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Synthesis and antiproliferative studies of 5-aryl-2-(3-thienylamino)-1,3,4-thiadiazoles

Germain Revelant [a], Charlène Gadais [a], Véronique Mathieu [b], Gilbert Kirsch [a], Stéphanie Hesse [a,*]

[a] Université de Lorraine, Laboratoire de Synthèse et Réactivité des Systèmes Moléculaires Complexes UMR-SRSMC 7565, Equipe Hécrin, 1 boulevard Arago, 57070 Metz Technopôle, France
[b] Laboratoire de toxicologie, Université Libre de Bruxelles, Brussels, Belgium

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ABSTRACT

A series of 5-aryl-2-(3-thienylamino)-1,3,4-thiadiazoles **3a**–**m** were synthesized in good yields in two steps starting from thiophen-3-isothiocyanates. Those compounds as well as the thiosemicarbazide intermediates **2a**–**m** were screened for their antiproliferative activity against a panel of six cancer cell lines. Among them, two 5-aryl-2-(3-thienylamino)-1,3,4-thiadiazoles (**3f** and **3i)** have shown very interesting results with IC_{50} <10 μM on three cell lines.

1,3,4-Thiadiazoles are heterocyclic scaffolds incorporated in many compounds presenting various pharmacological activities such as antimicrobial,[1] antitubercular,[2] anti-oxydant,[3] anti-leishmanial,[4] or anti-inflammatory[5] agents. Especially, 1,3,4-thiadiazole core is found in several marketed drugs such as acetazolamide, a carbonic anhydrase inhibitor used in treatment of glaucoma or sulfamethizole, an antibacterial agent. 1,3,4-Thiadiazole derivatives have also been investigated for their antiproliferative properties.[6] For example, 2-(4-fluorophenylamino)-5-(2,4-dihydroxy-phenyl)-1,3,4-thiadiazole (FABT), a promising anticancer compound for treating malignant tumors of the nervous system, decreased cell division and inhibited cell migration.[7] In the same way, Kumar et al. have synthesized and evaluated a series of 5-(3-indolyl)-2-substituted-1,3,4-thiadiazoles against six human cancer cell lines (prostate (PC3, DU145 and LnCaP), breast (MCF7 and MDA-MB-231) and pancreatic (PaCa2) cancer cell lines). Most of those compounds showed cytotoxic effect and the authors have demonstrated the importance of substituents at C-2 such as 3,4-dimethoxyphenyl or 4-benzyloxy-3-methoxyphenyl which significantly increased activity against all the cancer cell lines and especially against MCF7.[8] They have also studied a series of 2-arylamino-5-aryl-1,3,4-thiadiazoles and screened them on the same six cell line panel. Compounds with trimethoxyphenyl at C-5 position displayed potent anticancer activity on MCF7 and PaCa2 cell lines with IC_{50} 4.3–9.2 μM.[9]

A variety of synthetic methods for the preparation of 1,3,4-thiadiazoles have been reported. Especially 2-amino-1,3,4-thiadiazoles have been prepared via dithiocarbamate,[10] thiosemicarbazone[11] or thiosemicarbazide[12] intermediates. Some one-pot procedure was also described and synthesis of 2-arylamino-5-aryl-1,3,4-thiadiazoles was achieved by refluxing aryl aldehydes, hydrazine hydrate, and aryl isothiocyanates in methanol followed by oxidative cyclization with ferric ammonium sulfate.[9] In continuation of our interest in the synthesis of new 3-thienylisothiocyanates and their use in organic transformations (syntheses of thienylimino-1,3-thiazolidin-4-ones[13] and 2-aminothieno[3,2-d]thiazoles[14]), we focused our attention on their use in the synthesis of 2-(3-thienylamino)-1,3,4-thiadiazoles. Those compounds were indeed poorly described in literature excepted three compounds related to 2-(3-benzothienyl)-1,3,4-thiadiazoles.[15] We first tried the one-pot procedure described by Kumar;[9] however, in our case, cyclization by FAS of the thiosemicarbazone formed in situ required 24 h of reflux to reach its completion. Moreover, we also observed formation of secondary products so that the expected compounds have to be purified by column chromatography and yields were moderate (Scheme 1).

Condensation of 5-phenylthiophene-3-isothiocyanate with benzaldehyde (or 4-fluorobenzaldehyde) and hydrazine gave the expected thiadiazoles **1a**–**b** after cyclization by FAS in 33% yield. The same reaction conducted on 5-(4-methylphenyl)thiophene-

* Corresponding author. Tel.: +33 3 87 54 71 97; fax: +33 3 87 31 58 01.
E-mail address: stephanie.hesse@univ-lorraine.fr (S. Hesse).

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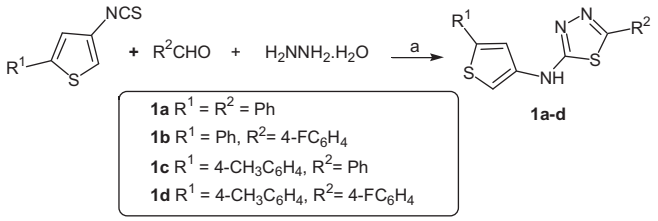
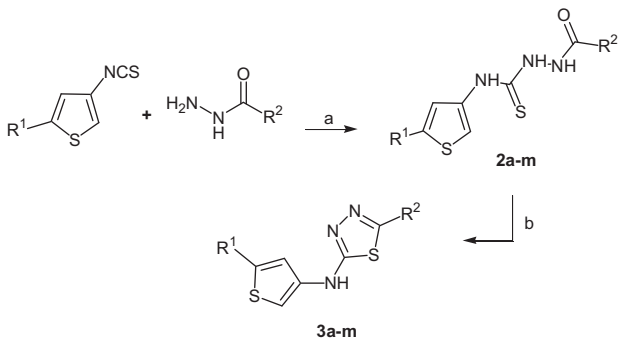


Scheme 1. Synthesis of 5-aryl-2-(3-thienylamino)-1,3,4-thiadiazoles **1a–d**. Reagents and conditions: (a) (i) methanol, reflux, 2 h; (ii) FAS, reflux, 24 h.



Scheme 2. Synthesis of 5-aryl-2-(3-thienylamino)-1,3,4-thiadiazoles **3a–m**. Reagents and conditions: (a) ethanol, reflux, 3 h; (b) concd H_2SO_4, rt, 1 h.

3-isothiocyanate led to thiadiazoles **1c–d** in respectively 46% and 25% yield.

We then turned our attention to a two-step process (Scheme 2) and synthesized thienylthiosemicarbazide intermediates **2a–m** by condensation of isothiocyanates with several arylhydrazides refluxing 3 h in ethanol. Most of the time, those compounds are then cyclized in acidic media in concentrated H_2SO_4,[16] PPA[17] or H_3PO_4.[18] The expected 5-aryl-2-(3-thienylamino)-1,3,4-thiadiazoles **3a–m** were obtained in good to excellent yields after 1 h at room temperature in concentrated H_2SO_4 (Table in Supplementary data section). This two-step procedure allowed formation of thiadiazoles[19] in 39–93% global yield.

The synthesized thienylthiosemicarbazide intermediates **2a–m** and thiadiazoles **3a–m** were screened in terms of antiproliferative activity against a panel of six cancer cell lines[20] (Table 1) which included three cell lines that display sensitivity to pro-apoptotic stimuli (human MCF-7 breast, human Hs683 anaplastic oligodendroglioma and mouse B16F10 melanoma cancer cell lines) and three cell lines that display various level of resistance to pro-apoptotic stimuli (human U373 glioblastoma, human SKMEL-28 melanoma and human A549 non-small-cell-lung cancer cell lines) by means of colorimetric MTT assay. Table 1 resumes IC_{50} concentrations obtained, that is, the concentration that decreased by 50% the global growth of the cell population after 72 h of exposure to the compound. Antiproliferative activities of thienylthiosemicarbazide intermediates **2a–m** ranged between 5 to >100 μM depending on the substituent and the cell line (Table 1). The most sensitive cell line is B16F10 with four compounds having IC_{50} <10 μM whereas almost all compounds displayed weak cytotoxic effect on U373 cell line (ten compounds with IC_{50} >40 μM). Thiosemicarbazide **2g** with a 5-(4-methylphenyl)thienyl substituent and a 4-methoxyphenyl substituent on the other side is the most potent one with a mean IC_{50} concentration of 11 μM on the six cancer cell lines under study. However, it should be noted that this compound is not selective as it showed toxicity to normal fibroblast cell lines (IC_{50} = 22 ± 1 μM on NHDF cell line and IC_{50} = 28 ± 3 μM on NHLF cell line). Thiosemicarbazide **2h** with phenyl (R^1) and 4-hydroxyphenyl (R^2) substituents was the less active one (IC_{50} >50 μM in five cell lines; mean IC_{50} concentration of 74 μM on the six cancer cell lines under study).

For most of thiosemicarbazides, the IC_{50} could be determined with values <50 μM in all six cell lines while the thiadiazoles **3a–m** presented different dose–response curves as compared to

Table 1
In vitro cytotoxic data of thienylthiosemicarbazide intermediates **2a–m** and 5-aryl-2-(3-thienylamino)-1,3,4-thiadiazoles **3a–m** against a panel of six cancer cell lines IC_{50} (μM)

R^1	R^2	Compound	H683	U373	A549	MCF-7	SKMEL-28	B16F10	Mean
4-OMeC$_6$H$_4$	4-MeC$_6$H$_4$	**2a**	30 ± 1	>100	35 ± 3	40 ± 2	22 ± 11	**7 ± 1**	>39 ± 13
C$_6$H$_5$	4-MeC$_6$H$_4$	**2b**	37 ± 2	62 ± 17	40 ± 2	30 ± 2	40 ± 3	24 ± 2	39 ± 5
4-MeC$_6$H$_4$	4-MeC$_6$H$_4$	**2c**	40 ± 1	38 ± 1	37 ± 1	29 ± 1	44 ± 2	20 ± 3	35 ± 4
C$_6$H$_5$	3,4,5-(OMe)$_3$C$_6$H$_2$	**2d**	42 ± 1	44 ± 1	39 ± 1	43 ± 1	55 ± 9	32 ± 2	43 ± 3
4-MeC$_6$H$_4$	3,4,5-(OMe)$_3$C$_6$H$_2$	**2e**	42 ± 1	47 ± 1	39 ± 1	31 ± 1	42 ± 2	13 ± 2	36 ± 5
C$_6$H$_5$	4-OMeC$_6$H$_4$	**2f**	35 ± 1	41 ± 2	36 ± 1	32 ± 1	30 ± 2	33 ± 1	35 ± 2
4-MeC$_6$H$_4$	4-OMeC$_6$H$_4$	**2g**	12 ± 1	19 ± 1	11 ± 1	**5 ± 1**	11 ± 1	**9 ± 1**	11 ± 2
C$_6$H$_5$	4-OHC$_6$H$_4$	**2h**	80 ± 1	>100	75 ± 1	78 ± 2	71 ± 3	40 ± 3	>74 ± 8
4-MeC$_6$H$_4$	4-OHC$_6$H$_4$	**2i**	49 ± 4	64 ± 3	44 ± 2	42 ± 1	46 ± 1	48 ± 3	49 ± 3
C$_6$H$_5$	3,5-(OMe)$_2$C$_6$H$_3$	**2j**	42 ± 1	40 ± 1	39 ± 1	38 ± 1	37 ± 1	30 ± 2	38 ± 2
4-MeC$_6$H$_4$	3,5-(OMe)$_2$C$_6$H$_3$	**2k**	37 ± 1	41 ± 1	32 ± 1	26 ± 2	38 ± 2	13 ± 2	31 ± 4
C$_6$H$_5$	3,4-(OMe)$_2$C$_6$H$_3$	**2l**	22 ± 2	59 ± 4	20 ± 1	21 ± 1	28 ± 2	**9 ± 1**	27 ± 7
4-MeC$_6$H$_4$	3,4-(OMe)$_2$C$_6$H$_3$	**2m**	39 ± 1	47 ± 1	36± 1	11 ± 1	19 ± 4	**7 ± 1**	27 ± 7
Mean			39 ± 4	>54 ± 7	37 ± 4	33 ± 5	37 ± 4	22 ± 4	
4-OMeC$_6$H$_4$	4-MeC$_6$H$_4$	**3a**	ND (10)	>100	ND (5)	>100	>100	ND (1)	ND (53±21)
C$_6$H$_5$	4-MeC$_6$H$_4$	**3b**	ND (5)	>100	ND (50)	34 ± 3	>100	**4 ± 1**	ND (64±19)
4-MeC$_6$H$_4$	4-MeC$_6$H$_4$	**3c**	ND (1)	ND (0.5)	ND (0.5)	ND (100)	>100	ND (1)	ND (34±21)
C$_6$H$_5$	3,4,5-(OMe)$_3$C$_6$H$_2$	**3d**	ND (5)	>100	ND (100)	>100	>100	ND (0.5)	ND (68±21)
4-MeC$_6$H$_4$	3,4,5-(OMe)$_3$C$_6$H$_2$	**3e**	ND (0.05)	ND (0.5)	ND (0.5)	ND (0.5)	ND (0.5)	12 ± 3	**ND (2 ± 2)**
C$_6$H$_5$	4-OMeC$_6$H$_4$	**3f**	**5 ± 1**	92 ± 7	**8 ± 1**	60 ± 7	>100	**6 ± 1**	>45 ±18
4-MeC$_6$H$_4$	4-OMeC$_6$H$_4$	**3g**	ND (0.5)	ND (0.5)	ND (0.5)	ND (0.5)	ND (100)	ND (1)	ND (17±17)
C$_6$H$_5$	4-OHC$_6$H$_4$	**3h**	26 ± 1	58 ± 2	15 ± 1	24 ± 1	57 ± 2	**8 ± 1**	31 ± 9
4-MeC$_6$H$_4$	4-OHC$_6$H$_4$	**3i**	17 ± 2	**8 ± 1**	**6 ± 1**	**8 ± 1**	14 ± 1	**6 ± 1**	**10 ± 2**
C$_6$H$_5$	3,5-(OMe)$_2$C$_6$H$_3$	**3j**	ND (50)	92 ± 4	ND (100)	ND (100)	>100	ND (50)	ND (80 ± 11)
4-MeC$_6$H$_4$	3,5-(OMe)$_2$C$_6$H$_3$	**3k**	ND (5)	ND (10)	ND (10)	ND (5)	>100	ND (1)	ND (22 ± 16)
C$_6$H$_5$	3,4-(OMe)$_2$C$_6$H$_3$	**3l**	ND (100)	>100	>100	92 ± 2	>100	ND (0.5)	ND (80 ± 18)
4-MeC$_6$H$_4$	3,4-(OMe)$_2$C$_6$H$_3$	**3m**	ND (5)	>100	ND (0.5)	ND (50)	>100	ND (5)	ND (43 ± 19)
Mean			ND (18 ± 8)	ND (>59 ± 13)	ND (>30 ± 12)	ND (>52 ± 12)	ND (>83 ± 10)	ND (7 ± 4)	

In vitro growth inhibitory IC_{50} concentrations were determined using the MTT colorimetric assay conducted once in sextuplicate. Bold values show IC_{50} less than 10 μM. ND: not determined because of 'plateau' phase, values in bracket correspond to the first concentration for which there is less than 50% of viable cells.

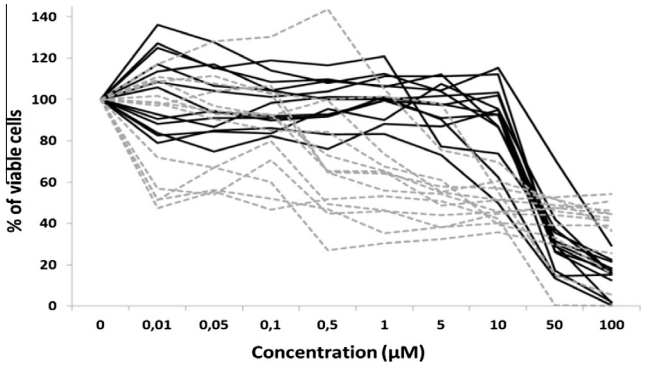


Figure 1. Dose–response curves of A549 NSCLC cells treated with **2a**–**m** compounds (black lines) or **3a**–**m** compounds (gray dotted lines) as established after MTT colorimetric assay.

2a–**m**, with a 'plateau' phase (Fig. 1, dotted gray lines as compared to black lines) indicating that increasing drug concentration, at least till 100 μM, did not lead to any improvement of the activity. Generally, thiadiazoles were nevertheless able to decrease partially the global growth of cancer cells. So, to evaluate the antiproliferative potential of thiadiazoles **3a**–**m**, we reported nevertheless in brackets in Table 1 the first tested concentration for which there remains less than 50% of viable cells after treatment. Marked differences of sensitivity between cell lines could be observed. Again the most sensitive cell line is B16F10 (five compounds with IC_{50} <12 μM; mean IC_{50} concentration of 7 μM considering all compounds under study) whereas ten thiadiazoles displayed rather no cytotoxic activity on SKMEL-28 cell line (IC_{50} >100 μM; mean IC_{50} concentration of >83 μM considering all compounds under study). Three thiadiazoles (**3f**, **3h** and **3i**) showed potent antiproliferative activity on the six cell line panel with classical dose-response curves. Thiadiazole **3f** containing a 5-phenylthienylamino substituent at C-2 and a 4-methoxyphenyl at C-5 exhibited growth inhibition on five cancer cell lines with IC_{50} below 10 μM for three cell lines (Hs 683, IC_{50} = 5 μM; A549, IC_{50} = 8 μM; B16F10, IC_{50} = 6 - μM). In the same way, thiadiazole **3i** containing a 5-(4-methylphenyl)thienylamino substituent at C-2 and a 4-hydroxyphenyl at C-5 showed growth inhibition on all cancer cell lines with IC_{50} below 10 μM for four cell lines (U373, IC_{50} = 8 μM; A549, IC_{50} = 6 - μM; MCF-7, IC_{50} = 8 μM; B16F10, IC_{50} = 6 μM). Thiadiazole **3h** displayed a mean IC_{50} concentration of 31 μM on the six cancer cell lines under study but only one IC_{50} <10 μM on B16F10 cell line. Thiadiazoles **3e** and **3g** with 5-(4-methylphenyl)thienylamino substituent at C-2 and respectively a 3,4,5-trimethoxyphenyl and a 4-methoxyphenyl at C-5 were also potent on almost all cancer cell lines with respectively mean IC_{50} concentration of 2 and 17 μM on the six cancer cell lines under study.

In conclusion, we have synthesized 26 new compounds and evaluated their in vitro antiproliferative properties on a panel of six cancer cell lines. Among them, two 5-aryl-2-(3-thienylamino)-1,3,4-thiadiazoles (**3f** and **3i)** have shown very interesting results with IC_{50} <10 μM on three cell lines and one thiosemicarbazide (**2g**) with a mean IC_{50} of 11 μM on the six cancer cell line panel. Presence of 5-(4-methylphenyl)thienylamino substituent at C-2 instead of phenylthienylamino seems to enhance activity in both series (mean IC_{50} concentrations on the six cancer cell line panel are always lower for compounds with 5-(4-methylphenyl)thienylamino substituent at C-2). Having a 4-methoxyphenyl group at C-5 gave potent compounds in both series (compounds **2g** and **3g**) whereas 4-hydroxyphenyl and 3,4,5-trimethoxyphenyl moieties at C-5 increased activity only in thiadiazole series (compounds **3e** and **3i**). Moreover, thiosemicarbazides and their corresponding 5-aryl-2-(3-thienylamino)-1,3,4-thiadiazoles might have different cellular targets as highlighted by the different dose-response curve profiles.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.04.043. These data include MOL files and InChiKeys of the most important compounds described in this article.

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19. *Spectral data of some potent derivatives:* 1-(4-Methoxyphenyl)-4-[5-(4-methylphenyl)-3-thienyl]thiosemicarbazide (**2g**). Yield 96%; beige solid; mp 209 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ_H 2.30 (s, 3H, CH_3), 3.82 (s, 3 H, OCH_3), 7.03 (d, 2H, 2 × CH, J = 7.5 Hz), 7.21 (d, 2H, 2 × CH, J = 7.5 Hz), 7.46 (d, 2H, 2 × CH, J = 7.5 Hz), 7.57 (s, 1H, CH), 7.66 (s, 1H, CH), 7.93 (d, 2H, 2 × CH, J = 7.5 Hz), 9.68 (s, 1H, NH), 9.88 (s, 1H, NH), 10.37 (s, 1H, NH); ^{13}C NMR (62,5 MHz, DMSO-d_6) δ_C 20.7, 55.4, 96.8, 101.8, 113.5, 120.9, 124.6, 124.8, 129.7, 129.8, 130.8, 131.5, 137.1, 137.8, 162.1, 165.7; HRMS (APCI): m/z calcd for $[C_{20}H_{17}N_3O_2S+H]^+$: 364.1114; found: 364.1120. 2-(5-Phenyl-3-thienylamino)-5-(4-methoxyphenyl)-1,3,4-thiadiazole (**3f**). Yield 88%; brown solid; mp 264 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ_H 3.81 (s, 3H, OCH_3), 7.05 (d, 2H, 2 × CH, J = 10 Hz), 7.32 (t, 1H, CH, J = 7.5 Hz), 7.37 (d, 1H, CH, J = 1.5 Hz), 7.43 (t, 2H, 2 × CH, J = 7.5 Hz), 7.54 (d, 1H, CH, J = 1.5 Hz), 7.63 (d, 2H, 2 × CH, J = 7.5 Hz), 7.78 (d, 2H, 2 × CH, J = 10 Hz), 10.81 (s, 1H, NH); ^{13}C NMR (62,5 MHz, DMSO-d_6) δ_C 55.4, 105.8, 114.6, 116.9, 122.9, 125.2, 127.9, 128.2, 129.2, 133.3, 138.8, 142.1, 157.5, 160.7, 163.1; HRMS (APCI): m/z calcd for $[C_{19}H_{15}N_3OS_2+H]^+$: 366.0729; found: 366.0730. 2-[5-(4-Methylphenyl)-3-thienylamino]-5-(4-hydroxyphenyl)-1,3,4-thiadiazole (**3i**). Yield 98%; brown solid; mp 240 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ_H 2.31 (s, 3H, CH_3), 6.87 (d, 2H, 2 × CH, J = 8.75 Hz), 7.23 (d, 2H, 2 × CH, J = 8.75 Hz), 7.30 (s, 1H, CH), 7.48 (s, 1H, CH), 7.51 (d, 2H, 2 × CH, J = 8.75 Hz), 7.66 (d, 2H, 2 × CH, J = 8.75 Hz), 10.01 (s, 1H, OH), 10.72 (s, 1H, NH); ^{13}C NMR (62,5 MHz, DMSO-d_6) δ_C 20.7, 105.0, 115.9, 116.3, 121.4, 125.0, 128.3, 129.7, 131.5, 137.4, 138.8, 142.2, 157.9,

159.3, 162.8; HRMS (APCI): m/z calcd for $[C_{19}H_{15}N_3OS_2+H]^+$: 366.0729; found: 366.0737.

20. Determination of the IC_{50} growth inhibitory concentrations were determined by means of the colorimetric MTT assay. Human cancer cell lines Hs683 and U373 (glioma), A549 (NSCLC), MCF-7 (breast cancer), SKMEL-28 (melanoma) and mouse B16F10 melanoma cells were purchased from the ATCC or ECACC and were cultivated in RPMI culture medium supplemented with 10% heat inactivated fetal bovine serum and antibiotics. Compounds were applied 72 h on cells after 24 h of seeding. Reduction of MTT into formazan cristals by succinate dehydrogenase is proportional to the number of viable cells and was evaluated by measurement of the optical density at 570 nm after their dissolution in DMSO.