The Pharmaceutical and Chemical Journal, 2021, 8(2):68-72

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Synthesis, Characterization and Anticancer activity of fused Pyrazolo Pyrimido benzothiazoles

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Abstract The compound 3-amino- 4-imino 6, 9 dimethyl- pyrazolo-[3, 4-*e*] pyrimido [2, 3-*b*] [1, 3] benzothiazole(IV) was obtained by the reaction of 3-Cyano -6, 9-dimethyl -2-methylthio -4-imino -4*H*-pyrimido [2, 1-*b*] [1, 3] benzothiazole (III) with Hydrazine hydrate (80%) in presence of anhydrous K_2CO_3 in DMF as solvent, and screened their *in-vitro* anticancer activity by the use of Developmental therapeutic program (DTP) as a computational technique. DTP selection process helps to design and study the anticancer agent, this research facility developed by National Cancer Institute, Maryland, USA. Selections of samples were carried by DTP process, screened their *in-vitro* anticancer activity against 60 human cancer cell lines, compounds exhibited better anticancer activity.

Keywords Pyrazolo Pyrimido Benzothiazoles, Anticancer activity, NCI Maryland USA.

1. Introduction

A survey of literature reveals that, pyrimidine, iminopyrimidine [1-3] and fused benzothiazole hetrocycles [4] exhibit effective pharmacological activity, such pharmacophore possesses better anticancer activity [5]. The development of drug has challenge to every researcher in the field of chemistry and pharmacy. The structure activity relationship (SAR), Toxicity risk assessment, PASS, molecular modeling [6] gives preliminary information about the target molecule that would be helpful for drug design. To investigate the anticancer activity of chemical entities, National Cancer Institute, Maryland, USA has developed program, known as Developmental Therapeutic program [7], where, the selected samples are screened against human 60 cancer cell lines. The submission of samples was carried out online to www.dtp.nci.nih.gov , hence it has the best computational tool for screening of anticancer activities.

In the present work, prepared fused substituted pyrimido benzothiazole and pyrazolo pyrimido benzothiazole after DTP selection process by NCI, Maryland USA. These samples were screened against human 60 cancer cell lines, and result obtained in the form of mean graph. The compound III (NSC code- **764277**) exhibited maximum in-vitro anticancer activity than compound IV (NSC code- **765944**) as shown in Table-01.

2. Materials and Methods

2.1. Experimental Section

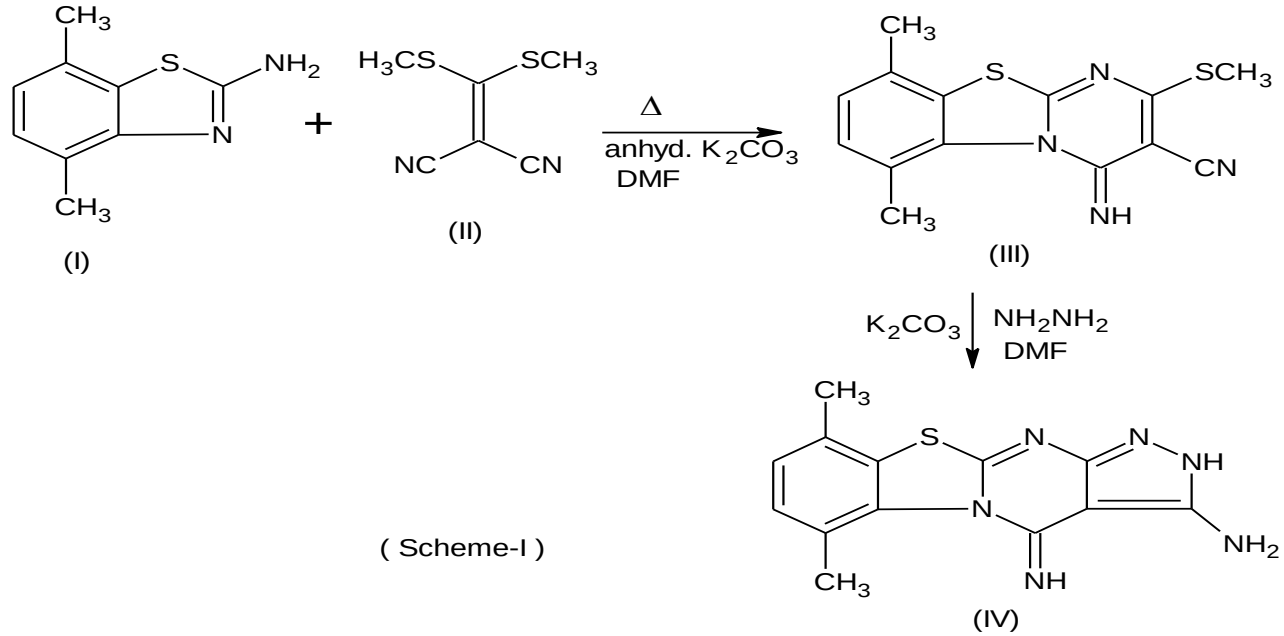
Melting points of all compounds were recorded in open capillary tubes and are uncorrected. All the reactions were monitored by TLC. IR spectra were recorded on SHIMADZU-FTIR spectrophotometer by using KBr pellets, ^{1}H-

NMR spectra were recorded on FT Gemini 300 MHz spectrometer using TMS as the internal standard. Mass spectra were recorded on GC-MS spectrometer using EI technique at 70 ev.

2.2. General Method:

1. **Synthesis of 3-cyano -6, 9-dimethyl -2-methylthio -4-imino -4*H*-pyrimido [2, 1-*b*] [1, 3] benzothiazole (III):** A mixture of 2- amino 4, 7 dimethyl benzothiazole [0.01mole], 3,3'- bis methylthio methylene malononitrile [0.01 mole] was refluxed for 6-7 hours in presence of anhydrous K_2CO_2 in DMF as solvent. Reaction mixture was monitored by TLC, then poured in ice cold water, solid get separated and recrystallised from ethanol and DMF.
2. **Synthesis of 3-amino- 4-imino 6, 9 dimethyl- pyrazolo-[3, 4-*e*] pyrimido [2, 3-*b*] [1, 3] benzothiazole(IV):** A mixture of 3-cyano -6, 9-dimethyl -2-methylthio -4-imino -4*H*-pyrimido [2, 1-*b*] [1, 3] benzothiazole [0.01mole] and Hydrazine hydrate (80%) was refluxed for 4-5 hours in presence of anhydrous K_2CO_3 and DMF, the reaction mixture was monitored by TLC. Then reaction mixture was poured in ice water, solid crude product obtained, then recrystalised by alcohol and DMF.

3. Reaction Scheme



(Scheme-I)

4. Spectral Discussion:

A. **Synthesis of 3-cyano -6, 9-dimethyl -2-methylthio -4-imino -4*H*-pyrimido [2, 1-*b*] [1, 3] benzothiazole (III):** Yield: 70 %, M.P: 230 ^{0}C, M.F: $C_{14}H_{12}N_4OS_2$, M. Wt: 300**. IR:** (KBr / cm^{-1}): 3489 (=NH), 2926, 2856 (CH), 2210 (-CN), 1647 (C=N)**. ^{1}H-NMR**: (60 MHz, DMSO) : δ2.2 (s 3H SCH_3) δ 2.4 (s 3H Ar-CH_3) δ 2.7 (s 3H Ar-CH_3) δ 6.5 (d 1H Ar-H) δ 6.8 (d 1H Ar-H) δ7.4 (s 1H =NH). **EI-MS:** (m/z: RA %): = 300 (M^{+} 15%) 253, 226, 200, 162, 136**. ^{13}C-NMR** in DMSO : δ: 14.1 (C_1-CH_3), δ19.0 (C_{10}), δ21 (C_{11}), δ117.2 (C_2), δ118.7 (C_3), δ119.7 (C_5, Ar-C), δ122.3 (C_6, Ar-C), δ123 (C_7, Ar-C), δ124 (C_8, Ar-C), δ127.4 (C_9, Ar-C), δ128 (C_{12}, Ar-C), δ130 (C_{13}, C=N) δ156 (C_{14}, CN), δ174 (C_4, C=NH).

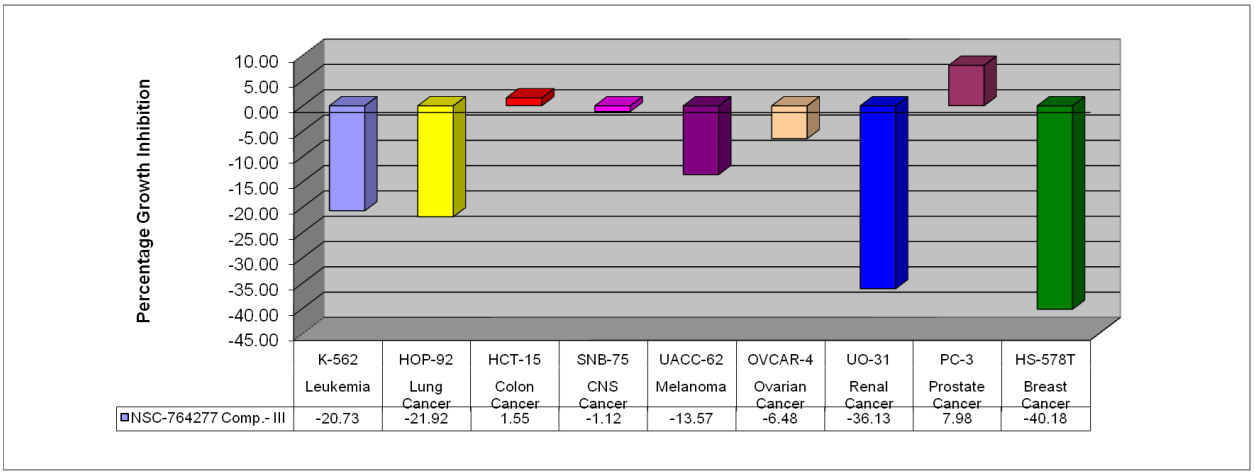
B. **Synthesis of 3-amino- 4-imino 6, 9 dimethyl- pyrazolo-[3, 4-*e*] pyrimido [2, 3-*b*] [1, 3[benzothiazole (IV):** Yield: 68%, M.p: 255 ^{0}C, M.F: $C_{13}H_{12}N_6S$, M.Wt: 284. IR: (KBr / cm^{-1}): 3406 cm^1 (=NH), 3329,3215 cm^1 , 1624 cm^1 (C=N). ^{1}H-NMR: (60 MHz, DMSO): **δ**2.4(s 3H Ar-CH_3), **δ** 2.5 (s 3H Ar-CH_3), **δ** 4.5 (s 1H NH), **δ** 4.4 (broad 2H NH_2), **δ** 6.8 (d 1H Ar-H), **δ** 7.6 (d 1H Ar-H), **δ** 8.2 (s 1H =NH) . EI-MS: (m/z: RA %): = 28 (30%).

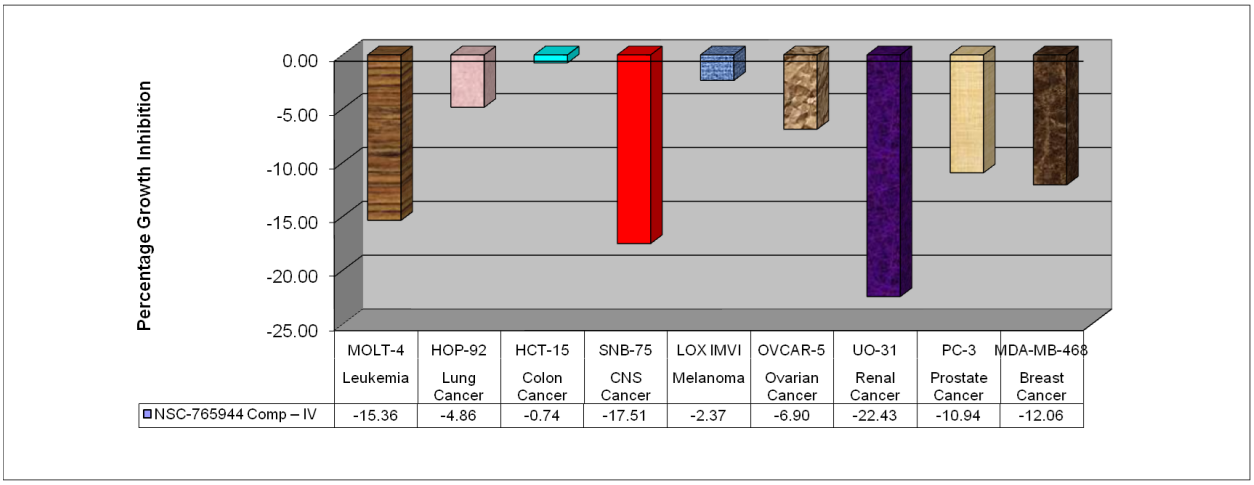
5. *In-vitro* anticancer activity

The *in- vitro* anticancer activity of synthesized compounds were carried out at National Cancer Institute of Maryland. USA. Three compounds were sent to NCI, Maryland, USA, by DTP processes, but only two compounds were selected by NCI for their *in- vitro* anticancer activity. The in-*vitro* anticancer activities of selected compounds were screened against 60 human cell lines at a single dose of 10µm against different types of cancer, Leukemia. Prostate Brest cancer, CNS, Colon and Melanoma cancer, non small cell lung, Renal cell lines (**Table No. 1**). Activity results for each compound were reported as in mean graph.

In mean graph, negative values project towards the right of the vertical line and it represent cellular sensitivities to the test agent that expected the mean and positive value project towards the left of the vertical line it represents cell lines are sensitivities to the test agent that are less than the average values. The compounds with cell lines appearing on the negative side in the mean graph exhibit inhibition (GI) of cancer cell to that of particular cancer.

6. Graphical Presentation of Anticancer Activity:





7. Result and Discussion

The present work reported synthesis of novel heterocyclic compounds containing fused pyrimido benzothiazole and fused pyrazolo pyrimido benzothiazole, **(Scheme-I)**. Structures of these compounds were sent to the National Cancer Institute Maryland, USA, online through Developmental Therapeutic Program by the website, www.dtp.nci.nih.gov. Our research group is collaborated to NCI, Maryland, and USA. Hence initially we submitted the structure of samples to DTP program for preliminary selection. Hence this is best tool in computational chemistry to develop new drugs.

The selected chemical entities which are better pharmacological active were tested at NCI laboratory and these samples were screened against human 60 cancer cell lines at single dose.

The *in-vitro* anticancer activity of samples in percentage growth of inhibition was calculated by, mean growth minus percentage growth of inhibition. Finally mean graph is obtained. In mean graph, value extended towards negative side; represent the cellular sensitivity to the test agent that means growth of inhibition is maximum. If the value projected towards positive side in mean graph, then growth of inhibition is less. Compound III exhibited maximum growth of inhibition against Leukemia, Lungs, Renal and Brest human cancer cell lines **(Table-1)**. And compound IV exhibited maximum growth of inhibition against Leukemia, CNS, Renal, Prostate and Breast human cancer cell lines **(Table-1).**

Table 1: *In-vitro* anticancer activity of compound III and IV.

Cancer Type ↓	**Selected Human Cancer Cell Line ↓**	**growth of inhibition ean graph**	
		Comp- III NSC-764277	**Comp–IV NSC-765944**
Leukemia	K-562	-20.73	-10.96
	MOLT-4	-7.12	-15.36
	RPMI-8226	-13.16	1.06
	SR	5.72	-14.25
Lung	HOP-92	-21.92	-4.86
	NCI-H23	-9.95	13.2
colon	HCT-15	1.55	-0.74
CNS	SNB-75	-1.12	-17.51
Melanoma	LOX IMVI	-0.78	-2.37
	UACC-62	-13.57	-1.61
Ovarian	OVCAR-4	-6.48	-0.65
	OVCAR-5	2.25	-6.90
Renal	CAKI-1	-10.93	0.00
	TK-10	0.00	-16.32
	UO-31	-36.13	-22.43
Prostate	PC-3	7.98	-10.94
Breast	MCF-7	-10.42	7.97
	HS-578T	-40.18	-1.20
	MDA-MB-468	13.33	-12.06
Mean Growth		**100.17**	**102.88**

8. Conclusion

The percentage growth of inhibition exhibited by fused pyrimido benzothiazole (III) was overall maximum than pyrazolo pyrimido benzothiazole against Leukemia, Lung, Renal, melanoma and Breast cancer of human 60 cancer cell lines.

These in-vitro anticancer activities were possible, due to sample selection by Developmental Therapeutic Program that was developed by National Cancer Institute Maryland, USA as online free accessible computational program.

Acknowledgement

Author thankful to NCI Maryland, USA for providing the *in vitro* anticancer activity, and the Director, IICT Hyderabad, Savitribai Phule Pune University Pune for providing the spectra. Author also thankful to the Principal Yeshwant Mahavidyalaya Nanded for providing the laboratory as well as spectral facility.

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