Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry

2016, 5 (2): 427-434

(International Peer Reviewed Journal)

An Expeditious Approach Towards Synthesis of Biologically Active Fused Benzo[4,5]Thiazolo[3,2-*a*]Pyrimido[4,5-*d*]Pyrimidines and Its Antioxidant Activity

B. D. Kalyankar[1], P. N. Ubale[2] and S. P. Vartale[1]*

1. PG Research Centre, Department of Chemistry, Yeshwant Mahavidyalaya Nanded-431602 (MS), **INDIA**
2. Late Babasaheb Deshmukh Gorthekar College Umri, Dist. Nanded-431807 (MS), **INDIA**

Email: spvartale@gmail.com

Accepted on 21st March 2016

ABSTRACT

Synthesis of novel biologically active fused benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidine derivatives (5a-h) have been Synthesized by condensation of 4-amino-2-methyl-6-(methylthio)pyrimidine-5-carbonitrile (3) with 2-amino 1/2/3/4-substituted benzothiazoles (4a-h) by using anhydrous K_2CO_3 as catalyst and solvent DMF. Compound (3) was prepared by reaction of acetamidine hydrochloride (1) and bis-(methylthio)methylene malanonitrile (2) with same reaction condition which is used for title compounds. The chemical structures of newly constructed derivatives were corroborated by IR, ^{1}H-NMR ^{13}C-NMR and Mass spectral analysis. Furthermore, these synthesized compounds were tested for antioxidant activity. The result of antioxidant activity reveals that most of the compounds show good to moderate activity. The major advantage of this protocol is short reaction time, operational simplicity and high yield.

Keywords: Acetamidine hydrochloride, 2-amino benzothiazole, Antioxidant activity and Bis-(methylthio) methylene malanonitrile.

INTRODUCTION

Heterocyclic chemistry is recently experiencing broad area of interest because of their potent biological activity [1-3]. They are widely distributed in nature and mostly found in bio-organic and medicinal chemistry with application in drug discovery. The heterocyclic compounds containing nitrogen and sulphur atoms serve as versatile moiety for drug designing. The most of the pharmaceutical drugs and biologically active agrochemicals are nitrogen and sulphur containing heterocycles. Pyrimido pyrimidines [4], benzothiazolopyrimidines [5] and pyrazolopyrimidines [6] undoubtedly belong to the most ubiquitous fused ring systems which plays very important role in the area of medicinal chemistry. Such fused heterocyclic scaffold diverts considerable attention of chemist due to their diverse biological activities and immense synthetic potential for the construction of many pharmacologically active heterocycles.

Pyrimido pyrimidines are analogues of folic acid as well as an important class of annelated uracils and their potent inhibitory properties regarding the tyrosine kinase domain of epidermal growth factor receptor

[7]. Survey of literature reveals that fused Pyrimido pyrimidine derivatives exhibit promising pharmacological applications such as antitumor [8], antioxidant [9], antiviral [10], antifungal [11], dihydrofolate reductase [12] and hepatoprotective activities [13]. Benzothiazole scaffold one of the privileged structure in pharmaceutical science and continuously drawing interest for development of novel drug due to its wide range of therapeutic importance such as antimicrobial [14], anticancer [15], antidiabetic [16], anthelmintic [17] anticonvulsant [18], antiviral [19], anti-inflammatory [20], antiallergic [21]. Literature survey shows that few references are available on synthesis and biological activity of heterocycles containing benzothiazole fused with pyrimidine ring [22-24]. Recently Sambhaji P. Vartale et al 2015 has reported synthesis and antimicrobial activity of fused benzo[4,5]thiazolo[3,2-a]pyrazolo[3,4-d]pyrimidine derivatives [25].

Biological importance of both benzothiazole and pyrimido pyrimidine stimulated us to develop new versatile heteroaromatic ring system in which benzothiazole ring is fused through the nitrogen atom with another biologically potent ring system such as pyrimido pyrimidine. Hence as a part of our ongoing research to develop a efficient method for the synthesis of benzo[4,5]thiazolo[3,2-*a*]pyrimido[4,5-*d*]pyrimidines via nucleophilic substitution-cyclization reaction in between 4-amino-2-methyl-6-(methylthio)pyrimidine-5-carbonitrile and 2-amino 1/2/3/4-substituted benzothiazoles with evaluation of their antioxidant activity.

MATERIALS AND METHODS

All the chemicals used in present work are of analytical grade and used without further purification. Electrothermal IA 9000 SERIES digital melting point apparatus was used to determine the melting points of synthesized compounds and were uncorrected. Purity of all the products was routinely checked by thin layer chromatography (TLC) on pre-coated sheets of silica gel-C plates of 0.25 mm thickness. Infrared spectra were recorded in Nujol or as KBr pallets on infrared spectrophotometer, nuclear magnetic resonance spectra were obtained on Brukner advance spectrophotometer 400 MHz in DMSO-d_6 using tetramethylsilane (TMS) as internal standard, Mass spectra were recorded on FT-VC-7070 H Mass spectrometer using the EI technique at 70 eV.

General procedure:

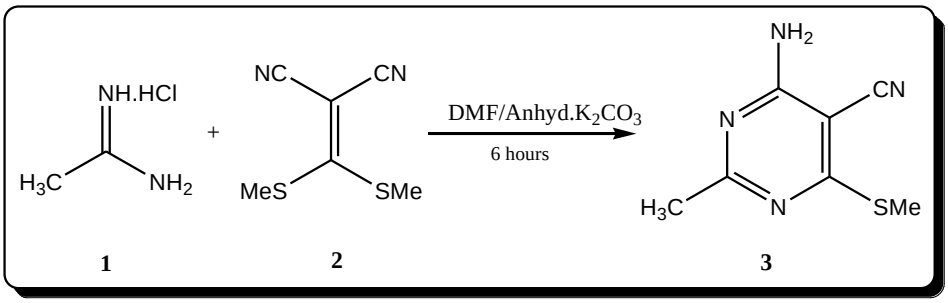
Synthesis of 4-amino-2-methyl-6-(methylthio)pyrimidine-5-carbonitrile (3): A mixture acetamidine hydrochloride (1) (0.01mol) and bis(methylthio)methylene malanonitrile (2) (0.01mol) in 10 ml of DMF and anhydrous potassium carbonate (10mg) was refluxed for 6 h. The reaction progress was monitored by thin layer chromatography (TLC) by using ethyl acetate: hexane (3:7) as irrigant. After completion of reaction, the reaction mixture was allowed to cool at room temperature and transferred in to ice cold water. The separated solid product was filtered, washed with water and recrystalized from ethanol to give pure compound (3).

Synthesis of benzo[4,5]thiazolo[3,2-*a*]pyrimido[4,5-*d*] pyrimidine derivatives (5a-h): As per scheme-2, a mixture of 4-amino-2-methyl-6-(methylthio)pyrimidine-5-carbonitrile (3) (0.001mol) and 2-amino 1/2/3/4 substituted benzothiazoles (4a-h) (0.001mol) were independently refluxed in 10 mL of DMF and anhydrous K_2CO_3 (10mg) for 6 h. After completion of reaction, the reaction mixture was allowed to cool at room temperature and transferred in to ice cold water. The separated solid product was filtered, washed with water and recrystalized from ethanol to give pure compound (5a-h).

RESULTS AND DISCUSSION

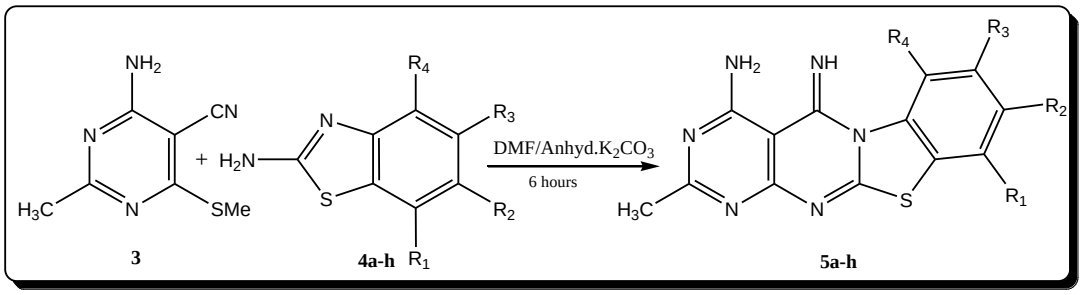
The therapeutic importance of these rings promoted us to develop new molecules in which substituents should be arranged by such pattern which displays higher pharmacological activities. Thus in present view we have synthesized a series of benzo[4,5]thiazolo[3,2-*a*]pyrimido[4,5-*d*]pyrimidines (5a-h). The synthetic route leading to title compound is summarized in scheme-1 and scheme-2. In our first scheme we

condensed acetamidine hydrochloride (1) and bis (methylthio) methylene malanonitrile (2) in DMF and catalytic amount of anhydrous K_2CO_3 to afford (3) **Scheme-1**.



Scheme-1. Formation of 4-amino-2-methyl-6-(methylthio)pyrimidine-5-carbonitrile.

The compound (3) posseses replaceable active thiomethyl group at 6-position and electron withdrawing nature of cyano group at 5-position. Due to the presence of thiomethyl group and cyano group on compound (3) which has susceptibility for nucleophilic substitution-cyclization. When compound (3) was condensed independently with 2-amino 1/2/3/4-substituted benzothiazoles (4a-h) under similar experimental condition to afford benzo[4,5]thiazolo[3,2-*a*]pyrimido[4,5-*d*]pyrimidine derivatives (5a-h) **Scheme-2.** The compounds Physical data are listed in table 1 and compound number and substituents position given in table 2.



Scheme-2. Formation of benzo[4,5]thiazolo[3,2-*a*]pyrimido[4,5-*d*]pyrimidine derivatives (5a-h).

Table 1. Physicochemical data

Com.No.	MolecularFormula	Mol. Wt.	Colour	Yield %	M.P °C
3	$C_7H_8N_4S$	180	Gray	72.17	135-36
5a	$C_{13}H_{10}N_6S$	282	Brown	65.04	185-87
5b	$C_{14}H_{12}N_6S$	296	Brown	78.96	161-62
5c	$C_{14}H_{12}N_6OS$	312	Brown	71.28	180-82
5d	$C_{13}H_9N_7O_2S$	327	Yellow	62.33	154-56
5e	$C_{13}H_9N_6SCl$	316	Yellow	59.08	193-95
5f	$C_{13}H_9N_6SBr$	360	Brown	64.00	164-66
5g	$C_{15}H_{14}N_6S$	310	Brown	72.80	212-14
5h	$C_{13}H_8N_6SCl_2$	350	Yellow	66.58	173-75

Table 2. Compound numbers and substituent's position

Comp. No.	R_1	R_2	R_3	R_4
5a	-H	-H	-H	-H
5b	-H	$-CH_3$	-H	-H
5c	-H	$-OCH_3$	-H	-H
5d	-H	$-NO_2$	-H	-H
5e	-H	- Cl	-H	-H
5f	-H	- Br	-H	-H
5g	-H	$-CH_3$	-H	$-CH_3$
5h	-H	-Cl	-Cl	-H

Spectral Analysis

4-amino-2-methyl-6-(methylthio)pyrimidine-5-carbonitrile (3): IR (KBr/cm^{-1}) 2206 (CN), 3359 (NH_2): ^{1}H-NMR (400 MHz,DMSO-d_6): δ 2.38 (s, 3H, CH_3), 2.53 (s, 3H, SCH_3), 7.5 (s, 2H, NH_2): ^{13}C-NMR (DMSO-d_6): δ 11.84 (SCH_3), 25.98(CH_3), 81.79 (C-CN), 114.54 (CN), 162.59 (C=N), 168.04 (C=N), 172.10 (C-SCH_3) EI-MS(m/z: RA%): 180 (M^+).

5-imino-2-methyl-5*H*-benzo[4,5]thiazolo[3,2-*a*]pyrimido[4,5-*d*]pyrimidine-4-amine (5a): IR (KBr/cm^{-1}) 1643 (C=N), 3271 (NH_2), 3394 (=NH): ^{1}HNMR (400 MHz,DMSO-d_6): δ 2.23 (s, 3H, CH_3), 6.6-6.8 (m, 4H, Ar-H), 7.11 (s, 2H, NH_2), 10.29 (s, 1H, =NH): EI-MS(m/z: RA%): 282 (M^+).

5-imino-9-methoxy-2-methyl-5*H*-benzo[4,5]thiazolo[3,2-*a*]pyrimido[4,5-*d*]pyrimidine-4-amine (5c): IR (KBr/cm^{-1}) 1656 (C=N), 3247 (NH_2), 3362 (=NH): ^{1}HNMR (400 MHz,DMSO-d_6): δ 2.33 (s, 3H, CH_3), 3.68 (s, 3H, OCH_3), 6.9-7.2 (m, 3H, Ar-H), 7.56 (s, 2H, NH_2), 9.42 (s, 1H, =NH): EI-MS (m/z: RA%): 312 (M^+).

5-imino-2-methyl-9-nitro-5*H*-benzo[4,5]thiazolo[3,2-*a*]pyrimido[4,5-*d*]pyrimidine-4-amine (5d): IR (KBr/cm^{-1}) 1642 (C=N), 3266 (NH_2), 3386 (=NH): ^{1}HNMR (400 MHz,DMSO-d_6): δ 2.51 (s, 3H, CH_3), 7.1-7.4 (m, 3H, Ar-H), 7.67 (s, 2H, NH_2), 8.97 (s, 1H, =NH): EI-MS (m/z: RA%): 327 (M^+).

5-imino-2,7,9-trimethyl-5*H*-benzo[4,5]thiazolo[3,2-*a*]pyrimido[4,5-*d*]pyrimidine-4-amine (5g): IR (KBr/cm^{-1}) 1631 (C=N), 3220 (NH_2), 3421 (=NH): ^{1}HNMR (400 MHz,DMSO-d_6): δ 2.48 (s, 3H, CH_3), 2.75 (s, 3H, CH_3), 2.90 (s, 3H, CH_3), 7.3-7.5 (dd, 2H, Ar-H), 7.74 (s, 2H, NH_2), 9.9 (s, 1H, =NH): EI-MS (m/z: RA%): 310 (M^+).

For the compound(3) IR spectrum (Fig.1), Mass Spectrum (Fig.2), ^{1}H NMR(Fig.3) and ^{13}C NMR spectrum (Fig.4) shown in respective figures.

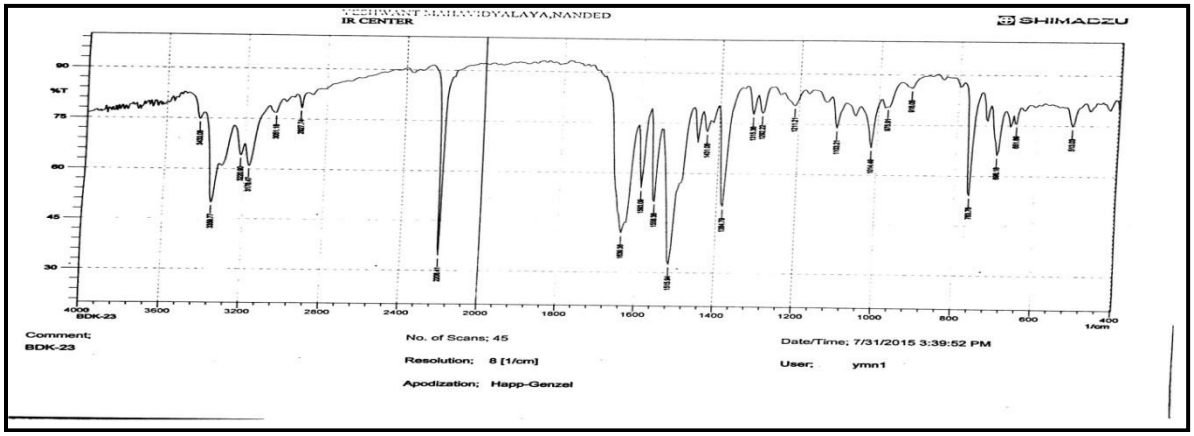


Fig.1 IR Spectrum of the compound (3)

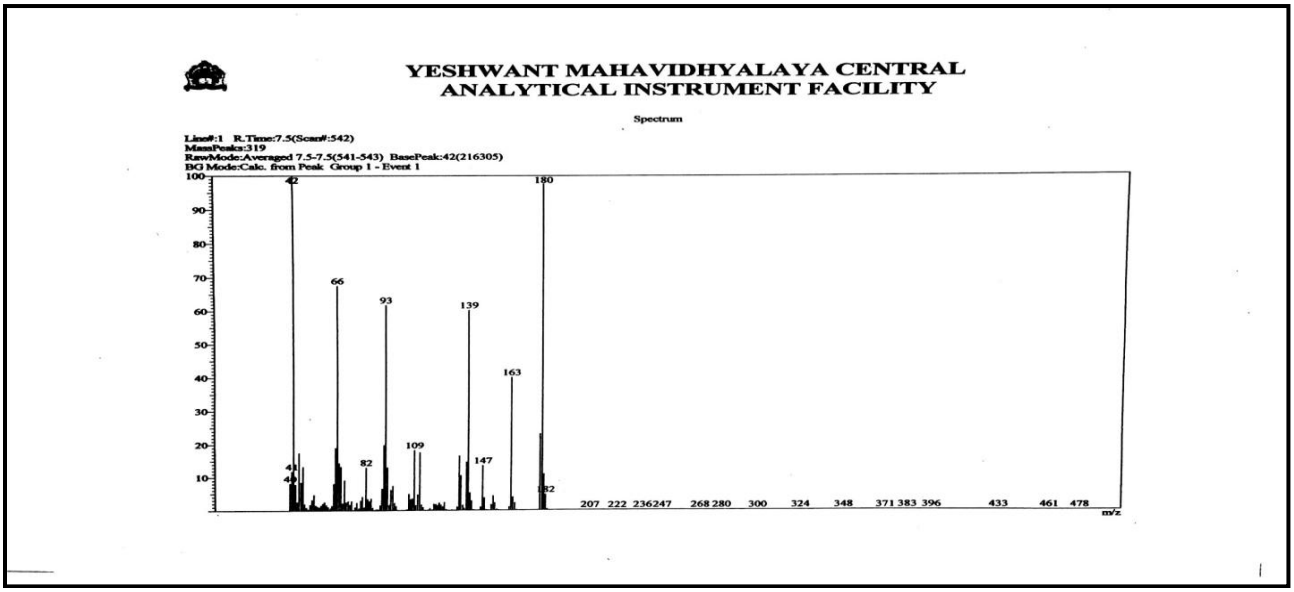


Fig.2 MASS Spectrum of Compound (3)

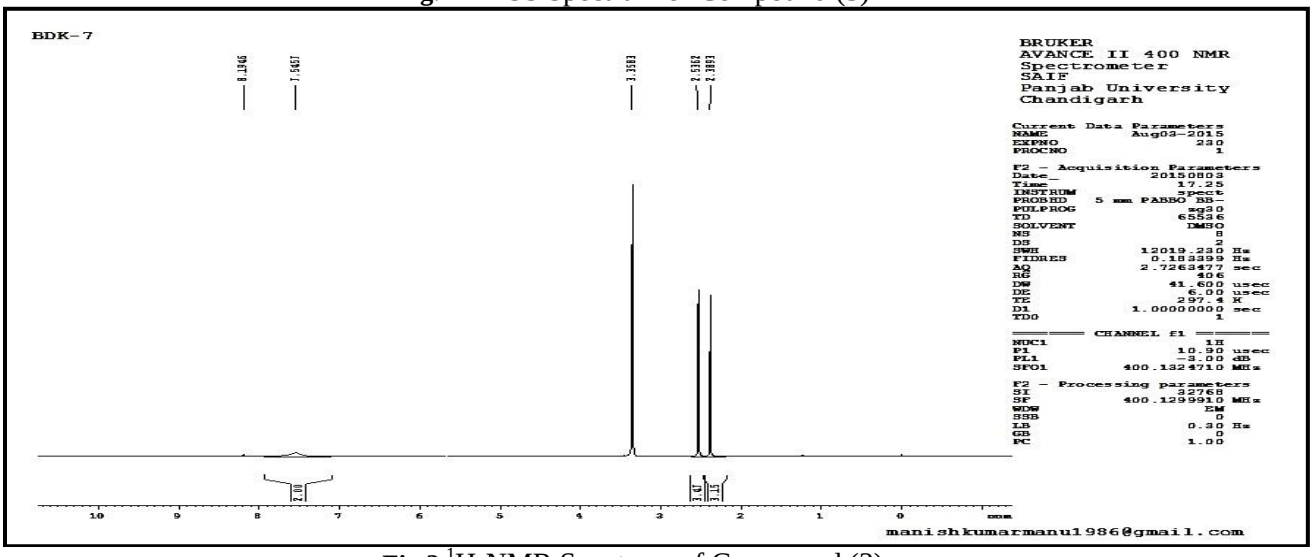


Fig.3 ^{1}H-NMR Spectrum of Compound (3)

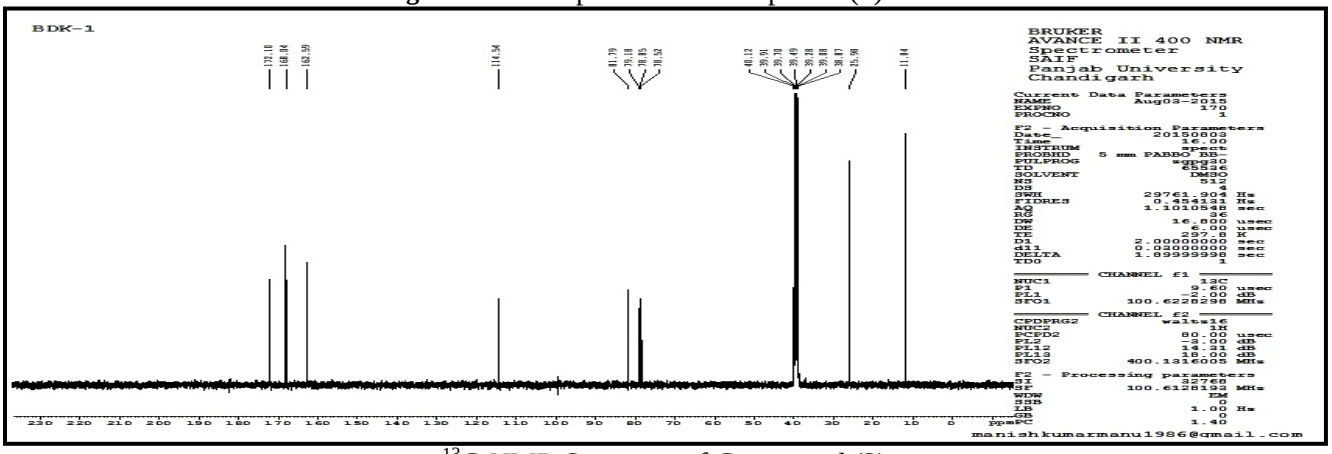


Fig.4 ^{13}C-NMR Spectrum of Compound (3)

The result of DPPH radical scavenging activity of newly synthesized benzo[4,5]thiazolo[3,2-*a*]pyrimido[4,5-*d*]pyrimidine derivatives are summarized in table-3. DPPH is relatively stable nitrogen centred free radical that easily accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH antioxidant assay is based on the ability of 1,1-diphenyl-2-picryl hydrazyl, a stable free radical to decolourise in the presence of antioxidants. When DPPH accepts an electron from antioxidant compound, the DPPH is decolourise which can be quantitatively measured from change in absorbance. Such reactivity has been widely used to test the ability of compounds to act as free radical scavengers. The overall DPPH radical scavenging activity of tested benzo[4,5]thiazolo[3,2-*a*]pyrimido[4,5-*d*]pyrimidine derivatives were in a range of 8.37-27.32 % as compared to the standardascorbic acid (90.15%). The highest DPPH radical scavenging activity was exhibited by **5b** whereas **5e** demonstrate lowest activity. From the result of present work, it can be concluded that benzo[4,5]thiazolo[3,2-*a*]pyrimido[4,5-*d*]pyrimidine derivatives are essential to boost the antioxidant activity.

APPLICATIONS

Antioxidant activity

DPPH radical scavenging assay: The DPPH radical scavenging assay has been used for preliminary screening of the sample for antioxidant activity. The proton radical scavenging action is known as an important mechanism of antioxidants. The odd electron in DPPH radical gives a strong absorption maximum at 517 nm and is purple in colour. The colour turns from purple to yellow when the odd electron of DPPH radical becomes paired with hydrogen from free radicals scavenging antioxidants to form reduced DPPH:H. 1ml (1 mM) of test Sample was added in to equal quantity of 0.1 mM solution of DPPH in ethanol. After 10 min of incubation at room temperature, the DPPH reduction was measured by reading the absorbance at 517 nm. Ascorbic acid was taken as standard reference. The result of DPPH reduction is summarized in table-3.

Table 3. Antioxidant activity of selected compounds

Sr. No.	Compounds	Antioxidant activity
		DPPH radical scavenging activity (%)
1	5a	22.57±0.27
2	5b	27.38±0.12
3	5c	21.94±0.76
4	5d	25.44±0.45
5	5e	08.32±0.09
6	Ascorbic acid	90.15±0.53

CONCLUSIONS

This work describe new method for the synthesis of novel heterocyclic compounds such as benzo[4,5]thiazolo[3,2-*a*]pyrimido[4,5-*d*]pyrimidine derivatives which were obtained by simple route with good product yield. The present protocol can be serve as reference to researcher and it may be applied for the synthesis novel series of fused benzothiazolopyrimidopyrimidines with further modification which may act as more potent therapeutic agent in future.

ACKNOWLEDGEMENTS

The authors are grateful to principal, Yeshwant Mahavidyalaya, Nanded, for providing laboratory facilities, and the Director, Panjab University, Chandigarh for providing spectra.

REFERENCES

[1] F A Attaby, S M Eldin, M A Razik, *Phosphorus Sulfur*, **1995**, 106, 21.

[2] S S Ghabrial, S M Eldin, *Egypt J. Pharm. Sci*, **1996**, 37, 375.
[3] S S Ghabrial, M Y Zaki, S M Eldin, *Indian J. Chem*, **1994**, 33B, 855.
[4] P Sharma, N Rane, V K Gurram, Synthesis and QSAR studies of pyrimido[4,5-d]pyrimidine-2,5-dione derivatives as potential antimicrobial agents, *Bioorg. and Med. Chem. Letters*, **2004**, 14, 4185.
[5] S Gupta, N Ajmera, N Gautam, R Sharma, D Gautam, Novel synthesis and biological activity study of pyrimido[2,1-b]benzothiazoles, *Indian J. Chem*, **2009**, 48B, 853.
[6] Aly A Aly, Iman A Gad El-Karim, Facile synthesis of new pyrazolopyrimidine derivatives of potential biosignificant interest, *J. Korean Chem. society*, **2011**, 55, 781.
[7] G W Rewcastle, A J Bridges, D W Fry, J R Rubin, W A Denny, *J. Med. Chem*, **1997**, 40, 1820.
[8] Y S Sanghhvi, S B Larson, S S Matsumoto, L D Nord, D F Smee, R C Willis, T H Avery, R K Robins, G R Revankar, *J. Med. Chem*, **1989**, 32, 629.
[9] J P De la Cruz, T Carrasco, G Ortega, F Sanchez De la Cuesta, *Lipids*, **1992**, 27, 192.
[10] R B Tenser, A Gaydos, K A Hay, *Antimiccrob. Agents Chemother*, **2001**, 45, 3657.
[11] Nizamuddin, M Mishra, M K Srivastava, M H Khan, *Indian J. Chem*, **2001**, 40, 49.
[12] J E Gready, C McKinlay, M G Gebauer, *Eur. J. Med. Chem*, **2003**, 38, 719.
[13] V J Ram, A Goel, S Sarkhel, P R Maulik, *Bioorg. Med. Chem*, **2002**, 10, 1275.
[14] B Rajeeva, N Srinivasulu, S Shantakumar, Synthesis and antimicrobial activity of some new 2-substituted benzothiazole derivatives, *E-Journal of Chem*, **2009**, 6, 775.
[15] S Kini, S Swain, A Gandhi, Synthesis and evaluation of novel benzothiazole derivatives against human cervical cancer cell lines, *Indian J. Pharm. Sci*, **2007**, 46-50.
[16] S Pattan, C Suresh, V Pujar, V Reddy, V Rasal, B Koti, Synthesis and antidiabetic activity of 2-amino[5(4-sulphonylbenzylidine)-2,4-thiazolidinenone]-7-chloro-6-flurobenzothiazole, *Indian J. Chem*., **2005**, 44B, 2404.
[17] M Sreenivasa, E Jaychand, B Shivakumar, K Jayrajkumar, J Vijaykumar, Synthesis of bioactive molecule flurobenzothiazole comprising potent heterocyclic moieties for anthelmintic activity, *Arch. Pharm. Sci. and Res*, **2009**, 1, 150.
[18] Nadeem Siddiqui *etal*, Design, synthesis and anticonvulsant screening of newer benzothiazole-semicarbazones, *Asian J. Biomedical and Pharma. Sci*, **2012**, 2, 8.
[19] S Akihama, M Okhude, A Mizno, Meiji Yakka, DiagaknKkenkyu Kiyo, *Chem. Abstr*, **1968**, 68, 10369.
[20] S N Sawhney, O P Bansal, *Indian J. Chem*, **1977**, 15B, 121.
[21] J H Musser, R E Brown, B Love, K Baily, H Jones, R Kahen, *J. Med. Chem*, **1984**, 27, 121.
[22] A Singh, A Bhal, *Indian J. Chem*, **1969**, 7, 302.
[23] H Reimlinger, M A Peiren, R Merenyi, *Chem. Ber*, **1975**, 108, 3894.
[24] J S D Gurinder, I O Dorcas, F Scheinmann, P A Bates, M B Hursthouse, *J. Chem. Soc. Perkin Trans I*, **1988**, 2993.
[25] S P Vartale, B D Kalyankar, P N Ubale, Facile synthesis of novel fused benzo[4,5]thiazolo[3,2-a]pyrazolo[3,4-d] pyrimidines and screening of their antimicrobial activity, *Indo American J. Pharm. Research*, **2015**, 5, 1373.

CITATIONS:

1. Akeel Ahamd, O.P. Pandey, Sarvesh K. Pandey and Nizamuddin, A high-atom economic and one-pot facile synthesis of active antimicrobial 2,4-disubstituted aryl-2*H*-pyrido / pyrimido[1,2-a]pyrimidines scafffold: a Michael addition approach, *Journal of Applicable Chemistry*, **2013**, 2 (4):958-966.
2. Sandeep Talari, Govindarajan. R, Divya Karunakaram, Srikanth Jupudi, Udhayavani.S, Screening of antimicrobial and anti-oxidant activity of newly synthesized 1- (4- (9- bromo- 6H- indolo [2, 3- b] quinoxalin- 6- yl)- 3- oxobutanoyl)- 3- substituted- 4, 5- dihydro- 1H- pyrazole- 4- carbaldehyde derivatives of Quinoxaline, *J. Applicable Chem.*, **2013**, 2 (2),236-245.
3. Monika Bansal, Ramandeep Kaur, Balbir kaur, Synthesis and antifungal evaluation of thiazolo[3,2-a]pyrimidine derivatives *J. Applicable Chem.*, **2013**, 2 (3), 391-397.
4. Ahmed S. Hamed, Synthesis, Characterization and Evaluation of Antibacterial Activity of Several New pyromillitimides Containing Benzothiazole Moiety, *J. Applicable Chem.*, **2015**, 4 (2), 450-455.
5. Vasu Namani, B. Bharath Kumar Goud, Y. Bharathi Kumari, Ramesh Kumbham, Synthesis And Biological Evaluation Of *N*-(Benzo[D]Thiazol-2-Yl)-6-Methoxy-5-(Phenylamino)Picolinamide Derivatives As Antimicrobial Agents, *J. Applicable Chem.*, **2015**, 4 (3), 840-846.

AUTHORS' ADDRESSES

1. **Dr. S. P. Vartale**
Associate Professor, PG Research Centre, Department of Chemistry,
Yeshwant Mahavidyalaya, Nanded-431602, (MS), India.
E-mail: spvartale@gmail.com, Mb No. 09822430549.
2. **B. D. Kalyankar**
Research Scholars**,** PG Research Centre, Department of Chemistry,
Yeshwant Mahavidyalaya, Nanded-431602, (MS), India.
E-mail: balajikalyankar888@gmail.com Mb No. 09766930888
3. **P. N. Ubale**
Assistant professor, Department of Chemistry,
Late Babasaheb Deshmukh Gorthekar College, Umri, Dist: Nanded-431807, (MS), India.
E-mail: pupatil2015@gmail.com Mb No.09404466447