RESEARCH ARTICLE

Facile Synthesis and Antioxidant Activity of Pyrimidine Derivatives via Thienyl Chalcones Under Phase Transfer Catalysis Method

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ABSTRACT

A series of pyrimidine derivatives and were synthesized by condensation of thienylchalcones and urea, thiourea under phase transfer catalysis conditions (PTC) with short reaction time and excellent yields. The mechanism of reaction of the synthetic compounds and their structures were established using physical analysis methods ^{1}H-NMR and IR. The compounds were also screened for radical scavenging activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. Compounds A6 and A8 showed radical scavenging stronger than the standard antioxidant and ascorbic acid. While the other compounds showed mild and varying activities.

Keywords: Antioxidant, Chalcones, DPPH, Phase-transfer catalysis, Pyrimidine.

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INTRODUCTION

One of the most important heterocyclic organic compounds that contain two nitrogen atoms is pyrimidine and is characterized by biological activities, such as antiviral, anticancer, antimicrobial, anti-inflammatory and antimalarial.[1-7] Pyrimidines can be considered as one of the most important pharmaceutical parts widely present in many natural products and an essential structure block of RNA and DNA. All chemical molecules containing pyrimidine have pharmacological properties.[8] Recently, anticancer drugs used in medical treatments as well as being evaluated in clinical studies have often been based on the pyrimidine structure, and new pyrimidine derivatives show favorable activities.[9-12]

Also, the most important compounds that are used as an intermediate for the preparation of heterocyclic organic compounds are chalcones, which are easy to prepare and have many biological activities including antibacterial, anti-inflammatory and antioxidant.[13-18]

It is worth noting that the thiophene group is found in a large number of biologically active molecules that have diverse biological activities.[19,20] The chalcones cyclization, leads to pyrimidines is an emerging field of heterocyclic chemistry in many years due to accessibility and diverse biological activities as antibacterial, antifungal, antiprotozoal, anti-inflammatory substances.

Pyrimidine derivatives are synthesized through many methods with some negative aspects like extended reaction times, complicated workup and lesser yields with the use of relatively high temperature.[21-24] In the present study and as a part of our project, we have planned to prepare 4,6-Diaryl-3,4-Dihydropyrimidine derivatives from chalcones bearing thiophene nucleus with thiourea and urea by using The liquid-liquid phase-transfer catalysis (L-L PTC) technique.[25-28] The (L-L PTC) technique has some drawbacks as efficiency, product purity, time and atom economy.

EXPERIMENTAL

Materials

^{1}H-NMR spectrums of synthetic compounds were recorded on NMR-Bruker 300 MH Ultra-shield. Melting points were obtained in an open capillary tube and were uncorrected. IR spectra were recorded on a Bruker –Alpha with Platinum-ART spectrometer (Germany). UV-Perkin Elmer Lambda25 UV-Vis spectrometer. DPPH (90%) (1,1-diphenyl-2-picrylhydrazyl) free radical, L-Ascorbic acid and tetra butyl ammonium bromide (TBAB) reagents were obtained from sigma Aldrich.

Methods

General procedure for the synthesis of thiophen-2-yl – chalcones(a-f)[29]:

The base-catalysed Claisen–Schmidt condensation were used for the synthesis of chalcones (Scheme 1).

General procedure for synthesis pyrimidine derivatives using (liquid-liquid) PTC technique (A1-A9)[30]:

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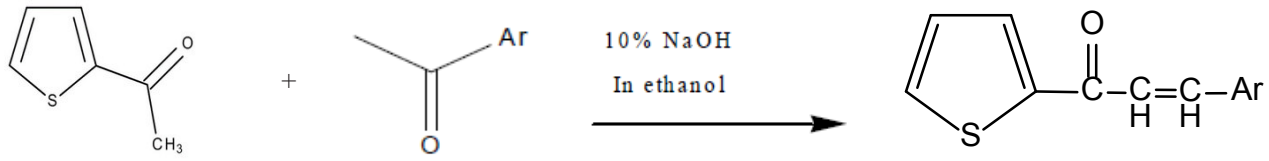
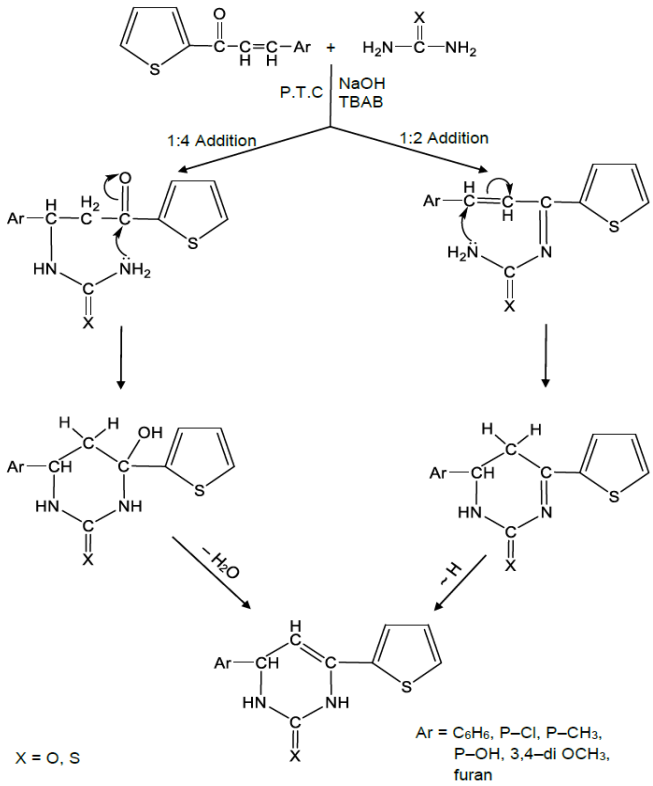


2-Acetylthiophene Aromatic aldehyde Thiophen-2-yl-Chalcones(a-f)
Ar = -C6H5,p-Cl. C6H5,p-CH3.C6H5.,p-OH.C6H5, 3,4-diOCH3.C6H5,furan.
Scheme 1 : Synthesis of Thienyl Chalcones Derivatives.



Scheme 2: Mechanism of pyrimidine derivatives synthesis.

The chalcones (a-f) (0.005 mole) was added To a solution of aqueous sodium hydroxide (6 mL, 50%), benzene (25 mL), tetra butyl ammonium bromide (TBAB) (0.2 g, 0.0015 mole) and urea or thiourea (0.005 mole).The mixture were stirred well for 30–60 minutes at 30–35ºC, The aqueous layer was separated and the organic layer was washed several time with cold water,(to get rid of the base and the catalyst), then dried with anhydrous MgSO4. the solvent was evaporated and the precipitate was recrystallized from suitable solvent. (Scheme 2).

Antioxidant Activity

1,1-diphenyl-2-picrylhydrazyl is a purplish-colored stable free radical in the presence of antioxidants, it can accept an electron or a hydrogen radical to become a magneto tropic molecule. Purple color of DPPH degrades. By using the spectrophotometric method this change can be monitored and used to estimate the radical scavenging activity, which is valent to the antioxidant activity of the prepared compounds. According to Brand William *et al.* (1995)[31] with some modifications, the free radical scavenging activity of DPPH has been determined. Briefly, the synthetic compounds were dissolved in a minimum volume of dimethylsulfoxide (DMSO) and diluted in methanol to 1-mg/mL. The different concentrations of compounds or ascorbic acid (62.5, 125, 250, and 500 µg/mL) in methanol (about 1-mL) was added to 3 mL of (0.5 mM) methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH). After incubating the absorbance at room temperature for 30 minutes, records was read against the blank at 517 nm.

The following equation is used to calculate the percentage of DPPH scavenging effect:

$$\% \text{ Inhibition} = A0 - A1/A0 \times 100$$

A0 = The absorbance of control.

A1 = The absorbance of sample test.

The (IC50) value (half of the maximum inhibitory concentration) is the sample concentration that can remove 50% of DPPH free radicals. The percentage of inhibition was plotted against four concentrations (62.5–500 µg/mL) of the prepared compounds to gain the inhibition curves. A straight-line equation was derived through a linear regression analysis. The (IC50) value determines the concentration of compounds required to inhibit half (50%) DPPH radicals.

RESULT AND DISCUSSION

Most heterocyclic compounds that contain Nitrogen and sulfur have received considerable attentiveness due to their wide range of pharmacological activity. Pyrimidine and their derivatives are important for drugs chemicals. Being a basic nucleus in DNA and RNA, pyrimidine is associated with diverse biological activities.[32] The reaction mechanism between chalcones compounds and nucleophiles can be accomplished through two pathways (Scheme 1). Thus, the reaction between thiourea, urea and chalcones containing various substituents was conducted in PTC media. The proposed mechanism involves the formation of anion from the deprotonation of the (-NH) group in urea or thiourea by the effect of the base, which is most likely because the negative charge on nitrogen is more stable. Through the simple Michael pathway (adding 1,4 nucleophile). The anion attacks the chalcone that produces the intermediary, which in turn cyclizes to provide pyrimidine derivatives (Scheme 2).[21-23]

Compound (A1) 4-phenyl-6-(thiophen-2-yl)-3,4-dihydropyrimidin-2-(1H) one.

White needles solid; m.p. 289–290°C; Yield 85%, reaction time; 30 min., solvent of crystallization: ethanol ; IR (cm^{-1}): 1640(C=O), 3438 (NH), 1601(C=C), 3086(=C-H), 2960(C-

H) ; ^{1}H-NMR (DMSO-d, 300 MHz): 4.7 (d, 1H, pyrimidine H-4), 5.71 (d, 1H, pyrimidine H-5), 6.76-7.60 (8H, m.Ar-H and thiophene-H),7,70 (brs, 2H,NH) (Figure 1).

Compound (A2) 4-(4-chlorophenyl)-6-(thiophen-2-yl)-3,4-dihydropyrimidin-2-(1H) one.

Yellow needles solid; m.p. 260–262˚C; Yield 83%, reaction time; 30 minutes, solvent of crystallization: ethanol, IR (cm^{-1}): 1638(C=O), 3436 (NH), 1603(C=C), 3105 (=C- H), 2946(C-H), 715(C-Cl); ^{1}H-NMR (DMSO-d, 300 MHz): 4.65 (d, 1H, pyrimidine H-4), 5.75 (d, 1H, pyrimidine H-5), 6.57-7.72 (7H,m. Ar-H and thiophene-H), 7.99(brs, 2H,NH).

Compound (A3) 4-(4-methylphenyl)-6-(thiophen-2-yl)-3,4-dihydropyrimidin-2-(1H) one.

Pale Yellow needles solid; m.p. 270–272˚C; Yield 80%, reaction time; 30 min., solvent of crystallization: ethanol IR (cm^{-1}): 1638(C=O), 3442 (NH), 1605(C=C), 3093 (=C- H), 3016(C-H); figure 2. 1H-NMR (DMSO-d, 300 MHz): 4.01 (d, 1H, pyrimidine H-4), 5.74 (d, 1H, pyrimidine H-5), 6.70-7.69 (7H, m.Ar-H and thiophene-H), 7.98 (brs, 2H,NH) (Figure 2).

Compound (A4) 4-(4-chlorophenyl)-6-(thiophen-2-yl)-3,4-dihydropyrimidin-2-(1H) thione.

Brown powder solid; m.p. 230–232˚C; Yield 77%, reaction time; 1 hour, solvent of crystallization: ethanol IR (cm-1): 1087(C=S), 3438 (NH), 1603(C=C), 3090 (=C- H), 2983(C-H), 716(C-Cl); ^{1}H-NMR (DMSO-d, 300 MHz): 5.26 (S, 1H, pyrimidine H-4), 5.74 (d, 1H, pyrimidine H-5), 6.79-7.99 (7H, m.Ar-H and thiophene-H), 8.29(brs, 2H,NH) (Figure 3).

Compound (A5) 4-(4-hydroxyphenyl)-6-(thiophen-2-yl)-3,4-dihydropyrimidin-2-(1H) one.

Brown powder solid; m.p. 208–210˚C; Yield 75%,, reaction time; 1 hour, solvent of crystallization: ethanol IR (cm-1): 1628(C=O), 2957 (NH), 1588(C=C), 2870(=C-H),3103(OH). (Figure 4) ; ^{1}H-NMR (DMSO-d, 300 MHz): 4.02 (d, 1H, pyrimidine H-4), 5.59 (d, 1H, pyrimidine H-5), 6.25-8.0 5(7H, m.Ar-H and thiophene-H),8.87 (brs, 2H,NH),9.05(brs,1H,OH).

Compound (A6) 4-(3,4-dimethoxyphenyl)-6-(thiophen-2-yl)-3,4-dihydropyrimidin-2-(1H) thione.

Yield 85%, m.p. 285–287°C, reaction time; 1 hour, solvent of crystallization: ethanol, yellow needles. : 3300 (NH), 1187(C=S), 1590,1H NMR (400 MHz, DMSO-d6): 3.75, 3.77 (2s, 6H, 2OCH3), 4.37 (d, pyrimidine H-4), 6.10(d, 1H, pyrimidine H-5), 6.65-8.33 (6H, m.Ar-H and thiophene-H),, 9.81 (brs, 1H, NH), 11.15 (brs, 1H, NH).

Compound (A7) 4-(4-methylphenyl)-6-(thiophen-2-yl)-3,4-dihydropyrimidin-2-(1H) thione.

Yellow powder solid; m.p. 250-252˚C; Yield 80%, reaction time; 30 minutes, solvent of crystallization: ethanol IR (cm-1):, 3090 (NH), 1565(C=C), 3007 (=C- H), 2917(C-H), 1183(C=S); 1H-NMR (DMSO-d, 300 MHz): 4.00 (d, 1H, pyrimidine H-4), 6.72 (d, 1H, pyrimidine H-5), 6.74-8.61 (7H,m.Ar-H and thiophene-H), 11.25(brs, 1H,NH), 12.09(brs, 1H,NH). (Figure 5).

Compound (A8) 4-phenyl-6-(thiophen-2-yl)-3,4-dihydropyrimidin-2-(1H) thione.

Yellow needles solid; m.p. 270-272˚C; Yield 80%, reaction time; 45 minutes, solvent of crystallization: ethanol IR (cm-1):, 3190 (NH), 1560(C=C), 1117(C=S) ; 1H-NMR (DMSO-d, 300 MHz): 4.44 (d, 1H, pyrimidine H-4), 6.05 (d, 1H, pyrimidine H-5), 6.51-7.61 (8H,m.Ar-H and thiophene-H), 8.25(brs, 2H,NH).

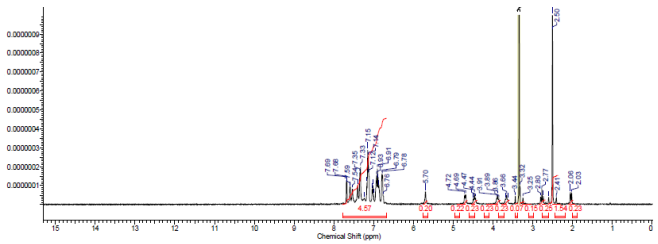


Figure 1: ^{1}H-NMR of compound A1.

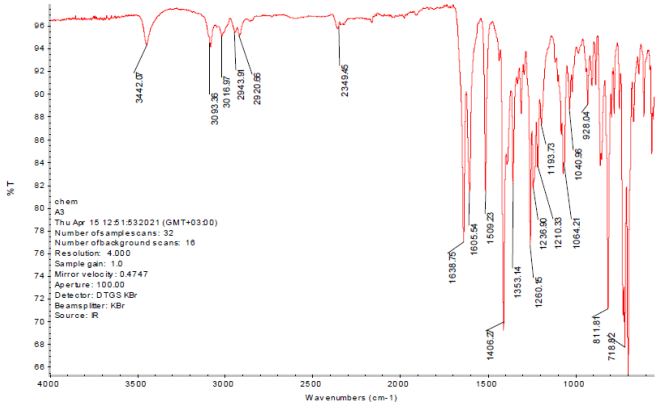


Figure 2: IR spectra of compound A1.

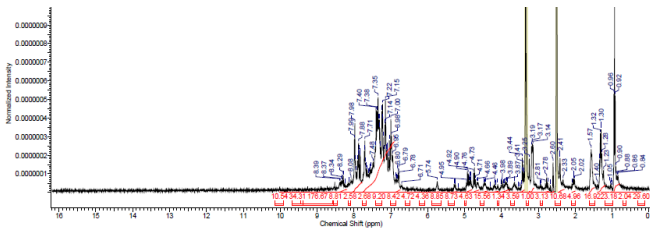


Figure 3: ^{1}H-NMR of compound A4

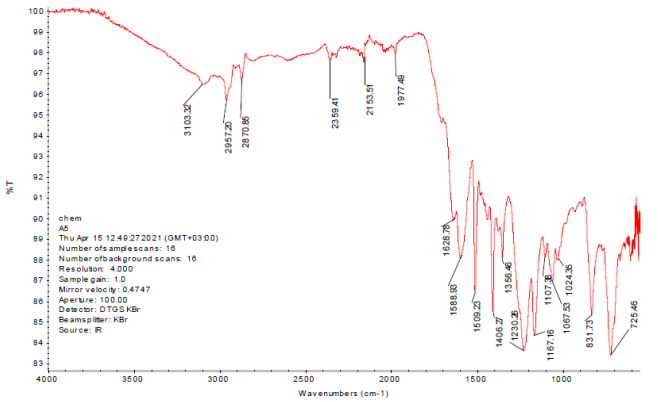


Figure 4: IR spectra of compound A5.

Table 1: The *in vitro* antioxidant activity of compounds(A1-A9) in DPPH method ranked (highest to lowest).

	DPPH inhibition (%)				
Compound	*500 µg/mL*	*250 µg/mL*	*125 µg/mL*	*62.5 µg/mL*	*IC50*
A6	48	40	33	24	14
A8	39	35	31	20	23
Ascorbic acid	96	95	92	64	109
A4	55	53	48	44	141
A3	73	72	71	70	176
A7	92	77	46	45	223
A1	48	14	1.0	2.0	266
A5	54	50	47	46	304
A2	77	73	71	70	1725
A9	70	58	56	50	9987

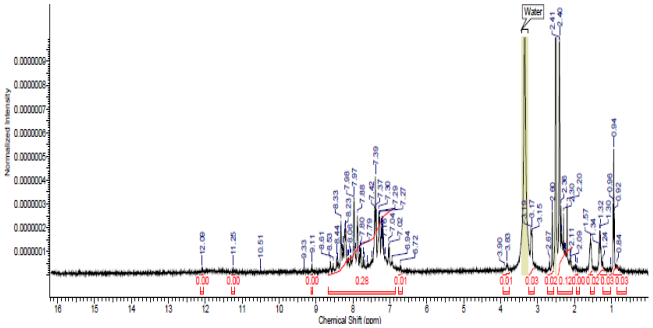


Figure 5: 1H-NMR of compound A7

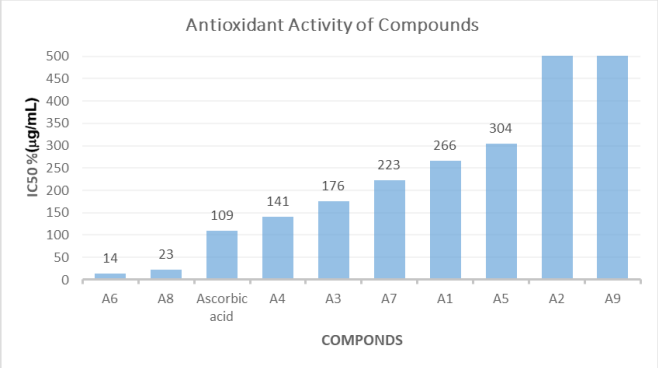


Figure 6 : Potential inhibition capacities of the synthesized compounds (A1-A9)

Compound (A9) 4-(furan-2-yl)-6-(thiophen-2-yl)-3,4-dihydropyrimidin-2-(1H) one.

Brown powder solid; m.p. 235-237˚C; Yield 70%, reaction time; 30 min., solvent of crystallization: ethanol ; IR (cm-1): 1640(C=O), 3430 (NH), 1601(C=C), 3080(=C-H), 2960(C-H) ; 1H-NMR (DMSO-d, 300 MHz): 4.7 (d, 1H, pyrimidine H-4), 5.61 (d, 1H, pyrimidine H-5), 6.56-7.83 (6H, m.Furan-H and thiophene-H),8,70 (brs, 2H,NH).

Antioxidant Activity of Synthetic Pyrimidine Derivatives

The formation of free radicals causes many chronic diseases such as cancer, diabetes, and cardiovascular disorders.[33] One of the most important characteristics of antioxidants is their ability to eliminate free radicals. According to scientific research, antioxidants reduce the risk of many chronic diseases. Many foods, fruits and vegetables contain natural antioxidants and have tremendous nutritional and therapeutic properties.[34] While the second type of antioxidants are those that are prepared in the laboratory The most important synthetic antioxidant are butylated hydroxyl anisole (BHA), butylated hydroxy toluene (BHT), and 1, 1-diphenyl-2-picrylhydrazyl (DPPH).

Radical scavenging activity of compounds (A1-A9) is summarized in Table 1. Better radical scavenging activity was observed for compound A6(IC50= 14 µg/mL) and A8 (IC50=23 µg/mL) than other compounds when compared to ascorbic acid (IC50= 109µg/mL), a known and potent antioxidant compound. Because of electron donating substituent such as methoxy group in A6 enhances the activity.[35] The compounds A3 and A4 showed moderate antioxidant, whereas the compounds A1, A5, and A7 exhibit mild activity. While the last two compounds A2 and A9 did not show any significant activity (Table 1 and Figure 6).

CONCLUSION

The present work is concentrating on a facile procedure for the synthesis of a series 4,6-diaryl-3,4-dihydropyrimidine-2(1H)-thione and 4,6- diaryl-3,4-dihydropyrimidine-2(1H)-one under PTC. This method depends on the reaction of thienylchalcone with urea and thiourea under basic conditions and provides products with good yield in a brief time. All synthetic compounds were studied for their antioxidant activity. It has been observed that the presence of substitutes affects the activity of the compound. All the compounds except A6, A8 exhibited lower inhibition. Electron donating methoxy group in A6 showed higher antioxidant activity compared to other compounds in the series.

REFERENCES

1. Okesli A, Khosla C, Bassik MC. Human pyrimidine nucleotide biosynthesis as a target for antiviral. chemotherapy. Curr. Opin. Biotechnol. 2017; 48: 127-134. doi: 10.1016/j.copbio.2017.03.010.
2. El-Gazzar AR, Hussein HA, Hafez HN. Synthesis and biological evaluation of thieno [2, 3-d] pyrimidine derivatives for anti-inflammatory, analgesic and ulcerogenic activity. Acta Pharm 2007; 57(4): 395–411. DOI: 10.2478/v10007-007-0032-6.

3. Sherif AF, Hayam MA, Heba A. Synthesis and biological evaluation of some novel polysubstituted pyrimidine derivatives as potential antimicrobial and anticancer agents. Arch. Pharm. 2009; 342(5): 299–310. DOI: 10.1002/ardp.200800223.
4. Hilmy KM, Khalifa MM, Hawata MA, Keshk RM, el- Torgman AA. Synthesis of new pyrrole [2,3-d] pyrimidine derivatives as antibacterial and antifungal agents. Eur.J. Med. Chem. 2010; 45(11): 5243–5250. DOI: 10.1016/j.ejmech.2010.08.043.
5. Verbitskiy EV, Baskakova SA, Kungurov NV, KravchenkoMA, Skornyakov SN, Pervova MG, Rusinov GL, Chupakhin ON, Charushin VN. Synthesis and biological evaluation of novel 5-aryl-4-(5-nitrofuran-2-yl)- pyrimidines as potential anti-bacterial agents. Bioorg.Med. Chem. Lett 2017; 27(13): 3003-3006. DOI: 10.1016/j.bmcl.2017.05.013.
6. Azeredo LFS, Coutinho JP, Jabor VA, Feliciano PR,Nonato MC, Kaiser CR, Menezes CMS, Hammes AS,Caffarena ER, Hoelz LV. Evaluation of 7-arylaminopyrazolo [1, 5-a] pyrimidines as anti-Plasmodium falciparum, antimalarial, and Pfdihydroorotatedehydrogenase inhibitors. Eur. J. Med.Chem. 2017; 126: 72-83. doi.org/10.1016/j.ejmech.2016.09.073.
7. Sun L, Jie W, Zhang L, Luo M, Sun D. Synthesis andantifungal activities of some novel pyrimidine derivatives. Molecules 2011; 16: 5618–5628. doi.org/10.3390/molecules16075618
8. Mohamed MS, Awad SM, Sayed AI. Synthesis of certain pyrimidine derivatives as antimicrobial agents and antiinflammatory agents. Molecules 2010; 15(3): 1882–1890. doi.org/10.3390/molecules15031882.
9. Selvam TP, James CR, Dniandev PV, Valzita SK, A mini review of pyrimidine and fused pyrimidine marketed drugs. Journal of Research in Pharmacy Practice. 2012; 2(4)
10. Tokunaga S, Takashima T, Kashiwagi S, Noda S, Kawajiri H, *et al*. () Neoadjuvant Chemotherapy with Nab-paclitaxel Plus Trastuzumab Followed by 5 Fluorouracil/Epirubicin/ Cyclophospha mide for HER2-positive Operable Breast Cancer: A Multicenter Phase II Trial.Anticancer Res. 2019; 39(4): 2053-2059. DOI: 10.21873/anticanres.13316.
11. Ghorab MM, Alsaid MS.Anticancer activity of some novel thieno [2, 3-d] pyrimidine derivatives. Biomedical Research. 2016; 27(1): 110-115.
12. Wilhelm M, Mueller L, Miller MC, Link K, Holdenrieder S, *et al*. Prospective, multicenter study of 5-fluorouracil therapeutic drug monitoring in metastatic colorectal cancer treated in routine clinical practice. Clin Colorectal Cancer. 2016 ; 15(4): 381-388. DOI: 10.1016/j.clcc.2016.04.001.
13. Kalirajan R, Sivakumar SU, Jubie S, Gowramma B, Suresh B.Synthesis and biological evaluation of some heterocyclic derivatives of chalcones. Int J ChemTech Res. 2009 ; 1:27–34.
14. Talia JM, Debattista NB, Pappano NB.New antimicrobial combinations substituted chalcones-oxacillin against methicillin resistant Staphylococcus aureus. Braz J Microbiol. 2011; 42:470–475. DOI:10.1590/S1517-83822011000200010.
15. Do TH, Nguyen DM, Truong VD, Do THT, Le MT, Pham TQ, Thai KM, Tran TD.Synthesis and selective cytotoxic activities on rhabdomyosarcoma and noncancerous cells of some heterocyclic chalcones. Molecules. 2016 ; 21(3):329. https ://doi.org/10.3390/ molecules2 10303 29.
16. Babasaheb PB, Sachin AP, Rajesh NG.Synthesis and biological evaluation of nitrogen containing chalcones as possible anti-inflammatory and antioxidant agents. Bioorg Med Chem Lett. 2010 ;20:730–733. DOI: 10.1016/j.bmcl.2009.11.068.
17. Vogel S, Barbic M, Jürgenliemk G, Heilmann J (2010) Synthesis, cytotoxicity, anti-oxidative and anti-inflammatory activity of chalcones and influence of A-ring modifications on the pharmacological effect. Eur J Med Chem 45:2206–2213. DOI: 10.1016/j.ejmech.2010.01.060.
18. Sivakumar PM, Prabhakar PK, Doble M.Synthesis, antioxidant evaluation, and quantitative structure–activity relationship studies of chalcones. Med Chem Res. 2011 ; 20:482–492. https:// doi.org/10.1007/s00044-010-9342-1.
19. Kulandasamy R, Adhikari AV, Stables JP.A new class of anticonvulsants possessing 6Hz activity: 3,4-dialkyloxy thiophene bishydrazones.Eur J Med Chem 2009;44(11):4376–4384. DOI: 10.1016/j.ejmech.2009.05.026.
20. Lu X, Wan B, Franzblau SG, You Q.Design, synthesis and antitubercular evaluation of new 2-acylated and 2-alkylated amino-5-(4-(benzyloxy) phenyl)thiophene-3-carboxylic acid derivatives. Part 1. Eur JMed Chem. 2011 ; 46(9):3551–3563. DOI: 10.1016/j.ejmech.2011.05.018.
21. Safaei-Ghomi, J.; Ghasemzadeh, M. A. Preparation of 4,6-Diaryl-3,4-Dihydropyrimidine-2(1H)-Thiones in an Ionic Liquid. Org. Prep. Proc. Int. 2012; 44, 527–531. DOI: 10.1080/00304948.2012.730944.
22. Al-Hajjar, F. H.; Al-Farkh, Y. A.; Hamoud, H. S. Synthesis and Spectroscopic Studies of the Pyrimidine-2(1H)Thione Derivatives. Can. J. Chem. 1979; 57, 2734–2742. DOI: 10.1139/v79-442.
23. Simon, D.; Lafont, O.; Farnoux, C. C.; Miocque, M. Obtention D' Heterocycles Par Action De Binucleophiles Diazotes Sur Des Chalcones: Influence Du Substituant En Position 2.J. Heterocycl. Chem. 1985; 22, 1551–1557. DOI: 10.1002/jhet.5570220617.
24. Sahu, M.; Siddiqui, N.; Sharma, V.; Wakode, S. 5,6-Dihydropyrimidine-1(2H)-Carbothioamides: Synthesis, in Vitro GABA-AT Screening, Anticonvulsant Activity and Molecular Modelling Study. Bioorg. Chem. 2018; 77, 56–67. DOI: 10.1016/j.bioorg.2017.12.031.
25. Asaad M. Mustafa, Mohammed S. Hussein, Alarqam Z. Tareq,Synthesis of some 2-Pyridones by Application of L-L Phase Transfer Catalysis Method. Materials Science Forum.2020; Vol. 1002, pp 448-456. DOI:10.4028/www.scientific.net/MSF.1002.
26. D. Albanese. Liquid–liquid phase transfer catalysis: basic principles and synthetic applications, Cata. Rev.: Sci. and Eng. 2003 ; 45:3-4 369-395.
27. G. Gokel, W. Weber. Phase transfer catalysis. Part I: General principles, J. Chem. Edu. 1978; 55 352.
28. A. J. Burke,, C. S. Marques, N. J. Turner, G. J. Hermann. Active Pharmaceutical Ingredients in Synthesis: Catalytic Processes in Research and Development''. 2018; first ed., Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.
29. Mazimba, O., Majinda, R.R., Masesane, I.B. An efficient synthesis of flavans from salicylaldehyde and acetophenone derivatives.Tetrahedr. 2011; Lett. 52 (50), 6716–6718.
30. E.Krein, Z.Aizenshat, Studies of heterocyclic compound. J. Org. Chem. 1993; (58) 6103 – 6108.
31. Brand-Williams W, Cuvelier ME, Berset C and Lebensm. W. Use of a free radical method to evaluate antioxidant activity. Technol. 1995; 28: 25-32.
32. Ghoneim K. M. and Youssef R., J. Indian Chem. Soc. 1986;(53) 914.
33. Boligon AA, Machado MM, Athayde ML. Technical evaluation of antioxidant activity. Med Chem (Los Angeles) Med Chem. 2014;4:517–522.

34. Sovilj MN. Critical review of supercritical carbon dioxide extraction of selected oil seeds. Acta Period. Technol. 2010;41:105–120.

35. Kikkeri N. Mohana a, Basavapatna N. Prasanna Kumar a,Lingappa Mallesha, Synthesis and biological activity of some pyrimidine derivatives. drug invention today. 2013;(5):216.