HL
http://heteroletters.org
ISSN: 2231 – 3087(print) / 2230 – 9632 (Online)
Vol. 1: (3), 2011, 221-226

SYNTHESIS AND THERAPEUTIC EVALUATION OF SOME FORMAZANS AS POTENTIAL ANTIMICROBIAL AGENTS

Anand K. Halve*, Amit Kankoriya, Sunil Kumar Samadhiya, Bhuwan Bhaskar Pant and Jitendra Kumar Gupta

** School of Studies in Chemistry, Jiwaji University Gwalior-474011, India*
E mail: halveanand@yahoo.co.in

ABSTRACT

A new series of formazan was synthesized and evaluated for antifungal and antibacterial sensitivity. The reaction of 2-methoxy-4-{[(3-nitrophenyl) imino] methyl} phenol **(3)** with diazonium salt was carried out in pyridine. The antimicrobial activity of the synthesized target compounds **(4)** was evaluated by screening on different human bacterial and fungal pathogens using disc diffusion and broth dilution methods respectively. All the synthesized compounds exhibited considerable inhibition against the human pathogens tested.

INTRODUCTION

In recent decades, the problem of microbial infections has reached alarming levels in the developing countries round the world. Studies on the influence of structure on activity showed that sometimes, minor changes in the nuclei enhance the pharmacological profile multifold than the parent molecule. The search for new, effective and safe nuclei has led to improvements in the existing drugs by increasing their potency, duration of action as well as minimizing their toxic effects. This is achieved by creating new biologically active agents by molecular modifications. Azomethines and their derivatives have been prominent research subject due to their striking complexometric behaviour and pharmacological characteristics. These properties allow them to play pivotal roles in various biological activities viz. anticancer, antitubercular, anti-HIV and antioxidant. In our ongoing research program we found that schiff bases and their derivatives[1-5] showed antimicrobial activity. Formazans possess biological[6], antimicrobial[7,8], antiviral[9-11], anticonvulsant[12] and anti HIV activities[13]. Sethi *et.al.* reported some formazans and tetrazolium indoles to act as CNS active agents[14]. A number of formazans have been claimed to possess promising antifertility activity[15]. Besides the biological activity their complexometric behaviour[16-18] has also been reported. Edwards *et.al.* used azo compound and formazans dyes for the determimation of mutagenicity[19]. Stellmach prepared formazans dyes by Ehrlich ascites tumor cells[20]. The antiviral activity of formazans particularly against *Ranikhet diseases virus* and *plant virus*[21] has also been reported. Formazans exhibit good analgesic[22] anti-inflammatory[23], anti-proliferative[24] and anti-parkinsonian activity[25].

With this background in mind the present study is designed in order to synthesize a new series of formazans as potential antimicrobial agents.

EXPERIMENTAL

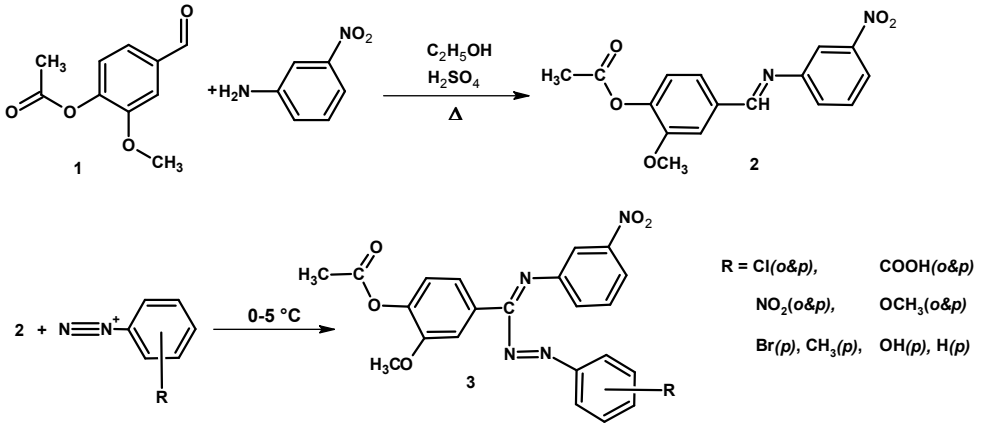
Materials and Methods

All the melting points were determined in open glass capillaries and are uncorrected. The progress of the reaction was monitored on silica gel plates. Spots were visualized by Iodine vapors and the purity of the compounds was ascertained by column chromatography. IR spectra were recorded on a Perkin-Elmer 398 spectrophotometer. The NMR spectra were recorded on a Bruker DRX-300 FT-NMR spectrometer. The mass spectra were obtained on a JEOL-SX-102 instrument using fast atom bombardment (FAB positive). Elemental analysis was performed on Carlo Erba 1108 analyzer. All chemicals and reagents were AR grade and were used after further purification. The synthetic route to the required compounds has been outlined in scheme 1.

General procedure for the synthesis of compounds

2-methoxy-4-{[(3-nitrophenyl)imino]methyl}phenylacetate (2): 4-hydroxy-3-methoxy benzaldehyde (0.005M) and *m*-nito aniline (0.005m) were dissolved in ethanol (12mL) and the contents were refluxed for three hours. The reaction mixture was cooled in ice and acidified with sulphuric acid as a condensing agent. The contents were scratched when solid separated. It was filtered under suction, washed with ethanol and recrystallised with glacial acetic acid.

2-methoxy-4-{[(3-nitrophenyl)imino][phenyldiazenyl]methyl}phenyl acetate (3): Aniline (0.01M) was dissolved in aqueous hydrochloric acid (7mL; 6N). The contents were cooled (0-2°C) and stirred. The cold solution of sodium nitrite (1gm in 4ml water) was slowly added to it by maintaining temperature (0-2°C). Cold diazotized solution was added drop wise to well cooled (0-2°C) and stirred mixture of 2-methoxy-4-{[(3-nitro phenyl) imino] methyl} phenol (0.01M) in pyridine (12mL). Stirring was further continued for one hour. The reaction mixture was kept in ice bath for four hours and then poured in ice water (200mL). The mixture was gently stirred which resulted in precipitation as colored solid. The solid was filtered, washed with water, dried and recrystallised with methanol.



Synthetic Scheme for Formazans

2-methoxy-4-{[(3-nitrophenyl)imino]methyl}phenyl acetate (2): Yield 89.4 %, m.p. 110°C; IR (KBr) cm^{-1} 1625.8(CH=N), 1763.9 (C=O), 1246.6 & 1029.6 (OCH3), 1528 & 1350.9 (NO_2); NMR (DMSO-d_6) δ; 2.3 $OCOCH_3$ (m) 3H, 3.8 OCH_3(s) 3H, 9.8CH=N(s) 1H, 7.3-7.8 Ar-H (m) 7H. MS(m/z)[M^+] 314. Anal. Calcd. for $C_{22}H_{18}N_4O_5$: C 63.79, H 4.24, N 14.84. Found C 63.83, H 4.28, N 14.89.

2-methoxy-4-{[(3-nitrophenyl)imino][phenyldiazenyl]methyl}phenyl acetate (3l): Yield 61.1 %, m.p. 80°C; IR(KBr) cm^{-1} 1634.8 (C=N), 1599.6 (N=N), 1762.1 (C=O), 1217.6 & 1037 (OCH_3), 1524.8 & 1349.9 (NO_2); NMR (DMSO-d_6) δ, 3.8 (3H) OCH_3(s), 2.3 (3H) CH_3(m), 7-7.8 (7H) ArH(m); MS (m/z) 418 [M^+] Anal. Calcd. for $C_{22}H_{18}N_4O_5$; Calcd (found); C 63.15 (63.03), H 4.34 (4.28), N 13.39 (13.30).

MICROBIOLOGY

Antibacterial activity

In disc-diffusion assay, few colonies of organisms were inoculated in 2–5 mL nutrient broth and grown for 2.5 h. The agar plates were dried and inoculated by spreading the bacterial suspension evenly over it. The sterile paper discs (6 mm) impregnated with fixed dose viz., 800 μg/mL of compound were placed on the pre inoculated surface. The disc-bearing plates were incubated at 37°C and examined at 48 h for zone of inhibition, if any, around the disc. Chloramphenicol was used as the standard drug. An additional negative control disc without any sample but impregnated with equivalent amount of solvent (DMF) was also used in the assay.

Antifungal activity

In broth dilution assay, solutions of different concentrations of the compounds were prepared in the range from 15.62 to 4000 μg/mL in DMF. Sabouraud's Dextrose treated with solutions of various concentrations of the title compound was employed as the media in sterile petriplates and suspension of fungal cultures was inoculated in them. The petridishes were incubated for 72 hrs at 37°C. Fluconazole was also screened under similar conditions as the reference drug. The dilution of a compound showing no visible growth of fungi was taken as minimum inhibitory concentration (MIC) of the compound.

RESULTS AND DISCUSSION

Antibacterial testing of the title compounds was performed by the disc diffusion assay using *S. aureus, E. coli, B. anthresis, S. flaxinerri & K. pneumonia* as the human bacterial pathogens. The results of antibacterial activity, in table- 1 indicate that all the synthesized compounds exhibited significant activity, for all the bacterial strains employed. *S. aureus* is the most inhibited bacteria and *B.anthresis* is the least inhibited one. The most potent compounds are 3a, 3b, 3i, 3j & 3k. Among the series of compounds the best growth inhibitory activity has been shown by 3b against *S. aureus*. Also 3a, 3i, 3k & 3l compounds have shown substantial activity against *S. aureus*. Compounds 3c, 3d, 3e, 3f, 3g, 3h & 3l have moderate inhibitory activity.

Antifungal activity was carried out by broth dilution method using *C. albicanes, A. fumigatus, C. neoformans, A. niger & P. Ittalecum* human fungal pathogens. Table 1 indicates that the entire series of test compounds shows moderate activity against all fungal pathogens. On the basis of our experiments and results we conclude that:

[I] Activity of the title compounds enhances on substitution.

[II] Title compounds show higher activity with chloro substitution at para & ortho positions respectively.

[III] Para substitution shows higher activity instead of ortho substitution.

[IV] Title compounds (3) shows satisfactory activity against bacterial pathogens as compared to their fungal counterparts.

ACKNOWLEDGEMENTS

Authors are thankful to Birla Institute of Medical Research and College of life sciences, Gwalior, for antimicrobial screening, CDRI, Lucknow for spectral analysis and ICMR New Delhi for financial support.

REFERENCES

1. Halve, A.K. Dubey, R. Bhadauria, D. (2007). N/C-4 substituted azetidin-2-ones: synthesis and preliminary evaluation as new class of antimicrobial agents, *Bioorgnic & Medicinal Chemistry Letters*, **17**: 341-345.
2. Halve, A.K. Bhadauria, D. Bhaskar, B. Dubey, R. Sharma, V. (2007). Synthesis and *in vitro* efficacy of some halogenated imine derivative as potential antimicrobial agents, *Indian Journal of Pharmaceutical Sciences*, **69**(5):680-682.
3. Halve, A.K. Kankoriya, A. (2010). Synthesis and Therapeutic Evaluation of Some New Thiosemicarbazones Along with N & S Heterocycles, *International Journal of Current Chemistry*, 1(2): 111-117.
4. Halve, A.K. Bhaskar, B. Sharma, V. Bhadauria, R. Kankoriya, A. Soni, A. Tiwari, K. (2008). Synthesis and in vitro antimicrobial studies of some new 3-[penyldiazzehyde] benzaldehyde N-phenyl thiosemicarbazone, *Journal of Enzyme inhibition and Medicinal Chemistry*, **23**(1):77-81.
5. Halve, A.K. Dubey, P.K. Kankoriya, A. Tiwari, K. (2009). 4-phenyldiazenyl 2-(phenylimino metyl) phenols; synthesis and *in vitro* biological evaluation as potential antibacterial agents, *Journal of Enzyme inhibition and Medicinal Chemistry*, **24**(1): 176-180.
6. Stefanescu, E. Dorneanu, M. Danila, C. Aprotosoaie, C. Grosu, G. Pavelescu, M. (1997). Hydrazones and formazans with possible biological activity. *Revista Medico-Chiruricala A Societatii de Medici si Naturalisti Din Iasi*, **101**(3-4): 178-82.
7. Desai, J.M. and Shah, V.H. (2003). Synthesis and antimicrobial profile of 5-imidazolinones, sulphonamides, azomethines, 2-azetidinones and formazans derived from 2-amino-3-cyano-5-(5'-chloro-3'-methyl-1'-phenyl pyra-zol-4'-yl vinyl)-7,7-dimethyl-6,7-dihydro benzo (b) thiophenes *Indian Journal of Chemistry*, **42B**(3), 631-635.
8. Desai, K.G. Desai, K.R. (2005). Synthesis of some novel pharmacologically active Schiff bases using microwave method and their derivatives formazans by conventional method *Indian Journal of Chemistry*, **44B**(10), 2097-2101.
9. Marjadi SI, Solanki JH and Patel AL, (2009). Synthesis and Antimicrobial Activity of Some New Formazan Derivatives, *E-Journal of Chemistry*, **6**(3), 844-848.
10. Mukerjee DD, Shukla SK, Chowdhary BL, (1981). Synthesis of Some New Formazans as Potential Antiviral Agents *Archiv der Pharmazie - Chemistry in Life Sciences*, **314**(12), 991-994.
11. Tiwari MM, Agarwal M, Saxena V, Bajpai SK, Joshi MM, (1995). Synthesis and antiviral activity of some new farmazans *Indian Journal of pharmaceutical Sciences*, **57**(3): 113-116.

12. Archana, Srivastava VK, Kumar A, (2003). Synthesis Of Newer Indolyl Thiadiazoles And Their Thiazolidinones And Formazans As Potential Anticonvulsant Agents, *Indian Journal of pharmaceutical Sciences*, **65**(4),358-362.
13. Zhao Q, Ernst JT, Hamilton AD, Debnath AK, Jiang S, (2002). XTT formazan widely used to detect cell viability inhibits HIV type 1 infection in vitro by targeting gp41. *AIDS Research and Human Retroviruses*, **18**(14), 989-997.
14. Sethi G, Gujrati VR, Nath C, Agarwal JC, Bhargava KP, Shanker K, (1983). Newer formazans and tetrazolium indoles as potential CNS-active agents, *Arzneimittel-Forschung/Drug Research.* **33**(9): 1218-1221.
15. Kidwai M, Negi N, Gupta SD, (1994). Synthesis and antifertility activity of 1,5-diaryl-3-(3'-indolyl)formazans *Chem Pharm Bull,* , **42**, 2363-2364.
16. Lipunova GN, pervova IG, lipunov IN, (1997). Synthesis and complex-forming properties of polymeric formazans *Journal of Polymer Science Part B: Polymer Chemistry*, **39**(9-10) 325-328.
17. Sigeikin GI, Lipunova GN, Pervova IG, (2006). Formazans and their metal complexes *Russian Chemical Reviews*, **75**(10), 885-900.
18. Gilroy JB, Ferguson MJ, McDonald R, Patrick BO, Hicks RG, (2007). Formazans as beta-diketiminate analogues. Structural characterization of boratatetrazines and their reduction to borataverdazyl radical anions *Chemical communications*, **14**(2): 126-128.
19. Edwards LC, Freeman HS, Claxton LD, (2004). Developing azo and formazan dyes based on environmental considerations: Salmonella mutagenicity *Mutation Research*, **26,546**(1-2): 17-28.
20. Stellmach J. (1984). Fluorescent redox dyes. 1. Production of fluorescent formazan by unstimulated and phorbol ester- or digitonin-stimulated Ehrlich ascites tumor cells, *Histochemistry*, **80**(2): 137-143.
21. Mishra VS, Dhar S, (1980). Synthesis of some newer formazans and tetrazolium salts and their effect on Ranikhet disease virus and the vaccinia virus. *Pharmazie*, **35**(10); 585-586.
22. Kalsi R, Pande K, Bhalla TN, Parmar SS, Barthwal JP, (1988). Novel formazans as potent anti-inflammatory and analgesic agents, *Pharmacology*, **37**(4); 218-224.
23. Kalsi R, Pande K, Bhalla TN, Barthwal JP, Gupta GP, Parmar SS. (1990), Anti-inflammatory activity of quinazolinoformazans, *Journal of Pharmaceutical Sciences,* **79**(4); 317-20.
24. Adler RA, Naumann SA, Mansouri A, Krieg RJ Jr, Latta K, Sanders KM, (1994) Anti-proliferative effects of deflazacort on Nb2 cells as quantitated by formazan production, *Life Sciences*, **55**(23): 1823-1831.
25. Kumar, P. Nath, C. and Shanker, K. (1985). Newer dopamine quinazolones as anti-parkinsonian agents, *Pharmazie*, **40**(4): 267-268.

Received on April 30, 2011.

Table 2 In-vitro antimicrobial activity of title compounds 3a-l.

Comp.	R	Bacterial Pathogens[a]					Fungal Pathogens[b]				
		S. aureus	*E. coli*	*B.anthresis*	*S. flaxinerri*	*K. pneumonia*	*C. albicanes*	*A. fumigatus*	*C. neoformans*	*A. niger*	*P. ittalecum*
3a	Cl	24	23	20	25	22	1000	500	1000	500	250
3b	Cl	25	23	21	26	23	500	500	500	500	500
3c	COOH	12	10	09	12	10	2000	2000	2000	1000	1000
3d	COOH	15	12	11	14	12	1000	2000	1000	1000	1000
3e	NO_2	15	12	10	15	13	- -	2000	- -	2000	1000
3f	NO_2	17	15	11	16	14	- -	2000	2000	2000	1000
3g	OCH_3	14	13	13	17	15	2000	500	2000	500	500
3h	OCH_3	18	16	14	19	17	1000	500	1000	500	500
3i	Br	21	20	20	22	20	1000	4000	1000	4000	2000
3j	CH_3	20	19	16	20	18	2000	2000	2000	2000	1000
3k	OH	22	20	16	23	21	4000	4000	4000	- -	4000
3l	H	10	08	06	05	06	4000	- -	4000	4000	2000
Chl	--	38	37	35	37	36	NA	NA	NA	NA	NA
Flu	--	NA	NA	NA	NA	NA	500	500	1000	500	250

a Inhibition zone diameter in millimeters at 800µg/mL concentration of compounds.
b The MIC value was defined as the lowest concentration of each compound in the tube(i.e. no turbidity) of inoculated fungi.
'NA' not applies.
'- -' no activity
Antibacterial susceptibility of compound was measured in the term of zone of growth inhibition.
Antifungal susceptibility of compound was measured in the term of serial dilution tube technique.