DESIGN, SYNTHESIS AND ANTI-CANCER ACTIVITY STUDIES OF SOME NOVEL 1,2,4 TRIAZOLE PYRIMIDINE DERIVATIVES

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Abstract

The quest for and development of combinational chemotherapeutic medicines with various modes of action and low side effects is an essential element of cancer treatment. Aside from developing wholly novel drugs with chemical properties that are obviously distinct from current ones, another strategy involves combining two or more pharmacophores into a single molecule. The method for making several 1,2,4-triazole derivatives is detailed in the scheme. By 5-Mercapto-3-pyrimidinyl-1,2,4 triazole with various substituted benzyl halides, a total of 7 distinct 1,2,4-triazole derivatives were obtained. Combustion Analysis, TLC, IR, MS, and other methods were used to confirm the physical and analytical properties of the newly synthesized 1,2,4- triazole derivatives. Compounds TP1-TP7 exhibit IC50 values ranging from 41.12 M to 61.11 M, with compound TP6 having the greatest activity against the murine melanoma (B16F10) cell line. TP 6 was discovered to have more strong anticancer action in anti-cancer screening. As a result, we believe that the findings of this study may pave the way for the creation of anticancer drugs with high efficacy and fewer side effects. As a result, 1,2,4-triazol-3-pyrimidine-based compounds may have a broad anticancer range and serve as a potential lead molecule in different malignancies.

Keywords: Synthesis, heterocyclic, 1,2,4 Triazole, Pyrimidine, derivatives, Cancer cell line

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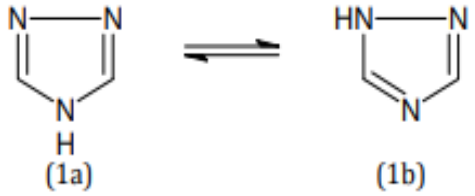
1. Introduction

Heterocycles are an important class of compounds, making up more than half of all known organic compounds [1]. Heterocycles are present in a wide variety of drugs, most vitamins, many natural products, biomolecules, and biologically active compounds, including antitumor, antibiotic, anti-inflammatory, antidepressant, antimalarial, anti-HIV, antimicrobial, antibacterial, antifungal, antiviral, antidiabetic, herbicidal, fungicidal, and insecticidal agents. Also, they have been frequently found as a key structural unit in synthetic pharmaceuticals and agrochemicals [2]. Some of these compounds exhibit a significant solvatochromic, photochromic, and biochemical-luminescence properties. Most of the heterocycles possess important applications in materials science such as dyestuff, fluorescent sensor, brightening agents, information storage, plastics, and analytical reagents. In addition, they have applications in supra molecular and polymer chemistry, especially in conjugated polymers. The medicinal chemists, the true utility of heterocyclic structures is the ability to synthesize one library based on one core scaffold and to screen it against a variety of different receptors, yielding several active compounds. Almost unlimited combinations of fused heterocyclic structures can be designed, resulting in novel polycyclic frameworks with the most diverse physical, chemical and biological properties. Therefore, efficient methodologies resulting in polycyclic structures from biologically active heterocyclic templates are always of interest to both organic and medicinal chemists [3]. The primary objective of medicinal chemistry is the design and discovery of new drug compounds.

1.21,2,4 Triazole and its derivatives

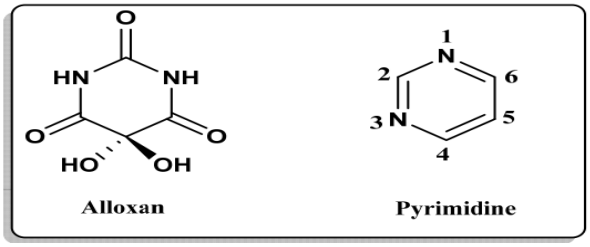
In the last few decades, the chemistry of 1, 2, 4-triazoles and their fused heterocyclic derivatives have received considerable attention owing to their synthetic and effective biological importance. 1, 2, 4- triazole moietiy has been incorporated into a wide variety of therapeutically interesting drug candidates including antifungal, antibacterial, analgesics and antiinflammatoriy, antineoplastic, antiviral, sedatives, anxiolytics, anti-convulsants, antimigraine, antihistaminics, CNS stimulants and other activities. [4- 17]



(1a) (1b)

1.3 Pyrimidine and its derivatives

Pyrimidines (“m-diazine”) were known as the breakdown products of uric acid. The first pyrimidine derivative to be isolated was alloxan (5,5-dihydroxypyrimidine-2,4,6(1H,3H,5H)-trione) in 1818 by Brugnatelli, oxidizing uric acid with nitric acid. [18] Pyrimidines are the heterocyclic aromatic compounds similar to benzene and pyridine containing two nitrogen atoms at positions 1 and 3 of thesix-membered rings. Pyrimidine has one axis of symmetry about the 2-5 axis; it has three different pairs of bond lengths and four different bond angles. Accordingly, in 1 H and 13CNMR spectra, the 1 H and 13C nuclei are found at three different chemical shifts. Symmetry is lost by unequal substitution at the 4 or/and 6 position. [19] Heterocyclic containing pyrimidine moiety are of great interest because they constitute an important class of natural and non-natural products, many of which exhibit useful biological activities and clinical applications.[20] Heterocyclic compounds containing pyrimidine moiety are gaining the focus in recent research due to their wide range of biological activities such as anti-inflammatory, antioxidant, antimicrobial, antitumor, antiviral, antidepressant, antiplatelet, antihypertensive and herbicidal. Additionally, thienopyrimidine-containing compounds are found in many pharmaceutical medications and as natural products.



Alloxan Pyrimidine

2. Materials and methods

2.1 Chemicals and equipment’s

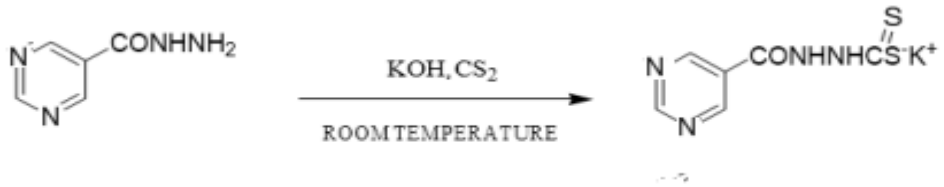
All the chemicals were purchased from sigma Aldrich U.S.A. Analytical TLC was performed on Precoated sheets of silica gel G/UV-254 of 0.2mm thickness (Macherey-Nagel, Germany) using analytical grade solvent and visualized with iodine spray (10% w/w I2 in silica gel) or UV light. We also used bioinformatics tools, biological databases like PDB (Protein Data Bank) and software’s like Autodock and ACD ChemSketch. The PDB (Protein Data Bank) is the single worldwide archive of Structural data of Biological macromolecules, established in Brookhaven National Laboratories (BNL). It contains Structural information of the macromolecules determined by X-ray crystallographic, NMR methods etc.

Melting point was determined in capillary tubes and is uncorrected. IR spectra were taken as KBr pellets for solids on Perkin Elmer Spectrum FT-IR. 1H NMR (400MHz) and 13C NMR (100 MHz) spectra were recorded in DMSO-d6 solution with TMS as an internal standard on Bruker instrument. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet). Coupling constant (J) is given in hertz. Mass spectra were recorded on a thermo Finnigan LCQ Advantage MAX 6000 ESI spectrometer.

2.2 General procedure for the synthesis of title compounds

Step I: Synthesis of Pyrimidine-5-dithiocarbazide potassium (I)

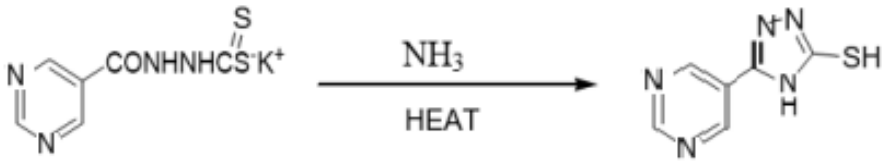
In 200 mL 100% ethanol, a solution of 8.4gm (0.15M) potassium hydroxide, 13.7gm (0.10M) pyrimidyl-2-carbohydrazide, and 11.4gm (0.15M) carbon disulfide was produced. The mixture was then stirred for 12-16 hours. It was then dried at 65°C after being diluted with 200 cc of dry ether. The salts were produced as stated above and yielded a practically quantitative yield, allowing them to be used without additional purification.



Pyrimidine-5-carbohydrazide **Pyrimidine-5- dithiocarbazide potassium (I)**

Step II: Synthesis of 5-Mercapto-3-pyrimidinyl-1,2,4 triazole (II)

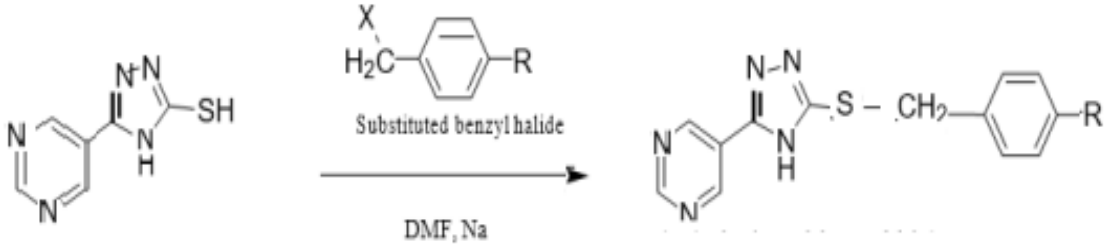
A suspension of I (24gm, 0.096M) in 20 ml (0.864M) Ammonia and water 40 ml, was refluxed with stirring for 3 to 4 hours. The resulting mixture was poured in ice cold water (100 ml). Obtained white precipitated was acidified with concentrated HCl, filtered and washed with cold water.



Pyrimidine-5- dithiocarbazide potassium **5-Mercapto-3-pyrimidinyl-1,2,4 triazole (II)**

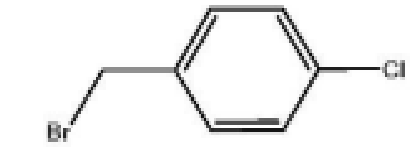
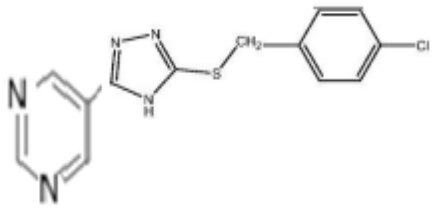
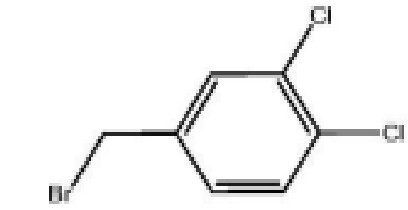
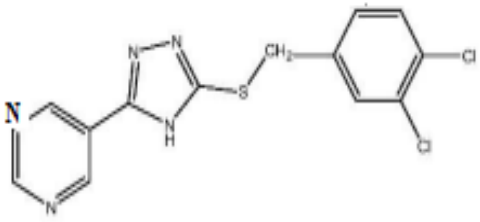
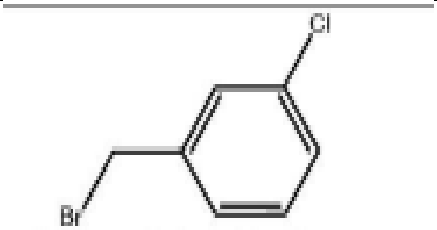
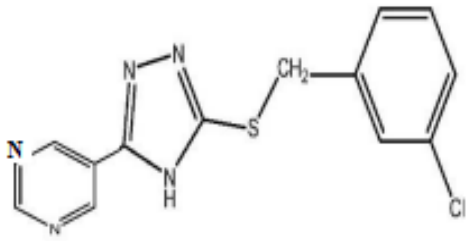
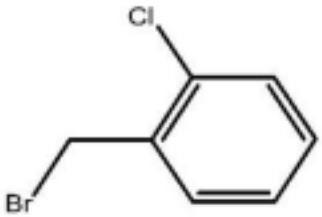
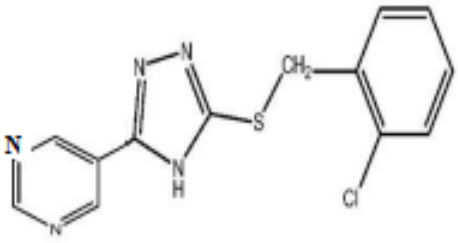
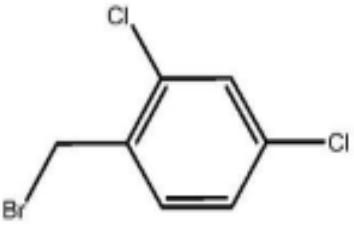
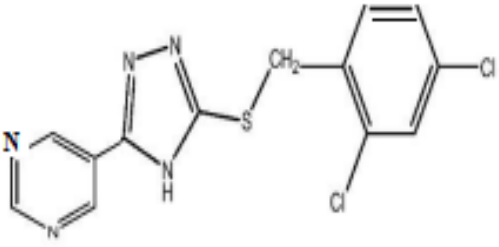
Step III: Synthesis of 5-(4-substituted benzylthio)-4H-1,2,4 triazol-3-yl) pyrimidine (III)

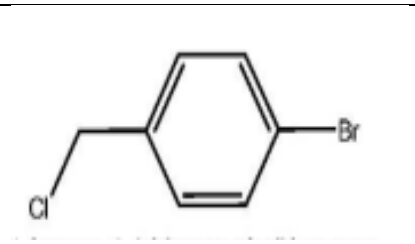
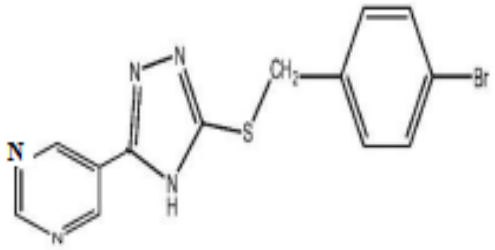
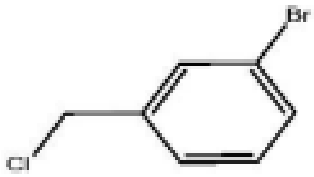
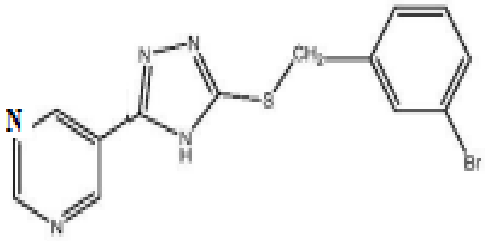
A mixture of II (0.006M), (0.69gm, 6M) in dry N,N-dimethyl formamide was added to a solution of sodium(0.14gm, 6M) in dry methanol. After 10 minutes of stirring at room temperature, benzyl halide (6M) was added. The resultant suspension was stirred with CaCl2 guar tube at room temperature 1-23 hours. The completion of reaction



5-Mercapto-3-pyrimidinyl-1,2,4 triazole5-(4-substituted benzylthio)-4H-1,2,4 triazol-3-yl) pyrimidine

Table1: List of synthesized 1,2,4-triazole derivatives in scheme with their different substituted benzyl halides used for the synthesis

Synthesized Compound Code	Substituents (Substituted benzyl halides)	1,2,4 Triazole Pyrimidine derivatives
TP1	 1-(bromomethyl)-4-chlorobenzene	
TP2	 4-(bromomethyl)-1,2-dichlorobenzene	
TP3	 1-(bromomethyl)-3-chlorobenzene	
TP4		
TP5		

TP6		
TP7		

2.3 Anti-Cancer Activity Screening (MTT Assay)

Each well of the first plate had the test ingredients removed. Then 50 l of MTT reagent (5 mg/ml) was added and incubated in the CO2 incubator for 2 hours at 37 °C. After discarding the MTT solution, 100 l of isopropanol was added. To dissolve the formations of purple crystal formazan, the plates were shaken. A microplate reader was used to measure the absorbance at a wavelength of 570 nm. The anticancer efficacy of several 1,2,4 triazole pyridine derivatives produced in vitro was tested using the cell viability assay technique (Prakash et al., 2017). Murine melanoma (B16F10) cancer cell lines were employed to investigate in vitro anticancer activities. The National Center for Cell Science in Pune provided cancer cell lines. The cells were plated or cultured for 24 hours in 96-multiwell plates (104 cells/well). Before being tested for anti-cancerous properties, all of the produced compounds were dissolved in dimethyl sulphoxide. All of the chemicals were applied to the cell monolayer in various concentrations. The vitality of the cells was tested in triplicate for 48 hours using the MTT assay in two distinct dosages of the produced compounds (100M and 10M). The MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide) test was used to determine the vitality of the cells. In this experiment, the MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide] was employed at a concentration of 5 mg/mL.

The anticancer efficacy of these drugs was determined using the IC50 method (the concentration that causes a 50 percent reduction of the cell growth). The vitality of the cells was tested in triplicate for 48 hours using the MTT assay in two distinct dosages of the produced compounds (100M and 10M). The MTT reagent [3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was used at a concentration of 5 mg/mL in the test.

3. Results

3.1 Physical and spectral data of synthesized 1,2,4-triazole derivatives.

The synthesis of 3-(5-(substituted-benzylthio)-4H-1,2,4-triazol-3-yl) pyrimidine derivatives is described in this paper. For the synthesis of chemicals, we employed pyidyl-2-carbohydrazide as a starting material. The method for making several 1,2,4- triazole derivatives is detailed in the scheme. By treating 5-mercapto-3-pyridyl-1,2,4-triazole with various substituted benzyl halides, a total of 7 distinct 1,2,4-triazole derivatives were obtained. Combustion Analysis, TLC, IR, MS, and other methods were used to confirm the physical and analytical properties of the newly synthesized 1,2,4- triazole derivatives.

Table 1: Physical and analytical data of Synthesized derivatives

Derivatives	Mol. formula	Mol. Weight	Melting point	Appearance	Solubility
TP1	$C_{14}H_{11}ClN_4S$	302	207 ºC	White Solid	Ethanol
TP2	$C_{14}H_{10}C_{l2}N_4S$	337	250 ºC	Creamy White Solid	DMF
TP3	$C_{14}H_{11}ClN_4S$	302	207ºC	Light Brown Solid	Ethanol
TP4	$C_{14}H_{11}ClN_4S$	302	207 ºC	Brown Solid	Ethanol
TP5	$C_{14}H_{10}C_{l2}N_4S$	337	250 ºC	Yellow Solid	Ethanol
TP6	$C_{14}H_{11}BrN_4S$	347	537ºC	White Solid	Water
TP7	$C_{14}H_{11}BrN_4S$	347	237 ºC	Brown Solid	Water

Table 2: Spectroscopic data of synthesized derivatives

Derivatives	IR(cm^{-1}) (KBr)	MASS (m/e)
TP1	2978.85(Ar-C-H str), 1630.41(Ar-C=C str), 1154.87(Ar–C–C str), 1595.76(C=Nstr), 1252.41(-C-N-str), 657.11(-C- S str), 735.83(C-Cl str)	301+
TP2	3088.25(Ar-C-H str), 1610.41(Ar-C=C str), 1173.78(Ar–C–C str), 1542.56(C=Nstr), 1200.41(-C-N-str), 647.12(-C-S str), 717.33(C-Cl str)	336+
TP3	3108.64(Ar-C-H str), 1684.40(Ar-C=Cstr), 1112.08 (Ar–C–C str), 1521.24(C=Nstr), 1221.56(-C-N-str), 612.41.11(-C-S str), 712.98(C-Cl str)	301+
TP4	2968.46(Ar-C-H str), 1598.35.47(ArC=C str),1175.47 (Ar–C–C str), 1500.36(C=Nstr), 1285.47(-C-N- str), 611.81(-C-Sstr), 765.79(C-Cl str)==	301+
TP5	2912.56(Ar-C-H str), 1623.56(Ar-C=C str), 1121.67(Ar–C–C str), 1521.12(C=Nstr), 1213.87(-C-N-str), 641.78(-C-S str), 735.13(C-Cl str)	336+
TP6	2890.45(Ar-C-H str), 1611.76(Ar-C=C str), 1108.45(Ar–C–C str), 1541.10(C=Nstr), 1286.45(-C-N- str), 698.34(-C-S str), 812.12(C-Br str)	346+
TP7	2890.45(Ar-C-H str), 1611.76(Ar-C=C str), 1108.45(Ar–C–C str), 1541.10(C=Nstr), 1286.45(-C-N-str), 698.34(-C-S str), 812.12(C-Br str)	346+

3.2 In vitro Anti-cancer activity:

The synthesized 1,2,4 triazole pyrimidine derivatives were tested for their in vitro anticancer activities against murine melanoma (B16F10) using the MTT assay. Table 5.1 summarized the test findings, which were reported as IC50 (M). The IC50 value is the average of three separate experiments and indicates the concentration of a substance that inhibits cell growth by 50% after 48 hours of incubation. Because the results indicate that all of the tested compounds have promise, they were chosen for the measurement of IC50 values, or the concentration required inhibiting cancer cells by 50% when treated with manufactured compounds.

Table 3: In vitro anticancer activity (IC50) of the synthesized compounds against cancer cell lines

Sr. No.	Compounds	B16F10 (µM)
1	TP1	58.50
2	TP2	52.35
3	TP3	57.70
4	TP4	50.25
5	TP5	61.11
6	TP6	41.12
7	TP7	45.60

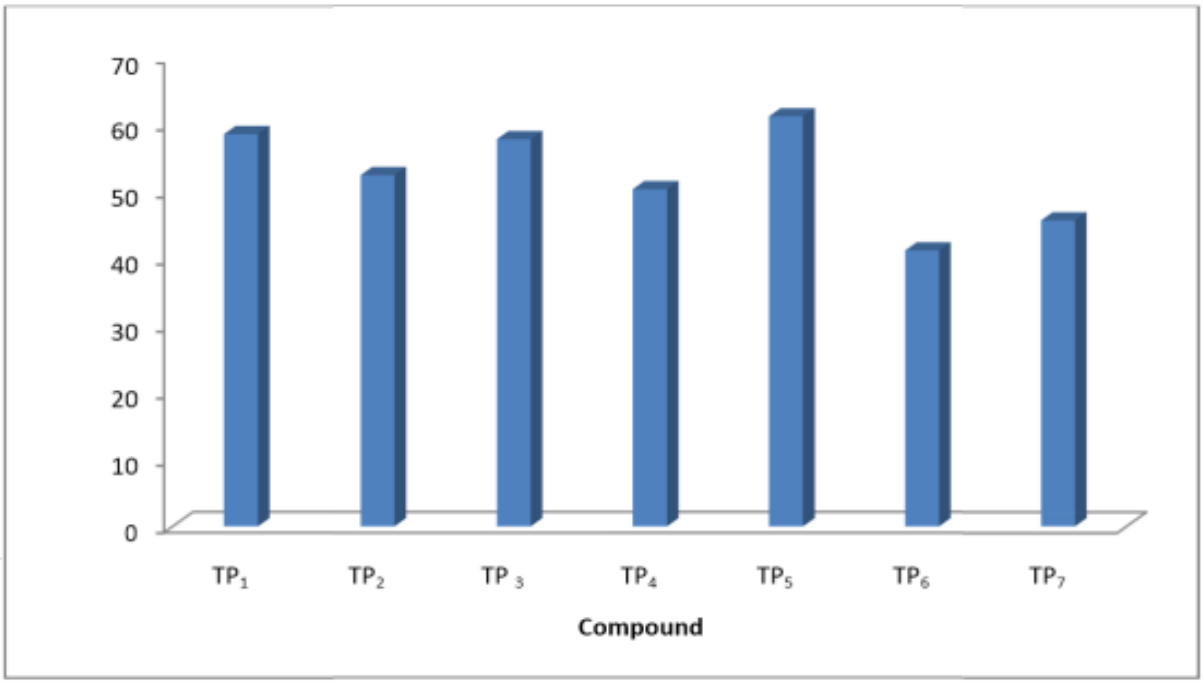


Figure 1: Graph for In vitro anticancer activity (IC50) of the compounds against cancer cell lines

4. Discussion

The quest for and development of combinational chemotherapeutic medicines with various modes of action and low side effects is an essential element of cancer treatment. Aside from developing wholly novel drugs with chemical properties that are obviously distinct from current ones, another strategy involves combining two or more pharmacophores into a single molecule. As a result, a single molecule having many pharmacophores, each with a unique mechanism of action, might be useful in cancer treatment. These combined pharmacophores may target the active site of several targets, allowing for drug resistance to be overcome and undesired side effects to be reduced.

Because of their synthetic and biological value, 1,2,4-Triazole-Pyrimidine hybrids and their fused heterocyclic derivatives have garnered great interest among the wide variety of heterocyclics being investigated for the expansion of novel components in medicinal chemistry. The triazole is an appealing bridge group because it may connect two pharmacophores to form novel bifunctional compounds and is nearly hard to hydrolyze, oxidize, or reduce.

The reaction of pyrimidine-5-carbohydrazide with carbon disulfide to create Pyrimidine-5-dithiocarbazide potassium yielded 1,2,4-triazole-pyrimidine hybrid compounds coupled with substituted benzyl group. After that, it was treated with an ammonia solution to produce 5-mercapto-substituted Hybrid of 1,2,4-triazole and pyrimidine. Finally, various benzyl derivatives were reacted with this to form a range of 1,2,4-Triazole-Pyrimidine hybrid compounds. The synthesis of 3-(5-(substituted -benzylthio) -4H-1,2,4- triazol -3-yl) pyrimidine derivatives is described in this research.

5. Conclusion

All the synthesized derivatives have anti-cancer properties. TP 6 was discovered to have more strong anticancer action in anti-cancer screening. As a result, we believe that the findings of this study may pave the way for the creation of anticancer drugs with high efficacy and fewer side effects. As a result, 1,2,4-triazol-3-pyrimidine-based compounds may have a broad anticancer range and serve as a potential lead molecule in different malignancies. Our findings show that 1,2,4-triazol-3-pyrimidine derivatives produced in the lab had greater anticancer activity in murine melanoma cell lines. As a result, the 1,2,4-triazol-3-pyrimidine compounds produced should be evaluated further in murine melanoma and other cancer cell lines.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors (Dr. Anup K Chakraborty, Dr. Snehal S. Manekar, Sushma M. Rathod, Manjusha U

Kakde, Krutika Surendra Sonar) declare no conflict of interest

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