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Synthesis and Antimicrobial Evaluation of Some New Fluoro-Formazans

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ABSTRACT

A series of fluoro-substituted formazan derivatives has been synthesized from the salts of diazonium chloride and fluoro schiff base in pyridine. The structures of newly synthesized formazans were established based on IR, ^{1}H NMR, and mass spectral data. All the synthesized compounds were screened for their antimicrobial activity. Some of the compounds showed very good activity compared to the standard drugs against all pathogenic bacteria and fungi.

Keywords: Fluoro-Schiff base, Formazans, Diazonium chloride, Antimicrobial activity

INTRODUCTION

Formazans are the important and distinct class of organic compounds. Their chemistry has attracted the interest of many research groups due to their wide biological and industrial application as well as their utility in analytical chemistry and synthesis of heterocyclic compounds [1]. formazans have been found to possess important biological activities such as anticancer [2], anticonvulsant [3], antihelmintic [4], antitubercular [5], antiviral [6], anti-inflammatory [7], anti-HIV [8], antimicrobial [9-10], analgesic [11], antiparkinsons [12], cardiovascular [13] and antiproliferative activity [14].

The chemistry of fluorine contains compounds has been tremendously developed. Fluorine has played a pivotal role in novel drug discovery for modulating physical and biological properties of the molecules [15-16]. Numerous drugs containing fluorine includes antipsychotic such as fluphenazine, HIV protease inhibitors such as tipranavir, antibiotics such as Ofloxacin and trovafloxacin and anesthetics such as haloethane [17-18]. Fluoroquinoline [19] are commonly used the family of broad-spectrum antibiotics. Due to these observations herein we reported eight substituted formazan derivatives (**3a-h**) by coupling fluoro-Schiff bases with appropriate aryl diazonium chloride in pyridine. All formazans were evaluated for antimicrobial activities.

EXPERIMENT

Chemicals and instrumentation

All the melting points were determined in open capillaries and are uncorrected. The purity of compounds was checked by TLC (0.5 mm thickness) using silica gel-G coated Al-

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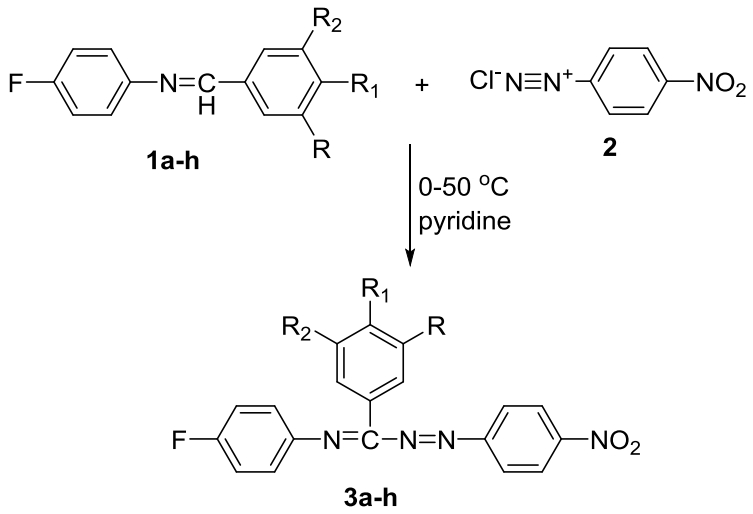
plates (Merck) and spots were visualized by exposing the dry plates in iodine vapors. IR spectra were recorded on a Perkin-Elmer spectrometer. ^{1}H NMR spectra were recorded on a Gemini 300 MHz instrument using DMSO-d6 as solvent and TMS as an internal reference. The mass spectra were recorded on EI-SHIMADZU-MS spectrometer. The elemental analysis (C, H, N) of compounds was performed on Perkin-Elmer CHNS 2400.

General procedure for preparation of fluoro-formazans (3a-h).

A cold solution of *p*-NO_2 aniline (0.01 mole) previously dissolved in aq. HCl (10 mL) was diazotized over crushed ice by dropwise addition of cold aq. The solution of $NaNO_2$ (0.7 g). Fluoro-Schiff base [20] (0.01 mole) was dissolved in dry pyridine (10 ml). The contents were cooled in the ice bath and stirred. To it a clear and cold solution of diazonium salt of *p*-NO_2 aniline was added dropwise for 10 min at low temperature (0-5^{o}C). The reaction mixture was kept in ice bath for 2 hours and then poured into ice water. The resulting solid was filtered, washed with water and crystallized from ethanol to get corresponding formazans (**3a-h**)



Scheme:- 1 synthesis of formazans 3a-h
a, R,R_1,R_2=H, **b**,R,R_2=H,R_1=Cl, **C**, R,R_2= H, R_1=F,
d, R,R_2=H,R_1=NO_2, **e**, R,R_2=H,R_1=OMe, **f**, R,R_1=OMe,R_2=H,
g, R,R_1=OMe,R_2=Br, **h**,R,R_1,R_2=OMe.

N-(4-fluorophenyl)-1-[(4-nitrophenyl)diazenyl]-1-phenyl methanimine-(3a) yield 85%, M.P. 210^{0}C. IR(KBr) 1570 Cm^{-1} (-N=N-), 1625 Cm^{-1},(-C=N-), 1H NMR (DMSO-d6): δ 6.30-7.10 (m 13H,3Ar-H), Anal calcd. For $C_{19}H_{13}FN_4O_2$ (348): C,65.51, H, 3.73, N, 16.09. found: C, 65.25, H, 3.50, N, 16.00.

1-(4-chlorophenyl)-N- (4-fluorophenyl)-1-[(4-nitrophenyl)diazenyl] methanimine-(3b) yield 80%, M.P. 215^{0}C. IR (KBr) 1575 Cm^{-1} (-N=N-), 1630 Cm^{-1},(-C=N-), 1H NMR (DMSO-d6): δ 6.35-7.21 (m 12H,3Ar-H), Anal calcd. For $C_{19}H_{12}N_4FClO_2$ (382.5): C, 59.60, H, 3.13, N, 14.64. found: C, 59.30, H, 3.05, N, 14.20.

N-1-bis(4-fluorophenyl)-1-[(4-nitrophenyl) diazenyl] methanimine-(3c) yield 75%, M.P. 302^{0}C. IR (KBr) 1585 Cm^{-1} (-N=N-), 1640 Cm^{-1},(-C=N-), 1H NMR (DMSO-d6): δ 6.41-7.38

(m 12H,3Ar-H), Anal calcd. For $C_{19}H_{12}F_2N_4O_2$ (366): C,62.29, H, 3.27, N, 15.30. found: C, 62.10, H, 3.27, N, 15.10.

N-(4-fluorophenyl)-1- (4-nitrophenyl)-1-[(4-nitrophenyl)diazenyl] methanimine-(3d) yield 70%, M.P. 220^{0}C. IR(KBr) 1590 Cm^{-1} (-N=N-), 1645 Cm^{-1},(-C=N-), 1H NMR (DMSO-d6): δ 7.10-8.35 (m 12H,3Ar-H), Anal calcd. For $C_{19}H_{12}FN_4O_4$ (393): C, 58.01, H, 3.05, N, 17.81. found: C, 57.75, H, 3.00, N, 16.90.

N-(4-fluorophenyl)-1-(4-methoxyphenyl)-1-[(4-nitrophenyl) diazenyl] methanimine-(3e) yield 80%, M.P. 230^{0}C. IR(KBr) 1570 Cm^{-1} (-N=N-), 1635 Cm^{-1},(-C=N-), 1H NMR (DMSO-d6): δ 6.85-7.35 (m 12H,3Ar-H), δ 3.09(S, 3H,OCH_3) Anal calcd. For $C_{20}H_{15}FN_4O_3$ (378): C, 63.49, H, 3.96, N, 14.81. found: C, 63.20, H,3.80, N,14.70.

1-(3,4-dimethoxyphenyl)-N-(4-fluorophenyl)-1-[(4-nitrophenyl) diazenyl] methanimine (3f) yield 75%, M.P. 225^{0}C. IR (KBr) 1565 Cm^{-1} (-N=N-), 1640 Cm^{-1},(-C=N-), 1H NMR (DMSO-d6): δ 7.30-8.34 (m 11H,3Ar-H), δ 3.88(S, 3H,OCH_3) Anal calcd. For $C_{21}H_{17}FN_4O_4$ (392): C, 64.28, H, 4.33, N, 14.28. found: C, 64.10, H, 4.20, N, 14.20.

1-(3-bromo-4,5-dimethoxyphenyl)-N-(4-fluorophenyl)-1-[(4-nitrophenyl) diazenyl] methanimine-(3g) yield 75%, M.P. 240^{0}C. IR(KBr) 1570 Cm^{-1} (-N=N-), 1670 Cm^{-1}, (-C=N), 1H NMR (DMSO-d6): δ 7.35-8.40 (m 10H,3Ar-H), δ 3.85(S, 3H,OCH_3), δ3.90(S, 3H, OCH_3) Anal calcd. For $C_{21}H_{16}BrFN_4O_4$ (471): C, 53.50, H, 3.39, N, 11.88. found: C, 53.21, H, 3.25, N, 11.30.

1-(3,4,5-trimethoxyphenyl)-N-(4-fluorophenyl)-1-[(4-nitrophenyl) diazenyl] methanimine-(3h) yield 70%, M.P. 250^{0}C. IR(KBr) 1565 Cm^{-1} (-N=N-), 1640 Cm^{-1}, (-C=N), ^{1}H NMR (DMSO-d6): δ 7.40-8.45 (m 10H,3Ar-H), δ 3.91(S, 9H,3OCH_3), Anal calcd. For $C_{22}H_{19}FN_4O_5$ (438): C, 60.27, H, 4.33, N, 12.78. found: C, 60.10, H, 4.15, N, 12.58

Antimicrobial activity

Antibacterial activity

The formazans derivatives **3a-h** were screened for their antibacterial activity against different types of bacterial strains i.e. gram-negative bacterial strains of *Klebsiella Pneumonia* (ATCC-700603), *Escherichia coli* (ATCC- 25922), *Pseudomonus aeruginosa* (ATCC-27853) and gram-positive bacteria *staphylococcus aureus* (ATCC- 25923) at a concentration of 50 µg mL^{-1}.

The cultures were diluted with 5% of autoclaved saline and the final volume was adjusted to a concentration of approximately 10^5-10^6 CFU ml^{-1}. The synthesized compounds were diluted with acetone for the antibacterial biological assay for agar disc diffusion method[21], the liquid form of test compound was soaked on to a disc (5mm) and then allowed to air dry, such that the disc became completely saturated with the test compound. The saturated chemical discs were introduced onto the upper layer of medium evenly loaded with the bacteria and incubated at 37^{0}C for 24 to 48 hours for better inhibition of bacteria. The zones of inhibition were measured after 24 to 48 hours. All the experiments were performed in triplicate and the results are expressed as zone of inhibition in mm. The zone of inhibition of the synthesized compounds **3a-h** was compared with zone of inhibition of standard antibiotics Ofloxacin (50 µg mL^{-1}).

Antifungal activity

The antifungal activity of synthesized compounds **3a-h** was tested against four pathogenic fungi, namely *Aspergillus niger* (MTCC-281), *Candida albricans* (ATCC-2091), *Penicillium citrinum* (NCIM-768) and *Fusarium oxysporum* (ATCC-7601) by the poison plate technique at a concentration of 50 µg mL^{-1}. Four kinds of fungi were incubated in potato dextrose agar medium (PDA) at 25± 1 ^{0}C for 5 days to obtain new mycelium for the antifungal assay and then mycelia disks of approximately 0.45 cm diameter cut from the culture medium were picked up with a sterilized inoculation needle and inoculated into the center of a PDA plate. The test compounds were dissolved in acetone (10 mL) then added to the PDA medium (90 mL). The final concentration of compounds in the medium was adjusted to 50 µg/mL. The inoculated plates were incubated at 25 ± 1 ^{0}C for 5 days. Acetone was diluted with sterilized distilled water and used as the control. While a 50 µg/mL of ketoconazole were used as the standard control for each treatment. Three replications of the experiments were performed. The radial growth of the fungal colonies was measured on the sixth day.

RESULT AND DISCUSSION

Chemistry

This paper describes a very straightforward methodology for effective synthesis of fluoro formazans from fluoro Schiff bases using diazotized salt of *p*-nitroaniline. Similar methodologies have been found to effective for synthesis of formazans [9-10].

Formazans **3a-h** were synthesized from fluoro-Schiff bases. Fluoro-schiff bases were dissolved in pyridine and cooled to 0-5^{o}C. The cold solution of diazotized p-nitro aniline was added with stirring into the cold solution of fluoro-Schiff bases within 10-15 min. The reaction mixture was kept in ice bath for 2hr and then poured into ice water and crystalized from ethanol (**Scheme 1**). The purity of synthesized formazans was checked by TLC and structure were assigned on the basis of elemental analysis and spectral data.

Formazans showed characteristics band in IR at in between 1500-1585 cm^{-1} due to N=N stretching vibration. In ^{1}H NMR spectrum the singlet due to azomethine at δ 8-9 did not appears which clearly indicate that formation of formazans, mass spectrum of formazan was in good agreement with their molecular weight.

Anti-microbial activity

Antibacterial activity

All the compounds were screened for their antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginasa*, *Klebsiela pneumonia* using Ofloxacin as the standard drug. The activity was determined using cup plate agar diffusion method by measuring the zone of inhibition in mm. The compounds were screened at the concentration of 50 µg mL^{-1} in DMSO. From the screening studies (**Table 1**), it is evident that the synthesized formazans derivatives **3b**, **3c**, **3f**, **3g**, and **3h** showed good antibacterial activity against all the tested organisms. It was further observed that the electron rich **3h** with three –OMe substituent, showed the best activity, and closely followed by **3f** which has two –OMe substituent. This observation leads to the conclusion that electron-rich formazans showed higher activity. Furthermore, the substituents like –NO_2, -F, and -Br substitution did not show any significant change in the level of activity against bacteria.

Table 1. Antibacterial activity (Zone of inhibition, mm) of compounds **3a-h**

Compound	Gram –ve bacteria		Gram + ve bacteria	
	K. pneumonie	***E. coli***	***P. aeruginosa***	***S. aureus***
3a	4	16	5	10
3b	10	28	9	32
3c	12	30	10	35
3d	5	22	7	26
3e	8	24	6	25
3f	11	29	10	31
3g	10	30	9	29
3h	13	35	13	32
Ofloxacin	12	30	10	30

Antifungal activity

All the compounds were screened for their antifungal activity against *Aspergillus niger*, *Candida albicans*, *Penicillium citrium* and *Fusarium oxysporum* using ketoconazole as standard drug. The activity was determined using cup plate agar diffusion method by measuring the zone of inhibition in mm. the compounds were screened at a concentration of 50 µg/mL in DMSO. From the screening studies, it was evident that the synthesized compounds **3a**, **3c**, **3f**, **3g** and **3h** showed good antifungal activity against the tested organisms. The difluorinated compound **3c** showed the highest activity followed by electron-rich substrate **3f**, **3g** and **3h**. When the fluorine atom was replaced by chloro or nitro substituent the activity against fungi decreased.

Table 2. Antifungal activity (zone of inhibition, mm) of compound **3a-3h**

Compound	Fungus			
	A. niger	***C. albicans***	***P.citrium***	***F. oxysporum***
3a	11	20	18	25
3b	8	20	16	22
3c	15	28	24	26
3d	6	19	14	23
3e	9	23	21	24
3f	12	25	21	25
3g	11	24	19	23
3h	13	26	21	25
Ketoconazole	12	25	20	25

CONCLUSION

In conclusion, synthesis of fluoro-formazans is reported by the reaction of diazonium salt with fluoro Schiff bases in good yields. The antimicrobial assay of these compounds revealed that compounds **3b**, **3c**, **3f**, **3g**, and **3h** showed maximum zone of inhibition against tested microorganism compared with the standard.

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