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Synthesis of novel [3,1]-benzothiazepine and [3,1]-benzoxazepine derivatives with antitumoral activity†

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A new method for the synthesis of [3,1]-benzothiazepines and [3,1]-benzoxazepines from the reaction of *C*-allylanilines and isothiocyanates or isocyanates without the need for the isolation of any intermediate is described. The compounds were obtained in good to moderate yields and some exhibited cytotoxic activity against tumor cell lines.

Benzothiazepines and benzoxazepines constitute important building blocks in pharmaceutical research while they can be found in many drugs and preclinical leads.[1]

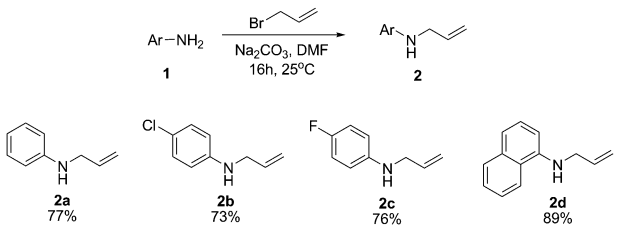
The synthesis of different isomeric forms of these compounds by varying the position of the heteroatoms in their structures together with their unique pharmacological properties make the development of new methods for the synthesis of medium-sized heterocycles containing N, S and O atoms a subject of great interest. Thus, several isomers of benzothiazepine[2] and benzoxazepine[3] rings were already described and some of them exhibited a wide variety of pharmacological effects.[4]

Herein, we wish to describe a new method for the synthesis of [3,1]-benzothiazepines and [3,1]-benzoxazepines for further evaluation of their antitumoral activities. The general method for the synthesis of [3,1]-benzoxazepines is based on the photochemical isomerization of quinoline *N*-oxides.[5] However, it requires a significant number of steps for preparation of the starting materials used in the cyclization event, and sometimes proceed with a lack of regioselectivity.[6] In the case of [3,1]-benzothiazepines derivatives, to our knowledge there are even few reports in the literature describing the synthesis of this class of compounds.[7]

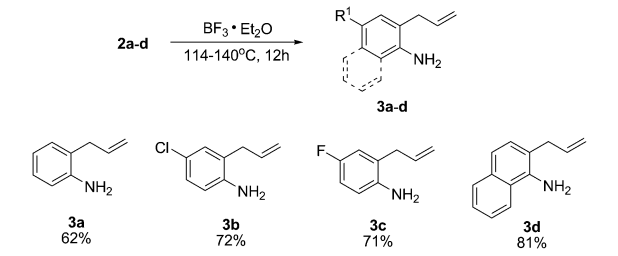
We initially focused on experiments to find a route which gave the required starting materials. Thus, allylation of commercially available anilines **1a–d** was performed following a literature procedure[8] to yield the corresponding *N*-allylanilines **2a–d** in good yields (Scheme 1).

Subsequent aza-Claisen rearrangement[9] at 114–140 °C without the use of any solvent gave the corresponding *C*-allylanilines **3a–d** in good yields (Scheme 2).

C-Allylanilines, **3** were then reacted with commercially available isothiocyanates, in dichloromethane to give *in situ* the corresponding thioureas. Subsequent addition of iodine[10] to the reaction mixture gave the corresponding [3,1]-benzothiazepines



Scheme 1 Synthesis of *N*-allylanilines **2a–d**.



Scheme 2 Synthesis of *C*-allylanilines **3a–d**.

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4 through a 7-*exo*-trig mode cyclization in good yields and without the isolation of any intermediate compound (Table 1).

The reaction yield does not seem to be strongly influenced by the electronic effects on the groups attached to the aromatic ring of both *C*-allylanilines 3 or isothiocyanates since the [3,1]-benzothiazepines bearing neutral, electron-withdrawing and electron-donating groups in the aromatic rings were compatible with the reaction conditions. The only exception occurred when **3a** was used as precursor, where a complex mixture of products was observed. Further attempts to purify **4a** from this mixture were unsuccessful (Table 1, entry 1).

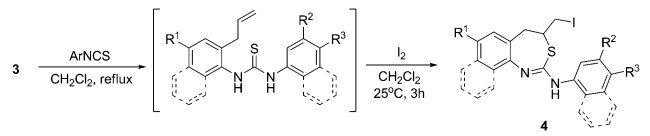
Next, we extended the method for the synthesis of the [3,1]-benzoxazepines derivatives, **5a–c**. In this case, the reaction solvent was replaced by tetrahydrofuran due to the low solubility of intermediates *N*,*N*′-diarylureas in dichloromethane.

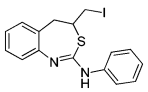
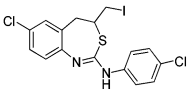
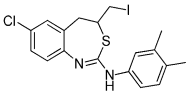
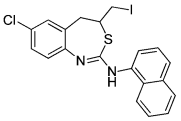
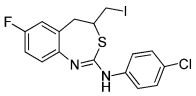
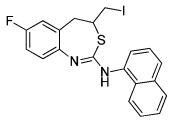
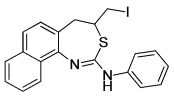
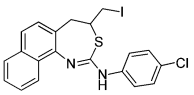
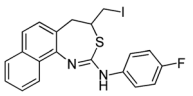
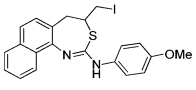
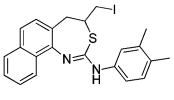
Thus, reaction of *C*-allylanilines, **3a–b** with phenylisocyanate led to the formation *in situ* of the corresponding *N*,*N*′-diarylureas after one hour. Subsequent cyclisation using iodine gave the corresponding [3,1]-benzoxazepines **5a–c** in moderate yields (Table 2). The reaction using isocyanates gave lower yields (38–56%) when compared to the reaction using thiocyanates. This result could be explained by the lower nucleophilic character of the oxygen atom from the carbonyl group of the synthesized ureas when compared to the sulfur atom present in the thioureas.

The cyclisation of ureas or thioureas could lead to two isomers being the C=N bond *endo* or *exo* (Fig. 1). For example, for compound **5a**, if the signal attributed to the proton bonded to nitrogen – Hb – presents an HMBC correlation with the carbon nuclei Cb from ring B, the obtained compound should have an *endo* C=N bond (Fig. 1, structure **5a**). However, if the hydrogen atom bonded to nitrogen –Ha – presents an HMBC correlation with the carbon nuclei Ca from ring A, the obtained compound should have an *exo* C=N bond (Fig. 1, structure **5a′**).

From the spectrum obtained for **5a**, ring A shows four different signals in the ^{1}H NMR spectrum at δ_H 7.74 (d, 8.1 Hz, 1H), 7.23 (d, 7.2 Hz, 1H), 7.14 (t, 8.1 Hz, 1H) and 6.93 (td, 7.5, 1.0 Hz, 1H). Conversely, ring B shows only three signals at δ_H 7.52 (dd, 8.7, 0.9 Hz, 2H), 7.34–7.26 (m, 2H) and 7.07–7.00 (m, 1H). HMBC spectrum shown a correlation between the signal at δ_H 8.85, corresponding to N–H and the signals at δ_C 152.0 and 120.6. The signal at δ_C 152.0 was attributed to C=N. In addition, the signal at δ_C 120.6 showed an HMQC correlation with the signal at δ_H 7.52, attributed to the proton at the *ortho*

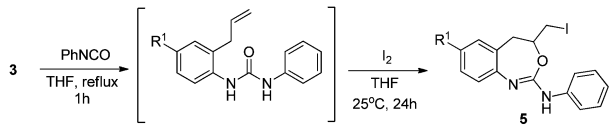
Table 1 Synthesis of [3,1]-benzothiazepines **4** through iodocyclization

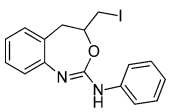
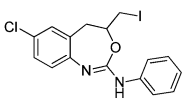
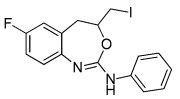


Entry	3	Ar	Time (h)	[3,1]-Benzothiazepine		Yield[a] (%)
1	**3a**	C_6H_5	72		**4a**	—[b]
2	**3b**	4-Cl-C_6H_4	99		**4b**	74
3	**3b**	3,4-Me_2-C_6H_3	72		**4c**	37
4	**3b**	$C_{10}H_7$	120		**4d**	63
5	**3c**	4-Cl-C_6H_4	72		**4e**	69
6	**3c**	$C_{10}H_7$	96		**4f**	76
7	**3d**	C_6H_5	72		**4g**	52
8	**3d**	4-Cl-C_6H_4	120		**4h**	77
9	**3d**	4-F-C_6H_4	48		**4i**	70
10	**3d**	4-MeO-C_6H_4	120		**4j**	64
11	**3d**	3,4-Me_2-C_6H_3	120		**4k**	74

[a] Isolated yield. [b] A complex mixture of products was obtained.

Table 2 Synthesis of [3,1]-benzoxazepines **5** through iodocyclization



Entry	3	[3,1]-Benzoxazepine, 5		Yield (%)
1	**3a**		**5a**	47
2	**3b**		**5b**	56
3	**3c**		**5c**	38

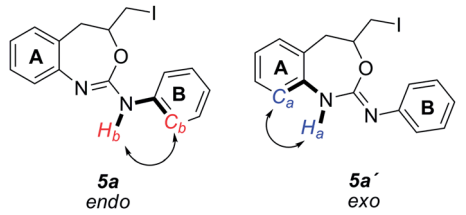


Fig. 1 HMBC correlation between the *endo* and *exo* compounds.

Table 3 IC_{50} and standard error values (μg mL^{-1}) of two different experiments for compounds **4h** and **4i**

Compound	HL-60	NCI-H292	HT-29
4h	8.2 ± 0.2	15.1 ± 1.6	14.8 ± 1.1
4i	2.1 ± 0.9*	12.4 ± 3.1	7.7 ± 0.8*
DOX[a]	0.02 ± 0.005	0.1 ± 0.05	0.4 ± 0.05

[a] Doxorubicin (DOX) was the positive control. *$p < 0.05$ compared compound **4i** to **4h** by *t* test.

position in ring B. In this way, the signal at δ_C 120.6 was attributed to the carbon at the *ortho* position in the ring B, confirming the structure of the obtained compound as **5a.**

The *in vitro* anticancer activity of benzoxathiepin,[11] benzodioxepin[12] and benzoxazepines[13] on MCF-7 cells has been previously described. Further studies of 4,1-benzoxazepines by microarray technology showed that the main molecular targets of some of these compounds are pro-apoptotic genes with protein kinase activity such as GP132, ERN1 or RAC1, which prevent the metastatic progression. These facts prompt us to submit the synthesized compounds to the MTT assay[14] for the evaluation of their cytotoxic effects on tumor cells.

The synthesized compounds were then screened at 25 μg mL^{-1} against HL-60 (human pro-myelocytic leukemia), NCI-H292 (human lung carcinoma) and HT-29 (human colon carcinoma) tumor cells lineages. The samples with growth inhibition over 90% were used to determine the IC_{50} values (concentration that causes 50% growth inhibition). Analogues **4h** and **4i** exhibited good cytotoxicities and their IC_{50} values (μg mL^{-1}) were determined. The results are described in Table 3.

From Table 3 it can be seen that compound **4h** displayed moderate cytotoxic activity against all tested cancer cell lines, being more active for HL-60 cells. Compound **4i** exhibited better antiproliferative activity against HL-60 and HT-29 cell lines,[15] being also more active for HL-60 with an IC_{50} = 2.1 μg mL^{-1} demonstrating the potential of the newly synthesized compounds as antitumoral agents.

Conclusions

In conclusion, the method demonstrates to be useful for the synthesis of [3,1]-benzothiazepines and [3,1]-benzoxazepines derivatives without the need of the isolation of any intermediate.

Compounds **4h** and **4i** exhibit good antitumoral activity for HL-60 cells and are promising intermediates for the synthesis of an array of more potent target structures while the iodine atom can be used as an additional point of diversity.

Acknowledgements

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