Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2014, 6(3):394-410
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Synthesis of novel 1,6-naphthyridines, pyrano[3,2-*c*]pyridines and pyrido[4,3-*d*]pyrimidines derived from 2,2,6,6-tetramethylpiperidin-4-one for *in vitro* anticancer and antioxidant evaluation

Hanan A. Soliman[a*], Mahmoud N. M. Yousif[a], Makarem M. Said[b], Nasser A. Hassan[a], Mamdouh M. Ali[c], Hanem M. Awad[d] and Farouk M. E. Abdel-Megeid[a]

[a]*Photochemistry Department, Chemical Industries Research Division, National Research Center, Dokki, Giza, Egypt*
[b]*Organic Chemistry Department, Faculty of Pharmacy, Cairo University, Egypt*
[c]*Biochemistry Department, Genetic Engineering and Biotechnology Division, National Research Center, Dokki, Giza, Egypt*
[d]*Tanning Materials and Leather Technology Department, Chemical Industries Research Division, National Research Center, Egypt*

ABSTRACT

Reaction of 2,2,6,6-tetramethylpiperidin-4-one (1) with different aromatic aldehydes afforded the diarylidenes 2a-f. Compound 1 reacted in one-pot reaction with aromatic aldehydes and malononitrile in ammonium acetate, and also with arylidene malononitrile in ammonium acetate to give the corresponding 1,6-naphthyridine-3-carbonitriles 3a-f and 4a-f, respectively. Compounds 3b, 4b were acetylated using acetic anhydride to give the mono- and tri-acetylated products 5a,b; 6a,b, respectively. Compound 1 was reacted with cyanoacetamide and aromatic aldehydes in ammonium acetate or ethyl cyanoactate and aromatic aldehydes in ammonium acetate to give 1,6-naphthyridine-3-carboxamides 7a-c or 1,6-naphthyridine-3-carboxylates 8a-c, respectively. On the other hand, reaction of compound 1 with aromatic aldehydes and malononitrile in triethylamine afforded the pyrano[3,2-c]pyridine-3-carbonitriles 9a-c. Also, compound 9c was acetylated with acetic anhydride to give the tri-acetylated product 10. Pyrido[4,3-d]pyrimidine-2-thione derivatives 11a-c were obtained by reaction of compound 1 with aromatic aldehydes and thiourea. Compounds 11a-c were reacted further with chloroacetic acid to give the corresponding 9-arylidene-pyrido[4,3-d]thiazolidino[3,2-a]pyrimidin-3-one derivatives 12a-c which in turn reacted with aromatic aldehydes to give the corresponding 2,9-diarylidenes 13a-c. Multi-component reaction of compound 1 with 4-chlorobenzaldehyde and 6-amino-S-methylthiouracil in DMF afforded the corresponding pyrimido[4,5-b][1,6]naphthyridine derivatives 14 which reacted with hydrazine hydrate to give the corresponding 2-hydrazinyl derivative 15. Antioxidant and anticancer results of the products are reported.

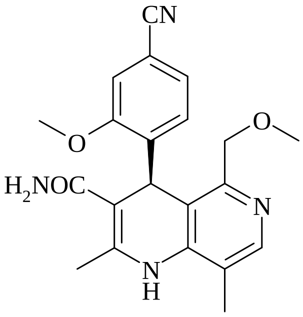
Keywords: 2,2,6,6-Tetramethylpiperidin-4-one, 1,6-Naphthyridines, Pyrano[3,2-*c*]pyridine, Pyrido[4,3-*d*] pyrimidine, Anticancer, Antioxidant.

INTRODUCTION

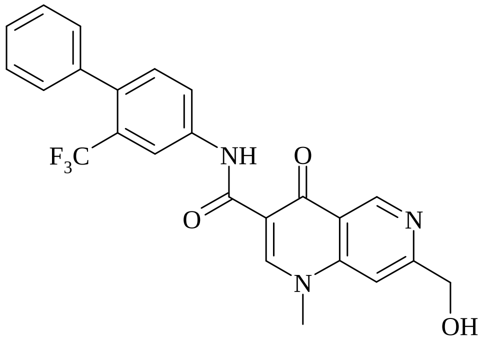
Sterically hindered amines such as 2,2,6,6-tetramethyl-piperidines (**1**) are potent and long acting ganglionic blocking agent with proven clinical efficacy in the treatment of hypertension [1]. Also such amines are used for spin labeling methods [2] and industrial use in a variety of gas treating processes [3]. Analogous compounds to 3,5-bis(benzylidene)-4-piperidones (**2**) were reported to show cytotoxic activity against leukemia cell lines and colon cancer [4-6]. It was thought that incorporation of active pharmacophore moieties in the structure of the newly prepared compounds to attain the target title compounds could enhance their biological activity.

Moreover, pyridine derivatives containing multi-functional groups were reported with anticancer [7-9], antioxidant [10-12] and antimicrobial activities [13]. Many papers described the synthesis of 1,6-naphthyridines [14-24] since they have a wide variety of biological properties [25-31]. They exhibited potential anticancer [20, 22] and antimicrobial [17] activities. Their carboxamide derivatives when tested for growth inhibitory properties against murine P388 leukemia, Lewis lung carcinoma (LLTC), and human Jurkat leukemia cell lines were found potent cytotoxins [16,18].

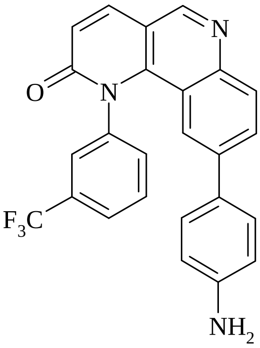
The following 1,6-naphthyridine derivatives were reported to exhibit remarkable biological activities. So, compound A, Bay 94-8862 is a potent non-steroidal antagonist of mineral corticoid receptor (MR) and is under investigation in a clinical phase II trial; while compound B is an efficient $hS1P_1$ (sphingosine-1-phosphate receptor) agonist; also, compound C (Torin 2) is highly potent and selective mammalian target of rapamycin (mTOR) inhibitor (Figure 1) [22].



A
BAY 94-8862



B
$hS1P_1$



C
Torin2

Figure 1

In view of the aforementioned activities, it seemed most interesting to study the chemical behavior of 2,2,6,6-tetramethyl-4-piperidone (**1**) and its derivatives 3,5-*bis*(arylidene)-2,2,6,6-tetramethyl piperidin-4-ones (**2a-f**) and synthesize some novel condensed title derivatives with the aim to evaluate their antioxidant and anticancer activities.

MATERIALS AND METHODS

All melting points are uncorrected and measured using Electro-thermal IA 9100 apparatus (Shimadzu, Tokyo, Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (Perkin-Elmer, Norwalk, CT, USA). ^{1}H NMR was determined on a Jeol-Ex-400 NMR spectrometer (Jeol, Tokyo, Japan) and chemical shifts were expressed as part per million; ppm (δ values) against TMS as internal standard. Mass spectra were recorded on VG 2AM-3F mass spectrometer (Thermo electron corporation, USA). Microanalyses were operated using Mario El Mentar apparatus and satisfactory results were within the accepted range (±0.30) of the calculated values. Follow up the reactions and checking the purity of the compounds was made by TLC on silica gel-protected aluminium sheets (Type 60 F254, Merck). All used chemicals were of reagent grade and were used as supplied directly unless otherwise stated. The reaction mixture was monitored with TLC [petroleum ether/chloroform (9:1) till the reaction was completed]. Compound **1** was prepared by literature procedure [36].

*General procedure for the synthesis of compounds **2a-f***
A mixture of compound **1** (1.55g, 0.01mol), 30 mL absolute ethanol, 10 mL piperidene, 5 mL acetic acid and 0.02 mol of the appropriate aldehyde (benzaldehyde, 2-chlorobenzaldehyde, 4-chlorobenzladehyde, 4-brombenzaldehyde, 4-methoxybenzaldehyde, or 4–(dimethylamino) benzaldehyde) was heated under reflux for 3 h. The solution was concentrated to one third of its volume then it was poured into water and the precipitate was filtered and crystallized from the proper solvent to give compounds **2a-e**.

*3,5-Dibenzylidene-2,2,6,6-tetramethylpiperidin-4-one (**2a**)*
Yield 53 %, m.p.120-122°C, solvent of crystallization, ethanol, orange powder. IR for compound **2a** (KBr) υ, cm^{-1}: 3423 (br NH), 1651 (C=O).

*3,5-Bis(2-chlorobenzylidene)-2,2,6,6-tetramethylpiperidin-4-one (**2b**)*
Yield 78 %, m.p.125-127°C, solvent of crystallization, ethanol, brown powder. IR for compound **2b** (KBr) υ, cm^{-1}: 3425 (br NH), 1657 (C=O).

*3,5-Bis(4-chlorobenzylidene)-2,2,6,6-tetramethylpiperidin-4-one (**2c**)*
Yield 61 %, m.p.150-152°C, solvent of crystallization, ethanol/dioxane, yellow powder. IR for compound **2c** (KBr) υ, cm^{-1}: 3425 (br NH), 1650 (C=O). ^{1}H NMR for compound **2c** (400 MHz, $CDCl_3$): δ_H 1.20 (s, 6H, $2CH_3$), 1.50 (s, 6H, $2CH_3$), 6.80-8.00 (m, 10H, 8 Ar+2CH=), 10.50 (s, 1H, NH). MS, *m/z* (%) (399, 1.6%, M^+), (401, 0.7%, M^{+2}), (403, 0.2%, M^{+4}).

*3,5-Bis(4-bromobenzylidene)-2,2,6,6-tetramethylpiperidin-4-one (**2d**)*
Yield 44 %, m.p.220-222°C, solvent of crystallization, ethanol/dioxane, pale yellow powder. IR for compound **2d** (KBr) υ, cm^{-1}: 3432 (br NH), 1648 (C=O).

*3,5-Bis(4-methoxybenzylidene)-2,2,6,6-tetramethylpiperidin-4-one (**2e**)*
Yield 63 %, m.p.105-107°C, solvent of crystallization, ethanol, brown powder. IR for compound **2e** (KBr) υ, cm^{-1}: 3422 (br NH), 1645 (C=O).

*3,5-Bis(4-(dimethylamino)benzylidene)-2,2,6,6-tetramethylpiperidin-4-one (**2f**)*
Yield 42 %, m.p.100-102°C, solvent of crystallization, ethanol, brown powder. IR for compound **2f** (KBr) υ, cm^{-1}: 3432 (br NH), 1705 (C=O).

*General procedure for the synthesis of compounds **3a-f***
Method A: a mixture of 0.01 mol of compound **1**, malononitrile (0.01mol., 0.66g), the appropriate aldehyde (0.02 mol) (benzaldehyde, 2-chlorobenzaldehyde, 4-chlorobenzladehyde, 4-brombenzaldehyde, 4-methoxybenzaldehyde, or 4–(dimethylamino)benzaldehyde), 50 mL acetic acid and excess ammonium acetate (10 g) in acetic acid was heated under reflux for 4 h. Then the reaction mixture was poured into water. The precipitate was filtered and crystallized from ethanol to give compounds **3a-f**.

Method B: compounds **2b** (0.01 mol.), malononitrile (0.66 g, 0.01 mol) and excess ammonium acetate were dissolved in 50 mL acetic acid. The reaction mixture was heated under reflux for 4 h. then the reaction mixture was poured into water. The precipitate was filtered and crystallized from ethanol to give compounds **3b**.

*2-Amino-8-benzylidene-5,5,7,7-tetramethyl-4-phenyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carbonitrile (**3a**)*
Yield 49 %, m.p.155-157°C, orange powder. IR for compound **3a** (KBr) υ, cm^{-1}: 3366-3058 (br, NH, NH_2), 2206 (CN).

*2-Amino-8-(2-chlorobenzylidene)-4-(2-chlorophenyl)-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carbonitrile (**3b**)*
Yield 51 % (method A) and 64 % (method B), m.p.205-207°C, orange powder. IR for compound **3b**(KBr) υ, cm^{-1}: 3395-3348 (br, NH, NH_2), 2212 (CN). ^{1}H NMR for compound **3b** (400 MHz, $CDCl_3$): δ_H 1.50-1.75 (br, 12H, $4CH_3$), 7.00-7.80 (m, 11H, 8H aromatic+NH+NH_2). MS, *m/z* (%) (462, 5.7%, M^+), (464, 2%, M^++2), (466, 1%, M^++4).

*2-Amino-8-(4-chlorobenzylidene)-4-(4-chlorophenyl)-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carbonitrile (**3c**)*
Yield 73 %, m.p.190-192°C, brown powder. IR for compound **3c** (KBr) υ, cm^{-1}: 3396-2963 (br, NH, NH_2), 2186 (CN).

*2-Amino-8-(4-bromobenzylidene)-4-(4-bromophenyl)-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carbonitrile (**3d**)*
Yield 62 %, m.p.198-200°C, yellow powder. IR for compound **3d** (KBr) υ, cm^{-1}: 3378-2966 (br, NH, NH_2), 2206 (CN).

*2-Amino-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carbonitrile (**3e**)*
Yield 64 %, m.p.165-167°C, brown powder. IR for compound **3e** (KBr) υ, cm^{-1}: 3390-2964 (br, NH, NH_2), 2208 (CN).

2-Amino-8-(4-(dimethylamino)benzylidene)-4-(4-(dimethylamino)phenyl)-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carbonitrile (3f)
Yield 52 %, m.p.185-187°C, brown powder. IR for compound **3f** (KBr) υ, cm^{-1}: 3331-3182 (br, NH, NH_2), 2208 (CN).

*General procedure for the synthesis of compounds **4a-f***
Compound **1** (1.55g, 0.01 mol.), 2-arylidenemalononitrile (0.01mol) and 3g ammonium acetate were dissolved in 50 mL acetic acid. The reaction mixture was heated under reflux for 5 h. The reaction mixture was concentrated to half of its volume then poured into water. The precipitate was filtered and crystallized from ethanol to give compound **4a-f** (brown powder).

*2-Amino-5,5,7,7-tetramethyl-4-phenyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carbonitrile (**4a**)*
Yield 51 %, m.p.180-182°C, IR for compounds **4a** (KBr) υ, cm^{-1}: 3396-3168 (br, NH,NH_2), 2210 (CN).

*2-Amino-4-(2-chlorophenyl)-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carbonitrile (**4b**)*
Yield 63 %, m.p.180-182°C, IR for compounds **4b** (KBr) υ, cm^{-1}: 3388-3345 (br, NH,NH_2), 2211 (CN). ^{1}H NMR for compound **4b** (400 MHz, $CDCl_3$): δ_H 1.2 (s, 2H, CH_2), 1.60-1.74 (br, 12H, $4CH_3$), 7.00-7.60 (m, 7H, 4H aromatic+NH+NH_2). MS, *m/z* (%) for compound **4b:** (340.2, 2.7%, M^+), (342, 1.1%, M^{+2}).

*2-Amino-4-(4-chlorophenyl)-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carbonitrile (**4c**)*
Yield 60 %, m.p.190-192°C, IR for compound **4c** (KBr) υ, cm^{-1}: 3396-3323 (br, NH,NH_2), 2209 (CN).

*2-Amino-4-(4-bromophenyl)-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carbonitrile (**4d**)*
Yield 58 %, m.p.175-177°C, IR for compound **4d** (KBr) υ, cm^{-1}: 3460-3376 (br, NH,NH_2), 2205 (CN).

*2-Amino-4-(4-methoxyphenyl)-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carbonitrile (**4e**)*
Yield 54 %, m.p.170-172°C, IR for compound **4e** (KBr) υ, cm^{-1}: 3396-3167 (br, NH,NH_2), 2207 (CN).

*2-Amino-4-(4-(dimethylamino)phenyl)-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carbonitrile (**4f**)*
Yield 52 %, m.p.180-182°C, IR for compound **4f** (KBr) υ, cm^{-1}: 3394-3200 (br, NH,NH_2), 2205 (CN).

*General procedure for the synthesis of compounds **5a,b***
A mixture of compound **3b** (0.01 mole) and 30 mL acetic anhydride was heated under reflux for 5 h. The reaction mixture was concentrated to half of its volume. Water was added and the formed solid was filtered off and crystallized from ethanol to give deep brown powder **5a**. The filterate was concentrated to half of its volume to give pale brown powder **5b** which was crystallized from ethanol.

*N-(8-(2-Chlorobenzylidene)-4-(2-chlorophenyl)-3-cyano-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-1,6-naphthyridin-2-yl)acetamide (**5a**)*
Yield 62%, m.p.160-162°C. IR for compound **5a** (KBr) υ, cm^{-1}: 2228 (CN), 1716 (CO). ^{1}H NMR for compound **5a** (400 MHz, $CDCl_3$): δ_H 1.70 (s, 6 H, $2CH_3$), 2.00 (s, 6H, $2CH_3$), 2.40-2.60 (br, 6H, 2 x $COCH_3$), 6.0-8.0 (m, 10H, 8H aromatic + 1 CH= + 1NH). MS, *m/z* (%) for compound 5**a** (546.45, M$^+$), (548.45, M$^+$+2), (550.45, M$^+$+4).

*N-Acetyl-N-(6-acetyl-8-(2-chlorobenzylidene)-4-(2-chlorophenyl)-3-cyano-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-1,6-naphthyridin-2-yl)acetamide (**5b**)*
yield 31%, m.p.130-132°C. IR for compound **5b** (KBr) υ, cm^{-1}: 2218 (CN), 1706 (CO). ^{1}H NMR for compound **5b** (400 MHz, $CDCl_3$): δ_H 1.24 (s, 6H, $2CH_3$), 2.00 (s, 6H, $2CH_3$), 2.40-2.70 (br, 9H, 3 x $COCH_3$), 7.10-7.90 (m, 9H, 8H aromatic+ 1 CH=). MS, *m/z* (%) (588.45, 24.8%, M$^+$), (590.45, 10.6%, M$^+$+2), (592.45, 5%, M$^+$+4).

*General procedure for the synthesis of compounds **6a,b***
Compound **4b** (0.01 mol.) in 30 mL acetic anhydride was refluxed for 5 h. The reaction mixture was concentrated to its half volume then poured into water. The precipitate was filtered and crystallized from ethanol to give compound **6a** as deep brown powder. The filtrate was concentrated to half of its volume to give compound **6b** as pale brown powder.

*N-(4-(2-Chlorophenyl)-3-cyano-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-1,6-naphthyridin-2-yl)acetamide (**6a**)*
Yield, 62%, m.p.170-172°C. IR for compound **6a** (KBr) υ, cm^{-1}: 3387 (NH), 2222 (CN), 1723 (CO). ^{1}H NMR for compound **6a** (400 MHz, $CDCl_3$): δ_H 1.30 (s, 3H, CH_3CO), 1.60-1.78 (br, 12H, $4CH_3$), 2.10 (s, 2H, CH_2), 7.00-7.60 (m, 6H, 4H aromatic+2NH). MS, *m/z* (%) for compound **6a** (382.87, M$^+$), (125, 100%, C_7H_5Cl).

*N-Acetyl-N-(6-acetyl-4-(2-chlorophenyl)-3-cyano-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-1,6-naphthyridin-2-yl)acetamide (**6b**)*
Yield 33%, m.p.145-147°C. IR for compound **6b** (KBr) υ, cm^{-1}: 2225 (CN), 1722 (CO). ^{1}H NMR for compound **6b** (400 MHz, $CDCl_3$): δ_H 1.24 (s, 6H, $2CH_3$), 2.0 (s, 6H, $2CH_3$), 2.10-2.60 (br, 9H, $3CH_3CO$), 2.70 (s, 2H, CH_2), 7.20-7.80 (m, 4H, 4H aromatic). MS, *m/z* (%) (467, 47%, M$^+$), (468, 20%, M^{+2}).

*General procedure for the synthesis of compounds **7a-c***
A mixture of compound **1** (0.01 mole), cyanoacetamide (0.01 mole), aromatic aldehyde (0.02 mole) and 8 gm ammonium acetate were heated under reflux in 50 mL acetic acid for 5 h. The reaction mixture was poured into water. The formed solid was collected and crystallized from the prober solvent to give compounds **7a-e**.

*2-Amino-8-benzylidene-5,5,7,7-tetramethyl-4-phenyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carboxamide (**7a**)*
Yield 61 %, m.p.270-272°C, solvent of crystallization=ethanol/dioxane, yellow powder. IR for compound **7a** (KBr) υ, cm^{-1}: 3409, 35220 ($2NH_2$), 3100 (NH), 1702 (C=O).

*2-Amino-8-(2-chlorobenzylidene)-4-(2-chlorophenyl)-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carboxamide (**7b**)*
yield 53 %, m.p.240-242°C, solvent of crystallization, ethanol, pale brown powder. IR for compound **7b** (KBr) υ, cm^{-1}: 3443 ($2NH_2$), 3062 (NH), 1672 (C=0). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.70 (s, 6H, $2CH_3$), 1.80 (s, 6H, $2CH_3$), 6.50-9 (m, 14 H, 8 Ar + CH= + 2 NH_2+NH). MS, *m/z* (%) (480.40, 11.7%, M$^+$), (482.40, 5%, M$^+$+2), (484.40, 2%, M$^+$+4).

*2-Amino-8-(4-chlorobenzylidene)-4-(4-chlorophenyl)-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carboxamide (**7c**)*
Yield 74 %, m.p.190-192°C, solvent of crystallization, ethanol, brown powder. IR for compound **7c** (KBr) υ, cm^{-1}: 3409, 3520 ($2NH_2$), 3100 (NH), 1702 (C=0).

*General procedure for preparation of compounds **8a-c***
A mixture of compound **1** (0.01 mole), ethylcyanoacetate (0.01 mole), aromatic aldehyde (0.02 mole) and 8 g ammonium acetate was heated under reflux in 50 mL acetic acid for 5 h. The reaction mixture was poured into water. The formed solid was collected and crystallized from ethanol to give compounds **8a-c** (brown powder).

*Ethyl 2-amino-8-benzylidene-5,5,7,7-tetramethyl-4-phenyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carboxylate (**8a**)*
Yield 65 %, m.p.165-167°C. IR for compound **8a** (KBr) υ, cm^{-1}: 3390, 3480 (NH_2), 3052 (NH), 1735 (C=0).

Ethyl 2-amino-8-(2-chlorobenzylidene)-4-(2-chlorophenyl)-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carboxylate (8b)
yield 51 %, m.p.195-197°C. IR for compound **8b** (KBr) υ, cm^{-1}: 3400, 3500 (NH_2), 3061 (NH), 1743 (C=0). ^1H NMR for compound **8b** (400 MHz, $CDCl_3$): δ_H 0.10 (t, 3H, CH_3), 1.20-2.00 (br, 12H, $4CH_3$), 3.70-4.20 (q, 2H, CH_2), 5.20 (br, CHAr), 6.80-8.80 (m, 12H, 8 Ar + =CHAr +NH_2+ NH). MS, *m/z* (%) (505.45, 30%, M^+), (507.45, 12%, M^++2), (509.45, 24%, M^++4).

*Ethy 2-amino-8-(4-chlorobenzylidene)-4-(4-chlorophenyl)-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carboxylate (**8c**)*
Yield 62 %, m.p.160-162°C. IR for compound **8c** (KBr) υ, cm^{-1}: 3410, 3492 (NH_2), 3043 (NH), 1727 (C=0).

*General procedure for preparation of compounds **9a-c***
A mixture of compound (0.01 mole), aromatic aldehyde (0.02 mole), malononitrile (0.01 mole) and 8 mL triethylamine were heated under reflux in 50 mL ethanol for 4 hours. Then the reaction mixture was heated in an oil bath at 160-180 °C for 1 h. The formed solid was collected and crystallized from ethanol to give compounds **9a-c**.

*2-Amino-8-benzylidene-5,5,7,7-tetramethyl-4-phenyl-5,6,7,8-tetrahydro-4H-pyrano[3,2-c]pyridine-3-carbonitrile (**9a**)*
Yield 70 %, m.p.200-202°C, yellow powder. IR for compound **9a** (KBr) υ, cm^{-1}: 3455, 3382 (br, NH_2), 3185 (NH), 2215 (CN).

*2-Amino-8-(2-chlorobenzylidene)-4-(2-chlorophenyl)-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-4H-pyrano[3,2-c]pyridine-3-carbonitrile (**9b**)*
Yield 82 %, m.p.220-222°C, brown powder. IR for compound **9b** (KBr) υ, cm^{-1}: 3500, 3392 (br, NH_2), 3200 (NH), 2186 (CN). ^1H NMR for compound **9b** (400 MHz, $CDCl_3$): δ_H 1.20-1.70 (brs, 12H, $4CH_3$), 5.30 (br, CHAr), 6.5-7.4 (m, 9H, 8 Ar + =CHAr), 8.40-8.50 (br, 3H, NH_2+NH). MS, *m/z* (%) for compound **9b** (465.39, 14.8%, M^+), (467.39, 6.3%, M^++2), (469.39, 3%, M^++4).

*2-Amino-8-(4-chlorobenzylidene)-4-(4-chlorophenyl)-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-4H-pyrano[3,2-c]pyridine-3-carbonitrile (**9c**)*
Yield 74 %, m.p.210-212°C, yellow powder. IR for compound **9c** (KBr) υ, cm^{-1}: 3430, 3378 (br, NH_2), 3237 (NH), 2195 (CN).

*N-Acetyl-N-(6-acetyl-8-(4-chlorobenzylidene)-4-(4-chlorophenyl)-3-cyano-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-4H-pyrano[3,2-c]pyridin-2-yl)acetamide (**10**)*
A solution of compound **9c** (0.01 mole) in 20 mL acetic anhydride was refluxed for 10 h, and then the reaction mixture was poured into water. The filtrate was concentrated then add hot water to give compound **10** (m.p.200-202°C, yield 18%, brown powder). IR for compounds **10** (KBr) υ, cm^{-1}: 3404 (br, NH), 2218 (CN), 1716 (CO). MS, m/z (%) (591.45, 18%, M^+), (593.45, 7%, M^++2), (595.45, 14%, M^++4). ^1H NMR for compound **10** (400 MHz, $CDCl_3$): δ_H 1.64 (s, 6H, $2CH_3$), 1.90 (s, 6H, $2CH_3$) 2.10-2.40 (br, 6H, 2 x $COCH_3$), 2.54 (s, 3H, $COCH_3$), 5.30 (s, 1H, CHAr), 7.25-8.10 (m, 9H, 8 Ar + 1ArCH).

*General procedure for the synthesis of compounds **11a-e***
A mixture of compound **1** (0.01 mole), aromatic aldehyde (0.02 mole) and thiourea (0.01 mole) in ethanolic potassium hydroxide (2 g potassium hydroxide in 100 mL ethanol) was refluxed for 3 h. The solvent was evaporated and the formed precipitate was washed several times with acidified cold water, filtered off and crystallized from ethanol to give compounds **11a-e** (brown powder).

*8-Benzylidene-5,5,7,7-tetramethyl-4-phenyl-3,4,5,6,7,8-hexahydropyrido[4,3-d]pyrimidine-2(1H)-thione (**11a**)*
Yield 31 %, m.p.170-172°C. IR for compound **11a** (KBr) υ, cm^{-1}: 3182, 3054 (NH), 1274 (C=S).

*8-(2-Chlorobenzylidene)-4-(2-chlorophenyl)-5,5,7,7-tetramethyl-3,4,5,6,7,8-hexahydropyrido[4,3-d]pyrimidine-2(1H)-thione (**11b**)*
Yield 54%, m.p.180-182°C. IR for compound **11b** (KBr) υ, cm^{-1}: 3062, 2965 (NH), 1260 (C=S).

*8-(4-Chlorobenzylidene)-4-(4-chlorophenyl)-5,5,7,7-tetramethyl-3,4,5,6,7,8-hexahydropyrido[4,3-d]pyrimidine-2(1H)-thione (**11c**)*
yield 62%, m.p.220-222°C. IR for compound **11c** (KBr) υ, cm^{-1}: 3094, 3050 (NH), 1282 (C=S). MS, *m/z* (%) for compound **11c** (457.43, 12.4%, M$^+$), (459.43, 5%, M$^+$+2), (461.43, 2%, M$^+$+4). ^{1}H NMR for compound **11c** (400 MHz, DMSO): δ_H 1.00 (s, 6H, 2CH$_3$), 1.90 (s, 6H, 2CH$_3$), 4.90 (s, 1H, CHAr), 7.30-7.80 (9H, 8H AB system + CH=), 13.10 (br, 3H, 3NH).

*8-(4-Bromobenzylidene)-4-(4-bromophenyl)-5,5,7,7-tetramethyl-3,4,5,6,7,8-hexahydropyrido[4,3-d]pyrimidine-2(1H)-thione (**11d**)*
Yield 52 %, m.p.225-227°C. IR for compound **11d** (KBr) υ, cm^{-1}: 3060, 2966 (NH), 1267 (C=S).

*8-(4-(Dimethylamino)benzylidene)-4-(4-(dimethylamino)phenyl)-5,5,7,7-tetramethyl-3,4,5,6,7,8-hexahydropyrido[4,3-d]pyrimidine-2(1H)-thione (**11e**)*
Yield 53 %, m.p.270-272°C. IR for compound **11e** (KBr) υ, cm^{-1}: 3091, 3047 (NH), 1262 (C=S).

*General procedure for the synthesis of compounds **12a-c***
A mixture of compound **11a-c** (0.01 mole) with chloroacetic acid (0.01 mole) in acetic acid (30 mL)/acetic anhydride (15 mL) mixture in the presence of fused anhydrous sodium acetate was refluxed for 3 h. The solution was cooled, gradually poured into water and the formed precipitate was washed several times with water, filtered off and recrystalized from prober solvent to give compounds **12a-c** (brown powder).

*9-Benzylidene-6,6,8,8-tetramethyl-5-phenyl-6,7,8,9-tetrahydro-2H-pyrido[4,3-d]thiazolo[3,2-a]pyrimidin-3(5H)-one (**12a**)*
Yield 42 %, m.p.145-147°C, solvent of crystallization, methanol. IR for compound **12a** (KBr) υ, cm^{-1}: 3020 (br, NH), 1701 (C=0).

*9-(2-Chlorobenzylidene)-5-(2-chlorophenyl)-6,6,8,8-tetramethyl-6,7,8,9-tetrahydro-2H-pyrido[4,3-d]thiazolo[3,2-a]pyrimidin-3(5H)-one (**12b**)*
Yield 51 %, m.p.140-142°C, solvent of crystallization, ethanol/water. IR for compound **12b** (KBr) υ, cm^{-1}: 3059 (br, NH), 1711 (C=0). MS, *m/z* (%) for compound **12b** (497.45, 11.7%, M$^+$), (499.45, 5%, M$^+$+2), (501.45, 1%, M$^+$+4). ^{1}H NMR for compound **12b** (400 MHz, CDCl$_3$): δ_H 1.70 (s, 6H, 2CH$_3$), 1.80 (s, 6H, 2CH$_3$), 2.40 (br, 2H, 8 CH$_2$S), 6.60 (s, 1H, CHAr), 6.90-9.80 (m, 10H, 8 Ar+ =CHAr+NH).

*9-(4-Chlorobenzylidene)-5-(4-chlorophenyl)-6,6,8,8-tetramethyl-6,7,8,9-tetrahydro-2H-pyrido[4,3-d]thiazolo[3,2-a]pyrimidin-3(5H)-one (**12c**)*
Yield 73 %, m.p.210-212°C, solvent of crystallization, ethanol/water. IR for compound **12c** (KBr) υ, cm^{-1}: 3022 (br, NH), 1721 (C=O).

*General procedure for the synthesis of compounds **13a-c***
Method A: a mixture of compound **12a-c** (0.01 mole) and aromatic aldehyde (0.01 mole) in acetic acid (30 mL)/ acetic anhydride (15 mL) mixture were refluxed for 3 h. The solution was cooled, gradually poured into cold water and the formed precipitate was filtered off and recrystalized from proper solvent to give compound **13a-c**.

Method B: a mixture of compound thiopyrimidine **11b** (0.01 mole), chloroacetic acid (0.01 mole), and aromatic aldehyde (0.01 mole) in acetic acid (30mL)/acetic anhydride (15mL) mixture in the presence of fused anhydrous sodium acetate were refluxed for 3 hours. The solution was cooled, gradually poured into cold water and the formed precipitate was filtered off and recrystalized from proper solvent to give compounds **13b**.

Method C: a mixture of compound **1** (0.01 mole), 2-chlorobenzaldehyde (0.03 mole), thiourea (0.01 mole) and chloroacetic acid (0.01 mole) in acetic acid (30mL)/ acetic anhydride (15 mL) mixture in the presence of fused anhydrous sodium acetate (2 g) was refluxed for 3 h. The solution was cooled, gradually poured into cold water and the formed precipitate was filtered off and recrystalized from proper solvent to give compounds **13b**.

*2,9-Dibenzylidene-6,6,8,8-tetramethyl-5-phenyl-6,7,8,9-tetrahydro-2H-pyrido[4,3-d]thiazolo[3,2-a]pyrimidin-3(5H)-one (**13a**)*
Yield 72 %, m.p.310-312°C, solvent of crystallization, ethanol, brown powder. IR for compounds **13a** (KBr) υ, cm^{-1}: 3127 (NH), 1720 (C=O).

*2,9-Bis(2-chlorobenzylidene)-5-(2-chlorophenyl)-6,6,8,8-tetramethyl-6,7,8,9-tetrahydro-2H-pyrido[4,3-d]thiazolo[3,2-a]pyrimidin-3(5H)-one (**13b**)*
Yield 51 % (method A), 62 % (method B), and 73 % (method C), m.p.300-302°C, solvent of crystallization, ethanol, brown powder. IR for compounds **13b** (KBr) υ, cm^{-1}: 3117 (NH), 1715 (C=O). ^{1}H NMR for compound **13b** (400 MHz, DMSO): δ_H 1.80-2.10 (brs, 12H, 4CH$_3$), 6.90-8.10 (m, 13H, 12Ar+ CH=), 12.90 (br, 1H, NH). MS, *m/z* (%) for compound **13b** (619.99, 1.7%, M$^+$), (621.99, 0.7%, M$^+$+2), (623.99, 0.1%, M$^+$+4).

*2,9-Bis(4-chlorobenzylidene)-5-(4-chlorophenyl)-6,6,8,8-tetramethyl-6,7,8,9-tetrahydro-2H-pyrido[4,3-d]thiazolo[3,2-a]pyrimidin-3(5H)-one (**13c**)*
Yield 73 %, m.p.340-342°C, solvent of crystallization, ethanol/dioxin, pale yellow powder. IR for compounds **13c** (KBr) υ, cm^{-1}: 3104 (NH), 1714 (C=O).

*9-(4-Chlorobenzylidene)-6,6,8,8-tetramethyl-2-(methylthio)-5-(4-chlorophenyl)-6,7,8,9-tetrahydropyrimido[4,5-b][1,6]naphthyridin-4-ol (**14**)*
A mixture of compound **1** (0.01 mole), aromatic aldehyde (0.02 mole) and *S*-methylaminothiouracil (0.01 mole) in 20 mL dimethyl formamide were heated under reflux for 10 h. The solution was cooled and poured into water. The formed precipitate was filtered off and crystalized from ethanol/dioxane to give compound **16** (m.p.305-307°C, yield 12%, brown powder). The structure of compound **14** was identified from IR, ^{1}H NMR, mass spectra. IR for compound **14** (KBr) υ, cm^{-1}: 3500 (OH), 3300 (NH), 1629, 1512 (C=N), 1488 (C=C). MS, *m/z* (%) (536.48, 0.79%, M$^+$), (538.48, 0.3%, M$^+$+2), (540.48, 0.1%, M$^+$+4). ^{1}H NMR for compound **14** (400 MHz, CDCl$_3$): δ_H 1.70 (s, 6H, 2CH$_3$), 2.40 (s, 6H, 2CH$_3$), 2.56 (s, 3H, CH$_3$S), 7.20-7.90 (m, 9H, 8 Ar + 1 ArCH=), 12.20 (br, 2H, 2NH).

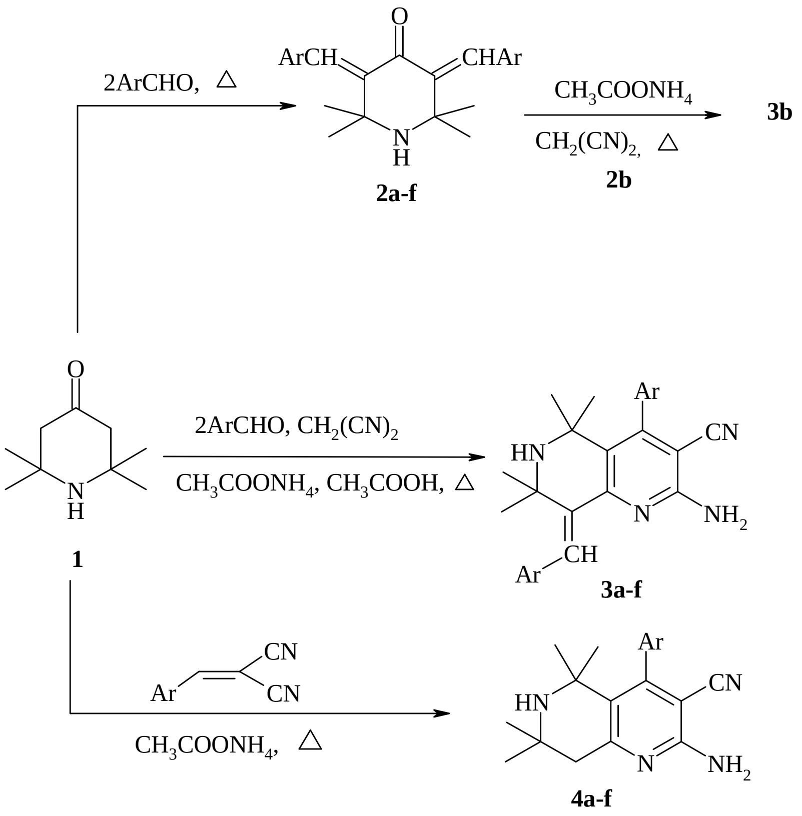
*9-(4-Chlorobenzylidene)-5-(4-chlorophenyl)-2-hydrazinyl-6,6,8,8-tetramethyl-6,7,8,9-tetrahydropyrimido[4,5-b][1,6]naphthyridin-4-ol (**15**)*
A solution of compound **14** (0.01 mole) and hydrazine hydrate (2 mL) in 50 mL ethanol was heated under reflux for 2 h. The reaction mixture was cooled and poured into water. The formed precipitate was filtered off and recrystalized from ethanol to give compound **15** (m.p.300-302°C, yield 33%, brown powder). The structure of compound **15** is identified from IR, mass, ^{1}H NMR spectra. Its IR (KBr) υ, cm^{-1}: 3500 (OH), 3398, 3444 (NH$_2$), 3181 (NH), 1614 (C=N), 1537 (C=C). MS, *m/z* (%) for compound **15** (520.44, 77.4%, M$^+$), (522.44, 33%, M$^+$+2), (524.44, 5%, M$^+$+4). ^{1}H NMR (400 MHz, CDCl$_3$): δ_H 1.70 (s, 6H, 2CH$_3$), 2.40 (s, 6H, 2CH$_3$), 2.50 (br, 2H, NH$_2$), 7.20-7.90 (m, 9H, 8 Ar + 1 ArCH=), 12.20 (br, 2H, 2NH).

RESULTS AND DISCUSSION

1. Chemistry

2,2,6,6-Tetramethyl-4-piperidone **1** contains two active methylene groups, it reacted with 2 moles of aromatic aldehydes namely, benzaldehyde, 2-chlorobenzaldehyde, 4-chlorobenzladehyde, 4-brombenzaldehyde, 4-methoxybenzaldehyde, and 4–(dimethylamino)benzaldehyde to produce 3,5-*bis*(arylidene)-2,2,6,6-tetramethyl piperidin-4-one **2a-f**, respectively in good yields (Scheme 1). Spectral data (^{1}H NMR, mass, IR) are in agreement with the proposed structures (*cf*. experimental).

Compound **1** was reacted in one-pot reaction with malononitrile and 2 moles of aromatic aldehyde in the presence of ammonium acetate to give the corresponding 2-amino-8-(arylidene)-4-(aryl)-5,5,7,7-tetramethyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carbonitriles **3a-f**. Compound **3b** was also prepared from the reaction of compound **2b** with malononitrile in ammonium acetate. The structures of compounds **3a-f** were elucidated from ^{1}H NMR, IR, and mass spectral data. The products revealed absorption bands for NH, NH$_2$, and CN groups and disappearance of CO group in the IR spectrum and their ^{1}H NMR spectra showed singlet signals for CH_3 and the signals corresponding to the aromatic protons and exchangeable protons corresponding to NH and NH_2.

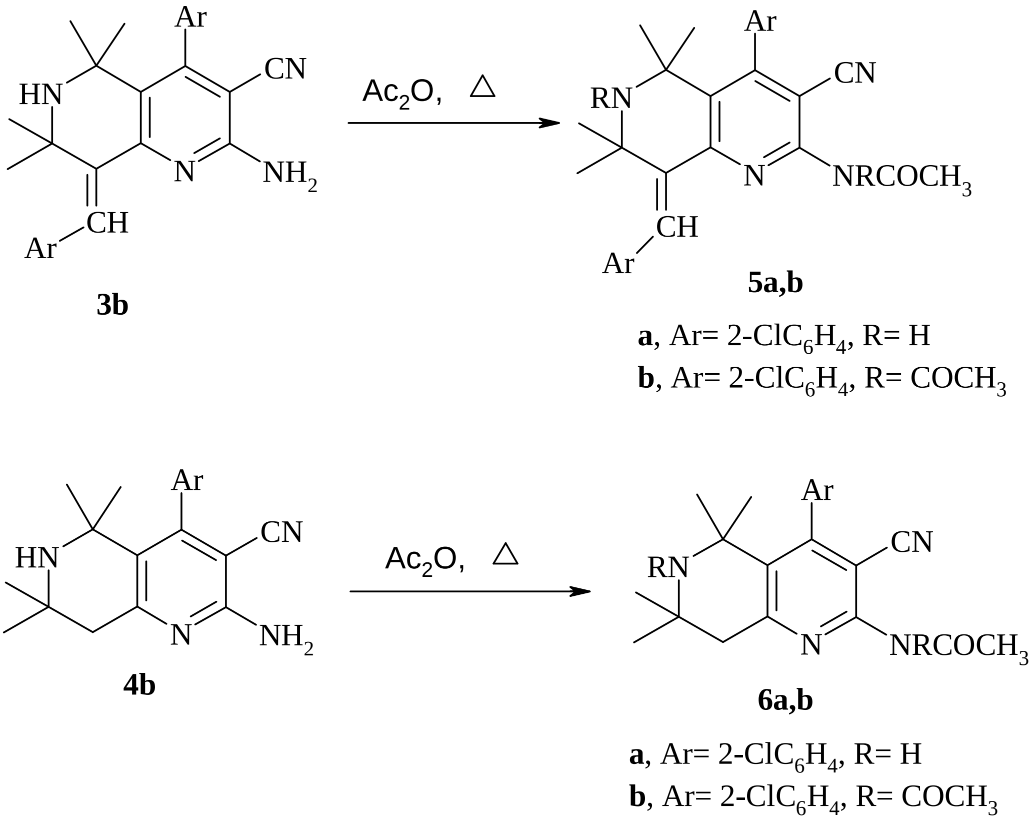


a, Ar= C_6H_5
b, Ar= 2-ClC_6H_4
c, Ar= 4-ClC_6H_4
d, Ar= 4-BrC_6H_4
e, Ar= 4-$CH_3OC_6H_4$
f, Ar= 4-$N(CH_3)_2C_6H_4$

Scheme 1

2-Amino-4-aryl-5,5,7,7-tetramethyl-5,67,8-tetrahydro-1,6-naphthynidine-3-carbonitriles **4a-f** were produced in one-step from the reaction of compound **1** with 2–arylidene malononitrile in the presence of ammonium acetate. The spectral data of compounds **4a-f** are compatible with the proposed structure.

Compounds **3b** or **4b** were reacted with acetic anhydride to afford mono and triacetylated compounds **5a,b** and **6a,b** (Scheme 2). Spectral data (^{1}H NMR, IR, mass spectra) of compounds **5a,b** and **6a,b** are in agreement with the suggested structures (*cf*. experimental).



3b

5a,b

a, Ar= 2-ClC$_6$H$_4$, R= H
b, Ar= 2-ClC$_6$H$_4$, R= COCH$_3$

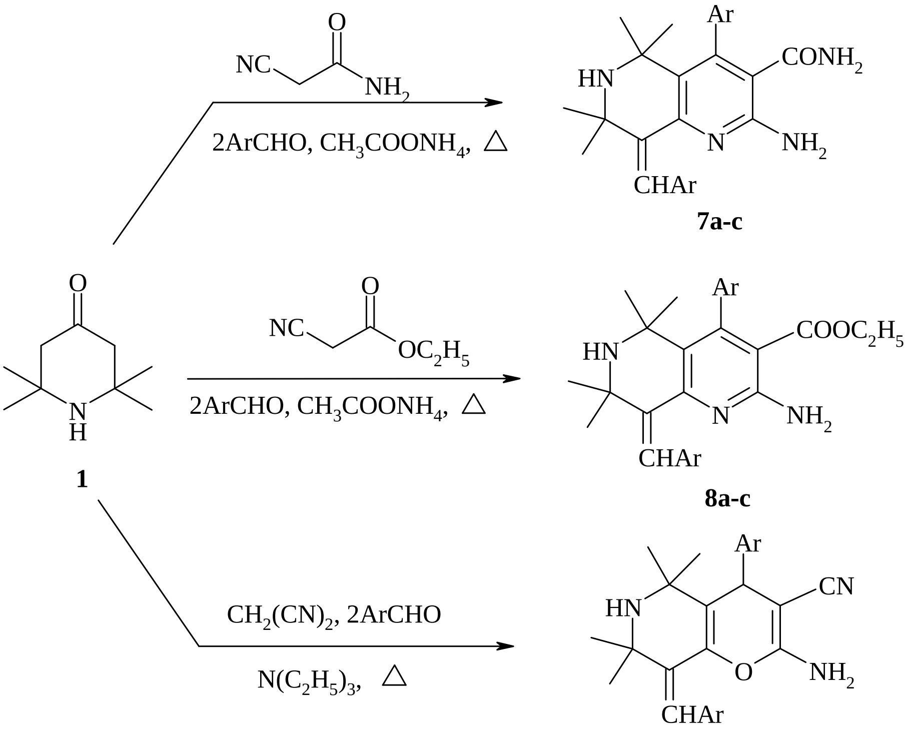
4b

6a,b

a, Ar= 2-ClC$_6$H$_4$, R= H
b, Ar= 2-ClC$_6$H$_4$, R= COCH$_3$

Scheme 2

Also, compound **1** was reacted with: 1) cyanoacetamide, aromatic aldehydes (benzaldehyde, 2-chlorobenzaldehyde, 4-chlorobenzladehyde, 4-brombenzaldehyde, 4-methoxybenzaldehyde, and 4–(dimethylamino)benzaldehyde) in the presence of ammonium acetate, or 2) with ethyl cyanoacetate, aromatic aldehydes, ammonium acetate or 3) with malononitrile, aromatic aldehydes in the prescence of triethylamine to give respectively 2-amino-8-arylidene-5,5,7,7-tetramethyl-4-aryl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carboxamides **7a-c**, ethyl 2-amino-8-arylidene-5,5,7,7-tetramethyl-4-aryl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carboxylates **8a-c** or 2-amino-8-arylidene-5,5,7,7-tetramethyl-4-aryl-5,6,7,8-tetrahydro-4*H*-pyrano[3,2-*c*]pyridine-3-carbonitriles **9a-c,** respectively (Scheme 3). The structures of compounds **7a-c**, **8a-c**, and **9a-c** were confirmed by spectral data (^{1}H NMR, IR, mass spectra) (*cf.* experimental).



1

7a-c

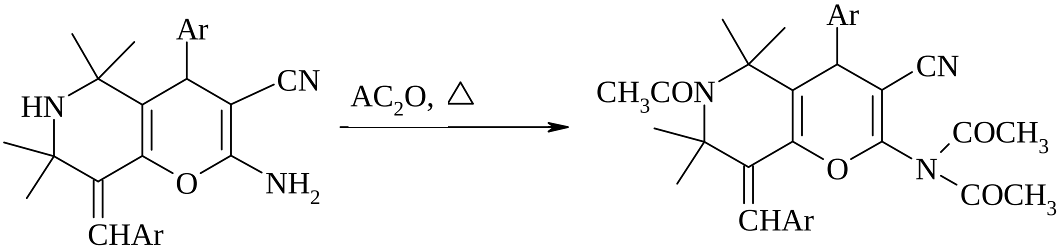
8a-c

9a-c

a, Ar= C_6H_5
b, Ar= 2-ClC_6H_4
c, Ar= 4-ClC_6H_4

Scheme 3

Compound **9c** was reacted with acetic anhydride to afford the triacetylated derivative **10** (Scheme 4). The structure of compound **10** was confirmed from spectral data.



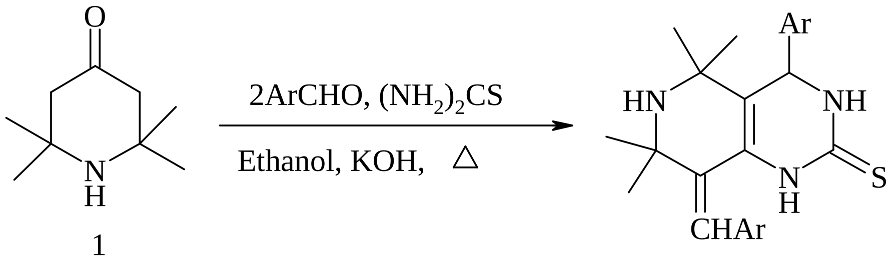
9c

10

Ar= 4-ClC_6H_4

Scheme 4

Compound **1** and 2 moles of different aromatic aldehydes (benzaldehyde, 2-chlorobenzaldehyde, 4-chlorobenzladehyde, 4-brombenzaldehyde, 4-methoxybenzaldehyde, and 4–(dimethylamino)benzaldehyde) reacted with thiourea in alcoholic potassium hydroxide solution under reflux to produce respectively 8-arylidene-4-(aryl)-5,5,7,7-tetramethyloctahydropyrido[4,3-*d*]pyrimidine-2(1*H*)-thione drevatives **11a-e** (Scheme 5). Spectral data of compounds **11a-c** are in agreement with the proposed structures (*cf*. experimental).

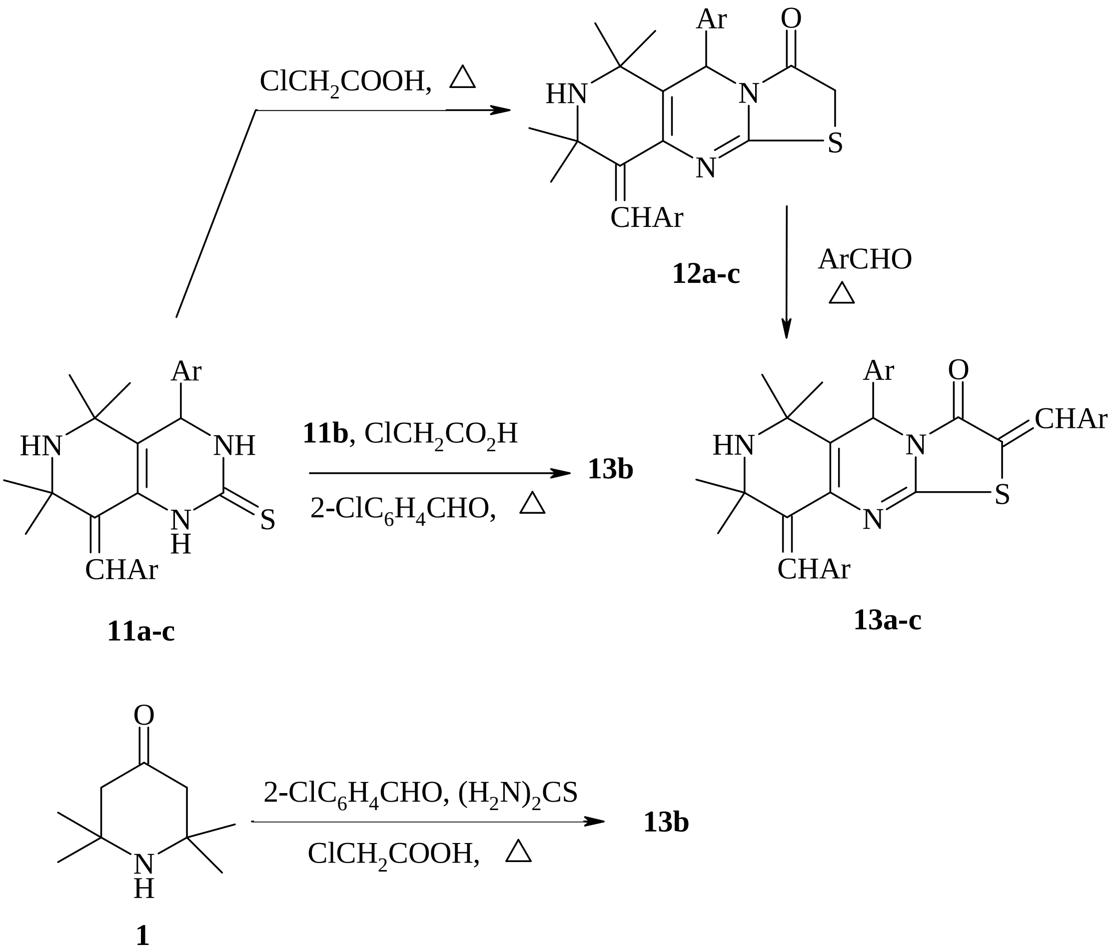


11a-e

a, Ar= C_6H_5
b, Ar= $2\text{-}ClC_6H_4$
c, Ar= $4\text{-}ClC_6H_4$
d, Ar= $4\text{-}BrC_6H_4$
e, Ar= $4\text{-}N(CH_3)_2C_6H_4$

Scheme 5

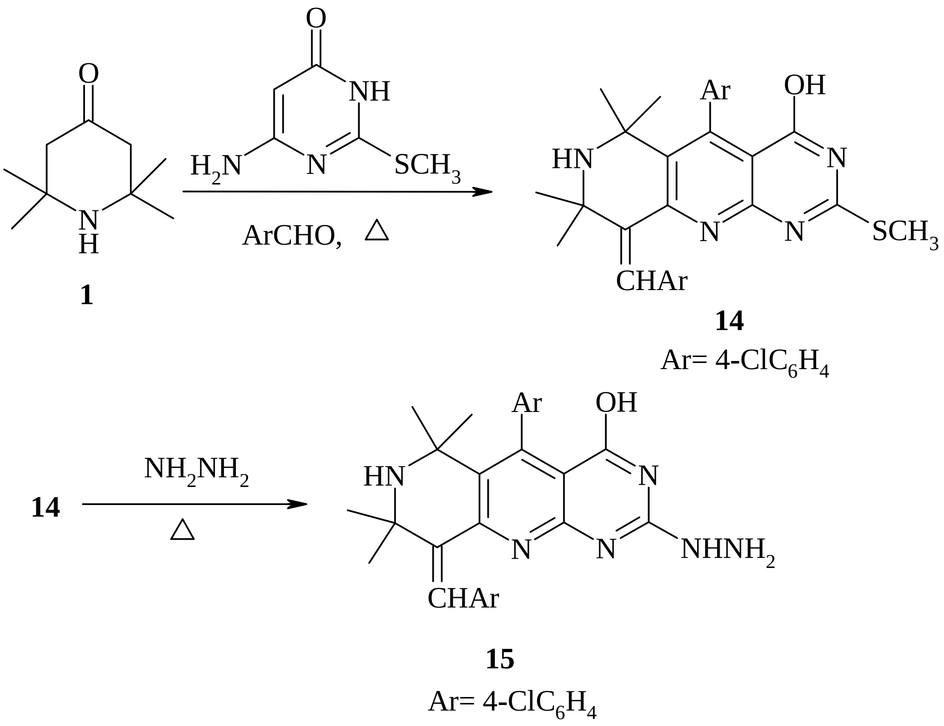
Compounds **11a-e** were reacted with chloroacetic acid to give 9-arylidene-6,6,8,8-tetramethyl-5-aryl-6,7,8,9-tetrahydro-2*H*-pyrido[4,3-*d*]thiazolo[3,2-*a*]pyrimidin-3(5*H*)-one **12a-c,** which were reacted with different aromatic aldehydes (benzaldehyde, 2-chlorobenzaldehyde, 4-chlorobenzladehyde) to produce 2,9-diarylidene-6,6,8,8-tetramethyl-5-aryl-6,7,8,9-tetrahydro-2*H*-pyrido[4,3-*d*]thiazolo[3,2-*a*]pyrimidin-3(5*H*)-one derivatives **13a-c**, respectively. Chemical proof for the structure of **13b** was reached by: a) The reaction of compound **1** with 3 moles of 2-chlorobenzaldehyde, thiourea and chloroacetic acid or b) The reaction of compound **11b** with one mole of 2-chlorobenzaldehyde and chloroacetic acid. Product **13b** obtained by the above methods are the same and identical (TLC) (*cf*. experimental).



a, Ar= C_6H_5
b, Ar= 2-ClC_6H_4
c, Ar= 4-ClC_6H_4

Scheme 6

Compound **1** was reacted with 4-chlorobenzaldehyde and 6-amino-2-*S*-methylthiouracil in DMF under reflux to give compound **14** in low yield which was reacted with hydrazine hydrate to give compound **15** (Scheme 7). ^{1}H NMR, mass, IR spectra for compound **14** and **15** are in agreement with the suggested structures (*cf*. experimental).



Scheme 7

2. **Biological evaluation**

The antiproliferative activity was measured *in vitro* using the Sulfo-Rhodamine-B stain (SRB) assay according to the previous reported standard procedure[32]. The antiproliferative activities were expressed by median growth inhibitory concentration (IC_{50}). As shown in table 1, the antiproliferative activity of the synthetic compounds was evaluated against human hepatocellular carcinoma HepG2, breast adenocarcinoma MCF-7 and human lung cancer A549 cell lines using SRB assay, in comparison with doxorubicin as reference drug. The results revealed that all compounds did not exert any activity against human lung cancer A549 cell line. However, many of the tested compounds showed remarkable anticancer activity against breast adenocarcinoma MCF-7 cell line. While compounds **4f** and **14** had no effect on the cancer cells, compounds **2f**, **11c** and **11d** were found to be more potent anticancer agents than the standard drug (IC_{50}: 4.00, 4.40, and 4.75 respectively versus 4.80 µg/mL for doxorubicin). The rest of the tested compounds revealed slight moderate activity. In the same time**,** evaluation the anticancer effect of the tested compounds against human hepatocellular carcinoma HepG2 revealed that compounds **3a**, **3d**, **4f**, **11a**, **11b**, **13b**, **11d** and **14** had no effect on the HepG2 cancer cell; however compounds **2f**, **3c**, **4d**, and **15** showed anticancer activity but less than the standard drug (IC_{50}: 9.00, 11.20, 9.70, and 8.60 respectively, versus 4.20 µg/mL for doxorubicin). The rest of the tested compounds revealed slight activity. From the foregoing results, the cytotoxicity and growth inhibitor activity of the synthesized compounds are more significant on breast carcinoma more than liver carcinoma.

Table 1. ***In vitro*** **cytotoxicity activity of the tested compounds as expressed as IC_{50} values in 3 different human cancer cell lines.**

Compound	**IC_{50} (µg/mL)**		
	HepG2	**MCF-7**	**A549**
2f	9.00±0.85	4.00±0.46	-
3a	-	38.60±4.00	-
3c	11.20±1.23	8.90±0.96	-
3d	-	35.75±4.00	-
3e	44.30±5.00	15.30±1.70	-
4a	21.60±2.20	23.00±2.40	-
4c	30.20±3.20	10.30±1.46	-
4d	9.70±1.00	6.10±0.60	-
4f	-	-	-
7b	19.90±2.00	18.30±1,80	-
8b	45.00±5.10	25.00±2.20	-
9c	39.10±4.30	19.60±2.00	-
10	46.30±4.62	16.60±1.85	-
11a	-	32.20±3.60	-
11b	-	28.00±2.90	-
11c	34.70±3.70	4.40±0.48	-
11d	-	4.75±0.50	-
11e	42.80±4.70	12.30±1.40	-
12b	46.60±5.35	6.20±0.65	-
13b	-	25.30±2.64	-
14	-	-	-
15	8.60±0.90	6.70±0.82	-
DMSO	-	-	-
Doxorubicin	4.20±0.44	4.80±0.50	5.30±0.60

Data were expressed as average of three independent experiments. - - no activity

Antioxidant activity of each compound and standards (ascorbic acid and rutin) were assessed based on the radical scavenging effect of stable DPPH free radical [33,34]. All experiments were conducted in triplicate (n = 3). All the values were represented as mean ± SD.

The synthesized compounds were examined *in vitro* for their antioxidant activities against DPPH radicals. The results obtained revealed that the activities of the tested compounds appeared in the following order: Vit. C > Rutin > **4e** > **3b** > **11b** > **2a** > **4b** > **2b** > **4c** > **2d** > **2e**. Compound **2c** did not show any antioxidant activity. In contrast, compound **6a** showed prooxidant activity. Comparing the activity of the eleven compounds to the standard antioxidants and well known potent DPPH inhibitors (rutin and vit C); it is clear that the best scavenging properties was gained by compounds **3b**, **4e**, and **11b**.

Eleven of the synthesized compounds were examined *in vitro* for their anti-tumor activities against breast carcinoma cell line (MCF-7) and liver carcinoma cell lines (HepG-2) and their cytotoxic activities against normal human skin fibroblast cell line (BJ-1) using MTT assay [35]. The percentage of the intact cells was measured and compared to the control. The activities of the derivatives against carcinoma cells and normal cells were compared with the cytotoxicity of Doxorubicin (the positive control).

The obtained results showed that only two compounds; **2c** and **2e** showed moderate cytotoxic activities against the HepG-2 and MCF7 with inhibition percentages of 46.91, 29.46 & and 38.775, 34.105, respectively. The other compounds either showed very week or no cytotoxicity against the two cancer cell lines. All of the twelve investigated compounds did not show any toxic effects against the normal cells (BJ-1). In the contrary, they all caused growth enhancement for the normal cells ranging from 31.2% to 77.5 % except for compounds **2c** and **2e** which showed very low growth enhancement (Table 2).

Table 2. *In vitro* antioxidant activity at concentration of 100 µM/mL and *in vitro* tumor growth inhibition activities at concentration of 200 µM.

Compound	% Antioxidant	BJ-1	HepG2	MCF-7
2a	24.93	-70.53	0	0
2b	11.24	-64.13	0	0
2c	0	-7.53	46.91	38.775
2d	7.04	-77.48	0	0
2e	4.22	-1.52	29.46	34.105
3b	52.42	-47.08	5.66	17.37
4b	20.92	-53.165	5.31	14.62
4c	8.10	-31.195	0	0
4e	55.73	-35.38	0	20.27
6a	-26.35	-58.88	0	12.92
11b	41.10	-51.78	14.56	0
Vit. C	77.12			
Rutin	57.13			

Acknowledgement

The authors extend their thanks to the National Research Center (Dokki, Cairo, Egypt) for the all given facilities.

REFERENCES

[1] F. H. Smirk, I. V. Hodge, *J. Chin. Pharm. Therap.*, **1960**, I, 610.
[2] a- J. F. W. Keana, *Chem. Rev.*, **1978**, 78, 37. b- O. Brede, D. Beckert, C. Wmdolph, H. A. Gottinger, *Phys. Chem. A.*, **1998**, 102, 1457-1464.
[3] a- R. Hook, *Ind. Eng. Chem. Res.*, **1997**, 36, 1779-1790. b- A. K. Chakraboty, G. Astarita, K. B. Bischoff, *Chem. Eng. Sci.*,**1985**, 41, 997-1003.
[4] H.N. Pati, U. Das, S. Das, B. Bandy, E. De Clercq, J. Balzarini, M. Kawase, H. Sakagami, J.W. Quail, J.P. Stables, J. R. Dimmock, *Eur. J. Med. Chem.* **2009**, 44, 54-62.
[5] U. Das, J. Alcorn, A. Shrivastav, R.K. Sharma, E. De Clercq, J. Balzarini, J. R. Dimmock, *Eur. J. Med. Chem.* **2007**, 42, 71-80.
[6] U. Das, H. N. Pati, H. Sakagami, K. Hashimoto, M. Kawase, J. Balzarini, E. De Clercq, J.R. Dimmock, *J. Med. Chem.* **2011**, 54, 3445-3449.
[7] S. Saponara, A. Ferrara, B. Gorelli, A. Shah, *Eur. J. Pharm.* **2007**, 563, 160.
[8] M. Kawase, A. Shah, H. Gaveriya, N. Motohashi, H. Sakagami, A. Vargae, J. Monar, *Bioorg. Med .Chem.* **2002**, 10, 1051.
[9] S. Tasaka, H. Ohmori, N. Gomi, M. Iino, T. Machida, A. Kuie, S. Naito, M. Kuwanoc, *Bioorg. Med. Chem. Lett.*, **2001**, 11, 275.
[10] T. Gunars, T. Dace, H. Zhanna, *Czech. J. Food Sci.* **2001**, 19(3), 81.
[11] D. Tirzite, A. Krauze, A. Zubareva, G. Tiritis and G. Duburs, *Chem. Heterocycl. Compd.* **2002**, 38(7), 795.
[12] A. Kumar, R. A. Mavrya, S. Sharma, M. Kumar, G. Bhatia, *Eur. J. Med. Chem.* **2010**, 45, 501.
[13] M. A. El-Hashash, M. El-Kady, *Rev. Roum. Chim.*, **1978**, 23, 581.
[14] M. E. Konshin, V. A. Khaldeeva, *Chem. Heterocycl. Compd.* **1973**, 9(10), 1262 – 1264.
[15] N. M. Fathy, A. S. Aly, F. Motti, F. M. E. Abdel-Megeid, *Egypt. J. Chem.* **1986**, 29, 609; *Chem. Abstr.* **1989**, 110, 23140w.
[16] L. W. Deady, T. Rodemann, L. Zhuang, B. C. Baguley and W. A. Denny, *J. Med. Chem.* **2003**, 46(6), 1049-54.
[17] T. Suresh, T. Dhananbal, R. N. Kumar and P. S. Mohan, *Indain J. Chem.*
[18] L. W. Deady, M. L. Rogers, L. Zhuang, B. C. Baguley and W. A. Denny, *Bioorg. Med. Chem.* **2005**, 13(4), 1341-55.
[19] Y. Zhou , J. A. Porco , Jr., J. K. Snyder, *Org. Lett.* **2007**, 9(3), 393–396.
[20] S. K. Srivastava, A. Jha, S. K. Agarwal, R. Mukherjee, A. C. Burman, *Anticancer Agents Med. Chem.* **2007**, 7(6), 685-709.
[21] Z-G. Han, G. Zhang, B. Jiang, N. Ma, F. Shi, and S-J. Tu, *J. Comb. Chem.* **2009**, 11(5), 809-812.
[22] B. Insuasty, D. Becerra , J. Quiroga, R. Abonia, M. Nogueras and J.Cobo, *Eur. J. Med. Chem.* **2013**, 60, 1-9.(and references cited therein).
[23] C. Li, X-Y. Mu, Y-L Li, Y. Liu and X-S. Wang, *ACS Comb. Sci.* **2013**, 15(5), 267-272.

[24] M. S. Moustafa, S. M. Al-Mousawi, N. M. Hilmy, Y. A. Ibrahim, J. C. Liermann, H. Meier, M. H. Elnagdi, *Molecules* **2013**, 18, 276-286.
[25] G. Falardeau, H. Lachance, A. S. Pierre, C. G. Yannopoulos, M. Drouin, J. Bédard, L. Chan, B*ioorg. Med. Chem. Lett*. **2005**, 15, 1693-1695.
[26] A. L. Ruchelman, P. J. Houghton, N. Zhou, A. Liu, L. F. Liu, E. J. LaVoie, J. *Med. Chem.* **2005**, 48, 792-804.
[27] Y. Zhou, A. B. Beeler, S. Cho, Y. Wang, S. G. Franzblau, J.K. Snyder, *J. Comb. Chem*. **2008**, 10, 534-540.
[28] B. A. Johns, J. G. Weatherhead, S. H. Allen, J. B. Thompson, E. P. Garvey, S. A. Foster, J. L. Jeffrey, W. H. Miller, *Bioorg. Med. Chem. Lett.* **2009**, 19, 1807-1810.
[29] S. Vanlaer, A. Voet, C. Gielens, M. de Maeyer, F. Compernolle, *Eur. J. Org. Chem*. **2009**, 643-654.
[30] K. Ramkumar, E. Serrao, S. Odde, N. Neamati, *Med. Res. Rev*. **2010**, 30, 890-954.
[31] H. Huang, Q. Chen, X. Ku, L. Meng, L. Lin, X. Wang, C. Zhu, Y. Wang, Z. Chen, M. Li, H. Jiang, K. Chen, J. Ding, H. Liu, *J. Med. Chem*. **2010**, 53, 3048-3064.
[32] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. Mcmahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Nat. Canc. Inst.* **1990**, 82, 1107-1112.
[33] E. J. Gamez, L. Luyengi, S. K. Lee, L. F. Zhou, H. H. Frog, A. D. Kinghorn, *J. Nat. Prod*. **1998**, 61, 706-708.
[34] R. M. Hussein, Y. E. Shahein, A. E. El Hakim, H. M. Awad, *J. Am. Sci*. **2010**, 6, 726-733.
[35] M. B. Hansen, S. E. Nielsen, K. Berg, *J. Immunol. Methods* **1989**, 119, 203.
[36] A. Wu, W. Yang, X. Pan, *Syn. Comm.*, **1996**, 26(19), 3565-3569.