Chemistry & Biology Interface, 2024, 14, 4, 95-117

RESEARCH PAPER ISSN: 2249 –4820

CHEMISTRY & BIOLOGY INTERFACE

An official Journal of ISCB, Journal homepage; www.cbijournal.com

"Synthesis and evaluation of a novel series of substituted thiazolo[4,5-e]indazol-2-aminederivatives as potential anticancer agents: In vitro cell culture analysis"

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Received; 17 July 2024, Accepted;20 August 2024

Abstract: A group of new analogs, based on fused thiazole and indazol-2-amine, were created and characterized using various methods such as ^{1}H NMR, ^{13}C NMR, IR, LCMS spectroscopy, and elemental analysisto ascertain the structural configuration and purity of compounds.The inhibitory effects of the synthesized compounds **7a-s** and **10a-o** were evaluated using human-origincancer cells such as MCF-7 (breast), ME-180 (cervical), and Hep-G2 (liver). Compound **7i** exhibited promising activity against the MCF-7, ME-180, and Hep-G2 cancer cell lines among the newly created compounds. The compounds **7a, 7e, 7i**, and **7o** showed excellent cytotoxicity against MCF-7 cells with IC_{50}values of 15.5±0.3, 12.8±0.6, 11.5±0.8, and 13.7±0.9 µM respectively. The compounds **7a, 7i, 7o,** and **7p** showed significant cytotoxicity against ME-180 cells with IC_{50}values of 11.6±0.1, 11.5±0.4, 11.5±0.9, and 12.4±0.8 µM respectively. On the other hand, the compounds **7d, 7i, and 7p**demonstrated the highest cytotoxic activity against Hep-G2 cells with IC_{50}values of 10.4±0.2, 12.4±0.5, and 10.6±0.4 µM respectively. The cytotoxicity of the synthesized compounds was compared with Adriamycin, asa reference compound. The outcome of the present investigation may provide a foundation for future studies in developing potent anticancer agents.

Keywords: Thiazolo and indazol-2-amine derivatives; anti-cancer; human cancer cells; cytotoxicity.

Introduction

Cancer is one of the leading causes of death in not only developing countries but also developed countries causing unprecedented social burden of treatment

costs and major health research is being explored by researchers all over the world[1]. As per a literature report in the American Cancer Society in 2019, about 1,762,450 cases of cancer were registered and 606,880 cancer deaths were projected to occur in the United States alone[2].

The Food and Drug Administration (FDA) approved about fifty-six new small molecules for cancer treatment from 2015 to 2020[3].Many natural and synthetic anticancer agents are available in the market and some are in clinical trials(**Fig. 1**).However, an excellent repertoire of activity profiles, fewer side effects,and off-target toxicities are the most important challenging tasks in designing novel anticancer agents. A small molecule drug to treat cancer involves major challenges like a progressive adaptive mutation in cancer cells, tumor heterogeneity, and evolving drug resistance mechanisms in cancer patients[4]. The WHO in 2020 has set a program to save 7.3 million lives by 2030 (WHO Report on Cancer)[5].

Owing to the aforementioned challenges in the mainstream of cancer management, there is a need for the development of new drug candidates that will be effective, possess minimum or no side effects, counteract emerging drug resistance, and be cost-effective. Moreover, strategic formulation of combination therapy is also one of the important areas of anticancer drug development, wherein the development of novel candidate drug molecules has paramount importance. [6-8].

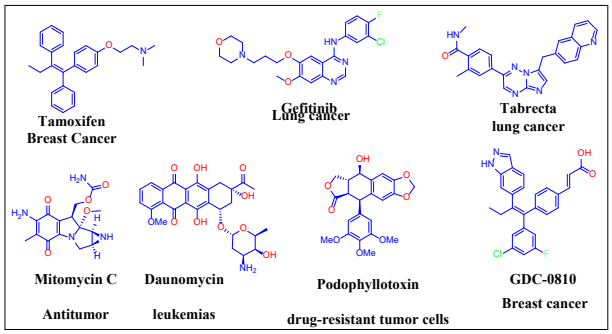


Fig 1. Structural peculiarities of natural and synthetic anticancer clinical drugs

Nitrogen-containing heterocyclic scaffolds, including indole, indazole, thiazole, and imidazole, hold significant prominence in the field of preclinical and clinical drug discovery, and have generated substantial recognition and priority within the global scientific community [9].

Among the prominent ring systems that are explored for the development of candidate drugs, indazole analogs have been reported to exhibit a wide range of pharmacological activities including anti-tumor[10], anti-HIV[11], anti-inflammatory[12], antidepressant[13], antimicrobial[14], Alzheimer's[15] and contraceptive activities[16]. 2H-indazoles have been shown to possess potent levels of affinity for 5-HT_{1A} receptors[17], estrogen receptors[18], and the imidazoline I_2 receptor[19] Of note, 2H-indazoles also act as kinase inhibitors[20].

On the other hand, the thiazole unit constitutes a key structural moiety in pharmaceutically relevant structures that have found applications in the treatment of allergies[21], hypertension[22],

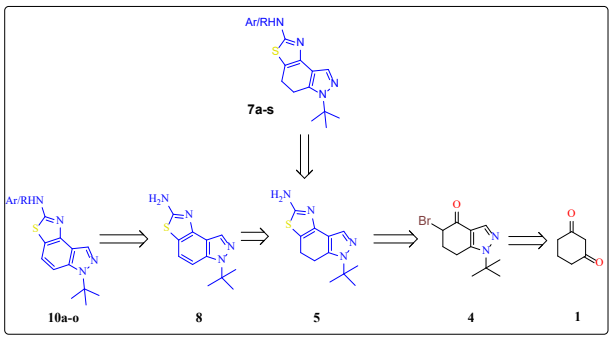
microbial infections[23], HIV infections[24], inflammations[25,26], schizophrenia[27], hypnotics[28], pain[29] and tuberculosis [30,31], Moreover, thiazole are also reported to target bacterial DNA gyrase B[32] and poliovirus RNA-dependent RNA polymerase [33].

Owing to their synthetic and biological significance, the chemistry of indazole and thiazole derivatives has stimulated increasing interest in developing new indazole-linked thiazole derivatives for managing varioushuman ailments. Furthermore, a comprehensive literature survey unveiled a limited number of reports on compounds incorporating indazole in combination with thiazole derivatives. [34,35].

In the pursuit of developing novel anticancer agents givenalleviate drug resistance and formulate a combination therapy, herein we report the synthesis of some novel structural hybrids by combining indazole and thiazole pharmacophores in a single molecular framework to explore their potential anticancer activities.

We merged previous research on anticancer agents with literature sources to find new potential bioactive compounds [36,37,38]. We aimed to combine thiazole groups with the indazole moiety (Scheme 1). We used diol **1** and NBS to create the reactive intermediate compound **5**, which was treated with hydrazine.

These intermediates were then used to prepare the desired compounds (**7a-s** and **10a-o**).



Scheme 1. Retro synthesis of thiazolo[4,5-e]indazol-2-amine derivatives (**7a-s** and **10a-o**).

Results and discussions

Chemistry

The synthetic route of novel substituted 4*H*-thiazole[4,5-e]indazol-2-amine and 6*H*-thiazole[4,5-e]indazol-2-amine derivatives is outlined in **schemes 1, 2**. The synthesis of the target compoundswas visualized to arise from the known cyclohexane-1,3-dione (**1**), which is transformed into 2-((dimethylamino) methylene)cyclohexane-1,3-dione (**2**) in an 85%, by the reaction of *N,N*-dimethylformamide - dimethyl acetal (DMF-DMA) and cyclohexane-1,3-dione (**1**)[39].2-((dimethylamino) methylene)cyclohexane-1,3-dione (**2**) could be converted into 1-(*tert*-butyl)-1,5,6,7-tetrahydro-4*H*-indazol-4-one (**3**) by the treatment of(**2**) with *t*-butyl hydrazine hydrochloride in the presence of catalytic PTSA/*t*-BuOHat 120 °C, in an 80% yield[40].

Further, the obtained compound **(3)** was then subjected tobromination with N-bromosuccinimide (NBS) and

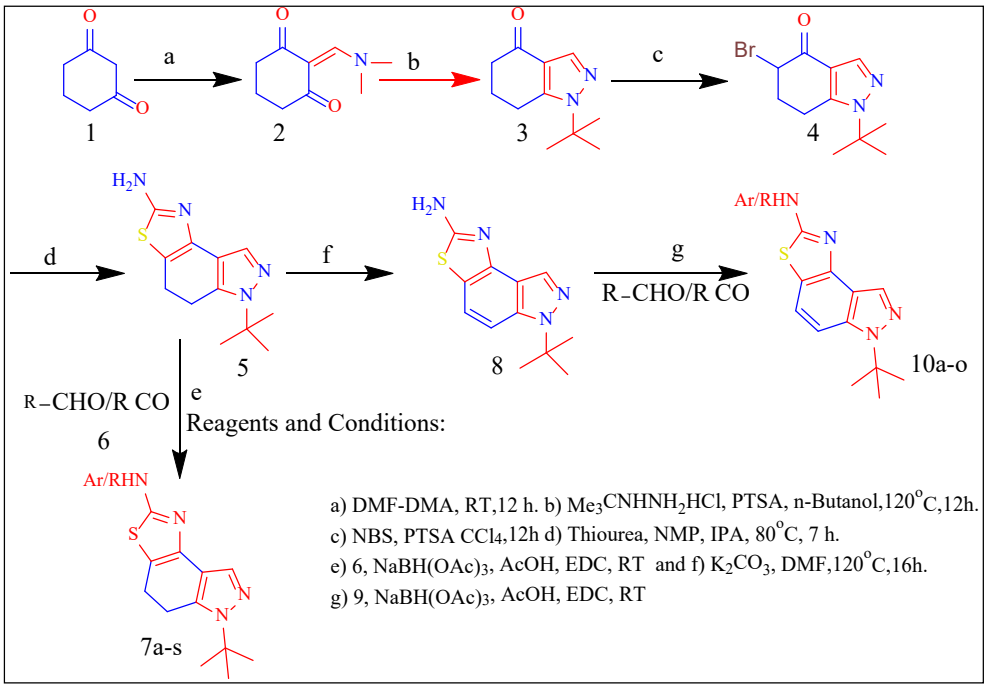
p-toluenesulfonic acid (*p*-TsOH) in the presence ofCCl_4gave 5-bromo-6, 7-dihydro-1-tert-butyl-1*H*-indazole-4(5*H*)-one **(4)** in a 70% yield[41]. The structures of **(4)** were confirmed based on the spectral analysis.

The compound **(4)**was transformed into the target intermediates **(5)**, which could be obtained via the cyclo-condensation reaction of thiourea with α, 5-bromo-6, 7-dihydro-1-tert-butyl-1*H*-indazole-4(5*H*)-one in the N-Methyl-2-pyrrolidone (NMP), isopropyl alcohol (IPA) provided the desired compound **5** in 85% yield**.** The structure of compound (**5**) was confirmed based on the spectral analysis and the purity of the obtained compound was 97.60% with LCMS chromatogram. Then, we move towards the synthesis of a series of proposed ligands (**7a-7s**), Target compounds substituted5, 6-dihydro-4H-thiazole [4, 5-e] indazol-2-amine(**7a–7s**) were prepared by the reductive amination of (**5a)** with numerous aldehydes/ ketones (**6**) and NaBH $(OAc)_3$, EDCI in AcOH at room temperaturein40-84 %yield. The series of substituted 5, 6-dihydro-4H-thiazole [4, 5-e] indazol-2-amine derivatives (**7a-7s**) was confirmed and characterized by using the ^{1}H, ^{13}C NMR, LCMS as well as elemental analysis. The purity of the final compound was also checked with an LCMS chromatogram, most of the compound shows 96 to 98%purity in the chromatogram. The compound N-(2-fluorobenzyl)-6-*t*-butyl-5, 6-dihydro-4H-thiazole [4, 5-e] indazol-2-amine **(7f),**The FTIR spectrum showed the peak at 3252 cm^{-1}corresponding to N-H proton. The ^{1}H-NMR spectrum showed the presence of signals for an NH (7.32 (s, 1H) group, the peak at δ1.55 (s, 9H,$(CH_3)_3$) belonging to 9H of methyl groups. The characteristic peak for benzylamine CH_2 proton appears at the signal atδ 4.48 (d, *J* = 5.6 Hz, 2H), the aromatic proton shows δ 7.93 (t, *J* = 6.0 Hz, 1H), 7.43(d, *J* = 7.2 Hz, 1H) and 7.30 (d, *J* = 4.4 Hz, 1H), 7.21-7.15 (m, 2H) belong to four protons of an aromatic ring. The ^{13}C NMR spectra show the characteristic peak at δ 167.35 corresponding to the C=N imine bond. The peak appears at δ 161.49 and shows coupling with C with J = 261.4 Hz belonging to the Carbon atom attached to the F atom. Lastly, the compound shown LCMS peak at m/z 357(M+1) indicates the formation of the **7f** compound with 97.67% purity on the LCMS chromatogram.

Alternatively, the substitutedaromatic novel aromatized series of 6H-thiazole [4, 5-e] indazol-2-amine derivatives**(10a-o)** was obtained viaaromatization of 5, 6-dihydro-4H-thiazole [4, 5-e] indazol-2-amine **(5)**with anhydrous K_2CO_3in DMF at 120°C gave (**8**) in 70% yields. Similarly, the final scaffold (**10a-o**) was obtained by the reduction amination of aldehyde/ketone with the NaBH$(OAc)_3$/ EDC in AcOH at room temperature afforded**(10a-o)**(40-84 % yield) **(Table 1).** The aromatic novel aromatized 6H-thiazole [4, 5-e] indazol-2-amine derivatives**(10a-o)** were confirmed by using the ^{1}H, ^{13}C NMR, LCMS, and elemental analysis. The purity of the final compounds **(10a-o)**was confirmed by LCMS chromatogram, most of the compounds show 95-98% chromatogram peak.

The N-(2-fluorobenzyl)-6-tert-butyl-6*H*-thiazolo **[4, 5-e]** indazol-2-amine(**10f**). The FTIR spectrum showed the peak at 3252 cm^{-1}corresponding to the N-H proton3267 cm^{-1} corresponding to the

N-H proton. In the ^{1}HNMR spectra, the peak appears at δ8.06 (s, 1H) belonging to the N-H proton. The signal at δ 4.68 doublet for 2H proton having coupling constant, J = 6.2 Hz for CH_2 proton. The singlet appears at δ1.71 for 9H of three methyl groups. The ^{13}C NMR spectra show the characteristic peak at δ168.50 corresponding to the C=N imine bond. The peak appears at δ 161.54 doublet with coupling constant $J_{C\text{-}F}$ = 291.1Hz belonging to the carbon atom attached to the F atom.Finally, the compound shown LCMS peak at m/z 355(M+1) indicates the formation of **10f** compound.At the beginning of our ongoing research, we decided to do modifications at NH_2 sites with numerous pharmacophore groups. The free NH of indazole has been blocked to find out the effect on the cytotoxic activity, therefore we have chosen the *t*-butyl hydrazine hydrate as the starting reagent (**Scheme 2**) for the preparation of desired intermediates (8) (Scheme 2). The modification at indazole sites and free NH of indazole is the ongoing research of our research group.

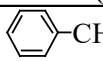
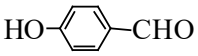
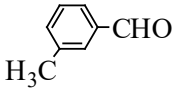
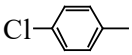
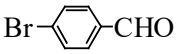
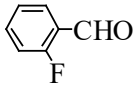
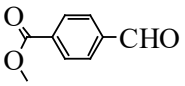
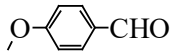
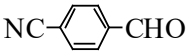
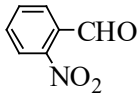
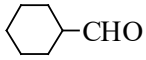
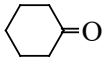
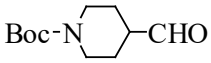
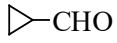
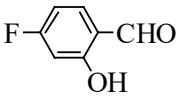
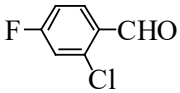
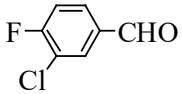
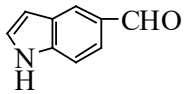


Scheme 2. Synthesis of substituted 6*H*-thiazole [4, 5-e] indazol-2-amine derivatives.

Biological Results

Cytotoxicity assay

Table 1. Synthesis of tricyclic thiazolopyrazole derivatives (7a - 7s to 10a-10o)

Entry	Product[a]	Aldehyde / Ketone **(6)**	Time (h)	Yield[b] (%)	Product[a]	Time (h)	Yield[b] (%)
1	7a		2	84	10a	3	70
2	7b		10	40	10b	12	35
3	7c		5	63	10c	6	50
4	7d		2	82	10d	3	65
5	7e		2	85	10e	3	68
6	7f		2	75	10f	4	60
7	7g		6	50	10g	---	---
8	7h		5	65	10 h	4	60
9	7i		2	70	10i	3	67
10	7j		4	60	10j	4	63
11	7k		2	82	10k	3	77
12	7l		12	50	10l	15	40
13	7m		12	55	10m	15	45
14	7n		4	60	10n	6	50
15	7o		2	80	10o	2	62
16	7p		5	60	10p	10	55
17	7q		4	65	10q	----	----
18	7r		4	62	10r	----	----
19	7s		6	68	10s	----	----

[a] All the products were characterized by ^{1}H NMR, LCMS, IR and ^{13}C NMR **[b]** Isolated yields

MTT assay was used for the evaluation of cytotoxic properties of the substituted dihydro 4H-thiazole [4, 5-e] indazol-2-amine(**7a–s**)andsubstituted 6H-thiazole [4, 5-e] indazol-2-aminederivatives **10a-p**.The growth inhibitory effect was assessed using three human cancer cell linessuch as MCF-7, ME-180, and Hep-G2.The results are summarized in **Table 2** and expressed in IC_{50} values. Adriamycin was used as a positive control for comparison purposes. All these compounds possess a commonlysubstituted 6H-thiazole [4, 5-e] indazol-2-amine nucleus.

It was found that the substituteddihydro 4H-thiazole [4, 5-e] indazol-2-amine **(7a–s)**and substituted6H-thiazole [4, 5-e] indazol-2-amine derivatives**10a-** have moderate to good cytotoxic activity against the selected human cancer cell lines.Concerning the MCF-7 and ME-180 cell line, compound **7a**demonstrated promising cytotoxic activity with IC_{50}values of 15.5±0.3 µM &and 11.6±0.1 µM respectively. Compound **7i** was found to have the highest activity against MCF-7, ME-180, and Hep-G2 cell lines with IC_{50} 11.5±0.8 µM, 11.5±0.4 µM & 12.4±0.5 µM respectively. For structure-activity relationship studies, it was revealed that compounds containing electron-withdrawing groups at the C-5-position of the thiazole ring demonstrated good cytotoxic activity. From IC_{50} values, we can assume that the synthesized derivatives showed very good to moderate cytotoxic activity against the examinedhuman cancer cell lines.

Table 2. Profile of cytotoxic activity of synthesized compounds by MTT assay at 0.01 mM (**7a-s**) and (**10a-o**).

Entry	Compound	IC_{50} (µM) MCF-7	ME-180	Hep-G2
1	7a	15.5±0.3	11.6±0.1	17.5±0.4
2	7b	35.3±0.5	30.7±0.7	20.9±0.3
3	7c	38.2±0.9	40.7±0.9	30.8±0.8
4	7d	20.1±0.4	18.5±0.5	10.4±0.2
5	7e	12.8±0.6	17.6±0.8	29.2±0.1
6	7f	21.7±0.5	17.6±0.1	28.9±0.3
7	7g	25.7±0.6	13.8±0.2	20.7±0.4
8	7h	20.1±0.5	17.8±0.3	29.5±0.1
9	7i	11.5±0.8	11.5±0.4	12.4±0.5
10	7j	29.1±0.9	29.1±0.3	51.2±0.5
11	7k	44.1±0.4	31.9±0.6	35.6±0.8
12	7l	16.7±0.4	28.6±0.6	40.4±0.8
13	7m	27.3±0.4	27.3±0.5	19.8±0.1
14	7n	54.8±0.3	22.6±0.8	33.4±0.9
15	7o	13.7±0.9	11.5±0.9	22.5±0.8
16	7p	18.2±0.7	12.4±0.8	10.6±0.4
17	7q	40.7±0.5	25.5±0.1	32.9±0.7
18	7r	38.9±0.4	50.2±0.3	21.5±0.2
19	7s	15.4±0.7	26.8±0.5	21.4±0.8
20	10a	48.8±0.7	41.5±0.1	30.9±0.3
21	10b	55.3±0.8	50.2±0.6	37.6±0.3
22	10c	28.4±0.8	30.3±0.5	26.6±0.3

23	10d	56.3±0.3	39.6±0.8	30.7±0.9
24	10e	50.2±0.7	29.8±0.3	40.5±0.1
25	10f	30.2±0.2	18.4±0.5	29.8±0.9
26	10h	46.1±0.9	32.7±0.7	22.66±0.5
27	10i	27.2±0.8	27.1±0.3	18.3±0.1
28	10j	52.4±0.4	40.8±0.8	25.5±0.2
29	10k	21.3±0.3	33.8±0.8	25.8±0.8
30	10l	35.7±0.8	48.2±0.3	64.4±0.6
31	10m	58.7±0.8	52.5±0.5	36.8±0.8
32	10n	64.8±0.5	38.8±0.2	37.4±0.8
33	10o	35.8±0.8	21.3±0.8	45.2±0.3
34	10p	58.2±0.9	42.2±0.5	59.4±0.3
35	Adriamycin (0.01 mM)	0.56±0.9	0.89±0.1	0.86±0.8

NR- No results, Results are expressed as the mean values from three independent experiments ± StandardDeviation (SD).

SAR Analysis

At the beginning of this SAR study, we had compounds of general formula **5 (Fig.2)** in hand, which display moderate or lower cytotoxicity activities compared to the standard. The cytotoxicity results of compounds (**7a-7s**) and the literature available concerning the structure-activity relationship suggest that the substituents on the indazole/thiazole ring play a significant role in the demonstration of anticancer activity[42]. While discussing the influence of substituents at the NH_2positions, we observed that substituents such as phenyl, cyclopropyl, and heteroaryl moiety greatly influence anticancer activity. However, most substituents like electron-withdrawing and electron-donating groups on the phenyl rings (analogues) showamoderate effect on the anticancer activity.

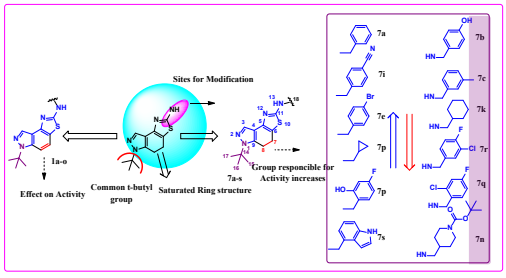


Fig 2. Schematic representation of SAR activity of compounds 7a-7s.

A schematic representation of the SAR activity of compounds 7a-7s has been shown in **Fig.2**.

The synthesized scaffold has an inbuilt indazole and thiazole central ring and numerous pharmacophore groups responsible for the enhancement of the cytotoxic activity (**Fig.3**).

The substituent on the phenyl moiety (e.g., phenyl 7a, phenyl CN 7i, and 7J) shows moderate to very good effects on the cytotoxic activity (IC_{50}15.5±0.3, 11.6±0.1, 17.5±0.4 μM (**7a**), 11.5±0.8, 11.5±0.4, 12.4±0.5 (**7i**),29.1±0.9, 29.1±0.3, 51.2±0.5 (**7J**) against MCF-7, ME-180, and Hep-G2 cells respectively (analogues **7a-7s**)[43]. Exploration around the phenyl moiety with EDG and EWG substituent, the modification at the 4-position was greatly favored compared to the 3- and 2-positions (**Table 1**). The compounds **7p 7q** and **7r** (Table 1, entry 16, 17, 18)have substituents fluorine atoms at the *para* position, and OH, and Cl atoms at the *ortho* and meta position, among these the molecule **7p** has substituent fluorine at *para* position show 18.2±0.7, 12.4±0.8, and 10.6±0.4 (%) cytotoxic activity against MCF-7, ME-180, and Hep-G2 cell lines and well-tolerated than the

7p and **7q**analogs [44]. The molecules **7b**,**7h**, and **7g** (Table 1 entry 2,7, 8)have electron-donating EDG substituents on the phenyl ring and exhibit moderate effects on their cytotoxic potency **(Table 1).**

The incorporation of the electron-withdrawing group (EWG) like CN, NO_2 (Table 1 entry 9,10) shows a good to moderate effect on the cancer potency (IC_{50}; 11.5±0.8, 11.5±0.4, 12.4±0.5 (**7i**)), and (IC_{50}; 29.1±0.9, 29.1±0.3, 51.2±0.5 (**7J**)) against MCF-7, ME-180, and Hep-G2 cell and the cytotoxic activities are compared to the standard Adriamycin (Table 2, entry 35).While, the replacement of cyclic aliphatic substituents with a three, five, and six-member ring such as cyclopropyl (**7o**), cyclopentyl (**7m**), cyclohexyl (**7l**), which having (IC_{50} 13.7±0.9, 11.5±0.9, 22.5±0.8), (IC_{50}; 27.3±0.4, 27.3±0.5, 19.8±0.1) and (IC_{50}; 16.7±0.4, 28.6±0.6, 40.4±0.8 potency against MCF-7, ME-180, and Hep-G2 cell line, the three-membered cyclic aliphatic substituentsprovide very good cytotoxic potency (Table 2, entry 15)[45]. The replacement of other heteroaromatic core scaffolds **7s** provides good cytotoxic potency, (IC_{50} 15.4±0.7, 26.8±0.5, 21.4±0.8) with the respective cell line (Table 2, entry 19). However, from the above results, our interest was shifted towards structural modification, improving or balancing the potency of compounds by reducing their molecular weight of the aromatic indazole-thiazole scaffold (Table 2.**10a-10p).** Unexpectedly, the compound (**10a-10p**) showed very weak cytotoxic activity. Based on these results, we assume that the saturated core ring is more potent than the aromatic scaffolds. The cytotoxic activity results and their comparative analysis show that molecules **7a**, **7e**, **7i, 7o, 7p**, and **7s** can be considered potential lead compounds for developing novel and effective anticancer agents.

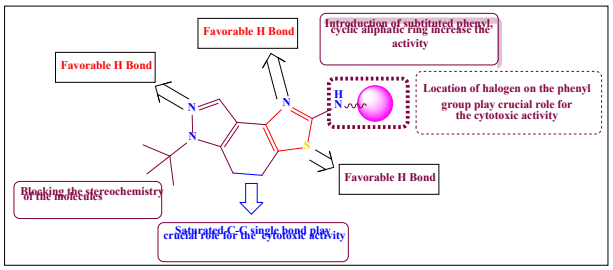


Fig. 3 Schematic representation of pharmacophore sites responsible for the manifestation of cytotoxic activity.

Conclusion

In the present investigation, we have attempted to synthesize and characterize novel substituted dihydro 4H-thiazole [4, 5-e] indazol-2-amine and substituted 6H-thiazole [4, 5-e] indazol-2-amine derivatives and evaluated their cytotoxicactivitiesagainst three human cancer cell lines (MCF-7, ME-180, and Hep-G2) by using MTT assay.The results revealed that the compounds **7a**, **7e**, **7i**, **7o**, **7p,** and **7s** were the most promising cytotoxic agents against the selected human cancer cell lines. Further research in the derivatization of such compounds, lead optimization, and testing them against tailored novel anti-cancer drug targets using state-of-the-art preclinical settings will be of interest with the hope of getting more selective target-oriented anti-cancer agents.

Experimental

Cell lines and chemicals

Human breast cancer cell line (MCF-7), human cervical cancer cell line (ME-180), and human liver hepatocellular cancer cell line (Hep-G2) were procured from National Centre for Cell Science (NCCS: A National Cell Line Facility) Pune (MS), India. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) were procured from Sigma-Aldrich Co. (St. Louis MO, USA). Other solvents, and reagents used were of AR grade and were obtained from commercial sources.

Synthesis

Synthesis of 2-(dimethylamino) methylene) cyclohexane-1,3-dione 2.

Into a round-bottom flask, 1,3-cyclohexanedione **1** (4.5 g, 40.17 mmol) and DMF-DMA (12 mL) were added. The reaction was stirred for 12 h and the progress of the reaction was checked by thin-layer chromatography (TLC). After the starting material was completed, DMF-DMA evaporated under reduced pressure, and the residue was crystallized from EtOAc (20 mL) to obtain title compound **2.** Yield: 85%. m.p.:164-168 °C; IR:ν/cm-^{1}:3060 (CH-aromatic), 2989 (CH-aliphatic), 1680 (C=O), 1345 (C-N); ^{1}HNMR (DMSO-d_6, 400 MHz) δ: 8.10 (s, 1H); 3.40 (s, 3H); 3.18 (s, 3H); 2.50-2.40 (t, 4H); 2.00-1.90 (m, 2H); MS: m/z 168 (M^+).

Synthesis of 1-tert-butyl-6, 7-dihydro-1H-indazol-4(5H)-one 3.

To the solution of 2-((dimethylamino) methylene) cyclohexane-1, 3-dione **2** (7.5 g, 44.91 mmol) was dissolved in an*n*-butanol (75 mL) with *t*-butyl hydrazine hydrochloride (4.57 g, 29.96 mmol), catalytic PTSA, the whole reaction mixture was stirred at 120°Cfor 12 h.The progress of the reaction was checked by thin-layer chromatography (TLC). After completion of the reaction, the excess *n*-butanol was removed under reduced pressure. The obtained residue was washed with the saturated solution of $NaHCO_3$and extracted withEtOAc (30 mL x 3).

Finally, the combined organic layers were washed with brine water (50:30mmL); and dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The obtained crude product was purified by crystallization (50 mL) to afford title compound **3**.Yield: 80%. m.p.:189-194°C; IR:ν/cm-^{1}:3060 (CH-aromatic), 2989 (CH-aliphatic), 1680 (C=O), 1585 (C=C), 1345 (C-N); ^{1}HNMR (DMSO-d_6, 400 MHz) δ 7.84 (s, 1H), 3.05-3.03 (m, 2H), 2.45-2.43 (m, 2H), 2.15–2.13 (m, 2H), 1.64 (s, 9H), HRMS (ESI-TOF) *m/z* [M + H]+ calcd for: $C_{11}H_{17}N_2O$; 193.1341& found 193.1357; LCMS: m/z 193 $(M+1)^+$.

Synthesis of 1-tert-butyl-5-bromo-6, 7-dihydro-1H-indazol-4(5H)-one 4.

The compound 1-*tert*-butyl-6,7-dihydro-1*H*-indazol-4(5H)-one **3** (4.5 g, 23.43 mmol) was dissolved in carbon tetrachloride (45 mL) and was added dropwise to a solution of N-bromosuccinimide(NBS;4.57 g, 29.96 mmol), PTSA, and the reaction mixture was stirred at room temperature for 12 h. The signs of progress of the reaction were checked by thin-layer chromatography (TLC). After completion of the reaction, the H_2O (100 mL) was added, the organic layer was separated, and the aqueous layer was extracted with CCl_4 (3×15

mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$solution (30 mL) and brine solution (40 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to afford title compound **4**.Yield: 70%.m.p.:266-271°C; IR:ν/cm-^{1}:3060 (CH-aromatic), 2989 (CH-aliphatic), 1680 (C=O), 1585 (C=C), 1345 (C-N), 675 (C-Br);^{1}HNMR (DMSO-d_6, 400 MHz) δ: 7.93 (s, 1H); 4.53 (t, *J* = 4 Hz, 1H); 3.26-3.10 (m, 2H); 2.51-2.47 (m, 2H); 1.69 (s, 9H),HRMS (ESI-TOF) *m/z* [M + H]+ calcd for $C_{11}H_{16}BrN_2O$; 271.0446 &found : 271.0462;LCMS: m/z 271 $(M+1)^+$.

Synthesis of 6-tert-butyl-5, 6-dihydro-4H-thiazolo [4, 5-e] indazol-2-amine 5.

A solution of 1-tert-butyl-5-bromo-6,7-dihydro-1*H*-indazol-4(5H)-one**4**(6.5 g, 23.98 mmol) and thiourea (1.87 g, 23.98 mmol) in NMP and IPA, (65 mL), was reflux at 80°C temperature for 7h. The progress of the reaction was checked by thin-layer chromatography (TLC). After the start material was converted, isopropanol was removed under reduced pressure, and a saturated solution of Na_2CO_3 (100 mL) was added to it. The resultant solid was filtered and dried. The crude product was washed with petroleum ether to obtain title compound **5**.Yield: 83 %. m.p.:245-250°C; IR:ν/cm-^{1}:3308 (NH_2), 3060 (CH-aromatic), 2989 (CH-aliphatic), 1680 (C=O), 1614 (C=N), 1585 (C=C), 1345 (C-N), 1310 (C-S), 675 (C-Br); ^{1}HNMR (DMSO-d_6, 400 MHz) δ: 7.28 (s, 1H); 6.80 (bs, 2H); 3.14 (t, *J* = 8.4 Hz, 2H); 2.84 (t, *J* = 8.4 Hz, 2H); 1.55 (s, 9H),HRMS (ESI-TOF) *m/z* [M + H]+ calcd for $C_{12}H_{17}N_4S$; 249.1174 & found: 249.1184 LCMS: m/z 249 $(M+1)^+$.

Synthesis of tricyclic thiazolopyrazole derivatives (7a – s).

A solution of 6-tert-butyl-5, 6-dihydro-4H-thiazole [4, 5-e] indazol-2-amine **5** (1equiv.) and appropriate aldehyde or ketone **6** (1.1eq) in dichloroethanesolvent along withacetic acid (2.5equiv.) was added at room temperature and stirred this reaction mixture for 0.5 to 3h. Later, a solution of sodium triacetoxyborohydride (2eq), EDCin acetic acid was added to the reaction mixtureat RT, and the reaction mixture was stirred for 2 to 12 h. After completion of the reaction, the crude product was purified by column chromatography to furnish pure product (**7a–s)**Yield varies between 40-84 %.

Analytical spectral data

***6-tert-butyl-N-benzyl-5, 6-dihydro-4H-thiazolo [4, 5-e] indazol-2-amine* (7a). Yield 0.104 gm, and 84%'s;**MP:145°C;Rf=0.35 (DCM: MeOH, 9:1);IR cm^{-1}(KBr): 696, 745, 830, 1033, 1202, 1361, 1524, 2979, 3155. ^{1}H NMR (DMSO-d_6, 400 MHz) δ:7.95 (*t*, *J* = 6.0 Hz, 1H),7.36-7.30 (m, 5H),7.24 (s, 1H),4.43 (d, *J* = 6.0 Hz, 2H),3.14 (t, *J* = 8.4 Hz, 2H),2.84 (t, *J* = 8.4 Hz, 2H),1.55 (s, 9H).^{13}C NMR (DMSO-d_6,100 MHz) δ:167.73, 141.46, 139.24, 136.59, 129.99, 128.37, 127.40, 126.88, 116.44, 108.63, 59.24, 47.71, 29.59, 23.34, 21.92. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{19}H_{23}N_4S$ 339.1643 found 339.1653; LCMS: m/z 339 $(M+1)^+$. Elemental Analysis for $C_{19}H_{23}N_4S$ Calc.: C, 67.42; H, 6.55; N, 16.55. Found: C, 67.58; H, 6.49; N, 16.76.

***4-((6-tert-butyl-5, 6-dihydro-4H-thiazolo [4, 5-e] indazol-2-ylamino) methyl) phenol (7b).* Yield 0.052**

gm, and 40%'s; MP:230°C;Rf=0.37 (DCM: MeOH, 8:2);IR cm^{-1} (KBr): 834, 974, 1228, 1384, 1542, 2980, 3190.^{1}H NMR (DMSO-d_6, 400 MHz) δ:9.28 (bs, 1H),7.80 (t, *J* = 6.0 Hz, 1H),7.31 (s, 1H),7.13(d, *J* = 8.4 Hz, 2H),6.68 (d, *J* = 8.4 Hz, 2H),4.27 (d, *J* = 5.6 Hz, 2H),3.12 (t, *J* = 8.0 Hz, 2H),2.82 (t, *J* = 8.0 Hz, 2H),1.54 (s, 9H).^{13}C NMR (DMSO-d_6, 100 MHz) *δ:* 167.69, 156.30, 143.55, 136.60, 132.80, 130.49, 128.03, 117.50, 114.74, 109.85, 59.23, 47.95, 29.59, 23.70, 21.94. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{19}H_{23}N_4OS$; 355.1593 found355.1609; LCMS: m/z $355(M+1)^+$. Elemental Analysis for $C_{19}H_{22}N_4OS$Calc.: C, 61.27; H, 5.68; N, 15.04. Found: C, 61.43; H, 5.61; N, 15.25.

N-(3-methylbenzyl)-6-tert-butyl-5, 6-dihydro-4H-thiazolo [4, 5-e] indazol-2-amine (7c). **Yield 0.075 gm, and 63%'s;** MP:192°C;Rf=0.4.1 (DCM: MeOH, 821);IR cm^{-1} (KBr): 772, 851, 1033, 1206, 1461, 1535, 1559, 2830, 2967, 3168. ^{1}H NMR (DMSO-d_6, 400 MHz) *δ:*7.93 (t, *J* = 6.0 Hz, 1H),7.32 (s, 1H),7.20 (d, *J* = 7.2 Hz, 2H),7.15 (t, *J* = 6.4 Hz, 1H),7.05 (s, 1H),4.38 (d, *J* = 5.6 Hz, 2H),3.14 (t, *J* = 8.4 Hz, 2H), 2.84 (t, *J* = 8.4 Hz, 2H),2.28 (s, 3H),1.55 (s, 9H). ^{13}C NMR (DMSO-d_6,100 MHz) δ:167.73, 141.52, 139.14, 137.28, 136.57, 129.97, 128.16, 127.96, 127.52, 124.48, 116.48, 108.59, 59.23, 47.71, 29.59, 23.35, 21.92, 21.03. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{20}H_{25}N_4S$; 353.1800 found 353.1818, LCMS: m/z $353(M+1)^+$. Elemental Analysis for $C_{20}H_{24}N_4S$Calc.: C, 68.15; H, 6.86; N, 15.89. Found: C, 68.05; H, 6.89; N, 15.99.

N-(4-chlorobenzyl)-6-tert-butyl-5, 6-dihydro-4H-thiazolo [4, 5-e] indazol-2-amine (7d). **Yield 0.112 gm, and 83%'s;** MP:164°C;Rf=0.25 (DCM: MeOH, 9:1);IR cm^{-1} (KBr): 821, 1015, 1204, 1368, 1472, 1524, 2826, 2979, 3205. ^{1}H NMR (DMSO-d_6, 400 MHz) δ:7.97 (t, *J* = 6.0 Hz, 1H),7.40-7.35 (m, 4H),7.31 (s, 1H), 4.42 (d, *J* = 6.0 Hz, 2H),3.14 (t, *J* = 8.4 Hz, 2H),2.84 (t, *J* = 8.4 Hz, 2H),1.55 (s, 9H).^{13}C NMR (DMSO-d_6,100 MHz) δ:167.54, 141.52, 138.38, 136.59, 131.41, 129.32, 128.25, 116.42, 108.82, 59.24, 46.51, 29.59, 23.33, 21.91. HRMS (ESI-TOF) *m/z* [M + H]+calcd for; $C_{19}H_{22}ClN_4S$; 373.1254 found 373.1270, LCMS: m/z $373(M+1)^+$. Elemental Analysis for $C_{19}H_{21}ClN_4S$ Calc.: C, 61.19; H, 5.68; N, 15.02. Found: C, 61.51; H, 5.57; N, 15.33.

N-*(4-bromobenzyl)-6-tert-butyl-5, 6-dihydro-4H-thiazolo [4, 5-e] indazol-2-amine (7e).*Yield 0.130 gm, and 85%'s;MP:166°C; Rf=0.40 (DCM: MeOH, 9:1);IR cm^{-1} (KBr): 820, 1011, 1066, 1205, 1471, 1524, 2824, 2968, 3206. ^{1}H NMR (DMSO-d_6, 400 MHz) δ:7.98 (t, *J* = 6.0 Hz, 1H),7.52 (d, *J* = 8.4 Hz, 2H),7.32 (s, 1H),7.30 (d, *J* = 4.4Hz, 2H),4.40 (d, *J* = 6.0 Hz, 2H),3.14 (t, *J* = 8.4 Hz, 2H),2.84 (t, *J* = 8.4 Hz, 2H),1.55 (s, 9H).^{13}C NMR (DMSO-d_6,100 MHz) δ:167.52, 141.51, 138.81, 136.59, 131.11, 129.98, 129.58, 119.88, 116.41, 108.83, 59.24, 46.96, 29.59, 23.33, 21.91. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{19}H_{22}BrN_4S$; 417.0749 & 417.0765, LCMS: m/z $417(M+1)^+$. Elemental Analysis for $C_{19}H_{21}BrN_4S$ Calc.: C, 54.68; H, 5.07; N, 13.42. Found: C, 54.83; H, 5.01; N, 13.59.

N-(2-fluorobenzyl)-6-tert-butyl-5, 6-dihydro-4H-thiazolo [4, 5-e] indazol-2-amine (7f). **Yield 0.098 gm, and 75%'s;**MP:183°C;Rf=0.52 (DCM: MeOH, 8:2);IR cm^{-1} (KBr): 817, 1033,

1188, 1319, 1456, 1542, 2222, 2947, 3252.,^{1}H NMR (DMSO-d_6, 400 MHz) δ:7.93 (t, J = 6.0 Hz, 1H),7.43(d, J = 7.2 Hz, 1H),7.32 (s, 1H),7.30 (d, J = 4.4 Hz, 1H),7.21-7.15 (m, 2H),4.48 (d, J = 5.6 Hz, 2H),3.14 (t, J = 8.4 Hz, 2H),2.84 (t, J = 8.4 Hz, 2H),1.55 (s, 9H).^{13}C NMR (DMSO-d_6,100 MHz) δ:167.35, 161.49, (d, J = 261.4 Hz), 159.06, 141.52, 136.58, 129.72, 128.96, 125.90, 124.25, 116.44, 115.18, 108.87, 59.24, 41.28, 29.59, 23.33, 21.90. LCMS: m/z357(M+1)$^{+}$. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{19}H_{22}FN_4S$; 357.1549 found; 357.1565, Elemental Analysis for $C_{19}H_{21}FN_4S$Calc.: C, 64.02; H, 5.94; N, 15.72. Found: C, 64.31; H, 5.79; N, 15.92.

Methyl4-((6-tert-butyl-5,6-dihydro-4H-thiazolo[4,5-e]indazol-2-ylamino) methyl)benzoate (7g). **Yield 0.073 gm, and 50%'s;**MP:151°C; Rf=0.42 (DCM: MeOH, 8:2);IR cm^{-1} (KBr): 753, 835, 1108, 1279, 1542, 1724, 2964, 3206. ^{1}H NMR (DMSO-d_6, 400 MHz) δ:8.05 (t, J = 6.0 Hz, 1H),7.92(d, J = 8.0 Hz, 2H),7.49 (d, J = 8.0 Hz, 2H),7.31 (s, 1H),4.52 (d, J = 6.0 Hz, 2H),3.83 (s, 3H),3.14 (t, J = 8.4 Hz, 2H),2.84 (t, J = 8.4 Hz, 2H),1.55 (s, 9H). ^{13}C NMR (DMSO-d_6,100 MHz) δ:167.56, 166.07, 145.11, 141.49, 136.59, 129.98, 129.20, 128.22,127.46, 116.38, 108.88, 59.23, 52.01, 47.30, 29.57, 23.32, 21.90. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{21}H_{25}N_4O_2S$; 397.1698 found 397.1714,LCMS: m/z397(M+1)$^{+}$.Elemental Analysis for $C_{21}H_{24}N_4O_2S$Calc.: C, 63.61; H, 6.10; N, 14.13. Found: C, 63.72; H, 6.11; N, 14.43.

N-(4-methoxybenzyl)-6-tert-butyl-5, 6-dihydro-4H-thiazolo [4, 5-e] indazol-2-amine (7h). **Yield 0.085 gm, and 65%'s;**MP:150°C; Rf=0.41 (DCM: MeOH, 8:1);IR cm^{-1} (KBr): 813, 1033, 1208, 1252, 1515, 2835, 2973, 3162. ^{1}H NMR (DMSO-d_6, 400 MHz) δ:7.87 (t, J = 6.0 Hz, 1H),7.32 (s, 1H),7.27(d, J = 8.4 Hz, 2H),6.88 (d, J = 8.4 Hz, 2H),4.34 (d, J = 6.0 Hz, 2H),3.72 (s, 3H),3.14 (t, J = 8.4 Hz, 2H),2.84 (t, J = 8.4 Hz, 2H),1.55 (s, 9H).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 167.69, 158.29, 141.52, 136.57, 131.06, 129.99, 128.73, 116.50, 113.64, 108.55, 59.23, 55.01, 47.25, 29.59, 23.36, 21.93. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{20}H_{25}N_4OS$; 369.1749 found 369.1765,LCMS: m/z 369 (M+1)$^{+}$. Elemental Analysis for$C_{20}H_{24}N_4OS$Calc.: C, 65.19; H, 6.56; N, 15.20. Found: C, 65.51; H, 6.47; N, 15.64.

4-((6-tert-butyl-5,6-dihydro-4H-thiazolo[4,5-e]indazol-2-ylamino) methyl)benzonitrile(7i). **Yield 0.093 gm, and 70%'s;**MP:182°C;Rf=0.41 (DCM: MeOH, 8:1); IR cm^{-1} (KBr): 786, 1025, 1225, 1362, 1520, 2923, 3209. ^{1}H NMR (DMSO-d_6, 400 MHz) δ:8.06 (t, J = 5.6 Hz, 1H),7.79 (d, J = 8.4 Hz, 2H),7.52 (d, J = 7.6 Hz, 2H),7.28 (s, 1H),4.52 (d, J = 6.0 Hz, 2H),3.12 (t, J = 8.0 Hz, 2H),2.83 (t, J = 8.4 Hz, 2H), 1.53 (s, 9H).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 167.43, 145.44, 141.47, 136.60, 132.27, 129.98, 128.12, 118.87, 116.33, 109.61, 109.04, 59.25, 47.16, 29.57, 23.30, 21.90.HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{20}H_{22}N_5S$; 364.1596 found 364.1636, LCMS: m/z 364 (M+1)$^{+}$.Elemental Analysisfor$C_{20}H_{21}N_5S$Calc. C, 66.09; H, 5.82; N, 19.27. Found: C, 66.36; H, 5.85; N, 19.53.

N-(2-nitrobenzyl)-6-tert-butyl-5, 6-dihydro-4H-thiazolo [4, 5-e] indazol-2-amine(7j). **Yield 0.085 gm, and 60%'s;**MP:145°C;Rf=0.44 (DCM:

MeOH, 8:2);IR cm^{-1} (KBr): 717, 784, 834, 1033, 1237, 1333, 1517, 2978, 3277. ^{1}H NMR (DMSO-d_6, 400 MHz) δ:8.07 (t, *J* = 5.6 Hz, 1H),8.02(d, *J* = 8.4 Hz, 1H),7.72 (t, *J* = 7.2 Hz, 1H),7.66 (d, *J* = 7.2 Hz, 1H),7.52 (t, *J* = 7.2 Hz, 1H), 7.30 (s, 1H), 4.75 (d, *J* = 6.0 Hz, 2H), 3.13 (t, *J* = 8.4 Hz, 2H),2.83 (t, *J* = 8.4 Hz, 2H),1.54 (s, 9H).). ^{13}C NMR ($CDCl_3$DMSO-d_6,100 MHz) δ: 167.11, 148.41, 141.32, 136.59, 134.47, 133.62, 130.04, 128.60, 128.33, 124.49, 116.25, 109.18, 59.24, 44.37, 29.58, 23.28, 21.87.HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{19}H_{22}N_5O_2S$; 384.1494 found 384.1510, LCMS: m/z384$(M+1)^+$.Elemental Analysis for $C_{19}H_{21}N_5O_2S$Calc.: C, 59.51; H, 5.52; N, 18.26. Found: C, 59.67; H, 5.41; N, 18.03.

6-tert-butyl-N-(cyclohexylmethyl)-5, 6-dihydro-4H-thiazolo [4, 5-e] indazol-2-amine (7k). **Yield 0.103 gm, and 82%'s;**MP:172°C;Rf=0.47 (DCM: MeOH, 8:1);IR cm^{-1} (KBr): 774, 999, 1204, 1367, 1522, 2848, 2924, 3230. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.42 (t, *J* = 5.6 Hz, 1H),7.31 (s, 1H), 3.14 (t, *J* = 8.4 Hz, 2H),3.03 (t, *J* = 6.4 Hz, 2H),2.84 (t, *J* = 8.4 Hz, 2H),1.75-1.64 (m, 5H), 1.56 (s, 9H),1.23-1.15 (m, 4H), 0.96 - 0.88 (m, 2H). ^{13}C NMR (DMSO-d_6,100 MHz) δ: 168.08, 141.56, 136.53, 129.96, 116.56, 107.79, 59.20, 51.06, 38.02, 30.48, 29.59, 26.04, 25.43, 23.39, 21.94.HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{19}H_{29}N_4S$; 345.2113 found 345.2139, LCMS: m/z345$(M+1)^+$. Elemental Analysis for $C_{19}H_{28}N_4S$Calc.: C, 66.24; H, 8.19; N, 16.26. Found: C, 66.01; H, 8.15; N, 16.51.

6-tert-butyl-N-cyclohexyl-5, 6-dihydro-4H-thiazolo [4, 5-e] indazol-2-amine (7l). **Yield 60 gm, and 50%'s;** MP:148 °C; Rf=0.45 (DCM: MeOH, 8:1);IR cm^{-1} (KBr): 696774, 999, 1204, 1367, 1522, 2848, 2924, 3230. ^{1}H NMR (DMSO-d_6, 400 MHz) δ:7.34 (t, *J* = 8.0 Hz, 1H),7.31 (s, 1H), 3.42-3.40 (m, 1H),3.14 (t, *J* = 8.4 Hz, 2H),2.83 (t, *J* = 8.4 Hz, 2H),1.71-1.64 (m, 4H), 1.56 (s, 9H), 1.35-1.14 (m, 6H). ^{13}C NMR (DMSO-d_6,100 MHz) δ:168.18, 141.53, 136.54, 129.96, 116.56, 107.85, 59.20, 48.02, 33.42, 29.59, 26.04, 25.43, 23.39, 21.96. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{18}H_{27}N_4S$; 331.1956 found 331.1996, LCMS: m/z331$(M+1)^+$.Elemental Analysis for $C_{18}H_{26}N_4S$Calc.: C, 65.42; H, 7.93; N, 16.95. Found, 65.64; H, 7.78; N, 16.81.

6-tert-butyl-N-cyclopentyl-5, 6-dihydro-4H-thiazolo [4, 5-e] indazol-2-amine (7m). **Yield 0.079 gm, and 55%'s;** MP:134°C;Rf=0.44 (DCM: MeOH, 8:1);IR cm^{-1} (KBr): 793, 1012, 1258, 1525, 2960, 3164. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.44 (t, *J* = 6.8 Hz, 1H),7.31 (s, 1H), 3.89-3.85 (m, 1H), 3.14 (t, *J* = 8.4 Hz, 2H),2.84 (t, *J* = 8.4 Hz, 2H),1.91-1.88 (m, 2H), 1.71-1.64 (m, 2H), 1.56 (s, 9H), 1.52-1.46 (m, 4H). ^{13}C NMR (DMSO-d_6,100 MHz) δ:168.18, 141.53, 136.54, 129.96, 116.56, 107.85, 59.20, 50.02, 35.42, 29.59, 25.43, 28.04, 21.96. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{17}H_{25}N_4S$; 317.1800 found 317.1840,LCMS: m/z317$(M+1)^+$. Elemental Analysis for $C_{17}H_{24}N_4S$Calc.: C, 64.52; H, 7.64; N, 17.70. Found:C, 64.75; H, 7.60; N, 17.93.

Tert-butyl 4-((6-tert-butyl-5, 6-dihydro-4H-thiazolo [4, 5-e] indazol-2-ylamino) methyl) piperidine-1-carboxylate (7n). **Yield 0.098 gm, and 60%'s;**MP:172°C;Rf=0.52 (DCM: MeOH, 8:1);IR cm^{-1} (KBr): 768, 994, 1161, 1243, 1409, 1523, 1694, 2846, 2975, 3240. ^{1}H NMR (DMSO-d_6, 400

MHz) δ:7.44 (t, *J* = 6.8 Hz, 1H),7.31 (s, 1H), 3.95-3.91 (m, 2H), 3.14(t, *J* = 8.4Hz, 4H),2.84 (t, *J* = 8.4 Hz, 2H), 2.69-2.62 (m, 2H),1.73-1.66 (m, 3H), 1.56 (s, 9H), 1.38 (s, 9H), 1.08-1.02 (m, 2H). ^{13}C NMR (DMSO-d_6,100 MHz) δ: 167.92, 153.80, 141.54, 136.52, 129.96, 116.50, 107.99, 78.38, 59.20, 49.95, 43.32, 35.47, 29.59, 29.21, 28.07, 23.36, 21.91. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{23}H_{36}N_5O_2S$; 446.2590 found 446.2606, LCMS: m/z446 $(M+1)^+$.Elemental Analysis for $C_{23}H_{35}N_5O_2S$Calc.: C, 61.99; H, 7.92; N, 15.72. Found, 61.80; H, 7.93; N, 15.89.

6-tert-butyl-N-(cyclopropylmethyl)-5, 6-dihydro-4H-thiazolo [4, 5-e] indazol-2-amine (7o). **Yield 0.089 gm, and 80%'s;**MP:186°C;Rf=0.46 (DCM: MeOH, 8:1);IR cm^{-1} (KBr): 724, 827, 994, 1216, 1366, 1545, 1559, 2963, 3147. ^{1}HNMR (DMSO-d_6, 400 MHz) δ:7.50 (t, *J* = 5.2 Hz, 1H),7.32 (s, 1H), 3.14 (t, *J* = 8.4 Hz, 2H),3.08 (t, *J* = 5.6 Hz, 2H),2.84 (t, *J* = 8.4 Hz, 2H),1.56 (s, 9H), 1.05-1.04 (m, 1H),1.56 (s, 9H), 0.45-0.43 (m, 2H), 0.22-0.21 (m, 2H). ^{13}C NMR (DMSO-d_6,100 MHz) δ: 167.69, 141.53, 136.52, 129.96, 116.56, 108.04, 59.20, 48.88, 29.59, 23.39, 21.94, 10.63, 3.37.HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{16}H_{23}N_4S$; 303.1643 & found 303.1657,LCMS: m/z303 $(M+1)^+$. Elemental Analysis for$C_{16}H_{22}N_4S$Calc.: C, 63.54; H, 7.33; N, 18.53. Found:C, 63.67; H, 7.28; N, 18.74.

2-((6-tert-butyl-5, 6-dihydro-4H-thiazolo [4,5-e] indazol-2-ylamino) methyl)-5-fluorophenol (7p). **Yield 0.082 gm, and 60%'s;**MP:205°C;Rf=0.47 (DCM: MeOH, 8:1);IR cm^{-1} (KBr): 782, 1188, 1367, 1541, 1595, 2947, 3265.^{1}H NMR (DMSO-d_6, 400 MHz)δ:10.74 (s, 1H), 7.99 (t, *J* = 6.0 Hz, 1H),7.34 (s, 1H), 7.21 (t, *J* = 7.2 Hz, 1H),6.60 (d, *J* = 4.0 Hz, 1H),6.58 (s, 1H), 4.32 (d, *J* = 6.0 Hz, 2H),3.15 (t, *J* = 8.4 Hz, 2H),2.84 (t, *J* = 8.4 Hz, 2H),1.56 (s, 9H); ^{13}C NMR (DMSO-d_6,100 MHz) δ:168.06, 163.22, 156.76, 140.62, 136.65, 130.61, 129.86, 121.83, 116.04, 108.58, 105.55, 103.00, 59.31, 42.56, 29.58, 23.31, 21.90. HRMS (ESI-TOF) *m/z* [M + H]+calcd forC$_{19}$H$_{22}$FN$_4$OS; 373.1498 found 373.1514, LCMS: m/z373$(M+1)^+$.Elemental Analysis for $C_{19}H_{21}FN_4OS$Calc.: C, 61.27; H, 5.68; N, 15.04. Found:C, 61.27; H, 5.68; N, 15.04.

N-(2-chloro-4-fluorobenzyl)-6-tert-butyl-5, 6-dihydro-4H-thiazolo [4,5-e] indazol-2-amine (7q). **Yield 0.093 gm, and 65%'s;**MP:117°C;Rf=0.44 (DCM: MeOH, 8:1);IR cm^{-1} (KBr): 860, 1033, 1212, 1542, 2972, 3149, 3749. ^{1}H NMR (DMSO-d_6, 400 MHz) δ:7.96 (t, *J* = 6.0 Hz, 1H),7.49 (d, *J* = 6.4 Hz, 1H), 7.44 (s, 1H),7.31 (s, 1H),7.21 (d, *J* = 8.4 Hz, 1H), 4.47 (d, *J* = 6.0 Hz, 2H),3.13 (t, *J* = 8.4 Hz, 2H),2.83 (t, *J* = 8.4 Hz, 2H),1.54 (s, 9H). ^{13}C NMR (DMSO-d_6,100 MHz) δ:167.18, 162.24, 141.79, 136.58, 133.05, 132.66, 130.65, 130.01, 116.37, 114.32, 114.11, 109.01, 59.24, 44.77, 29.58, 23.32, 21.90. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{19}H_{21}ClFN_4S$; 391.1159 found 391.1181,LCMS: m/z391 $(M+1)^+$.Elemental Analysis for$C_{19}H_{20}ClFN_4S$Calc.: C, 58.38; H, 5.16; N, 14.33. Found:C, 58.57; H, 5.11; N, 14.47.

N-(3-chloro-4-fluorobenzyl)-6-tert-butyl-5, 6-dihydro-4H-thiazolo [4, 5-e] indazol-2-amine (7r). **Yield 0.86 gm, and 60%'s;**MP:142°C; Rf=0.43 (DCM: MeOH, 8:1);IR cm^{-1} (KBr): 782,

937, 1262, 1437, 1575, 2892, 2968, 3195; ^{1}H NMR (DMSO-d_6, 400 MHz) δ:8.05 (t, *J* = 6.0 Hz, 1H),7.36 (s, 1H), 7.31 (s, 1H),7.30 (d, *J* = 8.4 Hz, 2H), 4.55 (d, *J* = 6.0 Hz, 2H),3.14 (t, *J* = 8.4 Hz, 2H),2.85 (t, *J* = 7.6 Hz, 2H),1.55 (s, 9H); ^{13}C NMR (DMSO-d_6,100 MHz) δ:167.18, 160.65, 141.79, 136.58, 133.05, 132.66, 131.75, 130.01, 116.37, 114.32, 113.110, 109.01, 59.24, 44.77, 29.58, 23.32, 21.90.HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{19}H_{21}ClFN_4S$; 391.1159 found 391.1182, LCMS: m/z391(M+1)$^{+}$.Elemental Analysisfor $C_{19}H_{20}ClFN_4S$Calc.: C, 58.38; H, 5.16; N, 14.33. Found:C, 58.56; H, 5.12; N, 14.47.

N-((1H-indol-4-yl) methyl)-6-tert-butyl-5, 6-dihydro-4H-thiazolo [4, 5-e] indazol-2-amine (7s). **Yield 0.091 gm, and 68%'s;**MP:230°C;Rf=0.46 (DCM: MeOH, 8:1);IR cm^{-1} (KBr): 792, 977, 1219, 1368, 1534, 2831, 2970, 3106, 3251. ^{1}HNMR(DMSO-d_6, 400 MHz) δ:11.02 (s, 1H), 7.89 (t, *J* = 6.0 Hz, 1H),7.50 (s, 1H),7.33 (d, *J* = 8.4 Hz, 2H), 7.31(s, 1H), 7.09 (d, *J* = 8.4 Hz, 1H), 6.37 (s, 1H), 4.46 (d, *J* = 6.0 Hz, 2H), 3.13 (t, *J* = 8.4 Hz, 2H),2.83 (t, *J* = 8.4 Hz, 2H),1.55 (s, 9H);^{13}C NMR (100 MHz, DMSO-d_6,100 MHz)δ: 162.94, 144.56, 135.67, 134.63, 133.62, 132.56, 131.45, 123.86, 122.76, 121.10, 120.65, 115.78, 109.26, 102.84, 60.44, 43.59, 29.28, 24.39, 22.28.HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{21}H_{24}N_5S$; 378.1752 378.1768, LCMS: m/z 378 (M+1)$^{+}$. Elemental Analysis for $C_{21}H_{23}N_5S$Calc.: C, 66.81; H, 6.14; N, 18.55. Found: C, 66.69; H, 6.09; N, 18.77.

Synthesis of 6-tert-butyl-6H-thiazolo [4, 5-e] indazol-2-amine 8.The compound 6-*tert*-butyl-5, 6-dihydro-4*H*-thiazolo [4, 5-e] indazol-2-amine **5** (9 g, 36.29 mmol) was dissolved in DMF (45 mL) then anhydrous K_2CO_3 (10.01 g, 72.58 mmol) were added to the reaction mixture and the reaction mixture was stirred at 120°C for 40 h. After complete conversion of the starting material, DMF was removed under reduced pressure, and the reaction mixture with quenched water and the formed precipitated solid was filtered and purified by silica gel column chromatography (using 2-5% MeOH in DCM) to afford title compound **8**. Yield: 70 %. ^{1}HNMR (DMSO-d_6, 400 MHz) δ: 8.00 (s, 1H); 7.58 (bs, 2H); 7.57 (d, *J* = 6.0 Hz, 2H); 7.50 (d, *J* = 8.8 Hz, 2H); 1.70 (s, 9H); LCMS: m/z 247 (M+1).

Synthesis of aromatized tricyclic thiazolopyrazole derivatives (10a – p).A solution of 6-*tert*-butyl-6*H*-thiazolo [4, 5-e] indazol-2-amine **8** (1equiv.) and substituted aldehyde or ketone **9** (1.1eq) in dichloroethane, along with acetic acid (2.5equiv.) was added at room temperature and stirred this reaction mixture for 0.5 to 3h. Later, the solution of sodium triacetoxyborohydride (2equiv.) and EDC in acetic acid was added to the reaction mixture at RT and the reaction mixture was stirred for 2 to 15 h. After completion of the reaction, the crude product was purified by column chromatography to furnish a pure product (**10a – p**).Yield varies between 35-77 %.

6-tert-butyl-N-benzyl-6H-thiazolo [4, 5-e] indazol-2-amine (10a). **Yield 0.097 gm, and 70%'s;**MP:176°C; Rf=0.41 (EtOAc:Hexane, 7:3);IR cm^{-1} (KBr): 691, 774, 987, 1187, 1322, 1451, 1551, 1592, 2866, 2948, 3012, 3265. ^{1}H NMR (DMSO-d_6, 400 MHz) δ:8.62 (t, *J* = 6.0 Hz, 1H),8.05 (s, 1H),7.24 (s, 1H),7.60 (d, *J* = 8.8 Hz, 1H), 7.52 (d, *J* = 8.8 Hz, 1H),

7.41 (d, *J* = 7.2 Hz, 2H), 7.35 (d, *J* = 7.2 Hz, 2H), 7.26 (t, *J* = 7.2 Hz, 2H), 4.64 (d, *J* = 6.2 Hz, 2H), 1.71 (s, 9H).^{13}C NMR (DMSO-d_6,100 MHz)*δ*:168.80, 144.66, 138.74, 137.74, 128.59, 128.34, 127.45, 127.04, 119.32, 118.38, 117.77, 105.62, 59.20, 47.57, 29.21. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{19}H_{21}N_4S$; 337.1487 found 337.1508,LCMS: m/z337(M+1)$^+$.Elemental Analysis for $C_{19}H_{20}N_4S$Calc.: C, 67.83; H, 5.99; N, 16.65. Found: C, 67.65; H, 5.91; N, 16.75.

4-(6-tert-butyl-6H-thiazolo [4, 5-e] indazol-2-ylamino) methyl) phenol (10b). **Yield 0.051 gm, and 35%'s;**MP:245°C;Rf=0.42 (EtOAc:Hexane, 7:3);IR cm^{-1} (KBr): 880, 928, 1231, 1329, 1531, 2980, 3115.^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.02 (bs, 1H), 8.81 (d, *J* = 6 Hz,2H), 8.65 (t, *J* = 6.0 Hz, 1H),8.08 (s, 1H),8.01 (d, *J* = 4.8 Hz, 3H),7.74 (d, *J* = 9.0 Hz, 1H),4.67 (d, *J* = 6.2 Hz, 2H),1.75 (s, 9H).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 167.69, 156.30, 143.55, 136.60, 132.80, 130.49, 128.03, 117.50, 114.74, 109.85, 59.80, 47.95, 29.85. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{19}H_{20}N_4OS$; 353.1436 found 353.4640, LCMS: m/z353(M+1)$^+$.Elemental Analysis for $C_{19}H_{20}N_4OS$Calc.: C, 64.75; H, 5.72; N, 15.90. Found: C, 64.80; H, 5.70; N, 15.99.

N-(3-methylbenzyl)-6-tert-butyl-6H-thiazolo [4, 5-e] indazol-2-amine (10c). **Yield 0.072 gm, and 50%'s;** MP:185°C;Rf=0.40 (EtOAc:Hexane, 7:3);IR cm^{-1} (KBr): 781, 995, 1219, 1542, 2966, 3142.^{1}H NMR (DMSO-d_6, 400 MHz) *δ*:8.58 (t, *J* = 6.0 Hz, 1H), 8.05 (s, 1H),7.60 (s, 1H),7.60 (d, *J* = 9.2 Hz, 1H),7.52 (d, *J* = 9.2 Hz, 1H),7.39 (d, *J* = 9.2 Hz, 1H),7.08 (s, 1H),4.60 (d, *J* = 5.6 Hz, 2H),1.71 (s, 9H).^{13}C NMR (DMSO-d_6,100 MHz) δ:168.80, 144.68, 138.61, 137.74, 137.42, 128.59, 128.26, 128.04, 127.69, 124.56, 119.30, 118.38, 117.76, 105.58, 59.20, 47.58, 29.21, 20.99. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{20}H_{23}N^4S$; 351.1643 found 351.1657, LCMS: m/z351(M+1)$^+$. Elemental Analysis for $C_{20}H_{22}N_4S$Calc.: C, 68.54; H, 6.33; N, 15.99. Found:C, 68.73; H, 6.12; N, 15.79.

N-(4-chlorobenzyl)-6-tert-butyl-6H-thiazolo [4, 5-e] indazol-2-amine (10d). **Yield 0.099 gm, and 65%'s;**MP:202°C; Rf=0.51 (EtOAc:Hexane, 7:3);IR cm^{-1} (KBr): 777, 924, 1210, 1363, 1542, 2972, 3201, 3748.^{1}H NMR (DMSO-d_6, 400 MHz) δ:8.64 (t, *J* = 5.6 Hz, 1H),8.01 (s, 1H),7.60 (d, *J* = 5.6 Hz, 1H),7.52 (d, *J* = 8.8 Hz, 1H),7.45–7.36 (m, 4H),4.63 (d, *J* = 5.6 Hz, 2H),1.71 (s, 9H).^{13}C NMR (DMSO-d_6,100 MHz) *δ*:168.66, 144.57, 137.87, 137.73, 131.61, 129.31, 128.58, 128.29, 119.38, 118.38, 117.78, 105.74, 59.22, 46.78, 29.21. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{19}H_{19}ClN_4S$; 370.1097 found 371.1139,LCMS: m/z371(M+1)$^+$.Elemental Analysis for $C_{19}H_{19}ClN_4S$Calc.: C, 61.53; H, 5.16; N, 15.11. Found:C, 61.13; H, 5.26; N, 15.38.

N-(4-bromobenzyl)-6-tert-butyl-6H-thiazolo [4, 5-e] indazol-2-amine (10e). **Yield 0.115 gm, and 68%'s;**MP:215°C; Rf=0.45 (EtOAc: Hexane, 7:3); IR cm^{-1} (KBr): 690, 776, 1012, 1188, 1362, 1536, 1560, 2850, 2920, 3241. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.64 (t, *J* = 5.6 Hz, 1H),8.02 (s, 1H),7.62 (d, *J* = 5.6 Hz, 1H),7.59 (d, *J* = 8.8 Hz, 1H),7.54 (d, *J* = 8.4 Hz, 2H),7.37 (d, *J* = 8.4 Hz, 2H),4.61 (d, *J* = 6.0 Hz, 2H),1.70 (s, 9H).^{13}C NMR (DMSO-d_6,100 MHz) δ: 168.65, 144.56,

138.30, 137.72, 131.20, 129.66, 128.57, 120.07, 119.37, 118.38, 117.77, 105.74, 59.21, 46.82, 29.21. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{19}H_{20}BrN_4S$; 415.3610 found 416.3654, LCMS: m/z415(M+1)$^+$.Elemental Analysis for $C_{19}H_{19}BrN_4S$Calc.: C, 54.94; H, 4.61; N, 13.49. Found:C, 54.49; H, 4.69; N, 13.33.

N-(2-fluorobenzyl)-6-tert-butyl-6H-thiazolo [4, 5-e] indazol-2-amine (10f). **Yield 0.88 gm, and 60%'s;** MP:205°C;Rf=0.47 (EtOAc: Hexane, 7:3);IR cm^{-1} (KBr): 783, 1187, 1321, 1543, 1594, 2946, 3267.^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.63 (t, J = 6.0 Hz, 1H),8.06 (s, 1H),7.61 (d, J = 5.6 Hz, 1H),7.54 (d, J = 8.8 Hz, 1H),7.52 (m, 1H),7.35 (m, 1H),7.20 (m, 2H),4.68 (d, J = 6.2 Hz, 2H),1.71 (s, 9H).^{13}C NMR (DMSO-d_6,100 MHz) δ: 168.50, 161.54 (d$J_{C\text{-}F}$ = 291.1Hz), 142.95, 137.77, 129.95, 129.39, 128.58, 125.11, 124.39, 118.79, 118.50, 117.30, 115.20, 106.19, 41.55, 29.19. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{19}H_{20}FN_4S$; 355.1393 found 355.1415, LCMS: m/z 355(M+1)$^+$. Elemental Analysis for $C_{19}H_{19}FN_4S$ Calc.: C, 64.38; H, 5.40; N, 15.81. Found: C, 64.55; H, 5.28; N, 15.99.

N-(4-methoxybenzyl)-6-tert-butyl-6H-thiazolo [4, 5-e] indazol-2-amine (10h). **Yield 0.090 gm, and 60%'s;** MP:185°C;Rf=0.55 (EtOAc:Hexane, 7:3);IR cm^{-1} (KBr): 775, 1248, 1542, 1594, 2920, 3245. ^{1}H NMR (DMSO-d_6, 400 MHz) δ:8.54 (t, J = 5.6 Hz, 1H),8.06 (s, 1H),7.60 (d, J = 8.8 Hz, 1H),7.51 (d, J = 8.8 Hz, 1H),7.34 (d, J = 8.4 Hz, 2H), 6.91s (d, J = 8.4 Hz, 2H),4.55 (d, J = 5.2 Hz, 2H),3.72 (s, 3H),1.71 (s, 9H).^{13}C NMR (DMSO-d_6,100 MHz) δ:168.72, 158.43, 144.68, 137.77, 130.57, 128.86, 128.58, 119.27, 118.37, 117.74, 113.75, 105.55, 59.20, 55.02, 47.11, 29.21. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{19}H_{20}FN_4S$; 355.1393 found 355.1418, LCMS: m/z367(M+1)$^+$.Elemental Analysis for$C_{20}H_{22}N_3OS_2$Calc.: C, 62.47; H, 5.77; N, 10.93. Found:C, 62.55; H, 5.62; N, 10.99.

4-((6-tert-butyl-6H-thiazolo [4, 5-e] indazol-2-ylamino) methyl) benzonitrile (10i). **Yield 0.100 gm, and 67%'s;** MP:158°C; Rf=0.46 (EtOAc:Hexane, 7:3);IR cm^{-1} (KBr): 779, 838, 1208, 1540, 2226, 2927, 3231. ^{1}H NMR (DMSO-d_6, 400 MHz) δ:8.73 (t, J = 5.6 Hz, 1H),8.03 (s, 1H),7.82 (d, J = 8.4 Hz, 1H),7.78 (d, J = 8.4 Hz, 1H),7.63–7.59 (m, 2H), 7.55–7.49 (m, 2H),4.75 (d, J = 5.6 Hz, 2H),1.70 (s, 9H).^{13}C NMR (DMSO-d_6,100 MHz) δ:166.66, 143.57, 138.87, 137.73, 130.61, 128.31, 127.58, 126.29, 119.38, 118.38, 117.78, 106.74, 59.22, 43.78, 28.22. HRMS (ESI-TOF) *m/z* [M + H]+calcd for 362.1439 found 362.1454, LCMS: m/z362(M+1)$^+$. Elemental Analysis for $C_{20}H_{19}N_5S$Calc.: C, 66.46; H, 5.30; N, 19.37. Found: C, 66.30; H, 5.59; N, 19.62.

N-(2-nitrobenzyl)-6-tert-butyl-6H-thiazolo [4, 5-e] indazol-2-amine (10j). **Yield 0.099 gm, and 63%'s;** MP:187°C; Rf=0.42 (EtOAc:Hexane, 7:3);IR cm^{-1} (KBr): 719, 784, 1033, 1207, 1362, 1521, 2919, 2937, 3142. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.72 (t, J = 6.0 Hz, 1H),8.07 (s, 1H),8.05 (d, J = 4.8 Hz, 1H),7.75 (d, J = 7.6 Hz, 1H),7.71 (d, J = 5.6 Hz, 1H),7.62 – 7.51 (m, 3H),4.95 (d, J = 6.0 Hz, 2H),1.70 (s, 9H).^{13}C NMR (DMSO-d_6,100 MHz) δ:168.50, 161.54, 142.95, 137.77, 129.95, 129.39, 128.58, 125.11, 124.39, 118.51, 118.50, 117.05, 115.20, 106.54, 42.55, 29.39.HRMS

(ESI-TOF) *m/z* [M + H]+calcd for $C_{19}H_{20}N_5O_2S$; 382.1338 found 382.1342, LCMS: m/z382(M+1)$^+$.Elemental Analysis for $C_{19}H_{19}N_5O_2S$Calc.: C, 59.82; H, 5.02; N, 18.36. Found: C, 59.36; H, 5.12; N, 18.25.

6-tert-butyl-N-(cyclohexylmethyl)-6H-thiazolo [4, 5-e] indazol-2-amine (10k).**Yield 0.110 gm, and 77%'s;** MP:160°C;Rf=0.49 (EtOAc: Hexane, 7:3);IR cm^{-1} (KBr): 776, 1001, 1207, 1253, 1360, 1540, 2848, 2917, 3244. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.13 (t, J = 5.6 Hz, 1H),8.03 (s, 1H),7.58 (d, J = 8.8 Hz, 1H),7.49 (d, J = 8.8 Hz, 1H),3.24 (t, J = 6.0 Hz, 2H),1.80–1.62 (m, 5H),1.71 (s, 9H),1.23 – 1.13 (m, 4H),1.03–0.95 (m, 2H). ^{13}C NMR (DMSO-d_6,100 MHz) δ:169.71, 144.36, 136.96, 132.96, 129.75, 128.58, 125.11, 122.10, 65.20, 53.06, 38.02, 30.48, 29.59, 25.43, 24.39. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{19}H_{27}N_4S$; 343.1956 found 343.1972, LCMS: m/z343(M+1)$^+$. Elemental Analysis for $C_{19}H_{26}N_4S$Calc.: C, 66.63; H, 7.65; N, 16.36. Found: C, 66.36; H, 7.55; N, 16.76.

6-tert-butyl-N-cyclohexyl-6H-thiazolo [4, 5-e] indazol-2-amine (10l).**Yield 0.054 gm, and 40%'s;** MP:156 °C; Rf=0.39 (EtOAc:Hexane, 7:3); IR cm^{-1} (KBr): 794, 1094, 1294, 1260, 1360, 1539, 1548, 1594, 2848, 2960, 3218. ^{1}H NMR (DMSO-d_6, 400 MHz) δ:8.54 (t, J = 6.8 Hz, 1H),8.15 (s, 1H), 8.10 (d, J = 8.6 Hz, 1H),7.83 (d, J = 8.6 Hz, 1H),3.54 (m, 1H),1.73-1.66 (m, 4H), 1.71 (s, 9H), 1.38-1.20 (m, 6H). ^{13}C NMR (DMSO-d_6,100 MHz) δ:172.18, 149.50, 137.54, 129.66, 126.56, 123.20, 121.02, 65.20, 50.42, 33.42, 28.57, 26.00, 23.50. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{18}H_{25}N_4S$; 329.1800 found 329.1816, LCMS: m/z329 (M+1)$^+$. Elemental Analysis for $C_{18}H_{24}N_4S$Calc.: C, 65.82; H, 7.36; N, 17.06. Found: C, 65.92; H, 7.49; N, 17.00.

6-tert-butyl-N-cyclopentyl-6H-thiazolo [4, 5-e] indazol-2-amine (10m). **Yield 0.058 gm, and 45%'s;** MP:144°C;Rf=0.39 (EtOAc: Hexane, 7:3);IR cm^{-1} (KBr): 778, 1248, 1312, 1524, 2830, 2922, 3239. ^{1}H NMR (DMSO-d_6, 400 MHz) δ:8.19 (t, J = 6.8 Hz, 1H),8.16 (s, 1H), 8.10 (d, J = 8.4 Hz, 1H), 8.03 (d, J = 8.4 Hz, 1H),3.86 (m, 1H),1.75-1.68(m, 6H), 1.71 (s, 9H), 1.38-1.22 (m, 2H). ^{13}C NMR (DMSO-d_6,100 MHz) δ:137.18, 149.58, 138.66, 128.66, 126.23, 123.20, 121.28, 65.50, 50.66, 34.23, 28.57, 26.68. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{17}H_{23}N_4S$; 315.1643 found 315.1659 , LCMS: m/z315 (M+1)$^+$.Elemental Analysis for $C_{17}H_{22}N_4S$Calc.: C, 64.93; H, 7.05; N, 17.82. Found: C, 64.66; H, 7.15; N, 17.57.

Tert-butyl4-((6-tert-butyl-6H-thiazolo [4, 5-e] indazol-2-ylamino) methyl) piperidine-1-carboxylate (10n).**Yield 0.091 gm, and 50%'s;**MP:180°C;Rf=0.51 (EtOAc:Hexane, 7:3);IR cm^{-1} (KBr): 794, 961, 1109, 1214, 1523, 1694, 2846, 2994, 3344. ^{1}H NMR (DMSO-d_6, 400 MHz) δ:8.09 (t, J = 5.6 Hz, 1H),8.05 (s, 1H),7.60 (d, J = 8.8 Hz, 1H),7.50 (d, J = 8.8 Hz, 1H),3.86 (t, J = 6.0 Hz, 2H),3.16 – 1.14 (m, 4H),1.80 – 1.63(m, 3H),1.72 (s, 9H), 1.53 (s, 9H),1.12 – 1.10 (m, 2H). ^{13}C NMR (DMSO-d_6,100 MHz) δ:172.54, 156.80, 145.50, 141.36, 136.52, 129.96, 126.23, 123.20, 121.28, 79.38, 59.32, 49.99, 43.47, 35.47, 29.59, 28.07, 23.52. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{23}H_{34}N_5O_2S$;

444.2433 found 444.2449, LCMS: m/z444 $(M+1)^+$.Elemental Analysis for $C_{23}H_{33}N_5O_2S$Calc.: C, 62.27; H, 7.50; N, 15.79. Found: C, 62.50; H, 7.79; N, 15.52.

6-tert-butyl-N-(cyclopropylmethyl)-6H-thiazolo [4, 5-e] indazol-2-amine (10o).**Yield 0.077 gm, and 62%'s;** MP:151°C;Rf=0.44 (EtOAc:Hexane, 7:3); IR cm^{-1} (KBr): 776, 1000, 1206, 1253, 1360, 1539, 1551, 1594, 2848, 2918, 3250. ^{1}H NMR (DMSO-d_6, 400 MHz) δ:8.23 (t, J = 5.6 Hz, 1H), 8.04 (s, 1H),7.59 (d, J = 8.8 Hz, 1H),7.49 (d, J = 8.8 Hz, 1H),3.28 (t, J = 6.4 Hz, 2H), 1.71 (s, 1H),1.12–1.10 (m, 1H),0.49 (q, J = 4.8 Hz, 2H),0.28 (q, J = 4.4 Hz, 2H). ^{13}C NMR (DMSO-d_6,100 MHz) δ:168.64, 144.86, 137.73, 128.59, 119.05, 118.32, 117.72, 105.34, 59.17, 48.73, 29.21, 10.56, 3.39. HRMS (ESI-TOF) *m/z* [M + H]+calcd for $C_{16}H_{21}N_4S$; 301.1487 found 301.1527, LCMS: m/z301 $(M+1)^+$. Elemental Analysis for $C_{16}H_{20}N_4S$Calc.: C, 63.97; H, 6.71; N, 18.65. Found: C, 63.86; H, 6.86; N, 18.76.

N-(2-chloro-4-fluorobenzyl)-6-tert-butyl-6H-thiazolo [4, 5-e] indazol-2-amine (10p).**Yield 0.089 gm, and 55%'s;** MP:165°C;Rf=0.42 (EtOAc: Hexane, 7:3);IR cm^{-1} (KBr): 779, 854, 1002, 1209, 1541, 2976, 3186.^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.63 (t, J = 5.2 Hz, 1H),8.06 (s, 1H),7.62 (d, J = 9.2 Hz, 1H),7.59 (d, J = 7.2 Hz, 1H),7.55(d, J = 8.8 Hz, 1H),7.53 (s, 1H),7.49 (d, J = 6.4 Hz, 1H),4.68(d, J = 5.6 Hz, 2H),1.71 (s, 1H); ^{13}C NMR (120 MHz, DMSO-d_6,) δ: 168.28, 160.67 (d, J_{C-F} = 251.2 Hz), 142.70, 138.16, 135.90, 133.70, 132.82, 131.45, 130.38, 120.64, 119.28, 116.43, 112.55, 109.76, 61.55, 41.38, 28.48(Cx3). HRMS (ESI-TOF) *m/z* [M + H]+calcd for$C_{19}H_{19}ClFN_4S$; 389.1003 found 389.1021,LCMS: m/z 389$(M+1)^+$.Elemental Analysis for $C_{19}H_{18}ClFN_4S$Calc.:C, 58.68; H, 4.67; N, 14.41. Found:C, 58.89; H, 4.58; N, 14.55.

Spectral data of the synthesized compounds is submitted in the supplementary information.

Cytotoxicity assay

The compounds' cytotoxic potential against selected cancer cell lines was determined using the MTT assay as previously reported with slight modifications[46-48].Inoculation of the cells at 1×10^5 cells/ml density was carried out in 96 well culture plates. The cells were treated with different concentrations of compound dissolved in 0.1 % DMSOand incubated for 24 h. The 20 μL MTT (2 mg/ml), was applied to each well after the incubation time and the cells were incubated further at 37°C for 4 hours. Further, formazan crystals were dissolved in isopropanol and the amount of formazan produced was estimated at 570 nm. The required concentration for inhibition of 50% cell viability has been calculated as IC_{50}.

Declaration of competing interest

The authors declare no competing interests.

Acknowledgments

RNG acknowledges the financial support from RashtriyaUchchatar Shiksha Abhiyan (RUSA) Phase-II grant of Savitribai Phule Pune University and the Government of India. SVG isthankful for the Comenius in University Bratislava Capacities and Competence in Research, Development, and Innovation,

ITMS2014+: 313021BUZ3, his Postdoctoral research program.

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