SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF 4'-BENZOPYRAN-BASED DERIVATIVES

D. S .Patel*, M. R. Panchal and J.R.Patel
Dr. Dinesh S .Patel
Professor and Principal
Shree P M Patel Institute of Post Graduate Studies and Research in Science, Anand-388001,
Gujarat (India).
Recognized Research Center of Sardar Patel University, V. V. Nagar.
For Ph D(Applied Chemistry),Ph. D (ForensicScience) & M. Phil (Chemistry)

Abstract: A series of eighteen 4'-Substituted-1,3,4,5-tetrahydro-2H-chromeno[4,3-d]pyrimidin-2-one was synthesized using 4-hydroxy Coumarin, aromatic aldehydes and urea as starting material. Furthermore, there has been some additional work investigating the effect of these derivatives on biological activity. Aiming to obtain novel compounds that exhibit biological activities. They were characterized using FTIR, ^{1}H NMR, ^{13}C NMR & elemental analysis. The compounds show antifungal and antibacterial (minimal inhibitory concentration) activities.

Keywords: 4'-Substituted-1,3,4,5-tetrahydro-2H-chromeno[4,3-d]pyrimidin-2-one, Antibacterial activity, antifungal activity.

1.INTRODUCTION

Mycobacterium tuberculosis is the most deadly bacterial pathogen and is responsible for million of deaths every year so it is interestingly to us to synthesized compounds having batter resistant power against above deadlly pathogens. [1-3] Dholakiya et al. have synthesized a series of 4'-aminochalcone based dibromomaleimides from 4'-acetyl-N-maleanilic acid precursor and reported antimicrobial activity.[4] A series of Coumarin derivatives was designed as potential antituberculosis agents. [5,6]

In the present research reference study on 3-heteroaryl-4-hydroxy coumarin derivatives were synthesized and evaluated in vitro for their preliminary antibacterial activities against four different pathogenic bacterial strains such as Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Pseudomonas aeruginosa. Antibacterial activity of each compound was compared with standard drug, Ampicillin. The compounds were interpreted by UV, IR, 1H NMR, mass spectroscopy and X-ray diffraction studies. Solvatochromic behaviour of these compounds was also investigated by UV–vis spectra. Zone of inhibition and minimum inhibitory concentration revealed that all the products exhibited greater antibacterial potential against all bacterial strains except 4g (reported compound in literature).3-Thiazolylazo and 3-(4-phenyl thiazolylazo) of 4-hydroxy coumarin, which have been exhibiting good zone of inhibition against both gram +ve strains and gram −ve strains, where as the compound pyrazolone azo analogue 4e (reported analogue in literature) has tremendous antibacterial activity. Finally we concluded that the compounds having thiazole, pyrazole and triazole nucleus in individual molecular structure clubbed with potent antibacterial pharmacophore of 4-hydroxy coumain showed antibacterial activities.[6-10] Chemists have long sought a patented molecule for the treatment of numerious Infections, a compound that will selectively activate toward the various bacterial and fungal strains without affecting the normal cells. A number of coumarin based derivatives (i.e., 1,3-diaryl-2-propen-1-ones) have demonstrated cytotoxic and antimicrobial properties because of their

preferential reactivity toward cellular thiols in contrast to amino and hydroxy groups found in nucleic acids.[1–4] Hence, these compounds may be free from the problems of mutagenicity that are associated with a number of alkylating agents used in chemotherapy.The affinity of thiol-alkylators for topoisomerase II, a regulator offers a means of selectivity. In the case of thiol-alkylators, such as a,b-unsaturated ketones, inhibition of tumor growth arises through nucleophilic addition involving reactive thiols of key regulatory enzymes. [4, 11]

As part of our research aims to synthesized compounds by environmental friendly route with improve biological activities including, antifungal and antibacterial. We attended to the bezopyrrole or commonly we call coumarin family. Recently several structurally interesting compounds with multicomponently synthesiee from 4-hydroxy coumarin, different aldehydes and ures have been promisingly prepared more over the concentarted Acidic condition by uses of some acids, chlotrosulphonic acid, sulfuric acid, hydrochloric acids and other lewis acids such ac $FeCl_3$, $AlCl_3$, BF_3,etc.

II. EXPERIMENTAL

2.1Materials and Instrumentation

All reagents were of analytical reagent grade and were used with further purification by their purification process. Here in this work we have extended the results and research on synthesis method develoment toward the minimum us of non polar solvents as per the green chemistry point of view and visionary approach towards the rescale upt valuable standard chemical derivatiization.Solvents employed were purified by standard procedure before to use. 4-hydroxy coumarin,was purchased from SD fine chem.pvt Ltd. Catalyst was prepared by standard process and supported material for catalyst was purchase from SD fine Chem Pvt. Ltd. [15] The melting points were determined in open capillary on electronic apparatus and are considering precise record of data. To monitor the reactions, as well as, to establish the identity and purity of reactants and products, thin layer chromatography was performed on microscopic glass slides (2x7.5 cm) coated with silica gel-G, using chloroform-methanol, as the solvent systems, Rf values and spots were visualized under UV radiation. Elemental analysis (C, H, N) were performed using a PerkinElmer, USA 2400-II CHN analyser. FTIR spectra (4000-400 cm-1) recorded on Simadzu 8400-S spectrophotometer using KBr disk. Nuclear magnetic resonance spectra were recorded on Bruker 400 MHz model spectrometer using DMSO as a solvent and TMS as internal reference (Chemical shifts in δ ppm).

2.2 Synthesis of catalyst as per literature reference (M0)

Synthesis of catalyst from mesh 60 silica gel with suport of literature method with dopped bromo thiomeleic anhydride at 1 hr reflux with ethanol in RB flask as given reference literature method of silicasulphuric acid catalyst then dry the reaction silica gel from solvent and used as catalyst ready for P^H sensitized heterocyclic ring formation reaction or preparation.

2.3 Synthesis of Derivatives

Synthesis of 4-phenyl-1,3,4,5-tetrahydro-2H-chromeno[4,3-d]pyrimidin-2-one (M1)

A powder funnel use to charge 4-hydroxy Coumarin (1.02 g, 1 mol), benzalaldehyde (1.50 g, 1mol), silica supported new acid catalyst (30mg, 0.02mol) and Urea(0.79l, 1mol) was heated at 50`C during 1 h in round bottom flask. After cooling the tube to room temperature, the mixture was poured to ice cube filled beaker and generated compounds settle down for 15 minutes filtered and the filtrate was concentrated in vacuo to afford the title compound (3.64gm 96%), as white solid, mp 150-160˚C.

Synthesis of 4-(p-tolyl)-1,3,4,5-tetrahydro-2H-chromeno[4,3-d]pyrimidine-2,5(1H)-dione (M2)

Similar to above process by using 4-hydroxy Coumarin (1 mol), 4-methyl benzalaldehyde (1mol), silica supported new acid catalyst (0.02mol) and Urea(1mol) as solid, mp 160-170 ˚C.

Synthesis of 4-(2,4-dichlorophenyl)-3,4-dihydro-2H-chromeno[4,3-d]pyrimidine-2,5(1H)-dione (M3)

Similar to above process by using 4-hydroxy Coumarin (1 mol), 2,4 dichloro benzalaldehyde (1mol), silica supported new acid catalyst (0.02mol) and Urea(1mol) as solid, mp 150˚C.

Synthesis of 4-(2-nitrophenyl)-3,4-dihydro-1H-chromeno[4,3-d]pyrimidine-2,5-dione (M4)

Similar to above process by using 4-hydroxy Coumarin (1 mol), 2-Nitro benzalaldehyde (1mol), silica supported new acid catalyst (0.02mol) and Urea(1mol) as solid, mp 150-160˚C.

Synthesis of 4-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromeno[4,3-d]pyrimidine-2,5(1H)-dione (M5)

Similar to above process by using 4-hydroxy Coumarin (1 mol), 3,4-dihydroxy benzalaldehyde (1mol), silica supported new acid catalyst (0.02mol) and Urea(1mol) as solid, mp 160-170 ˚C.

Synthesis of 4-Ethyl-3,4-dihydro-2H-chromeno[4,3-d]pyrimidine-2,5(1H)-dione (M6)

Similar to above process by using 4-hydroxy Coumarin (1 mol), Propionaldehyde (1mol), silica supported new acid catalyst (0.02mol) and Urea(1mol) as solid, mp 150-160 ˚C.

Synthesis of4-isopropyl-3,4-dihydro-1H- chromeno[4,3-d]pyrimidine-2,5-dione (M7)

Similar to above process by using 4-hydroxy Coumarin (1 mol), Isobutraldehyde (1mol), silica supported new acid catalyst (0.02mol) and Urea(1mol) as solid, mp 140 ˚C.

Synthesis of 4-(3-bromophenyl)-3,4-dihydro-1H-chromeno[4,3-d]pyrimidine-2,5-dione (M8)

Similar to above process by using 4-hydroxy Coumarin (1 mol), 3-Bromo benzalaldehyde (1mol), silica supported new acid catalyst (0.02mol) and Urea(1mol) as solid, mp 160-170 ˚C.

Synthesis of 4-(3-nitrophenyl)-3,4-dihydro-1H- chromeno[4,3-d]pyrimidine-2,5-dione (M9)

Similar to above process by using 4-hydroxy Coumarin (1 mol), 3-Nitro benzalaldehyde (1mol), silica supported new acid catalyst (0.02mol) and Urea(1mol) as solid, mp 150 ˚C.

Synthesis of 4-(methyl)-3,4-dihydro-1H- chromeno[4,3-d]pyrimidine-2,5-dione (M10)

Similar to above process by using 4-hydroxy Coumarin (1 mol), acetaldehyde (1mol), silica supported new acid catalyst (0.02mol) and Urea(1mol) as solid, mp 160-170 ˚C.

Synthesis of 4-(cinnamyl)-3,4-dihydro-2H-chromeno[4,3-d]pyrimidine-2,5(1H)-dione (M11)

Similar to above process by using 4-hydroxy Coumarin (1 mol), Cinnamldehyde (1mol), silica supported new acid catalyst (0.02mol) and Urea(1mol) as solid, mp 140-150 ˚C.

Synthesis of 4-anisyl-3,4-dihydro-2H-chromeno[4,3-d]pyrimidine-2,5(1H)-dione (M12)

Similar to above process by using 4-hydroxy Coumarin (1 mol), anisaldehyde (1mol), silica supported new acid catalyst (0.02mol) and Urea(1mol) as solid, mp 150-160 ˚C.

Synthesis of 4-(furfuryl)-3,4-dihydro-2H-chromeno[4,3-d]pyrimidine-2,5(1H)-dione (M13)

Similar to above process by using 4-hydroxy Coumarin (1 mol), furfuraldehyde(1mol), silica supported new acid catalyst (0.02mol) and Urea(1mol) as solid, mp 162-170 ˚C.

Synthesis of 4-(2-floro-phenyl)-3,4-dihydro-2H-chromeno[4,3-d]pyrimidine-2,5(1H)-dione (M14)

Similar to above process by using 4-hydroxy Coumarin (1 mol), 4-floro benzalaldehyde (1mol), silica supported new acid catalyst (0.02mol) and Urea(1mol) as solid, mp 170-180 ˚C.

Synthesis of 4-(4-hydroxyphenyl)-3,4-dihydro-2H-chromeno[4,3-d]pyrimidine-2,5(1H)-dione (M15)

Similar to above process by using 4-hydroxy Coumarin (1 mol), 4-hydroxy benzalaldehyde (1mol), silica supported new acid catalyst (0.02mol) and Urea(1mol) as solid, mp 150-160 ˚C.

Synthesis of 4-(3-methoxyphenyl)-3,4-dihydro-2H-chromeno[4,3-d]pyrimidine-2,5(1H)-dione by one step (M16)

Similar to above process by using 4-hydroxy Coumarin (1 mol), 3-methoxy benzalaldehyde (1mol), silica supported new acid catalyst (0.02mol) and Urea(1mol) as solid, mp 140-150 ˚C.

Synthesis of 4-(2-hydroxyphenyl)-3,4-dihydro-2H-chromeno[4,3-d]pyrimidine-2,5(1H)-dione by one step (M17)

Similar to above process by using 4-hydroxy Coumarin (1 mol), 2-hydroxy benzalaldehyde (1mol), silica supported new acid catalyst (0.02mol) and Urea(1mol) as solid, mp 160-170 ˚C.

Synthesis of 3,4-dihydro-2H-chromeno[4,3-d]pyrimidine-2,5(1H)-dione by one step (M18)

Similar to above process by using 4-hydroxy Coumarin (1 mol), formaldehyde (1mol), silica supported new acid catalyst (0.02mol) and Urea(1mol) as solid, mp 160-170 ˚C.

III ANTIMICROBIAL STUDIES

3.1 Antibacterial Studies:

All the synthesized compounds were tested for their antibacterial activity (MIC) in vitro by the broth dilution method with bacteria E-coli MTCC 443, P. aeruginosa MTCC 1688, S. aureus MTCC 96, S.

pyogenus MTCC 442 C taking Ampicilin, Chloramphenicol, Ciprofloxacin, Gentamycin, Norfloxacin as standard drugs. [20]

3.2 Antifungal Studies

All the synthesized compounds were tested for their antifungal activity (MIC) in vitro by the broth dilution method with fungi C albicans & A. clavatus, taking Nystatin and Greseofulvin as standard drugs. [16]

IV RESULT AND DISCUSSION

All the compound are derivative with different colour on choice of different aldehydes and stable in air. They were insoluble in water but soluble in organic solvents like $CHCl_3$, DMF and DMSO.

4.1 IR & NMR Spectral Studies

The important infrared spectral bands and their tentative assignments for benzopyran and its derivatives were recorded as KBr disks. The ^{1}H NMR spectra revealed signals 3.35 δ ppm for DMSO solvent.^{1}H NMR data of compound M9 revealed singlet at 2.58 δ ppm for – CH_3 group.The ^{1}H NMR data of compounds M9 & M12 revealed signals between 6.2-7.9 δ ppm for aromatic protons. The IR spectra of compounds M1 to M18 revealed a characteristic bands between 3020-3090 cm^{-1} confirming the presence of (C=C) groups. The ^{1}H NMR data of compounds revealed signals between 2.48-2.53 δ ppm & 2.74-2.80 δ ppm for asymmetric aliphatic (C=C) of Chalcones. IR spectrum of the compounds showed a characteristic bands between 1580-1716 cm^{-1} confirming the presence of C=O groups and C-Br present between 537- 655$cm^{-1}$of benzopyran derivates M8.. ^{13}C NMR spectra of compound M12 showed characteristic peak around 35, 39, 54, 104, 113, 115, 118, 123, 127, 131,152, 157, 165 δ ppm. IR spectrum of the compound M9 showed a characteristic band at 1400cm^{-1} confirming the presence of -NO_2 group. ^{13}C NMR spectra of compounds M9 showed characteristic peak around 36.25, 39.41, 46.82, 103.19, 105, 115, 116, 118,120,123,124, 129, 130, 131,132,133, 134, 143, 147, 152, 158, 164, 166 and 191 [21]

4.2 Antibacterial Studies

The antibacterial activity of compounds was studies with four pathogenic bacteria. Ampicilin, Chloramphenicol, Ciprofloxacin, Gentamycin, Norfloxacin were used as references for inhibitory activity against bacteria. All compounds showed quite good antibacterial activity. When compared with the compound M 12, the activity was comparable with Ampicilin in the case of E. coli and S.aureus, While the compound M14 was more active than Ampicilin and nearly comparable to chloramphenicol in case of E. coli. In case of S. aureus the activity of compounds M 9 & M 12 were comparable to ampicillin. In case of S.pyogenus the activity of compounds M 12, M13 & M14 were slightly comparable to ampicillin. In case of E. coli the activity of compound M13 was slightly comparable to Ampicilin.

4.3 Antifungal Studies

The antifungal activity of compounds was studies with two pathogenic fungi. Nystatin and greseofulvin were used as references for inhibitory activity against fungi. All compounds showed quite good antifungal activity. When compared with the compounds M 12 & M14, the activities were comparable with greseofulvin & nystatin respectively in the case of C. albicans, While the compounds M9, M14 & M15 were more active than greseofulvin. In case of A. clavatus the activity of compound M13 was slightly comparable to both the standard drugs.

V CONCLUSION

The present investigation revealed benzopyran based derivative as potential leads for development of new antibacterial drugs. Compound M13 proved at least as potent as the reference drug Ampicillin and Chloramphenicol. We can also conclude from the result of antifungal activity of compound M 12 & M14 comparable with the standard drugs greseofulvin & nystatin respectively in the case of C.*albicans*. 4'-aminochalcone-based dibromomaleimides are more bactericides than maleimide itself.

VI ACKNOWLEDGMENTS

The author wish thank to the Department of Applied Chemistry, Anand recognized affilaited center of Sardar Patel University, Vallabh Vidyanagar for providing laboratory facilities and our institutes"s microbiology Department, Anand for antimicrobial activity determinations. Analytical facilities providing

by NFDD, Rajkot and Central Salt & Marine Chemicals Research Institute, Bhavnagar, Gujarat, India are gratefully acknowledged.

References

[1] Kasimogullari, B. O., Cesur. Z. 2004. Molecules, 9:894.
[2] Guzel, O, Terzioglu. N, Çapan, G, Salman, A. 2006. ARKIVOC.12:98.
[3] Castagnolo,D, logu, A. D, Radi, M, Bechi, B., Mantti, F. Magnani, M Supino,S. Meleddu, R, Chisu, L. Botta, M. 2008. Bioorga. and Medic. Chem.16:8587
[4] Patel, J. R, Dholakiya,B. Z. 2011. Der pharma chemica. 3(1):458-466.
[5] Mali,H.M, Sabale,S.S, Degani, M. S, Borkute, R, Choudhari, A. S, Sarkar, D, Krishna, V.S Sriram,D. 2018. Future medicinal chemistry.10:2431-2444.
[5] H. Wang, M. Yang, Y. Allan, N. Z. Chen, J. L.V. Edmond, L. F. Liu. 2001. Biochemistry. 40:3316.
[6] Sahoo, J., Kumar Mekap, S. and Sudhir Kumar, P., 2015. Synthesis, spectral characterization of some new 3-heteroaryl azo 4-hydroxy coumarin derivatives and their antimicrobial evaluation. Journal of Taibah University for Science, 9(2):187-195.
[7] Jung, J.C. and Park, O.S., 2009. Synthetic approaches and biological activities of 4-hydroxycoumarin derivatives. Molecules, 14(11):4790-4803.
[8] Singh, I.P., Bharate, S.B. and Bhutani, K.K., 2005. Anti-HIV natural products. Current science,269-290.
[9] Neyts, J., Clercq, E.D., Singha, R., Chang, Y.H., Das, A.R., Chakraborty, S.K., Hong, S.C., Tsay, S.C., Hsu, M.H. and Hwu, J.R., 2009. Structure− activity relationship of new anti-Hepatitis C virus agents: Heterobicycle− coumarin conjugates. Journal of medicinal chemistry, 52(5):1486-1490.
[10] Hassan, M.Z., Osman, H., Ali, M.A. and Ahsan, M.J., 2016. Therapeutic potential of coumarins as antiviral agents. European journal of medicinal chemistry, 123:236-255.
[11] Gurubasavaraja Swamy, P.M. Agasimundin, Y.S. 2008. Acta Pharmaceutica Sciencia,50:197-202.
[12] Prasad, Y. R. Kumar, P. P. Kumar, P. R. Rao, A. S. 2008 e-J.Chem, 5:144-148.
[13] Hwang, D. Hyun, J. Jo, G. Koh, D. Lim, Y. 2010. MRC Latters.DOI: 10.1002/mrc.2707
[14] Perrin, D. D. Armarego, W. L. F. 1988. Purification of Laboratory Chemicals, Pergamon Press, New York
[15] M. Dubernet, V. Caubert, J. Guillard, M-C. Viaud-Massuard,2005 Tetrahedron,61:4585-4593.
[16] Dimmock, J. R. Jha, A. Zello, G. A. Allen, T. M. Santos, C. L. Clercq, E. D. Balzarini, J Manavanthu, E. K, Stables, J. P. 2003.Pharmazie.58:227.
[17] Lee, H. S. Yu, J. S. Lee, C. K. 2009.Bull. Korean Chem. Soc. 30(10):2351-2354.
[18] Stewart, S. G. Polomska, M. E. Lim, R. W. 2007.Tetrahedron Lett. 48:2241-2244.
[19] Cheng, C-F. Lai, Z-C. Lee, Y-J. 2008, Tetrahedron.64:4347-4353.
[20] Rattan,A. 2000. Antimicrobials in Laboratory Medicine, B. Churchill Livingstone, New Delhi, 85.
[21] Silverstein R.M and Webster, F. X.1997. Spectrometric identification of organic compounds, John Wiley & Sons, Canada, Sixth edition:79-223.

Table 1. Antibacterial activity of the compounds

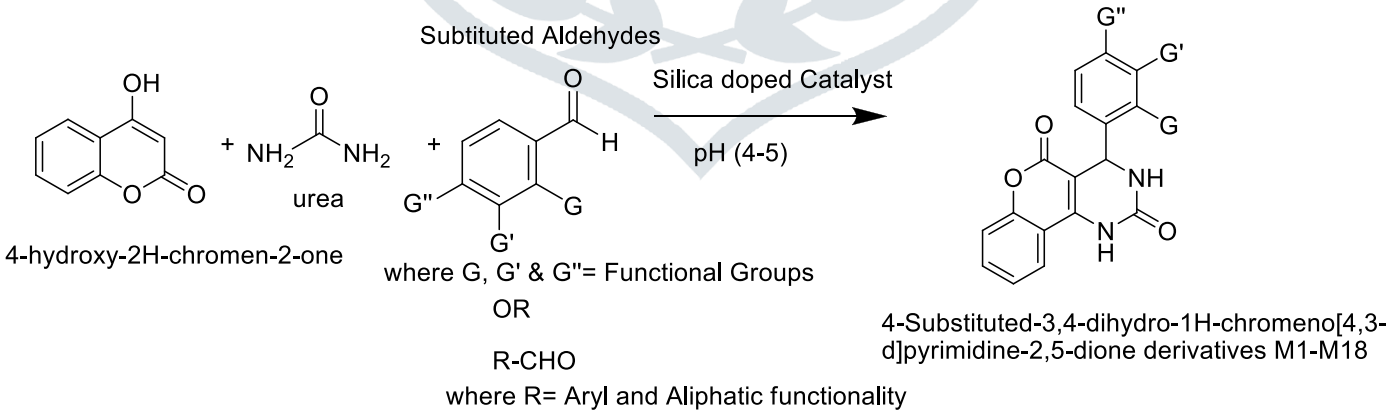
		Minimal Inhibitory Concentration MIC (µg/ml)		
		Bacterial Species		
	E. coli	P. aeruginosa	S. aureus	S. pyogenus
Compounds	MTCC443	MTCC1688	MTCC96	MTCC442
M1	250	200	250	250

M2	100	200	250	200
M3	200	250	100	200
M4	200	200	200	200
M5	250	500	500	500
M6	300	250	300	300
M7	500	500	500	500
M8	400	400	400	400
M9	60	60	60	60
M10	200	200	200	200
M11	100	100	100	100
M12	100	100	100	100
M13	100	100	200	200
M14	60	50	62.5	60
M15	50	50	50	50
M16	250	250	250	250
M17	200	200	200	200
M18	200	200	200	200
Standard drugs				
Ampicillin	100	100	250	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	25	25	50	50

Table 7. Antifungal activity of the compounds

	Minimal Inhibitory Concentration MIC (µg/ml)	
	Fungal Species	
	C. albicans	A. clavatus
Compounds	MTCC227	MTCC1323
M1	250	500
M2	500	1000

M3	1000	200
M4	100	1000
M5	250	500
M6	1000	500
M7	500	400
M8	400	500
M9	100	100
M10	250	200
M11	1000	1000
M12	100	100
M13	250	200
M14	100	100
M15	250	250
M16	1000	1000
M17	300	300
M18	500	500
Standard drugs		
Nystatin	100	100
Greseofulvin	500	100



Scheme 1. General reaction scheme of multicomponent synthesis using efficient catalyst