SYNTHESIS OF SOME NEWER HETEROCYCLIC DERIVATIVES AND THEIR ANTI-FUNGAL ACTIVITIES

Panchal Bhavik[1], Shanta Raj Lakshmi[2]*, Kushan Parikh[3], Dhruvi Chaudhari[4]

Abstract

A number of 3- amino methylene- [2'-(2"- substitutedbenzyliden-4"- thiazolidin-3"-yl)-1',2',3',- thiadiazol-5'-yl] benzopyrone(7 and its derivative) was synthesized through the interaction of the mercapto acetic acid and anhydrous zinc dichloride with 3 amino methylene [2'-(substituted benzylidene)-5'-yl] amino benzopyrone (6 and its derivative) and compound (6) was formed by reaction of compound 5 i.e. 3 Amino methylene- (2 amino-1,3,4 thiadiazol-5-yr-benzopyrone with different aldehydes respectively. In mixing of oil of vitriol(H2SO4) with 3- Amino-2-benzopyrone acetyl thio semi corbazide substance 5 has been created. Elemental analyses and spectrum research were used to establish the compounds' structures. The effectiveness of the newly created compounds as antifungal agents against Aspergillus flavus, Aspergillus niger, and Aspergillus fumigatus Candida krusei, Candida albicans, and Candida parapsilosis C. glabrata HO5 was assessed.

Keywords: Anti-Fungal activities, Heterocyclic.

[1]Department of Chemical Science, Parul Institute of Applied Science, Parul University, Vadodara, Gujarat, India. Email: panchalbhavik830@gmail.com
[2]*Department of Chemical Science, Parul Institute of Applied Science, Parul University, Vadodara, Gujarat, India.
[3]Department of Chemical Science, Parul Institute of Applied Science, Parul University, Vadodara, Gujarat, India.
[4]Department of Chemical Science, Parul Institute of Applied Science, Parul University, Vadodara, Gujarat, India.

***Corresponding Author:** Shanta Raj Lakshmi
*Department Of Chemical Science, Parul Institute of Applied Science, Parul University, Vadodara, Gujarat, India.

DOI: 10.48047/ecb/2023.12.si5a.0357

INTRODUCTION

Coumarin,(benzopyran-2-one) a component of warfarin, clover leaf and tonka beans, is a straightforward heterocycle that contains oxygen. It is generally known that coumarin is used in the perfume industry as a chemical because of its powerful odour. This natural product first isolated from tonka beans by VOGEL .300 or more coumarin collected from natural sources due to A vast number of significant biological effects, including analgesic1-2, ONS2-4, depression antitumor5, ant-inflammotory6-7, photosensitizing antifungal antimicrobial, tuberculostatic, psychotropic, HIV proliferation, etc., are present in coumarin. Novobiocin and other relatively new antibiotics like coumermycin A1, clorobiocin, and novobiocin also have the coumarin structure. Although many other kinds of heterocycles with coumarin rings have been created during this work.

CHEMISTRY

By using the Tripathy and Mukherjee method, compound (1) (3-acetamidocoumarin) was created. Compound (2) (3-amino-2-benzopyrone), which was created by Acid hydrolysis using ethanol as solvent in presence of conc HCl with the addition of sodium bi carbonate . Ethyl choro acetate and anhydrous potassium carbonate (K2CO3) were combined to generate compound (3) 3 Amino-2-bezethyl acetate. Following treatment with Thiourea this compound 3 yields (compound 4) 3-Amino-2-benzopyrone acetyl thiosemicorbazide and in presence of oil of vitriol (H2SO4) 3 Amino methylene- (2 amino-1,3,4 thiadiazol-5-yr-benzopyrone formed. Compound 5's interaction with different arylaldehydes produced compound (6)and its derivatives , which is 3 amino methylene [2'-(substituted benzylidene) -5'-yl] amino benzopyrone. In the end, compound 6 and its derivatives with mercaptoacetic acid in presence of Anh. zinc di chloride produced 3- amino methylene -[2'-(2''- substitutedbenzyliden-4''- thiazolidin-3''-yl)-1',2',3',- thiadiazol-5'-yl] benzopyrone.

EXPERIMENTAL

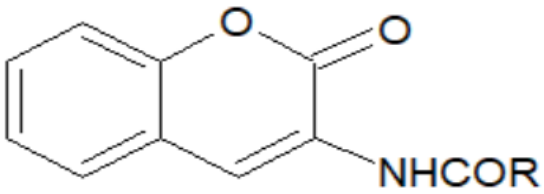
METTLER TOLEDO melting point equipment was used to measure Physical properties like liquefaction point and IR spectra captured on KBr pallets by SHIMADZU IR instrument, Tetramethyl silane was used as internal reference standard while recording H^1NMR spectra on Brucker DRX-400 FTNMR equipment using $CDcl_3$/DMSO d_6 as solvent. chemical shift value was recorded as S (parts per million) Mass spectrum E1 instrument were used to determine mass spectra of compounds.

TLC was used on silica gel G plates with 0.7 mm thickness to identifying the homogeneity of all newly synthesized compounds, At central Drug Research Institute at Lucknow, India, The elemental analysis (C.H.N.) of all substance was done using Carlo-Erba- 1108 elements analyzer.

3-Acetamidocoumarin(I)

n-acetyl glycine (0.512 mol) was suspended in dry benzene (150 ml) to get initially 40% concentration it's also containing triethylamine (0.25 mol) and benzene sulphonyl chloride (0.15 mol) . The components are mix well continuously at room temperature until the crystals of n-acetyl glycine disappeared and the triethylamine salts separating out, after which the residue was filtered and washed with benzene (50 ml) to produce the intermediate. Then allow this intermediate to reflex with aldehyde for 2 to 4 hrs till mass get clear. Afterwards the solution was then concentrated until it was completely dry, and the resulting residue was treated with ice-cold alcohol (Et-OH) before being filtered. The obtained crystals are recrystallized using ethayl alcohol to produce compound (1) with 72% yield.

Liquefaction point is between 208.5 to 210^0c.



3- Amino-2-benzopyrone (2)

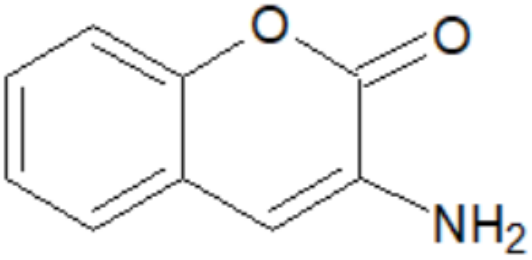
The compound 3-Acetamidocoumarin on simple acid hydrolysis in presence of water and conc Hcl treated synthesise the 3 amino-2-benzopyrone (2)

Firstly solid of substance one is dissolved in ethyl alcohol and water mixture with ration of 5[5:45] and con HCl approx 20 ml 36% added in the mixture drop by drop and allow mass to refluxed for 15-20 min. after it the solution was concentrated on steam bath, diluted with water, and to the clearsolution which was obtained now PH of rexn mass converted into nuteral using sodium carbonate 20% solution and on cooling solid gets following out which was filtered and wash with ethyl alcohol and then dried in Petry dish to obtained 3amino-2-benzopyronw with 58% yield .

IR (KBR)1110, 1715, 3250, Cm^{-1}

H^1NMR in ($CdCl_3$& DMSO-d_6) S (PPM);

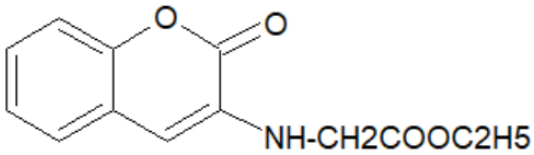
7.78 (bs, hH, NH_2 exchangeable with D_2o) .725-800 (M,5H, Aromatic -H)[MS]:[M] $^+$ a+ M/Z 161.



3- Amino-2-benzopyrone Ethyl acetate (3)

compound 3- Amino-2-benzopyrone (2)(0.45M) was added into (500 ml) dimethyl ketone and allow mass to stirr for 30 min at 20^0c now into this clear solution add (0.18M) anhydrous washing soda Na2CO3(5.0g) and 1.3 times mole equivant of ((0.58 mol) chloro ethyl acetate drop by drop. . The reaction mixture was then refluxed for about 20 to 24 hours. Excess of dimethyl ketone recover in heidolph Rota evaporator and remains bottom mass was poured into cold water of 1^0c so solid perticles fall out which was filtered and recrystallized by ethyl alchohol compound 3.

Liquefaction point is between 91^0 to 93^0c .
Output is around 52%
IR (KBR) 1123 (C-O-C), 1718 (C=O), 2829 (CH_2) 3260 (NH) Cm^{-1};
H-NMR ($CDCL_3$+$DMSOd_6$) 9.68 (SS,14, $NHCH_2$) Exchangeable With D_2O 730-805 (M, SH, Aromatic -H)

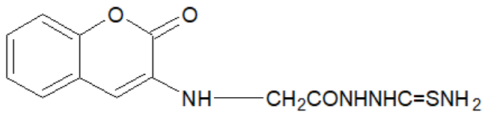


Elemental Analysis: calculated %(Ç, H, N) -61.35,5.583, 5.83.
Practically %(C ,H, N) -61.48, 5.75, 5.60.
MS: $[M]^+$ m/z 235.

3- Amino-2-benzopyrone acetyl thiosemicorbazide (4)

Make 20% solution of White solid crystal of amino thiourea in ethanol, so take 40 g of amino thiourea approx. (0.43 mole) in 200 gm of ethyl alcohol added drop by drop into to(0.45 mole) substance 3 which already taken in 500 ml 3 neck RBF & allow mass to reflex for 15 hrs and Excess of ethyl alcohol recover in Heidolph Rota evaporator and remains viscus bottom mass was poured into cold water to furnish compound 4

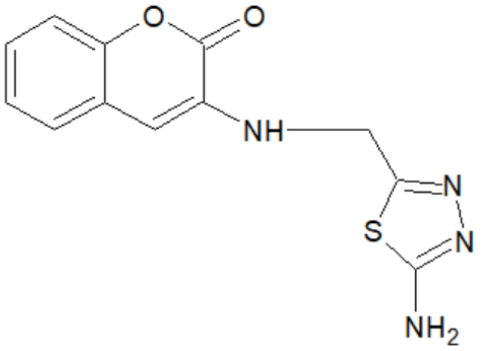
H- M.A. 222C h field 85%
IR (KBr):(C-O-C)-1113, (C=S)-1080 , (-0) 1318 ,
Elemental Analysis: calculated % (Ç, H,N)-47.10, 4.23, 20.11
Practically % (C,H,N)-46.99 4.20, 20.17
MS :$[M]^+$ m/z =280.



3 Amino methylene- (2 amino-1,3,4 thiadiazol-5-yr-benzopyrone (5)

The process of cyclisation started in presence of H3PO4 As catalyst, 100 ml of oil of vitriol(H2SO4) was added to compound 4 (0.5 mol). Then in stirring condition at temperature of 30 to 35 ^{o}c reaction solution was kept for 12 to 14 hrs , then again down the temperature up to 5^0c and maintain mass for 2.00 hrs then after reaction mass add slowly into crushed ice and allow to settle for 1 hrs and then neutralized the reaction mass with liquid ammonia and then filter. This solid mass crystallised to produces the compound 5 yield 63%.

IR(KBr) [(C-C-C)-690, (C-O-C)-1140, (C-N)-1260,(N-1)-1530, (CHOC of Ar)1580, (C=N)-1648 ,(C=O)-1713 ,(CH_2)-2832 .]



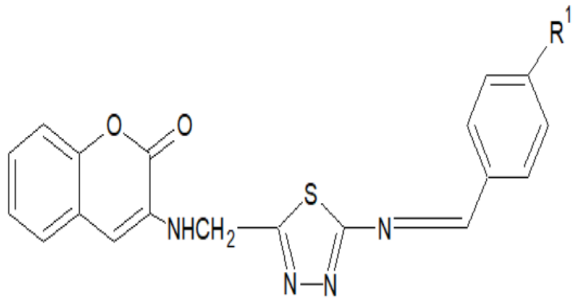
3 amino methylene [2'-(substituted benzylidene) -5'-yl] amino benzopyrone(6)

20%solution of 3 Amino methylene- (2 amino-1,3,4 thiadiazol-5-yr-benzopyrone (5) (0.40mole) Was prepare in ethyl alcohol and transfer mass into 250 ml 3 neck RB Fin which 5 ml of con hcl already taken , then after methoxy aromatic aldehyde solution (0.45 mole) was added in to the above mass within 1.00 hrs time period and allow mass to reflex for 14 hrs and durine this period of time rexn constantly moniter by TLC or GC at the

end of rexn spot of aromatic aldehyde completely disapper now after The excess of ethyl alcohol was recover in Heidolph Rota evaporator and the solid mass quinch with ice cold water and allow mass to settle for 1 hrs then after filtered the solid mass allow to dry in ROTA at 90 ^{0}c at 10 mbar vaccume to give compound 6 and its derivatives.

M.P. 173 ^{0}C,
Yield in % is 29.
IR (KBr)[(C-S-C)-678 , (C-O-C)-1107, (C-N)-1258, (N-N)-1532, (C-C of aromatic)-1570-59, (C=N)-1595, (C=O)-1715 ,(CH_2)-2910, (Ar-CH)-3040, (NH)-3340, (OH)-3550], H-NMR (COD_3 + DMSO+Dr) S... 4.32 (d-2H, NH CH_2), 4.88 (d, IH, N=CH-Ar) 8.30(M-9h, Ar. M), 9.60 S S, IH exchangeable with D_2O Elemental analysis: Calculated % (C.N.N.)-60.31, 3.70, 14.81, Practically % (C,H,N.):-60.5, 3.40, 14.90
MS: [M] + m/z= 378.

Physical evidences and qualitative evidences of 3 amino methylene [2'-(substituted benzylidene)-5'-yl] amino benzopyrone given below



3 amino methylene [2'-(substituted benzylidene)-5'-yl] aminobenzopyrone

Compound	R	M.P.	Yield	Recrystallisation	Molecular	Theoritical(T) practical(p)	Elemental Analysis (%) Calculated/Found		
		(C)	(%)	Solvent	Formula		C	H	N
6a.	CH3	155	60	Ethanol/Water	$C_{19}H_{14}N_4O_2S$	(T) (P)	62.98 62.68	3.86 3.54	15.46 15.72
6b.	m-OH	172	54	Acetone	$C_{19}H_{14}N_4O_3S$	(T) (P)	60.31 60.05	3.70 3.50	14.81 14.69
6c.	m-OH	200	50	Methanol/Water	$C_{19}H_{14}N_4O_3S$	(T) (P)	60.31 60.06	3.70 3.92	14.81 14.55
6d.	m-$N(CH_3)_2$	188	57	DMG	$C_{20}H_{19}N_5O_2S$	(T) (P)	61.00 61.37	4.83 4.58	17.81 17.54
6e.	p-OH	193	60	Acetic Acid	$C_{19}H_1N_4O_4S$	(T) (P)	57.58 57.75	4.04 3.92	14.14 14.00
6f.	p-OCH_3	194	56	Ethanol/water	$C_{19}H_{16}N_4O_3S$	(T) (P)	60.00 60.28	4.21 4.00	14.73 14.99
6g.	m-OCH_3	211	58	Benzene	$C_{19}H_{16}N_4O_3S$	(T) (P)	60.00 60.38	4.21 4.05	14.73 15.02

3- amino methylene- [2'-(2"- substituted benzylidene-4"- thiazolidin-3"-yl)-1',2',3',-thiadiazol-5'-yl] benzopyrone (7)
substance 6[0.1 mole] was taken into 150 ml DMS and pinch of Anhydrous zinc di chloride added into to above reaction mass after that at 25 ^{0}c mercapto acetic acid [0.2 mole] added within 40 to 6o mins and after addition reflex the reaction mixture for 22 hrs and continuously monitor the reaction with TLC after that cool the rexn mass and pour into crushed ice so solide got separating out filter the solid and recrystalised with ethyl alcohol.

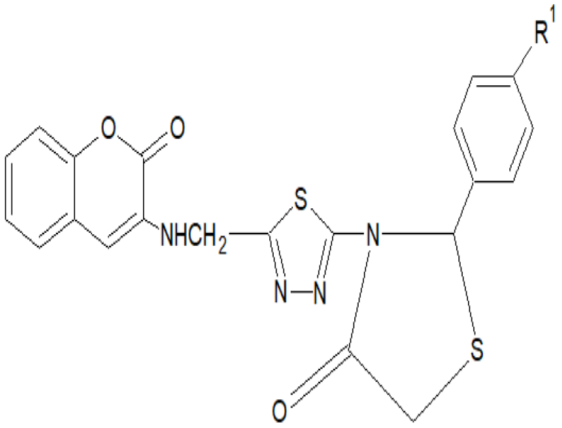
Compound (7) M-P 188^{0}C, yield 47% IR (KBr)[(C-S-C)-850, (C-O-C)-1109, (C-N)-1243, (N-N)-1524, (C-C of a r o m a t i c)-1560, (C=N)-1607, (C-O)-1766 of Bitathiolactam), (C-O of benzopyrone)-1710, (CH_2)-2920 , (NH)-3335,

(OH)-3367
H^1- NMR (COS_3+ DMSO-do) S (inPPM): 3.45 (S,CH_2 of thia-azolidinone) + 4.55 (d.2H) $NHCH_2$) 6.32 (S.IH.Ch-Aromatic), 7.40-8.15 (m.9H Ar-h), 9 -H5 (SS.IH, NHEM) Exchangeable with D_20), 12.35 (SS.IH.OH-Aromatic, exchangeable with D_20).

Elemental analysis: Calculated% (C,H,N)-55.67; 3.34,12.40
Practically % (C,H,N)-55.53, 3.29,12.53
MS: $[M]^+$m/z =452.

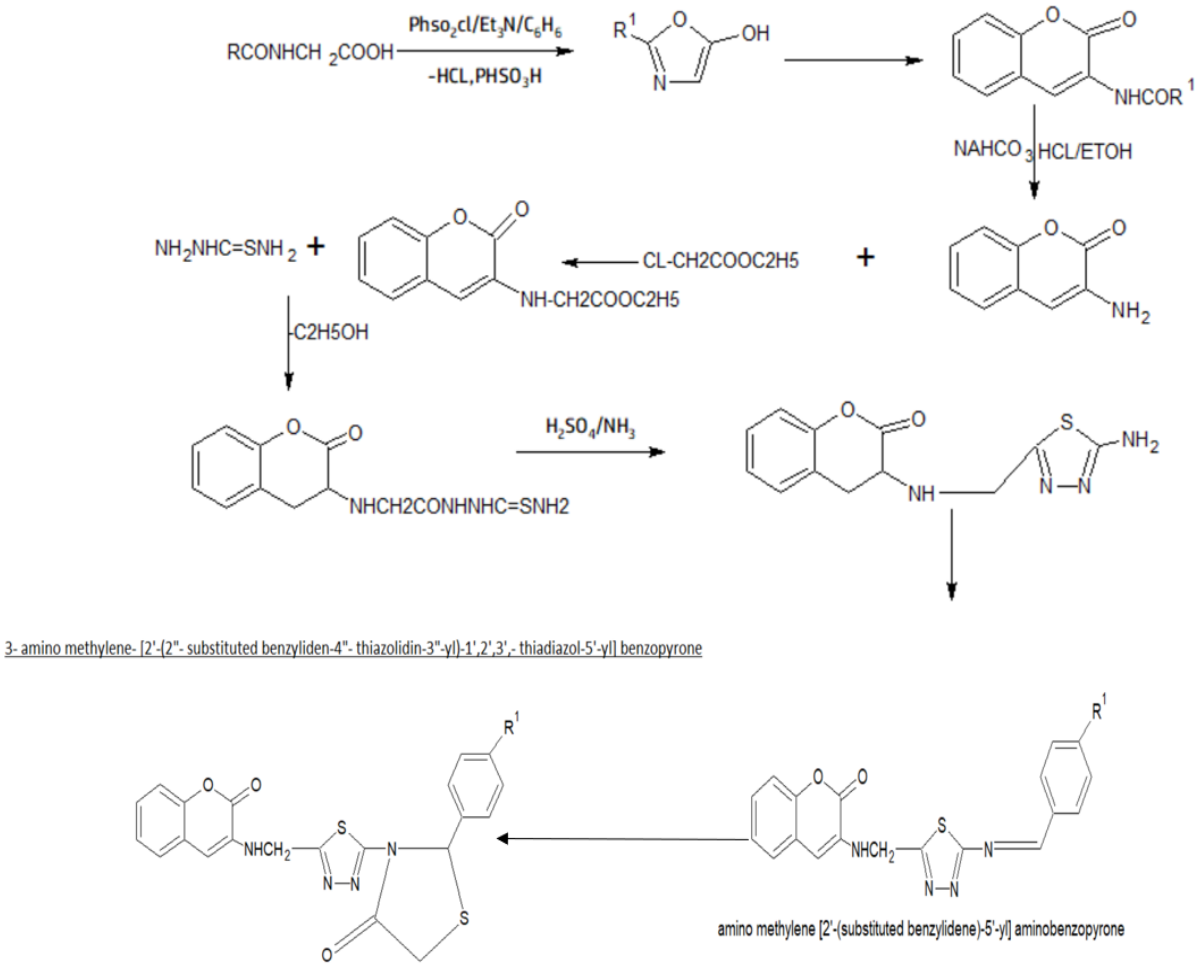
Physical evidences and qualitative evidences of 3- amino methylene- [2'-(2"- substitutedbenzyliden-4"- thiazolidin-3"-yl)-1',2',3',- thiadiazol-5'-yl] benzopyrone (7) given below

3- amino methylene- [2'-(2"- substituted benzyliden-4"- thiazolidin-3"-yl)-1',2',3',- thiadiazol-5'-yl] benzopyrone



Compound	R	M.P. (C)	Yield (%)	Recryatailisation Solvent	Mulecnlar Formula	Theoritical(T) practical(p)	Elemental Analysis (%) Calculated/Found		
							C	H	N
7a.	CH3	151	60	Benzene	$C_{21}H_{16}N_4SO_3$	(T)	62.8	1.13	15.42
						(P)	62.58	4.29	15.07
7b.	m-OH	183	8	Ethanol/Water	$C_{21}H_{16}N_4SO_4$	(T)	55.75	3.53	12.38
						(P)	55.53	3.29	12.53
7c.	o-OH	202	55	Aceticacid	$C_{21}H_{16}N_4SO_4$	(T)	60	4.69	15.5
						(P)	59.95	4.41	15.87
7d	m-$N(CH_3)$	204	57	Methanol	$C_{23}H_{21}N_5SO_3$	(T)	61.74	4.9	15.65
						(P)	61.95	4.41	15.87
7e	p-OH, $N(CH_3)$ 2	214	55	Hexane	$C_{22}H_{18}N_4SO_5$	(T)	58.66	4	12.44
						(P)	58.75	3.82	12.1
7f	o-OCH_3	196	59	DMG	$C_{22}H_{18}N_4SO_4$	(T)	60.82	4.14	12.9
						(P)	60.63	4.05	12.33
7g.	m-OCH_2	205	60	Methanol	$C_{22}H_{18}N_4SO_4$	(T)	60.82	4.14	12.9
						(P)	61.02	4.03	12.69

- **DETAIL REACTION CHEMISTRY IS GIVEN BELOW: -**



RESULTS & DISCUSSION

THE POISENED FOOD TECHNIQUE (Gehlot and Vohra, 1998)

Standard drug like fluconazole and Griseofulvin used to identifying the Antifungal Activities of synthesized sample compounds ,10% solution of DIMETHYLSULPHOXIDE prepare in methanol, after that 0.1 gm of substance which have to test and reference compound also dissolved in 10 ml of above prepare solution and make up it with 990 ml czapex dax medium so finally we gets the concentration of 0.1gm per lit.now approx. 20 gm of this prepapre solution pored into 9 centtimeter glass sterile petri dish and settling apply on it.

This sterile plates inoculated with 5 mm plugs of mycelia fungal taken from the freshly growing cultures now incubation at 25^0c taken for 8*24 hrs,after this incubation periods diameter of colony formation taken, Ave inhibition calculation take place with formula (C-T)100/C

Where,

T stands for diameter of fungal colony in tested compound

C stand for diameter of fungal colony in reference standard compound

DISC-DIFFUSION ANTIBIOTIC SENSITIVITY TEST OR AGAR DIFFUSION TEST (Pai and Platt, 1995)

Agar disk-diffusion method developed in 1940, it's a official method used in many clinical trials, Every culture was kept on Sbouraud dextrose agar medium kept at 30^0c.this fungi were cultivated overnight in sabouraudbroth,centrifuged to extract the plates ,and then resuspended in clean phosphate buffered saline in order to prepare a homogeneous solution for disc testing, a sterile handheld homogenizer was used to homogenize the fungus plates, then using a bacterial spread to ensure a uniform growth, this suspension was plated on a Sbouraud dextrose agar medium. Various test substance and common medications were impregnated with sterile 6 mm whattmann filter paper discs at a concentration of 100 mg/L

After that, these dishes were positioned in the middle of a medium sabouroud agar plate.one control disc on each of these plates is soaked in

10%dmso/methanol solution, incubation of these plates take place at 30^0c. for each test substance and each standard medication employed, three replicates were used, this plates were taken out after 48 hrs,the radius of the inhibitory zone was measured, and average was computed.

Antifungal activity# [diameter of inhibition zone] (mm)					
Compounds	**Candida albicans**	**Candida albicans ATCC**	**Candida Krusel GO3**	**Candida glabratu HO5**	**Candida Purapsiolsis 22019**
Control	0	0	0	0	0
Fluconazole*	28	27	18	16	21
Griseofulvin*	25	27	19	17	23
5	13	-	-	12	-
6A	16	-	-	14	-
6B	23	-	14	-	12
6C	17	-	8	-	-
6D	18	-	-	-	11
6E	17	-	11	-	-
6F	21	-	1	10	13
6G	16	-	9	-	-
7A	15	-	-	17	-
7B	33	34	17	-	23
7C	23	13	19	-	19
7D	21	15	-	-	-
7E	18	-	9	-	-
7F	32	-	24	13	24
7G	23	-	8	8	16

Concentration was 100 mg/L,
10%DMSO in methanol,
no inhibition zone,
std drug use as reference.

The thiodiazole ring containing substance 05 ,which was tested for antifungal eddicacy, demonstrated the least amount of fungal inhibition of all compound .conversion of substance 05 into various arylidene derivarives substance (6A to 6G),which is connected to the structure activity of the investigated compounds, boosts the antifungal activity in all of the derivatives.

Among this sevan arylidene derivatives of compoung (6A to 6G) the antifungal activities was increased higher by substance 6B with orthohydroxy- phenyl ring, and substance 6C eith orthomethoxy phenyl ring then compare to other variants.

The antifungal activities of the substances increased to the antifungal spectra of the compounded being winded by further cycalization of arylidene substanves 6A to 6G into there corresponding thiozolidinone derivatizes substance s 7A to 7G. Therefore, the thiozolidinone rings frame may be to blame for the rise in activities of thse substances.

Its noteworthy to note that substance with an ortho or pera hydroxyphenyl or methoxy phenyl group as a substituents additionally exhibited a remarkable increses in antifungal activities. Additionally it was shown that the ortho derivatives 7B and 7F had storngrt antifungal activities then pera isomers.

The conclusion that follows is that:- Compound 5 with thiodiazole ring displayed least antifungal activities ,it appears that replacement with anoethomethoxyphenyl group(o-{ome-PH})or orthohydroxy phenyl group (o-{oh-ph}) at position 2^{nd}in thiozolidinone ring as helpful for antifungal activities.

The antifungal activities of Variously substituted Benzylidines (6A to 6G) was mild to moderate. These benzylidine cogeners are cyclized to compound 7A to 7G,which improve the antifungal activities,

Concussion

Table display every pharmacological finding from the current study. The refrence drugs, fluconazole and griseofulvin, as well as compound five ,six A to six G and sevan A to sevan G were all screened for antifungal efficacy against various strains of candida and aspergillus spp.at concentration of 0.1 gm/L, in the series two most powerful chemicals were discovered to be 7B and 7F, ortho hydroxy phenyl (O-{OH-PH} Group containe compound 7B had impressive performance, contrarily compound 7F shoen superior efficacy against C.ALB, C.KRUSEI GO3, A.NIGER ans A. FLAVUS while being equally powerful againts C.PARAPSILOSIS 22019. It also demonstrated superior activities agints fluconazole and griseofulvin. compering these two compound to the refrence medications, these two compounds demonstrated stronger antifungal efficacy.

References:

1. Mohamed HM, Abd EL-Wahab HF, EL-Agrody AM, Bedair AH, Eid FA, Khafagy MM, et al. Synthesis and characterization of new diiodocoumarin derivatives with promising antimicrobial activities. Beilstein Journal of Organic Chemistry. 2011;7:1688-1696
2. Tripathy R. P., Tripathy R., Bhaduri A. P., Singh S. N., Chatterjee R. K., and Murthy P. K. (2000), Antifilarial activity of some 2H-1-benzopyran-2-ones (coumar-ins). Acta Tropica76, 101-106.
3. Tyagy Y. K., Kumar A., Raj H. G., Vohra P., Grupta G., Kumari R., Kumar P., and Grupta R. K. (2005), Syn-thesis of novel amino and acetyl amino-4-methylcoumarins and evaluation of their antioxidant activity. Eur. J. Med. Chem. 40, 413-420.
4. Rajanarendar, E, Karunakar, D, and Srinivas, M. Indian J. Chem, (2005), 44, 643-648
5. Souza S. M., Delle Monache F., and Smaˆnia A. Jr. (2005), Antibacterial activity of coumarins. Z. Natur-forsch.60c, 693-700.
6. Kiran, Y.A. Chandrashekhar, D. Kulkarni, M.G., and Kulkarni V.M. Indian J. Chem, (2003), 42, 1548, 1550
7. Pineo G. and Hull R. D. (2003), Coumarin therapy inthrombosis. Hematol. Oncol. Clin. North America17,201-216
8. Satyanarayana VSV, Sreevani P, Sivakumar A, Vijayakumar V. Synthesis and antimicrobial activity of new Schiff bases containing coumarin moiety and their spectral characterization. Arkivok. 2008;xvii:221-223. DOI: 10.3998/ark.5550190.0009.h21
9. Miky. J.A.A., Salehm, N.M., Shemeiss, N.A. M.M. and Fadi-Allah, M. Chem Abstr, (2000), 132, 7841.
10. Sharma, P. and Pritmani, S. Indian J. Chem, (1999), 38, 1139-3342
11. Rama Ganesh CK, Yadav DB, Venkatesh KB. Synthesis and biological evaluation of some innovative coumarin derivatives containing thiazolidin-4-one ring. Indian Journal of Chemistry. 2010;49B:1151-1154
12. Elgamal, M.H.A. Shalaby, N.M.N. and Shabon. M.A. Indian J. Chem, (1998), 37, 662-8.
13. Bhaskar, S.B. Mane, D.V. Shinde, D.B. Shingare, M.S., Deokate, A.S. and Gangawane, L.V. Chem., Abstr, (1997), 126, 2120.
14. Gulati S, Wakode S, Kaur A, Anand K. Synthesis, biological activity and recent advancement of enzothiazoles: A Classical Review. World Journal of Pharmacy and Pharmaceutical Sciences. 2017;6(6):1842-1869
15. Guillaumet, G. Hretanim coddert, G. Averbeck. D. and Averbeck, S. Eun. J. Med. Chem, (1990), 25, 45-51.
16. Patel RM, Patel NJ. In vitro antioxidant activity of coumarin compounds by DPPH, super oxide and nitric oxide free radical scavenging methods. Journal of Advanced Pharmacy Education & Research. 2011;1:52-68