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Original Article

DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL CINNOLO PIPERAZINE DERIVATIVES

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ABSTRACT

Objective: To design and synthesize a series of substituted 4-(p-amino piperazine) cinnoline-3- carboxamide derivatives and evaluate for anti-microbial activity.

Method: A novel series of substituted 4-(p-amino piperazine) cinnoline-3- carboxamide (4a-g) derivatives were synthesized by reacting substituted 4-amino cinnoline 3-carboxamide (3a-g) with DMF and o-chloro piperazine. Substituted 4-amino cinnoline 3-carboxamide (3a-g) were synthesized by reaction of substituted phenyl hydrazono cyano acetamide (2a-g) with anhydrous $AlCl_3$ and chlorobenzene in nitrogenous environment. Substituted phenyl hydrazono (cyano) acetamide was synthesized by reaction of substituted aniline diazonium chloride (1a-g) with CH_3COONa and ethanol. Substituted aniline diazonium chloride were synthesized by substituted aniline with conc HCl. and sodium nitrite .The synthesized compounds were characterized by IR, NMR and Mass spectral data. The synthesized compounds were screened for their antibacterial and antifungal activity against 4 pathogenic bacteria and 2 pathogenic funguses.

Results: The compound 4a, 4c and 4g shows potent antimicrobial activity in comparison to standard drugs while other compounds showed moderate activity. Further all the compounds are obtained in good purity.

Conclusion: All the compounds synthesized were checked for their purity and spectral analysis shows their structural confirmation. some compounds shows potent antimicrobial activity.

Keywords: Cinnoline derivatives, Anti-bacterial, Anti-fungal.

INTRODUCTION

The main objective of organic and medicinal chemistry is the synthesis, characterization and pharmacological evaluation of molecules having highly therapeutic and efficacy in nature. Now a days increasing the resistance of many organisms, we have to synthesized the more active new molecules against the resistance microbes, particularly the bacteria, virus and fungus is the major area in the antimicrobial research. The aim of this research is to synthesize and characterize the biological active molecule for resistant bacteria and fungus [1]. The substituted cinnoline derivatives were reported for various pharmacological activities including antimicrobial, insecticidal [2], antitumor, and antifungal [3].

Microbial development of resistance, as well as economic incentives, has resulted in research and development in the search for new antibiotics in order to maintain a pool of effective drugs at all times. While the development of resistant strains is inevitable, the slack ways that we administer and use antibiotics has greatly exacerbated the process [4].In spite of the large number of antibiotics and chemotherapeutics available for medical use, the emergence of old and new antibiotic resistant bacterial strains in the last decades constitutes a substantial need for the new class of antibacterial agents [5].

The progress achieved in the synthesis of heterocyclic compounds with biological potential is due to improvement of methodological study of tested substances too. The progress achieved in the synthesis of heterocyclic compounds with biological potential is due to improvement of methodological study of tested substances too. Cinnoline ring is a versatile lead molecule [5] that has been investigated widely in medicinal chemistry due to its important pharmacological activities.

The nucleus gives out different biological activities as anti-microbial, anti-tubercular, anti-malarial, anti-hypertensive, antidepressant, anti-pyretic, analgesic, anesthetic etc. [6-10]

MATERIALS AND METHODS

All the melting points were determined by open capillary method and are uncorrected. The purity of compound was monitored by TLC on silica gel coating aluminium plate using U.V. light as visualizing agent. The I.R. spectra (KBr in cm^{-1}) were recorded on Perkin-Elmer Spectrophotometer in the range of 4000-400 cm^{-1}. The $_1H$ NMR Spectra were recorded on Varion 500 MHz NMR Spectrophotometer using DMSO-4f as a solvent and TMS as an internal standard (chemical shift in δ ppm. Mass spectra were obtained by MS (EI) JEOL GC MATE700 EV spectrometer.

Experimental procedure

Synthesis: Substituted Cinnolo piperazine were synthesized by three steps:

(1) synthesis of substituted phenyl hydrazono (cyno) acetamide (2a-g) then (2) synthesis of substituted 4-amino cinnoline-3-carboxamide (3a-g) and finally (3) synthesis of substituted 4-(p-amino piperazine) Cinnoline -3-carboxamide (4a-g):

General procedure for the preparation of substituted phenyl hydrazono (cyano) acetamide (2a-g):

The substituted aniline (0.195 mole) was dissolved a mixture of conc HCl (7.5ml), water (7.5ml) and cooled to 0 to 5 °C in an ice bath. To this a cold saturated solution of sodium nitrite (0.19 mole) was added slowly. Soon after the addition, the fumes of nitrous acid were liberated; a pinch of sulphamic acid / thiourea is added, stirred till the fumes were ceased. The diazonium salt thus formed was filtered in to a cooled solution of cyano acetamide (0.195 mole) in water (350ml) 10 gm CH_3COONa and 15 ml alcohol. The mixture was kept stirring up to 6 hrs at room temperature; the solid was collected and recrystallized from methanol. 1_{a-g} 1-a M.P. 150°C (76.18%),R_f value 0.72, 1b- M.P. 182 ^{0}C (68.42%) R_f value 0.65, 1c - M.P. 164°C (72.16%) R_f value 0.54. 1d -M.P. 174°C (79.24%) R_f value 0.45, 1e-M.P. 150°C (70.28%) R_f value 0.62, 1f- M.P. 174°C (76.32%) R_f value 0.71, 1g- M.P. 132°C (80.22%) R_f value 0.53.

General procedure for the preparation of substituted phenyl 4-amino cinnoline 3-carboxamide (3a-e)

To the anhydrous $AlCl_3$ (0.111mole) chlorobenzene 150 ml was added and nitrogen gas was passed for half an hour. This mixture was added to the substituted phenyl hydrazono cyano acetamide then nitrogen was passed for 10 min, the mixture was then refluxed for 2hrs. It was cooled; dilute HCl (20ml) was added to it. It was then heated on water bath cooled, filtered, washed twice with dilute NaOH solution and filtered. The product was recrystallized from methanol, water 10:1.

$2_{a\text{-}g.}$ 2-a M.P. 216°C (67.18%) R_f value 0.65, 2b- M.P. 225^{0}C (70.28%) R_f value 0.71, 2c - M.P. 220°C (72.18%) R_f value 0.50, 2d -M.P. 222°C (74.26%) R_f value 0.48, 2e-M.P. 186°C (69.28%) R_f value 0.64, 2f-M.P. 227°C (71.56%) R_f value 0.67, 2g- M.P. 217°C (73.22%) R_f value 0.47.

Synthesis of substituted 4-(p-amino phenyl piperazine) Cinnoline -3-carboxamide (4a-g)

Substituted 4-amino cinnoline –3-carboxamide (0.05mole) was taken in 25 ml of DMF and o-chloro piperazine (0.01 mole) was added and refluxed for 2hrs. The mixture was poured in to the crushed ice and filtered, and recrystallised with alcohol.

4c 6-chloro-4-(p-amino piperazine) Cinnoline -3-carboxamide

$C_{13}H_{15}ClN_6O$;306.8, Yield 68.26%; M.P.190^{0}C,R_f value 0.50. IR (KBr) (cm-1) 1640 (N-H), 830(C-N), 2450 (N-H aliphatic), 1400 (C-C), 1700 (C=C),730(C-Cl), 1576 (N=N), 840(C-N) 1H- NMR(DMSO- 4f) (shift in ppm), δ 7.52-7.72(3H,m,ArH), 5.98(2H,s,NH_2),4.0(1H,s,NH),2.62-2.98(6H,m,3CH_2),2.0 (2H,s,NH),3.99(1H,s,CH). MS (ESI) m/z calculated for ($C_{13}H_{15}Cl\ N_6O$) 306.1(100%).

4b 8-chloro-4-(p-amino piperazine) Cinnoline -3-carboxamide

$C_{13}H_{15}ClN_6O$; 306.8, Yield 69.27%; M.P.172^{0}C M.P.190^{0}C,R_f value 0.50, IR (KBr) (cm-1) 1610 (N-H), 815(C-N), 2415 (N-H aliphatic), 1425 (C-C), 1750 (C=C),715 (C-Cl), 1555 (N=N), 865(C-N) 1H- NMR(DMSO- 4f) (shift in ppm), δ 7.52-7.70(3H,m,ArH), 5.97(2H,s,NH_2),4.0(1H,s,NH),2.62-2.98(6H,m,3CH_2),2.0 (2H,s,NH),3.99(1H,s,CH). MS(ESI) m/z calculated for ($C_{13}H_{15}Cl\ N_6O$) 306.1(100%).

4c 7-chloro-4-(p-amino piperazine) Cinnoline -3-carboxamide

$C_{13}H_{15}ClN_6O$; 306.8, Yield 66.60%; M.P.250^{0}C,R_f value 0.56, IR (KBr) (cm-1) 1632 (N-H), 824(C-N), 2425 (N-H aliphatic), 1420 (C-C), 1710 (C=C),715(C-Cl), 1545 (N=N), 342(C-N), 1H- NMR(DMSO- 4f) (shift in ppm), δ 8.25-7.70(3H,m,ArH), 5.97(2H,s,NH_2),4.0(1H,s,NH),2.66-2.98(6H,m,3CH_2),2.0 (2H,s,NH), 3.99 (1H,s,CH). MS (ESI) m/z calculated for ($C_{13}H_{15}Cl\ N_6O$) 306.1(100%).

4e 6-bromo-4-(p-amino piperazine) Cinnoline -3-carboxamide

$C_{13}H_{15}BrN_6O$; 351.2, Yield 67.86%; M.P.246^{0}C, Rf value 0.70, IR (KBr) (cm-1) 1626 (N-H), 834(C-N), 2434 (N-H aliphatic), 1420 (C-C), 1740 (C=C),732(C-Br), 1560 (N=N), 846(C-N) 1H- NMR(DMSO- 4f) (shift in ppm), δ 7.73 (3H,m,Ar-H), 5.97(2H,s,NH),2.62-2.98(6H,m,CH_2),2.0(2H,s,NH), MS (ESI) m/z calculated for ($C_{13}H_{15}BrN_6O$) 350 (100%)

4E **8-bromo-4-(p-amino piperazine) Cinnoline -3-carboxamide**

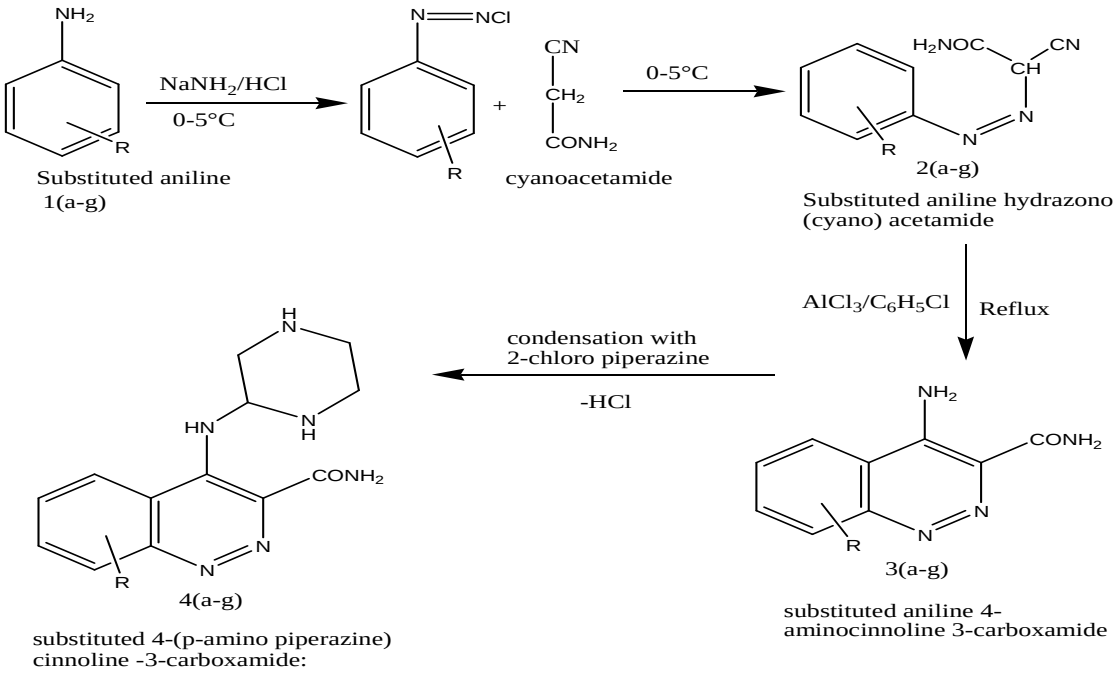
$C_{13}H_{15}BrN_6O$; 351.2, Yield 72.16%; M.P.240^{0}C,R_f value 0.54, IR (KBr) (cm-1) 1654 (N-H), 812(C-N), 2423 (N-H aliphatic), 1437 (C-C), 1740 (C=C),750(C-Br), 1576 (N=N), 840(C-N) $_1$H- NMR(DMSO- 4f) (shift in ppm), δ 7.73 (3H,m,Ar-H), 5.97(2H,s,NH),2.62-2.98(6H,m,CH_2),2.0(2H,s,NH), MS (ESI) m/z calculated for ($C_{13}H_{15}BrN_6O$) 350 (100%)

4f 6,7,8- bromo-4- (p-amino piperazine) Cinnoline -3-carboxamide

$C_{13}H_{13}Br_3N_6O$; 509.8, Yield 70.86%; M.P.228^{0}C, Rf value 0.54, IR (KBr) (cm-1) 1640 (N-H), 824(C-N), 2450 (N-H aliphatic), 1410 (C-C), 1730 (C=C),755 (C-Br), 1576 (N=N), 854(C-N) $_1$H- NMR(DMSO- 4f) (shift in ppm), δ 7.73(H,s,Ar-H), 5.97(2H,s,NH_2), 4.0(2H,s,NH), 2.62-2.98 (6H,m,CH_2), 2.0(2H,s,NH) MS (ESI) m/z calculated for ($C_{13}H_{13}Br_3N_6O$) 350 (100%)

4g 7-bromo-4-(p-amino piperazine) Cinnoline -3-carboxamide

$C_{13}H_{15}BrN_6O$; 351.2, Yield 72.16%; M.P.198 ^{0}C,R_f value 0.38, IR (KBr) (cm-1) 1646 (N-H), 823(C-N), 2450 (N-H aliphatic), 1420 (C-C), 1748 (C=C),735(C-Br), 1556 (N=N), 847(C-N) $_1$H- NMR(DMSO- 4f) (shift in ppm), δ 7.73 (3H,m,Ar-H), 5.97(2H,s,NH),2.62-2.98(6H,m,CH_2),2.0(2H,s,NH), MS (ESI) m/z calculated for ($C_{13}H_{15}BrN_6O$) 350 (100%)



SCHEME

Where R= o-Chloro (4a), p-chloro (4b), m-Chloro (4c), o-bromo (4d),p-bromo (4e), 2,4,6 tribromo (4f), m-bromo (4g)

Antimicrobial activity

The *In-Vitro* anti-microbial screening of the newly synthesized substituted 4-(p-amino piperazine) Cinnoline -3-carboxamide (4a-g) was carried out against Gram-positive organisms *Bacillus subtilis* (AL009126) and *Staphylococcus aureus* (BX571856) and two gram-negative bacteria: *Escherichia coli* (AE014075) and *Pseudomonas aeruginosa* (AE004091) were used to investigate the antibacterial activity and *Aspergillus niger* (CM000170) and *Candida albicans* (EAL02784) for the antifungal activity of the compounds by conventional tube dilution method [12] and compared with that of the standard drugs Norfloxacin and Griseofulvin respectively. MIC of each drug was defined as the lowest concentration that produces no visible turbidity after incubation time. Dilutions of each drug were prepared with tube dilution techniques for MIC. A stock solution of drug with concentration 1000 µg/10µl was prepared in DMF. The tubes were incubated for 24 hrs and observed for turbidity. The antimicrobial activity of the compounds **(4a-g)** is given in table-1-4. The antimicrobial activity was assayed biologically using disk diffusion method. In this method, wells of standard diameter were made in the nutrient agar medium for the antibacterial and antifungal activity respectively containing standard microbial inoculums. The test compounds were introduced into the wells and diameter of the zone of inhibition was measured by antibiotic zone reader. The test was carried out in triplicates and average value of these as a zone of inhibition. The final values of the compounds were tested at a concentration of 25 µg/ml and 50 µg/ml in dimethyl formamide as control against all the organisms.

Norfloxacin and griseofulvin were used as standards for comparison of antibacterial and antifungal activities, respectively at the same concentration as samples taken. The zone of inhibition was compared with the standard drugs after 24 h of incubation at 37 °C for antibacterial activity and 72 h at 25° C for antifungal activity.

Table 1: *In -Vitro* anti-bacterial activity data in zone of inhibition (mm) by disc diffusion method

S. No.	Micro- organisms	Zone of inhibition (mm) compounds (10 µg/disc)							Standard (Norfloxacin)
		4A	4B	4C	4D	4E	4F	4G	
1	*Bacillus subtilis*	21	13	31	14	10	7	18	30
2	*Staphylococcus aureus*	29	-	29	14	17	-	22	35
3	*Escherichia coli*	19	16	23	18	15	9	18	22
4	*Pseudomonas aeruginosa*	20	18	21	16	-	6	18	22

Table 2: *In -Vitro* anti-bacterial activity data in Minimal Inhibitory Concentration (MIC)

S. No.	Micro-organisms	MIC value (µg/ml)						
		4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**
1	*Bacillus subtilis*	25	25	50	25	50	12.5	25
2	*Staphylococcus aureus*	25	25	25	25	25	12.5	25
3	*Escherichia coli*	25	50	25	12.5	25	25	25
4	*Pseudomonas aeruginosa*	25	25	50	12.5	50	25	25

Table 3: *In -Vitro* anti-fungal activity data in zone of inhibition (mm) by disc diffusion method:

S. No.	Micro-organisms	Zone of inhibition (mm) compounds (10 µg/disc)							Standard (Griseofulvin)
		4a	4b	4c	4d	4e	4f	4g	
1	*Aspergillus niger*	25	12	26	19	18	-	25	30
2	*Candida albicans*	24	21	24	17	16	8	22	28

Table 4: *In -Vitro* anti-fungal activity data in Minimal Inhibitory Concentration (MIC):

S. No.	Micro-organisms	MIC value (µg/ml)						
		4a	4b	4c	4d	4e	4f	4g
1	*Aspergillus niger*	50	25	50	25	25	12.5	25
2	*Candida albicans*	25	50	25	25	50	25	25

RESULTS AND DISCUSSION

Interaction of substituted aniline with sodium nitrite with the presence of conc. HCl gives diazonium salt (2a-g) which was then reacted with chloro benzene and anhydrous $AlCl_3$ to give substituted aniline 4-amino cinnoline 3-carboxamide (3a-g). This substituted 4-amino cinnoline –3-carboxamide was reacted with DMF and o-chloro piperazine to form substituted 4-(p-amino piperazine) Cinnoline -3-carboxamide (4a-g). The physical parameters like molecular weight, solubility, melting point, and R_f of the synthesized compounds were determined. The melting points of the synthesized compounds determined and were uncorrected, and melting range was found to be between, 130-282°C.The purity of the synthesized compounds was established by single spot on the TLC Plate and all the compounds found to be pure. The structures of the synthesized compounds were confirmed by IR, NMR, and mass spectral analysis.

All the Mass spectra of the synthesized compound were found to be corresponding with its m/z mass. The results from antibacterial studies could be seen that all the synthesized derivatives showed moderate to good activity against the used gram positive and gram negative bacteria. Compound 4a, 4c, 4g demonstrated moderate to good activity against *Bacillus subtilis, Escherichia coli, Staphylococcus aureus and. Pseudomonas aeruginosa* with zone of inhibition (6-29 mm). The MIC of the synthesized compounds against *Bacillus subtilis, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa*. determined by tube assay method was found to be in the range of 12.5-50 µg/ml. Table 1 & 2. Evaluation of the results from anti-fungal studies showed that synthesised substituted Cinnolo piperazine derivatives exhibits moderate to good anti-fungal activity against, *Aspergillus niger*, *Candida albicans,* with zone of inhibition (8-25mm).The MIC of the synthesized compounds against

Aspergillus niger, *Candida albicans,* determined by tube assay method, was found to be in the range of 12.5-50 µg/ml. Table 3 & 4.

CONCLUSION

Some novel cinnoline derivatives were synthesized with the aim to get better yield and faster reaction and to get more potent drug for the treatment of microbial infectious diseases. Further studies on its possible mechanism and *in vivo* trials in experimental animals to broaden their Pharmacological assessment, may provide a new analogue that can overcome drug resistance, prolonged treatment, complex drug regimen and side effects involved in the treatment of infectious diseases [13].

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