Bioorganic & Medicinal Chemistry Letters xxx (xxxx) xxxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Ultrasounds-mediated 10-seconds synthesis of chalcones as potential farnesyltransferase inhibitors

Germain Homerin[a], Adrian Sorin Nica[a,b], Amaury Farce[c], Joëlle Dubois[d], Alina Ghinet[a,b,e,*]

[a] Yncréa Hauts-de-France, Laboratory of Sustainable Chemistry and Health, Health & Environment Department, Team Sustainable Chemistry, Ecole des Hautes Etudes d'Ingénieur (HEI), UCLille, 13 rue de Toul, F-59046 Lille, France
[b] Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1167 – RID-AGE – Facteurs de risque et déterminants moléculaires des maladies liées au vieillissement, F-59000 Lille, France
[c] Faculté des Sciences Pharmaceutiques et Biologiques de Lille, F-59006 Lille Cedex, France
[d] Institut de Chimie des Substances Naturelles, UPR2301 CNRS, Centre de Recherche de Gif, Avenue de la Terrasse, F-91198 Gif-sur-Yvette Cedex, France
[e] 'Al. I. Cuza' University of Iasi, Faculty of Chemistry, Bd. Carol I, nr. 11, 700506 Iasi, Romania

ARTICLE INFO

Keywords:
Farnesyltransferase
Inhibitor
Chalcone
Claisen-Schmidt
Ultrasounds

ABSTRACT

A broad range of chalcones and derivatives were easily and rapidly synthesized, following Claisen-Schmidt condensation of (hetero)aryl ketones and (hetero)aryl aldehydes using a ultrasound probe. A comparison was made with classical magnetic stirring experiments, and an optimization study was realized, showing lithium hydroxide to be the best basic catalyst of the studied condensations. By-products of the reactions (β-hydroxy-ketone, diketones, and cyclohexanols) were also isolated. All compounds were evaluated *in vitro* for their ability to inhibit human farnesyltransferase, a protein implicated in cancer and rare diseases and on the NCI-60 cancer cell lines panel. Molecules showed inhibitory activity on the target protein and cytostatic effect on different cell lines with particular activity against MCF7, breast cancer cells.

Chalcones are particularly easily synthesized and of great interest in medicinal chemistry. With a broad spectrum of pharmacological activities, chalcones were found to be mainly active against inflammation,[1,2] tuberculosis,[3] HIV,[4] malaria[5] and cancer.[6–9] A literature review dedicated to chalcones over the past ten years reveals almost 13,000 references with more than 1,500 references that describe the antitumor activity of reported chalcones. This biological activity seems the most investigated for such derivatives. The reported mechanisms of antitumor action are numerous, chalcones acting as inhibitors of: tubulin polymerization,[10–15] HDAC,[16] EGFR-tyrosine kinase (EGFR TK) phosphorylation,[17,18] thioredoxin reductases (TrxRs),[19] PI3K/Akt/mTOR pathway,[20] farnesyltransferase,[9] etc. The most documented is the antimitotic activity of chalcones involving the inhibition of tubulin polymerization. On the contrary, the activity of chalcones on human farnesyltransferase was very little explored and one study has been described by our group.[9] The best farnesyltransferase inhibitor identified in the study was compound **A** (Fig. 1).[9] Farnesyltransferase (FTase), heterodimeric metalloenzyme, is a prenyltransferase involved in the thiolinkage of a farnesyl (C_{15}) isoprenoid coming from the biosynthetic pathway of cholesterol, a critical metabolite for the prenylation of proteins. This process is a critical mediator in several diseases. Indeed, preventing the farnesylation process can constitute an approach in the treatment of cancers, acquired immunodeficiency syndrome (AIDS) or progeria.[21] Progeria or Hutchinson-Gilford syndrome is an extremely rare disease and has a prevalence of one case in five million births. The appearance of the newborn baby is normal at birth; an accelerated aging is noted a year after. Currently, only farnesyltransferase inhibitors were efficient on progeria models by blocking the progerin prenylation. Five phases I/II and II clinical trials using Lonafarnib (molecule **B**, Fig. 1), known farnesyltransferase inhibitor previously developed for oncology with moderate results, alone or in combination with zoledronic acid, pravastatin or with everolimus are currently ongoing in the hope of finding a treatment.[22] First results showed additional bone mineral density benefit for patients but likely no added cardiovascular benefit with the addition of pravastatin and zoledronic acid.[23] The discovery of new efficient farnesyltransferase inhibitors remains a significant challenge in the fight against this extremely rare disease but also other diseases.[21]

In this study, the investigation of chalcones bearing different heterocyclic units (thiophene, pyridine and ferrocene) than phenothiazine

* Corresponding author at: Yncréa Hauts-de-France, Laboratory of Sustainable Chemistry and Health, Health & Environment Department, Team Sustainable Chemistry, Ecole des Hautes Etudes d'Ingénieur (HEI), UCLille, 13 rue de Toul, F-59046 Lille, France.
E-mail address: alina.ghinet@yncrea.fr (A. Ghinet).

https://doi.org/10.1016/j.bmcl.2020.127149
Received 21 February 2020; Received in revised form 23 March 2020; Accepted 27 March 2020

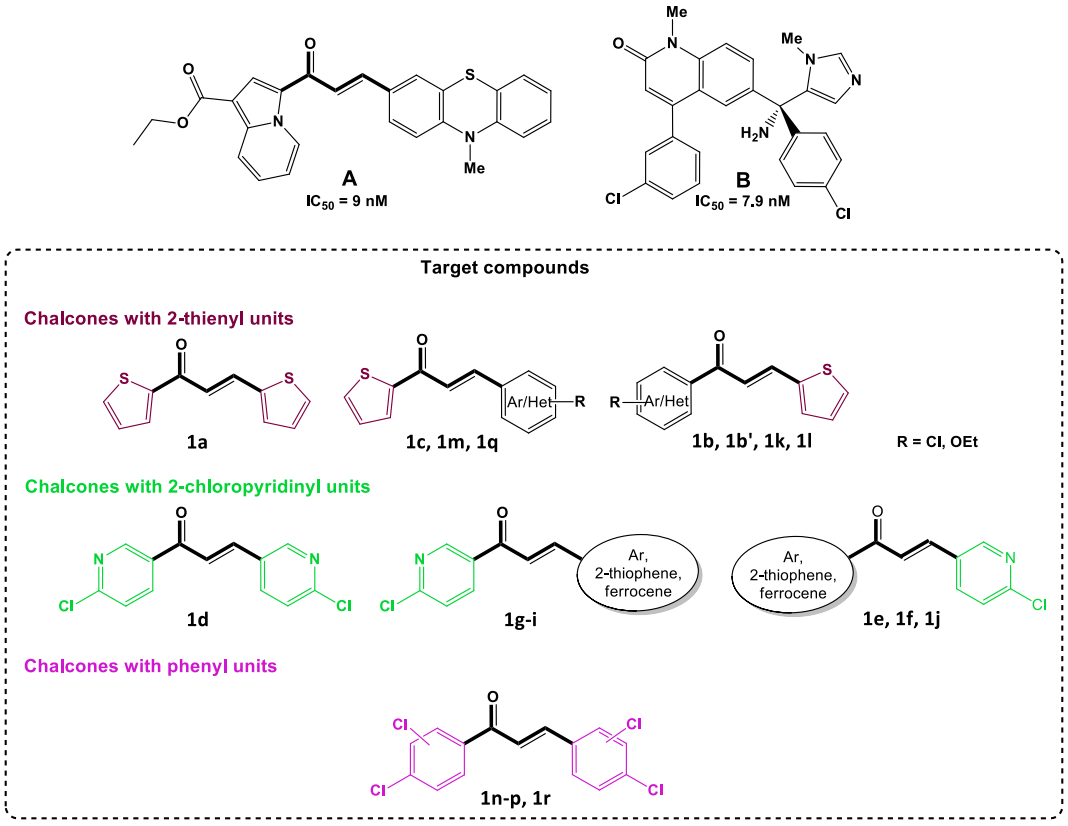


Fig. 1. Structure of reported human FTase inhibitors (indolizine-chalcone (A) and Lonafarnib (B)) and of target chalcones **1.**

investigated previously (e.g. compound **A**) as potential human farnesyltransferase inhibitors is reported (Fig. 1). Moreover, it has been described that chalcones were powerful chelators of zinc cation (Zn^{2+}).[24] This property is important in the inactivation of farnesyltransferase because the enzyme needs Zn^{2+} to perform its functions. Chalcone derivatives could inhibit Zn^{2+} metalloenzymes such as human FTase.

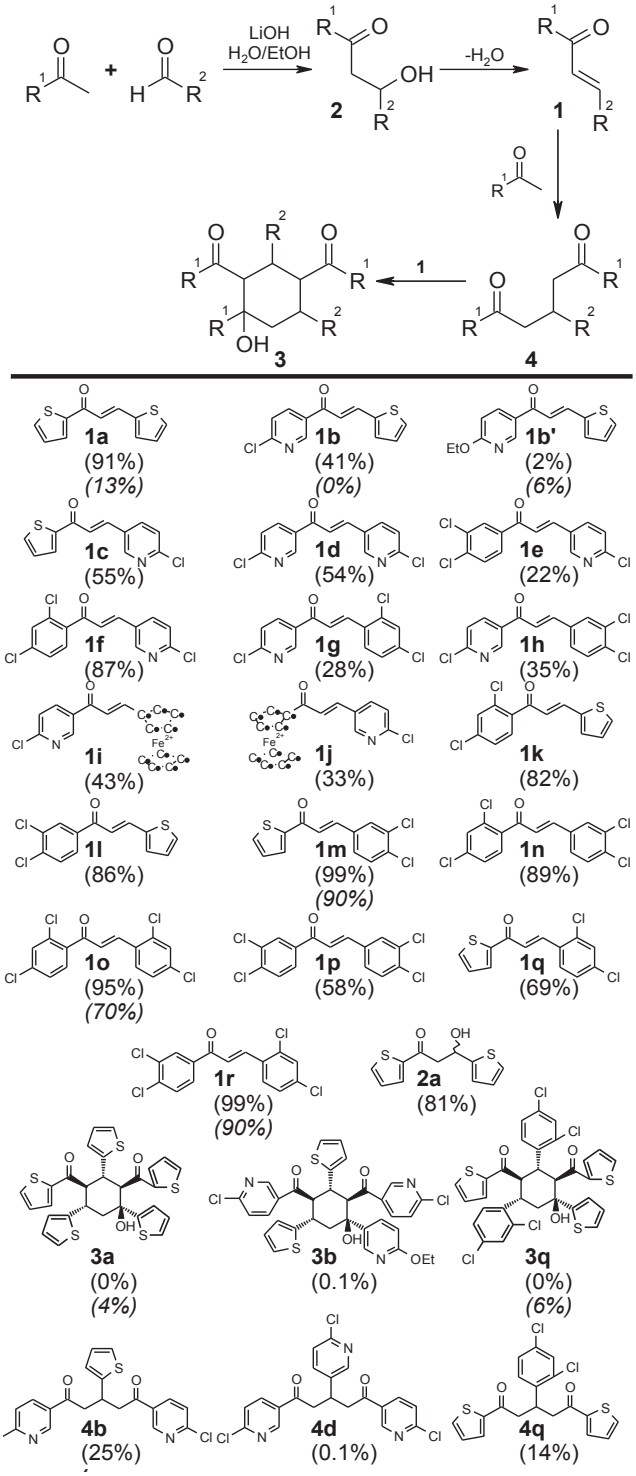
Chalcones of this study were prepared by Claisen-Schmidt condensation of corresponding (hetero)aryl ketones and (hetero)aryl aldehydes (Scheme 1).[25] According to the literature, bis(thienyl) chalcone **1a** can be obtained from 1-thiophen-2-yl-ethanone and thiophene-2-carbaldehyde using strong bases such as alkali or alkaline earth hydroxides.[2] However, this method suffers from drawbacks: it is very sensitive to reaction conditions such as dilution of the medium or base quantity. As an example, β-hydroxy-ketone **2a** was obtained when water was used as the solvent,[26,27] and cyclohexanol **3a** was isolated in presence of a large amount of base in low diluted medium.[28,29] In our hands, using a classical magnetic stirring and sodium hydroxide in *tert*-butanol, only 13% yield was achieved in the best case for the synthesis of chalcone **1a**, which was hard to separate from by-products such as cyclohexanol **3a** (see supplementary material section). Moreover, using 1-(6-chloro-3-pyridinyl)-1-ethanone and thiophene-2-carbaldehyde in ethanol and water, the substitution of the chlorine by an ethoxy group led to **1b′** and **3b′** in very low yield, and as the only isolated compounds of a complex mixture.

In this work, the sonication of the mixture was tested instead of classical magnetic steering. Globally, the results were comparable except in the case of chalcones **1a** and **1b**, but the reaction time was decreased from hours to seconds. Different bases were next selected and tested for the Claisen-Schmidt condensation of 1-thiophen-2-yl-ethanone and thiophene-2-carbaldehyde (Table 1). The blank gave no reaction. In the case of cesium carbonate, the reaction was not complete even after 60 s of sonication, and the use of cesium fluoride led to a complex mixture. Alkali hydroxides (NaOH and LiOH) were more suitable than other tested bases and led to the desired chalcone **1a** in good yield after 10 s of sonication. However, for the synthesis of **1b**, the use of NaOH provided only ethoxy-pyridine **1b′** in poor yield. The same reaction performed using LiOH afforded the desired chalcone **1b**. Consequently, the following experiments were realized using LiOH as the base.

A screening of different solvents was next performed (Table 2). Mixtures of water and ethanol provided only the desired chalcone **1a** (Table 2, entries 6–8), and a 10 time up-scaled experiment was realized with success (Table 2, entry 9). Water alone does not enable the dehydration of **2a**, ethanol alone led to the formation of by-product **3a**, while non-polar solvents are not suitable for the reaction.

A second screening was realized to determine the relative amounts of water, ethanol and lithium hydroxide needed, and to determine the settings of sonication like amplitude, duration, and temperature (Table 3). The amount of lithium hydroxide, the amplitude and duration of the sonication, and the temperature of the medium do not seem to play a role in the reaction progress. The dilution and the relative quantity of ethanol in water, on the contrary, are of great importance. β-Hydroxy-ketone **2a** was the major product when water alone was used as the solvent (Table 3, entries 1–7 and 16), except in diluted conditions (Table 3, entry 9), where chalcone **1a** was obtained as the major product. The dilution factor was raised and chalcone **1a** was obtained in good yields when water was used (Table 3, entries 9–11, 13 and 15). With less than 50% of water in the mixture, the conversion was medium (Table 3, entries 8, 12, and 14), and undesired products were formed. Finally, conditions were chosen as follows: i) sonication of the medium was preferred to classical magnetic stirring; ii) the solvent was a mixture of water and ethanol (1:1); iii) one equivalent of lithium hydroxide was optimal; iv) a concentration of 0.02 $mol.l^{-1}$ was preferred. In these conditions, chalcone **1a** was isolated in 91% yield (10–15% was obtained using classical magnetic stirring).

The above described parameters were applied to corresponding ketones and aldehydes, and chalcones **1a**-**1q** were obtained in medium to high yields (see Scheme 1). Interestingly, 6-chloropyridine chalcone



Scheme 1. Claisen-Schmidt condensation of (hetero-)aryl ketones and (hetero-) aryl aldehydes, and compounds synthesized in this study (yields are given within brackets, relatively to aldehyde and ketone reagents; regular writing correspond to ultrasounds mediated experiments, and italic writing correspond to classical stirring experiments).

1b was obtained in 31% yield only under sonication while it was not possible to isolate it when realizing the reaction under magnetic stirring. Also, lower amount of by-product 6-ethoxypyridine **1b′** was formed (6% yield under magnetic stirring and 2% yield under sonication). Consequently, sonication induced less substitution of the chlorine, however, di-keto-aryl compound **4b** and cyclohexanol **3b** were also isolated.

Table 1
Influence of the base on the Claisen-Schmidt condensation of 1-thiophen-2-yl-ethanone and thiophene-2-carbaldehyde (1 equiv of base, 0.89 mmol).[a]

Entry	Water/ EtOH (mL)	Base	Duration (s)	**2a**/**1a**/**4a**/**3a** (^{1}H NMR yield determined relatively to ketone reagent)
1	0.4/10.0	–	60	0/0/0/0
2	0.4/10.0	NaOH	10	0/ > **90**/0/0
3	0.2/10.0	LiOH	10	0/ > **90**/0/0
4	2.9/10.0	Cs_2CO_3	60	0/8/36/12
5	1.4/10.0	CsF	60	_[b]

[a] ^{1}H NMR of the crude was realized immediately after ultrasonic probe mediated condensation of 1-thiophen-2-yl-ethanone and thiophene-2-carbaldehyde.
[b] Complex mixture.

Table 2
Influence of the solvent on the Claisen-Schmidt condensation of 1-thiophen-2-yl-ethanone and thiophene-2-carbaldehyde (1 equiv LiOH, 0.32 mmol, 0.25 mol/L).[a]

Entry	Solvent	T_i - T_f (°C)	Energy (J)	**2a**/**1a**/**3a** (^{1}H NMR yield relatively to ketone reagent)
1	H_2O	22–26	181	> **90**/0/0
2	EtOH	23–28	288	0/**67**/15
3	DMF	23–32	306	0/0/0
4	THF	23–30	264	0/0/0
5	2-Me-THF	23–30	256	0/0/0
6	H_2O:EtOH (1/1)	26–32	356	0/ > **90**/0
7	H_2O:EtOH (1/3)	25–31	290	13/**77**/0
8	H_2O:EtOH (3/1)	27–32	354	0/ > **90**/0
9	Upscale of entry 6 (10X)	28–30	377	0/ > **90**/0

T_i = initial medium temperature; T_f = final medium temperature.
[a] ^{1}H NMR of the crude was realized immediately after ultrasonic-probe mediated condensation of 1-thiophen-2-yl-ethanone and thiophene-2-carbaldehyde.

If 6-chloropyridyl chalcones **1b-1j** were obtained in medium yields (31–43%) due to purification issues, other aryl and thienyl chalcones **1a** and **1 k-1r** were obtained in very good yields (91–99%). Most of the time, by-products of general formula **3** and **4** were observed in very low quantity in the crude. Using 1-(6-chloro-pyridin-3-yl)-ethanone and 6-chloro-pyridine-3-carbaldehyde, the isolation in very low yield (0.1%) of compound **4d** succeeded but not compound **3d**. In the same light, using 1-thiophen-2-yl-ethanone and 2,4-dichloro-benzaldehyde, compound **4q** (14% yield) was isolated but not compound **3q** (which was isolated from the corresponding experiment realized under magnetic stirring). For the other examples, only the chalcone was isolated, except for the synthesis of chalcone **1j**, where enone **5** was isolated (Scheme 2). This compound was also observed as traces in the crude of the syntheses of chalcones **1c-1f** (from 6-chloro-pyridine-3-carbaldehyde), but was not isolated despite efforts.

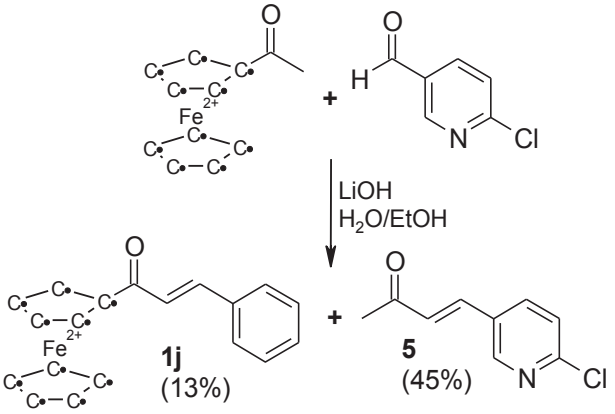
In order to understand the formation of enone **5**, the ultra-sonication of 6-chloro-pyridine-3-carbaldehyde alone with lithium hydroxide was performed. The Cannizzaro reaction products[30] alcohol **6**[31] and acid **7** were obtained (Scheme 3). The formation of compound **5** is thus difficult to explain however oxidations and/or reductions of the reagents and/or the solvents would be a track to follow.

The geometry of cyclohexanols **3** was then investigated. Spectroscopic studies for **3a**, **3b** and **3q** (^{1}H, ^{13}C, 2D-NMR) were undertaken. Results were in accordance with Gezegen's cyclohexanol[28] (see Supplementary Data for spectra), not with Shan's product.[29] It confirmed that **3b** has the same geometry as **3a** and **3q**, and it showed the position of the ethyl moiety, which is located on the pyridine next to the hydroxyl group (Fig. 2).

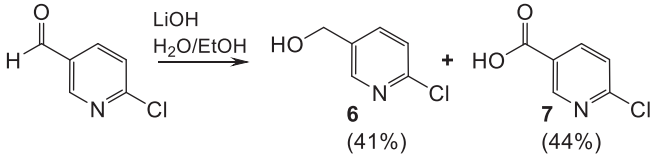
Table 3
Screening of the optimal volumes of water and EtOH, and number of equivalents of LiOH on the Claisen-Schmidt condensation of 1-thiophen-2-yl-ethanone and thiophene-2-carbaldehyde.[a]

Entry	Water (mL)	EtOH (mL)	Quantities of reagents (mmol)	LiOH (equiv)	Amplitude	Duration (s)	T_i - T_f (°C)	Energy (J)	**2a**/**1a**/**4a**/**3a** (^{1}H NMR yield relatively to ketone reagent)
1	12.5	0	3.2	1	0.3	10	25–28	350	> **90**/0/0/0
2	12.5	0	3.2	0.1	0.3	10	23–27	340	> **90**/0/0/0
3	12.5	0	3.2	0.01	0.3	10	24–28	362	> **90**/0/0/0
4	12.5	0	3.2	1	0.3	5	21–26	173	> **90**/0/0/0
5	12.5	0	3.2	1	0.3	20	25–38	699	> **90**/0/0/0
6	12.5	0	3.2	1	0.2	10	23–29	283	> **90**/0/0/0
7	12.5	0	3.2	1	0.4	10	23–29	279	> **90**/0/0/0
8	0	12.5	0.32	1	0.3	10	23–28	288	0/**67**/0/15
9	12.5	0	0.32	1	0.3	10	22–26	181	0/ > **90**/0/0
10	6.25	6.25	0.32	1	0.3	10	26–32	356	0/ > **90**/0/0
11	62.5	62.5	3.2	1	0.3	10	28–30	377	0/ > **90**/0/0
12	3.1	9.4	0.32	1	0.3	10	25–31	290	13/**77**/0/0
13	9.4	3.1	0.32	1	0.3	10	27–32	354	0/ > **90**/0/0
14	0	12.5	0.32	0.1	0.3	10	23–31	310	0/**67**/0/15
15	6.25	6.25	0.32	0.1	0.3	10	27–32	342	0/ > **90**/0/0
16	12.5	0	0.32	0.1	0.3	10	23–27	354	> **90**/0/0/0

T_i = initial medium temperature; T_f = final medium temperature.
[a] ^{1}H NMR of the crude was realized immediately after ultrasonic-probe mediated condensation of 1-thiophen-2-yl-ethanone and thiophene-2-carbaldehyde.



Scheme 2. Synthesis of enone **5** as a by-product of the Claisen-Schmidt condensation of 1-(ferrocenyl)-ethanone and 6-chloro-pyridine-3-carbaldehyde.



Scheme 3. Cannizzaro reaction of 6-chloro-pyridine-3-carbaldehyde.

It is to be noted that a degradation of compound **1o** was observed over time (the efforts to isolate the degradation product did not succeed). Finally, desired chalcones were all obtained in better yields using ultra-sonication and lithium hydroxide compared to the classical procedure.

Chalcones **1a-r**, β-hydroxy-ketone **2a**, cyclohexanol **3** and diketones **4** were evaluated *in vitro*, according to a previously described protocol,[32] for their ability to inhibit human FTase (Table 4). DMSO and chaetomellic acid A were used as negative and positive controls, respectively. First, it is to be noted that the determination of chalcone **1b** activity was not possible due to intrinsic fluorescence at the test wavelength. Ferrocenyl compounds **1i** and **1j** displayed a similar activity with IC_{50} values in the low micromolar range and were the most active chalcones in the current study. Chalcones bearing a thienyl unit, except inactive chalcones **1c** and **1m**, were all from medium to highly active. Chalcone **1l** was the most active in the thienyl-containing series (IC_{50} = 7.4 μM, Table 4). All molecules decorated with two chloropyridine units (*e. g.* chalcone **1d**) or a chloropyridine and a phenyl unit (*e. g.* chalcones **1e-h**) were completely inactive against human FTase. By-products (cyclohexanols **3a**, **3b** and **3q** and diketones **4b**, **4d** and **4q**) were also unable to inhibit the protein probably due to their very bulky structure preventing them to reach the binding site and interact with farnesyltransferase. Symmetric tetrachloro-substituted chalcones **1o** and **1p** displayed modest inhibitory activity, the 2,4-dichlorophenyl substitution being preferred for the activity over the 3,4-dichlorophenyl ring. (**1o**: IC_{50} = 38.7 μM; **1p**: IC_{50} = 96.6 μM, Table 4). Dissymmetric tetrachloro-substituted chalcones **1n** and **1r** interacted completely different with FTase. The 2,4-dichlorophenylcarbonyl unit and the 3,4-dichlorophenyl ring placed next to the ethylenic bridge in chalcone **1n** were not tolerated to inhibit the protein while the switching of the two rings (placement of the 2,4-dichlorophenyl next to the ethylenic bridge and the 3,4-dichlorophenyl next to the carbonyl function in chalcone **1r**) resulted in favorable interaction (**1r**: IC_{50} = 8.7 μM, Table 4).

Finally, the most active compound in this study was not a chalcone but the β-hydroxy-ketone **2a** (IC_{50} = 564 nM, Table 4) obtained as intermediate in the synthesis of chalcone **1a**. Compared to structurally closed chalcone **1a**, β-hydroxy-ketone **2a** was 17.8 times more active than its congener (**1a**: IC_{50} = 12 μM, Table 4).

Fourteen molecules **1b-h**, **1l-m**, **1p**, **1r**, **3a**, **3q** and **4q** have been selected by the National Cancer Institute (NCI), Germantown, for evaluation of their antiproliferative potential against the NCI-60 cancer cell lines, including multidrug-resistant (MDR) tumor cell lines (HCT-15: colorectal adenocarcinoma; NCI/ADR-RES: human ovary adenocarcinoma; RXF 393: human kidney poorly differentiated hypernephroma; MCF7: human breast adenocarcinoma and SF-539: human CNS glioblastoma). Resumed representative biological efficacy is described in Table 5. All selected molecules underwent the NCI 60 cell one-dose screen and were tested at a 10 μM concentration.

Farnesyltransferase inhibitors generally display a cytostatic effect rather than cytotoxic activity. This behavior was also registered in this study. Unfortunately, the most active chalcones against FTase **1i** and **1j** and β-hydroxy-ketone **2a** were not selected by the NCI for biological evaluation. Nevertheless, the other selected molecules displayed selective cytostatic effect. It is to be noted that no molecule was active against any SNC, renal and prostate cancer cells from the NCI-60 panel (see Supplementary information section for full one dose graphs for tested molecules). The most sensitive cancer cell line to chalcones was

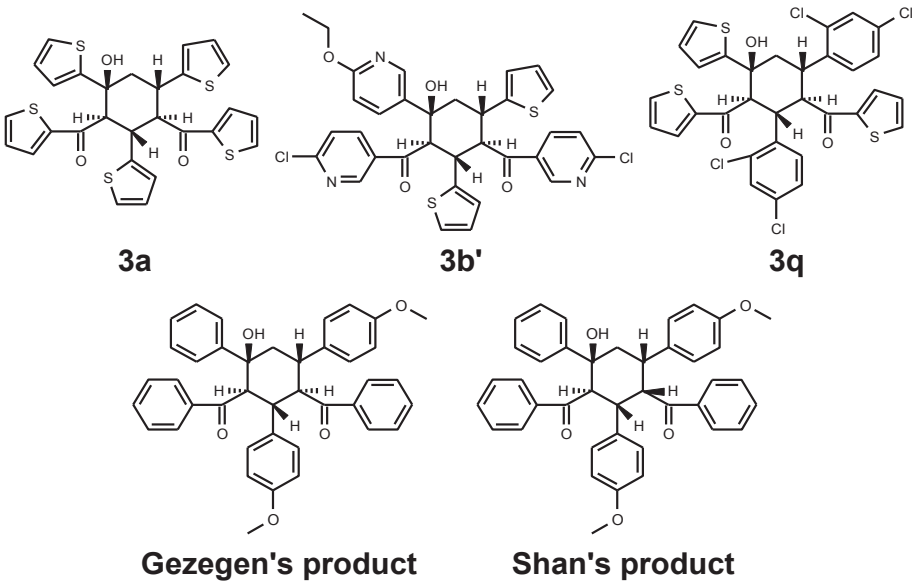


Fig. 2. Structure of cyclohexanols **3a**, **3b′** and **3q** compared to literature equivalents.

Table 4
Inhibitory activity of studied compounds on human FTase.[a]

Entry	Compound	% Inhibition of FTase at 100 μM	IC_{50} (μM)[b]	R^2
1	**1a**	100	12.0	0.9946
2	**1b**	–[c]	–[c]	–[c]
3	**1b′**	86	24.9	0.9228
4	**1c**	28	–	–
5	**1d**	26	–	–
6	**1e**	54	–	–
7	**1f**	53	–	–
8	**1g**	39	–	–
9	**1h**	22	–	–
10	**1i**	64	1.0	0.9464
11	**1j**	97	4.6	0.9818
12	**1k**	70	20.8	0.9823
13	**1l**	98	7.4	0.9493
14	**1m**	47	–	–
15	**1n**	53	–	–
16	**1o**	69	38.7	0.9815
17	**1p**	60	96.6	0.9090
18	**1q**	84	25.8	0.9415
19	**1r**	74	8.7	0.9867
20	**2a**	86	0.6	0.8356
21	**3a**	2	–	–
22	**3b′**	24	–	–
23	**3q**	11	–	–
24	**4b**	19	–	–
25	**4d**	4	–	–
26	**4q**	0	–	–
27	**Chaetomellic acid A**	100	0.18	0.9898

[a] Inhibition of human farnesyltransferase *in vitro* at 100 μM concentration of tested compound.
[b] IC_{50} values are indicated as the mean of two independent experiments each realized in duplicate.
[c] Intrinsic fluorescence at the test wavelength (data not reliable).

MCF7 (breast cancer). Seven chalcones (**1c**, **1e-h**, **1l** and **1 m**) inhibited the growth of multi-drug resistant MCF7 breast cancer cells.

Chalcones **1c** and **1m** were the most active compounds on MCF7 cells displaying cell growth inhibition of 84.3% and 87.1% at 10 μM concentration. Leukemia K-562, colon cancer HCT-116 and melanoma LOX IMVI cells were also sensitive after treatment with chalcones. Chalcone **1f** bearing a 2,4-dichlorophenyl and a chloropyridine rings was the most cytostatic agent in the current study (Table 5). The chemical agent induced excellent cell growth inhibition on K-562 and CCRF-CEM leukemia cells (92.3% and 93% inhibition, respectively), on LOX IMVI melanoma cells (93.5% inhibition) and on MCF7 breast cancer cells (82.9% inhibition). A cytotoxic effect on SW-620 colon cancer cells was also registered for chalcone **1f** (33.2% reduction of pre-existing tumor cells). Chalcones **1c**, **1g** and **1 m** were less active than chalcone **1f** on the NCI-60 panel but have demonstrated growth inhibition greater than 50% against four different cell lines. It is interesting to note that chalcones **1f** and **1g** have similar structures the only difference being the positioning of the chloropyridine and 2,4-dichlorophenyl units. The positioning of the chloropyridine next to the carbonyl group in chalcone **1f** is more favorable for antiproliferative activity while the positioning of the same motif next to the ethylenic bridge in chalcone **1g** greatly decreased the activity. The same observation applies to chalcones **1l** and **1m** having the left and the right units switched. When the heterocyclic unit (in this case 2-thienyl) was placed next to the carbonyl in chalcone **1m**, the antiproliferative activity was stronger than that of the analogue chalcone **1l** where the thienyl was placed next to the ethylenic linkage (Table 5). Chalcone **1l** has thus completely lost the activity against the leukemia cells and induced 72.5% inhibition only on MCF7 cells.

Differences between IC_{50} on human FTase in the (sub)micromolar range and IC_{50} on cancer cell growth in the micromolar or ten micromolar range is characteristic to potent FTIs and was reported previously on different series of compounds.[33]

In the case of the presence of two different heterocyclic units in the structure of chalcones (e.g. chalcones **1b** and **1c**), the best cell growth inhibition was induced by chalcone **1c** bearing the thienyl unit next to the carbonyl instead of chloropyridine in the structure of chalcone **1b** (Table 5). The presence of two identical chloropyridines in chalcone **1d** completely abolished the biological activity.

The absence of a heterocyclic ring in chalcones **1p** and **1r** bearing two chloro-substituted phenyl units was detrimental for the antiproliferative activity. Moreover, bulkier derivatives **3a**, **3q** and **4q** obtained as by-products in the synthesis of target chalcones **1a** and **1q**, respectively, were not active on the NCI-60 panel (Table 5).

Chalcones **1i-l**, **1p** and **1q** and β-hydroxy-ketone **2a** have been docked in the binding site of human farnesyltransferase in order to understand the binding mode and highlight the structural elements necessary for interaction with the protein (Fig. 3).

Chalcone **1i** has two-thirds of the solutions that superimposed with the highest score, as represented in Fig. 3(a). The chloropyridine unit of

Table 5
Results of the *in vitro* human cancer cell growth inhibition for selected compounds **1b-h**, **1l-m**, **1p-r**, **3a**, **3q** and **4q**[a,b]

	Compound	1b	1c	1d	1e	1f	1g	1h	1l	1m	1p	1r	3a	3q	4q
Cell type	Cell line	GI%[a,b]													
Leukemia	SR	17.5	n.t.[d]	n.t.	n.t.	n.t.	**55.7**	n.t.	–	**78.0**	6.1	2.8	7.9	10.0	9.7
	K-562	2.9	**65.8**	6.1	48.3	**92.3**	18.9	25.3	1.7	**71.6**	9.7	2.4	14.5	16.5	25.0
	CCRF-CEM	8.6	38.6	3.1	26.6	**93.0**	6.5	15.2	–	31.3	3.7	–	5.0	22.3	7.9
	RPMI-8226	**95.8**	n.t.	n.t.	n.t.	n.t.	8.9	n.t.	–	**66.9**	7.2	5.4	17.7	26.8	17.3
Non-Small Cell Lung Cancer	NCI-H522	18.0	37.2	5.3	45.8	**61.3**	24.2	17.0	8.6	24.7	8.1	11.4	15.3	18.5	17.3
Colon Cancer	KM12	–[c]	**57.8**	1.0	44.4	23.5	3.6	32.4	2.8	15.4	–	0.3	3.4	4.7	4.8
	SW-620	–	47.6	–	40.1	**100.0**[e]	7.9	17.6	5.5	29.6	–	–	–	12.7	4.4
	HCT-116	8.9	35.5	–	**55.1**	**63.3**	**56.6**	19.3	2.2	39.8	6.1	13.9	6.1	21.2	34.7
Melanoma	LOX IMVI	–	**85.0**	–	37.6	**93.5**	**65.1**	21.9	–	45.7	–	–	7.8	3.1	10.9
Ovarian Cancer	OVCAR-3	–	45.8	–	43.5	21.5	–	11.2	–	6.8	–	–	–	11.5	–
Breast cancer	MCF7	32.3	**84.3**	8.8	**66.4**	**82.9**	**63.4**	**69.8**	**72.5**	**87.1**	14.2	28.3	12.2	18.3	10.2

[a] Data obtained from NCI's *in vitro* 60-cell one dose screen (experiments conducted with 10 μM concentration of tested molecule).
[b] GI% is the percentage of growth inhibition of tumor cells.
[c] No inhibition effect.
[d] Not tested.
[e] Cytotoxic effect: growth percent of −33.2% on SW-620 cells induced by chalcone **1f**.

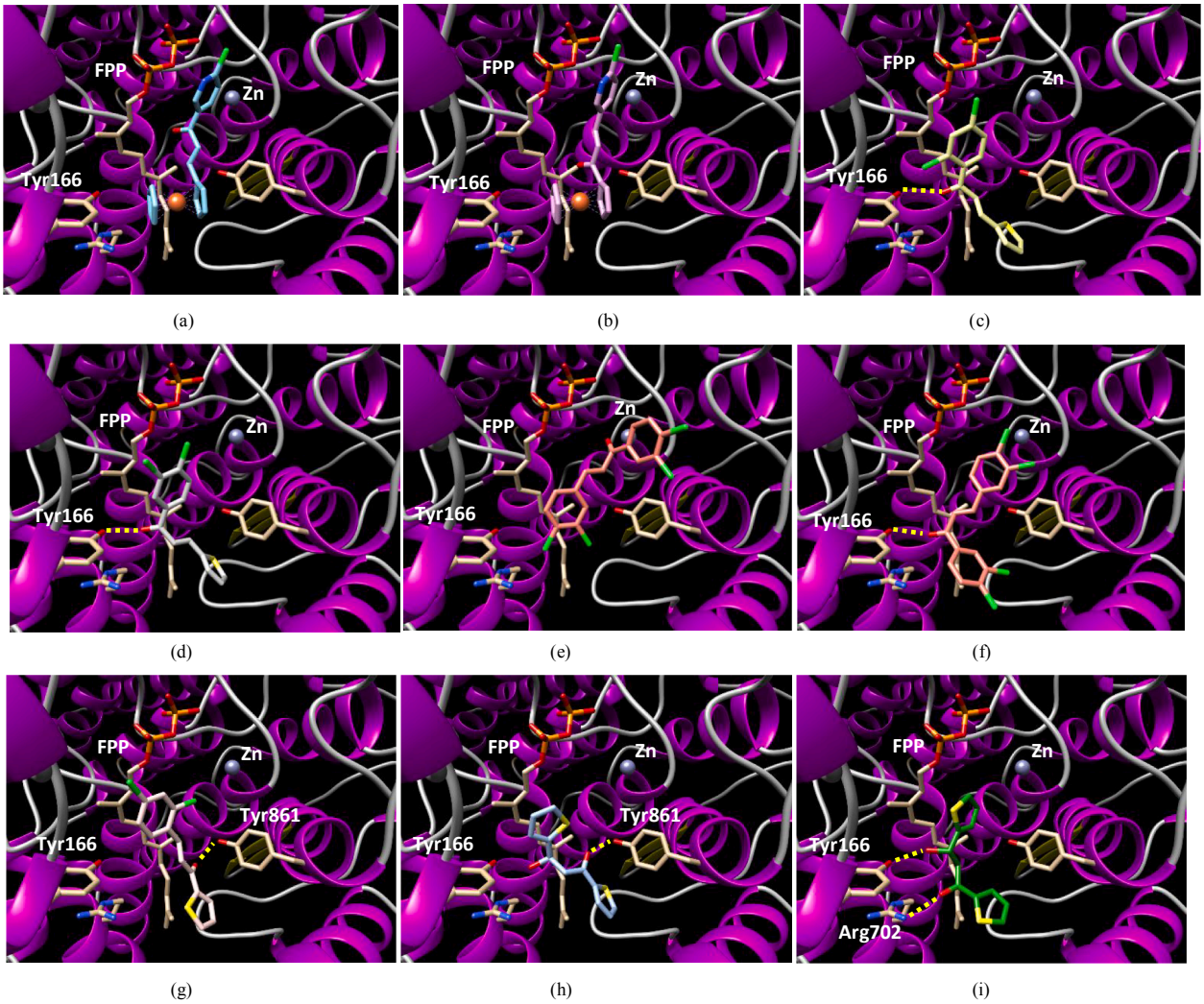


Fig. 3. Docking of synthesized molecules in the active site of human farnesyltransferase: chalcone **1i** (a), chalcone **1j** (b), chalcone **1k** (c), chalcone **1l** (d), chalcone **1p** (e-f), chalcone **1q** (g) and β-hydroxy-ketone **2a** (enantiomer *R* (h); enantiomer *S* (i)). Yellow dashed lines represent hydrogen bonds between investigated molecules and aminoacids present in the active site of human farnesyltransferase.

chalcone **1i** is positioned in front of the zinc of the protein while the ferrocene unit occupies the pocket formed by amino acids Tyr861, Arg702 and Tyr166, also known as the A_2 pocket.[34] Chalcone **1j**, reverse analogue of chalcone **1i**, also interacts with the zinc via the chloropyridine unit while the ferrocene occupies the same pocket as in chalcone **1i** (Fig. 3(b)). The positioning of the ferrocene from chalcones **1i** and **1j** agrees with our previous studies on ketones bearing bulky lipophilic groups such as paracyclophane, adamantane, noradamantane or ferrocene located in the A_2 binding site of farnesyltransferase.[34]

Chalcone **1k** has twelve solutions superimposed with the best score (Fig. 3(c)). In this configuration, it attaches to the Tyr166 via a hydrogen bond between the carbonyl of the chalcone and the phenol of the amino acid. The other solutions for chalcone **1k** are placed in the opposite direction with the thienyl unit in place of the 2,4-dichlorophenyl ring and do not make any single interaction. This may explain the moderate IC_{50} value of 20.8 μM obtained for this compound on FTase.

Chalcone **1l**, structurally very closed to chalcone **1k**, interacts in similar way (Fig. 3(d)). The hydrogen bond is conserved between the carbonyl of the chalcone linkage and the phenol of the Tyr166 and the chlorine atoms are slightly closer for interaction with the zinc atom compared to chalcone **1k**.

Tetrachloro-substituted chalcone **1p** places less well, with two solutions (Fig. 3(e) and (f)) and at least two other smaller groups that are less well defined. The conformation with the highest score does not form hydrogen bond but is correctly placed to interact with the zinc cation (Fig. 3(e)). The second conformation is placed conversely and forms a hydrogen bond with Tyr166 (Fig. 3(f)). However, the very small number of solutions can justify the modest activity of this molecule on the FTase (IC_{50} = 96.6 μM).

Chalcone **1q** behaves differently and forms a hydrogen bond via its carbonyl group but with the Tyr861 (Fig. 3(g)) and is fixed in the active site by stacking with the farnesyl moiety.

The best farnesyltransferase inhibitor identified in the current study was β-hydroxy-ketone **2a** synthesized as racemate. The both enantiomers R and S of **2a** were investigated in the binding site of the protein (Fig. 3(h) and (i)). The enantiomer *R* has two conformations which are positioned in the same place and which maintain a hydrogen bond with Tyr861 via the carbonyl unit (Fig. 3(h)). The difference between the two conformations retrieved for (*R*)-**2a** is the change of side of the 2-thienyl rings. The enantiomer *S* is even better positioned and has a single solution for 26 of the 30 conformations. The hydrogen bond with Tyr166 is still present and there is a second interaction with Arg702.

The cyclohexanol derivatives (*e.g.* compound **3q**) narrowly enter in the pocket of farnesyltransferase. There is no solution that stands out from the different loose conformations (data not shown) which may explain the absence of the biological activity of these bulky derivatives.

To conclude, an ultra-fast green Claisen-Schmidt synthesis of a series of chalcones has been developed under sonication in a mixture of ethanol/water and using lithium hydroxide as base. The chemical transformations were compared with corresponding classical magnetic stirring reactions and allowed to reduce the reaction duration from hours to 10 s with similar to superior results. In some cases, by-products were detected in the medium and were purified and identified as cyclohexanol or bisketone derivatives.

Synthesized molecules **1–4** have been evaluated on human farnesyltransferase inhibitors *in vitro*. Chalcones **1** were globally able to inhibit the protein and allowed to establish additional structure–activity relationships in the series: i) the ferrocenyl moiety was tolerated and provided the most active chalcones in this study with IC_{50} in the low micromolar range (*e.g.* **1i** and **1j**); ii) the presence of two pyridine units (*e. g.* chalcone **1d**) or a chloropyridine and a phenyl unit (*e. g.* chalcones **1e-h**) in the structure of chalcones abolished the activity; iii) thienyl-substituted chalcones conserved an inhibitory potential; iv) symmetric tetrachloro-substituted chalcones **1o** and **1p** displayed modest activity; v) placement of the 2,4-dichlorophenyl next to the ethylenic bridge and the 3,4-dichlorophenyl next to the carbonyl function in chalcone **1r** resulted in favorable interaction; vi) bulky by-products **3** and **4** were not active on FTase; vii) the β-hydroxy-ketone **2a** was the most active compound in the current study.

Compounds were also tested for their ability to prevent cancer cell growth and underwent the one-dose screen at the National Cancer Institute (NCI) at 10 μM concentration. Molecules showed a cytostatic effect on different cell lines with particular activity against MCF7, breast cancer cells.

The β-hydroxy-ketone **2a** deserves further biological investigation on progeria cells. Additional pharmacomodulations are expected on this scaffold and could lead to a new generation of potent farnesyltransferase inhibitors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors gratefully acknowledge Interreg NWE for financial support of this work which is part of the RIVER project. Acknowledgements are also addressed to Yncréa Hauts-de-France, HEI, for financial support of this work, especially for the investments allocated to our team.

The authors also acknowledge the National Cancer Institute (NCI) for the biological evaluation of compounds on their 60-cell panel: the testing was performed by the Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis (the URL to the Program's website: http://dtp.cancer.gov).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127149.

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