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Research Article

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Trypanocidal activity of a thioacyl-thiosemicarbazide derivative associating both immunostimulating thalidomide and anti-parasitic thiosemicarbazide pharmacophores

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ABSTRACT

African trypanosomiasis remains a life-threatening disease and there is nowadays an urgent need for improved therapeutic agents for this pathology. In this context, in order to create novel anti-protozoa prototype containing both a trypanocidal thiosemicarbazide moiety and an immuno-potentiating thalidomide-like, a hybrid structure was designed on the basis of a convergent synthesis process, synthesized and assayed for its potential trypanocidal activity. Initial biological results are very promising. The structure of the target compound was ascertained on the basis of ^{13}C-NMR, IR spectroscopy and semi-empirical AM1/PM3 quantum-mechanical calculations.

Keywords: Trypanocidal activity, immunostimulating thalidomide, thioacyl-thiosemicarbazide, thiosemicarbazide pharmacophore, Schotten-Baumann thioacylation reaction.

INTRODUCTION

African trypanosomiasis sometimes also referred to as sleeping sickness is a parasitic disease affecting humans and other animals. It is caused by a protozoa of the species *Trypanosoma brucei*. There are two types that can infect humans, *Trypanosoma brucei gambiense (T.b.g)* and *Trypanosoma brucei rhodesiense* (*T.b.r.*) [1] *T.b.g* causes over 98% of the reported cases. Both are usually transmitted by the bite of an infected tsetse fly. The disease is endemic in several regions of sub-Saharan Africa with the population at risk being about 70 million in 36 countries. As of 2010, it caused around 9,000 deaths per year. An estimated 30,000 people are currently infected with some 7000 new infections in 2012. [1, 2] Treatment of the first stage is performed using medications such as pentamidine or suramin. [1] Treatment of the second stage involves, eflornithine or a combination of nifurtimox and eflornithine for *T.b.g.* [3]. While melarsoprol works for both, it is typically only used for *T.b.r.* due to serious side effects [1]. These treatments are expensive and indeed often accompanied by severe side-effects. There is therefore an urgent need for improved therapeutic agents to eradicate this life-threatening parasite.

The design of our target molecule was based on the following premise. On the one hand, thiosemicarbazides, thiosemicarbazones as well as their metal complexes have been extensively studied over the recent years due to their wide variety of biological activities [1-16]. Certain drugs show even enhanced pharmacological potency when administered as their metal chelates presumably owing to their higher bioavailability under such a form. On the other hand, the phthalimide pharmacophore, as found for example in thalidomide, [17-22] appears to be a highly

druggable pharmacophore. Numbers of phthalimide derivatives have been synthesized with interesting biological activities [23-27]. Based on the canonical structure of Relacatib [28] used for the treatment of malaria, some antitrypanosomial molecules were successfully designed incorporating indeed the phthalimide pharmaphoric template (*Cfr* Figure **1**).

EXPERIMENTAL SECTION

General conditions

Melting points were determined using an electrothermal melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 457 spectrometer using KBr dispersion disks. Wave numbers are expressed in cm-1. ^{1}H- and ^{13}C-NMR spectrum was recorded at ambient temperature on a Bruker 400 spectrometer. Compounds were dissolved in $CDCl_3$ or DMSO-d_6. Chemical shifts are expressed in the δ scale with TMS (tetramethylsilane) as internal standard. Thin layer chromatography (TLC) analyses were performed on Merck TLC plates (silica gel, 60 F 254, E. Merck, Darmstadt, Germany, ref. 5735). For TLC, all the compounds reported were routinely checked in two standard solvents, *i. e.* acetone/toluene/cyclohexane (solvent A, 5:2:3, v/v/v) and ethyl acetate/n-hexane (solvent B, 4:6, v/v). The reverse-phase thin layer chromatography conditions were: HPTLC plates RP-18 F-254 S (Merck), methanol: water (75/25, v/v). All compounds reported were found homogenous under such TLC and HPTLC conditions. All reagents were purchased from Aldrich (Milwaukee, Wisconsin, USA). All solvents were of the ACS reagent grade (Aldrich).

(R, S)-2-(1, 3-dioxoisoindolin-2-yl)-2-phenylacetonitrile (2)

A solution of mandelonitrile (13.3 g, 0.1 mol) in absolute ethanol (100 mL) was saturated for 1 h with a steady stream of ammonia gas. After 24h, the solvent was removed *in vacuo* using a rotary evaporator to give a reddish oily residue which was immediately treated with phthalic anhydride (14.8 g, 0.1mol) dissolved in glacial acetic acid (100 ml). A sample of the above oil on standing in a refrigerator gave crystals (mp = 56-57°C). The reaction medium was stirred and refluxed for 4 h, allowed to cool at room temperature and treated with 500 mL of ice-cold water to give a precipitate, which was collected on a Büchner funnel and washed first with cold distilled water and then with diethyl ether. After drying, this crystalline material was recrystallized from 95% ethanol to give long white needles (yield = 75 %; mp = 126-127°C). IR (KBr) 3090, 3020, 2240 (CN), 1770, 1740 (phthalimido C=O), 1610 cm-1. ^{1}H-NMR (DMSO-d6) 7.90-8.15 (m, 4H, phthalimido H), 7.51-7.06 (m, 5H, benzyl aromatic H), 6.90 (s, ^{1}H, CH). ^{13}C-NMR (DMSO-d6) 168.25, 168.07 (phthalimido C=O), 133.74 (*ipso* aromatic C), 127.62-132.28 (6 peaks, aromatic CH), 116.40 (nitrile C), 39.46 (CH).

(R,S)Benzyl2-(1,3-dioxoisoindolin-2-yl)-phenylethanedithioate (3)

A clear solution of (R,S)-2-(1,3-dioxoisoindolin-2-yl)-2-phenylacetonitrile (2.62 g, 10 mmol) and phenylmethane thiol (2.48 g, 20 mmol) in dried dichloromethane (freshly redistilled from phosphorous pentoxide, 25 mL) maintained between -10°C - 0°C was saturated with dry hydrogen chloride gas for 1hr and the resulting reaction medium was kept in a refrigerator for 48h after which time it was evaporated *in vacuo* without external heating. The residue so obtained was treated at room temperature with dry pyridine (10 mL) beforehand saturated with dry hydrogen sulfide and dried triethylamine (TEA, redistilled from KOH, 0.5 mL). The resulting solution which rapidly turned from light yellow to a deep red color was treated for 4h by a steady stream of hydrogen sulfide dried by passing it through a short column packed with anhydrous calcium chloride. The reaction mixture was kept overnight in a refrigerator and filtered from insoluble material with a Büchner funnel. The resulting solution was poured onto ice, triturated with a glass rod to give a yellow precipitate which was recrystallized from 95 % ethanol to give 1.41 g of the title compound (35 % yield) as a deep canary yellow crystalline material. Mp = 120 - 122°C (lit. mp = 121 - 122°C). ^{1}H-NMR ($CDCl_3$) 8.3 - 7.2 (14 H, unresolved mult.), 4.95 (1H, s), 4.50 (2H, s) ppm; ^{13}C-NMR 229.81 (dithioester C=S), 168.29 and 165.43 (phthalimido C=0), 127.24 - 132.87 (9 peaks, aromatic CH), 139.62 and 143.10 (aromatic ipso carbons), 71.15 (CH), 42.66 (CH_2); IR (KBr) 3090, 2920, 1780, 1725 cm-1.

(R,S)-1-(2-(1,3-Dioxoisoindolin-2-yl)-2-phenylethanethioyl)-4-phenylthiosemicarbazide (1)

A solution of the above compound (350 mg, 0.86 mmol) and 4-phenylthiosemicarbazide (500 mg, 2.98 mmol) in analytical grade methanol (20 mL) was refluxed for 24h until TLC analysis indicated total consumption of the starting dithioester reagent. Simultaneously, we observed that the bright yellow color of the solution was slowly fading. The resulting solution was then kept in a refrigerator for several days until crystallization was complete. The light grey white-off crystalline material was then collected on a Büchner funnel and washed with a small amount of cold diethylether to remove any unpleasant odor of phenylmethanethiol left over. This material was recrystallized from a small amount of 95 % ethanol to give 320 mg of the title compound (yield = 83%) as a white crystalline material. ^{13}C-NMR 179.37 (enolized thiohydrazide C=S), 166.98 and 166.04 (phthalimido C=0), 156.30 (thiosemicarbazide C=S), 139.25 and 145.05 (aromatic *ipso* carbons), 116.76 - 131.02 (9 peaks, aromatic CH), 52.61 (CH) ppm; IR (KBr) 3080, 2910, 1780, 1725 cm-1.

***N*-Thiobenzoyl-4-phenylthiosemicarbazide (4)**

A solution of 4-phenylthiosemicarbazide (1.65g, 10 mmol) and a slight excess of S-thiobenzoyl-thioglycolic acid (2.15g, 10.2 mmol) in 70 ml of methanol was stirred and refluxed for 24h. The bright red colour of the dithioester was slowly discharged. The reaction mixture which was kept overnight in a refrigerator deposited the title compound as a nice yellow crystallization from ethanol. ^{13}C-NMR (DMSO-d6) 164.07(enolized thiobenzoyl C=S), 157.52 (enolized thioureide C=S), 140.53, 130.30, (*ipso* C), 130.19, 129.23, 129.11, 126.74, 122.65, 117.50. The product gave a positive yellow color test upon exposure to an aqueous $FeCl_3$ solution.

RESULTS AND DISCUSSION

Based on the implementation of these two molecular requirements in a single molecular entity, we designed a hybrid molecule containing both molecular recognition elements in the hope to create a novel anti-protozoa molecule associating both the trypanocidal thiosemicarbazide moiety and an immuno-potentiating activity relying on the thalidomide-like structure (*cfr* Figure **1**).

The approach of summing up two pharmacophores to generate a new hybrid chemical entity is often called "convergent synthesis" in drug design. Along this line, this paper reports our efforts to synthesize the target molecule **(1)**, its spectral characterization along with a first pharmacological evaluation based on some validated classical biological assays.

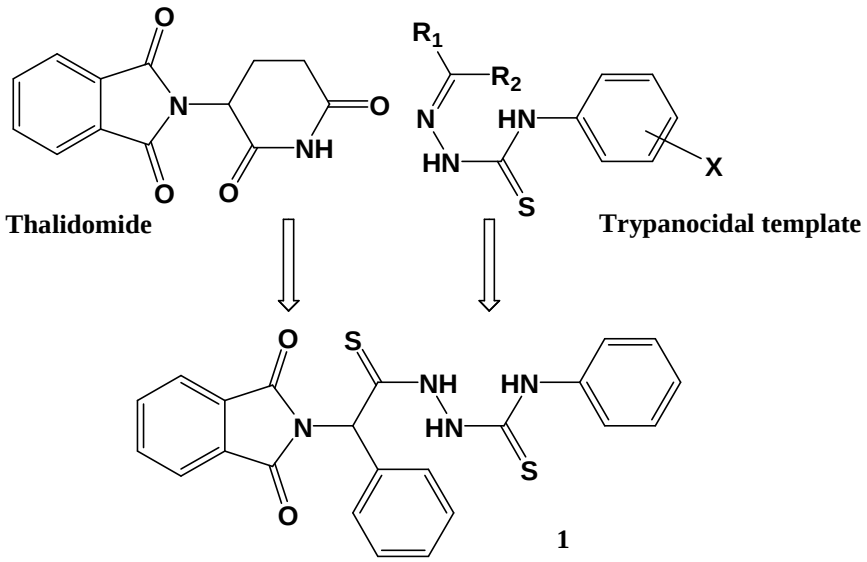


Figure 1: Design of our target hybrid molecule conceived from the immuno-stimulating thalidomide and trypanocidal arylthiosemicarbazone templates

Based on the exceptional reactive characteristics of the nitrogen in 1-position of 4-phenylthiosemicarbazide, it was anticipated that aminolysis of a thioacylating species such as *S*-benzyl *N*-phthalimidodithiophenylglycinate would readily take place. We had then to design a straightforward access to the dithioester species. This was accomplished using a pathway already delineated by Poupaert *et al*. in the 1970's [18]. Treatment of mandelonitrile by gas ammonia in absolute ethanol followed by direct action of phthalic anhydride in acetic acid gave rise to the nitrile precursor. Under the carefully controlled conditions of the well-known thioimidate Pinner's synthesis and subsequent base-catalyzed thiohydrolysis of the imidothioester intermediate, we obtained in 67 % yield our thioacylating reagent as a fine canary yellow crystalline material. The benzyl dithioester was then treated by 4-phenylthiosemicarbazide to yield our target compound as a racemic modification. Unfortunately, in our hands, attempts to produce the single enantiomeric species of this compound starting from a resolved (*R*)-phenylglycine precursor proved unsuccessful.

It should be noted that our target compound represents a druggable platform in that it respects the nowadays commonly accepted Lipinski's ‘’Rule of Five ’’ [29]. In particular, the LogP is 4.39 (<5), the molecular mass is 446.54 (<500) and the molecule contains only 8 heteroatoms (< 10), among which 3 donor moieties. Moreover, as the number of rotatable bonds is inferior to 10 (8 actually) and the polar surface is under 140 Å^2, we can state that our target molecule conforms to Weber's rule, which suggests that this compound has a high probability of good oral bioavailability in rats and humans.

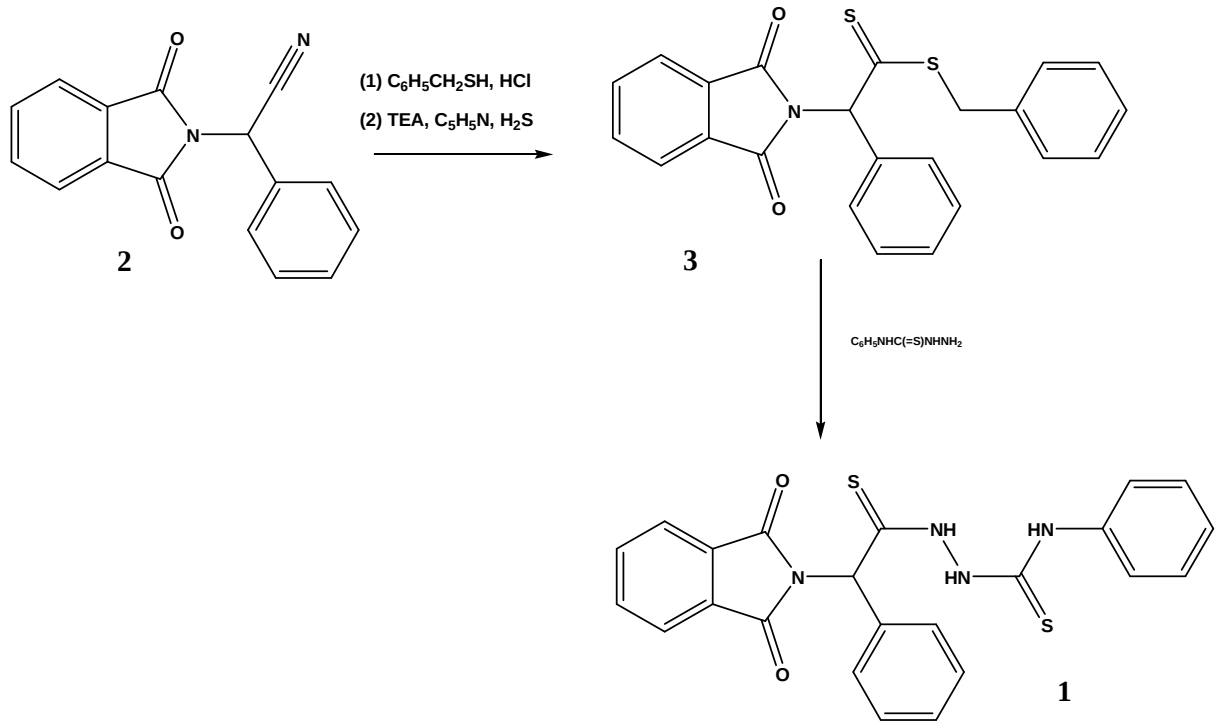


Figure 2. Access route to the trypanocidal target compound 1

As to the exact structure of our target compound, particularly perplexing was the ^{13}C-NMR spectrum which showed two resonances for C=S carbons at somewhat unexpected chemical shift figures. Simulation using ChemDraw prediction module gave a chemical shift of 203 ppm for the C=S in 2-position; however no signal could be found in this area of the actual spectrum. To solve this puzzling problem, we synthesized a simplified model compound featuring a concise representative of *N*-thioacylthiosemicarbazide (as illustrated in the Figure **3**) by reacting a thioacylating dithiobenzoic ester species with 4-phenylthiosemicarbazide in a schotten- baumann thioacylation process [30]. This model compound behaved as our target compound in that sense that it did not show any resonance in the forecasted region of the spectrum (around above 200 ppm). The only reasonable explanation we could think of, was that this exceptional feature was due potentially to extensive enolisation of the thiocarbonyl residues to form a conjugated system (Figure **4**). Examination of an ESP 3D-contour map obtained using semi-empirical quantum mechanics calculations (AM1 method) revealed the existence of two electron-rich regions, one featuring the phthalimide system (left-hand blue region) and another one displaying a large negative continuum (right-hand blue region) which is consistent with a large π-orbitals overlap between the two sulfur atoms (see illustration at Figure **3**).

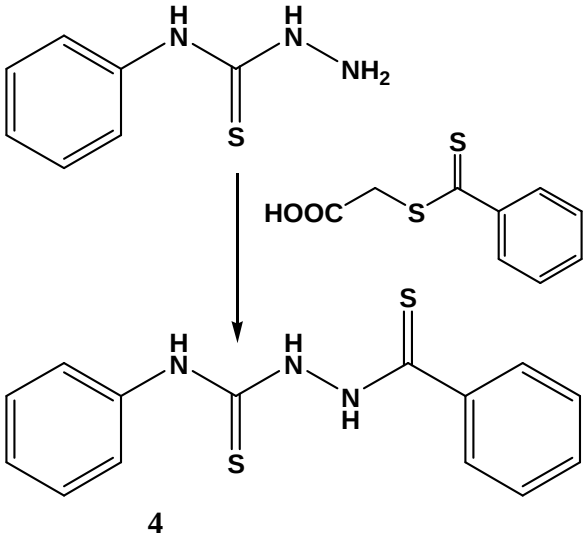
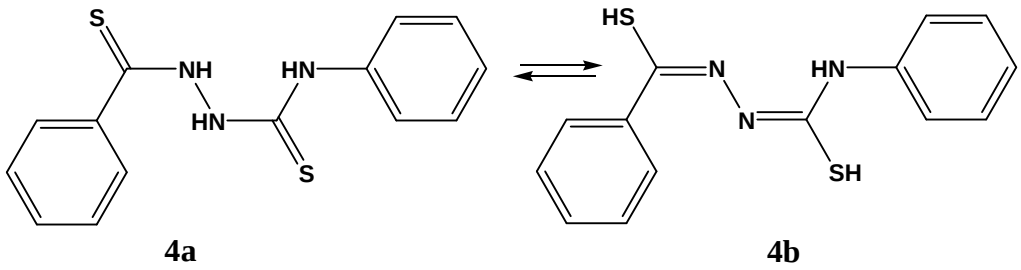


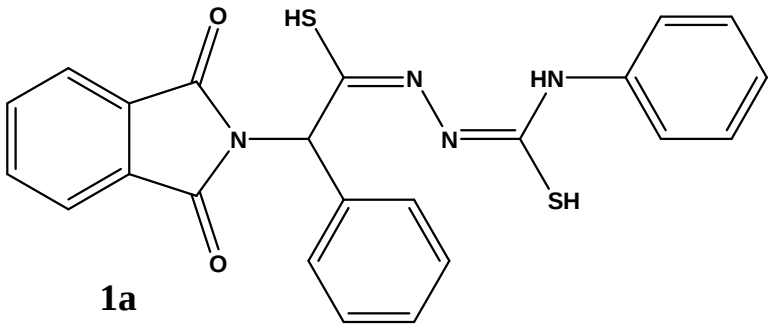
Figure 3: Synthesis of a model compound of *N*-thioacylthiosemicarbazide



4a **4b**

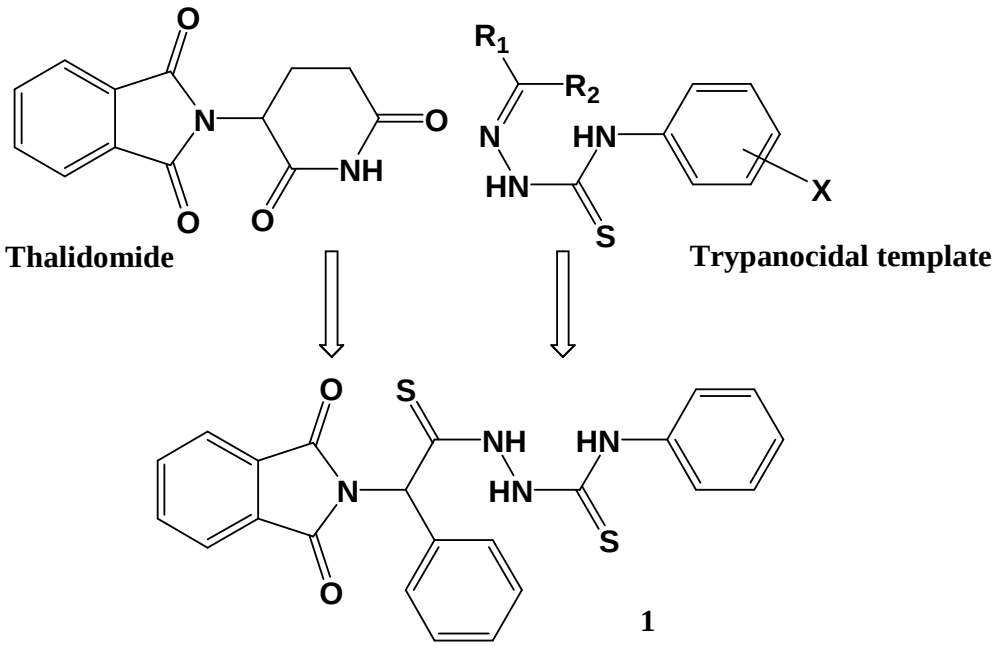
Figure 4: Tautomeric equilibrium between 4a & 4b

The ^{13}C-NMR spectrum of **1** can be thus rationalized in terms of a chemical exchange process, which refers to any process in which a nucleus exchanges between two or more environments in which its NMR parameters (*e.g.* chemical shift, scalar coupling, or relaxation) will differ. This description applies in particular to tautomeric equilibria. We are here typically facing a situation of fast chemical exchange between **1** and the enolized tautomeric **1a.** Consequently a single resonance is observed, whose chemical shift for the thione and sulfhydryl species is the weight average of the chemical shifts of the individual states. This situation is illustrated in the Figure **5.**



1a

Figure 5 : Tautomeric conjugated form 1a



1

Figure 1: Design of our target hybrid molecule conceived from the immuno-stimulating thalidomide and trypanocidal arylthiosemicarbazone templates

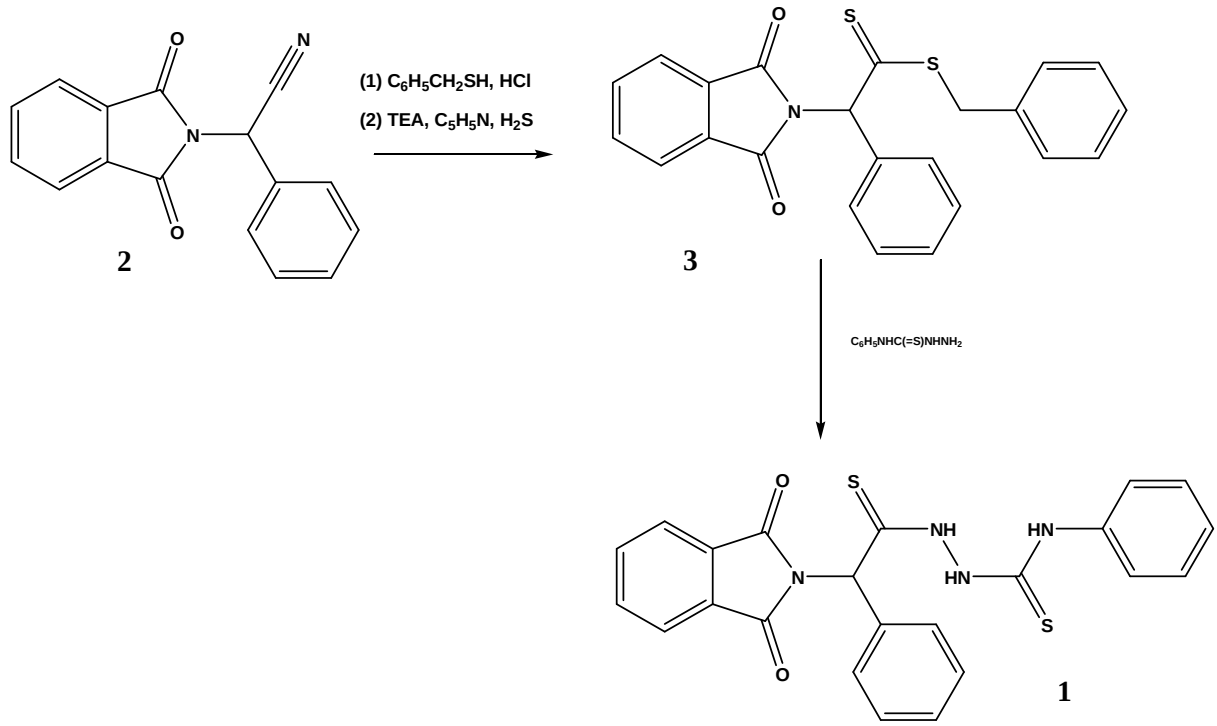


Figure 2. Access route to the trypanocidal target compound 1

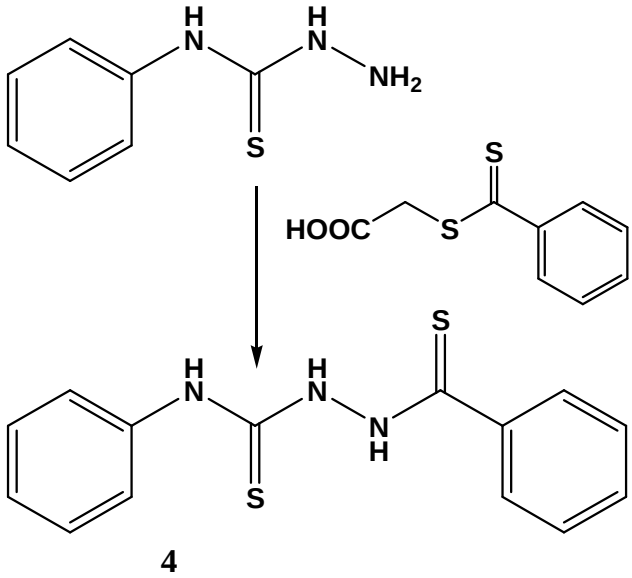


Figure 3: Synthesis of a model compound of *N*-thioacylthiosemicarbazide

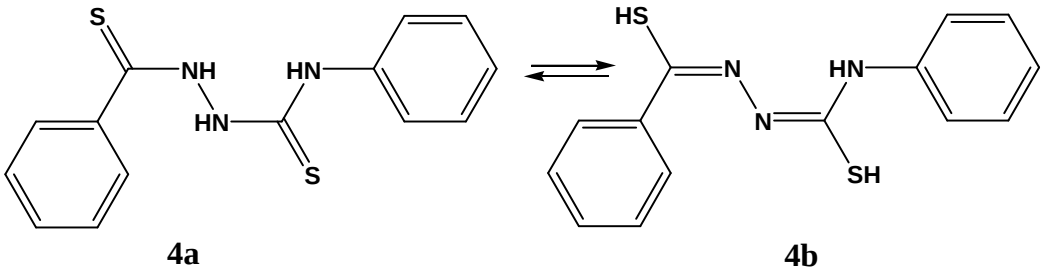
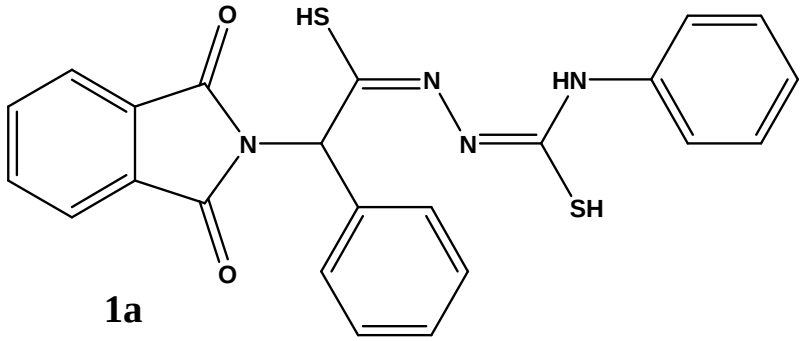


Figure 4: Tautomeric equilibrium between 4a & 4b



1a

Figure 5 : Tautomeric conjugated form 1a

CONCLUSION

A concise synthesis of a hybrid pharmacological probe was designed on the basis of the association of the phtalimide thalidomide's pharmacophore with that of 4-phenylthiosemicarbazide trypanocidal moiety in an effort to create a compound with reinforced activity in the treatment of the African trypanosomiasis. The strategy is based on a Schotten-Baumann thioacylation reaction. [30]

Our endeavours along this line were altogether successful as preliminary results were indicative that both IC_{50} concentrations against *T.b.r.* and in the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) tests were in the sub-micromolar range. Additional work is now being performed in the line of new chemotherapeutic treatments of this disease based on novel modes of action.

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The authors wish to acknowledge the generous contribution of the CTB/BTC (Coopération Technique Belge, Brussels, Belgium) both in terms of fellowship (U.C.K.) and financial support.

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