Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(8):957-963

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis and screening of pyrazole based cinnoline derivatives for its anti-tubercular and anti-fungal activity

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ABSTRACT

A Series of 4-methyl-3-[5-(substituted phenyl)-4, 5-dihydro-1H-Pyrazol-3-yl] cinnoline-6-Sulfonamide were synthesized from 4-methyl-3-acetylcinnoline-6-Sulfonamido chalcones and hydrazines. The structure of the synthesized compounds were characterized by UV, IR, NMR & Mass spectral data, and evaluated for their in vitro anti-tubercular and anti-fungal activity. Compound CN-5a was most potent against Mycobacterium tuberculosis H37Rv, and all the analogues showed good anti-fungal activity against various pathogenic fungi.

Key words: Cinnoline, Pyrazole, Chalcones, Anti-tubercular, Anti- fungal.

INTRODUCTION

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), is a tenacious and remarkably successful pathogen that has latently infected a one-third of the world population. It can be cured, the therapy takes at least 6-9 months and laborious and lengthy treatment brings with it dangers of non-compliance, significant toxicity and drug resistance and fungi causes many infections Aspergillosis, Candidoses, etc. Many of these diseases are fatal if untreated, and treatment has been complicated by the resistance of the microorganisms to the widely used drugs. The increasing emergence of drug resistance highlights the need to develop some novel active Anti-tubercular and Anti-fungal drugs. Cinnoline is a versatile lead molecule that has been investigated widely in medicinal chemistry due to its important pharmacological activities[1-11]. It have been reported to exhibit anti-microbial, anti-tubercular, anti-cancer, anti-malarial, anti-hypertensive, anti-pyretic, anti-thrombolytic, analgesic, anti-diabetic, anti-depressant, cardiotonic, anaesthetic, anxiolytic etc. Cinnoline ring system is an isosteric relative to either Quinoline or Isoquinoline [12], therefore, in many cases the synthesized compounds were designed as analogues of Quinoline or isoquinoline. Cinoxacin is a cinnoline analogue of the Quinoline antibacterials used for urinary tract infection. Most of azoles are used as effective anti-fungal agents[13]. This prompted us in the synthesis of new congeners as analogs of 4-methyl cinnoline with sulphonamide group and fusing pyrazole hoping to get more potent anti mycobacterial and anti fungal activity.

EXPERIMENTAL SECTION

Synthesis of Phenyl hydrazano acetyl acetone-4-Sulfonamide [14]

Sodium nitrite (7.4gm,0.1mol) dissolved in 26ml of water was added to a suspension of Sulfanilamide (10gm,0.1mol) in 1N HCl (200 ml), and the mixture was stirred for 1hr at 0-5˚C and filtered to obtain the clear diazonium salt. The diazonium salt obtained was then added to a well stirred solution of ethanol (30ml), water

(500ml) and acetyl acetone (10.01gm,0.1mol) at 0ºC with stirring. Sodim acetate was then added to keep the mixture alkaline to litmus after 3 hour stirring at 0ºC the crude product was filtered, washed with water and air dried. Recrystallisation from ethanol afforded yellow needles of purified Phenyl hydrazano acetyl acetone- 4-Sulfonamide.

Synthesis of 4-methyl 3-acetyl Cinnoline 6-Sulfonamide

The Phenyl hydrazano acetyl acetone-4-Sulfonamide (10g, 0.05 mole) was added to the Polyphosphoric acid (16gm, 7.216 ml,0.03 mole) in small lots over 30 mins while maintaining the temperature between 60-65^{0}C. The reaction was maintained for an additional 2 hour and monitored by TLC. After the completion of reaction, ice cold water (200 ml) was added carefully to decompose the black residue at 0-5^{0}C.The product was then extracted with ethyl acetate. Ethyl acetate layer was then treated with Charcoal and concentrated to get the crude product as a brownish black residue. Recrystallisation from methanol to obtained as light yellow crystals of 4-methyl 3-acetyl Cinnoline 6-Sulfonamide.

Synthesis of 1-(4-methyl Cinnoline-3-yl)-3-(substituted phenyl) prop-2-en-1-one 6-Sulfonamide [Cinnoline based chalcone]

The product obtained from step 3(2.03gm,0.01mole) and aromatic aldehyde in same ratio(0.01mole) in ethanol(50ml) was cooled at 0-5^{o}C and added (5-10ml) 40% NaOH solution till precipitated and washed with ice water. Few drops N/20 dilute HCl was added for complete precipitation and filtered, washed with ice water and recystallised from alcohol to afford the compound (CN-1 -11).

Synthesis of 3-(4'-methyl-(3"-Cinnolinyl)-5-(substituted phenyl)-1H-Pyrazoline 6-Sulfonamide (CN 1a-11a)

The compound CN-1-11 (0.01mole) in 20ml acetic acid was taken and hydrazine hydrate (0.01mole) was added to it and refluxed for 10 hour. The contents were poured into ice, filtered and the product isolated, crystallized from ethanol to afford the compound (CN-1a-11a).

The purity of the products were confirmed by a single spot on the TLC plate and solvent system used was Benzene:Ethyl acetate (8:2). Melting point was determined and uncorrected.

4-methyl-3-[5-(4-nitrophenyl)-4,5-dihydro-1H-Pyrazol-3-yl]Cinnoline-6-sulfonamide

IR(KBr,cm1),3126.04(NH), 2925(CH_3),1503.24(C=N),1356.08(NO_2),1302.08(S=0), 1164.79(C-N), 846.59(p-subs benzene), 681.713(C-S), 1HNMR(δppm); 8.37-8.63(m, ArH), 2.35(s,2H in CH_2), 2.0(s,2H in NH_2), 2.55(s,3H,CH_3), MS (m/z): 198.99.

4-methyl-3-[5-(4-hydroxyphenyl)-4,5-dihydro-1H-Pyrazol-3-yl]Cinnoline-6-sulfonamide

3126.04(N-H), 2925(CH3), 1503.24(C=N), 3000(Br OH group) 1302.08(S=0), 1164.79(C-N), 800-900(p-subs benzene), 681.713(C-S), 1HNMR(δppm): 8.21-8.34(m, ArH) 7.2(s,NH) 1.9(s, 2H inCH2) 2.35(s,3H inCH3) 2(s,2H in NH2) 6.68-6.95(m, ArH). MS (m/z)m+:389

4-methyl-3-[5-(4-chlorophenyl)-4,5-dihydro-1H-Pyrazol-3-yl]Cinnoline-6-sulfonamide

IR(KBr,cm1): 3126.04(N-H), 2925(CH_3), 1503.24(C=N), 1400.07(C-Cl), 1164.79(C-N) 810.17(p-subs benzene), 681.713(C-S). 1HNMR(δppm); 8.1-8.03(m, ArH), 2.35(s,2H in CH_2), 2.0(s,2H in NH_2), 2.55(s,3H,CH_3), MS (m/z)m+: 367.99.

4-methyl-3-[5-(4-methoxyphenyl)-4,5-dihydro-1H-Pyrazol-3-yl]Cinnoline-6-sulfonamide

IR(KBr,cm1): 3126.04(N-H), 2925(CH_3), 1503.24(C=N), 1312.57(OCH_3), 1164.79(C-N) 810.17(p-subs benzene), 681.713(C-S), 1HNMR(δppm): 8.21-8.55(m,4H,CH in Cinnoline) 7(s,NH) 1.9(s,2H,CH2) 2.35(s,3H,CH3) 2.0(s,2H,NH2) 3.7(s,3H,OCH_3). MS (m/z) 398

4-methyl-3-[5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1H-Pyrazol-3-yl]Cinnoline-6-sulfonamide

IR(KBr,cm1): 3126.04(N-H),3111.58(OHGroup), 2925(CH3), 1503.24(C=N) 1334.45(OCH3 Bend),1188.9(C-C),1164.79(C-N),810.17(p-subsbenzene),681.713(C-S), 1HNMR(δppm): 8.21-8.55(m,CH in Cinnoline) 7(s,NH) 1.9(s,2H, CH_2) 2.35(s,3H,OCH_3) 2(s,2H,NH_2) 2.2(s,3H,CH_3)5 (s,1H,OH in aromatic), 6.56-6.75(m,4H,ArH). MS (m/z)414

4-methyl-3-{5-[(E)-2-phenylethenyl]-4,5-dihydro-1H-Pyrazol-3-yl}Cinnoline-6-sulfonamide
IR(KBr,cm1): 3126.04(N-H) 2925(CH_3) 1631(C=C) 1503.24(C=N) 1188.9(C-C) 1164.79(C-N) 710.14(Mono subs benzene) 681.713(C-S).1HNMR(δppm): 8.1-8.4(m, ArH) 7.2(s,NH) 1.9(s, 2H inCH2) 2.35(s,3H inCH3) 2(s,2H in NH2) 6.68-6.95(m, ArH). MS (m/z)m+:393

4-methyl-3-[5-(4-dimethylphenyl)-4,5-dihydro-1H-Pyrazol-3-yl]Cinnoline-6-sulfonamide
IR(KBr,cm1): 3200(Secondary amine), 3126.04(N-H), 2925(CH_3),1503.24(C=N),1188.9(C-C) 1164.79(C-N),846.597(p-subsbenzene),681.713(C-S),1HNMR(δppm)8.21-8.55(m,ArH)7(NH) 1.9(s,2H,CH2) 2.35(s,3H,CH3) 2(s,2H,NH2) 6.54-6.94(m,4H,ArH). MS (m/z)411

4-methyl-3-[5-(3-fluorophenyl)-4,5-dihydro-1H-Pyrazol-3-yl]Cinnoline-6-sulfonamide
IR(KBr,cm1): 3200(Secondary amine) 3126.04(N-H) 2925(CH_3) 1503.24(C=N) 1400(C-F) 1188.9(C-C) 1164.79(C-N) 730-750(m-subs benzene) 681.713(C-S). 1HNMR(δppm): 8.29-8.3(m, ArH) 7.2(s,NH) 1.89(s, 2H inCH2) 2.31(s,3H inCH3) 2(s,2H in NH2) 6.68-6.95(m, ArH). MS (m/z)m+:386

4-methyl-3-[5-(2-chlorophenyl)-4,5-dihydro-1H-Pyrazol-3-yl]Cinnoline-6-sulfonamide
IR(KBr,cm1): 3126.04(N-H) 2925(CH_3) 1503.24(C=N) 1401.03(C-Cl) 1188.9(C-C) 1164.79(C-N) 681.713(C-S) 670.142(O-subs benzene). 1HNMR(δppm): 8.01-8.14(m, ArH) 7.2(s,NH) 1.91(s, 2H inCH2) 2.36(s,3H inCH3) 2(s,2H in NH2) 6.68-6.95(m, ArH). MS (m/z)m+:402

4-methyl-3-[5-(3-chlorophenyl)-4,5-dihydro-1H-Pyrazol-3-yl]Cinnoline-6-sulfonamide
IR(KBr,cm1): 3126.04(N-H) 2925(CH3) 1503.24(C=N) 1401.03(C-Cl) 1188.9(C-C) 1164.79(C-N) 730-780(m-subs benzene) 681.713(C-S). 1HNMR(δppm): 8.1-8.3(m, ArH) 7.(s,NH) 1.99(s, 2H inCH2) 2.35(s,3H inCH3) 2(s,2H in NH2) 6.68-6.95(m, ArH). MS (m/z)m+:402

4-methyl-3-[5-(2-nitrophenyl)-4,5-dihydro-1H-Pyrazol-3-yl]Cinnoline-6-sulfonamide
3126.04(N-H) 2925(CH3) 1530.24(NO_2) 1503.24(C=N) 1188.9(C-C) 1164.79(C-N) 1188.9(C-C) 680-749(o-subs benzene) 681.713(C-S) 1HNMR(δppm): 8.21-8.34(m, ArH) 7.(s,NH) 1.87(s, 2H inCH2) 2.3(s,3H inCH3) 2(s,2H in NH2) 6.8-6.9(m, ArH). MS (m/z)m+:.m/z(m+)=413

SCHEME:

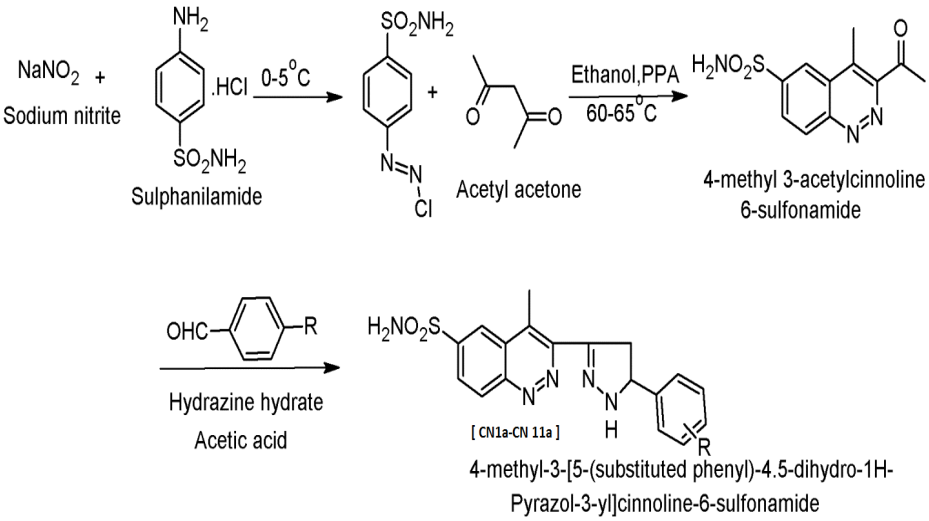
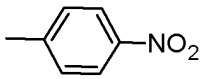
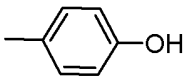
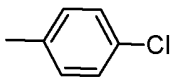
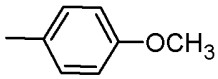
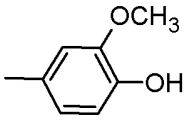
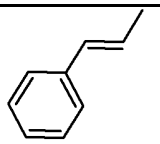
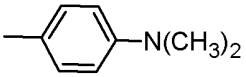
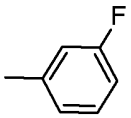
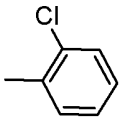
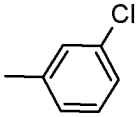
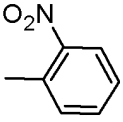


Table:1 Physicochemical properties of synthesised compounds

S.N	Compound code	R	Molecular formula	Molecular weight (g)	Percentage yield (%)	Colour	Solubility	Melting Point (^{0}C)	R_f value
1	CN-1a		$C_{18}H_{16}N_6O_4S$	412.42	92.5%	Yellow	DMSO	82^{o}C	0.68
2	CN-2a		$C_{18}H_{17}N_5O_3S$	388.42	86.1%	Yellow	DMSO	110^{o}C	0.72
3	CN-3a		$C_{18}H_{16}N_5O_2S$	366.42	87.2%	Yellow	DMSO	85^{o}C	0.71
4	CN-4a		$C_{19}H_{19}N_5O_3S$	397.45	90.7%	Yellow	DMSO	87^{o}C	0.63
5	CN-5a		$C_{19}H_{19}N_5O_4S$	413.45	92.2%	Yellow	DMSO	82^{o}C	0.52
6	CN-6a		$C_{20}H_{19}N_5O_2S$	393.46	86.1%	Golden brown	DMSO	80^{o}C	0.67
7	CN-7a		$C_{20}H_{22}N_6O_2S$	410.49	91.4%	Yellow	DMSO	210^{o}C	0.75
8	CN-8a		$C_{18}H_{16}FN_5O_2S$	385.42	76.2%	Yellow	DMSO	92^{o}C	0.62
9	CN-9a		$C_{18}H_{16}ClN_5O_2S$	401.87	82.4%	Yellow	DMSO	85^{o}C	0.66
10	CN-10a		$C_{18}H_{16}ClN_5O_2S$	401.87	74.2%	Brownish yellow	DMSO	87^{o}C	0.56
11	CN-11a		$C_{18}H_{16}N_6O_4S$	412.42	89.5%	Yellow	DMSO	112^{o}C	0.58

ANTI-FUNGAL SCREENING

Anti tubercular screening: Resazurin dye reduction assay method[15]

Mycobacterium tuberculosis H37Rv was grown in Middle brook 7H9 broth (Difco, USA) containing 0.05% Tween80, 0.5% Glycerol and 5% OADC supplement (Becton Dickinson, USA). The culture was grown till log-phase and was diluted to McFarland 1 standard with the same medium. From these 50 micro liters was added to 450 micro liter of fresh medium in 2ml eppendorf tubes. Stock solution of the test compounds was prepared in DMF/DMSO. And the compounds were tested at 1, 10 & 100 micro gram per ml concentrations. And Rifampacin 1µg/ml was used as positive control, Control tubes had the same volume of DMF/DMSO without any compound.

After incubation at 37 ºC for 7 days, 30 microlitre of 0.01% resazurin (Sigma, St.Louis.MO.USA) in water was added to each well. Resazurin, a redox dye, is blue in the oxidized state and turns pink when reduced by growth of viable cells. The control tubes showed change of color from blue to pink after 24 hours at 37ºC.Compounds in the well which remained blue were considered to inhibitory to M.tuberculosis at their respective concentrations.

Anti fungal screening [15,16]

Disc diffusion method

Sabourands dextrose broth medium was prepared and transferred into sterile Petri plates aseptically (thickness of 5-6mm). The plates were allowed to dry at room temp. The plates were inverted to prevent condensate falling on the agar surface. The layers of the medium are uniform in thickness, is done by placing the plates on a leveled surface. Standardized fungal inoculums of Aspergillus niger, Aspergillus fumigatus, Aspergillus parasiticus, Candida albicans, Monascus ruber, Streptomyces griseus were applied to the plates and spreaded uniformly over the surface of medium by using a sterile Non-absorbent cotton swab and finally the swab was passed around the edge of the medium. The inoculated plates were closed with the lid and allowed to dry at room temperature. The sample impregnated discs (10µg /disc) in dimethyl sulphoxide and standard clotrimazole 10µg/disc were placed on the inoculated agar medium. All petri plates were incubated at 27˚C -28˚C for 48 hrs. After the incubation diameter of zone of inhibition produced by the sample were measured.

Anti fungal screening: Serial dilution method

The serial dilutions of known concentration of compound solution are made from the stock (100 mg/ml) by using Sabourands dextrose broth using the method described below.The tubes were labeled 1 to 8 and 1 ml of Sabourands dextrose broth were added to the first 5 tubes and 8th tube, then added 0.5ml of Sabourands dextrose broth to 6th and 7th tubes. One ml of different synthesized compounds was added to the 1st tube, mixed and transfer 1ml serially up to tube 5.From the 5th tube transfer 1ml to 6th tube. Mixed and transfer 0.5 ml to the 7th tube. Each tube, 1 to 7 contains 1ml diluted extract.The 8th tube was the control. With a standardized micro pipette, add a drop of the diluted broth culture approximately 0.01ml of the test organism to all tubes, including the control, gently mixed and incubated at 26-28 0 C 48 hrs. The highest dilution of particular compounds showing no turbidity was observed and recorded. This was taken as the end point, and this dilution was considered to contain the concentration of drug equivalent to MIC.

Table:1 Anti-fungal activity of the synthesized compounds by Disc Diffusion method

S.N	Micro organisms	Zone of inhibition (in mm) Compound (100 µg/disc)											
		CN 1a	CN 2a	CN 3a	CN 4a	CN 5a	CN 6a	CN 7a	CN 8a	CN 9a	CN 10a	CN 11a	Std*
1	*C.albicans*	10	9	9	9	11	8	8	10	10	11	9	11
2	*S.griseus*	10	7	8	8	9	6	6	7	8	8	9	10
3	*A.niger*	9	9	9	8	10	9	11	10	9	9	8	11
4	*A.fumigalis*	8	8	7	7	9	7	7	8	8	7	6	9
5	*M.ruber*	8	7	7	6	8	8	6	7	7	8	8	9

**Clotrimazole*

Table:2 Anti-fungal activity of the synthesized compounds by Serial Dilution method

S.N	Micro organisms	MIC VALUES (µg/ml)										
		CN 1a	CN 2a	CN 3a	CN 4a	CN 5a	CN 6a	CN 7a	CN 8a	CN 9a	CN 10a	CN 11a
1	*C.albicans*	2.5	1.2	2.5	2.5	2.5	5	5	2.5	2.5	2.5	1.2
2	*S.griseus*	1.2	2.5	2.5	2.5	2.5	5	2.5	2.5	5	2.5	1.2
3	*A.niger*	2.5	2.5	2.5	5	2.5	1.2	1.2	2.5	2.5	2.5	2.5
4	*A.fumigalis*	5	5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	5	1.2
5	*M.ruber*	2.5	2.5	2.5	2.5	2.5	1.2	1.2	2.5	5.0	2.5	2.5

ANTI-TUBERCULAR SCREENING

Table:3 *In vitro* anti-mycobacterial activity of the synthesized compounds against *Mycobacterium tuberculosis* $H_{37}R_V$.

S.No	Compound code	Concentration of synthesized compounds		
		1µg/ml	10µg/ml	100µg/ml
1	CN-5a	N	N	P
2	CN-2a	N	N	N
3	*RIF	P	P	P

** Rifampicin N - Negative P- Positive*

RESULTS AND DISCUSSION

Provoked by the biological activity of the Cinnoline and in view of ongoing search for the most potent anti-malarial agent, some novel 3, 7 Di substituted derivatives of Cinnoline have been synthesized and their anti-tubercular and anti-fungal activity was studied**.** The synthesized compounds CN-2a and CN-5a were tested for activity against Mycobacterium tuberculosis H37Rv using Resazurin assay method at the concentration of 1µg/ml, 10µg/ml and 100µg/ml in DMSO. Rifampicin 1µg/ml was used as a standard. The anti-mycobacterial activity of the synthesized compounds was evaluated by the change in colour of resazurin from Pink to blue by Oxido reductase with in viable cell. Among these two compounds, compound CN-5a inhibited the growth of Mycobacterium at 100µg/ml.

Evaluation of the results from anti-fungal studies showed that synthesised Cinnoline derivatives exhibits moderate to good anti-fungal activity against *Candida albicans Aspergillus fumigatus, Streptomyces griseus, Aspergillus niger*, *Aspergillus fumigalis*, *Monascus ruber* with zone of inhibition was found to be in the range of (6-11mm). Out of the compounds, CN-5a, CN-7a, CN-11a were showed good activity against *Candida albicans, Streptomyces griseus,* with zone of inhibition was found to be in the range of 11mm. The MIC of the synthesized compounds against *Candida albicans Aspergillus fumigalis, Streptomyces griseus, Aspergillus niger, Aspergillus fumigalis*, *Monascus ruber* was determined by serial diluton method, was found to be in the range of 1.2-2.5µg/ml.

CONCLUSION

In Summary, some novel substituted Cinnoline derivatives have been synthesized and evaluated for its anti-tubercular and anti-fungal activity. All derivatives demonstrated significant anti-tubercular and anti-fungal activity amongst; compound CN-5a was found to be most potent compound with promising activity against resistant strains of *Mycobacterium tuberculosis* H37Rv and fungus. Taking into account the significant activities of the examined compounds, it is believed that further optimization of these identified chemical leads can probably lead to the development of more active molecules. Further studies on its possible mechanism and invivo 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.

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