

Synthesis, characterization and biological activities of substituted cinnoline culphonamides

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Abstract

Background: The Cinnoline moiety and Sulphonamide moiety both have good antimicrobial properties.

Methods: In the present study both the moieties were condensed to synthesize substituted cinnoline sulphonamide derivatives using intramolecular cyclization followed by diazotization in order to get synergistic activity.

Results: These derivatives particularly halogen substituted cinnoline derivatives showed potent antimicrobial activity.

Conclusion: Further investigations are required to find out possible mechanism of action.

Keywords: Cinnoline, Antibacterial, Antifungal

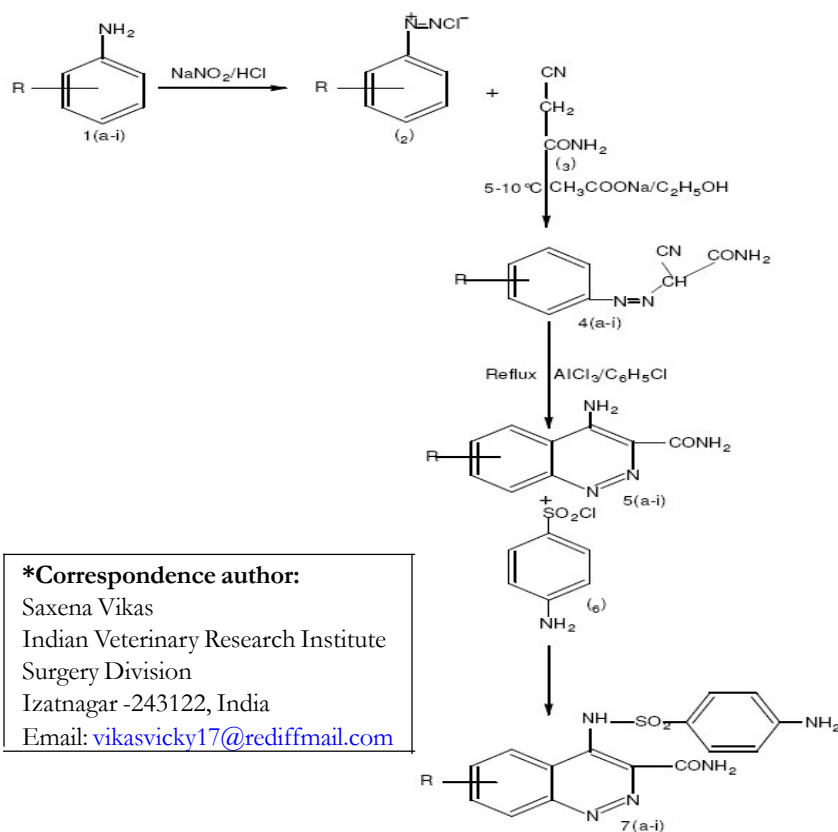
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Introduction

A simple and good yielding method for the synthesis of substituted cinnoline sulphonamide derivatives by the condensation of p-amino-benzene sulphonyl chloride with various substituted 4-amino cinnoline

3-carboxamides which was obtained by the intramolecular cyclization of the hydrazone which in turn obtained by coupling with cyanoacetamide in aqueous ethanolic solution containing sodium acetate followed by diazotisation. They were characterized by spectral data. All the synthesized compounds were screened for their anti-microbial activity. Among the compounds tested Bromo and Chloro substituted products showed significant antimicrobial activity.

Figure 1: Scheme



[In 1 (a-j) R : a = o-nitro, b = p-chloro, c = o-methyl, d = p-nitro, e = p-bromo, f = 3,4-dinitro, g = m-chloro, h = o-fluoro, i = 2,3-di-chloro]

Materials and Methods

All the reactions were carried out under prescribes laboratory conditions. The products were purified by recrystallisation. Melting points were determined in open capillaries and are uncorrected. Purity of the compounds was verified by pre coated TLC plates (E. MERCH kieselgel 60 F254). IR spectra were recorded using KBr pellets on a NICOLETT-IMPACT-400 CDCl₃ Spectro- photometer. ¹H NMR on a JEOL-JMS D-300 spectrometer in using TMS as internal standard and mass spectra were recorded on SHIMADZU GC-MS at 70ev

spectrometer. The new series of title, compounds were synthesized diazotization of substituted anilines 1a-i followed 2 by coupling with cyanoacetamide 3 in aqueous ethanolic solution containing sodium acetate, afforded the corresponding hydrazone 4a-i. Intramolecular cyclization of the hydrazone with anhydrous AlCl₃ in chlorobenzene resulted in the formation of substituted phenyl 4-amino cinnoline 3-carboxamide 5a-i. Crude p-acetamido benzene sulphonyl chloride was treated with dilute ammonia followed by hydrolysis. P-amino benzene sulphonyl chloride 6 obtained was refluxed with DMF and substituted 4- amino cinnoline -3-carboxamide, resulted in the formation of substituted 4-(p-amino phenyl sulphonamide) cinnoline 3-carboxamide 7a-i (Scheme-1). Physical data of final products is given in Table-1.

Table 1: Physical data of compounds prepared

Compound	R ₁	R ₂	Physical state	M.P (°C)	Yield (%)	Mol Formula (M.wt)
7a	NO ₂	H	Pale green crystals	192	68	C ₁₅ H ₁₂ N ₆ SO ₅
7b	Cl	H	Creamish white crystals	282	66	C ₁₅ H ₁₂ ClN ₅ SO ₃
7c	CH ₃	H	White crystals	252	66	C ₁₆ H ₁₅ N ₅ SO ₃
7d	H	NO ₂	Creamish white crystals	248	67	C ₁₅ H ₁₂ N ₆ SO ₅
7e	H	Br	Pale brown crystals	242	79	C ₁₅ H ₁₂ BrN ₅ SO ₃
7f	NO ₂	NO ₂	Pale yellow crystals	232	65	C ₁₅ H ₁₁ NaN ₆ S ₂ O ₈
7g	Cl	H	Pale orange crystals	230	69	C ₁₅ H ₁₂ ClN ₅ SO ₃
7h	F	H	Pale yellow crystals	219	70	C ₁₅ H ₁₁ FCIN ₅ SO ₃
7i	Cl	Cl	Pale yellow crystals	198	70	C ₁₅ H ₁₁ Cl ₂ N ₆ SO ₅

Synthesis of substituted phenyl hydrazono (cyano) acetamide (4a-i)

The substituted aniline (0.195 mole) was dissolved a mixture of conc Hcl (7.5ml) and water (7.5ml) and cooled to 0° to 5° c in an ice bath. To this a cold saturated solution of sodium nitrite (0.19mole) 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 containing cyanoacetamide (0.195 mole), water (350ml), 10 gm of CH₃COONa and 15 ml of alcohol. The mixture was kept stirring up to 6 hrs at room temperature, the solid was collected and recrystallized from methanol.

Synthesis of substituted 4-amino cinnoline 3-carboxamide (5a-i)

To the anhydrous AlCl₃ (0.111mole) the chlorobenzene 150ml 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.

Synthesis of p-aminobenzene sulphonyl chloride⁶

Transferred 20 gm of crude p-acetamido benzene sulphonyl chloride to a 500ml flask added 10ml of conc Hcl and 30ml of water. Boiled the mixture gently under reflux for 30-45min. The solution, when cooled to room temperature no solid amide was deposited, if a solid separated, heated for a further short period. Treated the cooled solution with 2 gm of activated charcoal, heated the mixture to boiling, and filtered under suction through a hardened filter paper. Place the filtrate (a solution of p-amino benzene sulphonyl chloride) in a litre beaker and

cautiously added 16 gm (approximately) of solid sodium bicarbonate in portions with stirring. After the evolution of gas was subsided, tested the suspension with litmus paper and if it is still acid added more sodium bicarbonate until neutral. Cooled in ice, filtered off the paminobenzene sulphonyl chloride under suction and dried. Recrystallised from water.

Synthesis of substituted 4-(p-amino phenyl-sulphonamide) cinnoline –3-carboxamide (7a-i)

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

Antimicrobial activity

The synthesized derivatives were screened for antibacterial and antifungal activity using disk diffusion

method (Modified Kirby-Bauer Method). Muller-Hinton broth and Sabouraud's agar media were used as growth media for bacteria and fungi respectively. *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger* were the organism used for the screening.

Results

IR spectra revealed the important functional groups. ¹H NMR spectra of the products indicate the formation of the substituted cinnoline sulphonamide derivatives. The mass spectral data indicated stable molecular ion peak for all the synthesized products. The halogenated compounds showed the expected isotopic ion peaks in the mass spectra. All the compounds were screened for their anti-bacterial activity against both gram-positive and gram-negative bacteria's and also for their anti-fungal activity against *C. albicans* and *A. niger* and found to be active as shown in the Table-2.

Table 2: Antibacterial and antifungal activities of the synthesized compounds

Sl.No.	Compound No.	Diameter of zone of inhibition (mm)					
		<i>P. aeruginosa</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>C. albicans</i>	<i>A. niger</i>
1	7a	12	14	16	15	18	17
2	7b	17	19	20	18	17	18
3	7c	13	16	15	16	20	22
4	7d	14	15	17	18	17	15
5	7e	15	14	12	13	16	17
6	7f	12	15	15	16	15	14
7	7g	19	20	22	19	19	20
8	7h	18	22	21	20	21	22
9	7i	13	14	16	15	18	17
10	Norfloxacin	20	23	24	22	-	-
11	Griseofulvin	-	-	-	-	22	24
12	DMF	-	-	-	-	-	-

-Indicates no inhibition

The antibacterial and antifungal activities were carried out by the disk diffusion method. It is concluded that substituted cinnoline sulphonamide derivatives can be synthesized successfully in the laboratory. The results from antimicrobial activities studies showed that some of the substituted cinnoline sulphonamide derivatives (7b, 7g, 7h) possess good antibacterial activities and compounds (7a, 7c, 7d, 7e, 7f, 7i) showed moderate to good activities when compared to the standard drug norfloxacin. Whereas compounds (7c, 7g, 7h) showed good antifungal activity and compounds (7a, 7b, 7d, 7e, 7f, 7i) showed moderate to good activities when compared

to the standard drug Griseofulvin. Spectral evidence for some of the representative substituted cinnolines sulphonamide derivatives are given below.

8-Nitro 4-(p-amino phenyl sulphonamide) cinnoline 3-carboxamide:7a

Pale green crystals m.p. 192° C (68.26%) IR (KBr, cm⁻¹): 3481 (-NH), 3361-3218 (-NH₂), 3106 (-CH), 1631 (-CO). ¹H NMR (CDCl₃) δ: 7.85 (3H, d), 7.5 (4H, m, Ar-H), 8.1 (1H, s, NH), 8.3 (2H, d, NH₂), 8.7 (2H, s, CONH₂). MASS: The peak at m/z 388 corresponds to the parent compound C₁₅H₁₂N₆SO₅. Base peak was observed at m/z

139 and the important fragment ion peaks are observed at m/z 156 and m/z 142.

6-chloro -4 (p-amino phenyl sulphonamide) cinnoline 3-carboxamide:7b

Creamish white crystals m.p. 288° C (66.28%), IR (KBr, cm^{-1}): 3464 (-NH), 3394- 3245 (-NH₂), 3108 (-CH), 1681(-CO), ¹H NMR (CDCl₃) δ : 7.9 (3H, d), 7.3 (4H, m, Ar-H), 8.0 (1H, s, -NH), 8.1 (2H, d, NH₂), 8.5 (2H, s, CONH₂). MASS: The peak at m/z 377 corresponds to the parent compound C₁₅H₁₂ClN₄SO₃. Base peak was observed at m/z 329 and the important fragment ion peaks are observed at m/z 156 and m/z 142.

8-Methyl 4-(p-amino phenyl sulphonamide) cinnoline3-carboxamide:7c

white crystals m.p. 252°C (66.60%), IR (KBr, cm^{-1}): 3465 (-NH), 3388-3290 (-NH₂), 3106 (-CH), 1629(-CO).¹H NMR (CDCl₃) δ : 7.9 (3H, d), 7.4 (4H, m, Ar-H), 2.3 (3H, s, CH₃), 8.1 (1H, s, -NH), 8.2 (2H, d, NH₂), 8.6 (2H, s, CONH₂). MASS: The peak at m/z 357 corresponds to the parent compound C₁₆H₁₅N₅SO₃. Base peak was observed at m/z 154 and the important fragment ion peaks are observed at m/z 156 and m/z 142.

Discussion

Evaluation of antimicrobial activity for substituted Cinnoline Sulphonamide derivatives exhibits potent antimicrobial activity. All derivatives demonstrated significantly activity, while some derivatives mainly halogen substituted derivatives not only demonstrated approximately same zone of inhibition as reference drug but also show potent activity on lesser concentration, this might be due to the reason that cinnoline ring system is identical to ring system found in ciprofloxacin and similarly it is proved that sulphonamides are active bacterial PABA (Para amino benzoic acid) inhibitor. In conclusion, the combination of two active moieties displayed profound antimicrobial activity. Further, it would be interesting to obtain the possible mechanism of action and their in vivo trial in experimental animals.

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