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SYNTHESIS AND BIOLOGICAL SCREENING OF NOVEL CINNOLINE DERIVATIVES FOR ANALGESIC ACTIVITY

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ABSTRACT

An attempt has been made to synthesize a series of Cinnoline derivatives by diazotization reaction of substituted anilines followed by Friedel –Craft acylation to form hydrazones and intramolecular cyclization to form cinnoline derivatives. The synthesized compounds were characterized by analytical techniques like TLC, IR, NMR spectral analysis. All the synthesized derivatives were screened for analgesic activity using acetic acid induced writhing test. Compounds S1 and S5 showed significant activity when compared to standard drug diclofenac sodium ($p< 0.01$).

KEYWORDS: Cinnoline, analgesic activity, Diclofenac sodium.

INTRODUCTION

Cinnoline is a 1,2-diazanaphthalene or benzo[c]-1,2-diazine (Hantsch-Widmann system), $C_8H_6N_2$ is a nitrogenous organic base, obtained from certain complex diazocompounds. Their system is an isosteric relative to either quinolone or isoquinoline. The synthesis of its nucleus was first carried out by V. Richter in1883, after whom this heterocyclic system is named. Cinnoline and its derivatives have received considerable interest due to their wide range of pharmacological profiles e.g. antibacterial, anti-tumor, analgesic, antifungal, anti-inflammatory, antihypertensive, anticonvulsant, anti-HIV, antianxiety, phosphodiesterase (PDE) inhibitors activities. Certain compounds of the cinnoline series have anti-thrombotic and anti-tuberculosis properties and also exhibit anaesthetizing and sedative activity, in addition to their use as agrochemicals. By considering the effectiveness and potential of this

category of compound hereby planning to synthesize some novel cinnoline derivatives and to screen them for analgesic activity.

MATERIALS AND METHODS

All the reagents and solvents used were from Central store, Pushpagiri college of pharmacy, Thiruvalla. Melting point were determined using open capillary tubes on electrical melting point apparatus and are uncorrected. IR (KBr) spectra were recorded on a Shimadzu Fourier transform infrared (FTIR) spectrometer (V_{max} in cm^{-1}). ^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker 300 MHz spectrometer using tetramethylsilane (TMS) as internal reference (chemical shift in delta ppm). Silica gel G coated plates were used for thin layer chromatography (TLC) and Ultraviolet lamp were used for visualization of thin layer chromatographic spots. ACD/Chemsketch software was used for drawing chemical structures as well as to calculate Log Pvalue.

SYNTHETIC PROCEDURE

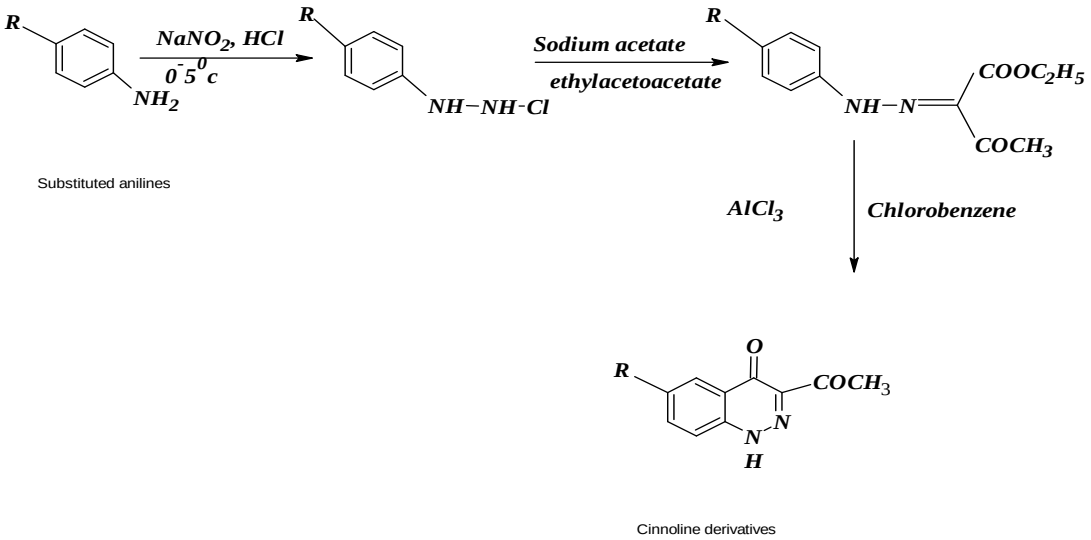
Synthesis of ethyl-3-oxo-2-(2-substituted phenyl hydrazinylidene) butanoate

Various substituted anilines (0.39 mol) were dissolved in a mixture of Concentrated hydrochloric acid (15 ml) and water (15 ml) and cooled to 0-5°C in ice bath, it was then added to a cold saturated solution of sodium nitrite (0.58 mol) with constant stirring. The diazonium salt thus formed was filtered into a cooled solution of ethylacetoacetate (0.39 mol) in ethanol and sodium acetate in water (to make it alkaline). The solid was collected and recrystallized from ethanol.

Synthesis of 3-acetyl cinnoline -4(1H)-one derivatives

To ethyl-3-oxo-2-(2-phenylhydrazinylidene) butanoate (0.01mol) was added anhydrous Aluminium chloride (0.02 mol). Chlorobenzene (30 ml) was added in order to dissolve the solids and the mixture was then refluxed for 1 hour. The complex formed was decomposed with concentrated hydrochloric acid (30 ml) and diluted with cold water. The product was filtered, washed with water, dried and recrystallized from ethanol.

SCHEME



R= 4-fluoro, 4-bromo, 2-nitro, 4-nitro, 2-chloro

SPECTRAL DATA OF THE SYNTHESIZED COMPOUNDS

3-acetyl-6-fluorocinnolin-4(1H)-one (S1)

IR (KBr cm^{-1}) :1539.6(Aromatic C=C stretching), 953.7(Aromatic C-H bending), 1333.8(C-F stretching), 1286.3 (C-N stretching), 3457.6(N-H stretching, ^{1}HNMR (300MHz, $CDCl_3$) δ ppm: 7.135-7.459 (Aromatic hydrogen), 13.929-14.122(H in NH group), 2.593(H in CH_3 group).

3-acetyl-6-bromocinnolin-4(1H)-one (S2)

IR (KBr cm^{-1}): 1534.2 (Aromatic C=C stretching), 3132.6 (Aromatic C-H bending), 3401.6 (N-H stretching), 610.2 (C-Br stretching), 1690.1 (C=O stretching), 1273.0 (C-N stretching), ^{1}HNMR (300MHz, $CDCl_3$) δ ppm: 7.322-7.592(Aromatic hydrogen), 13.865-14.056(H in NH group), 2.596 (H in CH_3 group).

3-acetyl-8-nitrocinnolin-4(1H)-one (S3)

IR (KBr cm^{-1}): 1575.2 (Aromatic C=C stretching), 734.94(Aromatic C-H bending), 1337.7 (N-O stretching), 1318.0 (C-N stretching), 3467.5 (N-H stretching), ^{1}HNMR (300MHz, $CDCl_3$) δ ppm: 7.17-7.79(Aromatic hydrogen), 13.911-15.274 (H in NH group), 2.672 (H in CH_3 group).

3-acetyl-6-nitrocinnolin-4(1H)-one (S4)

IR (KBr cm^{-1}): 1539.6(Aromatic C=C stretching), 850.7(Aromatic C-H bending), 1607.0(N-O stretching), 1690.0 (C=O stretching), 1211.0 (C-N stretching), 3467.2 (N-H stretching),

^{1}HNMR (300 MHz, $CDCl_3$) δ ppm: 7.272-8.333(Aromatic hydrogen), 12.713(H in NH group), 2.622 (H in CH_3 group).

3-acetyl-8-chlorocinnolin-4(1H)-one (S5)

IR (KBr cm^{-1}): 1547.5 (Aromatic C=C stretching), 953.71(Aromatic C-H bending), 609.3(C-Cl stretching), 1298.1(C-N stretching), 3492.3(N-H stretching), ^{1}HNMR (300 MHz, $CDCl_3$) δ ppm: 7.175-7.981(Aromatic hydrogen), 13.749-14.342(H in NH group), 2.619(H in CH_3 group).

STATISTICAL ANALYSIS

Values are represented as mean ± standard error mean for groups of six animals. The results were analysed by One-way analysis of varience (ANOVA) followed by Dunnett's multiple comparison test. Difference between groups were considered significant at $p<0.01$ levels. The statistical analysis was carried out using Graph Pad Instat 3.10 software.

ACUTE ORAL TOXICITY STUDIES

Acute oral toxicity study of the synthesized compounds was conducted as per OECD guidelines 423 in wistar rat. The compound showed toxic effects at a dose of 2000 mg/kg so the safe dose of the drug is 300 mg/kg. So, $1/5^{th}$ dose i.e., 60 mg/kg was selected for in-vivo screening studies.

ANIMALS USED FOR THE STUDY

Albino mice were used to carry out the activities. The animals had free access to standard commercial diet and water *ad libidum* and were housed in cages under standard laboratory conditions i.e, 12; 12-hour light/dark cycle at 25±2°C. Humidity should be at least 30% and preferably not exceed 70%. The experiments were carried out as per the guidelines of CPCSEA, New Delhi, India and approved by the Institutional Animal Ethical Committee (IAEC) (Re.No.IAEC No. PCP/IAEC/2018-1/5).

EXPERIMENTAL PROCEDURE

The method used to determine the analgesic activity was based on acetic acid induced writhing in mice (Rajiv. K. Tonk *et al*, 2012). Swiss albino mice (35-40 g) were divided in seven groups containing six animals in each group (one standard, one control and five test groups). The test as well as the standard were administered orally by suspending the compounds in tween 80. The standard was used Diclofenac sodium (10 mg /kg). The control

group was treated orally with vehicle 1% w/v tween 80. After 1 hr, the animals were given an intraperitoneal injection of 0.6% w/v solution of acetic acid (1 ml/100g body weight) as writhing inducing agent. The number of writhings were counted for 10 min for each animal of a group (n=6) after acetic acid injection. Analgesic activity was measured as percentage decrease in writhings (% protection) in comparison to control. The percentage protection was calculated using the formula.

Percentage protection (%)= [1-(number of writhings in test/number of writhings in control)] *100.

RESULTS AND DISCUSSION

Table No. 1: Physicochemical parameters of the synthesized cinnoline derivatives.

Sample code	State	Color	Molecular Formula	Molecular Weight (g/mol)	M.P (°C)	Yield % w/w	R_f Value *		Log P value
							Intermediate compound	Final cinnoline derivative	
S1	Solid Powder	Brown	$C_{10}H_7N_2O_2F$	206.173	137	64.5	0.58	0.63	0.38±0.68
S2	Solid Powder	Light Yellow	$C_{10}H_7N_2O_2Br$	267.079	204	70.4	0.49	0.53	1.10±0.68
S3	Solid Powder	Light Yellow	$C_{10}H_7N_3O_4$	233.18	101	72.8	0.11	0.14	0.58±0.66
S4	Solid Powder	Dark Yellow	$C_{10}H_7N_3O_4$	233.18	120	75.4	0.1	0.12	0.06±0.91
S5	Solid Powder	Brown	$C_{10}H_7N_2O_2Cl$	222.628	220	84.6	0.48	0.55	0.84±0.65

*Mobile phase – Benzene: ethyl acetate-8:2

By Acetic acid induced writhing method

Table No.2: Analgesic property of S1-S5 using Acetic acid induced writhing.

Sl.No.	Sample	Number of Writhing	Percentage inhibition
1.	S1	35.16±0.65**	51.94%
2.	S2	67.33±0.84	7.96%
3.	S3	65.16±0.83	10.93%
4.	S4	56.16±0.60	23.23%
5.	S5	25.5±0.61**	65.14%
6.	Control	73.16±1.621	Nil
7.	Standard (Diclofenac sodium 10mg/Kg)	14.33±0.76**	80.41%

Each value represents Mean ± SEM, n = 6, ** = p< 0.01 vs control (One- way ANOVA followed by Dunnett's test).

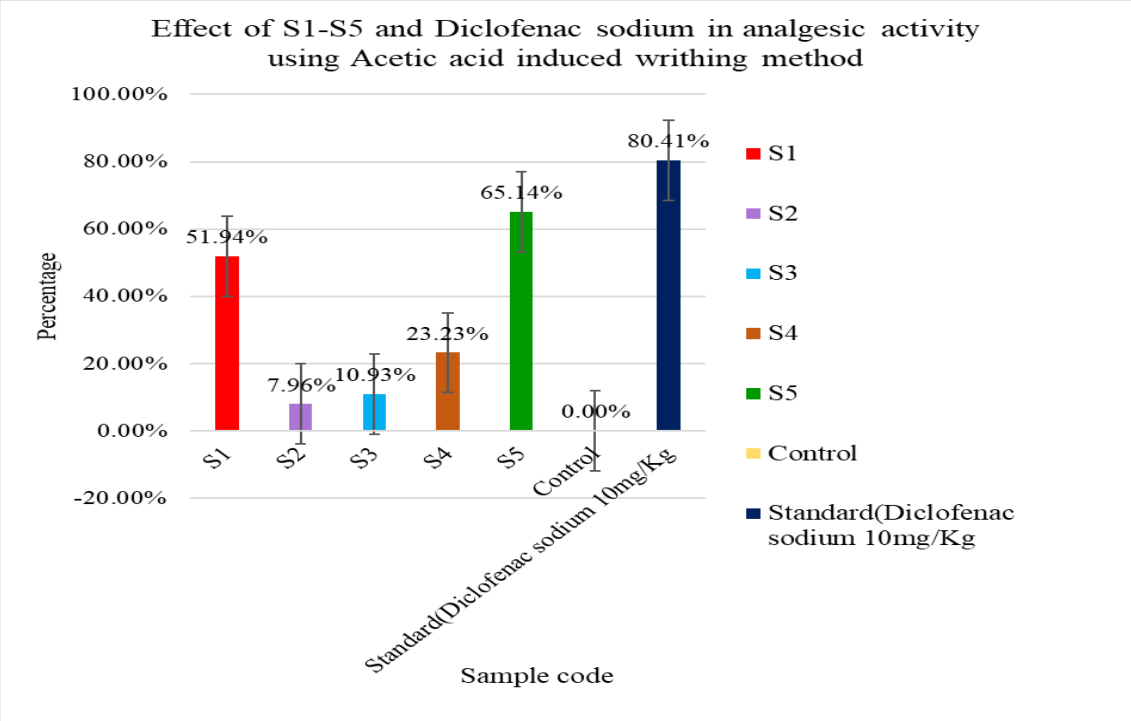


Figure No. 1: Effect of S1-S5 and Diclofenac sodium in analgesic activity using Acetic acid induced writhing.

Cinnoline derivatives were prepared by diazotization method by reacting various substituted anilines with a mixture of Conc.Hcl and water and cooled to 0-5°C in ice bath and it was then added to a cold saturated solution of Sodium nitrite. The diazonium salt was then treated with ethyl acetoacetate and then refluxed with chlorobenzene and aluminium chloride and further acid decomposition with Conc.HCl and dilution with cold water yields the product. Further recrystallized from ethanol. Cinnoline derivatives yield was found to be in the range of 64.5-84.6% w/w. Structures of the synthesized compounds were confirmed by measuring the physicochemical properties, IR and NMR spectral datas. R_f values of the synthesized compounds were in the range of 0.11-0.63 which indicates the purity of the compounds. Log P value of the synthesized cinnoline derivatives were found by ACD/ChemSketch software. The novel cinnoline derivatives were screened for analgesic activity by screening model-acetic acid induced writhing method. After administration of Cinnoline derivatives, it reduces the pain perception. Cinnoline derivatives showed significant decrease in pain perception by acetic acid induced writhing method. In this model Compounds S1(3-acetyl-6-fluorocinnolin-4(1H)-one) and S5 (3-acetyl-8-chlorocinnolin-4(1H)-one) showed significant activity when compared to standard drug Diclofenac sodium (10mg/kg). But compounds S2, S3, S4 showed least activity.

CONCLUSION

Analgesic activity of the synthesized compounds was evaluated by acetic acid induced writing model. The activity was studied at the dose level of 60 mg/kg body weight and their effects were recorded. The number of writhings observed were recorded in the Table No.2. It is observed that the compounds S1 and S5 containing Fluorine and Chlorine substitutions attached to the cinnoline nucleus showed significant analgesic activity and this may be due to the electron withdrawing nature of the substituents. The obtained results establish the fact that cinnoline can be used as a rich source for further development in medicinal field by fusing with different substituents.

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