Heterocyclic Letters
Vol. 12| No.4|737-744|Aug-Oct|2022
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

SYNTHETIC DEVELOPMENT AND ANTIOXIDANT POTENTIAL OF 4-METHYL-8-(METHYLTHIO)-2, 6-DIOXO-2,6-DIHYDRO-1H-PYRIMIDO[1,2-A]PYRIMIDINE-7-CARBONITRILE AND ITS ANALOGUES.

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ABSTRACT:
The reaction of 2-amino-6-methylpyrimidin-4-ol with ethyl 2-cyano-3,3-bis(methylthio) acrylate in N,N-dimethyl formamide in the presence of anhydrous potassium carbonate led to the formation of 4-methyl-8-(methylthio)-2,6-dioxo-2,6-dihydro-1H-pyrimido[1,2-a]pyrimidine-7-carbonitrile as a parent compound then reacted with some carbon and nitrogen nucleophiles such as active methylene compounds and substituted anilines to afford the corresponding 8-substituted derivatives. The structure of the obtained products were confirmed by IR, NMR and Mass spectral data. The reaction mechanism was also studied. Furthermore, their potential as antioxidant agents were evaluated using DPPH and hydroxyl free radical scavenging assay.

KEY WORDS:
Ethyl 2-cyano-3,3-bis(methylthio)acrylate, 2-amino-6-methylpyrimidin-4-ol, N,N-dimethyl formamide, anhydrous potassium carbonate, antioxidant activity.

INTRODUCTION:

Pyrimidine is a nitrogen containing electron rich heterocycle. Synthetic versatility of pyrimidine enables the creation of structurally varied derivatives which includes numerous pyrimido core containing pyrimidine analogs [1-3].The chemistry of pyrimidine and its derivatives has been studied extensively since the past century due to their diverse applications [4]. Due to the wide range of biological and pharmacological actions that pyrimidine exhibits, it is regarded as a crucial heterocyclic moiety. Nucleotides, nucleic acids, vitamins, coenzymes, purines and uric acids are examples of naturally occurring molecules that contain this six-membered 1, 3-diazine ring with nitrogen at the 1 and 3 positions. The numerous medicinal uses of pyrimidine may be explained by the fact that it is a component of DNA and RNA. [5].

It includes antiviral [6], antifungal [7], anticancer [8] antibacterial [9], ant tuberculosis [10], anti-inflammatory [11], antiparkinsonian [12] anti-HIV [13] and analgesic [14]. Owing to their beneficial biological applications, the use of pyrimido-pyrimidines in multicomponent reactions for the development of new heterocyclic compounds can definitely provide further opportunities. However, these compounds have not been tested for their antioxidant activity.
In the present article we reported the synthesis of 4-methyl-8-(methylthio)-2,6-dioxo-2,6-dihydro-1H-pyrimido[1,2-a]pyrimidine-7-carbonitrile (3) and its 8-substituted derivatives from 2-amino-6-methylpyrimidin-4-ol with ethyl 2-cyano-3,3-bis(methylthio) acrylate in one-pot cost effective method and evaluated their anti-oxidant activity.

EXPERIMENTAL:

All the chemicals used in present works are from analytical grade and used without further purification. Melting points of the products were determined in open capillary tubes on an electro thermal melting point apparatus and were uncorrected. The progress of reactions and the purity of the isolated compounds were monitored by TLC on UV-active silica gel plate (Merck). IR spectra were recorded on Shimadzu FT-IR spectrophotometer, 1HNMR spectra were obtained on Bruker advance spectrophotometer 500 MHz in DMSO-d6 using TMS as an internal standard. Mass spectrums were analyzed on GC-MS spectrometer using the ESI technique.

General Procedure for the synthesis of 4-methyl-8-(methylthio)-2,6-dioxo-2,6-dihydro-1H-pyrimido[1,2-a]pyrimidine-7-carbonitrile (3): A mixture of 2-amino-6-methylpyrimidin-4-ol **(1)** (1 mmol) and ethyl 2-cyano-3,3-bis(methylthio)acrylate **(2)** (1 mmol) in 25 ml of N,N-dimethyl formamide (DMF) and anhydrous potassium carbonate (1.38 g, 1 equivalent) was refluxed for 4-5 hours. The development of reaction was supervised by thin layer chromatography (TLC). Reaction content was cooled at room temperature and transferred in to ice cold water. Solid obtained was filtered, wash down with water and recrystallized from ethanol to obtain compound **(3)**. MF: $C_{10}H_8N_4O_2S$, Yield: 83%, MP: 145 - 147°C. IR spectrum, ν, cm–1: 3252 (NH), 2211 (-CN), 1631, 1671 (CO str). 1 H NMR spectrum, δ, ppm: 10.16 br.s (1H, NH), 5.97 s (1H, CH), 2.53 s (3H, SCH3). 13C NMR spectrum, δC, ppm: 15,24,61,91,104,148,158,165,168,174. Mass spectrum: m/z 249 (Irel 100%) [M+1].

General Procedure for the synthesis of 8-substituted pyrimido-pyrimidine derivatives 4a – 4d and 5a – 5d: A mixture of 1 mmol of compound 3, 1 mmol of the corresponding CH acid or substituted aniline, and 1 equivalent of anhydrous potassium carbonate in DMF was heated with continuous stirring for 4–5.5 h. After completion of the reaction, the mixture was cooled, and the solid product was collected by filtration, washed with water, and recrystallized from ethanol. The IR, ^{1}H and ^{13}CNMR, and mass spectra of 4a–4d and 5a–5d were in well agreement with the assigned structures and consistent with literature data for structurally related compounds [19-27]

2-(7-cyano-4-methyl-2,6-dioxo-2,6-dihydro-1H-pyrimido[1,2-a]pyrimidin-8-yl) malononitriles (4a): M.F. $C_{12}H_6N6O_2$, Yield: 80%, MP: 155 - 157°C. IR spectrum, ν, cm–1: 3326 (NH str), 2207 (-CN str), 1600 (CO str). ^{1}H NMR spectrum, δ, ppm: 11.49 br. s (1H, NH), 4.2 s (1H, -CH), 5.21 s (1H, =CH), 2.07 s (3H, SCH3). Mass spectrum: m/z 267 (Irel 100%) [M + 1].

1-ethyl 3-methyl 2-(7-cyano-4-methyl-2,6-dioxo-2,6-dihydro-1H-pyrimido[1,2-a]pyrimidin-8-yl)malonate (4b): $C_{15}H_{14}N_4O_6$, Yield: 72%, MP: 154 - 156°C. IR spectrum, ν, cm–1: 3320 (NH str), 2220 (-CN str), 1630 (CO str). ^{1}H NMR spectrum, δ, ppm: 10.85 br. s (1H, NH), 4.4 s (1H, -CH), 5.67 s (1H, =CH), 2.03 s (3H, SCH3). Mass spectrum: m/z 347 (Irel 100%) [M + 1].

Methyl 2-cyano-2-(7-cyano-4-methyl-2,6-dioxo-2,6-dihydro-1H-pyrimido[1,2-a]pyrimidin-8-yl)acetate (4c): M.F.$C_{13}H_9N_5O_4$, Yield: 72%, MP: 155 - 157°C. IR spectrum, ν, cm–1: 3290 (NH str), 2198 (-CN str), 1610 (CO str). ^{1}H NMR spectrum, δ, ppm: 10.90 br. s (1H, NH), 4.12 s (1H, -CH), 5.16 s (1H, =CH), 2.0 s (3H, SCH3). Mass spectrum: m/z 300 (Irel 100%) [M + 1].

Diethyl 2-(7-cyano-4-methyl-2,6-dioxo-2,6-dihydro-1H-pyrimido[1,2-a]pyrimidin-8-yl)malonate (4d): M.F. $C_{16}H_{16}N_4O_6$,Yield: 75%, MP: 158 - 160°C. IR spectrum, ν, cm–1: 3330 (NH str), 2210 (-CN str), 1620 (CO str). ^{1}H NMR spectrum, δ, ppm: 11.55 br. s (1H, NH), 4.34 s (1H, -CH), 5.70 s (1H, =CH), 2.05 s (3H, SCH3). Mass spectrum: m/z 361 (Irel 100%) [M + 1]

4-methyl-8-((4-nitrophenyl)amino)-2,6-dioxo-2,6-dihydro-1H-pyrimido[1,2-a]pyrimidine-7-carbonitrile (5a): M.F. $C_{15}H_{10}N_6O_4$, Yield: 78%, MP: 150 - 152°C. IR spectrum, ν, cm–1: 3325, 3192 (NH str), 2207 (-CN str), 1676, 1738 (CO str). ^{1}H NMR spectrum, δ, ppm: 10.93 br. s (1H, NH), 4.3 s (1H, aniline NH str) 6.7 – 7.6 m (4H, Ar-H), 5.2 s (1H, =CH), 2.3 s (3H, SCH3). Mass spectrum: m/z 339 (Irel 100%) [M + 1]

4-methyl-2,6-dioxo-8-(p-tolylamino)-2,6-dihydro-1H-pyrimido[1,2-a]pyrimidine-7-carbonitrile (5b): M.F. $C_{16}H_{13}N_5O_2$, Yield: 75%, MP: 149 - 151°C. IR spectrum, ν, cm–1: 3323, 3190 (NH str), 2209 (-CN str), 1678, 1732 (CO str). ^{1}H NMR spectrum, δ, ppm: 10.84 br. s (1H, NH), 4.5 s (1H, aniline NH str) 6.6 – 7.7 m (4H, Ar-H), 5.3 s (1H, =CH), 2.2 s (3H, SCH3). Mass spectrum: m/z 308 (Irel 100%) [M + 1]

8-((4-methoxyphenyl)amino)-4-methyl-2,6-dioxo-2,6-dihydro-1H-pyrimido[1,2-a] pyrimidine-7-carbonitrile (5c): M.F. $C_{16}H_{13}N_5O_3$, Yield: 78%, MP: 155 - 158°C. IR spectrum, ν, cm–1: 3320, 3185 (NH str), 2210 (-CN str), 1650, 1725 (CO str). ^{1}H NMR spectrum, δ, ppm: 11.04 br. s (1H, NH), 4.02 s (1H, aniline NH str) 6.7 – 7.7 m (4H, Ar-H), 5.4 s (1H, =CH), 2.1 s (3H, SCH3). Mass spectrum: m/z 324 (Irel 100%) [M + 1]

8-((4-chlorophenyl)amino)-4-methyl-2,6-dioxo-2,6-dihydro-1H-pyrimido[1,2-a]pyrimidine-7-carbonitrile (5d): M.F. $C_{15}H_{10}N_5ClO_2$, Yield: 70%, MP: 153 - 155°C. IR spectrum, ν, cm–1: 3290, 3198 (NH str), 2207 (-CN str), 1670, 1710 (CO str). ^{1}H NMR spectrum, δ, ppm: 11.30 br. s (1H, NH), 4.4 s (1H, aniline NH str) 6.7 – 7.6 m (4H, Ar-H), 5.3 s (1H, =CH), 2.3 s (3H, SCH3). Mass spectrum: m/z 328 (Irel 100%) [M + 1]

RESULT AND DISCUSSION:

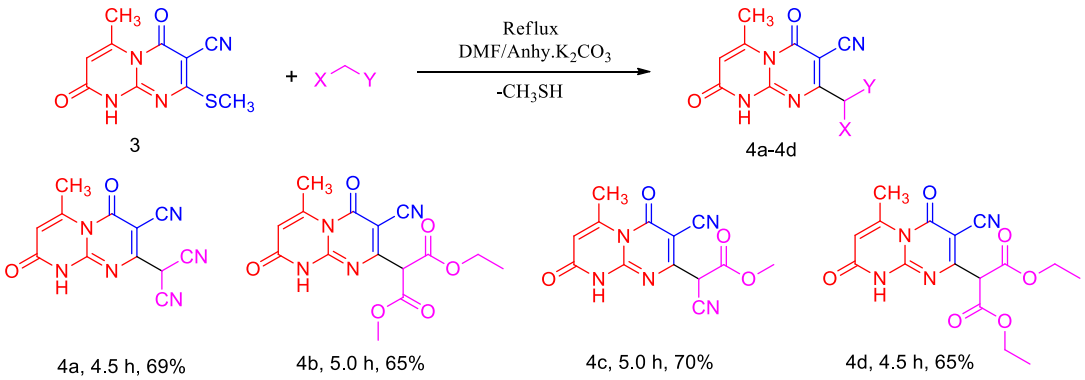
Primarily, we focused on the synthesis of 4-methyl-8-(methylthio)-2,6-dioxo-2,6-dihydro-1H-pyrimido[1,2-a] pyrimidine-7-carbonitrile (3) as the parent compound. It was synthesized from reacting the equimolar amount of 2-amino-6-methylpyrimidin-4-ol (1) with ethyl 2-cyano-3,3-bis(methylthio) acrylate (2) in N,N-dimethyl formamide in the presence of anhydrous potassium carbonate as shown in scheme-1.

Scheme-1

The synthetic details are given in experimental. The structure of the compound **(3)** was assigned on the basis of spectral data. IR spectrum showed absorption at 2211cm^{-1} and 1631, 1671 cm^{-1} which can be assigned to -CN and two -CO stretching frequencies respectively. The ^{1}H NMR spectrum of the compound was recorded in 300 MHz, $CdCl_3$ solvent, shows δ values, singlet at 2.34 and 2.53 ppm assignable to –CH_3 and –SCH_3 group, singlet at 5.97 ppm due

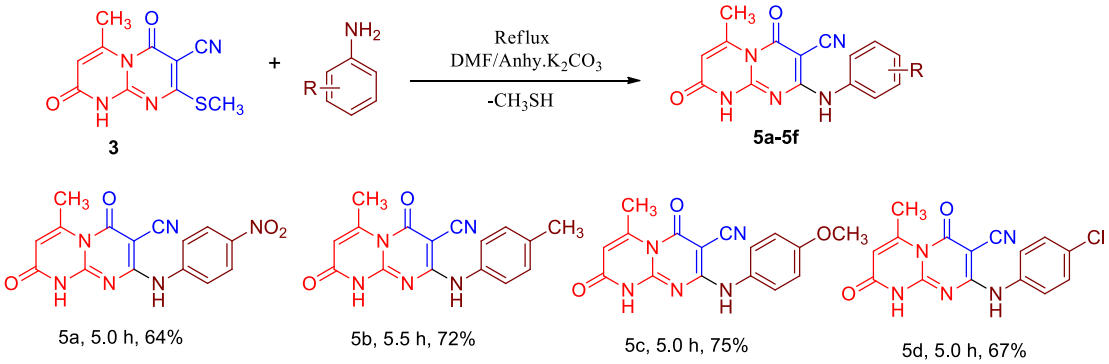
to -CH=C< (ethylene) proton present in ring and singlet at 10.16 ppm due to ring –NH (sec. amide) proton respectively. ^{13}C NMR (300 MHz, $CdCl_3$) spectrum of the compound gives below peaks δ: 24, 61, 91,104, 148, 165, 168, 174 ppm. Total eight peaks which are equivalent to number of carbon atoms in the compound **(3)** and mass spectrum exhibits molecular ion peak at m/z 249 (M+1) which corresponds to its molecular weight. As the number of nitrogen atoms in compound **(3)** increases, the ring pi-electrons become less energetic towards electrophilic substitution reaction while nucleophilic substitution becomes easier. Moreover compound **(3)** possess a dynamic methylthio group at 8- position which is a good leaving group and activated by adjacent electronegative ring nitrogen atom and negative inductive effect (- I effect) of cyano (-CN) group. Therefore, it can be presumed that compound 3 is a potential precursor for the synthesis of 8-substituted derivatives. In fact, 8-substituted derivatives of compound 3 were obtained by reacting it with different nucleophiles such as active methylene compounds and substituted anilines. Accordingly, the reactions of 3 with diethyl malonate, ethyl acetoacetate, ethyl cyanoacetate, and malononitrile in dimethylformamide in the presence of anhydrous potassium carbonate afforded the corresponding 8-substituted derivatives 4a–4d as shown in Scheme 2 given above.

Scheme-2



Under similar experimental conditions, compound 3 reacted with substituted anilines to produce 8-substituted derivatives 5a – 5d (Scheme-3).

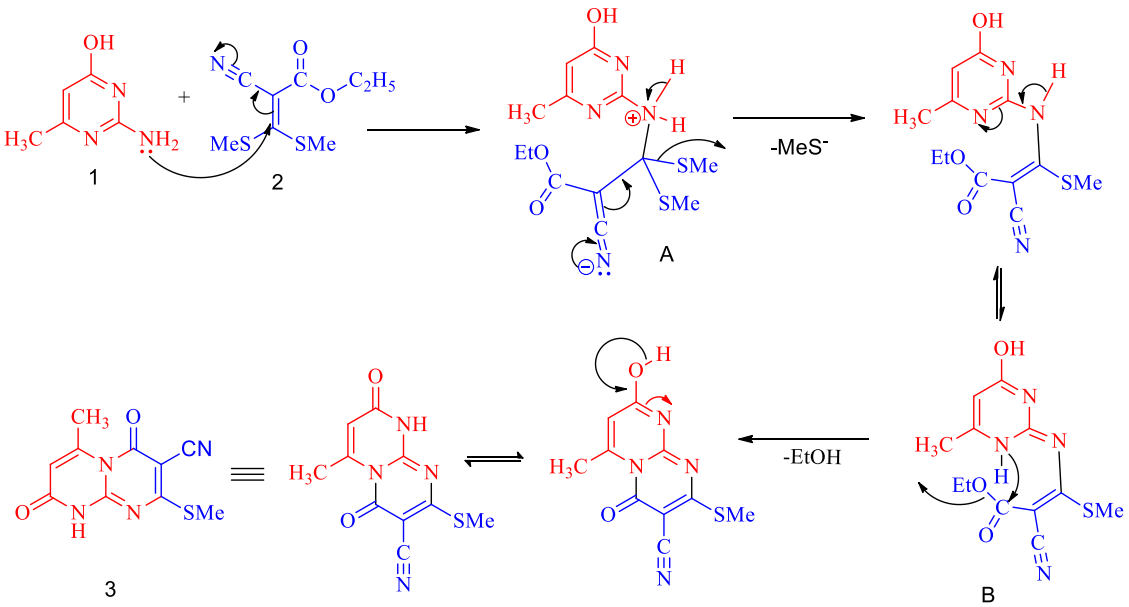
Scheme-3



A tentative mechanism was proposed for the formation of parent compound 3 (Scheme 4). Initially, nucleophilic addition of 2-amino-6-methylpyrimidin-4-ol **(1)** to ethyl 2-cyano-3,3-bis(methylthio) acrylate (2) gives zwitterion intermediate **A** [28] which is stabilized via elimination of methane thiolate anion and deprotonation followed by proton migration to produce intermediates B. Finally, intramolecular Michael addition between the NH and C=O groups of **B** leads to the formation of compound 3 [29-30]. In the next stage, nucleophilic

substitution of the 8-methylsulfanyl group in molecule 3 by different nucleophiles to yield final compounds 4 and 5 respectively.

Scheme-4



Antioxidant activity.

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay: The antioxidant properties of newly synthesized compounds 4a– 4d and 5a–5d were evaluated by the DPPH radical scavenging assay [15-16] using 1 mM ascorbic acid as the reference compound. Reaction mixtures were prepared by adding a solution of individual compound 4a–4d or 5a–5d in absolute ethanol to an equal volume of a 0.1 mM solution of DPPH radical. The mixture was incubated at room temperature for 20 min, and the absorbance at λ 517 nm was measured with a UV-Vis spectrophotometer. It is important to note that highest DPPH radical scavenging activity was exhibited by compound 3 is 45.10 % whereas compounds 5d showed the weakest activities by scavenging 14.5% of DPPH (Table 1).

Hydroxyl radical scavenging assay. The well-known Fenton reaction was used for measuring the OH radical scavenging activity [17-18]. In a typical Fenton reaction, 60 µL of FeCl2 (1 mM), 90 µL of 1,10-phenanthroline (1 mM), 2.4 mL of phosphate buffer (pH 7.8), and 150 µL of 0.17 M H2O2 were mixed together along with 1.5 mL of 1 mM solution of compound 3–5. After incubation at room temperature for 5 min, the absorbance at λ 560 nm was recorded. Ascorbic acid (1 mM) was used as reference compound. The results are shown in Table 1. Ascorbic acid as the reference compound showed a scavenging activity of 89.5%. Compound 3 showed highest OH radical scavenging activity of 42.1%. Compounds 4a, 4c, 4d, 5a and 5d showed good OH radical scavenging activities. Based on the performance results of both radical scavenging assays, it can be said that newly synthesized compounds 3, 4a– 4d and 5a–5d showed moderate to good antioxidant activity. It can also be said that quantitative difference in the antioxidant properties of the newly synthesized compounds were influenced by the nature of substituents attached to the parent compound.

Table 1. Antioxidant activity of compounds 3, 4a–4d, and 5a–5d according to DPPH and OH radical scavenging assays.

Sr. No	*Compound*	*DPPH radical scavenging activity (%)*	*OH radical scavenging activity (%)*
1	**3**	45.10±0.15	42.1 ± 0.547
2	**4a**	31.35±0.28	34.7 ± 0.921
3	**4b**	23.34±0.76	27.2± 0.723
4	**4c**	34.01±0.45	34.9+ 0.842
5	**4d**	23.16±0.55	36.9 ± 0.254
6	**5a**	22.43±0.08	39.8+ 0.346
7	**5b**	30.46±0.67	35.1 ± 0.329
8	**5c**	31.80±0.92	38.4.+ 0.419
9	**5d**	14.50±0.62	27.2 ± 0.275
10	**Ascorbic Acid**	**78.48±0.13**	**89.5 ± 0.240**

CONCLUSION:

In summary, a new series of 4-methyl-8-(methylthio)-2,6-dioxo-2,6-dihydro-1H-pyrimido[1,2-a]pyrimidine-7-carbonitrile (3) from 2-amino-6-methylpyrimidin-4-ol with ethyl 2-cyano-3,3-bis(methylthio) acrylate and its 8-substituted derivatives (4a – 4d & 5a – 5d) were synthesized in one-pot cost effective method and evaluated their anti-oxidant activity by using DPPH and hydroxyl free radical scavenging assay. The synthesized compounds can be easily isolated by simple workup technique, less expensive, short time, requires ambient reaction condition, and gives good yield.

ACKNOWLEDGMENT:

The authors duly acknowledge the Principal, Yeshwant Mahavidyalaya, Nanded, for providing laboratory facilities. The authors also express their gratitude to the Central Instrumentation Facility (CIF), Pune and Vishnu Chemicals Ltd, Hyderabad for providing spectral data and Department of Botany, Indira Gandhi Mahavidyalaya, Nanded for evaluation of antioxidant activity.

CONFLICT OF INTEREST: The authors declare the absence of conflict of interest.

REFERENCES:

i. Khalifa, N. M., Nossier, E. S., Al-Omar, M. A., & Amr, A. E. (2016). Synthesis, reactions, and antimicrobial activity of some novel fused thiazolo [3, 2-a] pyrimidine-5H-indeno [1, 2-d] pyrimidine derivatives. *Russian Journal of General Chemistry*, *86*(8), 1948-1953.

ii. Vartale, S. P., Sontakke, S. G., & Ubale, P. N. (2017). Synthesis of N-Substituted Pyrazolo Pyrimido Pyrimidines and Their Antioxidant Evaluation. *Heterocyclic Letters*, *7*(4), 1073-1077.

iii. Fang, Y., Xu, J., Li, Z., Yang, Z., Xiong, L., Jin, Y., & Chang, S. (2018). Design and synthesis of novel pyrimido [5, 4-d] pyrimidine derivatives as GPR119 agonist for treatment of type 2 diabetes. *Bioorganic & medicinal chemistry*, *26*(14), 4080-4087.

iv. Vartale, S. P., Halikar, N. K., Pawar, Y. D., & Tawde, K. V. (2016). Synthesis and evaluation of 3-cyano-4-imino-2-methylthio-4H-pyrido [1, 2-a] pyrimidine derivatives as potent antioxidant agents. *Arabian Journal of Chemistry*, *9*, S1117-S1124.

v. Mahapatra, A., Prasad, T., & Sharma, T. (2021). Pyrimidine: A review on anticancer activity with key emphasis on SAR. *Future Journal of Pharmaceutical Sciences*, *7*(1), 1-38.

vi. Seenaiah, D., Reddy, P. R., Reddy, G. M., Padmaja, A., & Padmavathi, V. (2014). Synthesis, antimicrobial and cytotoxic activities of pyrimidinyl benzoxazole, benzothiazole and benzimidazole. *European Journal of Medicinal Chemistry*, *77*, 1-7.

vii. Chen, Q., Zhu, X. L., Jiang, L. L., Liu, Z. M., & Yang, G. F. (2008). Synthesis, antifungal activity and CoMFA analysis of novel 1, 2, 4-triazolo [1, 5-a] pyrimidine derivatives. *European journal of medicinal chemistry*, *43*(3), 595-603.

viii. Grivsky, E. M., Lee, S., Sigel, C. W., Duch, D. S., & Nichol, C. A. (1980). Synthesis and antitumor activity of 2, 4-diamino-6-(2, 5-dimethoxybenzyl)-5-methylpyrido [2, 3-d] pyrimidine. *Journal of medicinal chemistry*, *23*(3), 327-329.

ix. Fathalla, O. A., Zeid, I. F., Haiba, M. E., & Soliman, A. M. (2009). Sh. I. Abd-Elmoez, WS El-Serwy. *World Journal of chemistry*, *4*(2), 127-132.

x. Holla, B. S., Mahalinga, M., Karthikeyan, M. S., Akberali, P. M., & Shetty, N. S. (2006). Synthesis of some novel pyrazolo [3, 4-d] pyrimidine derivatives as potential antimicrobial agents. *Bioorganic & medicinal chemistry*, *14*(6), 2040-2047.

xi. Trivedi, A. R., Dholariya, B. H., Vakhariya, C. P., Dodiya, D. K., Ram, H. K., Kataria, V. B., ... & Shah, V. H. (2012). Synthesis and anti-tubercular evaluation of some novel pyrazolo [3, 4-d] pyrimidine derivatives. *Medicinal Chemistry Research*, *21*(8), 1887-1891.

xii. Tozkoparan, B., Ertan, M., Kelicen, P., & Demirdamar, R. (1999). Synthesis and anti-inflammatory activities of some thiazolo [3, 2-a] pyrimidine derivatives. *Il Farmaco*, *54*(9), 588-593.

xiii. Amr, A. E. G. E., Sayed, H. H., & Abdulla, M. M. (2005). Synthesis and reactions of some new substituted pyridine and pyrimidine derivatives as analgesic, anticonvulsant and antiparkinsonian agents. *Archiv der Pharmazie: An International Journal Pharmaceutical and Medicinal Chemistry*, *338*(9), 433-440.

xiv. Wadwale, N. B., Prasad, D., Jadhav, A. H., Karad, A. R., Khansole, G. S., Choudhare, S. S., ... & Bhosale, V. N. (2021). Synthetic Development and Assessment of Antioxidant Activity of Imino [1, 2, 4] triazolo [1, 5-a] pyrimidine-6-carbonitrile and Its Derivatives. *Russian Journal of Organic Chemistry*, *57*(12), 2031-2038.

xv. Hour, M. J., Huang, L. J., Kuo, S. C., Xia, Y., Bastow, K., Nakanishi, Y., ... & Lee, K. H. (2000). 6-Alkylamino-and 2, 3-Dihydro-3 '-methoxy-2-phenyl-4-quinazolinones and related compounds: Their synthesis, cytotoxicity, and inhibition of tubulin polymerization. *Journal of medicinal chemistry*, *43*(23), 4479-4487.

xvi. Vartale, S. P., Halikar, N. K., Pawar, Y. D., & Tawde, K. V. (2016). Synthesis and evaluation of 3-cyano-4-imino-2-methylthio-4H-pyrido [1, 2-a] pyrimidine derivatives as potent antioxidant agents. *Arabian Journal of Chemistry*, *9*, S1117-S1124.

xvii. El-Mekabaty, A. (2015). Synthesis and antioxidant activity of some new heterocycles incorporating the pyrazolo-[3, 4-D] pyrimidin-4-one moiety. *Chemistry of Heterocyclic Compounds*, *50*(12), 1698-1706.

xviii. Khansole, G. S., Angulwar, J. A., & Bhosale, V. N. (2018). A Facile Synthesis of Some New Pyrimido [2, 3-d]-1, 3, 4-Thiadiazoles with their Biological Potential. *Indian Journal of Heterocyclic Chemistry*, *28*(02), 267-274.

xix. Vartale, S. P., Kadam, D. B., Halikar, N. K., & Pund, M. M. (2013). An efficient method for synthesis of novel iminothiazolopyrimidines and plausible antioxidant potential. *International Journal of Drug Development and Research*, *5*(1), 128-134.

xx. Vartale, S. P., Halikar, N. K., Sirsat, S. B., & Pawar, Y. D. (2013). An Efficient Synthesis of Some Novel 3-Cyano-4-imino-2-(methylthio) 4H-pyrido [1, 2-a] pyrimidine and Their Derivatives. *Journal of Heterocyclic Chemistry*, *50*(2), 351-354.

xxi. Kalyankar, B. D., Ubale, P. N., & Vartale, S. P. (2014). A Convenient Route for Synthesis and Antimicrobial Evaluation of Bis(diimino Benzothiazolo Pyrimido Pyrimidines). *Oriental Journal of Chemistry*, *30*(4), 1877.

xxii. Padghan, S. D., Bhosale, R. S., Ghule, N. V., Puyad, A. L., Bhosale, S. V., & Bhosale, S. V. (2016). Hydrogen sulfate ion sensing in aqueous media based on a fused pyrimido benzothiazole derivative. *RSC advances*, *6*(41), 34376-34380.

xxiii. Badne, S. G., Swamy, D. K., Bhosale, V. N., & Kuberkar, S. V. (2011). Novel synthesis and biological activity of 2-substituted derivatives of 3-cyano-4-imino-2-methylthio-8-methoxy-4H-pyrimido [2, 1-b][1, 3] benzothiazole and 3-amino-4-imino-8-methoxy-2H-pyrazolo [3′, 4′: 4, 5] pyrimido [2, 1-b][1, 3] benzothiazole. *Journal of Heterocyclic Chemistry*, *48*(4), 849-855.

xxiv. Kategaonkar, A. H., Sadaphal, S. A., Shelke, K. F., Shingate, B. B., & Shingare, M. S. (2009). Microwave assisted synthesis of pyrimido [4, 5-d] pyrimidine derivatives in dry media.

xxv. Moghaddam, F. M., Khodabakhshi, M. R., & Aminaee, M. (2014). Highly efficient synthesis of pyrimido [4, 5-d] pyrimidine-2, 4-dione derivatives catalyzed by iodine. *Tetrahedron Letters*, *55*(34), 4720-4723.

xxvi. Bacelar, A. H., Carvalho, M. A., & Proença, M. F. (2010). Synthesis and in vitro evaluation of substituted pyrimido [5, 4-d] pyrimidines as a novel class of Antimycobacterium tuberculosis agents. *European journal of medicinal chemistry*, *45*(7), 3234-3239.

xxvii. Cruz, J. S., & de Aguiar, A. P. (2021). Overview of the Biological Activities of Pyrimido [4, 5-d] pyrimidines. *Mini Reviews in Medicinal Chemistry*, *21*(15), 2138-2168.

xxviii. Pingle, M. S., Vartale, S. P., Bhosale, V. N., & Kuberkar, S. V. (2006). A convenient synthesis of 3-cyano-4-imino-2-methylthio-4H-pyrimido [2, 1-b][1, 3] benzothiazole and its reactions with selected nucleophiles. *Arkivoc*, *10*, 190-198.

xxix. Baheti, K. G., & Kuberkar, S. V. (2003). Novel Synthesis of 3-Amino-4-oxo-(2H)-pyrazolo [3′, 4′: 4, 5] pyrimido-[2, 1-b] benzothiazole and its 2-and 3-Substituted Derivatives. *Journal of heterocyclic chemistry*, *40*(3), 547-551.

xxx. Waghmare, G. S., Junne, S. B., Shinde, S. D., Waghamare, A. S., & Kuberkar, S. V. (2013). Synthesis and characterization of fused pyrimido-pyrimidine dicarbonitriles and their antibacterial activity. *Chemical Science Transactions*, *2*(1), 1-4.

Received on August 2, 2022.