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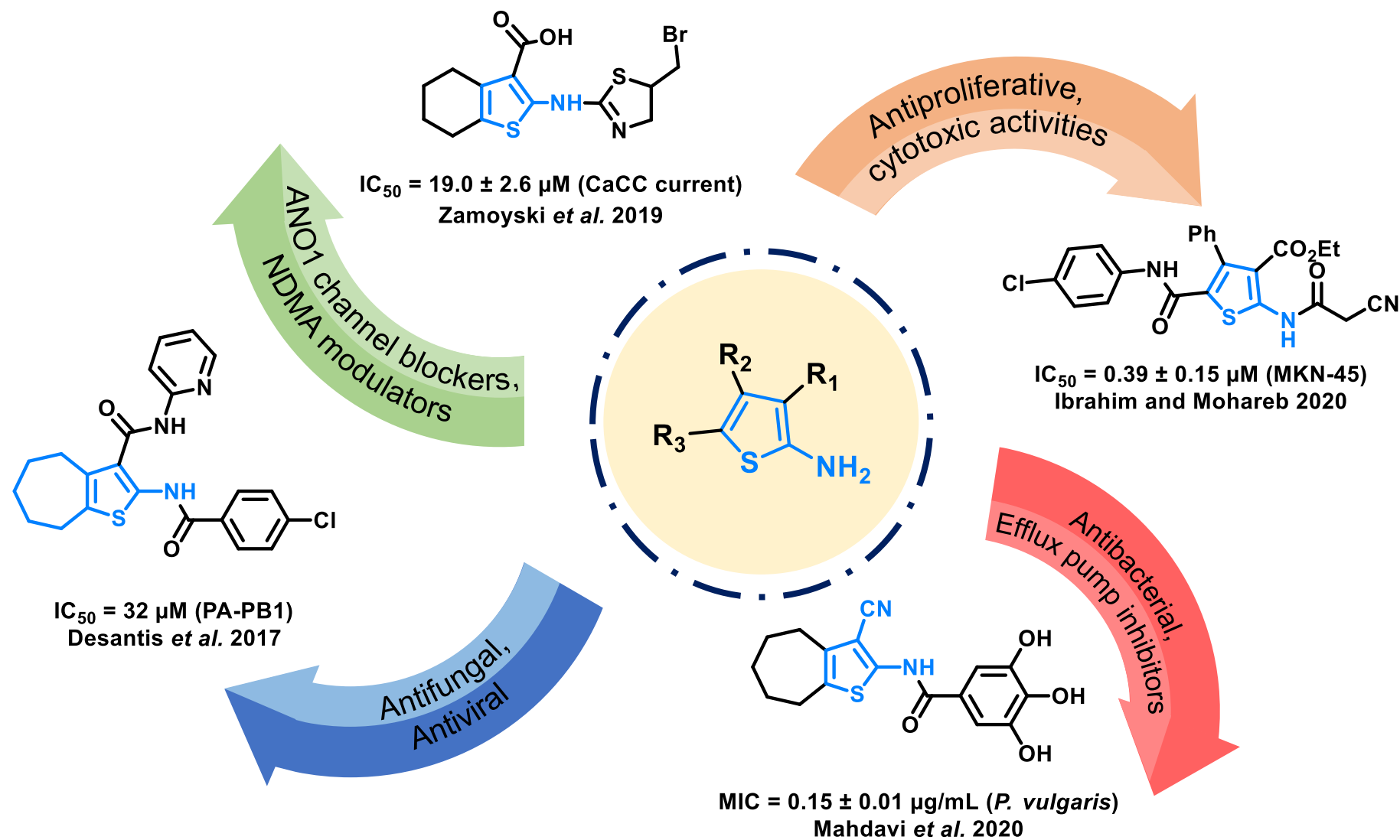
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Recent contribution of medicinally active 2-aminothiophenes: a privileged scaffold for drug discovery

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Abstract	2
List of abbreviations.....	3
1. Introduction	4
2. Biological and pharmacological attributes of 2-ATs	7
2.1. Antiprotozoal activity	7
2.2. Antiproliferative Activity	10
2.3. Antiviral Activity.....	21
2.4. Antibacterial Activity	24
2.5. Antifungal Activity.....	33
2.6. Channels inhibitors activity	37
2.7. Cannabinoid type-1 receptor modulator activity	42
3. Conclusion.....	50
Authors contributions.....	50
Acknowledgments	51
Declaration of competing interest	51
References	51

Abstract

In medicinal chemistry, 2-aminothiophene is a central five-membered heterocyclic core that is mostly synthesized using Gewald methodology. Its incorporation into a molecule can confer broad biological activities, making 2-aminothiophene an attractive scaffold for drug discovery. Another interesting feature of 2-aminothiophene moiety is its ability to act as synthon for the synthesis of biological active thiophene-containing heterocycles, conjugates or hybrids. Compounds from the 2-aminothiophene family are promising selective inhibitors and modulators in medicinal chemistry, and even exhibit effective pharmacological properties in the various clinical phases of appropriate diseases. Likewise, the biological actions of 2-aminothiophenes or their 2-N-substituted analogs are still being investigated because of their diverse mechanisms of action (*e.g.*, pharmacophore and pharmacokinetic properties). In this review, we focus on the structure-activity relationship, the synthesis and the biological activities of 2-aminothiophene derivatives, including antiprotozoal, antiproliferative, antiviral, antibacterial, antifungal, channel and cannabinoid receptor inhibitors. Most perspective drug-candidate hits were selected for discussion and described, along with additional synthetic pathways. Since there has been several contributions in this field recently, we emphasized on the literature dedicated to 2-aminothiophenes and 2-N-substituted derivatives which have been published from 2017 to 2022.

Keywords: 2-aminothiophenes; synthetic methodology; drug discovery; biological applications; structure-activity relationship; inhibitor

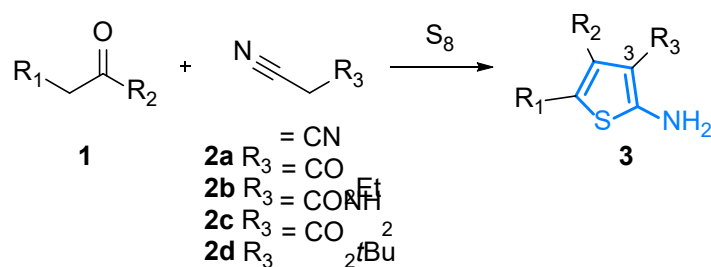
45 List of abbreviations

- | | |
|--|---|
| 46 2-AT: 2-aminothiophene | 65 EWG: Electron-withdrawing group |
| 47 ADME: Absorption distribution | 66 FT-IR: Fourier transform infrared |
| 48 metabolism and excretion | 67 GBM: Glioblastoma |
| 49 ANO: Anoctamin | 68 HER2: Human Epidermal Growth |
| 50 Boc ₂ O: Di-tert-butyl dicarbonate | 69 Factor Receptor-2 |
| 51 CaCC: Calcium-activated chloride | 70 IC ₅₀ : Half-maximal inhibitory |
| 52 channels | 71 concentration |
| 53 CB ₁ : Cannabinoid type-1 | 72 ICP-MS: Inductively coupled plasma mass |
| 54 CC ₅₀ : Half-maximal cytotoxic | 73 spectrometry |
| 55 concentration | 74 MCR: Multicomponent reaction |
| 56 DABCO: 1,4-Diazabicyclo[2.2.2]octane | 75 MDR: Multidrug resistant |
| 57 DBU: 1,8-Diazabicyclo(5.4.0)undec-7-ene | 76 RdRp: RNA-dependent RNA polymerase |
| 58 DCM: Dichloromethane | 77 SAR: Structure activity relationship |
| 59 DSC: Differential Scanning Calorimetry | 78 SEM: Scanning electron microscope |
| 60 EC ₅₀ : Half maximal effective | 79 TBATB: Tetrabutylammonium tribromide |
| 61 concentration | 80 TFA: Trifluoroacetic acid |
| 62 EDG: Electron-donating group | 81 VSM: Vibrating sample magnetometer |
| 63 EFGR: Epidermal growth factor receptor | 82 XRD: X-ray powder diffraction |
| 64 EtBr: Ethidium bromide | |

1. Introduction

Heterocyclic rings are favored scaffold for many years in medicinal chemistry, and drug discovery due to their importance in properties they confer. They account for more than 85% of all biologically active compounds.¹ S-heterocycles, and more specifically 2-aminothiophenes (2-ATs) are five-membered heteroaromatic core that have a significant impact on biological systems and can be used as synthons to develop potent new medicines. Moreover, they are considered as important in heterocyclic chemical libraries and when used as a building block are responsible for many biological activities.²⁻⁴ Small organic molecules are required in drug discovery and medicinal chemistry on a continuous basis. To do so, chemists inspire their chemical series over described methodologies and they evaluate potential biological activities of newly synthesized derivatives.

2-ATs are 5-membered aromatic scaffold whose synthesis has been firstly depicted by Campaigne and Foye in the 1950s before being democratized by Gewald in the 1960s.⁵⁻⁷ Gewald reaction frequently occurs with a carbonyl derivative **1** with a nitrile **2** in the presence of a sulfur source to afford **3**. Typically, methodologies result in the formation of trisubstituted thiophene ring with an electron-withdrawing groups (EWG) – especially negative mesomeric effect such as carboxamide, ester or nitrile group on C3 position (Scheme 1).⁸⁻¹²



Scheme 1. Synthesis of 2-ATs 3 following Gewald methodology.

Since then, many enhancements were published following many methodologies.^{4,13–22} 2-ATs continue to raise a large interest in both organic and medicinal chemistry. To our knowledge, 2-AT is absent of natural compounds, hence it must be obtained synthetically.²³ Multiples synthetic routes are described alongside literature, by means of heterogeneous catalysis,²⁴ homogeneous catalysis,²⁵ multicomponent reactions^{26,27} or post-core synthesis modification.²⁸ Medicinal chemists made thereafter their contribution thanks to structure-activity relationship (SAR) to allow a better understanding of 2-ATs biological properties. Considering biological evaluations, 2-ATs are known to confer antiprotozoal,^{29,30} antiproliferative,^{31–34} antiviral,^{32,35} antibacterial^{17,30–32} or antifungal activities.^{39,40} Among FDA-approved 2-ATs, we can highlight **RS-C-5966451**, described for its broad-spectrum antiviral properties;⁴¹ **PD 81723**, which is an adenosine A1 receptor agonist used to reduce necrosis, apoptosis and inflammation for renal ischemia-reperfusion;^{42,43} **Tinoridine**, as a non-steroidal anti-inflammatory;⁴⁴ and **T-62**, known as a former adenosine A1 receptor agonist and more recently for post herpetic neuralgia application (Figure 1).⁴⁵

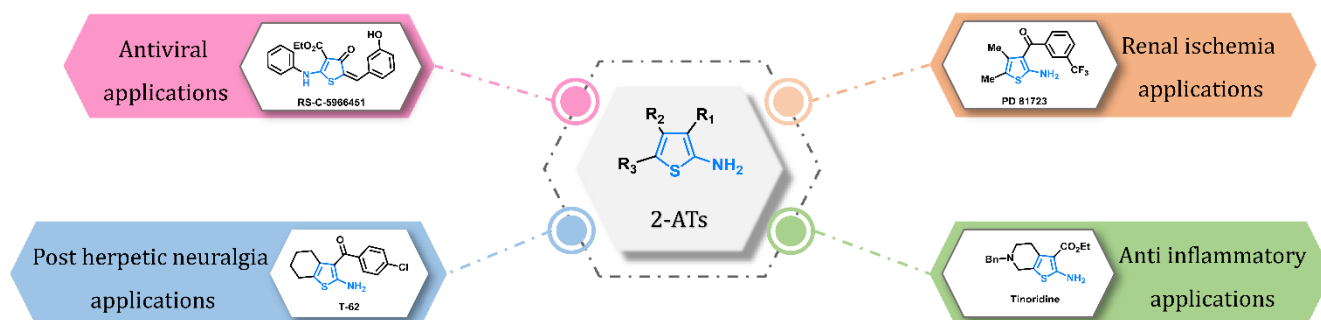


Figure 1. 2-ATs drugs used in the last decade.

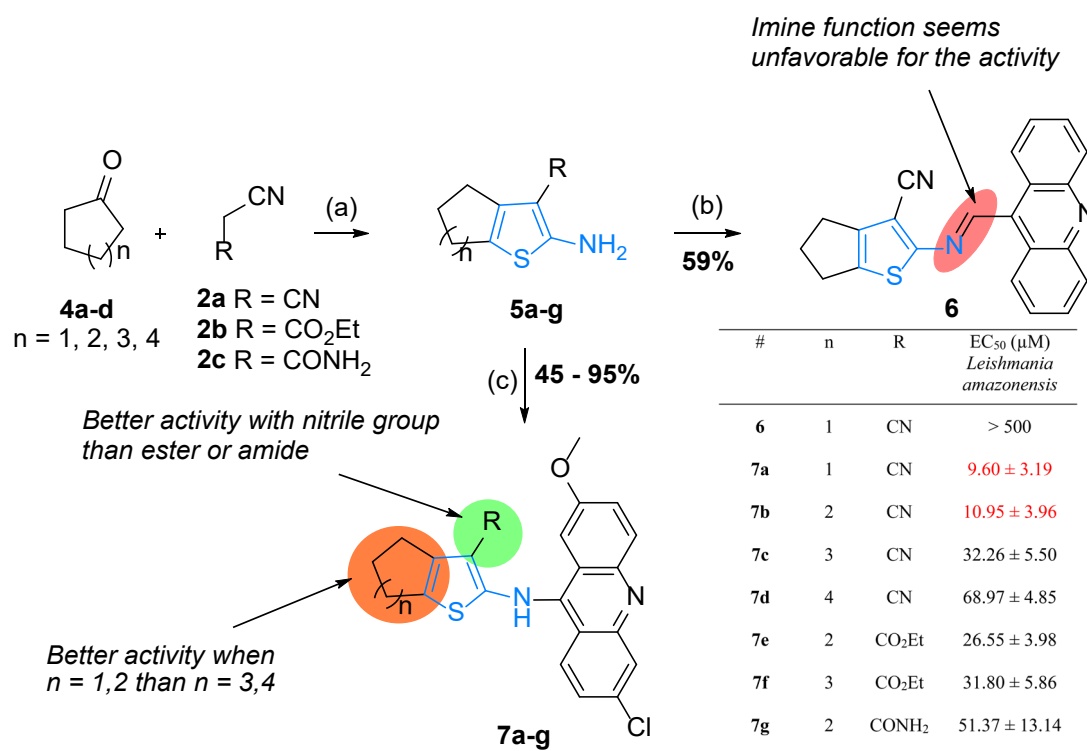
Nevertheless, we noticed that while reviews summarizing biological activities or synthesis of 2-ATs are explored in the literature,^{13,46–49} there are many recent examples describing biological activities for the last 5 years (2017–2022). In this review will be provided a comprehensive overview of the research dealing with 2-ATs over the years 2017–2022. Each synthesis of medicinally active differently substituted 2-ATs derivatives will be emphasized. Whereupon, their biological activities will also be an important point. Biological results will be summed up with the help of tabular representation, and best compounds will be put forward. Finally, the thorough elucidation of 2-ATs biological activities compared with the reference drug also allowed a proficient assessment of the SAR studies.

2. Biological and pharmacological attributes of 2-ATs

2.1. Antiprotozoal activity

Parasitic diseases are infections caused by single-celled eukaryotes and are responsible for important mortality. It does affect more than 500 million people all around the world.⁵⁰ The lack of effective antiprotozoal and drug resistance encouraged researchers to search for new drugs against protozoan parasites.⁵¹

Following a Gewald synthesis, De Lima Serafim *et al.* described in 2018 the synthesis of 2-ATs (**6**, **7a-g**) whose synthesis is disclosed in scheme 2. Cyclic ketones **4a-d** reacted with the appropriate malononitriles **2a-c** to afford **5a-g** with no reported yields.⁵² On one hand imine **6** was synthesized in the presence of acridine-9-carboxaldehyde and 2-AT **5a** in ethanol at 60°C for 5 hours in 59% yield after filtration and washes with cold ethanol. On the other hand **5a-g** reacted with 6,9-dichloro-2-methoxy-acridine in a mixture of ethanol and acetic acid at room temperature for 4 hours to give N-substituted **7a-g** in 45–95% yields after filtration and washes with cold ethanol.



Scheme 2. (a) morpholine, S₈ in EtOH, 5–10°C, 3h; (b) acridine-9-carboxaldehyde in EtOH, 60°C, 5h; (c) 6,9-dichloro-2-methoxy-acridine, AcOH in EtOH, r.t., 4h.

These compounds (**6**, **7a-g**) have been evaluated on promastigotes forms of *Leishmania amazonensis* (IFLA/BR/67/PH8) and except for **6**, the compounds **7a-g** exhibited good antiprotozoal properties (EC₅₀ range 9.60–429 μM, Scheme 2). **7a** and **7b** displayed best results (EC₅₀ range 9.60 ± 3.19 μM and 10.95 ± 3.96 μM, respectively, Table 1), suggesting that the nature of the cycloalkyl influenced the activity. Cyclopentyl and cyclohexyl derivatives showed better results than cycloheptyl and cyclooctyl. Moreover, the presence of the nitrile group increased the global biological activity (EC₅₀ = 9.60 ± 3.19 and 10.95 ± 3.96 μM for **7a** and **7b** against 26.55 ± 3.98 μM and 51.37 ± 13.14 μM for **7e** and **7g**). The authors also investigated the mechanism of action for **7a** and **7b** and observed that they were bound to parasite DNA with K_b = 1.10⁴ M⁻¹. As neither compound demonstrated DNA fragmentation, the authors hypothesized compounds **7a** and **7b** might be responsible for replicative enzymes inhibition that should be the reason for the antiprotozoal behaviors.

Duarte and co-workers carried out antiprotozoal studies on commercially available compounds (**5a,b,g**) in 2018 (Figure 2).⁵³ **5b** was shown to reduce hypnospore viability of *Perkinsus* spp. by 38% at the concentration of 500 μ M (Table 1). **5a** and **5g** did not exhibit antiprotozoal activities against *Perkinsus*. Hypnospore is a life stage of *Perkinsus* parasites responsible for high mortality of bivalves in the entire world, including oysters.

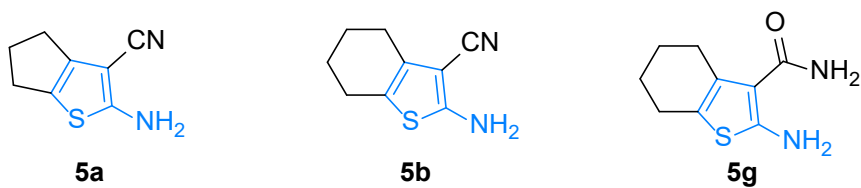
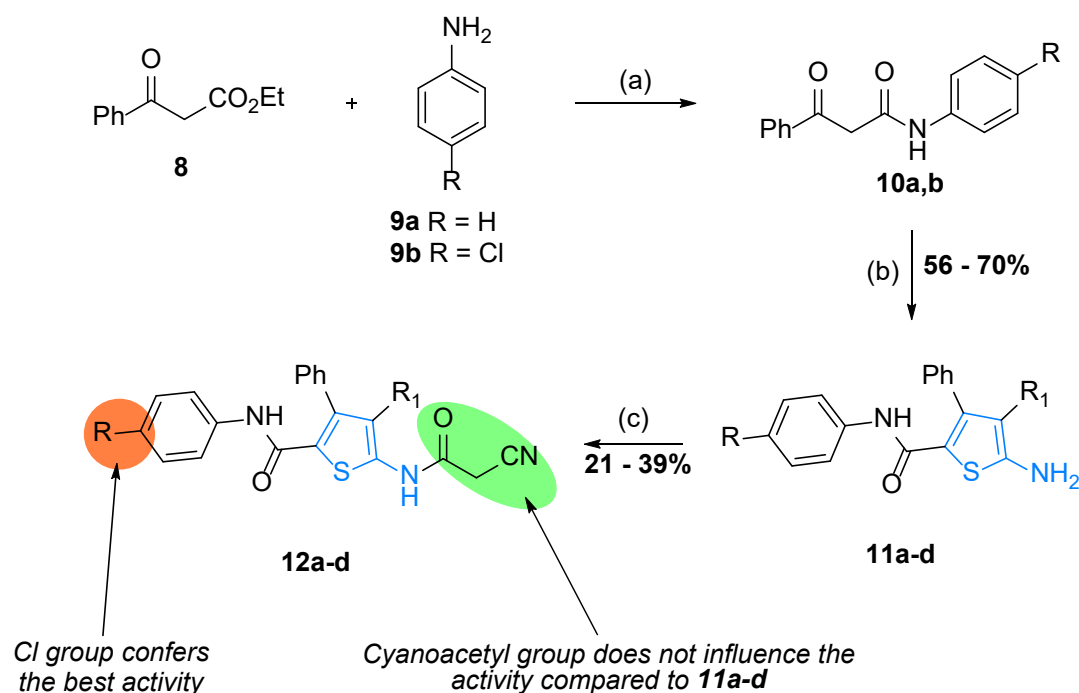


Figure 2. Structures of 5a, 5b and 5g.

2.2. Antiproliferative Activity

Cancer treatments significantly improved in the past decades. However, toxicity of several cures is still a hindrance which requires constant development of new antiproliferative drugs. Hence, the search for novel therapeutic approaches, such as compounds from natural sources, has become a topic of great interest.^{54–56}

In 2020, Ibrahim and Mohareb designed a novel 2-ATs series (**12a-d**). Their synthesis is pictured in scheme 3.¹¹ First step is the synthesis of intermediate **10a** and **10b** following an amide formation using ethyl benzoyl acetate **8** and anilines **9a,b** without mentioned yields. Then, Gewald reaction allowed formation of **11a-d** in 56–70% yields in the presence of malonodinitrile **2a** or ethyl cyanoacetate **2b** with **10a** or **10b** in ethanol, triethylamine and elemental sulfur S₈ after precipitation in cold diluted aqueous hydrochloride solution and filtration. The final step consisted in reaction of **11a-d** in presence of malonodinitrile **2a** in DMF to afford **12a-d** in 21–39% yields after precipitation in cold water, filtration and recrystallization from ethanol. Antiproliferative activity of all molecules have been evaluated on 6 cancer cells lines: A549 (lung), H460 (lung), HT29 (colon), MKN-45 (gastric), U87MG (glioblastoma) and SMMC-7721 (liver). Compounds **11c**, **11d**, **12b** and **12d** showed particular good activities on all cancer cell lines (IC₅₀ range 0.19–9.62 μ M; Scheme 3, Table 1). Results were similar for **11a-d** and **12a-d**: the addition of the acyl group on the amine function did not influence the activities much. Compounds **11c**, **11d**, **12b** and **12d** also showed better antiproliferative activities than foretinib (reference molecule), against U87MG (glioblastoma) and SMMC-7721 (liver) cancer cell lines, are thus promising lead as antiproliferative molecules for these types of cancers.

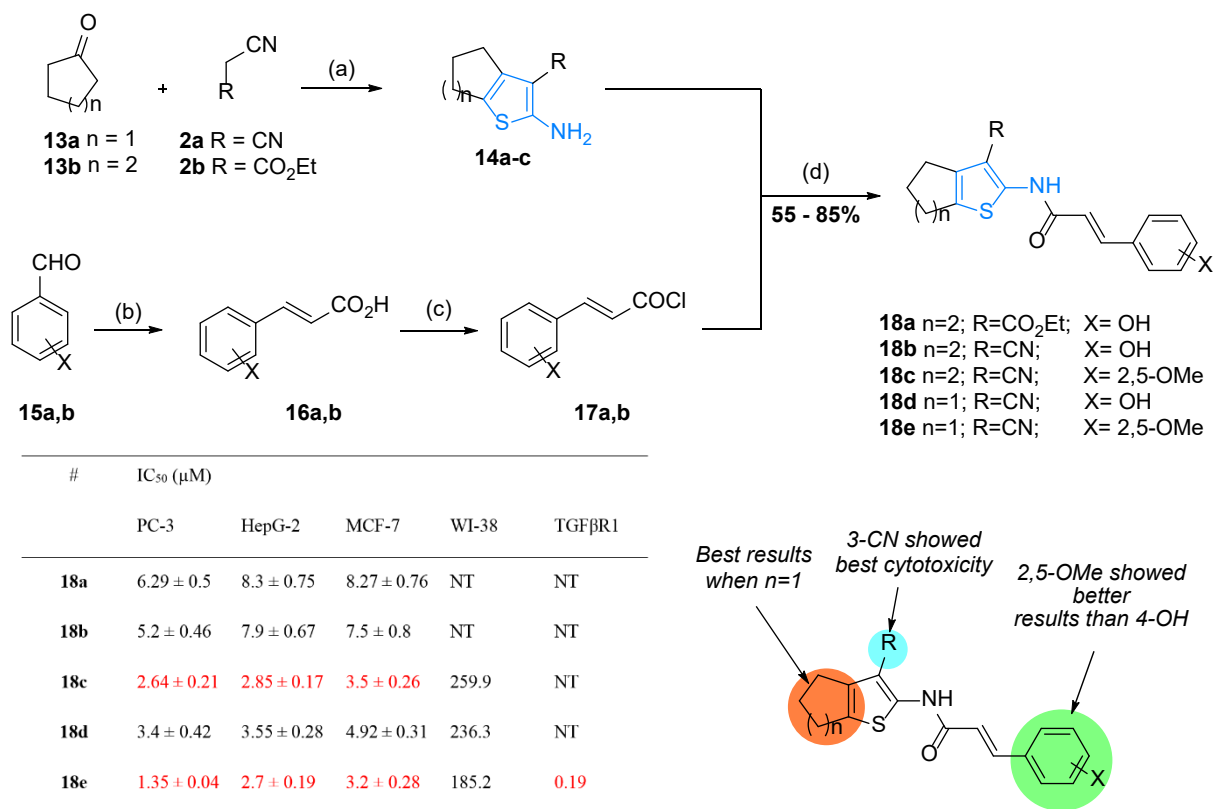


#	R	R ₁	IC ₅₀ ± SEM (μM)					
			A549	H460	HT29	MKN-45	U87MG	SMMC-7721
11a	H	CN	8.28 ± 4.18	9.62 ± 2.70	8.39 ± 2.73	7.09 ± 2.35	8.93 ± 2.73	5.09 ± 1.63
11b	H	CO ₂ Et	6.80 ± 1.42	8.59 ± 1.80	7.49 ± 2.73	8.09 ± 2.04	7.42 ± 2.69	8.58 ± 2.68
11c	Cl	CN	0.70 ± 0.29	0.76 ± 0.38	0.65 ± 0.27	0.42 ± 0.23	0.59 ± 0.18	0.69 ± 0.28
11d	Cl	CO ₂ Et	0.23 ± 0.08	0.38 ± 0.27	0.19 ± 0.08	0.26 ± 0.11	0.39 ± 0.21	0.42 ± 0.27
12a	H	CN	7.59 ± 2.31	8.06 ± 3.19	6.59 ± 1.80	9.14 ± 2.59	6.28 ± 1.49	7.93 ± 1.31
12b	H	CO ₂ Et	0.32 ± 0.20	0.49 ± 0.21	0.63 ± 0.32	0.59 ± 0.23	0.62 ± 0.18	0.53 ± 0.27
12c	Cl	CN	1.40 ± 0.69	1.04 ± 0.73	0.86 ± 0.32	0.59 ± 0.32	0.86 ± 0.52	0.39 ± 0.18
12d	Cl	CO ₂ Et	0.60 ± 0.24	0.43 ± 0.26	0.62 ± 0.30	0.39 ± 0.15	0.43 ± 0.26	0.73 ± 0.31
Foretinib			0.08 ± 0.01	0.18 ± 0.03	0.15 ± 0.023	0.03 ± 0.0055	0.90 ± 0.13	0.44 ± 0.062

Scheme 3. (a) 120°C; (b) malonodinitrile **2a** or ethyl cyanoacetate **2b**, Et₃N, S₈ in EtOH, r.t.; (c) malonodinitrile **2a** in DMF, r.t.

In 2020, Ismail *et al.* described the synthesis of N-substituted-2-ATs (**18a-e**) as antiproliferative analogs of tranilast (reference compound), an FDA-approved drug targeting the catalytic kinase domain of TGFβR1.⁵⁷ Although 4 series have been designed, we have focused on the 2-ATs series, synthesized proceeding the pathway shown in scheme 4. First, the

reaction with cyclopentanone **13a** or cyclohexanone **13b**, in presence of malonodinitrile **2a** or ethyl cyanoacetate **2b** with elemental sulfur S₈ in a mixture of ethanol/morpholine afforded **14a-c**. Subsequently, the next step followed a Knoevenagel reaction, in the presence of **15a,b** with malonic acid and piperidine in ethanol under reflux and led to **16a,b**. Then, **16a,b** reacted with POCl₃ and triethylamine to give the cinnamoyl chloride derivatives **17a,b**. Yields are not mentioned for both **14a-c** and **17a-b** synthesis. In the last step, both reagents **14a-c** and **17a,b** were allowed to react together in acetone in the presence of K₂CO₃ under reflux for 5–8 hours to afford amides **18a-e** in 55–85% yields after crystallization from ethanol.



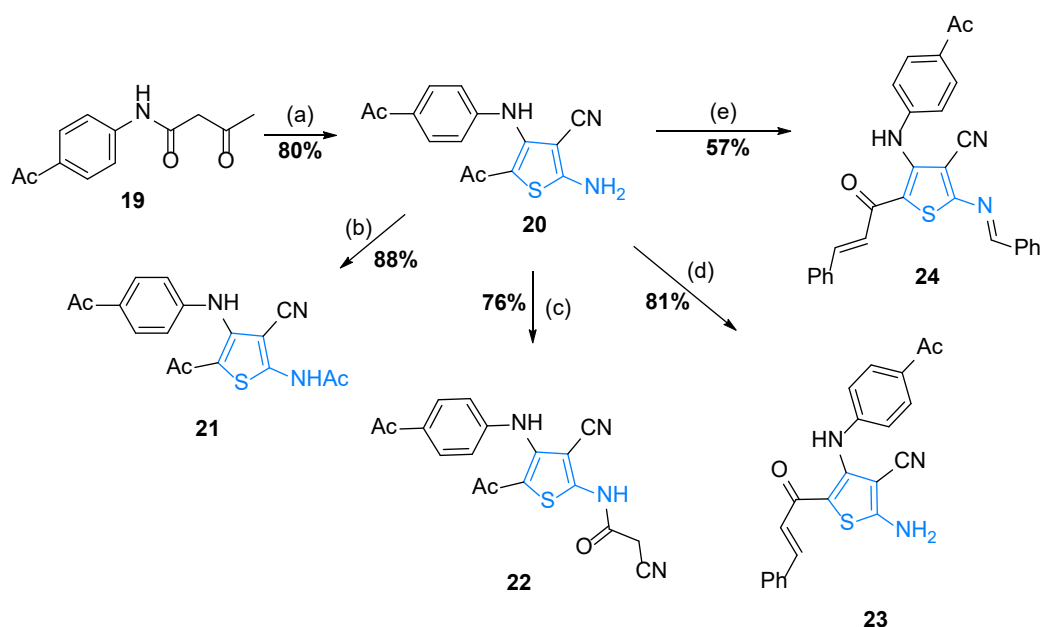
Scheme 4. (a) malonodinitrile **2a** or ethyl cyanoacetate **2b**, morpholine, S₈ in EtOH under reflux for 4h then 24h at 0°C; (b) malonic acid, piperidine in EtOH, under reflux, 5h; (c) POCl₃ NEt₃ under reflux for 20 min; (d) K₂CO₃ in acetone under reflux, 5 – 8h.

Four cell lines have been chosen: PC-3 (prostate carcinoma), HepG-2 (hepatocellular carcinoma), MCF-7 (breast adenocarcinoma) and WI-38 (normal cell lines) to determine their

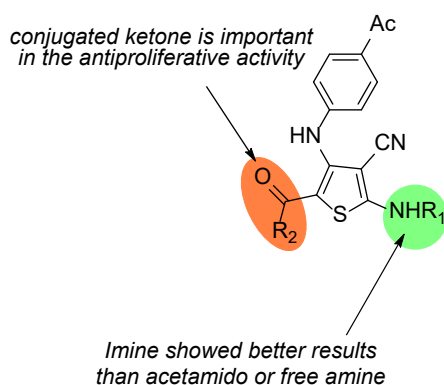
antiproliferative activities following the MTT assay. The MTT assays have been realized on 5 compounds (**18a-e**) and showed good IC_{50} on cancer cell lines (IC_{50} range 1.35–8.3 μ M, scheme 4). MTT evaluations on normal human cell lines (WI-38) have been realized for the 3 best compounds (**18c-e**) to conclude they were not acutely toxic (IC_{50} range 185.2–259.9 μ M). Compounds **18c-e** displayed selectivity toward cancer cell lines by inhibiting TGF β R1 with an IC_{50} comprised between 0.087 and 3.276 μ M. TGF β R1 is a key protein in cell proliferation. Thereafter, TGF β R1 inhibition assay has been realized on the best compound **18e** following TGF β 1 ELISA assays to determine the potency of this molecule on the targeted protein (IC_{50} = 0.09 μ M; Table 1). For comparison, galunisertib (reference compound) showed an IC_{50} of 0.17 μ M on TGF β R1. Concerning SAR studies, antiproliferative properties were proved to be better when a cyano group was grafted on C3 position. The biological activities were good when the cycloalkyl was a cyclopentyl and when the phenyl moiety was substituted in positions 2 and 5 by methoxy groups. The most effective derivative **18e** has been docked in TGF β R1 binding site and exhibited the good docking score of - 6.92 kcal/mol. Moreover, *in silico* ADME properties of compounds **18a-e** have been predicted using SwissADME software to be drug like compatible.

In 2020, Khalifa and Algothami developed a novel series of 2-ATs (**20–24**) as antiproliferative molecules on HepG2 and MCF-7 cancer cell lines.⁵⁸ The synthesis is described in scheme 5 and started with reaction of the amide **19** with malonodinitrile **2a**, elemental sulfur S₈ and triethylamine in DMF at 60°C to give **20** in 80% yield after precipitation in cold water and washes with ethanol. Afterwards, 2-AT **20** has been used as a central scaffold to synthesize 4 derivatives. N-acetylated **21** was obtained by treatment of **20** with acetic anhydride in acetic acid under reflux for 5 hours in 88% yield after recrystallization from DMF. **20** was fused in solvent-free conditions with ethyl cyanoacetate **2b** to afford **22** in 76% yield after recrystallization from ethanol. The α - β -unsaturated ketone **23** was synthesized by reaction of

20 with 1 equivalent of acetaldehyde in ethanol in 81% yield after recrystallization from 1,4-dioxane. Compound 24 was obtained in the same condition using 2 equivalents of acetaldehyde in 57% yield after purification by recrystallization from 1,4-dioxane.



#	Cytotoxicity IC ₅₀ (μM)	
	HEPG2	MCF-7
20	91.46 ± 4.9	88.48 ± 4.7
21	64.61 ± 3.8	66.23 ± 4.1
22	41.39 ± 3.2	56.07 ± 3.9
23	67.50 ± 3.9	73.62 ± 4.5
24	19.14 ± 1.9	26.16 ± 2.1
Doxorubicin	4.50 ± 0.3	4.17 ± 0.2

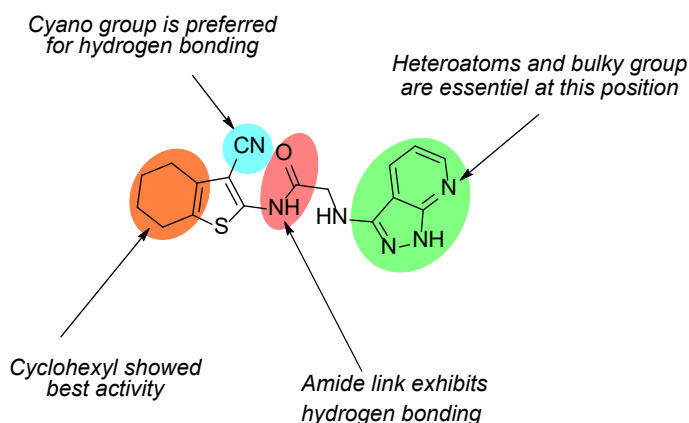
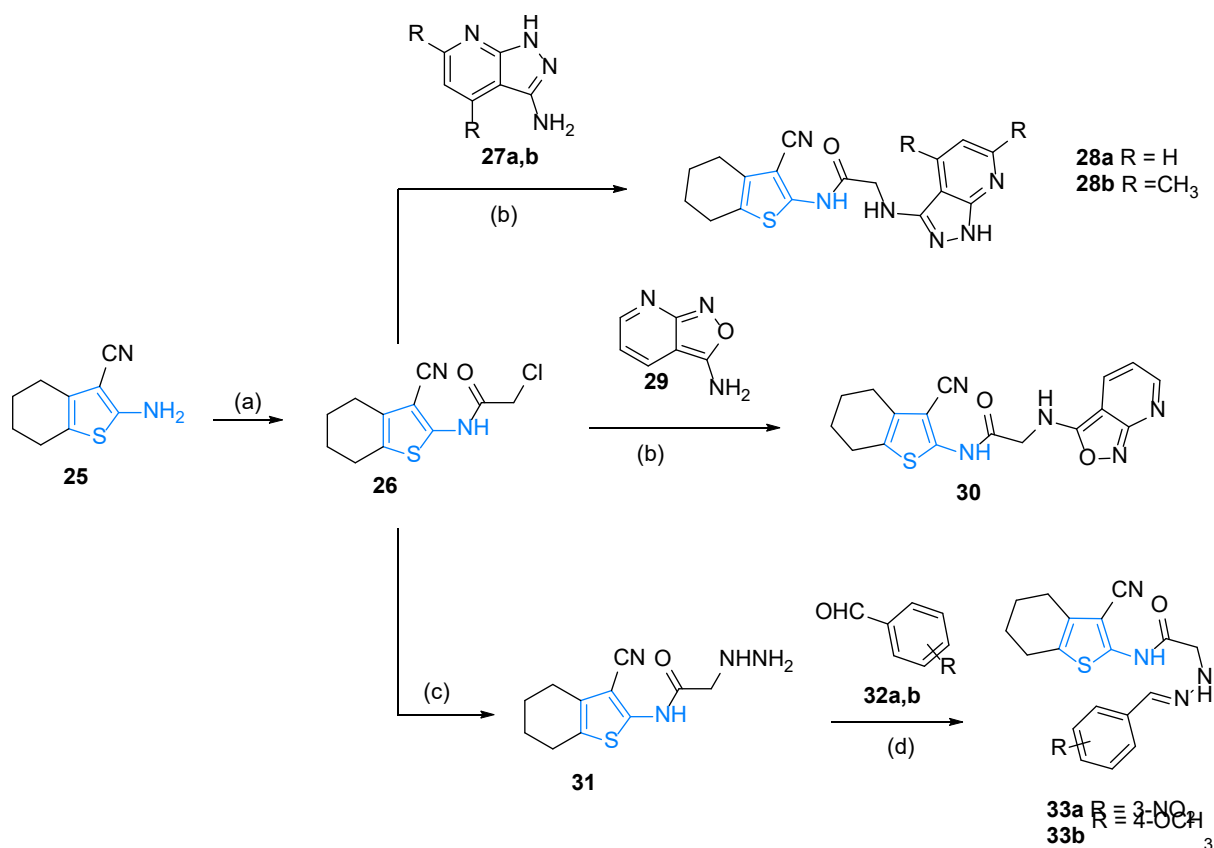


235
 236 **Scheme 5.** (a) malonodinitrile **2a**, *S*₈, *NEt*₃ in DMF, 60°C; (b) *Ac*₂*O* in *AcOH*, reflux, 5h; (c) ethyl
 237 cyanoacetate **2b**, fusion methodology; (d) acetaldehyde (1eq.) in *EtOH*; (e) acetaldehyde (2 eq.) in *EtOH*.

238 The authors compared their series (**20–24**) with activities of doxorubicin (reference
 239 compound) on HEPG2 (IC₅₀ range 19.14–91.46 μM) and MCF-7 (IC₅₀ range 26.16–88.48 μM)
 240 cancer cell lines (Scheme 5). Best compound **24** showed a slightly lower activity than
 241 doxorubicin (IC₅₀ range 19.14–26.16 μM for **24** and 4.17–4.50 μM for doxorubicin, Table 1),

making it a good starting point for further development as antiproliferative drugs on these two types of cancers. Concerning the SAR studies, only C2 and C5 positions can be discussed because C3 and C4 groups were conserved for **20–24**. Imine function in C2 seemed to be important for the antiproliferative activity ($IC_{50} = 19.14 \pm 1.9$ for **24** on HEPG2 against IC_{50} range 41.39–91.46 μM for **20–23** on HEPG2) as well as the bulky conjugated ketone in C5 position ($IC_{50} = 19.14 \pm 1.9$ μM for **24**, 67.50 ± 3.9 μM for **23** against IC_{50} range 41.39–91.46 μM for **20–22**).

In 2021, Elrayess and his team worked on synthesis of a series composed of 5 different 2-ATs derivatives (**28a,b**, **30** and **33a,b**) as potent epidermal growth factor receptor (EGFR) and human EGFR-related receptor 2 (HER2) targeting lung cancer.⁵⁹ The synthesis is illustrated in scheme 6. First, commercially available **25** reacted with chloroacetyl chloride in acetone at 0–5°C for 4h to give the N-substituted 2-AT **26**. Then, the nucleophilic substitution occurred between **26** and **27a,b** with triethylamine in DCM under reflux for 6–9 hours to give **28a,b**. In the same manner, reaction between **26** and **29** yielded **30**. Chloroacetyl **26** reacted with hydrazine hydrate in ethanol under reflux for 4 hours to afford compound **31**. Finally, desired products **33a,b** were obtained by reaction of **31** with **32a,b** using acetic acid in methanol under reflux for 5 hours. No yield mentioned for all steps.



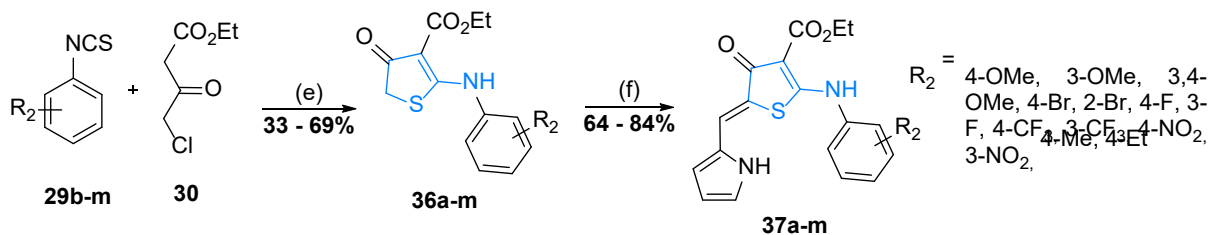
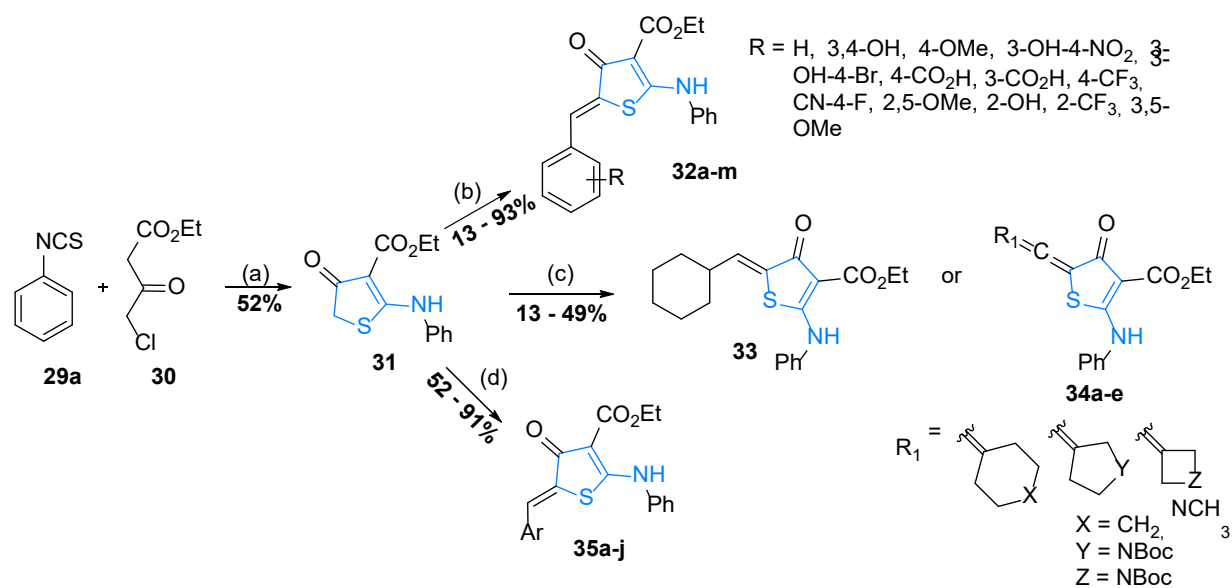
#	IC ₅₀ (nM)		
	H1299	EGFR	HER2
28a	12.50	0.47	0.14
28b	13.68	-	-
30	18.41	-	-
33a	18.53	-	-
33b	18.47	-	-
Gefitinib	40	1.9	-
Imatinib	-	0.11	0.06

Scheme 6. (a) chloroacetyl chloride in acetone, 0–5°C, 4h; (b) NEt₃ in DCM, reflux, 6 – 9h; (c) hydrazine hydrate in EtOH, reflux, 4h; (d) AcOH in MeOH, reflux, 5h.

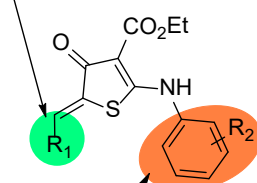
The series was evaluated on H1299 lung cancer cell line and displayed good results (IC₅₀ range 12.50–18.53 nM for **28a,b, 30** and **33a,b** and 40 nM for gefitinib the reference compound, Scheme 6). Compound **28a** showed the best activity on lung cancer cell lines H1299 with an IC₅₀ of 12.5 nM. Moreover, **28a** exhibited a significant inhibition with IC₅₀ of 0.47 and 0.14 nM on EGFR and HER2, respectively (Table 1). As an example, these values were comparable

to the FDA-approved drug imatinib (IC_{50} range 0.06–0.11 nM). Results were shown to be better than gefitinib (reference EGFR inhibitor, IC_{50} = 1.9 nM), and neratinib (reference EGFR and HER2 inhibitor). Also, to validate their model, authors realized a molecular docking that could explain the reason for the high affinity with both proteins. They found strong interactions between **28a** and important residues in the active site of both proteins. For comparison, **28a** exhibited a good glide energy model of – 77.03 kcal/mol on HER2 whereas neratinib value was inferior with – 52.6 kcal/mol. Finally, drug likeness of the molecule has been confirmed with *in silico* ADME prediction parameters and molecules showed good results as lead. Considering the SAR studies, authors noticed that the presence of cyclohexyl thiophene was determining because all the compounds exhibited activity (**28a,b**, **30** and **33a,b**). Moreover, the nitrile group grafted on C3 position allowed hydrogen bonding in the targeted protein, as well as the amide bond formed with amine function on C2 position. Also, compounds containing bulky heteroaryls showed best results (IC_{50} range 12.50–13.68 nM for **28a,b** against 18.41–18.53 nM for **30**, **33a,b**).

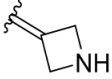
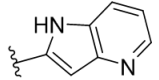
In 2022, Hwang *et al.* synthesized a series of 43 novel 2-ATs (**32a-m**, **33**, **34a-e**, **35a-j** and **37a-m**) as antiproliferative compounds aiming ribonuclease L (RNase L). RNase L is an endoribonuclease playing an important role in innate immunity, apoptosis, cell growth and differentiation. Its action consists in regulation and expression of cellular RNA.⁶⁰ The synthesis is shown in scheme 7. Reaction of commercially available thioisocyanates **29a** or **29b-m** with chloroketone **30** and sodium hydride in 1,4-dioxane for 30 min at room temperature afford 2-ATs **31** or **36a-m** in 33–69% yields after filtration and recrystallization from ethanol. The second step consisted in the reaction in Knoevenagel conditions between 2-ATs **31** or **36a-m** with the corresponding ketone or aldehyde, piperidine in ethanol under reflux to give the desired products **32a-m**, **33**, **34a-e**, **35a-j** or **37a-m** in 13–93% yields after purification by filtration and washes with ethanol or flash silica gel chromatography.



Cycloalkyl groups show better RNase-L binding results



Presence of substituent globally decrease the activity on RNase-L binding

#	R	Ar	R ₁	R ₂	Growth inhibition rate at 10 μ M (%) [IC ₅₀ in μ M] on human cancer cell lines			
					JAR	K562	A549	MCF7
32i	3-CN, 4-F	-	-	-	99.9 \pm 0.3 [1.5 \pm 0.1]	N.D. [1.6 \pm 0.3]	N.D. [8.0 \pm 0.8]	N.D. [5.2 \pm 2.0]
33	-	-	-	-	95.2 \pm 4.5 [3.7 \pm 0.8]	N.D. [5.9 \pm 1.1]	N.D. [18.0 \pm 0.8]	45.3 \pm 2.8 [6.4]
34e	-	-		-	70.2 \pm 5.3 [8.4 \pm 2.2]	N.D. [4.5 \pm 0.9]	N.D. [17.0 \pm 0.5]	40.4 \pm 1.6 [18.1]
35j	-		-	-	74.8 \pm 3.1 [9.6 \pm 0.9]	N.D. [6.6 \pm 0.7]	N.D. [13.6 \pm 0.4]	42.2 \pm 2.9 [N.D.]
37m	-	-	-	3-NO ₂	57.4 \pm 12.5 [10.1 \pm 2.9]	N.D. [87.2 \pm 0.5]	N.D. [23.9 \pm 3.8]	27.4 \pm 0.9 [20.7]

Scheme 7. (a) NaH, 1,4-dioxane, 40°C, 1h; (b) aryl aldehyde, piperidine, EtOH, reflux, 4 – 6h; (c) corresponding ketone or aldehyde, piperidine, EtOH, reflux, 4 – 24h; (d) piperidine, corresponding aldehydes, EtOH, reflux, 4 – 8h; (e) NaH, 1,4-dioxane, 40°C, 1h; (f) pyrrole-2-carbaldehyde, piperidine, EtOH, reflux, 2h.

From all the 43 derivatives synthesized, several compounds showed particularly good activities as antiproliferative compounds (Scheme 7). Human cancer cell lines chosen were: JAR (choriocarcinoma), K562 (leukemia), A549 (lung carcinoma) and MCF7 (breast adenocarcinoma). Compound **37m** showed fair activity on JAR and MCF7 cancer cell lines with a respective growth inhibition at 10 μ M of 57% and 27% (IC₅₀ range 10.1–20.7 μ M). Derivatives **34e** and **35j** showed good results on JAR and MCF7 cancer cell lines with growth inhibition of respectively 70% and 75% on JAR (IC₅₀ range 8.4–9.6 μ M) and 40% and 42% on MCF7 (IC₅₀ = 18.1 μ M for **34e**). Compounds **32i** and **33** displayed the best results with a growth inhibition on JAR of 95% for **33** and 100% for **32i** at 10 μ M (IC₅₀ range 1.5–3.7 μ M, Table 1). Globally compounds **32i** and **33** were the most promising leads for antiproliferative drugs development. Moreover, the antiproliferative property could be attributed to RNase L which is involved in apoptosis induction, as suggested by Hwang and co-workers. Considering the SAR studies, the authors evaluated the difference of RNase L thermal shift induced by the presence

311 of 60 μM of synthesized compounds. The authors observed that the presence of cycloalkyl
312 group was important in the antiproliferative activity *via* RNase-binding results (**33** and **34a-e**).
313 However, the presence of substituents on the phenyl group grafted on the amine did not show
314 importance in the binding affinity with RNase-L (**37a-m**).

315 In antibacterial part (Section 2.4.), **54d** will be briefly discussed for its antiproliferative activity
316 on HeLa cancer cell line. As these works are mainly about antibacterial compounds, they are
317 not pictured here.

2.3. Antiviral Activity

Viral pathogens are simple structures, unable to replicate itself without using the host's cellular machinery. Hence, these pathogens are obligate intracellular pathogens. Moreover, recent examples have highlighted the importance to develop an arsenal of antivirals to be able to fight back against novel viral strains.^{61,62}

In 2017, Desantis *et al.* investigated cycloheptathiophene-3-carboxamide scaffolds (**38** and **39**) as influenza polymerase subunits disruptors (Figure 3).¹⁸ The authors proposed the inhibition of RdRp (RNA-dependent RNA polymerase), which is a heterotrimeric complex composed of three distinct subunits: polymerase acidic protein (PA), polymerase basic protein 1 (PB1) and polymerase basic protein 2 (PB2). The strategy developed by the authors is to inhibit the PA-PB1 subunit to propose anti-influenza compounds.

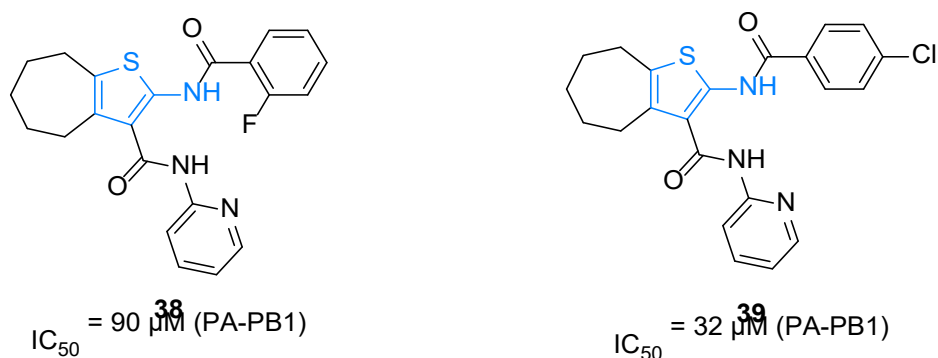
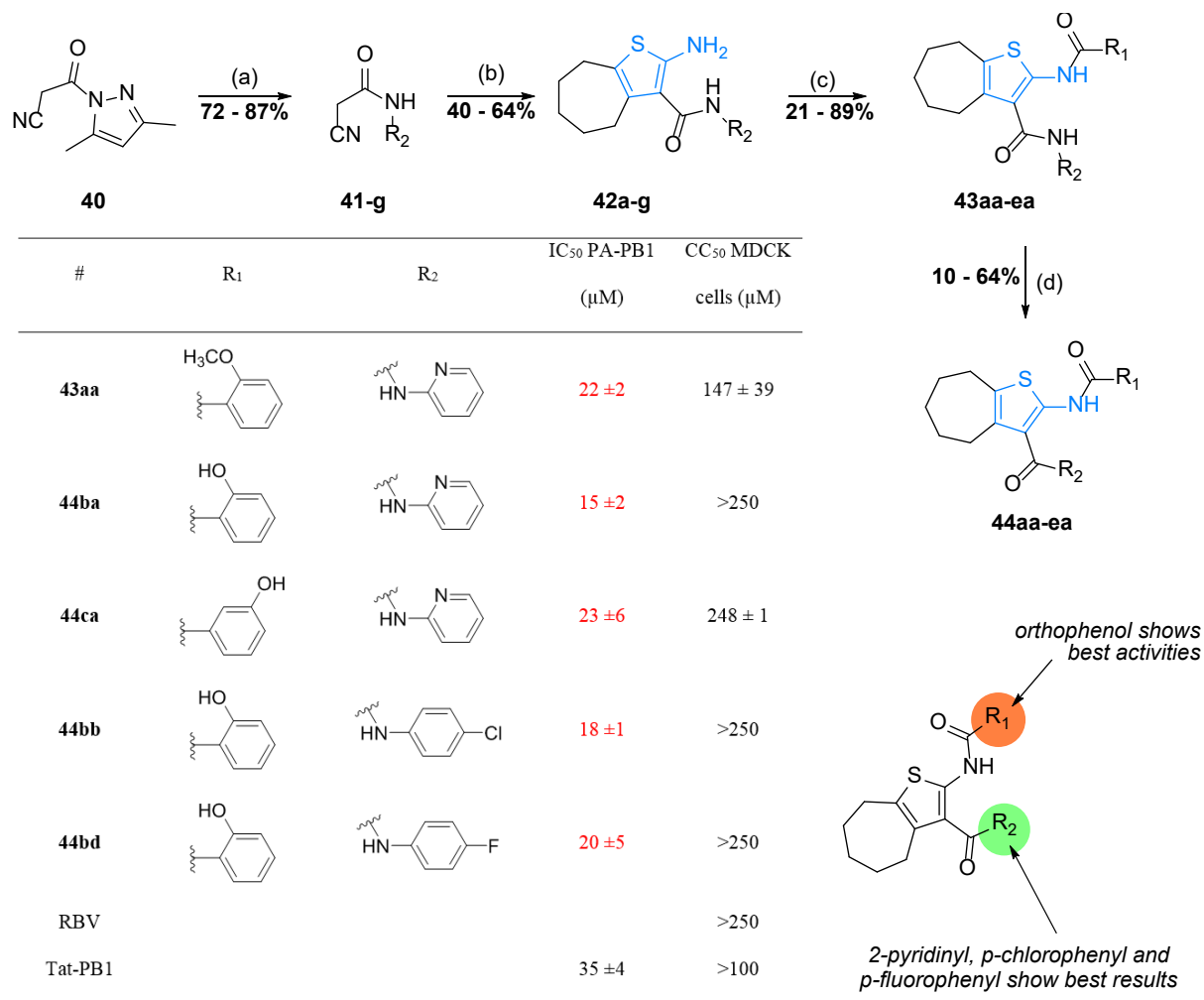


Figure 3. Known antiviral derivatives used as hits by Desantis *et al.*¹⁸

Based on **38** and **39** scaffolds that showed good activities (IC_{50} range 32–90 μM ; EC_{50} range 18–145 μM), the authors designed a large series of 21 new molecules (**44aa-ea**) whose synthesis is pictured in scheme 8. First, compound **40** reacted with substituted amines in toluene under reflux to afford **41a-g** in 72–87% yields after purification by precipitation and washes with Et₂O. Then, reaction of **41a-g** with cycloheptanone, ammonium acetate and acetic acid in benzene under reflux followed by addition of elemental sulfur S₈ with diethylamine in ethanol at 40–50°C led to **42a-g** in 40–64% yields and used without further purification. The third step

consisted in substitution of the amine function of **42a-g** with the corresponding acyl chloride in pyridine at room temperature to give **43aa-ea** with 21–89% yields after precipitation in cold water and filtration. The last step was the demethylation of **43aa-ea** with BBr₃ in DCM at room temperature to form the desired products **44aa-ea** in 10–64% yields after crystallization from ethanol/water.



Scheme 8. (a) R₂NH₂ in toluene, reflux; (b) cycloheptanone, NH₄OAc, AcOH in benzene, reflux then S₈, diethylamine in EtOH, 40 – 50 °C; (c) R₁COCl in pyridine, r.t.; (d) BBr₃, DCM, r.t.

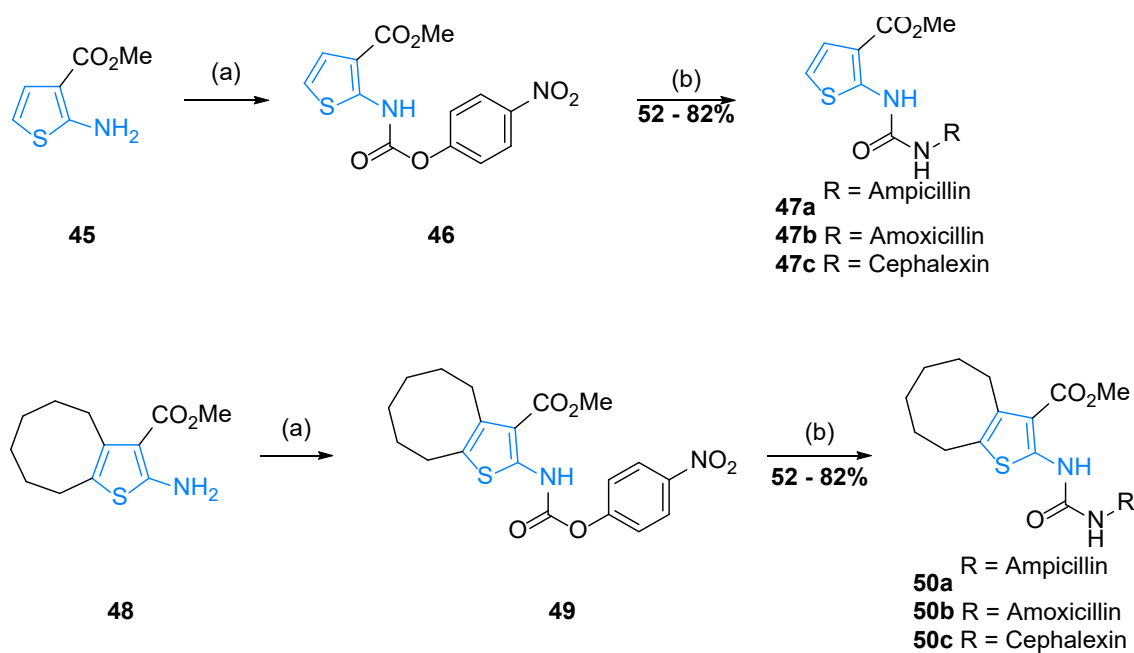
5 compounds (**43aa**, **44ba**, **44ca**, **44bb** and **44bd**) showed an IC₅₀ against PA-PB1 below 25 μM (Scheme 8). Among these 5 derivatives, **44bb** showed best activities with IC₅₀ on PA-

349 PB1 subunit of $18 \pm 1 \mu\text{M}$ (Table 1), which was better than RBV (1-D-ribofuranosyl-1,2,4-
350 triazole-3-carboxamide) and its IC_{50} of $35 \pm 4 \mu\text{M}$ (reference compound targeting RNA viruses'
351 polymerase). Moreover, none of the 5 compounds showed cytotoxicity with MTT assays on
352 MDCK cells (CC_{50} range $147 - >250 \mu\text{M}$). Considering the SAR studies, the derivatives having
353 *o*-phenol moiety were shown to be best (IC_{50} range $18-22 \mu\text{M}$). Presence of a 2-pyridinyl (**43aa**,
354 **44ba** and **44ca**) or *p*-halogeno group (**44bb** and **44bd**) as substituents on R_2 position, increased
355 antiviral activities. This study confirmed PA-PB1 as targets for innovative and potent anti-flu
356 agents.

2.4. Antibacterial Activity

The emergence of antibiotic-resistant organisms is a major public well-being concern, especially in health care centers.⁶³ Bacteria have succeeded in establishing resistance against antibiotics, making them resistant toward various classes of antibacterial compounds. The emergency to develop novel series possessing antimicrobial activities has never been more important.^{64,65} The World Health Organization (WHO) started to consider the bacterial resistance seriously, notifying the death toll would reach million in 2050, against 700 000 deaths in 2015⁶⁶ and 1.3 million in 2019.⁶⁷ To survive and adapt to environmental changes, bacteria developed different resistance mechanisms and changed in response to the use of antibiotics.

In 2018, Wagh and his team synthesized two series of 2-ATs as novel antibacterial compounds (**47a-c** and **50a-c**).⁶⁸ To enhance the global biological activity, they decided to couple 2-ATs with antibiotics, whose synthesis is disclosed in scheme 9. Ampicillin, amoxicillin and cephalexin were chosen as β -lactamine antibiotics. The first series has been formed from 2-AT **45**, using 4-nitrophenylchloroformate as coupling reagent and sodium hydrogen carbonate in acetonitrile at 0°C to room temperature for 8 hours to afford carbamate **46** in no specified yield. Then, **46** reacted with the corresponding aminopenicillins with diisopropylethylamine in DMF at room temperature for 2 hours to give **47a-c** in 52–82% yields. The second series was synthesized in the same manner from 2-AT **48** to give the desired products **50a-c** in 52–82% yields for the second step.



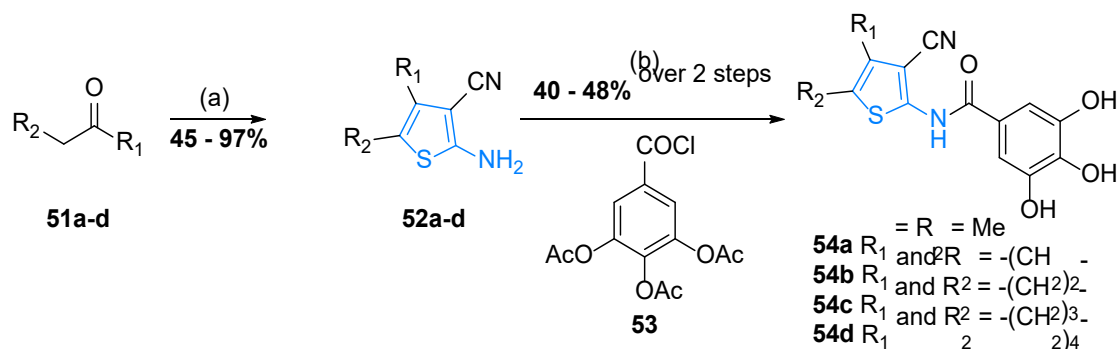
#	MIC ($\mu\text{g/mL}$) or EC ₅₀ (μM)						
	<i>S. aureus</i> MIC	EC ₅₀	<i>B. subtilis</i> MIC	EC ₅₀	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>Mtb</i> H ₃₇ Ra
47a	0.28	0.16	0.55	0.21	>10	>10	>50
47b	0.29	0.15	0.32	0.15	>10	>10	>50
47c	0.75	0.26	3.14	0.21	>10	>10	>50
50a	0.28	0.15	2.3	0.28	>10	>10	23.26
50b	0.28	0.16	2.57	0.25	>10	>10	>50
50c	0.46	0.18	2.35	0.26	>10	>10	0.782
Ampicillin	0.28	0.15	0.28	0.15	0.88	>10	/
Amoxicillin	0.29	0.15	0.32	0.15	0.32	>10	/
Cephalexin	2.39	0.77	1.87	0.25	4.94	>10	/
Rifampicin	/	/	/	/	/	/	0.042

Scheme 9. (a) 4-nitrophenylchloroformate, NaHCO₃ in ACN, 0°C – r.t., 8h; (b) aminopenicillins, DIPEA, DMF, r.t., 2h.

They observed the antimicrobial activities through minimal inhibitory concentration (MIC) and half-maximal effective concentration (EC₅₀) on 4 different strains: *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Mycobacterium tuberculosis* (*Mtb*). Generally, antimicrobial activities

stayed close to that of reference antibiotics (Scheme 9). The authors showed that several compounds showed better antimicrobial behaviors than free reference antibiotics (Ampicillin, amoxicillin and cephalixin): **47c**, **50c** (MIC of 0.75 $\mu\text{g/mL}$ for **47c** and 0.46 $\mu\text{g/mL}$ for **50c** compared to 2.39 $\mu\text{g/mL}$ for cephalixin on both *S. aureus* and *B. subtilis* strains, Table 1). EC_{50} were also improved (EC_{50} of 0.26 μM for **47c** and 0.18 μM for **50c** compared to 0.77 μM for cephalixin on *S. aureus*). On *B. subtilis*, **50c** also showed a better EC_{50} (EC_{50} of 0.21 μM against 0.25 μM for cephalixin). Other derivatives kept same activities range on different bacterial strains. Considering the antitubercular activity, the authors observed that **50c** showed a good activity with MIC of 0.78 $\mu\text{g/mL}$ on *Mtb* H₃₇ RA strain. They also conducted toxicity evaluations: none of the tested compounds exhibited cytotoxic activities on human cancer cell lines (Hela, PC-3 and THP-1). Computational predictions were realized. Compound **50c** has been shown to fit in the PBP pocket from penicillin G-binding site. Probably, the 2-AT moiety did not add too much steric hindrance that would lower the activity. Rather, the 2-AT scaffold contributed to the global biological activity by interacting with residues of the binding domain, such as serine in position 398 (Ser398). Finally, compound **50c** was shown to be suitable for further development as antitubercular drugs.

In 2020, Mahdavi *et al.* worked on a novel 2-ATs series (**54a-d**) as antibacterial, synthesized in two steps as depicted in scheme 10.⁶⁹ The first step was the synthesis following classical Gewald conditions consisting in the reaction of malonodinitrile **2a** with ketones **51a-d** to afford 2-ATs **52a-d** in 45–97% yield after filtration and recrystallization from ethanol. Secondly, **52a-d** reacted with acyl chloride **53** and triethylamine in DCM at 0°C for 2 hours followed by the addition of hydrazine hydrate in DCM at room temperature for 1 hour (cleavage of the acetates of the gallic acid moiety) to give amides **54a-d** in 40–48% yields after recrystallization from methanol/water.



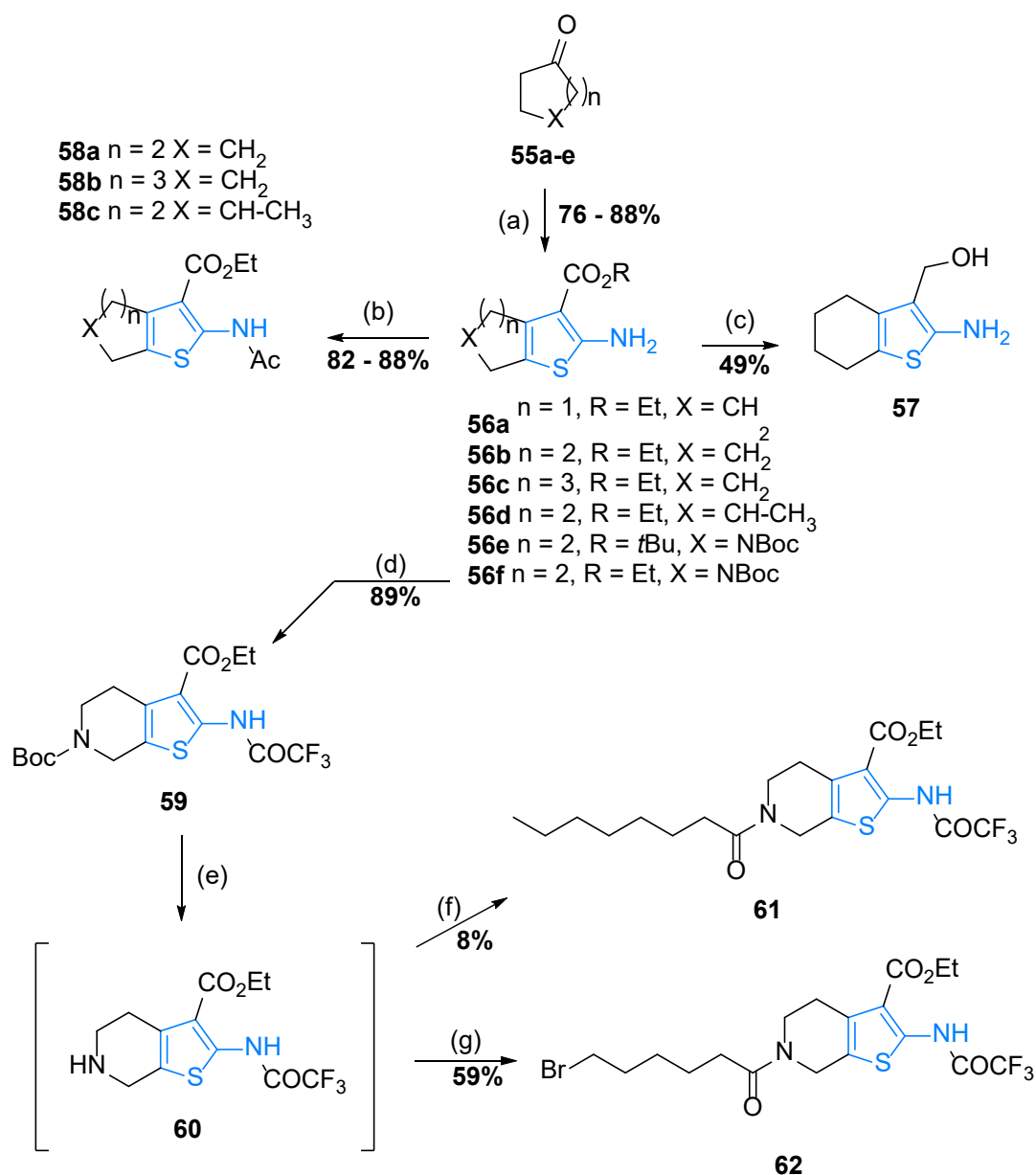
#	Gram-positive MIC (µg/mL)			Gram-negative MIC (µg/mL)			
	<i>S. aureus</i>	<i>S. coagulase</i>	<i>E. faecalis</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. vulgaris</i>	<i>P. aeruginosa</i>
54a	0.41 ± 0.14	0.15 ± 0.01	0.25 ± 0.00	NA	0.25 ± 0.00	0.17 ± 0.07	NA
54b	0.21 ± 0.07	0.10 ± 0.03	0.25 ± 0.00	NA	0.17 ± 0.07	0.17 ± 0.07	NA
54c	0.21 ± 0.07	0.15 ± 0.01	0.17 ± 0.07	NA	0.41 ± 0.14	0.10 ± 0.03	NA
54d	0.21 ± 0.07	0.15 ± 0.01	0.25 ± 0.00	NA	0.17 ± 0.07	0.15 ± 0.01	NA
Chloramphenicol	0.03 ± 0.01	0.41 ± 0.14	0.05 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.01

Scheme 10. (a) malonodinitrile **2a**, *S*₈, morpholine in EtOH, r.t.; (b) **53**, NEt₃, DCM, 0°C, 2h then hydrazine hydrate, r.t., 1h.

Products (**54a-d**) were evaluated on 3 different Gram-positive strains: *S. aureus*, *Staphylococcus coagulase* (*S. coagulase*) and *Enterococcus faecalis* (*E. faecalis*); and 4 Gram-negative strains: *E. coli*, *Klebsiella pneumoniae* (*K. pneumoniae*), *Proteus vulgaris* (*P. vulgaris*) and *P. aeruginosa* (Scheme 10). Chloramphenicol, a known bacteriostatic antibacterial has been chosen as reference control. Good antibacterial activities were observed on all Gram-positive strains (MIC range 0.10–0.41 µg/mL for **54a-d** compared to 0.03–0.41 µg/mL for chloramphenicol). Considering Gram-negative strains, all products were shown to be active on *K. pneumoniae* and *P. vulgaris* (MIC range 0.10–0.41 µg/mL for **54a-d** compared to 0.03 µg/mL for chloramphenicol); but inactive on *E. coli* and *P. aeruginosa*. In conclusion, results were better than chloramphenicol (reference compound) only for *S. coagulase* (MIC range 0.10–0.15 µg/mL for **54a-d** against 0.41 µM for chloramphenicol), making this series interesting for further development against this bacterial strain specifically (Table 1). All compounds were also evaluated following MTT assay on HeLa, HCT116 and FT cancer cell

lines. Compound **54d** showed a good IC₅₀ on HeLa cell line with 3.2 μ M, making it interesting for further development for cervical cancer. SAR studies were not examined by the authors.

In 2020, da Cruz *et al.* described the synthesis of 2-ATs (**56a-f**, **58a-c**, **57**, **59**, **61** and **62**) as *S. aureus* efflux pump inhibitors (Scheme 11).⁷⁰ By analogy with former works,^{71,72} they decided to synthesize analogs to inhibit NorA efflux pumps of *S. aureus* 1199B strain. The first step was the formation of N-substituted 2-ATs following Gewald conditions. Ketones **55a-e** reacted with alkyl cyanoacetates **2b** or **2d**, elemental sulfur S₈ and morpholine in ethanol to give N-acetyl-2ATs **56a-f** in 76–88% yields after filtration and washes with water. Then, the second step was the Bouveault-Blanc ester reduction of **56a** in the presence of metallic sodium in dry methanol to afford **57** in 49% yield after purification on silica gel column chromatography. In another step, the reaction of **56b-d** with anhydride acetic in 1,4-dioxane at 80–90°C for 3 to 4 hours led to **58a-c** in 82–88% yields after filtration and washes with water. In the last step, **56f** reacted with trifluoroacetic anhydride and trimethylamine in DCM at 0–5°C for 2 hours to give **59** in 89% yield after washes with cold ethanol. The intermediate **60** was obtained from **59** in the presence of a mixture of DCM and TFA (8/2) at 0–5°C for 1 hour and further used without prior purification. **60** reacted with octanoyl chloride and DIPEA in DCM at 0–5°C for 1–2 hours to afford **61** in 8% yield after purification after washes with ice cold ethanol. Reaction with **60** and 6-bromo hexanoyl chloride gave **62** in 59% yield after washes with cold ethanol.



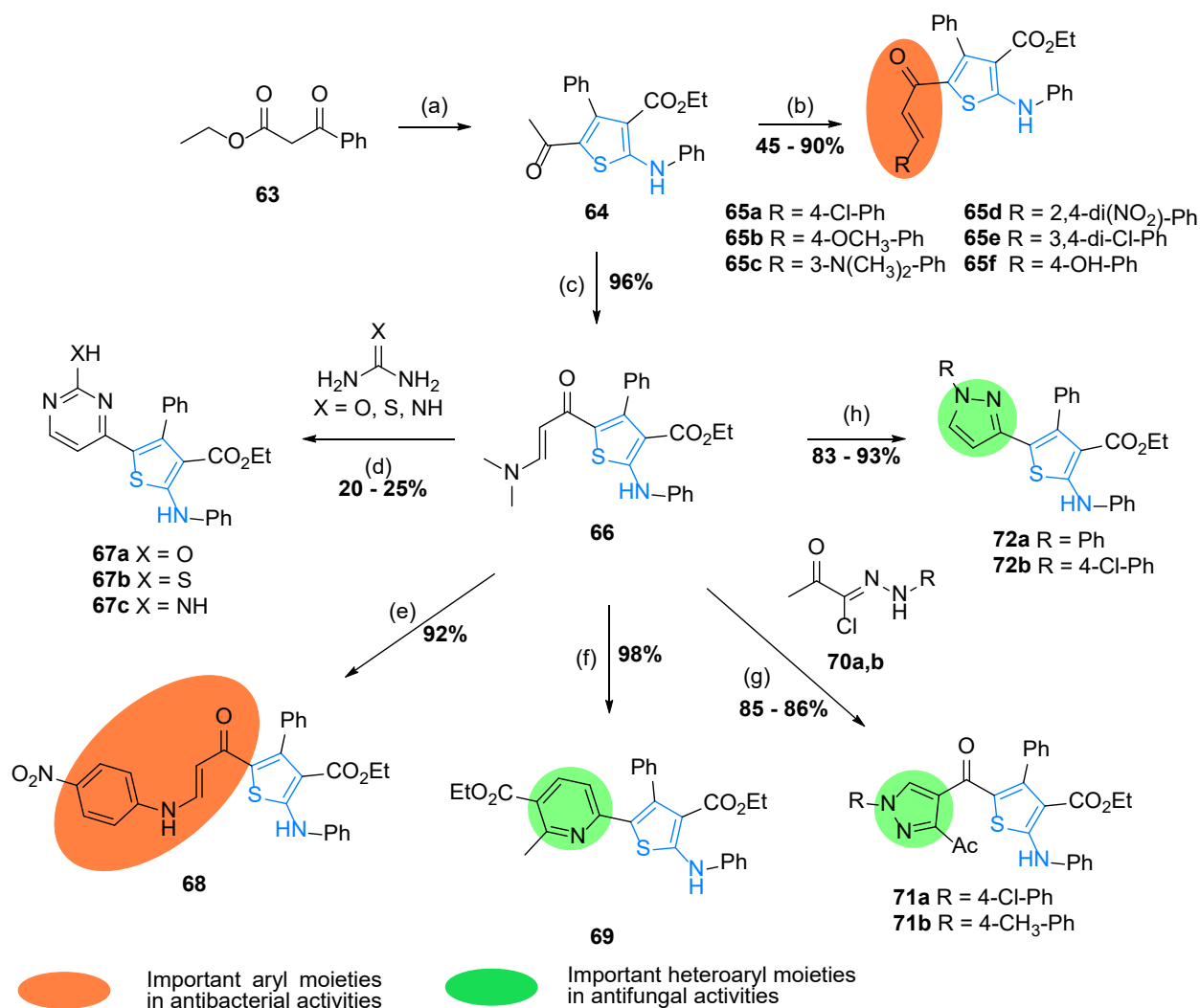
443

444 **Scheme 11.** (a) ethyl cyanoacetate **2b** or tert-butyl cyanoacetate **2d**, S_8 , morpholine in EtOH, 50–60°C, 2 –
 445 3h; (b) acetic anhydride in 1,4-dioxane, 80 – 90°C, 3 – 4h; (c) Na(s) in MeOH, 2h; (d) trifluoroacetic
 446 anhydride in DCM, 0 - 5°C, 2h; (e) DCM/TFA (8/2), 0 – 5°C, 1h; (f) DIPEA, octanoyl chloride in DCM, 0 –
 447 5°C, 1 – 2h; (g) DIPEA, 6-bromo hexanoyl chloride in DCM, 0 – 5°C, 1 – 2h.

448 These compounds have been evaluated in the presence of ciprofloxacin against
 449 ciprofloxacin-resistant SA-1 strain and with erythromycin against erythromycin-resistant RN-
 450 4220 strain. Compounds **58b**, **59**, **61** and **62** showed a partial restoration of sensitivity on SA-1
 451 strain toward ciprofloxacin by reducing the MIC by 4 to 8-fold. As comparison, reserpine, a

known NorA efflux pump inhibitor restore the activity by 16-fold. These four compounds (**58b**, **59**, **61** and **62**) were evaluated following the ethidium bromide (EtBr) accumulation and efflux assays as NorA efflux pump inhibitors. **62** was shown to have similar results as reserpine for accumulation assay (125% EtBr accumulation at 50 μ M for **62** and 110% for reserpine, Table 1). EtBr is a NorA efflux pump substrate, its accumulation in the bacteria indicates an inhibition of the efflux pump. **61** was the only one to show an inhibition of NorA efflux pump by preventing EtBr efflux by 85% over 30 minutes at 50 μ M (Table 1).

In 2021, Asiri *et al.* described the design, the synthesis and the antimicrobial activity of novel 2-ATs (**65a-f**, **67a-c**, **68**, **69**, **71a,b** and **72a,b**).⁷³ The first step of the synthesis pictured in scheme 12 was inspired by Mabkhot methodology⁷⁴ and consisted in the reaction of **63** with potassium carbonate and phenyl isothiocyanate followed by addition of chloroacetone to afford 2-AT **64** in 95% yield after filtration and recrystallization from ethanol. **65a-f** were synthesized by condensing corresponding aldehydes with **64** in EtOH with triethylamine under reflux for 8 hours in 45–90% yields. Reaction of **64** with DMA in DMF under reflux for 8 hours yielded to **66** in 96%. **66** is a central scaffold used to form different series. **67a-c** were synthesized by reaction of ureas with **66** in 20–25% yields. **66** reacted with 4-nitroaniline under reflux for 6 hours to give **68** in 92% yield. Pyridine core synthesis on **66** with AcOH/AcONH₄ and ethyl acetoacetate under reflux for 3 hours led to **69** in 98% yield. As well, **66** reacted with **70a,b** in dry benzene/TFA to afford **71a,b** in 85–86% yields. Finally, reaction of **66** with corresponding hydrazine derivatives under reflux for 6 hours to lead to **72a,b** in 83–93% yields. All the products were purified by filtration followed by recrystallization from ethanol.



#	Gram-positive MIC (μg/mL)		Gram-negative MIC (μg/mL)	
	<i>S. pneumoniae</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
65a	19.5 ± 0.44	29.8 ± 0.58	12.3 ± 0.25	17.6 ± 0.19
65b	18.9 ± 0.63	17.9 ± 0.49	11.4 ± 0.27	10.2 ± 0.41
65c	16.3 ± 0.52	19.6 ± 0.58	12.5 ± 0.39	14.8 ± 0.47
65d	16.5 ± 0.47	18.9 ± 0.53	11.3 ± 0.45	9.9 ± 0.41
65e	11.3 ± 0.52	10.7 ± 0.39	9.7 ± 0.42	8.9 ± 0.31
65f	26.7 ± 0.74	21.4 ± 0.58	18.7 ± 0.68	19.5 ± 0.64
67a	16.3 ± 0.42	19.1 ± 0.51	11.4 ± 0.36	10.7 ± 0.31
67b	12.3 ± 0.58	12.7 ± 0.37	9.8 ± 0.34	11.3 ± 0.39
67c	16.3 ± 0.42	19.1 ± 0.51	11.4 ± 0.36	10.7 ± 0.31
68	26.3 ± 0.73	20.9 ± 0.61	12.6 ± 0.54	11.2 ± 0.44
69	18.2 ± 0.68	18.9 ± 0.64	10.8 ± 0.41	11.1 ± 0.43
71a	14.6 ± 0.58	14.3 ± 0.58	10.2 ± 0.31	8.8 ± 0.24
71b	12.3 ± 0.58	12.7 ± 0.37	9.8 ± 0.34	11.3 ± 0.39
72a	12.3 ± 0.58	12.7 ± 0.37	11.6 ± 0.36	10.9 ± 0.21
72b	18.2 ± 0.68	18.9 ± 0.64	10.8 ± 0.41	11.1 ± 0.43
Ampicillin	23.8 ± 0.2	32.4 ± 0.3	-	-
Gentamicin	-	-	17.3 ± 0.1	19.9 ± 0.3

Scheme 12. (a) K₂CO₃, PhNCS in DMF, 25°C then chloroacetone; (b) RCHO, NEt₃ in EtOH, reflux, 8h; (c)

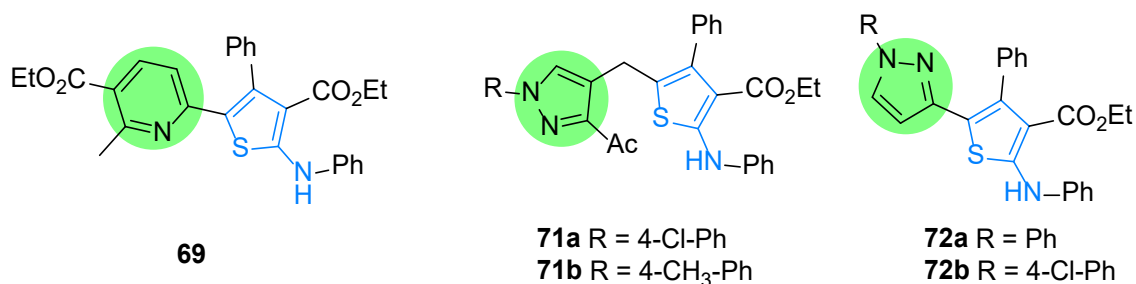
476 DMF/DMA, reflux, 8h; (d) EtOH, reflux, 6h; (e) 4-nitroaniline, reflux, 6h; (f) Ethyl acetoacetate, AcOH,
477 AcONH₄, reflux, 3h.; (g) dry benzene, NEt₃, reflux, 6h; (h) RNHNH₂, reflux, 6h.

478 These series have been evaluated as antifungal (See section antifungal below) and
479 antimicrobial (*Streptococcus pneumoniae* (*S. pneumoniae*), *B. subtilis* for Gram-positive and
480 *P. aeruginosa*, *E. coli* for Gram-negative strains, Scheme 12). Among these 15 different
481 molecules, 4 displayed a particularly interesting biological activity. Compounds **65e** and **67b**
482 showed better antibacterial activities than ampicillin and gentamicin on both Gram-positive
483 (MIC range 10.7–11.3 µg/mL for **65e** and 12.3–12.7 µg/mL for **67b**; Table 1) and Gram-
484 negative strains (MIC range 8.9–9.7 µg/mL for **65e** and 9.8–11.3 µg/mL for **67b**). Compounds
485 with α,β -unsaturated ketone with phenyl moiety (compounds **65** and **68**) exhibited better
486 antibacterial properties than antifungals.

2.5. Antifungal Activity

Fungal infections are associated with significant morbidity and mortality. Existing antifungal agents are frequently limited by dose-limiting toxicity, drug interactions or drug resistance, necessitating the development of new antifungals, particularly those with novel mechanisms of action.^{75,76}

Firstly, previously described compounds (**69**, **71a,b** and **72a,b**) by Asiri *et al.*⁷³ (see scheme 12) were evaluated on 4 fungal strains: *Aspergillus fumigatus* (*A.fumigatus*), *Syncephalastrum racemosum* (*S. racemosum*), *Geotrichum candidum* (*G. candidum*) and *Candida albicans* (*C. albicans*). These 3 derivatives showed better antifungal activities than amphotericin B (the reference antifungal) especially against *G. candidum* and *C. albicans* (MIC range 11.2–19.1 µg/mL against 19.7–28.7 µg/mL for amphotericin B, Figure 4, Table 1). The authors noticed that the nature of heterocycle was important in the antifungal activity.



#	Fungal strains			
	<i>A. fumigatus</i>	<i>S. racemosum</i>	<i>G. candidum</i>	<i>C. albicans</i>
65a	22.3 ± 0.25	16.5 ± 0.25	25.8 ± 0.58	12.3 ± 0.35
65b	23.4 ± 0.65	16.1 ± 0.53	10.8 ± 0.36	11.9 ± 0.34
65c	24.3 ± 0.68	24.5 ± 0.64	25.8 ± 0.58	14.3 ± 0.58
65d	18.9 ± 0.63	19.6 ± 0.68	14.8 ± 0.65	13.4 ± 0.65
65e	17.7 ± 0.48	15.4 ± 0.38	12.9 ± 0.47	13.2 ± 0.58
65f	15.7 ± 0.36	11.2 ± 0.33	17.3 ± 0.44	13.3 ± 0.36
67a	18.2 ± 0.56	13.7 ± 0.39	19.1 ± 0.45	18.2 ± 0.44
67b	15.7 ± 0.47	16.6 ± 0.62	12.6 ± 0.38	12.7 ± 0.67
67c	18.2 ± 0.56	13.7 ± 0.39	19.1 ± 0.45	18.2 ± 0.44
68	12.6 ± 0.38	16.5 ± 0.25	25.8 ± 0.58	10.9 ± 0.4
69	26.3 ± 0.73	20.9 ± 0.61	12.6 ± 0.54	11.2 ± 0.44
71a	16.3 ± 0.35	14.8 ± 0.46	15.3 ± 0.52	14.3 ± 0.58
71b	15.7 ± 0.47	16.6 ± 0.62	12.6 ± 0.38	12.7 ± 0.37
72a	15.9 ± 0.62	18.9 ± 0.58	19.1 ± 0.54	15.8 ± 0.38
72b	26.3 ± 0.73	20.9 ± 0.61	12.6 ± 0.54	11.2 ± 0.44
Amphotericin B	23.7 ± 0.1	19.7 ± 0.2	28.7 ± 0.2	25.4 ± 0.1

Figure 4. Structures of compounds **69**, **71a,b** and **72a,b** and antifungal activities.

In 2021, Luna *et al.* developed a series of 17 different 2-ATs (**75a-q**) as antifungal compounds bearing an imine function.⁷⁷ To do so, they designed their series based on a 2-AT named 6CN10 (Figure 5). The synthesis is further described in scheme 13.

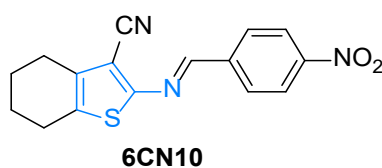
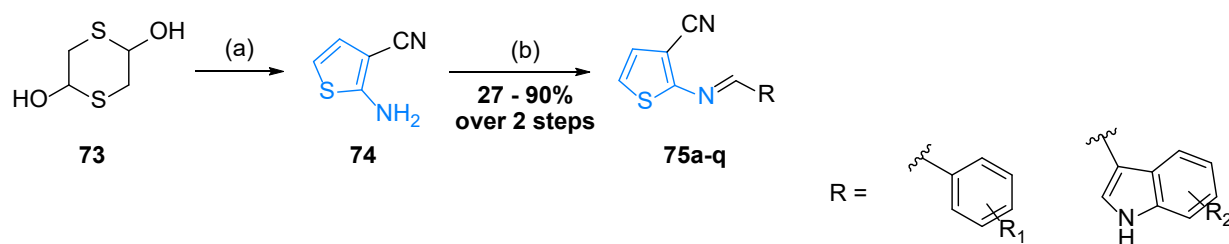


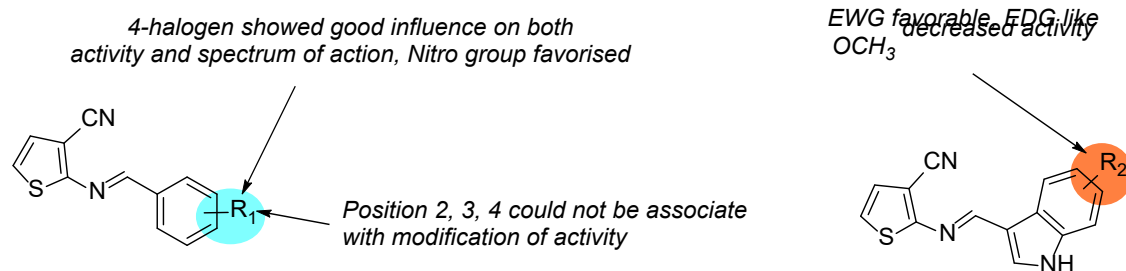
Figure 5. Structure of **6CN10**.

Desired Schiff bases **75a-q** were synthesized under microwave irradiation in presence of commercially available 1,4-dithiane-2,5-diol **73** with malonodinitrile **2a** and sodium bicarbonate in ethylene glycol at 80°C for 8 minutes to give transitorily **74**. Imination of **74**

was realized with corresponding aryl aldehydes and acetic acid at 50°C for 2 minutes to afford **75a-q** in 27–90% yield after purification by precipitation in water/methanol.



#	R ₁	R ₂	<i>C. parapsilosis</i> ATCC 22019	<i>E. floccosum</i> 6999	<i>E. floccosum</i> 6754	<i>T. tonsurans</i> 700	<i>T. tonsurans</i> 2822	<i>T. mentagrophytes</i> 5431	<i>T. mentagrophytes</i> 5432	<i>T. rubrum</i> 6753
75e	2,6-Cl	-	G	G	G	64	256	G	G	32
75f	2-NO ₂	-	128	32	G	G	G	G	G	G
75g	3- NO ₂	-	G	64	G	G	G	G	G	128
75k	4- NO ₂	-	1024	1024	512	512	G	G	G	64
75l	3,4,5- OCH ₃	-	G	256	G	128	G	256	128	64
75m	H	-	G	16	512	32	32	G	256	G
75o	4-Br	-	128	512	512	64	1024	128	1024	32
75p	-	4-NO ₂	G	1024	G	128	512	G	512	64



Scheme 13. (a) malonodinitrile **2a**, NaHCO₃ in ethylene glycol, M.W., 80°C, 8 min; (b) aryl aldehyde in AcOH, M.W., 50°C, 2 min. G: bacteria growth observed at 1024 µg/mL

These products have been evaluated on 8 different fungal strains: *Candida parapsilosis* (*C. parapsilosis*) ATCC 22019, *Epidermophyton floccosum* (*E. floccosum*) 6999 and 6754, *Trichophyton tonsurans* (*T. tonsurans*) 700 and 2822, *Trichophyton mentagrophytes* (*T. mentagrophytes*) 5431 and 5432 and *Trichophyton rubrum* (*T. rubrum*) 6753 (Scheme 13). Among 17 derivatives, 8 exhibited good antifungal activity (**75e-g**, **75k-m**, **75o,p**). **75e,f**, **75m** and **75o** showed correct antifungal activities (MIC range 16–32 µg/mL) on 4 fungal strains (*E. floccosum* 6999, *T. tonsurans* 700 and 2822 and *T. rubrum* 6753). Among the 8 best

derivatives, none exhibited antifungal activities on *C. parapsilosis*, *E. floccosum* 6754 and both *T. mentagrophytes* 5431 and 5432. Considering the SAR, when a halogen was grafted on position 4 of the phenyl moiety, compounds showed broad activities on several strains (750, Table 1). The benzylidene moiety demonstrated best antifungal activities but no substituent was attributed as determining for the action. Considering the indolyl substituent, EWG groups presented best results. Presence of electron-donating group (EDG) was associated to a loss of the activity when methoxy group was present on indolyl group.

In 2022, da Silva Oliveira *et al.* presented the possibility to insert 6CN (Figure 6) inside chitosan polymers (6CN-chitosan).⁷⁸ The polymer was prepared following a solvent casting method between a 1% polymer solution (w/v) and a 1% chitosan solution in acetic acid (v/v) under stirring for 24 hours at room temperature. These 6CN-chitosan films were characterized by FT-IR, DSC, TGA, XRD and SEM. These films were evaluated as antifungal on *C. albicans*, *C. tropicalis* and *C. parapsilosis*. On films with the highest percentage of 6CN (1.28 mg/mL), antifungal activity was maximal with the diameter of the zone of inhibition of 1.5 mm around the film, used as the discs (Table 1).

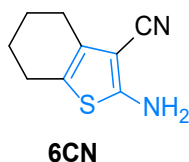


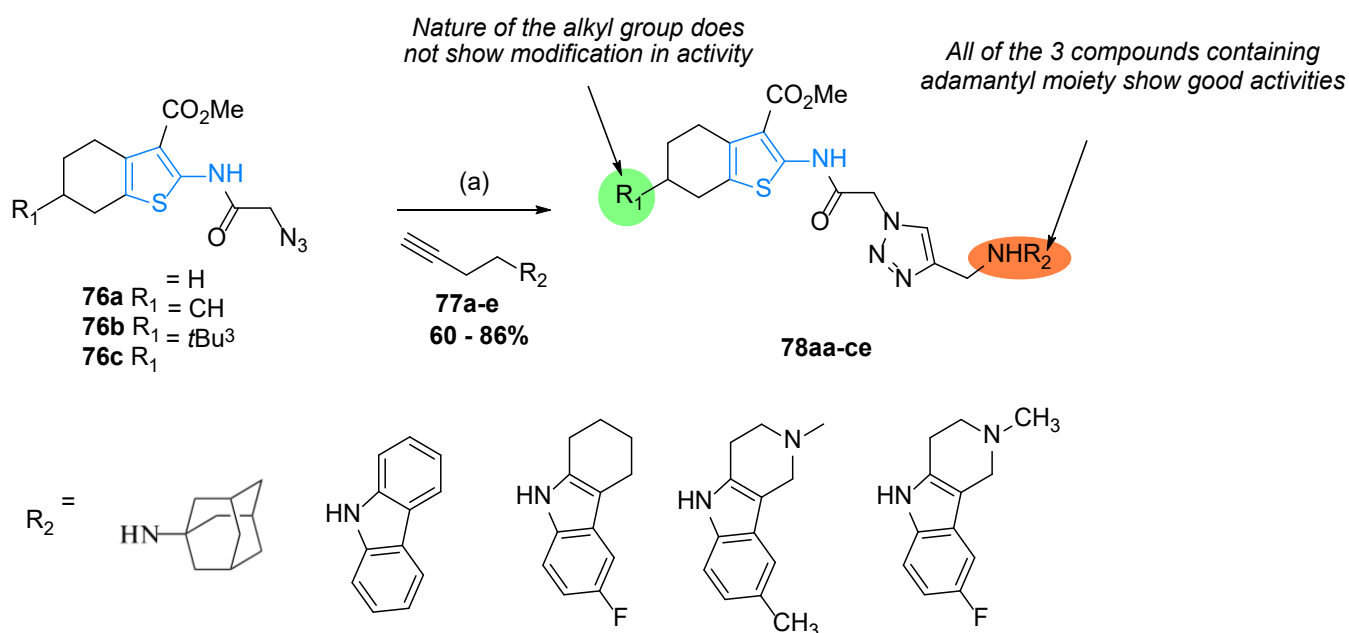
Figure 6. Structure of 6CN.

6CN was inserted between two sheets of chitosan and the authors suggested that the structure realized the following interactions: nitrile function of 6CN interacted with the amine function of chitosan and interactions were observed between the polymer carbonyl and the 6CN amino group on position 2.

2.6. Channels inhibitors activity

T-type calcium channels are attractive targets for potential treatment of epilepsy inflammatory or neuropathic pain, insomnia, Parkinson's disease, and cancer. Three isoforms of these T-type calcium channels are expressed in peripheral and central nerve and responsible for different biophysical functions.^{79–81}

In 2020, Zamoyski *et al.* synthesized new N-substituted 2-ATs (**78aa-ce**) as calcium-activated chloride channels (CACC) modulators (Scheme 14).⁸² The synthesis followed a Huisgen methodology in the presence of 2-AT **76a-c** and alkynes **77a-e** to afford triazoles **78aa-ce** in 60–86% yields after purification on flash chromatography.⁸³



Scheme 14. (a) Cu_2SO_4 , sodium ascorbate, DCM/ H_2O , 3h, 40°C.

All 3 compounds containing adamantyl moiety exhibited biological effect on the targeted channel: **78ca** displayed potentiation on CaCC of 35% at 10 μM , **78aa** showed an IC_{50} of $1.6 \pm 0.4 \mu\text{M}$ and **78ba** an IC_{50} of $5.4 \pm 0.5 \mu\text{M}$ whereas the other derivatives showed no effect on the protein. Adamantane moiety could be crucial in the biological activity because all compounds possessing one can modulate the activity of CACC. The same team worked on other 2-ATs

derivatives (**79–84**, Figure 7). The authors used previously reported 2-ATs whose synthesis was not depicted. They evaluated the biological activity of **80–84** and compared them to the known CaCC_{inh} A01 **79** that is a good CaCC inhibitor ($IC_{50} = 1.7 \mu M$).⁸⁴ The tested compounds (**79–84**) showed good results (IC_{50} range on CaCC channels 4.6–49.7 μM). Compound **80** demonstrated best results with good inhibition at 1.0 μM and an IC_{50} of 4.6 μM (Table 1). Compound **81** illustrated antagonistic activity on the channel by increasing CaCC current by 40% at the concentration of 10 μM .

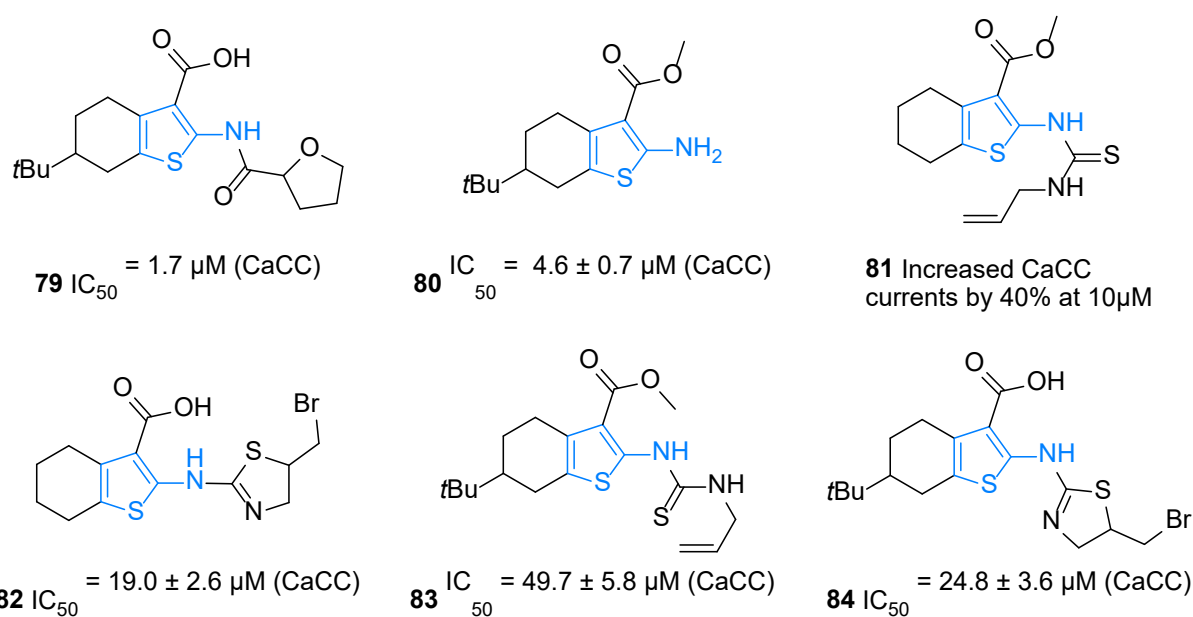
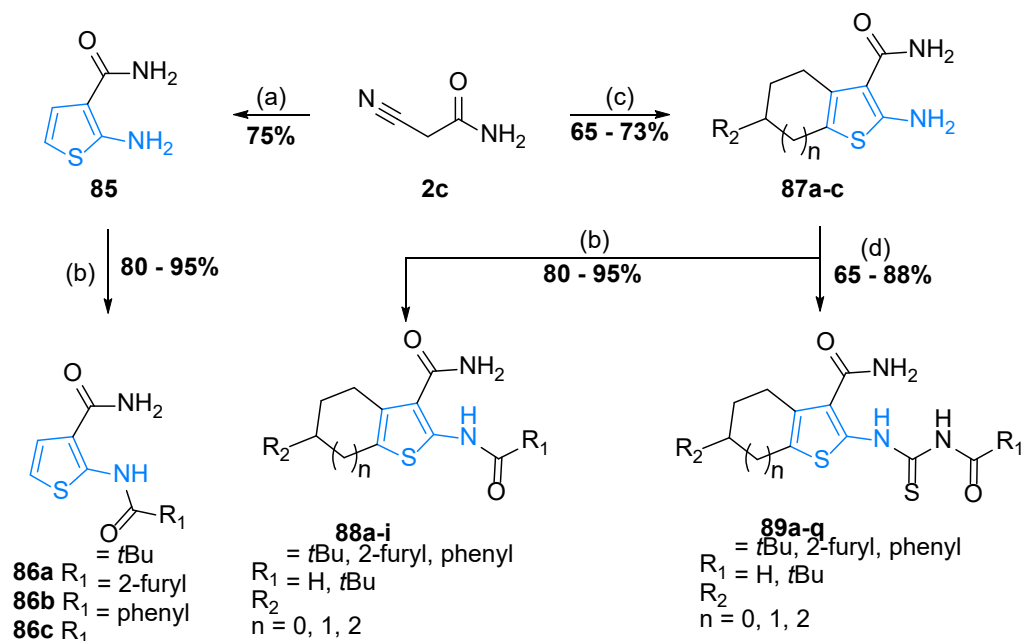


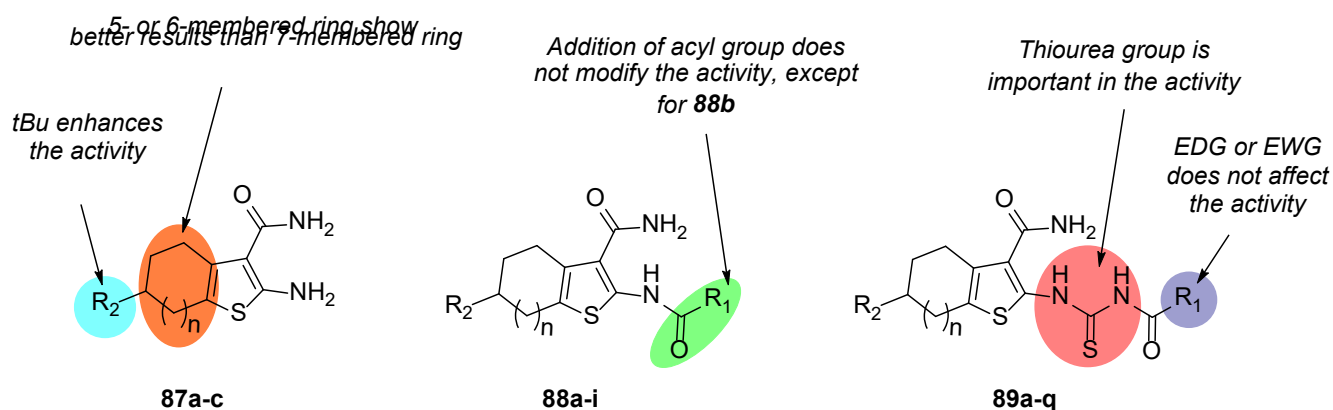
Figure 7. Structures and activities of compounds **79–84**.

In 2020, Choi and his team developed the synthesis of 2-ATs (**86a-c**, **88a,i** and **89a-q**) as a potential anti-glioma, inhibitors of Anoctamin-1 (ANO1) channels (CaCCs), known to be overexpressed in these cancers.⁸⁵ The synthesis for this series is shown in scheme 15. All the 2-ATs derivatives were synthesized in classical Gewald way. On the one hand, starting with commercially available 2-cyanoacetamide **2c** with 1,4-dithiane-2,5-diol **73** and triethylamine in ethanol at 70°C for 6 hours afforded **85** in 75% yield after crystallization with Et₂O in DCM. Then, **85** reacted with corresponding acyl chloride in pyridine at room temperature for 1 hour to yield **86a-c** in 80–95% after purification on flash silica gel chromatography. On the other

576 hand, reaction of cyanoacetamide **2c** with the corresponding ketone with elemental sulfur S₈,
577 morpholine in ethanol at 70°C for 12 hours gave 2-ATs **87a-c** in 65–73% yields after
578 crystallization with Et₂O in DCM. Thereafter, **87a-c** was poured with various acyl chloride in
579 pyridine at room temperature for 1 hour to form **88a-i** in 80–95% after purification on flash
580 chromatography. Finally, **89a-q** were prepared from **87a-c**, acyl chloride and ammonium
581 thiocyanate under reflux for 2 hours in 65–88% yields after purification on flash silica gel
582 chromatography.



#	R_1	n	R_2	% inhibition	IC ₅₀ ANO1(μM)	IC ₅₀ ANO2(μM)
87c	-	1	<i>t</i> Bu	80.09 ± 0.75	2.95 ± 0.05	NT
88b	<i>t</i> Bu	2	H	67.94 ± 0.78	4.79 ± 1.10	NT
88c	<i>t</i> Bu	1	<i>t</i> Bu	86.87 ± 2.01	3.10 ± 1.23	15.43 ± 1.23
89a	Ph	0	H	70.50 ± 0.00	2.83 ± 0.96	NT
89c	Ph	1	<i>t</i> Bu	70.28 ± 8.76	3.09 ± 1.07	NT
89o	2-ClPh	0	H	76.70 ± 10.75	2.29 ± 0.30	NT
89q	4-ClPh	0	H	72.25 ± 11.78	2.36 ± 0.79	7.43 ± 0.67



583

584 **Scheme 15.** (a) 1,4-dithiane-2,5-diol, NEt_3 in $EtOH$, 70°C, 6h; (b) acyl chloride, pyridine, r.t., 1h; (c) S_8 ,

585 morpholine in $EtOH$, 70°C, 12h; (d) acyl chloride, NH_4SCN , reflux, 2h.

586 In total, 33 compounds were synthesized and evaluated as ANO1 inhibitors. Seven
587 derivatives (**87c**, **88b**, **88c**, **89a**, **89c**, **89o**, **89q**) have shown good results, with inhibition in the
588 range of 67–87% for IC₅₀ comprised between 2.29 and 4.79 μ M on ATP-induced ANO1
589 chloride currents following FLIPR format (Scheme 15). Compounds **88c** and **89q** showed
590 interesting results on ANO1 and were selective to ANO1 over ANO2 (Table 1). These two
591 compounds reduced proliferation and invasion of glioblastoma (GBM) cells more than
592 reference compounds. Moreover, **87c** showed synergy against GBM cells in the presence of
593 temozolomide following Chou-Talalay method. Considering the SAR studies, the authors
594 observed best biological results were obtained when R₂ was substituted with *tert*-butyl group.
595 Also, the presence of the 5- or 6-membered ring was favorable to the activity (**87c**, **88c** and
596 **89c**). Globally, the addition of an acyl group on amine function did not modify the biological
597 activity. However, when a thiourea function was added, the activity was enhanced. However,
598 presence of EDG or EWG did not modify the biological activity.

2.7. Cannabinoid type-1 receptor modulator activity

The cannabinoid type-1 (CB₁) receptor is an interest target because it is involved in a wide range of neuronal and physiological processes, such as memory, appetite, anxiety and depression.

Nguyen and co-workers described in 2019 novel series based on 2-ATs (**94a-r**) and inspired by diarylurea series they previously reported.⁸⁶ Their goal was to realize the synthesis of allosteric inhibitors of human CB₁ receptors. Based on PSNCBAM-1 (**90**) structure (Figure 8), they designed their series of 21 compounds (**94a-r**) whose preparation is disclosed in scheme 16.

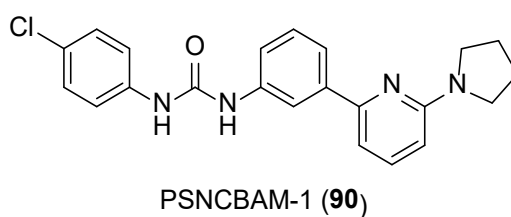
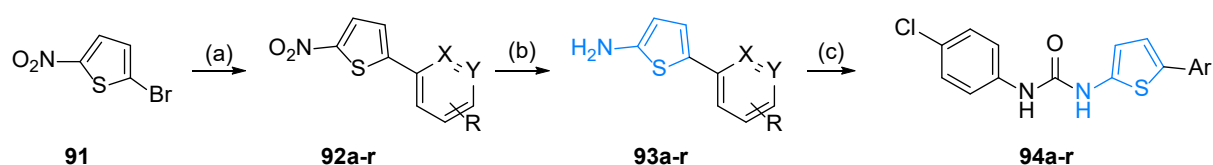


Figure 8. Structure of PSNCBAM-1.

The first step was the Suzuki-Miyaura coupling in the presence of nitrothiophene **91** with the corresponding phenyl boronic acid derivatives, palladium tetrakis and aqueous solution of sodium carbonate in dimethoxyethane under reflux for 16 hours to give **92a-r** in 8–85% yields after purification on flash silica gel chromatography. The second step was the reduction of the nitro function of **92a-r** in the presence of hydrazine hydrate with Raney nickel in ethanol at 60°C for 1 hour to afford 2-ATs **93a-r** in 12% to quantitative yields after purification on flash silica gel chromatography or directly used as crude material. The last step was the formation of the urea moiety from **93a-f** with 4-chlorophenyl isocyanate in chloroform at 60°C for 16 hours to yield **94a-r** in 7–88% after filtration and washes with DCM.



$R = \text{H, F, Cl, Ac, CO}_2\text{Me}$
 $\text{SO}_2\text{Me, OMe, Me, NMe}_2$
 $X, Y = \text{CH or N}$

#	Ar	IC ₅₀ (nM)		
		hCB ₁ Calcium assay	hCB ₁ [³⁵ S]GTPγS binding assay	mCB ₁ [³⁵ S]GTPγS binding assay
90	-	33 ± 8	455 (307 – 673)	288 (152 – 589)
94a		6.8 ± 1	524 (283 – 969)	63 (42 – 92)
94b		39 ± 6	425 (281 – 644)	N.D.
94c		40 ± 2	537 (319 – 904)	174 (78 – 399)
94d		22 ± 4	84 (45 – 157)	138 (39 – 468)
94e		21 ± 2	272 (181 – 410)	363 (159 – 813)

Conserved by analogy
with reference compound

Presence of thiophene or thiazole
improve potency when pyridinyl or non
aromatic ring decrease potency

Best results when Ar = Ph

Show better results when phenyl
group were substituted by +M
groups, especially halogens (F, Cl)

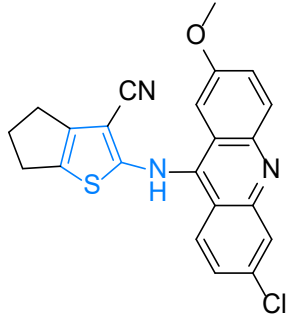
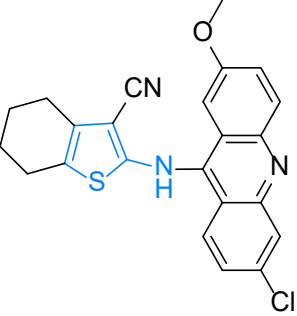
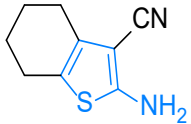
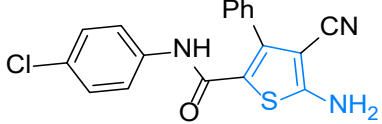
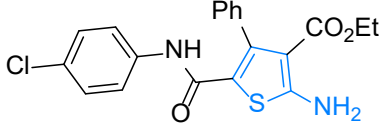
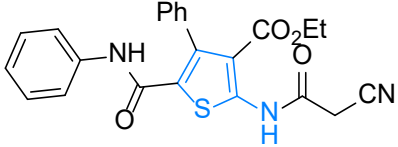
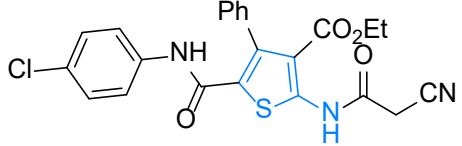
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620 **Scheme 16.** (a) phenyl boronic acid derivatives, Pd(PPh₃)₄, aq. NaHCO₃, DME, reflux, 16h; (b) hydrazine
621 hydrate, Raney-Ni, EtOH, 60°C, 1h; (c) 4-ClPhNCO, CHCl₃, 60°C, 16h.

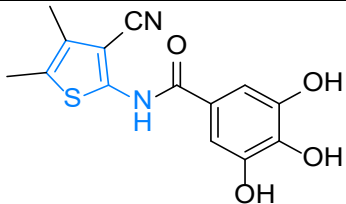
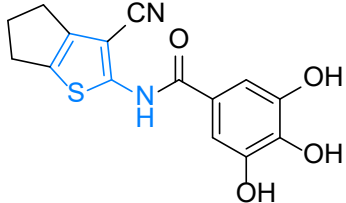
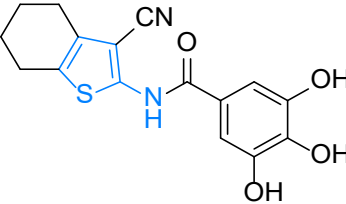
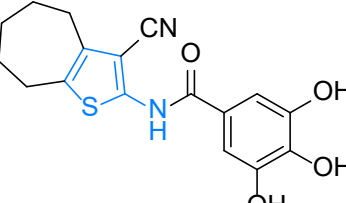
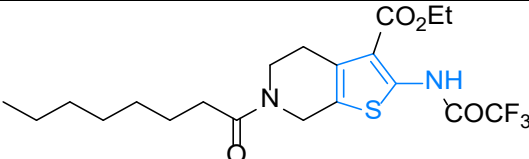
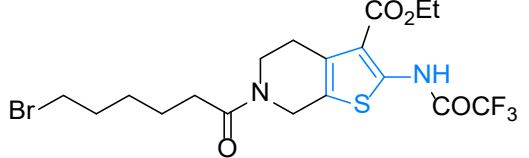
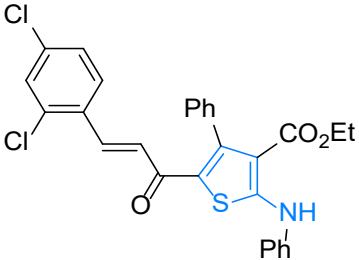
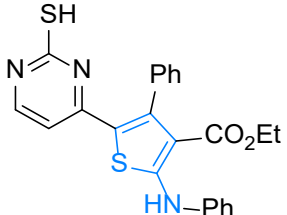
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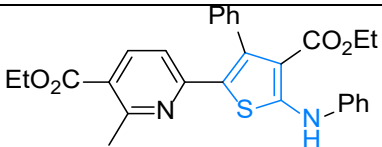
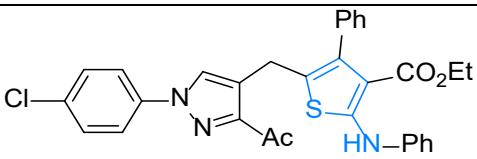
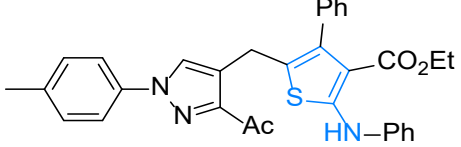
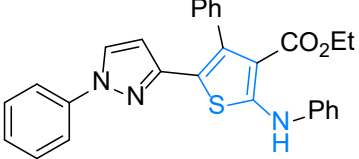
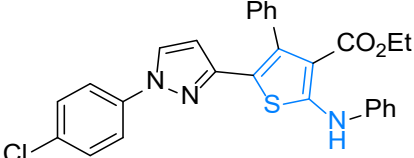
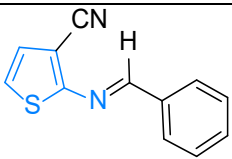
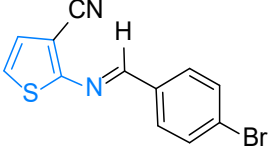
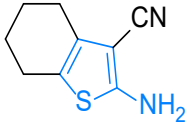
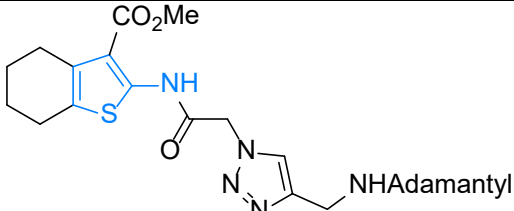
623 Among the 20 synthesized products (IC₅₀ range on hCB₁ with calcium assay 6.8–501 nM;
624 IC₅₀ range on hCB₁ with [³⁵S]GTPγS binding experiment 63–3467 nM), 5 compounds showed
625 a particular good activity (**94a-e**, IC₅₀ range on hCB₁ with calcium assay 6.8–40 nM; IC₅₀ range
626 on hCB₁ with [³⁵S]GTPγS binding assay 63–363 nM, Scheme 16). Compound **94a** gave best
627 results and was chosen by the authors to realize *in vivo* evaluations on mice (Table 1). **94a**
628 showed a good stability in rat liver microsomes (T_{1/2} = 65 ± 19 min). They also reported a good

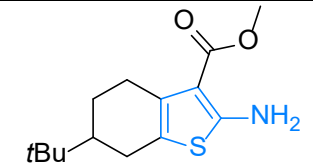
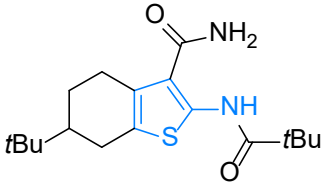
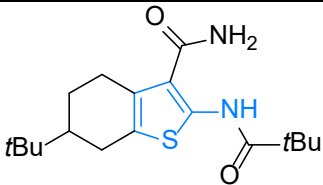
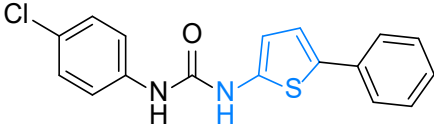
629 example of a compound that exhibited a great *in vitro* result but difficult to concretize because
630 of low blood solubility and blood brain barrier permeation. The authors offered exhaustive SAR
631 studies, and explained the replacement of the phenyl ring by a thiophenes ring demonstrated
632 considerable improvement in CB₁ inhibitory activity, like thiazole (IC₅₀ range 6.8–79 nM).
633 However, the activity decreased when cycloalkyl or pyridinyl was present (IC₅₀ range 233 –
634 >10 000 nM). Dealing with the aryl group grafted on the thiophenes moiety, they observed that
635 halogeno substituted phenyl and more generally positive mesomeric effect groups, showed
636 better results (IC₅₀ range 21–111 nM) than, acetyl, ester, sulfonate groups (negative mesomeric
637 effect groups; IC₅₀ range 108–501 nM).

Antiprotozoal activity					
Cpd	Structure	Activity	Mechanism of action	Yield (%)	Ref
7a		EC ₅₀ = 9.60 ± 3.19 μM (parasite population)	Inhibition of replicative enzymes hypothesized by the authors	87%	52
7b		EC ₅₀ = 10.95 ± 3.96 μM (Parasite population)	Inhibition of replicative enzymes hypothesized by the authors	95%	52
5b		Reduction of the hypno-spore viability of <i>Perkinsus</i> by 38% at the concentration of 500 μM	N.D.	N.D.	53
Antiproliferative activity					
11c		IC ₅₀ = 0.59 ± 0.18 μM (U87MG, glioblastoma)	N.D.	70%	11
11d		IC ₅₀ = 0.39 ± 0.21 μM (U87MG, glioblastoma)	N.D.	56%	11
12b		IC ₅₀ = 0.62 ± 0.18 μM (U87MG, glioblastoma)	N.D.	39%	11
12d		IC ₅₀ = 0.43 ± 0.26 μM (U87MG, glioblastoma)	N.D.	26%	11

18e		$IC_{50} = 0.09 \pm 0.16 \mu M$ (TGF β R1)	Inhibition of TGF β R1	55%	57
24		$IC_{50} = 19.14 \pm 1.9 \mu M$ (HEPG2, liver cancer)	N.D.	57%	58
28a		$IC_{50} = 12.50 \text{ nM}$ (H1299); 0.47 nM (EGFR); 0.14 nM (HER2)	Inhibition of EFGR and HER2 (confirmed by molecular docking)	N.D.	59
32i		$IC_{50} = 1.5 \pm 0.1 \mu M$ (JAR, choriocarcinoma)	RNase-L binding	13%	87
33		$IC_{50} = 3.7 \pm 0.8 \mu M$ (JAR, choriocarcinoma)	RNase-L binding	49%	87
Antiviral activity					
44bb		$IC_{50} = 18 \pm 1 \mu M$ (PA-PB1)	PA-PB1 inhibition	64%	18
Antibacterial activity					
50c		$EC_{50} = 0.18 \mu M$ (<i>S. aureus</i>); $0.782 \mu M$ (<i>Mtb</i> H37 Ra)	Penicillin G binding site	52%	68

54b		MIC = 0.15 ± 0.01 $\mu\text{g/mL}$; $0.49 \mu\text{M}$ (<i>S. coagulase</i>)	N.D.	48%	69
54b		MIC = 0.10 ± 0.03 $\mu\text{g/mL}$; $0.32 \mu\text{M}$ (<i>S. coagulase</i>)	N.D.	43%	69
54c		MIC = 0.15 ± 0.01 $\mu\text{g/mL}$; $0.45 \mu\text{M}$ (<i>S. coagulase</i>)	N.D.	45%	69
54d		MIC = 0.15 ± 0.01 $\mu\text{g/mL}$; $0.43 \mu\text{M}$ (<i>S. coagulase</i>)	N.D.	40%	69
61		85% EtBr efflux reduction over 30 minutes at $50\mu\text{M}$	NorA efflux pump inhibition	59%	70
62		125% EtBr accumulation at $50\mu\text{M}$	NorA efflux pump inhibition	8%	70
65e		MIC = 8.9 ± 0.31 $\mu\text{g/mL}$; $17.0 \mu\text{M}$ (<i>E. coli</i>)	N.D.	88%	73
67b		MIC = 9.8 ± 0.34 $\mu\text{g/mL}$; $22.6 \mu\text{M}$ (<i>P. aeruginosa</i>)	N.D.	20%	73
Antifungal activity					

69		$11.2 \pm 0.44 \mu\text{g/mL}$; $23.0 \mu\text{M}$ (<i>C. albicans</i>)	N.D.	98%	73
71a		$14.3 \pm 0.58 \mu\text{g/mL}$; $25.7 \mu\text{M}$ (<i>C. albicans</i>)	N.D.	86%	73
71b		$12.6 \pm 0.38 \mu\text{g/mL}$; $23.5 \mu\text{M}$ (<i>G. candidum</i>)	N.D.	85%	73
72a		$15.8 \pm 0.38 \mu\text{g/mL}$; $33.9 \mu\text{M}$ (<i>C. albicans</i>)	N.D.	83%	73
72b		MIC = $11.2 \mu\text{g/mL}$; $22.4 \mu\text{M}$ (<i>C. albicans</i>)	N.D.	93%	73
75m	 Chemical Formula: $\text{C}_{12}\text{H}_8\text{N}_2\text{S}$ Molecular Weight: 212,2700	MIC = $16 \mu\text{g/mL}$; $75.3 \mu\text{M}$ (<i>E. floccosum</i> 6999)	N.D.	56%	77
75o	 Chemical Formula: $\text{C}_{12}\text{H}_7\text{BrN}_2\text{S}$ Molecular Weight: 291,1660	MIC = $32 \mu\text{g/mL}$; $101 \mu\text{M}$ (<i>T. rubrum</i> 6753)	N.D.	45%	77
5b	 incorporated in chitosan polymers (1.28 mg/mL)	1.5 mm of inhibition around chitosan film (<i>C. tropicalis</i>)	N.D.	N.D.	78
Channels inhibitory activity					
78aa		$\text{IC}_{50} = 1.6 \pm 0.4 \mu\text{M}$ (CaCC)	CaCC channel inhibition	85%	82

80		$IC_{50} = 4.6 \pm 0.7 \mu M$ (CaCC)	CaCC channel inhibition	N.D.	84
88c		$86.87 \pm 2.01\%$; $IC_{50} = 3.10 \pm 1.23 \mu M$ (ANO1); $15.43 \pm 1.23 \mu M$ (ANO2)	ANO1/ANO2 chloride current inhibition	89%	85
88q		$72.25 \pm 11.78\%$; $IC_{50} = 2.36 \pm 0.79 \mu M$ (ANO1); $7.43 \pm 0.67 \mu M$ (ANO2)	ANO1/ANO2 chloride current inhibition	79%	85
Cannabinoid type-1 inhibitor activity					
94a		$IC_{50} = 6.8 \pm 1 \mu M$ (hCB1) With calcium assay	hCB1 inhibitor	57%	86

639 N.D.: not described

3. Conclusion

We reported here the synthesis and biological properties of 2-ATs described over the last 5 years (2017–2022) with a particular interest in promising biological applications. The 2-AT scaffold has recently demonstrated wide applications in medicinal chemistry. As previously described, its lead-likeness and ability to fight against various infections, regardless they are viral, fungal, bacterial or protozoal is an important tool in drug discovery. 2-ATs could also possess anticancer properties and further development as antiproliferative compounds seems interesting and crucial in cancer chemotherapy. 2-AT potential as channel inhibitors as CaCC modulators is also interesting for the discovery of treatments of neurological diseases such as Parkinson's. SAR studies were shown to be almost systematically realized when a series was synthesized to provide maximal lead information for further development following the “hit to lead” process for preclinical studies or *in vivo* evaluations.

Over the past 5 years, synthesized 2-ATs were N-substituted and sometimes conjugated with antibiotics or incorporated in chitosan polymers to enhance biological properties. Some studies added *in silico* ADME predictions to predict potential toxicity or water solubility based on literature. It would be appropriate to realize this experiment in preclinical phase to enrich the knowledge about biological applications of this scaffold. Also, we discussed about predicting tools such as molecular docking is time-saving in drug discovery. All of these data may help in further development for 2-AT moiety.

Authors contributions

ZB was responsible for the management of the work, overall supervision. The authors contributed to the data preparation and drafted and revised the manuscript. All authors have read and approved the final manuscript.

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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.

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