Synthesis of New Drug Carriers of Metronidazoleas Antifungal Agents

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Abstract

New drug carriers were synthesised through etherification with sugar moiety and metronidazole drug. The synthetic pathway was started with two sugars, β-D-glucose and β-D-fructose, which were protected as peracetate and benzoate respectively, then converted to bromo sugar. The bromo sugars were subjected to coupling with metronidazole drug via Willemenson reaction to afford protected drug carriers. All prepared compounds were characterised via FT-IR, ^{1}H-NMR and ^{13}C-NMR and HR-MS spectroscopy. These new carriers were tested against four types of fungi kandida yeast. Some of tested compounds showed moderate activity while the other showed weak inhibition. Interestingly, two carrier drugs showed high activity against fungi.

Keywords: *Prodrug, metronidazole, nucleosides, antifungal.*

Introduction

Since 1958, when Albert[(1)] initially coined the term prodrug and used to refer to pharmacologically compounds, the carrier-linked prodrugs are imparting some desirable properties to the drugs such as increased lipid or water solubility or site-directed delivery or decreased toxicity and prolonged or shortened action[(2)].

Meteronidazole, 2-methyl-5-nitroimidazole-1-ethanol (flagyl), is the most useful of a group of antiprotozoal drugs that have synthesized in several laboratories. This drug is accommodating amebicidal activity; it is valuable against both intestinal and hepatic amebiasis. It has also been explored of the use in the treatment of such other protozoal diseases as giardiasis[(3-6)]. Because of its bactericidal action, it became an important agent for treatment of serious infections (e.g. septicemia, pneumonia and meningitis) caused by anaerobic bacteria[(7-9)].

Since meteronidazole is sparingly soluble in water, our target is to link meteronidazole with sugar as carrier, which called a promoiety through glycoside linkage to increase their solubility in water. The gclycosidase enzyme found in colon bectria, allows hydrolysis of glycoside derivatives of drug in the colon, that is the side of action, and provides higher concentrations of active drug.[(10,11)]

Nucleosides, both of natural and synthetic origin have at least some biological activity.[(12)] Such as potential anti-viral[(13)] fungicidal, and anticancer agents[(14,15)]. Nucleoside analogues play significant role in several established chemotherapies (anticancer, antiviral and antibacterial).[(16-19)]

A new methodology to synthesis a new modified prodrugs using two kinds of protected sugars and meteronidazole was developed in this article. The new prodrugs were examined against fungi to evaluate them as antimicrobial and antifungal agents. Expectedly, the new compounds showed higher activity against fungi.

General experimental: All chemicals were

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purchased from Sigma-Aldrich unless otherwise stated. ^{1}H-NMR spectra were measured on a Bruker Avance 500 MHz spectrometer or or a Bruker Avance DPX 400 NMR spectrometer. ^{13}C-NMR spectra were measured on a Bruker Avance 500 MHz spectrometer(Isfahan University of Technology (IUT), Iran) and Sharif University of Technology (SUT).

Preparation of β-D Glucose Penta Acetate[20] (2): β-D glucose (0.0055 mole) and (0.00975 mole) an hydrous sodium acetate were dissolved in (6mL) acetic anhydride, and then heated under reflux for 2hrs. The resulting mixturewas poured on to (50mL) of ice-cold water, thenfiltered and recrystallized form ethanol to afford a white crystal (2) m.p: (131-132 °C); IR (thin film) cm^{-1} 2918 (m) (CH aliphatic, stretch), 1720 (s) (C=O ester, stretch); ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 2.2 (5x 3H, s, $5CH_3$), 4.2 (2H, s, CH_2), 4.6 (2 x 1H, d, 2CH), 5.1 (2 x 1H, d, 2CH), 6.21 (1H, d, CH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C25 ($5CH_3$), 64 (CH_2), 71 (2*C*H), 75 (2*C*H), 175 (5*C*O); HRMS(EI$^+$) found 390.2303, $C_{16}H_{22}O_{11}$ requires 390.2301.

General Procedure for Synthesis of 1-Bromo Acetylated Sugar[21](3): The acetylated sugar (1) (0.38 g 0.0024 mole) was dissolved in (3 ml) of (50%) hydrogen bromide in glacial acetic acid, which was added drop wise at (0°C). The solution was kept at (0°C) for one hour, and then poured in (35ml) chloroform. After a final wash with ice-water (20 ml),the organic phase was dried over anhydrous $MgSO_4$, and solvent was removed to give compound (**3**) as syrup. The isolated sugar bromide (3) was used directly for the nucleoside analogues synthesis; IR (thin film) cm^{-1} 2820 (m) (CH aliphatic, stretch), 1720 (s) (C=O ester, stretch); ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 2.1 (4x 3H, s, $5CH_3$), 4.1 (2H, s, CH_2), 4.56 (2 x 1H, d, 2CH), 5.1 (2 x 1H, d, 2CH), 6.01 (1H, d, CH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 20 ($4CH_3$), 60 (CH_2), 69 (2*C*H), 73 (2*C*H), 175 (5*C*O); HRMS(EI$^+$) found 410.2303, $C_{14}H_{19}O_9Br$ requires 410.2301.

General procedure for the preparation of protected prodrug of1-(`2,`3,`4,`6-Tetra O-acetyl –β-D-glucoPyranosyl) – ethyl -2-methyl-5-nitro imidazole(5)[22]: β-D-1-Bromo-glucose penta acetate **2** (3.5 gm, 0.1 mol), silver oxide (25 gm, 0.11 mole), calcium sulphate (10 gm, preheated for 2 hour. at 240ºC. to ensure the complete absence of water, Iodine (5 g) then was added as a catalyst. Flagyl (Metronidazole) (4.3 g, 0.1 mol) was dissolved in absolute ethanol (10 ml) and was added through dropping funnel to the stirred reaction mixture over a period of about 1 hr. The stirring is continued for 24 hr. The resulting mixture was filtered off through and the residue is washed well with chloroform. The filtrate and washings are combined together, and concentrated under reduced pressure. The residue was re-crystallized from chloroform to give brown dark powder of compound (5) in yield 75% (m.p. 197-199 °C), RF (0.75) (benzene: methanol, 6:4); IR (thin film) cm^{-1} 2918 (m) (CH aliphatic, stretch), 1720 (s) (C=O ester, stretch), 1612 (C=N, stretch); ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 1.7 (3H, s, CH_3), 2.2 (4x 3H, s, $4CH_3$), 4.2 (2H, s, CH_2), 3.6 (2H, t, CH_2O), 3.8 (2H, t, CH_2N), 4. 6 (2 x 1H, d, 2CH), 5.1 (2 x 1H, d, 2CH), 6.21 (1H, d, CH), 7.5 (1H, s, CH=N); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 12 (*C*H$_3$), 22 (4*C*H$_3$), 65 (*C*H$_2$), 67 (*C*H$_2$), 71 (2*C*H), 75 (2*C*H), 128 (*C*H), 140 (*C*-NO$_2$), 158 (*C*H=N), 172 (4*C*O); HRMS(EI$^+$) found 459.2303, $C_{20}H_{19}O_{12}$ requires 459.2301.

hydrolysis of protected prodrug (6)[22]: A solution of (0.0026mole) of the blocked nucleoside analogue in (7mL) of (0.1 M) methanolic sodium methoxide was heated under reflux for 30 min. The reaction mixture was neutralized with acetic acid and concentrated to dryness. The residue was portioned between water and chloroform. The aqueous phase evaporated to dryness under vacuum to give free prodrug (**6**); (m.p. 197-199 °C), RF (0.75) (benzene: methanol, 6:5); IR (thin film) cm^{-1}3220 (s) (OH Stretch), 2918 (m) (CH aliphatic, stretch), 1612 (C=Nimidazo, stretch); ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H1.7(3H, s, CH_3), 4.4 (2H, s, CH_2), 3.5 (2H, t, CH_2O), 3.7 (2H, t, CH_2N), 4. 6 (2 x 1H, d, 2CH), 5.1 (2 x 1H, d, 2CH), 6.21 (1H, d, CH), 7.5 (1H, s, CH=N); ^{13}C-NMR (125 MHz, $CDCl_3$) ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 12 (*C*H$_3$), 65 (*C*H$_2$), 67 (*C*H$_2$), 70 (4*C*OH), 74 (2*C*H), 77 (2*C*H), 128 (*C*H), 140 (*C*-NO$_2$), 158 (*C*H=N), 172 (4*C*O); HRMS(EI$^+$) found 451.2303, $C_{20}H_{19}O_{12}$ requires 451.2301.

Protection and Preparation of Sugar Moiety: 1,3,4,6-tetra-O-benzoyl-β-D-fructofuranose (8)[23]: Benzoyl chloride (7 ml) was added dropwise to anhydrous-D-fructose (2 g, 11.11 mmol) that suspended in mixture of chloroform (30 ml) and dry pyridine (5 ml), after that the mixture was heated at 318–338 K with continuous stirring for 4 h. The mixture was poured over ice-water, and then extracted with $CHCl_3$ (3x15 ml). The organic layer was washed with 5 % HCl solution (10 ml). The organic layer was naturalized with 5 % of sodium carbonate solution (10 ml), after that the organic layer was dried over sodium sulphate and the

solvent was concentrated under vacuum to give a syrup that crystallized from absolute ethanol to afford white crystals (m.p. 394–395 K); IR (thin film) cm^{-1} 3064 (m) (CH aromatic, stretch), 2918 (m) (CH aliphatic, stretch), 1720 (s) (C=O ester, stretch), 1655 (m) (C=C aromatic, stretch); ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 3.0-3.1 (2H, s, CH_2OBz), 3.3-3.4 (2H, s, 2CH), 7.3-7.4 (10H, m, 10CH aromatic), 7.8-7.9 (10H, m, 10CH aromatic); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 64 (*C*H_2O), 70 (2*C*H), 92 (2*C*O), 130 (8*C*H aromatic), 134 (8*C*H aromatic), 136 (4*C*H aromatic), 138 (4*C*C=O), 170 (4*C*=O); HRMS(EI$^+$) found 612.2303, $C_{34}H_{28}O_{11}$ requires 612.2301.

1,3,4,6- Tetra-O-benzoyl-β-D-fructofuranosyl bromide(9)[21]: Hydrogen bromide in glacial acetic acid (45%) (5 ml) was added to tetrabenzoyl fructofuranose 4 (2 g, 3.36 mmole), then (5 ml) of glacial acetic acid was added, the mixture was stirred for 30 min. at room temperature and left for 6 h. at room temperature, and mixture was neutralized with sodium bicarbonate solution, then extracted with chloroform (3 x 15 ml). The organic layers were dried over sodium sulphate, filtered and evaporated in vacuo to give brown syrup; IR (thin film) cm^{-1} 3217 (broad s) (OH, stretch), 3064 (m) (CH aromatic, stretch), 2918 (m) (CH aliphatic, stretch), 1720 (s) (C=O ester, stretch), 1655 (m) (C=C aromatic, stretch); ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 2.1-2.3 (1H, s, 1OH), 3.0-3.1 (2H, s, CH_2OBz), 3.3-3.4 (2H, s, 2CH), 7.3-7.4 (10H, m, 10CH aromatic), 7.5-7.6 (4H, m, 4CH aromatic);^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 64 (2*C*H_2O), 70 (2*C*H), 80 (2*C*Br), 130 (10*C*H aromatic), 134 (5*C*H aromatic), 138 (4*C*C=O), 170 (4*C*=O); HRMS(EI$^+$) found 570.2303, $C_{27}H_{23}O_9Br$ requires 570.2301.

General procedure for the preparation of protected prodrug of1-(-(´1, ´3, ´4, ´6-tetra benzoyl-β-D-fructofuranosyl)-) – ethyl -2-methyl-5-nitro imidazole(10)[22]: 1,3,4,6-Tetra-O-benzoyl-β-D-fructofuranose **2** (3.5 g, 0.1 mol), silver oxide (25 g, 0.11 mol), calcium sulphate (10 g, preheated for 2 h at 513 K) and dry, absolute ethanol (10 ml) to ensure total absence of water, Iodine (5 g) was later added as a catalyst. metronidazole(4.3 g, 0.1 mol), was dissolved in absolute ethanol (10 ml) and added through dropping funnel to the stirred reaction mixture over a period of about 1 h. The stirring continued for 24 h. The reaction mixture then was filtered and the residue was washed well with chloroform. The filtrate and washings were combined and concentrated under reduced pressure. The residue was re-crystallised from chloroform to give brown dark powder in the yield 75 % (m.p. 470–472 K), R_f = 0.75 (benzene:methanol = 6:4); IR (thin film) cm^{-1} 3070 (m) (CH aromatic, stretch), 2925 (m) (CH aliphatic, stretch), 1720 (s) (C=O ester, stretch), stretch), 1450 (m) (C=C aromatic, stretch), 1612 (m) (C=N, stretch; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H1.8 (3H, s, CH_3), 3.0 (2H, s, CH_2OBz), 3.3 (2H, s, CH_2CO), 3.5 (2H, s, 2CH), 7.2-7.3 (10H, m, 10CH aromatic), 7.5-7.6 (5H, m, 5CH aromatic), 7.5-7.6 (H,s, CH=N); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C12 (CH_3), 45 (*C*H_2CO), 64 (2*C*H_2O), 70 (2*C*H), 92 (2*C*O), 119 (2*C*H aromatic), 128 (2*C*H aromatic), 130 (8*C*H aromatic), 134 (8*C*H aromatic), 136 (4*C*H aromatic), 138 (4*C*C=O), 147 (2*C*=N aromatic), 170 (4*C*=O); HR-MS(EI$^+$) found 782.125 $C_{40}H_{36}O_{14}N_3$ requires 782.123.

Hydrolysis of protected prodrug(11)[23]: A solution of (0.0026mole) of the blocked prodrug **7** in (7mL) of (0.1 M) methanolic sodium methoxide was refluxed with stirring for (0.5 h). The mixture was neutralized with acetic acid and evaporated to dryness. The residue was portioned between water and chloroform. The aqueous phaseevaporated to dryness under vacuum to obtain free prodrug (6). The residue was re-crystallised from chloroform to give brown dark powder in the yield 80 % (m.p. 320-333 K), R_f = 0.85 (benzene:methanol = 6:5); IR (thin film) cm^{-1} 3225 (OH, stretch), 3070 (m) (CH aromatic, stretch), 2925 (m) (CH aliphatic, stretch), 1450 (m) (C=C aromatic, stretch), 1612 (m) (C=N, stretch; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 1.8 (3H, s, CH_3), 4.1 (2H, s, CH_2N), 3.3 (2H, s, CH_2CO), 3.5 (2H, s, 2CH), 7.2-7.3 (1H, s, CH aromatic), 7.5-7.6 (H,s, CH=N); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 12 (CH_3), 45 (*C*H_2CO), 64 (2*C*H_2O), 70 (2*C*H), 80 (2*C*O), 85 (*C*NO_2), 119 (2*C*H aromatic), 147 (2*C*=N aromatic); HR-MS(EI$^+$) found 470.125 $C_{19}H_{24}O_{11}N_3$ requires 470.123.

Results and Discussion

To incorporate molecules containing several reactive centers, and adopt different conformation in solution like mono saccharides in a synthetic organic reaction, it is essential to block the unwanted reactive sites, and also restrict these molecules in one conformation which were required to the synthetic project successfully. β-D-glucose(**1**) was protected as a stable ester derivative using acetic anhydride in presence of sodium acetate afforded β - D-glucose penta acetate (**2**) Figure 1, then fully protected glucose treated with hydrogen bromide undergo specific displacement reaction at numeric center to give axial (α-anomer) halide, the α-halo anomer is substantially preferred,[24]the short mechanism for

formation of α -bromo sugar**3**[25] illustrated in Figure 2. Then, α -bromo sugar**3** was coupled with metronidazole (**4**) as nucleobase through nucleophilic substitution of hydroxyl group of metronidazole (**4**) on sugar bromide (**3**) afforded new blocked nucleoside (**5**) analogue,which after hydrolysedgave analogue **6** free hydroxyl groups. The FT-IR spectrum of this analogue (**6**) showed stretching band at 3230 cm^{-1} (stretch OH), and this was good evidence to form this compound.

The second sugar used in this article was β-D-fructose, which protected their hydroxyls at C-1, C-3, C-4 and C-6 leaving hydroxyl group at C-2 free for further chemical modification.[26]In same manner, per benzoate fructose (**8**) was turned to α-bromo sugar (**9**) as syrup, which coupled with metronidazole (**4**) as nucleobase through nucleophilic substitution of hydroxyl group of metronidazole (**4**) on sugar bromide (**9**) yielded new blocked nucleoside analogue (**10**), which after hydrolysis gave pro drug (**11**).

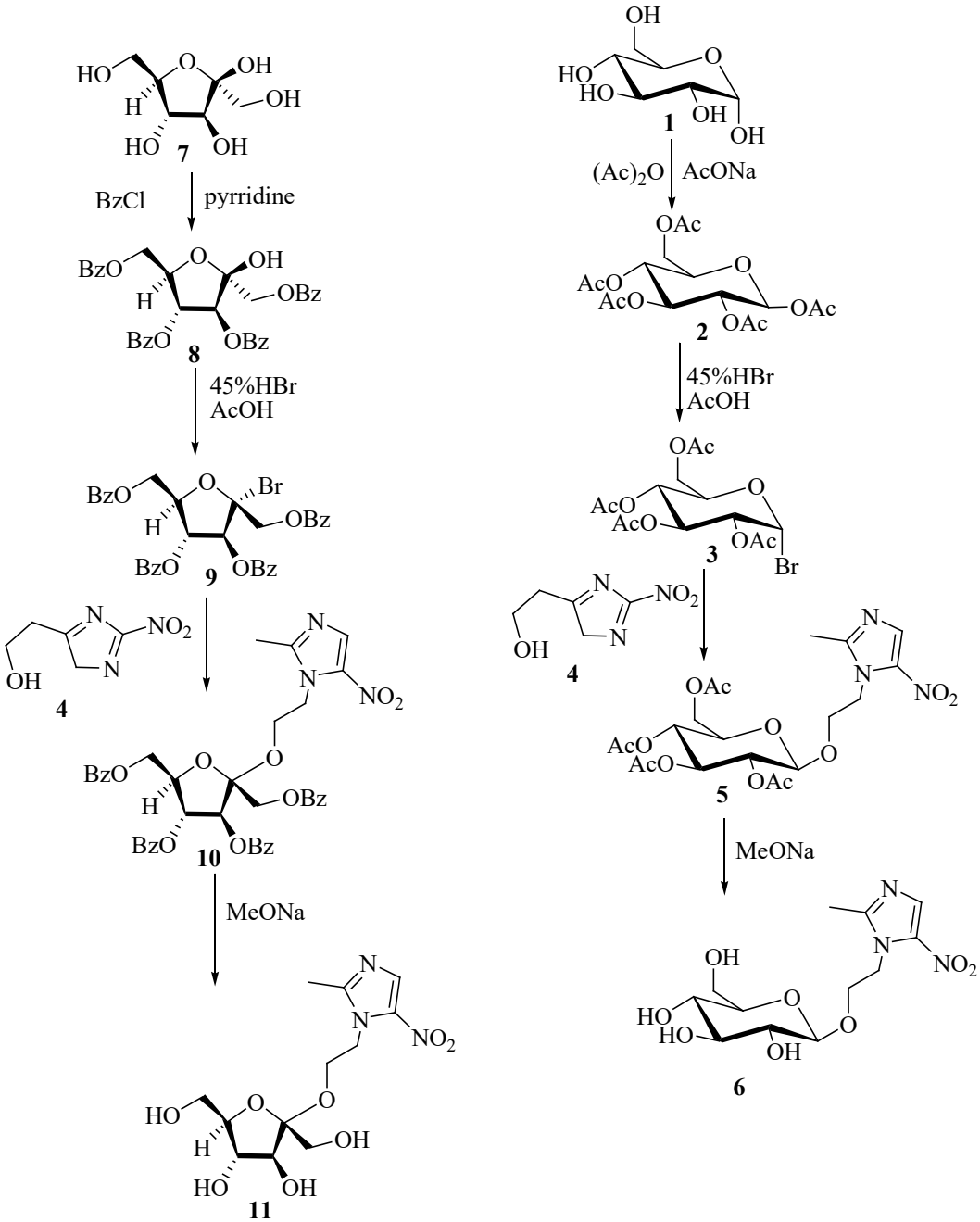

Figure 1: Total synthesis of prod rug 6 and 11

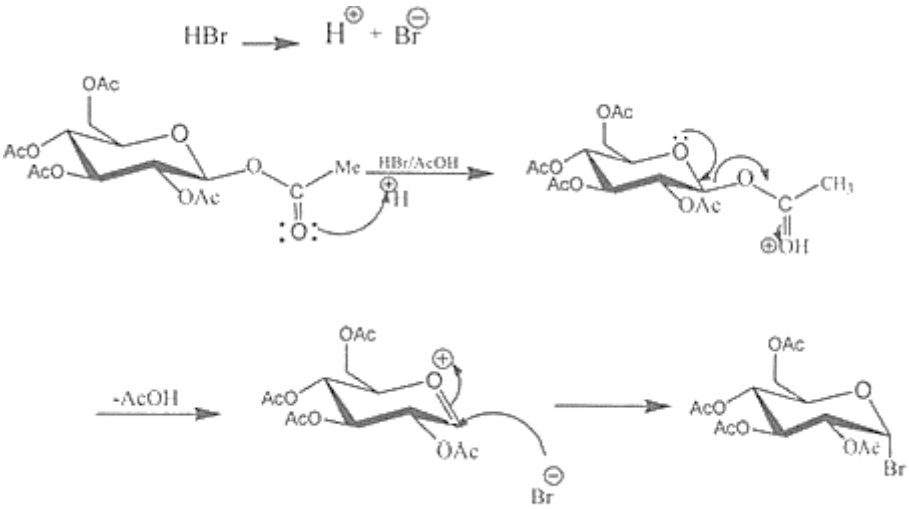

Figure 2: Mechanism for formation of α -bromo sugar

Antifungal activity of compounds 2-10: Since the metronidazole derivatives and prodrugs have been reported in the literature as antifungal [3] activity, most of these prepared compounds were examined to inhibit fungal spore germination and hyphal growth in this article. Aliquots of solutions of eight purified compounds 2, 3 and 5-10 along with compound 4 (metronidazole) were arrayed on silica TLC plates (Figure 3) and either visualized with a vanillin-H_2SO_4 rising reagent (left panel) or exposed to a fungal overlay bioassay (right). Antifungal activity was obvious as white zones sparkly an inhibition of spore germination and hyphal growth, only compound 4and compounds (6,11) exhibited significantanti-fungal activity while no such activity was observed with compounds (5-7) and 3,2. The smallest amount inhibitor concentration (MIC) observed for compound 2 was 3 ng/spt, 10-fold under that necessary for compounds 6 and 11 (30 ng/spot), indicating the importance of metronidazole moeity in sugar for full antifungal activity. However, the lack of antifungal activity with compounds 5-7 suggests that sugar is not sufficient for activity.

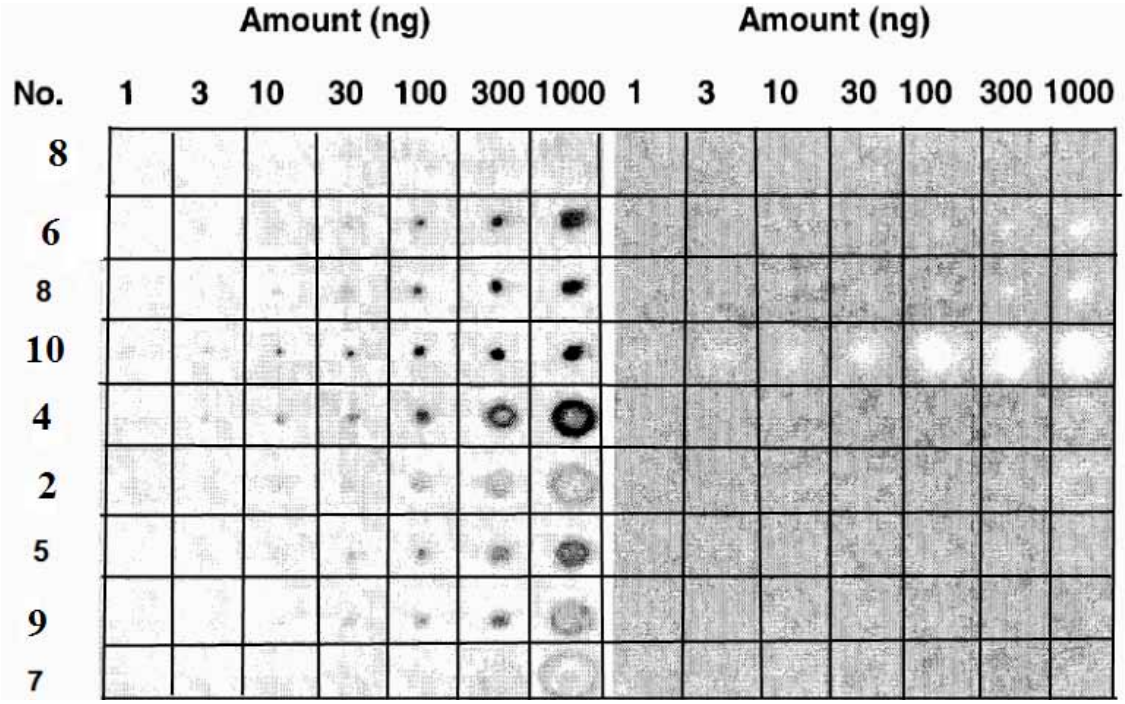

Figure 3: Antifungal activity assay of compounds 2-11

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