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Anticancer activities of some newly synthesized pyridine, pyrane, and pyrimidine derivatives

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Abstract—A series of pyridine, pyrane, and pyrimidine derivatives (**2**–**11**) were newly synthesized using nitrobenzosuberone **1** as a starting material. The antitumor activities of the synthesized compounds were evaluated utilizing 59 different human tumor cell lines, representing leukemia, melanoma, lung, colon, brain, ovary, breast, prostate as well as kidney. Some of the tested compounds especially **2**, **3**, **4c**, **6**, **7**, **9b**, **10a**, and **11** exhibited better in vitro antitumor activities at low concentration ($\log_{10}$ $GI_{50} = -4.7$) against the used human tumor cell lines. Additionally, compounds **3**, **4c**, **6**, **7**, and **9b** were highly selective to inhibit leukemia cell lines. The detailed synthesis, spectroscopic data and antitumor properties for the synthesized compounds were reported.

1. Introduction

In a previous work we reported that certain of our newly substituted heterocyclic compounds exhibited antiparkinsonian,[1] antitumor,[2–4] antimicrobial,[5–7] and anti-inflammatory[8,9] activities. Pyrazoles present an interesting group of compounds many of which possess widespread pharmacological properties such as analgesic, antipyretic, and antirheumatic activities.[10,11] In addition, the pharmacological and antitumor activities of many compounds containing heterocyclic ring have been reviewed.[2–14] Recently, the heterocyclic nitrogen derivatives exhibited a general ionophoric potency for divalent cations[15] and used a novel thiocyanate-selective membrane sensor.[16] In view of these reports and in continuation of our previous works in heterocyclic chemistry, we have herein synthesized some new derivatives containing heterocyclic ring fused with substituted benzosuberone structure for the evaluation of their anticancer activity.

2. Results and discussion

2.1. Chemistry

3-Nitro-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-5-one (**1**) was synthesized according to the reported procedure[17] and used as starting material. It was reacted with sulfur powder and ethyl cyanoacetate in the presence of diethylamine in ethanol as catalyst to afford the corresponding thiophene amino ester **2**, which was heated with phenylisothiocyanate on a water bath at 80 °C without solvent to afford the corresponding *N*-phenyl pyrimidine **3**. Condensation of compound **1** with arylmethylene cyanoacetamide in the presence of triethylamine or ammonium acetate in refluxing ethanol afforded the corresponding cyanopyridone derivative **4**, but compound **1** was reacted with malononitrile in the presence of β-alanine as catalyst to give ylidene malononitrile **5** according to a literature procedure.[18] Condensation of compound **5** with *p*-chlorobenzaldehyde or *p*-chlorophenylmethylene cyanoacetamide in the presence of ammonium acetate in refluxing glacial acetic acid afforded the corresponding cyanoaminopyridine (**6**) and dibenzodicarbonitrile derivative (**7**), respectively (Scheme 1).

Reaction of nitrobenzosuberone **1** with aromatic aldehydes, namely, *p*-chloro-, *p*-bromo-, *p*-nitro- or 3,4,5-trimethoxybenzaldehyde in acetic acid in the presence of concentrated sulfuric acid as a catalyst or in piperidine

Keywords: Nitrobenzosuberone; Pyranes; Pyrimidinethiones; Pyrazoles; Anticancer activity.
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† Prof. Dr. Hammam was deceased during the course of this work.

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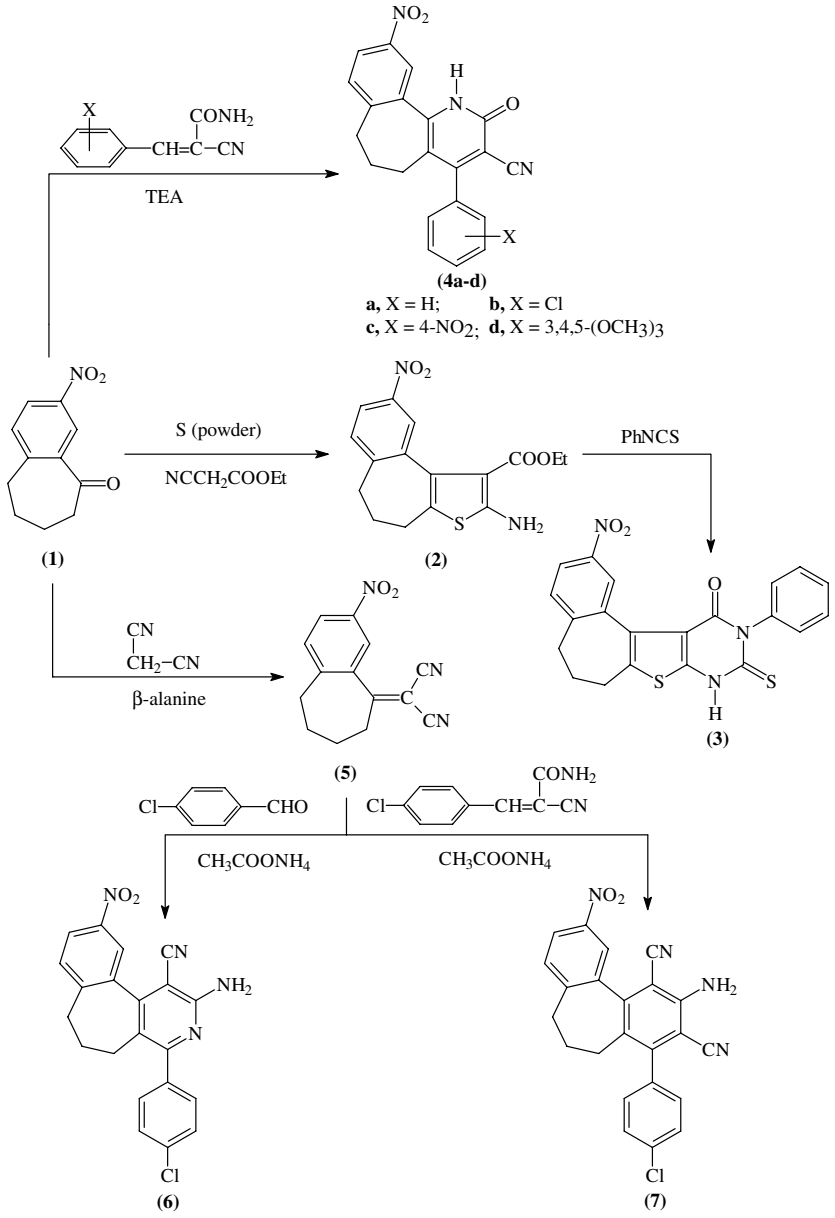


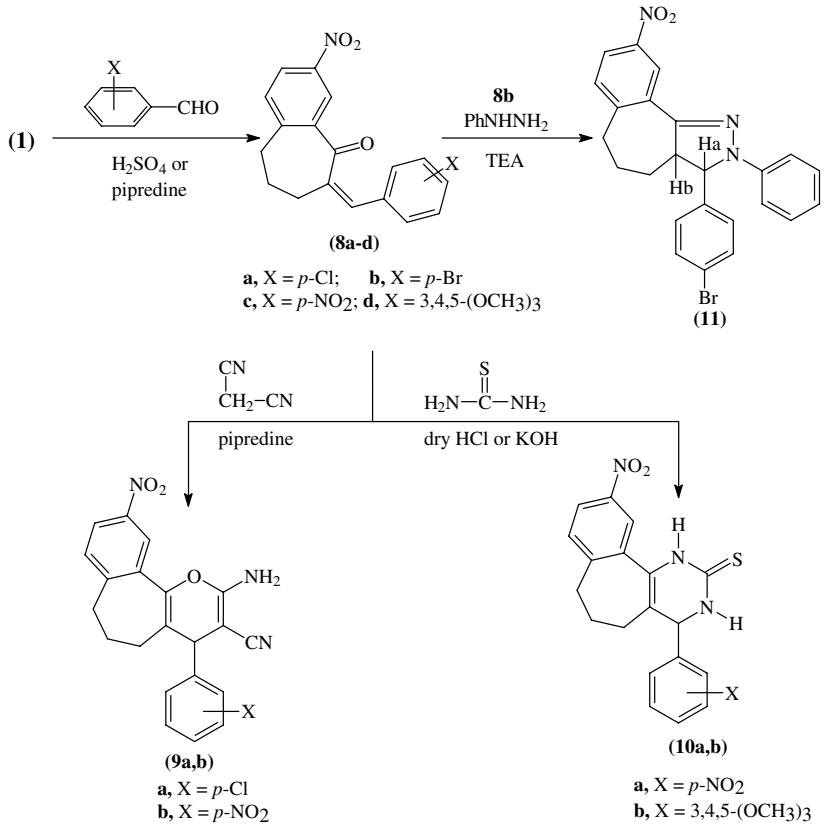
Scheme 1.

without solvent gave the corresponding arylmethylene derivatives **8a**–**d**, respectively. Compounds **8a**,**c** were condensed with malononitrile in refluxing ethanol in the presence of pipridene as a catalyst to give cyanoaminopyrane derivatives **9a**,**b**, respectively. Thioxopyrimidine derivatives **10a**,**b** were obtained from the reaction of the arylmethylene derivatives **8c**,**d** with thiourea in refluxing ethanolic potassium hydroxide according to a published procedure[19] or in ethanol with dry hydrogen chloride gas. Condensation of compound **8b** with phenyl hydrazine in absolute ethanol in the presence of triethylamine as a catalyst afforded the corresponding pyrazole derivative **11** (Scheme 2).

2.2. Antitumor screening

2.2.1. Antitumor activity. Antitumor activity screening for the synthesized compounds utilizing 59 different human tumor cell lines, representing leukemia, melanoma, and cancers of the lung, colon, brain, ovary, breast, prostate as well as kidney, was carried out according to the previously reported standard procedure.[20–22] The obtained results (Table 1) represent concentrations of the used investigated compounds resulting in growth inhibition of 50% (GI_{50}) for the tested human tumor cell lines. From the in vitro observed data it has been noticed that, the selected compounds **2**, **3**, **4c**, **6**, **7**, **9b**, **10a**, and **11** seem to be the most active prepared derivatives against all the tested cell lines.

2.2.2. Structural–activity relationship (SAR). From the above-obtained results (Table 1), we can conclude that cyanopyridine, pyrimidine, and pyrane moieties fused to nitrobenzosuberone ring are essential for antitumor activities. In the present work, we can suggest that the anticancer activity is due to:



Scheme 2.

(i) The presence of nitrogen heterocyclic rings.
(ii) The most active compounds being **3**, **4c**, **6**, **7**, and **9b** against leukemia cell lines.
(iii) The presence of the nitrile groups (CN) generally enhancing the activity.
(iv) The difference in activity between the compounds which is due to the indicated subsistents in the phenyl group of the molecule.

3. Experimental

3.1. Chemistry

Melting points were determined on open glass capillaries using an Electrothermal IA 9000 digital melting point apparatus and are uncorrected. Elemental analyses were performed on Elementar, Vario EL, Microanalytical Unit, National Research Centre, Cairo, Egypt and were found within ±0.4% of the theoretical values. Infrared (IR) spectra were recorded on Carlzeise Spectrophotometer model 'UR 10' spectrophotometer using the KBr disc technique. ^{1}H NMR spectra were recorded on Varian Gemini 200 MHz spectrometer (DMSO-d_6 or $CDCl_3$) and the chemical shifts are given in δ (parts per million) downfield from tetramethylsilane (TMS) as an internal standard. Splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet. The mass spectra (MS) were measured using a Finnigan SSQ 7000 mass spectrometer. Followup of the reactions and checking the purity of the compounds were made by TLC on silica gel-precoated aluminum sheets (Type 60 F254, Merck, Darmstadt, Germany) and the spots were detected by exposure to UV lamp at λ_{254} nanometer for few seconds. The starting material, 3-nitro-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-5-one (**1**), was prepared according to the reported procedures.[17]

3.1.1. Synthesis of 2-amino-5-nitro-8,9-dihydro-5*H*-benzo[3,4]cyclohepta[1,2-*b*]thiophen-3-ethyl carboxylate (2). To a mixture of compound **1** (2.05 g, 10 mmol), ethyl cyanacetate (1.13 g, 10 mmol), and sulfur powder (0.32 g, 10 mmol) in ethanol (100 ml), diethyl amine (25 ml) was added. The reaction mixture was stirred at room temperature for 6 h, left overnight, poured onto cold water, the formed solid was filtered off, dried, and crystallized from ethanol to give the corresponding amino ester derivative **2**, in 90% yield; mp 85–86 °C; IR (KBr, cm^{-1}): 3350–3125 (NH_2) and 1735 (C=O, ester); ^{1}H NMR ($CDCl_3$): δ 7.65–7.15 (m, 3H, Ar-H), 4.20 (s, 2H, NH_2, exchangeable with D_2O), 3.85 (q, 2H, CH_2), 3.15–2.35 (m, 6H, $3CH_2$ of cycloheptene ring), 1.35 (t, 3H, CH_3); MS, *m*/*z* (%): 332 [M^+] (35), 287 [M^+−OEt] (100), 286 [M^+−NO_2] (90), 197 [286—COOEt, NH_2] (65). Anal. Calcd for $C_{16}H_{16}N_2O_4S$ (332.38): C, 57.81; H, 4.85; N, 8.42. Found: C, 57.76; H, 4.81; N, 8.39.

3.1.2. Synthesis of 10-mercapto-2-nitro-11-phenyl-5,6,7,11-tetrahydro-12*H*-benzo[6′,7′]-cyclohepta[1′,2′:4,5]-thieno[2,3-*b*]pyridin-12-one (3). A mixture of compound **2** (3.32 g, 10 mmol) and phenyl isothiocyanate (1.35 g, 10 mmol) was heated on water bath at 80 °C without

Table 1. Concentrations resulting in growth inhibition of 50% ($\log_{10}$ GI_{50}) of in vitro human tumor cell lines

Panel/cell line	Compound							
	2	**3**	**4c**	**6**	**7**	**9b**	**10a**	**11**
Leukemia								
CCRF-CEM	−4.0	−5.3	−5.3	−5.3	−5.3	−5.3	−4.0	−4.0
HL-60 (TB)	−4.0	−5.3	−5.3	−5.3	−5.3	−5.3	−4.0	−4.0
K-562	−4.0	−5.3	−5.3	−5.3	−5.3	−5.3	−4.0	−4.0
MOLT-4	−4.0	−5.3	−5.3	−5.3	−5.3	−5.3	−4.0	−4.0
RPMI-8226	−4.0	−5.3	−5.3	−5.3	−5.3	−5.3	−4.0	−4.0
SR	−4.0	−5.3	−5.3	−5.3	−5.3	−5.3	−4.0	−4.0
Non-small cell lung cancer								
A549/ATCC	−4.3	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
EKVX	−4.3	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
HOP-62	−4.3	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
HOP-92	−4.3	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
NCI-H226	−4.3	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
NCI-H23	−4.3	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
NCI-H322M	−4.3	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
NCI-H460	−4.3	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
NCI-H522	−4.3	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
Colon cancer								
COLO 205	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
HCC-2998	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
HCT-116	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
HCT-15	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
HT29	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
KM12	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
SW-620	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
CNS cancer								
SF-268	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
SF-295	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
SF-539	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
SNB-19	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
SNB-75	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
U 251	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
Melanoma								
LOXIMVI	−4.0	−4.3	−4.3	−4.0	−4.3	−4.0	−4.0	−4.0
MALME-3M	−4.0	−4.3	−4.3	−4.0	−4.3	−4.0	−4.0	−4.0
M 14	−4.0	−4.3	−4.3	−4.0	−4.3	−4.0	−4.0	−4.0
SK-MEL-2	−4.0	−4.3	−4.3	−4.0	−4.3	−4.0	−4.0	−4.0
SK-MEL-28	−4.0	−4.3	−4.3	−4.0	−4.3	−4.0	−4.0	−4.0
SK-MEL-5	−4.0	−4.3	−4.3	−4.0	−4.3	−4.0	−4.0	−4.0
UACC-257	−4.0	−4.3	−4.3	−4.0	−4.3	−4.0	−4.0	−4.0
UACC-62	−4.0	−4.3	−4.3	−4.0	−4.3	−4.0	−4.0	−4.0
Ovarian cancer								
IGROVI	−4.0	−4.3	−4.3	−4.0	−4.3	−4.3	−4.0	−4.0
OVCAR-3	−4.0	−4.3	−4.3	−4.0	−4.3	−4.3	−4.0	−4.0
OVCAR-4	−4.0	−4.3	−4.3	−4.0	−4.3	−4.3	−4.0	−4.0
OVCAR-5	−4.0	−4.3	−4.3	−4.0	−4.3	−4.3	−4.0	−4.0
OVCAR-8	−4.0	−4.3	−4.3	−4.0	−4.3	−4.3	−4.0	−4.0
SK-OV-3	−4.0	−4.3	−4.3	−4.0	−4.3	−4.3	−4.0	−4.0
	−4.0	−4.3	−4.3	−4.0	−4.3	−4.3	−4.0	−4.0
Renal cancer								
786-0	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
A 498	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
ACHN	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
CAKI-1	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
RXF-393	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
SN12C	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
TK-10	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
UO-31	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
Prostate cancer								
PC-3	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
Du-145	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0

Table 1 (*continued*)

Panel/cell line	Compound							
	2	**3**	**4c**	**6**	**7**	**9b**	**10a**	**11**
Breast cancer								
MCF 7	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
NCI/ADR-RES	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
MDA-MB-231/ATCC	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
HS 578T	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
MDA-MB-435	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
BT-549	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0
T-47D	−4.0	−4.3	−4.3	−4.3	−4.3	−4.3	−4.0	−4.0

solvent for 3 h. The residue was triturated with ethanol, the formed solid was collected by filtration, washed with cold ethanol, dried, and crystallized from methanol to give the corresponding pyrimidine thione derivative (**3**), in 60% yield; mp 123–125 °C; IR (KBr, cm^{-1}): 3375 (NH) and 1660 (C═O); ^{1}H NMR ($CDCl_3$): δ 11.30 (s, 1H, NH, exchangeable with D_2O), 8.00–7.20 (m, 8H, Ar-H), 3.10–2.20 (m, 6H, $3CH_2$ of cycloheptene ring); ^{13}C NMR: δ 22.55, 31.05, 41.80 ($3\times CH_2$), 121.25, 121.80, 127.95, 133.00, 141.95, 146.65 (O_2N–Ph–C), 135.35, 152.50, 137.25, 169.25 (thiophene-C), 120.05, 122.60, 127.00, 131.65 (Ph–C), 159.75 (C═O), 178.35 (C═S); MS, *m/z* (%): 421 [M^+] (12), 375 [M^+-NO_2] (24), 344 [M^+-Ph] (36), 254 [298—CS] (100). Anal. Calcd for $C_{21}H_{15}N_3O_3S_2$ (421.50): C, 59.84; H, 3.58; N, 9.96. Found: C, 59.77; H, 3.54; N, 9.93.

3.1.3. Synthesis of 10-nitro-2-oxo-4-(substituted phenyl)-6,7-dihydro-1*H*,5*H*-benzo[6,7]-cyclohepta[1,2-*b*]pyridine-3-carbonitrile derivatives (4a–d). *Method A*:[3,4,14] A mixture of compound **1** (1.02 g, 5 mmol) and arylmethylene cyanoacetamide derivatives (5 mmol) and triethylamine (0.3 ml) in ethanol (50 ml) was heated under reflux for 4 h. The reaction mixture was cooled and poured onto water, the obtained solid was filtered off, dried, and crystallized from the proper solvent to give the corresponding cyanopyridone derivatives (**4a**–**d**), respectively.

Method B:[4,14] A mixture of **1** (1.02 g, 5 mmol), aromatic aldehyde, namely, benzaldehyde, *p*-chloro-, *p*-nitro- or 3,4,5-trimethoxybenzaldehyde (5 mmol), ethyl cyanoacetate (0.56 g, 5 mmol), and ammonium acetate (0.77 g, 10 mmol) in ethanol (50 ml) was refluxed for 3 h. The reaction mixture was poured onto water, the formed solid was collected by filtration, dried, and crystallized from the proper solvent to give compounds (**4a**–**d**), respectively.

The products were identified by their mp and R_f-values in comparison with authentic samples previously obtained by method A.

3.1.3.1. 10-Nitro-2-oxo-4-phenyl-6,7-dihydro-1*H*,5*H*-benzo[6,7]cyclohepta[1,2-*b*]pyridine-3-carbonitrile (4a). Yield 68% [A], 76% [B]; mp >300 °C (dioxane); IR (KBr, cm^{-1}): 3388 (NH), 2221 (CN) and 1655 (C═O); ^{1}H NMR (DMSO-d_6) δ: 12.90 (s, 1H, NH, exchangeable with D_2O), 8.30–7.50 (m, 8H, Ar-H), 2.70–2.00 (m, 6H, $3CH_2$ of cycloheptene ring); MS, *m/z* (%): 357 [M^+] (14), 311 [M^+-NO_2] (19), 285 [311—CN] (25), 280 [M^+-Ph] (8), 234 [280—NO_2] (10) and at 208 [234—CN] (100). Anal. Calcd for $C_{21}H_{15}N_3O_3$ (357.36): C, 70.58; H, 4.23; N, 11.76. Found: C, 70.52; H, 4.18; N, 11.73.

3.1.3.2. 4-(*p*-Chlorophenyl)-10-nitro-2-oxo-6,7-dihydro-1*H*,5*H*-benzo[6,7]cyclohepta[1,2-*b*]-pyridine-3-carbonitrile (4b). Yield 56% [A], 66% [B]; mp >300 °C (dioxane); IR (KBr, cm^{-1}): 3388 (NH), 2219 (CN) and 1645 (C═O); ^{1}H NMR (DMSO-d_6) δ: 13.00 (br s, 1H, NH, exchangeable with D_2O), 8.50–7.20 (m, 7H, Ar-H) and 2.90–2.20 (m, 6H, $3CH_2$ of cycloheptene ring); MS, *m/z* (%): 391 [M^+] (26), 393 [M^++2] (8) due to the presence of chlorine atom, 310 [M^+-Cl, NO_2] (34), 280 [$M^+-Cl-Ph$] (10) and at 238 [280—CONH] (100). Anal. Calcd for $C_{21}H_{14}ClN_3O_3$ (391.81): C, 64.37; H, 3.60; N, 10.72. Found: C, 64.33; H, 3.55; N, 10.69.

3.1.3.3. 10-Nitro-4-(*p*-nitrophenyl)-2-oxo-6,7-dihydro-1*H*,5*H*-benzo[6,7]cyclohepta[1,2-*b*]-pyridine-3-carbonitrile (4c). Yield 85% [A], 72% [B]; mp >300 °C (dioxane); IR (KBr, cm^{-1}): 3370 (NH), 2225 (CN) and 1660 (C═O); ^{1}H NMR ($CDCl_3$) δ: 12.70 (br s, 1H, NH, exchangeable with D_2O), 8.30–7.20 (m, 7H, Ar-H), 2.75–2.30 (m, 6H, $3CH_2$ of cycloheptene ring); ^{13}C NMR: δ 22.85, 30.85, 41.95 ($3\times CH_2$), 120.75, 121.75, 122.80, 126.75, 128.15, 132.65, 137.60, 142.65, 146.15, 146.65 ($2\times O_2N$–Ph–C), 114.10, 115.65, 125.45, 168.25 (pyridone-C), 115.45 (CN), 160.10 (C═O); MS, *m/z* (%): 402 [M^+] (45), 310 [M^+-2NO_2] (32), 280 [M^+-NO_2-Ph] (42), 238 [280—CONH] (100) and at 192 [238—NO_2] (85). Anal. Calcd for $C_{21}H_{14}N_4O_5$ (402.67): C, 66.64; H, 3.50; N, 13.91. Found: C, 66.58; H, 3.45; N, 13.87.

3.1.3.4. 10-Nitro-2-oxo-4-(3′,4′,5′-trimethoxyphenyl)-6,7-dihydro-1*H*,5*H*-benzo[6,7]cyclohepta[1,2-*b*]pyridine-3-carbonitrile (4d). Yield 75% [A], 64% [B]; mp >300 °C (dioxane); IR (KBr, cm^{-1}): 3360 (NH), 2222 (CN) and 1655 (C═O); ^{1}H NMR ($CDCl_3$): δ 12.60 (br s, 1H, NH, exchangeable with D_2O), 8.10–7.40 (m, 3H, Ar-H), 6.60 (s, 2H, Ar-H), 3.90 (s, 9H, $3OCH_3$) and 2.80–2.10 (m, 6H, $3CH_2$ of cycloheptene ring); MS, *m/z* (%): 447 [M^+] (12), 354 [M^+-3OCH_3] (17), 308 [354—NO_2] (10), 280 [$M^+-Ph(OCH_3)_3$] (6) and at 238 [280—CONH] (100). Anal. Calcd for $C_{24}H_{21}N_3O_6$ (447.45): C, 64.42; H, 4.73; N, 9.39. Found: C, 64.37; H, 4.68; N, 9.35.

3.1.4. Synthesis of 3-nitro-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-5-ylidene malononitrile (5). A mixture of compound **1** (2.05 g, 10 mmol), malononitrile (0.66 g, 10 mmol), and β-alanine (50 mg) as catalyst in ethanol

(50 ml) was refluxed for 12 h. After cooling, the formed solid was collected by filtration, dried and crystallized from ethanol. The product was separated, identified as 3-nitro-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-5-ylidene malononitrile (**5**) in 80% yield, mp 120 °C [lit. 122 °C].[18]

3.1.5. Synthesis of 2-amino-4-(*p*-chlorophenyl)-10-nitro-6,7-dihydro-5*H*-benzo[3,4]cyclohepta[1,2-*c*]pyridine-1-carbonitrile (6). A mixture of compound **5** (1.15 g, 5 mmol), *p*-chlorobenzaldehyde (0.7 g, 5 mmol), and ammonium acetate (0.77 g, 10 mmol) in glacial acetic acid (50 ml) was heated under reflux for 5 h. The reaction mixture was cooled, poured onto ice-water, the obtained solid was filtered off, dried, and crystallized from ethanol to give cyanoaminopyridine derivative **6**, in 75% yield; mp 152–154 °C (EtOH) IR (KBr, cm^{-1}): 3390, 3320 (NH_2) and 2208 (CN); ^1H NMR (DMSO-d_6) δ: 8.20–8.00 (m, 3H, Ar-H of NO_2–Ph), 7.50–7.00 (m, 4H, Ar-H for Cl-phenyl), 4.20 (s, 2H, NH_2, exchangeable with D_2O) and 2.50–1.80 (m, 6H, $3CH_2$ of cycloheptene ring); ^{13}C NMR: δ 23.15, 30.65, 42.05 ($3\times CH_2$), 121.15, 123.25, 128.10, 132.85, 138.35, 142.95 (O_2N–Ph–C), 127.80, 128.65, 130.10, 139.05 (Cl–Ph–C), 116.35, 124.05, 150.60, 158.90, 159.15 (pyridine-C), 116.90 (CN); MS, *m*/*z* (%): 390 [M^+] (45), 392 [M^++2] (14) due to the presence of chlorine atom, 309 [M^+-Cl, NO_2] (54), 279 [M^+-PhCl] (100) and at 233 [279−NO_2] (65). Anal. Calcd for $C_{21}H_{15}ClN_4O_2$ (390.83): C, 64.53; H, 3.86; N, 14.33. Found: C, 64.47; H, 3.80; N, 14.29.

3.1.6. Synthesis of 2-amino-4-(*p*-chlorophenyl)-10-nitro-6,7-dihydro-5*H*-dibenzo[*a*,*c*]-cyclohepten-1,3-dicarbonitrile (7). To a mixture of compound **5** (1.15 g, 5 mmol) and *p*-chlorophenylmethylene cyanoacetamide (1.03 g, 5 mmol) in glacial acetic acid (50 ml), ammonium acetate (0.77 g, 10 mmol) was added. The reaction mixture was heated under reflux for 3 h, after cooling, the formed solid was filtered off, dried, and crystallized from ethanol to give compound **7**, in 80% yield; mp 273–275 °C (EtOH); IR (KBr, cm^{-1}): 3420–3390 (NH_2) and 2220 (CN); ^1H NMR ($CDCl_3$) δ: 8.40–8.25 (m, 3H, Ar-H, for NO_2–Ph), 7.50–7.35 (m, 4H, Ar-H for Cl-phenyl), 5.30 (s, 2H, NH_2, exchangeable with D_2O) and 2.70–1.90 (m, 6H, $3CH_2$ of cycloheptene ring); MS, *m*/*z* (%): 414 [M^+] (19), 416 [M^++2] (6), due to the presence of chlorine atom, 333 [M^+-Cl, NO_2] (24), 303 [M^+-PhCl] (86) and at 231 [303—CN, NO_2] (100). Anal. Calcd for $C_{23}H_{15}ClN_4O_2$ (414.85): C, 66.65; H, 3.64; N, 13.51. Found: C, 66.60; H, 3.58; N, 13.47.

3.1.7. Synthesis of 6-(substituted benzylidine)-3-nitro-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-5-one (8a–d). *Method A:*[3] To a mixture of nitrobenzosuberone **1** (2.05 g, 10 mmol) and aromatic aldehydes, namely, *p*-chloro-, *p*-bromo-, *p*-nitro-, and 3,4,5-trimethoxybenzaldehyde (10 mmol) in glacial acetic acid (20 ml), concentrated sulfuric acid (6 ml) was added. The reaction mixture was stirred at room temperature for 6 h, then left to cool overnight at −5 °C, and poured onto crushed ice (100 g). The formed precipitate was collected by filtration, washed with water, dried, and crystallized from the proper solvent to give the corresponding arylmethylene derivatives (**8a–d**), respectively.

Method B:[3] A mixture compound **1** (2,05 g, 10 mmol) and previously described aromatic aldehydes (10 mmol) was heated at 110 °C without solvent for 15 min, few drops of piperidine (~3 ml) were added with stirring. After cooling, the reaction mixture was dissolved in acetic acid and poured into cooled water, the formed precipitate was collected by filtration, washed with water, dried, and crystallized from the proper solvent to give (**8a–d**), respectively.

The products were identified by their mp and R_f-values in comparison with authentic samples previously obtained by method A.

3.1.7.1. 6-(*p*-Chlorobenzylidene)-3-nitro-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-5-one (8a). Yield 55% [A], 68% [B]; mp 203–205 °C (EtOH); IR (KBr, cm^{-1}): 1655 (C=O); ^1H NMR ($CDCl_3$) δ: 8.20 (s, 1H, benzylic proton), 8.00–7.60 (m, 3H, Ar-H), 7.50–7.30 (m, 4H, Ar-H), 3.00–2.10 (m, 6H, $3CH_2$ of cycloheptene ring); MS, *m*/*z* (%): 327 [M^+] (32), 329 [M^++2] (11), due to the presence of chlorine atom, 216 [$M^+-C_6H_4Cl$] (100), 188 [216—CO] (85), 170 [216—Cl] (54) and at 142 [188—Cl] (65). Anal. Calcd for $C_{18}H_{14}ClNO_3$ (327.76): C, 65.96; H, 4.31; N, 4.27. Found: C, 65.91; H, 4.24; N, 4.22.

3.1.7.2. 6-(*p*-Bromobenzylidene)-3-nitro-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-5-one (8b). Yield 75% [A], 62% [B]; mp 156–158 °C (EtOH); IR (KBr, cm^{-1}): 1667 (C=O); ^1H NMR ($CDCl_3$) δ: 8.30 (s, 1H, benzylic proton), 8.50–7.70 (m, 3H, Ar-H), 7.50–7.30 (m, 4H, Ar-H), 3.10–2.20 (m, 6H, $3CH_2$ of cycloheptene ring); MS, *m*/*z* (%): 372 [M^+] (100), 374 [M^++2] (94), due to the presence of bromine atom, 216 [$M^+-C_6H_4Br$] (34), 188 [216—CO] (12), 170 [216—Br] (14) and at 142 [188—Br] (75). Anal. Calcd for $C_{18}H_{14}BrNO_3$ (372.22): C, 58.10; H, 3.79; N, 3.76. Found: C, 58.04; H, 3.73; N, 3.72.

3.1.7.3. 6-(*p*-Nitrobenzylidene)-3-nitro-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-5-one (8c). Yield 76% [A], 65% [B]; mp 186–188 °C (EtOH); IR (KBr, cm^{-1}): 1660 (C=O); ^1H NMR ($CDCl_3$) δ: 8.10 (s, 1H, benzylic proton), 8.30–7.80 (m, 3H, Ar-H), 7.50–7.30 (m, 4H, Ar-H), 3.00–2.10 (m, 6H, $3CH_2$ of cycloheptene ring); MS *m*/*z* (%): 338 [M^+] (22), 216 [$M^+-C_6H_4NO_2$] (24), 188 [216—CO] (100), 170 [216—NO_2] (14) and at 142 [188—NO_2] (75). Anal. Calcd for $C_{18}H_{14}N_2O_5$ (338.32): C, 63.90; H, 4.17; N, 8.28. Found: C, 63.83; H, 4.13; N, 8.22.

3.1.7.4. 3-Nitro-6-(3′,4′,5′-trimethoxybenzylidene)-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-5-one (8d). Yield 74% [A], 68% [B]; mp 196–198 °C (EtOH); IR (KBr, cm^{-1}): 1655 (C=O); ^1H NMR (DMSO-d_6) δ: 8.30 (s, 1H, benzylic proton), 8.50–7.70 (m, 3H, Ar-H), 6.70 (s, 2H, Ar-H), 3.90 (s, 9H, $3OCH_3$), 3.00–2.00 (m, 6H, $3CH_2$ of cycloheptene ring); MS, *m*/*z* (%): 383 [M^+] (34), 216 [$M^+-C_6H_2(OCH_3)_3$] (18), 188 [216—CO] (90), 170 [216—$3OCH_3$] (44) and at 142 [188—$3OCH_3$] (100). Anal. Calcd for $C_{21}H_{21}NO_6$ (383.40): C, 65.78; H, 5.52; N, 3.65. Found: C, 65.73; H, 5.48; N, 3.60.

3.1.8. Synthesis of 2-amino-4-(substituted phenyl)-10-nitro-4,5,6,7-tetrahydrobenzo[6,7]-cyclohepta[1,2-*b*]pyran-3-carbonitrile derivatives (9a,b). *Method A:*[14] A mixture of compounds **8a**,**c** (5 mmol), malononitrile (0.33 g, 5 mmol), and few drops of piperidine in absolute ethanol (100 ml) was stirred at room temperature for 2 h. The solvent was concentrated under reduced pressure; the formed solid was filtered off, dried, and crystallized from the proper solvent to give the corresponding cyanoaminopyrane (**9a**,**b**), respectively.

Method B:[4] A mixture of compounds **8a**,**c** (5 mmol), malononitrile (0.33 g, 5 mmol), and sodium acetate anhydrous (2 g) in glacial acetic acid (25 ml) was refluxed for 4 h. The reaction mixture was poured onto crushed ice, the obtained precipitate was collected by filtration, dried, and crystallized from the proper solvent to give (**9a**,**b**), respectively.

The products were identified by their mp and R_f-values in comparison with authentic samples previously obtained by method A.

3.1.8.1. 2-Amino-4-(*p*-chlorophenyl)-10-nitro-4,5,6,7-tetrahydrobenzo[6,7]cyclohepta[1,2-*b*]-pyran-3-carbonitrile (9a). Yield 75% [A], 60% [B]; mp 248–250 °C (EtOH); IR (KBr, cm^{-1}): 3420, 3380 (NH_2) and 2220 (CN); ^1H NMR (DMSO d_6) δ: 8.30–8.20 (m, 3H, Ar-H), 7.50–7.40 (m, 4H, Ar-H), 6.80 (s, 2H, NH_2, exchangeable with D_2O), 4.20 (s, 1H, pyran-H), 2.50–1.50 (m, 6H, $3CH_2$ of cycloheptene ring); ^{13}C NMR: δ 23.25, 29.95, 41.55 ($3\times CH_2$), 120.90, 123.30, 128.15, 133.05, 138.75, 143.15 (O_2N–Ph–C), 45.70, 109.35, 111.80, 147.60, 157.65 (pyrane-C), 127.55, 128.45, 130.25, 138.45 (Cl–Ph–C), 116.65 (CN); MS, *m*/*z* (%): 393 [M^+] (8), 395 [M^++2] (3), due to the presence of chlorine atom, 282 [M^+-PhCl] (65), 240 [282—CN, NH_2] (100), 236 [282—NO_2] (45) and at 195 [282—CN, NH_2] (64). Anal. Calcd for $C_{21}H_{16}ClN_3O_3$ (393.83): C, 64.04; H, 4.10; N, 10.67. Found: C, 63.98; H, 4.04; N, 10.63.

3.1.8.2. 2-Amino-10-nitro-4-(*p*-nitrophenyl)-4,5,6,7-tetrahydrobenzo[6,7]cyclohepta[1,2-*b*]-pyran-3-carbonitrile (9b). Yield 80% [A], 74% [B]; mp 178–180 °C (EtOH); IR (KBr, cm^{-1}): 3480, 3390 (NH_2) and 2210 (CN); ^1H NMR ($CDCl_3$) δ: 8.20–7.20 (m, 7H, Ar-H), 4.60 (s, 2H, NH_2, exchangeable with D_2O), 4.30 (s, 1H, pyran-H), 2.60–1.80 (m, 6H, $3CH_2$ of cycloheptene ring); MS, *m*/*z* (%): 404 [M^+] (45), 282 [M^+-PhNO_2] (45), 240 [282—CN, NH_2] (74), 236 [282—NO_2] (100) and at 195 [282—CN, NH_2] (24). Anal. Calcd for $C_{21}H_{16}N_4O_5$ (404.38): C, 62.37; H, 3.98; N, 13.85. Found: C, 62.32; H, 3.93; N, 13.79.

3.1.9. Synthesis of 10-nitro-4-(substituted phenyl)-1,3,4,5,6,7-hexahydro-2*H*-benzo[6,7]-cyclohepta[1,2-*d*]pyrimidine-2-thione derivatives (10a,b). *Method A:*[3] To a mixture of compounds **8c**,**d** (10 mmol) and thiourea (0.76 g, 10 mmol) in absolute ethanol (50 ml), dry hydrogen chloride gas was bubbled at 0 °C for 1 h. The reaction mixture was heated on a water bath for 10 h, the solvent was concentrated under reduced pressure, poured onto crushed ice followed by neutralization with sodium bicarbonate. The obtained precipitate was collected by filtration, dried, and crystallized from the proper solvent to give (**10a**,**b**), respectively.

Method B:[14] A mixture of compounds **8c**,**d** (10 mmol) and thiourea (0.76 g, 10 mmol) in ethanolic potassium hydroxide (2 g KOH in 100 ml ethanol) was refluxed for 3 h. The reaction mixture was acidified with hydrochloric acid (1 N), the obtained precipitate was filtered off, washed with water, dried, and crystallized from the proper solvent to give (**10a**,**b**), respectively.

The products were identified by their mp and R_f-values in comparison with authentic samples previously obtained by method A.

3.1.9.1. 10-Nitro-4-(*p*-nitrophenyl)-1,3,4,5,6,7-hexahydro-2*H*-benzo[6,7]cyclohepta[1,2-*d*]-pyrimidine-2-thione (10a). Yield 76% [A], 62% [B]; mp 174–176 °C (EtOH); IR (KBr, cm^{-1}): 3423, 3385 (2NH); ^1H NMR (DMSO-d_6) δ: 10.50, 9.09 (2s, 2H, 2NH, exchangeable with D_2O), 8.10–7.70 (m, 3H, Ar-H), 7.10–6.70 (m, 4H, Ar-H), 5.40 (s, 1H, pyrimidine-H) and 3.15–2.00 (m, 6H, $3CH_2$ of cycloheptene ring); ^{13}C NMR (DMSO-d_6): δ 22.40, 29.55, 38.95 ($3\times CH_2$), 120.65, 121.65, 122.65, 126.70, 128.10, 133.05, 137.55, 142.70, 146.25, 146.75 ($2\times O_2N$–Ph–C), 66.35, 112.10, 125.75 (pyrimidine-C), 180.10 (C=S); MS, *m*/*z* (%): 396 [M^+] (65), 350 [M^+-NO_2] (43), 304 [M^+-NO_2] (100), 274 [$M^+-C_6H_4NO_2$] (6) and at 228 [274—NO_2] (86). Anal. Calcd for $C_{19}H_{16}N_4O_4S$ (396.42): C, 57.57; H, 4.07; N, 14.13. Found: C, 57.51; H, 3.98; N, 14.08.

3.1.9.2. 10-Nitro-4-(3′,4′,5′-trimethoxyphenyl)-1,3,4,5,6,7-hexahydro-2*H*-benzo[6,7]cyclohepta[1,2-*d*]-pyrimidine-2-thione (10b). Yield 66% [A], 65% [B]; mp 278–280 °C (EtOH); IR (KBr, cm^{-1}): 3380 (2NH), ^1H NMR (DMSO-d_6) δ: 10.10, 9.00 (2s, 2H, 2NH, exchangeable with D_2O), 8.20–7.50 (m, 3H, Ar-H), 6.80 (s, 2H, Ar-H), 5.50 (s, 1H, pyrimidine-H), 3.90 (s, 9H, $3OCH_3$) and 3.20–1.90 (m, 6H, $3CH_2$ of cycloheptene ring); MS, *m*/*z* (%): 441 [M^+] (66), 348 [M^+-3OCH_3] (32), 274 [$M^+-C_6H_2(OCH_3)_3$] (16), 228 [274—NO_2] (26) and at 213 [274—$CSNH_2$] (100). Anal. Calcd for $C_{22}H_{23}N_3O_5S$ (441.51): C, 59.85; H, 5.25; N, 9.51. Found: C, 59.78; H, 5.20; N, 9.43.

3.1.10. Synthesis of 3-(*p*-bromophenyl)-9-nitro-2-phenyl-2,3,3a,4,5,6-hexahydrobenzo-[6,7]cyclohepta[1,2-*c*]pyrazole (11). To a mixture of compound **8b** (1.86 g, 5 mmol) and phenyl hydrazine (0.54 g, 5 mmol) in absolute ethanol (50 ml), few drops of triethylamine were added as a catalyst. The reaction mixture was refluxed for 3 h, after cooling, the formed solid was collected by filtration, dried, and crystallized from ethanol to give the corresponding pyrazole derivative **11**, in 76% yield; mp 177–179 °C (AcOH); IR (KBr, cm^{-1}): 1596 (C=N); ^1H NMR ($CDCl_3$) δ: 8.00–6.80 (m, 12H, Ar-H); 4.70 (d, 1H, Ha), 4.20–3.50 (m, 3H, Hb + CH_2 of cycloheptene ring) and 2.40–2.10 (m, 4H, CH_2 of cycloheptene ring); MS, *m*/*z* (%): 462 [M^+] (100), 464 [M^++2] (98) due to the presence of bro-

mine atom, 381 [M^+−HBr] (14), 306 [M^+−PhBr] (20) and at 229 [306—Ph] (18). Anal. Calcd for $C_{24}H_{20}BrN_3O_2$ (462.34): C, 62.35; H, 4.36; N, 9.10. Found: C, 62.28; H, 4.30; N, 9.00.

3.2. Anticancer activity

Some of the synthesized compounds were selected and screened for their anticancer activity. Each compound was tested at five different concentrations against 60 cell lines of nine types of human cancers, namely, leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer.

Results are expressed as $\log_{10}GI_{50}$, which is the drug concentration (*M*) causing a 50% reduction in the net protein increase in control cells during the drug incubation[23] (Table 1).

Some of the synthesized compounds showed good anticancer activity at low concentration compared with 5-fluorodeoxyuridine $\log_{10}$ $GI_{50} = -4.7$ as reference control.

4. Conclusion

In our previous works,[3,4] we reported that fused pyrimidine derivatives were proved to be active anticancer agents. In the present work, we can suggest that the anticancer activity is due to the presence of these nitrogen heterocyclic rings represented by **2**, **3**, **4c**, **6**, **7**, **9b**, **10a**, and **11** and the difference in activity between them is due to the various indicated substituents in the phenyl group of the molecule.

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References and notes

1. Amr, A. E.; Hegab, M. I.; Ibrahim, A. A.; Abdalah, M. M. *Monatsch. Chem.* **2003**, *134*, 1395.
2. Abou-Ghalia, M. H.; Amr, A. E. *Amino Acids* **2004**, *26*, 283.
3. Hammam, A. G.; Fahmy, A. F. M.; Amr, A. E.; Mohamed, A. M. *Indian J. Chem.* **2003**, *42B*, 1985.
4. Hammam, A. G.; Sharaf, M. A.; Abdel Hafez, N. A. *Indian J. Chem.* **2001**, *40B*, 213.
5. Amr, A. E.; Mohamed, A. M.; Ibrahim, A. A. *Z. Naturforsch.* **2003**, *58b*, 861.
6. Amr, A. E.; Abdel-Salam, O. I.; Attia, A.; Stibor, I. *Collect. Czech. Commun.* **1999**, *64*, 288.
7. Attia, A.; Abdel-Salam, O. I.; Amr, A. E.; Stibor, I.; Budesinsky, M. *Egypt. J. Chem.* **2000**, *43*, 187.
8. Amr, A. E.; Abdulla, M. M. *Indian J. Heterocycl. Chem.* **2002**, *12*, 129.
9. Abou-Ghalia, M. H.; Amr, A. E.; Abdalah, M. M. *Z. Naturforsch.* **2003**, *58b*, 903.
10. Fylaktakidou, C. K.; Hadjipavlou, J. D. L.; Litinas, E. K.; Nicolaides, N. D. *Curr. Pharm. Des.* **2004**, *10*, 3813.
11. Jung, J. C.; Watkins, E. B.; Avery, M. A. *Heterocycles* **2005**, *65*, 77.
12. Amr, A. E. *Indian J. Heterocycl. Chem.* **2000**, *10*, 49.
13. Hammam, A. G.; Ali, M. I. *J. Chem. Eng. Data* **1981**, *26*, 99.
14. Hammam, A. G.; Abdel Hafez, N. A.; Midura, W. H.; Mikolajczyk, M. *Z. Naturforsch.* **2000**, *55b*, 417.
15. Hassan, S. S. M.; Abou-Ghalia, M. H.; Amr, A. E.; Mohamed, A. H. K. *Talanta* **2003**, *60*, 81.
16. Hassan, S. S. M.; Abou-Ghalia, M. H.; Amr, A. E.; Mohamed, A. H. K. *Anal. Chem. Acta* **2003**, *482*, 9.
17. Smith, P. A.; Berry, W. L. *J. Org. Chem.* **1961**, *26*, 27.
18. Schneller, S. W.; Moore, D. R. *J. Org. Chem.* **1977**, *39*, 1433.
19. Yousif, N. M.; Gad, F. A.; Fahmy, A. F.; Amine, M. S.; Sayed, H. H. *Phosphorus, Sulfur Silicon Relat. Elem.* **1996**, *117*, 11.
20. Ally, M. C.; Scudiero, D. A.; Monks, P. A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. A.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589.
21. Grever, M. R.; Schepartz, S. A.; Chabner, B. A. *Semin. Oncol.* **1992**, *19*, 622.
22. Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91.
23. Chemical structure of interest to the division Nancita R. Lomox and Dr. V.L. Naryanan, Drug Synthesis and Chemistry Branch Development Therapeutic Program, Vol. III, 1983.