Hydrazonoyl halides as precursors for synthesis of novel bioactive thiazole and formazan derivatives

Thoraya A. Farghaly[a]* and Eman M.H. Abbas[b]

[a]*Department of Chemistry, Faculty of Science, University of Cairo, Giza, Egypt*

[b]*Department of Chemistry, Natural and Microbial Products, National Research Center, Dokki, Cairo, Egypt*

A new series of substituted 4-methyl-5-(arylazo)-2-[*N*′-(6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)-hydrazino]-thiazoles and 5-(arylhydrazono)-2-[*N*′-(6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)-hydrazino]-thiazol-4-one were prepared by reaction of hydrazonoyl chlorides with a thiosemicarbazone derivative. The tautomeric structures of the products were studied using electronic absorption and NMR spectroscopy. The site-selectivities of the reactions of hydrazonoyl halides with benzocyclohepten-5-one hydrazone were also investigated. In addition, the antimicrobial activity of some of the products was evaluated. Many derivatives have promising activities against antibacterial and antifungal species.

Keywords: thiosemicarbazone, hydrazone, thiazole, hydrazonoyl chlorides, formazan, antimicrobial activity

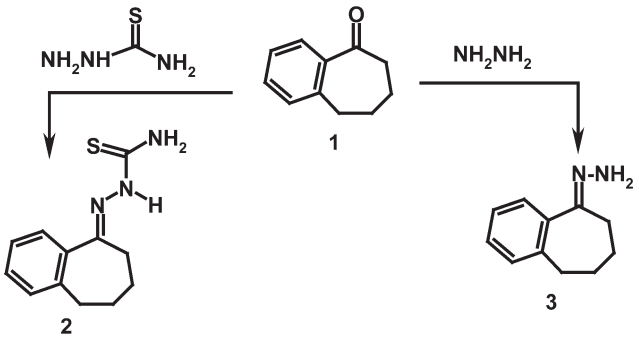
Our previous work with benzocycloheptenone (benzosuberone) has led to the synthesis of bioactive novel heterocyclic compounds.[1–3] Thiazole derivatives exhibit a number of biological properties, such as antimicrobial,[4] antiprotozoal,[5] anti-inflammatory,[4] anticonvulsant,[6] cardiotonic,[7] analgesic, antithermic[8] and anticancer[9] properties. In addition to these findings, formazans and heterocyclic hydrazones are known for their diverse biological activities.[10–12] As part of our ongoing interests in the reactions of hydrazonoyl halides with several heterocyclic compounds to synthesise bioactive compounds,[13–15] we investigated the reaction of hydrazonoyl halides with benzocyclohepten-5-one thiosemicarbazones and benzocyclohepten-5-one hydrazone to give new derivatives of thiazole and substituted formazan which were expected to be biologically active. We also elucidated the actual tautomeric structures of such thiazole derivatives as they can have one or more of eight possible tautomeric forms (Scheme 2 and Fig. 1).

Results and discussion

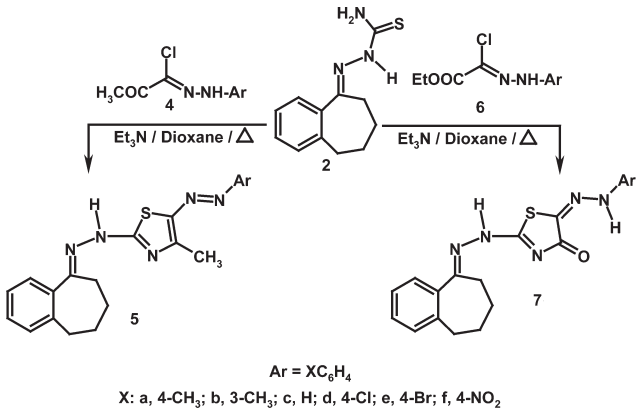
The required starting compounds, benzocycloheptenone thiosemicarbazone **2** and benzocycloheptanone hydrazone **3** were prepared according to the method reported previously[16,17] (Scheme 1).

Reaction of benzocycloheptenone thiosemicarbazone **2** with α-keto-hydrazonoyl chlorides **4a–d** in dioxane in the presence of triethylamine as a basic catalyst can lead to one or more of three tautomeric structures **5A–C** (Scheme 2). To elucidate the actual tautomeric form(s) of these compounds, their electronic absorption spectra were first studied. The spectra of the compounds in dioxane showed in each case two absorption bands in the regions 450–442 and 302–288 nm (Table 1). This absorption pattern is analogous to that reported for the azo chromophore.[18] Furthermore, the spectrum of **5c**, taken as a typical example of the series studied, in solvents of different polarities, showed little, if any, shift (Table 1). The small shift in λ_{max} of **5c** in different solvents is due to solute–solvent interaction. This finding excludes the hydrazone tautomeric form **5A** (Fig. 1). From our point of view, the most stable structure is likely to be **5C** due to the aromaticity of the thiazole ring. This choice is also based on the ^{13}C NMR spectral data of compounds **5Cc** and **5Cd** which revealed a signal near 170 ppm for the carbon 2 in thiazole ring (S–C=N) while carbon 2 in thiazole ring of compound **5B** appears upfield of this value.[19]

On the other hand, reaction of hydrazonoyl chlorides **6** with thiosemicarbazone derivative **2** afforded the hydrazone tautomeric structures **7B** (Scheme 2 and Fig. 1). The structure of these products was established on the basis of spectral data and absorption spectra. For example, the mass spectra of the latter products revealed in each case, the molecular ion peaks at the expected *m/z* values and their elemental analysis data were consistent with their assigned structures. Their IR spectra showed, in each case, one carbonyl band in the region 1712–1663 cm^{-1} and two NH bands in the region 3228–3151 and 3166–3066 cm^{-1}. The observed wavenumber of the CO stretching band in the compounds **7** seems to result from the possible strong hydrogen bonding with the hydrazone NH and conjugation with the C=N double bond as required by the hydrazone form **7B** (Fig. 1). The electronic absorption spectral data of the studied compounds **7** are summarised in Table 1. Each of the compounds **7** in dioxane exhibits two characteristic absorption bands in the regions 391–366 and 306–296 nm. Such an absorption pattern is similar to that of the typical hydrazone chromophore.[20] Furthermore, the spectra of **7c**, taken as a



Scheme 1 Synthesis of compounds **2** and **3**.



Scheme 2 Reaction of compounds **2** with hydrazonoyl chlorides **4** and **6**.

* Correspondent. E-mail: thoraya-f@hotmail.com

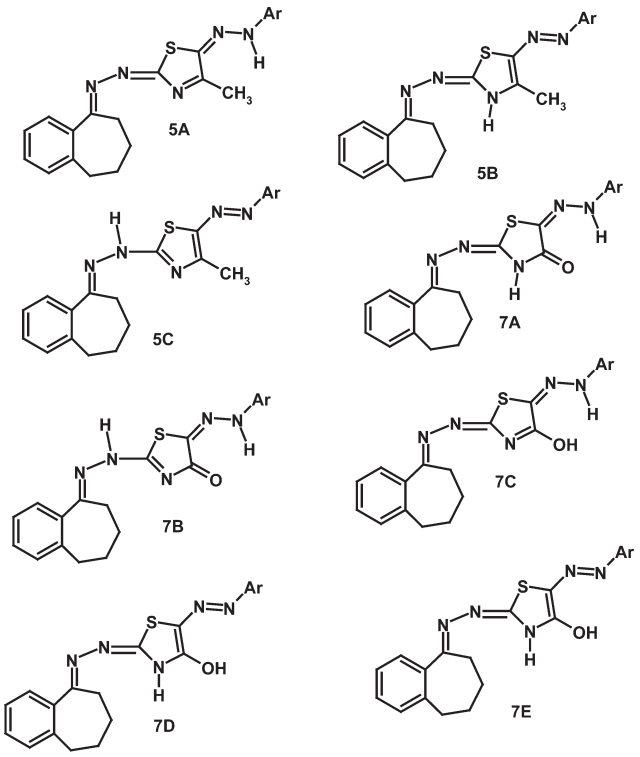
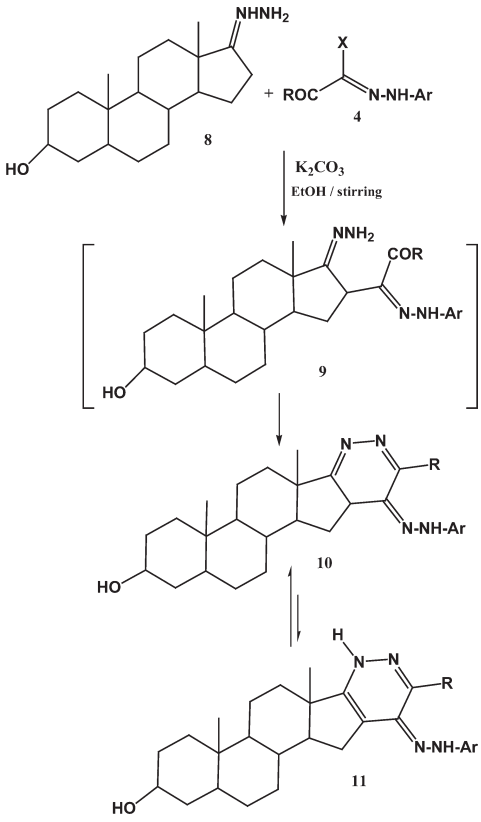


Fig. 1 Tautomeric structure of compounds **5** and **7**.



Scheme 3

typical example of the series studied, in solvents of different polarities showed little, if any, shift (Table 1). This finding while it suggests that compounds **7** exist in one tautomeric form, it excludes the azo tautomeric forms **7D** and **7E** and the enol-hydrazone form **7C** (Fig. 1). ^{13}C NMR spectrum of **7f**, taken as a typical example of the series prepared, revealed the signal of the carbonyl carbon of the pyrimidinone ring residue at δ 179.52 ppm. Such a chemical shift value indicated the presence of a carbonyl group adjacent to the N=C group.[21] On the basis of all the above data, the isolated products were assigned the tautomeric form **7B**.

Recently, it was reported that reaction of hydrazone derivative **8** with hydrazonoyl chlorides **4** at room temperature in the presence of potassium carbonate gave the product **10** *via* intermediate **9** (Scheme 3).[22]

Following the above report, we investigated the site-selectivity of the reaction of hydrazonoyl halides **4** with hydrazone **3**. Thus, hydrazone **3** reacted with hydrazonoyl chlorides **4a–g** in ethanol in the presence of potassium carbonate at room temperature or in ethanol in the presence of triethylamine as a basic catalyst under reflux to give in all cases only one isolable product (as indicated by TLC) and identified as a member of the series **12** (Scheme 4). All attempts to cyclise these products to give compound **13** failed. The structure of compounds **12** was established on the basis of spectral data (mass, IR and ^{1}H NMR) and elemental analysis. For example, IR spectra of the products **12** showed absorption bands at ν 3380–3265, 3224–3150 and 1685–1666 cm^{-1} attributed to the two NH and carbonyl group, respectively. ^{1}H NMR spectra of compounds **12** revealed three singlet signals at δ 2.36–2.46, 8.88–9.12 and 11.70–12.28 ppm for the acetyl and two NH groups, respectively. The above spectral data indicated that the reaction of hydrazonoyl halides with hydrazone **3** proceeds *via* elimination of HX from NH_2 group to give compounds **12** rather than the different reaction course reported in the steroid series **9**[22] to give compounds **14** and/or **15**.

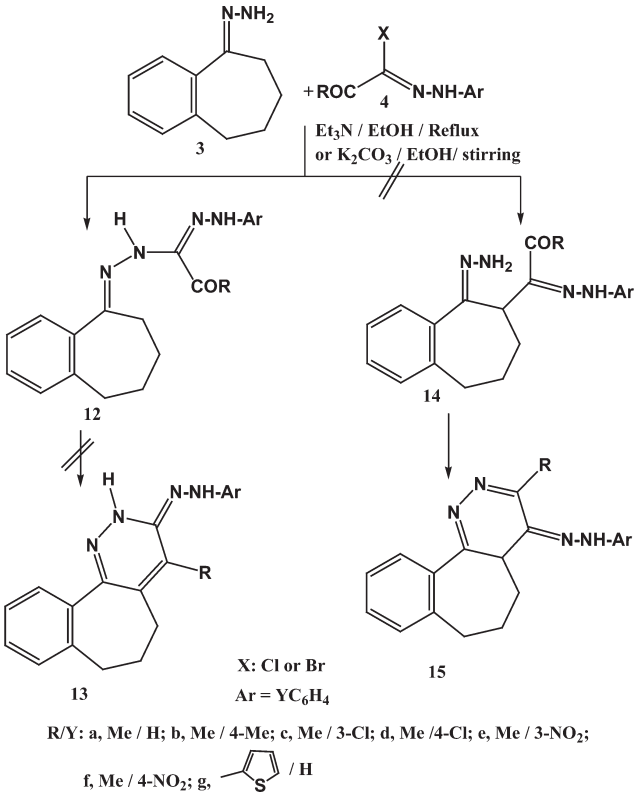
Table 1 UV spectra of compounds **5** and **7** in dioxane

Compd	λ_{max} (log ε)
5a	442 (4.83), 292 (4.94)
5b	444 (4.80), 296 (4.77)
5c[a]	444 (4.61), 288 (4.69)
5d	450 (4.36), 302 (4.92)
7c[b]	366 (4.25), 298 (4.82)
7d	373 (4.35), 296 (4.68)
7e	369 (4.50), 306 (4.84)
7f	391 (4.60), 298 (5.01)

[a] Solvent: λ_{max} (log ε): acetic acid 438 (5.08), 306 (4.80); DMF 454 (4.31), 300 (4.90); ethanol 434 (4.84), 298 (5.08).
[b] Solvent: λ_{max} (log ε): acetic acid 376 (4.21), 288 (4.35); DMF 365 (4.05), 289 (4.31); ethanol 366 (4.28), 298 (4.56).

Biological screening

In vitro anti-microbial screening of the series of compounds (**5**, **7** and **12**) prepared in this study was carried out using four fungal strains, including *Aspergillus fumigatus (RCMB 002003)* (AF), *Geotrichum candidum (RB 052006)* (GC), *Candida albicans (RCMB 005002) (CA)* and *Syncephalastrum racemosum (RCMB 005003)* (SR), and four bacteria species, including *Gram positive Bacteria, Staphylococcus aureus (RCMB 000106) (SA)* and *Bacillus subtilis (RCMB 000107) (BS), Gram negative Bacteria, Pseudomonas aeruginosa (RCMB 000102 (PA))* and *Escherichia coli (RCMB 000103) (EC).* Data in Table 2 revealed that most of the compounds have superior antibacterial potency relative to antifungal and antibacterial potency. Of the thiazole derivatives **5a,b** and **7a–d**, compounds **5a** and **7d** are highly active against all microorganisms used. However, compound **5b** has excellent activity against all microorganisms except *Syncephalastrum racemosum (SR)* and *Pseudomonas aeruginosa (PA)*. These results indicated that the presence of the electron donating group of the phenyl group at position 5 of the thiazole ring increases



Scheme 4 Reaction of hydrazone derivative **3** with hydrazonoyl halides.

the activity. On the other hand, for the formazan derivatives, the most reactive compounds were **12a** and **12b** against all microorganisms except *SR* and *PA*. While, compound **12c** shows significant activity against gram positive bacteria and gram negative bacteria.

Conclusion

In conclusion, we report the synthesis of new derivatives of thiazole and formazan incorporated with the benzocycloheptene unit *via* reaction of thiosemicarbazone and hydrazone derivatives. The structures of the newly synthesised compounds were established on the basis of spectral data (mass, IR, ^{1}H NMR, ^{13}C NMR and UV) and elemental analyses. Also, some of the newly synthesised compounds were evaluated for antimicrobial activity and the results showed that some compounds showed excellent activity against the tested microorganisms.

Experimental

All melting points were determined on an electrothermal Gallenkamp apparatus. Solvents were distilled and dried by standard literature procedures prior to use. The IR spectra were measured on a Pye-Unicam sp 300 instrument in potassium bromide disks. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian spectrometer (300 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) and the chemical shifts were related to those of the solvent DMSO-d_6. The mass spectra were recorded on GCMS-Q1000-EX Shimadzu and GCMS 5988-A HP spectrometers, the ionising voltage was 70 eV. Elemental analyses were carried out by the Microanalytical Center of Cairo University, Giza, Egypt. Hydrazonoyl halides **4** and **6** were prepared as previously described.[23,24]

Table 2 Anti-microbial activities of compounds **5**, **7** and **12**

Microorganism	**5a**	**5b**	**7c**	**7d**	**7e**	**12a**	ST (30 μg mL^{-1})	
Fungi							Itraconazole	Clotrimazole
AF	22.1	21.5	15.2	21.3	14.2	23.8	28.5	26.1
GC	19.6	18.5	17.4	18.2	11.5	21.2	27.1	23.1
CA	19.5	16.5	13.2	14.5	12.0	20.1	26.1	18.3
SR	NA	10.6	NA	9.4	NA	NA	22.3	20.5
Gram positive bacteria							Penicillin G	Streptomycin
SA	23.1	22.0	16.2	22.9	10.5	20.8	29.4	25.1
BS	26.4	23.6	19.8	22.1	13.2	25.0	32.5	29.1
Gram negative bacteria							Penicillin G	Streptomycin
PA	NA	13.4	NA	21.0	NA	NA	28.3	24.3
EC	22.6	20.5	10.1	15.7	8.6	23.0	33.5	25.6

The experiment was carried out in triplicate and average zone of inhibition was calculated; NA, no activity.

Table 3 Anti-microbial activities of compounds **5**, **7** and **12**

Microorganism	**12b**	**12c**	**12d**	**12e**	**12f**	**12g**	ST (30 μg mL^{-1})	
Fungi							Itraconazole	Clotrimazole
AF	23.4	NA	18.5	NA	14.6	10.4	28.5	26.1
GC	23.1	NA	17.4	NA	19.0	9.8	27.1	23.1
CA	16.2	NA	15.2	NA	11.2	10.1	26.1	18.3
SR	NA	NA	NA	NA	NA	NA	22.3	20.5
Gram positive bacteria							Penicillin G	Streptomycin
SA	22.8	22.6	16.2	NA	10.6	9.5	29.4	25.1
BS	28.3	23.4	14.3	NA	12.1	12.1	32.5	29.1
Gram negative bacteria							Penicillin G	Streptomycin
PA	NA	21.9	NA	NA	NA	NA	28.3	24.3
EC	23.1	19.9	NA	NA	8.0	NA	33.5	25.6

The experiment was carried out in triplicate and average zone of inhibition was calculated; NA, no activity.

Reaction of hydrazonoyl chlorides **4** *or* **6** *with thiosemicarbazone derivative* **2***; general procedure*

Triethylamine (0.35 mL) was added to a stirred solution of thiosemicarbazone derivative **2** (0.47 g, 2 mmol) and the appropriate hydrazonoyl chloride **4** or **6** (2 mmol) in dioxane (30 mL), and the mixture was refluxed for 5 h. The precipitated triethylamine hydrochloride was filtered off, and the filtrate was evaporated under reduced pressure. The residue was triturated with methanol. The solid product, so formed in each case, was collected by filtration, washed with water, dried, and crystallised from the appropriate solvent to afford the corresponding thiazole derivatives **5** or **7**, respectively.

4-Methyl-5-(4-methylphenylazo)-2-[N′-(6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)-hydrazino]-thiazole (**5Ca**): Pink solid, (82% yield), m.p. 80–82 °C (EtOH); IR (KBr) ν_{max} 3359 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.17–1.76 (m, 4H, $2CH_2$), 2.26 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.79–2.91 (m, 4H, $2CH_2$), 7.12 (d, J = 8Hz, 2H, ArH), 7.23–7.38 (m, 4H, ArH), 7.59 (d, J = 8Hz, 2H, ArH), 10.42 (s, 1H, NH); MS *m/z* (%) 390 (M^++1, 15), 389 (M^+, 35), 232 (31), 158 (42), 130 (54), 129 (50), 117 (50), 104 (39), 91 (100), 77 (46). Anal. Calcd for $C_{22}H_{23}N_5S$ (389.52): C, 67.84; H, 5.95; N, 17.98. Found: C, 67.65; H, 5.84; N, 17.72%.

4-Methyl-5-(3-methylphenylazo)-2-[N′-(6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)-hydrazino]-thiazole (**5Cb**): Dark orange crystals, (80% yield), m.p. 70–73 °C (EtOH); IR (KBr) ν_{max} 3190 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.62–1.78 (m, 4H, $2CH_2$), 2.29 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 2.65–2.90 (m, 4H, $2CH_2$), 6.81–7.63 (m, 8H, ArH), 10.43 (s, 1H, NH); MS *m/z* (%) 390 (M^++1, 24), 389 (M^+, 44), 231 (24), 158 (48), 130 (60), 116 (48), 91 (100), 77 (48). Anal. Calcd for $C_{22}H_{23}N_5S$ (389.52): C, 67.84; H, 5.95; N, 17.98. Found: C, 67.75; H, 6.0; N, 17.80%.

4-Methyl-5-(phenylazo)-2-[N′-(6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)-hydrazino]-thiazole (**5Cc**): Dark red solid, (80% yield), m.p. 100–102 °C (EtOH); IR (KBr) ν_{max} 3359 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.15–1.75 (m, 4H, $2CH_2$), 2.49 (s, 3H, CH_3), 2.79–3.09 (m, 4H, $2CH_2$), 6.98–7.62 (m, 9H, ArH), 10.10 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 16.94 ($\underline{C}H_3$), 22.68, 26.04, 29.95, 32.33 ($4\underline{C}H_2$), 113.33, 114.95, 124.25, 126.72, 128.39, 129.38, 129.77, 130.18, 130.36, 138.32, 138.55 (10 ArC + =$\underline{C}$), 140.51 (N–C=C), 144.26 ($\underline{C}$=N), 171.45 (S–C=N). MS *m/z* (%) 376 (M^++1, 15), 375 (M^+, 42), 374 (30), 342 (21), 158 (28), 139 (56), 130 (51), 117 (34), 115 (30), 91 (37), 77 (100). Anal. Calcd for $C_{21}H_{21}N_5S$ (375.49): C, 67.17; H, 5.64; N, 18.65. Found: C, 67.08; H, 5.49; N, 18.52%.

5-(4-Chlorophenylazo)-4-methyl-2-[N′-(6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)-hydrazino]-thiazole (**5Cd**): Dark red solid, (82% yield), m.p. 110–112 °C (EtOH/dioxane); IR (KBr) ν_{max} 3254 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.64–1.76 (m, 4H, $2CH_2$), 2.59 (s, 3H, CH_3), 2.79–2.91 (m, 4H, $2CH_2$), 7.24–7.62 (m, 8H, ArH), 10.57 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 15.98 ($\underline{C}H_3$), 22.45, 25.25, 29.16, 31.27 ($4\underline{C}H_2$), 113.57, 115.49, 124.15, 126.20, 128.23, 130.01, 130.28, 131.28, 133.31, 138.01, 138.45 (10 ArC + =$\underline{C}$),140.11 (N–C=C), 142.35 ($\underline{C}$=N), 170.28 (S–C=N). MS *m/z* (%) 411 (M^++2, 26), 410 (M^++1, 25), 409 (M^+, 66), 408 (58), 375 (32), 158 (66), 143 (39), 130 (84), 116 (100), 111 (65), 89 (35), 77 (31). Anal. Calcd for $C_{21}H_{20}ClN_5S$ (409.94): C, 61.53; H, 4.92; N, 17.08. Found: C, 61.41; H, 4.83; N, 17.00%.

5-(Phenylhydrazono)-2-[N′-(6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)-hydrazino]-thiazol-4-one (**7Bc**): Yellow solid, (88% yield), m.p. 185–186 °C (EtOH); IR (KBr) ν_{max} 3200, 3151 (2NH), 1708 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.14–1.66 (m, 4H, $2CH_2$), 2.63–2.84 (m, 4H, $2CH_2$), 7.12–7.53 (m, 9H, ArH), 10.09 (s, 1H, NH), 10.38 (s, 1H, NH); MS *m/z* (%) 377 (M^+, 28), 376 (14), 159 (39), 130 (72), 120 (31), 91 (89), 90 (39), 77 (100). Anal. Calcd for $C_{20}H_{19}N_5OS$ (377.46): C, 63.64; H, 5.07; N, 18.55. Found: C, 63.55; H, 5.00; N, 18.41%.

5-[(4-Chlorophenyl)-hydrazono]-2-[N′-(6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)-hydrazino]-thiazol-4-one (**7Bd**): Red solid, (91% yield), m.p. 125–126 °C (EtOH); IR (KBr) ν_{max} 3228, 3166 (2NH), 1663 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.17–1.72 (m, 4H, $2CH_2$), 2.65–2.69 (m, 4H, $2CH_2$), 7.15–7.58 (m, 8H, ArH), 10.08 (s, 1H, NH), 10.40 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 20.54, 25.17, 28.29, 30.98 ($4\underline{C}H_2$), 113.48, 124.99, 126.16, 126.28, 127.59, 128.09, 129.68, 130.54, 131.25, 137.12, 139.49 (10 ArC + =$\underline{C}$), 154.28 ($\underline{C}$=N), 172.11 (S–C=N), 178.97 ($\underline{C}$=O). MS *m/z* (%) 413 (M^++2, 38), 412 (M^++1, 37), 411 (M^+, 100), 285 (52), 200 (26), 158 (32), 143 (62), 126 (63), 128 (34), 125 (51), 116 (53), 111 (43), 91 (30), 75 (37). Anal. Calcd for $C_{20}H_{18}ClN_5OS$ (411.91): C, 58.32; H, 4.40; N, 17.00. Found: C, 58.21; H, 4.20; N, 16.92%.

5-[(4-Bromo-phenyl)-hydrazono]-2-[N′-(6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)-hydrazino]-thiazol-4-one (**7Be**): Yellow solid, (87% yield), m.p. 130–132 °C (EtOH); IR (KBr) ν_{max} 3200, 3151 (2NH), 1708 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.25–1.66 (m, 4H, $2CH_2$), 2.65–3.05 (m, 4H, $2CH_2$), 7.12–7.27 (m, 8H, ArH), 10.09 (s, 1H, NH), 10.44 (s, 1H, NH); MS *m/z* (%) 457 (M^++2, 53), 456 (M^++1, 45), 455 (M^+, 30), 454 (43), 284 (30), 200 (33), 199 (40), 170 (72), 156 (42), 129 (100), 104 (32), 91 (57), 77 (60). Anal. Calcd for $C_{20}H_{18}BrN_5OS$ (455.04): C, 52.64; H, 3.98; N, 15.35. Found: C, 52.45; H, 4.08; N, 15.18%.

5-[(4-Nitro-phenyl)-hydrazono]-2-[N′-(6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)-hydrazino]-thiazol-4-one (**7Bf**): Orange solid, (80% yield), m.p. 122 °C (EtOH/dioxane); IR (KBr) ν_{max} 3151, 3066 (2NH), 1712 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.07–1.83 (m, 4H, $2CH_2$), 2.64–3.06 (m, 4H, $2CH_2$), 7.13–8.29 (m, 8H, ArH), 10.10 (s, 1H, NH), 11.0 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 21.48, 25.77, 28.13, 31.40 ($4\underline{C}H_2$), 113.95, 125.82, 126.54, 126.85, 127.82, 128.54, 129.10, 129.62, 131.05, 138.27, 139.36 (10 ArC + =$\underline{C}$), 155.94 ($\underline{C}$=N), 171.0 (S–C=N), 179.52 ($\underline{C}$=O). MS *m/z* (%) 423 (M^++1, 37), 422 (M^+, 90), 259 (34), 156 (46), 143 (77), 130 (100), 116 (96), 91 (57), 89 (50), 77 (61), 76 (56). Anal. Calcd for $C_{20}H_{18}N_6O_3S$ (422.46): C, 56.86; H, 4.29; N, 19.89. Found: C, 56.64; H, 4.08; N, 19.71%.

Reaction of hydrazonoyl chlorides **4** *with hydrazone derivative* **3**

Method A: Triethylamine (0.35 mL) was added to a stirred solution of hydrazone derivative **3** (0.35g, 2 mmol) and the appropriate hydrazonoyl chloride **4** (2 mmol) in absolute ethanol (30 mL), and the mixture was refluxed for 5 h. The precipitated triethylamine hydrochloride was filtered off, and the filtrate was evaporated under reduced pressure. The residue was triturated with methanol. The solid product, so formed in each case, was collected by filtration, washed with water, dried, and crystallised from ethanol to afford the corresponding formazan derivatives **12**.

Method B: A mixture of hydrazone derivative **3** (0.87 g, 0.005 mol) and hydrazonoyl halide (0.005 mol) in absolute ethanol (30 ml) containing K_2CO_3 (0.69 g, 0.005 mol) was stirred for 24 h, at room temperature. The reaction mixture was poured onto ice/water and the resulting solid was collected by filtration and crystallised from ethanol to afford the corresponding formazan derivatives **12** which are identical in all respects with those produced by method A.

3-Acetyl-5-phenyl-1-[N′-(6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)]-1,2-dihydroformazan (**12a**): Red solid, (86% yield), m.p. 160 °C; IR (KBr) ν_{max} 3380, 3224 (2NH), 1670 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.59–1.77 (m, 4H, $2CH_2$), 2.36 (s, 3H, CH_3), 2.51–2.74 (m, 4H, $2CH_2$), 6.81–7.52 (m, 9H, ArH), 8.93 (s, 1H, NH), 11.78 (s, 1H, NH); MS *m/z* (%) 335 (M^++1, 7), 334 (M^+, 11), 206 (43), 158 (23), 143 (31), 128 (32), 115 (34), 104 (31), 93 (48), 77 (100). Anal. Calcd for $C_{20}H_{22}N_4O$ (334.42): C, 71.83; H, 6.63; N, 16.75. Found: C, 71.65; H, 6.50; N, 16.58%.

3-Acetyl-5-(4-methylphenyl)-1-[N′-(6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)]-1,2-dihydroformazan (**12b**): Red crystals, (95% yield), m.p. 132 °C; IR (KBr) ν_{max} 3332, 3224 (2NH), 1666 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.60–1.75 (m, 4H, $2CH_2$), 2.22 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 2.56–2.70 (m, 4H, $2CH_2$), 6.91 (d, J = 9Hz, 2H, ArH), 7.06 (d, J = 9Hz, 2H, ArH), 7.22–7.46 (m, 4H, ArH), 8.88 (s, 1H, NH), 11.70 (s, 1H, NH); MS *m/z* (%) 349 (M^++1, 9), 348 (M^+, 33), 190 (25), 160 (40), 159 (23), 158 (23), 121 (30), 119 (23), 106 (23), 91 (100), 77 (42). Anal. Calcd for $C_{21}H_{24}N_4O$ (348.44): C, 72.39; H,6.94; N, 16.08. Found: C, 72.17; H, 6.87; N, 16.12%.

3-Acetyl-5-(3-chlorophenyl)-1-[N′-(6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)]-1,2-dihydroformazan (**12c**): Orange solid, (84% yield), m.p. 86–88 °C; IR (KBr) ν_{max} 3320, 3150 (2NH), 1685 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.50–1.74 (m, 4H, $2CH_2$), 2.36 (s, 3H, CH_3), 2.57–2.70 (m, 4H, $2CH_2$), 6.85–7.38 (m, 8H, ArH), 8.95 (s, 1H, NH), 11.83 (s, 1H, NH); MS *m/z* (%) 370 (M^++2, 20), 369 (M^++1, 21), 368 (M^+, 63), 367 (55), 269 (26), 240 (27), 209 (40), 160 (100), 140 (72), 130 (71), 111 (54), 91 (43), 77 (32). Anal. Calcd for $C_{20}H_{21}ClN_4O$ (368.86): C, 65.12; H, 5.74; N, 15.19. Found: C, 65.03; H, 5.64; N, 15.06%.

3-Acetyl-5-(4-chlorophenyl)-1-[N′-(6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)]-1,2-dihydroformazan (**12d**): Red crystals, (90% yield), m.p. 140–142 °C; IR (KBr) ν_{max} 3265, 3210 (2NH), 1685 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.59–1.76 (m, 4H, $2CH_2$), 2.36 (s, 3H, CH_3), 2.57–2.72 (m, 4H, $2CH_2$), 7.06 (d, J = 9Hz, 2H, ArH), 7.20 (d, J = 9Hz, 2H, ArH), 7.23–7.49 (m, 4H, ArH), 8.93 (s, 1H, NH), 11.77 (s, 1H, NH); MS *m/z* (%) 370 (M^++2, 5), 369 (M^++1, 11), 368 (M^+, 22), 210 (24), 160 (42), 140 (29), 130 (24), 105 (100), 77 (15).

Anal. Calcd for $C_{20}H_{21}ClN_4O$ (368.86): C, 65.12; H, 5.74; N, 15.19. Found: C, 65.08; H, 5.58; N, 15.27%.

3-Acetyl-5-(3-nitrophenyl)-1-[N′-(6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)]-1,2-dihydroformazan (**12e**): Brown solid, (85% yield), m.p. 130–131 °C; IR (KBr) ν_{max} 3354, 3221 (2NH), 1684 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.60–1.75 (m, 4H, $2CH_2$), 2.39 (s, 3H, CH_3), 2.59–2.71 (m, 4H, $2CH_2$), 7.22–7.77 (m, 8H, ArH), 8.99 (s, 1H, NH), 12.01 (s, 1H, NH); MS *m/z* (%) 379 (M^+, 38), 378 (69), 349 (50), 333 (63), 279 (75), 250 (75), 223 (69), 191 (88), 151 (81), 101 (100), 77 (63). Anal. Calcd for $C_{20}H_{21}N_5O_3$ (379.41): C, 63.31; H, 5.58; N, 18.46. Found: C, 63.08; H, 5.42; N, 18.28%.

3-Acetyl-5-(4-nitrophenyl)-1-[N′-(6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)]-1,2-dihydroformazan (**12f**): Red crystals, (95% yield), m.p. 190–192 °C; IR (KBr) ν_{max} 3290, 3159 (2NH), 1678 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.58–1.88 (m, 4H, $2CH_2$), 2.36 (s, 3H, CH_3), 2.51–2.71 (m, 4H, $2CH_2$), 7.07 (d, J = 9Hz, 2H, ArH), 7.21–7.53 (m, 4H, ArH), 8.13 (d, J = 9Hz, 2H, ArH), 9.12 (s, 1H, NH), 12.28 (s, 1H, NH); MS *m/z* (%) 379 (M^+, 89), 378 (66), 221 (32), 160 (92), 144 (33), 130 (100), 115 (39), 91 (45), 77 (33). Anal. Calcd for $C_{20}H_{21}N_5O_3$ (379.41): C, 63.31; H, 5.58; N, 18.46. Found: C, 63.08; H, 5.42; N, 18.28%.

3-Thienyl-5-phenyl-1-[N′-(6,7,8,9-tetrahydro-benzocycloheptен-5-ylidene)]-1,2-dihydroformazan (**12g**): Red crystals, (90% yield), m.p. 170–172 °C; IR (KBr) ν_{max} 3321, 3205 (2NH), 1601 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.59–1.77 (m, 4H, $2CH_2$), 2.51–2.74 (m, 4H, $2CH_2$), 6.87–8.13 (m, 12H, ArH), 9.21 (s, 1H, NH), 12.02 (s, 1H, NH); MS *m/z* (%) 403 (M^++1, 2), 402 (M^+, 6), 245 (19), 159 (19), 111 (81), 110 (100), 104 (16), 91 (13), 77 (39). Anal. Calcd for $C_{23}H_{22}N_4OS$ (402.51): C, 68.63; H, 5.51; N, 13.92. Found: C, 68.48; H, 5.35; N, 13.88%.

Agar diffusion well method to determine the antimicrobial activity

The microorganism inoculums were uniformly spread using a sterile cotton swab on a sterile Petri dish containing malt extract agar (for fungi) and nutrient agar (for bacteria). Each sample (100 μL) was added to each well (6 mm diameter holes cut in the agar gel, 20 mm apart from one another). The systems were incubated for 24–48 h at 37 °C (for bacteria) and at 28 °C (for fungi). After incubation, microorganism growth was observed. Inhibition of the bacterial and fungal growth were measured in mm. Tests were performed in triplicate.[25]

Received 30 July 2012; accepted 18 September 2012
Paper 1201441 doi: 10.3184/174751912X13491094428620
Published online: 12 November 2012

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