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Synthesis of Novel Clubbed Triazolyl Indeno[1,2-*C*]Isoquinolines As Potent Anticancer Agents

John Thomas*[1], Mahendra Shiradkar[2] Jisha James [3]Golwala Dharmesh[1]

1. Research Development and Innovation centre (RDIC), C.U. Shah University, Wadhwancity 363030

2.Amneal Pharmaceuticals, Aurangabad 431009

3. KVM College of Pharmacy, Kokkothamangalam P.O.Cherthala 688529

ABSTRACT

A variety of novel clubbed triazolyl indeno[1,2-*c*]isoquinolines **14-21** derivatives were synthesized in good yields and characterized by IR, 1H NMR, mass spectral and elemental analyses. All the newly synthesized compounds were evaluated for their *in-vitro* anticancer activity and topoisomerase I inhibition. Several compounds showed interesting cytotoxic activities when compared with the Doxorubicin as a reference drug.

Keywords: Clubbed triazoles, topoisomerase

*Corresponding Author Email: thomasjohnthomas1@gmail.com

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INTRODUCTION

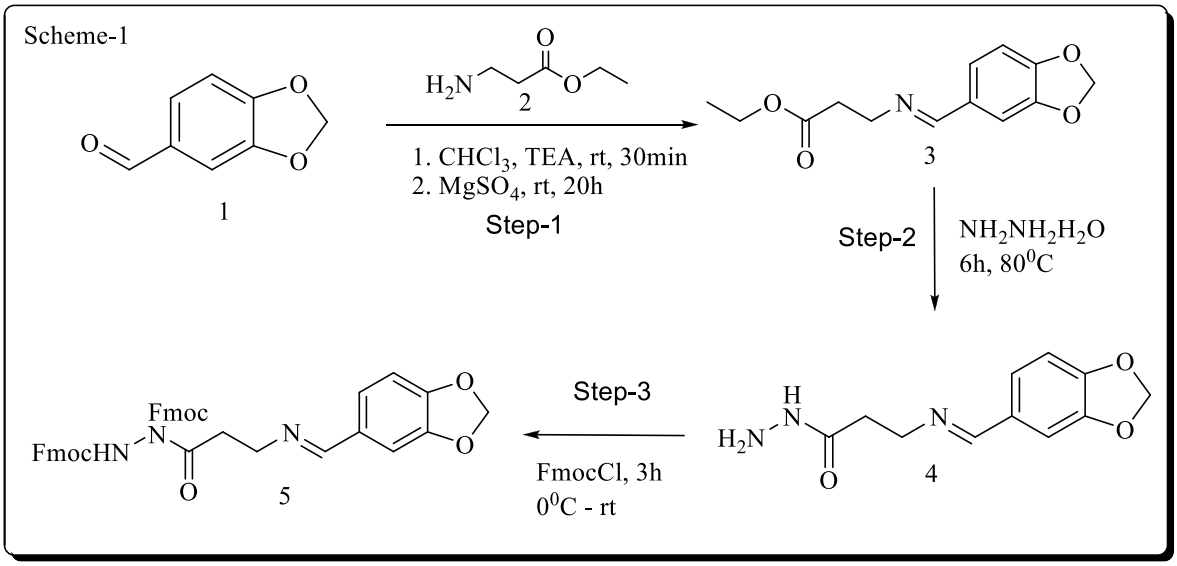
The incidence and mortality of cancer patients have become one of the important issues discussed worldwide. Therefore, identification of novel potent, selective, and less toxic anticancer agents remains one of the most pressing health problems [1, 2].

Camptothecins (CPT), an alkaloid obtained from bark of Chinese camptotheca tree was the first to show the potent cytotoxicity by inhibiting the enzyme known as topoisomerase 1. Unfortunately camptothecin derivatives are not ideal drug molecules. They are compromised by their inability to completely stabilize the ternary (DNA-enzyme-drug) complex, necessitating long infusion times to achieve maximum activity [3]. Furthermore, the camptothecins are inherently unstable and suffer from lactone ring opening (which is favored at physiological pH) to form a hydroxy-acid that has a high affinity for human serum albumin [4-7]. Additionally, certain cancers have been found to be unresponsive to camptothecin treatment and have either developed Top1 mutations limiting the sensitivity of the enzyme to the drug or have evolved P-glycoprotein drug efflux pumps to remove the drug from the cancer cell [8,9]. As a result of the pharmacokinetic problems with the camptothecins, there is great interest in the development of noncamptothecin Top1 inhibitors as anticancer agents The limitations of camptothecins, which include instability due to lactone ring opening and rapid reversibility of top1 inhibition upon drug removal. Therefore, a series of indenoisoquinolines were synthesized as top1 inhibitors and as cytotoxic agents in human cancer cell cultures [10]. Triazoles are class of compounds with many potential pharmacological activities [11]. For effective Top1 inhibition, a stable ternary complex consisting of DNA, Top1, and the drug molecule is required The present investigation was undertaken in order to determine the effect of clubbing dimethoy indenoisoquinolines with triazoles on cytotoxicity and Top1 inhibition.

RESULTS AND DISCUSSION

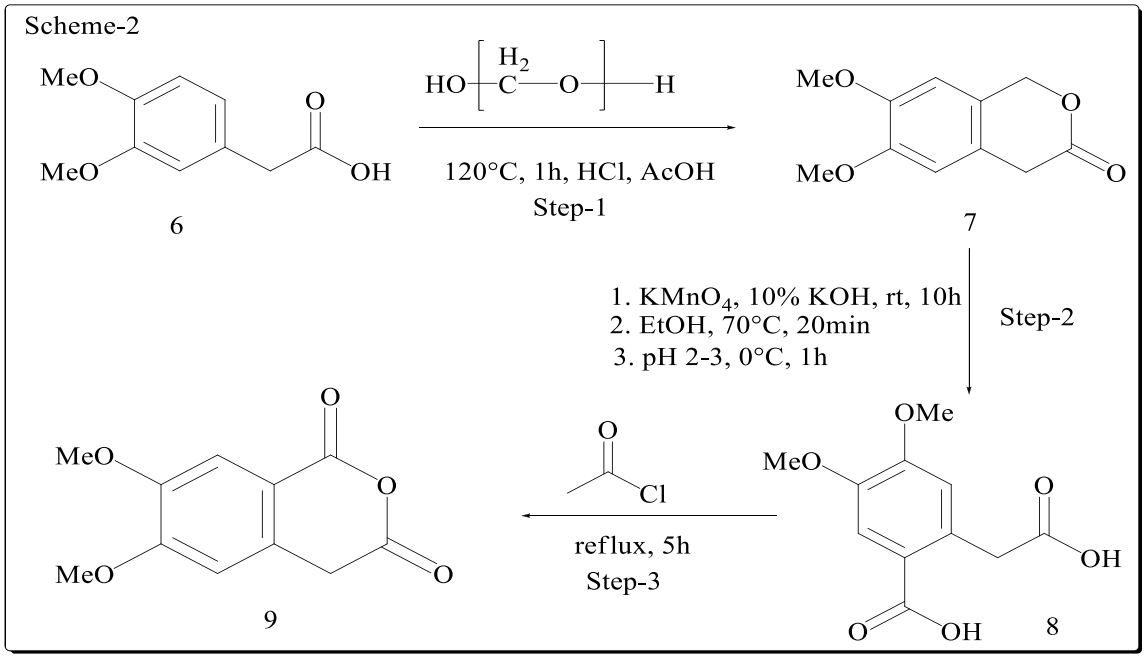
Chemistry

The designed lead compound of clubbed indenoisoquinolines with triazole was depicted in **Scheme 1-3**. The condensation of piperonal **1** with Ethyl 3-aminopropanoate **2** yield (*E*)-ethyl 3-((benzo[*d*][1,3]dioxol-6-yl)methyleneamino)propanoate **3,** which on treatment with hydrazine hydrate gives imine **4**. The reaction of compound **4** under McMurry conditions with Fmoc-Cl to afford the Fmoc-protected imine **5**, as shown in **Scheme 1**.



Scheme 1

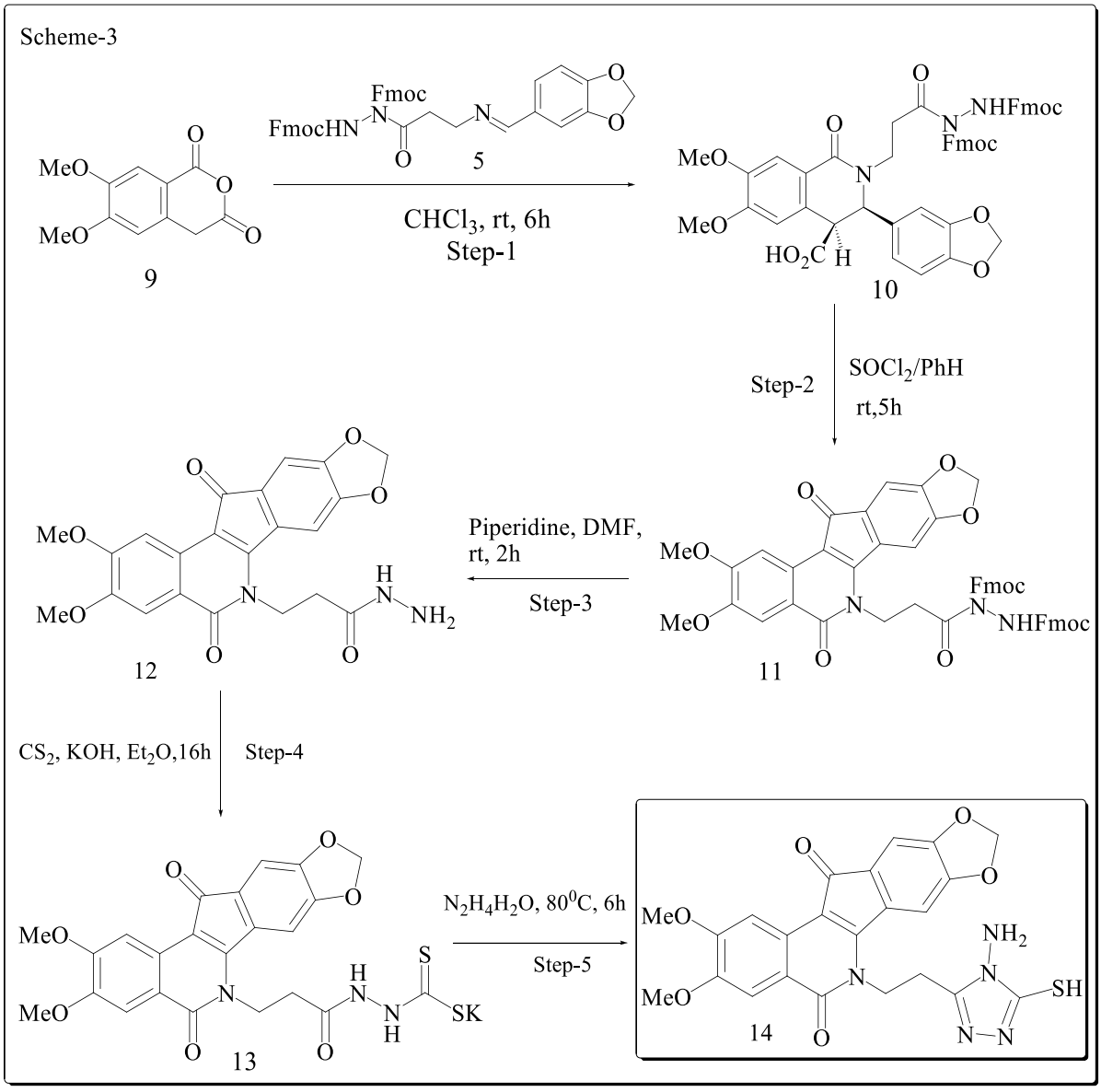
Treatment of **6** with paraformaldehyde gave compound **7** in good yield. Compound **7** on treatment with $KMno_4$ and 10% KOH gave homophthalic acid **8**. The condensation of homophthalic acid **8** with acetyl chloride furnished homophthalic anhydride **9**, as shown in **Scheme 2**.



Scheme 2

Condensation of imine **5** with 4,5-dimethoxyhomophthalic anhydride **9** gave cis Carboxylic acid intermediate **10**, whose relative configuration was determined by 1H NMR. This carboxylic acid **10** was subjected to oxidative Friedel craft's ring closure with thionyl chloride to provide the desired indeno[1,2-c]isoquinoline **11**. The compound **12** was expected to be prepared from global deprotection of Fmoc-protected compound **11**, Deprotection of Fmoc groups in **12** under basic

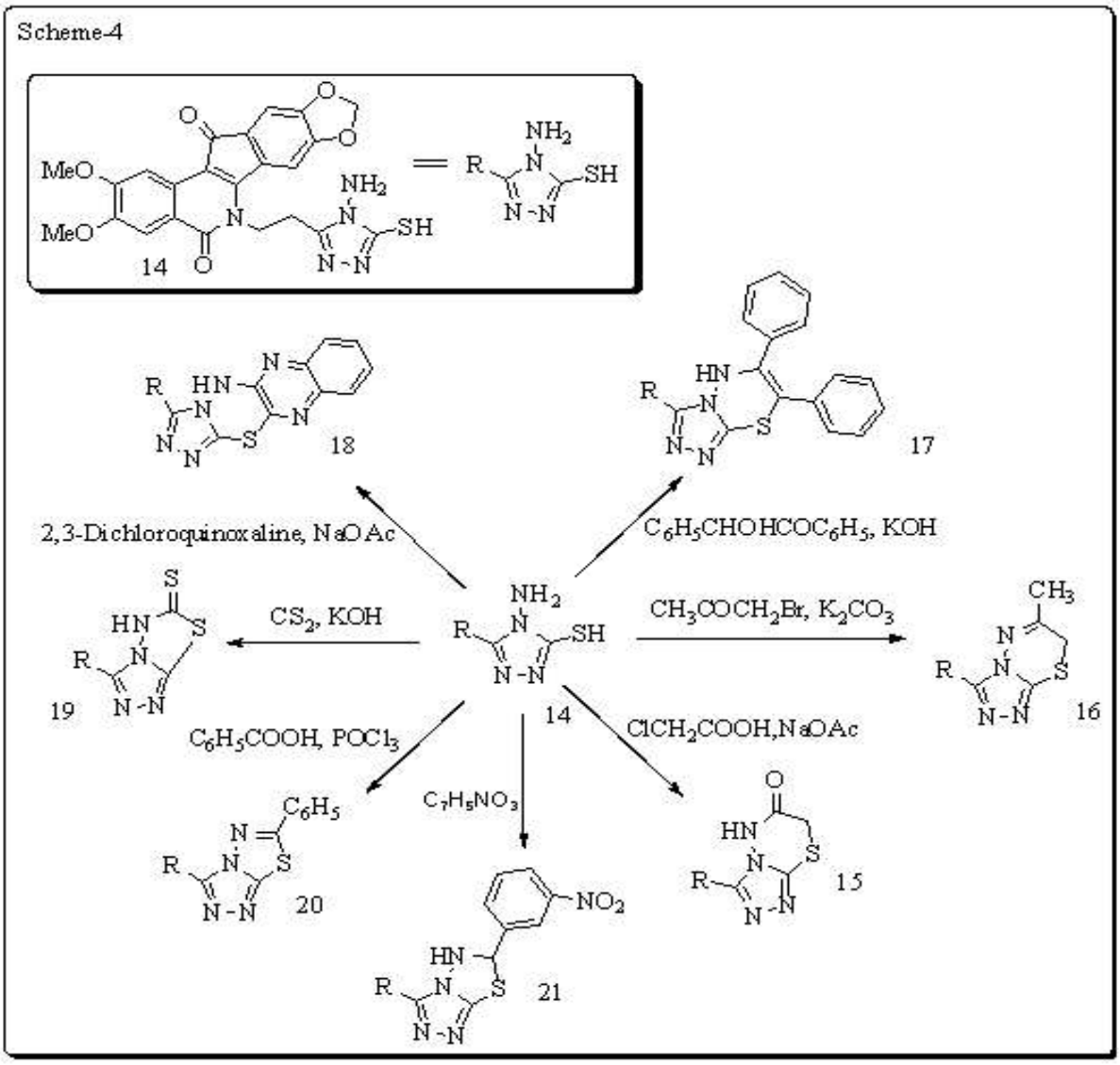
conditions, however, gave predominantly the oxidatively cleaved product **13**. The compound **13** on further reaction with alcoholic potassium hydroxide-carbon disulfide followed by cyclization with hydrazine hydrate gave triazolyl-indenoisoquinoline **14** as shown in **Scheme 3**. Thus, we have considered **14** as a lead molecule and subsequent structural modifications were carried out on the SH and NH_2 groups.



Scheme 3

The starting material triazolyl-indenoisoquinoline **14** was prepared in good yield: by the reaction of the indenoisoquinoline **12** with hydrazine hydrate via intermediate potassium dithiocarbazinate **13**. The latter compound is useful intermediate for the synthesis of triazolothiadiazoles, and triazolothiadiazines as shown in **Scheme 4**. The amino and mercapto groups are ready-made nucleophilic centers for the synthesis of condensed heterocyclic rings. The absence of N-H, and S-

H absorptions in the IR spectra confirms that the cyclo condensation to form the title compounds has taken place.



Scheme 4

Thus, an attempt to prepare the compound **15** by the treatment of **14** in ethanol with choroacetic acid in presence fused sodium acetate afforded **15** in good yield, whose ^{1}H NMR spectrum showed a signal corresponding to the amino group at 8.09 ppm, a singlet assigned to CH_2 at 4.48 ppm. Heating of **14** under reflux for 8 hr with p-bromophenacyl bromide in ethanol furnished **16** in 48% yield, whose IR and ^{1}H NMR spectra confirmed the success of the cyclization by the disappearance of the signals corresponding to the SH and NH_2 protons and appearance of a singlet at δ 1.12 ppm assigned to the methyl protons and to CH_2 at 3.85 ppm. Treatment of **14** with benzoin in ethanolic KOH gave **17** in good yield. The IR and 1H NMR spectra confirmed the structure. Reaction of **14** with 2, 3-dichloroquinoxaline in presence of NaOAc in ethanol afforded

18 under conventional heating for 8-10 h in 56% yield, respectively. Its 1H NMR spectrum showed the presence of a singlet at δ 4.03 corresponding to the NH proton. Heating compound **14** under reflux for 2 h with carbon disulfide in methanolic KOH furnished **19** in 51% yield. Its ^{1}H NMR spectrum showed a singlet at δH 2.03 due to the NH group. Reaction of **14** with p-toluic acid in phosphoryl chloride for 2 hrs caused a ring closure to form the thiadiazole ring of **20** in 55% yield. The IR and 1H NMR spectra of **20** confirmed the success of the cyclization by the disappearance of the signals corresponding to the SH and NH_2 protons and appearance of multiplet at δ 7.01- 8.08 assigned to the aromatic protons. Treatment of **14** with m-nitrobenzaldehyde in ethanol for 4hr furnished **21** in 50% yield.

Biological testing

The triazolyl-indenoisoquinoline derivatives were examined for *in-vivo* anticancer activity against the human cancer cell lines compared with the Doxorubicin (ADR) as a reference drug. From the Microcare Laboratories Surat, in which the activity of each compound was evaluated in approximately 05 different cancer cell lines of diverse tumor origins. The GI50 values obtained with selected cell lines are summarized in **Table 1**. The relative potencies of the compounds in the production of Top1- mediated DNA cleavage are also listed in **Table 1**, with the ability of the compounds to produce Top1-mediated DNA cleavage expressed semiquantitatively as follows: : + and ++: weak activity; +++: moderate activity; ++++: similar activity as 1 µM camptothecin. As a positive control, relaxation assay was carried out with Camptothecin (CPT), an amide alkaloid which makes complete inhibition of relaxation of plasmid DNA (Figure 1&2).

From the 21 synthesized compounds, 09 compounds were screened for their in vitro anticancer activities against human liver (HOP62), ovarian (OVKAR3), lung (ZR751), breast (HL60) and leukemia (DU145) cancer cell lines. Results are reported in the form of GI50, Table: 1. four compounds (12, 14, 15, and 16) showed significant activity (below 20) against all cell lines. While compound 17 was found to active against Liver, Ovarian and Leukemia cell lines. Compound 20 was found to active against Ovarian, breast and Leukemia cell lines. Compound 18 were active against two cancer cell lines of breast, and leukemia, liver and lung, breast and lung, liver and ovarian respectively. Compound 21 were found to selective towards the ovarian cancer while compound 19 was selective to liver cancer.

Some of the compounds tested are inhibitors of the enzyme very few of them are able to inhibit complete relaxation of the plasmid DNA by the Topoisomerase IB. However, the compounds BAB-12, 14, 15 and 16 (Top ++++) demonstrated the greatest ability to inhibit Top1. Compound 17 showed moderate top-I activity (top +++) while compound 18, 19 and 20 showed poor Top1

inhibition as well as cytotoxicity. Compound 21 exhibited no Top-I activity. Finally other biological targets besides Top1 are clearly involved in the activities of the selected indenoisoquinolines. From the activities reported in Table 1, it can be inferred that the introduction of bulky substituent (compound 18, 19, 20) on the triazole ring of the basic moiety has a negative effect on their ability to inhibit Top1. Compounds 12, 14, 15 and 16 (Top ++++) demonstrated the greatest ability to inhibit Top1.

Table 1: *In-vitro* cytotoxic activity and Topoisomerase-I Inhibitory Activity of the synthesized compounds 12-21

Compd.	**Cytotoxicity (GI50 in lM)[a]**					**Top 1 cleavage[b]**	
	HOP62 Lung	**OVKAR3 Ovarian**	**ZR751 Breast**	**HL60 Leukemia**	**DU145 Prostate**	**SRB**	**TOPO-I**
12	2.0	0.1	0.5	2.8	3.7	*****	++++
14	2.5	0.1	0.6	3.26	4.3	*****	++++
15	9.5	7.66	5.49	8.26	9.56	*****	++++
16	6.8	1.56	6.45	9.05	8.3	*****	++++
17	>80	19.2	18.14	>80	15.9	***	++
18	7.3	46.7	>80	15.9	21.3	**	+
19	5.12	58.2	>80	23.5	71.2	*	+
20	6.2	9.1	>80	26.4	13.4	***	+
21	>80	8.3	23.1	>80	30.23	*	nt
ADR	0.1	0.5	0.8	0.6	0.1	*****	nt
CPT	2.5	1.85	2.8	3.65	9.56	*****	++++

[a]The cytotoxicity GI50 values are the concentrations corresponding to 50% growth inhibition.

[b]The compounds were tested at concentrations ranging up to 10 µM. The activity of the compounds to produce Top1-mediated DNA cleavage was expressed semiquantitatively as follows: +: weak activity; ++ and +++: modest activity; ++++: similar activity as 1 µM camptothecin; +++++: greater activity than 1 µM camptothecin.

Lane no. **1 2 3 4 5 6 7 8 9 10 11 12**

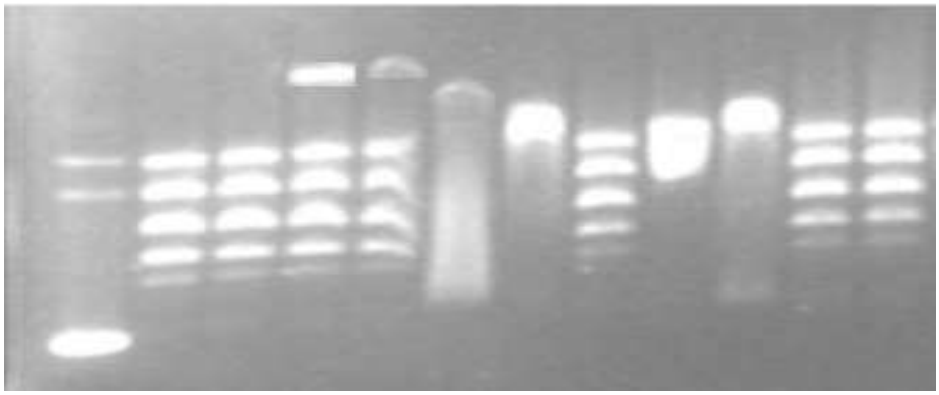


Figure 1: Comparison of the Top1-mediated DNA cleavages of 12 compounds

Lane 1: Plasmid DNA[pBS (SK+)]

Lane 2: pBS(SK+)DNA + LdTOPIB

Lane 3: pBS(SK+)DNA + LdTOPIB+ DMSO

Lane 4: pBS(SK+)DNA + LdTOPIB+ BAB12 Lane5: pBS(SK+)DNA + LdTOPIB+ BAB14

Lane 6: pBS(SK+)DNA + LdTOPIB+ BAB15

Lane 7: pBS(SK+)DNA + LdTOPIB+ BAB16 Lane 8: pBS(SK+)DNA + LdTOPIB+ BAB17

Lane 9: pBS(SK+)DNA + LdTOPIB+ BAB18 Lane 10: pBS(SK+)DNA + LdTOPIB+ BAB19

Lane 11: pBS(SK+)DNA + LdTOPIB+ BAB20

Lane 12: pBS(SK+)DNA + LdTOPIB+ BAB21

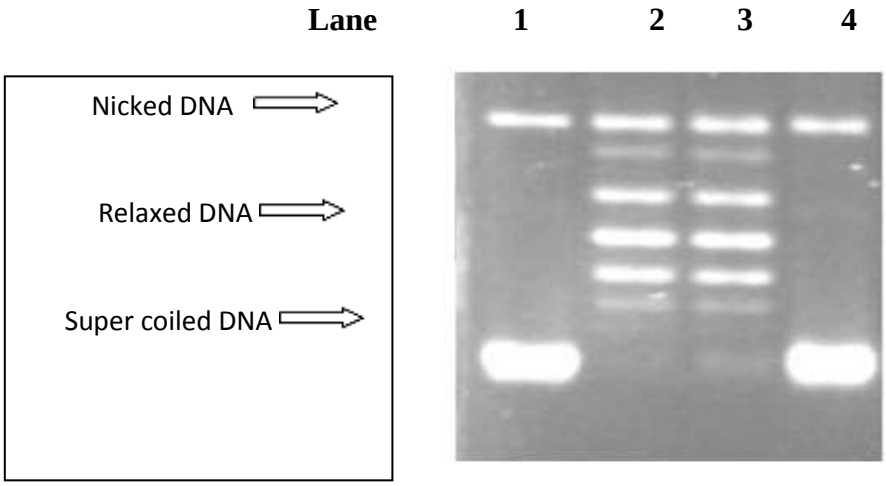


Figure 2: Comparison of the Top1-mediated DNA cleavages of standard compounds

Lane 1: DNA control

Lane 2: Enzyme control

Lane 3: DMSO control

Lane 4: Plasmid DNA + Enzyme + Camptothecin (positive inhibitor)

CONCLUSION

The objective of the present study was to synthesize and investigate the anticancer activity as well as topoisomerase–I inhibitory activity of novel triazolyl-indenoisoquinolines derivatives containing a thiadiazine and thiadiazoles moieties. Several compounds have been shown to exhibit significant anticancer activity such as the triazole derivatives having 12 and 14, free amino group.

Experimental

Chemistry

The melting points were recorded on electrothermal apparatus and are uncorrected. IR spectra were recorded on Shimazu model IRAFFINITY-1 in KBr. 1H NMR spectra were recorded on a

Varian Murcury 300 MHz instrument using DMSO-d_6 as solvent using TMS as internal standard; the chemical shifts (δ) are reported in ppm and coupling constants (J) are given in Hz. Mass spectra were recorded on a Finning LCQ mass spectrometer. Elemental analyses were performed on a Heracus CHN-Rapid Analyser. Analyses indicated by the symbols of the elements of functions were within ±/0.4% of the theoretical values. The purity of the compounds was checked on Merck precoated silica gel 60 F-254.

(*E*)-ethyl 3-((benzo[*d*][1,3]dioxol-6-yl)methyleneamino)propanoate 3

Triethylamine (0.60mL, 0.0060mol) was added all at once to the well stirred suspension of Ethyl 3-aminopropanoate **2** (0.656g, 0.0056mol) in $CHCl_3$ (15 mL) under nitrogen and the mixture was stirred at ambient temperature for 30 minutes. Piperonal **1** (0.840g, 0.0056mol) and $MgSO_4$ (0.750g) were added sequentially and the suspension was stirred at ambient temperature for 20 h. The slurry was filtered and concentrated to a white suspension. The suspension was triturated with Et_2O (10 mL), TEA salts removed via filtration, and the clear filtrate concentrated to afford imine **3** as a clear oil. M.P.: 178-180 ^{0}C; yield: 58%. IR (KBr, Cm^{-1}): 1735 (C=O ester), 1606 (C=N), 1577, 1508, 1448 (C=C, aromatic); ^{1}H NMR (300MHz, DMSO-d_6) δ (ppm): 1.29(t, 3H, CH_3), 2.65 (t, 2H, CH_2), 3.79 (t, 2H, CH_2), 4.21 (q, 2H, CH_2), 6.12 (s, 2H, CH_2), 6.85 – 7.78 (m, 3H, ArH), 8.95 (s, 1H, CH); Anal. calcd for $C_{13}H_{15}NO_4$: C, 62.64, H, 6.07, N, 5.62 Found: C, 62.69; H, 6.01; N, 5.58.

(*E*)-3-((benzo[*d*][1,3]dioxol-6-yl)methyleneamino)propanehydrazide 4

Compound **3** (0.498g, 0.002mol) and hydrazine hydrate (0.125 mL, 0.0025mol, 99%) were mixed and the mixture was refluxed for 10 min. To it ethanol (10 mL) was added till both the layers were miscible. Then refluxing was continued for 6 hr at 80 ^{0}C. The excess of ethanol and unreacted hydrazine hydrate were distilled out and the contents poured into a beaker. The solid obtained was purified by recrystallization from ethanol to get pure white crystalline compound **4**; M.P.: 210-213 ^{0}C; yield: 76%; IR (KBr, Cm^{-1}): 3312, 3282 (NH.NH_2), 1674 (C=O), 1603 (C=N), 1508, 1450 (C=C, aromatic); ^{1}H NMR (300MHz, DMSO-d_6) δ (ppm): 2.00 (s, 2H, NH_2), 2.54 (t, 2H, CH_2), 3.84 (t, 2H, CH_2), 6.07 (s, 2H, CH_2), 6.95 (d, 1H, ArH), 7.25 (d, 1H, ArH), 7.38 (s, 1H, ArH), 8.84 (s, 1H, CH); MS (m/z, %): 235 (M+, 100), 236 (M+1); Anal. calcd for $C_{11}H_{13}N_3O_3$: C, 56.16; H, 5.57; N, 17.86; Found: C, 56.23; H, 5.64; N, 17.78.

(*E*)-bis((9*H*-fluoren-9-yl)methyl)1-(3-(benzo[*d*][1,3]dioxol-5-ylmethyleneamino) propanoyl)hydrazine-1,2-dicarboxylate-5.

(E)-3-((benzo[d][1,3]dioxol-6-yl)methyleneamino)propanehydrazide **4** (0.540g, 0.0023mol) was suspended in aqueous sodium bicarbonate (5mL) with stirring. The resulting solution was cooled

to 5° and Fmoc-Cl (0.6g, 0.0024mol) is added slowly as a solution in 1,4-dioxane (also cooled). The resulting mixture is stirred at 0° for 3 h and allowed to warm to room temperature overnight. Water is then added and the aqueous layer is extracted 2 times with EtOAc (2×5mL). The organic layer is back extracted twice with saturated sodium bicarbonate solution. The combined aqueous layers are acidified to a pH of 1 with 10% HCl, then extracted 3 times with EtOAc (3×7mL). The combined organic layers were dried over dry $MgSO_4$, and concentrated in vacuo. The resulting residue can then be purified by column chromatography to get the White solid compound **5**; M.P.: 184-186 ^{0}C; yield: 93%. Column chromatography solvent/recrystallization: Chloroform: ethyl acetate (95:5) M.P.: 184-186 ^{0}C; Mol. Formula: $C_{41}H_{33}N_3O_7$; Mol. Wt.: 679.72; Yield: 93%; ^{1}H NMR (300MHz, DMSO-d_6) δ (ppm): 2.46 (t, 2H, CH_2), 3.83 (t, 2H, CH_2), 4.41 (t, 2H, CH-Fmoc), 4.82 (d, 4H, CH_2-Fmoc), 6.07 (s, 2H, CH_2), 6.95 (d, 1H, ArH), 7.26-7.89 (m, 18H, ArH), 8.00 (s,1H, NH), 8.89 (s, 1H, CH).

6, 7-dimethoxy-1*H*-isochromen-3(4*H*)-one 7

A mixture of 2-(3,4-dimethoxyphenyl) acetic acid **6** (1.96g, 0.01mol) and paraformaldehyde (1.18g, 0.0246mol) heat at 120°C for 1 hour, add 5 ml of concentrated HCl in 10 ml of AcOH and evaporate to dryness. Add 10 ml of H_2O, and extract three times with 20 ml of CH_2Cl_2. Wash the organic phases with 10 ml of 0.5 N $NaHCO_3$ and dry on Na_2SO_4. Evaporate to dryness. Allow to stand for 2 hours in Et_2O to provide white crystals of **7**; Solvent for recrystallization: ethanol. M.P.: 106-108 ^{0}C; Mol. Formula: $C_{11}H_{12}O_4$; Mol. Wt.: 208.21; yield: 71%; ^{1}H NMR (300MHz, DMSO-d_6) δ (ppm): 3.64 (s, 2H, CH_2), 3.89 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 5.26 (s, 2H, CH_2), 6.71 (s, 1H ArH), 6.83 (s, 1H ArH).

4,5-dimethoxyhomophthalic acid 8

Add dropwise 400 ml of a 10% solution of $KMnO_4$ to a solution of 6, 7-dimethoxy-1H-isochromen-3(4H)-one **7** (5.2g, 0.025mol) in 30 ml of 10% KOH stir at room temperature for 10 hours. Add 15 ml of EtOH and heat at 70°C for 20 minutes. Concentrate the reaction medium to two-thirds. Acidify to pH 2-3 (check with pH paper) using concentrated HCl. Allow to crystallize at 0 ^{0}C for 1 hour. Filter and wash twice with 10 ml of H_2O to get the light yellow colour product **8**; Column chromatography solvent/recrystallization: chloroform: ethyl acetate (97:3); M.P.: 178 - 182 °C; Mol. Formula: $C_{11}H_{12}O_6$; Mol. Wt.: 240.21; yield: 78%; IR (KBr, Cm^{-1}): 3300 (O–H stretch), 1685, 1705 (C=O), 1296 (C–O), 1419, 948 (O–H bends); ^{1}H NMR (300MHz, DMSO-d_6) δ (ppm): 3.74 (s, 2H, CH_2), 3.96 (s, 6H, OCH_3), 6.98 (s, 1H Ar), 7.66 (s, 1H Ar), 11.00 (s, 2H, OH).

6, 7-dimethoxyisochroman-1,3-diones (Homophthalic anhydride derivative) 9

Dissolve 4, 5-dimethoxyhomophthalic acid **8** (3.6g, 0.015mol) in 30 ml of acetyl chloride. Heat the mixture under reflux for 5 hrs. Add 40 ml of Et_2O and Filter. Wash twice with 3 ml of Et_2O then twice with 10 ml of pentane to get a solid yellow powder **9**. The product is used as it is for the subsequent reactions. M.P.: 140-144 ^{0}C; yield: 63%. IR (KBr, Cm^{-1}): 1780, 1740 (C=O), 1500, 1465 (C=C, aromatic), 1201 (C-O-C), ^{1}H NMR (300MHz, DMSO-d_6) δ (ppm): 3. 49 (s, 2H, CH_2), 3.90 (s, 6H, OCH_3), 6.67 (s, 1H Ar), 7.41 (s, 1H Ar); MS (m/z, %): 52, 65, 78 (100), 90, 106, 118, 136, 222 (M^+), 223 (M+1); Anal. calcd for $C_{11}H_{10}O_5$: C, 59.46; H, 4.54 Found: C, -59.40; H, 4.58.

(3*R*,4*S*)-3-(benzo[*d*][1,3]dioxol-5-yl)-2-(3-(1,2-bis(((9*H*-fluoren-9-yl)methoxy) carbonyl)hydrazinyl)-3-oxopropyl)-6,7-dimethoxy-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylicacid-10.

4,5-Dimethoxyhomophthalic anhydride **9** (2.22g, 0.01mol) was added to a chloroform (60 mL) solution of the imine **5** (6.79 g, 0.01mol) and the mixture was stirred at room temperature for 6hrs. After the complete disappearance of the starting material (TLC), the white precipitate formed in the reaction was filtered off, washed with chloroform (5 mL) and dried to give pure white powder of isoquinolones **10**. M.P.: 256-258 °C; yield: 43-89%; IR (KBr, Cm^{-1}): 3292 (O–H stretch), 2950, 1716, 1644, (C=O), 1242(C–O), 1489, 928 (O–H bends); ^{1}H NMR (300MHz, DMSO-d_6) δ (ppm): 2.48 (t, 2H, CH_2), 3.48 (t, 2H, CH_2), 3.89 (s, 6H, OCH_3), 4.39 (t, 2H, CH-Fmoc), 4.65 (s, 1H, CH-isoquinolones), 4.94 (d, 4H, CH_2-Fmoc), 5.60 (s, 1H, CH-isoquinolines), 6.05 (s, 2H, CH_2), 6.75-7.90 (m, 21H, ArH), 8.00 (s, 1H, NH), 11.07 (s, 1H, OH); Anal. calcd for $C_{52}H_{43}N_3O_{12}$: C, 69.25; H, 4.81; N, 4.66; Found: C, 69.20; H, 4.86; N, 4.60.

di(9*H*-9-fluorenylmethyl)1-[3-(2,3-dimethoxy-5,12-dioxo-6,12-dihydro-5*H*-[1,3] dioxolo[4',5':5,6]indeno[1,2-*c*]isoquinolin-6-yl)propanoyl]-1,2-hydrazinedi carboxylate 11

Thionyl chloride (30 mL) was added drop wise to the acid **10** (1.80g, 0.002mol) and the resulting mixture were stirred at room temperature for 5 h. Benzene (20mL) was added to the red solution and it was concentrated under reduced pressure. Chloroform was added to the residue and the solution passed through a short column of silica gel, eluting with chloroform: methanol (95:5). The resulting product was crystallized from chloroform-ethyl acetate to obtain pure light yellow powder of indenoisoquinolines **11**. Column chromatography solvent/recrystallization: chloroform: methanol (95:5)/ chloroform-ethyl acetate (97:3); M.P.: 304-308 °C; Mol. Formula: $C_{52}H_{39}N_3O_{11}$, Mol. Wt.: 888.18; yield: 20-72%; ^{1}H NMR (300MHz, DMSO-d_6) δ (ppm): 2.70 (t, 2H, CH_2), 3.89 (s, 6H, OCH_3), 4.28 (t, 2H, CH-Fmoc), 4.58 (t, 2H, CH_2), 4.85 (d, 4H, CH_2-Fmoc), 6.08 (s, 2H, CH_2), 7.05-7.90 (m, 20H, ArH), 8.02 (s, 1H, NH).

3-(2,3-dimethoxy-5,12-dioxo-6,12-dihydro-5*H*-[1,3]dioxolo[4',5':5,6]indeno [1,2-*c*]iso quinolin-6-yl)propanohydrazide 12

The compound **11** (2.22g, 0.0025mol) was dissolved in a solution of 20% piperidine in DMF. (approximately 10 mL/gm). The mixture was stirred for 2 hrs at room temperature and concentrated in vacuo. Filter the product and wash it several times with DMF to get the product **12**. Recrystallization, by dissolving in chloroform and slowly adding methanol, afforded red solid. The residue can then be purified by column chromatography. Column chromatography solvent/recrystallization: methanol: chloroform (1:99); M.P.: 296-298; Mol. Wt.: 437.40; yield: 93%; IR (KBr, Cm^{-1}): 3250-3280 ($-NH_2$); 3120 (-NH); 1680, 1640 (C=O), 1465 (-CN); ^1H NMR (300MHz, DMSO-d_6) δ (ppm): 2.07 (s, 2H, NH_2), 2.81 (t, 2H, CH_2), 3.83 (s, 6H, OCH_3), 4.52 (t, 2H, CH_2), 6.04 (s, 2H, CH_2), 7.08-7.47 (m, 4H, ArH), 8.01 (s, 1H, NH); MS (m/z, %): 87, 71, 185, 320, 437 (M+), 438 (M+1); Anal. calcd for $C_{22}H_{19}N_3O_7$: C, 60.41; H, 4.38; N, 9.61; O, 25.60; Found: C, 60.36; H, 4.45; N, 9.68.

6-[2-(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)ethyl]-2,3-dimethoxy-6,12-dihydro-5*H*-[1,3]dioxolo[4',5':5,6]indeno[1,2-*c*]isoquinoline-5,12-dione 14

Potassium hydroxide (1.68g, 0.03mol) was dissolved in absolute ethanol (50 mL). The solution was cooled in an ice bath and indenoisoquinolines **12** (4.37g, 0.01mol) was added with stirring. To this, carbon disulfide (2mL, 0.025mol) was added in small portions with constant stirring. The reaction mixture was stirred continuously for 16 hr at RT. The precipitated potassium dithiocarbazinate was collected by filtration, washed with anhydrous ether and dried in vacuum. The potassium salt thus obtained was used in the next step without further purification. A suspension of potassium dithiocarbazinate 13 (0.02 mol) and hydrazine hydrate (99%, 0.04 mol) in ethanol (50 mL) was refluxed for 10-15 hr with occasional shaking. The colour of the reaction mixture changed to light green with evolution of hydrogen sulfide gas. A homogenous mixture was obtained during the reaction process. The reaction mixture was cooled to RT and diluted with cold water (20 mL). On acidification with dil. HCl, the required triazole was precipitated as white precipitate. It was filtered, washed with cold water, dried and purified by recrystallization from DMSO. The compound was found homogeneous on TLC analysis using toluene: ethyl acetate: formic acid (5:4:1, v/v/v) as solvent system. Column chromatography solvent: The residue was dissolved in a minimum amount of chloroform and subjected to column chromatography on silica gel (150 g) ethyl acetate as eluent. M.P.: 300-302 ^oC; Mol. Wt.: 493.49; yield: 55%; IR (KBr, Cm^{-1}): 3327 (NH_2), 2584 (SH), 1678 (C=O), 1594 (C=N), 1333 (C-N); ^1H NMR (300MHz, DMSO-d_6) δ (ppm): 2.33 (t, 2H, CH_2), 2.86 (t, 2H, CH_2), 3.65 (s, 6H, OCH_3), 5.07 (s, 2H, NH_2), 6.14 (s, 2H,

CH_2), 7.10 (s, 1H, ArH), 7.15 (s, 1H, ArH), 7.31 (s, 1H, ArH), 7.46 (s, 1H, ArH), 13.15 (br s, 1H, SH); MS (m/z, %): 493 (M+), 495 (M+2); Anal. calcd for $C_{23}H_{19}N_5O_6S$: C, 55.98; H, 3.88; N, 14.19; Found: C, 55.94; H, 3.95; N, 14.26.

2,3-dimethoxy-6-[2-(6-oxo-6,7-dihydro-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-3-yl)ethyl]-6,12-dihydro-5*H*-[1,3]dioxolo[4',5':5,6]indeno[1,2-*c*]isoquinoline-5,12-dione 15

A solution of compound **14** (0.493g, 0.001mol) in ethanol 10 mL was taken. Chloroacetic acid (0.001 mol, 0.095mL) was added in one shot followed by freshly prepared fused sodium acetate (0.001mol, 0.082gm). This reaction mixture was refluxed for 10hrs. The reaction mixture was then cooled and poured on to crushed ice. The solid thus separated was filtered, washed thoroughly with water and recrystallized from ethanol to get off white solid; M.P. 262-264^{0}C; yield 56%; Mol wt: 533.51; IR (KBr, Cm^{-1}): 3330 (NH), 3015 (arom.CH.), 1675 (C=O), 1557 (C=N), 1501, 1495 (C=C, aromatic); 1351 (C-N); 1210 (Ph-O-C), 629 (C-S-C); ^{1}H NMR (300MHz, DMSO-d_6) δ (ppm): 2.85 (t, 2H, CH_2), 3.28 (t, 2H, CH_2), 3.90 (s, 6H, OCH_3), 4.48 (s, 2H, CH_2), 5.84 (s, 2H, CH_2), 7.10 (s, 1H, ArH), 7.21 (s, 1H, ArH), 7.46 (s, 1H, ArH), 7.68 (s, 1H, ArH), 8.09 (s, 1H, NH); MS (m/z, %): 533 (M+), 534 (M+1); Anal. Calcd for $C_{25}H_{19}N_5O_7S$: C, 56.28; H, 3.59; N, 13.13%, Found: C, 56.18; H, 3.64; N, 13.15.

2,3-dimethoxy-6-[2-(6-methyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl) ethyl]-6,12-dihydro-5*H*-[1,3]dioxolo[4',5':5,6]indeno[1,2-*c*]isoquinoline-5,12-dione 16

Compound **14** (0.493g, 0.001mol) mixed with p-bromophenacyl bromide (0.277g, 0.001mol) in ethanol 10mL. The reaction mixture was heated under reflux for 8hrs. After cooling the solvent was removed under reduce pressure and product was added in cold water and neutralized with aqueous potassium carbonate. The solid thus separated was filtered, washed thoroughly with water and recrystallised from ethanol to get orange red solid. M.P. 298-300^{0}C; yield 48%; Mol wt: 531.54; IR (KBr, Cm^{-1}): 3035 (arom.CH.), 1715 (C=O), 1625 (C=N), 1558, 1502, 1480 (C=C, aromatic); 1306 (C-N); 1211 (Ph-O-C), 611 (C-S-C); ^{1}H NMR (300MHz, DMSO-d_6) δ (ppm): 1.12 (s, 3H, CH_3), 2.65 (t, 2H, CH_2), 3.05 (t, 2H, CH_2), 3.56 (s, 6H, OCH_3), 3.85 (s, 2H, CH_2), 5.82 (s, 2H, CH_2), 7.12 (s, 1H, ArH), 7.19(s, 1H, ArH), 7.33(s, 1H, ArH), 7.48 (s, 1H, ArH); MS (m/z, %): 531 (M+), 532 (M+1); Anal. Calcd for $C_{26}H_{21}N_5O_6S$: C, 58.75; H, 3.98; N, 13.18%, Found: C, 58.70; H, 4.01; N, 13.22.

6-[2-(6,7-diphenyl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)ethyl]-2,3-dimethoxy-6,12-dihydro-5*H*-[1,3]dioxolo[4',5':5,6]indeno[1,2-*c*]isoquinoline-5,12-dione 17

A mixture of compound **14** (0.493g, 0.001mol), benzoin (0.212g, 0.001mol) and 15mL of 2N KOH solution in 10mL ethanol was heated under reflux for 8hr. After cooling the solvent was

removed under reduced pressure and the solid product formed was washed thoroughly with water and recrystallized from ethanol to yield brown solid. M.P. 218-220^{0}C; yield 48%; Mol wt: 669.71; IR (KBr, Cm^{-1}): 3378 (NH), 3010 (arom.CH.), 1698 (C=O), 1620 (C=N),1538, 1498, 1448 (C=C, aromatic), 1392 (C-N), 1208 (Ph-O-C), 678 (C-S-C); ^{1}H NMR (300MHz, DMSO-d_6) δ (ppm): 2.16 (s, 1H, NH), 2.58 (t, 2H, CH_2), 3.20 (t, 2H, CH_2), 3.59 (s, 6H, OCH_3), 6.02 (s, 2H, CH_2), 7.10 (s, 1H, ArH), 7.13 (s, 1H, ArH), 7.30-7.85 (m, 12H, ArH); Anal. Calcd for $C_{37}H_{27}N_5O_6S$: C, 66.36; H, 4.06; N, 10.46%, Found: C, 66.41; H, 4.08; N, 10.49.

2,3-dimethoxy-6-[2-(5*H*-[1,2,4]triazolo[3',4':2,3][1,3,4]thiadiazino[5,6-*b*] quinoxalin-3-yl)ethyl]-6,12-dihydro-5*H*-[1,3]dioxolo[4',5':5,6]indeno[1,2-*c*]isoquinoline-5, 12-dione 18

A mixture of compound **14** (0.493g, 0.001mol), 2,3-dichloroquinoxazline (0.199g, 0.001mol) and fused sodium acetate (0.164g, 0.002mol) was added in 10mL ethanol and the reaction mixture was heated under refluxed for 8-10hrs. After cooling the solvent was removed under reduced pressure and the solid product formed was recrystallized from ethanol to get a cream colour solid; M.P. 264-266^{0}C; yield 56%; Mol wt: 621.62; IR (KBr, Cm^{-1}): 3326 (NH), 1681 (C=O), 1605 (C=N), 1591, 1509, 1449 (C=C,aromatic), 1330 (C-N); 1248 (Ph-O-C), 621 (C-S-C); ^{1}H NMR (300MHz, DMSO-d_6) δ (ppm): 2.83 (t, 2H, CH_2), 3.22 (d, 4H, CH_2), 3.57 (t, 2H, CH_2), 3.83 (s, 6H, OCH_3), 4.03 (s, 1H, NH), 5.43 (t, 2H, CH), 6.11 (s, 2H, CH_2), 7.11 (s, 1H, ArH), 7.18 (s, 1H, ArH), 7.36 (s, 1H, ArH), 7.58 (s, 1H, ArH); Anal. Calcd for $C_{31}H_{23}N_7O_6S$: C, 59.90; H, 3.73; N, 15.77%, Found: C, 59.84; H, 3.75; N, 15.78.

2,3-dimethoxy-6-[2-(6-thioxo-5,6-dihydro-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-3-yl)ethyl]-6,12-dihydro-5*H*-[1,3]dioxolo[4',5':5,6]indeno[1,2-*c*]isoquinoline-5, 12-dione 19

Carbon disulfide (0.15 mL, 0.0015mol) was added drop wise with constant stirring to the solution of comp **14** (0.493g, 0.001mol) in 1 gm methanolic KOH solution and the reaction mixture was heated under reflux for 2hrs. The mixture was cooled and then poured on to crushed ice and acidified with dilute HCl. The solid thus separated was filtered, washed thoroughly with water and recrystallized from aqueous ethanol to yield an off white colour solid. M.P. 214-216^{0}C; yield 51%; Mol wt: 535.55; IR (KBr, Cm^{-1}): 3314 (NH), 3012 (arom.CH.), 1678 (C=O), 1605 (C=N), 1501, 1448 (C=C, aromatic); 1333 (C-N); 1280 (C=S); 1249 (Ph-O-C), 610 (C-S-C); ^{1}H NMR (300MHz, DMSO-d_6) δ (ppm): 2.03 (s, 1H, NH), 2.81 (t, 2H, CH_2), 3.36 (t, 2H, CH_2), 3.83 (s, 6H, OCH_3), 5.84 (s, 2H, CH_2), 7.13 (s, 1H, ArH), 7.21 (s, 1H, ArH), 7.36 (s, 1H, ArH), 7.47 (s, 1H, ArH); Anal. Calcd for $C_{24}H_{17}N_5O_6S_2$: C, 53.82; H, 3.20; N, 13.08%, Found: C, 53.86; H, 3.22; N, 13.12.

2,3-dimethoxy-6-[2-(6-phenyl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)ethyl]-6,12-dihydro-5*H*-[1,3]dioxolo[4',5':5,6]indeno[1,2-*c*]isoquinoline-5,12-dione 20

A mixture of compound **14** (0.493g, 0.001mol), and p-toluic acid (0.001mol) in phosphoryl chloride 10mL was heated under reflux for 2 hrs. After cooling, the solvent was removed under reduce pressure and an ice –water mixture was added to the residue with stirring. The reaction mixture was neutralized with ammonium hydroxide or aqueous potassium carbonate solution and the solid ppt formed was filtered and recrystallized from ethanol to get an off white colour solid. M.P. 198-200^{0}C; yield 55%; Mol wt: 579.58; IR (KBr, Cm^{-1}): 3031 (arom.CH), 1697 (C=O), 1597 (C=N), 1509, 1489 (C=C, aromatic); 1338 (C-N); 1203 (Ph-O-C), 616 (C-S-C); ^{1}H NMR (300MHz, DMSO-d_6) δ (ppm): 2.82 (t, 2H, CH_2), 3.37 (t, 2H, CH_2), 3.84 (s, 6H, OCH_3), 6.12 (s, 2H, CH_2), 7.09 (s, 1H, ArH), 7.14 (s, 1H, ArH), 7.30 (s, 1H, ArH), 7.01-8.08 (m, 6H, ArH); Anal. Calcd for $C_{30}H_{21}N_5O_6S$: C, 62.17; H, 3.65; N, 12.08, Found: C, 62.12; H, 3.68; N, 12.05%.

2,3-dimethoxy-6-2-[6-(3-nitrophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]ethyl-6,12-dihydro-5*H*-[1,3]dioxolo[4',5':5,6]indeno[1,2-*c*]isoquinoline-5,12-dione 21

A mixture of compound **14** (0.493g, 0.001mol), and m-nitrobenzaldehyde (0.001mol) in 10mL ethanol was prepared and heated under reflux for 4hrs. After cooling, the solvent was removed under reduced pressure and an ice cold water mixture was added to the residue with stirring. The solid separated was filtered wash thoroughly with water and recrystallized from ethanol to get yellow colour solid. M.P. 208-210^{0}C; yield 50%; Mol wt: 626.60; IR (KBr, Cm^{-1}): 3230 (NH), 3063 (arom.CH), 1662 (C=O), 1619 (C=N), 1558, 1502, 1496, 1446 (C=C, aromatic); 1532 (C-NO_2), 1336 (C-N); 1241 (Ph-O-C), 620 (C-S-C); ^{1}H NMR (300MHz, DMSO-d_6) δ (ppm): 1.98 (s, 1H,NH); 2.85 (t, 2H, CH_2), 3.31 (t, 2H, CH_2), 3.86 (s, 6H, OCH_3), 5.01 (s, 1H, CH), 6.14 (s, 2H, CH_2), 7.21 (s, 1H, ArH), 7.41 (s, 1H, ArH), 7.42-8.38 (m, 6H, ArH); Anal. Calcd for $C_{30}H_{22}N_6O_8S$: C, 57.50; H, 3.54; N, 13.41; Found: C, 57.46; H, 3.57; N, 13.46.

BIOLOGICAL TESTING

Anticancer activity

Potential cytotoxicity effect of the newly synthesized compounds in four concentrations, were evaluated in the Microcare Laboratories, Surat, by SRB assay [14]. The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 100 µL at plating densities as shown in the study details above, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37° C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, one 96 well plate containing 5*103cells/well was fixed in situ with TCA, to represent a measurement of the cell population at the time of drug addition (Tz). Experimental drugs were

initially solubilized in dimethyl sulfoxide at 100mg/ml and diluted to 1mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquote of frozen concentrate (1mg/ml) was thawed and diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml with complete medium containing test article. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µl of medium, resulting in the required final drug concentrations i.e.10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml.

After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells * 100.

Topoisomerase-I inhibition

The topoisomerase I activity of the 9 compounds which were found to be cytotoxic in SRB assay was determined by a DNA relaxation assay on plasmid DNA of the recombinant *Leishmania topoisomerase* I as per following procedure [15].

Plasmid DNA and Enzyme.

DNA used was negatively supercoiled pBluescript (SK+) isolated from E.coli host cells (DH5α) using standard purification protocol (Novagen).

Relaxation Assay.

DNA topoisomerase I was assayed by measuring the decreased mobility of the relaxed isomers of supercoiled pBluescript (SK+) DNA in an agarose gel. The standard topoisomerase assay mixture (25 µl) contained 50 mM Tris-HCl (pH = 7.5), 5% glycerol, 50 mM KCl, 0.5 mM dithiothreitol (DTT), 10 mM $MgCl_2$, 30 µg/ml bovine serum albumin (BSA), 0.5 µg supercoiled DNA and 2 units of enzyme (1 unit is defined as the amount of DNA required to convert 50% of 0.5 µg supercoiled DNA substrate into the relaxed form under standard assay conditions) in absence and

presence of varying drug concentrations. Reactions were performed at 37 °C for 30 min, and then terminated by adding 10 mM EDTA, 0.5% SDS, 0.25 µg/ml bromophenol blue and 15% (v/v) glycerol. The samples were electrophoresed in a horizontal 1% agarose gel in TAE buffer (40 mM tris/acetate, 2 mM EDTA, pH = 8) at room temperature. The gels were stained with ethidium bromide (0.5 µg/ml), de-stained in water and photographed under UV illumination. Percentage relaxation was measured by microdensitometry of negative photographs of supercoiled monomer DNA band fluorescence after ethidium bromide staining.

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