SYNTHESIS AND PRELIMINARY CYTOTOXIC EVALUATION OF NOVEL 3,4-DIHYDRO-2*H*-1,2,4-BENZOTIADIAZINE-1,1-DIOXIDE DERIVATIVES

Jelem Restrepo,[1] Yelsi Pérez,[1] José Salazar,[1] Jaime Charris[2], Francisco Arvelo[3] and Simón E. López[1]*
[1]Laboratorio de Química Medicinal y Heterociclos, Departamento de Química, Universidad Simon Bolívar, Valle de Sartenejas, Baruta, Caracas 1080-A, Apartado 89000, Venezuela. [2]Unidad de Síntesis de Medicamentos, Laboratorio de Síntesis Orgánica, Facultad de Farmacia, Universidad Central de Venezuela, Caracas, Venezuela. [3]Laboratorio de Cultivo de Tejidos y Biología de Tumores, Instituto de Biología Experimental, Universidad Central de Venezuela, Los Chaguaramos, Caracas, Venezuela.

Abstract : The preparation of novel 3,4-dihydro-2*H*-1,2,4-benzothiadiazine-1,1-dioxide derivatives through the condensation of halogenated 2-aminobenzenesulfonamides and benzaldehydes using sodium hydrogen sulfite is described. Contrary to previous reports for non substituted 2-aminobenzenesulfonamides, sodium hydrogen sulfite does not effect the dehydrogenation of 3,4-dihydro compounds to the corresponding 3,4-unsaturated 2*H*-1,2,4-benzothiadiazines. The preliminary cytotoxic evaluation of some of these new compounds toward several human tumor cell lines is also reported.

Introduction

1,2,4-Benzothiadiazine heterocyclic ring system has been extensively investigated during the last 50 years, mainly because of its variety of biological activities, which includes diuretic (1), antihypertensive (2), potassium channel openers (3), α_1-adrenoreceptor antagonists (4), phosphodiesterase (PDE_4) inhibitors (5), free radical scavengers (6), allosteric modulators of AMPA/kainate receptors (7), bone regeneration (8), and prolylendopeptidase inhibitors (9). Structural similarity to other important pharmacophores, such as 4-quinazolinones (10) has recently impulse its preparation through modern combinatorial chemistry techniques (11).

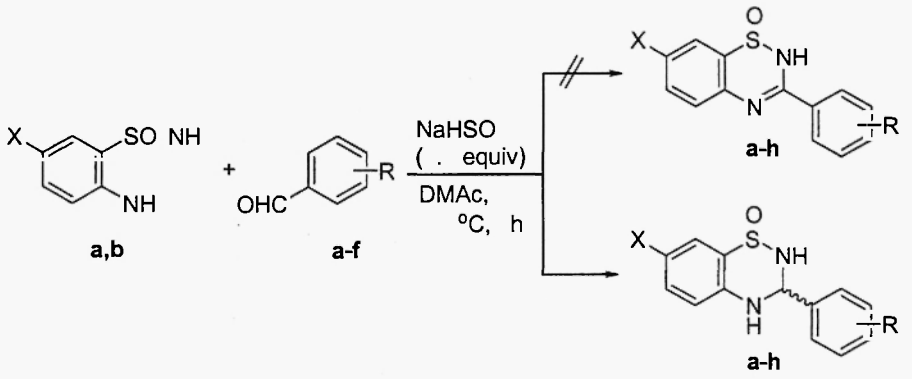
3-substituted alkyl and aryl 1,2,4-benzothiadiazine 1,1-dioxides have been synthesized for over a hundred years ago. Initial reports from Ekbom (12) and Freeman (13) consisted in their preparation by condensation of a 2-amino-benzenesulfonamide with an alkyl-orthoformate. Other reported procedures includes acylation of the above with reactive carboxylic acid derivatives followed by a cyclodehydration promoted by heating with base (13,14), direct cyclocondensation with amidines at elevated temperatures (15), and cyclodehydrogenation with aldehydes in the presence of sodium hydrogen sulfite (16). During our research program, directed toward the preparation of novel compounds of the 4(3*H*)-quinazolinone (17) and 1,8-naphthyridine-4-one (18) type with potential cytotoxic-antitumoral activity, we decided to explore the synthesis and biological testing of new 3-aryl-substituted 1,2,4-benzothiadiazine 1,1-dioxides as their isostere analogs.

Results and Discussions

Chemistry

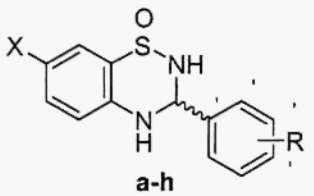
Initially interested in the preparation of 2*H*-1,2,4-benzothiadiazine 1,1-dioxide derivatives **3**, we attempted a cyclodehydrogenation procedure with an appropriate 5-halo-substituted 2-aminobenzenesulfonamide **1** and different benzaldehydes **2** using sodium hydrogen sulfite in dimethylacetamide (Scheme 1). Despite this methodology has been used successfully with non substituted 2-aminobenzenesulfonamides (16), and in the preparation of their isostere counterparts 4(3*H*)-quinazolinones (16,17), it failed for all our cases (Table 1) to generate the 3,4-double bond of desired 2*H*-1,2,4-benzothiadiazines **3**, even at higher temperatures (up to 180°C) or longer heating times (12 h).

Somehow, the halogen located at C-5 of the sulfonamide affects the double bond generation, cause only a 5% of the unsaturated compound was detected by ^{1}HNMR –typically characterized by a broad singlet NH signal at around 12 ppm- at longer reaction times. Standard procedures for the preparation of 3-alkyl or aryl 3,4-dihydro-1,2,4-benzothiadiazine 1,1-dioxides are the condensation of 2-aminobenzenesulfonamides with aldehydes either in acid or basic media (7,13), as well as their condensation with the corresponding hemiacetal precursor (7). Our results show that different 2-aryl 3,4-dihydro-benzothiadiazines **4a-h** should be obtained in good yields using sodium hydrogen sulfite as the activator for the condensation, but not as a dehydrogenating agent if the starting 2-aminobenzenesulfonamide is substituted with an halogen at C-5. Perhaps the electronic nature of this substituent should affect the dehydrogenation step of the reaction, and changing it to an electron donating group (*ie.* alkoxy) may help to elicit an answer to this question; an ongoing study on this matter is being doing in our labs and will be published elsewhere.



Scheme-1

Table-1 : Yields of synthesis compounds



Compd. No.	X	R	Yield (%)	Mol. formula
4a	Br	4'-Br	64	$C_{13}H_{10}Br_2N_2O_2S$
4b	Cl	3'-Cl	82	$C_{13}H_{10}Cl_2N_2O_2S$
4c	Cl	3'-F	50	$C_{13}H_{10}ClFN_2O_2S$
4d	Cl	3'-OMe	68[a]	$C_{14}H_{13}ClN_2O_3S$
4e	Cl	3'-Me	52	$C_{14}H_{13}ClN_2O_2S$
4f	Cl	3,4-$(OMe)_2$	70	$C_{15}H_{15}ClN_2O_4S$
4g	Br	3'-OMe	59	$C_{14}H_{13}BrN_2O_3S$
4h	Br	3,4-$(OMe)_2$	74[b]	$C_{15}H_{15}BrN_2O_4S$

[a] When the reaction was performed at higher temperatures (180°C), a comparable result was obtained (67 % yield) and nothing of **3**d was detected.[b] Prolonging the reaction time up to 12h only generates a 5% ratio of the unsaturated 2*H*-1,2,4-benzothiadiazine 1,1-dioxide **3h** (detected by ^{1}HNMR).

Preliminary Biological Evaluation

3,4-dihydro-1,2,4-benzothiadiazine 1,1-dioxide derivatives **4b**, **4c**, **4d** and **4f** were evaluated. The cellular responses through the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] (MTT) (20) method were measured in Lovo (colon carcinoma), X-17 (colon carcinoma), PC-3 (prostate carcinoma), MCF-7 (human breast carcinoma), FOG (cerebral tumor) and human fibroblast cells at the concentration of 10 ppm (μg/ml) for 24 h (the cytotoxic drug adriamycin was used for comparison). Results indicated some selectivity but only a moderate cytotoxicity for compounds **4d** and **4f** against the PC-3 prostate carcinoma cell line (Table 2). Despite this is only a preliminary test, a more extensive study, including all the compounds synthesized and new polysubstituted derivatives is underway. Due biological activity is known to be strongly affected by chirality, it is obvious that resolution of the chiral center at C-3 should clear the results here observed. A recent study of Canazza and coworkers on the resolution of the chiral center of 3,4-dihydro-3-methyl-1,2,4-benzothiadiazine must be taken into account to solve this mattern (21).

Table-2 : Inhibition of Cellular Proliferation (%)

Compd. No.	Lovo	X-17	PC-3	MCF-7	FOG	Fibroblast
4b	2.19	0	4.3	16.0	9.0	19.67
4c	15.8	0	19.1	11.0	5.0	0
4d	28.7	0	38.08	13.0	11.0	24.7
4f	12.5	0	37.6	8.0	14	28.8
Adriamycin	63.0	59.0	78.3	72.0	70.0	66.0

Experimental

Melting points were determined in a Fischer-Johns micro hot-stage apparatus and are uncorrected. NMR spectra were obtained on a JEOL Eclipse Plus spectrometer in hexadeuterated dimethylsulfoxide, operating at 400 MHz (^{1}H, internal standard TMS); δ values in ppm relative to the internal standard are given. The IR spectra were recorded as potassium bromide discs using a Shimadzu CW/IR 470 spectrometer. Elemental analyses were performed by Atlantic Microlab Inc. (Norcross, GA, USA); results fell in the range of ± 0.4% of the required theoretical values. Silica gel plates ALUGRAM® SIL G/UV254 (Macherey-Nagel GmbH & Co., Germany) were used for TLC testing. Reagents were obtained from Aldrich (Milwaukee, MI, USA) or Merck (Darmstadt, Germany) and used without further purification. 5-halo substituted 2-aminobenezenesulfonamides **1a,b** were prepared from the appropriate aniline following the procedure described in the literature (19).

7-Halo-(3-substituted-phenyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (4a-h);

General Procedure

Sodium hydrogen sulfite (0.156g, 1.5 mmol) is added to a solution of 5-halo-2-amino-benzenesulfonamide (1 mmol) and the corresponding substituted benzaldehyde (1 mmol) in dimethylacetamide (2 mL). The mixture is heated with stirring at 160 °C for 3h, cooled at room temperature and then poured into crushed ice-water. The precipitated solid is collected by filtration and then washed several times with cool water. Compounds were recrystallized from dimethylformamide-water.

7-Bromo-3-(4-bromo-phenyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (4a). Yield 64%. M.p.244-46 °C. ^{1}H-NMR δ 8.01 (1H, d, J=12.0 Hz, NH); 7.63 (6H, m, Harom); 7.56 (1H, bs, NH); 7.48 (1H, dd, J= 2.5, 9.0 Hz, Harom$_6$); 6.89 (1H, d, J=9.0 Hz, Harom$_5$); 5.80 (1H, d, J= 12.0 Hz, H-C$_3$). IR (KBr): 1302, 1155 (SO_2) cm^{-1}. *Anal.* Calcd. for $C_{13}H_{10}Br_2N_2O_2S$: C, 37.34; H, 2.41; N, 6.70. Found: C, 37.15 ; H, 2.52 ; N, 6.82.

7-Chloro-3-(3-chloro-phenyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (4b). Yield 82%. M.p.195-6 °C. ^{1}H-NMR δ 8.01 (1H, d, J=12.0 Hz, NH); 7.79 (1H, s, Harom); 7.68 (1H, bs, NH); 7.62 (1H, d, J=6.6 Hz, Harom); 7.54 (3H, m, Harom); 7.40 (1H, dd, J= 1.5, 8.9 Hz, Harom$_6$); 6.93 (1H, d, J=8.9 Hz, Harom$_5$); 5.83 (1H, d, J= 12.0 Hz, H-C$_3$). IR (KBr): 1290, 1150 (SO_2) cm^{-1}. *Anal.* Calcd. for $C_{13}H_{10}Cl_2N_2O_2S$: C, 47.43; H, 3.06; N, 8.51. Found: C, 47.52 ; H, 3.10 ; N, 8.42.

7-Chloro-3-(3-fluoro-phenyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (4c). Yield 50%. M.p.188-90 °C. ^{1}H-NMR δ 8.11 (1H, d, J=12.0 Hz, NH); 7.67 (1H, s, NH); 7.58 (1H, bs, NH); 7.53 (4H, m, Harom); 7.40 (1H, dd, J= 2.5, 8.9 Hz, Harom$_6$); 7.30 (1H, m, Harom); 6.93 (1H, d, J=8.9 Hz, Harom$_5$); 5.83 (1H, d, J= 12.0 Hz, H-C$_3$). IR (KBr): 1285, 1155 (SO_2) cm^{-1}. *Anal.* Calcd. for $C_{13}H_{10}ClFN_2O_2S$: C, 49.93; H, 3.22; N, 8.96. Found: C, 49.75 ; H, 3.15 ; N, 9.03.

7-Chloro-3-(3-methoxy-phenyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (4d). Yield 68%. M.p.258-9 °C. ^{1}H-NMR δ 7.93 (1H, d, J=12.0 Hz, NH); 7.52 (2H, m, NH, Harom); 7.37 (2H, m, Harom); 7.29 (1H, s, Harom); 7.21 (1H, d, J=7.3 Hz, Harom); 7.01 (1H, dd, J=2.5, 8.9 Hz, Harom$_6$); 6.96 (1H, d, J=8.9 Hz, Harom$_5$); 5.75 (1H, d, J= 12.0 Hz, H-C$_3$); 3.81 (3H, s, OCH_3). IR (KBr): 1290, 1150 (SO_2) cm^{-1}. *Anal.* Calcd. for $C_{14}H_{13}ClN_2O_3S$: C, 51.77; H, 4.03; N, 8.63. Found: C, 51.62 ; H, 4.08 ; N, 8.52.

7-Chloro-3-(3-methyl-phenyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (4e). Yield 52%. M.p.190-91 °C. ^{1}H-NMR δ 8.02 (1H, d, J=12.0 Hz, NH); 7.62 (1H, bs, NH); 7.53 (1H, d, J=2.5 Hz, Harom$_8$); 7.49 (1H, s, Harom); 7.44 (1H, d, J=7.7 Hz, Harom); 7.38 (1H, dd, J= 2.5, 8.9 Hz, Harom$_6$); 7.34 (1H, d, J=7.7 Hz, Harom); 7.26 (1H, d, J=7.7 Hz, Harom); 6.93 (1H, d, J=8.9 Hz, Harom$_5$); 5.73 (1H, d, J= 12.0 Hz, H-C$_3$); 2.35 (3H, s, CH_3). IR (KBr): 1280, 1160 (SO_2) cm^{-1}. *Anal.* Calcd. for $C_{14}H_{13}ClN_2O_2S$: C, 54.46; H, 4.24; N, 9.07. Found: C, 54.61; H, 4.18 ; N, 9.15.

7-Chloro-3-(3,4-dimethoxy-phenyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (4f). Yield 70%. M.p. 253-4 °C. ^{1}H-NMR δ 7.97 (1H, d, J=12.0 Hz, NH); 7.58 (1H, bs, NH); 7.54 (1H, d, J=2.2 Hz, Harom$_8$); 7.38 (1H, dd, J= 2.2, 8.8 Hz, Harom$_6$); 7.33 (1H, s, Harom); 7.17 (1H, d, J=8.4 Hz, Harom); 7.00 (1H, d, J=8.4 Hz); 6.93 (1H, d, J=8.8 Hz, Harom$_5$); 5.70 (1H, d, J= 12.0 Hz, H-C$_3$); 3.78 (6H, s, OCH_3). IR (KBr): 1295, 1145 (SO_2) cm^{-1}. *Anal.* Calcd. for $C_{15}H_{15}ClN_2O_4S$: C, 50.78; H, 4.26; N, 7.90. Found: C, 50.62 ; H, 4.19 ; N, 7.98.

7-Bromo-3-(3-methoxy-phenyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (4g). Yield 59%. M.p.225-27 °C. ^{1}H-NMR δ 8.04 (1H, d, J=12.0 Hz, NH); 7.64 (2H, m, Harom, NH); 7.48 (1H, dd, J=2.2, 8.8 Hz, Harom$_6$) 7.37 (1H, t, J=8.0 Hz, Harom); 7.29 (1H, s, Harom); 7.22 (1H, d, J=8.0 Hz, Harom); 7.02 (1H, dd, J=2.2, 8.0 Hz, Harom); 6.89 (1H, d, J=8.8 Hz, Harom$_5$); 5.75 (1H, d, J= 12.0 Hz, H-C$_3$); 3.79 (3H, s, OCH_3). IR (KBr): 1290, 1158 (SO_2) cm^{-1}. *Anal.* Calcd. for $C_{14}H_{13}BrN_2O_3S$: C, 45.54; H, 3.55; N, 7.59. Found: C, 45.69 ; H, 3.61 ; N, 7.41.

7-Bromo-3-(3,4-dimethoxy-phenyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (4h). Yield 74%. M.p.260-1 °C. ^{1}H-NMR δ 7.98 (1H, d, J=12.1 Hz, NH); 7.64 (1H, d, J=2.2 Hz, Harom); 7.58 (1H, bs, NH); 7.47 (1H, dd, J= 2.5, 8.9 Hz, Harom$_6$); 7.32 (1H, d, J=1.9Hz, Harom); 7.17 (1H, dd, J=1.9, 8.4 Hz, Harom); 7.00 (1H, d, J=8.4 Hz, Harom); 6.87 (1H, d, J=8.9 Hz, Harom$_5$); 5.70 (1H, d, J= 12.0 Hz, H-C$_3$); 3.79 (3H, s, OCH_3); 3.78 (3H, s, OCH_3). IR (KBr): 1292, 1158 (SO_2) cm^{-1}. *Anal.* Calcd. for $C_{15}H_{15}BrN_2O_4S$: C, 50.74; H, 4.26; N, 7.90. Found: C, 50.87 ; H, 4.35 ; N, 7.74.

Acknowledgements

Authors would like to thank Fonacit (Caracas, Venezuela. Project SI-99000020) and Decanato de Investigación y Desarrollo (Universidad Simón Bolívar, Caracas) for their financial support, as well as to Laboratorio de Resonancia Magnetica Nuclear (Laboratorio B, Universidad Simón Bolívar, Caracas) for the spectral facilities. JR thanks Fonacit (Caracas, Venezuela) for a Doctoral Fellowship. YP thanks Decanato de Estudios Profesionales (Universidad Simón Bolívar, Caracas) for an undergraduate research financial support.

References

1. (a) F.C. Novello, J.M. Sprague, *J. Am. Chem. Soc.* **79**, 2028 (1957). (b) J.-H. Short, U. Biermacher, *J. Am. Chem. Soc.* **82**, 1132 (1960).
2. A.A. Rubin, F.E. Roth, M.M. Winbury, J.G. Topliss, M.H. Sherlock, N. Sperber, J. Black, *Science* **138**, 2067 (1961).
3. (a) B. Pirotte, P. De Tullio, P. Lebrun, M.-H. Antoine, J. Fontaine, B. Masereel, M. Schynts, L. Dupont, A. Herchuelz, J. Delarge, *J. Med. Chem.* 3211 (1993). (b) P. De Tullio, B. Pirotte, P. Lebrun, J. Fontaine, L. Dupont, M.-H. Antoine, R. Ouedraogo, S. Khelili, C. Magetto, B. Masereel, O. Diouf, T. Podona, J. Delarge, *J. Med. Chem.* **39**, 937 (1996). (c) B. Pirotte, R. Ouedraogo, P. De Tullio, S. Kheilili, F. Somers, S. Boverie, L. Dupont, J. Fontaine, J. Damas, P. Lebrun, *J. Med. Chem.* **43**, 1456 (2000). (d) P. De Tullio, B. Becker, S. Boverie, M. Dabrowski, P. Wahl, M.-H. Antoine, F. Somers, S. Sebille, R. Ouedraogo, J.B. Hansen, *J. Med. Chem.* **46**, 3342 (2003). (e) S. Boverie, M.-H. Antoine, F. Somers, B. Becker, S. Sebille, R. Ouedraogo, S. Counerotte, B. Pirotte, P. Lebrun, P. De Tullio, *J. Med. Chem.* **48**, 3492 (2005).
4. J.-W. Chern, P.-L. Tao, K.-C. Wang, A. Gutcait, S.-W. Liu, M.-H. Yen, S.-L. Chien, J.-K. Rong, *J. Med. Chem.* **41**, 3128 (1998).
5. A. Tait, A. Luppi, A. Hatzelmann, P. Fossa, L. Mosti, *Bioorg. Med. Chem.* **13**, 1393 (2005).
6. A. Tait, S. Ganzerli, M. Di Bella, *Bioorg. Med. Chem.* **3**, 12587 (1996).
7. D. Braghiroli, G. Puia, G. Canazza, A. Tait, C. Parenti, G. Losi, M. Baraldi, *J. Med. Chem.* **45**, 2356 (2002).
8. Fujisawa Pharmaceutical, Co. EP268990, 1998.
9. Chinoin Gyogyszer es Vegyeszet. WO 9707116, 1997.
10. (a) S. Makino, N. Suzuki, E. Nakanishi, T. Tsuji, *Synlett* 1670 (2000). (b) J.P. Mayer, G.S. Lewis, M.J. Curtis, J.M. Zhang, *Tetrahedron Lett.* **38**, 8445 (1997).
11. S. Makino, E. Nakanishi, T. Tsuji, *J. Comb. Chem.* **5**, 73 (2003).
12. B. Ekbom, *Svensk Vetenskakad. Handl.* **27**, 3; *Beilstein*, 4th Ed., **27**, 570; **14**, 682 (1902).
13. J.H. Freeman, E.C. Wagner, *J. Org. Chem.* **16**, 815 (1951).
14. (a) Y. Imai, H. Koga, *J. Polym. Sci. Polym. Chem. Ed.* **11**, 289 (1973). (b) L. Raffa, *Farmaco (Pavia) Ed. Sci.* **12**, 279 (1957); *C.A.* **54**, 24788 (1960).
15. S. Tanimoto, S. Shimojo, R. Oda, *Yuki Gosei Kagaku Kyokaishi* **26**, 151 (1968); *C.A.* **69**, 36089 (1968).
16. Y. Imai, S. Sato, R. Takasawa, M. Ueda, *Synthesis*, 35 (1981).

17. (a) S.E. López, M.E. Rosales, N. Urdaneta, M.V. Godoy, J. Charris, *J. Chem. Res. (S)*, 258 (2000). (b) S.E. López, M.E. Rosales, C.E. Canelon, E.A. Valverde, R.C. Narváez, J.E. Cariz, F.A. Giannini, R.D. Enriz, M. Carrasco, S. Zacchino, *Heterocycl. Commun.*, **5**, 473 (2001).

18. S.E. López, M.E. Rosales, J. Salazar, N. Urdaneta, R. Ferrer, J.E. Angel, J.E. Charris, *Heterocycl. Commun.* **9**, 345 (2003).

19. Y. Girard, J. Atkinson, J. Rokach, *J. Chem. Soc. Perkin Trans. I*, 1043 (1978).

20. (a) F. Denizot, R. Lang, *J. Immunol. Methods*, **89**, 271 (1986). (b) T. Mosman, *J. Immunol. Methods*, **65**, 55 (1983).

21. G. Cannazza, D. Braghiroli, M. Baraldi, C. Parenti, *J. Pharm. Biomed. Anal.* **23**, 117 (2000).

Received on January 24, 2006