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The expedient synthesis of 1,5-benzothiazepines as a family of cytotoxic drugs

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Abstract—The expedient synthesis of 1,5-benzothiazepines using LaY zeolite under stirring condition is reported and synthesized compound screened for cytotoxic activity. The reaction produces the product in relatively low yields and requires a long time when they were performed in various solvents under conventional and microwave irradiation. Thus, the procedure provides a simple and green synthetic methodology under environmentally friendly conditions.
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The versatile chemotherapeutic applications of 1,5-benzothiazepines especially that of *diltiazem* in the treatment of ailments of the cardiovascular system such as coronary vasodilation,[1] hypertension,[2] etc. enthused great interest in a detailed study of this class of compounds. While carrying out drug design, it was found that an important number of fluorinated 1,4- and 1,5-benzodiazepines had been introduced as pharmacological and cardiovascular agents, such as *fluorodiazepam, triflubazam*, etc. Incorporation of fluorine atoms on 1,5-benzothiazepines or analogous nuclei enhances pharmacological properties by increasing the solubility in lipid materials and fat deposits in the body when compared to their non-fluorinated analogs.[3] It was also reported that Cl,[4] Me,[5] CF_3[6] or a free COOH group[7] when present on different positions in the 1,5-benzothiazepine nucleus act as potential pharmacophores. A series of 1,5-benzothiazepines bearing fluorine and 4-fluorophenyl groups have been found to be effective for treatment of cancer metastasis[8] and recently 8-fluoro-1,5-benzothiazepine reported from our laboratory has been found to be a promising anti-AIDS agent in preliminary screening.[9]

RE exchanged Y Zeolites are reported as effective catalysts in organic chemistry and their specificity in gas phase transformations is greatly utilized in industry.[10] Several organic reactions like alkylation, polymerization, cyclization,[11] photoreduction,[12] or preparation of nitroalkenes[13] occur in gas phase or with reactants sorbed within zeolite in inert solvent. Recently, several reports on the use of acidic zeolites (HY) in macrolactonization,[14] acetalization,[15] acetylation,[16] and gem-diacetalization,[17] as well as the synthesis and application of the first organic-functionalized zeolite-beta[18] prompted us to investigate the new catalytic possibilities of Y zeolite.

It has been extensively studied by various workers that the reaction between α,β-unsaturated carbonyl compounds and 2-aminobenzenethiols takes place in two steps[19,20] involving the previous formation of the Michael adduct as an intermediate[20,21] which readily undergoes dehydrative cyclization to give 1,5-benzothiazepines. It was observed that the formation of the intermediate and the final cyclized product is affected by the reaction conditions,[22] catalyst,[23] and type of groups or substituents present[24] on the compounds (Scheme 1).

The use of various zeolite catalysts in organic transformations is a growing area of interest.[25,26] The Na form of faujasites (Y), a class of zeolite catalysts, is almost neutral in nature. However, a basic character can be introduced by increasing the alumina content of the catalyst framework, thereby increasing the population of counter cations and their radii.[27,28] Furthermore, the

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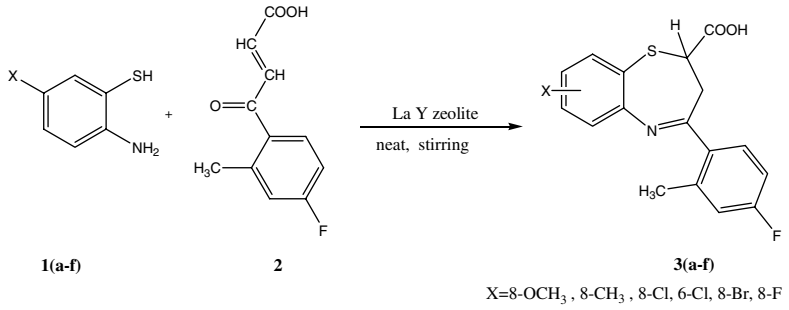

Scheme 1.

electrostatic interaction between the metal cation and the organic guests within the zeolite supercage has been well studied.[29,30] In this context, we have initiated a program exploiting the interactions of exchanged rare-earth cations of the faujasites with the included organic guest molecules in the supercages. Our main aim is to identify the conditions in which such an interaction could create sufficient polarization of the organic guests inside the supercages to facilitate chemical transformations. In line with our expectations, when 2-aminobenzenethiols (**1a**–**f**) with α,β-unsaturated ketones (**2**) were stirred at room temperature in the presence of LaY zeolite, the 8-substituted-2-carboxy-2,3-dihydro-1,5-benzothiazepines **3a**–**f** were produced in 72–94% yield (Scheme 1).

Traditional methods applied for the syntheses of 2-carboxy-2,3-dihydro-1,5-benzothiazepines involve the use of volatile organic solvents under reflux and strong acids/bases as catalyst, giving low yields due to the extended reaction time which favors the formation of the corresponding disulfides.[31,32]

As part of a program aimed at achieving simple and environmentally compatible synthetic methodologies in search of medicinally important heterocycles, we have investigated the catalytic activity of LaY zeolite for organic syntheses and synthesized compounds were tested for cytotoxic activities first on a cell line of human tumor HL-60 (human promyelocytic leukemia). Hence, in an attempt toward a non-traditional approach to the experimental setup of organic reactions, and our continuing interest in the synthesis of fluorine containing biodynamic heterocycles and as an extension of our work on the reactions of 2-aminobenzenethiols with α,β-unsaturated ketones and analogous compounds[33,9] under solvent-free and ecofriendly conditions, we report herein, for the first time, the synthesis of title compounds **3a**–**f** under solventless conditions (Scheme 1).

As an initial attempt to find an optimal reaction condition, a variety of experimental conditions were examined for title compound **3a** by changing catalyst, reaction medium (MW irradiation, conventional, and stirring), and temperature (Table 1). It was found that more acidic LaY[34] zeolite is the best choice of catalyst for the preparation of 2-carboxy-2,3-dihydro-1,5-benzothiazepines compared with CeY, NaY, and HY[34] zeolites (Table 1). Since the number and strength of acid site in zeolite increase with metal cation exchanged in the order of $H^+ < Na+ < Ce^2 + < La^{3+}$, the increase in the yield of **3a** according to this order suggests that acid sites on zeolite work as active sites for this reaction (Table 1). For checking the catalyst amount's effect on reaction conditions, we have tried the reaction taking different

Table 1. Comparative study of the synthesis of **3a** (X = OMe)

Entry	Medium	Reaction conditions	Reaction temperature (°C)	Time (min)	Yield[b] (%)
1	Ethanol + dry HCl gas	Reflux	78	420	62
2	Toluene + TFA	Reflux	120	720	46
3	Methanol + gl. AcOH	Reflux	115	420	44
4	LaY zeolite[a]	MW	130	13	70
5	Montmorillonite KSF	MW	138	12	68
6	CeY zeolite[a]	MW	130	16	58
7	Neat + DMF (2–3 drops)	MW	140	10	59
8	PTSA	MW	125	12	54
9	**LaY zeolite[a]**	**Stirring**	**100**	**8**	**94**
10	LaY zeolite[a]	Stirring	80	14	65
11	LaY zeolite[a]	Stirring	60	17	56
12	LaY zeolite[a]	Stirring	rt	18	50
13	Montmorillonite KSF	Stirring	100	15	54
14	CeY zeolite[a]	Stirring	100	12	72
15	NaY zeolite[a]	Stirring	100	15	64
16	HY zeolite[a]	Stirring	100	18	50

[a] 100 wt % catalyst used that means that the substrate:catalyst weight ratio is 1:1.
[b] Isolated yields.

amount's of catalyst and found that 2 g of catalyst is sufficient for activation of the synthesis of **3a**. Higher amount of catalyst produced lesser yield (Table 2).

To check the general applicability of reaction in commonly available monomode reactor, reaction was performed under three types of MW reactor and results found with high reproducibility (Table 3).

As usual in the catalytic reaction, the increase of the reaction temperature accelerated the conversion of the substrate. It was also observed that MW irradiation using different monomode reactor with focused rays and a much more homogeneous electromagnetic field and classical heating gave lower yield with higher reaction time compared to reactions realized under stirring conditions and interphase catalysis. In view of all these results, we have synthesized all the compounds (**3a–f**) using LaY zeolite under stirring at 100 °C (Table 4).

The phototoxicity of title compounds was investigated first on a cell line of human tumor HL-60 (human promyelocytic leukemia). Table 5 shows the extent of cell survival expressed as GI50, which is the concentration, expressed in mM, that induces 50% of inhibition of cell growth, after irradiation at different UVA doses. Doxorubicin was used as positive control.

Control experiments with UVA light or compounds alone were carried out without significant cytotoxic effects (data not shown). The results, shown in Table 5, indicate that compound **3a** is not active, instead **3e**,**f** exhibited the highest activity. Interestingly, substitution for a methyl group leads to an inactive derivative **3b**. From this preliminary screening the most active compounds were also evaluated on a human intestinal adenocarcinoma cell line (LoVo) and one line of immortalized, not tumorigenic, human keratinocytes (NCTC 2544). From Table 6 it appears that the

Table 2. Effect of LaY catalyst amounts on synthesis of **3a** (X = OMe)

Entry	LaY catalyst amount (g)	Time (min)	Yield[a] (%)
1	0	20	No reaction
2	0.5	15	Traces of product
3	1.0	12	20
4	1.5	11	58
5	2.0	8	94
6	3.0	8	92

[a] Isolated yields.

Table 3. Synthesis of **3a** using different available MW monomode reactor

Entry	Medium	MW reactor	Reaction temperature (°C)	Time/min	Yield[a] (%)
1	LaY zeolite	CEM	130	12.5	70
		Prolobo	130	13	70
		Biotage	130	13	69
2	Montmorillonite KSF	CEM	138	12	68
		Prolobo	138	12	68
		Biotage	138	13	68
3	CeY zeolite	CEM	130	15	58
		Prolobo	130	16	58
		Biotage	130	16.5	57
4	Neat + DMF (2–3 drops)	CEM	140	10	58
		Prolobo	140	10	59
		Biotage	140	10.5	59
5	PTSA	CEM	125	12.5	52
		Prolobo	125	12	54
		Biotage	125	12	52

[a] Isolated yield.

Table 4. Synthesis of 8-substituted-2-carboxy-4-(4-fluoro-2-methylphenyl)-2,3-dihydro-1,5-benzothiazepines (**3a–f**) using LaY zeolite[a]

Cmpd	X	Reaction time (min)	Yield[b] (%)	Mp (°C)	Lit. mp (°C)
3a	8-OCH_3	8	94	180	178[37]
3b	8-CH_3	10	75	155	157[37]
3c	8-Cl	12	72	189	186[37]
3d	6-Cl	10	84	195	192[37]
3e	6-Br	13	85	165	166[37]
3f	6-F	15	82	139	138[38]

[a] 100 wt % catalyst used that means that the substrate: catalyst weight ratio is 1:1.
[b] Isolated yields.

Table 5. Photocytotoxicity of test compounds against HL-60 cell line as Compounds, GI50 (mM)[a]

Compounds	GI_{50}[a] (μm)	
	1.25[b] J cm^{-2}	2.5 J cm^{-2}
3a	>10	10
3b	>10	10
3c	>10	6.8 ± 2.0
3d	>10	6.9 ± 1.9
3e	2.5 ± 0.6	0.9 ± 0.2
3f	6.6 ± 0.9	0.8 ± 0.1
Doxorubicin	2.36	1.45

[a] Concentration of compound required to inhibit the cell growth by 50% after 72 h of exposure as determined by MTT assay.
[b] UVA dose expressed in J cm^{-2} as measured at 365 nm with a Cole–Parmer radiometer.

Table 6. Photocytotoxicity of test compounds against NCTC 2544 and LoVo cell lines[a]

Compounds	Cell line GI_{50}[b] (μm)			
	NCTC 2544		LoVo	
	1.25[c] J cm^{-2}	2.5 J cm^{-2}	1.25[b] J cm^{-2}	2.5 J cm^{-2}
3f	7.0 ± 1.3	2.6 ± 0.7	3.8 ± 0.8	1.94 ± 0.2
3e	>20	>20	>20	13.2 ± 1.9
3d	>20	6.9 ± 2.2	16.8 ± 2.1	9.80 ± 1.3
Doxorubicin	3.04	1.32	8.54	5.48

[a] Human cell lines: NCTC 2544 Human keratinocytes; LoVo intestinal adenocarcinoma.
[b] Concentration of compound required to inhibit the cell growth by 50% after 72 h of exposure as determined by MTT assay.
[c] UVA dose expressed in J cm^{-2} as measured at 365 nm with a Cole–Parmer radiometer.

Table 7. Percentage of HL-60 in the different phases of the cell cycle[a]

Treatment	G1	G2	S	Apoptotic cells[b]
Non-irradiated cells	39.0	9.0	51.7	0.8
UVA irradiated cells without drug (2.5 J cm^{-2})				
24 h	35.9	12.4	50.1	8.2
48 h	49.6	10.2	40.5	10.9
72 h	34.8	11.9	55.1	9.1
3a *2.5 m MCUVA (2.5 J cm^{-2})*				
24 h	28.9	10.8	58.4	13.5
48 h	44.5	10.3	45.0	28.7
72 h	43.5	12.0	43.8	42.5
3a *5.0 mMCUVA (2.5 J cm^{-2})*				
24 h	33.9	11.8	55.3	20.4
48 h	51.7	11.2	37.8	28.2
72 h	58.7	5.9	35.4	47.9

[a] The percentage of each phase of the cell cycle (G1, S, and G2/M) was calculated on living cells.

[b] The percentage of apoptotic cells is referred to cells population characterized by the appearance of a sub G1 peak.

phototoxicity of the most active compounds, in particular **3f**, is higher in the tumor cell lines in comparison to the normal ones (NCTC 2544). In preliminary experiments devoted to the search for a possible molecular target, compound **3f** was evaluated for its potential capability to induce single strand breaks in a plasmid DNA, as a model.

The obtained results (data not shown) indicate that **3f**, after irradiation in the presence of DNA, is not able to induce any significant damage to DNA thus suggesting that another target at cellular level may be involved in its phototoxic effect. In parallel to the cytotoxic evaluation, flow cytometry was employed to study cell cycle variations upon irradiation. The effects of the most active compound **3f** were evaluated after 24, 48, and 72 h from irradiation in the leukemic cell line. The percentage of the cells in the different phases of the cell cycle is shown in Table 7.

It can be observed that treatment with **3f** in combination with UVA induces a reduction of the S phase at 48 and 72 h after irradiation especially for the highest dose utilized. This is accompanied by a concomitant block in G1 phase. This event is followed at 48 and 72 h after the irradiation by massive induction of apoptosis, as observed by the appearance of a sub G1 peak (apoptotic cells) that refers to cells with DNA content lower than G1.[35,36] In fact, apoptosis induces the activation of endogenous nucleases, which are responsible for nucleic acid degradation.

It can be concluded that this economically, environmentally benign procedure[40,41] has several advantages such as (1) short reaction times, (ii) no excess of reactants is demanded, (iii) no solvent is employed, (iv) cheap catalyst is applied, (v) no work up is needed, (vi) thermally stable crystalline inorganic materials are safe to handle and environmentally benign. Their recyclable nature and ease of manipulation, entailing a simple filtration step, illustrate some of the added advantages of zeolite catalysts. Thus, our method, compared with the reported ones, shows very important advantages from both ecological and economic point of view.

Human promyelocytic leukemia cells (HL-60) were grown in RPMI-1640 medium (Sigma Co Mo, USA), human keratinocytes (NCTC 2544) were grown in DMEM (Sigma Co Mo USA), and intestinal adenocarcinoma cells (LoVo) were grown in Ham's F12 medium (Sigma Co Mo, USA) all supplemented with 115 units/mL of penicillin G (Invitrogen, Milano, Italy), 115 μg/mL streptomycin (Invitrogen, Milano, Italy), and 10% fetal bovine serum (Invitrogen, Milano, Italy). Individual wells of a 96-well tissue culture microtiter plate (Falcon BD) were inoculated with 100 mL of complete medium containing 8×10^3 HL-60 cells or 5×10^3 NCTC 2544 and LoVo cells. The plates were incubated at 37 °C in a humidified 5% incubator for 18 h prior to the experiments. After medium removal, 100 mL of the drug solution, dissolved in DMSO and diluted with Hanks' Balanced Salt Solution (HBSS pH 7.2), was added to each well and incubated at 37 °C for 30 min and then irradiated. Doxorubicin was used as positive control. After irradiation, the solution was replaced with the medium, and the plates were incubated for 72 h. Cell viability was assayed by the MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] test, as described previously.[42,43]

For flow cytometric analysis of DNA content, 5×10^5 HL-60 cells in exponential growth were treated at different concentrations of the test compounds for 24, 48, and 72 h. After the incubation period, the cells were centrifuged and fixed with ice-cold ethanol (70%), treated with lysis buffer containing RNAseA, and then stained with propidium iodide. Samples were analyzed on a Becton Coulter Epics XL-MCL flow cytometer. For cell cycle analysis, DNA histograms were analyzed using Multi Cycle for Windows (Phoenix Flow Systems, San Diego, CA).

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40. *Experimental*: All melting points were taken on a Büchi-Tottoli capillary apparatus and are uncorrected; Microwave-assisted reactions were carried out on monomode reactor, Maxidigest MX 350 Prolabo (50 W). All reactants were purchased from Aldrich Chemical Co. and were used as received. All the products were characterized by comparison of their ^1H NMR spectral data and mixed mp with the reported ones.[37] The starting materials 5-substituted-2-aminobenzenethiols[38] (**1a**–**f**) and 3-(4-fluoro-2-methylbenzoyl)-2-propenoic acid (**2**)[39] were prepared by literature-reported methods.
41. *Synthesis of 8/6-substituted-2-carboxy-2,3-dihydro-1,5-benzothiazepines* (**3a**–**f**): (i) *Conventional method*: (a) Using ethanol + dry hydrogen chloride gas: An equimolar mixture of **1** (1 mmol) and **2** (1 mmol) was refluxed for 7–10 h with dry ethanol (25 mL) saturated with hydrogen chloride gas, whereupon the reaction mixture changed from yellow to dark green. Excess of solvent was concentrated by distillation under reduced pressure. The product so obtained was recrystallized from methanol to give light green crystals of compounds **3**. Similarly **3** was prepared by reflux with toluene and trifluoroacetic acid (2–3 drops)–methanol containing traces of glacial acetic acid to check the most effective method of synthesis; (ii) *MW mediated method*: (a) Using different solid supports such as montmorillonite KSF, Neat in the presence of few drops of DMF, *p*-toluenesulfonic acid (PTSA): An equimolar mixture of 5-methoxy-2-aminobenzenethiol **1** (1 mmol) and 3-(4-fluoro-2-methyl benzoyl)-2-propenoic acid **2** (1 mmol) was introduced in a beaker and dissolved in acetone (15 mL). Inorganic solid support (20% by weight of the reactants) was then added and swirled for a while followed by removal of the solvent under gentle vacuum. The dry free flowing powder thus obtained was placed into a sealed vessel and irradiated inside the microwave oven.

After completion of the reaction (monitored by TLC) the recyclable inorganic solid support was separated by filtration after eluting the product with methanol. The combined filtrate was concentrated under reduced pressure and the residue was purified by column chromatography with silica gel (*n*-hexane/EtOAc = 20:1) giving pure product (**3**); (iii) *Using stirring method*: To a stirred heterogeneous mixture of 5-substituted-2-aminobenzenethiols (**1a**–**f**) and 3-(4-fluoro-2-methylbenzoyl)-2-propenoic acid (1 mmol), 2 g of LaY zeolite was added at 80 °C. The resulting mixture was stirred at the same temperature for 8–15 min (Table 4). After the completion of reaction (TLC analysis), reaction mixture was filtered and zeolite was washed with water (and/or methanol). When catalyst was reused, it was dried in air overnight at 300 °C. Alternatively the reaction mixture was directly applied on a silica gel column and eluted with hexane/CH_2Cl_2 (1:9) to afford the pure product (**3a**–**f**).

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