SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NEW 1-[4-(4-FLUOROBENZOYLAMINO)-BENZOYL]-4-SUBSTITUTED THIOSEMICARBAZIDES

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SUMMARY

1-Aroyl-4-substituted thiosemicarbazides were obtained by the addition of 4-(4-fluorobenzoylamino)benzoylhydrazine to methyl, ethyl, propyl, allyl, cyclohexyl, phenyl and phenethyl isothiocyanates. The structures of the synthesized compounds were confirmed using UV, IR, ^{1}H-NMR (for compounds 4c, 4g) and mass (for compounds 4b, 4c, 4g) spectral methods together with elemental analyses. None of the synthesized compounds has been reported previously.

KEY WORDS

substituted thiosemicarbazide, synthesis, spectral methods, microbiology

INTRODUCTION

Thiosemicarbazide derivatives are of interest due to their bioactivity, including antifungal /1,2/, antitubercular /3-5/, antiviral /6/ and anticonvulsant /7/ properties. In a previous study /8/, we described the preparation of 1-[4-(benzoylamino)benzoyl]-4-substituted thiosemicarbazides which showed antifungal activity. We report here on

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the synthesis, structure and microbiological evaluation of 1-[4-(4-fluorobenzoylamino)benzoyl]-4-substituted thiosemicarbazides.

MATERIALS AND METHODS

Chemicals and instruments

Chemicals used in the experiments were purchased from Merck and Sigma companies. All melting points were determined on a Buchi-530 melting point apparatus (open capillaries) and uncorrected. UV spectra were determined on a Shimadzu UV 2100S spectrophotometer (approx. 1 mg/100 ml ethanol). IR spectra were run on a Perkin-Elmer 298 spectrophotometer as KBr pellets. Mass spectra were taken on a Kratos MS-9/50 double focusing mass spectrometer. ^{1}H-NMR spectra were obtained on a Bruker AC 200L spectrophotometer at 200 MHz using DMSO as the internal reference. Elemental analyses were run on a Carlo Erba 1106.

Ethyl 4-(4-fluorobenzoylamino)benzoate [1] (76%) was prepared from 4-fluorobenzoylchloride and ethyl *p*-aminobenzoate (see Fig. 1). It was crystallized from ethanol. M.p. 174°C.

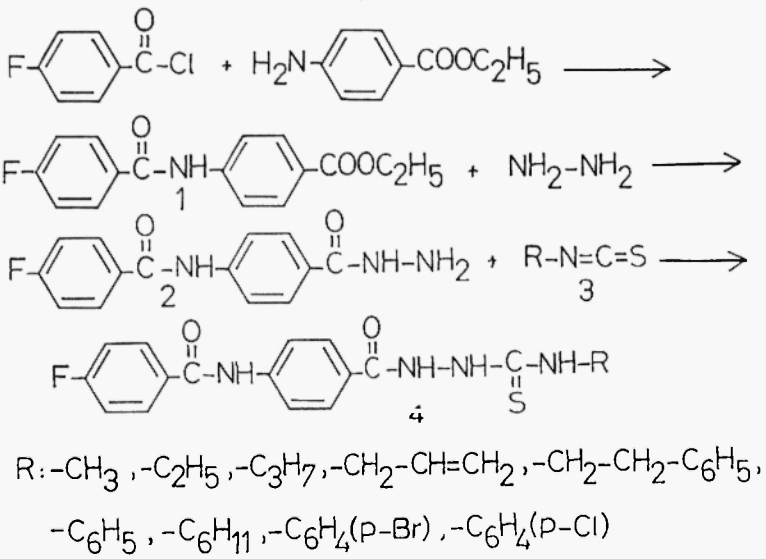

Fig. 1: Synthetic route to the title compounds.

4-(4-Fluorobenzoylamino)benzoylhydrazine [**2**] (75.8%) was prepared by heating a mixture of **1** (0.02 mol) and hydrazine hydrate (64%, 18 ml) under ruflex for 1 h at 110-130°C. The hot reaction mixture was set aside to cool to room temperature. The precipitate formed was filtered and washed with water. The crude product was purified by washing in ethanol. It was crystallized from ethanol.

1-[4-(4-Fluorobenzoylamino)benzoyl]-4-substituted thiosemicarbazides [**4**] were prepared from a mixture of hydrazide [**2**] (0.005 mol) and ethanol (80 ml), warmed until dissolved. The isothiocyanate [**3**] (0.003 mol) was added and the mixture was refluxed for 2-2.5 h. The precipitate which formed after cooling was collected by filtration and washed with water. Crystallization from ethanol afforded the pure compounds.

1-[4-(4-Fluorobenzoylamino)benzoyl]-4-methyl thiosemicarbazide [**4a**] (62%): M.Wt. 346.39; M.p. 223-225°C; UV 287.2, 238.6, 205.3 nm; IR 1685, 1640, 1180 cm^{-1}; Analysis for $C_{16}H_{15}FN_4O_2S$ (%, calculated/found): 55.48/54.11 (C), 4.36/4.72 (H), 16.17/15.66 (N).

1-[4-(4-Fluorobenzoylamino)benzoyl]-4-ethyl thiosemicarbazide [**4b**] (67%): M.Wt. 360.42; M.p. 220°C; UV 285.2, 210.2 nm; IR 1670, 1640, 1180 cm^{-1}; Analysis for $C_{17}H_{17}FN_4O_2S$ (%, calculated/found): 56.65/56.92 (C), 4.75/5.08(H), 15.54/15.27 (N).

1-[4-(4-Fluorobenzoylamino)benzoyl]-4-propyl thiosemicarbazide [**4c**] (42%): M.Wt. 374.44; M.p. 210-213°C; UV 289, 203.6 nm; IR 1655, 1650, 1180 cm^{-1}; ^{1}H-NMR δ 0.83 (t,3H, $-CH_3$), 1.50 (m, 2H, $-CH_2-$), 3.40 (q,2H, $-CH_2-$), 7.35-7.95 (Ar-H), 8.06 (s, 1H, NH-C_3H_7), 9.16 (s, 1H- CONHNH), 10.19 (s, 1H, CONH), 10.45 (s,1H, CONHNH); Analysis for $C_{18}H_{19}FN_4O_2S$ (%, calculated/found): 57.73/57.46 (C), 5.11/5.05 (H), 14.96/14.94 (N).

1-[4-(4-Fluorobenzoylamino)benzoyl]-4-cyclohexyl thiosemicarbazide [**4d**] (64%): M.Wt. 414.5; M.p. 222°C; UV 287.2, 242.8, 205.6 nm; IR 1660, 1180 cm^{-1}; Analysis for $C_{21}H_{23}FN_4O_2S$ (%, calculated/found): 60.85/60.95 (C), 5.59/5.76 (H), 13.51/13.90 (N).

1-[4-(4-Fluorobenzoylamino)benzoyl]-4-phenethyl thiosemicarbazide [**4e**] (50%): M.Wt. 436.51; M.p. 218°C; UV 288.6, 207.0 nm; Analysis for $C_{23}H_{21}FN_4O_2S$ (%, calculated/found): 63.28/62.97 (C), 4.84/5.16 (H), 12.83/13.32 (N).

1-[4-(4-Fluorobenzoylamino)benzoyl]-4-allyl thiosemicarbazide [**4f**] (63%): M.Wt. 372.43; M.p. 198-200°C; UV 286.6, 204.2 nm. IR 1670, 1645, 1180 cm^{-1}; ^{1}H-NMR δ 4.15 (t,2H, allyl CH_2), 5.01-5.191 (m, 2H, CH_2 vinyl), 5.75-5.94 (m, 1H, vinyl CH), 7.38-7.90 (Ar-H),

8.08 (s,1H, NH-C_3H_5), 9.22 (s,1H, CONHNH), 10.18 (s,1H, CONH), 10.39 (s,1H, CONHNH).

1-[4-(4-Fluorobenzoylamino)benzoyl]-4-phenyl thiosemicarbazide [4g] (36%): M.Wt. 408.46; M.p. 240°C; UV 281.2, 207.0 nm; IR 1655, 1645,1185 cm^{-1}; ^{1}H-NMR δ 7.11-8.09 (Ar-H), 9.59 (s,1H,-NH), 9.73 (s,1H, CONHNH), 10.37 (s,1H, CONH), 10.41 (s,1H, CONHNH). Mass (EI) m/e 258 (% 7.97), 242 (% 22.65), 135 (% 35.13) 123 (% 100) 95 (% 36.83), 77 (% 95.85), 51 (% 42.30). Analysis for $C_{21}H_{17}FN_4O_2S$ (%, calculated/found): 61.75/61.62 (C), 4.19/4.23 (H), 13.71/13.68 (N).

1-[4-(4-Fluorobenzoylamino)benzoyl]-4-*p*-bromophenyl thiosemicarbazide [4h] (50%): M.Wt. 487.36; M.p. 218°C; IR 1660, 1650, 1180 cm^{-1}; Analysis for $C_{21}H_{16}BrFN_4O_2S$ (%, calculated/found): 51.75/ 50.80 (C), 3.31/3.52 (H), 11.50/12.12 (N).

1-[4-(4-Fluorobenzoylamino)benzoyl]-4-*p*-chlorophenyl thiosemicarbazide [4i] (64%): M.Wt. 442.90; M.p. 230°C; IR 1655, 1650, 1190 cm^{-1}; Analysis for $C_{21}H_{16}ClFN_4O_2S$(%, calculated/found): 56.94/ 55.40 (C), 3.64/3.66 (H), 12.65/11.55 (N).

Testing for antimicrobiological activity

Chemical substances to be tested were dissolved in dimethyl sulfoxide (DMSO; Sigma D-8779) in 10 mg/ml quantities. This solution was placed onto 6 mm diameter paper disks capable of holding 0.01 ml liquid and these disks were dried in a vacuum desiccator. Control disks were saturated with DMSO only. Microorganisms were suspended in physiologic serum at a value of 0.5 Macfarland; yeast (0.01 ml) was inoculated in Saboraud dextrose-agar and bacteria were inoculated in Mueller-Hinton agar. The yeasts tested were *Candida albicans* ATCC 10231 and 3 *Candida albicans* and 2 *Candida tropicalis* produced in our laboratory; the bacteria tested were *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29953 and 3 *Escherichica coli*, 3 *Staphylococcus aureus* and *2 Pseudomonas aeruginosae* produced in our laboratory.

Comparison was made using Nystatin, Ketokonazole, imipenem and cefazolin disks. Yeasts were incubated for 48 h at 30°C and bacteria were incubated for 24 h at 37°C.

RESULTS AND DISCUSSION

Thiosemicarbazides [**4a-i**] were prepared as described above (Fig. 1).

The UV spectrum of **4g** containing an aromatic ring showed two maxima at 207 and 281.2 nm. **4a** and **4d** showed three maxima at 205.3-205.6, 238.6-242.8 and 287.2 nm. The IR spectra of **4a-i** exhibited characteristic NH stretching bands in the 3380-3220 cm^{-1} region. The absorption bands associated with other functional groups appeared in the expected regions. ^{1}H-NMR spectra of the compounds **4c**, **4f**, **4g** were consistent with their proposed structure, showing four low field singlets that could be assigned to the amide (9.16, 9.22, 9.73) and thiosemicarbazide (8.06, 10.19, 10.45, 8.08, 10.18, 10.39, 9.59, 10.37, 10.41 ppm) NH protons.

The effects of the compounds against microorganisms (yeast and bacteria) were tested using a disk diffusion method /9/. No inhibition zone was observed around the disk with any compound for the bacteria and yeast tested.

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