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SYNTHESIS AND ANTIMICROBIAL TESTING OF 2-AMINO-4- (*p*-FLUORO-*m*-NITROANILINO) -6-SUBSTITUTED-*s*-TRIAZINES

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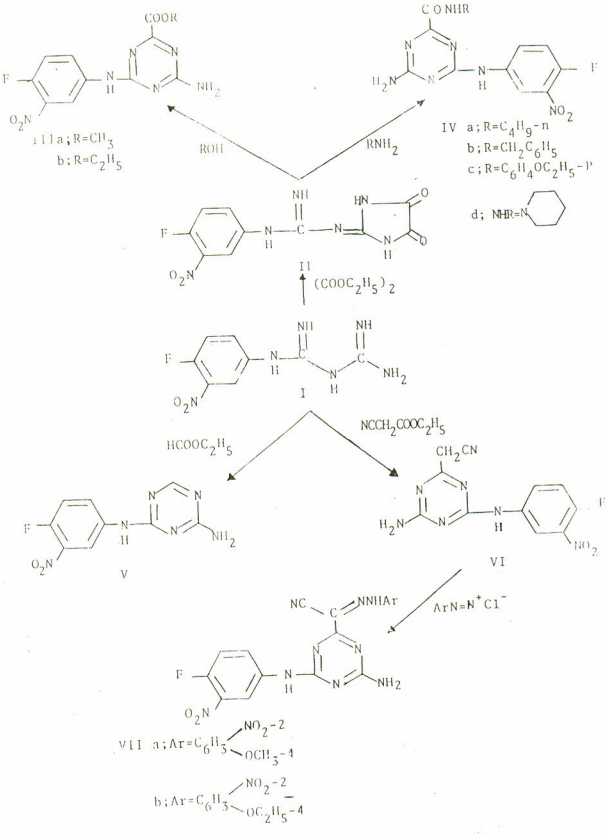
1 – (*p*-Fluoro-*m*-nitrophenyl) biguanide (1) was treated with diethyl oxalate to obtain 1-(*p*-fluoro-*m*-nitrophenyl)-3-(4, 5-dioxo-2-imidazolidinylidene) guanidine (II) as intermediate, which upon treatment with alcohols or amines afforded 2-amino-4-(*p*-fluoro-*m*-nitroanilino)-*s*-triazine-6-carboxylic acid esters ($III_{a,b}$) and acid amides (IV_{a-d}) respectively. Treatment of I with ethyl formate or ethyl cyanoacetate gave 2-amino-4-(*p*-fluoro-*m*-nitroanilino)-*s*-triazine (V) and its 6-acetonitrile derivative (VI). Coupling of VI with *p*-alkoxy-*o*-nitrophenyldiazonium chlorides yielded the corresponding azo derivatives ($VII_{a,b}$). All compounds have been tested against *Staph. aureus, Pseudomonas aeruginosa* and *Candida albicans*. Compounds $III_{a,b}$ exhibited a significant bactericidal activity.

Key words: *s*-Triazines, Biguanides, Synthesis.

INTRODUCTION

Many triazine derivatives reported to posses broad spectrum biological activity. In particular, 2-amino-4-(arylamino)-*s*-triazines with fluoro, methylthio and trifluoromethyl functional groups were found to exhibit antimicrobial activity [1, 2], The presence of a nitro group in the aryl moiety also caused a significant increase in the antimicrobial activity of *s*-triazines [3]. On these observations, this paper reports the synthesis of certain new *s*-triazine derivatives having both fluoro and nitro groups in the same molecule. We also report the results of antimicrobial testing.

The arylbigaunide (1) was prepared using the method of Overberger and Shapiro [4], by heating equimolecular amounts of *p*-fluoro-*m*-nitroaniline and dicyandiamide in aqueous hydrochloric acid. Reaction of arylbiguanides with diethyl oxalate was investigated by Furukawa [5, 6] who found that the substituted *s*-triazine were formed through the intermediate 1-aryl-3-(4, 5-dioxo-2-imidazolidinylidene) guanidine derivatives. In the present work, the 1-(*p*-fluoro-*m*-nitroanilino)-3-(4, 5-dioxo-2-imidazolidinylidene) guanidine (II) was obtained by reacting the arylbiguanide I with diethyl oxalate in anhydrous methanol. When compound II was boiled with alcohols or amines, it was converted into the *s*-triazine derivatives ($III_{a,b}$) and (IV_{a-d}) respectively. Treatment of compound I with ethyl formate afforded 2-amino-4-(*p*-fluoro-*m*-nitroanilino)-*s*-triazine (V). On the other hand, 2-amino-4-(*p*-fluoro-*m*-nitroanilino)-*s*-triazine-6-acetonitrile (VI) was obtained by heating of compound I with ethyl cyanoacetate. The coupling of diazonium salts with the active methylene compound was investigated in the present work. Thus, coupling of *p*-alkoxy-*o*-nitrophenyldiazonium chlorides with compound VI afforded the corresponding azo derivatives ($VII_{a,b}$), Scheme 1.



Scheme 1

Antimicrobial activity of the prepared compounds were tested using the standardized disc method of Bondi *et al* [7]. Compounds $III_{a,b}$ exhibited promising antibacterial effects against *St. aureus* and *Ps. aeruginosa*, while compounds IV_{a-d} and V had moderate effects against *St. aureus*. Compounds VI and $VII_{a,b}$ showed no antibacterial activity against the organisms. None of the compounds showed activity against *Candida albicans*. The results are presented in (Table 1).

Table 1. Antimicrobial activity of compounds at 100mg/ disc concentration

Comp.	*Staph aureus*	*PS. aerugi-nosa*	*Candida albicans*
III			
III_a	$(+)_2$	$(+)_2$	ϕ
b	$(+)_2$	$(+)_2$	ϕ
IV_a	$(+)_1$	ϕ	ϕ
b	$(+)_1$	ϕ	ϕ
c	$(+)_1$	ϕ	ϕ
d	$(+)_1$	ϕ	ϕ
V	$(+)_1$	ϕ	ϕ
VI	$(+)_1$	ϕ	ϕ
VIIa	ϕ	ϕ	ϕ
b	ϕ	ϕ	ϕ

ϕ = Inactive (no inhibition zone)

$(+)_1$ = Moderately active (inhibition zone 15-20 mm)

$(+)_2$ = Active (inhibition zone 20 - 25 mm)

EXPERIMENTAL

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. IR spectra were recorded on a Pye-Unicam SP 1000 spectrophotometer in KBr. ^{1}H NMR spectra were determined on a IBM FT-200 spectrometer in (DMSO-d_6). Mass spectra were determined on a Beckman Du-8 spectrometer. Elemental analysis were performed by the microanalysis unit, Cairo University. The Analytical results obtained for all compounds were within ± 0.4% of the theoretical values.

1 – (p-fluoro-m-nitrophenyl) biguanide (1). Dicyandiamide (2.52 g, 0.03 mol) was added to a solution of *p*-fluoro-*m*-nitroaniline (4.68, 0.03 mol) in a mixture of concentrated hydrochloric acid (3.3 g, 0.03 mol) and water (12.5 ml). The reaction mixture was heated on a steam bath, for 3 hr. then left overnight at ambient temperature. The separated solid product was collected, dried and recrystallized from water to give 6.9 g (83%) of the HCl salt of compound I, mp 208°C. Anal. ($C_8H_9FN_6O_2$. HCl) C, H, N.

To obtain the free base the hydrochloride salt (5.5 g) in was dissolved warm water (30 ml), and sodium hydroxide solution (3 ml, 30%) added gradually with stirring. The separated solid was collected, washed with water, dried and recrystallized from aqueous methanol gave 4.5 g (95%) of free base of 1, m.p 184°C. Mass spectrum: m/z 240 (M); UV: λ max at pH 7 252, 207 nm; ^{1}H NMR, δ 3.35 (s, 2H, C = NH), 7.22 (brS, 2H, NH_2), 7.90 (m, 3H, ArH), 9.96 (s, 1H, NH). Anal. ($C_8H_9FN_6O_2$) C, H, N.

1 – (p-fluoro-m-nitroanilino)-3-(4,5-dioxo-2-imidazolidinylidene) guanidine (II). A mixture of compound I (2.4 g, 0.01 mol) and diethyloxalate (1.5 g, 0.01 mol) in methanol (10 ml) was warmed at 40° for 30 min. The reaction mixture was set aside at room temperature overnight and the yellow precipitate collected by filtration under suction and washed thoroughly with methanol to yield 2.88 g (98%) of analytically pure II, mp 205-206°C. IR 1650 (C = O), 1730 (C = O) and 3325 (NH) cm-1. Anal. ($C_{10}H_7FN_6O_4$) C, H, N.

*Methyl 2-amino-4-(p-fluoro-m-nitroanilino)-s-triazine-6-carboxylate (III*a–). A suspension of II (294 mg, 1 mmol) in anhydrous methanol (10 ml) was heated under reflux for 5 hrs till a clear solution was obtained. The solid separated by cooling was collected, dried and recrystallized from ethanol to give 0.26 g (86%) of III_a, mp 235°; mass spectrum m/z 308 (M+H); ^{1}H NMR, δ 3.85 (s, 3H, OCH_3), 8.6-7.4. (m, 5H, NH_2 and ArH), 10.42 (s, 1H, NH). Anal. ($C_{11}H_9FN_6O_4$) C, H, N.

*Ethyl 2-amino-4-(p-fluoro-m-nitroanilino)-s-triazine-6-carboxylate (III*b–). Prepared by the same procedure for preparation of III_a in 78% yield, mp 212°, Mass spectrum, m/z 322 (M+H); UV λ max at pH 7, 266 nm. Anal. ($C_{12}H_{11}FN_6O_4$) C, H, N.

2-Amino-4-(p-fluoro-m-nitroanilino)-s-triazine-6-carboxamides (IV_{a-d}): General method. A solution of II (1 mmol), in an excess of the appropriate amine (2 ml), was heated on water bath for 5 hrs. The solids separated by cooling were collected, dried, and recrystallized from aqueous ethanol.

IV_a; was obtained in 65% yeild, mp 204°C.Anal. ($C_{14}H_{16}FN_7O_3$) C, H, F.

IV_b; 62% yield, mp 212°C. IR 1640 (C = O) cm^{-1} Anal. ($C_{17}H_{14}FN_7O_3$) C, H, F.

IV_c; 63% yield, mp 245°C. Anal. ($C_{18}H_{16}FN_7O_4$) C, H, F.

IV_d; 60% yield, mp 145°C. Anal. ($C_{15}H_{16}FN_7O_3$) C, H, F

2-Amino-4-(p-fluoro-m-nitroanilino)-s-triazine (V). Ethyl formate (0.37 g, 5 mmol) was added to a solution of 1 (1.2 g, 5 mmol) in methanol (10 ml). The reaction mixture was heated under reflux for ½ hr, and set aside at room temperature for overnight. The precipitated solid was collected, dried, and recrystallized from ethanol to give 0.88 g (71%) of V, mp 260°C. Mass spectrum m/z 250 (M); UV ± max at pH 7 278 nm. Anal. ($C_9H_7FN_6O_2$) C, H, N.

2-Amino-4-(p-fluoro-m-nitroanilino)-s-triazine-6-acetonitrile (VI). A mixture of compound I (1.2 g, 5 mmol) and ethyl cyanoacetate (0.57 g, 5 mmol) in methanol (10 ml) was heated under reflux for 5 hrs. The solid separated by cooling was collected, dried, recrystallized from ethanol gave 1.13 g (78%) of VI mp 154°C. Mass spectrum m/z 289 (M); UV λ max at pH 7 277 nm; ^{1}H NMR δ 4.0 (s, 2H, CH_2 CN), 8.2-7.4 (m, 5H, NH_2 + ArH), 10.12 (s, 1H, NH), IR 2260 (CN), 3350 and 3200 (NH) cm^{-1} Anal. ($C_{11}H_8FN_7O_2$) C, H, N.

2-Amino-4-(p-fluoro-m-nitroanilino)-6-[q (p-alkoxy-o-nitro-phenylhydrazono) acetonitrile]-s-triazines ($VII_{a,b}$). A solution of the 4-alkoxy-2-nitroaniline (0.84 g, 5 mmol) in glacial acetic acid (10 ml) and concentrated hydrochloric acid (15 ml) was cooled and diazotized with a solution of sodium nitrite (0.35 g, 5 mmol) in water (25 ml). The cold diazonium salt was added gradually with continuous stirring to a cold solution of compound VI (1.44 g, 5 mmol) in ethanol (50 ml), sodium acetate (5 g) and water (5 ml). The solid products were collected by filteration, washed with water, dried, and recrystallized from acetic acid.

VII_a; was obtained in 85% yield, mp 210°. Mass spectrum m/z 468 (M) ^{1}H NMR δ 3.93 (s, 3H, OCH_3), 7.9-7.4 (m, 8H, NH_2+ ArH) . Anal. ($C_{18}H_{13}FN_{10}O_5$) C, H, N.

VII_b; 88% yield, mp 215°C. Anal. ($C_{19}H_{15}FN_{10}O_5$) C, H, N.

Biological screening. The Antimicrobial effect of the prepared compounds on different strains of various organisms was studied according to the following method: Filter paper discs were moisted with solutions of the test compounds (100 ug/disc) in DIMF and carefully placed on an agar culture plates that had been inoculated separately with Staph. aureus (NCTC 7447), *Pseudomonas aeruginosa* and *Candida albicans* (ATCC 753). The plates were incubated at 37° for 24 hours, and the zone of inhibition around the disc measured to the nearest mm. The results are shown in Table 1.

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