Synthesis and Antimicrobial Evaluation of New 6 and7 Substituted Derivatives of Coumarin

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Abstract

A series of benzohydrazide derivatives attached to coumarin moiety at position 6 and 7 have been synthesized. The reaction of coumarin derivatives (coumarin I and II) with p-nitrophenyl hydrazine yield Schiff bases (compound1a and IIa_1).These Schiff bases were refluxed with benzoyl chloride to give benzohydrazide derivatives of coumarin substituted at its 6 or 7 nucleus position (Ia_1 and IIa_1).The reaction and the purity of the products were checked by thin layer chromatography (TLC). The structures of the final compounds and their intermediates were confirmed by their melting points, infra red spectroscopy, and elemental microanalysis(CHN).

Compounds (Ia1 and IIa1) were evaluated for their preliminary antibacterial and antifungal activities. Compound (IIa_1) has good antibacterial activity against *Staphylococcus aureus* other than bacterial species, and was equivalent to ofloxacin as (standard drug).

Key word: Coumarin, Schiff base, benzohydrazide derivatives, antimicrobial activity.

تحضير والتقييم البايولوجي لمشتقات الكومارين المعوضة في الموقعين ٦و٧ كمضادات للمايكروبات

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الخلاصة

تم تخليق سلسلة من مركبات البنزوهايدرازايد المتصلة بنواة الكومارين في الموقعين ٦و٧ . ان تفاعل مشتقات الكومارين (مركب (I) و (II) مع (*p*-Nitrophenyl hydrazine) اعطت قواعد شف, مركب ((1a) و(IIa)).لقد استعملت هده القواعد مع كلوريد البنزويل ليعطي مشتقات البنزوهايدرازايد للكومارين المعوض على نواته في الموقعين ٦و٧ (II_{a1} ,I_{a1}). لقد جرت مراقبة جميع التفاعلات والتاكد من نقاوة المركبات بواسطة كروموتغرافيا الطبقة الرقيقة, كما تم متابعة سير المركبات الوسطية والمركبات النهائية واثبات هويتها من خلال قياس درجات الانصهار والتحليل الطيفي للاشعة تحت الحمراء, والتحليل الدقيق للعناصر.لقد تم تقييم الفعالية المبدئية للمركبين(II_{a1},I_{a1}) ضد الجراثيم والفطريات,حيث وجد ان المركب (II_{a1}) يمتلك فعالية جيدة ضد *Staohylococcus aureus* دون غيرها من اصناف البكتريا وكان مكافئا لفعاية الاوفلوكساسين كدواء قياسي.

الكلمات المفتاحية: الكومارين، قواعد شف ، مشتقات البنزوهايدرازايد ، الفعالية المضادة للجراثيم .

Introduction

Coumarins (2H-1benzopyran-2ones) are important oxygen containing fused heterocycles used in drugs and dyes. Coumarins bound their class name to "coumarou" the vernacular name of *Tonaka bean*, from which coumarin itself was isolated in 1820.Coumarin and its derivatives represent one of the most active classes of compounds possessing a wide spectrum of biological activity [(1-3)].Novobiocin and chlorobiocin are established antimicrobials containing a coumarin skeleton [(4)].Many of these compounds have been proven to be active as,antibacterial[(5-7)], antifungal[(8)],anti-Inflamatory[(9)],anticoagulant[(10)] and anti tumor agents[(11)].

Azomethine group (C=N) containing compounds typically known as Schiff bases have been synthesized by condensation of primary amines with active carbonyl form. Schiff bases from a significant class of compounds in medicinal and pharmaceutical chemistry with several biological applications that induce antibacterial and antifungal activities [(12-16)]. It's known that Schiff bases react smoothly with acid chloride to give the corresponding addition products [(17-21)].

Experimental section:

1. Chemicals:

The specific chemicals used in this work are supplied from HIMEDIA, Fluka and SIGMA companies.All the solvents were of annular grade and used without further purification.

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***2. Detection Equipments**:*

Melting points were determined by capillary tube method on Banested (UK). The FTIR spectra were recorded on FTIR spectrophotometer, Shimadzu (Japan) at the college of Science(Al-Mustansaryia University).The CHN microanalysis were carried out using Euro A(Elemental Analyzer), (Italy) performed in the college of Science-Al-Mustansaryia University. Thin layer chromatography (TLC) was run on silica gel GF_{254} (60), Merck (Germany) to check both purity of compounds and their reactions progress. The antimicrobial study of the synthesized final products was done in Al-Kindy college of Medicine-University of Baghdad.

Synthesis:

A. Synthesis of 4-methyl -2 – oxo -2H-chromene-6-carbaldehyde (compound I):

Methyl acetoacetate (0.28 mole, 30ml)was mixed with re-distilled trimethyl orthoformate (0.27mole,30 ml) in dry methanol (25)ml and then conc. HCl (0.3ml) was added. The mixture was distilled immediately to obtain methyl-β-methoxy crotonate. Later polyphosphoric acid 100gm was added to a mixture of *p*-hydroxy benzaldehyde (0.05mole ,6.2 gm) and methyl-β- methoxy – crotonate (0.055 mole, 8 gm).The mixture was stirred for 90 minutes on steam bath at 80-85 °C and then poured into ice and water. The solid product was collected, washed and dried. Re-crystallization was from ethanol [22].

Percent yield, physical appearance, m.p., R_f values are listed in table (1),
While the IR characteristic absorption bands are listed in table (2).

***B. Synthesis of 4- methyl -6 - (2 - (4-nitrophenyl) hydrazano) methyl) -2H-chromene-2-one (compound Ia*)**

Equimoles of compound (I) (0.01mole ,1.88gm) and *p*-nitrophenyl hydrazine (0.01mole,1.53gm) were dissolved in 25 ml of methanol, to the resulting mixture 4-5 drops of piperidine was added and the mixture was refluxed for 10 hours. After completing the reaction, the mixture was poured into crushed ice. The product obtained was filtered and purified from methanol [23].

Percent yield, physical appearance, m.p., and R_f values are listed in table (1) and the IR characteristic absorption bands are listed in table (2) and the elemental micro analysis data are listed in table (3).

C. Synthesis of N- (chloro (4- methyl -2 - oxo-2H- chromene - 6 -yl) methyl)-N'-(4-nitrophenyl) benzohydrazide (compound I_{a1}).

Benzoyl chloride (0.01 mole, 1,2ml) was added drop wise to a solution of equimolar quantity of compound (Ia), (0,01 mole,3.23gm),dissolved in 1,4 dioxane(15 ml) and the mixture was refluxed for 6-8 hrs. Later the mixture was poured into ice with stirring. The product was filtered and purified from ethanol [24], Percent yield, physical appearance, m.p.,and R_f values are listed in table (1),IR characteristic absorption bands are listed in table (2) and the elemental microanalysis data are listed in table (3).

D. Synthesis of 4-methyl-2-oxo-2H-chromene-7-carbaldehyd (compound II).

A flask containing (45) ml conc. sulfuric acid was immersed in an ice bath and the temperature was kept below 10°C. A solution of (0,1 mole,12.2 gm) of 3-hydroxy benzaldehyde in (0.1 mole, 1.2 ml) of methyl acetoacetate was then added, and the temperature should be kept below 10°C during the time of addition. Later the reaction mixture was kept at R.T for 48 hrs. after which, it was poured into a flask containing a mixture of ice and water, and the formed solid product was filtered off, washed with water, and re-crystallized from ethanol [25].

Percent yield, physical appearance, m.p., and R_f values are listed in table(1), while the IR characteristic absorption bands are listed in table (2).

E. Synthesis of 4-methyl-7-((2-(4-nitrophenyl) hydrazono) methyl)-2H-chromen-2-one (compound IIa).

To a solution of compound (II), (0.01 mole,1.88gm) in absolute ethanol (25 ml), an equimolecular amount of *p*-nitropheny hydrazine was added(0.01 mole,1.53 gm), to the above solution and a catalytic amount of piperidine (4-5) drops. The reaction mixture was heated under reflux for 10 hrs. It was then cooled at R.T, poured into crushed ice, filtered and purified from methanol [26].

Percent yield, physical appearance, m.p., and R_f values are listed in table (1), While the IR characteristic absorption bands are listed in table (2).

F. Synthesis of N-(Chloro(4-methyl-2-oxo-2H-chromene-7-yl)methyl)-N'-(4-nitro phenyl) benzohydrazide (compound II a_1):

Compound (IIa), (0.01 mole,3.23 gm) was dissolved in 1,4-dioxane (15 ml) and to which (0.01 mole,1.2 ml) of benzoyl chloride was added dropwise, and the mixture was refluxed for 6-8 hrs. Afterwhich, the mixture was poured into ice with stirring. The product

obtained was filtered and purified from ethanol [24].

Percent yield, physical appearance ,m.p., and the R_f values are listed in table (2).The elemental microanalysis data are listed in table (3).

Table (1) The physical appearance, percent yield, melting points and R_f values of the synthesized compounds and their intermediates.

Compd.	Physical appearance	Yield %	Observed melting point °C	Rf value	Solvent system
I	Dark brown crystal	63%	185-188	0.8	Ethanol 8: Benzene 2
Ia	Light brown crystal	77%	207-210	0.88	Petroleum ether(40-60°C) 5: Ethyl acetate 5
Ia_1	Light walnut powder	89%	110-112	0.84	Ethanol 8: Benzene 2
II	Dark brown crystal	58%	194-196	0.89	Petroleum ether(40-60°C) 5: Ethyl acetate 5
IIa	Light brown powde	77% 93	203-205	0.86	Ethanol 8: Benzene 2
IIa_1	Light walnut powder		115-118	0.83	Ethanol 8: Benzene 2

Table (2) The IR characteristic absorption bands of the synthesized compounds

Compd.	IR characteristic absorption bands v` (cm^{-1})
I	(1724) cm^{-1} for C=O of pyran ring and (1710)cm^{-1} for C=O stretching of aldehyde group
Ia	(1600) cm^{-1} for C=N stretching, (3400)cm^{-1} for N-H stretching and (1446) cm^{-1} and (1370) $cm^{-1}$for NO_2 stretching
Ia_1	(1687) cm^{-1} C=O of amide group and (781) cm^{-1} for C-Cl
II	(1707)cm^{-1} for C=O of pyran ring and (1695)cm^{-1} for C=O stretching of aldehyde group
IIa	(1579) cm^{-1} for C=N stretching,(3392)cm^{-1} for NH-stretching and (1516) cm^{-1} and (1385) cm^{-1} for NO_2 stretching
IIa_1	(1687) cm^{-1} for C=O of amide group and (808) cm^{-1} for C-Cl

Table (3) The elemental microanalysis of the products Ia_1 and IIa_I

Compd.	Value type	C%	H%	N%	M.wt.
Ia_1	Calculated Observed	62.1 59.3	3.89 3.84	9.07 9.11	463.5
IIa_1	Calculated Observed	62.1 59.72	3.89 3.87	9.07 9.34	463.5

Antimicrobial Activity

A preliminary antibacterial and antifungal activity have been carried out according to Well Diffusion method.

The prepared compounds have been studied for their antimicrobial activity *in vitro* against three tested bacteria (*Staphylcoccus aureus*, *Streptococcus spp*., as Gram positive bacteria and *Proteus spp*., As Gram negative bacteria, and fungi (*Candida spp*.), were clinically activated and maintained on nutrient agar medium for testing antibacterial activity and sabouraud dextreose agar medium for antifungal activity. Ofloxacin was used a standard drug for antifungal activity. The plates were incubated at 30°C for 72 hrs., for

the (fungal *spp*.), or 37°C for 24 hrs., for the bacterial *spp*.)[27].

The antimicrobial activity was evaluated by measuring the diameter of inhibition zone around the disk in (mm), as shown in table (4) and (5) respectively.

Table (4) The antibacterial activity of the tested compounds.

Compd.	Concentration µg/ml	Zone of inhibition in mm	Zone of inhibition in mm	Zone of Inhibition in mm.
		Staphylococcus aureus spp.	*Streptococcus spp.*	*Proteus spp.*
Ia1	3 µg/ml 25 µg/ml 60 µg/ml	1 5 9	2 4 8	No activity No activity No activity
IIa1	3 µg/ml 25 µg/ml 60 µg/ml	4 8 14	2 6 12	No activity No activity No activity
Ofloxacin	3 µg/ml 26 µg/ml 60 µg/ml	8 10 16	7 13 18	6 11 17

Table (5) The antifungal activity of tested compounds

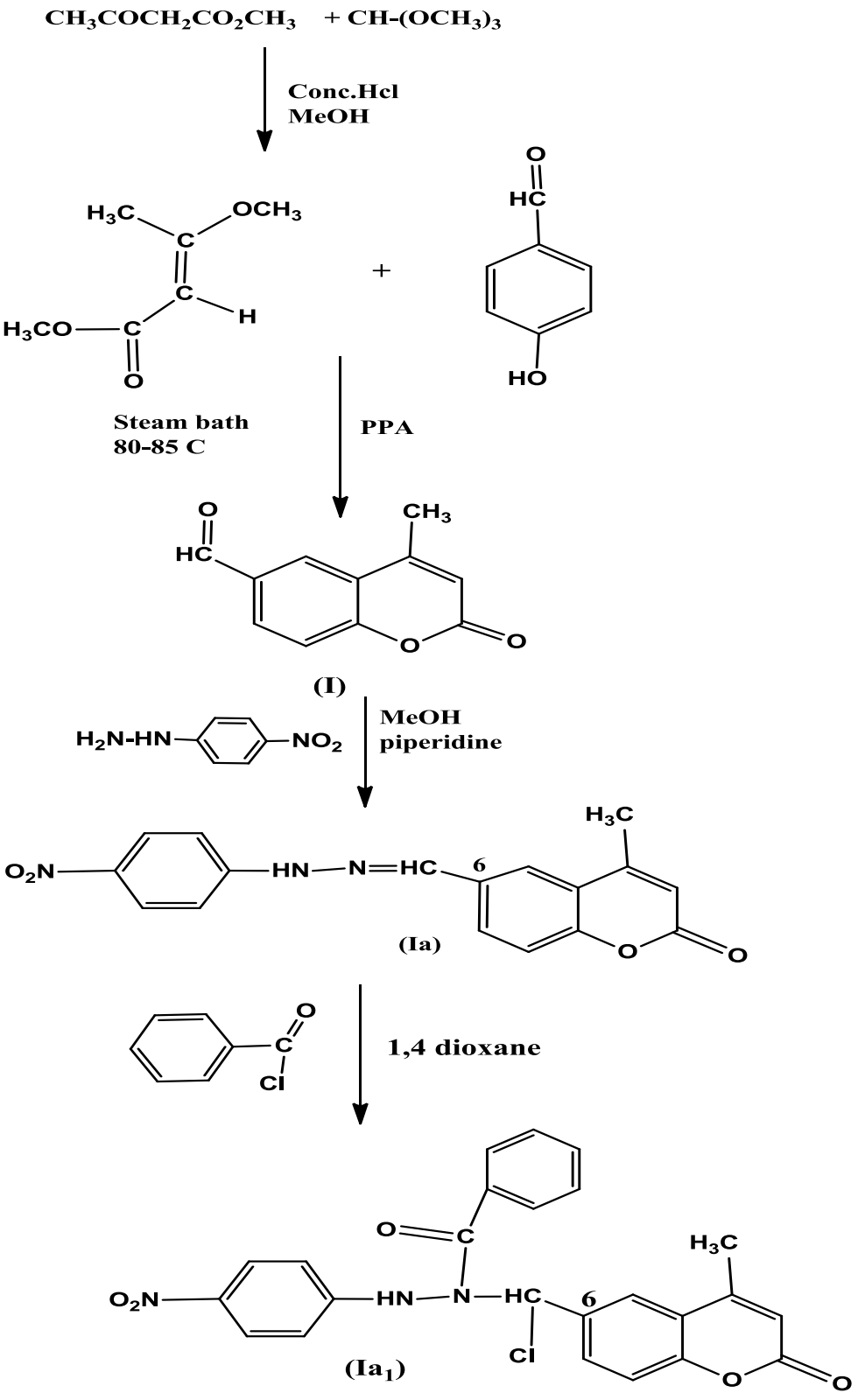
Compd.	Concentration µg/ml	Zone of inhibition in mm *Candida spp.*
Ia1	3 µg/ml	7
	25 µg/ml	9
	60 µg/ml	16
IIa1	3 µg/ml	6
	25 µg/ml	4
	60 µg/ml	12
Ketoconazole	3	11
	25	27
	60	30

Results and Discussion

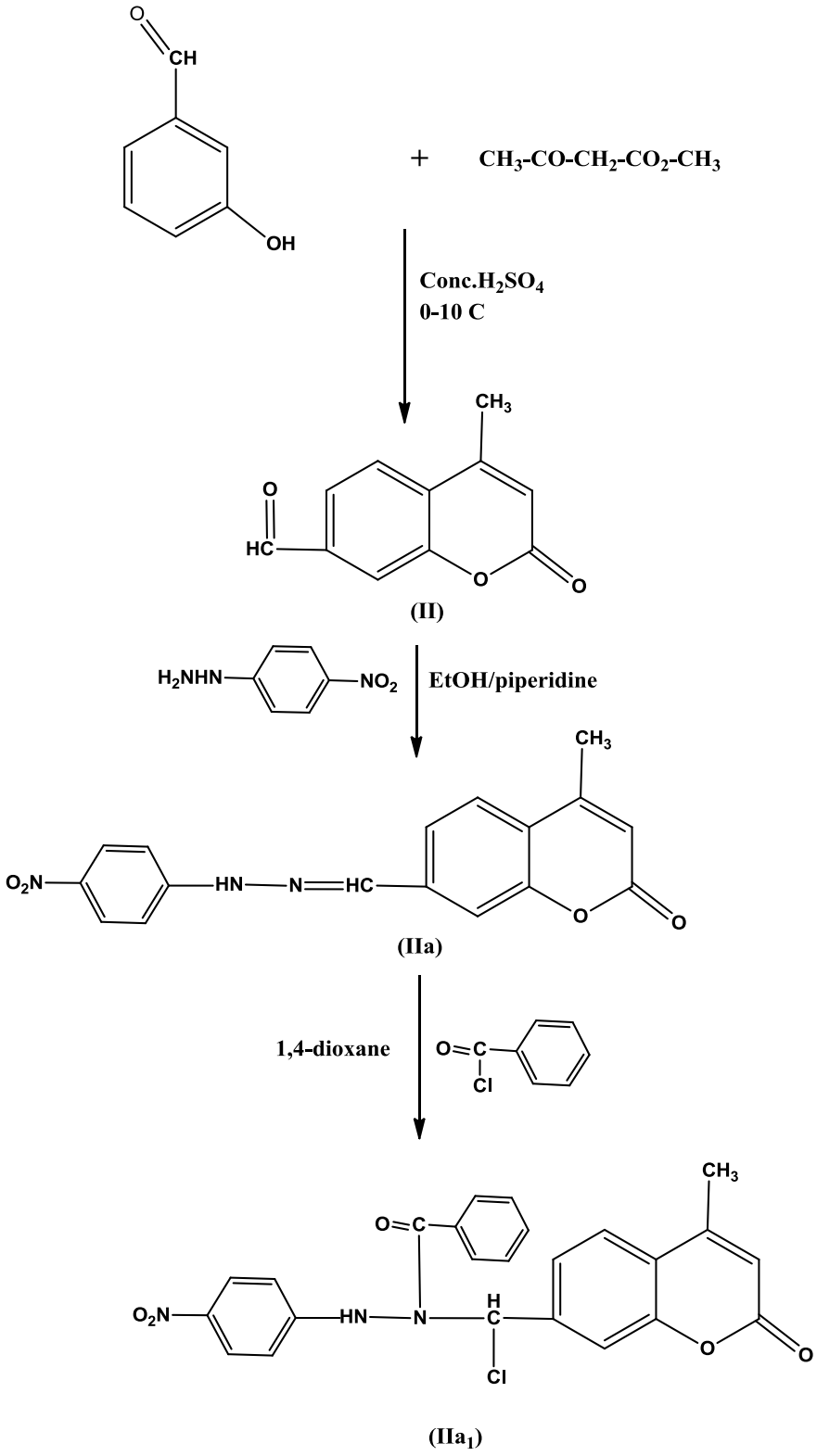
The present investigation involves the synthesis of new series of compounds involving the coumarin moiety as shown in scheme 1 and scheme 2; the pathway of synthesis of coumarin derivatives that have a characteristic aldehyde group substituted at the 6 or 7 positions of the coumarin nucleus as shown in compound (I) and (II).

Schiff bases (1a,IIa) were prepared by condensation of compound (I) and (II) with *p*-nitrophenyl hydrazine respectively using piperidine as catalyst; piperidine will increase the nucleophilicty of the amine group, and the reaction when followed by IR, showed the appearance of a characteristic absorption bands for (C=N) and (N-H), and disappearance of the band for (C=O) of aldehyde group as shown in table 2. The reactions between benzoyl chloride and Schiff bases are type from addition to the (C=N) moiety, and the reactions when followed by IR; illustrated the disappearance of (C=N) band, and the appearance of (C=O) of amide band and the appearance of (C-Cl) band as shown in table 2.

The antimicrobial activities (antibacterial and antifungal activities) have been carried out according to Well Diffusion methodology using ofloxacin and ketonazole as a standards respectively. All the results are fixed in table 4 and table 5.



Scheme 1: pathway of synthesis of compound (Ia_1)



Scheme 2: Pathway of synthesis of compound ($IIa_{1)}$

References

1. H.Zuo, G.Jose,Z Boli, B Hynumoon, D-Soo shin, and M Ghate, Microwave-assisted synthesis of fluorinated coumarino sulfonamide, Arkivok 2008,2,193-189
2. S Lee; K Sivakumar, W Seobshin, F Xie, and Q Wang, Synthesis and angiogenesis activity of coumarin derivatives, Biorg. and Medi. Chemi. Letters, 2006;16; 4596-4599 .

3. K Moghadam, and M Mohseni , A route to synthesis of novel coumarins, Montash Chem., 2004; 135: 817-821

4. SV Dekic, VS Dekick, BR Dekic andMS Dekick, Synthesis of new condensed and cyclized coumarin derivatives, Chem. Pap., 2007; 61(3) 233-235.

5. AM El-saghier and A Khodiyar, New synthetic approaches to condensed and spirocoumarins: Coumarin-3 thiocarba-xomide as building block for the synthesis of condensed and Spiro coumarins Phosphprous, Sulfur and Silicon ,2000;160:105-119.

6. B Musicki ,AM Periers, P Laurin;et.al., Improved antibacterial activity of coumarin antibiotics bearing 5',5'-dialyl noviose: biological activity of R479115, Bioorg.Med.Lett.,2000;10:1695-1699.

7. J Azizian, Ali A Mohammadi, I Bidar, and P Mirazaei, $KAl(SO_4)_2.12H_2O$(alum) a reusable catalyst for the synthesis of some 4-substiuted coumarins via Pechmann reaction under solvent free conditions, Montash Chem.,2008;139: 805-808 .

8. VS Sayanarayan, P Sreevani, and A Sivaumar, Synthesis and antimicrobial activity of new Schiff bases containing coumarin moiety and their spectral characterization, Arkivoc,2008; 17:221-233.

9. Garazad, MM,Muzychka,O.V,Vovk, A.I, I.V., Ogorodnichuk , A.S., Modified coumarins 27. Synthesis and antioxidant activity of 3- substiuted 5,7- dihydroxy-4-methyl coumarins. ,Chem. of Nat. Comp. ,2007;43,(1):19-23.

10. Smitha, and R Sanjeeva, $ZrCl_4$-catalyzed Pechman reaction: Synthesis of coumarins under solvent free conditions., Synthetic Comunications,2004;34(21): 3997-4003.

11. Hamadi, C Lidrissi, M Saoud and AR Nievas , H Zarrouk, Synthesis of some biologically coumarin derivatives, Chem. Hetero. Comp. 2006; 42 (3): 320-325

12. Abu-Hussen, A. A. A., J.Coord.Chem., 2006;59, 157.

13. Singh,K., Barwa ,M.S .,Tyagi, P., Synthesis, Charecterization and biological studies of Co(II),Ni(II),Cu(II) and Zn(II) complexes with bi-denntate Schiff base derived by heterocyclic ketone , Eur.J. Med. Chem., 2006;41(1),147-153

14. Yang,W.,Ruan, Z., Wang, Y., Van Kirk, K., Ma,Z., Arey, B.J., Cooper,C.B., Seehala,R., Feyen, J.H.M and Dickson, J.K., Discovery and structure activity relationships of tri-substituted pyrimidines /pyridines as novel calcium channel sensing receptor antagonists, J.Med .Chem. 2009;52:1204.

15. Mladenova, R.; Ignatova,M,;Manolova, N.; Petrova, T.; Rashkov I., Synthesis, structural features and evaluation of antibacterial activities of two Schiff bases derived from 3,4-dihydroxybenzhdrazide., Eur.Polym.J.,2002;38:989-999.

16. Gillespie, R.J., Bamford, S.J., Botting ,R.,Comer,M.,Denny,.Gaur, S.Griffin., Antagonists of the human A2A Adenosine receptors.4.design, synthesis and preclinical evaluation of 7- aryltriazolo [4,5-d] pyrimidines. ,J.Med.Chem.,2009;52:33.

17. K.M.Hello, T.A.Mussa and K.J.Al-Adeily;Al-Qadissia J. for pure Sci., 2006;11:1 .

18. A.S.Hameed," Synthesis and comparative study of new liquid crystalline compounds containing heterocyclic units"., PhD thesis, university of Al-Nahrain –Baghdad-Iraq. (1999).

19. W.Qin,Sha Long, Mauro panunzio, Schiff bases: A short survey on an evergreen chemistry tool, Molecules,2013;18:12264-12289

20. Patai S.," The Chemistry of the Carbon-Nitrogen Double Bond", John Wiely and Sons,New York,1970,68.

21. Scholz M., Schumke A., and M.G. Numchstolt, j.Chem., 1962;2: 309

22. E.Brand and M.Sand berg, Org.Synth.,Coll., 1943;2:49.

23. 23.ZA Sizova; AA Karasev; LL Lukatskaya; MI Rubtsov; AO Dosohenko, Theoretical and Experimental Chemistry, 2002; 38(3): 168-172.

24. Hansa Parekh; Ashutosh Joshi and Ketan Hirpani, Indian. J. Heterocycyclic. Chem.,2004;13:221-224

25. 25.Ahluwalia V.K., Bhagat P., Agarwal R., Chandra R., Intermediates for Organic Synthesis, I.K International, New Delhi, 2005 p.276.

26. Wadher S.J., Karande N.A., Borkar D,S, Yeole P.G., Synthesis and Biological Evaluation of Schiff bases as Antimicrobial Agents, International J.Chem.Tech. Research., 2009;1(4): 1297-1302.

27. Kuete V.,Tangmouo. J.G., Penlap. V., Mofo.f., Lontsi.D: Antimicrobial Activity of Tridesmostemon Omphalocarpoides, J. Ethnopharmacology.2006;104:5-11.