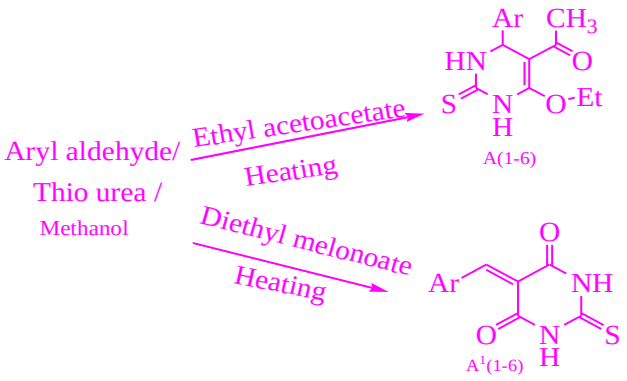
Dihydropyrimidine thione derviatives: Preparation, *In silico* studies, *In vitro* Antioxidantal and Antibacterial activity

Divya Puskuri[1], Badithapuram Vinitha[1], Velidandi Amarnath[1,2]* and Siddoju Kavitha[1]*
1. Department of Chemistry, Chaitanya Deemed to be University, Warangal, Telangana, 506001, INDIA
2. Department of Physical Sciences, Kakatiya Institute of Technology and Sciences, Warangal, INDIA
*velidandi@yahoo.co.in; kavithavbr@gmail.com

Abstract
Thioxo pyrimidine derivatives play a vital role in the preparation of antimicrobial, antitumor, antiulcer and anticonvulsant drugs. Herein, we discussed the synthesis, in silico studies, anti-microbial activity and anti-oxidant activity of A (1-6), A[1] (1-6) compounds. Titled *compounds are non-polar. Results of biological studies indicate that these precursors can be used as anti bacterial, antioxidant and anti cancer agents.*

Keywords: 2-thioxo-tetrahydropyrimidine, 2-thioxo-1, 2, 3, 4-tetrahydropyrimidine, Antibacterial activity, Anti oxidant activity.



Graphical abstract

Introduction
Thio pyrimidine compounds are an important part of making many drugs[8]. A powerful pyrimidine moiety with a thiazole ring has been made to cure viral diseases. QSAR research supports the antimicrobial activities of two substances among the series. Pyrimidine with ethyl group is effective against bacteria[10,11]. In the pyrimidine series of fourteen compounds, one molecule acts as a COX-2 inhibitor[5]. The antimicrobial effects of eleven thiopyrimidine substances, with methoxy groups were more effective than substances with a chloro or bromo group on three Gram-negative and four Gram-positive bacteria culture[2]. A sequence of fourty three spiropyrimidine substances, SAR studies and docking studies shows that these substances act as urea inhibitors[7].

Thiopyrimidines were prepared and tested for anti-inflammatory, analgesic and protein kinase (CDK-5, GSK-3) inhibiting effects[12]. Using domino [3+3], [4+2] and [5+1] cyclisations, synthesised bioactive thiopyrimidine, these biomolecule inhibitors targeting proteins were involved in cell division and act as antimicrobial drugs[4,13]. Simple Michael addition used in preparation of pyrimido thiadiazine shows antimicrobial activity against two pathogenic fungi and four bacterial species[14]. The studies on newer pyrimidine substances demonstrate that four are effective against bacteria and one is effecitve against fungi. The ring system of substances alters the activity[6].

Nitrogen containing heterocyclic compounds are available naturally; these are having more medical applications[3]. Chalcones and thiourea, in methanol solvent mixture were heated for two hours to produce novel thioxypyrimidines with good yields. Four compounds in the series show excellent antibacterial and antioxidantal activity among the twelve compounds[1].

Newer phthalazine-piperazine-1, 2, 4-oxadiazole derivatives were produced with 78% of yields. One of the compounds is active among the series resulted from ADMET studies, *in vitro* anticancer activity against three human cancer cell lines, molecular docking studies and EGFR receptor studies[9]. In this study, we described titled compounds preparation, *in silico*, *in vitro* antibacterial, as well as antioxidant studies.

Material and Methods
Characterisation of compounds by 400 MHz Bruker Avance spectrometer for NMR spectra and Mass spectra (electrospray ionization) was recorded on an Agilent Technologies QTOF 6530 instrument. *In silico* studies were performed by OSIRIS and PASS softwares. The synthesis of titled compounds from aryl aldehyde, thio urea, ethyl aceto acetate / diethyl melonoate was taken along with methanol as solvent in round bottom flask, stir the mixture at 60-70° C for two hours, pour the reaction mixture in to ice cold water. Precipitate formed was filtered and recrystallised with ethanol giving good yield.

A1) 1-(4-(2-chloroquinolin-3-yl)-6-ethoxy-2-thioxo-1,2,3, 4-tetrahydropyrimidin-5-yl)ethanone: ^{1}HNMR (400 MHz, (DMSO-d 6 , δ, ppm):13.76(br, 1H, NH), 8.27 (s,1H, Ar,H), 8.03-7.59(m, 4H, Ar-H), 4.16 (d, 1H,CH-NH), 4.01 (q, 2H, CH_2), 2.27 (S,3H,CH_3), 2.0(d,1H,Ar-NH), 1.21(T,3H,CH_3). Chemical Formula: $C_{17}H_{16}ClN_3O_2S$. m/z: $[M^+ +K]$=401; % of yield: 90.

A2) 3-(5-acetyl-6-ethoxy-2-thioxo-1,2,3,4-tetrahydro pyrimidin-4-yl)-4H-chromen-4-one: ^{1}HNMR (400 MHz,

(DMSO-d6, δ, ppm): 13.76(br, 1H, NH), 8.08-7.55 (m,4H,Ar-H), 7.10(S,1H,CHO), 4.01(q,2H,CH_2), 3.99(d,1H,CHN), 2.27(S,3H,CH_3), 2.0(br,1H,NH), 1.21 (t,3H,CH_3). Chemical Formula: $C_{17}H_{16}N_2O_4S$. m/z: [M^+] = 344.08. % of yield: 91.

A3) 1-(4-(2-butyl-4-chloro-1H-imidazol-5-yl)-6-ethoxy-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)ethanone: ^{1}H NMR (400 MHz, (DMSO-d6, δ, ppm):13.76(br,1H,NH), 13.00(s,1H,NH), 4.59(d,1H,CH), 4.01(q,1H,CH_2), 2.87 (t,2H,CH_2), 2,27(S,3H,CH_3), 2.0(d,1H,NH), 1.59 (m,2H,CH_2), 1.31(m,2H,CH_2), 1.21(t,3H,CH_3), 0.90 (t,3H,CH_3). Chemical Formula: $C_{15}H_{21}ClN_4O_2S$. m/z: [M^+] =356.87; % of yield: 88.

A4) 1-(6-ethoxy-4-(thiazol-2-yl)-2-thioxo-1,2,3,4-tetra hydropyrimidin-5-yl)ethanone: ^{1}HNMR (400 MHz, (DMSO-d 6 , δ, ppm):13.76(br,1H,NH), 7.53(d,1H,CH), 7.31(d,1H,CH), 4.59(d,1H,CH), 4.01(q,2H,CH_2), 2.27(s,3H,CH_3), 2.0(br,1H,NH), 1.21(t,3H,CH_3). Chemical Formula: $C_{11}H_{13}N_3O_2S_2$. m/z [M^+]= 283.04; % of yield: 89.

A5) 1-(6-ethoxy-4-(pyridin-2-yl)-2-thioxo-1,2,3,4-tetra hydropyrimidin-5-yl)ethanone: ^{1}HNMR (400 MHz, (DMSO-d 6 , δ, ppm):13.76(br,1H,NH),7.49-8.46(m,4H.Ar-H), 4.59(d,1H,CH), 4.01(q,2H,CH_2), 2.27(s,3H,CH_3), 2.0 (br,1H,NH),1.21(t,3H,CH_3). Chemical Formula: $C_{13}H_{15}N_3O_2S$. m/z[M^+]=277.09; % of yield: 90.

A6) 1-(6-ethoxy-4-(thiophen-2-yl)-2-thioxo-1,2,3,4-tetra hydropyrimidin-5-yl)ethanone: ^{1}HNMR (400 MHz, (DMSO-d 6 , δ, ppm): 13.76(br,1H,NH), 7.40(d,1H,CH), 6.93(t,1H,CH), 6.83(d,1H,CH), 4.44(d,1H,CH), 4.01 (q,2H,CH2), 2.27(s,3H,CH_3), 2.0(d,1H,NH), 1.21 (t,3H,CH_3). Chemical Formula: $C_{12}H_{14}N_2O_2S_2$. m/z= [M^++1] 283.05; % of yield: 92.

A^{1}1) 5-((2-chloroquinolin-3-yl)methylene)-2-thioxodi hydropyrimidine-4,6(1H,5H)-dione: ^{1}HNMR (400 MHz, (DMSO-d 6 , δ, ppm):8.67(s,1H,CH), 8.26(s,1H,CH), 7.60-8.06(m,4H,Ar-H),8.0(s,2H,(NHx2). Chemical Formula: $C_{14}H_8ClN_3O_2S$. m/z[M^++2]=319; % of yield: 89

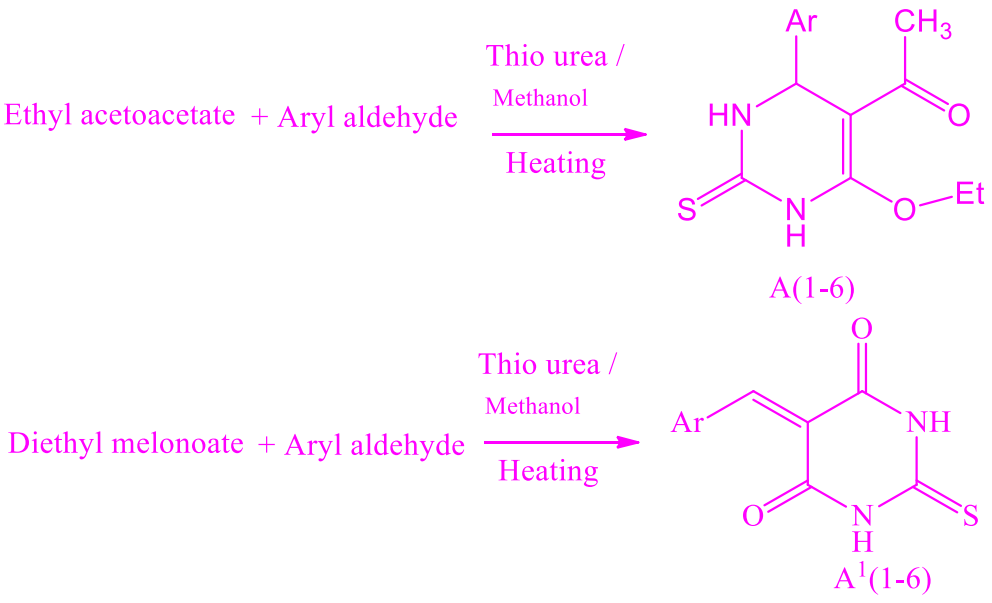
A^{1}2) 5-((4-oxo-4H-chromen-3-yl)methylene)-2-thioxodi hydropyrimidine-4,6(1H,5H)-dione: ^{1}HNMR (400 MHz, (DMSO-d 6, δ, ppm):7.55-8.08(m,4H,Ar-H), 8.0(s,2H, (NHx2), 7.87(s,1H,CH), 6.72(s,1H,CH). Chemical Formula: $C_{14}H_8N_2O_4S$. m/z[M^+]=300.02; % of yield: 87

A^{1}3) 5-((2-butyl-4-chloro-1H-imidazol-5-yl)methylene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione: ^{1}HNMR (400 MHz, (DMSO-d 6 , δ, ppm): 13.00(br,1H,NH), 8.01(s,1H,CH),8.0(s,2H,(NHx2),2.87(t,2H,CH_2),1.59(m,2, CH_2),1.31(m,2H,CH_2),0.90(t,3H,CH_3). Chemical Formula: $C_{12}H_{13}ClN_4O_2S$. Exact Mass: m/z[M^+]= 312.04; % of yield: 88.

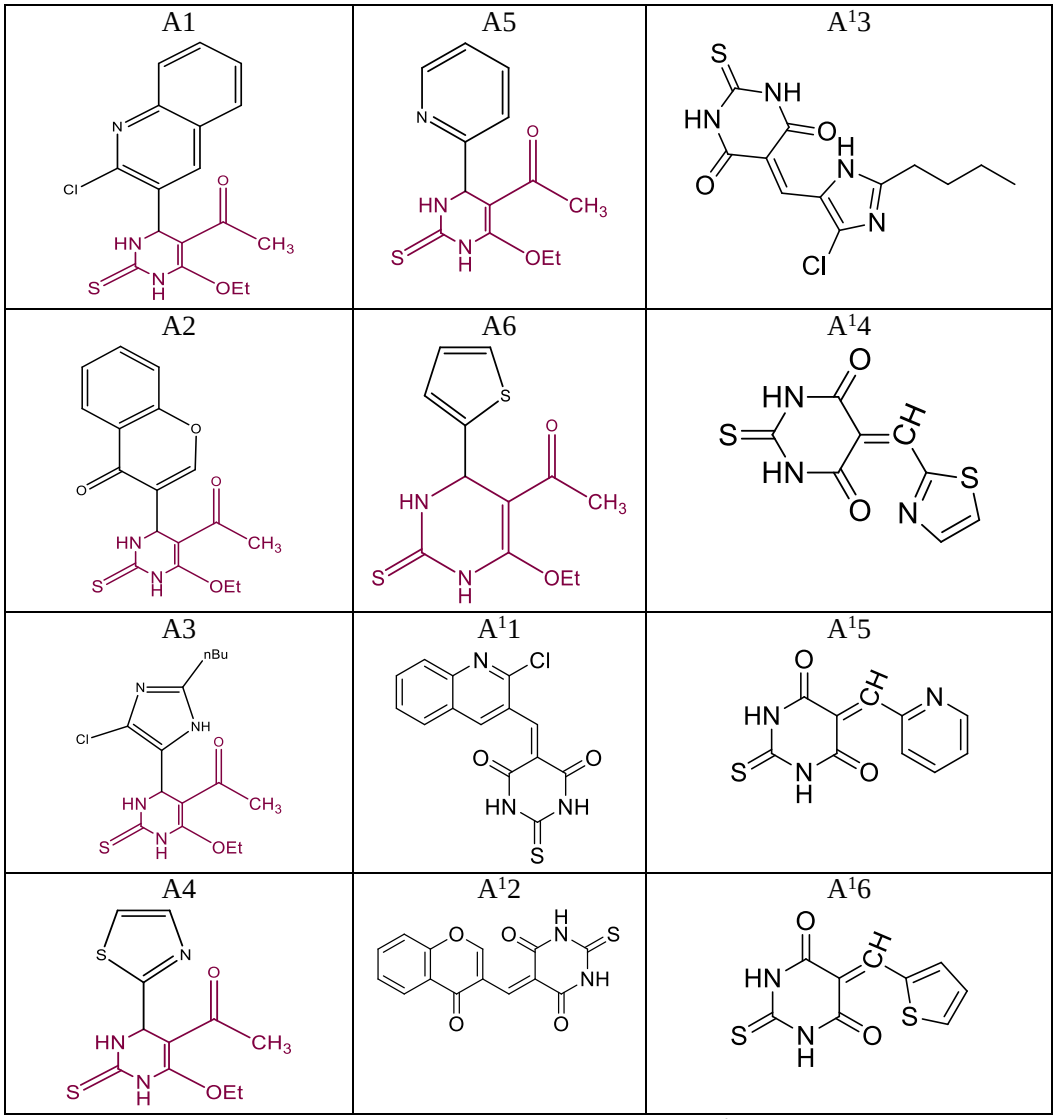
A^{1}4) 5-(thiazol-2-ylmethylene)-2-thioxodihydropyrimi dine-4,6(1H,5H)-dione: ^{1}HNMR (400 MHz, (DMSO-d 6 , δ, ppm):8.12(d,1H,CH), 8.02(d,1H,CH), 8.01(s,1H,=CH), 8.0(s,2H.(NHx2). Chemical Formula: $C_8H_5N_3O_2S_2$. Exact Mass: m/z[M^+]= 238.98; % of yield: 89.

A^{1}5) 5-(pyridin-2-ylmethylene)-2-thioxodihydropyrimi dine-4,6(1H,5H)-dione: ^{1}HNMR (400 MHz, (DMSO-d 6 , δ, ppm):8.45(d,1H,CH), 8.22(s,1H,CH), 8.0(s,2H,(NHx2), 7.43(d,1H,CH), 7.41(t,1H,CH). 7.29(t,1H,CH). Chemical Formula: $C_{10}H_7N_3O_2S$. m/z=[M^++NH_4]=252; % of yield: 88.

A^{1}6)5-(thiophen-2-ylmethylene)-2-thioxodihydropyrimi dine-4,6(1H,5H)-dione: ^{1}HNMR (400 MHz, (DMSO-d 6 , δ, ppm):8.11(d,1H,CH), 8.01(s,1H,CH), 8.0(s,2H,(NH)2), 7.74(d,1H,CH), 7.52(t,1H,CH). Chemical Formula: $C_9H_6N_2O_2S_2$. m/z. [M^++Acetonitrile]=279; % of yield: 89.



Scheme 1: Synthesis of A(1-6) & A^1(1-6)



Structured Formulae of A (1 to 6) and A[1] (1 to 6)

Table 1
Interaction of Compounds binding energies with PROTIEN
(TOPOISOMERASE -II DNA-1AB4)

S.N.	Binding Energy	Inhibition Constant (micro molar)	No. of Hydrogen bonds	Residues involved in the hydrogen bonding
A1	-6.53 Kcal/mol	16.37 µM	-	-
A2	-6.83 Kcal/mol	9.77 µM	-	-
A3	-6.63 Kcal/mol	13.87 µM	-	-
A4	-5.86 Kcal/mol	50.41 µM	-	-
A5	-5.64 Kcal/mol	73.70 µM	-	-
A6	-5.76 Kcal/mol	59.77 µM	-	-

Table 2
Interactions of A-Series with TYROSYL-TRNA SYNTHETASE 5USF

A1	-6.91 Kcal/mol	8.63 µM	1	PHE112 (2.34)
A2	-7.13 Kcal/mol	5.97 µM	2	GLU66 (2.16) , ASP108 (2.09)
A3	-5.97 Kcal/mol	7.81 µM	1	ASP197 (1.95)
A4	-5.57 Kcal/mol	83.30 µM	1	THR107 (2.00)
A5	-5.59 Kcal/mol	79.55 µM	1	THR107 (2.00)
A6	-5.72 Kcal/mol	64.47 µM	1	THR107 (2.12)

Results and Discussion

Aryl aldehyde, thio urea, methanol, ethyl acetoacetate [EAA] / di ethyl melanoate [DEM] were mixed and heated for two hours, pour the reaction mixture in to ice cold water, precipitate was formed. Compounds A1, $A^1$1 having quinoline, A2, $A^1$2 having chromone, A5, $A^1$5 having pyridine and A6, $A^1$6 having thiophene were formed with good yields.

"A2" is having good binding energy- 6.83 Kcal/mol, Inhibition Constant 9.77 µM as per table 1. As per table 2, "A2" is having good binding energy- 7.13 Kcal/mol, Inhibition Constant 5.97 µM. As per table 3, $A^1$2 is having good binding energy- 8.56 Kcal/mol, Inhibition constant 530.85 nM.

Anti oxidantal activity of A(1-6), A^1(1-6) study shows that A1, A3, $A^1$2 are having excellent activity by DPPH method. In antibacterial activity, A1, A3, A5 are showing good activity against *Staphylococous aures* (Gram+ve) (14mm), (13mm), (13mm), (standard *streptomycin* with 20mm). A2, A3, A4, A5 are showing more antimicrobial activity against *Salmonella typhi* (Gram-ve) (15mm), (16 mm), (14 mm), (14 mm), (standard *streptomycin* with 19mm). A1, A3, A4 are showing antibacterial activity against *Bacillus* (Gram+ve) (14 mm), (15 mm), (16 mm) (standard *streptomycin* with 20mm).

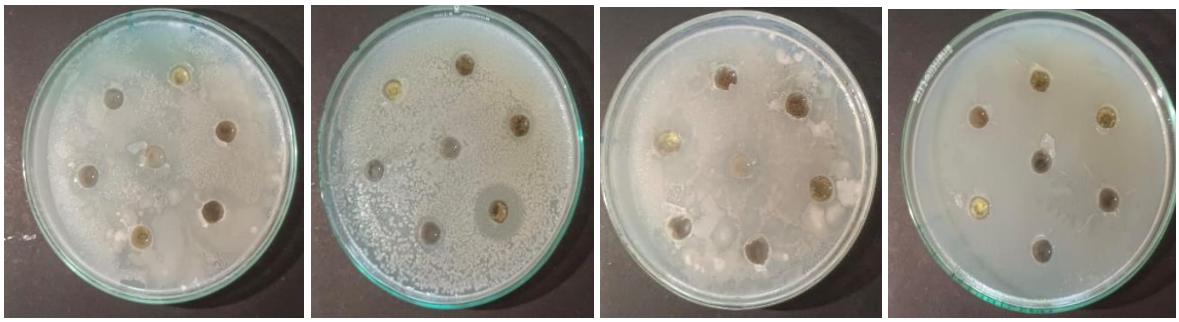
Antioxidantal study of A (1-6), A^1 (1-6): The titled compounds A (1-6), A^1 (1-6) were tested for their *in vitro* antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay method in methanol (95%) as a blank, DPPH solution as a control and ascorbic acid as a reference. The absorbance was measured at wavelength 517 nm[13].

Antimicrobial Activity using Agar-plate Assay: Gram positive and Gram negative bacteria are subcultured in nutrient-brath media (overnight at 37°c). Each strain was adjusted at a concentration of 10 cells/ml using 0.5 MC Farland standard. 100µl of each extract (20% w/v) was added to respective cells. Put the plate in refrigerator for 30 minutes. Incubate the plate at 37°c for 18 hours. DMSO at a concentration of 10% was employed as negative control. Anti bacterial method is performed by agar diffusion method[13].

Table 3
Interactions of $A^1$1-SERIES with EGFR 4 H J0)

S.N.	Binding Energy	Inhibition Constant	No. of Hydrogen bonds	Residues involved in the hydrogen bonding (Å)
$A^1$1	-8.00 Kcal/mol	1.36 µM	1	ASP831 (1.88)
$A^1$2	-8.56 Kcal/mol	530.85 nM	-	-
$A^1$3	-6.81 Kcal/mol	10.22 µM	1	ASP831 (1.79)
$A^1$4	-6.83 Kcal/mol	9.84 µM	-	-
$A^1$5	-7.12 Kcal/mol	6.01 µM	-	-
$A^1$6	-7.10 Kcal/mol	6.23 µM	1	THR830 (1.94)

Fig. 1: Antioxidant studies on A (1 to 6) and A^1 (1 to 6)



Staphylococcus areus *Salmonella typhi* *E. coli.* *Bacillus*

Conclusion

In this work, newer thio pyrimidine derivatives were prepared with easy procedure with good yields. Among the twelve compounds, three are showing good anti-oxidant activity and four are showing good antibacterial activity. In future, we are planning *in vitro* and *in vivo* anti cancer studies on tilted compounds.

References

1. Balije Rakesh et al, Preparation, *In Silico* Studies, *In Vitro* Antibacterial and Antioxidantal Activity of 4,6-Disubstituted Dihydropyrimidine Thiones, *Russ J Bioorg Chem*, https://doi.org/10.1134/S1068162023010247 **(2022)**

2. Basile L., Alvarez S., Blanco A., Santagati A., Granata G., Di Pietro P., Guccione S. and Muñoz-Fernández M.Á., Sulfonilamido thiopyrimidone and thiopyrimidone derivatives as selective COX-2 inhibitors: synthesis, biological evaluation and docking studies, *Eur J Med Chem*, **57**, 149-61, doi: 10.1016/j.ejmech.2012.09.005 **(2012)**

3. Ebenezer O., Jordaan M.A., Carena G., Bono T., Shapi M. and Tuszynski J.A., An Overview of the Biological Evaluation of Selected Nitrogen-Containing Heterocycle Medicinal Chemistry Compounds, *Int J Mol Sci*., **23**, 8117, doi: 10.3390/ijms23158117 **(2022)**

4. Fang Z., Zheng S., Chan K.F., Yuan W., Guo Q., Lui H.K., Lu Y., Leung Y.C., Chan T.H., Wong K.Y. and Sun N., Design, synthesis and antibacterial evaluation of 2,4-disubstituted-6-thiophenyl-pyrimidines, *Eur J Med Chem*., **1**, 141-153, doi: 10.1016/j.ejmech.2018.10.039 **(2019)**

5. Kilaru R.B. et al, Identification of substituted [3,2-a] pyrimidines as selective antiviral agents: Molecular modeling study, *Antiviral Research*, **95**, 118–127, doi.org/10.1016/j.antiviral.2012.05.010 **(2012)**

6. Mohamed M.S., Awad S.M. and Sayed A.I., Synthesis of certain pyrimidine derivatives as antimicrobial agents and anti-inflammatory agents, *Molecules*, **15(3)**, 1882-90, doi: 10.3390/molecules15031882 **(2010)**

7. Mohammed E.F., Youssef R., Abdelfettah Z., Ahmed T., Youssef B. and ElMokhtar E., Synthesis and Antibacterial Activity of New Spiro [thiadiazoline-(pyrazolo[3,4-d]pyrimidine)] Derivatives, *Journal of Chemistry*, doi.org/10.1155/2015/982404 **(2015)**

8. Perez C., Pauli M. and Bazerque P., An Antibiotic Assay by Agar Well Diffusion Method, *Acta Biologiae et Medicinae Experimentalis*, **15**, 113-115 **(1990)**

9. Samala Raju, Nukala Satheesh Kumar, Thirukovela Narasimha Swamy, Dasari Gouthami and Bandari Srinivas, One-Pot Synthesis of Some New Phthalazine-Piperazine-1,2,4-Oxadiazole Hybrids: Anticancer Evaluation, Molecular Docking and ADMET Studies, *Polycyclic Aromatic Compounds*, DOI: doi: 10.1080/10406638.2022.2158884 **(2022)**

10. Shahbaz S. et al, 5-Acetyl-6-methyl-4-aryl-3,4-dihydropyrimidin-2(1H)-ones: As potent urease inhibitors; synthesis, *in vitro* screening and molecular modeling study, *Bioorganic Chemistry*, **76**, 37–52, doi.org/10.1016/j.bioorg.2017.10.021 **(2018)**

11. Solankee A., Patel K. and Patel R.A., Facile Synthesis and Studies of Some New Chalcones, *E-Journal of Chemistry*, **9(4)**, 1897-1905 **(2012)**

12. Sondhi S.M., Goyal R.N., Lahoti A.M., Singh N., Shukla R. and Raghubir R., Synthesis and biological evaluation of 2-thiopyrimidine derivatives, *Bioorg Med Chem*, **2**, 3185-3195, doi: 10.1016/j.bmc.2005.02.047 **(2005)**

13. Voskoboynik O.Y., Kolomoets O.S., Berest G.G., Nosulenko I.S., Martynenko Y.V. and Kovalenko S.I., Preparation and biological properties of 2-thio-containing pyrimidines and their condensed analogs, *Chem Heterocycl Compd (N Y)*, **53**, 256-272, doi: 10.1007/s10593-017-2048-2 **(2017)**

14. Zaki Y.H., Gomha S.M. and Mohamed A.M.G., Utility of 2-thioxo-pyrido[2,3-d]pyrimidinone in synthesis of pyridopyrimido [2,1-b][1,3,5]-thiadiazinones and pyridopyrimido[2,1-b][1,3] thiazinones as antimicrobial agents, *Chemistry Central Journal*, **11**, 57, https://doi.org/10.1186/s13065-017-0286-0 **(2017)**.

(Received 09th July 2025, accepted 17th September 2025)