European Journal of Medicinal Chemistry 118 (2016) 21–26

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Synthesis and preliminary evaluation of 3-thiocyanato-1*H*-indoles as potential anticancer agents

Margiani P. Fortes [a], Paulo B.N. da Silva [b], Teresinha G. da Silva [c], Teodoro S. Kaufman [d, *], Gardenia C.G. Militão [b], Claudio C. Silveira [a, **]

[a] Departamento de Química, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil
[b] Universidade Federal de Pernambuco, Departamento de Fisiologia e Farmacologia, CCB R. Prof. Moraes Rego, Cidade Universitária, 50670-901 Recife, PE, Brazil
[c] Universidade Federal de Pernambuco, Departamento de Antibióticos, CCB R. Prof. Moraes Rego, Cidade Universitária, 50670-901 Recife, PE, Brazil
[d] Instituto de Química Rosario (IQUIR, CONICET-UNR), Suipacha 531, 2000 Rosario, Argentina

ARTICLE INFO

Article history:
Received 20 February 2016
Received in revised form
14 April 2016
Accepted 15 April 2016
Available online 19 April 2016

Keywords:
3-Thiocyanato-1*H*-indoles
Bioactive heterocycles
Cytotoxic compounds
HL60 and HEP-2
NCI-H292 and MCF-7 cells

ABSTRACT

A novel series of twenty 3-thiocyanato-1*H*-indoles, carrying diversification at positions N-1, C-2 and C-5 of the heterocyclic core, were synthesized; their antiproliferative activity against four human cancer cell lines (HL60, HEP-2, NCI-H292 and MCF-7) was evaluated, employing doxorubicin as positive control. Indole, *N*-methylindole and 2-(4-chlorophenyl)-*N*-methylindole demonstrated to be essentially inactive, whereas several of their congener 3-thiocyanato-1*H*-indoles displayed good to excellent levels of potency ($IC_{50} \leq 6$ μM), while being non-hemolytic.

N-Phenyl-3-thiocyanato-1*H*-indole and 1-methyl-2-(4-chlorophenyl)-3-thiocyanato-1*H*-indole showed good to high potency against all the cell lines. On the other side, the *N*-(4-chlorophenyl)-, 2-(4-chlorophenyl)- and 2-phenyl- 3-thiocyanato-1*H*-indole derivatives were slightly less active against the test cell lines.

Overall, these results suggest that the indole-3-thiocyanate motif can be suitably decorated to afford highly cytotoxic compounds and that the substituted indole can be employed as a useful scaffold toward more potent compounds.

1. Introduction

One of the most fruitful paradigms for the discovery of new bioactive chemical entities is to start with established structural cores, known to be part of other bioactive molecules [1]. The indole skeleton is a privileged scaffold [2]; hence, there is an enhanced likelihood that indole derivatives will have biological activity. Therefore, this heterocyclic motif is one of the most important morphological sub-units for the discovery of new drug candidates.

Indoles are the most abundant heterocycles among biologically active natural products, pharmaceuticals and agrochemicals. Many of them possess interesting biological and pharmacological properties [3]; thus, analogs of the aplicyanines A, B and E (**1a**–**c**) [4], 3-indolyl benzimidazoles (**2**) [5], tricyclic and tetracyclic cycloalkanoindoles (**3**) [6], and the 2-acyl-1*H*-indole-4,7-diones (**4**) [7] are cytotoxic and antiproliferative.

Historically, natural products have inspired the synthesis of new pharmacological agents. However, naturally-occurring thiocyanates are rather scarce [8]. Hence, not surprisingly, there have been few reports on bioactive natural thiocyanates.

Further, most of the natural thiocyanates are terpenoids [9], but linear alkanes as the thiocyanatins [10] and aminoacid derivatives as psammaplin B [11], have also been disclosed. In addition, the thiocyanato group occurs in certain anticancer natural products formed by deglycosylation of cruciferous glucosinolates [12].

The naturally-occurring fasicularin (**5**), is bioactive in a DNA repair-deficient strain of yeasts lacking the RAD 52 gene, and cytotoxic against Vero cells, acting as a guanidine alkylating agent [13], while the related cylindricines were toxic in the brine shrimp assay [14].

The inclusion of the thiocyanato functionality in potentially bioactive compounds is infrequent and only scattered reports are

* Corresponding author.
** Corresponding author.
E-mail addresses: kaufman@iquir-conicet.gov.ar (T.S. Kaufman), silveira@quimica.ufsm.br (C.C. Silveira).

http://dx.doi.org/10.1016/j.ejmech.2016.04.039
0223-5234/

available [15]. Simple aromatic thiocyanates (**6**) are active against *Trypanosoma cruzi*, the causative agent of Chagas' disease [16], whereas others (**7**) hold promise for the treatment of leishmanial infections [17].

In addition, benzyl thiocyanate was isolated from *Lepidium sativum*; its chlorinated derivatives (**8**) were shown to cause mitotic arrest, acting as a sulfhydryl alkylating reagent [18], and 2,5,6-substituted imidazo[2,1-*b*][1,3,4] thiadiazole derivatives (**9**) have been found to be potent antiproliferative agents [19].

On the other hand, isatin thiocyanates (**10**) have shown significant in vitro anticancer activity, whilst thiocyanate-substituted derivatives exhibited growth inhibition against melanoma UACC903 cells [20]. Further, indoles bearing benzimidazole carbamate (**11**) and glyoxylamide moieties have been successfully explored as antiproliferative agents [21], and different thiocyanates have been tested as cytotoxics [22] Fig. 1.

Finally, in spite that to date the bioactivity of 3-thiocyanato-1*H*-indole derivatives has not been explored, these compounds have been employed as synthetic intermediates toward complex bioactive compounds [21a]; some of the latter have been shown to act as estrogen receptor ligands, non-nucleoside reverse transcriptase inhibitors and agents for treatment of respiratory syncytial virus infection [23].

Considering our previous work with indole [24] and thiocyanato [25] derivatives, and from a combined literature analysis on bioactive compounds carrying these structural features, we conjectured that the combination of a suitable decorated indole nucleus with the thiocyanato functionalization could afford novel cytotoxic compounds. Therefore, herein we report the synthesis and biological activity results of the first generation of 3-thiocyanato-1*H*-indole derivatives, as antiproliferative agents.

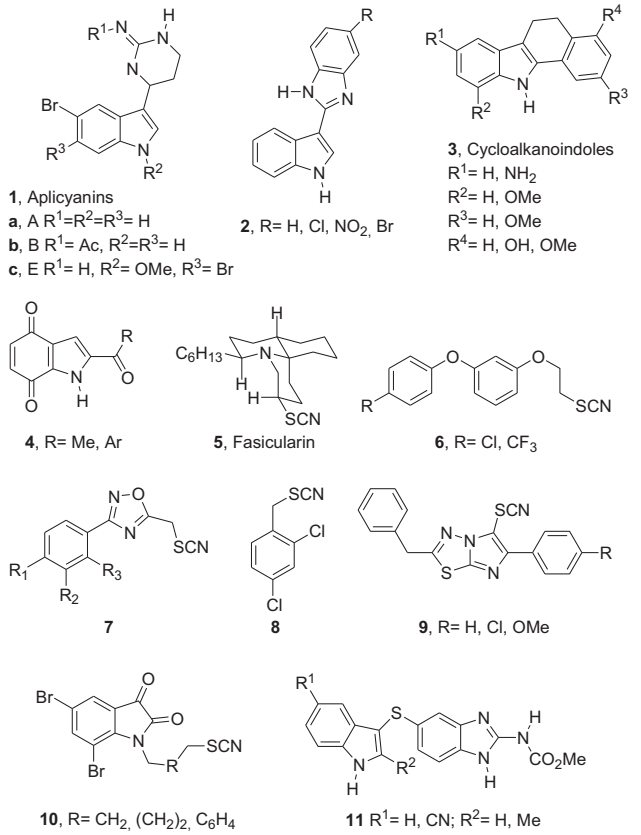


Fig. 1. Selected bioactive simple indole and thiocyanato derivatives.

2. Results and discussion

2.1. Chemistry

The complete set of compounds submitted to the cytotoxicity tests is detailed in Table 1. All of them were obtained in good overall yields and in few reaction steps. Compounds **12a**–**e** and **13a**–**e** were prepared and fully characterized as previously reported [25].

On the other hand, the 2-arylindoles **20a**–**e**, which were employed as precursors of the 2-aryl-3-thiocyanato-1*H*-indole derivatives **14a**–**e** and **15a**–**e**, were synthesized by means of two different strategies, disclosed by the groups of Rossi and Samsoniya, respectively, as shown in Scheme 1.

In the first method [26], the anion of the starting acetophenones **16a**–**c** (**17a**–**c**) was prepared by reaction of the corresponding ketones with potassium *tert*-butoxide in anhydrous DMSO, and coupled to 2-iodoaniline (**18**) through a photostimulated (λ = 254 nm) $S_{RN}1$ reaction at room temperature. The resulting ketones **19a**–**c** spontaneously cyclized *in situ*, to afford the target 2-arylindoles **20a**–**c** in 45–60% yield, under acid-free conditions.

The second procedure [27], which entailed the acetic acid-promoted condensation of acetophenones **16d,e** with phenylhydrazine (**21**) and the subsequent Fischer synthesis-type polyphosphoric acid (PPA)-mediated [3,3]-sigmatropic rearrangement of the resulting phenylhydrazone intermediates **22d,e**, followed by ammonia elimination and aromatization, afforded the expected 2-arylindoles **20d** and **20e** in 28% and 20% yield, respectively.

Next, the indoles **20a**–**e** were *N*-methylated by treatment with KOH in DMSO, followed by MeI quench, furnishing the corresponding derivatives **23a**–**e** in 80–94% yield. The final stage comprised the desired thiocyanation, which was accomplished by exposure of the indoles to the NH_4SCN-oxone reagent system and resulted in 80–98% yields of the 3-thiocyanato-1*H*-indoles **15a**–**e**. Analogously, submission of the indole derivatives **20a**–**e** to thiocyanation under similar conditions furnished 82–98% yield of the expected 3-thiocyanato-1*H*-indoles **14a**–**e**.

The structures of the synthesized compounds were established on the basis of exhaustive infrared, ^{1}H and ^{13}C NMR and mass spectral analyses. They were also characterized by their melting point. The infrared and ^{13}C NMR spectra provided the most useful confirmatory evidences. For instance, the IR spectra of compounds in series **14** and **15** evidenced the stretching absorption of the C≡N triple bond of the thiocyanates as sharp signals at 2156 ± 10 cm^{-1}.

On the other side, in their ^{13}C NMR spectra, it was observed that the resonance of C-3, which supported the SCN moiety, appeared as a signal at δ 111.7–112.2 ppm in the nitrogen unsubstituted derivatives (**14a**–**e**); however, this resonance experienced a slight upfield shift (δ 110.4–110.6 ppm) when the nitrogen atom was alkylated (**15a**–**e**).

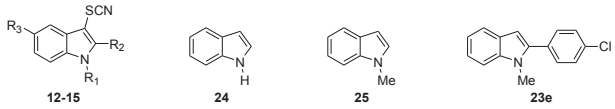
In addition, the diagnostic signal of the carbon atom of the thiocyanato moiety also appeared in a very narrow region (δ 86.5–90.8 ppm). With the exception of **14d**, these resonances were shifted slightly upfield in the nitrogen-unsubstituted compounds compared with their *N*-alkyl congeners (**15a**–**e**).

2.2. Biological activity

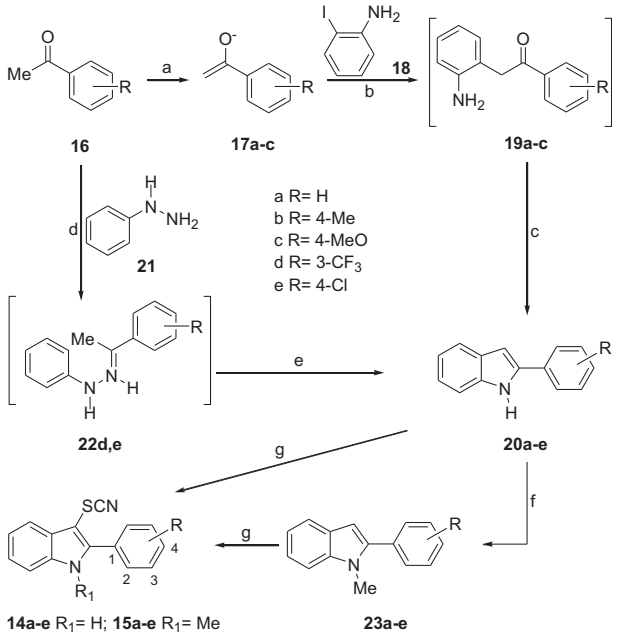
The antiproliferative activity of the 3-thiocyanato-1*H*-indole derivatives was assessed by investigating their effects on a panel of four different cell lines, including HL60 (human promyelocytic leukemia), MCF-7 (human breast adenocarcinoma), NCI-H292 (human lung cancer), and HEP-2 (human cervical carcinoma).

The MTT colorimetric assay was employed to assess the potency of the compounds. In this assay, the formazan product resulting from the reduction of MTT is dissolved and quantitated

Table 1
Chemical structures of the 3-thiocyanato-1*H*-indole derivatives synthesized for testing.



Compound no	R_1	R_2	R_3	Compound no	R_1	R_2	R_3
12a	H	H	H	**14a**	H	C_6H_5	H
12b	H	H	4-Me−C_6H_4	**14b**	H	4-Me−C_6H_4	H
12c	H	H	OMe	**14c**	H	4-MeO−C_6H_4	H
12d	H	H	Br	**14d**	H	3-CF_3−C_6H_4	H
12e	H	H	CN	**14e**	H	4-Cl−C_6H_4	H
13a	Ph	H	H	**15a**	Me	C_6H_5	H
13b	Me	H	4-Me−C_6H_4	**15b**	Me	4-Me−C_6H_4	H
13c	4-MeO−C_6H_4	H	H	**15c**	Me	4-MeO−C_6H_4	H
13d	Me	H	H	**15d**	Me	3-CF_3−C_6H_4	H
13e	4-Cl−C_6H_4	H	H	**15e**	Me	4-Cl−C_6H_4	H
24	Indole			**23e**	2-(4-Chlorophenyl)-*N*-methylindole		
25	*N*-Methylindole						



Scheme 1. *Reagents and conditions*: a) K^{t}BuO, DMSO, r.t., 15 min; b) 2-iodoaniline; c) hν (254 nm, 400 W), r.t., 3 h (R = 4-H, 4-Me, 4-MeO); d) $PhNHNH_2$, AcOH (cat.), EtOH, reflux, 6 h; e) 1. PPA, 80 °C, 30 min; 2. 1 M NaOH (R = 3-CF_3, 4-Cl); f) 1. KOH, DMSO, r.t.; 2. MeI, r.t.; g) NH_4SCN, oxone, MeOH, r.t. 0.3–1 h.

spectrophotometrically. The results are summarized in Table 2, where Doxorubicin was used as a positive control (entry 24).

Owing to the wide range of molecular weights of the tested compounds (174.2–332.3) and also taking into account that doxorubicin is a complex, high molecular weight small organic molecule (MW = 543.5), the potencies of these thiocyanates were compared taking into account their micromolar concentrations. The 50% inhibitory concentrations (IC_{50}) and their 95% confidence intervals were calculated by non-linear regression.

At the beginning of the study, it was demonstrated that indole (**24**), *N*-methylindole (**25**) and 2-(4-chlorophenyl)-*N*-methylindole (**23e**) were essentially inactive under the test conditions (entries 1–3). Next, a general analysis of the performance of the cell lines and the tested compounds was carried out, according to the sensitivity of the former and the substitution pattern of the latter.

Compounds with $IC_{50} \leq 3$ μM were judged as highly active, whereas thiocyanates bearing IC_{50} values in the range 3–6 μM were regarded as having good activity. On the other hand, compounds with IC_{50} between 6 μM and 12 μM were considered of moderate activity, while indole derivatives displaying IC_{50} values in the range 12–24 μM were regarded as bearing low activity. Finally, the thiocyanates exhibiting $IC_{50} > 24$ μM were deemed inactive. Under these conditions, it was concluded that the HL60 cell line was the most sensitive one, since all the tested compounds exhibited good to high levels of potency ($IC_{50} = 0.63–5.63$ μM).

However, since the HL60 cells were also highly sensitive to doxorubicin ($IC_{50} = 0.02$ μM), thiocyanato derivatives with high potency towards HL60 were observed as being more than 50 times less potent than doxorubicin. Conversely, the MCF-7 cell line was the least sensitive. Not a single compound was highly potent, a few exhibited good activity (**13a**, **13e**, **14e** and **15e**) and many indole derivatives displayed low activity (**12a**, **12d**, **14a**, **15a** and **15d**) or were inactive (**13d**, **14b** and **14c**).

The HEP-2 cells were slightly more sensitive. Few thiocyanates were highly potent (**13a**, **15e**) while many compounds exhibited low activity (**12a**, **12b**, **12d** and **15c**) or were inactive (**12c**, **13b**, **13d** and **15d**). Finally, the remaining cell line (NCI-H292) exhibited a quite similar profile, with more compounds with high (**13a**, **14e** and **15e**) and good (**13c**, **13e**, **14a** and **15a**) potency, and a smaller number of inactive substituted thiocyanate-1*H*-indoles (**12c** and **15d**).

Analysis of the series of compounds **12**–**15** brought about additional conclusions. The series of compounds **12a**–**e** (entries 4–8) comprises the 3-thiocyanato-1*H*-indole derivatives carrying only C-5 substituents. The profile of potency of the unsubstituted 3-thiocyanato-1*H*-indole **12a** improved very slightly upon introduction of an electron withdrawing group (CN) at C-5 (**12e**). However, the presence of substituents able to release electrons resulted in low general performances, as those observed with the 5-tolyl and 5-bromo derivatives **12b** and **12d**; furthermore, compound **12c** which carries an electron donating group (MeO) exhibited the worst profile.

On the other hand, the series of compounds **13a**–**e** (entries 9–13) includes the 3-thiocyanato-1*H*-indole derivatives displaying substitution of the nitrogen atom. In this case, the introduction of an unsubstituted phenyl moiety considerably improved the activity (**13a** vs **12a**), resulting in the *N*-phenyl-3-thiocyanato derivative **13a**, that exhibited good potency against MCF-7, being highly active

Table 2
Cytotoxic activity of the 3-thiocyanato-1*H*-indole derivatives against four different cell lines, and their hemolytic activity against human erythrocytes.[a]

Entry no	Compound no[b]	IC_{50} and confidence intervals, in μM			
		HL60	HEP-2	NCI-H292	MCF-7
1	**24**	>50	>50	>50	NT
2	**25**	>50	>50	>50	NT
3	**23e**	20.7 (14.8–30.5)	>50	>50	NT
4	**12a**	3.21 (2.58–3.96)	18.9 (16.1–22.3)	14.9 (12.2–18.3)	14.7 (10.3–20.9)
5	**12b**	2.53 (1.74–3.75)	15.7 (13.2–18.7)	21.5 (6.73–9.65)	6.85 (5.45–8.63)
6	**12c**	5.63 (3.36–9.40)	40.7 (36.2–45.9)	27.8 (25.0–31.0)	6.2 (1.17–33.1)
7	**12d**	3.00 (2.64–3.39)	19.9 (12.1–32.9)	13.7 (10.7–17.6)	22.3 (10.7–46.2)
8	**12e**	5.62 (5.11–6.17)	11.89 (9.78–14.5)	7.22 (6.67–7.83)	8.73 (6.97–10.9)
9	**13a**	1.43 (1.19–1.71)	2.79 (1.56–4.95)	2.07 (1.95–2.19)	3.95 (3.07–5.07)
10	**13b**	4.05 (2.83–5.81)	24.2 (13.2–44.2)	20.9 (14.1–30.9)	NT
11	**13c**	1.42 (1.35–1.53)	10.8 (7.5–26.7)	5.99 (5.1–7.02)	NT
12	**13d**	1.54 (1.06–2.18)	35.6 (18.1–70.1)	21.9 (15.7–30.8)	>50
13	**13e**	3.58 (3.33–4.10)	8.49 (5.51–13.2)	3.47 (2.94–4.03)	6.81 (3.72–12.4)
14	**14a**	0.63 (0.20–2.19)	5.03 (4.31–5.83)	5.43 (4.79–6.11)	13.46 (9.50–19.2)
15	**14b**	2.27 (2.04–2.49)	10.21 (7.90–12.9)	17.3 (11.2–26.7)	47.43 (21.4–104.7)
16	**14c**	4.45 (3.95–4.99)	9.1 (7.9–8.16)	10.16 (7.09–14.5)	25.68 (17.9–35.0)
17	**14d**	0.69 (0.37–1.31)	4.1 (3.45–5.34)	2.07 (1.66–2.54)	4.96 (3.74–6.60)
18	**14e**	1.08 (0.80–1.40)	8.74 (4.53–6.04)	7.28 (6.49–8.18)	3.96 (2.63–6.04)
19	**15a**	1.39 (0.90–1.74)	8.09 (6.16–10.7)	4.23 (3.44–5.18)	17.81 (11.7–27.2)
20	**15b**	1.50 (1.01–2.23)	3.62 (2.67–4.92)	9.90 (8.52–11.5)	11.8 (7.03–19.7)
21	**15c**	1.35 (1.29–1.39)	22.01 (13.9–35.0)	11.58 (8.28–16.1)	NT
22	**15d**	2.56 (2.38–2.79)	37.3 (24.8–56.5)	44.23 (16.4–119.7)	21.0 (9.08–48.7)
23	**15e**	1.10 (0.79–1.53)	2.80 (2.0–3.75)	2.07 (1.87–2.28)	4.05 (1.32–12.4)
24	**Doxorubicin** (positive control)	0.02 (0.02–0.04)	1.29 (0.55–2.58)	0.37 (0.18–0.55)	0.55 (0.37–0.92)

[a] Data are presented as IC_{50} values obtained by non-linear regression. NT = Not tested.
[b] The series of compounds **12**–**15** were not hemolytic, with EC_{50} > 500 μM.

against the other cell lines.

However, *para*-substitution of the phenyl moiety either with chlorine (**13e**) or methoxy (**13c**) groups resulted in compounds with lower potency with regards of the *N*-phenyl derivative (**13a**). Changing to an *N*-alkyl derivative was of no help, as the *N*-methyl substituted compound **13d** exhibited a poor profile, being inactive against HEP-2 and MCF-7 cells. In addition, as expected, the performance of the indoles could not be improved by introduction of a 5-tolyl substitution. Comparison of the potency against HL60, HEP-2 and NCI-H292 and their confidence intervals suggested that the resulting *N*-methylindole **13b** exhibited an activity pattern with slightly lower quality than that of its non-methylated 5-tolyl congener **12b**.

Compounds **14a**–**e** (entries 14–18) helped to establish the effect of the presence of a C-2 substituent. The introduction of a phenyl group resulted in a general increase of potency with regards to the unsubstituted congener (**14a** vs **12a**), which was more evident against HL60, HEP-2 and NCI-H292. However, lower activities were observed when the phenyl moiety was substituted with a 4-methyl motif, such as in the 2-tolyl derivative **14b**, and the performance of the tested compound worsened by addition of a 4-methoxy group as example of an electron releasing substituent (**14c**), which became the worst compound of this series. Both, **14b** and **14c** were considered inactive against the MCF-7 cells.

Slight improvements, compared with the unsubstituted **14a**, could be achieved by adding 4-Cl and 3-CF_3 moieties as electron withdrawing groups (**14d** and **14e**, respectively), concluding that the 2-(3-trifluoromethyl-phenyl) substitution pattern (**14d**) despite leading to a moderately active compound against the MCF-7 cell line, was the best overall choice within this series.

Finally, the lot of compounds **15a**–**e** (entries 19–23), which embraces the *N*-methylated 2-substituted 3-thiocyanato-1*H*-indole derivatives, was analyzed. It was observed that introduction of a C-2 phenyl ring increased the potency of the resulting *N*-methylated indole derivative (**15a** vs **13d**); however, the overall performance of the *N*-methyl,2-phenyl indole **15a** was slightly lower than that of its non-methylated congener (**14a**). In addition, no clear trend could be found when the *N*-methylated compounds **15b** (which carries a 2-tolyl moiety) and **15c** were compared with their respective non-methylated precursors **14b** and **14c**.

Interestingly, *N*-methylation deeply affected the general performance of the 3-trifluoromethylated compound (**15d** vs **14d**), which became inactive against both, HEP-2 and NCI-H292 cells, and exhibited low activity against the MCF-7 cell line. Conversely, *N*-methylation notably improved the potency profile of **14e**. The resulting chloro-derivative **15e** displayed good potency against MCF-7, while being highly active against the other cells. The profile of activity of **15e** was very similar to that of the *N*-phenyl derivative **13a**, carrying no substituent on C-2, being a strong improvement over that of **15a**, unsubstituted on the heterocyclic nitrogen atom.

In order to demonstrate that the cytotoxic effects presented by the substituted indoles do not relate to unspecific cellular membrane disruption, and as a measure of their toxicity to mammalian cells, the hemolytic activity of the 3-thiocyanato-1*H*-indole derivatives was assessed, against the highly sensitive human erythrocytes. The release of hemoglobin was monitored by measuring the absorbance at 540 nm, against negative (erythrocytes in saline solution, without test compound) and positive (0.1% w/v Triton X-100 in saline solution) controls.

No hemolytic activity was observed among the compounds, under test concentrations up to 500 μM, suggesting that the mechanism of cytotoxicity is not a result of membrane damage. As a result of this screen, it may be concluded that the whole cell panel was more sensitive only to the *N*-phenyl indole **13a** and the *N*-methylated 2(4-chlorophenyl) derivative **15e**, whereas compounds **13e** [3(4-chlorophenyl)], **14a** (2-phenyl) and **14e** [2(4-chlorophenyl)] can also be considered promising compounds, displaying suitable potency against at least three of the cell lines.

3. Conclusions

In summary, a series of 3-thiocyanato-1*H*-indole derivatives,

carrying three different points of diversification, has been designed and synthesized, following simple and straightforward protocols. All the synthesized compounds were spectroscopically characterized and screened for their growth inhibitory activity against a panel of four different human cancer cell lines, including HL60, NCI-H292, Hep-2 and MCF-7.

Indole (**24**), *N*-methylindole (**25**) and 2-(4-chlorophenyl)-*N*-methylindole (**23e**) were essentially inactive, while all of the 3-thiocyanato-1*H*-indole derivatives displayed good to high potency toward at least one of the test cells, suggesting that the thiocyanate moiety plays a key role in the observed bioactivity. Further, many of the tested compounds exhibited interesting levels of growth inhibitory activity against the whole panel and all of them demonstrated to be non-lytic at doses >500 μM in the hemolytic activity test. The 3-thiocyanato-1*H*-indoles **13a** (*N*-phenyl) and **15e** [*N*-methylated 2(4-chlorophenyl) derivative] showed the best profile of potency.

Against HL60, they were 55–71.5 times less potent than doxorubicin; however, confronted with the HEP-2, NCI-H292 and MCF-7 cells, the most active compounds were only 2.2–6.8 times less potent than this reference compound.

These results, which suggest that the 3-thiocyanato-1*H*-indole motif can be used as a versatile and valuable scaffold to afford potent cytotoxic compounds, represent an important advance in this field. Therefore, with the hypothesis that suitably decorated 3-thiocyanato-1*H*-indole can afford more potent compounds, further studies involving redesign of the active moieties are in progress; their results will be disclosed in due time.

4. Experimental section

4.1. In vitro cytotoxicity

4.1.1. Cell lines

The tumor cell lines employed, HL60 (human promyelocytic leukemia), MCF-7 (human breast adenocarcinoma), NCI-H292 (human lung cancer), and HEP-2 (human cervical carcinoma) were obtained from the cell bank of Rio de Janeiro, Brazil, and were maintained at 37 °C with 5% CO_2 in RPMI 1640 or DMEM media supplemented with 10% fetal bovine serum and 1% antibiotics.

4.1.2. Cytotoxicity testing

The MTT analysis method, based on the conversion of 3-(4,5-dimethyl-2-thiazole)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) to blue formazan by the mitochondrial enzymes present only in metabolically active cells, was employed [28]. The NCI-H292, HEP-2 and MCF-7 cells were seeded at 1×10^5 cells/mL in 96-well plate, while the HL60 cell line was seed at 3×10^5 cells/mL. All compounds were solubilized in DMSO and diluted with culture medium to achieve a final concentration of 1% DMSO.

The plates were incubated for 72 h at 37 °C in an incubator containing a 5% CO_2 atmosphere. After 72 h, 25 μL of a MTT solution was added to each well, and the plates were incubated for additional 3 h. Subsequently, the media was removed, the precipitate was dissolved in DMSO, and the absorbance was read in a plate reader, at 595 nm. Each sample was tested in duplicate. The 50% inhibitory concentrations (IC_{50}) and their 95% confidence intervals were calculated by non-linear regression using GraphPad Prism.

4.1.3. Hemolytic assay

Some compounds are cytotoxic due to unspecific cell membrane damage which led to immediate cell death. In order to exclude compounds with direct cell membrane damage properties we used the hemolytic assay.

The hemolytic assay was performed with erythrocytes obtained from peripheral blood of healthy volunteers. The protocol was approved by the Ethics Committee of the Federal University of Pernambuco. The erythrocytes were separated from plasma by centrifugation at 1500 rpm for 5 min. For the experiment 100 μL of a 2% erythrocytes suspension in 0.85% NaCl containing 10 mM $CaCl_2$ was added at each well.

A solution of Triton X-100 (1% w/v) was used as positive control (100%); saline (0.85% NaCl containing 10 mM $CaCl_2$) was employed as a negative control (blank) and the samples were tested at a concentration of 500 μM. After incubation at room temperature for 1 h the supernatant was removed and the liberated hemoglobin was measured spectrophotometrically as the absorbance at 540 nm. Each sample was tested in triplicate. Compounds with EC_{50} > 500 μM were considered non-hemolytic. The percentage of hemolysis [Hemolysis (%)] was calculated using Eq. (1)

$$\text{Hemolysis (\%)} = [(A_{sample} - A_{blank})/(A_{100\%} - A_{blank})] \times 100 \qquad (1)$$

where A_{sample}, A_{blank} and $A_{100\%}$ are the absorbances of the sample, and the negative and positive controls, respectively [29].

Acknowledgments

The authors are grateful to FAPERGS, CNPq (P. 473139/2013-8) and CAPES for financial support and scholarships. TSK also thanks ANPCyT (PICT 2014-0445), CONICET and SECTeI.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.04.039.

References

[1] a) M.E. Welsch, S.A. Snyder, B.R. Stockwell, Privileged scaffolds for library design and drug discovery, Curr. Opin. Chem. Biol. 14 (2010) 347–361;
b) R.W. DeSimone, K.S. Currie, S.A. Mitchell, J.W. Darrow, D.A. Pippin, Privileged structures: applications in drug discovery, Comb. Chem. High. Throughput Screen 7 (2004) 473–494.

[2] a) M. Bandini, A. Eichholzer, Catalytic functionalization of indoles in a new dimension, Angew. Chem. Int. Ed. 48 (2009) 9608–9644;
b) F.R. de Sá Alves, E.J. Barreiro, C.A. Fraga, From nature to drug discovery: the indole scaffold as a 'privileged structure', Mini Rev. Med. Chem. 9 (2009) 782–793.

[3] a) R.J. Sundberg, The Chemistry of Indoles. Best Synthetic Methods Series, Academic Press, New York, 1996;
b) H. Johansson, T. Bøgeløv Jørgensen, D.E. Gloriam, H. Bräuner-Osborne, D.S. Pedersen, 3-Substituted 2-phenyl-indoles: privileged structures for medicinal chemistry, RSC Adv. 3 (2013) 945–960;
c) M. Inman, C.J. Moody, Indole synthesis – something old, something new, Chem. Sci. 4 (2013) 29–41.

[4] M. Sisa, D. Pla, M. Altuna, A. Francesch, C. Cuevas, F. Albericio, M. Alvarez, Total synthesis and antiproliferative activity screening of (±)-aplicyanins A, B and E and related analogues, J. Med. Chem. 52 (2009) 6217–6223.

[5] Y. Radha, A. Manjula, B. Madhava Reddy, B. Vittal Rao, Synthesis and biological activity of novel benzimidazoles, Indian J. Chem. B 50 (2011) 1762–1773.

[6] B.-C. Hong, Y.-F. Jiang, Y.-L. Chang, S.-J. Lee, Synthesis and cytotoxicity studies of cyclohepta[*b*]indoles, benzo[6,7]cyclohepta[1,2-*b*]indoles, indeno[1,2-*b*]indoles, and benzo[*a*]carbazoles, J. Chin. Chem. Soc. 53 (2006) 647–662.

[7] S. Mahboobi, A. Sellmer, E. Eichhorn, T. Beckers, H.-H. Fiebig, G. Kelter, Synthesis and cytotoxic activity of 2-acyl-1*H*-indole-4,7-diones on human cancer cell lines, Eur. J. Med. Chem. 40 (2005) 85–92.

[8] a) H. He, J.D. Faulkner, J.S. Shumsky, K. Hong, J. Clardy, A sesquiterpene thiocyanate and three sesquiterpene isothiocyanates from the sponge *Trachyopsis aplysinoides*, J. Org. Chem. 54 (1989) 2511–2514;
b) A.T. Pham, T. Ichiba, W.Y. Yoshida, P.J. Scheuer, T. Uchida, J. Tanaka, T. Higa, Two marine sesquiterpene thiocyanates, Tetrahedron Lett. 52 (1991) 4843–4846.

[9] a) A. Srikrishna, S.J. Gharpure, Enantiospecific total synthesis of both enantiomers of 2-thiocyanato neopupukeanane from (*R*)-carvone, J. Chem. Soc. Perkin Trans. 1 (2000) 3191–3193;
b) N. Fusetani, H.J. Wolstenholme, K. Shinoda, N. Asai, S. Matsunaga, H. Onuki, H. Hirota, Two sesquiterpene isocyanides and a sesquiterpene thiocyanate from the marine sponge *Acanthella cf. cavernosa* and the nudibranch *Phyllidia*

ocellata, Tetrahedron Lett. 33 (1992) 6823–6826.
[10] R.J. Capon, C. Skene, E.H.-T. Liu, E. Lacey, J.H. Gill, K. Heiland, T. Friedel, The isolation and synthesis of novel nematocidal dithiocyanates from an australian marine sponge *Oceanapia sp.* J. Org. Chem. 66 (2001) 7765–7769.
[11] C. Jiménez, P. Crews, Novel marine sponge derived amino acids 13. Additional psammaplin derivatives from *Psammaplysilla purpurea*, Tetrahedron 47 (1991) 2097–2102.
[12] M. Burow, A. Bergner, J. Gershenzon, U. Wittstock, Glucosinolate hydrolysis in *Lepidium sativum* – identification of the thiocyanate-forming protein, Plant Mol. Biol. 63 (2007) 49–61.
[13] S. Dutta, H. Abe, S. Aoyagi, C. Kibayashi, K.S. Gates, DNA damage by fasicularin, J. Am. Chem. Soc. 127 (2005) 15004–15005.
[14] S.M. Weinreb, Studies on total synthesis of the cylindricine/fasicularin/lepadiformine family of tricyclic marine alkaloids, Chem. Rev. 106 (2006) 2531–2549.
[15] a) F. Yan, R.V. Bikbulatov, V. Mocanu, N. Dicheva, C.E. Parker, W.C. Wetsel, P.D. Mosier, R.B. Westkaemper, J.A. Allen, J.K. Zjawiony, B.L. Roth, Structure-based design, synthesis, biochemical and pharmacological characterization of novel salvinorin a analogues as active state probes of the κ-opioid receptor, Biochemistry 48 (2009) 6898–6908;
b) A.K. Gadad, M.N. Noolvi, R.V. Karpoormath, Synthesis and anti-tubercular activity of a series of 2-sulfonamido/trifluoromethyl- 6-substituted imidazo [2,1-*b*]-1,3,4-thiadiazole derivatives, Bioorg. Med. Chem. 12 (2004) 5651–5659.
[16] G. García Liñares, S. Gismondi, N. Osa Codesido, S.N.J. Moreno, R. Docampo, J.B. Rodríguez, Fluorine–containing aryloxyethyl thiocyanate derivatives are potent inhibitors of *Trypanosoma cruzi* and *Toxoplasma gondii* proliferation, Bioorg. Med. Chem. Lett. 17 (2007) 5068–5071.
[17] a) D.M. Cottrell, J. Capers, M.M. Salem, K. DeLuca-Fradley, S.L. Croft, K.A. Werbovetz, Antikinetoplastid activity of 3-aryl-5-thiocyanatomethyl-1,2,4-oxadiazoles, Bioorg. Med. Chem. 12 (2004) 2815–2824;
b) C. Havens, N. Bryant, L. Asher, L. Lamoreaux, S. Perfetto, J. Brendle, K. Werbovetz, Cellular effects of leishmanial tubulin inhibitors on *L. donovani*, Mol. Biochem. Parasitol. 110 (2000) 223–236.
[18] a) R.-L. Bai, C. Duanmu, E. Hamel, Mechanism of action of the antimitotic drug 2,4-dichlorobenzyl thiocyanate: alkylation of sulfhydryl group(s) of β-tubulin, Biochim. Biophys. Acta 994 (1989) 12–20;
b) M. Saarivirta, The formation of benzylcyanide, benzylthiocyanate, benzylisothiocyanate and benzylamine from benzylglucosinolate in *Lepidium*, Planta Med. 24 (1973) 112–119.
[19] S. Kumar, V. Gopalakrishnan, M. Hegde, V. Rana, S.S. Dhepe, S.A. Ramareddy, A. Leoni, A. Locatelli, R. Morigi, M. Rambaldi, M. Srivastava, S.C. Raghavan, S.S. Karki, Synthesis and antiproliferative activity of imidazo[2,1-*b*][1,3,4] thiadiazole derivatives, Bioorg. Med. Chem. Lett. 24 (2014) 4682–4688.
[20] P. Sai Prathima, P. Rajesh, J. Venkateswara Rao, U. Sai Kailash, B. Sridhar, M. Mohan Rao, "On water" expedient synthesis of 3-indolyl-3-hydroxy oxindole derivatives and their anticancer activity in vitro, Eur. J. Med. Chem. 84 (2014) 155–159.
[21] a) Q. Guan, C. Han, D. Zuo, M. Zhai, Z. Li, Q. Zhang, Y. Zhai, X. Jiang, K. Bao, Y. Wu, W. Zhang, Synthesis and evaluation of benzimidazole carbamates bearing indole moieties for antiproliferative and antitubulin activities, Eur. J. Med. Chem. 87 (2014) 306–315;
b) H.E. Colley, M. Muthana, S.J. Danson, L.V. Jackson, M.L. Brett, J. Harrison, S.F. Coole, D.P. Mason, L.R. Jennings, M. Wong, V. Tulasi, D. Norman, P.M. Lockey, L. Williams, A.G. Dossetter, E.J. Griffen, M.J. Thompson, An orally bioavailable, indole-3-glyoxylamide based series of tubulin polymerization inhibitors showing tumor growth inhibition in a mouse xenograft model of head and neck cancer, J. Med. Chem. 58 (2015) 9309–9333.
[22] a) M.N. Noolvi, H.M. Patel, N. Singh, A.K. Gadad, S.S. Cameotra, A. Badiger, Synthesis and anticancer evaluation of novel 2-cyclopropylimidazo[2,1-*b*] [1,3,4]-thiadiazole derivatives, Eur. J. Med. Chem. 46 (2011) 4411–4418;
b) S.S. Karki, K. Panjamurthy, S. Kumar, M. Nambiar, S.A. Ramareddy, K.K. Chiruvella, S.C. Raghavan, Synthesis and biological evaluation of novel 2-aralkyl-5-substituted-6-(4′-fluorophenyl)-imidazo[2,1-*b*][1,3,4]thiadiazole derivatives as potent anticancer agents, Eur. J. Med. Chem. 46 (2011) 2109–2116.
[23] a) P. Rhoennstad, E. Kallin, T. Apelqvist, M. Wennerstaal, A. Cheng, Novel estrogen receptor ligands, Patent WO2009/127686, 2009;
b) S. Feng, L. Gao, D. Hong, L. Wang, H. Yun, S.-H. Zhao, Novel indazoles for the treatment and prophylaxis of respiratory syncytial virus infection, Patent WO 2014/9302, 2014;
c) T. Williams, X.-F. Zhang, Non-nucleoside reverse transcriptase inhibitors, Patent WO 2007/021629, 2007.
[24] a) G.M. Martins, G. Zeni, D.F. Back, T.S. Kaufman, C.C. Silveira, Expedient iodocyclization approach toward polysubstituted 3*H*-benzo[*e*]indoles, Adv. Synth. Catal. 357 (2015) 3255–3261;
b) M.M. Bassaco, M.P. Fortes, T.S. Kaufman, C.C. Silveira, Metal-free synthesis of 3,5-disubstituted 1*H*- and 1-aryl-1*H*-pyrazoles from 1,3-diyne-indole derivatives employing two successive hydroaminations, RSC Adv. 5 (2015) 21112–21124;
c) M.M. Bassaco, M.P. Fortes, D.F. Back, T.S. Kaufman, C.C. Silveira, An eco–-friendly synthesis of novel 3,5-disubstituted-1,2-isoxazoles in PEG-400, employing the Et3N-promoted hydroamination of symmetric and unsymmetric 1,3-diyne-indole derivatives, RSC Adv. 4 (2014) 60785–60797;
d) C.C. Silveira, S.R. Mendes, M.A. Villetti, D.F. Back, T.S. Kaufman, Ce^{III}-promoted oxidation. efficient aerobic one-pot eco-friendly synthesis of oxidized bis(indol-3-yl)methanes and cyclic tetra(indolyl) dimethanes, Green Chem. 14 (2012) 2912–2921.
[25] a) M.P. Fortes, M.M. Bassaco, T.S. Kaufman, C.C. Silveira, A convenient eco-friendly system for the synthesis of 5-sulfenyl tetrazole derivatives of indoles and pyrroles employing $CeCl_3.7H_2O$ in PEG-400, RSC Adv. 4 (2014) 34519–34530;
b) B.L. Kuhn, M.P. Fortes, T.S. Kaufman, C.C. Silveira, Facile, efficient and eco-friendly synthesis of 5-sulfenyl tetrazole derivatives of indoles and pyrroles, Tetrahedron Lett. 55 (2014) 1648–1652.
[26] a) M.T. Baumgartner, M.A. Nazareno, M.C. Murguía, A.B. Pierini, R.A. Rossi, Reaction of *o*-iodoaniline with aromatic ketones in DMSO. Synthesis of 2-aryl or 2-hetaryl indoles, Synthesis (1999) 2053–2056;
b) S.M. Barolo, A.E. Lukach, R.A. Rossi, Syntheses of 2-substituted indoles and fused indoles by photostimulated reactions of *o*-iodoanilines with carbanions by the $S_{RN}1$ mechanism, J. Org. Chem. 68 (2003) 2807–2811.
[27] a) I.S. Chikvaidze, N.N. Barbakadze, S.A. Samsoniya, Some new derivatives of 5-aryl-, 2,5-diaryl- and 2-ethoxycarbonyl-5-aryl-indoles, Arkivoc vi (2012) 143–154;
b) V. Aksenov, A.N. Smirnov, I.V. Magedov, M.R. Reisenauer, N.A. Aksenov, I.V. Aksenova, A.L. Pendleton, G. Nguyen, R.K. Johnston, M. Rubin, A. De Carvalho, R. Kiss, V. Mathieu, F. Lefranc, J. Correa, D.A. Cavazos, A.J. Brenner, B.A. Bryan, S. Rogelj, A. Kornienko, L.V. Frolova, Activity of 2-aryl-2-(3-indolyl) acetohydroxamates against drug-resistant cancer cells, J. Med. Chem. 58 (2015) 2206–2220.
[28] a) P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. Mcmahon, D. Vistica, J.T. Warren, H. Bodesch, S. Kenney, M.R. Boyd, New colorimetric cytotoxicity assay for anticancer – drug screening, J. Nat. Cancer Inst. 82 (1990) 1107–1112;
b) T. Mossman, Rapid colorimetric assay for cellular growth and survival. Application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63.
[29] L.V. Costa-Lotufo, G.M.A. Cunha, P.A.M. Farías, G.S.B. Viana, K.M.A. Cunha, C. Pessoa, M.O. Moraes, E.R. Silveira, N.V. Gramosa, V.S.N. Rao, The cytotoxic and embriotoxic effects of kaurenoic acid, a diterpene isolated from *Copaifera langsdorfii* óleo-resin, Toxicon 40 (2002) 1231–1234.