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Synthesis and Pharmacological Evaluation of New 4-Aryl-3,4,5,6,7,8-hexahydroquinazolin-2(1*H*)-thiones.

C.M Aslak, B. Jayalakshmi*, A.Balasubramaniam and N. Senthilkumar.

Department of pharmaceutical chemistry, JKK Munirajah Medical Research Foundation, College of Pharmacy, Komarapalayam - 638 183 Tamilnadu, India

ABSTRACT

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New 4-aryl substituted quinazolin-2-thiones were synthesized and confirmed by spectral studies like IR and NMR. Then screened for antimicrobial, analgesic, anti-inflammatory and CNS depressant activities. All the derivatives were showing the pharmacological activity mainly due to the presence of basic nucleus, quinazolin-2-thione and was modified with various substituents at 4th position with different aryl substituents. Among all the substituents, the halogen substituted compounds are comparatively more active.

Keywords: Quinazolinones, cyclohexanone, chalcones, Aldol condensation, cylization.

INTRODUCTION

The quinazoline skeleton is most common in several naturally occurring alkaloids showing a wide range of biological activities which are useful in developing chemotherapeutic agents against many diseases and hence the exploration of this skeleton as privileged new chemical entities in drug discovery and research[1-2]. Quinazolinone is a building block for approximately 150 naturally occurring alkaloids isolated from a number of families of the plant kingdom, from animals, micro organisms, marine source and synthetically[3].

Interest in the medicinal chemistry of quinazoline derivatives was stimulated in the early 1950's with the elucidation of a quinazoline alkaloid. From an Asian plant *Dichroa febrifugea* which is an ingredient of a traditional Chinese herbal remedy, effective against malaria. And also Luotonine-A from *peganum nigellastrum* and 2-(4-Hydroxy butyl) quinazolin-4-one from *Dichora febrifuga,* Bouchrdatine from *Bouchardatia neurococca*[3-4].

2- Methyl-4-(3H) - quinozolinone can be obtained from the micro organism *Bacillus cereus*. The ever growing resistance to antibiotics leads to continuous screening for new biologically effective substance of natural and synthetic origin[6].

Several marine alkaloids with interesting biological activities possessing DHPM (Dihydro pyrimidinedione) nucleus have also been isolated. "Hexahydro quinazolinone" is cyclohexanone fused DHPM. Several marine alkaloids having the DHPM core unit are showing interesting biological activities such as calcium channel blockers, anti hypertensives and α –10- adreno receptor antagonists. The structurally rather simple DHPM, monastrol specifically inhibits the mitotic kinesin Eg5 motor protein and can be considered as a new lead for the development of anticancer drugs.[7]

The first quinazoline was synthesised in the late 1860's from anthranilic acid and cyanogens to give 2- Cyanoquinazoline[3-5]. As our interest in search for bioactive heterocycles, synthetically accessible heterocyclic template (Quinazoline) capable of bearing some potential pharmacophores to elicit and enhance the inherent biological activity.

Due to the availability of the quinazoline skeleton in a large variety of naturally occurring compounds, it has great importance to chemists as well as biologists. It is also found in clinically useful molecules having diverse biological activities such as antiviral, antimalarial, anticonvulsant, hypnotic, antibacterial, diuretic, hypoglycaemic, antihypertensive and antitumoral. Literature study reveals that, quinazolin-2(3H)-ones have been prepared under thermal conditions through multistep reactions[1].

MATERIALS AND METHODS

All the chemicals were of synthetic grade and commercially procured from M/S Thomsons India Scientific Company, Coimbatore. Melting points were determined in open capillary method. Purity of the compounds was checked on Silica Gel TLC plates, electronic balance used was Type-BL 220H SHIMADZU. IR spectra were recorded on FTIR-8400S SHIMADZU, the pellets were prepared in KBr press model M-15 Technosearch Instruments.

***Address for Correspondence:**

B. Jayalakshmi, Asst. Prof., Department of Pharmaceutical Chemistry, JKK Munirajah Medical Research Foundation College of Pharmacy,

Komarapalayam - 638 183, Tamilnadu, India

E-mail : bjayavasan@gmail.com

Procedure

Step I: Synthesis of 2-Benzyliden cyclohexanone (AI-EI)[12]

Cyclohexanone (0.01 mol) and an aldehyde (0.01 mol) were taken in a clean and dry reaction vessel and dissolved in methanol. To this solution alcoholic KOH was added. Then the reaction mixture was allowed to stand at room temperature for an hour with occasional shaking. A yellow crystalline solid that resulted was filtered and washed with small quantities of methanol and dried. The product was purified by recrystallization from methanol to get a yellow crystalline solid. (A1) ^{1}HNMR($CDCl_3$) 7.75(s, 1H,aliph), 7.35-7.54(m, 5H, Ar-H), 2.9-3.02(t, 2H), 1.77-1.85(t, 2H), 3.31-3.38(p, 4H) (Cyclic).

Step II:Synthesis of cyclized compounds (AII-EII) [13]

A mixture of AI (0.01 mol), thiosemi carbazide hydrochloride (0.01mol) and an alcoholic solution of KOH were taken into a reaction flask and heated under reflux for 3hr . The excess of solvent was removed by distilation. The concentrate was then diluted with cold water and cooled further. The solid mass thus resulted was filtered, washed with small portions of cold water and dried. It was purified by recrystallisation from alcohol to get colourless crystalline solid.

(AII) ^{1}H NMR($CDCl_3$), δ9.41(s, 1H), 7.2-7.8(m, 5H,Ar-H); 6.34(s, 1H, at S), 2.04(s, 2H,at 1°N) & 1.35-1.97(m, 8H) Cyclic; ^{13}C NMR δ 144.01- Aryl C-S (at C_2), 132.88--C-C=C(at 5,10), 130.87, 128.91, 127.52- Aromatic or Hetero aromatic, 77.46- C-N(at 4), 77.24,77.03,76.61- Cycloalkane (at 6,7,8,9)

Animals and Instruments used:

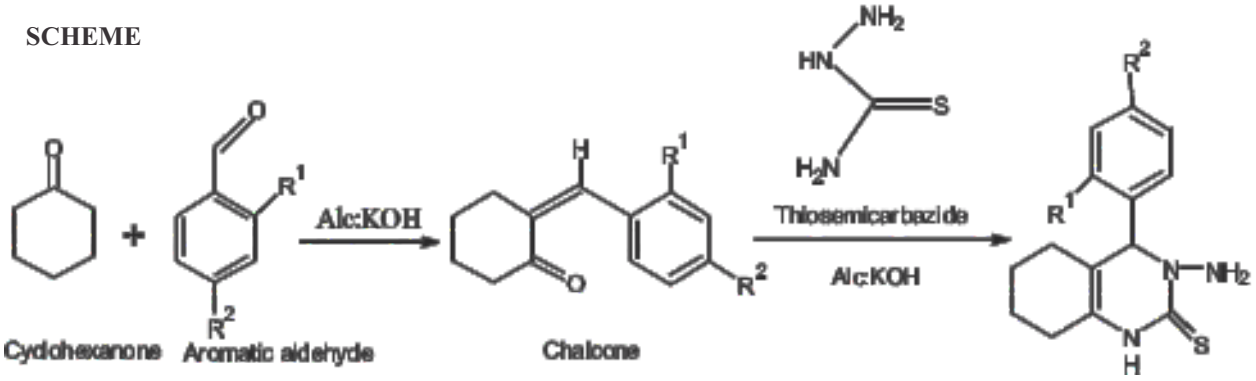
Adult Wistar rats of either sex weighing between 120-150 g were used for the study. All experimental procedures were carried out in strict accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision on experiments on Animals (CPCSEA) and were approved by the Institutional Animal Ethics Committee proposal number JKKMMRFCP/IAEC/2010/007.

Edema was produced by using Carrageenan, foot volume measured in Plethismograph. Analgesic activity was measured in Eddy's hot plate and Locomotor activity was measured in actophotometer.

Pharmacological screening

The synthesized compounds were screened for antimicrobial, analgesic, anti inflammatory and CNS depressant activity.

SCHEME



Compounds	R^1	R^2
A-II	H	H
B-II	H	OCH_3
C-II	H	Cl
D-II	Cl	H
E-II	H	$N(CH_3)_2$

The test dose for the synthesized compounds were fixed as 20mg/kg by acute oral toxicity studies. Indomethacine is taken as the standard drug for analgesic and anti inflammatory activity and Chlorpromazine as standard drug for CNS depressant activity.

Antibacterial activity

The antibacterial activity of the synthesized compounds was determined by the standard Kirby-Bauer disk diffusion method. The test organism chosen were *Escherichia coli* (ATCC25922) and *Staphylococcus aureus* (ATCC25923) the concentration of the sample compounds was 2, 5 and 10mg. Ciprofloxacin was used as standard drug. The results are reported in Table 3.

Analgesic activity by Eddy's hot plate

Adult Wistar rats of either sex weighed and numbered, the basal reaction-time was noted by observing hind paw licking or jump response (whichever appears first) in animals when placed on the hot plate maintained at constant temperature (55ºC) was taken. Drug was given by oral route to animals and noted the reaction time of animals on the hot plate at 15, 30, 60 and 90 min after the drug administration. The percent increase in reaction-time (as index of analgesia) was calculated at each time interval[8], the results are shown in Table 4.

Anti inflammatory activity by paw edema method

Adult Wistar rats of either sex with a body weight between 100 and 150g were used to study anti inflammatory activity. The animals were starved overnight. The test drug was suspended in 1%CMC (control) and given orally. Thirty minutes later, the rats were challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the plantar side of the left hind paw. The paw volume was measured plethysmographically after injection. Then the paw volume was again measured after 1h, 2h and 3h time intervals[8-10]. (Table 5)

Table 1: Physical Data of Compounds AI-EI

Compound	Mol Formula	Melting Range	% yeild	IR
A-I	$C_{13}H_{14}O$	87-90°C	72.64	3475-(OH-tautomeric) 2925.15 (Ar-CH-str), 1658.84 (-C=O), 1577.82(-C=C-str)
B-I	$C_{14}H_{16}O_2$	79-84°C	69.2	3453-(OH-tautomeric) 2925.15 (Ar-CH str), 1654.98(C=O), 1596.15(C=C str),1250.88 (-C-O-C-)
C-I	$C_{13}H_{14}OC$	I72-75°C	73.67	3449 (OH-tautomeric) 2926.11(Ar-CHstr), 1605.79(C=O),1488.13 (-C=C-str)
D-I	$C_{13}H_{14}OCl$	83-86°C	59.76	1612.54(-C=O), 2926.11(Ar-CHstr), 1464.98 (-C=C-)
E-I	$C_{15}H_{19}NO$	74-77°C	51.88	1635.69(C=O),1582.65(C=C),2927 .13(Ar-CHstr) 1283.67(-C-N-)

Recrytalisation solvent: Methanol

Table 2: Physical Data of Compounds AII-EII

Compound	Mol Formula	Melting Range	% yeild	IR
A-II	$C_{14}H_{17}N_3S$	115-118°C	61.69	3422.8(-NH_2), 3257.88(NH), 2983.01(Ar-CHstr), 1547(C=C), 1375(C=S)
B-II	$C_{15}H_{19}N_3OS$	108-112°C	67.34	3449.80(-NH_2),1171.79(-C=S), 1244.13(-C-O-C-)
C-II	$C_{14}H_{16}N_3SCl$	107-110°C	62.45	3437.26(-NH_2),3281.99(-NH), 1089.82(-C=S),
D-II	$C_{14}H_{16}N_3SCl$	117-120°C	64.48	1612.54(-C=O), 2926.11(Ar-CHstr), 1464.98 (-C=C-)
E-I	$C_{16}H_{22}N_4S$	105-107°C	53.35	3450.77(NH_2),1182.40(C=S), 1363.72(-C-N)

Recrytalisation solvent: Ethanol

Table 3: Antimicrobial activity of synthesized compounds

Compound	Zone of inhibition(mm) against E.coli		
	2mg	5mg	10mg
A-II	15	17	21
B-II	-	17	20
C-II	15	16	21
D-II	20	25	30
E-II	-	10	12

- Indicates no activity

CNS depressant activity by actophotometer

All the male Wistar rats were weighed and marked, Weight was found to be 115-155gm. The normal value of locomotor activity was determined. Then drug suspension (20mg/kg) was administered orally, After 30 minutes of administration individual rats were placed in activity cage and the reading was noted. All the readings were recorded to do the statistical analysis and the significance[11,12]. The results are shown in Table 6.

RESULTS AND DISCUSSION

In this study we have prepared new 4-aryl-3,4,5,6,7,8-hexahydroquinazolin-2(1h)-thiones. The initial step in the synthetic method involved the synthesis of five different chalcones from cyclohexanone and different substituted benzaldehyde, followed by cyclization with thiosemicarbazide. Physical properties were studied and the structures were conformed by the spectral study (IR, NMR). All the fives synthesized compounds were studied for their pharmacological activity, Analgesic, Anti inflammatory and CNS depressant activity, the results are tabulated.

Table 4: Analgesic activity of synthesized compounds

Compd Name	Before admn (paw licking)	After administration (paw licking response) 30mts	60mts	90mts
Control	9.4±1.3	9.5±1	9.4±1.34	9.4±1.34
A-II	9.3±1.34	12.0±1.56**	14.0±1.41**	13.33±1.64*
*B-II	9.3±1.33	11.3±1.33***	12.0±1.0***	11.6±1.34***
C-II	9.3±1.33	11.6±1.34**	15.66±3.19**	14.3±1.33***
D-II	8.6±1.34	12.3±1.28**	12.50±2.0***	11.3±1.33**
*E-II	8.6±1.34	10.6±1.46**	12.0±1.0***	13.0±1.58***
Control	9.4±1.3	9.5±1	9.4±1.34	9.4±1.34
Std	9.3±1.34	12.2±1.28**	15.8±1.33**	13.8±1.58***

n = 6 animals in each group: *p<0.05, ** p<0.01., ***p<0.001 when compared with control.

Table 5: Anti inflammatory activity of synthesized of compounds

Compounds	After 1 hr	After 2 hr	After 3 hr
Control	38.9±5.6	53.5±6.8	65.1±4.7
A-II	16.2±3.4**	19.9±2.4**	24.9±8.2*
B-II	17.7±4.1*	21.3±2.3*	26.6±8.1*
C-II	14.44±3.3**	17.6±1.6**	22.4±8.7**
D-II	15.2±3.1**	19.1±2.1	*24.3±7.9**
E-II	19.1±5.2*	23.3±6.7*	28.7±4.9*
Standard	14.4±3.2**	17.2±1.4**	21.4±8.4**

n = 6 animals in each group: *p<0.05, ** p<0.01., ***p<0.001 when compared with control.

Table 6: CNS depressant activity of synthesized compounds

Compd Name	Before admn	After administration 30mts	60mts	90mts	120mts	150mts	180mts
Control	151± 3.18	145± 2.42	145± 2.98	148±2.76	150± 5.68	151±1.66	151±1.62
A-II	151± 11.56	109.33±6.01**	87± 3.46***	108.6± 5.04**	118.33± 4.33*	126.33± 4.26	137.33± 7.75
B-II	136.33±3.18	95.67± 2.41***	67± 2.89***	100.33± 2.96***	111± 5.78***	124.70± 1.33	134.33± 1.67
C-II	151± 5.78	90± 1.73***	66± 6.11***	96± 1.73***	102.33± 3.84***	118 ± 3.51***	128.3± 1.45**
D-II	176.33±3.18	105± 2.08**	64± 1.51***	116.67± 2.34***	127± 1.0***	140.3± 3.48***	151± 2.89***
E-II	136± 2.89	89± 1.16**	*72.33± 2.3***	95.33± 2.19***	115.67± 2.41***	126.67± 1.20	132.33± 2.41
Std	151± 2.81	87± 1.26	60.33±2.66	93± 2.29	100.66 ± 2.81	115±1.40	128.33± 2.81

n = 6 animals in each group: *p<0.05, ** p<0.01., ***p<0.001 when compared with control.

Fig. 1: Statistical data for Analgesic activity

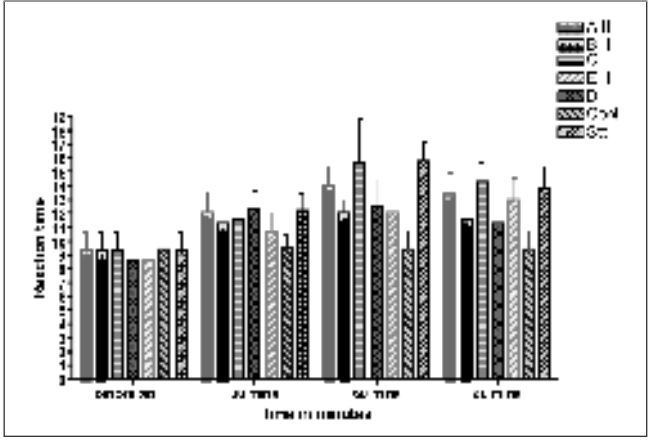


Fig. 2: Statistical data for anti-inflammatory activity

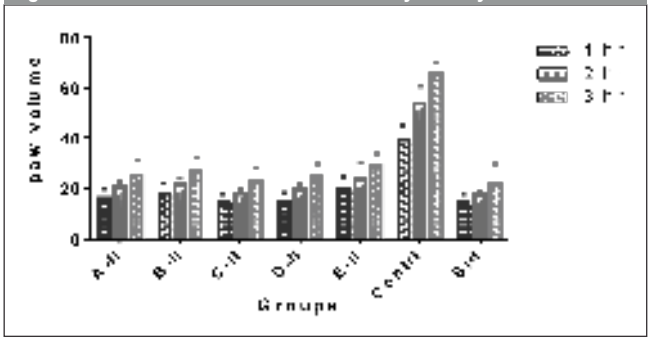
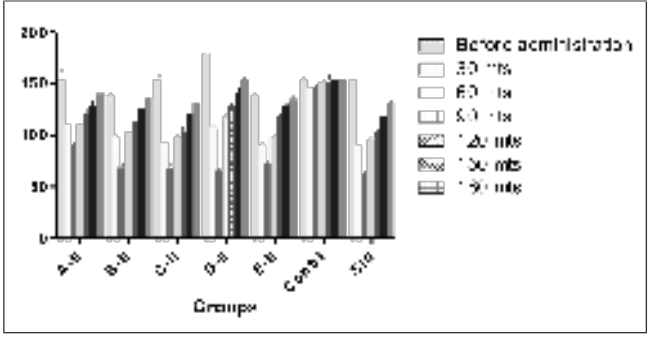


Fig. 3: Statistical data for locomotor activity



CONCLUSION

All the synthesized compounds were purified by column chromatography and they were confirmed by physicochemical and spectral analysis. Then the compounds were subjected to their antibacterial activity, and was found to be more effective against gram negative bacteria, the decreasing order was as follows, DII > AII > CII > BII > EII. The synthesized compounds were screened for analgesic, anti inflammatory and locomotor activity for its pharmacological effects by *in vivo* methods. Analgesic activity was found to be more for C-II, All compounds showing locomotor activity by CNS depression and was more for D-II. All the compounds shows anti inflammatory activity and more activity was found in C-II.

In short, all the derivatives are showing the pharmacological activity mainly due to the presence of basic nucleus, quinazolin-2-thione and was modified with various substituents at 4th position with different aryl substituents. Among all the substituents the halogen substituted compounds are comparatively more active than the others.

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