UGC Minor Research Project report

on

Synthesis Characterization and Biological evaluation of novel amides and sulphanamides of piperazine containing quinoline and piperazine nucleus (MRP(S)-0371/13-14/KABA023/UGC- SWRO)

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Submitted to

UGC

South Western Regional Office Bengaluru.

DECLARATION

I declare that the Minor Research Project entitled **"Synthesis Characterization and Biological evaluation of novel amides and sulphanamides of piperazine containing quinoline and piperazine nucleus" (MRP(S)-0371/13-14/KABA023/UGC- SWRO)** submitted to the UGC is based on the results of the studies carried out by me independently. The results of this work or any part thereof are not previously submitted elsewhere for any other funding agency.

Date : 15-05-2016

Place : Bengaluru

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Place: Bangalore

Date: 15-05-2016 Smt. M.R.Chaya

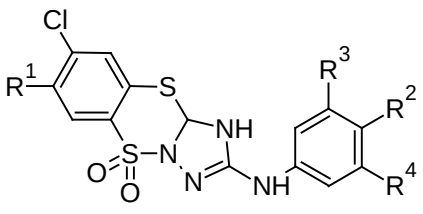
CONTENTS

Synthesis Characterization and Biological evaluation of novel amides and sulphanamides of piperazine containing quinoline and piperazine nucleus

Chapter 1: Literature survey

Some of the important health related problems faced by the humanity in the 21st century arises due to life style related diseases. The poor physical activity, adulterated or contaminated food consumption, poor nutritional content in the diet, pollution, pesticide usage in food crop production etc have induced various types of diseases such as diabetes, cardiac problems, cancer,. AIDS etc are prominent diseases. In this chapter, we have discussed the literature reports on the synthesis of new sulfonamides, their synthesis from different routes containing different heterocyclic nucleus and different substituted sulfonylchlorides and evaluation of these sulfonamide derivatives for their potential activities.

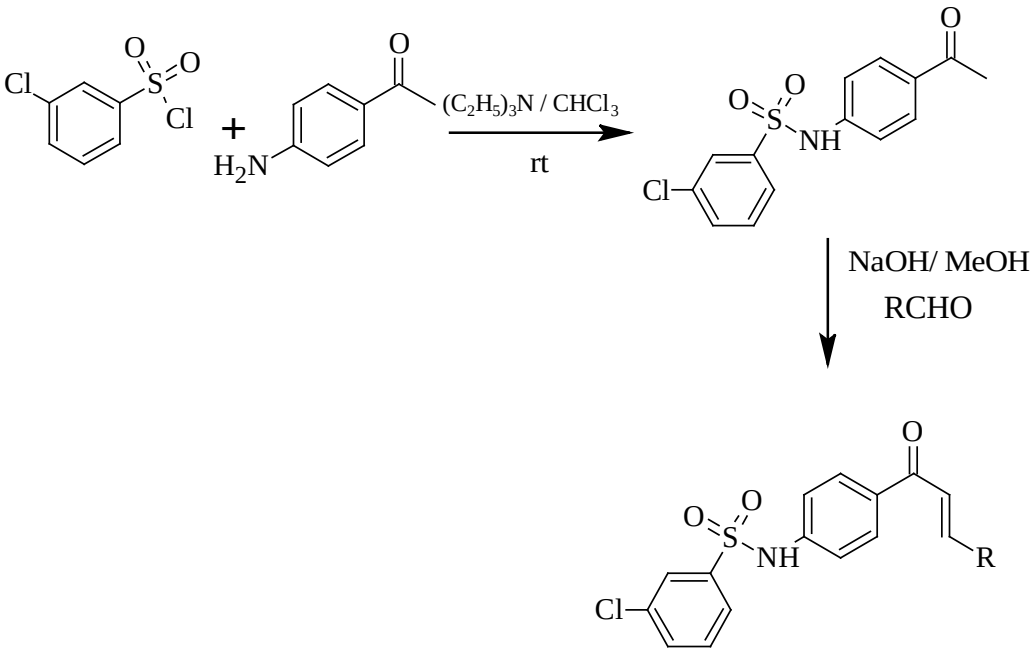
Pomarnacka[1] reported the synthesis of [1-(6-chloro-1, 1-dioxo-1, 4, 2-benzodithiazin-3-yl)-4-arylsemicarbazides **(1)]** and was screened for their anti-tumour activity using human cell lines. The tested compounds are relatively show highest sensitivity towards Leukaemia (SR, CCRF-CEM), ovarian cancer (IGROV1, OVCAR-3), CNS cancer (SF-539) and lung cancer (HOP-92).[1]



(1)

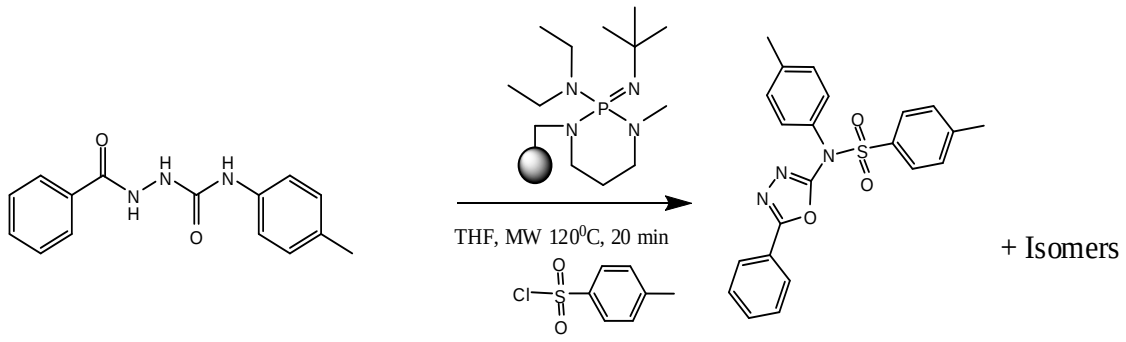
	R^1	R^2	R^3	R^4
a	CH_3	H	H	H
b	CH_3	CH_3	H	H
c	CH_3	Cl	H	H

A series of sulfonamide chalcone **(2)** were synthesized by J N Dominguez et al, these synthesized compounds were examined for their ability to inhibit the β-hematin formation *in vitro* and their activity against malarial parasites *Plasmodium falciparum*. Compound **(2)** shows reasonably good activity against malarial parasites.[2]



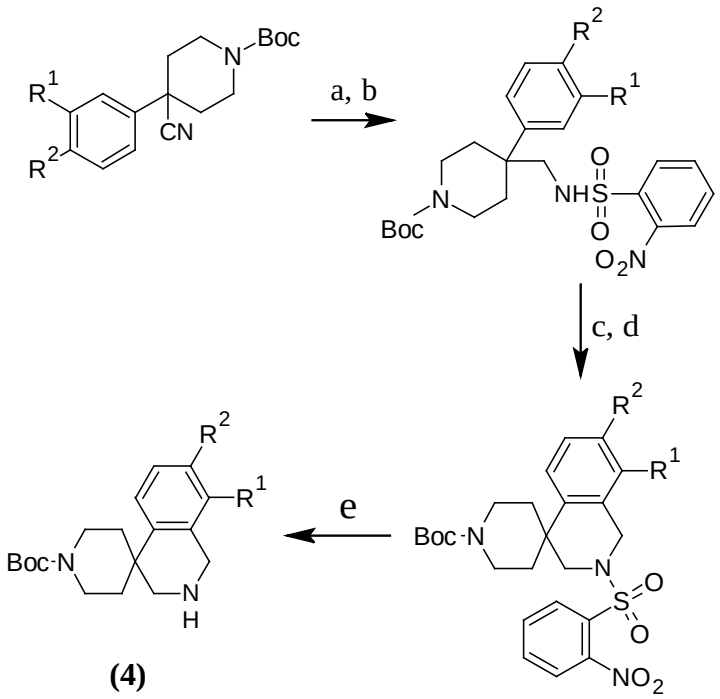
(2)

I R Baxendale et al.,[3] reported the one pot synthesis of 2-aminosulfomamide-1,3,4-oxadiazoles **(3)** using polymer supported reagents and microwave heating in good yields.



(3)

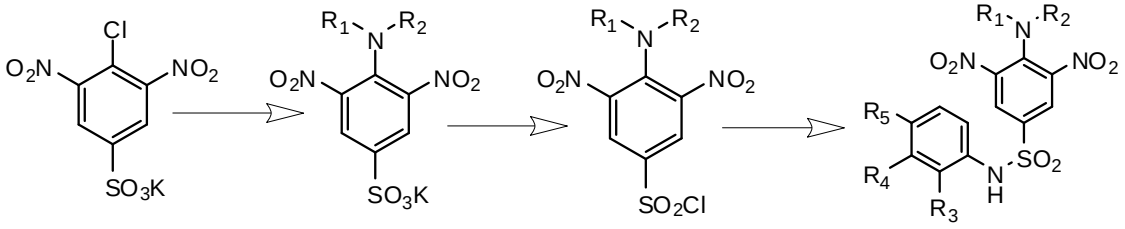
A series of 2-nitrophenyl sulfonamides were prepared by adopting Pictet-Spengler reaction in good yield by Liu et al.[4]. They have also reported that 2-nitrophenyl group **(4)** can be selectively removed in presence of Boc using LiOH and mercaptoacetic acid.



a) BH_3, THF, 40°C; **b)** 2-nitrophenylsulfonylchloride, TEA, MDC;

c) $(CH_2O)n$, AcOH - H_2SO4 (4:1); **d)** Boc_2O, NaOH, EtOAc; **e)** $HSCH_2COOH$, LiOH, DMF

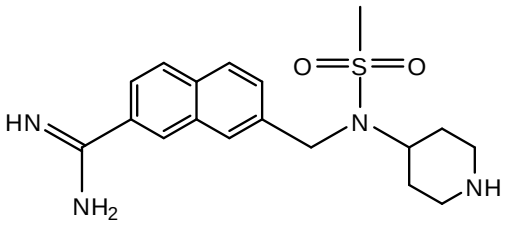
Various N^1-(4-substituted)phenyl-3,5-dinitro-N^4,N^4-disubstituted sulfonamides **(5)** were prepared by George et al. The compounds were tested for *in vitro* anti kinetoplastid activity. It has been reported that compound with $R_1 = R_2 = nBu$, $R_3 = R_5 = H$, $R_4 = OH$ is a promising candidate for further studies.[5]



(5)

a) Secondary amine MeOH, Reflux; b) PCl_5, MDC; c) $ArNH_2$, Pyridine, 40^oC

In search of oral anti-coagulants, Ishihara et al., synthesized different sulfonyl derivatives **(6) - (10)**. Synthesized compounds were tested for their potency to inhibit human factor Xa in purified enzyme system. Out of the synthesized compound, **(10)** with X,Y = -CH=CH-, R^1 = 4-pyridyl and $R^2 = SO_2Me$ exhibited good factor Xa inhibitory activity.[6]



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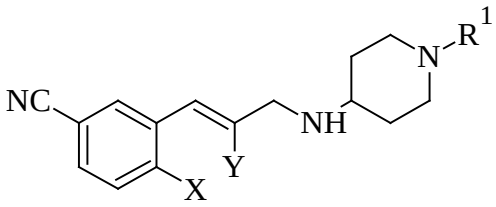

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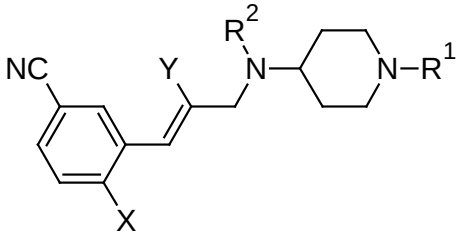

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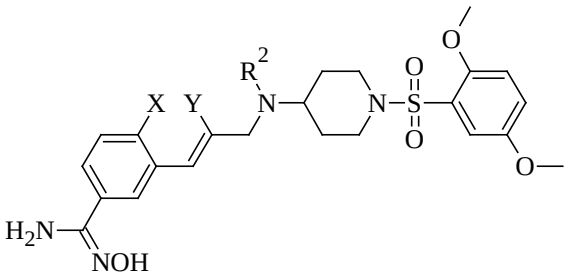

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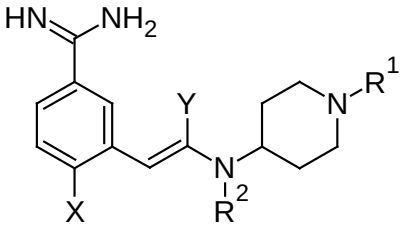
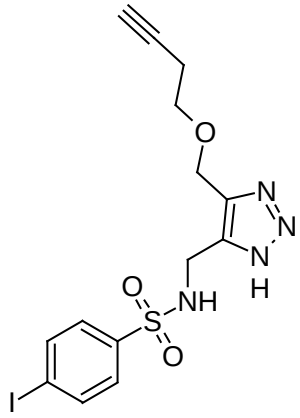
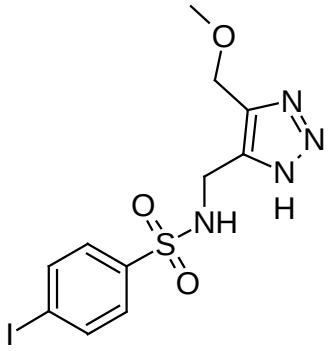


(10)

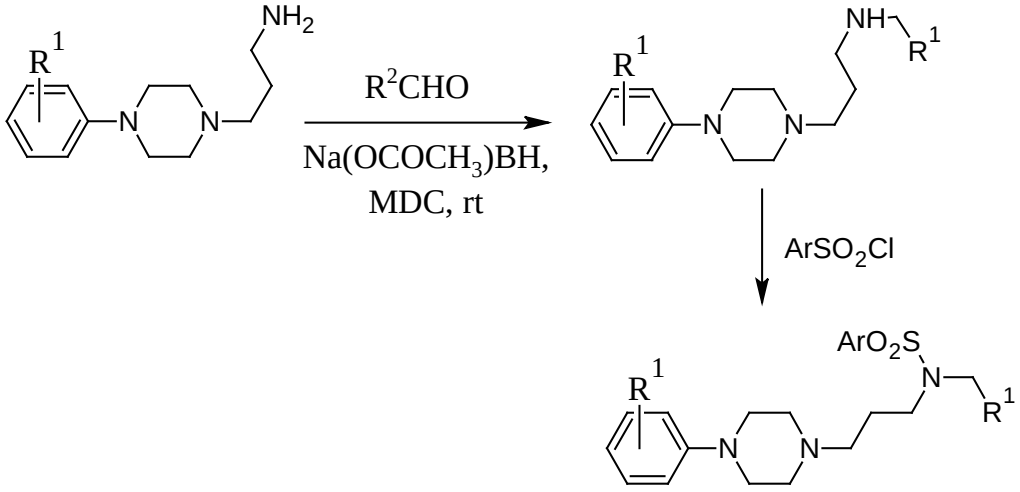
Two sulfonamide-1,2,3-triazole analog compounds **(11)** and **(12)** were identified from click chemistry library compounds and subjected to VIM-2 nitrocefin screening (assay). It has been reported that compounds **11** and **12** were found to be competitive inhibitors of VIM-2 indicating that they can act as good antibacterial agents.[7]





(11) **(12)**

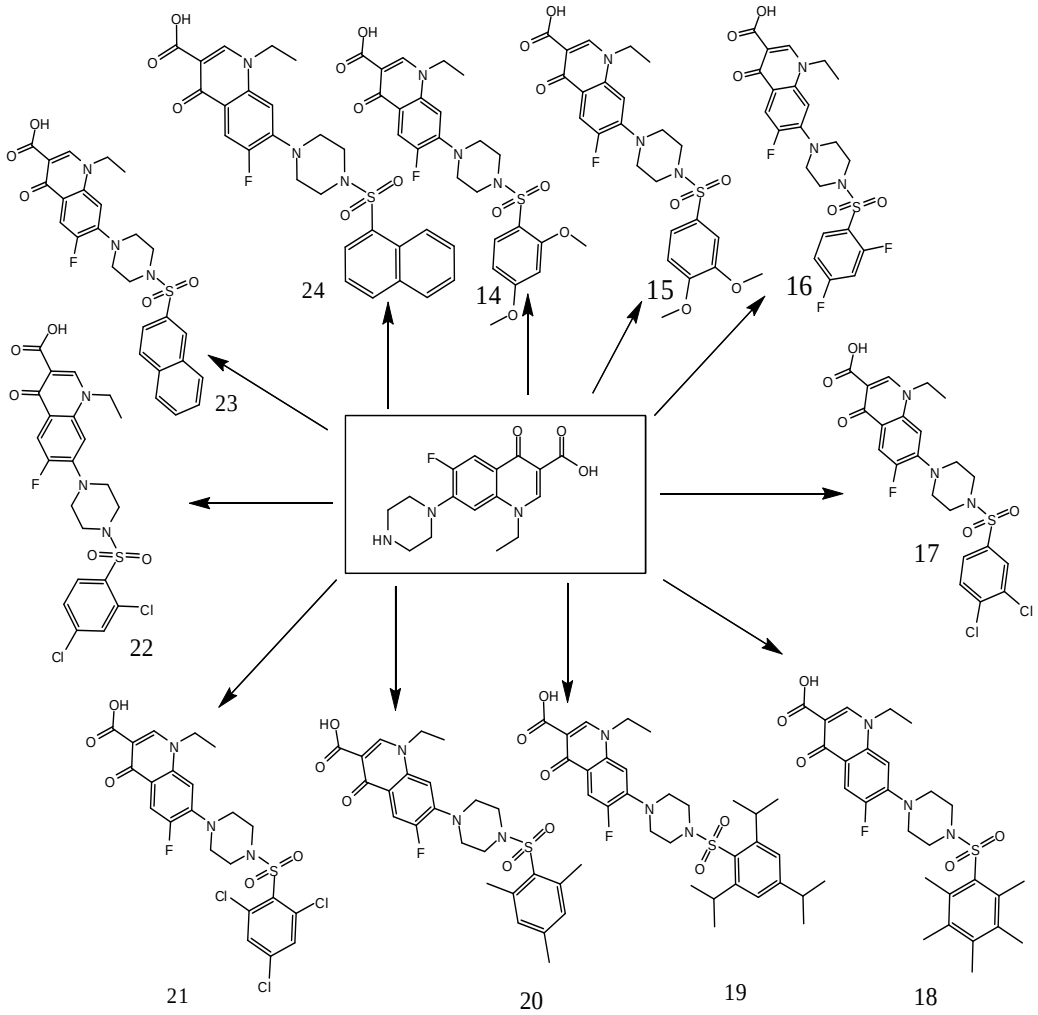
A series of compounds (phenylpiperazinyl-propyl)arylsulfonamides **(13)** were prepared by Euna Yoo et al., All the newly synthesized compounds were evaluated against human recombinant $5HT_{2A}$ serotonin receptor in stable CHO cell line. Synthesized compounds **(13)** with R^1 = H, Ar = 2-Napthyl and R^1 = 4-F, Ar = 2-napthyl, R^2 = Cyclohexyl showed good affinity with IC50 values in the nano molar range.[8]



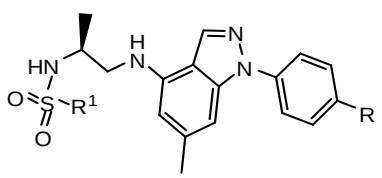
(13)

R_1 = Cyclopropyl, Cyclohexyl, H, ; Ar = 2- napthyl,

A series of areneosulfonamide derivatives were prepared by Abdel-Aziz et al., All the newly synthesized compounds **(14) - (24)** were checked for their anti-bacterial activity and 2D-QSAR studies results are also reported.[9]



Molecules containing sulfonamide group was prepared by Hawa Diallo et al.[10] that binds to glucocorticoid receptors and thereby affecting inflammatory disease like asthma, allergic rhinitis, COPD and rheumatoid arthritis. Compounds **(22) - (26)** were synthesized by different routes and examined for their activities. Reported **(26)** series compounds showed good selectivity in binding to glucocorticoid receptor (GR).[10]



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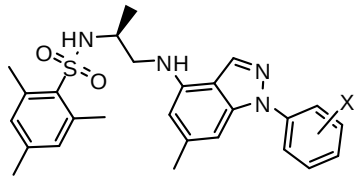

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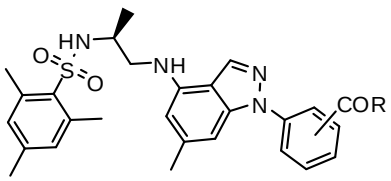

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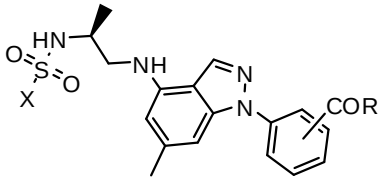

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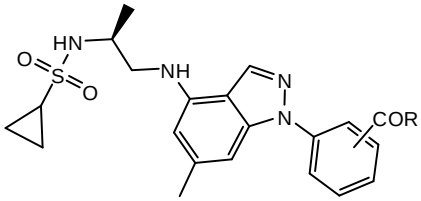
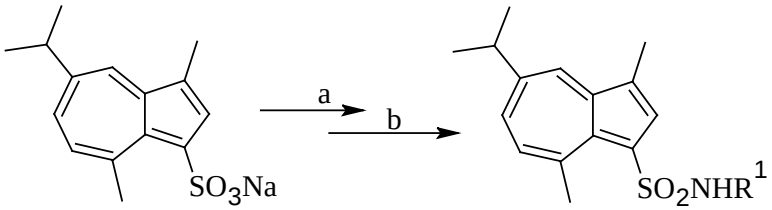


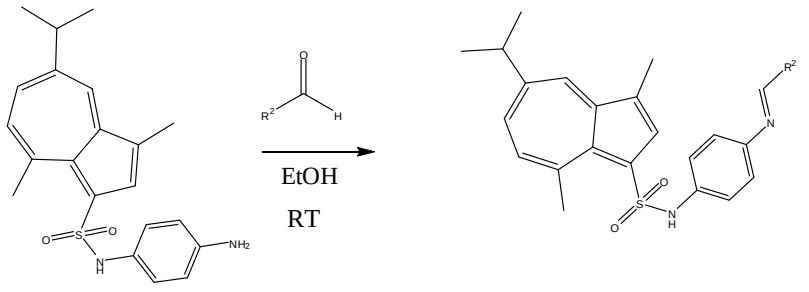
(29)

Different N-substituted-5-isopropyl-3, 8-dimethylazulene-1-sulfonamides **(30) (31) (32)** were prepared by Lu-Yun Zhang et al., By adopting different methods, most of the synthesized compounds showed attractive activity against gastric ulcer. It is also reported that 3- series compounds **(32)** showed potent antigastric ulcer activity superior to sodium guaiazazulene sulfonate, it is also reported that aryl amino substituted compounds are more active than alkyl amino substituted compounds.[11]



(30)

a) $COCl_2$, Cat, DMF, MDC, Pyridine; **b)** Amine, TEA ,Pyridine



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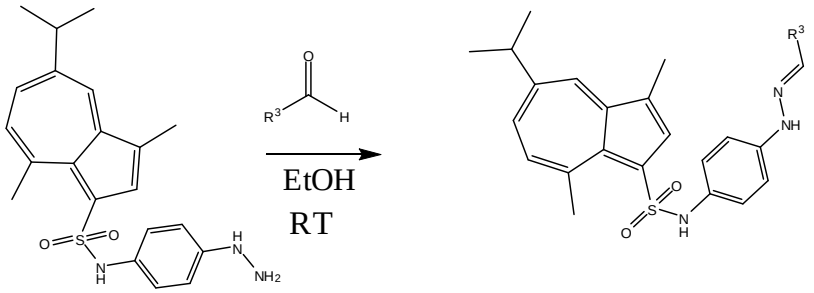
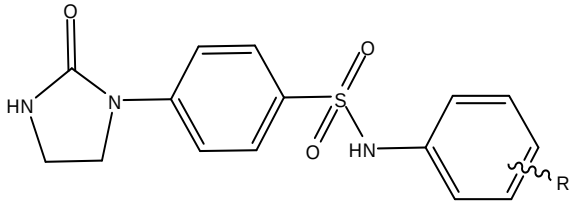


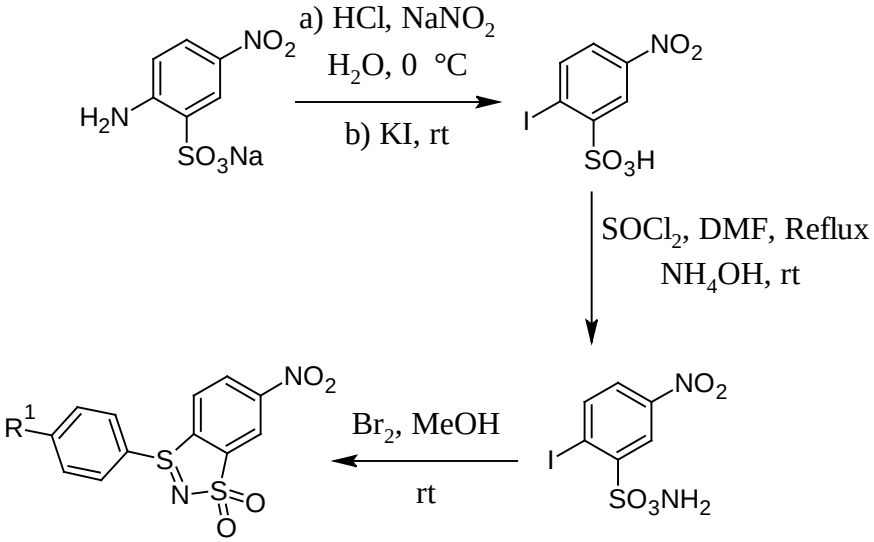
(32)

Various phenyl-4-(2-oxoimidazolidin-1-yl)benzenesulfonamides **(33)** were prepared and evaluated for their antiproliferative activity against human cancer cell lines H29, colon carcinoma, M21, skin melanoma and MCF-7 breast carcinoma cells. Most of the compounds of the series inhibited the cell growth by 50% (IC_{50}).[12]



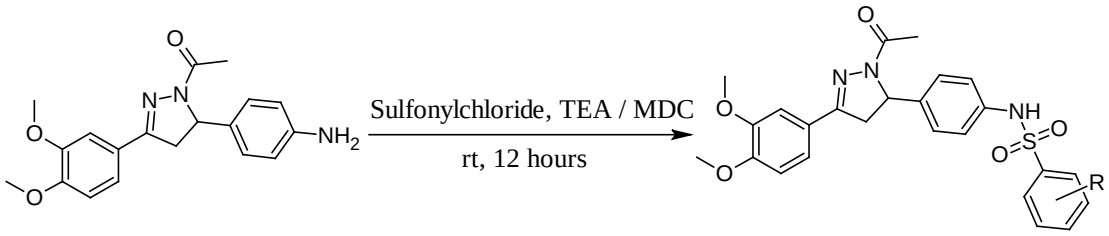
(33)

6-nitrobenzo[1,3,2]dithiazolium ylide 1,1-dioxido derivatives **(34)** prepared and evaluated for the COX-2 and 5-LOX inhibitor activities. The compounds exhibited potent anti-inflammatory activity.[13]



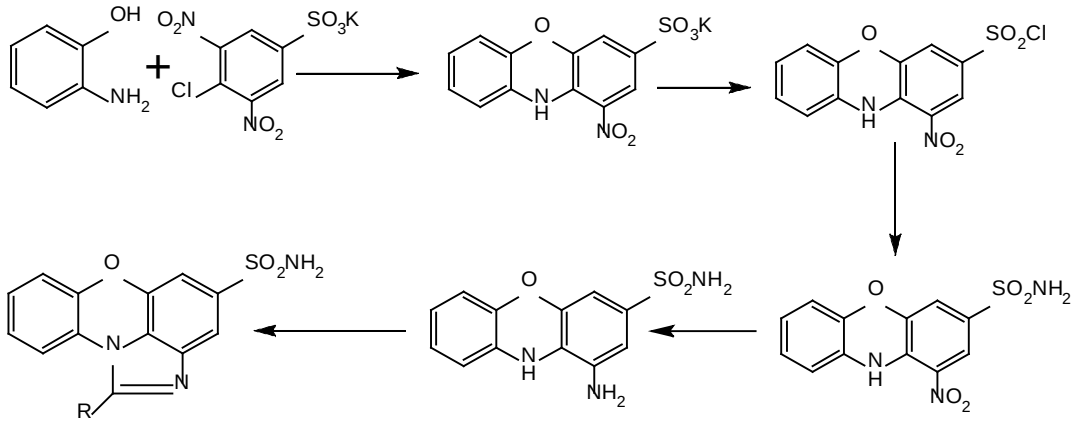
(34) R_1= -O-CH_3, - F

1-Acetyl-3-(3,4-dimethoxyphenyl)-5-(4-(3-(sulfonamidophenyl)-4,5-dihydropyrazole derivatives **(35)** were prepared and evaluated for their antimicrobial and anti-inflammatory activities. Newly synthesized compounds showed good antibacterial and antifungal activity and they have not shown any anti-inflammatory activities.[14]



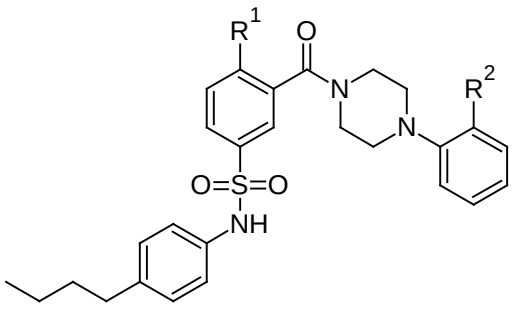
(35)

A series of novel imidazophenoxazine-4-sulfonamides were synthesized by Reddy et al,. and tested for phosphodiesterase 4 (PDE4) inhibitor property *in vitro* at 30 μM, compound **(36)** with R = 2-hydroxyphenyl, R = 3-bromophenyl and R = 5-bromo-2-flurophenyl have shown good activity.[15]



(36)

One-pot synthetic strategy was developed by Y L Yang et al., [16] for the preparation of the sulfonamide-type inhibitors of isocitrate dehydrogenase isoform (IDH) mutants (IDH1m and IDH2m). Sulfonamide analogues **(37)** were synthesized and their inhibition of IDH1m and IDH2m was further evaluated via *in vitro* and ex vivo assays. Compounds **(37)** with R^1= Me, R^2 = OMe, Cl are known to show the best inhibition activity against IDH1m.[16]



(37)

From the above data, it is clearly evident that a wide range of substituted/series of sulfonamide compounds can be used as antibacterials, antifungals and antimalarials, and in the treatment of leukemia, ovarian cancer, gastric ulcer, inflammation etc., Their biological activities depends on the structure, reactive group, their binding affinity and selectivity for particular receptors to treat chronic diseases and therapeutic uses. The results promote us to extend the concepts of sulfonamide substituent derivatives to pyrimidine and piperazines derived compounds and modify their side groups in order to improve their biological activity. There are no reports on the synthesis of halogen substituted pyridine and piperazine derivatives and their biological activity studies. In the chapter 2, we synthesized piperazine methanone substituted derivatives such as i) **Quinoline amides , Quinoline piperazine methanone and Quinoline** sulphonyl piperazine methanone The prospective utility of these substituted pyridine and piperazine derivatives were examined for antibacterial and anthelmintic studies of few prospective molecules was taken up the details are given in chapter 3

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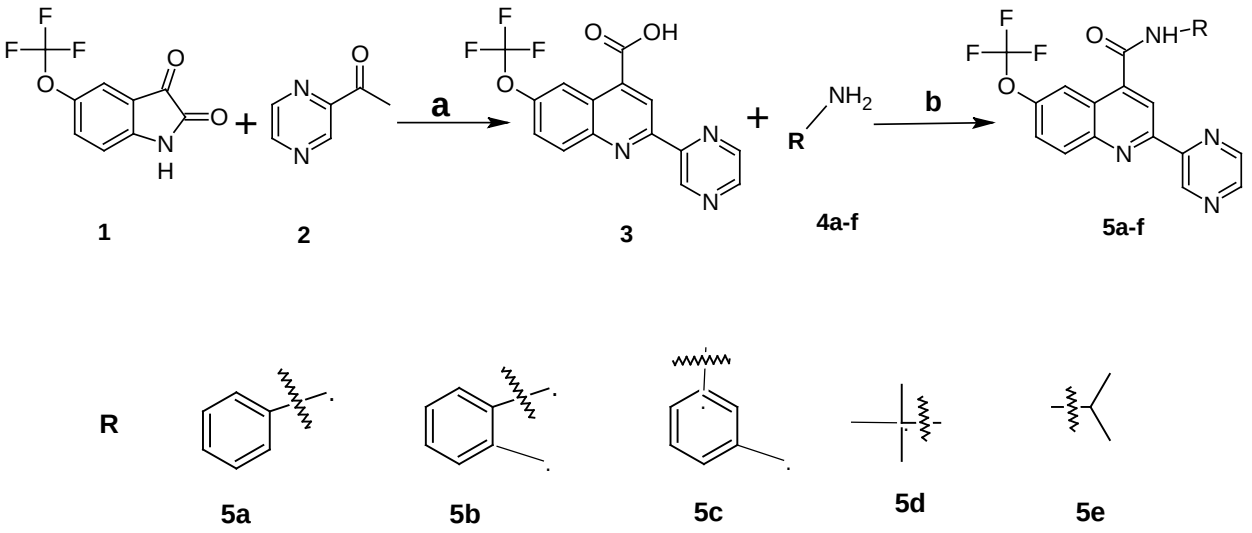
Chapter 2

Synthesis and Characterization amides and sulphanamides containing piperazine and quinoline nucleus

Introduction:

Substituted Quinolines exhibit different biological activity such as antibacterial1[-3], analgesic[4], anticancer[5], diuretic[6], anticonvulsant[7], insecticidal[8], antifungal, photosynthesis inhibiter[9] and antiviral activity[10-11]. *N*-Formyl hydroxylamide derivatives acts as a potent peptide deformylase inhibiter[12]. In addition to that, carboxylic amides and sulphanamides with a wide spectrum of biological effects including antitubercular and anti-inflammatory activity[13]. Although the preparation of amides using conventional methods is well documented[14], clean and lesser time consumption methods are less common. It has been reported that secondary and tertiary amides of quinolines were synthesized by adopting microwave irradiation method[15]. Amides and sulphanamides of quinoline derivatives promising structural moiety for drug designing and acts as component in a number of useful drugs and are associated with many biological and therapeutical activities. Condensed quinolines derivatives have been reported as anticancer[16-19], anti-microbial[20-22] analgesic, anti-inflammatory, ulcerogenic, anti-viral, anti-tumour, antioxidant[23, 24], antifungal[25], anti-HIV-1[26], anthelmintic agents[27] and also used as drugs for COX-2[28] and dynamin inhibitors[29].

Prompted by the above data we planned to synthesize new series of amides and sulphanamides containing quinoline nucleus and evaluate their pharmacological potential. Synthetic route of novel compounds are shown scheme 1, scheme 2 and scheme 3.



Scheme 1

Reagents and conditions; a : NaOH , THF and H_2O , 65^oC, **b**: EDC, TEA and T_3P

1. Procedure for the preparation for 2-pyrazin-2-yl-6-(trifluoromethoxy)quinoline-4-Carboxylic acid (3).

5-(Trifluoromethoxy)isatin (0.01 mol) was taken in THF and water mixture, to this added sodium hydroxide solution (0.02 mol) drop wise, stirred the contents for 20 min . To this solution added 2-Acetylpyrazine (0.01 mol) and stirred the contents for 2 h. Reaction was monitored by TLC, reaction mixture was neutralized by adding 1.5 M HCl solution, yellow precipitate was filtered and dried to get title compound 3.

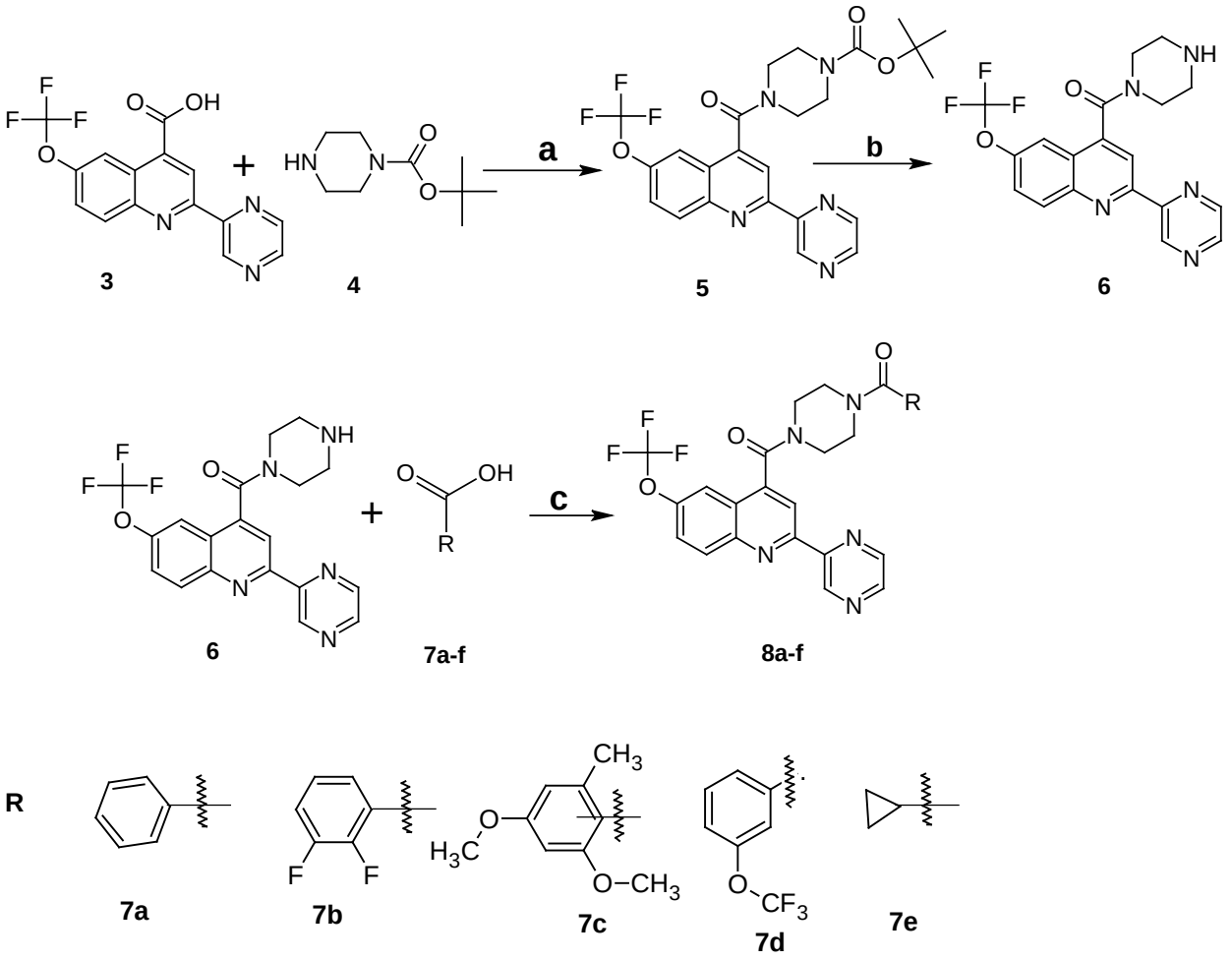
^{1}H-NMR (DMSO-d_6) δ ppm : 14.24 (s, 1H, -COOH), 9.65 (s, 1H, Ar-H), 8.91 (s, 1H, Ar-H), 8.80- 8.76 (m, 3H, Ar-H), 8.28 (d, 1H, Ar-H), 7.8 (dd, Ar-H). Yield : 75%

2. General procedure for the preparation of N-Substituted-2-pyrazin-2-yl-6-(trifluoromethoxy)quinoline-4-carboxamide (5a-f).

Compound 2-pyrazin-2-yl-6-(trifluoromethoxy)quinoline-4-carboxylic acid (0.01 mol) is dissolved in ethylene dichloride , to this solution added triethyl amine (0.02 mol) and stirred

the contents for 10 min. To the stirred solution different amines (0.01 mol)are added followed by the addition of T_3P (0.02 mol).

Reaction was monitored by TLC, organic layer was washed with water and layers are separated. The organic layer was dried with anhydrous $MgSO_4$. Crude final compounds were purified by column chromatography by using petroleum ether and ethyl acetate. Structures were confirmed by ^1H NMR, HPLC and CHN analysis.



Scheme 2

Reagents and conditions; **a:** T3P, TEA, 65°C, **b:** TFA, 40°C, 1 h, **c:** EDC, TEA and T_3P, 65°C

1. Procedure for the preparation for 2-pyrazin-2-yl-6-(trifluoromethoxy)quinoline-4-N-Boc piperazine intermediate (5).

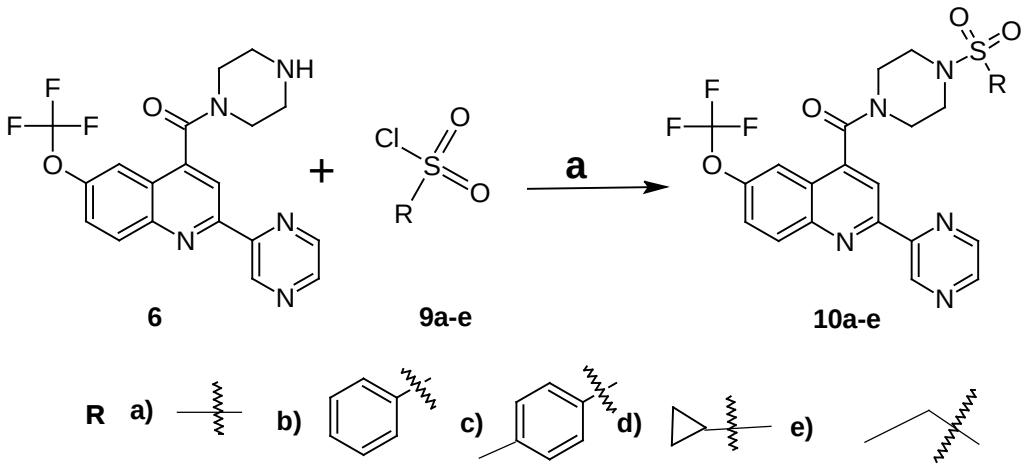
Compound 2-pyrazin-2-yl-6-(trifluoromethoxy) quinoline-4-carboxylic acid (3) (0.01 mol) was dissolved in ethylene dichloride, to this solution added triethyl amine (0.02 mol) and stirred the contents for 10 min. To the stirred solution, N-Boc piperazine (0.01 mol) was added followed by the addition of T_3P (0.02 mol). Reaction mixture was stirred at 65^oC for 6h.

Reaction was monitored by TLC, organic layer was washed with water and layers are separated. The organic layer was dried with anhydrous $MgSO_4$. Crude intermediate was purified by column chromatography by using petroleum ether and ethyl acetate to get pale yellow colored title compound **5.** Yield is 76%.

2. Procedure for the preparation for 2-pyrazin-2-yl-6-(trifluoromethoxy) quinoline-4-piperazine intermediate (6).

Intermediate 2-pyrazin-2-yl-6-(trifluoromethoxy) quinoline-4-N-Boc piperazine (0.01 mol) (5) was dissolved in ethylene dichloride , solution is cool into 0 ^oC to this added trifluoro acetic acid (TFA) (0.04 mol) and stirred the reaction mixture for 1 h at 40^oC.

Reaction was monitored by TLC, organic layer was removed by vacuum, and crude mass was purified by column chromatography by using neutral alumina and pet ether and ethyl acetate as eluent. The title intermediate compound (**6)** was recrystallised by ethanol to get off white solid.



Scheme 3

Reagents and conditions**: a:** EDC, TEA and RT

General Procedure for the preparation for amides of 2-pyrazin-2-yl-6-(trifluoromethoxy) quinoline-4-piperazine intermediate (8a-f).

Compound 2-pyrazin-2-yl-6-(trifluoromethoxy)quinoline-4-piperazine intermediate (0.01 mol) is dissolved in ethylene dichloride , to this solution added triethyl amine (0.02 mol) and stirred the contents for 10 min. To the stirred solution, different carboxylic acids (7a-f) (0.011 mol) were added followed by the addition of T_3P (0.02 mol). Reaction mixture was stirred at 65 ^oC for 6h.

Reaction was monitored by TLC, organic layer was washed with water and layers are separated. The organic layer was dried with anhydrous $MgSO_4$. Crude final compounds were purified by column chromatography by using petroleum ether and ethyl acetate to get final compounds (8a-f).

General Procedure for the preparation for sulphanamides of 2-pyrazin-2-yl-6-(trifluoromethoxy) quinoline-4-piperazine intermediate (10a-e).

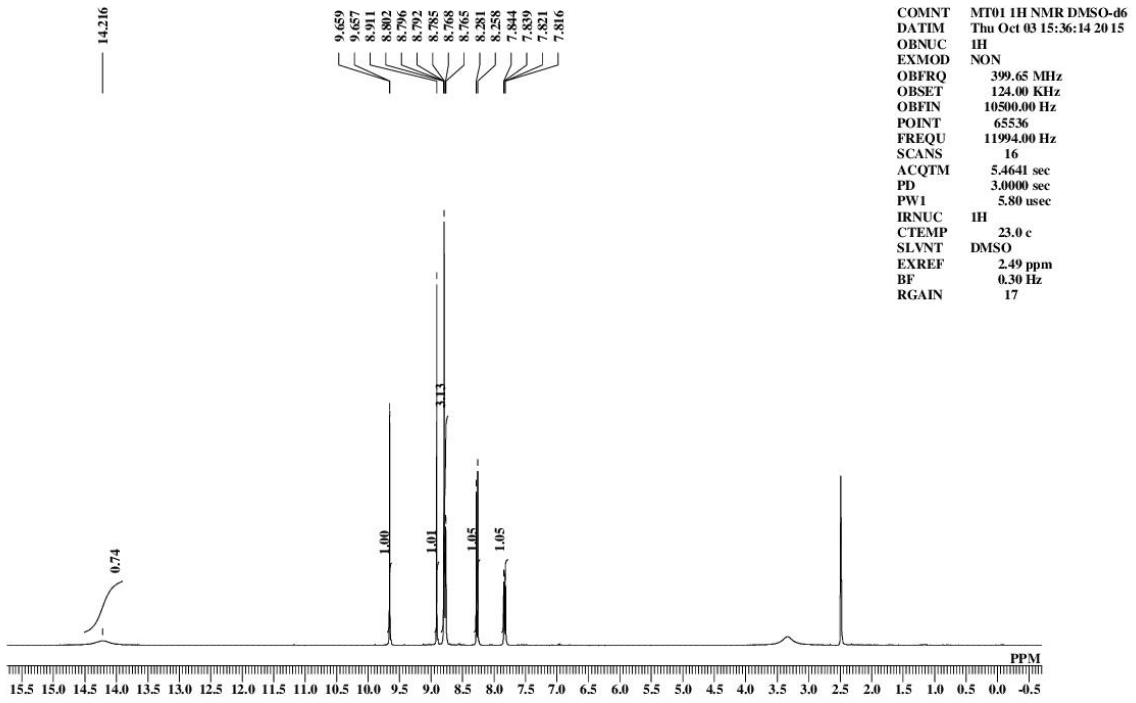
Compound 2-pyrazin-2-yl-6-(trifluoromethoxy)quinoline-4-piperazine intermediate (6) (0.01 mol) is dissolved in ethylene dichloride , to this solution added triethyl amine (0.02 mol) and

stirred the contents for 10 min. To the stirred solution, different sulphonyl chlorides (9a-e) (0.011 mol) were added. Reaction mixture was stirred at 65 ^{o}C for 6h.

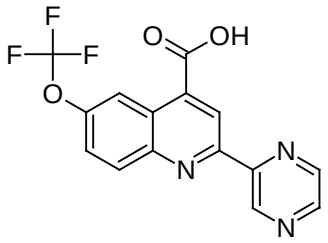
Reaction was monitored by TLC, organic layer was washed with water and layers are separated. The organic layer was dried with anhydrous $MgSO_4$. Crude final compounds were purified by column chromatography by using petroleum ether and ethyl acetate to get final compounds (10a-e).

MATERIALS AND METHODS

Chemicals were purchased from Merck India, Spectrochem and Sigma–Aldrich. Solvents and chemicals were used will be AR grade. The purity of the compounds were confirmed by thin layer chromatography using pre coated TLC plates and solvent systems are dichloromethane / methanol (9:1) and petroleum ether / ethyl acetate (6:4) and further purification was done using column chromatography. Melting points were determined in one end open capillary tubes on a liquid paraffin bath. Mass spectra, ^{1}H Nuclear Magnetic Resonance spectra and ^{13}C Nuclear Magnetic Resonance spectra were recorded for the compounds on Agilent Mass spectrometer, Bruker model avance II (400 MHz, ^{1}H NMR) and Bruker model avance II (100 MHz, ^{13}C NMR) instruments respectively. Chemical shifts were reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard.



^{1}H NMR spectra of 2-pyrazin-2-yl-6-(trifluoromethoxy)quinoline-4-carboxylic acid (3).



VarioMICRO CHNS Analysis Report for 2-pyrazin-2-yl-6-(trifluoromethoxy)quinoline-4-carboxylic acid (3).

Elemental

Statistic report

Name	C [%]	H [%]	N [%]
01C,A5510	53.73	2.41	12.53
01C,A5510	53.71	2.39	12.51
Mean value [%]	53.72	2.40	12.52
Deviation, abs. [%]	0.02	0.02	0.01

Name: eassuperuser, Access: varioMICRO superuser 24/02/2016 09:30:05 AM

varioMICRO V1.3.2 20-Mar-07, CHNS Mode, Ser. No.: 15062021

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Chapter 3

Biological evaluation of amides and sulphanamides containing piperazine and quinoline nucleus

Antibacterial Activity:

Recent discoveries of new infectious diseases and re-emergence of several infectious diseases because of increased bacterial and fungal resistance resulted in urge for studies concentrating towards the advance of new antimicrobials agents. Considering the failure in effectiveness of proven molecules, optimization of structural functionality in new molecules for the identification of better antimicrobials is of great importance in research now a days.

Hence, in this section in view of identifying new effective antibacterial agents, antibacterial evaluation have been done illustrated for all the three series of newly synthesized **(5a-e)**, **(8a-e)**, and **(10a-e)** effectively.

Material and Methods:

In vitro antibacterial activity of all the series of synthesized compounds was studied against Gram positive *Staphylococcus aureus* (NCIM-5022), *Bacillus cereus* (NCIM-5232) and Gram negative *Escherichia coli* (NCIM-5051), *Pseudomonas aeruginosa* (NCIM-2242) bacterial strains, procured form CSIR-National Chemical Laboratory (NCL) Pune in DMSO by Disc Diffusion Method on nutrient agar medium. Sterile discs of 3 mm diameter and Standard drug Ciprofloxacin were procured from Himedia, Mumbai.

Experimental Procedure:

Disc diffusion method:

Disc diffusion method[2] was incorporated for the study for all the four series of newly synthesized compounds for their antibacterial activity. Each petri plates with sterile nutrient agar medium[3] (Mueller-Hinton agar) (15 mL) were uniformly inoculated with cultures of

Gram positive and Gram negative bacterial strains respectively. The test compounds **were** prepared at concentration 10 and 20 µg 50 μL^{-1} using dimethylsulfoxide (DMSO) accoding to the Minimum Inhibitory Concentration (MIC) values obtained from microdilution method as given in **Table 1**. Five sterile discs (3 mm DIA) of 12 batches were dispensed to screw capped bottles to which 3 mL of 10 and 20 µg 50 μL^{-1} test samples were added respectively. The treatment also included equivalent amount of dimethylsulfoxide as negative control and standard Ciprofloxacin (CIPRO) 10 µg 50 μL^{-1} as poititve control for comparison. For each treatment, triplicate were maintained and incubated at 37 ± 2 °C for 24 h and zone of inhibition was measured in mm. Similarly evaluation of antibacterial activity of all the other series **(8a-e)** and **(10a-e)** of compounds were done and were reported in **Table 2**

Minimum inhibitory constant [MIC]:

A series of test tubes containing 2.5, 5, 10 and 20 µg mL^{-1} concentration of all the sereies of synthesized compounds with Mueller Hinton broth was immunized with required amount of the bacterial strains to obtain the microorganism suspension, which would contain 105 colony units per milliter. One positive control tube was prepared with the addition of standard Ciprofloxacin and another blank tube without microorganisms. All the tubes were incubated at 37 ^{o}C for 24 h, after incubation period UV-visible spectrometer was used to record the turbidity produced in each test tubes. The minimum inhibitory concentration was considered to be concentrations at which turbidity was minimal or same as blank.

Statistical Analysis

The data of antibacterial study was expressed as Mean ± S.E of triplicates. The difference in values at $p \leq 0.01$ was considered as statistically significant. The analysis of variance (ANOVA) was performed using ezANOVA (version 0.98) software to determine the mean and standard error of the inhibition zone in antibacterial activity.

Table 1: Antibacterial activity Minimum Inhibitory Concentration (MIC) values of **(5a-e)**, **(8a-e)** and **(10a-e)** .

Bacterial Stains	Conc. (µg 50 µL-1)	Scheme 1 Comps (5a-e)	Scheme 2 Comps (8a-e)	Scheme 3 Comps (10a-e)
		Bacterial Growth		
E. coli	2.5	+	+	+
	5.0	+	+	+
	10	+	-	-
	20	-	-	-
P. aeruginosa	2.5	+	+	+
	5.0	+	+	+
	10	-	+	-
	20	-	-	-
S. aureus	2.5	+	+	+
	5.0	+	+	+
	10	-	-	+
	20	-	-	-
B. cereus	2.5	+	+	+
	5.0	+	+	+
	10	-	+	+
	20	-	-	-

+: Presence of Growth; -: Absence of Growth

Table 2: Antibacterial activity of **(5a-e) , (8a-e) and (10a-e).**

Code	Conc. (µg 50 µL^{-1})	*E. coli* (Mean±SE)	*P. aeruginosa* (Mean±SE)	*S. aureus* (Mean±SE)	*B. cereus* (Mean±SE)
CIPRO	10	13.00±0.58	14.17±0.17	15.17±0.44	12.33±0.33
5a	10	4.67±0.33**	2.17±0.17**	5.83±0.44**	2.67±0.17**
	20	6.67±0.32**	3.83±0.44**	6.97±0.03**	3.17±0.44**
5b	10	6.50±0.29**	2.50±0.29**	3.83±0.17**	4.50±0.29**
	20	8.17±0.17**	3.83±0.60**	4.57±0.30**	6.60±0.29**
5c	10	6.15±0.22**	2.33±0.33**	5.83±0.44**	2.44±0.41**
	20	8.11±0.38**	5.50±0.29**	6.97±0.03**	5.20±0.17**
5d	10	4.50±0.21**	2.83±0.44**	3.83±0.17**	3.50±0.29**
	20	6.50±0.20**	4.50±0.29**	6.57±0.30**	4.11±0.29**
5e	10	3.50±0.22**	3.33±0.44**	4.17±0.38**	5.44±0.29**
	20	5.51±0.29**	5.33±0.33**	6.50±0.29**	6.57±0.30**
8a	10	5.58±0.25**	3.67±0.33**	5.50±0.29**	3.50±0.29**
	20	7.57±0.26**	5.50±0.29**	7.83±0.60**	5.67±0.13**
8b	10	5.83±0.44**	2.67±0.17**	2.33±0.33**	5.67±0.83**
	20	7.97±0.03**	4.17±0.44**	4.50±0.29**	7.50±0.99**
8c	10	3.83±0.17**	4.50±0.29**	2.83±0.44**	5.17±0.17**
	20	5.80±0.70**	6.67±0.33**	4.50±0.29**	8.17±0.22**
8d	10	5.50±0.29**	3.67±0.17**	4.50±0.21**	2.83±0.44**
	20	6.17±0.44**	5.64±0.33**	6.50±0.20**	4.50±0.29**
8e	10	6.67±0.33**	3.50±0.58**	5.50±0.29**	3.67±0.33**
	20	8.12±0.44**	5.17±0.33**	6.57±0.30**	4.50±0.29**
10a	10	5.58±0.25**	3.67±0.33**	5.50±0.29**	3.50±0.29**
	20	7.57±0.26**	5.50±0.29**	7.83±0.60**	5.67±0.13**
10b	10	5.83±0.44**	2.67±0.17**	2.33±0.33**	5.67±0.83**
	20	7.97±0.03**	4.17±0.44**	4.50±0.29**	7.50±0.99**
10c	10	3.83±0.17**	4.50±0.29**	2.83±0.44**	5.17±0.17**
	20	5.80±0.70**	6.67±0.33**	4.50±0.29**	8.17±0.22**
10d	10	5.50±0.29**	3.67±0.17**	4.50±0.21**	2.83±0.44**
	20	6.17±0.44**	5.64±0.33**	6.50±0.20**	4.50±0.29**
10e	10	6.67±0.33**	3.50±0.58**	5.50±0.29**	3.67±0.33**
	20	8.12±0.44**	5.17±0.33**	6.57±0.30**	4.50±0.29**
Control	-	-	-	-	-

Values are the mean ± standard error of mean (SEM) of clear zone. Symbols represent statistical significance, **p≤0.01 as compared with the control group.

CIPRO: Standard Ciprofloxacin. Control: Dimethyl sulfoxide

Results and Discussion:

Chemicals were purchased from Merck India, Spectrochem and Sigma–Aldrich. Solvents and chemicals were used will be AR grade. The purity of the compounds were confirmed by thin layer chromatography using pre coated TLC plates coated with silica gel with the thickness of 0.2 mm and solvent systems are dichloromethane / methanol (9:1) and petroleum ether / ethyl acetate (6:4) according to their solubility condition. Further purification was done using column chromatography by using 60-120 and 230-400 silica gel. Some of the basic nature final compounds were purified by using neutral alumina column chromatography. Majority of the reaction carried out under inert atmosphere by using argon and nitrogen gas to improve the rate of reaction. Regularly reaction was monitored to avoid the formation side product to improve the yield and reduce the purification cost respectively. Melting points were determined in one end open capillary tubes on a liquid paraffin bath. Mass spectra, ^{1}H Nuclear Magnetic Resonance spectra and ^{13}C Nuclear Magnetic Resonance spectra were recorded for the compounds on Agilent Mass spectrometer, Bruker model avance II (400 MHz, ^{1}H NMR) and Bruker model avance II (100 MHz, ^{13}C NMR) instruments respectively. Chemical shifts were reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard.

Detailed minimum inhibitory concentration examination of all the three series of test samples for activity at different concentrations (2, 5, 10 and 15 µg 50 μL^{-1}) against *C. indicum*, *M. gypseum*, *T. equinum* and *C. albican* fungal strains was done and the, data's given in **Table 1**. Minimum inhibitory concentrations (MICs) obtained by micro dilution method were observed to be 10 µg and 15 µg 50 μL^{-1}. Further all the four series of compounds were screened at 10 and 15 µg 50 μL^{-1} against all the fungal strains.

Analysis of screening data of all the series of compounds revealed that tested,

- **5a-e** showed moderate to good activity against *C. indicum, and C. albicans*, less activity against *M. gypseum* and *T. equinum* data given in **Table 2**.
- **8a-e** showed less activity against *C. indicum, M. gypseum, T. equinum* and *C. albicans,* data given in **Table 2**
- **10a-e** showed moderate activity against all the tested fungal strains, data given in **Table 2**

Conclusion:

It is evident from the tabulated data that all the tested series of compounds showed less to significant activity against all the tested strains compared to standard drug. Electron donating groups are attached to the carboxylic acid leads to decrease the acidity.