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SYNTHESIS AND ANTIVIRAL ACTIVITY OF SOME N-BENZENESULPHONYLBENZIMIDAZOLES.

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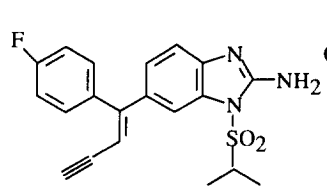
Abstract. Some N-sulphonylated benzimidazoles were synthesized as potential antiviral agents. Compound **16b** and, to a lesser extent, **19b** showed activity against two RNA viruses at micromolar concentrations.

Arylsulfones are an important class of non-nucleoside antiviral agents. Following Enviroxime and Enviradene [1,2] several analogues were synthesized and evaluated for their antiviral activity.Vinylacetylene benzimidazoles[3,4] **1** were found to be potent inhibitors of poliovirus in tissue culture and to have a mechanism of action directed towards the inhibition of viral RNA synthesis. Aryl indolyl [5,6,7] and aryl pyrrolyl [7,8] sulfones were also intensely studied. 5-chloro-3-(phenylsulfonyl) indole-2-carboxamide L-737-126 (**2**) and its derivative **3** appeared to be good selective HIV-1 RT inhibitors. Pyrrole derivative **4** was highly potent, showing EC 50s in the submicromolar range against HIV-1. In previous work[9] we have synthesized some N-benzensulfonyl 2-(2 or 3-pyridylethyl) - benzimidazoles.



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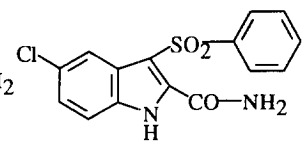

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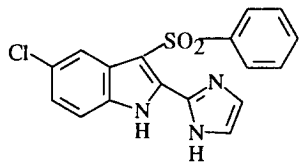

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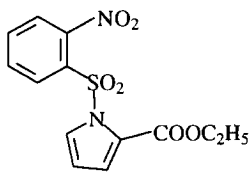

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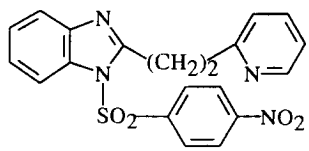

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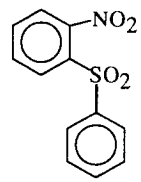

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One of them (**5**) showed a good antiviral activity against Coxsackievirus B5. We therefore decided to synthesize other N- sulfonylated benzimidazoles, in which some structural modifications have been performed.

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SAR studes on diaryl sulfones [8,10] revealed that the presence of a nitro group ortho to the SO_2 was crucial for activity as appeared in the 2-nitrophenyl phenyl sulfone **6** (NPPS)[10].

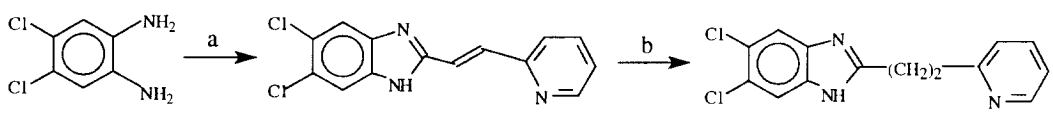
Thus, it appeared interesting to study the replacement of the nitro group from para to ortho position of the benzenesulfonyl substituent. In view of the antiviral efficacy of several 5,6-dichlorobenzimidazoles [11-14] we expanded our research on this nucleous. Moreover, considering the favourable influence of a 2-alkylthio substituent [11,13] we replaced in some derivatives the ethylenic group between the two heterocycles, with a thioether linkage. Additional modification included substitution of the ethylenic bridge by a sulfur atom. The aim of this work is to evaluate if the changes on the structure **5** are useful for biological activity.We report herein the synthesis of some 2-substituted -benzimidazole-N-sulfonylated and their antiviral activity.

Chemistry

The compounds **5**[9],**7**[9], **8**[15],**9**[16] were reported in literature.

Scheme 1 illustrates the synthetic route used to prepare the 2-(2-pyridylethyl)-5,6-dichlorobenzimidazole **12.** Condensation of 4,5-dichloro-1,2-phenylenediamine **10** and (E) -3 - (2-pyridyl) acrylic acid[17] (1 equiv), in PPA (10 g) at 170°C, afforded **11** in good yield, by application of Hein's benzimidazole synthesis[18]. The vinyl double bond of **11** was hydrogenated (1 atm) in ethanol, in the presence of 10% palladium on charcoal.

Scheme 1

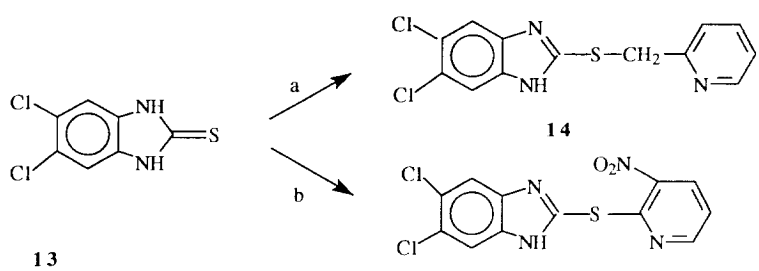


10 **11** **12**

a. (E)-3-(2-pyridyl) acrylic acid, PPA, 170°C, 25% NH_4OH to pH 8; b. H_2, Pd/C, EtOH.

The reaction of 5,6-dichlorobenzimidazole-2-thione **13**[11] respectively with 2-picolyl chloride hydrochloride (1 equiv) and with 2-chloro-3-nitropyridine (1 equiv) in 95% ethanol, in the presence of aqueous sodium hydroxide gave the corresponding 2-(2-pyridinylmethylthio)-1H-benzimidazole **14** and 2-(3-nitro-2-pyridinylthio)-1-H-benzimidazole **15** in 65%, 55% yields, as shown in Scheme 2[19].

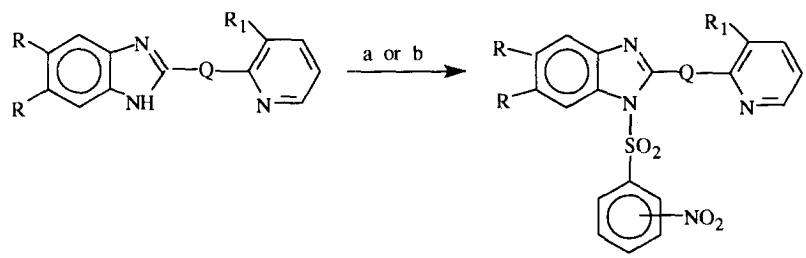
Scheme 2



a. 2-pycolyl chloride hydrochloride, NaOH, H_2O, EtOH. b. 2-chloro-3-nitropyridine, NaOH, H_2O, EtOH.

N-Benzenesulfonylated derivatives **16b**, **17a**, **17b**, **18a**, **19a**, **19b**, **20a**, **20b** and **21a**[20]were obtained in good yields (62-80%) by treating the compounds **7**, **8**, **9**, **12**, **14**, **15** with o- or p-nitrobenzenesulfonyl chloride in pyridine at 0°C, as shown in Scheme 3.

Scheme 3



7: R = R = H, Q = - $(CH_2)_2$ -
8: R = R_1 = H, Q = -S-CH_2-
9: R = H, R_1 = NO_2 , Q = S
12: R = Cl, R_1 = H, Q = -$(CH_2)_2$-
14: R = Cl, R_1 = H, Q = -S-CH_2-
15: R = Cl, R_1 = NO_2, Q = S

16b: R = R_1 = H, Q = -$(CH_2)_2$-
17a: R = R_1 = H, Q = -S-CH_2-
17b: R = R_1 = H, Q = -S-CH_2-
18a: R = H, R_1 = NO_2, Q = S
19a: R = Cl, R_1 = H, Q = -$(CH_2)_2$-
19b: R = Cl ,R_1 = H, Q= -$(CH_2)_2$-
20a: R = Cl, R_1= H, Q= -S-CH_2-
20b: R = Cl, R_1 = H, Q= -S-CH_2-
21a: R = Cl, R_1 = NO_2, Q = S

(a) 4-nitrobenzenesulfonylchloride; (b) 2-nitrobenzenesulfonylchloride (1,2 equiv.), pyridine 0°C, then 10 h r. t.

Table 1. Antiviral activity against RNA and DNA viruses.

No.	Conc. µg/ mL	Coxsackie virus B5 $TCID_{50}$[a]	Mumps virus TCID[a]	Herpes simplex1 PFU[b]	Vaccinia virus PFU[b]
Controls		$10^{6.3}$	$10^{4.3}$	3.8 X 10^6	8.5 X 10^3
Guanidine	80	$10^{2.6}$			
	3	$10^{4.5}$			
5	5	$10^{2.5}$	n.d.	n.d.	n.d;
16b	5	$10^{4.3}$	$10^{2.6}$	1.8x10^6	7.5x10^2
17a	4	$10^{5.4}$	n.d.	n.d.	n.d.
17b	2	$10^{5.8}$	n.d.	n.d.	n.d.
18a	3	$10^{5.5}$	n.d.	n.d.	n.d.
19a	2	$10^{6.6}$	n.d.	n.d.	n.d.
19b	2	10^5	$10^{3.6}$	4.7x10^6	1.2x10^2
20a	3	$10^{5.3}$	n.d.	n.d.	n.d.
20b	3	$10^{5.7}$	n.d.	n.d.	n.d.
21a	3	$10^{5.4}$	n.d.	n.d.	n.d.

a Total cell infectious $dose_{50}$ / mL[21,22]
b Plaque forming unit /mL

First all the derivatives were tested against Coxackievirus B5, an RNA virus belonging to the Picornaviridae family, by means of viral yield assays.The compounds **5**[9], **16b** and **19b** were also tested against another RNA virus (Mumps virus, belonging to the Paramyxoviridae family) and against two DNA viruses, Herpes simplex-1 virus and poxvirus vaccinia. In both cases, virus yield assays were used as above, but in the case of HSV-1 and vaccinia virus, titration of virus yield was carried out with plaque assay. Briefly, 10-fold dilutions of the cell lysates were seeded on to 24 h grouth VERO cells; after 1 h adsorption , culture medium was added containing 0.2% of human γ-globulins. 48 h later, medium was removed, cells fixed for 15 min with methonol and stained with Giemsa stain 1:10 for 20 min and the plaques counted. The titre, expressed as PFU/ mL, represented the plaque number multiplicated for the reciprocal of the dilution factor (Tables 1).
Two tests were carried out to determine the toxic activity on VERO cells: namely, the MTT test[23] for cell viability and the Lowry' s method[24]for protein content.(Table 2).

Table 2. Cytotoxicity on Vero cell cultures and Antiviral Activity against COXB5

Compound	Conc. μg/mL	Lowry Test[a]	MTT/Test[b]	IC_{50} (μg/mL)[c]
Controls		855.7	1.57	
Guanidine	80	825.7 (96.5)	1.41(90.1)	
	3	839.4 (98.1)	1.45(92.3)	
5				4
16b	10	627.8 (73.3)	0.82 (52.2)	5
	5	759.5 (88.7)	1.23 (78.8)	
17a	4	734.2 (85.8)	1.12 (71.5)	
	2	744.3 (87.0)	1.44 (91.7)	
17b	2	724.1 (84.6)	1.02 (64.9)	
	1	774.7 (90.5)	1.42 (90.4)	
18a	5	508.9 (59.5)	0.80 (50.9)	
	3	678.6 (79.3)	1.36 (86.9)	
19a	2	784.8 (91.7)	0.89 56.7)	
	1	845.6 (98.8)	1.4 (89.2)	
19b	2	784.8 (91.7)	1.36 (86.6)	8
	1	840.5 (98.2)	1.49 (94.9)	
20a	5	540.8 (63.2)	0.89 (56.7)	
	3	663.4 (77.5)	1.23 (78.8)	
20b	5	495.8 (57.9)	0.83 (52.9)	
	3	740.1 (86.5)	1.12 (71.5)	
21a	5	456.1 (53.3)	0.79 (50.3)	
	3	595.6 (69.6)	1.27 (80.9)	

a % Protein content versus controls
b The optical density is proportional to cell number
c Inhibitory concentration required to reduce virus plaque formation by 50%.

Results and Discussion: The compounds were tested against RNA (Coxsackie B5, Mumps) and DNA (Herpes simplex, Vaccinia) viruses. Concentrations used in the antiviral assays did not demonstrate a significant reduction of the protein content (Lowry test) and of the cell viability (MTT test). The derivatives **5**, **16b** and **19b**

showed good antiviral activity against Coxsackievirus B5 with IC_{50} values of 4, 5 and 8 μg/mL respectively. **5** and **16b** were active at lower concentration than the cytotoxic concentration, while for **19b** antiviral activity was not well separated from cytotoxicity. Both **16b** and **19b** inhibited effeciently Mumps virus growth (1.7 and 0.7 log respectively). All the compouds were inactive against DNA viruses; in fact, although the exact mechanism of action remains unclear, probably, they inhibit RNA replication and, specifically they selectively inhibit (+)-strand RNA synthesis[4,25]The structural analogy of our derivatives with Enviradene and Enviroxime led us to suppose the same mechanism of action. From the results it is evident the importance of the bridge between the two heterocycles.In fact only the ethylenic chain led to active derivatives (**5**, **16b** and **19b**). Of the 5,6-dichloro derivatives, **19b** showed any activity , while the parent **19a** was inactive.In this case the position of the NO_2 group in the benzenesulphonyl moiety was crucial; a NO_2 group ortho to the SO_2 was useful for antiviral activity. At present, experiments are being carried out to assess the mechanism of action of compound **16b** and if it selects resistent mutants. Further studies, focused on the modifications of the bridge between two heterocycles are in progress.

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19 The derivatives **14** and **15** were purified by column chromatography eluted with 6:4 ethyl acetate-petroleum ether.

20 Melting point, ^{1}H NMR and elemental analysis data for compounds **16b, 17a, 17b, 18a, 19a,19b,20a, 20b** and **21a** are available from the authors upon request.

21 $TCID_{50/ml}$ means total cell infection dose 50/ ml: it is the virus dilution able, in theory, to infect the 50% cell cultures. It is one of the units of measure used to quantify viral titre and it is obtained by the end point titration of the virus stock, while PFU/ ml (plaque forming unit/ ml) is another unit of measure obtained by plaque titration assay. The value of $TCID_{50/ml}$ is obtained by extrapolation from different values in a 96 well cell culture plate by means of the Reed and Muench formula which provides the exact exponent of the 10 fold dilution.

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