JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2010. Vol. 4(2), p. 797-801

Antimicrobial activity of 1-Substituted phenyl-3-Substituted phenyl-4-(*O*-carboxyphenyl) Formazans

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(Received: 01 April 2010; accepted: 11 May 2010)

Novel series of 1-substituted phenyl-3-substituted phenyl-4-(*o*-carboxyphenyl) formazans (3a-3n) were synthesized by coupling aryl diazonium salts of various aromatic substituted amines with *N*-*o*-carboxyphenyl-2-substituted phenyl azomethines (2a-2f). The later were prepared by condensation of anthranilic acid with various substituted aldehydes. The structures of all these compounds were established on the basis of elemental analysis, IR and ^{1}HNMR and mass spectral data. All the synthesized compounds have been screened for their antimicrobial activity against gram-positive, gram-negative and fungal pathogens. The synthesized compounds were found to have significant effect against the tested organisms.

Key words: Synthesis, Anthranilic acid, Formazans, Azomethines, Antimicrobial activity.

Formazans show promising anti-fertility[1], antiparkinsonian[2-3], anticancer[4], antibacterial[5-7], antifungal[5-7], antiviral[8-9], antidepressant[10], MAO inhibitory[10], analgesic[11-12] and anti-inflammatory[11-12] activities. All these valid observations led us to synthesize some new formazan derivatives to explore their possible biological activities.

MATERIAL AND METHODS

Melting points of the newly synthesized compounds were determined by open capillary method and uncorrected. Purity of the compound was checked by TLC using silica gel-G plate, chloroform: benzene (8.5:1.5) as mobile phase and iodine vapours as detecting agent. IR spectra (KBr, cm^{-1}) were recorded on Perkin Elmer FTIR spectrophotometer. ^{1}H NMR spectra were recorded on Brucker AMX 400 MHz instrument using TMS as internal standard. Mass spectra were recorded on Agilent LC-MSD spectrometer. The synthesis of 1-sub stituted phenyl-3-substituted phenyl-4-(*o*-carboxyphenyl) formazans is given in the Scheme 1. The physical data are given in Table 1.

Synthesis of *N*-*(o*-*c*arboxyphenyl)-*N*-substituted phenyl azomethines (2a-2f)

A mixture of anthranilic acid (0.1 mole) and aldehyde or substituted aldehyde (0.1 mole) was refluxed for 4 hrs in alcohol and poured into ice-cold water. The resulting mass was filtered, dried and recrystallized from DMSO.

Synthesis of 1-substituted phenyl-3-substituted phenyl-4-(*o* -carboxyphenyl) formazans (3a-3n)

The diazonium salts derived from the respective amines (0.01 mole) were added with stirring to *N*-*o*-carboxyphenyl-*N*-substituted phenyl azomethine (0.01 mole) in pyridine at 0^0-

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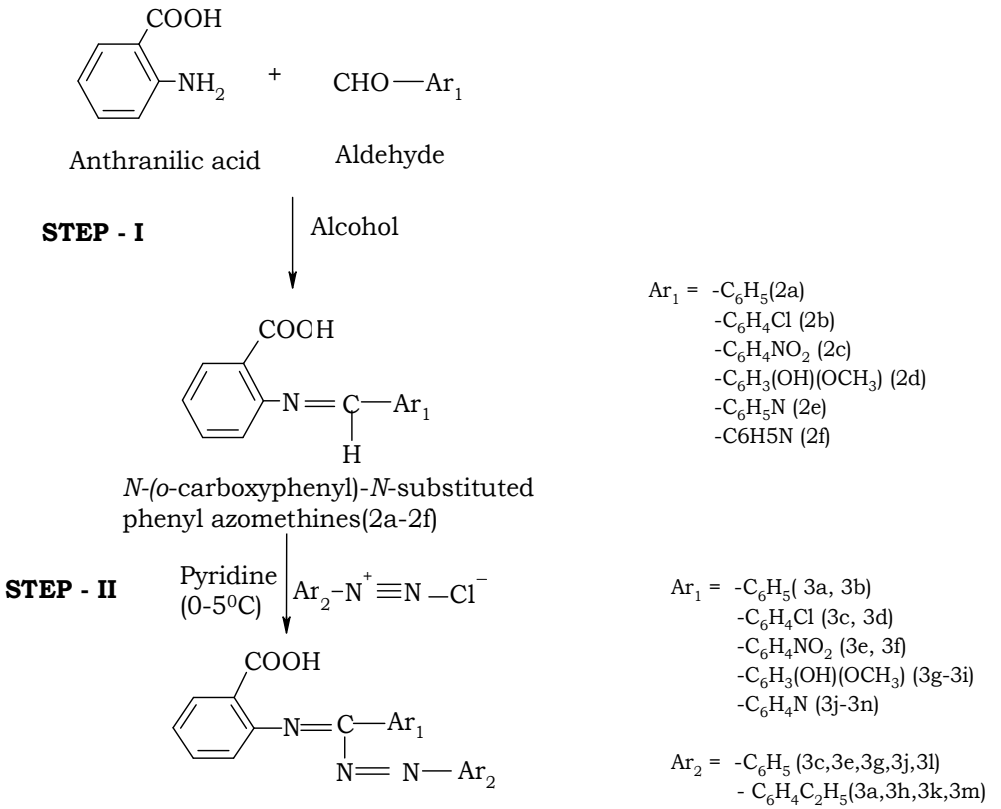
5^0C for 30 minutes. The mixture was added to ice-cold water. The resultant product was filtered, dried and crystallized from DMSO.

Antimicrobial screening

Screening of formazans for antibacterial activity by Disc diffusion Method[13-19]

All the synthesized compounds of present study were screened for *in-vitro* antibacterial activity against six different strains of bacteria i.e. Gram negative organisms like *Escherichia coli* MTCC 1687, *Pseudomonas aeruginosa* MTCC 1035 and Gram positive organisms i.e *Bacillus subtilis* MTCC 441, *Bacillus cereus* MTCC 430, *Staphylococcus aureus* MTCC 737, *S. epidermidis* MTCC 3086 by paper disc method.

Whatman filter paper grade-1 disc of 5 mm diameter was sterilized by autoclaving for 15 min at 121^0C. The sterile discs were impregnated with different synthesized compounds which were dissolved in DMF at the concentration level of 2 mg/disc, 5 mg/disc and 10 mg/disc. The nutrient agar of 20 ml was placed in a flat bottomed petridish. When solidified 4 ml of second nutrition solution seeded with test bacteria was poured evenly on to the first layer (40 – 48^0C). As soon as the second layer was solidified, the impregnated discs were placed on the medium suitably spaced apart and plates were incubated at 5^0C for 1 hr, to permit good diffusion and transferred to an incubator at 37^0C ± 1^0C for 18-24 hrs. The inhibition zones caused by various synthesized compounds and standard drugs (Ciprofloxacin, Cefetoxime) on the micro organisms were examined and results were given in the Table 2.



Scheme 1.

Screening of formazans for Antifungal activity by Disc Diffusion method[13-19]

All synthesized compounds of present study were screened for *in-vitro* anti fungal activity against four organisms i.e *Aspergillus niger* MTCC 2638, *Saccharomyces cerevisiae* MTCC 170, *Candida albicans* MTCC 3018, *Candida glabrata* MTCC 3019 by the paper disc method. What man filter paper grade-1 disc of 5 mm diameter was sterilized by autoclaving for 15 min at 121^{0}C. The sterile discs were impregnated with different synthesized compounds.

All test compounds were dissolved in DMF at the concentration level of 2 mg/disc, 5 mg/disc and 10 mg/disc. The nutrient agar of 20 ml was placed in a flat bottomed petridish. When solidified 4 ml of second nutrition solution seeded with test bacteria was poured evenly on to the first layer (40-48^{0}C). As soon as the second layer was solidified, the impregnated discs were placed on the medium suitably spaced apart and plates were incubated at 5^{0}C for 1 hr, to permit good diffusion and transferred to an incubator at 25^{0}C ± 1^{0}C for 72 hrs. The inhibition zones caused by various synthesized compounds and standard drugs (Fluconazole and Clotrimazole) on the micro organisms were examined and results were given in Table 2.

RESULTS AND DISCUSSION

All synthesized compounds (10mg/ml) were evaluated for their *in vitro* antimicrobial activity against bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis,*

Table1. Physical data of Synthesized Compounds

Compd.	Ar_1	Ar_2	Physical State	M.f (M.W)	m.p. (^{0}C)	Yield (%)
3a	C_6H_5	$C_6H_4C_2H_5$	Black Sticky	$C_{22}H_{19}N_3O_2$ (357.4)	290	57.6
3b	C_6H_5	$C_6H_4CH_3$	Brown amorphous	$C_{21}H_{17}N_3O_2$ (343.4)	186	67.2
3c	C_6H_4Cl	C_6H_5	Blackish brown amorphous	$C_{20}H_{14}N_3O_2Cl$ (363.8)	202	44
3d	C_6H_4Cl	$C_6H_4CH_3$	Black crystalline	$C_{21}H_{16}N_3O_2Cl$ (377.8)	148	60
3e	$C_6H_4NO_2$	C_6H_5	Black amorphous	$C_{20}H_{14}N_4O_4$ (374.3)	170	54.6
3f	$C_6H_4NO_2$	$C_6H_4CH_3$	Black crystalline	$C_{21}H_{16}N_4O_4$ (388.4)	150	54.9
3g	C_6H_3 (OH) (OCH_3)	C_6H_5	Black Sticky	$C_{21}H_{17}N_3O_4$ (375.4)	178	63.3
3h	C_6H_3 (OH) (OCH_3)	$C_6H_4C_2H_5$	Brown amorphous	$C_{23}H_{21}N_3O_4$ (403.5)	272	67
3i	C_6H_3 (OH) (OCH_3)	$C_6H_4CH_3$	Brown amorphous	$C_{22}H_{19}N_3O_4$ (389.4)	154	55
3j	C_6H_4N	C_6H_5	Yellow amorphous	$C_{21}H_{18}N_4O_2$ (358.3)	270	45
3k	C_6H_4N	$C_6H_4C_2H_5$	Brown amorphous	$C_{20}H_{16}N_4O_2$ (344.4)	162	47.2
3l	C_6H_4N	C_6H_5	Yellow amorphous	$C_{19}H_{14}N_4O_2$ (330.3)	188	58.3
3m	C_6H_4N	$C_6H_4C_2H_5$	Yellow amorphous	$C_{21}H_{18}N_4O_2$ (358.3)	272	60.2
3n	C_6H_4N	$C_6H_4CH_3$	Brown amorphous	$C_{20}H_{16}N_4O_2$ (344.4)	190	53.1

Table 2. Antimicrobial screening

Compd.	Zone of inhibition (mm)									
	Antibacterial activity						Antifungal activity			
	B.s	*B.c*	*E.c*	*S.a*	*S.e*	*P.s*	*C.a*	*C.g*	*A.n*	*S.c*
3a	26	19	19	16	14	17	8	25	-	13
3b	8	16	17	15	12	18	10	22	-	16
3c	13	12	-	17	12	12	-	14	-	-
3d	21	21	-	-	9	14	-	27	-	-
3e	21	20	18	22	16	16	17	19	-	-
3f	9	12	11	16	13	8	13	13	-	-
3g	13	11	6	13	-	14	7	17	14	12
3h	6	7	8	6	10	9	-	15	30	10
3i	11	13	6	11	8	10	9	22	11	14
3j	9	6	9	6	8	17	-	16	30	12
3k	11	11	10	9	12	15	6	17	11	14
3l	-	-	-	-	-	6	-	9	-	-
3m	11	10	-	-	-	9	-	17	-	10
3n	13	11	8	13	8	9	8	22	10	12
Std.1	20	17	22	20	20	21	19	22	17	18
Std.2	21	17	21	19	25	17	20	20	-	16

(-) indicates no zone of inhibition

B.s : Bacillus subtilis *C.a : Candida albicans* *B.c : Bacillus cereus*
C.g : Candida glabrata *E.c : Escherichia coli* *A.n : Aspergillus niger*
S.a: *Staphylococcus aureus,* *S.c : Saccharomyces cerevisiae* *S.e: Staphylococcus epidermidis*
P.s:Pseudomonas aeruginosa

For antibacterial activity: Std.1- Ciprofloxacin, Std.2-CefetoximeFor antifungal activity: Std.1-Fluconazole, Std.2-Clotrimazole

Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis and the fungal strains *Aspergillus niger*, *Saccharomyces cerevisiae, Candida albicans* and *Candida glabrata* by disk diffusion method. Ciprofloxacin and Cefetoxime were used as standard drugs for antibacterial and Fluconazole and Clotrimazole were used as standard drugs for antifungal studies respectively.

Compounds 3a and 3e exhibited broad spectrum of activity. Compounds 3b, 3d, 3f, 3g, 3i, 3k, 3n possesses moderate antibacterial activity.

It is evident from the screening data of antifungal activity, that compounds 3a was effective against *Saccharomyces cerevisiae* and *Candida glabrata*, 3d was effective against *Candida glabrata*, 3e was effective against *Candida albicans* and *Candida glabrata*, 3g was effective against *Saccharomyces cerevisiae, Candida glabrata* and *Aspergillus niger* and 3h was effective against *Aspergillus niger* and *Candida glabrata.*

CONCLUSION

In the present study potentially therapeutic carboxyphenyl formazans were synthesized and were characterized. All synthesized compounds were subjected to *in-vitro* antibacterial and *in-vitro* antifungal activities and the results were presented in Tables. The activity of the compounds depends upon the nature and position of the substituents at the aryl moiety. The presence of certain substituents especially nitro, dimethylamino, methoxy, methyl groups when attached to the phenyl ring augmented the activity remarkably. In conclusion the present work provides excellent approach for the synthesis of potent antimicrobial formazans derivatives.

ACKNOWLEDGEMENTS

The authors are greatful to Chalapathi Institute of Pharmaceutical Sciences, Guntur for providing necessary facilities to carry out this research work.

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