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Antimicrobial activities of some newly synthesized substituted Benzosuberone and its related derivatives.

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Abstract

A series of substituted benzosuberone scaffold fused with pyrazole, isoxazole, pyrimidine and triazole moieties were synthesized and evaluated as antimicrobial agents. Some reactions were performed under microwave irradiation, resulted in short time reactions and surprisingly high yields. Some of the obtained products showed remarkable antimicrobial activities and the most active products 7b, 9, 13 and 14 were further tested to evaluate their Minimum Inhibitory Concentration (MIC) value at 125 μg/mL. The structures of new compounds were proved by their elemental analyses and spectral data.

Keywords: Substituted benzosuberone, Pyrimidine, Pyrazole, Triazole, Antimicrobial activity.

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Introduction

According to World Health Organization, the continuous use of antibiotics in treating infections has led to the emergence of Multidrug Resistance (MDR) among the various strains of microorganisms [1,2]. The important class of pyrazoles have been reported to be of broad antimicrobial [3-5], antitubercular [6], antiviral [7] and anticancer [8,9] activities. Their activities to treat inflammation, convulsion, and depression were also published [10-13]. In light of the aforementioned findings and our interest in the synthesis of novel heterocycles of biological importance [14-20], we have synthesized some new fused pyrazole, isoxazole and triazol moieties with benzosuberone and 3-nitrobenzosuberone to evaluate their anticipated antimicrobial activities.

Material and Methods

Chemistry

All melting points were measured on a Gallenkamp melting point apparatus (Weiss Gallenkamp, London, UK). The infrared spectra were recorded in potassium bromide disks on a PyeUnicam SP 3300 and Shimadzu FT IR 8101 PC infrared spectrophotometers (PyeUnicam Ltd. Cambridge, UK and Shimadzu, Tokyo, Japan, respectively). The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer (Varian, Palo Alto, CA, USA). ^{1}H spectra were run at 300 MHz and ^{13}C spectra were run at 75.46 MHz in dimethylsulfoxide (DMSO-d_6) (Sigma-Aldrich, St. Louis, MO, USA). Chemical shifts are given in parts per million and were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer (Shimadzu, Tokyo, Japan) at 70 eV. Microwave irradiations were carried out in a StartSYNTH Microwave apparatus (ATS Scientific Inc). Elemental analyses were carried out at the Micro-analytical Centre of Cairo University, Giza, Egypt and recorded on Elementar-VarioEL (ELTRA GmbH, Haan, Germany) automatic analyzer. Compounds 1b and 16a-c were prepared by following the reported procedures in the literature [19-22]. The *in vitro* antimicrobial screening was performed by Chemistry of Natural and Microbial Products Dept., National Research Centre, Cairo-12622, Cairo, Egypt.

Synthesis of 9-nitro-3-phenyl-2,3,3a,4,5,6-hexahydro-2H-benzo[6,7]cyclohepteno[2,1-c]-pyrazole 3

To a mixture of compound 2b (0.29 g, 1 mmol) and hydrazine hydrate (0.4 mL, 12 mmol, 99%) in absolute ethanol (10 mL), few drops of triethylamine were added. The reaction mixture was refluxed for 4 h. After cooling, the reaction mixture was poured onto ice-water. The obtained solid was filtered off,

washed with cold water, dried and crystallized from ethanol to afford the title compound 3 as gray crystals. Yield (0.25 g, 81%); mp 135-138°C; IR (KBr, cm^{-1}) : v 3218 (NH), 1620 (C=N). ^{1}H NMR (DMSO-d_6): δ 1.68-1.79 (m, 3H, CH_2 + H_a) 1.98-2.46 (m, 4H, $2CH_2$), 3.26 (d, 1H, H_b), 7.11-8.46 (m, 8H, Ar-H), 10.55 (br.s, 1H, NH, D_2O-exchangable). ^{13}C NMR (DMSO-d_6): δ 24.15, 32.90, 113.07, 121.11, 122.87, 127.50, 127.94, 128.33, 129.45, 129.8, 131.1,135.65, 142.71, 145.31, 147.88. MS m/z (%): 307 [M^+, 20], 230 (35), 115 (100), 77 (80). Anal. Calcd. For $C_{18}H_{17}N_3O_2$ (307.33): C, 70.34; H, 5.58; N, 13.67. Found: C, 70.56, H, 5.72, N, 13.44.

Synthesis of 2-acetyl-9-nitro-3-phenyl-2,3,3a,4,5,6-hexahydro-2H-benzo[6,7] cyclohepteno-[2,1-c] pyrazole 4

A mixture of compound 2b (0.29 g, 1 mmol) and hydrazine hydrate (0.4 mL, 12 mmol, 99%) in glacial acetic acid (10 ml) was refluxed for 3 h. After cooling, the formed precipitate was collected by filtration, dried and crystallized from ethanol to give the title compound 4 as orange crystals. Yield (0.32 g, 91%); mp 150-153°C; IR (KBr, cm^{-1}): v 1725 (C=O), 1643 (C=N). ^{1}H NMR (DMSO-d_6): δ 1.58 (s, 3H, CH_3), 1.73-1.85 (m, 3H, CH_2+ H_a), 1.97-2.36 (m, 4H, $2CH_2$), 3.42 (d, 1H, H_b), 7.30-8.36 (m, 8H, Ar-H); MS m/z (%): 349 [M^+, 15], 306 (35), 228 (40), 187 (50), 77 (70). Anal. Calcd. For $C_{20}H_{19}N_3O_3$ (349.14): C, 68.75; H, 5.48; N, 12.03. Found: C, 68.31; H, 5.27; N, 12.30.

Synthesis of 9-substituted 3-phenyl-3,4,5,6-tetrahydro-2H-benzo[6,7]cyclohepteno[4,3-c]-isoxazole 6a,b

To a solution of 2a,b (1 mmol) and hydroxylamine hydrochloride (0.07 g, 1 mmol) in absolute ethanol (10 mL), potassium carbonate anhydrous (0.14 g, 1 mmol) was added. The resulting mixture was heated under reflux temperature for 5-8 h and allowed to cool then diluted with water (30 mL). The solid products that formed were collected by filtration, washed with water, dried and crystallized from the proper solvents to afford the corresponding isoxazole derivatives 6a,b, respectively. 3-Phenyl-3,4,5,6-tetrahydro-2H-benzo[6,7]cyclohepteno[4,3-c]isoxazole 6a. Yield (0.22 g, 84%); mp 110-112°C (EtOH/dioxin, pale yellow crystals); IR (KBr, cm^{-1}): v 2915-2850 ($3CH_2$).^{1}H NMR (DMSO-d_6): δ 1.79-3.21 (m, 6H, $3CH_2$), 6.84 -7.63 (m, 8H, Ar-H). ^{13}C NMR (DMSO-d_6): δ25.33, 31.29, 38.12,101.05, 126.5, 127.60, 127.90, 128.83, 129.01, 129.75, 131.21, 135.13, 135.67, 138.28, 155.35, 166.35. MS m/z (%): 261 [M^+, 75], 188 (100), 142 (61), 77 (85). Anal. Calcd. For $C_{18}H_{15}NO$ (261.32): C, 82.73; H, 5.79; N, 5.36 Found: C, 82.25; H, 5.62; N, 5.37.

9-Nitro-3-phenyl-3,4,5,6-tetrahydro-2H-benzo[6,7] cyclohepteno [4,3-c] isoxazole 6b. Yield (0.28 g, 91%); mp: 120-122°C (Dioxan, gray crystals); IR (KBr, cm^{-1}): v 2935-2858 ($3CH_2$). 1H NMR (DMSO-d_6): δ 2.4 4-3.35 (m, 6H, $3CH_2$), 6.79-8.34 (m, 8H, Ar-H). MS m/z (%): 306 [M+, 46], 259 (15), 128 (51), 117 (61), 104 (36), 91 (29), 80 (100), 64 (77). Anal. Calcd. For C18H14N2O3 (306.32): C, 70.58; H, 4.61; N, 9.15. Found: C, 70.37; H, 4.48; N, 9.42.

Synthesis of benzo[d]imidazo[1,2-a]pyrimidines 7a,b

Method A: A mixture of compounds 2a,b (1 mmol) and 2-aminobezimidiazole (0.13 g, 1 mmol) in anhydrous dimethylformamide (10 mL) was refluxed for 24 h. After cooling, the reaction mixture was diluted with water (30 mL), the obtained solid product was collected by filtration, washed with water, dried and crystallized from the proper solvents to afford the corresponding pentacyclic dihydropyrimidine derivatives 7a,b, respectively.

Method B: To a solution of benzylidene benzosuberones 2a,b (1 mmol) in anhydrous dimethyl-formamide (10 mL), 2-aminobenzimidazole (0.13 g, 1 mmol) was added. The reaction mixture was subjected to microwave irradiation for 25 minutes and temperature 120°C, then left to cool and diluted with cold water. The formed solid product was collected by filtration, washed with EtOH, dried, and crystallized from the proper solvents to afford pentacyclic dihydropyrimidine derivatives 7a,b.

Method C: A mixture of benzaldehyde (0.11g, 1 mmol), appropriate benzosuberones 1a,b (1 mmol) and 2-aminobenzimidazole (0.13 g, 1 mmol) in anhydrous dimethylformamide (10 mL), was heated under temperature 120°C using microwave irradiation for 30 minutes, then left to cool and diluted with cold water. The solid product that formed was filtrated off, washed with ethanol, dried, and finally recrystallized from the proper solvents to afford pentacyclic dihydropyrimidine derivatives 7a,b. The products that prepared by methods B and C were identical in all respects (mp, TLC and spectra) with that prepared from method A.

8-Phenyl-1,9,10,11- tetrahydrobenzo [6`,7`] cyclohepteno [d]-1H-benzo [d]imidazo-[1,2-a]-pyrimidine 7a. Yield: (Method A: 0.25g, 68%; Method B: 0.28g, 75%; Method C: 0.4g, 90%); mp: 175-178°C white crystals (EtOH/dioxan). IR (KBr, cm^{-1}): v 3431(NH), 1625 (C=N). ^{1}H NMR (DMSO-d_6): δ 1.61-2.25 (m, 6H, $3CH_2$), 5.63 (s, 1H, CH), 6.58 (s, 1H, NH-D_2O-exchangeable), 7.02- 8.61(m, 12H, Ar-H). MS m/z (%): 363 [M^+, 100], 172 (35), 115 (17), 91 (10), 77 (25). Anal. Calcd. For $C_{25}H_{21}N_3$ (363.45): C, 82.61; H, 5.82; N, 11.56. Found: C, 82.14; H, 5.65 N, 11.90.

8-Phenyl-14-nitro-1,9,10,11- tetrahydrobenzo [6`,7`] cyclohepteno [d]-1H-benzo [d]imidazo-[1,2-a]pyrimidine 7b. Yield: (Method A: 0.17 g, 65%; Method B: 0.21 g, 77%; Method C: 0.3 g, 90%); mp: 205-207°C yellow crystals (EtOH/ dioxan). IR (KBr, cm^{-1}): v 3433 (NH), 1603 (C=N). ^{1}H NMR (DMSO-d_6): δ 1.99-2.36 (m, 6H, $3CH_2$), 5.59 (s, 1H, CH-pyrimidine), 7.41-8.57 (m, 12H, Ar-H), 8.57 (s, 1H, NH-D2O-exchangeable). ^{13}C NMR (DMSO-d_6): $^{\delta}$24.70, 26.12, 39.80, 56.44, 108.50, 115.20, 121.46, 123.67, 125.43, 126.01, 126.75, 127.12, 127.74, 129.36, 134.21, 136.21, 137.07, 138.90, 139.79, 141.11, 143.62, 154.66. MS m/z (%): 408 [M^+, 15], 292 (28), 172 (10), 128 (14), 115 (20), 91 (18), 80 (98), 64

(100). Anal. Calcd. For $C_{25}H_{20}N_4O_2$ (408.45): C, 73.51; H, 4.94; N, 13.72. Found C, 73.31; H, 4.80; N, 14.13.

Synthesis of 6-(4-chlorophenyl)-11-nitro-6,7,8,9,10,11,13-hexahydrobenzo[6`, 7`]cyclohepta-[d]-[1,2,4]-triazolo[4,3-a]pyrimidine 9

To a stirred solution of nitrobenzosuberone 1b (1 mmol), 4-chlorobenzaldehyde (0.14 g, 1 mmol) in dimethylformamide (10 mL), 2-aminotriazole (0.085 g, 1 mmol) was added. The reaction mixture was refluxed for 9 h, allowed to cool and diluted with water (50 mL). The solid product formed was collected by filtration, washed with water. The crude product was purified by recrystallization from dioxan to give compound 9 as white crystals. Yield: 84%; mp: >300°C (Dioxan); IR (KBr, cm^{-1}): v 3331 (NH), 1630 (C=N). ^1H NMR (DMSO-d_6): δ 1.71-2.65 (m, 6H, $3CH_2$), 6.01 (s, 1H, CH-pyrimidine), 7.20-8.12 (m, 7H, Ar-H), 8.24 (s, 1H, CH-triazole), 8.58 (s, 1H, NH-D_2O- exchangeable). MS m/z (%): 393 [M^+, 100], 347 (35), 237(20), 111 (50), 77 (25). Anal. Calcd. For $C_{20}H_{16}ClN_5O_2$ (393.83): C, 60.99; H, 4.09; N, 17.78. Found: C, 60.75; H, 3.97; N, 17.92.

Synthesis of triazolo[3,4-b]quinazoline derivatives 13-15

Method A: To a stirred solution of the appropriate cycloalkanones 10, 11 or 12 (1 mmol) and 4-chlorobenzaldehyde (0.14 g, 1 mmol) in DMF (10 mL), 2-aminotriazole (0.085 g, 1 mmol) was added. The reaction mixtures were refluxed for 8-12 h (monitored by TLC), allowed to cool and 50 mL water was then added. The solid products that formed were collected by filtration, washed with water. The crude products were purified by recrystallization from dioxan to give 13, 14 and 15, respectively.

Method B: A mixture of 4-chlorobenzaldehyde (0.14 g, 1 mmol), the appropriate cycloalkanone 10, 11 or 12 (1 mmol) and 2-aminotriazole (0.085 g, 1 mmol) in DMF (10 mL), was heated at 120°C using microwave irradiation for 30 minutes then left to cool and diluted with cold water. The solid product that formed was filtrated off, washed with ethanol, dried, and finally recrystallized from dioxan to afford 13, 14 and 15, respectively. The products that prepared by method B were identical in all respects (mp, TLC and spectra) with that prepared from method A.

5-(4-Chlorophenyl)-7-methyl-5,6,7,8,9,10-hexahydro-[1,2,4]triazolo[3,4-b]-quinazoline 13. Yield: (Method A: 0.22 g, 75%; Method B: 0.27 g, 89%); mp: 270-272°C as white crystals. IR (KBr, cm^{-1}): v 3341 (NH), 1635 (C=N). ^1H NMR (DMSO-d_6): δ 1.29 (d, 3H, CH_3), 1.81-2.45 (m, 6H, $3CH_2$), 3.30 (m, 1H, CH), 5.71 (s, 1H, CH), 7.30-7.60 (m, 4H, Ar-H), 8.30 (s, 1H, CH), 8.32 (s, 1H, NH-D_2O- exchangeable). MS m/z (%): 300 [M^+, 100], 300 [M^+] (100), 189 (50), 120 (20), 113 (70), 77 (30). Anal. Calcd. For $C_{16}H_{17}ClN_4$ (300.79): C, 63.89; H, 5.70; N, 18.63. Found C, 62.98; H, 5.53; N, 19.18.

5-(4-Chlorophenyl)-5,6,7,8,9,10,11,13-hexahydrocyclohepta[d][1,2,4]triazolo[4,3-a]pyrimidine 14. Yield: (Method A: 0.18 g, 60%; Method B: 0.25 g, 83%); mp: >300°C as white crystals. IR (KBr, cm^{-1}): v 3335 (NH), 1640 (C=N). ^1H NMR (DMSO-d_6): δ 1.30-2.13 (m, 10H, $5CH_2$), 5.57 (s, 1H, CH-pyrimidine), 7.10-7.4 (m, 4H, Ar-H), 8.15 (s, 1H, NH-D_2O- exchangeable), 8.34 (s, 1H, CH-triazole). MS m/z (%): 301 [M^++ 1, 100], 189 (60), 111 (70), 70 (30), 55(20). Anal. Calcd. For $C_{16}H_{17}ClN_4$ (300.79): C, 63.89; H, 5.70; N, 18.63. Found C, 63.68; H, 5.53; N, 18.78.

5-(4-Chlorophenyl)-5,7,8,9,10,11,12,14-octaahydrocycloocta[d][1,2,4]triazolo [4,3-a]-pyrimidine 15. Yield: (Method A: 0.20 g, 63%; Method B: 0.24g, 76%); mp: > 300°C as white crystals. IR (KBr, cm^{-1}): v 3330 (NH), 1633 (C=N). ^1H NMR (DMSO-d_6): δ 1.30-2.13 (m, 10H, $5CH_2$), 5.57 (s, 1H, CH-pyrimidine), 7.14-7.43 (m, 4H, Ar-H), 8.34 (s, 1H, CH-triazole), 8.55 (s, 1H, NH, D_2O-exchangeable). MS m/z (%): 314 [M^+, 100], 203 (40), 111 (70), 84(35), 77 (60). Anal. Calcd. For $C_{17}H_{19}ClN_4$ (314.81): C, 64.86; H, 6.08; N, 17.80. Found C, 64.98; H, 6.13; N, 17.72.

Synthesis of 3-acetyl-1-(substituted phenyl)-4,5,6-trihydrobenzo[6,7]cyclohepteno[1,2-c]-pyrazol 18a-c

To a stirred sodium ethoxide solution [prepared from sodium metal (0.11 g) and absolute ethanol (20 mL)], benzosuberone 1a (0.32 g, 2 mmol) was added. The mixture was stirred for 30 min, and then appropriate hydrazonoyl halides 16a-c (2 mmol) was added. The reaction mixture was stirred at room temperature for 24 h. The formed solid was collected by filtration, washed with water, dried and crystallized from ethanol to afford the corresponding pyrazole derivatives 18a-c, respectively.

3-Acetyl-1-phenyl-4,5,6-trihydrobenzo[6,7]cyclohepteno[1,2-c]pyrazol 18a. Yield: (85%); mp: 240-242°C as yellow powder. IR (KBr, cm^{-1}): v 1670 (C=O). ^1H NMR (DMSO-d_6): δ 1.75-2.30 (m, 6H, $3CH_2$), 3.43 (s, 3H, CH_3), 6.96-7.30 (m, 9H, Ar-H). ^{13}C NMR (DMSO-d_6): δ 23.13, 26.60, 32.0, 38.20, 116.44, 120.20, 126.50, 128.90, 135.11, 139.67, 141.28, 150.13, 196.10, 195.25. MS m/z (%):302 [M^+, 45], 259 (25), 183 (30), 77 (100). Anal. Calcd. For $C_{20}H_{18}N_2O$ (302.14): C, 79.44; H, 6.00; N, 9.26. Found: C, 79.06; H, 5.82; N, 9.53.

3-Acetyl-1-tolyl-4,5,6-trihydrobenzo[6,7]cyclohepteno[1,2-c]pyrazol 18b. Yield: (70%); mp: 260-262°C as golden powder. IR (KBr, cm^{-1}): v 1670 (C=O). 1H NMR (DMSO-d_6): δ 1.75-2.30 (m, 6H, $3CH_2$), 2.55 (s, 3H, CH_3), 3.23 (s, 3H, CH_3), 6.96-7.30 (m, 8H, Ar-H).MS m/z (%):316 [M^+, 70], 273(25), 183 (40), 91 (100). Anal. Calcd. For $C_{21}H_{20}N_2O$ (316.40): C, 79.72; H, 6.37; N, 8.85. Found C, 79.33; H, 6.18; N, 9.11.

3-Acetyl-1-(4-chlorophenyl)-4,5,6-trihydrobenzo[6,7]cyclohepteno[1,2-c]pyrazol 18c. Yield: (80%); mp: 275-277°C as yellow powder. IR (KBr, cm^{-1}): v 1670 (C=O). ^1H NMR (DMSO-d_6): δ 1.75-2.20 (m, 6H, $3CH_2$), 3.13 (s, 3H, CH_3), 6.86-7.20 (m, 8H, Ar-H). MS m/z (%): 336 [M^+, 45], 293 (45), 183 (15), 111 (100). Anal. Calcd. For $C_{20}H_{17}ClN_2O$ (336.81): C, 71.32; H, 5.09; N, 8.32. Found C, 71.19; H, 4.94; N, 8.56.

Antimicrobial activity

Strains used: The antimicrobial activity of the synthesized compounds was screened against common pathogenic and food spoilage microorganisms were selected for their relevance in bakery products and other food: gram-positive (*Staphylococcus aureus* ATCC 29213 and *Bacillus subtilits* ATCC 6633) and gram-negative (*Escherichia coli* ATCC 2592 and *Pseudomonas aeruginosa* ATCC 27953) bacteria, yeast (*Candida albicans* NRRL Y-477) and filamentous fungi (*Aspergillus niger* ATCC 1015 and *Aspergillus flavus* ATCC 16883).

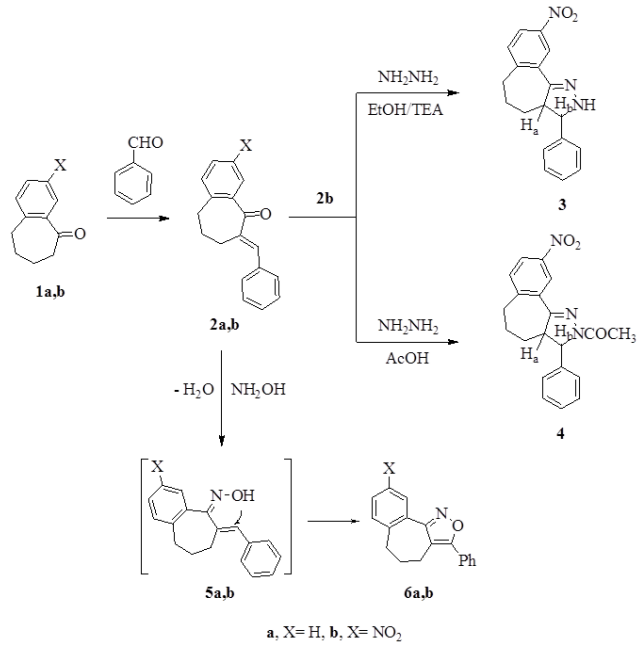

Figure 1. *Reaction on 6-Benzylidene-3-nitro-6,7,8,9-tetrahydrobenzo[6,7] cyclohepten-5-one.*

Bioassay: Chemical compounds were individually tested against a panel of gram positive and gram negative bacterial pathogens, yeast and fungi. Antimicrobial tests were carried out by the agar well diffusion method [23] using 100 µL of suspension containing 1×10^8 CFU/mL of pathological tested bacteria, 1×10^6 CFU/ mL of yeast and 1×10^4 spore/ml of fungi spread on nutrient agar (NA), plates each containing 25 mL of the respective, Sabourand Dextrose Agar (SDA), and Potato Dextrose Agar (PDA) media, respectively, to reach a final concentration of 0.015 (OD600). After the media had cooled and solidified, wells (10 mm in diameter) were made in the solidified agar and loaded with 100 µL of tested compound solution prepared by dissolving 10 mg of the chemical compound in one ml of dimethyl sulfoxide (DMSO).

The inculcated plates were then incubated for 24 h at 37, 26 and 28°C for bacteria, yeast and for 48 h for fungi, respectively. Negative controls were prepared using DMSO employed for dissolving the tested compound. Vebromycine (10 mg/ml) and Ketoconazole (10 mg/ml) were used as standard for antibacterial and antifungal activity respectively. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the standard. The observed zone of inhibition is presented in Table 1. Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated.

Minimal inhibitory concentration (MIC) measurement

The bacteriostatic activity of four active compounds 7b, 9, 13 and 14 was then evaluated using the two fold serial dilution technique (Table 2) [24]. Two fold serial dilutions of the tested compounds solutions were prepared using the proper nutrient broth. The final concentration of the solutions were 1000, 500, 250, and 125 µg/mL. The tubes were then inoculated with the test organisms, grown in their suitable broth at 37°C for 24 hours for tested microorganisms (1×10^8 CFU/mL for bacteria, 1×10^6 CFU/mL of yeast and 1×10^4 spore/ml for fungi), each 5 ml received 0.1 ml of the above inoculum and incubated at 37°C for 24 hours. The lowest concentration showing no growth was taken as the Minimum Inhibitory Concentration (MIC).

Results and Discussion

Chemistry

6- Benzylidene-3- nitro-6,7,8,9- tetrahydrobenzo [6,7] cyclohepten-5- one 2 was synthesized as starting material by reacting of 3-nitro-6,7,8,9-tetrahydro-5H-benzocyclohepten-5-one 1a,b with benzaldehyde according to the reported procedures (Figure1) [21,22]. Condensation of 2b with hydrazine hydrate in ethanol containing a catalytic amount of triethylamine gave 9-nitro-3-phenyl-2,3,3a,4,5,6-hexahydrobenzo[6,7]cyclohepteno[2,1-c]pyrazole 3 (Figure 1). Meanwhile, reaction of 2b with hydrazine hydrate in glacial acetic acid afforded 2-acetyl-9-nitro-3-phenyl-2,3,3a,4,5,6-hexahydro-2H-benzo[3,4]cyclohepteno[2,1-c]pyrazole 4. The benzosuberone derivatives 2a and 2b were reacted with hydroxylamine hydrochloride, afforded the corresponding 3-phenyl-3,4,5,6-tetrahydro-2H-benzo[6,7]cyclohepteno[4,3-c]isoxazole 6a as pale yellow crystals and 9-nitro-3-phenyl-3,4,5,6-tetrahydro-2H-benzo[6,7]cyclohepteno[4,3-c]isoxazol 6b as gray crystals in 84% and 91% yield, respectively (Figure 1). This reaction of 6-benzylidene-6,7,8,9-tetrahydro-3-nitrobenzo[7]annulen-5-ones 2a and 2b with hydroxylamine proceeds by initial condensation, followed by cyclization of intermediate 5a,b via nucleophilic addition to give 6a,b as presented in Figure 1.

Treatment of benzylidene derivatives 2a,b with 2-aminobenzimidazole in refluxing DMF afforded pentacyclic dihydropyrimidines (DHPs) 7a,b, respectively (Method A), which were obtained also by using microwave irradiation (Method B). One pot synthesis, as alternative method, was applied to prepare the dihydropyrimidines 7a,b by heating the mixture of 1a,b with benzaldehyde and 2-aminobenzimidazole in DMF using microwave irradiation (Method C) (Figure 2). The usage of microwave in the synthesis of 7a,b (methods B

and C) has proved to be time saving and surprisingly increasing the yield. Treatment of 1b with 4-chlorobenzaldehyde and 2-aminotriazole in one pot reaction in DMF under reflux afforded the cyclized tetracyclic dihydropyrimidine derivative 9 via the intermediate 8 (Figure 2).

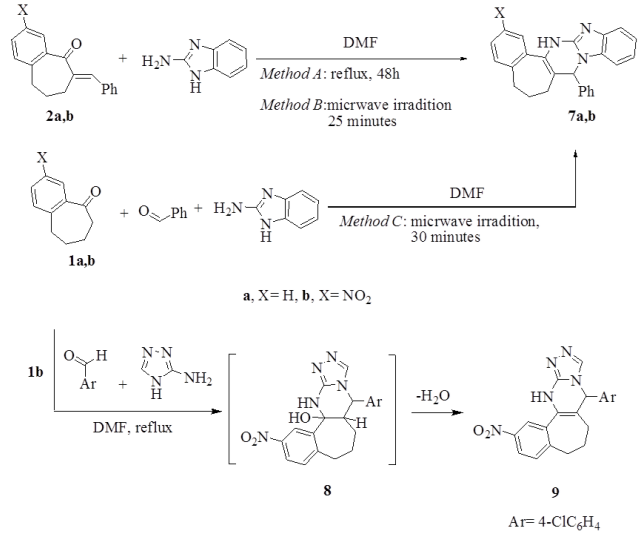

Figure 2. *One pot synthesis of dihydropyrimidines 7a,b.*

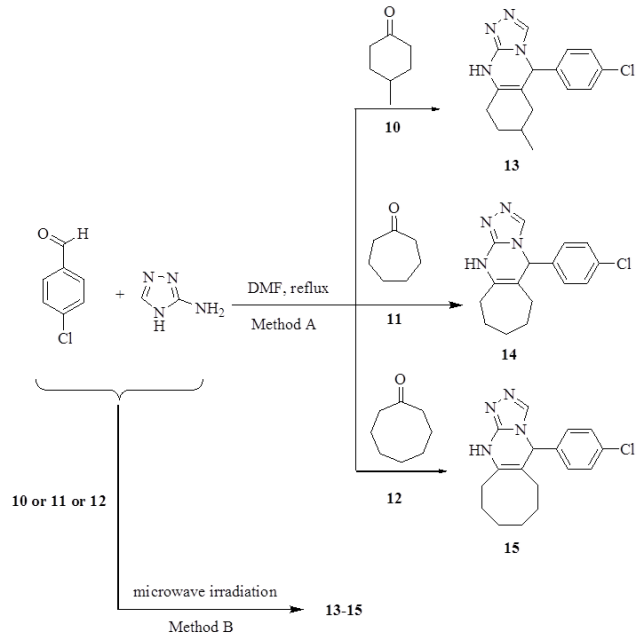

Figure 3. *One pot synthesis of tricyclic dihydropyrimidines.*

We have extended our synthetic strategy towards polycyclic dihydropyrimidines via three components one pot synthesis. To obtain these targets we have used monocyclic ketone instead of benzosuberone with 4-chlorobenzaldehyde and 2-aminotriazole. Thus, the reaction of 4-chlorobenzaldehyde with 2-aminotriazole and cycloalkanone derivatives 10-12, in refluxing DMF for 8-12 h, afforded the corresponding tricyclic dihydropyrimidines 13-15, in 75%, 60% and 63% yield, respectively (Method A, Figure 3). In light of the aforementioned data, we have used the microwave irradiation as a tool in performing this one pot reaction. The same one pot reactions were performed in DMF under microwave irradiation at 120°C for 30 minutes to give the corresponding tricyclic dihydropyrimidines 13-15, in 84%, 89%, 83% and 76% yield respectively (Method B, Figure 3).

The reaction of benzosuberone 1a with (1-(2-(4-substituted phenyl)hydrazono)-1-chloropropan-2-ones 16a-c in the presence of sodium ethoxide in absolute ethanol at room temperature with stirring afforded the corresponding pyrazolo derivatives 18a-c, via the intermediates 17A and 17B (Figure 4).

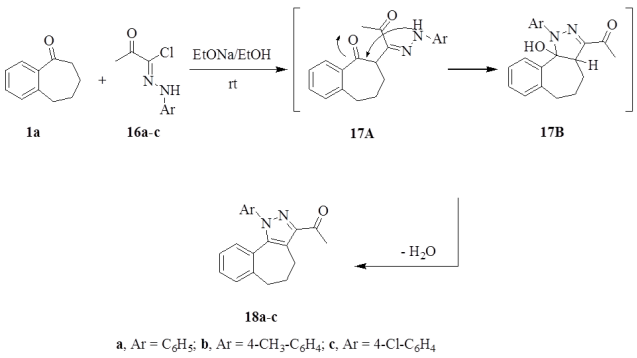

Figure 4. *Synthesis of pyrazolo derivatives 18a-c, via the intermediates 17A and 17B.*

Antimicrobial evaluation

The *in vitro* antimicrobial activity of the synthesized products was performed against the tested pathogens represented by bacteria, yeast and fungi in comparison with the standard antibiotics used vebromycine and ketoconazole (Table 1). Compounds 7b, 9, 13, 14 and 15 have shown a strong inhibitory effect against gram-positive bacteria (*S. aureus*). Meanwhile, compounds 4, 13, 14 and 16 were shown to be active against *B. subtilits*. In case of gram-negative bacteria, all the tested products showed a remarkable activity against *E. coli*. The tested products have shown a strong to moderate effect against most of. Products 3, 4, 9, 13, 14, 15 and 18a have shown a strong activity against *P. aeruginosa*.

The antifungal activities of compounds 4, 6b, 7b and 9 showed to be strong against *C. albicans*. Whereas, compounds 4, 6b, 7b, 9 and 18a have a higher effect than the standard (Ketoconazole), in case of filamentous fungi (*A. niger* and *A. flavus*). Also, the broad spectrum effect of compounds 3 and 4 against all tested pathogens: gram-positive bacteria, gram-negative bacteria, and fungi, indicated that these compounds can be used in the treatment of the tested pathogens. The Minimum Inhibitory Concentration (MIC) of the tested products 7b, 9, 13 and 14 is presented in Table 2. The MIC of the compounds 13 and 14 was 125 µg/mL against *S. aureus* and was 250 µg/mL against *B. sutbtilis*. MIC was 250 µg/mL for the tested compounds 7b and 9 for their antibacterial (*S. aureus* and *E. coli*) and antifungal (*C. Albicans* and *A. flavus*) activities. Finally, the structure-activity relationships (SAR) of these synthesized compounds (polycyclic derivatives) can be

attributed to their ability to inhibit the cell growth by inhibiting the protein synthesis [25,26].

Table 1. *Antimicrobial activity expressed as inhibition diameter zones in millimeters (mm) of chemical compounds against the pathological strains based on well diffusion assay.*

Compound no	Bacteria				Yeast	Fungi	
	Gram +ve		Gram -ve				
	S. aureus	*B. subtilis*	*E. coli*	*P. aeroginosa*	*C. Albicans*	*A. Niger*	*A. flavus*
3	12	18	18	18	18	15	21
4	20	22	20	20	30	25	28
6b	15	19	25	N.A.	30	28	25
7b	25	15	25	N.A.	25	20	27
9	25	N.A.	25	15	25	20	26
13	30	26	15	25	15	N.A	17
14	30	25	15	24	N.A.	N.A.	N.A.
15	30	28	N.A.	20	N.A.	N.A.	N.A.
18a	N.A.	N.A.	20	15	22	20	26
18b	20	N.A.	15	N.A.	19	N.A.	15
18c	N.A.	N.A.	15	N.A.	17	N.A.	15
Vebromycine	20	22	25	24	N.A.	N.A.	N.A.
Ketoconazole	N.A.	N.A.	N.A.	N.A.	N.A.	22	23
The experiment was carried out in triplicate and the average zone of inhibition was calculated							

Table 2. *Minimum inhibitory concentration (MIC, μg/ml) against the pathological strains based on two fold serial dilution technique.*

Compound no.	Gram +ve		Gram -ve		Yeast	Fungus	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeroginosa*	*C. Albicans*	*A. Niger*	*A. flavus*
7b	250	1000	250	-	250	500	250
9	250	-	250	1000	250	500	250
13	125	250	1000	250	1000	-	500
14	125	250	1000	250	-.	-	-
Vebromycine	125	125	125	125	-	-	-
Ketoconazole	-	-	-	-	125	125	-
The experiment was carried out in triplicate and the average zone of inhibition was calculated							

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