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Synthesis of Some Thienyl-Triazine Derivatives and Antimicrobial Activity

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Some 4-(1-aryl-ethylideneamino)-5-phenyl-1,4-dihydro-2*H*-[1,2,4]triazine-3-thione derivatives were synthesized and evaluated for their antimicrobial activity. The reaction of 2-acetylfuran or 2-acetylthiophene with thiocarbohydrazide yielded the N-(1-arylethylidene) thiocarbohydrazides which furnished the thienyl-triazine derivatives by reacting with phenacyl bromides. The chemical structures of the compounds were elucidated by IR, ^{1}H NMR and FAB$^+$-MS spectral data and elemental analyses. Microdilution broth susceptibility assay was used for the antimicrobial activity evaluation of the compounds against the strains *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia spp.*, *Salmonella thyphimurium*, *Proteus vulgaris*, *Listeria monocytogenes*, *Candida glabrata*, *Candida albicans*, *Candida tropicalis*. Chloramphenicol and ketoconazole were used as control drugs. A significant level of activity was observed.

Key Words: Triazine, Thiophene, Thiocarbohydrazide, Antimicrobial activity.

INTRODUCTION

Rapid development of resistance to currently available antimicrobial agents becomes a major threat to human health. Current reports propose that bacteria and fungi are developing resistance to existing drugs. Many researchers are reported their results on this subject[1-4]. One desirable long-term solution is the discovery of new drug candidates[5]. In view of the above, the design and synthesis of effective and potent antimicrobial agents is an important area in medicinal chemistry[1-5]. The NCNN group is an essential part of various heterocycles bearing high biological activities. In recent years, there has been a great interest in the synthesis of heterocyclic compounds containing 1,2,4-triazine ring which is bearing NCNN group because of their biological importance[6-16]. Some 1,2,4-triazine derivatives have played a vital role as antimicrobial compounds[17-20]. Ceftriaxone is a well known

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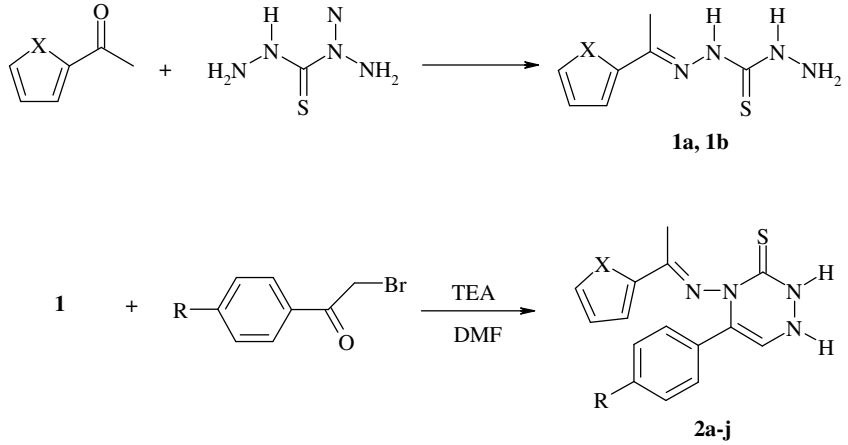
antibiotic, contains 1,2,4-triazine ring[17,18]. There is an interest in the design and synthesis of compounds structurally based on this ring system because of their higher antimicrobial activity than that of reference drug and antifungal activity comparable with reference drug[19,20]. Additionally, 1,2,4-triazines, which are regarded as 6-aza analogues of pyrimidine bases, in general, have a great biological importance[21]. Encouraged by the above-mentioned findings, it would be interesting to investigate the synthesis and antimicrobial activity of some new 1,2,4-triazine derivatives.

EXPERIMENTAL

All reagents were used as purchased from commercial suppliers without further purification. Melting points were determined by using an Electrothermal 9100 digital melting point apparatus and were uncorrected (Electrothermal, Essex, UK). The compounds were checked for purity by TLC on silica gel 60 F_{254}. Spectroscopic data were recorded on the following instruments: IR, Shimadzu 435 IR spectrophotometer (Shimadzu, Tokyo, Japan); ^{1}H NMR, Bruker 500 MHz NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) in DMSO-d_6 using TMS as internal standard; MS-FAB, VG Quattro mass spectrometer (Fisons Instruments Vertriebs GmbH, Mainz, Germany), Elemental analyses were performed on a Perkin-Elmer EAL 240 elemental analyser (Perkin-Elmer, Norwalk, CT, USA).

General procedure for the synthesis of the compounds

N-(1-Arylethylidene)thiocarbohydrazides (1a-b): Equimolar amounts of 2-acetylthiophene or 2-acetylfuran (10 mmol) and thiocarbohydrazide (1.06 g, 10 mmol) in absolute ethanol (50 mL) was refluxed for 4 h. The obtained solid was filtered off and crystallized from ethanol-water to give **1a,b** (**Scheme-I**).



Scheme-I: Synthetic route of the title compounds

4-(1-Arylethylideneamino)-5-aryl-1,4-dihydro-2*H*-[1,2,4]triazine-3-thiones (2a-j): A mixture of **1a** or **1b** (5 mmol) and phenacyl bromide (5 mmol) in DMF

(50 mL) containing few drops of piperidine was refluxed for 6 h. The mixture was cooled and poured onto ice-water. The solid obtained was filtered off and crystallized from ethanol to give **2-a-j** (**Scheme-I**, Table-1).

TABLE-1
PHYSICAL CHARACTERISTICS OF THE SYNTHESIZED COMPOUNDS

Compound	X	R	m.p. (ºC)	Yield (%)	m.f.	m.w.
2a	O	H	119-121	75	$C_{15}H_{14}N_4OS$	298
2b	O	Cl	159-160	65	$C_{15}H_{13}N_4OSCl$	332
2c	O	CH_3	113-115	70	$C_{16}H_{16}N_4OS$	312
2d	O	OCH_3	80-83	68	$C_{16}H_{16}N_4O_2S$	328
2e	O	NO_2	159-160	77	$C_{15}H_{13}N_5O_3S$	343
2f	S	H	66-68	63	$C_{15}H_{14}N_4S_2$	314
2g	S	Cl	160-163	59	$C_{15}H_{13}N_4S_2Cl$	348
2h	S	CH_3	118-120	65	$C_{16}H_{16}N_4S_2$	328
2i	S	OCH_3	68-71	75	$C_{16}H_{16}N_4OS_2$	344
2j	S	NO_2	178-180	78	$C_{15}H_{13}N_5O_2S_2$	359

2a-j: IR (KBr, ν_{max}, cm^{-1}): 3225-3175 (NH), 1620-1435 (C=C and C=N).

4-[1-(2-Furyl)ethylideneamino]-5-phenyl-1,4-dihydro-2*H*-[1,2,4]triazine-3-thione (2a): ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.35 (3H, s, CH_3), 5.50-5.55 (2H, m, two NH of triazine), 6.30 (1H, s, triazine C_6-H), 6.60-6.70 (1H, m, furan C_4-H), 6.80-6.85 (1H, m, furan C_3-H), 7,15-7.50 (5H, m, phenyl protons), 7.80 (1H, m furan C_5-H). For $C_{15}H_{14}N_4OS$ calcd. (%): 60.38 C, 4.73 H, 18.78 N; found. (%): 60.40 C, 4.71 H, 18.76 N. MS-FAB$^+$: m/z: 299 [M + 1].

4-[1-(2-Furyl)ethylideneamino]-5-(4-chlorophenyl)-1,4-dihydro-2*H*-[1,2,4]triazine-3-thione (2b): ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.30 (3H, s, CH_3), 5.40-5.50 (2H, m, two NH of triazine), 6.45 (1H, s, triazine C_6-H), 6.60-6.65 (1H, m, furan C_4-H), 6.85 (1H, d [J = 3.40 Hz], furan C_3-H), 7.50 and 7.70 (4H, two d [J = 6.67 Hz and J = 6.69 Hz], phenyl protons), 7.80-7.90 (1H, m, furan C_5-H). For $C_{15}H_{13}N_4OSCl$ calcd. (%): 54.13 C, 3.94 H, 16.06 N; found. (%): 54.16 C, 3.98 H, 16.10 N. MS-FAB$^+$: m/z: 333 [M + 1].

4-[1-(2-Furyl)ethylideneamino]-5-(4-methylphenyl)-1,4-dihydro-2*H*-[1,2,4]triazine-3-thione (2c): ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.30 (3H, s, CH_3), 2.40 (3H, s, phenyl-CH_3), 5.50-5.60 (2H, m, two NH of triazine), 6.30 (1H, s, triazine C_6-H), 6.60-6.65 (1H, m, furan C_4-H), 6.80 (1H, d [J = 3.40 Hz], furan C_3-H), 7.20 and 7.50 (4H, two d [J = 8.06 Hz and J = 8.09 Hz], phenyl protons), 7.75-7.80 (1H, m furan C_5-H). For $C_{16}H_{16}N_4OS$ calcd. (%): 61.52 C, 5.16 H, 17.93 N; found. (%): 61.55 C, 5.20 H, 17.91 N. MS-FAB$^+$: m/z: 313 [M + 1].

4-[1-(2-Furyl)ethylideneamino]-5-(4-methoxyphenyl)-1,4-dihydro-2*H*-[1,2,4]triazine-3-thione (2d): ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.40 (3H, s, CH_3), 3.90-4.00 (3H, m, OCH_3), 5.30-5.35 (2H, m, two NH of triazine), 6.50 (1H, s, triazine C_6-H), 6.70-6.80 (1H, m, furan C_4-H), 7.00-7.10 (1H, m, furan C_3-

H), 7,00-7.65 (4H, m, phenyl protons), 7.85-7.90 (1H, m, furan C_5-H). For $C_{16}H_{16}N_4O_2S$ calcd. (%): 58.52 C, 4.91 H, 17.06 N; found. (%): 58.50 C, 4.94 H, 17.08 N. MS-FAB$^+$: m/z: 329 [M + 1].

4-[1-(2-Furyl)ethylideneamino]-5-(4-methoxyphenyl)-1,4-dihydro-2*H*-[1,2,4]triazine-3-thione (2e): ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.35 (3H, s, CH_3), 5.50 (2H, s, two NH of triazine), 6.60 (1H, s, triazine C_6-H), 6.70-6.75 (1H, m, furan C_4-H), 6.85 (1H, d [J = 3.43 Hz], furan C_3-H), 7.80-7.85 (1H, m furan C_5-H), 7.95 and 8.30 (4H, two d [J = 8.87 Hz and J = 8.86 Hz], phenyl protons). For $C_{15}H_{13}N_5O_3S$ calcd. (%): 52.47 C, 3.82 H, 20.40 N; found. (%): 52.50 C, 3.85 H, 20.36 N. MS-FAB$^+$: m/z: 344 [M + 1].

4-[1-(2-Thienyl)ethylideneamino]-5-phenyl-1,4-dihydro-2*H*-[1,2,4]triazine-3-thione (2f): ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.40 (3H, s, CH_3), 5.40-5.50 (2H, m, two NH of triazine), 6.40-6.45 (1H, m, triazine C_6-H), 6.90-7.15 (1H, m, thiophene C_4-H), 7.35-7.75 (6H, m, thiophene C_3-H and phenyl protons), 7.95-8.00 (1H, m, thiophene C_5-H). For $C_{15}H_{14}N_4S_2$ calcd. (%): 57.30 C, 4.49 H, 17.82 N; found. (%): 57.34 C, 4.51 H, 17.85 N. MS-FAB$^+$: m/z: 315 [M + 1].

4-[1-(2-Thienyl)ethylideneamino]-5-(4-chlorophenyl)-1,4-dihydro-2*H*-[1,2,4]triazine-3-thione (2g): ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.45 (3H, s, CH_3), 5.40-5.50 (2H, m, two NH of triazine), 6.45-6.55 (1H, m, triazine C_6-H), 7.05-7.15 (1H, m, thiophene C_4-H), 7.35-7.80 (5H, m, thiophene C_3-H and phenyl protons), 7.95 (1H, s, thiophene C_5-H). For $C_{15}H_{13}N_4S_2Cl$ calcd. (%): 51.64 C, 3.76 H, 16.06 N; found. (%): 51.67 C, 3.77 H, 16.10 N. MS-FAB$^+$: m/z: 349 [M + 1].

4-[1-(2-Thienyl)ethylideneamino]-5-(4-methylphenyl)-1,4-dihydro-2*H*-[1,2,4]triazine-3-thione (2h): ^{1}H NMR (500 MHz)(DMSO-d_6) δ (ppm): 2.35-2.45 (6H, m, two CH_3), 5.35-5.50 (2H, m, two NH of triazine), 6.30-6.40 (1H, m, triazine C_6-H), 7.05-7.10 (1H, m, thiophene C_4-H), 7.20-7.75 (5H, m, thiophene C_3-H and phenyl protons), 7.95-8.00 (1H, s, thiophene C_5-H). For $C_{16}H_{16}N_4S_2$ calcd. (%): 58.51 C, 4.91 H, 17.06 N; found. (%): 58.50 C, 4.91 H, 17.07 N. MS-FAB$^+$: m/z: 329 [M + 1].

4-[1-(2-Thienyl)ethylideneamino]-5-(4-methoxyphenyl)-1,4-dihydro-2*H*-[1,2,4]triazine-3-thione (2i): ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.35-2.40 (3H, m, CH_3), 3.90 (3H, s, OCH_3), 5.40-5.50 (2H, m, two NH of triazine), 6.40-6.45 (1H, m, triazine C_6-H), 7.10-7.20 (1H, m, thiophene C_4-H), 7.30-7.85 (5H, m, thiophene C_3-H and phenyl protons), 7.95-8.00 (1H, s, thiophene C_5-H). For $C_{16}H_{16}N_4OS_2$ calcd. (%): 55.79 C, 4.68 H, 16.27 N; found. (%): 55.80 C, 4.68 H, 16.29 N. MS-FAB$^+$: m/z: 345 [M + 1].

4-[1-(2-Thienyl)ethylideneamino]-5-(4-methoyphenyl)-1,4-dihydro-2*H*-[1,2,4]triazine-3-thione (2j): ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.40 (3H, s, CH_3), 5.50-5.60 (2H, m, two NH of triazine), 6.70 (1H, s, triazine C_6-H), 7.10 (1H, m, thiophene C_4-H), 7.40 (1H, d [J = 1.00 Hz], thiophene C_3-H), 7,95 and 8.30 (4H, two d [J = 6.95 Hz and J = 6.95 Hz], phenyl protons). 8.10 (1H, d [J = 1.07 Hz], thiophene C_5-H). For $C_{15}H_{13}N_5O_2S_2$ calcd. (%): 50.13 C, 3.65 H, 19.48 N; found. (%): 50.15 C, 3.66 H, 19.50 N. MS-FAB$^+$: m/z: 360 [M + 1].

Microbiology

Antibacterial and antifungal activity assay: The study was designed to compare MICs obtained by the CLSI reference M7-A7 broth microdilution method[22,23]. Twice MIC readings were performed by each chemical agent. For both the antibacterial and antimycotic assays the compounds were dissolved in DMSO. Further dilutions of the compounds and standard drugs in test medium were prepared at the required quantities of 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.5625 µg/mL concentrations with Mueller-Hinton broth and Sabouroud dextrose broth. All the compounds were tested for their *in vitro* growth inhibitory activity against human pothogenic as Gram-positive bacteria *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212 and *Bacillus subtilis* isolated obtained from Faculty of Pharmacy Anadolu University, Eskisehir, Turkey), as Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 35218, *Escherichia coli* ATCC 25922, *Salmonella thyphimurium* NRRL B-4420, *Proteus vulgaris* NRLL B-123, *Listeria monocytogenes* and yeast *Candida albicans* (isolated obtained from Faculty of Medicine Osmangazi University, Eskisehir, Turkey), *Candida tropicalis* (isolated obtained from Faculty of Medicine Osmangazi University, Eskisehir, Turkey) and *Candida globrata* ATCC 36583.

The cultures were obtained from Mueller-Hinton broth (Difco) for the bacterial strains after overnight incubation at 35 ± 1 ºC. The yeasts were maintained in Sabouroud dextrose broth (Difco) after overnight incubation 35 ± 1 ºC. The inocula of test microorganisms adjusted to match the turbidity of a Mac Farland 0.5 standard tube as determined with a spectrophotometer and the final inoculum size was 0.5-2.5 × 10^5 cfu/mL for antibacterial and antifungal assays. Testing was carried out in Mueller-Hinton broth and Sabouroud dextrose broth (Difco) at pH 7 and the two-fold serial dilutions technique was applied. The last well on the microplates was containing only inoculated broth was kept as controls and the last well with no growth of microorganism was recorded to represent the MIC expressed in mg/mL. Every experiment in the antimicrobial assays was replicated twice in order to define the MIC values. In order to ensure that the solvent per se had no effect on bacteria or yeast growth, a control test was also performed containing inoculated broth supplemented with only DMSO at the same dilutions used in our experiments and found inactive in culture medium. Chloramphenicol and ketokanozole were used as control drugs. The observed data on the antibacterial and antifungal activity of the compounds and the control drugs are given in Table-2.

RESULTS AND DISCUSSION

The structures of compounds **2a-j** were confirmed by elemental analyses, MS-FAB, IR and ^{1}H NMR spectral data. All compounds gave satisfactory elemental analysis. Mass spectra [MS(FAB)] of compounds showed (M + 1) peak, in agreement with their molecular formula. IR spectra of compounds showed NH bands at 3225-3175 cm^{-1} and C=C, C=N bands at 1620-1435 cm^{-1} regions, respectively.

TABLE-2
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF THE COMPOUNDS AS MIC VALUES (μg/mL)

Compound	A	B	C	D	E	F	G	H	I	J	K	L	M
2a	25	25	25	25	50	50	100	25	6.25	25	50	50	50
2b	12.5	25	50	12.5	12.5	50	50	25	12.5	25	50	50	50
2c	50	25	100	50	50	50	100	25	6.25	25	50	50	50
2d	12.5	12.5	50	25	25	50	50	12.5	12.5	25	50	50	50
2e	25	25	100	25	25	50	50	25	6.25	25	50	50	50
2f	25	25	100	25	50	50	50	25	6.25	50	50	50	50
2g	25	25	50	25	12.5	25	50	25	12.5	25	50	50	50
2h	25	25	50	25	25	25	100	12.5	12.5	25	50	50	25
2i	12.5	12.5	50	12.5	12.5	25	50	6.25	12.5	25	50	50	50
2j	12.5	12.5	50	12.5	12.5	25	50	6.25	12.5	25	50	50	50
Ref. 1	12.5	12.5	50	12.5	12.5	50	50	12.5	12.5	12.5	–	–	–
Ref. 2	–	–	–	–	–	–	–	–	–	–	50	25	50

A: *E. coli* (35218), **B:** *E. coli* (25922), **C:** *P. vulgaris*, **D:** *S. thyphimurium*, **E:** *K. pneumoniae*, **F:** *L. monocytogenes*, **G:** *P. aeruginosa*, **H:** *S. aureus*, **I:** *E. faecalis*, **J:** *B. subtilis*, **K:** *C. albicans*, **L:** *C. globrata*, **M:** *C. tropicalis*, Ref. 1: Chloramphenicol, Ref. 2: Ketoconazole.

In the 500 MHz ^{1}H NMR spectrum of compounds, the CH_3 protons of ethylidene were observed as singlet or multiplet at 2.30-2.45 ppm. The protons of two NH groups on triazine appeared at 5.30-5.60 ppm. The C_6 proton of triazine was observed at 6.30-6.70 ppm. All the other aromatic and aliphatic protons were observed at expected regions.

The result of antimicrobial screening of newly prepared compounds **2a-j** expressed as the MIC is summarized in Table-2. The important results were observed at antimicrobial activity screening. The antimicrobial assessment revealed that the compounds posses stronger, similar or moderate activity when compared with reference agents. The MIC values are generally within the range of 6.25-50.00 μg/mL against all evaluated strains. Some of the compounds were highly effective against *E. faecalis*, *L. monocytogenes*, *S. aureus*, *P. vulgaris* and *C. tropicalis*. When compared with chloramphenicol; especially **2a**, **2c**, **2e**, **2f** showed stronger, the other compounds similar to reference agent against *E. faecalis*. Compounds **2g**, **2h**, **2i**, **2j** showed better and the other compounds similar activity against *L. monocytogenes* when compared with chloramphenicol. While the compounds **2i** and **2j** showed stronger activity against *S. aureus*, **2d** and **2h** similar to reference agent. Compound **2h** was more effective than ketoconazole, the other compounds similar against *C. tropicalis*. Compounds **2c** showed better and **2b**, **2d**, **2g**, **2h**, **2i**, **2j** similar activity against *P. vulgaris* when compared with chloramphenicol.

REFERENCES

1. D.T.W. Chu, J.J. Plattner and L. Katz, *J. Med. Chem*., **39**, 3853 (1996).
2. K.M. Overbye and J.F. Barrett, *Drug Discov. Today*, **10**, 45 (2005).
3. C. Walsh, *Nature*, **406**, 775 (2000).
4. J. Travis, *Science*, **264**, 360 (1994).
5. D. Niccolai, L. Tarsi and R.J. Thomas, *Chem. Comm*., **24**, 2333 (1997).
6. W.P. Heilman, R.D. Heilman, J.A. Scozzie, R.J. Wayner, J.M. Gullo and Z.S. Ariyan, *J. Med. Chem*., **22**, 671 (1979).
7. V.L. Rusinov, A.V. Myasnikov, T.L. Pilicheva, O.N. Chupakhim, E.A. Kiprianova and A.D. Garagulya, *Pharm. Chem. J*., **24**, 52 (1990).
8. E.S.H. El Ashry, N. Rashed, M. Taha and E. Ramadan, *Adv. Heterocycl. Chem*., **59**, 39 (1994).
9. E.S.H. El Ashry, N. Rashed, A. Mousaad and E. Ramadan, *Adv. Heterocycl. Chem*., **61**, 207 (1994).
10. L.C. March, G.S. Bajwa, J. Lee, K. Wasti and M.M. Joullie, *J. Med. Chem*., **19**, 845 (1976).
11. A.M. Omar, N.H. Eshba and H.M. Aboushleib, *J. Heterocycl. Chem*., **23**, 1731 (1986).
12. K. Ramasamy, B.G. Ugarkar, P.A. Mc Kernan, R.K. Robins and G.R. Revankar, *J. Med. Chem*., **29**, 2231 (1986).
13. T.E. Ali, S.A. Abdel-Aziz, H.M. El-Shaaener, F.I. Hanafy and A.Z. El-Fauomy, *Phosphorus Sulfur Silicon Rel. Elem.*, **183**, 2139 (2008).
14. M.F. Reich, P.F. Fabio, V.J. Lee, N.A. Kuck and R.T. Testa, *J. Med. Chem*., **32**, 2474 (1989).
15. W.W. Paudler and J.M. Barton, *J. Org. Chem*., **31**, 1720 (1966).
16. S. Nagai, T. Ueda, A. Nagatsu, N. Murakami, J. Sakakibara and M. Murata, *Heterocycles*, **44**, 117 (1997).
17. R. Reiner, U. Weiss, U. Brombacher, P. Lanz, M. Montavon, A. Furlenmeier, P. Angehrn and P.J. Probst, *J. Antibiot*., **33**, 783 (1980).
18. P. Angehrn, P.J. Probst, R. Reiner and R.L. Then, *Antimicrob. Ag. Chemother*., **18**, 913 (1980).
19. J.C. Hegde, K.S. Girisha, A. Adhikari and B. Kalluraya, *Eur. J. Med. Chem*., **43**, 2831 (2008).
20. M.A.M. Taha, *Monatsh. Chem*., **138**, 505 (2007).
21. E.A. Falco, E. Pappas and G.H. Hitchings, *J. Am. Chem. Soc*., **78**, 1938 (1956).
22. Clinical and Laboratory Standards Institute, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Aproved Standard M7-A7, CLSI, Wayne, edn. 7, PA (2006).
23. Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing; M100-S16, 16th Informational Supplement, CLSI, Wayne, PA (2006).

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