ISSN 2321-807X

Synthesis, Characterization and Antimicrobial Activityof Some Novel1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile Derivatives

Assist. Prof. Dr.Monther Faisal Mahdi

Department of pharmaceutical Chemistry, College of Pharmacy,Al-Mustanisriyah University

Email dr.monther.f71@uomustansiriyah.edu.iq ;dr.monther.f71@gmail.com

Abstract:

The present research work deals with synthesize a series of various substituted benzothiazole containing 6-amino- 4-(substituted aryl)-tetrahydropyrano[2,3-*c*]pyrazole-5-carbonitrilic by reacting 2-mercaptobenzothiazole with hydrazine hydrate to synthesize hydrazine derivatives of benzothiazole (1), which then reacted with β- keto ester to develop pyrazole derivative (2), and then finally reacted with malononitrile and different aromatic aldehydes to obtain the final compounds (3a-3f). The structures of the intermediates and final compounds are assigned by using FTIR and ^{1}H-NMR. The final compounds were screened for their antibacterial activity against four tested bacteria as G^{+} and G^{-}using a well diffusion method. The results of antibacterial activity exhibited that the species *Klebsiella pneumonia* was the most effected types, while *Pseudomonas aeruginosa* was least the effected in general.

Keywords: 2-mercaptobenzothiazole; pyranopyrazole; malononitrile; antibacterial.

Council for Innovative Research

Peer Review Research Publishing System

Journal: Journal of Advances in Chemistry

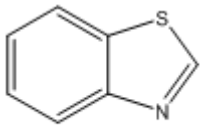
Vol 11, No. 6

editorjaconline@gmail.com, www.cirjac.com

ISSN 2321-807X

Introduction

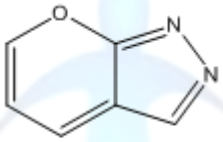
There are many biologically active bicyclic nucleus containing two heteroatoms. Benzothiazole (I) is used as a chemical intermediate in many organic synthesis. It is a component of cyanine dyes and a precursor of rubber accelerators. It is also used as a flavoring substance[1].



(I)

Benzothiazole derivatives are interesting compounds and their chemistry has received considerable attention [2&3] . Several derivatives are reported to have useful pharmacological effects, such as antimicrobial,[4-6] anti-inflammatory,[7&8]& antitumor.[9&10]

Pyranopyrazole (II) is fused heterocyclic comprised of pyran& pyrazole rings as an important scaffold which plays a significant role in pharmaceutical field & biologically active compounds.[11&12]



(II)

Compounds bearing pyranopyrazole system have been found to have many biological activities, which includes, antimicrobial,[13] antifungal,[14] anti-inflammatory,[15] analgesic,[16] anticancer,[17] & inhibitors of human Chk1 kinase.[18] Furthermore, some of these derivatives are commonly in employment like cosmetics & pigments.[19]

These high profile applications and biological significance of benzothiazole and pyranopyrazole containing compounds and the speedy development of bacterial resistant to conventional antibiotics[20] impelled us to continue working on the synthesis of new analogues of 1,4-dihydropyrano[2,3-c] pyrazole-5- carbonitrile and preliminary pharmacological evaluated as an antimicrobial agents.

EXPERIMENTAL

Materials and Instruments: Melting points were determined by usingGallenkampelectrical melting pointapparatus and are uncorrected. Materials and reagents were obtained from commercial suppliers (Merck grade) and were used without further purification. IR spectra were recorded in KBr discs on a Bruker analyzer. ^{1}H- NMR spectra were recorded on a Bruker (400 MHz) spectrometer, (chemical shifts in δ, ppm) in DMSO-d_6 using TMS as internal standard. The progress of the reaction was monitored by TLC using Silica Gel G (Merck).

2-hydrazino-1,3-benzothiazole (1)[21]

A mixture of 2-mercaptobenzothiazole (29.9 mmole, 5g) and hydrazine hydrate (29.9 mmole, 1.497g) was added and mixed thoroughly. The mixture was air dried and subjected to microwave irradiation for 1 minute (completion of reaction as indicated by TLC). The reaction mixture was cooled to room temperature and the separated solid was extracted with ethanol on standing the filtrated afforded colorless crystalline solid. The product was recrystallized from ethanol, $C_7H_7N_3S$,yield 89%. m.p 202-204^0 C.

1-(benzo[d]thiazol-2-yl)-3-methyl-1H-pyrazol-5(4H)-one(2)[22]

Method a : β- ketoester (5mmole, 0.655g) was added drop wise to corresponding 2- hydrazinobenzothiazole(5 mmole,0.825g), the mixture was irradiated with microwave (300 W) for 10min, the cold reaction mixture was treated with ethanol. The solid product was filtered; dried and recrystallized.

Method b: Solution of 2- hydrazinobenzothiazole (5 mmole, 0.825g) in ethanol (1ml) was added drop wise to the corresponding β- keto ester (5 mmole, 0.655g) contained in a 25ml conical flask. The mixture was irradiated in the water bath of an ultrasonic cleaner for 25min.The cold reaction mixture was treated with ethanol. The solid product was filtered and dried.

1-(benzo[*d*]thiazol-2-yl)-3-methyl-1*H*-pyrazol-5(4*H*)-one(2): $C_{11}H_9N_3OS$**,** orange powder; yield 90.13%; mp.150-152 ^{0}C**; IR (KBr, cm^{-1}):** 3113 ν(C-H , aromatic),2962,2895 ν(C-H , aliphatic), 2839 ν(CH_3) ,1737 ν(CO, ester), 1637 ν (C=N), 1595, 1496 ν(C=C), 1246 ν(C-N), 1076,1033,665 ν(C-S-C) ;**^{1}H-NMR (DMSO-d_6, 400 MHz) δ:** 2.007 (3H, s, CH_3), 2.238 (2H, s, CH_2), 7.266- 7.413 (2H ,d,Ar-H), 7.677 (2H ,d,Ar-H)

ISSN 2321-807X

Typical procedure for the synthesis of6-amino-1-(3a,7a-dihydrobenzo[*d*]thiazol-2-yl)-3-methyl-1,4,5,6-tetrahydropyrano[2,3-*c*]pyrazole-5-carbonitrilic derivatives (3a-3f)

Method a: Introduced(1.3 mmole,0.3 g) of compound **(2)** in to a clean, dry round bottom flask and dissolved in ethanol(4 ml).Then added (1.3 mmole,0.085g) malononitrile, and different aldehyde(1.3 mmole) with constant stirring. The reaction mixture was allowed to stir with slow addition of about 3 drops of piperidine and stirring was continued for 2 hrs. The solid thus separated was filtered off, washed with distilled water, driedand recrystallized from methanol.

Microwave method b [23]:

A mixture of (2) (1.3 mmole, 0.3 g), malononitrile (1.3mmole, 0.085g), substituted aromatic aldehyde (1.3 mmole) and sodium acetate (3 mmole, 0.231 g) in 10ml ethanol vessel. Introduced into microwave vessel, the reaction mixture was irradiated by300W for 6-10 min at 130^{o}C .the formed solid was filtered, washed with water and recrystallized from ethanol.

6-amino-1-(3a,7a-dihydrobenzo[*d*]thiazol-2-yl)-3-methyl-1,4,5,6-tetrahydropyrano[2,3-*c*]pyrazole-5-carbonitrilic (3a):yellow powder; **$C_{21}H_{15}N_5OS$**; yield 87.3%; mp.125^{0}C**; IR (KBr, cm^{-1}):** 3335 v(NH_2),, 3115 v(C-H , aromatic), 2960,2897v(C-H , aliphatic) , 2189 v (CN),1600 v (C=N), 1494 v(C=C), 1247 v(C-O-C) ;**^{1}H- NMR (DMSO-d_6, 400 MHz) δ:** 1.646 (3H, s, CH_3), ,3.008 (1H ,s, CH) , 6.899(2H,s,NH_2) , 7.270-7.399 (4H, m, benzothiazole ring) ,7.497-7.704 (5H , m, Ar-H).

6-amino-4-(4-chlorophenyl)-1-(3a,7a-dihydrobenzo[*d*]thiazol-2-yl)-3-methyl-1,4,5,6-tetrahydropyrano[2,3-*c*]pyrazole-5-carbonitrilic (3b) : $C_{21}H_{14}ClN_5OS$;pale brown powder; yield 79.04 %; mp.126-129^{0}C**; IR (KBr, cm^{-1})** 3385 v(NH_2), 3113 v(C-H , aromatic), 2960,2895 v(C-H , aliphatic) , 2227 v (CN), 1610 v (C=N), 1583 v(C=C), 1244 v(C-O-C);**^{1}H- NMR (DMSO-d_6, 400 MHz) δ:** 1.658 (3H, s, CH_3), 3.008 (1H ,s, CH) , 7.707(2H,s,NH_2), 7.327-7.564 (8H , m, Ar-H).

6-amino-1-(3a,7a-dihydrobenzo[*d*]thiazol-2-yl)-4-(4-hydroxyphenyl)-3-methyl-1,4,5,6-tetrahydropyrano[2,3-*c*]pyrazole-5-carbonitrilic (3c):$C_{21}H_{15}N_5O_2S$; brown powder; yield 87%;mp.120 ^{0}C**; IR (KBr, cm^{-1})**:3405 v(NH_2), 3196 v(C-H , aromatic), 2949,2897 v(C-H , aliphatic) , 2207 v (CN), 1614 v (C=N), 1518 v(C=C), 1247 v(C-O-C);**^{1}H NMR (DMSO-d_6, 400 MHz) δ:** 1.646 (3H, s, CH_3), 3.012 (1H ,s, CH) , 6.741(2H,s,NH_2), 7.1757.466 (4H, m, benzothiazole ring) ,7..685-7.872 (4H , m, Ar-H), 8.068(IH,s,OH).

6-amino-1-(3a,7a-dihydrobenzo[*d*]thiazol-2-yl)-4-(4-methoxyphenyl)-3-methyl-1,4,5,6-tetrahydropyrano[2,3-*c*]pyrazole-5-carbonitrilic (3d): $C_{22}H_{17}N_5O_2S$ dark brown powder; yield 71.44 %; mp.100-102 ^{0}C**; IR (KBr, cm^{-1})**: 3405 v(NH_2), 3066 v(C-H , aromatic), 2935,2897 v(C-H , aliphatic) ,2202 v (CN), 1608 v (C=N), 1512 v(C=C), 1253 v(C-O-C) ;**^{1}H NMR (DMSO-d_6, 400 MHz) δ:** 1.645 (3H, s, CH_3), 3.013 (1H ,s, CH) , 3.346 (3H,s,OCH_3) , 8.403 (2H,s,NH_2), 6.727-7.012 (4H, m, benzothiazole ring) ,7.497-8.041 (4H , m, Ar-H).

6-amino-1-(3a,7a-dihydrobenzo[*d*]thiazol-2-yl)-4-(4-nitrophenyl)-3-methyl-1,4,5,6-tetrahydropyrano[2,3-*c*]pyrazole-5-carbonitrilic (3e):$C_{23}H_{20}N_6OS$; dark brown crystals ; yield 83 .64%; mp.155^{0}C**; IR (KBr, cm^{-1}):** 3333 v(NH_2), 3113 v(C-H , aromatic), 2960,2897 v(C-H , aliphatic), 2854 v(CH_3) , 2195 v(CN), 1622 v (C=N), 1600 v(C=C), 1523,1348 v(NO_2), **^{1}H NMR (DMSO-d_6, 400 MHz) δ:** 1.618 (3H, s, CH_3), 3.013 (1H ,s,CH) , 8.403 (2H,s,NH_2), 6.727-7.012 (4H, m, benzothiazole ring) ,7.497-8.041 (4H , m, Ar-H).

6-amino-1-(3a,7a-dihydrobenzo[*d*]thiazol-2-yl)-4-(4-dimethylamino)phenyl)-3-methyl-1,4,5,6-tetrahydropyrano[2,3-*c*]pyrazole-5-carbonitrilic (3f):$C_{21}H_{14}N_6O_3S$; red crystals ; yield 94.84%; mp.173^{0}C**; IR (KBr, cm^{-1}):**3387,3306 v(NH_2), 3173 v(C-H , aromatic), 2956,2883 v(C-H , aliphatic), 2820 v(CH_3) , 2189 v(CN), 1645 v (C=N), 1606 v(C=C), 1396 v(N-CH_3), 1253 v(C-O-C) **^{1}H NMR (DMSO-d_6, 400 MHz) δ:** 1.645 (3H, s, CH_3), 2.201 (1H,t,CH) ,3.013 (1H ,d, CH) , 3.124 (6H,s,N(CH_3)$_2$) , 8.403 (2H,s,NH_2), 6.727-7.012 (4H, m, benzothiazole ring) ,7.497-8.041 (4H , m, Ar-H).

Antimicrobial activity:

The MIC were determined following the recommendations of the Clinical and Laboratory Standards Institute[24].

Bacterial strains:

Four species of bacteria were used in this study, two of them are gram positive (*Staphylococcus aureus* and *streptococcus pneumonia*), the others are gram negative (*Klebsiella pneumonia* and *pseudomonas aeruginosa*), the microorganisms were available in the bacterial labs of Department of biology/College of science /AL-mustansiriyah University. The species had previously been characterized by VITEK 1.

The synthesized compounds have been studied for their antimicrobial activity in vitro against four tested bacteria at concentrations of 62.5,125,250, and 500µg/ml, the DMSO which used pure, Ciprofloxacin and Ceftriaxone were used as a

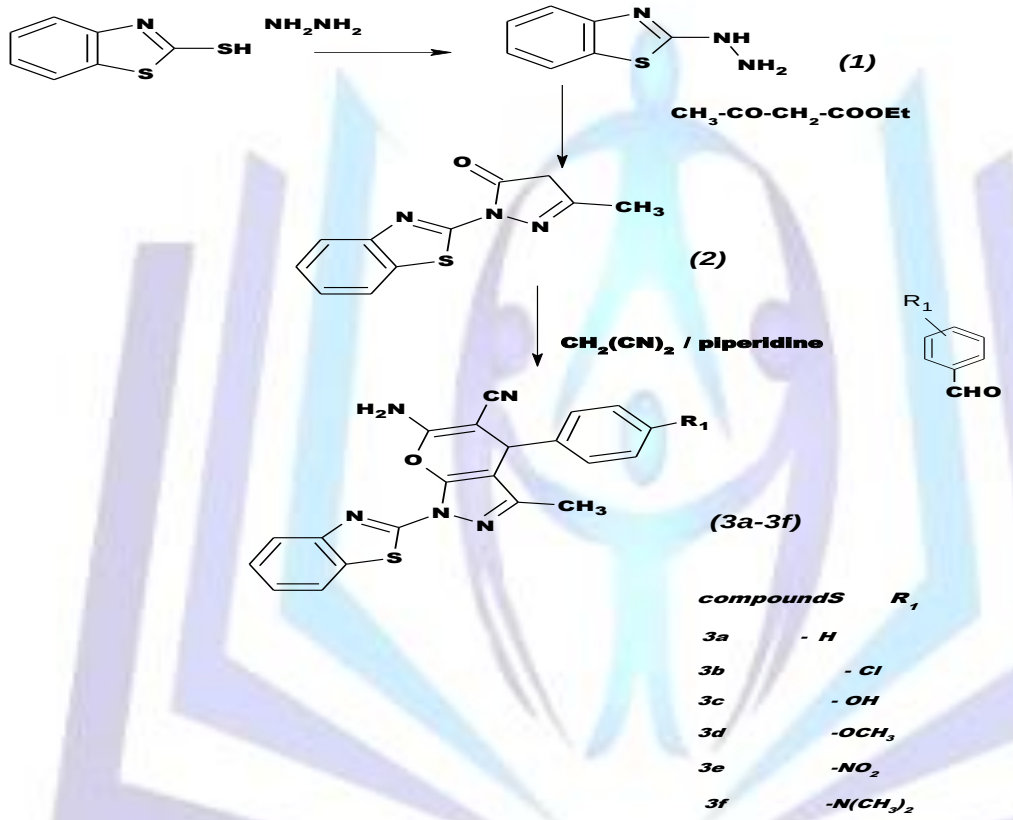
reference drug for antibacterial activity. They are clinically activated and maintained on nutrient agar medium for testing antibacterial.

Sensitivity Assay:

Well diffusion assay was carried out by using bacterial suspension of about (1.5×106 CFU/ml) with turbidity standard (number 0.5). This was used to inoculate by swabbing the surface of Mueller Hinton agar (MHA) plates. Excess liquid was air-dried under a sterile hood. In each agar plate of tested bacteria five wells were made and (100µl) of each concentration was added in it. The plates were incubated at 37 °C for 24 hours. The assessment of antibacterial and activity was based on measurement of the diameter of inhibition zone in (mm) formed around the well.

Results and Discussion

A simple, straightforward and highly efficient multicomponent one-pot synthesis of a pharmaceutically interesting diverse kind of functionalized 2-amino-3-cyano-4*H*-pyrans annulated heterocycles. The reaction occurs via tandem knoevenagel-cyclocondensation of aromatic aldehydes, malononitrile, and C-H-activated acidic compounds in ethanol at room temperature as shown in this scheme.



Scheme: Synthesis of final compounds (3a -3f)

The FT-IR spectrum of compound **(2)** clearly shows the disappearance of the band of SH group in starting material **(1)** which is a good indication for successful condensation. The spectrum also shows absorption bands at (1737 cm-1) referred to (C=O) stretching vibration of ketone, the spectrum also shows an absorption band at (3319, 3201 cm-1) referring to ($NHNH_2$) band Stretching, also an absorption band at (1651 cm-1) due to (C=N) stretching vibration.

The synthesis of 2-aminopyridine-3-carbonitrile derivatives has been accomplished *via* one-pot reaction, involving condensation of **1-(benzo[d]thiazol-2-yl)-3-methyl-1H-pyrazol-5(4H)-one** , several aromatic aldehydes and malononitrile in the presence of catalytic amount of piperidineby modified Knoevenagel reaction using microwave irradiation. The corresponding reactions proceeded smoothly and in excellent yields (85-95%).

The FT-IR spectrum of compound **(3a-3f),** shows absorption band at (2189-2227 cm^{-1}) which corresponds to (CN) stretching vibration which is a good indication for successful condensation, the spectrum also shows a band at (1600-1645 cm^{-1}) due to (C=N) stretching vibration, (3306-3405 cm^{-1})) for (NH_2) stretching vibration, The success of the reaction has been confirmed by disappearance of the (C=O) absorption band in the compound **(2)**.

The homogeneous mixture turns to precipitate by the addition of ethanol and led to the isolation of pureproducts in good yields. Under similar conditions, aromatic aldehydes bearing electron withdrawing & electron donating groups afford the corresponding 2-aminopyridine-3-carbonitrile derivatives in high yields and purity During the work-up of the reaction

mixture, first itwas cooled, and then adds ethanol to get precipitate. Filter it, dry and then recrystallize it with ethanol. Reaction time, % yield and melting points were depicted;all the products were characterized and confirmed by IR,and ^{1}H NMR data.

The results of antibacterial activity exhibited that the species *Klebsiella pneumonia* was the most effected as displayed in the diameter of inhibition zone of all concentrations used in the current study, while *pseudomonas aeruginosa* was less affected in general, illustrated in the following table.

Table: Antibacterial activity of DMSO, 2-mercaptobenzothiazole, Ciprofloxacin Ceftriaxone and compounds (Va-f) against tested bacteria

Compound No.	Concentration (µg/ml)	Inhibition Zone (mm)			
		Staphylococcus aureus	*streptococcus pneumonia*	*Klebsiella pneumoniae*	*pseudomonas aeruginosa*
Va	500.0	-	-	19	-
	250.0	-	4	21	-
	125.0	11	-	13	-
	62.5	11	19	11	-
Vb	500.0	16	10	19	-
	250.0	-	4	17	-
	125.0	-	-	20	-
	62.5	11	11	14	-
Vc	500.0	29	16	14	14
	250.0	18	-	13	14
	125.0	17	14	14	13
	62.5	10	12	12	18
Vd	500.0	17	16	12	-
	250.0	14	10	16	-
	125.0	11	13	16	-
	62.5	-	12	11	-
Ve	500.0	12	10	10	-
	250.0	-	13	10	-
	125.0	-	10	14	-
	62.5	-	10	13	-
Vf	500.0	27	11	29	-
	250.0	30	13	30	-
	125.0	32	-	25	-
	62.5	30	-	22	-
Ciprofloxacin	500.0	25	28	30	18
	250.0	25	15	32	23
	125.0	25	15	29	19
	62.5	25	14	24	13
Ceftriaxone	500.0	32	26	10	20
	250.0	28	19	-	19
	125.0	24	17	10	9
	62.5	28	16	4	14

DMSO	pure	-	-	-	-

The concentration 500 (µg/ml) was the most for killing bacteria, the results also showed that the others concentrations were less active against bacteria.

Conclusion:

In conclusion I have demonstrated a highly efficient method for the synthesis of novel pyranopyrazole derivatives using microwave method, the significant advantages of this procedure are simplicity, low cost and higher yields. These newly synthesized compounds were screened for antibacterial activity, and among them nitro containing derivative is the only one which exhibited antibacterial activity against *pseudomonas aeruginosa* and n-dimethyl derivative is the best one against *Klebsiella pneumonia*.

Acknowledgments

The author is thankful to assistant professor Dr. Ali H. Alwan Department of biology/College of science/Al-mustansiriyah University for his help in studying the antibacterial activity; also I'm grateful to the staff members and Colleagues of the department of Pharmaceutical Chemistry/ College of pharmacy/ Al-mustansiriyah University.

References

1. FEMA (1997) Monograph 3256: Benzothiazole, Flavor and Extract Manufacturers' Association, Washington, DC, 5 pp.
2. Vedavathi M., B. Somasheker, G. M. Sreenivasa, E. Jayachandran. Synthesis, characterization and anti-microbial activity of flouro benzothiazole incorporated with 1,3,4- thiadiazole. J Pharm. Sci. & Res.2010, 2(1), pp: 53-63.
3. Katritzky, A. R. Preface. New ring systems from 1,2-benzisothiazole-1,1-dioxides and related compounds. Tetrahedron, 1996, 52 (9), xiii.
4. Alang, G.; kaur, R.; Kaur, G.; Singh, A.; Singla, P. Synthesis and antibacterial activity of some new benzothiazole derivatives. Acta Pharm. Sci. 2010, 52, 213-218.
5. C. Franchini, M. Muraglia, F. Corbo, M. A. Florio, A. D. Mola, A. Rosato, R. Matucci, M. Nesi, F.V. Bambeke, C. Vitali, "Synthsis and Biological evaluation of 2-Mercapto-1,3-benzothiazole derivatives with potential antimicrobial activity" Arch. Pharm. Chem. Life Sci. 2009, 342, 605-613.
6. H. Kaur, S. Kumar, I. Singh, K. K. Saxena, A. Kumar, "Synthesis, Characterization and Biological activity of various substituted benzothiazole derivatives" Digest J. Nanomaterials and Biostructures. 2010, 5(1), 67-76.
7. Gurupadayya BM, Gopal M, Padmashali B, Vaidya VP, Synthesisand biological activities of fluorobenzothiazoles. IntJ HeterocyclicChem, 2005; 15: 169-172.
8. Srivastava SK, Yadav R, Srivastava SD. Synthesis and biological activity of 4-oxothiazolidines and their 5-arylidenes. Indian J Chem. 2004; 43B: 399–405.
9. Devmurari, V.P.; Shivanand, P.; Goyani, M.B.; Nandanwar, R.R.; Jivani, N.P.; Perumal, P. Synthesis and anticancer activity of some novel 2-substituted benzothiazole derivatives. *Int. J.Chem. Tech. Res*. **2010**, *2*, 681-689.
10. Repicky, A.; Jantova, S.; Cipak, L. Apoptosis induced by 2-acetyl-3-(6-methoxybenzothiazo)-2-yl-aminoacylonitrile in human leukemia cell involves ROS- mitochondrial mediated death signaling and activation of p38 MAPK. *Cancer Lett*. **2009**, *277*, 55-63.
11. Shaaani A, Sarvary A, Rezayan AH, Keshipour S; Synthesis of fully substituted pyrano [2,3-*c*] pyrazole derivatives via multicomponent reaction of isocyanides. Tetrahedron, 2009; 65: 3492-3495.
12. Tomar I, Mishra R; The molecule of diverse biological and medicinal importance. International Journal of Pharmaceutical Sciences and Research, 2011; 2:758-771.
13. Fadda AA, Abdel-Rahman AH, Hamed EA, Khalil EH; Utility of Enaminonitriles in Heterocyclic Synthesis: Synthesis and Antimicrobial Activity of Some New Azole and Azine Derivatives. American Journal of Organic Chemistry, 2012; 2: 7-13.
14. Ramiz, M. M. M.; Abdel Hafiz, I. S.; Abdel Reheim, M. A. M.; Gaber, H. M. Pyrazolones as Building Blocks in Heterocyclic Synthesis: Synthesis of New Pyrazolopyran, pyrazolopyridazine and Pyrazole Derivatives of Expected Antifungicidal Activity. *J. Chin. Chem. Soc*. 2012, 59, 72-80.
15. Zaki, M. E. A.; Saliman, H. A.; Hiekal, O. A.; Rashad, A. E. Pyrazolopyranopyrimidines as a Class of Anti-Inflammatory Agents. Z. Naturforsch., C: Biosci. 2006, 61, 1-5.

16. Kuo, S. C.; Huang, L. J.; Nakamura, H. Studies on heterocyclic compounds. Synthesis and analgesic and antiinflammatory activities of 3,4-dimethyl-pyra- 2,3-c]pyrazol-6-one derivatives. *J. Med. Chem.* 1984, 27, 539-544.

17. Peng-Cheng L, Zhu Hai L, Huan-Qiu L, Sun J, Zhou Y; synthesis and biological evaluation of pyrazolederivatives containing thiourea skeleton as anticancer agents. Bioorg Med Chem., 2010; 18: 4606–4614.

18. Foloppe, N.; Fisher, L. M.; Howes, R.; Potter, A.; Robertson, A.G.S.; Surgenor, A. E. Identification of chemically diver Chk1inhibitors by receptor-based virtual screening. Bioorg. Med. Chem. 2006, 14, 4792-4802.

19. Hafez, E. A. A.; Elnagdi, M. H.; Elagamey, A. G. A.; Ei-Taweel, F. M. A. A. Nitriles in Heterocyclic Synthesis: Novel Synthesis of Benzo[c]coumarin and of Benzo[c]pyrano[3,2-c]quinoline Derivatives. *Heterocycles 1987,* 26, 903-907.

20. E. N. Lorenzón, et al. Effects of Dimerization on the Structure and Biological Activity of Antimicrobial Peptide Ctx-Ha. Antimicrobial Agents and Chemotherapy.May 2015, Volume 59, Issue 5.

21. Rajiv Dua , S.K.Sonwane, S.K.Srivastava and S.D. Srivastava " *Geener and expeditious synthesis of 2-azetidinone derivative from 2- mercaptobenzothiazole and their pharmacological screening of the synthesized compounds using microwave irradiation"* world journal of chemistry 5(1): 52-56,2010.

22. AamalA.Al-Mutairi, FatmaE.M.Ei-Baih And Hassan M. Al-Hazimi" *Microwave Versus Ultrasound Assisted Synthesis Of Some New Heterocycles Based On Pyrazolone Moiety"* journal of Saudi chemical society (2010),14 ,287-299.

23. N.C.Desai, H.M. Satodiya, K.M.Rajpara, V.V.Joshi, H.V .Vaghani *"a microwave –assisted facile synthesis of novel coumarin derivatives containing cyanopyridine and furan as antimicrobial agents"* journal of Saudi chemical society (2014).

24. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standards, 6th ed. Document M7-A6. Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute, Wayne, PA, 2006.