International Journal of Organic Chemistry, 2024, 14, 107-122
https://www.scirp.org/journal/ijoc
ISSN Online: 2161-4695
ISSN Print: 2161-4687

New 4-imino-4*H*-Chromeno[2,3-*d*] Pyrimidin-3(5*H*)-Amine: Synthesis, Cytotoxic Effects on Tumoral Cell Lines and *in Silico* ADMET Properties

Marwa Dhiabi[1,2], Sirine Karoui[1,2], Mehdi Fakhfakh[1], Souhir Abid[3], Emmanuelle Limanton[2,4], Rémy Le Guével[5], Thierry Charlier[5,6], Ludovic Paquin[2,4], Jean-Pierre Bazureau[2,4*], Houcine Ammar[1]

[1]Laboratoire de Chimie Appliquée: Hétérocycles, Corps et Polymères, Faculté des Sciences de Sfax, Université de Sfax, Sfax, Tunisie
[2]Institut des Sciences Chimiques de Rennes, Université de Rennes, Campus de Beaulieu, Rennes Cedex, France
[3]Department of Chemistry (Science and Arts): Al Qurayat, Al-Jouf University, Al Jawf, Saudi Arabia
[4]S2Wave Platform, ScanMAT UAR CNRS, Université de Rennes, Campus de Beaulieu, Rennes Cedex, France
[5]ImPACcell Platform, Biosit, SFR UMS CNRS, Inserm, Campus de Villejean, Rennes, France
[6]Institut de Recherche en Santé, Environnement et Travail, IRSET Inserm UMR_S, Rennes, France
Email: *jean-pierre.bazureau@univ-rennes1.fr

How to cite this paper: Dhiabi, M., Karoui, S., Fakhfakh, M., Abid, S., Limanton, E., Le Guével, R., Charlier, T., Paquin, L., Bazureau, J.-P. and Ammar, H. (2024) New 4-imino-4*H*-Chromeno[2,3-*d*]Pyrimidin-3(5*H*)-Amine: Synthesis, Cytotoxic Effects on Tumoral Cell Lines and *in Silico* ADMET Properties. *International Journal of Organic Chemistry*, **14**, 107-122.
https://doi.org/10.4236/ijoc.2024.143006

Received: September 3, 2024
Accepted: September 22, 2024
Published: September 25, 2024

Abstract

The synthesis of new 4-imino-4*H*-chromeno[2,3-*d*]pyrimidin-3(5*H*)-amine in four steps including one step under microwave dielectric heating is reported. The structural identity of the synthesized compounds was established according to their spectroscopic analysis, such as FT-IR, NMR and mass spectroscopy. These new compounds were tested for their antiproliferative activities on seven representative human tumoral cell lines (Huh7 D12, Caco2, MDA-MB231, MDA-MB468, HCT116, PC3 and MCF7) and also on fibroblasts. Among them, only the compounds **6c** showed micromolar cytotoxic activity on tumor cell lines ($1.8 < IC_{50} < 6$ μM) and no toxicity on fibroblasts ($IC_{50} > 25$ μM). Finally, *in silico* ADMET studies ware performed to investigate the possibility of using of the identified compound **6c** as potential anti-tumor compound.

Keywords

2-Amino-4*H*-Chromene, 4*H*-Chromeno[2,3-*d*]Pyrimidin-3(5*H*)-Amine, Microwave Irradiation, Tumoral Cell Line, *in Silico* ADMET

1. Introduction

Among heterocyclic compounds, the 2-amino-4*H*-chromenes and their 4*H*-chromeno[2,3-*d*]pyrimidin derivatives represent very attractive products known for their broad spectrum of biological activities. They are referenced as potential anti-tumor agents [1] [2], anti-bacterial [3]-[5], anti-inflammatory agents [6] and also exhibited significant selective activity against bacteria and fungi [7]. It is interesting to point out that this type of structure is also present in natural products. As examples, 4*H*-chromene appears in *Wisteria sinensis* plant [8] and in the leaves of *Calyptranthes tricona* [9]. Among the 2-amino-4*H*-chromene products, those which have been a most advanced stage of pharmacological development are respectively drug-candidate LY290181 as potent antiproliferative compound blocking cells in G_2/M phase of the cell cycle [10], *Crolibulin*™ (EPC 2407) in clinical phase I/II trials for treatment of aggressive solid tumors [11] and HA14.1 as tubulin inhibitor in pre-clinal and clinal phase I trials for cancer [12].

As an extension of our previous studies concerning the use of 2-amino-4*H*-chromene as starting platform [13] for the construction of fused heterocyclic systems with biological applications, we reported herein the synthesis of 4*H*-chromeno[2,3-*d*]pyrimidin-3(5*H*)-amine, explored *in vitro* the cytotoxic activities on a panel of tumoral cell lines and determined *in silico* their physicochemical and ADME (Absorption, Distribution, Metabolism, Excretion) properties.

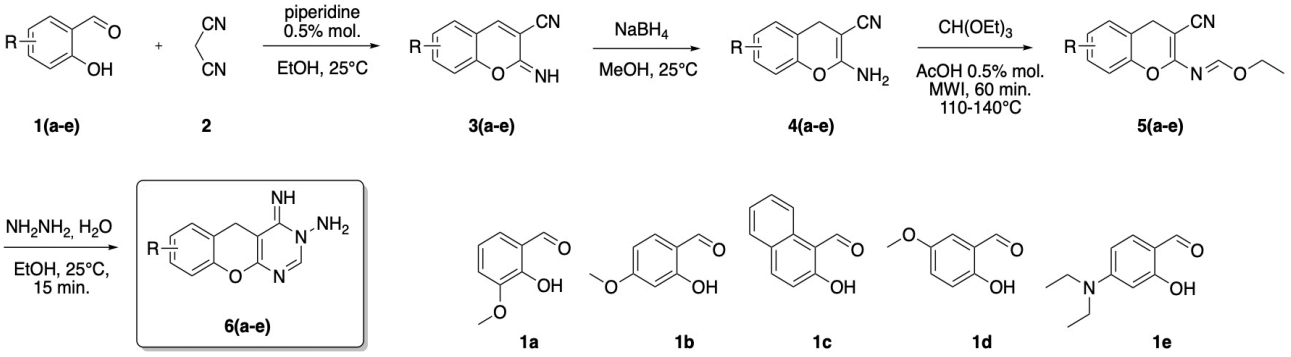
2. Results and Discussion

2.1. Chemistry

The *β*-enaminonitrile moiety which is directly integrated in the 2-amino-4*H*-chromen-4-carbonitrile platform offers a wide diversity of molecular reactivity. The simultaneous presence of the carbonitrile function in position C-4 as electrophilic center and amino function in position C-2 as nucleophilic center allows heterocyclization reactions. The methodology adopted for the preparation of 2-amino-4*H*-chromen-4-carbonitrile **4(a-e)** is outlined in Scheme 1 according to procedures reported in literature [13].

To enrich the molecular diversity of this platform, we initially used 2-hydroxy-3-methoxybenzaldehyde **1a** or orthovanilin, 2-hydroxy-4-methoxybenzaldehyde **1b**, 2-hydroxynaphtaldehyde **1c**, 2-hydroxy-5-methoxybenzaldehyde **1d** and 4-(diethylamino)salicylaldehyde **1e**. To introduce the pyrimidine fragment on the 4*H*-chromene skeleton of **4**, we transformed the 2-amino function of **4** into imidate **5**. After addition of ethyl orthoformate (6 equivalents) to **4** in the presence of glacial acetic acid 0.5% mol. as catalyst, the resulting mixture was submitted to microwave dielectric heating at 110˚C - 140˚C for 60 min. After cooling down to room temperature, we observed that imidate **5** is not soluble in the crude reaction mixture which facilitates recovery by a simple filtration. Due to the success of this step for heating under microwave irradiation, we investigated access to 4-imino-4,5-dihydro-3*H*-chromeno[2,3-*d*]pyrimidin-3-amine **6** by aza-annulation of

imidate **5** with hydrazine. Initial attempts after a set of experiments under microwave irradiation showed that the desired compound **6** was progressively decomposed, even we used low irradiation temperature (50˚C - 60˚C) and short irradiation time (30 - 45 min.). Applying a method described in literature [14] with slight modifications, treatment of imidate **5** with 6 equivalents of aqueous solution of hydrazine at room temperature in absolute ethanol afforded compound **6** as precipitate after only 15 min. The desired compound **6** was finally collected by simple filtration. According to this simple protocol, the compounds **6(a-e)** were synthesized in yields (Table 1) ranging from 59% to 90%.



Scheme 1. Route used for the preparation of ethyl *N*-(3-cyano-4*H*-chromen-2-yl)formimidate derivatives **5(a-e)** and 4-imino-4*H*-chromeno[2,3-d]pyrimidin-3(5*H*)-amine **6(a-e)**.

Table 1. Results for the preparation of ethyl *N*-(3-cyano-4*H*-chromen-2-yl)formimidate derivatives **5(a-e)** and 4-imino-4*H*-chromeno[2,3-*d*]pyrimidin-3(5*H*)-amine **7(a-e)**.

Compound	R	React. Time (min.)	React. Temp. (˚C)	Yield (%)[a]	Overall yield (%)[b]	d of CH_2 (ppm)
5a	8-MeO	60	110	73	54	3.66
5b	7-MeO	60	140	80	59	3.59
5c	5,6-$(CH=CH)_2$	60	130	80	69	3.97
5d	6-MeO	60	140	70	37	3.65
5e	7-Et_2N	60	110	62	26	3.58
6a	8-MeO	15	25	70	38	3.63
6b	7-MeO	15	25	81	48	3.57
6c	5,6-$(CH=CH)_2$	15	25	90	62	3.93
6d	6-MeO	15	25	70	26	3.63
6e	7-Et_2N	15	25	59	15	3.31

[a]Isolated yield; [b]Overall yield calculated from compound **3**.

The structure assignment of the new compounds **6(a-e)** was evidenced by FTIR (Fourier-transform infrared spectroscopy) with NH band (ν_{NH} 3190 cm^{-1}), NH_2 band (3000 < ν < 3400 cm^{-1}); by ^1H NMR in DMSO-d_6 (see Table 1 for CH_2 signal of compounds **5** and **6**) and also ^{13}C NMR. In mass spectrometry analysis (HRMS), the $[M+H]^+$ molecular ion signals for all compounds **6(a-e)** were readily obtained as base signals.

2.2. Antitumor Evaluations of Compounds 4(a-e) and 6(a-e)

Table 2. Results for antiproliferative activity of the 2-amino-4H-chromene-3-carbonitrile **4(a-e)** and the 4-imino-4*H*-chromeno [2,3-*d*]pyrimidin-3(5*H*)-amine **7(a-e)**.

Compounds		% of survival[a] and IC_{50} (mM) of selected compounds[b]							
4 and **6**	R	Huh7 D12	Caco2	MDA-MB231	HCT 116	PC3	MDA MB468	MCF7	Fibro-blast
4a	8-MeO	100	89	104	94	95	102	106	NT
4b	7-MeO	96	84	92	82	93	98	96	NT
4c	5,6-$(CH=CH)_2$	95	88	103	81	98	94	97	NT
4d	6-MeO	103	88	100	75	92	89	96	NT
4e	7-Et_2N	87	93	90	76	97	85	89	NT
6a	8-MeO	89	87	88	85	97	96	91	NT
6b	7-MeO	100	92	79	77	95	95	76	NT
6c	5,6-$(CH=CH)_2$	8 (3)	49 (3)	17 (3)	2 (2.1)	27 (7)	11 (1.8)	31 (6)	(>25)
6d	6-MeO	89	95	97	67 (>25)	86	88	89	(>25)
6e	7-Et_2N	81	104	85	61 (>25)	83	62 (>25)	78	(>25)
DMSO		100 (>25)	100 (>25)	100 (>25)	100 (>25)	100 (>25)	100 (>25)	100 (>25)	(0.00)
Roscovitine[c]		12 (15)	21 (13)	23 (14)	8 (14)	27 (9)	8 (8)	20 (9)	(13)
Doxorubicine[c]		61 (0.06)	64 (0.03)	37 (0.02)	22 (0.03)	41 (0.07)	26 (0.07)	37 (0.09)	(0.01)
Taxol[c]		44	62	32	7	35	22	23	NT

[a]Percentage of survival measured at 25 mM (after a single dose, triplicate); [b]IC50 values in brackets are expressed in mM and are the average of three assays, standard error ±0.5 Mm; [c]Used as positive control.

Based on the antitumor activities reported in literature of bioactive compounds incorporating chrome or chromeno-pyrimidine moieties [1] [15]-[17], the 2-amino-4*H*-chromene intermediates **4(a-e)** and their 4*H*-chromeno[2,3-*d*]pyrimidin derivatives **6(a-e)** were evaluated on a panel of seven tumoral cell lines and diploid skin fibroblasts as normal cell lines for control. The roscovitin [18], doxorubicin [19] and taxol [20] compounds were used in all experiments as reference

standards because they are wide-spectrum anticancer agents. For these *in vitro* antitumor assays, the used cell lines represented commons forms of human cancers, *i.e.,* hepatocellular carcinoma (Huh7 D12), colon adenocarcinoma (Caco2), breast carcinoma (MDA-MB231), colon carcinoma (HCT116), triple negative breast cancer (MDA-MB468), breast cancer (MCF7), prostate adenocarcinoma (PC3).

The protocol for evaluation of antiproliferative activity takes place in two phases. The first phase is an evaluation of the target compound at a 25 μM single dose. When compounds exhibited a strong inhibitory activity with a percentage of survival is < 50% for the tumoral cell, the compound was used for a more detailed study in the second phase. Using different concentrations (0.1 - 25 μM), a study of full dose-response and survival curves was realized for determination of IC_{50} (concentration of the compound that kills 50% of the tumoral cell after 48 h incubation). Results for cytotoxic assays of compounds **4(a-e)** and **6(a-e)** are reported in Table 2. Analysis of the results in this table showed that most of the compounds **4** and **6** were inactive on the seven tumoral cell lines (percentage of survival is > 50% in phase 1 or IC_{50} > 25 μM in phase 2). The most interesting compound is **6c** because IC_{50} < 10 μM on the seven tumoral cell lines and does not present toxic effect on fibroblasts. Indeed, compound **6c** has interesting cytotoxic activities on Huh7 D12, Caco2 and MDA-MB231 with IC_{50} 3 μM, on HCT116 (IC_{50} 2.1 μM), on PC3 (IC_{50} 7 μM), on MDA-MB468 (IC_{50} 1.8 μM) and MCF7 (IC_{50} 6 μM). It is noteworthy that this compound **6c** does not present toxic effects on fibroblasts. In terms of cytotoxicity efficiency, we can notice that **6c** is better than roscovitine and taxol on the seven tumoral cell lines but significantly less than doxorubicin. However, the latter is toxic towards fibroblasts.

2.3. *In silico* Physicochemical, ADME and Drug-Likeness Properties of Compound 6c

For the *in silico* physicochemicals properties of the bioactive compound **6c** and also for the ADME pharmacokinetic properties, we used respectively the SwissADME [21] server platform [22] and the pkCSM web server [23]. The drug-likeness of compound **6c** based on Lipinski's rule of five (Ro5) [24] [25] was also predicted with the SwissADME web server.

In drug development, effective binding to the target is not only essential but also ensures oral bioavailability and drug-likeness properties based on Lipinski's rule of five (Ro5) which includes molecular weight (MW), the octanol-water partition coefficient (log $P_{o/w}$), the number of hydrogen bond acceptors (HBA), the number of hydrogen bond donors (HBD) and the topological polar surface area (*t*PSA). We find that these values are within the recommended range (Table 3) and it can be concluded that **6c** had drug-ability characteristics according to Lipinski's rules (violation = 0). Compound **6c** presents favorable bioavailability score (0.55) that means prediction of good suitability for oral drug applications [26].

Table 3. *In silico* physicochemical properties and drug-likeness properties of compound **6c**.

Parameters	Compound **6c**	Range
Molecular weight (MW) (g/mol)	264.28	180 < MW < 500
# Heavy atoms	20	20 < atoms > 70
# Aromatic heavy atoms	16	-
Fraction Csp^3	0.07	-
# Rotatable bonds (RB)	0	<15
# H-bond acceptors (HBA)	3	<10
# H-bond donors (HBD)	2	<5
Molar refractivity (MR)	75.93	40 < MR < 130
Topological Polar Surface Area, *t*PSA (Å^2)	76.92	*t*PSA < 140 Å^2
Lipophilicity Log $P_{o/w}$	1.93	−0.4 < Log $P_{o/w}$ < +5.6
Water solubility Log S (Ali), class	Soluble	-
Drug likeness (Lipinski Ro5), # violations	Yes, 0	-
Bioavailability Score	0.55	-
Lead-likeness, # violations	Yes, 0	-
Synthetic accessibility	3.10	from 0 to 10, 10 is very difficult

Csp^3 = Fraction of carbon atoms in the sp3 hybridization; H = Hydrogen, # = number.

Predictive ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) analyses offer valuable insights into have compound believe in the human body (Table 4 and Table 5). Compound **6c** is predicted to have a good Caco2-permeability (1.248). The compound potential for distribution within the body was evaluated such as steady-state volume of distribution (VDss); in our case, **6c** has VDss of 0.22. P-glycoprotein (P-gp) acts as a biological barrier [27], limiting the absorption of a compound from the gut by pumping xenobiotics out of cells to protect against toxic substances. Compound **6c** is a substrate of P-gp and not a P-gp I/II inhibitor. Another aspect of **6c** is their lower ability to traverse the blood-brain barrier (BBB), as evidenced by negative log BB (−0.155) but it can be distributed into the central nervous system, as evidenced by log PS value > −2 (log PS −2.152), which may have a potential neurological effect.

Table 4. ADME pharmacokinetic properties of compound **6c**.

Parameters	Compound **6c**	Range
	Absorption	
Water solubility (log mol/L)	−2.78	Class of solubility: soluble < −2
Caco-2 permeability (log Papp in 10^{-6} cm/s)	1.248	high Caco-2 permeability > 0.90
GI absorption	95.82	poorly absorption when < 30%
Skin permeability log Kp (cm/s)	−2.76	low skin permeability if log K_p < −2.5
P-gp substrate (Yes/No)	Yes	-
P-gp I inhibitor (Yes/No)	No	-
P-gp II inhibitor (Yes/No)	No	-
	Distribution	
VDss (human) (Log L/kg)	0.22	low if log VDss < −0.15, high if log VDss < 0.45
Fraction unbound (human)	0.129	-
BBB permeant	−0.155	if log BB > 0.3 readily cross the BBB, if log BB < −1 poorly
CNS permeability (log PS)	−2.152	if log PS > −2 penetrate the CNS; if log < −3 unable
	Metabolism	
CYP2D6 substrate	No	-
CYP3A4 substrate	Yes	-
CYP1A2 inhibitor	Yes	-
CYP2C19	Yes	-
CYP2C9 inhibitor	Yes	-
CYP2D6 inhibitor	No	-
CYP3A4 inhibitor	No	-
	Excretion	
Total real clearance (log ml/min/kg)	0.716	-
Renal OCT2 substrate	No	-

Table 5. Toxicity properties of compound **6c**.

Parameters	Compound **6c**	Range
Max. recommended tolerated dose MRTD (human) (log mg/kg/day)	−0.153	if MRTD < ou = 0.477 low toxicity, if MRTD > 0.477 high
hERG I inhibitor	No	-
hERG II inhibitor	No	-
Oral rat acute toxicity (LD_{50}) (mol/kg)	2.762	-
Oral rat chronic toxicity (LOAEL) (log mg/kg/day)	0.97	-
AMES toxicity	Yes	-
Hepatoxicity	No	-
Skin sensitization	No	-

After distribution, metabolism (Table 4) was conducted by assessing the interaction of compound **6c** with cytochrome P450 isoforms, considering their role as substrate or inhibitor: **6c** serve as substrate of CYP3A4, indicating possible modulation of metabolic pathways mediated by CYP3A4. Regarding excretion kinetics, compound **6c** was not predicted as substrate of oral organic cation transporter 2 (OCT2) and exhibited a total real clearance of 0.716 log ml/min/kg, indicating its efficient elimination from the body.

The toxicity assessments outlined in Table 5 provide insights into the safety profiles of compound **6c**. The value of oral rat acute toxicity (LD_{50} 2.762 mol/kg) indicate immediate toxic effects and concerning the oral rat chronic toxicity (LOAEL 0.97 log mg/kg/day), the use of **6c** suggests a potential long-term health risk associated with their usage, even at low dose of this potential cancer agent. On the contrary, **6c** is not an inhibitor of hERG I/II and has no impact on cardiac health. Moreover, the absence of hepatotoxicity and skin sensitization are positive findings but **6c** is predicted as potential mutagenic compound and may act as a carcinogen.

3. Conclusion

In conclusion, a new series of 4-imino-4*H*-chromeno[2,3-*d*]pyrimidin-3(5*H*)-amine **6(a-e)** were synthesized in 5 steps. It was possible to prepare the imidates **5(a-e)** under microwave dielectric heating. The structure of imidates **5(a-e)** and new 4-imino-4*H*-chromeno[2,3-*d*]pyrimidin-3(5*H*)-amine **6(a-e)** was characterized on the basis of their infrared (FTIR), NMR spectral data. The 2-amino-4*H*-chromene intermediates **4(a-e)** and their 4*H*-chromeno[2,3-*d*]pyrimidin derivatives **6(a-e)** were evaluated on a panel of seven tumoral cell lines representing commons forms of human cancers and compared to diploid skin fibroblasts as

normal cell lines for control. According to the results obtained, compounds **4(a-e)**, **6(a-b)** and **6(d,e)** exhibited low cytotoxic effects on tumoral cell lines compared to the three references (roscovitine, doxorubicine and taxol). Only the 4-imino-4*H*-benzo[5,6]-chromeno[2,3-*d*]pyrimidin-3(5*H*)-amine **6c** showed interesting results on the 7 tumoral cell lines ($1.8 < IC_{50} < 6$ μm). This can be attributed to the dihydronaphto group. The predicted *in* silico ADMET profile of this compound **6c** was in line with Lipinski's rule of five (Ro5). This study will be an important guide for future *in vivo* studies and this structure can be considered interesting for further modification as very active anti-tumor agent.

4. Experimental Section

4.1. Chemistry Section

4.1.1. General Information

Thin-layer chromatography (TLC): on 0.2-mm precoated plates of silica gel 60 F-254 (Merck) with appropriate eluent. Visualization: with ultraviolet light (254 and 365 nm) or with a fluorescence indicator. Infrared (IR) spectra: recorded on a Jasco FT-IR 420 spectrophotometer apparatus using potassium bromide pellets. ^{1}H NMR spectra: recorded on BRUKER AC 300 P (300 MHz) spectrometer. ^{13}C NMR spectra: recorded on BRUKER AC 300 P (75 MHz) spectrometer. Chemical shifts: expressed in parts per million downfield. Data are given in the following order: d value, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; quint: quintuplet, m, multiplet; br, broad), number of protons, coupling constants *J* is given in Hertz. The high resolution mass spectra (HRMS): recorded in positive mode using direct Electrospray infusion, respectively on a Waters Q-Tof 2 or on a Thermo Fisher Scientific Q-Exactive spectrometers at the "Centre Régional de Mesures Physiques de l'Ouest" CRMPO (CRMPO platform, ScanMAT UAR 2025 CNRS, Rennes, France). Melting points: determined on a Kofler melting point apparatus and were uncorrected. Reactions under microwave irradiation (S2Wave platform ScanMAT UAR 2025 CNRS, Rennes): realized in the Anton Paar Monowave 300® microwave reactor (Anton Paar France) using borosilicate glass vials of 10 ml equipped with snap caps (at the end of the irradiation, cooling reaction was realized by compressed air). The microwave instrument consists of a continuous focused microwave power output from 0 to 800W. All the experiments were performed using stirring option. The target temperature was reached with a ramp of 3 minutes and the chosen microwave power stay constant to hold the mixture at this temperature. The reaction temperature is monitored using calibrated infrared sensor and the reaction time included the ramp period. The microwave irradiation parameters (power and temperature) were monitored by the Monowave software package for the Anton Paar Monowave 300® reactor.

4.1.2. General Procedure Under Microwave Irradiation for the Synthesis of Ethyl N-(3-Cyano-N-Substituted-4H-Chromen-2-yl)formimidate 5(a-e)

In a 20 ml glass tube were placed successively 2-amino-4*H*-chromen-3-

carbonitrile **4** (2.5 mmol), commercial triethyl orthoformate (1.59 g, 15 mmol, 6 equiv) and glacial acetic acid (0.5% mol, 7 µl, 1.25 µmol). The glass tube was sealed with a snap cap and placed in the Microwave® 300 Anton Paar microwave cavity (P = 800 Watt). The reaction mixture under vigorous stirring (550 rpm) was irradiated at 110˚C -140˚C for 60 min. After microwave dielectric heating, the crude reaction mixture was allowed to cool down to room temperature. After standing, the insoluble compound **5** was collected by filtration on a Buchner funnel (porosity N˚ 4) and washed with Et_2O (2 × 10 ml). Then the desired product **5** was dried under high vacuum at 25˚ (10^{-2} Torr) for 1 h to give a yellowish powder and was further used without purification.

*Ethyl N-(3-cyano-8-methoxy-4H-chromen-2-yl)formimidate (**5a**):*

Yield: 73%. Mp = 153˚C - 155˚C. IR (KBr, ν cm^{-1}): 1674 (C=N), 2213 (C≡N). ^{1}H NMR (DMSO-d_6) δ 1.33 (t, 3H, J = 7.1 Hz, CH_3); 3.64 (s, 2H, CH_2); 3.81 (s, 3H, MeO); 4.33 (q, 2H, J = 7.1 Hz, =C-CH_2); 6.73 (d, 1H, J = 7.6 Hz, H-7, Ar); 6.95 (d, 1H, J = 8.2 Hz, H-5, Ar); 7.07 (t, 1H, J = 7.9 Hz, H-6, Ar); 8.41 (s, 1H, CH).^{13}C NMR (DMSO-d_6) δ 14.3 (CH_3, C-13); 25.7(CH_2, C-4); 56.3 (CH_3O, C-8'); 64.3 (CH_2, C-12);74.3 (C-3, C=); 111.5 (C-7,Ar); 118.9 (CN); 119.1 (C-10,C=); 120.3 (C-5,Ar); 125.3 (C-6,Ar); 139.0 (C-9, C=); 148.0 (C-8, C=); 158.6 (C-2,C=); 160.9 (C-11). HRMS (ES^+, MeOH/CH_2Cl_2: 9:1), *m/z:* 281. 0896 found (calculated for $C_{14}H_{14}N_2O_3Na$ $[M+Na]^+$ requires 281.08966).

*Ethyl N-(3-cyano-7-methoxy-4H-chromen-2-yl)formimidate (**5b**):*

Yield: 80%. Mp = 133˚C - 135˚C. IR (KBr, ν cm^{-1}): 1689 (C=N), 2209 (C≡N). ^{1}H NMR (DMSO-d_6) δ 1.32 (t, 3H, J = 7.1 Hz, CH_3); 3.59 (s, 2H, CH_2); 3.74 (s, 3H, MeO); 4.32 (q, 2H, J = 7.1 Hz, =C-CH_2); 6.68 (d, 1H, J = 2.5 Hz, H-8, Ar); 6.74 (dd, 1H, J = 8.4 Hz, J = 2.6 Hz, H-6, Ar); 7.11 (d, 1H, J = 8.4 Hz, H-5, Ar); 8.56 (s, 1H, CH).^{13}C NMR (DMSO-d_6) δ 14.3 (CH_3, C-13); 25.0 (CH_2, C-4); 55.9 (C-3, C=); 64.2 (CH_2, C-12); 75.1 (CH_3O, C-7'); 102.1 (C-5, Ar); 109.9 (C-6, Ar); 112.0 (C-10, Ar); 119.0 (CN); 129.9 (C-8, Ar); 150.3 (C-9, Ar); 158.4 (C-7, Ar); 159.5 (C-11); 161.4 (C-2, Ar). HRMS (ES^+, MeOH/CH_2Cl_2 9:1), *m/z*: 281.0898 found (calculated for $C_{14}H_{14}N_2O_3Na$ $[M+Na]^+$ requires 281.09021).

*Ethyl N-(2-cyano-1H-benzo[f]chromen-3-yl)formimidate (**5c**):*

Yield: 80%. Mp = 133˚C - 135 ˚C. IR (KBr, ν cm^{-1}): 1674 (C=N), 2212 (C≡N). ^{1}H NMR (DMSO-d_6) δ 1.35 (t, 3H, J = 7.1 Hz, CH_3); 3.97 (s, 2H, CH_2); 4.35 (q, 2H, J = 7.0 Hz, =C-CH_2); 7.29 (d, 1H, J = 8.9 Hz, H-8, Ar); 7.53 (t, 1H, J = 7.4 Hz, H-5", Ar); 7.63 (t, 1H, J = 7.6 Hz, H-6", Ar); 7.84 (d, 1H, J = 8.4 Hz, H-7, Ar); 7.90 (d, 1H, J = 9Hz, H-6', Ar); 7.94 (d, 1H, J = 7.7 Hz, H-5', Ar); 8.63 (s, 1H, CH). ^{13}C NMR (DMSO-d_6) δ 14.3 (CH_3, C-13); 23.8 (CH_2, C-4); 64.3 (C-3, C=); 75.0 (CH_2, C-12); 111.0 (C-8, Ar); 117.5 (C-10, Ar); 119.1 (CN); 123.5 (C-5", Ar); 125.8 (C-6", Ar); 127.8 (C-7, Ar); 128.7 (C-6', Ar); 129.4 (C-5', Ar); 131.0 (C-5, Ar); 131.1 (C-6, Ar); 146.8 (C-9, Ar); 158.2 (C-2, Ar); 161.6 (C-11). HRMS (ES^+, MeOH/CH_2Cl_2 9:1), *m/z*: 301.0949 found (calculated for $C_{17}H_{14}N_2O_2Na$ $[M+Na]^+$ requires 301.0953).

*Ethyl N-(3-cyano-6-methoxy-4H-chromen-2-yl)formimidate (**5d**):*

Yield: 70%. Mp = 197˚C - 199˚C; IR (KBr, ν cm^{-1}): 1648 (C=N), 2207 (C≡N). ^{1}H NMR (DMSO-d_6) δ 1.32 (t, 3H, J = 7.1 Hz, CH_3); 3.65 (s, 2H, CH_2); 3.75 (s, 3H, MeO); 4.32 (q, 2H, J = 7.0 Hz, =C-CH_2); 6.77 (s, 1H, H-5, Ar); 6.82 (d, 1H, J = 8.9 Hz, H-7, Ar); 7.02 (d, 1H, J = 8.9 Hz, H-8, Ar); 8.54 (s, 1H, CH). ^{13}C NMR (DMSO-d_6) δ 14.3 (CH_3, C-13); 26.0 (CH_2, C-4); 56.0 (C-3, C=); 64.2 (CH_2, C-12); 73.8 (CH_3O, C-6'); 113.3 (C-5, Ar); 114.5 (C-10, Ar); 117.9 (C-7, Ar); 119.1 (CN); 119.2 (C-8, Ar); 143.6 (C-9, Ar); 156.6 (C-6, Ar); 158.8 (C-2, Ar); 161.4 (C-11). HRMS (ES$^+$, MeOH/CH_2Cl_2 9:1), *m/z*: 281.0901 found (calculated for $C_{14}H_{14}N_2O_3Na$ $[M+Na]^+$ requires 281.0902).

*Ethyl N-(3-cyano-7-(diethylamino)-4H-chromen-2-yl)formimidate (**5e**):*

Yield: 62%. Mp = 110˚C - 112˚C. IR (KBr, ν cm^{-1}): 1650 (C=N), 2213 (C≡N). ^{1}H NMR ($CDCl_3$) δ 1.18 (t, 6H, J = 7.0 Hz, 2-CH_3); 1.41 (t, 3H, J = 7.1 Hz, CH_3); 3.34 (q, 4H, J = 7.0 Hz, 2-CH_2-N); 3.58 (s, 2H, CH_2); 4.44 (q, 2H, J = 7.1 Hz, CH_2CH_3); 6.24 (d, 1H, J = 2.1 Hz, H-8, Ar); 6.46 (dd, 1H, J = 8.5 Hz, J = 2.2 Hz, H-6, Ar); 6.93 (d, 1H, J = 8.5 Hz, H-5, Ar); 8.35 (s, 1H, CH).^{13}C NMR (DMSO-d_6) δ 12.5 (CH_3, C-7c, C-7d); 13.9 (CH_3, C-13); 44.4 (C-7a, C-7b, CH_2); 63.8 (CH_2CH_3, C-12); 75.6 (C-3); 99.1 (C-8, Ar); 103.4 (C-10, Ar); 109.0 (C-6, Ar); 119.2 (CN); 129.0 (C-5, Ar); 148.0 (C-9, Ar); 150.6 (C-7, Ar); 158.3 (C-11); 158.6 (C-2, Ar). HRMS (ES$^+$, MeOH/CH_2Cl_2 9:1), *m/z*: 355.1169 found (calculated for $C_{19}H_{16}N_4O_2Na$ $[M+Na]^+$ requires calculated for 355.11710).

4.1.3. General Procedure for the Preparation of 4-Imino-4H-Chromeno[2,3-d]pyrimidin-3(5H)-Amine 6(a-e)

To a vigorous stirred solution (550 rpm) of ethyl *N*-(3-cyano-4*H*-chromen-2-yl) formimidate **5** (5 mmol) in absolute ethanol (20 ml) was added portion wise hydrazine monohydrate (1.5 g, 30 mmol, 6 equiv.). After 3 min., a precipitate appeared and mixing was pursued at 25˚C until complete precipitation (15 min.). The insoluble material was collected by filtration in a Büchner funnel (porosity N˚4) and the solid product was washed with cooled ethanol (2 × 10 ml) to give the desired product **6**. Drying under high vacuum at 25˚ (10^{-2} Torr) for 1h produced **6** as a yellowish powder which was recrystallized from ethanol.

*4-Imino-9-methoxy-4H-chromeno[2,3-d]pyrimidin-3(5H)-amine (**6a**):*

Yield: 70%, Mp > 250˚C. IR (KBr, ν cm^{-1}): 1648 (C=N), 3212 (NH), 3296/3331 (NH_2). ^{1}H NMR (DMSO-d_6) δ 3.47 (s, 1H, NH); 3.63 (s, 2H, CH_2); 3.81 (s, 3H, MeO); 5.70 (s, 2H, NH_2); 6.79 (d, 1H, J = 7.4 Hz, H-8, Ar); 6.92 (d, 1H, J = 7.8 Hz, H-6, Ar); 7.03 (t, 1H, J = 7.9 Hz, H-7, Ar); 8.01 (s, 1H, CH). ^{13}C NMR (DMSO-d_6) δ 23.7 (CH_2, C-5); 56.2 (CH_3, C-10); 94.9 (C-4a, C=); 111.2 (C-8, Ar); 120.2 (C-5a, Ar); 120.9 (C-6, Ar); 120.5 (C-7, Ar); 139.7 (C-9a, Ar); 148.1 (C-4, Ar); 150.4 (C-2, Ar); 158.9 (C-9, Ar). HRMS (ES$^+$, MeOH/CH_2Cl_2 9:1), *m/z*: 245.1038 found (calculated for $C_{12}H_{13}N_4O_2$ $[M+H]^+$ requires 245.1039).

*4-Imino-8-methoxy-4H-chromeno[2,3-d]pyrimidin-3(5H)-amine (**6b**):*

Yield: 81%. Mp ≥ 260˚C. IR (KBr, ν cm^{-1}): 1654 (C=N), 3208/3212 (NH/NH_2). ^{1}H NMR (DMSO-d_6) δ 3.57 (s, 2H, CH_2); 3.74 (s, 3H, MeO); 5.70 (s, 2H, NH_2); 6.64 (s, 1H, H-9, Ar); 6.70 (d, 1H, J = 6.4 Hz, H-7, Ar); 7.15 (d, 1H, J = 8.4 Hz, H-

6, Ar); 8.02 (s, 1H, CH). HRMS (ES^+, MeOH/CH_2Cl_2 9:1), m/z: 245.1034 found (calculated for $C_{12}H_{13}N_4O_2$ $[M+H]^+$ requires 245.1039).

*4-Imino-4H-benzo[5,6]-chromeno[2,3-d]pyrimidin-3(5H)-amine (**6c**):*

Yield: 90%. Mp ≥ 260˚C. IR (KBr, ν cm^{-1}): 1650 (C=N), 3179 (NH), 3281/3301 (NH_2). ^1H NMR (DMSO-d_6) δ 3.93 (s, 2H, CH_2); 5.78 (s, 2H, NH_2); 7.30 (d, 1H, J = 8.9 Hz, H-9); 7.53 (t, 1H, J = 7.1 Hz, H-6", Ar); 7.66 (t, 1H, J = 8.1Hz, H-7", Ar); 7.88 (d, 1H, J = 8.9 Hz, H-8, Ar); 7.95 (d, 1H, J = 8.0 Hz, H-7', Ar); 8.01 (d, 1H, J = 8.4Hz, H-6', Ar); 8.10 (s, 1H, CH). ^{13}C NMR (DMSO-d_6) δ 21.8 (CH_2, C-5); 95.0 (C-4a, C=); 111.9 (C-9, Ar); 117.7 (C-5a, C=); 123.7 (C-8, Ar); 125.4 (C-6', Ar); 127.6 (C-7', Ar); 128.7 (C-6", Ar); 129.1 (C-7", Ar); 130.7 (C-6, C=); 131.8 (C-7, C=); 147.4 (C-2, Ar); 150.4 (C-4, C=); 155.6 (C-9a, C=). HRMS (ES^+, MeOH/CH_2Cl_2 9:1), *m/z*: 265.1085 found (calculated for $C_{15}H_{13}N_4O$ $[M+H]^+$ requires 265.10894).

*4-Imino-7-methoxy-4H-chromeno[2,3-d]pyrimidin-3(5H)-amine (**6d**):*

Yield: 70%. Mp ≥ 260˚C. IR (KBr, ν cm^{-1}): 1650(C=N), 3179(NH), 3281/3301 (NH_2). ^1H NMR (DMSO-d_6) δ 3.63 (s, 2H, CH_2); 3.73 (s, 3H, MeO); 5.69 (s, 2H, NH_2); 6.81-6.78 (m, 2H, H-8, H-6, Ar); 7.00 (d, 1H, J = 9.6 Hz, H-9, Ar); 8.01 (s, 1H, CH). ^{13}C NMR (DMSO-d_6) δ 24.0 (CH_2, C-5); 55.8 (CH_3, C-7a); 94.3 (C-4a, C=); 113.9 (C-8, Ar); 114.1 (C-6, Ar); 117.7 (C-9, Ar); 120.3 (C-5a, C=); 144.1 (C-9a, C=); 150.5 (C-4, C=); 156.1 (C-2, Ar). HRMS (ES^+, MeOH/CH_2Cl_2 9:1), *m/z:* 245.1031 found (calculated for $C_{12}H_{13}N_4O_2$ $[M+H]^+$ requires 245.10385).

*N-diethylamino-4-imino-4H-chromeno[2,3-d]pyrimidin-3(5H)-amine (**6e**):*

Yield: 59%. Mp 212˚C; IR (KBr, ν cm^{-1}): 1645 (C=N), 3250 (NH), 3279/3320 (NH_2). ^1H NMR (DMSO-d_6) δ 1.07 (t, 6H, 2-CH_3); 3.33 (q, 6H, 3-CH_2); 3.49 (s, 1H, NH); 5.69 (s, 2H,NH_2); 6.26 (s, 1H, H-9, Ar); 6.45 (d, 1H, ^3J = 6 Hz, H-7, Ar); 7.01 (d, 1H, J = 6 Hz, H-6, Ar); 8.01 (s, 1H, CH).^{13}C NMR (DMSO-d_6) δ 12.8 (CH_3, C-8"', C-8"); 22.7 (CH_2, C-5); 44.2 (CH_2, C-8', C-8"); 95.4 (C-4a, C=); 99.2 (C-9, Ar); 105.1 (C-5a, C=); 108.9 (C-7, Ar); 130.1 (C-6, Ar); 147.9 (C-2, Ar); 150.1 (C-4, C=); 151.1 (C-9a, C=); 156.2 (C-8, C=). HRMS (ES^+, MeOH/CH_2Cl_2 9:1), *m/z:* 268.1658 found (calculated for $C_{15}H_{20}N_5O$ $[M+H]^+$ requires 286.16679).

4.2. Biology Section for Antiproliferative Assays

4.2.1. Cell Culture

Skin diploid fibroblastic cells were provided by BIOPREDIC International Company (Rennes, France). Huh-7D12 (Ref ECACC: 01042712), Caco2 (Ref ECACC: 86010202), MDA-MB-231 (Ref ECACC: 92020424), MDA-MB-468, HCT-116 (Ref ECACC: 91091005), PC3 (Ref ECACC: 90112714), and MCF7 cell lines were obtained from the ECACC collection. Cells were grown according to ECACC recommendations [28] in DMEM for Huh-D12, MDA-MB-231, MDA-MB-468 and fibroblast, in EMEM for Caco2, in McCoy's for HCT-116 and RPMI for PC3 at 37˚C and 5% CO_2. All culture media with 10% of FBS, 1% of penicillin-streptomycin and 2 mM glutamine.

4.2.2. Protocol for Antiproliferative Assays

Chemicals were solubilized in DMSO at a concentration of 10 mM (stock

solution) and diluted in culture medium to the desired final concentrations. The dose effect cytotoxic assays (IC_{50} determination) were performed by increasing concentrations of each chemical (final concentrations: 0.1 µM, 0.3 µM, 0.9 µM, 3 µM, 9 µM and 25 µM). The toxicity test of the chemicals on these cells was as follows: 2×10^3 cells for HCT-116 cells or 4×10^3 for the other cells were seeded in 96-multi-well plates in triplicate and left for 24 h for attachment, spreading and growing. Then, cells were exposed for 48 h to increasing concentrations of chemicals, ranging from 0.1 µM to 25 µM, in a final volume of 120 µL of culture medium. After 48 h of treatment, cells were washed in PBS and fixed in cooled 90% ethanol/5% acetic acid solution for 20 min. The nuclei were stained with Hoechst 3342 (B2261 Merck Sigma-Aldrich) and counted. Image acquisition and analysis were performed using automated imaging analysis with a Cellomics Arrayscan VTI/HCS Reader (Thermo/Scientific, Waltham, MA, USA). The survival percentages were calculated as the percentage of cell number after chemical treatment over cell number after DMSO treatment. The IC_{50} were graphically determined.

4.3. *In Silico* Pharmacokinetics

The SMILES of compound **6c** was used for *in silico* ADME (absorption, distribution, metabolism, and excretion) screening on SwissADME server [22], which was performed at default parameters. Also, the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) study of **6c** were calculated using the pkCSM server (http://biosig.unimelb.edu.au/pkcsm/, [23]).

Author Contributions

Organic synthesis, S.K., M.D. and M.F.; supervision for organic synthesis, H.A., S.A., L.P. and J-P.B.; hygiene and security training for organic synthesis, technical formation for microwave irradiation synthesis, E.L.; tumoral cell lines assays, R.L.G.; tumoral cell lines assays, R.L.G.; T.C., supervision of tumoral cell lines assays; *in silico* ADME prediction on SwissADME and pkCSM-pharmacokinetics servers, S.K. and J-P.B.; supervision of project, H.A. and J-P.B.; writing-article and editing, J-P.B., funding acquisition of Canceropôle Grand-Ouest contract, J-P.B.; funding acquisition for junior and senior mobility of Campus France, H.A. and J-P.B. All authors have read and agreed to the publication version of the manuscript.

Funding

This research work was funded by the "Ministère de l'Enseignement Supérieur et de la Recherche de la République Tunisienne" (PhD fellowship for S.K. and M.D.). The biological assays for this research program were supported by the "Canceropôle Grand-Ouest" in "Molecules Marines, Metabolisme et Cancer 3MC" network. This project has received funding for mobility of Tunisian junior and senior researchers (S.K., H.A.) and French senior researchers (L.P., E.L. and J-P.B.) by Campus France *via* PHC Utique 2021-23 contract (Campus France code 46156NL/CMCU code 21G1202) and also funding for mobility of M.D. by

University of Sfax.

Acknowledgments

The authors are grateful to the assistance of the staff (N. Le Yondre, P. Jéhan, F. Lambert) of CMRPO analytical chemistry core facility for HRMS analysis (CRMPO platform UAR 2025 CNRS, Université de Rennes 1, Bât. 11A, Campus de Beaulieu, Rennes, France).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Okasha, R., Alblewi, F., Afifi, T., Naqvi, A., Fouda, A., Al-Dies, A., *et al.* (2017) Design of New Benzo[*h*]chromene Derivatives: Antitumor Activities and Structure-Activity Relationships of the 2,3-Positions and Fused Rings at the 2,3-Positions. *Molecules*, **22**, Article 479. https://doi.org/10.3390/molecules22030479

[2] Kalla, R.M.N., Choi, J., Yoo, J., Byeon, S.J., Heo, M.S. and Kim, I. (2014) Synthesis of 2-Amino-3-Cyano-4h-Chromen-4-Ylphosphonates and Their Anticancer Properties. *European Journal of Medicinal Chemistry*, **76**, 61-66. https://doi.org/10.1016/j.ejmech.2014.02.025

[3] Baral, N., Mohapatra, S., Raiguru, B.P., Mishra, N.P., Panda, P., Nayak, S., *et al.* (2018) Microwave-Assisted Rapid and Efficient Synthesis of New Series of Chromene-Based 1,2,4-Oxadiazole Derivatives and Evaluation of Antibacterial Activity with Molecular Docking Investigation. *Journal of Heterocyclic Chemistry*, **56**, 552-565. https://doi.org/10.1002/jhet.3430

[4] Nawaz, M., Abbasi, M.W. and Hisaindee, S. (2016) Synthesis, Characterization, Anti-Bacterial, Anti-Fungal and Nematicidal Activities of 2-Amino-3-Cyanochromenes. *Journal of Photochemistry and Photobiology B: Biology*, **164**, 160-163. https://doi.org/10.1016/j.jphotobiol.2016.09.032

[5] Boukattaya, F., Daoud, A., Boeda, F., Pearson-Long, M.S.M., Gharsallah, N., Kadri, A., *et al.* (2019) Synthesis and Biological Evaluation of 3-Cyano-4h-Chromene Derivatives Bearing Carbamate Functionality. *Medicinal Chemistry*, **15**, 257-264. https://doi.org/10.2174/1573406414666181009124449

[6] Thomas, N. and Zachariah, S.M. (2013) In Silico Drug Design and Analysis of 4-Phenyl-4*H*-Chromene Derivatives as Anticancer and Anti-Inflammatory Agents. *International Journal of Pharmaceutical Sciences Review and Research*, **22**, 50-54.

[7] Banaee, M., Shahafve, S., Vaziriyan, M., Taheri, S. and Haghi, N. (2016) Effects of Sewage Effluent on Blood Biochemical Parameters of Common Carp (*Cyprinus carpio*): A Case Study of Behbahan, Khuzestan Province. *Journal of Chemical Health Risks*, **6**, 161-173.

[8] Menut, C., Bessiere, J.M., Ntalani, H., Verin, P., Henriques, A.T. and Limberger, R. (2000) Two Chromene Derivatives from Calyptranthes Tricona. *Phytochemistry*, **53**, 975-979. https://doi.org/10.1016/s0031-9422(99)00601-9

[9] Joulain, D. and Tabacchi, R. (1994) Two Volatile B-Chromenes Fromwisteria Sinensis Flowers. *Phytochemistry*, **37**, 1769-1770. https://doi.org/10.1016/s0031-9422(00)89610-7

[10] Panda, D., Singh, J.P. and Wilson, L. (1997) Suppression of Microtubule Dynamics

by Ly290181. *Journal of Biological Chemistry*, **272**, 7681-7687. https://doi.org/10.1074/jbc.272.12.7681

[11] Selvam, T.P., James, C.R., Dniandev, P.V. and Valzita, S.K. (2012) A Mini Review of Pyrimidine and Fused Pyrimidine Marketed Drugs. *Pharmaceutical Research*, **2**, 1-9.

[12] Grée, D., Vorin, S., Manthati, V.L., Caijo, F., Viault, G., Manero, F., *et al.* (2008) The Synthesis of New, Selected Analogues of the Pro-Apoptotic and Anticancer Molecule HA 14-1. *Tetrahedron Letters*, **49**, 3276-3278. https://doi.org/10.1016/j.tetlet.2008.03.070

[13] Dhiabi, M., Bouattour, A., Fakhfakh, M., Abid, S., Paquin, L., Robert, T., *et al.* (2023) Practical Approach to N-Benzyl Derivatives of 2-Amino-8-Methoxy-4h-Chromene-3-Carbonitrile by Reductive Amination: Exploration of Their Effects against Protein Kinases and in Silico ADME Profiling. *Journal of Molecular Structure*, **1274**, Article 134319. https://doi.org/10.1016/j.molstruc.2022.134319

[14] Mohammed, F.K. and Badrey, M.G. (2011) Synthesis of Pyrimidines and Heteroannulated Pyrimidine Ring Systems. *Journal of the Korean Chemical Society*, **55**, 218-229. https://doi.org/10.5012/jkcs.2011.55.2.218

[15] Alblewi, F.F., Okasha, R.M., Hritani, Z.M., Mohamed, H.M., El-Nassag, M.A.A., Halawa, A.H., *et al.* (2019) Antiproliferative Effect, Cell Cycle Arrest and Apoptosis Generation of Novel Synthesized Anticancer Heterocyclic Derivatives Based 4h-benzo[h]chromene. *Bioorganic Chemistry*, **87**, 560-571. https://doi.org/10.1016/j.bioorg.2019.03.059

[16] Kandeel, M.M., Kamal, A.M., Abdelall, E.K.A. and Elshemy, H.A.H. (2013) Synthesis of Novel Chromene Derivatives of Expected Antitumor Activity. *European Journal of Medicinal Chemistry*, **59**, 183-193. https://doi.org/10.1016/j.ejmech.2012.11.011

[17] Badrey, M.G. and Gomha, S.M. (2012) 3-Amino-8-Hydroxy-4-Imino-6-Methyl-5-Phenyl-4,5-Dihydro-3h-Chromeno [2,3-D]pyrimidine: An Effecient Key Precursor for Novel Synthesis of Some Interesting Triazines and Triazepines as Potential Anti-Tumor Agents. *Molecules*, **17**, 11538-11553. https://doi.org/10.3390/molecules171011538

[18] Cicenas, J., Kalyan, K., Sorokinas, A., Stankunas, E., Levy, J., Meskinyte, I., Stankevicius, V., Kaupinis, A. and Valius, M. (2015) Roscovitine in Cancer and Other Diseases. *Annals of Translational Medicine*, **3**, 135.

[19] Rivankar, S. (2014) An Overview of Doxorubicin Formulations in Cancer Therapy. *Journal of Cancer Research and Therapeutics*, **10**, 853-858. https://doi.org/10.4103/0973-1482.139267

[20] Wani, M.C., Taylor, H.L., Wall, M.E., Coggon, P. and McPhail, A.T. (1971) Plant Antitumor Agents. VI. Isolation and Structure of Taxol, a Novel Antileukemic and Antitumor Agent from Taxus Brevifolia. *Journal of the American Chemical Society*, **93**, 2325-2327. https://doi.org/10.1021/ja00738a045

[21] Daina, A., Michielin, O. and Zoete, V. (2017) Swissadme: A Free Web Tool to Evaluate Pharmacokinetics, Drug-Likeness and Medicinal Chemistry Friendliness of Small Molecules. *Scientific Reports*, **7**, Article 42717. https://doi.org/10.1038/srep42717

[22] Institute of Bioinformatics (2024) Swiss ADME of Swiss. http://www.swissadme.ch/

[23] ADME Profiles (Absorption, Distribution, Metabolism, and Excretion) Was Run on the pkCSM Web Server. https://biosig.lab.uq.edu.au/pkcsm/

[24] Lipinski, C.A., Lombardo, F., Dominy, B.W. and Feeney, P.J. (2001) Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug

Discovery and Development Settings. *Advanced Drug Delivery Reviews*, **46**, 3-26. https://doi.org/10.1016/s0169-409x(00)00129-0

[25] Lipinski, C.A. (2004) Lead- and Drug-Like Compounds: The Rule-of-Five Revolution. *Drug Discovery Today*: *Technologies*, **1**, 337-341. https://doi.org/10.1016/j.ddtec.2004.11.007

[26] Martin, Y.C. (2005) A Bioavailability Score. *Journal of Medicinal Chemistry*, **48**, 3164-3170. https://doi.org/10.1021/jm0492002

[27] Artursson, P., Neuhoff, S., Matsson, P. and Tavelin, S. (2007) Passive Permeability and Active Transport Models for the Prediction of Oral Absorption. *Comprehensive Medicinal Chemistry II*, **5**, 259-278. https://doi.org/10.1016/b0-08-045044-x/00126-7

[28] Nakabayashi, H., Taketa, K., Miyano, K., Yamane, T. and Sato, J. (1982) Growth of Human Hepatoma Cells Lines with Differentiated Functions in Chemically Defined Medium. *Cancer Research*, **42**, 3858-3863.