FINAL REPORT - UGC Annexure-IX

Major Research Project

PROJECT TITLE

"Development and Investigation of O/N/S heterocyclic Scaffolds as Potent Antimicrobial and Antitubercular Agents"

P.I. Dr. K. D. Ladva

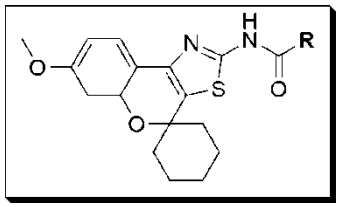
Department of Chemistry

Shree Manibhai Virani & Smt. Navalben Virani Science College

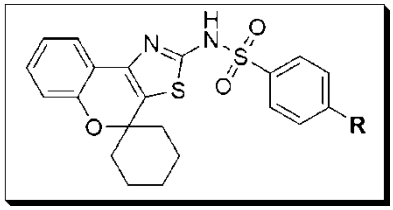
'Yogidham Gurukul', Kalawad Road, Rajkot - 360 005 (Gujarat)

PART-A : Synthesis and characterization of Amide &Sulfonamide derivatives bearing Spirochromeno thiazole scaffold

SCHEME: A.1 : Synthesis and characterization of Amide derivatives bearing Spirochromeno thiazole scaffold

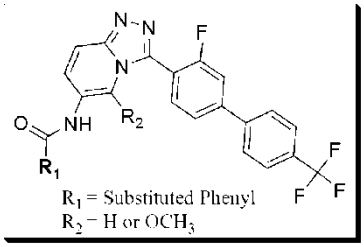


SCHEME: A.2 : Synthesis and characterization of Sulfonamide derivatives bearing Spirochromeno thiazole scaffold

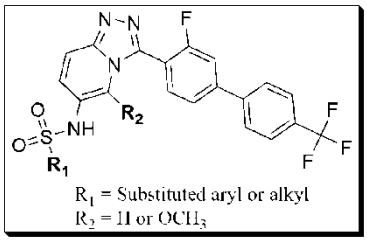


PART- B : Synthesis and characterization of Amide & Sulfonamide derivatives bearing [1,2,4]triazolo[4,3-a]pyridine scaffold

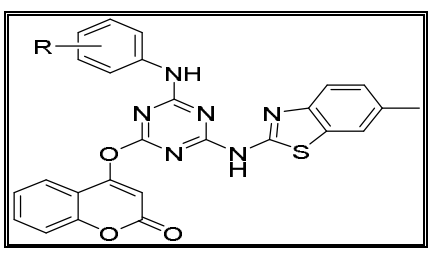
SCHEME- B.1 : Synthesis and characterization of Amide derivatives bearing [1,2,4]triazolo[4,3-a]pyridine scaffold



SCHEME-B.2 : Synthesis and characterization of Sulfonamide derivatives bearing [1,2,4]triazolo[4,3-a]pyridine scaffold



PART-C : Synthesis and characterization of coumarin, 2-aminobenzothiazole and arylamino substituted 1,3,5- triazines



13. Achievements from the Project:

Among the tested compounds, following compounds have shown comparable/ higher antimicrobial activity with that of displayed by known antibiotics and hence these compounds can be considered as potential antimicrobial agents; after detailed biological activity screening/ profiling.

Potent Antibacterial & Antifungal Agents

Part/ Scheme	**Compounds more active than Ampicillin as an Antibacterial agents**				**Compounds more active than Nystatin as an Antifungal agents**		
Part A	E.C.	P.A.	S.A.	S.P.	C.A.	A.N.	A.C.
A1	**1,6**	**1,6,9**	**1,2,6,8,9,10**	**6**	**1**	**6**	**-**
A2	**18,21**	**18,22**	**15,16,18,19,21**	**18**	**-**	**18**	**-**
Part B	E.C.	P.A.	S.A.	S.P.	C.A.	A.N.	A.C.
B1	**29,35**	**35**	**28,29,35**	**-**	**-**	**-**	**-**
B2	**41**	**40,41,51**	**39-47, 49, 51-53,56**	**41**	**-**	**-**	**41,42,51**

The gram positive and gram negative bacteria used were *Escherichia coli (MTCC443), Pseudomonas Aeruginosa (MTCC1688)*, *Streptococcus pyogenus (MTCC442), B.subtilis (MTCC2423)* and *Staphylococcus aureus(MTCC96)* and the fungi used were *Candida albicans (MTCC227), Aspergillus Niger(MTCC282) and AspergillusClavatus (MTCC1323)*.

Part/Scheme	**Compound No.**	**R**	**Gram-positive bacteria**	**Gram-negative bacteria**	**Fungi**
			B.subtilis	***E.coli***	***A.niger***
Part C C1	**AYC-69**	**H**	**3.00**	**4.25**	**3.75**
	AYC-75	**4-Cl**	**3.75**	**4.25**	**3.75**
	AYC-76	**3-NO_2**	**4.75**	**3.50**	**5.75**
	AYC-79	**2-CH_3**	**3.25**	**4.25**	**3.25**
	AYC-80	**2,4-$(CH_3)_2$**	**3.25**	**4.75**	**3.25**

14. Summary of the Findings (in 500 words): Please refer **Annexure-IX.14 & 15**

15. Contribution to the Society (Give details):

More than 60 new chemical entities have been found to be potent anti bacterial and anti fungal agent of total new compounds synthesized under this project.

Detailed pharmacological investigation of these 60 NCEs will give at least one or two **Lead molecules** for the design and development of new broad spectrum antibiotics.

Please refer **Annexure-IX.14 & 15**

16. Whether any Ph.D. Enrolled/Produced out of the Project: **01**

Name: Mr. Kalpesh Menpara; Regi. No.: SU/PG/Ph.D. 3936; Saurashtra University, Rajkot

17. No. of Publications out of the Project (Please attach): **02** [copy enclosed]

Dr. Kartik D. Ladva
Principal Investigator

Dr. A. U. Patel
Principal
Shree Manibahi Virani And
Smt. Navalben Virani Science College,
Rajkot.

Annexure-IX.14 & 15

Summary and Achievement of the Research Project

PART A: Synthesis and Characterization of amide and sulfonamide derivatives bearing Spirochromanothiazole scaffold.

INTRODUCTION

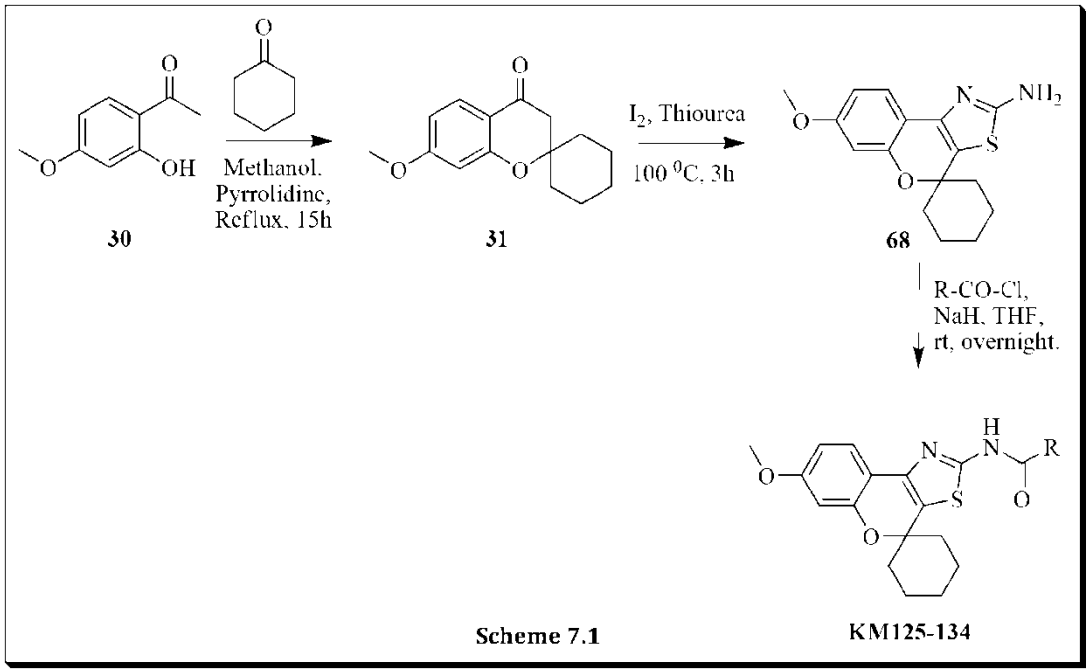
Spirochromanone has recently been given particular interest due to various biological properties of these compounds. Alves et al have isolated three new heterocyclic compounds from the branch of *Piper montealegreanum*: two of them were flavonoids (chromones) and one was phenylpropanoid. Bhilabutra et al have reported that bioactive secondary metabolites isolated from *periconia siamensis* CMUGE015 displayed very good antimicrobial acivity: one of them was 6-hydroxy-2-methyl-4-chromanone. HIV /AIDS *pandenics* is a serious threat to health and development of mankind, and searching for anti HIV agents remains actual. Considerable progress has been made in recent years in the field of drug development against HIV. Literature describes recent progress in the discovery that naturally occurring benzopyrene derivatives, (+) – *Calanolide A* and (-) – *Calanolide A* as a potent anti HIV derivatives. Gambogic acid (GA) is a major active ingredient of gambose. In the chemical structure of GA, there is an intriguing 4-oxo-tricyclo[4.3.1.0]decan-2-one scaffold is found in a growing class of biologically active natural products isolated from plant of the *genus Garcinia*, it exhibits interesting antibacterial activity and cytotoxicity. Wang et al have synthesized some derivatives of GA and cytotoxicity of those derivatives was evaluated. In 2006, three new caged xanthones, 7-methoxydesoxymorellin, 2-isoprenyl forbesione and 8, 8a-epoxymorellic acid, together with nine known caged xenthons were isolated from the EtOAc extract of resin and fruits of *Garcinia hunburyi*. Most of the isolated compounds showed significant cytotoxicities against a panel of mammalian cancer cell lines. Compound-3 exhibited anti-HIV activities in the reverse transcriptase (RT) assay . An urgent need for the identification and total synthesis of new antimalarial inspired a persistent quest to unravel the elusive structures of Robustadial A & B,

natural products isolated from the antimalarial chines herbal medicinal extract of *Eucalyptus robusta* leaves. It was the spirocyclic derivative of chromane.

In a systematic screening effort, extracts of marine fungi from Malaysia were investigated for antimicrobial activity and potentially active secondary metabolites. On the basis of primary screening results, five marine fungi, *Fasciatispora nypae*, *caryosporella rhizophorae*, *Melaspilea mangrovei*, *Leptosphaeria sp.* and *ascomycete strain* 19 (NF) were selected for further investigation to confirm their biological activity. Only *F. nypae* showed wider range of antifungal and antibacterial activity as compared to remaining fungal strains under investigation. Therefore, bioactivity-guided fractionation was undertaken to isolate the active principles, resulting in the characterization of 2,2,7- trimethyl-2H-chroman-5-ol which had antimicrobial activity towards test microorganisms .

Because of these interesting biological activities, numerous synthetic routes to synthesized substituted 2-aminothiazoles have been reported. Modern synthetic methods for accessing substituted 2-aminothiazoles can be roughly classified as heterocyclization of substituted or unsubstituted thiourea with α-haloketones. Although different substituted α-haloketones are not readily available and can be prepared in situ (e.g. from Iodine).

REACTION SCHEME A.1



Scheme 7.1

Table A.1 Characteristics physical data of amide derivatives (KM125-134).

Sr. No.	Compound Code	R	M.P. (°C)	Yield (%)
1	KM125	2,5-difluoro phenyl	142-143	66
2	KM126	2-trifluoromethyl phenyl	156-157	62
3	KM127	4-n-butoxy phenyl	138-139	69
4	KM128	4-ethyl phenyl	159-160	68
5	KM129	4-n-pentyl phenyl	117-118	65
6	KM130	2,4,5-trifluoro phenyl	175-176	58
7	KM131	Isobutyryl	165-166	52
8	KM132	4-morpholino phenyl	99-100	61
9	KM133	4-trifluoromethyl phenyl	123-124	68
10	KM134	Phenyl	135-136	71

All compounds are either crystalline or amorphous solid.

EXPERIMENTAL A.1

Preparation of 7-methoxyspiro[chroman-2,1'-cyclohexan]-4-one (31) : 2-Hydroxy-4-methoxy acetophenone **30** (10.0g, 60.2mmol) and cyclohexanone (5.9g, 60.2mmol) were dissolved in anhydrous methanol (100 mL). Pyrrolidine (4.3g, 60.2mmol) was added and the reaction mixture was allowed to reflux at 80 °C overnight under N_2. The mixture was then concentrated and water (100 mL) and EtOAc (200 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated by rotary evaporation. The crude material was purified by column chromatography (EtOAc/Hexane, 1:9) to yield 7-methoxyspiro[chroman-2,1'-cyclohexan]-4-one **31** (8.6g, 58% yield) as a colorless solid. **M.P.:** 73.5-74.1 °C; **IR (KBr) cm^{-1}:** 1620, 1597, 1507, 1466; MS: m/z 247 (M+H)+.

Preparation of 7-methoxyspiro[chromeno [4,3-d] thiazole-4,1'-cyclohexan]-2-amine (68) : Thiourea (3.71g, 48.7mmol) and iodine (3.4g, 13.4mmol) were mixed with 7-methoxyspiro[chroman-2,1'-cyclohexan]-4-one **31** (3g, 12.2mmol). The reaction mixture was heated at 100 °C for 3 h. The residue was washed with EtOAc (3 x 25 mL), dissolved in water (50 mL) and heated for 30 min and cooled. The brown solid was filtered, dried, and recrystallized from 9:1 EtOH–H_2O and dried under vacuum to afford the hydro iodide salt of **68**. The salt was taken in dichloromethane (100 mL) and upon a quick free-basing by washing with 5% aqueous NaOH solution and evaporating the dichloromethane layer to yield 7-methoxyspiro[chromeno[4,3-

d]thiazole-4,1'-cyclohexan]-2-amine **68** (2.95g, 80% yield) as a brown solid. **M.P.:** 247.5-248.9 °C; **IR (KBr) cm^{-1}**: 3320, 1510, 1460, 1045; MS: m/z 303 (M+H)+.

General Procedure for the Preparation of Substituted-N-(7-methoxyspiro [chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)benzamide : To a stirred solution of 7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexan]-2-amine **68** (0.3g, 0.99mmol) in dry THF (3 mL) was added sodium hydride (60% dispersion in mineral oil) (0.06g, 2.5mmol) under nitrogen atmosphere at 0 ºC followed by the addition of substituted benzoyl chloride (1.5 mol), and the mixture was stirred at room temperature for overnight. The mixture was then concentrated and water (25 mL) and EtOAc (50 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 25 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated by rotary evaporation. The crude material was purified by column chromatography (EtOAc/Hexane, 3:7) to yield Substituted-N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)benzamide. All the compounds were found to be brown solid. 10 new compounds (**KM125-KM134**) were synthesized in similar manner and characteristic physical data of all the final compounds were shown in Table A.1.

SPECTRAL CHARACTERIZATION A.1

2,5-difluoro-N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)benzamide (KM-125) : Yield: 66 %. **^{1}H NMR (400 MHz, DMSO-d^6):** δ =1.37-1.80(8H, m), 2.09(2H, m), 3.77(3H, s), 6.606(2H, d), 7.444(1H, d), 7.468(1H, d), 7.503(1H, d), 7.622(1H, broad), 12.95(1H, s)ppm; **MS:** m/z 443 $(M+H)^+$; **IR (KBr) Cm^{-1}:** 3431, 2925, 1669, 1541, 1499, 1150

N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)-2-(trifluoro methyl)benzamide (126): Yield: 62%. **^{1}H NMR (400 MHz, DMSO-d6):** δ =1.31-1.79(8H, m), 2.11(2H, m), 3.75(3H, s), 6.611(2H, d), 7.440(1H, d), 7.474(1H, t), 7.497(1H, t), 7.763(1H, d), 7.801(1H, d), 12.98(1H, s)ppm; **MS:** m/z 475 $(M+H)^+$; **IR (KBr) Cm^{-1}:** 3435, 2941, 1665, 1531, 1485, 1147

4-butoxy-N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl) benzamide (KM-127) : Yield: 57 %. **^{1}H NMR (400 MHz, $CDCl_3$):** δ =0.995-1.044(5H, m), 1.542(2H, m), 1.650-1.876(8H, m), 2.241(2H, m), 3.864(3H, s), 4.077(2H, t), 6.613-6.669(1H, s), 6.955(2H, d), 7.040(1H, d), 8.087(2H, d), 8.208(1H, d), 12.98(1H, s)ppm; **MS:** m/z 479 $(M+H)^+$; **IR (KBr) Cm^{-1}:** 3429, 2927, 1673, 1554, 1470, 1133

4-ethyl-N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl) benzamide (KM-128) : Yield: 68 %. **^{1}H NMR (400 MHz, $CDCl_3$):** δ =1.288(3H, t), 1.354-1.929(8H, m), 2.242(2H, m), 2.749 (2H, q), 3.837(3H, s), 6.534-6.588(1H, 35d), 7.347(2H, d), 7.551(1H, d), 7.951(2H, d), 8.158(1H, d), 10.8 (1H, broad) ppm; **MS:** m/z 436 $(M+H)^+$; **IR (KBr) Cm^{-1}:** 3441, 2958, 1677, 1538, 1491, 1168

N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)-4-pentyl benzamide (KM-129) : Yield: 65 %. **^{1}H NMR (400 MHz, $CDCl_3$):** δ =0.903-0.948(3H, t), 1.272-1.378(6H, m), 1.660-1.901(8H, m), 2.246(2H, m), 2.709(2H, t), 3.861(3H, s), 6.612-6.660(1H, 35d), 7.309(2H, d), 7.374(1H, d), 7.691(1H, d), 8.079-8.169(2H, dd), 12.98(1H, s)ppm; **MS:** m/z 477 $(M+H)^+$; **IR (KBr) Cm^{-1}:** 3435, 2928, 1655, 1545, 1479, 1146

2,4,5-trifluoro-N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl) benzamide (KM-130) : Yield: 58 %. **^{1}H NMR (400 MHz, DMSO-d^6):** δ =1.274-1.439(2H, m), 1,644-1.922(6H, m), 2.246(2H, m), 3.850(3H, s), 6.598(1H, s), 6.616-6.622(1H, d), 7.034-7.163(1H, m), 7.597(1H, d), 7.857-8.002(1H, m), 12.96(1H, s)ppm; **MS:** m/z 461 $(M+H)^+$; **IR (KBr) Cm^{-1}:** 3437, 2935, 1653, 1577, 1490, 1153

N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)isobutyramide (KM-131) : Yield: 52 %. **^{1}H NMR (400 MHz, DMSO-d^6):** δ =1.282(6H, d), 1.327-1.902(8H, m), 2.206(2H, m), 2.628(1H, m), 3.837(3H, s), 6.560-6.565(1H, d), 6.583(1H, s), 7.542(1H, d), 9.109(1H, broad)ppm; **MS:** m/z 373 $(M+H)^+$; **IR (KBr) Cm^{-1}:** 3432, 2941, 1679, 1535, 1495, 1158

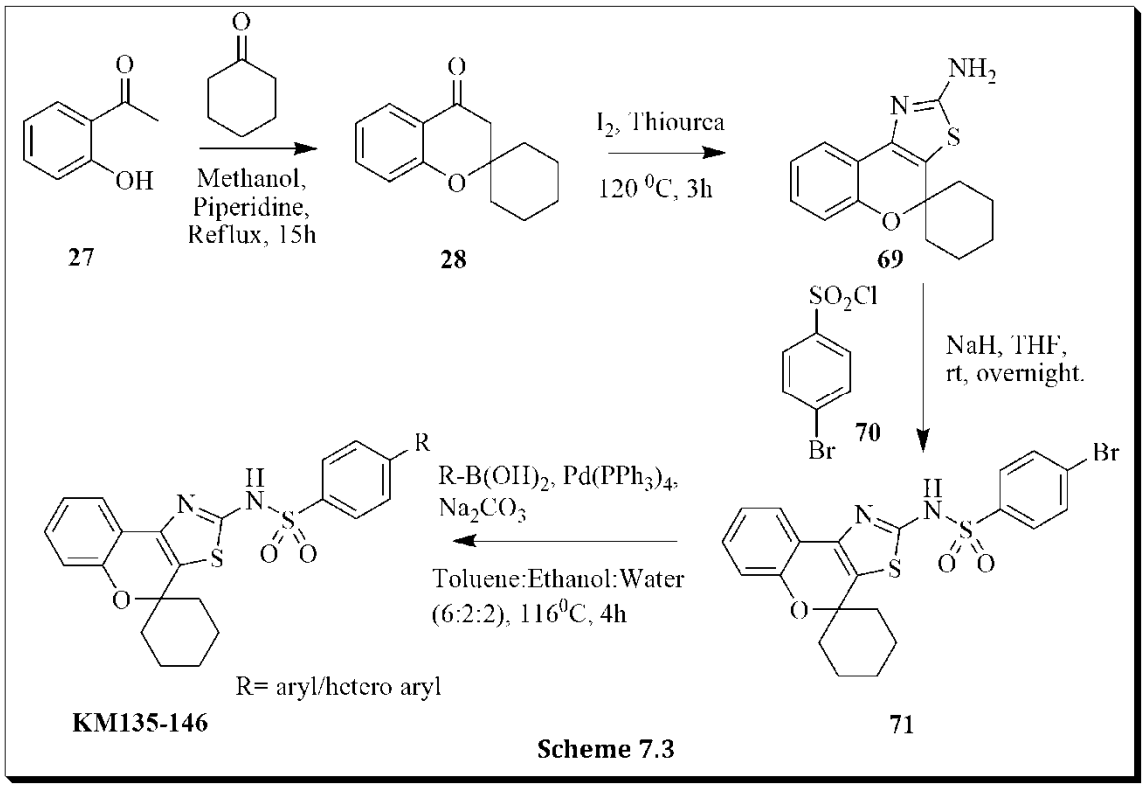
N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)morpholine-4-carboxamide (132) : Yield: 61 %. **^{1}H NMR (400 MHz, DMSO-d6):** δ =1.322-1.941(8H,

m), 2.216(2H, m), 3.321(4H, t), 3.753(4H, t), 3.864(3H, s), 6.660-6.675(2H, d), 7.341(2H, d), 7.541(1H, s), 7.959(2H, d), 11.98(1H, s)ppm; **MS:** m/z 492 $(M+H)^+$; **IR (KBr) Cm^{-1}:** 3438, 2932, 1679, 1543, 1482, 1148;

N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)-4-(trifluoro methyl)benzamide (133) : Yield: 68 %. **^{1}H NMR (400 MHz, DMSO-d6):** δ =1.364-1.923(8H, m), 2.236(2H, m), 3.877(3H, s), 6.539(1H, d), 6.581(1H, s), 7.537(2H, d), 7.553(1H, d), 7.951(2H, d), 10.8 (1H, broad)ppm; **MS:** m/z 475 $(M+H)^+$; **IR (KBr) Cm^{-1}:** 3447, 2912, 1650, 1561, 1478, 1163

N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl) benzamide (134) : Yield: 71 %. **^{1}H NMR (400 MHz, DMSO-d6):** δ =1.351-1.919(8H, m), 2.232(2H, m), 3.869(3H, s), 6.539(1H, d), 6.578(1H, s), 7.551(1H, d), 7.769(5H, m), 11.92 (1H, broad) ppm; **MS:** m/z 407 $(M+H)^+$; **IR (KBr) Cm^{-1}:** 3422, 2936, 1644, 1556, 1483, 1147

REACTION SCHEME A.2



27 28 69 70 71 KM135-146

Scheme 7.3

Table A.2 Characteristics physical data of sulfonamide derivatives (KM135-146).

Sr. No.	Compound Code	R	M.P. (°C)	Yield (%)
1	KM135	4-methoxy phenyl	203-204	67
2	KM136	3-fluoro 4-methyl phenyl	216-217	55
3	KM137	p-tolyl	195-196	61
4	KM138	2,4,5-trifluoro phenyl	187-188	67
5	KM139	4-t-butyl phenyl	172-173	54
6	KM140	3,4-dimethoxy phenyl	125-126	49
7	KM141	2-chloro 5-pyridyl	166-167	51
8	KM142	3,4-(methylenedioxy) phenyl	158-159	43
9	KM143	4-(N,N-dimethylamino) phenyl	184-185	66
10	KM144	2-methoxy phenyl	170-171	48
11	KM145	2,3-dimethoxy phenyl	163-164	57
12	KM146	2,5-dimethoxy phenyl	153-154	63

All compounds are either crystalline or amorphous solid.

EXPERIMENTAL PROTOCOL A.2

Preparation of spiro[chroman-2,1'-cyclohexan]-4-one (28) : 2-Hydroxyacetophenone **27** (5.0g, 36.7mmol) and cyclohexanone (3.61g, 36.7mmol) were dissolved in anhydrous methanol (50 ml). Pyrrolidine (2.87g, 40.4mmol) was added and the reaction mixture was allowed to reflux at 80 °C for overnight under N_2. The mixture was then concentrated and water (50 mL) and EtOAc (50 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated by rotary evaporation. The crude material was purified by column chromatography (EtOAc/Hexane, 1:9) to yield spiro[chroman-2,1'-cyclohexan]-4-one **28** (5.16g, 65% yield) as a colorless viscous oil.

Preparation of spiro[chromeno[4,3-d]thiazole-4,1'-cyclohexan]-2-amine (69) :

Thiourea (7.26g, 95.4mmol) and iodine (6.66g, 26.2mmol) were added in viscous oil of spiro[chroman-2,1'-cyclohexan]-4-one **28** (5.16g, 23.9mmol). The reaction mixture was heated at 100 °C for 3h. The residue was washed with EtOAc (3 x 25 mL), dissolved in water (50 mL) and heated for 30 min and cooled. The brown solid was filtered, dried, and recrystallized from 9:1 EtOH–H_2O and dried under vacuum to afford the hydro iodide salt of 3. The salt was taken in dichloromethane (100 mL) and upon a quick free-basing by washing with 5% aqueous NaOH solution and

evaporating the dichloromethane layer to yield spiro[chromeno[4,3-d]thiazole-4,1'-cyclohexan]-2-amine **69** (5.2g, 80%) as a brown solid.

Preparation of 4-bromo-N-(spiro[chromeno [4,3-d] thiazole-4,1'-cyclohexane]-2-yl) benzenesulfonamide (71) : To a stirred solution of spiro[chromeno[4,3-d]thiazole-4,1'-cyclohexan]-2-amine **69** (5.0g, 18.4mmol) in dry THF (50 mL) was added sodium hydride (60% dispersion in mineral oil) (0.7g, 27.5mmol) under nitrogen atmosphere at 0 °C followed by the addition of 4-bromo benzenesulfonyl chloride **70** (5.63g, 22mmol), and the mixture was stirred at room temperature for overnight. The mixture was then concentrated and water (50 mL) and EtOAc (100 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated by rotary evaporation. The crude material was purified by column chromatography (EtOAc/Hexane, 3:7) to yield 4-bromo-N-(spiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)benzenesulfonamide **71** (4.42g, 49% yield) as a brown solid.

General Procedure for the Preparation of substituted-N-(spiro[chrom- eno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)biphenyl-4-sulfonamide (KM135-KM146): To a stirred solution of 4-bromo-N-(spiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)benzenesulfonamide **71** (0.3g, 0.61mmol) in Toluene (3 mL), substituted aryl/heteroaryl boronic acid (0.92mmol) in ethanol (1 mL) and sodium carbonate (0.32g, 3.1mmol) in water (1 mL) were added to the reaction mixture. Degassed the reaction mixture by N_2 for 10 min followed by the addition of tetrakis triphenylphosphinopalladium (0) (0.035g, 5%). The reaction mixture was heated to reflux for 4h. Water (25 mL) and EtOAc (25 mL) were added into the reaction mixture. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 25 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated by rotary evaporation. The crude material was purified by column chromatography (EtOAc/Hexane, 3:7) to yield the substituted-N-(spiro[chromeno[4,3-d]thiazole-4,1'-cyclohexa- ne]-2-yl)biphenyl-4-sulfonamide as a brown solid. 12 new compounds (**KM135-KM146**) were synthesized in similar manner.

SPECTRAL CHARACTERIZATION A.2

4'-methoxy-N-(spiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)biphenyl-4-sulfonamide (KM-135): Yield: 67 %. **^{1}H NMR (400 MHz, DMSO-d^6):** δ =1.42-1.75(8H, m), 2.01(2h, m), 3.80(3H, s), 6.98(1H, d0, 7.020(1H, t), 7.045(2H, d), 7.250(1H, t), 7.522(1H, d), 7.666(2H, d), 7.802(2H, d), 7.869(2H, d), 13.55(1H, broad)ppm; **MS:** m/z 520 $(M+H)^+$; **IR (KBr) Cm^{-1}:** 3252, 2936, 1727, 1634, 1540, 1435, 1298, 1140, 951.

3'-fluoro-4'-methyl-N-(spiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)bi phenyl-4-sulfonamide (KM-136) : Yield: 55 %. **^{1}H NMR (400 MHz, DMSO-d^6):** δ =1.31(3H, s), 1.47-1.74(8H, m), 2.01(2H, m), 6.985(1H, t), 7.250(1H, t), 7.504(2H, d), 7.540(1H, d), 7.636(2H, d), 7.826(2H, d), 7.895(2H, d), 13.55(1H, broad)ppm; **MS:** m/z 522 $(M+H)^+$; **IR (KBr) Cm^{-1}:** 3237, 2941, 1631, 1555, 1272, 1137, 954, 842

3',4'-dimethoxy-N-(spiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)biphenyl-4-sulfonamide (KM-140) : Yield: 49 %. **^{1}H NMR (400 MHz, DMSO-d^6):** δ =1.239-1.689(8H, m), 2.011(2H, m), 3.798(3H, s), 3.841(3H, s), 6.984(1H, t), 7.054(1H, d), 7.229(1H, d), 7.278(1H, s), 7.525-7.625(5H, m), 7.854(2H, d), 13.55(1H, broad)ppm; **MS:** m/z 549 $(M+H)^+$; **IR (KBr) Cm^{-1}:** 3529, 3055, 2937, 1629, 1547, 1495, 1142, 950.

4-(benzo[d][1,3]dioxol-5-yl)-N-(spiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)benzenesulfonamide (KM-142) : Yield: 43 %. **^{1}H NMR (400 MHz, DMSO-d^6):** δ =1.32-1.72(8H, m),2.01(2H, m), 6.082(2H, s), 6.985(1H, d), 7.019(1H, d), 7.227(1H, m), 7.321(1H, s), 7.520(1H, d), 7.539(1H, t), 7.615(1H, t), 7.783(2H, d), 7.853(2H, d), 13.55(1h, broad)ppm; **MS:** m/z 533 $(M+H)^+$; **IR (KBr) Cm^{-1}:** 3324, 2936, 1628, 1546, 1479, 1229, 1143, 950, 759

2'-methoxy-N-(spiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)biphenyl-4-sulfonamide (KM-144) : Yield: 48 %. **^{1}H NMR (400 MHz, DMSO-d^6):** δ =1.242-1.692(8H, m), 2.016(2H, m), 3.768(3H, s), 6.987(1H, t), 7.023(1H, t), 7.130(1H, d), 7.252(1H, t), 7.320(1H, d), 7.378(1H, t), 7.532(1H, d), 7.617(1H, d), 7.778(2H, d), 7.861(2H, d), 13.56(1H, broad) ppm; **MS:** m/z 520 $(M+H)^+$; **IR (KBr) Cm^{-1}:** 3060, 2936, 1629, 1543, 1493, 1299, 1144, 950, 841, 757

2',5'-dimethoxy-N-(spiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)biphenyl-4-sulfonamide (KM-145)

Yield: 57 %. **^{1}H NMR (400 MHz, DMSO-d^6):** δ =1.352-1.696(8H, m), 2.017(2H, m), 3,559(3H, s), 3.849(3H, s), 6.928(1H, d), 7.007(1H, t), 7.109(1H, d), 7.143(1H, t), 7.236(1H, t), 7.532(2H, d), 7.666(2H, d), 7.903(2H, d)ppm; **MS:** m/z 549 $(M+H)^+$; **IR (KBr) Cm^{-1}:** 3056, 2936, 1629, 1547, 1438, 1265, 1146, 950, 753

PART B : Synthesis and characterization of sulfonamide derivatives bearing [1,2,4]triazolo[4,3-a]pyridine scaffold

INTRODUCTION : Sulfonamides constitute an important class of compounds that exhibit a broad spectrum of biological activities like antibacterial, antitumour, diuretic, hypoglycaemic, etc. A number of sulfonamides reported for their antitumour activity, which led to the discovery of a novel sulfonamide N-[2-[(4-hydroxyphenyl)amino]-3-pyridinyl]-4-methoxybenzenesulfonamide (E7010), which inhibits tubulin polymerization (Fig. 5.1). This compound causes cell cycle arrest and apoptosis in M phase and is shown to exhibit microtubule assembly owing to its reversible binding to the colchicine binding site on tubulin. Compound E7010 also exhibited good in vivo antitumour activity against various rodent tumour and human tumourxenografts. [98]

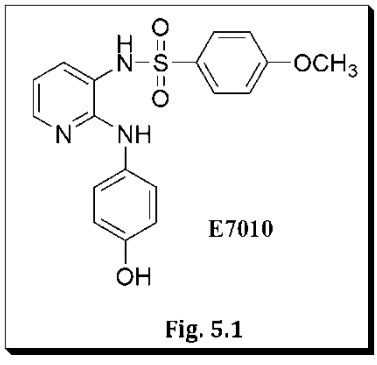


Fig. 5.1

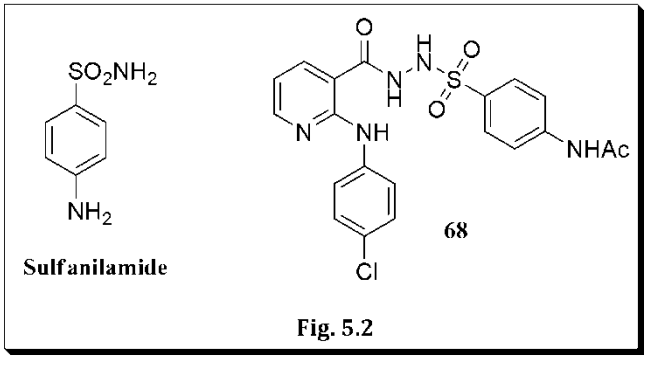
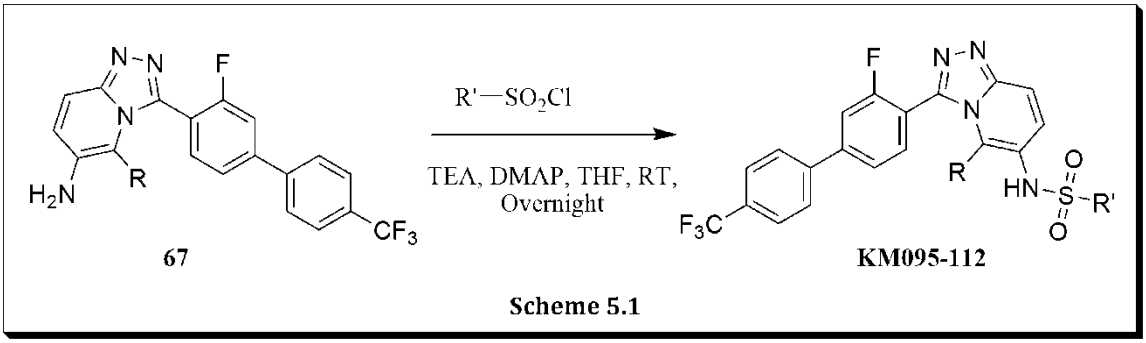


Fig. 5.2

A. Kamal et al. [98] describes a new class of 2-anilino substituted nicotinyl arylsulfonyl hydrazides as potential anticancer and antibacterial agents. A series of N'-1-[2-anilino-3-pyridyl]carbonyl-1-benzenesulfonohydrazide derivatives described

and among these compound **68** showed 50% growth inhibitory activity in leukemia, melanoma, lung cancer, colon cancer, renal cancer and breast cancer cells with GI50 value of 3.2-9.6 µM (Fig. 5.2). The synthesized compounds were also evaluated for their antibacterial activity against various Gram-positive and Gram-negative strains of bacteria. Most of these compounds showed better inhibitory activity in comparison to the standard drugs.

REACTION SCHEME B.2



Scheme 5.1

Table B.2 Characteristics data showing melting point and % yield of sulfonamides

Sr. No.	Compound Code	R	R_1	M.P. (°C)	Yield (%)
1	KM095	H	2-chloro 5-pyridyl	283-284	82
2	KM096	H	2-chlor 4-methyl 5-pyridyl	264-265	76
3	KM097	H	3-bromo 2-chloro 5-pyridyl	285-286	62
4	KM098	H	8-quinolinyl	287-288	48
5	KM099	H	2-trifluoromethoxy phenyl	209-210	67
6	KM100	H	4-trifluoromethoxy phenyl	212-213	54
7	KM101	H	4-isopropyl phenyl	269-270	71
8	KM102	H	2-difluoromethyl phenyl	225-226	64
9	KM103	H	n-butyl	242-243	81
10	KM104	H	2-propyl	253-254	78
11	KM105	H	3-chloro propyl	194-195	65
12	KM106	H	Methyl	277-278	73
13	KM107	$-OCH_3$	3-bromo 2-chloro 5-pyridyl	202-203	43
14	KM108	$-OCH_3$	4-trifluoromethoxy phenyl	179-180	54
15	KM109	$-OCH_3$	2-trifluoromethoxy phenyl	236-237	59
16	KM110	$-OCH_3$	n-butyl	255-256	47
17	KM111	$-OCH_3$	2-propyl	198-199	53
18	KM112	$-OCH_3$	3-chloro propyl	282-283	61

All compounds are either crystalline or amorphous solid.

EXPERIMENTAL PROTOCOL B.2

General procedure for the preparation of N-(3-(3-fluoro-4'-(trifluoromethyl)biphenyl-4-yl)-[1,2,4]triazolo[4,3-a]pyridin-6-yl)substituted sulfonamide and N-(3-(3-fluoro-4'-(trifluoromethyl)biphenyl-4-yl)-5-methoxy-[1,2,4]triazolo[4,3-a]pyridin-6-yl)substituted sulfonamide : To a stirred solution of amine derivatives **67** (0.2g, 0.54mmol) in dry THF (2 mL), triethylamine (0.22ml, 1.6mmol) and catalytic 4-(dimethylamino)pyridine (DMAP) were added to the reaction mixture followed by the addition of substituted aryl/alkyl sulfonyl chloride (0.64mmol) at room temperature under N_2. The reaction mixture was stirred at room temperature for overnight. The mixture was then concentrated and water (25 mL) and EtOAc (25 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 25 mL). The combined organic layers were washed with 1N HCl, dried over sodium sulfate, filtered, and concentrated by rotary evaporation. The crude material was purified by column chromatography (EtOAc/Hexane, 3:7) to yield the compounds N-(3-(3-fluoro-4'-(trifluoromethyl)biphenyl-4-yl)-[1,2,4]triazolo[4,3-a]pyridin-6-yl)substitu ted sulfonamide and N-(3-(3-fluoro-4'-(trifluoromethyl)biphenyl-4-yl)-5-methoxy-[1,2,4]triazolo[4,3-a] pyridin-6-yl)substituted sulfonamide as a off white solid. 18 new compounds (**KM095-KM112**) were synthesized in similar manner.

SPECTRAL CHARACTERIZATION B.2

6-chloro-N-(3-(3-fluoro-4'-(trifluoromethyl)biphenyl-4-yl)-[1,2,4]triazolo[4,3-a]pyridin-6-yl)pyridine-3-sulfonamide (KM-095)

Yield: 51 %. **^{1}H NMR (400 MHz, DMSO-d^6):**δ =7.426(1H, dd), 7.737(1H, d), 7.790(1H, dd), 7.859(1H, d), 7.870(2H, dd), 7.894(1H, d), 8.039(2H, dd), 8.168(1H, dd), 8.301(1H, t), 8.761(1H, s), 8.771(1H, s), 10.88(1H, s)ppm;**MS:** m/z 548 $(M+H)^+$;**IR (KBr) Cm^{-1}:** 3088, 1621, 1561, 1450, 1359, 1172, 1116, 832

6-chloro-N-(3-(3-fluoro-4'-(trifluoromethyl)biphenyl-4-yl)-[1,2,4]triazolo[4,3-a]pyridin-6-yl)-4-methylpyridine-3-sulfonamide (KM-096) : Yield: 76 %. **^{1}H NMR (400 MHz, DMSO-d^6):**δ =2.661(3H, s), 7.486(1H, d), 7.726(1H, s), 7.776(1H, d), 7.836(1H, d), 7.853(2H, d), 7.892(1H, d), 8.031(2H, d), 8,287(1H, t), 8.730(1H, s),

8.759(1H, s), 11.06(1H, s)ppm;**MS:** m/z 562 $(M+H)^+$;**IR (KBr) Cm^{-1}:** 3088, 1620, 1570, 1449, 1326, 1164, 1117, 967, 831

5-bromo-6-chloro-N-(3-(3-fluoro-4'-(trifluoromethyl)biphenyl-4-yl)-[1,2,4]triazolo[4,3-a]pyridin-6-yl)pyridine-3-sulfonamide (KM-097): Yield: 62 %. **^{1}H NMR (400 MHz, DMSO-d^6):**δ =7.460(1H, d), 7.787(1H, d), 7.845(1H, d), 7.857(2H, d), 7.901(1H, d), 8.036(2H, d), 8.306(1H, t), 8.544(1H, s), 8.717(1H, s), 8.843(1H, s), 10.92(1H, broad)ppm;**MS:** m/z 627 $(M+H)^+$;**IR (KBr) Cm^{-1}:** 1621, 1595, 1326, 1168, 1069, 965, 829

N-(3-(3-fluoro-4'-(trifluoromethyl)biphenyl-4-yl)-[1,2,4]triazolo[4,3-a]pyridin-6-yl)quinoline-8-sulfonamide (KM-098): Yield: 48 %. **^{1}H NMR (400 MHz, DMSO-d^6):**δ =7.452(1H, d), 7.721(1H, d), 7.731(1H, d), 7.746(2H, d), 7.773(1H, d), 7.836(2H, d), 8.006(2H, d), 8.218(1H, d), 8.309(1H, d), 8.458(1H, d), 8.553(2H, d), 9.167(1H, d)ppm;**MS:** m/z 564 $(M+H)^+$;**IR (KBr) Cm^{-1}:** 3241, 2927, 1618, 1565, 1322, 1163, 893.

N-(3-(3-fluoro-4'-(trifluoromethyl)biphenyl-4-yl)-[1,2,4]triazolo[4,3-a]pyridin-6-yl)-2-(trifluoromethoxy)benzenesulfonamide (KM-099) : Yield: 67 %. **^{1}H NMR (400 MHz, DMSO-d^6):**δ =7.487(1H, d), 7.735(1H, d), 7.757(1H, d), 7.810(2H, d), 7.847(2H, d0, 7.968(1H, d), 8.026(2H, d), 8.044(1H, d), 8.292(1H, t), 8.550(1H, s)ppm;**MS:** m/z 597 $(M+H)^+$;**IR (KBr) Cm^{-1}:** 3419, 2918, 1622, 1503, 1373, 1327, 1110, 823

N-(3-(3-fluoro-4'-(trifluoromethyl)biphenyl-4-yl)-[1,2,4]triazolo[4,3-a]pyridine-6-yl)-4-(trifluoromethoxy)benzenesulfonamide (KM-100) : Yield: 54 %. **^{1}H NMR (400 MHz, DMSO-d^6):**δ =7498(1H, d), 7.749(1H, d), 7.770(1H, d), 7.786(1H, d), 7.817(2H, d), 7.853(2H, d), 8.001(1H, d), 8.032(2H, d), 8.052(1H, d), 8.297(1H, t), 8.579(1H, s)ppm;**MS:** m/z 597 $(M+H)^+$;**IR (KBr) Cm^{-1}:** 3432, 2918, 1620, 1502, 1324, 1111, 822

N-(3-(3-fluoro-4'-(trifluoromethyl)biphenyl-4-yl)-[1,2,4]triazolo[4,3-a]pyridin-6-yl)-4-isopropylbenzenesulfonamide (KM-101) : Yield: 71 %. **^{1}H NMR (400 MHz, DMSO-d^6):**δ =1.29(6H, d), 3.44(1H, m), 7,62(1H,d), 7.79(H, d), 7.84(1H, d), 7.86(2H, d), 7.92(1H, d), 8.04(2H, d), 8.31(1H, t), 8.76(1H, s), 10.16(1H, broad)ppm;**MS:** m/z 538 $(M+H)^+$;**IR (KBr) Cm^{-1}:** 3092, 2969, 1621, 1516, 1325, 1164, 1124, 795

3-chloro-N-(3-(3-fluoro-4'-(trifluoromethyl)biphenyl-4-yl)-[1,2,4]triazolo[4,3-a]pyridin-6-yl)propane-1-sulfonamide (KM-105) : Yield: 65 %. **^{1}H NMR (400 MHz, DMSO-d^6):**δ =2.176(2H, p), 3.390(2H, t), 3.742(2H, t), 7.61(1H, d), 7.72(1H, d),7.79(1H, d), 7.85(2H, d), 7.94(1H, d), 8.04(2H, d), 8.32(1H, d), 8.81(1H, s),

10.26(1H, s)ppm;**MS:** m/z 514 $(M+H)^+$;**IR (KBr) Cm^{-1}:** 3092, 1623, 1564, 1449, 1399, 1155, 827

N-(3-(3-fluoro-4'-(trifluoromethyl)biphenyl-4-yl)-[1,2,4]triazolo[4,3-a]pyridin-6-yl)methanesulfonamide (KM-106) : Yield: 73 %. **^{1}H NMR (400 MHz, DMSO-d^6):**δ =3.312(3H, s), 7.60(1H, d), 7.76(1H, d), 7.79(1H, d), 7.86(2H, d), 7.93(1H, d), 8.04(2H, d), 8.32(1H, t), 8.81(1H, s), 10.04(1H, s) ppm;**MS:** m/z 451 $(M+H)^+$; **IR (KBr) Cm^{-1}:** 3246, 1623, 1564, 1455, 1396, 1168, 1134, 834, 789

5-bromo-6-chloro-N-(3-(3-fluoro-4'-(trifluoromethyl)biphenyl-4-yl)-5-methoxy-[1,2,4]triazolo[4,3-a]pyridin-6-yl)pyridine-3-sulfonamide (KM-107) : Yield: 43 %. **^{1}H NMR (400 MHz, DMSO-d^6):**δ =4.11(3H, s, -OCH3), 7.523(1H, d), 7.616(1H, d), 7.794(1H, d), 7.851(1H, d), 7.870(2H, d), 7.888(1H, d), 8.046(2H, d), 8.316(1H, t), 8.536(1H, s), 8.715(1H, s)ppm;**MS:** m/z 657 $(M+H)^+$;**IR (KBr) Cm^{-1}:** 3082, 1628, 1572, 1446, 1321, 1174, 1127, 987, 821

N-(3-(3-fluoro-4'-(trifluoromethyl)biphenyl-4-yl)-5-methoxy-[1,2,4]triazolo[4,3-a]pyridin-6-yl)-4-(trifluoromethoxy)benzenesulfonamide (KM-108) : Yield: 54 %. **^{1}H NMR (400 MHz, DMSO-d^6):**δ =3.928(3H, s, -OCH3), 7.530(1H, d), 7.573(2H, d), 7.625(1H, d), 7.786(1H, d), 7.838(1H, d), 7.867-7.888(4H, d), 8.036-8.057(3H, d), 8.299(1H, t)ppm;**MS:** m/z 627 $(M+H)^+$; **IR (KBr) Cm^{-1}:** 3191, 3071, 1623, 1563, 1447, 1326, 1260, 1163, 831, 697

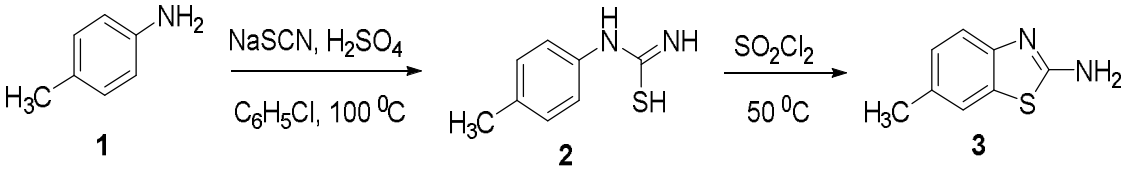
PART- C : Synthesis and characterization of coumarin, 2-aminobenzothiazole and arylamino substituted 1,3,5- triazines

INTRODUCTION : Our group is involved in the development of various synthetic methodologies for the synthesis of triazines containing various heterocycle and other substituents biological interest. Substitution of Chloro functionality in the cyanuric chloride offers various triazines. Reports reveal that coumarin, 2-amino-6-methyl benzothiazole and aryl amines substituted triazines which might have potential biological activities were less studied. Very promising results may obtain with these modifications to 1,3,5-triazine skeleton. As discussed in introduction, the tremendous biological potential of 1,3,5-triazines, coumarin and benzothiazole

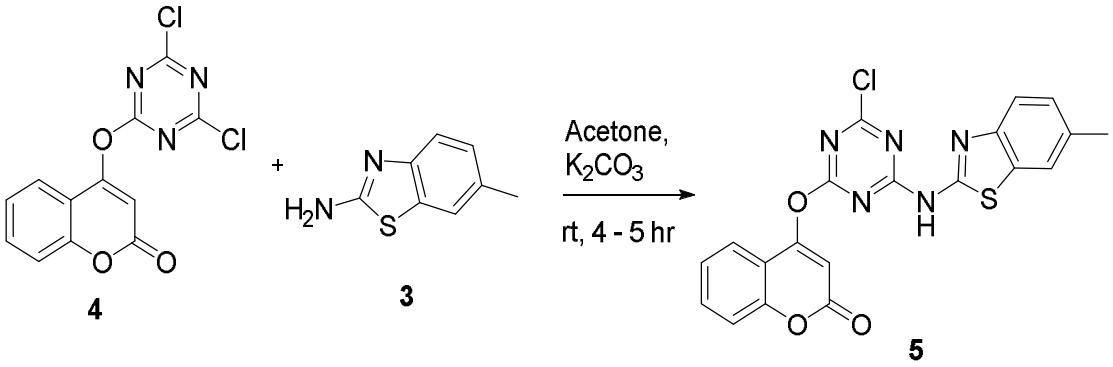
heterocycles motivated us to combine all three functionality in triazine for biological interest. For this modification, 2-amino-6-methyl benzothiazole was required as a precursor which was synthesized by the reported procedure in literature.[1]

To synthesize the desired compounds we have utilized cyanuric chloride as main synthon, as the removal of chlorine functionality using various nucleophiles under basic conditions was well studied. The reaction of cyanuric chloride with coumarins afforded 4-((4,6-dichloro-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one. Which on reaction with 2-amino-6-methyl benzothiazole and followed by aromatic amines in specific reaction condition afforded the novel highly substituted 1,3,5-triazines. The newly synthesized compounds were characterized by IR, Mass, ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis. All the synthesized compounds were screened for *in vitro* antimicrobial activity against Gram-positive *Bacillus subtilis* and *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and fungi *Aspergillus niger*.

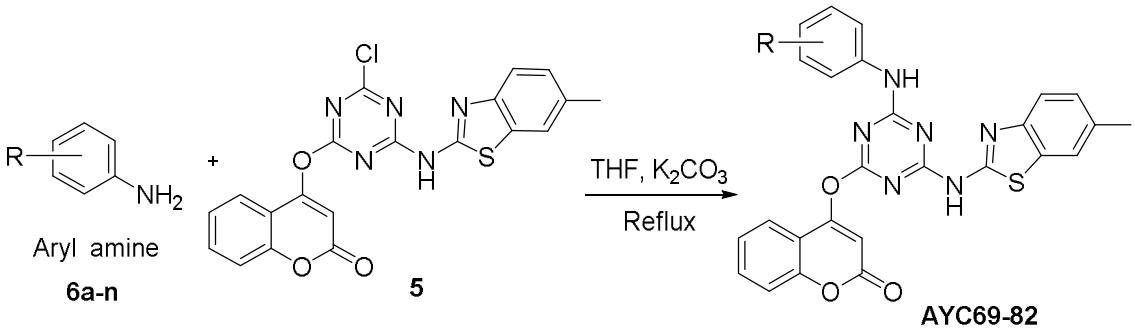
Scheme-C.1: Synthesis of 2-amino-6-methyl benzothiazole 3.



Scheme-C.2: Synthesis of 4-((4-chloro-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2*H*-chromen-2-one.



Scheme-C.3: Synthesis of coumarin, 2-aminobenzothiazole and arylamino substituted 1,3,5- triazines.



Initially, the reaction of *p*-Toluidine with sodium thiocyanate and sulfuric acid under reflux condition afforded the intermediate **2** which on intramolecular cyclisation using sulfuryl chloride afforded the 2-amino-6-methyl benzothiazole **3** (**Scheme C.1**) in good yield and used for further reaction without purification.

Further to synthesize the intermediate 4-((4-chloro-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one **5,** the reaction of 2-amino-6-methyl benzothiazole with **4** was carried out with stirring at room temperature using acetone as solvent and potassium carbonate as base (**Scheme C.2**). The desired compounds 4-((4-((6-methylbenzo[d]thiazol-2-yl)amino)-6-(arylamino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one **AYC69-82** were synthesized by the reaction of aromatic amines **6a-n** with compound **5** using tetrahydrofuran under reflux condition using K_2CO_3 in catalytic amount (**Scheme C.3**).. In all reaction steps, the work up of products was very easy and simple to give analytically pure compounds.

Table C : Physical properties of compounds AYC-69-82

Compound No.	R	Yields (%)	Melting range
AYC-69	H	72	190-192
AYC-70	4-OCH_3	75	202-204
AYC-71	3-Cl	78	180-182

AYC-72	4-NO_2	70	198-200
AYC-73	4-Br	75	208-210
AYC-74	4-F	75	192-194
AYC-75	4-Cl	78	196-198
AYC-76	3-NO_2	71	208-210
AYC-77	4-CH_3	73	195-197
AYC-78	2-OCH_3	74	202-204
AYC-79	2-CH_3	76	208-210
AYC-80	2,4-$(CH_3)_2$	74	220-222
AYC-81	2-F	70	180-182
AYC-82	2-Br	71	216-218

4-((4,6-dichloro-1,3,5-triazin-2-yl)oxy)-2*H*-chromen-2-one was synthesized by reported process as discussed in chapter 3. ^{1}H NMR of 4-((4-chloro-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one showed -CH_3 proton at 2.43 and -CHAr at 5.65 δ ppm, aromatic proton between 7.33 to 8.39 while NH at 12.58 δ ppm. IR signal appeared at 1742 due to presence of C=O group. These data confirmed the formation of compound **5**. ^{1}H NMR signal of 4-((4-((6-methylbenzo[d]thiazol-2-yl)amino)-6-(phenylamino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one **AYC-69 (R=H)** showed –CH_3 proton at 2.30 δ ppm, ArH at 5.78, aromatic protons between 7.01 to 7.88, -NH at 9.82 and -NH at 12.01 δ ppm which resemble to the formation of trisubstituted 1,3,5-triazine. The IR signals of C=O and NH were observed at 1719 and 3272 cm-^{1}, respectively. The ^{13}C NMR data of compounds **AYC-71 (R=3-Cl)** also suggests the formation of desired compound. The physical properties of newly synthesized compounds are depicted in Table C

Among the tested compounds, compound AYC-76 exhibited good inhibition against Gram- positive *B.Subtilis*, and Gram-negative *E.coli* bacteria and Fungi at 4.75, 3.50 and 5.75 mm, respectively. *A.Niger*. While compound AYC-80 was potent against only Gram-negative *E. coli* bacteria. Comopound AYC-75 showed inhibition against *B.Subtiils*, *E.Coli* bacteria and *A.Niger* fungi. However, all compounds were inactive

against Gram-positive *S.aureus* bacteria strain. Remaining compounds have shown moderate inhibition against microbial strains.

EXPERIMENTAL SECTION C

Synthesis of 2-amino-6-methyl benzothiazole (3) : To a stirred solution of *p*-Toluidine (10.7 gm, 0.1 mol) in acetone 70 ml was added Con. Sulfuric acid (3.0 ml, 0.05 mol) at 0-5 ^{0}C over a period of 10 min. then stirred at room temperature and sodium thiocyanate was added (9.0 gm, 0.1 mol). The reaction mixture was then heated on oil batch for 3 hr at 100 ^{0}C. The reaction mixture contains thiourea, which was cooled to rt and 10.8 ml Sulfuryl chloride was added over a period of 20 min by maintain the reaction temp. below 50 ^{0}C. the solvent was then removed by filtration and solid residue was then added to water and solution was basified using ammonium hydroxide solution 90 ml. the separated product was filtered and dried to afford benzothiazole. The product was crystallized out using ethanol to get analytically pure product. Yield 62%, mp 130-132 °C.

Synthesis of 4-((4-chloro-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2*H*-chromen-2-one 5: The the solution of compound 4 (9.4 gm, 30 mmol) and 10 % $NaHCO_3$ 13 ml solution was added the solution of benzothiazle (5 gm, 30 mmol) in acetone 20 ml with stirring at room temperature over a period of 30 min. The reaction mixutre was further stirred for 3 to 4 hr. The reaction was being monitored by TLC. After completion of the reaction, the reaction mixutre was poured in to crushed ice. The separated product was filtered off and dired to yield the desired product.

Spectral data of 4-((4-chloro-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2*H*-chromen-2-one (5) : Yellow color solid; R*f*: 0.21; IR (KBr cm^{-1}): 3252, 2962, 2920, 2240, 1603, 1509, 1371, 802; ^{1}H NMR (400 MHz, DMSO-d_6); δ ppm 2.43 (S, 3G, CH_3), 5.61(s, 1H, ArH) 7.33-8.39 (m, ArH), 12.58 (1H, NH); Mass (m/z): 437[m+1]; Anal. Calcd. for $C_{20}H_{12}ClN_5O_3S$ Calculated C:54.86, H: 2.76, N:15.99. Found C: 54.85, H: 2.74, N: 15.92 %.

General synthesis of 4-((4-((6-methylbenzo[d]thiazol-2-yl)amino)-6-(arylamino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one AYC69-82.: The mixture of 4-((4-chloro-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2*H*-chromen-2-one (500 mg, 1.3 mmol), various aryl amines (1.3 mmol), catalytic amount of K_2CO_3 and

THF was heated under reflux condition for 7-8 hr. After completion of the reaction, it was poured in to crushed ice. The separated product was filtered, dried to yield the desired products **AYC69-82** with good yields.

Spectral data of the synthesized compounds AYC69-82.: 4-((4-((6-methylbenzo[d]thiazol-2-yl)amino)-6-(phenylamino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one AYC-69: Cream solid; R*f*: 0.21; IR (KBr cm^{-1}): 3272, 2961, 2862, 2239, 1719, 1510, 1181, 1137, 1030, 812, 763; ^{1}H NMR(400 MHz, DMSO-d_6); δ ppm 2.36 (s, 3H, CH_3), 5.78 (s, 1H, ArH), 7.01-7.88 (m, 12H, ArH), 9.82 (s, 1H, NH), 12.01 (s, 1H, NH); Mass (m/z): 494; Anal. Calcd. for $C_{26}H_{18}N_6O_3S$ Calculated C:63.15, H: 3.67, N:16.99. Found C: 63.14, H: 3.65, N: 16.98 %.

4-((4-((4-methoxyphenyl)amino)-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one AYC-70 : Yellow solid; R*f*: 0.22; IR (KBr cm^{-1}): 3452, 3450, 2852, 2735, 1781, 1618, 1534, 1407, 1312, 898, 751; Mass (m/z): 524; Anal. Calcd. for $C_{27}H_{20}N_6O_4S$ Calculated C:61.82, H: 3.84, N: 16.02. Found C: 61.79, H: 3.85, N: 16.02 %.

4-((4-((3-chlorophenyl)amino)-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one AYC-71 : Yellow solid; R*f*: 0.21; IR (KBr cm^{-1}): 3260, 2959, 2919, 2240, 1751, 1505, 1521, 1410, 1354, 805, 761; Mass (m/z): 528 [m+1]; Anal. Calcd. for $C_{26}H_{17}ClN_6O_3S$ Calculated C:59.04, H: 3.24, N: 15.89. Found C: 59.01, H: 3.25, N: 15.87 %.

4-((4-((6-methylbenzo[d]thiazol-2-yl)amino)-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one AYC-72: Yellow solid; R*f*: 0.20; IR (KBr cm^{-1}): 3355, 3240, 2922, 2825, 1727, 1612, 1511, 1352, 1244 1120, 854, 711; ^{1}H NMR(400 MHz, DMSO-d_6); δ ppm 2.42 (S, 3H, CH_3), 5.78 (s, 1H), 6.58-7.95 (m, 12H, ArH), 10.28 (s, 1H, NH), 12.11 (s, 1H, NH); ^{13}C NMR (100 MHz); 17, 97, 109, 111, 112, 112, 118, 119, 121, 128, 131, 132, 138, 149, 150, 152, 161, 163, 170, 178. Mass (m/z): 539; Anal. Calcd. for $C_{26}H_{17}N_7O_5S$ Calculated C: 57.88, H: 3.18, N: 18.17. Found C: 57.89, H: 3.17, N: 18.15 %.

4-((4-((4-bromophenyl)amino)-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one AYC-73 : Yello solid; R*f*: 0.22; IR (KBr cm^{-1}): 3275, 2961, 2920, 2240, 1727, 1487, 1180, 1136, 807; Mass (m/z): 572[m+1]; Anal.

Calcd. for $C_{26}H_{17}BrN_6O_3S$ Calculated C:54.46, H: 2.99, N: 14.66. Found C: 54.49, H: 2.99, N: 14.64 %.

4-((4-((4-fluorophenyl)amino)-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one AYC-74 : Yellow solid; R*f*: 0.22; IR (KBr cm^{-1}): 3365, 2959, 2919, 2240, 1506, 1410, 1354, 1211, 1180, 805, 761; Mass (m/z): 512 [m+1]; Anal. Calcd. for $C_{26}H_{17}FN_6O_3S$ Calculated C: 60.93, H: 3.34, N: 16.40. Found C: 60.90, H: 3.38, N: 16.36 %.

4-((4-((4-chlorophenyl)amino)-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one AYC-75 : Yellow solid; R*f*: 0.22; IR (KBr cm^{-1}): 3265, 2959, 2819, 2245, 1706, 1410, 1351, 1211, 1180, 815, 751; Mass (m/z): 528 [m+1]; Anal. Calcd. for $C_{26}H_{17}ClN_6O_3S$ Calculated C: 59.04, H: 3.24, N: 15.89. Found C: 59.01, H: 3.21, N: 15.88 %.

4-((4-((6-methylbenzo[d]thiazol-2-yl)amino)-6-((3-nitrophenyl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one AYC-76 : Yellow color solid; R*f*: 0.21; IR (KBr cm^{-1}): 3372, 2863, 2813, 2239, 1711, 1520, 1434, 1280, 1137, 815, 752; Mass (m/z): 539; Anal. Calcd. for $C_{26}H_{17}N_7O_5S$ Calculated C: 57.88, H: 3.18, N: 18.17. Found C: 57.89, H: 3.15, N: 18.15 %.

4-((4-((6-methylbenzo[d]thiazol-2-yl)amino)-6-(p-tolylamino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one AYC-77 : Cream solid; R*f*: 0.23; IR (KBr cm^{-1}): 3272, 2919, 2863, 2239, 1719, 1510, 1234, 1180, 1137, 812, 762; Mass (m/z): 508; Anal. Calcd. for $C_{27}H_{20}N_6O_3S$ Calculated C: 63.77, H: 3.96, N: 16.53. Found C: 63.79, H: 3.95, N: 16.52 %.

4-((4-((2-methoxyphenyl)amino)-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one AYC-78 : Yellow solid; R*f*: 0.23; IR (KBr cm^{-1}): 3356, 3240, 2932, 2835, 1764, 1610, 1504, 1440, 871, 725; Mass (m/z): 524; Anal. Calcd. for $C_{27}H_{20}N_6O_4S$ Calculated C: 61.82, H: 3.84, N: 16.02. Found C: 61.80, H: 3.84, N: 16.01 %.

4-((4-((6-methylbenzo[d]thiazol-2-yl)amino)-6-(o-tolylamino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one AYC-79 : Yellow solid; R*f*: 0.23; IR (KBr cm^{-1}): 3257, 3250, 2872, 2837, 1726, 1614, 1541, 1462, 1374, 1151, 818, 724; Mass (m/z): 508; Anal. Calcd. for $C_{27}H_{20}N_6O_3S$ Calculated C: 63.77, H: 3.96, N: 16.53. Found C: 63.75, H: 3.94, N: 16.51 %.

4-((4-((2,4-dimethylphenyl)amino)-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one AYC-80 : Yellow solid; R*f*: 0.23; IR (KBr cm^{-1}): 3365, 3212, 2851, 2762, 1708, 1557, 1414, 1332, 1121, 865, 745; ^{1}H NMR (400 MHz, DMSO-d_6); δ ppm 2.27 (s, 3H, CH_3), 3.09 (s, 6H, $2CH_3$), 5.81 (s, 1H, ArH), 6.73-7.81 (m, 11H, ArH), 9.82 (s, 1H, NH), 11.91 (s, 1H, NH); Mass (m/z): 522 [m+1]; Anal. Calcd. for $C_{28}H_{22}N_6O_3S$ Calculated C:64.35, H: 4.24, N: 16.08. Found C: 64.32, H: 4.21, N: 16.07%

4-((4-((2-fluorophenyl)amino)-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one AYC-81 : Yellow solid; R*f*: 0.23; IR (KBr cm^{-1}): 3321, 3243, 2927, 2765, 1989, 1725, 1532, 1515, 1422, 1311, 835, 702; Mass (m/z): 512 [m+1]; Anal. Calcd. for $C_{26}H_{17}FN_6O_3S$ Calculated C:60.93, H: 3.34, N: 16.40. Found C: 60.92, H: 3.35, N: 16.38 %

4-((4-((2-bromophenyl)amino)-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one AYC-82 : Yellow solid; R*f*: 0.22; IR (KBr cm^{-1}): 3315, 3280, 2922, 2815, 1701, 1571, 1412, 1425 1367, 854, 722; Mass (m/z): 573 [m+1]; Anal. Calcd. for $C_{26}H_{17}BrN_6O_3S$ Calculated C:54.46, H: 2.99, N: 14.66. Found C: 54.45, H: 2.93, N: 14.65 %.

COMMENTS OF THE MIDTERM EVALUATION COMMITTEE WHICH WAS HELD FROM 01.02.2014 TO 08.02.2014

CHEMISTRY/POLYMER SCIENCE (08.02.2014)

100	41-297/2012 (SR)	Dr. G. Alagumuthu Deptt. of Chemistry Sri Paramakalyani College Alwarkurichi, Tirunelveli	Progress is satisfactory
101	41-298/2012 (SR)	Dr. K.D. Ladva Deptt. of Chemistry Shri M.V & Smt. N.V. Virani Science, College Rajkot	Progress is good
102	41-299/2012 (SR)	Dr. Sairabanu A. Farokhi Deptt. of Chemistry Govt. First Grade College Davangere,Karnataka	Progress is satisfactory
103	41-300/2012 (SR)	Dr. Hitendrakumar Mangubhai Patel Deptt. of Chemistry Vitthalbhai Patel and Rajrathan, P.T.Patel Science College Vallabh Vidyanagar	Progress is satisfactory
104	41-301/2012 (SR)	Prof. (DR.) P.N. Bhosale Deptt. of Chemistry Shivaji University Kolhapur,	Progress is good
105	41-302/2012 (SR)	Prof. Mahulikar Pramod Pandurang Deptt. of Chemistry North Maharashtra University Jalgaon	Absent
106	41-303/2012 (SR)	Dr. S. Nagarajan Deptt. of Chemistry Annamalai University Annamalai Nagar	Absent
107	41-304/2012 (SR)	Dr.(Mrs.) Meera Sharma Deptt. of Chemistry Agra College Agra	**NOT SATISFACTORY:** 1. Progress unsatisfactory 2. Could not explain objectives. 3. No clue of what chemistry is all about. 4. Does not know what actually she is doing.
108	41-305/2012 (SR)	Dr. Savita Sanjay Desai Deptt. of Chemistry Devchand College Arujunnagar, Kolhapur	Progress is satisfactory
109	41-306/2012 (SR)	Dr. A.B. Zade Deptt. of Chemistry Laxminarayan Institute of Technology Nagpur	Progress is satisfactory

Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry

2014, 3 (2): 535-540

(International Peer Reviewed Journal)

Synthesis, Characterization And Biological Evaluation Of Novel Amides Containing Spiro [Chromeno[4,3-D]Thiazole-4,1'-Cyclohexan]-2-Amine Derivatives

Kalpesh Menpara, Dharmesh Pansuriya, Naresh Kachhadiya, Jignesh Menpara and Kartik Ladva*

*Chemical Research Laboratory, Shree M.&N.Virani Science College, Saurashtra University, Rajkot (Gujarat) – 360 005, **INDIA**

Email: kdladva@vsc.edu.in

Accepted on 11th February 2014

ABSTRACT

A series of novel N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)alkyl/aryl amide derivatives were synthesized for evaluation of their antimicrobial activity. The newly synthesized compounds were characterized by spectroscopic studies such as IR, ^{1}H NMR and LC-Mass analysis. All the synthesized compounds were screened for their in vitro antimicrobial activity. Some of the compounds showed good biological activity.

Keywords: Antimicrobial activity, spiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane, spectroscopic studies, potent antimicrobial derivatives.

INTRODUCTION

Heterocyclic compounds are one of the main groups of organic compounds possessing wide range of applications in various areas of science and high technologies. Many heterocyclic compounds are natural compounds that can also be formed by biosynthesis. From the important group of heterocyclic compounds a growing interest is given to the thiazole derivatives, especially after the identification thiazole ring in the structures of some active compounds and alkaloids (thiazole in vitamin B1 and carboxylase, thiazolidine in penicillin, etc.). It is known about forty alkaloids bearing thiazole ring: antibiotic coumermycine and acidomycine, anthelmintic micothiazole, macrocyclic alkaloids tantazole, sisomycine, etc. Reduced thiazoles serve in the study of polypeptides and proteins and occur as structural units in compounds of biological significance, for example; firefly luciferins, antibiotics bacitracin A and thiostrepton. Equally some derivatives of the 2-aminothiazoles are used as fungicides, pesticides, and bactericides; other possesses mitodepressive and mitostatic properties, and a large range of 2-amino (and hydrazino) 5-nitrothiazoles (nitridazole) are devoid of schistosomicidal activity. Fused thiazoleamine continues to attract considerable attention because of their great particular usefulness primarily, due to a very wide spectrum of biological activities. Thiazole core unit were found to show interesting biological activities such as anti-anoxic (AA) [1], allosteric enhancer of adenosine A1 receptors [2], mycobacterium tuberculosis methionine amino peptidases [3], anti-helicobacter pylori (H-pylori) agent [4] and adenosine A2B receptor

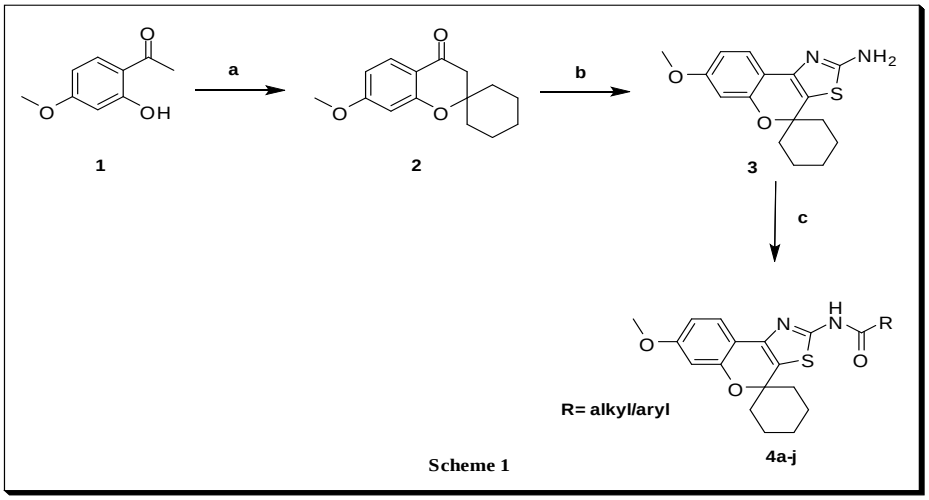
antagonist [5] etc. In the view of biological importance of 2-aminothiazole derivatives we aimed the synthesis of a series of novel N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)alkyl/aryl amide.

MATERIALS AND METHODS

All chemicals were purchased from commercial suppliers. Pyrrolidine and THF were purified by distilling from sodium spheres through a Vigereaux column. The progress of reaction was monitored by Analytical TLC in EtOAc-Hexane solvent system on precoated plates (silica gel 60, F254) and visualized with UV light. Column chromatography was performed with silica gel 60(60-120 mesh). NMR spectra (^{1}H at 400 MH_z) were recorded using $CDCl_3$ or DMSO-d^6 as a solvent. Infrared spectra were determined on a Shimadzu FT-IR. The specifications of the LC/MS are as follows: electrospray (+) ionization, mass range 100-1500 Da, 20-V cone voltage, and Xterra MS C_{18} column (2.1 mm x 50 mm x 3.5 µm). Melting points were determined using Lab India V10 Thermovar apparatus and were uncorrected.

Reagents:(**a**) cyclohexanone, pyrrolidine, methanol, reflux; (**b**) thiourea, iodine, 120 ºC; (**c**) R-CO-Cl, Sodium hydride (60% dispersion in mineral oil), anhydrous THF, RT.

Synthesis of 7-methoxyspiro[chroman-2,1'-cyclohexan]-4-one(2). 1-(4-Methoxy-2-hydroxypheny1) ethanone (10.0g, 60.2mmol) and cyclohexanone (5.9g, 60.2mmol) were dissolved in anhydrous methanol (100 mL). Pyrrolidine (4.3g, 60.2mmol) was added and the reaction mixture was allowed to reflux at 80 ºC overnight under N_2. The mixture was then concentrated and water (100 mL) and EtOAc (200 mL) were added. The layers were formed and the aqueous layer was extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated by rotary evaporation. The crude material was purified by column chromatography (EtOAc/Hexanes, 1:9) to yield 7-methoxyspiro[chroman-2,1'-cyclohexan]-4-one **2** (8.6g, 58% yield) as a colorless solid. M.P.: 73.5-74.1 °C; IR (KBr cm^{-1}): 1620, 1597, 1507, 1466; MS: m/z 247 $(M+H)^+$.



Scheme 1

Synthesis of 7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexan]-2-amine(3). Thiourea (3.71g, 48.7mmol) and iodine (3.4g, 13.4mmol) were added in 7-methoxyspiro[chroman-2,1'-cyclohexan]-4-one **2** (3.0g, 12.2mmol) . The reaction mixture was heated at 100ºC for 3 h. The residue was washed with EtOAc (3 x 25 mL), dissolved in water (50 mL) and heated for 30 min. and cooled. The brown solid was filtered, dried, and recrystallized from 9:1 EtOH–H_2O and dried under vacuum to afford the hydroidide salt of **3**. The salt was taken in dichloromethane (100 mL) and upon a quick free-basing by washing with 5% NaOH and evaporating the dichloromethane layer to yield 7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-

cyclohexan]-2-amine **3** (2.95g, 80% yield) as a brown solid. M.P.: 247.5-248.9 °C; IR (KBr cm^{-1}): 3320, 1510, 1460, 1045; MS: m/z 303 $(M+H)^+$.

General Procedure for the Preparation of Substituted-N-(7-methoxyspiro [chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)benzamide (4a-j) To a stirred solution of 7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexan]-2-amine **3** (0.3g, 0.99mmol) in dry THF (3 mL) sodium hydride (60% dispersion in mineral oil) (0.06g, 2.5mmol)was added under nitrogen atmosphere at 0 ºC followed by the addition of substituted benzoyl chloride (1.5 mol), and the mixture was stirred at room temperature for overnight. The mixture was then concentrated and water (25 mL) and EtOAc (50 mL) were added. The layers were formed and the aqueous layer was extracted with EtOAc (2 x 25 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated by rotary evaporation. The crude material was purified by column chromatography (EtOAc/Hexanes, 3:7) to yield Substituted-N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)benzamide**4a-j**. All the compounds were found to be brown solid. Characteristic physical data of all the final compounds are represented in table 1.

Table 1: Characteristics physical data of amide derivatives **4a-j**.

Sr. No.	Compound Code	R	M.P. (°C)	Yield (%)
1	4a	2,5-difluoro phenyl	142-143	66
2	4b	2-trifluoromethyl phenyl	156-157	62
3	4c	4-n-butoxy phenyl	138-139	69
4	4d	4-ethyl phenyl	159-160	68
5	4e	4-n-pentyl phenyl	117-118	65
6	4f	2,4,5-trifluoro phenyl	175-176	58
7	4g	Isobutyryl	165-166	52
8	4h	4-morpholino phenyl	99-100	61
9	4i	4-trifluoromethyl phenyl	123-124	68
10	4j	Phenyl	135-136	71

All compounds are either crystalline or amorphous solid.

2,5-difluoro-N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)benzamide(4a) ^{1}H NMR (400 MHz, DMSO-d^6): δ =1.37-1.80(8H, m), 2.09(2H, m), 3.77(3H, s), 6.606(2H, d), 7.444(1H, d), 7.468(1H, d), 7.503(1H, d), 7.622(1H, s), 12.95(1H, s) ppm.; IR (KBr cm^{-1}): 3431, 2925, 1669, 1541, 1499, 1150; MS: m/z 443 $(M+H)^+$.

N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)-2-(trifluoromethyl) benzamide (4b) ^{1}H NMR (400 MHz, DMSO-d^6): δ =1.31-1.79(8H, m), 2.11(2H, m), 3.75(3H, s), 6.611(2H, d), 7.440(1H, d), 7.474(1H, t), 7.497(1H, t), 7.763(1H, d), 7.801(1H, d), 12.98(1H, s)ppm. IR (KBr cm^{-1}): 3435, 2941, 1665, 1531, 1485, 1147; MS: m/z 475 $(M+H)^+$.

4-butoxy-N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)benzamide(4c) ^{1}H NMR (400 MHz, $CDCl_3$): δ =0.995-1.044(5H, m), 1.542(2H, m), 1.650-1.876(8H, m), 2.241(2H, m), 3.864(3H, s), 4.077(2H, t), 6.613-6.669(1H, 3sd), 6.955(2H, d), 7.040(1H, d), 8.087(2H, d), 8.208(1H, d), 12.98(1H, s) ppm. IR (KBr cm^{-1}): 3429, 2927, 1673, 1554, 1470, 1133; MS: m/z 479 $(M+H)^+$.

4-ethyl-N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)benzamide(4d) ^{1}H NMR (400 MHz, $CDCl_3$): δ =1.288(3H, t), 1.354-1.929(8H, m), 2.242(2H, m), 2.749 (2H, q), 3.837(3H, s), 6.534-6.588(1H, d), 6.583(1H, s), 7.347(2H, d), 7.551(1H, d), 7.951(2H, d), 10.8 (1H, broad)ppm.IR (KBr cm^{-1}): 3441, 2958, 1677, 1538, 1491, 1168; MS: m/z 436 $(M+H)^+$.

N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)-4-pentylbenzamide(4e) ^{1}H NMR (400 MHz, $CDCl_3$): δ =0.903-0.948(3H, t), 1.272-1.378(6H, m), 1.660-1.901(8H, m), 2.246(2H, m), 2.709(2H, t), 3.861(3H, s), 6.612-6.660(1H, 35d), 7.309(2H, d), 7.374(1H, d), 7.691(1H, d), 8.079-8.169(2H, dd), 12.98(1H, s) ppm. IR (KBr cm^{-1}): 3435, 2928, 1655, 1545, 1479, 1146; MS: m/z 477 $(M+H)^+$.

2,4,5-trifluoro-N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)benzamide(4f)^{1}H NMR (400 MHz, DMSO-d^6): δ =1.274-1.439(2H, m), 1,644-1.922(6H, m), 2.246(2H, m), 3.850(3H, s), 6.598(1H, s), 6.616-6.622(1H, d), 7.034-7.163(1H, m), 7.597(1H, d), 7.857-8.002(1H, m), 12.96(1H, s) ppm. IR (KBr cm^{-1}): 3437, 2935, 1653, 1577, 1490, 1153; MS: m/z 461 $(M+H)^+$.

N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)isobutyramide(4g) ^{1}H NMR (400 MHz, DMSO-d^6): δ =1.282(6H, d), 1.327-1.902(8H, m), 2.206(2H, m), 2.628(1H, m), 3.837(3H, s), 6.560-6.565(1H, d), 6.583(1H, s), 7.542(1H, d), 9.109(1H, broad)ppm. IR (KBr cm^{-1}): 3432, 2941, 1679, 1535, 1495, 1158; MS: m/z 373 $(M+H)^+$.

N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)morpholine-4-carboxamide (4h) ^{1}H NMR (400 MHz, DMSO-d^6): δ =1.322-1.941(8H, m), 2.216(2H, m), 3.321(4H, t), 3.753(4H, t), 3.864(3H, s), 6.660-6.675(2H, d), 7.341(2H, d), 7.541(1H, s), 7.959(2H, d),11.98(1H, s)ppm.IR (KBr cm^{-1}): 3438, 2932, 1679, 1543, 1482, 1148; MS: m/z 492 $(M+H)^+$.

N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)-4-(trifluoromethyl) benzamide (4i)^{1}H NMR (400 MHz, DMSO-d^6): δ =1.364-1.923(8H, m), 2.236(2H, m), 3.877(3H, s), 6.539(1H, d), 6.581(1H, s), 7.537(2H, d), 7.553(1H, d), 7.951(2H, d), 10.8 (1H, broad) ppm. IR (KBr cm^{-1}): 3447, 2912, 1650, 1561, 1478, 1163; MS: m/z 475 $(M+H)^+$.

N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl) benzamide (4j)^{1}H NMR (400 MHz, DMSO-d^6): δ =1.351-1.919(8H, m), 2.232(2H, m), 3.869(3H, s), 6.539(1H, d), 6.578(1H, s), 7.551(1H, d), 7.769(5H, m), 11.92 (1H, broad) ppm. IR (KBr cm^{-1}): 3422, 2936, 1644, 1556, 1483, 1147; MS: m/z 407 $(M+H)^+$.

RESULTS AND DISCUSSION

Chemistry: As delineated in synthetic **Scheme 1**, 7-methoxyspiro[chroman-2,1'-cyclohexan]-4-one **2** were prepared by Chandrasekhar's enamine-mediated crossed Aldol condensation method [6-9]. This efficient one-pot transformation employs freshly distilled catalytic pyrrolidine to condense reagent 2-hydroxy 4-methoxy acetophenone **1** with cyclohexanone; subsequent Michael addition of the phenoxy moiety to the newly generated enone delivers chromanone **2** in good yields. The formation of 7-methoxyspiro[chroman-2,1'-cyclohexan]-4-one **2** was evident from the IR and Mass spectrometry. Condensation of **2** with thiourea in the presence of iodine at 120 ºC led to formation of 7-methoxyspiro [chromeno[4,3-d]thiazole-4,1'-cyclohexan]-2-amine **3** [2,5,10-11]. The target compounds**4a-j** was obtained with variable yield by reacting primary amine **3** with various aryl or alkyl acid chlorides. In this preparation various bases like potassium carbonate, cesium carbonate, DBU, and 60% sodium hydride in mineral oil in the final step were used. Among them 60% sodium hydride in mineral oil with anhydrous THF shows best result. Based on the data given, the reaction appears to be compatible with both aryl and alkyl substitutions. All the reactions have provided the products in 52-71% yields. The compounds were purified by column chromatography using 30% ethyl acetate in hexanes as the eluent.

Biological activities

Antibacterial and antifungal activities: The newly synthesized derivatives were evaluated for their in vitro antibacterial activity against gram positive staphylococcus aureus and streptococcus pyogenes, gram negative Escherichia coli and pseudomonas aeruginosa, and antifungal activity against candida albicans and aspergillusniger by micro broth dilution method [12-14]. The standard strains used for screening antibacterial and antifungal activities were procured from institute of microbial technology (IMTECH), Chandigarh, India. The MIC values are given in Table-2. The standard drugs used for antibacterial activity were ampicillin and ciprofloxacin and nystatin for antifungal activity. Mueller Hinton Broth was used as neutriant medium for bacteria and Sabouraud Dextrose Broth for fungal to grow. Inoculums size for test strain was adjusted to 10^8 CFU mL^{-1} by comparing the turbidity. The serial dilutions were prepared in primary and secondary screening. The target compounds and standard drugs were dissolved in DMSO-water at a concentration of 2.0 mg mL^{-1}. In primary screening, 500 µg mL^{-1}, 250 µg mL^{-1}, and 125 µg mL^{-1} concentrations of the synthesized drugs were taken. Data were not taken for the initial solution because of the high DMSO concentration (10%). The actively synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. In secondary screening, the drugs found active in primary screening were similarly diluted to obtain 100 µg mL^{-1}, 50 µg mL^{-1}, and 25 µg mL^{-1}, 12.5 µg mL^{-1}, and 6.25 µg mL^{-1} concentrations. The inoculated wells were incubated overnight at 37ºC in a humid atmosphere overnight. The highest dilution showing at least 99% inhibition zone is taken as MIC. The MIC values revealed that some of the newly synthesized compounds showed moderate to good inhibition. Compounds 4b, 4f and4iexhibited good activities against all the four bacterial strains. The MIC values of antifungal activity revealed that compound 4a, 4f exhibited good activity against C. albicans and A. niger fungal strain. Rest of all compounds did not exhibit comparable activity against both the fungal strains.

Table 2: Antibacterial and antifungal activity of amide derivatives **4a-j.**

	Antibacterial MIC (µg mL^{-1})				Antifungal MIC (µg mL^{-1})		
Compounds	E. coli MTCC 443	P. aeruginosa MTCC 1688	S. aureus MTCC 96	S. pyogenes MTCC 442	C. albicans MTCC 227	A. niger MTCC 282	A. clavatus MTCC 1323
Ampicillin	100	100	250	100	-	-	-
Ciprofloxacin	25	25	50	50	-	-	-
Nystatin	-	-	-	-	100	100	100
4a	62.5	62.5	100	125	100	500	500
4b	125	125	200	250	500	500	1000
4c	250	250	250	500	1000	500	500
4d	200	200	250	500	1000	1000	1000
4e	250	125	250	500	1000	500	1000
4f	62.5	62.5	62.5	100	200	100	500
4g	500	250	250	500	1000	1000	500
4h	125	100	100	200	500	1000	1000
4i	100	62.5	125	200	1000	1000	500
4j	200	200	125	250	500	500	1000

APPLICATIONS

In the present study the derivatives which we have synthesized were screened for their antimicrobial activity, which are promising as active pharmacophore. Further studies are undergoing to explore the scope of the various biological activities.

CONCLUSIONS

An efficient method for preparing Spiro[chromeno[4,3-d]thiazole-4,1'-cyclohexan]-2-amine Derivatives was described and the structure of synthesized compounds was determine by IR, ^{1}H NMR, and LC-Mass spectroscopic analysis and evaluated for their in vitro antimicrobial activity by broth dilution method.

ACKNOWLEDGEMENTS

We gratefully acknowledge financial support for this work, given by the University Grant commition, New Delhi.

REFERENCES

[1] Mitsure Ohkubo, Atsushi kurjo, isao nakanishi, *Chem. Pharm. Bull.*, **1995**, 43(9), 1497-1504.
[2] Mahendra D. Chordia, Lauren J. Murphree, *Bio-Organic Medicinal Chemistry Letter*, **2002**, 12, 1563-66.
[3] Shridhar Bhat, Omonike Olaleye *Bio-organic medicinal chemistry*, **2012**, 20, 4507-4513.
[4] Yousuke katsura, Tetsuo tomishi, *Journal of medicinal chemistry*, **2000**, 43, 3315-3321.
[5] Jianping cai, Adrian wai-hing cheang, et al., US2006/0030589A1
[6] S.Chandrasekhar, K. Vijeender, K.V. Reddy, *Tetrahedron Lett.* **2005**, *46*, 6991–6993.
[7] Jacquot, yves; Xicluna, Alain; et al. *Medicinal chemistry research*, 2013, 22(2), 681-691.
[8] A.K.Tripathi, S. Koul et al. *Indian journal of chemistry, Section B,* **2009**, 48(2), 301-304.
[9] Brian S. Brown et al. Antagonists of the vanilloid receptor subtype 1(VR1) and uses thereof. US2007/0249614A1.
[10] L.C.King, R.J. Hlavacek, *J. Am. Chem. Soc.* **1950**, 72, 3722.
[11] L.A.Popova, N.Y. Yurashevich, V.A. Knizhnikov, *Russ. J. Org. Chem.* **2004**, 40, 311.
[12] National committee for clinical laboratory standards. Methods for dilution, antimicrobial susceptibility tests for bacteria that grow aerobically approved standard, (M7A5), 5th ed.; national committee for clinical laboratory standards: Wayne, PA, **2000**.
[13] S.Shadomy, In Manual of Clinical microbiology; albert, B. Ed.; ASM press: Washington, DC, **1991**, p. 1173.
[14] A.Rattan, antimicrobial in laboratory medicine; BI Churchill Livingstone; India, **2000**; p. 85.

Journal of Chemistry and Chemical Sciences, *Vol.8(6), 940-947, June 2018* *ISSN 2229-760X (Print)*
(An International Research Journal), *www.chemistry-journal.org* *ISSN 2319-7625 (Online)*

Design, Synthesis, Characterization and Antimicrobial screening of 4-((4-((6-methylbenzo[d]thiazol-2-yl)amino)-6-(phenylamino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one derivatives

Archana B. Pandit and Kartik D. Ladva

Department of Chemistry,
Shree Manibhai Virani & Smt. Navalben Virani Science College-Autonomous,
Kalawad Road, Rajkot-360005, INDIA.
email: kdladva@vsc.edu.in, aycholera@vsc.edu.in

(Received on: May 28, 2018)

ABSTRACT

We have described an easy and conventional method for the synthesis of novel 4-hydroxy coumarin, 2-amino-6-methyl benzothiazole and arylamino bearing 1,3,5-triazine derivatives with good to high yields. The reaction of cyanuric chlorides with coumarin afforded the intermediates under basic condition. Followed by reaction with benzothiazole derivative and different amines under developed reaction conditions yielded the desired trisubstituted 1,3,5-triazines **6a-n**. Among the synthesized compounds, some compounds were screened against Gram positive and Gram negative bacteria and fungi and examined zone of inhibition. Out of them, compound **6h** has found significant against four microorganisms up to the inhibition of 1.75 to 5.75 mm.

Keywords: Cyanuric chloride, Benzothiazole, 4-Hydroxy coumarin, antimicrobial screening.

INTRODUCTION

The thiazoles and benzathiazoles are found in a wide variety of bioactive molecules and natural products.[1] The terrestrial and marine organisms / microorganisms have been a prominent source of these heterocycles. These naturally occurring secondary metabolites or polyketides are often bioactive and a large bulk of literature is being published related to their isolation, chemistry and biology. Thiazole and its derivatives have been of great scientific

exploitation and interest as these are accompanied with almost all the biological and pharmacological activities, like antibacterial, antiprotozoal, antimalarial, anticancer,[2] treat allergies,[3] genemodulating activities, antischizophrenia, antihypertension,[4] anti-inflammation,[5] anti-HIV infections[6] and many more.

In 2015, Kumar *et al.*, have developed two new series of s-triazine derivatives appended with benzimidazoles and benzothiazole derivatives and structure–activity relationships on anticancer activity of these compounds were examined (**Figure 1, a**). *In vitro* inhibitory activity against the growth of six cancer cell lines, viz., MCF-7, MDAMB-231, PC-3, DU-145, HT-29 and HGC-27 was evaluated for synthesized analogues.[7] Moreover, Padalkar and coworkers have synthesized some new benzimidazole, benzoxazole and benzothiazole derivatives and screened for antimicrobial activity (**Figure 1, b**).[8] The reaction of DIPOD 5 with different o-phenylenediamine or o-amino phenol or o-amino thiophenol in ethanol gave benzimidazole, benzoxazole and benzothiazole. Novel heterocycles showed excellent broad-spectrum antimicrobial activity against bacterial strain (*Escherichia coli*, *Staphylococcus aureus*) and fungal strain (*Candida albicans*, *Aspergillus niger*) cultures. Some 1-{4-Chloro-6-[3-(6-methoxy -benzothiazol-2-ylazo)-2,6-dimethyl-quinolin-4-yloxy]-[1,3,5] triazin-2-yl}-(substituted phenyl)-urea (**Figure 1, c**)were synthesized and studied for their microbial activity by Mistry and coworkers.[9] Sareen et al have demonstrated that cyanuric chloride has been reacted selectively with nucleophilic reagents, 6-fluoro-2aminobenzothiazole, phenyl thioureas and different substituted thioureas to give 2-(6-fluorobenzothiazole-2'-ylamino)-4-(phenylthioureido)-6-(substituted thioureido)-1,3,5-triazine (**Figure 1, d**). These compounds were evaluated for their antimicrobial activity.[10]

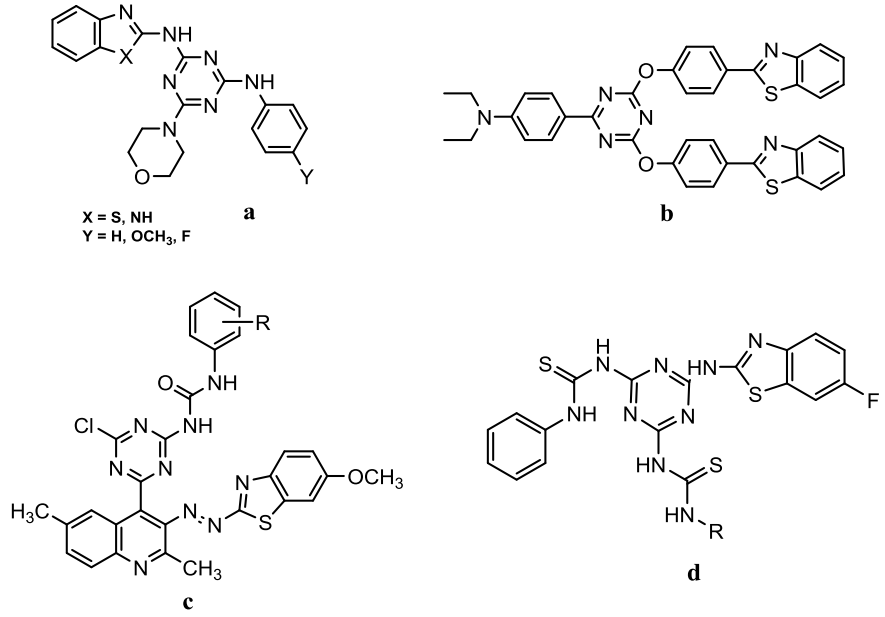


Figure 1. Biologically active compounds.

Reports reveal that coumarin, 2-amino-6-methyl benzothiazole and aryl amines substituted triazines which might have potential biological activities were less studied. Very promising results may obtain with these modifications to 1,3,5-triazine skeleton. As discussed above, and our ongoing interest to synthesize novel heterocycles,[11] the tremendous biological potential of 1,3,5-triazines, coumarin and benzothiazole heterocycles motivated us to combine all three functionality in triazine for biological interest. For this modification, 2-amino-6-methyl benzothiazole was required as a precursor which was synthesized by the reported procedure in literature.[12]

MATERIAL AND METHODS

Experimental section

^{1}HNMR (400 MHz) and ^{13}CNMR (100 MHz) spectra were recorded in DMSO, and TMS was used as an internal reference on a Bruker AVANCE II spectrometer. Mass spectra were determined using direct inlet probe on a GCMS-Agilent mass spectrometer. IR spectra were recorded on KBr discs, using FTIR-Bruker spectrophotometer. Melting points were measured in open capillaries and are uncorrected. Chemicals were purchased from Loba, Molychem, Himedia, Spectrochem, Sigma aldrich and are used without purifications.

Synthesis of 4-((4-chloro-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2*H*-chromen-2-one: The solution of compound 4 (9.4 gm, 30 mmol) and 10 % $NaHCO_3$ 13 ml solution was added the solution of benzothiazle (5 gm, 30 mmol) in acetone 20 ml with stirring at room temperature over a period of 30 min. The reaction mixutre was further stirred for 3 to 4 hr. The reaction was being monitored by TLC. After completion of the reaction, the reaction mixutre was poured in to crushed ice. The separated product was filtered off and dired to yield the desired product.

Spectral data of 4-((4-chloro-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2*H*-chromen-2-one: Yellow color solid; R*f*: 0.21; IR (KBr cm^{-1}): 3252, 2962, 2920, 2240, 1603, 1509, 1371, 802; ^{1}H NMR (400 MHz, DMSO-d_6); δ ppm 2.43 (S, 3G, CH_3), 5.61(s, 1H, ArH) 7.33-8.39 (m, ArH), 12.58 (1H, NH); Mass (m/z): 437[m+1]; Anal. Calcd. for $C_{20}H_{12}ClN_5O_3S$ Calculated C:54.86, H: 2.76, N:15.99. Found C: 54.85, H: 2.74, N: 15.92 %.

General synthesis of 4-((4-((6-methylbenzo[d]thiazol-2-yl)amino)-6-(arylamino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one: The mixture of 4-((4-chloro-6-((6-methylbenzo[d] thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2*H*-chromen-2-one (500 mg, 1.3 mmol), various aryl amines (1.3 mmol), catalytic amount of K_2CO_3 and THF was heated under reflux condition for 7-8 hr. After completion of the reaction, it was poured in to crushed ice. The separated product was filtered, dried to yield the desired products **6a-n** with good yields.

Spectral data of the synthesized compounds 6a-n

4-((4-((6-methylbenzo[d]thiazol-2-yl)amino)-6-(phenylamino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one (6a) : Cream solid; R*f*: 0.21; IR (KBr cm^{-1}): 3272, 2961, 2862, 2239,

1719, 1510, 1181, 1137, 1030, 812, 763; ^{1}H NMR(400 MHz, DMSO-d_6); δ ppm 2.36 (s, 3H, CH_3), 5.78 (s, 1H, ArH), 7.01-7.88 (m, 12H, ArH), 9.82 (s, 1H, NH), 12.01 (s, 1H, NH); Mass (m/z): 494; Anal. Calcd. for $C_{26}H_{18}N_6O_3S$ Calculated C:63.15, H: 3.67, N:16.99. Found C: 63.14, H: 3.65, N: 16.98 %.

4-((4-((4-methoxyphenyl)amino)-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one (6b) : Yellow solid; R*f*: 0.22; IR (KBr cm^{-1}): 3452, 3450, 2852, 2735, 1781, 1618, 1534, 1407, 1312, 898, 751; Mass (m/z): 524; Anal. Calcd. for $C_{27}H_{20}N_6O_4S$ Calculated C:61.82, H: 3.84, N: 16.02. Found C: 61.79, H: 3.85, N: 16.02 %.

4-((4-((3-chlorophenyl)amino)-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one (6c) : Yellow solid; R*f*: 0.21; IR (KBr cm^{-1}): 3260, 2959, 2919, 2240, 1751, 1505, 1521, 1410, 1354, 805, 761; Mass (m/z): 528 [m+1]; Anal. Calcd. for $C_{26}H_{17}ClN_6O_3S$ Calculated C:59.04, H: 3.24, N: 15.89. Found C: 59.01, H: 3.25, N: 15.87 %.

4-((4-((6-methylbenzo[d]thiazol-2-yl)amino)-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one (6d): Yellow solid; R*f*: 0.20; IR (KBr cm^{-1}): 3355, 3240, 2922, 2825, 1727, 1612, 1511, 1352, 1244 1120, 854, 711; ^{1}H NMR(400 MHz, DMSO-d_6); δ ppm 2.42 (S, 3H, CH_3), 5.78 (s, 1H), 6.58-7.95 (m, 12H, ArH), 10.28 (s, 1H, NH), 12.11 (s, 1H, NH); ^{13}C NMR (100 MHz); 17, 97, 109, 111, 112, 112, 118, 119, 121, 128, 131, 132, 138, 149, 150, 152, 161, 163, 170, 178. Mass (m/z): 539; Anal. Calcd. for $C_{26}H_{17}N_7O_5S$ Calculated C: 57.88, H: 3.18, N: 18.17. Found C: 57.89, H: 3.17, N: 18.15 %.

4-((4-((4-bromophenyl)amino)-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one (6e) : Yello solid; R*f*: 0.22; IR (KBr cm^{-1}): 3275, 2961, 2920, 2240, 1727, 1487, 1180, 1136, 807; Mass (m/z): 572[m+1]; Anal. Calcd. for $C_{26}H_{17}BrN_6O_3S$ Calculated C:54.46, H: 2.99, N: 14.66. Found C: 54.49, H: 2.99, N: 14.64 %.

4-((4-((4-fluorophenyl)amino)-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one (6f) : Yellow solid; R*f*: 0.22; IR (KBr cm^{-1}): 3365, 2959, 2919, 2240, 1506, 1410, 1354, 1211, 1180, 805, 761; Mass (m/z): 512 [m+1]; Anal. Calcd. for $C_{26}H_{17}FN_6O_3S$ Calculated C: 60.93, H: 3.34, N: 16.40. Found C: 60.90, H: 3.38, N: 16.36 %.

4-((4-((4-chlorophenyl)amino)-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one (6g) : Yellow solid; R*f*: 0.22; IR (KBr cm^{-1}): 3265, 2959, 2819, 2245, 1706, 1410, 1351, 1211, 1180, 815, 751; Mass (m/z): 528 [m+1]; Anal. Calcd. for $C_{26}H_{17}ClN_6O_3S$ Calculated C: 59.04, H: 3.24, N: 15.89. Found C: 59.01, H: 3.21, N: 15.88 %.

4-((4-((6-methylbenzo[d]thiazol-2-yl)amino)-6-((3-nitrophenyl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one (6h) : Yellow color solid; R*f*: 0.21; IR (KBr cm^{-1}): 3372, 2863, 2813, 2239, 1711, 1520, 1434, 1280, 1137, 815, 752; Mass (m/z): 539; Anal. Calcd. for $C_{26}H_{17}N_7O_5S$ Calculated C: 57.88, H: 3.18, N: 18.17. Found C: 57.89, H: 3.15, N: 18.15 %.

4-((4-((6-methylbenzo[d]thiazol-2-yl)amino)-6-(p-tolylamino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one (6i) : Cream solid; R*f*: 0.23; IR (KBr cm^{-1}): 3272, 2919, 2863, 2239, 1719, 1510, 1234, 1180, 1137, 812, 762; Mass (m/z): 508; Anal. Calcd. for $C_{27}H_{20}N_6O_3S$ Calculated C: 63.77, H: 3.96, N: 16.53. Found C: 63.79, H: 3.95, N: 16.52 %.

4-((4-((2-methoxyphenyl)amino)-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one (6j) : Yellow solid; R*f*: 0.23; IR (KBr cm^{-1}): 3356, 3240, 2932, 2835, 1764, 1610, 1504, 1440, 871, 725; Mass (m/z): 524; Anal. Calcd. for $C_{27}H_{20}N_6O_4S$ Calculated C: 61.82, H: 3.84, N: 16.02. Found C: 61.80, H: 3.84, N: 16.01 %.

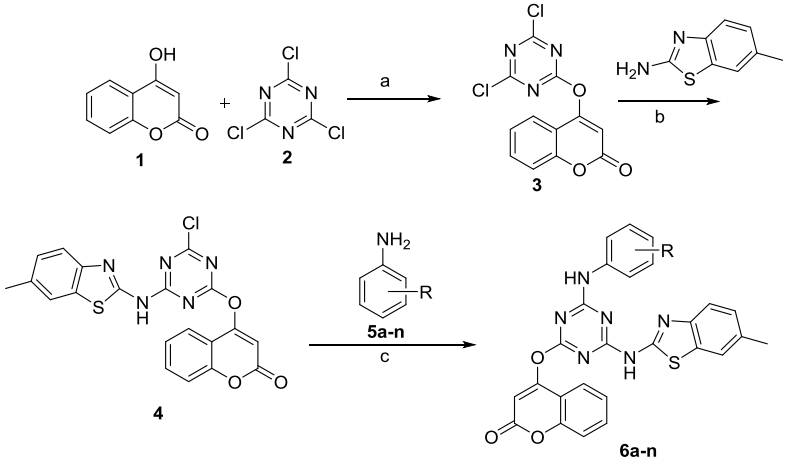
4-((4-((6-methylbenzo[d]thiazol-2-yl)amino)-6-(o-tolylamino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one (6k) : Yellow solid; R*f*: 0.23; IR (KBr cm^{-1}): 3257, 3250, 2872, 2837, 1726, 1614, 1541, 1462, 1374, 1151, 818, 724; Mass (m/z): 508; Anal. Calcd. for $C_{27}H_{20}N_6O_3S$ Calculated C: 63.77, H: 3.96, N: 16.53. Found C: 63.75, H: 3.94, N: 16.51 %.

4-((4-((2,4-dimethylphenyl)amino)-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one (6l): Yellow solid; R*f*: 0.23; IR (KBr cm^{-1}): 3365, 3212, 2851, 2762, 1708, 1557, 1414, 1332, 1121, 865, 745; ^{1}H NMR (400 MHz, DMSO-d_6); δ ppm 2.27 (s, 3H, CH_3), 3.09 (s, 6H, $2CH_3$), 5.81 (s, 1H, ArH), 6.73-7.81 (m, 11H, ArH), 9.82 (s, 1H, NH), 11.91 (s, 1H, NH); Mass (m/z): 522 [m+1]; Anal. Calcd. for $C_{28}H_{22}N_6O_3S$ Calculated C:64.35, H: 4.24, N: 16.08. Found C: 64.32, H: 4.21, N: 16.07 %.

4-((4-((2-fluorophenyl)amino)-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one (6m) : Yellow solid; R*f*: 0.23; IR (KBr cm^{-1}): 3321, 3243, 2927, 2765, 1989, 1725, 1532, 1515, 1422, 1311, 835, 702; Mass (m/z): 512 [m+1]; Anal. Calcd. for $C_{26}H_{17}FN_6O_3S$ Calculated C:60.93, H: 3.34, N: 16.40. Found C: 60.92, H: 3.35, N: 16.38 %

4-((4-((2-bromophenyl)amino)-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one (6n) : Yellow solid; R*f*: 0.22; IR (KBr cm^{-1}): 3315, 3280, 2922, 2815, 1701, 1571, 1412, 1425 1367, 854, 722; Mass (m/z): 573 [m+1]; Anal. Calcd. for $C_{26}H_{17}BrN_6O_3S$ Calculated C:54.46, H: 2.99, N: 14.66. Found C: 54.45, H: 2.93, N: 14.65 %.

RESULTS AND DISCUSSION



Scheme 1: Synthesis of Coumarin, benzothiazole and amino bearing 1,3,5-triazines. a) Acetone, 10 % $NaHCO_3$ solution, stirring at 0-5 ^{0}C, 2-3hr. b) Acetone, K_2CO_3 (1 eq.), stirring at 0-5 ^{0}C to rt, 4-5 hr. c) THF, cat K_2CO_3, reflux 7- 8 hr.

The reaction of 4-hydroxy coumarin **1** with cyanuric chloride **2** was carried out using reported procedure. To synthesize the intermediate 4-((4-chloro-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one **4,** the reaction of 2-amino-6-methyl benzothiazole with **3** was carried out with stirring at room temperature using acetone as solvent and potassium carbonate as base (**Scheme 1**). The desired compounds 4-((4-((6-methylbenzo[d]thiazol-2-yl)amino)-6-(arylamino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one **6a-n** were synthesized by the reaction of aromatic amines **5a-n** with compound **4** using tetrahydrofuran under reflux condition using K_2CO_3 in catalytic amount. In all reaction steps, the work up of products was very easy and simple to give analytically pure compounds.

Table 1. Physical properties of compounds 6a-n.

Entry	R_1	Yields (%)	Melting range
6a	H	72	190-192
6b	4-OCH_3	75	202-204
6c	3-Cl	78	180-182
6d	4-NO_2	70	198-200
6e	4-Br	75	208-210
6f	4-F	75	192-194
6g	4-Cl	78	196-198
6h	3-NO_2	71	208-210
6i	4-CH_3	73	195-197
6j	2-OCH_3	74	202-204
6k	2-CH_3	76	208-210
6l	2,4-$(CH3)_2$	74	220-222
6m	2-F	70	180-182
6n	2-Br	71	216-218

4-((4,6-dichloro-1,3,5-triazin-2-yl)oxy)-2*H*-chromen-2-one was synthesized by reported process as discussed in chapter 3. ^{1}H NMR of 4-((4-chloro-6-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one showed -CH_3 proton at 2.43 and -CHAr at 5.65 δ ppm, aromatic proton between 7.33 to 8.39 while NH at 12.58 δ ppm. IR signal appeared at 1742 due to presence of C=O group. These data confirmed the formation of compound **5**. ^{1}H NMR signal of 4-((4-((6-methylbenzo[d]thiazol-2-yl)amino)-6-(phenylamino)-1,3,5-triazin-2-yl)oxy)-2H-chromen-2-one **6a** showed –CH_3 proton at 2.30 δ ppm, ArH at 5.78, aromatic protons between 7.01 to 7.88, -NH at 9.82 and -NH at 12.01 δ ppm which resemble to the formation of trisubstituted 1,3,5-triazine. The IR signals of C=O and NH were observed at 1719 and 3272 cm-^{1}, respectively. The ^{13}C NMR data of compounds **6d** also suggests the formation of desired compound. The physical properties of newly synthesized compounds are depicted in **Table 1**. Among the synthesized compounds, some compounds have been screened for their antimicrobial activity and data are shown in **Table 2**.

Table 2. Antimicrobial activity of selected compounds.

Entry	Gram positive		Gram negative		Fungi
	B.subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. niger*
6a	3.00	-	4.25	-	**3.75**
6g	**3.75**	-	**4.25**	1.75	**3.75**
6h	**4.75**	-	**3.50**	**1.75**	**5.75**
6k	3.25	-	4.25	-	3.25
6l	3.25	-	**4.75**	-	3.25

*Zone of inhibition in mm, *Concentration 1000 microgram per ml, - = not active.

Among the tested compounds, compound **6h** exhibited good inhibition against Gram positive *B. Subtilis*, and Gram negative *E. coli* bacteria and Fungi at 4.75, 3.50 and 5.75 mm, respectively. *A. Niger*. While compound **6l** was potent against only Gram negative *E. coli* bacteria. Comopound 7g showed inhibition against *B. Subtiils, E. Coli* bacteria and *A. Niger* fungi However, all compounds were inactive against Gram positive *S. aureas* bacteria strain. Remaining compounds have shown moderate inhibition against microbial strains

CONCLUSION

We have demonstrated an easy and conventional method for the synthesis of novel 4-hydroxy coumarin and benzothiazole bearing 1,3,5-triazine derivatives with good to high yields. The present process comprises easy and clean workup which gave desired product with good purity. Among all compounds, five compounds were screened against gram positive and gram negative bacteria and fungi and examined zone of inhibition. Compound **6h** was found active against gram positive and gram negative bacteria and fungi. However, all compounds have moderate inhibition against fungi *A. Niger*.

ACKNOWLEDGMENT

The authors are thankful to Shree M. & N. Virani Science College for providing laboratory facility and Biotechnology Dept. of Pramukh swami science & H. D. Patel arts college, Kadi for providing biological activity.

REFERENCES

1. Xinjiang, W.; Fekadu, K.; Volker, M.-S.; *Mutat. Res*., 589, 81-102 (2005**)**.
2. Hutchinson, I.; Jennings, S.A.; Vishnuvajjala, B.R.; Westwell, A.D.; Stevens, M.F.G.; *J. Med. Chem*., 45, 744-747, (2002).
3. (a) Hargrave, K.D.; Hess, F.K.; Oliver, J. T. *J. Med. Chem*., 26, 1158-1163, (1983); (b) Gronowitz, S.; Gronowitz, S.; (Ed.), The Chemistry of Heterocyclic Compounds: Thiophene and its Derivatives, vol. 44, Wiley, New York, NY, USA, 1991. Part 3, (Chapter 2).

4. (a) Patt, W. C.; Hamilton, H. W.; Taylor, M. D.; Ryan, M. J.; Taylor, D. G. J.; Connolly, C. J. C.; Doherty, A. M.; Klutchko, S. R.; Sircar, I.; Steinbaugh, B. A. *J. Med. Chem*., 35, 2562-2572, (1992); (b) Jaen, J. C.; Wise, L. D.; Caprathe, B. W.; Tecle, H.; Bergmeier, S.; Humblet, C. C.; Heffner, T. G.; Meltzner, L. T.; Pugsley, T. A. *J. Med. Chem*., 33, 311-317, (1990).
5. Sharma, R. N.; Xavier, F. P.; Vasu, K. K.; Chaturvedi, S. C.; Pancholi, S. S. *J. Enzyme Inhib. Med. Chem*., 24, 890-897 (2009).
6. Kempf, D.J.; Sham, H.L.; Marsh, K.C.; Flentge, C.A.; Betebenner, D.; Green, B.E.; McDonald, E.; Vasavanonda, S.; Saldivar, A.; Wideburg, N.E.; Kati, W.M.; Ruiz, L.; Zhao, C.; Fino, L.M.; Patterson, J.; Molla, A.; Plattner, J.J.; Norbeck, D.W. *J. Med. Chem*., 41, 602-617 (1998).
7. Kumar, G. J.; Kumar, S. N.; Thummuri, D.; Adari, L. B.D.; Naidu, V. G. M.; Srinivas, K.; Rao, V. J. *Med Chem Res*, 24, 12, 3991–4001 (2015).
8. Padalkar, V. S.; Gupta, V. D.; Phatangare, K. R.; Patil, V. S.; Umape, P. G.; Sekar, N. *Journal of Saudi Chemical Society*, 18 (3), 262-268 (2014).
9. Mistry, M. H.; Parmar, S. J.; Desai, G. C. *J. Chem. Pharm. Res*., 3(4), 831-837 (2011).
10. Murhekar, M. M.; Padghan, P. D.; Mhaske, S. S.; Khadsan, R. E.; *Der Pharma Chemica*, 3 (6), 243-246, (2011).
11. Pandit, A. B.; Ladva, Savant, M. M.; K. D., *Journal of Het. Chem.*, 2018, https://doi.org/10.1002/jhet.3128.
12. Allen, C. F. H. and Allan, V. J. *Org. Synth.* 22, 16 (1942).