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

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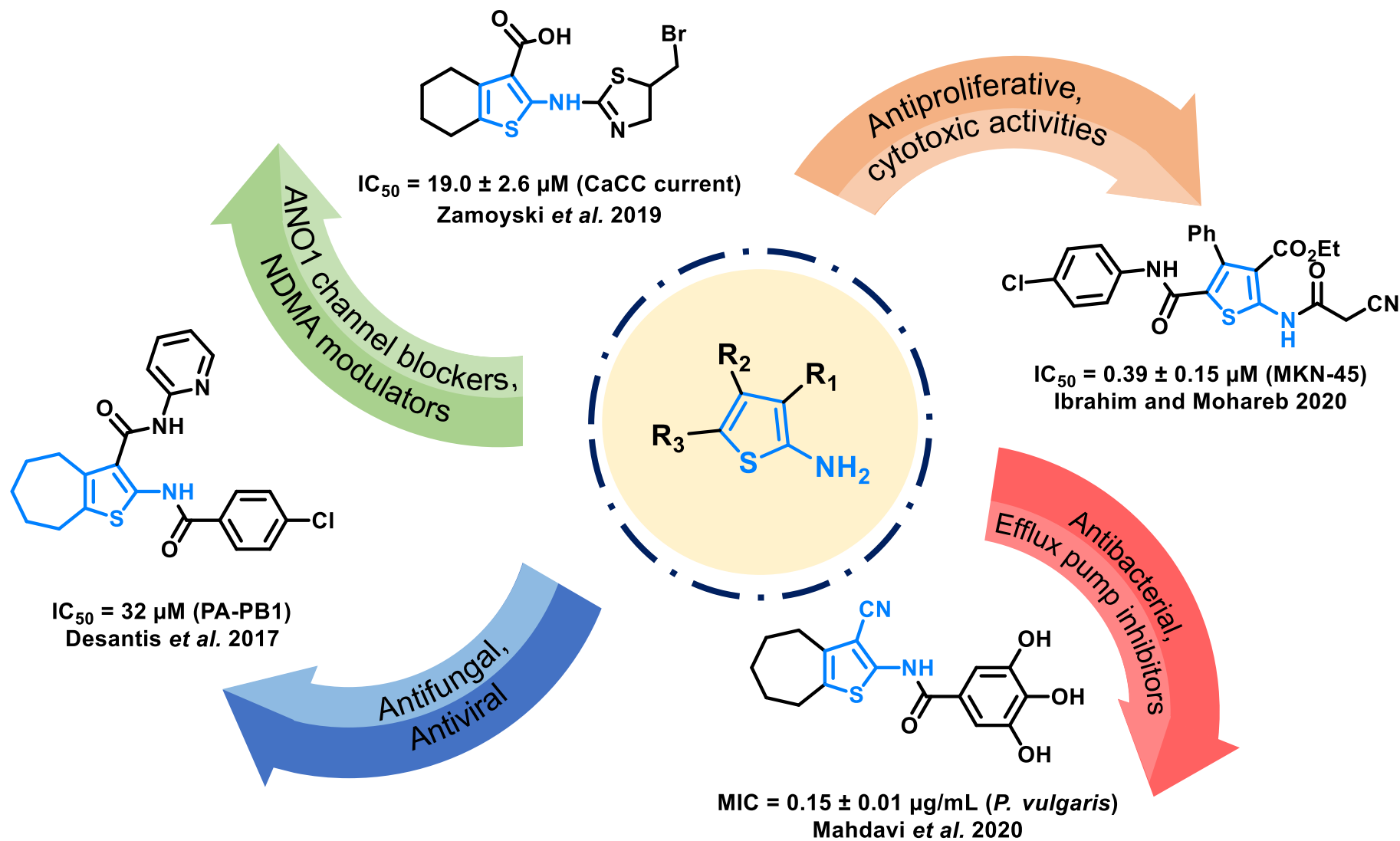
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1    Recent contribution of medicinally active 2-aminothiophenes:  
2                                    a privileged scaffold for drug discovery

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## 24 Abstract

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

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

44

## 45 List of abbreviations

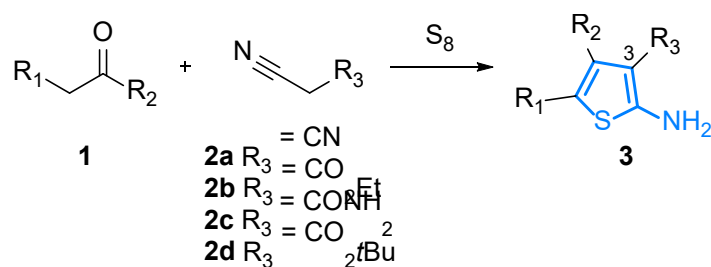
- 46 2-AT: 2-aminothiophene
- 47 ADME: Absorption distribution  
48 metabolism and excretion
- 49 ANO: Anoctamin
- 50 Boc<sub>2</sub>O: Di-tert-butyl dicarbonate
- 51 CaCC: Calcium-activated chloride  
52 channels
- 53 CB<sub>1</sub>: Cannabinoid type-1
- 54 CC<sub>50</sub>: Half-maximal cytotoxic  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

## 84 1. Introduction

85 Heterocyclic rings are favored scaffold for many years in medicinal chemistry, and drug  
86 discovery due to their importance in properties they confer. They account for more than 85%  
87 of all biologically active compounds.<sup>1</sup> S-heterocycles, and more specifically 2-aminothiophenes  
88 (2-ATs) are five-membered heteroaromatic core that have a significant impact on biological  
89 systems and can be used as synthons to develop potent new medicines. Moreover, they are  
90 considered as important in heterocyclic chemical libraries and when used as a building block  
91 are responsible for many biological activities.<sup>2-4</sup> Small organic molecules are required in drug  
92 discovery and medicinal chemistry on a continuous basis. To do so, chemists inspire their  
93 chemical series over described methodologies and they evaluate potential biological activities  
94 of newly synthesized derivatives.

95 2-ATs are 5-membered aromatic scaffold whose synthesis has been firstly depicted by  
96 Campaigne and Foye in the 1950s before being democratized by Gewald in the 1960s.<sup>5-7</sup>  
97 Gewald reaction frequently occurs with a carbonyl derivative **1** with a nitrile **2** in the presence  
98 of a sulfur source to afford **3**. Typically, methodologies result in the formation of trisubstituted  
99 thiophene ring with an electron-withdrawing groups (EWG) – especially negative mesomeric  
100 effect such as carboxamide, ester or nitrile group on C3 position (Scheme 1).<sup>8-12</sup>

101



102

103

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

104 Since then, many enhancements were published following many methodologies.<sup>4,13–22</sup> 2-

105 ATs continue to raise a large interest in both organic and medicinal chemistry. To our

106 knowledge, 2-AT is absent of natural compounds, hence it must be obtained synthetically.<sup>23</sup>

107 Multiples synthetic routes are described alongside literature, by means of heterogeneous

108 catalysis,<sup>24</sup> homogeneous catalysis,<sup>25</sup> multicomponent reactions<sup>26,27</sup> or post-core synthesis

109 modification.<sup>28</sup> Medicinal chemists made thereafter their contribution thanks to structure-

110 activity relationship (SAR) to allow a better understanding of 2-ATs biological properties.

111 Considering biological evaluations, 2-ATs are known to confer antiprotozoal,<sup>29,30</sup>

112 antiproliferative,<sup>31–34</sup> antiviral,<sup>32,35</sup> antibacterial<sup>17,30–32</sup> or antifungal activities.<sup>39,40</sup> Among FDA-

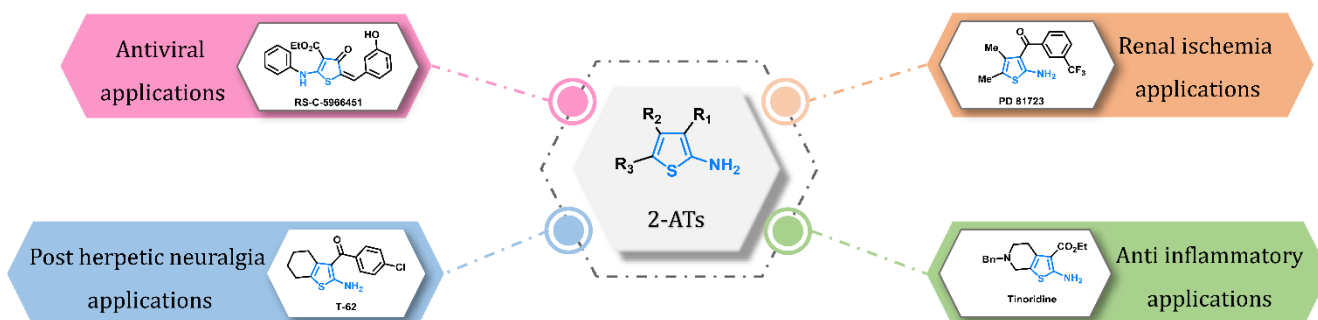
113 approved 2-ATs, we can highlight **RS-C-5966451**, described for its broad-spectrum antiviral

114 properties;<sup>41</sup> **PD 81723**, which is an adenosine A1 receptor agonist used to reduce necrosis,

115 apoptosis and inflammation for renal ischemia-reperfusion;<sup>42,43</sup> **Tinoridine**, as a non-steroidal

116 anti-inflammatory;<sup>44</sup> and **T-62**, known as a former adenosine A1 receptor agonist and more

117 recently for post herpetic neuralgia application (Figure 1).<sup>45</sup>



118

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

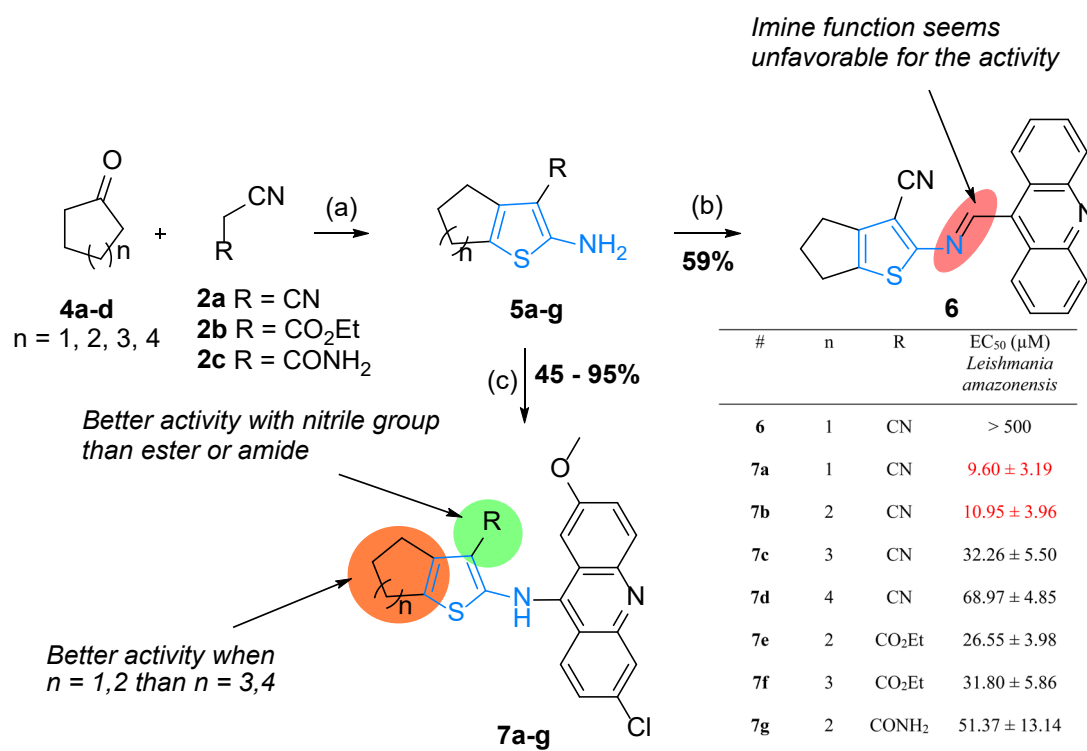
119 Nevertheless, we noticed that while reviews summarizing biological activities or synthesis  
 120 of 2-ATs are explored in the literature,<sup>13,46-49</sup> there are many recent examples describing  
 121 biological activities for the last 5 years (2017–2022). In this review will be provided a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

## 128 2. Biological and pharmacological attributes of 2-ATs

### 129 2.1. Antiprotozoal activity

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

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

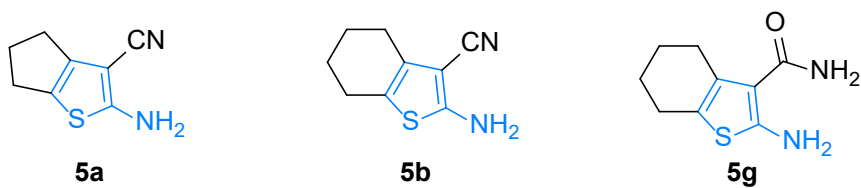


142

143 **Scheme 2.** (a) morpholine, S<sub>8</sub> in EtOH, 5–10°C, 3h; (b) acridine-9-carboxaldehyde in EtOH, 60°C, 5h; (c)  
 144 6,9-dichloro-2-methoxy-acridine, AcOH in EtOH, r.t., 4h.

145 These compounds (**6**, **7a-g**) have been evaluated on promastigotes forms of *Leishmania*  
 146 *amazonensis* (IFLA/BR/67/PH8) and except for **6**, the compounds **7a-g** exhibited good  
 147 antiprotozoal properties (EC<sub>50</sub> range 9.60–429 μM, Scheme 2). **7a** and **7b** displayed best results  
 148 (EC<sub>50</sub> range 9.60 ± 3.19 μM and 10.95 ± 3.96 μM, respectively, Table 1), suggesting that the  
 149 nature of the cycloalkyl influenced the activity. Cyclopentyl and cyclohexyl derivatives showed  
 150 better results than cycloheptyl and cyclooctyl. Moreover, the presence of the nitrile group  
 151 increased the global biological activity (EC<sub>50</sub> = 9.60 ± 3.19 and 10.95 ± 3.96 μM for **7a** and **7b**  
 152 against 26.55 ± 3.98 μM and 51.37 ± 13.14 μM for **7e** and **7g**). The authors also investigated  
 153 the mechanism of action for **7a** and **7b** and observed that they were bound to parasite DNA  
 154 with K<sub>b</sub> = 1.10<sup>4</sup> M<sup>-1</sup>. As neither compound demonstrated DNA fragmentation, the authors  
 155 hypothesized compounds **7a** and **7b** might be responsible for replicative enzymes inhibition  
 156 that should be the reason for the antiprotozoal behaviors.

157 Duarte and co-workers carried out antiprotozoal studies on commercially available  
158 compounds (**5a,b,g**) in 2018 (Figure 2).<sup>53</sup> **5b** was shown to reduce hypnosporic viability of  
159 *Perkinsus* spp. by 38% at the concentration of 500  $\mu$ M (Table 1). **5a** and **5g** did not exhibit  
160 antiprotozoal activities against *Perkinsus*. Hypnosporic is a life stage of *Perkinsus* parasites  
161 responsible for high mortality of bivalves in the entire world, including oysters.

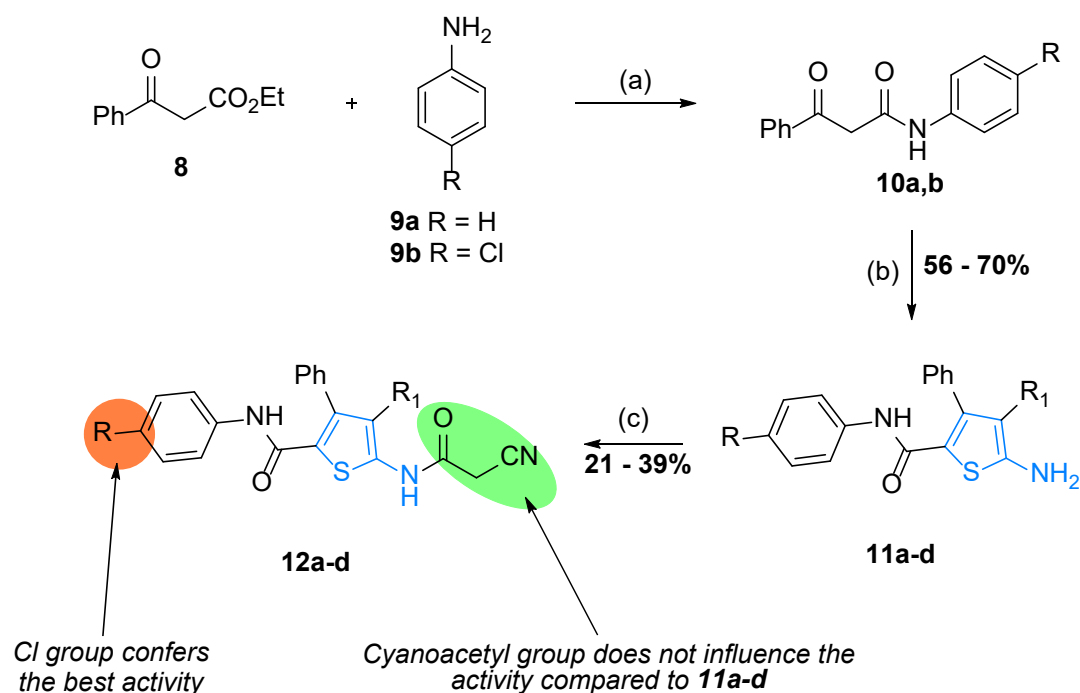


*Figure 2. Structures of 5a, 5b and 5g.*

## 164 2.2. Antiproliferative Activity

165 Cancer treatments significantly improved in the past decades. However, toxicity of several  
166 cures is still a hindrance which requires constant development of new antiproliferative drugs.  
167 Hence, the search for novel therapeutic approaches, such as compounds from natural sources,  
168 has become a topic of great interest.<sup>54-56</sup>

169 In 2020, Ibrahim and Mohareb designed a novel 2-ATs series (**12a-d**). Their synthesis is  
170 pictured in scheme 3.<sup>11</sup> First step is the synthesis of intermediate **10a** and **10b** following an  
171 amide formation using ethyl benzoyl acetate **8** and anilines **9a,b** without mentioned yields.  
172 Then, Gewald reaction allowed formation of **11a-d** in 56–70% yields in the presence of  
173 malonodinitrile **2a** or ethyl cyanoacetate **2b** with **10a** or **10b** in ethanol, triethylamine and  
174 elemental sulfur S<sub>8</sub> after precipitation in cold diluted aqueous hydrochloride solution and  
175 filtration. The final step consisted in reaction of **11a-d** in presence of malonodinitrile **2a** in  
176 DMF to afford **12a-d** in 21–39% yields after precipitation in cold water, filtration and  
177 recrystallization from ethanol. Antiproliferative activity of all molecules have been evaluated  
178 on 6 cancer cells lines: A549 (lung), H460 (lung), HT29 (colon), MKN-45 (gastric), U87MG  
179 (glioblastoma) and SMMC-7721 (liver). Compounds **11c**, **11d**, **12b** and **12d** showed particular  
180 good activities on all cancer cell lines (IC<sub>50</sub> range 0.19–9.62 μM; Scheme 3, Table 1). Results  
181 were similar for **11a-d** and **12a-d**: the addition of the acyl group on the amine function did not  
182 influence the activities much. Compounds **11c**, **11d**, **12b** and **12d** also showed better  
183 antiproliferative activities than foretinib (reference molecule), against U87MG (glioblastoma)  
184 and SMMC-7721 (liver) cancer cell lines, are thus promising lead as antiproliferative molecules  
185 for these types of cancers.

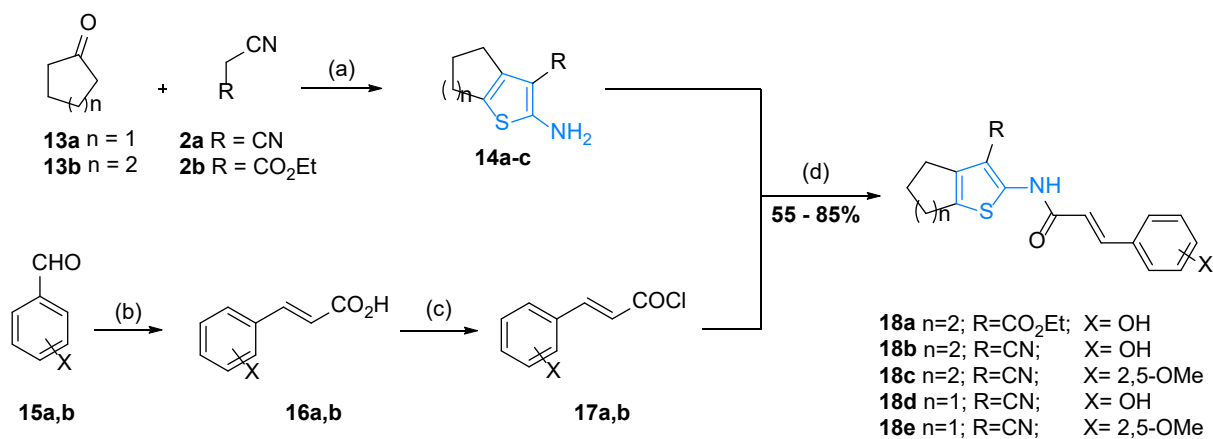


#	R	R <sub>1</sub>	IC <sub>50</sub> ± SEM (μM)					
			A549	H460	HT29	MKN-45	U87MG	SMMC-7721
11a	H	CN	8.28 ± 4.18	9.62 ± 2.70	8.39 ± 2.73	7.09 ± 2.35	8.93 ± 2.73	5.09 ± 1.63
11b	H	CO <sub>2</sub> Et	6.80 ± 1.42	8.59 ± 1.80	7.49 ± 2.73	8.09 ± 2.04	7.42 ± 2.69	8.58 ± 2.68
11c	Cl	CN	0.70 ± 0.29	0.76 ± 0.38	0.65 ± 0.27	0.42 ± 0.23	<b>0.59 ± 0.18</b>	0.69 ± 0.28
11d	Cl	CO <sub>2</sub> Et	0.23 ± 0.08	0.38 ± 0.27	0.19 ± 0.08	0.26 ± 0.11	<b>0.39 ± 0.21</b>	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 <sub>2</sub> Et	0.32 ± 0.20	0.49 ± 0.21	0.63 ± 0.32	0.59 ± 0.23	<b>0.62 ± 0.18</b>	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 <sub>2</sub> Et	0.60 ± 0.24	0.43 ± 0.26	0.62 ± 0.30	0.39 ± 0.15	<b>0.43 ± 0.26</b>	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

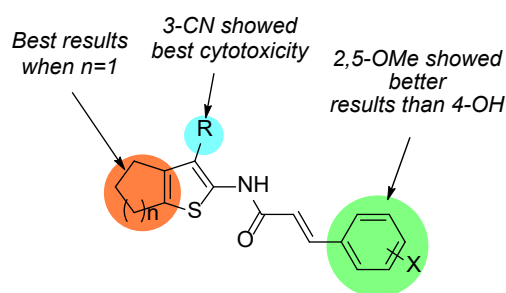
187 **Scheme 3.** (a) 120°C; (b) malonodinitrile **2a** or ethyl cyanoacetate **2b**, Et<sub>3</sub>N, S<sub>8</sub> in EtOH, r.t.; (c)  
 188 malonodinitrile **2a** in DMF, r.t.

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

193 reaction with cyclopentanone **13a** or cyclohexanone **13b**, in presence of malonodinitrile **2a** or  
 194 ethyl cyanoacetate **2b** with elemental sulfur S<sub>8</sub> in a mixture of ethanol/morpholine afforded  
 195 **14a-c**. Subsequently, the next step followed a Knoevenagel reaction, in the presence of **15a,b**  
 196 with malonic acid and piperidine in ethanol under reflux and led to **16a,b**. Then, **16a,b** reacted  
 197 with POCl<sub>3</sub> and triethylamine to give the cinnamoyl chloride derivatives **17a,b**. Yields are not  
 198 mentioned for both **14a-c** and **17a-b** synthesis. In the last step, both reagents **14a-c** and **17a,b**  
 199 were allowed to react together in acetone in the presence of K<sub>2</sub>CO<sub>3</sub> under reflux for 5–8 hours  
 200 to afford amides **18a-e** in 55–85% yields after crystallization from ethanol.



#	IC <sub>50</sub> (μM)				
	PC-3	HepG-2	MCF-7	WI-38	TGFβR1
<b>18a</b>	6.29 ± 0.5	8.3 ± 0.75	8.27 ± 0.76	NT	NT
<b>18b</b>	5.2 ± 0.46	7.9 ± 0.67	7.5 ± 0.8	NT	NT
<b>18c</b>	2.64 ± 0.21	2.85 ± 0.17	3.5 ± 0.26	259.9	NT
<b>18d</b>	3.4 ± 0.42	3.55 ± 0.28	4.92 ± 0.31	236.3	NT
<b>18e</b>	1.35 ± 0.04	2.7 ± 0.19	3.2 ± 0.28	185.2	0.19



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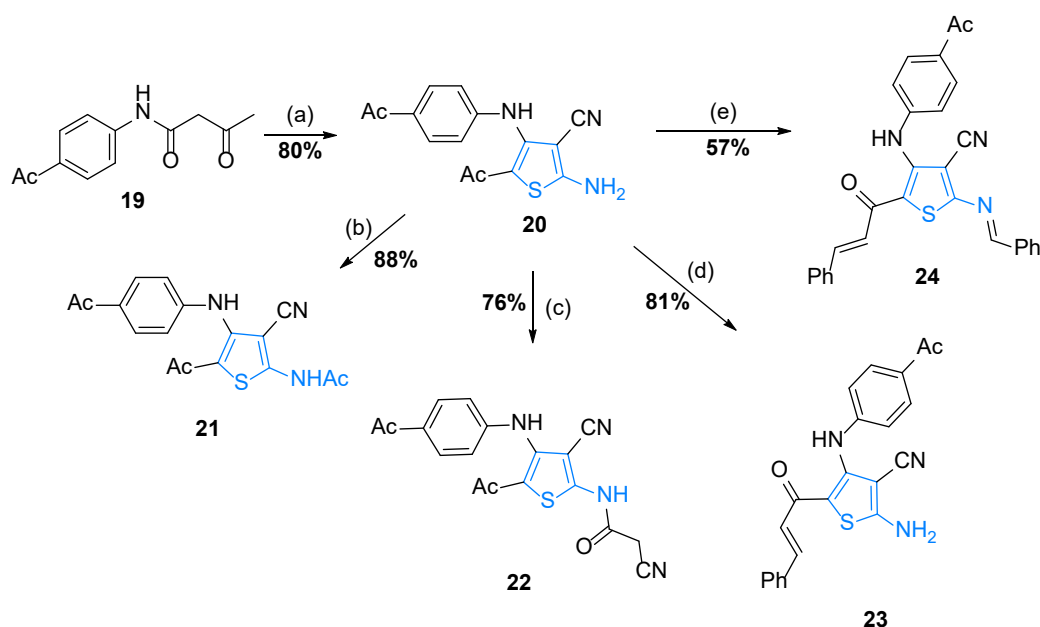
202 **Scheme 4.** (a) malonodinitrile **2a** or ethyl cyanoacetate **2b**, morpholine, S<sub>8</sub> in EtOH under reflux for 4h then  
 203 24h at 0°C; (b) malonic acid, piperidine in EtOH, under reflux, 5h; (c) POCl<sub>3</sub> NEt<sub>3</sub> under reflux for 20 min;  
 204 (d) K<sub>2</sub>CO<sub>3</sub> in acetone under reflux, 5 – 8h.

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

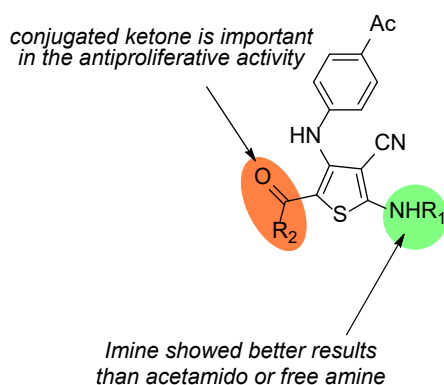
207 antiproliferative activities following the MTT assay. The MTT assays have been realized on 5  
208 compounds (**18a-e**) and showed good  $IC_{50}$  on cancer cell lines ( $IC_{50}$  range 1.35–8.3  $\mu$ M, scheme  
209 4). MTT evaluations on normal human cell lines (WI-38) have been realized for the 3 best  
210 compounds (**18c-e**) to conclude they were not acutely toxic ( $IC_{50}$  range 185.2–259.9  $\mu$ M).  
211 Compounds **18c-e** displayed selectivity toward cancer cell lines by inhibiting TGF $\beta$ R1 with an  
212  $IC_{50}$  comprised between 0.087 and 3.276  $\mu$ M. TGF $\beta$ R1 is a key protein in cell proliferation.  
213 Thereafter, TGF $\beta$ R1 inhibition assay has been realized on the best compound **18e** following  
214 TGF $\beta$ 1 ELISA assays to determine the potency of this molecule on the targeted protein ( $IC_{50}$  =  
215 0.09  $\mu$ M; Table 1). For comparison, galunisertib (reference compound) showed an  $IC_{50}$  of 0.17  
216  $\mu$ M on TGF $\beta$ R1. Concerning SAR studies, antiproliferative properties were proved to be better  
217 when a cyano group was grafted on C3 position. The biological activities were good when the  
218 cycloalkyl was a cyclopentyl and when the phenyl moiety was substituted in positions 2 and 5  
219 by methoxy groups. The most effective derivative **18e** has been docked in TGF $\beta$ R1 binding site  
220 and exhibited the good docking score of - 6.92 kcal/mol. Moreover, *in silico* ADME properties  
221 of compounds **18a-e** have been predicted using SwissADME software to be drug like  
222 compatible.

223 In 2020, Khalifa and Algothami developed a novel series of 2-ATs (**20–24**) as  
224 antiproliferative molecules on HepG2 and MCF-7 cancer cell lines.<sup>58</sup> The synthesis is described  
225 in scheme 5 and started with reaction of the amide **19** with malonodinitrile **2a**, elemental  
226 sulfur S<sub>8</sub> and triethylamine in DMF at 60°C to give **20** in 80% yield after precipitation in cold  
227 water and washes with ethanol. Afterwards, 2-AT **20** has been used as a central scaffold to  
228 synthesize 4 derivatives. N-acetylated **21** was obtained by treatment of **20** with acetic anhydride  
229 in acetic acid under reflux for 5 hours in 88% yield after recrystallization from DMF. **20** was  
230 fused in solvent-free conditions with ethyl cyanoacetate **2b** to afford **22** in 76% yield after  
231 recrystallization from ethanol. The  $\alpha$ - $\beta$ -unsaturated ketone **23** was synthesized by reaction of

232 **20** with 1 equivalent of acetaldehyde in ethanol in 81% yield after recrystallization from 1,4-  
 233 dioxane. Compound **24** was obtained in the same condition using 2 equivalents of acetaldehyde  
 234 in 57% yield after purification by recrystallization from 1,4-dioxane.



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

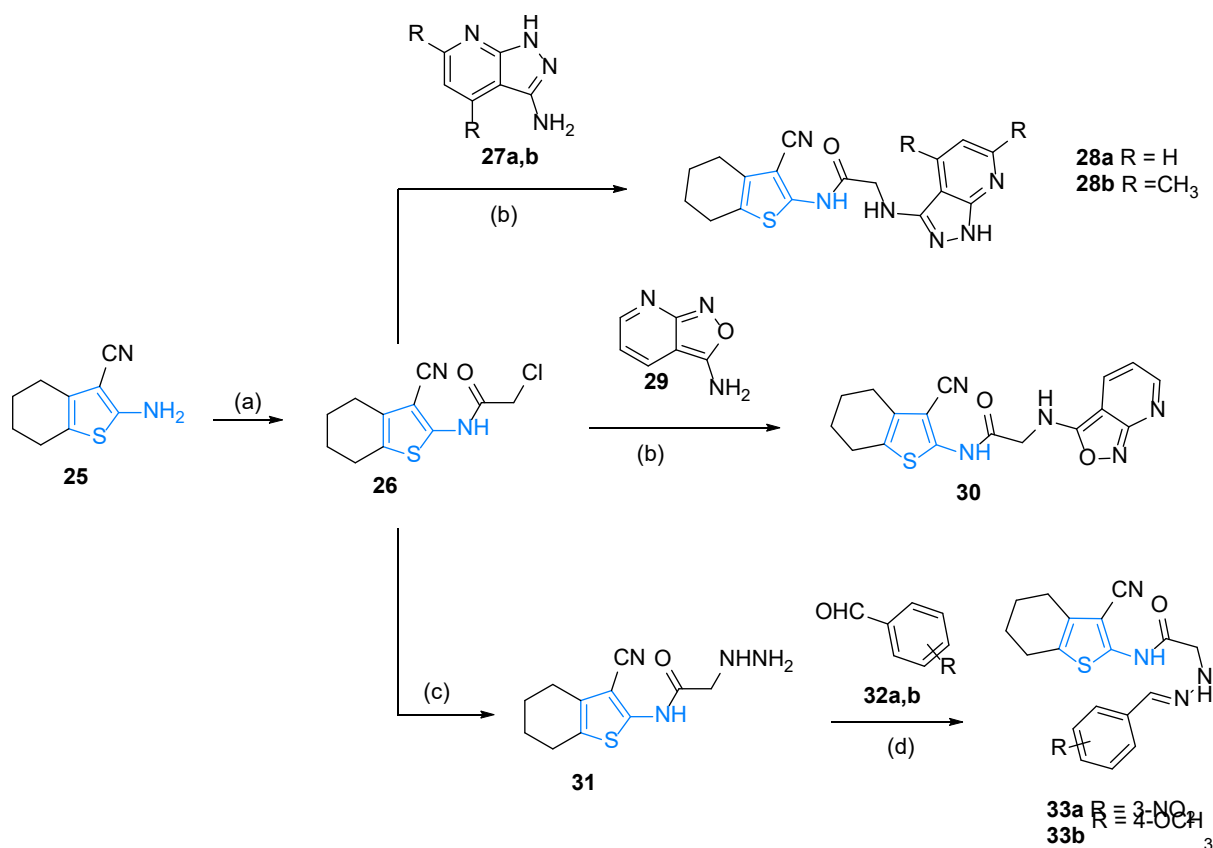


235  
 236 **Scheme 5.** (a) malonodinitrile **2a**, S<sub>8</sub>, NEt<sub>3</sub> in DMF, 60°C; (b) Ac<sub>2</sub>O in AcOH, reflux, 5h; (c) ethyl  
 237 cyanoacetate **2b**, fusion methodology; (d) acetaldehyde (1eq.) in EtOH; (e) acetaldehyde (2 eq.) in EtOH.

238 The authors compared their series (**20–24**) with activities of doxorubicin (reference  
 239 compound) on HEPG2 (IC<sub>50</sub> range 19.14–91.46 μM) and MCF-7 (IC<sub>50</sub> range 26.16–88.48 μM)  
 240 cancer cell lines (Scheme 5). Best compound **24** showed a slightly lower activity than  
 241 doxorubicin (IC<sub>50</sub> range 19.14–26.16 μM for **24** and 4.17–4.50 μM for doxorubicin, Table 1),

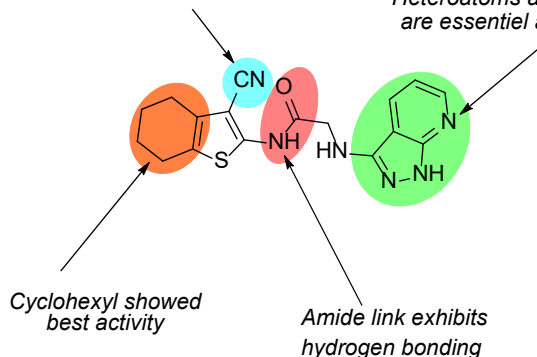
242 making it a good starting point for further development as antiproliferative drugs on these two  
243 types of cancers. Concerning the SAR studies, only C2 and C5 positions can be discussed  
244 because C3 and C4 groups were conserved for **20–24**. Imine function in C2 seemed to be  
245 important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

249 In 2021, Elrayess and his team worked on synthesis of a series composed of 5 different 2-  
250 ATs derivatives (**28a,b**, **30** and **33a,b**) as potent epidermal growth factor receptor (EGFR) and  
251 human EGFR-related receptor 2 (HER2) targeting lung cancer.<sup>59</sup> The synthesis is illustrated in  
252 scheme 6. First, commercially available **25** reacted with chloroacetyl chloride in acetone at 0–  
253 5°C for 4h to give the N-substituted 2-AT **26**. Then, the nucleophilic substitution occurred  
254 between **26** and **27a,b** with triethylamine in DCM under reflux for 6–9 hours to give **28a,b**. In  
255 the same manner, reaction between **26** and **29** yielded **30**. Chloroacetyl **26** reacted with  
256 hydrazine hydrate in ethanol under reflux for 4 hours to afford compound **31**. Finally, desired  
257 products **33a,b** were obtained by reaction of **31** with **32a,b** using acetic acid in methanol under  
258 reflux for 5 hours. No yield mentioned for all steps.



Cyano group is preferred for hydrogen bonding

Heteroatoms and bulky group are essential at this position



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

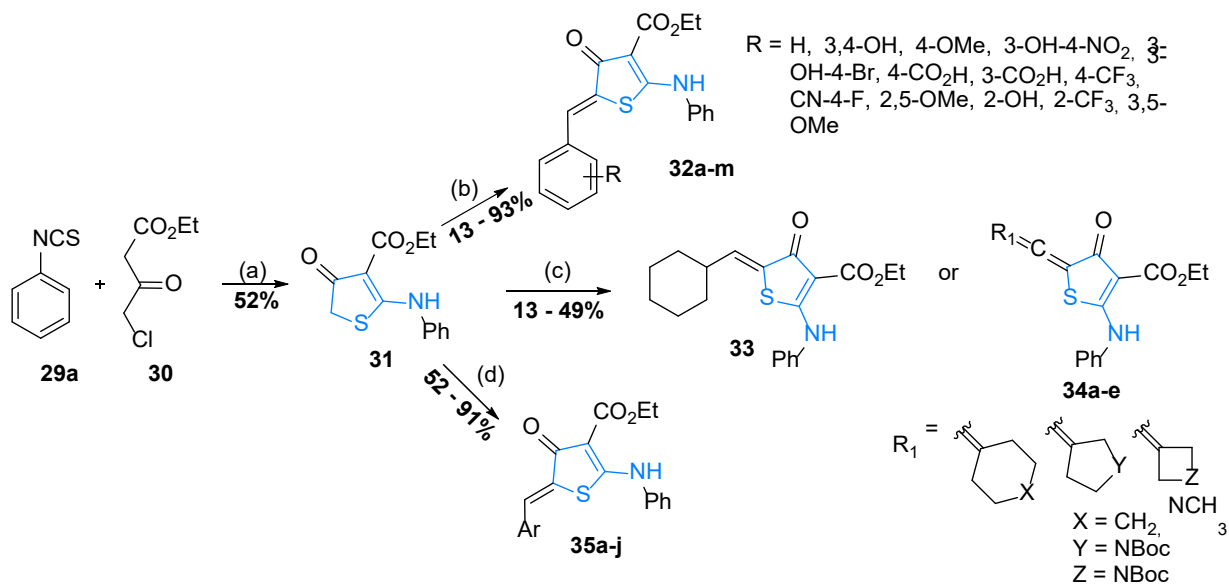
259

260 **Scheme 6.** (a) chloroacetyl chloride in acetone, 0–5°C, 4h; (b) NEt<sub>3</sub> in DCM, reflux, 6 – 9h; (c) hydrazine  
 261 hydrate in EtOH, reflux, 4h; (d) AcOH in MeOH, reflux, 5h.

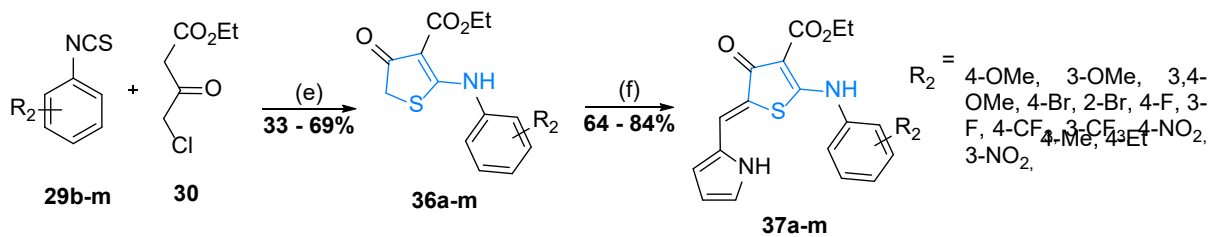
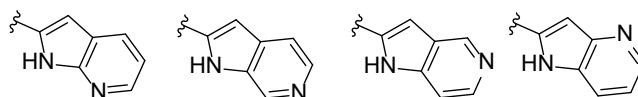
262 The series was evaluated on H1299 lung cancer cell line and displayed good results (IC<sub>50</sub>  
 263 range 12.50–18.53 nM for **28a,b, 30** and **33a,b** and 40 nM for gefitinib the reference compound,  
 264 Scheme 6). Compound **28a** showed the best activity on lung cancer cell lines H1299 with an  
 265 IC<sub>50</sub> of 12.5 nM. Moreover, **28a** exhibited a significant inhibition with IC<sub>50</sub> of 0.47 and 0.14  
 266 nM on EGFR and HER2, respectively (Table 1). As an example, these values were comparable

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

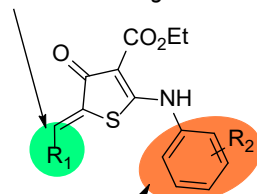
281 In 2022, Hwang *et al.* synthesized a series of 43 novel 2-ATs (**32a-m**, **33**, **34a-e**, **35a-j** and  
282 **37a-m**) as antiproliferative compounds aiming ribonuclease L (RNase L). RNase L is an  
283 endoribonuclease playing an important role in innate immunity, apoptosis, cell growth and  
284 differentiation. Its action consists in regulation and expression of cellular RNA.<sup>60</sup> The synthesis  
285 is shown in scheme 7. Reaction of commercially available thioisocyanates **29a** or **29b-m** with  
286 chloroketone **30** and sodium hydride in 1,4-dioxane for 30 min at room temperature afford 2-  
287 ATs **31** or **36a-m** in 33–69% yields after filtration and recrystallization from ethanol. The  
288 second step consisted in the reaction in Knoevenagel conditions between 2-ATs **31** or **36a-m**  
289 with the corresponding ketone or aldehyde, piperidine in ethanol under reflux to give the desired  
290 products **32a-m**, **33**, **34a-e**, **35a-j** or **37a-m** in 13–93% yields after purification by filtration and  
291 washes with ethanol or flash silica gel chromatography.



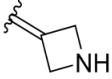
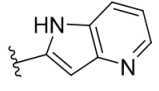
Ar = 2-furyl, 2-thiophenyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyridinyl, 3-indolyl,



Cycloalkyl groups show better RNase-L binding results



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

#	R	Ar	R <sub>1</sub>	R <sub>2</sub>	Growth inhibition rate at 10 $\mu$ M (%) [IC <sub>50</sub> in $\mu$ M] on human cancer cell lines			
					JAR	K562	A549	MCF7
<b>32i</b>	3-CN, 4-F	-	-	-	<b>99.9 <math>\pm</math> 0.3</b> <b>[1.5 <math>\pm</math> 0.1]</b>	N.D. [1.6 $\pm$ 0.3]	N.D. [8.0 $\pm$ 0.8]	N.D. [5.2 $\pm$ 2.0]
<b>33</b>	-	-	-	-	<b>95.2 <math>\pm</math> 4.5</b> <b>[3.7 <math>\pm</math> 0.8]</b>	N.D. [5.9 $\pm$ 1.1]	N.D. [18.0 $\pm$ 0.8]	45.3 $\pm$ 2.8 [6.4]
<b>34e</b>	-	-		-	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]
<b>35j</b>	-		-	-	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.]
<b>37m</b>	-	-	-	3-NO <sub>2</sub>	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]

293

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

298 From all the 43 derivatives synthesized, several compounds showed particularly good  
 299 activities as antiproliferative compounds (Scheme 7). Human cancer cell lines chosen were:  
 300 JAR (choriocarcinoma), K562 (leukemia), A549 (lung carcinoma) and MCF7 (breast  
 301 adenocarcinoma). Compound **37m** showed fair activity on JAR and MCF7 cancer cell lines  
 302 with a respective growth inhibition at 10  $\mu$ M of 57% and 27% (IC<sub>50</sub> range 10.1–20.7  $\mu$ M).  
 303 Derivatives **34e** and **35j** showed good results on JAR and MCF7 cancer cell lines with growth  
 304 inhibition of respectively 70% and 75% on JAR (IC<sub>50</sub> range 8.4–9.6  $\mu$ M) and 40% and 42% on  
 305 MCF7 (IC<sub>50</sub> = 18.1  $\mu$ M for **34e**). Compounds **32i** and **33** displayed the best results with a growth  
 306 inhibition on JAR of 95% for **33** and 100% for **32i** at 10  $\mu$ M (IC<sub>50</sub> range 1.5–3.7  $\mu$ M, Table 1).  
 307 Globally compounds **32i** and **33** were the most promising leads for antiproliferative drugs  
 308 development. Moreover, the antiproliferative property could be attributed to RNase L which is  
 309 involved in apoptosis induction, as suggested by Hwang and co-workers. Considering the SAR  
 310 studies, the authors evaluated the difference of RNase L thermal shift induced by the presence

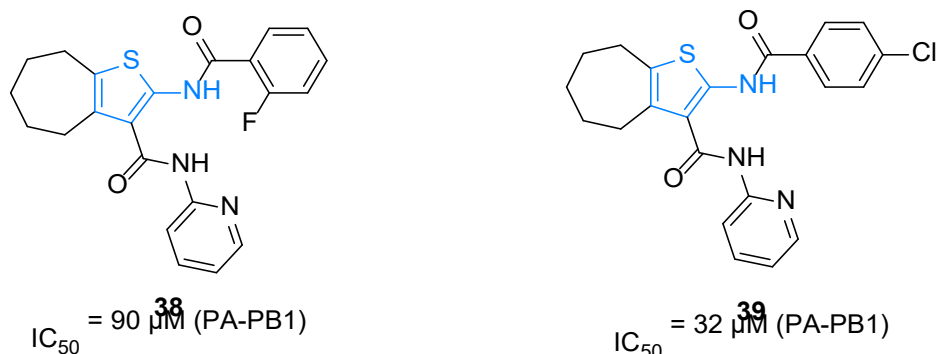
311 of 60  $\mu\text{M}$  of synthesized compounds. The authors observed that the presence of cycloalkyl  
312 group was important in the antiproliferative activity *via* RNase-binding results (**33** and **34a-e**).  
313 However, the presence of substituents on the phenyl group grafted on the amine 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.

## 318 2.3. Antiviral Activity

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

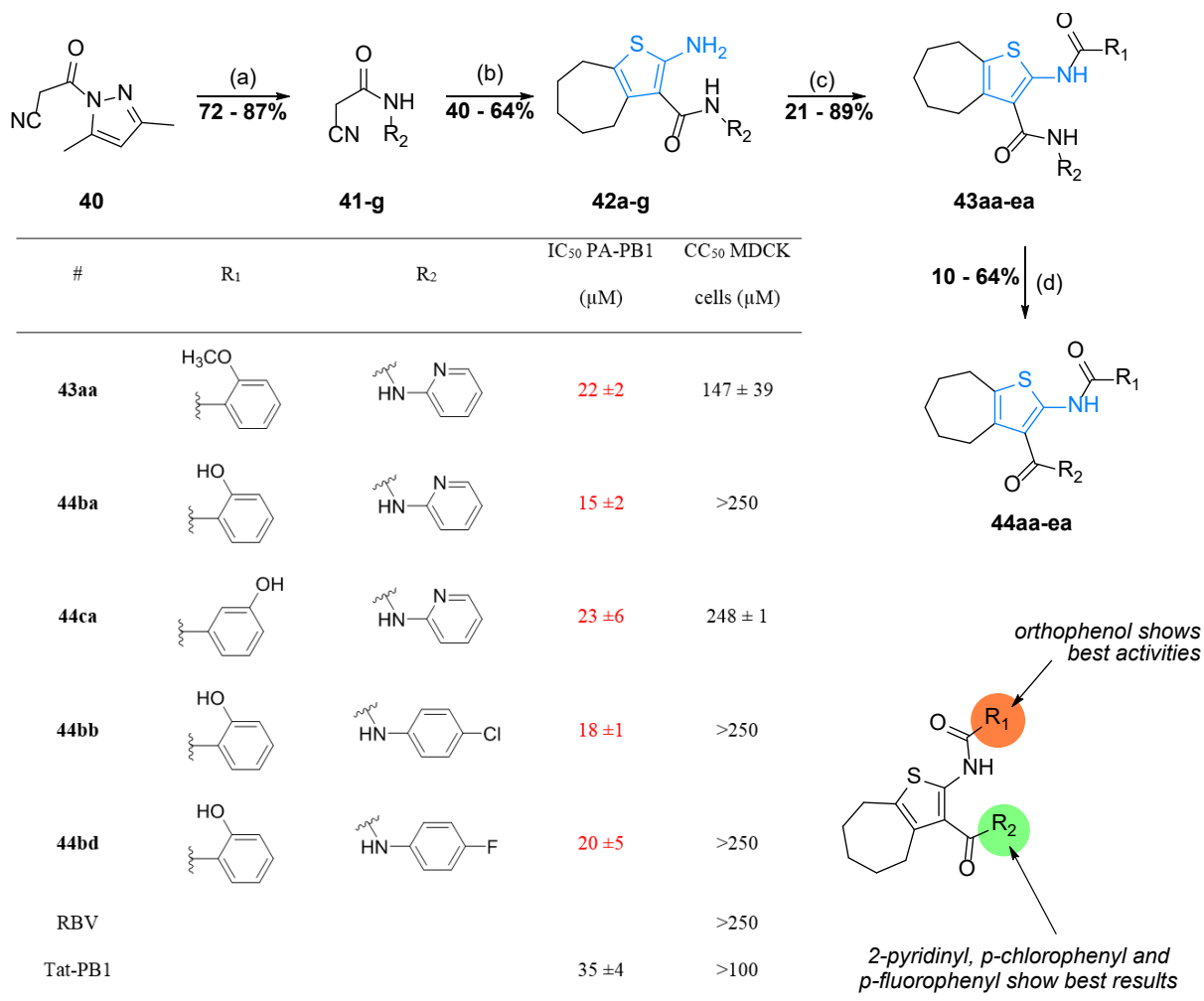
323 In 2017, Desantis *et al.* investigated cycloheptathiophene-3-carboxamide scaffolds (**38** and  
324 **39**) as influenza polymerase subunits disruptors (Figure 3).<sup>18</sup> The authors proposed the  
325 inhibition of RdRp (RNA-dependent RNA polymerase), which is a heterotrimeric complex  
326 composed of three distinct subunits: polymerase acidic protein (PA), polymerase basic protein  
327 1 (PB1) and polymerase basic protein 2 (PB2). The strategy developed by the authors is to  
328 inhibit the PA-PB1 subunit to propose anti-influenza compounds.



330 *Figure 3. Known antiviral derivatives used as hits by Desantis et al.*<sup>18</sup>

331 Based on **38** and **39** scaffolds that showed good activities ( $IC_{50}$  range 32–90  $\mu M$ ;  $EC_{50}$  range  
332 18–145  $\mu M$ ), the authors designed a large series of 21 new molecules (**44aa-ea**) whose  
333 synthesis is pictured in scheme 8. First, compound **40** reacted with substituted amines in toluene  
334 under reflux to afford **41a-g** in 72–87% yields after purification by precipitation and washes  
335 with Et<sub>2</sub>O. Then, reaction of **41a-g** with cycloheptanone, ammonium acetate and acetic acid in  
336 benzene under reflux followed by addition of elemental sulfur S<sub>8</sub> with diethylamine in ethanol  
337 at 40–50°C led to **42a-g** in 40–64% yields and used without further purification. The third step

338 consisted in substitution of the amine function of **42a-g** with the corresponding acyl chloride in  
 339 pyridine at room temperature to give **43aa-ea** with 21–89% yields after precipitation in cold  
 340 water and filtration. The last step was the demethylation of **43aa-ea** with BBr<sub>3</sub> in DCM at room  
 341 temperature to form the desired products **44aa-ea** in 10–64% yields after crystallization from  
 342 ethanol/water.



343

344 **Scheme 8.** (a) R<sub>2</sub>NH<sub>2</sub> in toluene, reflux; (b) cycloheptanone, NH<sub>4</sub>OAc, AcOH in benzene, reflux then S<sub>8</sub>,  
 345 diethylamine in EtOH, 40 – 50 °C; (c) R<sub>1</sub>COCl in pyridine, r.t.; (d) BBr<sub>3</sub>, DCM, r.t.

346

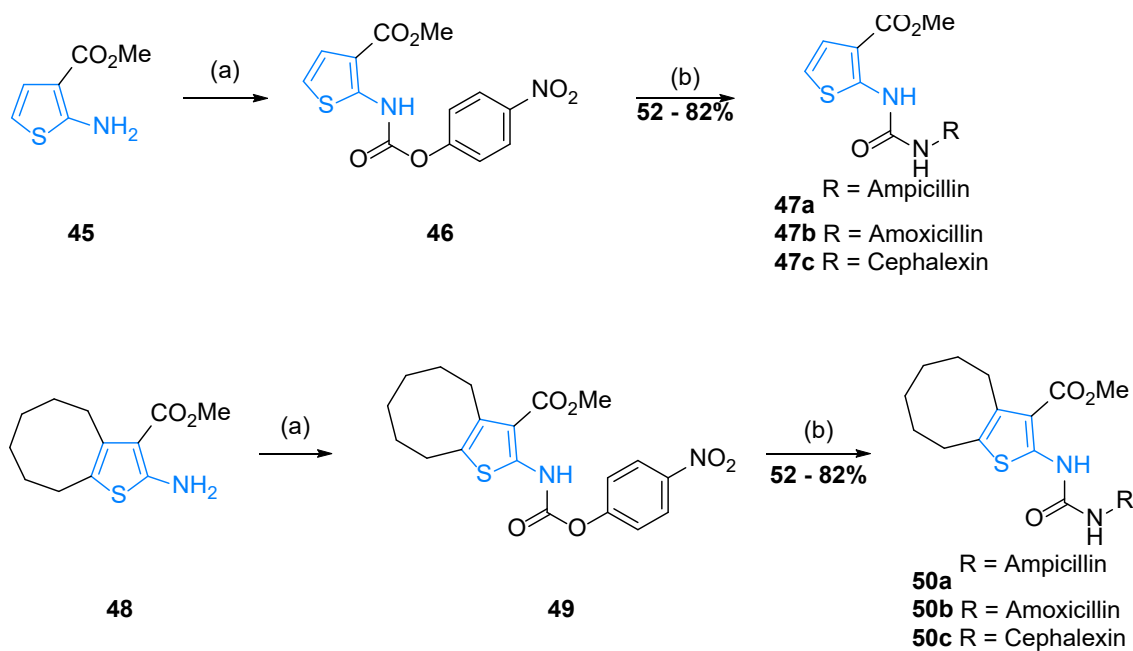
347 5 compounds (**43aa**, **44ba**, **44ca**, **44bb** and **44bd**) showed an IC<sub>50</sub> against PA-PB1 below  
 348 25 μM (Scheme 8). Among these 5 derivatives, **44bb** showed best activities with IC<sub>50</sub> on PA-

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  $\text{IC}_{50}$  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 ( $\text{CC}_{50}$  range  $147 - >250 \mu\text{M}$ ). Considering the SAR studies, the derivatives having  
353 *o*-phenol moiety were shown to be best ( $\text{IC}_{50}$  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  $\text{R}_2$  position, increased  
355 antiviral activities. This study confirmed PA-PB1 as targets for innovative and potent anti-flu  
356 agents.

## 357 2.4. Antibacterial Activity

358 The emergence of antibiotic-resistant organisms is a major public well-being concern,  
359 especially in health care centers.<sup>63</sup> Bacteria have succeeded in establishing resistance against  
360 antibiotics, making them resistant toward various classes of antibacterial compounds. The  
361 emergency to develop novel series possessing antimicrobial activities has never been more  
362 important.<sup>64,65</sup> The World Health Organization (WHO) started to consider the bacterial  
363 resistance seriously, notifying the death toll would reach million in 2050, against 700 000  
364 deaths in 2015<sup>66</sup> and 1.3 million in 2019.<sup>67</sup> To survive and adapt to environmental changes,  
365 bacteria developed different resistance mechanisms and changed in response to the use of  
366 antibiotics.

367 In 2018, Wagh and his team synthesized two series of 2-ATs as novel antibacterial  
368 compounds (**47a-c** and **50a-c**).<sup>68</sup> To enhance the global biological activity, they decided to  
369 couple 2-ATs with antibiotics, whose synthesis is disclosed in scheme 9. Ampicillin,  
370 amoxicillin and cephalexin were chosen as  $\beta$ -lactamine antibiotics. The first series has been  
371 formed from 2-AT **45**, using 4-nitrophenylchloroformate as coupling reagent and sodium  
372 hydrogen carbonate in acetonitrile at 0°C to room temperature for 8 hours to afford  
373 carbamate **46** in no specified yield. Then, **46** reacted with the corresponding aminopenicillins  
374 with diisopropylethylamine in DMF at room temperature for 2 hours to give **47a-c** in 52–  
375 82% yields. The second series was synthesized in the same manner from 2-AT **48** to give the  
376 desired products **50a-c** in 52–82% yields for the second step.



377

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

378 **Scheme 9.** (a) 4-nitrophenylchloroformate, NaHCO<sub>3</sub> in ACN, 0°C – r.t., 8h; (b) aminopenicillins, DIPEA,

379 DMF, r.t., 2h.

380 They observed the antimicrobial activities through minimal inhibitory concentration (MIC)

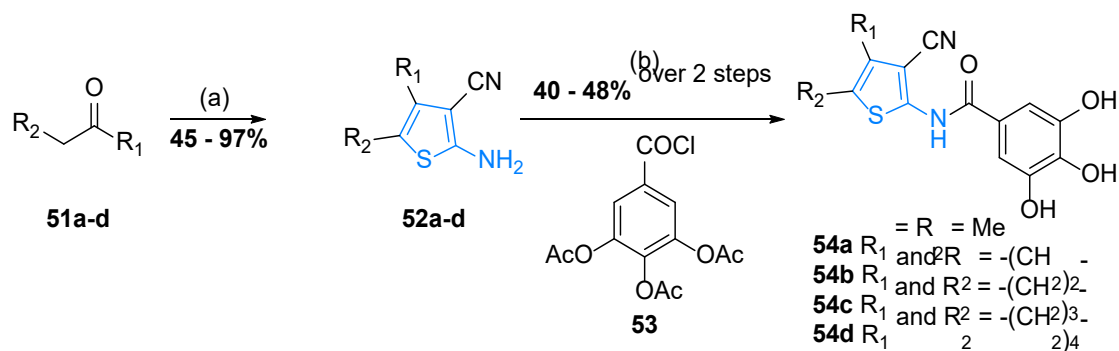
381 and half-maximal effective concentration (EC<sub>50</sub>) on 4 different strains: *Staphylococcus aureus*

382 (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa*

383 (*P. aeruginosa*) and *Mycobacterium tuberculosis* (*Mtb*). Generally, antimicrobial activities

384 stayed close to that of reference antibiotics (Scheme 9). The authors showed that several  
385 compounds showed better antimicrobial behaviors than free reference antibiotics (Ampicillin,  
386 amoxicillin and cephalexin): **47c**, **50c** (MIC of 0.75  $\mu\text{g/mL}$  for **47c** and 0.46  $\mu\text{g/mL}$  for **50c**  
387 compared to 2.39  $\mu\text{g/mL}$  for cephalexin on both *S. aureus* and *B. subtilis* strains, Table 1).  $\text{EC}_{50}$   
388 were also improved ( $\text{EC}_{50}$  of 0.26  $\mu\text{M}$  for **47c** and 0.18  $\mu\text{M}$  for **50c** compared to 0.77  $\mu\text{M}$  for  
389 cephalexin on *S. aureus*). On *B. subtilis*, **50c** also showed a better  $\text{EC}_{50}$  ( $\text{EC}_{50}$  of 0.21  $\mu\text{M}$  against  
390 0.25  $\mu\text{M}$  for cephalexin). Other derivatives kept same activities range on different bacterial  
391 strains. Considering the antitubercular activity, the authors observed that **50c** showed a good  
392 activity with MIC of 0.78  $\mu\text{g/mL}$  on *Mtb* H<sub>37</sub> RA strain. They also conducted toxicity  
393 evaluations: none of the tested compounds exhibited cytotoxic activities on human cancer cell  
394 lines (Hela, PC-3 and THP-1). Computational predictions were realized. Compound **50c** has  
395 been shown to fit in the PBP pocket from penicillin G-binding site. Probably, the 2-AT moiety  
396 did not add too much steric hindrance that would lower the activity. Rather, the 2-AT scaffold  
397 contributed to the global biological activity by interacting with residues of the binding domain,  
398 such as serine in position 398 (Ser398). Finally, compound **50c** was shown to be suitable for  
399 further development as antitubercular drugs.

400 In 2020, Mahdavi *et al.* worked on a novel 2-ATs series (**54a-d**) as antibacterial, synthesized  
401 in two steps as depicted in scheme 10.<sup>69</sup> The first step was the synthesis following classical  
402 Gewald conditions consisting in the reaction of malonodinitrile **2a** with ketones **51a-d** to afford  
403 2-ATs **52a-d** in 45–97% yield after filtration and recrystallization from ethanol. Secondly, **52a-**  
404 **d** reacted with acyl chloride **53** and triethylamine in DCM at 0°C for 2 hours followed by the  
405 addition of hydrazine hydrate in DCM at room temperature for 1 hour (cleavage of the acetates  
406 of the gallic acid moiety) to give amides **54a-d** in 40–48% yields after recrystallization from  
407 methanol/water.



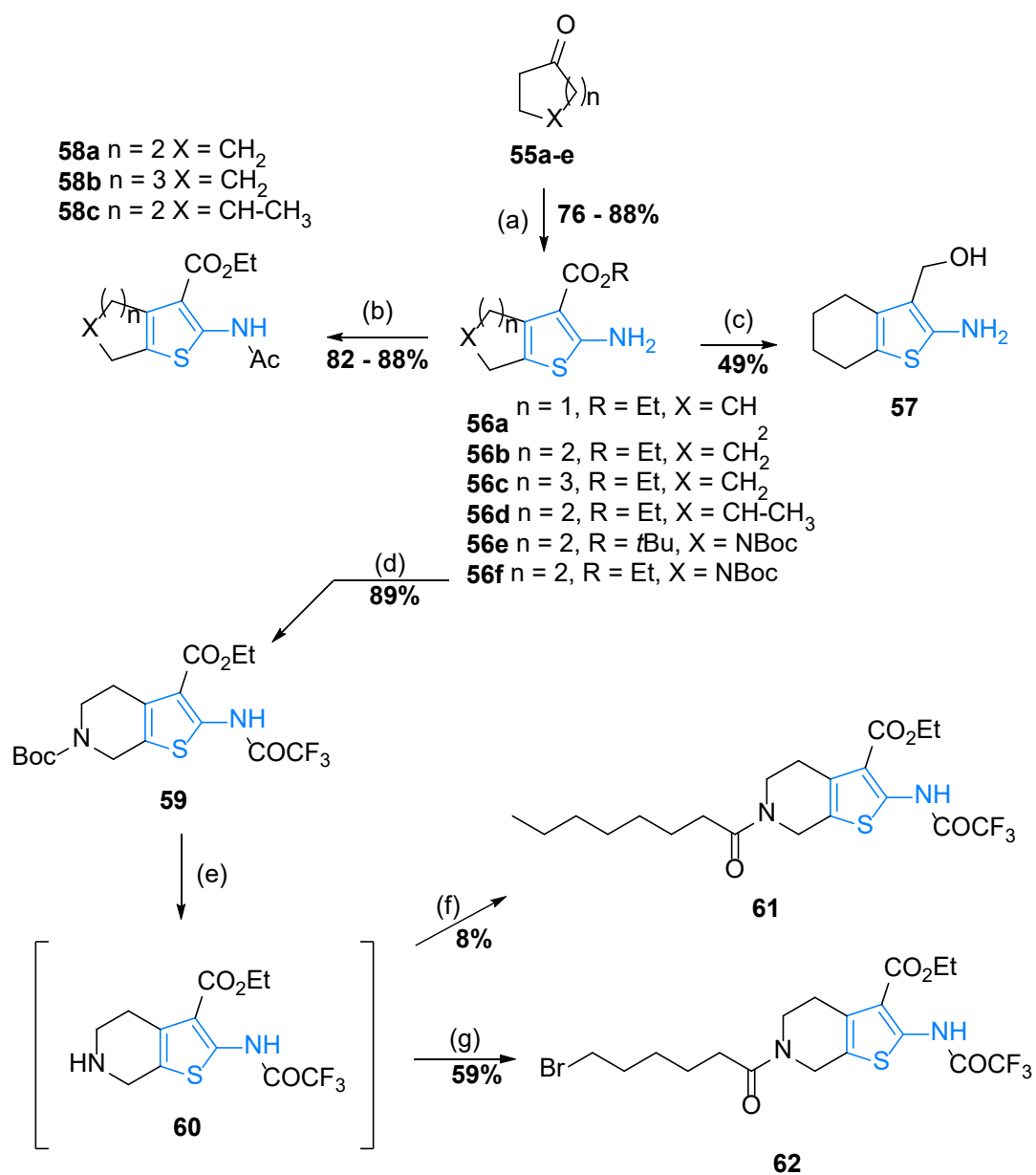
#	Gram-positive MIC ( $\mu\text{g/mL}$ )			Gram-negative MIC ( $\mu\text{g/mL}$ )			
	<i>S. aureus</i>	<i>S. coagulase</i>	<i>E. faecalis</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. vulgaris</i>	<i>P. aeruginosa</i>
<b>54a</b>	0.41 $\pm$ 0.14	0.15 $\pm$ 0.01	0.25 $\pm$ 0.00	NA	0.25 $\pm$ 0.00	0.17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

409 **Scheme 10.** (a) malonodinitrile **2a**, *S*<sub>8</sub>, morpholine in EtOH, r.t.; (b) **53**, NEt<sub>3</sub>, DCM, 0°C, 2h then hydrazine  
 410 hydrate, r.t., 1h.

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

424 lines. Compound **54d** showed a good IC<sub>50</sub> on HeLa cell line with 3.2 μM, making it interesting  
425 for further development for cervical cancer. SAR studies were not examined by the authors.

426 In 2020, da Cruz *et al.* described the synthesis of 2-ATs (**56a-f**, **58a-c**, **57**, **59**, **61** and **62**) as  
427 *S. aureus* efflux pump inhibitors (Scheme 11).<sup>70</sup> By analogy with former works,<sup>71,72</sup> they  
428 decided to synthesize analogs to inhibit NorA efflux pumps of *S. aureus* 1199B strain. The first  
429 step was the formation of N-substituted 2-ATs following Gewald conditions. Ketones **55a-e**  
430 reacted with alkyl cyanoacetates **2b** or **2d**, elemental sulfur S<sub>8</sub> and morpholine in ethanol to  
431 give N-acetyl-2ATs **56a-f** in 76–88% yields after filtration and washes with water. Then, the  
432 second step was the Bouveault-Blanc ester reduction of **56a** in the presence of metallic sodium  
433 in dry methanol to afford **57** in 49% yield after purification on silica gel column  
434 chromatography. In another step, the reaction of **56b-d** with anhydride acetic in 1,4-dioxane at  
435 80–90°C for 3 to 4 hours led to **58a-c** in 82–88% yields after filtration and washes with water.  
436 In the last step, **56f** reacted with trifluoroacetic anhydride and trimethylamine in DCM at 0–  
437 5°C for 2 hours to give **59** in 89% yield after washes with cold ethanol. The intermediate **60**  
438 was obtained from **59** in the presence of a mixture of DCM and TFA (8/2) at 0–5°C for 1 hour  
439 and further used without prior purification. **60** reacted with octanoyl chloride and DIPEA in  
440 DCM at 0–5°C for 1–2 hours to afford **61** in 8% yield after purification after washes with ice  
441 cold ethanol. Reaction with **60** and 6-bromo hexanoyl chloride gave **62** in 59% yield after  
442 washes with cold ethanol.



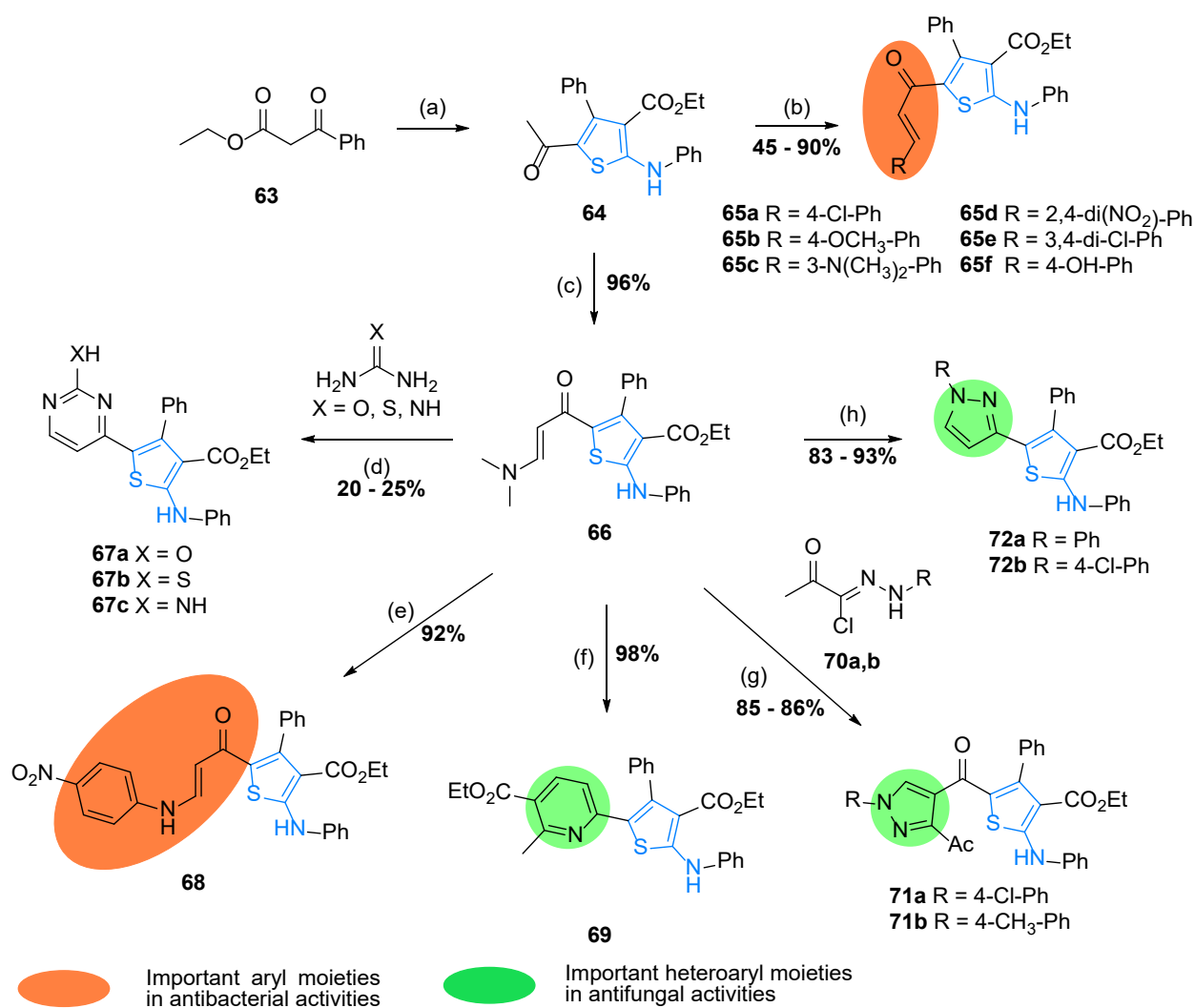
443

444 **Scheme 11.** (a) ethyl cyanoacetate **2b** or tert-butyl cyanoacetate **2d**, *S<sub>8</sub>*, 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

452 known NorA efflux pump inhibitor restore the activity by 16-fold. These four compounds (**58b**,  
453 **59**, **61** and **62**) were evaluated following the ethidium bromide (EtBr) accumulation and efflux  
454 assays as NorA efflux pump inhibitors. **62** was shown to have similar results as reserpine for  
455 accumulation assay (125% EtBr accumulation at 50  $\mu$ M for **62** and 110% for reserpine, Table  
456 1). EtBr is a NorA efflux pump substrate, its accumulation in the bacteria indicates an inhibition  
457 of the efflux pump. **61** was the only one to show an inhibition of NorA efflux pump by  
458 preventing EtBr efflux by 85% over 30 minutes at 50 $\mu$ M (Table 1).

459 In 2021, Asiri *et al.* described the design, the synthesis and the antimicrobial activity of  
460 novel 2-ATs (**65a-f**, **67a-c**, **68**, **69**, **71a,b** and **72a,b**).<sup>73</sup> The first step of the synthesis pictured  
461 in scheme 12 was inspired by Mabkhot methodology<sup>74</sup> and consisted in the reaction of **63** with  
462 potassium carbonate and phenyl isothiocyanate followed by addition of chloroacetone to afford  
463 2-AT **64** in 95% yield after filtration and recrystallization from ethanol. **65a-f** were synthesized  
464 by condensing corresponding aldehydes with **64** in EtOH with triethylamine under reflux for 8  
465 hours in 45–90% yields. Reaction of **64** with DMA in DMF under reflux for 8 hours yielded to  
466 **66** in 96%. **66** is a central scaffold used to form different series. **67a-c** were synthesized by  
467 reaction of ureas with **66** in 20–25% yields. **66** reacted with 4-nitroaniline under reflux for 6  
468 hours to give **68** in 92% yield. Pyridine core synthesis on **66** with AcOH/AcONH<sub>4</sub> and ethyl  
469 acetoacetate under reflux for 3 hours led to **69** in 98% yield. As well, **66** reacted with **70a,b** in  
470 dry benzene/TFA to afford **71a,b** in 85–86% yields. Finally, reaction of **66** with corresponding  
471 hydrazine derivatives under reflux for 6 hours to lead to **72a,b** in 83–93% yields. All the  
472 products were purified by filtration followed by recrystallization from ethanol.



473

#	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>
<b>65a</b>	19.5 ± 0.44	29.8 ± 0.58	12.3 ± 0.25	17.6 ± 0.19
<b>65b</b>	18.9 ± 0.63	17.9 ± 0.49	11.4 ± 0.27	10.2 ± 0.41
<b>65c</b>	16.3 ± 0.52	19.6 ± 0.58	12.5 ± 0.39	14.8 ± 0.47
<b>65d</b>	16.5 ± 0.47	18.9 ± 0.53	11.3 ± 0.45	9.9 ± 0.41
<b>65e</b>	11.3 ± 0.52	10.7 ± 0.39	9.7 ± 0.42	8.9 ± 0.31
<b>65f</b>	26.7 ± 0.74	21.4 ± 0.58	18.7 ± 0.68	19.5 ± 0.64
<b>67a</b>	16.3 ± 0.42	19.1 ± 0.51	11.4 ± 0.36	10.7 ± 0.31
<b>67b</b>	12.3 ± 0.58	12.7 ± 0.37	9.8 ± 0.34	11.3 ± 0.39
<b>67c</b>	16.3 ± 0.42	19.1 ± 0.51	11.4 ± 0.36	10.7 ± 0.31
<b>68</b>	26.3 ± 0.73	20.9 ± 0.61	12.6 ± 0.54	11.2 ± 0.44
<b>69</b>	18.2 ± 0.68	18.9 ± 0.64	10.8 ± 0.41	11.1 ± 0.43
<b>71a</b>	14.6 ± 0.58	14.3 ± 0.58	10.2 ± 0.31	8.8 ± 0.24
<b>71b</b>	12.3 ± 0.58	12.7 ± 0.37	9.8 ± 0.34	11.3 ± 0.39
<b>72a</b>	12.3 ± 0.58	12.7 ± 0.37	11.6 ± 0.36	10.9 ± 0.21
<b>72b</b>	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

474

475 *Scheme 12.* (a) K<sub>2</sub>CO<sub>3</sub>, PhNCS in DMF, 25°C then chloroacetone; (b) RCHO, NEt<sub>3</sub> in EtOH, reflux, 8h; (c)

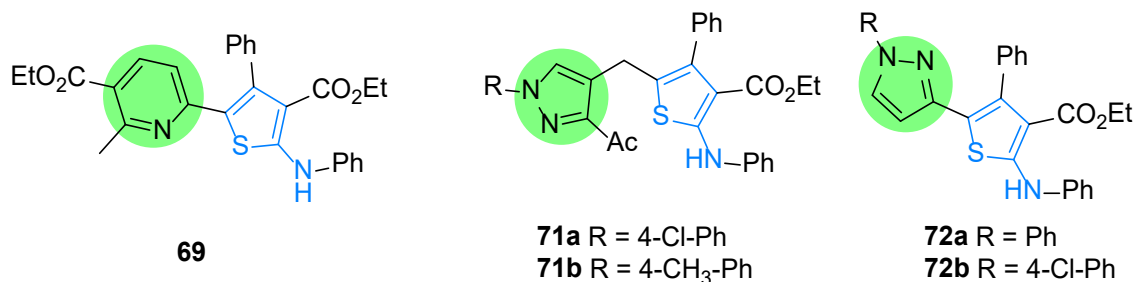
476 DMF/DMA, reflux, 8h; (d) EtOH, reflux, 6h; (e) 4-nitroaniline, reflux, 6h; (f) Ethyl acetoacetate, AcOH,  
477 AcONH<sub>4</sub>, reflux, 3h.; (g) dry benzene, NEt<sub>3</sub>, reflux, 6h; (h) RNHNH<sub>2</sub>, reflux, 6h.

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  $\alpha,\beta$ -unsaturated ketone with phenyl moiety (compounds **65** and **68**) exhibited better  
486 antibacterial properties than antifungals.

## 487 2.5. Antifungal Activity

488 Fungal infections are associated with significant morbidity and mortality. Existing  
489 antifungal agents are frequently limited by dose-limiting toxicity, drug interactions or drug  
490 resistance, necessitating the development of new antifungals, particularly those with novel  
491 mechanisms of action.<sup>75,76</sup>

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



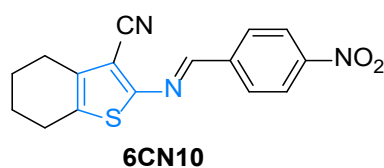
499

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

500

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

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

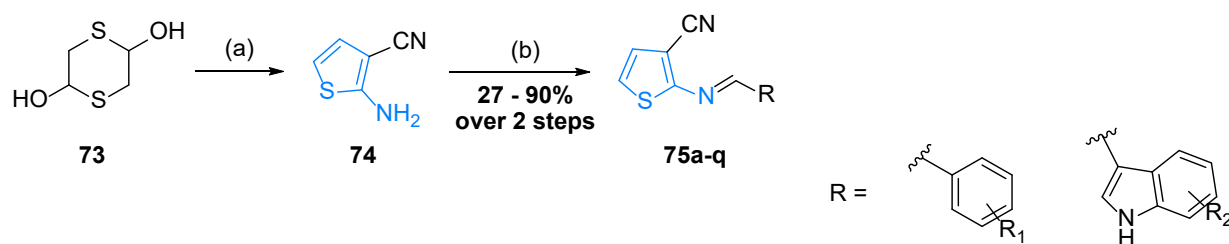


505

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

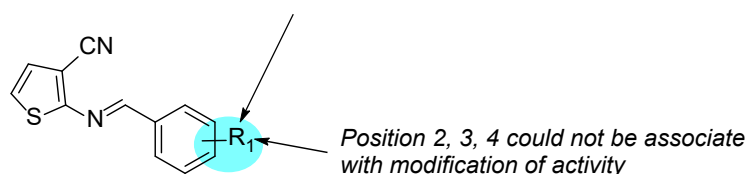
507 Desired Schiff bases **75a-q** were synthesized under microwave irradiation in presence of  
 508 commercially available 1,4-dithiane-2,5-diol **73** with malonodinitrile **2a** and sodium  
 509 bicarbonate in ethylene glycol at 80°C for 8 minutes to give transiently **74**. Imination of **74**

510 was realized with corresponding aryl aldehydes and acetic acid at 50°C for 2 minutes to afford  
 511 **75a-q** in 27–90% yield after purification by precipitation in water/methanol.

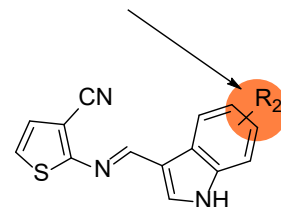


#	R <sub>1</sub>	R <sub>2</sub>	<i>C. parapsilosis</i> ATCC 22019	<i>E. floccosum</i> 6999	<i>E. floccosum</i> 6754	<i>T. tonsurans</i> 700	<i>T. tonsurans</i> 2822	<i>T. mentagrophytes</i> 5431	<i>T. mentagrophytes</i> 5432	<i>T. rubrum</i> 6753
<b>75e</b>	2,6-Cl	-	G	G	G	64	256	G	G	32
<b>75f</b>	2-NO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

4-halogen showed good influence on both activity and spectrum of action, Nitro group favored



EWG favorable, FDG like decreased activity  
OCH<sub>3</sub>

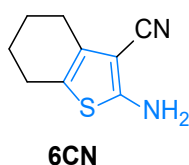


512  
 513 **Scheme 13.** (a) malonodinitrile **2a**, NaHCO<sub>3</sub> in ethylene glycol, M.W., 80°C, 8 min; (b) aryl aldehyde in  
 514 AcOH, M.W., 50°C, 2 min. G: bacteria growth observed at 1024 µg/mL

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

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

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



537

538

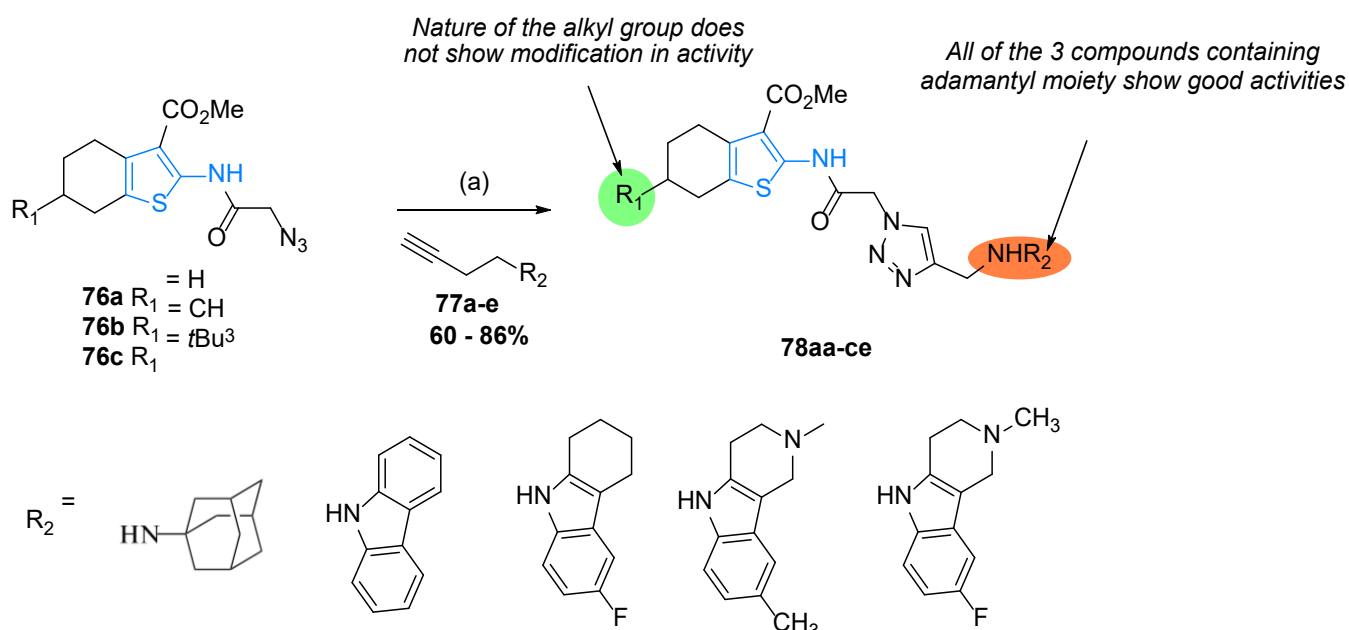
*Figure 6. Structure of 6CN.*

539 **6CN** was inserted between two sheets of chitosan and the authors suggested that the  
540 structure realized the following interactions: nitrile function of **6CN** interacted with the amine  
541 function of chitosan and interactions were observed between the polymer carbonyl and the **6CN**  
542 amino group on position 2.

## 543 2.6. Channels inhibitors activity

544 T-type calcium channels are attractive targets for potential treatment of epilepsy  
545 inflammatory or neuropathic pain, insomnia, Parkinson's disease, and cancer. Three isoforms  
546 of these T-type calcium channels are expressed in peripheral and central nerve and responsible  
547 for different biophysical functions.<sup>79-81</sup>

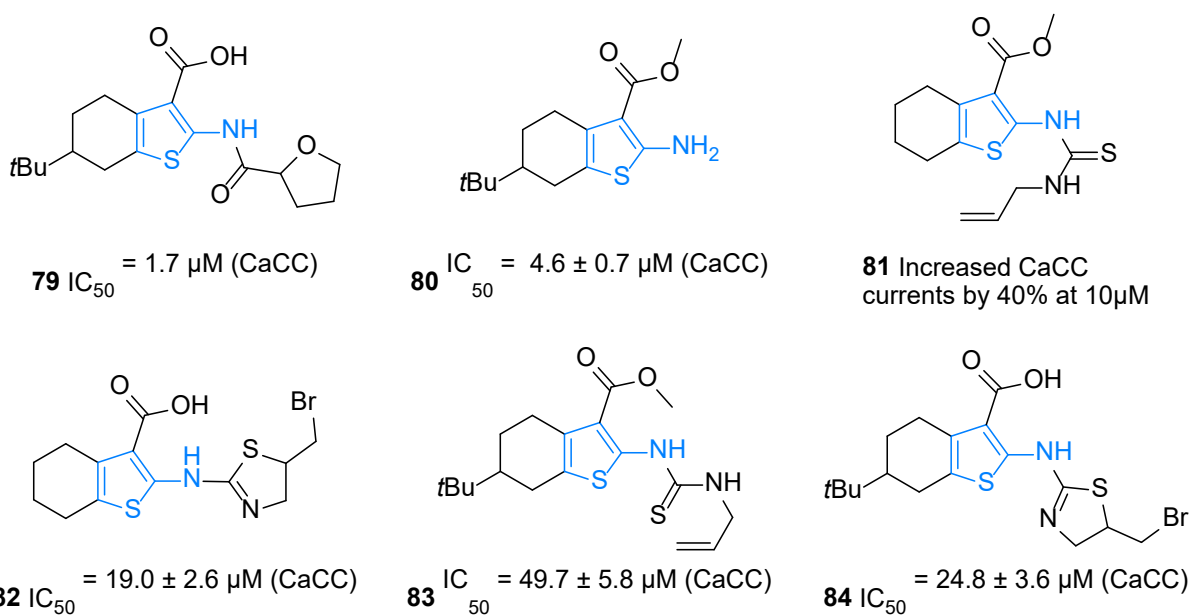
548 In 2020, Zamoyski *et al.* synthesized new N-substituted 2-ATs (**78aa-ce**) as calcium-  
549 activated chloride channels (CACC) modulators (Scheme 14).<sup>82</sup> The synthesis followed a  
550 Huisgen methodology in the presence of 2-AT **76a-c** and alkynes **77a-e** to afford triazoles **78aa-**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



553 **Scheme 14.** (a) Cu<sub>2</sub>SO<sub>4</sub>, sodium ascorbate, DCM/H<sub>2</sub>O, 3h, 40°C.

554 All 3 compounds containing adamantyl moiety exhibited biological effect on the targeted  
555 channel: **78ca** displayed potentiation on CaCC of 35% at 10μM, **78aa** showed an IC<sub>50</sub> of 1.6 ±  
556 0.4μM and **78ba** an IC<sub>50</sub> of 5.4 ± 0.5 μM whereas the other derivatives showed no effect on the  
557 protein. Adamantane moiety could be crucial in the biological activity because all compounds  
558 possessing one can modulate the activity of CACC. The same team worked on other 2-ATs

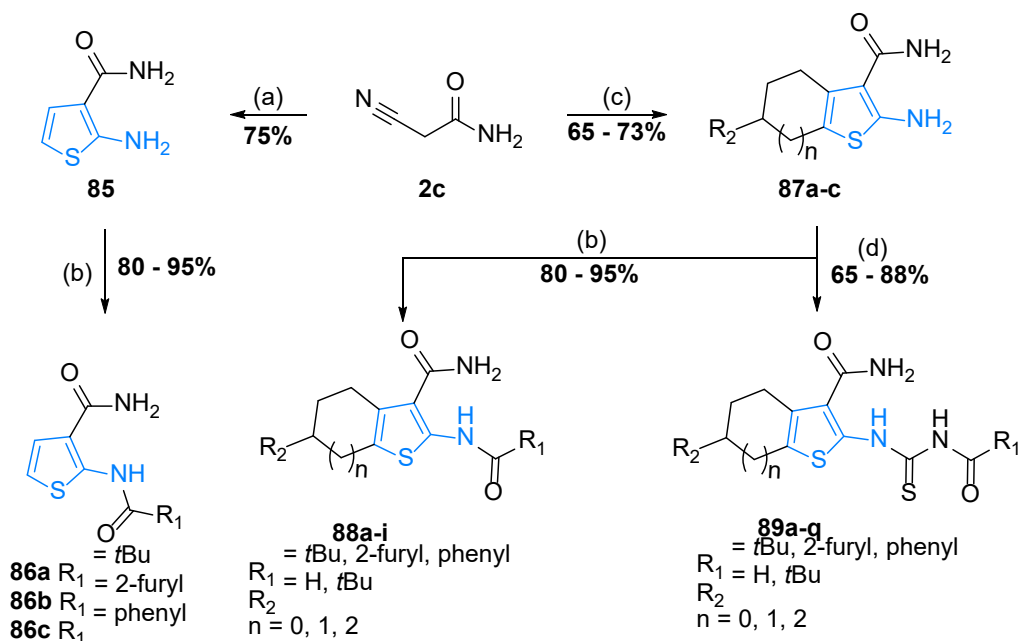
559 derivatives (**79–84**, Figure 7). The authors used previously reported 2-ATs whose synthesis was  
 560 not depicted. They evaluated the biological activity of **80–84** and compared them to the known  
 561 CaCC<sub>inh</sub> A01 **79** that is a good CaCC inhibitor ( $IC_{50} = 1.7 \mu\text{M}$ ).<sup>84</sup> The tested compounds (**79–**  
 562 **84**) showed good results ( $IC_{50}$  range on CaCC channels 4.6–49.7  $\mu\text{M}$ ). Compound **80**  
 563 demonstrated best results with good inhibition at 1.0  $\mu\text{M}$  and an  $IC_{50}$  of 4.6  $\mu\text{M}$  (Table 1).  
 564 Compound **81** illustrated antagonistic activity on the channel by increasing CaCC current by  
 565 40% at the concentration of 10  $\mu\text{M}$ .



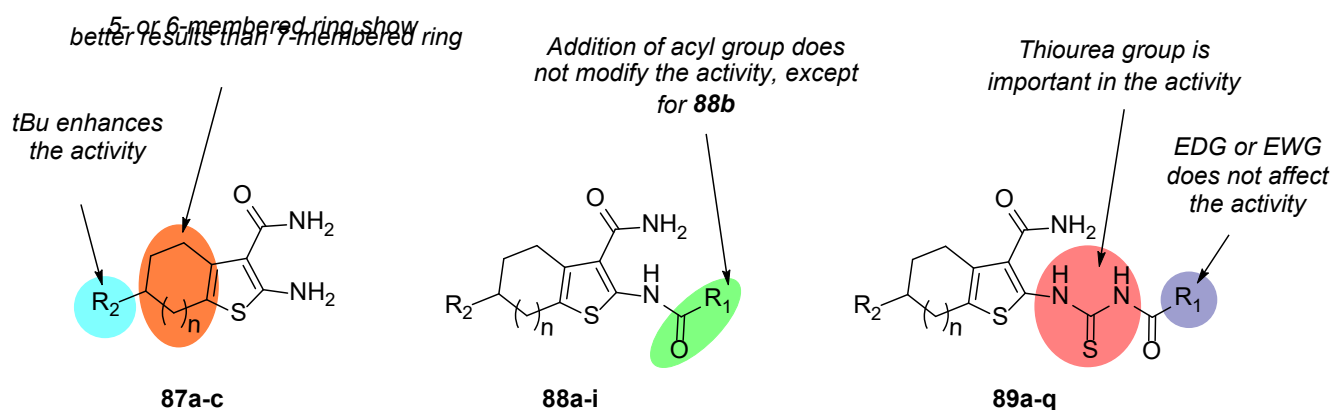
567 **Figure 7.** Structures and activities of compounds **79–84**.

568 In 2020, Choi and his team developed the synthesis of 2-ATs (**86a-c**, **88a,i** and **89a-q**) as a  
 569 potential anti-glioma, inhibitors of Anoctamin-1 (ANO1) channels (CaCCs), known to be  
 570 overexpressed in these cancers.<sup>85</sup> The synthesis for this series is shown in scheme 15. All the  
 571 2-ATs derivatives were synthesized in classical Gewald way. On the one hand, starting with  
 572 commercially available 2-cyanoacetamide **2c** with 1,4-dithiane-2,5-diol **73** and triethylamine  
 573 in ethanol at 70°C for 6 hours afforded **85** in 75% yield after crystallization with Et<sub>2</sub>O in DCM.  
 574 Then, **85** reacted with corresponding acyl chloride in pyridine at room temperature for 1 hour  
 575 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 S<sub>8</sub>,  
577 morpholine in ethanol at 70°C for 12 hours gave 2-ATs **87a-c** in 65–73% yields after  
578 crystallization with Et<sub>2</sub>O 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( $\mu\text{M}$ )	ANO2( $\mu\text{M}$ )
<b>87c</b>	-	1	<i>t</i> Bu	80.09 $\pm$ 0.75	2.95 $\pm$ 0.05	NT
<b>88b</b>	<i>t</i> Bu	2	H	67.94 $\pm$ 0.78	4.79 $\pm$ 1.10	NT
<b>88c</b>	<i>t</i> Bu	1	<i>t</i> Bu	<b>86.87 <math>\pm</math> 2.01</b>	<b>3.10 <math>\pm</math> 1.23</b>	<b>15.43 <math>\pm</math> 1.23</b>
<b>89a</b>	Ph	0	H	70.50 $\pm$ 0.00	2.83 $\pm$ 0.96	NT
<b>89c</b>	Ph	1	<i>t</i> Bu	70.28 $\pm$ 8.76	3.09 $\pm$ 1.07	NT
<b>89o</b>	2-ClPh	0	H	76.70 $\pm$ 10.75	2.29 $\pm$ 0.30	NT
<b>89q</b>	4-ClPh	0	H	<b>72.25 <math>\pm</math> 11.78</b>	<b>2.36 <math>\pm</math> 0.79</b>	<b>7.43 <math>\pm</math> 0.67</b>



583

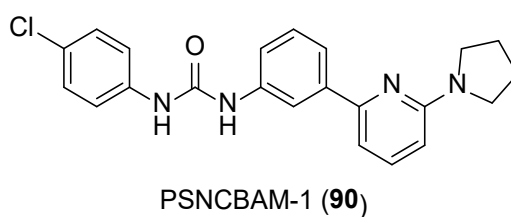
584 **Scheme 15.** (a) 1,4-dithiane-2,5-diol,  $\text{NEt}_3$  in EtOH, 70°C, 6h; (b) acyl chloride, pyridine, r.t., 1h; (c)  $S_8$ ,585 morpholine in EtOH, 70°C, 12h; (d) acyl chloride,  $\text{NH}_4\text{SCN}$ , reflux, 2h.

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 IC<sub>50</sub> comprised between 2.29 and 4.79 μM 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 R<sub>2</sub> 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

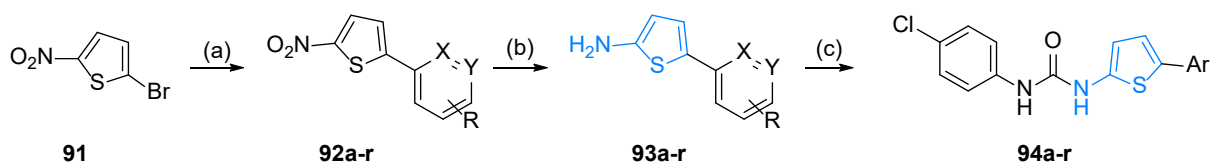
The cannabinoid type-1 (CB<sub>1</sub>) receptor is an interest target because it is involved in a wide range of neuronal and physiological processes, such as memory, appetite, anxiety and depression.

Nguyen and co-workers described in 2019 novel series based on 2-ATs (**94a-r**) and inspired by diarylurea series they previously reported.<sup>86</sup> Their goal was to realize the synthesis of allosteric inhibitors of human CB<sub>1</sub> 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.



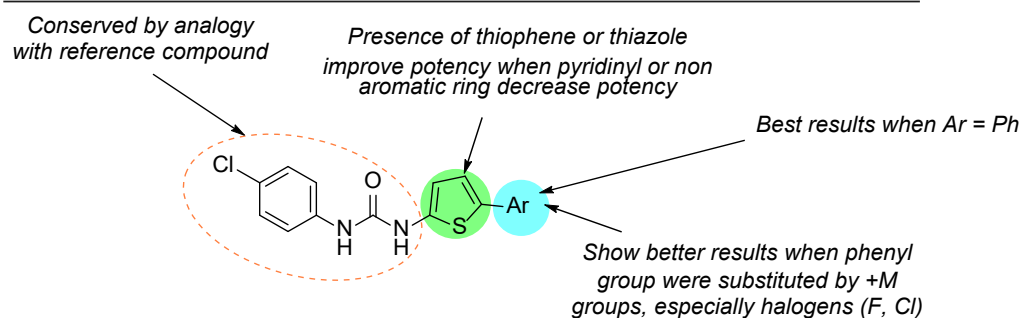
*Figure 8. Structure of PSNCBAM-1.*

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 = H, F, Cl, Ac, CO<sub>2</sub>Me  
 SO<sub>2</sub>Me, OMe, Me, NMe<sub>2</sub>  
 X, Y = CH or N

#	Ar	IC <sub>50</sub> (nM)		
		hCB <sub>1</sub> Calcium assay	hCB <sub>1</sub> [ <sup>35</sup> S]GTPγS binding assay	mCB <sub>1</sub> [ <sup>35</sup> 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)



619

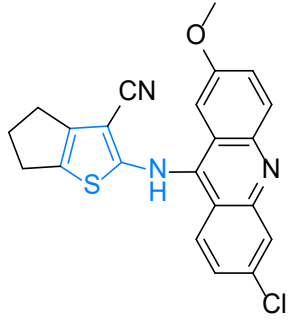
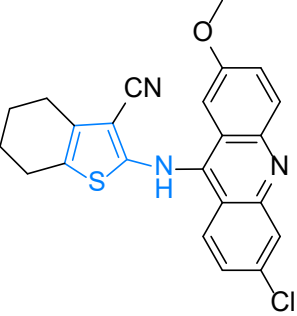
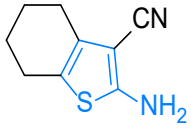
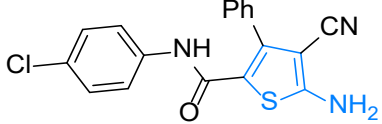
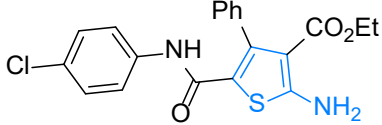
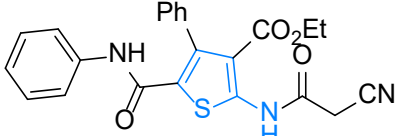
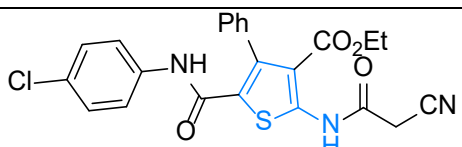
620 **Scheme 16.** (a) phenyl boronic acid derivatives, Pd(PPh<sub>3</sub>)<sub>4</sub>, aq. NaHCO<sub>3</sub>, DME, reflux, 16h; (b) hydrazine  
 621 hydrate, Raney-Ni, EtOH, 60°C, 1h; (c) 4-ClPhNCO, CHCl<sub>3</sub>, 60°C, 16h.

622

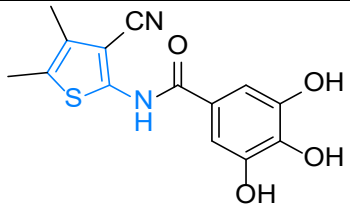
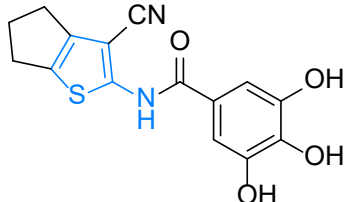
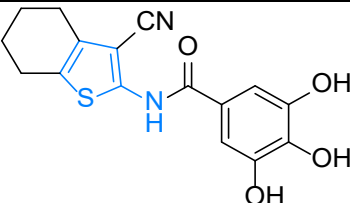
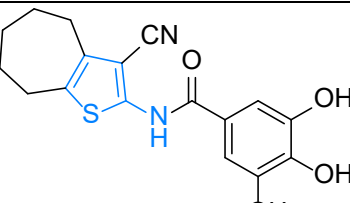
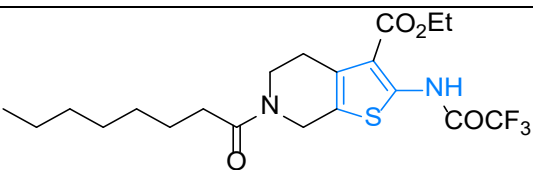
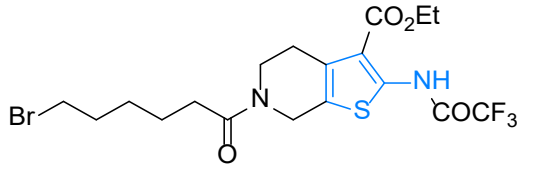
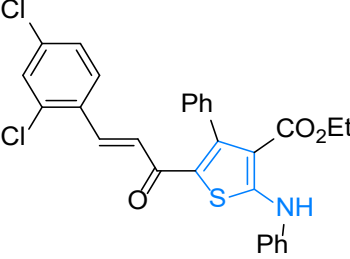
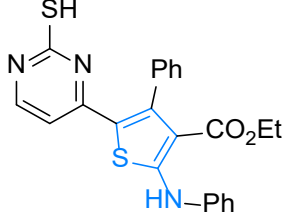
623 Among the 20 synthesized products (IC<sub>50</sub> range on hCB<sub>1</sub> with calcium assay 6.8–501 nM;  
 624 IC<sub>50</sub> range on hCB<sub>1</sub> with [<sup>35</sup>S]GTPγS binding experiment 63–3467 nM), 5 compounds showed  
 625 a particular good activity (**94a-e**, IC<sub>50</sub> range on hCB<sub>1</sub> with calcium assay 6.8–40 nM; IC<sub>50</sub> range  
 626 on hCB<sub>1</sub> with [<sup>35</sup>S]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<sub>1/2</sub> = 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 CB<sub>1</sub> inhibitory activity, like thiazole (IC<sub>50</sub> range 6.8–79 nM).  
633 However, the activity decreased when cycloalkyl or pyridinyl was present (IC<sub>50</sub> 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 (IC<sub>50</sub> range 21–111 nM) than, acetyl, ester, sulfonate groups (negative mesomeric  
637 effect groups; IC<sub>50</sub> range 108–501 nM).

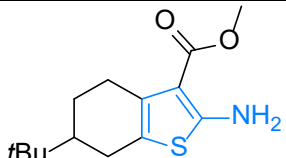
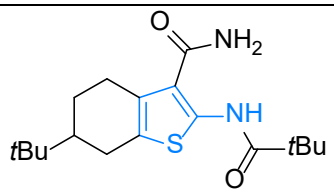
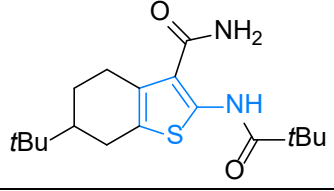
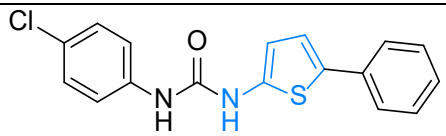
638 **Table 1.** Compounds with best activities, their mechanisms of action and their yields.

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

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

54b		MIC = $0.15 \pm 0.01$ $\mu\text{g/mL}$ ; $0.49 \mu\text{M}$ ( <i>S. coagulase</i> )	N.D.	48%	69
54b		MIC = $0.10 \pm 0.03$ $\mu\text{g/mL}$ ; $0.32 \mu\text{M}$ ( <i>S. coagulase</i> )	N.D.	43%	69
54c		MIC = $0.15 \pm 0.01$ $\mu\text{g/mL}$ ; $0.45 \mu\text{M}$ ( <i>S. coagulase</i> )	N.D.	45%	69
54d		MIC = $0.15 \pm 0.01$ $\mu\text{g/mL}$ ; $0.43 \mu\text{M}$ ( <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 = $8.9 \pm 0.31$ $\mu\text{g/mL}$ ; $17.0 \mu\text{M}$ ( <i>E. coli</i> )	N.D.	88%	73
67b		MIC = $9.8 \pm 0.34$ $\mu\text{g/mL}$ ; $22.6 \mu\text{M}$ ( <i>P. aeruginosa</i> )	N.D.	20%	73
<b>Antifungal activity</b>					

69		11.2 ± 0.44 µg/mL; 23.0 µM ( <i>C. albicans</i> )	N.D.	98%	73
71a		14.3 ± 0.58 µg/mL; 25.7 µM ( <i>C. albicans</i> )	N.D.	86%	73
71b		12.6 ± 0.38 µg/mL; 23.5 µM ( <i>G. candidum</i> )	N.D.	85%	73
72a		15.8 ± 0.38 µg/mL; 33.9 µM ( <i>C. albicans</i> )	N.D.	83%	73
72b		MIC = 11.2 µg/mL; 22.4 µM ( <i>C. albicans</i> )	N.D.	93%	73
75m	 Chemical Formula: C <sub>12</sub> H <sub>8</sub> N <sub>2</sub> S Molecular Weight: 212,2700	MIC = 16 µg/mL; 75.3 µM ( <i>E. floccosum</i> 6999)	N.D.	56%	77
75o	 Chemical Formula: C <sub>12</sub> H <sub>7</sub> BrN <sub>2</sub> S Molecular Weight: 291,1660	MIC = 32 µg/mL; 101 µM ( <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
<b>Channels inhibitory activity</b>					
78aa		IC <sub>50</sub> = 1.6 ± 0.4 µM (CaCC)	CaCC channel inhibition	85%	82

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

639 N.D.: not described

### 640 3. Conclusion

641 We reported here the synthesis and biological properties of 2-ATs described over the last 5 years  
642 (2017–2022) with a particular interest in promising biological applications. The 2-AT scaffold has  
643 recently demonstrated wide applications in medicinal chemistry. As previously described, its lead-  
644 likeness and ability to fight against various infections, regardless they are viral, fungal, bacterial or  
645 protozoal is an important tool in drug discovery. 2-ATs could also possess anticancer properties and  
646 further development as antiproliferative compounds seems interesting and crucial in cancer  
647 chemotherapy. 2-AT potential as channel inhibitors as CaCC modulators is also interesting for the  
648 discovery of treatments of neurological diseases such as Parkinson's. SAR studies were shown to be  
649 almost systematically realized when a series was synthesized to provide maximal lead information for  
650 further development following the “hit to lead” process for preclinical studies or *in vivo* evaluations.

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

### 657 Authors contributions

658  
659 ZB was responsible for the management of the work, overall supervision. The authors  
660 contributed to the data preparation and drafted and revised the manuscript. All authors have  
661 read and approved the final manuscript.

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663

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666 Region for the financial support.

## 667 Declaration of competing interest

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

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