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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

N-acylhydrazones containing thiophene nucleus: a new anticancer class

Laura N. F. Cardoso[1,2] · Thais C. M. Nogueira[1] · Felipe A. R. Rodrigues[3] · Augusto Cesar Aragão Oliveira[3] · Maria Claudia dos Santos Luciano[3] · Claudia Pessoa[3,4] · Marcus V. N. de Souza[1]

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Abstract In this study, we present a series of *N*-acylhydrazones containing thiophene nuclei as a new anticancer class. Fifty-seven compounds in this series were evaluated for their activity against four human cancer cell lines. Cytotoxicity (IC_{50}) ranged from 0.82 to 12.90 µM. The compound (*E*)-*N*′-(2-hydroxy-3-methoxybenzylidene)thiophene-2-carbohydrazide displayed good cytotoxic activity in all cell lines ($IC_{50} = 0.82–5.36$ µM) and yielded the best result in this series; therefore, it is an important lead compound in this new class.

Keywords Thienyl derivatives · *N*-acylhydrazones · Cancer · Antitumor

Introduction

Sulfur is an important chemical element present in various synthetic and natural products, which plays a critical role in earth processes. In the field of drug discovery, there are several classes that incorporate sulfur into their structure, resulting in a wide range of biological applications, such as cancer. Note that cancer is the leading cause of death worldwide; it accounted for 8.2 million deaths in 2012 (Ferlay et al. 2013).

Because of the importance of a thiophene nucleus to drug discovery and the continuous search for effective and safe anticancer agents (Fig. 1) (Palkowitz et al. 1997; Romagnoli et al. 2014), this study presents a series of *N*-acylhydrazones containing a thiophene nucleus designed by molecular hybridization (Schemes 1 and 2).

The series was tested against three human cancer cell lines with good results (Table 1). The choice of thiophene *N*-acylhydrazones derivatives was based on the importance of these as functional groups and their involvement in various pharmacological activities such as anticancer agents (Montenegro et al. 2012).

Results and discussion

Chemistry

All series **I** (**1a–u**), **II** (**2a–i**), and **III** (**3a–j**) were previously synthesized in three steps by our research group (Scheme 1) and evaluated as antituberculosis class (Cardoso et al. 2014). The series **IV** (**4a–k**) were synthesized by the same strategy (Scheme 1) and the series **V** (**5a–e**) were synthesized using the derivatives from series **II** and **IV** (Scheme 2) (Cardoso et al. 2016).

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✉ Marcus V. N. de Souza
marcos_souza@far.fiocruz.br

[1] FioCruz - Fundação Oswaldo Cruz, Instituto de Tecnologia em Fármacos-Far-Manguinhos, Rua Sizenando Nabuco, 100, Manguinhos, Rio de Janeiro, RJ 21041-250, Brazil

[2] Instituto de Química, Cidade Universitária, Universidade Federal do Rio de Janeiro, Avenida Athos da Silveira Ramos, 149 Bloco A - 7° Andar, Rio de Janeiro, RJ 21941-909, Brazil

[3] Laboratório de Oncologia Experimental, Universidade Federal do Ceará, Fortaleza, CE, Brazil

[4] FioCruz - Fundação Oswaldo Cruz/ Ceará, Av. Santos Dumont, 5753 - Papicu, Fortaleza, CE 60176-032, Brazil

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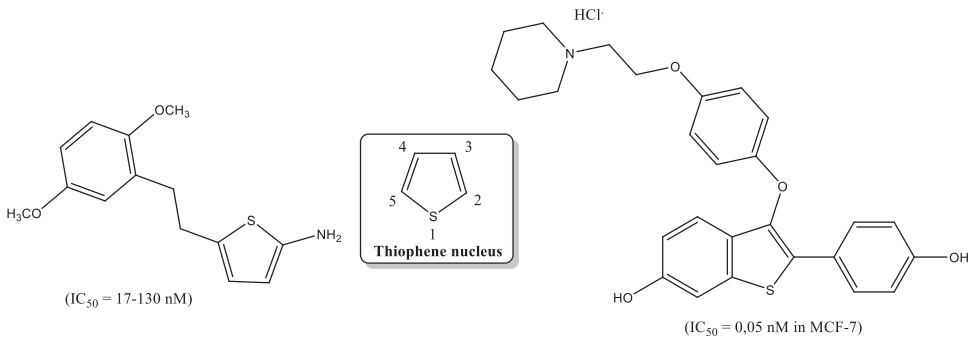
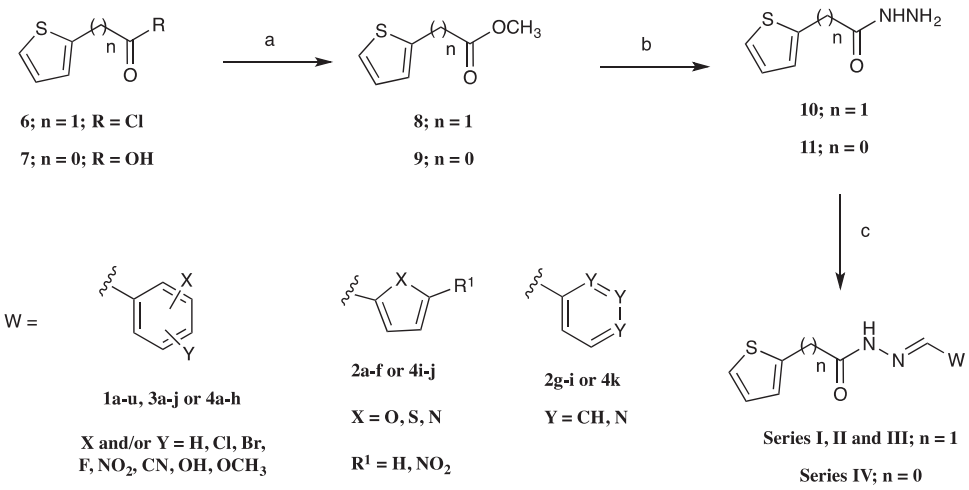
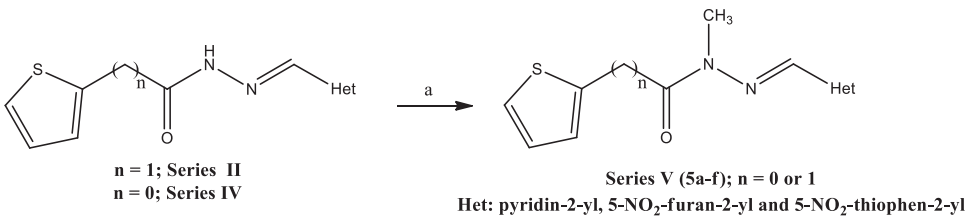


Fig. 1 Synthesized thiophene derivatives identified with anticancer activity



Scheme 1 Reagents and conditions: **a** (**6**); MeOH, r.t, 4 h, 100%; (**7**); $SOCl_2$, MeOH, 0 °C-r.t., 3 h, 96%; **b** $N_2H_4{\cdot}H_2O$ (55%), EtOH, 80 °C, 2 h, 75% (**8**), 18 h, 80% (**9**); **c** EtOH, appropriate aldehyde, r.t, 1–72 h, 52–87%



Scheme 2 Reagents and conditions: **a** K_2CO_3, CH_3I, acetone, 40 °C, 2–24 h, 65–89%

Cytotoxicity against cancer cell lines

All compounds in series **I**, **II**, **III**, **IV**, and **V** were tested in vitro against three cancer cell lines: SF-295 (glioblastoma), OVCAR-8 (human ovary), and HCT-116 (colon) (National Cancer Institute, Bethesda, MD) at 5 μg/mL using an MTT assay. The compounds were then classified by the percentage of growth inhibition (GI) displayed, i.e., at least one active cell line (100% GI), moderately active (75% < GI < 100%), or inactive (GI < 50%).

Compounds which presented more than 96% GI in 5 μg/mL were selected to determine the concentrations (μM) that induced 50% cell GI (IC_{50}) in vitro anticancer activity against four human cancer cell lines: OVCAR-8 (human ovary), SF-295 (glioblastoma), HCT-116 (colon) and HL-60 (leukemia), using the MTT assay. The concentrations (μM) that induced 50% cell GI (IC_{50}) are in Table 1.

Initially, we synthesized series **I** and **II**; based on their large cytotoxicity to human cells, found in our previous study (Cardoso et al. 2014), we now suggest an evaluation of their potential activity against cancer cell lines. Because of poor results from the first synthesized series (**I** and **II**), we propose a new series (**III**) by maintaining important substituents and their positions, although we include other substituents at different positions in benzene.

Table 1 Cytotoxic activity of compounds with GI > 96% [IC_{50} (μM)] on tumor cell lines

Compounds	IC_{50}			
	HCT-116	OVCAR-8	HL-60	SF-295
2a (5-NO_2-furan-2-yl)	5.43	3.39	6.86	12.90
2b (5-NO_2-thien-2-yl)	6.02	1.13	6.57	10.37
3a (R = 2,3-diOH)	5.36	5.26	1.24	5.71
3e (R = 2-OH, 4-Me)	10.73	7.32	5.43	6.82
3j (R = 2-OH, 5-NO_2)	6.71	5.80	12.21	10.00
4a ($n = 0$; R = H; Ar; R^1 = OH)	3.30	2.56	1.29	4.32
4b ($n = 0$; R = H; Ar; R^1 = 2,3-diOH)	1.46	0.83	0.51	1.34
4c ($n = 0$; R = H; Ar; R^1 = 2,4-diOH)	3.57	2.28	1.04	1.43
4d ($n = 0$; R = H; Ar; R^1 = 2-OH, 3-OMe)	3.11	2.97	0.82	5.36
4e ($n = 0$; R = H; Ar; R^1 = 2-OH, 4-OMe)	4.00	2.17	0.92	1.49
4f ($n = 0$; R = H; Ar; R^1 = 2-OH, 4-Me)	6.86	4.15	1.38	5.49
4g ($n = 0$; R = H; Ar; R^1 = 2-OH, 5-Me)	5.99	1.67	1.93	6.66
4h ($n = 0$; R = H; Ar; R^1 = 2-OH, 5-NO_2)	9.44	3.81	1.76	5.80
5a ($n = 0$; R = CH_3; Het; 5-NO_2-furan-2-yl)	9.70	10.55	8.44	11.24
Doxorubicin	0.23	0.49	0.04	0.42

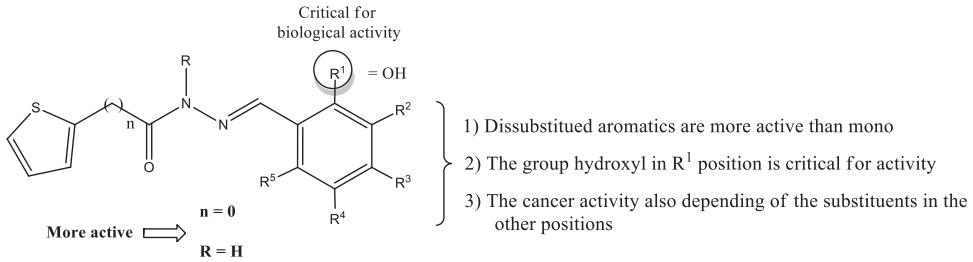


Fig. 2 Structure–activity relationship (SAR) for the *N*-acylhydrazones containing the thiophene ring (series **I**, **II**, **III**, **IV**, and **V**)

From this results (series **III**), we can confirm the importance of the hydroxyl group at the *ortho* position of benzene (Ma et al. 2014). This result suggests that the mechanism of action of this class is based on the formation of complexes; hydroxyl groups located in the *ortho* position in *N*-acylhydrazone systems are good metal ligands for iron, zinc, and copper, which are essential for cell replication (Li et al. 2014; Gup et al. 2015; Rodríguez-Argüelles et al. 2009).

According to the results from the firsts series (**I**, **II**, and **III**), we applied strategies from medicinal chemistry, such as conformational retention, to synthesized series **IV** and **V**. Furthermore, these series (series **IV** and **V**) were helpful to identify the presence of conformers, *syn/anti*, as discussed previously (Cardoso et al. 2014).

When the methylene group between *N*-acylhydrazones and the thiophene ring (**4a–k**) was removed, more derivatives displayed good activity. Furthermore, when the hydrogen from NH in *N*-acylhydrazone was substituted with a methyl group (**5a–e**) the number of active derivatives decreased (Table 1).

The retention of conformation, *syn/anti*, through substitution of hydrogen for methyl groups in the nitrogen of *N*-acylhydrazone, generally increased the activity (Cardoso et al. 2014). However, the undefined conformation complicated the molecule-enzyme interaction, the results from series **V** show the opposite.

The structure–activity relationship (SAR) analysis indicated that the number, position, and type of substituents attached to the aromatic ring are critical for biological activity. Considering that, the disubstituted derivatives were more active than the respective mono. Moreover, the derivatives without the methylene group between *N*-acylhydrazone and the thiophene ring were more active than their counterparts in the methylene group. Another important observation was the retention of conformation, *syn/anti*, where the derivatives without retention were more active than the ones with retention (Fig. 2).

Conclusion

A series of 57 *N*-acylhydrazones containing a thiophene ring were evaluated as a new anticancer class. Compound **4d** showed good cytotoxic activity compared with the reference drug doxorubicin and is an important lead

compound in this class. The SAR analysis of this class gave us important information for further studies. For example, hydroxyl groups located at the *ortho* position in *N*-acylhydrazone systems are good ligands for metals this may indicate that the action mechanism of this class is based on the formation of complexes, which could inactivate enzymes involved in abnormal cell division. Furthermore, the retention of conformation generally decreases the activity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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