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RESEARCH ARTICLE

Synthesis and Antitumor Activity Evaluation of 2-Aminothiazoles Appended 5-methylisoxazoline and Pyridine-piperazine Hybrid Molecules

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Abstract: A highly efficient and milder protocol for the syntheses of novel series of 2-aminothiazoles bearing 5-methylisoxazoline and pyridine-piperazine hybrid molecules has been developed. The target compounds **13a-e** were screened for their *in vitro* cytotoxicity activity against various tumor cell lines including MCF-7 (human breast adenocarcinoma), HCT-116 (colorectal carcinoma), Jurkat (human T-cell leukemia) and THP-1 (human acute monocytic leukemia). The bioactive assay showed most of the new compounds exhibited promising results in comparison with the parental Sunitinib. The synthesized compounds could well be used in the future as lead anticancer drugs in drug development studies. The synthesized compounds were fully characterized by IR, ^{1}H NMR, ^{13}C NMR, elemental analysis and mass spectral data.

Keywords: Hybrid drugs, antiproliferative activity, 2-aminothiazole, pyridine-piperazine scaffold, 5-methylisoxazoline, anticancer drug.

1. INTRODUCTION

In recent days, the model of "hybrid drugs" has acquired recognition in medicine and this concept was originated from combination therapies which were conventionally applied to cure unresponsive patients [1]. Current literature shows that hybrid drugs have gained significant role in the treatment of different health problems like systemic heart diseases [2], antiparasitic activities [3], and anti-tumour reagents [4]. A review article was published by Rejniak, Katarzyna A., and Alexander RA Anderson [5], on mathematical modelling approaches to design hybrid molecules for tumour growth inhibition. Different types of hybrid molecules are conjugate hybrid, cleavage hybrid, fused hybrid and merged hybrid [4]. Hybrid molecules are designed by chemical hybridization wherein two drug pharmacophores are incorporated in a single molecule with an objective to put forth twin drug action. For example, one of the pharmacophores possibly will target explicitly tumour vessels whereas another may be the active agent [6]. Hence, hybrid molecules are also known as multifunctional or conjugated drugs. Hybrid molecules may also exhibit synergetic effect compared to the individual pharmacophores [7]. Optimisation of drug delivery to the target and reduction in systemic toxicity can be achieved from hybrids with the simultaneous release of two active reagents by the action of specific enzymes [8]. The other probable salient advantages of hybrids are reduced side effects due to optimal dose usage, improved efficacy, counterbalancing side effects of one pharmacophore by another, improved drug bioavailability and transport across membranes of cell organelles, protection of active substances from enzymatic degradation, minimization of risk of drug-drug interactions and avoiding potential drug resistance [4]. Most of the hybrid heterocyclic compounds with different heterocyclic moieties act as potent antitumor agents in cancer chemotherapy and showed good anticancer activities against a panel of human cancer cell lines [9-18].

Cancer is a genetic disease in which abnormal cells divide without control and can invade nearby tissues. Cancer cells can also extent to other parts of the body through the blood and lymph systems. Based on the origin, there are several main types of cancer. Carcinoma begins in the skin or in tissues. Sarcoma is a cancer that begins in bone, cartilage, fat and muscle blood vessels. Leukemia cancer starts in blood-forming tissue, such as the bone marrow, and causes large numbers of abnormal blood cells production. Lymphoma and multiple myeloma are cancers which starts in the cells of the immune system. Malignancy cancer starts in the central nervous system. As per the World Health Organization (WHO) reports cancer is a fast growing disease in 21st century with an estimation of 13.1 million deaths in 2030 [19,

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20]. Cancer is the second leading cause of death in high-income countries (following cardiovascular diseases) and the third leading cause of death in low- and middle-income countries (following cardiovascular diseases and infectious and parasitic diseases). According to estimates from the International Agency for Research on Cancer (IARC), there were 14.1 million new cancer cases in 2012 worldwide, of which 8 million occurred in economically developing countries, which contain about 82% of the world's population. These estimates do not include non-melanoma skin cancers, which are not tracked in cancer registries. The corresponding estimates for total cancer deaths in 2012 were 8.2 million (about 22,000 cancer deaths a day) – 2.9 million in economically developed countries, and 5.3 million in economically developing countries. However, the estimated future cancer burden will probably be considerably larger due to the adoption of lifestyles that are known to increase cancer risk, such as smoking, poor diet, physical inactivity, and fewer pregnancies, in economically developing countries. A tour-de-force development in anticancer research results vast number of anticancer drugs. Based on the mode of action these anticancer drugs are broadly categorized into three main groups [21].

a) Genotoxic agents.

b) Antimetabolites.

c) Mitotic spindle inhibitors.

In view of the seriousness of the dreadful disease and improved drug resistance among the cancer cells, there is a continuous need for the development of new anticancer drugs [21].

"Thiazole, a nitrogen-sulfur containing" heterocycle is widely used as an important synthetic intermediate for the preparation of large number of pharmaceutical drug products. A number of drugs including Famotidine, Cefdinir and Meloxcam are widely distributed in the market which contains 2-aminothiazole (2-AT) core as an active pharmacophore. Substituted aminothiazole compounds play a vital role in the drug development and have a diverse range of biological properties [22-28] including potential antitumor activity [29-32]. Anticancer drugs Dabrafenib, Dasatinib and Bleomycin are marketed widely in the world which contain thiazole as an important structural element. The groove-binding agents Dactinomycin, Netropsin and Thia-netropsin are well known anticancer drugs with thiazole ring as an important structural element [33]. It is evident from the literature, aminothiazoles acts as ligands for estrogen receptors and novel class of adenosine receptor antagonists [34, 35]. It is evident from the literature, amide containing heterocycles are reported as a class of compounds displaying potential biological activities, which consist of a vast number of natural and synthetic products and are extremely versatile building blocks for the manufacturing of bioactive compounds in pharmaceutical drug development [36, 37].

Piperazine is a well-known six membered nitrogen containing heterocyclic that can be found in a vast number of marketed drug products including anticancer drugs. The piperazine scaffold and its analogues hybrid systems have gained much attention of the scientific community due to its potential biological properties [38-43], especially antitumor activity [44]. Slight structural alteration on the piperazine pharmacophore facilitates as indispensable anchors for the development of new chemical entities with a range of different biological targets in medicinal chemistry [45, 46]. Louis J. Lombardo and his co-workers reported the synthesis of N-(2-Chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-methylpyrimidin-4-ylamino) thiazole-5-carboxamide (BMS-354825), a dual Src/Abl Kinase Inhibitor with potent antitumor activity, which is widely used in the cancer treatment with a brand name Sprycel (Dasatinib monohydrate) which contain piperazine-thiazole scaffold [47]. Peter L. Toogood and his team invented the best cancer drug, 6-Acetyl-8-cyclopentyl-5-methyl-2-{[5-(1-piperazinyl)-2-pyridinyl] amino}pyrido[2,3-d]pyrimidin-7(8H)-one, brand name Ibrance (Palbocilcib) that contains piperazine-pyridine heterocyclic system [48].The well-known marketed alpha blockers Terazosin, and Doxazosin comprise of the piperazine structure. On the other hand, a large number of piperazine scaffolds are reported with alpha blocking activity [49-52].

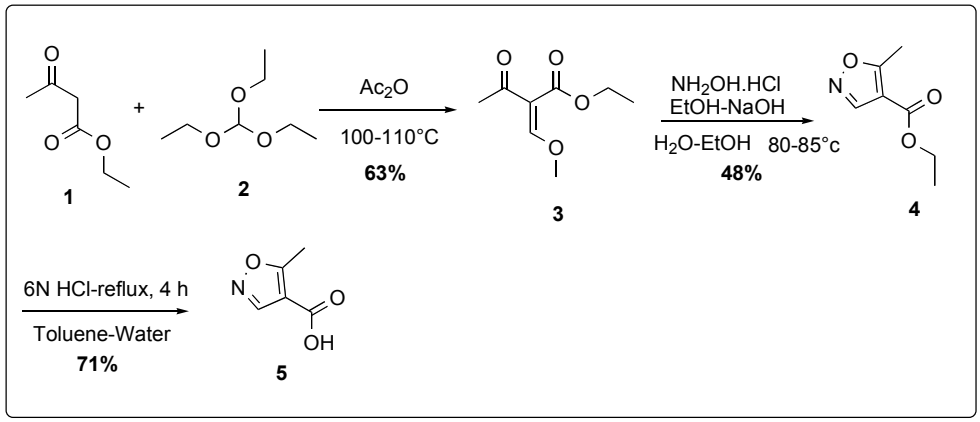
Isoxazoline is an important class of nitrogen and oxygen containing five membered heterocycles belongs to azole family. Substituted isoxazoles play a vital role in the drug development and have a diverse range of biological properties [53-58] including antitumor activity [59].

Aforementioned endings in combining features of more than one biologically active pharmacophores in a single molecule may result in prominent pharmacological activity while retaining high diversity and biological significance [60-64]. On the basis of high-throughput screening, it was envisaged to synthesize conjugate derivatives with amino-thiazole, piperazine, pyridine and methyl isoxazoline moieties in the same scaffold. The present study describes the synthesis, characterization and antitumor activity evaluation of 2-aminothiazoles bearing 5-methylisoxazoline and pyridine-piperazine scaffolds.

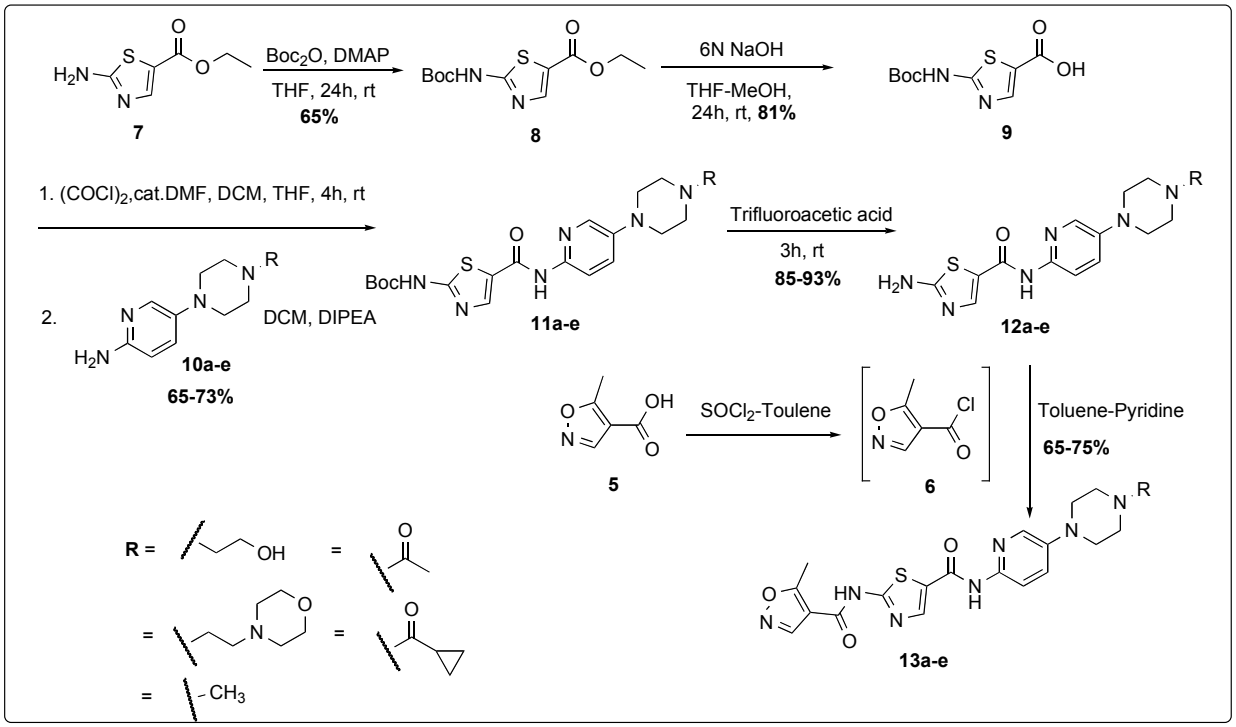
2. RESULTS AND DISCUSSION

2.1. Chemistry

The key intermediate **5** for this study was prepared as shown in Scheme **1**. The first step of the synthesis involves the condensation of ethyl acetoacetate **1** and triethylorthoformate **2** in presence of acetic anhydride resulted (Z)-ethyl 2-(ethoxymethylene)-3-oxobutanoate **3** as a pale yellow liquid in 63% yield. Further cyclization of **3** in presence of aqueous hydroxylamine hydrochloride and alcoholic NaOH resulted in ethyl 5-methylisoxazole-4-carboxylate **4** as a dark brown liquid in 48% yield. Further hydrolysis of **4** in aqueous hydrochloric acid for 4h at reflux, yielded the key intermediate 5 as a pale yellow solid, in 71% yield [65]. Further chlorination with thionyl chloride [66] in refluxing toluene and catalytic DMF result acid chloride **6.** Further coupling of *in situ* generated acid chloride **6** with different aryl amines **12a-e** yielded [67] the corresponding piperazine scaffolds **13a-e** in 69-75% yields. All these newly synthesized compounds were purified by column chromatography and characterized by Mass, ^{1}H NMR and ^{13}C NMR.



Scheme (1). Synthetic route for key starting material (5).



Scheme (2). Synthesis of 2-aminothiazoles appended 5-methylisoxazoline and pyridine-piperazine derivatives (13a-e).

The five stage synthetic route for the target compounds **13a-e** is depicted in Scheme **2**. Briefly, the first step of the synthesis involves the Boc protection of amine function in Ethyl 2-aminothiazole-5-carboxylate **7** resulted compound **8** in 65% yield, which was further hydrolyzed with aqueous NaOH in THF/MeOH gave acid **9** in 81% yield [67]. Boc acid **9** was chlorinated with oxalyl chloride followed by *in situ* coupling with aryl amine, **10a-e** gave thiazole coupled piperazine pyridine intermediates, **11a-e** in 67-73%yield [66]. Deprotection of Boc function of **11a-e** in trifluoroacetic acid yielded aminothiazole conjugates **12a-e** in 85-93% yield [32]. Finally, coupling of **12a-e** with 5-methylisoxazole-4-carboxylic acid **5** *via in situ* acid chloride **6,** in pyridine and refluxing toluene [66], gave the final compounds **13a-e** in 69-75% yields (Table **2**) as off white solid. All these newly synthesized compounds were purified by column chromatography and characterized by Mass, ^{1}H NMR and ^{13}C NMR.

2.1. Antiproliferative Activity

Antiproliferative activity of the final compounds, **13a-e** have been evaluated *in vitro* against four tumor cell lines, namely MCF-7 (human breast adenocarcinoma), HCT-116 (colorectal carcinoma), Jurkat (human T-cell leukemia) and THP-1 (human acute monocytic leukemia) using Sunitinib as standard by following the MTT assay method [68].

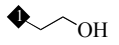
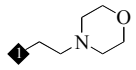
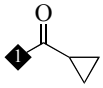
In view of the structural diversity of 2-aminothiazoles bearing 5-methylisoxazoline and pyridine-piperazine scaffolds, we mainly investigated the influence of **R** functional group on piperazine heterocycles. All the synthesized compounds showed potent activities against tested cancel cell lines. Among the screened compounds, the rate of inhibition was observed in the following order **13a > 13b > 13c > 13d > 13e** (Table **1**) against THP 1 cancer cell lines. Among these, compound **13a** and **13b** showed highest inhibition of

Table 1. ***In vitro*** **cytotoxicity data of compounds 13a-e on MCF-7, HCT-116, Jurkat and THP-1 tumor cell lines.**

Compound	IC_{50}[a] (μM/L) ± SE			
	MCF-7	HCT-116	Jurkat	THP-1
13a	6.4 ± 0.31	5.9 ± 0.29	8.3 ± 0.92	2.9 ± 0.32
13b	7.2 ± 0.29	4.9 ± 0.22	9.4 ± 0.25	3.2 ± 0.24
13c	8.4 ± 0.18	4.8 ± 0.28	7.4 ± 0.86	3.9 ± 0.54
13d	7.1 ± 0.16	5.7 ± 0.29	6.9 ± 0.75	5.9 ± 0.26
13e	8.3 ± 0.24	6.7 ± 0.28	9.9 ± 0.52	6.8 ± 0.51
Sunitinib	6.3± 0.22	4.7 ± 0.32	6.4 ± 0.92	3.5 ± 0.56

[a] Concentration inhibiting fifty percent of cell growth for 72 h exposure period of tested samples. Assay was done in triplicate and mean values are presented.

Table 2. Yields of 2-aminothiazoles appended 5-methylisoxazoline and pyridine-piperazine derivatives (13a-e).

S. No.	R	Product	Yield* (%)
1		**13a**	69
2		**13b**	71
3		**13c**	70
4		**13d**	75
5		**13e**	73

proliferation activity against THP 1 cancer cell lines with IC_{50} values 2.9 ± 0.32 and 3.2 ± 0.24, when compared to reference standard drug Sunitinib. The better antiproliferative activity of compound **13a** and **13b** is due to the presence of electron donating groups like 2-hydroxyethyl in **13a** and 6-memebered morphine ring in **13b**. The observed antiproliferation activity data showed that compounds containing electron donating groups on aryl-piperazine ring are more active compared to electron withdrawing carbonyl functional groups on aryl-piperazine heterocyclic systems [69]. The better activity by the compounds **13a** and **13b** could be attributed to the cell permeability factor which is usually enhanced by electron donating functional group like 2-hydroxyethyl functional substitution on aryl-piperazine heterocyclic systems [49].

3. EXPERIMENTAL

All of the chemicals were obtained from commercial sources and used without further purification. Melting points were determined in open glass capillaries on a Fisher–Johns melting point apparatus and are uncorrected. NMR (^{1}H 400 MHz; ^{13}C 100 MHz) spectra were recorded at room temperature in DMSO and $CDCl_3$ as solvent and TMS as an internal standard (δ = 0 ppm), and the values were reported in the following order: chemical shift (δ in ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, qq = quartet of quartet), coupling constants (J in Hz), and integration. All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F254 (mesh); spots were visualized under UV light at 254 nm.

3.1. Typical Experimental Procedure for the Key Compounds

3.1.1. 5-methylisoxazole-4-carboxylic acid (5)

To the stirred solution of 6N HCl (20.0 mL), ethyl 5-methylisoxazole-4-carboxylate **4** (2.0 g, 0.0064 mol) was added at 5-10°C and heated for 4 hours at 90-95°C. Reaction completion was confirmed by TLC and charged with toluene (30.0 mL) at 90-95°C. Further cooled to 25-30°C and stirred for 5 hours, filtered the precipitated solid as a creamy solid. Creamy solid charged in to a stirred 10% aqueous toluene (150 ml) and heated to reflux, cooled to 40-45°C, filtered and dried to give 5-methylisoxazole-4-carboxylic acid compound **5** as off white solid 1.14 g (71%). MR: 136-138 °C; ^{1}H NMR spectrum (400 MHz, DMSO-d6), δ, ppm (J, Hz): 8.53 (s, 1H, thiazole-H), 2.74 (s, 3H, -CH_3);^{13}C NMR spectrum (100 MHz, DMSO-d6), δ, ppm:169.7 (isoxazole-C), 167.9 (acid-C), 159.3 (isoxazole-C), 106.3 (isoxazole-C), 14.3 (CH_3); MS (ESI) m/z 127.9 [M + H], 149.9 [M + Na].

3.1.2. Ethyl 2-((tert-butoxycarbonyl)amino)thiazole-5-carboxylate (8)

To the stirred solution of ethyl 2-amino-4-methylthiazole- 5-carboxylate **7** (5.0 g, 0.0290 mol) in dry THF (65.0 mL), Boc-anhydride (7.6 g, 0.0348 mol) and catalytic amount of 4-DMAP (177 mg, 0.0145 mol) were added at 25-30°C. The reaction mass warmed up to 40-45°C and stirred for 8 hours. The reaction mixture was concentrated. The residue was dissolved in ethyl acetate (150.0 mL), and washed with 2N HCl, DM water, 10% aqueous NaCl solution, dried over sodium sulfate and concentration resulted creamy solid. Further recrystallization in ethyl acetate and hexane resulted **8** (5.18 g, 65%) as a pale yellow solid. ^{1}H NMR spectrum (400 MHz, DMSO-d6), δ, ppm (J, Hz): 11.3 (s, 1H, N-H), 8.12 (s, 1H, thiazole), 4.21-4.11 (q, 2H), 1.49 (s, 9H, Boc-H), 1.28-1.23 (t, 3H); ^{13}C NMR spectrum (100 MHz, DMSO-d6), δ, ppm: 168.3 (ester-C), 163.2 (thiazole-C), 158.3 (amide-C), 131.2 (thiazole-C), 119.9 (thiazole-C), 69.8 (Boc-C), 59.8 (CH_2), 20.5 (3C, Boc-CH_3), 15.2 (CH_3); MS (ESI) m/z 273.1 $[M + H]^+$.

3.1.3. 2-((tert-butoxycarbonyl)amino)thiazole-5-carboxylic acid (9)

To the stirred solution of ester **8**, (4.5 g, 0.0165 mol) in 1:1 (72.0 mL) THF-Methanol, 3.3 g of NaOH dissolved in 45.0 mL water was added at 25-30°C. The reaction mass was warmed to 60-65°C and stirred for 3 hours. Solvent was distilled under vacuum and charged with 45.0 mL water. The reaction mass was cooled to 0-5°C and adjusted the reaction mass pH to 3.2 with citric acid (15.8 g, 0.0826) and stirred for 1h. The precipitated solid was filtered, washed with water, 20% acetone-MTBE solution, yielded the compound **9** (3.24 g, 81%) as a pale yellow solid. ^{1}H NMR spectrum (400 MHz, DMSO-d6), δ, ppm (J, Hz): 12.5 (bs, 1H, acid), 10.9 (s, 1H, NH), 8.4 (s, 1H, thiazole), 1.49 (s, 9H, Boc-H), ^{13}C NMR spectrum (100 MHz, DMSO-d6), δ, ppm: 171.3 (acid-C), 165.3 (thiazole-C), 160.5 (amide-C), 134.2 (thiazole-C), 119.6 (thiazole-C), 70.2 (Boc-C), 20.5 (3C, Boc-CH_3); MS (ESI) m/z 245.1 $[M + H]^+$, 267.2 [M + Na].

3.1.4. tert-butyl (5-((5-(4-(2-hydroxyethyl)piperazin-1-yl)pyridin-2-yl)carbamoyl)thiazol-2-yl)carbamate (11a)

To the stirred suspension of **9** (2.75 g, 0.01126 mol) in dichloromethane, 2 M oxalyl chloride (6.7 mL, 0.0135 mol) and DMF (0.12 mL) were added at 0-5°C and warmed to 25-30°C and stirred for 2 hours. The reaction mass was concentrated and the obtained residue was dissolved in ethyl acetate (50.0 mL) and washed with 2N HCl, DM water, 10% aqueous NaCl solution, dried over sodium sulfate and concentrated, resulted pale yellow residual mass, treated with ethyl acetate and hexane resulted acid chloride (2.8 g, 95%) as a pale yellow solid, suspended in toluene (42.0 mL) and cooled to 15-20°C and charged commercially available 2-(4-(6-aminopyridin-3-yl)piperazin-1-yl)ethanol **10a** (2.36 g, 0.01062 mol), diisopropylethylamine (4.6 mL, 0.0266 mol) and catalytic DMAP. Reaction mass was heated for 3 hours at reflux. Cooled the reaction mass to 25-30°C filtered the solid **11a** (3.2 g, 67%) as a pale yellow solid.

^{1}H NMR spectrum (400 MHz, DMSO-*d*6), δ, ppm (*J*, Hz): 11.32 (s, 1H, Boc-NH), 10.98 (bs, 1H, amide-N<u>H</u>), 8.92 (s, 1H, thiazole-H), 7.69 (d, 1H, pyridine-H), 7.36-7.34 (m, 2H, pyridine-H), 4.41 (t, 1H, -O<u>H</u>), 3.54-3.51 (m, 6H, -$CH_2C\underline{H_2}OH$ & piperazine-CH_2), 2.39-2.32 (m, 6H, -$CH_2C\underline{H_2}OH$ & piperazine-CH_2) 1.42 (s, 9H, Boc-H); ^{13}C NMR spectrum (100 MHz, DMSO-*d*6), δ, ppm: 160.6 (thiazole-C), 159.1 (amide-C), 153.2 (Boc amide-C), 141.9 (pyridine-C), 136.9 (thiazole-C), 135.2 (pyridine-C), 129.5 (pyridine-C), 127.9 (pyridine-C), 125.3 (thiazole-C), 112.3 (pyridine-C), 71.2 (Boc-CH), 59.7 (CH_2), 58.9 (CH_2), 51.1 (piperazine-2C), 39.7 (piperazine-2C), 29.3 (CH_3-3C); MS (ESI) *m/z* 449.1$[M + H]^+$; *Anal*. Calcd % for $C_{20}H_{28}N_6O_4S$: C 53.56; H 6.29; N 18.74. Found: C 53.45; H 6.31; N 18.63.

Following the same procedure as illustrated for **11a** the other Boc protected 2-aminothiazoles **11b-e** were prepared by coupling **9** with corresponding amino pyridines **10b-e**.

3.1.5. 2-amino-N-(5-(4-(2-hydroxyethyl)piperazin-1-yl)pyridin-2-yl)thiazole-5-carboxamide (12a)

To the stirred solution of trifluoroacetic acid (22.0 mL), compound **11a** (2.75 g, 0.00613) was added at 0-5°C. The reaction mass warmed to 25-30°C and stirred for 2 hours. The reaction mass was concentrated and charged with water (25.0 mL) and 10% $NaHCO_3$ solution (50.0 mL) and stirred for 3 hours. Filtered the solid and washed the wet cake with 10% acetone water solution, obtained the compound **12a** as a pale yellow solid (1.9 g). Further recrystallization in 85% Methanol/water (160.0 mL), obtained the compound **12a** (1.82 g, 86%) as an off white solid.

^{1}H NMR spectrum (400 MHz, DMSO-*d*6), δ, ppm (*J*, Hz): 10.91 (bs, 1H, amide-N<u>H</u>), 8.26 (s, 1H, thiazole-H), 7.74 (d, 1H, pyridine-H), 7.30-7.23 (m, 2H, pyridine-H), 7.12 (bs, 2H, NH_2), 4.32 (t, 1H, -O<u>H</u>), 3.59-3.63 (m, 6H, -$CH_2C\underline{H_2}OH$ & piperazine-CH_2), 2.45-2.41 (m, 6H, -$CH_2C\underline{H_2}OH$ & piperazine-CH_2); ^{13}C NMR spectrum (100 MHz, DMSO-*d*6), δ, ppm: 165.3 (thiazole-C), 159.7 (amide-C), 141.3 (pyridine-C), 138.9 (thiazole-C), 134.6 (pyridine-C), 131.9 (pyridine-C), 129.4 (pyridine-C), 127.6 (thiazole-C), 115.3 (pyridine-C), 60.8 (CH_2), 59.3 (CH_2), 51.6 (piperazine-2C), 39.1 (piperazine-2C); MS (ESI) *m/z* 349.1 $[M + H]^+$; *Anal*. Calcd % for $C_{15}H_{20}N_6O_2S$: C 51.71; H 5.79; N 24.12. Found: C 51.68; H 5.77; N 24.17.

Following the same procedure as depicted for **12a** the other derivatives **12b-e** were prepared from the corresponding Boc protected 2-aminothiazoles **11b-d**.

3.1.6. N-(5-((5-(4-(2-hydroxyethyl)piperazin-1-yl)pyridin-2-yl)carbamoyl)thiazol-2-yl)-5-methylisoxazole-4-carboxamide (13a)

5-methylisoxazole-4-carboxylic acid **5** (1.0 g, 0.00786 mol) and catalytic amount of DMF (0.05) were added to the stirred solution of thionyl chloride (3.0 mL) at 25-30°C [46]. Reaction mass was heated under reflux for 3 hours and concentrated. The residue was distilled through a fractionating column by collecting the acid chloride (0.59 g, 52%) at 68-71°C in 11 mm Hg vacuum. Acid chloride fraction (0.59 g) was taken in toluene (12.0 mL) and cooled to 5-10°C and charged with 5-amino-N-(5-(4-(2-hydroxyethyl)piperazin-1-

yl)pyridin-2-yl)thiazole-2-carboxamide **12a** (1.41 g, 0.0040 mol). Pyridine (1.8 mL, 0.0223) added slowly to the stirred reaction mass at 5-10°C, over 1-2 minutes. Reaction mass was heated to reflux and stirred for 3 hours and concentrated. The residue was purified by column chromatography using methanol/dichloromethane/triethyl amine eluent, yielded the target compound **13a** as an off white solid (1.27 g, 69%).

MR: 265-267°C; ^{1}H NMR spectrum (400 MHz, DMSO-*d*6), δ, ppm (*J*, Hz): 11.47 (bs, 1H, amide-NH), 9.88 (s, 1H, amide-NH), 8.49 (s, 1H, thiazole-H), 8.22 (s, 1H, isoxazole-H), 7.39 (t, 1H, pyridine-H), 7.30-7.23 (m, 2H, pyridine-H), 4.46 (t, 1H, -OH), 3.54-3.51 (m, 6H, -CH_2CH_2OH & piperazine-CH_2), 2.48-2.46 (m, 4H, piperazine-CH_2), 2.44-2.42 (m, 2H, -CH_2CH_2OH), 2.40 (s, 3H, ioxazole-CH_3); ^{13}C NMR spectrum (100 MHz, DMSO-*d*6), δ, ppm: 165.1 (isoxazole-C), 162.5 (amide-C), 162.3 (thiazole-C), 159.9 (amide-C), 156.9 (isoxazole-C), 140.8 (pyridine-C), 138.8 (thiazole-C), 133.5 (pyridine-C), 132.4 (pyridine-C), 129.0 (pyridine-C), 128.1 (thiazole-C), 126.9 (pyridine-C), 98.8 (isoxazole-C), 60.1 (CH_2), 58.5 (CH_2), 52.7 (piperazine-2C), 43.6 (piperazine-2C), 18.29 (CH_3); MS (ESI) *m/z* 458.1 [M + H] $^+$; *Anal*. Calcd % for $C_{20}H_{23}N_7O_4S$: C 52.51; H 5.07; N 21.43. Found: C 52.49; H 5.12; N 21.45.

Following the same procedure as illustrated for **13a** the other 2-aminothiazoles appended 5-methylisoxazoline and pyridine-piperazine derivatives **13b-e** were prepared by coupling **5** with corresponding amines **12b-e**. The physical, spectral, and analytical data for these compounds are mentioned as follows.

3.1.6.1. 5-methyl-N-(5-((5-(4-(2-morpholinoethyl)piperazin-1-yl)pyridin-2-yl)carbamoyl)thiazol-2-yl)isoxazole-4-carboxamide (13b)

(1.25 g, 71%); MR: 279-281°C; ^{1}H NMR spectrum (400 MHz, DMSO-*d*6), δ, ppm (*J*, Hz): 11.49 (bs, 1H, amide-NH), 9.85 (s, 1H, amide-NH), 8.71 (s, 1H, thiazole-H), 8.24 (s, 1H, isoxazole-H), 7.43 (t, 1H, pyridine-H), 7.31-7.24 (m, 2H, pyridine-H), 3.55-3.52 (m, 6H, morpholine & piperazine-CH_2), 3.34-3.36 (m, 2H, piperazine-CH_2), 3.15-3.11 (m, 4H, piperazine-CH_2), 2.49-2.45 (m, 4H, morpholine-CH_2), 2.43 (t, 2H, methylene -CH_2), 2.40 (t, 2H, methylene -CH_2), 2.15 (s, 3H, -CH_3); ^{13}C NMR spectrum (100 MHz, DMSO-*d*6), δ, ppm: 165.1 (isoxazole-C), 162.6 (amide-C), 162.4 (thiazole-C), 159.9 (amide-C), 156.9 (isoxazole-C), 140.9 (pyridine-C), 138.8 (thiazole-C), 133.5 (pyridine-C), 132.4 (pyridine-C), 129.0 (pyridine-C), 128.1 (thiazole-C), 127.0 (pyridine-C), 97.0 (isoxazole-C), 66.4 (morpholine-2C), 61.3 (morpholine-2C), 60.23 (methylene-1C), 58.5 (methylene-C), 52.7 (piperazine-2C), 43.6 (piperazine-2C), 18.3 (CH_3); MS (ESI) *m/z* 527.1 [M + H] $^+$; *Anal*. Calcd % for $C_{24}H_{30}N_8O_4S$: C 54.74; H 5.74; N 21.28. Found: C 54.71; H 5.69; N 21.31.

3.1.6.2. 5-methyl-N-(5-((5-(4-methylpiperazin-1-yl)pyridin-2-yl)carbamoyl)thiazol-2-yl)isoxazole-4-carboxamide (13c)

(1.31 g, 70%); MR: 243-246°C; ^{1}H NMR spectrum (400 MHz, DMSO-*d*6), δ, ppm (*J*, Hz): 11.15 (bs, 1H, amide-NH), 9.90 (s, 1H, amide-NH), 8.69 (s, 1H, thiazole-H), 8.23 (s, 1H, isoxazole-H), 7.40-7.38 (m, 1H, pyridine-H), 7.29-7.23 (m, 2H, pyridine-H), 2.86-2.80 (m, 4H, piperazine-CH_2), 2.79-2.78 (m, 4H, piperazine-CH_2), 2.40 (s, 3H, ioxazole-CH_3), 2.23 (s, 3H, N-CH_3); ^{13}C NMR spectrum (100 MHz, DMSO-*d*6), δ, ppm: 165.6 (isoxazole-C), 163.0 (amide-C), 162.7 (thiazole-C), 160.4 (amide-C), 157.4 (isoxazole-C), 154.3 (pyridine-C), 141.3 (thiazole-C), 139.2 (pyridine-C), 134.0 (pyridine-C), 132.9 (pyridine-C), 129.4 (thiazole-C), 128.6 (pyridine-C), 99.5 (isoxazole-C), 61.1 (piperazine-2C), 43.7 (piperazine-2C), 38.5 (N-CH_3), 16.0 (CH_3); MS (ESI) *m/z* 428.1 [M + H] $^+$; *Anal*. Calcd % for $C_{19}H_{21}N_7O_3S$: C 53.38; H 4.95; N 22.94. Found: C 53.29; H 4.87; N 23.03.

3.1.6.3. N-(5-((5-(4-acetylpiperazin-1-yl)pyridin-2-yl)carbamoyl)thiazol-2-yl)-5-methylisoxazole-4-carboxamide (13d)

(1.37 g, 75%); MR: 257-259°C; ^{1}H NMR spectrum (400 MHz, DMSO-*d*6), δ, ppm (*J*, Hz): 11.31 (bs, 1H, amide-NH), 9.89 (s, 1H, amide-NH), 8.44 (s, 1H, thiazole-H), 8.22 (s, 1H, isoxazole-H), 7.41-7.39 (m, 1H, pyridine-H), 7.30-7.24 (m, 2H, pyridine-H), 3.44 (m, 4H, piperazine-CH_2), 2.75-2.73 (m, 4H, piperazine-CH_2), 2.40 (s, 3H, ioxazole-CH_3), 2.24 (s, 3H, acetyl-CH_3); ^{13}C NMR spectrum (100 MHz, DMSO-*d*6), δ, ppm: 167.0 (isoxazole-C), 165.1 (acetyl-C), 162.6 (amide-C), 162.5 (thiazole-C), 159.9 (amide-C), 156.9 (isoxazole-C), 140.8 (pyridine-C), 138.8 (thiazole-C), 133.5 (pyridine-C), 132.4 (pyridine-C), 129.0 (pyridine-C), 128.1 (thiazole-C), 127.0 (pyridine-C), 98.4 (isoxazole-C), 45.2 (piperazine-2C), 44.7 (piperazine-2C), 25.6 (acetyl-CH_3), 18.3 (CH_3); MS (ESI) *m/z* 456.1 [M + H] $^+$; *Anal*. Calcd % for $C_{20}H_{21}N_7O_4S$: C 52.74; H 4.65; N 21.53. Found: C 52.69; H 4.69; N 21.61.

3.1.6.4. N-(5-((5-(4-(cyclopropanecarbonyl)piperazin-1-yl)pyridin-2-yl)carbamoyl)thiazol-2-yl)-5-methylisoxazole-4-carboxamide (13e)

(1.31 g, 73%); MR: 259-261°C; ^{1}H NMR spectrum (400 MHz, DMSO-*d*6), δ, ppm (*J*, Hz): 11.51 (bs, 1H, amide-NH), 9.91 (s, 1H, amide-NH), 8.75 (s, 1H, thiazole-H), 8.25 (s, 1H, isoxazole-H), 7.47-7.41 (m, 1H, pyridine-H), 7.35-7.31 (m, 2H, pyridine-H), 3.56-3.51 (m, 4H, piperazine-CH_2), 2.89-2.85 (m, 4H, piperazine-CH_2), 2.40 (s, 3H, ioxazole-CH_3), 1.48-1.46 (m, 1H, cyclopropyl-CH), 0.89-0.79 (m, 4H, cyclopropyl-CH_2); ^{13}C NMR spectrum (100 MHz, DMSO-*d*6), δ, ppm: 170.4 (isoxazole-C), 165.6 (acetyl-C), 163.0 (amide-C), 162.8 (thiazole-C), 160.4 (amide-C), 157.4 (isoxazole-C), 141.3 (pyridine-C), 139.3 (thiazole-C), 134.0 (pyridine-C), 132.9 (pyridine-C), 129.4 (pyridine-C), 128.6 (thiazole-C), 127.4 (pyridine-C), 98.6 (isoxazole-C), 60.4 (piperazine-2C), 58.6 (piperazine-2C), 26.0 (CH_3), 18.7 (cyclopropyl-CH), 13.1 (cyclopropyl-2C); MS (ESI) *m/z* 482.0 [M + H] $^+$; *Anal*. Calcd % for $C_{20}H_{23}N_7O_4S$: C 54.88; H 4.81; N 20.36. Found: C 54.85; H 4.86; N 20.43.

4. SCREENING OF ANTIPROLIFERATIVE ACTIVITY

Antiproliferative activity of the final compounds **13a-e** has been evaluated *in vitro* against four tumor cell lines, by

using following MTT assay method [68] (MTT; 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide). All cancer cell lines (MCF-7, HCT-116, Jurkat and THP-1) were cultured in Roswell Park Memorial Institute (RPMI) 1640 Medium with heat inactivated Fetal Bovine Serum (FBS) (10%) and 1% Pen/Strep in a humidified atmosphere of 95% air and 5% CO_2 at 37°C for 72 hours. Primarily, each test compound of 10.0 mg was dissolved in 1.0 mL of DMSO and further diluted to obtain required experimental stock concentrations from 0.01 to 0.1% and obtained the final volume of 250.0 mL. Briefly, cells were seeded in 96 well microtiter plates at a density of 2 X 10^6 cells per well with 0.1 mL of RPMI medium. After 72 hours the inhibition of cell proliferation was determined by treating cells against tested compounds **13a-e** at different concentrations and the cell viability was assessed after 48 h, by adding 10 µL per well of MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide). The plates were incubated at 37°C for additional 5 hours. Discarded the medium and the obtained formazan blue in the cells, was dissolved with DMSO for 10 minutes with gentle agitation. The rate of colour production was measured at 570 nm in a spectrophotometer. The assay was done in triplicate and average values were recorded.

The concentrations that cause 50% of cell proliferation inhibition, IC_{50} values were determined for each compound from a regression equation, a plot of drug concentration versus percentage loss of viability and the results were summarized in Table **1**.

CONCLUSION

In conclusion, we have successfully achieved two important aspects of this work. One is a highly efficient and milder protocol for the synthesis of 2-aminothiazoles bearing 5-methylisoxazoline and pyridine-piperazine scaffolds **13a-e** in good yields. The second one is the coupling of different pharmacophores, each endowed with diverse biological properties resulted in hybrid molecules with significant antitumor activity. Interestingly, all these new compounds are active and showed moderate to good activity against tested cancel cell lines. The observed activity profile suggested that the presence of electron donating functional group enhanced the anticancer activity compared to electron withdrawing groups on piperazine heterocyclic. In conclusion, based on the observed antiproliferative activity data compounds **13a** and **13b** could be further developed in to potential anticancer drugs.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article. Spectra of key compounds were attached as a separate file.

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