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Research Article

IN-VITRO ANTI-MICROBIAL ACTIVITY OF NOVEL DERIVATIVE OFAZO DYE FROM CYANO ESTER

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ABSTRACT

The object of the work was to find out and develop the novel potent anti-microbial activity of a series of novel azo dye derivatives form cyano ester. The synthesized coupled azo dye compounds were screened anti-microbial activity and compare efficiency against the standard drugs like ampicillin, and fluconazole. All the synthesized compounds were characterized by respective IR, H-NMR,spectroscopy. Most of the synthesized compounds (G1, G2 andG5) show moderate to good anti-microbial activity.

Keywords: Coupled azo dye, Cyano ethylacetate, antibacterial and antifungal.

INTRODUCTION

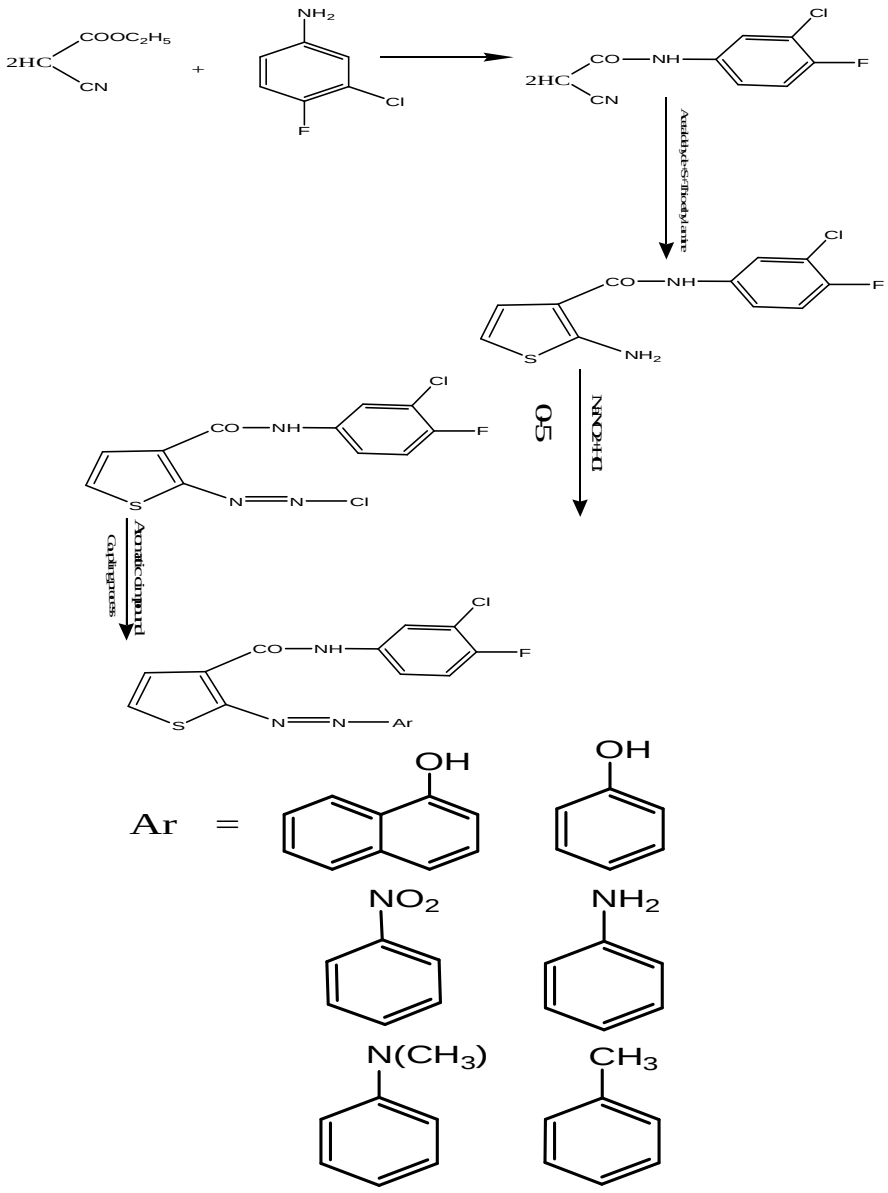
Thiophene nucleus represents a very important field in drug discovery, which is present in many natural and synthetic products with a wide range of pharmacological activities[1-7]. Basically sulfur& Nitrogen nucleus containing heterocyclic families are very interesting due to their versatile pharmacological activities, such as anti-tumour, diuretics, fungicides, bactericides, antihelmintic, antiallergic, anti-ulcer and local analgesic[8-10]. Especially in the sense of design of new drugs. As like that of thiophene, Azo dyes compounds are also have a plenty of applications in industry and photodynamic therapy as well as photosensitive species in photographic or electro photographic systems and are dominant organic photoconductive materials[11]. So far various new thiophenes have been synthesized and screened for different pharmacological activity. The encouraging results made us the impetus to continue the investigation. Furthermore, the various changes in the structure of these thiophene nucleus compounds are worth studying in order to synthesize less toxic and more potent drugs. We decided to combine azo group with thiophene heterocyclic nucleus hope that the resulting novel heterocyclic compounds would be more biologically active than thiophen or azo group nucleus containing drugs. By considering these potential antimicrobial effects, a new series of compounds were synthesized via gewald reaction and their antimicrobial properties were evaluated.

MATERIAL AND METHODS

The chemicals and reagents were procured from Sigma lab grade source. All the solvents used from commercial sources and redistilled before use. All synthesized compounds melting points were determined on a Buchiaratus and are uncorrected. The Infra red spectra (in KBr pellets) were recorded on a JASCO spectrometer frequencies are expressed in cm^{-1}. $_1H$ NMR spectra were recorded on a Bruker Advance 400 spectrometer operating at 400.00 MHz 13C NMR spectra were recorded on a Bruker Advance 300 spectrometer operating at 300.00MHz. The chemical shifts are reported in ppm (δ) relative to tetra methyl silane. The purity of the compounds was

checked by thin layer chromatography (TLC) silica gel plates using ethyl acetate: hexane or methylene dichloride: methanol as eluent and spots were eloped in ultraviolet.

SCHEME



EXPERIMENT

Step -I

N-(3chloro-4flurophenyl)-2-cyano acetatamide

One mole of Cyano ethylacetate was refluxed for 2 hours with 3 chloro 4fluro aniline in a round bottom flask, amide linkage taking place between the cyano ethyl acetate and 3 chloro 4 fluroaniline, after removal of 1 mole of ethanol.

STEP- II

2-amino-N-(3-chloro-4-fluorophenyl) thiophene-3-carboxamide

The first step product has been used as a raw material for second step, along with acetaldehyde sulphur,and thioethyl amine. This reaction is called as gewald reaction. End of this step we got 2 amino thiophene derivative.

STEP-III

2-(2-chlorodiazenyl)-N-(3chloro-4-fluorophenyl) thiophene-3-carboxamide

Now we used the second step product used in this diazodisation process, 1 mole of second step product was reacted with sodium nitrite and hydrochloric acid at the temperature of 0-5˚C. Finally formed thiophene diazonium salt separate by vacuum pump.

STEP-4

Derivative of 2-(2-chlorodiazenyl)-N-(3chloro-4-fluorophenyl) thiophene-3-carboxamide

In this step we prepared 6 different novel thiophene azo derivative was prepared by reaction between thiophene diazonium salt with different aromatic compound such as napthol, phenol, aniline, dimethylaniline, nitrobenzene, toluene.

ANTI-MICROBIAL ACTIVITY

The synthesized compounds were screened against four bacteria, and two fungal organisms. Anti-microbial activity of the synthesized drug has been carried out by paper disc diffusion method[12]. Micro-organism used in the microbial study was B.subtilis, E.coli, P.aeuruginosa, P.aeuruginosa, A.Fumigatus, and A.flavus. The sterilized medium was inoculated with the suspension of the micro-organism and poured into a petridish to give a depth of 3-4mm. The paper impregnated with the synthesised compounds (10, 50, 100µg ml^{-1} in dimethyl formamide) was placed on the solidified medium. The plates were pre-incubated for 1 hour at RT and incubated at 37°C for 24 and 48 hour for anti-bacterial and anti-bacterial and anti-fungal activities, respectively. Ampicillin (10, 50,100µg/disc) and Fluconazole (50µg/disc) were used as standard for anti-bacterial and anti-fungal activities.

RESULTS AND CONCLUSION

The results reveal that synthesized six new compounds, which contain thiophen azo nucleus was conformed by sending sample in IR, and NMR spectrascopical studies. At last we performed the anti-bacterial and anti-fungal activity of our synthesized compound was compared with the standard drug ampicillin and fluconazole respectively. The results predicted that our synthesized compound posses anti-microbial activity against the various micro-organisms, like B.subtilis, E.coli, P.aeuruginosa, S.aureus, A.Fumigatus, A.flavus. Compound G1, G2, G5 shown the maximum activity due to the presence of electron releasing group such as hydroxyl, and nitro function group. Compound G6 shown mild activity when compare to the other compounds.

ACKNOWLEDGEMENTS

I would like to thank friends to motivate me to do this project.

Table 1: Characterization of Synthesised Compounds

S.No	Synthesized compound identification name	Molecular Formula	Molecular Weight	Yield of Synthesized Compound (%)
1	G1(Napthol)	$C_{21}H_{13}ClFN_3O_2S$	425	65
2	G2(Phenol)	$C_{17}H_{11}ClFN_3OS$	360	72
3	G3(Aniline)	$C_{17}H_{12}ClFN_4OS$	374	54
4	G4(dimethyl amine)	$C_{19}H_{16}ClFN_4OS$	402	68
5	G5(Nitro benzene)	$C_{17}H_{10}ClFN_4O_3S$	404	80
6	G6(Toluene)	$C_{18}H_{13}ClFN_3OS$	373	78

Table 2: IR Spectroscopy Studys

IR Spectroscopy (cm-1)

Compound name	C=C	C-N	C=O	N-H	OH	N=N	-CH_3	C-Cl	C-F
G1	1490	1255	1595	3280	3643	1517	-	720	995
G2	1540	1280	1640	3320	3682	1520	-	715	1050
G3	1655	1245	1635	3305	-	1490	-	738	1136
G4	1530	1265	1600	3298	-	1550	2930	795	1100
G5	1537	1290	1650	3254	-	1570	-	810	1050
G6	1520	1230	1700	3315	-	1536	2850	764	1095

Table 3: NMR Spectroscopy Result

Compound Name	Aromatic Region	Aliphatic Region
G1	6.2-7.6(11-H,m,Ar-H) 6.2(1-H d,Ar-H) 6.7(4-H,m,Ar-H) 7.4(3-H,m,Ar-H) 7.6(3-H,q,Ar-H) 8.1(1-H,d,N-H)	5.0(1-H,s,OH)
G2	6.7-8.2(9-H,m,Ar-H) 6.7(2-H d,Ar-H) 7.0(4-H,m,Ar-H) 8.2(3-H,q,Ar-H) 8.3(1-H,s,N-H)	5.2(1-H,s,OH)
G3	6.3-8.9(9-H,m,Ar-H) 6.3(1-H d,Ar-H) 6.6(5-H,m,Ar-H) 8.5(3-H,m,Ar-H) 8.9(1-H,d,N-H)	4.5(2-H,s,NH_2)
G4	6.5-8.0(16-H,m,Ar-H) 6.9(4-H q, Ar-H) 7.2(2-H,m,Ar-H) 7.6(3-H,qAr-H) 8.1(1-H,s,N-H)	2.9(6-H,s,$(CH3)_2$)
G5	6.9-8.5(9-H,m,Ar-H) 6.9(3-H q, Ar-H) 7.4(4-H,m,Ar-H) 8.0(1-H,s,N-H) 8.5(2-H,d,Ar-H)	-
G6	6.5-6.7(2-H,d,Ar-H) 6.8(3-H q,Ar-H) 7.3(4H,m,Ar-H) 7.9(1H,S,N-H)	2.4(3-H,s,CH3)

Table 4: Zone of inhibition of synthesized compound (concentration µg/ml^{-1})

	BACTERIA				FUNGUS	
	B.subtilis	**E.coli**	**P.aeuruginosa**	**S.aureus**	**A.Fumigatus**	**A.flavus**
Compound	**10 50 100**	**10 50 100**	**10 50 100**	**10 50 100**	**50**	**50**
G1	**14 17 20**	**13 17 21**	**12 16 18**	**17 16 21**	**15**	**14**
G2	**13 14 18**	**11 1319**	**09 16 18**	**14 15 18**	**14**	**13**
G3	**12 13 14**	**09 10 14**	**07 11 13**	**09 11 14**	**09**	**10**
G4	**11 12 14**	**08 10 13**	**07 11 12**	**08 11 13**	**09**	**09**
G5	**12 15 17**	**10 12 17**	**08 11 15**	**10 12 15**	**12**	**11**
G6	**08 10 11**	**05 07 11**	**05 09 10**	**07 08 08**	**07**	**06**
Standard						
	Ampicillin				**Fluconazole**	
	16 20 24	**14 20 24**	**15 21 24**	**21 22 25**	**17**	**17**

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