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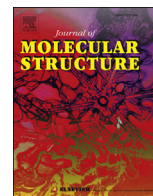
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## Synthesis and evaluation of new isatin-aminorhodanine hybrids as PIM1 and CLK1 kinase inhibitors

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### ABSTRACT

A series of new hybrid isatin-aminorhodanine molecules was prepared by microwave activation under mild conditions. Unexpected condensation of isatin derivatives at C-5 position of aminorhodanine was highlighted and confirmed by NMR and X-ray analyses. The inhibitory activity of these compounds was evaluated towards eight protein kinases, CDK's-2,-5,-9; PIM-1, CLK-1, Haspin, GSK3 $\beta$  and DYRK1A, whose signaling pathway dysregulation is associated to multifactorial diseases such as cancer, inflammatory, cardiovascular, and neurodegenerative diseases. Compound **31** bearing a bromo substituent has shown a potent and selective Pim1 kinase inhibitor activity.

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## 1. Introduction

Complex diseases are multifactorial disorders involving various potential targets associated with their pathogenesis. For instance, cardiovascular diseases, Alzheimer's disease, cancer, complications of diabetes, malaria, and tuberculosis imply an extremely complex multifactorial pathoetiology leading to a complex pathogenesis. As a result, conventional treatments are usually not effective and suffer major failures against these diseases due to the development of multidrug resistance, adverse drug reactions and the lack of clinical specificity of a targeted single drug treatment.

One of the alternatives of these conventional treatments consists on a new therapy based on the combination of two or more targeted therapeutic pharmacophores, with different mechanisms of action, into a single so-called hybrid molecule in the aim to create synergistic or additive pharmacological activities. The

molecular hybridization allows to access to compounds with improved affinity and efficiency and reduced side effects in comparison to the parent drugs. Furthermore, the selectivity profile and modes of action can be tuned out through a suitable molecular design.

Several numbers of successful examples of pharmacophore hybridization strategies for drug design, discovery and development have been reported [1]. Hybridization concept was adopted to treat cancer and other multifactorial diseases in order to limit the use of chemotherapeutic agents, which are often limited because of the chemoresistance and/or undesirable toxic effects caused by the destruction of nonspecific cells at an effective dose of drugs.

Isatin derivatives have been identified as natural scaffolds present in various traditional Chinese medicines with potent biological activity on different protein kinases [2] (Fig. 1). For example, isatin-based sunitinib maleate (Sutent<sup>®</sup>) was approved by the FDA for the treatment of advanced renal carcinoma and gastrointestinal stromal tumours. The hybrid anti-cancer agent, (Z)-N1-(3-((1H-pyrrol-2-yl)methylene)-2-oxindolin-5-yl)-N8-hydroxyoctanediamide, with an isatin fragment based on kinase and histone deacetylase inhibitors, was also reported as an effective potential chemical probe for angiogenesis [3]. The main molecular mechanism of isatin

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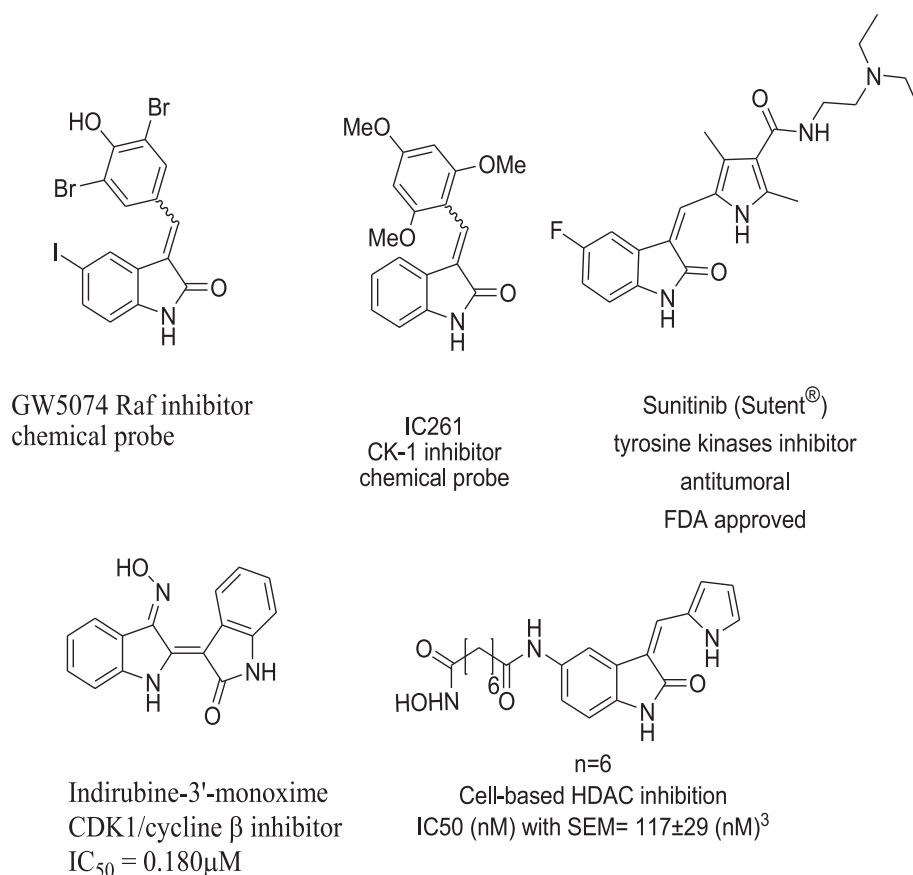


Fig. 1. Some isatin-based inhibitors.

derivatives is based on tyrosine kinase inhibition (TKIs) and inhibition of cyclin-dependent kinases (CDKs) by binding to the ATP pocket and/or caspase inhibition [4].

On the other hand, molecules bearing a rhodanine scaffold have been recognized as antineoplastic agents with a broad spectrum of activities against many cancer cell lines and several other biological activities [5] such as antidiabetic, antibacterial, antifungal, anti-convulsant, inflammatory and antiviral. The rhodanine derivatives are also known as inhibitors of many targets, such as HCV protease NS3 or NS5b [6] β-lactamase [7] UDP-N-acetylmuramate/L-alanine ligase, penicillin<sup>3</sup> binding protein, histidine decarboxylase [8] cyclooxygenase, 5-lipoxygenase, cathepsin D, mannosyl transferase protein and JNK transferase [9]. They exhibit also submicromolar inhibitory activity against PDE4 [6], Jnk Stimulatory Phosphatase-1 (JSP-1) [10] and Plasmodium falciparum Enoyl acyl Carrier Protein Reductase (PfENR) [11] (Fig. 2). Despite this, only the rhodanine-

based Epalrestat has been approved for the treatment of diabetic complications as aldose reductase inhibitor (Fig. 2) [12].

Although, some studies suggest that rhodanines are likely to be pan assay interference compounds (PAINS) due to their possible Michael acceptor functionality [13], experimental confirmations still essential and the positive aspect of such covalent modifiers was not be discarded as evidenced by numerous recent works reported in literature [14].

Recently, several isatin-rhodanine hybrids have been prepared and screened for their bioactivities. Some of them showed potent inhibitor activity against UDP-Galactopyranose Mutase UGM [15] (Fig. 3-A), β-lactamase [16] (Infectious disease) (Fig. 3-B), and Histone acetyltransferases (wide range of diseases) [17] (Fig. 3-C).

In light of these foregoing finding, we were interested on the design, synthesis and evaluation of a new series of hybrid molecules, by combining the two pharmacophoric elements, N-

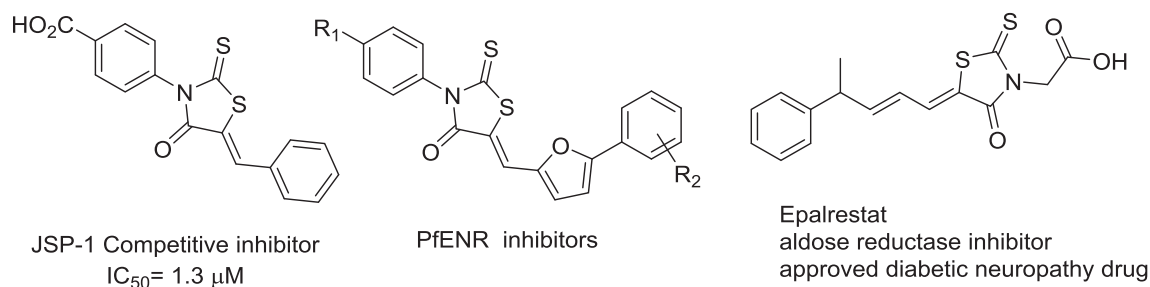


Fig. 2. Some rhodanine-based inhibitors.

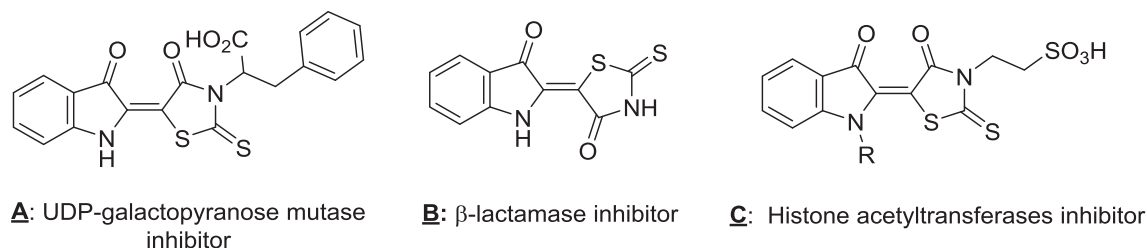


Fig. 3. Some biological active isatin-rhodanine hybrids.

aminorhodanine and isatin which are present in a number of natural and synthetic agents, in a single chemical framework. In this paper, we describe efforts towards the preparation, identification and preliminary evaluation as kinase inhibitors of new series of isatin-aminorhodanine hybrids.

## 2. Experimental section

### 2.1. Materials and methods

All reagents were purchased from Acros (Geel, Belgium), Aldrich (Saint Louis, MI, USA) and were used without further purification. Reactions under microwave irradiations were realized in the Monowave 300 Anton-Paar (Courtabœuf, France), using borosilicate glass vials of 10 mL equipped with snap caps (at the end of the irradiation, cooling reaction was realized by compressed air). The microwave instrument consists of a continuous focused microwave power output from 0 to 300 W. All the experiments were performed using a stirring option. The target temperature was reached with a ramp of 2 min and the chosen microwave power stayed constant to hold the mixture at this temperature. The reaction temperature is monitored using calibrated infrared sensor and the reaction time included the ramp period.

Melting points were determined on a Kofler melting point apparatus (Wagner & Munz, Munich, Germany), and were uncorrected.

$^1\text{H}$  NMR spectra were recorded on BRUKER AC 300P (300 MHz) spectrometer (Bruker, Bremen, Germany),  $^{13}\text{C}$  NMR spectra on BRUKER AC 300 P (75 MHz, Bruker) spectrometer. Chemical shifts are expressed in part per million downfield from tetramethylsilane as an internal standard. Data are given in the following order:  $\delta$  value, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), number of protons, coupling constants  $J$  is given in Hz.

High-resolution mass spectra (HMRS) were recorded on a Bruker Micro-Tof-Q II (Bruker) or on a Waters Q-Tof 2 at the CRMPO (Centre Régional de Mesures Physiques de l'Ouest, Rennes, France) using positive ion Electro-Spray Ionization (ESI, Waters, Manchester, UK).

For X-ray diffraction experiment, an appropriate single crystal of the title compound was selected and X-ray-data were collected using a STOE IPDS II single crystal X-ray diffractometer equipped with a two-dimensional image plate detector and with graphite monochromated  $\text{MoK}\alpha$  radiation ( $\lambda = 0.71073 \text{ \AA}$ ). SHELXS [18] program was used to solve the structure by direct methods and SHELXL [19] program was used for the refinement through the full-matrix least-squares method. These two programs are implemented in the WinGX [20] software. All atoms except hydrogen atoms were refined anisotropically. Hydrogen atoms were fixed geometrically at their calculated positions. Reflections with  $I \geq 2\sigma(I)$  were used in the structure determination. Structural results were presented using Mercury program [21].

### 2.2. Protein kinase assays

Kinase enzymatic activities were assayed in 384-well plates using the ADP-Glo™ assay kit (Promega, Madison, WI): a high-throughput screening method to measure kinase activity by quantifying the amount of ADP produced (described by Zegzouti et al., 2009) [22]. Briefly, the reactions were carried out in a final volume of 6  $\mu\text{L}$  for 30 min at 30 °C in appropriate kinase buffer, with either protein or peptide as substrate in the presence of 10  $\mu\text{M}$  ATP. After that, 6  $\mu\text{L}$  of ADP-Glo™ Kinase Reagent was added to stop the kinase reaction. After an incubation time of 50 min at room temperature (RT), 12  $\mu\text{L}$  of Kinase Detection Reagent was added for 1 h at RT. The transmitted signal was measured using the Envision (PerkinElmer, Waltham, MA) microplate luminometer and expressed in Relative Light Unit (RLU). Peptide substrates were obtained from Proteogenix (Schiltigheim, France). The following buffer was used for all the kinases tested: (Buffer "A") 10 mM  $\text{MgCl}_2$ , 1 mM EGTA, 1 mM DTT, 25 mM Tris-HCl pH 7.5, 50  $\mu\text{g}/\text{mL}$  heparin.

The following kinases were analyzed during this study:

*Hs\_CDK2/CyclinA* (cyclin-dependent kinase-2, human, kindly provided by Dr. A. Echalié-Glazer, Leicester, UK) was assayed in buffer A with 0.8  $\mu\text{g}/\mu\text{L}$  of histone H1 as substrate;

*Hs\_CDK5/p25* (human, recombinant, expressed in bacteria) was assayed in buffer A with 0.8  $\mu\text{g}/\mu\text{L}$  of histone H1 as substrate;

*Hs\_CDK9/CyclinT* (human, recombinant, expressed by baculovirus in Sf9 insect cells) was assayed in buffer A with 0.27  $\mu\text{g}/\mu\text{L}$  of the following peptide: YSPTSPSYSPTSPSYSPTSPSKKKK, as substrate;

*HsHASPIN-kd* (human, kinase domain, amino acids 470 to 798, recombinant, expressed in bacteria) was assayed in buffer A with 0.007  $\mu\text{g}/\mu\text{L}$  of Histone H3 (1–21) peptide (ARTKQTARKSTGG-KAPRKQLA) as substrate;

*Hs\_PIM1* (human proto-oncogene, recombinant, expressed in bacteria) was assayed in buffer A with 0.8  $\mu\text{g}/\mu\text{L}$  of histone H1 (Sigma #H5505) as substrate;

*Mm\_CLK1* (from *Mus musculus*, recombinant, expressed in bacteria) was assayed in buffer A with 0.027  $\mu\text{g}/\mu\text{L}$  of the following peptide: GRSRSRSRSR as substrate;

*Rn\_DYRK1A-kd* (*Rattus norvegicus*, amino acids 1 to 499 including the kinase domain, recombinant, expressed in bacteria, DNA vector kindly provided by Dr. W. Becker, Aachen, Germany) was assayed in buffer A with 0.033  $\mu\text{g}/\mu\text{L}$  of the following peptide: KKISGRLSPIMTEQ as substrate;

*Ssc\_GSK-3 $\alpha/\beta$*  (glycogen synthase kinase-3, porcine brain, native, affinity purified) isoforms were assayed in buffer A with 0.010  $\mu\text{g}/\mu\text{L}$  of GS-1 peptide, a GSK-3-selective substrate (YRRAAVPPSPSLSRHSSPHQSPeDEEE).

### 2.3. General procedure for the condensation reaction

A mixture of aminorhodanine (1 mmol), isatin (1 mmol) and 5  $\mu\text{L}$  of acetic acid in 2 mL of distilled ethanol was placed in a

cylindrical quartz reactor ( $\varnothing = 4$  cm). The reactor was introduced into a monomode microwave (Anton Paar) apparatus, for 5 min at 100 °C and 50 Watts. The crude reaction mixture was allowed to cool down at room temperature and ethanol (10 mL) or mixture of H<sub>2</sub>O/EtOH (10 mL) was directly added in the cylindrical quartz reactor. The resulting precipitated product was filtered off and was purified by recrystallization from ethanol if necessary.

**(Z)-3-((2-oxoindolin-3-ylidene)amino)-2-thioxothiazolidin-4-one (3a)**

Compound **3a** was obtained in 83% yield (0.78 g) from 3-aminorhodanine **1** and isatin **2a** as an orange powder, mp. 220 °C.

<sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  11.25 (s, 1H, NH), 8.84 (d, *J* = 7.9 Hz, 1H, CH<sub>ar</sub>), 7.44 (t, *J* = 7.7 Hz, 1H, CH<sub>ar</sub>), 7.12 (t, *J* = 7.7 Hz, 1H, CH<sub>ar</sub>), 6.97 (d, *J* = 7.8 Hz, 1H, CH<sub>ar</sub>), 5.98 (s, 2H, NH<sub>2</sub>).

<sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  192.4, 68.5, 164.0, 145.2, 133.7, 129.1, 128.3, 126.0, 122.7, 120.4, 111.3.

HRMS *m/z*: calcd for C<sub>11</sub>H<sub>7</sub>N<sub>3</sub>O<sub>2</sub>NaS<sub>2</sub> [M+Na]<sup>+</sup>: 276.99797, found: 276.9977; *m/z*: calcd for C<sub>11</sub>H<sub>7</sub>N<sub>3</sub>O<sub>2</sub>S<sub>2</sub>K [M+K]<sup>+</sup>: 315.96167, found: 315.9611; *m/z*: calcd for C<sub>11</sub>H<sub>7</sub>N<sub>3</sub>O<sub>2</sub>Na<sub>2</sub>S<sub>2</sub> [M-H+2Na]<sup>+</sup>: 322.97751, found 322.9769.

**(Z)-3-amino-5-(1-methyl-2-oxoindolin-3-ylidene)-2-thioxothiazolidin-4-one (3b)**

Compound **3b** was prepared in 58% yield (0.10 g) from 3-aminorhodanine **1** and N-methyl isatin **2b** as an orange powder, mp. 235 °C.

<sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.87 (d, *J* = 7.9 Hz, 1H, CH<sub>ar</sub>), 7.53 (t, *J* = 7.5 Hz, 1H, CH<sub>ar</sub>), 7.18 (t, *J* = 8.4 Hz, 2H, CH<sub>ar</sub>), 5.99 (s, 2H, NH<sub>2</sub>), 3.24 (s, 3H, Me).

<sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  192.2, 166.9, 163.9, 146.1, 133.6, 129.8, 127.9, 124.9, 123.3, 119.7, 110.1, 26.9.

HRMS *m/z*: calcd for C<sub>12</sub>H<sub>9</sub>N<sub>3</sub>O<sub>2</sub>NaS<sub>2</sub> [M+Na]<sup>+</sup>: 314.00339, found: 314.0031; *m/z*: calcd for C<sub>12</sub>H<sub>9</sub>N<sub>3</sub>O<sub>2</sub>S<sub>2</sub>K [M+K]<sup>+</sup>: 329.97732, found: 329.9773; *m/z*: calcd for C<sub>12</sub>H<sub>9</sub>N<sub>3</sub>O<sub>2</sub>Na<sub>2</sub>S<sub>2</sub> [M-H+2Na]<sup>+</sup>: 337.32254, found 337.3219.

**(Z)-3-amino-5-(5-fluoro-2-oxoindolin-3-ylidene)-2-thioxothiazolidin-4-one (3c)**

Compound **3c** was prepared in 67% yield (0.120 g) from 3-aminorhodanine **1** and 5-fluoro isatin **2c** as an orange powder, mp. 220 °C.

<sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  11.31 (s, 1H, NH), 8.62 (d, *J* = 8.7 Hz, 1H, CH<sub>ar</sub>), 7.51–7.35 (m, 1H, CH<sub>ar</sub>), 6.95–7.01 (m, 1H, CH<sub>ar</sub>), 6.01 (s, 2H, NH<sub>2</sub>).

<sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  192.2 (C=S), 168.5 (C<sub>8</sub>=O), 164.1 (C<sub>13</sub>=O), 158.0, 141.5, 130.9, 125.2, 121.1, 120.1, 114.7, 112.1.

HRMS *m/z*: calcd for C<sub>11</sub>H<sub>8</sub>N<sub>3</sub>O<sub>2</sub>FNaS<sub>2</sub> [M+Na]<sup>+</sup>: 317.97832, found: 317.9780; *m/z*: calcd for C<sub>11</sub>H<sub>8</sub>N<sub>3</sub>O<sub>2</sub>FS<sub>2</sub>K [M+K]<sup>+</sup>: 333.95225, found: 333.9517; *m/z*: calcd for C<sub>11</sub>H<sub>8</sub>N<sub>3</sub>O<sub>2</sub>FNa<sub>2</sub>S<sub>2</sub> [M-H+2Na]<sup>+</sup>: 340.96809, found 340.9674.

**(Z)-3-amino-5-(5-fluoro-1-methyl-2-oxoindolin-3-ylidene)-2-thioxothiazolidin-4-one (3d)**

Compound **3d** was prepared in 69% yield (0.884 g) from 3-aminorhodanine **1** and 5-fluoro N-methyl isatin **2d** as an orange powder, mp. 250 °C.

<sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.95–8.42 (m, 1H, CH<sub>ar</sub>), 7.35 (dt, *J* = 8.7, 4.3 Hz, 1H, CH<sub>ar</sub>), 7.13 (dd, *J* = 8.7, 4.5 Hz, 1H, CH<sub>ar</sub>), 5.94 (s, 2H, NH<sub>2</sub>), 3.23 (s, 1H, Me).

<sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  192.0 (C=S), 165.4, 164.1, 159.6, 157.3, 142.5, 131.7, 124.2, 120.4, 119.7, 114.7, 111, 31.15.

HRMS *m/z*: calcd for C<sub>12</sub>H<sub>8</sub>FN<sub>3</sub>O<sub>2</sub>NaS<sub>2</sub> [M+Na]<sup>+</sup>: 331.99397, found: 331.9937; *m/z*: calcd for C<sub>12</sub>H<sub>8</sub>FN<sub>3</sub>O<sub>2</sub>S<sub>2</sub>K [M+K]<sup>+</sup>: 347.96790, found: 347.9679; *m/z*: calcd for C<sub>12</sub>H<sub>8</sub>FN<sub>3</sub>O<sub>2</sub>Na<sub>2</sub>S<sub>2</sub> [M-H+2Na]<sup>+</sup>: 354.98374, found 354.9831.

**(Z)-3-amino-5-(5-methoxy-2-oxoindolin-3-ylidene)-2-thioxothiazolidin-4-one (3e)**

Compound **3e** was prepared in 46% yield (0.135 g) from 3-

aminorhodanine **1** and 5-methoxy isatin **2e** as a brown powder, mp. 220 °C.

<sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  11.01 (s, 1H, NH), 7.28 (d, *J* = 2.6 Hz, 1H, CH<sub>ar</sub>), 7.17 (dd, *J* = 8.6, 2.7 Hz, 1H, CH<sub>ar</sub>), 6.88 (d, *J* = 8.6 Hz, 1H, CH<sub>ar</sub>), 5.72 (s, 2H, NH<sub>2</sub>), 3.79 (s, 3H, OMe).

<sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  195.5, 170.9, 168.7, 163.0, 155.5, 141.1, 123.9, 123.0, 118.8, 113.2, 108.7, 56.3.

HRMS *m/z*: calcd for C<sub>12</sub>H<sub>9</sub>N<sub>3</sub>O<sub>3</sub>NaS<sub>2</sub> [M+Na]<sup>+</sup>: 329.99830, found: 329.9982; *m/z*: calcd for C<sub>12</sub>H<sub>9</sub>N<sub>3</sub>O<sub>3</sub>S<sub>2</sub>K [M+K]<sup>+</sup>: 345.97224, found: 345.9717; *m/z*: calcd for C<sub>12</sub>H<sub>8</sub>N<sub>3</sub>O<sub>3</sub>Na<sub>2</sub>S<sub>2</sub> [M-H+2Na]<sup>+</sup>: 351.98025, found 351.9797.

**(Z)-3-amino-5-(5-methoxy-1-methyl-2-oxoindolin-3-ylidene)-2-thioxothiazolidin-4-one (3f)**

Compound **3f** was prepared in 55% yield (0.99 g) from 3-aminorhodanine **1** and 5-methoxy N-methyl isatin **2f** according to the standard procedure described above as an orange powder, mp > 300 °C.

<sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.57 (s, 1H, CH<sub>ar</sub>), 7.21–6.99 (m, 2H, CH<sub>ar</sub>), 5.94 (s, 2H, NH<sub>2</sub>), 3.83 (s, 3H, OMe), 3.22 (s, 3H, Me).

<sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  192.0, 166.8, 163.8, 155.9, 140.2, 129.9, 125.6, 120.4, 119.0, 114.3, 110.4, 56.3, 26.9.

HRMS *m/z*: calcd for C<sub>13</sub>H<sub>11</sub>N<sub>3</sub>O<sub>3</sub>NaS<sub>2</sub> [M+Na]<sup>+</sup>: 344.01395, found: 344.01375; *m/z*: calcd for C<sub>13</sub>H<sub>11</sub>N<sub>3</sub>O<sub>3</sub>S<sub>2</sub>K [M+K]<sup>+</sup>: 360.46730, found: 360.4667; *m/z*: calcd for C<sub>13</sub>H<sub>11</sub>N<sub>3</sub>O<sub>3</sub>Na<sub>2</sub>S<sub>2</sub> [M-H+2Na]<sup>+</sup>: 367.34854, found 367.3479.

**(Z)-3-amino-5-(5-nitro-2-oxoindolin-3-ylidene)-2-thioxothiazolidin-4-one (3g)**

Compound **3g** was prepared in 55% yield (0.068 g) from 3-aminorhodanine **1** and 5-nitro N-isatin **2g** according to the standard procedure described above as a brown powder, mp. 255 °C.

<sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  11.88 (s, 1H, NH), 9.66 (d, *J* = 2.4 Hz, 1H, CH<sub>ar</sub>), 8.30 (dd, *J* = 8.7, 2.4 Hz, 1H, CH<sub>ar</sub>), 7.10 (d, *J* = 8.7 Hz, 1H, CH<sub>ar</sub>), 6.06 (s, 2H, NH<sub>2</sub>).

<sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  190.9, 168.5, 163.7, 150.0, 142.7, 132.8, 128.7, 123.4, 120.1.

HRMS *m/z*: calcd for C<sub>11</sub>H<sub>6</sub>N<sub>4</sub>O<sub>4</sub>NaS<sub>2</sub> [M+Na]<sup>+</sup>: 344.97227, found: 344.9724; *m/z*: calcd for C<sub>11</sub>H<sub>6</sub>N<sub>4</sub>O<sub>4</sub>S<sub>2</sub>K [M+K]<sup>+</sup>: 360.94621, found: 360.9459; *m/z*: calcd for C<sub>11</sub>H<sub>5</sub>N<sub>4</sub>O<sub>4</sub>Na<sub>2</sub>S<sub>2</sub> [M-H+2Na]<sup>+</sup>: 366.95421, found 366.9539.

**(Z)-3-amino-5-(1-methyl-5-nitro-2-oxoindolin-3-ylidene)-2-thioxothiazolidin-4-one (3h)**

Compound **3h** was prepared in 92% yield (0.806 g) from 3-aminorhodanine **1** and 5-nitro isatin **2h** as a yellow powder, mp. 350 °C.

<sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.66–8.56 (m, 1H, CH<sub>ar</sub>), 7.59–7.28 (m, 1H, CH<sub>ar</sub>), 7.13 (dd, *J* = 8.7, 4.5 Hz, 1H, CH<sub>ar</sub>), 5.94 (s, 2H, NH<sub>2</sub>), 3.23 (s, 3H, CH<sub>3</sub>).

<sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  191.1, 168.7, 167.3, 150.6, 143.0, 142.7, 133.5, 128.8, 122.8, 119.6, 110.2, 27.3.

HRMS *m/z*: calcd for C<sub>12</sub>H<sub>8</sub>N<sub>4</sub>O<sub>4</sub>NaS<sub>2</sub> [M+Na]<sup>+</sup>: 358.98847, found: 358.9884; *m/z*: calcd for C<sub>12</sub>H<sub>8</sub>N<sub>4</sub>O<sub>4</sub>S<sub>2</sub>K [M+K]<sup>+</sup>: 374.96240, found: 374.9618; *m/z*: calcd for C<sub>12</sub>H<sub>8</sub>N<sub>4</sub>O<sub>4</sub>Na<sub>2</sub>S<sub>2</sub> [M-H+2Na]<sup>+</sup>: 382.31954, found 382.3189.

**(Z)-3-amino-5-(5-chloro-2-oxoindolin-3-ylidene)-2-thioxothiazolidin-4-one(3i)**

Compound **3i** was prepared in 80% yield (0.06 g) from 3-aminorhodanine **1** and 5-chloro N-methylisatin **2i** as an orange powder, mp. 311 °C.

<sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  11.35 (s, 1H, NH), 8.83 (s, 1H, CH<sub>ar</sub>), 7.46 (d, *J* = 8.4 Hz, 1H, CH<sub>ar</sub>), 6.96 (d, *J* = 8.4 Hz, 1H, CH<sub>ar</sub>), 6.00 (s, 2H, NH<sub>2</sub>).

<sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  191.9, 168.2, 163.9, 143.9, 132.7, 131.1, 127.5, 126.6, 124.8, 121.7, 112.6.

HRMS *m/z*: calcd for C<sub>11</sub>H<sub>6</sub>N<sub>3</sub>O<sub>2</sub>ClNaS<sub>2</sub> [M+Na]<sup>+</sup>: 382.31954, found: 382.3192; *m/z*: calcd for C<sub>11</sub>H<sub>6</sub>N<sub>3</sub>O<sub>2</sub>ClS<sub>2</sub>K [M+K]<sup>+</sup>:

349.92270, found: 349.9226;  $m/z$ : calcd for  $C_{11}H_6N_3O_2ClNa_2S_2 [M-H+2Na]^+$ : 356.93853, found 356.9379.

**(Z)-3-amino-5-(5-chloro-1-methyl-2-oxoindolin-3-ylidene)-2-thioxothiazolidin-4-one (3j)**

Compound **3j** was prepared in 75% yield (0.830 g) from 3-aminorhodanine **1** and 5-chloro N-methylisatin **2j** as an orange powder, mp > 300 °C.

$^1H$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  8.89 (s, 1H,  $CH_{ar}$ ), 7.57 (d,  $J=8.7$  Hz, 1H,  $CH_{ar}$ ), 7.17 (d,  $J=8.4$  Hz, 1H,  $CH_{ar}$ ), 5.96 (s, 2H,  $NH_2$ ), 3.25 (s, 3H,  $CH_3$ ).

$^{13}C$  NMR (75 MHz, DMSO)  $\delta$  191.6, 166.6, 163.9, 144.7, 132.6, 131.8, 127.2, 123.7, 120.9, 111.4, 96.05 ( $CCl_4$ ), 27.1.

HRMS  $m/z$ : calcd for  $C_{12}H_8N_3O_2ClNaS_2 [M+Na]^+$ : 347.96442, found: 347.9642;  $m/z$ : calcd for  $C_{12}H_8N_3O_2ClS_2K [M+K]^+$ : 363.93835, found: 363.9378;  $m/z$ : calcd for  $C_{12}H_8N_3O_2ClNa_2S_2 [M-H+2Na]^+$ : 370.95418, found 370.9541.

**(Z)-3-amino-5-(5-bromo-2-oxoindolin-3-ylidene)-2-thioxothiazolidin-4-one (3k)**

Compound **3k** was prepared in 60% yield (0.032 g) from 3-aminorhodanine **1** and 5-bromo isatin **2k** as a brown powder, mp > 300 °C.

$^1H$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  11.25 (s, 1H, NH), 9.02 (s, 1H,  $CH_{ar}$ ), 7.60 (d,  $J=8.2$  Hz, 1H,  $CH_{ar}$ ), 6.95 (d,  $J=8.4$  Hz, 1H,  $CH_{ar}$ ), 5.96 (s, 2H,  $NH_2$ ).

$^{13}C$  NMR (75 MHz, DMSO- $d_6$ )  $\delta$  191.9, 168.1, 164.0, 144.1, 135.5, 131.2, 130.2, 124.4, 122.1, 114.2, 113.1.

HRMS  $m/z$ : calcd for  $C_{11}H_6N_3O_2BrNaS_2 [M+Na]^+$ : 377.89825, found: 377.8980;  $m/z$ : calcd for  $C_{11}H_6N_3O_2BrS_2K [M+K]^+$ : 398.87219, found: 398.8720;  $m/z$ : calcd for  $C_{11}H_6N_3O_2BrNa_2S_2 [M-H+2Na]^+$ : 400.88802, found 400.8880.

**(Z)-3-amino-5-(5-bromo-2-oxoindolin-3-ylidene)-2-thioxothiazolidin-4-one (3l)**

Compound **3l** was prepared in 55% yield (0.102 g) from 3-aminorhodanine **1** and 5-bromo N-methylisatin **2l** as a brown powder, mp > 300 °C.

$^1H$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  8.92 (s, 1H,  $CH_{ar}$ ), 7.41 (d,  $J=8.5$  Hz, 1H,  $CH_{ar}$ ), 7.05 (d,  $J=8.5$  Hz, 1H,  $CH_{ar}$ ), 5.89 (s, 2H,  $NH_2$ ).

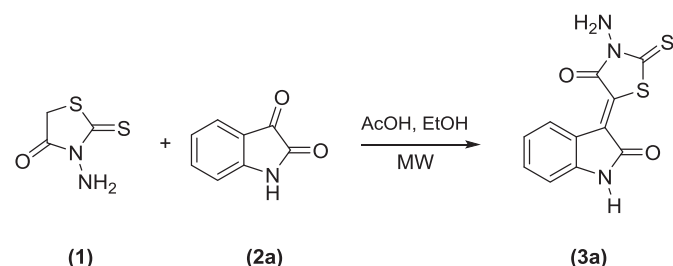
$^{13}C$  NMR (75 MHz, DMSO- $d_6$ )  $\delta$  190.5, 168.2, 165.6, 147.6, 136.6, 130.9, 131.8, 125.5, 122.6, 116.2, 115.5, 0.27.7.

HRMS  $m/z$ : calcd for  $C_{12}H_8N_3O_2BrNaS_2 [M+Na]^+$ : 391.91390, found: 391.9137;  $m/z$ : calcd for  $C_{12}H_8N_3O_2BrS_2K [M+K]^+$ : 407.88784, found: 407.8873;  $m/z$ : calcd for  $C_{12}H_8N_3O_2BrNa_2S_2 [M-H+2Na]^+$ : 414.90367, found 414.9301.

### 3. Results and discussion

#### 3.1. Chemistry

Initially, the microwave-assisted Knoevenagel condensation of N-aminorhodanine (**1**) with isatin (**2a**) using a solvent-free procedure was studied (Scheme 1, Table 1). Optimization of the



**Scheme 1.** General synthesis of isatin-aminorhodanine (**3a**) under microwave irradiations.

**Table 1**  
Optimization of operating conditions for the preparation of (**3a**) under microwave irradiations.

Entry	T (°C)	P (W)	t (min)	Solvent	Conversion <sup>a</sup> (%)
1	100	50	5	EtOH	100
2	100	100	5	EtOH	100
3	150	100	5	EtOH	95
4	100	50	5	Solvent Free	85
5	–	–	24h	EtOH	95

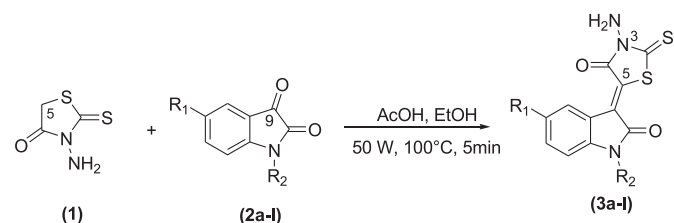
<sup>a</sup> Conversions were calculated by  $^1H$  NMR.

microwave dielectric heating reaction conditions, using continuous irradiation led to the setting of the reaction temperature, and showed that the irradiation of an equimolar mixture of (**1**) and (**2a**) in ethanol at 100 °C (50 W) for 5 min (entry 1) allows their full conversion into product (**3a**). Similar result was obtained for an irradiation power of 100 W (entry 2), however further increase of the reaction temperature induces a decrease of conversion (entry 3). Besides that, the reaction leads to a conversion of 85% when it was carried out in solvent free media (entry 4). Finally, to appreciate the time and energy savings brought by this method, we realized the same reaction by conventional heating. After 24 h in reflux of ethanol, the conversion rate was 95% (entry 5).

Special attention was focused on the determination of the structure of compounds obtained from both methods, i.e. microwave activation and conventional heating in order to ensure that they are similar.  $^1H$  NMR,  $^{13}C$  NMR and DEPT experiments in DMSO- $d_6$  and  $D_2O$  confirmed the absence of methylenic proton in favor of  $NH_2$  signals. The  $D_2O$ -exchangeable singlet signal was observed at 5.98 ppm due to  $NH_2$  proton (see supplementary file). One can conclude that the condensation reaction occurred at C5 position of the aminorhodanine and not at the primary amine to achieve the Schiff's base derivative.

With these optimized conditions, i.e. (1)/(2a-1) = 1:1, ethanol, 100 °C, 50 W, 5 min, we tested the scope of this method with several structurally varied isatin derivatives (**2a-l**) (Scheme 2) and the results are collected in Table 2. An assembly of 12 compounds was synthesized in good to excellent yields using this protocol. They were obtained pure after simple precipitation in ethanol or a mixture of EtOH/ $H_2O$ .

The structures of these compounds were confirmed by  $^1H$  NMR,  $^{13}C$  NMR and High-resolution mass spectroscopy (HMRS). Furthermore, X-ray single crystal analysis of compound (**3c**) was carried out to confirm its structure. All crystallographic data and refinement details are collected in Table 3 and the X-Ray diffraction structure of (**3c**) is shown in Fig. 4.<sup>1</sup>



**Scheme 2.** Synthesis of isatin-aminorhodanine derivatives.

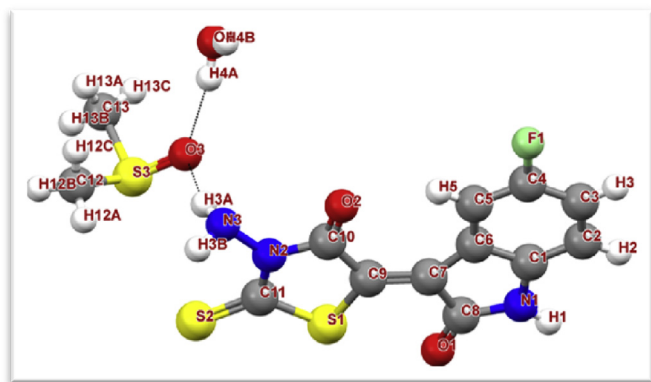
<sup>1</sup> The supplementary crystallographic data have been deposited at the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ (UK), Tel.: (p44) 1223/336 408, fax: (p44) 1223/336 033, E-mail [deposit@ccdc.cam.ac.uk](mailto:deposit@ccdc.cam.ac.uk), World Wide Web: <http://www.ccdc.cam.ac.uk> (deposition No. CCDC 1878827).

**Table 2**  
Yields of synthesized isatin-aminorhodanine hybrids (**3a-l**).

Product	R <sub>1</sub>	R <sub>2</sub>	Yield (%)
3a	H	H	83
3b	H	CH <sub>3</sub>	56
3c	F	H	67
3d	F	CH <sub>3</sub>	69
3 <sup>e</sup>	OCH <sub>3</sub>	H	46
3f	OCH <sub>3</sub>	CH <sub>3</sub>	55
3g	NO <sub>2</sub>	H	80
3h	NO <sub>2</sub>	CH <sub>3</sub>	92
3i	Cl	H	80
3j	Cl	CH <sub>3</sub>	75
3k	Br	H	60
3l	Br	CH <sub>3</sub>	55

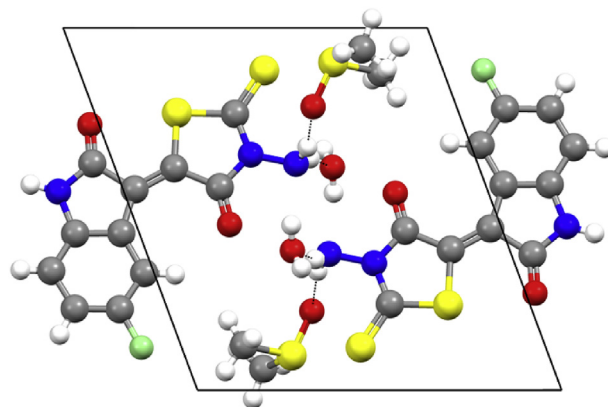
**Table 3**  
Crystal data and structure refinement details.

Empirical formula	C <sub>13</sub> H <sub>14</sub> N <sub>3</sub> O <sub>4</sub> S <sub>3</sub> F
Formula weight	391.45
Crystal size (mm)	0.28 × 0.21 × 0.18
Temperature (K)	293
Crystal system, space group	Triclinic, P-1
Unit cell dimensions	
a (Å)	7.0766 (10)
b (Å)	11.4780 (14)
c (Å)	11.6411 (17)
α (°)	68.865 (10)
β (°)	84.260 (12)
γ (°)	72.319 (10)
Wavelength (Å)	0.71073
Volume (Å <sup>3</sup> )	840.2 (2)
Z, calculated density (mg/m <sup>3</sup> )	2/1.547
θ range for data collection	3.02–25.05
Limiting indices	−8 ≤ h ≤ 8, −13 ≤ k ≤ 13, −13 ≤ l ≤ 13
Reflections collected/total/unique	7253/2980/1915
Refinement method	full-matrix least-squares on F <sup>2</sup> data
Parameters	223
Goodness of fit on F <sup>2</sup>	1.080
Final R indices	
R <sub>1</sub>	0.0611
wR <sub>2</sub>	0.0982
R indices (all data)	
R <sub>1</sub>	0.1031
wR <sub>2</sub>	0.2105



**Fig. 4.** General view of molecule 3c with atomic numbering.

X-Ray diffraction results agree well with those given by the NMR spectroscopy. This compound crystallizes in the triclinic system. The stereochemistry of arylidene double bond C (7) = C (9) was explicitly assigned the “cis” isomer in DMSO media. A view of the



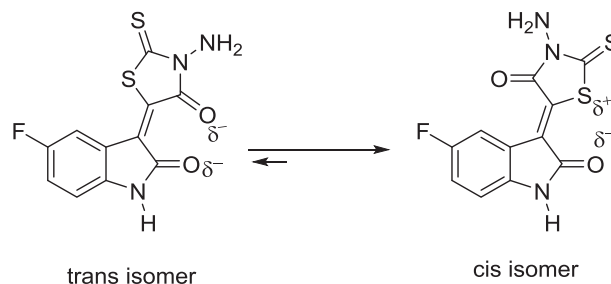
**Fig. 5.** Crystal-packing view in the unit cell along a axis.

unit cell contents for (**3c**) is shown in Fig. 5. This configuration may be ascribed to the favorable electrostatic interaction between the C8-carbonyl oxygen atom of the isatin core and the partially positively charged S1 of the aminorhodanine heterocycle (Scheme 3). Similar results have been reported by Sun et al. for 3-substituted indolin-2-ones derivatives [23].

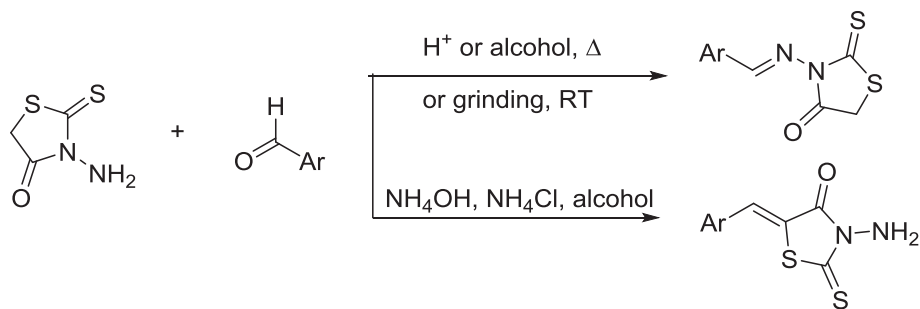
Encouraged by the success of the above reaction, we became interested in the mechanism. Several condensation examples of N-aminorhodanine with aldehyde or ketone derivatives have been reported in literature [24] Depending on the reaction conditions, N-aminorhodanine can react with aldehyde or ketone derivatives either at the C-5 position through a condensation reaction to form the 5-(aryl)alkylidene compound [25,26] or with primary amine group to conduct to the Schiff base derivative. The former reaction, known as Girard's method is usually favored in ammoniacal medium while heating in alcohols or in the presence of acid catalysis as glacial acetic acid or hydrochloric acid gives usually the Schiff base compounds as illustrated in Scheme 4. Likewise, solvent-free mechanochemical route has been also used to prepare Schiff base derivatives of 3-aminorhodanine [27].

The dual reactivity of 3-aminorhodanine was ascribed to its two tautomeric forms I and II (Scheme 5). According to Stainier-Lapiere works [28] in alcoholic media, 3-aminorhodanine tends to react in the “hydrazine” form I to give the Schiff base compound. In contrast, basic media, as ammonia, promotes displacement of the equilibrium to the right side with the formation of the ammonium salt of the thio form II. The occurrence of a tautomeric thio form (form II) explains the formation of the enolic form IV and its subsequent Knoevenagel condensation with isatin.

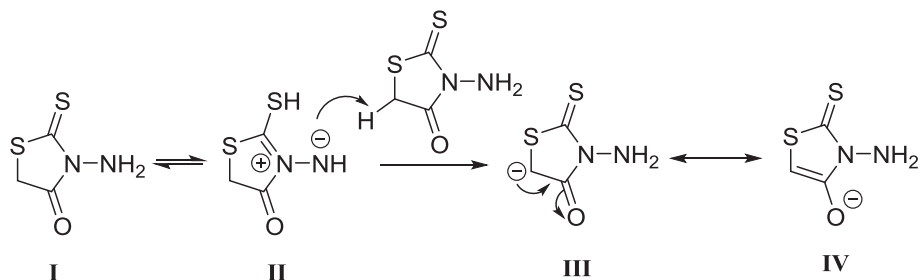
It is worth mentioning that the opposite behavior was obtained under our acid catalysis operating conditions whether under microwaves irradiation or by conventional heating, since only the condensation product at C-5 position of the aminorhodanine was



**Scheme 3.** Isomerism interpretation of compound 3e.



**Scheme 4.** Condensation reactions of 3-aminorhodanine with aldehyde.



**Scheme 5.** Tautomeric forms of 3-aminorhodanine.

obtained. Furthermore, the reaction of aminorhodanine with isatin **2a** or 5-fluoroisatin **2c** in reflux of ethanol conducts also to the same 5-substituted derivatives of 3-aminorhodanine and not to the claimed Schiff base compounds reported in literature [29,30].

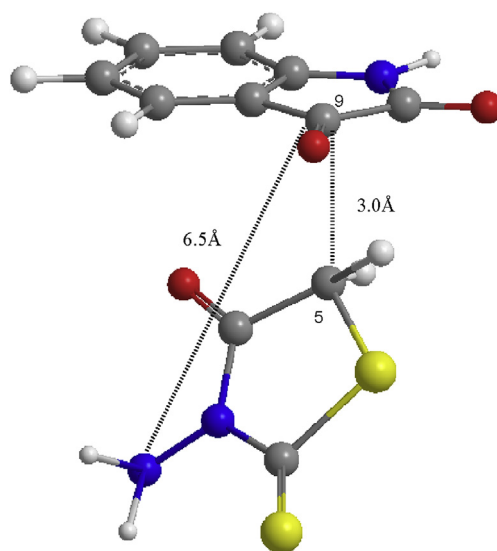
Thus, it seems that this unexpected reactivity is rather related to the nature of used ketone derivative, and more particularly to the steric hindrance of isatin, than to the operating conditions used to carry out the condensation reaction. Preliminary theoretical calculations carried out by the MM2 method confirmed that the most favorable approach between isatin and aminorhodanine takes place through a transition state where the C9 carbon of the carbonyl function of isatin is at a distance of 6.5 Å from the nitrogen atom of the aminorhodanine and at only 3.0 Å of the C5 atom of the aminorhodanine (Scheme 6). Further theoretical calculations are underway to explain this feature.

### 3.2. Biology

In the first instance, the series of compounds **3a–l** were tested for their ability to inhibit eight kinases (*Mm*CLK1, *Hs*HASPIN, *Hs*CDK2/CyclinA, *Hs*CDK5/p25, *Hs*CDK9/CyclinT, *Ssc*GSK3 $\alpha/\beta$ , *Rn*DYRK1A and *Hs*PIM1). This class of enzymes catalyzes the protein phosphorylation on serine, threonine and tyrosine residues. Kinases play numerous crucial roles in various cellular processes (e.g. cell signaling, cell division cycle) and are frequently deregulated in human diseases. Relative kinases inhibition percentages of each compound were measured at a molar concentration of 10  $\mu$ M. This class of *in vitro* kinase assays is summarized in Table 4.

Results showed that the main inhibition activity of the tested compounds was focused on three proteins kinases (*Mm*CLK1, *Hs*CDK2/CyclinA and *Hs*PIM1). Compounds **3g** and **3k** exhibit an additional significant activity for *Ssc*GSK3 $\alpha/\beta$  and *Hs*DYRK1A kinases. The IC<sub>50</sub> values of these derivatives for *Mm*CLK1 and *Hs*PIM1 kinases were then determined from the dose response curves (Sigma-Plot). Results are summarized in Table 5.

The SAR study revealed that the nature of both substituent in position 1 and 5 of isatin core play a key role on the kinase



**Scheme 6.** Lowest energy transition state for isatin – aminorhodanine approach calculated by MM2 method.

inhibition activity. Compounds bearing a bromine atom in position 5 (**3k** and **3l**) are the most active towards pim1 kinase. Compound **3k**, bearing a secondary amine, shows a substantial additional activity for CLK1 kinase.

It is worth mentioning that compound **3l** (N-methylated isatin) have a very selective and potent inhibitor activity against PIM1 kinase with sub-micro molar inhibition (IC<sub>50</sub> = 0.77  $\mu$ M) while **3e** and **3k** derivatives are mildly active toward PIM1 kinase with IC<sub>50</sub> values in the micro-molar range ranging from 5.29 to 1.8  $\mu$ M, respectively. Other substitution with halogens or small polar groups does not improve inhibition activity towards these two kinases.

Molecular recognitions including drug-protein interactions play



**Table 4**

Percentage of kinase inhibition of hybrids (3a–l). The table displays the remaining activities detected after treatment with 10  $\mu\text{M}$  of the tested compounds. Results are expressed in % of inhibition, i.e. 0% of inhibition is measured in the absence of inhibitor. ATP concentration used in the kinase assays was 10  $\mu\text{mol/L}$  (values are means,  $n = 2$ ). Kinases are from human origin (*Homo sapiens*) unless specified: *Mm*, *Mus musculus*; *Rn*, *Rattus norvegicus*; *Ssc*, *Sus scrofa*.

(% Inhibition at 10 $\mu\text{M}$ )											
Product	R <sub>1</sub>	R <sub>2</sub>	<i>Mm</i> CLK1	HASPIN	CDK2/CyclinA	CDK5/p25	CDK9/CyclinT	<i>Ssc</i> GSK3 $\alpha/\beta$	<i>Rn</i> DYRK1A	PIM1	
3a	H	H	0	0	5	0	1	0	0	30	
3b	H	CH <sub>3</sub>	3	0	4	5	0	0	0	33	
3c	F	H	34	0	30	0	1	0	0	3	
3d	F	CH <sub>3</sub>	29	0	19	0	0	6	0	0	
3e	OCH <sub>3</sub>	H	78	16	8	0	0	0	38	18	
3f	OCH <sub>3</sub>	CH <sub>3</sub>	24	0	9	0	0	1	0	0	
3g	NO <sub>2</sub>	H	16	39	19	8	19	75	23	23	
3h	NO <sub>2</sub>	CH <sub>3</sub>	4	2	32	0	0	0	0	0	
3i	Cl	H	43	6	25	10	0	4	0	21	
3j	Cl	CH <sub>3</sub>	59	0	43	0	7	1	0	0	
3k	Br	H	94	10	53	0	21	55	64	33	
3l	Br	CH <sub>3</sub>	0	9	1	0	0	23	0	93	

**Table 5**

Dose-dependent effect of isatinylidene aminorhodanine (3a–l) on the inhibition activity of *Mm*CLK1 and PIM1 protein kinases<sup>a</sup>.

Product	R <sub>1</sub>	R <sub>2</sub>	IC <sub>50</sub> ( $\mu\text{M}$ )/ <i>Mm</i> CLK1	IC <sub>50</sub> ( $\mu\text{M}$ )/PIM1
3a	H	H	>10 <sup>b</sup>	>10
3b	H	CH <sub>3</sub>	>10	>10
3c	F	H	>10	>10
3d	F	CH <sub>3</sub>	>10	>10
3e	OCH <sub>3</sub>	H	>10	5.29
3f	OCH <sub>3</sub>	CH <sub>3</sub>	>10	>10
3g	NO <sub>2</sub>	H	NT <sup>c</sup>	NT
3h	NO <sub>2</sub>	CH <sub>3</sub>	>10	>10
3i	Cl	H	NT	NT
3j	Cl	CH <sub>3</sub>	>10	>10
3k	Br	H	4.67	1.8
3l	Br	CH <sub>3</sub>	>10	0.77

<sup>a</sup>The IC<sub>50</sub> values calculated from the dose-response curves are reported in  $\mu\text{M}$ . ATP concentration used in the kinase assays was 10  $\mu\text{mol/L}$  (values are means,  $n = 3$ ).

<sup>b</sup> > 10, inhibitory but IC<sub>50</sub> > 10  $\mu\text{M}$ .

<sup>c</sup> NT not tested.

a key role in this process. Therefore, determination of the binding mode and affinity is crucial to understand the interaction mechanisms and to the design new compounds with better and more specific inhibitory properties. A molecular docking study to identify the active sites of the kinases that interact with the synthesized molecules is currently underway.

Regarding these results, compound **3l** appears to be a potent and selective agent for PIM1 kinase, and could be particularly interesting in the development of new selective inhibitors of PIM1 kinase in targeted therapy domain. Compound **3k**, that inhibits both CLK1 and PIM1 kinases is interesting as a hybrid molecule for multifactorial disease. These preliminary results open up promising ways for the development of new, more active and selective molecules in the fight against multifactorial diseases. Further biological investigations are underway.

#### 4. Conclusion

In conclusion, we have developed a simple, effective, ecological, fast and energy efficient new procedure for the preparation of new isatin-aminorhodanine-based hybrid agents. The structure and stereochemistry of these hybrids were determined through NMR and X-Ray diffraction analyses. Regardless the used operating conditions, unexpected Knoevenagel condensation of isatin derivatives on C-5 position of aminorhodanine was obtained.

The evaluation of the inhibitory potential of these derivatives

towards eight kinases showed that the brominated hybrids (**3k**) and (**3l**) are the most active against the PIM1 and CLK1 kinases. The methylation of the nitrogen atom of the isatin allows the brominated hybrid (**3l**) to gain in selectivity. These promising results motivate us to refine our structures to further enhance their activity and selectivity towards a wider range of kinases.

#### Funding

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#### Conflicts of interest

The authors declare that they have no conflict of interest.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.molstruc.2019.04.122>.

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