymerization inhibitory assay

Literature survey reveals that the development of new anticancer agents by targeting the microtubules was considered as one of the outstanding goals,[32] because they had key involvement in the regulation of cellular functions like organelle

transport, cell division, motility and upkeep of signal transduction etc. Many indole and isoxazole derivatives have also been reported as tubulin polymerization inhibiting agents.[33–35]

The compounds **7e**, **7f** and **7g** which were shown more anticancer activity and **7l** which has shown less activity were screened for their efficacy in inhibiting tubulin polymerization by employing tubulin assembly assay using Combretastatin A4 (CA-4) as the reference drug.[36] According to the results (**Table 2**) the compounds **7e** and **7f** have exhibited more inhibition than standard with IC_{50} values 0.82 and .044 μM respectively. On the other hand, the compound **7g** has exhibited less activity compared to the reference drug.

Table 2. Tubulin polymerization inhibitory activity

Compound	IC_{50}(μM)*
7e	0.82±0.02
7f	0.44±0.01
7g	2.34±0.05
CA-4	1.10±0.01

*The values are indicated as the mean±SD

EXPERIMENTAL

General information

All the readily accessible chemicals were utilized without further purification. The purity of all compounds was analysed using Merck 60F254 silica gel TLC plates. The melting points were calculated on a hot stage melting point apparatus Ernst Leitz Wetzlar, Germany and were uncorrected. The ^1H &^{13}C NMR spectra of synthesized compounds were recorded using Mercuryplus spectrometer (operating at 400 MHz for ^1H & 100 MHz for ^{13}C) and the chemical shifts were referenced to the TMS. ESI (electrospray ionization) mass spectra (at an ionising voltage of 70 eV) were achieved by Shimadzu QP5050A quadrupole-based mass spectrometer.

Synthesis of 1-(prop-2-yn-1-yl)-1H-indole-3-carbaldehyde (3): In a 250 mL RBF, a mixture of 1H-indole-3-carbaldehyde (1) (0.027 mol), Cs2CO3 (0.054 mol) and propargyl bromide (0.0378 mol) in DMF (50 mL) was stirred at RT for 3 h. The progress of reaction as analyzed by the TLC, the reaction mixture was then poured into ice-cold water (50 mL). The obtained precipitate was filtered, washed with excess of water and dried under vacuum for 3 h. Yield: 76%.

Synthesis of (Z)-5-((1-(prop-2-yn-1-yl)-1H-indol-3-yl)methylene)thiazolidine-2,4-dione (5): In a 250 mL RBF, A mixture of the 1-(prop-2-yn-1-yl)-1H-indole-3-carbaldehyde (3) (0.02 mol), thiazolidine-2,4-dione (0.02 mol) and piperidine (0.002 mol) in EtOH (40 mL) was refluxed for 21 hours. The progress of the reaction as analyzed by TLC, the reaction mixture was then cooled to RT for overnight. Later the resulting precipitate was filtered, washed with cold ethanol and dried and finally recrystallized using EtOH. Yield: 74%.

General method for the preparation of indole-thiazolidine-2,4-dione-isoxazoles (7a-7n): Synthesis of (Z)-5-((1-((3-phenylisoxazol-5-yl)methyl)-1H-indol-3-yl)methylene) thiazolidine-2,4-dione (7a). The benzaldehyde (6a), NH_2OH. HCl (1.1 mmol) and NaOH (1.1 mmol) in 3 mL of equal ratio of H_2O-^{t}BuOH solvent media were stirred at at RT for 2 h. The in situ generation of benzaldoxime as analyzed by TLC, chloramine-T trihydrate (1.1 mmol) was then added in portion wise slowly for 20 min. Later, to this resulting mixture, the $CuSO_4.5H_2O$ (0.1 mmol), sodium ascorbate (0.2 mmol) and intermediate 5 (1 mmol) were added and the pH of reaction mixture was attuned to 6 by adding addition few drops of NaOH (1M) and the stirring was sustained at RT for further 12 h. After completion of the reaction as checked with TLC, the reaction mixture was then decanted into cold water (15 mL) and dilute NH4OH (3 mL) was added to it, in order to eradicate all needless Cu-salts. The crude product was separated from the filtration which was subsequently subjected to 60-120 mesh size silica gel column chromatography using hexane/ethyl acetate (7:3) mobile phase to afford pure product 7a.

Characterization data:

(Z)-5-((1-((3-phenylisoxazol-5-yl)methyl)-1H-indol-3-yl)methylene)thiazolidine-2,4-dione (**7a**): Yield: 76%; ^1H NMR (400 MHz, DMSO-d_6; δ in ppm): 11.98 (s, 1H, -N*H*), 7.71 (d, J =8.0 Hz, 2H), 7.68 (s, 1H), 7.62 (d, *J*=8.0 Hz, 1H), 7.55-7.36 (m, 3H), 7.30-7.24 (m, 2H), 7.17 (s, 1H), 7.08 (t, *J*=8.0 Hz, 1H), 6.87 (s, 1H), 5.50 (s, 2H, N-C*H*$_2$), ^{13}C NMR (100 MHz, DMSO-d_6; δ in ppm): 168.87, 162.01, 160.54, 157.83, 139.71, 136.23, 131.38, 130.66, 129.91, 129.13, 128.39 (2C), 127.33 (2C), 124.71, 124.03, 122.19, 121.49, 114.98, 111.88, 98.27, 42.20; ESI-MS m/z: 402 [M+H]. Anal. Cal for $C_{22}H_{15}N_3O_3S$: C, 65.82; H, 3.77; N, 10.47. found: C, 65.85; H, 3.74; N, 10.45.

(Z)-5-((1-((3-(p-tolyl)isoxazol-5-yl)methyl)-1H-indol-3-yl)methylene)thiazolidine-2,4-dione (**7b**): Yield: 82%; ^1H NMR (400 MHz, DMSO-d_6; δ in ppm): 11.95 (s, 1H, -N*H*), 7.68 (s, 1H), 7.60 (d, *J*=8.8 Hz, 1H), 7.51 (d, *J*=8.0 Hz, 2H), 7.42 (d, *J*=8.0 Hz, 1H), 7.30 (d, *J*=8.8 Hz, 2H), 7.19 (t, *J*=8.0 Hz, 1H), 7.16 (s, 1H), 7.06 (t, *J*=8.0 Hz, 1H), 6.88 (s, 1H), 5.51 (s, 2H, N-C*H*$_2$), 2.37(s, 3H, -CH_3); ^{13}C NMR (100 MHz, DMSO-d_6; δ in ppm): 168.66, 162.12, 160.43, 157.23, 139.87, 138.93, 137.21, 131.03, 130.54, 129.23, 128.98 (2C), 127.11(2C), 124.22, 124.07, 121.89, 120.72, 114.21, 111.63, 98.53, 42.27, 21.89; ESI-MS m/z: 416 [M+H]. Anal. Cal for $C_{23}H_{17}N_3O_3S$: C, 66.49; H, 4.12; N, 10.11. found: C, 66.53; H, 4.16; N, 10.08.

(Z)-5-((1-((3-(3,5-dimethylphenyl)isoxazol-5-yl)methyl)-1H-indol-3-yl)methylene) thiazolidine-2,4-dione (**7c**): Yield: 84%; ^1H NMR (400 MHz, DMSO-d_6; δ in ppm): 11.92 (s, 1H, -N*H*), 7.69 (s, 1H), 7.61 (d, *J*=8.0 Hz, 1H), 7.50 (s, 2H), 7.40 (d, *J*=8.0 Hz, 1H), 7.27 (s, 1H), 7.18 (t, *J*=8.0 Hz, 1H), 7.15 (s, 1H), 7.04 (t, *J*=8.0 Hz, 1H), 6.86 (s, 1H), 5.50 (s, 2H, N-C*H*$_2$), 2.31(s, 6H, 2-CH_3); ^{13}C NMR (100 MHz, DMSO-d_6; δ in ppm): 168.45, 162.13, 160.88, 157.56, 139.16, 138.22, 137.59 (2C), 132.12, 131.09, 130.53, 128.66, 127.41 (2C), 124.62, 124.03, 121.78, 121.31, 114.32, 111.41, 98.64, 42.39, 21.27 (2C); ESI-MS m/z: 430 [M+H]. Anal. Cal for $C_{24}H_{19}N_3O_3S$: C, 67.12; H, 4.46; N, 9.78. found: C, 67.18; H, 4.49; N, 9.75.

(Z)-5-((1-((3-(4-methoxyphenyl)isoxazol-5-yl)methyl)-1H-indol-3-yl)methylene) thiazolidine-2,4-dione (**7d**): Yield: 86%;

^{1}H NMR (400 MHz, DMSO-d_6; δ in ppm): 11.94 (s, 1H, -N*H*), 7.71 (d, *J*=8.8 Hz, 2H), 7.67 (s, 1H), 7.64 (d, *J*=8.2 Hz, 1H), 7.44 (d, *J*=8.2 Hz, 1H), 7.24 (t, *J*=8.2 Hz, 1H), 7.18 (s, 1H), 7.05 (t, *J*=8.2 Hz, 1H), 7.00 (d, *J*=8.8 Hz, 2H), 6.89 (s, 1H), 5.53 (s, 2H, N-C*H*$_2$), 3.87 (s, 2H, O-C*H*$_3$); ^{13}C NMR (100 MHz, DMSO-d_6; δ in ppm): 168.23, 162.11, 160.79, 158.23, 157.23, 139.23, 138.23, 130.65, 129.81(2C), 128.64, 124.32, 124.04, 121.91, 121.73, 121.21, 114.88 (2C), 114.23, 111.23, 98.56, 56.23, 42.53; ESI-MS m/z: 432 [M+H]. Anal. Cal for $C_{23}H_{17}N_3O_4S$: C, 64.03; H, 3.97; N, 9.74. found: C, 64.07; H, 3.93; N, 9.77.

(Z)-5-((1-((3-(3,5-dimethoxyphenyl)isoxazol-5-yl)methyl)-1H-indol-3-yl)methylene) thiazolidine-2,4-dione (**7e**): Yield: 88%; ^{1}H NMR (400 MHz, DMSO-d_6; δ in ppm): 11.91 (s, 1H, -N*H*), 7.68(s, 1H), 7.63 (d, *J*=8.2 Hz, 1H), 7.43 (d, *J*=8.2 Hz, 1H), 7.25 (t, *J*=8.2 Hz, 1H), 7.19 (s, 1H), 7.13 (s, 1H), 7.06 (t, *J*=8.2 Hz, 1H), 6.98 (s, 2H), 6.89 (s, 1H), 5.50 (s, 2H, N-C*H*$_2$), 3.83 (s, 6H, 2O-C*H*$_3$); ^{13}C NMR (100 MHz, DMSO-d_6; δ in ppm): 168.58, 162.15, 160.36, 159.43(2C), 158.17, 139.22, 138.19, 134.31, 130.48, 129.66, 124.68, 124.02, 121.88, 121.50, 114.52, 111.72, 110.32(2C), 104.63, 98.54, 56.34 (2C), 42.61; ESI-MS m/z: 462 [M+H]. Anal. Cal for $C_{24}H_{19}N_3O_5S$: C, 62.46; H, 4.15; N, 9.11. found: C, 62.49; H, 4.18; N, 9.07.

(Z)-5-((1-((3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl)methyl)-1H-indol-3-yl)methylene) thiazolidine-2,4-dione (**7f**): Yield: 86%; ^{1}H NMR (400 MHz, DMSO-d_6; δ in ppm): 11.95 (s, 1H, -N*H*), 7.68 (s, 1H), 7.63 (d, *J*=8.0 Hz, 1H), 7.42 (d, *J*=8.0 Hz, 1H), 7.23 (t, *J*=8.0 Hz, 1H), 7.19 (s, 1H), 7.07 (t, *J*=8.0 Hz, 1H), 7.00 (s, 2H), 6.87 (s, 1H), 5.52 (s, 2H, N-C*H*$_2$), 3.87(s, 3H, OCH$_3$), 3.83 (s, 6H, 2OCH$_3$), ^{13}C NMR (100 MHz, DMSO-d_6; δ in ppm): 168.30, 162.66, 160.43, 159.22, 153.65 (2C),141.82, 139.15, 138.54, 130.62, 129.49, 125.66, 124.42, 124.05, 121.57, 121.12, 114.64, 111.98, 108.28 (2C), 98.62, 56.66, 55.55(2C), 42.53; ESI-MS m/z: 492 [M+H]. Anal. Cal for $C_{25}H_{21}N_3O_6S$: C, 61.09; H, 4.31; N, 8.55. found: C, 61.12; H, 4.35; N, 8.50.

(Z)-5-((1-((3-(4-chloro-3,5-dimethoxyphenyl)isoxazol-5-yl)methyl)-1H-indol-3-yl)methylene) thiazolidine-2,4-dione (**7g**): Yield: 82%; ^{1}H NMR (400 MHz, DMSO-d_6; δ in ppm): 11.96 (s, 1H, -N*H*), 7.68(s, 1H), 7.63 (d, *J*=8.4 Hz, 1H), 7.42 (d, *J*=8.4 Hz, 1H), 7.25 (t, *J*=8.4 Hz, 1H), 7.19 (s, 1H), 7.06 (t, *J*=8.4 Hz, 1H), 7.01 (s, 2H), 6.87 (s, 1H), 5.50 (s, 2H, N-C*H*$_2$), 3.84 (s, 6H, 2OCH$_3$), ^{13}C NMR (100 MHz, DMSO-d_6; δ in ppm): 168.69, 162.31, 160.41, 159.13, 158.65 (2C), 139.26, 138.88 (2C), 131.08, 129.66, 124.58, 124.12, 121.88, 121.59, 120.43, 114.33, 111.88, 106.26(2C), 98.65, 56.23 (2C), 42.65; ESI-MS m/z: 496 [M+H]. Anal. Cal for $C_{24}H_{18}ClN_3O_5S$: C, 58.12; H, 3.66; N, 8.47. found: C, 58.15; H, 3.69; N, 8.44.

(Z)-5-((1-((3-(4-chlorophenyl)isoxazol-5-yl)methyl)-1H-indol-3-yl)methylene) thiazolidine-2,4-dione (**7h**): Yield: 80%; ^{1}H NMR (400 MHz, DMSO-d_6; δ in ppm): 11.95 (s, 1H, -N*H*), 7.73 (d, *J*=8.8 Hz, 2H), 7.68 (s, 1H), 7.63 (d, *J*=8.0 Hz, 1H), 7.50 (d, *J*=8.8 Hz, 2H), 7.43 (d, *J*=8.0 Hz, 1H), 7.27 (t, *J*=8.0 Hz, 1H), 7.16 (s, 1H), 7.08 (t, *J*=8.0 Hz, 1H), 6.89 (s, 1H), 5.50 (s, 2H, N-C*H*$_2$), ^{13}C NMR (100 MHz, DMSO-d_6; δ in ppm): 168.33, 162.41, 160.38, 159.63, 139.76, 138.32, 136.41, 132.44, 130.89, 129.21, 128.71 (2C), 127.33(2C), 124.53, 124.03, 121.89, 121.13, 114.76, 111.63, 98.63, 42.55; ESI-MS m/z: 436 [M+H]. Anal. Cal for $C_{22}H_{14}ClN_3O_3S$: C, 60.62; H, 3.24; N, 9.64. found: C, 60.58; H, 3.20; N, 9.67.

(Z)-5-((1-((3-(3,5-dichlorophenyl)isoxazol-5-yl)methyl)-1H-indol-3-yl)methylene) thiazolidine-2,4-dione (**7i**): Yield: 78%; ^{1}H NMR (400 MHz, DMSO-d_6; δ in ppm): 11.96 (s, 1H, -N*H*), 7.77 (s, 1H), 7.69 (s, 1H), 7.64 (d, *J*=8.0 Hz, 1H), 7.46 (d, *J*=8.0 Hz, 1H), 7.39 (s, 2H), 7.25 (t, *J*=8.0 Hz, 1H), 7.18 (s, 1H), 7.07 (t, *J*=8.0 Hz, 1H), 6.89 (s, 1H), 5.50 (s, 2H, N-C*H*$_2$), ^{13}C NMR (100 MHz, DMSO-d_6; δ in ppm): 168.16, 162.29, 160.32, 159.48, 139.39, 138.22, 134.98 (2C), 132.87, 131.98, 130.32, 129.43, 126.78 (2C), 124.65, 124.03, 121.93, 121.23, 114.43, 111.65, 98.88, 42.38; ESI-MS m/z: 470 [M+H]. Anal. Cal for $C_{22}H_{13}Cl_2N_3O_3S$: C, 56.18; H, 2.79; N, 8.93. found: C, 56.15; H, 2.75; N, 8.96.

(Z)-5-((1-((3-(4-bromophenyl)isoxazol-5-yl)methyl)-1H-indol-3-yl)methylene) thiazolidine-2,4-dione (**7j**): Yield: 76%; ^{1}H NMR (400 MHz, DMSO-d_6; δ in ppm): 11.92 (s, 1H, -N*H*), 7.68 (s, 1H), 7.64 (d, *J*=8.0 Hz, 1H), 7.58-7.50 (m, 4H), 7.42 (d, *J*=8.0 Hz, 1H), 7.23 (t, *J*=8.0 Hz, 1H), 7.17 (s, 1H), 7.05 (t, *J*=8.0 Hz, 1H), 6.87 (s, 1H), 5.50 (s, 2H, N-C*H*$_2$), ^{13}C NMR (100 MHz, DMSO-d_6; δ in ppm): 168.77, 162.52, 160.54, 159.38, 139.65, 138.44, 132.12(2C), 130.87, 129.54, 128.32, 126.65 (2C), 125.59, 124.42, 124.04, 121.87, 121.23, 114.63, 111.93, 98.97, 42.79; ESI-MS m/z: 479 [M+H] & 481 [M+3H] . Anal. Cal for $C_{22}H_{14}BrN_3O_3S$: C, 55.01; H, 2.94; N, 8.75. found: C, 55.03; H, 2.91; N, 8.72.

(Z)-5-((1-((3-(4-fluorophenyl)isoxazol-5-yl)methyl)-1H-indol-3-yl)methylene) thiazolidine-2,4-dione (**7k**): Yield: 74%; ^{1}H NMR (400 MHz, DMSO-d_6; δ in ppm): 11.96 (s, 1H, -N*H*), 8.25 (d, *J*=8.8 Hz, 2H), 7.90 (d, *J*=8.8 Hz, 2H), 7.70 (s, 1H), 7.66 (d, *J*=8.0 Hz, 1H), 7.43 (d, *J*=8.0 Hz, 1H), 7.27 (t, *J*=8.0 Hz, 1H), 7.19 (s, 1H), 7.09 (t, *J*=8.0 Hz, 1H), 6.89 (s, 1H), 5.50 (s, 2H, N-C*H*$_2$), ^{13}C NMR (100 MHz, DMSO-d_6; δ in ppm): 168.88, 163.73, 162.32, 160.23, 159.43, 158.65, 139.97, 138.76, 132.91, 131.23 (2C), 129.78, 127.38, 124.66, 124.12, 122.63, 121.17, 117.32 (2C), 114.36, 111.66, 98.89, 42.67; ESI-MS m/z: 420 [M+H]. Anal. Cal for $C_{22}H_{14}FN_3O_3S$: C, 63.00; H, 3.36; N, 10.02. found: C, 63.02; H, 3.38; N, 10.06.

(Z)-5-((1-((3-(4-nitrophenyl)isoxazol-5-yl)methyl)-1H-indol-3-yl)methylene) thiazolidine-2,4-dione (**7l**): Yield: 72%; ^{1}H NMR (400 MHz, DMSO-d_6; δ in ppm): 11.96 (s, 1H, -N*H*), 8.35(d, *J*=8.4 Hz, 2H), 8.10 (d, *J*=8.4 Hz, 2H), 7.69 (s, 1H), 7.64 (d, *J*=8.0 Hz, 1H), 7.44 (d, *J*=8.0 Hz, 1H), 7.24 (t, *J*=8.0 Hz, 1H), 7.19 (s, 1H), 7.09 (t, *J*=8.0 Hz, 1H), 6.90 (s, 1H), 5.55 (s, 2H, N-C*H*$_2$), ^{13}C NMR (100 MHz, DMSO-d_6; δ in ppm): 168.41, 162.54, 160.55, 159.38, 148.35, 139.98, 138.35, 135.28, 130.66, 128.49, 127.32 (2C), 125.54 (2C), 124.98, 124.23, 122.55, 121.09, 114.87, 111.76, 98.29, 42.67; ESI-MS m/z: 447 [M+H]. Anal. Cal for $C_{22}H_{14}N_4O_5S$: C, 59.19; H, 3.16; N, 12.55. found: C, 59.17; H, 3.13; N, 12.59.

(Z)-5-((1-((3-(3-nitrophenyl)isoxazol-5-yl)methyl)-1H-indol-3-yl)methylene) thiazolidine-2,4-dione (**7m**): Yield: 70%; ^{1}H NMR (400 MHz, DMSO-d_6; δ in ppm): 11.96 (s, 1H, -N*H*), 8,53 (s, 1H), 8.33-8.23 (m, 3H), 7.69 (s, 1H), 7.64 (d, *J*=8.4 Hz, 1H), 7.43 (d, *J*=8.4 Hz, 1H), 7.28 (t, *J*=8.4 Hz, 1H), 7.19 (s,

1H), 7.09 (t, *J*=8.4 Hz, 1H), 6.89 (s, 1H), 5.53 (s, 2H, N-C*H*$_2$), ^{13}C NMR (100 MHz, DMSO-d$_6$; δ in ppm): 168.13, 162.32, 160.87, 159.54, 148.21, 139.98, 138.23, 134.32, 131.65, 130.65, 129.66, 128.23, 127.31, 124.54, 124.24, 124.12, 121.87, 121.21, 114.65, 111.48, 98.66, 42.69; ESI-MS m/z: 447 [M+H]. Anal. Cal for $C_{22}H_{14}N_4O_5S$: C, 59.19; H, 3.16; N, 12.55. found: C, 59.16; H, 3.13; N, 12.58.

(Z)-4-(5-((3-((2,4-dioxothiazolidin-5-ylidene)methyl)-1H-indol-1-yl)methyl)isoxazol-3-yl)benzonitrile (**7n**): Yield: 76%; ^{1}H NMR (400 MHz, DMSO-d$_6$; δ in ppm): 11.94 (s, 1H, -N*H*), 7.68 (s, 1H), 7.63 (d, *J*=8.0 Hz, 1H), 7.55(d, *J*=8.0 Hz, 2H), 7.43 (d, *J*=8.0 Hz, 1H), 7.30 (d, *J*=8.0 Hz, 2H), 7.24 (t, *J*=8.0 Hz, 1H), 7.18 (s, 1H), 7.07 (t, *J*=8.0 Hz, 1H), 6.89 (s, 1H), 5.51 (s, 2H, N-C*H*$_2$), ^{13}C NMR (100 MHz, DMSO-d$_6$; δ in ppm): 168.57, 162.41, 160.67, 159.98, 139.55, 138.23, 134.40, 133.51(2C), 130.87, 129.65, 128.54 (2C), 124.17, 124.08, 122.09, 121.65, 119.76, 116.09, 114.43, 111.54, 98.66, 42.76; ESI-MS m/z: 427 [M+H]. Anal. Cal for $C_{23}H_{14}N_4O_3S$: C, 64.78; H, 3.31; N, 13.14. found: C, 64.82; H, 3.29; N, 13.15.

MTT Assay

Individual wells of a 96-well tissue culture micro titer plate were inoculated with 100 µL of complete medium containing 1×10^4 cells. Then the plates were incubated at 37 °C in a humidified 5% CO_2 incubator for 18 hours prior to the experiment. After medium removal, 100 µL of fresh medium containing the test compounds and etoposide at different concentrations such as 0.5, 1 and 2 µM were added to each well and incubated at 37 °C for 24 hours. Then the medium was discarded and replaced with 10 µL MTT dye. Plates were incubated at 37 °C for 2 hours. The resulting formazan crystals were solubilized in 100 µL extraction buffer. The optical density (O.D) was read at 570 nm with micro plate reader (Multi-mode Varioskan Instrument-Themo Scientific). The percentage of DMSO in the medium never exceeded 0.25%.

***In vitro* tubulin polymerization inhibitory assay**

Tubulin polymerization assay was performed using a commercial kit (cytoskeleton, cat.#BK011P) where tubulin isolated from porcine brain tissue was used. The tubulin reaction mixture containing 2.0 mM $MgCl_2$, 0.5 mM EGTA, 80.0 mM PIPES (pH 6.9), 1 mM GTP, and 10.2% glycerol was prepared. Then, 5 µL of the tested compounds at the indicated concentrations were added, and the mixture was pre-warmed to 37 °C for 1 min. after that the reaction was initiated by the addition of 55 µL tubulin solution. The fluorescence intensity was recorded at every 60 sec for 90 min in a multifunction microplate reader. IC_{50} values were calculated from area under the curve.

CONCLUSION

New indole-thiazolidine-2,4-dione coupled isoxazoles (**7a-n**) were synthesized regio-selectively and tested for *in vitro* anticancer activity against A549 (lung), MCF-7 (breast), and SKOV3 (ovarian) human cancer cell lines by employing MTT assay. Among all the compounds, **7e**, **7f** and **7g** where shown more activity than etoposide and they were active selectively towards A549 cell line. Further *in vitro* tubulin polymerization assay performed on **7e**, **7f** and **7g** which revealed that compounds **7e** and **7f** have exhibited potency than standard combretastatin A-4.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest (financial or academic) for this work.

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