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Synthesis and pharmacological evaluation of 6-sulphnamido cinnoline for Pharmacological and antibacterial activity

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A B S T R A C T

Drug discovery, synthesis and development multidisciplinary, creative, complex and highly regulated process Heterocyclic compound are organic compound containing oxygen, sulphur and nitrogen. New drug synthesis not only done loping new chemical entities but also important for therapeutic need .In view of the general observation that pharmacological activity is invariably associated with a large variety of heterocyclic compounds, the present investigation of heterocycles belonging to class of cinnoline was undertaken. These compounds are reported to possess a wide spectrum of biological activity which includes antibacterial, antifungal, anticonvulsant, anticancer, anti-inflammatory, antihypertensive and analgesic activities. The current study involves the synthesis of 6-sulphonamido-cinnolines and their purification and characterized reaction .and were screened for antibacterial anti-oxidant and in vitro anti-inflammatory activity using bovine serum albumin denaturation model Sulphanilamide was treated with sodium nitrite in presence of conc. HCl at 0-5 °C to form diazonium salt, which on treatment with cyanoacetamide gave aryl hydrazine (cyano) acetamide. It was allowed to react with anhydrous $AlCl_3$ in the presence of chlorobenzene to form 4-amino-6-sulphonamido-3-cinnolino carboxamides. This on treatment with form amide gave sulphonamido-cinnolino-pyramidine.6-sulphonamide cinnoline derivative identified and characterized by physical methods like m.p, TLC, and spectral method UV, IR, and NMR data. The newly synthesized compounds were investigated for their microbial susceptibility using in vitro model for against bacillus subtillis (gram +ve) Escherichia coli (gram - ve) the compounds were also subjected for bovine serum albumin denaturation which was considered as in vitro anti-inflammatory model. The newly synthesized compounds 4, 5, 9, 15 and 17 exhibited good antibacterial activity. Other compounds exhibited weak activity. Compounds 5, 6, 7 and 13 showed good anti-inflammatory activity. Other synthesized compounds (4, 12 and16) exhibited weak activity.

Keywords: Sulphanilamide, anti-bacterial, anti-inflammatory, anti-oxidant

A R T I C L E I N F O

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1. Introduction

Cinnoline is an aromatic heterocyclic compound with the formula $C_8H_6N_2$ and can also be called as benzo derivative of pyridazine [1]. It is isomeric with phthalazine. Though it does not occur in nature, it is a vigorously developing branch of organic chemistry. Although cinnoline belong to a family of well known heteocycles, Heterocyclic compound are organic compound containing oxygen, sulphur and nitrogen. New drug synthesis not only done loping new chemical entities but also important for therapeutic need. The interest in the study of its derivatives continues. Recent studies have shown that cinnoline and their derivatives exhibit various biological activity [2] such as antihypertensive [3], antithrombocytic [4], antitumour[5], anti-inflammatory [6], anticancer [7] bactericidal activity. Heterocycles play an important role in biology and are an important constituent of various biomolecules like DNA, RNA, vitamins and amino acids.

The well known biological importance of sulphonamide derivatives as antibacterial, anticancer, anti-malarial, and anti-tubercular agents prompted us to introduce a sulphonamido group into the cinnoline ring hoping to get compound to enhance potency and synergistic effect[8]. Although sulphonamides have provided medicinal science with some of the most potent weapons for the effective conquest of many diseases of bacterial origin, there is still a long list of bacterial infections uninfluenced by the newer chemotherapeutic agents. Among them, leprosy and tuberculosis continue to constitute new challenges in chemotherapy. The present study focuses on an exploratory investigation in cinnolines by synthesizing novel sulphonamido cinnoline.

Synthesis of Cinnoline by Following Name Reactions:

a. Von Richter's synthesis
b. Busch and klett's synthesis
c. Borsche's synthesis
d. Baumgarten's synthesis
e. Widman-stoermer's synthesis

Synthesis of Cinnolines:

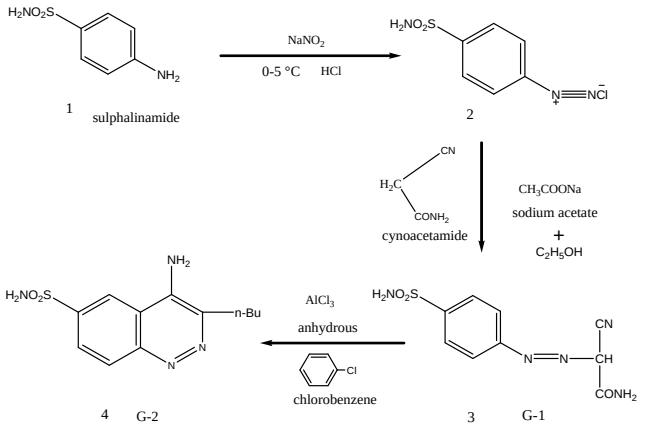
a. Von Richter's synthesis
b. Busch and klett's synthesis
c. Borsche's synthesis
d. Baumgarten's synthesis
e. Widman-stoermer's synthesis

2. Materials and Methods

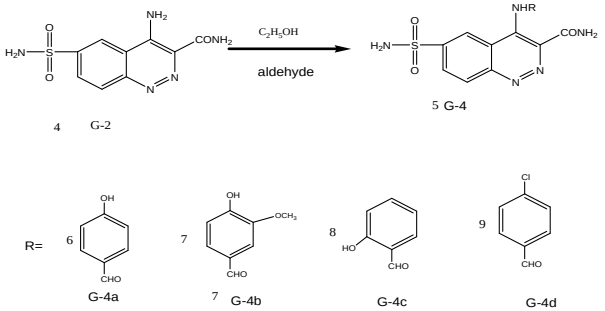
I. Synthesis of Diazonium Salt Solution:

Ice cold solution of Sodium nitrite (8.058g, 116 mmol) dissolved in 25 ml water was added drop wise to an ice-cold suspension of sulphanilamide (20.66g, 116 mmol) in Conc. HCl (29 ml) at 0-5 °C with stirring . The stirring continued for further 15 min. and the resulting light yellow diazonium chloride solution (I) was immediately use for next step.

Scheme-1



Scheme-2



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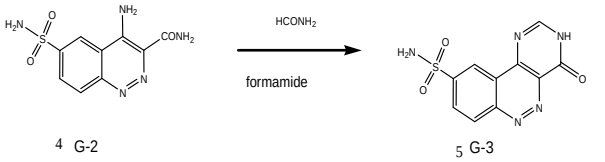

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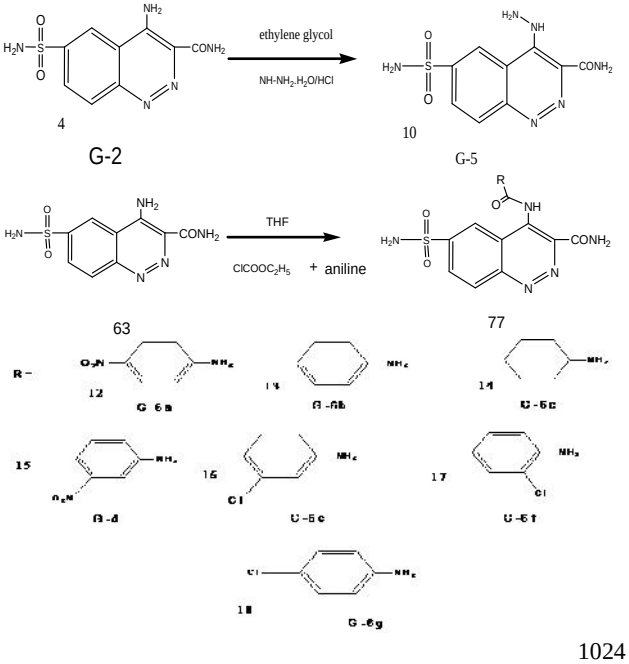

II. Synthesis of 2-Cyano-2-(4-sulfamoyl-phenylazo)-acetamide: Procedure:

In a 250 ml of RBF was placed a solution of Sodium acetate (9.56g, 116 mmol) in 35 ml water. To this was added a solution of cyanoacetamide (9.81g 116 m.mol) in a mixture of water (170 ml) and ethanol (120 ml). The previously mixture was cooled to <5 °C in a ice salt bath. After cooling the diazonium salution (**2)** prepared in the previous step was added slowly with continuous Stirring maintain the temperature 0-5 °C during addition. Approximately 15 min repeated for the addition stirring was continued for further two hrs at room temperature & the precipitate was filtered. Wash with water, ethanol, The crude product was finally dried at 50 °C in a vacuum oven.The crude product recrytalised from 80% (H2O+DMF) the yield of the recrystalised product 80%

(5g) (MP = 276-278 °C) The R_f value of the product was found to be 0.52 mobile phase (EA; n-hexane ::2:1)

III Synthesis of substituted 4-amino, 6 sulphonamido,3-cinnolino carboxamide:

In a 250 ml of RBF fitted with condenser with guard tube was placed 2-Cyano-2-(4-sulfamoyl-phenylazo)-acetamide (9.25g, 25 mmol) in aluminum chloride (anhydrous) (6.6g, 50 mmol) and 30 ml chloro benzene stirring under reflux for 12 hour in anhydrous condition. After it was cooled, poured into a beaker containing crushed ice (50 g) and 25 ml conc. HCl with stirring. The residue was filtered, washed with pet ether (60:80) and made alkaline by the addition of 5% sodium carbonate solution. The pure base was filtered and washed with water 2-3times. The solid obtained was dissolve in Dimethyl form amide (DMF) and water mixture .The mixture was extracted with ethyl acetate and evaporated to get the product(63) .The title compound obtained was recrytallized by DMF. (MP=140-150 °C, colour = yellowish brown). The R_f value of the product was found to be 0.68 (Mobile phase EA; n-hexane: 2:1)

IV Synthesis o1-Hydroxy-1, 2-dihydro-2, 4, 9, 10-tetra azaphenanthrene-6-sulfonic acid amide (G-3) :

A mixture of substituted 4-amino cinnoline -3-carboxamide **(G-2)**(1.2g, 5 mmol) was refluxed for 6 hr with formamide (15 ml) after it was cooled the solid obtained. The solid was s dissolve in water extracted with ethyl acetate and evaporated to get the product **(G-3)** .The product was obtained in 70% as a light yellow solid mass and recrystallized by DMF.

Synthesis of 6 Sulphonamido Cinnoline Shiff Base

An equimolar ratio of 6-sulphonamido cinnoline **((G-2) (**1g 37mmol)and aromatic aldehydes(37 mmol),without α hydrogen atom was placed in a RBF fitted with reflux condenser and guard tube. To the mixture was added 2 to 3 drops of glacial acetic acid and dry ethanol 25 ml(as solvent) and refluxed for 4 hrs, cooled & filtered to obtained the crud product. The precipitate was recryctallised by DMF .derivatives G-5(a-d) was prepared by this method. As mentioned on scheme no.2

V Synthesis of 6-Sulphonamido Cinnolin Urea

Take solution of 6-sulphonamido cinnoline (**G-2)** (0.1g, 37mmol) in tetra hydro furan 10ml at 0^0C was added triethyl amine (0.034ml, 37mmol) and ethyl chloroformate (0.038 ml, 37 mmol). After being stirred at 0^0C for 1 hour, substituted amine (0.048ml, 37 mmol) were added. The reaction mixture was stirred over night at room temperature. The reaction mixture was partitioned between ethyl acetate and water, extracted with ethyl acetate, dried over $MgSO_4$, evaporated to gate the product **G-6** the recrystallized by DMF. The yield of the recrystallized product 60% (60 mg)

(a). Antimicrobial Activity

Study of Anti-Microbial Activity by Agar Diffusion Method

In our current study, anti anti-microbial activity was carried out by agar diffusion method. Here responses of micro-organisms to the synthesized compounds were measured and compared with the response of the standard reference drug. The standard drug used in the present work was Amoxicillin. [33]

Micro-Organisms Used

The two micro-organisms were used are (Gram+ve). *Bacillus subtillis* and (Gram–ve) *Escherichia coli*

Preparation of Test solutions

Each test compound was dissolved in Dimthyl formamide to get a concentration of 50 µg/ml. this concentration was used for testing anti-microbial activity.

Preparation of Muller Hinton Agar media

The beef extract was taken in 1000 ml beaker and made-up the volume upto 1000 ml with distilled water. To this mixture known quantity of beef infusion, agar, starch and Casamino acids were added and dissolved by heating the mixture. The pH was adjusted to 7.3 and finally the media was sterilized by autoclaving at 121 °C for 15 minutes at 20 psi pressure. Afterwards the mixture was cooled to 45 °C and then inoculums were added to the sterile borer. 0.1 ml of test solution and standard solution at a concentration of 50µg/ml were taken. A standard Amoxicillin was maintained with same concentration in each plate and a control having only DMF in one plate. Then the petri dishes were incubated at 37°C for 24 hours and zone of inhibition was observed and measured for each sample.

Preparation of Inoculum:

The suspensions of all the organisms were prepared as per the standard procedure (McFarland Nephelometer Standard). A 24 hours culture was used for the preparation of bacterial suspension. Suspensions of organisms were made in sterile isotonic solution of Sodium Chloride (0.9% w/v).

(a). *In-vitro* Denaturation of protein assay by bovine serum albumin

Based on a specific biochemical or cellular mechanism have been developed for the initial screening of the anti-inflammatory compounds. Denaturation of proteins is one of the causes for inflammation which is well documented. A number of anti-inflammatory drugs are known to inhibit the denaturation of proteins and an *in vitro* test which causes denaturation of proteins may be used as a preliminary screen for anti-inflammatory activity.

Percentage inhibition of denaturation was calculated from the formula

$$\% \text{ Inhibition} = \frac{(\text{Abs. of control} - \text{Abs. of sample}) \times 100}{\text{Abs. of control}}$$

G-1 Synthesis of 2-Cyano-2-(4-sulfamoyl-phenylazo)-acetamide:

3456.56, 3402.50, (NH str.), 29.12(C-HAr.Str.), 2222.7 (C≡N)1672.26,1614(C=O str), 1591.33, 1558.59, (C=C.), 1336.18, 11551(SO_2). 7.75 (s, CH, 4H at I.II.III), 12.1 (s, SO_2NH_2,2H, at VII), 7.25(s. 1H,CH at V (Aromatic protons). 7.53&7.93(s.2H,$CONH_2$ at VI)

G-2 4-Amino, 6 sulphonamido,3-cinnolino carboxamide: 3450.12, 3311.50, (NH str.), 2924.12 (C-H Ar.Str.), 1639, 56(C=Ostr),1561.12(C=C), 1394.58, 1155.54. (SO_2). 14.10 (s, SO_2NH_2, 2H at I.), 8.1725 (S,NH_2,2H at V), 7.7521-7.80 (M, 2H CONH2 at VI), 7.5788& 7.6001 (d,2H,CHatIII), 7.318&7.3399(d 1H CH at IV)

1-Hydroxy-1, 2-dihydro-2, 4, 9,10-tetraazaphenanthrene-6-sulfonic acid amide

G-3 3311.12, 3156.52, (NH str.), 2922.26 (C-Ar.Str.), 1675. (C=Ostr), 1559.56,1475,(C=CAr.Ben..),1591.33(N-H).ben) 1320, 1155.54.(SO_2 **G-4a** 35.25 (O-H) 3383.26, 3259.81 (NH str.), 1649.46, (C=O), 15.99.33, (C=C Ar str.), 1435.23 (C=N str.), 1398,1313.51,1119 (SO_2)

G-5 a 3414.25(O-H) , 3348.85, (NH str.), 2962.40(C-H Ar.), 2848.96(C-H Str.)1683.91, 1653.96 (C=O), 1595 (C=C Ar ben.), 1290.37,1151.40 (SO_2).

G-5. 3525.99, 3383.26,(NH str.), 2918.40(C-H Ar.), 2848.96(C-H Str.)1649.19, (C=Ostr), 1590.04 (C=C), 1244.13 (N-N str.), 1396.44,1155.40 (SO_2).G-4d 3446.91, 3345.54, (NH str.), 2924.18(C-H), 1672.34, (C=Ostr), 1489.10(C=N),1595.18 (C=C), 1323.56, 1157.33 (C-C str), 835.21,790.74 (C-Cl).

G 6-a 3356.25, 3427.85, (NH str.), 1653.26, 1629.96 (C=O), 1597.12(C=C), 1510.11, 1510.22(N=O), 1309.71, 1111.03 (SO2**)**

G-6d3309.25, 3267.85, (NH str.), 1734.26, 1629.96 (C=O) 1604.23 (C=C), 1595.11 ,1527.67(N=O), 1348.29, 1150 (SO_2).

G-6e3423.76, 3348.54, (NH str.), 2928.04(C-H, Ar), 2870.17(C-H), 1653.05(C=O) , 1558.54.1492.95, (C=C Ar ben.), 1263.42 (C-N), 1307.36, 1089.82 (SO_2), 827.26,752 (C-Cl).

G-6E 3446.91,3340.82 (NH str.), 3082.25(C-H), 1687.77, 1654.98(C=O),1600.97.1543.10 (C=C Ar str.), 1261.42 (C-N), 1226.71 (C-Cstr.), 1319.35,1157.33 (SO_2) 752.26 (C-Cl).

3. Results and Discussion

6-sulphonamido cinnolines was obtained in good yield by Friedel-Craft reaction involving cyclisation .Sulphanilamide (1) with sodium nitrite in presence of Conc. HCl at 0-5°C form diazonium salt (**2**), which on treatment with cyanoacetamide gives aryl hydrazine (cyano) acetamide (**3).** This was reacted with anhydrous $AlCl_3$ in the presence of chlorobenzene to form 4-amino-6-sulphonamido-3-cinnolino carboxamides (**4).**Which was further refluxed with formamide to give sulphonamido cinnolino- pyramidine**(5).** 4-amino-6-sulphonamido-3-cinnolino carboxamides in THF was stirred with various anilines(**a-g)** which gave different urea derivativesG-**6(a-g).** Sulphonamido cinnoline**(4**) in the presence of alcohol with 2-3 drops of glacial acetic acid was refluxed with various aldehydes**(a-d**)to give different schiff's base G-4**(a-d**).4-Hydrazino-6-sulfamoyl-cinnoline-3-carboxylic acid amide**(5**) was synthesized by sulphonamido cinnoline(63) with hydrazine hydrate in presence of ethylene glycol as a solvent.

Synthesis of 6-sulphonamide cinnoline derivatives (**4)** identified and characterized by physical methods and spectral method UV, IR, and NMR spectra. TLC were derivatized using either Ninhydrin, P- anisaldehyde, Ehrlich's.The amine group was identify by chemical test (diazotization), All the compounds were confirmed by elemental analysis (**table no-3**), zpart from this presence of peak and other functional groups were identified by I R such as 2222.07 cm^{-1} (C≡N) in IR spectra, (**scheme no.** 1 and figure no-1) conversion of nitrile to amine group was confired by peak 3433.41 cm^{-1} (figure no. 2)and 1664 cm^{-1} (C=O) str, sulphanilamido proton signal in ^{1}H NMR-spectra(δ) 14.1(s .NH_2,2H atI)., and 8.17 (s, $CONH_2$,2H at IV) was identified (figure no. 13).

The disappearence of methylene proton of diazo nitrile acetamide (3) in ^{1}H NMR may indicate the formation of cyclic procduct (4). 3375.54 cm^{-1} (NH str.) broad peak indicates the formation of tricyclic ring 5 (scheme 2 figure no. 3) 3525 cm^{-1} (O-H) str. due to hydroxyl peak (scheme no 3 , figure no 4) indicates the formation of cinnoline schiff's base(5) ,752 cm^{-1} (C-Cl) due to halogen peak (scheme 5 figure no. 8) Indicates the formation of cinnoline Schiff's base and 1320, 1150 cm^{-1} (SO_2) (scheme. no. 14, figure no. 1) indicates the presence of sulphonamide group (62). 1597.11 ,1450 (N=O) due to nitro group (scheme no.5 figure no 9,10).indicates the formation of cinnoline urea derivatives (6a, 6d and 6g), 827.26 ,752cm^{-1} (Cl) due to halogen peak (scheme no.5, figure no. 10,11,) confirmed the synthesis of cinnoline urea derivatives(6a, 6d and 6g).

Pharmacologacal evaluaction.

- All the synthesized compounds were tested for antibacterial activity as compared with standard drug Amoxicillin against *Bacillus subtillis* and *Escherichia coli.* 4, 5, 9, 15 and 17 showed good activity and other compounds exhibited weak activity.
- All the compounds were Screened for *in-vitro* anti-inflammatory activity of BSA using Ibuprofen as standard drug, Compounds 5, 6,7, and 13 showed good anti-inflammatory activity. Other synthesized compounds (4, 12 and16) exhibited weak activity.

Attempt was made to explore structure activity relationship of synthesized 6- sulphonamido cinnoline derivatives. Sulphonamido cinnoline urea derivatives were tasted against different microbes namely *E. coli* and *B. subtillis.* The presence electro withdrawing group like nitro at Meta position demonstrated very good activity against (E. coli) however this compound was mild activity against *B. subtillis.*

Cinnoline Schiff's base derivatives was tested against different microbes namely *E. coli* and *B. subtillis* .The presence of electron donating group like Hydroxy at ortho

position demonstrated very good activity against (E. coli) however this compound showed mild activity against B.subtillis. Sulphonamido-Cinnolino-pyrimidine derivatives (5) Substituted pyrimidine ring a cinnoline molecules demonstrated good activity against (E. coli) however this compound was showed mild activity against B. subtillis. Cinnoline pyrimidine derivatives (5) were evaluated for against Bovine serum Denaturation. Substituted pyrimidine ring in cinnoline molecule demonstrated good activity. Cinnoline schiff's base derivatives against BSD present electron donating group like (OH) at ortho and Meta position demonstrated very good activity.

Table 1: Physica Properties of Derivatives

Comp. code	Mol. Formula	Element analysis	Mol. Wt.	M.P (°C)	% Yield	R_f value
G-3	$C_{10}H_7O_3N_5S$	C=43.32, H=2.54, N=25.26, O=17.31, S=11.56	277.26	260	61.78	0.68
G-4a	$C_{16}H_{13}N_5O_4S$	C=51.75, H=3.53, N= 18.86, O=17.23, S-=8.63	371.37	120	37.08	0.71
G-4b	$C_{17}H_{15}O_5N_5S$	C=50.87,H=3.77,N=17.47,O=19.93, S=7.93	401.08	185	43.9	0.80
G-5	$C_9H_{10}O_3N_6S$	C=38.29,H=3.57,N= 29.77, O= 17,S-=11.36	282.00	255	57	0.50
G-4c	$C_{16}H_{13}O_4N_5S$	C=51.75, H= 3.53, N=18.18, O=17.23, S= 8.63.	341.28	144	54	0.68
G-4d	$C_{16}H_{12}O_3N_5SCl$	C=49.30,O=3.18,Cl= 9.09, N=17.97, O=13.31,S= 8.23	371.03	146	57	0.62
G-6a	$C_{16}H_{13}O6N_7S$	C=44.55, H=3.03, N=22.73, O- 22.25, S=7.43.	431.06	190	59	0.58
G-6b	$C_{16}H_{14}O_4N_6S$	C=49.74, H=3.64, N= 21.75, O=16.56, S= 8.30.	386.39	135	78	0.56
G-6c	$C_{16}H_{13}O_6N_7S$	C=48.97, H=5.14, N=21.42, O=16.31, S=8.17	392.43	210	56	0.70
G-6d	$C_{16}H_{13}O_6N_7S$	C=44.55, H= 3.03, N=22.73, O=22.25, S=7.43.	431.64	130	61	0.65
G- 6e	$C_{16}H_{13}O_4N_6SCl$	C=45.66, H=3.11,Cl=8.42, O=19.97, S=15.21, Cl=7.26	420.64	120	54	0.58
G-6f	$C_{16}H_{13}O_4N_6SCl$	C=45.66, H=3.11,Cl=8.42, O=19.97, S=15.21, Cl=7.26	420.64	125	56	0.62
G-6g	$C_{16}H_{13}O_4N_6SCl$	C=45.66, H=3.11,Cl=8.42, O=19.97, S=15.21, Cl=7.26	420.64	115	66	0.54

Table 2: Anti microbial activity of 6-sulphonamido cinnolines derivatives

Sr. No	Compound Code	Zone of Inhibition	
		Bacillus subtillis **(Gram +ve)**	***Escheria coli*** **(Gram-ve)**
		50 µg	**50 µg**
01.	4 (G-2)	2.5 mm	2.3 mm
02.	5 (G-03)	2.8 mm	2.9 mm
03.	6 (G-4a)	2.5 mm	3.5 mm
04.	7 (G-4b)	2.1 mm	2.4mm
05.	10 (G-5)	2.1 mm	2.6 mm
06.	8 (G-4c)	2.6 mm	2.8mm
07.	9 (G-4d)	3 mm	3.1 mm
08	12 (G-6a)	2.2 mm	2.7mm
09	13 (G-6b)	2.1 mm	1.6mm
10	14 (G-6c)	2.8 mm	2.2 mm
11	15 (G-6d)	3.5 mm	1.9 mm
12	16 (G-6e)	1.9 mm	1.6 mm
13	17 (G-6f)	2.6 mm	3.5 mm
18	Amoxicillin	8.5 mm	9.1 mm

Table 3: Bovine serum protein Denaturation activity of 6-sulphonamidoCinnolines

Sr. No	Compound Code	IC_{50} value
01.	4(G-2)	Above 300
02.	5 (G-3)	Above 400
03.	10(G-6)	Nil
04.	6 (G-4a)	Above 350
05.	7 (G-4b)	Nil
06.	8 (G-4c)	Above 300
07.	12 (G-6a)	NIL
08	13 (G-6b)	Below 400
12	Ibuprofen	125

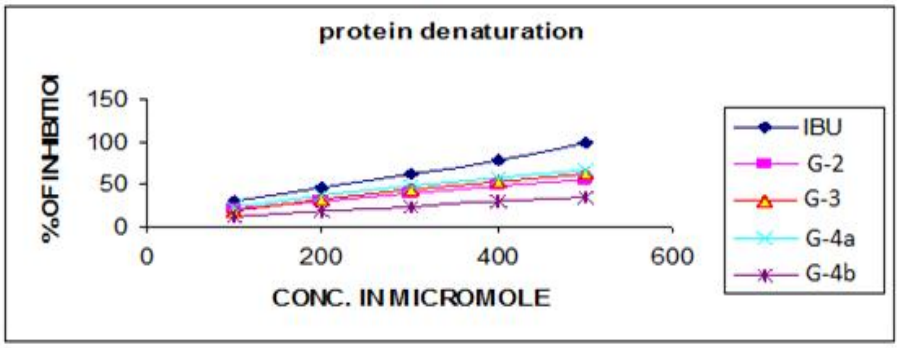

Figure 1

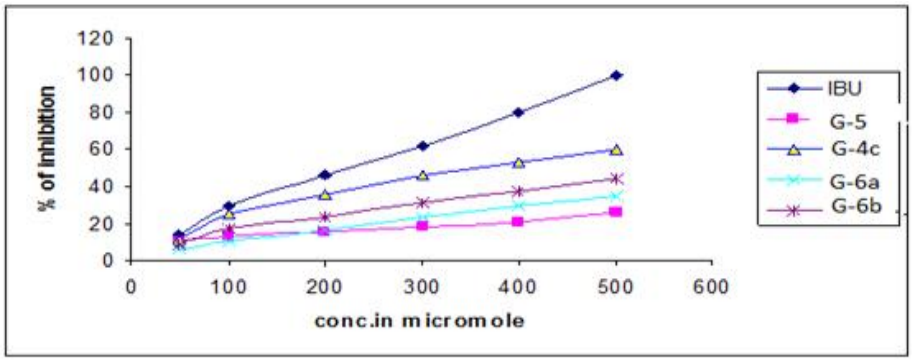

Figure 2

4. Conclusion

Meloxicam can pass through the buccal mucosa, Meloxicam patches were found to be ideal for immediate as well as delayed release of drug. The adhesive nature of the polymers was taken advantage for better delivery of the meloxicam. Mucoadhesive patches of meloxicam containing 10 mg of drug were prepared successfully using HPMC polymer for Film I and HPMC-eudragit RS 100 polymers for Film II with an intention to obtain immediate as well as delayed release of meloxicam. The *in vitro* release of meloxicam from HPMC films was about 91% in 20 minutes in Sorensen's phosphate buffers, pH 6.2. About 86% of the drug was released from HMPC-eudragit films in 20 minutes. The release kinetics indicated first order release of drug from Film I, where as zero order release from Film II. Hixon-Crowell cube root law was applied to test the release mechanism. A plot of ($M_0^{1/3}$ - $M^{1/3}$) versus time, t, gave a straight line for both HPMC (Film I) as well as HPMC-eudragit (Film II) films indicating that the release was dissolution rate limited.

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