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SYNTHESIS OF NEW 1,2,4-TRIAZOLE DERIVATIVES OF NALIDIXIC ACID AS POTENTIAL ANTIBACTERIAL AND ANTIFUNGAL AGENTS

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تم فى هذا البحث تحضير مجموعة من مشتقات حمض الناليدكسيك والتى تم فيها استبدال مجموعة الكربوكسيل فى الوضع ٣ بحلقة ١، ٢، ٤ ترايازول ثم تفاعل هذا المركب الوسيط الجديد مع الألدهيدات العطرية لينتج قواعد شيف او تفاعله مع الأكيل هاليد المناسب لتحضير مشتقات ألكيل ثيو. كذلك تفاعل بعض قواعد شيف مع كلوريد البنزيل لتحضير مشتقات ألكيل ثيو لهذه القواعد. وقد تم التحقق من التركيب البنائى ودرجة النقاوة للمركبات المحضرة بواسطة التحاليل الدقيقة للعناصر والقياسات الطيفية مثل الأشعة تحت الحمراء ، الرنين النووى المغناطيسى وكذلك مطياف الكتلة. تم اختبار فاعلية المركبات المحضرة وحمض الناليدكسيك كمضادات للبكتريا بالمقارنة بعقار الأمبيسيللين المائى كعقار مرجعى. وقد تم أيضا تحديد أقل تركيز تثبيطى للمركبات ذات الفاعلية العالية. ايضا تم اختبار المركبات المستهدفة كمضادات للفطريات بالمقارنة بالكلوتريمازول كعقار مرجعى. وأظهرت النتائج أن معظم المركبات المختبرة لها فاعلية عالية كمضادات للبكتريا أعلى من عقار الأمبيسيللين ومتقاربة من حمض الناليدكسيك ، بينما معظم المركبات المختبرة أعطت نتائج ضعيفة بالمقارنة مع عقار الكلوتريمازول كمضادات للفطريات.

Triazole and triazole fused heterocyclic ring systems possess diverse applications in the fields of medicine, agriculture and industry. A new series of nalidixic acid derivatives having 1,2,4-triazole moiety at position 3 were synthesised to achieve enhanced biological activity and wide spectrum of activity. Nalidixic acid was first converted into its methyl ester which upon hydrazinolysis afforded nalidixic acid hydrazide. Condensation of the hydrazide with CS_2/KOH furnished the potassium dithiocarbazate salt, which cyclized to the 3-[4-amino-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl]-1-ethyl-7-methyl-1H-[1,8]naphthyridin-4-one, ***(4)****, on*

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*refluxing with hydrazine hydrate. Condensation of the key intermediate **4** with aryl aldehydes afforded Schiff's bases **5a-f**, while its reaction with alkyl or aralkyl halides gave compounds **6a-e**. Furthermore, compounds **5a,e** were reacted with benzyl chloride to afford **7a,b**. The chemical structure of the target compounds was confirmed by IR, ^{1}H-NMR, FAB-MS, EI-MS spectra and elemental analyses. The title compounds and the starting Nalidixic acid; were tested for their in-vitro antibacterial and antifungal activities. Most of the tested compounds showed comparable antibacterial activity with those of Nalidixic acid and higher activity than ampicillin. The tested compounds and Nalidixic acid showed non or moderate antifungal activity in comparison to clotrimazole as a reference drug.*

INTRODUCTION

The 1,8-naphthyridine nucleus (the main skeleton of nalidixic acid) is one of the frequently found heterocyclic moiety among different biologically active compounds[1-7]. Nalidixic acid (trade name NegGram) is the basis for quinoline antibiotics which is effective against both Gram-positive and Gram-negative bacteria. It was introduced for the treatment of urinary tract infections caused, for example, by *Escherichia coli*, *Proteus*, *Shigella*, *Enterobacter*, and *Klebsiella*[1&2]. It failed to achieve adequate concentrations in the plasma or tissues for the treatment of systemic infections following oral or potential administration but got concentrated in the urine, where they could be effective for eradicating urinary tract infections[1]. Structure activity relationship (SAR) of compounds based on nalidixic acid, have led to a large group of synthetic antibacterial agents collectively known as the quinolones[2,8&9]. Resistance was found to emerge rapidly, even while on therapy due to the increasing of resistance of many infections by Gram negative and Gram positive bacteria. The emergence of multidrug resistance is a problem of ever increasing significance[10]. Organisms including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermids* (MRSE), vancomycin-resitant *enterococci* (VRE), penicillin and cephalosporin-resistant strepto-cocci are continuously challenging chemists, physicians and patients. Consequently, the search of new chemotherapeutic agents constitutes real challenge facing microbiologists, pharmacologists as well as medicinal chemists.

Currently, 1,2,4-triazole nucleus has been incorporated in a wide variety of pharmacologically active compounds. It represents one of the most biologically active classes of compounds, possessing a wide

spectrum of activities. Also, it has been observed that 1,2,4-triazole moiety has a great versatility in fusing to various ring systems and the N-bridged heterocycles derived from them are associated with diverse pharmacological activity such as antibacterial, antifungal, hypoglycemic, antihypertensive and analgesic properties[11-13].

In the present work, these encouraging facts mentioned above about biological activity associated with both 1,2,4-triazoles and nalidixic acid moieties[14] prompted us to synthesize some nalidixic acid derivatives carrying the biodynamic heterocyclic system (1,2,4-triazole) at position-3 with an objective to obtain biheterocycles of enhanced biological activity. The compounds so obtained were screened for their *in-vitro* activity against Gram-positive, Gram-negative bacteria and different phytopathogenic fungi species.

EXPERIMENTAL

Chemistry

Materials and equipments

Melting points were determined on an electrothermal melting point apparatus (Stuart Scientific, model SMP1, UK), and were uncorrected. Precoated silica gel plates (kieselgel 0.2 mm, 60G F_{254}, Merck) were used for thin layer chromatography. A developing solvent system of chloroform/methanol (9:1) was used and the spots were detected by ultraviolet lamp (Spectroline, model CM-10, U.S.A.).

IR spectra (KBr discs) were recorded on a Shimadzu spectrophotometer (IR-470), Faculty of Pharmacy, Assiut University, Assiut. ^{1}H-NMR Spectra were performed on a Varian EM-360 60 MHz NMR spectrometer, Faculty of Pharmacy, Assiut University, Assiut. Chemical shifts are expressed in δ-value (ppm) relative to TMS as an internal standard, using DMSO-d_6 or $CDCl_3$ as solvents and deuterium oxide was used for the detection of the exchangeable protons. Mass spectra were recorded on Jeol JMS mass spectrometer, at Assiut University Central Lab., Assiut University, Assiut. Elemental analyses were performed on a Perkin-Elmer 240 elemental analyzer at the unit of microanalysis, Faculty of Science, Assiut University, Assiut, Egypt.

Nalidixic acid acid was offered by El-Nasr chemical industry co. (Cairo/ Egypt). Other materials used for the synthesis of the different intermediates required in this work are commercially available from Aldrich and include alkyl, aralkyl halides and (un)substituted benzaldehydes. All other chemicals and solvents are of the reagent grade.

The following starting materials were prepared according to reported methods: Methyl 1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylate (**2**)[15] and 1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid hydrazide (**3**)[16].

3-[4-Amino-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]-1-ethyl-7-methyl-1*H*-1,8-naphthyridin-4-one (4)

To a stirred ice-cooled solution of 1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid hydrazide (**3**) (2.46 g, 0.01 mol) and potassium hydroxide (0.84 g, 0.015 mol) in absolute ethanol (60 mL), carbon disulfide (1 mL, 0.015 mol) was added. The reaction mixture was stirred at room temperature for 20 h whereupon a yellow precipitate of the corresponding potassium dithiocarbazate was separated. Dry ether (50 mL) was then added to complete the precipitation. The product was filtered off, washed several times with dry ether and dried. The salt was then suspended in hydrazine hydrate 97% (1 mL, 0.02 mol) with stirring and heated under reflux for 2 hrs. The reaction mixture was cooled, diluted with ice-cold water (50 mL) and neutralized with 10% hydrochloric acid[11]. The precipitate obtained was filtered, washed thoroughly with cold water and crystallized from dimethylformamide / water affording 2.4 g (80%) of a white solid: m.p. 258-260°C.

IR, υ^{-} cm^{-1} (KBr): 3455, 3250 (NH_2, NH), 3130 (Ar. CH), 2920 (aliph. CH), 1614 (C=O), 1558 (C=N), 1527 (N-H), 1251 (C=S).

^{1}H-NMR, δ ppm (DMSO-d_6): 1.50 (t, 3H, CH_2-C<u>H</u>$_3$); 2.80 (s, 3H, C7-C<u>H</u>$_3$); 4.80 (q, 2H, C<u>H</u>$_2$-CH_3); 6.13 (br. s, 2H, N<u>H</u>$_2$); 7.92 (d, 1H, C6-<u>H</u>); 9.13 (d, 1H, C5-<u>H</u>); 9.30 (s, 1H, C2-<u>H</u>); 15.16 (br. s, 1H, N<u>H</u>). Anal. Calcd for $C_{13}H_{14}N_6OS$ (M W 302.09): %C 51.64, %H 4.67, %N 27.80, %S 10.61. Found: %C 51.59, %H 4.24, %N 27.62, %S 10.91.

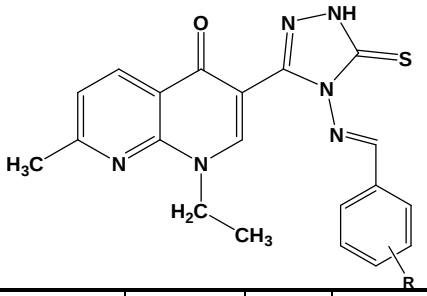
Synthesis of 3-{[4-(un)substituted benzylidene-amino]-5-thioxo-4,5-dihydro-1*H*-(1,2,4)triazol-3-yl}-1-ethyl-7-methyl-1*H*-[1,8]naphthyridin-4-one derivatives (5a-f)

To a suspension of 3-[4-amino-5-thioxo-4,5-dihydro-1*H*-(1,2,4)-triazole-3-yl]-1-ethyl-7-methyl-1*H*-1,8-naphthyridin-4-one, compound **4** (1.51 g, 0.005 mol) in absolute ethanol (25 mL), the appropriate (un)substituted aryl aldehyde (0.005 mol) was added, treated with concentrated HCl (0.5 mL) and refluxed for 20 hrs[17]. The reaction mixture was cooled to the ambient temperature and the formed precipitate was filtered and recrystallized from ethanol to yield the compounds **(5a-f)**. The physicochemical data are listed in Table I.

3-{[4-Benzylidene-amino]-5-thioxo-4,5-dihydro-1*H*-(1,2,4)triazol-3-yl}-1-ethyl-7-methyl-1*H*-[1,8]naphthyridin-4-one (5a)

^{1}H-NMR (DMSO-d_6, δ ppm): 1.50 (t, 3H, N-CH_2C<u>H</u>$_3$); 2.86 (s, 3H, C7-C<u>H</u>$_3$); 4.81 (q, 2H, N-C<u>H</u>$_2$-CH_3); 7.76-8.46 (m, 6H, C_6<u>H</u>$_5$ and C6-<u>H</u>); 9.00 (d,1H, C5-<u>H</u>); 9.30 (s,1H, C2-<u>H</u>); 10.86 (s, 1H, N=C<u>H</u>); 15.53 (br. s, 1H, N<u>H</u> exchanged by D_2O).

Table I: Physicochemical data of compounds (**5 a-f**).



Comp. No.	R	M. formula (M.W.)	m.p. (°C)	Yield (%)	Microanalyses Calcd. / Found			
					C%	H%	N%	S%
5a	H	$C_{20}H_{18}N_6OS$ (390.13)	300-302	75	61.52 62.38	4.65 4.64	21.52 21.41	8.21 8.40
5b	*p*-Br	$C_{20}H_{17}BrN_6OS$ (468.04)	270-2	73	51.18 51.55	3.65 3.92	17.91 17.89	6.83 6.82
5c	*p*-Cl	$C_{20}H_{17}ClN_6OS$ (424.09)	275-7	68	56.53 56.29	4.03 4.03	19.78 20.37	7.55 7.70
5d	*o*-OCH_3	$C_{21}H_{20}N_6O_2S$ (420.14)	255-7	71	59.98 59.30	4.79 5.28	19.91 20.12	7.63 8.12
5e*	*p*-OCH_3	$C_{21}H_{20}N_6O_2S$ 1/2 H_2O (429.14)	298-300	70	58.72 58.20	4.69 4.34	19.57 19.96	7.45 7.63
5f	*p*-CH_3	$C_{21}H_{20}N_6OS$ (404.49)	230-2	60	62.36 61.36	4.98 5.21	20.78 20.78	7.93 8.29

* It was further confirmed by FAB MS *m/z*, (%): 421.18 $[M+1]^+$ (2.2%), 287.93 (100%) 214.01 (36.8%), 185.95 (46.4%), 92.94 (49.4%) and 78.95 (86.2)

3-{[4-(*p*-Bromo-benzylidene)-amino]-5-thioxo-4,5-dihydro-1*H*-(1,2,4)triazol-3-yl}-1-ethyl-7-methyl-1*H*-[1,8]naphthyridin-4-one (5b)

^{1}H-NMR (DMSO-d_6, δ ppm): 1.53 (t, 3H, N-$CH_2C\underline{H}_3$); 2.86 (s, 3H, C7-$C\underline{H}_3$); 4.90 (q, 2H, N-$C\underline{H}_2$-CH_3); 8.03 (d, 1H, C6-<u>H</u>); 8.35 (s, 4H, $C_6\underline{H}_4$); 9.20 (d, 1H, C5-<u>H</u>); 9.46 (s, 1H, C2-<u>H</u>); 10.80 (s, 1H, N=C<u>H</u>); 15.47 (br. s, 1H, N<u>H</u> exchanged by D_2O).

3-{[4-(*p*-Chloro-benzylidene)-amino]-5-thioxo-4,5-dihydro-1*H*-(1,2,4)triazol-3-yl}-1-ethyl-7-methyl-1*H*-[1,8]naphthyridin-4-one (5c)

^{1}H-NMR (DMSO-d_6, δ ppm): 1.54 (t, 3H, N-$CH_2C\underline{H}_3$); 2.83 (s, 3H, C7-$C\underline{H}_3$); 4.88 (q, 2H, N-$C\underline{H}_2$-CH_3); 7.80-8.56 (m, 5H, $C_6\underline{H}_4$ and C6-<u>H</u>); 9.0 (d,1H, C5-<u>H</u>); 9.30 (s,1H,C2-<u>H</u>); 10.93 (s,1H,N=C<u>H</u>); 15.56 (br. s, 1H, N<u>H</u> exchanged by D_2O).

3-{[4-(*o*-Methoxy-benzylidene)-amino]-5-thioxo-4,5-dihydro-1*H*-(1,2,4)triazol-3-yl}-1-ethyl-7-methyl-1*H*-[1,8]naphthyridin-4-one (5d)

^{1}H-NMR (DMSO-d_6, δ ppm): 1.50 (t, 3H,N-$CH_2C\underline{H}_3$); 2.80 (s, 3H, C7-$C\underline{H}_3$); 4.16 (s, 3H, $OC\underline{H}_3$); 4.80 (q, 2H, N-$C\underline{H}_2$-CH_3); 7.20-8.46 (m, 5H, $C_6\underline{H}_4$ and C6-H); 8.93 (d, 1H, C5-H); 9.25 (s, 1H, C2-H); 11.2 (s, 1H, N=CH); 15.53 (br. s, 1H, NH exchanged by D_2O).

3-{[4-(*p*-Methoxy-benzylidene)-amino]-5-thioxo-4,5-dihydro-1*H*-(1,2,4)triazol-3-yl}-1-ethyl-7-methyl-1*H*-[1,8]naphthyridin-4-one (5e)

^{1}H-NMR (DMSO-d_6, δ ppm): 1.50 (t, 3H, N-$CH_2C\underline{H}_3$); 2.83 (s, 3H, C7-$C\underline{H}_3$); 4.13 (s, 3H, $OC\underline{H}_3$); 4.86 (q, 2H, N-$C\underline{H}_2$-CH_3); 7.56 (d, 1H, C6-H); 7.93 and 8.37(dd, 4H, C_6H_4); 9.10 (d, 1H, C5-H); 9.35(s, 1H, C2-H); 10.56 (s, 1H, N=CH); 15.37 (br. s, 1H, NH exchanged by D_2O).

FAB MS *m/z*, (%): 421.18 $[M+1]^+$ (2.2%), 287.93 (100%), 214.01 (36.8%), 185.95 (46.4%), 92.94 (49.4%) and 78.95 (86.2).

3-{[4-(*p*-Methyl-benzylidene)-amino]-5-thioxo-4,5-dihydro-1*H*-(1,2,4)triazol-3-yl}-1-ethyl-7-methyl-1*H*-[1,8]naphthyridin-4-one (5f)

^{1}H-NMR (DMSO-d_6, δ ppm): 1.56 (t, 3H, N-$CH_2C\underline{H}_3$); 2.55 (s, 3H, p-$C\underline{H}_3$-ph); 2.86 (s, 3H, C7-$C\underline{H}_3$); 4.86 (q, 2H, N-$C\underline{H}_2$-CH_3); 7.53-8.43 (m, 5H, $C_6\underline{H}_4$ and C6-H); 8.93 (s, 1H, C2-H); 9.25 (d,1H, C5-H); 10.85 (s, 1H, N=CH); 12.50 (br. s, 1H, NH exchanged by D_2O).

Synthesis of 3-[4-amino-5-alkylthio-4*H*-(1,2,4)triazol-3-yl]-1-ethyl-7-methyl-1*H*-[1,8]naphthyridin-4-one derivatives (6a-e)

The appropriate alkyl or aralkyl halide (0.0024 mol) in absolute ethanol (30 mL) was added to 3-[4-amino-5-thioxo-4,5-dihydro-1*H*-(1,2,4)-triazol-3-yl]-1-ethyl-7-methyl-1*H*-1,8-naphthyridin-4-one,compound **4** (0.6 g, 0.002 mol) dissolved in a least amount of 10% NaOH[18]. The reaction mixture was refluxed for 15 hrs, and then concentrated under reduced pressure. The residue obtained was filtered washed with water and crystallized from ethyl acetate to yield compounds **(6a-e)**. The physicochemical data are listed in Table II.

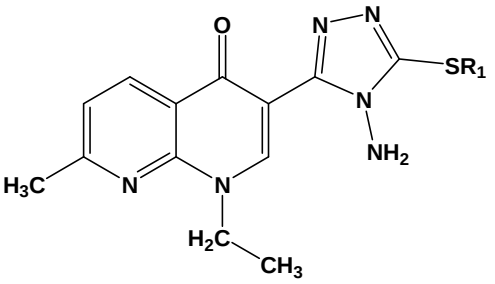
3-[4-Amino-5-methylthio-4*H*-(1,2,4)triazol-3-yl]-1-ethyl-7-methyl-1*H*-[1,8]naphthyridin-4-one (6a)

IR, υ^- cm^{-1} (KBr)**:** 3400 and 3260 (NH_2), 3010 (Ar. CH), 2950 (aliph. CH), 1619 (C=O), 1566 (NH, C=N).

^{1}H-NMR ($CDCl_3$ + DMSO-d_6 (4:1), δ ppm): 1.65 (t, 3H, CH_2-$C\underline{H}_3$); 2.93 (s, 3H, C7-CH_3); 3.00 (s, 3H, S-CH3); 5.00 (q, 2H, N-$C\underline{H}_2$-CH_3); 6.40 (br. s, 2H, NH_2, exchanged by D_2O); 8.00 d, 1H, C5-H); 9.26 (s, 1H, C2-H); 9.36 (d, 1H, C6-H).

FAB-MS, *m/z*, %: 317.29$[M+1]^+$, (95%), 302.27 (22.7%), 214.24 (17.6%), 185.26 (50%), 93.10 (100%).

Table II: Physicochemical data of compounds (**6a-e**).



Comp. No.	R_1	M. Formula* (M.W.)	m.p. (^oC)	Yield (%)	Microanalyses Calcd/ Found			
					C%	H%	N%	S%
6a	CH_3	$C_{14}H_{16}N_6OS$ 1.5 H_2O (343.12)	188-190	65	48.96 48.22	4.70 4.55	--	9.32 8.86
6b	C_2H_5	$C_{15}H_{18}N_6OS$ 1.5 H_2O (357.12)	170-172	67	50.40 49.87	5.08 6.09	--	8.95 8.58
6c	n-C_3H_7	$C_{16}H_{20}N_6OS$ 1.5 H_2O (371.16)	177-179	65	51.73 51.46	5.43 5.98	22.63 22.81	8.61 9.57
6d	$CH_2CH=CH_2$	$C_{16}H_{18}N_6OS$ 0.5 H_2O (351.14)	167-169	62	54.68 54.04	5.17 5.28	--	9.10 8.54
6e	$CH_2C_6H_5$	$C_{20}H_{20}N_6OS$ 1.5 H_2O (419.15)	166-168	79	57.26 57.56	4.81 5.39	20.08 19.91	7.63 7.47

* All compounds are further confirmed by EI and FAB MS (see the exp. part).

3-[4-Amino-5-ethylthio-4*H*-(1,2,4)-triazol-3-yl]-1-ethyl-7-methyl-1*H*-[1,8]naphthyridin-4-one (6b)

^{1}H-NMR ($CDCl_3$, δ ppm): 2.16-1.36 (m, 6H, $2CH_2$-C<u>H</u>$_3$); 2.96 (s, 3H, C7-CH_3); 3.53 (q, 2H, S-C<u>H</u>$_2$-CH_3); 5.00 (q, 2H, N-C<u>H</u>$_2$-CH_3)6.28 (br. s, 2H, NH_2, exchanged by D_2O); 7.93 (d, 1H, C5-H); 9.20(s, 1H, C2-H); 9.36(d, 1H, C6-H).

EI-MS, *m/z*, %: 330 ($M^{\cdot+}$, 90%), 314 ($M^{\cdot+}$ - NH_2, 97%), 214 (67%), 186 (100%).

3-[4-Amino-5-n-propylthio-4*H*-(1,2,4)triazol-3-yl]-1-ethyl-7-methyl-1*H*-[1,8]naphthyridin-4-one (6c)

^{1}H-NMR ($CDCl_3$, δ ppm): 1.10 (t, 3H, CH_2-CH_2-C<u>H</u>$_3$); 1.46-2.26 (m, 5H, CH_2-C<u>H</u>$_3$ and CH_2-C<u>H</u>$_2$-CH_3);

3.00 (s, 3H, C7-CH_3); 3.36 (t, 2H, C$\underline{H_2}$-CH_2-CH_3); 4.95 (q, 2H, C$\underline{H_2}$-CH_3); 6.23 (s, 2H, NH_2, exchanged by D_2O); 7.86 (d, 1H, C5-H); 9.13 (s, 1H, C2-H); 9.30 (d, 1H, C6-H).

FAB-MS, *m/z*, %: 345.19$[M+1]^+$, (100%), 330.16(19.8%), 214.19 (18.7%), 186.16 (21%), 93.07 (26.2%).

3-[4-Amino-5-allylthio-4*H*-(1,2,4)-triazol-3-yl]-1-ethyl-7-methyl-1*H*-[1,8]naphthyridin-4-one (6d)

IR, υ^- cm^{-1} (KBr)**:** 3380 and 3260 (NH_2), 3055 (Ar. CH), 2950 (aliph. CH), 1620 (C=O), 1569 (NH, C=N).

^{1}H-NMR ($CDCl_3$, δ ppm): 1.68 (t, 3H, CH_2-C$\underline{H_3}$); 2.95 (s, 3H, C7-CH_3); 4.16 (d, 2H, s-C$\underline{H_2}$-CH=); 5.00 (q, 2H, C$\underline{H_2}$-CH_3); 5.56-5.86 (dd, 2H, CH=C$\underline{H_2}$); 6.26 (br. s, 2H, NH_2, exchanged by D_2O); 6.95-6.05 (m, 1H, CH_2-C$\underline{H}$=); 7.90 (d, 1H, C5-H); 9.20 (s, 1H, C2-H); 9.36(d, 1H, C6-H).

EI-MS, *m/z*, %: 342 ($M^{\cdot+}$, 53.1%), 326 ($M^{\cdot+}$ - NH_2, 75.7%), 214 (100%), 186 (81%).

3-[4-Amino-5-benzylthio-4*H*-(1,2,4)triazol-3-yl]-1-ethyl-7-methyl-1*H*-[1,8]naphthyridin-4-one (6e)

IR, υ^- cm^{-1} (KBr): 3400 and 3265 (NH_2), 3050 (Ar. CH), 2950 (aliph. CH), 1617 (C=O), 1558 (NH, C=N), 782 and 708 (monosubstituted benzene).

^{1}H-NMR ($CDCl_3$, δ ppm): 1.76 (t, 3H, CH_2-C$\underline{H_3}$); 3.00 (s, 3H, C7-CH_3); 5.30-4.63 (m, 4H, C$\underline{H_2}$-CH_3 and C$\underline{H_2}$-ph); 6.16 (brs, 2H, NH_2, exchanged by D_2O); 8.33-7.70 (m, 6H, C6H5 and C5-H); 9.22 (s, 1H, C2-H); 9.36(d, 1H, C6-H).

EI-MS, *m/z*, %: 392 ($M^{\cdot+}$, 15.3%), 376 ($M^{\cdot+}$ - NH_2, 36.4%), 214 (40.7%), 186 (96.6%), 91 (100%).

Synthesis of 3-{[4-(un)substituted benzylidene-amino]-5-benzylthio-4*H*-(1,2,4) triazol-3-yl]}-1-ethyl-7-methyl-1*H*-[1,8]naphthyridin-4-one derivatives (7a,b)

Benzyl chloride (0.15 g, 0.0012 mol) in absolute ethanol (30 mL) was added to the appropriate Schiff's bases (**5a,e)** (0.001 mol) dissolved in a least amount of 10% NaOH and refluxed for 15 hrs. The solid obtained was filtered, washed with water and crystallized from methanol as white solids.

3-{[4-Benzylidene-amino]-5-benzylthio-4*H*-(1,2,4)triazol-3-yl]}-1-ethyl-7-methyl-1*H*-[1,8]naphthyridin-4-one (7a): Yield; (69%), m.p. 208-210°C.

IR, υ^- cm^{-1} (KBr): 3025 (Ar. CH), 2940 (aliph. CH), 1616 (C=O), 1582 (C=C, C=N), 746, 690 (monosubstituted benzene).

^{1}H-NMR, δ ppm (DMSO-d_6): 1.53 (t, 3H, CH_2-C$\underline{H_3}$); 2.86 (s, 3H, C7-CH_3); 5.25-4.60 (m, 4H, C$\underline{H_2}$-CH_3 and C$\underline{H_2}$-ph); 8.50-7.70 (m, 11H, 2$C_6\underline{H_5}$ and N=C$\underline{H}$); 9.03 (d, 1H, C5-$\underline{H}$); 9.32 (s, 2H, C2-$\underline{H}$ and C6-$\underline{H}$).

EI-MS, *m/z* (%): 480 [M^+, 28.4%], 186 [100%], 214 [93.9%], 91 [70.1%]. Anal. Calcd. for $C_{27}H_{24}N_6OS.H_2O$ (M.W. 498.18): %C 65.03, %H 4.85, %N 16.86, %S 6.41. Found: %C 65.11, %H 5.42, %N 16.98, %S 7.08.

3-{[4-(p-Methoxy)benzylidene-amino]-5-benzylthio-4*H*-(1,2,4)-triazol-3-yl]}-1-ethyl-7-methyl-1*H*-[1,8]naphthyridin-4-one (7b): Yield; (65%), m.p. 229-231°C.

IR, υ cm^{-1} (KBr): 3030 (Ar. CH), 2950 (aliph. CH), 1616 (C=O), 1540 (C=C, C=N), 1250 (SP^2 C-O), 1168, 1027 (SP^3 C-O), 820 (p-disubstituted benzene), 787, 691 (monosubstituted benzene).

^{1}H-NMR, δ ppm (DMSO-d_6): 1.55 (t, 3H, CH_2-C<u>H</u>$_3$); 2.92 (s, 3H, C7-CH_3); 4.16 (s, 3H, O-C<u>H</u>$_3$); 5.30-4.56 (m, 4H, C<u>H</u>$_2$-CH_3 and C<u>H</u>$_2$-ph); 8.55-7.45 (m, 10H, C_6<u>H</u>$_5$, C_6<u>H</u>$_4$ and N=C<u>H</u>); 9.56-8.96 (m, 3H, C2-<u>H</u>, C5-<u>H</u> and C6-<u>H</u>).

Anal. Calcd. for $C_{28}H_{26}N_6O_2S$. 1/2 H_2O (M.W. 519.18): %C 64.71, %H 5.05, %N 16.18, %S 6.15. Found: %C 64.21, %H 5.61, %N 16.51, %S 6.86.

Antimicrobial screening

Antibacterial activity

The antibacterial activity of all target compounds **(4, 5a-f, 6a-e** and **7a,b)** was investigated *in-vitro* at the department of microbiology and immunology, faculty of medicine, Assiut University. The title compounds were tested against methicillin resistant *Staphylococcus aureus* (MRSA), *Bacillus cereus*, *Escherichia coli*, and *Klebsiella pneumoniae* (clinical isolates obtained from Infection Control Unit, Assiut University Hospital, Faculty of Medicine, Assiut University) using agar cup diffusion method[19&20] for susceptibility screening, and two-fold dilution method[20] for MIC determination. Ampicillin was used as a reference antibiotic, and DMSO was used as a solvent control.

Agar cup diffusion method

38 Gm of Mueller-Hinton agar medium (MH) (Hi-Media, M 001) were added to 1 L of distilled water, heated to boiling to dissolve the ingredients completely, and sterilized by autoclaving at 121°C for 30 mins. High density inocula were made by diluting 3-5 well isolated colonies grown overnight on selective media in 5 mL of distilled water to prepare a suspension equivalent in density to 0.5 McFarland Barium Sulfate standard unit with average turbidity 10^8 CFU mL^{-1}[20]. The sterile petri dishes were seeded with 100 µL of the microorganism; a specified amount of the molten MH agar medium (45-50°C) was poured into the seeded Petri dishes to give a depth of 3-4 mm and allowed to solidify. Cylindrical plugs were removed from the agar using sterile cork borer. 100 µL of the tested compounds (20 mg/mL in DMSO), the blank solvent, and ampicillin sodium (20 mg/mL in DMSO) were added to the wells in triplicate. The seeded plates were incubated at 37°C for 24 hrs then the average diameters of the inhibition zones were measured in millimeters (Table III).

Minimum inhibitory concentration

The MIC values were determined using two fold-dilution method[21] for compounds having moderate to strong antibacterial activity. The squares of inhibition zone diameters were plotted against log concentrations of the tested compounds, extrapolation

Table III: Antibacterial Activity of The Tested Compounds (expressed as the inhibition zone diameter and as MIC µM/ mL).

Compd. No.	MRSA		*Bacillus cereus*		*E. coli*		*Klebsiella Pneumoniae*	
	Inhibition zone (mm)	MIC µM/ml	Inhibition zone (mm)	MIC µM/ml	Inhibition zone (mm)	MIC µM/ml	Inhibition zone (mm)	MIC µM/ml
4	15		13		11		17	
5a	21	75	23	69.1	24	65	26	35
5b	21	70	23	61	25	50	24	57
5c	20	70	22	60	26	35	27	30
5d	16		15		14		12	
5e	21		-ve		-ve		-ve	
5f	-ve		-ve		-ve		-ve	
6a	9		-ve		-ve		-ve	
6b	28	25	22	60	27	30	27	30
6c	18		15		13		9	
6d	20	69.1	22	72	21	75	23	70
6e	20	70	23	69.1	22	71	25	40
7a	-ve		6		7		-ve	
7b	10		-ve		10		7	
Ampicillin	20	69	22	60	20	70	23	50
Nalidixic acid	27	30	20	67	28	25	30	20

of the resulting straight line to intersect with log concentration scale in the curve corresponded to log MIC, and MIC was obtained as antilog[22] (Table III).

Antifungal activity

Organisms and culture conditions

The synthesized compounds (**4, 5a-f, 6a-e, 7a** and **7b**) were tested for their antifungal activity *in-vitro*, in comparison with clotrimazole as a reference drug and nalidixic acid as a reference starting material, using the standard agar cup diffusion method[22] at the Assiut University Mycological Center (AUMC), Faculty of Science, Assiut University.

Seven pathogenic, phytopathogenic or food poisoning fungal species were used in the present study: *Trichophyton rubrum* (AUMC 1804), *Candida albicans* (AUMC 5109), *Fusarium oxysporium* (AUMC 3224), *Penicillium chrysogenum* (AUMC 277), *Aspergillus terreus* (AUMC 2726), *Drechslera spicifier* (AUMC 5110) and *Micosporum gypseum* (AUMC 5095).

Materials and method[23]

Spore suspension in sterile malt extract broth media was prepared from 2-5 days old culture of the test fungi growing on malt extract agar as in case of *Aspergillus terreus* and *Penicillium chrysogenium*, sabouraud

agar as in case of *Candida albicans*, *Drechslera spicifier*, *Micosporum gypseum* and *Trichophyton rubrum*, or on potato dextrose agar as in case of *Fusarium oxysporium*. The final spore concentration was $5x10^4$ spores/ ml. About 15 ml of growth medium was introduced on sterilized Petri dishes of 9 cm diameter and inoculated with 1 ml spore suspension. Plates were shaken gently to homogenize the inoculum. Antifungal activity of the tested compounds was performed by the standard agar cup diffusion method as follow: Cylindrical plugs were removed from the agar using a sterile cork bore. 100 µl of the tested compounds (100 µmol/ ml in DMSO) and the blank solvent were added to each well in triplicate. The seeded plates were incubated at 28±2°C for 1-7 days according to fungi used. After the specified time for incubation the average diameter of inhibition zones was measured in millimeters (Table IV). Solution of nalidixic acid and clotrimazole (100 µmol /ml in DMSO) were used as reference starting compound and as standard antifungal agent.

Table IV: Antifungal activity of the tested compounds (expressed as the diameter of the inhibition zone* in mm).

Fungi / Comp. No.	*Trichophyton Rubrum*	*Candida Albicans*	*Fusarium Oxysporium*	*Penicillium chrysogenium*	*Drechlera spicifer*	*Aspergillus terreus*	*Microsporum gypseum*
4	0	0	0	0	22	0	0
5a	0	0	0	0	0	0	22
5b	0	0	0	0	0	0	0
5c	0	0	0	0	0	0	0
5d	20 p.i	0	0	0	0	0	0
5e	0	0	0	0	0	0	22
5f	0	0	0	0	0	0	0
6a	0	0	0	0	0	0	0
6b	0	0	0	0	0	0	20
6c	0	0	0	0	0	0	0
6d	0	0	0	0	0	0	0
6e	0	0	0	0	0	0	0
7a	0	0	0	0	0	0	0
7b	0	0	0	0	0	0	24
Nalidixic acid	18 p.i	0	0	0	12 p.i	0	16
Clotrimazole	58	35	25	55	30	27	58

*****Average of three determinations
p.i partial inhibition

RESULTS AND DISCUSSION

Chemistry

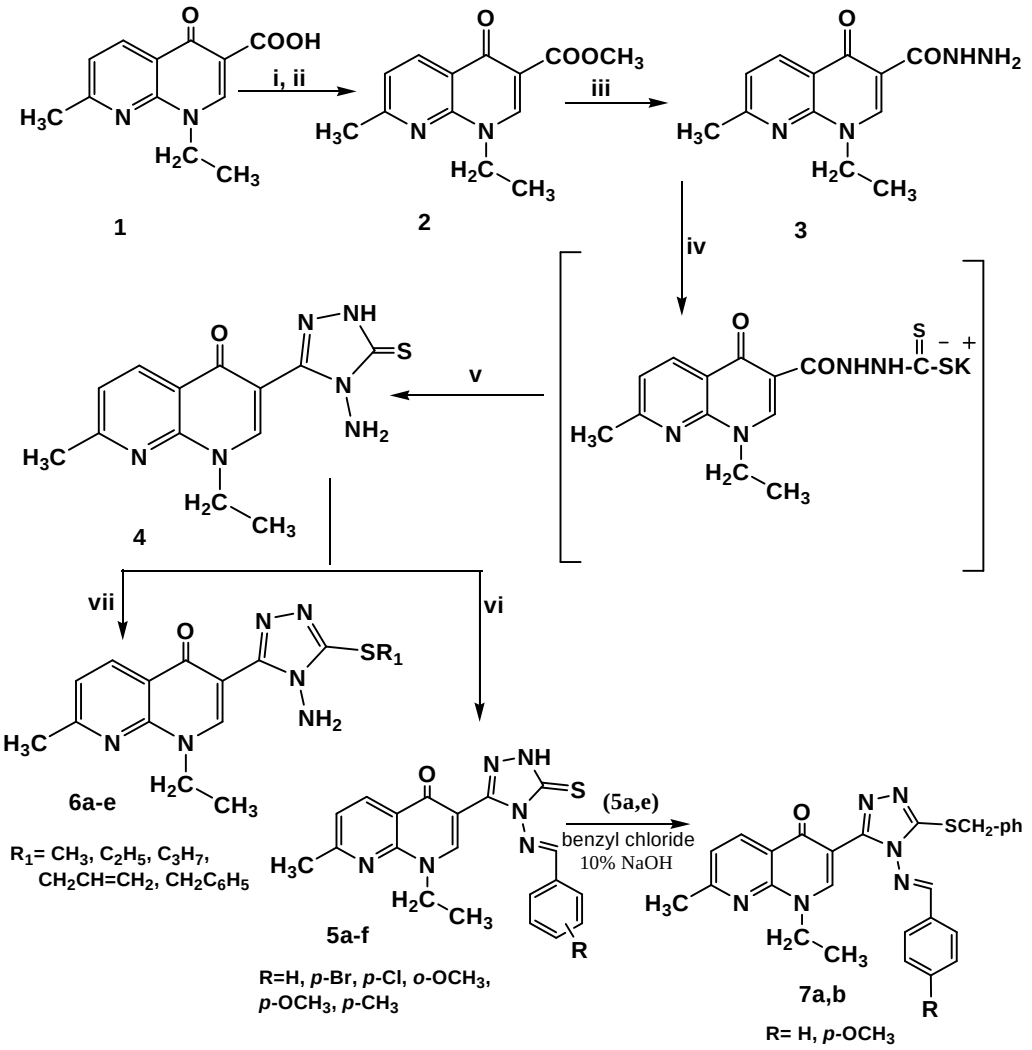
Nalidixic acid hydrazide **3** was prepared in analogy to the reported procedure[15-16] by refluxing the corresponding methyl ester **2** with hydrazine hydrate in methanol (Scheme 1). The structure of compounds **2** and **3** was confirmed by matching its physical data with the reported.

The key intermediate in this work, compound **4**; 3-[4-amino-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl]-1-ethyl-7-methyl-1H-[1,8]naphthyridin-4-one; was prepared by the condensation of the hydrazide **3** with CS_2 and ethanolic KOH to afford the corresponding potassium dithio-carbazate salt. The salt was cyclized, at refluxing temperature, with hydrazine hydrate 97% to furnish the triazole **4**, which is a new compound. The structure of compound **4** was confirmed by IR, ^{1}H-NMR spectra as well as elemental analysis. IR spectrum of compound **4** showed a broad band at 3455 cm^{-1} (NH_2, NH) stretching, and a medium band at 1251 cm^{-1} (C=S) stretching which revealed that compound **4** present in the thione form in the solid state[24]. Also, the absence of absorption at about 2600-2550 region cited for SH group have proved that the compound was in the thionic form.

The Schiff's bases (compounds **5a-f)** were prepared by reaction of the triazole **4** with one equivalent of (un)substituted benzaldehydes in ethanol and few drops of concentrated HCl acid as catalysis (Scheme 1).

Comparative study of the ^{1}H-NMR spectra of these Schiff's bases with compound **4** easily revealed the disappearance of the broad singlet at δ 6.13 ppm corresponding to NH_2 group present in compound **4** and appearance of N=CH signals at 10.56-11.20 ppm in addition to the marked change in the aromatic protons number and splitting pattern, which proofs the introduced aromatic moiety. A representative FAB-MS spectrum was done for compound **5e** (M.F.= $C_{21}H_{20}N_6O_2S$, M.Wt.= 420.14) showed the adduct ion peak [M^++1] at (*m/z* 421.18; intensity 2.2%), as well as prominent peaks at *m/z*: 287.93 (100%), 214.01 (36.8%), 185.95 (46.4%), 92.94 (49.4%) and 78.95 (86.2%).

Reaction of compound **4** with equimolar amount of the appropriate alkyl or aralkyl halide in presence of NaOH in ethanol at refluxing temperature, afforded the S-alkyl derivatives (compounds **6a-e**) Scheme 1. It was reported that S-alkylation supercsedes the N-alkylation due to of the difference in nucelophilicity between the sulfur and nitrogen atoms[25&26]. The IR spectra of compounds **6a-e** showed a broad band at 3400-3260 cm^{-1} (stretching) and at 1568 cm^{-1} (bending) of the NH_2. The ^{1}H-NMR spectra showed the presence of a broad singlet at δ 6.16-6.40 ppm corresponding to NH_2 protons and the absence of the broad singlet at δ 12.5- 15.37 (NH). Moreover the S-alkyl groups give a



Scheme 1

Reagents and conditions: (i) $ClCOOCH_2CH_3$, Triethylamine, $CHCl_3$, 5-10°C; (ii) CH_3OH; (iii) $N_2H_4.H_2O$ 80%, CH_3OH, reflux, 6 hrs; (iv) CS_2, KOH, EtOH, 24°C, 20 hrs; (v) $N_2H_4.H_2O$ 97%, reflux, 2 hrs; (vi) appropriate (un)substituted benzaldehydes, EtOH, reflux, 20 hrs; (vii) appropriate alkyl or aralkyl halide, 10% NaOH, EtOH, reflux, 15 hrs.

pattern in the ^{1}H-NMR spectra in accordance with the expected structures of such compounds.

A representative EI-MS spectrum was done for compound **6d** showed the molecular ion peak M^+ at (*m/z* 342; intensity 53.1%), corresponding to the molecular weight of the compound and prominent peaks at (m/z 326; 75.7%), (*m/z* 214; 100%) and (*m/z* 186; 81%).

Moreover, treatment of the Schiff's bases **5a,e** with benzyl chloride afforded S-benzyl derivatives **(7a,b)** of the two Schiff's bases. The structures of the formed Schiff's bases were verified by IR, ^{1}H-NMR, EI or FAB-MS and elemental analyses. IR and ^{1}H-NMR showed neither NH nor NH_2 and appearance of N=CH in addition to aromatic protons (exp. Part).

Antimicrobial activities

Antibacterial activity

The newly synthesized compounds **4, 5a-f, 6a-e** and **7a,b** were tested for their *in-vitro* antibacterial activity against methicillin resistant *Staphylococcus aureus* (MRSA) and *Bacillus cereus* as representatives of Gram-positive strains and *Escherichia coli* and *Klebsiella pneumoniae* as representatives of Gram-negative ones using nalidixic acid and ampicillin as reference drugs. The results revealed that most of the newly synthesized compounds exhibited promising antibacterial activity against all the test organisms (Table III). The synthesized Schiff's bases, compounds **5a-c** and the S-alkyl derivatives compounds **6b, d,** and **6e** were the most potent against all strains. Their antibacterial potency was superior to that of ampicillin against all the tested strains and higher than that of nalidixic acid against *Bacillus cereus*. They showed comparable antibacterial activity to nalidixic acid against methicillin resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* and *Klebsiella pneumoniae*.

In general, screening results revealed that replacement of the carboxylic acid group at position 3- of nalidixic acid with the triazole group (compound **4)**, causes marked decrease in the antibacterial activity. Among the Schiff's bases, (compounds **5d,e)**, having methoxy group in the benzylidene moiety, did not exhibit enhanced activity compared to the other bases having no substituent or substituted by halogen. Substitution by methyl group led to loss of antibacterial activity, compound **5f**.

Also, it has been observed that alkylation of compound **4** with methyl group, (compound **6a),** decreases the activity and alkylation with n- propyl (compound **6c),** slightly increases it, while alkylation with ethyl, allyl or benzyl group showed higher activity than ampicillin, with the most active one is (compound **6b**). In conclusion, the substitution of the carboxylic group of nalidixic acid by unsubstituted 1,2,4-triazole moiety or S-alkyl derivatives of Schiff's bases decreases the antibacterial activity. In

general anticipation of SAR can not be attained.

Antifungal activity

Unfortunately, results of the antifungal activity (Table IV) revealed that all the test compounds and nalidixic acid were inactive against *C. albicans, F. oxysporium, P. chrysogenum* and *A. terreus.* Also they were inactive against *D. spicifier* except nalidixic acid which showed partial inhibition 40% relative to clotrimazole. The test compounds were inactive against *T. rubrum* except compounds **5d** which showed 34% inhibition and nalidixic acid showed 31% inhibition relative to the standard. It was also noticed that many compounds showed moderate activity against *M. gypseum* (compounds **6b, 5a, 5d, 5e** and **7b** showed 34,38,34,38,41% inhibition respectively), while nalidixic acid showed weak inhibition (27.6%).

From the above results, it was clear that conversion of compound **4** to Schiff's bases (**5a, 5d** and **5e)** and some S-alkylation (**6b** and **7b**) is critical for the antifungal activity, with compound **7b** was the most active one.

Also, screening tests are quiet consistent with the reported literature that nalidixic acid has weak or no antifungal activity[27&28].

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