SUMMARY OF FINAL PROJECT COMPLETION REPORT

UGC-MAJOR RESEARCH PROJECT

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NEW DELHI – 110 002

Project Title: ***"Synthesis of a new class of Bis-heterocyclic compounds using greener synthetic routes and evaluation of their anticancer activity"***

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A DETAILED REPORT ABOUT RESEARCH WORK DONE

1.0 INTRODUCTION

Synthesis and biological evaluation of new heterocyclic rings is a very attractive area, polyfunctionalized heterocyclic compounds play an important role in the drug discovery process. Heterocyclic compounds have manifold applications in pharmacy, medicine, agriculture, and allied fields.

Certain possible modifications on the heterocyclic ring by the addition of diverse substituents may lead to new products with better biological profiles. As a result of the biological activity exhibited by the heterocyclic molecules, the development of new chemical entities (NCEs) is the focus of intense activity in pharmaceutical industry.

." Heterocyclic compounds are very widely distributed in nature and are very essential to living organisms. They play a vital role in the metabolism of all the living cells. Therefore, heterocyclic compounds occupying chief position in the medicinal chemistry. Heterocycles are present in a wide variety of drugs, most vitamins, many natural products, biomolecules and biologically active compounds, including antitumor, antibiotic, anti-inflammatory, antidepressant, antimalarial, anti-HIV, antibacterial, antifungal, antiviral, antidiabetic, herbicidal, fungicidal and insecticidal agents. Also, they have been frequently found as a key structural unit in synthetic pharmaceuticals and agrochemicals. Besides the vast distribution of heterocycles in natural products, they are also the major components of biological molecules such as DNA and RNA. DNA is without doubt the most important macromolecule of life.

Not only one heterocyclic moiety containing Heterocyclic compounds, but also two heterocyclic moieties containing molecules (Bis- heterocyclic compounds) are found to exhibit very good biological properties. Bis-heterocyclic compounds are gaining increased interest in the recent past as the dimeric analogues have proven to be having better and potential biological activity than the corresponding monomer. Bis-heterocyclic compounds also show biological activities such as antimicrobial, antifungal, anti-inflammatory, antiviral, and anti-HIV. As a result, many research groups are turning their attention to the synthesis of bis-heterocycles.

We have synthesized novel aurones, benzofurans, biaryl systems, coumarin, flavonols, spirochromones, triazoles, bistriazoles and flavanone by both conventional and green methods.

The synthesized compounds were evaluated with various biological activities such as anti bacterial, antifungal, anti-inflammatory, anti-oxidant, anticancer activity.

2.0 Materials and Methods

All the Suzuki reactions were performed under nitrogen atmosphere using oven dried apparatus. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F_{254}), visualizing with ultraviolet light. Column chromatography was performed on silica gel (60–120 mesh) using distilled hexane, ethyl acetate. ^{1}H NMR and ^{13}C NMR spectra were determined in $CDCl_3$ solution by using 400 and 100 MHz spectrometers, respectively (Instrument Bruker Avance II 400 MHz). Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet) as well as bs (broad singlet). Coupling constants (*J*) are given in hertz. Mass spectra were recorded on GCMS-QP 1000 EX mass spectrometer. Infrared spectra were recorded on a Shimadzu FT-IR-8400s spectrometer. Melting points were determined using Stuart SMP3 melting-point apparatus and are uncorrected. CEM discover microwave reaction vessel equipped with a magnetic stirrer was used for microwave irradiated reactions. The antioxidant property was carried out by using Shimadzu UV-2450 spectrophotometer and the Perkin Elmer Lambda 750 UV-Visible Spectrophotometer was used to calculate the percentage inhibition for anti inflammatory activity.

3.0 SYNTHESIS

3.1 SCHEME 1

INTRODUCTON

Aurone derivatives are versatile biodynamic agents that can be used to design and develop new potentially useful therapeutic agents. Literature survey based on the framework reveals that aurone ring containing moiety, exhibit as potent antileishmanial agents. The molecule contains a benzofuran element associated with a benzylidene linked in position. In aurones, a chalcone-like group is closed into a 5-membered ring instead of the 6-membered ring more typical of flavonoids. Recent investigation has shown that these compounds have good biological activities and are better than flavones and chalcones. Aurones are a class of flavonoids called anthochlor pigments and occurs rarely in nature[1]. It is present in fruits and flowers where they act as phytoalexins against infections and gives contribution to yellow pigmentation of plant parts. Aurones contribute to bright yellow coloration of flowers of some well known ornamental plants such as Snapdragon and Cosmos.

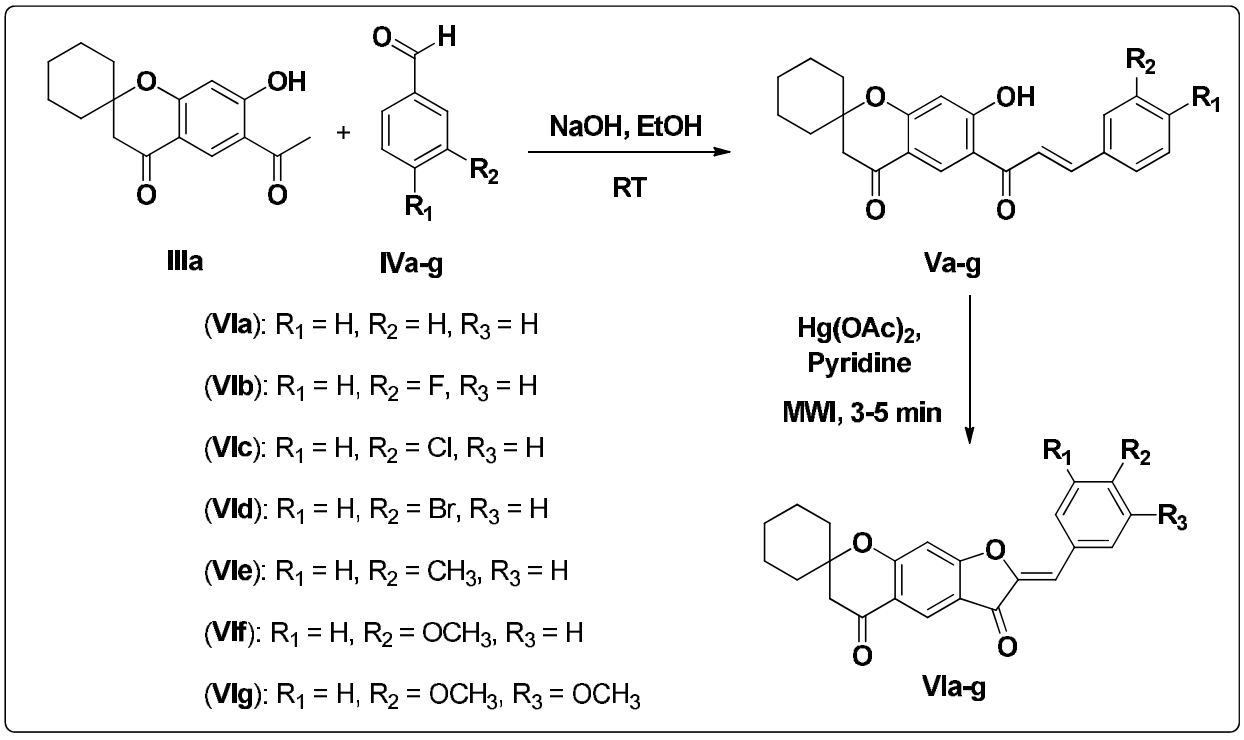
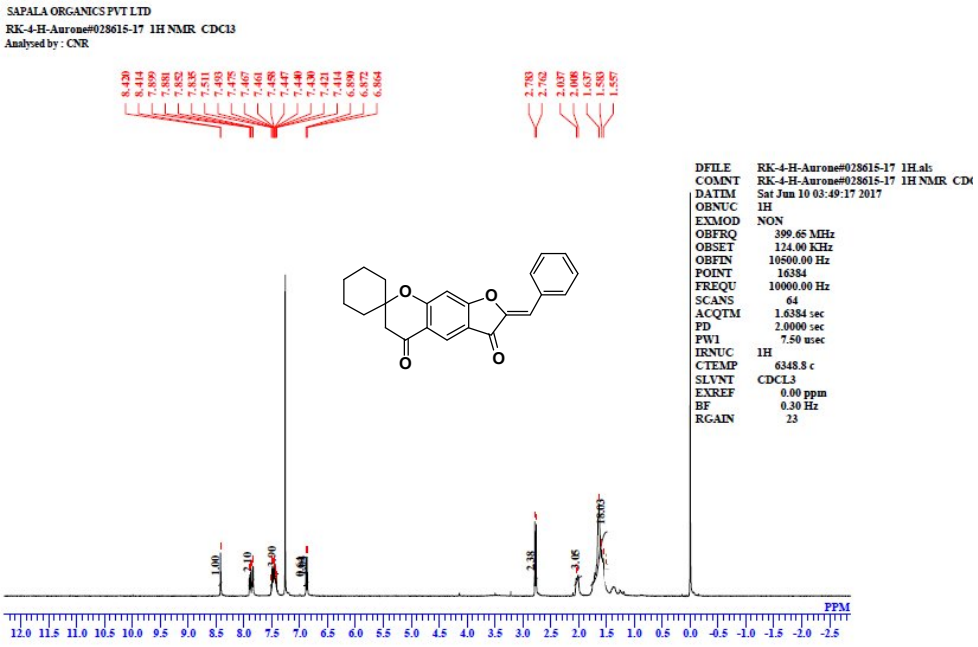


Fig 1

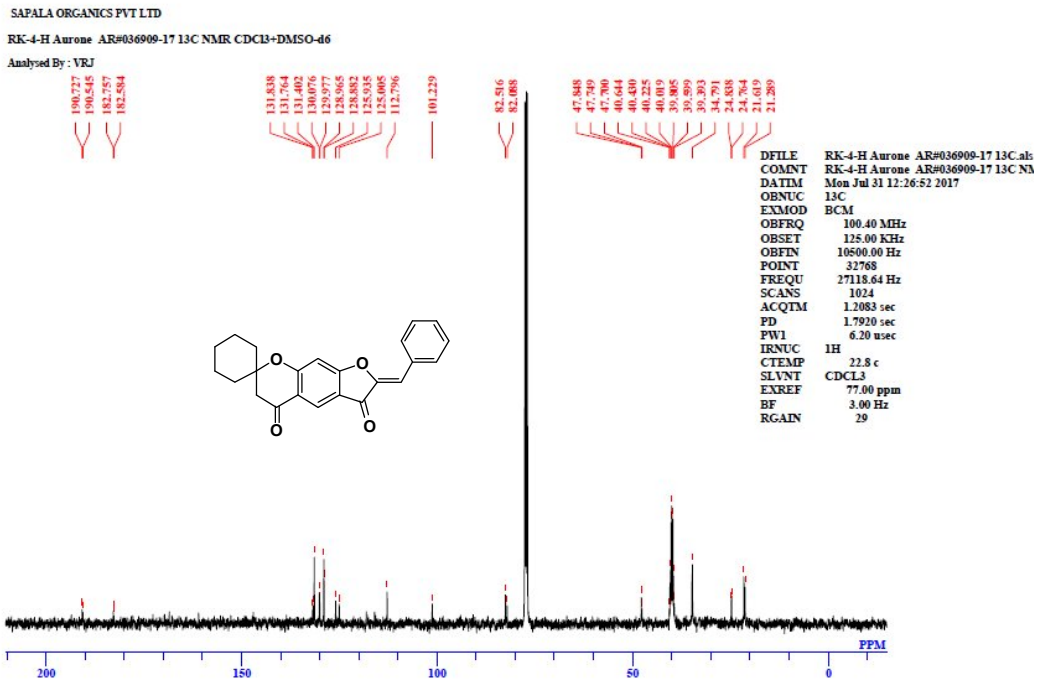
Russian Journal of General Chemistry, 2018, Vol. 88, No. 5, pp. 1015–1019

IF: 0.481, Springer, SCI expanded, SCOPUS.

SPECTRAL ANALYSIS



^{1}H NMR of (*Z*)-2'-benzylidenespiro[cyclohexane-1,7'-furo[3,2-*g*]chromene]-3',5'(2'*H*,6'*H*)-dione (**VIa**)



^{13}C NMR of (*Z*)-2'-benzylidenespiro[cyclohexane-1,7'-furo[3,2-*g*]chromene]-3',5'(2'*H*,6'*H*)-dione (**VIa**)

Antibacterial activity: The synthesized compounds **6a–6g** was evaluated for *in vitro* antibacterial activity using the cup-plate agar diffusion method. Streptomycin was used as a standard (40 μg/mL). The compounds **6a–6g** were tested against representative Gram-positive organisms, *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 737), and Gram-negative organisms, *Escherichia coli* (MTCC 443) and *Pseudomonas aeruginosa* (MTCC 741) (Table 1). The cup-plate agar diffusion method was recommended by National Committee for Clinical Laboratory (NCCL) standards.

Antifungal activity: All synthesized compounds **6a– 6g** were tested for *in-vitro* antifungal activity against the fungal strains *Aspergillus flavus* (ATCC-9643) and *Fusarium oxysporum* (ATCC-48112), by the poison plate technique at the concentration of 50 μg/mL. Zones of inhibition were compared against the standard drug Ampothericin-B. The antifungal screening data revealed that the compounds **6a**, **6c**, **6e**, **6g** had pronounced activity against the tested organisms.

Table 1: Antimicrobial activity of aurones **6a–6g**

Compound	Zone of inhibition, mm					
	gram-positive bacteria		gram-negative bacteria			
	Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Aspergillus flavus*	*Fusarium oxysporum*
6a	19.5	18.0	22.5	20.5	5.5	4.5
6b	13.5	14.0	15.9	16.0	4.5	6.5
6c	12.5	11.5	13.0	14.0	2.5	3.0
6d	13.5	14.5	12.0	15.0	6.0	4.5
6e	19.0	18.5	21.0	20.0	4.0	8.5
6f	19.0	21.0	22.5	21.0	6.5	7.5
6g	21	19.5	22.5	21.0	4.5	6.5
Streptomycin	21	20.0	23.0	21.0	-	-
Amphotericin B	-	-	-	-	12.0	15.0

Conclusion: Synthesis of novel hybrid spirochromanone molecules containing aurones under microwave irradiation. The relatively inexpensive method proceeded via a facile, two-steps synthesis.

3.2 SCHEME 2

INTRODUCTION

Chromones are important oxygen containing heterocycles which possess variety of biological activities such as antiarrhythmic, anti-HIV, ACC inhibitor, vanilloid receptor antagonist, and antiviral. Chroman-4-ones fused with Spiro substitution possess diverse pharmaceutical actions viz. antihistamine[2], antioxidant and anti-inflammatory[3] (Ashok et al., 2016) activities. Flavonoid framework is endowed with various activities, such as antibacterial, antiprotozoal, oestrogenic, anticancer or anticarcinogenic.

In a view of the biological significance of spirochromanones and flavonols, and in continuation of our research works on the synthesis of such biologically active chroman-4-one derivatives. Further, all the newly synthesized compounds were evaluated for their *in vitro* antimicrobial activity against different pathogenic strains.

In a view of the biological significance of spirochromanones and flavonols and in connection with our search on the design and synthesis of pharmacologically important new heterocycles linked to Chromanone, it was thought worthwhile to synthesize the titled compounds.

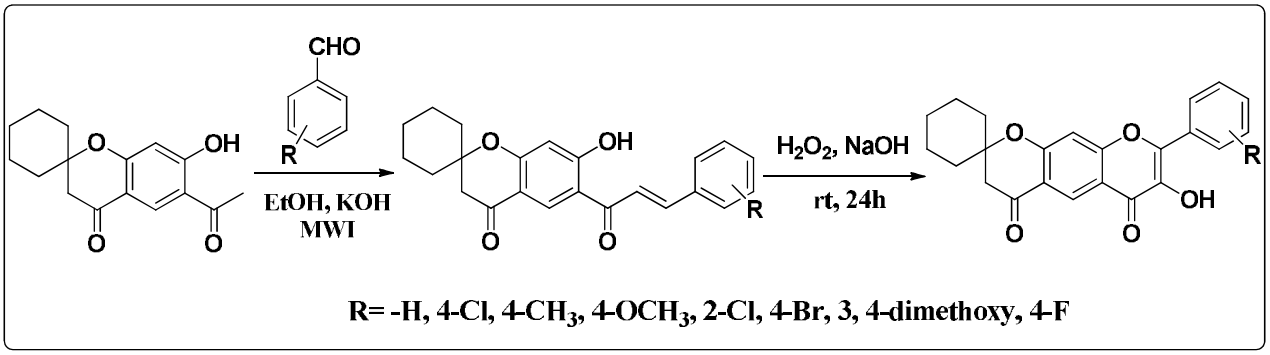
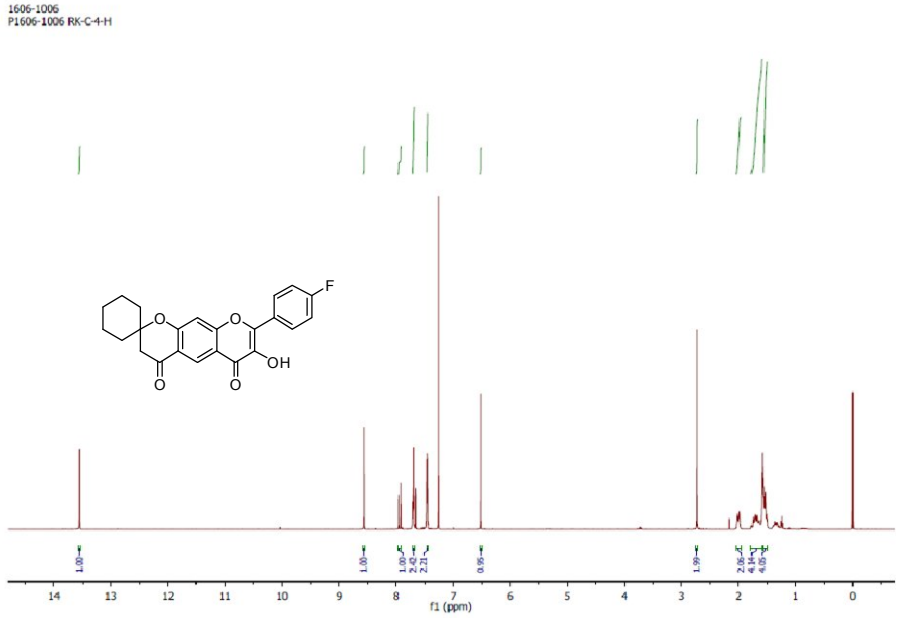


Fig- 2

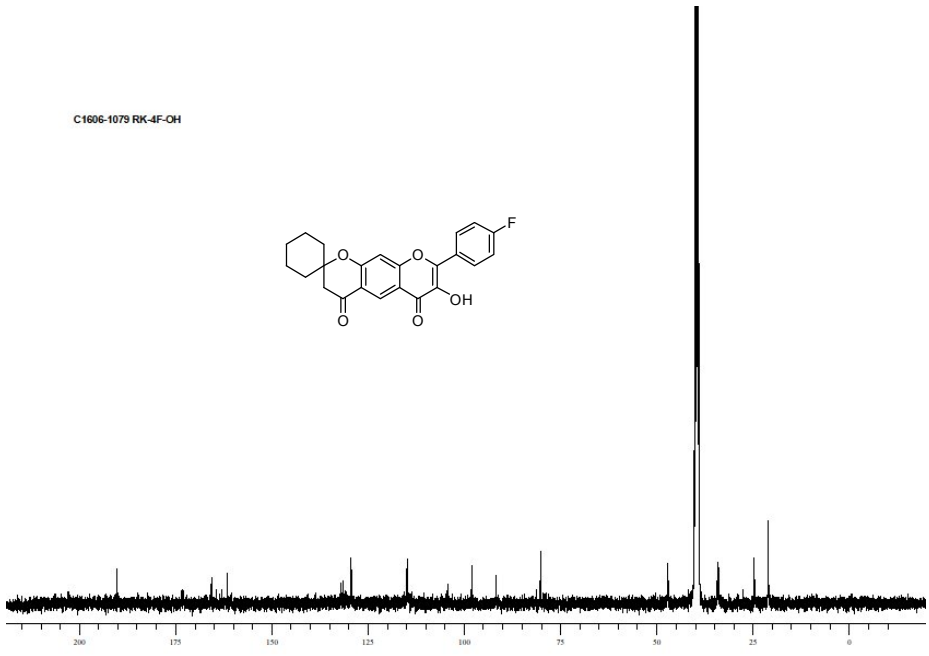
Chemistry of Heterocyclic Compounds 2017, 53(11), 1187–1191

IF: 0.815, Springer, SCI expanded, SCOPUS

SPECTRAL ANALYSIS



^{1}H NMR of 8'-(4-fluorophenyl)-7'-hydroxy-3'*H*-spiro[cyclohexane-1,2'-pyrano[3,2-*g*]chromene]-4',6'-dione **(5b):**



^{13}C NMR of 8'-(4-fluorophenyl)-7'-hydroxy-3'*H*-spiro[cyclohexane-1,2'-pyrano[3,2-*g*]chromene]-4',6'-dione **(5b):**

Antibacterial activity

The newly synthesized compounds **5a**–**g** were screened *in vitro* for their antibacterial activity by the cup-plate agar diffusion method. Antibacterial activity was tested against two Gram-positive bacterial strains (*Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 737)) and two Gram-negative bacterial strains (*Pseudomonas aeruginosa* (MTCC 741), *Escherichia coli* (MTCC 443)) at a concentrations 40 μg/ml. Nutrient agar medium was used for the antibacterial screening. The zone of inhibition (in mm) was compared with standard drug streptomycin sulfate. The investigation of antibacterial activity revealed that compounds **5a**,**c**,**d** showed excellent growth inhibition against *Bacillus subtilis* as compared to streptomycin (18.5–19.5 *vs* 20 mm) at concentration 40 μg/ml.

In the case of *Staphylococcus aureus*, compound **5d** was found to be exceedingly potent in comparison with the standard (18 *vs* 21 mm). Compounds **5a**–**d** showed moderate to good activity compared to the standard streptomycin (23 mm) against *Escherichia coli*. Compounds **5e**, **f** showed moderate activity comparable with that of streptomycin against *Escherichia coli*.

Table 2 Antimicrobial activity of compounds **5a**–**g** expressed as zone of inhibition (mm)

Compound	Bacteria strains				Fungal strains	
5a	*S. aureus*	B.subtilis	E.coli	P.aeruginosa	A.flavus	F.oxysporum
5b	17.0	19.0	19.0	22.0	4.5	6.5
5c	16.0	17.5	18.5	15.0	5.0	4.5
5d	15.5	18.5	18.5	21.0	3.5	5.0
5e	16.0	15.0	17.5	18.5	4.0	7.5
5f	14.5	15.5	14.0	16.0	5.5	6.5
5g	15.5	11.0	13.0	16.5	2.0	4.5
Streptomycin	21.0	20.0	21.0	23.0	-	-
Amphotericin-B	-	-	-	-	12.0	15.0

Conclusion: A novel series of spirochromone-flavonol derivatives were synthesized in good yields without isolating chalcones. All the newly synthesized compounds were screened for *in vitro* antimicrobial activity. All the compounds were shown moderate to good antimicrobial activity.

3.3 SCHEME 3:

INTRODUCTION

Benzofurans are interesting oxygen containing heterocycles which are ubiquitous in nature and show a wide range of biological activities such as analgesics, antidepressants, potent antitumor agents. Chromanone derivatives, in particular 2-spiro-chroman-4(1*H*)-ones are embodied in many bio-active molecules and possess various biological activities which include antiarrhythmic, anti-HIV[4], antidiabetic, ACC inhibitor, vanilloid receptor antagonist, growth hormone secretagogues, histamine receptor antagonist and antiviral. Hybrid compounds containing benzofuran and spirochromanone moieties, called spirofurochromanone, due to combined effect may exhibit better biological profile. Microwave assisted organic synthesis leads to rate enhancement with excellent reproducibility, improved yields and less side reactions compared to conventional heating[5].

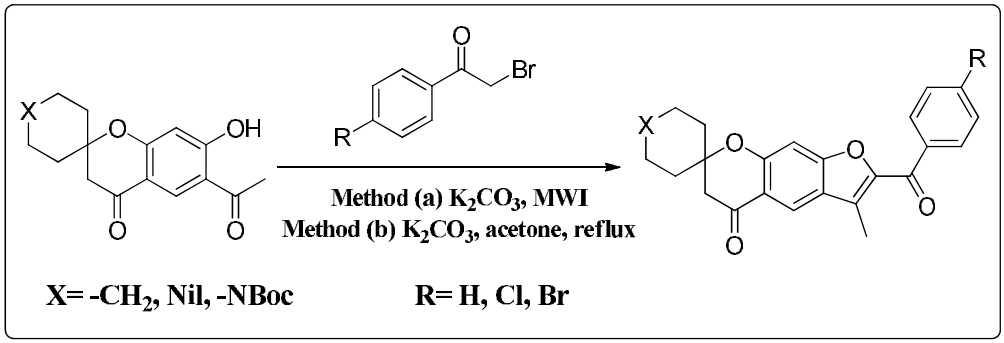
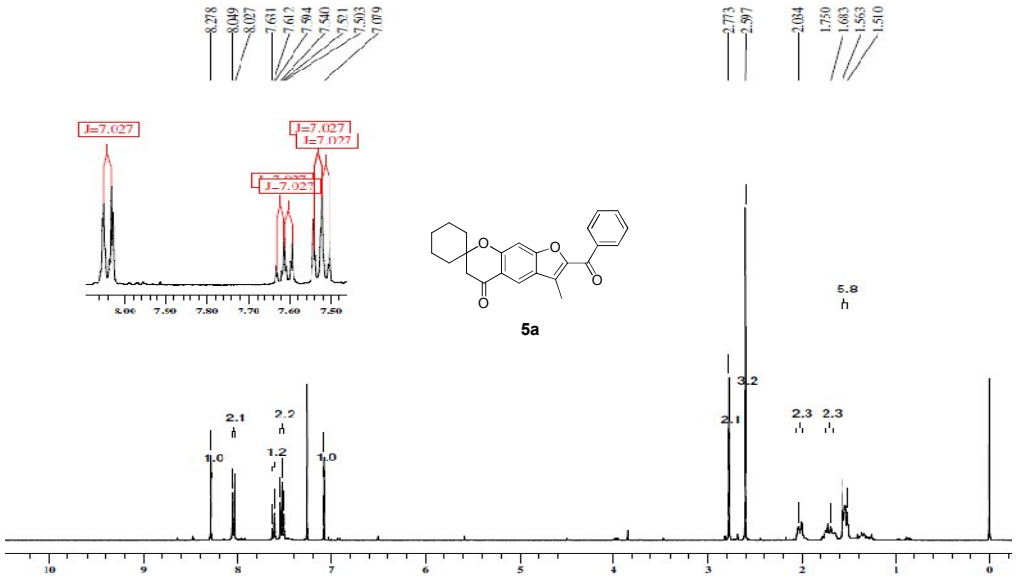


Fig 3

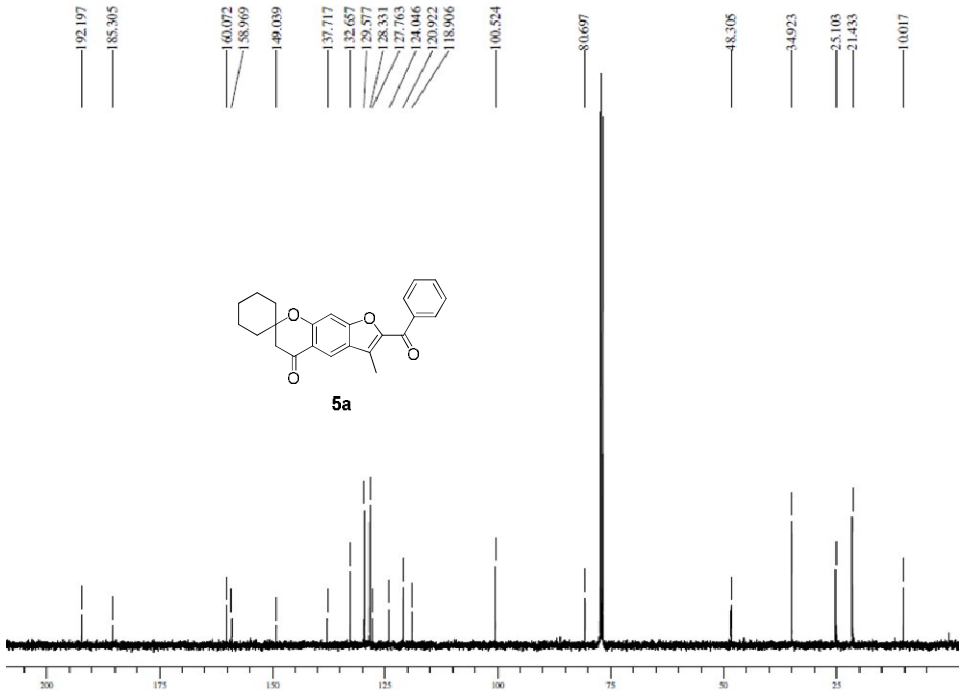
RSC Advances 7(41):25710-25724

IF: 2.936, Royal Society of Chemistry, SCI expanded, SCOPUS

SPECTRAL ANALYSIS



^{1}H NMR of **7'-benzoyl-6'-methyl-3'*H*-spiro[cyclohexane-1,2'-cyclopenta[*g*]chromen]-4'(8'*H*)-one (*5a*)**



^{13}C NMR of **7'-benzoyl-6'-methyl-3'*H*-spiro[cyclohexane-1,2'-cyclopenta[*g*]chromen]-4'(8'*H*)-one (*5a*)**

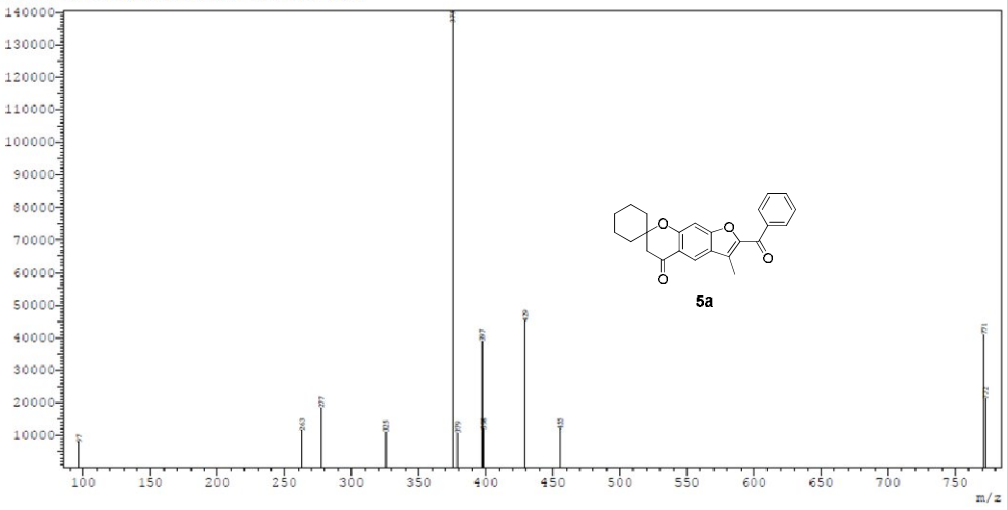
SHIMADZU
LabSolutions

MASS REPORT (IICT - NPL)

Sample Name : GSR-H-6-374
Data File : 260716.37.lcd
Date Acquired : 7/28/2016 6:31:42 PM

MS Spectrum
GSR-H-6-374

AveragedESI Positive+
Spectrum Mode:Averaged 0.110-0.310(45-125)



5a

Mass of **7'-benzoyl-6'-methyl-3'*H*-spiro[cyclohexane-1,2'-cyclopenta[*g*]chromen]-4'(8'*H*)-one (*5a*)**

3.4 SCHEME 4

Biaryl units as molecular components in pharmaceuticals, herbicides and natural products, as well as in engineering materials, has attracted enormous interest and their syntheses have been widely carried out and have yielded innumerable compounds with diverse biological activities such as: anti-inflammatory, antimicrobial, antifungal, antiproliferative, antidiabetic, immunosuppressant, analgesic, antioxidant.[6] The Suzuki cross-coupling reaction, which represents an attractive and alternative methods that use organometallic species for the construction of unsymmetrical biaryl compounds, involves air and moisture stable organoboranes that possess relatively low toxicity and also has broad functional group tolerance. Catalysts used in the Suzuki reaction have been traditionally based on homogeneous palladium phosphine complexes

The study of Suzuki reactions in water or aqueous-organic mixtures has received attention due to the ability of the base to dissolve in water for activating arylboronic acids, and a suitable amount of water is known for improving the reactivity of Suzuki reactions (APOS)[7].

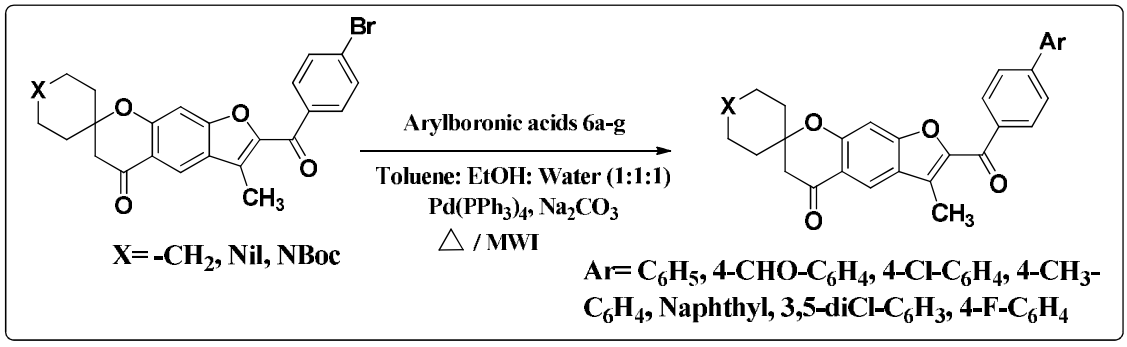
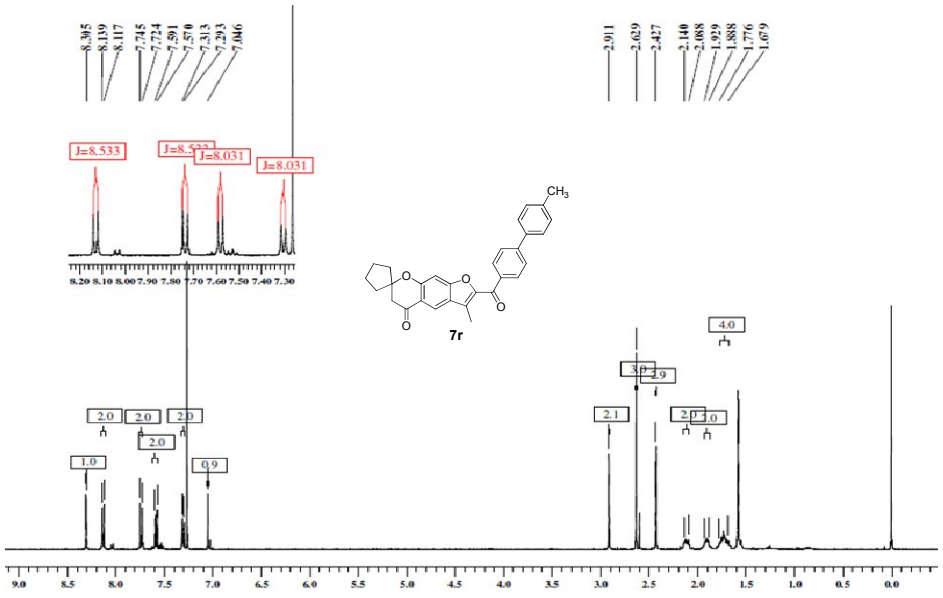


Fig 4

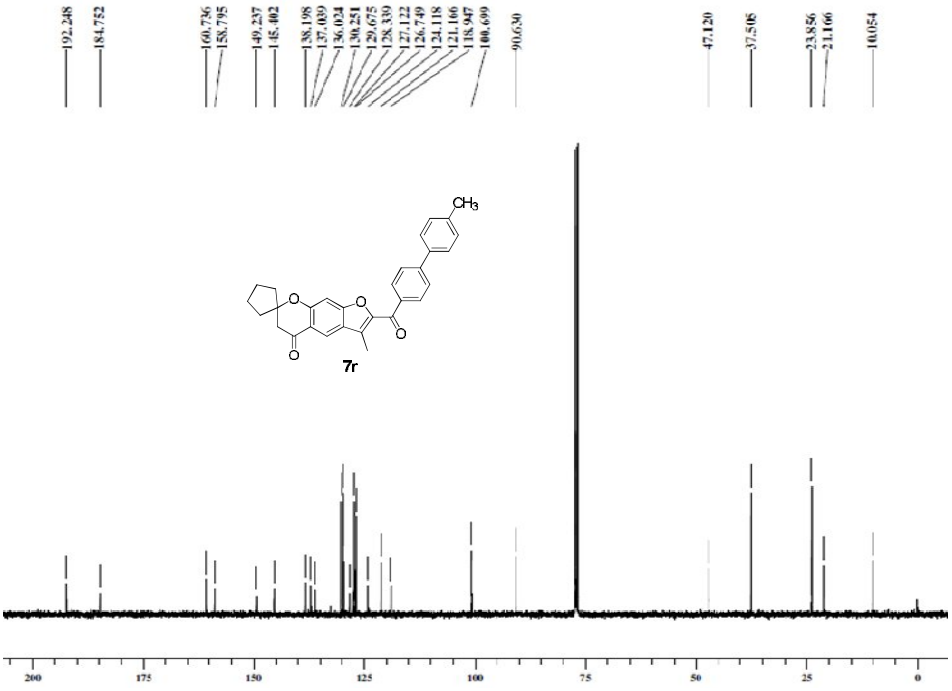
RSC Advances 7(41):25710-25724

IF: 2.936, Royal Society of Chemistry, SCI expanded, SCOPUS

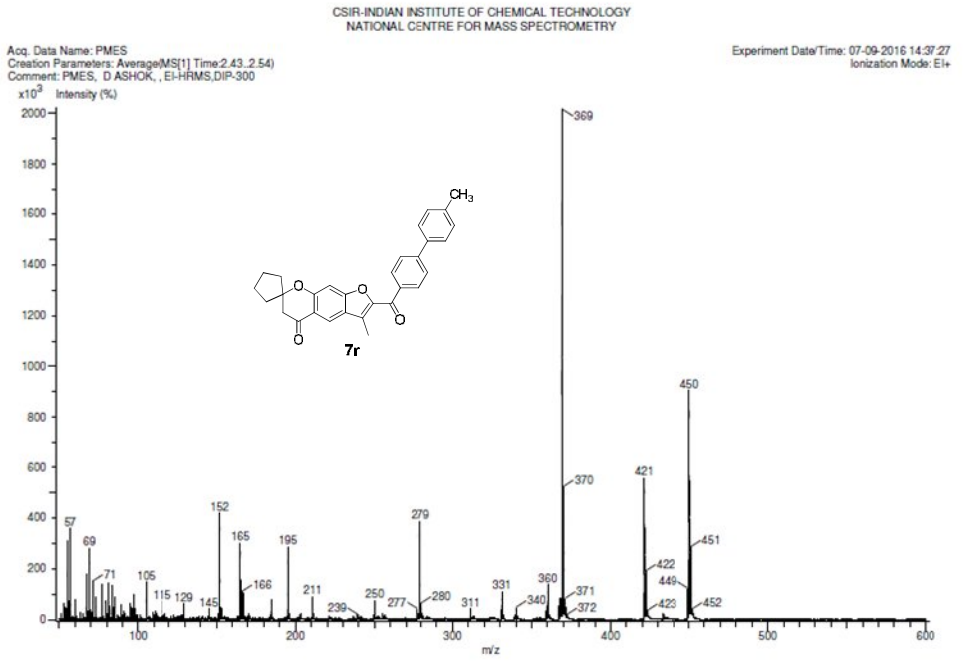
SPECTRAL ANALYSIS



^{1}H NMR of 3'-methyl-2'-(4'-methyl-[1,1'-biphenyl]-4-carbonyl)spiro[cyclopentane-1,7'-furo[3,2-*g*]chromen]-5'(6'*H*)-one (7r)



^{13}C NMR of 3'-methyl-2'-(4'-methyl-[1,1'-biphenyl]-4-carbonyl)spiro[cyclopentane-1,7'-furo[3,2-*g*]chromen]-5'(6'*H*)-one (7r)



Mass of 3'-methyl-2'-(4'-methyl-[1,1'-biphenyl]-4-carbonyl)spiro[cyclopentane-1,7'-furo[3,2-*g*]chromen]-5'(6'*H*)-one (7r)

BIOLOGICAL ACTVITY OF SCHEME 3 AND SCHEME 4

Anti- inflammatory studies

The anti-inflammatory activity of the precursors **5a-i** and synthesized compounds **7a-u** were tested for their *invitro* anti-inflammatory activity by using inhibition of albumin denaturation technique, for the determination of IC_{50} values (concentration of an inhibitor where the response (or binding) is reduced by half). The results of the activity were compared with the standard drug aspirin. The IC_{50} values are listed in **Table 4**. The synthesized compounds have IC_{50} values in the micromolar range, varying from 77.11 to 172.04. Among them, ten compounds **5a**, **5b**, **5d, 5e,7g, 7h**, **7j**, **7l**, **7n** and **7q** with IC_{50} values varying from 77.11 to 116.10 μM possess relatively better inhibitory efficiency compared to that of standard Aspirin (IC_{50} = 116.48 μM).

Anti oxidant studies

DPPH radical-scavenging assay

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical-scavenging assay is usually used to evaluate the abilities of new compounds to capture free radicals by producing the reduced form DPPH-H through a hydrogen-donating action. It was performed in order to determine the antioxidant potential.

DPPH is a stable nitrogen-centered free radical. Its reaction rates correlate directly with antioxidant activity, the higher the rate, the more effective the antioxidant**.** A freshly prepared DPPH solution shows a deep purple color with an absorption maximum at 517 nm. When the purple color changes to yellow, it leads to decreased absorbance. This is because of the antioxidant molecule reducing the DPPH free radical through donation of hydrogen atom. Instantaneous or concomitant decrease in absorbance would be indicative of potent antioxidant activity by the compound.

Hydrogen peroxide scavenging assay

Several reactive species are known to produce in excess during the inflammatory processes, the ROS peroxyl radical ($ROO^{\cdot}$), $HO^{\cdot}$, $O_2^{\cdot -}$, H_2O_2, and HOCl play important roles in their pathophysiological conditions, Hydrogen peroxide (H_2O_2) is a biologically important, non-radical reactive oxygen species (ROS) that can influence several cellular processes, which makes

them potential targets for the therapy of inflammation. In vitro antioxidant activities of synthesized compounds were evaluated against hydrogen peroxide and were compared with standard ascorbic acid. Our results reveal that these compounds exhibit better radical scavenging activities.

Table 3 Anti-inflammatory activity of compounds **5a-i** and **7a-u**

Compound	Anti-inflammatory activity[a] IC_{50} (μM/mL)	Compound	Anti-inflammatory activity[a] IC_{50} (μM/mL)
5a	**110.58 ± 0.78**	7**g**	**97.39** ± 0.76
5b	**77.94 ± 1.00**	**7h**	**77.11** ± 0.66
5c	132.0 ± 1.02	7i	142.31 ± 0.64
5d	**105.61 ± 0.59**	**7j**	**108.97** ± 0.85
5e	**98.46 ± 0.90**	7k	120.48 ± 0.88
5f	159.17 ± 1.00	**7l**	**108.49** ± 0.74
5g	127.55 ± 0.86	7m	152.77 ± 0.89
5h	140.48 ± 1.29	**7n**	**116.10** ± 1.10
5i	152.81 ± 0.92	7o	130.92 ± 0.94
7a	154.07 ± 0.71	7p	137.42 ± 0.76
7b	118.3 ± 0.95	**7e**	**102.82** ± 0.70
7c	164.09 ± 1.15	7r	172.04 ± 0.93
7d	140.85 ± 1.02	7s	168.42 ± 0.74
7e	170.41 ± 0.74	7t	145.33 ± 0.91
7f	128.99 ± 0.87	7u	127.59 ± 0.75
Aspirin	116.48 ± 0.98		

[a] Values are mean ± SD of three replicates

Table 4 Antioxidant DPPH activity of compounds **5a–i** and **7a–u**

Compound	DPPH[a] IC_{50} (μg/mL)	Compound	DPPH[a] IC_{50} (μg/mL)
5a	8.16 ± 1.41	7g	3.93 ± 1.06
5b	6.84 ± 1.51	7h	63.92 ± 1.02
5c	8.81 ± 1.24	7i	56.17 ± 1.17
5d	2.64 ± 1.14	7j	10.16 ± 1.25
5e	10.16 ± 1.39	7k	6.84 ± 1.32
5f	8.81 ± 1.06	7l	4.86 ± 1.56
5g	12.4 ± 0.98	7m	6.84 ± 1.38
5h	8.81 ± 1.15	7n	37.04 ± 1.43
5i	3.93 ± 1.07	7o	66.2 ± 1.55
7a	39.64 ± 1.16	7p	39.64 ± 1.17
7b	6.84 ± 1.31	7q	76.64 ± 0.93
7c	11.03 ± 1.89	7r	47.5 ± 1.01
7d	13.5 ± 1.04	7s	47.49 ± 1.23
7e	11.03 ± 1.12	7t	80.59 ± 1.19
7f	6.84 ± 1.29	7u	66.16 ± 1.32
Ascorbic acid	145.4 ± 0.27		

[a] Values are mean ± SD of three replicates

Table 5 Antioxidant H_2O_2 activity of compounds **5a–i** and **7a–u**

S. No	Compound	IC50[a] Value (µg/ml)	Compound	IC50[a] Value (µg/ml)
1	5a	112.05±0.88	7g	96.65± 0.79
2	5b	73.67± 0.15	7h	44.73± 0.49
3	5c	25.12±0.26	7i	42.26 ±0.41
4	5d	83.14 ± 0.13	7j	80.05 ± 0.16
5	5e	98.55± 0.18	7k	42.26 ±0.41
6	5f	41.11 ±1.23	7l	82.21 ± 0.61
7	5g	68.6±0.54	7m	78.05±0.65
8	5h	77.05±0.34	7n	43.04± 0.50
9	5i	51.42±0.69	7o	49.51± 0.17
10	7a	103.95± 0.9	7p	93.12±0.23
11	7b	70.93 ± 0.38	7q	35.51 ±0.07
12	7c	32.11 ±0.38	7r	35.51 ±0.07
13	7d	62.05± 0.74	7s	62.03± 0.77
14	7e	51.21 ± 0.35	7t	78.05 ± 0.22
15	7f	37.11±0.41	7u	76.5 ± 0.24
			Ascorbic acid	77.13 ± 0.87

[a] Values are mean ± SD of three replicates

In vitro cytotoxicity screening:

The synthesized benzo furanone compounds on the cell lines of HeLa and report its anticancer activity, nearly 36 compounds were selected. These were tested for in vitro cell viability assay employing MTT (3-[4, 5-dimethylthiozol-2-yl]-2, 5-diphenyltetrazolium bromide) protocol to check the cell cytotoxicity. The cell lines of HeLa were obtained and cultured in T_{75} flasks containing DMEM with high glucose medium supplemented with 10% Foetal Bovine Serum (FBS), antibiotic solution (1% v/v Penicillin-Streptomycin) and incubated at 37˚C with 5% CO_2.

The seeding of cells was performed with an initial count of $3x10^3$ cells in a 96 well plate and left for 12hrs-24hrs incubation. The cells in the well plate were treated with various concentrations of the test compound in triplicates. The untreated cells were maintained as control. Upon incubation for 36hrs under standard tissue culture conditions, the MTT dye 20µl was added and further incubated for 4 hrs. The media in the well plates was discarded and 100µl DMSO was added and incubated for 20 minutes by covering the well plates with aluminium foil. After incubation the absorbance was determined at 620nm.

The IC_{50} of the standard compound (Curcumin) and the synthesized chemical compounds is tabulated as shown below. The comparative study of the chemical compounds with curcumin helps to analyze the anticancer activity.

Table 6: IC_{50} of the synthesized compounds and the standard compound curcumin

Compound	IC50	Compound	IC50
1	98.804	19	2.975
2	6.831	20	39.723
3	51.289	21	4.819
4	5.405	22	0.769
5	9.346	23	31.179
6	7.117	24	16.498
7	7.021	25	5.199
8	7.277	26	86.118
9	5.801	27	0.38
10	62.811	28	58.549

11	3.161	29	6.51
12	67.758	30	0.315
13	8.236	31	0.272
14	6.921	32	87.267
15	3.535	33	25.858
16	0.315	34	3.635
17	66.941	35	0.136
18	0.248	36	7.97
Curcumin			

Conclusion: We herein report the selective synthesis of monospiro Chromanone derivatives **3a-c** from which a series of spirofurochromanones were synthesized following microwave irradiation method and their anti-inflammatory and antioxidant activities were determined. The reaction protocol requires cheap starting materials and is carried out under mild conditions. Compounds **5a**, **5b**, **5c**, **5d**, **5e**, **7g**, **7h**, **7j**, **7l**, **7n** and **7q** were found to be promising with IC_{50} values ranging from 77.11 to 116.10 μM/mL indicates that they are potential anti-inflammatory agents.

Most of the compounds have shown better cytotoxicity than standard compound curcumin. Among them few have shown vital activity with IC_{50} ranges from 0.1362-0.315 μM. So, it was found that the synthesized compounds have demonstrated potent cytotoxicity.

3.5 SCHEME 5

INTRODUCTION

Chalcones are an important subclass of flavonoids that involve of "open-chain flavonoids" in which the two aromatic rings are fused by a three-carbon α, β-unsaturated carbonyl system[8].

Chalcones play an important role as precursors and intermediates in all flavonoid synthetic pathways. The usage of microwave energy is one of the sustainable methods to quicken the synthesis of organic molecules which may interest various researchers and has merits over conventional heating such as short reaction time, solvent free condition, no side product, high yield, easy workup procedure. Therefore, there is scope to develop a new method for the synthesis of flavanone from by using an inexpensive, safe, simple and eco-friendly catalyst i.e. trifluoroacetic acid (TFA).

Flavonoids, a class of plant secondary metabolites, are built around a phenylbenzopyrone structure[9]. According to their different skeletons, they are classified into flavones, flavanones, chalcones, flavonols, isoflavones, aurones, etc[10].

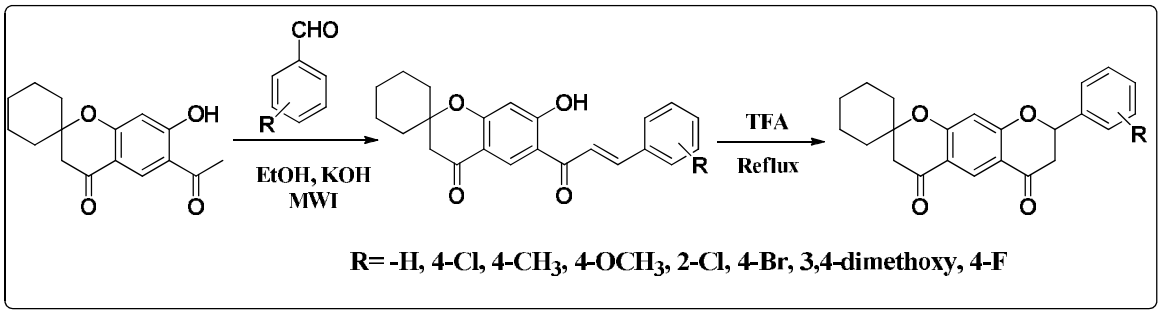
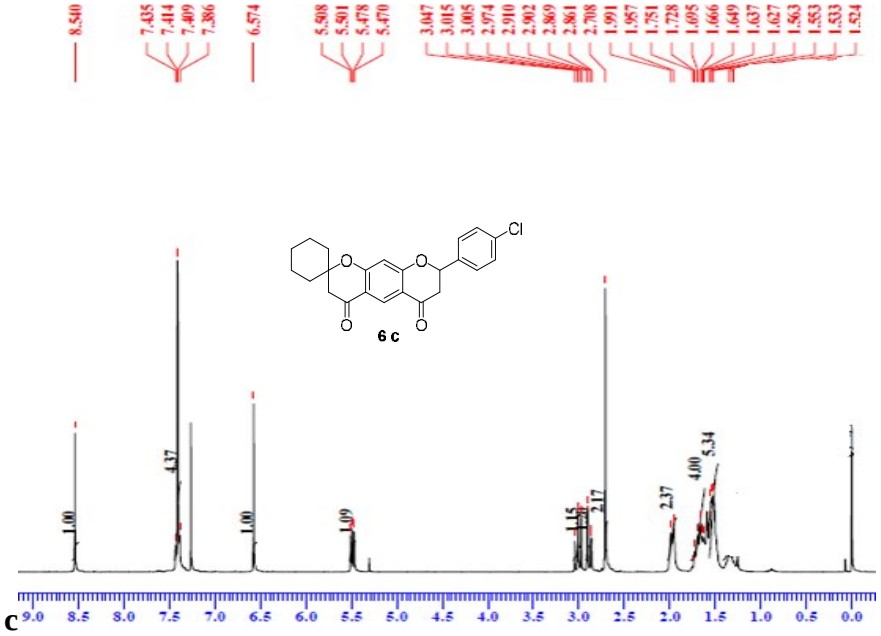


Fig 5

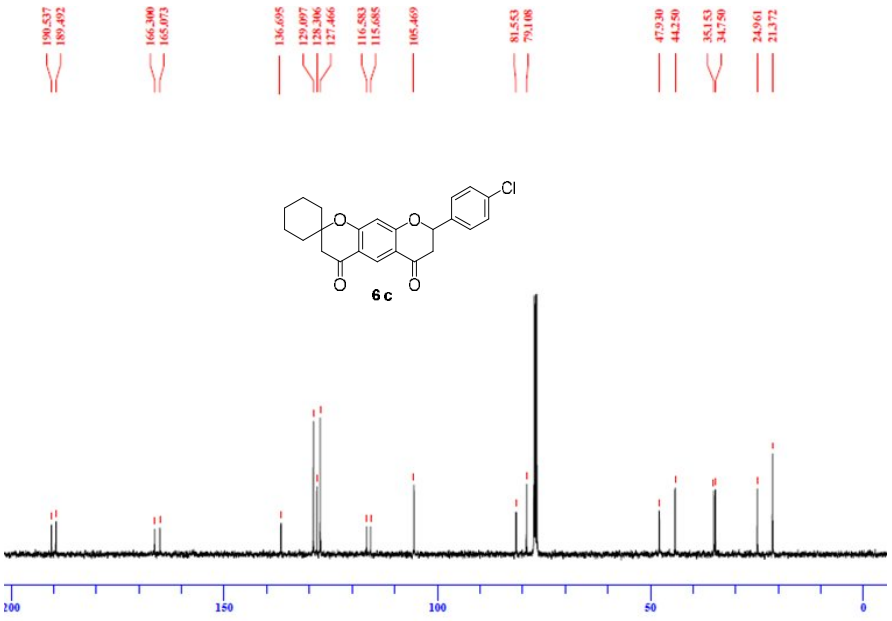
***Chemistry of Heterocyclic Compounds* 2017, *53*(5), 511–517**

IF: 0.815, Springer, SCI expanded, SCOPUS

SPECTRAL ANALYSIS



^{1}H NMR of 8'-(4-chlorophenyl)-7',8'-dihydro-3'*H*-spiro[cyclohexane-1,2'-pyrano[3,2-*g*]chromene]-4',6'-dione (6c)



^{13}C NMR of 8'-(4-chlorophenyl)-7', 8'-dihydro-3'*H*-spiro[cyclohexane-1,2'-pyrano[3,2-*g* chromene]-4',6'-dione (6c)

Anti Bacterial activity

All the synthesized compounds **5a**–**g** and **6a**–**g** were assessed for their *in vitro* antibacterial activity against two Gram-positive bacterial strains *Bacillus subtilis* and *Staphylococcus aureus*, two Gram-negative bacterial strains *Escherichia coli* and *Pseudomonas aeruginosa* and for their anifungal activity against three yeasts *Aspergillus niger*, *Penicillium italicum*, and *Fusarium oxysporum* using agar well diffusion method and compared with the well known commercially available standard drugs ampicillin and griseofulvin

The antibacterial activity was determined using agar disc diffusion method by measuring the zone of inhibition in mm. The compounds were screened at the concentration of 50 μg/ml in acetone.

Table 7: Antibacterial activity of compounds **5a**–**g** and **6a**–**g**

Compound	Zone of inhibition, mm			
	Gram positive bacteria		Gram negative bacteria	
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
5a	14	04	03	13
5b	28	13	12	26
5c	25	11	0	24
5d	26	09	08	26
5e	12	04	04	12
5f	18	08	06	15
5g	20	07	07	17
6a	13	03	05	12
6b	27	10	08	26
6c	25	09	07	25
6d	26	08	07	23
6e	11	05	03	11
6f	14	07	06	13
6g	16	06	07	14
Ampicllin	30	12	10	30

Antifungal activity

The antifungal activity was determined using the poison food plate method by measuring the zone of inhibition in mm (Table 3, Fig. 2). The compounds were screened at the concentration of 50 μg/ml in acetone.

Table 8: Antifungal activity of compounds **5a**–**g** and **6a**–**g**

Compound	Zone of inhibition, mm		
	Aspergillus niger	*Penicillium italicum*	*Fusarium oxysporum*
5a	05	11	14
5b	08	15	19
5c	09	16	18
5d	08	14	17
5e	05	08	11
5f	05	11	12
5g	06	14	15
6a	06	10	13
6b	08	17	18
6c	09	18	20
6d	07	15	17
6e	03	09	10
6f	06	11	13
6g	07	10	13
Griseofulvin	12	20	25

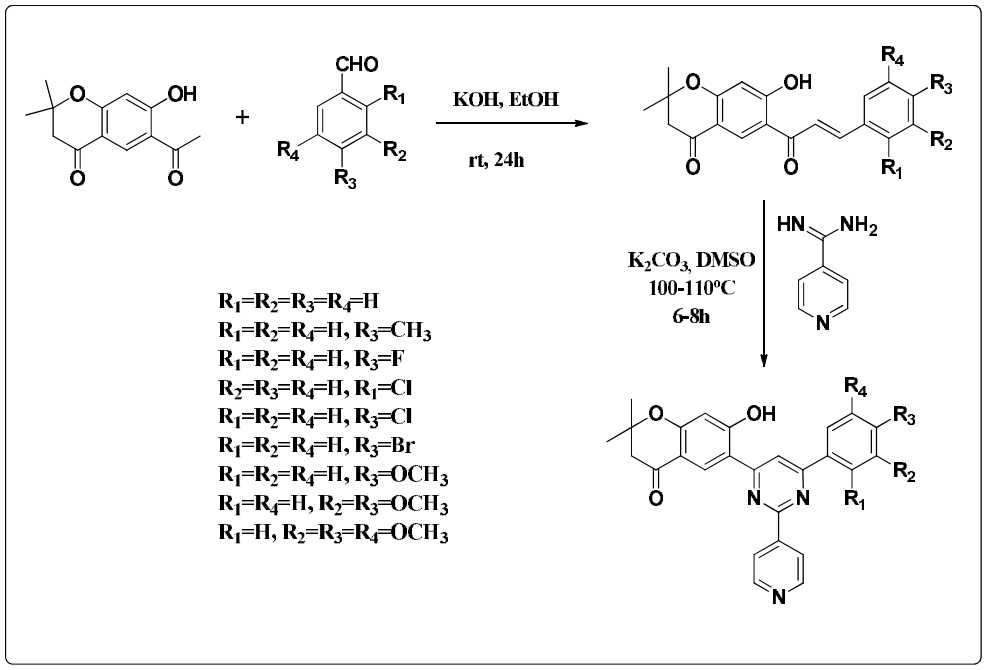
Conclusion: A novel series of spiro[chromene-2,1'-cyclohexan]-4(3*H*)-one derivatives containing either a chalcone or flavanone fragment in their molecules was synthesized. The catalytic efficiency of TFA in the chalcone–flavanone transformation has been demonstrated.

3.6 SCHEME 6

INTRODUCTION

Chalcones demonstrate a wide variety of anticancer, anti-inflammatory, anti-invasive, and antimicrobial activities. Pyrimidine derivatives demonstrated antitumor, antimicrobial, antimalarial and anticonvulsant activities.

In the present study we synthesized pyrimidines[11] derivatives by the Michael addition of chalcone scaffolds to Chromanone. The synthesized chalcones and pyrimidines were evaluated for their *in vitro* antimicrobial activity.



Russian Journal of General Chemistry, 2016, Vol. 86, No. 6, pp. 1396–1404.

IF: 0.481, Springer, SCI expanded, SCOPUS.

Antibacterial activity: The synthesized compounds **4a–4i** and **6a–6i** were evaluated for in vitro antibacterial activity using broth dilution method [22]. Penicillin and Streptomycin were used as standard drugs for comparison. The minimum inhibitory concentrations (MIC) of synthesized compounds **4a–4i** and **6a–6i** were tested against three representative Gram-positive organisms such as *Bacillussubtilis* (MTCC 451), *Staphylococcus aureus* (MTCC 114), *Staphylococcus epidermidis* (MTCC 2896) and Gram-negative organisms such as *Escherichia coli* (MTCC 493), *Pseudomonas aeruginosa* (MTCC 771), and *Klebsiella pneumonia* (MTCC 654) by broth dilution method recommended by National Committee for Clinical Laboratory (NCCL) standards

Anti-fungal activity: All synthesized compounds **4a– 4i** and **6a–6i** were tested for *in-vitro* antifungal activity against the fungal strains *Candida albicans* (MTCC 227), *Saccharomyces cerevisiae* (MTCC 45), *Aspergillus niger* (MTCC 298), and *Aspergillus flavus* (MTCC 254) by Agar Well Diffusion Method atthe concentration 100 μg/mL. Zone of inhibition (in mm) was compared with standard drug Ampothericin- B.

Table 9

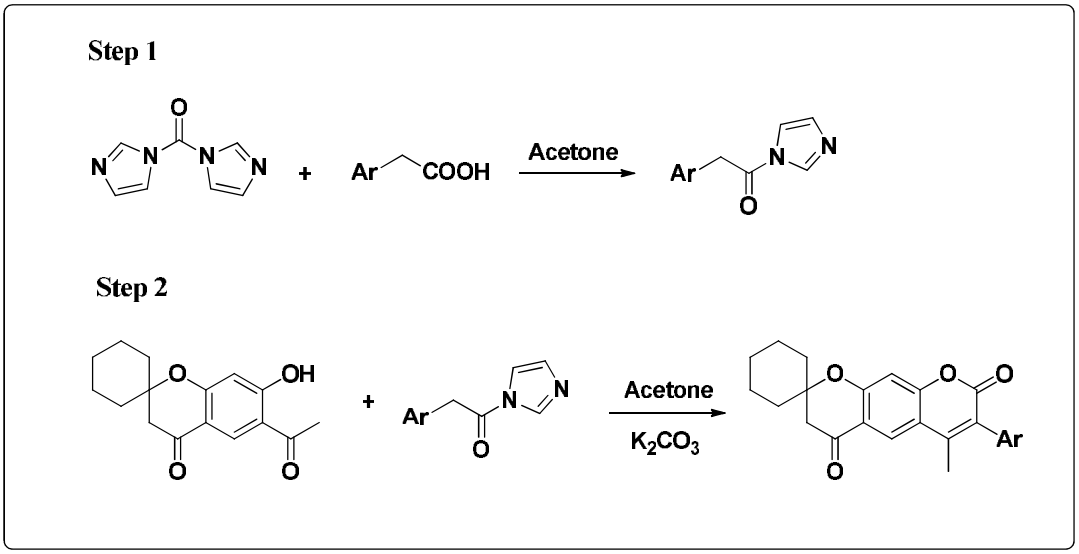
Comp. no.	Zone of inhibiton, mm			
	Candida albicans	*Sacharromyces cerevisiae*	*Aspergillus niger*	*Aspergillus flavus*
4a	18.0	17.0	16.0	19.0
4b	0.0	0.0	0.0	7.0
4c	17.0	6.0	14.0	18.0
4d	8.0	6.0	0.0	0.0
4e	17.0	16.0	19.0	18.0
4f	8.0	6.0	10.	8.0
4g	16.0	19.0	15.0	15.0
4h	17.0	11.0	19.0	16.0
4i	8.0	10.0P	6.0	7.0
6a	6.0	10.0	11.0	10.0
6b	8.0	7.0	0.0	6.0
6c	17.0	16.0	15.0	18.-0
6d	8.0	8.0	10.0	8.0
6e	11.0	7.0	6.0	14.0
6f	15.0	17.0	16.0	18.0
6g	10.0	8.0	11.0	16.0
6h	7.0	10.0	8.0	6.0
6i	7.0	6.0	6.0	10.0
Ampothericin-B	23.5	22.0	25.0	25.0

Conclusion: We have synthesized novel 2,4,6-trisubstituted pyrimidinesbased on chroman-4-one chalcones conjugated with isonicotinimidamide·HCl. The synthesized compounds **4b**, **4d**, **4e**, **4g–4i**, **6g–6i** demonstrated very high antibacterial activity and **4a**, **4c**, **4e**, **4g**, **4h**, **6c**, and **6f** compounds showed the most promising antifungal activity.

3.7 SCHEME 7

INTRODUCTION

Coumarin derivatives have found applications as antibiotics, antimicrobial, antioxidant, anti-inflammatory, anticancer agents[12] and as HIV proliferators. Microwave assisted and phase transfer catalyzed pathways to effect the above mentioned reactions to form the coumarin derivatives with considerable yield. Concerning the instability of the acid chloride intermediate in the synthesis of coumarin derivatives from o-hydroxyacetophenone, using thionylchloride, we decided to investigate the potential use of CDI activated phenyl acetic acids as acylating agents. The synthesized aryl coumarins are expected to exhibit anticancer agents. The biological evaluation is in progress.



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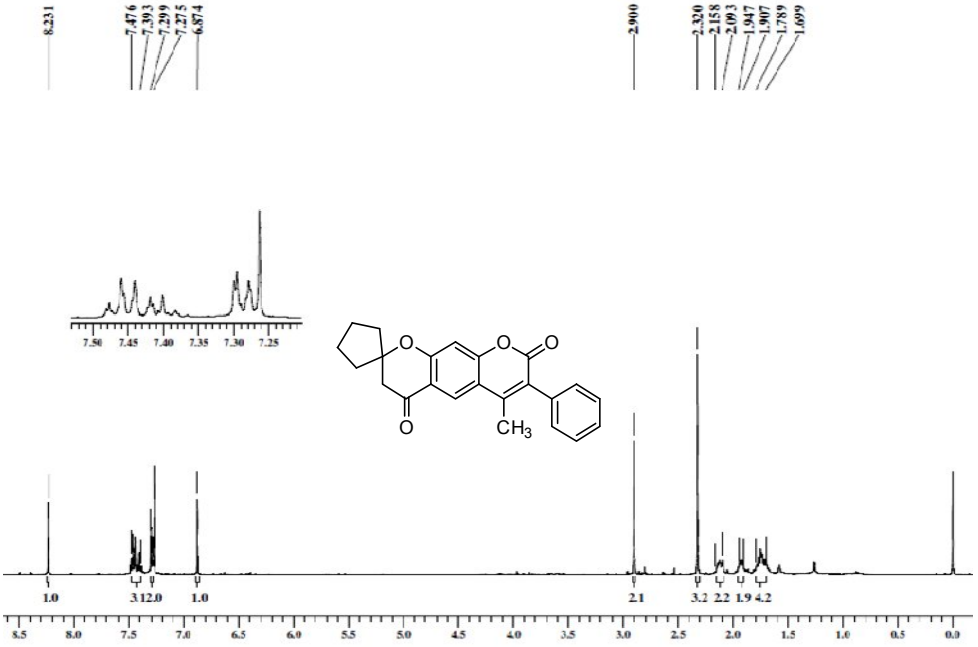
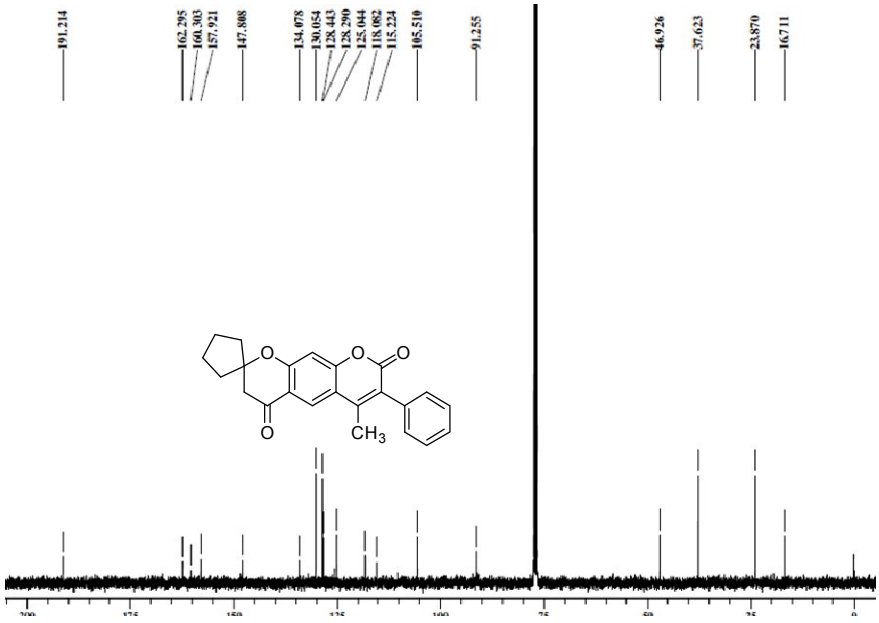
Conclusion: Novel spirochromanone fused coumarin were synthesized by using CDI and acetone as solvent and K_2CO_3 as base. This reaction is performed under mild conditions. The combination of reagent CDI along with acetone is unreported.

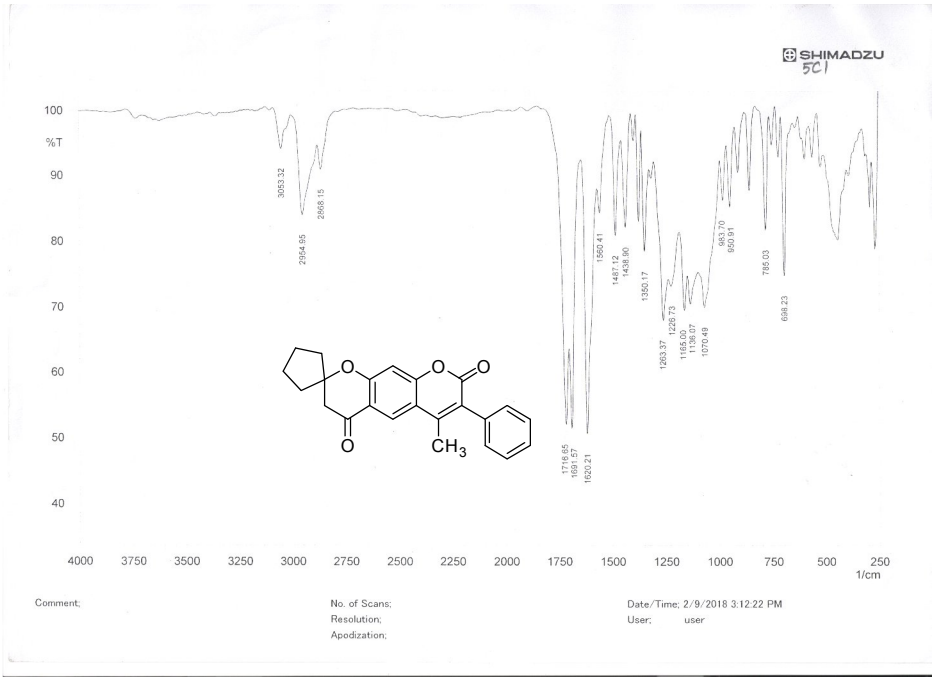
SPECTRAL ANALYSIS



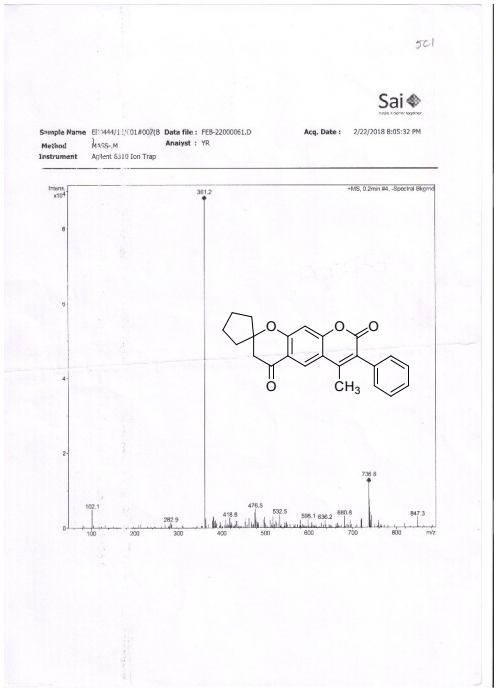
^{1}H NMR of 6'-methyl-7'-phenyl-3'H-spiro[cyclopentane-1,2'-pyrano[3,2-g]chromene]-4',8'-dione



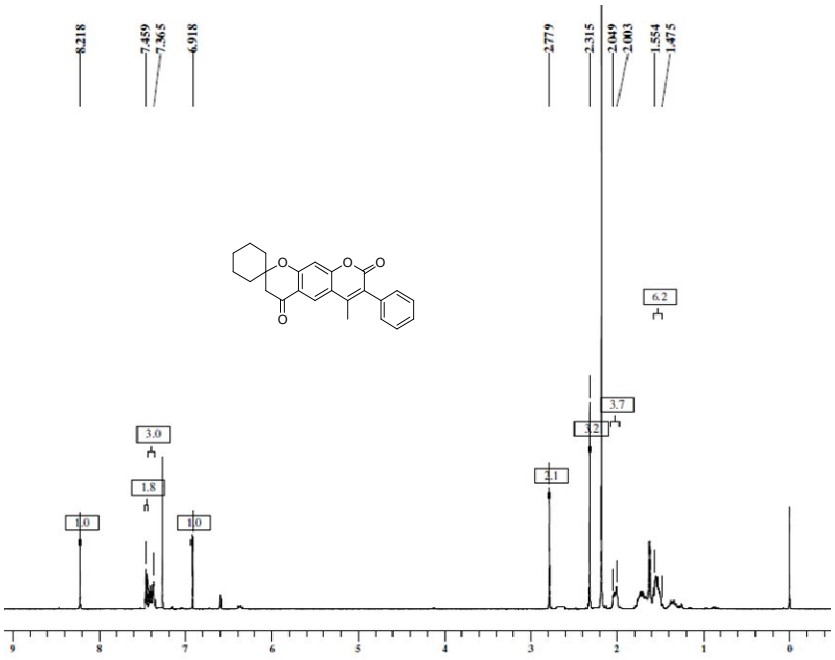
^{13}C NMR of 6'-methyl-7'-phenyl-3'H-spiro[cyclopentane-1,2'-pyrano[3,2-g]chromene]-4',8'-dione



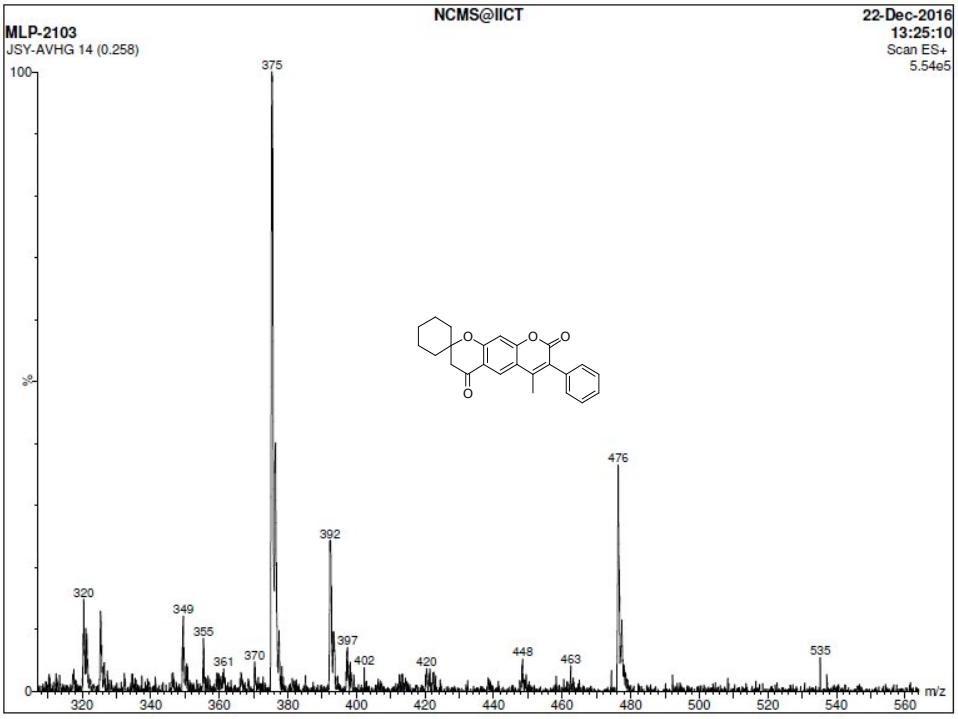
IR of 6'-methyl-7'-phenyl-3'H-spiro[cyclopentane-1,2'-pyrano[3,2-g]chromene]-4',8'-dione



Mass of 6'-methyl-7'-phenyl-3'H-spiro[cyclopentane-1,2'-pyrano[3,2-g]chromene]-4',8'-dione



^{1}H NMR of 6'-methyl-7'-phenyl-3'*H*-spiro[cyclohexane-1,2'-pyrano[3,2-*g*]chromene]-4',8'-dione



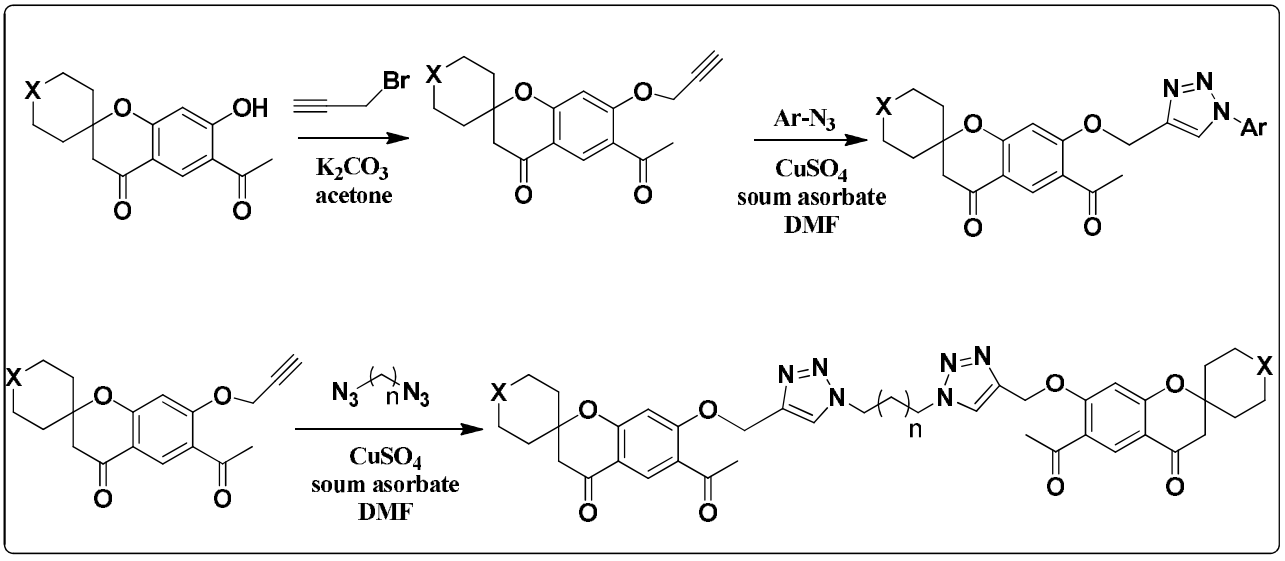
Mass of 6'-methyl-7'-phenyl-3'*H*-spiro[cyclohexane-1,2'-pyrano[3,2-*g*]chromene]-4',8'-dione

3.8 SCHEME 8

INTRODUCTION

1,2,3-Triazole is one of a pair of isomeric chemical compounds with molecular formula $C_2H_3N_3$, called triazoles, which have a five-membered ring of two carbon atoms and three nitrogen atoms. 1, 2, 3-Triazole is a basic aromatic heterocycles. Substituted 1, 2, 3-triazoles can be produced using the azide alkyne Huisgen cycloaddition in which an azide and an alkyne undergo a 1, 3-dipolar cycloaddition reaction. It is a surprisingly stable structure compared to other organic compounds with three adjacent nitrogen atoms.

Compounds of the 1, 2, 3-triazole series have various chemical, biological, and technical characteristics[13]. In medicinal chemistry greatest attention has been paid to the synthesis of 1, 2, 3-triazoles condensed with other heterocycles and investigation of their biological activity. Even in 1935 research was started on the possibility of using 1, 2, 3-triazolo[4,5-d]pyrimidines (8-azapurines) as chemotherapeutic agents for the treatment of various diseases and particularly malignant tumors. The search for new biologically active compounds in the series of condensed 1, 2, 3-triazoles is continuing to the present day. Thus, for example, substances acting against the hepatitis C virus and compounds inhibiting benzodiazepine and adenosine receptors were found.



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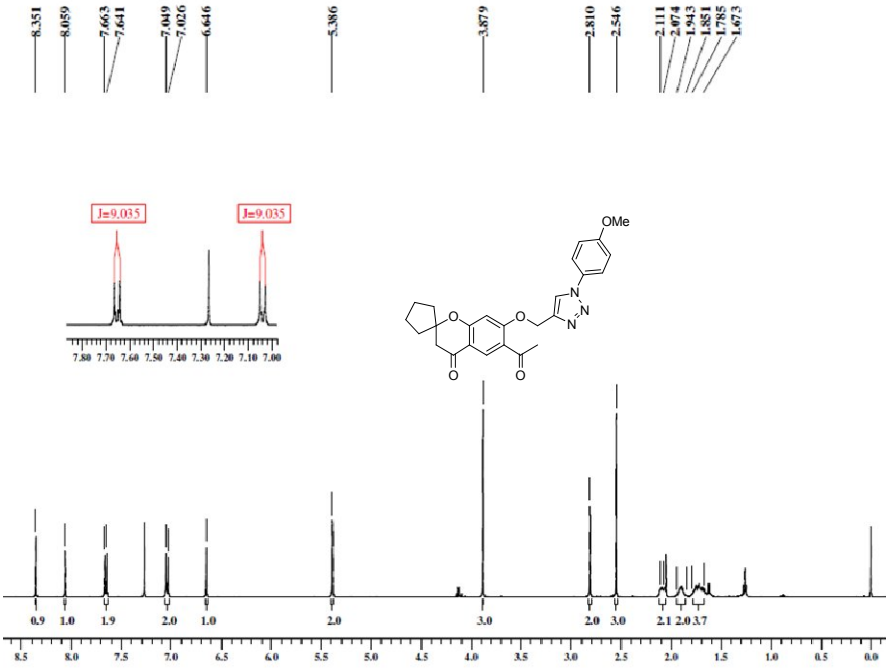
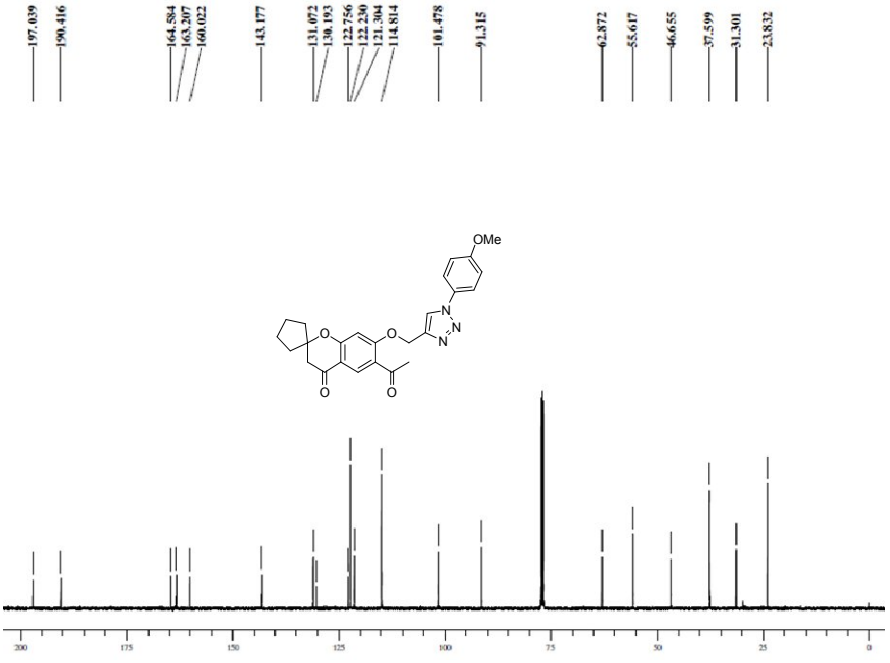
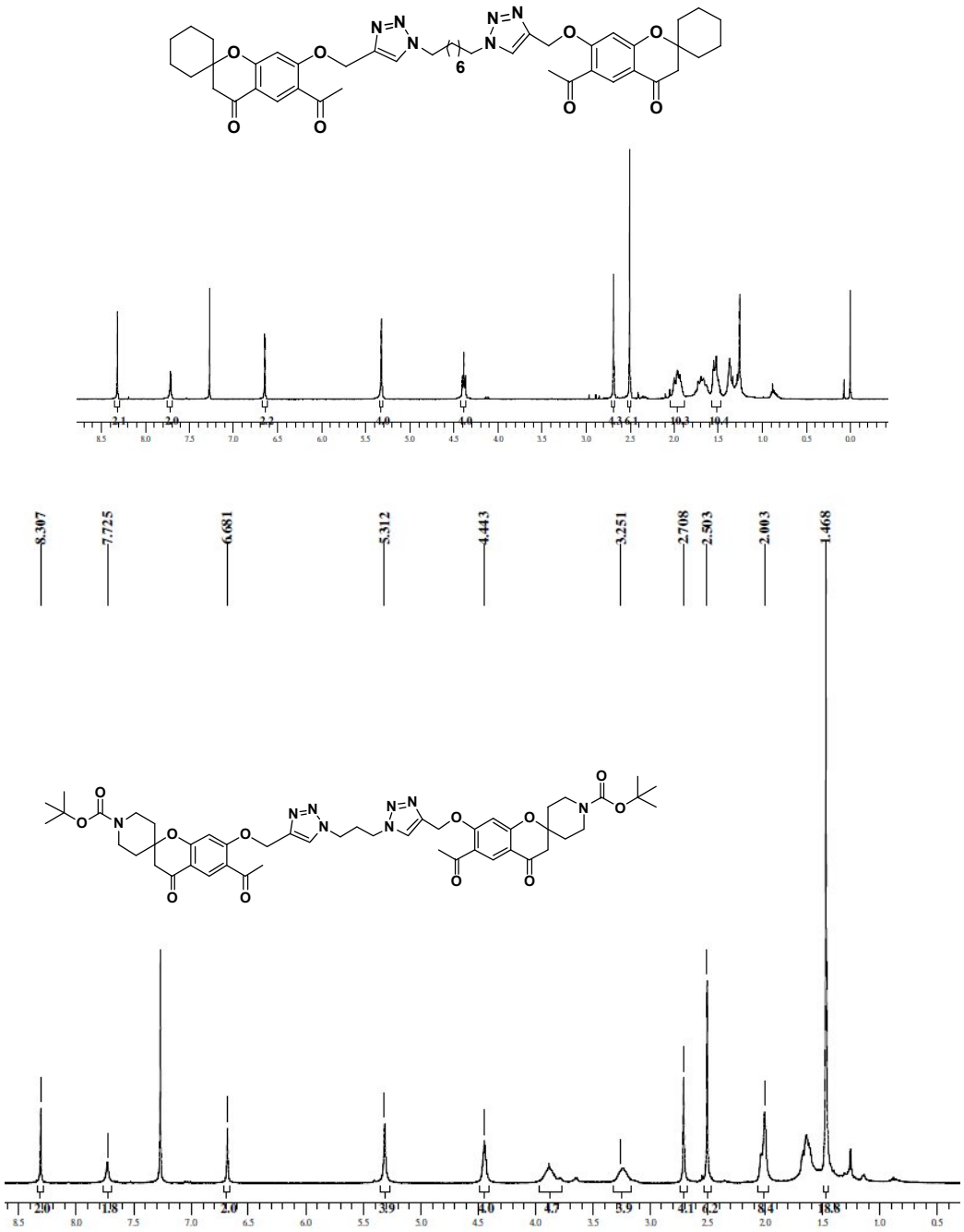
SPECTRAL ANALYSIS



^{1}H NMR of 6-acetyl-7-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)spiro[chroman-2,1'-cyclopentan]-4-one



^{13}C NMR of 6-acetyl-7-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)spiro[chroman-2,1'-cyclopentan]-4-one

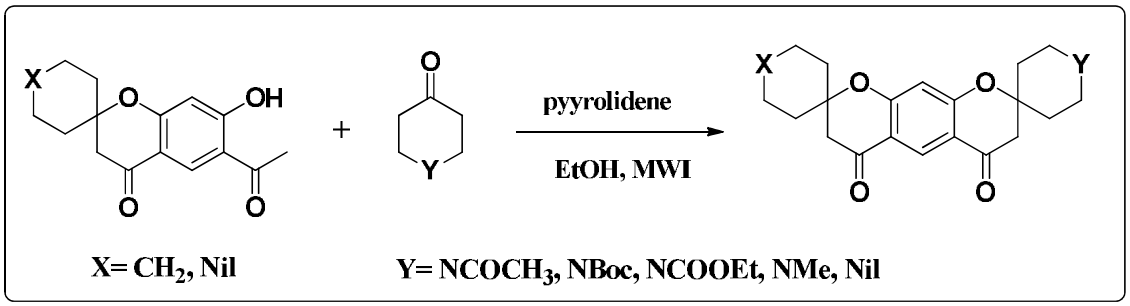


Conclusion: A new series of 1,2,3- triazoles and bis-1,2,3-triazoles are synthesized and are obtained in good yields.

3.9 SCHEME 9

INTRODUCTION

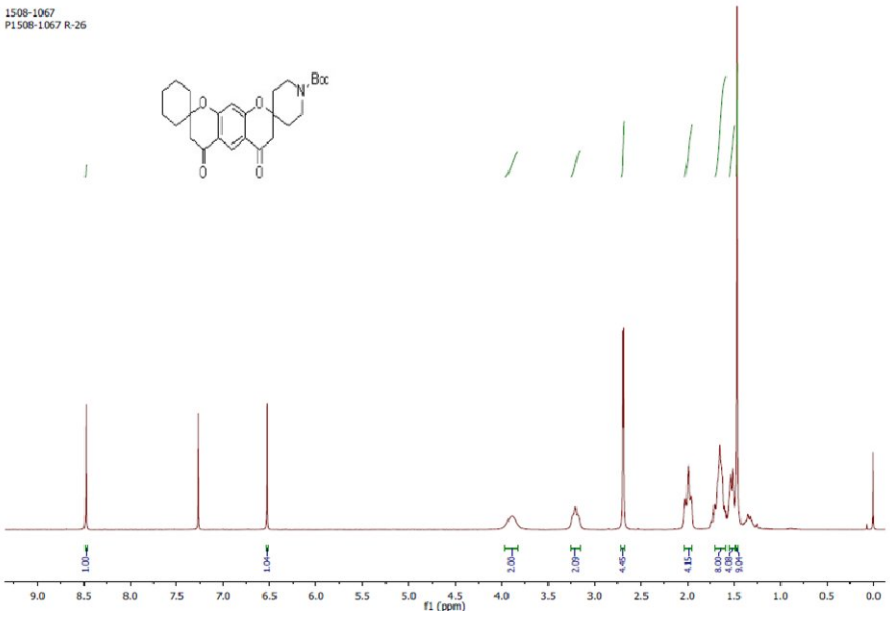
2-spiro-chroman-4(1H)-ones serve as an important precursor for the synthesis of other medicinally important compounds such as rotenoids and xanthones. Recently, these structural scaffolds have been assigned as privileged structures for drug development. Newly designed and synthesized bis-spirochromanone derivatives are evaluated for the antimicrobial and antioxidant activities. All the synthesized bis-spirochromanones were screened for their *in vitro* antibacterial activity against two gram positive bacterial strains *Bacillus faecalis*, *Staphylococcus aureus* and two gram negative bacterial strains *Klebsiella pneumonia*, *Escherichia coli* by the disc diffusion method at different concentrations (20 µg/mL & 40 µg/mL) and exhibited very good antibacterial activity. All the bis-spirochromanones were tested for their antifungal activity against two fungal strains, *Fusarium oxysporum,* and *Aspergilus flavus* by the poison plate technique.



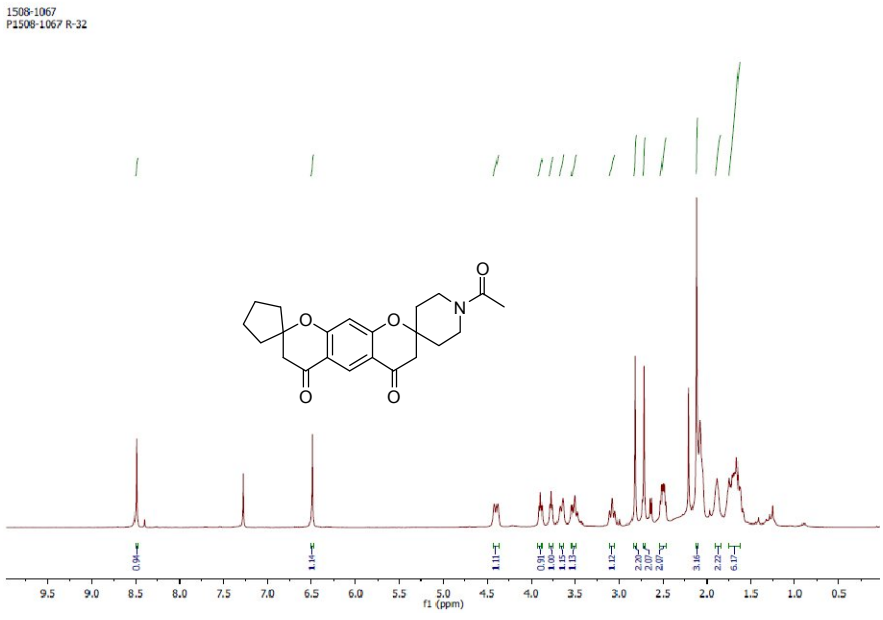
Manuscript under preparation

Conclusion: A series of new Bis spirochromanones are synthesized by MWI using pyyrolidene in good yields.

SPECTRAL ANALYSIS



^{1}H NMR of tert-butyl 4',6'-dioxo-3',4',6',7'-tetrahydrodispiro[cyclohexane-1,2'-pyrano[3,2-*g*]chromene-8',4''-piperidine]-1''-carboxylate (6b)



^{1}H NMR of 1''-Acetyldispiro[cyclopentane-1,2'-pyrano[3,2-*g*]chromene-8',4''-piperidine]-4',6'(3'*H*,7'*H*)-dione (7a)

4.0 REFERENCES

1. (a) Ashok, D., Lakshmi Vijaya, B., Sidda, R., Ganesh, A., and Shaik, A., *Chem. Heterocycl. Compd*., **2015**, vol. 51, p. 462. doi 10.1007/S10593-015-1719-0. (b) Ashok, D., Srinivas, G., Vikas Kumar, A., Mohan Gandhi, D., and Srinivas Reddy, M., *Russ. J. Gen.Chem.,* **2015**, vol. 85, p. 708. doi 10.1134/S1070363215030305.
2. Meltzer, E. O. *Allergy Asthma Proc.* **2005**, *26*, 445.
3. Ashok, D.; Mohan Gandhi, D.; Vikas Kumar, A.; Srinivas, G.; Srinivas Reddy, M.; Sree Kanth, S.; Vijjulatha, M. *Med. Chem. Res.* **2016**, *25*, 2882.
4. J. D. Flavin M T R, A. Khilevich, A. Kucherenko, A. K. Sheinkman, V. Vilaychack, L. Lin, W. Chen, E. M. Greenwood, T. Pengsuparp, J. M. Pezzuto,T. M. Hughes SHF, M. Cibulski, W. A. Boulanger, R. L. Shone and Z. Q. J Xu, *Med. Chem.,* **1997**, 39.
5. D. Ashok, K. Sudershan and M. Khalilullah, *Green Chem. Lett. Rev.,* **2012**, 5, 121.
6. Nam T. S. Phan, D. H. Brown and P. Styring; *Tetrahedron Lett* **2004**, 4,7915.
7. Q N. Chun Liu, Fanying Bao and Jieshan Qiu, *Green Chem.,* **2011**, 13,1270.
8. Dhar, D. N. *The Chemistry of Chalcones and Related Compounds*; John Wiley & Sons: New York, **1981**.
9. (a) Grassi, D.; Desideri, G.; Croce, G.; Tiberti, S.; Aggio, A.; Ferri, C. *Curr. Pharm. Des.* **2009**, *10*, 1072. (b) Harborne, J. B.; Williams, C. A. *Phytochemistry* **2000**, *55*, 481.
10. Bravo, L. *Nutr. Rev.* **1986**, *56*, 317.
11. Maryanoff, B.E., O'Neill, J.C., McComsey, D.F., Yabut, S.C., Luci, D.K., Jordan, A.D., Masucci, J.A.Jr., Jones, W.J., Abad, M.C., Gibbs, A.C., and Petrounia, I., *Med. Chem. Lett.,* **2011**, vol. 14, no. 2(7), p. 538. DOI: 10.1021/ml200070g.
12. Kamal, A.; Adil, S. F.; Tamboli, J. R.; Siddardha, B.; Murthy, U. S. N. Lett. Drug Des. Discovery **2009**, 6, 201.
13. Totobenazara J, Burke AJ, New click chemistry methods for 1,2,3-triazoles synthesis: recent advances and applications. Tetrahedron Lett **2015** 56: 2853-2859.

(PRINCIPAL INVESTIGATOR)